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Diadromie, dispersion et histoire évolutive des complexes "Caridina nilotica" et "Caridina weberi" (Crustacea - Decapoda - Atyidae) dans les systèmes insulaires de l'Indo-Pacifique

Valentin De Mazancourt

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MUSEUM NATIONAL D'HISTOIRE NATURELLE
Ecole Doctorale Sciences de la Nature et de l'Homme – ED 227

Année 2018

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THESE

Pour obtenir le grade de

DOCTEUR DU MUSEUM NATIONAL D'HISTOIRE NATURELLE

Spécialité : Systématique

Présentée et soutenue publiquement par

Valentin Seizilles de Mazancourt

Le 12 octobre 2018

Diadromie, dispersion et histoire évolutive des complexes *Caridina nilotica* et *Caridina weberi* (Crustacea : Decapoda : Atyidae) dans les systèmes insulaires de l'Indo-Pacifique

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Sous la direction de **: M. Philippe Keith, Professeur**

JURY :

M. von RINTELEN Thomas	**Conservateur, Museum für Naturkunde, Berlin (Allemagne)**	**Rapporteur**
M. POUPIN Joseph	**Chargé de Recherche, HDR, Ecole Navale, Brest (29)**	**Rapporteur**
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Introduction.

Introduction.

Où l'on présente ***la famille des Atyidae****, avec leur systématique, leur distribution géographique et quelques éléments de biologie,* ***le genre*** **Caridina***, avec sa systématique complexe,* ***la diadromie*** *avec ses différents cas particuliers et les techniques de sclérochronologie appliquées à son étude,* ***la zone Indo-Pacifique****, avec sa géographie, sa géologie, sa biogéographie et sa courantologie****, les systèmes insulaires****, en détaillant la typologie des îles, leurs caractéristiques et leurs différents habitats aquatiques,* ***la taxonomie intégrative*** *et ses applications, et enfin,* ***les objectifs de la thèse****.*

Where are presented the ***family Atyidae*** *with their taxonomy, geographic distribution and a few details about their biology,* ***genus*** **Caridina***, with its complex taxonomy,* ***diadromy*** *with its different particular cases and the sclerochronology techniques applied to its study,* ***the Indo-Pacific area****, with its geography, geology, biogeography and currentology,* ***island systems****, detailing islands typology, their characteristics and the different aquatic habitats,* ***integrative taxonomy*** *and its applications, and finally,* ***the aims of the thesis****.*

1. Introduction

1.1 Préambule

Peu connues et pourtant présentes en nombre dans presque tous les habitats dulcicoles tropicaux, toujours au cœur des préoccupations des hydrobiologistes et des écologues, par exemple comme bio-indicateurs pour des études d'écotoxicologie, les crevettes d'eau douce sont un modèle particulièrement intéressant. Elles soulèvent en effet de nombreuses questions ; pourquoi et comment ces organismes primairement marins se sont-ils adaptés à la vie en eau douce ? Comment une telle diversité d'espèces a-t-elle pu se mettre en place ? Pourquoi certaines espèces sont-elles restreintes à une île, voire à un bassin versant de cette île tandis que d'autres sont largement réparties à l'échelle d'océans entiers ? De fait, les crevettes d'eau douce ont depuis longtemps attisé la curiosité des scientifiques, ayant été étudiées par les plus éminents carcinologistes (Henri Milne Edwards, Eugène Louis Bouvier, Lipke Holthuis, Théodore Monod…). Cependant, l'extraordinaire diversité caractéristique de ce groupe est la source d'une grande confusion taxonomique, rendant hasardeuse toute conclusion biologique quant aux espèces mal délimitées. De plus, avec les menaces grandissantes qui pèsent sur les milieux naturels, et en particulier les eaux douces tropicales (prélèvements d'eau excessifs, pollution d'origine anthropique, eutrophisation, artificialisation des cours d'eau, etc.), il devient urgent de parfaire la connaissance de ces espèces – à commencer par leur taxonomie si l'on veut pouvoir mettre en place leur protection.

1.2 La famille des Atyidae

1.2.1 Systématique

Les Atyidae De Haan, 1849 sont une famille de crustacés de l'ordre des Decapoda Latreille, 1802 appartenant à l'infra-ordre des Caridea Dana, 1852 et représentant la seule famille de la super-famille des Atyoidea De Haan, 1849.

Initialement composée des genres *Atya* Leach, 1816, *Caridina* H. Milne Edwards, 1837, *Ephyra* Roux, 1831 et *Oplophorus* H. Milne Edwards, 1837 lors de sa description par De Haan (1849), sa composition a fluctué avec le temps (Tableau 1.1). Lors de la première révision de la famille, Kingsley (1879) y reconnaît deux sous-familles, celle des Atyinae, caractérisée par l'absence d'exopodites aux péréiopodes, et celle des Ephyrinae, possédant des exopodites. Par la suite, Ortmann (1894) reprend cette séparation en

deux sous-familles, renommant les Ephyrinae en Xiphocarinae. Lors de la révision majeure de la famille dans sa monographie dédiée, Bouvier (1925) propose de classer les Atyidae en 4 groupes : La « forme Acanthéphyroïde », composée uniquement du genre *Xiphocaris*, la « série paratyenne », la « série caridinenne » et la série « caridellienne », basées notamment sur des caractères branchiaux. Cette division reste inchangée jusqu'en 1970 où Monod & Cals distinguent la « série typhlatyenne » de la « série paratyenne ». En 1986, Holthuis érige ces groupes en sous-familles, proposant Atyinae, Caridellinae, Paratyinae, Typhlatyinae et Xiphocaridinae. Le genre *Xiphocaris* est exclu de la famille des Atyidae par Chace (1992) qui établit pour eux la famille des Xiphocarididae Ortmann, 1894 au sein de la super-famille des Nematocarcinoidea Smith, 1884. Holthuis (1993) présente la famille des Atyidae avec ses quatre sous-familles. Après une étude de phylogénie moléculaire, Sket & Zaksek (2009) font de la sous-famille des Typhlatyinae un synonyme des Paratyinae. La reconstruction phylogénétique de la famille réalisée par Rintelen *et al.* (2012) partage désormais en cinq groupes que les auteurs n'ont pas décrits comme sous-famille, faute de synapomorphies évidentes : l'« *Atya* group », le « *Caridella* group » (auquel appartient le genre *Caridina*), le « *Paratya* group », le « *Typhlatya* group » et les Syncaridinae. C'est cette classification qui sera suivie dans le reste du mémoire.

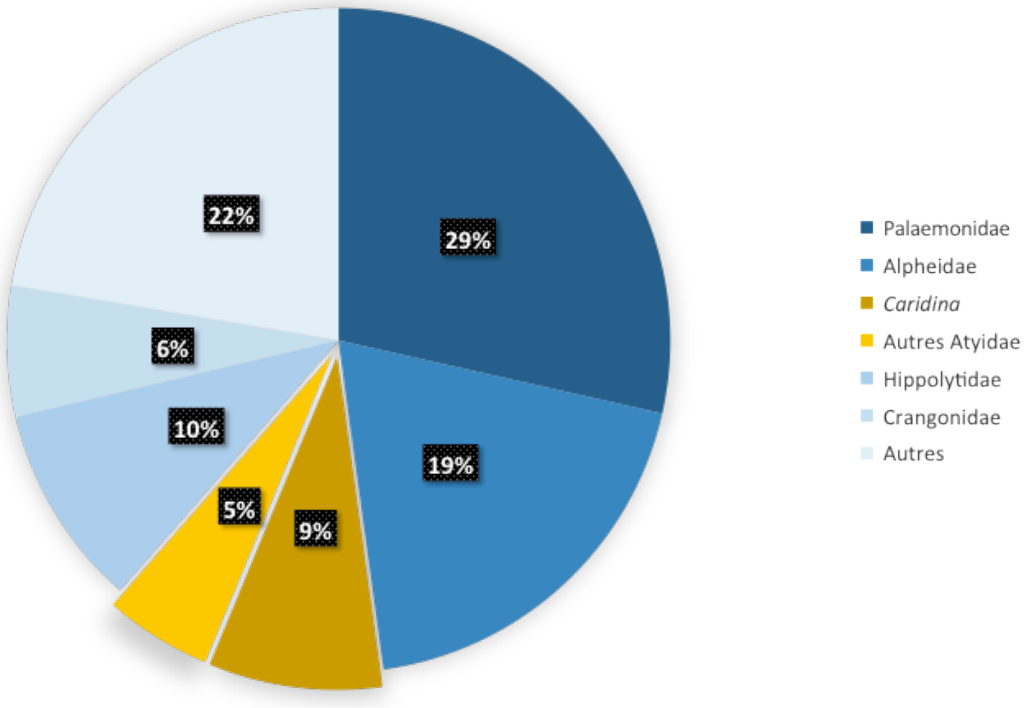


Figure 1.1 : Répartition du nombre d'espèce par famille et pour le genre *Caridina* au sein des Caridea

Figure 1.1: Distribution of the number of species for each family of Caridea and for genus *Caridina*

Seuls cinq espèces fossiles appartenant à trois genres sont reconnues comme appartenant à la famille des Atyidae. Il s'agit de *Delclosia roselli* (Vía, 1971), *D. martinelli* Rabadà, 1990 (toutes deux du Crétacé inférieur d'Espagne), *Atyoida roxoi* Beurlen, 1950 (du Crétacé inférieur du Brésil), *A. tremembeensis* Beurlen , 1950 (Oligocène supérieur ou Miocène du Brésil) et *Caridina nitida* A. Milne Edwards, 1879 (Oligocène inférieur de France).

Composée de 44 genres et près de 500 espèces, il s'agit de la 3e famille la plus diversifiée parmi les Caridea, derrière les Palaemonidae et les Alpheidae (De Grave & Fransen, 2011) (Figure 1.1).

D'après les dernières phylogénies de l'infra-ordre, la famille des Atyidae est un des groupes les plus basaux, ayant divergé très tôt dans l'histoire des Caridea (Bracken *et al.*, 2009 ; Davis *et al.*, 2018), ce qui confirme les observations de l'organisation des pièces buccales qui a semblé primitive à différents auteurs (Fryer, 1960 ; Felgenhauer & Abele, 1985).

Tableau 1.1 : Historique de la systématique des Atyidae. Rouge : genres nouvellement décrits, Bleu : genres déjà décrits déplacés d'une sous-famille à une autre.

Tableau 1.1: Systematic history of the Atyidae. Red: newly described genera, blue: genera already described moved from a subfamily to another.

De Haan, 1849	Bouvier, 1925	Monod & Cals, 1970	Holthuis, 1993	Sket & Zakšek, 2009	von Rintelen *et al.* 2012 (et depuis)
Atya Leach, 1816 ; *Caridina* H. Milne Edwards, 1837 ; *Ephyra* Roux, 1831 ; *Oplophorus* H. Milne Edwards, 1837	**Série caridinienne** *Atya* ; *Ortmannia* Rathbun, 1901 ; *Caridina* ; *Micratya* Bouvier, 1913	**Série caridinienne** *Atya* ; *Ortmannia* ; *Caridina* ; *Micratya* ; *Neocaridina* Kubo, 1938 ; *Potimirim* Holthuis, 1954	**Atyinae De Haan, 1849** *Atya* ; *Atyoida* ; *Caridina* ; *Micratya* ; *Neocaridina* ; *Potimirim*	**Atyinae** *Atya* ; *Atyoida* ; *Caridina* ; *Micratya* ; *Neocaridina* ; *Potimirim*	***Atya* group** *Atya* ; *Atyoida* ; *Micratya* ; *Potimirim* ; *Atyopsis* Chace, 1983 ; *Australatya* Chace, 1983 ; *Jonga* Hart, 1961
	Série caridellienne *Caridinopsis* Bouvier, 1912 ; *Limnocaridella* Bouvier, 1913 ; *Limnocaridina* Calman, 1899 ; *Caridella* Calman, 1906 ; *Atyella* Calman, 1906	**Série caridellienne** *Caridinopsis* ; *Limnocaridella* ; *Limnocaridina* ; *Caridella* ; *Atyella* ; *Caridinides* Calman, 1926 ; *Halocaridina* Holthuis, 1963 ; *Parisia* Holthuis, 1956	**Caridellinae Holthuis, 1986** *Caridinopsis* ; *Limnocaridella* ; *Limnocaridina* ; *Caridella* ; *Atyella* ; *Caridinides* ; *Halocaridina* ; *Parisia* ; *Halocaridinides* Fujino & Shokita, 1975 ; *Edoneus* Holthuis, 1978 ; *Pycneus* Holthuis, 1986	**Caridellinae** *Caridinopsis* ; *Limnocaridella* ; *Limnocaridina* ; *Caridella* ; *Atyella* ; *Caridinides* ; *Halocaridina* ; *Parisia* ; *Halocaridinides* ; *Edoneus* ; *Pycneus*	***Caridella* group** *Caridinopsis* ; *Limnocaridina* ; *Caridella* ; *Atyella* ; *Parisia* ; *Halocaridinides* ; *Edoneus* ; *Pycneus* ; *Lancaris* Cai & Bahir, 2005 ; *Marosina* Cai & Ng, 2005 ; *Paracaridina* Liang, Guo & Tang, 1999 ; *Pycnisia* Bruce, 1992 ; *Sinodina* Liang & Cai, 1999 *Neocaridina* ; *Caridina* ; *Elephantis* Castelin, Marquet & Klotz, 2013
	Série paratyenne *Atyaephyra* de Brito Capello, 1867 ; *Troglocaris* Dormitzer, 1853 ; *Paratya* Miers, 1882 ; *Syncaris* Holmes, 1900 ; *Palaemonias* Hay, 1902 ; *Dugastella* Bouvier, 1912	**Série paratyenne** *Atyaephyra* ; *Troglocaris* ; *Paratya* ; *Syncaris* ; *Palaemonias* ; *Dugastella*	**Paratyinae Holthuis, 1986** *Atyaephyra* ; *Troglocaris* ; *Paratya* ; *Syncaris* ; *Palaemonias* ; *Dugastella*	**Paratyinae** *Atyaephyra* ; *Troglocaris* ; *Paratya* ; *Syncaris* ; *Palaemonias* ; *Dugastella* ; *Antecaridina* ; *Typhlatya* ; *Typhlopatsa* ;	***Paratya* group** *Atyaephyra* ; *Troglocaris* ; *Paratya* ; *Palaemonias* ; *Dugastella* ; *Gallocaris*
		Série typhlatyenne *Antecaridina* Edmonson, 1954 ; *Typhlatya* Creaser, 1936 ; *Spelaeocaris* Matjašič, 1956 ; *Typhlopatsa* Holthuis, 1956 ; *Stygiocaris* Holthuis, 1960	**Typhlatyinae Holthuis, 1986** *Antecaridina* ; *Typhlatya* ; *Spelaeocaris* ; *Typhlopatsa* ; *Stygiocaris*	*Stygiocaris* ; *Gallocaris* Sket & Zakšec, 2009	***Typhlatya* group** *Antecaridina* ; *Typhlatya* ; *Stygiocaris* ; *Halocaridina* ; *Halocaridinides* ; *Monsamnis* Richard, De Grave & Clark, 2012 (?)
					Syncaridinae von Rintelen *et al.* 2012 *Syncaris*
	Forme acanthéphyroïde *Xiphocaris* Martens, 1872	**Forme acanthéphyroïde** *Xiphocaris*			***Incertae sedis*** *Archaeatya* Villalobos, 1960 ; *Atydina* Cai, 2010 ; *Jolivetya* Cals, 1986 ; *Limnocaridella* Bouvier, 1914 ; *Mancicaris* Liang, Guo & Tang, 1999 ; *Puteonator* Gurney, 1987 ; *Typhlocaridina* Liang & Yan, 1981 ; *Typhlopatsa* Holthuis, 1956

1.2.2 *Distribution géographique*

Les représentants de la famille des Atyidae vivent dans les eaux douces tropicales et tempérées et présentent une répartition mondiale, avec des représentants sur tous les continents (à l'exception de l'Antarctique) (De Grave *et al.*, 2008) (Figure 1.2).

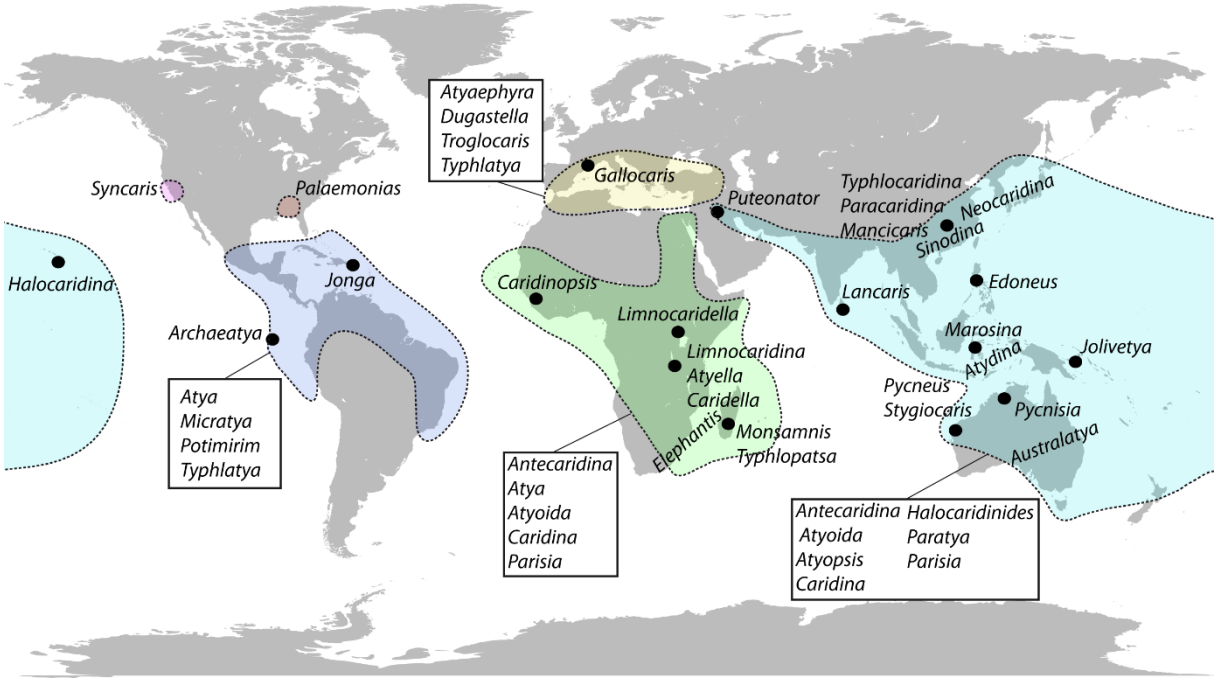


Figure 1.2 : Aire de répartition des différents genres d'Atyidae. Les points noirs indiquent les genres endémiques d'une localité

Figure 1.2: Geographical distribution of atyid genera. Dots indicate genera endemic to a locality.

On distingue dans leur répartition 6 zones d'endémisme :

- La zone **Indo-Pacifique**, où l'on peut identifier notamment :
  - L'Asie orientale (genres *Mancicaris*, *Paracaridina*, *Neocaridina, Sinodina*, *Typhlocaridina*)
  - L'Australie (genres *Australatya*, *Pycneus*, *Pycnisia*, *Stygiocaris*)
  - L'Indonésie / Philippines (genres *Atydina*, *Edoneus*, *Jolivetya*, *Marosina*)
- La zone **Afrotropicale** (genres *Caridinopsis*, *Limnocaridella*, *Limnocaridina*, *Atyella*, *Caridella*, *Elephantis*, *Monsamnis*, *Typhlopatsa*)
- La zone **Méditerranéenne** (genres *Atyaephyra*, *Dugastella*, *Troglocaris*, *Gallocaris*)
- La zone **Est Nord-Américaine** (genre *Palaemonias*)
- La zone **Ouest Nord Américaine** (genre *Syncaris*)

- La zone **Néotropicale** (genres *Archaeatya*, *Jonga*, *Micratya*, *Potimirim*)

Outre les genres endémiques des zones pré-citées, certains possèdent une large distribution, se répartissant sur plusieurs aires d'endémisme. C'est le cas des genres amphi-Atlantiques *Atya* et *Typhlatya* et des genres Indo-Pacifique *Antecaridina*, *Atyoida*, *Caridina*, *Halocaridinides* et *Parisia*.

1.2.3 Biologie

Les membres de la famille des Atyidae sont notamment caractérisés par la présence de soies au bout de leurs pinces qui jouent un rôle dans la prise de nourriture. On peut distinguer deux grands modes de prise alimentaires chez les Atyidae :

- Le mode filtreur, que l'on retrouve surtout dans le groupe des *Atya*, où la crevette se tient face courant, les pinces ouvertes pour que leurs longues soies collectent la matière organique en suspension (Fryer , 1977 ; Felgenhauer & Abele, 1985),
- Le mode détritivore-collecteur, que l'on retrouve chez la plupart des autres Atyidae, où la crevette « brosse » le substrat avec ses touffes de soies pour arracher le biofilm ou la matière organique particulaire (Yam, 2016) (Figure 1.3).

Ces différents modes de nutrition induisent des adaptations morphologiques particulières. Par exemple, les espèces filtreuses possèdent des soies plus longues et de natures plus diverses que les espèces détritivores (Fryer, 1977 ; Yam, 2016). Certaines espèces détritivores possèdent des griffes en plus des touffes de soies de manière à avoir un grattage plus efficace des surfaces dures comme chez *Caridina wyckii* (Hickson, 1888) ou chez les espèces du genre *Limnocaridina* qui ont connu une radiation dans le Lac Tanganyika, donnant lieu à toutes sortes d'adaptations à une grande diversité de modes de nutrition (Fryer, 2006).

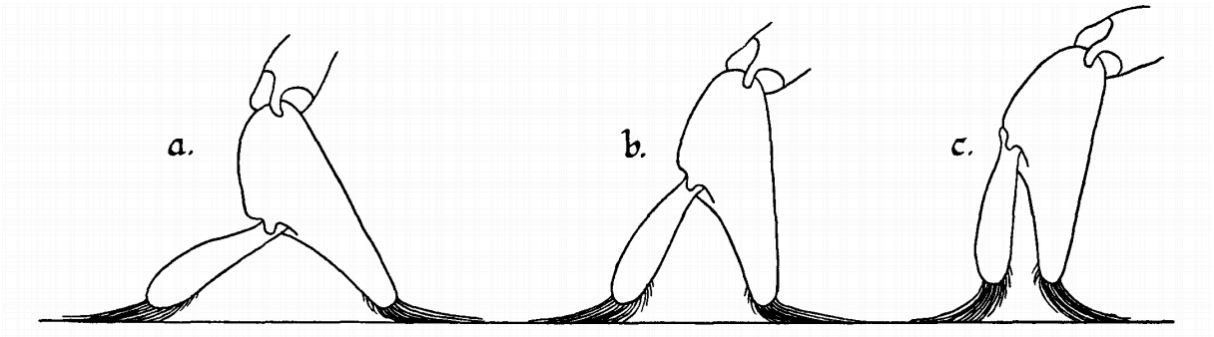


Figure 1.3 : Séquence de fermeture de la pince dans le cas d'un Atyidae détritivore (Fryer, 1960).

Figure 1.3: Closing sequence of the chela in a detritivore Atyidae (Fryer, 1960).

Qu'ils soient filtreurs ou détritivores, les Atyidae occupent une place essentielle dans les écosystèmes dont ils font partie en permettant de remobiliser la matière organique issue de la décomposition des végétaux, en assurant un rôle de nettoyage du milieu en éliminant les dépôts de sédiments après les crues (Pringle *et al.*, 1993), et en limitant la prolifération des algues (Pringle, 1996 ; Burns & Walker, 2000 ; March & Pringle, 2003).

D'un point de vue écologique, la distribution spatiale des Atyidae est fortement influencée par la quantité d'oxygène dissous, la température et la vitesse du courant (Hunte, 1978). Ce sont également des organismes très sensibles à la pollution aux métaux lourds (A. Kumar *et al.*, 2010a ; A. Kumar *et al.*, 2010b) et aux pesticides (Mensah *et al.*, 2011), faisant d'eux de bons candidats comme marqueurs de la qualité du milieu (Koji *et al.*, 2017) mais les rendant très sensibles à tout impact d'origine anthropique. Les Atyidae sont une proie de choix pour de nombreux prédateurs, comme par exemple la perche du Nil dans les lacs africains (Hughes, 1992 ; Goudswaard *et al.*, 2006), les anguilles, mulets et Eleotridae dans les rivières des îles des Caraïbes (Covich *et al.*, 2009) auxquels s'ajoutent les Kuhliidae dans les îles du Pacifique (Keith *et al.*, 2010). Les Atyidae peuvent être localement très abondants, dépassant parfois les 50 individus au m^2 (March *et al.* 2003a), notamment dans les stations d'altitude où leurs principaux prédateurs sont absents, ils peuvent représenter la part la plus importante de la biomasse.

En plus de leur importance écologique, les Atyidae peuvent constituer une ressource pour l'Homme, étant localement consommés par les populations et se faisant de plus en plus présents sur le marché de l'aquariophilie ornementale (De Grave *et al.*, 2008). Les prélèvements dans ce dernier cadre peuvent d'ailleurs parfois mettre en péril le maintien des populations, notamment chez les espèces endémiques lacustres de Sulawesi (Rintelen & Cai, 2009).



Figure 1.4 : Spécimen mâle de *Caridina typus* de Nouvelle Calédonie (Photo N. Charpin).

Figure 1.4: Male specimen of *Caridina typus* from New Caledonia (Credit N. Charpin).

1.3 Le genre *Caridina*

1.3.1 Systématique

Parmi les Atyidae, un genre est particulièrement diversifié, comptant près de 300 espèces décrites, il s'agit du genre *Caridina* H. Milne Edwards, 1837. Décrit initialement pour deux espèces *Caridina typus* (Figure 1.4) et *Caridina longirostris*, il deviendra le deuxième genre comportant le plus d'espèces chez les Caridea (derrière le genre *Alpheus* avec 307 espèces valides (d'après la base de données en ligne WoRMS, juin 2018 : http://www.marinespecies.org/aphia.php?p=taxdetails&id=240672), si bien que près d'une espèce décrite sur 10 est une *Caridina* (De Grave & Fransen, 2011). Depuis les années 1980, le nombre d'espèces décrites augmente de façon exponentielle et la tendance ne montre pas de ralentissement, ce qui laisse présager d'un grand nombre d'espèces encore non décrites (Figure 1.5).

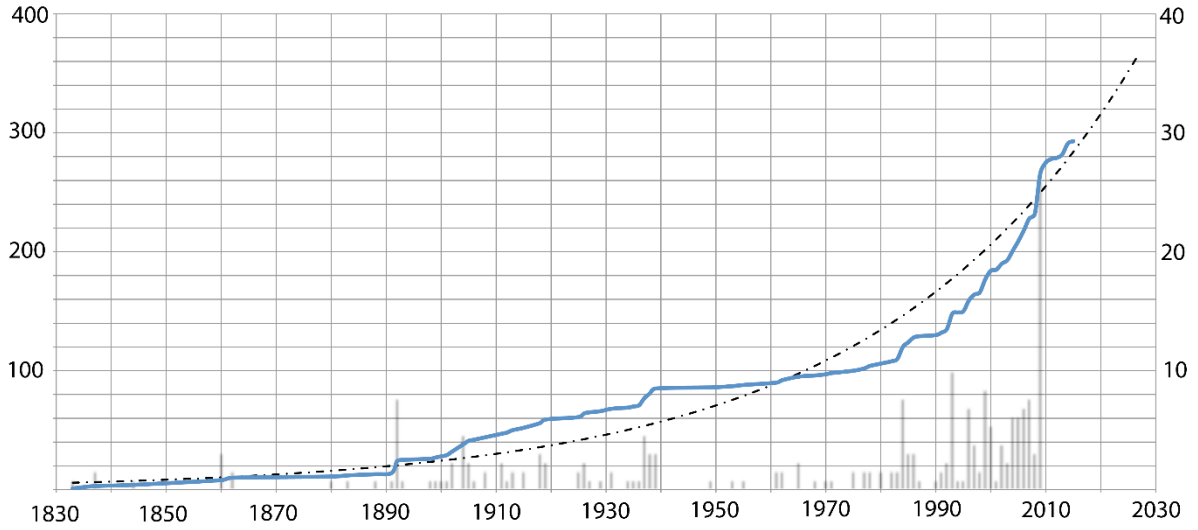


Figure 1.5 : Courbe du nombre d'espèces de *Caridina* cumulé par année (axe de gauche), courbe de tendance exponentielle (R^2 = 0,91) et histogramme du nombre d'espèces décrites chaque année (axe de droite).

Figure 1.5: Graph of the cumulative number of *Caridina* species described each year (left axis), exponential trend (R^2 = 0,91) and histogram of the number of species described each year (right axis).

D'un point de vue morphologique, le genre *Caridina* est caractérisé par (voir Figure 1.6 pour les termes anatomiques) :

- Des yeux bien développés ou réduits et peu pigmentés.
- Un rostre généralement denté sur les deux bords.
- Une carapace avec une épine antennaire et une épine ptérygostomienne pour quelques espèces.

- Le carpe de la seconde paire de péréiopodes plus long que large, généralement peu creusé antérieurement.
- La portion palmaire des pinces distincte, avec un dactyle bien plus court que le propode.
- La présence d'épipodes sur les 4 premiers péréiopodes.
- Le 3e maxillipède avec 2 arthrobranchies.
- Le 1er péréiopode avec une seule arthrobranchie.
- L'absence d'exopodes aux péréiopodes.

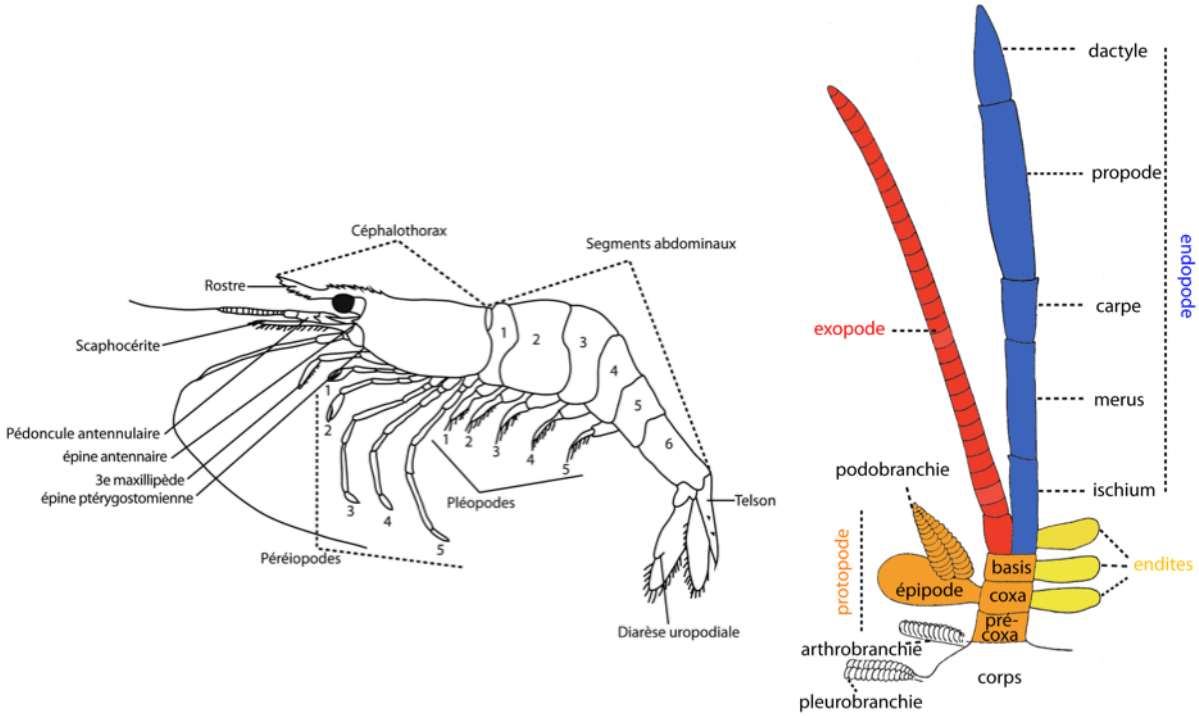


Figure 1.6 : Morphologie d'un Atyidae, d'après Rintelen & Cai (2009) et représentation schématique d'un péréiopode, d'après Holthuis (1993).

Figure 1.6: Morphology of an Atyidae from Rintelen & Cai (2009) and schematic representation of a pereiopod from Holthuis (1993).

Le genre *Caridina* a une répartition Indo-Ouest-Pacifique et Africaine, poussant jusqu'aux côtes Atlantiques de l'Afrique et le delta du Nil (Figure 1.7). Ce sont des espèces à affinité tropicale vivant dans les lacs et les cours d'eau permanents, et certaines sont cavernicoles (Par exemple, *C. rubella*, *C. troglophila*, *C. demenica*, etc.). Ce genre est représenté dans le registre fossile par l'espèce *C. nitida* † A. Milne Edwards, 1879 décrite des marnes lacustres d'Aix-en-Provence (Oligocène).

Bouvier écrivait en 1905 : « Il n'est pas facile de réunir en groupes et de caractériser nettement les 40 espèces qui sont réunies actuellement dans le genre Caridine ; elles

sont fort voisines les unes des autres, encore que leurs types extrêmes semblent au premier abord très dissemblables, et ce n'est pas sans une analyse délicate qu'on arrive à trouver leurs traits distinctifs. ». Il fut néanmoins le premier à rassembler les espèces de caridines en groupes basés sur des caractères morphologiques communs, dans sa monographie (Bouvier, 1925). Il en distingue cinq :

- 1. **Le groupe du *C. nilotica***, caractérisé par des pédoncules antennulaires égalant ou dépassant les ¾ de la longueur de la carapace, le 6e segment abdominal égalant ou dépassant les ⅔ de la longueur de la carapace, un rostre long, infléchi vers le haut et de 8 à 10 épines uropodiales.
- 2. **Le groupe du *C. laevis***, caractérisé par des pédoncules antennulaires ne dépassant pas les ¾ de la longueur de la carapace, le 6e segment abdominal inférieur aux ⅔ de la longueur de la carapace, un rostre plus court que les pédoncules antennulaires et de 9 à 12 épines uropodiales.
- 3. **Le groupe du *C. africana***, caractérisé par des soies intermédiaires du telson plus longues et plumeuses que les latérales et un rostre égalant la longueur des pédoncules antennulaires.
- 4. **Le groupe du *C. brevirostris***, caractérisé par des pédoncules antennulaires inférieurs ou égaux aux ⅔ de la carapace, le 6e segment abdominal égalant la moitié de la longueur de la carapace, un rostre égalant la longueur des pédoncules antennulaires et de 15 à plus de 20 épines uropodiales.
- 5. **Le groupe du *C. typus***, caractérisé par une forme trapue, des espèces de grande taille.

Du fait de la confusion taxonomique régnant au sein du genre et du nombre important d'espèces décrites par la suite, certains de ces groupes ont été modifiés, abandonnés ou combinés et de nouveaux ont été formés, toujours basés sur des critères de ressemblance morphologique. On peut citer par exemple le groupe *C. serrata* (Cai & Ng, 1999 ; Karge & Klotz, 2007 ; Klotz & Rintelen, 2014), le groupe *C. gracilirostris* (Cai & Ng, 2007), le groupe *C. babaulti* (Karge & Klotz, 2007), le groupe des espèces endémiques lacustres de Sulawesi (Karge & Klotz, 2007 ; Rintelen *et al*., 2007, 2009), le groupe *C. nilotica* (Johnson, 1963 ; Karge & Klotz, 2007), le groupe *C. typus* (Karge & Klotz, 2007) et le groupe *C. weberi* (Cai & Shokita, 2006a). Certains groupes, issus du regroupement d'anciennes sous-espèces d'une même espèce, sont volontiers traités en tant que complexes d'espèces, comme les complexes *C. gracilirostris* (Cai & Ng, 2001), *C. indistincta* (Chenoweth & Hughes, 2003), *C. brevicarpalis* (Short, 2009), ***C. nilotica*** (Johnson, 1963 ; Holthuis, 1978a ; Jalihal *et al.*, 1984 ; Choy, 1991 ; Richard & Clark, 2005), et ***C. weberi*** (Richard & Chandran, 1994). **Le présent mémoire portera sur ces deux derniers complexes, largement représentés dans la zone Indo-Pacifique et formant la grande majorité des spécimens collectés par l'équipe porteuse de la thèse au cours de ses différentes expéditions.**

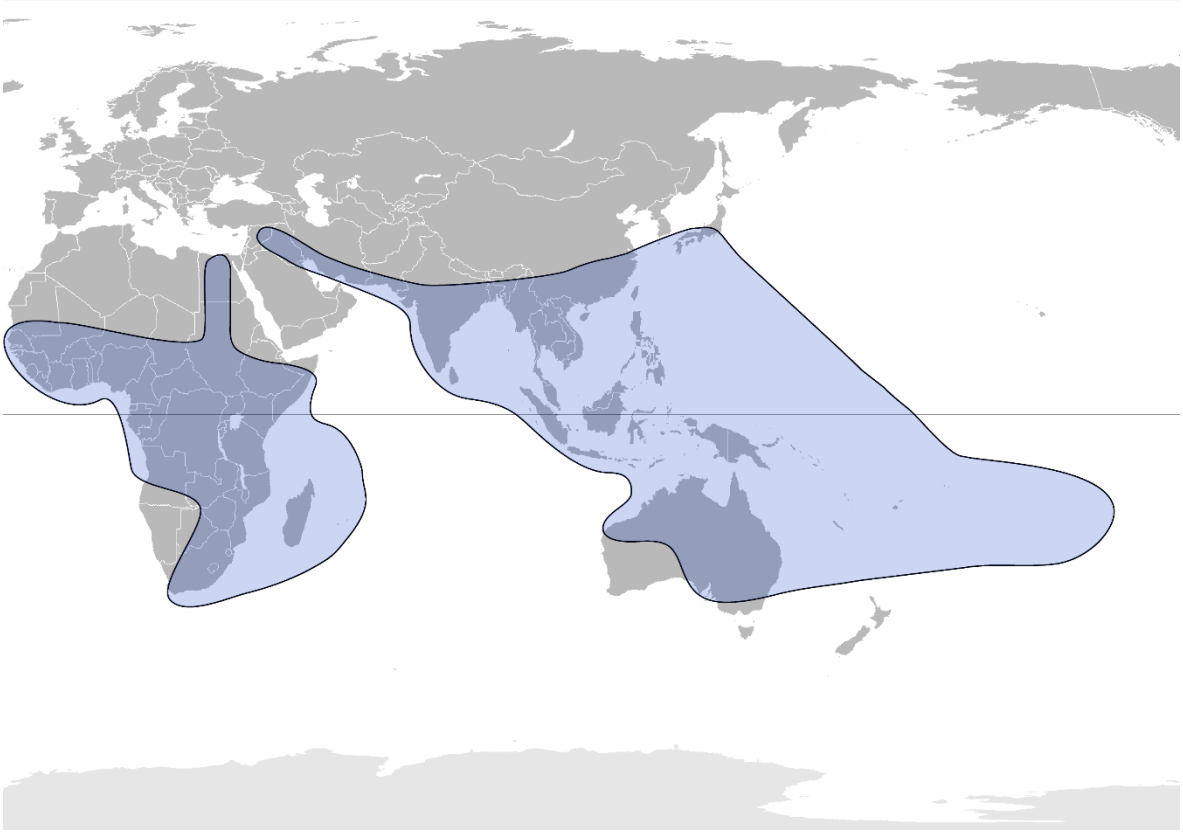


Figure 1.7 : Répartition mondiale du genre *Caridina*.

Figure 1.7: World distribution of genus *Caridina*.

1.3.2 Les complexes C. nilotica *et* C. weberi

Au sens strict, le complexe *C. nilotica* comporte 22 taxons différents qui ont été à un moment ou un autre des sous-espèces de l'espèce *C. nilotica* (Tableau 1.2). L'espèce *Pelias niloticus* est décrite par Polydore Roux en 1833 à partir de spécimens qu'il a collecté dans le Nil. Hilgendorf (1878) la transfère dans le genre *Caridina* en tant que *Caridina nilotica*. En 1908, De Man dresse une liste de 9 variétés de *C. nilotica* à partir de taxons déjà existant où de nouveaux taxons qu'il décrit à cette occasion. Plus tard, Woltereck (1937) reconnaît 19 sous-espèces valides de *C. nilotica*. Johnson (1963) révise ce complexe en mettant la plupart des sous-espèces en synonymie avec *C. simoni* ou *C. wyckii* qu'il érige au rang d'espèce. Certaines anciennes sous-espèces sont par la suite revalidées et considérées en tant qu'espèces distinctes de *C. nilotica* tandis que d'autres sont mises en synonymie avec d'autres espèces. En 2015, le complexe *C. nilotica* au sens strict est composé de 17 espèces valides et l'espèce *C. nilotica* ne comporte plus de sous-espèce (Base WoRMS). Au sens plus large, il regroupe toutes les espèces correspondant à la description du groupe donnée par Bouvier (1925). Les représentants de ce complexe se retrouvent dans un quadrilatère circonscrit entre l'Algérie (H. Milne Edwards, 1837), l'Afrique du Sud (De Man, 1908), Fidji (Edmonson, 1935) et le Japon (Kubo, 1938). La forme typique du complexe, *C. nilotica* est quant à elle limitée au bassin du Nil (De Grave, 2013)

Tableau 1.2 : Historique de la systématique des sous-espèces de *Caridina nilotica* (modifié d'après Karge & Klotz, 2007). Jaune : *C. nilotica*, Bleu : sous-espèces de *C. nilotica*, Vert : autres espèces, Orange : autres sous-espèces.

Table 1.2:Systematic history of the subspecies of *Caridina nilotica* (modified from Karge & Klotz, 2007). Yellow: *C. nilotica*, Blue: subspecies of *C. nilotica*, Green: other species, Orange: other subspecies.

Jusqu'en 1900	Jusqu'en 1920	Jusqu'en 1930	Woltereck, 1937	Jusqu'en 1980	Jusqu'en 2005	Karge & Klotz, 2007	En 2015
C. longirostris H. Milne Edwards, 1837	*C. nilotica* var. *longirostris* H. Milne Edwards, 1837	*C. nilotica* var. *longirostris* H. Milne Edwards, 1837	*C. nilotica longirostris* H. Milne Edwards, 1837		*C. longirostris* H. Milne Edwards, 1837	*C. longirostris* H. Milne Edwards, 1837	*C. longirostris* H. Milne Edwards, 1837
C. leucosticta Stimpson, 1860		*C. nilotica typica* (P. Roux, 1833)	*C. nilotica leucosticta* Stimpson, 1860			*C. leucosticta* Stimpson, 1860	*C. leucosticta* Stimpson, 1860
C. grandirostris Stimpson, 1860			*C. nilotica grandirostris* Stimpson, 1860			*C. grandirostris* Stimpson, 1860	*C. grandirostris* Stimpson, 1860
Pelias niloticus P. Roux, 1833	*C. nilotica typica* (P. Roux, 1833)		*C. nilotica typica* (P. Roux, 1833)		*C. nilotica typica* (P. Roux, 1833)	*C. nilotica typica* (P. Roux, 1833)	*C. nilotica* (P. Roux, 1833)
Atya wyckii Hickson, 1888	*C. nilotica* var. *wyckii* (Hickson, 1888)	*C. nilotica* var. *wyckii* (Hickson, 1888)	*C. nilotica wyckii* (Hickson, 1888)	*C. wyckii* (Hickson, 1888)		*C. wyckii* (Hickson, 1888)	*C. wyckii* (Hickson, 1888)
	C. nilotica macrophora Kemp, 1918	*C. nilotica typica* (P. Roux, 1833)	*C. nilotica macrophora* Kemp, 1918			*C. nilotica macrophora* Kemp, 1918	*C. macrophora* Kemp, 1918
		C. nilotica var. *styliformis* Bouvier, 1925	*C. nilotica styliformis* Bouvier, 1925	*C. xiphias* Bouvier, 1925		*C. xiphias* Bouvier, 1925	*C. xiphias* Bouvier, 1925
		C. nilotica var. *xiphias* Bouvier, 1925	*C. nilotica xiphias* Bouvier, 1925				
	C. nilotica var. *brachydactyla* De Man, 1908	*C. nilotica* var. *brachydactyla* De Man, 1908	*C. nilotica brachydactyla* De Man, 1908	*C. simoni* Bouvier, 1904	*C. nilotica typica* (P. Roux, 1833)	*C. brachydactyla* De Man, 1908	*C. brachydactyla* De Man, 1908
	C. brachydactyla peninsularis Kemp, 1918		*C. nilotica brachydactyla* f. *peninsularis* Kemp, 1918	*C. simoni peninsularis*	*C. peninsularis* Kemp, 1918	*C. peninsularis* Kemp, 1918	*C. peninsularis* Kemp, 1918
C. wyckii var. *gracilipes* De Man, 1892	*C. nilotica* var. *gracilipes* De Man, 1892	*C. nilotica* var. *gracilipes* De Man, 1892	*C. nilotica gracilipes* De Man, 1892	*C. simoni* Bouvier, 1904		*C. gracilipes* De Man, 1892	*C. gracilipes* De Man, 1892
C. wyckii var. *paucipara* De Man, 1892	*C. nilotica* var. *paucipara* De Man, 1892	*C. nilotica* var. *paucipara* De Man, 1892	*C. nilotica paucipara* De Man, 1892			*C. nilotica paucipara* De Man, 1892	*C. africana* Kingsley, 1883
	C. nilotica var. *natalensis* De Man, 1908	*C. nilotica* var. *natalensis* De Man, 1908	*C. nilotica natalensis* De Man, 1908			*C. nilotica natalensis* De Man, 1908	*C. brachydactyla* De Man, 1908
	C. nilotica var. *bengalensis* De Man, 1908	*C. nilotica* var. *bengalensis* De Man, 1908	*C. nilotica bengalensis* De Man, 1908	*C. simoni* Bouvier, 1904		*C. gracilipes* De Man, 1892	*C. gracilipes* De Man, 1892
	C. nilotica var. *minahassae* De Man, 1908	*C. nilotica* var. *minahassae* De Man, 1908	*C. nilotica minahassae* De Man, 1908	*C. wyckii* (Hickson, 1888)	*C. nilotica typica* (P. Roux, 1833)	*C. nilotica minahassae* De Man, 1908	*C. wyckii* (Hickson, 1888)
	C. simoni Bouvier, 1904	*C. nilotica* var. *simoni* Bouvier, 1904	*C. nilotica simoni* Bouvier, 1904	*C. simoni* Bouvier, 1904		*C. simoni* Bouvier, 1904	*C. simoni Bouvier, 1904*
	C. aruensis J. Roux, 1911	*C. nilotica* var. *aruensis* J. Roux, 1911	*C. nilotica aruensis* J. Roux, 1911	*C. simoni aruensis* J. Roux, 1911	*C. nilotica typica (P. Roux, 1833)*	*C. nilotica aruensis* J. Roux, 1911	
		C. nilotica var. *brevidactyla* J. Roux, 1920	*C. nilotica brevidactyla* J. Roux, 1920			*C. nilotica brevidactyla* J. Roux, 1920	*C. brachydactyla* De Man, 1908
		C. nilotica var. *meridionalis* J. Roux, 1926	*C. nilotica meridionalis* J. Roux, 1926	*C. simoni meridionalis* J. Roux, 1926		*C. nilotica meridionalis* J. Roux, 1926	*C. meridionalis* J. Roux, 1926
				C. nilotica var. *chauhani* Chopra & Tiwari, 1949		*C. chauhani* Chopra & Tiwari, 1949	*C. chauhani* Chopra & Tiwari, 1949
				C. nilotica var. *zeylanica* Arudpragasam & Costa, 1962		*C. zeylanica* Arudpragasam & Costa, 1962	*C. zeylanica* Arudpragasam & Costa, 1962
				C. nilotica elongapoda Liang & Yan, 1977	*C. elongapoda* Liang & Yan, 1977	*C. elongapoda* Liang & Yan, 1977	*C. elongapoda* Liang & Yan, 1977

C. nilotica
Sous-espèces de*C. nilotica*
Autres espèces
Autres sous-espèces

Le complexe *C. weberi*, quant à lui, est composé de 7 taxons reconnus comme sous-espèces de *C. weberi* à un moment donné (Tableau 1.3). De Man (1892b) décrit l'espèce *Caridina weberi* à partir de spécimens collectés par le Pr. Max Weber dans différentes îles d'Indonésie (Flores, Sulawesi et Selayar) et sa variété *C. weberi* var. *sumatrensis* à Sumatra. Par la suite, de nouvelles variétés sont décrites : la variété *celebensis* de Sulawesi par Schenkel (1902), la variété *papuana* de Papouasie par Nobili (1905), les variétés *keiensis* des îles Kei et *longicarpus* de Nouvelle Calédonie par Jean Roux (1911, 1926), et Bouvier (1925), reprennant la suggestion de Roux (1911) fait de l'espèce *C. parvirostris* de Florès une variété de *C. weberi*. Johnson (1963) met en synonymie les variétés *celebensis* et *parvirostris* avec la sous-espèce typique *C. weberi weberi* et considère la variété *sumatrensis* comme une sous-espèce de *C. weberi*. Cai & Ng (2001) considèrent les variétés non examinées par Johnson comme des sous-espèces valides. Depuis De Grave & Fransen (2011), toutes les sous-espèces de *C. weberi* sont considérées comme des espèces valides, à l'exception de *C. weberi keiensis* dont le statut est douteux et *C. weberi* var. *celebensis* dont le nom, pré-occupé par une variété de *C. serratirostris* n'est pas disponible. En 2015, le complexe *C. weberi* au sens strict est composé de 5 espèces valides et l'espèce *C. weberi* ne comporte pas de sous-espèce (Base WoRMS). Au sens plus large, il regroupe les espèces morphologiquement proches de *C. weberi* (comme *C. multidentata* (De Man, 1892a) ; *C. laoagensis* (Chace, 1997), *C. okinawa* (Cai & Shokita, 2006a) ou *C. lobocensis* (Cai *et al.*, 2009)). Les représentants de ce complexe se rencontrent depuis les côtes occidentales de l'océan Indien (Bouvier, 1925) jusqu'aux îles Marquises (Edmonson, 1935).

Tableau 1.3 : Historique de la systématique des sous-espèces de *Caridina weberi*. Jaune : *C. weberi*, Bleu : sous-espèces de *C. weberi*, Vert : autres espèces.

Table 1.3: Systematic history of the subspecies of *Caridina weberi*. Yellow: *C. weberi*, Blue: subspecies of *C. weberi*, Green: other species.

De Man, 1892	Woltereck, 1937	Johnson, 1963	Cai & Ng, 2001	En 2015
C. weberi De Man, 1892	*C. weberi* var. *typica* Bouvier, 1925	*C. weberi weberi* De Man, 1892	*C. weberi weberi* De Man, 1892	*C. weberi* De Man, 1892
	C. weberi var. *celebensis* Schenkel, 1902			
C. parvirostris De Man, 1892	*C. weberi* var. *parvirostris* De Man, 1892			*C. parvirostris* De Man, 1892
	C. weberi var. *keiensis* J. Roux, 1911	*C. weberi* var. *keiensis* J. Roux, 1911	*C. weberi keiensis* J. Roux, 1911	*C. weberi* De Man, 1892
	C. weberi var. *longicarpus* J. Roux, 1926	*C. weberi* var. *longicarpus* J. Roux, 1926	*C. weberi longicarpus* J. Roux, 1926	*C. longicarpus* J. Roux, 1926
	C. weberi var. *papuana* Nobili, 1905	*C. weberi* var. *papuana* Nobili, 1905	*C. weberi papuana* Nobili, 1905	*C. papuana* Nobili, 1905
C. weberi var. *sumatrensis* De Man,1892	*C. weberi* var. *sumatrensis* De Man,1892	*C. weberi sumatrensis* De Man,1892	*C. weberi sumatrensis* De Man,1892	*C. sumatrensis* De Man, 1892

C. weberi
Sous-espèces de *C. weberi*
Autres espèces

1.3.3 Les espèces du genre Caridina dans la zone d'étude

Sont listées ci-dessous (Tableau 1.4) les espèces valides du genre *Caridina* signalées dans la zone d'étude (systèmes insulaires de la zone Indo-Pacifique) avant le début de la thèse (2015). Les espèces marquées d'une astérisque (*) font partie du complexe *C. nilotica*, celles marquées de deux astérisques (**), du complexe *C. weberi*. Par souci de clarté, les noms d'auteurs des espèces ne sont pas indiqués dans le tableau, ils seront détaillés par la suite pour les espèces révisées dans ce travail.

Tableau 1.4 : Liste des espèces recensées par localité avant 2015 (voir Figure 1.15 pour les localités).

Table 1.4: List of species reported by locality before 2015 (see Figure 1.15 for localities).

OCEAN INDIEN OCCIDENTAL					
Afrique du Sud (Emmerson, 2016)	**Comores et Mayotte (Keith *et al.*, 2006)**	**Madagascar (Short & Doumenq, 2003 ; Cumberlidge *et al.*, 2017)**	**Maurice (Short & Doumenq, 2003 ; Keith *et al.*, 2006)**	**La Réunion (Keith *et al.*, 2006)**	**Les Seychelles (Keith *et al.*, 2006)**
Caridina africana *C. belazoniensis* *C. serratirostris* *C. susuroflabra*	*C. longirostris** *C. serratirostris* *C. typus*	*C. angulata* *C. brevirostris brevipes* *C. brachydactyla** *C. calmani*	*C. longirostris** *C. mauritii* *C. richtersi* *C. serratirostris* *C.*	*C. longirostris** *C. serratirostris* *C. typus*	*C. longirostris** *C. serratirostris* *C. similis* *C. typus*

C. togoensis *C. typus* *C. umtatensis*		*C. crurispinata* *C. edulis* *C. gracilirostris* *C. hova* *C. isaloensis grandidieri* *C. isaloensis isaloensis* *C. japonica* *C. lamiana* *C. lipalmaria* *C. longirostris** *C. nilotica** *C. norvestica* *C. parvocula* *C. petiti* *C. serratirostris* *C. steineri* *C. troglophila* *C. typus* *C. unca* *C. xiphias**	*spathulirostris* *C. typus*		
OCEAN INDIEN ORIENTAL					
Andaman (Tiwari & Pillai, 1971 ; Costa, 1984)			**Sri Lanka (De Silva, 1982)**		
*C. brachydactyla** *C. gracilirostris* *C. prashadi* *C. serratirostris* *C. sumatrensis*** *C. typus*			*C. costai* *C. fernandoi* *C. gracilirostris* *C. pristis cruszi* *C. pristis pristis* *C. propinqua* *C. simoni** *C. typus* *C. zeylanica**		

INSULINDE		
Indonésie	**Philippines (Cai & Anker, 2004 ; Cai & Shokita, 2006b ; Cai *et al.*, 2009)**	**Malaisie**
Ambon (J. Roux, 1904) *C. typus* *C. brevicarpalis* **Aru (J. Roux, 1911, 1920 ; Woltereck, 1937)** *C. aruensis** *C. brachydactyla** *C. brevidactyla** *C. gracilirostris* *C. opaensis* *C. serratirostris* *C. typus* **Bacan (J. Roux, 1928)** *C. multidentata* **Bali (Woltereck, 1937)** *C. brevicarpalis* *C. nilotica** **Bornéo (Choy, 1992 ; Wowor *et al.*, 2004)** *C. bruneiana* *C. gracilipes** **Buru (J. Roux, 1928 ; Woltereck, 1937)** *C. gracilipes** *C. typus*	**Bohol** *C. batuan* *C. boholensis* *C. camaro* *C. elongapoda** *C. endehensis* *C. gracilirostris* *C. laoagensis* *C. liaoi* *C. lobocensis* *C. typus* *C. valencia* *C. villadolidi* **Busuanga** *C. peninsularis** *C. typus* **Cebu** *C. cebuensis* *C. elongapoda** *C. laoagensis* *C. peninsularis** **Luzon** *C. buhi* *C. celebensis* *C. elongapoda** *C. endehensis* *C. gracilirostris*	**Péninsule Malaise et Singapour (Wowor *et al.*, 2004)** *C. bakoensis* *C. bruneiana* *C. celebensis* *C. gracilipes** *C. gracilirostris* *C. elongapoda** *C. endehensis* *C. excavatoides* *C. johnsoni* *C. malayensis* *C. peninsularis** *C. propinqua* *C. serratirostris* *C. sumatrensis*** *C. temasek* *C. thambipilaii* *C.* aff. *tonkinensis* *C. typus* **Pulau Tioman (Yeo *et al.*, 1999)** *C. celebensis* *C. elongapoda** *C. typus*

Buton (Klotz & Rintelen, 2013) *C. butonensis* **Ceram (J. Roux, 1928 ; Woltereck, 1937)** *C. brevidactyla** *C. gracilirostris* *C. papuana*** *C. weberi*** **Flores (De Man, 1892b ; Woltereck, 1937)** *C. brachydactyla** *C. endehensis* *C. gracilirostris* *C. parvirostris*** *C. serratirostris* *C. typus* *C. weberi*** **Halmahera (Cai & Ng, 2001)** *C. brachydactyla** *C. brevidactyla** *C. gracilirostris* *C.* cf. *pareparensis* *C. villadolidi* *C. weberi*** **Java (J. Roux, 1932 ; Woltereck, 1937)** *C. brevicarpalis* *C. nilotica** *C. laevis* *C. typus* *C. weberi*** **Kei (J. Roux, 1911 ; Woltereck,**	**Mindanao** *C. brevicarpalis* *C. celebensis* *C. elongapoda** *C. endehensis* *C. gracilipes** *C. gracilirostris* *C. laoagensis* *C. mindanao* *C. propinqua* *C. serratirostris* **Mindoro** *C. brevicarpalis* *C. elongapoda** *C. endehensis* *C. laoagensis* *C. villadolidi* **Palawan** *C. brevicarpalis* *C. celebensis* *C. elongapoda** *C. laoagensis* *C. palawanensis* *C. peninsularis** *C. rubella* *C. sumatrensis*** *C. serratirostris* *C. typus* **Panay** *C. elongapoda** *C. laoagensis*	

1937) *C. brachydactyla** *C. jeani* *C. mertoni* *C. typus* *C. weberi keiensis*** **Lombok (Woltereck, 1937)** *C. gracilipes** *C. typus* **Peleng (Rintelen *et al.*, 2008)** *C. thomasi* **Selayar (De Man, 1892b ; Woltereck, 1937)** *C. brachydactyla** *C. gracilipes** *C. gracilirostris* *C. serratirostris* *C. typus* *C. weberi*** **Sulawesi (Rintelen *et al.*, 2008 ; Cai & Ng, 2009 ; Rintelen & Cai, 2009 ; Klotz & Rintelen, 2013 ; Annawaty & Wowor, 2015)** *C. acutirostris* *C. appendiculata* *C. brachydactyla** *C. brevicarpalis* *C. boehmei* *C. buehleri* *C. caerulea* *C. celebensis* *C. dali* *C. dennerli*	**Samar** *C. gortio* *C. minidentata* *C. samar* *C. typus*	

C. ensifera *C. glaubrechti* *C. gracilipes** *C. gracilirostris* *C. holthuisi* *C. kaili* *C. lanceolata* *C. laroeha* *C. leclerci* *C. lingkonae* *C. linduensis* *C. loehae* *C. longidigitata* *C. longifrons* *C. mahalona* *C. masapi* *C. cf. multidentata* *C. neglecta* *C. opaensis* *C. pareparensis* *C. parvidentata* *C. parvula* *C. profundicola* *C. sarasinorum* *C. schenkeli* *C. spinata* *C. spongicola* *C. striata* *C. sulawesi* *C. tenuirostris* *C. towutensis* *C. typus* *C. villadolidi* *C. weberi*** *C. woltereckae*		

*C. wyckii**		
Sumatra (De Man, 1892b ; J. Roux, 1932)		
*C. brevidactyla**		
C. multidentata		
*C. sumatrensis***		
C. gracilirostris		
*C. weberi***		
Sumba (Holthuis, 1978a)		
C. brevicarpalis		
C. celebensis		
C. gracilirostris		
*C. longirostris**		
C. serratirostris		
C. sundanella		
C. typus		
*C. weberi***		
Ternate (De Man, 1902)		
C. serratirostris		
Timor (De Man, 1893)		
C. timorensis		
C. typus		
*C. wyckii**		
Waigeo (J. Roux, 1928)		
*C. brevidactyla**		
C. brevicarpalis		
C. gracilirostris		
C. mertoni		
C. typus		

MELANESIE				
Fidji (Choy, 1991)	**Nouvelle Calédonie (Marquet *et al.*, 2003)**	**Nouvelle Guinée**	**Salomon (Edmonson, 1935 ; Johnson, 1963)**	**Vanuatu (Keith *et al.*, 2010)**
C. brevicarpalis *C. devaneyi* *C. fijiana* *C. japonica* *C. longirostris** *C. nilotica** *C. nudirostris* *C. serratirostris* *C. typus* *C. vitiensis* *C. weberi***	*C. gracilirostris* *C. imitatrix* *C. leucosticta** *C. longirostris** *C. nilotica** *C. novaecaledoniae* *C. serratirostris* *C. sp. 1* *C. sp. 2* *C. typus* *C. vitiensis* *C. weberi***	**Manus (J. Roux, 1934)** *C. brevidactyla** *C. papuana*** *C. typus* **Nouveau Hannovre (J. Roux, 1934)** *C. brevidactyla** *C. papuana*** **Nouvelle Bretagne (J. Roux, 1934)** *C. serratirostris* **Nouvelle Guinée + Papouasie (Holthuis, 1982 ; Short, 2009 ; Karge *et al.*, 2010)** *C. buergersi* *C. brevicarpalis* *C. cognata* *C. demani* *C. elisabethae* *C. fecunda* *C. gracilipes** *C. gracilirostris* *C. longirostris** *C. nilotica**	*C. vitiensis* *C. weberi***	*C. brevicarpalis* *C. gracilirostris* *C. gueryi* *C. longirostris** *C. serratirostris* *C. typus* *C. weberi***

		*C. papuana***		
		C. rouxi		
		C. serratirostris		
		C. typus		
		C. vitiensis		
		*C. weberi***		
		*C. wyckii**		
		C. sp. 1		
		C. sp. 2		
		C. sp. 3		
		C. sp. 4		
		C. sp. 5		
		Nouvelle Irlande (J. Roux, 1934)		
		C. buehleri		

AUSTRALIE (Davie, 2002)			
Australie Occidentale	**Queensland**	**Nouvelle Galles du Sud**	**Territoire du Nord**
C. spelunca	*C. celebensis* *C. confusa* *C. gracilirostris* *C. indistincta indistincta* *C. indistincta sobrina* *C. nilotica** *C. serratirostris* *C. spinula* *C. thermophila* *C. typus* *C. zebra*	*C. mccullochi*	*C. gracilirostris* *C. nilotica**

<table>
<tr><th colspan="4">POLYNESIE (Keith et al., 2013)</th></tr>
<tr><th>Cook</th><th>Polynésie Française</th><th>Samoa</th><th>Futuna</th></tr>
<tr><td>C. weberi**</td><td>Australes
C. rapaensis
C. serratirostris
C. weberi**

Marquises
C. weberi**

Société
C. serratirostris
C. weberi</td><td>C. buehleri
C. serratirostris
C. typus
C. weberi**</td><td>C. serratirostris
C. typus
C. weberi**</td></tr>
<tr><th colspan="4">MICRONESIE</th></tr>
<tr><th>Guam (Leberer & Cai, 2003)</th><th>Micronésie</th><th colspan="2">Palau (Bright, 1979 ; Cai & Ng, 2007)</th></tr>
<tr><td>C. brachydactyla*
C. mertoni
C. typus</td><td>Kosrae (Benstead et al., 2009)
C. typus
C. weberi**

Pohnpei (Maciolek & Ford, 1987)
C. brachydactyla*
C. longirostris*
C. typus
C. vitiensis
C. weberi**

Yap (Bright, 1989)
C. brachydactyla*
C. gracilirostris
C. serratirostris
C. typus</td><td colspan="2">C. brachydactyla*
C. gracilirostris
C. serratirostris
C. typus</td></tr>
</table>

ASIE DE L'EST	
Japon (Ryukyu) (Cai & Shokita, 2006a)	**Taiwan (Hung *et al.*, 1993)**
C. celebensis	*C. brevicarpalis*
C. gracilirostris	*C. fasciata*
*C. grandirostris**	*C. formosae*
C. laoagensis	*C. japonica*
*C. leucosticta**	*C. longirostris**
C. prashadi	*C. pseudodenticulata*
C. propinqua	*C. serrata*
C. macrodentata	*C. serratirostris*
C. multidentata	*C. typus*
C. okinawa	*C. villadolidi*
C. rubella	*C. weberi***
C. serratirostris	
C. typus	

1.4 Cycles de vie et Diadromie

1.4.1 Diversité des cycles de vie chez les Atyidae

Trois types de développement larvaire ont été identifiés chez les Atyidae (Shokita, 1973, 1981 ; Hayashi & Hamano, 1984 ; Shy *et al.*, 1987 ; Lai & Shy, 2009) : Le type « commun », caractérisé par des petits œufs et 9 à 12 stades planctoniques, le type « suppression complète » caractérisé par de gros œufs et l'absence de stades planctoniques, et un type intermédiaire, dit « abrégé », caractérisé par des œufs de taille intermédiaire et 4 à 7 stades planctoniques (Tableau 1.5).

1.4.2 L'amphidromie, cas particulier de la diadromie

Les espèces présentant un développement de type commun ont très souvent une phase larvaire marine et sont dits amphidromes. L'amphidromie (Myers, 1949) est un cas particulier de la diadromie, que l'on divise habituellement en trois catégories :

- L'**anadromie**, qui s'applique aux espèces marines qui remontent les rivières pour s'y reproduire et y pondre (par exemple, les saumons, les éperlans, les lamproies, les esturgeons ou les aloses).

- La **catadromie**, qui s'applique aux espèces fluviatiles qui migrent en mer pour s'y reproduire et y pondre (par example, l'anguille).
- L'**amphidromie**, qui s'applique aux espèces qui vivent et se reproduisent en eau douce mais dont les larves migrent jusqu'à un environnement salin (marin ou saumâtre) pour s'y métamorphoser puis regagner les eaux douces au stade de juvénile ou de post-larve (par exemple, les gobies Sycidiinae ou les caridines) (McDowall, 2007) (Figure 1.8).

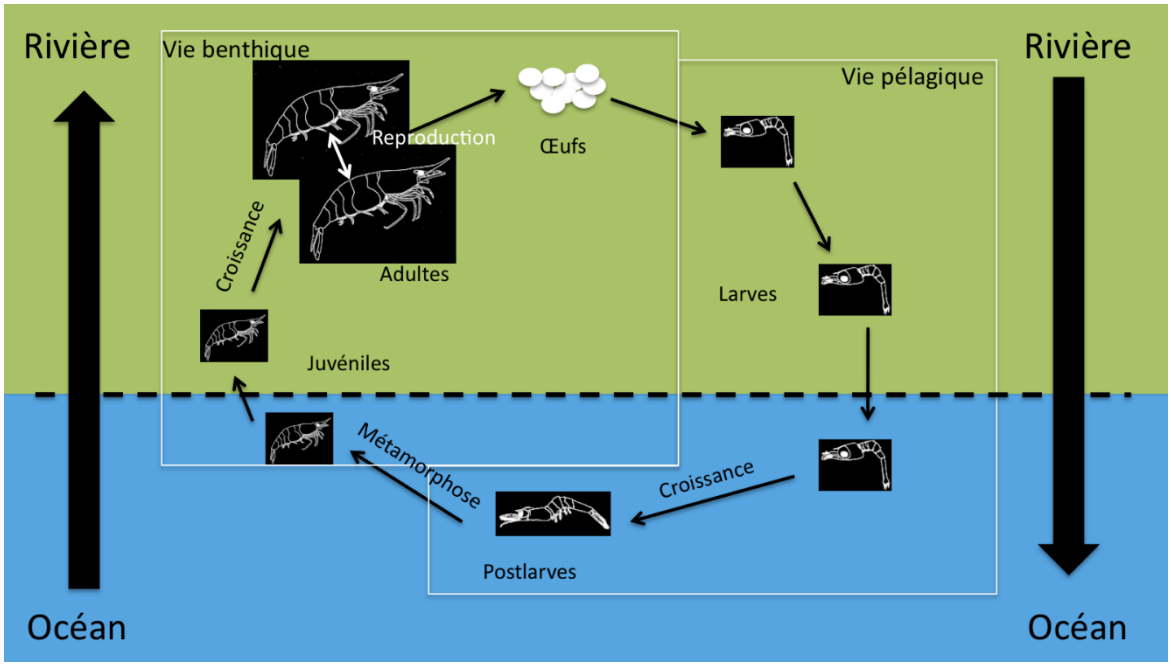


Figure 1.8 : Cycle de vie amphidrome dans le genre *Caridina*.

Figure 1.8: Amphidromous lifecycle within genus *Caridina*.

Cette dernière catégorie a pu apparaître indépendamment chez des espèces de poissons, de mollusques gastéropodes et de crustacés décapodes (Pyron & Covich, 2003 ; Thuesen *et al.*, 2011 ; Bauer, 2013) qui se rencontrent dans les cours d'eau tropicaux ou subtropicaux et particulièrement ceux des îles océaniques.

En effet, l'amphidromie présente l'avantage de favoriser la dispersion des espèces *via* le milieu marin (Page & Hughes, 2007 ; Bauer, 2013) et la colonisation des habitats nouvellement apparus, comme les rivières des îles volcaniques par exemple. Un autre avantage de ce mode de vie et une plus forte résilience des espèces face à l'instabilité qui caractérise l'environnement des îles tropicales (sécheresse, crues, cyclones, éruptions volcaniques, etc.) grâce au maintien d'un stock de larves en milieu marin, prêtes à

Tableau 1.5 : Synthèse des données sur le développement de quelques espèces d'Atyidae. En bleu, espèces du complexe *C. nilotica* ; en rouge, espèces du complexe *C. weberi*

Table 1.5: Data synthesis on the development of some species of Atyidae. In blue, species of the *C. nilotica* complex ; in red, species of the *C. weberi* complex

Espèce	Nb stades zoé	Durée développement	Taille œuf	N œufs (moy)	Développement (Lai & Shy, 2009)	Milieu de croissance	Références
Lancaris singhalensis (Sri Lanka)	0	0 j	1,80x1,24		Suppression complète	douce	Benzie & Silva, 1983
Neocaridina ishigakiensis (Japon)	0	0j	1,07x0,67		Suppression complète	douce	Shokita, 1976
N. davidi	0	0j	1,00x0,80		Suppression complète	douce	Pantaleao, 2015
N. denticulata (Japon)	0	0j	1,60x1,05	100-140	Suppression complète	douce	Mizue & Iwamoto, 1961
Caridina brevirostris (Ryukyu)	1	0j	1,2x0,8	13-30	Suppression complète	douce	Shokita, 1973
C. laevis (Trivandrum)	2	3-4j	0,95x0,56	30-45	Abrégé	douce	Nair, 1949
C. mccullochi (Australie)	2	1j	0,77x0,62	44	Abrégé	douce	Benzie, 1982
C. aruensis (Australie)	4	12 j	0,64x0,40	74	Abrégé	douce	Glaister, 1976
Halocaridina rubra (Hawaii)	4	14-17j	0,93x0,71	12	Abrégé	salé (15‰)	Couret & Wong, 1978
C. nilotica (Egypte)	5	12 j	0,73x0,5	43-147	Abrégé	douce	Gurney, 1927
C. propinqua	5	4 j	0,49x0,36		Abrégé	douce	Babu, 1963
C. pseudodenticulata (Taiwan)	5	15-17j	0,77x0,50		Abrégé	douce	Lai & Shy, 2009
Caridina sp. (Cochin)	5	11j			Abrégé	salé (16-17‰)	Lakshmi, 1975
C. babaulti basrensis (Irak)	6	14j			Abrégé	douce	Salman, 1987
C. nilotica (Lac Victoria)	6				Abrégé	douce	Daday, 1907
C. gracilirostris (Cochin)	6	9-15 j	0,32x0,28	>500	Commun (amphidrome)	salé (15-16‰)	Thomas et al., 1973 ; Pillai, 1975 ; Heerbrandt & Lin, 2006
Atyaephyra desmaresti (Algérie)	6-7	18-24j	0,60x0,40	950-1250	Commun	douce	Gauthier, 1924
C. gracilipes	8		0,45x0,30		Commun (amphidrome)		Zhang & Sun, 1979
C. longirostris (Cochin)	8	15 j	0,35x0,21	435-1325	Commun (amphidrome)	salé (6-10‰)	Pillai, 2002
Paratya australiensis	8	28-45j	0,59x0,36	200-1200	Commun	douce	Walsh, 1993
C. multidentata (Japon)	9	24-38j	0,50x0,31	747-4391	Commun (amphidrome)	salé (16,9‰)	Hayashi & Hamano, 1984
C. weberi (Aurangabad)	9	26 j	0,47x0,30		Commun (amphidrome)	douce (peu de succès)	Chinnaya, 1974
Atya scabra (Brésil)	10	53j			Commun (amphidrome)	salé (18‰)	Abrunhosa & Moura, 1988
Micratya poeyi (Jamaïque)	11	53-74j	0,42x0,32		Commun (amphidrome)	salé (32‰)	Hunte, 1979b
Atya innocous (Jamaïque)	12	76-119j			Commun (amphidrome)	salé (30‰)	Hunte, 1979a
Atyoida serrata (Réunion)		18j			Commun (amphidrome facultatif ?)	douce	Bordage, 1908
C. leucosticta (Japon)		14-26j			Commun (amphidrome)	salé (17‰)	Nakahara et al, 2005
C. serratirostris (Japon)		34-46j			Commun (amphidrome)	salé (25,5‰)	Nakahara et al, 2005
C. typus (Japon)		18-48j			Commun (amphidrome)	salé (25,5‰)	Nakahara et al, 2005
Atyoida bisulcata (Hawaii)		21j	0,72x0,48	1000-3000	Commun (amphidrome)	salé (35‰)	Couret & Wong, 1978

recoloniser les habitats dépeuplés par les perturbations de l'environnement (McDowall, 2007).

La dispersion marine des larves de caridines est dépendante de différents facteurs la favorisant ou la perturbant comme des barrières physiques (masses continentales, courants océaniques, grandes profondeurs, etc.) ou physico-chimiques (salinité, température) (Borkin, 1991 ; Hare & Cowen, 1996 ; Grant & Bowen, 1998). Ainsi, suivant les barrières ou les voies de dispersion existantes ou passées, la distribution géographique des espèces amphidromes peut être expliquée. De nombreuses interrogations demeurent cependant quant à la phase planctonique. Par exemple, on ignore à quelle profondeur évoluent les larves, leur régime alimentaire ou encore la durée de la vie planctonique.

1.4.3 La sclérochronologie comme étude de l'amphidromie

Certaines techniques ont été utilisées pour l'étude de l'amphidromie, notamment la sclérochronologie. Il s'agit de l'étude physico-chimique de parties dures (généralement calcifiées) d'organismes pour obtenir des informations sur leur vie, notamment grâce à l'observation de stries de croissances (Buddemeier *et al.*, 1974). Cette technique a été appliquée, entre autres, chez les poissons, où on étudie l'otolithe (une concrétion de carbonate de calcium présente dans l'oreille interne) (Pannella, 1971, 1974) et chez les mollusques, où l'on étudie la coquille ou l'opercule pour les gastéropodes (Gröcke & Gillikin, 2008). Initialement limitée à la détermination de l'âge par comptage de stries, la sclérochronologie peut fournir des informations détaillées sur la vie de l'organisme grâce à des analyses microchimiques des structures calcifiées (Radtke *et al.*, 1988). Par exemple, les otolithes peuvent servir de « boîtes noires » en enregistrant les variations de la composition chimique des milieux traversés au cours de la vie du poisson, permettant de repérer la transition entre le milieu marin et la rivière chez les espèces amphidromes (Radtke *et al.*, 1988 ; Shen *et al.*, 1998). Il en va de même pour l'opercule des gastéropodes amphidromes du genre *Neritina* (Abdou, 2016). Aucune structure de ce type n'avait été mise en évidence chez les crustacés jusqu'à quelques travaux récents (Leland *et al.*, 2011 ; Kilada *et al.*, 2012 ; Kilada & Acuna, 2015 ; Kilada *et al.*, 2015 ; Kilada & Ibrahim, 2016 ; Kilada & Driscoll, 2017) utilisant des stries de croissances annuelles repérées dans l'épaisseur de l'endo-cuticule des pédoncules oculaires et de certains ossicules gastriques chez différentes espèces de décapodes marins. **Nous**

tenterons dans le présent mémoire d'appliquer les techniques de sclérochronologie à l'étude de l'amphidromie dans le genre *Caridina*.

1.5 La zone Indo-Pacifique

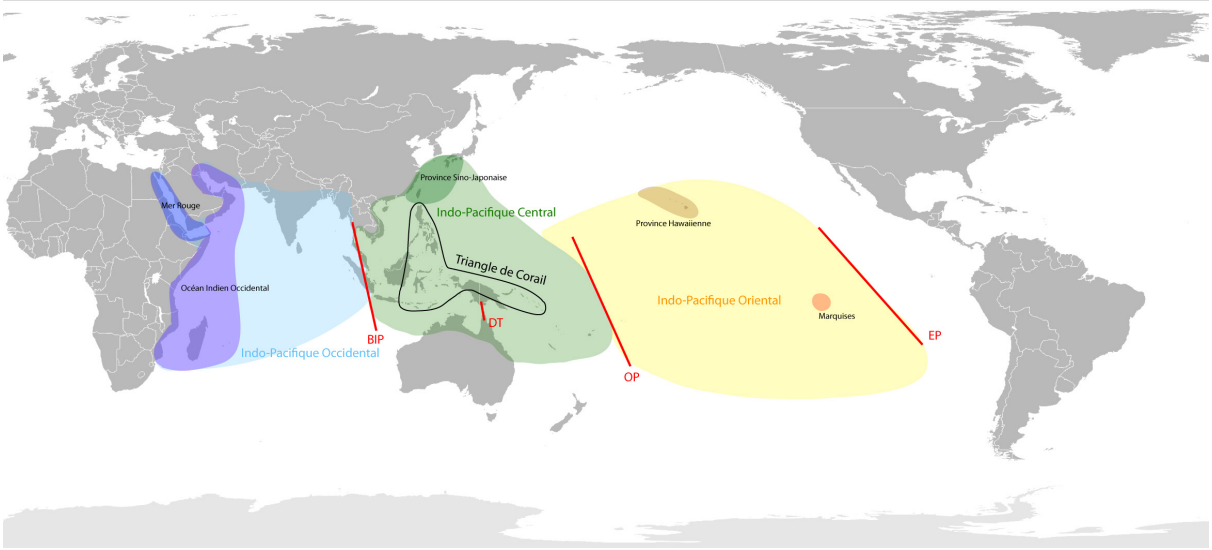


Figure 1.9 : Carte de la zone Indo-Pacifique, ses trois domaines et cinq provinces (aires colorées) et les barrières (lignes rouges) impactant les espèces amphidromes (BIP : Barrière Indo-Pacifique ; DT : Détroit de Torres ; OP : Ouest Pacifique ; EP : Est Pacifique).

Figure 1.9: Map of the Indo-Pacific area, its three realms and five provinces (colored areas) and barriers (red lines) impacting amphidromous species (BIP: Indo-Pacific Barrier; DT: Torres Strait; OP: West Pacific; EP: East Pacific).

1.5.1 Géographie

La région Indo-Pacifique (ou Indo-Ouest-Pacifique), notre zone d'étude, est une immense entité biogéographique s'étendant sur plus de 220° de longitude et 60° de latitude, comprenant les régions tropicales des océans Indien et Pacifique et leurs innombrables îles. La région est subdivisée en trois domaines (Spalding *et al.*, 2007) (Figure 1.9) :

- L'Indo-Pacifique **Central**, qui comprend l'Insulinde, la Mélanésie et la Micronésie
- L'Indo-Pacifique **Oriental**, qui comprend la Polynésie et Clipperton
- L'Indo-Pacifique **Occidental**, qui comprend les régions tropicales de l'océan Indien et la mer Rouge.

1.5.2 Géologie

L'océan Indien est l'héritier de la Tethys, un océan séparant les continents du Nord et ceux du Sud entre le Crétacé et le Miocène, qui s'est refermé lors de la collision de l'Inde et de l'Arabie avec la plaque Eurasienne. Etant le plus récent des océans, l'océan

Indien possède un système d'extension océanique actif avec trois dorsales séparant les plaques Indo-australienne, Africaine et Antarctique se rejoignant au niveau de la jonction triple de Rodrigues, et une zone de subduction formant la Fosse de la Sonde (ou Fosse de Java, 7 725m de profondeur maximale), marquant la limite entre les plaques Indo-australienne et Eurasienne (Bird, 2003). Plusieurs points chauds volcaniques existent dans l'océan Indien, à l'origine de chaînes de monts sous-marins comme les rides de Chagos-Laccadive et du 90° Est, émergeant parfois pour former des îles comme pour le point chaud de la Réunion et de l'Île Maurice ou celui des Comores (Müller *et al.*, 1993) (Figure 1.10).

L'océan Pacifique est issu de la Panthalassa, l'océan entourant la Pangée jusqu'à son éclatement au début du Mésozoïque. S'étendant sur neuf plaques tectoniques, l'océan Pacifique possède une géologie très complexe. Au Sud et à l'Est, une dorsale marque la limite entre les plaques Pacifique d'une part et Juan de Fuca, Cocos, Nazca et Antarctique de l'autre. Partout ailleurs, les limites sont marquées par des zones de subduction avec des fosses doublées d'arcs volcaniques. On peut citer notamment la Fosse des Tonga (10 882m de profondeur maximale) couplée à l'arc de Tonga-Kermadec et la Fosse des Nouvelles Hébrides (7 600m de profondeur maximale) couplée à l'arc du Vanuatu séparant toutes deux les plaques Pacifique et Indo-australienne ; la Fosse des Philippines (10 540m de profondeur maximale) couplée à l'arc des Philippines et la Fosse de Ryukyu (5 212m de profondeur maximale) couplée à l'arc des Ryukyu, séparant toutes deux les plaques Eurasienne et des Philippines ; ou encore la Fosse des Mariannes/Izu-Ogasawara (11 034m de profondeur maximale) couplée à l'arc Izu-Bonin-Mariannes séparant les plaques Pacifique et des Philippines (Bird, 2003). Les plaques de l'océan Pacifique sont également percées de plusieurs points chauds, notamment celui des Carolines, celui des Samoa, celui de Hawaii, celui des Marquises, celui de la Société, celui de Pitcairn ou celui des Australes, tous à l'origine d'archipels ou de chaînes de monts sous-marins (Figure 1.10).

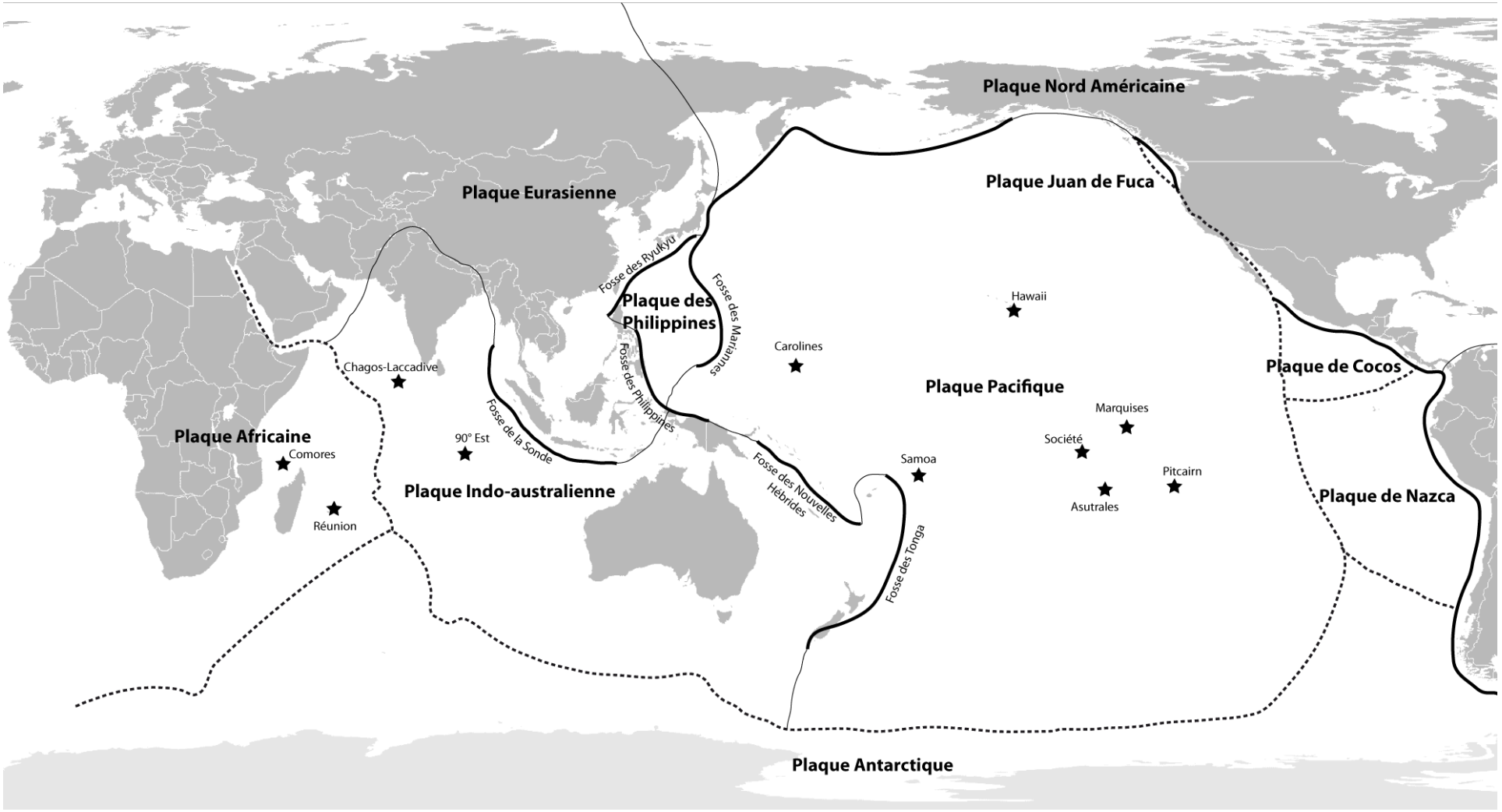


Figure 1.10 : Géologie de la zone Indo-Pacifique, les limites de plaques sont indiquées par les lignes ; pointillés : dorsales ; épaisse : subduction. Les étoiles noires indiquent les points chauds.

Figure 1.10: Geology of the Indo-Pacific region, plate limits are represented by full lines; dashed lines: ridges; thick lines: subduction zones. Black stars indicate volcanic hotspots.

1.5.3 Biogéographie

La zone Indo-Pacifique a été définie avant tout selon des critères biogéographiques. Incroyablement riche en espèces (plus de 6000 espèces de mollusques, 800 d'échinodermes, 500 de coraux scléractiniaires et 4000 de poissons, d'après Briggs (1995) mais largement supérieur actuellement grâce à de grandes expéditions réalisées et l'apparition de nouvelles techniques de séquençage, voir Albano *et al.* (2011)), une certaine homogénéité existe au sein de la zone du fait d'espèces à large répartition et l'absence de barrières continentales. Il existe cependant des différences en terme de diversité d'espèces suivant les régions de cette zone, la plus forte diversité (tous groupes taxonomiques confondus) se retrouvant dans le « Triangle de Corail » (Figure 1.9), une zone circonscrite entre les Philippines, la péninsule malaise et les îles Salomon (Veron *et al.*, 2009) avec une diminution de la diversité à mesure que l'on s'en éloigne, corrélée à la distance (Briggs, 1999). Plusieurs provinces biogéographiques ont été identifiées au sein de la zone Indo-Pacifique par un taux d'endémisme des poissons marins supérieur à 10% (Bowen *et al.*, 2016) : la mer Rouge, l'océan Indien Occidental, la province Indo-Polynésienne, la province Sino-Japonaise, la province Hawaiienne et les Marquises. Des barrières à la dispersion ont pu être mises en évidence par des différences de diversité génétique ou de composition des faunes. Bowen *et al.* (2016) ont ainsi identifié la barrière de la mer Rouge, la barrière Est-Pacifique et la barrière Indo-Pacifique. Ces barrières affectant la distribution des espèces marines sont de différentes natures. La barrière de la mer Rouge est due à des différences de salinité et de température couplées à une connexion étroite, la barrière Est-Pacifique est due à l'isolement géographique et l'absence d'habitats favorables, et la barrière Indo-Pacifique est due à la présence de plateaux continentaux (Sunda, qui joignait l'Asie à la Malaisie, Sumatra, Bornéo et Java, et Sahul, qui joignait l'Australie à la Papouasie *via* le pont du détroit de Torres) ayant émergé à plusieurs reprises en fonction des variations du niveau marin avec le climat.

Ces barrières concernent surtout les faunes marines, mais d'autres ont été identifiées pour les faunes terrestres, notamment au niveau de l'archipel malais, où différentes lignes ont été proposées pour séparer les provinces Indo-malaise et Australasienne, notamment (Figure 1.11) :

- La ligne de Wallace (Wallace, 1863) passant à l'Est des Philippines, entre les îles de Bornéo et Sulawesi au Nord et entre Lombok et Bali au Sud, construite à partir d'observations sur la faune.
- La ligne de Huxley (Huxley, 1868) reprenant la ligne de Wallace mais passant à l'Ouest des Philippines, construite à partir de la distribution des espèces d'oiseaux.
- La ligne de Lydekker (Lydekker, 1896), passant entre les Moluques et la Papouasie au Nord et entre les îles Tanimbar et Aru au Sud, construite à partir de la distribution des espèces de mammifères.
- La ligne de Weber (M. Weber, 1902), passant entre Halmahera et Sulawesi au Nord, à l'Ouest de Buru et entre les îles Tanimbar et Babar, construite à partir de la distribution des poissons d'eau douce.

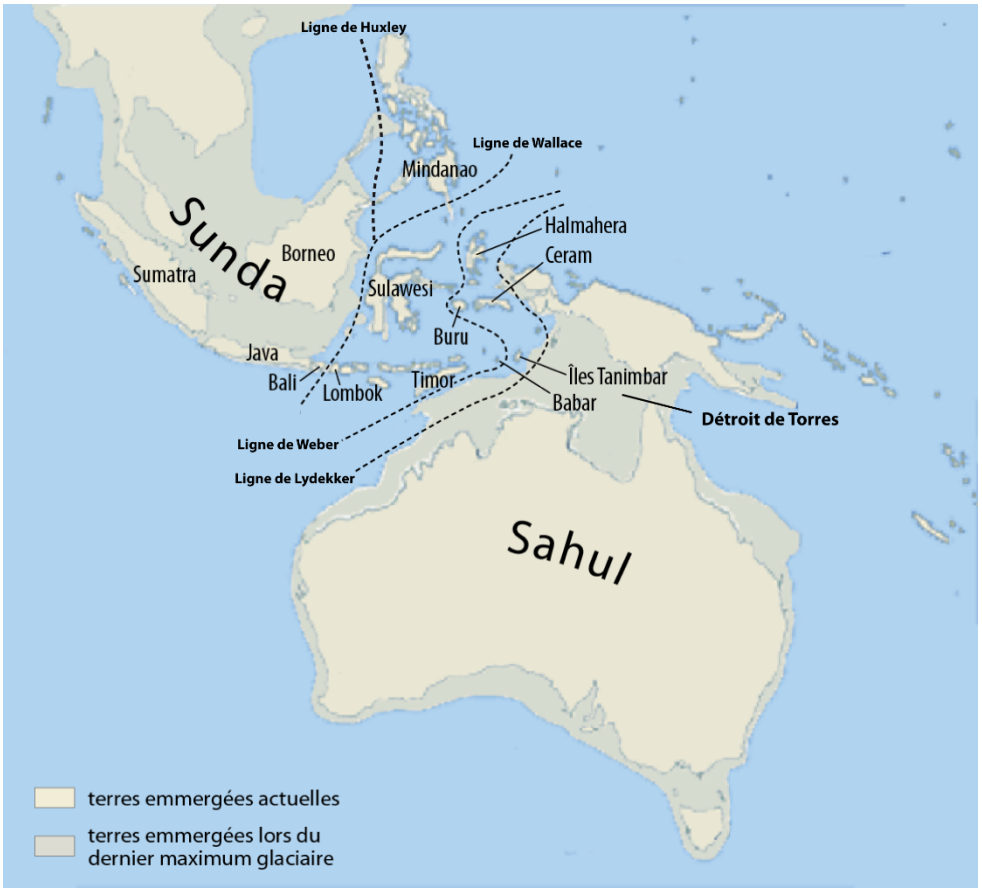


Figure 1.11 : Carte de l'archipel Indo-australien avec 4 lignes délimitant les provinces terrestres Indo-malaise et Australienne et montrant les ponts continentaux ayant existé lors du dernier maximum glaciaire. Modifié d'après Wikimedia Commons.

Figure 1.11: Map of the Indo-Australian Archipelago with 4 lines delimiting Indo-Malaysian and Australian terrestrial provinces and showing continental bridges having existed during the last glacial maximum. Adapted from Wikimedia Commons.

Chez les espèces amphidromes, les barrières à la dispersion peuvent être différentes de celles influençant la répartition des espèces terrestres, du fait de la phase de dispersion océanique des larves. Ainsi, des études de génétique des populations ont montré l'influence des barrières Indo-Pacifique (séparant l'Ouest Pacifique de l'océan Indien) et Est-Pacifique (séparant l'Ouest Pacifique de l'Est Pacifique) chez les gobies du genre *Sicyopterus* (Lord *et al.*, 2012), les crevettes de l'espèce *Macrobrachium lar* (Castelin *et al.*, 2013a), les poissons du genre *Eleotris* (Mennesson & Keith, 2017 ; Mennesson *et al.*, 2018) et les gastéropodes du genre *Neritina* (Crandall *et al.*, 2010 ; Abdou, 2016), ou encore la barrière du détroit de Torres (entre l'Australie et la Papouasie-Nouvelle Guinée) chez les gobies de l'espèce *Sicyopus zosterophorum* (Taillebois *et al.*, 2013a) (Figure 1.9).

1.5.4 Courantologie

Dans le cas des espèces amphidromes, les courants marins peuvent avoir un impact considérable sur leur répartition (selon la profondeur où circulent les larves), pouvant servir de voies de dispersion comme de barrières. Les courants marins de surface de la zone Indo-Pacifique ont une direction globale Est-Ouest, mus par les vents dominants et dirigés par la force de Coriolis. On trouve ainsi les courants Nord et Sud-Equatorial dans les deux océans, séparés par le contre-courant Equatorial. Au Nord de l'Equateur, les courants tournent vers la droite en rencontrant un obstacle alors qu'ils tournent à gauche dans l'hémisphère Sud, formant de grands gyres océaniques dans les deux cas. Le courant Sud-Nord formant la partie Ouest du gyre subtropical du Pacifique Nord est appelé Kuroshio et réchauffe les côtes de Taiwan, de Chine et du Japon. Le courant Nord-Sud formant la partie Ouest du gyre subtropical du Pacifique Sud est appelé le Courant Est-Australien. Le courant Nord-Sud formant la partie Ouest du gyre de l'océan Indien est appelé courant des Aiguilles (Agulhas current) (Figure 1.12).

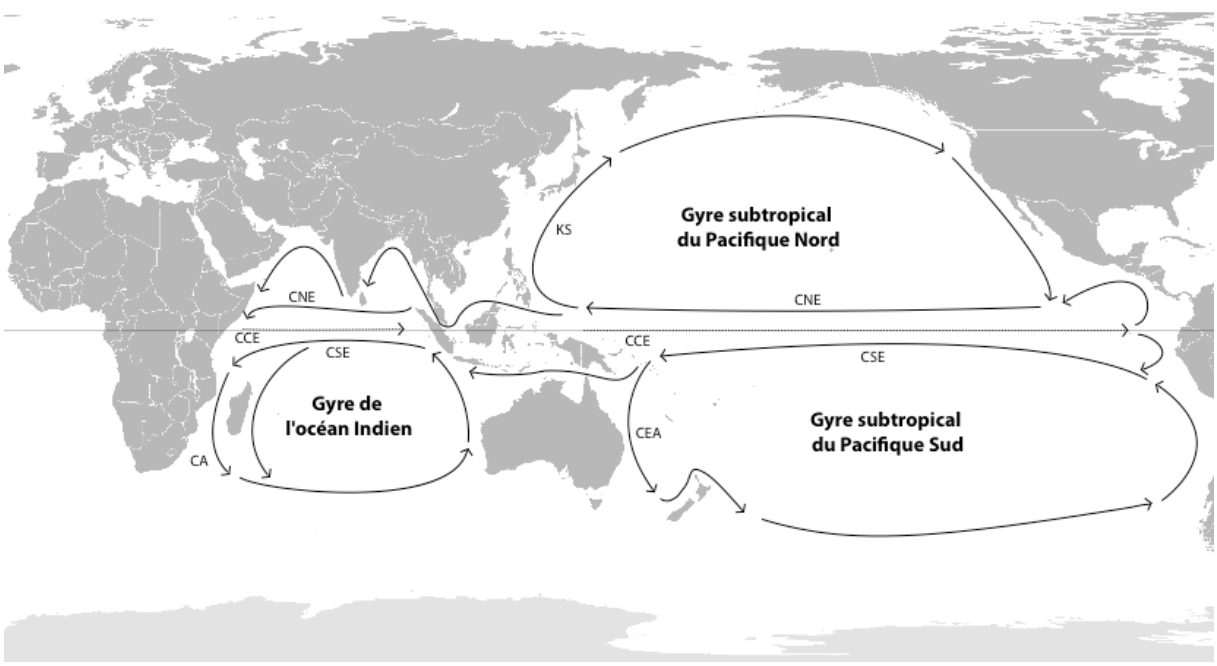


Figure 1.12 : Carte des courants principaux de la zone Indo-Pacifique. CNE : Courant Nord Equatorial ; CSE : Courant Sud Equatorial ; CCE : Contre-courant Equatorial ; CEA : Courant Est-Australien ; KS : Kuroshio ; CA : Courant des Aiguilles.

Figure 1.12: Map of the main oceanic currents of the Indo-Pacific region. CNE: North Equatorial Current; CSE: South Equatorial Current; CCE: Equatorial Counter-Current; CEA: East-Australian Current; KS: Kuroshio; CA: Agulhas Current.

1.6 Unité et diversité des systèmes insulaires

D'après la Convention des Nations Unies sur le droit de la mer de 1982 (Multilatéral, 1994), « une île est une étendue naturelle de terre entourée d'eau qui reste découverte à marée haute. » (Partie VIII, Article 121, alinéa 1, p. 48). Il s'agit donc d'espaces plus ou moins exigus, circonscrits dans l'espace par un environnement aquatique et donc soumis à son influence, notamment climatique. Leur superficie peut varier de quelques mètres carrés à 7,6 millions de kilomètres carrés (On considèrera dans ce mémoire l'Australie comme étant la plus grande des îles plutôt que le plus petit des continents).

1.6.1 Typologie des îles

Selon les mécanismes à l'origine de leur formation, les îles peuvent être classées en plusieurs types. Premièrement, les îles de type continental, qui sont issues de la fragmentation d'un continent soit par transgression marine suite à un réchauffement climatique (Par exemple, la Nouvelle Guinée et la Tasmanie, séparées de l'Australie par les détroits de Torres et de Bass ou Bornéo et Sumatra, séparées de l'Asie par la formation du détroit de Malacca et de la mer de Java et la transgression de la mer de Chine méridionale) soit par fracture d'une plaque continentale (Par exemple, l'Australie

ou la Nouvelle Zélande, séparées du Gondwana et Madagascar, séparée de l'Afrique). Dans les deux cas, les îles continentales sont susceptibles de posséder une faune et une flore ancienne qui ont évolué indépendamment depuis leur isolement, provoquant un fort taux d'endémisme.

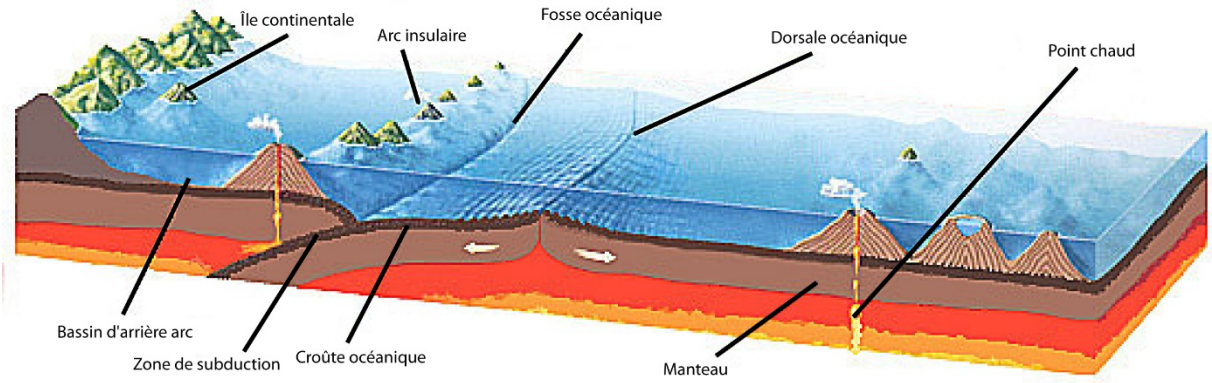


Figure 1.13 : Représentation schématique des différents mécanismes de formation des îles.

Figure 1.13: Schematic representation of the different island-formation mechanisms.

Deuxièmement, les îles océaniques, formées par une accrétion de roche volcanique qui finit par atteindre la surface de la mer. Suivant le type de volcanisme, on en distingue 3 types (Figures 1.13 et 1.15) ; les îles de zones de convergence, formées lorsqu'une plaque tectonique plonge sous une autre (Par exemple, le Vanuatu, les îles Salomon, les îles Mariannes, etc.) ou par les mécanismes d'accrétion sédimentaire par l'accumulation de sédiments marins à l'endroit où la plaque océanique plonge sous une autre plaque (Par exemple, Nias et les autres îles au large de la côte Est de Sumatra) ; les îles des zones d'extension, formées par l'émergence de volcans en zone de dorsale océanique (Par exemple, l'île de l'Ascension ou l'Islande) ; les îles intra plaque (ou de point chaud), formées par l'émergence d'un volcan au niveau d'un point chaud stationnaire par rapport au mouvement de la plaque tectonique. Ce type de volcanisme a formé de nombreux archipels avec différents types d'îles à mesure que l'on s'éloigne de la position actuelle du point chaud. En effet, du fait de l'érosion et de l'ajustement isostatique, les îles se transforment (Figure 1.14). Une île nouvellement formée par l'émergence d'un volcan va rapidement voir un récif corallien frangeant se former. A mesure que l'île s'érode et s'enfonce avec le plancher océanique du fait de la subsidence, un lagon commence à apparaître entre le récif désormais appelé barrière et l'île, qui finira par disparaître, ne laissant visible que le récif et un lagon en son centre, c'est-à-dire un atoll. Le stade final d'évolution de ces îles est atteint lorsque le récif n'émerge plus et devient

un mont sous-marin que l'on appelle guyot. Au contraire, du fait de mouvements tectoniques ou isostatiques ou de baisse du niveau de la mer, un atoll peut se retrouver surélevé au dessus du niveau marin, entraînant la disparition du lagon (Par exemple, Nauru ou Niue).

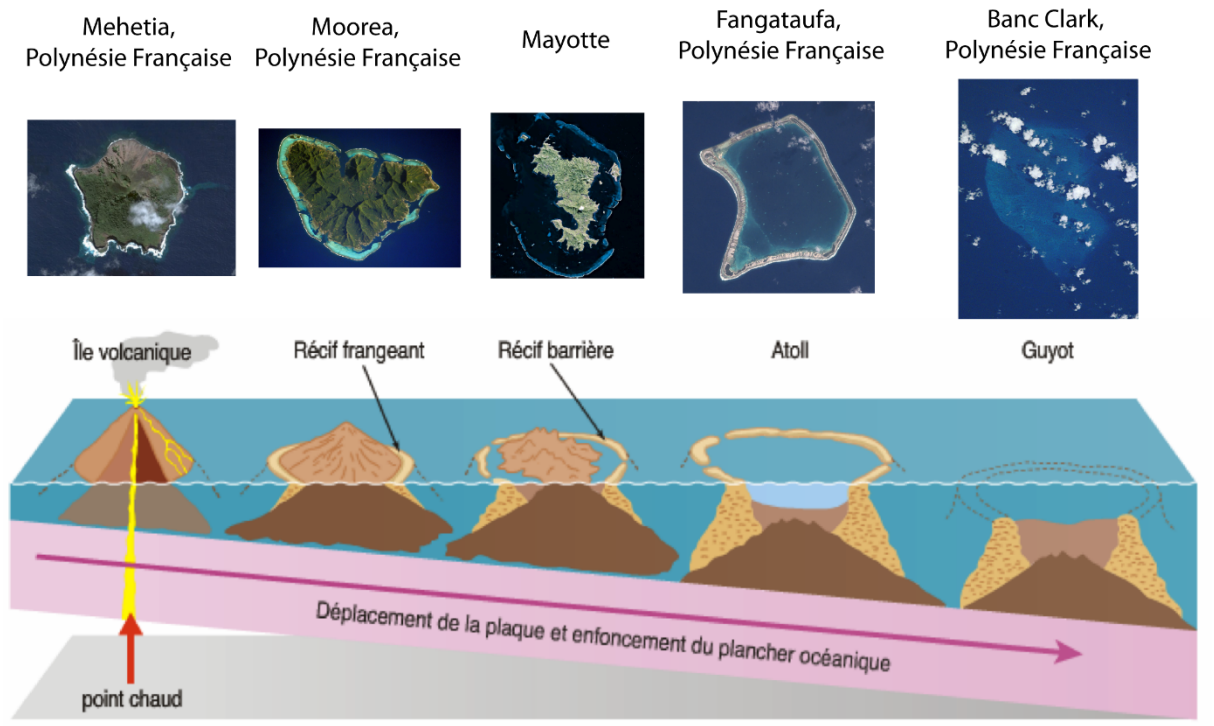


Figure 1.14 : Formation et évolution géomorphologique temporelle d'une île volcanique avec vues satellites en illustrations.

Figure 1.14: Formation and geomorphological evolution of a volcanic island with satellite views as illustrations.

En pratique, on distingue les îles hautes des îles basses. Les premières se caractérisent justement par une altitude maximale plus élevée du fait de la persistance du socle volcanique ou sédimentaire, alors que les secondes présentent un relief peu marqué et sont souvent issues de formations coralliennes. Ces différences topographiques entraînent des différences climatiques. En effet, les îles basses ont généralement un climat plus aride et sont dépourvues d'eaux douces permanentes en surface, contrairement aux îles hautes plus humides et qui possèdent généralement un réseau hydrographique développé.

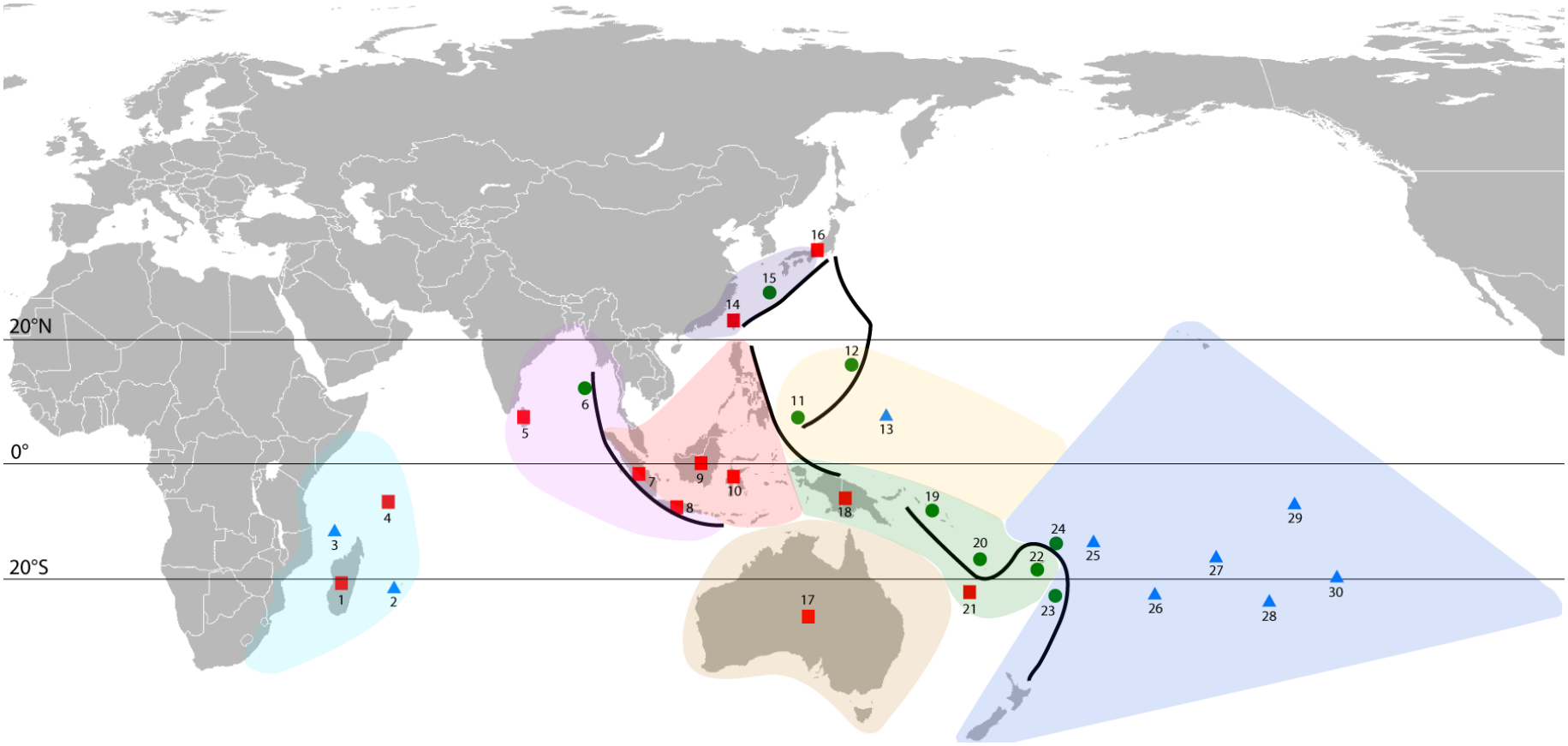


Figure 1.15 : Carte des différents types d'îles (Carrés rouges : îles continentales ; Triangles bleus : volcanisme de point chaud ; Ronds verts : volcanisme d'arc) au sein des différentes aires géographiques de la zone Indo-Pacifique (Bleu clair : Océan Indien occidental ; Mauve : Océan Indien oriental ; Rouge : Insulinde ; Vert : Mélanésie ; Jaune : Micronésie ; Bleu foncé : Polynésie ; Brun : Australie ; Gris : Asie de l'est) avec les fosses océaniques (lignes noires). 1 : Madagascar ; 2 : Mascareignes ; 3 : Comores ; 4 : Seychelles ; 5 : Sri Lanka ; 6 : Andaman ; 7 : Sumatra ; 8 : Java ; 9 : Bornéo ; 10 : Sulawesi ; 11 : Palau ; 12 : Mariannes ; 13 : Micronésie ; 14 : Taiwan ; 15 : Ryukyu ; 16 : Japon ; 17 : Australie ; 18 : Nouvelle Guinée ; 19 : Salomon ; 20 : Vanuatu ; 21 : Nouvelle Calédonie ; 22 : Fidji ; 23 : Tonga ; 24 Wallis et Futuna ; 25 : Samoa ; 26 : Cook ; 27 : Société ; 28 : Australes ; 29 : Marquises ; 30 : Gambier.

Figure 1.15: Map of the different island types (red squares: contiental islands; blue triangles: hotspot volcanism; green circles: arc volcanism) in the different areas of the Indo-Pacific region (light blue: Western Indian Ocean; mauve: Eastern Indian Ocean; red: Malay Archipelago; green: Melanesia; yellow: Micronesia; dark blue: Polynesia; Brown: Australia; gey: East Asia) with oceanic trenches (black lines). 1 : Madagascar ; 2 : Mascarenes ; 3 : Comoros ; 4 : Seychelles ; 5 : Sri Lanka ; 6 : Andaman ; 7 : Sumatra ; 8 : Java ; 9 : Borneo ; 10 : Sulawesi ; 11 : Palau ; 12 : Marianas ; 13 : Micronesia ; 14 : Taiwan ; 15 : Ryukyu ; 16 : Japan ; 17 : Australia ; 18 : New Guinea ; 19 : Solomon ; 20 : Vanuatu ; 21 : New Caledonia ; 22 : Fiji ; 23 : Tonga ; 24 Wallis and Futuna ; 25 : Samoa ; 26 : Cook ; 27 : Society ; 28 : Austral ; 29 : Marquesas ; 30 : Gambier.

1.6.2 Les différents habitats aquatiques

Les écosystèmes dulcicoles des îles tropicales se présentent sous différentes formes, lacs, marais, eaux souterraines, ou encore rivières (Figure 1.16). Ces milieux sont soumis à de fortes perturbations, comme les sécheresses, les crues, les éruptions volcaniques, etc. et forment des unités discontinues, séparées les unes des autres (par le milieu marin dans le cas des bassins versants). Les cours d'eau peuvent être plus ou moins longs selon la taille de l'île allant de ruisseaux de quelques mètres de long jusqu'à de véritables fleuves de plusieurs kilomètres (Par exemple le Diahot en Nouvelle Calédonie ou le Sigatoka à Fidji). On distingue les cours d'eau rapides et montagneux des cours d'eau lents de plaine (devenant éventuellement des trous bleus ou des marais). Leur débit est sujet à de fortes variations temporelles (pouvant être très important en période de crue ou nul dans le cas des rivières non permanentes) et spatiales (suivant la pente, le type de substrat rocheux ou le couvert végétal). Une zonation altitudinale en cinq zones a été proposée (Keith *et al.*, 2010) dont les limites varient selon les îles étudiées, à savoir, la zone des sources, le cours supérieur, le cours moyen, la partie basse et le cours inférieur. Une sous zonation a pu être déterminée pour les quatre derniers étages en fonction de la vitesse du courant. On identifie ainsi des zones calmes (de 0 à 30 cm/s), des zones à courant moyen (de 30 à 75 cm/s) et des zones à courant rapide (75 à >100 cm/s).

En pratique, la zonation altitudinale est simplifiée en trois étages fonctionnels :

- **Le cours supérieur**, caractérisé par une forte pente (généralement >10%), un courant rapide et un substrat composé de gros blocs rocheux.
- **Le cours moyen**, caractérisé par une pente modérée (<10%) et un substrat composé de galets et de blocs, voire de sable dans les zones calmes. Cet étage est généralement séparé du précédent par un accident topographique de type cascade.
- **Le cours inférieur**, caractérisé par une pente très faible (voire nulle) avec peu de courant et un substrat de graviers dont la granulométrie diminue en s'approchant de l'estuaire. On y distingue l'estuaire, zone influencée par le milieu marin (saumâtre, influence de la marée) de la zone proprement limnique.

Certaines espèces peuvent être restreintes à une zone particulière du cours d'eau en fonction de leurs adaptations à ses caractéristiques (substrat, profondeur, vitesse du courant, salinité, etc.).

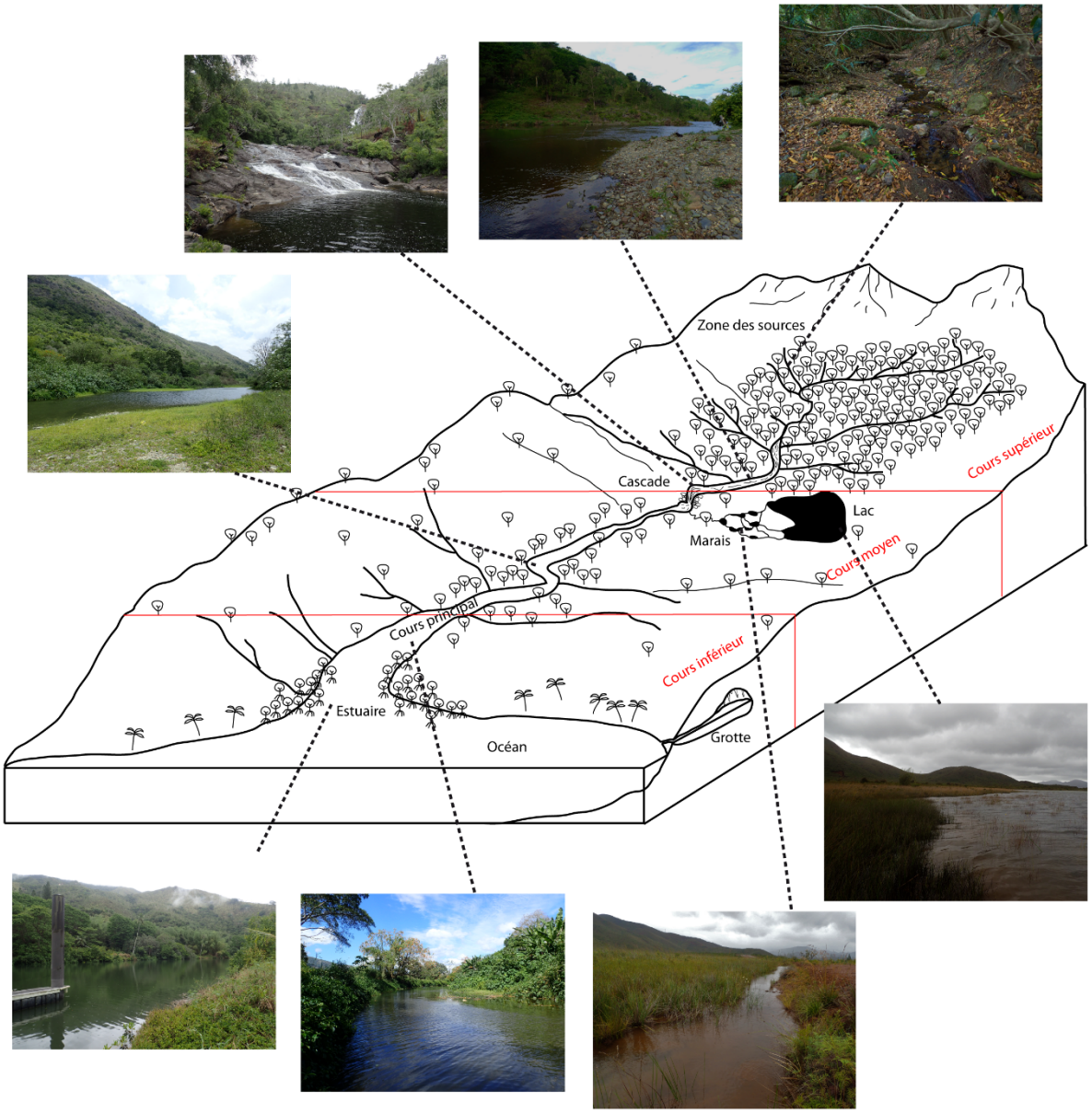


Figure 1.16 : Représentation schématique des différents habitats aquatiques d'une île tropicale avec illustrations de ces milieux en Nouvelle Calédonie.

Figure 1.16: Schematic representation of the different aquatic habitats of a tropical island with illustrations of these environments from New Caledonia.

Tout ce qui vient d'être exposé dans les deux sous-parties précédentes (1.5 et 1.6) explique une grande partie de la distribution actuelle dans les rivières et de l'évolution des espèces, de même que la mise en place de la biodiversité du genre *Caridina* dans les îles de l'Indo-Pacifique.

1.7 La Taxonomie intégrative

La taxonomie (Candolle, 1813) est une discipline de la biologie dont le but est de classer les êtres vivants en *taxons*, des entités permettant de les classer et de les reconnaître. Traditionnellement, ce sont des caractères morphologiques, anatomiques ou éthologiques qui sont utilisés en suivant le Code International de Nomenclature (Teletchea, 2010). L'unité de base de la taxonomie est l'espèce. Différentes définitions du concept d'espèce ont été proposées suivant les méthodes et les groupes taxonomiques sur lesquels se sont concentrés les auteurs. A l'origine, les espèces étaient construites selon le concept morphologique, c'est-à-dire des individus partageant une ressemblance physique. Ce concept ne prenait pas en compte la notion d'évolution et était lié à une vision fixiste de la diversité du vivant (Bocquet *et al.*, 1976). Le concept le plus connu est le concept biologique, selon lequel une espèce est composée de populations interfécondes donnant une descendance viable et fertile et reproductivement isolées des autres (Mayr, 1942). Selon le concept écologique, les membres d'une même espèce doivent partager une même niche écologique et des environnements similaires (Hutchinson, 1968 ; Van Valen, 1976). D'après le concept évolutif, une espèce est une entité qui maintient son identité spatiale et temporelle par rapport aux autres, avec son historique (passé et futur) propre (Simpson, 1951 ; Wiley, 1978 ; Mayden, 1997). Templeton (1989, 1998) définit l'espèce selon le concept phénotypique comme un ensemble d'individus présentant un phénotype cohérent avec des échanges génétiques et démographiques. Le concept phylogénétique stipule qu'une espèce est composée d'un ensemble d'individus partageant un ancêtre commun et des caractères dérivés propres (synapomorphies) (Hennig, 1966). Il peut devenir le concept phénétique lorsque la reconstruction phylogénétique employée pour identifier les espèces se base sur la distance entre les taxons (Michener, 1970 ; Sokal & Crovello, 1970 ; Sneath & Sokal, 1973). Enfin, selon le concept génotypique, les membres d'une même espèce appartiennent à un même cluster génotypique (Mallet, 1995).

Ces nombreux concepts sont difficiles à unifier (De Queiroz, 2007 ; Dubois, 2011) car chacun trouve son application avec des méthodes de délimitation d'espèces différentes. Un morphologiste utilisera plutôt le concept morphologique, un généticien, le concept génotypique, ou un écologue, le concept écologique. Certaines visions se sont opposées, comme celle de la taxonomie dite « classique » (basée sur des critères morphologiques) et celle de la taxonomie moléculaire (basée sur des critères

génétiques) (Tautz *et al.*, 2003). L'utilisation de marqueurs moléculaires pour compléter ou remettre en question les hypothèses basées sur la morphologie a en effet alimenté de vifs débats entre les partisans de chaque méthode (Pires & Marinoni, 2010 ; Teletchea, 2010).

La tendance est actuellement à combiner des données issues de différentes méthodes pour tester les hypothèses taxonomiques (Page *et al.*, 2005), comme la biogéographie, l'écologie, la cytogénétique ou des données liées à la reproduction ou au développement (Dayrat, 2005 ; DeSalle *et al.*, 2005 ; Padial *et al.*, 2009 ; Padial *et al.*, 2010 ; Schlick-Steiner *et al.*, 2010 ; Pante *et al.*, 2015a) dans une approche que l'on appelle la **taxonomie intégrative** (Dayrat, 2005 ; Will *et al.*, 2005 ; Esselstyn, 2007 ; Padial *et al.*, 2009). Selon cette approche, une hypothèse de délimitation d'espèces n'est valide que si au moins deux jeux de données indépendants s'accordent à la valider (Padial *et al.*, 2010 ; Schlick-Steiner *et al.*, 2010 ; Pante *et al.*, 2015a) (Figure 1.17).

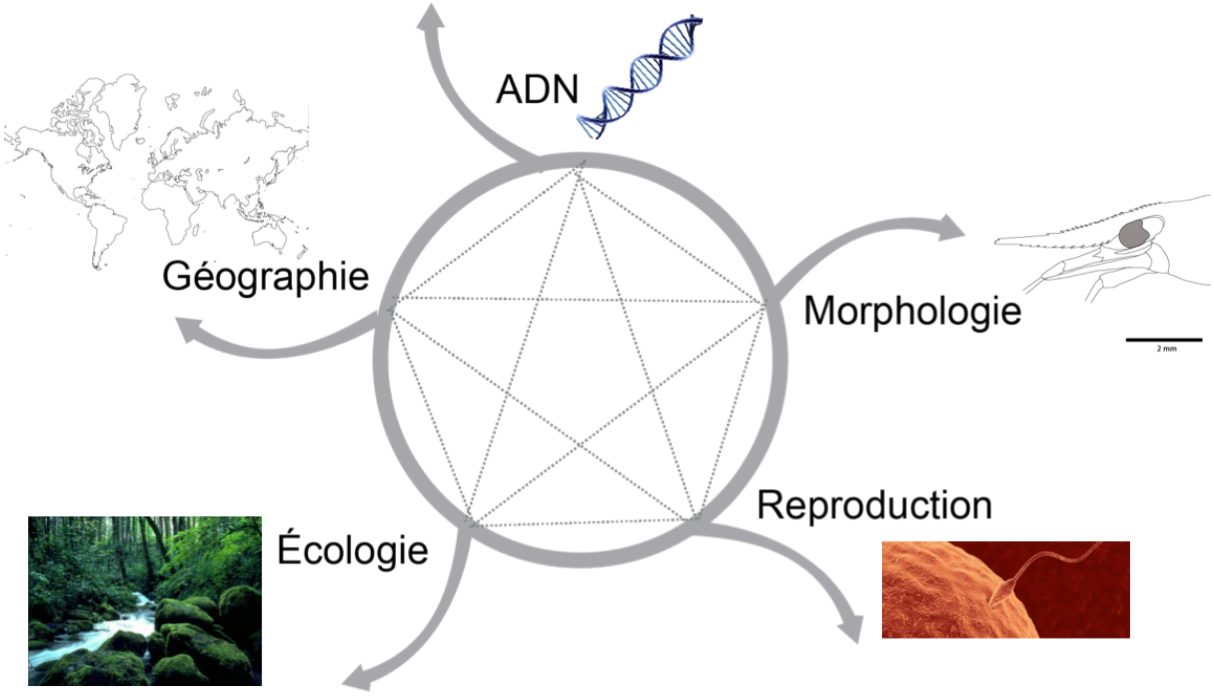


Figure 1.17 : Représentation schématique de l'approche de taxonomie intégrative selon DeSalle *et al.*, 2005 et Padial *et al.*, 2010. Les pointillés relient deux types de jeux de données entre eux. Une espèce est reconnue lorsque deux sources sont congruentes et permettent de sortir du cercle (flèches).

Figure 1.17: Schematic representation of the integrative taxonomy approach according to DeSalle *et al.*, 2005 and Padial *et al.*, 2010. Dashed lines link two types of dataset. A specied is recignised when two different sources of data are congruent and allow to leave the circle (arrows).

De plus en plus de taxonomistes choisissent cette méthode pour décrire de nouvelles espèces (Pante *et al.*, 2015b) et certains la considèrent comme l'avenir de la discipline (Padial *et al.*, 2010). **C'est cette approche qui sera utilisée tout au long du présent mémoire.**

1.8 Objectifs de la thèse

La difficulté taxonomique du genre *Caridina*, et en particulier au sein des complexes *C. nilotica* et *C. weberi* est apparue très tôt aux yeux des différents auteurs qui s'y sont intéressés, comme en témoigne le florilège suivant :

- « The family Atyidae, although a small one, comprises a considerable number of ill-defined species and genera, since most authors in creating such did not investigate their relations to those already known. » (*La famille des Atyidae, bien qu'étant petite, comprend un nombre considérable d'espèces et de genres mal définis, parce qu'en les créant, la plupart des auteurs n'ont pas étudié leur relation avec ceux déjà connus*.) (Ortmann, 1894).

- « Our knowledge of this species (*C. nilotica*) and its varieties appears still much confused and unsatisfactory. » (*Notre connaissance de cette espèce (*C. nilotica*) et ses variétés apparaît toujours très confuse et peu satisfaisante.*) (De Man, 1908).

- « Le *C. Weberi* (...) présente des différences locales assez considérables qui ont servi à reconnaître dans l'espèce un certain nombre de formes décrites sous le nom de variétés. Ces formes sont mal définies pour la plupart, et semblent reliées entre elles par des intermédiaires. » (Bouvier, 1925).

- « As is clearly shown by the present material, local populations may show a remarkable constancy in certain characters, while if representatives of various populations of a whole species are considered, these characters vary greatly, often linking two forms, which at first sight seem to represent distinct species. » (*Comme il apparaît clairement dans le présent matériel, des*

populations locales peuvent présenter une remarquable constance pour certains caractères, alors que si on considère des représentants de différentes populations d'une même espèce, ces caractères varient grandement, reliant souvent deux formes qui à première vue semblaient représenter des espèces différentes.) (Holthuis, 1965).

- « Bis heute ist die gültige Zuordnung vieler Arten, insbesondere der früheren Unterarten von *C. nilotica*, sehr umstritten. Die heute noch anerkannten Unterarten von *C. nilotica*, sehr stellen fast ausschließlich vereinzelte Funde von sehr spezifischen Fangorten dar, die nur sehr schwer mit neuem Belegmaterial verifiziert werden dürften. » (*A ce jour, la validité de nombreuses sous-espèces de* C. nilotica*, et en particulier des plus anciennes, est très controversée. Les sous-espèces de* C. nilotica *qui sont reconnues aujourd'hui ne sont représentées presque que par quelques spécimens provenant de localités très spécifiques et difficiles à retrouver.*) (Karge & Klotz, 2007).

La science a heureusement avancé depuis l'époque des premières études sur ce genre et de nouveaux outils sont apparus comme la génétique, qui, combinée avec les méthodes classiques de morphologie et les connaissances sur l'écologie de ces animaux dans une approche de taxonomie intégrative, permettent d'avoir un nouveau regard sur leur taxonomie. Il apparaît urgent, en effet, de clarifier la taxonomie de ce groupe qui a laissé perplexe des générations de grands naturalistes. Si les espèces sont mal définies, les identifications seront hasardeuses et toute étude (écologique, physiologique, embryologique, etc.) menée sur un organisme dont la détermination n'est pas certaine perd en valeur scientifique car elle n'est pas reproductible avec certitude, et perd en intérêt pour les gestionnaires qui ne connaîtront pas les traits de vie des espèces sur lesquels ils devront travailler. **C'est pourquoi, dans le présent travail, nous nous efforcerons de résoudre les problèmes taxonomiques existant au sein des complexes *C. nilotica* et *C. weberi* en combinant des données provenant de différentes sources, pour pouvoir étudier plus aisément leur histoire évolutive, leurs traits d'histoire de vie dont l'amphidromie est un des plus importants, et**

tenter d'éclaircir les zones d'ombres qui obscurcissent encore notre connaissance de la biologie de ces animaux.

Dans un **premier chapitre**, nous exposerons la méthodologie employée tout au long de ce travail, ainsi que l'échantillonnage du matériel biologique étudié.

Dans un **second chapitre**, nous verrons comment certains caractères morphologiques traditionnellement utilisés pour séparer les espèces de caridines sont rendus caducs par l'approche de taxonomie intégrative (un article publié).

Dans un **troisième chapitre**, nous nous intéresserons à la systématique du complexe *C. nilotica* dans trois régions distinctes de la zone Indo-Pacifique : 1. l'océan Indien occidental, avec la revalidation d'une espèce commune dans le cours inférieur des rivières de cette zone (un article publié), 2. la Micronésie, avec la description de deux espèces nouvelles se rencontrant dans le cours supérieur (deux articles publiés), et 3. l'Insulinde/Mélanésie avec une révision des espèces vivant dans le cours inférieur (un article publié).

Dans un **quatrième chapitre**, c'est sur la systématique du complexe *C. weberi* que nous nous pencherons dans deux régions distinctes de la zone Indo-Pacifique : 1. la Mélanésie, avec la mise en synonymie de deux espèces du cours inférieur (un article publié), et 2. la Polynésie, avec une révision des espèces confondues avec *C. weberi* trouvées dans le cours supérieur des rivières de cette zone (un article en préparation).

Dans un **cinquième chapitre**, nous chercherons à expliquer l'existence des deux complexes grâce à l'étude de leur phylogénie, afin de rendre compte de leurs liens de parenté et de leur position au sein du genre *Caridina*, de leur biogéographie, afin de mettre en évidence des aires d'endémisme, des barrières et des voies de dispersion, et l'étude des habitats préférentiels des espèces qui les composent, tout en replaçant les évènements évolutifs dans un contexte paléobiogéographique (un article soumis).

Dans un **sixième chapitre**, nous étudierons les possibilités d'application de méthodes de sclérochronologie pour étudier l'amphidromie dans le genre *Caridina*, ainsi que l'ultrastructure de la cuticule d'une espèce du complexe *C. weberi* (un article en préparation).

Enfin, dans un **septième et dernier chapitre**, nous dresserons le bilan de notre étude en discutant de ses apports à la connaissance du genre *Caridina*, sa biodiversité, sa systématique, sa biologie et son écologie, pour la replacer dans un contexte de conservation et de gestion de ces animaux et de leur milieu, et en examinant la possibilité d'étendre ce travail à d'autres taxons proches et (presque !) tout aussi problématiques.

Aims of the thesis

The present work aims to resolve the taxonomic complexity within *C. nilotica* and *C. weberi* complexes by combining data coming from different sources in order to ease the study of their evolutionary history, their life traits, including amphidromy and improve our global knowledge of their biology.

In a first chapter, we will expose the material and the methodology used in this work.

In a second chapter, we will see how integrative taxonomy can expose the invalidity of morphological characters commonly used to separate species.

In a third chapter, we will focus on the systematics of the *C. nilotica* complex in three different geographical areas of the Indo-Pacific region: Western Indian Ocean, Micronesia and Malay Archipelago/Melanesia.

In a fourth chapter, we will study the taxonomy of the *C. weberi* complex in two regions: Melanesia and Polynesia.

In a fifth chapter, we will try to explain the existence of the two complexes from their phylogeny in order to establish their relationships and their position within *Caridina* genus and study their biogeography and evolutionary history.

In a sixth chapter, we will investigate the possibilities of using sclerochronological methods to study the amphidromy in *Caridina* and present an ultrastructural study of the cuticle of a species belonging to the *C. weberi* complex.

Finally, in a seventh chapter, we will draw the conclusions of this work by discussing its inputs to the knowledge of the genus *Caridina*, its biodiversity, its systematics, its

biology and its ecology and by replacing it in a perspective of conservation and management of these species and their environment, and by considering the possibilities to extend this work to other problematic taxa.

Chapitre premier,
Matériel et Méthodes.

Material and Methods.

Où l'on présente ***l'échantillonnage*** *du matériel biologique ayant servi au présent travail, avec la liste des spécimens collectés par l'équipe, ceux envoyés par des collaborateurs étrangers et les spécimens empruntés dans diverses collections de musées, ainsi que les* ***méthodes*** *employées dans le cadre de la taxonomie intégrative, avec* ***les études morphologiques et statistiques*** *et les techniques de* ***biologie moléculaire*** *pour le barcoding et la phylogénie.*

Where are presented ***the sampling*** *of biological material used in the present work, with a list of specimens collected by the team, those sent by foreign co-workers and those lended by different museums, as well as* ***the methods*** *used in the framework of integrative taxonomy, with* ***morphological and statistical studies*** *and* ***molecular biology*** *techniques for barcoding and phylogeny*

2. Chapitre 1 : Matériel et Méthodes

2.1 Abréviations utilisées dans le mémoire

ACP/PCA : Analyse en Composantes Principales

ADN/DNA : Acide désoxyribonucléique

AFD : Analyse Factorielle Discriminante

BPBM : Bernice P. Bishop Museum, Hawaii, Etats-Unis

B : Nombre de bootstrap

BI : Bayesian Inference

BSA : Bovine Serum Albumine

BSE : Electrons rétro-diffusés

DCR : D. Christopher Rogers

Dd : Nombre de dents dorsales du rostre

DMSO : Diméthylsulfoxyde

Dv : Nombre de dents ventrales du rostre

EDX : Analyse dispersive en énergie

GM : Gérard Marquet

IUCN/UICN : Union Internationale pour la Conservation de la Nature

MCC : Contre-Courant des Marquises

MEB/SEM : Microscope Electronique à Balayage

MET/TEM : Microscope Electronique à Transmission

MGF : Mitochondrial Genome Fragment

ML : Maximum Likelihood

MNHN : Muséum national d'Histoire naturelle, Paris, France

MZB : Museum Zoologicum Bogoriense, Bogor, Indonésie

MYA : Million Years Ago

MYR : Million d'années

NGS : Séquençage Nouvelle Génération

NMB : Naturhistorisches Museum Basel, Bâle, Suisse

NHM : Natural History Museum, Londres, Royaume-Uni

P1 (P2, P3, P5) : Péréiopode 1 (2, 3, 5)

P1d (P2d, P3d, P5d) : Dactyle du péréiopode 1 (2, 3, 5)

P1p (P2p, P3p, P5p) : Propode du péréiopode 1 (2, 3, 5)

P1ca (P2ca, P3ca, P5ca) : Carpe du péréiopode 1 (2, 3, 5)

P1ch (P2ch) : Pince du préiopode 1 (2)

P1dp (P2dp) : Rapport entre la longueur du dactyle et celle de la paume du Péréiopode 1 (2)

P3pd (P5 pd) : rapport entre la longueur du propode et celle du dactyle du Péréiopode 3 (5)

PCR : Réaction en chaîne par polymérase

PK : Philippe Keith

Pl1 (2) : Premier (Deuxième) pléopode mâle

PP : Probabilité postérieure

RC : Rapport entre la longueur du rostre et la longueur de la carapace

Rndd : Rapport entre la longueur de la partie dorsale inerme du rostre et celle de la partie dentée

SEC : Courant Sud-Equatorial

SECC : Contre-Courant Sud-Equatorial

SSM : Service de Systématique Moléculaire

STCC : Contre-Courant Sud-Tropical

USNM : National Museum of Natural History, Smithsonian Institution, Washington DC, Etats-Unis

VM : Valentin de Mazancourt

WK : Werner Klotz

ZMA/RMNH : Naturalis Biodiversity Center, Leiden, Pays-Bas

ZRC : Zoological Reference Collection, Singapour

2.2 Echantillonnage

2.2.1 Spécimens récents

L'équipe de l'UMR BOREA spécialisée dans les milieux insulaires tropicaux a acquis une grande expérience des missions de terrain au cours des 20 dernières années. Grâce à ces échantillonnages extensifs des rivières des îles de la zone Indo-Pacifique, de très nombreux spécimens de *Caridina* ont pu être mis à notre disposition. À ces échantillonnages réalisés par l'équipe s'ajoutent les spécimens récoltés par des

collaborateurs étrangers, aimablement mis à notre disposition pour compléter notre couverture de la zone Indo-Pacifique (Figure 2.1, Détails en annexes).

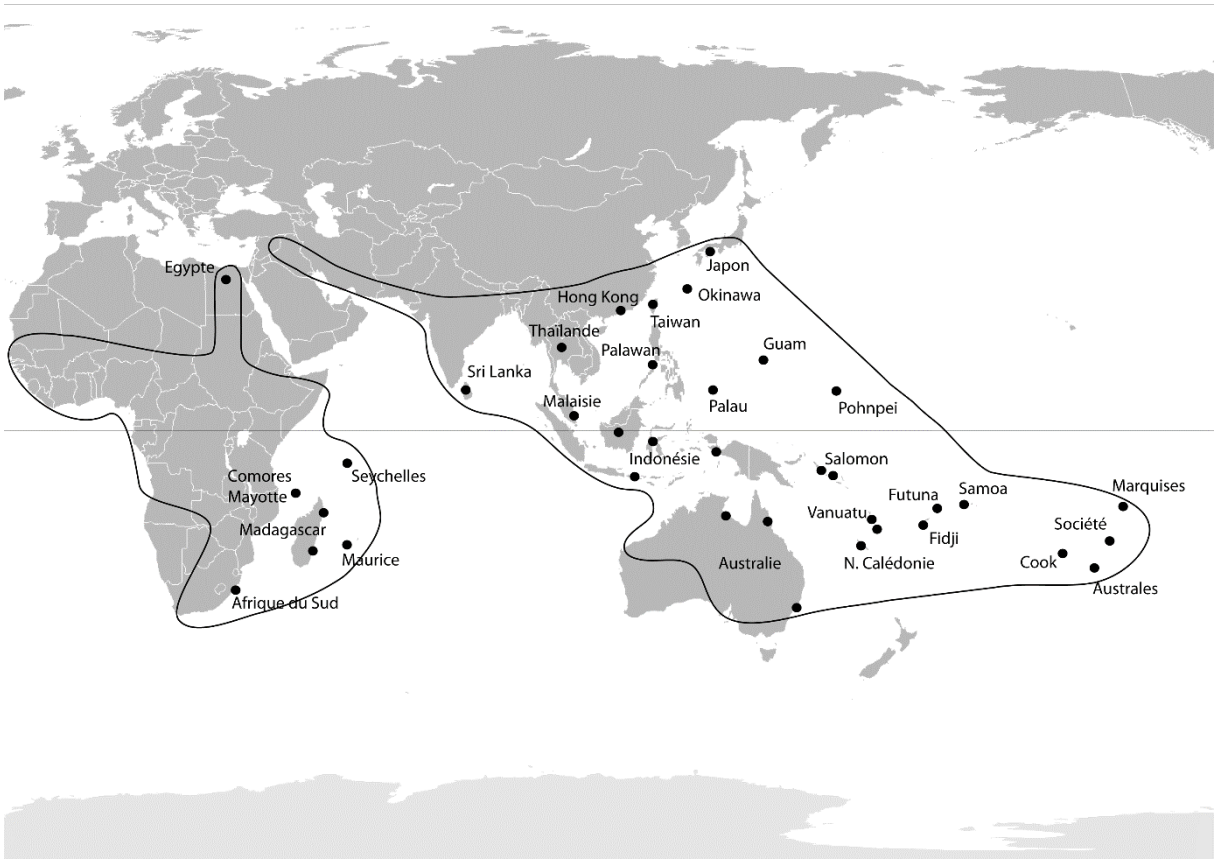


Figure 2.1 : Carte des localités d'où des spécimens récemment collectés ont pu être examiné. L'aire de répartition du genre *Caridina* est représentée à titre indicatif (ligne noire).

Figure 2.1: Map of the localities from where specimens recently sampled could be examined. Distribution area of *Caridina* is figured for information (black line).

Les spécimens ont été récoltés grâce à deux méthodes employées en fonction des conditions de pêche. La première, utilisée en routine par l'équipe de l'UMR BOREA est la pêche électrique (Lamarque, 1975), une méthode qui consiste à tétaniser temporairement les animaux grâce à un courant électrique circulant entre deux électrodes immergées et à les recueillir dans un filet en aval de la zone de pêche. Différents appareils ont été utilisés par l'équipe, notamment le « Martin-pêcheur » (Gosset *et al.*, 1971), le Dekka 3000 (Dekka Ltd, Leutkirch, Allemagne) ou encore les Samus 725MS et 1000 (Samus Special Electronics, http://www.electro-fisher.com). Lorsque la conductivité de l'eau est trop élevée (eau saumâtre) ou trop basse (rivières sur substrat granitique), ces appareils sont inopérant. Dans ces cas, une deuxième

méthode est utilisée, l'échantillonnage au filet, qui consiste à draguer les plantes et le substrat avec une épuisette pour capturer les animaux qui s'y cachent. Les deux méthodes présentent des avantages et des inconvénients. Si la pêche électrique est bien plus efficace pour obtenir un échantillon représentatif du peuplement de la rivière, il nécessite de transporter un équipement parfois lourd et encombrant et de savoir régler ses paramètres pour ne pas blesser les organismes. D'un autre côté, la pêche à l'épuisette ne demande pas d'entraînement ni de matériel particulier mais ne permet pas d'atteindre les animaux cachés dans les recoins inaccessibles et peut endommager l'habitat. Un emploi raisonné de ces deux méthodes dans les bonnes conditions d'utilisations permet, au cours d'une même mission, d'optimiser les collectes tout en minimisant l'impact sur l'environnement.

Dès la récolte, les spécimens sont triés par morpho-espèces puis sont anesthésiés et préservés dans de l'éthanol (70% à 100% selon la disponibilité pendant les missions). Certains sont d'abord photographiés en aquarium pour conserver une trace de leur coloration et sont clairement identifiés afin d'assurer la traçabilité. Pour chaque station de collecte, la température l'eau, le pH et la conductivité sont mesurés et les coordonnées GPS et l'altitude sont notées.

2.2.2 Spécimens de musées

Afin de valider les identifications, **les spécimens types de 38 espèces ou sous-espèces** (16 du complexe *C. nilotica*, 7 du complexe *C. weberi* et 16 autres**) ont été empruntés à 9 musées différents** (Muséum national d'Histoire naturelle, Paris ; Naturhistorisches Museum, Bâle (Suisse) ; Naturalis Biodiversity Center, Leiden (Pays-Bas) ; Natural History Museum, Londres (Royaume-Uni) ; Magyar Természettudományi Múzeum, Budapest (Hongrie) ; Zoological Research Collection, National University, Singapour ; Museum Zoologicum Bogoriense , Bogor (Indonésie) ; Bernice Pauahi Bishop Museum, Hawaii (Etats-Unis) ; Smithsonian Institution, National Museum of Natural History, Washington (Etats-Unis)). À ces spécimens types s'ajoutent des spécimens non-types également conservés dans différents musées, ayant servi à compléter notre échantillonnage avec des localités peu prospectées par l'équipe (comme l'Indonésie ou Fidji, par exemple), ou proches des localités types d'espèces dont nous n'avons pu obtenir de spécimens types (Tableau 2.1).

Tableau 2.1 : Liste des espèces dont des spécimens de collection (types ou non) ont été examinés au cours du présent travail avec indication du musée où les spécimens sont conservés.

Table 2.1: List of species of which collection specimens (types or not) were examined in the present work with indication of the repository.

Complexe *C. nilotica* (s. s.)

Atya wyckii (syntypes)	Paris
Caridina aruensis (Syntypes)	Bâle + Paris
C. brachydactyla subsp. *peninsularis* (Lectotype + paralectotypes)	Londres
C. brachydactyla subsp. *uncata* (Holotype + paratypes)	Washington
C. grandirostris (non-types)	Singapour
C. leucosticta (non-types)	Singapour
C. longirostris (Lectotype + paralectotypes)	Paris
C. nilotica (non-types)	Paris
C. nilotica var. *brachydactyla* (Lectotype + paralectotypes)	Leiden + Paris
C. nilotica var. *brevidactyla* (Syntypes)	Bâle
C. nilotica var. *meridionalis* (Lectotype + paralectotypes)	Bâle
C. nilotica var. *minahassae* (Syntypes)	Paris
C. nilotica var. *natalensis* (Lectotype + paralectotypes)	Leiden
C. simoni (Lectotype + paralectotypes)	Paris
C. wyckii var. *gracilipes* (Paralectotypes)	Leiden + Bâle

Complexe *C. weberi* (s. s.)

C. parvirostris (Syntypes)	Paris
Caridina weberi (Syntypes)	Paris
C. weberi var. *celebensis* (Holotype)	Bâle
C. weberi var. *keiensis* (Lectotype + paralectotypes)	Bâle
C. weberi var. *longicarpus* (Lectotype + paralectotypes)	Bâle
C. weberi var. *papuana* (Lectotype + paralectotypes)	Budapest
C. weberi var. *sumatrensis* (Holotype)	Paris

Autres espèces

Caridina appendiculata (Lectotype + paralectotypes)	Leiden
C. buehleri (Holotype)	Bâle
C. coulaborensis (Holotype + paratypes)	Bâle
C. fritzi (Holotype + paratypes)	Bâle
C. gracilirostris (Paralectotypes)	Paris
C. japonica (Holotype)	Paris
C. mccullochi (Syntypes)	Bâle
C. mertoni (Syntypes)	Paris + Bâle
C. neglecta (Holotype + paratypes)	Bogor + Singapour
C. novaecaledoniae (Lectotype + paralectotypes)	Bâle
C. rapaensis (Holotype)	Hawaii
C. riverstoni (Paratypes)	Bâle
C. solamulieres (Holotype)	Bâle
C. sundanella (Syntypes)	Bâle
C. vitiensis (non-types)	Leiden
C. vitiensis canacorum (Syntypes)	Bâle

2.3 Etudes morphologiques

2.3.1 Observations, prises de vues et mesures

Dès la récolte, les spécimens sont examinés sur le terrain avec une loupe de poche pour repérer des caractères morphologiques significatifs et les trier en morpho-espèces. De retour au laboratoire, les spécimens sont observés sous la loupe binoculaire (Olympus SZX7) et sont photographiés grâce à une caméra montée dessus (Olympus DP-20) Les images sont ensuite analysées grâce au logiciel AnalySIS Work (v. 5.0 ; Olympus Soft Imaging System) qui permet d'effectuer des mesures et comptages. Différents appendices sont ensuite prélevés, montés entre lame et lamelle puis observés au microscope (Olympus BX51). Grâce à une caméra (Leica MC170 HD) montée sur le microscope, les appendices sont pris en photo à différents grossissements. Les images sont ensuite analysées grâce au logiciel Leica Application Suite (v. 4.9 ; Leica Microsystems) qui permet de mesurer les segments des appendices observés. Les caractères observés et mesurés sont ceux définis par les auteurs ayant travaillé précédemment sur la systématique de ce groupe (voir par exemple De Man (1908) et Roux (1926)).

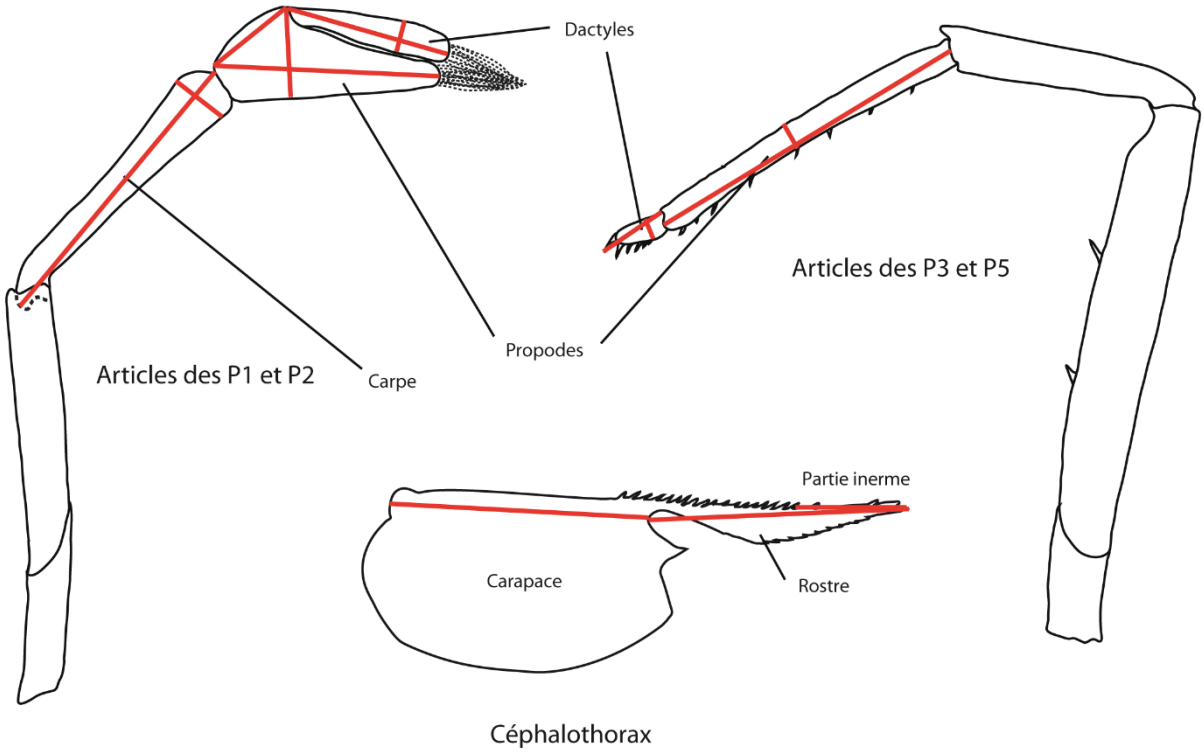


Figure 2.2 : Méthode de mesure des articles des péréiopodes et du céphalothorax.

Figure 2.2: Measuring method of the articles of the pereiopods and the cephalothorax

Outre les mesures, les microphotographies servent également à réaliser des dessins pour les descriptions d'espèces. Pour cela, la méthode employée est celle du « Digital inking » (Coleman, 2003, 2006) qui consiste à tracer des lignes vectorielles sur des empilements d'images (stacks) haute résolution grâce au logiciel Adobe Illustrator (CS3 ou CS6) depuis une tablette graphique Wacom PTZ-1230.

2.3.2 Etude biométrique

Les données issues des analyses d'images sont entrées dans un tableur Excel (Microsoft) indiquant pour chaque spécimen étudié son espèce, sa provenance, un identifiant et une série de 17 rapports de mesures et 5 comptages (Tableau 2.2).

Tableau 2.2 : Liste des rapports et comptages utilisés pour l'étude biométrique.

Tableau 2.2: List of ratios and counts used in the biometry study.

Péréiopode 1	Longueur/ Largeur du dactyle Longueur / Largeur de la pince Longueur / Largeur du carpe Longueur du dactyle / Longueur du propode
Péréiopode 2	Longueur/ Largeur du dactyle Longueur / Largeur de la pince Longueur / Largeur du carpe Longueur du dactyle / Longueur du propode
Péréiopode 3	Longueur / Largeur du dactyle Longueur / Largeur du propode Longueur du propode / Longueur du dactyle Nombre d'épines sur le dactyle (épine terminale incluse)
Péréiopode 5	Longueur / Largeur du dactyle
	Longueur / Largeur du propode Longueur du propode / Longueur du dactyle Nombre de soies spiniformes sur le dactyle (épine terminale non incluse)
Rostre	Longueur du rostre / Longueur de la carapace Longueur de la partie inerme du rostre / Longueur de la partie dentée Nombre de dents sur le bord dorsal Nombre de dents sur le bord ventral Nombre de dents dorsales en arrière de la première dent ventrale
Diarèse uropodiale	Nombre de spinules

À ces rapports et comptages s'ajoutent des caractères plus qualitatifs liés au telson (présence ou absence de projection, forme de la bordure distale), à la carène pré-anale (présence ou absence d'épine, forme) et au premier pléopode des mâles (présence ou absence d'appendice sur l'endopode, forme de l'endopode).

Toutes les mesures obtenues servent à comparer nos valeurs avec celles obtenues par les précédents auteurs pour la même espèce, à caractériser l'espèce par rapport aux autres et à donner l'amplitude des variations de ses caractères.

Au total, 957 spécimens d'Atyidae ont fait partie de l'étude morphologique.

2.3.3 Etude statistique

Afin d'identifier des groupes parmi un ensemble de spécimens de plusieurs espèces mélangées, il est possible de réaliser des analyses multivariées à partir des données biométriques obtenues sur ces spécimens. Nous avons employé cette technique dans les cas où l'étude de la morphologie était le seul outil disponible pour séparer les espèces (par exemple, dans le cas de spécimens anciens de collection dont l'habitat est inconnu et pour lesquels l'étude génétique a échoué), en reprenant la méthode suivie par Konan *et al.* (2010) avec le logiciel Statistica (v. 10.0 ; Statsoft).

Une Analyse en Composantes Principales (ACP) permet de visualiser la répartition des individus dans un plan formé par deux axes correspondant à des composantes principales, auxquelles les différentes variables contribuent plus ou moins. Les coordonnées de chaque individu dépendent de leur score pour chaque composante principale. Des nuages de points peuvent ainsi être identifiés et servir à identifier les espèces. Dans cette étude, les données utilisées dans les ACP sont les variables biométriques obtenues selon la méthode du paragraphe précédent.

Dans le cas où des spécimens sont attribués à des espèces avec certitude (c'est-à-dire pour lesquels au moins deux sources de données s'accordent à valider les hypothèses d'espèces), il est possible d'affiner l'analyse pour attribuer des spécimens non identifiés à l'une ou l'autre. Une Analyse Factorielle Discriminante (AFD) est d'abord réalisée en n'incluant que les spécimens identifiés avec certitude. Cette analyse permet de maximiser les différences entre des groupes connus a priori et de repérer les variables les plus discriminantes en maximisant leur poids dans l'analyse. Ces variables sont donc sélectionnées pour réaliser une ACP incluant les spécimens non identifiés qui

permettra d'affiner les hypothèses d'identification en fonction de la proximité des individus avec un nuage de points identifié (donc une espèce) ou un autre.

Ce même logiciel permet de tester des corrélations de variables entre elles (voir chapitre suivant).

2.4 Etudes génétiques

2.4.1 Barcoding moléculaire

Afin de faciliter l'identification des spécimens, nous avons séquencé de manière systématique deux fragments d'ADN mitochondrial, le 16S (ARN ribosomique, petite sous-unité) et le COI (gène codant pour la sous-unité 1 de la protéine cytochrome c oxydase). Ces deux marqueurs sont largement utilisés et permettent une comparaison avec la plupart des séquences obtenues dans le genre *Caridina* accessibles dans GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

Les protocoles utilisés ont évolué au cours des 3 années ayant été progressivement optimisés. Ceux présentés dans ce chapitre sont les versions finales utilisées en fin de thèse, d'où de légères différences avec ceux employés dans les divers chapitres.

Les spécimens sélectionnés pour l'étude génétique se voient attribuer un numéro identifiant unique de type CA + 4 chiffres (Par exemple, CA1000, CA1001, CA1002, etc.) permettant de relier les informations morphologiques aux séquences pour un même spécimen.

Les ADN totaux sont extraits à partir d'un morceau de muscle abdominal ou d'un pléopode (suivant la taille du spécimen) grâce au robot semi-automatique Ependorf ep-Motion 5075 (protocole détaillé en annexe) qui permet d'obtenir 200 µl d'ADN en suspension dans un tampon pour 96 spécimens en une fois. Les réactions d'amplification par PCR (Polymerase Chain Reaction) sont réalisées dans des volumes de 20 µl contenant environ 3 ng d'ADN, 2,5 mM de $MgCl_2$, 0,26 mM de chaque nucléotide, 0,3 µM de chaque amorce, 5% de DMSO, 1 ng de BSA et 1,5 unité de polymérase QBIOTAQ (MPBiomedicals). **Les amorces utilisées ont été nouvellement créées à partir de celles proposées par Palumbi (1996) pour le marqueur 16S (16Sar-Lmod : TACTTCTGCCTGTTTATCAAAAA / 16Sbmod : GGTCTGAACTCAAATCATGTAAA) et par Folmer *et al.* (1994) pour le marqueur COI (LCOI1490mod : TGTCAACTAA-**

TCATAAAGATATTGG / HCO2198mod : GGGTGTCCAAAAAACCAAAATAA). Les protocoles d'amplification utilisés sont : 4 minutes de dénaturation initiale à 94°C, 45 cycles de 30s de dénaturation à 94°C, 30s d'hybridation des amorces à 55°C (pour le 16S) ou 48°C (pour le COI) et 40s d'élongation à 72°C, avec une phase finale d'extension de 7min à 72°C. Le séquençage des produits d'amplification a été confié à l'entreprise Eurofins Genomics (https://www.eurofinsgenomics.eu) avec la méthode Sanger et une lecture bidirectionnelle afin de s'assurer de la bonne lecture de chaque nucléotide. Les séquences sont ensuite vérifiées et nettoyées manuellement grâce au logiciel Geneious (R7, Biomatters) pour corriger les erreurs de lecture lors du séquençage ou repérer les contaminations, et sont alignées grâce au logiciel MEGA7 (S. Kumar *et al.*, 2016). Dans le même logiciel, des analyses en Neighbor-Joining (NJ) permettent de visualiser des clusters de séquences servant de base aux hypothèses d'espèces en fonction des regroupements de spécimens. Toutes les séquences ainsi que les fichiers traces, les informations sur la localité et des photos des spécimens sont déposées dans un projet BOLD (http://www.boldsystems.org/) intitulé CARIT (CARidina Integrative Taxonomy).

2.4.2 Séquençage de spécimens anciens

Pour s'assurer de la bonne identification d'un clade, l'idéal est d'avoir une séquence du spécimen type pour voir à quel cluster elle se rattache, ne laissant aucun doute quant à l'identité des spécimens compris dans ce cluster. Les spécimens types des espèces sur lesquelles porte le présent travail ayant généralement été collectés il y a près d'un siècle, les techniques ont dû être adaptées pour pouvoir obtenir de l'ADN en quantité et qualité suffisantes pour le séquençage.

Les ADN totaux de spécimens anciens sont obtenus grâce à un protocole d'extraction manuelle au CTAB qui permet d'obtenir 25 µl d'ADN concentré en suspension dans un tampon (protocole détaillé en annexe). Des PCR quantitatives en temps réel sont réalisées pour vérifier que les extraits contiennent de l'ADN en quantité suffisante pour être amplifié.

Le fragment COI ne possédant pas de région conservée suffisamment longue pour que des amorces s'y fixent, **de nouvelles amorces ont été créées pour amplifier un fragment plus court du marqueur 16S (332 paires de bases) situé dans la région utilisée pour le barcoding (16S-Car-81F : AGGTAGCATAATAAATAGTC / 16S-Car-413R : CTGTTATCCCTAAAGTAAC). Si l'amplification n'a pas fonctionné, une autre**

amorce reverse est employée pour tenter d'amplifier un fragment plus court pour les spécimens concernés (16S-Car-22R : CTTTATAGGGTCTTATCGT). Le milieu de réaction et le protocole d'amplification sont les mêmes que pour le barcoding du 16S. Le protocole de séquençage Sanger utilisé par Eurofins n'étant pas optimisé pour des séquences aussi courtes, les séquençages ont été réalisés au Service de Systématique Moléculaire du MNHN grâce à un séquenceur ABI 3130 (Applied Biosystems). L'emploi de cette méthode nécessite de purifier les produits d'amplification (purification ExoSap), d'effectuer une réaction de séquençage puis de purifier à nouveau par un passage sur gel Sephadex avec formamide avant la lecture par l'appareil (détail en annexe). Les séquences obtenues sont nettoyées selon la méthod explicitée précédemment et alignées avec les séquences obtenues par le barcoding.

Au total, 1151 spécimens récents et 151 spécimens anciens d'Atyidae ont fait partie de l'étude génétique (Tableau 2.3).

Tableau 2.3 : Nombre de spécimens récents (en noir) et anciens (en bleu) étudiés génétiquement pour chaque localité.

Table 2.3: Number of recent (in black) and old (in blue) specimens studied genetically for each locality.

Pays	Archipel	Île	Nombre
Algérie			3
Egypte			
Kenya			4
Tanzanie			3
Afrique du Sud			62 +4
Comores		*Mohéli*	4
Mayotte			
Madagascar			83+4
Maurice			8
Seychelles		*Mahé*	12
		Praslin	11
		Silhouette	16
Total Seychelles			39
Sri Lanka			13
Thaïlande			2
Malaisie		*Penang*	1
		Tioman	6
Total Malaisie			6+1
Indonésie		*Aru*	12
		Bali	1
		Bornéo	2

	Flores	**14**
	Java	**1**
	Kei	**13**
	Lombok	1
	Nias	**3**
	Obira	**1**
	Papua	20
	Sulawesi	6**+23**
	Sumba	**2**
Total Indonésie		29**+70**
Philippines	*Luzon*	**1**
	Palawan	2
Total Philippines		2**+1**
Chine	*Hong Kong*	4
Taiwan		3
Japon	*Okinawa*	20
	Shikoku	8
Total Japon		28
Palau	*Babeldaob*	51
Guam		10**+2**
Micronésie	*Pohnpei*	55
Papouasie N. Guinée	*Nouvelle Irlande*	**1**
Salomon	*Choiseul*	81
	Kolombangara	45
	Malaita	21
	Vella Lavella	30
Total Salomon		177
Vanuatu	*Aneityum*	11
	Efate	36
	Epi	20
	Gaua	10
	Malekula	16
	Santo	27
	Tanna	12
	Inconnu	7
Total Vanuatu		139
Australie		72**+8**
Nouvelle Calédonie	*Grande Terre*	230**+36**
	Lifou	3
Total Nouvelle Calédonie		233**+36**
Fidji	*Taveuni*	9
	Ovalau	**6**
	Vanua Levu	**7**
	Viti Levu	**1**
Total Fidji		9**+14**

Wallis et Futuna		*Futuna*		16
Samoa		*Savai*	4	
		Upolu	14	
Total Samoa				18
Cook		*Mitiaro*	2	
		Rarotonga	6	
Total Cook				8
Polynésie Française	Australes	*Rapa*	5	
		Rurutu	4	
	Total Australes		9	
	Marquises	*Hiva Oa*	4	
		Nuku Hiva	4	
		Ua Pou	9	
	Total Marquises		17	
	Société	*Huahiné*	4	
		Moorea	4	
		Tahiti	4	
	Total Société		12	
Total Polynésie Française				38
Elevage				6
TOTAL				**1151+151**

2.4.3 Obtention de génomes mitochondriaux complets

L'utilisation de génomes mitochondriaux complets pour produire des phylogénies se développe de plus en plus, notamment grâce à la baisse des coûts des Séquençages Nouvelle Génération (NGS) permettant de les obtenir. Au Service de Systématique Moléculaire du MNHN, une méthode de multiplexage avant séquençage Ion-Torrent a été développée et permet d'obtenir des génomes mitochondriaux complets pour moins de 10€ (Hinsinger *et al.*, 2015).

Un alignement a été construit à partir des génomes mitochondriaux d'Atyidae disponibles sur GenBank afin d'identifier des zones conservées pour définir de nouvelles amorces pour amplifier plusieurs longs fragments du génome mitochondrial. **En plus des 4 utilisées pour le barcoding, 8 nouvelles amorces ont été définies** à partir du génome mitochondrial de *Caridina gracilipes* (Xu *et al.*, 2016) :

- tRNA-Leu1540F : AAAATGGCAGATCATTGCATAG
- tRNA-Lys2292R : GACCTACACTTACTTTCAGTCA
- tRNA-Ala4486F : TTATAGTCAAGTATGACATTTGGG
- tRNA-Glu4760R : ACCTTTAGTTTTACAATGAAAATG

- tRNA-Glu4760F : CATTTTCATTGTAAAACTAAAGGT
- tRNA-His6639F : CACAAACCAGCATTTTTATTAAAC
- ND4-7817R : TATATTTTAATTTTACTGAGGTTTTG
- 12S-12639R : AAGGTGAGATAAGTCGTAACA

Après avoir testé ces différentes amorces sur des espèces de *Caridina* phylogénétiquement éloignées les unes des autres, 4 « cocktails d'amorces » (pour maximiser les chances d'amplification du fragment, plusieurs amorces Forward ou Reverse peuvent être utilisées en mélange, donnant des fragments de taille différente en fonction du couple qui a amplifié avec succès) ont été retenus pour amplifer le mitogénome en 4 fragments (Figure 2.3) :

- **Fragment 1** (depuis LCOI1490mod ou tRNA-Leu1540F jusqu'à tRNA-Glu4760R, 3252-4776 paires de bases)
- **Fragment 2** (depuis tRNA-Ala4486F jusqu'à ND4-7817R, 3360 paires de bases)
- **Fragment 3** (depuis tRNA-His6639F jusqu'à 12S-12639R ou 16Sar-Lmod, 5238-6031 paires de bases)
- **Fragment 4** (depuis 16Sbmod jusqu'à HCO2198mod ou tRNA-Lys2292R, 4950-6555 paires de bases)

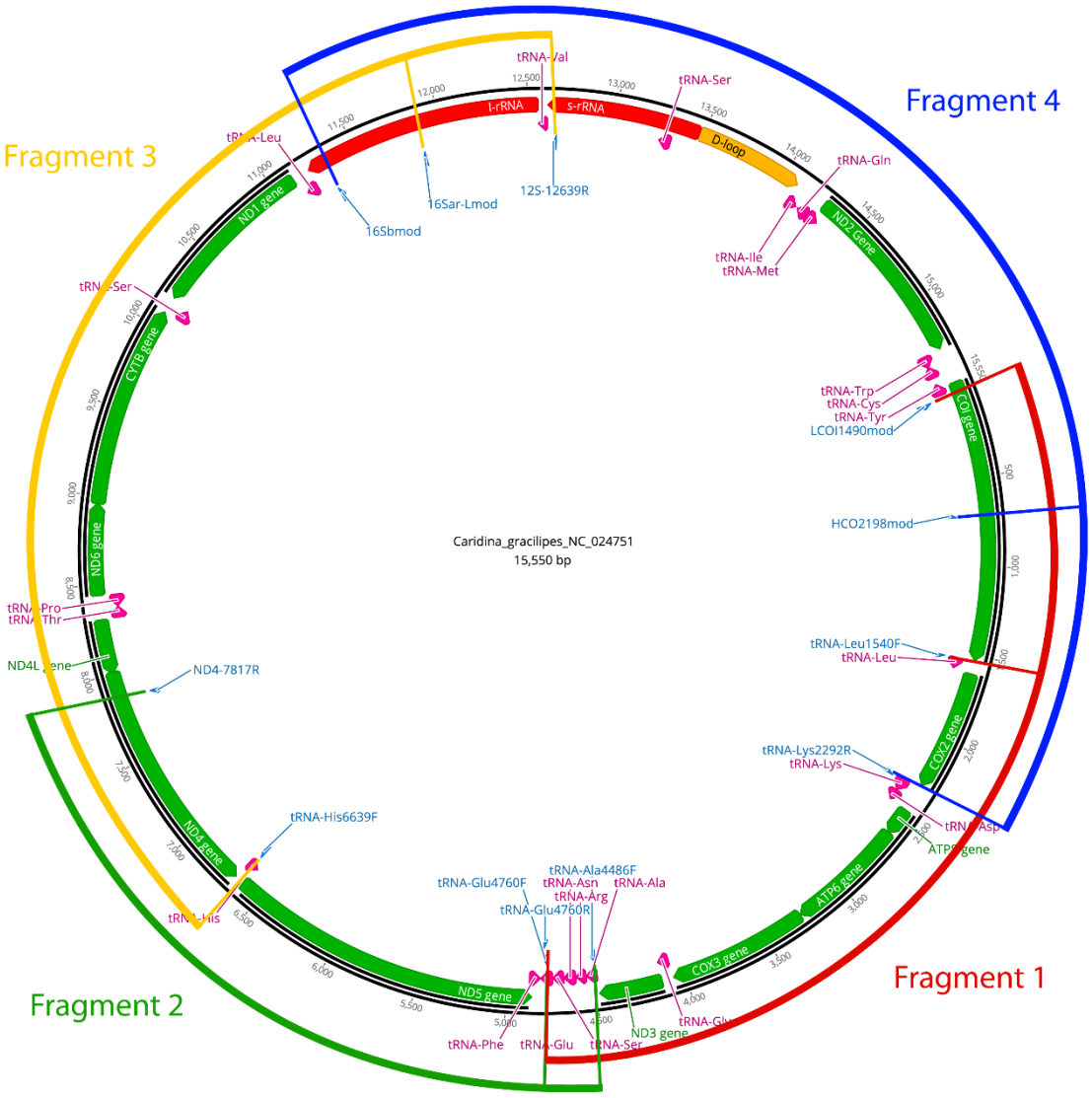


Figure 2.3 : Cartographie du génome mitochondrial de *Caridina gracilipes* publié par Xu *et al.* (2016). Les positions et noms des amorces sont représentés en bleu.

Figure 2.3: Map of the mitochondrial genome of *Caridina gracilipes* published by Xu *et al.* (2016). Positions and names of the primers are figured in blue.

Les réactions d'amplification ont lieu dans 20 µl contenant 2,5 mM de $MgCl_2$, 0,3 mM de chaque nucléotide, 0,3 µM de chaque amorce, 3.5% de DMSO, 1 ng de BSA, et 1 unité de Taq polymérase LongAmp. Le protocole d'amplification est : 30s de dénaturation initiale à 94°C, 60 cycles de 20s de dénaturation à 94°C, 30s d'hybridation des amorces à 50°C et 15min d'élongation à 65°C, et une phase d'élongation finale de 15min à 65°C.

Les produits d'amplification sont ensuite répartis dans des banques mutualisées avec d'autres utilisateurs de la plateforme à raison d'un spécimen (donc 4 fragments) par banque. Les produits multiplexés sont ensuite fractionnés, les fragments mesurant entre 100 et 400 paires de bases sont sélectionnés. Les fragments de chaque banque

sont marqués avec une sonde spécifique à chaque banque puis sont mélangés dans une puce qui sera lue par le séquençeur Ion Torrent PGM du Service de Systématique Moléculaire. Les très nombreuses séquences obtenues (ou reads) sont triées informatiquement par le logiciel Geneious grâce à leur sonde et sont réparties dans leur banque d'origine respective. Les reads d'une banque donnée sont ensuite alignés sur une séquence de référence de l'espèce correspondante (par exemple une séquence obtenue pour le barcoding) puis la séquence consensus est allongée de manière itérative en alignant toujours plus de reads jusqu'à revenir à la séquence de départ (le génome étant circulaire).

2.5 Mise en œuvre de l'approche de taxonomie intégrative

En combinant les études morphologique, génétique et les informations sur l'habitat recueillies sur le terrain lors de l'échantillonnage, nous avons à notre disposition 3 jeux de données différents pour tester des hypothèses d'espèces.

Une première étude morphologique va nous permettre d'identifier les spécimens récoltés en fonction des espèces signalées dans la région de collecte par de précédents auteurs. Ensuite, plusieurs spécimens de chaque espèce identifiée morphologiquement sont sélectionnés pour l'étude de barcoding. Les séquences obtenues sont ensuite comparées avec celles de notre base de référence et celles des spécimens types dont des séquences ont pu être obtenues.

Ainsi, plusieurs cas de figures apparaissent :

- Des spécimens identifiés comme appartenant à la même espèce d'après l'étude morphologique se retrouvent groupés dans un même cluster génétique.

  → L'hypothèse d'espèce est validée, il reste à vérifier l'identité de l'espèce à l'aide des séquences de spécimens types ou de caractères morphologiques identifiés par l'auteur comme diagnostiques lors de la description de l'espèce ou encore à décrire l'espèce si aucune description existante ne correspond.

- Des spécimens identifiés comme appartenant à des espèces différentes d'après l'étude morphologique sont séparés dans des clusters génétiques différents.

  → Les hypothèses d'espèces sont validées, il faut encore vérifier les identités respectives de ces espèces ou les décrire si elles ont nouvelles.

- Des spécimens identifiés comme appartenant à la même espèce d'après l'étude morphologique se retrouvent répartis dans plusieurs clusters génétiques distincts.

  → L'hypothèse d'espèce est invalidée, au moins partiellement si l'un des clusters correspond effectivement à l'espèce identifiée par la morphologie. Il faut alors revenir à l'étude de la morphologie pour trouver des caractères permettant de séparer les groupes identifiés par le barcoding pour vérifier leur identité ou les décrire comme espèces nouvelles.

- Des spécimens identifiés comme appartenant à des espèces différentes d'après l'étude morphologique se trouvent groupés dans un même cluster génétique.

  → Les hypothèses d'espèces sont invalidées, il faut alors revenir à l'étude de la morphologie pour rendre compte de la variabilité de certains caractères et en identifier de plus constants. Après vérification, les deux espèces peuvent éventuellement être mises en synonymie.

Dans tous les cas, les données liées à l'habitat (altitude, substrat, hydrologie…) des spécimens sont prises en compte pour appuyer les hypothèses d'espèces.

Tous ces cas de figures ont été rencontrés au cours de la présente étude et chacun sera illustré par des exemples au sein des complexes *C. nilotica* et *C. weberi* dans les chapitres suivants.

2.6 Analyses phylogénétiques

Pour étudier les liens de parenté entre les espèces identifiées par la taxonomie intégrative, nous avons réalisé plusieurs études phylogénétiques à partir des données moléculaires.

Les premières reconstructions phylogénétiques réalisées au cours de ce travail ont été produites à partir de la concaténation des deux marqueurs utilisés pour le barcoding grâce au logiciel MEGA7. Les modèles d'évolution moléculaire les mieux adaptés ont été calculés indépendamment pour le 16S et pour les 3 positions de codon du COI à l'aide du programme PartitionFinder v. 2.0 (Lanfear *et al.*, 2012). En appliquant les modèles sélectionnés, les analyses ont ensuite été réalisées indépendamment selon deux méthodes, le Maximum de vraisemblance (ML) et l'Inférence bayésienne (BI) implémentées respectivement dans les programmes RaxML (Stamatakis, 2006) et

MrBayes (Huelsenbeck & Ronquist, 2001) sur la plateforme en ligne CIPRES (Miller *et al.*, 2010). La robustesse des nœuds des arbres obtenus est évaluée en ML par la méthode du bootstrap (Felsenstein, 1985) avec 1000 réplicats, et en BI par le calcul des probabilités postérieures (probabilité d'obtenir un nœud sachant les données).

L'utilisation de deux marqueurs et de deux méthodes indépendantes permet d'obtenir une meilleure résolution relative des relations phylogénétiques qu'avec l'étude de barcoding, mais qui reste objectivement faible. Un jeu de données comprenant plus de gènes et provenant de génomes différents (nucléaire et mitochondrial) permet d'améliorer la précision de l'arbre.

Pour ce faire, 6 marqueurs nucléaires utilisés dans la littérature sur des espèces d'Atyidae ont été testés pour notre groupe taxonomique (Tableau 2.4).

Tableau 2.4 : Comparaison des 6 marqueurs nucléaires testés pour l'étude phylogénétique. Les marqueurs mitochondriaux 16S et COI sont présentés à titre de comparaison.

Table 2.4: Comparison of 6 nuclear markers tested for the phylogenetic study. Mitochondrial 16S and COI markers are presented for information.

Marqueur	Distance Intra-Atyidae (p-distance)	Distance Atyidae / Palaemonidae (p-distance)	Longueur du marqueur	Séquence *Caridina spp.* (Atyidae)	Séquence *Atyopsis spp.* (Atyidae)	Séquence *Macrobrachium rosenbergii* (Palaemonidae)
18S	0,40%	9%	1790 pb	JF346236	JF346252	GQ131934
28S	7,80%	26,50%	887 pb	FN995576	FN995557	EF540848
Enolase	11,20%	17,45%	367 pb	JF346267	JF346286	JF346284
H3	9,80%	13,50%	327 pb	JF346303	JF346322	JF346320
NaK	15,70%	17,70%	654 pb	JF346339	JF346358	JF346356
PEPCK	9,80%	19,65%	540 pb	JF346375	JF346394	JF346392
16S	*17,90%*	*37,90%*	*475 pb*	*KF023111*	*EF489996*	*KJ544684*
COI	*18,30%*	*20,85%*	*569 pb*	*LC072109*	*KP759381*	*KP756692*

Les marqueurs nucléaires ont tous un taux d'évolution plus faible que les marqueurs mitochondriaux (p-distances toujours inférieures), sauf le 28S pour lequel la distance entre les Atyidae et les Palaemonidae était plus grande que pour le COI. De fait, les marqueurs nucléaires sont efficaces pour étudier les relations phylogénétiques anciennes mais ne résoudront pas les nœuds les plus récents.

Le 18S a été éliminé d'office car trop peu variables pour être informatif pour une analyse phylogénétique à cette échelle. L'amplification des marqueurs restants a été testée sur des spécimens appartenant à des espèces éloignées phylogénétiquement grâce aux amorces suivantes dans les mêmes conditions d'amplification que pour le barcoding :

- 28S (Littlewood, 1994 ; Rintelen *et al.*, 2012) :
    - 28S-F-Cru ACCCGCCTAATTTAAGCATAT
    - 28S-R-Met GCTATCCTGAGGGAAACTTC
- Enolase (Tsang *et al.*, 2011) :
    - EA2 AGTTGGCTATGCAGGARTTYATGAT
    - ES2 ACCTGGTCGAATGGRTCYTC
- H3 (Colgan *et al.*, 2000) :
    - H3F ATGGCTCGTACCAAGCAGACVGC
    - H3R ATATCCTTRGGCATRATRGTGAC
- NaK (De Grave *et al.*, 2014) :
    - N79 GAYAARWCCTCYGAAGGTTGGAA
    - N610 RGGAGGATCRATCATRGACAT
- PEPCK (Tsang *et al.*, 2008) :
    - PEPCK for GTAGGTGACGACATTGCYTGGATGAA
    - PEPCK for2 GCAAGACCAACCTGGCCATGATGAC
    - PEPCK rev GAACCAGTTGACGTGGAAGATC
    - PEPCK rev3 CGGGYCTCCATGCTSAGCCARTG

Finalement, seuls les marqueurs 28S et H3 ont été retenus car les autres étaient trop variables pour permettre l'amplification de tout notre échantillonnage taxonomique avec un seul couple d'amorces.

Combinées à ces marqueurs nucléaires, des données issues du séquençage des mitogénomes ont permis d'obtenir un jeu de données concaténé de près de 5400 paires de bases, dont les détails seront précisés dans le cinquième chapitre.

Chapitre second,
L’ « Effet Pinocchio ».

The “Pinocchio-shrimp Effect”.

Où l’on voit comment l’approche de taxonomie intégrative du genre Caridina *invalide des caractères morphologiques traditionnellement utilisés pour séparer des espèces.*

Where we see how integrative taxonomy of the genus Caridina *invalidates morphological characters traditionally used to separate species.*

3. Chapitre 2 : L'« Effet Pinocchio » ou comment la morphologie peut être influencée par l'environnement chez une espèce du genre *Caridina*

Le rostre a toujours été un caractère privilégié pour séparer des espèces de crevettes, et en particulier dans le genre *Caridina*. Cependant, par l'approche de taxonomie intégrative mise en œuvre dans le présent travail, nous avons montré que ce caractère (ainsi que d'autres) n'était pas si informatif et qu'il faut l'employer avec précaution et surtout pas comme seul caractère pour diagnostiquer une espèce. Cette étude a fait l'objet d'un article publié en 2017 dans le *Journal of Crustacean Biology, 37 (3)* dont le texte suit.

The rostrum has always been a character of choice to separate species of shrimps, and particularly in the genus *Caridina*. However, thanks to the integrative taxonomy approach implemented in the present work, we showed that this character (among others) was not so informative and was to use with caution and above all, not as single character to diagnose a species. This study was subject of an article published in 2017 in the *Journal of Crustacean Biology, 37 (3)* of which the text follows.

Journal of Crustacean Biology 37(3), 249-257

The "Pinocchio-shrimp effect": first evidence of variation in rostrum length with the environment in *Caridina* H. Milne-Edwards, 1837 (Decapoda: Caridea: Atyidae)

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3.1 Abstract

External morphology has always been the first criterion used to separate species of shrimps, especially in the freshwater genus *Caridina* H. Milne Edwards, 1837, but more doubts have been expressed regarding the relevance of some of the morphological characters. We collected 27 specimens of *Caridina* from seven different localities during fieldwork conducted on the island of Pohnpei (Federated States of Micronesia). After genetic verification that they all belonged to the same species, 19 morphological variables were measured and correlated with the elevation of the collecting stations using correlation analyses. We provide evidence that the length of the rostrum showed strong negative correlation with the elevation. This could be explained either by the physical stress exerted on the rostrum by the stronger water currents in the stations at a higher elevation, as a defence against predators in the lower stations, or a combination of both possibilities. The taxonomy of these shrimps is thus challenging and should not rely only on rostrum length, but on other characters such as the number of teeth on the

dorsal margin of the carapace, which is not correlated with rostrum length and therefore, with the environment.

Key words: correlation analysis, effect of elevation, freshwater shrimps, Micronesia, morphology, taxonomy

3.2 Introduction

The freshwater shrimp genus *Caridina* H. Milne Edwards, 1837 is one of the most diverse of Caridea (De Grave *et al.*, 2015) with 298 described species thus far (World Register of Marine Species (WoRMS), December 2016; http://www.marinespecies.org/), and an important ecological component of tropical freshwater ecosystems (Pringle *et al.*, 1993 ; Covich *et al.*, 1999). This high diversity is a challenge for taxonomists, as the boundaries among species are not always clear from a morphological point of view as well as from a molecular perspective. The main morphological character used to identify the species of *Caridina* is generally the length, shape, and armature of the rostrum. This character, however, has been proven to be plastic in shrimps, tending to vary among individuals within a single species (De Grave, 1999), sometimes in adaptation to environmental constraints (Jugovic *et al.*, 2010). There is therefore a need to be very cautious and proceed with an integrative approach when dealing with the taxonomy of caridean shrimps (Page *et al.*, 2005 ; Page & Hughes, 2011).

A survey of the freshwater fauna conducted on the island of Pohnpei (Federated States of Micronesia) by a team from the Muséum national d'Histoire naturelle (MNHN), Paris collected a large number of atyid shrimps from different rivers and habitats (Keith *et al.*, 2012). Some of these specimens showing a long rostrum were attributed to *Caridina brachydactyla* De Man, 1908, whereas others, showing a short rostrum, were identified as *Caridina mertoni* Roux, 1911, following the previous identifications made from Guam, another Micronesian island, by Leberer & Cai (2003). A molecular analysis (see below) of these specimens indicated that they belong to the same species, referred to here to as *Caridina sp.* We therefore treated them as such to test the hypothesis that morphological variation is related to environmental factors. We performed a correlation

analysis of 19 morphological variables on 27 specimens with the elevation of the station where the specimens were collected.

3.3 Materials and Methods

3.3.1 Sampling

A total of 14 stations were sampled using electrofishing (see Ng, 2017) in the four main streams of Pohnpei, Federated States of Micronesia, and 27 shrimp specimens were captured in seven of these stations at different altitudes. Out of these seven stations, three were located below the first major waterfall in each of the rivers (Table 3.1; Fig. 3.1).

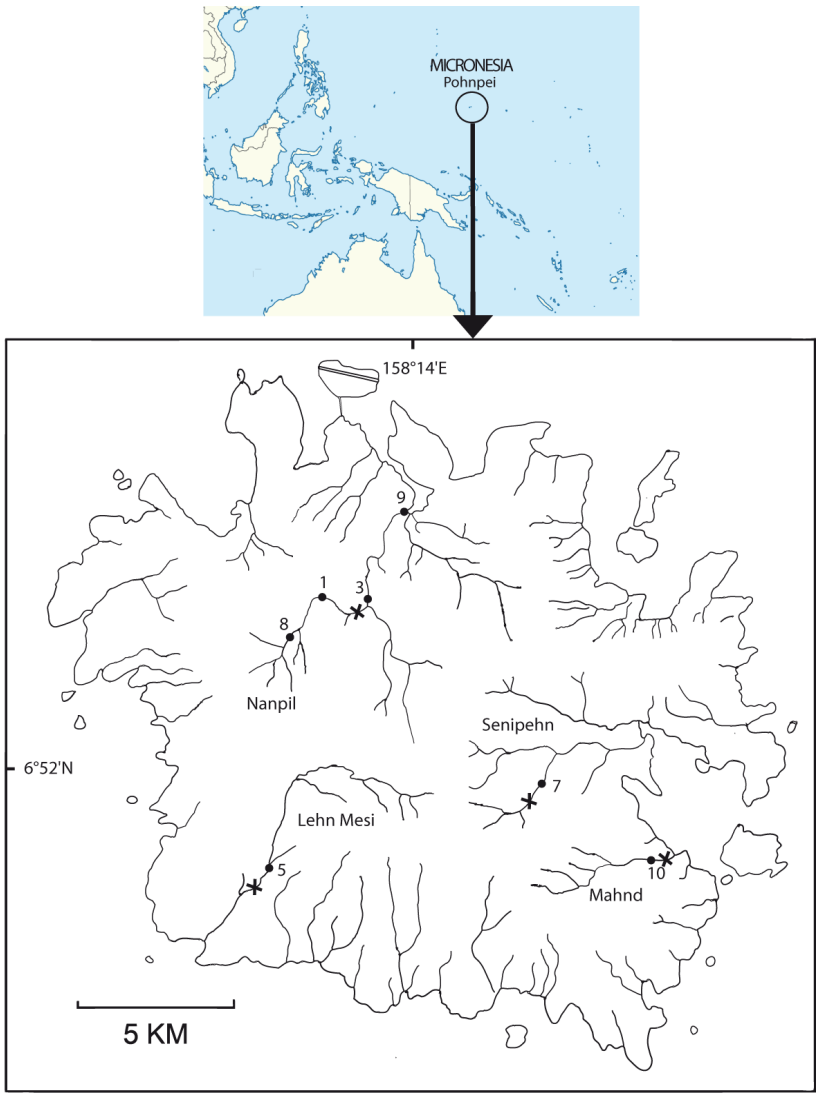


Figure 3.1 : Pohnpei Island, Federated States of Micronesia, showing sampling stations (see Keith *et al.*, 2012). Crosses indicate the position of the first major waterfall for each sampled river.

Table 3.1 : Specimens studied with GenBank numbers for 16S and COI sequences and locality information. Stations marked with an asterisk are above the first major waterfall (see Fig. 1).

Specimen of *Caridina sp.*	Station number	River	Elevation	Coordinates
CA1242	7	Senipehn	95 m	06°51.906N, 158°16.010E
CA1243	10*	Mahnd	107 m	06°50.221N, 158°17.212E
CA1244	9	Nanpil	5 m	06°56.609N, 158°13.550E
CA1246	3	Nanpil	52 m	06°55.111N, 158°12.878E
CA1247	5*	Lehn Mesi	139 m	06°51.107N, 158°11.896E
CA1248	8*	Nanpil	180 m	06°54.252N, 158°11.491E
CA1328	3	Nanpil	52 m	06°55.111N, 158°12.878E
CA1334	8*	Nanpil	180 m	06°54.252N, 158°11.491E
CA1335	8*	Nanpil	180 m	06°54.252N, 158°11.491E
CA1336	8*	Nanpil	180 m	06°54.252N, 158°11.491E
CA1337	9	Nanpil	5 m	06°56.609N, 158°13.550E
CA1338	9	Nanpil	5 m	06°56.609N, 158°13.550E
CA1339	9	Nanpil	5 m	06°56.609N, 158°13.550E
CA1340	1*	Nanpil	127 m	06°55.242N, 158°12.265E
CA1341	1*	Nanpil	127 m	06°55.242N, 158°12.265E
CA1396	1*	Nanpil	127 m	06°55.242N, 158°12.265E
CA1397	8*	Nanpil	180 m	06°54.252N, 158°11.491E

CA1398	8*	Nanpil	180 m	06°54.252N, 158°11.491E
CA1399	8*	Nanpil	180 m	06°54.252N, 158°11.491E
CA1400	9	Nanpil	5 m	06°56.609N, 158°13.550E
CA1401	9	Nanpil	5 m	06°56.609N, 158°13.550E
CA1402	5*	Lehn Mesi	139 m	06°51.107N, 158°11.896E
CA1403	5*	Lehn Mesi	139 m	06°51.107N, 158°11.896E
CA1404	10*	Mahnd	107 m	06°50.221N, 158°17.212E
CA1405	10*	Mahnd	107 m	06°50.221N, 158°17.212E
CA1406	7	Senipehn	95 m	06°51.906N, 158°16.010E
CA1407	7	Senipehn	95 m	06°51.906N, 158°16.010E

3.3.2 Molecular analysis

We used the same protocol given by Mazancourt *et al.* (2017b) to obtain sequences of 16S rRNA and COI gene for all the 27 specimens studied to perform a phylogenetic analysis.

3.3.3 Morphological analysis

Each specimen was dissected and the appendages prepared for microscopic observation. Microphotographs were taken using an Olympus UTV1X-2 camera mounted on an Olympus BX51 stereomicroscope. The images obtained were treated using the AnalySIS Work v5.0 software (Olympus Soft Imaging System) to measure 19 morphological variables (Table 3.2). Correlation analyses were performed using Statistica v.10 software (StatSoft), providing 2D scatterplots, regression, and r coefficients.

Table 3.2 : List of the 19 morphological variables measured.

Structure	Variable	Remarks
Pereiopod 1	1. Dactylus length-to-width ratio	-
	2. Dactylus length to propodus length ratio	-
	3. Chela length-to-width ratio	-
	4. Carpus length-to-width ratio	Width measured at widest part
Pereiopod 2	5. Dactylus length-to-width ratio	-
	6. Dactylus length to propodus length ratio	-
	7. Chela length-to-width ratio	-
	8. Carpus length-to-width ratio	Width measured at widest part
Pereiopod 3	9. Dactylus length-to-width ratio	Length including terminal spine
	10. Propodus length-to-width ratio	Width measured in the middle portion
	11. Propodus length to dactylus length ratio	-
Pereiopod 5	12. Dactylus length-to-width ratio	Length including terminal spine
	13. Propodus length-to-width ratio	Width measured in the middle
	14. Propodus length to dactylus length ratio	-
Rostrum	15. Rostrum length to carapace length ratio	Rostrum length from the orbit to tip
	16. Unarmed part length to armed part length ratio	-
	17. Number of teeth on dorsal margin	Including post-orbital teeth but not apical teeth
	18. Number of teeth on ventral margin	-
Cephalothorax	19. Carapace length	Laterally, from the orbit to the posterior margin

3.4 Results

3.4.1 Sampling

The species of fishes, particularly the shrimp predators, were reported for each station. For stations 1, 5, 8, and 10, which were located above the first waterfall of each river, the only predator able to climb up was the eel *Anguilla marmorata* Quoy &

Gaimard, 1826; The flagtails *Kuhlia marginata* (Cuvier, 1829) and *K. rupestris* (Lacépède, 1802) were present in station 3; for the station 7, *K. rupestris* and *A. marmorata* in station 7, and *A. marmorata*, *K. marginata*, and *Eleotris fusca* (Forster, 1801) in station 9.

3.4.2 Molecular analysis

After a molecular analysis based on two mitochondrial genes (16S and COI) (Fig. 3.2), we found that all of our specimens were all very close genetically (> 1% p-distances).

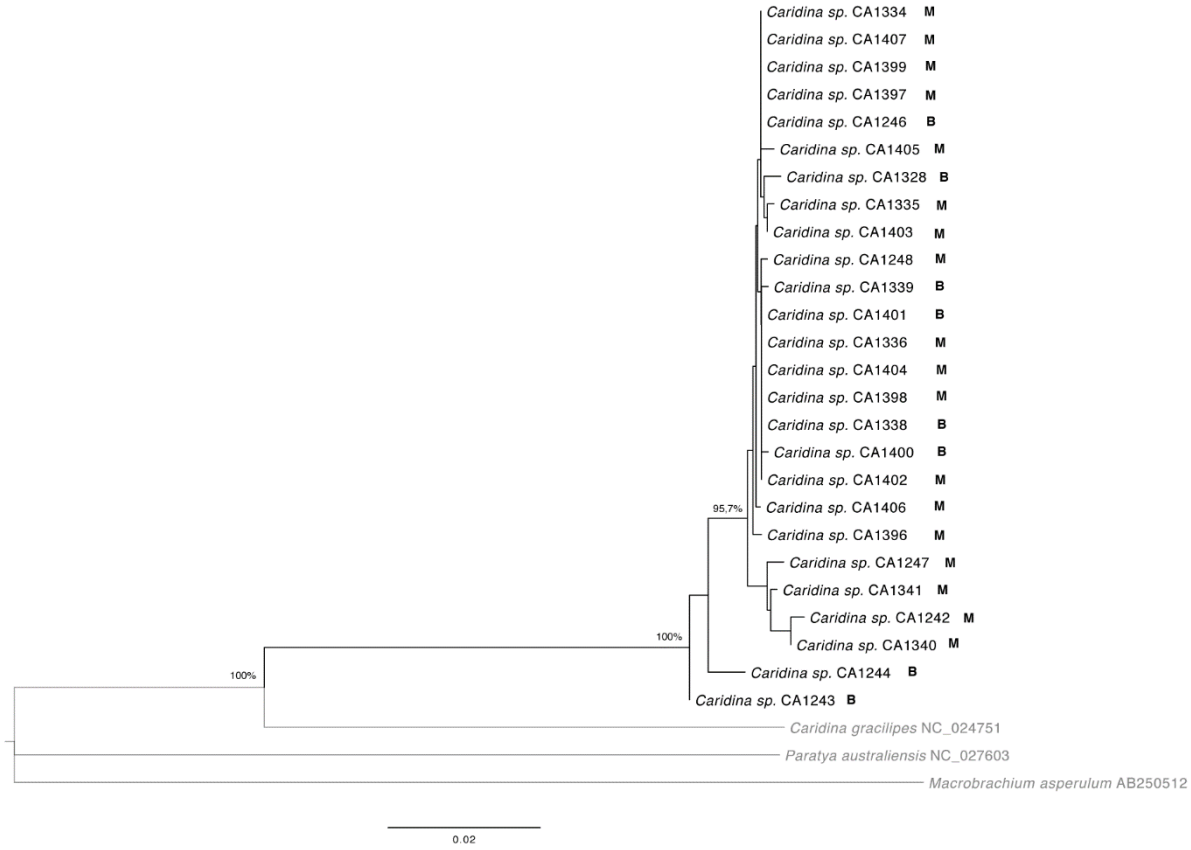


Figure 3.2 : NJ tree of concatenated COI and 16S of the specimens studied. The numbers at nodes indicate bootstrap numbers. The letter M indicates specimens identified as *Caridina mertoni* because of their short rostrum and the letter B indicates specimens identified as *Caridina brachydactyla* because of their long rostrum.

3.4.3 Morphological analysis

Of the 19 variables tested, five showed a significant correlation ($r > 0.5$) with elevation (Fig. 3.3). These variable were, from the highest to the lowest absolute r value, rostrum length to carapace length ratio (variable 15; Fig. 3b, $r = -0.9277$), number of teeth on ventral margin of rostrum (variable 18; Fig. 3d, $r = -0.6480$), unarmed part of

rostrum length to armed part length ratio (variable 16; Fig. 3e, r = –0.6868), pereiopod 2 carpus length-to-width ratio (variable 8; Fig. 3j, r = –0.6318), and pereiopod 3 propodus length-to-width ratio (variable 10; Fig. 3o, r = –0.5302).

Variables from rostrum/cephalothorax

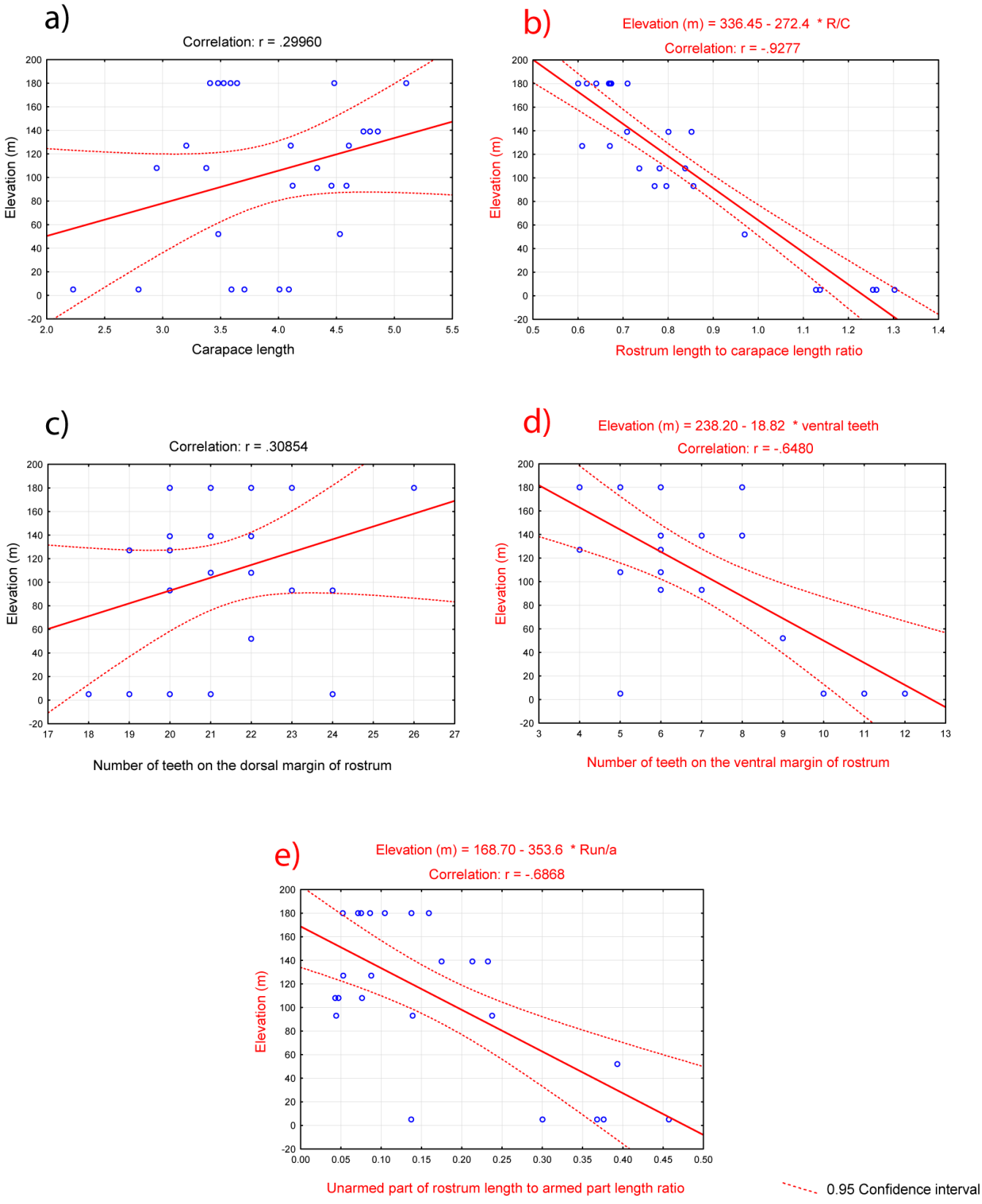


Figure 3.3 : Correlation scatterplots of the 19 morphological variables with the elevation (in m); a-e, rostrum/cephalothorax; f-i, pereiopod 1; j-m, pereiopod 2; n-p, pereiopod 3; q-s, pereiopod 5. Above scatterplots, r coefficient, and equation of the regression line present for variables with significant correlations. Dashed lines indicate the 0.95 confidence interval.

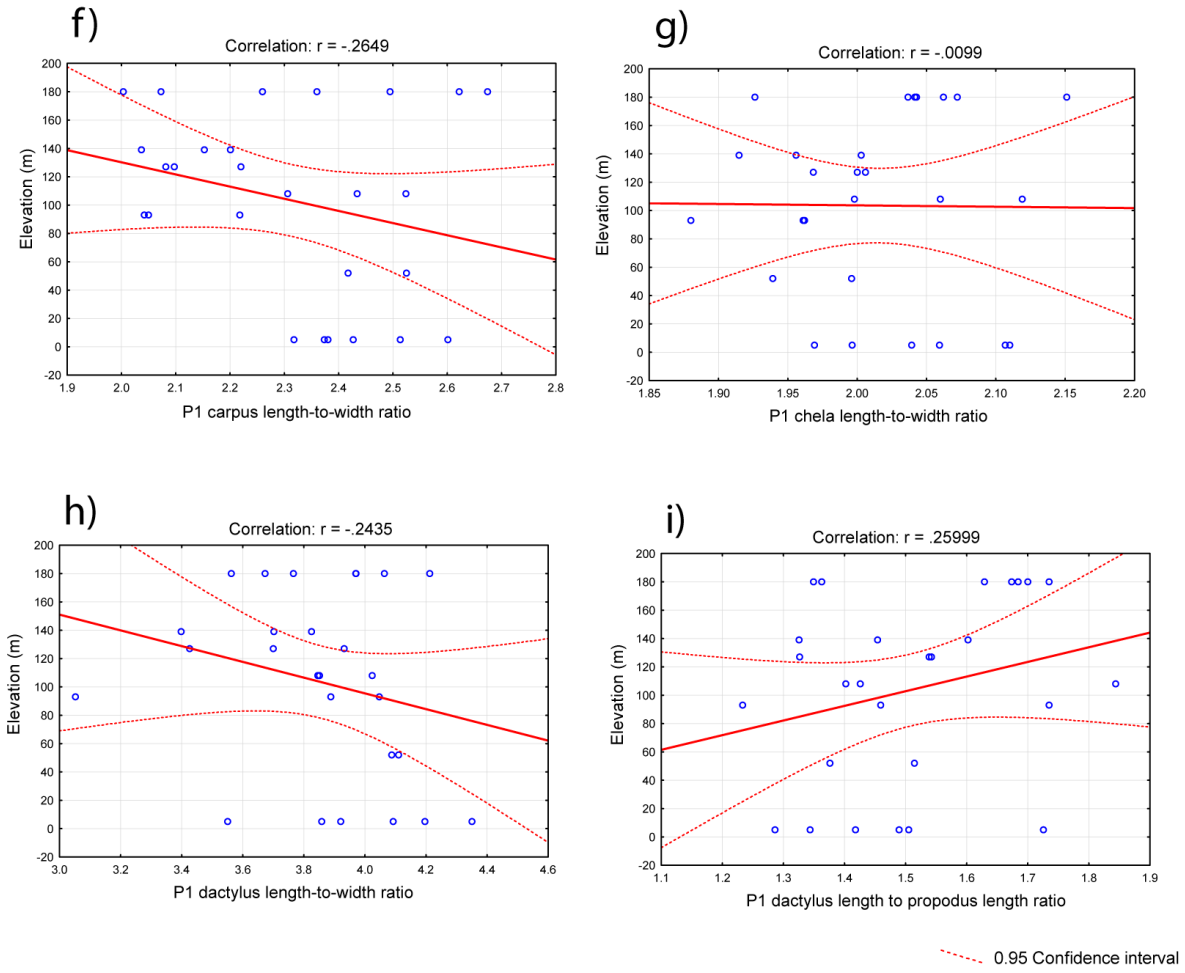


Figure 3.3 (Continued)

In order to refine the analysis, we hypothesized that the variables related to the rostrum would be linked to its length instead of elevation. To test this hypothesis, another correlation analysis was performed on the three concerned variables (16, 17, and 18) with rostrum length to carapace length ratio (Fig. 3.4).

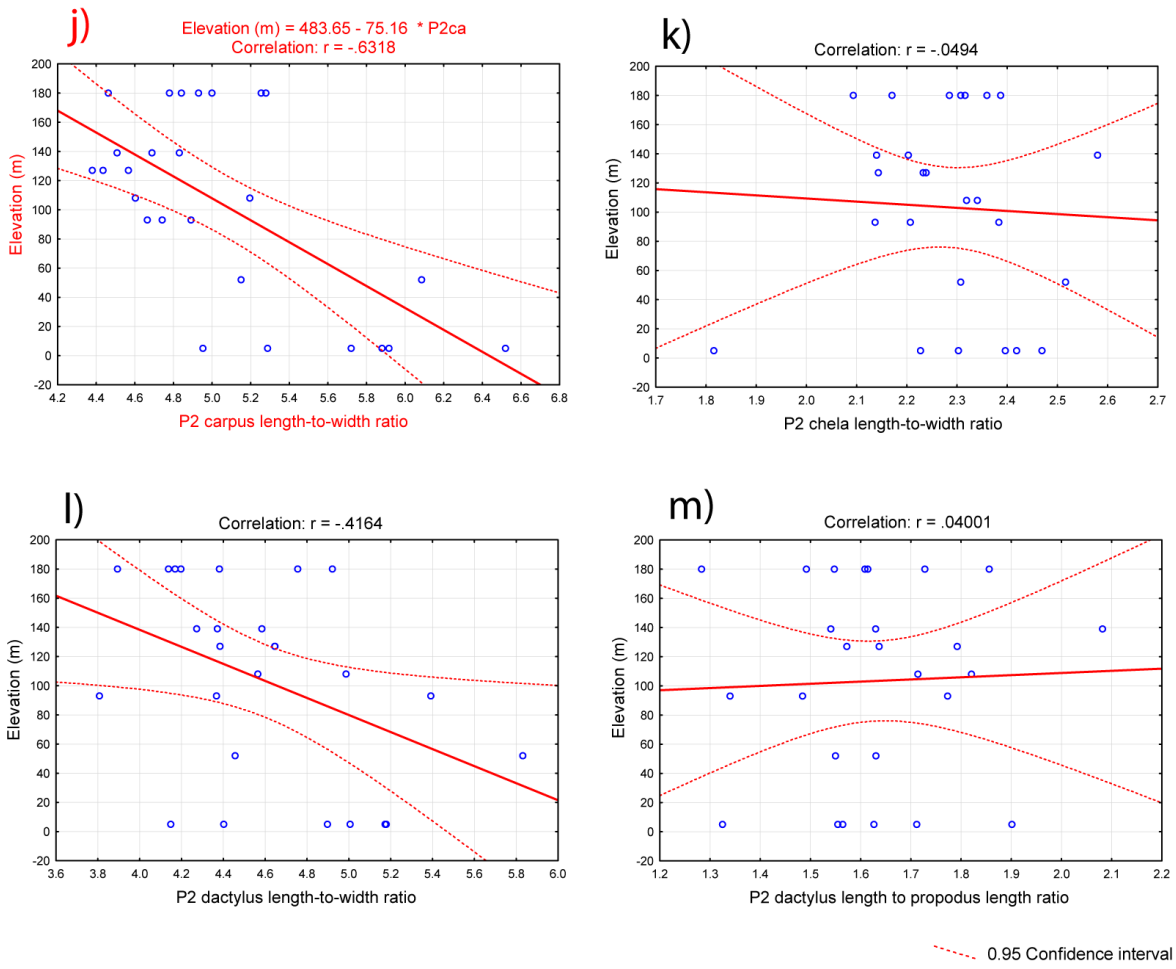


Figure 3.3 (Continued)

We found that there was a strong correlation between rostrum length and variables 18 and 16 (Fig. 4b, r = 0.67166, and 4c, r = 0.81086, respectively). Out of the 19 variables tested, three are directly linked to elevation (variable 15, rostrum length to carapace length ratio; variable 8, pereiopod 2 carpus length-to-width ratio; and variable 10, pereiopod 3 propodus length-to-width ratio), with only the former showing a strong correlation ($r > \pm 0.9$; Fig. 3.5).

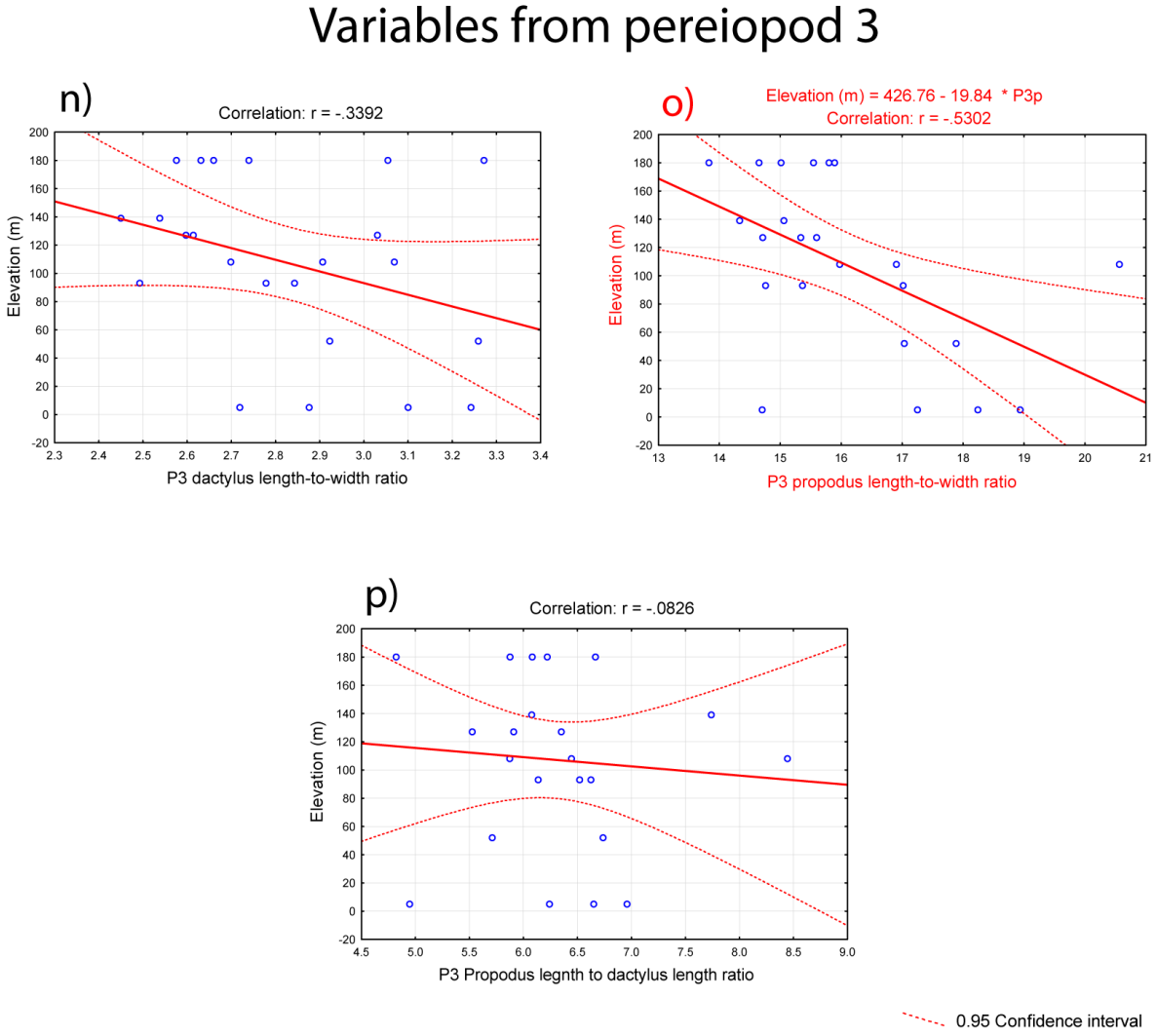


Figure 3.3 (Continued)

These correlations show that the specimens living at higher altitudes had a shorter rostrum (and therefore, a shorter unarmed dorsal margin and fewer ventral teeth), a shorter pereiopod 2 carpus, and a shorter pereiopod 3 propodus than specimens living at lower altitudes.

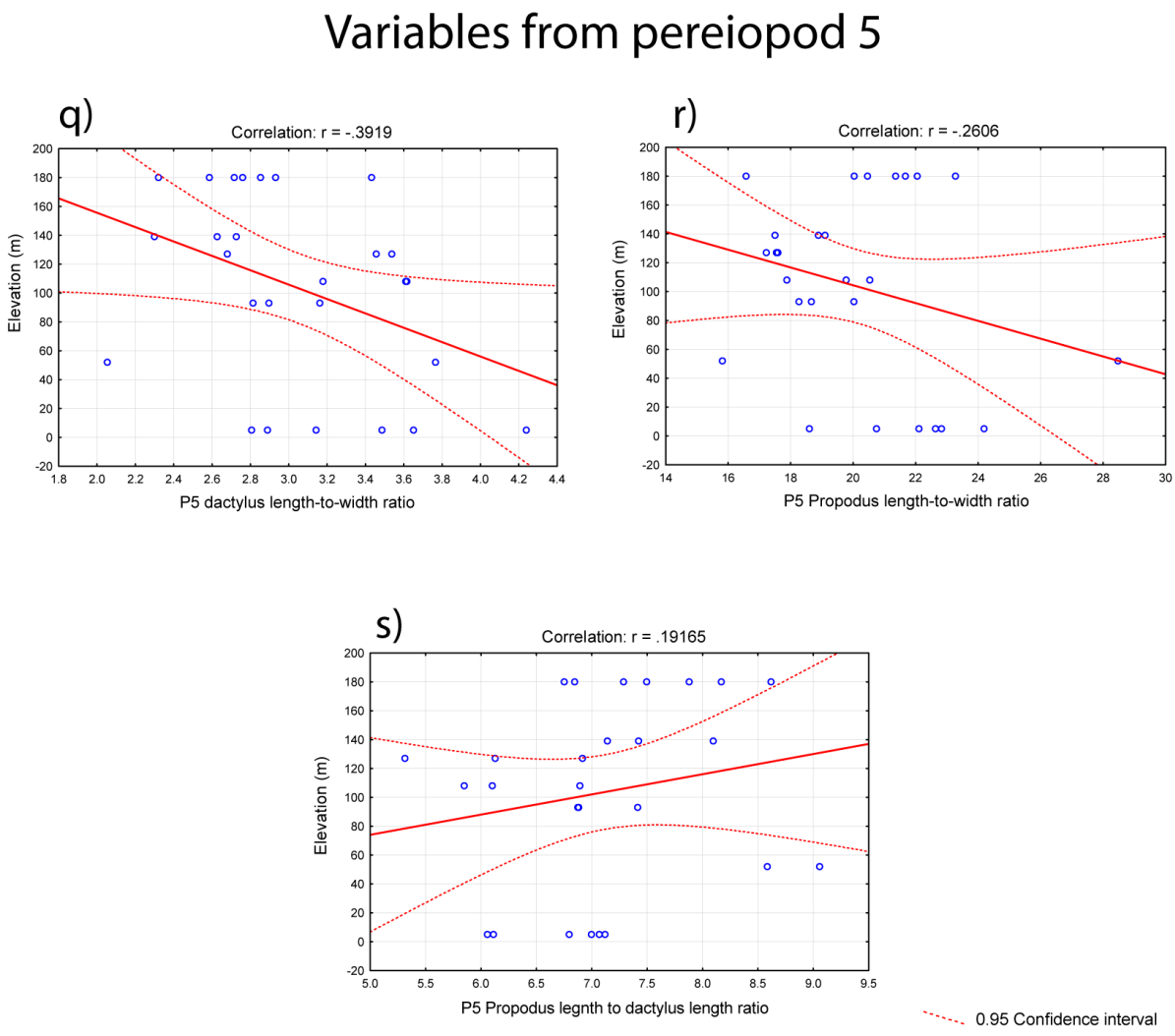


Figure 3.3 (Continued)

3.5 Discussion

This is the first time that variation in a morphological character, the length of the rostrum in this case, is demonstrated to be linked to an environmental factor in *Caridina*. The elevation that was tested in this study is most likely not linked directly to the morphological changes observed. Indeed, several parameters can be shown to be related to elevation. The speed of the current increases with elevation, which would result in stronger mechanical forces being applied on the rostrum, limiting its growth.

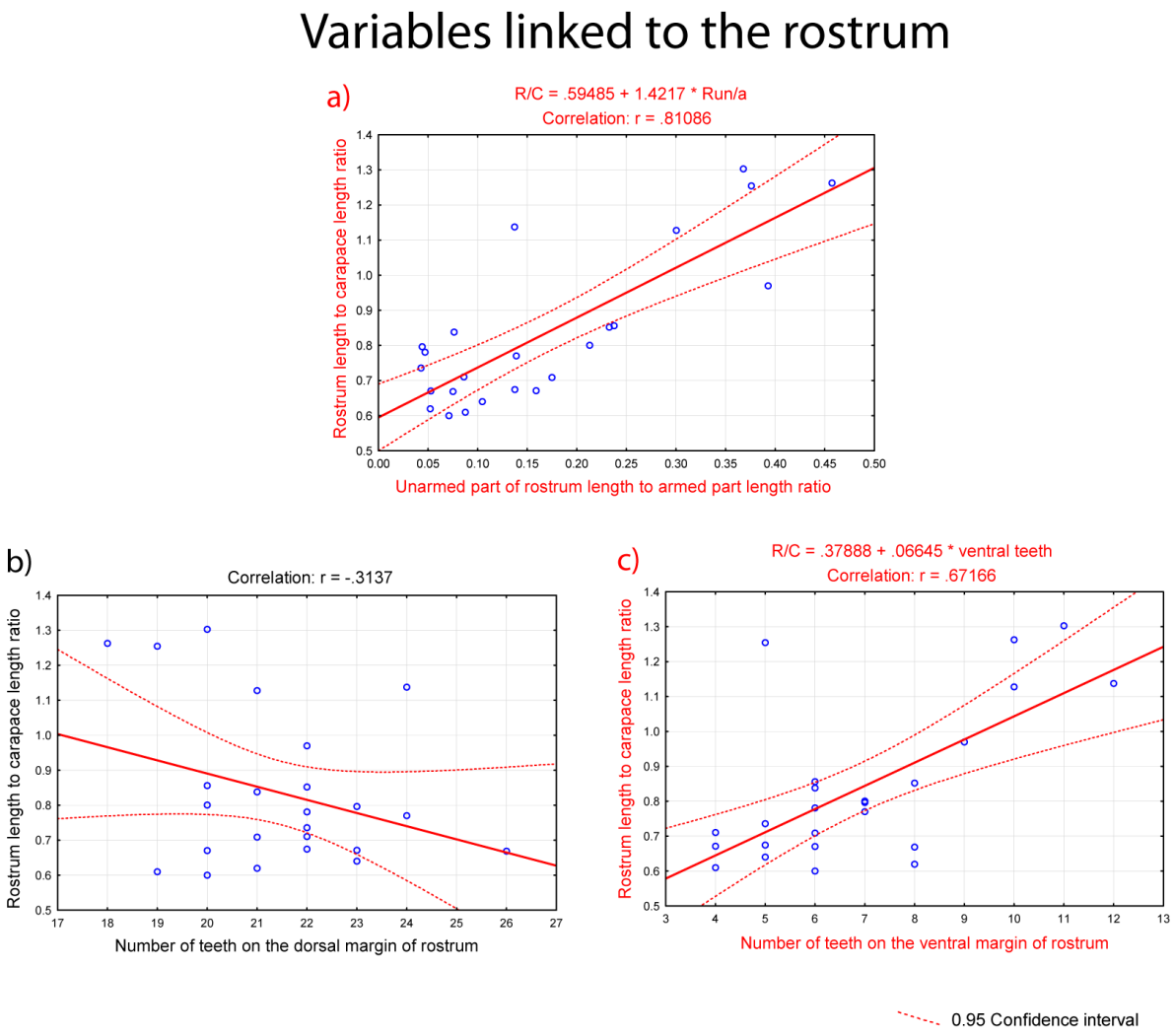


Figure 3.4 : Correlation scatterplots of the three rostrum-related variables with the length of the rostrum. Equation of the regression line present when correlations are significant. Dashed lines indicate the 0.95 confidence interval.

Furthermore, a longer rostrum tends to be more fragile and breaks easily, and might offer more current resistance, hindering movements in fast-flowing areas. The flow is certainly slower in the estuary at sea level, and little stress is applied on the rostrum, hence its long shape, whereas higher in the course, the slope of the stream is more important and the current faster. This phenomenon has been shown in another freshwater shrimp *Macrobrachium australe* (Guérin , 1838), with a longer rostrum in lentic habitats in contrast to a shorter rostrum in lotic habitats (Zimmermann *et al.*, 2012). This would also be valid for the other two morphological variables linked with elevation (i.e., pereiopod 2 carpus length and pereiopod 3 propodus length), the higher

in the stream, the shorter and broader the legs, presumably an adaptation to better resist stronger currents.

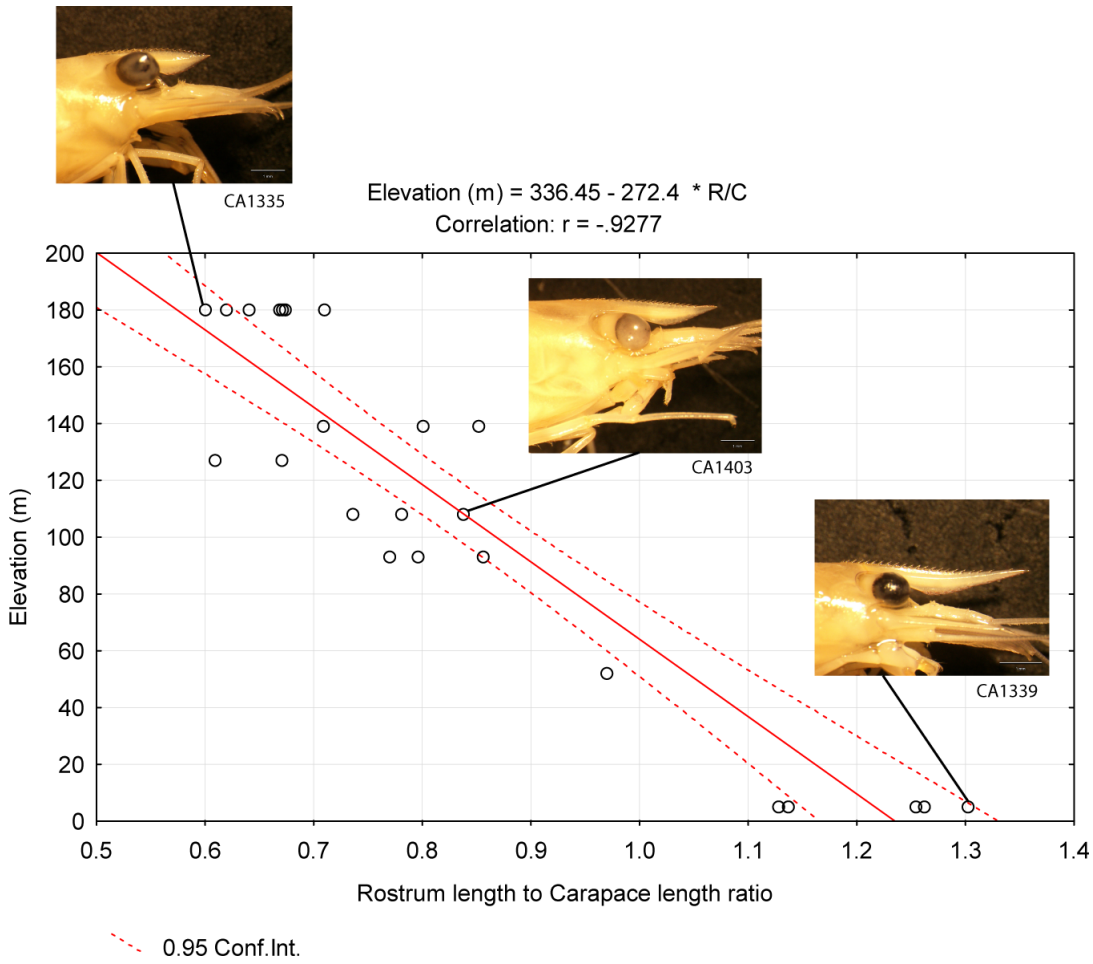


Figure 3.5 : Correlation scatterplot of the rostrum length over carapace length with elevation. Some of the specimens are illustrated to show the variation.

Morphological adaptations to hydrodynamic parameters have been highlighted in crustaceans before, such as the evolution towards an elongated shape in shrimp-like crustaceans (Spaargaren, 1999). We can argue, however, that there can be areas at higher altitudes of the stream with little or no current, as the slope is not steady along all of its length. Another parameter linked to the altitude is temperature. The higher the elevation of the stream, the cooler the water, because of lower air temperatures as well as thicker vegetation cover providing more shade. Temperature could influence the morphology as growth would be slower at lower temperatures due to a lower metabolism of the animal (Lee & Fielder, 1981), resulting in a shorter rostrum. This variation with temperature has been shown in freshwater shrimp species of *Macrobrachium* (Dimmock *et al.*, 2004). Unfortunately, the water temperature at each station was not measured in our study so no correlation could be tested. The

temperature difference between the estuary and the upper course in such tropical island can be up to more than 5°C (PK, unpublished data), which could significantly impact the metabolism and the growth of organisms living there. The amount of food available could also limit the growth of the shrimp. This species is a detritivore feeding in particular on decaying plants, which would tend to accumulate in downstream areas of slow current to the detriment of upstream fast-flowing zones. These last two parameters have indeed been demonstrated to significantly affect the size of atyid shrimps (Pérez-Reyes *et al.*, 2015).

The sediment size of the substrate also varies with slope and current speed. The substrate is composed of large blocks in steep-slope, fast-flowing areas, but replaced with sand or gravel in areas with a gentler slope (Keith *et al.*, 2010). This would imply that the shrimp could use the spaces between the rocks to hide from predators and a long rostrum would be undesirable for crawling in these narrow spaces. A long rostrum does not therefore hinder the shrimp living lower in the stream where the sediment provides fewer hideouts. On the contrary, as indicated by Jugovic *et al.* (2010) and Ocasio-Torres *et al.* (2015), a long rostrum could be a protection against predators, making the shrimp more difficult to swallow. Predator pressure would be greater in the lower course than in the upper course, because many shrimp-eating fishes like species of Eleotridae or Kuhliidae are present in the lower course of rivers (Covich *et al.*, 2009 ; Keith *et al.*, 2010) and are unable to climb waterfalls. This limitation in the distribution and mobility of predators affects the distribution of others organisms. Atyids are more abundant in the areas situated above waterfalls where predators are less numerous (Covich *et al.*, 2009). We found the individuals with the longest rostra in the three stations (3, 7, and 9) where the most predators were present, which were all below the first waterfall and at the lowest altitudes. This kind of defensive feature is supposedly present in some species of the freshwater gastropods *Clithon* Montfort, 1810, in which specimens encountered in brackish water exhibit spikes on their shell, whereas specimens of the same species living higher in the river have smooth shells (Haynes, 2005). These spikes could be a protection against predators (Eichhorst, 2016). Eels, which are also known to be shrimp predators, however, are able to crawl upward these waterfalls (Keith *et al.*, 2010), which would still make some kind of defensive adaptation useful for prey living there. This kind of morphological adaptation against predation could be chemically induced by kairomones from predators, as shown in *Xiphocaris*

elongata (F. E. Guérin-Méneville, 1857) (Ocasio-Torres *et al.*, 2014) and species of *Troglocaris* Dormitzer, 1853 (Jugovic *et al.*, 2010). If the presence of a long rostrum was only linked to the presence or absence of predators, one would expect only two classes of length, short and long, but we nevertheless showed that there is a continuum following a gradient, which is not related to the abundance of predators in the sampled stations.

To ease future citations of the phenomenon we describe here, we decided to name the elongation of the rostrum at lower altitudes in freshwater shrimps the "Pinocchio-shrimp effect". This name is in relation to the commercial name of *Caridina gracilirostris* in the aquarium trade, known for having a long and slender rostrum. This is not to be confused with the "Pinocchio effect" *sensu* Chapman (1990), Zelditch *et al.* (2004), and Zimmerman *et al.* (2012), which refers to Procrustes-based morphometric analyses in which a variation that is localized to a single landmark can be smeared out across many in the superimposition consensus.

This observed variation of the morphology of this species with environmental parameters shows that some of the characters widely used in the taxonomy of *Caridina* might not be as reliable as it was thought. We found here that rostrum length, but also the length of the unarmed portion of the dorsal part of the rostrum and the number of ventral teeth are highly plastic in this species, and presumably in others. We nevertheless found that the number of dorsal teeth seems surprisingly unrelated to the length of the rostrum (Fig. 3.4a), and therefore with the elevation, making it a more reliable taxonomic character. We strongly suggest to always perform some type of molecular work to studying and describing a new species of *Caridina* in order to unravel this kind of variation within a species, focus on the morphology, and uncover any type of variation. This would provide enough characters to confidently identify the species in the field. It is likely that the different described species in the genus exhibit the same kind of polymorphism that has been shown here. Species with a short rostrum like *C. mertoni* and *C. sundanella* Holthuis, 1978 need to be studied in order to ensure that other species with a long rostrum do not actually belong to species having such polymorphism. The taxon investigated in this study is probably neither *C. brachydactyla* nor *C. mertoni* so an in-depth taxonomic study should follow.

3.6 Acknowledgements

We would like to thank Magalie Castelin, Laura Taillebois, and Philippe Gerbeaux for their help collecting specimens in the field, the Conservation Society of Pohnpei and particularly Eugene Joseph (Director), Francesca Sohl Obispo, Relio Lengsi, and Iakop loanis, and the Office of fisheries and aquaculture for their help with processing the research permits. Brian Lynch was an incredible source of knowledge on the biodiversity of Pohnpei. He also facilitated the organization of various meetings with a number of local stakeholders, including assistance with some aspects of the logistics in the field. This work was supported by the Muséum national d'Histoire naturelle (MNHN), Paris, the AIMARA Association, and the French Ichthyological Society. We finally thank two anonymous reviewers for their helpful comments on the manuscript.

Chapitre troisième, Taxonomie intégrative du complexe *Caridina nilotica*.

Integrative taxonomy of the C. nilotica *complex.*

Où l'on révise la taxonomie de quelques espèces du complexe Caridina nilotica *dans trois zones géographiques distinctes ; Premièrement, l'océan Indien occidental, avec la revalidation d'une espèce en synonymie avec* Caridina brachydactyla *largement répartie dans le cours inférieur des rivières de la zone ; deuxièmement, la description de deux nouvelles espèces vivant dans les rivières des îles de Micronésie et troisièmement,, l'Insulinde/Mélanésie, avec l'étude des espèces confondues avec* Caridina longirostris *rencontrées dans le cours inférieur des rivières.*

Where we revise the taxonomy of some species of the Caridina nilotica *complexe in three different geographic areas; First, the Indian Ocean, with the revalidation of a species synonymised with* Caridina brachydactyla *with a wide repartition in the lower course of the rivers of the area; second, the description of two new species living in the rivers of Micronesian islands; and third, the Malay Archipelago/Melanesia, with the study of species confused with* Caridina longirostris *from the lower course of rivers.*

4. Chapitre 3 : Taxonomie intégrative du complexe *Caridina nilotica*

4.1 Introduction

Forts des résultats présentés dans le chapitre précédent, il apparaît évident que l'emploi de la morphologie seule ne permet pas de fournir une taxonomie correcte pour les espèces du genre *Caridina*. Nous avons donc décidé d'appliquer l'approche de taxonomie intégrative aux espèces du complexe *C. nilotica* récoltées au cours de différentes missions de l'équipe dans plusieurs régions de la zone Indo-Pacifique.

Nous illustrerons cette étude en 3 parties correspondant à 3 zones géographiques.

La première se concentre sur une espèce du cours inférieur des rivières se jetant dans l'océan Indien occidental qui a eu plusieurs noms avant d'être mise en synonymie avec *Caridina brachydactyla*. En étudiant morphologiquement les spécimens types des différentes espèces concernée et génétiquement des spécimens nouvellement collectés dans la zone, nous avons revalidé cette espèce sous le nom de *C. natalensis*. Ce travail a fait l'objet d'un article accepté en 2018 dans *Zootaxa* dont le texte constitue la première partie de ce chapitre.

Pour la seconde, nous avons décrit deux espèces appartenant à ce complexe provenant de 3 îles de Micronésie : *C. variabilis* à Palau et Guam et *C. variabilirostris* à Pohnpei. Du fait de leur variabilité morphologique due à leur diversité d'habitats occupés tout au long du cours des rivières, des spécimens appartenant à ces deux espèces avaient été identifiés comme appartenant à des espèces distinctes en fonction de la longueur de leur rostre. Ce travail a fait l'objet de deux articles publiés en 2018, le premier dans *Zootaxa 4377(1)* et le second dans le *European Journal of Taxonomy 453* dont les textes consituent la deuxième partie de ce chapitre.

Pour la dernière partie, nous nous sommes intéressés à 4 espèces vivant dans le cours inférieur des rivières de l'Insulinde et de la Mélanésie, habituellement identifiées comme *Caridina longirostris*. Ce travail a fait l'objet d'un article accepté en 2018 dans *Invertebrate Systematics* dont le texte constitue la troisième partie de ce chapitre.

Considering the results presented in the previous chapter, it appears clearly that the using only morphological data cannot provide a satisfactory taxonomy for species of the genus *Caridina*. We thus decided to apply the integrative taxonomy approach to the species belonging to the *C. nilotica* complex collected during different fieldtrips by the team in several areas of the Indo-Pacific region.

We will illustrate this study in 3 parts corresponding to 3 geographic areas.

The first one focuses on a species of the lower course of rivers draining to the Western Indian Ocean, which had several different names before being synonymised with *Caridina brachydactyla*. By studying the morphology of type specimens of the different species concerned and the DNA of newly collected specimens from the area, we revalidated this species under the name *C. natalensis*. This work was subject of an article accepted in 2018 in *Zootaxa* of which the text forms the first part of this chapter.

For the second one, we described two species belonging to this complex coming from 3 Micronesian islands: *C. variabilis* in Palau and Guam and *C. variabilirostris* in Pohnpei. Because of their morphological variability due to their occupied habitat diversity all over the rivers course, specimens belonging to these species were previously identified as distinct species according to the rostrum length. This work was subject of two articles published in 2018, the first one in *Zootaxa 4377(1)* and the second one in the *European Journal of Taxonomy 453* of which the texts forms the second part of this chapter.

For the last part, we focussed on 4 species living in the lower course of the rivers of the Malay Archipelago and Melanesia, usually identified as *Caridina longirostris*. THis work was subject of an article accepted in 2018 in *Invertebrate Systematics* of which the text forms the third part of this chapter.

4.2 Revalidation de *Caridina natalensis* De Man, 1908 (Crustacea: Decapoda: Atyidae) dans le Sud-Ouest de l'océan Indien.

Zootaxa, in press

Revalidation of *Caridina natalensis* De Man, 1908 (Crustacea: Decapoda: Atyidae) in the South Western Indian Ocean.

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4.2.1 Abstract

Numerous specimens of a freshwater shrimp with small eggs belonging to the *Caridina nilotica* complex collected in the South Western Indian Ocean were studied and compared with recent and old collection specimens genetically (16S mitochondrial analysis for recent and type specimens) and morphologically. The results revealed that, in the Indian Ocean, what has been identified by several authors under various species

names of the complex *C. nilotica*, was in fact *C. natalensis* De Man, 1908. This valid species is re-described and compared with closely related species, often confused with it in this area: *C. brachydactyla* De Man, 1908, *C. brevidactyla* Roux, 1920, *C. gracilipes* De Man, 1892 and *C. longirostris* H. Milne Edwards, 1837.

Keywords: *Caridina*, Integrative taxonomy, 16S, Morphology, South Africa, Seychelles, Comoros, Madagascar, Mayotte

4.2.2 Introduction

From 1991 to 2010, several South West Indian Ocean islands were prospected by field trips conducted by the Muséum national d'Histoire naturelle (MNHN) in Mascarenes archipelago (Reunion, Mauritius and Rodrigues; see Keith *et al.* 1999 and Keith *et al.* 2006), in Comoros (Anjouan, Mohéli) and Mayotte (see Keith *et al.* 2006) and in Seychelles (La Digue, Mahé, Praslin and Silhouette; see Keith *et al.* 2006). In 2008 and 2010 the MNHN conducted also freshwater inventories in the Northeastern rivers of Madagascar, for the DIAMSOI (Diversité des AMphihalins du Sud-Ouest de l'Océan Indien) program. Recently two of us (GM and MM, in February 2018) collected various *Caridina* from South Africa. During these prospections, numerous specimens of freshwater shrimps with small eggs belonging to the "*Caridina nilotica* complex" were collected.

De Man (1908) compared all the specimens he received from all the Indo-Pacific area, related to the *C. nilotica* complex with type material and recognized nine varieties. Four of those with small eggs were then reported as occurring in the Indian Ocean: *C. nilotica brachydactyla*, *C. n. longirostris*, *C. n. gracilipes* and *C. n. natalensis*. Later, Roux (1920) described *C. n. brevidactyla* and reported it from the Indian Ocean (1929). The first three are considered valid species (Cai & Ng, 2001 ; Cai & Shokita, 2006b ; Richard & Clark, 2014), respectively *C. brachydactyla* De Man, 1908, *C. longirostris* H. Milne Edwards, 1837 and *C. gracilipes* De Man, 1892, whereas *C. n. natalensis* and *C. n. brevidactyla* are currently junior synonyms of *C. brachydactyla* (Richard & Clark, 2010).

Caridina brachydactyla was originally described from Mbawa (Flores) and from Palopo, (Celebes) (De Man, 1908). According to Lenz (1910) *C. brachydactyla* occurs in

Madagascar, Mohéli and Mayotte (Comoros Islands). Costa (1980) identified *C. brachydactyla brachydactyla* from Seychelles, Mauritius, Comoros and Reunion islands. More recently, Richard & Clark (2010) have identified *C. brachydactyla*, for the first time, from Africa (Tanzania, South Africa).

Caridina longirostris was originally described supposedly from the river Macta, near Oran, Algeria, however, authors like Bouvier (1925) have suggested that this locality indication is erroneous. The types would more likely be coming from Egypt (unpublished data). Richters (1880) identified *C. longirostris* from Seychelles; Holthuis (1965) identified *C. longirostris* from Madagascar, so did Gurney (1984) and Short & Doumenq (2003). Keith *et al.* (2006) reported *C. longirostris* from Comoros, Mascarenes archipelago and Seychelles.

Caridina gracilipes was originally described from Maros (Celebes). According to Lenz (1910) *C. gracilipes* occurs in Mohéli and Madagascar.

Caridina n. natalensis De Man, 1908 was described from specimens collected in the river Umgeni, Natal (South Africa) in November 1894. They were wrongly referred by Weber (1897) to *C. wyckii* (Hickson, 1888) and considered by De Man (1908) as a distinct variety of *C. nilotica*, *C. nilotica natalensis*. Lenz (1912) also identified *C. nilotica var. natalensis* near Amawzentoti (Natal, correct spelling: Amanzimtoti) and Bouvier (1925) in the river Tiwi, 20km south of Mombasa (Kenya) and in the river Imanambo in Madagascar. In his key for the South African species and varieties, Barnard (1950) listed *C. nilotica var. natalensis* with small eggs (0.42-0.46 mm major axis). Recently, according to Richard & Clark (2010), *C. n. natalensis* was not considered as a valid species but as a junior synonym of *C. brachydactyla*. However, Klotz & De Grave (2015) predicted that *C. n. natalensis* should be resurrected for certain African populations, but they were lacking data to effectively do it.

Caridina n. brevidactyla Roux, 1920 described from the Aru Islands, Indonesia, occurs also in Madagascar according to Roux (1929). Richard & Clark (2010) considered that *C. nilotica brevidactyla* was also a junior synonym of *C. brachydactyla* in contrast of Cai & Ng (2001) who considered it as a valid species.

Comparing the new material collected by the MNHN in the South West Indian Ocean to the old collections, the first aim of the present work is to review the status of *C. n.*

natalensis, currently a junior synonym of *C. brachydactyla*, and provide additional taxonomic information about their respective morphologies and distributions. The second aim of this study is to re-examine existing material and descriptions related to all the species of the *C. nilotica* complex that have historically been reported as occurring in the Western Indian Ocean.

4.2.3 Materials and Methods

Field collection data

All specimens were collected in Indian Ocean islands by electrofishing (portable Dekka 3,000 electric device, Germany) and by hand nets in South Africa. In the field, caught specimens were fixed in 75%. All material has been deposited in the collections of the Muséum national d'Histoire naturelle (MNHN) or Albany Museum in Grahamstown (South Africa).

Abbreviations for museums

Muséum national d'Histoire naturelle, Paris: MNHN; Naturhistorisches Museum Basel, Basel: NMB; Rijksmuseum van Natuurlijke Historie (now in the Naturalis Biodiversity Center, Leiden): RMNH; Zoological Museum Amsterdam (now in the Naturalis Biodiversity Center, Leiden): ZMA.

Abbreviations for morphological analyses

The following abbreviations are used in the present text: cl, carapace length (mm): measured from the post-orbital margin to the posterior margin of the carapace. P1: first pereiopod. P2: second pereiopod. P3: third pereiopod. P5: fifth pereiopod. Pl1: first pleopod. PlI2: second pleopod.

Morphological comparison.

The rostrum, the general cephalon, the pereiopods 1, 2, 3 and 5 and the abdomen were observed using a stereoscopic microscope. The proportions of the various joints of the appendages were measured using microphotographs and the AnalySIS Works software (Olympus). Drawings were made using the "Digital Inking" method (Coleman, 2003, 2006) by tracing vectorial paths on high-resolution photographs using Adobe Illustrator (CS6) and a WACOM PTZ-1230 graphic tablet.

DNA extraction, amplification and sequencing.

For recent specimens, DNA was extracted from abdominal tissues using the semi-automatic Ependorf ep-Motion 5075 robot. Fragments of the mitochondrial 16S rRNA (~ 520 bp) were amplified using the newly designed primers 16Sar-Lmod (TACTTCTGCCTGTTTATCAAAAA) and 16Sbmod (GGTCTGAACTCAAATCATGTAAA). DNA amplification was performed in 20µl PCR reactions, containing approximately 3 ng of template DNA, 2.5 mM MgCl2, 0.26 mM of each nucleotide, 0.3 µM of each primer, 5% DMSO, 1 ng of BSA and 1.5 units of QBIOTAQ polymerase (MPBiomedicals). Amplification products were generated by an initial denaturation step of 4 min at 94°C followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 52°C for 40 sec, extension at 72°C for 60 sec and a final extension step at 72°C for 7 min.

For old collection specimens (Type and non-type specimens of *C. brachydactyla*, *C. brevidactyla*, *C. gracilipes*, *C. longirostris* and *C. natalensis*), a CTAB protocol was used to extract DNA from pleopods. A shorter fragment of the 16S rRNA (332 bp) was amplified using the newly designed primers 16S–Car–81F (AGGTAGCATAATAAATAGTC) and 16S–Car–413R (CTGTTATCCCTAAAGTAAC). If these primers could not amplify successfully, another Reverse primer was designed and used to try amplifying a shorter fragment (141 bp): 16S–Car–222R (CTTTATAGGGTCTTATCGT). DNA amplification was performed in 20µl PCR reactions, containing 2.5 mM MgCl2, 0.26 mM of each nucleotide, 0.3 µM of each primer, 1 ng of BSA and 1.5 units of QBIOTAQ polymerase (MPBiomedicals). Amplification products were generated by an initial denaturation step of 4 min at 94°C followed by 45 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 40 sec and a final extension step at 72°C for 7 min.

PCR products were sequenced using the same primers and in both directions to insure the accuracy of base calls. Chromatograms were edited using Geneious v.8 software (http://www.geneious.com/ Kearse *et al.* 2012). All new sequences were deposited in GenBank (Table 1, Numbers MH644413 to MH644429).

Table 4.2.1 : List of the specimens used in the genetic study.

Species	Locality	DNA voucher	Museum n°	Type status	GenBank n°	Reference
C. brachydactyla	Bali	CA037	NMB 1054a		MH497490	de Mazancourt *et al*, in press
	Sulawesi	CA1129	WK 63-10	Topotypical	MH497500	de Mazancourt *et al*, in press
		CA1130	WK 63-10-1	Topotypical	MH497501	de Mazancourt *et al*, in press
		CA1131	WK 64-10-3	Topotypical	MH497502	de Mazancourt *et al*, in press
C. brevidactyla	Solomon Islands	CA1302	MNHN-IU-2018-175		MH497513	de Mazancourt *et al*, in press
	Vanuatu	CA1232	MNHN-IU-2018-191		MH497508	de Mazancourt *et al*, in press
C. gracilipes	Borneo	CA1673	MNHN-IU-2018-209		MH497535	de Mazancourt *et al*, in press
	Flores	CA029	NMB 1061f		MH497489	de Mazancourt *et al*, in press
	Sulawesi	CA023	ZMA.CRUS.D.102635 1	Paralectotype	MH497485	de Mazancourt *et al*, in press
		CA024	ZMA.CRUS.D.102635 2	Paralectotype	MH497486	de Mazancourt *et al*, in press
		CA025	ZMA.CRUS.D.102635 3	Paralectotype	MH497487	de Mazancourt *et al*, in press
		CA027	ZMA.CRUS.D.102635 5	Paralectotype	MH497488	de Mazancourt *et al*, in press
	Taiwan	CA1674	MNHN-IU-2018-208		MH497536	de Mazancourt *et al*, in press
C. natalensis	Comoros	CA1343	MNHN-IU-2018-78		MH644416	This study
		CA1344	MNHN-IU-2018-77		MH644417	This study
	Madagascar	CA1035	MNHN-IU-2018-79		MH644413	This study
		CA1036	MNHN-IU-2017-1455		MH644414	This study

	CA1037	MNHN-IU-2018-80		MH644415	This study
	CA1184	MNHN-IU-2018-99		MH644424	This study
	CA1185	MNHN-IU-2018-100		MH644425	This study
Mayotte	CA1206	MNHN-IU-2017-1456		MH644418	This study
	CA1207	MNHN-IU-2017-1457		MH644419	This study
Seychelles	CA1186	MNHN-IU-2017-1447		MH644426	This study
	CA1187	MNHN-IU-2017-1448		MH644427	This study
	CA1194	MNHN-IU-2017-1446		MH644420	This study
	CA1195	MNHN-IU-2017-1445		MH644421	This study
	CA1196	MNHN-IU-2017-1450		MH644422	This study
	CA1197	MNHN-IU-2017-1449		MH644423	This study
South Africa	CA2079	MNHN-IU-2018-61	Topotypical	MH644428	This study
	CA2083	MNHN-IU-2018-66	Topotypical	MH644429	This study

Molecular analyses

DNA sequences were aligned using MEGA7 software (S. Kumar *et al.*, 2016) with Muscle algorithm (Edgar, 2004). Using Bayesian information criterion in jModelTest (Guindon & Gascuel, 2003 ; Darriba *et al.*, 2012) we retained the GTR + G model. Best-scoring ML trees were estimated using RAxML HPC2 v.8.2.10 (Stamatakis, 2014) and best-scoring Bayesian Inference trees were estimated using MrBayes v.3.2.6 (Ronquist & Huelsenbeck, 2003), both methods implemented in the Cyber Infrastructure for Phylogenetic Research (CIPRES) portal v.3.1. (Miller *et al.*, 2010) (https://www.phylo.org/) with the previously determined model, running for 10,000,000 generations, a sampling frequency of 2,000 and a burn in of 25%. Support for nodes was determined using posterior probabilities calculated by MrBayes implemented in CIPRES. One hundred independent searches, each starting from distinct

random trees, were conducted. Robustness of the nodes was assessed using non–parametric bootstrapping (Felsenstein, 1985) with 1,000 bootstrap replicates. We considered a group to be 'moderately supported' if it had a bootstrap support value (B) between 75 and 89% or a posterior probability (PP) between 0.80 and 0.94 and 'highly supported' when B ≥ 90% or PP ≥ 0.95.

4.2.4 Results

Molecular results

Due to their old age, no DNA in sufficient quantity and quality could be extracted using our method for the type specimens of *C. brachydactyla*, *C. brevidactyla*, *C. longirostris* and *C. natalensis*, thus, no 16S sequence was amplified for these specimens. However, the approach was successful for type specimens of *C. gracilipes* and non-type specimen of *C. brachydactyla*. Furthermore, we tried to obtain recent specimens as close as possible to the type locality for *C. brachydactyla* (Palopo, Sulawesi, Indonesia) and *C. natalensis* (Durban, South Africa). Four major clades can be separated in the 16S tree. The first one (A in Fig. 4.2.1), highly supported (B = 95% PP = 0.99) comprises the specimens of *C. brevidactyla* and is separated from all the others grouped in a moderately supported clade (PP = 0.89). Within this clade, the specimens of *C. brachydactyla* (B) cluster together in a highly supported clade (B = 99% PP = 1) away from the others grouped in a moderately supported clade (PP = 0.84). This clade is split in two, the first one (C), highly supported (B = 100% PP = 1), comprises the type and non-type specimens of *C. gracilipes* and the other one (D), moderately supported (PP = 0.84) with all the specimens of *C. natalensis* from different Indian Ocean localities.

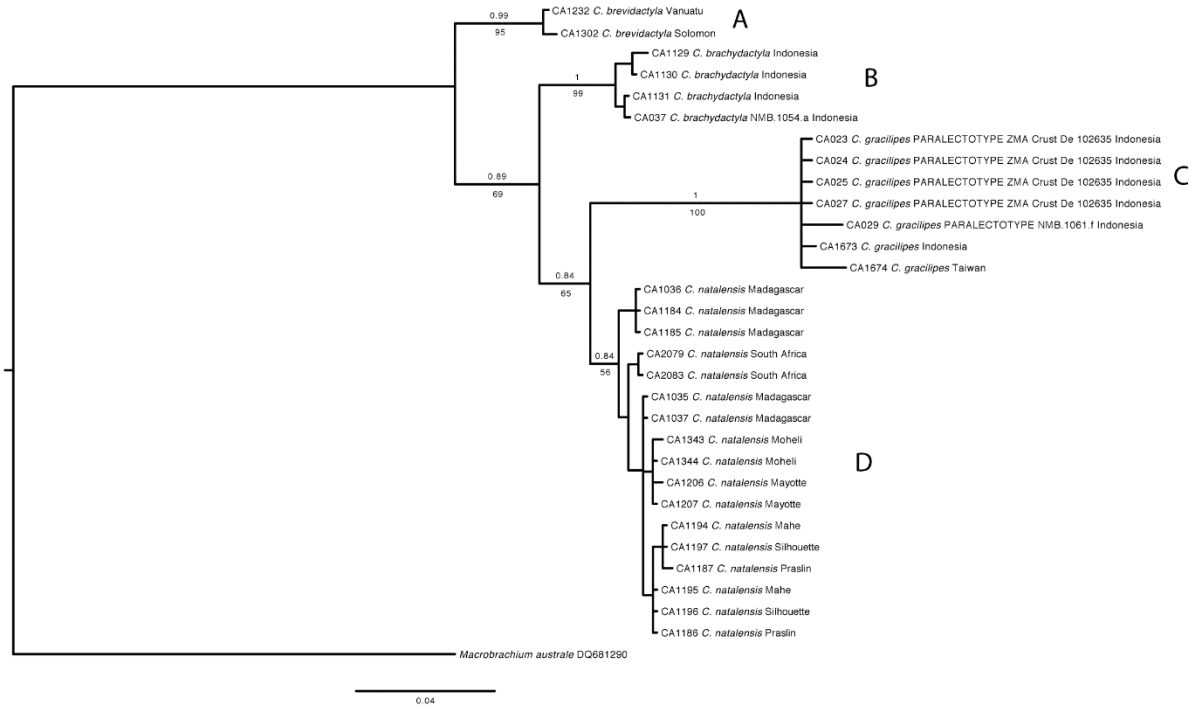


Figure 4.2.1 : Bayesian 16S rRNA phylogenetic tree showing the relationships of some of the species discussed in this study. Numbers above branches indicate bayesian posterior probabilities; numbers under branches indicate Maximum Likelihood bootstrap values.

Morphological comparison

Our morphological study, confirming the molecular study results, reveals that what has been attributed by several authors to various species names is in fact *C. natalensis* De Man, 1908. Until now, *C. natalensis* was considered a synonym of *C. brachydactyla*.

Detailed description

Infraorder Caridea Dana, 1852

Family Atyidae De Haan, 1849

Genus *Caridina* H. Milne Edwards, 1837

***Caridina natalensis* De Man, 1908**

Caridina nilotica var. natalensis – De Man, 1908: 262–263 pl.20, fig.3, 3a, 3b; Lenz, 1912: 5; Bouvier, 1925: 154–155 fig. 320; Barnard, 1950: 655 (key).

Caridina longirostris – Richters, 1880:162–163 ; Holthuis, 1965: 20–23 fig. 6 ; Keith *et al.* 2006: 50–51.

Caridina nilotica var. brachydactyla – Lenz, 1910: 568; Roux, 1929: 303–304 tab.1 no.15.

Caridina nilotica var. gracilipes – Lenz, 1910: 568.

Caridina brachydactyla brachydactyla – Costa 1980: 673–700.

Material examined

Type material:

Caridina nilotica var. *natalensis* De Man, 1908: **SOUTH AFRICA.** Lectotype (designated by Richard & Clark, 2010). ZMA.CRUS.D.102886, 1♀ ovig. cl 6.4mm; Umgeni River, Durban, Natal, coll. M. Weber, 1898, det. J.G. De Man. Paralectotypes. ZMA.CRUS.D.102886, 2♀ ovig. cl 5.7–6.4mm; same data.

Caridina nilotica var. *brachydactyla* De Man, 1908: **INDONESIA.** Lectotype (designated by Richard & Clark, 2010). RMNH.CRUS.D.977, 1♀ ovig. cl 4.8mm; Near Reo, Flores, coll. M. Weber. Paralectotypes. RMNH.CRUS.D.2552, 2♀ ovig. cl 5.3–5.4mm; river near Palopo Luwu, Sulawesi, coll. M. Weber..

Caridina brevidactyla Roux, 1920: **INDONESIA.** Lectotype (present designation). NMB 4.VI.b 1, 1♂ cl 4,8mm; Sungi Manumbai, Wokam Island, Aru, April 14–15,1908. Paralectotypes. NMB 4.VI.a, 1♂ cl 3.3mm; Aru, Udjir Island, April 15, 1908, NMB 4.VI.b, 2♀ cl 5.8–6.4mm; Sungi Manumbai, Wokam Island, Aru, April 14–15,1908. NMB 00004 VIc, Wokamar, Wokam Island, Aru, April 17, 1908, 1♀ ovig. 5,7mm; NMB 4.VI.d; 2♀ ovig. cl 5.7mm, Seltutti, Kobroor Island, Aru, May 2, 1908.

Caridina gracilipes De Man, 1892: **INDONESIA.** Paralectotypes. ZMA.CRUS.D.102635, 1♂ cl 4.1mm (DNA: CA025), 1♀ ovig. cl 5.9mm (DNA: CA023), 3♀ cl 5.1mm (DNA: CA027), 5.2mm (DNA:CA024) and 5.6mm; in a rice field, Makassar, Sulawesi, coll. M. Weber, 1888. NMB 1061e, 1♂ 3.8mm; 2♀ cl 5.8–5.9mm; same data.

Caridina longirostris H. Milne Edwards, 1837: **ALGERIA.** Paralectotypes. MNHN-IU-2013-19419, 3♂ cl 2.9–3.1mm; from the river Macta, near Oran. MNHN IU-2013-19418, 2♂ 3.2–3.6mm; same data.

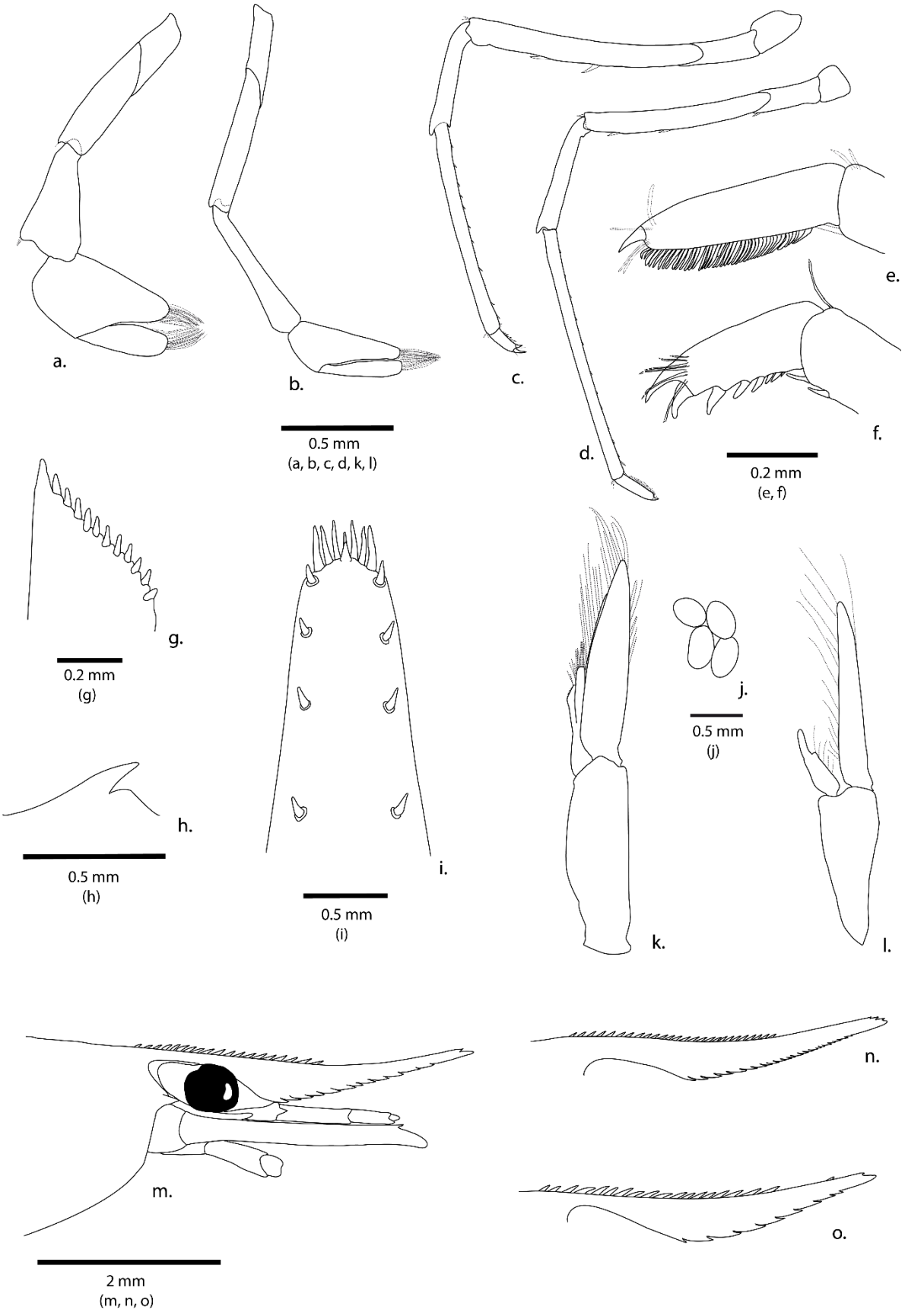


Figure 4.2.2 : *Caridina natalensis*; a. first pereiopod; b. second pereiopod; c. third pereiopod; d. fifth pereiopod; e. dactylus of fifth pereiopod; f. dactylus of third pereiopod; g. uropodal diaeresis; h. pre-anal carina; i. telson; j. eggs; k. second male pleopod; l. first male pleopod; m. anterior region of cephalothorax: n, o. rostrum variations.

Non- types :

Caridina natalensis **KENYA.** MNHN-IU-2015-1854, Tiwi river, 20km south of Mombasa. **TANZANIA.** NHM 1982.576, 3♀ ovig. cl 5.5–7.2mm, Ruo Lukuledi, Tanganyika. **SOUTH AFRICA.** MNHN-IU-2018-61, 1♂ cl 3.3mm (DNA: CA2079) and MNHN-IU-2018-62, 1♀ ovig. cl 6.0mm, River Mbanyana, 32°13.123'S 28°55.031'E, altitude 9m, coll. Maliwa L., Mlambo M., Marquet G. & Tiberghien, P., February 11, 2018; MNHN-IU-2018-63, 1♀ ovig. cl 6.9mm and MNHN-IU-2018-64, 1♀ ovig. cl 6.8mm, River Umtata, altitude 2m, 31°34.144'S, 28°45.523'E, coll. Maliwa L., Mlambo M., Marquet G. & Tiberghien, P., February 13, 2018; MNHN-IU-2018-65, 1♀ ovig. cl 6.3mm and MNHN-IU-2018-66, 1♀ ovig. cl 6.3mm (DNA: CA2083), River Mzumbe, altitude 6m, 30°36.151'S 30°32.817'E, coll. Maliwa L., Mlambo M., Marquet G. & Tiberghien, P., February 16, 2018; MNHN-IU-2018-67, 1♀ovig. cl 6.8mm and MNHN-IU-2018-68, 1♀ovig. cl 7.0mm, River iLovu, altitude 8m, 17°05.858'S 30°49.390'E, coll. Maliwa L., Mlambo M., Marquet G. & Tiberghien, P., February 17, 2018. **MAYOTTE.** MNHN-IU-2018-69, 1♀ovig cl 6.1mm, River Bouyoni, altitude 14m, 12°43.966'S 45°8.250'E, coll. ARDA, May 15, 2006; MNHN-IU-2018-70, 1♀ cl 4.5mm and MNHN-IU-2018-71, 1♀ cl 6.0mm, River Dembeni, altitude 19m, 12°50.402'S 45°10.461'E, coll. ARDA, November 12, 2003; MNHN-IU-2018-72, River Kwalé, altitude 39m, 12°48'092'S 45°11.520'E, coll. ARDA, November 10, 2003; MNHN-IU-2018-73, 1♀ cl 4.6mm, MNHN-IU-2018-74, MNHN-IU-2018-76 and MNHN-IU-2018-75, 1♀ cl 5.2mm, River Soulou, altitude 14m, 12°45.812'S 45°5.951'E, coll. ARDA, November 9, 2003. **COMOROS.** MNHN-IU-2018-77, 1♀ ovig. cl 3.8mm (DNA: CA1344), River Bwanifunge, Moheli Island, altitude 39m, 12°19.642'S, 43°40.240'E, coll. A. Abdou December 30, 2013; MNHN-IU-2018-78, 1♀ ovig. cl 4.5mm (DNA: CA1343), River Mdjawashe, Moheli Island altitude 18m, 12°21.386'S, 43°41.944'E, coll. A. Abdou, December 31, 2013. **MADAGASCAR.** MNHN-IU-2018-79, 1♀ ovig. cl 4.1mm (DNA: CA1035), MNHN-IU-2018-80, 1♀ ovig. 4.8mm (DNA: CA1037), MNHN-IU-2018-81, 1♀ cl 4.3mm and MNHN-IU-2018-82, 1♂ cl 4.8mm, River Ambodiforaha, altitude 9m, 15°43.054'S 49°57.637'E, coll. R. Fara, H. Grondin, G. Marquet, T. Robinet, May 15, 2010; MNHN-IU-2018-87, 1♀ ovig cl 3.5mm, MNHN-IU-2018-83, 1♀ ovig cl 5.2mm, MNHN-IU-2018-88 1♀ cl 4.0mm, MNHN-IU-2018-85, 1♀ cl 4.0mm, MNHN-IU-2018-86, 1♀ cl 4.3mm and MNHN-IU-2018-84, 1♂ cl 4.0mm, River Andempona, Ampanasanovy estuary, altitude 0m, 14°35.298'S 50°10.105'E, coll. C. Ellien, R. Fara, E. Feunteun, N. Mary, T. Robinet, July 04, 2008; MNHN-IU-2018-89, 1♀ ovig. cl 4.7mm, MNHN-IU-2018-90, 1♀ cl

4.7mm, MNHN-IU-2018-92, 1♀ cl 4.9mm, MNHN-IU-2018-91, 1♀ cl 5.5mm, MNHN-IU-2018-93, 1♂ cl 3.8mm and MNHN-IU-2018-94, 1♂ cl 4.0mm, River Ankavia, altitude 23m, 14°59.488'S 50°11.095'E, coll. C. Ellien, R. Fara, E. Feunteun, N. Mary, T. Robinet, July 03, 2008; MNHN-IU-2018-95, 1♀ ovig. cl 4.0mm, MNHN-IU-2018-96, 1♀ cl 4.8mm, MNHN-IU-2018-97, 1♀ cl 5.5mm and MNHN-IU-2018-98, 1♂ cl 3.0mm, River Fanambana, altitude 11m, 14°33.517'S 50°10.017'E, coll. C. Ellien, R. Fara, E. Feunteun, N. Mary, T. Robinet, July 4, 2008; MNHN-IU-2018-99, 1♀ovig cl 3.9mm (DNA: CA1184) and MNHN-IU-2018-100, 1♀ cl 4.8mm (DNA: CA1185), unknown locality, coll. C. Ellien, R. Fara, E. Feunteun, N. Mary, T. Robinet. **SEYCHELLES ISLANDS.** MNHN-IU-2017-1445, 1♀ ovig. cl 4.8mm (DNA: CA1195) and MNHN-IU-2017-1446, 1♀ cl 4.8mm (DNA: CA1194), unknown river, Mahé Island, coll. ARDA, November 24, 2004; MNHN-IU-2017-1447, 1♀ ovig. cl 4.5mm (DNA: CA1186) and MNHN-IU-2017-1448, 1♂ cl 4mm (DNA: CA1187), River Nouvelle Découverte, Praslin Island, altitude 8m, 4°19.304'S 55°42.351'E, coll. ARDA, October 08, 2003.; MNHN-IU-2017-1449, 1♀ ovig. cl 4.9mm (DNA: CA1197) and MNHN-IU-2017-1450, 1♂ cl 4.1mm (DNA: CA1196), River Grande Barbe, Silhouette Island, 4°30.052'S 55°13.537'E, coll. ARDA, November 20, 2004. **MAURITIUS.** MNHN-IU-2017-1451, 1♀ ovig. cl 4.2mm, MNHN-IU-2017-1452, 1 juvenile cl 2.3mm, MNHN-IU-2017-1454, 1 juvenile cl 2.4mm and MNHN-IU-2017-1453, 1 juvenile cl 2.6mm; Forested lake, saline, near Camp Poorun, altitude 11m, 20°10.517'S 57°45.767'E, coll. C. R. Turner, December 11, 2005.

Diagnosis.

Carapace (Fig. 4.2.2m, n, o) smooth, glabrous, with sharp antennal spine placed at lower orbital angle. Pterygostomian margin rounded. Rostrum rather constantly long, 0.8–1.3 of cl, straight or curved up distally, reaching well beyond scaphocerite. 15–26 dorsal teeth, leaving distally unarmed part of rostrum 0.4–1.2 times that of armed part, except for one to three subapical tooth, 1 to 2 post-orbital teeth present. 7–23 teeth present on the ventral margin extending from highest part of the rostrum either to tip or with short distal part unarmed. Number of dorsal teeth on the rostrum before the most proximal ventral tooth 10–14. Rostral formula: (1–2) 15–26 + 1–3 / 7–23.

First pereiopod (Fig. 4.2.2a): Stout, chela about 1.8–2.4 times as long as wide, dactylus 2.5–6.0 times as long as wide, 0.8–1.6 length of palm; carpus 1.7–3.3 times as long as wide with shallow excavation on anterior margin.

Second pereiopod (Fig. 4.2.2b): More slender and longer than first pereiopod. Chela 1.9–3.0 times as long as wide, dactylus 3.5–7.0 times as long as wide, 1.1–1.9 times length of palm; carpus 4.3–6.0 times as long as wide.

Third pereiopod (Fig. 4.2.2c): Dactylus (Fig. 4.2.2d): 2.9–4.0 times as long as wide (terminal spine included) with 6–9 spineson flexor margin in addition to the terminal one; propodus 12.7–18.6 times as long as wide, 4.1–6.6 times as long as dactylus.

Fifth pereiopod (Fig. 4.2.2e): Dactylus (Fig. 4.2.2f): 3.0–6.01 times as long as wide with 31–61 spiniform setae on flexor margin; propodus 15.2–22.0 times as long as wide, 4.3–5.7 as long as dactylus.

First male pleopod (Fig. 4.2.2k): Endopod leaf-like with a developped appendix interna in males.

Second male pleopod (Fig. 4.2.2l): Appendix masculina on second pleopod reaching 0.52 times length of endopod; appendix interna reaching about 0.84 times length of appendix masculina.

Egg size (Fig. 4.2.2j): 0.35–0.46 × 0.22–0.29 mm.

Preanal carina (Fig. 4.2.2h): with a strong spine.

Telson (Fig. 4.2.2i): ending triangular with a posteromedian projection; 1 pair of longer lateral simple setae and 2 to 3 pairs of shorter intermediate simple setae shorter or slightly longer.

Uropodal diaeresis (Fig. 4.2.2g): with 9–14 short spinules.

Colour pattern. Colours (Fig. 4.2.3) vary and tend to match the substrate the shrimp lives on. The body can vary from hyaline to brownish with red dots, with sometimes a large red border on the ventral side of the rostrum. An oblique brown or carmine red band on the cephalothorax is very characteristic. The coloration is lost on preserved animals.

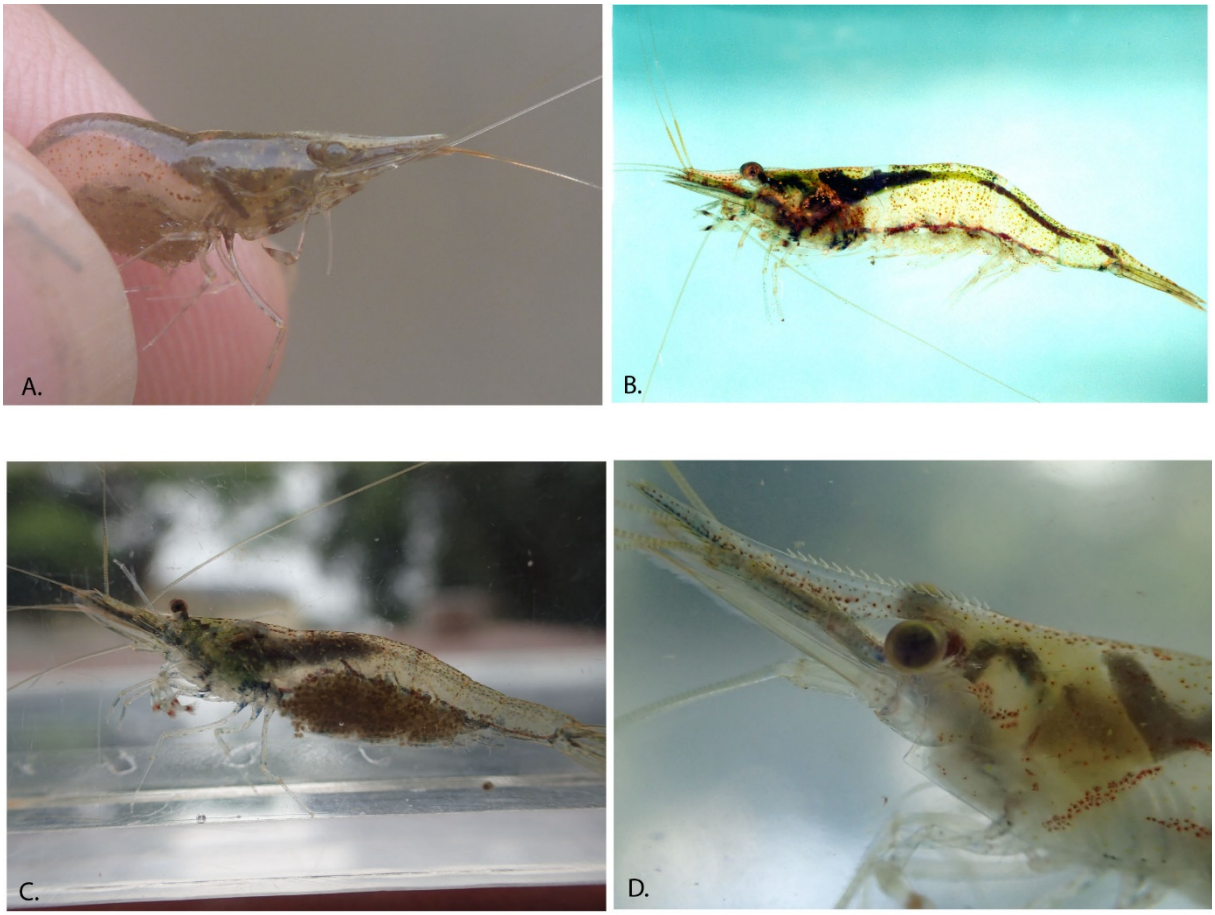




Figure 4.2.3 : a, b, c, d: Views of living specimens of *Caridina natalensis* showing their coloration; a. Specimen from Madagascar (G. Marquet); b. Specimen from Seychelles (P. Keith); c, d. Specimen from South Africa (G. Marquet). e: Example of habitat where *C. natalensis* lives; Iharaka river, Madagascar (G. Marquet).

Habitat. All specimens were collected in the lower course of rivers, (altitude 5–25 m), among macrophytes in running water.

Distribution. *C. natalensis* occurs in Comoros (Anjouan, Mohéli), Mayotte, Seychelles (La Digue, Mahé, Praslin and Silhouette), Mauritius, in Madagascar and South Africa. Present before in Reunion Island (Costa 1980; Kiener & Duchochois 1981) this species seems now absent despite extensive prospection made there since (Fig. 4.2.4).

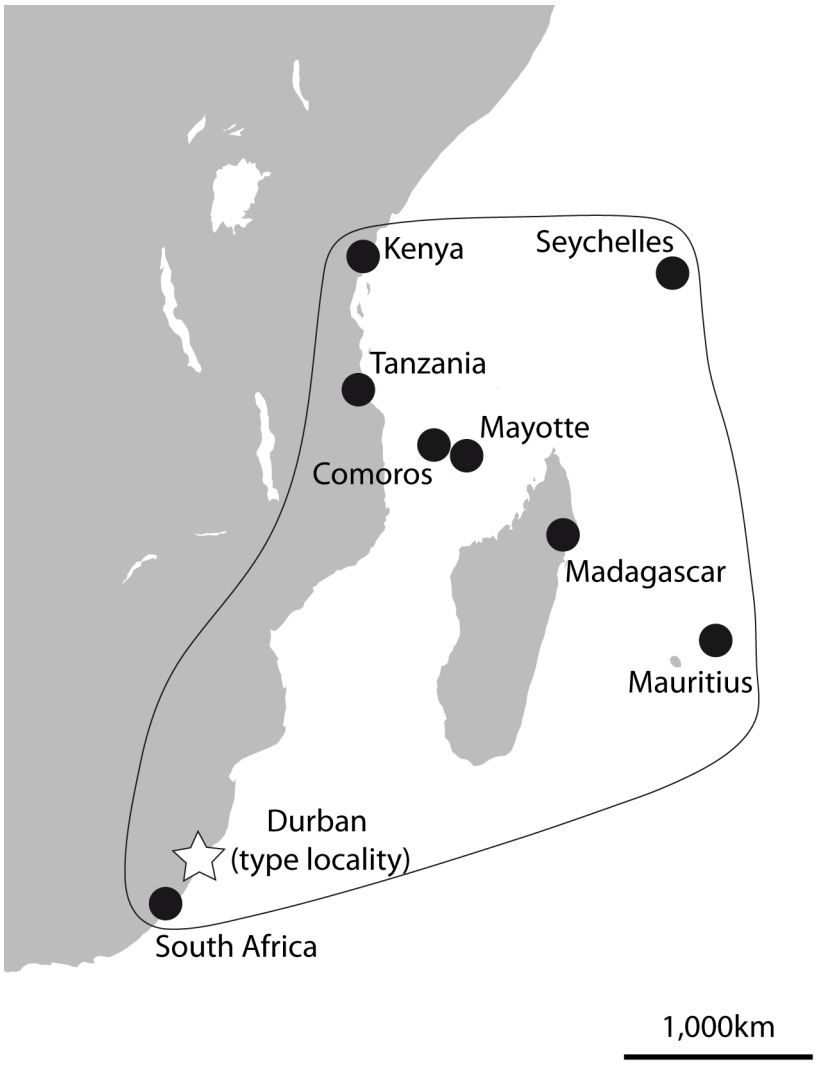


Figure 4.2.4 : Distribution of *Caridina natalensis* with sampling localities of the specimens of this species examined for the study (black circles) and its type locality (white star).

Key for studied species

1.1 Preanal carina with spine.....2

1.2 Preanal carina unarmed.....*C. brevidactyla*

2.1 P1 carpus greater than 1.9 times as long as wide and P2 carpus mostly than 4.3.....3

2.2 P1 carpus less than 1.8 (1.4–1.8) times as long as wide and P2 carpus than 4.2.....*C. longirostris*

3.1 P1 Chela greater than 2.1 times (2.1–2.3) as long as wide; P3 dactylus with mostly 5-6 spines (5–7) on flexor margin in addition to the terminal one.....*C. brachydactyla*

P1 Chela less than 2.1 times as long; P3 dactylus with mostly 6–9 spines on flexor margin in addition to the terminal one..... 4

4.1 P5 propodus 4.3–5.7 as long as dactylus.....*C. natalensis*

4.2 P5 propodus mostly 3.4–4.1 (3.4–5.8) as long as dactylus.....*C. gracilipes*

4.2.5 Discussion

Caridina natalensis*, not a junior synonym of *C. brachydactyla

The molecular and morphological analyses of specimens collected from a large geographic range, covering the entire known distribution of *C. brachydactyla* (*sensu* Richard & Clark (2010)) in the Indian Ocean and including localities close to most of the type localities of its synonyms, suggest that *C. n. natalensis* is a valid species. These results disprove Richard and Clark (2010) study, which based on old collection specimens (from which molecular studies are difficult to do), thus using morphology only, recognized *C. n. natalensis* and *C. n. brevidactyla* as junior synonyms of *C. brachydactyla*. Indeed, Richard and Clark (2010) found that the dactylus to propodus ratios of the first and second pereiopods in *C. n. natalensis* fell into the range calculated for *C. brachydactyla*. They also showed that the Natal specimens differed from

topotypical specimens of *C. brachydactyla* in having the appendix interna slightly turned down towards the exopod, whereas it was straight in the Indonesian material. This was regarded by the authors as a non-significant character for the separation of species. Here, the molecular results show that the specimens from the Southwestern Indian Ocean (here attributed to *C. natalensis* - type locality: Umgeni River, Durban, South Africa) are genetically highly distinct from the specimens collected in Indonesia (here attributed to *C. brachydactyla* - type locality: Sulawesi, Indonesia) and to specimens from Melanesia (here attributed to C. *brevidactyla* - type locality: Aru Island, Indonesia). The morphological examination of the specimens used in the molecular tree allowed finding new characters that are stable within genetic clusters but clearly different among clusters. Having examined the type specimens of *C. brachydactyla*, we show that it can be separated from *C. natalensis* by P1 chela greater than 2.1 times as long as wide (vs mostly 1.8–2.1) and P3 dactylus with mostly 5–6 spines on flexor margin in addition to the terminal one (vs 6–9). Having also examined the type specimens of *C. n. brevidactyla*, we show that it can be separated from *C. natalensis* by the tooth on the preanal carina that is present in *C. natalensis* but absent in *C. brevidactyla*. Indeed, in a not yet published work, we show based on many specimens from the Pacific Ocean that *C. brevidactyla* is also a valid species (see de Mazancourt *et al*. unpublished).

Taxonomy of Caridina nilotica *complex in the Indian Ocean*

Of the 298 known species of *Caridina* (WoRMs database as of May 2018), the complex *C. nilotica*, which includes 22 described varieties and/or species (Karge & Klotz, 2007) has a very confuse systematic (Johnson, 1963). Although, assumed to be broadly distributed across the Indo-West Pacific (from Africa to Fiji), *C. nilotica* complex remains ill defined and subject to many confusions. In this study, we focussed on the Indian Ocean members of the *C. nilotica* complex. Since the studies of De Man (1908) and Roux (1920), six varieties were generally reported in the Western Indian Ocean: *C. n. brachydactyla*; *C. n. longirostris*; *C. n. gracilipes; C. n. natalensis* and *C. n. brevidactyla.* Here, based on a large geographic range, covering the entire known distribution of *C. nilotica* complex in the Indian Ocean and including localities close to most of the type localities of its varieties, molecular and morphological analyses suggest that the only

variety of *C. nilotica* that occurs in the Indian Ocean is *C. n. natalensis*, that we here resurrect as a valid species.

As demonstrated above, the 16S molecular tree showed that *C. brachydactyla* only occurs in Indonesia, and *C. brevidactyla* only occurs in the West Pacific. Moreover, the inclusion of the paralectotypes of *C. gracilipes* in the molecular tree also suggested that this species is absent from the Western Indian Ocean. Indeed, in this study, none of the specimens collected in the Indian Ocean and morphologically resembling *C. brachydactyla, C. brevidactyla* or *C. gracilipes*, fell into the genetic clusters containing specimens of each of these three species. On the contrary, all of these specimens collected in the Indian Ocean formed a single clade, genetically highly differentiated from *C. brachydactyla, C. brevidactyla* and *C. gracilipes,* and morphologically close to the type specimen of *C. natalensis*.

In this study, we have compared the specimens of *C. natalensis* with all the species of the *C. nilotica* complex that have been historically reported as occurring in the Western Indian Ocean, namely: *C. n. gracilipes*, *C. n. brevidactyla*, *C. n. brachydactyla* and *C. n. longirostris*. These comparisons showed that not only these species do not occur in the Western Indian Ocean, but they also are distinguishable from *C. natalensis*. Morphologically, *C. gracilipes*, can be separated from *C. natalensis* by the P5 propodus mostly 3.3–4.1 as long as dactylus (vs 4.3–5.7). As mentioned earlier, *C. brevidactyla* and *C. brachydactyla* can be morphologically separated from *C. natalensis.* Finally, although the type specimens of *C. n. longirostris* could not be genetically analyzed, they were morphologically easy to separate from *C. natalensis* (or from other variants of *C. nilotica* complex) because of their P1 and P2 carpus respectively 1.7–2.3 (vs 1.4–1.8) and 4.3–8.0 (vs 3.9–4.7), P3 dactylus stouter, dactylus 2.9-4.0 (vs 4.0-4.8) times as long as wide, with propodus 4.1-6.6 (vs 3.4–4.0) times as long as dactylus, and P5 dactylus stouter with propodus 4.3–5.7 (vs 3.2–3.7) times as long as dactylus.

4.2.6 Conclusion

Further studies should be led on a larger geographic coverage in the Indian Ocean to determine more precisely the northern limit of *C. natalensis* distribution range. This species is abundant in lower course of rivers from South Africa, Madagascar, Comoros,

Mayotte and Seychelles, however, it has a critical status in Mascarene archipelago. Indeed *C. natalensis* seems to have now disappeared from Reunion Island (Keith *et al.* 2006) like *Macrobrachium hirtimanus* (Olivier, 1811).

4.2.7 Acknowledgements

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4.3 Description de deux nouvelles espèces de *Caridina* en Micronésie (partie 1 : *C. variabilis*)

Zootaxa 4377(1), 39-50

Description of a new species of *Caridina* (Crustacea: Decapoda: Atyidae) from two Micronesian islands (Guam and Babeldaob).

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4.3.1 Abstract

During field trips to Guam and Babeldaob Islands in Micronesia, freshwater shrimps were sampled and assigned either to *Caridina brachydactyla* De Man, 1908 or *C. mertoni* J. Roux, 1911 (Decapoda: Atyidae), following previous inventories. In combining morphological data with a genetical analysis, it appeared that all the specimens

belonged to a new species, *Caridina variabilis sp. nov.*, here described with its distribution. The status of this new species is clarified and finally, neither *C. brachydactyla*, nor *C. mertoni* occur in these two islands. DNA sequences of 16S were obtained from the syntypes of *C. mertoni*.

Key words: *Caridina*, New species, Micronesia, Integrative taxonomy, 16S, Morphology.

4.3.2 Introduction

Micronesia is a vast expanse of more than 2000 Pacific islands and atolls stretching from the Caroline and Mariana islands in the West, to the Marshall, Nauru and Kiribati islands in the East (Fig. 4.3.1).

The Mariana Islands is a crescent-shaped archipelago comprising the summits of fifteen mostly dormant volcanic mountains. At the southern end of the chain, Guam (formally the Territory of Guam) is an unincorporated and organized territory of the United States of America. It is located at 13°28'N and 144°45'E, and is 50 km long and 6 to 19 km wide, with an area of 549 km^2. The highest point is Mount Lamlam at 407 meters above sea level. It belongs to the Mariana ridge, which was created by the colliding Pacific and Philippine Sea tectonic plates (Tracey *et al.*, 1964)

The Caroline Islands consist of a chain of seamounts, atolls and high islands extended south-eastward to the Marshall Islands. At the western edge of the Caroline Islands the Republic of Palau comprises a curved archipelago of approximately 350 islands lying between 4 and 8 degrees north latitude and 131 – 135 degrees east longitude. The high island of Babeldaob covers 334 km^2 accounting for over 80% of Palau's landmass, with a highest elevation point of only 230 m. Palau, aged of 30 Ma, is the crest of the Palau-Kyushu ridge (Kobayashi, 2004).

The freshwater shrimp genus *Caridina* H. Milne Edwards, 1837, comprising 298 species (WoRMS database) and mostly present in the Indo-Pacific region, is the most diversified genus of the Atyidae (De Grave *et al*, 2015) and an important ecological component in the tropical streams (Covich *et al.*, 1999; Pringle *et al.*, 1993). Its high diversity combined with the lack of informative morphological characters has led to a

confused taxonomy (Richard & Clark, 2009). Indeed, until recently, the taxonomy of the genus was mainly based on morphological characters. Some have been proven highly variable within a species (e.g. rostrum shape and indentation or coloration) and so taxonomically non-informative, making it difficult to establish boundaries between them (Mazancourt *et al.*, 2017a). Thus, there is a need for an integrative and standardized approach to improve the group's systematics, focusing on informative morphological features and using molecular characters (Page *et al.*, 2005; Page & Hughes, 2011).

From the Mariana Islands (with no island specified), Bouvier (1925) reported six specimens belonging to *C. nilotica brachydactyla* De Man, 1908 (A. Marche coll.) with a short rostrum armed to the tip on both margins. Specimens are deposited in MNHN (MNHN-IU-2015-1824). Leberer & Nelson (2001) collected *C. longirostris* H. Milne Edwards, 1837 and *C. nilotica* (P. Roux, 1833) from two rivers of Guam. Reviewed later by Leberer & Cai (2003), *C. longirostris* became *C. brachydactyla* and *C. nilotica* became *C. mertoni* Roux, 1911 in the Pacific. Recently one of us (DCR in March 2015) collected various *Caridina* from Guam, with some specimens bearing a long rostrum and attributed to *C. brachydactyla*, whereas others, with a short rostrum, were identified as *C. mertoni* following the previous identifications made by Leberer & Cai, 2003.

Bright (1979) reported *C. brachydactyla* from Palau among seven Atyid species, depositing them at the USNM (USNM 172589 and USNM 172590). Holthuis (year unknown) named specimens from Palau, based on material collected by H. A. Fehlmann on the 30 October 1956 from Arakitaoch stream and deposited in USNM as *C. brachydactyla uncata* (holotype USNM 105430, paratypes UNSNM 105434) but never described or published this taxon.

Keith *et al.* (2011), reviewing the freshwater decapods of Palau conducted a survey from 22 February through 5 March 2011, identifying some specimens as *C. brachydactyla* or as *C. mertoni*.

As we examined more and more specimens from Palau and Guam, we gradually started to question the validity of these two species from Micronesia. Consequently, we here examined recent specimens and Museum collections in combining morphological data with a 16S mtDNA analysis.

4.3.3 Materials and methods

Collection of specimens

Rivers and sites surveyed are indicated in Figure 4.3.1 and Table 4.3.1. Specimens from Guam were collected by net. Specimens from Palau were collected by electrofishing (portable Dekka 3,000 electric device, Germany). All material preserved in 75%-95% alcohol has been deposited in the collections of the Muséum national d'Histoire naturelle in Paris (MNHN, specimens n° MNHN-IU-2017-2082 to MNHN-IU-2017-2106).

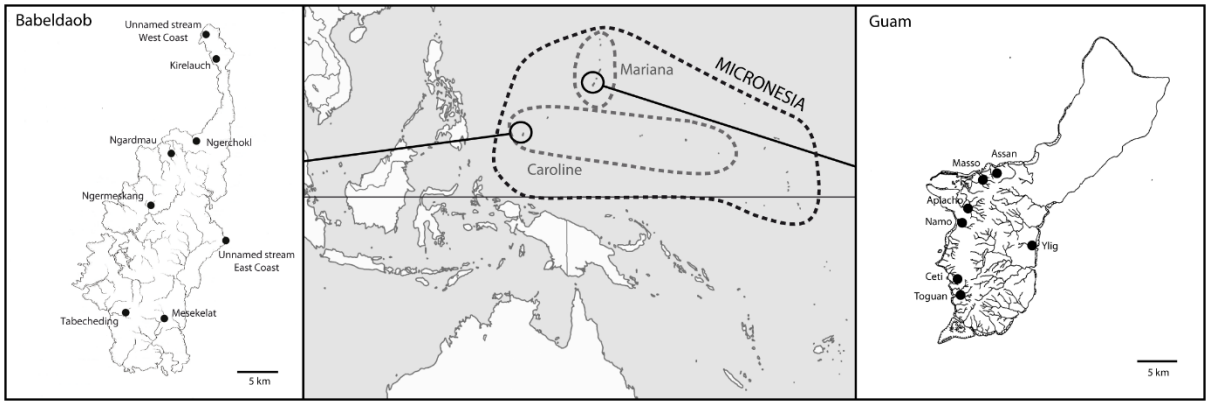


Figure 4.3.1: Rivers and sites surveyed in Babeldaob and Guam (black dots: prospected sites).

Table 4.3.1: Sampling localities data.

Island	Dates	Coordinates	Rivers	Altitude (m)
Babeldaob (P. Keith, P. Gerbeaux, G. Marquet, L. Taillebois, M. Castelin coll.)	27/02/2011	07°27.169'N 134°31.748'E	Tabecheding	28
	27/02/2011	07°36.527'N 134°36.958'E	Ngerchokl	36
	28/02/2011	07°41.417'N 134°37'754E	Kirelauch	2
	28/02/2011	07°43.056'N 134°36.924'E	Unnamed West Coast stream	10
	28/02/2011	07°29.909'N 134°38.099'E	Unnamed East Coast stream	7
	01/03/2011	07°32.484'N 134°34.670'E	Ngermeskang	75
	02/03/2011	07°35.482'N	Ngardmau	39

		134°35.573'E	(waterfall)	
	03/03/2011	07°26.305'N 134°34.328'E	Mesekelat	20
Guam (D. Christopher Rogers, A. Farahi, T. Jones coll.)	30/01 – 03/02/2011	13°28.168'N 144°42.762'E	Assan	11-16
	30/01 – 03/02/2011	13°27.200'N 144°41.871'E	Masso	34
	30/01 – 03/02/2011	13°24.345'N 144°41.187'E	Aplacho	58-75
	30/01 – 03/02/2011	13°23.904'N 144°39.959'E	Namo	4
	30/01 – 03/02/2011	13°19.514'N 144°40.183'E	Ceti	197
	30/01 – 03/02/2011	13°17.117'N 144°39.955'E	Toguan	15-41
	30/01 – 03/02/2011	13°23.589'N 144°45.636'E	Ylig	2

DNA extraction, amplification and sequencing

For recent specimens, DNA was extracted from abdominal tissues using the semi-automatic Ependorf ep-Motion 5075 robot. Fragments of the mitochondrial 16S rRNA (~ 520 bp) were amplified using the primers 16Sa-L (CGCCTGTTTATCAAAAACAT) and 16Sb-H2 (CTCCGGTTTGAACTCAGATCA) (Palumbi, 1996). DNA amplification was performed in 25µl PCR reactions, containing approximately 3 ng of template DNA, 2.5 mM MgCl2, 0.26 mM of each nucleotide, 0.3 µM of each primer, 5% DMSO, 1 ng of BSA and 1.5 units of QBIOTAQ polymerase (MPBiomedicals). Amplification products were generated by an initial denaturation step of 4 min at 94°C followed by 35 cycles of denaturation at 94°C for 30s, annealing at 52°C for 40 s, extension at 72°C for 60 s and a final extension step at 72°C for 7 min.

For old collection specimens (Syntypes of *C. mertoni*), a CTAB protocol was used to extract DNA from pleopods. A shorter fragment of the 16S rRNA (332 bp) was amplified using two newly designed primers: 16S-Car-81F (AGGTAGCATAATAAATAGTC) and 16S-Car-413R (CTGTTATCCCTAAAGTAAC). DNA amplification was performed in 25µl PCR

reactions, containing 2.5 mM MgCl2, 0.26 mM of each nucleotide, 0.3 µM of each primer, 1 ng of BSA and 1.5 units of QBIOTAQ polymerase (MPBiomedicals). Amplification products were generated by an initial denaturation step of 4 min at 94°C followed by 45 cycles of denaturation at 94°C for 30s, annealing at 55°C for 30 s, extension at 72°C for 40 s and a final extension step at 72°C for 7 min.

PCR products were sequenced using same primers and in both directions to insure the accuracy of base calls. Chromatograms were edited using Geneious v.8 software (http://www.geneious.com/ Kearse et al, 2012). All sequences were deposited in GenBank (Numbers MG707138 to MG707171). Sequences for the species *Caridina brachydactyla*, *Caridina elongapoda* and *Paratya australiensis* published by Page et al (2007) were included in our analysis, *Paratya australiensis* sequence being used as outgroup.

Molecular analyses

DNA sequences were aligned using MEGA7 software (S. Kumar *et al.*, 2016) with Muscle algorithm (Edgar, 2004). Using Bayesian information criterion in PartitionFinder (Lanfear *et al.*, 2012) we retained the GTR + G + I model. Best-scoring ML trees were estimated using RAxML HPC2 v.8.2.10 (Stamatakis, 2014) implemented in the Cyber Infrastructure for Phylogenetic Research (CIPRES) portal v.3.1. (Miller *et al.* 2010) (https://www.phylo.org/). One hundred independent searches, each starting from distinct random trees, were conducted. Robustness of the nodes was assessed using non-parametric bootstrapping (Felsenstein, 1985) with 1,000 bootstrap replicates. We considered a group to be 'moderately supported' if it had a bootstrap support value (B) between 75 and 89% and 'highly supported' when B ≥ 90%.

To visualize genealogical relationships among haplotypes from our two sampling localities (Guam and Palau), a median-joining network was built using Network 4.1.1.2 (Bandelt *et al.*, 1999) with equal weights for variable sites. A maximum parsimony algorithm was applied to simplify the complex branching pattern and generate a network representing all of the most parsimonious intraspecific phylogenies.

Abbreviations for museums

Muséum national d'Histoire naturelle, Paris: MNHN; Naturhistorisches Museum Basel, Basel: NMB; National Museum of Natural History, Smithsonian Institution, Washington, DC: NMNH; Rijksmuseum van Natuurlijke Historie (now in the Naturalis Biodiversity Center, Leiden): RMNH; Bernice Pauahi Bishop Museum, Honolulu: BPBM; Zoological Research Collection, Singapore: ZRC.

Abbreviations for morphological analyses

The following abbreviations are used in the present text: cl, carapace length (mm): measured from the post-orbital margin to the posterior margin of the carapace. P1: first pereiopod. P2: second pereiopod. P3: third pereiopod. P5: fifth pereiopod. Pl1: first pleopod. Pl2: second pleopod.

Morphological comparison

The rostrum, the general cephalon, the pereiopods 1, 2, 3 and 5 and the abdomen were observed using a stereoscopic microscope. The proportions of the various joints of the appendages were measured using microphotographs and the AnalySIS Works software (Olympus). Drawings were made using the "Digital Inking" method (Coleman, 2004; 2006) by tracing vectorial paths on stacks of high-resolution photographs using Adobe Illustrator (CS6) and a WACOM PTZ-1230 graphic tablet.

PCA for some museum specimens

Some museum specimens, for which DNA could not be retrieved for a genetic analysis, but still having all their pereiopods, were measured and included in a PCA with our recent sequenced specimens and old specimens that yielded DNA. We followed the method proposed by Konan *et al.* (2010) using Statistica v.10 software (Statsoft). The same 19 ratios and counts used in previous work (Mazancourt *et al.*, 2017a) were used for the analysis.

Old specimens without genetic data included in the analysis:

Palau. Holotype USNM 105430 *C. brachydactyla uncata* one ovigerous female cl 3.5mm;

Mariana Islands. MNHN-IU-2015-1824 *C. brachydactyla* (A. Marche coll.)

Indonesia. Aru Island, Syntypes *C. mertoni*. NMB 693a one male cl 2.7 mm; NMB 693b one male 3.1 mm; NMB 693c one non-ovigerous female, Flores Island, Lectotype *C. brachydactyla* ZMA 977 one ovigerous female cl 4.8 mm, Sulawesi, Paralectotypes ZMA 2552 two ovigerous female cl; 5.3-5.4 mm.

4.3.4 Results

Phylogenetic analyses

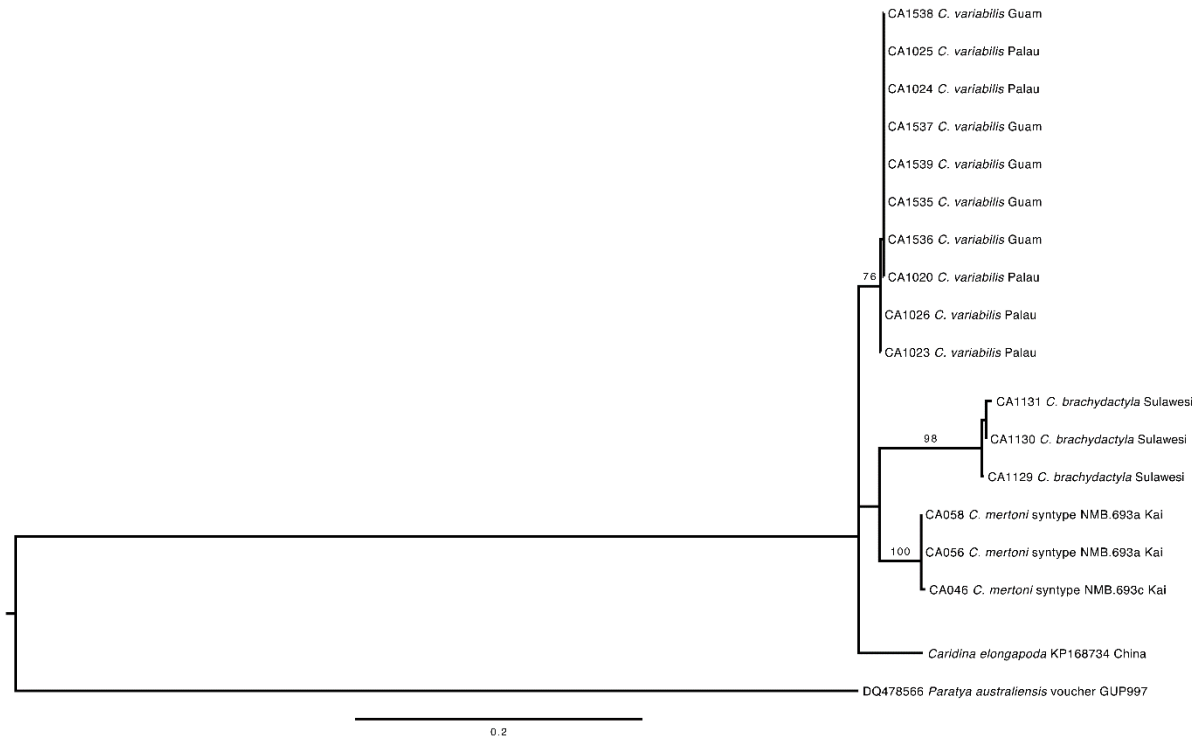


Figure 4.3.2: Maximum likelihood phylogenetic tree (16S mtDNA) of the specimens. CAXXXX numbers are lab IDs of the specimens.

A total of 31 recent specimens were sequenced, including 23 of the new species (10 from Guam, 13 from Palau), 5 recent specimens of *C. mertoni* from Kolombangara Island, 3 specimens of *C. brachydactyla* from type locality, Sulawesi and a shorter fragment could be retrieved from 3 old collection specimens (*C. mertoni* syntypes from Kai Island). All of the specimens from Palau and Guam fall in a same moderately supported clade, with little genetic structure and are well distinct from *C. brachydactyla* specimens and *C.*

mertoni syntypes. We considered this genetic group to be a new species that we describe below (Figure 4.3.2).

Haplotypes network

There is a difference in haplotype diversity between the two localities. Indeed, there are 9 haplotypes found in Palau (vs only one in Guam). One of the haplotypes, also the most frequent, is shared between the two islands (Figure 4.3.3).

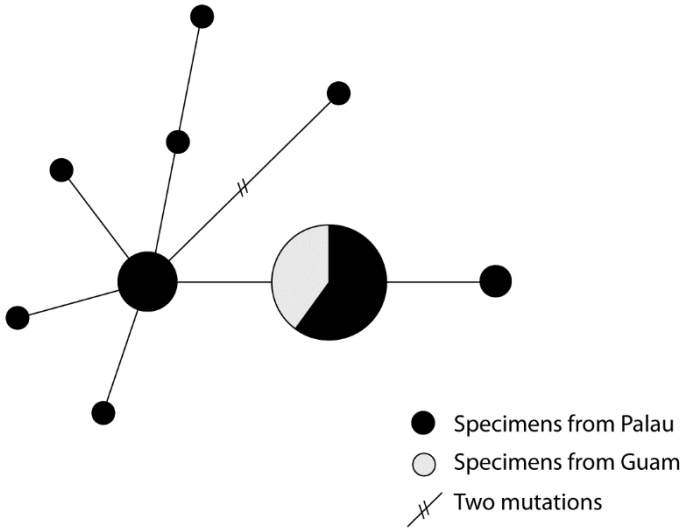


Figure 4.3.3: Haplotypes network of the specimens sequenced

Taxonomy

Atyidae De Haan, 1849

***Caridina* H. Milne Edwards, 1837**

Caridina variabilis, n. sp.

(Fig. 4.3.4)

Material examined.

Holotype: **PALAU.** MNHN-IU-2017-2082 (DNA: CA1026) 1♂ cl 3.4 mm, Tabecheding River 27/02/2011, 07°27.169N, 134°31.748E.

Paratypes: **PALAU.** One male cl 2.4 mm (MNHN-IU-2017-2083, DNA: CA1032) and three non-ovigerous females cl 4.1 mm (MNHN-IU-2017-2084 DNA: CA1029) 4.8 mm (MNHN-IU-2017-2085 DNA: CA1025) & cl. 4.9 mm (MNHN-IU-2017-2086 DNA: CA1030), Tabecheding river 27/02/2011, 07°27.169N, 134°31.748E. 2♀ ovig cl 4.0 mm (MNHN-

IU-2017-2087 DNA: CA1149) & 4.4 mm (MNHN-IU-2017-2088 DNA: CA1148), Ngerchokl River 27/02/2011, 07°36.527N, 134°36.958E. 1♀ ovig cl 3.9 mm (MNHN-IU-2017-2089 DNA: CA1111), Kirelauch River 28/02/2011, 07°41.417N, 134°37.754E. 2♂ cl 3.2 mm (MNHN-IU-2017-2090) & 3.3 mm (MNHN-IU-2017-2091), West Coast stream Northern Tip 28/02/2011, 07°43.056N, 134°36.924E. 1♀ cl 3.8 mm (MNHN-IU-2017-2092 DNA: CA1106) and 2♀ ovig cl 4.2 mm (MNHN-IU-2017-2093 DNA: CA1104) & 3.8 mm (MNHN-IU-2017-2094 DNA: CA1107), small stream East Coast 07°29.909N, 134°38.099E. 1♀ ovig cl 4.2 mm (MNHN-IU-2017-2095 DNA: CA1020), Ngermeskang River 01/03/2011, altitude: 75m, 07°32.484N, 134°34.670E. 1♀ ovig cl 4.4 mm (MNHN-IU-2017-2096 DNA: CA1203), Ngardmau Waterfall 02/03/2011, 07°35.482N, 134°35.573E. 1♀ cl 3.6 mm (MNHN-IU-2017-2097 DNA: CA1204), Mesekelat River 03/03/2011, 07°26.305N, 134°34.321E.

PALAU. Not *C. b. uncata* Holthuis: USNM 105430: holotype, 1♀ ovig cl 3.5 mm Arakitaoch River; USNM 105434: paratype, 1♀ cl 4.4 mm.

Not *C. brachydactyla*: USNM 172589: 1♂ cl 2.5 mm and 1♀ cl 3.0 mm; USNM 172590: 1♀ cl 5.5 mm.

GUAM. Assan River: DCR (D. Christopher Rogers collections) 9♂ cl 2.7 mm (MNHN-IU-2017-2098 DNA: CA1535), 3.5 mm (MNHN-IU-2017-2099 DNA: CA1536), 2.3 mm (MNHN-IU-2017-2100 DNA: CA1537), 2.7 mm (MNHN-IU-2017-2101 DNA: CA1539), 2.8 mm (MNHN-IU-2017-2102 DNA: CA1540), 2.4 mm (MNHN-IU-2017-2103 DNA: CA1541), 2.2 mm (MNHN-IU-2017-2104 DNA: CA1542), 2.7 mm (MNHN-IU-2017-2113 DNA: CA1543), 2.3 mm (MNHN-IU-2017-2105 DNA: CA1544) and 1♀ ovig cl 4.5 mm (MNHN-IU-2017-2106 DNA: CA1538). BPBM 4227 1♀ ovig cl 4.4 mm; 13° 28' 18.24"N, 144° 42' 48.66"E; 03/02/2011; DCR: 14♂ cl 2.1-3.4 mm, 6♀ cl 2.8-3.3 mm, and 4♀ ovig cl 3.4-4.3 mm; 13° 28' 18.24"N, 144 ° 42' 48.66"E. 02/02/2011. Masso River: DCR: 9♂ cl 2.2-3.6 mm, 3♀ cl 2.7-3.2 mm, and 13♀ ovig cl 3.4-4.5 mm; 13° 27' 32.85"N, 144° 41' 54.26"E; 1 February 2011. Namo River: 2♂ cl 2.4-2.8 mm, 2♀ cl 2.6-3.0 mm, and 11♀ ovig cl 3.4-4.2 mm; 13° 23' 58.59"N, 144° 39' 58.81"E; 01/02/2011. DCR: 6♂ cl 2.4-3.1 mm; 13° 23' 57.16"N, 144° 40' 05.22"E; 02/02/2011. Ceti River: 1♀ cl 2.3 mm, and 7♀ ovig cl 3.1-4.1 mm; 13° 19' 34.66"N, 144° 40' 09.12"E; 31/01/2011. Toguan River: 29♂ cl 2.0-3.4 mm, 16♀ cl 2.4-3.5 mm, and 34♀ ovig cl 3.5-4.3 mm; 13° 17' 17.94"N, 144° 39' 55.77"E; 31/01/2011. DCR: 8♂ cl 2.2-3.4 mm, 1♀ cl 2.6 mm, and 9♀ ovig cl 3.6-4.0 mm;

13° 17' 15.79"N, 144° 40' 02.51"E; 02/02/2011. Ylig River: 1♂ cl 2.4 mm, and 3♀ ovig cl 3.3-4.1 mm; 13° 23' 46.21"N, 144° 45' 54.85"E; 30/01/2011.

MARIANA ISLANDS (Island not specified). Not *C. brachydactyla*: MNHN-IU-2015-1824: 2♀ ovig cl 4.2-5.2 mm.

Comparative material

- *Caridina brachydactyla* De Man, 1908

Type material: **INDONESIA:** *Caridina nilotica* var. *brachydactyla*: lectotype, Flores, Reo, RMNH Crust D. 977, 1♀ ovig cl 4.8 mm; paralectotypes Flores, near Mbawa, RMNH 2552, 2♀ ovig (cl 5.3-5.4 mm).

Other material: **INDONESIA:** *C. brachydactyla*: NMB 1054a, Bali, 1♀ cl 5.8 mm. Sulawesi, Palopo, Macaui, 63.10, 2♂ cl 2.7-3.7 mm; 1♀ ovig cl 4.3 mm and 1♀, cl 4.8 mm; Palopo, Tojo, 64.10.3 1♀ ovig cl 4.0 mm.

- *Caridina mertoni* Roux, 1911

Type material: **INDONESIA**; Cotypes: Grand Kai, NMB 693a: 2♂ cl 2.7-3.8 mm and 1♀ cl 4.1 mm; NMB 693b: 1♂ cl 3.1 mm and 1♀ ovig cl 4.8 mm; NMBc 1♀ cl 4.3 mm. MNHN-IU-2015-1819 1♂ cl 3.8 mm; MNHN-IU-2015-1820 1♂ cl 3.4 mm.

Other material: **SOLOMON ISLANDS**: Kolombangara. 3♂ cl 3.9 (MNHN-IU-2017-2107 DNA: CA1506), 3.0 mm (MNHN-IU-2017-2108 DNA: CA1507), 3.7 mm (MNHN-IU-2017-2109 DNA: CA1505), 1♀ ovig cl 3.9 mm (MNHN-IU-2017-2110), Manolu River 10/11/2015 08°05.312S 157°00.813E; 1♀ cl 6.2 mm (MNHN-IU-2017-2111 DNA: CA1514) and 1♀ ovig cl 4.1 mm (MNHN-IU-2017-2112 DNA: CA1517), Sulumuni River 12/11/2015 08°02.253'S 157°09.257'E.

- *Caridina elongopoda* Liang & Yan, 1977

HONG-KONG: Pak Tam Chung and Kai Sai Chau, 3♂ cl 2.8-3.5 mm and 1♀ cl 4.2 mm. W. Klotz coll. **MALAYSIA:** ZRC 1998.0865 Pulau Tioman, Sungai Asah, 24/06/1997 1♂ cl 4.2 mm, 1♀ cl 4.3 mm, 1♀ ovig cl 4.3 mm Ng et al. coll..

- *Caridina peninsularis* Kemp, 1918

Type material: *Caridina brachydactyla* subsp. *peninsularis* Kemp, 1918: **MALAYSIA;** lectotype Botanical garden, Penang Island, coll. N. Annandale, Feb. 1916, MNHN-IU-2015-1749: 1♂ cl 3.2 mm; paralectotypes same data as lectotype, MNHN-IU-2015-1750: 1♀ ovig cl 5.4 mm; NHM 1919.11.1.12-21 (1761124): 1♀ cl 3.4 mm; 1♀ ovig cl 5.2 mm; 1♂ cl 3.9 mm.

Other material: **SINGAPORE;** Tanglin (incorrectly spelt Tangtum in NHM register and on label, see Richards & Clark, 2014), coll. Bedford & Lanchester, det. D.S. Johnson, 1958, NHM reg. 1958.8.7.14–17 (1749569): 1♀ ovig cl 5.1 mm; 1♂ cl 4.2 mm.

Description

Cephalothorax. Rostrum (Fig. 4.3.4m): very variable in length, 0.7-1.5 of cl, reaching to scaphocerite apex, armed dorsally with 16-24 teeth, distal unarmed portion 0.1-0.7 times that of armed portion, with one or two subapical teeth, 1-4 teeth on carapace posterior to orbital margin, ventral margin with 4-15 teeth. Number of dorsal teeth before the most proximal ventral tooth 10-17. Rostral formula: (16-24 (1-4) + 1-2 / 4-15). Antennular spine acute, placed slightly below orbital angle. Pterygostomian margin rectangular.

Eyes well developed, anterior end reaching to 0.7 length of antennular peduncle basal antennomere. *Antennular peduncle* (Fig. 4.2.4m, n, cephalothorax view, and o, general view) 0.9 times as long as carapace. Anterolateral angle reaching 0.29 length of the second antennomere; second antennomere distinctly longer than third. Stylocerite reaching to 0.8 length of antennular peduncle basal antennomere. Scaphocerite extending slightly beyond antennular peduncleapex, length about 2.8 times width. Scaphocerite anteriolateral spine well developed, about level with antennular peduncle distal margin, both overreached by the lamella. Antennal peduncle near the base of the scaphocerite with a strong and slender lateral spine.

Mandibles dimorphic; left mandible (Fig. 4.3.4q) more developed, corpus large, robust with three strong sharp teeth separated by ridged gap; incisor and molar processes separated by two patches of long setae; molar process ridged. Right mandible (Fig. 4.3.4p) with six sharp incisor teeth, medially with two groups of long setae; molar process narrow, elongate, ridged. Maxillula (Fig. 4.3.4r). Lower lacinia with margin

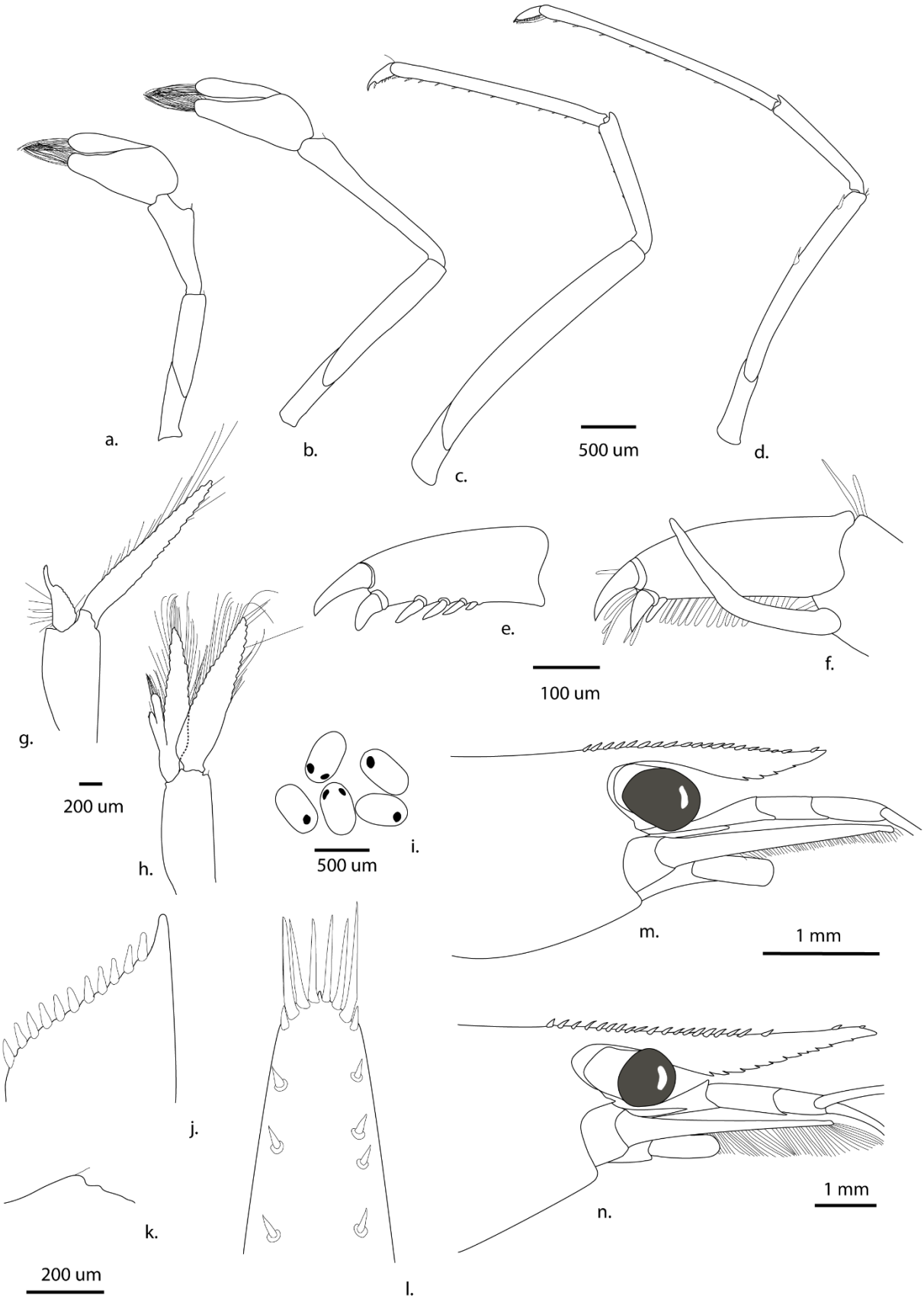


Figure 4.3.4: a. First pereiopod; b. Second pereiopod 2; c. Third pereiopod; d. Fifth pereiopod; e. Dactylus of third pereiopod; f. Dactylus of fifth pereiopod; g. Male first pleopod; h. Male second pleopod; i. Eggs; j. Uropodial diaeresis; k. Pre-anal carina; l. Telson; m., n. Cephalothorax (to be continued next page).

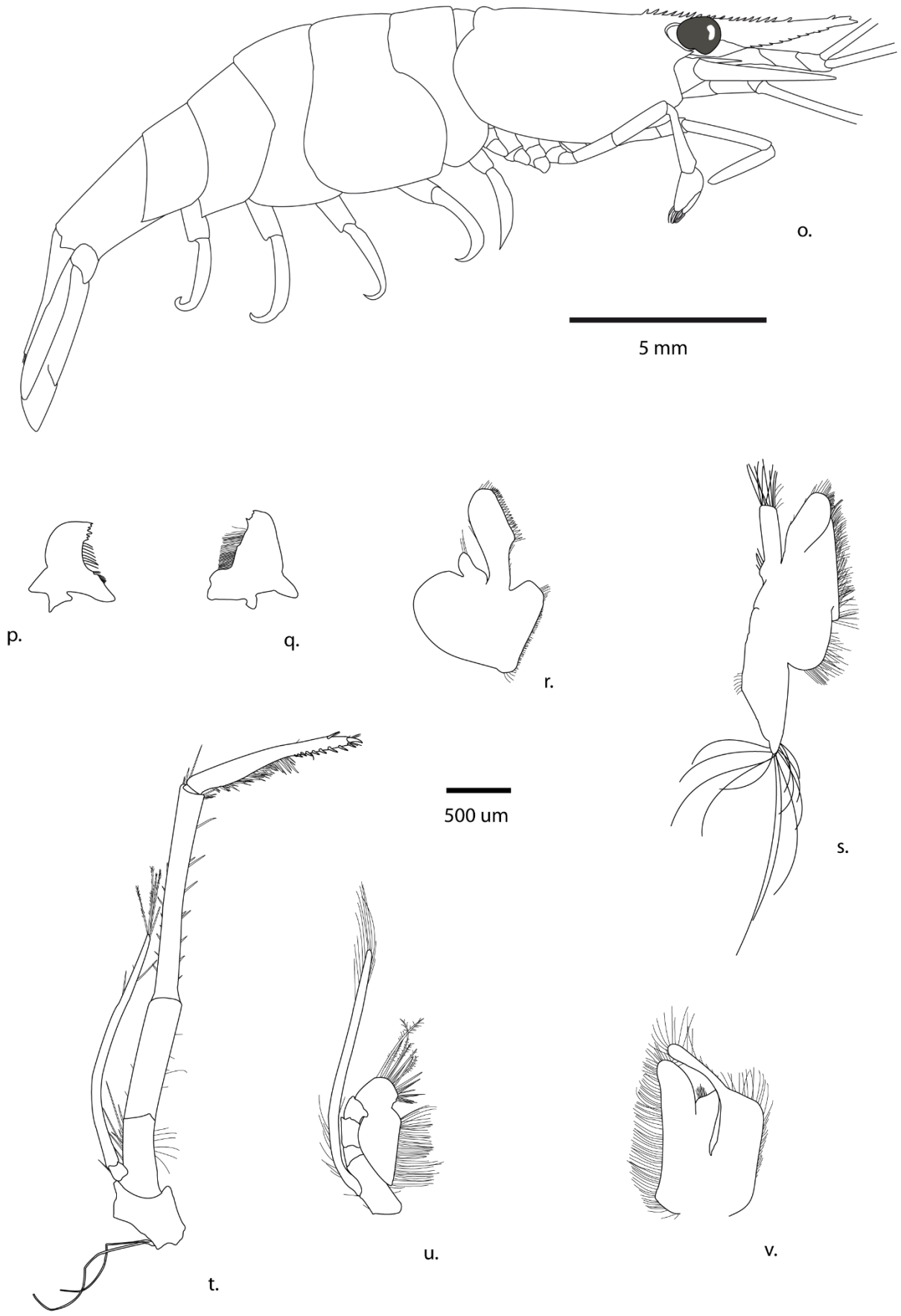


Figure 4.3.4 (continued) : o. General appearance; p. Right mandible; q. Left mandible; r. First maxilla; s. Second maxilla; t. Third maxilliped; u. Second maxilliped; v. First maxilliped.

broadly rounded, bearing several rows of setae. Upper lacinia elongate, with medial margin bearing a number of distinct teeth, palp setose.

Maxilla (Fig. 4.3.4s) upper and middle endite with marginal and submarginal plumose setae. Lower endite with simple setae; palp narrow, shorter than upper endite cleft with a few setae. Scaphognathite fringed with long setae, tapering posteriorly with some long, curved setae at posterior end.

First maxilliped (Fig. 4.3.4v) endopodite ultimate segment medial margin with long denticulate setae, lower portion with transverse rows of simple setae. Palp elongate, setose. Exopod flagellum long and narrow distally with marginal plumose setae. Caridean lobe large, with marginal setae.

Second maxilliped (Fig. 4.3.4u) endopodite ultimate and penultimate antennomeres fused, reflected against basal antennomeres. Ultimate, penultimate and basal antennomeres medial margins with long setae of various types; flagellum very long, slender with marginal plumose setae distally; podobranch well developed with reduced branchial lamella.

Third maxilliped (Fig. 4.3.4t) with terminal article reaching third antennular peduncle antennomere apex; distal antennomere about 10 times as long as wide, slightly shorter than the penultimate, ending in large hamulate apical spine surrounded by simple setae. Penultimate antennomere about eight times as long as wide, with group of transverse rows of simple setae. Exopod flagellum well developed, about a third the length of endopodite second article, distal margin with long plumose setae.

Pereiopods. Pereiopods I-IV with epipods.

P1 (Fig. 4.3.4a): chela about 1.8-2.3 times as long as wide, movable finger 3.1-4.6 times as long as wide, 1.1-1.7 times length of palm; carpus 2.1-3.5 times as long as wide.

P2 (Fig. 4.3.4b) more slender and longer than first pereiopod with chela 2.1-3.0 times as long as wide: movable finger 4-5.4 times as long as wide, 1.3-1.9 times length of palm; carpus slender 5.2-7.8 times as long as wide.

P3 (Fig. 4.3.2c): slender, dactylus (Fig. 4.3.4e) 2.8-4.0 times as long as wide, including terminal spine), ending in two strong claws, flexor margin with 5-8 spines in parallel to

the terminal spine; propodus 14.1-24.7 times as long as wide, 4.5-7.3 times as long as dactylus.

P5 (fig. 4.3.4d): dactylus (Fig. 4.3.4f) 3.0-4.3 as long as wide, ending in two large claws, with a long distal propodus seta and with 13-30 spines on flexor margin; propodus 15.4-32.0 times as long as wide, 5.1-7.9 times as long as dactylus.

Abdomen. Third abdominal somite (Fig. 4.3.4o) with moderately convex dorsal profile. Sixth abdominal somite about 0.65 of carapace, 2.1 times as long as fifth somite, slightly shorter than telson. Telson (Fig. 4.3.4l) three times as long as wide, with four pairs of dorsal spinules and one pair of dorsolateral spinules; posterior margin, with or without a median process, exhibits variations. Process mostly triangular with four to five intermediate spines shorter or subequal to lateral spines, rarely rounded, with ten intermediate subequal spines.

Pl1 (Fig. 4.3.4g): Endopod foliform with a developed appendix interna in males.

Pl2 (Fig. 4.3.4h): Appendix masculina on second pleopod reaching 0.62 times length of endopod; appendix interna reaching about 0.58 times appendix masculina length.

Preanal carina (Fig. 4.3.4k) unarmed.

Uropodal diaeresis (Fig. 4.3.4j) with 10-14 spinules.

Egg size (Fig. 4.3.4i): 0.41-0.48 × 0.24-0. 31 mm.

Type Locality

Palau, Babeladob Island, Tabecheding River, 07°27.169N, 134°31.748E.

Habitat

This species is found in rivers from lower to higher elevations in flowing water between riffles. The specimens are found in roots, leaf pack or aquatic macrophytes (in Guam).

Etymology

The new species is named *variabilis* for its variable rostrum length.

Colour pattern

Unknown

Distribution

This new species has only been found in Guam and Babeldaob islands so far.

Remarks

Caridina variabilis n. sp. is most similar to *C. mertoni*, *C. brachydactyla*, *C. elongapoda* Liang & Yan, 1977 and *C. peninsularis* Kemp, 1918. This new species displays a variable rostrum length among specimens. When the rostrum is short the general appearance is like *C. mertoni*, whereas when the rostrum is long the general appearance is of *C. brachydactyla*. In *C. variabilis* n. sp. the antennal spine is placed below the orbital angle, the P5 dactylus is biunguiculate with 13-30 setae vs. 32-42 in *C. brachydactyla* and there is a very large propodus seta, whereas in *C. mertoni*, the antennal spine is somewhat fused with the orbital angle, possesses an unguiculate dactylus with 30-40 setae and has no prominent propodus seta. *Caridina variabilis* n. sp. differs from *C. brachydactyla* by the absence of spine on the preanal carina, a shorter distal unarmed portion of the rostrum 0.1-0.7 (vs 0.6-1.6) times that of armed portion, a shorter unarmed portion of the rostrum 0-0.35 (vs 0.3-0.8) times that of the armed portion, by a longer P5 propodus, which is 5.1-7.9 time as long as the dactylus (vs 3.9-5.7) and a lower number of spines (13-30) on the P5 dactylus (32-42 in *C. brachydactyla*).

This new species differs from *C. elongapoda* with the preanal carina lacking a spine. But, the P3 propodus is longer 14.1-24.7 (vs 10.8-17.0), the P5 dactylus shorter 3.0-4.3 (vs 4.3-5.6) with fewer setae on the P5 dactylus 13-30 (vs 33-44) and with larger eggs 0.41-0.48 × 0.24-0. 31 mm (vs 0.39-0.40 × 0.22-0.23).

It also differs from *C. peninsularis* by a lower number of dorsal rostrum teeth (16-24 vs 20-35), a shorter P3 dactylus (2.8-4.0 vs 4.4-5.8) and a P5 dactylus biunguiculate with 13-30 setae (vs unguiculate and 25-39 setae).

PCA for some museum specimens

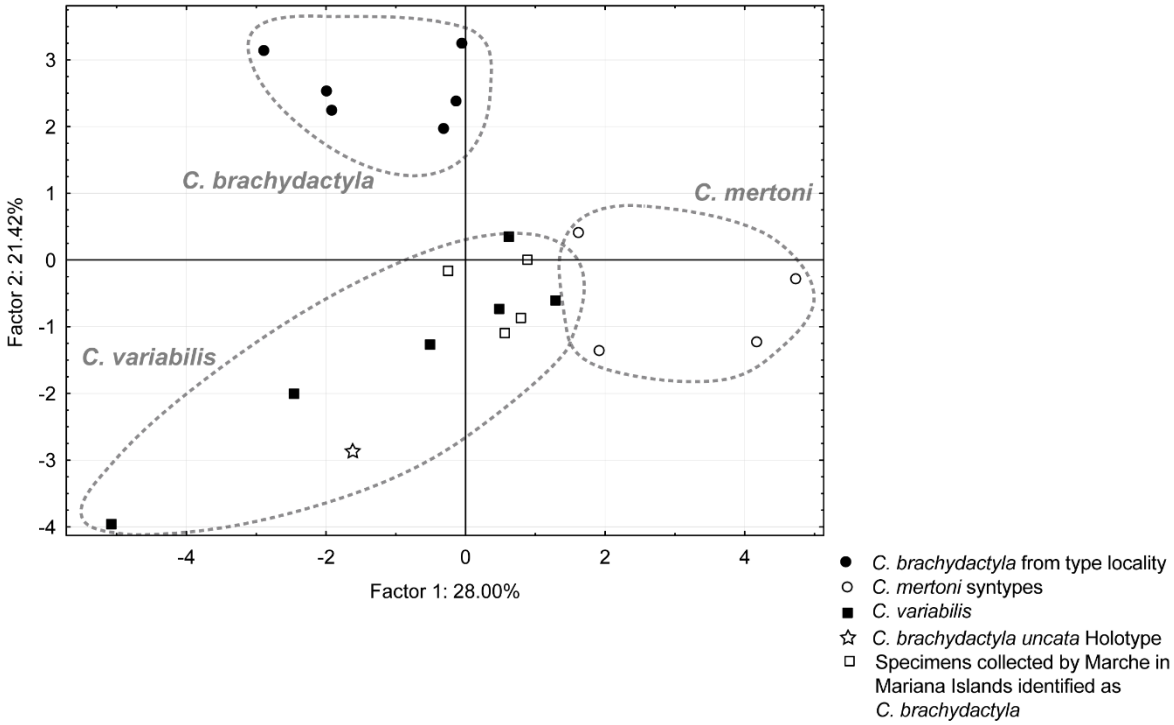


Figure 4.3.5: Scatterplot of the specimens projected on a plane defined by the first two factors of the PCA.

The first two principal components (Fig. 4.3.5) accounted for 49.42% of the total variance (28% for Factor 1 and 21.42% for Factor 2). With the first component, *C. mertoni* specimens are well differentiated from the two other groups. With the second component, *C. brachydactyla* specimens are well differentiated from the two other groups. Factor 1 was mainly correlated with variables related to the second pereiopod (Carpus length-width ratio, score: -0.166; Dactylus length-width ratio, score: -0.149; Chela length-width ratio, score: -0.146), the rostrum (Rostrum length to Carapace length ratio, score: -0.152) and the first pereiopod (Chela length-width ratio, score: -0.150). Factor 2 was mainly correlated with variables related to the fifth pereiopod (Propodus length to dactylus length ratio, score: -0.211; Propodus length to width ratio, score: -0.197), the rostrum (Unarmed dorsal part to armed dorsal part ratio, score: 0.157; Number of dorsal spines, score: -0.152) and the first pereiopod (Dactylus length to propodus length ratio, score: -0.155; Carpus length to width ratio, score: -0.154) (Table 4.3.2).

Table 4.3.2: Contribution of the factors to the axes of the PCA.

	Factor 1	Factor 2
P3pd	-0,017046124	-0,126414132
P3d	-0,092709362	-0,057535322
P3p	-0,089083174	-0,130935196
P1d	-0,1185254	-0,056499047
P1ch	**-0,150481595**	0,100526984
P1ca	-0,098238878	**-0,154429328**
P1dp	-0,006782501	**-0,15497317**
P2d	**-0,148922607**	0,028800419
P2ch	**-0,145582132**	0,119759603
P2ca	**-0,16555737**	-0,10088558
P2dp	0,059041331	-0,041298277
P5pd	0,018408878	**-0,211356127**
P5d	-0,068281129	0,055620433
P5p	-0,048772544	**-0,197269865**
RC	**-0,151842227**	-0,003800791
Rndd	-0,108112767	**0,157452229**
Dd	0,029568368	**-0,152496848**
Dv	-0,134018739	0,003451246

4.3.5 Discussion

Holthuis (Bayer & Fehlmann, 1960) identified some specimens of this species from Palau as *C. brachydactyla uncata* assigning types, but never described the taxon nor published the name. As these specimens were not described, we chose another name for this species and to stabilize the taxonomy, we chose to designate as holotype the specimen MNHN-IU-2017-2082 from which a DNA sequence was obtained (GenBank n° MG707146).

During the surveys of Palau and Guam, some specimens showing a long rostrum were initially attributed morphologically to *Caridina brachydactyla* De Man, 1908 whereas others, showing a short rostrum, were initially identified as *Caridina mertoni*

Roux, 1911. In fact all specimens belonged to the new species described here. Indeed rostrum length, a character widely used in *Caridina* taxonomy might not be as reliable as it was thought, depending on the species, as it is highly plastic and varies with environmental parameters (Mazancourt *et al.*, 2017a).

Thereby, we think that a new species description must be inevitably accompanied by a genetic sequence. Informative morphological features must be linked to molecular characters.

Despite the long distance (1296 km) between Babeldaob and Guam, our analyses showed that some haplotypes are shared (Fig. 4.3.4), and thus, genetic exchanges occur between populations from these two islands. These exchanges agree well with the geological history of the islands (Kobayashi *et al.* 2004). Formerly (Fig. 4.3.6, left), Palau was close to Guam, aligned to form the proto-Kyushu Palau Ridge, which was an active volcanic chain at a period from 40 to 30 Ma (Fig. 4.3.6 left). Between 30 to 15 Ma (Fig. 4.3.6, center), the Shikoku Parece Vela Basin opened, its spreading centre remains as the extinct Parece Vela Rift situated at the centre of the basin. Consequently, the Mariana Islands, including Guam, drifted eastwards whereas Palau remained, with active volcanism. Later, by a complex movement of tectonic plates, Guam moved further away from Palau (Fig. 4.3.6, right) to its current location (1296 km northeast).

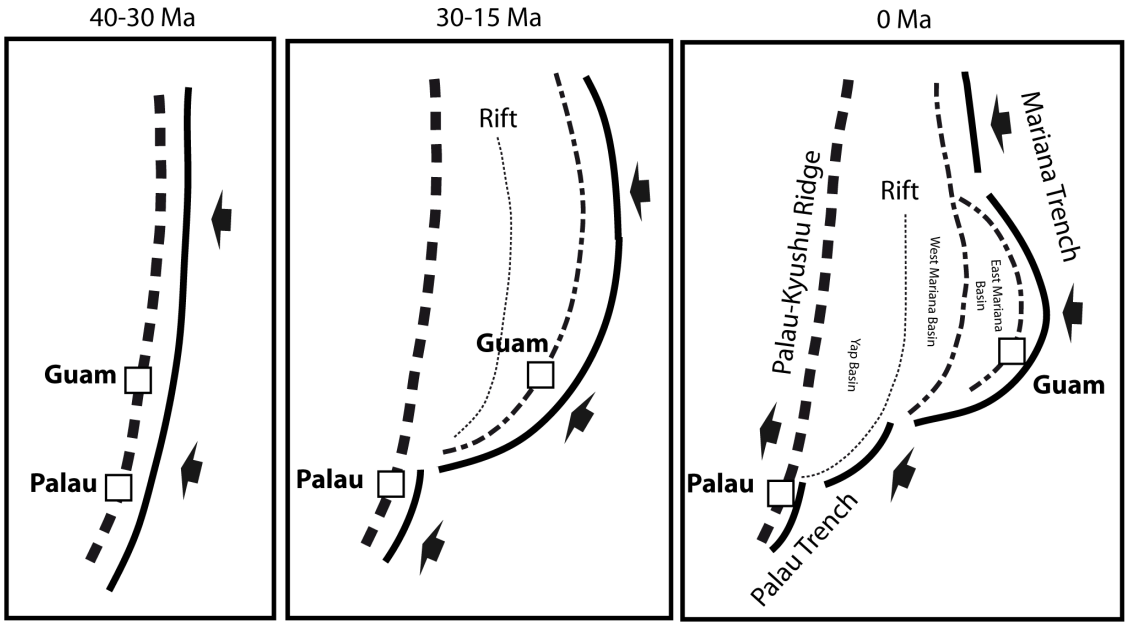


Figure 4.3.6: Schematic representation of the geological history of Micronesia (redrawn from Kobayashi *et al.* 2004).

4.3.6 Acknowledgements

The authors wish to thank Dr Urs Wuest and Edi Stöckli (NMB), Paula Martin-Lefèvre and Dr Laure Corbari (MNHN), Dr Rafael Lemaitre (USNM), Dr Charles Fransen and Karen van Dorp (ZMA), Dr Holly Bollick (BPBM) and Dr Werner Klotz for providing the specimens used in this study. We would like to thank the Ministry of Natural Resources, Environment, & Tourism Koror, Republic of Palau, and in particular Helena Rebelkuul from the Bureau of Marine Resources, and Kashgar Rengulbai (and his assistant Gwen) from the Bureau of Agriculture (BOAGRI), for their help with processing the research permits. Kashgar's assistance with some aspects of the logistics in the field was invaluable. He has been for us a wonderful guide and an incredible source of knowledge on the biodiversity of Palau and Babeldaob in particular. He also facilitated the organisation of various meetings with a number of local stakeholders, including Dr Yimnang Golbuu from the Palau International Coral Reef Centre, staff at the Environmental Quality Protection Board, at the Palau Conservation Society, at the Coral Reef Research Foundation and at The Nature Conservancy. We are also grateful to the staff from BOAGRI who accompanied us on the Nergmeskang River survey. Finally, a special thought to G. Bright, P. Gerbeaux, M. Castelin and L. Taillebois. We are grateful to Tahzay Jones and Anne Farahi of the United States of America National Park Service for funding DCR's trip to Guam. We are especially grateful to Brent Tibbatts of the Guam Department of Agriculture, Division of Aquatic and Wildlife Resources for permits, help in the field and advice on good collection locations. Many thanks to Jasmine Richard and Kristina von Rintelen for their helpful comments on the manuscript.

4.4 Description de deux nouvelles espèces de *Caridina* en Micronésie (partie 2 : *C. variabilirostris*)

European Journal of Taxonomy, 453, 1-16

Caridina variabilirostris (Crustacea: Decapoda: Atyidae) a new species of freshwater shrimp from Pohnpei (Micronesia).

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4.4.1 Abstract

Recently, the status of a new species of atyid shrimp from Pohnpei (Micronesia) was discussed in relation to *C. brachydactyla* De Man 1908 and *C. mertoni* J. Roux, 1911. By combining morphological data with a phylogenetic analysis with closely related species, this species is here described as *Caridina variabilirostris* sp. nov. Notes on its ecological distribution are also provided. The new species is characterized by a highly variable rostrum and is present in rivers all over Pohnpei Island. The status of this new species is clarified and it is shown that neither *C. brachydactyla* De Man 1908 nor *C. mertoni* J. Roux, 1911 occur on Pohnpei Island.

Key words: 16S, Molecular, Integrative taxonomy, Island, Morphology

4.4.2 Introduction

Micronesia is a vast expanse of more than 2,000 Pacific islands and atolls stretching from the Caroline and Mariana islands in the West, to the Marshall, Nauru and Kiribati islands in the East. The Caroline Islands consist of a chain of seamounts, atolls and high islands extended southeastward to the Marshall Islands. The high island of Pohnpei (formely known as Ponape) is situated at 6°54'N latitude and 158° 14'E longitude (Fig. 4.4.1). It belongs to Pohnpei State, one of the four states in the Federated State of Micronesia. The island of Pohnpei covers 345 km^2. The highest elevation point is 791m. Aged of 8.7 Ma, this volcanic island was not only the remnant of a hotspot trace but was also produced in a fracture-induced subduction-related tectonic environment (Rehman *et al.*, 2013).

The freshwater shrimp genus *Caridina*, comprising 298 species (WoRMS database, as of May 2018) and mostly present in the Indo-Pacific region, is the most diversified genus of the Atyidae (De Grave *et al*. 2015) and an important ecological component in the tropical streams (Covich *et al*. 1999; Pringle *et al*. 1993). Its high diversity combined with the lack of informative morphological characters has led to a confused taxonomy (Richard & Clark 2009). Indeed, until recently, the taxonomy of the genus was mainly based on morphological characters. Some have been proven highly variable within a species (e.g. rostrum shape and indentation or coloration) and so taxonomically non-informative, making it difficult to establish boundaries between species (Rintelen & Cai 2009; Mazancourt *et al.* 2017a). Thus, there is a need for an integrative and standardized approach to improve the group's systematics, focusing on informative morphological features and using molecular characters (Page *et al*. 2005; Page & Hughes 2011).

Maciolek & Ford (1987) recorded *Caridina brachydactyla* De Man, 1908 among seven Atyidae species in Pohnpei rivers. Later this species was not collected neither by Nelson *et al*. (1966) nor by Buden *et al.* (2001). Keith *et al.* (2012), reviewing the freshwater decapods of Ponhpei conducted a survey from 7th March through 17th March 2012, identified the *Caridina* material belonging to *C. nilotica* complex as *C. brachydactyla* or *C. mertoni* Roux, 1911 (following the previous identifications made from Guam, another Micronesian island by (Leberer & Cai, 2003).

One of the aims of the Muséum national d'Histoire naturelle (MNHN) in Paris is to carry out faunistic inventories of rivers in tropical islands in order to establish a better protection of these fragile ecosystems and, in this context, to clarify taxonomy of poorly known organisms. As we examined more and more specimens from Pohnpei, we gradually started to question the validity of some species from this island. Consequently, we here re-examined our specimens collected in 2012 in combining morphological data with a 16S mtDNA analysis and found out that neither *C. brachydactyla*, nor *C. mertoni* occur in Pohnpei but are confused until now with one undescribed species. Recently, Mazancourt *et al.* (2017a) highlighted the "Pinocchio-shrimp effect", in this species, which has a variable length of the rostrum depending on the altitude.

Detailed description of this new species is given as well as its ecological distribution. The position of this species is clarified by comparing it with *C. brachydactyla* and *C. mertoni.*

4.4.3 Materials and methods

***Collection of specimens*.**

Rivers and sites surveyed in March 2012 are indicated in Figure 4.4.1 and Table 4.4.1. Specimens from Pohnpei were collected by electrofishing (portable Dekka 3,000 electric device, Germany). All material preserved in 75%-95% alcohol has been deposited in the collections of the Muséum national d'Histoire naturelle in Paris (MNHN, specimens n° MNHN-IU-2018-231 to MNHN-IU-2018-256)

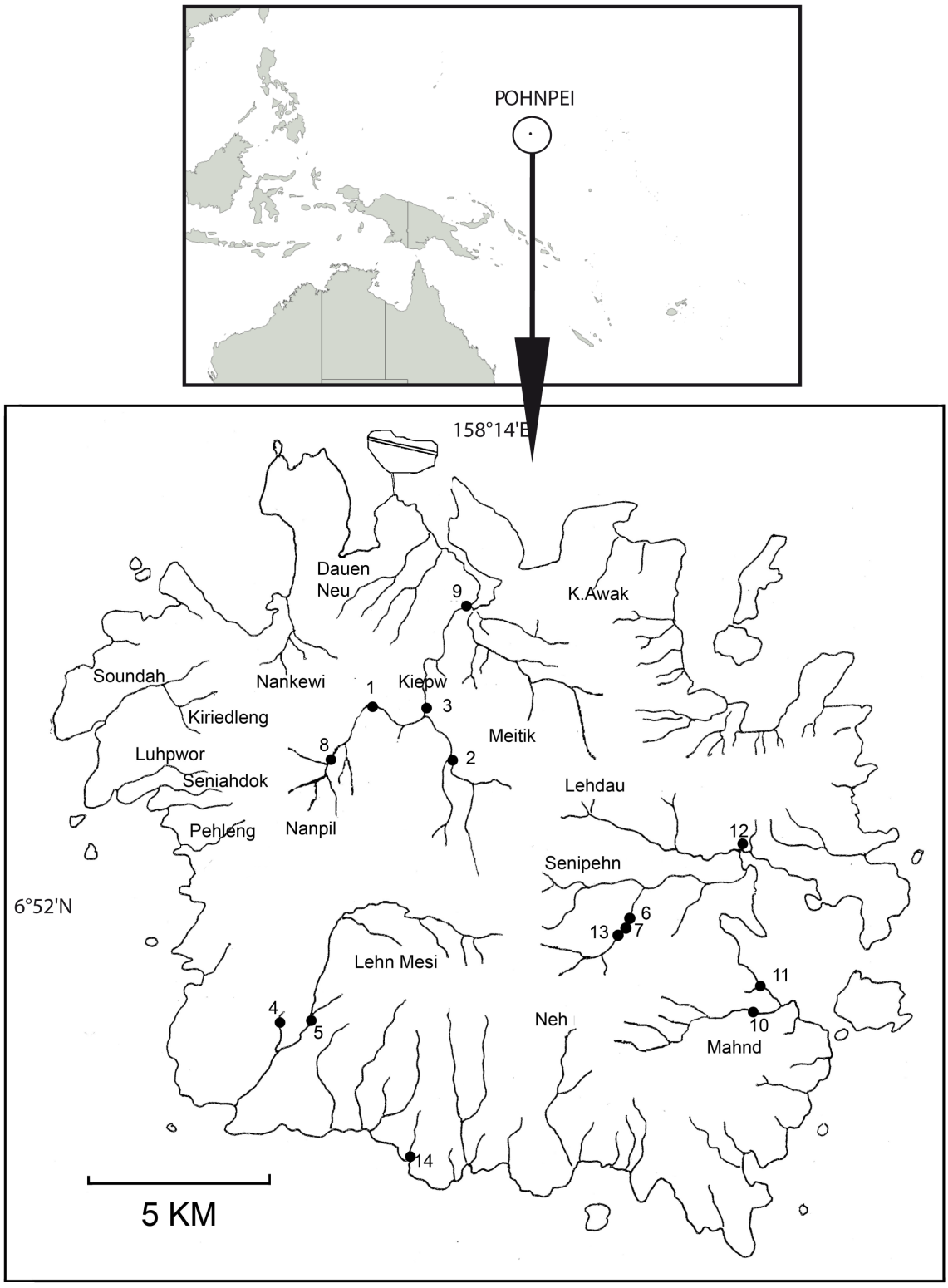


Figure 4.4.1: Map of Pohnpei Island showing sampling stations (black dots) with their number (refers to Table 4.4.1).

Table 4.4.1: Sampling localities data.

	Dates	Coordinates	Rivers	Altitude (m)
Station 1	09/03/2012	06°55.242N 158°12.265E	Nanpil	127
Station 2	09/03/2012	06°54.498N 158°13.270E	Nanpil	93
Station 3	09/03/2012	06°55.111N 158°12.878E	Nanpil	52
Station 4	10/03/2012	06°51.055N 158°10.890E	Lehn Mesi	197
Station 5	10/03/2012	06°51.107N 158°11.896E	Lehn Mesi	139
Station 6	12/03/2012	06°51.895N 158°15.810E	Senipehn	-
Station 7	12/03/2012	06°51.906N 158°16.010E	Senipehn	95
Station 8	13/03/2012	06°54.252N 158°11.491E	Nanpil	180
Station 9	13/03/2012	06°56.609N 158°13.550E	Nanpil estuary	5
Station 10	14/03/2012	06°50.221N 158°17.212E	Mahnd	107
Station 11	14/03/2012	06°51.120N 158°17.854E	"River 1" estuary	5
Station 12	14/03/2012	06°53.221N 158°17.44E	"Petroglyphe River"	5
Station 13	15/03/2012	06°51.795N 158°15.622E	Senipehn	119
Station 14	10/03/2012	06°48.579N 158°12.639E	"River 2" estuary	5

DNA extraction, amplification and sequencing

For recent specimens, DNA was extracted from abdominal tissues using the semi-automatic Ependorf ep-Motion 5075 robot. Fragments of the mitochondrial 16S rRNA (~ 520 bp) were amplified using the primers 16Sa-L (CGCCTGTTTATCAAAAACAT) and 16Sb-H2 (CTCCGGTTTGAACTCAGATCA) (Palumbi 1996). DNA amplification was performed in 25µl PCR reactions, containing approximately 3 ng of template DNA, 2.5 mM MgCl2, 0.26 mM of each nucleotide, 0.3 µM of each primer, 5% DMSO, 1 ng of BSA and 1.5 units of QBIOTAQ polymerase (MPBiomedicals). Amplification products were generated by an initial denaturation step of 4 min at 94°C followed by 35 cycles of denaturation at 94°C for 30s, annealing at 52°C for 40 s, extension at 72°C for 60 s and a final extension step at 72°C for 7 min.

For old collection specimens (Syntypes of *C. mertoni*), a CTAB protocol was used to extract DNA from pleopods. A shorter fragment of the 16S rRNA (332 bp) was amplified using two newly designed primers: 16S-Car-81F (AGGTAGCATAATAAATAGTC) and 16S-Car-413R (CTGTTATCCCTAAAGTAAC). DNA amplification was performed in 25µl PCR reactions, containing 2.5 mM MgCl2, 0.26 mM of each nucleotide, 0.3 µM of each primer, 1 ng of BSA and 1.5 units of QBIOTAQ polymerase (MPBiomedicals). Amplification products were generated by an initial denaturation step of 4 min at 94°C followed by 45 cycles of denaturation at 94°C for 30s, annealing at 55°C for 30 s, extension at 72°C for 40 s and a final extension step at 72°C for 7 min.

PCR products were sequenced using same primers and in both directions to insure the accuracy of base calls. Chromatograms were edited using Geneious v.8 software (http://www.geneious.com/ Kearse *et al*., 2012). All sequences were deposited in GenBank (Numbers MH476222 to MH476227). A sequence retrieved from GenBank for *Paratya australiensis* published by Page *et al.* (2007a) was included in our analysis, used as outgroup.

A total of 17 recent specimens were sequenced (Table 4.4.2): 6 of the new species, of which 2 firstly identified as *C. brachydactyla* with a long rostrum and 4 as *C. mertoni* with a short rostrum; 5 of *C. mertoni* from Kolombangara Island; 3 type specimens of *C. variabilis* and 3 of *C. brachydactyla* from type locality, Sulawesi. In addition, 3 old collection specimens, syntypes of *C. mertoni* from Kai Island were included (Mazancourt *et al.*, 2018b).

Molecular analyses

DNA sequences were aligned using MEGA7 software (Kumar *et al.*, 2016) with Muscle algorithm (Edgar, 2004). Using Bayesian information criterion in PartitionFinder (Lanfear *et al.*, 2012) we retained the HKY + G + I model. Best-scoring Maximum Likelihood (ML) trees were estimated using RAxML HPC2 v.8.2.10 (Stamatakis, 2014) implemented in the Cyber Infrastructure for Phylogenetic Research (CIPRES) portal v.3.1. (Miller et al., 2010) (https://www.phylo.org/). One hundred independent searches, each starting from distinct random trees, were conducted. Robustness of the nodes was assessed using non-parametric bootstrapping (Felsenstein, 1985) with 1,000 bootstrap replicates. Best-scoring Bayesian Inference trees were estimated using MrBayes v.3.2.6 (Ronquist & Huelsenbeck, 2003) also implemented in CIPRES with the previously determined model, running for 10,000,000 generations, a sampling frequency of 2,000 and a burn in of 25%. Support for nodes was determined using posterior probabilities calculated by MrBayes.

Abbreviations for museums

Muséum national d'Histoire naturelle, Paris: MNHN; Naturhistorisches Museum Basel, Basel: NMB; natural History Museum, London: NHM; Rijksmuseum van Natuurlijke Historie (now in the Naturalis Biodiversity Center, Leiden): RMNH; Werner Klotz's collection: WK; Zoological Reference Collection, National University of Singapore, Singapore: ZRC.

Abbreviations for morphological analyses

The following abbreviations are used in the present text: cl, carapace length (mm): measured from the post-orbital margin to the posterior margin of the carapace. P1: first pereopod. P2: second pereopod. P3: third pereopod. P5: fifth pereopod. Pl1: first pleopod. Pl2: second pleopod.

Morphological comparison

The rostrum, the general cephalon, the pereopods 1, 2, 3 and 5 and the abdomen were observed using a stereoscopic microscope. The proportions of the various joints of the appendages were measured using microphotographs and AnalySIS Works software (Olympus). Drawings were made using the "Digital Inking" method (Coleman 2003; 2006) by tracing vectorial paths on stacks of high-resolution photographs using Adobe Illustrator (CS6) and a WACOM PTZ-1230 graphic tablet.

4.4.4 Results

Collection of specimens

About 50 specimens were collected by electro-fishing in the different rivers prospected in Pohnpei. From all of these, 26 were sequenced, 18 having a short rostrum and 8 having a long one. A representative sample of 6 specimens is shown here in the genetic study.

Phylogenetic analyses

After checking that consensus trees obtained from the two different methods were congruent, we decided to show the Bayesian consensus tree with both the ML bootstrap values and Bayesian posterior probabilities on branches (Figure 4.4.2). The specimens are grouped in four distinct and moderately supported clades. The most basal comprises the specimens of *C. brachydactyla* from Sulawesi (type locality), then, the type specimens of *C. variabilis*, and finally, two sister clades, one comprising recent and old specimens of *C. mertoni* and the other the specimens from Pohnpei. Due to the low support values, we cannot discuss the affinities of the different species but we can confidently assert that the terminal clades are distinct species and consider the last one to be a new species that we describe below.

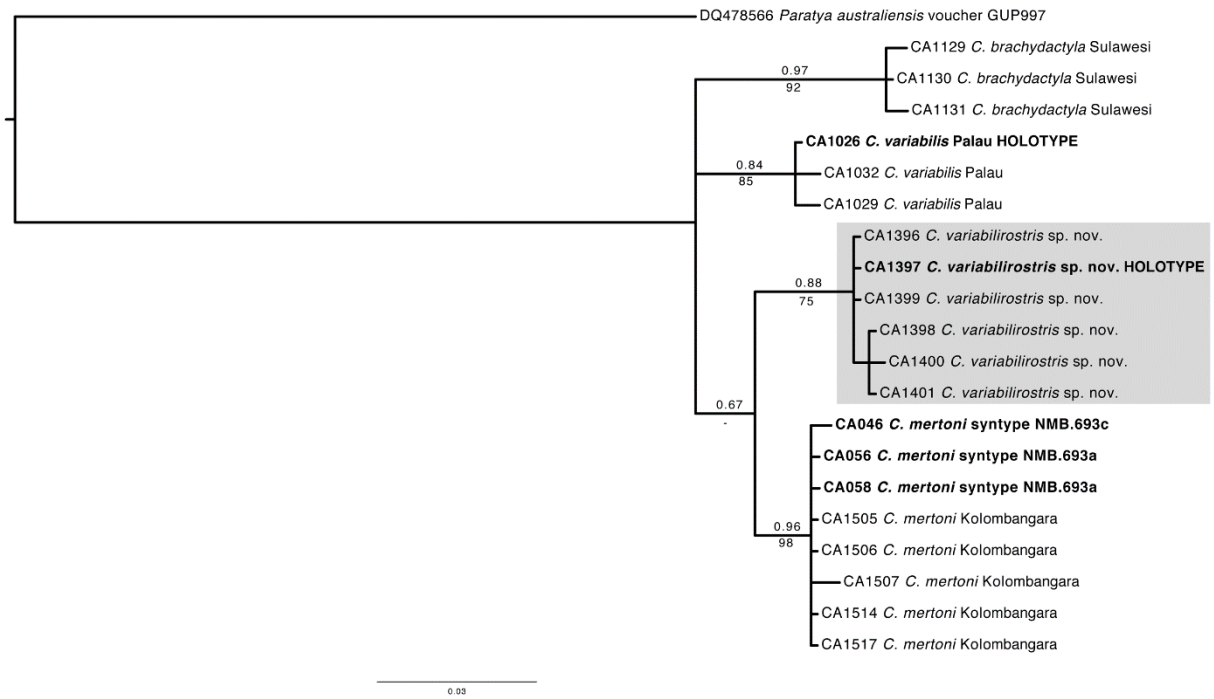


Figure 4.4.2: Bayesian Inference tree of the 16S sequences of the specimens. Numbers on branches indicate bootstrap values. Letters "M" indicate specimens firstly identified as Caridina mertoni and letters "B" indicate specimens firstly identified as Caridina brachydactyla.

Table 4.4.2: Specimens used in the genetic analysis.

Specimen n°	Species	Locality	Firstly identified as	GenBank n°	Reference
CA1396	*C. variabilirostris*	Pohnpei (St. 1)	*C. mertoni*	MH476222	This study
CA1397	*C. variabilirostris*	Pohnpei (St. 8)	*C. mertoni*	MH476223	This study
CA1398	*C. variabilirostris*	Pohnpei (St. 8)	*C. mertoni*	MH476224	This study
CA1399	*C. variabilirostris*	Pohnpei (St. 8)	*C. mertoni*	MH476225	This study
CA1400	*C. variabilirostris*	Pohnpei (St. 9)	*C. brachydactyla*	MH476226	This study
CA1401	*C. variabilirostris*	Pohnpei (St. 9)	*C. brachydactyla*	MH476227	This study
CA1026	*C. variabilis*	Palau		MG707146	Mazancourt *et al.* 2018
CA1029	*C. variabilis*	Palau		MG707148	Mazancourt *et al.* 2018
CA1032	*C. variabilis*	Palau		MG707147	Mazancourt *et al.* 2018
CA1129	*C. brachydactyla*	Indonesia		MG707169	Mazancourt *et al.* 2018
CA1130	*C. brachydactyla*	Indonesia		MG707170	Mazancourt *et al.* 2018
CA1131	*C. brachydactyla*	Indonesia		MG707171	Mazancourt *et al.* 2018
CA046	*C. mertoni*	Indonesia		MG707138	Mazancourt *et al.* 2018
CA056	*C. mertoni*	Indonesia		MG707139	Mazancourt *et al.* 2018

CA058	*C. mertoni*	Indonesia	MG707140	Mazancourt *et al.* 2018
CA1505	*C. mertoni*	Solomon Islands	MG707141	Mazancourt *et al.* 2018
CA1506	*C. mertoni*	Solomon Islands	MG707142	Mazancourt *et al.* 2018
CA1507	*C. mertoni*	Solomon Islands	MG707143	Mazancourt *et al.* 2018
CA1514	*C. mertoni*	Solomon Islands	MG707144	Mazancourt *et al.* 2018
CA1517	*C. mertoni*	Solomon Islands	MG707145	Mazancourt *et al.* 2018
GUP997	*Paratya australiensis*	Australia	DQ478566	Page *et al.* 2007

Taxonomy

Atyidae De Haan, 1849

***Caridina* H. Milne Edwards, 1837**

Caridina variabilirostris, n. sp.

(Fig. 4.4.3, 4.4.4)

Material examined.

Holotype

POHNPEI (Station 8): ♂, cl 3.5 mm, Nanpil river, 06°54.252′ N, 158°11.491′ E, alt. 180 m, 13 Mar. 2012 (MNHN-IU-2018-231, DNA: CA1397).

Paratypes

POHNPEI – **Nanpil river (Station 1)**: 1 ♂, cl 3.2 mm, 06°55.242′ N, 158°12.265′ E, alt. 127 m, 9 Mar. 2012, (MNHN-IU-2018-232); 1 ♀, cl 4.6 mm, same data as for previous (MNHN-IU-2018-233); 1 ♀ ovig., cl 4.1 mm, same data as for previous (MNHN-IU-2018-234, DNA: CA1396). – **Nanpil river (Station 3)**: 1 ♂, cl 3.4 mm 06°55.111′ N, 158°12.878′ E, alt. 52 m, 9 Mar. 2012 (MNHN-IU-2018-235) ; 1 ♀ ovig., cl 4.5 mm, same data as for previous (MNHN-IU-2018-236). – **Lehn Mesi river (Station 5)**: 2 ♀♀, cl 4.7 mm (MNHN-IU-2018-237) and 4.8 mm (MNHN-IU-2018-238) ; 1 ♀ ovig., cl 4.7 mm (MNHN-IU-2018-239), 06°51.107′ N, 158°11.896′ E, alt. 139 m, 10 Mar. 2012. –

Senipehn river (Station 7): 1 ♀, 06°51.906′ N, 158°16.010′ E, alt. 95 m, 12 Mar. 2012, cl 4.4 mm (MNHN-IU-2018-240) ; 2 ♀♀, ovig., cl. 4.1 mm (MNHN-IU-2018-241) and 4.5 mm (MNHN-IU-2018-242), same data as for previous. – **Nanpil river (Station 8)**: 3 ♂♂, cl 3.4 mm (MNHN-IU-2018-243, DNA: CA1399), 3.5 mm (MNHN-IU-2018-244) and 3.6 mm (MNHN-IU-2018-245, DNA: CA1398), 06°54.252′ N, 158°11.491′ E, alt. 180 m, 13 Mar. 2012; 1 ♀, cl 4.4 mm (MNHN-IU-2018-246), same data as for previous ; 1 ♀ ovig., cl 5.1 mm (MNHN-IU-2018-247), same data as for previous. – **Nanpil estuary (Station 9)**: 2 ♂♂, cl 2.2 mm (MNHN-IU-2018-248, DNA: CA1401) and 2.7 mm (MNHN-IU-2018-249), 06°56.609′ N, 158°13.550′ E, alt. 5 m, 13 Mar. 2012; 2 ♀♀, 3.5 mm (MNHN-IU-2018-250, DNA: CA1400) and 3.7 mm (MNHN-IU-2018-251), same data as for previous ; 2 ♀♀ ovig., cl 4.0 mm (MNHN-IU-2018-252) and 4.4 mm (MNHN-IU-2018-253), same data as for previous. – **Mahnd river (Station 10)**: 2 ♂♂, cl 2.9 mm (MNHN-IU-2018-254) and 3.3 mm (MNHN-IU-2018-255), 06°50.221′ N, 158°17.212′ E, alt. 107 m, 14 Mar. 2012 ; 1 ♀ ovig., cl. 4.3 mm (MNHN-IU-2018-256), same data as previous.

Comparative material

- *Caridina brachydactyla* De Man, 1908

Lectotype

INDONESIA – 1 ♀ ovig., cl 4.8 mm, Flores, Reo, RMNH (Crus.D.977).

Paralectotypes

INDONESIA – 2 ♀♀ ovig., cl 5.3–5.4 mm, Flores, near Mbawa, RMNH (Crus.D.255).

Non types

INDONESIA – 1 ♀, cl 5.8 mm, Bali, NMB (1054a); 2 ♂♂, cl 2.7 mm and 3.7 mm (DNA: CA1130), Sulawesi, Palopo, Macaui, WK (63.10); 2 ♀♀ ovig., cl 4.3 mm (DNA: CA1129) and 4.0 mm (DNA: CA1131), and 1 ♀, cl 4.8 mm, Palopo, Tojo, WK (64.10).

- *Caridina mertoni* Roux, 1911

Syntypes

INDONESIA – 2 ♂♂, cl 2.7 mm and 3.8 mm (DNA: CA056) and 1 ♀, cl 4.1 mm (DNA: CA058), Grand Kai, Elat, NMB (693a); 1 ♂, cl 3.1 mm and 1♀ ovig., cl 4.8 mm Grand Kai, Warka, NMB (693b); 1 ♀, cl 4.3 mm (DNA: CA046), Grand Kai, Enralang NMB (693c); 1 ♂, cl 3.8 mm, Grand Kai, Elat, MNHN (MNHN-IU-2015-1819); 1 ♂, cl 3.4 mm, Grand Kai, Elat, MNHN (MNHN-IU-2015-1820).

Non types

KOLOMBANGARA (SOLOMON ISLANDS) – **Manolu river:** 1 ♂, cl 3.9 mm, 08°05.312' S, 157°00.813' E, 10 Nov. 2015, (MNHN-IU-2017-2107 DNA: CA1506); 1 ♂, cl 3.0 mm, same data as for previous (MNHN-IU-2017-2108 DNA: CA1507); 1 ♂, cl 3.7 mm, same data as for previous (MNHN-IU-2017-2109 DNA: CA1505); 1 ♀ ovig., cl 3.9 mm, same data as for previous (MNHN-IU-2017-2110). – **Sulumuni river:** 1♀, cl 6.2 mm, 08°02.253' S, 157°09.257' E, 12 Nov. 2015 (MNHN-IU-2017-2111 DNA: CA1514); 1 ♀ ovig., cl 4.1 mm , same data as for previous (MNHN-IU-2017-2112 DNA: CA1517).

- *Caridina elongapoda* Liang & Yan, 1977

Non types

HONG-KONG: 2 ♂♂, cl 2.8-3.0 mm, Pak Tam Chung (WK 22 09), W. Klotz leg; 1 ♀, cl 4.2 mm and 1 ♂, cl 3.5 mm, Kai Sai Chau (WK 14 11), W. Klotz leg.

MALAYSIA – 1 ♂, cl 4.2 mm, Pulau Tioman, Sungai Asah, 24 Jun. 1997, Ng *et al.* leg., ZRC (1998.0865); 1 ♀, cl 4.3 mm, same data as for previous, ZRC (1998.0865); 1 ♀ ovig., cl 4.3 mm, same data as for previous, ZRC (1998.0865).

- *Caridina peninsularis* Kemp, 1918

Lectotype

MALAYSIA – 1 ♂, cl 3.2 mm, Penang Island, Botanical garden, Feb. 1916, N. Anandale leg., MNHN (MNHN-IU-2015-1749);

Paralectotypes

MALAYSIA – 1 ♀ ovig., cl 5.4 mm, same data as lectotype, MNHN (MNHN-IU-2015-1750); 1 ♀, cl 3.4 mm, same data as for previous, NHM (1919.11.1.12-21 (1761124)); 1 ♀ ovig., cl 5.2 mm, same data as for previous, NHM (1919.11.1.12-21 (1761124)); 1♂ cl 3.9 mm, same data as for previous, NHM (1919.11.1.12-21 (1761124)).

Non types

SINGAPORE – 1 ♀ ovig., cl 5.1 mm, Tanglin (incorrectly spelt Tangtum in NHM register and on label, see Richard & Clark, 2014), Bedford & Lanchester leg., D.S. Johnson det., 1958, NHM (1958.8.7.14–17 (1749569)); 1 ♂, cl 4.2 mm, same data as for previous, NHM (1958.8.7.14–17 (1749569)).

Description

Cephalothorax. Rostrum (Fig. 4.4.3a; b; c): very variable in length, 0.6-1.3 of cl, reaching to scaphocerite apex, armed dorsally with 18-26 teeth, distal unarmed portion 0.0-0.4 times that of armed portion, with one or two subapical teeth, 1-4 teeth on carapace posterior to orbital margin, ventral margin with 4-12 teeth. Number of dorsal teeth behind the most proximal ventral tooth 12-18. Rostrum formula (1-4) 18-26+1-2/4-12. Antennular tooth acute, placed slightly below orbital angle. Pterygostomian margin rectangularly rounded.

Eyes well developed, anterior end reaching to 0.7 length of antennular peduncle basal antennomere. Antennular peduncle 0.7 times as long as carapace. Anterolateral angle reaching 0.25 length of the second antennomere; second antennomere distinctly longer than third. Stylocerite reaching to 0.7 length of antennular peduncle basal antennomere.

Mouthparts. Mandibles dimorphic; left mandible (Fig. 4.4.4b) more developed, corpus large, robust with five strong sharp teeth separated by ridged gap; incisor and molar processes separated by a patch of long simple setae. Right mandible (Fig. 4.4.4c) with five sharp incisor teeth, medially with a group of long setae; molar process narrow, elongate, ridged. First maxilla (Fig. 4.4.4d) having a lower lacinia with margin broadly rounded, bearing several rows of plumose setae. Upper lacinia elongate, with medial margin bearing a number of distinct teeth and simple setae, palp bearing long simple setae. Second maxilla (Fig. 4.4.4e) upper and middle endite with marginal and

submarginal simple or slightly plumose setae. Lower endite with simple setae; palp narrow, shorter than upper endite cleft with a few setae. Scaphognathite fringed with long simple setae, tapering posteriorly with some long, curved simple setae at posterior end. First maxilliped (Fig. 4.4.4h) endopodite ultimate segment medial margin with long plumose setae. Palp elongate, setose. Exopod flagellum long and narrow distally with marginal plumose setae. Caridean lobe large, with marginal setae. Second maxilliped (Fig. 4.4.4g) endopodite ultimate and penultimate antennomeres fused, reflected against basal antennomeres. Ultimate, penultimate and basal antennomeres medial margins with long setae of various types; flagellum very long, slender with marginal plumose setae distally. Third maxilliped (Fig. 4.4.4f) with terminal article reaching third antennular peduncle antennomere apex; distal antennomere about 10 times as long as wide, slightly shorter than the penultimate, ending in large hamulate apical spine surrounded by simple setae. Penultimate antennomere about eight times as long as wide, with group of transverse rows of simple setae. Exopod flagellum well developed, about a third the length of endopodite second article, distal margin with long plumose setae.

Pereopods. Pereopods I-IV with epipods pereopod. P1 (Fig. 4.4.3d): chela about 1.9-2.2 times as long as wide, movable finger 3.6-4.4 times as long as wide, 1.2-1.7 times length of palm; carpus 2.0-2.7 times as long as wide. P2 (Fig. 4.4.3e) more slender and longer than first pereopod with chela 2.1-2.6 times as long as wide: movable finger 3.9-5.8 times as long as wide, 1.3-2.0 times length of palm; carpus slender 4.4-6.5 times as long as wide. P3 (Fig. 4.4.3f): slender, dactylus (Fig. 4.4.3g) 2.4-3.3 times as long as wide, including terminal spine), flexor margin with 4-6 spines in parallel to the terminal one; propodus 13.8-20.5 times as long as wide, 4.8-8.4 times as long as dactylus. P5 (fig. 4.4.3h): dactylus (Fig. 4.4.3i) 2.1-4.2 as long as wide with 18-29 spinules on flexor margin; propodus 15.8-28.4 times as long as wide, 5.3-8.6 times as long as dactylus.

Abdomen. Third abdominal (Fig. 4.4.4a) somite with moderately convex dorsal profile. Sixth abdominal somite about 0.68 of carapace, 1.76 times as long as fifth somite, slightly shorter than telson. Telson (Fig. 4.4.3k) with four pairs of dorsal spines and one pair of dorsolateral spines; posterior margin, with or without a median process, exhibits variations, triangular or rounded with four to five intermediate simple setae longer or equal than lateral ones.

Pl1 (Fig. 4.4.3m): Endopod foliform with a developed appendix interna in males. Pl2 (Fig. 4.4.3n): Appendix masculina on second pleopod reaching 0.52 times length of endopod; appendix interna reaching about 0.85 times appendix masculina length.

Preanal carina (Fig. 4.4.3l) unarmed.

Uropodal diaeresis (Fig. 4.4.3j) with 10-15 spinules.

Ovigerous female egg size (Fig. 4.4.3o): 0.39-0.49 x 0.24-0. 29 mm.

Habitat

This species is found among macrophytes in flowing fresh water of rivers of Pohnpei all over the course, showing good adaptability to different temperatures and hydrological conditions. It is more abundant in higher elevations due to reduced predator pressure.

Etymology

The new species is named *variabilirostris* for its variable rostrum length.

Colour pattern

The colour of the body is hyaline with many reds dots. An oblique red band on the cephalothorax is very characteristic (Fig. 4.4.5).

Distribution

This new species is currently only known from Pohnpei Island.

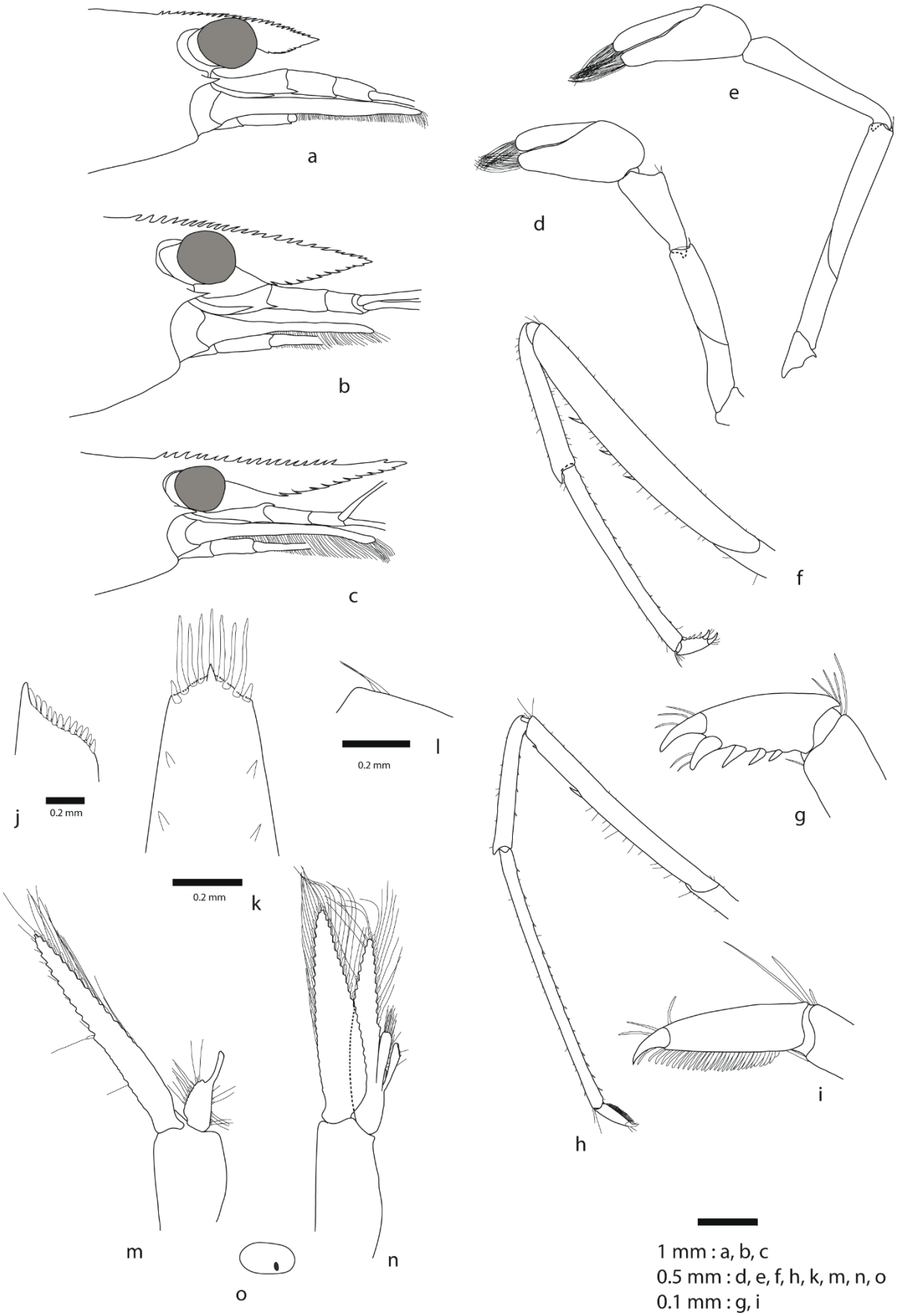


Figure 4.4.3: *Caridina variabilirostris* sp. nov., showing the variation in rostrum length related to the altitude (a–c), drawings made from different specimens. a, m–n. ♂, cl 3.5 mm (MNHN-IU-2018-244). a. Rostrum. m. First pleiopod. n. Second pleiopod. b. ♀, cl 5.6 mm, rostrum (MNHN-IU-2018-252). c, j, o. ♀, cl 4.0 mm (MNHN-IU-2018-253) c. Rostrum. j. Uropodial diaeresis. o. Eggs. d–i, k. ♀, cl 3.5 mm (MNHN-IU-2018-250). d. First pereopod. e. Second pereopod. f. Third pereopod. g. Dactylus of third pereopod. h. Fifth pereopod. i. Dactylus of fifth pereopod. k. Telson. l. ♀, cl 4.3 mm, pre-anal carina (MNHN-IU-2018-256).

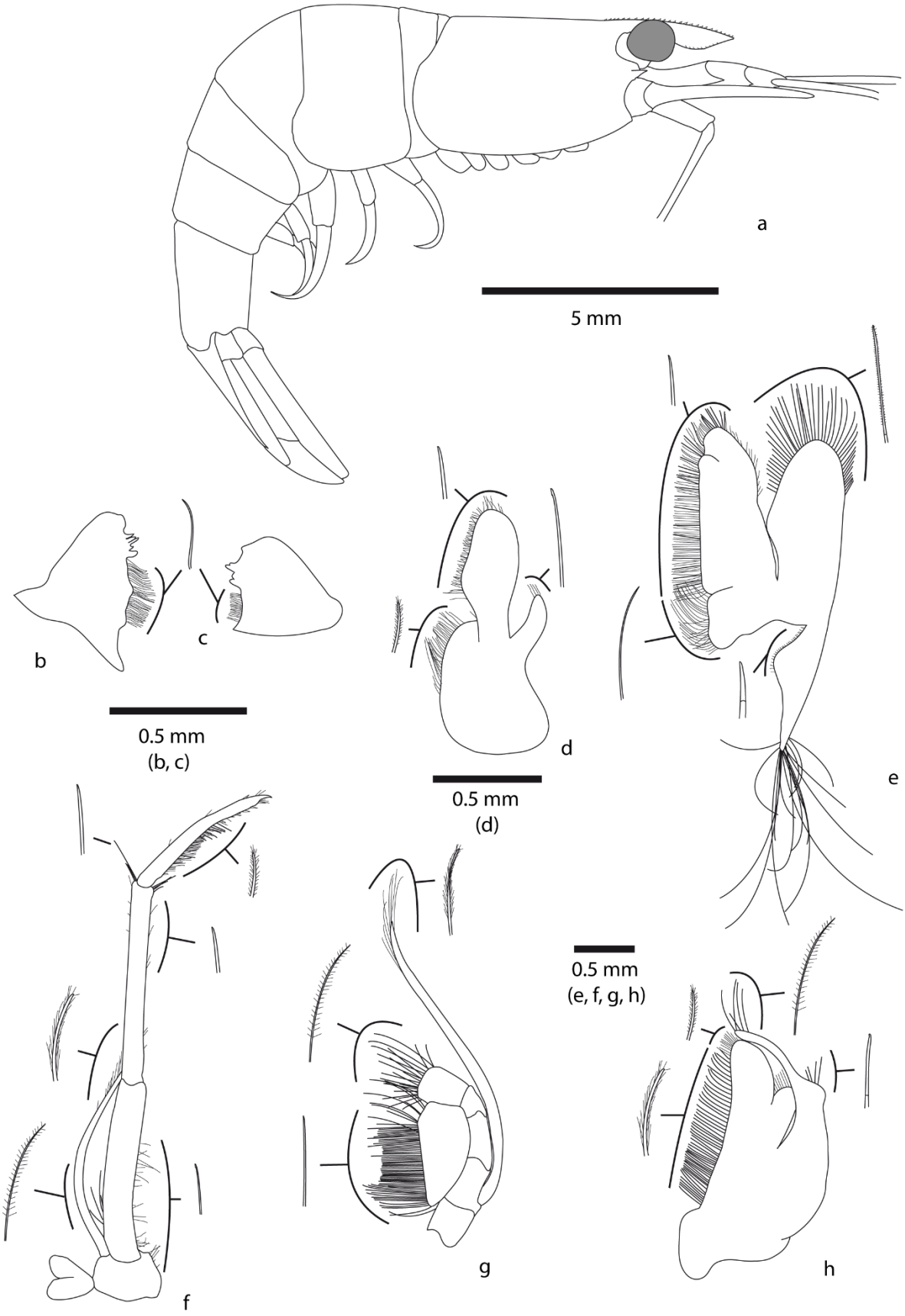


Figure 4.4.4: ***Caridina variabilirostris*** **sp. nov., showing drawings made from different specimens: a. ♂, cl 3.5 mm, general appearance (MNHN-IU-2018-244). c-d. ♀, cl 3.5 mm (MNHN-IU-2018-250). c. Right mandible. d. First maxilla. b, e-h. ♀, cl 4.8 mm, (MNHN-IU-2018-238). b. Left mandible. e. Second maxilla. f. Third maxilliped. g. Second maxilliped. h. First maxilliped.**



Figure 4.4.5: *Caridina variabilirostris* sp. nov. live coloration (Photos: P. Keith).

Remarks

Caridina variabilirostris n. sp. is most similar to *C. mertoni*, *C. brachydactyla*, *C. elongapoda* Liang & Yan, 1977 and *C. peninsularis* Kemp, 1918. This new species displays a variable rostrum length among specimens. When the rostrum is short the general appearance is like *C. mertoni*, whereas when the rostrum is long the general appearance is of *C. brachydactyla*. In *C. variabilirostris* n. sp. the antennal spine is placed below the orbital angle, the P5 dactylus with 18-29 spinules whereas in *C. mertoni*, the antennal spine is somewhat fused with the orbital angle and P5 dactylus with 24-43 spinules. *C. variabilirostris* n. sp. differs from *C. brachydactyla* by the absence of spine on the preanal carina, a shorter distal unarmed portion of the rostrum 0.0-0.4 (vs 0.4-1.6) times that of armed portion, by a longer P5 propodus, which is 5.3-8.6 time as long as the dactylus (vs 3.9-5.7) and a lower number of spinules on the P5 dactylus (32-42 in *C. brachydactyla*).

This new species resembles *C. elongapoda* with its preanal carina lacking a spine. But the P5 dactylus is shorter 2.1-4.2 (vs 4.3-5.6) with fewer spinules on the P5 dactylus 18-29 (vs 33-44) and the species has larger eggs 0.39-0.49 x 0.24-0. 29 mm (vs 0.39-0.40 x 0.22-0.23).

It also differs from *C. peninsularis* by a lower number of dorsal rostrum teeth (18-26 vs 20-35), a lower P3 propodus-dactylus ratio (4.8-8.4 vs 4.4-5.8) and a P5 dactylus with 18-29 spinules vs 25-38.

This species looks very much like *C. variabilis* de Mazancourt, Rogers & Keith, (2018) from Guam and Palau with the preanal always non-armed, the number of teeth and their placement on the rostrum, the proportions between the joints of pereiopods, eggs size. But the P5 dactylus has a single terminal spine and short distal propodus seta (vs two strong distal spines and a very long distal propodus seta) the posterior margin of the telson with intermediate setae longer or equal than lateral ones (vs shorter or equal than lateral ones).

4.4.5 Discussion

Recently Mazancourt *et al.* (2017a) highlighted the “Pinocchio shrimp effect” on this new species. Indeed some specimens showing a long rostrum were attributed morphologically to *C. brachydactyla* whereas others, showing a short rostrum, were

identified as *C. mertoni*. In fact all specimens belong to this new species. Indeed the rostrum length widely used in *Caridina* taxonomy might not be as reliable as it was thought. It is highly plastic and varies with environmental parameters.

Despite an increasing use of integrative taxonomy for the systematics of *Caridina* (and shrimps in general), some authors keep describing new species based not only on morphology alone, but on a single character. For example, different species described by Richard & Clark (2009; 2014) based on rostrum morphology proved to be synonyms, and the differences observed were finally intra-specific variation (see Wood *et al.*, 2018; Mazancourt *et al.* unpublished)

We thus advocate to always perform some molecular work prior to study a new species in order to unveil this kind of variation within a species, focus on the morphology and expose the variation when describing it. Alternatively, if retrieving molecular data is not possible, using a combination of reliable characters seems sounder than a single one. This would allow to provide characters to identify confidently the species when encountered in the field. It is likely that different species described in this genus are finally the same one, exhibiting the same kind of polymorphism as we showed here.

4.4.6 Acknowledgements

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4.5 Taxonomie intégrative des espèces confondues avec *C. longirostris* dans l'Indo-Pacifique Central

Invertebrate Systematics, in press

Integrative taxonomy helps separate four species of freshwater shrimps commonly overlooked as *Caridina longirostris* (Crustacea: Decapoda: Atyidae) in Indo-West Pacific islands.

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4.5.1 Abstract

Caridina longirostris described by H. Milne Edwards (1837) from specimens supposedly from La Macta River, near Oran (Algeria) is an emblematic species of the "*C. nilotica* complex". Until now this species was thought to have a wide distribution in the Indo-Pacific region. Recently, numerous specimens identified as *C. longirostris* were collected

from various Indo-West Pacific localities. In the context of an integrative taxonomy research, old collection specimens and newly collected ones were morphologically and genetically studied. Four species allied to *C. longirostris* are here identified: *C. appendiculata*, *C. brevidactyla*, *C. gracilipes* and *C. meridionalis*. Detailed re-descriptions of these four species are given as well as their geographical and ecological distributions. *Caridina nilotica* var. *brevidactyla* is revalidated as the species *C. brevidactyla*, and *Caridina coulaborensis*, *C. fritzi* and *C. solamulieres* are considered junior synonyms of *C. meridionalis*. The position of these species in the troublesome "*C. nilotica* complex" is clarified and some species are synonymised. A key is provided to facilitate identification.

Additional keywords: *Caridina*, Indo-Pacific region, Integrative taxonomy, morphology, 16S rRNA

4.5.2 Introduction

One of the aims of the Muséum national d'Histoire naturelle (MNHN), Paris, is to undertake faunistic inventories of rivers in tropical islands in order to establish a better protection of these fragile ecosystems and, in this context, to clarify taxonomy of poorly known species. The freshwater shrimp *Caridina* H. Milne Edwards, 1837, comprising 302 valid species (WoRMS database as of June 2018) and mainly present in the Indo-Pacific region, is the most diversified genus of the Atyidae (De Grave *et al.* 2015) and an important ecological component in tropical streams (Covich *et al.* 1999; Pringle *et al.* 1993). Their high diversity combined with the lack of informative morphological characters has led to a confused taxonomy (Richard & Clark, 2009). Indeed, until recently, their taxonomy was mainly based on morphological characters. Some have been proven highly variable within a species (e.g. rostrum shape and indentation or coloration; Mazancourt *et al.* 2017a) and so taxonomically non-informative, making it difficult to establish speciation (Rintelen & Cai, 2009). There is thus a need for an integrative and standardized approach to improve the systematic of the group, focusing on informative morphological features and using molecular characters (Page *et al.* 2005; Page and Hughes 2011).

To illustrate this problem, we here focus on *Caridina longirostris,* a species belonging to troublesome "*C. nilotica* complex", described in 1837 by H. Milne Edwards from specimens from La Macta river, near Oran, Algeria. The taxonomic complexity within *Caridina* has led previous authors to create groups of species based on shared morphological characters. For example, Bouvier (1925) defined 5 species groups: *C. nilotica* group, *C. laevis* group, *C. africana* group, *C. brevirostris* group and *C. typus* group. Some of these have been further divided as new species were described, such as the *C. serrata* group (Cai & Ng, 1999 ; Karge & Klotz, 2007), *C. gracilirostris* group (Cai & Ng, 2007), and *C. weberi* group (Cai & Anker, 2004 ; Cai *et al.*, 2009), while others still exist, like the *C. nilotica* and *C. typus* groups (Karge & Klotz, 2007). According to Bouvier (1925) *C. nilotica* group is characterized by the following: 1) antennular peduncles usually equal or exceed three quarters of the carapace and the 6th abdominal segment by two thirds; 2) rostrum usually long, curved upwards; 3) uropodal diaeresis with 8–10 short spiniform setae. Until 1900, *C. longirostris* was one of eight species belonging to the "*C. nilotica* complex" that includes 22 different taxa (Karge & Klotz, 2007).

Caridina longirostris has been reported as a widely distributed species ranging from the Indian Ocean including Madagascar (Holthuis, 1965) and Reunion Island (Keith *et al.* 2006) and the West Pacific Ocean including New Caledonia (Holthuis, 1969 ; Choy & Marquet, 2002 ; Marquet *et al.*, 2003), Fiji (Choy, 1991), Vanuatu (Keith *et al.*, 2010), Australia (Page *et al.*, 2007a), Papua New Guinea (Short, 2009), Pohnpei (Maciolek & Ford, 1987), Sumba (Holthuis, 1978a), Philippines (Chace, 1997) and Taiwan (Hung *et al.*, 1993). However, this species does not occur in Central Pacfic (Keith & Marquet, 2011 ; Keith *et al.*, 2013).

Numerous *Caridina* specimens were collected from New Caledonia by the Pedcal Expedition in 1991 and by Chloe Expeditions between 1997 and 2002. Similarly, rivers on the islands of Efate, Santo and Malekula (Vanuatu) were sampled in July 2003 and November 2008 by the Vanuatu Environment Unit and the MNHN. Part of this material was identified as *C. longirostris* (Marquet *et al.*, 2003; Keith *et al.*, 2010). More recently, additional specimens identified as *Caridina longirostris* were collected during trips to Micronesia including Babeldaob, Palau in February-March 2011 and Pohnpei in March 2012) and Melanesia including Taveuni, Fiji in February 2013; Efate and Epi, Vanuatu in November 2014; Aneityum, Vanuatu in June 2015; Choiseul in October 2014,

Kolobangara in November 2015 and Vella Lavella in October 2016, all Solomon Islands and New Caledonia in September and November 2016 and November 2017.

Therefore aim of the present study was to combine morphological data with a 16S rRNA analysis, so using integrative taxonomy, to re-examine all old (1991–2008) and recent (2011–2017) specimens from Micronesia and Melanesia and material deposited in five museums to established the distribution of *C. longirostris*.

4.5.3 Materials and methods

Abbreviations for museums

Muséum national d'Histoire naturelle, Paris: MNHN; Naturhistorisches Museum Basel, Basel: NMB; Museum für Naturkunde, Berlin: ZMB; Rijksmuseum van Natuurlijke Historie (now in the Naturalis Biodiversity Center, Leiden): RMNH; Zoölogisch Museum Amsterdam (now in the Naturalis Biodiversity Center, Leiden): ZMA. Bernice Pauahi Bishop Museum, Honolulu: BPBM. Werner Klotz's collection: WK.

Morphological comparison

The rostrum, the general cephalon, the pereiopods 1, 2, 3 and 5 and the abdomen were observed using a stereoscopic microscope. The proportions of the various joints of the appendages were measured using microphotographs and the AnalySIS Works software (Olympus). Drawings were made using the "Digital Inking" method (Coleman 2003, 2006) by tracing vectorial paths on high-resolution photographs using Adobe Illustrator (CS6) and a WACOM PTZ–1230 graphic tablet.

Abbreviations for morphological analyses

The following abbreviations are used in the present text: cl, carapace length (mm): measured from the post-orbital margin to the posterior margin of the carapace. P1: first pereiopod. P2: second pereiopod. P3: third pereiopod. P5: fifth pereiopod. Pl1: first male pleopod. Pl2: second male pleopod.

DNA extraction, amplification and sequencing

For recent specimens, DNA was extracted from abdominal tissues using the semi-automatic Ependorf ep-Motion 5075 robot. Fragments of the mitochondrial 16S rRNA (~ 520 bp) were amplified using newly designed primers, adapted from Palumbi (1996) to our taxa: 16Sar-Lmod (TACTTCTGCCTGTTTATCAAAAA) and 16Sbmod (GGTCTGAACTCAAATCATGTAAA). DNA amplification was performed in 20µl PCR reactions, containing approximately 3ng of template DNA, 2.5mM MgCl2, 0.26mM of each nucleotide, 0.3µM of each primer, 5% DMSO, 1ng of BSA and 1.5 units of QBIOTAQ polymerase (MPBiomedicals). Amplification products were generated by an initial denaturation step of 4min at 94°C followed by 35 cycles of denaturation at 94°C for 30s, annealing at 52°C for 40s, extension at 72°C for 60s and a final extension step at 72°C for 7 min.

For old collection specimens (Types and non-types of *C. appendiculata*, *C. brachydactyla*, *C. brevidactyla*, *C. coulaborensis*, *C. gracilipes*, *C. fritzi*, *C. longirostris*, *C. meridionalis*, *C. peninsularis* and *C. solamulieres*), a CTAB protocol was used to extract DNA from pleopods. A shorter fragment of the 16S rRNA (332 bp) was amplified using the primers 16S-Car-81F (AGGTAGCATAATAAATAGTC) and 16S-Car-413R (CTGTTATCCCTAAAGTAAC)(de Mazancourt *et al.* 2018). If these primers could not amplify successfully, another Reverse primer was used to try to amplify a shorter fragment (141 bp): 16S-Car-222R (CTTTATAGGGTCTTATCGT). DNA amplification was performed in 20µl PCR reactions, containing 2.5mM MgCl2, 0.26mM of each nucleotide, 0.3µM of each primer, 1ng of BSA and 1.5 units of QBIOTAQ polymerase (MPBiomedicals). Amplification products were generated by an initial denaturation step of 4min at 94°C followed by 45 cycles of denaturation at 94°C for 30s, annealing at 55°C for 30s, extension at 72°C for 40s and a final extension step at 72°C for 7 min.

PCR products were sequenced using the same primers and in both directions to ensure the accuracy of base calls. Chromatograms were edited using Geneious v.8 software (http://www.geneious.com/ Kearse *et al.* 2012). All sequences were deposited in GenBank (Numbers MH497484 to MH497558).

Molecular analyses

DNA sequences were aligned using MEGA7 software (Kumar *et al.*, 2015) with Muscle algorithm (Edgar, 2004). Using Bayesian information criterion in jModelTest (Darriba *et al.*, 2012; Guindon & Gascuel, 2003) we retained the GTR + G model. Best-scoring ML trees were estimated using RAxML HPC2 v.8.2.10 (Stamatakis, 2014) and best-scoring Bayesian Inference trees were estimated using MrBayes v.3.2.6 (Ronquist & Huelsenbeck, 2003), both methods implemented in CIPRES with the previously determined model, running for 10,000,000 generations, a sampling frequency of 2,000 and a burn in of 25%. Support for nodes was determined using posterior probabilities calculated by MrBayes implemented in the Cyber Infrastructure for Phylogenetic Research (CIPRES) portal v.3.1. (Miller *et al.*, 2010; https://www.phylo.org/). One hundred independent searches, each starting from distinct random trees, were conducted. Robustness of the nodes was assessed using non-parametric bootstrapping (Felsenstein, 1985) with 1,000 bootstrap replicates. We considered a group to be 'moderately supported' if it had a bootstrap support value (B) between 75 and 89% and 'highly supported' when B ≥ 90%. For the analysis, 11 recent specimens of *C. brevidactyla* from different localities were selected and 2 sequences retrieved from GenBank, 4 recent specimens of *C. leucosticta* from Okinawa and mainland Japan, 4 specimens of *C. grandirostris* from Taiwan and Okinawa, 3 recent specimens from Sulawesi, 1 old collection specimen from Bali and 1 sequence retrieved from GenBank for *C. brachydactyla*, 11 recent specimens from different localities, 4 type specimens from Sulawesi, 1 old collection specimen from Flores and 2 sequences retrieved from GenBank for *C. gracilipes*, 5 type specimens for *C. coulaborensis*, 2 type specimens for *C. fritzi*, 6 recent specimens and 1 sequence retrieved from GenBank for *C. meridionalis*, 22 specimens from different localities for *C. appendiculata*, 2 sequences of *C. sp. indistincta E* retrieved from GenBank and finally, 1 old collection specimen of *C. longirostris* from Fiji (see details in Table 4.5.1).

Table 4.5.1: List of the specimens used in the genetic study.

Species	Locality	Previous ID	DNA voucher	Museum n°	Type status	GenBank n°	Reference
C. appendiculata	Australia		CA1708	MNHN-IU-2018-126		MH497543	This study
		C. sp. indistincta E	GU0170			AY795051	Page *et al*, 2005
			CA1665	MNHN-IU-2018-121		MH497530	This study
			CA1667	MNHN-IU-2018-118		MH497531	This study
			CA1668	MNHN-IU-2018-117		MH497532	This study
		C. sp. indistincta E	GUC186			DQ478526	Page *et al*, 2007
			CA1669	MNHN-IU-2018-125		MH497533	This study
			CA1670	MNHN-IU-2018-124		MH497534	This study
	Micronesia		CA1333	MNHN-IU-2018-129		MH497517	This study
			CA1330	MNHN-IU-2018-130		MH497516	This study
	Palau		CA1021	MNHN-IU-2018-127		MH497499	This study
	Solomon Islands		CA1494	MNHN-IU-2018-135		MH497525	This study
			CA1493	MNHN-IU-2018-		MH497524	This study

				133		
	Vanuatu		CA1216	MNHN-IU-2018-151	MH497504	This study
			CA1139	MNHN-IU-2018-152	MH497503	This study
			CA1218	MNHN-IU-2018-153	MH497505	This study
			CA1219	MNHN-IU-2018-154	MH497506	This study
			CA1220	MNHN-IU-2018-155	MH497507	This study
			CA1264	MNHN-IU-2018-143	MH497511	This study
			CA1268	MNHN-IU-2018-144	MH497512	This study
			CA1489	MNHN-IU-2018-140	MH497523	This study
			CA1378	MNHN-IU-2018-142	MH497521	This study
			CA1259	MNHN-IU-2018-156	MH497509	This study
			CA1261	MNHN-IU-2018-157	MH497510	This study
C. brachydactyla	Bali		CA037	NMB 1054a	MH497490	This study
	Sulawesi	Topotypical	CA1129	WK 63-10	MH497500	This study

		Topotypical	CA1130	WK 63-10-1	MH497501	This study
		Topotypical	CA1131	WK 63-10-3	MH497502	This study
C. brevidactyla	Fiji	*C. longirostris*	CA017	RMNH.CRUS.D.54674	MH497484	This study
			CA1426	MNHN-IU-2018-159	MH497522	This study
	New Caledonia		CA1785	MNHN-IU-2018-166	MH497546	This study
			CA1788	MNHN-IU-2018-167	MH497547	This study
			CA1007	MNHN-IU-2018-161	MH497498	This study
	Papua New Guinea	*C. nilotica brevidactyla*	GUC358		DQ478539	Page *et al*, 2007
		C. nilotica brevidactyla	GUC529		DQ478540	Page *et al*, 2007
	Solomon Islands		CA1305	MNHN-IU-2018-168	MH497514	This study
			CA1345	MNHN-IU-2018-172	MH497518	This study
			CA1346	MNHN-IU-2018-171	MH497519	This study
			CA1940	MNHN-IU-2018-185	MH497558	This study
			CA1302	MNHN-IU-2018-175	MH497513	This study

	Vanuatu		CA1307	MNHN-IU-2018-188		MH497515	This study
			CA1232	MNHN-IU-2018-191		MH497508	This study
C. gracilipes	?	*C. nilotica gracilipes*				NC_024751	Xu *et al*, 2016
	Australia		CA1689	MNHN-IU-2018-201		MH497538	This study
			CA1690	MNHN-IU-2018-202		MH497539	This study
			CA1695	MNHN-IU-2018-205		MH497541	This study
			CA1700	MNHN-IU-2018-203		MH497542	This study
			CA1694	MNHN-IU-2018-199		MH497540	This study
	Borneo		CA1673	WK		MH497535	This study
	Flores		CA029	NMB 1061f 1		MH497489	This study
	Lombok		CA1348	MNHN-IU-2018-207		MH497520	This study
	Sulawesi		CA023	ZMA.CRUS.D.102635 1	Paralectotype	MH497485	This study
			CA024	ZMA.CRUS.D.102635 2	Paralectotype	MH497486	This study
			CA025	ZMA.CRUS.D.102635 3	Paralectotype	MH497487	This study

			CA027	ZMA.CRUS.D.102635 5	Paralectotype	MH497488	This study
				ZMB 29016		FN995358	Rintelen *et al*, 2012
	Sri Lanka		CA1825	MNHN-IU-2018-210		MH497549	This study
			CA1826	MNHN-IU-2018-211		MH497550	This study
			CA1823	MNHN-IU-2018-212		MH497548	This study
	Taiwan		CA1674	WK		MH497536	This study
C. grandirostris	Indonesia	*C. brachydactyla*	GUCKZ252			DQ478528	Page *et al*, 2007
	Japan		CA1893	ZRC 2004.0530 1		MH497553	This study
			CA1891	ZRC 2004.0519 3		MH497551	This study
			CA1892	ZRC 2004.0519 4		MH497552	This study
	Taiwan		CA1676	WK		MH497537	This study
C. leucosticta	Japan		CA1912	MNHN-IU-2018-229		MH497556	This study
			CA1913	MNHN-IU-2018-230		MH497557	This study
			CA1894	ZRC 2004.0530 2		MH497554	This study
			CA1896	ZRC 2004.0530 4		MH497555	This study
C. meridionalis	New Caledonia	*C. coulaborensis*	CA064	NMB 1057a 2	Holotype	MH497497	This study

		CA1572	MNHN-IU-2018-226		MH497526	This study
		CA1573	MNHN-IU-2018-227		MH497527	This study
		CA1575	MNHN-IU-2018-219		MH497528	This study
	C. cf. meridionalis	GUC467			DQ478492	Page *et al*, 2007
		CA1726	MNHN-IU-2018-217		MH497544	This study
		CA1772	MNHN-IU-2018-228		MH497545	This study
	C. coulaborensis	CA048	NMB 1057d 2	Paratype	MH497491	This study
	C. coulaborensis	CA049	NMB 1057e 1	Paratype	MH497492	This study
	C. coulaborensis	CA050	NMB 1057e 2	Paratype	MH497493	This study
	C. coulaborensis	CA051	NMB 1057e 3	Paratype	MH497494	This study
	C. fritzi	CA052	NMB 1058c 1	Paratype	MH497495	This study
	C. fritzi	CA053	NMB 1058c 2	Paratype	MH497496	This study
		CA1610	MNHN-IU-2018-222		MH497529	This study

4.5.4 Results

Molecular results

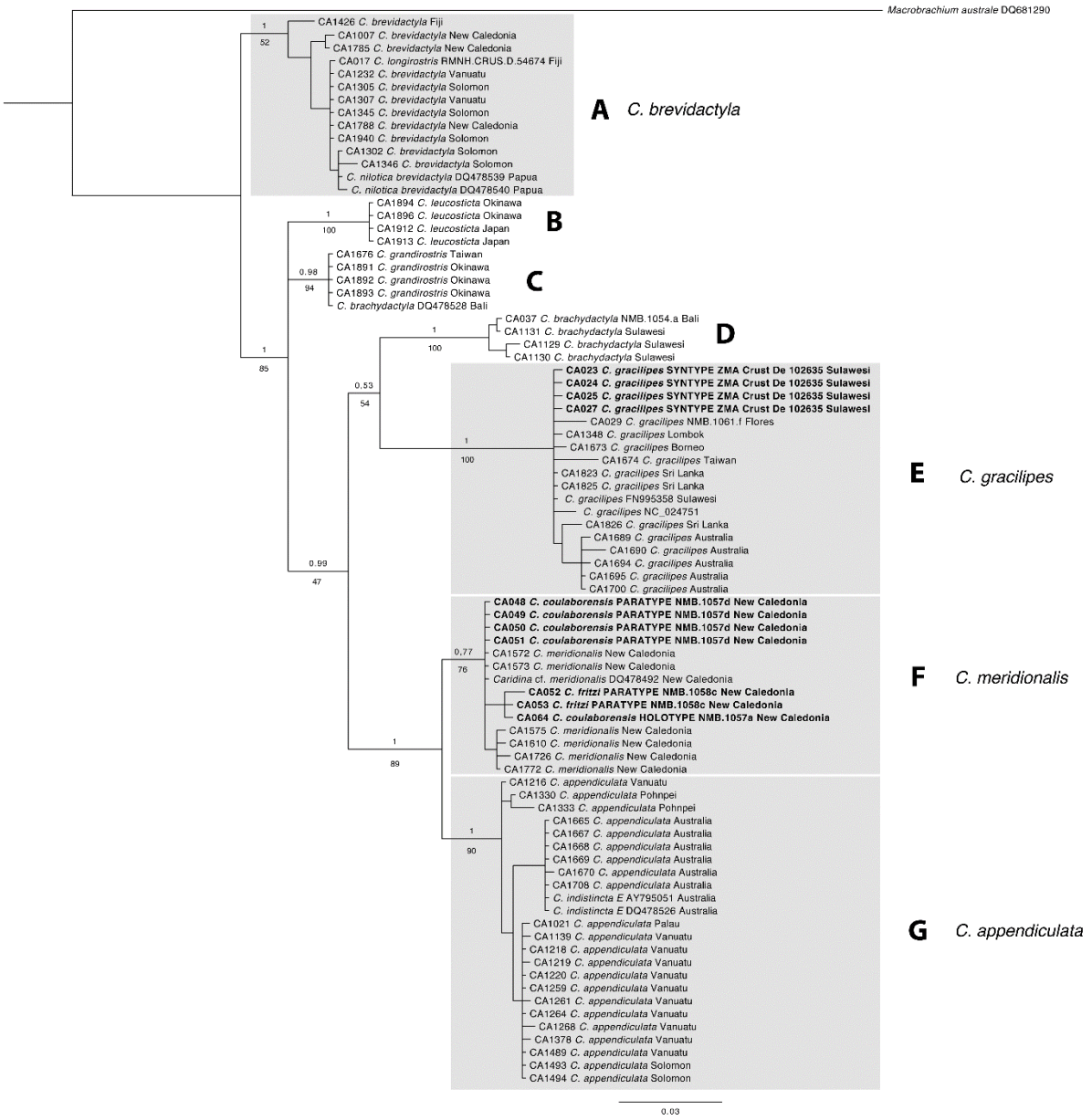


Figure 4.5.1: Bayesian 16S rRNA phylogenetic tree showing the relationships of some of the species discussed in this study. Numbers above branches indicate bayesian posterior probabilities; numbers under branches indicate Maximum Likelihood bootstrap values.

Due to their old age, no DNA in sufficient quantity and quality could be extracted using our method for the type specimens of *C. appendiculata*, *C. brachydactyla*, *C. brevidactyla*, *C. longirostris*, *C. meridionalis*, *C. peninsularis* and *C. solamulieres*; thus, no 16S sequence was amplified for these specimens. However, the approach was successful for type specimens of *C. coulaborensis*, *C. fritzi*, *C. gracilipes* and non-type specimen of *C. brachydactyla* from Indonesia and *C.* "*longirostris*" from Fiji. Furthermore, we tried to obtain recent specimens as close as possible to the type locality for *C. brachydactyla* (Palopo, Sulawesi, Indonesia) and *C. meridionalis* (New Caledonia). The genetic analysis shows 7 well-defined clades (Fig. 4.5.1). The first

clade (A in Fig. 4.5.1) is well supported in the Bayesian analysis (PP = 1) and comprises all the specimens identified as *C. brevidactyla* and a specimen refered as *C. longirostris* from Fiji. All the others are grouped in a clade sister–group to *C. brevidactyla* (Clade A). This clade is partially unresolved with a polytomy comprising a clade with the specimens of *C. leucosticta* (Clade B, PP = 1; B = 100), another with the specimens of *C. grandirostris* and a GenBank sequence of a specimen refered as *C. brachydactyla* (Clade C, PP = 0.98; B = 94), and a third with all the remaining specimens. This third clade, well supported in the Bayesian analysis (PP = 0.99), is composed of two clades, themselves each divided in two. On one side, a clade composed of recent and old specimens of *C. brachydactyla* (Clade D, PP = 1; B = 100) is sister-group to another with recent, old and type specimens of *C. gracilipes* (Clade E, PP = 1; B = 100). On the other side, a clade composed of recent specimens of *C. meridionalis*, type specimens of *C. coulaborensis* and type specimens of *C. fritzi* (Clade F, PP = 0.77; B = 76) is sister-group to the specimens of *C. appendiculata* and 2 specimens refered as *C. indistincta E* altogether (Clade G, PP = 1; B = 90).

Morphological study

The morphological examination of many specimens previously identified as *C. longirostris* from different localities allowed 5 species to be distinguished with support from the molecular data (Fig. 4.5.1): *C. longirostris*, *C. appendiculata* (Clade G), *C. brevidactyla* (Clade A), *C. gracilipes* (Clade E) and *C. meridionalis* (Clade F). An identification key and the diagnoses of these 5 species are given in the discussion below.

4.5.5 Discussion

This study provided molecular data from old type specimens of *C. gracilipes*, *C. coulaborensis* and *C. fritzi*, which will help for stabilize the taxonomy of this group. Indeed, the position of the type specimens of *C. gracilipes* in this phylogenetic analysis (Fig. 4.3.1) allowed the assignment of other specimens to this species.

Jean Roux (1926) described *C. nilotica meridionalis* from 7 localities in New Caledonia. These type specimens have been separated by Richard and Clark (2014) into five species: *C. meridionalis*, *C. peninsularis* and three new species: *C. coulaborensis*, *C.*

fritzi and *C. solamulieres*. Numerous specimens were collected in New Caledonia by the Pedcal Expedition in 1991, by Chloe Expeditions between 1997 and 2002, by two of us (VM and GM) in September 2016 and by one of us (VM) in November 2016 and November 2017. By examining them and considering the molecular results (Clade F, Fig. 4.5.1), *C. coulaborensis, C. fritzi and C. solamulieres* are considered to be junior synonyms of *C. meridionalis*. Indeed, specimens captured near the estuary, show a long rostrum with a distal unarmed end, rather characteristic of *C. coulaborensis*, whereas specimens captured in middle course of rivers possess a shorter rostrum with teeth present on the dorsal margin to the tip, rather characteristic of *C. meridionalis* or *C. fritzi*. The single specimen of *C. solamulieres* would be an aberrant specimen of *C. meridionalis*, with an unusually short rostrum. Indeed, when visiting the type locality (VM & GM, 2016), no specimen with this morphology was found.

Some specimens that were incorrectly identified before their sequence was submitted to GenBank can now be attributed to the correct species. RMNH.CRUS.D.54674 from Fiji, identified as "*C. longirostris*", was nested within our *C. brevidactyla* specimens. Furthermore, the GenBank sequence DQ478528 attributed to "*C. brachydactyla*" and collected in Bali, is *C. grandirostris*, together with sequences of "*C. indistincta E*" from Australia are assigned to *C. appendiculata*. Some sequences, however, provided valuable information about the geographical range of species, such as the two sequences of *C. brevidactyla* from specimens collected in Papua New Guinea, a locality not sampled in the present study.

The shorter DNA fragments obtained for old collection specimens introduced a significant amount of missing data to the matrix, which explains the lower support values in the tree for some of the clades that contained such sequences. However, the analyses made without the short sequences yielded the same topology.

The analysis was made from a single marker, the 16S rRNA. In these conditions, the phylogenetic relationships shown in the tree are to be taken with caution. Using more markers and combining mitochondrial and nuclear data would allow getting a more resolved and more supported tree. However, this analysis showed that the species are distinct, each of them being clearly separated from the others, a result unlikely to be disproved by further analyses.

The C. nilotica *complex*

Taxa assigned to the "*C. nilotica* complex" display incredible morphological variation between populations of the same species, so the characters given here take into account this intra-specific variability. Some characters frequently used to separate *Caridina* species are 1) the rostrum, 2) the proportions between the joints of pereiopods, 3) the presence or the absence of an appendix interna on the endopod of the first male pleopod, 4) the presence of a postero-median projection at the distal margin of the telson, 5) a preanal carina with or without a spine, or 6) the size of eggs. Generally, species should be discriminated based on a combination of characters instead of a single one. All the species treated in the present study are part of this complex that we consider to be biologically valid and not only an artificial group, as Bouvier (1925) meant it to be. Shared morphological characters are indeed present, such as the slender morphology, with a mostly long rostrum (longer than the antennular peduncle), the antennal spine ventral to the inferior orbital angle, a long antennular peduncle (subequal to carapace length), long leg segments, a typical dorsal hump over the third abdominal somite, a long sixth abdominal somite (always more than half of carapace length), a small pre-anal carina bearing sometimes an acute spine, a moderate number of spinules on the uropodal diaeresis (<15), few medium to short terminal simple setae on the telson and a rounded endopod of the first male pleopod with or without an appendix on the subdistal outer margin or even placed at the distal end.

The rostrum

The rostrum length plasticity in atyid shrimps (Jugovic *et al.*, 2010) is, according to Mazancourt *et al.* (2017a) linked to the environment. In the present study, specimens of *C. meridionalis* captured near the estuary, showed a long rostrum with a remarkable proportion of the rostrum unarmed between proximal and apical teeth, while specimens caught in the middle course of rivers showed a shorter rostrum with teeth present on the dorsal margin to the tip. But, the number of teeth and their placement on the rostrum (Richard & Clark, 2014) remains an important identification character. For example, *C. appendiculata* was moved by Cai & Ng (2007) from the *C. gracilirostris* species group to the *C. nilotica* species group due to the presence of postorbital teeth on the rostrum, a diagnostic character of this group. Indeed, *C. appendiculata* (1–3), *C.*

longirostris (1–2), *C. brevidactyla* (1–3), *C. gracilipes* (1–2) and *C. meridionalis* (1–3) have postorbital teeth like all *C. nilotica* group species. *Caridina neglecta* however, is maintained in its original species group because it does not possess postorbital teeth, a diagnostic character of species in the *C. gracilirostris* group. The rostrum has always some subapical teeth: one for *C. longirostris*, 1–2 for *C. gracilipes*, and 1–3 for *C. appendiculata*, *C. brevidactyla* and C. *meridionalis.* The number of dorsal teeth on the rostrum is greater for *C. brevidactyla* (16–30), lower for *C. appendiculata* (12–18) and *C. longirostris* (14–17), intermediate for *C. gracilipes* (12–23) and *C. meridionalis* 16–26. The number of dorsal teeth on the rostrum before the first ventral tooth is greater for *C. brevidactyla* (11–15), *C. gracilipes* (9–15) and *C. meridionalis* (10–15) lower for *C. appendiculata* (8–12) and *C. longirostris* (9–12).

The proportions between pereiopod joints

In our study, P1 and P2 carpus length (respectively 1.4–1.8 and 4.0–4.2) was used to distinguish *C. longirostris* from all the other studied species. It is greater for *C. brevidactyla* (2.5–3.6 and 5.6–7.5), *C. appendiculata* (1.9–2.8 and 4.4–6.3) for *C. gracilipes* (1.7–2.6 and 4.4–6.3) and *C. meridionalis* (2.3–3.5 and 4.1–6.2).

The presence or the absence of an appendix interna on the endopod of the male first pleopod

We agree with Richard & Clark that identifying species based on the presence or absence of appendix interna seemed unwarranted. For example, in *C. gracilipes* the appendix interna on the endopod of the male first pleopod can be present or absent (our observation). However, *C. appendiculata*, *C. brevidactyla* and *C. meridionalis* always show an appendix interna unlike *C. longirostris* in which it is always absent. The presence or absence of the appendix interna in adult specimens could be linked to seasonal variation, depending on the breeding period. During the winter, when the conditions are less favorable, the appendix interna would disappear, to grow again when the temperature rises in the summer. This could explain the absence of this organ in *C. longirostris*, since the species is known from a single collecting event, supposedly during the winter (see below) in an area where seasonality is more marked than the rest of the

geographical range of *Caridina* (Egypt). However, ovigerous females are common among the type material of *C. longirostris*, raising doubts about the breeding condition of this population/species during winter. Same thing for *C. gracilipes*, in our material, males without appendix interna were found along with ovigerous females. Furthermore, what would be the evolutionary advantage of reducing and growing the appendix interna several times during the life of the animal? Would some species be able to breed without it? Further investigations should be made to understand the mechanisms of gain and loss of this feature in species where its presence is variable like *C. gracilipes*.

The telson

The telsons of *C. appendiculata*, *C. brevidactyla*, *C. longirostris* and *C. gracilipes* have a posterior margin ending in a median point. *Caridina meridionalis* however, is variable in that the posterior margin of the telson does not always end in a medial point. Although the median point is absent in *C. longirostris*, the posterior margin of the telson is sharply triangular compared to relatively triangular in the other species.

The preanal carina spine

Caridina appendiculata, *C. gracilipes, C. meridionalis* and *C. longirostris* all possess a spine on the preanal carina, which is absent in *C. brevidactyla*. This character is regarded as a significant character.

Egg size

Although all studied species were considered to have small eggs, their size is regarded as a significant character. *Caridina meridionalis* had the largest eggs at 0.34–0.75 × 0.24–0.48mm, then intermediate for *C. brevidactyla* (0.37–0.47 × 0.22–0.30mm), *C. appendiculata* (0.38–0.46 × 0.23–0.28mm), *C. gracilipes* (0.32–0.46 × 0.18–0.27mm), and the smallest for *C. longirostris* (0.33–0.39 × 0.21–0.26mm). The size of eggs of *C. meridionalis* is however smaller near the estuary and larger in the middle course of rivers. Also differences in egg size between riverine and lacustrine specimens were reported for *C. gracilipes* (Han *et al.*, 2011).

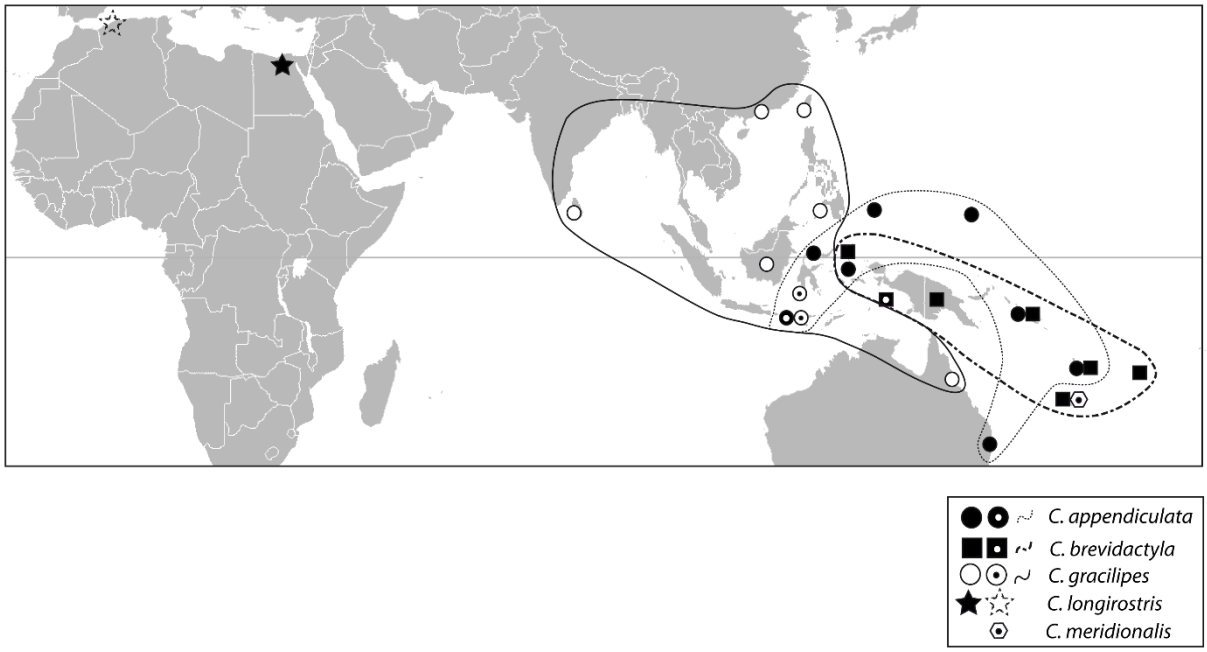


Figure 4.5.2: Distribution map of the five species re–described in this study. Shapes marked with a dot indicate type localities. For *C. longirostris*, the dashed star indicates the previously accepted type locality; the full star indicates the new proposed type locality.

Distribution

The present study considers that *C. longirostris* is not present in the following localities (Fig. 4.5.2).

Micronesia: This species was reported by Leberer & Nelson (2001) from Guam and Pohnpei by Maciolek & Ford (1987). Recently a new species, *C. variabilis* de Mazancourt, Rogers and Keith, 2018 was described from Palau and Guam and another from Pohnpei currently under consideration (Mazancourt *et al.* in press).

Melanesia: Short (2009) described *C. longirostris* with a rostral formula (5–30/2–22 vs 14–17/10–15) and P1 carpus (2–3 times long than broad vs 1.4–1.8), which suggests that this species does not occur in the Mimika Region, New Guinea. Three allied species occur in the Solomon Islands including *C. appendiculata* (Kolobangara), *C. brevidactyla* (Choiseul, Kolobangara, Vella Lavella) and a new species. *Caridina longirostris* is confused in Vanuatu (Keith *et al.*, 2010) until now with *C. appendiculata* or *C. brevidactyla*, in New Caledonia (Holthuis, 1969; Choy & Marquet, 2002; Marquet *et al.*,

2003) with *C. brevidactyla* and *C. meridionalis* J. Roux, 1926 and in Fiji (Choy, 1991) with *C. brevidactyla*.

Australia: According to Page *et al.* (2007), *C. longirostris* does not occur in Australia. Specimen DQ478497 identified as *C. gracilirostris* from north Johnstone River is in fact *C. gracilipes*.

West Pacific: The report of Chace (1997) from Philippines is either *C. elongapoda* Liang and Yan, 1977 or *C. gracilipes* (see Cai & Shokita 2006). The specimens reported by Holthuis (1978a) from Sumba and by Schenkel (1902) from Sulawesi (Indonesia) and by Hung *et al.* (1993) from Taiwan are *C. gracilipes*. The latter species is a common atyid shrimp in fresh and brackish waters of Taiwan (Ueno, 1935 ; H. P. Yu, 1974 ; Shy & Yu, 1998). Characters described by Hung *et al.* (1993) are 14–24 dorsal teeth on rostrum (13–20 in *C. gracilipes* vs 14–17 in *C. longirostris*), P1 carpus more than 2 times long as wide (1.7–3.4 in *C. gracilipes* vs 1.4–1.8 in *C. longirostris*), P2 carpus more than 5 times long as wide (4.4–6.4 in *C. gracilipes* vs 3.9–4.7 in *C. longirostris*).

In summary, *C. longirostris* is not present in Indo-West Pacific islands but some morphologically close species were identified: *C. appendiculata*, *C. brevidactyla*, *C. gracilipes* and *C. meridionalis* (see Fig. 2 for the distribution range of these species).

Key for the studied species

1.1 Preanal carina without spine.....*C. brevidactyla*

1.2 Preanal carina with spine.....2

2.1 P1 carpus mostly 1.7–1.8 times as long as wide and P2 carpus, mostly 3.9–4.2.....*C. longirostris*

2.2 P1 carpus mostly 1.9–3.6 times as long as wide and P2 carpus, mostly 4.3–6.5.....3

3.1 Relatively large eggs 0.34–0.75 × 0.24–0.48mm. Telson mostly rounded without a median process.....*C. meridionalis*

3.2 Small eggs 0.32–0.47 × 0.18–0.30mm, Telson always with a posterior margin triangular, ending in a posteromedian projection.....4

4.1 P3 propodus mostly 4.6–7.1 times as long as dactylus, P5 propodus mostly 4.5–6.2 times as long as dactylus.....*C. appendiculata*

4.2 P3 propodus mostly 3.5–4.5 times as long as dactylus, P5 propodus mostly 3.3–4.2 times as long as dactylus.....*C. gracilipes*

Taxonomy

Atyidae De Haan, 1849

***Caridina* H. Milne Edwards, 1837**

***Caridina longirostris* H. Milne Edwards, 1837**

(Figure 4.5.3)

Caridina longirostris – H. Milne Edwards 1837: 363; De Man, 1892b: 395–397, fig. 29l, m; 1908: 264–265, plate XX, fig. 5, 5a, 5b mm; Bouvier, 1905: 78, 1925: 149–151, fig. 313–314 (partim) 1925 : 150, fig. 314; Richard & Clark, 2009: fig. 4d, e, f.

Not *Caridina longirostris* – Bouvier, 1925: 151

Not *Caridina longirostris* – Bouvier 1925: 151 (partim, various localities); Holthuis, 1969: 94–97 (New Caledonia); 1978: 36 (Sumba); Maciolek & Ford, 1987: 628 (Pohnpei); Choy, 1991: 354 (Fiji); Hung *et al.,* 1993: 493–494, fig. 8 (Taiwan); Chace, 1997: 14, fig. 6, 7 (Philippines, Borneo, Sulawesi); Leberer & Nelson, 2001: 389 (Guam); Liang, 2004: 195, fig. 94 (China); Choy & Marquet, 2002: 217 (New Caledonia); Marquet *et al.*, 2003: 60–61 (New Caledonia); Page *et al.*, 2007: 647 (Australia); Short, 2009: 42, fig. 31 (Papua); Keith *et al.*, 2010: 42–43 (Vanuatu).

Material examined

Type material: Paralectotypes. MNHN Na 746, 3♂ cl 2.9–3.1mm; MNHN–IU–2013–19418, 2♂ 3.2–3.6mm; MNHN IU–2013–19417, 2♀ ovig. cl 3.1–3.3mm; from the River Macta, near Oran, Algeria.

Other material: **INDONESIA.** Not *C. nilotica longirostris* MNHN–IU–2015–1741, 1♂ cl 2.2mm; MNHN–IU–2015–1891, 1♂ cl 2.3mm, Makassar, Sulawesi.

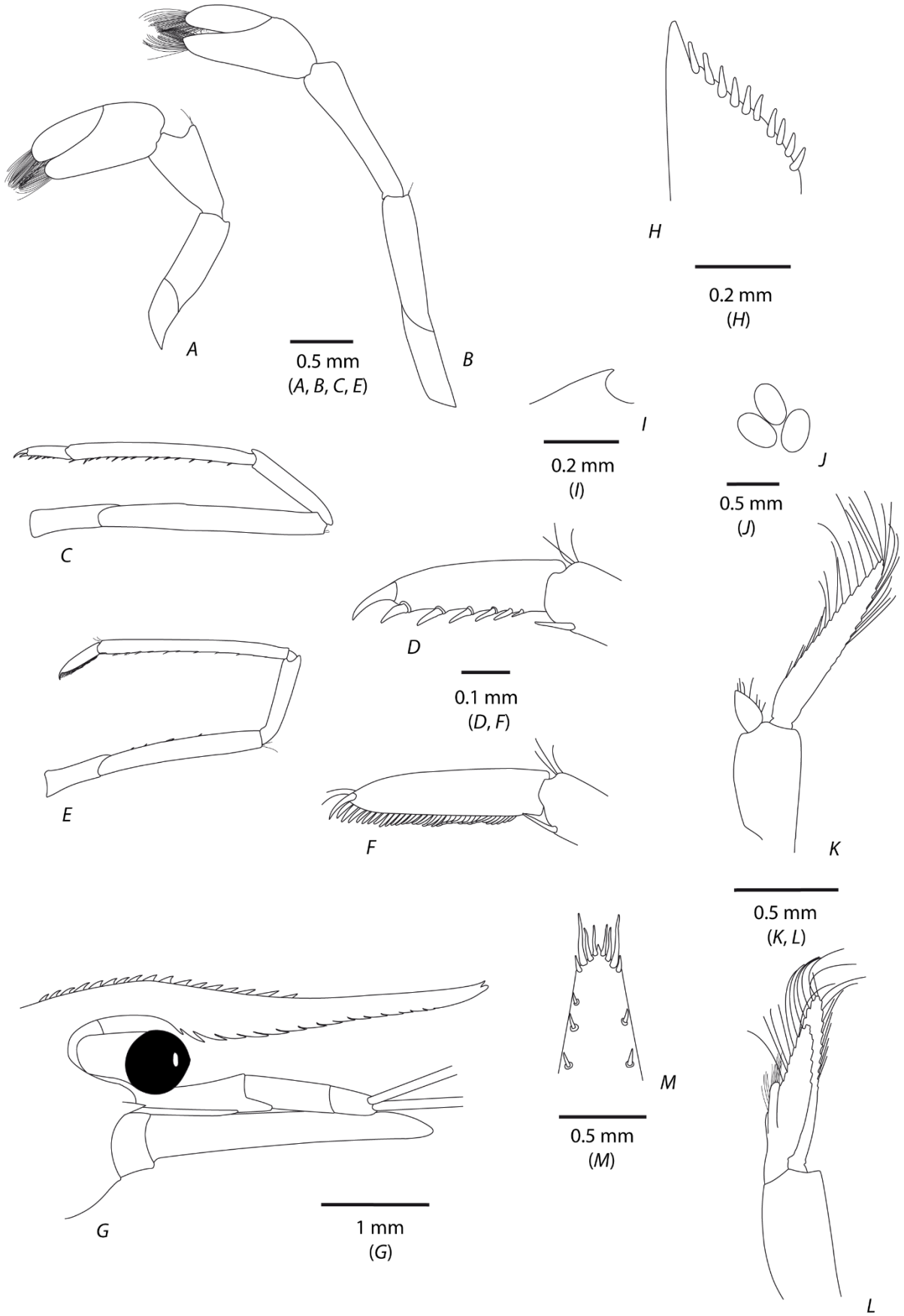


Figure 4.5.3: *Caridina longirostris*; (*A*) first pereiopod; (*B*) second pereiopod; (*C*) third pereiopod; (*D*) dactylus of third pereiopod; (*E*) fifth pereiopod; (*F*) dactylus of fifth pereiopod; (*G*) anterior region of cephalothorax; (*H*) uropodal diaeresis; (*I*) pre-anal carina; (*J*) eggs; (*K*) first male pleopod; (*L*) second male pleopod; (*M*)

Diagnosis

Carapace (Fig. 4.5.3g): Smooth, glabrous, with sharp antennal spine placed at lower orbital angle. Pterygostomian margin blunt. Rostrum long, 1.0–1.2 of cl, curved up distally, reaching well beyond scaphocerite. 14–17 dorsal teeth, leaving distally unarmed part of rostrum 0.7–1.1 times that of armed part, except for one or two subapical tooth, 1 to 2 post-orbital teeth present. 10–15 teeth present on the ventral margin extending from highest part of the rostrum either to tip or with short distal part unarmed. Number of dorsal teeth on the rostrum before the most proximal ventral tooth 9–12. Rostral formula: (1–2) 14–17 + 1–2 / 10–15.

First pereiopod (Fig. 4.5.3a): Stouter than second pereiopod; chela about 1.6–2.1 times as long as wide, dactylus 2.2–3.3 times as long as wide, 1.0–1.5 length of palm; carpus 1.4–1.8 times as long as wide with shallow excavation on anterior margin.

Second pereiopod (Fig. 4.5.3b): More slender and longer than first pereiopod; chela 1.9–2.4 times as long as wide, dactylus 3.0–3.6 times as long as wide, 1.3–1.6 times length of palm; carpus 3.9–4.7 times as long as wide.

Third pereiopod (Fig. 4.5.3c, d): Dactylus 4.0–4.8 times as long as wide (terminal spine included) with 7–9 spiniform setae on flexor margin in addition to the terminal one; propodus 11.3–14.3 times as long as wide, 3.4–4.0 times as long as dactylus.

Fifth pereiopod (Fig. 4.5.3e, f): Dactylus 4.5–5.1 times as long as wide with 32–38 spiniform setae on flexor margin; propodus 13.4–16.1 times as long as wide, 3.2–3.7 as long as dactylus.

Telson (Fig. 4.5.3m): Ending sharply triangular with a posteromedian projection; 1 pair of longer lateral simple setae and two pairs of clearly shorter intermediate simple setae.

First male pleopod (Fig. 4.5.3k): Endopod leaf-like without a developed appendix interna.

Second male pleopod (Fig. 4.5.3l): Appendix masculina on second pleopod reaching 0.62 times length of endopod; appendix interna reaching about 0.58 times length of appendix masculina.

Preanal carina (Fig. 4.5.3i) with a spine.

Uropodal diaeresis (Fig. 4.5.3h): with 9–12 short spinules.

Eggs (Fig. 4.5.3j): Size 0.36–0.40 × 0.22–0.30mm.

Colour pattern

Unknown

Distribution

According to Bouvier (1925), "Jusqu'ici les seuls représentants connus de cette variété sont les nombreux exemplaires types du *Caridina longirostris* qui, d'après H. Milne-Edwards, furent capturés dans la rivière de la Macta, près d'Oran, par M. Roux. Or, on ne connaît en Algérie qu'un Atyidé, *l'Atyaëphyra Desmaresti*, si bien qu'on peut dire, presque sans aucun doute que les types de Milne-Edwards proviennent d'une région tout autre que l'Algérie." ("Until now, the only representatives known for this variety are the numerous type specimens of *Caridina longirostris* which, according to H. Milne Edwards, were captured in La Macta River, near Oran, by M. Roux. However, we only know one Atyidae from Algeria, *Atyaëphyra Desmaresti*, so we can say almost without a doubt that Milne Edwards' type specimens come from a region other than Algeria."). He believed that they would have come from the Celebes area, as he found specimens identified as *C. nilotica gracilipes* by Schenkel (1902) from Makassar (Celebes Island) to be morphologically close to the type specimens (Bouvier, 1925). After having examined these specimens, we concluded that they were young individuals of *C. gracilipes*. Thus, the exact type locality remained unknown. We hypothesized that if the locality was erroneous on the specimen label, maybe the collector was not. The "M. Roux" indicated by H. Milne Edwards was his contemporary Jean Louis Florent Polydore Roux (1792–1833), more commonly referred to as Polydore Roux. The life and times of Polydore Roux are well documented including two published letters (1833, 1834a) addressed to a M. le baron de Férussac, an obituary (Barthélémy, 1834) and a biographical account (P.-M. Roux (son of Polydore) 1834). Accordingly, Polydore Roux was appointed the conservateur du Cabinet d'histoire naturelle de Marseille on the 1st June 1819. Consequently it would have been relatively easy for him to travel to Algeria and collect at "Rivière de la Macta, près d'Oran", but there is no mention of P. Roux visiting Algeria although there are several references that confirm he left France on 6 September 1831 with Baron Karl von Hugel and the pair arrived in Egypt towards the end of that month.

They both travelled widely in the region including up the Nile to Nubia (now in Sudan), along the coast of the Red Sea and part of Arabia; collecting specimens along the way. While in Egypt, P. Roux (1833) addressed a letter to M. le Baron de Férussac which was published by the Annales des Sciences Naturelles and figured *Pelias* (now *Caridina*) *niloticus* and *Palaemon niloticus* (now *Macrobrachium niloticum*). According to Richard and Clark (2005) the figured type specimen is no longer extant as P.-M. Roux later sold his father's collections to dealers such as Dupont in Paris. Furthermore, Baron Karl von Hugel and Polydore parted company in Egypt with P. Roux travelling up the Nile to Kené (now Qena or Qiná) and catching a caravan to Cosseyyre (Al Qusayr or Quseir) on the Red Sea. Here he caught a steamship to Bombay (now Mumbai). On 8 March 1832 he sailed through the Strait of Bab el Mandael on the coast of Abyssinia and a few days later arrived in Bombay. According to P.-M. Roux (1834), Polydore explored the chain of Ghats and collected freshwater Crustacea before he died of the plague in Bombay on 12 April 1833. We think that he may have collected other species of shrimps in Egypt that somehow ended later in the collections of the MNHN in Paris. There was at this time a locality named Arawat el Macta (Linant de Bellefonds, 1843), now called Hawwarat Al Maqta: 29°15.471'N 30°53.785'E) in the Faiyum Governorate crossed by a channel linking the Lake Qarun to the Nile River. We know that Roux visited the Faiyum Oasis in the winter 1831 (P.-M. Roux, 1834), so it is not unlikely that he collected shrimps from this locality that he preserved with the indication "El Macta". Henri Milne Edwards, examining specimens from this collect in 1837 would have assumed that the specimens came from the well-known Macta River in Algeria (where took place a military battle between French troops and the Algerian resistance just two years before), unaware of the existence of such a name in Egypt and was not able to get the locality corrected by P. Roux who died in 1833 in India. The probability is indeed much higher that *C. longirostris* is present in Egypt than in Algeria since *Caridina* exists in the Nile watershed, about a thousand kilometers of desert away from Oran. A new field trip to Hawwarat Al Maqta would allow to confirm or not the presence of *C. longirostris* in this locality and validate it as the type locality. Considering the locality, the only congeneric species present would be *C. nilotica*, a species that can morphologically be separated from *C. longirostris*. Furthermore, new samples of this species would allow to verify the morphological data provided here and obtain molecular data to place this species in the phylogeny and ascertain its validity.

Habitat

Unknown

Remarks

The paralectotypes re-examined for this study fit well with the description given by De Man (1908): rostrum long, reaching well beyond scaphocerite, curved up distally with presence of 0–2 postorbital teeth (vs 1–2 for present study), 14–21 dorsal teeth (vs 14–17 for present study) and 11–17 ventral teeth (vs 10–15 for present study); P1 carpus 1.5–1.9 times as long as wide (vs 1.4–1.8 for present study); P2 carpus 3.4–4.7 times as long as wide (vs 3.9–4.7 for present study). P3 dactylus with 7–10 spiniform setae (vs 7–9 for present study) propodus 3.4–3.7 as long as dactylus (vs 3.4–4.0 for present study); P5 dactylus with about 30–39 spiniform setae (vs 32–38 for present study), propodus 14.7–18 times long as width (vs 13.4–16.1 for present study) and propodus 3.2–3.5 times as long as dactylus (vs 3.2–3.7 for present study); uropodal diaresis 11–13 spinules (vs 9–12 for present study); small eggs 0.33–0.39 × 0.21–0.26mm (vs 0.36–0.40 × 0.22–0.30mm for present study). Some values given by Bouvier (1925) are also similar to the *C. longirostris* material examined for the present study: P3 dactylus with 7–10 spines (vs 7–9 for present study), P5 dactylus with about 29–38 spiniform setae (vs 32–38 for present study), uropodal diaeresis with 11–13 spinules (vs 9–12 for present study), small eggs 0.33–0.39 × 0.20–0.22mm (vs 0.36–0.40 × 0.22–0.30mm for present study). Also, our specimens fit well with the brief description given by Richard & Clark, (2009: 13) with a rostral formula (1–2) 14–20/13–16 (vs (1–2) 14–17/10–15 for present study), a telson which terminates in a sharply triangular posterior margin with a posteromedian projection and 3 pairs of distal spiniform setae, the lateral pair of which is distinctly longer than intermediates (see also Bouvier 1925: 150, fig. 315), and a preanal carina having a distinct spine.

Thus the morphology of *C. longirostris* is defined by its short P1 and P2 carpus respectively 1.4–1.8 and 3.9–4.7 times as long as wide. Consequently, *C. longirostris* could be separated from all the members of the *C. nilotica* group studied here: *C. appendiculata, C. brevidactyla*, *C. gracilipes* and *C. meridionalis* (see below).

According to Schenkel (1902: fig. 5a, pl. 8) examining specimens collected in Makassar, Sulawesi, the P1 of *C. gracilipes* appears similar to that of *C. longirostris* (De

Man, 1892b, fig. 20l, tab. XXIV). In addition, Schenkel (1902) indicates further similarities between these two species including P1 carpus 1.5 times as long as wide (vs 1.4–1.8 for *C. longirostris*, present study) and P5 dactylus with 27 spiniform setae (vs 32–38 for *C. longirostris*, present study). According to Bouvier (1925), specimens of *C. gracilipes* reported by Schenkel (1902) from Makassar include two young specimens of *C. longirostris* (MNHN–IU–2015–1741 and MNHN–IU–2015–1891, the P5 propodus being 3.5 times as long as dactylus like *C. longirostris* (vs 3.2–3.7 for *C. longirostris*, present study). These two specimens of Bouvier were re-examined. They are not *C. longirostris* because of their longer P1 and P2 carpus 2.1–2.2 (vs 1.4–1.8 for *C. longirostris*, present study) and 5.0–5.1 (vs 3.9–4.7 for *C. longirostris*, present study). The P3 propodus dimension of the Makassar specimens are 10.1–12.2 and are within the range variation of *C. gracilipes* (vs 10.2–16.0) but not for *C. appendiculata* (vs 13.8–22.7) and *C. brevidactyla* (vs 13.2–24.9). These two specimens from Makassar are considered to be juveniles of *C. gracilipes*.

***Caridina appendiculata* Jalihal and Shenoy, 1998**

(Figure 4.5.4)

Caridina appendiculata – Jalihal & Shenoy, 1998: 128; Klotz *et al.*, 2007: 7–9, figs. 3 (part), 4.

Caridina sp. E – Page *et al.*, 2005, 2007; Cook *et al.*, 2011.

Material examined

Type material: Lectotype (designated by Cai & Ng, 2007). ZMA.CRUS.D.102646, 1♂ cl 3.1mm; *Caridina gracilirostris* De Man, 1892, syntypes, river near Bari, Flores, Indonesia, coll. M. Weber, 1888. Paralectotypes. 1♂ cl 2.9mm, 3♀ cl 2.2–4.3mm; same data as lectotype.

Other material: **AUSTRALIA.** MNHN–IU–2018–117, 1♂ cl 2.8mm (DNA: CA1668) and MNHN–IU–2018–118, 1♂ cl 4.0mm (DNA: CA1667); River Hastings, Bain Bridge crossing near Crossroads, 31°26.150'S 152°42.283'E, coll. B. Mos, July 9, 2016. MNHN–IU–2018–119, 1♀ cl 3.1mm, MNHN–IU–2018–120, 1♀ cl 3.5mm, MNHN–IU–2018–121, 1♀ cl 4.8mm (DNA: CA1665), MNHN–IU–2018–122, 1♀ cl 5.4mm and MNHN–IU–2018–123, 1♂ cl 3.1mm; River Clarence, near Cowper/Brushgrove, 29°34.067'S 153°04.633'E, coll. B. Mos, July 2 2016. MNHN–IU–2018–124, 1♂ cl 2.8mm (DNA: CA1670) and MNHN–IU–2018–125, 1♂ cl 3.5mm (DNA: CA1669); Warrell Creek, 30°45.583'S 152° 53.217'E, coll. B. Mos, July 9 2016. **INDONESIA.** SULAWESI: ZMB 29000, 4♂ cl 3.4–3.7mm, 2♀ ovig. cl 4.3–5.1mm; Luwuk Peninsula, freshwater spring in Malontong, west of Ampana, brackish water pool, 0°53.125'S 121°31.371'E, coll. M. Glaubrecht, T. von Rintelen & K. Zitzler , May 27, 2005. OBIRA: ZMB 29001, 2♂ cl 3.4–3.5mm; West of Laiwui; 1°21.613'S 127°36'421. September 09, 2005. **PALAU.** MNHN–IU–2018–127, 1♀ ovig. cl 3.9mm (DNA: CA1021); Babeldaob, small stream East Coast, near the estuary, altitude 0m, 07°29.909'N, 134°38.099'E, coll. P. Keith, P. Gerbeaux, G. Marquet, L. Taillebois & M. Castelin, March 2, 2011. **MICRONESIA.** MNHN–IU–2018–128, 1♀ ovig. cl 3.2mm and MNHN–IU–2018–129, 1♀ ovig. cl 3.6mm (DNA: CA1333); River Nanpil, estuary, Pohnpei Island, altitude 5m, 06°56.609'N, 158°13.550'E, coll. P. Keith, P. Gerbeaux, G. Marquet, L. Taillebois & M. Castelin, March 13, 2012. MNHN–IU–2018–130, 1♀ ovig. cl 3.0mm (DNA: CA1330), MNHN–IU–2018–131, 1♀ ovig. cl 3.4mm and MNHN–IU–2018–132, 1♀ cl

3.1mm; unnamed river, estuary, Pohnpei Island, altitude 5m, 06°48.579'N, 158°12.639E, coll. P. Keith, P. Gerbeaux, G. Marquet, L. Taillebois & M. Castelin, March 15, 2012. **SOLOMON ISLANDS**. KOLOBANGARA: MNHN–IU–2018–133, 1♂ cl 3.5mm (DNA: CA1493) and MNHN–IU–2018–134, 1♂ cl 3.6mm; River Zamba, altitude 0m, 08°05.934'S 157°00.830'E, coll. P. Keith, G. Marquet & C. Lord, November 9, 2015. MNHN–IU–2018–135, 1♀ ovig. cl 4.0mm (DNA: CA1494), MNHN–IU–2018–136, 1♀ ovig. cl 4.1mm and MNHN–IU–2018–137, 1♂ cl 3.3mm; River Vanga, altitude 5m, 07°54.825'S 156°57.762'E, coll. P. Keith, G. Marquet & C. Lord, November 18, 2015. **VANUATU.** ANEITYUM: MNHN–IU–2018–138, 1♀ cl 3.7mm; River Inuje, altitude 0m, 20°13.321'S 169° 45.899'E. coll. C. Lord & G. Segura, June 25, 2015. MNHN–IU–2018–139, 1♀ 3.8mm and MNHN–IU–2018–140, 1♂ 2.5mm (DNA: CA1489) River Inwe Lengei, altitude 9m, 20°13.915'S 169°47.385'E, coll. C. Lord & G. Segura, June 24, 2015. EPI: MNHN–IU–2018–141, 1♀ ovig. cl 4.5mm; River Buavinai, 16°48.189'S 168°11.079'E, coll. P. Keith, G. Marquet & M. Mennesson, October 27, 2014; . EFATE: MNHN–IU–2018–146, 1♀ ovig. cl 4.6mm, MNHN–IU–2018–143, 1♀ ovig. cl 4.7mm (DNA: CA1264), MNHN–IU–2018–144, 1♀ ovig. cl 4.7mm (DNA: CA1268), MNHN–IU–2018–145, 1♀ ovig. cl 4.8mm, MNHN–IU–2018–147, 1♀ cl 4.7mm, MNHN–IU–2018–148, 1♀ cl 4.9mm, MNHN–IU–2018–149, 1♂ cl 3.4mm and MNHN–IU–2018–150, 1♂ cl 3.8mm; Creek Ai, altitude: 2m, 17°36.627'S 168°14.525'E, coll. P. Keith, G. Marquet & M. Mennesson, November 09, 2014. MNHN–IU–2018–156, 1♀ ovig. cl 4.2mm (DNA: CA1259) and MNHN–IU–2018–157, 1♀ ovig. cl 4.6mm (DNA: CA1261); Creek Marona, altitude 5m, 17°33.860'S 168°17.140'E, coll. P. Keith, G. Marquet & M. Mennesson, November 9, 2014. SANTO: MNHN–IU–2018–158, 1♀ cl 4.2mm; Jackie's Blue Hole, 15°18.788'S 167°10.208'E, coll. P. Keith & G. Marquet, July 22, 2003.

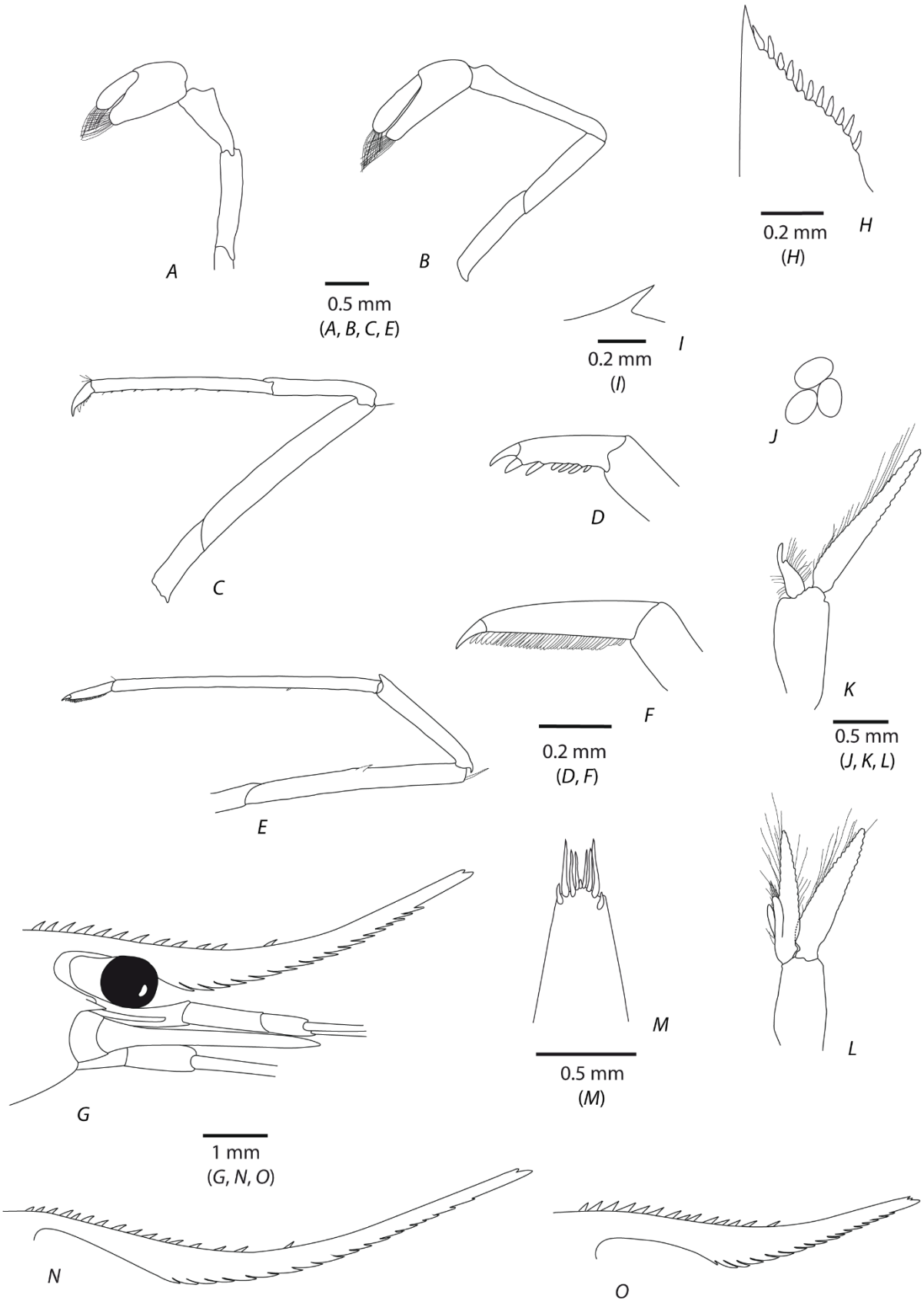


Figure 4.5.4: *Caridina appendiculata*; (*A*) first pereiopod; (*B*) second pereiopod; (*C*) third pereiopod; (*D*) dactylus of third pereiopod; (*E*) fifth pereiopod; (*F*) dactylus of fifth pereiopod; (*G*) anterior region of cephalothorax; (*H*) uropodal diaeresis; (*I*) pre-anal carina; (*J*) eggs; (*K*) first male pleopod; (*L*) second male pleopod; (*M*) telson; (*N-O*) rostrum variations.

Diagnosis

Carapace (Fig. 4.5.4g, n, o) smooth, glabrous, with sharp antennal spine placed at lower orbital angle. Pterygostomian margin blunt. Rostrum long, 1.3–2.1 of cl, curved up distally, reaching well beyond scaphocerite. 12–18 dorsal teeth somewhat irregular spaces, leaving distally unarmed part of rostrum 0.5–1.2 times that of armed part, except for 1–3 subapical teeth, 1 to 3 post-orbital teeth present. 8–25 teeth present of the ventral margin extending from proximal end either to tip or with short distal part unarmed. Number of dorsal teeth on the rostrum before the first ventral tooth 8–12. Rostral formula: (1–3) 12–18 + 1–3 / 8–25.

First Pereiopod (Fig. 4.5.4a): Slender, chela about 1.7–2.4 times as long as wide, dactylus 3.1–4.3 times as long as wide, 1.1–1.7 length of palm; carpus 1.9–2.8 times as long as wide with shallow excavation on anterior margin.

Second pereiopod (Fig. 4.5.4b): More slender and longer than first pereiopod: chela 2.0–2.7 times as long as wide, dactylus 3.3–5.5 times as long as wide, 1.1–1.6 times length of palm; carpus 4.8–7.1 times as long as wide.

Third pereiopod (Fig. 4.5.4c, d): Dactylus 2.9–4.1 times as long as wide, terminal spine included) with 6–8 spines on flexor margin in addition to the terminal one; propodus 13.8–22.7 times as long as wide, 4.4–7.1 times as long as dactylus.

Fifth pereiopod (Fig. 4.5.4e, f): Dactylus 3.8–5.2 long as wide with 33–44 spiniform setae on flexor margin; propodus 13.7– 26.2 times as long as wide, 4.3–6.2 as long as dactylus.

Telson (Fig. 4.5.4m): ending in a posteromedian projection; 3 pairs of distal simple setae, lateral pair slightly longer than intermediate.

Preanal carina (Fig. 4.5.4i) with a prominent fingerlike backward striking tooth.

First male pleopod (Fig. 4.5.4k): Endopod leaf-like with well developed appendix interna.

Second male pleopod (Fig. 4.5.4l): Appendix masculina on second pleopod reaching 0.61–0.66 times length of endopod; appendix interna reaching 0.70–0.71 times length of appendix masculina.

Uropodal diaeresis (Fig. 4.5.4h) with 8–12 short spinules.

Eggs (Fig. 4.5.4j) size 0.38–0.46 × 0.23–0.28mm.

Colour pattern

Colour varies in live specimens and tends to match their background. The colour of the body is hyaline with many reds dots (Fig. 4.5.8a, b).

Distribution

Caridina appendiculata is now known from Australia (present study), Indonesia (Flores (Cai and Ng 2007), Obira (present study), and Sulawesi (Klotz *et al.* 2007)) Solomon Islands (Kolobangara, present study), Micronesia (Pohnpei, present study), Palau (present study) and Vanuatu (Aneityum, Efate, Epi and Santo, present study) (Fig. 4.5.2).

Habitat

All specimens were collected in a typical brackish water environment (brackish water pool or colonize the lower part of rivers, near the estuary).

Remarks

Our specimens of *C. appendiculata* agree well with the descriptions by Klotz *et al.* (2007) of this species from Sulawesi (Indonesia): long rostrum reaching well beyond scaphocerite 1.3–2.2 (vs 1.3–2.1 for present study), curved up distally with presence of 2–3 postorbital teeth (vs 1–3 for present study), 9–20 dorsal teeth (vs 12–18 for present study) and 13–29 ventral teeth (vs 8–25 for present study); P1 carpus 1.8–3.2 (vs 1.9–2.8 for present study); P2 carpus 5.0–7.7 (vs 4.8–7.1 for present study). P3 dactylus with 6–8 spines (vs 6–8 for present study) propodus 4.5–7.1 as long as dactylus (vs 4.4–7.1 for present study); P5 dactylus with about 37 spiniform setae (vs 33–44 for present study), propodus 16.0–26.7 times long as width (vs 13.7–26.2 for present study) and propodus 4.5–6.0 times as long as dactylus (vs 4.3–6.2 for present study); Pl1 endopod with a long appendix interna; preanal carina with a prominent fingerlike backward striking tooth; telson ending in a median point, 3 pairs of distal spiniform setae, lateral pair slightly longer than intermediate; uropodal diaeresis 9–13 spinules (vs 8–12 for present study) and egg size 0.41–0.46 × 0.16–0.26mm (vs 0.38–0.46 × 0.23–0.28mm for present study).

Our specimens with a long and upcurved rostrum and apical teeth were for a long time associated with the *C. gracilirostris* species group. According to Cai and Ng (2007), *C. appendiculata* was moved from the *C. gracilirostris* species group to the *C. nilotica* species group due to the presence of postorbital teeth (1–3) on the rostrum, a diagnostic character of the latter. Moreover, our specimens differed from this group by the larger number of teeth on the dorsal margin of the rostrum, 12–18 (vs 3–11 in the *C. gracilirostris* group), a lower number of ventral teeth, 8–25 (vs 19–36 in the *C. gracilirostris* group).

Comparison

In *C. nilotica* species group, *C. appendiculata* resembles *C. longirostris*, *C. brevidactyla*, *C. gracilipes* and *C. meridionalis*, particularly by the long rostrum with subapical teeth.

Caridina appendiculata can be distinguished from *C. longirostris* the carpus length in P1, 1.9–2.8 (vs 1.4–1.8 in *C. longirostris*); the carpus length in P2, 4.8–7.1 (vs 3.9–4.7 in *C. longirostris*), P3 with stouter dactylus 2.9–4.1 times as long as wide (vs 4.0–4.8 in *C. longirostris*); P3 propodus 4.4–7.1 times as long as dactylus (vs 3.4–4.0 in *C. longirostris*); P5 stouter with propodus 4.3–6.2 times as long as dactylus (vs 3.2–3.7 in *C. longirostris*).

Although difficult, *C. appendiculata* can be differenciated from *C. brevidactyla* by the prominent spine on the preanal carina (vs absent in *C. brevidactyla*); the lower number of teeth on the proximal part of the dorsal margin of the rostrum being 12–18 somewhat irregular spaced (vs 16–30 more closely set in *C. brevidactyla*); a lower number of dorsal teeth behind the first ventral teeth 8–12 (vs 11–15 in *C. brevidactyla*); a shorter P1 carpus, 1.9–2.8 (vs 2.5–3.6 in *C. brevidactyla*); a slightly lower number of spiniform setae on the dactylus of P5, 33–44 (vs 35–53 in *C. brevidactyla*); a more slender appendix masculina on second pleopod reaching 0.61–0.66 times length of endopod (vs 0.54–0.55 in *C. brevidactyla*); and a shorter appendix interna reaching 0.70–0.71 times length of appendix masculina (vs 0.77–0.81 in *C. brevidactyla*).

Caridina appendiculata differs from *C. gracilipes* in a higher number of ventral teeth on rostrum, 8–25 (vs 7–17 in *C. gracilipes*); a lower number of dorsal teeth behind the first ventral teeth, 8–12 (vs 9–15 in *C. gracilipes*); a more slender P3 propodus, 13.8–

22.7 times as long as wide (vs 10.2–16.0 in *C. gracilipes*); a slightly lower number of spiniform setae on the P5 dactylus, 33–44 (vs 34–58 in *C. gracilipes*) and a more slender P5 propodus, 4.3–6.2 times as long as dactylus (vs 3.3–4.5 in *C. gracilipes*).

Specimens of *C. appendiculata* examined for the present study only differ from *C. meridionalis* in a lower number of dorsal teeth on the rostrum, 12–18 (vs 16–26 in *C. meridionalis*); a lower number of dorsal teeth behind the first ventral teeth, 8–12 (vs 10–15 in *C. meridionalis*); a slightly lower number of spiniform setae on the P5 dactylus, 33–44 (vs 42–62 in *C. meridionalis*) and smaller eggs 0.38–0.46 × 0.23–0.28mm (vs 0.34–0.75 × 0.24–0.48mm in *C. meridionalis*).

***Caridina brevidactyla* Roux, 1920**

(Figure 4.5.5)

Caridina nilotica var. brevidactyla – J. Roux, 1920: 320–321, 1926a: 204–206.

Caridina brevidactyla – Cai & Ng, 2001: 671, fig. 5.

Material examined

Type material: Lectotype (here designated). NMB 4 VI b1, 1♂ cl 4.8mm; Sungi Manumbai, Wokam Island, Aru, April 14–15, 1908. Paralectotypes. NMB 4 VI a, 1♂ cl 3.3mm; Udjir Island, April 15, 1908. NMB 4 VI b, 2♀ cl 5.8–6.4mm; Sungi Manumbai, Wokam Island, Aru, April 14–15, 1908. NMB 4 VI c, 1♀ cl 5.7mm; Wokamar, Wokam Island, Aru; April 17, 1908. NMB 4 VI d, 2♀ ovig. cl 5.7mm; Kobroor Island, Seltutti, Aru; May 2, 1908.

Other material: **FIJI.** OVALAU: RMNH.CRUS.D.54674, 1♀ cl 4.6mm; River Lovoni, 17°41'20"S 178°47'45"W, coll. S. C. Choy, July 26, 1980. . TAVEUNI: MNHN–IU–2018–159, 1♂ cl 3.1mm (DNA: CA1426); Bouma National Heritage Park, altitude 13m, 16°49.599'S 179°52.557'W, coll. P. Keith & G. Marquet, February 26, 2013. UNKNOWN ISLAND: BPBM 3947, 1♀ cl 4.3mm; unknown locality, coll. C. H. Edmonson, 1933. **NEW CALEDONIA.** MNHN–IU–2018–160, 1♀ ovig. cl 4.3mm, MNHN–IU–2018–161, 1♀ cl 4.5mm (DNA: CA1007), MNHN–IU–2018–162, 1♀ cl 4.6mm and MNHN–IU–2018–163, 1♀ cl 5.0mm; River Tibarama, altitude 5m, 20°56.300'S 165°22.700'E, coll. G. Marquet & L. Taillebois, October 23, 2010. MNHN–IU–2018–164, 1♂ cl 4.0mm and MNHN–IU–2018–165, 1♂ cl 4.0mm; River Poueo, altitude 0m, 21°32.966'S 165°30.062'E, coll. G. Marquet & V. de Mazancourt, September 28, 2016. **SOLOMON ISLANDS.** CHOISEUL: MNHN–IU–2018–168, 1♀ ovig. cl 5.3mm (DNA: CA1305); River Gu'ma, altitude 50m, 07°01.764'S 156°49.899'E, coll. P. Keith, G. Marquet & M. Mennesson, October 17, 2014. MNHN–IU–2018–169, 1♀ ovig cl 5.0mm, MNHN–IU–2018–170, 1♀ ovig cl 5.1mm, MNHN–IU–2018–171, 1♀ ovig cl 5.7mm (DNA: CA1346) and MNHN–IU–2018–172, 1♀ ovig cl 5.8mm (DNA: CA1345); River Lokasereke, altitude 45m, 06°58.024'S 156°47.861'E, coll. P. Keith, G. Marquet & M. Mennesson, October 13, 2014. MNHN–IU–2018–173, 1♀ cl 4.4mm and MNHN–IU–2018–174, 1♂ cl 3.3mm; River Lopakare, upstream, altitude 50m, 07°01.613'S 156°45.567'E, coll. P. Keith, G. Marquet & M. Mennesson, October 20, 2014. MNHN–IU–

2018–175, 1♀ ovig. cl 5.0mm (DNA: CA1302), MNHN–IU–2018–176, 1♀ ovig. cl 5.3mm and MNHN–IU–2018–177, 1♂ cl 3.1mm; River Vorama, altitude 15m, 06°58.687'S 156°46.746'E, coll. P. Keith, G. Marquet & M. Mennesson, October 11, 2014. KOLOBANGARA: MNHN–IU–2018–178, 1♀ ovig. cl 4.2mm and MNHN–IU–2018–179, 1♀ ovig. cl 4.9mm; River Lodumoe, altitude 0m, 07°50.961'S 157°04.320'E, coll. P. Keith, G. Marquet & C. Lord, November 16, 2015. MNHN–IU–2018–180, 1♀ ovig. cl 3.7mm, MNHN–IU–2018–181, 1♀ ovig. cl 3.9mm and MNHN–IU–2018–182, 1♂ cl 3.8mm; River Vanga, altitude 5m, 07°54.825'S 156°57.762'E, coll. P. Keith, G. Marquet & C. Lord, November, 18, 2015. VELLA LAVELLA: MNHN–IU–2018–183, 1♀ ovig cl 4.6mm and MNHN–IU–2018–184, 1♂; River Maravari, 07°51.703'S 156°41.748'E, coll. P. Keith & C. Lord, October 31, 2016. MNHN–IU–2018–186, 1♀ ovig. cl 4.0mm and MNHN–IU–2018–187, 1♂ cl 4.2mm; River Wariassi, coll. P. Keith & C. Lord, October 29, 2016. **VANUATU.** EPI: MNHN–IU–2018–188, 1♀ cl 5.1mm (DNA: CA1307); River Buavinai, 16°48.189'S 168°11.079'E, coll. P. Keith, G. Marquet & M. Mennesson, October 27, 2014. EFATE: MNHN–IU–2018–189, 1♂ cl 3.0mm and MNHN–IU–2018–190, 1♂ cl 3.2mm; Mele stream, altitude 32m, 17°40.545'S 168°15.558'E, coll. P. Keith, G. Marquet & M. Mennesson, November 9, 2014. MALEKULA: MNHN–IU–2018–191, 1♀ ovig. cl. 4.3mm and MNHN–IU–2018–192, 1♀ cl 4.0mm; River Brenwe, 16°07.986'S 167°17.148'E, coll. P. Keith, L. Taillebois & C. Lord, November 20, 2008. SANTO: MNHN–IU–2018–193, 1♀ ovig. cl 5.0mm and MNHN–IU–2018–194, 1♀ ovig. cl 5.1mm; Jackie's Blue Hole, altitude 0m, 15°18.788'S 167°10.208'E, coll. P. Keith & G. Marquet, July 22, 2003.

Comparative material

Caridina grandirostris Stimpson, 1860

JAPAN. ZRC 2004–0519, 1♂ cl 3.4mm, 1♂ cl 3.8mm (DNA: CA1892), 1♀ ovig. cl 6.1mm and 1♀ cl 4.7mm (DNA: CA1891); upstream of Tima River, about 1–2 km from river mouth, Okinawa Island, Ryukyu Islands, 26°33.42'N 128°04.60'E, Cai, Ng, Taruse and Islam coll., June 11, 2000. ZRC 2004–0530, 1♀ ovig. cl. 5.0mm (DNA: CA1893); freshwater stream draining to a small patch of mangrove, Oura River, Okinawa Island, Ryukyu Islands, 26°33.48'N 128°02.48'E, coll. Cai *et al*, June 11, 2000. **TAIWAN.** WK, 1♂ cl 3.1mm (DNA: CA1676); Pingtung, river near Jialeshui Coastal rock formations, altitude 2m, 21°59.305'N 120°49.956'E, coll. W. and M. Klotz and M.-C. Liu, July 25, 2013.

Caridina leucosticta Stimpson, 1860

JAPAN. ZRC 2004–0530, 1♂ cl 3.1mm (DNA: CA1894), 1♂ cl 3.6mm, 1♂ cl 3.8mm, 1♀ ovig. cl 5.3mm and 1♀ ovig. cl 6.1mm (DNA: CA1896); freshwater stream draining to a small patch of mangrove, Oura River, Okinawa Island, Ryukyu Islands, 26°33.48'N 128°02.48'E, coll. Cai *et al*, June 11, 2000.

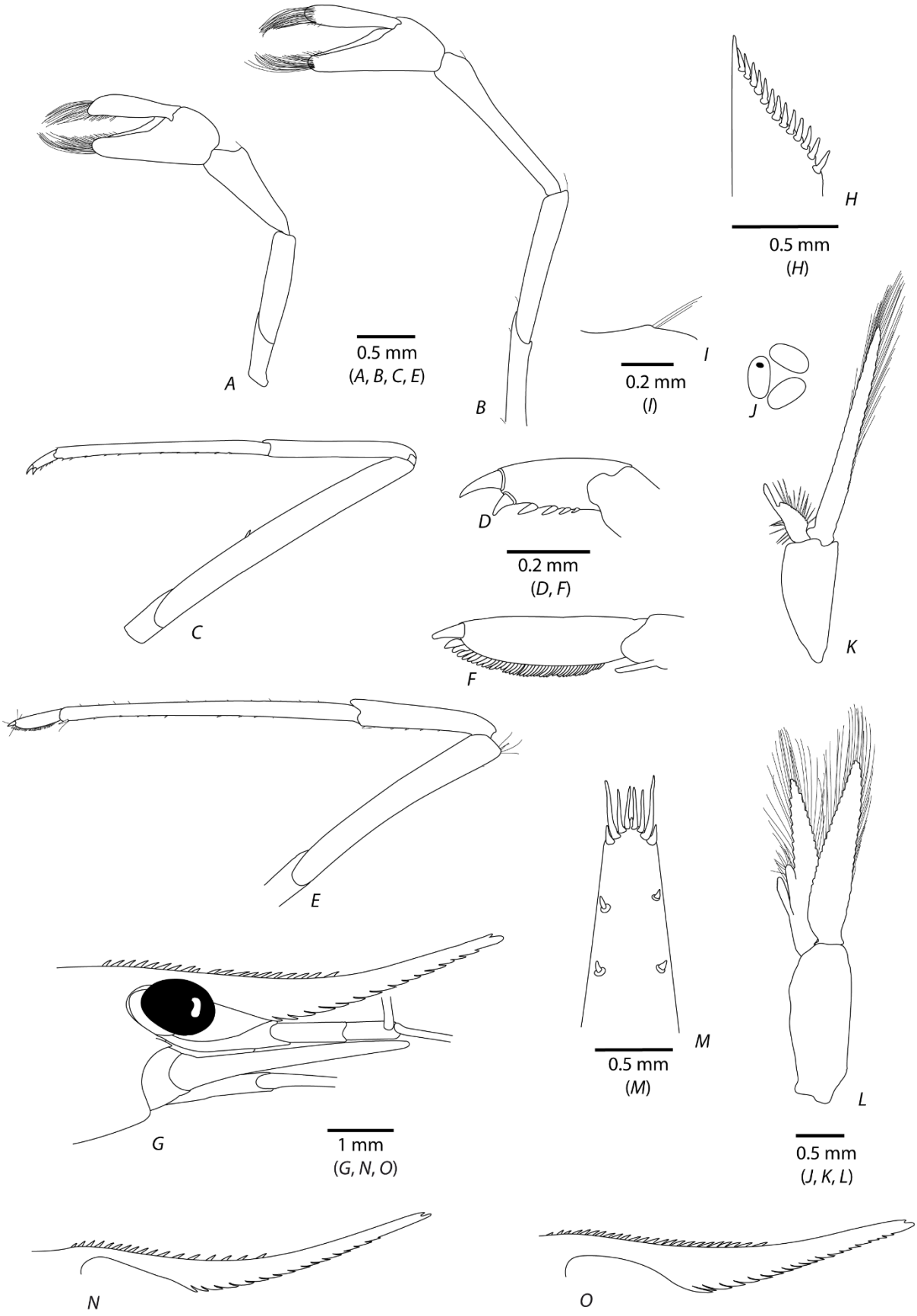


Figure 4.5.5: ***Caridina brevidactyla*****; (*A*) first pereiopod; (*B*) second pereiopod; (*C*) third pereiopod; (*D*) dactylus of third pereiopod; (*E*) fifth pereiopod; (*F*) dactylus of fifth pereiopod; (*G*) anterior region of cephalothorax; (*H*) uropodal diaeresis; (*I*) pre-anal carina; (*J*) eggs; (*K*) first male pleopod; (*L*) second male pleopod; (*M*) telson; (*N-O*) rostrum variations.**

Diagnosis

Carapace (Fig. 4.5.5g, n, o): Smooth, glabrous, with sharp antennal spine placed at lower orbital angle. Pterygostomian margin rounded. Rostrum long, 1.1–2.1 of cl, curved up distally, reaching well beyond scaphocerite. 16–30 dorsal teeth regular spaces, leaving distally unarmed part of rostrum 0.6–1.3 times that of armed part, except for 1–3 subapical teeth, 1 to 3 post-orbital teeth present. 12–24 teeth present of the ventral margin extending from proximal end either to tip or with short distal part unarmed. Number of dorsal teeth on the rostrum before the first ventral tooth 11–15. Rostral formula: (1–3) 16–30 + 1–3 / 12–24.

Pereiopod 1 (Fig. 4.5.5a): Slender, chela about 2.1–2.5 times as long as wide, dactylus 3.4–4.8 times as long as wide, 1.1–1.9 length of palm; carpus 2.5–3.6 times as long as wide with shallow excavation on anterior margin.

Pereiopod 2 (Fig. 4.5.5b): More slender and longer than first pereiopod, chela 2.6–3.5 times as long as wide, dactylus 4.6–6.8 times as long as wide, 1.3–2.1 times length of palm; carpus 5.6–7.5 times as long as wide.

Pereiopod 3 (Fig. 4.5.5c, d): Dactylus 2.8–4.6 times as long as wide, terminal spine included) with 4–8 spines on flexor margin in addition to the terminal one; propodus 13.2–24.9 times as long as wide, 4.7–8.4 times as long as dactylus.

Pereiopod 5 (Fig. 4.5.5e, f): Dactylus 3.6–7.4 long as wide with 35–53 spiniform setae on flexor margin; propodus 19.1– 29.1 times as long as wide, 4.7–6.2 as long as dactylus.

Telson (Fig. 4.5.5m) ending in a posteromedian projection; 3–4 pairs of distal simple setae, lateral pair longer than intermediate.

First male pleopod (Fig. 4.5.5k): Endopod leaf–like with well developped appendix interna.

Second male pleopod (Fig. 4.5.5l): Appendix masculina on second pleopod reaching 0.54–0.55 times length of endopod; appendix interna reaching 0.77–0.81 times length of appendix masculina.

Preanal carina (Fig. 4.5.5i): Without a spine.

Uropodal diaeresis (Fig. 4.5.5h): with 9–13 spinules.

Eggs (Fig. 4.5.5i): Size: 0.37–0.47 × 0.22–0.30mm.

Colour pattern

The colour of the body is hyaline with many reds dots. An oblique carmine red band on the cephalothorax is characteristic (Fig. 4.5.8c, d).

Distribution

Caridina brevidactyla is now known from Indonesia (Aru Island (Roux 1920), Halmahera (Cai & Ng 2001)), Solomon Islands (Kolobangara, Vella Lavella, present study), Vanuatu (Efate, Epi, Malekula and Santo, present study), New Caledonia and Fiji (present study) (Fig. 4.5.2).

Habitat

The specimens were collected in the lower reach of the rivers, rarely found in brackish conditions.

Remarks

The specimens examined for the present study fit well with the descriptions by Cai & Ng (2001) of this species from Halmahera (Indonesia): the long rostrum reaching well beyond scaphocerite curved up distally with the presence of 2–3 postorbital teeth (vs 1–3 for the present study); 19–25 dorsal teeth (vs 16–30 for the present study) and 18–23 ventral teeth (vs 12–24 for the present study); P1 carpus 2.2 (vs 2.5–3.6 for the present study); P2 carpus 5.8 (vs 5.6–7.5 for the present study); P3 dactylus with 5–6 spines (vs 4–8 for the present study); propodus 6.2 as long as dactylus (vs 4.7–8.4 for the present study); P5 dactylus with about 57 spiniform setae (vs 38–53 for the present study); propodus 6 times as long as dactylus (vs 4.7–6.2 for the present study); Pl1 endopod with a long appendix interna (vs Pl1 endopod with a long appendix interna for the present study); preanal carina without spine (vs without for the present study); telson ending in a median point, 7 distal simple setae, lateral pair longer than intermediate (vs telson ending in a median point, 3–4 pairs of distal simple setae, lateral pair longer than intermediate for the present study); uropodal diaeresis with 12 spinules (vs 9–13 for the present study); eggs size 0.41 × 0.25mm (vs 0.37–0.46 × 0.22–0.30mm for the present study).

The syntypes have an equal amount of specimens showing a preanal carina with a spine and without a spine and are likely a mix of species. For example the specimen NMB 4 VI c with 17 dorsal teeth on the rostrum somewhat irregularly spaced, 17 ventral teeth and 9 dorsal teeth on the rostrum behind the first ventral one is probably *C. appendiculata*.

Comparison

In *C. nilotica* species group, *C. brevidactyla* resembles *C. longirostris, C. appendiculata* (see above), *C. grandirostris*, *C. gracilipes* and *C. meridionalis* particularly by the long rostrum with subapical teeth.

Caridina brevidactyla is distinct from *C. longirostris*: the spine on the preanal carina present in *C. longirostris* is absent in *C. brevidactyla*, P1 carpus is longer, 2.5–3.6 (vs 1.4–1.8 in *C. longirostris*); P2 carpus longer 5.6–7.5 (vs 3.9–4.7 in *C. longirostris*); P3 dactylus stouter, 2.8–4.6 times as long as wide (vs 4.0–4.8 in *C. longirostris*); a longer propodus, 4.7–8.4 times as long as wide (vs 3.4–4.0 in *C. longirostris*); P5 stouter with propodus 4.7–6.2 times as long as dactylus (vs 3.2–3.7 in *C. longirostris*).

Choy (1991) described *C. longirostris* from Fiji (specimens RMNH 54674, 54691, 54733) and considered the specimens identified as *C. nilotica brachydactyla* (specimens BPBM S3941, S3947) collected by Edmondson (1935) as belonging to this species . Re-examination of all these specimens show that they belong to *C. brevidactyla* with the spine absent from the preanal carina (vs spine present on the preanal carina in *C. longirostris* and *C. brachydactyla*) and numerous dorsal teeth on the rostrum, 20–24 (vs 14–17 in *C. longirostris* and 16–22 in *C. brachydactyla*). Also some specimens from Vanuatu (Keith *et al.*, 2010) and New Caledonia (Marquet *et al.*, 2003) were confused with *C. longirostris*.

Specimens of *C. grandirostris* examined for the present study compare well with the material from Japan re-described by Cai *et al.* (2006) and this species is here reported from Taiwan (specimen wk04–13g3) for the first time. *Caridina brevidactyla* can be distinguished from *C*. *grandirostris* by the absence of a spine on the preanal carina (vs present in *C*. *grandirostris*); P1 carpus being longer, 2.5–3.6 (vs 2.4–2.8 in *C. grandirostris*).

Specimens of *C. leucosticta* examined for the present study compare well with the material from Ryukyu Islands, Japan and re-described by Cai *et al.* (2006) and like *C. brevidactyla*, the spine on the preanal carina is absent. However, *217C. brevidactyla*, when compared with *C. leucosticta*, have a longer P1 carpus, 2.5–3.6 (vs 2.2–2.4 in *C. leucosticta*); a slender P3 propodus, 13.2–24.9 times as long as wide (vs 12.4–15.7 in *C. leucosticta*) and a slender P5 propodus 19.1– 29.1 times as long as wide (vs 18.5–19.9 in *C. leucosticta*).

Specimens of *C. brevidactyla* examined for the present study are similar to *C. gracilipes* but differ in the absence of a tooth on the preanal carina (vs present in *C. gracilipes*); by the P3 propodus slender 4.7–8.4 times as long as dactylus (vs 3.5–4.9 in *C. gracilipes*) and by a P3 propodus slender 13.2–24.9 times long as width (vs 10.2–16.0 in *C. gracilipes*).

Our specimens of *C. brevidactyla* resemble *C. meridionalis* but can be distinguished by the tooth on the preanal carina always being absent (vs present in *C. meridionalis*); by a longer P2 carpus, 5.6–7.5 (vs 4.1–6.2 in *C. meridionalis*); by a slender P5 propodus, 4.7–6.2 times as long as dactylus (vs 3.2–5.3 in *C. meridionalis*), by a slender P5 propodus 19.1–29.1 times as long as wide (vs 15.3–22.7 in *C. meridionalis*) and by smaller eggs, 0.37–0.47 × 0.22–0.30mm (vs 0.34–0.75 × 0.24–0.48mm in *C. meridionalis*).

According to Richard & Clark (2010), *C. nilotica var. brevidactyla* is a junior synonym of *C. brachydactyla*. Nevertheless, agreeing with Cai & Ng (2001) we consider *C. brevidactyla* as a valid species. Indeed, the spine on the preanal carina present in *C. brachydactyla* is absent in *C. brevidactyla*.

***Caridina gracilipes* De Man, 1892**

(Figure 4.5.6)

Caridina Wyckii var. gracilipes – De Man, 1892b: 387, fig. 29a-e.

Caridina wyckii var. gracilipes – Schenkel, 1902: 498.

Caridina nilotica var. gracilipes – De Man, 1908: 270, pl. XX, fig. 7a-b; Kemp 1918: 275.

Caridina gracilipes – Cai, 2014: 208–209, figs 1–3; Cai & Shokita, 2006: 250.

Caridina gracilipes – Richard & Clark, 2014: 310–314, fig. 4-5.

Caridina longirostris – Bouvier, 1925: 151.

Caridina longirostris – Holthuis, 1978: 36.*C. nilotica var. gracilipes*, Roux, 1926a: 203–204 (partim).

Caridina brachydactyla – Richard & Clark, 2010: 308–310, fig. 3.

Material examined

Type material: **INDONESIA**. *Caridina gracilipes* De Man, 1892. Paralectotypes. ZMA.CRUS.D.102635, 1♂ cl 4.1mm (DNA: CA025), 1♀ ovig. cl 5.9mm (DNA: CA023), 3♀ cl 5.1mm (DNA: CA027), 5.2mm (DNA:CA024) and 5.6mm; in a rice field, Makassar, Sulawesi, coll. M. Weber, 1888. NMB 1061e, 1♂ 3.8mm; 2♀ cl 5.8–5.9mm; same data.

Other material: **AUSTRALIA.** MNHN–IU–2018–196, 1♀ cl 6.3mm and MNHN–IU–2018–197, 1♂ cl 3.7mm; River Mulgrave, near Aloomba, altitude 8m, 17°08.783'S 145°52.783'E, coll. B. Mos, June 07, 2016. MNHN–IU–2018–198, 1♀ ovig cl 4.6mm; Captain Cook Highway crossing, altitude 7m, 16°22.217'S 145°24.683'E, coll. B. Mos, June 14, 2016. MNHN–IU–2018–200, 1♀ ovig. cl 4.7mm; River Johnstone, near Innisfail altitude 7m; 17°30.317'S 145°59.517'E, coll. B. Mos June 11, 2016. MNHN–IU–2018–201, 1♂ cl 3.4mm (DNA: CA1689); River Barron, Kamerunga, altitude 6m, 16°52.450'S 145°40.817'E, coll. B. Mos June 08, 2016. MNHN–IU–2018–203, 1♂ cl 4.5mm (DNA: CA1700); Gordon's Creek, Cairns, altitude 15m, 16°57.967'S 145°43.833'E, coll. B. Mos, June 03, 2016. MNHN–IU–2018–204, 1♂ cl 2.9mm; River Mowbray, upstream of Diggers Bridge, altitude 18m, 16°33.800'S 145°27.850'E, coll. B. Mos, June 14, 2016. MNHN–IU–

2018–206, 1♀ ovig. cl 6.5mm; Tributary of Moody's Creek, near Mooroobool, altitude 18m, 16°56.550'S 145°43.700'E, coll. B. Mos, June 03, 2016. **INDONESIA.** NMB 1061f, 1♂ cl 2.9mm; 1♀ cl 4.5mm (DNA : CA029) and 1♀ cl 4.9mm; Nusa Tenggara Timu, Konga, Flores Island. MNHN–IU–2018–207, 1♂ cl 4.4mm (DNA: CA1348); Lombok Island. Not *C. longirostris* NMB 990a, 1♂ cl 4.1mm and 1♀ ovig. cl 5.9mm; Laiwuhi Brook, S. of Melolo, East Sumba, Sumba Island, coll. Sutter, May 31, 1949.

TAIWAN. WK, 1♂ cl 3.6mm (DNA: CA1674); Pingtung, river near Jialeshui Coastal rock formations, altitude 2m, 21°59.305'N 120°49.956'E, coll. W. and M. Klotz and M.-C. Liu, July 25, 2013.

BORNEO. WK, 1♀ cl 4.7mm (DNA: CA1673); from ornamental shrimp wholesaler in Singapore.

SRI LANKA. MNHN–IU–2018–210, 1♀ ovig cl 4.8mm (DNA: CA1825) and MNHN–IU–2018–211, 1♂ cl 3.2mm; Kandalama Reservoir, near to the shore in dead aquatic plants, altitude 184m, 07°52.612'N 80°41.503'E, coll. W. Klotz, February 8, 2017. MNHN–IU–2018–212, 1♀ cl 4.2mm (DNA: CA1823) and MNHN–IU–2018–213, 1♂ cl 2.8mm; small stream near Kekirawa, altitude 130m, 08°2.050'N 80°35.999'E, coll. W. Klotz February 8, 2017. MNHN–IU–2018–214, 1♀ ovig. cl 3.9mm and MNHN–IU–2018–215, 1♂ cl 2.6mm; brackish lagoon, near Maggona, 06°29.920'N 79°58.849'E, coll. W. Klotz, February 13, 2017.

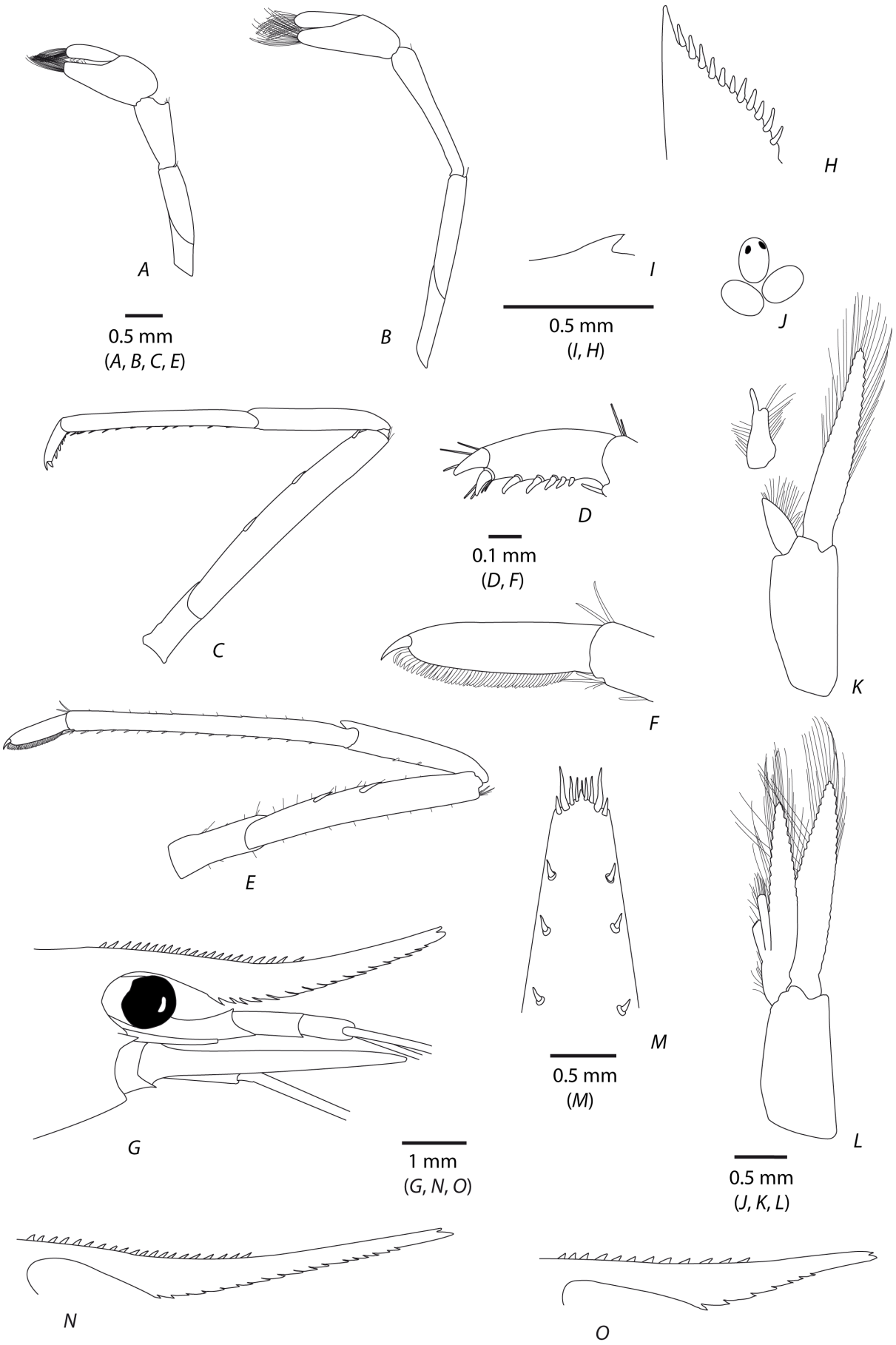


Figure 4.5.6: ***Caridina gracilipes*****; (*A*) first pereiopod; (*B*) second pereiopod; (*C*) third pereiopod; (*D*) dactylus of third pereiopod; (*E*) fifth pereiopod; (*F*) dactylus of fifth pereiopod; (*G*) anterior region of cephalothorax; (*H*) uropodal diaeresis; (*I*) pre-anal carina; (*J*) eggs; (*K*) first male pleopod; (*L*) second male pleopod; (*M*) telson; (*N-O*) rostrum variations.**

Diagnosis

Carapace (Fig. 4.5.6g, n, o): Smooth, glabrous, with sharp antennal spine placed at lower orbital angle. Pterygostomian margin blunt. Rostrum long, 0.8–1.5 of cl, curved up distally, reaching well beyond scaphocerite. 12–23 dorsal teeth closely set, leaving distally unarmed part of rostrum 0.5–1.2 times that of armed part, except for one or two subapical teeth, 1 to 2 post–orbital teeth present. 7–17 teeth present of the ventral margin extending from proximal end either to tip or with short distal part unarmed. Number of dorsal teeth on the rostrum behind the first ventral tooth 9–15. Rostral formula: (1–2) 12–23 + 1–2 / 7–17.

First pereiopod (Fig. 4.5.6a): Chela about 1.8–2.5 times as long as wide, dactylus 2.8–4.6 times as long as wide, 0.8–1.4 length of palm; carpus 1.8–2.6 times as long as wide with shallow excavation on anterior margin.

Second pereiopod (Fig. 4.5.6b): More slender and longer than first pereiopod, Chela 2.4–2.8 times as long as wide, dactylus 3.8–5.1 times as long as wide, 1.1–1.7 times length of palm; carpus 4.4–6.3 times as long as wide.

Third pereiopod (Fig. 4.5.6c, d): Dactylus3.2–4.6 times as long as wide, terminal spine included) with 7–9 spines on flexor margin in addition to the terminal one; propodus 10.2.2–16.0 times as long as wide, 3.3–4.5 times as long as dactylus.

Fifth pereiopod (Fig. 4.5.6e, f): Dactylus 4.4–6.6 long as wide with 34–58 spiniform setae on flexor margin; propodus 15.1–19.9 times as long as wide, 3.4–5.8 as long as dactylus.

Telson (Fig. 4.5.6m): Broad, posterior margin triangular, ending in a posteromedian projection; 1 pair of long lateral spiniform setae and 2–3 pairs of intermediate simple setae clearly shorter than lateral simple setae.

Firs male pleopod (Fig. 4.5.6k): Endopod leaf–like with or without appendix interna in males.

Second male pleopod (Fig. 4.5.6l): Appendix masculina on second pleopod reaching 0.62 times length of endopod; appendix interna reaching 0.73 times length of appendix masculina.

Preanal carina (Fig. 4.5.6i): With a spine.

Uropodal diaeresis (Fig. 4.5.6h): With 8–13 short spinules.

Eggs (Fig. 4.5.6j): Size: 0.32–0.46 × 0.18–0.27mm.

Colour pattern

The colour of the body is hyaline with many reds dots. An oblique punctiform band on the cephalothorax is visible (Fig. 4.5.8e, f).

Distribution

Caridina gracilipes is known from Australia (present study), Borneo (Wowor *et al.*, 2004; present study), Indonesia (Flores, Selayar, Sulawesi (De Man, 1892b) and Lombok (present study)), India (Kemp, 1918), Peninsular Malaysia (Wowor *et al.*, 2004), Philippines (Cai & Shokita, 2006), Singapore (Cai *et al.*, 2007), Southern China (Kemp, 1918; Cai, 2014), Sri Lanka (present study), Taiwan (Cai & Shokita, 2006; present study), Vietnam (Li & Liang, 2002) (Fig. 4.5.2).

Habitat

Caridina gracilipes can be found in lowlands rivers in freshwater, near estuaries in brackish water or in lakes.

Remarks

Specimens of *C. gracilipes* examined for the present study fit well with the descriptions by De Man (1908) of this species: the long rostrum reaching well beyond scaphocerite, curved up distally with presence of 1–2 postorbital teeth (vs 2 by De Man, 1908); 13–20 dorsal teeth (vs 13–20 by De Man, 1908) and 9–17 ventral teeth (vs 11–17 by De Man, 1908); P1 carpus 1.7–3.4 (vs 2–2.3 by De Man 1908); P2 carpus 4.4–6.4 (vs 5.4–5.5 by De Man, 1908); P3 dactylus with 6–9 spines (vs 9–10 by De Man, 1908); propodus 3.5–5.1 as long as dactylus (vs 3.8–4.4 by De Man, 1908); P5 dactylus with about 28–58 spiniform setae (vs 46–57 by De Man, 1908); propodus 14.3–27.4 times long as width (vs 17–19 by De Man, 1908) and propodus 3.2–5.4 times as long as dactylus (vs 3.7–3.8 by De Man, 1908).

Our specimens agree with the description given by Richard & Clark (2014), with a bifid slender rostrum 0.8–1.7 of cl (vs 1.0–1.1 by Richard & Clark, 2014); 13–20 dorsal teeth (vs 12–24 by Richard & Clark, 2014) and 9–17 ventral teeth (vs 12–21 by Richard

& Clark, 2014); P1 carpus 1.7–3.4 (vs 1.9–2.2 by Richard & Clark, 2014); P2 carpus 4.4–6.4 (vs 5.5–6.3 by Richard & Clark, 2014); P3 propodus 3.5–5.1 as long as dactylus (vs 3.5–5.3 by Richard & Clark, 2014); P5 dactylus 3.2–5.4 times long as wide (vs 3.3–4.3) with 28–58 spiniform setae (vs 55–70 by Richard & Clark, 2014); small eggs 0.32–0.42 × 0.20–0.18mm (vs 0.35–0.39 × 0.19–0.23mm by Richard & Clark, 2014); preanal carina with a spine;diaeresis with 9–13 spinules (vs 9–12 by Richard & Clark, 2014). Our material also compare well with the study of Cai & Shokita (2006) with 13–20 dorsal teeth rostrum (vs 11–27 by Cai & Shokita, 2006) and 9–17 ventral teeth (vs 8–18 by Cai & Shokita, 2006); P1 carpus 1.7–3.4 (vs 2.0–2.5 by Cai & Shokita, 2006); P2 carpus 4.4–6.4 (vs 5.4–6.3 by Cai & Shokita, 2006); P3 propodus 3.5–5.1 as long as dactylus (vs 3.8–4.4 by Cai & Shokita, 2006); P5 dactylus 3.6–5.3 times long as wide (vs 3.1–3.8 by Cai & Shokita, 2006) with 28–58 spiniform setae (vs 36–57 by Cai & Shokita, 2006); smaller eggs (0.32–0.43 × 0.18–0.25mm vs 0.42–0.53 × 0.23–0.33mm by Cai & Shokita, 2006); preanal carina with a spine and diaeresis with 9–13 spinules (vs 8–12 by Cai & Shokita, 2006).

Comparison

In comparison with *C. longirostris*, *C. gracilipes* has a similar rostral formula, (1–2) 12–23 + 1–2 / 7–17 (vs (1–2) 14–17 + 1 / 14–17 for *C. longirostris*) but the number of dorsal teeth on the rostrum behind the first ventral tooth is greater, 9–15 (vs 9–12 for *C. longirostris*); P1 and P2 carpus are respectively longer, 1.7–2.6 and 4.4–6.3 (vs 1.4–1.8 and 3.9–4.7 for *C. longirostris*) and P5 propodus 3.3–4.5 times as long as dactylus (vs 3.2–3.7for *C. longirostris*).

Caridina gracilipes is distinguishable from *C. meridionalis* by the P1 carpus being shorter, 1.7–2.6 (vs 2.3–3.5 in *C. meridionalis*) and smaller eggs, 0.32–0.46 × 0.18–0.27mm (vs 0.34–0.75 × 0.24–0.48mm in *C. meridionalis*).

***Caridina meridionalis* Roux, 1926**

(Figure 4.5.7)

Caridina nilotica var. meridionalis – J. Roux, 1926a: 207, partim.

Caridina meridionalis – Richard & Clark, 2014: 321–323, figs. 11-12.

Caridina coulaborensis – Richard & Clark, 2014: 324–326, fig. 13.

Caridina fritzi – Richard & Clark, 2014: 326–329, fig. 14.

Caridina solamulieres – Richard & Clark, 2014: 329–331, fig. 15.

Not *Caridina nilotica var. meridionalis* – Roux, 1926b: 246–247.

Material examined

Type material: Caridina meridionalis Roux, 1926. **NEW CALEDONIA**. Lectotype (designated by Richard & Clark, 2014). NMB 1056a, 1♂ cl 2.7mm; near Pemboa, coll. F. Sarasin and J. Roux, 1 May 1911; Paralectotypes. NMB 1056b. 1♀ ovig cl 4.2mm and 1♀ cl 4.1mm; same data. *Caridina coulaborenis* Richard and Clark, 2014. **NEW CALEDONIA.** Holotype. NMB 1057a, 1♂ cl 4.1mm (DNA: CA064); River Coula, near Boréaré, coll. F. Sarasin and J. Roux, February 6, 1912; Paratype 1♀ ovig. cl 5.0mm; Paratype. NMB 1057b, 1♀ cl 5.8mm; Paratypes. NMB 1057c, 1♂ 4.3mm and 1♀ cl 5.0mm; Koné, coll. F. Sarasin and J. Roux, August 09, 1911; Paratypes. NMB 1057d, 1♂ cl 2.6mm and 1♀ cl 4.4mm (DNA: CA048); La Foa, coll. F. Sarasin and J. Roux, January 16, 1912. *Caridina fritzi* Richard and Clark, 2014. **NEW CALEDONIA.** Holotype. NMB 1058a, 1♂ cl 2.8mm; River Coula, near Boréaré, coll. F. Sarasin and J. Roux, February 6, 1912; Paratypes. NMB 1058c, 2♂ cl 2.5mm (DNA: CA052) and 2.6mm (DNA: CA053); Coindé, coll. F. Sarasin and J. Roux, January 12, 1912. *Caridina solamulieres* Richard and Clark, 2014. **NEW CALEDONIA.** Holotype. NMB 1059a 1♀ cl 4.5mm; Tiouaka, coll. F. Sarasin and J. Roux, August 20, 1911.

Other material: **NEW CALEDONIA.** MNHN–IU–2018–216, 1♀ ovig. cl 4.2mm; River Iouanga, 20°41.032'S 164°23.676'E, coll. V. de Mazancourt, November 09, 2016. MNHN–IU–2018–217, 1♂ cl 3.7mm (DNA: CA1726); River Hienghène, 20°44.058'S 164°54.061E, coll. V. de Mazancourt, November 14, 2016. MNHN–IU–2018–218, 1♀ ovig. cl 4.0mm,

MNHN–IU–2018–219, 1♀ ovig. cl 5.0mm (DNA: CA1575) and MNHN–IU–2018–220, 1♂ cl 3.7mm; River Dumbea, altitude 4m, 22°08.332'S 166°28.013'E, coll. G. Marquet & V. de Mazancourt, November 16, 2016. MNHN–IU–2018–221, 1♀ ovig. cl 5.6mm and MNHN–IU–2018–222, 1♀ ovig. cl 6.5mm (DNA: CA1610); River La Foa, altitude 6m, 21°39.051'S 165°55.202'E, coll. G. Marquet & V. de Mazancourt, November 27, 2016. MNHN–IU–2018–223, 1♀ ovig. cl 5.3mm (DNA: CA1621); River Courrie, altitude 6m, S21°34.676' E165°29.716'E, coll. G. Marquet & V. de Mazancourt, November 28, 2016. MNHN–IU–2018–224, 1♂ cl 4.3mm; River Coula, altitude 240m, 21°21.656'S 165°20.370'E, coll. G. Marquet & V. de Mazancourt, November 26, 2016. MNHN–IU–2018–225, 1♂ cl 3.9mm; River La Couvelée, altitude 21m, 22°07.778'S 166°28.014'E, coll. G. Marquet & V. de Mazancourt, November 16, 2016.

C. nilotica var. gracilipes. NMB 1061h, 1♂ cl 3.7mm; River La Foa, coll. F. Sarasin and J. Roux, January 16, 1912; NMB 1061g, 1♀ ovig. cl 4.5mm, 2♀ cl 3.6-4.4mm and 2♂ cl 2.9–3.4mm; Koné, coll. Sarasin and Roux, August 9, 1911.

Caridina wyckii var. gracilipes De Man, 1892 **NEW CALEDONIA.** BPBM S5060, 1♀ cl 4.2mm, coll. F. X. Williams, 1940.

Comparative material: Caridina brachydactyla De Man, 1908 *Type material*: **INDONESIA.** *Caridina nilotica var. brachydactyla* De Man, 1908 Lectotype. RMNH.CRUS.D.977, 1♀ ovig cl 4.8mm; River near Reo, Flores Island, coll. M. Weber. Paralectotypes. RMNH.CRUS.D.2552, 2♀ ovig cl 5.3–5.4mm; river near Palopo, Luwu, Sulawesi, coll. M. Weber.

Other material: **INDONESIA.** *C. brachydactyla*: NMB 1054a, 1♀ cl 5.8mm (DNA: CA037); Bali, coll. P. Wirz, 1926. WK 63–10, 2♂ cl 2.7mm (DNA: CA1129) and 3.7mm (DNA: CA1130) 1♀ ovig cl 4.3mm and 1♀, cl 4.8mm; Palopo, Macaui, Sulawesi, 02°55.210'S 120°10.758'E, coll. W. Klotz, 2010; WK 64–10–3 1♀ ovig cl 4.0mm (DNA: CA1131), Palopo, Tojo, Sulawesi, coll. W. Klotz, 2010.

Not *Caridina nilotica var. meridionalis*. **AUSTRALIA.** NMB 1054d, 1♂ cl 4.7mm, 1♀ ovig cl 5.6mm and 2♀ cl 5.8–6.5mm; Cari Creek, Cook Town, N. Queensland, 1923.

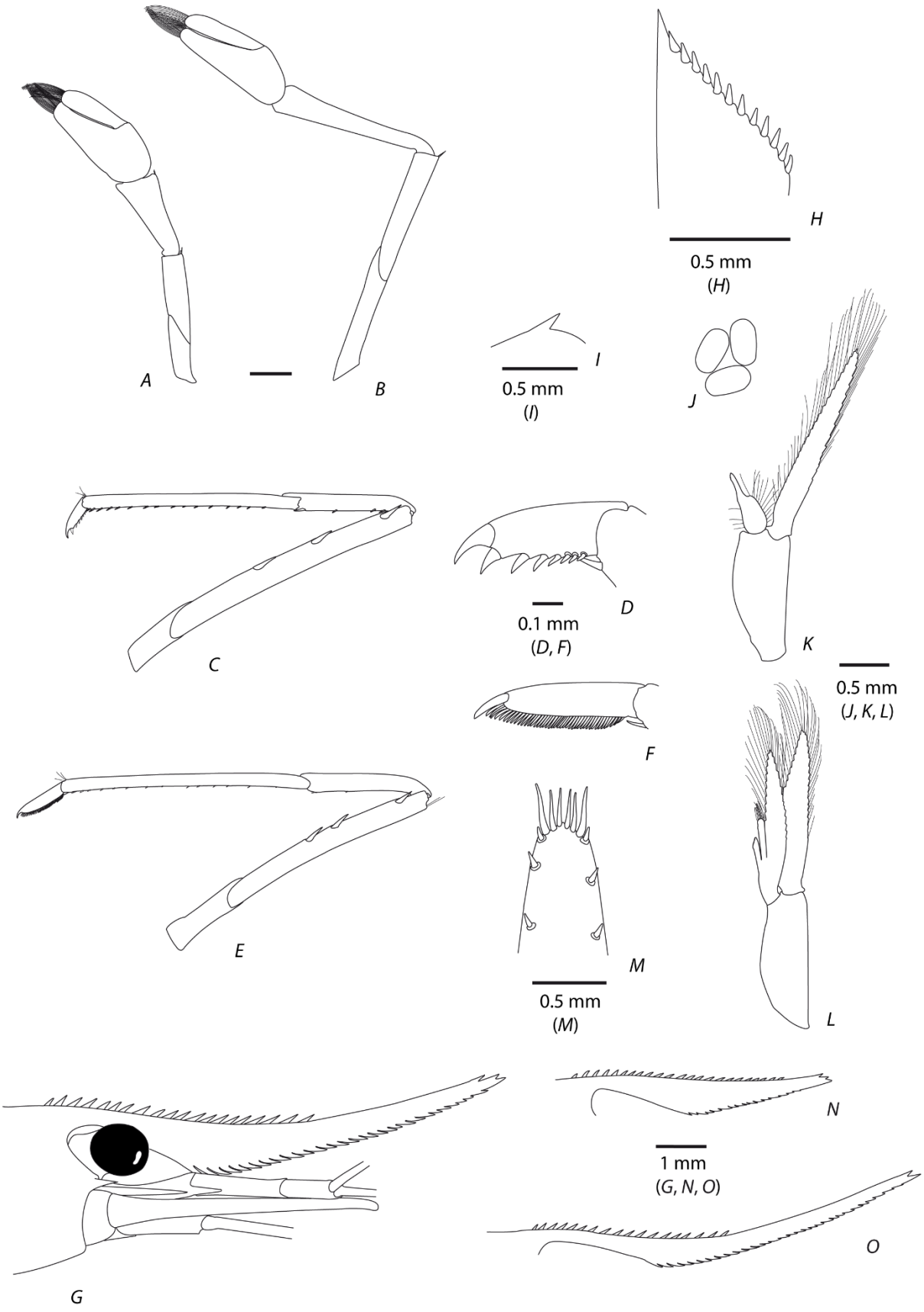


Figure 4.5.7: *Caridina meridionalis*; (*A*) first pereiopod; (*B*) second pereiopod; (*C*) third pereiopod; (*D*) dactylus of third pereiopod; (*E*) fifth pereiopod; (*F*) dactylus of fifth pereiopod; (*G*) anterior region of cephalothorax; (*H*) uropodal diaeresis; (*I*) pre-anal carina; (*J*) eggs; (*K*) first male pleopod; (*L*) second male pleopod; (*M*) telson; (*N-O*) rostrum variations.

Diagnosis

Carapace (Fig. 4.5.7g, n, o): Smooth, glabrous, with sharp antennal spine placed at lower orbital angle. Pterygostomian margin blunt. Rostrum variable, 0.8–2.1 of cl, reaching sometimes well beyond scaphocerite. 16–26 dorsal teeth, leaving distally unarmed part of rostrum 0.8–2.1 times that of armed part, except for 1–3 subapical teeth, 1–3 post–orbital teeth. 5–33 teeth on the ventral margin extending from proximal end either to tip or with short distal part unarmed. Number of dorsal teeth on the rostrum behind the first ventral tooth 10–15. Rostral formula: (1–3) 16–26 + 1–3 / 5–33.

First pereiopod (Fig. 4.5.7a): Slender, chela about 2.0–2.7 times as long as wide, dactylus 2.8–46 times as long as wide, 1.1–1.7 length of palm; carpus 2.3–3.5 times as long as wide with shallow excavation on anterior margin.

Secoond pereiopod (Fig. 4.5.7b): More slender and longer than first pereiopod; chela 2.1–3.4 times as long as wide, dactylus 3.5–5.7 times as long as wide, 1.2–1.9 times length of palm; carpus 4.1–6.2 times as long as wide.

Third pereiopod (Fig. 4.5.7c, d): Dactylus 3.2–4.3 times as long as wide, terminal spine included) with 6–9 spines on flexor margin in addition to the terminal one; propodus 12.4–19.8 times as long as wide, 3.9–5.8 times as long as dactylus.

Fifth pereiopod (Fig. 4.5.7e, f): Dactylus 4.4–6.6 times as long as wide with 42–58 spiniform setae on flexor margin; propodus 15.3–22.7 times as long as wide, 3.2–5.3 as long as dactylus.

Telson (Fig. 4.5.7m): Variable, mostly rounded without a median process, rarely triangular with a median process; 3–4 pairs of distal simple setae, intermediate setae slightly shorteror slightly longer than the lateral setae.

Preanal carina (Fig. 4.5.7i): Armed with a spine.

First male pleopod (Fig. 4.5.7k): Leaf–like endopod with well developped appendix interna.

Second male pleopod (Fig. 4.5.7l): Appendix masculina on second pleopod reaching 0.56 times length of endopod; appendix interna reaching 0.85 times length of appendix masculina.

Uropodal diaeresis (Fig. 4.5.7h): With 7–13 spinules.

Eggs (Fig. 4.5.7j): Size: 0.34–0.75 × 0.24–0.48mm.

Colour pattern

Colour varies in live specimens and tends to match their background. The colour of the body is hyaline with many reds dots. The coloration is lost on preserved material (Fig. 4.5.8g, h).

Distribution

Caridina meridionalis is endemic to New Caledonia (J. Roux, 1926a; present study) (Fig. 4.5.2).

Habitat.

All specimens were collected in rivers, from the estuary, in brackish water to the upper middle course, in freshwater.

Remarks

Our specimens of *C. meridionalis* agree with those of Richard & Clark (2014) by slender rostrum, length 0.8–2.1 times length of the carapace (vs 0.75–1 by Richard & Clark, 2014); with 16–26 dorsal teeth (vs 11–27 by Richard & Clark, 2014) and 5–33 ventral teeth (vs 4–17 by Richard & Clark, 2014); P1 carpus 2.3–3.5 (vs 1.8–2.4 by Richard & Clark, 2014); P2 carpus 4.1–6.2 (vs 4.5–5.5 by Richard & Clark, 2014); P3 propodus 3.9–5.8 times as long as dactylus (vs 5.0–7.0 by Richard & Clark, 2014); P5 dactylus 4.4–6.6 times long as wide (vs 2–4.2 by Richard & Clark, 2014) with 42–62 spiniform setae (vs 45–60 by Richard & Clark, 2014); larger eggs 0.34–0.75 × 0.24–0.48mm (vs 0.60–0.65 × 0.35–0.43mm by Richard & Clark, 2014); preanal carina with a spine and diaeresis with 7–13 spinules (vs 9–14 by Richard & Clark, 2014).

Jean Roux (1926b) described *C. meridionalis* from Australia. Agreeing with Richard & Clark (2014), these Australian specimens differ from the type specimens of *C. meridionalis* with respect to the following characters, short P1 carpus, 2.1–2.4 (vs 2.3–3.5 for types); short P3 dactylus, 3.1–3.4 (vs 3.2–4.3 for types); smaller eggs, 0.40–0.43 × 0.18–0.27mm (vs 0.34–0.75 × 0.24–0.48mm for types) and its triangular posterior margin with median process, bearing 1 pair of long lateral simple setae and 2 pairs

intermediate simple setae that are shorter than the laterals (vs mostly rounded without a median process rarely triangular with a median process; 3–4 pairs of distal spiniform setae, intermediate spiniform setae slightly shorter or slightly longer than the lateral spiniform setae). Contrary to Richard & Clark (2014), who considered them to be *C. brachydactyla*, we identified them as *C. gracilipes* because of their short P1 chela, 2–2.1 (vs 2.1–2.3 in *C. brachydactyla*); short P2 chela 2.4–2.9 (vs 2.7–3.2 in *C. brachydactyla*); P3 dactylus with 8–9 spines on the flexor margin in addition to the terminal one (vs 5–7 in *C. brachydactyla*) and P5 dactylus with 50–67 spiniform setae on the flexor margin (vs 32–42 in *C. brachydactyla*).

We observed a great variation in egg size (Table 4.3.2), with the specimens with the biggest eggs collected in the higher altitudes. This phenomenon has been shown in *Paratya australiensis* (Hancock, 1998) and is supposed to have appeared to maintain the population in the higher altitudes, preventing the drift of the larvae down to the sea.

Table 4.5.2: Comparison of the egg size of different specimens of *C. meridionalis*.

Specimen	Altitude (m)	Development stage	Size (mm)
NMB 1056b Lectotype of *C. meridionalis*	250	early stage	0.57–0.60 × 0.35–0.40
NMB 1061g 4	11	early stage	0.39–0.42 × 0.26–0.29
CA1575	0	early stage	0.46–0.50 × 0.26–0.27
CA1576	0	early stage	0.46–0.50 × 0.27–0.29
CA1609	0	eyes visible	0.45–0.49 × 0.26–0.28
CA1610	0	early stage	0.43–0.44 × 0.26–0.27
CA1621	0	early stage	0.35–0.40 × 0.24–0.26
CA1725	4	eyes visible	0.40–0.45 × 0.24–0.26
NMB 1057a Paratype of *C. coulaborensis*	250	early stage	0.66–0.76 × 0.42–0.48
NMB 1057c 1 Paratype of *C. coulaborensis*	250	early stage	0.72–0.76 × 0.45–0.49



Figure 4.5.8: Views of live specimens showing their coloration; (*A*, *B*) *C. appendiculata*; (*C*, *D*) *C. brevidactyla*; (*E*, *F*). *C. gracilipes*; (*G*, *H*) *C. meridionalis*. Credits: Philippe Keith: (*A*, *B*, *C*); Valentin de Mazancourt: (*D*, *G*, *H*); Werner Klotz: (*E*, *F*).

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4.6 Conclusion

Grâce à notre approche de taxonomie intégrative, une partie de la complexité taxonomique du complexe *C. nilotica* a été levée pour les espèces présentes dans la zone étudiée. Les différents cas de figure exposés en introduction de ce chapitre ont été illustrés ; des espèces validées par la taxonomie intégrative ; des espèces cryptiques mises en évidence ; ou encore des espèces confondues, séparées par cette approche. Il conviendra cependant d'étendre ce travail en appliquant la même méthode aux espèces vivant dans d'autres zones géographiques où le complexe est présent.

Nous avons vu que les espèces de ce complexe, que l'on pensait initialement limitées au cours inférieur des rivières, peuvent finalement se rencontrer à tous les niveaux des cours d'eau. En effet, le complexe ayant été caractérisé par un rostre long, et ce caractère étant fortement influencé par l'environnement (voir l'Effet Pinocchio du chapitre précédent), seuls les individus à rostre long étaient considérés comme faisant partie du complexe *C. nilotica*. Par conséquent, le complexe *C. nilotica* nécessite d'être redéfini et son statut monophylétique évalué pour qu'il ait une signification biologique et évolutive.

Thanks to our approach of integrative taxonomy, some of the taxonomic complexity of the *C. nilotica* complex has been cleared up for the species present in the studied region. The different cases exposed in introduction of this chapter were illustrated; species validated by integrative taxonomy; cryptic species exposed; or species previously confused, separated by this approach. It will be necessary, however, to extend this work using the same method to species living in other geographical areas where the complex is present.

We saw that species of this complex, that we first thought to be limited to the lower course of the rivers could be collected all along the river courses. Indeed, the complex was characterised by a long rostrum, and this character being strongly influenced by the environment (see the « Pinocchio-shrimp Effect » of the previous chapter), only the specimens with a long rostrum were considered members of the *C. nilotica* complex. Therefore, *C. nilotica* complex needs to be redefined and its monophyletic status assessed for it to have a biological and evolutionary meaning.

Chapitre quatrième, Taxonomie intégrative du complexe *C. weberi*.

Integrative taxonomy of the C. weberi *complex.*

Où l'on révise la taxonomie de quelques espèces du complexe Caridina weberi *dans deux zones géographiques distinctes ; premièrement, la Mélanésie, avec la mise en synonymie de deux espèces vivant dans le cours inférieur des rivières de la zone, et deuxièmement, la Polynésie, avec la révision des espèces confondues avec* Caridina weberi *rencontrées dans le cours supérieur des rivières.*

Where we revise the taxonomy of some species of the Caridina weberi *complex in two geographic areas; first, Melanesia, with the synonymy of two species living in the lower course of the rivers in the area; and second, Polynesia, with a revision of the species confused with* Caridina weberi *from the higher course of the rivers.*

5. Chapitre 4 : Taxonomie intégrative du complexe *Caridina weberi*

5.1 Introduction

De même que pour le complexe *C. nilotica*, les espèces du complexe *C. weberi* sont sujettes à une certaine confusion taxonomique, nécessitant de réviser leur systématique à travers le prisme de la taxonomie intégrative.

Nous présenterons dans ce chapitre deux exemples de problèmes rencontrés dans ce complexe et les solutions qui y ont été apportées.

Dans un premier temps, il s'agit de la mise en synonymie de *C. gueryi* décrite du Vanuatu avec *C. buehleri* décrite de Nouvelle Irlande, deux espèces du complexe *C. weberi sensu lato* vivant dans le cours inférieur des rivières de Mélanésie.

Dans un second temps, nous ferons la révision des espèces du complexe *C. weberi* dans les îles de Polynésie, avec les descriptions de trois nouvelles espèces anciennement confondues avec *C. weberi*.

Ces travaux ont fait l'objet de deux articles, le premier publié en 2017 dans *Invertebrate Systematics 31(2)* et le second en préparation, dont les textes constituent les deux parties de ce chapitre.

In the same way as for the *C. nilotica* complex, there is a certain taxonomic complexity among the species of the *C. weberi* complex, with a need to revise their systematics through an integrative taxonomy approach.

We will present in this chapter two examples of issues met in this complex and the solutions we raised to solve them.

Firstly, it is about the synonymisation of *C. gueryi* described from Vanuatu with *C. buehleri*, described from New Ireland, two species of the *C. weberi* complex *sensu lato* living in the lower course of the rivers of Melanesia.

Secondly, we will revise the species of the *C. weberi* complex in Polynesian islands, with the description of three new species formerly confused with *C. weberi*.

These studies were subject of two publications, the first one published in 2017 in *Invertebrate Systematics 31(2)* and the second one in preparation, of which the texts form the two parts of this chapter.

5.2 *Caridina gueryi*, synonyme junior de *C. buehleri* en Mélanésie

Invertebrate Systematics 31(2), 220-230

When molecules and morphology work together: lines of evidence for the validity of *Caridina buehleri* Roux, 1934 (Crustacea: Decapoda: Atyidae) and for *C. gueryi* Marquet, Keith & Kalfatak, 2009 as its junior synonym.

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5.2.1 Abstract

The taxonomy of the freshwater shrimps *Caridina* (Atyidae) is very complex and confused mostly because the morphological characters that have traditionally been used for species delimitation and identification are highly plastic. There is thus a need for an integrative approach of their taxonomy. A total of 42 specimens belonging to either

Caridina buehleri Roux, 1934 or *Caridina gueryi* Marquet, Keith & Kalfatak, 2009 were examined. We combined here morphological data from 12 specimens from the whole distribution range of the species, including type specimens with 16S mtDNA analyses from 7 freshly sampled specimens, in order to verify the specific status of *Caridina buehleri*, from Papua New-Guinea, Central Sulawesi, Western Samoa, Solomon Islands, and *C. gueryi* from Vanuatu. The comparison of 24 morphological characters showed that morphological variations of character traits, between *C. gueryi* and *C. buehleri*, are indeed widely overlapping and that no morphological feature can effectively split specimens into two clear groups. Molecular characters corroborated these results, as specimens from both groups where only separated by 2% p-distance, a genetic distance that is coherent with their potentially high dispersal abilities. We thus consider *C. gueryi* as a junior synonym of *C. buehleri*. Finally, *C. buehleri* is characterized mainly by a styliform and characteristically indented rostrum and a long stylocerite. Detailed re-descriptions of the type specimens of *C. buehleri* and *C. gueryi* are given as well as their geographical and ecological distribution.

Key words: Freshwater shrimp, Indo-Pacific, Integrative taxonomy, 16S.

5.2.2 Introduction

One of the aims of the Muséum national d'Histoire naturelle (MNHN) in Paris is to carry out faunistic inventories of rivers in tropical islands in order to establish a better protection of these fragile ecosystems and, in this context, to clarify taxonomy of poorly known organisms.

The freshwater shrimp *Caridina* H. Milne-Edwards, 1837, comprising 298 species (WoRMS database as of Oct. 2016) and mostly present in the Indo-Pacific region, is the most diversified genus of the family Atyidae (De Grave *et al.*, 2015) and an important ecological component in the tropical streams (Pringle *et al.*, 1993 ; Covich *et al.*, 1999). Their high diversity combined with the lack of informative morphological characters has led to a confused taxonomy (Richard & Clark, 2009). Indeed, until recently, their taxonomy was mainly based on morphological characters. Some have been proven highly variable within a single species (e.g. rostrum shape and indentation or coloration)

and so taxonomically non-informative, making it difficult to identify boundaries among species (Rintelen & Cai, 2009). There is thus a need for an integrative and standardized approach to improve the systematic of the group, focusing on informative morphological features and using molecular characters (Page *et al.*, 2005 ; Page & Hughes, 2011). To illustrate this problem, we here focused on two species that are morphologically very close: *Caridina buehleri* Roux, 1934 and *Caridina gueryi* Marquet, Keith & Kalfatak, 2009.

Roux (J. Roux, 1934) described *Caridina buehleri* from a single non-ovigerous female from New Ireland (Papua New-Guinea) as possessing a distinctively long stylocerite and a long and pointed rostrum. In 2007, this species has been reported from Luwuk Peninsula, Central Sulawesi, Indonesia and re-described with more morphological characters but without examining the type specimen (Klotz *et al.* 2007). *Caridina buehleri* was later reported from Upolu Island (Western Samoa) (Keith *et al.* 2013) and collected by some of the authors (P. Keith and G. Marquet) in Kolobangara Island (Solomon Islands) in 2015.

Caridina gueryi, was described after examination of specimens collected from Vanuatu (Santo and Malekula islands) by the Vanuatu Environment Unit (VEU) and the MNHN (Keith *et al.* 2010) but they were not compared to the holotype of *C. buehleri*, despite showing strong similarities between the two species.

As we collected more and more specimens from different localities, we started to raise doubts regarding the validity of *C. gueryi*, as the specimens identified as such fitted well with the description of *C. buehleri*. Consequently, we here examined type specimens of these two species and combined morphological data with a 16S mtDNA analysis in order to clarify their respective status.

5.2.3 Materials and methods

Abbreviations for Museums

Muséum national d'Histoire naturelle, Paris: MNHN; Naturhistorisches Museum Basel, Basel: NMB; Museum für Naturkunde, Berlin: ZMB.

Abbreviations for morphological analyses

The following abbreviations are used in the present text: CL, carapace length (mm): measured from the post-orbital margin to the posterior margin of the carapace; TL, total length (mm): measured from the tip of the rostrum to the posterior margin of the telson; P1: first pereiopod; P2: second pereiopod; P3: third pereiopod; P5: fifth pereiopod; PlI: first pleopod.

Molecular analyses

DNA extraction, amplification and sequencing

DNA was extracted from abdominal tissues using the semi-automatic Ependorf ep-Motion 5075 robot. Fragments of the mitochondrial 16S rRNA (~ 520 bp) were amplified using the primers 16Sa-L (CGCCTGTTTATCAAAAACAT) and 16Sb-H2 (CTCCGGTTTGAACTCAGATCA) (Palumbi, 1996). DNA amplification was performed in 25µl PCR reactions, containing approximately 3 ng of template DNA, 2.5 mM MgCL2, 0.26 mM of each nucleotide, 0.3 µM of each primer, 5% DMSO, 1 ng of BSA and 1.5 units of QBIOTAQ polymerase (MPBiomedicals). Amplification products were generated by an initial denaturation step of 4 min at 94°C followed by 30 cycles of denaturation at 94°C for 30s, annealing at 51°C for 60 s and extension at 72°C for 60 s. PCR products were sequenced using same primers and in both directions to insure the accuracy of base calls. Chromatograms were edited using BioEdit Sequence Alignment Editor Version 7.0.4.1 (Hall, 1999). All sequences were deposited in GenBank (GenBank Accession N°s: KY350238-KY350244, Table 5.2.1). Sequences for the genera *Caridina* and *Paratya* published by Page *et al.*, (Page *et al.*, 2007a) were included in our analysis, *Paratya* sequences being used as outgroup.

Table 5.2.1: GenBank accession numbers for the obtained sequences.

Accession number	Specimen ID	Collection ID	Locality	Date	Collectors
KY350238	CA1015	IU-MNHN-2015-23	Vanuatu, Malekula Island, Brenwe, -16.126545 167.279658	20 Nov. 2008	P. Keith, L. Taillebois, C. Lord
KY350239	CA1016	IU-MNHN-2015-23	Vanuatu, Malekula Island, Brenwe, -16.126545 167.279658	20 Nov. 2008	P. Keith, L. Taillebois, C. Lord
KY350240	CA1017	IU-MNHN-2015-23	Vanuatu, Malekula Island, Brenwe, -16.126545 167.279658	20 Nov. 2008	P. Keith, L. Taillebois, C. Lord
KY350241	CA1161	ZMB 29002	Indonesia, Sulawesi, Malontong, 0.885502 121.522850	27 May 2005	M. Glaubrecht, T. von Rintelen & K. Zitzler
KY350242	CA1162	ZMB 29002	Indonesia, Sulawesi, Malontong, 0.885502 121.522850	27 May 2005	M. Glaubrecht, T. von Rintelen & K. Zitzler
KY350243	CA1163	ZMB 29002	Indonesia, Sulawesi, Malontong, 0.885502 121.522850	27 May 2005	M. Glaubrecht, T. von Rintelen & K. Zitzler
KY350244	CA1519	IU-MNHN-2015-19	Solomon Islands, Kolobangara Island, Jack Harbour, -8.051389 157.182222	11 Nov. 2015	P. Keith, G. Marquet, C. Lord

Sequence and phylogenetic analyses.

DNA sequences were aligned using MEGA6 software (Tamura *et al.*, 2013) with Muscle algorithm (Edgar, 2004).

Since non-coding markers can be difficult to align, two datasets were produced; a first one was obtained by analyzing our alignment through GBLOCKS (Talavera & Castresana, 2007) in order to remove poorly aligned portions of sequences, and a second by leaving the sequences as they were after the alignment.

Using Bayesian information criterion in PartitionFinder (Lanfear *et al.*, 2012) we retained the GTR + G + I model for both datasets. Best-scoring ML trees were estimated for each dataset using RAxML HPC2 v.8.2.4 (Stamatakis, 2006) on Teragrid v.7.2.7, implemented in the Cyber Infrastructure for phylogenetic Research (CIPRES) portal v.3.1. (Miller *et al.*, 2010) (https://www.phylo.org/). One hundred independent searches, each starting from distinct random trees, were conducted. Robustness of the nodes was assessed using non-parametric bootstrapping (Felsenstein, 1985) with 1,000 bootstrap replicates. Bayesian trees were calculated using MrBayes software (Huelsenbeck & Ronquist, 2001) with the previously determined model, running for 5,000,000 generations, a sampling frequency of 1,000 and a burn in of 25%. Support for nodes was determined using posterior probabilities (PP) calculated by MrBayes. We considered a group to be 'moderately supported' if it had a bootstrap support value (B) between 75 and 89% and posterior probability (PP) between 0.95 and 0.97, and 'highly supported' when B ≥ 90% and PP ≥ 0.98.

Morphological comparisons

The rostrum, the general cephalothorax, the pereiopods 1, 2, 3 and 5 and the abdomen were observed using a stereoscopic microscope. The proportions of the various joints of the appendages were measured using microphotographs and the AnalySIS Works software (Olympus).

The examinations were made on the type specimens of *C. buehleri* and *C. gueryi,* and on a total of 12 specimens grouped by locality (6 from Sulawesi, 2 from Kolobangara and

4 from Malekula). The ranges were then compared side by side and a global range of all the specimens was given.

Drawings were made using the "Digital Inking" method (Coleman 2003; Coleman 2006) by tracing vectorial paths on high resolution photographs using Adobe Illustrator (CS6) and a WACOM PTZ-1230 graphic tablet.

SEM micrographs were obtained from ethanol preserved specimens, some treated with 10% bleach for a night in order to remove the setae.

5.2.4 Results

Molecular results.

A 16S gene fragment was successfully sequenced for seven specimens (Table 5.2.1). Sequences included 490 bp, containing 10 variable sites. After alignment with 87 atyid sequences (83 *Caridina* and 4 *Paratya* as outgroup) from Page *et al.* (Page *et al.*, 2007a), the 16S datasets of 94 sequences of 513 bp included 193 variable sites, of which 174 were phylogenetically informative. After removal of ambiguous blocks, 374 bp of sequence remained to be used in phylogenetic analyses (73% of the original 513 positions), of which 131 bp were variable and 112 bp phylogenetically informative.

No supported conflict was found between full-length and reduced datasets, however, both the BI and ML trees produced using the full-length dataset (Figure 5.2.1) were overall more supported than the trees produced using the reduced dataset (Appendix 1). Therefore only the results obtained for the full-length dataset are presented here.

In each of the BI and ML analysis, the consensus tree showed *C. buehleri* and *C. gueryi* as a monophyletic and highly supported group (PP=0.99; B=97%), which was well differentiated from all other *Caridina* analyzed (p-distance to the closest relative: 12.3%), and appeared as belonging to the *C. weberi* species complex. Within this monophyletic group, sequences from *C. buehleri* and *C. gueryi* were clustered in 2 well supported genetic groups; the first one (PP=0.94; B=100%) including 2 specimens identified as *C. buehleri* from Indonesia, and the second one (PP=0.99; B=100%) including 3 specimens identified as *C. gueryi* from Vanuatu, 1 specimen identified as *C. buehleri* from Indonesia and 1 specimen identified as *C. buehleri* too, from Solomon Islands. Clade 1 is 2% (p-distance) distant from Clade 2.

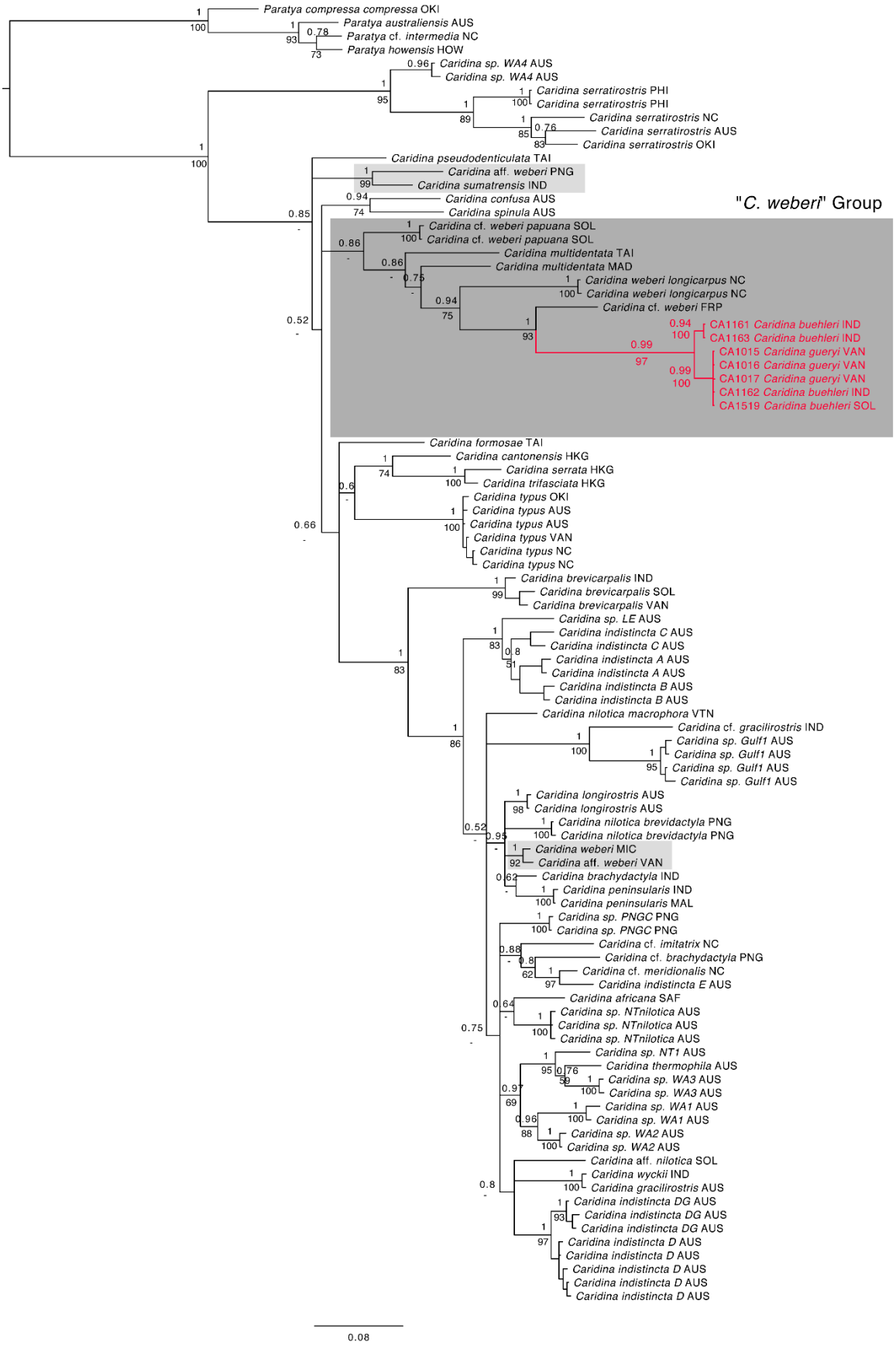


Figure 5.2.1: Molecular phylogeny obtained from the full dataset showing the position of *Caridina buehleri* (16S). Numbers above branches are the Bayesian posterior probabilities; numbers under branches are the bootstrap from the Maximum Likelihood analysis. Dark grey shading indicates the genetic "*C. weberi* species complex" and light grey shading indicates taxa morphologically close to the "*C. weberi* species complex". Locality codes: AUS = Australia; FRP = French Polynesia; HKG = Hong Kong; HOW = Lord Howe Island; IND = Indonesia; MAD = Madagascar; MAL = Malaysia; MIC = Micronesia; NC = New Caledonia; OKI = Okinawa; PHI = Philippines; PNG = Papua New Guinea; SAF = South Africa; SOL = Solomon Islands; TAI = Taiwan; VAN = Vanuatu; VTN = Vietnam.

Taxonomy

Atyidae De Haan, 1849

***Caridina* H. Milne Edwards, 1837**

***Caridina buehleri* Roux, 1934**

(Figure 5.2.2)

Caridina buehleri Roux, 1934: 219, Figs 1–5 [type locality: Bimoun, New Ireland west coast, Papua New Guinea].

Caridina gueryi Marquet, Keith & Kalfatak, 2009:159–166, Figs 1–3 [type locality: unnamed river (15.29590S; 167.16E), Santo, Vanuatu].

Material examined

Type material: *Caridina buehleri* Roux, 1934. Holotype NMB 822 Papua New Guinea, New Ireland, Bimoun, one non ovigerous female, CL 7 mm.

Caridina gueryi Marquet, Keith & Kalfatak, 2009. Vanuatu, Santo island Holotype: MNHN-IU-2015-1768, ovigerous female, CL 6.4 mm, unnamed river, 17°50 7S, 145° 31W, 22-07-2003, coll. Keith & Marquet; Allotype: MNHN-IU-2015-1769, 16-236, male, TL 14.5 mm, CL 3.2 mm 22-07-2003, coll. Keith & Marquet; Paratypes: MNHN-IU-2015-1770, 12 females, MNHN-IU-2015-1771, one ovigerous female TL 23.1 mm, Mantatas river 23-07-2003 coll. Keith Marquet, MNHN-Na 16-239, one ovigerous female TL 18.9 mm Sarataka river, 25-07-2003 coll. Keith & Marquet.

Other material: *Caridina buehleri* Roux, 1934. ZMB 29002 Indonesia Central Sulawesi, Luwuk Peninsula, freshwater spring in Malontong, West of Ampana, brackish water pool; 0°53.125'S 121°31.371'E (field code 70-05); coll. M. Glaubrecht, T. von Rintelen & K. Zitzler, 27-05-2005, det. Klotz/Karge. 1 male (CL 2.9 mm), 1 female (CL 3.9 mm) and 3 ovigerous females (CL 4.3–6.4mm). IU-MNHN-2015-17 Solomon Islands, Kolobangara Island, Vagé river; 08°05.112’S 156°59.867’E; coll. P. Keith, C. Lord & G. Marquet, 10-11-2015, det. Marquet : 3 ovigerous females (CL 3.7 mm; 3.2 mm; 3.5 mm). IU-MNHN-2015-

18 Liva river; 08°03.859’S 157°10.684’E; 11-11-2015, det. Marquet: 3 ovigerous females (CL 3.5 mm; 3.8 mm; 4.2 mm). IU-MNHN-2015-19 Jack Harbour river; 08°03.085’S 157°10.945’E; 11-11-2015, det. Marquet: 3 ovigerous females (CL 4.4 mm; 3.4 mm; 4.3 mm) and 1 non-ovigerous female (CL 4 mm). IU-MNHN-2015-20 Pipiro river; 07°53.083’S 157°08.195’E; 13-11-2015, det. Marquet: 2 ovigerous females (CL 3.8 mm; 4.2 mm). IU-MNHN-2015-21 Vanga I river; 07°55.088’S 156°57.624’E; 18-11-2015, det. Marquet. 2 ovigerous females (CL 4.4 mm; 4.6 mm). IU-MNHN-2015-22 Vanga II river; 07°54.825’S 156°57.762’E; 18-11-2015, det. Marquet: 4 ovigerous females (CL 4.1 mm; 3.2 mm; 4 mm; 4.2 mm).

Caridina gueryi IU-MNHN-2015-23 Marquet, Keith & Kalfatak, 2009 Vanuatu, Malakula Island, Brenwe river, 16°07.849’S 167°17.342’E; coll. Kalfatak, Keith & Lord, 20-11-2008, det. Marquet : 4 ovigerous females (CL 5–6 mm).

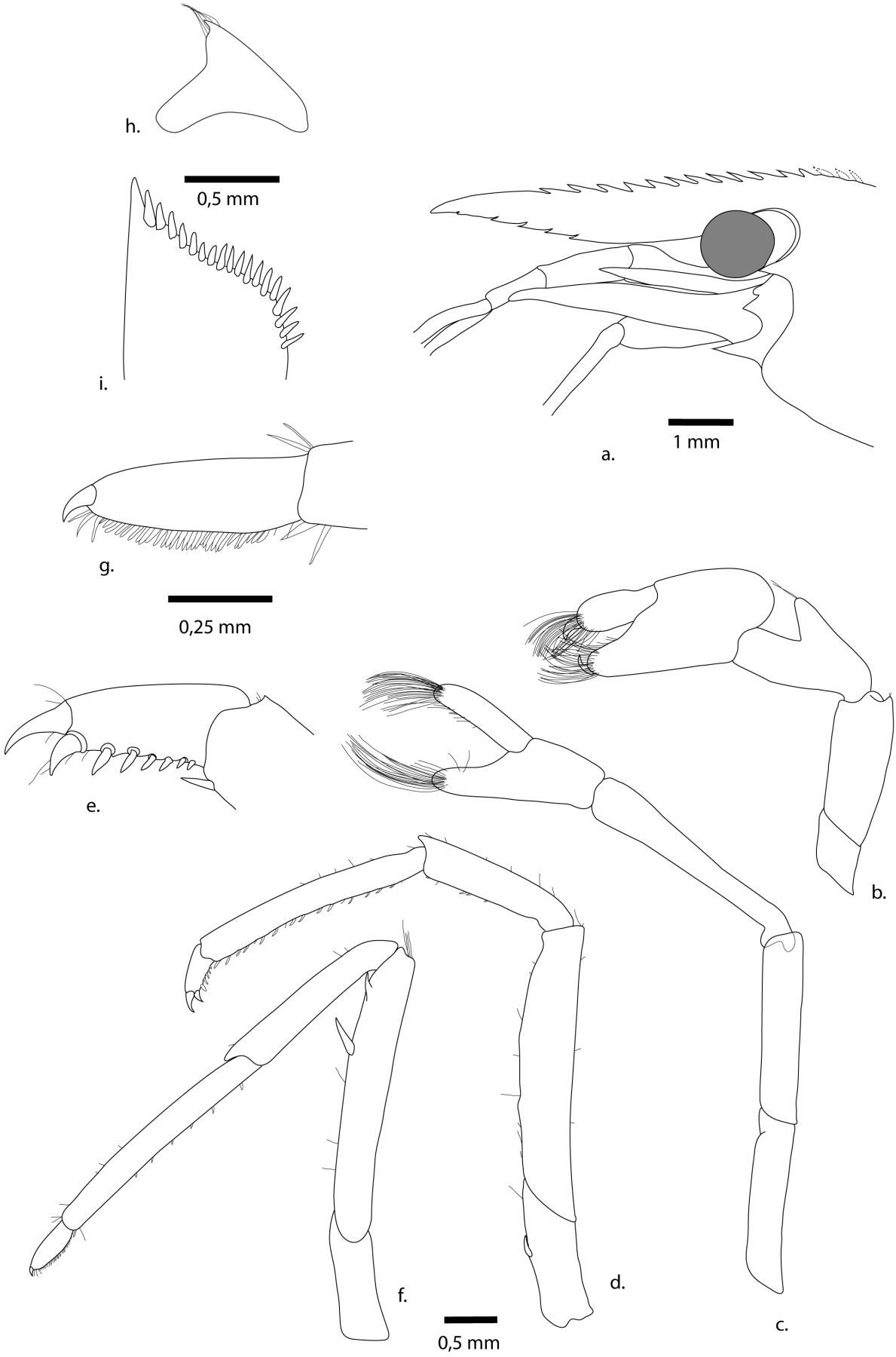


Figure 5.2.2: *Caridina buehleri*, holotype female (cl 7 mm): a. cephalothorax; b. first pereiopod; c. second pereiopod; f. fifth pereiopod; g. dactylus of fifth pereiopod. *Caridina gueryi* holotype female (cl 6.4 mm) d. third pereiopod; e. dactylus of third pereiopod; h. preanal carina; i. uropodal diaeresis.

Diagnosis

Carapace smooth, glabrous, with sharp antennal spine placed at lower orbital angle. Pterygostomial margin blunt. Rostrum long, 0.5–1.4 of CL, styliform, reaching to the end of scaphocerite, curved upwards, distal margin unarmed without apical teeth, armed with 8 to 16 teeth on basal part of dorsal margin, 1–4 of them situated on carapace behind orbital margin, ventral margin with 2–7 teeth. Stylocerite sharp, long, reaching to about half of second segment of peduncle.

First pereiopod stout, chela about 1.9–2.7 times as long as wide, dactylus 1.3–2.9 times as long as wide, 0.4–0.8 length of palm; tips of both fingers with prominent claws, carpus 1.2–1.7 times as long as wide.

Second pereiopod more slender and longer than P1; chela 2.2–3.2 times as long as wide, dactylus 3.0–4.8 times as long as wide, 1.0–1.6 times length of palm, tips of fingers rounded, carpus slender 4.4–7.0 times as long as wide.

Third pereiopod slender, dactylus 2.7–4.0 times as long as wide, terminal spiniform setae included) with 5–8 spiniform setae on flexor margin in addition to the terminal one; propodus 8.0–14.0 times as long as wide, 3.0–5.3 times as long as dactylus.

Fifth pereiopod with dactylus 3.3–5.6 as long as wide with 26–36 spiniform setae on flexor margin; propodus 9.4–20.5 times as long as wide, 3.8–6.2 as long as dactylus.

Endopod of first pleopod with well developed appendix interna in male specimens; Appendix masculina on second pleopod reaching to about 0.6 times length of endopod; appendix interna reaching about 2/3 of appendix masculina. Telson ending in broadly convex margin bearing a median projection; distal margin with 5–10 long plumose setae flanked by one pair of stout spiniform setae. Uropodal diaeresis with 16–20 short cuspidate teeth. Pre-anal carina high, without a spine. Eggs size: 0.36–0.49 x 0.23–0.32 mm.

Distribution

C. buehleri is known from Indonesia (Sulawesi), Papua New Guinea (New Ireland), Vanuatu (Santo, Malekula), Western Samoa (Upolu) and Solomon islands (Kolobangara) (Figure 5.2.3).



Figure 5.2.3: Collecting sites of *Caridina buehleri* in Pacific Ocean.

Habitat

All specimens were collected in a typical brackish water environment (brackish water pools or lower part of rivers, near the estuary). The species is rarely found over the superior limit of the tide-influenced zone, and was not found in the true freshwater zone.

Live colour pattern

This species shows a rather specific pattern of seven white stripes (one on each somite and one on the cephalothorax), upon a varying colour that tends to match the environment, ranging from bright yellow to black, with reddish dots (Figure 5.2.4).

Morphological comparison

A total of 24 variables were obtained for each group and were compared in Table 2. It appears that all of the variations of the measures are globally overlapping, and no significant differences were observed.

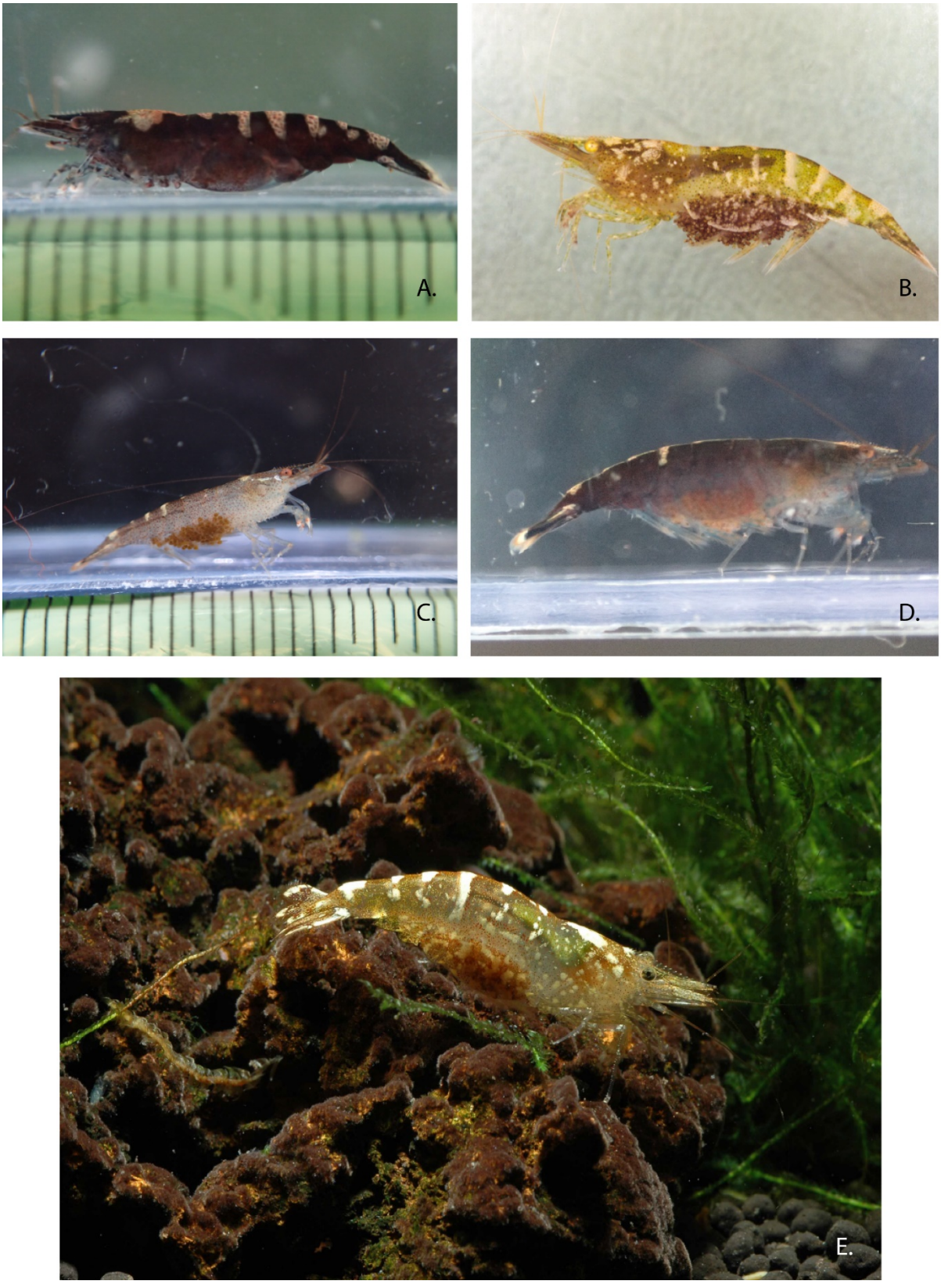


Figure 5.2.4: A, C, D = *Caridina buehleri* from Kolobangara, Solomon Islands (Photo P. Keith) respectively specimen CA1519, and 2 not sequenced specimens and; B = *Caridina gueryi* from Santo, Vanuatu (Photo P. Keith), not sequenced; E = *Caridina buehleri* from Sulawesi, Indonesia (Photo H. Evers), not sequenced.

Table 5.2.2: Comparison of the type specimens of *C. buehleri* and *C. gueryi* with specimens from Sulawesi, Kolobangara and Malekula.

	Rostrum times length of cl	**Dorsal teeth (Ventral teeth)**	**Unarmed distal portion of dorsal margin / armed portion**	**Teeth placed on carapace**
C. buehleri				
Holotype cl 7 mm	1.3	15 (5)	0.5	4
C. gueryi				
Holotype cl 6.4 mm	0.8	12 (6)	0.7	3
Allotype cl 3.2 mm	0.9	16 (5)	0.3	3
Sulawesi specimens N = 6 (Klotz *et al.*, 2007)	0.7 - 1.4	13 – 15 (4 – 7)	0.33	3 - 4
Kolobangara specimens N = 2	0.5	12 – 14 (2)	0.4	3 - 4
Malekula specimens N = 4	0.7	14 – 16 (3 – 5)	0.2 – 0.5	3 – 4
Global range	**0.5 – 1.4**	**12 – 16 (2 – 7)**	**0.2 – 0.7**	**3 - 4**

	P1 Dactylus times as long as wide	**P1 Chela times as long as wide**	**P1 Carpus times as long as wide**	**P1 Dactylus times length of palm**
C.buehleri				
Holotype cl 7 mm	2.1	2.2	1.6	0.7
C. gueryi				
Holotype cl 6.4 mm	2.9	2.1	1.5	0.7
Allotype cl 3.2 mm	2.5	2.3	1.7	0.7
Sulawesi specimens N = 6 (Klotz *et al.*, 2007)	1.3 – 2.3	1.9 – 2.3	1.2 – 1.7	0.4 – 0.7
Kolobangara specimens N = 2	2.4 – 2.8	2.2 – 2.4	1.4 – 1.7	0.5 -0.7
Malekula specimens N = 4	2.3 – 2.7	2.2 – 2.4	1.5 – 1.7	0.6 – 0.8
Global range	**1.3 – 2.9**	**1.9 – 2.4**	**1.2 – 1.7**	**0.4 – 0.8**

	P2 Dactylus times as long as wide	P2 Chela times as long as wide	P2 Carpus times as long as wide	P2 Dactylus times length of palm
C.buehleri				
Holotype cl 7mm	4.0	2.7	4.4	1.4
C. gueryi				
Holotype cl 6.4 mm	4.7	3.0	6.3	1.5
Allotype cl 3.2 mm	6.1	3.2	7.0	1.5
Sulawesi specimens N = 6 (Klotz *et al.*, 2007)	3.0 – 4.8	2.2 – 3.0	5.0 – 6.7	1.0 – 1.5
Kolobangara specimens N = 2	4.0 – 4.4	2.9 – 3.0	5.6 – 5.9	1.2 – 1.4
Malekula specimens N = 4	4.2 – 4.6	2.6 – 3.0	5.5 – 5.6	1.4 – 1.6
Global range	**3.0 – 4.8**	**2.2 – 3.2**	**4.4 – 7.0**	**1.0 – 1.6**

	P3 Dactylus times as long as wide	P3 Propodus times as long as wide	P3 Propodus times as long as dactylus	P3 Spines on flexor margin
C. buehleri				
Holotype cl 7 mm	Missing	Missing	Missing	Missing
(Roux, 1934)	(3.3)	(8.0)	(3.0)	(8)
C. gueryi				
Holotype cl 6.4 mm	3.4	13.7	5.3	8
Allotype cl 3.2 mm	3.1	10.7	4.1	7
Sulawesi specimens N = 6 (Klotz *et al.*, 2007)	3.3 – 4.0	10.1 – 14.0	3.9 – 5.3	5 - 6
Kolobangara specimens N = 2	3.0	9.3 -10.1	4.1 – 4.4	6
Malekula specimens N = 4	2.9 – 3.1	8.1 – 10.5	3.5 – 4.6	7
Global range	**2.9 – 4.0**	**8.0 – 14.0**	**3.0 – 5.3**	**5 - 8**

	P5 Dactylus times as long as wide	P5 Propodus times as long as wide	P5 Propodus times as long as dactylus	P5 Spines on flexor margin
C. buehleri				
Holotype cl 7 mm	3.6	9.4	3.8	30
C. gueryi				
Holotype cl 6.4 mm	Missing	Missing	Missing	Missing
(Marquet *et al.*, 2009)	(5.6)	(15.9)	(5.0)	36
Allotype cl 3.2 mm	Missing	Missing	Missing	Missing
Sulawesi specimens N = 6 (Klotz *et al.*, 2007)	3.5 – 4.4	12.8 – 20.5	4.5 – 6.2	26 - 29
Kolobangara specimens N = 2	3.7 – 3.9	12.8 – 14.0	4.1 – 4.4	28 - 33
Malekula specimens N = 4	3.3	12.3	4.5	29
Global range	**3.3 – 5.6**	**9.4 – 20.5**	**3.8 – 6.2**	**26 - 36**

	Uropodial diaeresis spinules	Egg size	Preanal carina	Appendix interna on Pl1
C. buehleri				
Holotype cl 7 mm	16 (left) – 17 (right)	Non ovigerous female	High, with no spine	NA
C. gueryi				
Holotype cl 6.4 mm	18	Male	High, with no spine	Well developed
(Marquet *et al.*, 2009)		(0.48 x 0.28)		
Sulawesi specimens N = 6 (Klotz *et al.*, 2007)	17 - 18	0.40 – 0.45 x 0.23 – 0.25	High with an indistinct tooth	Well developed
Kolobangara specimens N = 2	17 - 19	0.39 – 0.44 x 0.24 – 0.26	High, with no spine	NA
Malekula specimens N = 4	17 - 18	0.43 – 0.49 x 0.27 – 0.32	High with no spine	NA
Global range	**16 - 19**	**0.36 – 0.49 x 0.23 – 0.32**	**High with no spine**	**Well developed**

5.2.5 Discussion

Caridina gueryi, a junior synonym of _C. buehleri_.

Genetic results showed that specimens attributed to both of the two species clustered in the same clade, and that the two groups were too close (2% p-distance) to support the hypothesis of separate species, and thus suggesting that one of the two species names has to be synonymized. After morphological examination of both the type specimens and the populations from the known distribution of the two species (Sulawesi, Vanuatu and Solomon Islands), we found that no morphological feature could split our specimens in two clear groups. Through the integrative taxonomy approach applied here, we regard both populations as belonging to *C. buehleri*, since no morphological or ecological difference was observed between them. Therefore, our results suggested that *C. gueryi* Marquet, Keith & Kalfatak, 2009 should be treated as a junior synonym of *C. buehleri* Roux, 1934.

Diagnostic morphological characters of _C. buehleri_

From our morphological observations we found 4 very specific characters that are diagnostic of this species and allow to separate it from all other *Caridina* species: 1) a long styliform rostrum, reaching the end of scaphocerite, distodorsal margin of the rostrum unarmed without apical teeth, with 8–16 teeth on basal part of dorsal margin, 1–4 of them situated on carapace behind orbital margin and 3–6 teeth on ventral margin; 2) the stylocerite is long, reaching at least about half of the second segment of antennular peduncle; 3) the first pereiopod is stout, with a very short dactylus and both fingers of chela with prominent distal claws; 4) a high pre-anal carina with an indistinct tooth. According to Richard & Clark (Richard & Clark, 2014), the number of teeth and their placement on the rostrum (character 1) remain an important identification character, in contrast to its length, which has been shown to be greatly plastic in atyid shrimps (Jugovic *et al.*, 2010). Indeed, we found that the disposition of the teeth on the rostrum of *C. buehleri* is quite characteristic (without an apical dorsal tooth and few ventral teeth) and cannot be confused with any other species of the genus. The long stylocerite reaching to the second segment of the antennular peduncle (character 2) is also found in *C. serratirostris* De Man, 1892, *C. celebensis* De Man, 1892, *C. lobocensis* Cai, Choy & Ng, 2009 and in all members of the *Caridina serrata* species group (Cai & Ng,

1999; Klotz & Rintelen, 2014). However, these species can be easily differentiated from *C. buehleri* by the rostrum denticulations and the proportions of the carpus of the first pereiopod for *C. serratirostris* and *C. celebensis*, the spines of the telson, the shape of the male first pleopod and the size of the eggs for species of the *C. serrata* group (Klotz *et al.* 2007) and the setae of the fifth pereiopod for *C. lobocensis*. The chelae of the first pereiopods exhibit a distinctive claw on both fingers (character 3). This character was not reported by Roux (1934) in the original description of *C. buehleri* nor in *C. gueryi*. It might thus be more widely distributed in this genus than it appears, as it is quite difficult to see, hidden in the setae. According to Klotz *et al.* (2007), *C. buehleri* shows a high pre-anal carina with an indistinct tooth (character 4). Marquet *et al.* (Marquet *et al.*, 2009) have reported a spine on this structure in *C. gueryi*. After re-examination of the holotype of *C. gueryi*, it appears that there is actually no tooth on the pre-anal carina and that the carina is similar to the one that *C. buehleri* possesses.

Perspectives on the taxonomy of Caridina: C. buehleri, part of the C. weberi complex?

Our single-gene phylogenetic analysis (Figure 5.2.1) corroborated the statement of Roux (1934), identifying *C. buehleri* as closely related to *C. weberi* De Man, 1892 and *C. multidentata* Stimpson, 1860, and more generally to species traditionally included in the *C. typus* group (*sensu* Bouvier, 1925). This whole group includes species with rather stout bodies, short antennular peduncle, short sixth abdominal segment, a high pre-anal carina and a high number of spinules on the uropodial diaeresis.

In the present work (Figure 5.2.1), this group was genetically supported by BI (PP = 0.99), thus suggesting the existence of a *C. weberi* species complex in the same way that there exist species complexes for the groups *C. nilotica* (Karge & Klotz, 2007), *C. serrata* ; (Klotz & Rintelen, 2014), and *C. gracilirostris*. Indeed our sequences clustered with *C.* cf. *weberi* from Moorea in both datasets, but also with *C. weberi longicarpus* from New Caledonia, *C. multidentata* from Taiwan, *C. multidentata* from Madagascar and *Caridina* cf. *weberi papuana* from Solomon Islands, in the BI analyses. *Caridina sumatrensis* from Indonesia and *Caridina* aff. *weberi* from Papua New Guinea are not supported in this group, but due to the low resolution of the node that bring them together with the "*C. weberi* complex", some further analyses are needed. *Caridina weberi* from Micronesia and *Caridina* aff. *weberi* from Vanuatu are far from this group in the tree and are most

likely a misidentification. Multi-locus phylogenetic approach combined with morphological and ecological data is highly needed to clarify the taxonomy of *Caridina*.

New insights on the biology of C. buehleri

Caridina buehleri exhibits a stout first pereiopod with both fingers showing a prominent claw (Figure 5.2.5). These features are also found in other species of *Caridina* such as *C. typus* (Klotz, pers. com.). Clawed fingers might prove advantageous in feeding on discrete particles, as shown in *Limnocaridina* species (Fryer, 2006) or small invertebrates, like oligochaetes or juvenile insect or larvae, while species without clawed finger tips are primarily feeding on detritus or single-celled periphyton (Klotz *et al.* 2007). This morphological feature could be linked to a predatory behaviour by scrapping fixed invertebrates that evolved as an adaptation to the strong competition in the part of the rivers where *C. buehleri* lives.

It is interesting to note that *C. buehleri* is a species inhabiting brackish water environment, and that ovigerous females carry small and numerous eggs, indicative of a prolonged larval development. A wide distribution range could therefore be expected (Rintelen *et al.*, 2008) rather than a scattered distribution as known until today. Further fieldworks are necessary to collect more specimens and get a better picture of the extent of the distribution range of *C. buehleri*, whose identification should be facilitated by this study.

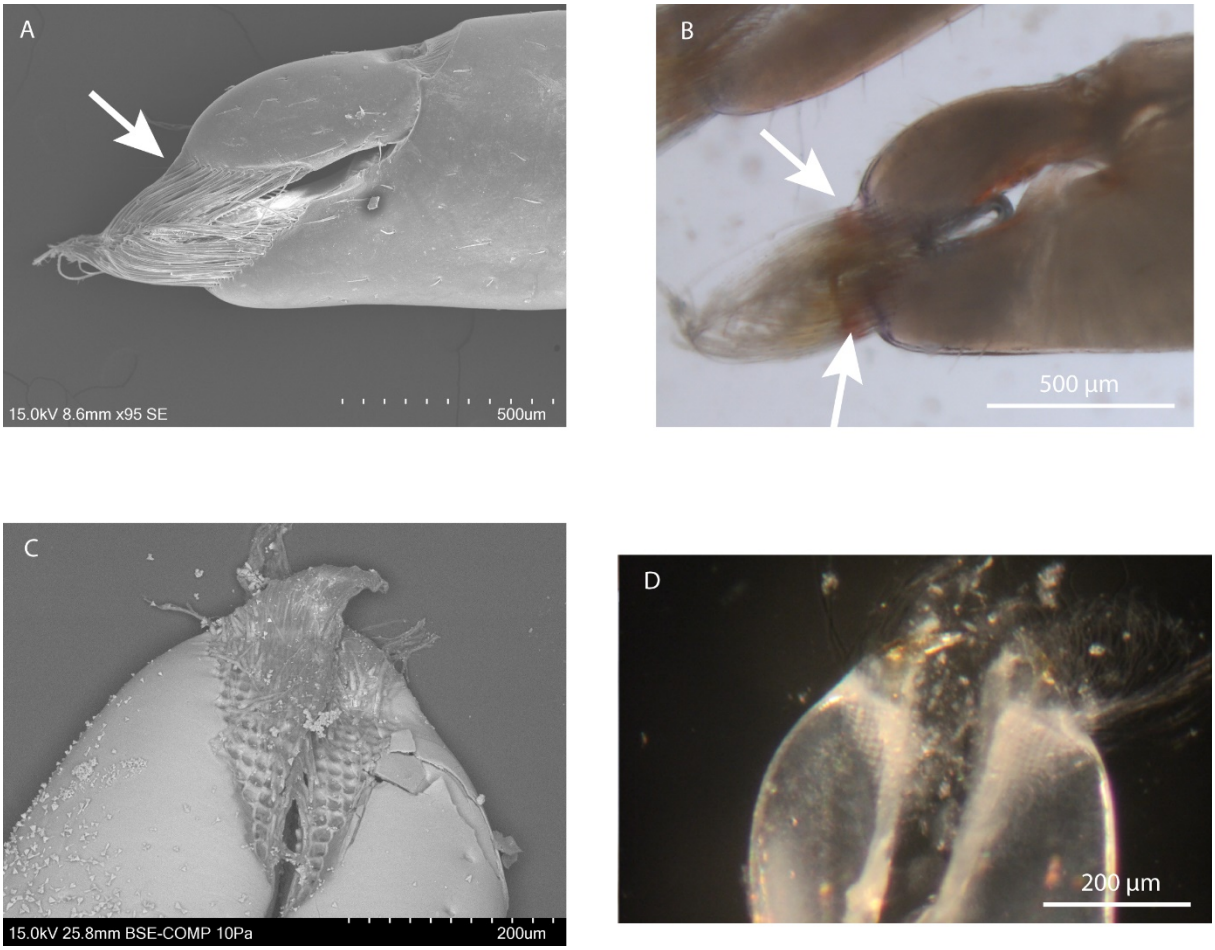


Figure 5.2.5: A = SEM micrograph of a first pereiopod of *Caridina buehleri*. B = Optical microscope photograph of a first pereiopod of *Caridina buehleri*. C = SEM micrograph of a first pereiopod of *Caridina buehleri* after bleach treatment. D = Optical microscope photograph of a first pereiopod of *Caridina buehleri* after bleach treatment.

5.2.6 Acknowledgements

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scientists operated under the “Forests, Mountains, Rivers” theme coordinated by Bruno Corbara, Philippe Keith and Jérôme Munzinger.

We would like to acknowledge the customary landowners, villages and tribes of Hunda-Kena, Jack Harbour, Poitete, Lodumoe and Vanga who welcomed us on Kolobangara Island and for allowing the expedition team to enter their customary lands. We would also like to thank KIBCA and ESSI for the meetings in the villages prior to the expeditions and for the organisation of the trip. Thanks to Rex Loka and Piokera Holland, our very efficient partners on the Kolobangara field work. Thanks also to the MNHN and the BOREA team.

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5.3 Révision des espèces du complexe *C. weberi* en Polynésie

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Revision of freshwater shrimps belonging to the *Caridina weberi* complex (Crustacea: Decapoda: Atyidae) from Polynesia with discussion on their biogeography.

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5.3.1 Abstract

Caridina weberi, an emblematic species of the '*C. weberi* complex', was described by De Man in 1892 from different localities in Indonesia. Until now, this species was thought to have a wide distribution in Polynesia. Numerous specimens identified as C. weberi were collected recently from various Polynesian islands. In the context of an integrative taxonomy, they were morphologically and genetically studied. Three new species allied to *C. weberi* were here described: *C. marquesensis* n. sp., *C. futunensis* n. sp. and *C. tupaia* n. sp.. The occurence of *C. rapaensis*, another species part of the '*C. weberi*' complex was confirmed. Descriptions or redescription of these species are given as well as their geographical and ecological distributions. The position of these species in the complex is clarified. A key is provided to facilitate identification.

Keywords: 16S, genetic, morphology, integrative taxonomy, freshwater shrimp.

5.3.2 Introduction

The freshwater shrimp genus *Caridina*, comprising 298 species (WoRMS database, as of May 2018) and mostly present in the Indo-Pacific region, is the most diversified genus of the Atyidae (De Grave *et al.*, 2015) and an important ecological component in the tropical streams (Pringle *et al.*, 1993 ; Covich *et al.*, 1999). Its high diversity combined with the lack of informative morphological characters has led to a confused taxonomy (Richard & Clark, 2009). Indeed, until recently, the taxonomy of the genus was mainly based on morphological characters. Some have been proven highly variable within a species (e.g. rostrum shape and indentation or coloration) and so taxonomically non-informative, making it difficult to establish boundaries between them (Rintelen & Cai, 2009 ; Mazancourt *et al.*, 2017a). Thus, there is a need for an integrative and standardized approach to improve the group's systematics, focusing on informative morphological features and using molecular characters (Page *et al.*, 2005 ; Page & Hughes, 2011).

To illustrate this problem, we focus here on species of the troublesome '*C. weberi* complex'. The taxonomic complexity within the genus *Caridina* has led previous authors to create groups of species based on morphological characters they share. For example,

Bouvier (1925) defined 5 species group: *C. nilotica* group, *C. laevis* group, *C. africana* group, *C. brevirostris* group and *C. typus* group. Some of these groups have been split to create new ones as new species were described, such as *C. serrata* (Cai & Ng, 1999 ; Karge & Klotz, 2007), *C. gracilirostris* (Cai & Ng, 2007) and *C. weberi* (Cai & Anker, 2004 ; Cai *et al.*, 2009) while others still exist to this day, like *C. nilotica* and *C. typus* groups (Karge & Klotz, 2007).

Polynesia forms a triangle between Hawaii, New Zealand and Easter Island (Figure 5.3.1). We can distinguish Northern Polynesia (Hawaii), Western Polynesia (Tuvalu, Tokelau, Wallis and Futuna, Tonga, Niu, Samoa islands, Cook Islands) and Eastern Polynesia (French Polynesia, Pitcairn Island and Eastern Island). The occurrence of *C. weberi* in Polynesia has been reported by Edmondson (1935), Adamson (1935, 1939), Marquet (1988, 1991), Fossati and Marquet (1998), Keith and Vigneux (2002), Keith *et al.* (2002), Keith and Marquet (2011), and Keith *et al.* (2013).

Streams of Austral, Society and Marquesas Islands (French Polynesia) were prospected by the Muséum national d'Histoire naturelle (MNHN, Paris) from 1983 to 2001 and recently in September and October 2017, rivers of Futuna (Territory of Wallis and Futuna islands) in October 2004, rivers of Rarotonga (Cook islands) in July 2010, rivers of Upolu (Samoa) in July 2008 and August 2014. One of the aims of the MNHN is to carry out faunistic inventories of rivers in tropical islands in order to establish a better protection of these fragile ecosystems and, in this context, to clarify the taxonomy of poorly known organisms. As we collected more and more specimens from different islands from Polynesia, we started to raise doubts regarding the validity of *C. weberi* in this area. Thereby, in combining morphological data with a 16S mtDNA analysis, we identified three new species and confirmed the presence of *C. rapaensis*. The aim of this study is to describe or re-describe them and to compare them to type specimens of species belonging to the *C. weberi* complex.

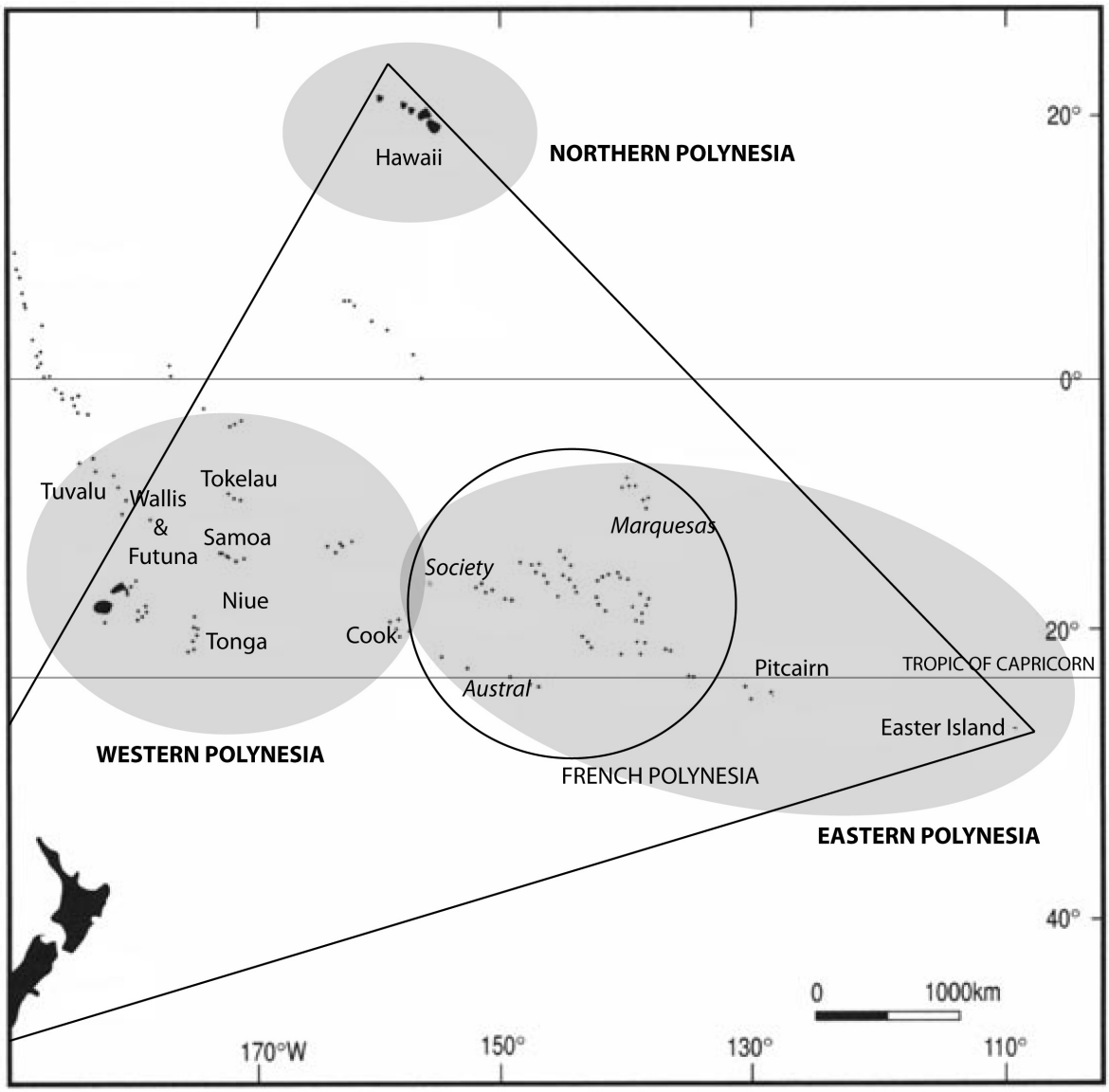


Figure 5.3.1: Map of the Polynesian triangle.

5.3.3 Material and methods

DNA extraction, amplification and sequencing

DNA was extracted from abdominal tissues using the semi-automatic Ependorf ep-Motion 5075 robot. Fragments of the mitochondrial 16S rRNA (~ 520 bp) were amplified using primers, adapted from Palumbi (1996) to our taxa: 16Sar-Lmod (TACTTCTGCCTGTTTATCAAAAA) and 16Sbmod (GGTCTGAACTCAAATCATGTAAA). DNA amplification was performed in 20µl PCR reactions, containing approximately 3ng of template DNA, 2.5mM MgCl2, 0.26mM of each nucleotide, 0.3µM of each primer, 5% DMSO, 1ng of BSA and 1.5 units of QBIOTAQ polymerase (MPBiomedicals). Amplification products were generated by an initial denaturation step of 4min at 94°C followed by 35

cycles of denaturation at 94°C for 30s, annealing at 52°C for 40s, extension at 72°C for 60s and a final extension step at 72°C for 7 min. PCR products were sequenced using the same primers and in both directions to ensure the accuracy of base calls. Chromatograms were edited using Geneious v.8 software (http://www.geneious.com/ Kearse *et al.*, 2012). All sequences were deposited in GenBank (Numbers pending, Table 5.3.1).

Molecular analyses

DNA sequences were aligned using MEGA7 software (S. Kumar *et al.*, 2016) with Muscle algorithm (Edgar, 2004). Using Bayesian information criterion in jModelTest (Guindon & Gascuel, 2003 ; Darriba *et al.*, 2012) we retained the GTR + G model. Best-scoring ML trees were estimated using RAxML HPC2 v.8.2.10 (Stamatakis, 2014) and best-scoring Bayesian Inference trees were estimated using MrBayes v.3.2.6 (Ronquist & Huelsenbeck, 2003), both methods implemented in CIPRES with the previously determined model, running for 5,000,000 generations, a sampling frequency of 1,000 and a burn in of 25%. Support for nodes was determined using posterior probabilities (PP) calculated by MrBayes implemented in the Cyber Infrastructure for Phylogenetic Research (CIPRES) portal v.3.1. (Miller *et al.*, 2010, https://www.phylo.org/). One hundred independent searches, each starting from distinct random trees, were conducted. Robustness of the nodes was assessed using non-parametric bootstrapping (Felsenstein, 1985) with 1,000 bootstrap replicates (B). A total of 30 specimens were included in the analysis, 27 initially identified as *C. weberi* collected in various Polynesian islands and 3 identified as *C. rapaensis* from Rapa (Table 5.3.1), plus a sequence from *Macrobrachium australe* published by Page *et al.* (2007b) to root the tree.

Table 5.3.1: List of the specimens used in the 16S phylogenetic study.

Species	Island	DNA voucher	Museum n°	Type status	GenBank n°	Reference
Caridina futunensis sp. nov.	Futuna (Wallis and Futuna Territory)	CA2031	MNHN-IU-2017-1459	Paratype	XXXXXXX	This study
		CA2032	MNHN-IU-2017-1460	Paratype	XXXXXXX	This study
		CA2033	MNHN-IU-2017-65	Paratype	XXXXXXX	This study
		CA2116	MNHN-IU-2018-209	Paratype	XXXXXXX	This study
		CA2117	MNHN-IU-2017-64	Paratype	XXXXXXX	This study
		CA2118	MNHN-IU-2018-195	Holotype	XXXXXXX	This study
		CA2119	MNHN-IU-2018-208	Paratype	XXXXXXX	This study
C. marquesensis sp. nov.	Hiva Oa (Fr. Polynesia)	CA2066	MNHN-IU-2017-68	Paratype	XXXXXXX	This study
		CA2067	MNHN-IU-2017-67	Paratype	XXXXXXX	This study
	Ua Pou (Fr. Polynesia)	CA2070	MNHN-IU-2018-257	Paratype	XXXXXXX	This study
	Nuku Hiva (Fr. Polynesia)	CA2074	MNHN-IU-2017-70	Paratype	XXXXXXX	This study
		CA2075	MNHN-IU-2017-69	Paratype	XXXXXXX	This study
C. rapaensis	Rapa (Fr. Polynesia)	CA2111	MNHN-IU-2016-11772	Topotype	XXXXXXX	This study
		CA2114	MNHN-IU-2016-11769	Topotype	XXXXXXX	This study
		CA2115	MNHN-IU-2016-11771	Topotype	XXXXXXX	This study
C. tupaia sp. nov.	Rarotonga (Cook Islands)	CA1046	MNHN-IU-2018-262	Paratype	XXXXXXX	This study
		CA1047	MNHN-IU-2018-263	Paratype	XXXXXXX	This study
		CA1048	MNHN-IU-2018-261	Paratype	XXXXXXX	This study
		CA1049	MNHN-IU-2018-264	Paratype	XXXXXXX	This study
	Upolu (Samoa)	CA1410	MNHN-IU-2016-11763	Paratype	XXXXXXX	This study
		CA1411	MNHN-IU-2016-11764	Paratype	XXXXXXX	This study
		CA1412	MNHN-IU-2016-11765	Paratype	XXXXXXX	This study
		CA1918	MNHN-IU-2016-11767	Paratype	XXXXXXX	This study
	Savai (Samoa)	CA1413	MNHN-IU-2016-11766	Paratype	XXXXXXX	This study
	Rurutu (Fr. Polynesia)	CA2054	MNHN-IU-2018-265	Paratype	XXXXXXX	This study
	Tahiti (Fr. Polynesia)	CA2058	MNHN-IU-2018-260	Holotype	XXXXXXX	This study
		CA2059	MNHN-IU-2018-267	Paratype	XXXXXXX	This study

	Moorea (Fr. Polynesia)	CA2062	MNHN-IU-2018-270	Paratype	XXXXXXX	This study
		CA2063	MNHN-IU-2016-11762	Paratype	XXXXXXX	This study
		GUC993			DQ478493	Page *et al.* 2007b
Macrobrachium australe	Papua	GU363			DQ681290	Page *et al.* 2007a

Collection of specimens

Specimens from Polynesia were collected by electrofishing (portable Dekka 3,000 electric device, Germany or SAMUS 1,000, Poland: http://www.electro-fisher.net/). All material preserved in 75%-95% alcohol has been deposited in the collections of the Muséum national d'Histoire naturelle in Paris (MNHN). A total of 42 recent specimens (33 identified as *C. weberi* from various Polynesian islands and 9 identified as *C. rapaensis* from Rapa and Rurutu islands) were morphologically studied. As comparative material, syntypes of *C. weberi* (MNHN-IU-2015-1755) and *C. parvirostris* (MNHN-IU-2015-1748 and MNHN-IU-2015-1754) preserved in the MNHN collections and type specimens of *C. rapaensis* (Holotype BPBM 53933 and paratype BPBM 53694) preserved in the Bernice Pauahi Bishop Museum collections (BPBM, Hawaii, USA) were examined.

Abbreviations for morphological analyses

The following abbreviations are used in the present text: cl, carapace length (mm): measured from the post-orbital margin to the posterior margin of the carapace. P1: first pereopod. P2: second pereopod. P3: third pereopod. P5: fifth pereopod. Pl1: first pleopod. Pl2: second pleopod.

Morphological comparison

The rostrum, the general cephalon, the pereopods 1, 2, 3 and 5 and the abdomen were observed using a stereoscopic microscope. The proportions of the various joints of the appendages were measured using microphotographs and AnalySIS Works software (Olympus). Drawings were made using the 'Digital Inking' method (Coleman, 2003, 2006) by tracing vectorial paths on stacks of high-resolution photographs using Adobe Illustrator (CS3) and a WACOM PTZ-1230 graphic tablet.

5.3.4 Results

Molecular analyses

As the trees obtained from the two methods were congruent, we present here the one obtained from the Bayesian Inference analysis with the posterior probabilities and the bootstrap numbers obtained from the Maximum Likelihood analysis (Figure 5.3.2). Four well-supported groups could be identified. The most basal one (A in Figure 5.3.2; PP = 0.9; B = 48; 0.6% p-distances intra-group maximum divergence) containing all the specimens from Futuna is separated (9.3% p-distance from the closest sequence) from the group containing all the others (PP = 0.97; B = 48). The second one (B in Figure 5.3.2; PP = 1; B = 100; 0.6% p-distances intra-group maximum divergence) clusters the three specimens of *C. rapaensis* from Rapa Island and is sister group (11.1% p-distance from the closest sequence) to a clade containing the two last ones (PP = 1; B = 79), one containing the specimens from Marquesas (Hiva Oa, Ua Pou and Nuku Hiva) (C in Figure 5.3.2; PP = 1; B = 99; 0.4% p-distances intra-group maximum divergence) and another containing specimens from Samoa, Cook and Society Islands (D in Figure 5.3.2; PP = 1; B = 99; 2.4% p-distances intra-group maximum divergence), separated by 12.1% (p-distance between the two the closest sequences). Within clade D, there seem to be a geographical structuration, with the specimens from Samoa all clustered in a well supported group (PP = 1; B = 99), suggesting an isolation of this population.

Each one of these clusters is homogenous enough and distant enough from its sister-group to be considered as a distinct and valid species.

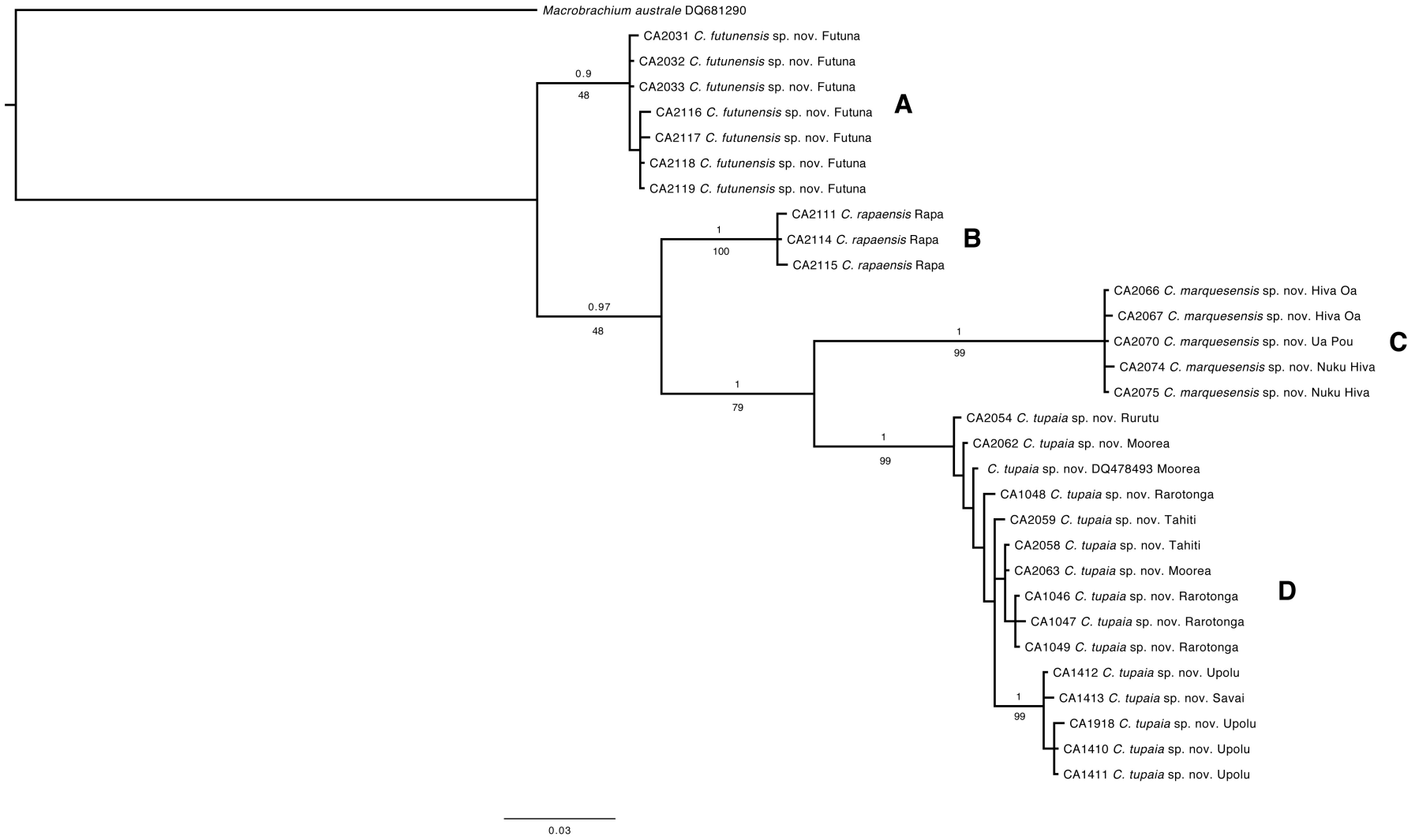


Figure 5.3.2: Bayesian Inference phylogenetic tree of the specimens studied. Numbers above branches indicate BI posterior probabilities, numbers under branches indicate ML bootstrap.

Morphological analyses

Measures and observations made on the specimens allowed us to confirm the molecular results in recognising 6 species including 3 new ones: *C. futunensis n. sp.*, *C. marquesensis n. sp.*, *C. tupaia n. sp.*, *C. rapaensis* Edmonson, 1935, *C. parvirostris* De Man, 1892, and *C. weberi* De Man, 1892. No molecular data could be obtained from specimens of *C. weberi* and *C. parvirostris* of which only type specimens were examined, but morphological studies allowed us to confidently consider them distinct. The descriptions (or re-descriptions) of the species present in Polynesia, as well as their identification key follow.

Taxonomy

Family **Atyidae De Haan, 1849**

Genus ***Caridina*** **H. Milne Edwards, 1837**

Caridina futunensis **n. sp**.

(Figure 5.3.3)

Caridina weberi – Keith and Marquet 2011: 42–43

Caridina weberi – Mary et al. 2006: 33

Material examined

Type material. Holotype: 1 ♀ ovig, cl 2.8 mm, 14 October 2004, 14°17.278'S 178°09.037'W, Leava river, Futuna, altitude 97m, G. Marquet, P. Keith & N. Mary coll., MNHN-IU-2018-195 (DNA: CA2118).

Paratypes: 1 ♀ ovig, cl 2.6 mm, same data as for holotype, MNHN-IU-2018-208 (DNA: CA2119); 1 ♀ ovig, cl 2.9 mm, same data as for holotype, MNHN-IU-2018-209 (DNA:

CA2116); 1 ♀ ovig, cl 3.1 mm, same data as for holotype, MNHN-IU-2017-1460 (DNA: CA2032); 1 ♀ ovig, 3.1 mm, same data as for holotype, MNHN-IU-2017-64 (DNA: CA2117); 1 ♀, cl 3.0 mm, same data as for holotype, MNHN-IU-2017-1459 (DNA: CA2031); 1 ♀, cl 3.6 mm, 12 October 2004, 14°17.738'S 178°08.423'W, Vainifao river, Futuna, altitude 150m, G. Marquet, P. Keith & N. Mary coll., MNHN-IU-2017-65 (DNA: CA2033).

Comparative material. Type material

Caridina parvirostris De Man, 1892.

Syntypes: 2 ♀, cl. 3.1–3.2 mm, river near Bombang, Flores Island, Indonesia, M. Weber coll., MNHN-IU-2015-1748; 1 ♀, cl. 4.2 mm, same data as for previous, MNHN-IU-2015-1754.

Caridina weberi De Man, 1892.

Syntypes: 2 ♂, cl 4.4–4.5 mm and 1 ♀ ovig, cl 6.1 mm, Kotting, Flores Island, Indonesia, M. Weber coll., MNHN-IU-2015-1755.

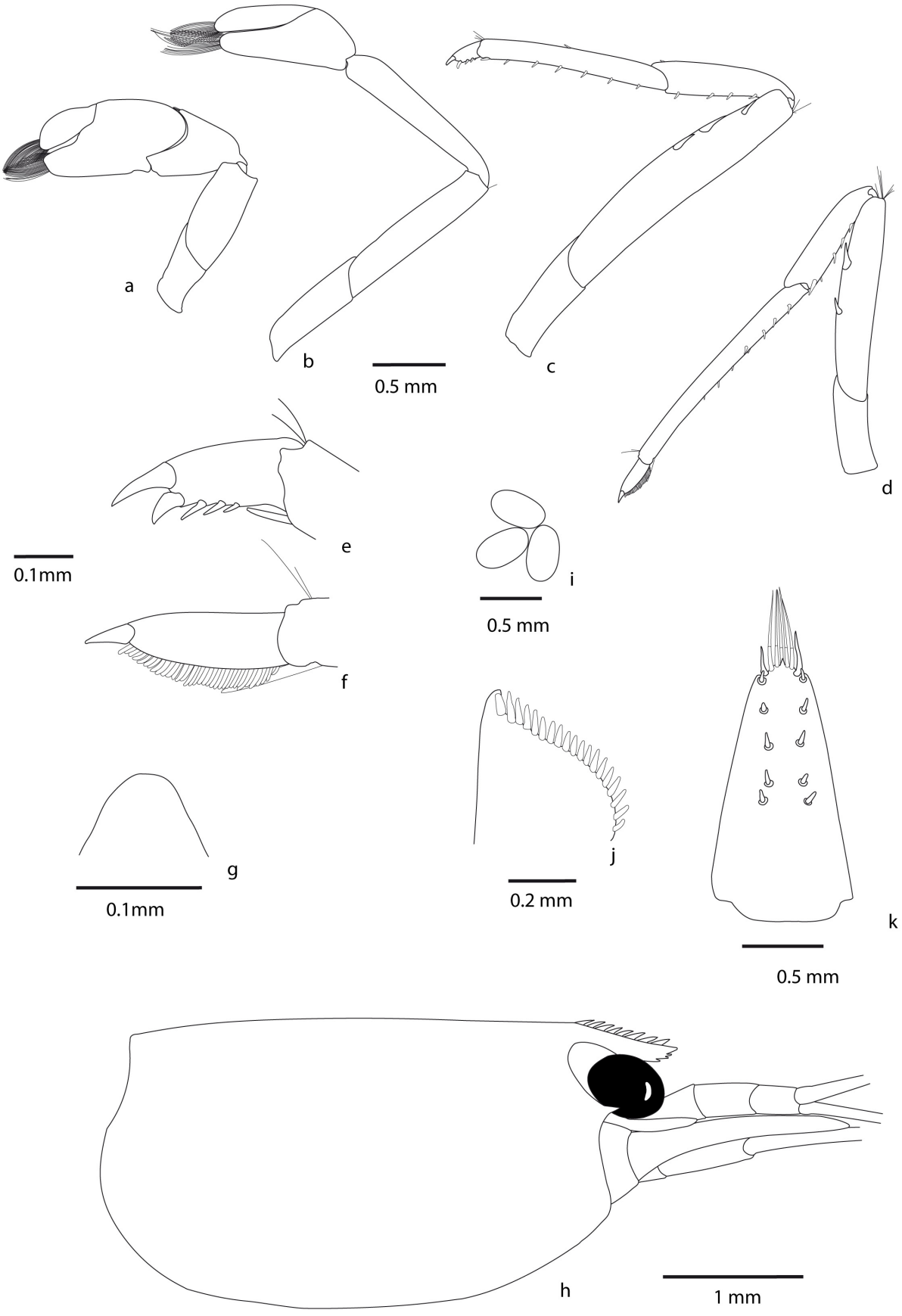


Figure 5.3.3: *Caridina futunensis n. sp*, Holotype (MNHN-IU-2018-195; DNA: CA2118), a. first pereiopod, b. second pereiopod, c. third pereiopod, d. fifth pereiopod, e. dactylus of third pereiopod, f. dactylus of fifth pereiopod, g. pre-anal carina, h. cephalothorax, i. eggs, j. uropodial diaeresis, k. telson.

Description

Cephalothorax. Rostrum (Figures 5.3.3h and 5.3.7F): always bent down, short, 0.2–0.3 of cl, reaching to base of first segment of antennular peduncle, armed dorsally with 5-10 teeth, 0 of them on carapace posterior to orbital margin, ventral margin with 0–3 teeth. Rostrum formula (0) 5–10/0–3. Suborbital angle indistinguishably fused with antennal spine. Pterygostomian margin rectangularly rounded..

Eyes well developed, anterior end reaching to 0.73 length of antennular peduncle basal antennomere. Antennular peduncle 0.47 times as long as carapace. Anterolateral angle reaching 0.20 length of the second antennomere, second antennomere distinctly shorter than third. Stylocerite reaching to 0.90 length of antennular peduncle basal antennomere. The scaphocerite reaches the tip of antennular peduncle, about 2.1 times longer than wide. Antero-lateral spine of the scaphocerite, well developped lies about level with distal margin of antennular peduncle, both being overreached by the lamella. There is a strong and slender outer spine on the antennal peduncle near the base of the scaphocerite.

Mouthparts. Left mandible, right mandible, first maxilla, second maxilla, first maxilliped, second maxilliped and third maxilliped are typical of the genus.

Pereiopods. Epipods on first four pereiopods.

Pereiopod 1 (Figure 5.3.3a): chela about 1.9–2.2 times as long as wide, movable finger 2.3–2.9 times as long as wide, 0.7–1.0 times length of palm, carpus 1.3–1.5 times as long as wide.

Pereiopod 2 (Figure 5.3.3b) More slender and longer than first pereiopod with chela 2.4–2.6 times as long as wide: movable finger 4.0–5.0 times as long as wide, 1.5–1.9 times length of palm, carpus slender 5.5–6.2 times as long as wide.

Pereiopod 3 (Figure 5.3.3c, e): stout, dactylus 2.6–3.9 times as long as wide (terminal spine included) with 5 spines on flexor margin in addition to the terminal spine, propodus 8.8–13.1 times as long as wide, 3.7–4.5 times as long as dactylus.

Pereiopod 5 (Figure 5.3.3d, f): dactylus 2.9–3.7 as long as wide with 25–38 spiniform setae on flexor margin, propodus 12.2–13.8 times as long as wide, 4.3–4.9 times as long as dactylus.

Abdomen. Third abdominal somite with moderately convex dorsal profile. Sixth abdominal somite about 0.5 of carapace, 1.3 times as long as fifth somite, shorter than telson Telson (Figure 5.3.3k) 2 times as long as wide, with four to six pairs of dorsal spinules and one pair of dorsolateral spinules, posterior margin with a median process, It is rounded with 5 intermediate plumose setae longer than lateral ones.

First male pleopod: Unknown (no males in the collections).

Second male pleopod: Unknown (no males in the collections).

Preanal carina (Figure 5.3.3g): High, unarmed.

Uropodal diaeresis (Figure 5.3.3j) with 17–22 spinules.

Eggs (Figure 5.3.3i): Size 0.43–0.53 × 0.25–0.30mm

Habitat (Figure 5.3.7G)

This new species prefers fresh and well-oxygenated running waters from the lower course to the higher course.

Etymology

This new species is named *futunensis*, from the name of the island, Futuna, where this new species occurs.

Colour pattern

Unknown

Distribution (Figure 5.3.8)

This species is known only from Futuna so far and seems to be endemic.

Remarks

This species looks like *C. parvirostris* by its bent rostrum with 5-10 dorsal teeth and no post orbital teeth (vs bent rostrum with 8–10 dorsal teeth and no postorbital teeth in *C. parvirostris*) but the P2 carpus is shorter 5.5-6.2 times as long as wide (vs 6.0–7.4 in *C. parvirostris*) and so is P2 chela 2.4–2.6 times as long as wide (vs 2.6–3.0 in *C. parvirostris*).

This species differs from *C. marquesensis n. sp.* and *C. tupaia n. sp.* by its shorter rostrum 0.2–0.3 of cl (vs 0.3–0.4 in *C. marquesensis n. sp.* and *C. tupaia n. sp.*), fewer dorsal teeth on the rostrum 5–10 (vs 10–13 in *C. marquesensis n. sp.* and 8–14 in *C. tupaia n. sp.*) and P5 dactylus with fewer spiniform setae on flexor margin 25–38 spines (vs 32–55 in *C. marquesensis n. sp.* and 29–54 in *C. tupaia n. sp.*) and by its telson with 5 intermediate setae longer than lateral ones (vs 6–14 in *C. marquesensis n. sp.* and 6–10 in *C. tupaia n. sp.*).

This species differs from *C. rapaensis* by its bent rostrum with more dorsal teeth (5–10) (vs bent rostrum with 0–5 in *C. rapaensis*) and its P5 dactylus ending in two large claws (vs one large claw in *C. rapaensis*).

Caridina marquesensis **n. sp.**

(Figure 5.3.4)

Caridina weberi – Edmonson 1935 : 10, fig.4h page 13.

Caridina weberi – Keith et al. 2013: 84–85 (partim)

Caridina weberi – Keith et al. 2002: 44–45 (partim)

Material examined

Type material. Holotype: 1 ♂, cl 2.9 mm, 9 October 2017, 09°49.750'S 135°04.418'W, Ta'ooa river, Hiva Oa Island, French Polynesia, altitude 220m, G. Marquet, P. Tiberghien, E. Feunteun, P. Gerbeaux & M. Gerbeaux coll., MNHN-IU-2017-66.

Paratypes: 1 ♀ ovig, cl 3.3 mm, same data as holotype, MNHN-IU-2017-67 (DNA: CA2067); 1 ♀, cl 2.8 mm, same data as holotype, MNHN-IU-2017-68 (DNA: CA2066); 1 ♀ ovig, cl 4.7 mm, 18 October 2017, 08°51.126'S 140°06.066'W, Taipivai river, Nuku Hiva Island, French Polynesia, altitude 130m, G. Marquet, P. Tiberghien, P. Keith & H. Lucien-Brun coll., MNHN-IU-2017-69 (DNA: CA2075); 1 ♀ ovig, cl 4.8mm, same data as previous, MNHN-IU-2017-70 (DNA: CA2074); 1 ♀, cl 3.2 mm, 14 October 2017, 09°25.452'S 140°03.409'W, unnamed rivulet, Ua Pou Island, French Polynesia, altitude 130m, G. Marquet, P. Tiberghien, P. Keith & H. Lucien-Brun coll., MNHN-IU-2017-71 (DNA: CA2071); 1 ♀, cl 3.4 mm, same data as previous, MNHN-IU-2018-257 (DNA: CA2070); 1 ♀ ovig, cl 5.2 mm, 21 February 2000, 09°24.180'S 140°03.060'W, Paaumea river, Ua Pou Island, French Polynesia, altitude 200m, P. Keith & E. Vigneux coll., MNHN-IU-2018-258; 1 ♀, cl 4.6 mm, same data as previous, MNHN-IU-2018-259.

Comparative material. Type material
Caridina weberi De Man, 1892.

Syntypes: 2 ♂, cl 4.4–4.5 mm and 1 ♀ ovig, cl 6.1 mm, Kotting, Flores Island, Indonesia, M. Weber coll., MNHN-IU-2015-1755.

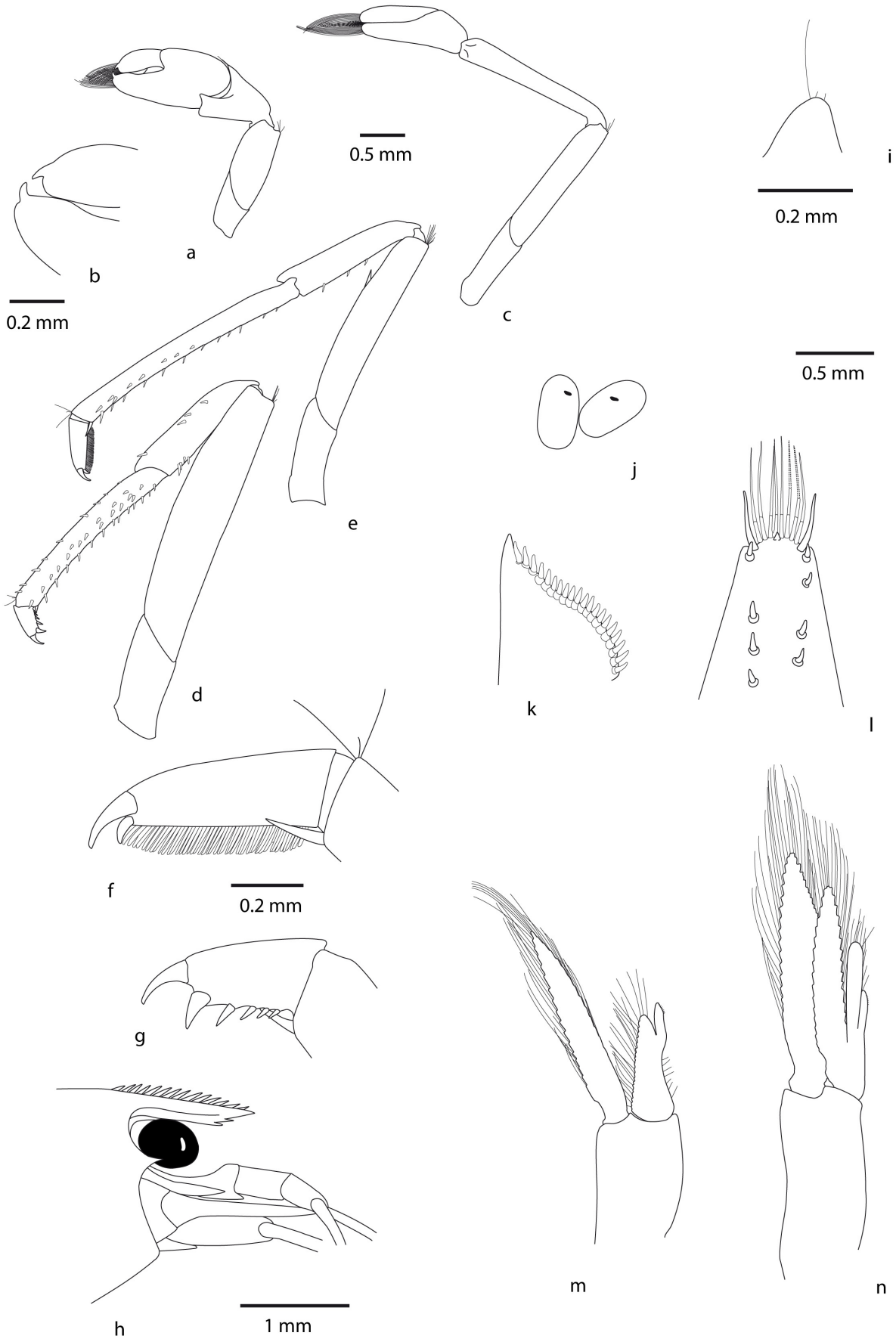


Figure 5.3.4: *Caridina marquesensis n. sp*. Paratype (MNHN-IU-2017-71; DNA: CA2071), a. first pereiopod; b. first pereiopod detail of the fingertips; c. second pereiopod; d. third pereiopod; e. fifth pereiopod; f. dactylus of fifth pereiopod; g. dactylus of third pereiopod; h. cephalothorax; i. preanal carina; k. uropodal diaeresis; l. telson. Paratype (MNHN-IU-2017-69; DNA: CA2075), j. eggs. Holotype (MNHN-IU-2017-66), m. first pleopod; n. second pleopod.

Description

Cephalothorax. Rostrum (Figure 5.3.4h): always bent down, short, 0.3–0.4 of cl, reaching to base or middle of second segment of antennular peduncle, armed dorsally with 10–13 teeth, 0–3 teeth on carapace posterior to orbital margin (mostly 2–3), ventral margin with 0–3 teeth. Rostrum formula (0–3) 10–13/0–3. Suborbital angle indistinguishably fused with antennular tooth. Pterygostomian margin rectangularly rounded.

Eyes well developed, anterior end reaching to 0.62 length of antennular peduncle basal antennomere. Antennular peduncle 0.50 times as long as carapace. Anterolateral angle reaching 0.20 length of the second antennomere, second antennomere distinctly shorter than third. Stylocerite reaching to 0.9 length of antennular peduncle basal antennomere. The scaphocerite reaches just slightly beyond the tip of antennular peduncle, about 2.1 times longer than wide. Antero-lateral spine of the scaphocerite, well developped lies about level with distal margin of antennular peduncle, both being overreached by the lamella. There is a strong and slender outer spine on the antennal peduncle near the base of the scaphocerite.

Mouthparts. Left mandible, right mandible, first maxilla, second maxilla, first maxilliped, second maxilliped and third maxilliped are typical of the genus.

Pereiopods. Epipods on first four pereiopods.

Pereiopod 1 (Figure 5.3.4a): chela about 2.0-2.2 times as long as wide, movable finger 2.2–3.1 times as long as wide, 0.6–0.9 times length of palm, carpus 1.3–1.7 times as long as wide. Tips of both fingers with prominent claws (Figure 4b).

Pereiopod 2 (Figure 5.3.4c) more slender and long than first pereiopod with chela 2.5–3.3 times as long as wide: movable finger 4.2–5.2 times as long as wide, 1.5–1.8 times length of palm, carpus slender 5.5–6.6 times as long as wide.

Pereiopod 3 (Figure 5.3.4d, g): stout, dactylus 2.7–3.1 times as long as wide including terminal spine) with 6–7 spines in parallel to the terminal one, propodus with numerous spines on its surface, 8.0–9.3 times as long as wide, 3.4–4.3 times as long as dactylus.

Pereiopod 5 (Figure 5.3.4e, f): dactylus 3.4–5.5 as long as wide, ending in two large claws with 32–55 spiniform setae on flexor margin, propodus 13.0–17.7 times as long as wide, 3.8–5.2 times as long as dactylus.

Abdomen. Third abdominal somite with moderately convex dorsal profile. Sixth abdominal somite about 0.37 of carapace, 0.7 times as long as fifth somite, shorter than telson. Telson (Figure 5.3.4l) 2.1 times as long as wide, with 4 to six pairs of dorsal spines and one pair of dorsolateral spines, posterior margin with a median process, It is rounded with 6–14 intermediate plumose setae longer than lateral ones

First male pleopod (Figure 5.3.4m): Endopod extending to half length of exopod, elongate, with a developed appendix interna near distal end of endopod.

Second male pleopod (Figure 5.3.4n): Appendix masculina reaching 0.72 times length of endopod, appendix interna reaching about 0.50 times appendix masculina length.

Preanal carina (Figure 5.3.4i): High, unarmed.

Uropodal diaeresis (Figure 5.3.4k): with 16–22 spinules.

Eggs (Figure 5.3.4j): Size 0.39–0.47 × 0.23–0. 27mm.

Habitat

This new species prefers fresh and well-oxygenated running waters from the lower course to the higher courses. In Nuku Hiva (Marquesas islands) *C. marquesensis n. sp.* lives in lentic habitat of the lower course, associated with accumulations of leaves, where it collects small detritus and eventually is able to swim (Fossati *et al.*, 2002).

Etymology

This new species is named *marquesensis,* from the name of the archipelago, Marquesas Islands, where it occurs.

Colour pattern (Figure 5.3.7C)

The live general colour of the body is bluish to greenish and slightly translucent. Numerous red spots are visible all over the body.

Distribution (Figure 5.3.8)

This species is known only from Marquesas islands (Nuku Hiva, Hiva Oa, Ua Pou Islands) so far and seems to be endemic.

Remarks

This new species was identified as *C. weberi* several times: Edmonson (1935), Adamson (1935, 1939), Marquet (1988, 1991), Keith and Vigneux (2002), Keith *et al.* (2002), Keith *et al.* (2013). Indeed this species looks like *C. weberi sensu stricto* by its straight or bent down rostrum, 0.3–0.4 of cl, (vs 0.4 in *C. weberi*) reaching to the base or middle of second segment of antennular peduncle with numerous dorsal teeth 10–13 (vs 10–19 in *C. weberi*), but differs by the presence of 0–3 dorsal teeth situated on carapace behind orbital margin (vs 0 in *C. weberi*). Also its P1 movable finger is shorter 0.6–0.9 times length of palm (vs 1.2–1.3 in *C. weberi*) as is its P1 carpus 1.3–1.7 times as long as wide (vs 1.9–2.0 in *C. weberi*), and its P2 carpus is longer, 5.5–6.6 times as long as wide (vs 4.9–5.0 in *C. weberi*). Furthermore, the posterior margin of the telson always shows only one pair of lateral spines (vs 2 in *C. weberi*).

This species differs from *C. tupaia n. sp.* by its rostrum with mostly 2–3 teeth on carapace posterior to orbital margin (vs mostly 0 in *C. tupaia n. sp.*), its tips of both P1 fingers with prominent claws (vs not in *C. tupaia n. sp.*) and its P5 dactylus ending in two large claws (vs one claw in *C. tupaia n. sp.*).

Caridina tupaia n. sp.

(Figure 5.3.5)

Caridina weberi – Edmonson 1935 : 8-9, fig3 (a-f) page 11, fig. 4g page 13.

Caridina cf. weberi – Page et al. 2007b: specimen voucher GU-993, sequence DQ478493.

Caridina weberi – Keith et al. 2013: 84–85 (partim)

Caridina weberi – Keith et al. 2002: 44–45 (partim)

Material examined

Type material. Holotype: 1 ♀, cl 4.1 mm, 23 October 2017, 17°49.719'S 149°17.067'W, Papehere river, Tahiti Island, French Polynesia, G. Marquet & P. Tiberghien coll., MNHN-IU-2018-260 (DNA: CA2058).

Paratypes: 1 ♂, cl 3.9 mm, 11 July 2010, 21°14.806'S 159°43.795'W, Avana river, Rarotonga Island, Cook Islands, altitude 5–10m, P. Keith, P. Gerbeaux & G. Marquet coll., MNHN-IU-2018-261 (DNA: CA1048); 1 ♂, cl 4.1 mm, same data as previous, MNHN-IU-2018-262 (DNA: CA1046); 1 ♂, cl 4.2 mm, same data as previous, MNHN-IU-2018-263 (DNA: CA1047); 1 ♀ ovig, cl 3.8 mm, same data as previous, MNHN-IU-2018-264 (DNA: CA1049); 1 ♂, cl 2.5 mm, 26 September 2017, 22°27.009'S 151°21.061'W, Puputa river, Rurutu Island, French Polynesia, G. Marquet & P. Tiberghien coll., MNHN-IU-2018-265 (DNA: CA2054); 1 ♀, cl 3.5 mm, same data as previous, MNHN-IU-2018-266 (DNA: CA2055); 1 ♀, cl 4.1 mm, same data as holotype, MNHN-IU-2018-267 (DNA: CA2059); 1 ♀ ovig, cl 4.2 mm, 1 July 2001, Mahidi river, Huahiné Island, French Polynesia, P. Keith & E. Vigneux coll., MNHN-IU-2018-268; 1 ♀, cl 4.5 mm, same data as previous, MNHN-IU-2018-269; 1 ♀, cl 2.8 mm, 5 October 2017, 17°32.785'S 149°48.484'W, Afareaitu river, Moorea Island, French Polynesia, altitude 109m, G. Marquet & P. Tiberghien coll., MNHN-IU-2018-270 (DNA: CA2062); 1 ♀ ovig, cl 3.9 mm, same data as previous, MNHN-IU-2016-11762 (DNA: CA2063); 1 ♀ ovig, cl 6.2 mm, 10 July 2014, 13°54.543'S 171°35.300'W, Falefa river, Upolu Island, Samoa, altitude <50m, P. Gerbeaux coll., MNHN-IU-2016-11763 (DNA: CA1410); 1 ♀ ovig, cl 5.5 mm, 8 July 2014, 13°53.642'S 171°39.913'W, Tuafaleloa river, Upolu Island, Samoa, altitude 100m, P. Gerbeaux coll.,

MNHN-IU-2016-11764 (DNA: CA1411); 1 ♀ ovig, cl 4.4 mm, 8 July 2014, 13°53.788'S 171°40.031'W, Tuafaleloa river, Upolu Island, Samoa, altitude 250m, P. Gerbeaux coll., MNHN-IU-2016-11765 (DNA: CA1412); 1 ♀, cl 5.0 mm, 12 July 2014, 13°43.741'S 172°18.832'W, Faleata river, Savaii Island, Samoa, altitude 195m, P. Gerbeaux coll., MNHN-IU-2016-11766 (DNA: CA1413); 1 ♀ ovig, cl 5.0 mm, 7 July 2014, 13°51.900'S 171° 41.196'W, Ma'epu river, Savaii Island, Samoa, altitude 20–50m, P. Gerbeaux coll., MNHN-IU-2016-11767 (DNA: CA1918).

Comparative material. Type material

Caridina weberi De Man, 1892.

Syntypes: 2 ♂, cl 4.4–4.5 mm and 1 ♀ ovig, cl 6.1 mm, Kotting, Flores Island, Indonesia, M. Weber coll., MNHN-IU-2015-1755.

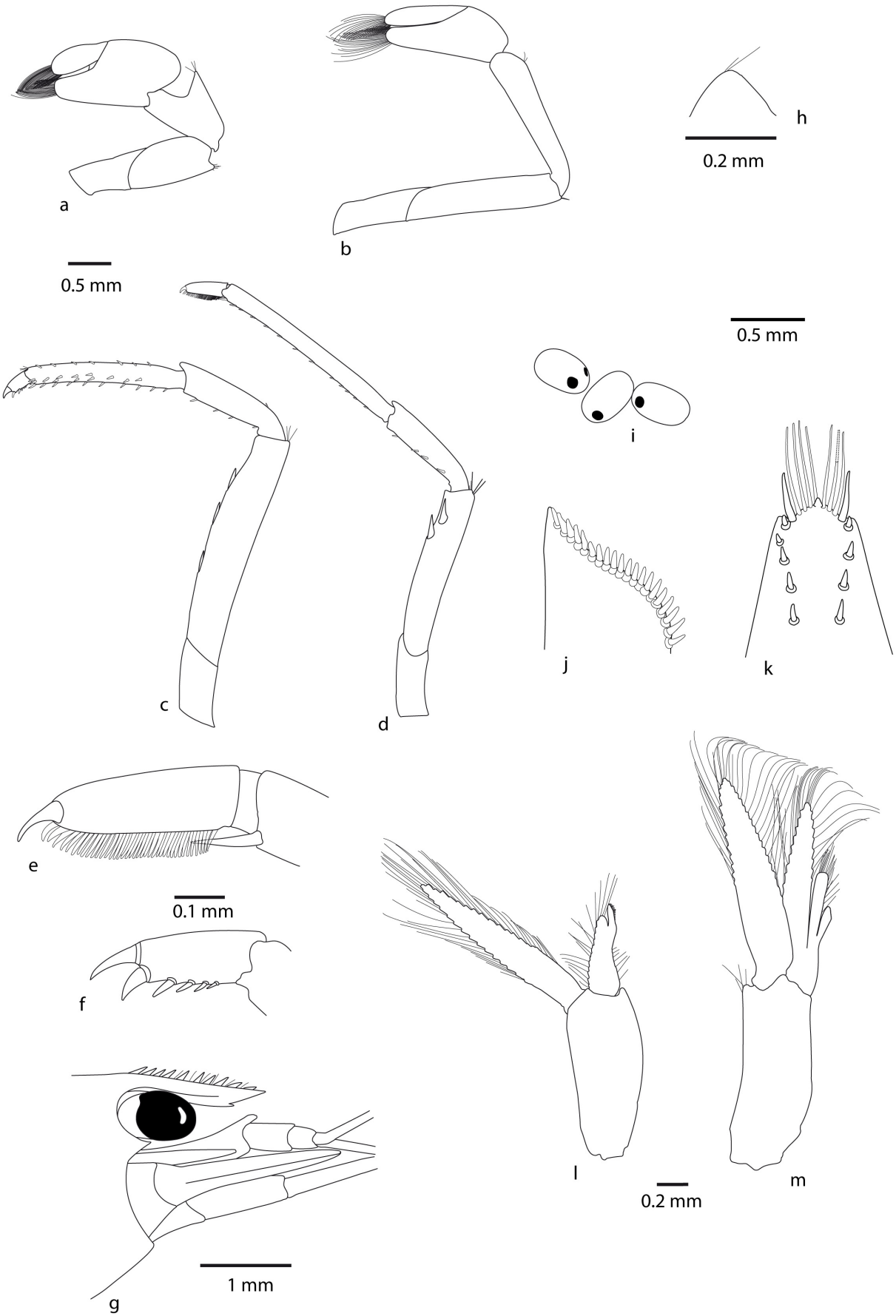


Figure 5.3.5: ***Caridina tupaia n. sp.*** **Holotype (MNHN-IU-2018-260; DNA: CA2058), a. first pereiopod; b. second pereiopod; c. third pereiopod; d. fifth pereiopod; e. dactylus of fifth pereiopod; f. dactylus of third pereiopod; g. cephalothorax; h. preanal carina; j. uropodal diaeresis; k. telson. Paratype (MNHN-IU-2018-268), i. eggs. Paratype (MNHN-IU-2018-261; DNA: CA1048), l. first pleopod; m. second pleopod.**

Description

Cephalothorax. Rostrum (Figure 5.3.5g): rarely straight, mostly bent down, short, 0.3–0.4 of cl, reaching to base or middle of second segment of antennular peduncle, armed dorsally with 8–14 teeth, 0–3 of them situated on carapace posterior to orbital margin (mostly 0), ventral margin with 1–3 teeth. Rostrum formula (0–3) 8–14/1–3. Suborbital angle indistinguishably fused with antennular tooth. Pterygostomian margin rectangularly rounded.

Eyes well developed, anterior end reaching to 0.77 length of antennular peduncle basal antennomere. Antennular peduncle 0.55 times as long as carapace. Anterolateral angle reaching 0.20 length of the second antennomere, second antennomere distinctly shorter than the third. Stylocerite reaching to 0.90 length of antennular peduncle basal antennomere. The scaphocerite reaches just slightly beyond the tip of antennular peduncle, about 3 times longer than wide. Antero-lateral spine of the scaphocerite, well developped lies about level with distal margin of antennular peduncle, both being overreached by the lamella. There is a strong and slender outer spine on the antennal peduncle near the base of the scaphocerite.

Mouthparts. Left mandible, right mandible, first maxilla, second maxilla, first maxilliped, second maxilliped and third maxilliped are typical of the genus.

Pereiopods. Epipods on first four pereiopods.

Pereiopod 1 (Figure 5.3.5a): chela about 1.9–2.1 times as long as wide, movable finger 2.2–3.3 times as long as wide, 0.7 1.0 times length of palm, carpus 1.3–1.7 times as long as wide.

Pereiopod 2 (Figure 5.3.5b) more slender and longer than first pereiopod with chela 2.4–2.8 times as long as wide: movable finger 3.8–4.9 times as long as wide, 1.3–1.7 times length of palm, carpus slender 4.7–6.1 times as long as wide.

Pereiopod 3 (Figure 5.3.5c, f): stout, dactylus 2.6–4.2 times as long as wide including terminal spine with 5–7 spines in parallel to the terminal one, propodus with numerous spines on its surface, 7.6–10.6 times as long as wide, 3.4–5.2 times as long as dactylus.

Pereiopod 5 (Figure 5.3.5d, e): dactylus 3.5–4.7 as long as wide with 29–54 spiniform setae on flexor margin, propodus 11.6–16.0 times as long as wide, 3.7–4.4 times as long as dactylus.

Abdomen. Third abdominal somite with moderarely convex dorsal profile. Sixth abdominal somite about 0.46 times carapace length, as long as fifth somite, shorter than telson . Telson (Figure 5.3.5k): 2.3 times as long as wide, with four or five pairs of dorsal spines and one pair of dorsolateral spines, posterior margin with a median process, It is rounded with 6–10 intermediate plumose setae longer than lateral ones.

First male pleopod (Figure 5.3.5l): Endopod extending to half length of exopod, elongate, with a developed appendix interna near distal end of endopod.

Second male pleopod (Figure 5.3.5m): Appendix masculina reaching 0.68 times length of endopod, appendix interna reaching about 0.40 times appendix masculina length

Preanal carina (Figure 5.3.5h): High, unarmed.

Uropodal diaeresis (Figure 5.3.5j): With 16–20 spinules.

Eggs (Figure 5.3.5i): Size 0.38–0.47 × 0.20–0. 30mm.

Habitat

This new species prefers fresh and well-oxygenated running waters from the lower course to the higher courses. Under the name of *C. weberi*, its microhabitat was intensely studied in Opunohu river (Moorea) with that of another sympatric species *Atyoida pilipes* (Newport, 1847). *C. tupaia n. sp.* were often found near leaf litter, tending to aggregate in on the bottom of pools where detritus accumulated. Both their leaf litter and ability to consume it in the laboratory indicate the possibility that this scraper increase rates of litter decomposition (Williams, 2002). Cheliped morphology and, consequently, differences in feeding methods are the main factors determining the microdistribution of *A. pilipes* and *C. weberi*. *A. pilipes* is filter feeding in faster flowing habitats, while *C. weberi* are scraping and sweeping detritus within and on leaf surfaces and also in other microhabiats on the stream channel bed (Resh *et al.*, 1990). *C. weberi*, whose microhabitat is in pools and slow water, may be less able to grasp the substrate in high velocity water, while *A. pilipes* which is often found in the faster moving water of

riffles may be better able to grasp the substrate (Feldman, 1996). These differences are likely a contributing factor in their distribution above and below barriers such as waterfalls and culverts, which require fighting current to move farther upstream. The climbing ability of these two species was tested by Falvo (2013) in rivers of Moorea (Opunohu and Afareaitu). *C. weberi* had the highest success rate and *A. pilipes* was the fastest climber. This latter is found at higher reaches of the streams than *C. weberi*. These species exhibited different climbing behaviours. *C. weberi* would consistently take breaks while climbing, only moving a few centimetres at a time, while *A. pilipes* would rarely stop once it started climbing. An examination of the distal portions of the pereiopods of *A. pilipes* and *C. weberi* indicates that *A. pilipes* is more strongly built and its appendages may also be better suited for gripping substrate and with standing current, explaining its ability to reach higher altitudes.

Etymology

This new species is named in honour of Tupaia, the Polynesian pilot of James Cook, on board of H.M.S. Endeavour for its ability to cross long distances between Polynesian islands.

Colour pattern (Figure 5.3.7A, B)

The live general colour of the body is yellowish and slightly translucent. Numerous red spots are visible all over the body. Sometimes, a lighter longitudinal dorsal band is present stretching from the rostrum to the telson.

Distribution (Figure 5.3.8)

Among the species studied here, this species seems to have the widest distribution in Polynesia (Upolu, Savaii (Samoa), Rarotonga (Cook Islands), Rurutu, Tahiti, Moorea, and Huahine (French Polynesia)).

Remarks

This new species was identified as *C. weberi* several times: Edmonson (1935), Adamson (1935, 1939), Marquet (1988, 1991), Keith and Vigneux (2002), Keith et al. (2002), Page *et al.* (2007a), Keith *et al.* (2013). Indeed this species looks like *C. weberi sensu stricto* by its straight or bent down rostrum, 0.3–0.4 of cl, (vs 0.4 in *C. weberi*) reaching to the base or middle of second segment of antennular peduncle with numerous dorsal teeth 8–14 (vs 10–19 in *C. weberi*), but differs by the presence of 0–3 dorsal teeth situated on carapace behind orbital margin (vs 0 in *C. weberi*). Also its P1 movable finger is shorter 0.7–1.0 times length of palm (vs 1.2–1.3 in *C. weberi*) as is its P1 carpus 1.3–1.7 times as long as wide (vs 1.9–2.0 in *C. weberi*). Furthermore, the posterior margin of the telson shows always only one pair of lateral spines (vs 2 in *C. weberi*).

This species is easily separated from *C. marquesensis n. sp.* by numerous characters (see previously).

Caridina rapaensis Edmonson, 1935

(Figure 5.3.6)

Caridina rapaensis – Edmonson 1935: 12, 14, fig. 5(a-h) page 15

Caridina rapaensis – Keith *et al.*, 2002: 40–41

Caridina rapaensis – Keith *et al.*, 2013: 78–79

Material observed

Type material. Holotype: 1 ♀, cl 4.7 mm, 1934, a freshwater stream, Rapa Island, French Polynesia, Mangarevan Expedition coll., BPBM 53933.

Paratype: 1 ♀, cl 5.0 mm, same data as holotype, BPBM 53694.

Other material. 1 ♂, cl 3.3 mm, 15 June 2001, 27°35.919'S 144°20.392'W, Panui stream, Rapa Island, French Polynesia, altitude 18m, G. Marquet coll., MNHN-IU-2016-11768; 1 ♂, cl 3.4 mm, same data as previous, MNHN-IU-2016-11769 (DNA: CA2114); 1 ♀, cl 4.8 mm, same data as previous, MNHN-IU-2016-11770; 1 ♀, cl 5.2 mm, same data as previous, MNHN-IU-2016-11771 (DNA: CA2115); 1 ♀, cl 6.0 mm, same data as previous, MNHN-IU-2016-11772 (DNA: CA2111); 2 ♂, cl 2.5–2.9 mm and 2 ♀ ovig, cl 4.0–4.6 mm, 2 May 1986, 22°26.240'S 151°22.243'W, Tevaovai stream, Rurutu Island, French Polynesia, altitude 52m, G. Marquet coll., MNHN-IU-2015-1921.

Comparative material. Type material

Caridina parvirostris De Man, 1892.

Syntypes: 2 ♀, cl. 3.1–3.2 mm, river near Bombang, Flores Island, Indonesia, M. Weber coll., MNHN-IU-2015-1748; 1 ♀, cl. 4.2 mm, same data as for previous, MNHN-IU-2015-1754.

Caridina weberi var. *keiensis* Roux, 1911

Lectotype: 1 ♀ ovig. cl 5.7mm, 5 June 1908, Warka, Grand Kei Island, Indonesia, H. Merton coll., NMB 6.IV.b.

Paralectotypes: 2 ♂ cl 2.6mm, 1 ♂ 6.IV.b, same data as for lectotype, NMB 6.IV.a; 1 ♀, cl 5.5mm and 1 ♂, cl 3.4mm, same data as for lectotype, NMB 6.IV.b.

Caridina weberi De Man, 1892.

Syntypes: 2 ♂, cl 4.4–4.5 mm and 1 ♀ ovig, cl 6.1 mm, Kotting, Flores Island, Indonesia, M. Weber coll., MNHN-IU-2015-1755.

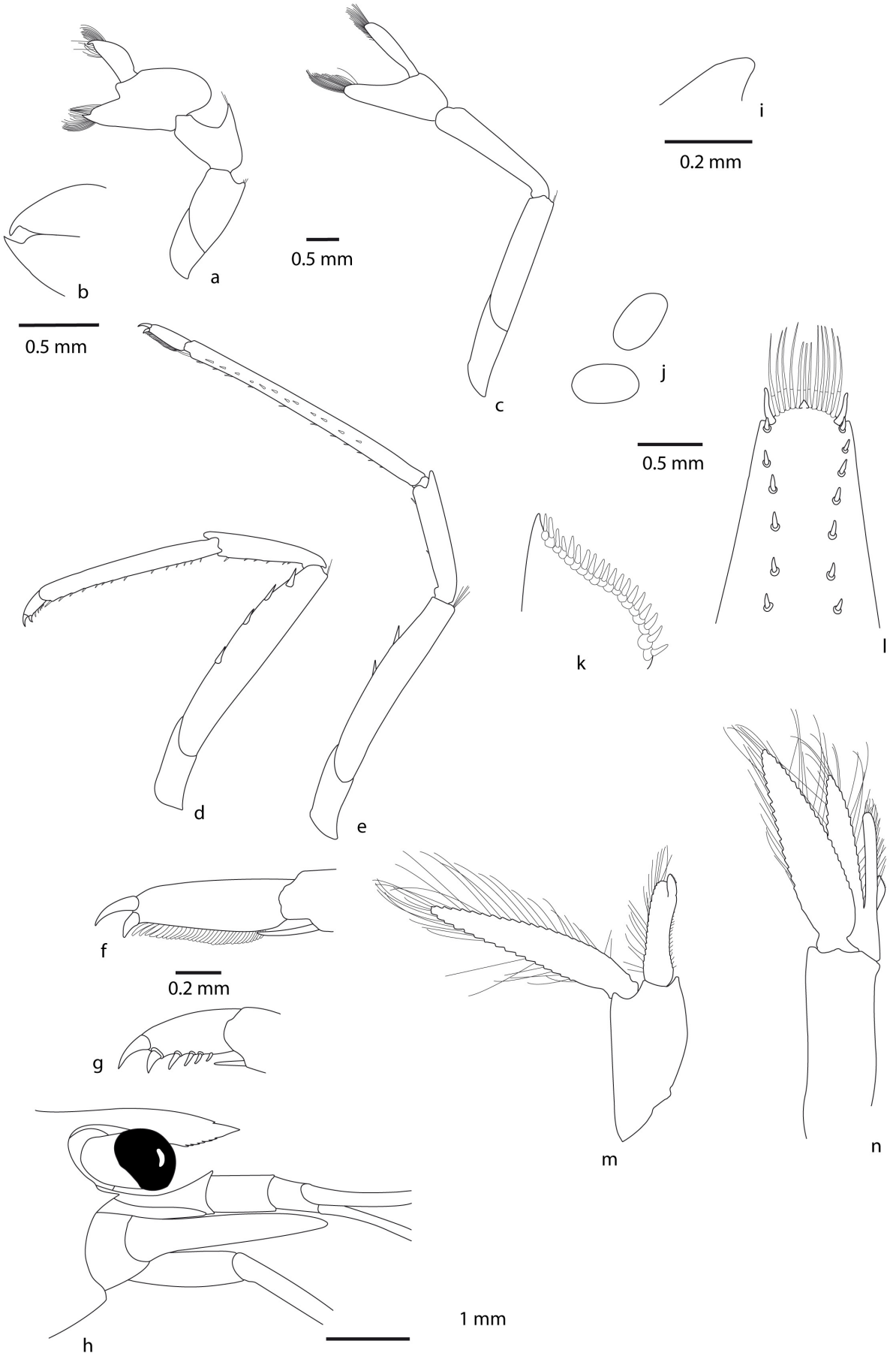


Figure 5.3.6: ***Caridina rapaensis.*** **(MNHN-IU-2016-11771; DNA: CA2115), a. first pereiopod; b. first pereiopod, detail of the fingertips; c. second pereiopod; d. third pereiopod; e. fifth pereiopod; f. dactylus of fifth pereiopod; g. dactylus of third pereiopod; h. cephalothorax; i. preanal carina; k. uropodal diaeresis; l. telson. (MNHN-IU-2015-1921: ♀ ovig, 4.0 mm), j. eggs. (MNHN-IU-2015-1921: ♀, cl 2.9 mm), m. first pleopod; n. second pleopod.**

Description

Cephalothorax. Rostrum (Figure 5.3.6h): always bent down, short, 0.3–0.5 of cl, reaching to the base of second segment of antennular peduncle, armed dorsally with 0-5 teeth, occupying the distal fourth of the border, no of them situated on carapace posterior to orbital margin, ventral margin with 1-6 teeth. Rostrum formula (0) 0–5/1–6. Suborbital angle indistinguishably fused with antennular tooth. Pterygostomian margin rounded.

Eyes well developed, anterior end reaching to 0.77 length of antennular peduncle basal antennomere. Antennular peduncle 0.44 times as long as carapace. Anterolateral angle reaching 0.20 length of the second antennomere, second antennomere distinctly shorter than the third. Stylocerite reaching to the end of antennular peduncle basal antennomere. The scaphocerite reaches just slightly beyond the tip of antennular peduncle, about 3.1 times longer than wide. Antero-lateral spine of the scaphocerite, well developped lies about level with distal margin of antennular peduncle, both being overreached by the lamella. There is a strong and slender outer spine on the antennal peduncle near the base of the scaphocerite.

Mouthparts. Left mandible, right mandible, first maxilla, second maxilla, first maxilliped, second maxilliped and third maxilliped are typical of the genus.

Pereiopods. Epipods on first four pereiopods.

Pereiopod 1 (Figure 5.3.6a): chela about 1.5–2.5 times as long as wide, movable finger 1.5–4.4 times as long as wide, 0.5–1.4 times length of palm, carpus 1.2–1.7 times as long as wide. Tips of both fingers with prominent claws (Figure 6b).

Pereiopod 2 (Figure 5.3.6c): more slender and long than first pereiopod with chela 2.0–2.7 times as long as wide: movable finger 3.2–4.7 times as long as wide, 1.3–1.9 times length of palm, carpus slender 4.2–5.2 times as long as wide.

Pereiopod 3 (Figure 5.3.6d, g): stout, dactylus 2.7–3.9 times as long as wide including terminal spine with 6–8 spines in parallel to the terminal one, propodus 9.0–12.8 times as long as wide, 3.9–4.9 times as long as dactylus.

Pereiopod 5 (Figure 5.3.6e, f): dactylus 3.1–4.9 as long as wide, ending in two large claws with 30–45 spiniform setae on flexor margin, propodus 11.7–19.0 times as long as wide, with numerous spines on its surface, 4.5-5.4 times as long as dactylus.

Abdomen. Third abdominal somite with moderarely convex dorsal profile. Sixth abdominal somite about 0.4 times carapace length, 1.2 times as long as fifth somite, shorter than telson. Telson (Figure 5.3.6k): 2.9 times as long as wide, with five or six pairs of dorsal spines and one pair of dorsolateral spines, posterior margin with a little median process, It is rounded with 5–13 intermediate plumose setae much longer than lateral ones

First male pleopod (Figure 5.3.6m): Endopod extending to 0.42 times length of exopod, rectangular, with a short appendix interna near distal end of endopod.

Second male pleopod (Figure 5.3.6n): Appendix masculina reaching 0.83 times length of endopod, appendix interna reaching about 0.34 times appendix masculina length

Preanal carina (Figure 5.3.6i): High, unarmed.

Uropodal diaeresis (Figure 5.3.6k): with 15–20 spinules.

Eggs (Figure 5.3.6j): Size 0.44–0.49 × 0.26–0.29mm.

Habitat

This new species prefers fresh and well-oxygenated running waters from the lower course to the middle course.

Colour pattern (Figure 5.3.7D, E)

The live general colour of the body is yellowish to greenish and slightly translucent. Numerous red spots are visible all over the body.

Distribution (Figure 5.3.8)

This species is known only from Austral Islands (Rurutu and Rapa islands) so far and seems to be endemic.

Remarks

Our specimens look like those described by Edmonson (1935): Rostrum always bent down, short, 0.3–0.5 of cl, reaching to base of second segment of of antennular peduncle (vs rostrum slender, slightly turned down, reaching to the middle of the second segment of the antennular peduncle for Edmonson (1935)), rostrum armed with 0–5 teeth on dorsal margin, occupying the distal fourth of the border, ventral margin with 1–6 teeth. (vs rostral teeth small and few, stronger above than below, those above occupying the distal fourth of the border, the first (basal) tooth below being posterior to the first one of the upper border for Edmonson (1935)), P1 finger 0.5–1.4 times length of palm (vs dactylus as long as upper border of palm for Edmonson (1935)), P3 dactylus with 6–8 spines on flexor margin in addition to the terminal spine (vs border dactylus provided with a stout tooth just proximal of the tip, and four other small spines for Edmonson (1935)) P5 dactylus ending in two large claws with 30–45 spiniform setae on flexor margin (vs dactylus of fifth leg bearing numerous short hairs and a strong supplementary tooth near the tip for Edmonson (1935)), posterior margin of the telson with a little median process, rounded with 5–13 intermediate plumose setae much longer than lateral ones (vs telson with 6 pairs of small spinelets on upper border, its posterior margin bearing a small median tooth for Edmonson (1935)), and uropodal diaeresis with 15–20 spinules (vs uropodal spinelets 20 in number for Edmonson (1935)).

This species differs from *C. parvirostris* by its bent rostrum with fewer dorsal teeth (0–5) (vs bent rostrum with 8–10 dorsal teeth in *C. parvirostris*) and its P2 carpus shorter 4.2–5.2 times as long as wide (vs 6.0–7.4 in *C. parvirostris*) and so is P2 chela 2.0–2.7 times as long as wide (vs 2.6–3.0 in *C. parvirostris*).

This species differs from *C. futunensis n. sp.* by its bent rostrum with fewer dorsal teeth (0–5) (vs bent rostrum with 5–10 in *C. futunensis n. sp.*) and its P5 dactylus ending in two large claws (vs one large claw in *C. futunensis n. sp.*).

C. rapaensis was reported by Shokita (2003) from Ryukyu Islands, but according to Cai and Shokita (2006a), these specimens actually belong to *C. prashadi* Tiwari & Pilai, 1971 described from Andaman Islands. Indeed, even if the rostrum formula is similar (0) 0–5/1–6 (vs (0)1–6/3–6 in *C. prashadi*), *C. rapaensis* can be distinguished easily from *C. prashadi* by its prominent claws on the tips of both P1 fingers (vs no prominent claws

in *C. prashadi*) and by its P5 dactylus ending in two large claws (vs one claw in *C. prashadi*) with 30–45 spiniform setae on flexor margin (vs 29 in *C. prashadi*).

C. rapaensis looks like *C. weberi* var. *keiensis* from Kei Islands (Indonesia) by its bent and short rostrum, its P1 chela bearing prominent claws and its P5 dactylus biunguiculate. However, they can be distinguished by the number of dorsal teeth on the rostrum 0–5 (vs 2–13 in *C. weberi* var. *keiensis*). Furthermore, considering the distance between Kei Islands and Polynesia and that *C. weberi* var. *keiensis* has never been collected in between, we consider it to be a distinct species.



Figure 5.3.7: Live colouration of the species: A, B. *C. tupaia n. sp.* (Credit GM). C. *C. marquesensis n. sp.* (Credit GM). D, E. *C. rapaensis* (Credit E. Vigneux). F. *C. futunensis n. sp.* (Credit VM). G. Habitat of *C. futunensis n. sp.* in Futuna (Credit A. Dutartre).

Key of the Polynesian species of the C. weberi complex

1. Rostrum armed dorsally with many teeth (10–19), relatively long (0.3–0.4 of cl, reaching to base or middle of antennular peduncle second antennomere).....3

2 Rostrum armed dorsally with few teeth (0–10), mostly relatively short (0.2–0.3 of cl, reaching to end of antennular peduncle basal antennomere).....4

3.1 Mostly 2–3 dorsal rostrum teeth situated on carapace behind orbital margin, tips of both P1 fingers with prominent claws, P5 dactylus ending in two large claws.....*C. marquesensis n. sp.*

3.2 Mostly 0 dorsal rostrum teeth, tips of both P1 fingers without prominent claws, P5 dactylus ending in one large claws.....*C. tupaia n. sp.*

4.1 Rostrum armed dorsally with 5–10 teeth, 0.2–0.3 of cl, reaching to end of antennular peduncle basal antennomere, tips of both P1 fingers without prominent claws.....*C. futunensis n. sp.*

4.2 Rostrum armed dorsally with 0–5 teeth on dorsal margin, 0.3–0.5 of cl, reaching to base or middle of antennular peduncle second antennomere), tips of both P1 fingers with prominent claws.....*C. rapaensis*

5.3.5 Discussion

Systematics

The taxonomy of the *C. weberi* complex is problematic because it is based essentially on the rostrum (variable in length and shape) and on teeth (number of dorsal teeth, dorsal teeth situated on carapace behind orbital margin). Curiously, *C. weberi* is reported from numerous localities in Indonesia, Philippines, Micronesia and Melanesia, but in the literature, put apart specimens described by Bouvier (1925) and Edmonson (1935), only two publications detailed the morphological characters of this species, the first one by Roux (1928), and the second one by Cai and Ng (2001). Specimens from Ceram (Indonesia) described as *C. weberi* by Roux (1928) look like our specimens of *C. tupaia n.*

sp. by its P1 chela about 1.9–2.1 times as long as wide (vs 2–2.2 in *C. tupaia n. sp.*), P1 movable finger 0.7–1.0 times length of palm; (vs 0.9–1.2 in *C. tupaia n. sp.*), its P2 carpus slender 4.7–6.1 times as long as wide (vs 5.5–7.5 in *C. tupaia n. sp.*), its P3 dactylus 2.6–4.2 times as long as wide (terminal spine included) (vs 3.1–3.8 in *C. tupaia n. sp.*) with 5–7 spines on flexor margin in addition to the terminal one (vs 6–7 in *C. tupaia n. sp.*), P3 propodus, 3.7–5.2 times as long as dactylus (vs 4.5–5.4 in *C. tupaia n. sp.*), its P5 dactylus 3.5–4.7 as long as wide (vs 4.4–5.0 in *C. tupaia n. sp.*) and its P5 propodus 11.6–16.0 times as long as wide (vs 11–15 in *C. tupaia n. sp.*), but its rostral formula is different: (0–3) 8–14/ 1–3 (vs (0–2)12–19/2–6 in *C. tupaia n. sp.*), its P1 carpus is shorter 1.3–1.7 times as long as wide.(vs 1.5–2.1 in *C. tupaia n. sp.*), so is its P2 chela 2.4–2.8 times as long as wide: (vs 2.8–3.1 in *C. tupaia n. sp.*), and its P3 propodus 7.6–10.7 times as long as wide (vs 11–16 in *C. tupaia n. sp.*), its P5 dactylus bears fewer spiniform setae on flexor margin 29–54 (vs 62–70 in *C. tupaia n. sp.*), and finally, its eggs are larger 0.38–0.47 × 0.20–0. 30 mm (vs 0.35–0.38 × 0.2–0.21 in *C. tupaia n. sp.*).

Our specimens of *C. tupaia n. sp.* also look like those from Halmahera described by Cai and Ng (2001) as *C. weberi* by its rostral formula (0–3) 8–14/1–3 (vs (0–2) 13–14/2–3 for Cai and Ng (2001)), P1 movable finger 0.7–1.0 times length of palm (vs 1.0 for Cai and Ng (2001)), carpus 1.3–1.7 times as long as wide (vs 1.3 for Cai and Ng (2001)), P5 dactylus with 29–54 spiniform setae on flexor margin (vs 55 for Cai and Ng (2001)) and P5 propodus 3.7–4.4 times as long as dactylus (vs 5 for Cai and Ng (2001)), and its eggs size 0.38–0.47 × 0.20–0. 30 mm (vs 0.4 × 0.2 for Cai and Ng (2001)). They differ from these specimens by the posterior margin of the telson that always shows one pair of lateral spines (vs 2 for Cai and Ng (2001); see Figure 3C, D, E, like *C. weberi* sensu stricto).

In this complex, as for others, it is necessary to always combine genetics with morphology and to collect specimens from the type locality in order to do a genetic study.

Distribution

Endemism in the Marquesas and Austral archipelagos, and in Futuna

The high rate of freshwater endemism is well known in Marquesas Islands where all the freshwater Gobiidae (Teleostei) are endemic. There is also one endemic freshwater Palaemonidae prawn (*Macrobrachium feunteuni* Keith and Vigneux, 2002) (Keith & Vigneux, 2002), one endemic Varunidae (Decapoda: Brachyura) *Ptychognathus crassimanus* Finnegan, 1931 (N. K. Ng, pers. comm.) and now an endemic *Caridina* shrimp, *C. marquesensis n. sp.*. This high rate of endemism is also well known in the Austral archipelago, and particularly in Rapa Island, with its endemic Gobiidae (Teleostei): *Sicyopterus rapa* Parenti & Maciolek, 1996 and *Stiphodon julieni* Keith, Watson & Marquet, 2002. *Stenogobius randalli* Watson, 1991 is restricted to Austral archipelago, and *Stiphodon discotorquatus* Watson, 1995 is restricted to Rarotonga (Cook), Rurutu and Tubuai (Austral Islands). *C. rapaensis* is restricted to Rapa and Rurutu. Compared to Marquesas and Austral archipelagos, Society shows another different type of fauna.

The type of life cycle for all native freshwater species in Polynesia is amphidromy. Indeed, river systems are colonised by crustaceans (Palaemonidae and Atyidae) with a life cycle typically adapted to the conditions that prevail in these distinctive insular habitats; i.e, young oligotrophic rivers, subject to extreme climatic and hydrological seasonal variations. These species spawn in freshwater, the free larvae drift downstream to the sea where they undergo a planktonic phase during several days to several months, before returning to the rivers to grow and reproduce. With this type of lifecycle, numerous authors suggest that some oceanic currents are biogeographic genetic break (Planes, 1993 ; Mennesson, 2016) and so generate endemic species. This could partially explain the patterns observed in Marquesas and Austral Islands. There are in our study area (Figure 5.3.8) two main currents: the South Equatorial Current (SEC) and the South Equatorial countercurrent (SECC). SEC is caused by the influence of South-East trade winds and flows from east to west. More southward, the SECC flows from West to East. Under the influence of SEC and SECC, a gyre is formed. The global westward trend of the currents in the area could partially explain the distribution of *C. tupaia n. sp.* ranging from Society to Samoa through Cook and Rurutu (Austral) by carrying the larvae that would thus supposedly live in depths within the influence of those surface currents.

In French Polynesia, Marquesas archipelago (Figure 5.3.8) is located in the Marquesas countercurrent (MCC), oriented from West to East. It is very regular throughout the year (Martinez *et al.*, 2009). Tuamotu, Gambier and Society archipelagos are located in the South Equatorial Current (SEC) oriented from Northeast to Southwest. Austral archipelago is located in South Equatorial Countercurrent (SECC) oriented from west to east. This climatolological schema will be of major importance for the establishment of species that have a planktonic dispersive larval phase, such as species of the genus Caridina. Indeed the opposition (Figure 5.3.8) between the MCC and the SEC could explain the strong divergence of the Marquesas population, the border between these 2 currents (current in opposite direction and bodies of water having different characters) constituting a barrier for larvae (Vermeij, 1987).

The same schema exists between the SEC and the SECC, explaining the divergence of the Austral population (Figure 5.3.8). So the two endemic areas in the north and South of French Polynesia could be explain by their provision of currents but also by their isolation. Indeed, the Marquesas Islands, which are almost exactly in the centre of the Pacific, are very isolated, some 4,828 km from the nearest American coast, 4,572 km from the western margin of the Pacific depression and 1,287 km from the nearest high islands of the Society archipelago. The Marquesas Islands chain (Craig *et al.*, 2001), with the putative hotspot to the southeast, comprises eleven islands from Eiao (average age 5.4 Ma) in the NW, to Fatu Hiva (average age 1.55 Ma) in the SE. Among all prospected islands of Austral archipelago, Rurutu is the oldest: 12.8–0.3 Ma (see Craig *et al.*, 2001), and Rapa is relatively young 5.0–4.1 Ma (Craig *et al.*, 2001) but the most isolated (500 km from the nearest island Raivavae).

Contrarily to *C. futunensis n. sp.*, *C. marquesensis n. sp.* or *C. rapaensis*, respectively endemic to Futuna, Marquesas and Austral Islands, *C. tupaia n. sp.* has a wide distribution in Polynesia: Upolu (Samoa), Rarotonga (Cook Islands), Rurutu, Tahiti, Moorea, and Huahine (French Polynesia). Its larvae are supposedly transported by the southwestward currents of the gyre to colonize these different lineaments and *C. tupaia n. sp.* is likely to have a greater distribution and reach other islands to the West, like Tonga or Melanesia. Genetic studies are in progress on specimens belonging to *C. weberi* complex from Fiji, New Caledonia, Vanuatu and Solomon Islands. Since *C. tupaia n. sp.* and *C. rapaensis* occur in sympatry in Rurutu Island but have different distribution areas,

there must be biological differences explaining that they are not constrained in the same way by the currents, such as differences in larvae circulation depths between the two species, duration of the marine larval phase, differences in habitat preferences that restrict the distribution. The explication could also be of historical nature, one species having colonised the islands earlier, when the currents and conditions were different than now, that prevent new species to arrive.

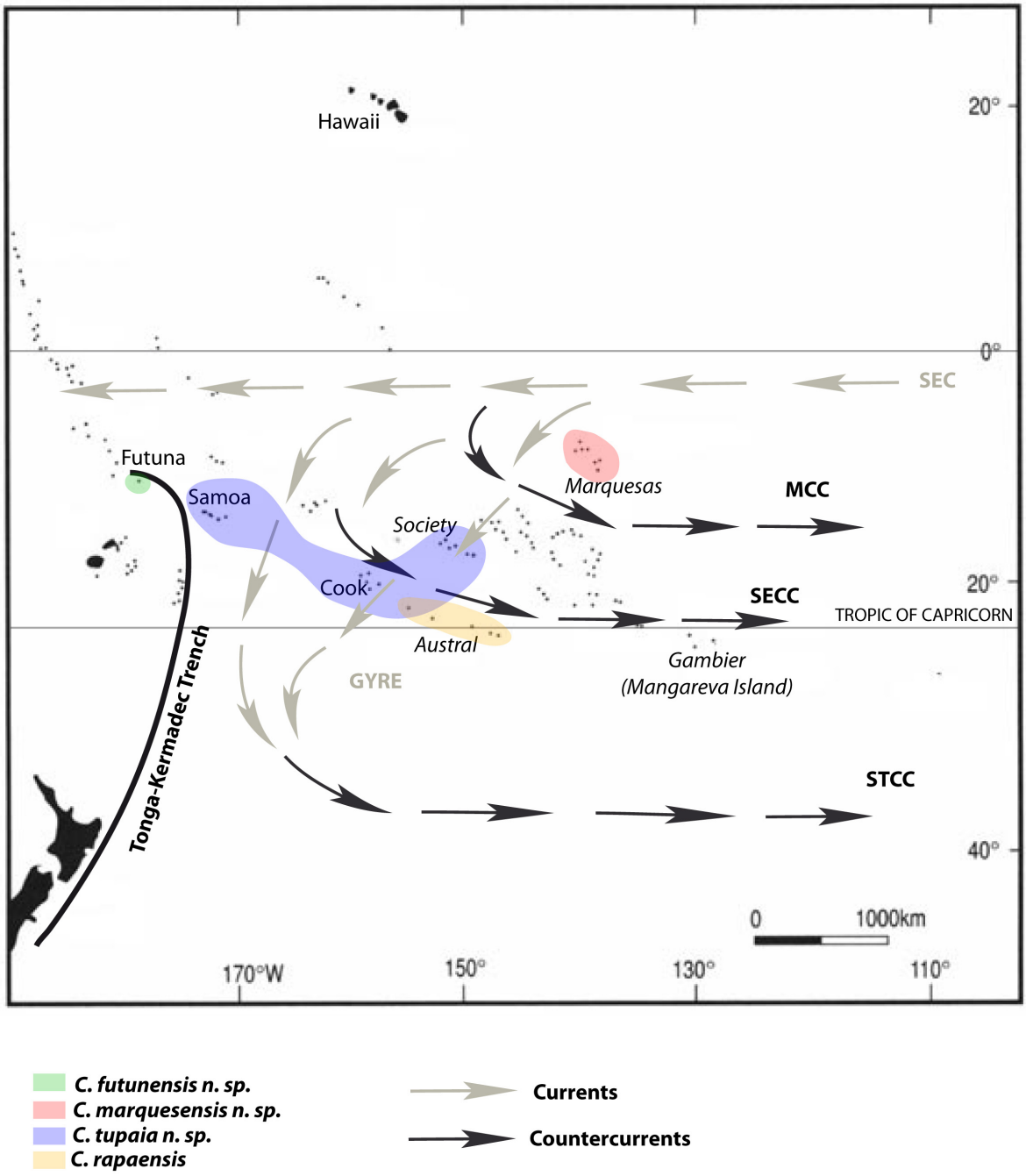


Figure 5.3.8: Distribution map of the four species studied with oceanic currents of Southern Polynesia represented (from Martinez *et al.*, 2009): SEC. South Equatorial Current. SECC. South Equatorial Countercurrent. MCC. Marquesas Countercurrent. STCC. South Tropical Countercurrent. GYRE. South Pacific Gyre.

The freshwater endemism is well known in Futuna Island with its endemic freshwater Gobiidae (Teleostei): *Akihito futuna* Keith, Marquet and Watson, 2008, *Stenogobius keletaona* Keith & Marquet, 2006, and *Smilosicyopus sasali* (Keith and Marquet, 2005) and with now an endemic *Caridina* shrimp, *C. futunensis n. sp.*. This endemism could be explained by the position of this island at the South of Vityaz trench, which would be – depending on the geological time – an obstacle to migration of larvae by its depth and by oceanic current variations. Indeed, Futuna (Keith *et al.*, 2013) appeared as strong volcanic activity occurred in the contact zone between the Pacific and the Australian plates, at the South of fossil Vityaz subduction, in the junction area between the Lau and North Fiji basins, near the North Fiji transform zone (Figure 5.3.9).

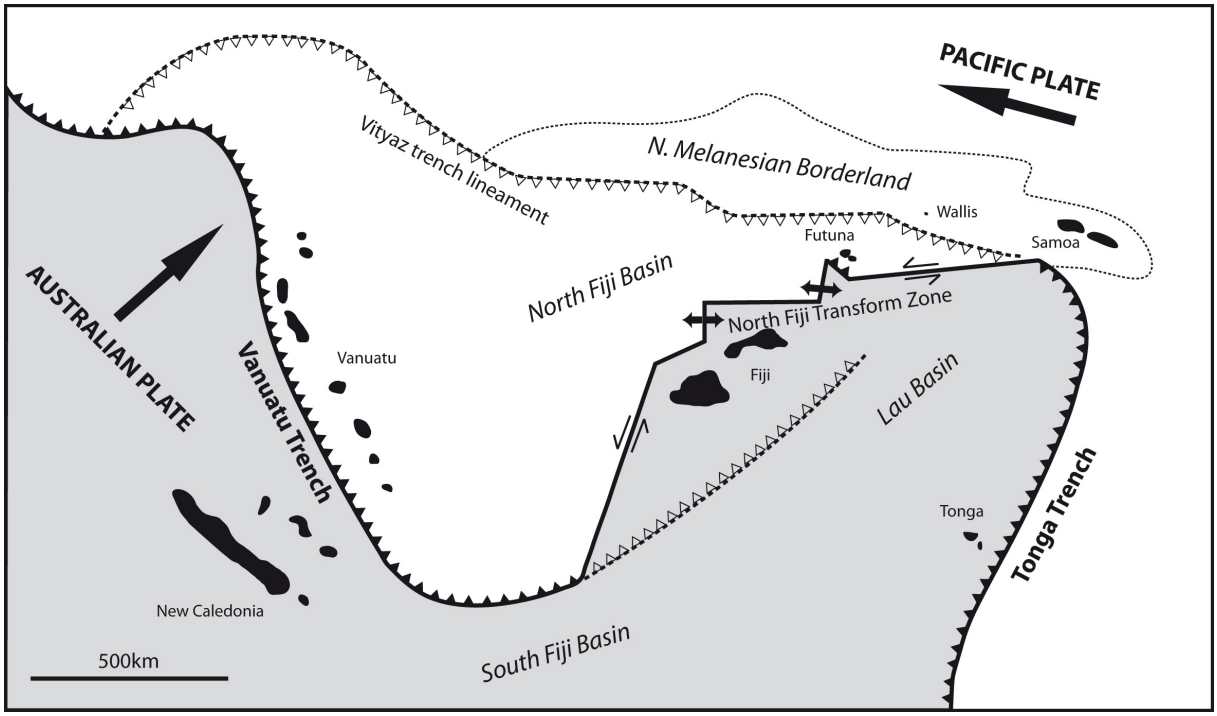


Figure 5.3.9: Map of the geology of the surroundings of Futuna Island.

The absence of Caridina *in the Further East and Further North of Polynesia*

Marquesas Islands represent the eastern limit in Polynesia of the *C. weberi* complex with the presence of *C. marquesensis n. sp.*. Further east, no species of *Caridina* occur (see Marquet, 1991) as it seems to not occur in Mangareva (Gambier archipelago). Situated 1,600 km from Tahiti (Society archipelago) and 1,060 km from Rapa (Austral archipelago), Mangareva (6.26-5.66 Ma, see Craig *et al.*, 2001), of the Gambier Islands, is a high island with small streams suitable for *Caridina* species, but none has been found so far (Keith *et al.*, 2013). Further north, the Hawaiian Islands are biogeographically the

most isolated in the world being about 3,500 to 4,000 km from the high islands of the Marquesas archipelago, but no *Caridina* species occurs in this archipelago. '*C. weberi*' recorded by Devick (1991) as an introduced species, was a misidentification of *Neocaridina denticulata sinensis* (Kemp, 1918) (see Englund and Cai, 1999). Among the Atyidae, only the genus *Atyoida* reaches both Mangareva and Hawaii.

Oceanic currents often act as dispersal barriers for organisms with planktonic larval stages (for example Lessios *et al.*, 1999, Teske *et al.*, 2008) and the position of Hawaii within the North Pacific gyre surrounded by westward currents makes it impossible for larvae coming from West Polynesia to reach the archipelago.

According to several authors studying the amphidromous lifecycle of different species, *C. gracilipes* (Zhang & Sun, 1979), *C. gracilirostris* (Thomas *et al.*, 1973 ; Pillai, 1975 ; Heerbrandt & Lin, 2006), *C. multidentata* from Japan (Hayashi & Hamano, 1984 ; Nakahara *et al.*, 2005), *C. typus* from Japan (Nakahara *et al.*, 2005), *C. leucosticta* from Japan (Nakahara *et al.*, 2005), and *C.* '*weberi*' from India (Chinnaya, 1974) we count 6 to 9 larval stages reared in the laboratory in 15 to 25.5% salt water with a larval development from 9 to 48 days. This short larval duration is related to that of other crustaceans like *Macrobrachium* species. For example, in *Macrobrachium australe*, Ito *et al.* (2002) managed to obtain 13 zoeal stages. The completion of the first 12 stages requires 50 to 69 days but the larvae can then survive to 150 days before dying for failing to find the environmental conditions required for their survival. For *M. lar*, Atkinson (1977) determined 11 zoeal stages representing a maximum duration of 89 days. Their relatively short larval duration could explain the absence of any *Caridina* in the far North and East of Polynesia. Indeed it does not allow the larvae to cross great distances from Tahiti or Rapa to Mangareva and from Marquesas islands to Hawaii without islands with permanent streams along their migration in this area of the Central Pacific. On the contrary, only robust crustacean species with supposedly long larval durations were able to reach Mangareva or Hawaii such as in genera *Atyoida* or *Macrobrachium* for Mangareva. Furthermore, Central Pacific waters are oligotrophic with little phytoplankton, which does not allow the larvae to get much food. Indeed, situated in 8° and 12° of latitude South, Marquesas archipelago still benefits from a remnant of equatorial wealth (2 to 4 mmol/m^3 of nitrates) but this effect is completely

faded south of 15°S; the waters of the whole Tuamotu-Society-Austral archipelagos are oligotrophic with nitrate levels lower than 0.2 mmol/m^3 (Rougerie & Wauthy, 1993).

The larval durations of the species studied in this work are unknown but are probably different from one to another, judging from the differences in geographical ranges, with endemic species like *C. futunensis n. sp.* or *C. marquesensis n. sp.* having a shorter larval duration (and likely fewer larval stages) than *C. tupaia n. sp.*. It would be interesting to study the larval phases of these species by rearing them in the laboratory in order to clarify these points.

Management and conservation of freshwater crustaceans

This first review of *C. weberi* complex in Polynesia gives us an understanding of how diverse and unique is its distribution, with species having a very restricted distribution (*C. futunensis n. sp.* for example, endemic to Futuna Island) in contrast to others, more widely distributed (*C. tupaia n. sp.*). An assessment of the status of freshwater fish in Oceania was made at a Red List workshop held at the IUCN Oceania in September 2011, but crustaceans were not included in this assessment as not enough information was currently available to determine their conservation status. Complementary genetic studies on Palaemonidae and Atyidae in this region could fill this gap. An assessment of the status of freshwater crustaceans in Oceania becomes urgent, particularly for endemic species, by the development of nuisances on this fauna like culverts and fords (that assist with water flow passage across roads), dams (for water supply or electricity generation), weirs (for water abstraction), artificial channels (in urban areas), diversions of rivers by convenience or irrigation.

5.3.6 Acknowledgements

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Chapitre cinquième, Phylogénie moléculaire des deux complexes.

Molecular phylogeny of the two complexes

Où l'on réalise une phylogénie à partir d'un grand jeu de données moléculaires provenant des génomes mitochondrial et nucléaire pour des spécimens des complexes C. nilotica *et* C. weberi *provenant de toute leur aire de distribution, qui permet d'étudier leur évolution grâce à une calibration temporelle, la reconstruction de caractères ancestraux centrés sur l'habitat et la biogéographie, et l'établissement de nouvelles hypothèses paléobiogéographiques pour la famille des Atyidae.*

Where we build a phylogeny from a large molecular dataset from mitochondrial and nuclear genomes of specimens of the C. nilotica *and* C. weberi *complexes collected from all their geographic range, allowing to study their evolution thanks to a time calibration, an ancestral characters states reconstruction focussed on habitat and biogeography and the elaboration of new palaeobiogeographic hypotheses for the family Atyidae.*

6. Chapitre 5 : Phylogénie moléculaire des deux complexes

Depuis une dizaine d'années et le développement des techniques de séquençage, plusieurs phylogénies utilisant des marqueurs essentiellement mitochondriaux et incluant des espèces du genre *Caridina* ont été construites, sans jamais atteindre un niveau de résolution satisfaisant à l'échelle du genre. Pour étudier les relations de parenté entre les complexes *C. nilotica* et *C. weberi* détaillés dans les chapitres précédents et comprendre leur évolution et la mise en place de leur biodiversité, nous avons réalisé une analyse phylogénétique à partir d'un grand jeu de données obtenu en partie par NGS (Séquençage Nouvelle Génération) sur 92 spécimens appartenant à 50 espèces. L'obtention d'une phylogénie soutenue nous a permis de réaliser différentes analyses pour nous éclairer sur l'évolution de ces taxons, comme une reconstruction des caractères ancestraux sur l'habitat et la biogéographie, ainsi qu'une datation de la phylogénie calibrée avec un fossile.

For a decade, because of the development of sequencing methods, several phylogenies based essentially on mitochondrial markers and including species of *Caridina* were built, without ever raching a satisfactory resolution at genus level. To study the relationships between *C. nilotica* and *C. weberi* complexes detailes in the previous chapters, and understand their evolution and the establishment of their biodiversity, we realised a phylogenetic analysis from a large dataset obtain partly through NGS (Next Generation Sequencing) on 92 specimens belonging to 50 species. The obtention of a supported phylogeny allowed us to realise different analyses to enlighten us on the evolution of these taxa, such as an ancestral characters states reconstruction of the habitat and biogeography, as well as a datation of the phylogeny calibrated with a fossil.

Molecular Phylogenetics and Evolution, in revision

The complex study of complexes: The first well-supported phylogeny of two species complexes within genus *Caridina* (Decapoda: Caridea: Atyidae) sheds light on evolution, biogeography, and habitat.

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6.1 Abstract

Atyid shrimps, a key component of tropical freshwater ecosystems, face multiple anthropogenic threats and thus need special attention. With more than 300 described species, the genus *Caridina* is the most speciose of all the Caridea infra-order. *Caridina* spp. occupy diverse habitats in tropical freshwaters of the Indo-West Pacific region. Several species complexes have been recognized, based on common morphological features, but little is known about how well these morphological characteristics align with phylogenetic characteristics. Furthermore, no phylogeny of the genus *Caridina* published so far has provided well-resolved and supported relationships among different species, thus impeding the possibility of proposing evolutionary hypotheses. In this study we used next generation sequencing (NGS) to provide new insights into the phylogenetic relationships among the genus *Caridina*, focusing on two complexes: '*Caridina nilotica*' and '*Caridina weberi*'. We collected 92 specimens belonging to these two groups from most of their known geographical range, representing 50 species, for which we sequenced seven mitochondrial genes and two nuclear markers using ion torrent NGS. We performed a phylogenetic analysis, which yielded the first well-supported tree for the genus *Caridina*. On this tree were mapped the geographic ranges and the habitats used by the different species, and a time calibration was tested. We found the driving factors that most likely account for separation of clades are differences in habitat and to a lesser extent geography. This work provides new insights into the taxonomy of this group and identifies opportunities for further studies in order to fill knowledge gaps that currently impede the management and conservation of atyid species.

Keywords: Amphidromy; Freshwater shrimp; Indo-Pacific; Mitochondrial genome; Molecular systematics; Taxonomy

6.2 Introduction

Atyidae is the most diversified decapod crustacean family in freshwaters worldwide, comprising nearly 500 species and showing a large variety of feeding and breeding

strategies in diverse habitats. The genus *Caridina* H. Milne Edwards, 1837, with around 300 described species, is the most speciose of the Atyidae and also the most diversified genus of shrimps (De Grave *et al.*, 2015). Currently, around one third of all known freshwater shrimp species are threatened or near-threatened as a result of human activity, primarily habitat destruction (De Grave *et al.*, 2015). Shrimp play important ecological roles in their natural habitats (e.g. nutrient cycling) and are important prey species for a variety of other organisms (Covich *et al.*, 1999 ; Crowl *et al.*, 2001). Freshwater shrimps are also an important food source for humans, especially in the Indo-Pacific where they often support the livelihoods of the poorest communities through fishing or aquaculture (Russi *et al.*, 2013). Declines in the abundance or extinctions of shrimp species could therefore have important implications for food security and the ecology of freshwater and brackish habitats, with the potential to hamper ecosystem services.

One obstacle to addressing the problem of rapid species loss is that conservationists cannot properly monitor species or implement conservation plans without good taxonomic information. There is considerable confusion surrounding the taxonomy of the genus *Caridina* that has, for example, hampered efforts to assess the impacts of wild harvest for the aquarium trade (Klotz & Rintelen, 2014). Confusion surrounding the taxonomy of the genus has been, in part, due to natural variability in traditionally retained characters that have been used to separate species (Mazancourt *et al.*, 2017a). Additionally, some specimens have been grouped in complexes based on common morphological features, but little work has been done to differentiate among closely related species. For example, Bouvier (1925), following the previous attempts of De Man (1892b) and Ortmann (1894), separated the 50 known species at that time into five species groups: *Caridina nilotica*, *C. laevis*, *C. africana*, *C. brevirostris*, and *C. typus*. As more species were described, species groups were modified, abandoned, or combined and new groups were created, including the *C. serrata* complex (Cai & Ng, 1999 ; Klotz & Rintelen, 2014), *C. gracilirostris* complex (Cai & Ng, 2007) and *C. weberi* complex (Mazancourt *et al.*, 2017b). Two of these, *C. nilotica* complex and *C. weberi* complex, are well represented in insular systems of the Indo-West Pacific region thanks to the great number of amphidromous representatives that allowed them to colonize new habitats quickly. *C. nilotica* complex exists in tropical freshwaters from Africa to Melanesia and Micronesia, whereas *C. weberi* complex representatives can be found further eastward in

Central Pacific Islands (Keith *et al.*, 2013). Few species from the *C. nilotica* or *C. weberi* complexes have been separated and described since the complexes were established.

To overcome the taxonomic complexity of the family Atyidae, molecular data is being increasingly used to uncover phylogenetic relationships (Page *et al.*, 2007a, 2007b ; Rintelen *et al.*, 2007 ; Rintelen *et al.*, 2012). Published phylogenies including species of *Caridina* have been predominantly built from a single locus (Rintelen *et al.*, 2008), although phylogenies built using two or three loci have become available in recent years (Bossuyt *et al.*, 2004 ; Page *et al.*, 2007a, 2007b ; Cook *et al.*, 2011 ; Rintelen *et al.*, 2012 ; Klotz & Rintelen, 2014). Despite this, no phylogeny of the genus *Caridina* published so far has provided well-resolved and supported relationships among different species, impeding the possibility of proposing evolutionary hypotheses. For example, the most comprehensive phylogeny of Atyidae built from one mitochondrial and two nuclear markers was unfortunately unable to provide reliable dating and biogeographic evolution at the generic level (Rintelen *et al.*, 2012). Greater understanding of the phylogenetic relationships and evolution of *Caridina* requires the combination of morphological and/or ecological data with DNA sequencing to improve the accuracy and reliability of species delineations (Rintelen *et al.*, 2008 ; Rintelen & Cai, 2009 ; Klotz & Rintelen, 2014 ; Mazancourt *et al.*, 2017a, 2018a ; Mazancourt *et al.*, 2018b).

The aim of this study was to (i) provide the first well-resolved partial phylogeny of *Caridina* focusing on two species complexes, *C. nilotica* and *C. weberi*, using next generation sequencing methods (NGS), and (ii) combine our DNA sequencing with ecological, habitat, and biogeographical data to better understand the evolutionary and phylogenetic relationships among *Caridina*. Large datasets obtained using NGS often provide better resolution of the phylogenetic relationships than analyses with single or few markers (Hillis, 1996). Using data from seven mitochondrial and two nuclear markers, we constructed a phylogenetic tree that provides novel insights into the evolution of this group and the family Atyidae. The implications of our phylogeny for the management of caridean shrimp are also discussed.

6.3 Material and methods

6.3.1 Specimens sampling

We obtained specimens of *Caridina* from most of its range thanks to numerous expeditions made by two of the authors (GM & PK), from the collections held at the Muséum national d'Histoire naturelle (Paris, France), as well as specimens obtained from a network of naturalists and scientists worldwide. A total of 92 specimens of atyid shrimps (89 *Caridina*, 2 *Paratya* and 1 *Elephantis*) representing 50 species (48 *Caridina*, 1 *Paratya* and 1 *Elephantis*) were collected from different localities of the Indo-West Pacific region (Figure 6.1, Table 6.1) mostly by electro-fishing or by hand net scooping in waterways. Specimens were then preserved in absolute ethanol and stored prior to morphological examination and tissue sampling. Identifications were made following keys provided by relevant publications focusing on the different prospected areas, comparisons with type specimens, and by DNA barcoding (see Mazancourt *et al.*, 2017a; 2017b; 2018a,b).

6.3.2 DNA extraction, amplification and sequencing

DNA was extracted from abdominal tissue using the semi-automatic Ependorf ep-Motion 5075 robot. Fragments of mitochondrial genome (MGF)(~3,200 bp, containing COII, ATP8, ATP6, COIII and ND3), 16S rRNA (~500 bp), COI (~640 bp), nuclear 28S rRNA (~1,090 bp) and nuclear Histone 3 (H3)(328 bp) were amplified by PCR using primers and amplification protocols detailed in Table 6.2. DNA amplifications were performed in 20 µl PCR reactions, containing approximately 3 ng of template DNA, 2.5 mM $MgCl_2$, 0.26 mM of each nucleotide, 0.3 µM of each primer, 5% DMSO, 1 ng of BSA and 1.5 units of QBIOTAQ polymerase (MPBiomedicals) for nuclear markers, 16S and COI or LongAmp Taq Polymerase (BioLabs) for MGF. PCR products were sequenced using an Ion-Torrent PGM platform following the method developed by Hinsinger et al. (2015). Contigs obtained after sequencing were assembled iteratively by mapping them to previously Sanger-sequenced or assembled sequences and each gene of the MGF was annotated and extracted separately in Geneious v.8 software (http://www.geneious.com: Kearse *et al.*, 2012). All sequences were deposited in GenBank (Numbers pending, Table 6.1).



Figure 6.1: Map of the sampling localities of the specimens used in the study.

Table 6.1: List of the specimens included in the phylogeny.

Species	DNA voucher	Country	16S	COI	Mt1 partiel	28S	H3
C. africana	CA1628	South Africa	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. appendiculata	CA1670	Australia	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1708		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1493	Solomon Islands	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1261	Vanuatu	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. brachydactyla	CA1131	Indonesia	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. brevicarpalis	CA1521	Solomon Islands	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. brevidactyla	CA1345	Solomon Islands	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1501		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	
C. buehleri	CA1519	Solomon Islands	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1520		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. cf. gracilirostris	CA1238	Indonesia	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. cf. jeani	CA1415	Samoa	XXXXXXXXXX		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1416		XXXXXXXXXX		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1370	Vanuatu	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1371		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1372		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1377		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. cf. multidentata	CA1053	Madagascar	XXXXXXXXXX		XXXXXXXXXX		
C. cf. nilotica 1	CA1707	Australia	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. cf. nilotica 2	CA1711	Australia	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1714		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. cf. vitiensis	CA1385	Fiji	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1003	New Caledonia	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1601		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. cf. weberi Pohnpei	CA1388	Micronesia	XXXXXXXXXX		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1390		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX		XXXXXXXXXX
C. cf. weberi sp. 1	CA1349	Solomon Islands	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. cf. weberi sp. 2	CA1516	Solomon Islands	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. cf. weberi sp. 3	CA1048	Cook Islands	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX		
	CA1410	Samoa	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1508	Solomon Islands	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. cf. weberi sp. 4	CA1509	Solomon Islands	XXXXXXXXXX		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1510			XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. cf. weberi sp. 5	CA1909	Solomon Islands	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. cf. weberi sp. 6	CA1902	Solomon Islands	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX		XXXXXXXXXX
	CA1903		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX		XXXXXXXXXX
C. confusa	CA1727	Australia	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1728		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX

C. elongapoda	CA1899	Malaysia	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. gracilipes	CA1348	Indonesia	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
		?	NC_024751	NC_024751	NC_024751	FN995565	FN995481
C. gracilirostris	CA1678	Australia	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. leucosticta	CA1912	Japan	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. lobocensis	CA1056	Palau	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1554		XXXXXXXXXX		XXXXXXXXXX	XXXXXXXXXX	
C. longicarpus	CA1557	New Caledonia	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1599		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1611		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. macrodentata	CA1374	Vanuatu	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1375		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. meridionalis	CA1585	New Caledonia	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	
	CA1620		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. mertoni	CA1505	Solomon Islands	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1506		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	
C. multidentata	CA1545	Aquarium trade (Taiwan)	XXXXXXXXXX		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1546		XXXXXXXXXX		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. natalensis	CA1343	Comoros	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. novaecaledoniae	CA1789	New Caledonia	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1792		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. papuana	CA1286	Solomon Islands	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1361		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. similis	CA1854	Seychelles	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. simoni	CA1831	Sri Lanka	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1833		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. sp. 1 Solomon	CA1326	Solomon Islands	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1499		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. sp. 2 Solomon	CA1285	Solomon Islands	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	
	CA1515		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	
C. sp. 3 Solomon	CA1685	Australia	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	
	CA1240	Indonesia	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1282	Solomon Islands	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. sp. Malanda	CA1736	Australia	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1737		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. sp. NT nilotica	CA1721	Australia	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX		XXXXXXXXXX
		Australia	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX		
	CA1103	Indonesia	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX		
C. sp. Santo	CA1132	Vanuatu	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX		XXXXXXXXXX
C. sp. WA4	CA1743	Australia		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1744		XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. typus	CA1568	New Caledonia	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
	CA1524	Solomon Islands	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX
C. variabilirostris	CA1404	Micronesia	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX		XXXXXXXXXX
	CA1249	Vanuatu	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX	XXXXXXXXXX

	CA1435		xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx
C. variabilis	CA1205	Palau	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx
C. wilkinsi	CA1746	Australia	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx
	CA1747		xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx
C. zebra	CA1731	Australia	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx
	CA1732		xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx
C. zeylanica	CA1835	Sri Lanka	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx
Elephantis jaggeri	CA1160	Madagascar	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx		xxxxxxxxxx
Halocaridina rubra		Hawaii	NC_008413	NC_008413	NC_008413	FN995585	FN995500
Macrobrachium bullatum		Australia	NC_027602	NC_027602	NC_027602	AY374147	
Neocaridina denticulata		China	NC_023823	NC_023823	NC_023823	FN995598	FN995513
Paratya australiensis		Australia	NC_027603	NC_027603	NC_027603	FN995610	FN995525
Paratya caledonica	CA1800	New Caledonia	xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx		xxxxxxxxxx
	CA1801		xxxxxxxxxx	xxxxxxxxxx	xxxxxxxxxx		xxxxxxxxxx

Table 6.2: List of the primers used to amplify the different markers.

Marker	Primers	Sequence	PCR protocol	Reference
COI (~640 bp)	LCOI1490mod	TGTCAACTAATCATAAAGATATTGG	Initial denaturation 94°C for 4 min, 45 cycles of 94°C for 30s, 48°C for 30s, 72°C for 40s, final extension 72°C for 7min.	Modified from Folmer*et al.*, 1994
	HCO2198mod	GGGTGTCCAAAAAACCAAAATAA		
16S rRNA (~500 bp)	16Sar-Lmod	TACTTCTGCCTGTTTATCAAAAA	Initial denaturation 94°C for 4 min, 45 cycles of 94°C for 30s, 55°C for 30s, 72°C for 40s, final extension 72°C for 7min.	Modified from Palumbi, 1996
	16Sbmod	GGTCTGAACTCAAATCATGTAAA		
Mitochondrial genome fragment (COII, ATP8, ATP6, COIII, ND3) (~3,200 bp)	tRNA-Leu1540F	AAAATGGCAGATCATTGCATAG	Initial denaturation 94°C for 30s, 60 cycles of 94°C for 20s, 50°C for 30s, 65°C for 15min, final extension 65°C for 15min.	This study
	tRNA-Glu4760R	ACCTTTAGTTTTACAATGAAAATG		
28S rRNA (~1,090 bp)	28S-F-Cru	ACCCGCCTAATTTAAGCATAT	Initial denaturation 94°C for 3min,	Rintelen

28S rRNA (~1,090 bp)	28S-F-Cru	ACCCGCCTAATTTA AGCATAT	35 cycles of 94°C for 30s, 60°C to 52°C (-1°C/cycle) for 30s, 72°C for 2min, final extension 72°C for 5min.	*et al.*, 2012
	28S-R-Met	GCTATCCTGAGGGA AACTTC		
Histone 3 (328 bp)	H3F	ATGGCTCGTACCAA GCAGACVGC	Initial denaturation 94°C for 3min, 35 cycles of 94°C for 30s, 50°C for 60s, 72°C for 60s, final extension 72°C for 5min.	Colgan *et al.*, 2000
	H3R	ATATCCTTRGGCAT RATRGTGAC		

6.3.3 Phylogenetic analyses

Six sequences retrieved from GenBank for species that had a mitochondrial genome and nuclear markers published, were included with sequences obtained from specimens (Table 6.1). *Macrobrachium bullatum* (Palaemonidae) from Australia was used as an extra-family outgroup and *Paratya australiensis* from Australia, *P. caledonica* from New Caledonia, and *Halocaridina rubra* from Hawaii were used as intra-family, extra-genus outgroups. Sequences from *Caridina* cf. *nilotica*, *C. gracilipes* and *Neocaridina denticulata* were added as comparison. The origin and identification of the GenBank sequences could not be ascertained, but including them allowed us to analyse all the atyid mitochondrial genomes published at that time and confirm their identification.

Alignments of the genes and gene fragments were conducted using Muscle algorithm (Edgar, 2004) implemented in MEGA7 software (S. Kumar *et al.*, 2016). Fast Neighbor-Joining analyses were performed on each alignment and the trees obtained were compared by eye. As the trees obtained from the different mitochondrial and nuclear markers were consistent, we concatenated all the alignments. In total, 98 specimens were included in the concatenated analysis, all of them having the MGF, 96 a 16S sequence, 90 a COI sequence, 85 a 28S sequence, and 85 a H3 sequence (Table 6.1).

The best substitution model was calculated independently for each marker of the concatenated alignment and each codon position for protein-coding genes (Table 6.3) using Bayesian Information Criterion in PartitionFinder (Lanfear *et al.*, 2012). Best-scoring ML trees were estimated using RAxML HPC2 v.8.2.10 (Stamatakis, 2014) and

best-scoring Bayesian Inference trees were estimated using MrBayes v.3.2.6 (Ronquist & Huelsenbeck, 2003), both methods implemented in the Cyber Infrastructure for Phylogenetic Research (CIPRES) portal v.3.1. (Miller *et al.*, 2010) (https://www.phylo.org) with the previously determined models applied to their respective subset, with two independent searches running for 10,000,000 generations, a sampling frequency of 2,000 and a burn in of 6% determined by checking trace files in the Tracer program of the BEAST package (Drummond *et al.*, 2012). Convergence was tested by comparing the two runs in Tracer. Support for nodes for Bayesian analysis was determined using posterior probabilities (PP) calculated by MrBayes. One hundred independent searches, each starting from distinct random trees, were conducted. Robustness of the nodes for ML analysis was assessed using non–parametric bootstrapping (Felsenstein, 1985) with 1,000 bootstrap replicates (B). We considered a node to be moderately supported with PP between 0.9 and 0.95 and B between 80 and 95. Below these values, we considered the node to be unsupported. At values above this range, we considered the node to be highly supported.

Table 6.3: Substitution models retained by PartitionFinder for each partition of the dataset.

Partition	Substitution Model	Partition Finder subset
16S	HKY+I+G	1
COI position 1	SYM+I+G	2
COI position 2	F81+I	3
COI position 3	GTR+G	4
COII position 1	SYM+I+G	5
COII position 2	GTR+I+G	6
COII position 3	GTR+I+G	7
ATP8 position 1	HKY+I+G	1
ATP8 position 2	GTR+G	8
ATP8 position 3	GTR+I+G	7
ATP6 position 1	GTR+I+G	9
ATP6 position 2	SYM+G	10
ATP6 position 3	GTR+I+G	9
COIII position 1	SYM+I+G	11
COIII position 2	GTR+I+G	6
COIII position 3	GTR+I+G	7
ND3 position 1	SYM+I+G	5

ND3 position 2	GTR+G	8
ND3 position 3	GTR+I+G	7
28S	GTR+I+G	12
H3 position 1	K80+I	13
H3 position 2	K80+I	13
H3 position 3	HKY+G	14

6.3.4 Time-calibrated tree

The concatenated dataset was used in Beast v.1.8.3 (Drummond *et al.*, 2012) to obtain a time-calibrated phylogenetic tree constraining the topology obtained in Bayesian Inference. The fossil shrimps *Delclosia roselli* (Vía, 1971) and *D. martinelli* Rabadá , 1993 from Early Cretaceous freshwater deposits (127 ± 2 MYA [million years ago]) in Spain were used as the calibration point, i.e. stem of the Atyidae family. Two separate analyses (to assess the results reproducibility) ran for 50,000,000 generations, sampling every 1,000 generations, burn-in of 2,000,000, Yule process speciation, an uncorrelated relaxed molecular clock (because evolutionary rates appear to very depending on the branches in the phylogenetic analysis) with a lognormal distribution, and a lognormal distribution around the calibration point. Priors for the calibration point were: mean = 0.01; standard deviation = 2.0; offset = 127.0. The other parameters were set to default.

6.3.5 Character mapping on the tree

Six different characters were mapped against the Bayesian tree obtained previously, reduced to one specimen per species, and the most parsimonious ancestral character states were reconstructed using Mesquite v.3.4 software (Maddison & Maddison, 2018) using a taxa-character matrix (55 taxa for 6 characters tested) (Supplementary material). Two biogeographic codings were tested, one with 31 freshwater ecoregions adapted from Abell *et al.* (2008) and one with 9 marine geographic provinces adapted from Longhurst (1998). The preferred habitat of the species was also tested with the Altitude coded in 5 characters: Estuary, Lower course, Middle course, Higher course and Headwater stream, the Substrate Type coded in 5 characters: Sand, Gravel, Pebbles, Boulders and Mud, the Hydrology coded in 2 characters: Lotic and Lentic, and the affinity with vegetation coded in 2 characters: Not in Plants and In Plants.

6.3.6 Historical biogeography analysis

A biogeographical analysis was performed on trees obtained from the time calibration using different programs implemented in RASP (Y. Yu *et al.*, 2015). To each taxa were assigned biogeographical ranges based on the terrestrial biogeographical realms recognised by the WWF (Global 200, see Olson *et al.*, 2001): Australasian, Indo-Malay, Oceanian, Afrotropical and Palearctic (see Supplementary material). A BioGeoBEARS analysis determined the best fit model to our data. Using Akaike Information Criterion, the model retained was Dispersal-Extinction-Cladogenesis (DEC) (Ree & Smith, 2008). Using Lagrange algorithm implemented in RASP, with a dispersal constraints matrix set to default, ancestral areas were reconstructed on the consensus tree.

6.4 Results

6.4.1 Phylogenetic analysis

A total of 9 sequences alignments were used in the analysis: 16S (546 bp); COI (620 bp); COII (674 bp); ATP8 (150 bp); ATP6 (676 bp); COIII (789 bp); ND3 (357 bp); 28S (1284 bp) and H3 (327 bp). Trees obtained in ML and Bayesian analyses showed no inconsistencies; only the Bayesian tree is presented here with ML bootstrap values plotted (Figure 6.2). Overall support for the tree was high. Most of the deep and the shallowest nodes were well supported in both methods, and only a few intermediate nodes showed low support (PP < 0.9, B < 80). Atyid sequences were separated in two highly supported clades. The first clade comprised species of *Paratya* and *Halocaridina rubra*, and the other clade included all of the other species. In this second clade, a first dichotomy excluded *Caridina sp. WA4* from Australia from all others. Within this clade emerged a strongly supported clade containing almost all the species of the *C. weberi* complex (node 1) and dispatched further into two highly supported clades. The tree was further divided into clades with low support; one clade comprised *Neocaridina denticulata*, *C. confusa*, *C. zebra* and *C. sp. Malanda*, another with *C. cf. weberi sp. 1* and *C. typus*, and a third with all the remaining species. Subsequent dichotomies separated *C. brevicarpalis*, *C. gracilirostris* and *C. cf. gracilirostris*, and all species belonging to the *C. nilotica* complex in a highly supported clade, respectively.

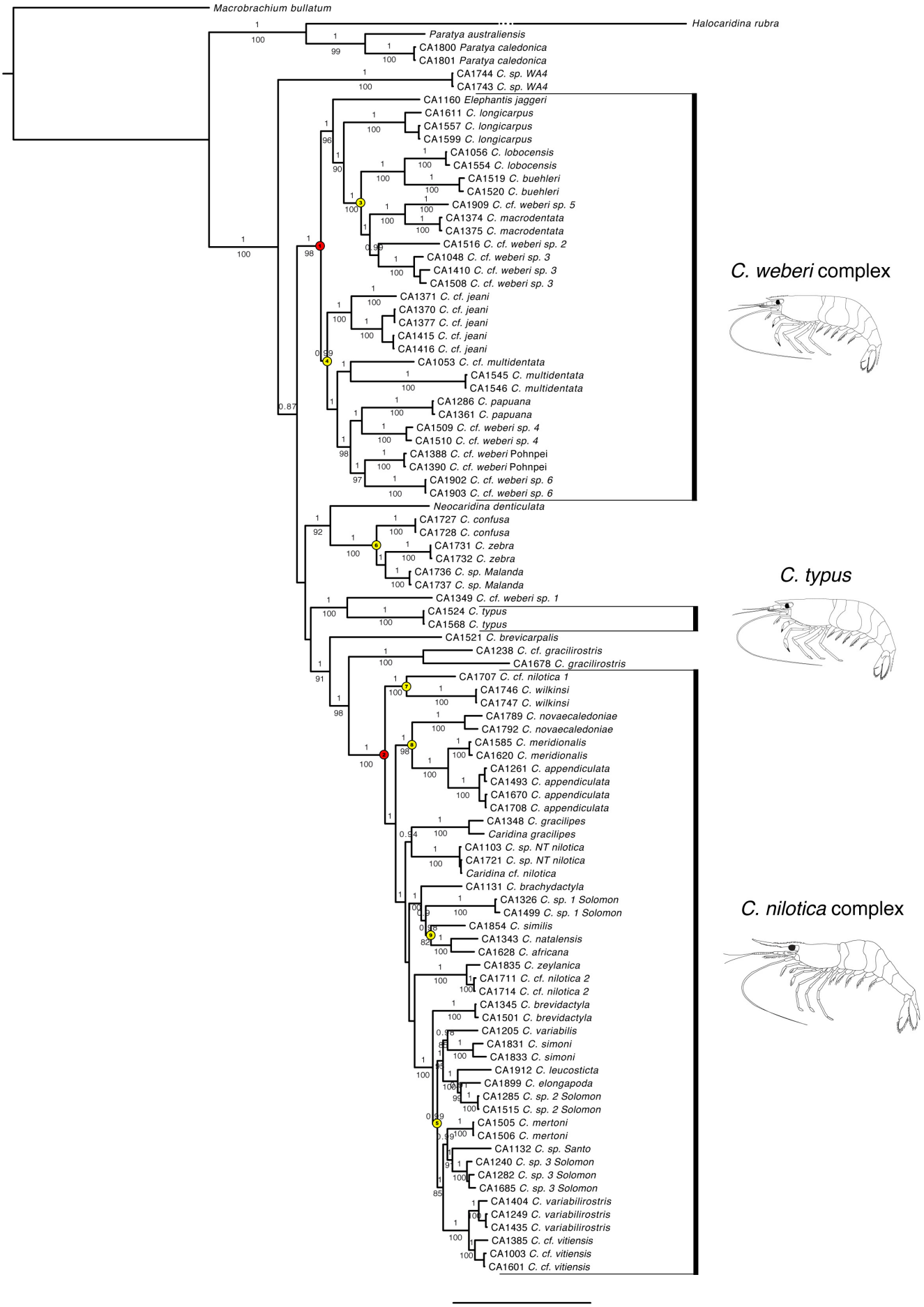

Macrobrachium bullatum
Halocaridina rubra
Paratya australiensis
CA1800 Paratya caledonica
CA1801 Paratya caledonica
CA1744 C. sp. WA4
CA1743 C. sp. WA4
CA1160 Elephantis jaggeri
CA1611 C. longicarpus
CA1557 C. longicarpus
CA1599 C. longicarpus
CA1056 C. lobocensis
CA1554 C. lobocensis
CA1519 C. buehleri
CA1520 C. buehleri
CA1909 C. cf. weberi sp. 5
CA1374 C. macrodentata
CA1375 C. macrodentata
CA1516 C. cf. weberi sp. 2
CA1048 C. cf. weberi sp. 3
CA1410 C. cf. weberi sp. 3
CA1508 C. cf. weberi sp. 3
C. weberi complex
CA1371 C. cf. jeani
CA1370 C. cf. jeani
CA1377 C. cf. jeani
CA1415 C. cf. jeani
CA1416 C. cf. jeani
CA1053 C. cf. multidentata
CA1545 C. multidentata
CA1546 C. multidentata
CA1286 C. papuana
CA1361 C. papuana
CA1509 C. cf. weberi sp. 4
CA1510 C. cf. weberi sp. 4
CA1388 C. cf. weberi Pohnpei
CA1390 C. cf. weberi Pohnpei
CA1902 C. cf. weberi sp. 6
CA1903 C. cf. weberi sp. 6
Neocaridina denticulata
CA1727 C. confusa
CA1728 C. confusa
CA1731 C. zebra
CA1732 C. zebra
CA1736 C. sp. Malanda
CA1737 C. sp. Malanda
C. typus
CA1349 C. cf. weberi sp. 1
CA1524 C. typus
CA1568 C. typus
CA1521 C. brevicarpalis
CA1238 C. cf. gracilirostris
CA1678 C. gracilirostris
CA1707 C. cf. nilotica 1
CA1746 C. wilkinsi
CA1747 C. wilkinsi
CA1789 C. novaecaledoniae
CA1792 C. novaecaledoniae
CA1585 C. meridionalis
CA1620 C. meridionalis
CA1261 C. appendiculata
CA1493 C. appendiculata
CA1670 C. appendiculata
CA1708 C. appendiculata
CA1348 C. gracilipes
Caridina gracilipes
CA1103 C. sp. NT nilotica
CA1721 C. sp. NT nilotica
Caridina cf. nilotica
CA1131 C. brachydactyla
CA1326 C. sp. 1 Solomon
CA1499 C. sp. 1 Solomon
CA1854 C. similis
CA1343 C. natalensis
CA1628 C. africana
CA1835 C. zeylanica
CA1711 C. cf. nilotica 2
CA1714 C. cf. nilotica 2
CA1345 C. brevidactyla
CA1501 C. brevidactyla
CA1205 C. variabilis
CA1831 C. simoni
CA1833 C. simoni
CA1912 C. leucosticta
CA1899 C. elongapoda
CA1285 C. sp. 2 Solomon
CA1515 C. sp. 2 Solomon
CA1505 C. mertoni
CA1506 C. mertoni
CA1132 C. sp. Santo
CA1240 C. sp. 3 Solomon
CA1282 C. sp. 3 Solomon
CA1685 C. sp. 3 Solomon
CA1404 C. variabilirostris
CA1249 C. variabilirostris
CA1435 C. variabilirostris
CA1385 C. cf. vitiensis
CA1003 C. cf. vitiensis
CA1601 C. cf. vitiensis
C. nilotica complex
0.3

Figure 6.2: (Previous page) Bayesian Inference Phylogram obtained from the concatenated dataset showing the position of the two species complexes and the type species of the genus, *Caridina typus*. Numbers above branches indicate Bayesian posterior probabilities (PP); numbers under branches indicate Maximum Likelihood bootstrap values (B). Support values under 0.8 in PP or 80% in B are not shown. Nodes highlighted with a colored circle are discussed. Nodes with a red circle correspond to the "*C. weberi*" and "*C. nilotica*" complexes.

6.4.2 Time calibration

The time calibration gave an origin date for the split of the two most basal atyid clades ranging from 140 to 127 MYA (Mean = 128.01), following the calibration given by the fossils *Delclosia* spp. (Figure 6.3). The origin of *Caridina* group was estimated to have occurred between 95 and 52 MYA (Mean = 72.68). The origin of *C. weberi* complex (Figure 6.3, Node 1) was dated to between 62 to 32 MYA (Mean = 45.68), whilst the origin of *C. nilotica* complex (Figure 6.3, Node 2) was estimated to have occurred more recently, between 47 to 27 MYA (Mean = 44.09). A rapid radiation of the *C. nilotica* complex seems to have occurred around 27 MYA.

6.4.3 Character mapping

The primary factor driving evolution in the character mapping analyses appeared to be habitat, with some secondary influence of geography. There were clear correlations between habitat-related characters and the phylogeny, especially for deeper nodes. For example, following ancestral states reconstructions, node 1 (Figures 6.2 and 6.4) corresponding to *C. weberi* complex, was likely to have originated in the higher course (Figure 6.4C), on gravel substrate (Figure 6.4D), in a lotic environment (Figure 6.4E), and with no vegetation (Figure 6.4F). In contrast, node 2 corresponding to *C. nilotica* complex appeared to have originated in the lower course (Figure 6.4C), on gravel substrate (Figure 6.4D), in a lentic environment (Figure 6.4E), and among vegetation (Figure 6.4F). Some characters may have changed or reverted in more shallow nodes. For example, nodes 3 and 4, both belonging to *C. weberi* complex, differed from their ancestor in having originated on boulder substrate (Figure 6.4D). Node 3 further differed in showing affinities to lentic environments (Figure 6.4E). For node 5, members of *C. nilotica* complex showed affinities with the higher course (Figure 6.4C), with the absence of vegetation (Figure 6.4F), and several of its subclades were correlated with a boulder substrate (Figure 6.4D).

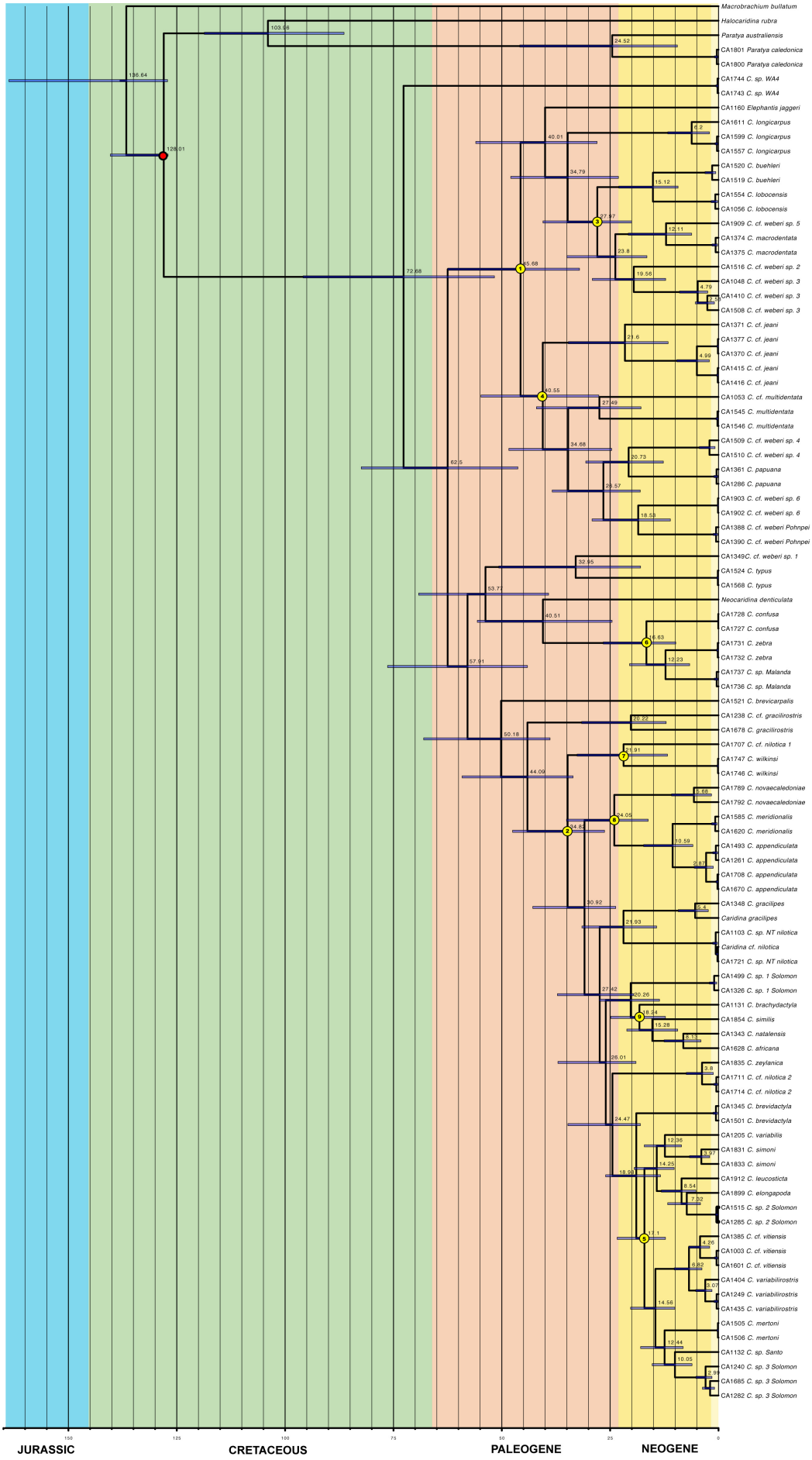


Figure 6.3: Time calibrated Bayesian tree obtained with Beast software. X axis in Myrs. Blue bars indicate confidence intervals; numbers indicate mean date of the node. The red dot indicates the calibration point

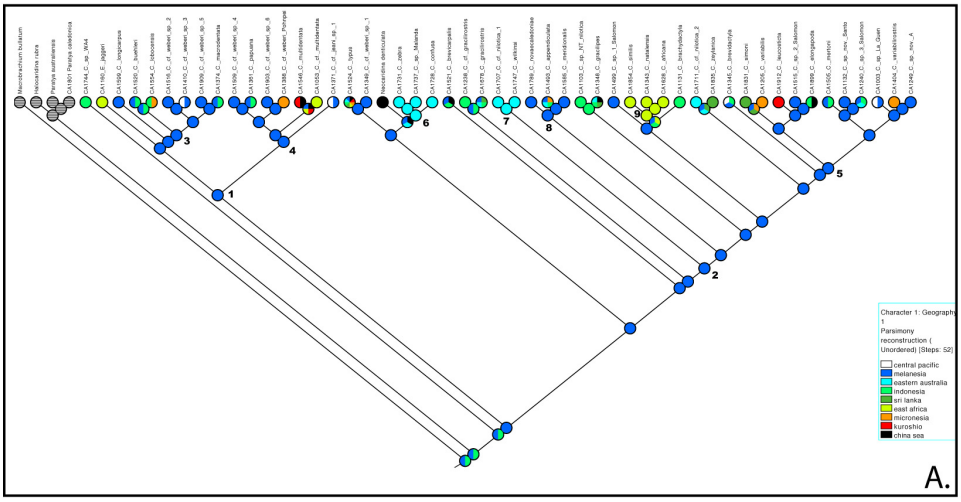
6.4.4 Historical biogeography analysis

To study more thoroughly the historical biogeography of the species, a statistical reconstruction of ancestral areas was performed (Figure 6.5). No clear biogeographic pattern appeared, with the Australasian realm being the ancestral range of most of the clades. Over the 193 nodes, the analysis found 39 dispersal events and 11 vicariance events, making dispersion more likely to occur than vicariance.

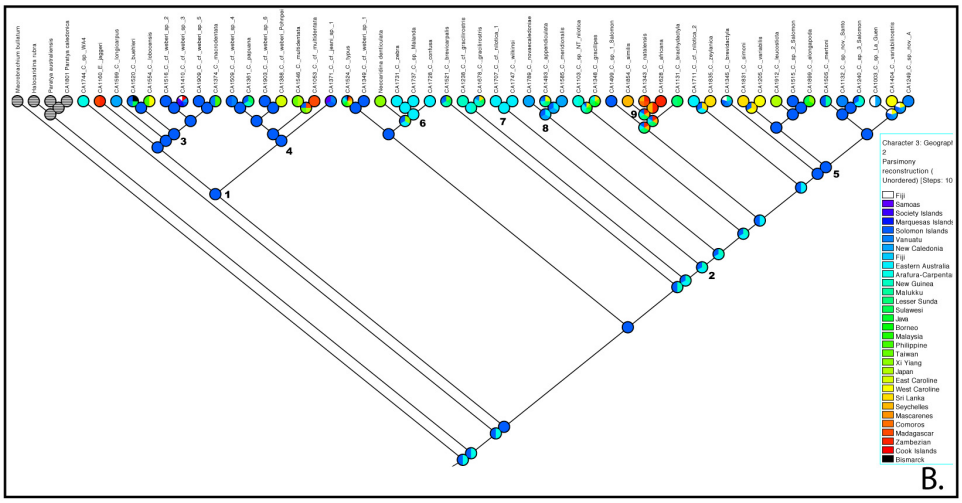
6.5 Discussion

6.5.1 A large dataset for robust analyses

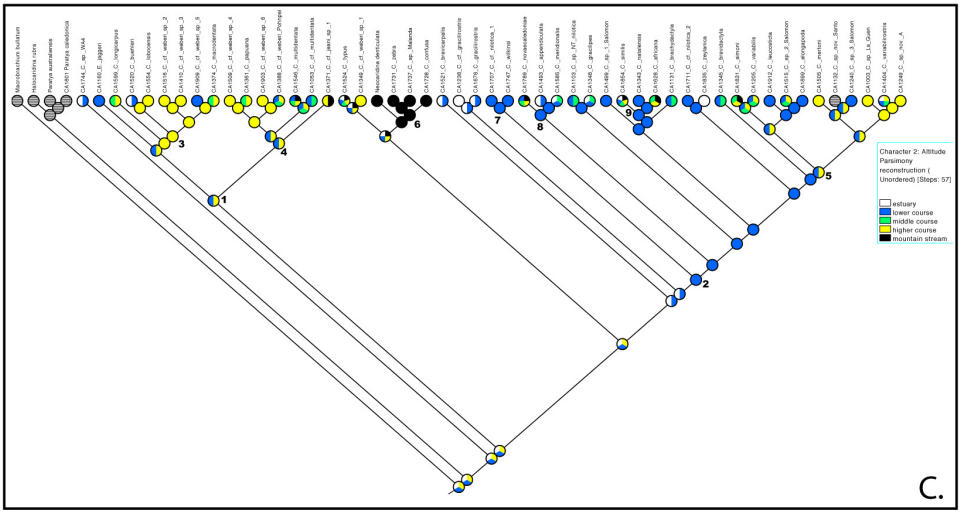
This study presents the first well resolved and well supported phylogenetic tree for the genus *Caridina* thanks to the large molecular dataset obtained by next generation sequencing (NGS) and the number of species included that have been well determined. Indeed, with nine different molecular markers from both mitochondrial and nuclear genomes, totalling around 5,420 bp for 98 specimens (of which 92 were newly sequenced), this is the most ambitious study made on this taxon to date. Obtaining such a large dataset was made possible by the increasingly affordable NGS technologies, namely the ion-torrent sequencing coupled with multiplexing. This method, developed by Hinsinger *et al.* (2015), allowed us to get fragments of the mitochondrial genome (MGF) for up to 40 specimens in a single sequencing, for prices comparable to regular Sanger sequencings. Furthermore, thanks to our network of researchers from all around the world and the numerous field trips made by the Muséum national d'Histoire naturelle, we could use a great number of specimens from many different localities, some of them with a poorly studied freshwater carcinofauna until now, such as the Solomon Islands or Micronesia. As expected with a dataset this large, the overall support of the tree was high, regardless of the reconstructing method used. This allowed us to have, for the first time, a clear representation of the phylogenetic relationships among the species of *Caridina*, showing that species complexes defined previously using morphological resemblances were monophyletic.



Geography 1 (Longhurst, 1998)

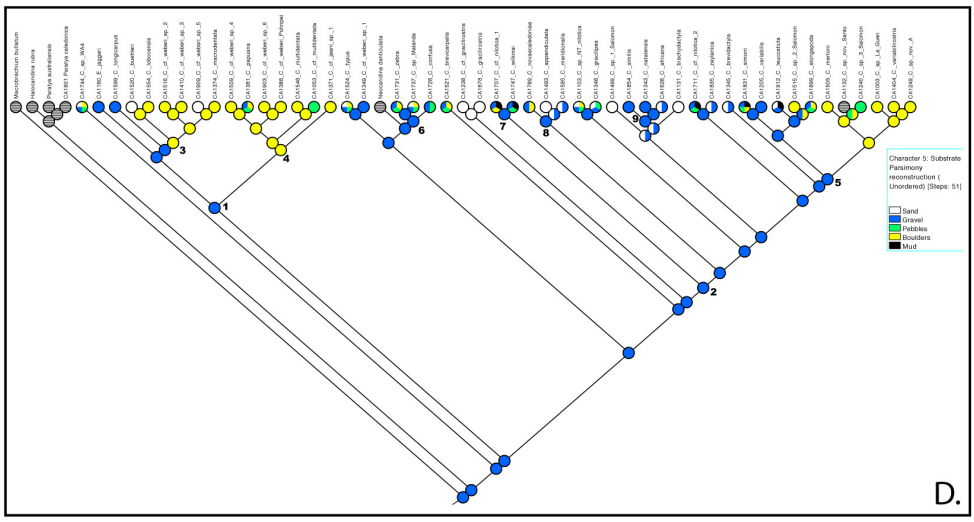


Geography 2 (Abell et al., 2008)

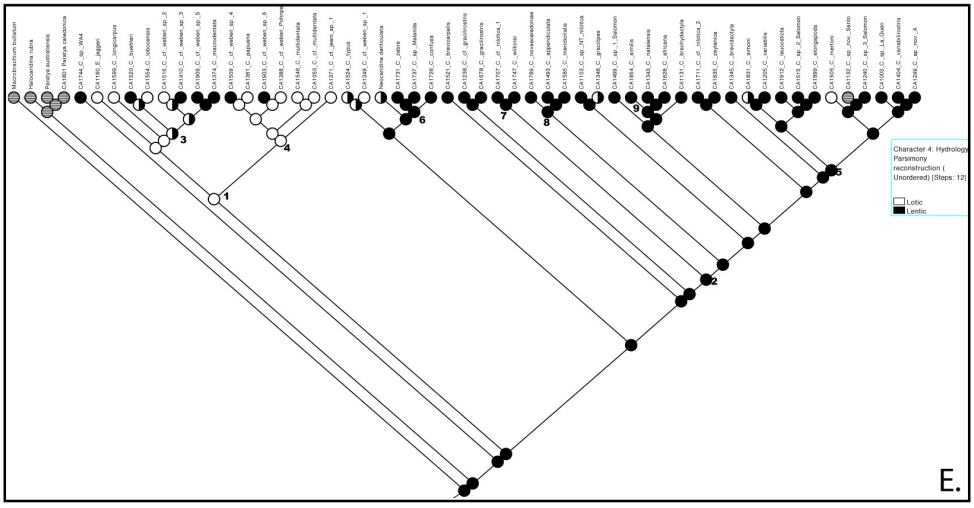


Altitude

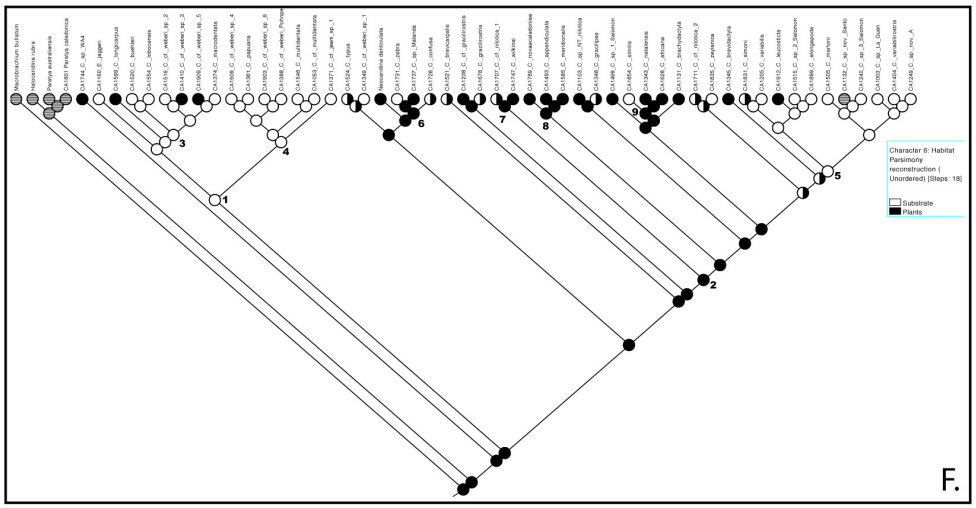
Figure 6.4: Character mapping on the Bayesian tree obtained in Figure 6.2. A: Geography coded following Longhurst's (1998) marine provinces; B: Geography coded following Abell et al.'s (2008) freshwater ecoregions; C: Altitude



Substrate type



Hydrology



Habitat

Figure 6.4 (continued): Character mapping on the Bayesian tree obtained in Figure 6.2. D: Substrate type; E: Hydrology; F: Habitat.

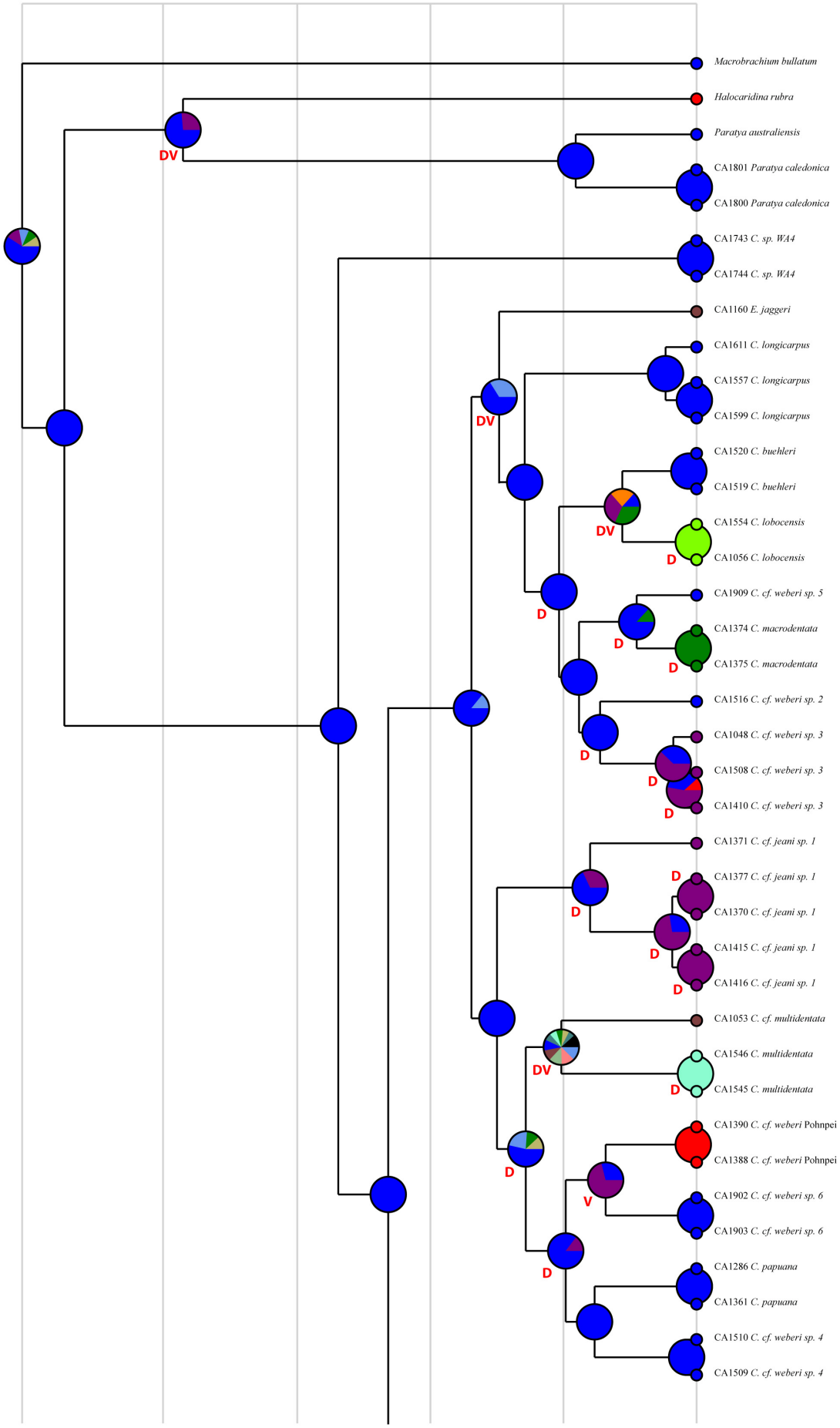
6.5.2 Time calibration

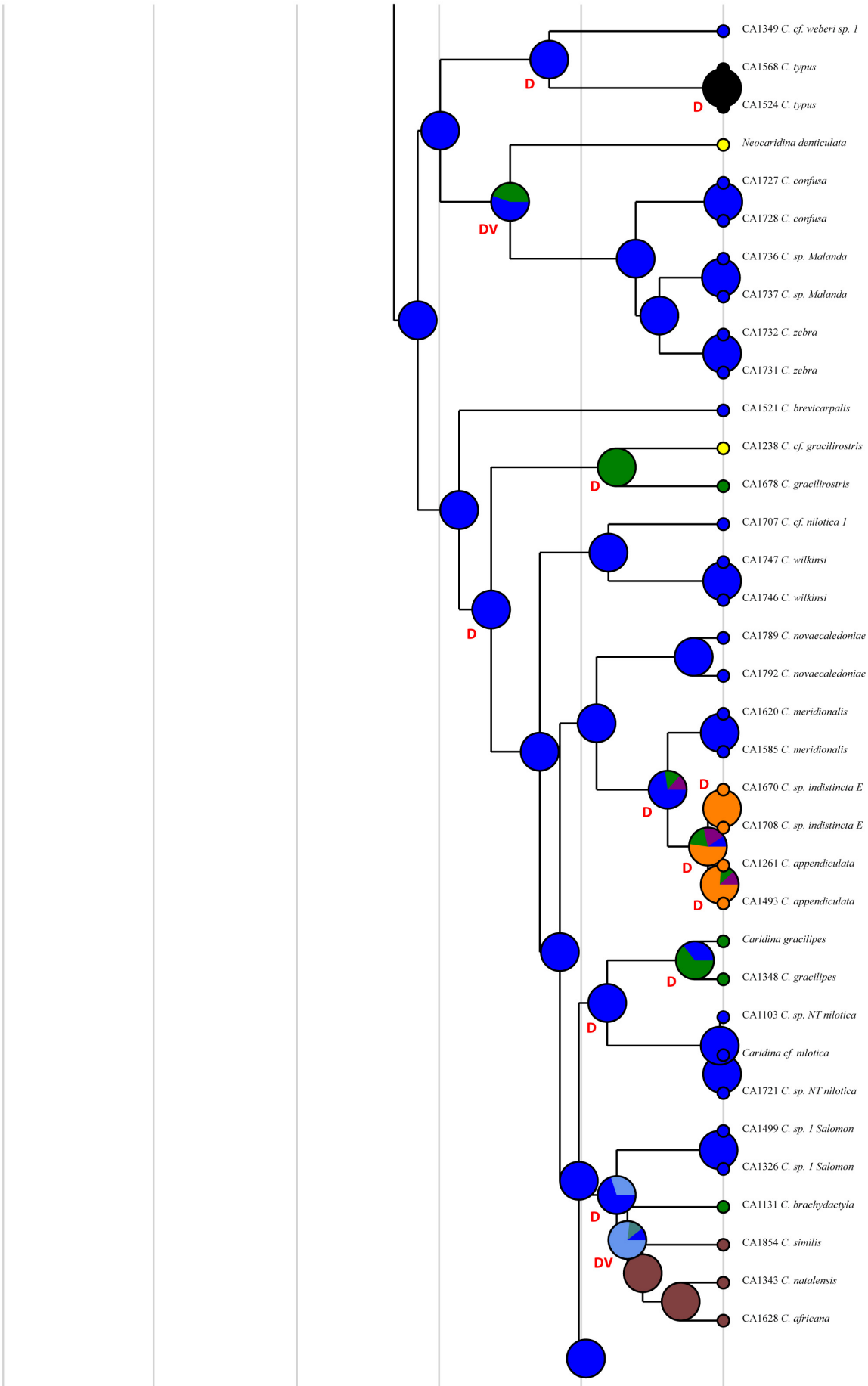
Until now, few studies have proposed dated phylogenies of atyid shrimps (Botello *et al.*, 2013 ; Jurado-Rivera *et al.*, 2017 ; Davis *et al.*, 2018) and only two have discussed *Caridina* (Rintelen *et al.*, 2012 ; Bernardes *et al.*, 2017). This can be explained by the lack of fossils attributed to this family. Caridean shrimps are quite rare in the fossil record (52 species according to Schweitzer *et al.*, 2010) and even scarcer in freshwater deposits. Five atyid species belonging to three genera exist in the fossil record, *Delclosia martinelli* (Via, 1971), *D. roselli* Rabadá, 1990, *Atyoida roxoi* Beurlen, 1950, *A. tremembeensis* Beurlen, 1950, and *Caridina nitida* A. Milne Edwards, 1879. A fourth species (and genus), currently *incertae sedis* could be added to this list, *Homelys minor* von Meyer, 1862, that Ortmann (1894) proposed as an Atyidae because of the small size of the specimens and the freshwater environment. It is doubtful that extant genera could be represented in the fossil record, the two species of fossil *Atyoida* Randall, 1840 described by Beurlen (1950) being separated by almost 100 million years. These fossils were identified as atyids because they resemble modern day *Potimirim* Holthuis, 1954 (known as *Atyoida* at the time of Beurlen) that live today in the same area. Additionally, *Caridina* was the default name of small freshwater shrimps at the time of A. Milne Edwards (see *Caridina desmarestii* (Millet, 1831) (Joly, 1843 ; A. Milne Edwards, 1879) now *Atyaephyra desmarestii* (Millet, 1831)). Regardless, because of their small size (3cm at most), morphological characters as demonstrated by Rabadá (1990), and age (oldest fossil atyid record), the two species of *Delclosia*, *D. roselli* (Via, 1971) and *D. martinelli* (Rabadá, 1990) from the Lower Cretaceous of Spain remain the most convincing fossil atyid, which is why we retained *Delclosia* as our calibration point.

Only one calibration point was used in our analysis given the limited availability of suitable fossils. Multiple calibration points can improve the reliability of molecular timing analyses (Lukoschek *et al.*, 2012 ; Hipsley & Müller, 2014). Despite this, we consider our results to be reliable because they are consistent with geographical events. For example, the clade containing all New Caledonian endemic species is posterior to the re-emergence of the island after its complete submersion, 37 MYA (Grandcolas *et al.*, 2008). Similarly, *C. variabilirostris*, a species endemic to Pohnpei Island, Micronesia appeared to have split from its sister-species between 10 and 3 MYA (Mean = 6.82 MYA)

in our analysis, congruent with the apparition of the island, between 3–8.6 MYA (Craig *et al.*, 2001). Furthermore, if the fossils we used for the calibration point were excluded from the Atyidae, the molecular clock would still give us a congruent timing. Substitution rates are sometimes used to calibrate a tree where no fossils exist or a geological event cannot be used. For *Caridina*, Bernardes *et al.* (2017), following Hurwood & Hughes (2001) who were following Knowlton *et al.* (1993), a substitution rate of 1.1–1.3% per MYR (million year) in COI was retained as originally calculated for alpheid shrimps based on the closing of the Isthmus of Panama. If we suppose this rate to be constant in caridean shrimps, our data agrees somewhat for intra-specific divergences. For example, the two sequences of *C. simoni* are separated by 5.1% divergence (K2P) in COI, corresponding to 3.97 MYA in the calibrated tree, which gives a rate of 1.28% per MYR. For *C. novaecaledoniae*, a COI divergence of 6% between the two sequences, corresponding to 5.68 MYA, gives a substitution rate of 1.06% per MYR.

An arising of the Atyidae in the Early Cretaceous agrees with most studies that have examined the origin of the family. For example, four out of six analyses by von Rintelen et al. (2012) suggested a Cretaceous origin of the family and a Paleogene radiation. Conversely, Jurado-Rivera *et al.* (2017) suggest a Permo-Triassic origin of the Atyidae, but only included fossils that are phylogenetically outside of the family as calibration points. Additionally, time-estimates could be over-estimated due to high mutation rates in the *Typhlatya* group (see the branch length of *H. rubra* on Figure 6.2). Likewise differences between the results of this study and Davis *et al.* (2018), which proposed the origin of Atyidae during the Early Jurassic without rapid diversification and an equal rate of speciation throughout the tree, may be explained by differences in methodology and underlying assumptions. Combining trees from different sources as used by Davis *et al.* (2018), in which original branch lengths are lost and recalculated, can lead to over-estimation of time-estimates (Bryant *et al.*, 2004).


Macrobrachium bullatum
Halocaridina rubra
Paratya australiensis
CA1801 Paratya caledonica
CA1800 Paratya caledonica
CA1743 C. sp. WA4
CA1744 C. sp. WA4
CA1160 E. jaggeri
CA1611 C. longicarpus
CA1557 C. longicarpus
CA1599 C. longicarpus
CA1520 C. buehleri
CA1519 C. buehleri
CA1554 C. lobocensis
CA1056 C. lobocensis
CA1909 C. cf. weberi sp. 5
CA1374 C. macrodentata
CA1375 C. macrodentata
CA1516 C. cf. weberi sp. 2
CA1048 C. cf. weberi sp. 3
CA1508 C. cf. weberi sp. 3
CA1410 C. cf. weberi sp. 3
CA1371 C. cf. jeani sp. 1
CA1377 C. cf. jeani sp. 1
CA1370 C. cf. jeani sp. 1
CA1415 C. cf. jeani sp. 1
CA1416 C. cf. jeani sp. 1
CA1053 C. cf. multidentata
CA1546 C. multidentata
CA1545 C. multidentata
CA1390 C. cf. weberi Pohnpei
CA1388 C. cf. weberi Pohnpei
CA1902 C. cf. weberi sp. 6
CA1903 C. cf. weberi sp. 6
CA1286 C. papuana
CA1361 C. papuana
CA1510 C. cf. weberi sp. 4
CA1509 C. cf. weberi sp. 4
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DV
DV
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CA1349 C. cf. weberi sp. 1
CA1568 C. typus
CA1524 C. typus
Neocaridina denticulata
CA1727 C. confusa
CA1728 C. confusa
CA1736 C. sp. Malanda
CA1737 C. sp. Malanda
CA1732 C. zebra
CA1731 C. zebra
CA1521 C. brevicarpalis
CA1238 C. cf. gracilirostris
CA1678 C. gracilirostris
CA1707 C. cf. nilotica 1
CA1747 C. wilkinsi
CA1746 C. wilkinsi
CA1789 C. novaecaledoniae
CA1792 C. novaecaledoniae
CA1620 C. meridionalis
CA1585 C. meridionalis
CA1670 C. sp. indistincta E
CA1708 C. sp. indistincta E
CA1261 C. appendiculata
CA1493 C. appendiculata
Caridina gracilipes
CA1348 C. gracilipes
CA1103 C. sp. NT nilotica
Caridina cf. nilotica
CA1721 C. sp. NT nilotica
CA1499 C. sp. 1 Salomon
CA1326 C. sp. 1 Salomon
CA1131 C. brachydactyla
CA1854 C. similis
CA1343 C. natalensis
CA1628 C. africana
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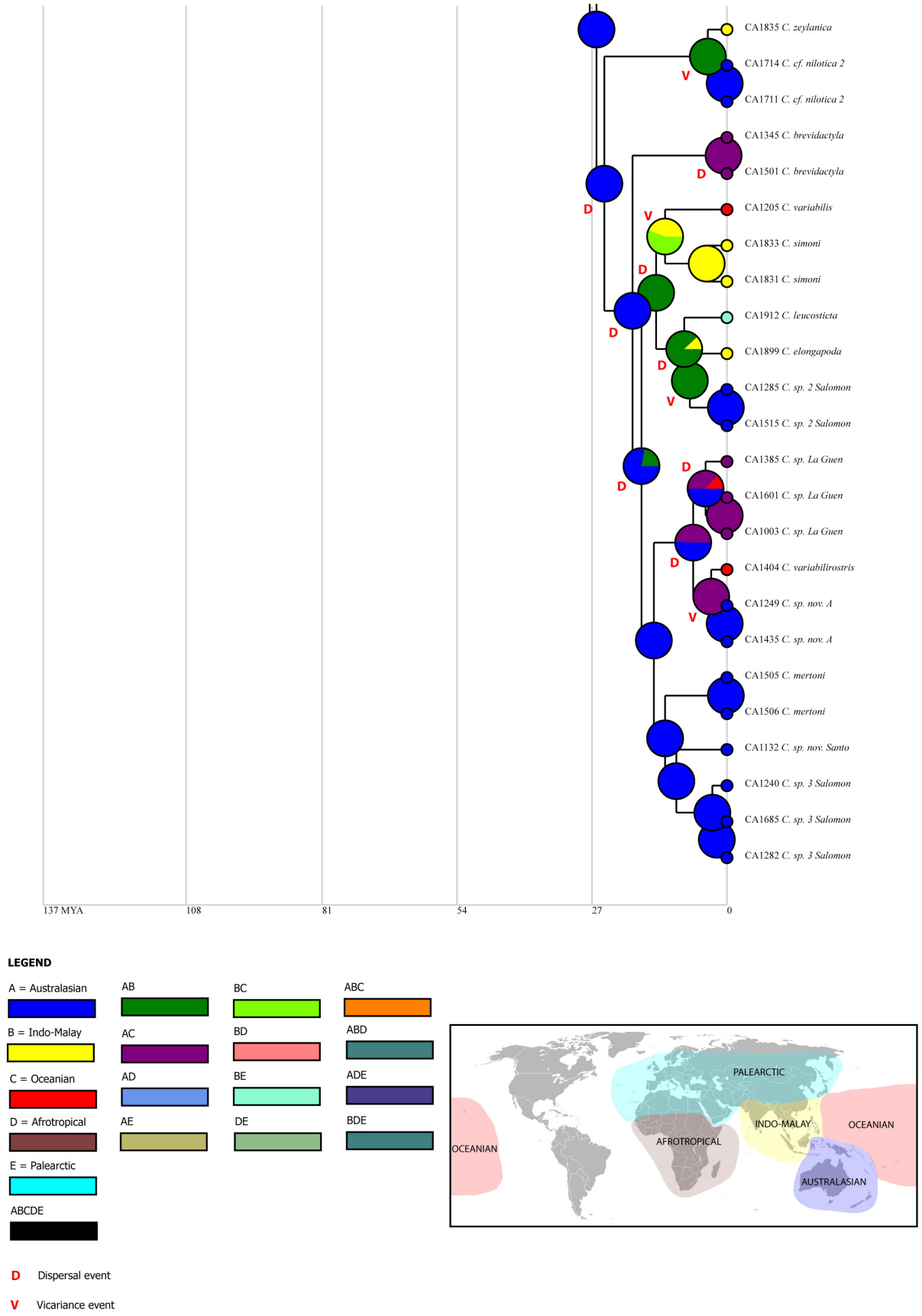


Figure 6.5: (And previous pages) Ancestral areas reconstruction based on the time calibration consensus tree.

6.5.3 Historical biogeography and character mapping

For the geographic coding, we first tried to use freshwater ecoregions (Abell *et al.*, 2008) but the great number of distinct regions made the analysis unreadable. As most caridean species are postulated to have colonized their range via marine larval dispersal, we tried to test marine biogeographic provinces based on plankton communities and currents (Longhurst, 1998). This approach was easier to read due to the fewer number of coded provinces, but could not highlight endemic taxa. Given the difficulties in our approaches, no biogeographic hypotheses can be proposed for the deeper nodes of the phylogeny due to a strong bias towards Melanesia, however, some of the terminal nodes can be explained by their geographical distribution. For example, nodes 6 and 7 clearly originate in Australia (Figure 6.4A), which supports the hypothesis that this continent was colonised in several events (Page *et al.*, 2007a, 2007b). Node 8, containing all the New Caledonian endemic species is likely to have originated there (33% likelihood, Figure 6.4B). Node 9, composed only of species living in the Indian Ocean region, would likely have originated there. It is interesting to note, however, that an Indonesian species, *C. brachydactyla* is sister to the clade delineated at Node 9, supporting the hypothesis of a colonization of the Indian Ocean region from Indonesia. The historical biogeography analysis was made on a shorter list of areas based on terrestrial biogeographical realms. It confirmed the results obtained for the marine and freshwater bioregions in showing no deep geographic structuration of the phylogeny and still with a bias towards Melanesia (here included among others in the Australasian realm). It furthered showed that dispersal seems to be far more common than vicariance to explain cladogenesis, even for deep nodes, suggesting that amphidromy could be an ancestral character in *Caridina*. It is important to note that the Indo-Malay region was not sampled as extensively as other regions in our study. The Indo-Malay region is a biodiversity hotspot for many taxa, including freshwater shrimps (De Grave *et al.*, 2015). Given this, it is possible that a significant proportion of species may be missing from our analyses, which could have biased our results. Whilst we included species from this region representing a broad range of geographical and ecological types, further samples are required to better understand the phylogeny and historical biogeography of the shrimp from the Indo-Malay region.

Our analyses provide new insights into the habitat and potential ecology of the common ancestors of the genus *Caridina*. According to the analyses, the common

ancestors of the *C. nilotica* and *C. weberi* complexes seem to have lived at low altitudes (67% of chances in the estuary or lower course, Figure 6.4C), on gravel substrate (Figure 6.4D) in a lentic environment (Figure 6.4E) and among vegetation (Figure 6.4F). Differences in the ecology of shrimps from complexes other than '*C. nilotica*' and '*C. weberi*' used here might provide further explanation for the separation of complexes that have been identified using genetics (this study) and morphology (Cai & Ng, 1999; 2007; Klotz & Rintelen, 2014). For example, in the lower reaches of rivers, there often is strong predation pressure (from Eleotridae, Kuhliidae, Anguillidae) but an abundance of food available, whereas in the higher course, there are almost no predators (except Anguillidae and Palaemonidae) but a strong competition for food with other atyids (namely the filter-feeding *Atya*-like species).

6.5.4 Differences between C. nilotica *and* C. weberi *complexes*

The analyses highlighted the genetic reality of some species complexes that were established using morphological characteristics, namely *C. weberi* and *C. nilotica* complexes on which this study focused. Both of these complexes were well separated from other taxa and well supported in the phylogeny regardless of the method used to differentiate them. As pointed out by previous authors (Page *et al.*, 2007a, 2007b ; Rintelen *et al.*, 2008 ; Rintelen *et al.*, 2012), genus *Caridina* appears polyphyletic, with genera *Elephantis* Castelin, Marquet & Klotz, 2013 and *Neocaridina* Kubo, 1938 nested in the middle of the *Caridina* species.

Our time calibration showed that *C. weberi* complex is older than *C. nilotica*. Ancestral states reconstruction and character mapping analyses showed that the separation of the clades was due to habitat at first (including altitude, habitat, and hydrology) and then geography was responsible for the diversification within the complexes. This was not a surprising result given environment has a profound influence on the morphology of atyid species (Jugovic *et al.*, 2010 ; Mazancourt *et al.*, 2017a). For example, as altitude decreases, along with changes in associated environmental factors (current flow, substrate type, etc.), the length of the rostrum and the segments of the legs tend to increase; a phenomenon known as the "Pinocchio-Shrimp effect" (Mazancourt *et al.*, 2017a). This effect is visible in both species complexes studied here. *C. weberi* complex displays robust morphology, with usually a short rostrum and stout legs, associated with their affinities to mostly lotic environment in high altitudes among

boulders with no vegetation (Figure 6.4). In contrast, *C. nilotica* complex species tend to display a slender morphology, with a long rostrum and long legs associated with their affinity to lentic environments on the lower course with gravel bed and the presence of aquatic vegetation (Figure 6.4). It is important to note, however, some species belonging to these complexes have adapted to different habitats than their ancestors, and this is associated with differences in their morphology. For example, species in *C. nilotica* complex that can live in the higher course, like *C. mertoni*, *C. variabilis* or *C. variabilirostris* (species of the node 5) often show a shorter rostrum (Mazancourt *et al.*, 2018a ; Mazancourt *et al.*, 2018b). Conversely, species of the *C. weberi* complex that are adapted to lower altitudes, like *C. buehleri* (species of node 3), can possess a longer rostrum than other species of this complex (Mazancourt *et al.*, 2017b). Likewise, *C. cf. weberi sp. 1* from the Solomon Islands, living in environments close to those of species of *C. weberi* complex exhibit a close morphology, which led to this species being included as part of the complex. *C. cf. jeani* that was thought to be close to *C. typus* because of its unarmed rostrum (Cai, 2010b) falls within the *C. weberi* complex in our analysis. Our results highlight the need for careful morphological examination to identify discriminating characters and prevent misidentifications.

6.5.5 Implications for conservation and management of Caridina

Our genetic analyses provide strong evidence for the existence of multiple valid and undescribed species within the *C. nilotica* and *C. weberi* species complexes. Currently, both '*C. nilotica*' and '*C. weberi*' are classified as "Least Concern" on the IUCN Red List (IUCN, 2018a,b). These classifications will need to be reassessed to account for differences in the vulnerability of the species identified in this study that are clustered within the two complexes. For example, our analysis indicated the presence of three undescribed species within the *C. nilotica* complex that are likely to be restricted to the Solomon Islands and/or nearby islands. Species with restricted distributions are generally more vulnerable to extinction compared to widely dispersed species (Purvis *et al.*, 2000). Similarly, our genetic data indicates the range of some species reported as widespread (e.g. *C. longirostris*) and in turn classified as "Least Concern" (IUCN, 2018c), may be smaller than currently thought due to difficulties in distinguishing among specimens with very similar morphology (Mazancourt *et al.*, in press). Given this, it is likely that the risk of extinction for many shrimp populations within the *'C. nilotica'* and *'C. weberi'* species complexes is greater than previously thought. This is concerning given

one third of caridean shrimps are already classed as 'Threatened' or 'Near Threatened' (De Grave *et al.*, 2015). Until full taxonomic assessments can be made, managers and conservationists should use a 'precautionary approach' in managing and protecting populations that may be closely related and have similar morphology to other wide-spread species.

6.6 Concluding remarks

This study reconstructing the phylogeny of genus *Caridina* provided a well-resolved and supported phylogenetic tree for the first time, with clades clearly identified that allowed us to propose novel evolutionary hypotheses. Our results highlight how inclusion of species complexes in phylogenies can provide new insights into evolutionary relationships and propose new avenues for distinguishing closely related species, as well as providing information crucial to conservation and management initiatives. To expand this work and improve the taxonomy of the whole genus, some species positions will need to be clarified (e.g. *C. typus*), which could be obtained with a larger dataset of complete mitochondrial genomes. The inclusion of comparatively unexplored species complexes in our phylogeny, such as Sulawesi, African, Chinese landlocked groups, *C. gracilirostris* complex, or the species close to *C. serratirostris*/*C. celebensis*, would likely add substantial information on the evolution of this group. Finally, the evolutionary hypotheses proposed here can be improved by refining time calibrations, but this is reliant on the discovery of new fossils or the improvement of existing techniques that do not rely on fossils.

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Chapitre sixième, Etude sclérochronologique préliminaire de l'amphidromie chez *C. multidentata*

Preliminary sclerochronological study of amphidromy in C. multidentata

Où l'on étudie la faisabilité d'une étude de l'amphidromie par l'observation de stries de croissance dans l'épaisseur de la cuticule des pédoncules oculaires de C. multidentata *en évaluant la conservation de cette structure à travers les cycles de mue et en étudiant sa composition au niveau microscopique et élémentaire.*

Where we investigate the feasibility of studying the amphidromy by observing growth bands in the thickness of the cuticle of the eyestalks of C. multidentata *by evaluating the conservation of this structure through the molt cycles and by studying its composition at the microscopic and elementary level.*

7. Chapitre 6 : Etude sclérochronologique préliminaire de l'amphidromie chez *C. multidentata*

Afin d'en apprendre plus sur la biologie des caridines, et notamment sur leur mode de vie amphidrome, nous avons tenté de développer une nouvelle technique de sclérochronologie appliquée à la cuticule des pédoncules oculaires, supposée être conservée d'une mue à l'autre chez les crustacés et présenter des stries de croissance (Kilada *et al.* 2012). Nous avons donc mené différentes expériences sur des spécimens vivants de *Caridina multidentata*, une espèce du complexe *C. weberi* disponible dans le commerce aquariophile, pour tester la faisabilité de l'étude sclérochronologique de cette structure.

In order to learn more about the biology of *Caridina* shrimps, and especially about their amphidromous lifestyle, we tried to develop a new method of sclerochronology applied to the eyestalk cuticle, supposedly conserved through the molts in crustaceans and showing growth bands (Kilada *et al.* 2012). We thus carried out several experiments on live specimens of *Caridina multidentata*, a species belonging to the *C. weberi* complex available from the aquarium trade, to test the feasibility of the sclerochronological study of this structure.

7.1 Introduction

Nous avons vu que les espèces de caridines étudiées sont supposées diadromes, mais on ignore encore tout de leur cycle de vie : où a lieu la reproduction (estuaire ou rivière), quelle est la durée de vie larvaire en mer, à quelle profondeur évoluent les larves, à quelle distance du lieu de naissance peuvent-elles migrer, quelles sont leurs adaptations physiologiques, etc. Pour pouvoir mettre en place la gestion et la conservation de ces espèces, l'utilisation d'outils comme la sclérochronologie peut permettre d'améliorer les connaissances sur leur biologie.

7.1.1 La sclérochronologie

La sclérochronologie, ou l'étude physico-chimique de parties dures (généralement calcifiées) d'organismes permet d'obtenir des informations sur leur vie, notamment grâce à l'observation de stries de croissances (Buddemeier *et al.*, 1974). Elle a été appliquée, entre autres, chez les poissons, où l'on étudie surtout l'otolithe (une concrétion de carbonate de calcium présente dans l'oreille interne) (Pannella, 1971, 1974) et chez les mollusques, où l'on étudie la coquille pour les bivalves, ou l'opercule pour les gastéropodes (Gröcke & Gillikin, 2008). Initialement limitée à la détermination de l'âge par comptage de stries, la sclérochronologie peut, de nos jours, aussi fournir des informations détaillées sur la vie de l'organisme grâce à des analyses microchimiques des structures calcifiées (Radtke *et al.*, 1988). En effet, chez les poissons, les otolithes sont des « boîtes noires » enregistrant les variations de la composition chimique des milieux traversés au cours de la vie de l'individu, et permettant de repérer par exemple la transition entre le milieu marin (zone de croissance) et la rivière (zone de reproduction) chez les espèces amphidromes (Radtke *et al.*, 1988 ; Shen *et al.*, 1998) ou encore les déplacements au sein du bassin versant (amont vers aval et inversement). Il en va de même pour l'opercule des gastéropodes amphidromes du genre *Neritina* (Abdou, 2016). Chez les crustacés, aucune structure de ce type n'avait été mise en évidence jusqu'à quelques travaux récents (Leland *et al.*, 2011 ; Kilada *et al.*, 2012 ; Kilada & Acuna, 2015 ; Kilada *et al.*, 2015 ; Kilada & Ibrahim, 2016 ; Kilada & Driscoll, 2017) utilisant des stries de croissance annuelles repérées dans l'épaisseur de l'endo-cuticule des pédoncules oculaires et de certains ossicules gastriques chez différentes espèces commerciales de décapodes marins (Homards, langoustes, crabes et crevettes).

Nous avons donc décidé de **tester la faisabilité de l'utilisation de pièces calcifiées conservées chez les caridines** pour étudier l'amphidromie et leurs traits de vie. Pour des questions pratiques, **l'étude s'est concentrée sur la cuticule des pédoncules oculaires**, plus facilement accessibles que les ossicules gastriques chez des espèces de cette taille. Tout d'abord, il s'agit de vérifier que la cuticule des pédoncules oculaire est bien conservée d'une mue à l'autre.

7.1.2 Le processus de mue chez les crustacés

Pour comprendre les implications de l'existence de structures calcifiées conservées chez les crustacés, il convient de faire ici quelques rappels sur la structure de la cuticule, ainsi que sur les mécanismes permettant la mise en place d'une nouvelle cuticule en remplacement de l'ancienne, c'est-à-dire la mue.

La cuticule correspond à l'exosquelette calcifié chez les crustacés (Richards, 1951). Elle est composée de quatre couches :

- L'épicuticule, la couche la plus externe et la plus fine, composée de lipoprotéines sclérotinisées (Travis, 1955) mais qui n'est pas minéralisée (Nagasawa, 2012).
- L'exocuticule, située sous la précédente, composée d'empilements de fibres chitino-protéiques dont l'orientation varie (Travis, 1955). Elle est indurée par sclérotisation (ou tannage) par des quinones et est calcifiée.
- L'endocuticule, située sous la précédente, est la couche la plus épaisse et la plus calcifiée, également composée de lamelles horizontales de fibres chitino-protéiques dont l'orientation varie à chaque couche. Elle n'est pas tannée, son induration est due à des sels de calcium (Travis, 1955).
- La couche membraneuse est la plus interne, en contact avec l'épiderme, elle est composée de chitine et n'est pas minéralisée (Travis, 1955).

Le minéral présent dans les trois couches supérieures de la cuticule est le carbonate de calcium, sous forme cristalline de calcite ou amorphe (Travis, 1963).

La présence de cet exosquelette rigide empêche une croissance continue. Pour parer à cela, le processus de mue, consistant en un renouvellement régulier de l'enveloppe externe pendant lequel l'animal croît a été développé par les organismes du groupe des Ecdysozoaires (comprenant entre autre les Nématodes, les Tardigrades et les Arthropodes).

Le cycle de mue chez les crustacés a été subdivisé en 5 stades ; A, B, C, D et E, possédant eux-mêmes d'autres subdivisions (Drach, 1939 ; Drach & Tchernigovtzeff, 1967) (Figure 7.1). Les stades A, B et C_1 à C_3 correspondent à différents niveaux de minéralisation de la cuticule directement après la mue et peuvent être regroupés comme faisant partie de l'étape de post-mue. Le stade C_4, où la cuticule est pleinement minéralisée correspond à l'inter-mue. C'est dans cet état que les crustacés passent la plus grande partie de leur vie. Pendant le stade D, une nouvelle cuticule se forme sous l'ancienne, commençant par l'épicuticule. Un espace dit « exuvial » va se former entre l'épicuticule nouvellement formée et l'ancienne endocuticule à mesure qu'elle est digérée par des enzymes et est peu à peu déminéralisée. La nouvelle exocuticule est sécrétée sous la nouvelle épicuticule. Ces différents changements précédant la mue correspondent à l'étape de pré-mue. Enfin, le stade E correspond à la mue, ou exuviation ou ecdysis, étape pendant laquelle l'ancienne cuticule (exuvie) est éliminée et où l'animal va pouvoir grandir.

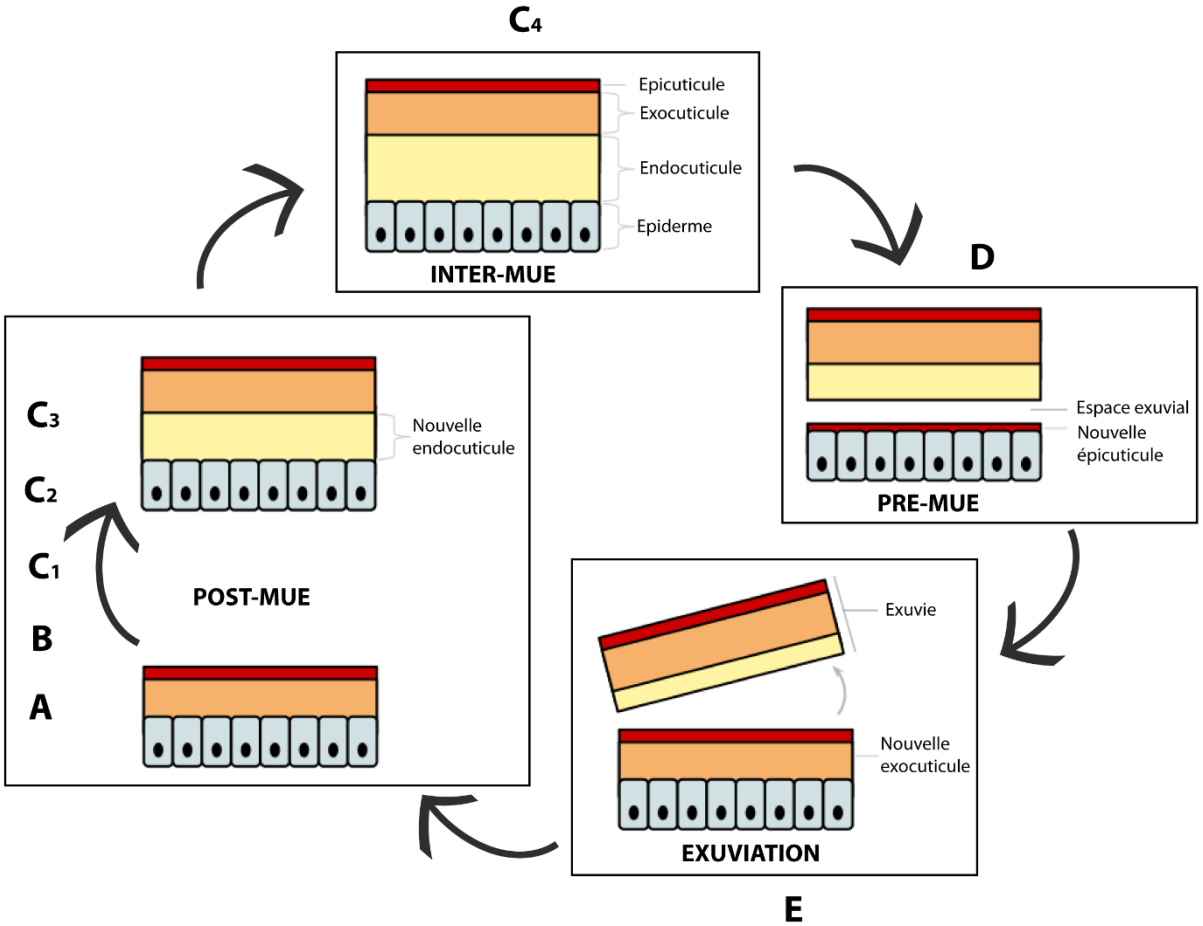


Figure 7.1 : Les 4 étapes principales du cycle de mue chez les crustacés.

Figure 7.1: The 4 main stages of the molt cycle in crustaceans.

7.2 Vérification de la conservation de la cuticule des pédoncules oculaires

La première étape pour tester la faisabilité de l'étude de la cuticule des pédoncules oculaires par sclérochronologie est de vérifier la conservation d'une mue à l'autre de cette structure suggérée par de précédents travaux. Pour ce faire, deux approches ont été employées, la première consistant à marquer la cuticule de spécimens maintenus en élevage avec un colorant spécifique du calcium et vérifier la persistance de la coloration entre les mues, et la seconde, consistant à observer la structure de la cuticule à différents moments du cycle de mue pour vérifier si une partie de l'ancienne cuticule est maintenue en place au moment de l'exuviation.

7.2.1 Maintien des spécimens vivants en aquarium

Une salle d'élevage climatisée à 21°C (± 3°C) a été mise en place dans les locaux de l'UMR BOREA, avec plusieurs aquariums ; un bac de 180l hébergeant des spécimens de *Caridina multidentata* (espèce du complexe *C. weberi*) des deux sexes et de toutes les tailles provenant du commerce aquariophile (Origine : Taiwan, M. Négrini, Pisciculture d'Estalens, http://www.estalens.fr/), et quatre bacs de 20l servant à isoler des individus pour les différentes expériences. Les conditions de maintenance sont les suivantes : Température : 27°C (± 3°C), pH : 8, GH : 12, Nitrates : 12,5-25 mg/l. Les variables physico-chimiques sont contrôlées régulièrement par dosages colorimétriques (Tetra GmbH) et de 20 à 30% (selon les mesures obtenues) du volume des bacs est remplacé toutes les deux semaines par de l'eau de conduite. Les masses filtrantes sont nettoyées avec l'eau du bac à chaque changement d'eau. Les aquariums ont un substrat composé de grains de quartz, ainsi que des racines et des plantes (*Ceratophyllum demersum*, *Anubias barteri* var. *nana* et *Egeria densa*) pour servir de cachettes aux crevettes et sont éclairés 10h par jour. L'oxygénation est effectuée par un bulleur dans le bac de maintenance et par l'agitation créée par l'eau à la sortie des filtres exhausteurs dans les bacs expérimentaux. La crépine de sortie du filtre interne du bac de maintenance est orientée vers le sol de l'aquarium pour mimer le courant existant dans l'habitat naturel de *C. multidentata*. Les crevettes sont nourries une fois par semaine avec des granulés adaptés (Novo Prawn ou Novo Crab, JBL).

7.2.2 Marquages vitaux des spécimens

Kilada *et al.* (2012) ont réalisé un marquage vital à la calcéine sur des homards et ont observé une marque fluorescente localisée à la base de l'endocuticule, au contact de

la couche membraneuse, laissant penser que les couches inférieures seraient conservées d'une mue à l'autre, expliquant la présence de stries de croissance par accumulation de nouvelles couches. Afin de vérifier la persistance des structures calcifiées d'une mue à l'autre chez *C. multidentata*, un marquage vital de ces structures a été réalisé au moment de la calcification de la cuticule en post-mue pour tenter de reproduire les résultats de Kilada *et al.* (2012). Si, après une seconde mue, le marquage est toujours visible en place, cela signifie que la structure est conservée.

Des individus en inter-mue ou pré-mue sont isolés dans un bac expérimental (20l) rempli d'eau du bac de maintenance et contenant de la calcéine (100 mg/l) ou de l'alizarine (50 mg/l). Il s'agit de deux colorants (fluorescent quand exposés aux rayonnements ultraviolets, pour le premier) se liant de manière spécifique et durable au calcium contenu dans les structures squelettiques au moment de leur calcification (Puchtler *et al.*, 1969 ; Meunier & Boivin, 1978). Deux tests sont réalisés ; pour le premier (expérience sur 1 cycle), les crevettes sont maintenues dans le bain de colorant pendant 48h après l'exuviation puis transférées pendant 24h dans un bac sans colorant pour rinçage (c'est-à-dire élimination du marquage non spécifique). Pour le second test (expérience sur 2 cycles), les crevettes sont maintenues dans le bain de colorant pendant 48h après l'exuviation puis transférées dans un bac sans colorant jusqu'à l'exuviation suivante. Pour chaque test, des réplicats sont réalisés pour deux catégories de taille des individus (Petits, pour une carapace de moins de 6mm et Grands, pour une carapace de plus de 6mm). A l'issue de chacun des tests, les individus sont anesthésiés par un passage au congélateur à -80°C pendant 10 minutes puis sont fixés dans un tampon cacodylate (0,08 M de cacodylate de sodium à pH 7,4) contenant 4% de glutaraldéhyde et 2% de paraformaldéhyde pendant au moins 3h. Au total, 5 spécimens ont été marqués à la calcéine (1 grand et 2 petits suivis sur 1 cycle ; 1 grand et 1 petit suivis sur 2 cycles), 6 l'ont été à l'alizarine (1 grand sur 1 cycle ; 3 petits sur 2 cycles ; 1 grand et 1 petit placés 24h en présence du colorant) et un petit spécimen non marqué a servi de témoin. Les exuvies sont récupérées après chaque exuviation et préservées de la même manière que les spécimens entiers. Après fixation, les pédoncules oculaires sont prélevés, inclus dans de la résine époxy puis poncés jusqu'à obtenir une vue en coupe transversale qui peut être observée au microscope photonique (Figure 7.2). A titre de comparaison, un spécimen témoin n'ayant pas été mis en présence de colorant a également été préparé.

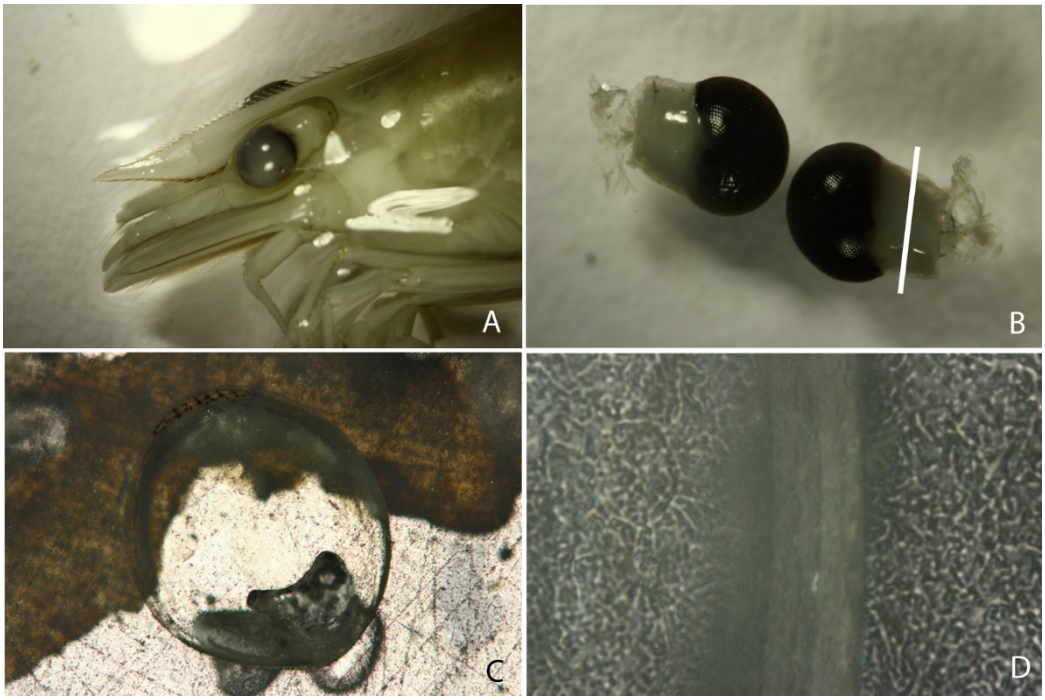


Figure 7.2 : Etapes de la préparation des pédoncules oculaires pour observation. A : Pédoncules en place sur l'animal. B : Pédoncules extraits avec visualisation du plan de coupe transversale (trait blanc). C : Pédoncule inclus dans la résine et vu en coupe après ponçage. D : Observation au microscope optique de l'épaisseur de la cuticule.

Figure 7.2: Steps of the preparation of eyestalks for observation. A: Eyestalks in place on the animal. B: Eyestalks extracted with the cross section plane figured (white line). C: Eyestalk included in resin seen in cross section after polishing. D: Observation in optical microscopy of the thickness of the cuticle.

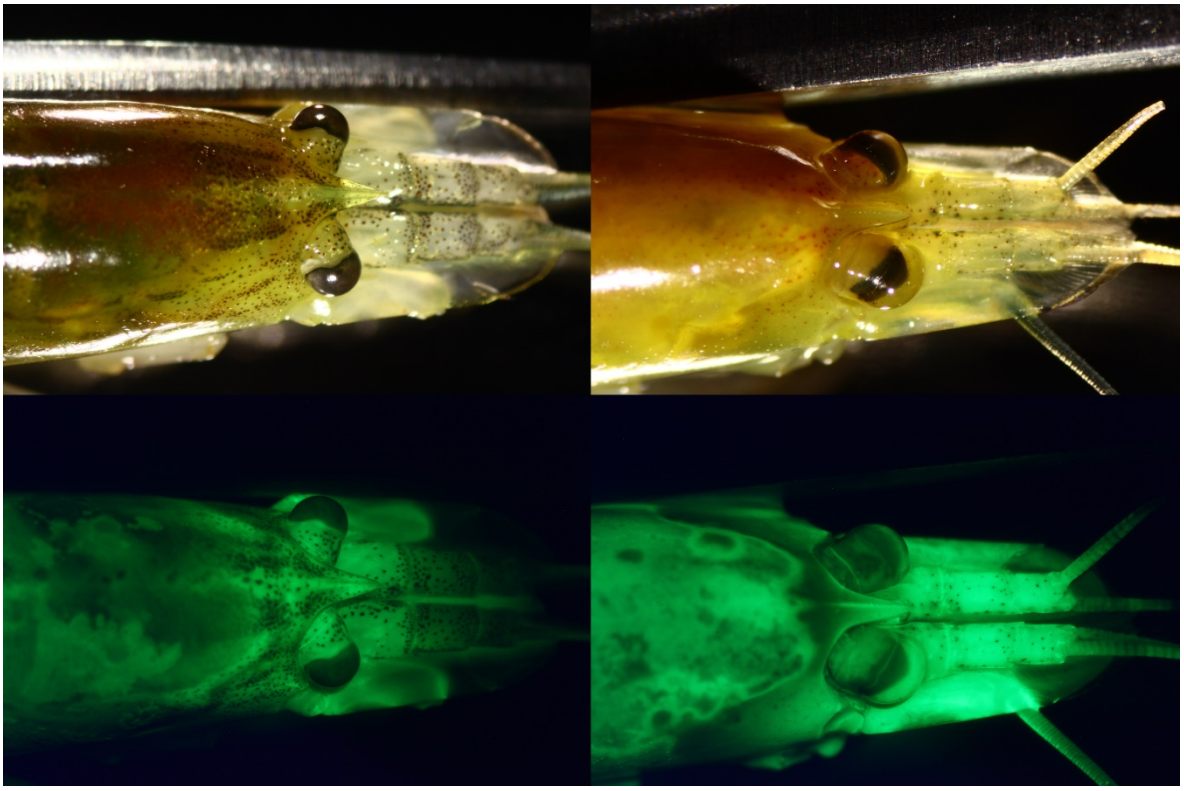


Figure 7.3 : Vues en lumière normale (en haut) et ultraviolette (en bas) de deux spécimens (grand à gauche, petit à droite) marqués à la calcéine sur 1 cycle de mue.

Figure 7.3: Views in normal (top) and ultraviolet light (bottom) of two specimens (left: large; right: small) marked with calcein over 1 molt cycle.

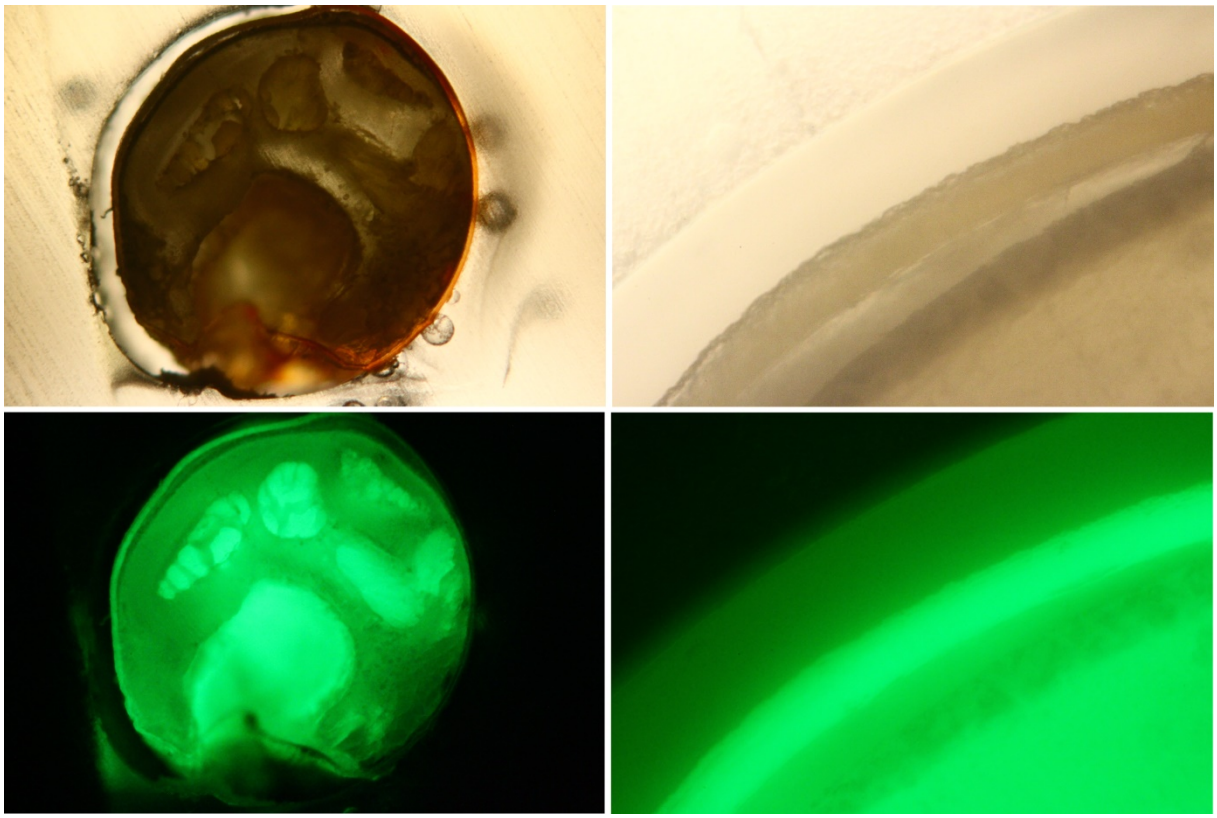


Figure 7.4 : Vues en lumière normale (en haut) et ultraviolette (en bas) d'une coupe transversale d'un pédoncule oculaire d'un grand spécimen marqué à la calcéine sur 1 cycle de mue.

Figure 7.4: Views in normal (top) and ultraviolet light (bottom) of a cross section of an eyestalk of a large specimen marked with calcein over 1 molt cycle.

Résultats pour les marquages à la calcéine

Après l'expérience réalisée sur 1 cycle de mue, la fluorescence est bien visible sur toute la surface du corps des individus marqués (Figure 7.3) tout comme dans l'épaisseur de la cuticule (Figure 7.4). On remarquera que les tissus semblent concentrer le colorant plus que la cuticule.

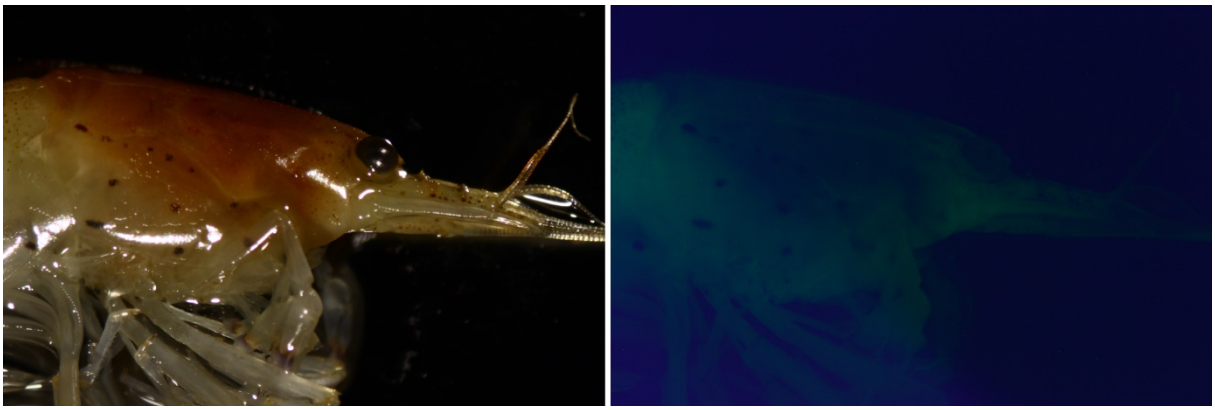


Figure 7.5 : Vues en lumière normale (gauche) et ultraviolette (droite, exposition plus longue que précédemment) d'un petit individu marqué à la calcéine après deux cycles de mue.

Figure 7.5: Views in normal (left) and ultraviolet light (right, longer exposure than previously) of a small specimen marked with calcein over 2 molt cycles.

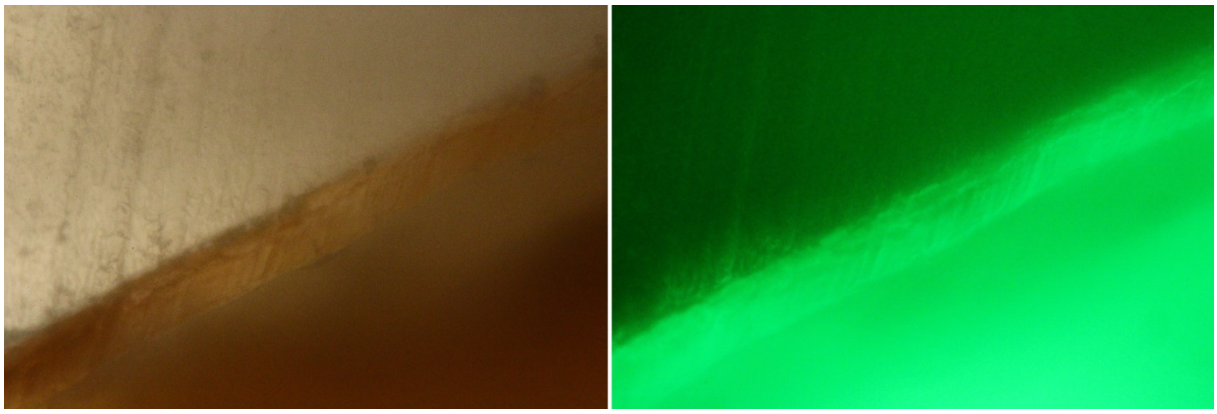


Figure 7.6 : Vues en lumière normale (gauche) et ultraviolette (droite) d'une coupe transversale d'un pédoncule oculaire d'un grand individu marqué à la calcéine sur 2 cycles de mue.

Figure 7.6: Views in normal (left) and ultraviolet light (right) of a cross section of an eyestalk of a large specimen marked with calcein over 2 molt cycles.

Après l'expérience réalisée sur 2 cycles de mue, on constate qu'une légère fluorescence est toujours visible extérieurement (Figure 7.5) de même que dans l'épaisseur de la cuticule (Figure 7.6).

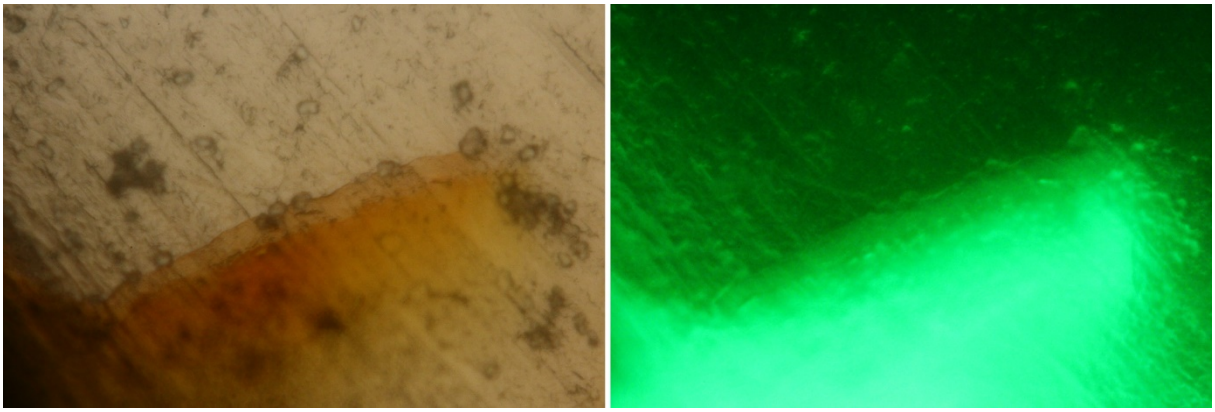


Figure 7.7 : Vues en lumière normale (gauche) et ultraviolette (droite, exposition plus longue que précédemment) d'une coupe transversale d'un pédoncule oculaire d'un petit individu non marqué à la calcéine.

Figure 7.7: Views in normal (left) and ultraviolet light (right, longer exposure than previously) of a cross section of an eyestalk of a small specimen not marked with calcein.

Cependant, l'observation de la cuticule des spécimens témoins a montré l'existence d'une légère auto fluorescence des tissus et de la cuticule dans les mêmes longueurs d'ondes que la calcéine, rendant l'interprétation du marquage plus compliquée (Figure 7.7).

D'après nos résultats, il semble donc qu'une partie au moins du calcium soit conservée puisqu'une fluorescence est toujours visible après un cycle complet (et donc une nouvelle calcification) réalisé en l'absence de calcéine. Cependant, contrairement aux résultats de Kilada *et al.* (2012) qui avaient également réalisé des marquages vitaux

à la calcéine pour valider leur méthode, la coloration n'est pas limitée aux couches les plus internes de la cuticule, mais semble diffuse et répartie de façon homogène dans toute son épaisseur. **Nos résultats mettent donc en doute la conservation d'une partie de la cuticule des pédoncules oculaires et laissent plutôt supposer une rétention de calcium dans les tissus qui serait remobilisé au moment de la calcification en post-mue.**

Résultats pour les marquages à l'alizarine

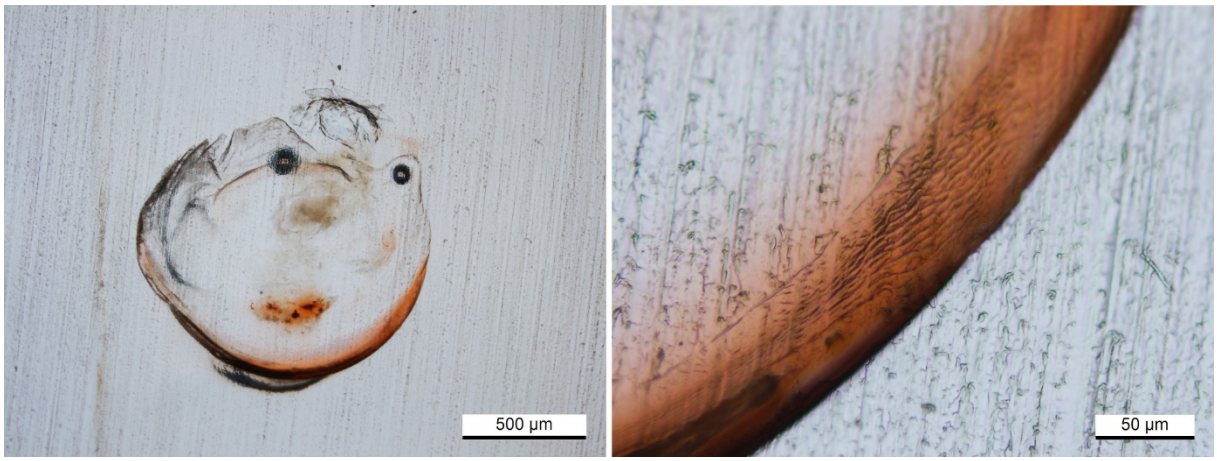


Figure 7.9 : Vues au microscope photonique à différents grossissements d'une coupe transversale de pédoncule oculaire d'un grand individu marqué à l'alizarine sur 1 cycle.

Figure 7.8: Views in photonic microscope at different magnifications of a cross section of an eyestalk of a large specimen marked with alizarin over 1 cycle.

Après l'expérience réalisée sur 1 cycle de mue, la coloration est bien visible sur toute l'épaisseur de la cuticule (Figure 7.8).

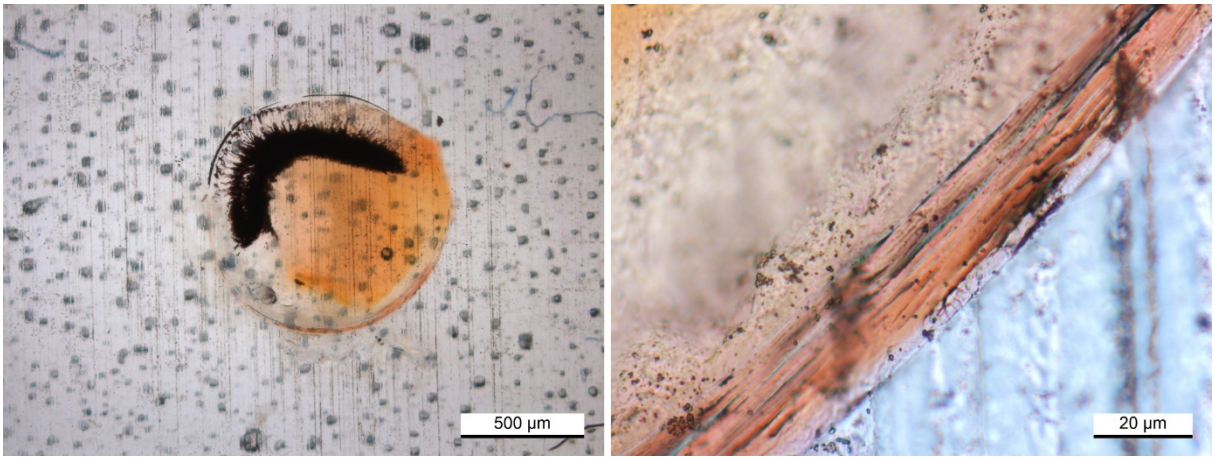


Figure 7.8 : Vues au microscope photonique à différents grossissements d'une coupe transversale de pédoncule oculaire d'un petit individu marqué à l'alizarine sur 2 cycles (contrastes augmentés).

Figure 7.9: Views in photonic microscope at different magnifications of a cross section of an eyestalk of a small specimen marked with alizarin over 2 cycles (enhanced contrasts).

Après l'expérience réalisée sur 2 cycles de mue, la coloration est plus légère mais toujours présente et semble absente des couches les plus externes de la cuticule (Figure 7.9), au moins pour deux des trois individus testés.

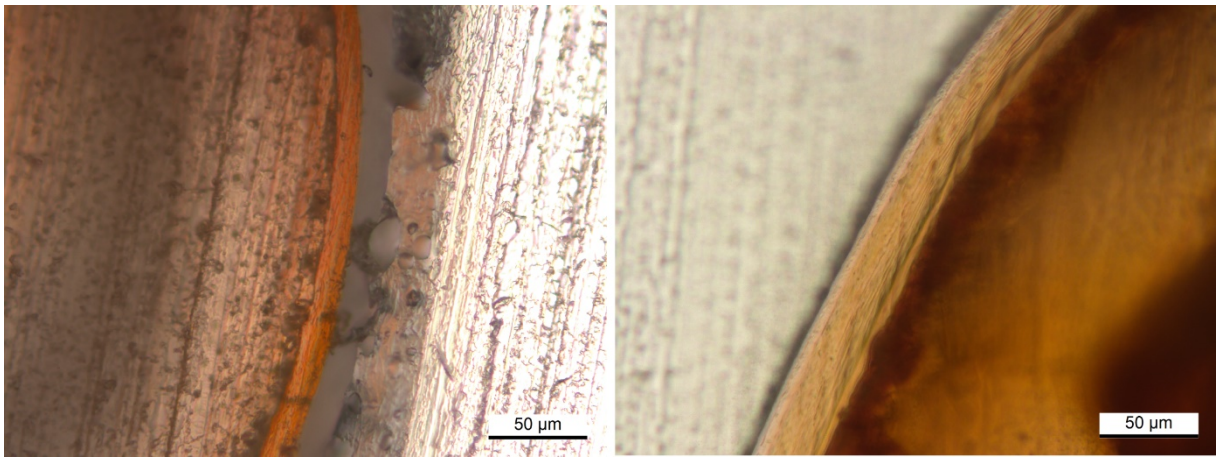


Figure 7.10 : Vues au microscope photonique à différents grossissements d'une coupe transversale de pédoncule oculaire d'un petit individu (gauche) et d'un grand individu (droite) placés dans un bain d'alizarine pendant 24h.

Figure 7.10: Views in photonic microscope at different magnifications of a cross section of an eyestalk of a small specimen (left) and a large specimen (right) placed in an alizarin bath for 24h.

A titre indicatif, deux individus en inter-mue (un grand et un petit) ont été placés dans un bain d'alizarine pendant 24h puis ont été prélevés, sans qu'ils y aient effectué une mue, et donc qu'une minéralisation ait pu avoir lieu. On constate qu'après 24h en présence d'alizarine et malgré l'absence de calcification de la cuticule, un marquage vif est apparent (Figure 7.10), laissant supposer une fixation passive du colorant.

Les expériences de marquages vitaux ne se sont donc pas révélées concluantes quant à la conservation d'une partie de la cuticule, les résultats de Kilada *et al.* (2012) présentant une rétention du marquage dans les couches inférieures de l'endo-cuticule n'ayant pu être reproduits sur notre modèle. Cependant, **il semble qu'une certaine proportion du calcium de la cuticule soit retenue à travers les mues selon un mécanisme qui reste à déterminer**. En effet, si toute la matière de la cuticule était éliminée au moment de l'exuviation, il ne resterait pas de traces de la coloration après une seconde mue en l'absence de colorant, or, selon nos résultats, une coloration réduite demeure.

Afin de vérifier la possibilité d'une conservation d'une partie de la cuticule et identifier ses différentes couches, nous avons voulu étudier la structure de la cuticule à différentes étapes du cycle de mue.

7.2.3 Etude de la cuticule à différents stades du cycle de mue

Dix spécimens (5 grands et 5 petits) ont été prélevés à chaque étape du cycle de mue (Inter-mue, pré-mue et post-mue, contrôlées en fonction de la teinte de la carapace et des yeux pour les deux premiers stades et prélevés juste après l'exuviation d'individus isolés pour la post-mue) et leurs exuvies ont été recueillies. Pour chaque individu prélevé, la longueur de la carapace (cl) a été mesurée. Les pédoncules oculaires ont été prélevés et fixés dans un tampon cacodylate (0,08 M de cacodylate de sodium à pH 7,4) contenant 4% de glutaraldéhyde et 2% de paraformaldéhyde pendant au moins 3h. Les pédoncules fixés ont ensuite subi une post-fixation au tétroxyde d'osmium (1% dans du tampon phosphate de Sorensen) pendant 30 minutes, puis une déshydratation par 4 bains successifs de 30 minutes à concentration croissante d'éthanol (50, 70, 90 et 100%) et une imprégnation par de la résine Spurr (10g ERL 4206, 6g DER 736, 0,4g S-1 et 26g NSA) en 3 bains : 1h dans un mélange à part égale de résine et d'éthanol, 1h dans de la résine, et 12h dans de la résine. Enfin, les pédoncules imprégnés sont inclus dans de la résine en laissant polymériser une nuit à 60°C. Les blocs contenant les pédoncules sont découpés grâce à une lame diamant 45° (Diatome) montée sur un ultramicrotome (Ultracut E, Reichert-Jung) pour obtenir des coupes transversales semi fines de 1,5µm d'épaisseur. Les coupes obtenues sont déposées sur une lame de verre et colorées par une solution de bleu de toluidine (1%) additionnée de borax (1%) pour être observées en microscopie photonique (microscope Olympus BX51) et photographiés grâce à une caméra (Leica MC170 HD) montée sur le microscope.

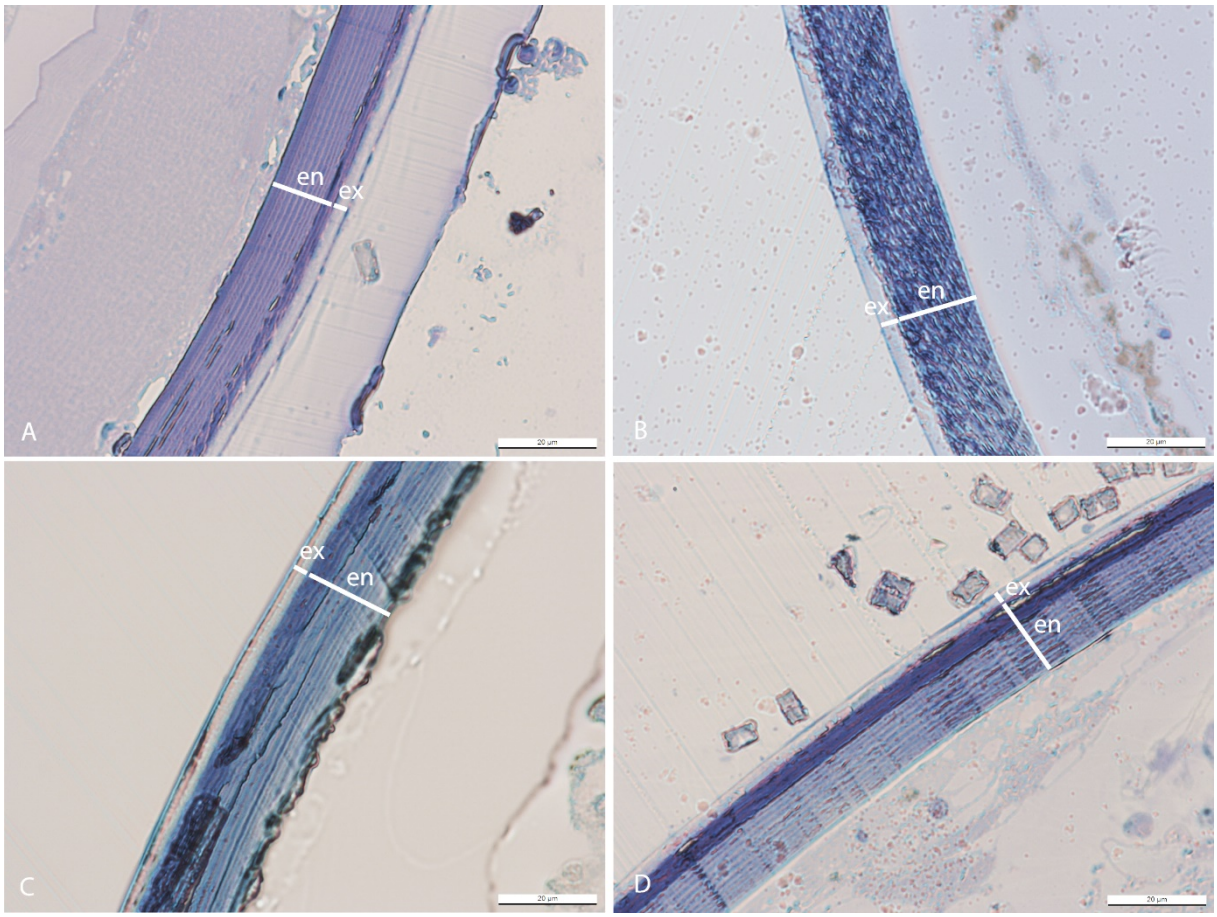


Figure 7.11 : Vues en microscopie photonique de coupes transversales de cuticule (exocuticule = ex ; endocuticule = en) de pédoncules oculaires provenant de spécimens en inter-mue, colorées au bleu de toluidine + borax. A, cl = 5mm ; B, cl = 6mm ; C, cl = 9 mm ; D, cl = 11mm.

Figure 7.11: Views in photonic microscope of cross sections of cuticle (exocuticle = ex; endocuticle = en) of eyestalks of specimens in inter-molt colored with toluidin blue + borax. A, cl = 5mm; B, cl = 6mm; C, cl = 9 mm; D, cl = 11mm.

Pour les spécimens en inter-mue, on distingue l'exocuticule, peu colorée de l'endocuticule, plus colorée et bien plus épaisse (Figure 7.11). Des stries sont clairement visibles dans l'endocuticule. Selon les individus observés, des couches de différentes nuances coloration peuvent être visibles dans l'endocuticule (Figure 7.11C et D par exemple). L'épicuticule, trop fine pour être visible, n'apparaît pas à ce grossissement. L'épaisseur de la cuticule ne semble pas dépendre de la taille du spécimen.

Pour les spécimens en pré-mue, la cuticule est dégradée, on ne distingue pas de différences de structure entre les différentes couches, la coloration étant homogène dans toute l'épaisseur (Figure 7.12).

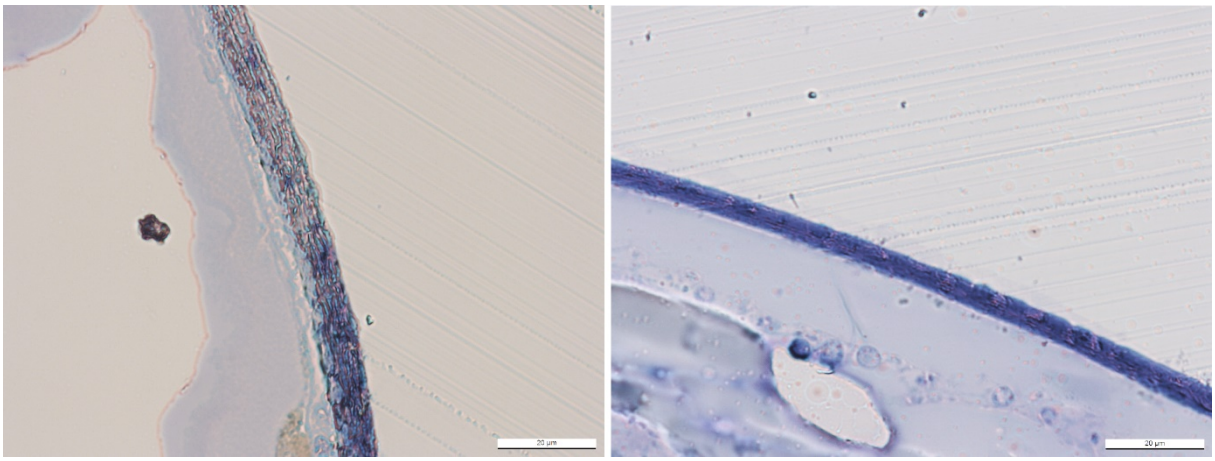


Figure 7.12 : Vues en microscopie photonique de coupes transversales de cuticule de pédoncules oculaires provenant de spécimens en pré-mue, colorées au bleu de toluidine + borax. Gauche, cl = 6mm ; droite, cl = 7mm.

Figure 7.12: Views in photonic microscope of cross sections of cuticle of eyestalks of specimens in pre-molt colored with toluidin blue + borax. Left, cl = 6mm; right, cl = 7mm.

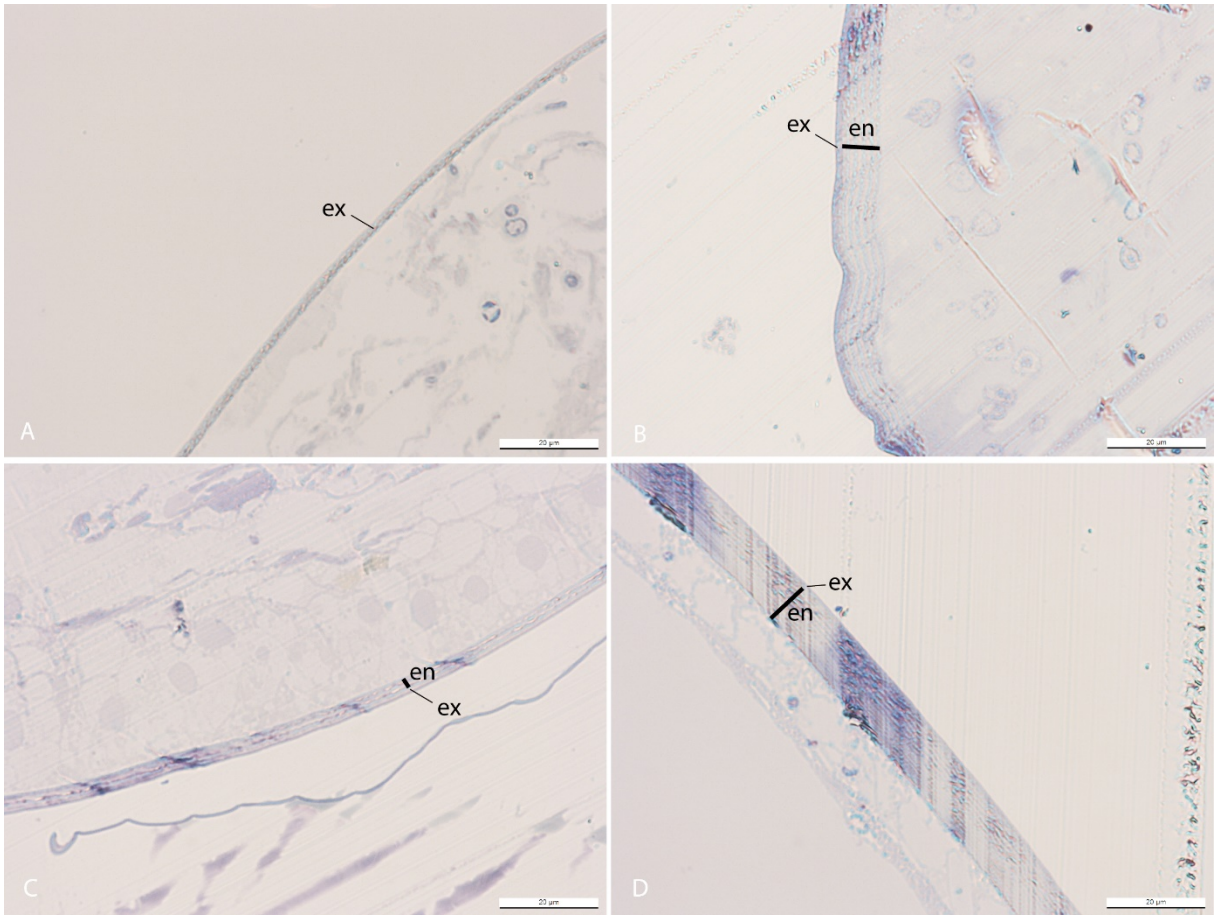


Figure 7.13 : Vues en microscopie photonique de coupes transversales de cuticule (exocuticule = ex ; endocuticule = en) de pédoncules oculaires provenant de spécimens en post-mue, colorées au bleu de toluidine + borax. A, cl = 5mm ; B, cl = 6mm ; C, cl = 8mm ; D, cl = 10mm.

Figure 7.13: Views in photonic microscope of cross sections of cuticle (exocuticle = ex; endocuticle = en) of eyestalks of specimens in post-molt colored with toluidin blue + borax. A, cl = 5mm; B, cl = 6mm; C, cl = 8mm; D, cl = 10mm.

Pour les spécimens en post-mue, l'aspect de la cuticule varie beaucoup selon les spécimens observés en fonction de la durée écoulée entre son exuviation et son prélèvement, difficilement contrôlable car l'exuviation advient généralement de nuit (Figure 7.13). Ainsi, on peut avoir une cuticule très mince lorsque l'exuviation vient d'avoir lieu (Figure 7.13A et B), plus épaisse mais encore souple car la minéralisation n'est pas achevée (Figure 7.13B) ou épaisse et minéralisée en fin de post-mue (Figure 7.13D).

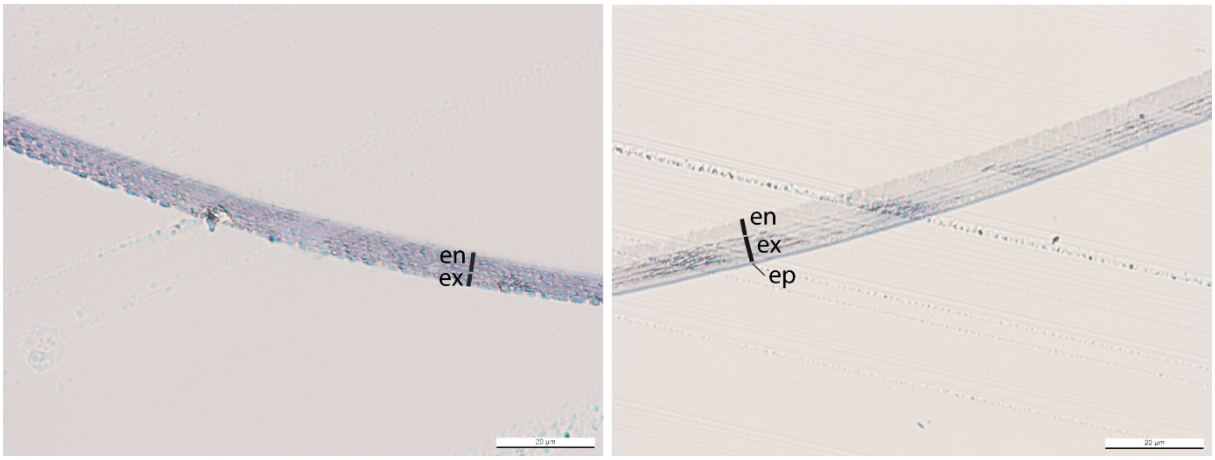


Figure 7.14 : Vues en microscopie photonique de coupes transversales de cuticule (épicuticule = ep ; exocuticule = ex ; endocuticule = en) de pédoncules oculaires provenant d'exuvies, colorées au bleu de toluidine + borax. Gauche, cl = 8mm ; droite, cl = 10mm.

Figure 7.14: Views in photonic microscope of cross section of cuticle (epicuticle = ep; exocuticle = ex; endocuticle = en) of eyestalks of exuviae colored with toluidin blue + borax. Left, cl = 8mm; right, cl = 10mm.

Pour les exuvies observées, la cuticule apparaît très mince et dégradée, avec une structuration peu marquée par la coloration (Figure 7.14).

Au cours de l'expérience, un des individus isolés est mort pendant l'exuviation. L'observation de sa cuticule a permis de nous éclairer sur les mécanismes de l'ecdysis chez *C. multidentata* (Figure 7.15).

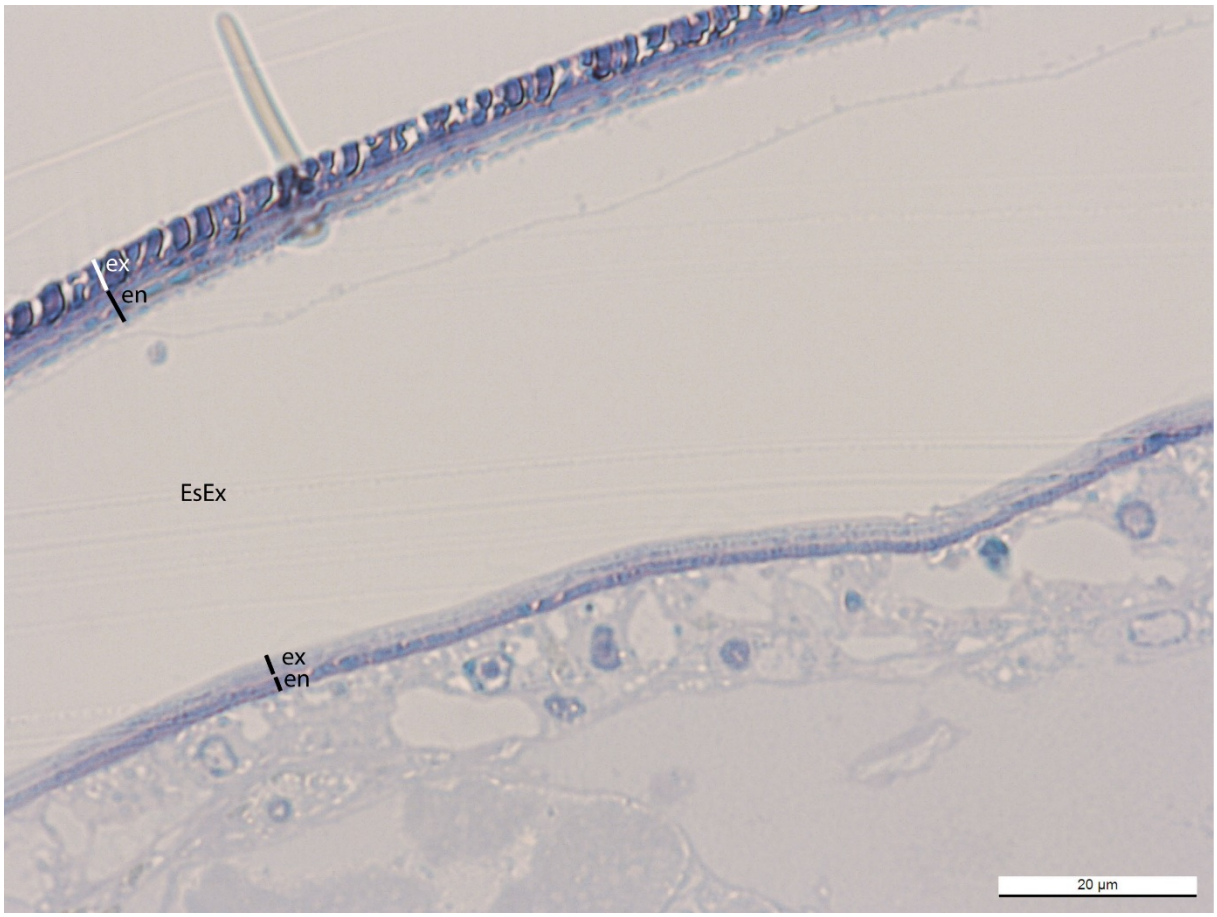


Figure 7.15 : Vue en microscopie photonique d'une coupe transversale de cuticule (exocuticule = ex ; endocuticule = en ; espace exuvial = EsEx) de pédoncule oculaire provenant d'un spécimen en cours d'exuviation (cl = 6mm), colorée au bleu de toluidine + borax.

Figure 7.15: View in photonic microscope of a cross section of cuticle (exocuticle = ex; endocuticle = en; exuvial space = EsEx) of an eyestalk of a specimen in ecdysis (cl = 6mm), colored with toluidin blue + borax.

On constate qu'au moment de l'exuviation, les couches supérieures de la nouvelle cuticule sont déjà formées, avec l'exocuticule visible (peu colorée) ainsi que les couches supérieures de l'endocuticule (aux nuances plus foncées). D'après cette observation, la conservation des couches inférieures de l'endocuticule suggérée par Kilada *et al.* (2012) semble impossible, la synthèse de la cuticule commençant par les couches externes. Il serait en revanche possible que le calcium provenant de l'ancienne cuticule ne soit remobilisé dans la nouvelle qu'après l'exuviation, ce qui expliquerait la localisation de la fluorescence dans les couches inférieures dans les résultats de Kilada *et al.* (2012). Les stries observées dans l'endocuticule à différents stades ne semblent pas être des stries de croissance. Il s'agit plutôt des couches chitino-protéiques qui ont un arrangement dit « en contreplaqué » (Plywood structures) ou de Bouligand (Bouligand, 1972) en rotation de 45° les unes par rapport aux autres, donnant une alternance de couches sombres et de couches claires vues en coupe perpendiculaire au plan des fibres.

Afin de vérifier les observations faites en microscopie photonique, des coupes semi-fines ont été préparées pour chaque étape du cycle de mue pour être observées au microscope électronique à balayage (MEB) en imagerie en électrons rétrodiffusés (Backscattered electrons : BSE). Cette méthode permet d'observer à la fois le relief et l'épaisseur de l'objet étudié et permet de contraster des zones de compositions chimiques différentes (Goldstein *et al.*, 1981). Avant observation, les coupes ont été montées sur des lamelles de verre rondes de 12mm de diamètre et immergées dans une solution d'acétate d'uranyle (2% dans de l'éthanol 50%) pendant 10 minutes. Les observations ont été réalisées grâce au MEB Hitachi SU3500 de la Plateforme de Microscopie Electronique et de Microanalyses du MNHN.

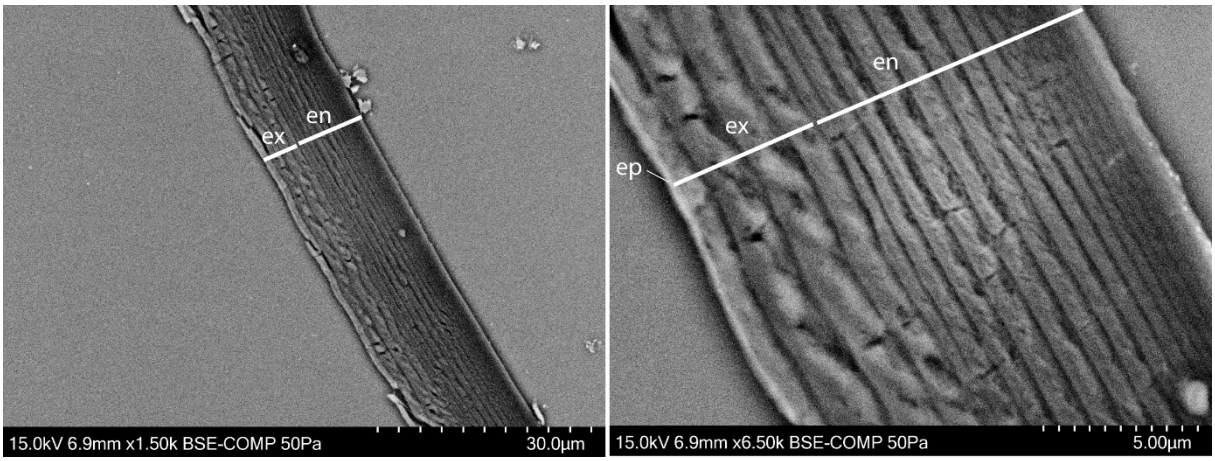


Figure 7.16 : Vues en BSE d'une coupe transversale de cuticule (épicuticule = ep ; exocuticule = ex ; endocuticule = en) de pédoncule oculaire provenant d'un spécimen en inter-mue (cl = 8mm) à différents grossissements.

Figure 7.16: Views in BSE of a cross section of cutcle (epicuticle = ep; exocuticle = ex; endocuticle = en) of an eyestalk of a specimen in inter-molt (cl = 8mm) at different magnifications.

Pour les spécimens en inter-mue, on distingue clairement les couches chitino-protéiques qui sont de plus en plus fines de l'extérieur vers l'intérieur. Il semble y avoir une discontinuité de tailles de couches entre l'exocuticule et l'endocuticule (Figure 7.16).

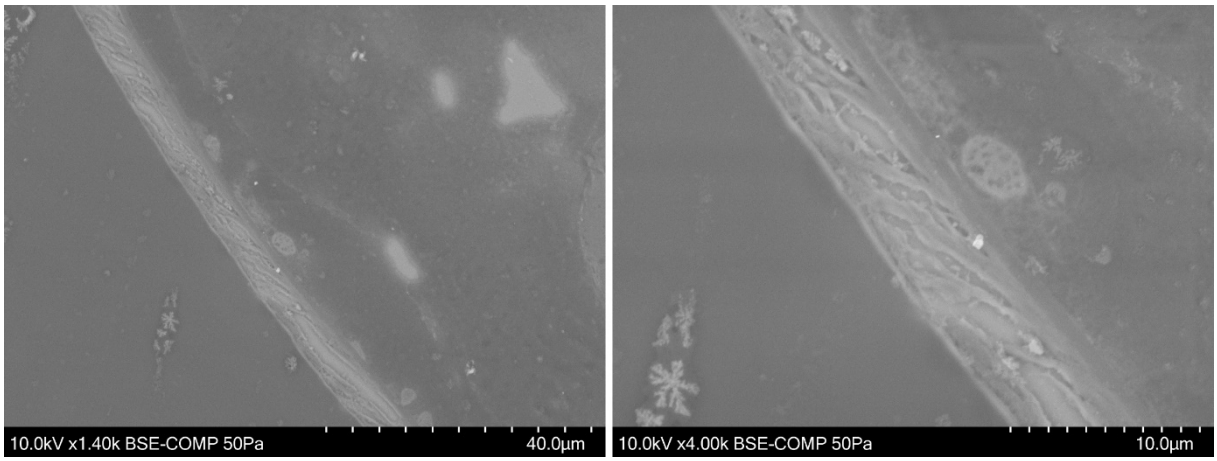


Figure 7.17 : Vues en BSE d'une coupe transversale de cuticule de pédoncule oculaire provenant d'un spécimen en pré-mue (cl = 6mm) à différents grossissements.

Figure 7.17: Views in BSE of a cross section of cuticle of an eyestalk of a specimen in pre-molt (cl = 6mm) at different magnifications.

Pour les spécimens observés en pré-mue, la cuticule apparaît amincie et dégradée (Figure 7.17).

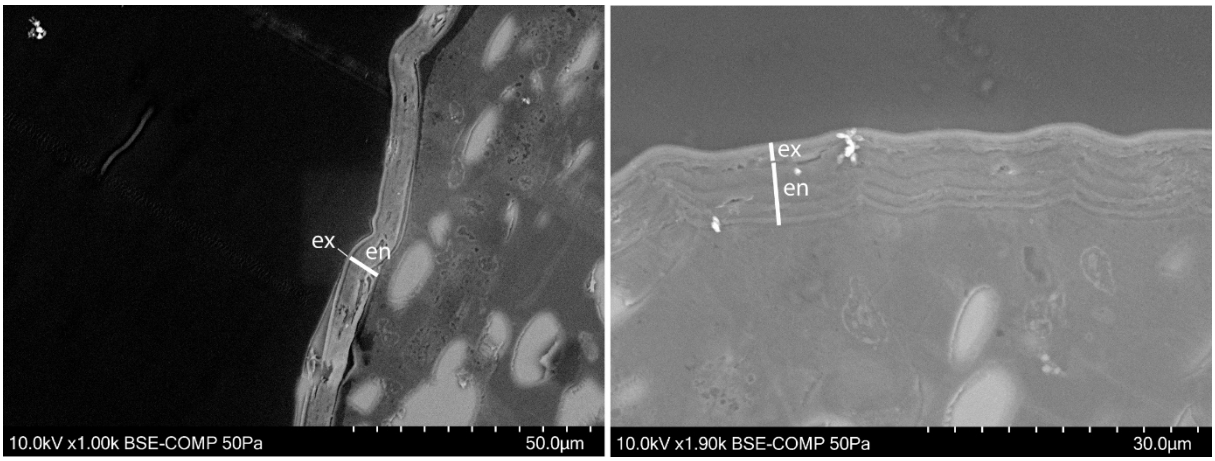


Figure 7.18 : Vues en BSE d'une coupe transversale de cuticule (exocuticule = ex ; endocuticule = en) de pédoncule oculaire provenant d'un spécimen en post-mue (cl = 6mm) à différents endroits.

Figure 7.18: Views in BSE of a cross section of cuticle (exocuticle = ex; endocuticle = en) of an eyestalk of a specimen in post-molt (cl = 6mm) at different locations.

En post-mue, l'endocuticule n'est pas encore complètement synthétisée et minéralisée, la cuticule est toujours souple (Figure 7.18).

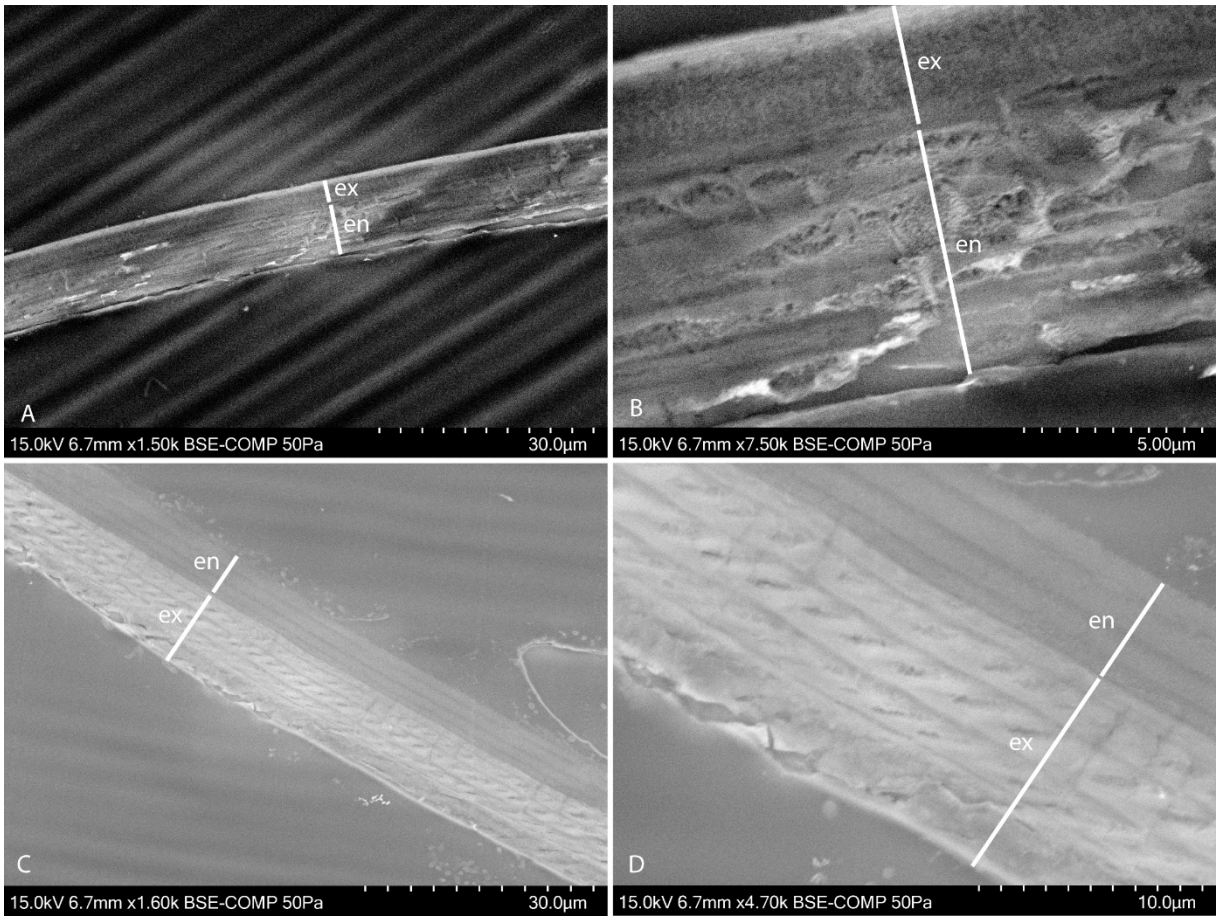


Figure 7.19 : Vues en BSE d'une coupe transversale de cuticule (exocuticule = ex ; endocuticule = en) de pédoncules oculaires provenant de deux exuvies. A et B : cl = 8mm (cf. Figure 7.14 gauche) ; C et D : cl = 10 mm (cf. Figure 7.14 droite).

Figure 7.19: Views in BSE of a cross section of cuticle (exocuticle = ex; endocuticle = en) of eyestalks of two exuviae. A and B: cl = 8mm (cf. Figure 7.14 left) ; C and D: cl = 10 mm (cf. Figure 7.14 right).

Pour les exuvies observées, on constate qu'une endocuticule dégradée, réduite à ses couches supérieures demeure (Figure 7.19).

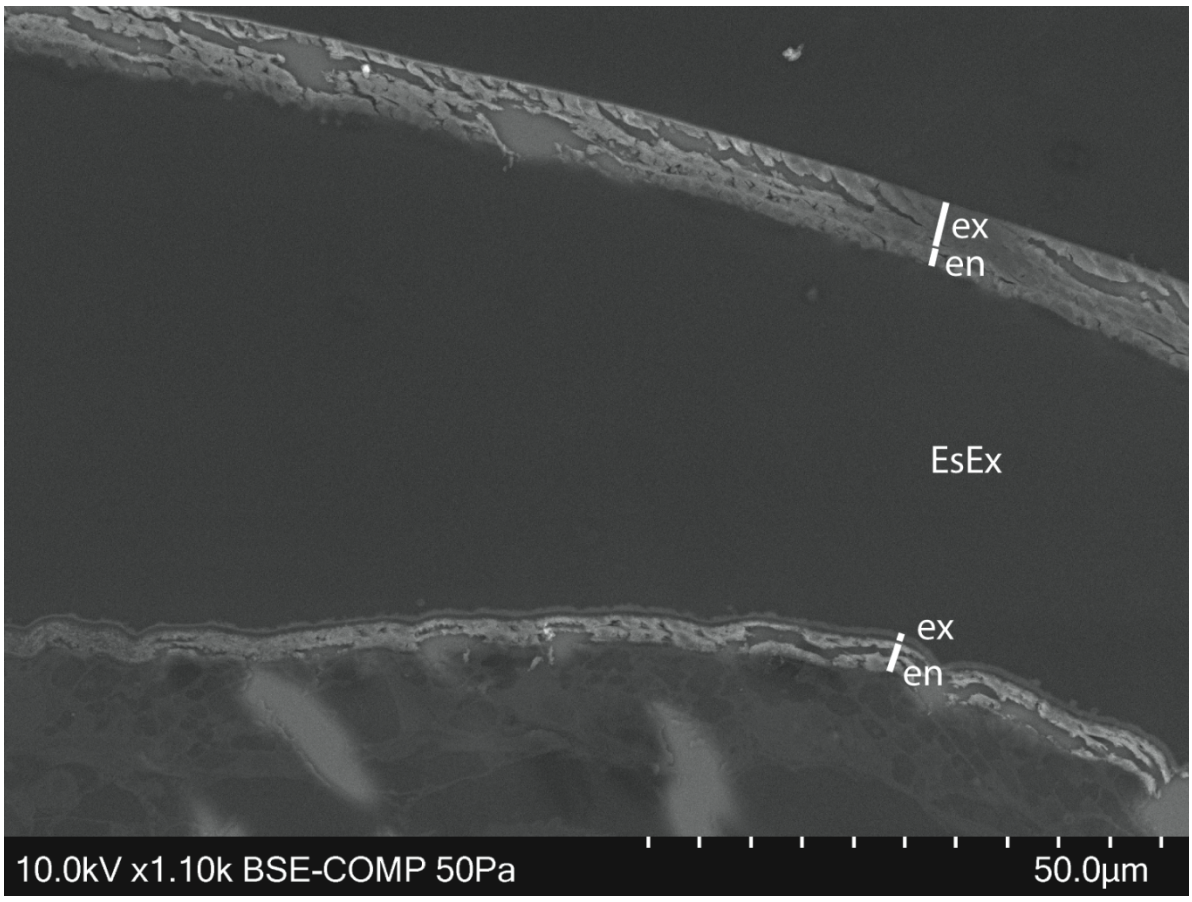


Figure 7.20 : Vue en BSE d'une coupe transversale de cuticule (exocuticule = ex ; endocuticule = en) de pédoncule oculaire provenant d'un spécimen en cours d'exuviation (cl = 6mm, cf. Figure 7.15).

Figure 7.20: Views in BSE of a cross section of cuticle (exocuticle = ex; endocuticle = en) of an eyestalk of a specimen in ecdysis (cl = 6mm, cf. Figure 7.15).

Le spécimen en cours d'exuviation observé précédemment a également été observé en BSE (Figure 7.20).

Les observations en BSE confirment donc celles faites en microscopie photonique.

Afin de s'assurer que les structures observées correspondent bien aux différentes couches de la cuticule, l'ultrastructure de la cuticule de spécimens en inter-mue a été observée en microscopie électronique à transmission.

7.3 Etude de la cuticule en inter-mue

7.3.1 Observations de l'ultrastructure au MET

Des coupes ultra-fines (80nm) de cuticule de pédoncules oculaires de spécimens en inter-mue ont été préparées et déposées sur des grilles de cuivre recouvertes d'une fine couche de carbone et plongées dans une solution d'acétate d'uranyle (2% dans de l'éthanol 50%) pendant 10 minutes. Les observations ont été réalisées soit sur le MET Hitachi H7100 soit sur le Hitachi H7700 (suivant les disponibilités), tous les deux à la Plateforme de Microscopie Electronique et de Microanalyses du MNHN.

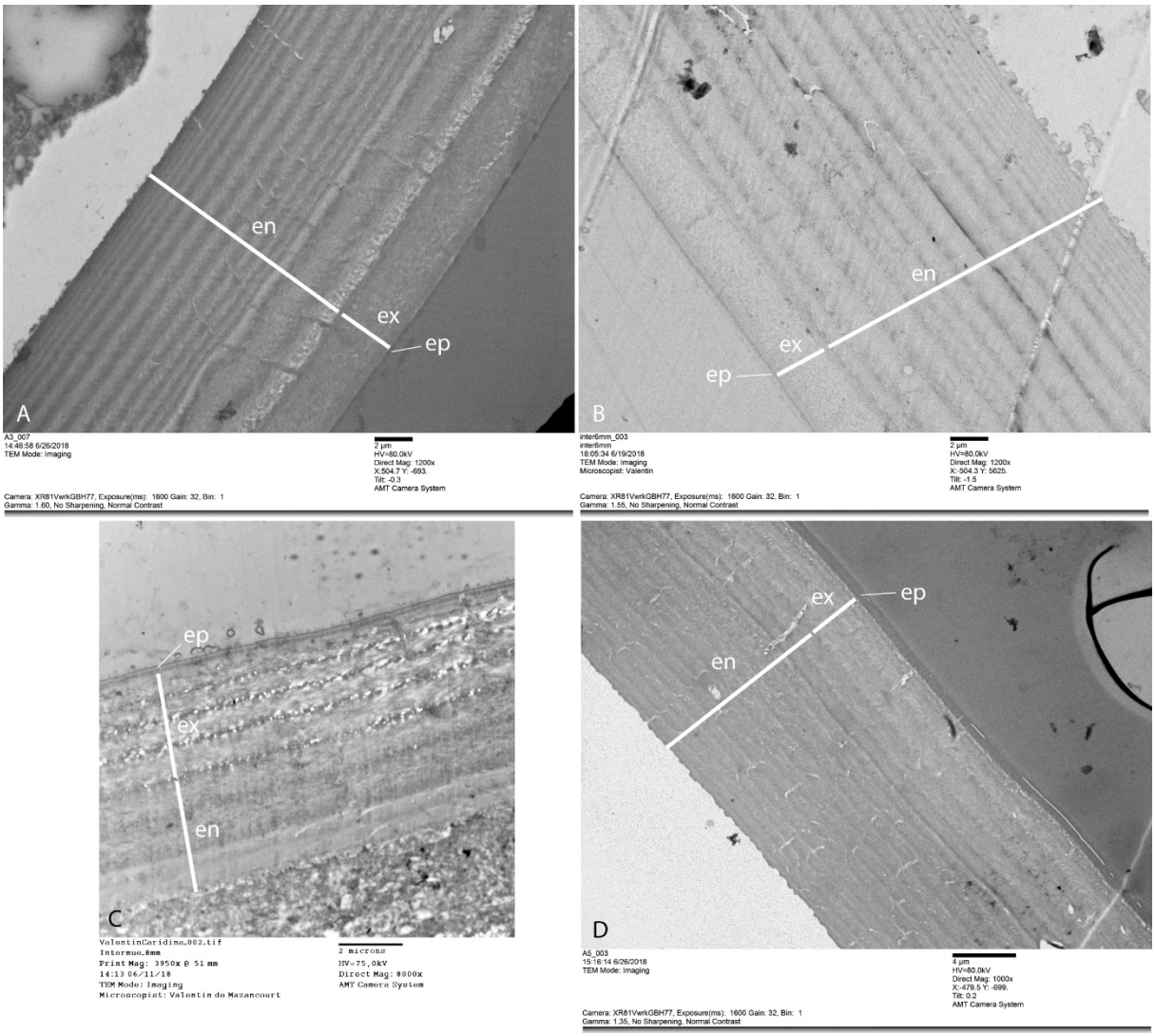


Figure 7.21 : Vues en MET de coupes transversales de cuticule (épicuticule = ep ; exocuticule = ex ; endocuticule = en) de pédoncules oculaires d'individus en inter-mue. A : cl = 5mm (cf. Figure 7.11A) ; B : cl = 6mm (cf. Figure 7.11B) ; C : cl = 8mm (cf. Figure 7.16) ; D : cl = 11mm (cf. Figure 7.11D).

Figure 7.21: Views in TEM of cross sections of cuticle (epicuticle = ep; exocuticle = ex; endocuticle = en) of eyestalks of specimens in inter-molt. A: cl = 5mm (cf. Figure 7.11A); B: cl = 6mm (cf. Figure 7.11B); C: cl = 8mm (cf. Figure 7.16); D: cl = 11mm (cf. Figure 7.11D).

On observe une différence structurale entre l'exocuticule et l'endocuticule (Figure 7.21), avec une endocuticule représentant 70 à 85% de l'épaisseur totale de la cuticule, sauf pour un individu (Figure 7.21C) où elle ne représente que 50% de l'épaisseur, laissant supposer une synthèse incomplète, le spécimen ayant probablement été prélevé peu de temps après la mue. A cette échelle, l'épicuticule est visible, plus sombre que l'exocuticule (Figure 7.22). Dans l'endocuticule mais aussi parfois dans l'exocuticule (Figure 7.21C et D), les stries correspondant aux couches chitino-protéiques en contreplaqué hélicoïdal sont apparentes et de taille irrégulière, semblant cependant plus rapprochées dans les couches inférieures que dans les couches supérieures. Une vue en coupe légèrement oblique par rapport à la perpendiculaire du plan des couches permet de voir l'organisation « en croissant » où la période entre deux stries sombres correspond à une rotation de 180°, soit 5 couches chitino-protéiques pivotant chacune de 45° par rapport à la précédente. Les stries sombres correspondent donc aux couches dont les fibres sont orientées perpendiculairement au plan de coupe et les stries claires correspondent au 3 couches où les fibres sont orientées parallèlement ou à 45° par rapport au plan de coupe (Figure 7.23).

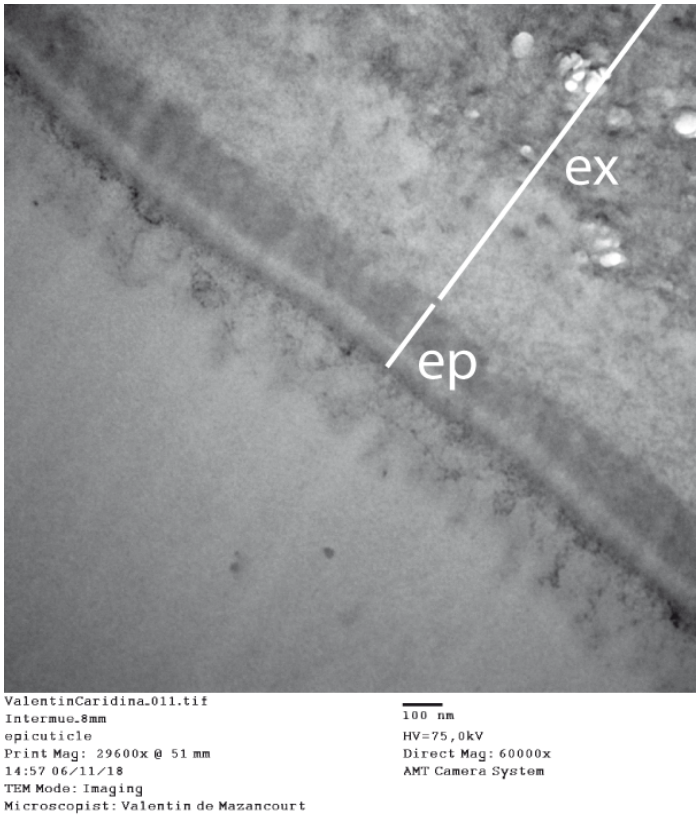


Figure 7.22 : Vue en MET d'une coupe transversale de cuticule de pédoncule oculaire d'un individu en inter-mue (cl = 8mm), détail de l'épicuticule (ep ; ex = exocuticule).

Figure 7.22: Views in TEM of a cross section of cuticle of an eyestalk of a specimen in inter-molt (cl = 8mm), detail of the epicuticle (ep; ex = exocuticule).

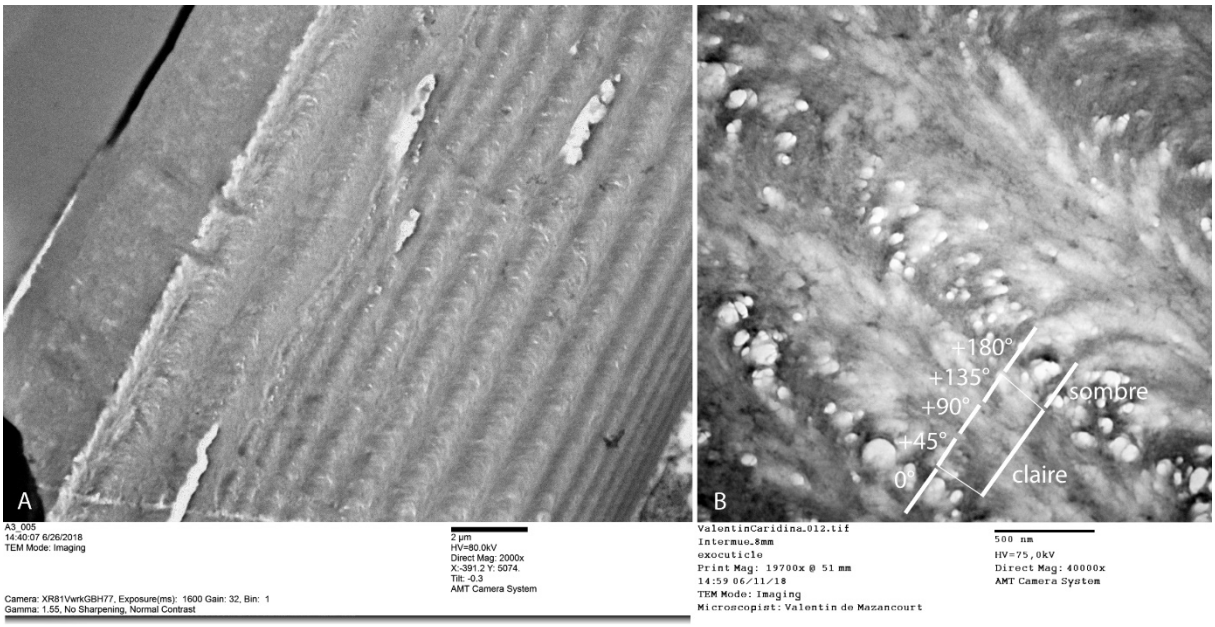


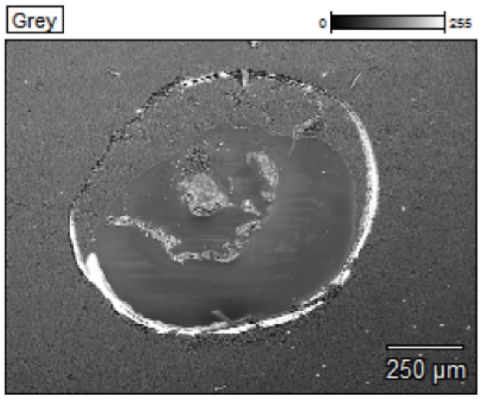
Figure 7.23 : Vue en MET de coupes légèrement obliques de cuticules de pédoncules oculaires d'individus en inter-mue (A : cl = 5mm ; B : cl = 8mm), détail des structures « en croissant » des fibres agencées en contreplaqué hélicoïdal.

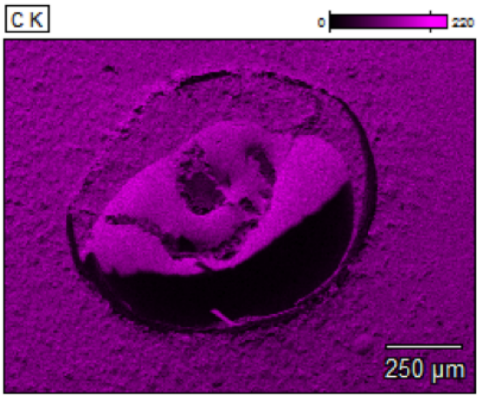
Figure 7.23: Views in TEM of slightly oblique sections of cuticle of eyestalks of specimens in inter-molt (A: cl = 5mm; B: cl = 8mm), detail of the "crescent" structures of fibers arranged in twisted plywood.

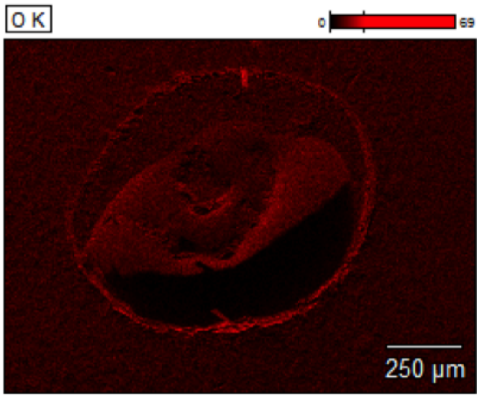
7.3.2 Etude de la composition chimique de la cuticule

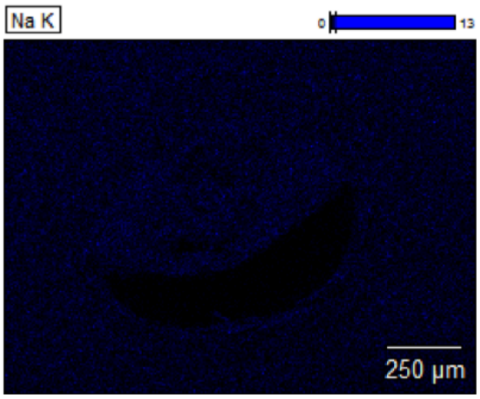
Afin d'obtenir plus d'informations sur la cuticule de *C. multidentata*, nous avons réalisé des analyses élémentaires en complément de l'étude de l'ultrastructure.

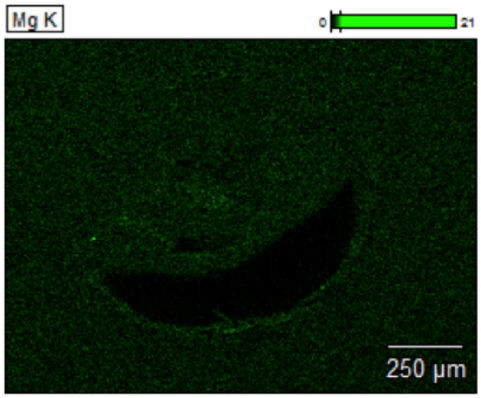
Des coupes de pédoncules oculaires inclus dans de la résine Epoxy ont été préparées pour une analyse élémentaire globale de la cuticule en MEB-EDX (Energie Dispersive de rayons X) grâce à une sonde montée sur le MEB Hitachi SU3500 de la Plateforme de Microscopie Electronique et de Microanalyses du MNHN.

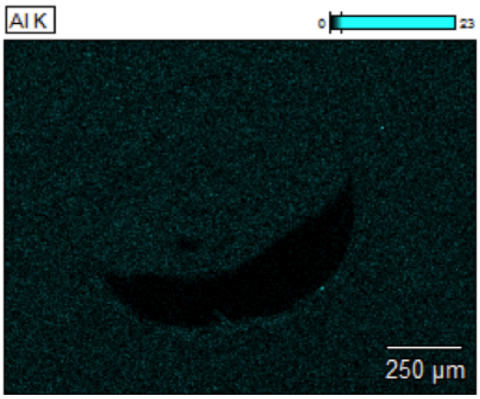

Grey
0
255
250 µm

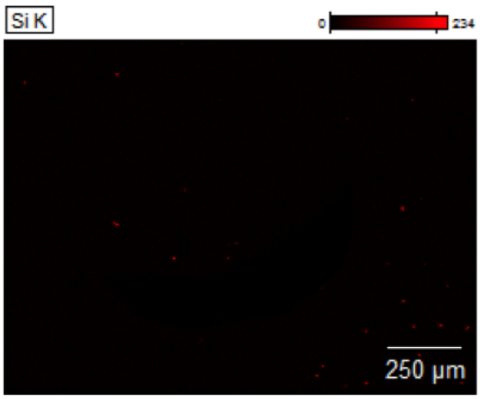

C K
0
220
250 µm

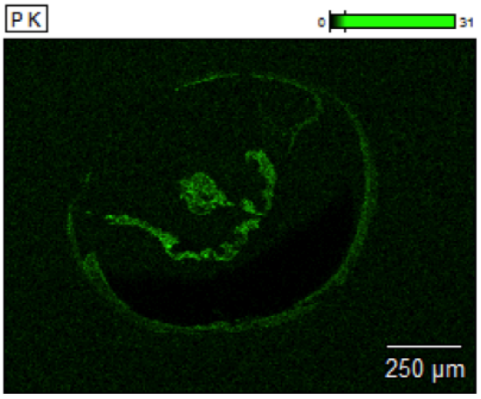

O K
0
69
250 µm


Na K
0
13
250 µm


Mg K
0
21
250 µm


Al K
0
23
250 µm


Si K
0
234
250 µm


P K
0
31
250 µm

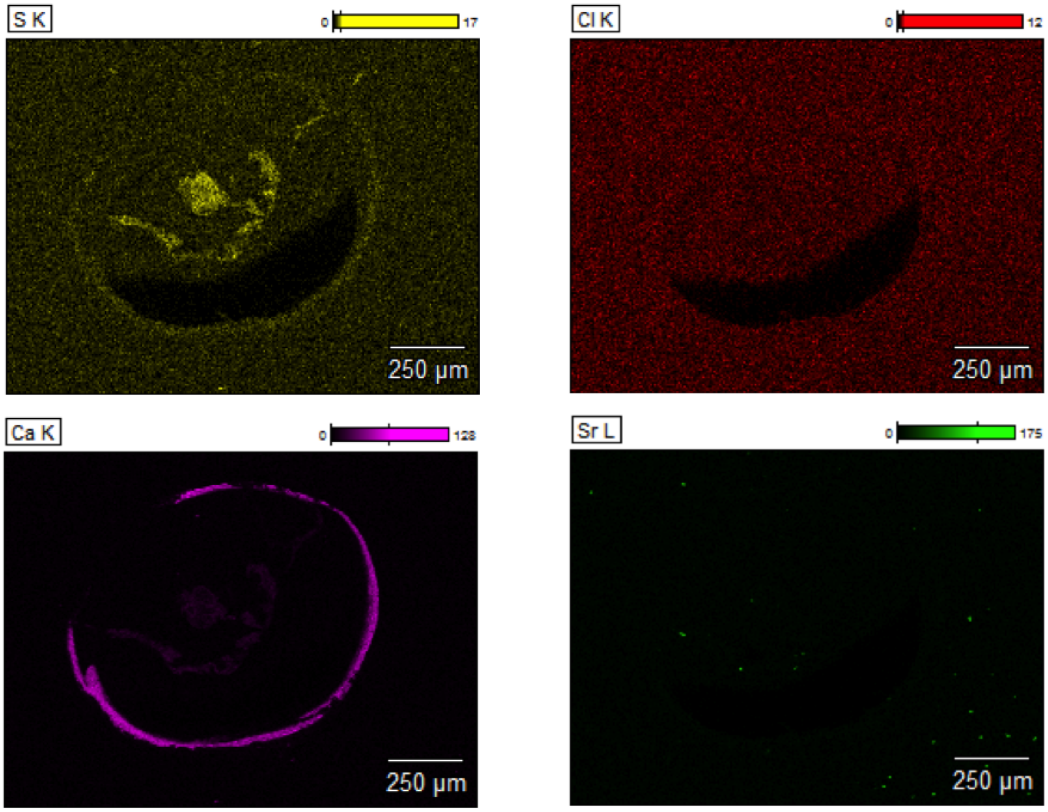


Figure 7.24 : (Et page précédente) Images spectrales en MEB EDX d'une coupe transversale de pédoncule avec la distribution des éléments pour le carbone (C), l'oxygène (O), le sodium (Na), le magnésium (Mg), l'aluminium (Al), le silicium (Si), le phosphore (P), le soufre (S), le chlore (Cl), le calcium (Ca), le strontium (Sr) et une vue en MEB (grey).

Figure 7.24: (And previous page) Spectral images of SEM EDX of a cross section of eyestalk with the elements distribution for carbon (C), oxygen (O), sodium (Na), magnesium (Mg), aluminium (Al), silicon (Si), phosphorus (P), sulfur (S), chlorine (Cl), calcium (Ca), strontium (Sr) and a view in SEM (grey).

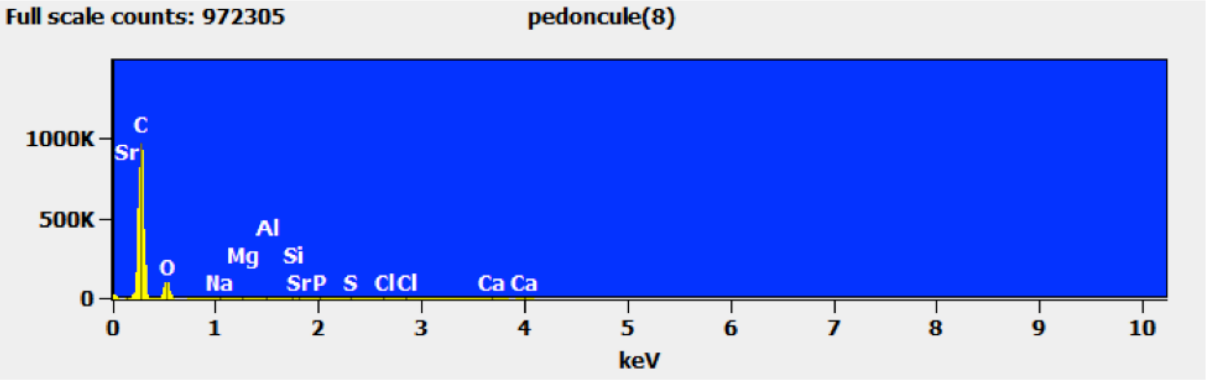


Figure 7.25 : Spectre EDX de la composition élémentaire calculé depuis les images élémentaires obtenues précédemment (cf. Figure 7.24).

Figure 7.25: EDX spectrum of the element composition calculated from the images obtained previously (cf. Figure 7.24).

Afin d'obtenir une meilleure résolution, et de réduire le bruit causé par les éléments contenus dans la résine, des analyses ont été faites à une échelle plus réduite, centrée sur l'épaisseur de la cuticule (Figure 7.26).

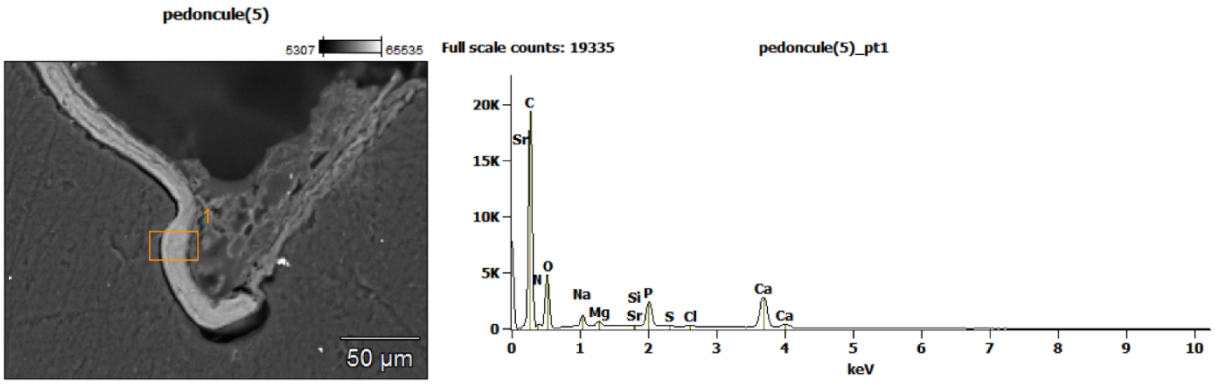


Figure 7.26 : Vue en MEB et spectre EDX de la région encadrée en orange d'une coupe longitudinale de cuticule d'un pédoncule oculaire.

Figure 7.26: Views in SEM and EDX spectrum of the region framed in orange in a longitudinal section of the cuticle of an eyestalk.

Les analyses montrent la présence dans la cuticule d'une grande quantité de carbone, d'oxygène, de phosphore et de calcium, ainsi que, dans une moindre mesure, de soufre de magnésium et de sodium (Figures 7.24, 7.25 et 7.26). D'après les analyses, le carbone est l'élément principal de la composition de la cuticule, suivi par l'oxygène, ce qui indique qu'elle est principalement minéralisée par du carbonate de calcium ($CaCO_3$). La présence de phosphate en quantité relativement importante pourrait également laisser supposer qu'une partie de la minéralisation est due à du phosphate de calcium, et à la présence de phospholipides (Travis, 1963). Il a été montré que sa présence sert à stabiliser le carbonate de calcium amorphe, évitant la nucléation et la formation de cristaux de calcite (Seidl *et al.*, 2011).

7.4 Conclusions et perspectives

Après les différentes expériences menées au cours de ces travaux, **il semble que les techniques de sclérochronologie utilisées sur les otolithes des poissons ou les opercules des gastéropodes pour étudier leur amphidromie ne soient pas applicables sur la cuticule des pédoncules oculaires des caridines.**

En effet, les différentes expériences de marquages vitaux ont montré que l'endocuticule n'était pas conservée d'une mue à l'autre, et que la rétention de la coloration observée dans toute la cuticule ou seulement sa partie inférieure (Kilada *et al.*, 2012) pourrait être causée par un stockage du calcium pendant la mue qui serait remobilisé par la suite. En effet, les caridines vivant en eau douce dans des environnements parfois très pauvres en minéraux (comme les rivières à substrat basaltique des îles volcaniques, ou à substrat granitique des îles continentales), il est

très probable que des mécanismes de recyclage du calcium aient évolué comme cela a pu être observé chez d'autres espèces de crustacés d'eau douce ou terrestres (Greenaway, 1985).

De même, l'observation de la structure de la cuticule à différents stades du cycle de mue permet d'affirmer que **l'endocuticule n'est pas conservée**, puisque seule l'épicuticule et l'exocuticule sont présentes au moment de l'exuvie (cf. Figures 7.15 et 7.20).

Les stries interprétées par Kilada *et al.* (2012) comme des stries de croissance sont en réalité les empilements des couches de fibres chitino-protéiques agencées à 45° les unes par rapport aux autres dans une structure dite « en contreplaqué hélicoïdal » (Figure 7.27). Leur nombre ne devrait, a priori, pas dépendre de l'âge ou du nombre de mues de l'animal, sauf si l'épaisseur de la cuticule est corrélée à la taille de l'animal et donc, supposément, à son âge, ce qui ne semble pas être le cas chez *C. multidentata* (cf. Figure 7.11). De plus, les espèces étudiées par Kilada *et al.* (2012) proviennent de zones tempérées avec une saisonnalité marquée, ce qui expliquerait des ralentissements de croissance et l'existence de stries, ce qui n'est pas le cas des caridines qui vivent en milieu tropical et connaissent peu de variations saisonnières. La détermination de l'âge par comptage de stries dans l'endocuticule des pédoncules oculaires semble donc inapplicable chez cette espèce.

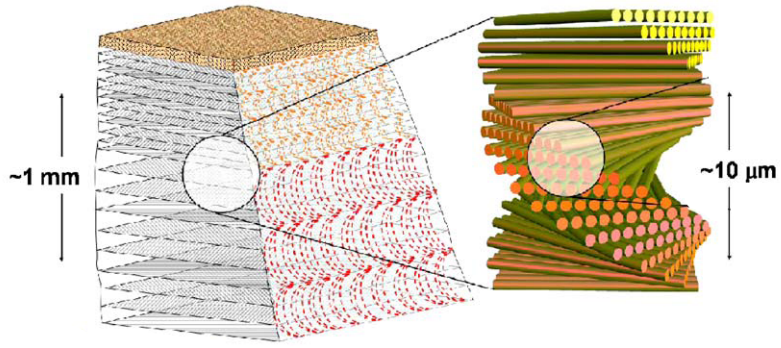


Figure 7.27 : Représentation schématique de l'arrangement en contreplaqué hélicoïdal des fibres chitino-protéiques de la cuticule de homard (modifié d'après Raabe *et al.* 2005)

Figure 7.27: Schematic representation of the twisted plywood organisation of the chitino-proteic fibers of a lobster cuticle (modified from Raabe *et al.* 2005)

Compte tenu de nos résultats, l'étude sclérochronologique n'a pas pu être réalisée complètement, mais de nouvelles données sur la cuticule de *C. multidentata* ont été obtenues.

Grâce à l'étude structurale, on sait que l'épaisseur de la cuticule en inter-mue est constituée de 70 à 85% d'endocuticule, de 10 à 25% d'exocuticule et 1 à 5% d'épicuticule, ces proportions restant relativement constantes quelle que soit la taille de l'individu étudié. La couche membraneuse semble absente chez notre modèle, du moins dans cette partie du corps, ce qui est en accord avec les résultats de Voss-Foucart & Jeuniaux (1978) selon lesquels cette couche n'est présente que chez les gros décapodes à cuticule fortement minéralisée. Les couches chitino-protéiques sont présentes dans l'endocuticule (et se distinguent difficilement de celles de l'exocuticule) et sont de taille irrégulière, mesurant jusqu'à 1µm au contact avec l'exocuticule ou 0,1µm pour les couches les plus internes. L'exocuticule peut contenir une ou plusieurs couches d'épaisseur plus constante et toujours supérieure à la plus épaisse des couches de l'endocuticule. Cette structuration de la cuticule est caractéristique des espèces nectobenthiques (c'est-à-dire pouvant se déplacer sur le fond comme dans la colonne d'eau) étudiées par Pütz & Buchholz (1991), dont notamment la crevette *Crangon crangon*. D'après ces mêmes travaux, une plus grande épaisseur des couches chitino-protéiques permet une plus forte minéralisation, ainsi, le type nectobenthique d'organisation de la cuticule est un compromis entre les cuticules de type benthique, fortement minéralisées et avec des couches épaisses et les cuticules de type pélagique, très peu minéralisées et avec des couches très fines et rapprochées. De fait, les caridines partagent bien leur temps entre la recherche de nourriture sur le substrat de la rivière et le déplacement en pleine eau (Obs. pers.), ce qui correspond à un mode de vie nectobenthique. **Sachant que des différences de mode de vie existent entre les espèces de caridines, il pourrait être intéressant de vérifier si l'organisation de la cuticule diffère entre les espèces plus benthiques et vivant dans un fort courant, comme celles du complexe *C. weberi* et celles plus pélagiques et vivant dans des zones où le courant est faible, comme celles du complexe *C. nilotica*.**

Une incertitude demeure cependant quant à l'interprétation de certaines images et à la délimitation entre l'exocuticule et l'endocuticule. Des colorants fluorescents spécifiques comme l'acridine orange ou le bleu alcyan permettraient de visualiser plus

clairement la frontière entre ces deux couches et de confirmer les différences observées (différence de coloration en microscopie optique et différences dans l'agencement des couches chitino-protéiques en microscopie électronique).

L'étude élémentaire de la cuticule de *C. multidentata* a permis de donner un aperçu global préliminaire de la composition de la cuticule et les proportions de chaque élément. Elle est composée principalement de carbone, combiné à de l'oxygène sous forme de carbonates et de protéines comme la chitine, de calcium sous forme de carbonate de calcium (soit en tant que cristaux de calcite soit amorphe) ou de phosphate de calcium, potentiellement de phospholipides, et d'autres éléments moins abondants comme le magnésium, le soufre ou le sodium. Cette composition globale est en accord avec différentes analyses effectuées en EDX sur la cuticule d'autres crustacés (Hild *et al.*, 2009 ; Seidl *et al.*, 2011). **Des analyses plus poussées à une échelle plus réduite mériteraient d'être réalisées afin d'étudier la distribution spatiale des divers éléments entre les couches de la cuticule pour en apprendre davantage sur leur degré de minéralisation respectif.**

ABSTRACT

After performing live staining experiments on specimens of *C. multidentata* kept in aquaria and observing cross sections of their eyestalks, we could not reproduce the results of Kilada *et al.* (2012) in finding a retention of the colorant in the lower parts of the endocuticle. We found that there was indeed a retention of the colorant but it was diffuse in all the thickness of the cuticle, suggesting that the calcium is resorbed and stored before the molt, and used again when mineralising the new cuticle in post-molt.

In order to see if any part of the eyestalk cuticle is conserved through the molts, we examined the ultrastructure of the cuticle at different stages of the molt cycle in photonic microscopy and back-scattered electron microscopy (BSE). The observation of the cuticle of a specimen in ecdysis demonstrated that no part of the old cuticle is conserved, since the epicuticle, the exocuticle and the upper parts of the endocuticle are already present when the old cuticle is shed.

As no sclerochronological analysis could be made on this structure, we decided to study the structure and the composition of the cuticle of *C. multidentata*. We observed in transmission electron microscopy (TEM) the ultrastructure of the cuticle of specimens in inter-molt and found that the cuticle was organised in 70-85% endocuticle, 10-25% exocuticle and 1-5% epicuticule, a structure consistent with that found in other nektobenthic crustaceans. As there are differences in lifestyle between the species of *Caridina*, it would be interesting to compare with the structure of the cuticle of a more pelagic species like those of the *C. nilotica* complex.

The striae interpreted by Kilada *et al.* (2012) as growth bands are visible in the cuticle but correspond to the twisted plywood organisation of the chitino-proteic fibers, whose number correlates more to the thickness of the cuticle than to the age of the shrimp, these two being apparently uncorrelated in this species.

To study the chemical composition of the cuticle, we performed energy-dispersive X-ray (EDX) element analysis on cross sections of eyestalk cuticle, showing that it was mainly composed of carbon, in the form of proteins and carbonates, of calcium and of phosphorus, and to a lesser extent, of magnesium, sodium and sulfur, agreeing with the composition of the cuticle of other crustaceans studied by previous authors. Performing such analyses at a finer scale would allow to study the chemical differences between the layers of the cuticle.

Chapitre septième,
Bilan et conclusions
de l'étude.

Synthesis and conclusions.

Où l'on dresse le bilan des travaux effectués dans la présente étude, des éléments nouveaux qui en résultent et des conclusions et perspectives que l'on peut en tirer sur la biologie et l'évolution des espèces des complexes C. nilotica *et* C. weberi*.*

Where we review the results obtained in the present study, the new elements that they brought and the conclusions and prospects that we can learn from it on the biology and evolution of the species of the C. nilotica *and* C. weberi *complexes.*

8. Chapitre 7 : Bilan et conclusions de l'étude

8.1 Résultats

L'étude de taxonomie intégrative a permis de résoudre une partie de la complexité des complexes *C. nilotica* et *C. weberi* en permettant de choisir des caractères morphologiques plus informatifs que ceux habituellement utilisés et en caractérisant chaque espèce par une séquence ADN.

8.1.1 Etude moléculaire

Au total, sur les 1151 spécimens récents étudiés génétiquement, **1682 séquences ont été obtenues pour 92 espèces** et déposées dans la base BOLD (http://www.boldsystems.org/) (Ratnasingham & Hebert, 2007) sous le projet CARIT (CARidina Integrative Taxonomy) :

- **890 séquences de 16S**
- **573 séquences de COI** (correspondant à 68 « BINs »)
- **116 de H3**
- **103 de 28S**

A ces chiffres s'ajoutent les génomes mitochondriaux dont **32 ont pu être obtenus complets et 97 partiels, représentant respectivement 21 et 45 espèces**.

Pour les spécimens anciens, des séquences courtes du marqueur 16S ont pu être **obtenues pour 33 spécimens**, dont notamment des spécimens types de *C. coulaborensis*, *C. fritzi, C. gracilipes* et *C. mertoni*, ce qui a permis de clarifier et stabiliser la systématique de ces espèces.

8.1.2 Liste des espèces

Notre étude a permis de réviser la liste des espèces présentes dans les zones prospectées, pour les deux complexes d'espèces mais également pour les autres espèces du genre *Caridina*. Nous présentons ci-après la liste ré-actualisée de ces espèces (Tableau 8.1). **Cette liste comprend 43 espèces nouvelles par rapport à la liste présentée en introduction de ce mémoire, dont 5 ont été décrites dans ce travail.**

Tableau 8.1 : Liste réactualisée des espèces de caridines dans les localités étudiées au cours du présent travail. Les noms en rouge correspondent aux espèces validées par notre travail en taxonomie intégrative. Une astérisque (*) indique l'appartenance au complexe *C. nilotica* redéfini et deux (**) indiquent l'appartenance au complexe *C. weberi* redéfini.

Table 8.1 : Updated list of *Caridina* species in localities studied in the present work. Names in red indicate species validated by our work in integrative taxonomy. One asterisk (*) indicates a species belonging to the redefined *C. nilotica* complex, and two (**) indicate a species belonging to the redefined *C. weberi* complex.

OCEAN INDIEN OCCIDENTAL					
Afrique du Sud	**Comores et Mayotte**	**Madagascar**	**Maurice**	**Réunion**	**Seychelles**
*Caridina africana**	*C. natalensis**	*C. angulata*	*C. mauritii*	*C. natalensis**	*C. natalensis**
C. belazoniensis	*C. cf. serratirostris*	*C. brevirostris brevipes*	*C. natalensis**	*C. cf. serratirostris*	*C. cf. serratirostris 2*
*C. natalensis**	*C. typus*	*C. calmani*	*C. richtersi*	*C. typus*	*C. similis**
C. cf. serratirostris		*C. crurispinata*	*C. cf. serratirostris*		*C. typus*
C. togoensis		*C. edulis*	*C. spathulirostris*		
C. typus		*C. hova*	*C. typus*		
		C. isaloensis grandidieri			
		C. isaloensis isaloensis			
		C. lamiana			
		C. lipalmaria			
		*C. cf. multidentata***			
		*C. natalensis**			
		*C. cf. nilotica**			
		C. norvestica			
		C. parvocula			
		C. petiti			
		C. cf. serratirostris			
		C. steineri			
		C. troglophila			
		C. typus			
		C. unca			
		*C. xiphias**			

OCEAN INDIEN ORIENTAL

Sri Lanka

C. costai

C. fernandoi

*C. gracilipes**

C. gracilirostris

C. pristis cruszi

C. pristis pristis

C. propinqua

*C. simoni**

C. typus

*C. zeylanica**

INSULINDE

Indonésie

Bornéo

C. bruneiana

*C. gracilipes**

Lombok

*C. gracilipes**

C. typus

Malaisie

Pulau Tioman

C. celebensis

*C. elongapoda**

C. typus

MELANESIE

Fidji

C. brevicarpalis

*C. brevidactyla**

C. devaneyi

C. fijiana

C. nudirostris

C. cf. serratirostris 1

C. typus

*C. vitiensis**

*C. tupaia***

Nouvelle Calédonie

*C. brevidactyla**

*C. longicarpus***

*C. meridionalis**

*C. novaecaledoniae**

C. rubella

C. cf. serratirostris 3

*C. tupaia***

Nouvelle Guinée

C. buergersi

*C. brevidactyla**

C. cf. brevicarpalis 1

C. cognata

C. demani

C. elisabethae

C. fecunda

*C. gracilipes**

Salomon

*C. appendiculata**

C. cf. brevicarpalis 3

*C. brevidactyla**

*C. buehleri***

C. celebensis

C. gracilirostris

*C. cf. jeani 1***

*C. mertoni**

Vanuatu

*C. appendiculata**

C. cf. brevicarpalis 2

*C. buehleri***

C. celebensis

*C. cf. jeani 1***

*C. cf. jeani 2***

*C. cf. jeani 3***

	C. typus *C. vitiensis**	*C. cf. gracilirostris* *C. sp. NTnilotica** *C. papuana*** *C. rouxi* *C. serratirostris* *C. cf. typus 1* *C. cf. typus 2* *C. vitiensis* *C. cf. weberi 6*** *C. wyckii** *C. sp. 1* *C. sp. 2* *C. sp. 3* *C. sp. 4* *C. sp. 5*	*C. neglecta* *C. cf. papuana*** *C. cf. serratirostris 5* *C. sp. 1** *C. sp. 2** *C. sp. 3** *C. tupaia*** *C. typus* *C. cf. weberi 1*** *C. cf. weberi 2*** *C. cf. weberi 4*** *C. cf. weberi 5*** *C. cf. weberi 7***	*C. cf. jeani 4*** *C. macrodentata*** *C. neglecta* *C. cf. serratirostris 4* *C. sp. nov. Santo** *C. typus* *C. variabilirostris** *C. cf. weberi 4***

AUSTRALIE

Queensland	Nouvelle Galles du Sud	Territoire du Nord
C. celebensis *C. confusa* *C. gracilipes** *C. gracilirostris* *C. sp. Gulf1* *C. indistincta A* *C. indistincta B* *C. indistincta C* *C. indistincta D* *C. cf. nilotica 1** *C. cf. nilotica 2** *C. cf. serratirostris* *C. sp. 3 (Salomon)** *C. spinula* *C. thermophila* *C. typus* *C. wilkinsi*	*C. appendiculata** *C. indistincta B* *C. indistincta C* *C. indistincta D* *C. mccullochi*	*C. sp. Gulf1* *C. sp. NTnilotica** *C. sp. NT2* *C. sp. WA4*

C. zebra		

POLYNESIE

Cook	Polynésie Française	Samoa	Futuna
*C. tupaia***	**Australes** *C. rapaensis*** *C. cf. serratirostris* *C. tupaia*** **Marquises** *C. marquesensis*** **Société** *C. cf. serratirostris* *C. tupaia*	*C. buehleri*** *C. cf. serratirostris 1* *C. cf. jeani 2* *C. tupaia**	*C. cf. serratirostris* *C. cf. jeani 2* *C. futunensis***

MICRONESIE

Guam	Pohnpei	Palau
*C. variabilis**	*C. appendiculata**	*C. appendiculata**
C. typus	*C. cf. serratirostris 7*	*C. lobocensis***
	C. typus	*C. cf. serratirostris 6*
	*C. variabilirostris**	*C. typus*
	*C. cf. weberi***	*C. variabilis**

ASIE DE L'EST

Japon (+ Okinawa)	Taiwan
C. celebensis	*C. brevicarpalis*
C. gracilirostris	*C. fasciata*
*C. grandirostris**	*C. formosae*
C. laoagensis	*C. gracilipes**
*C. leucosticta**	*C. grandirostris**

C. prashadi	*C. multidentata***
C. propinqua	*C. pseudodenticulata*
C. macrodentata	*C. serrata*
*C. multidentata***	*C. serratirostris*
C. okinawa	*C. typus*
C. rubella	*C. villadolidi*
C. serratirostris	*C. weberi***
C. typus	

Il apparaît ainsi que des zones peu prospectées jusqu'à cette étude comme les îles Salomon ou le Vanuatu se révèlent abriter une très forte biodiversité pour les caridines, tandis que des zones que l'on pensait riches en espèces endémiques comme la Nouvelle Calédonie possèdent en réalité une diversité moins importante que ce que laissait croire la morphologie seule, du fait d'espèces polymorphes, généralement influencées par l'environnement. De même, des espèces que l'on pensait largement réparties ont vu leur répartition géographique modifiée, comme c'est le cas avec *C. weberi*, absent de Polynésie, ou l'espèce *C. longirostris*, probablement limitée à l'Afrique du Nord alors que plusieurs auteurs lui donnaient une répartition Indo-Pacifique.

8.2 Apports à la taxonomie

En s'intéressant plus précisément aux complexes *C. nilotica* et *C. weberi*, de nombreuses différences ont pu être notées permettant de dépasser les groupes artificiels proposés par de précédents auteurs, et de les caractériser comme groupes monophylétiques ayant un sens évolutif.

Ainsi, les espèces du complexe *C. nilotica* se caractérisent par une morphologie plutôt gracile, avec un rostre parfois long (pouvant dépasser le pédoncule antennulaire mais parfois plus court selon l'environnement (voir Mazancourt *et al.*, 2017a) et courbé vers le haut et toujours denté sur son bord supérieur, avec la présence de dents apicales, l'épine antennaire est distincte de l'angle orbital inférieur, le pédoncule antennulaire est long (à peu près aussi long que la longueur de la carapace), le bord ptérygostomien est arrondi, les péréiopodes sont généralement longs et minces, on retrouve une bosse dorsale typique au niveau du 3^{e} segment abdominal, le 6^{e} segment abdominal est long

(toujours plus de la moitié de la longueur de la carapace), la carène pré-anale est petite et peut présenter une forte projection en forme d'épine, la diarèse uropodiale porte un nombre modéré de spinules (toujours inférieur à 15), le telson porte un nombre réduit de soies terminales simples et modérément longues, et l'endopode du premier pléopode mâle est arrondi, avec ou sans appendice interne situé sur le bord externe en position subdistale voire distale (Mazancourt *et al.*, 2018a, b, in press).

Les espèces du complexe *C. weberi*, quant à elles, présentent une morphologie robuste, avec un rostre habituellement court (plus court que le pédoncule antennulaire) et droit ou courbé vers le bas, avec ou sans dents sur le bord dorsal, sans dents apicales, l'épine antennaire est fusionnée avec l'angle orbital inférieur, le pédoncule antennulaire est court (environ la moitié de la longueur de la carapace), le bord ptérygostomien est variable, les péréiopodes sont courts et trapus, le carpe du péréiopode 1 est souvent profondément échancré, pas de bosse dorsale au niveau de l'abdomen, le 6^{e} segment abdominal est court (moins de la moitié de la longueur de la carapace), la carène pré-anale est haute, sans projection ou avec une petite épine, la diarèse uropodiale porte un grand nombre de spinules (toujours supérieur à 15), le telson porte de nombreuses soies terminales plumeuses, et l'endopode du premier pléopode mâle est long, subrectangulaire, avec un appendice interne court situé sur le bord externe en position subdistale (Mazancourt *et al.*, 2017b, en révision).

Les différences de morphologie entre les deux complexes peuvent s'expliquer par des différences en terme d'habitats occupés. En effet, nous avons vu que les espèces du complexe *C. nilotica* présentaient plus d'affinités avec les milieux lentiques du cours inférieur, tandis que celles du complexe *C. weberi* se rencontraient plutôt dans les zones lotiques (Mazancourt *et al.*, en révision). Bouvier (1925) avait interprété certaines modifications morphologiques tels, entre autres, « la réduction de l'abdomen qui devient plus court et large », « la réduction en longueur et l'épaississement des pattes ambulatoires », l'augmentation du nombre d'épines uropodiales et « la réduction longitudinale des appendices céphaliques » comme des adaptations à la marche, et donc un mode de vie benthique. Il justifie cette réduction globale de la taille des appendices par la limitation de la gêne qu'ils représentent « lorsque l'animal fouit ou se déplace parmi les obstacles du fond », et l'augmentation du nombre d'épines uropodiales car elles servent de « soutien qui donnent plus de rigidité aux rames ». De fait, les espèces du

complexe *C. weberi* semblent présenter les caractéristiques d'une adaptation à la marche, ce qui est en accord avec les habitats qu'elles occupent où le fort courant contrarie la nage. Le phénomène inverse a été observé chez les espèces de l'*Atya*-group des Caraïbes, où des espèces dont l'ancêtre avait probablement une morphologie robuste se sont adaptées aux milieux calmes dont la niche écologique était inoccupée (Page *et al.*, 2008).

Suivant toujours l'approche de taxonomie intégrative, étant donné que des caractères morphologiques, des différences d'habitat et de mode de vie permettent de caractériser les deux complexes, et en accord avec les données moléculaires qui en font deux groupes monophylétiques, ils pourront être considérés comme des taxons valides et être formellement décrits.

8.3 Apports à la biogéographie

Le fait de pouvoir délimiter les espèces de manière plus précise permet de mieux connaître leur distribution, en identifiant celles qui sont endémiques et celles qui ont une plus large répartition.

8.3.1 Facteurs influençant la répartition des espèces

En étudiant les aires de distribution des espèces largement réparties, des différences apparaissent, laissant supposer que des facteurs différents limitent ou favorisent la répartition d'espèces différentes.

Par exemple, aucune espèce du complexe *C. nilotica* ne semble aller plus à l'est que les îles Fidji, alors que certaines espèces du complexe *C. weberi* ont pu évoluer en Polynésie. L'espèce *C. appendiculata* du complexe *C. nilotica*, présente depuis l'Indonésie jusqu'en Australie (Nouvelles Galles du Sud) en passant par la Micronésie, les îles Salomon et le Vanuatu est absente de Nouvelle Calédonie et du Queensland. *C. variabilis*, espèce du complexe *C. nilotica* présente à Palau et à Guam est absente de Pohnpei. Un certain nombre d'espèces présentes aux îles Salomon ne se retrouvent pas au Vanuatu, et vice-versa.

Plusieurs facteurs peuvent expliquer ces différences de distribution.

Les espèces de caridines à large répartition étant amphidromes, leurs larves sont soumises à l'influence des courants lors de leur phase planctonique marine, de la même manière que pour les espèces marines à larves pélagiques. Ainsi, suivant la direction des courants, les larves peuvent être entraînées vers des îles présentant des habitats favorables à leur établissement – le courant sert donc de voie de dispersion dans ce cas – ou bien vers le large, où elles ne pourront jamais compléter leur métamorphose – le courant agit donc comme une barrière dans ce cas. Cependant, l'influence de ce facteur sur une espèce donnée est difficile à évaluer car on ne connaît pas la profondeur à laquelle évoluent les larves dans la colonne d'eau, sachant que des courants de directions différentes suivant la profondeur peuvent exister. Même s'il a été montré que les larves sont phototrophes (Benzie, 1982 ; Heerbrandt & Lin, 2006), laissant penser que les larves se dirigent activement vers les eaux de surface, la profondeur de nage des larves reste à étudier, comme ce fut le cas pour les anguilles par exemple (Tsukamoto *et al.*, 2003).

Les traits de vie de la phase larvaire peuvent également expliquer les différences de répartition. Une espèce possédant une phase larvaire longue avec de nombreux stades zoé pourra rester plus longtemps en mer, et donc être entraînée plus loin par le courant qu'une espèce à durée larvaire plus courte. De la même manière, des larves plus lécithotrophes possédant des réserves nutritives plus importantes pourront plus facilement traverser les étendues océaniques parfois pauvres en matière organique que des larves planctotrophes. La salinité requise pour le développement des larves est également un caractère à prendre en compte. Par exemple, il a été montré que les larves de *C. leucosticta* se développent dans un environnement saumâtre et ne s'éloignent pas de l'embouchure des rivières (Yatsuya *et al.*, 2013 ; Fujita *et al.*, 2016) tandis que *C. typus* et *C. multidentata* se développent dans un environnement pleinement marin (Fujita *et al.*, 2016), ce qui pourrait expliquer une plus large répartition de ces dernières par rapport à la première. Lorsque les conditions ne sont pas favorables à leur croissance, certaines larves sont capables de la ralentir, le temps de retrouver un environnement plus adéquat. Cette capacité peut être plus ou moins développée selon l'espèce, expliquant des différences de répartition.

La géologie pourrait avoir un impact important dans la biogéographie des espèces amphidromes. Par exemple, les fosses océaniques semblent jouer le rôle de barrière à la

dispersion chez des poisons marins (Last & White, 2011 ; Last *et al.*, 2011) et chez des espèces amphidromes de poissons (Mennesson, 2016) et de gastéropodes (Abdou, 2016). La présence de barrières continentales est également un obstacle à la dispersion des larves, certaines ayant apparu ou disparu au cours des variations du niveau marin avec les glaciations (voir la barrière Indo-Pacifique (Lord *et al.*, 2012) ou le pont du détroit de Torres (Taillebois *et al.*, 2013b), par exemple). Les points chauds peuvent également jouer un rôle dans la répartition des espèces. En effet, ils sont à l'origine d'alignements d'îles pouvant servir de relais pour la progression des espèces. A mesure que les îles s'éloignent de leur point chaud, elles perdent en altitude jusqu'à finir par disparaître sous la surface de l'océan. Les îles hautes possèdent des rivières avec une forte pente lorsqu'elles sont jeunes alors qu'elles perdent en relief avec le temps (Dupon *et al.*, 1993). Ainsi, une île qui a pu abriter une espèce à un instant donné peut ne plus présenter l'habitat favorable à l'établissement de cette espèce à mesure qu'elle change de morphologie, ce qui conduit à la formation d'une barrière à la dispersion.

L'écologie semble avoir son importance quant à la distribution des espèces. Certaines espèces peuvent être limitées à un habitat bien particulier et soumis à une forte compétition. Si une espèce est déjà établie dans cet habitat, il peut être difficile pour une autre de venir le coloniser, les espèces s'excluent donc mutuellement (Hardin, 1960). De même, l'absence d'habitat adéquat pour une espèce constitue en soi une barrière. Si, par exemple, une espèce est adaptée aux zones lentiques du cours inférieur et que les rivières d'une île donnée ne présentent pas ce faciès, l'espèce ne pourra pas s'y installer et s'en servir comme point de départ pour coloniser de nouvelles îles.

L'histoire évolutive des espèces peut également expliquer des différences de répartition. Par exemple, une espèce apparue assez tôt a pu profiter de certaines conditions favorables à sa dispersion (courants, îles relai, etc.) et qui ont disparu suite à son établissement, empêchant l'arrivée d'autres espèces apparues plus tard (Planes & Galzin, 1997).

Suivant les espèces, différents facteurs peuvent jouer, pouvant même combiner leurs effets. Ainsi, en reprenant les exemples cités au début de cette partie, les différences de répartition entre les complexes *C. nilotica* et *C. weberi* dans le Pacifique peuvent s'expliquer par une origine plus ancienne du complexe *C. weberi* (Mazancourt *et al.*, en révision), dont les espèces auraient pu bénéficier de courants qui ont disparu

lorsque le complexe *C. nilotica* est arrivé dans la région. Pour *C. appendiculata*, il pourrait s'agir d'exclusion écologique, sa niche étant occupée par *C. meridionalis* en Nouvelle Calédonie et *C. gracilipes* dans le Queensland, expliquant son absence dans ces deux zones. La répartition de *C. variabilis* à Palau et Guam mais absente de Pohnpei peut s'expliquer par la géologie, les deux premières îles étant séparées de la troisième par une fosse océanique, mais aussi par les courants océaniques jouant un rôle de barrière pour les larves, ou par exclusion écologique, sa niche étant occupée par *C. variabilirostris* à Pohnpei. La différence de faune observée entre le Vanuatu et les îles Salomon peut être expliquée par la présence de forts courants entre les deux archipels (Ceccarelli *et al.*, 2013) limitant les échanges de populations et favorisant les spéciations.

8.3.2 Biodiversité dans la zone Indo-Pacifique

Le genre *Caridina* demeure un genre très diversifié, et particulièrement pour les deux complexes étudiés dans notre étude, en ce qui concerne la zone Indo-Pacifique. Cependant, il existe des différences régionales en terme de biodiversité pour ces groupes. Nous proposons ici une cartographie des nombres d'espèces endémiques par localité à partir de données publiées et de nos résultats (Figure 8.1).

La plus forte biodiversité se retrouve au niveau du Triangle de Corail, entre les Philippines, l'Indonésie et la Papouasie, et semble décroître à mesure que l'on s'en éloigne. Certaines îles d'origine continentale comme Madagascar ou le Sri Lanka concentrent des espèces endémiques. Des îles d'origine relativement ancienne comme la Nouvelle Calédonie, Fidji ou Maurice présentent également un nombre non négligeable d'espèces endémiques. En revanche, les îles les plus récentes et souvent éloignées comme en Polynésie, en Micronésie ou dans l'océan Indien présentent peu d'espèces endémiques. De même, les zones situées aux latitudes extrêmes de la répartition du genre comme le Japon ou le Sud de l'Australie ont un endémisme faible.

Certains des « hotspots » présentés ici dans le genre *Caridina* ont été mis en évidence par de précédents travaux chez les crevettes d'eau douce, comme par exemple Madagascar (De Grave *et al.*, 2008 ; De Grave *et al.*, 2015 ; Cumberlidge *et al.*, 2017) ou encore Sulawesi (De Grave *et al.*, 2008 ; Klotz & Rintelen, 2013 ; De Grave *et al.*, 2015).

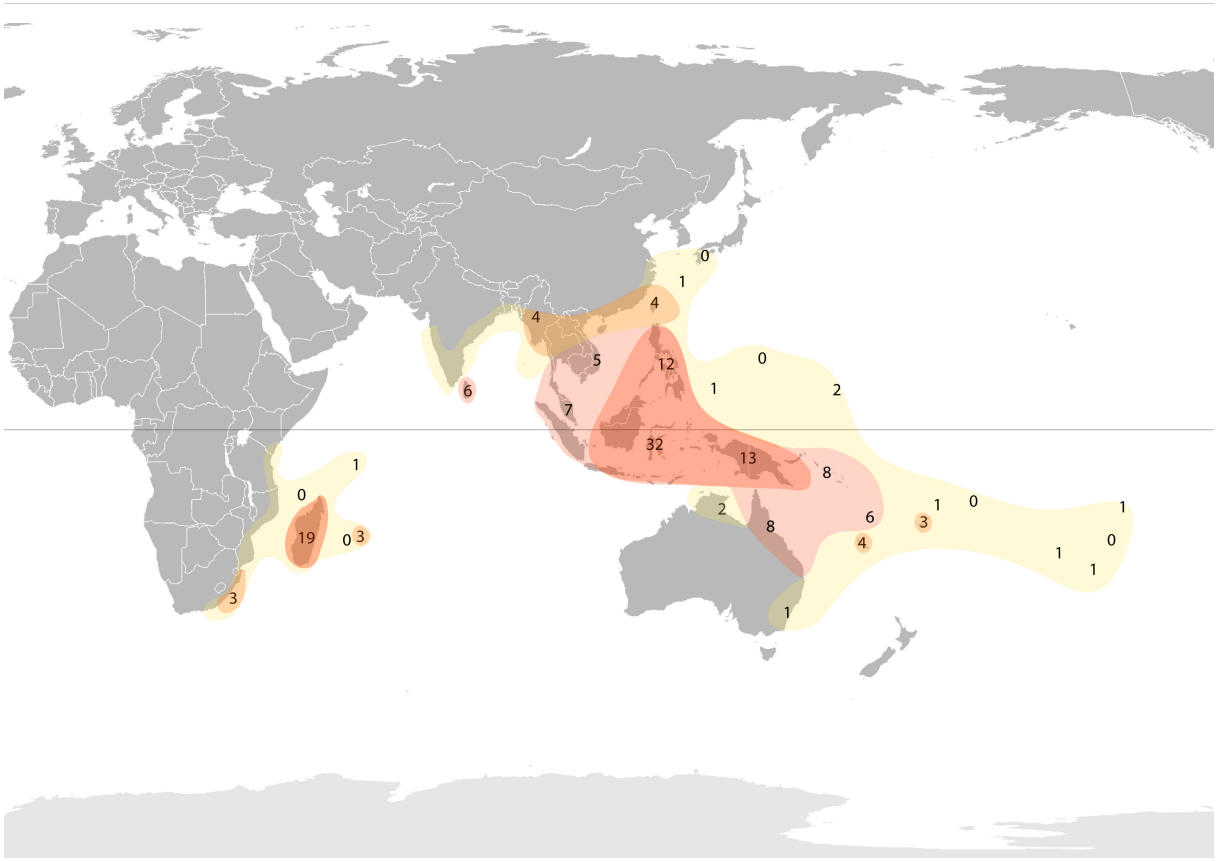


Figure 8.1 : Carte du nombre d'espèces endémique par localité. Aire en jaune : 0 à 2 espèces endémiques ; aire en orange : 3 à 4 espèces endémiques ; aire en rose : 5 à 10 espèces endémiques ; aire en rouge : plus de 10 espèces endémiques.

Figure 8.1: Map of the number of endemic species for each locality. Area in yellow: 0 to 2 endemic species; area in orange: 3 to 4 endemic species; area in pink: 5 to 10 endemic species; area in red: more than 10 endemic species.

Il semble donc que la biodiversité soit liée à l'ancienneté du milieu, sa taille et à la latitude. Elle sera maximale dans une grande île d'origine continentale proche de l'équateur, et minimale dans une petite île volcanique récente ou dans une zone éloignée de l'équateur (Paulay, 1994 ; Willig *et al.*, 2003). En effet, si le milieu est ancien, on peut supposer que les caridines y sont arrivées il y a longtemps et qu'elles ont donc eu le temps de se spécialiser à différents habitats et de former de nouvelles espèces. Ces observations sont en accord avec les résultats de Chen & He (2009), qui, d'après le modèle qu'ils ont développé, ont montré que le nombre de spéciations dans les îles, et donc leur biodiversité, augmente avec le temps, alors que le nombre de colonisations diminue. D'après cette même étude, le nombre d'espèces endémiques est corrélé à la superficie de l'île, ce que semblent confirmer nos observations, pour les grandes îles comme Madagascar, le Sri Lanka ou la Nouvelle Calédonie.

Pour les espèces amphidromes, il est intéressant de noter que même si le motif est globalement similaire, quelques différences existent (Figure 8.2).

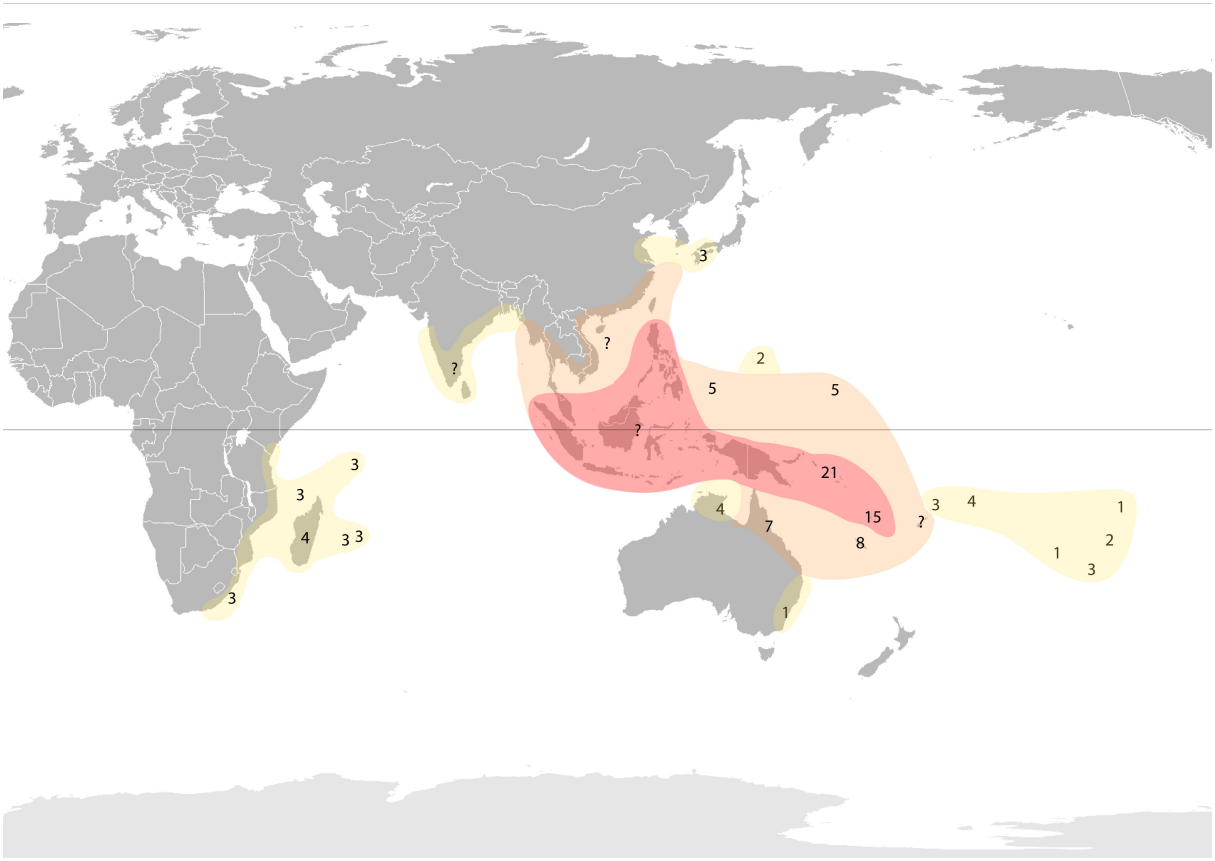


Figure 8.2 : Carte du nombre d'espèces amphidromes supposé par localité, d'après les données de ce travail. Les zones marquées d'un point d'interrogation n'ont pas été prospectées pour cette étude. Aire en jaune : 0 à 4 espèces amphidromes ; aire en orange : 5 à 10 espèces amphidromes ; aire en rouge : plus de 10 espèces amphidromes.

Figure 8.2: Map of the number of amphidromous species for each locality, from data obtained in the present work. Areas with a question mark have not been prospected for this study. Area in yellow: 0 to 4 amphidromous species; area in orange: 5 to 10 amphidromous species; area in red: more than 10 amphidromous species.

La zone de plus forte diversité se trouve au niveau des îles Salomon et du Vanuatu, et incluant potentiellement l'Indonésie, les Philippines et la Papouasie. A mesure que l'on s'en éloigne, le nombre d'espèces diminue pour atteindre son niveau le plus faible en Polynésie comme généralement aux limites de l'aire de répartition. On constate que des aires d'endémisme importantes comme Madagascar ou le Sri Lanka ne sont pas particulièrement riches en espèces amphidromes.

8.4 Habitat et écologie des caridines

L'habitat s'est révélé être un critère particulièrement important pour notre étude. En effet, nous avons montré que c'était le premier facteur de diversification dans le groupe, avant la biogéographie, et que l'habitat peut être un critère de détermination de l'espèce (Mazancourt *et al.*, en révision).

Les espèces vivant en rivière sont en effet soumises à des facteurs différents de ceux s'appliquant à leurs ancêtres marins. Des gradients abiotiques et biotiques s'appliquent ainsi sur tout le cours des rivières. On peut noter :

- Un gradient de **courant**, principalement lié à l'altitude (courant rapide en altitude et lent vers l'estuaire) mais pouvant également présenter des variations locales suivant la configuration du cours d'eau (zones de rapides ou vasques calmes).
- Un gradient de **température** et donc d'oxygénation (proche de la source, les eaux sont fraîches et brassées, tandis qu'elles sont plus chaudes au niveau de l'embouchure).
- Un gradient de **taille et profondeur de la rivière** (un filet d'eau proche de la source jusqu'à une large embouchure au niveau de l'estuaire).
- Un gradient de **salinité**, limité à la zone d'estuaire, l'influence du milieu marin diminuant progressivement à mesure qu'on s'éloigne de l'embouchure, jusqu'à disparaître totalement.
- Un gradient de **granulométrie** (les ruisseaux de montagne ont la roche mère comme substrat, puis, en perdant de l'altitude, des blocs, des galets, des graviers, du sable et de la vase en estuaire).
- Un gradient de **matière organique** (les cours d'eau proches de la source présentent une eau très claire avec peu de nutriments, tandis qu'en estuaire, les eaux se sont chargées de débris organiques et de matière en suspension).
- Un gradient de **végétation** (les rives du cours inférieur sont bordées de végétation alors que les ruisseaux d'altitude courent sur de la roche nue).
- Un gradient de **prédation** (la majorité des prédateurs se rencontrent proche de l'embouchure et sont incapables de franchir les cascades, réduisant la pression de prédation dans le cours supérieur).

Tous ces facteurs combinés permettent l'existence d'une grande variété de micro-habitats auxquels les espèces de caridines se sont adaptées. On remarque l'existence d'une zonation verticale des espèces en fonction des variations des facteurs liés à l'altitude influençant l'habitat, ainsi que des variantes horizontales, liées au faciès de la rivière, selon que le mode est lotique (avec un courant rapide) ou lentique (avec un courant lent), suivant la configuration du cours d'eau à un étage donné (Figure 8.3).

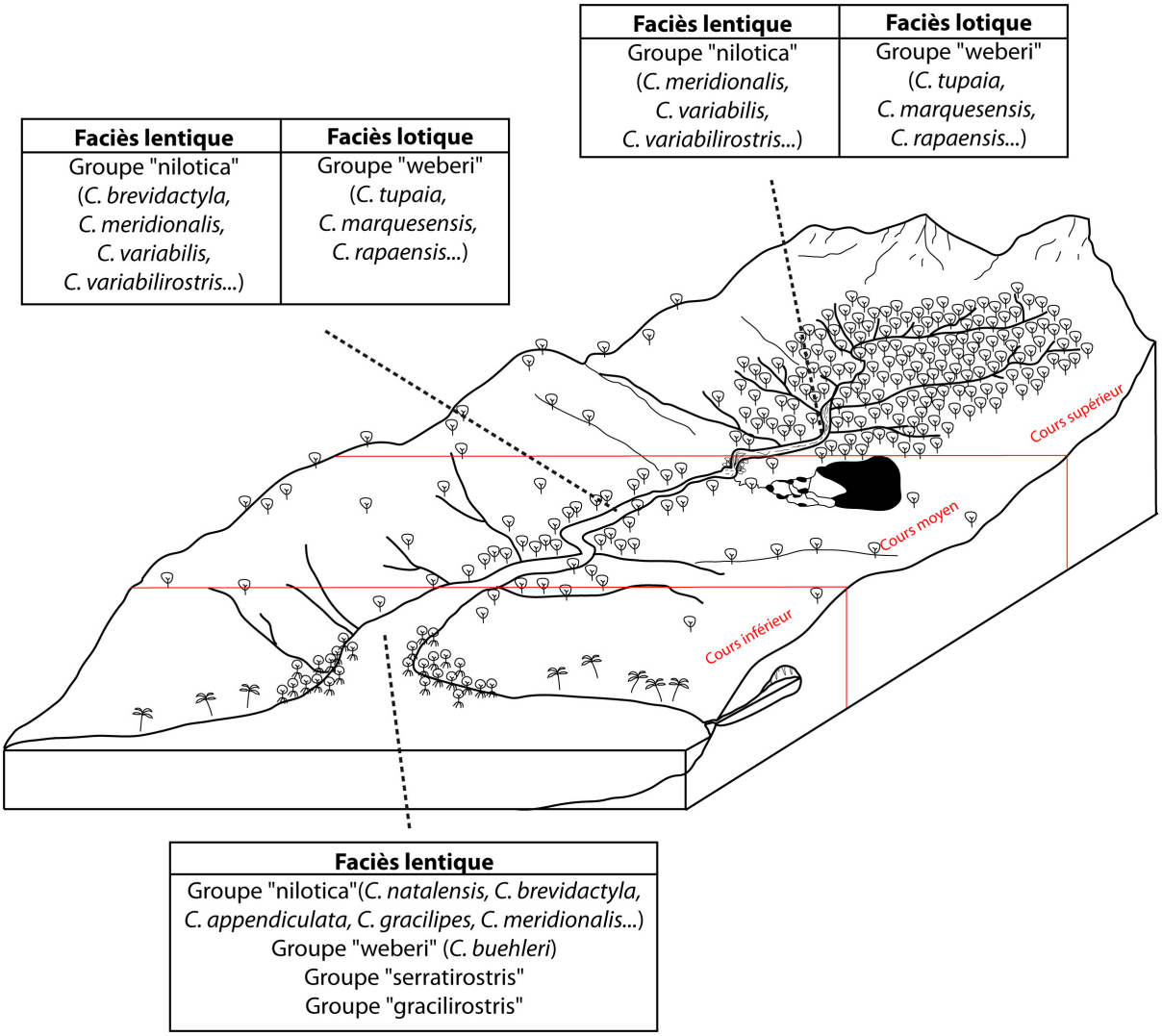


Figure 8.3 : Zonation altitudinale d'une rivière tropicale représentant les différents groupes d'espèces présents aux trois étages principaux (cours inférieur, cours moyen et cours supérieur) en fonction du mode lotique ou lentique.

Figure 8.3: Altitudinal zonation of a tropical river representing the different species groups present in the three main levels (lower course, middle course and higher course) depending on lotic or lentic mode.

Comme observé chez les poissons dans des rivières tropicales en milieu contiental par Pouilly *et al.* (2006) et par Lorion *et al.* (2011) et en milieu insulaire par Keith *et al.* (2015), un gradient altitudinal de biodiversité semble exister dans les rivières, avec une diversité d'espèces maximale dans le cours inférieur, se réduisant avec l'altitude. Cette différence peut s'expliquer par la plus grande abondance de matière organique dans les zones inférieures, permettant l'établissement d'une plus forte biomasse, mais aussi par la plus forte compétition entre les membres d'un même niveau trophique et par la plus forte prédation, deux raisons qui demandent aux espèces de s'adapter et donc stimulent la diversification. La proximité par rapport à l'océan peut également présenter l'avantage de réduire la distance que les larves des espèces amphidromes auront à

parcourir, dans un sens comme dans l'autre. En altitude, au contraire, les ressources sont plus limitées mais la pression de prédation est moins forte, ce qui conduit généralement à une faible diversité mais de fortes abondances, une espèce parfaitement adaptée étant alors largement dominante en nombre d'individus dans le milieu. Certaines d'espèces vivant dans ces milieux ont adopté un cycle de vie complet en eau douce, avec une durée larvaire réduite, voire un développement direct, évitant les aléas de la migration.

Néanmoins, cette zonation peut être parfois nuancée. Dans certains cas, la nature du substrat peut également jouer un rôle, comme par exemple en Nouvelle Calédonie, où les roches ultramafiques du sud de l'île entraînent la présence de concentrations élevées en métaux (nickel, chrome, cobalt, etc.) dans l'eau des rivières, ce qui est toxique pour la plupart des espèces, mais certaines tolérantes ou spécialisées peuvent s'y développer (Marquet *et al.*, 2003). Ces rivières sur substrat ultramafique présentent alors une faune différente de celle des rivières sur substrat sédimentaire, et avec une zonation altitudinale différente.

Des études précises de préférences de micro-habitats sont désormais faisables, maintenant que la confusion taxonomique au sein des complexes *C. nilotica* et *C. weberi* a été levée, et permettront d'obtenir de précieuses informations sur leur écologie pour pouvoir mettre en place une meilleure gestion des habitats et une meilleure protection de ces espèces.

8.5 Gestion et conservation des espèces et de leur milieu

La confusion taxonomique a été jusqu'à présent un frein à la mise en place de la conservation des espèces du genre *Caridina*. En effet, certaines espèces mal définies, comme *C. longirostris* ou *C. weberi* étaient réputées être largement réparties, or, elle se sont révélées renfermer plusieurs espèces à répartition plus restreinte, voire endémiques et ont vu leur propre répartition réduite. De même, une meilleure connaissance de leur systématique permet de mieux cerner l'habitat des espèces, puisque chacune d'elle est adaptée à un environnement différent, alors qu'elles étaient supposées écologiquement plastiques. Ces nouvelles connaissances impactent la façon dont ces espèces sont évaluées, car une espèce à répartition réduite et à préférences

écologiques particulière est plus vulnérable qu'une espèce largement répartie et peu exigeante en terme d'habitat (Purvis *et al.*, 2000).

Les caridines sont en effet menacées par les activités humaines. Elles peuvent l'être indirectement, par la disparition de leur habitat empêchant le maintien de populations viables, comme par exemple la disparition des rivières pérennes suite à une trop forte déforestation (Keith *et al.*, 2006), ou par réduction du débit du fait de prélèvements d'eau pour l'agriculture, l'industrie ou le tourisme, causant la disparition des zones lotiques (Keith *et al.*, 2013), ou directement, par la pollution et l'eutrophisation des rivières (pesticides et engrais pour l'agriculture, accidents industriels, braconnage par empoisonnement, utilisation des rivières comme décharge, colmatage par sédimentation trop importante due à la déforestation, etc.) (Boseto *et al.*, 2007 ; Keith *et al.*, 2013), par la mise en place de barrières réduisant ou supprimant la connectivité des habitats, notamment avec le milieu marin (barrages, radiers et autres ouvrages routiers mal conçus, canalisation des cours d'eau, etc.) (Dudgeon, 2000 ; March *et al.*, 2003b), par l'introduction d'espèces prédatrices ou entrant en compétition avec les espèces indigènes (De Grave *et al.*, 2015), ou encore par le prélèvement d'individus pour la consommation (Holthuis, 1980) ou le commerce aquariophile (De Grave *et al.*, 2015). Le changement climatique pourra également avoir un impact sur les caridines, notamment en modifiant les courants océaniques et donc la dispersion des larves des espèces amphidromes (Keith *et al.*, 2015). Par mesure de précaution, il convient de maintenir les rivières dans un bon état écologique (végétation des rives abondante, débit suffisant, bonne qualité de l'eau) et de garantir la connectivité biologique tout au long des cours d'eau (par la réalisation d'ouvrages de franchissement des barrages et la prise en compte de ces aspects lors de la construction de routes les traversant) pour permettre aux espèces migratrices de se développer et se déplacer librement dans les rivières, dans les sens amont-aval et aval-amont.

Le présent travail a permis de mettre en évidence de nouvelles espèces confondues avec d'autres et ainsi d'obtenir des informations plus précises quant aux répartitions et traits de vie de ces espèces. Ces informations permettront de ré-évaluer avec plus de fiabilité leur statut de conservation et leur classification dans la liste de rouge de l'IUCN. Par exemple, l'espèce *C. longirostris* y est évaluée comme « Préocupation mineure » du fait de sa répartition qui était supposée très large. Or, nous avons montré que dans

toutes les localités de l'Indo-Pacifique où cette espèce a été signalée, il s'agit d'autres espèces (*C. appendiculata*, *C. brevidactyla*, *C. gracilipes*, etc.) et que *C. longirostris* n'a en fait jamais été retrouvée depuis sa description et n'est connue que de la localité type (supposément en Egypte) (Mazancourt *et al.*, in press). Cette espèce semble donc bien plus vulnérable qu'on ne le pensait avant notre étude.

8.6 Etude des traits de vie du genre *Caridina*

Si l'observation de la cuticule du pédoncule antennulaire de *C. multidentata* n'a pas permis d'en apprendre plus sur les milieux traversés par les individus au cours de leur vie, nous avons néanmoins pu décrire pour la première fois l'ultrastructure de la cuticule de cette espèce. Les résultats obtenus par Kilada *et al.* (2012) sur les pédoncules oculaires n'ont pas pu être reproduits sur notre modèle, cependant, d'autres structures potentiellement conservées d'une mue à l'autre pourraient être étudiées, comme les ossicules gastriques, également étudiés par Kilada *et al.* (2012).

La manière la plus efficace d'en apprendre plus sur les traits de vie de ces espèces serait peut-être de les élever en aquarium, mais le cycle de vie amphidrome pose des problèmes de changement de conditions de maintenance entre celles dans lesquelles les adultes évoluent et celles dans lesquelles les larves se développent. Si la durée larvaire et la salinité favorable au développement des larves étaient connues, des réponses pourraient être apportées aux questionnements d'ordre biogéographique des espèces étudiées. De même, un marquage vital des larves permettrait de rechercher des structures calcifiées conservées sur les individus ayant atteint l'âge adulte.

8.7 Perspectives

Du fait des nombreuses missions de terrain réalisées dans la zone, ce travail a principalement porté sur la Mélanésie. Cependant, peu de spécimens provenant d'Insulinde, zone présentant la plus forte diversité du genre *Caridina*, ont été étudiés. De même, des zones particulièrement intéressantes d'un point de vue biogéographique comme les îles Fidji et Tonga, à la frontière entre les provinces Mélanésienne et Polynésienne, ou l'archipel des Andamans, proche de la barrière Indo-Pacifique

mériteraient d'être prospectées. L'inclusion de spécimens collectés dans ces zones permettra certainement de nous éclairer sur l'histoire évolutive des deux complexes.

Bien que se concentrant sur les complexes *C. nilotica* et *C. weberi*, l'étude moléculaire a également inclus des spécimens d'espèces extérieures aux deux complexes, ce qui a permis la mise en évidence de problèmes taxonomiques similaires à ceux présentés dans ce travail. Par exemple, l'espèce *C. serratirostris*, réputée largement répartie dans l'Indo-Pacifique s'est révélée être un complexe d'espèces, avec quasiment une espèce différente pour chaque localité. *Caridina typus* renferme plusieurs espèces cryptiques (Bernardes *et al.*, 2017), tout comme le complexe *C. gracilirostris*. Il serait intéressant d'inclure dans une phylogénie des représentants des grandes radiations endémiques dans le genre *Caridina*, comme celle des lacs anciens de Sulawesi (Indonésie), la radiation chinoise, ou encore les nombreuses espèces africaines et malgaches, afin de déterminer l'origine de ces radiations.

Des spécimens appartenant à d'autres genres d'Atyidae ont également été étudiés. Par exemple, dans le genre *Paratya*, en Nouvelle Calédonie, des espèces réputées distinctes d'après leur morphologie se sont révélées être une seule et même espèce polymorphe, tandis qu'une autre espèce, à morphologie peu variable cachait une espèce cryptique. Les genres *Atyoida* et *Atyopsis*, à répartition Indo-Pacifique, mériteraient d'être redéfinis, de nombreuses espèces cryptiques ayant pu être mises en évidence dans l'un comme dans l'autre, avec des répartitions géographiques parfois complexes. Des études plus poussées des groupes fournis ici en exemple, comme une phylogénie globale des Atyidae, permettra d'en apprendre plus sur la biogéographie de la région, comme sur l'histoire évolutive de la famille des Atyidae.

The present study allowed us to obtain a large dataset of DNA sequences, including barcodes as well as partial and complete mitochondrial genomes for a total of 92 species. The integrative taxonomy approach led us to recognize 43 new species that were unknown before the begining of the study (Table 8.1), especially in Melanesia.

We were able to spot morphological, ecological and molecular characters to characterize the two species complexes studied that can lead to treat them as valid taxa. Species of the *C. weberi* complex show a robust morphology, with a set of morphological features adapted to their life in fast-flowing areas, whereas species of the *C. nilotica* complex are more gracile, adapted to the lentic areas of the rivers.

Being able to separate species more precisely, we obtained a better knowledge of their biogeography. We listed the factors influencing their distribution: oceanic currents, biology of the larval stages, geology, ecology and evolutionary history. Most of the time, a combination of these factors explain the range of the species of *Caridina*. Differences in biodiversity pattern were observed (Figures 8.1 and 8.2) with a maximum biodiversity around the Indo-Australian Archipelago, decreasing as one moves away from it, with several biodiversity hotspots begin observed for large continental islands such as Madagascar, Sri Lanka or New Caledonia for example. Differences were observed between biodiversity patterns of endemic species and amphidromous species.

Biotic and abiotic factors related to the habitat are very important for freshwater shrimps. A set of altitudinal gradients was recognized: a gradient of current speed, of temperature, of river width and depth, of salinity, of sediment size, of organic matter, of vegetation and of predator pressure. Depending of these factors, and the local configuration of the river (lotic or lentic mode) there is a structure of species assemblages (Figure 8.3), with the greatest biodiversity in the lower course.

Our results have implications for the conservation of the species. With a better species delineation, we have a better knowledge of their distribution range and their biology, factors taken into account for the assessment of their conservation status. *Caridina* shrimps are indeed threatened by human activities like deforestation, pollution, excessive water removal, introduction of invasive species, or improper damming of the rivers.

We could not study amphidromy using the cuticle of the eyestalk but we obtained new data on the ultrastructure of the cuticle of *C. multidentata* observed for the first time. Other structures could be conserved throughout molt cycles, like gastric ossicles, and should be investigated as candidates for future sclerochronological studies. Rearing the larvae in aquaria could be a great way to study the biology of the species of *Caridina*.

As future prospects, this study could be extended to other localities currently poorly known, such as Fiji and Tonga, Madagascar, Indonesia or Andaman, to other species complexes of the same genus like species allied to *C. typus*, *C. serratirostris*, *C. serratirostris* or *C. brevicarpalis*, and to other genera of Atyidae like *Paratya* in New Caledonia or *Atyoida* and *Atyopsis* in the different sampled localities. A broader phylogeny of the whole family Atyidae using the same methods could provide new insights on the biogeography of the Indo-Pacific and the evolutionary history of the family.

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Annexes.

Annexes

Annexe 1 : Liste des missions réalisées par l'équipe d'accueil ayant fourni des spécimens au présent travail

Lieu et nom de la mission	Dates	Participants
Nouvelle Calédonie PEDCAL	septembre 1991	B. Séret, G. Dingerkus, E. Guilbert, G. Marquet et P. Lamarque
Nouvelle Calédonie Chloé 1	avril 1999	P. Keith, G. Marquet, E. Vigneux (+ E. Feunteun
Nouvelle Calédonie Chloé 2	octobre/novembre 1999	P. Keith, G. Marquet, E. Vigneux
Polynésie Française	février 2000	P. Keith et E. Vigneux
Nouvelle Calédonie Chloé 3	juillet 2000	P. Keith, G. Marquet, E. Vigneux
Rapa (Polynésie Française)	juin 2001	G. Marquet et J.-C. Guéry
Polynésie Française	juillet 2001	P. Keith et E. Vigneux
Efate/Santo (Vanuatu)	juillet 2003	P. Keith et G. Marquet
Seychelles/Mayotte	novembre 2003	H. Grondin, P. Bosc, P. Valade et P. Keith
Wallis et Futuna	octobre 2004	N. Mary, A. Dutartre, P. Keith, G. Marquet et P. Sasal
Mohéli (Comores) Conservation Internationale	octobre 2005	P. Keith, G. Marquet, A. Abdou et Y. Ibrahim

Santo (Vanuatu) La Planète Revisitée	novembre 2006	P. Keith, P. Gerbeaux, C. Lord et D. Kalfatak
Madagascar DIAMSOI 2008	juin/juillet 2008	N. Mary, E. Feunteun, C. Ellien et T. Robinet
Malekula (Vanuatu)	novembre 2008	P. Keith, C. Lord, G. Ségura, P. Gaucher et D. Kalfatak
Nouvelle Calédonie Côte Oubliée	novembre 2008	P. Keith, C. Lord, G. Ségura, P. Gaucher et C. Flouhr
Madagascar DIAMSOI 2010	mai 2010	G. Marquet, H. Grondin, T. Robinet *et al.*
Rarotonga (Cook)	juillet 2010	P. Keith, P. Gerbeaux et G. Marquet
Nouvelle Calédonie RAP	octobre 2010	G. Marquet, L. Taillebois *et al.*
Papouasie Lengguru	octobre/novembre 2010	P. Keith, P. Gaucher et G. Segura
Palau	mars 2011	P. Keith, P. Gerbeaux, G. Marquet, L. Taillebois et M. Castelin
Mayotte	mai 2011	H. Grondin, E. Feunteun et G. Marquet
Pohnpei	mars 2012	P. Keith, P. Gerbeaux, G. Marquet, L. Taillebois et M. Castelin
Silhouette (Seychelles)	novembre 2012	P. Keith
Taveuni (Fidji)	février 2013	P. Keith, P. Gerbeaux et G. Marquet
Mohéli (Comores)	décembre 2013	A. Abdou
Upolu/Savai (Samoa)	juillet 2014	P. Gerbeaux et J. Goodman
Choiseul (Salomon)	octobre 2014	P. Keith, G. Marquet et P. Gerbeaux
Epi/Gaua/Efate (Vanuatu)	octobre/novembre 2014	G. Marquet, A. Acou, D. Kalfatak et M. Mennesson

Tanna/Aneityum (Vanuatu)	juin 2015	C. Lord, G. Segura et D. Kalfatak
Kolombangara (Salomon)	novembre 2015	P. Keith, G. Marquet et C. Lord
Nouvelle Calédonie Mission Œil/Province Sud	septembre 2016	G. Marquet, P. Tiberghien et V. de Mazancourt
Vella Lavella/Ranongga (Salomon)	octobre 2016	P. Keith et C. Lord
Nouvelle Calédonie La Planète Revisitée	novembre 2016	V. de Mazancourt
Nouvelle Calédonie La Planète Revisitée	novembre 2017	V. de Mazancourt et N. Charpin
Polynésie Française	septembre/octobre 2017	G. Marquet, P. Tiberghien, E. Feunteun, P. Gerbeaux, M. Gerbeaux, P. Keith et H. Lucien-Brun
Afrique du Sud	février 2018	P. Kubheka, L. Maliwa, M. Mlambo, G. Marquet, P. Tiberghien, N. McClurg et T. McClurg

Annexe 2 : Liste des missions réalisées par des collaborateurs internationaux ayant fourni des spécimens au présent travail

Lieu de collecte	Dates de collecte	Participants	Envoyé par
Thaïlande	25 février 1994	W. D. Shepard	D. C. Rogers
Tioman (Malaisie)	26 juin 1997	D. C. Yep *et al.*	W. Klotz
Madagascar	mai 2004	H. Grondin, P. Bosc et T. Hoareau	H. Grondin
Okinawa (Ryukyu)	août 2004	J. L. Furnish	D. C. Rogers
Okinawa (Ryukyu)	avril 2005	W. D. Shepard	D. C. Rogers
Sulawesi (Indonésie)	27 mai 2005	M. Glaubrecht, T. von Rintelen & K. Zitzler	W. Klotz
Maurice	décembre 2005	C. R. Turner	D. C. Rogers
Egypte			W. Klotz
Hong Kong	2009	W. Klotz	W. Klotz
Sulawesi (Indonésie)	2010	H. G. Evers	W. Klotz
Bornéo (vendeur aquariophile à Singapour)	2011	W. Klotz	W. Klotz
Hong Kong	2011	W. Klotz	W. Klotz
Nouvelle Calédonie	8 au 18 novembre 2012	N. Mary	N. Mary
Taiwan	2013	W. Klotz	W. Klotz

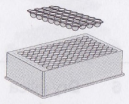
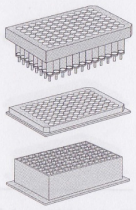
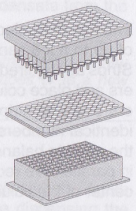
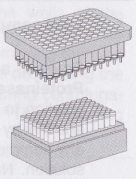
Palawan (Philippines)	8 février 2015	M. Guitz, C. J. Consul et C. Edong	D. C. Rogers
Malaita (Salomon)	juin 2015	D. Boseto *et al.*	D. Boseto
Guam	19 mars 2015	D. C. Rogers	D. C. Rogers
Afrique du Sud	1 février 2016	A. Holland	A. Holland
Australie	mai/juin + août 2016	B. Mos, B. Kroll et D. Wilson	B. Mos
Sri Lanka	6 au 14 février 2017	W. Klotz	W. Klotz
Japon	mars + juin 2017	M. Saito	M. Saito
Mitiaro (Cook)	7 mai 2017	P. Hosie	P. Hosie

Annexe 3 : Protocole d'extraction au robot Ependorf

NucleoSpin® 96 Tissue, vacuum processing
(For details on each step see section 5.4.)

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
- Set incubator or oven to 56°C.
- Preheat Elution Buffer BE to 70°C.

1	**Lyse** samples	**2 x 0.5 cm mouse tail** **or** **up to 20 mg tissue,** **10^6 cultured cells, or bacteria** **180 µl T1** **25 µl Proteinase K** **Mix** **56°C, ≥6 h**	 Round-well Block with Cap Strips
2	**Adjust** DNA binding condition	**200 µl BQ1** **200 µl ethanol (90-100%)** **Mix**	
3	**Load** samples	**Transfer samples to NucleoSpin® Tissue Binding Plate**	
4	**Bind** DNA to silica membrane	**- 0.2 bar*** **5 min**	NucleoSpin® Tissue Binding Plate and MN Wash Plate on MN Square-well Block *(optional)*
5	**Wash** silica membrane	**600 µl BW** **- 0.2 bar*** **5 min** **900 µl B5** **- 0.2 bar*** **5 min** **900 µl B5** **- 0.2 bar*** **5 min**	 NucleoSpin® Tissue Binding Plate and MN Wash Plate on MN Square-well Block *(optional)*
6	**Dry** silica membrane	**Remove MN Wash Plate** **- 0.6 bar*** **10 min**	
7	**Elute** DNA	**100 µl BE (70°C)** **- 0.4 bar*** **2 min** *Optional: Repeat elution step once.*	 NucleoSpin® Tissue Binding Plate on Rack of Tube Strips

Plaque 96 puits à ADN placée à l'étuve pendant une nuit

Ajout de 200 µl du mélange par puits manuellement

Les tampons BQ1, B5, BE, BW sont ajoutés dans les cuves du robot epmotion

Récupération sur une nouvelle plaque des ADN totaux élués

Annexe 4 : Protocole d'extraction au CTAB

1. Extraction

- Plonger les morceaux de tissus dans 600 µl de CTAB préchauffé à 60°C au bain-marie
- Ajouter 5 µl de protéinase K
- Placer les tubes au thermomixeur toute la nuit à 55°C

2. Purification au CIA

- Ajouter 500 µl de CIA (96 % Chloroforme 4 % Isoamyle Alcool)
- Mélanger manuellement pendant 2 minutes
- Centrifuger à 4°C et à 15 000 g pendant 10 minutes
- Récupérer la phase aqueuse surnageante

3. Précipitation

- Ajouter 250 µl d'isopropanol froid et 1 µg de RNA carrier
- Laisser une nuit au congélateur à -20°C

4. Récupération du culot

- Centrifuger à 4°C et à 15 000 g pendant 10 minutes
- Jeter le surnageant
- Ajouter 500 µl d'éthanol 95 % froid
- Centrifuger à 4°C et à 15 000 g pendant 10 minutes
- Jeter le surnageant
- Laisser sécher les tubes sous la hotte
- Re-suspendre le culot dans 25 µl de TAE préchauffé
- Mélanger 15 minutes au thermomixeur 55°C

Annexe 5 : Protocole de séquençage

1. *Purification ExoSap*

Mix ExoSap :

- 0,15 µl d'Exonucléase 1
- 0,2 µl de Phosphatase alcaline
- 1 µl de tampon de Phosphatase.

Par puits :

- 1,35 µl de mix
- 8,65 µl de produit de PCR

Au thermocycleur, 30 minutes à 37°C (réaction enzymatique) puis 15 minutes à 80°C (dénaturation des enzymes).

2. *Réaction de Séquençage*

Mix de séquençage, par puits :

- 6,1 µl d'eau distillée
- 2 µl de tampon
- 0,5 µl d'amorce (1,5 pM)
- 0,4 µl de BigDye
- 1 µl de produit de PCR purifié

La réaction de séquençage se fait au thermocycleur : 1 min à 95°C puis 15 cycles [10 s à 95°C → 5 s à 50°C → 1 min 15 s à 60°C] puis 5 cycles [10 s à 95°C → 5 s à 50°C → 1 min 30 s à 60°C] puis 5 cycles [10 s à 95°C → 5 s à 50°C → 2 min à 60°C]

3. *Purification sur Sephadex*

- Mélanger 3 g de Sephadex avec 50 ml d'eau distillée
- Déposer 300 µl de mélange dans chaque puits de la plaque de purification
- Laisser reposer 30 min
- Centrifuger 2 min à 2000 rpm
- Déposer le produit de séquençage au centre de la colonne de Sephadex
- Centrifuger 2 min à 2300 rpm
- Si besoin, re-centrifuger 1 min

Ajouter 10 µl de formamide dans chaque puits.

Annexe 6 : Codage utilisé pour la biogéographie et les reconstructions de caractères ancestraux

	Code	Character
Geography (Longhurst, 1998)	0	central pacific
	1	melanesia
	2	eastern australia
	3	indonesia
	4	sri lanka
	5	east africa
	6	micronesia
	7	kuroshio
	8	china sea
Geography (Abell et al. 2008)	0	Fiji
	1	Wallis-Futuna
	2	Samoas
	3	Society Islands
	4	Tubuai Island
	5	Marquesas Islands
	6	Rapa
	7	Solomon Islands
	8	Vanuatu
	9	New Caledonia
	a	Bismarck
	B	Eastern Australia
	C	Arafura-Carpentaria
	D	New Guinea
	E	Malukku
	F	Lesser Sunda
	G	Sulawesi
	H	Java
	J	Borneo
	K	Malaysia
	M	Philippine
	N	Taiwan
	P	Xi Yiang
	Q	Japan
	R	East Caroline
	S	West Caroline
	T	Sri Lanka
	U	Seychelles
	V	Mascarenes
	W	Comoros
	X	Madagascar
	Y	Zambezian
	Z	Cook Islands
Geography (Olson et al. 2001)	A	Australasian
	B	Indo-Malay
	C	Oceanian
	D	Afrotropical
	E	Palearctic
Altitude	0	Estuary
	1	Lower course
	2	Middle course
	3	Higher course
	4	Mountain stream

	Code	Character
Hydrology	0	Lotic
	1	Lentic
Substrate	0	Sand
	1	Gravel
	2	Pebbles
	3	Boulders
	4	Mud
Habitat	2	Plants
	3	Naked substrate

	Geography (Longhurst, 1998)	Altitude	Geography 2 (Abell et al. 2008)	Hydrology	Substrate	Habitat	Geography (Olson et al. 2001)
CA1003_C._sp._La_Guen'	0&1	3	0&9	1	3	3	AC
CA1048_C._cf._weberi_sp._3'	0&1	3	0&2&3&5&7&9&Z	1	3	2	AC
CA1053_C._cf._multidentata'	5	1&2	X	0	2	3	D
CA1056_C._lobocensis'	3&6	3	M&S	0	2	3	BC
CA1103_C._sp._NT_nilotica'	3	1&2	C&D	1	?	?	A
CA1131_C._brachydactyla'	3	1	F&G	1	0	2	AB
CA1132_C._sp._nov._Santo'	1	?	8	?	?	?	A
CA1160_E._jaggeri'	5	1	X&Y	0	1	3	D
CA1205_C._variabilis'	6	1&2&3	S	1	1	3	C
CA1238_C._cf._gracilirostris'	3	0	D	1	0	2	B
CA1240_C._sp._3_Salomon'	1&2&3	1	7&B&D	1	2	3	A
CA1249_C._sp._nov._A'	1	3	8	1	3	3	A
CA1261_C._appendiculata'	1&3&6	0&1	7&8&E&F&G&R&S	1	0	2	ABC
CA1282_C._sp._3_Salomon'	1&2&3	1	7&B&D	1	2	3	A
CA1285_C._sp._2_Salomon'	1	1&2&3	7	1	3	3	A
CA1286_C._papuana'	1	3	7&D	0	3	3	A
CA1326_C._sp._1_Salomon'	1	1	7	1	0	2	A
CA1343_C._natalensis'	5	1	U&W&X&Y	1	1	2	D
CA1345_C._brevidactyla'	0&1&3	1&2	0&7&8&9&E	1	0&1	2	AC
CA1348_C._gracilipes'	2&3&4&8	0&1	B&F&G&J&N&T	1	?	?	AB
CA1349_C._cf._weberi_sp._1'	1	3	7	0	1	3	A
CA1361_C._papuana'	1&3	3	7&D	0	3	3	A
CA1370_C._cf._jeani_sp._1'	0&1	3&4	2&7&8	0	3	3	AC
CA1371_C._cf._jeani_sp._1'	0&1	3&4	2&7&8	0	3	3	AC
CA1374_C._macrodentata'	1&3	2&3	8&M	1	3	3	AB
CA1375_C._macrodentata'	1&3	2&3	8&M	1	3	3	AB
CA1377_C._cf._jeani_sp._1'	0&1	3&4	2&7&8	0	3	3	AC
CA1385_C._sp._La_Guen'	0&1	3	9&A	1	3	3	AC
CA1388_C._cf._weberi_Pohnpei'	6	1&2&3	R	0	3	3	C
CA1390_C._cf._weberi_Pohnpei'	6	1&2&3	R	0	3	3	C
CA1404_C._variabilirostris'	6	0&1&2&3	S	1	3	3	C
CA1410_C._cf._weberi_sp._3'	0&1	3	0&2&3&5&7&9&Z	1	3	2	AC
CA1415_C._cf._jeani_sp._1'	0&1	3&4	2&7&8	0	3	3	AC
CA1416_C._cf._jeani_sp._1'	0&1	3&4	2&7&8	0	3	3	AC
CA1435_C._sp._nov._A'	1	3	8	1	3	3	A
CA1493_C._appendiculata'	1&3&6	0&1	7&8&E&F&G&R&S	1	0	2	ABC
CA1499_C._sp._1_Salomon'	1	1	7	1	0	2	A
CA1501_C._brevidactyla'	0&1&3	1&2	0&7&8&9&E	1	0&1	2	AC
CA1505_C._mertoni'	1&3	3	7&E	0	3	3	A
CA1506_C._mertoni'	1&3	3	7&E	0	3	3	A
CA1508_C._cf._weberi_sp._3'	0&1	3	0&2&3&5&7&9&Z	1	3	2	AC
CA1509_C._cf._weberi_sp._4'	1	3	7&8	1	3	3	A
CA1510_C._cf._weberi_sp._4'	1	3	7&8	1	3	3	A
CA1515_C._sp._2_Salomon'	1	1&2&3	7	1	3	3	A

CA1516_C._cf._weberi_sp._2'	1	3	7	1	3	3	A
CA1519_C._buehleri'	1&3	0&1	7&8&G&a	1	0	2	A
CA1520_C._buehleri'	1&3	0&1	7&8&G&a	1	0	2	A
CA1521_C._brevicarpalis'	1&3	0&1	7&8&D&E&G	1	1&2	3	A
CA1524_C._typus'	1&2&3&4&5&6&7&8	0&1&2&3&4	7&8&9&B&C&D&E&F&G&H&J&K&M&N&P&Q&R&S&T&U&V&W&X&Y&a	0&1	0&1&2&3	2&3	ABCDE
CA1545_C._multidentata'	7&8	1	N&Q	0	3	3	BE
CA1546_C._multidentata'	7&8	1	N&Q	0	3	3	BE
CA1554_C._lobocensis'	3&6	3	M&S	0	3	3	BC
CA1557_C._longicarpus'	1	2&3	9	0	1	2	A
CA1568_C._typus'	1&2&3&4&5&6&7&8	1&2&3&4	7&8&9&B&C&D&E&F&G&H&J&K&M&N&P&Q&R&S&T&U&V&W&X&Y&a	0&1	0&1&2&3	2&3	ABCDE
CA1585_C._meridionalis'	1	0&1&2	9	1	0&1	2	A
CA1599_C._longicarpus'	1	2&3	9	0	1	2	A
CA1601_C._sp._La_Guen'	0&1	3	0&9	1	3	3	AC
CA1611_C._longicarpus'	1	2&3	9	0	1	2	A
CA1620_C._meridionalis'	1	0&1&2	9	1	0&1	2	A
CA1628_C._africana'	5	2&3&4	Y	1	0&1	2	D
CA1670_C._sp._indistincta_E'	2	0&1	B	1	0	2	ABC
CA1678_C._gracilirostris'	1&2&3	0&1	8&B&D&E&G	1	0	2	AB
CA1685_C._sp._3_Salomon'	1&2&3	1	7&B&D	1	0	2	A
CA1707_C._cf._nilotica_1'	2	1	B	1	1&3&4	2&3	A
CA1708_C._sp._indistincta_E'	2	0&1	B	1	0	2	ABC
CA1711_C._cf._nilotica_2'	2	1	B	1	1&2&4	2&3	A
CA1714_C._cf._nilotica_2'	2	1	B	1	1&4	2&3	A
CA1721_C._sp._NT_nilotica'	3	1&2	C&D	1	0&1&2&3	2	A
CA1727_C._confusa'	2	4	B	1	1&2	2&3	A
CA1728_C._confusa'	2	4	B	1	1&2	2&3	A
CA1731_C._zebra'	2	4	B	1	1&2&3	3	A
CA1732_C._zebra'	2	4	B	1	1&2&3	3	A
CA1736_C._sp._Malanda'	2	4	B	1	0&1&2&3	2	A
CA1737_C._sp._Malanda'	2	4	B	1	0&1&3	2	A
CA1743_C._sp._WA4'	3	0&1	C	1	0&1&2&3	2	A
CA1744_C._sp._WA4'	3	0&1	C	1	0&1&2&3	2	A
CA1746_C._wilkinsi'	2	1	B	1	1&2&4	2	A
CA1747_C._wilkinsi'	2	1	B	1	1&2&4	2	A
CA1789_C._novaecaledoniae'	1	1&2&3&4	9	1	1&3	2	A
CA1792_C._novaecaledoniae'	1	1&2&3&4	9	1	1&3	2	A
CA1800_Paratya_caledonica	1	1&2&3&4	9	0&1	1&3	2	A
CA1801_Paratya_caledonica	1	1&2&3&4	9	0&1	1&3	2	A
CA1831_C._simoni'	4	1	T	1	?	?	B
CA1833_C._simoni'	4	1	T	1	?	?	B
CA1835_C._zeylanica'	4	1	T	1	?	?	B

CA1854_C._similis'	5	0&1&2&3&4	U	1	1	3	D
CA1899_C._elongapoda'	3&8	1	G&K&P	1	?	?	B
CA1902_C._cf._weberi_sp._6'	1	3	7	1	3	3	A
CA1903_C._cf._weberi_sp._6'	1	3	7	1	3	3	A
CA1909_C._cf._weberi_sp._5'	1	1	7	1	0	2	A
CA1912_C._leucosticta'	7	1	Q	?	?	?	BE
Caridina_cf._nilotica'	3	?	C&D	?	?	?	A
Caridina_gracilipes	2&3&4&8	?	B&F&G&J&N&T	?	?	?	AB
Halocaridina_rubra	?	?	?	?	?	?	C
Macrobrachium_bullatum	?	?	?	?	?	?	A
Neocaridina_denticulata	8	?	P	?	?	?	B
Paratya_australiensis	2	?	B	?	?	?	A

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When molecules and morphology work together: lines of evidence for the validity of *Caridina buehleri* Roux (Crustacea : Decapoda : Atyidae) and for *C. gueryi* Marquet, Keith & Kalfatak as its junior synonym

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Abstract. The taxonomy of the freshwater shrimps *Caridina* (Atyidae) is very complex and confused mostly because the morphological characters that have traditionally been used for species delimitation and identification are highly plastic. There is thus a need for an integrative approach to their taxonomy. In total, 42 specimens belonging to either *Caridina buehleri* Roux, 1934 or *Caridina gueryi* Marquet, Keith & Kalfatak, 2009 were examined. We combined here morphological data from 12 specimens from the whole distributional range of the species, including type specimens with *16S* mtDNA analyses from seven freshly sampled specimens, to verify the specific status of *Caridina buehleri* from Papua New-Guinea, Central Sulawesi, Western Samoa and the Solomon Islands, and *C. gueryi* from Vanuatu. The comparison of 24 morphological characters showed that morphological variations of character traits between *C. gueryi* and *C. buehleri* are widely overlapping and that no morphological feature can effectively split specimens into two clear groups. Molecular characters corroborated these results, as specimens from both groups were only separated by a 2% p-distance, a genetic distance that is coherent with their potentially high dispersal abilities. We thus consider *C. gueryi* as a junior synonym of *C. buehleri*. Finally, *C. buehleri* is characterised mainly by a styliform and characteristically indented rostrum and a long stylocerite. Detailed re-descriptions of the type specimens of *C. buehleri* and *C. gueryi* are given, as well as their geographical and ecological distribution.

Additional keywords: freshwater shrimp, Indo-Pacific, integrative taxonomy, *16S*.

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Introduction

One of the aims of the Muséum national d'Histoire naturelle (MNHN) in Paris is to carry out faunistic inventories of rivers on tropical islands to establish better protection of these fragile ecosystems and to clarify the taxonomy of poorly known organisms.

The freshwater shrimp *Caridina* H. Milne-Edwards, 1837, comprising 298 species (World Register of Marine Species (WoRMS) database as of October 2016; http://www.marinespecies.org/) and mostly present in the Indo-Pacific region, is the most diversified genus of the family Atyidae (De Grave *et al.* 2015) and an important ecological component in tropical streams (Covich *et al.* 1999; Pringle *et al.* 1993). Their high diversity combined with the lack of informative morphological characters has led to a confused taxonomy (Richard and Clark 2009). Indeed, until recently their taxonomy was mainly based on morphological characters. Some are highly variable within a single species (e.g. rostrum shape and indentation or colouration) and so are taxonomically non-informative, making it difficult to identify boundaries between species (von Rintelen and Cai 2009). There is thus a need for an integrative and standardised approach to improve the systematics of the group, focusing on informative morphological features and using molecular characters (Page *et al.* 2005; Page and Hughes 2011). To illustrate this problem, we here focussed on two species that are morphologically very similar: *Caridina buehleri* Roux, 1934 and *Caridina gueryi* Marquet, Keith & Kalfatak, 2009.

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Roux (1934) described *Caridina buehleri* from a single non-ovigerous female from New Ireland (Papua New Guinea) as possessing a distinctively long stylocerite and a long and pointed rostrum. In 2007, this species was reported from Luwuk Peninsula, Central Sulawesi, Indonesia, and re-described with more morphological characters, but without examining the type specimen (Klotz *et al*. 2007). *Caridina buehleri* was later reported from Upolu Island (western Samoa) (Gerbeaux *et al*. 2014) and collected by some of the authors (P. Keith and G. Marquet) on Kolobangara Island (Solomon Islands) in 2015.

Caridina gueryi was described after examination of specimens collected from Vanuatu (Santo and Malekula islands) by the Vanuatu Environment Unit (VEU) and the MNHN (Keith *et al*. 2010), but they were not compared with the holotype of *C. buehleri*, despite strong similarities between the two species.

As we collected more and more specimens from different localities, we started to doubt the validity of *C. gueryi*, as the specimens identified as such fitted well with the description of *C. buehleri*. Consequently, we examined the type specimens of these two species and combined morphological data with a *16S* mtDNA analysis to clarify their status.

Materials and methods

Abbreviations used for museums are as follows: MNHN, Muséum national d'Histoire naturelle, Paris; NMB, Naturhistorisches Museum Basel, Basel; ZMB, Museum für Naturkunde, Berlin.

Abbreviations used in the morphological analyses are as follows: CL, carapace length (mm) measured from the post-orbital margin to the posterior margin of the carapace; TL, total length (mm) measured from the tip of the rostrum to the posterior margin of the telson; P1, first pereiopod; P2, second pereiopod; P3, third pereiopod; P5, fifth pereiopod; PlI, first pleopod.

Molecular analyses

DNA extraction, amplification and sequencing

DNA was extracted from abdominal tissues using a semi-automatic Ependorf ep-Motion 5075 robot. Fragments of mitochondrial *16S* rRNA (~520 bp) were amplified using the primers 16Sa-L (CGCCTGTTTATCAAAAACAT) and 16Sb-H2 (CTCCGGTTTGAACTCAGATCA) (Palumbi 1996). DNA amplification was performed in 25-µL PCR reactions containing ~3 ng of template DNA, 2.5 mM of $MgCl_2$, 0.26 mM of each nucleotide, 0.3 µM of each primer, 5% dimethyl sulfoxide (DMSO), 1 ng of bovine serum albumin (BSA) and 1.5 units of QBIOTAQ polymerase (MPBiomedicals, Santa Ana, CA). Amplification products were generated by an initial denaturation step of 4 min at 94°C followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 51°C for 60 s and extension at 72°C for 60 s. Polymerase chain reaction products were sequenced using the same primers and in both directions to ensure the accuracy of base calls. Chromatograms were edited using BioEdit Sequence Alignment Editor Version 7.0.4.1 (Hall 1999). All sequences were deposited in GenBank (GenBank accession nos KY350238–KY350244; Table 1). Sequences for the genera *Caridina* and *Paratya* published by Page *et al*. (2007) were included in our analysis, *Paratya* sequences being used as outgroup.

Sequence and phylogenetic analyses

DNA sequences were aligned using MEGA6 software (Tamura *et al.* 2013) with the Muscle algorithm (Edgar 2004).

Given that non-coding markers can be difficult to align, two datasets were produced: the first was obtained by analysing our alignment through GBLOCKS (Talavera and Castresana 2007) to remove poorly aligned portions of sequences, and the second by leaving the sequences as they were after the alignment.

Using the Bayesian information criterion in PartitionFinder (Lanfear *et al.* 2012), we retained the GTR+G+I model for both datasets. Best-scoring maximum likelihood (ML) trees were estimated for each dataset using RAxML HPC2 v.8.2.4 (Stamatakis 2006) on Teragrid v.7.2.7, implemented in the Cyber Infrastructure for Phylogenetic Research (CIPRES) portal v.3.1 (Miller *et al*. 2010) (https://www.phylo.org/). One hundred independent searches, each starting from distinct random trees, were conducted. Robustness of the nodes was assessed using non-parametric bootstrapping (Felsenstein 1985) with 1000

Table 1. GenBank accession numbers for the obtained sequences
CL, carapace length

Accession number	Specimen ID	Collection ID	Locality	Date	Collectors
KY350238	CA1015	IU-MNHN-2015–23	Vanuatu, Malekula Island, Brenwe, 16° 7′35.56″S; 167°16′46.77″E	20 Nov. 2008	P. Keith, L. Taillebois, C. Lord
KY350239	CA1016	IU-MNHN-2015–23	Vanuatu, Malekula Island, Brenwe, 16.126545 167.279658	20 Nov. 2008	P. Keith, L. Taillebois, C. Lord
KY350240	CA1017	IU-MNHN-2015–23	Vanuatu, Malekula Island, Brenwe, 16° 7′35.56″S; 167°16′46.77″E	20 Nov. 2008	P. Keith, L. Taillebois, C. Lord
KY350241	CA1161	ZMB 29002	Indonesia, Sulawesi, Malontong, 0°53′7.81″S; 121°31′22.26″E	27 May 2005	M. Glaubrecht, T. von Rintelen and K. Zitzler
KY350242	CA1162	ZMB 29002	Indonesia, Sulawesi, Malontong, 0°53′7.81″S; 121°31′22.26″E	27 May 2005	M. Glaubrecht, T. von Rintelen and K. Zitzler
KY350243	CA1163	ZMB 29002	Indonesia, Sulawesi, Malontong, 0°53′7.81″S; 121°31′22.26″E	27 May 2005	M. Glaubrecht, T. von Rintelen and K. Zitzler
KY350244	CA1519	IU-MNHN-2015–19	Solomon Islands, Kolobangara Island, Jack Harbour, 8° 3′5.10″S; 157°10′56.70″E	11 Nov. 2015	P. Keith, G. Marquet, C. Lord

bootstrap replicates. Bayesian trees were calculated using MrBayes software (Huelsenbeck and Ronquist 2001) with the previously determined model, running for 5 000 000 generations, with a sampling frequency of 1000 and burn-in of 25%. Support for nodes was determined using posterior probabilities (PP) calculated by MrBayes. We considered a group to be 'moderately supported' if it had a bootstrap support value (B) between 75% and 89% and posterior probability (PP) between 0.95 and 0.97, and 'highly supported' when B ≥90% and PP ≥0.98.

Morphological comparisons

The rostrum, the general cephalothorax, pereiopods 1, 2, 3 and 5, and the abdomen were observed using a stereoscopic microscope. The proportions of the various joints of the appendages were measured using microphotographs and the AnalySIS Works software (Olympus, Shinjuku, Tokyo, Japan).

Examinations were made on the type specimens of *C. buehleri* and *C. gueryi*, and on a total of 12 specimens grouped by locality (six from Sulawesi, two from Kolobangara and four from Malekula). The ranges were then compared side by side and a global range of all the specimens was given.

Drawings were made using the 'digital inking' method (Coleman 2003, 2006) by tracing vectorial paths on high resolution photographs using Adobe Illustrator (CS6) and a WACOM MPTZ-1230 graphic tablet.

Scanning electron microscopy (SEM) images were obtained from ethanol-preserved specimens, some treated with 10% bleach overnight to remove the setae.

Results

Molecular results

A *16S* gene fragment was successfully sequenced for seven specimens (Table 1). Sequences included 490 bp containing 10 variable sites. After alignment with 87 atyid sequences (83 *Caridina* and four *Paratya* as outgroup) from Page *et al.* (2007), the *16S* datasets of 94 sequences of 513 bp included 193 variable sites, of which 174 were phylogenetically informative. After removal of ambiguous blocks, 374 bp of sequence remained to be used in phylogenetic analyses (73% of the original 513 positions), of which 131 bp were variable and 112 bp were phylogenetically informative.

No significant incongruence was found between full-length and reduced datasets; however, both the Bayesian inference (BI) and ML trees produced using the full-length dataset (Fig. 1) were overall more supported than the trees produced using the reduced dataset (available as 'Supplementary material'). Therefore, only the results obtained for the full-length dataset are presented here.

In each of the BI and ML analysis, the consensus tree showed *C. buehleri* and *C. gueryi* as a monophyletic and highly supported group (PP = 0.99; B = 97%), which was well differentiated from all other *Caridina* analysed (p-distance to the closest relative: 12.3%), and appeared as belonging to the *C. weberi* species-complex. Within this monophyletic group, sequences from *C. buehleri* and *C. gueryi* were clustered in two well-supported genetic groups: the first one (PP = 0.94; B = 100%) including two specimens identified as *C. buehleri* from Indonesia; and the second one (PP = 0.99; B = 100%) including three specimens identified as *C. gueryi* from Vanuatu, one specimen identified as *C. buehleri* from Indonesia and one specimen identified as *C. buehleri* from the Solomon Islands. Clade 1 had a 2% p-distance from Clade 2.

Taxonomy

Family **ATYIDAE** De Haan

Genus ***Caridina*** H. Milne Edwards

Caridina buehleri Roux

(Fig. 2)

Caridina buehleri Roux, 1934: 219, figs 1–5 (type locality: Bimoun, New Ireland west coast, Papua New Guinea).

Caridina gueryi Marquet, Keith & Kalfatak, 2009: 159–166, figs 1–3 (type locality: unnamed river (15°17′45.30″S; 167°9′35.98″E), Santo, Vanuatu).

Material examined

Holotype. 1 non-ovigerous ♀, CL 7 mm, coll. F. Speiser, H. Hediger and A. Bühler. Papua New Guinea: New Ireland: Bimoun (NMB 822).

Types. *Caridina gueryi* Marquet, Keith & Kalfatak, 2009, Vanuatu: Santo Island:. *Holotype*: ovigerous ♀, CL 6.4 mm, unnamed river (15°17′45.30″S; 167°9′35.98″E), 22.vii.2003, coll. P. Keith and G. Marquet (MNHN-IU-2015–1768). *Allotype*: MNHN-Na 16–236, ♂, TL 14.5 mm, CL 3.2 mm, same data as holotype (MNHN-IU-2015–1769). *Paratypes*: 12 ♀ (MNHN-IU-2015–1770); one ovigerous ♀, TL 23.1 mm, Mantatas River (15°9′38.05″S; 166°57′4.32″E), 23.vii.2003, coll. P. Keith and G. Marquet (MNHN-IU-2015–1771); one ovigerous ♀, TL 18.9 mm, Sarataka River (15°30′4.54″S; 167°9′15.59″E), 25.vii.2003, coll. P. Keith and G. Marquet (MNHN-Na 16–239).

Other material. *Caridina buehleri* Roux, 1934, **Indonesia:** central Sulawesi: Luwuk Peninsula, freshwater spring in Malontong, west of Ampana, brackish water pool, 0°53.125′S 121°31.371′E (field code 70–05), 1 ♂ (CL 2.9 mm), 1 ♀ (CL 3.9 mm), 3 ovigerous ♀ (CL 4.3–6.4 mm), coll. M. Glaubrecht, T. von Rintelen and K. Zitzler, 27.v.2005, det. W. Klotz and A. Karge (ZMB 29002). **Solomon Islands:** Kolobangara Island: Vagé River, 08°05.112′S 156°59.867′E, 3 ovigerous ♀ (CL 3.7 mm; 3.2 mm; 3.5 mm), coll. P. Keith, C. Lord and G. Marquet, 10.xi.2015, det. G. Marquet (IU-MNHN-2015–17); Liva River: 08°03.859′S 157°10.684′E, 3 ovigerous ♀ (CL 3.5 mm; 3.8 mm; 4.2 mm), 11.xi.2015, det. G. Marquet (IU-MNHN-2015–18); Jack Harbour River: 08°03.085′S 157°10.945′E, 3 ovigerous ♀ (CL 4.4 mm; 3.4 mm; 4.3 mm) and 1 non-ovigerous ♀ (CL 4 mm), 11.xi.2015, det. G. Marquet (IU-MNHN-2015–19); Pipiro River: 07°53.083′S 157°08.195′E, 2 ovigerous ♀ (CL 3.8 mm; 4.2 mm), 13.xi.2015, det. G. Marquet (IU-MNHN-2015–20); Vanga I River: 07°55.088′S 156°57.624′E, 2 ovigerous ♀ (CL 4.4 mm; 4.6 mm), 18.xi.2015, det. G. Marquet (IU-MNHN-2015–21); Vanga II River: 07°54.825′S 156°57.762′E, 4 ovigerous ♀ (CL 4.1 mm; 3.2 mm; 4 mm; 4.2 mm), 18.xi.2015, det. Marquet (IU-MNHN-2015–22). *Caridina gueryi* Marquet, Keith & Kalfatak, 2009, **Vanuatu:** Malakula Island: Brenwe River, 16°07.849′S 167°17.342′E, 4 ovigerous ♀ (CL 5–6 mm), coll. Kalfatak, Keith and Lord, 20.xi.2008, det. Marquet (IU-MNHN-2015–23).

Diagnosis

Carapace smooth, glabrous, with sharp antennal spine placed at lower orbital angle. Pterygostomial margin blunt. Rostrum long, 0.5–1.4 of CL, styliform, reaching the end of scaphocerite, curved upwards, distal margin unarmed without apical teeth, armed with 8 to 16 teeth on basal part of dorsal margin, 1–4 of

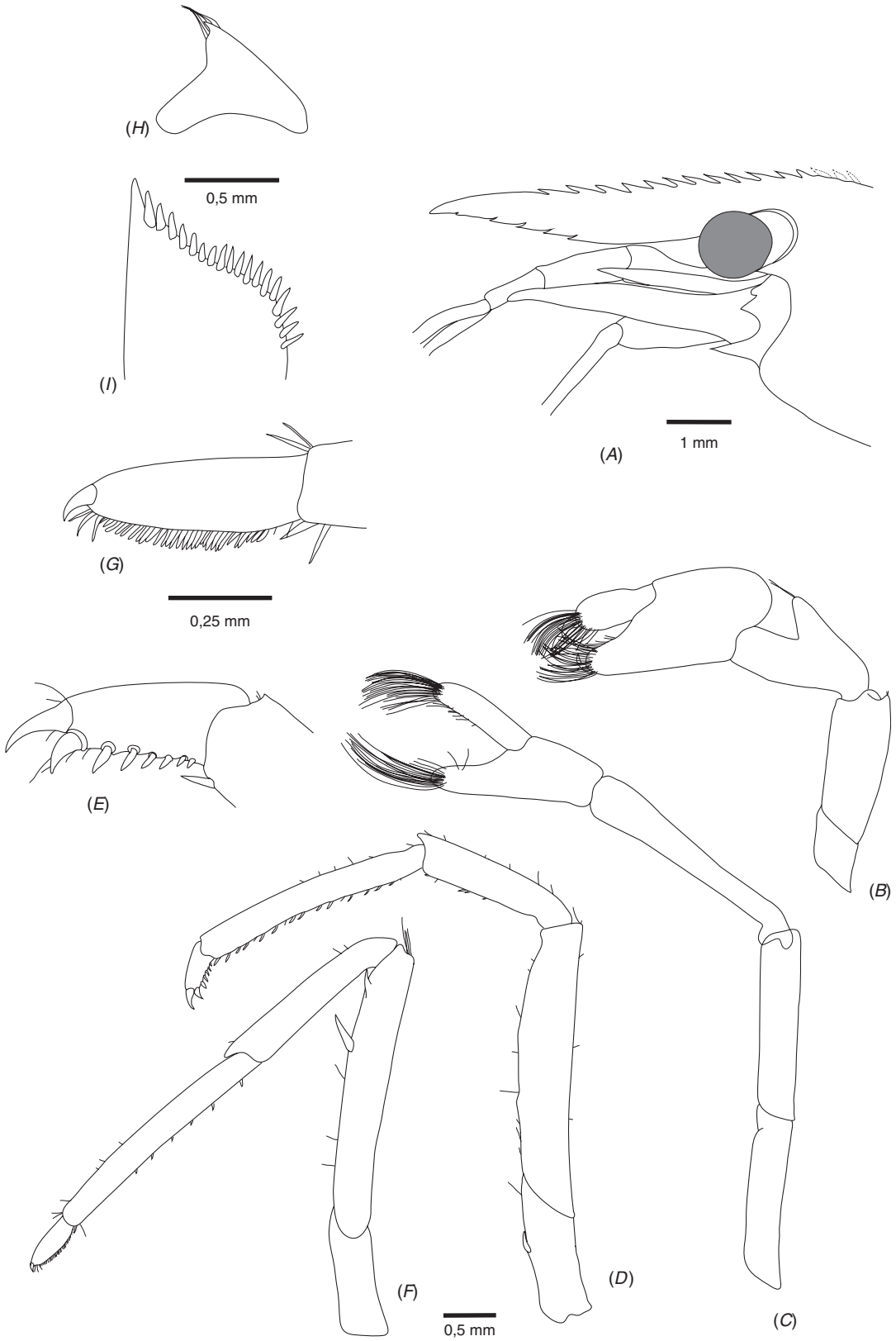


Fig. 1. *Caridina buehleri*, holotype, female (carapace length, CL 7 mm): (*A*) cephalothorax; (*B*) first pereiopod; (*C*) second pereiopod; (*F*) fifth pereiopod; (*G*) dactylus of fifth pereiopod. *Caridina gueryi* holotype female (CL 6.4 mm): (*D*) third pereiopod; (*E*) dactylus of third pereiopod; (*H*) pre-anal carina; (*I*) uropodial diaeresis.



Fig. 2. Collecting sites of *Caridina buehleri* in Pacific Ocean.

them situated on carapace behind orbital margin, ventral margin with 2–7 teeth. Stylocerite sharp, long, reaching to about halfway along second segment of peduncle.

First pereiopod stout, chela ~1.9–2.7× as long as wide, dactylus 1.3–2.9× as long as wide, 0.4–0.8× length of palm; tips of both fingers with prominent claws, carpus 1.2–1.7× as long as wide.

Second pereiopod more slender and longer than P1; chela 2.2–3.2× as long as wide, dactylus 3.0–4.8× as long as wide, 1.0–1.6× length of palm, tips of fingers rounded, carpus slender 4.4–7.0× as long as wide.

Third pereiopod slender, dactylus 2.7–4.0× as long as wide, terminal spiniform setae included) with 5–8 spiniform setae on flexor margin in addition to the terminal one; propodus 8.0–14.0× as long as wide, 3.0–5.3× as long as dactylus.

Fifth pereiopod with dactylus 3.3–5.6× as long as wide with 26–36 spiniform setae on flexor margin; propodus 9.4–20.5× as long as wide, 3.8–6.2 as long as dactylus.

Endopod of first pleopod with well-developed appendix interna in male specimens; appendix masculina on second pleopod ~0.6× length of endopod; appendix interna ~2/3 length of appendix masculina. Telson ending in broadly convex margin bearing a median projection; distal margin with 5–10 long plumose setae flanked by one pair of stout spiniform setae. Uropodal diaeresis with 16–20 short cuspidate teeth. Pre-anal carina high, without a spine. Egg size: 0.36–0.49 × 0.23–0.32 mm.

Distribution

Caridina buehleri is known from Indonesia (Sulawesi), Papua New Guinea (New Ireland), Vanuatu (Santo, Malekula), western Samoa (Upolu) and the Solomon Islands (Kolobangara) (Fig. 3).

Habitat

All specimens were collected in typical brackish water environments (i.e. brackish water pools or lower parts of rivers, near the estuary). The species is rarely found over the superior limit of the tide-influenced zone, and was not found in the true freshwater zone.

Live colour pattern

This species shows a rather specific pattern of seven white stripes (one on each somite and one on the cephalothorax), on varying colour that tends to match the environment, ranging from bright yellow to black with reddish dots (Fig. 4).

Morphological comparison

In total, 24 variables were obtained for each group (compared in Table 2). It appears that all of the variations of the measures are globally overlapping, and no significant differences were observed.

Discussion

Caridina gueryi*, a junior synonym of* C. buehleri

Genetic results showed that specimens attributed to the two species clustered in the same clade, and the two groups were too close (2% p-distance) to support the hypothesis of separate species, thus suggesting that one of the species names should be synonymised. After morphological examination of the type specimens and populations from the known distribution of the two species (Sulawesi, Vanuatu and the Solomon Islands), we found that no morphological feature could split the specimens into two clear groups. Therefore, according to the integrative taxonomy approach applied here, we regard both populations as belonging to *C. buehleri*, as no morphological, ecological or genetic differences could be detected between them. Therefore, we suggest that *C. gueryi* Marquet, Keith & Kalfatak, 2009 should be treated as a junior synonym of *C. buehleri* Roux, 1934.

Diagnostic morphological characters of C. buehleri

From our morphological observations we found four specific characters diagnostic of this species that delimit it from all other *Caridina* species: (1) a long styliform rostrum, reaching the end of scaphocerite, distodorsal margin of the rostrum unarmed without apical teeth, with 8–16 teeth on basal part of dorsal margin, 1–4 of them situated on carapace behind orbital margin and 3–6 teeth on ventral margin; (2) a long stylocerite, reaching at least halfway along the second segment of antennular peduncle;

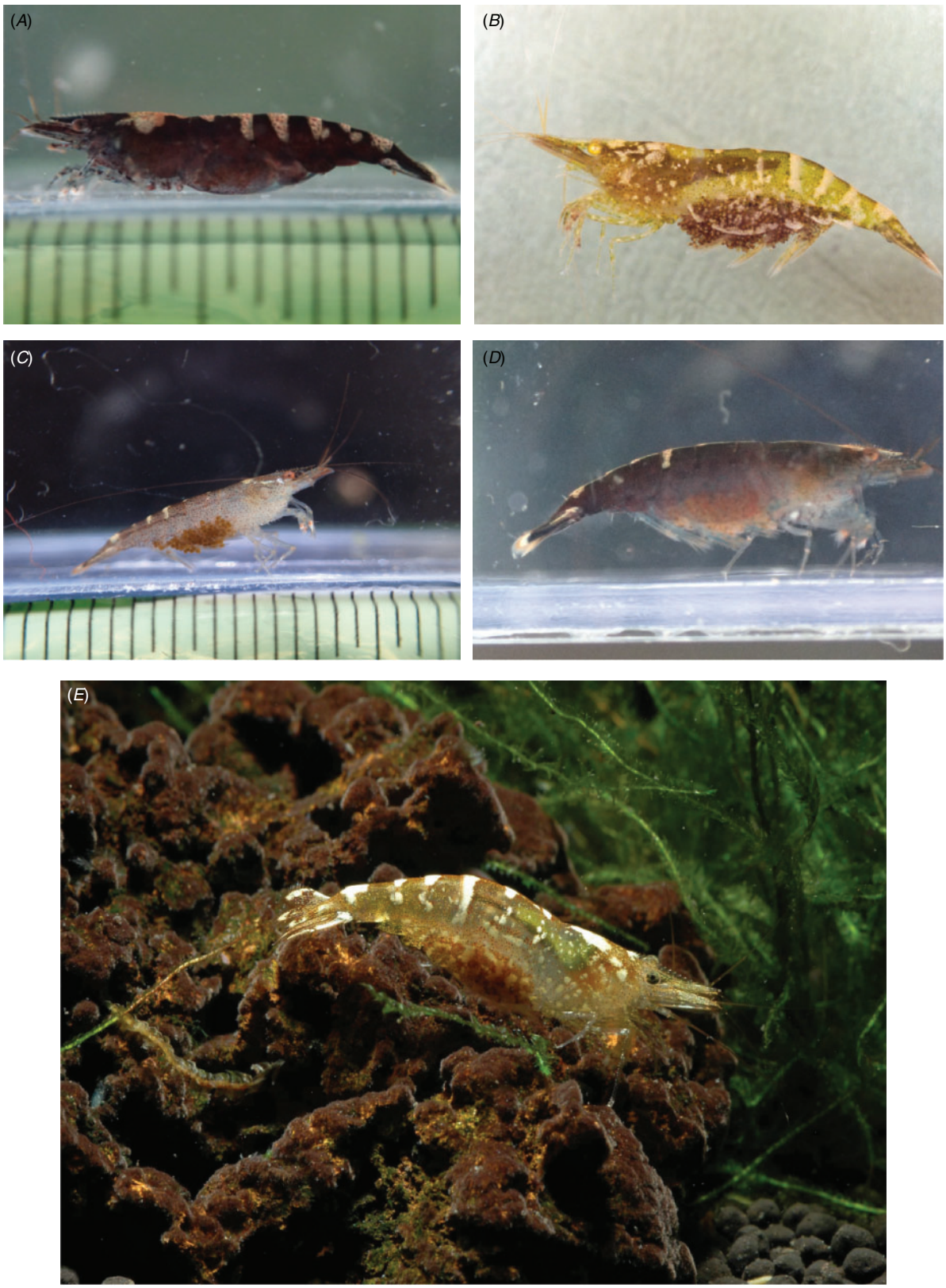


Fig. 3. (*A*) *Caridina buehleri* from Kolobangara, Solomon Islands (Photo P. Keith); (*C*) *C. buehleri* specimen CA1519; and (*D*) two not sequenced *C. buehleri* specimens; (*B*) *C. gueryi* from Santo, Vanuatu (Photo P. Keith), not sequenced; (*E*) *C. buehleri* from Sulawesi, Indonesia (Photo H. Evers), not sequenced.

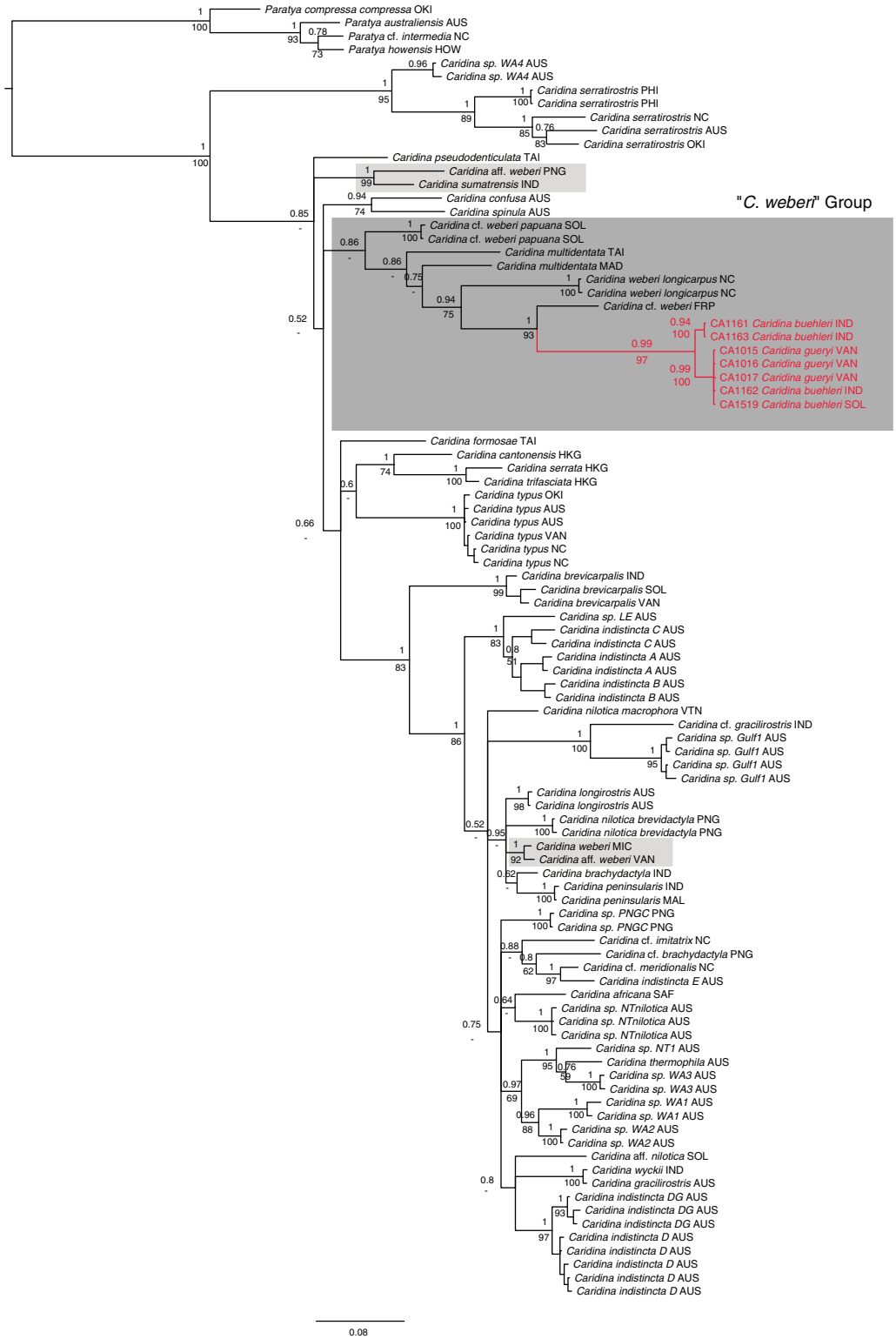


Fig. 4. Molecular phylogeny obtained from the full dataset showing the position of *Caridina buehleri* (16S). Numbers above branches are the Bayesian posterior probabilities; numbers under branches are the bootstrap from the maximum likelihood analysis. Dark grey shading indicates the genetic '*C. weberi* species-complex' and light grey shading indicates taxa morphologically close to the *C. weberi* species-complex. Locality codes: AUS, Australia; FRP, French Polynesia; HKG, Hong Kong; HOW, Lord Howe Island; IND, Indonesia; MAD, Madagascar; MAL, Malaysia; MIC, Micronesia; NC, New Caledonia; OKI, Okinawa; PHI, Philippines; PNG, Papua New Guinea; SAF, South Africa; SOL, Solomon Islands; TAI, Taiwan; VAN, Vanuatu; VTN, Vietnam.

Table 2. Comparison of the type specimens of *Caridina buehleri* and *C. gueryi* with specimens from Sulawesi, Kolobangara and Malekula
CL, carapace length; P1, first pereiopod; P2, second pereiopod; P3, third pereiopod; P5, fifth pereiopod; PII, first pleopod

	Rostrum times length of CL	Dorsal teeth (ventral teeth)	Unarmed distal portion of dorsal margin/armed portion	Teeth placed on carapace
C. buehleri holotype CL 7 mm	1.3	15 (5)	0.5	4
C. gueryi holotype CL 6.4 mm, allotype CL 3.2 mm	0.8, 0.9	12 (6), 16 (5)	0.7, 0.3	3, 3
Sulawesi specimens $n = 6$ (Klotz *et al*. 2007)	0.7–1.4	13–15 (4–7)	0.33	3–4
Kolobangara specimens $n = 2$	0.5	12–14 (2)	0.4	3–4
Malekula specimens $n = 4$	0.7	14–16 (3–5)	0.2–0.5	3–4
Global range	0.5–1.4	12–16 (2–7)	0.2–0.7	3–4
	P1 dactylus times as long as wide	P1 chela times as long as wide	P1 carpus times as long as wide	P1 dactylus times length of palm
C. buehleri holotype CL 7 mm	2.1	2.2	1.6	0.7
C. gueryi holotype CL 6.4 mm, allotype CL 3.2 mm	2.9 2.5	2.1, 2.3	1.5, 1.7	0.7, 0.7
Sulawesi specimens $n = 6$ (Klotz *et al*. 2007)	1.3–2.3	1.9–2.3	1.2–1.7	0.4–0.7
Kolobangara specimens $n = 2$	2.4–2.8	2.2–2.4	1.4–1.7	0.5–0.7
Malekula specimens $n = 4$	2.3–2.7	2.2–2.4	1.5–1.7	0.6–0.8
Global range	1.3–2.9	1.9–2.4	1.2–1.7	0.4–0.8
	P2 dactylus times as long as wide	P2 chela times as long as wide	P2 carpus times as long as wide	P2 dactylus times length of palm
C. buehleri holotype CL 7 mm	4.0	2.7	4.4	1.4
C. gueryi holotype CL 6.4 mm, allotype CL 3.2 mm	4.7, 6.1	3.0, 3.2	6.3, 7.0	1.5, 1.5
Sulawesi specimens $n = 6$ (Klotz *et al*. 2007)	3.0–4.8	2.2–3.0	5.0–6.7	1.0–1.5
Kolobangara specimens $n = 2$	4.0–4.4	2.9–3.0	5.6–5.9	1.2–1.4
Malekula specimens $n = 4$	4.2–4.6	2.6–3.0	5.5–5.6	1.4–1.6
Global range	3.0–4.8	2.2–3.2	4.4–7.0	1.0–1.6
	P3 dactylus times as long as wide	P3 propodus times as long as wide	P3 propodus times as long as dactylus	P3 spines on flexor margin
C. buehleri holotype CL 7 mm (Roux 1934)	Missing (3.3)	Missing (8.0)	Missing (3.0)	Missing (8)
C. gueryi holotype CL 6.4 mm, allotype CL 3.2 mm	3.4, 3.1	13.7, 10.7	5.3, 4.1	8, 7
Sulawesi specimens $n = 6$ (Klotz *et al*. 2007)	3.3–4.0	10.1–14.0	3.9–5.3	5–6
Kolobangara specimens $n = 2$	3.0	9.3–10.1	4.1–4.4	6
Malekula specimens $n = 4$	2.9–3.1	8.1–10.5	3.5–4.6	7
Global range	2.9–4.0	8.0–14.0	3.0–5.3	5–8
	P5 dactylus times as long as wide	P5 propodus times as long as wide	P5 propodus times as long as dactylus	P5 spines on flexor margin
C. buehleri holotype CL 7 mm	3.6	9.4	3.8	30
C. gueryi holotype CL 6.4 mm (Marquet *et al.* 2009), allotype CL 3.2 mm	Missing (5.6), Missing	Missing (15.9), Missing	Missing (5.0), Missing	Missing (36), Missing
Sulawesi specimens $n = 6$ (Klotz *et al*. 2007)	3.5–4.4	12.8–20.5	4.5–6.2	26–29
Kolobangara specimens $n = 2$	3.7–3.9	12.8–14.0	4.1–4.4	28–33
Malekula specimens $n = 4$	3.3	12.3	4.5	29
Global range	3.3–5.6	9.4–20.5	3.8–6.2	26–36
	Uropodial diaeresis spinules	Egg size	Pre-anal carina	Appendix interna on Pl1
C. buehleri holotype CL 7 mm	16 (left)–17 (right)	Non-ovigerous female	High, with no spine	NA
C. gueryi holotype CL 6.4 mm (Marquet *et al.* 2009)	18	Male (0.48 × 0.28)	High, with no spine	Well developed
Sulawesi specimens $n = 6$ (Klotz *et al*. 2007)	17–18	0.40–0.45 × 0.23–0.25	High with an indistinct tooth	Well developed
Kolobangara specimens $n = 2$	17–19	0.39–0.44 × 0.24–0.26	High, with no spine	NA
Malekula specimens $n = 4$	17–18	0.43–0.49 × 0.27–0.32	High with no spine	NA
Global range	16–19	0.36–0.49 × 0.23–0.32	High with no spine	Well developed

(3) the first pereiopod is stout, with a very short dactylus and both fingers of chela with prominent distal claws; and (4) a high pre-anal carina with an indistinct tooth. According to Richard and Clark (2014), the number of teeth and their placement on the rostrum (character 1) are important identification characters, in contrast to the rostrum length, which has been shown to be greatly plastic in atyid shrimps (Jugovic *et al.* 2010). Indeed, we found that the disposition of the teeth on the rostrum of *C. buehleri* is quite characteristic (without an apical dorsal tooth and few ventral teeth) and cannot be confused with any other species of the genus. A long stylocerite reaching the second segment of the antennular peduncle (character 2) is also found

in *C. serratirostris* De Man, 1892, *C. celebensis* De Man, 1892, *C. lobocensis* Cai, Choy & Ng, 2009 and in all members of the *Caridina serrata* species-group (Cai and Ng 1999; Klotz and von Rintelen 2014). However, these species can be easily differentiated from *C. buehleri* by the rostrum denticulation and the proportion of the carpus of the first pereiopod for *C. serratirostris* and *C. celebensis*; the spines of the telson, shape of the male first pleopod and the size of the eggs for species of the *C. serrata*-group (Klotz *et al*. 2007); and the setae of the fifth pereiopod for *C. lobocensis*. The chelae of the first pereiopods exhibit a distinctive claw on both fingers (character 3). This character was not reported by Roux (1934) in the original description of *C. buehleri*, nor in *C. gueryi*. This character might thus be more widely distributed in this genus than it appears, as it is hidden in the setae and so is quite difficult to see. According to Klotz *et al*. (2007), *C. buehleri* shows a high pre-anal carina with an indistinct tooth (character 4). Marquet *et al*. (2009) reported a spine on this structure in *C. gueryi*. After re-examination of the holotype of *C. gueryi*, it appears that there is actually no tooth on the pre-anal carina and the carina is similar to the one possessed by *C. buehleri*.

Perspectives on the taxonomy of Caridina*:* C. buehleri*, part of the* C. weberi*-complex?*

Our single-gene phylogenetic analysis (Fig. 1) corroborated the statement of Roux (1934), identifying *C. buehleri* as closely related to *C. weberi* De Man, 1892 and *C. multidentata* Stimpson, 1860, and more generally to species traditionally included in the *C. typus*-group (*sensu* Bouvier 1925). The *C. weberi* group includes species with rather stout bodies, short antennular peduncle, short sixth abdominal segment, a high pre-anal carina and a high number of spinules on the uropodial diaeresis.

In the present work (Fig. 1), this group was genetically supported by BI (PP = 0.99), thus suggesting the existence of a *C. weberi* species-complex in the same way that there exist species-complexes for the groups *C. nilotica* (Karge and Klotz 2008), *C. serrata* (Cai and Ng 1999; Klotz and von Rintelen 2014) and *C. gracilirostris* (Cai and Ng 2007). Indeed, in the BI analyses, our sequences clustered with *C.* cf. *weberi* from Moorea in both datasets, but also with *C. weberi longicarpus* from New Caledonia, *C. multidentata* from Taiwan, *C. multidentata* from Madagascar and *Caridina* cf. *weberi papuana* from the Solomon Islands. *Caridina sumatrensis* from Indonesia and *Caridina* aff.

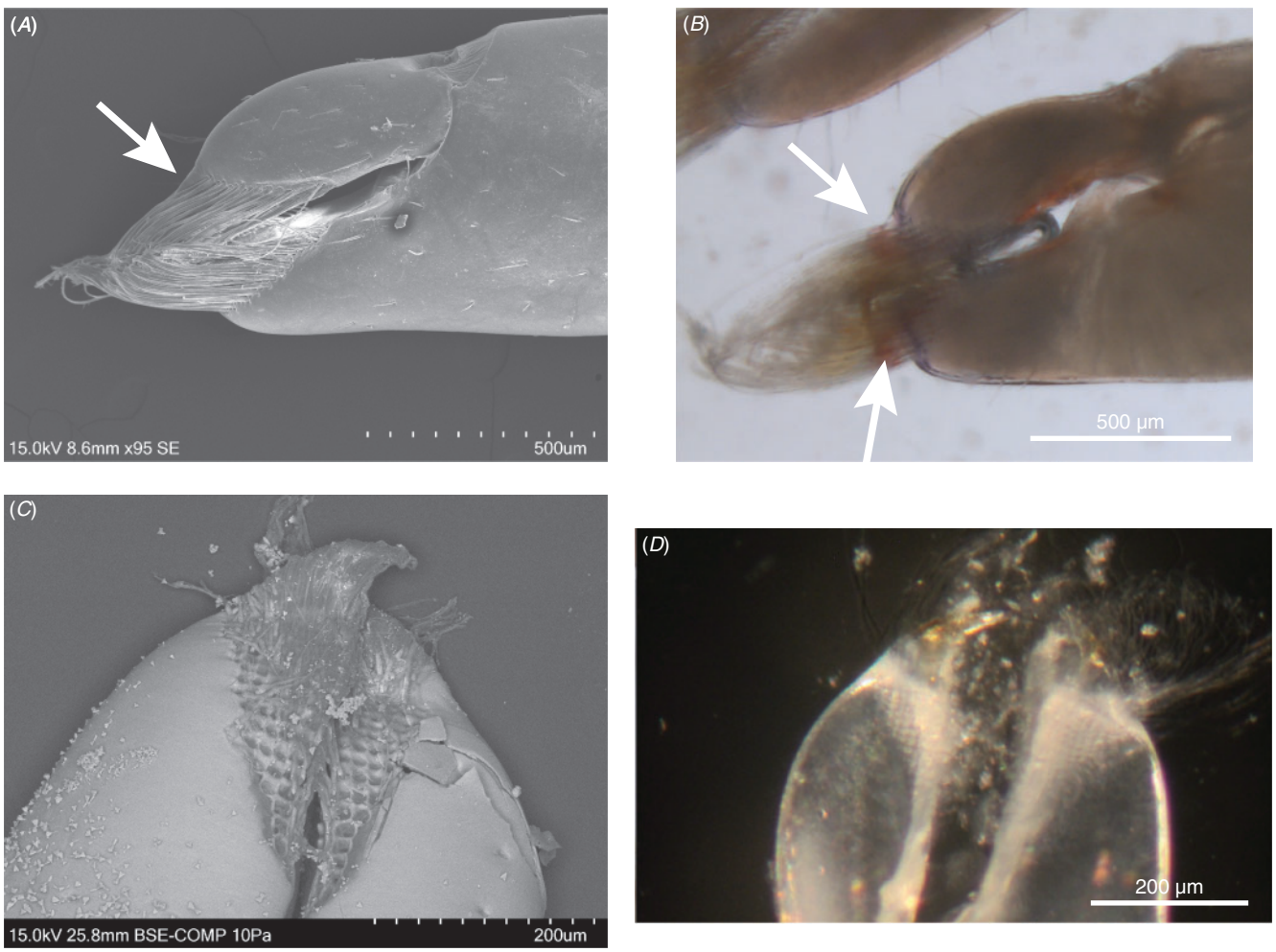


Fig. 5. (*A*) SEM image of a first pereiopod of *Caridina buehleri*. (*B*) Optical microscope photograph of a first pereiopod of *Caridina buehleri*. (*C*) SEM image of a first pereiopod of *Caridina buehleri* after bleach treatment. (*D*) Optical microscope photograph of a first pereiopod of *Caridina buehleri* after bleach treatment.

weberi from Papua New Guinea are not supported in this group, but due to the low resolution of the node that brings them together with the '*C. weberi*-complex', some further analyses are needed. *Caridina weberi* from Micronesia and *Caridina* aff. *weberi* from Vanuatu are far from this group in the tree and are most likely misidentifications. A multi-locus phylogenetic approach combined with morphological and ecological data is highly needed to clarify the taxonomy of *Caridina*.

New insights on the biology of C. buehleri

Caridina buehleri exhibits a stout first pereiopod with both fingers showing a prominent claw (Fig. 5). These features are also found in other species of *Caridina* such as *C. typus* (W. Klotz, pers. comm.). Clawed fingers might prove advantageous when feeding on discrete particles, as has been shown in *Limnocaridina* species (Fryer 2006), or small invertebrates such as oligochaetes or juvenile insects, while species without clawed finger tips primarily feed on detritus or single-celled periphyton (Klotz *et al*. 2007). This morphological feature could be linked to a predatory behaviour of scrapping sessile invertebrates in the part of the rivers where *C. buehleri* lives.

It is interesting to note that *C. buehleri* is a species inhabiting a brackish water environment, and that ovigerous females carry numerous small eggs, which is indicative of prolonged larval development (Hayashi and Hamano 1984). As a prolonged larval development implies a longer marine dispersal phase, because this species is most likely amphidromous, a wide distribution range could be expected (von Rintelen *et al.* 2008) rather than a scattered distribution as is currently known. Further fieldwork is necessary to collect more specimens and get a better picture of the extent of the distribution range of *C. buehleri*, whose identification should be facilitated by this study.

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The "Pinocchio-shrimp effect": first evidence of variation in rostrum length with the environment in *Caridina* H. Milne-Edwards, 1837 (Decapoda: Caridea: Atyidae)

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ABSTRACT

External morphology has always been the first criterion used to separate species of shrimps, especially in the freshwater genus *Caridina* H. Milne-Edwards, 1837, but more doubts have been expressed regarding the relevance of some of the morphological characters. We collected 27 specimens of *Caridina* from seven different localities during field work conducted on the island of Pohnpei (Federated States of Micronesia). After genetic verification that they all belonged to the same species, 19 morphological variables were measured and correlated with the elevation of the collecting stations using correlation analyses. We provide evidence that the length of the rostrum showed strong negative correlation with the elevation. This could be explained either by the physical stress exerted on the rostrum by the stronger water currents in the stations at a higher elevation, as a defence against predators in the lower stations, or a combination of both possibilities. The taxonomy of these shrimps is thus challenging and should not rely only on rostrum length, but on other characters such as the number of teeth on the dorsal margin of the carapace, which is not correlated with rostrum length and therefore, with the environment.

Key Words: correlation analysis, effect of elevation, freshwater shrimps, Micronesia, morphology, taxonomy

INTRODUCTION

The freshwater shrimp genus *Caridina* H. Milne-Edwards, 1837 is one of the most diverse of Caridea (De Grave *et al.*, 2015) with 298 described species thus far (World Register of Marine Species (WoRMS), December 2016; http://www.marinespecies.org/), and an important ecological component of tropical freshwater ecosystems (Pringle *et al.*, 1993; Covich *et al.*, 1999). This high diversity is a challenge for taxonomists, as the boundaries among species are not always clear from a morphological point of view as well as from a molecular perspective. The main morphological character used to identify the species of *Caridina* is generally the length, shape, and armature of the rostrum. This character, however, has been proven to be plastic in shrimps, tending to vary among individuals within a single species (De Grave, 1999), sometimes in adaptation to environmental constraints (Jugovic *et al.*, 2010). There is therefore a need to be very cautious and proceed with an integrative approach when dealing with the taxonomy of caridean shrimps (Page *et al.*, 2005; Page & Hughes, 2011).

A survey of the freshwater fauna conducted on the island of Pohnpei (Federated States of Micronesia) by a team from the Muséum national d'Histoire naturelle (MNHN), Paris collected a large number of atyid shrimps from different rivers and habitats (Keith *et al.*, 2012). Some of these specimens showing a long rostrum were attributed to *Caridina brachydactyla* De Man, 1908, whereas others, showing a short rostrum, were identified as *Caridina mertoni* Roux, 1911, following the previous identifications made from Guam, another Micronesian island, by Leberer & Cai (2003). A molecular analysis (see below) of these specimens indicated that they belong to the same species, referred to here to as *Caridina sp.* We therefore treated them as such to test the hypothesis that morphological variation is related to environmental factors. We performed a correlation analysis of 19 morphological variables on 27 specimens with the elevation of the station where the specimens were collected.

MATERIALS AND METHODS

Sampling

A total of 14 stations were sampled using electrofishing (see Ng *et al.*, 2017) in the four main streams of Pohnpei, Federated States

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Table 1. Specimens studied with locality information. Stations marked with an asterisk are above the first major waterfall (see Fig. 1).

Specimen of *Caridina sp.*	Station number	River	Elevation	Coordinates
CA1242	7	Senipehn	95 m	06°51.906N, 158°16.010E
CA1243	10*	Mahnd	107 m	06°50.221N, 158°17.212E
CA1244	9	Nanpil	5 m	06°56.609N, 158°13.550E
CA1246	3	Nanpil	52 m	06°55.111N, 158°12.878E
CA1247	5*	Lehn Mesi	139 m	06°51.107N, 158°11.896E
CA1248	8*	Nanpil	180 m	06°54.252N, 158°11.491E
CA1328	3	Nanpil	52 m	06°55.111N, 158°12.878E
CA1334	8*	Nanpil	180 m	06°54.252N, 158°11.491E
CA1335	8*	Nanpil	180 m	06°54.252N, 158°11.491E
CA1336	8*	Nanpil	180 m	06°54.252N, 158°11.491E
CA1337	9	Nanpil	5 m	06°56.609N, 158°13.550E
CA1338	9	Nanpil	5 m	06°56.609N, 158°13.550E
CA1339	9	Nanpil	5 m	06°56.609N, 158°13.550E
CA1340	1*	Nanpil	127 m	06°55.242N, 158°12.265E
CA1341	1*	Nanpil	127 m	06°55.242N, 158°12.265E
CA1396	1*	Nanpil	127 m	06°55.242N, 158°12.265E
CA1397	8*	Nanpil	180 m	06°54.252N, 158°11.491E
CA1398	8*	Nanpil	180 m	06°54.252N, 158°11.491E
CA1399	8*	Nanpil	180 m	06°54.252N, 158°11.491E
CA1400	9	Nanpil	5 m	06°56.609N, 158°13.550E
CA1401	9	Nanpil	5 m	06°56.609N, 158°13.550E
CA1402	5*	Lehn Mesi	139 m	06°51.107N, 158°11.896E
CA1403	5*	Lehn Mesi	139 m	06°51.107N, 158°11.896E
CA1404	10*	Mahnd	107 m	06°50.221N, 158°17.212E
CA1405	10*	Mahnd	107 m	06°50.221N, 158°17.212E
CA1406	7	Senipehn	95 m	06°51.906N, 158°16.010E
CA1407	7	Senipehn	95 m	06°51.906N, 158°16.010E

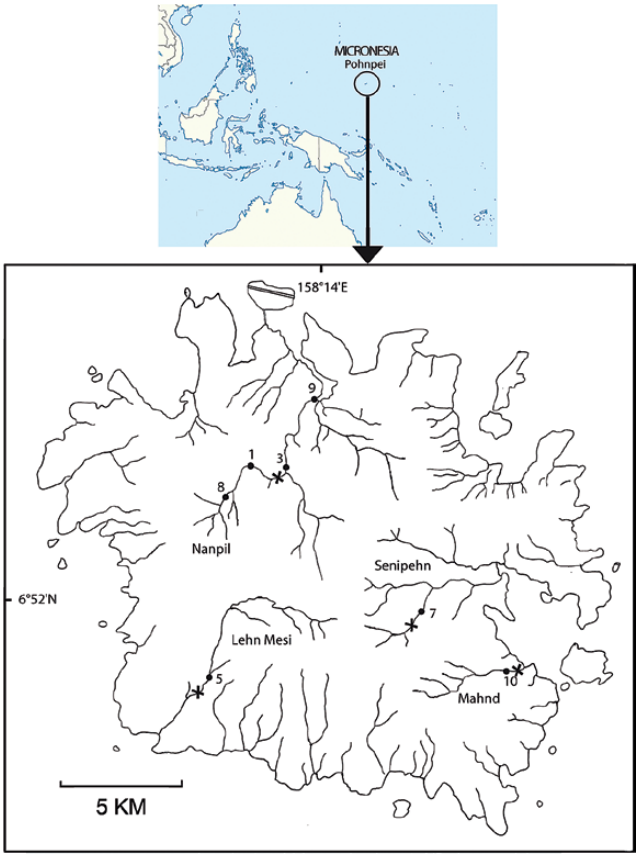


Figure 1. Pohnpei Island, Federated States of Micronesia, showing sampling stations (see Keith et al., 2012). Crosses indicate the position of the first major-waterfall for each sampled river.

of Micronesia, and 27 shrimp specimens were captured in seven of these stations at different altitudes. Out of these seven stations, three were located below the first major waterfall in each of the rivers (Table 1; Fig. 1).

Molecular analysis

We used the same protocol given by Mazancourt *et al.* (2017) to obtain sequences of 16S rRNA and COI gene for all the 27 specimens studied to perform a phylogenetic analysis.

Morphological analysis

Each specimen was dissected and the appendages prepared for microscopic observation. Microphotographs were taken using an Olympus UTV1X-2 camera mounted on an Olympus BX51 stereomicroscope. The images obtained were treated using the AnalySIS Work v5.0 software (Olympus Soft Imaging System) to measure 19 morphological variables (Table 2). Correlation analyses were performed using Statistica v.10 software (StatSoft), providing 2D scatterplots, regression, and r coefficients.

RESULTS

Sampling

The species of fishes, particularly the shrimp predators, were reported for each station. For stations 1, 5, 8, and 10, which were located above the first waterfall of each river, the only predator

Table 2. List of the 19 morphological variables measured.

Structure	Variable	Remarks
Pereiopod 1	1. Dactylus length-to-width ratio	-
	2. Dactylus length to propodus length ratio	-
	3. Chela length-to-width ratio	-
	4. Carpus length-to-width ratio	Width measured at widest part
Pereiopod 2	5. Dactylus length-to-width ratio	-
	6. Dactylus length to propodus length ratio	-
	7. Chela length-to-width ratio	-
	8. Carpus length-to-width ratio	Width measured at widest part
Pereiopod 3	9. Dactylus length-to-width ratio	Length including terminal spine
	10. Propodus length-to-width ratio	Width measured in the middle portion
	11. Propodus length to dactylus length ratio	-
Pereiopod 5	12. Dactylus length-to-width ratio	Length including terminal spine
	13. Propodus length-to-width ratio	Width measured in the middle
	14. Propodus length to dactylus length ratio	-
Rostrum	15. Rostrum length to carapace length ratio	Rostrum length from the orbit to tip
	16. Unarmed part length to armed part length ratio	-
	17. Number of teeth on dorsal margin	Including post-orbital teeth but not apical teeth
	18. Number of teeth on ventral margin	-
Cephalothorax	19. Carapace length	Laterally, from the orbit to the posterior margin

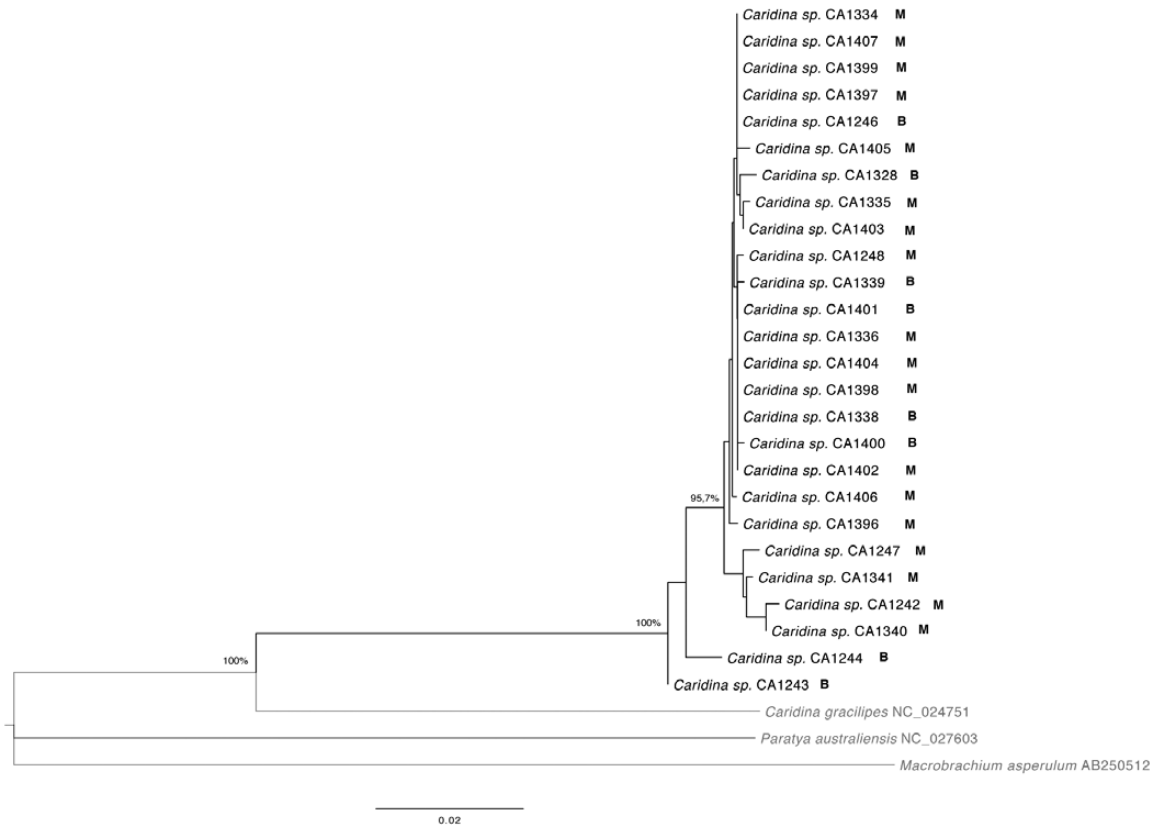


Figure 2. NJ tree of concatenated COI and 16S of the specimens studied. The numbers at nodes indicate bootstrap numbers. The letter M indicates specimens identified as *Caridina mertoni* because of their short rostrum and the letter B indicates specimens identified as *Caridina brachydactyla* because of their long rostrum.

able to climb up was the eel *Anguilla marmorata* Quoy & Gaimard, 1826.; The flagtails *Kuhlia marginata* (Cuvier, 1829) and *K. rupestris* (Lacépède, 1802) were present in station 3; for the station 7, *K. rupestris* and *A. marmorata* in station 7, and *A. marmorata*, *K. marginata*, and *Eleotris fusca* (Forster, 1801) in station 9.

Molecular analysis

After a molecular analysis based on two mitochondrial genes (16S and COI) (Fig. 2), we found that all of our specimens were all very close genetically (> 1% p-distances).

Morphological analysis

Of the 19 variables tested, five showed a significant correlation ($r > 0.5$) with elevation (Fig. 3). These variable were, from the highest to the lowest absolute r value, rostrum length to carapace length ratio (variable 15; Fig. 3b, $r = -0.9277$), number of teeth on ventral margin of rostrum (variable 18; Fig. 3d, $r = -0.6480$), unarmed part of rostrum length to armed part length ratio (variable 16; Fig. 3e, $r = -0.6868$), pereiopod 2 carpus length-to-width ratio (variable 8; Fig. 3j, $r = -0.6318$), and pereiopod 3 propodus length-to-width ratio (variable 10; Fig. 3o, $r = -0.5302$).

In order to refine the analysis, we hypothesized that the variables related to the rostrum would be linked to its length instead of elevation. To test this hypothesis, another correlation analysis was performed on the three concerned variables (16, 17, and 18) with rostrum length to carapace length ratio (Fig. 4).

We found that there was a strong correlation between rostrum length and variables 18 and 16 (Fig. 4b, $r = 0.67166$, and 4c, $r = 0.81086$, respectively). Out of the 19 variables tested, three are directly linked to elevation (variable 15, rostrum length to carapace length ratio; variable 8, pereiopod 2 carpus length-to-width ratio; and variable 10, pereiopod 3 propodus length-to-width ratio), with only the former showing a strong correlation ($r > \pm 0.9$; Fig. 5).

These correlations show that the specimens living at higher altitudes had a shorter rostrum (and therefore, a shorter unarmed dorsal margin and fewer ventral teeth), a shorter pereiopod 2 carpus, and a shorter pereiopod 3 propodus than specimens living at lower altitudes.

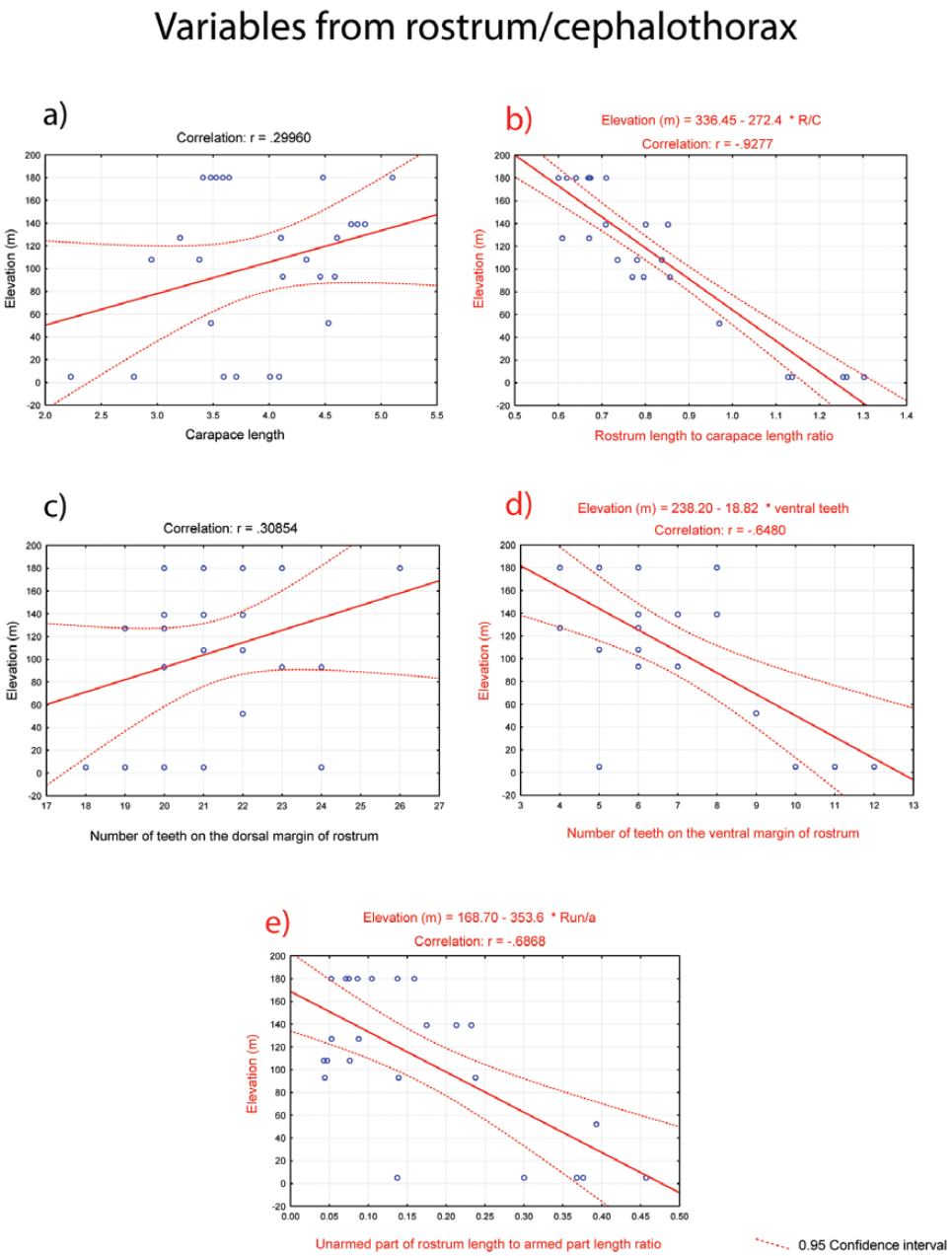


Figure 3. Correlation scatterplots of the 19 morphological variables with the elevation (in m); **a-e**, rostrum/cephalothorax; **f-i**, pereiopod 1; **j-m**, pereiopod 2; **n-p**, pereiopod 3; **q-s**, pereiopod 5. Above scatterplots, r coefficient, and equation of the regression line present for variables with significant correlations. Dashed lines indicate the 0.95 confidence interval.

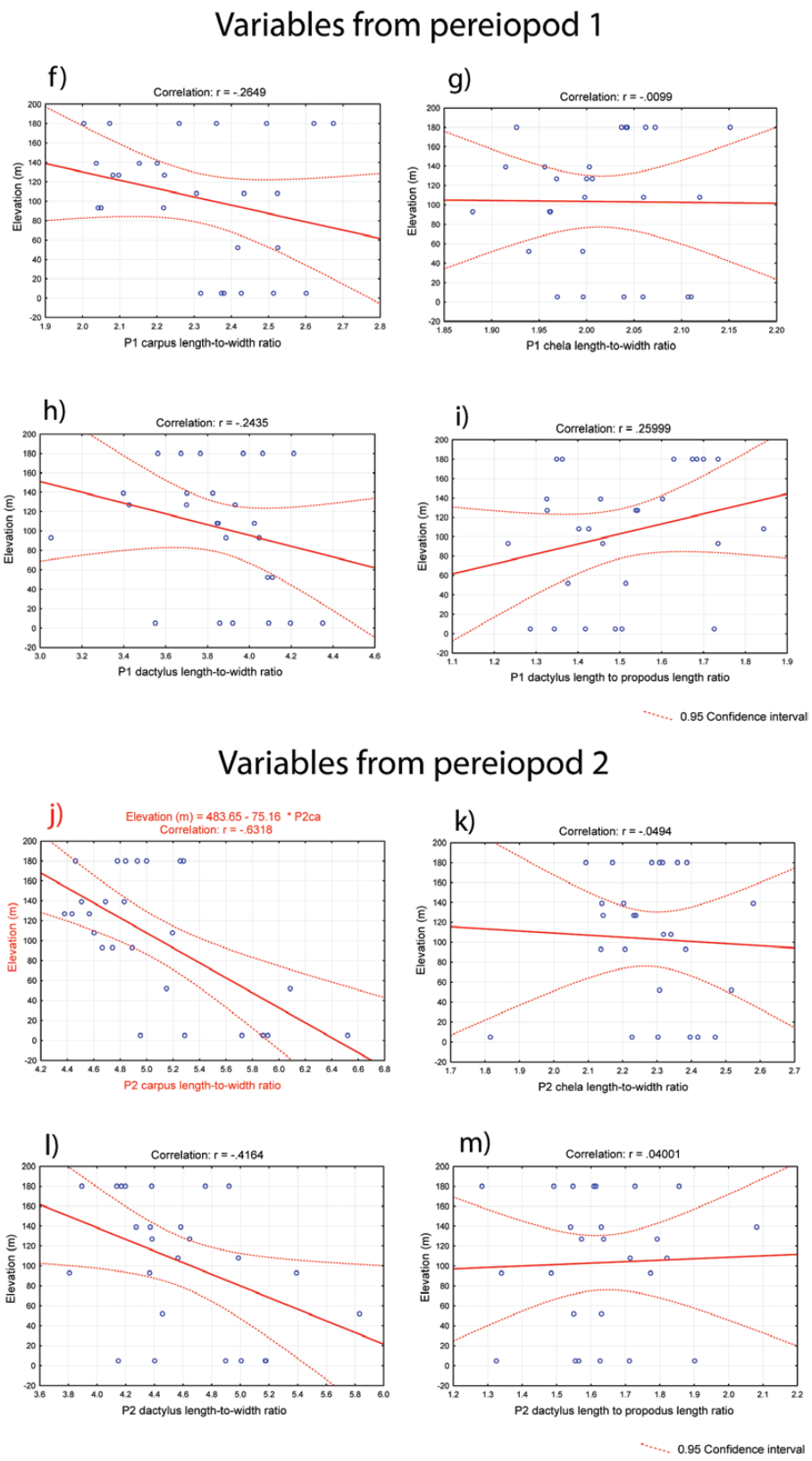


Figure 3. *Continued*

DISCUSSION

This is the first time that variation in a morphological character, the length of the rostrum in this case, is demonstrated to be linked to an environmental factor in *Caridina*. The elevation that was tested in this study is most likely not linked directly to the morphological changes observed. Indeed, several parameters can be shown to be related to elevation. The speed of the current increases with elevation, which would result in stronger mechanical forces being

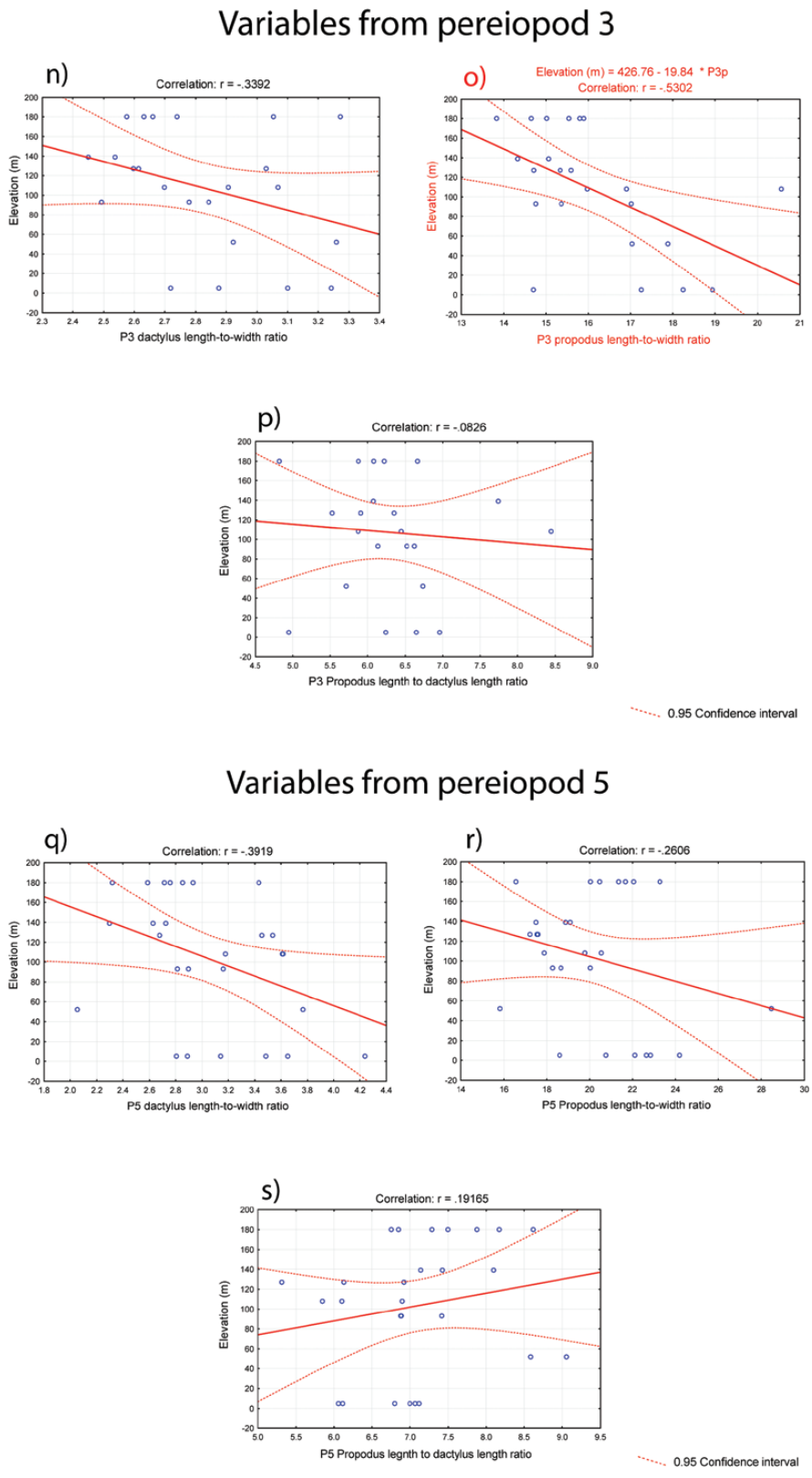


Figure 3. *Continued*

applied on the rostrum, limiting its growth. Furthermore, a longer rostrum tends to be more fragile and breaks easily, and might offer more current resistance, hindering movements in fast-flowing areas. The flow is certainly slower in the estuary at sea level, and little stress is applied on the rostrum, hence its long shape, whereas higher in the course, the slope of the stream is more important and the current faster. This phenomenon has been shown in another freshwater shrimp *Macrobrachium australe* (Guérin, 1838), with a longer rostrum in lentic habitats in contrast to a shorter rostrum in lotic habitats (Zimmermann *et al.*, 2012). This would also be valid for the other two morphological variables linked with elevation (i.e., pereiopod 2 carpus length and pereiopod 3 propodus

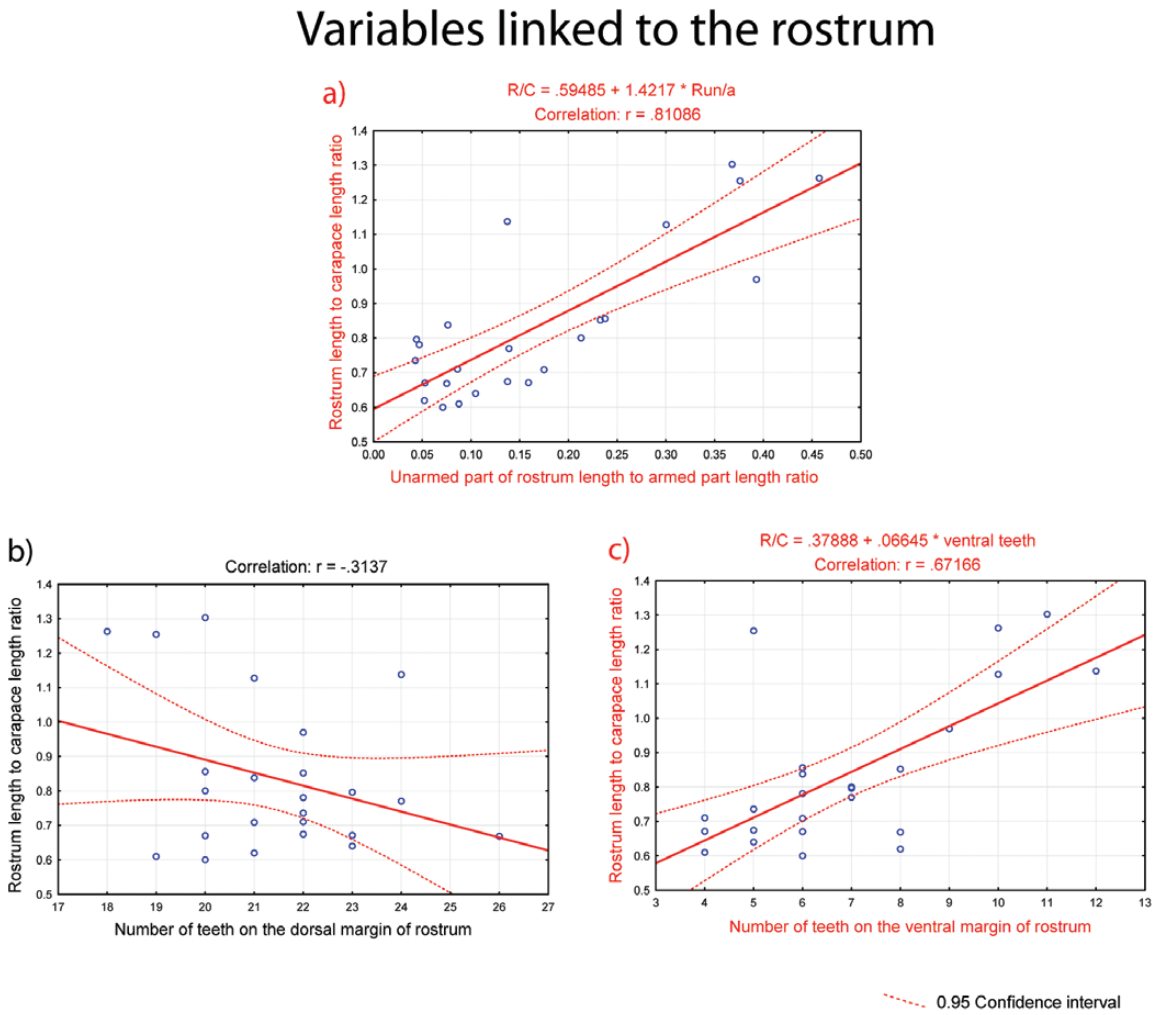


Figure 4. Correlation scatterplots of the three rostrum-related variables with the length of the rostrum. Equation of the regression line present when correlations are significant. Dashed lines indicate the 0.95 confidence interval.

length), the higher in the stream, the shorter and broader the legs, presumably an adaptation to better resist stronger currents.

Morphological adaptations to hydrodynamic parameters have been highlighted in crustaceans before, such as the evolution towards an elongated shape in shrimp-like crustaceans (Spaargaren, 1999). We can argue, however, that there can be areas at higher altitudes of the stream with little or no current, as the slope is not steady along all of its length. Another parameter linked to the altitude is temperature. The higher the elevation of the stream, the cooler the water, because of lower air temperatures as well as thicker vegetation cover providing more shade. Temperature could influence the morphology as growth would be slower at lower temperatures due to a lower metabolism of the animal (Lee & Fielder, 1981), resulting in a shorter rostrum. This variation with temperature has been shown in freshwater shrimp species of *Macrobrachium* (Dimmock *et al.*, 2004). Unfortunately, the water temperature at each station was not measured in our study so no correlation could be tested. The temperature difference between the estuary and the upper course in such tropical island can be up to more than 5°C (PK, unpublished data), which could significantly impact the metabolism and the growth of organisms living there. The amount of food available could also limit the growth of the shrimp. This species is a detritivore feeding in particular on decaying plants, which would tend to accumulate in downstream areas of slow current to the detriment of upstream fast-flowing zones. These last two parameters have indeed been demonstrated to significantly affect the size of atyid shrimps (Pérez-Reyes *et al.*, 2015).

The sediment size of the substrate also varies with slope and current speed. The substrate is composed of large blocks in steep-slope, fast-flowing areas, but replaced with sand or gravel in areas with a gentler slope (Keith *et al.*, 2010). This would imply that the shrimp could use the spaces between the rocks to hide from predators and a long rostrum would be undesirable for crawling in these narrow spaces. A long rostrum does not therefore hinder the shrimp living lower in the stream where the sediment provides fewer hideouts. On the contrary, as indicated by Jugovic *et al.* (2010) and Occasio-Torres *et al.* (2015), a long rostrum could be a protection against predators, making the shrimp more difficult to swallow. Predator pressure would be greater in the lower course than in the upper course, because many shrimp-eating fishes like species of Eleotridae or Kuhliidae are present in the lower course of rivers (Covich *et al.*, 2009; Keith *et al.*, 2010) and are unable to climb waterfalls. This limitation in the distribution and mobility of predators affects the distribution of others organisms. Atyids are more abundant in the areas situated above waterfalls where predators are less numerous (Covich *et al.*, 2009). We found the individuals with the longest rostra in the three stations (3, 7, and 9) where the most predators were present, which were all below the first waterfall and at the lowest altitudes. This kind of defensive feature is supposedly present in some species of the freshwater gastropods *Clithon* Montfort, 1810, in which specimens encountered

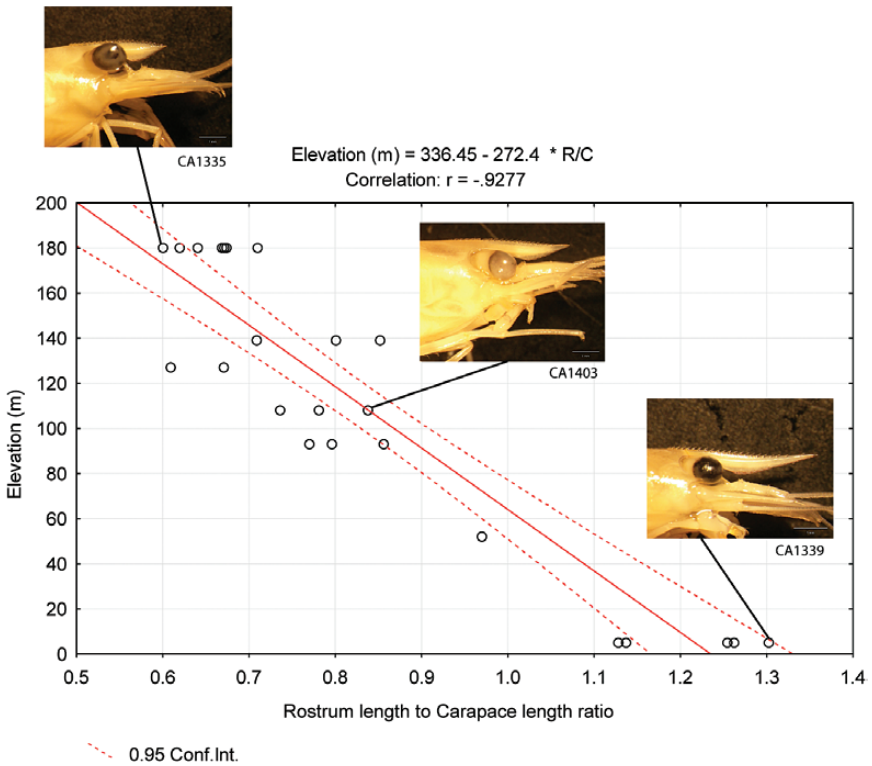


Figure 5. Correlation scatterplot of the rostrum length over carapace length with elevation. Some of the specimens are illustrated to show the variation.

in brackish water exhibit spikes on their shell, whereas specimens of the same species living higher in the river have smooth shells (Haynes, 2005). These spikes could be a protection against predators (Eichhorst, 2016). Eels, which are also known to be shrimp predators, however, are able to crawl upward these waterfalls (Keith *et al.*, 2010), which would still make some kind of defensive adaptation useful for prey living there. This kind of morphological adaptation against predation could be chemically induced by kairomones from predators, as shown in *Xiphocaris elongata* (Guérin-Méneville, 1857) (Ocasio-Torres *et al.*, 2014) and species of *Troglocaris* Dormitzer, 1853 (Jugovic *et al.*, 2010). If the presence of a long rostrum was only linked to the presence or absence of predators, one would expect only two classes of length, short and long, but we nevertheless showed that there is a continuum following a gradient, which is not related to the abundance of predators in the sampled stations.

To ease future citations of the phenomenon we describe here, we decided to name the elongation of the rostrum at lower altitudes in freshwater shrimps the "Pinocchio-shrimp effect". This name is in relation to the commercial name of *Caridina gracilirostris* in the aquarium trade, known for having a long and slender rostrum. This is not to be confused with the "Pinocchio effect" *sensu* Chapman (1990), Zelditch *et al.* (2004), and Zimmerman *et al.* (2012), which refers to Procrutes-based morphometric analyses in which a variation that is localized to a single landmark can be smeared out across many in the superimposition consensus.

This observed variation of the morphology of this species with environmental parameters shows that some of the characters widely used in the taxonomy of *Caridina* might not be as reliable as it was thought. We found here that rostrum length, but also the length of the unarmed portion of the dorsal part of the rostrum and the number of ventral teeth are highly plastic in this species, and presumably in others. We nevertheless found that the number of dorsal teeth seems surprisingly unrelated to the length of the rostrum (Fig. 4a), and therefore with the elevation, making it a more reliable taxonomic character. We strongly suggest to always perform some type of molecular work to studying and describing a new species of *Caridina* in order to unravel this kind of variation within a species, focus on the morphology, and uncover any type of variation. This would provide enough characters to confidently identify the species in the field. It is likely that the different described species in the genus exhibit the same kind of polymorphism that has been shown here. Species with a short rostrum like *C. mertoni* and *C. sundanella* Holthuis, 1978 need to be studied in order to ensure that other species with a long rostrum do not actually belong to species having such polymorphism. The taxon investigated in this study is probably neither *C. brachydactyla* nor *C. mertoni* so an in-depth taxonomic study should follow.

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Description of a new species of *Caridina* (Crustacea: Decapoda: Atyidae) from two Micronesian islands (Guam and Babeldaob)

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Abstract

During field trips to Guam and Babeldaob Islands in Micronesia, freshwater shrimps were sampled and assigned either to *Caridina brachydactyla* De Man, 1908 or *C. mertoni* J. Roux, 1911 (Decapoda: Atyidae), following previous inventories. In combining morphological data with a genetical analysis, it appeared that all the specimens belonged to a new species, *Caridina variabilis sp. nov.*, here described with its distribution. The status of this new species is clarified and finally, neither *C. brachydactyla*, nor *C. mertoni* occur in these two islands. DNA sequences of 16S were obtained from the syntypes of *C. mertoni*.

Key words: *Caridina*, New species, Micronesia, Integrative taxonomy, 16S, Morphology

Introduction

Micronesia is a vast expanse of more than 2000 Pacific islands and atolls stretching from the Caroline and Mariana islands in the West, to the Marshall, Nauru and Kiribati islands in the East (Fig. 1).

The Mariana Islands is a crescent-shaped archipelago comprising the summits of fifteen mostly dormant volcanic mountains. At the southern end of the chain, Guam (formally the Territory of Guam) is an unincorporated and organized territory of the United States of America. It is located at 13°28'N and 144°45'E, and is 50 km long and 6 to 19 km wide, with an area of 549 km^2. The highest point is Mount Lamlam at 407 meters above sea level. It belongs to the Mariana ridge, which was created by the colliding Pacific and Philippine Sea tectonic plates (Tracey *et al*, 1964)

The Caroline Islands consist of a chain of seamounts, atolls and high islands extended south-eastward to the Marshall Islands. At the western edge of the Caroline Islands the Republic of Palau comprises a curved archipelago of approximately 350 islands lying between 4 and 8 degrees north latitude and 131–135 degrees east longitude. The high island of Babeldaob covers 334 km^2 accounting for over 80% of Palau's landmass, with a highest elevation point of only 230 m. Palau, aged of 30 Ma, is the crest of the Palau-Kyushu ridge (Kobayashi, 2004).

The freshwater shrimp genus *Caridina* H. Milne Edwards, 1837, comprising 298 species (WoRMS database: http://www.marinespecies.org/aphia.php?p=taxdetails&id=240672) and mostly present in the Indo-Pacific region, is the most diversified genus of the Atyidae (De Grave *et al*, 2015) and an important ecological component in the tropical streams (Covich *et al.*, 1999; Pringle *et al.*, 1993). Its high diversity combined with the lack of informative morphological characters has led to a confused taxonomy (Richard & Clark, 2009). Indeed, until recently, the taxonomy of the genus was mainly based on morphological characters. Some have been proven highly variable within a species (e.g. rostrum shape and indentation or coloration) and so taxonomically non-informative, making it difficult to establish boundaries between them (de Mazancourt *et al*., 2017). Thus, there is a need for an integrative and standardized approach to improve the group's systematics, focusing on informative morphological features and using molecular characters (Page *et al.*, 2005; Page & Hughes, 2011).

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From the Mariana Islands (with no island specified), Bouvier (1925) reported six specimens belonging to *C. nilotica brachydactyla* De Man, 1908 (A. Marche coll.) with a short rostrum armed to the tip on both margins. Specimens are deposited in MNHN (MNHN-IU-2015-1824). Leberer & Nelson (2001) collected *C. longirostris* H. Milne Edwards, 1837 and *C. nilotica* (P. Roux, 1833) from two rivers of Guam. Reviewed later by Leberer & Cai (2003), *C. longirostris* became *C. brachydactyla* and *C. nilotica* became *C. mertoni* Roux, 1911 in the Pacific. Recently one of us (DCR in March 2015) collected various *Caridina* from Guam, with some specimens bearing a long rostrum and attributed to *C. brachydactyla*, whereas others, with a short rostrum, were identified as *C. mertoni* following the previous identifications made by Leberer & Cai, 2003.

Bright (1979) reported *C. brachydactyla* from Palau among seven Atyid species, depositing them at the USNM (USNM 172589 and USNM 172590). Holthuis (year unknown) named specimens from Palau, based on material collected by H. A. Fehlmann on the 30 October 1956 from Arakitaoch stream and deposited in USNM as *C. brachydactyla uncata* (holotype USNM 105430, paratypes UNSNM 105434) but never described or published this taxon.

Keith *et al*. (2011), reviewing the freshwater decapods of Palau conducted a survey from 22 February through 5 March 2011, identifying some specimens as *C. brachydactyla* or as *C. mertoni*.

As we examined more and more specimens from Palau and Guam, we gradually started to question the validity of these two species from Micronesia. Consequently, we here examined recent specimens and Museum collections in combining morphological data with a 16S mtDNA analysis.

Material and methods

Collection of specimens. Rivers and sites surveyed are indicated in Figure 1 and Table 1. Specimens from Guam were collected by net. Specimens from Palau were collected by electrofishing (portable Dekka 3,000 electric device, Germany). All material preserved in 75%–95% alcohol has been deposited in the collections of the Muséum national d'Histoire naturelle in Paris (MNHN, specimens n° MNHN-IU-2017-2082 to MNHN-IU-2017-2106).

DNA extraction, amplification and sequencing. For recent specimens, DNA was extracted from abdominal tissues using the semi-automatic Ependorf ep-Motion 5075 robot. Fragments of the mitochondrial 16S rRNA (~ 520 bp) were amplified using the primers 16Sa-L (CGCCTGTTTATCAAAAACAT) and 16Sb-H2 (CTCCGGTTTGAACTCAGATCA) (Palumbi, 1996). DNA amplification was performed in 25µl PCR reactions, containing approximately 3 ng of template DNA, 2.5 mM MgCl2, 0.26 mM of each nucleotide, 0.3 µM of each primer, 5% DMSO, 1 ng of BSA and 1.5 units of QBIOTAQ polymerase (MPBiomedicals). Amplification products were generated by an initial denaturation step of 4 min at 94°C followed by 35 cycles of denaturation at 94°C for 30s, annealing at 52°C for 40 s, extension at 72°C for 60 s and a final extension step at 72°C for 7 min.

For old collection specimens (Syntypes of *C. mertoni*), a CTAB protocol was used to extract DNA from pleopods. A shorter fragment of the 16S rRNA (332 bp) was amplified using two newly designed primers: 16S-Car-81F (AGGTAGCATAATAAATAGTC) and 16S-Car-413R (CTGTTATCCCTAAAGTAAC). DNA amplification was performed in 25µl PCR reactions, containing 2.5 mM MgCl2, 0.26 mM of each nucleotide, 0.3 µM of each primer, 1 ng of BSA and 1.5 units of QBIOTAQ polymerase (MPBiomedicals). Amplification products were generated by an initial denaturation step of 4 min at 94°C followed by 45 cycles of denaturation at 94°C for 30s, annealing at 55°C for 30 s, extension at 72°C for 40 s and a final extension step at 72°C for 7 min.

PCR products were sequenced using same primers and in both directions to insure the accuracy of base calls. Chromatograms were edited using Geneious v.8 software (http://www.geneious.com/ Kearse *et al*, 2012). All sequences were deposited in GenBank (Numbers MG707138 to MG707171). Sequences for the species *Caridina elongapoda* and *Paratya australiensis* published by Page *et al.* (2007) were included in our analysis, *Paratya australiensis* sequence being used as outgroup.

Molecular analyses DNA sequences were aligned using MEGA7 software (Kumar *et al.*, 2016) with Muscle algorithm (Edgar, 2004). Using Bayesian information criterion in PartitionFinder (Lanfear *et al.*, 2012) we retained the GTR + G + I model. Best-scoring ML trees were estimated using RAxML HPC2 v.8.2.10 (Stamatakis, 2014) implemented in the Cyber Infrastructure for Phylogenetic Research (CIPRES) portal v.3.1. (Miller *et al*., 2010) (https://www.phylo.org/). One hundred independent searches, each starting from distinct random trees, were conducted. Robustness of the nodes was assessed using non-parametric bootstrapping (Felsenstein, 1985) with

1,000 bootstrap replicates. We considered a group to be 'moderately supported' if it had a bootstrap support value (B) between 75 and 89% and 'highly supported' when B ≥ 90%.

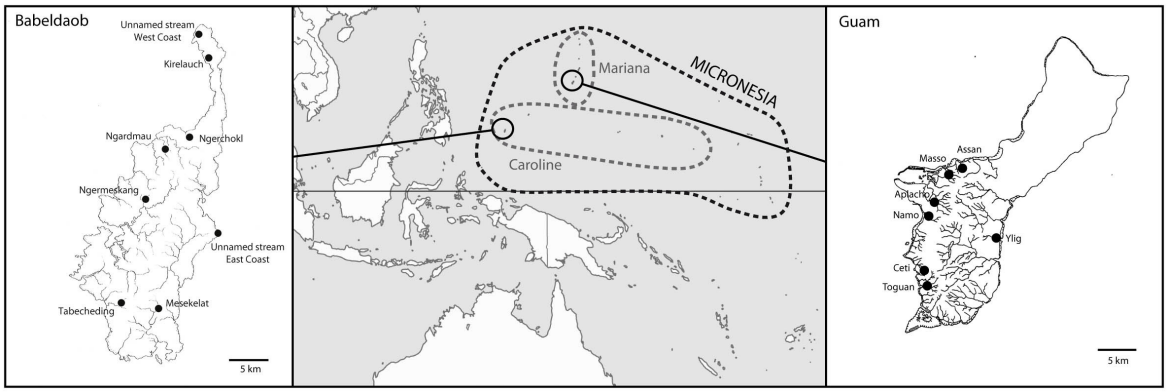


FIGURE 1. Rivers and sites surveyed in Babeldaob and Guam (black dots: prospected sites)

TABLE 1. Sampling localities data.

Island	Dates	Coordinates	Rivers	Altitude (m)
Babeldaob	27/02/2011	07°27.169'N 134°31.748'E	Tabecheding	28
(P. Keith, P. Gerbeaux, G. Marquet, L. Taillebois, M. Castelin coll.)	27/02/2011	07°36.527'N 134°36.958'E	Ngerchokl	36
	28/02/2011	07°41.417'N 134°37'754E	Kirelauch	2
	28/02/2011	07°43.056'N 134°36.924'E	Unnamed West Coast stream	10
	28/02/2011	07°29.909'N 134°38.099'E	Unnamed East Coast stream	7
	01/03/2011	07°32.484'N 134°34.670'E	Ngermeskang	75
	02/03/2011	07°35.482'N 134°35.573'E	Ngardmau (waterfall)	39
	03/03/2011	07°26.305'N 134°34.328'E	Mesekelat	20
Guam	30/01–03/02/2011	13°28.168'N 144°42.762'E	Assan	11–16
(D. Christopher Rogers, A. Farahi, T. Jones coll.)	30/01–03/02/2011	13°27.200'N 144°41.871'E	Masso	34
	30/01–03/02/2011	13°24.345'N 144°41.187'E	Aplacho	58–75
	30/01–03/02/2011	13°23.904'N 144°39.959'E	Namo	4
	30/01–03/02/2011	13°19.514'N 144°40.183'E	Ceti	197
	30/01–03/02/2011	13°17.117'N 144°39.955'E	Toguan	15–41
	30/01–03/02/2011	13°23.589'N 144°45.636'E	Ylig	2

To visualize genealogical relationships among haplotypes from our two sampling localities (Guam and Palau), a median-joining network was built using Network 4.1.1.2 (Bandelt *et al*. 1999) with equal weights for variable sites. A maximum parsimony algorithm was applied to simplify the complex branching pattern and generate a network representing all of the most parsimonious intraspecific phylogenies.

Abbreviations for museums. Muséum national d'Histoire naturelle, Paris: MNHN; Naturhistorisches Museum Basel, Basel: NMB; National Museum of Natural History, Smithsonian Institution, Washington, DC: NMNH; Rijksmuseum van Natuurlijke Historie (now in the Naturalis Biodiversity Center, Leiden): RMNH; Bernice Pauahi Bishop Museum, Honolulu: BPBM; Zoological Research Collection, Singapore: ZRC

Abbreviations for morphological analyses. The following abbreviations are used in the present text: cl, carapace length (mm): measured from the post-orbital margin to the posterior margin of the carapace. P1: first pereiopod. P2: second pereiopod. P3: third pereiopod. P5: fifth pereiopod. Pl1: first pleopod. Pl2: second pleopod.

Morphological comparison. The rostrum, the general cephalon, the pereiopods 1, 2, 3 and 5 and the abdomen were observed using a stereoscopic microscope. The proportions of the various joints of the appendages were measured using microphotographs and the AnalySIS Works software (Olympus). Drawings were made using the "Digital Inking" method (Coleman, 2004; 2006) by tracing vectorial paths on stacks of high-resolution photographs using Adobe Illustrator (CS6) and a WACOM MPTZ-1230 graphic tablet.

PCA for some museum specimens. Some museum specimens, for which DNA could not be retrieved for a genetic analysis, but still having all their pereiopods, were measured and included in a PCA with our recent sequenced specimens and old specimens that yielded DNA. We followed the method proposed by Konan *et al* (2010) using Statistica v.10 software (Statsoft). The same 19 ratios and counts used in previous work (de Mazancourt *et al*, 2017) were used for the analysis.

Old specimens without genetic data included in the analysis:

Palau. Holotype USNM 105430 *C. brachydactyla uncata* one ovigerous female cl 3.5mm;

Mariana Islands. MNHN-IU-2015-1824 *C. brachydactyla* (A. Marche coll.)

Indonesia. Aru Island, Syntypes *C. mertoni*. NMB 693a one male cl 2.7 mm; NMB 693b one male 3.1 mm; NMB 693c one non-ovigerous female, Flores Island, Lectotype *C. brachydactyla* ZMA 977 one ovigerous female cl 4.8 mm, Sulawesi, Paralectotypes ZMA 2552 two ovigerous female cl; 5.3–5.4 mm.

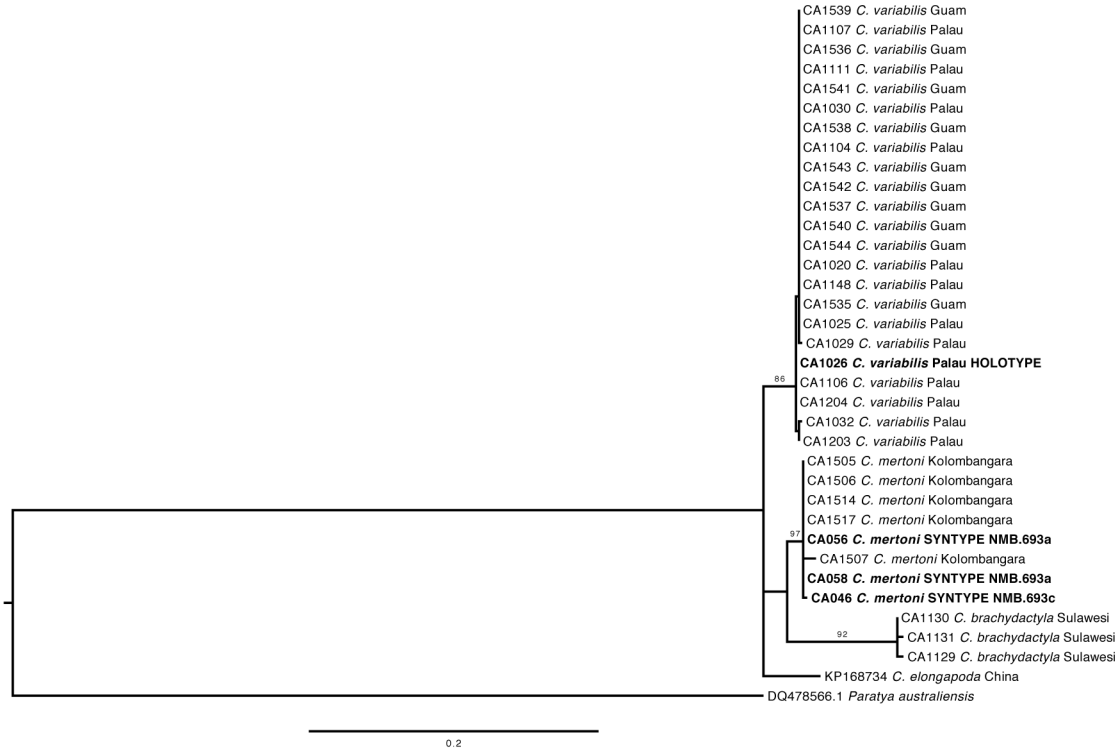


FIGURE 2. Maximum likelihood phylogenetic tree (16S mtDNA) of the specimens. CAXXXX numbers are lab IDs of the specimens.

Results

Phylogenetic analyses. A total of 31 recent specimens were sequenced, including 23 of the new species (10 from Guam, 13 from Palau), 5 recent specimens of *C. mertoni* from Kolombangara Island, 3 specimens of *C.*

brachydactyla from type locality, Sulawesi and a shorter fragment could be retrieved from 3 old collection specimens (*C. mertoni* syntypes from Kai Island). All of the specimens from Palau and Guam fall in a same moderately supported clade, with little genetic structure and are well distinct from *C. brachydactyla* specimens and *C. mertoni* syntypes. We considered this genetic group to be a new species that we describe below.

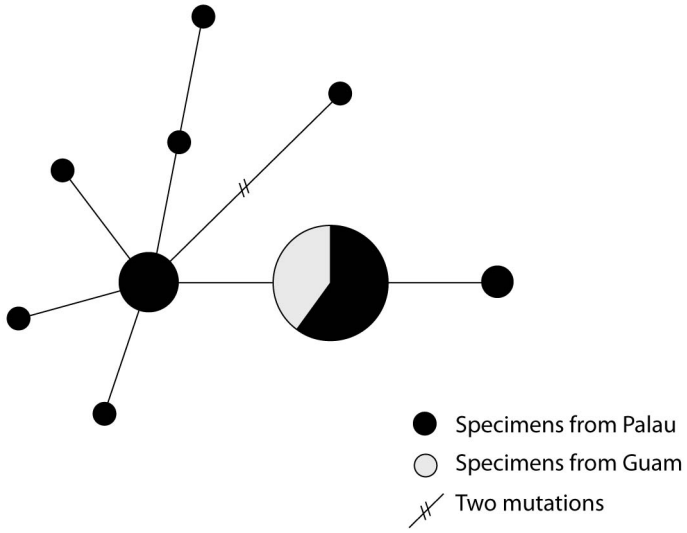


FIGURE 3. Haplotypes network of the specimens sequenced.

Haplotypes network. There is a difference in haplotype diversity between the two localities. Indeed, there are 9 haplotypes found in Palau (vs only one in Guam). One of the haplotypes, also the most frequent, is shared between the two islands.

Taxonomy

Atyidae De Haan, 1849

Caridina H. Milne Edwards, 1837

Caridina variabilis, n. sp.

(Fig. 4)

Material examined. Holotype: PALAU. MNHN-IU-2017-2082 (DNA: CA1026) 1♂ cl 3.4 mm, Tabecheding River 27/02/2011, 07°27.169N, 134°31.748E.

Paratypes: PALAU. One male cl 2.4 mm (MNHN-IU-2017-2083, DNA: CA1032) and three non-ovigerous females cl 4.1 mm (MNHN-IU-2017-2084 DNA: CA1029) 4.8 mm (MNHN-IU-2017-2085 DNA: CA1025) & cl. 4.9 mm (MNHN-IU-2017-2086 DNA: CA1030), Tabecheding river 27/02/2011, 07°27.169N, 134°31.748E. 2♀ ovig cl 4.0 mm (MNHN-IU-2017-2087 DNA: CA1149) & 4.4 mm (MNHN-IU-2017-2088 DNA: CA1148), Ngerchokl River 27/02/2011, 07°36.527N, 134°36.958E. 1♀ ovig cl 3.9 mm (MNHN-IU-2017-2089 DNA: CA1111), Kirelauch River 28/02/2011, 07°41.417N, 134°37.754E. 2♂ cl 3.2 mm (MNHN-IU-2017-2090) & 3.3 mm (MNHN-IU-2017-2091), West Coast stream Northern Tip 28/02/2011, 07°43.056N, 134°36.924E. 1♀ cl 3.8 mm (MNHN-IU-2017-2092 DNA: CA1106) and 2♀ ovig cl 4.2 mm (MNHN-IU-2017-2093 DNA: CA1104) & 3.8 mm (MNHN-IU-2017-2094 DNA: CA1107), small stream East Coast 07°29.909N, 134°38.099E. 1♀ ovig cl 4.2 mm (MNHN-IU-2017-2095 DNA: CA1020), Ngermeskang River 01/03/2011, altitude: 75m, 07°32.484N, 134°34.670E. 1♀ ovig cl 4.4 mm (MNHN-IU-2017-2096 DNA: CA1203), Ngardmau Waterfall 02/03/2011, 07°35.482N, 134°35.573E. 1♀ cl 3.6 mm (MNHN-IU-2017-2097 DNA: CA1204), Mesekelat River 03/03/2011, 07°26.305N, 134°34.321E.

PALAU Not *C. b. uncata* Holthuis: USNM 105430: holotype, 1♀ ovig cl 3.5 mm Arakitaoch River; USNM 105434: paratype, 1♀ cl 4.4 mm.

Not *C. brachydactyla* : USNM 172589: 1♂ cl 2.5 mm and 1♀ cl 3.0 mm; USNM 172590: 1♀ cl 5.5 mm.

GUAM. Assan River: DCR (D. Christopher Rogers collections) 9♂ cl 2.7 mm (MNHN-IU-2017-2098 DNA: CA1535), 3.5 mm (MNHN-IU-2017-2099 DNA: CA1536), 2.3 mm (MNHN-IU-2017-2100 DNA: CA1537), 2.7 mm (MNHN-IU-2017-2101 DNA: CA1539), 2.8 mm (MNHN-IU-2017-2102 DNA: CA1540), 2.4 mm (MNHN-IU-2017-2103 DNA: CA1541), 2.2 mm (MNHN-IU-2017-2104 DNA: CA1542), 2.7 mm (MNHN-IU-2017-2102 DNA: CA1543), 2.3 mm (MNHN-IU-2017-2105 DNA: CA1544) and 1♀ ovig cl 4.5 mm (MNHN-IU-2017-2106 DNA: CA1538). BPBM 4227 1♀ ovig cl 4.4 mm; 13° 28' 18.24"N, 144° 42' 48.66"E; 03/02/2011; DCR: 14♂ cl 2.1–3.4 mm, 6♀ cl 2.8–3.3 mm, and 4♀ ovig cl 3.4–4.3 mm; 13° 28' 18.24"N, 144 ° 42' 48.66"E. 02/02/2011. Masso River: DCR: 9♂ cl 2.2–3.6 mm, 3♀ cl 2.7–3.2 mm, and 13♀ ovig cl 3.4–4.5 mm; 13° 27' 32.85"N, 144° 41' 54.26"E; 1 February 2011. Namo River: 2♂ cl 2.4–2.8 mm, 2♀ cl 2.6–3.0 mm, and 11♀ ovig cl 3.4–4.2 mm; 13° 23' 58.59"N, 144° 39' 58.81"E; 01/02/2011. DCR: 6♂ cl 2.4–3.1 mm; 13° 23' 57.16"N, 144° 40' 05.22"E; 02/02/2011. Ceti River: 1♀ cl 2.3 mm, and 7♀ ovig cl 3.1–4.1 mm; 13° 19' 34.66"N, 144° 40' 09.12"E; 31/01/2011. Toguan River: 29♂ cl 2.0–3.4 mm, 16♀ cl 2.4–3.5 mm, and 34♀ ovig cl 3.5–4.3 mm; 13° 17' 17.94"N, 144° 39' 55.77"E; 31/01/2011. DCR: 8♂ cl 2.2–3.4 mm, 1♀ cl 2.6 mm, and 9♀ ovig cl 3.6–4.0 mm; 13° 17' 15.79"N, 144° 40' 02.51"E; 02/02/2011. Ylig River: 1♂ cl 2.4 mm, and 3♀ ovig cl 3.3–4.1 mm; 13° 23' 46.21"N, 144° 45' 54.85"E; 30/01/2011.

MARIANA ISLANDS (Island not specified): Not *C. brachydactyla*: MNHN-IU-2015-1824: 2♀ ovig cl 4.2–5.2 mm.

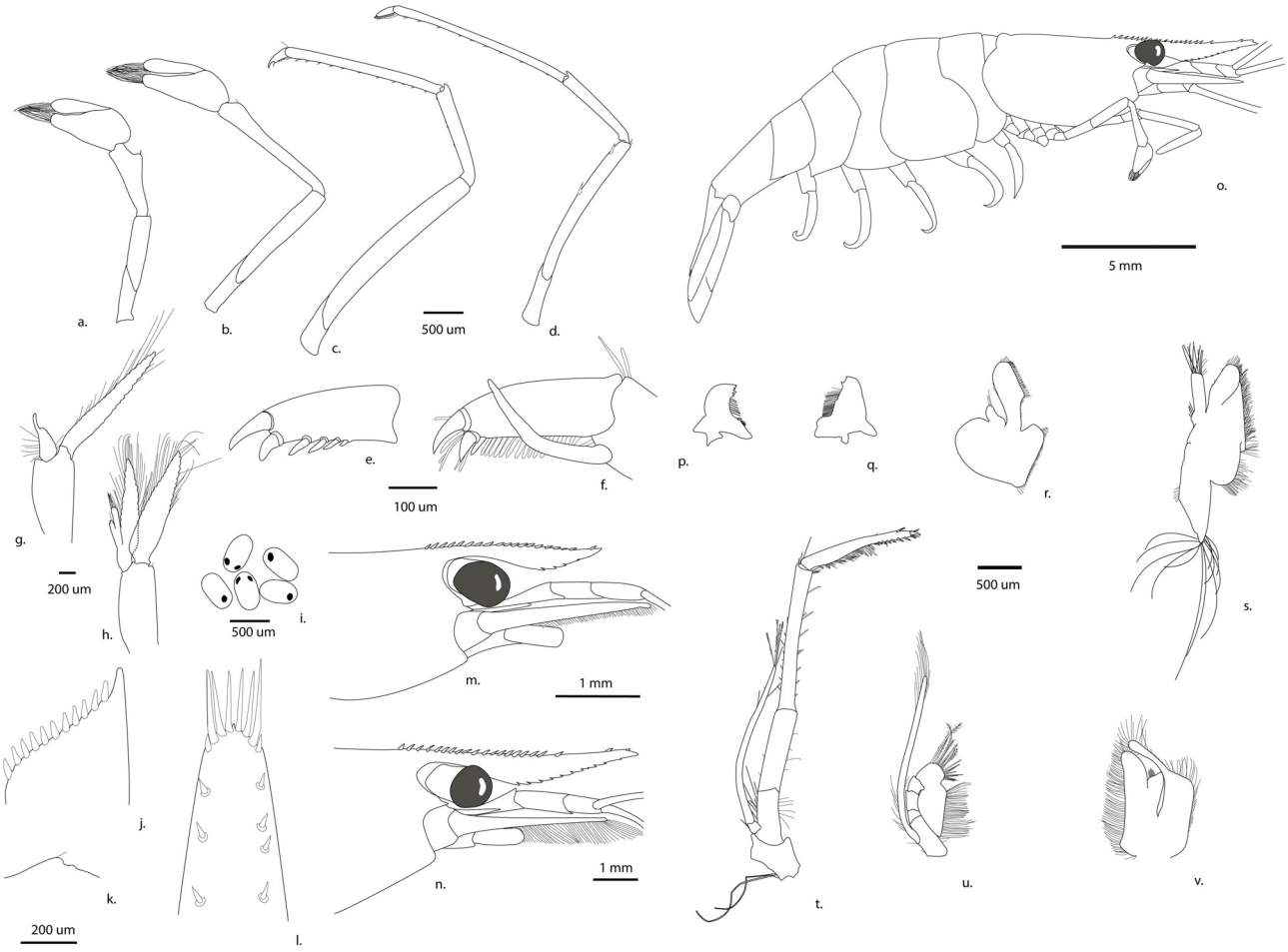


FIGURE 4. a. First pereiopod; b. Second pereiopod 2; c. Third pereiopod; d. Fifth pereiopod; e. Dactylus of third pereiopod; f. Dactylus of fifth pereiopod; g. Male first pleopod; h. Male second pleopod; i. Eggs; j. Uropodial diaeresis; k. Pre-anal carina; l. Telson; m., n. Cephalothorax; o. General appearance; p. Right mandible; q. Left mandible; r. First maxilla; s. Second maxilla; t. Third maxilliped; u. Second maxilliped; v. First maxilliped.

Comparative material.

- *Caridina brachydactyla* De Man, 1908

Type material: INDONESIA*: Caridina nilotica var. brachydactyla:* lectotype, Flores, Reo, RMNH Crust D. 977, 1♀ ovig cl 4.8 mm; paralectotypes Flores, near Mbawa, RMNH 2552, 2♀ ovig (cl 5.3–5.4 mm).

Other material: INDONESIA: *C. brachydactyla*: NMB 1054a, Bali, 1♀ cl 5.8 mm. Sulawesi, Palopo, Macaui, 63.10, 2♂ cl 2.7–3.7 mm; 1♀ ovig cl 4.3 mm and 1♀, cl 4.8 mm; Palopo, Tojo, 64.10.3 1♀ ovig cl 4.0 mm.

- *Caridina mertoni* Roux, 1911

Type material: INDONESIA; Cotypes: Grand Kai, NMB 693a: 2♂ cl 2.7–3.8 mm and 1♀ cl 4.1 mm; NMB 693b: 1♂ cl 3.1 mm and 1♀ ovig cl 4.8 mm; NMBc 1♀ cl 4.3 mm. MNHN-IU-2015-1819 1♂ cl 3.8 mm; MNHN-IU-2015-1820 1♂ cl 3.4 mm.

Other material: SOLOMON ISLANDS: Kolombangara. 3♂ cl 3.9 (MNHN-IU-2017-2107 DNA: CA1506), 3.0 mm (MNHN-IU-2017-2108 DNA: CA1507), 3.7 mm (MNHN-IU-2017-2109 DNA: CA1505), 1♀ ovig cl 3.9 mm (MNHN-IU-2017-2110), Manolu River 10/11/2015 08°05.312S 157°00.813E; 1♀ cl 6.2 mm (MNHN-IU-2017-2111 DNA: CA1514) and 1♀ ovig cl 4.1 mm (MNHN-IU-2017-2112 DNA: CA1517), Sulumuni River 12/11/2015 08°02.253'S 157°09.257'E.

- *Caridina elongopoda* Liang & Yan, 1977

HONG-KONG: Pak Tam Chung and Kai Sai Chau, 3♂ cl 2.8–3.5 mm and 1♀ cl 4.2 mm. W. Klotz coll. MALAYSIA: ZRC 1998.0865 Pulau Tioman, Sungai Asah, 24/06/1997 1♂ cl 4.2 mm, 1♀ cl 4.3 mm, 1♀ ovig cl 4.3 mm Ng *et al.* coll..

- *Caridina peninsularis* Kemp, 1918

Type material: *Caridina brachydactyla* subsp. *peninsularis* Kemp, 1918: MALAYSIA; lectotype Botanical garden, Penang Island, coll. N. Anandale, Feb. 1916, MNHN-IU-2015-1749: 1♂ cl 3.2 mm; paralectotypes same data as lectotype, MNHN-IU-2015-1750: 1♀ ovig cl 5.4 mm; NHM 1919.11.1.12-21 (1761124): 1♀ cl 3.4 mm; 1♀ ovig cl 5.2 mm; 1♂ cl 3.9 mm.

Other material: SINGAPORE; Tanglin (incorrectly spelt Tangtum in NHM register and on label, see Richards & Clark, 2014), coll. Bedford & Lanchester, det. D.S. Johnson, 1958, NHM reg. 1958.8.7.14–17 (1749569): 1♀ ovig cl 5.1 mm; 1♂ cl 4.2 mm.

Description. *Cephalothorax*. Rostrum (Fig. 4m): very variable in length, 0.7–1.5 of cl, reaching to scaphocerite apex, armed dorsally with 16–24 teeth, distal unarmed portion 0.1–0.7 times that of armed portion, with one or two subapical teeth, 1–4 teeth on carapace posterior to orbital margin, ventral margin with 4–15 teeth. Number of dorsal teeth before the most proximal ventral tooth 10–17. Rostral formula: (16-24 (1-4) + 1-2 / 4-15). Antennular spine acute, placed slightly below orbital angle. Pterygostomian margin rectangular.

Eyes well developed, anterior end reaching to 0.7 length of antennular peduncle basal antennomere. *Antennular peduncle* (Fig. 4m, 4n, cephalothorax view, and 4o, general view) 0.9 times as long as carapace. Anterolateral angle reaching 0.29 length of the second antennomere; second antennomere distinctly longer than third. Stylocerite reaching to 0.8 length of antennular peduncle basal antennomere. Scaphocerite extending slightly beyond antennular peduncleapex, length about 2.8 times width. Scaphocerite anteriolateral spine well developed, about level with antennular peduncle distal margin, both overreached by the lamella. Antennal peduncle near the base of the scaphocerite with a strong and slender lateral spine.

Mandibles dimorphic; left mandible (Fig. 4q) more developed, corpus large, robust with three strong sharp teeth separated by ridged gap; incisor and molar processes separated by two patches of long setae; molar process ridged. Right mandible (Fig. 4p) with six sharp incisor teeth, medially with two groups of long setae; molar process narrow, elongate, ridged. Maxillula (Fig. 4r). Lower lacinia with margin broadly rounded, bearing several rows of setae. Upper lacinia elongate, with medial margin bearing a number of distinct teeth, palp setose.

Maxilla (Fig. 4s) upper and middle endite with marginal and submarginal plumose setae. Lower endite with simple setae; palp narrow, shorter than upper endite cleft with a few setae. Scaphognathite fringed with long setae, tapering posteriorly with some long, curved setae at posterior end.

First maxilliped (Fig. 4v) endopodite ultimate segment medial margin with long denticulate setae, lower portion with transverse rows of simple setae. Palp elongate, setose. Exopod flagellum long and narrow distally with marginal plumose setae. Caridean lobe large, with marginal setae.

Second maxilliped (Fig. 4u) endopodite ultimate and penultimate antennomeres fused, reflected against basal antennomeres. Ultimate, penultimate and basal antennomeres medial margins with long setae of various types;

flagellum very long, slender with marginal plumose setae distally; podobranch well developed with reduced branchial lamella.

Third maxilliped (Fig. 4t) with terminal article reaching third antennular peduncle antennomere apex; distal antennomere about 10 times as long as wide, slightly shorter than the penultimate, ending in large hamulate apical spine surrounded by simple setae. Penultimate antennomere about eight times as long as wide, with group of transverse rows of simple setae. Exopod flagellum well developed, about a third the length of endopodite second article, distal margin with long plumose setae.

Pereiopods. Pereiopods I–IV with epipods.

P1 (Fig. 4a): chela about 1.8–2.3 times as long as wide, movable finger 3.1–4.6 times as long as wide, 1.1–1.7 times length of palm; carpus 2.1–3.5 times as long as wide.

P2 (Fig. 4b) more slender and longer than first pereiopod with chela 2.1–3.0 times as long as wide: movable finger 4–5.4 times as long as wide, 1.3–1.9 times length of palm; carpus slender 5.2–7.8 times as long as wide.

P3 (Fig.2c): slender, dactylus (Fig. 4e) 2.8–4.0 times as long as wide, including terminal spine), ending in two strong claws, flexor margin with 5–8 spines in parallel to the terminal spine; propodus 14.1–24.7 times as long as wide, 4.5–7.3 times as long as dactylus.

P5 (fig. 4d): dactylus (Fig. 4f) 3.0–4.3 as long as wide, ending in two large claws, with a long distal propodus seta and with 13–30 spines on flexor margin; propodus 15.4–32.0 times as long as wide, 5.1–7.9 times as long as dactylus.

Abdomen. Third abdominal somite (Fig. 4o) with moderately convex dorsal profile. Sixth abdominal somite about 0.65 of carapace, 2.1 times as long as fifth somite, slightly shorter than telson. Telson (Fig. 4l) three times as long as wide, with four pairs of dorsal spinules and one pair of dorsolateral spinules; posterior margin, with or without a median process, exhibits variations. Process mostly triangular with four to five intermediate spines shorter or subequal to lateral spines, rarely rounded, with ten intermediate subequal spines.

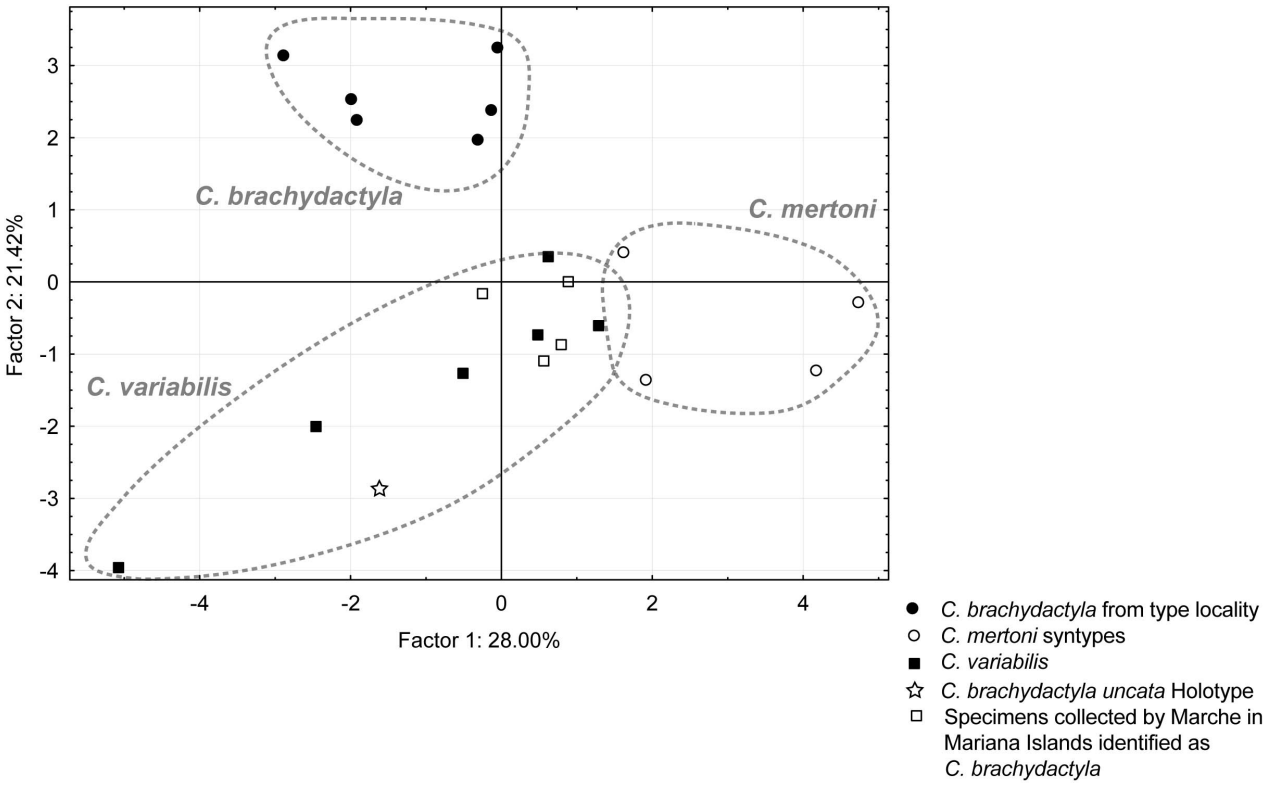


FIGURE 5. Scatterplot of the specimens projected on a plane defined by the first two factors of the CPA.

Pl1 (Fig. 4g): Endopod foliform with a developed appendix interna in males.

Pl2 (Fig. 4h): Appendix masculina on second pleopod reaching 0.62 times length of endopod; appendix interna reaching about 0.58 times appendix masculina length.

Preanal carina (Fig. 4k) unarmed.

Uropodal diaeresis (Fig. 4j) with 10–14 spinules.

Egg size (Fig. 4i): 0.41–0.48 × 0.24–0. 31 mm.

Type Locality. Palau, Babeladob Island, Tabecheding River, 07°27.169N, 134°31.748E.

Habitat. This species is found in rivers from lower to higher elevations in flowing water between riffles. The specimens are found in roots, leaf pack or aquatic macrophytes (in Guam).

Etymology. The new species is named *variabilis* for its variable rostrum length.

Colour pattern. Unknown

Distribution. This new species has only been found in Guam and Babeldaob islands so far.

Remarks. *Caridina variabilis* **n. sp.** is most similar to *C. mertoni*, *C. brachydactyla*, *C. elongapoda* Liang & Yan, 1977 and *C. peninsularis* Kemp, 1918. This new species displays a variable rostrum length among specimens. When the rostrum is short the general appearance is like *C. mertoni*, whereas when the rostrum is long the general appearance is of *C. brachydactyla*. In *C. variabilis* **n. sp.** the antennal spine is placed below the orbital angle, the P5 dactylus is biunguiculate with 13–30 setae vs. 32–42 in *C. brachydactyla* and there is a very large propodus seta, whereas in *C. mertoni*, the antennal spine is somewhat fused with the orbital angle, possesses an unguiculate dactylus with 30–40 setae and has no prominent propodus seta. *Caridina variabilis* **n. sp.** differs from *C. brachydactyla* by the absence of spine on the preanal carina, a shorter distal unarmed portion of the rostrum 0.1–0.7 (vs 0.6–1.6) times that of armed portion, a shorter unarmed portion of the rostrum 0–0.35 (vs 0.3–0.8) times that of the armed portion, by a longer P5 propodus, which is 5.1–7.9 time as long as the dactylus (vs 3.9–5.7) and a lower number of spines (13–30) on the P5 dactylus (32–42 in *C. brachydactyla*).

This new species differs from *C. elongapoda* with the preanal carina lacking a spine. But, the P3 propodus is longer 14.1–24.7 (vs 10.8–17.0), the P5 dactylus shorter 3.0–4.3 (vs 4.3–5.6) with fewer setae on the P5 dactylus 13–30 (vs 33–44) and with larger eggs 0.41–0.48 × 0.24–0. 31 mm (vs 0.39–0.40 × 0.22–0.23).

TABLE 2. Contribution of the factors to the axes of the PCA.

	Factor 1	Factor 2
P3pd	-0,017046124	-0,126414132
P3d	-0,092709362	-0,057535322
P3p	-0,089083174	-0,130935196
P1d	-0,1185254	-0,056499047
P1ch	-0,150481595	0,100526984
P1ca	-0,098238878	-0,154429328
P1dp	-0,006782501	-0,15497317
P2d	-0,148922607	0,028800419
P2ch	-0,145582132	0,119759603
P2ca	-0,16555737	-0,10088558
P2dp	0,059041331	-0,041298277
P5pd	0,018408878	-0,211356127
P5d	-0,068281129	0,055620433
P5p	-0,048772544	-0,197269865
RC	-0,151842227	-0,003800791
Rndd	-0,108112767	0,157452229
Dd	0,029568368	-0,152496848
Dv	-0,134018739	0,003451246

It also differs from *C. peninsularis* by a lower number of dorsal rostrum teeth (16–24 vs 20–35), a shorter P3 dactylus (2.8–4.0 vs 4.4–5.8) and a P5 dactylus biunguiculate with 13–30 setae (vs unguiculate and 25–39 setae).

PCA for some museum specimens. The first two principal components (Fig. 5) accounted for 49.42% of the total variance (28% for Factor 1 and 21.42% for Factor 2). With the first component, *C. mertoni* specimens are well

differentiated from the two other groups. With the second component, *C. brachydactyla* specimens are well differentiated from the two other groups. Factor 1 was mainly correlated with variables related to the second pereiopod (Carpus length-width ratio, score: -0.166; Dactylus length-width ratio, score: -0.149; Chela length-width ratio, score: -0.146), the rostrum (Rostrum length to Carapace length ratio, score: -0.152) and the first pereiopod (Chela length-width ratio, score: -0.150). Factor 2 was mainly correlated with variables related to the fifth pereiopod (Propodus length to dactylus length ratio, score: -0.211; Propodus length to width ratio, score: -0.197), the rostrum (Unarmed dorsal part to armed dorsal part ratio, score: 0.157; Number of dorsal spines, score: -0.152) and the first pereiopod (Dactylus length to propodus length ratio, score: -0.155; Carpus length to width ratio, score: -0.154).

Discussion

Holthuis (1960) identified some specimens of this species from Palau as *C. brachydactyla uncata* assigning types, but never described the taxon nor published the name. As these specimens were not described, we chose another name for this species and to stabilize the taxonomy, we chose to designate as holotype the specimen MNHN-IU-2017-2082 from which a DNA sequence was obtained (GenBank n° MG707146).

During the surveys of Palau and Guam, some specimens showing a long rostrum were initially attributed morphologically to *Caridina brachydactyla* De Man, 1908 whereas others, showing a short rostrum, were initially identified as *Caridina mertoni* Roux, 1911. In fact all specimens belonged to the new species described here. Indeed rostrum length, a character widely used in *Caridina* taxonomy might not be as reliable as it was thought, depending on the species, as it is highly plastic and varies with environmental parameters (de Mazancourt *et al*, 2017).

Thereby, we think that a new species description must be inevitably accompanied by a genetic sequence. Informative morphological features must be linked to molecular characters.

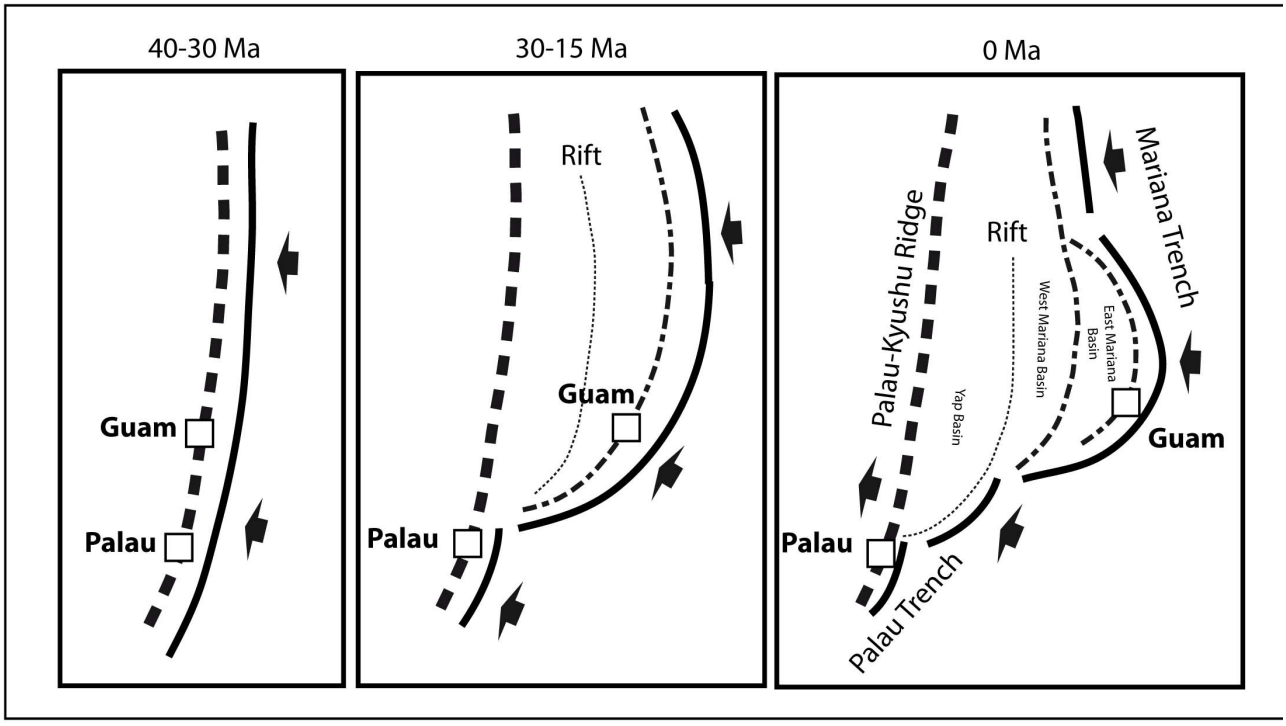


FIGURE 6. Schematic representation of the geological history of Micronesia (redrawn from Kobayashi *et al.*, 2004).

Despite the long distance (1296 km) between Babeldaob and Guam, our analyses showed that some haplotypes are shared (Fig. 4), and thus, genetic exchanges occur between populations from these two islands. These exchanges agree well with the geological history of the islands (Kobayashi *et al*, 2004). Formerly (Fig. 6, left), Palau was close to Guam, aligned to form the proto-Kyushu Palau Ridge, which was an active volcanic chain at a

period from 40 to 30 Ma (Fig. 6 left). Between 30 to 15 Ma (Fig. 6, center), the Shikoku Parece Vela Basin opened, its spreading centre remains as the extinct Parece Vela Rift situated at the centre of the basin. Consequently, the Mariana Islands, including Guam, drifted eastwards whereas Palau remained, with active volcanism. Later, by a complex movement of tectonic plates, Guam moved further away from Palau (Fig. 6, right) to its current location (1296 km northeast).

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Research article

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Caridina variabilirostris (Crustacea: Decapoda: Atyidae), a new species of freshwater shrimp from Pohnpei (Micronesia)

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Abstract. Recently, the status of a new species of atyid shrimp from Pohnpei (Micronesia) was discussed in relation to *C. brachydactyla* De Man, 1908 and *C. mertoni* J. Roux, 1911. By combining morphological data with a phylogenetic analysis with closely related species, this species is here described as *Caridina variabilirostris* sp. nov. Notes on its ecological distribution are also provided. The new species is characterized by a highly variable rostrum and is present in rivers all over Pohnpei Island. The status of this new species is clarified and it is shown that neither *C. brachydactyla* De Man 1908 nor *C. mertoni* J. Roux, 1911 occur on Pohnpei Island.

Keywords. 16S, molecular, integrative taxonomy, island, morphology.

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Introduction

Micronesia is a vast expanse of more than 2000 Pacific islands and atolls stretching from the Caroline and Mariana Islands in the West, to the Marshall, Nauru and Kiribati Islands in the East. The Caroline Islands consist of a chain of seamounts, atolls and high islands extended southeastward to the Marshall Islands. The high island of Pohnpei (formely known as Ponape) is situated at 6°54′ N latitude and 158°14′ E longitude (Fig.1). It belongs to Pohnpei State, one of the four states in the Federated State of Micronesia. The island of Pohnpei covers 345 km^2. The highest elevation point is 791 m. Aged of 8.7 Ma, this volcanic island was not only the remnant of a hotspot trace, but was also produced in a fracture-induced subduction-related tectonic environment (Rehman *et al.* 2013).

The freshwater shrimp genus *Caridina* Milne Edwards, 1837, comprising 298 species (WoRMS database, as of May 2018) and mostly present in the Indo-Pacific region, is the most diversified genus of the Atyidae (De Grave *et al.* 2015) and an important ecological component in the tropical streams (Covich *et al.* 1999; Pringle *et al.* 1993). Its high diversity combined with the lack of informative morphological characters has led to a confused taxonomy (Richard & Clark 2009). Indeed, until recently, the taxonomy of the genus was mainly based on morphological characters. Some of those have been proven highly variable within a species (e.g., rostrum shape and indentation or coloration) and so taxonomically non-informative, making it difficult to establish boundaries between species (Rintelen & Cai 2009; Mazancourt *et al.* 2017). Thus, there is a need for an integrative and standardized approach to improve the group's systematics, focusing on informative morphological features and using molecular characters (Page *et al.* 2005; Page & Hughes 2011).

Maciolek & Ford (1987) recorded *Caridina brachydactyla* De Man, 1908 among seven species of Atyidae in rivers on Pohnpei. Later, this species was not collected either by Nelson *et al.* (1996) or by Buden *et al.* (2001). Keith *et al.* (2012), reviewing the decapods of Ponhpei, conducted a freshwater survey from 7 to 17 March 2012, identified the material of *Caridina* belonging to the complex of *C. nilotica* as *C. brachydactyla* or *C. mertoni* Roux, 1911 (following the previous identifications made from Guam, another Micronesian island, by Leberer & Cai 2003).

One of the aims of the Muséum national d'Histoire naturelle (MNHN) in Paris is to carry out faunistic inventories of rivers in tropical islands in order to establish a better protection of these fragile ecosystems and, in this context, to clarify taxonomy of poorly known organisms. As we examined more and more specimens from Pohnpei, we gradually started to question the validity of some species from this island. Consequently, we here re-examined our specimens collected in 2012 in combining morphological data with a 16S mtDNA analysis and found that neither *C. brachydactyla* nor *C. mertoni* occur in Pohnpei, but have been confused until now with one undescribed species. Recently, Mazancourt *et al.* (2017) highlighted the "Pinocchio-shrimp effect", in this species, which has a variable length of the rostrum depending on the altitude.

Detailed description of this new species is given as well as its ecological distribution. The position of this species is clarified by comparing it with *C. brachydactyla* and *C. mertoni.*

Material and methods

Abbreviations for collections

MNHN = Muséum national d'Histoire naturelle, Paris
NHM = Natural History Museum, London
NMB = Naturhistorisches Museum Basel, Basel
RMNH = Rijksmuseum van Natuurlijke Historie (now the Naturalis Biodiversity Center, Leiden)
WK = Werner Klotz's collection
ZRC = Zoological Reference Collection, National University of Singapore, Singapore

Abbreviations for morphological analyses

cl = carapace length (mm): measured from post-orbital margin to posterior margin of the carapace
P1 = first pereopod
P2 = second pereopod
P3 = third pereopod
P5 = fifth pereopod
Pl1 = first pleopod
Pl2 = second pleopod

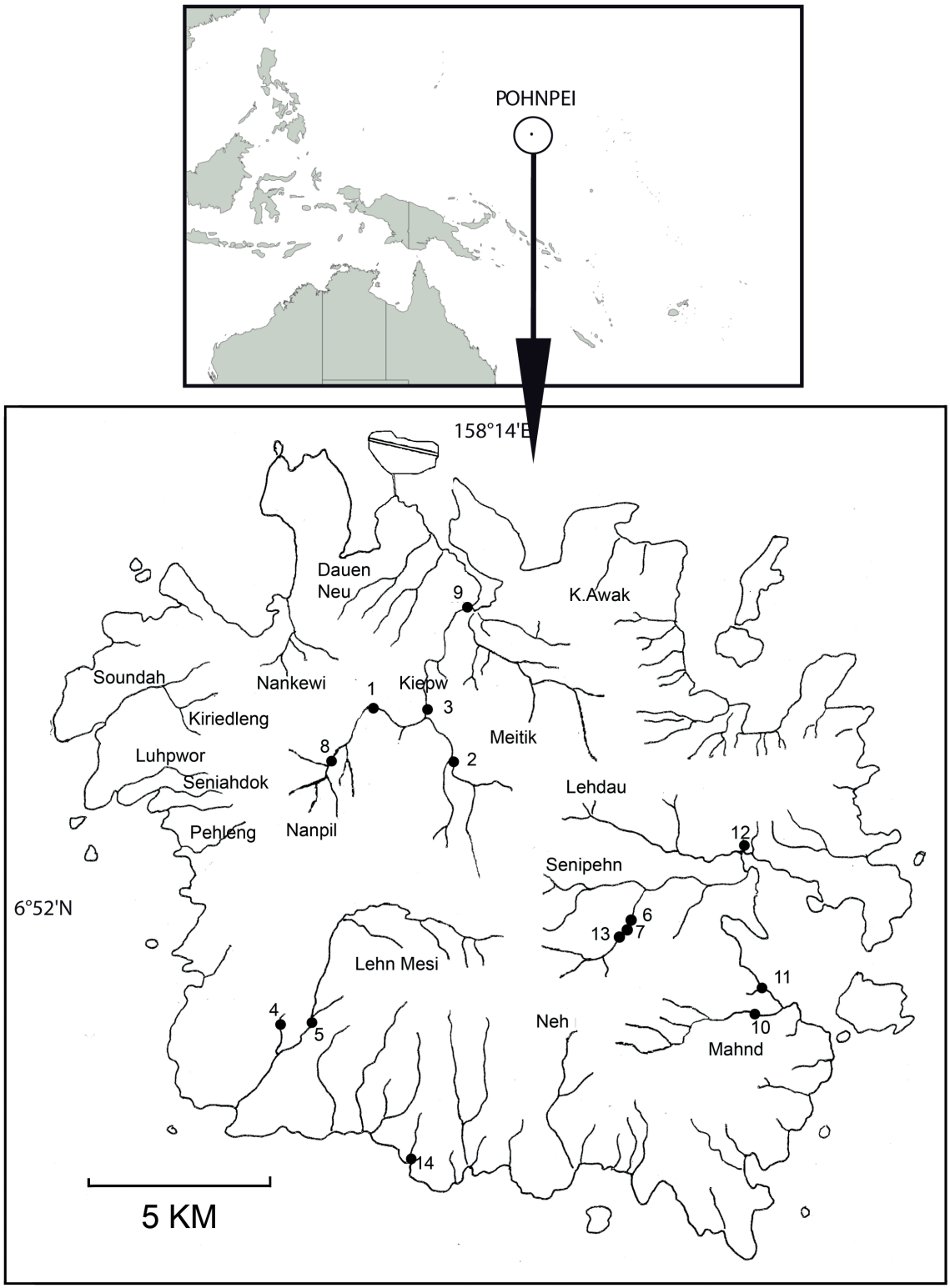


Fig. 1. Map of Pohnpei Island showing sampling stations (black dots) with their number (refers to Table 1).

Table 1. Sampling localities data.

	Dates	Coordinates	Rivers	Altitude (m)
Station 1	9 Mar. 2012	06°55.242′ N, 158°12.265′ E	Nanpil	127
Station 2	9 Mar. 2012	06°54.498′ N, 158°13.270′ E	Nanpil	93
Station 3	9 Mar. 2012	06°55.111′ N, 158°12.878′ E	Nanpil	52
Station 4	10 Mar. 2012	06°51.055′ N, 158°10.890′ E	Lehn Mesi	197
Station 5	10 Mar. 2012	06°51.107′ N, 158°11.896′ E	Lehn Mesi	139
Station 6	12 Mar. 2012	06°51.895′ N, 158°15.810′ E	Senipehn	–
Station 7	12 Mar. 2012	06°51.906′ N, 158°16.010′ E	Senipehn	95
Station 8	13 Mar. 2012	06°54.252′ N, 158°11.491′ E	Nanpil	180
Station 9	13 Mar. 2012	06°56.609′ N, 158°13.550′ E	Nanpil estuary	5
Station 10	14 Mar. 2012	06°50.221′ N, 158°17.212′ E	Mahnd	107
Station 11	14 Mar. 2012	06°51.120′ N, 158°17.854′ E	"River 1" estuary	5
Station 12	14 Mar. 2012	06°53.221′ N, 158°17.44′ E	"Petroglyphe River"	5
Station 13	15 Mar. 2012	06°51.795′ N, 158°15.622′ E	Senipehn	119
Station 14	10 Mar. 2012	06°48.579′ N, 158°12.639′ E	"River 2" estuary	5

Collection of specimens

Rivers and sites surveyed in March 2012 are indicated in Figure 1 and Table 1. Specimens from Pohnpei were collected by electrofishing (portable Dekka 3000 electric device, Germany). All material preserved in 75%–95% alcohol has been deposited in the collections of the Muséum national d'Histoire naturelle in Paris (MNHN, specimens n° MNHN-IU-2018-231 to MNHN-IU-2018-256).

DNA extraction, amplification and sequencing

For recent specimens, DNA was extracted from abdominal tissues using the semi-automatic Eppendorf ep-Motion 5075 robot. Fragments of the mitochondrial 16S rRNA (~ 520 bp) were amplified using the primers 16Sa-L (CGCCTGTTTATCAAAAACAT) and 16Sb-H2 (CTCCGGTTTGAACTCAGATCA) (Palumbi 1996). DNA amplification was performed in 25 µl PCR reactions, containing approximately 3 ng of template DNA, 2.5 mM $MgCl_2$, 0.26 mM of each nucleotide, 0.3 µM of each primer, 5% DMSO, 1 ng of BSA and 1.5 units of QBIOTAQ polymerase (MPBiomedicals). Amplification products were generated by an initial denaturation step of 4 min at 94°C followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 40 s, extension at 72°C for 60 s and a final extension step at 72°C for 7 min.

For old collection specimens (syntypes of *C. mertoni*), a CTAB protocol was used to extract DNA from pleopods. A shorter fragment of the 16S rRNA (332 bp) was amplified using two newly designed primers: 16S-Car-81F (AGGTAGCATAATAAATAGTC) and 16S-Car-413R (CTGTTATCCCTAAAGTAAC).

DNA amplification was performed in 25µl PCR reactions, containing 2.5 mM $MgCl_2$, 0.26 mM of each nucleotide, 0.3 µM of each primer, 1 ng of BSA and 1.5 units of QBIOTAQ polymerase (MPBiomedicals). Amplification products were generated by an initial denaturation step of 4 min at 94°C followed by 45 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 40 s and a final extension step at 72°C for 7 min.

PCR products were sequenced using the same primers and in both directions to insure the accuracy of base calls. Chromatograms were edited using Geneious v. 8 software (Kearse *et al.* 2012). All sequences were deposited in GenBank (Numbers MH476222 to MH476227). A sequence retrieved from GenBank for *Paratya australiensis* published by Page *et al.* (2007) was included in our analysis, used as outgroup.

A total of 17 recent specimens were sequenced (Table 2): six of the new species, of which two firstly identified as *C. brachydactyla* with a long rostrum and four as *C. mertoni* with a short rostrum; five of *C. mertoni* from Kolombangara Island; three type specimens of *C. variabilis* Mazancourt, Rogers & Keith, 2018 and three of *C. brachydactyla* from the type locality, Sulawesi. In addition, three old collection specimens, syntypes of *C. mertoni* from Kai Island were included (Mazancourt *et al.* 2018).

Molecular analyses

DNA sequences were aligned using MEGA7 software (Kumar *et al.* 2016) with Muscle algorithm (Edgar 2004). Using Bayesian information criterion in PartitionFinder (Lanfear *et al.* 2012), we retained the HKY + G + I model. Best-scoring Maximum Likelihood (ML) trees were estimated using RAxML HPC2 v. 8.2.10 (Stamatakis 2014) implemented in the Cyber Infrastructure for Phylogenetic Research (CIPRES) portal v. 3.1. (Miller *et al.* 2010). One hundred independent searches, each starting from

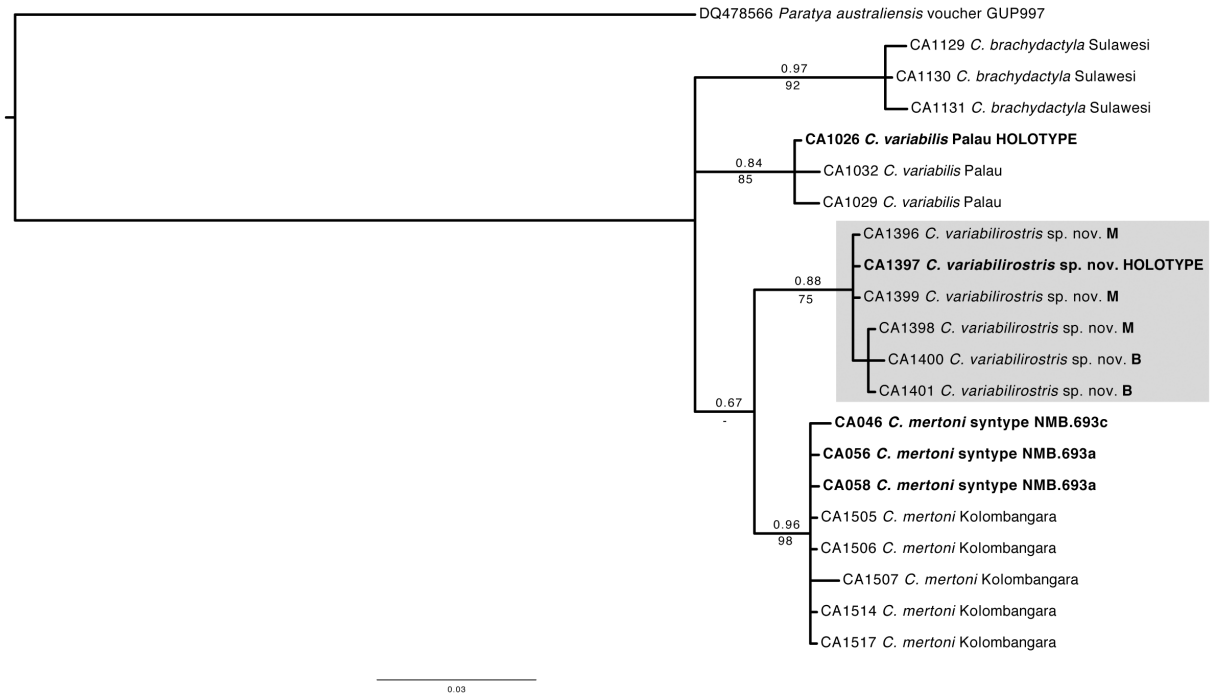


Fig. 2. Neighbour-Joining tree of the 16S sequences of the specimens. Numbers on branches indicate bootstrap values. Letters 'M' indicate specimens firstly identified as *Caridina mertoni* and letters 'B' indicate specimens firstly identified as *Caridina brachydactyla*.

Table 2. Specimens used in the genetic analysis.

Specimen n°	Species	Locality	Firstly ident. as	GenBank n°	Reference
CA1396	*C. variabilirostris*	Pohnpei (St. 1)	*C. mertoni*	MH476222	this study
CA1397	*C. variabilirostris*	Pohnpei (St. 8)	*C. mertoni*	MH476223	this study
CA1398	*C. variabilirostris*	Pohnpei (St. 8)	*C. mertoni*	MH476224	this study
CA1399	*C. variabilirostris*	Pohnpei (St. 8)	*C. mertoni*	MH476225	this study
CA1400	*C. variabilirostris*	Pohnpei (St. 9)	*C. brachydactyla*	MH476226	this study
CA1401	*C. variabilirostris*	Pohnpei (St. 9)	*C. brachydactyla*	MH476227	this study
CA1026	*C. variabilis*	Palau		MG707146	Mazancourt *et al*. 2018
CA1029	*C. variabilis*	Palau		MG707148	Mazancourt *et al*. 2018
CA1032	*C. variabilis*	Palau		MG707147	Mazancourt *et al*. 2018
CA1129	*C. brachydactyla*	Indonesia		MG707169	Mazancourt *et al*. 2018
CA1130	*C. brachydactyla*	Indonesia		MG707170	Mazancourt *et al*. 2018
CA1131	*C. brachydactyla*	Indonesia		MG707171	Mazancourt *et al*. 2018
CA046	*C. mertoni*	Indonesia		MG707138	Mazancourt *et al*. 2018
CA056	*C. mertoni*	Indonesia		MG707139	Mazancourt *et al*. 2018
CA058	*C. mertoni*	Indonesia		MG707140	Mazancourt *et al*. 2018
CA1505	*C. mertoni*	Solomon Islands		MG707141	Mazancourt *et al*. 2018
CA1506	*C. mertoni*	Solomon Islands		MG707142	Mazancourt *et al*. 2018
CA1507	*C. mertoni*	Solomon Islands		MG707143	Mazancourt *et al*. 2018
CA1514	*C. mertoni*	Solomon Islands		MG707144	Mazancourt *et al*. 2018
CA1517	*C. mertoni*	Solomon Islands		MG707145	Mazancourt *et al*. 2018
GUP997	*Paratya australiensis*	Australia		DQ478566	Page *et al*. 2007

distinct random trees, were conducted. Robustness of the nodes was assessed using non-parametric bootstrapping (Felsenstein 1985) with 1000 bootstrap replicates. Best-scoring Bayesian Inference trees were estimated using MrBayes v. 3.2.6 (Ronquist & Huelsenbeck 2003) also implemented in CIPRES with the previously determined model, running for 10 000 000 generations, a sampling frequency of 2000 and a burn in of 25%. Support for nodes was determined using posterior probabilities calculated by MrBayes.

Morphological comparison

The rostrum, general cephalon, pereopods 1, 2, 3 and 5 and abdomen were observed using a stereoscopic microscope. The proportions of the various joints of the appendages were measured using microphotographs and AnalySIS Works software (Olympus). Drawings were made using the "Digital Inking" method (Coleman 2003, 2006) by tracing vectorial paths on stacks of high-resolution photographs using Adobe Illustrator (CS6) and a WACOM MPTZ-1230 graphic tablet.

Results

Collection of specimens

About 50 specimens were collected by electro-fishing in the different rivers prospected in Pohnpei. Of these, 26 were sequenced, 18 with a short rostrum and eight with a long one. A representative sample of six specimens is shown here in the genetic study.

Phylogenetic analyses

After checking that consensus trees obtained from the two different methods were congruent, we decided to show the Bayesian consensus tree with both the ML bootstrap values and Bayesian posterior probabilities on branches. The specimens are grouped in four distinct and moderately supported clades. The most basal comprises the specimens of *C. brachydactyla* from Sulawesi (type locality), then, the type specimens of *C. variabilis* and, finally, two sister clades, one comprising recent and old specimens of *C. mertoni* and the other the specimens from Pohnpei. Due to the low support values, we cannot discuss the affinities of the different species, but we can confidently assert that the terminal clades are distinct species and consider the last one to be a new species that we describe below.

Taxonomy

Class Malacostraca Latreille, 1802
Order Decapoda Latreille, 1802
Family Atyidae De Haan, 1849
Genus *Caridina* H. Milne Edwards, 1837

Caridina variabilirostris sp. nov.
urn:lsid:zoobank.org:act:63F36D6C-9049-4D5E-902E-D38A3BCA3B0A
Figs 3–4

Etymology

The new species is named *variabilirostris* for its variable rostrum length.

Material examined

Holotype

POHNPEI (Station 8): ♂, cl 3.5 mm, Nanpil river, 06°54.252′ N, 158°11.491′ E, 180 m a.s.l., 13 Mar. 2012 (MNHN-IU-2018-231, DNA: CA1397).

Paratypes

POHNPEI – **Nanpil river (Station 1)**: 1 ♂, cl 3.2 mm, 06°55.242′ N, 158°12.265′ E, 127 m a.s.l., 9 Mar. 2012, (MNHN-IU-2018-232); 1 ♀, cl 4.6 mm, same data as for preceding (MNHN-IU-2018-233); 1 ♀ ovig., cl 4.1 mm, same data as for preceding (MNHN-IU-2018-234, DNA: CA1396). – **Nanpil river (Station 3)**: 1 ♂, cl 3.4 mm 06°55.111′ N, 158°12.878′ E, 52 m a.s.l., 9 Mar. 2012 (MNHN-IU-2018-235); 1 ♀ ovig., cl 4.5 mm, same data as for preceding (MNHN-IU-2018-236). – **Lehn Mesi river (Station 5)**: 2 ♀♀, cl 4.7 mm (MNHN-IU-2018-237) and 4.8 mm (MNHN-IU-2018-238); 1 ♀ ovig., cl 4.7 mm (MNHN-IU-2018-239), 06°51.107′ N, 158°11.896′ E, 139 m a.s.l., 10 Mar. 2012. – **Senipehn river (Station 7)**: 1 ♀, 06°51.906′ N, 158°16.010′ E, 95 m a.s.l., 12 Mar. 2012, cl 4.4 mm (MNHN-IU-2018-240); 2 ♀♀, ovig., cl. 4.1 mm (MNHN-IU-2018-241) and 4.5 mm (MNHN-IU-2018-242), same data as for preceding. – **Nanpil river (Station 8)**: 3 ♂♂, cl 3.4 mm (MNHN-IU-2018-243, DNA: CA1399), 3.5 mm (MNHN-IU-2018-244) and 3.6 mm (MNHN-IU-2018-245, DNA: CA1398), 06°54.252′ N, 158°11.491′ E, 180 m a.s.l., 13 Mar. 2012; 1 ♀, cl 4.4 mm (MNHN-IU-2018-246), same data as for preceding; 1 ♀ ovig., cl 5.1 mm (MNHN-IU-2018-247), same data as for preceding. – **Nanpil estuary (Station 9)**: 2 ♂♂, cl 2.2 mm (MNHN-IU-2018-248, DNA: CA1401) and 2.7 mm (MNHN-IU-2018-249), 06°56.609′ N, 158°13.550′ E, 5 m a.s.l., 13 Mar. 2012; 2 ♀♀, 3.5 mm (MNHN-IU-2018-250, DNA: CA1400) and 3.7 mm (MNHN-IU-2018-251), same data as for preceding; 2 ♀♀ ovig., cl 4.0 mm (MNHN-IU-2018-252) and 4.4 mm (MNHN-IU-2018-253), same data as for preceding. – **Mahnd river (Station 10)**: 2 ♂♂, cl 2.9 mm (MNHN-IU-2018-254) and 3.3 mm (MNHN-IU-2018-255), 06°50.221′ N, 158°17.212′ E, 107 m a.s.l., 14 Mar. 2012; 1 ♀ ovig., cl. 4.3 mm (MNHN-IU-2018-256), same data as for preceding.

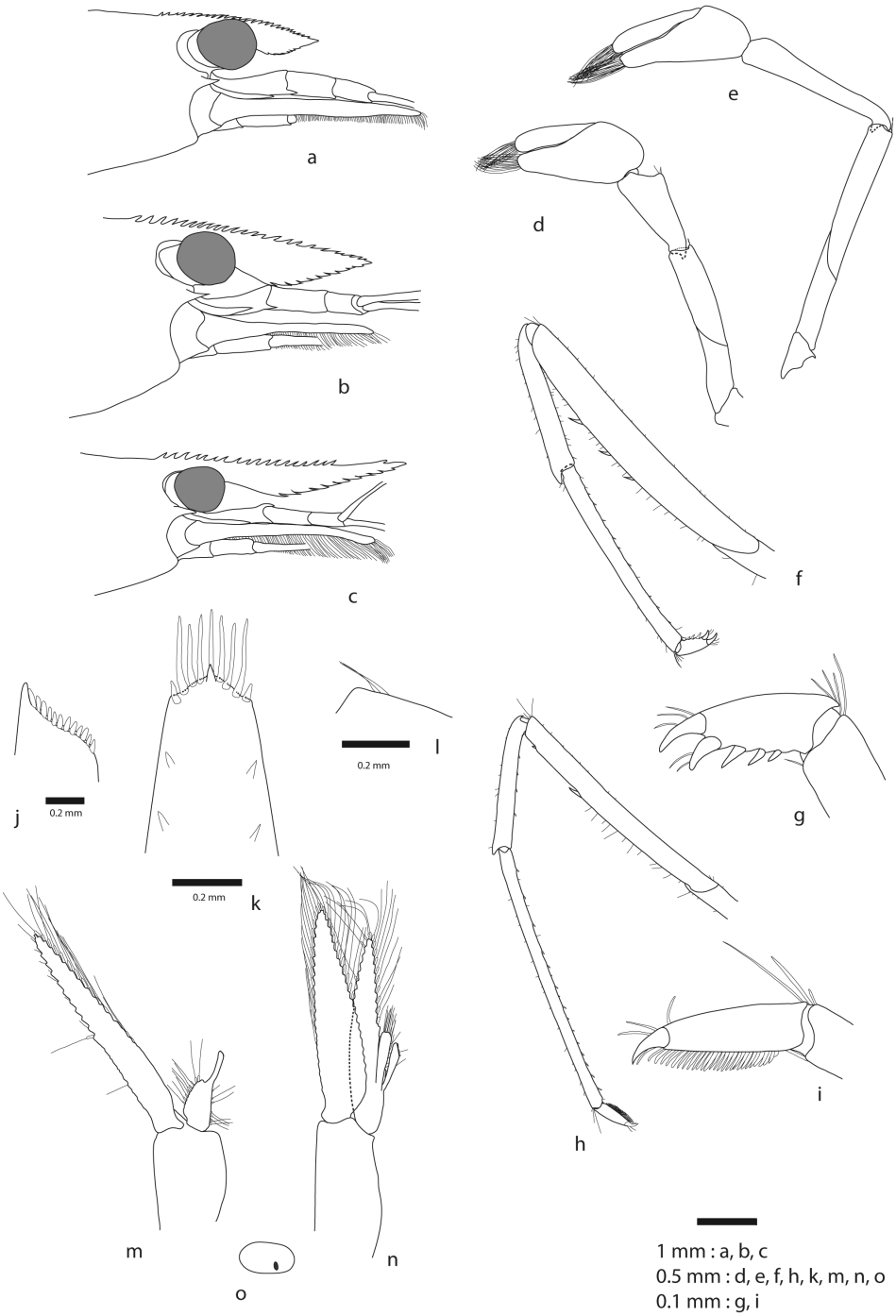


Fig. 3. *Caridina variabilirostris* sp. nov., showing the variation in rostrum length related to the altitude (a–c), drawings made from different specimens. **a, m–n.** ♂, cl 3.5 mm (MNHN-IU-2018-244). **a.** Rostrum. **m.** First pleiopod. **n.** Second pleiopod. **b.** ♀, cl 5.6 mm, rostrum (MNHN-IU-2018-252). **c, j, o.** ♀, cl 4.0 mm (MNHN-IU-2018-253) **c.** Rostrum. **j.** Uropodial diaeresis. **o.** Eggs. **d–i, k.** ♀, cl 3.5 mm (MNHN-IU-2018-250). **d.** First pereopod. **e.** Second pereopod. **f.** Third pereopod. **g.** Dactylus of third pereopod. **h.** Fifth pereopod. **i.** Dactylus of fifth pereopod. **k.** Telson. l. ♀, cl 4.3 mm, pre-anal carina (MNHN-IU-2018-256). of third pereopod. **h.** Fifth pereopod. **i.** Dactylus of fifth pereopod. **k.** Telson. **l.** ♀, cl 4.3 mm, pre-anal carina (MNHN-IU-2018-256).

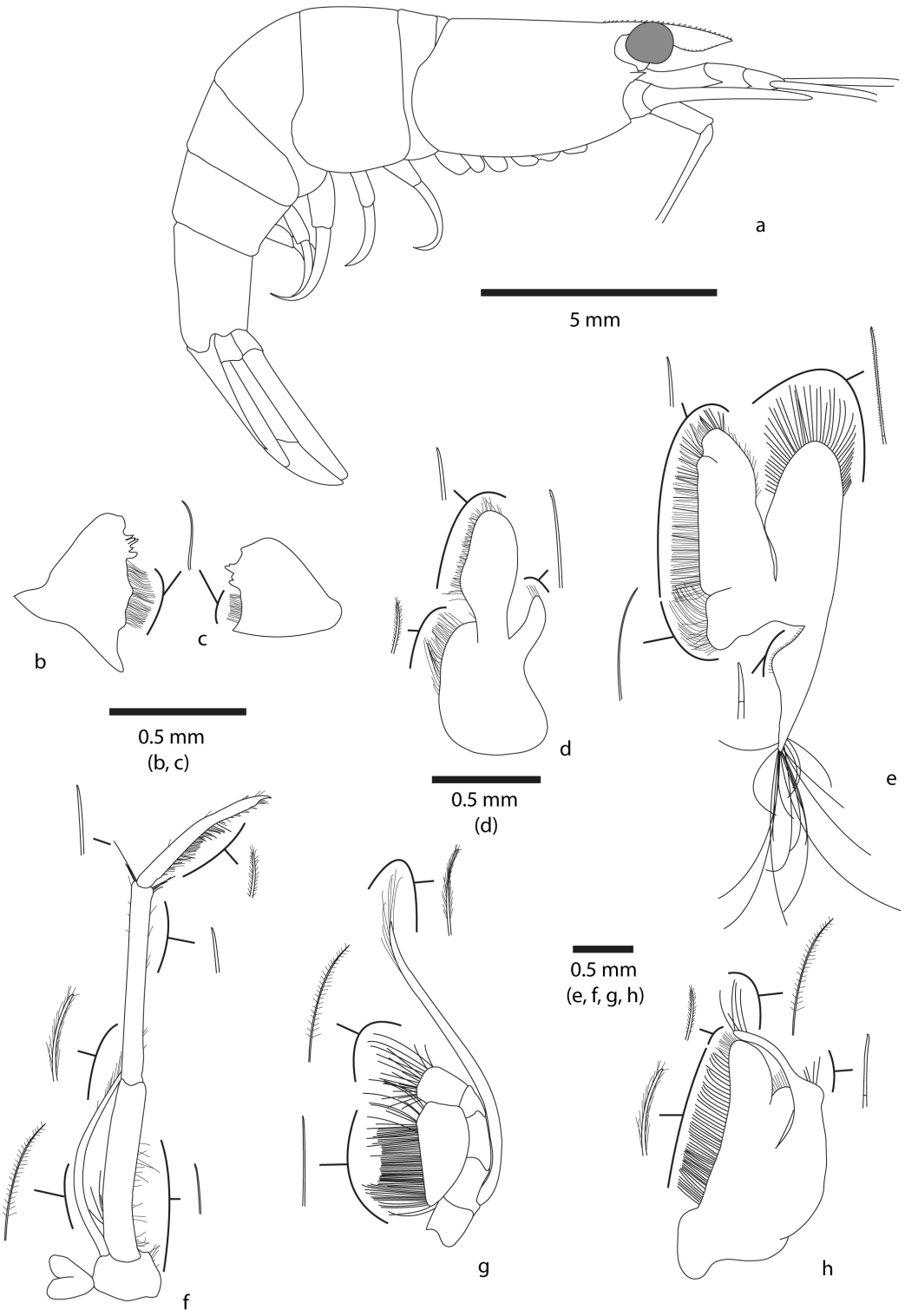


Fig. 4. *Caridina variabilirostris* sp. nov., showing drawings made from different specimens: **a.** ♂, cl 3.5 mm, general appearance (MNHN-IU-2018-244). **c–d.** ♀, cl 3.5 mm (MNHN-IU-2018-250). **c.** Right mandible. **d.** First maxilla. **b, e–h.** ♀, cl 4.8 mm, (MNHN-IU-2018-238). **b.** Left mandible. **e.** Second maxilla. **f.** Third maxilliped. **g.** Second maxilliped. **h.** First maxilliped.

Comparative material

Caridina brachydactyla De Man, 1908

Lectotype

INDONESIA: 1 ♀ ovig., cl 4.8 mm, Flores, Reo (RMNH Crus.D.977).

Paralectotypes

INDONESIA: 2 ♀♀ ovig., cl 5.3–5.4 mm, Flores, near Mbawa (RMNH Crus.D.255).

Non types

INDONESIA: 1 ♀, cl 5.8 mm, Bali (NMB 1054a); 2 ♂♂, cl 2.7 mm and 3.7 mm (DNA: CA1130), Sulawesi, Palopo, Macaui (WK 63.10); 2 ♀♀ ovig., cl 4.3 mm (DNA: CA1129) and 4.0 mm (DNA: CA1131), and 1 ♀, cl 4.8 mm, Palopo, Tojo (WK 64.10).

Caridina mertoni Roux, 1911

Syntypes

INDONESIA: 2 ♂♂, cl 2.7 mm and 3.8 mm (DNA: CA056) and 1 ♀, cl 4.1 mm (DNA: CA058), Grand Kai, Elat (NMB 693a); 1 ♂, cl 3.1 mm and 1 ♀ ovig., cl 4.8 mm, Grand Kai, Warka (NMB 693b); 1 ♀, cl 4.3 mm (DNA: CA046), Grand Kai, Enralang, (NMB 693c); 1 ♂, cl 3.8 mm, Grand Kai, Elat (MNHN-IU-2015-1819); 1 ♂, cl 3.4 mm, Grand Kai, Elat (MNHN-IU-2015-1820).

Non types

KOLOMBANGARA (SOLOMON ISLANDS) – **Manolu river**: 1 ♂, cl 3.9 mm, 08°05.312′ S, 157°00.813′ E, 10 Nov. 2015 (MNHN-IU-2017-2107, DNA: CA1506); 1 ♂, cl 3.0 mm, same data as for preceding (MNHN-IU-2017-2108, DNA: CA1507); 1 ♂, cl 3.7 mm, same data as for preceding (MNHN-IU-2017-2109, DNA: CA1505); 1 ♀ ovig., cl 3.9 mm, same data as for preceding (MNHN-IU-2017-2110). – **Sulumuni river**: 1 ♀, cl 6.2 mm, 08°02.253′ S, 157°09.257′ E, 12 Nov. 2015 (MNHN-IU-2017-2111, DNA: CA1514); 1 ♀ ovig., cl 4.1 mm, same data as for preceding (MNHN-IU-2017-2112, DNA: CA1517).

Caridina elongapoda Liang & Yan, 1977

Non types

HONG-KONG: 2 ♂♂, cl 2.8–3.5 mm, Pak Tam Chung, W. Klotz leg. (WK 22 09); 1 ♀, cl 4.2 mm and 1 ♂, cl 3.5 mm, Kai Sai Chau, W. Klotz leg. (WK 14 11).

MALAYSIA: 1 ♂, cl 4.2 mm, Pulau Tioman, Sungai Asah, 24 Jun. 1997, Ng *et al.* leg. (ZRC 1998.0865); 1 ♀, cl 4.3 mm, same data as for preceding (ZRC 1998.0865); 1 ♀ ovig., cl 4.3 mm, same data as for preceding (ZRC 1998.0865).

Caridina peninsularis Kemp, 1918

Lectotype

MALAYSIA: 1 ♂, cl 3.2 mm, Penang Island, Botanical garden, Feb. 1916, N. Anandale leg. (MNHN-IU-2015-1749).

Paralectotypes

MALAYSIA: 1 ♀ ovig., cl 5.4 mm, same data as lectotype (MNHN-IU-2015-1750); 1 ♀, cl 3.4 mm, same data as for preceding (NHM 1919.11.1.12-21 (1761124)); 1 ♀ ovig., cl 5.2 mm, same data as for preceding (NHM 1919.11.1.12-21 (1761124)); 1 ♂, cl 3.9 mm, same data as for preceding (NHM 1919.11.1.12-21 (1761124)).

Non types

SINGAPORE: 1 ♀ ovig., cl 5.1 mm, Tanglin (incorrectly spelt "Tangtum" in NHM register and on label, see Richard & Clark 2014), 1958 Bedford and Lanchester leg., D.S. Johnson det. (NHM 1958.8.7.14–17 (1749569)); 1 ♂, cl 4.2 mm, same data as for preceding (NHM 1958.8.7.14–17 (1749569)).

Description

Cephalothorax. Rostrum (Fig. 3a–c): very variable in length, 0.6–1.3 of cl, reaching to scaphocerite apex, armed dorsally with 18–26 teeth, distal unarmed portion 0.0–0.4 times that of armed portion, with one or two subapical teeth, 1–4 teeth on carapace posterior to orbital margin, ventral margin with 4–12 teeth. Number of dorsal teeth behind most proximal ventral tooth 12–18. Rostrum formula (1–4) 18-26+1-2/4-12. Antennular tooth acute, placed slightly below orbital angle. Pterygostomian margin rectangularly rounded.

Head. Eyes well developed, anterior end reaching to 0.7 length of antennular peduncle basal antennomere. Antennular peduncle 0.7 times as long as carapace. Anterolateral angle reaching 0.25 length of second antennomere; second antennomere distinctly longer than third. Stylocerite reaching to 0.7 length of antennular peduncle basal antennomere.

Mouthparts. Mandibles dimorphic; left mandible (Fig. 4b) more developed, corpus large, robust with five strong sharp teeth separated by ridged gap; incisor and molar processes separated by patch of long simple setae. Right mandible (Fig. 4c) with five sharp incisor teeth, medially with group of long setae; molar process narrow, elongate, ridged. First maxilla (Fig. 4d) having a lower lacinia with margin broadly rounded, bearing several rows of plumose setae. Upper lacinia elongate, with medial margin bearing a number of distinct teeth and simple setae, palp bearing long simple setae. Second maxilla (Fig. 4e) upper and middle endite with marginal and submarginal simple or slightly plumose setae. Lower endite with simple setae; palp narrow, shorter than upper endite cleft with few setae. Scaphognathite fringed with long simple setae, tapering posteriorly with some long, curved simple setae at posterior end. First maxilliped (Fig. 4h) endopodite ultimate segment medial margin with long plumose setae. Palp elongate, setose. Exopod flagellum long and narrow distally with marginal plumose setae. Caridean lobe large, with marginal setae. Second maxilliped (Fig. 4g) endopodite ultimate and penultimate antennomeres fused, reflected against basal antennomeres. Ultimate, penultimate and basal antennomeres medial margins with long setae of various types; flagellum very long, slender with marginal plumose setae distally. Third maxilliped (Fig. 4f) with terminal article reaching third antennular peduncle antennomere apex; distal antennomere about 10 times as long as wide, slightly shorter than penultimate, ending in large hamulate apical spine surrounded by simple setae. Penultimate antennomere about eight times as long as wide, with group of transverse rows of simple setae. Exopod flagellum well developed, about a third the length of endopodite second article, distal margin with long plumose setae.

Pereopods. Pereopods I–IV with epipods pereopod. P1 (Fig. 3d): chela about 1.9–2.2 times as long as wide, movable finger 3.6–4.4 times as long as wide, 1.2–1.7 times length of palm; carpus 2.0–2.7 times as long as wide. P2 (Fig. 3e) more slender and longer than first pereopod with chela 2.1–2.6 times as long as wide: movable finger 3.9–5.8 times as long as wide, 1.3–2.0 times length of palm; carpus slender 4.4–6.5 times as long as wide. P3 (Fig. 3f): slender, dactylus (Fig. 3g) 2.4–3.3 times as long as wide, including terminal spine), flexor margin with 4–6 spines parallel to terminal one; propodus 13.8–20.5 times as long as wide, 4.8–8.4 times as long as dactylus. P5 (Fig. 3h): dactylus (Fig. 3i) 2.1–4.2 as long as wide with 18–29 spinules on flexor margin; propodus 15.8–28.4 times as long as wide, 5.3–8.6 times as long as dactylus.

Abdomen. Third abdominal (Fig. 4a) somite with moderately convex dorsal profile. Sixth abdominal somite about 0.68 of carapace, 1.76 times as long as fifth somite, slightly shorter than telson. Telson

(Fig. 3k) with four pairs of dorsal spines and one pair of dorsolateral spines; posterior margin, with or without median process, exhibits variations, triangular or rounded with four to five intermediate simple setae longer or equal than lateral ones.

Pl1 (Fig. 3m). Endopod foliform with a developed appendix interna in males. Pl2 (Fig. 3n): appendix masculina on second pleopod reaching 0.52 times length of endopod; appendix interna reaching about 0.85 times appendix masculina length.

Preanal carina (Fig. 3l). Unarmed.

Uropodal diaeresis (Fig. 3j). With 10–15 spinules.

Ovigerous female egg size (Fig. 3o). 0.39–0.49x0.24–0. 29 mm.

Habitat

This species is found among macrophytes in flowing fresh water of the rivers of Pohnpei all along the course, showing good adaptability to different temperatures and hydrological conditions. It is more abundant in higher elevations due to reduced predator pressure.

Colour pattern (Fig. 5)

The colour of the body is hyaline with many reds dots. An oblique red band on the cephalothorax is very characteristic.



Fig. 5. *Caridina variabilirostris* sp. nov., live coloration (Photos: P. Keith)

Distribution

This new species is currently known from Pohnpei Island only.

Remarks

Caridina variabilirostris sp. nov. is most similar to *C. mertoni*, *C. brachydactyla*, *C. elongapoda* Liang & Yan, 1977 and *C. peninsularis* Kemp, 1918. The new species displays a variable rostrum length among specimens. When the rostrum is short the general appearance is like *C. mertoni*, whereas when the rostrum is long, the general appearance is of *C. brachydactyla*. In *C. variabilirostris* spec. nov. the antennal spine is placed below the orbital angle, the P5 dactylus with 18–29 spinules whereas in *C. mertoni*, the antennal spine is somewhat fused with the orbital angle and P5 dactylus with 24–43 spinules. *C. variabilirostris* spec. nov. differs from *C. brachydactyla* by the absence of spine on the preanal carina, a shorter distal unarmed portion of the rostrum 0.0–0.4 (vs 0.4–1.6) times that of armed portion, by a longer P5 propodus, which is 5.3–8.6 time as long as the dactylus (vs 3.9–5.7) and a lower number of spinules on the P5 dactylus (32–42 in *C. brachydactyla*).

This new species resembles *C. elongapoda* in its preanal carina lacking a spine, but it has larger eggs 0.39–0.49 × 0.24–0. 29 mm (vs 0.39–0.40 × 0.22–0.23), and the P5 dactylus is shorter 2.1–4.2 (vs 4.3–5.6) and with fewer spinules 18–29 (vs 33–44). It also differs from *C. peninsularis* by a lower number of dorsal rostrum teeth (18–26 vs 20–35), a lower P3 propodus-dactylus ratio (4.8–8.4 vs 4.4–5.8) and a P5 dactylus with 18–29 spinules vs 25–38.

The new species looks very much like *C. variabilis* from Guam and Palau with the preanal always non-armed, the number of teeth and their placement on the rostrum, the proportions between the joints of pereiopods, and the egg size (Mazancourt *et al.* 2018). But the P5 dactylus has a single terminal spine and a short distal propodus seta (vs two strong distal spines and a very long distal propodus seta), and the posterior margin of the telson with intermediate setae longer or equal than lateral ones (vs shorter or equal than lateral ones).

Discussion

Recently Mazancourt *et al.* (2017) highligted the "Pinocchio shrimp effect" on this new species. Indeed some specimens with a long rostrum were attributed morphologically to *C. brachydactyla* whereas others, with a short rostrum, were identified as *C. mertoni*. In fact all specimens belong to this new species. Indeed the rostrum length widely used in the taxonomy of *Caridina* might not be as reliable as it was thought. It is highly plastic and varies with environmental parameters.

Despite an increasing use of integrative taxonomy for the systematics of *Caridina* (and shrimps in general), some authors keep describing new species based not only on morphology alone, but even on a single character. For example, different species described by Richard & Clark (2009, 2014) based on rostrum morphology proved to be synonyms, and the differences observed were after all recognized as intra-specific variation (see Wood *et al.* 2018; Mazancourt *et al.* unpublished).

We thus advocate to always perform some molecular work prior to studying a new species in order to unveil this kind of variation within a species, focus on the morphology and expose the variation when describing it. Alternatively, if retrieving molecular data is not possible, using a combination of reliable characters seems more sound than a single one. This would allow to provide characters to identify confidently the species when encountered in the field. It is likely that different species described in this genus will prove to be the same one, exhibiting the same kind of polymorphism as we showed here.

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The complex study of complexes: The first well-supported phylogeny of two species complexes within genus *Caridina* (Decapoda: Caridea: Atyidae) sheds light on evolution, biogeography, and habitat☆

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Taxonomy

ABSTRACT

Atyid shrimps, a key component of tropical freshwater ecosystems, face multiple anthropogenic threats and thus need special attention. With more than 300 described species, the genus *Caridina* is the most speciose of all the Caridea infra-order. *Caridina* spp. occupy diverse habitats in tropical freshwaters of the Indo-West Pacific region. Several species complexes have been recognized, based on common morphological features, but little is known about how well these morphological characteristics align with phylogenetic characteristics. Furthermore, no phylogeny of the genus *Caridina* published so far has provided well-resolved and supported relationships among different species, thus impeding the possibility of proposing evolutionary hypotheses. In this study we used next generation sequencing (NGS) to provide new insights into the phylogenetic relationships among the genus *Caridina*, focusing on two complexes: '*Caridina nilotica*' and '*Caridina weberi*'. We collected 92 specimens belonging to these two groups from most of their known geographical range, representing 50 species, for which we sequenced seven mitochondrial genes and two nuclear markers using ion torrent NGS. We performed a phylogenetic analysis, which yielded the first well-supported tree for the genus *Caridina*. On this tree were mapped the geographic ranges and the habitats used by the different species, and a time calibration was tested. We found the driving factors that most likely account for separation of clades are differences in habitat and to a lesser extent geography. This work provides new insights into the taxonomy of this group and identifies opportunities for further studies in order to fill knowledge gaps that currently impede the management and conservation of atyid species.

1. Introduction

Atyidae is the most diversified decapod crustacean family in freshwaters worldwide, comprising nearly 500 species and showing a large variety of feeding and breeding strategies in diverse habitats. The genus *Caridina* H. Milne Edwards, 1837, with around 300 described species, is the most speciose of the Atyidae and also the most diversified genus of shrimps (De Grave et al., 2015). Currently, around one third of all known freshwater shrimp species are threatened or near-threatened as a result of human activity, primarily habitat destruction (De Grave et al., 2015). Shrimp play important ecological roles in their natural habitats (e.g. nutrient cycling) and are important prey species for a variety of other organisms (Covich et al., 1999; Crowl et al., 2001). Freshwater shrimps are also an important food source for humans, especially in the Indo-Pacific where they often support the livelihoods of the poorest communities through fishing or aquaculture (Russi et al., 2013). Declines in the abundance or extinctions of shrimp species could therefore have important implications for food security and the ecology of freshwater and brackish habitats, with the potential to hamper ecosystem services.

One obstacle to addressing the problem of rapid species loss is that conservationists cannot properly monitor species or implement

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Fig. 1. Map of the sampling localities of the specimens used in the study.

conservation plans without good taxonomic information. There is considerable confusion surrounding the taxonomy of the genus *Caridina* that has, for example, hampered efforts to assess the impacts of wild harvest for the aquarium trade (Klotz and von Rintelen, 2014). Confusion surrounding the taxonomy of the genus has been, in part, due to natural variability in traditionally retained characters that have been used to separate species (de Mazancourt et al., 2017b). Additionally, some specimens have been grouped in complexes based on common morphological features, but little work has been done to differentiate among closely related species. For example, Bouvier (1925), following the previous attempts of De Man (1892) and Ortmann (1894), separated the 50 known species at that time into five species groups: *Caridina nilotica*, *C. laevis*, *C. africana*, *C. brevirostris*, and *C. typus*. As more species were described, species groups were modified, abandoned, or combined and new groups were created, including the *C. serrata* complex (Cai & Ng, 1999; Klotz and von Rintelen, 2014), *C. gracilirostris* complex (Cai & Ng, 2007) and *C. weberi* complex (de Mazancourt et al., 2017a). Two of these, *C. nilotica* complex and *C. weberi* complex, are well represented in insular systems of the Indo-West Pacific region thanks to the great number of amphidromous representatives that allowed them to colonize new habitats quickly. *C. nilotica* complex exists in tropical freshwaters from Africa to Melanesia and Micronesia, whereas *C. weberi* complex representatives can be found further eastward in Central Pacific Islands (Keith et al., 2013). Few species from the *C. nilotica* or *C. weberi* complexes have been separated and described since the complexes were established.

To overcome the taxonomic complexity of the family Atyidae, molecular data is being increasingly used to uncover phylogenetic relationships (Page et al., 2007a,b; von Rintelen et al., 2007; 2012). Published phylogenies including species of *Caridina* have been predominantly built from a single locus (von Rintelen et al., 2008), although phylogenies built using two or three loci have become available in recent years (von Rintelen et al., 2012; Bossuyt et al., 2004; Page et al., 2007a,b; Cook et al., 2011; Klotz and von Rintelen, 2014). Despite this, no phylogeny of the genus *Caridina* published so far has provided well-resolved and supported relationships among different species, impeding the possibility of proposing evolutionary hypotheses. For example, the most comprehensive phylogeny of Atyidae built from one mitochondrial and two nuclear markers was unfortunately unable to provide reliable dating and biogeographic evolution at the generic level (von Rintelen et al., 2012). Greater understanding of the phylogenetic relationships and evolution of *Caridina* requires the combination of morphological and/or ecological data with DNA sequencing to improve the accuracy and reliability of species delineations (von Rintelen et al., 2008; von Rintelen & Cai, 2009; Klotz & von Rintelen, 2014; de Mazancourt et al., 2017a; 2018a,b).

The aim of this study was to (i) provide the first well-resolved partial phylogeny of *Caridina* focusing on two species complexes, *C. nilotica* and *C. weberi*, using next generation sequencing methods (NGS), and (ii) combine our DNA sequencing with ecological, habitat, and biogeographical data to better understand the evolutionary and phylogenetic relationships among *Caridina*. Large datasets obtained using NGS often provide better resolution of the phylogenetic relationships than analyses with single or few markers (Hillis, 1996). Using data from seven mitochondrial and two nuclear markers, we constructed a phylogenetic tree that provides novel insights into the evolution of this group and the family Atyidae. The implications of our phylogeny for the management of caridean shrimp are also discussed.

2. Material and methods

2.1. Specimens sampling

We obtained specimens of *Caridina* from most of its range thanks to numerous expeditions made by two of the authors (GM & PK), from the collections held at the Muséum national d'Histoire naturelle (Paris, France), as well as specimens obtained from a network of naturalists and scientists worldwide. A total of 92 specimens of atyid shrimps (89 *Caridina*, 2 *Paratya* and 1 *Elephantis*) representing 50 species (48 *Caridina*, 1 *Paratya* and 1 *Elephantis*) were collected from different

Table 1
List of the specimens included in the phylogeny.

Species	DNA voucher	Country	16S	COI	COII	ATP8	ATP6	COIII	ND3	28S	H3
C. africana	CA1628	South Africa	MK189899	MK190058	MK189648	MK189557	MK190240	MK190149	MK189819	MK189980	MK189729
C. appendiculata	CA1670	Australia	MH497534	MK190059	MK189649	MK189558	MK190241	MK190150	MK189820	MK189981	MK189730
	CA1708		MK189903	MK190063	MK189653	MK189562	MK190245	MK190154	MK189824	MK189985	MK189733
	CA1493	Solomon Islands	MH497524	MK190038	MK189624	MK189533	MK190216	MK190125	MK189795	MK189956	MK189711
	CA1261	Vanuatu	MH497510	MK190018	MK189601	MK189510	MK190193	MK190102	MK189772	MK189935	MK189689
C. brachydactyla	CA1131	Indonesia	MH497502	MK190011	MK189594	MK189503	MK190186	MK190095	MK189765	MK189930	MK189682
C. brevicarpalis	CA1521	Solomon Islands	MK189887	MK190049	MK189636	MK189545	MK190228	MK190137	MK189807	MK189968	MK189719
C. brevidactyla	CA1345	Solomon Islands	MH497518	MK190024	MK189607	MK189516	MK190199	MK190108	MK189778	MK189941	MK189694
	CA1501		MK189881	MK190040	MK189626	MK189535	MK190218	MK190127	MK189797	MK189958	
C. buehleri	CA1519	Solomon Islands	KY350244	MK190047	MK189634	MK189543	MK190226	MK190135	MK189805	MK189966	MK189717
	CA1520		MK189886	MK190048	MK189635	MK189544	MK190227	MK190136	MK189806	MK189967	MK189718
C. cf. gracilirostris	CA1238	Indonesia	MK189858	MK190015	MK189598	MK189507	MK190190	MK190099	MK189769	MK189932	MK189686
C. cf. jeani	CA1415	Samoa	MK189877		MK189621	MK189530	MK190213	MK190122	MK189792	MK189953	MK189708
	CA1416		MK189878		MK189622	MK189531	MK190214	MK190123	MK189793	MK189954	MK189709
	CA1370	Vanuatu	MK189867	MK190028	MK189611	MK189520	MK190203	MK190112	MK189782	MK189945	MK189698
	CA1371		MK189868	MK190029	MK189612	MK189521	MK190204	MK190113	MK189783	MK189946	MK189699
	CA1377		MK189871	MK190032	MK189615	MK189524	MK190207	MK190116	MK189786	MK189949	MK189702
C. cf. multidentata	CA1053	Madagascar	MK189853		MK189591	MK189500	MK190183	MK190092	MK189762		
C. cf. nilotica 1	CA1707	Australia	MK189902	MK190062	MK189652	MK189561	MK190244	MK190153	MK189823	MK189984	MK189732
C. cf. nilotica 2	CA1711	Australia	MK189904	MK190064	MK189654	MK189563	MK190246	MK190155	MK189825	MK189986	MK189734
	CA1714		MK189905	MK190065	MK189655	MK189564	MK190247	MK190156	MK189826	MK189987	MK189735
C. cf. vitiensis	CA1385	Fiji	MK189872	MK190033	MK189616	MK189525	MK190208	MK190117	MK189787	MK189950	MK189703
	CA1003	New Caledonia	MK189851	MK190007	MK189589	MK189498	MK190181	MK190090	MK189760	MK189928	MK189680
	CA1601		MK189896	MK190055	MK189645	MK189554	MK190237	MK190146	MK189816	MK189977	MK189726
C. cf. weberi Pohnpei	CA1388	Micronesia	MK189873		MK189617	MK189526	MK190209	MK190118	MK189788	MK189951	MK189704
	CA1390		MK189874	MK190034	MK189618	MK189527	MK190210	MK190119	MK189789		MK189705
C. cf. weberi sp. 1	CA1349	Solomon Islands	MK189865	MK190026	MK189609	MK189518	MK190201	MK190110	MK189780	MK189943	MK189696
C. cf. weberi sp. 2	CA1516	Solomon Islands	MK189885	MK190046	MK189633	MK189542	MK190225	MK190134	MK189804	MK189965	MK189716
C. cf. weberi sp. 3	CA1048	Cook Islands	MK189852	MK190008	MK189590	MK189499	MK190182	MK190091	MK189761		
	CA1410	Samoa	MK189876	MK190036	MK189620	MK189529	MK190212	MK190121	MK189791	MK189952	MK189707
	CA1508	Solomon Islands	MK189882	MK190043	MK189629	MK189538	MK190221	MK190130	MK189800	MK189961	MK189714
C. cf. weberi sp. 4	CA1509	Solomon Islands	MK189883		MK189630	MK189539	MK190222	MK190131	MK189801	MK189962	MK189715
	CA1510			MK190044	MK189631	MK189540	MK190223	MK190132	MK189802	MK189963	
C. cf. weberi sp. 5	CA1909	Solomon Islands	MK189927	MK190088	MK189678	MK189587	MK190270	MK190179	MK189849	MK190005	MK189758
C. cf. weberi sp. 6	CA1902	Solomon Islands	MK189925	MK190086	MK189676	MK189585	MK190268	MK190177	MK189847		MK189756
	CA1903		MK189926	MK190087	MK189677	MK189586	MK190269	MK190178	MK189848		MK189757
C. confusa	CA1727	Australia	MK189907	MK190067	MK189657	MK189566	MK190249	MK190158	MK189828	MK189988	MK189737
	CA1728		MK189908	MK190068	MK189658	MK189567	MK190250	MK190159	MK189829	MK189989	MK189738
C. elongapoda	CA1899	Malaysia	MK189924	MK190085	MK189675	MK189584	MK190267	MK190176	MK189846	MK190004	MK189755
C. gracilipes	CA1348	Indonesia	MH497520	MK190025	MK189608	MK189517	MK190200	MK190109	MK189779	MK189942	MK189695
		?	NC_024751	NC_024751	NC_024751	NC_024751	NC_024751	NC_024751	NC_024751	FN995565	FN995481
C. gracilirostris	CA1678	Australia	MK189900	MK190060	MK189650	MK189559	MK190242	MK190151	MK189821	MK189982	MK189731
C. leucosticta	CA1912	Japan	MH497556	MK190089	MK189679	MK189588	MK190271	MK190180	MK189850	MK190006	MK189759
C. lobocensis	CA1056	Palau	MK189854	MK190009	MK189592	MK189501	MK190184	MK190093	MK189763	MK189929	MK189681
	CA1554		MK189891		MK189640	MK189549	MK190232	MK190141	MK189811	MK189972	
C. longicarpus	CA1557	New Caledonia	MK189892	MK190051	MK189641	MK189550	MK190233	MK190142	MK189812	MK189973	MK189723
	CA1599		MK189895	MK190054	MK189644	MK189553	MK190236	MK190145	MK189815	MK189976	MK189725
	CA1611		MK189897	MK190056	MK189646	MK189555	MK190238	MK190147	MK189817	MK189978	MK189727
C. macrodentata	CA1374	Vanuatu	MK189784	MK190030	MK189613	MK189522	MK190205	MK190114	MK189784	MK189947	MK189700
	CA1375		MK189785	MK190031	MK189614	MK189523	MK190206	MK190115	MK189785	MK189948	MK189701
C. meridionalis	CA1585	New Caledonia	MK189814	MK190053	MK189643	MK189552	MK190235	MK190144	MK189814	MK189975	
	CA1620		MK189818	MK190057	MK189647	MK189556	MK190239	MK190148	MK189818	MK189979	MK189728
C. mertoni	CA1505	Solomon Islands	MG707141	MK190041	MK189627	MK189536	MK190219	MK190128	MK189798	MK189959	MK189713
	CA1506		MG707142	MK190042	MK189628	MK189537	MK190220	MK190129	MK189799	MK189960	
C. multidentata	CA1545	Aquarium trade (Taiwan)	MK189809		MK189638	MK189547	MK190230	MK190139	MK189809	MK189970	MK189721
	CA1546		MK189810		MK189639	MK189548	MK190231	MK190140	MK189810	MK189971	MK189722
C. natalensis	CA1343	Comoros	MH644416	MK190023	MK189606	MK189515	MK190198	MK190107	MK189777	MK189940	MK189693
C. novaecaledoniae	CA1789	New Caledonia	MK189838	MK190077	MK189667	MK189576	MK190259	MK190168	MK189838	MK189998	MK189747
	CA1792		MK189839	MK190078	MK189668	MK189577	MK190260	MK190169	MK189839	MK189999	MK189748
C. papuana	CA1286	Solomon Islands	MK189775	MK190021	MK189604	MK189513	MK190196	MK190105	MK189775	MK189938	MK189691
	CA1361		MK189781	MK190027	MK189610	MK189519	MK190202	MK190111	MK189781	MK189944	MK189697
C. similis	CA1854	Seychelles	MK189845	MK190084	MK189674	MK189583	MK190266	MK190175	MK189845	MK190003	MK189754
C. simoni	CA1831	Sri Lanka	MK189842	MK190081	MK189671	MK189580	MK190263	MK190172	MK189842	MK190000	MK189751
	CA1833		MK189843	MK190082	MK189672	MK189581	MK190264	MK190173	MK189843	MK190001	MK189752
C. sp. 1 Solomon	CA1326	Solomon Islands	MK189776	MK190022	MK189605	MK189514	MK190197	MK190106	MK189776	MK189939	MK189692
	CA1499		MK189796	MK190039	MK189625	MK189534	MK190217	MK190126	MK189796	MK189957	MK189712
C. sp. 2 Solomon	CA1285	Solomon Islands	MK189774	MK190020	MK189603	MK189512	MK190195	MK190104	MK189774	MK189937	
	CA1515		MK189803	MK190045	MK189632	MK189541	MK190224	MK190133	MK189803	MK189964	
C. sp. 3 Solomon	CA1685	Australia	MK189822	MK190061	MK189651	MK189560	MK190243	MK190152	MK189822	MK189983	
	CA1240	Indonesia	MK189770	MK190016	MK189599	MK189508	MK190191	MK190100	MK189770	MK189933	MK189687
	CA1282	Solomon Islands	MK189773	MK190019	MK189602	MK189511	MK190194	MK190103	MK189773	MK189936	MK189690
C. sp. Malanda	CA1736	Australia	MK189832	MK190071	MK189661	MK189570	MK190253	MK190162	MK189832	MK189992	MK189741

(*continued on next page*)

Table 1 (*continued*)

Species	DNA voucher	Country	16S	COI	COII	ATP8	ATP6	COIII	ND3	28S	H3
	CA1737		MK189833	MK190072	MK189662	MK189571	MK190254	MK190163	MK189833	MK189993	MK189742
C. sp. NT nilotica	CA1721	Australia	MK189827	MK190066	MK189656	MK189565	MK190248	MK190157	MK189827		MK189736
		Australia	NC_030219	NC_030219	NC_030219	NC_030219	NC_030219	NC_030219	NC_030219		
	CA1103	Indonesia	MK189764	MK190010	MK189593	MK189502	MK190185	MK190094	MK189764		
C. sp. Santo	CA1132	Vanuatu	MK189766	MK190012	MK189595	MK189504	MK190187	MK190096	MK189766		MK189683
C. sp. WA4	CA1743	Australia		MK190073	MK189663	MK189572	MK190255	MK190164	MK189834	MK189994	MK189743
	CA1744		MK189835	MK190074	MK189664	MK189573	MK190256	MK190165	MK189835	MK189995	MK189744
C. typus	CA1568	New Caledonia	MK189813	MK190052	MK189642	MK189551	MK190234	MK190143	MK189813	MK189974	MK189724
	CA1524	Solomon Islands	MK189808	MK190050	MK189637	MK189546	MK190229	MK190138	MK189808	MK189969	MK189720
C. variabilirostris	CA1404	Micronesia	MK189790	MK190035	MK189619	MK189528	MK190211	MK190120	MK189790		MK189706
	CA1249	Vanuatu	MK189771	MK190017	MK189600	MK189509	MK190192	MK190101	MK189771	MK189934	MK189688
	CA1435		MK189794	MK190037	MK189623	MK189532	MK190215	MK190124	MK189794	MK189955	MK189710
C. variabilis	CA1205	Palau	MK189768	MK190014	MK189597	MK189506	MK190189	MK190098	MK189768	MK189931	MK189685
C. wilkinsi	CA1746	Australia	MK189836	MK190075	MK189665	MK189574	MK190257	MK190166	MK189836	MK189996	MK189745
	CA1747		MK189837	MK190076	MK189666	MK189575	MK190258	MK190167	MK189837	MK189997	MK189746
C. zebra	CA1731	Australia	MK189830	MK190069	MK189659	MK189568	MK190251	MK190160	MK189830	MK189990	MK189739
	CA1732		MK189831	MK190070	MK189660	MK189569	MK190252	MK190161	MK189831	MK189991	MK189740
C. zeylanica	CA1835	Sri Lanka	MK189844	MK190083	MK189673	MK189582	MK190265	MK190174	MK189844	MK190002	MK189753
Elephantis jaggeri	CA1160	Madagascar	KF468795	MK190013	MK189596	MK189505	MK190188	MK190097	MK189767		MK189684
Halocaridina rubra		Hawaii	NC_008413	NC_008413	NC_008413	NC_008413	NC_008413	NC_008413	NC_008413	FN995585	FN995500
Macrobrachium bullatum		Australia	NC_027602	NC_027602	NC_027602	NC_027602	NC_027602	NC_027602	NC_027602	AY374147	
Neocaridina denticulata		China	NC_023823	NC_023823	NC_023823	NC_023823	NC_023823	NC_023823	NC_023823	FN995598	FN995513
Paratya australiensis		Australia	NC_027603	NC_027603	NC_027603	NC_027603	NC_027603	NC_027603	NC_027603	FN995610	FN995525
Paratya caledonica	CA1800	New Caledonia	MK189840	MK190079	MK189669	MK189578	MK190261	MK190170	MK189840		MK189749
	CA1801		MK189841	MK190080	MK189670	MK189579	MK190262	MK190171	MK189841		MK189750

localities of the Indo-West Pacific region (Fig. 1, Table 1) mostly by electro-fishing or by hand net scooping in waterways. Specimens were then preserved in absolute ethanol and stored prior to morphological examination and tissue sampling. Identifications were made following keys provided by relevant publications focusing on the different prospected areas, comparisons with type specimens, and by DNA barcoding (see de Mazancourt et al., 2017a; 2017b; 2018a,b).

2.2. DNA extraction, amplification and sequencing

DNA was extracted from abdominal tissue using the semi-automatic Eppendorf ep-Motion 5075 robot. Fragments of mitochondrial genome (MGF) (~3200 bp, containing COII, ATP8, ATP6, COIII and ND3), 16S rRNA (~500 bp), COI (~640 bp), nuclear 28S rRNA (~1090 bp) and nuclear Histone 3 (H3) (328 bp) were amplified by PCR using primers and amplification protocols detailed in Table 2. DNA amplifications were performed in 20 µl PCR reactions, containing approximately 3 ng of template DNA, 2.5 mM $MgCl_2$, 0.26 mM of each nucleotide, 0.3 µM of each primer, 5% DMSO, 1 ng of BSA and 1.5 units of QBIOTAQ polymerase (MPBiomedicals) for nuclear markers, 16S and COI or LongAmp Taq Polymerase (BioLabs) for MGF. PCR products were sequenced using an Ion-Torrent PGM platform following the method developed by Hinsinger et al. (2015). Contigs obtained after sequencing were assembled iteratively by mapping them to previously Sanger-sequenced or assembled sequences and each gene of the MGF was annotated and extracted separately in Geneious v.8 software (http://www.geneious.com/: Kearse et al., 2012).

All sequences were deposited in GenBank (Table 1).

2.3. Phylogenetic analyses

Six sequences retrieved from GenBank for species that had a mitochondrial genome and nuclear markers published, were included with sequences obtained from specimens (Table 1). *Macrobrachium bullatum* (Palaemonidae) from Australia was used as an extra-family outgroup and *Paratya australiensis* from Australia, *P. caledonica* from New

Table 2
List of the primers used to amplify the different markers.

Marker	Primers	Sequence	PCR protocol	Reference
COI (~640 bp)	LCOI1490mod HCO2198mod	3'-TGTCAACTAATCATAAAGATATTGG-5' 3'-GGGTGTCCAAAAAACCAAAATAA-5'	Initial denaturation 94 °C for 4 min, 45 cycles of 94 °C for 30 s, 48 °C for 30 s, 72 °C for 40 s, final extension 72 °C for 7 min.	Modified from Folmer et al. (1994)
16S rRNA (~500 bp)	16Sar-Lmod 16Sbmod	3'-TACTTCTGCCTGTTTATCAAAAA-5' 3'-GGTCTGAACTCAAATCATGTAAA-5'	Initial denaturation 94 °C for 4 min, 45 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 40 s, final extension 72 °C for 7 min.	Modified from Palumbi (1996)
Mitochondrial genome fragment (COII, ATP8, ATP6, COIII, ND3) (~3200 bp)	tRNA-Leu1540F tRNA-Glu4760R	3'-AAAATGGCAGATCATTGCATAG-5' 3'-ACCTTTAGTTTTACAATGAAAATG-5'	Initial denaturation 94 °C for 30 s, 60 cycles of 94 °C for 20 s, 50 °C for 30 s, 65 °C for 15 min, final extension 65 °C for 15 min.	This study
28S rRNA (~1090 bp)	28S-F-Cru 28S-R-Met	3'-ACCCGCCTAATTTAAGCATAT 3'-GCTATCCTGAGGGAAACTTC	Initial denaturation 94 °C for 3 min, 35 cycles of 94 °C for 30 s, 60 °C to 52 °C (−1 °C/cycle) for 30 s, 72 °C for 2 min, final extension 72 °C for 5 min.	von Rintelen et al. (2012)
Histone 3 (328 bp)	H3F H3R	3'-ATGGCTCGTACCAAGCAGACVGC 3'-ATATCCTTRGGCATRATRGTGAC	Initial denaturation 94 °C for 3 min, 35 cycles of 94 °C for 30 s, 50 °C for 60 s, 72 °C for 60 s, final extension 72 °C for 5 min.	Colgan et al. (2000)

Table 3
Substitution models retained by PartitionFinder for each partition of the dataset.

Partition	Substitution Model	Partition Finder subset
16S	HKY + I + G	1
COI position 1	SYM + I + G	2
COI position 2	F81 + I	3
COI position 3	GTR + G	4
COII position 1	SYM + I + G	5
COII position 2	GTR + I + G	6
COII position 3	GTR + I + G	7
ATP8 position 1	HKY + I + G	1
ATP8 position 2	GTR + G	8
ATP8 position 3	GTR + I + G	7
ATP6 position 1	GTR + I + G	9
ATP6 position 2	SYM + G	10
ATP6 position 3	GTR + I + G	9
COIII position 1	SYM + I + G	11
COIII position 2	GTR + I + G	6
COIII position 3	GTR + I + G	7
ND3 position 1	SYM + I + G	5
ND3 position 2	GTR + G	8
ND3 position 3	GTR + I + G	7
28S	GTR + I + G	12
H3 position 1	K80 + I	13
H3 position 2	K80 + I	13
H3 position 3	HKY + G	14

Caledonia, and *Halocaridina rubra* from Hawaii were used as intra-family, extra-genus outgroups. Sequences from *Caridina cf. nilotica*, *C. gracilipes* and *Neocaridina denticulata* were added as comparison. The origin and identification of the GenBank sequences could not be ascertained, but including them allowed us to analyse all the atyid mitochondrial genomes published at that time and confirm their identification.

Alignments of the genes and gene fragments were conducted using Muscle algorithm (Edgar, 2004) implemented in MEGA7 software (Kumar et al., 2016). Fast Neighbor-Joining analyses were performed on each alignment and the trees obtained were compared by eye. As the trees obtained from the different mitochondrial and nuclear markers were consistent, we concatenated all the alignments. In total, 98 specimens were included in the concatenated analysis, all of them having the MGF, 96 a 16S sequence, 90 a COI sequence, 85 a 28S sequence, and 85 a H3 sequence (Table 1).

The best substitution model was calculated independently for each marker of the concatenated alignment and each codon position for protein-coding genes (Table 3) using Bayesian Information Criterion in PartitionFinder (Lanfear et al., 2012). Best-scoring ML trees were estimated using RAxML HPC2 v.8.2.10 (Stamatakis, 2014) and best-scoring Bayesian Inference trees were estimated using MrBayes v.3.2.6 (Ronquist and Huelsenbeck, 2003), both methods implemented in the Cyber Infrastructure for Phylogenetic Research (CIPRES) portal v.3.1. (Miller et al. 2010) (https://www.phylo.org/) with the previously determined models applied to their respective subset, with two independent searches running for 10,000,000 generations, a sampling frequency of 2000 and a burn in of 6% determined by checking trace files in the Tracer program of the BEAST package (Drummond et al., 2012). Convergence was tested by comparing the two runs in Tracer. Support for nodes for Bayesian analysis was determined using posterior probabilities (PP) calculated by MrBayes. One hundred independent searches, each starting from distinct random trees, were conducted. Robustness of the nodes for ML analysis was assessed using non–parametric bootstrapping (Felsenstein, 1985) with 1000 bootstrap replicates (B). We considered a node to be moderately supported with PP between 0.9 and 0.95 and B between 80 and 95. Below these values, we considered the node to be unsupported. At values above this range, we considered the node to be highly supported.

2.4. Time-calibrated tree

The concatenated dataset was used in BEAST v.1.8.3 (Drummond et al., 2012) to obtain a time-calibrated phylogenetic tree constraining the topology obtained in Bayesian Inference. The fossil shrimps *Delclosia roselli* (Via, 1971) and *D. martinelli* Rabadá, 1993 from Early Cretaceous freshwater deposits (127 ± 2 MYA [million years ago]) in Spain were used as the calibration point, i.e. stem of the Atyidae family. Two separate analyses (to assess the results reproducibility) ran for 50,000,000 generations, sampling every 1000 generations, with a burn-in of 2,000,000, under Yule process speciation, and using an uncorrelated relaxed molecular clock (because evolutionary rates appear to vary depending on the branches in the phylogenetic analyses) with a lognormal distribution, and a lognormal distribution around the calibration point. Priors for the calibration point were: mean = 0.01; standard deviation = 2.0; offset = 127.0. The other parameters were set to default. Convergence was assessed by comparing the two runs in Tracer. The trees were summed using the TreeAnnotator program from the Beast package in a maximum clade credibility tree.

2.5. Character mapping on the tree

Six different characters were mapped against the Bayesian tree obtained previously, reduced to one specimen per species, and the most parsimonious ancestral character states were reconstructed using Mesquite v.3.4 software (Maddison & Maddison, 2018) using a taxa-character matrix (55 taxa for 6 characters tested) (Supplementary material). Two biogeographic codings were tested, one with 31 freshwater ecoregions adapted from Abell et al. (2008) and one with 9 marine geographic provinces adapted from Longhurst (1998). The preferred habitat of the species was also tested with the Altitude coded in 5 characters: Estuary, Lower course, Middle course, Higher course and Headwater stream, the Substrate Type coded in 5 characters: Sand, Gravel, Pebbles, Boulders and Mud, the Hydrology coded in 2 characters: Lotic and Lentic, and the affinity with vegetation coded in 2 characters: Not in Plants and In Plants.

2.6. Historical biogeography analysis

A biogeographical analysis was performed on trees obtained from the time calibration using different programs implemented in RASP (Yu et al. 2015). To each taxa were assigned biogeographical ranges based on the terrestrial biogeographical realms recognised by the WWF (Global 200, see Olson et al. 2001): Australasian, Indo-Malay, Oceanian, Afrotropical and Palearctic (see Supplementary material). A BioGeoBEARS analysis determined the best fit model to our data. Using Akaike Information Criterion, the model retained was Dispersal-Extinction-Cladogenesis (DEC) (Ree et al. 2008). Using Lagrange algorithm implemented in RASP, with a dispersal constraints matrix set to default, ancestral areas were reconstructed on the Bayesian tree.

3. Results

3.1. Phylogenetic analysis

A total of 9 sequences alignments were used in the analysis: 16S (546 bp); COI (620 bp); COII (674 bp); ATP8 (150 bp); ATP6 (676 bp); COIII (789 bp); ND3 (357 bp); 28S (1284 bp) and H3 (327 bp). Trees obtained in ML and Bayesian analyses showed no inconsistencies; only the Bayesian tree is presented here with ML bootstrap values plotted (Fig. 2). Overall support for the tree was high. Most of the deep and the shallowest nodes were well supported in both methods, and only a few intermediate nodes showed low support (PP < 0.9, B < 80). Atyid sequences were separated in two highly supported clades. The first clade comprised species of *Paratya* and *Halocaridina rubra*, and the other clade included all of the other species. In this second clade, a first

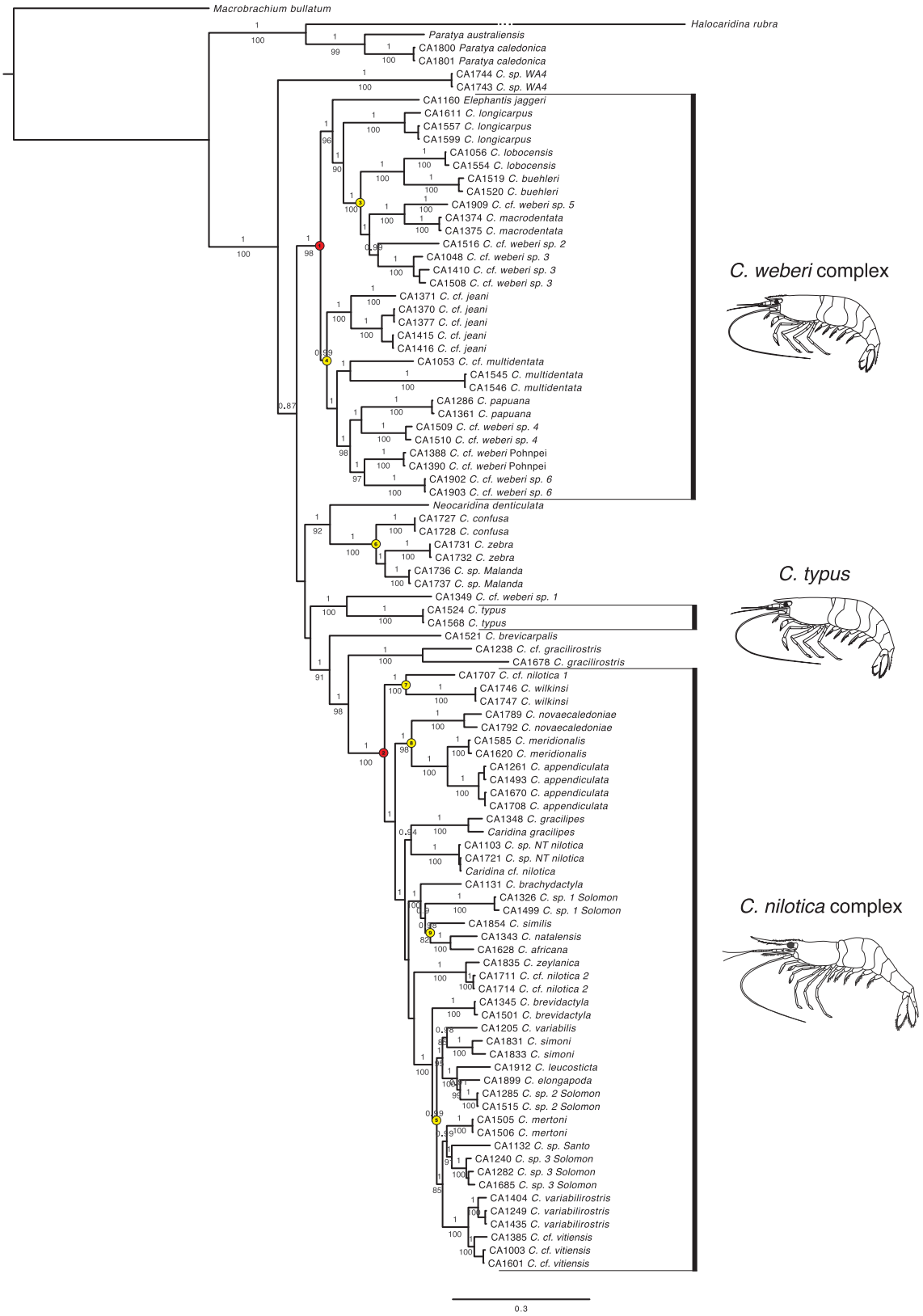


Fig. 2. Bayesian Inference Phylogram obtained from the concatenated dataset showing the position of the two species complexes and the type species of the genus, *Caridina typus*. Numbers above branches indicate Bayesian posterior probabilities (PP); numbers under branches indicate Maximum Likelihood bootstrap values (B). Support values under 0.8 in PP or 80% in B are not shown. Nodes highlighted with a colored circle are discussed. Nodes with a red circle correspond to the "*C. weberi*" and "*C. nilotica*" complexes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

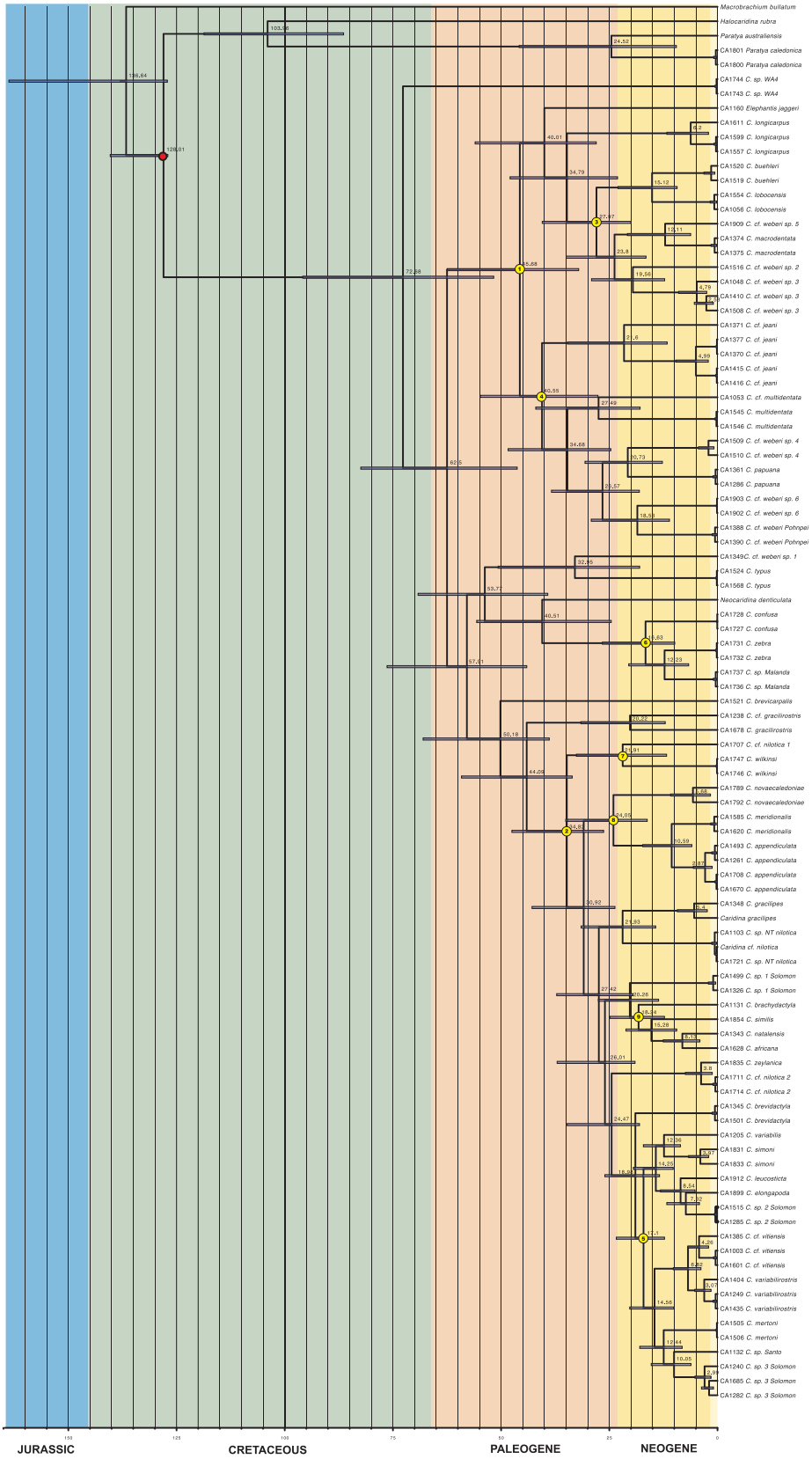


Fig. 3. Time calibrated Bayesian tree obtained with Beast software. Blue bars indicate confidence intervals; numbers indicate mean date of the node. The red dot indicates the calibration point. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

dichotomy excluded *Caridina* sp. *WA4* from Australia from all others. Within this clade emerged a strongly supported clade containing almost all the species of the *C. weberi* complex (node 1) and dispatched further into two highly supported clades. The tree was further divided into clades with low support; one clade comprised *Neocaridina denticulata*, *C. confusa*, *C. zebra* and *C. sp. Malanda*, another with *C. cf. weberi* sp. *1* and *C. typus*, and a third with all the remaining species. Subsequent dichotomies separated *C. brevicarpalis*, *C. gracilirostris* and *C. cf. gracilirostris*, and all species belonging to the *C. nilotica* complex in a highly supported clade, respectively.

3.2. Time calibration

The time calibration gave an origin date for the split of the two most basal atyid clades ranging from 140 to 127 MYA (Mean = 128.01), following the calibration given by the fossils *Delclosia* spp. (Fig. 3). The origin of *Caridina* group was estimated to have occurred between 95 and 52 MYA (Mean = 72.68). The last common ancestor between *C. nilotica* and *C. weberi* complexes existed around 82 to 44 MYA (Mean = 62.5). The origin of *C. weberi* complex (Fig. 3, Node 1) was dated to between 62 and 32 MYA (Mean = 45.68), whilst the origin of *C. nilotica* complex (Fig. 3, Node 2) was estimated to have occurred more recently, between 47 and 27 MYA (Mean = 44.09). A rapid radiation of the *C. nilotica* complex seems to have occurred around 27 MYA.

3.3. Character mapping

The primary factor driving evolution in the character mapping analyses appeared to be habitat, with some secondary influence of geography (Fig. 4A and B). There were clear correlations between habitat-related characters and the phylogeny, especially for deeper nodes. For example, following ancestral states reconstructions, node 1 (Figs. 2 and 4) corresponding to *C. weberi* complex, was likely to have originated in the higher course (Fig. 4C), on gravel substrate (Fig. 4D), in a lotic environment (Fig. 4E), and with no vegetation (Fig. 4F). In contrast, node 2 corresponding to *C. nilotica* complex appeared to have originated in the lower course (Fig. 4C), on gravel substrate (Fig. 4D), in a lentic environment (Fig. 4E), and among vegetation (Fig. 4F). Some characters may have changed or reverted in more shallow nodes. For example, nodes 3 and 4, both belonging to *C. weberi* complex, differed from their ancestor in having originated on boulder substrate (Fig. 4D). Node 3 further differed in showing affinities to lentic environments (Fig. 4E). For node 5, members of *C. nilotica* complex showed affinities with the higher course (Fig. 4C), with the absence of vegetation (Fig. 4F), and several of its subclades were correlated with a boulder substrate (Fig. 4D).

3.4. Historical biogeography analysis

To study more thoroughly the historical biogeography of the species, a statistical reconstruction of ancestral areas was performed (Fig. 5). The Australasian realm appeared to be the ancestral range of most of the clades. Over the tree, the analysis found divergences within biogeographic realm more common than vicariance events. Dispersal is frequent, while only one extinction event was found for the species close to *C. multidentata*.

4. Discussion

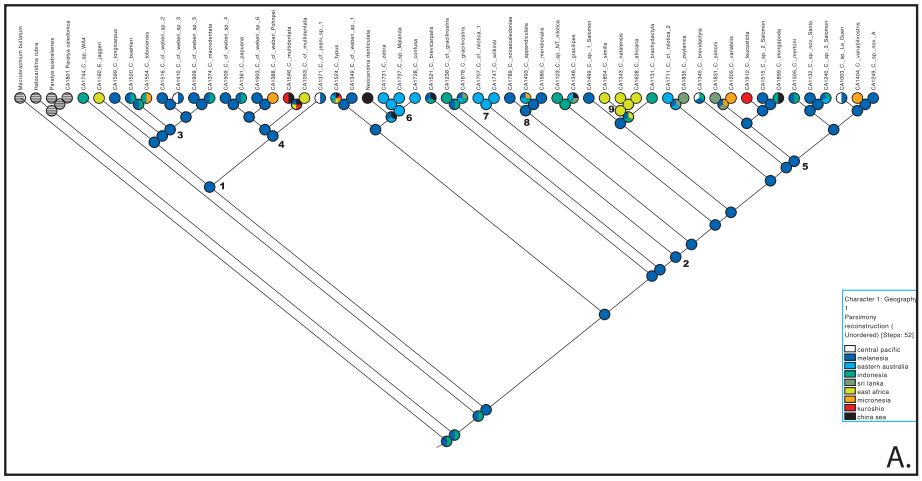
4.1. A large dataset for robust analyses

This study presents the first well resolved and well supported phylogenetic tree for the genus *Caridina* thanks to the large molecular dataset obtained by next generation sequencing (NGS) and the number of species included that have been well determined. Indeed, with nine different molecular markers from both mitochondrial and nuclear genomes, totalling around 5420 bp for 98 specimens (of which 92 were newly sequenced), this is the most ambitious study made on this taxon to date. Obtaining such a large dataset was made possible by the increasingly affordable NGS technologies, namely the ion-torrent sequencing coupled with multiplexing. This method, developed by Hinsinger et al. (2015), allowed us to get fragments of the mitochondrial genome (MGF) for up to 40 specimens in a single sequencing, for prices comparable to regular Sanger sequencings. Furthermore, thanks to our network of researchers from all around the world and the numerous field trips made by the Muséum national d'Histoire naturelle, we could use a great number of specimens from many different localities, some of them with a poorly studied freshwater carcinofauna until now, such as the Solomon Islands or Micronesia. As expected with a dataset this large, the overall support of the tree was high, regardless of the reconstructing method used. This allowed us to have, for the first time, a clear representation of the phylogenetic relationships among the species of *Caridina*, showing that species complexes defined previously using morphological resemblances were monophyletic.

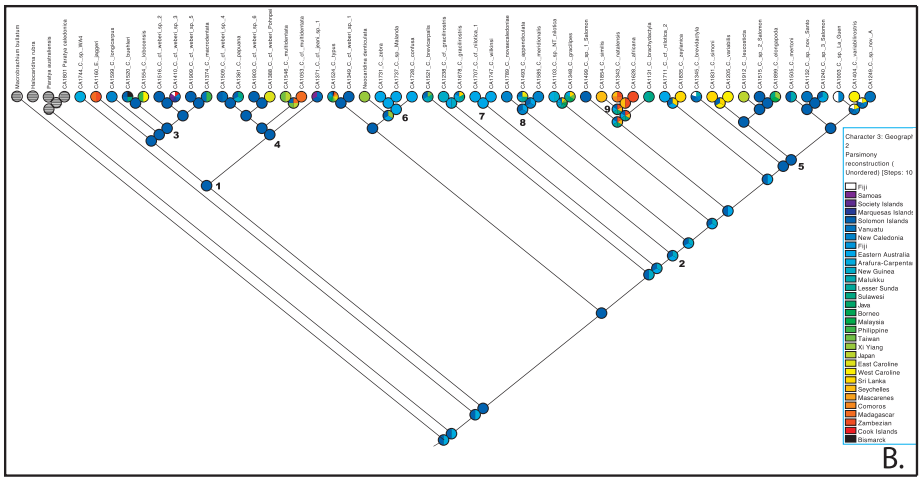
4.2. Time calibration

Until now, few studies have proposed dated phylogenies of atyid shrimps (Botello et al., 2013; Jurado-Rivera et al., 2017; Davis et al., 2018) and only two have discussed *Caridina* (von Rintelen et al., 2012; Bernardes et al., 2017). This can be explained by the lack of fossils attributed to this family. Caridean shrimps are quite rare in the fossil record (52 species according to Schweitzer et al., 2010) and even scarcer in freshwater deposits. Five atyid species belonging to three genera exist in the fossil record, *Delclosia martinelli* (Via, 1971), *D. roselli* Rabada, 1990, *Atyoida roxoi* Beurlen, 1950, *A. tremembeensis* Beurlen, 1950, and *Caridina nitida* A. Milne Edwards, 1879. A fourth species (and genus), currently *incertae sedis* could be added to this list, *Homelys minor* Meyer, 1862, that Ortmann (1894) proposed as an Atyidae because of the small size of the specimens and the freshwater environment. It is doubtful that extant genera could be represented in the fossil record, the two species of fossil *Atyoida* Randall, 1840 described by Beurlen (1950) being separated by almost 100 million years. These fossils were identified as atyids because they resemble modern day *Potimirim* Holthuis, 1954 (known as *Atyoida* at the time of Beurlen) that live today in the same area. Additionally, *Caridina* was the default name of small freshwater shrimps at the time of A. Milne Edwards (see *Caridina desmarestii* (Millet, 1831) (Joly, 1843, A. Milne Edwards, 1879) now *Atyaephyra desmarestii* (Millet, 1831)). Regardless, because of their small size (3 cm at most), morphological characters as demonstrated by Rabadá (1990), and age (oldest fossil atyid record), the two species of *Delclosia*, *D. roselli* (Via, 1971) and *D. martinelli* (Rabadá, 1990) from the Lower Cretaceous of Spain remain the most convincing fossil atyid, which is why we retained *Delclosia* as our calibration point.

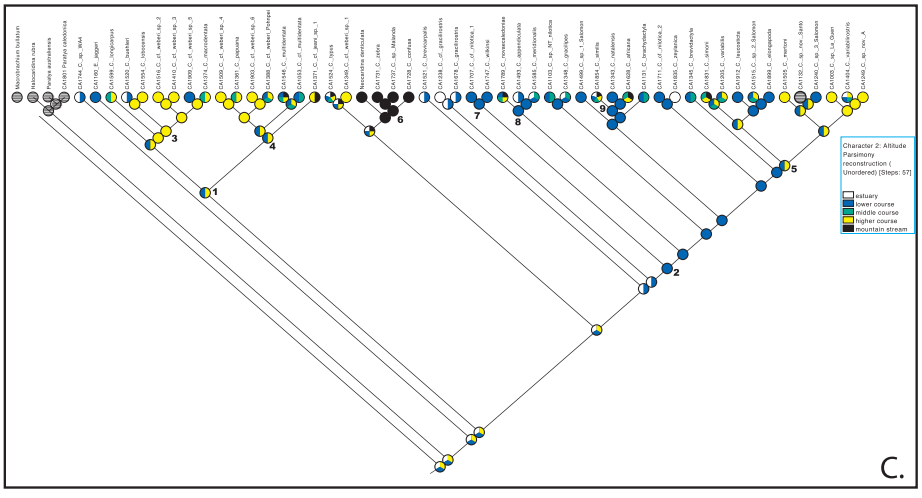
Only one calibration point was used in our analysis given the limited availability of suitable fossils. Multiple calibration points can improve the reliability of molecular timing analyses (Lukoschek et al., 2012; Hipsley & Müller, 2014). Despite this, we consider our results to be reliable because they are consistent with geographical events. For example, the clade containing all New Caledonian endemic species is posterior to the re-emergence of the island after its complete submersion, 37 MYA (million years ago) (Grandcolas et al., 2008). Similarly, *C. variabilirostris*, a species endemic to Pohnpei Island, Micronesia appeared to have split from its sister-species between 10 and 3 MYA (Mean = 6.82 MYA) in our analysis, congruent with the apparition of the island, between 3 and 8.6 MYA (Craig et al., 2001). Furthermore, if the fossils we used for the calibration point were excluded from the Atyidae, the molecular clock would still give us a congruent timing. Substitution rates are sometimes used to calibrate a tree where no fossils exist or a geological event cannot be used. For *Caridina*, Bernardes et al. (2017), following Hurwood & Hughes (2001) who were



Geography 1 (Longhurst, 1998)

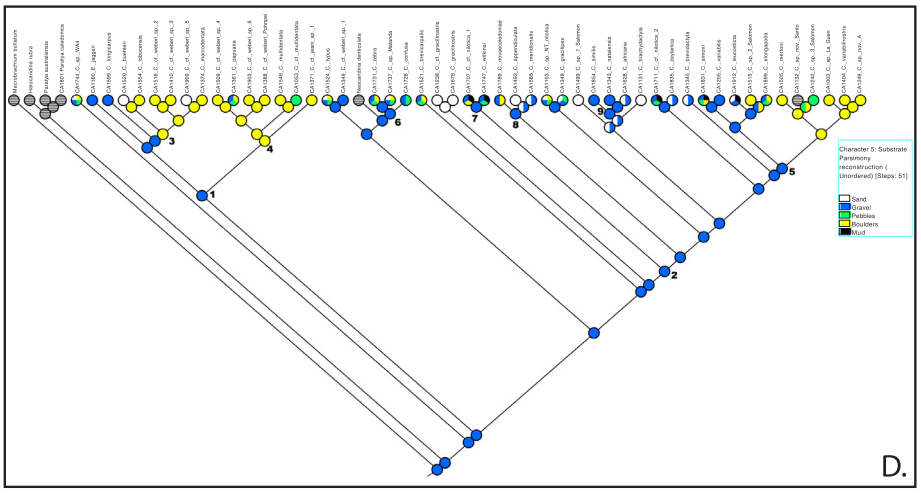


Geography 2 (Abell et al., 2008)

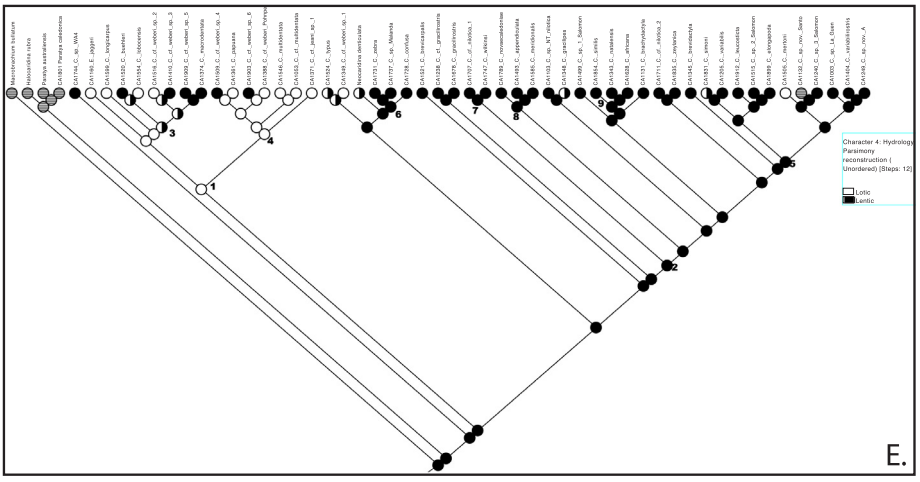


Altitude

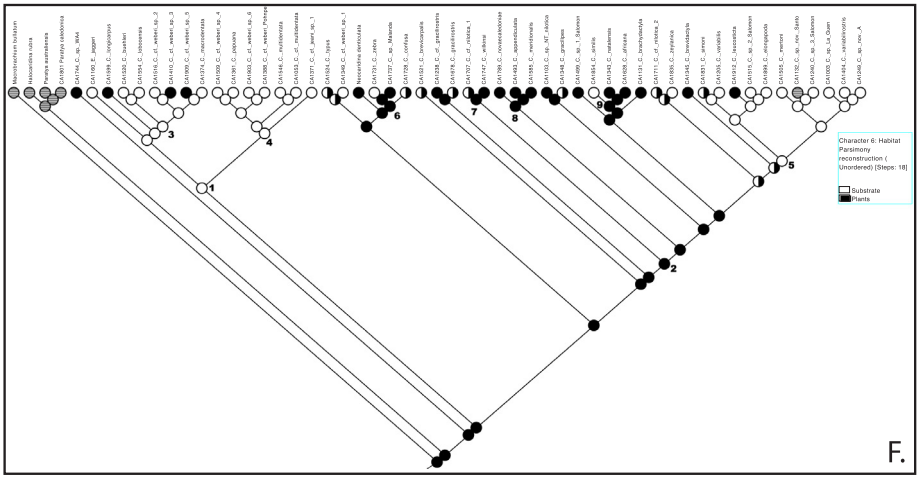
Fig. 4. Character mapping on the Bayesian tree obtained in Fig. 2. (A) Geography coded following Longhurst's (1998) marine provinces; (B) Geography coded following Abell et al.'s (2008) freshwater ecoregions; (C) Altitude; (D) Substrate type; (E) Hydrology; (F) Habitat.



Substrate type



Hydrology



Habitat

Fig. 4. (*continued*)

following Knowlton et al. (1993), a substitution rate of 1.1–1.3% per MYR (million years) in COI was retained as originally calculated for alpheid shrimps based on the closing of the Isthmus of Panama. If we suppose this rate to be constant in caridean shrimps, our data agrees somewhat for intra-specific divergences. For example, the two sequences of *C. simoni* are separated by 5.1% divergence (K2P) in COI, corresponding to 3.97 MYA in the calibrated tree, which gives a rate of 1.28% per MYR. For *C. novaecaledoniae*, a COI divergence of 6%

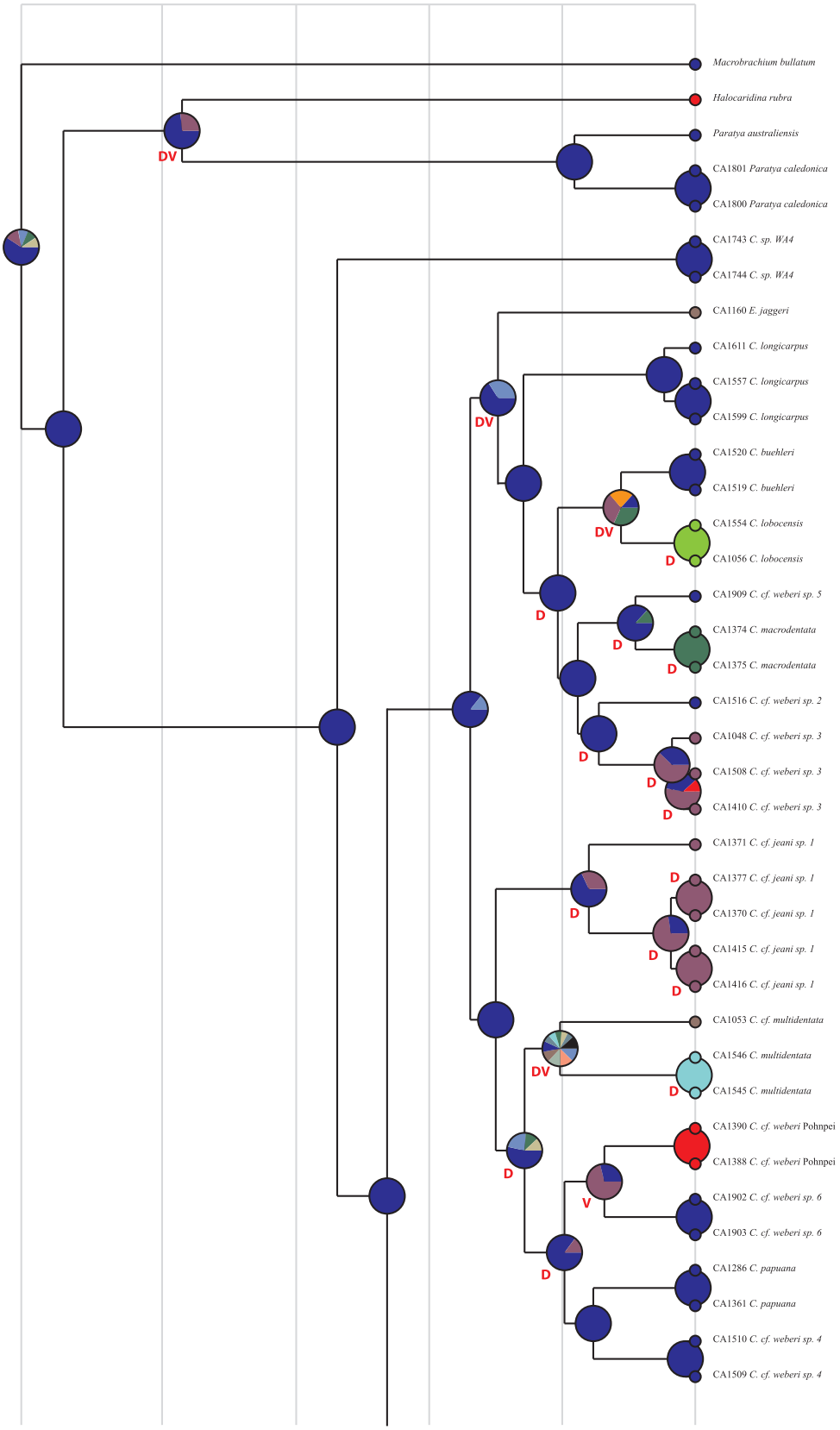


Fig. 5. Ancestral areas reconstruction based on the time calibration consensus tree.

between the two sequences, corresponding to 5.68 MYA, gives a substitution rate of 1.06% per MYR.

An arising of the Atyidae in the Early Cretaceous agrees with most studies that have examined the origin of the family. For example, four out of six analyses by von Rintelen et al. (2012) suggested a Cretaceous origin of the family and a Paleogene radiation. Conversely, Jurado-Rivera et al. (2017) suggest a Permo-Triassic origin of the Atyidae, but only included fossils that are phylogenetically outside of the family as

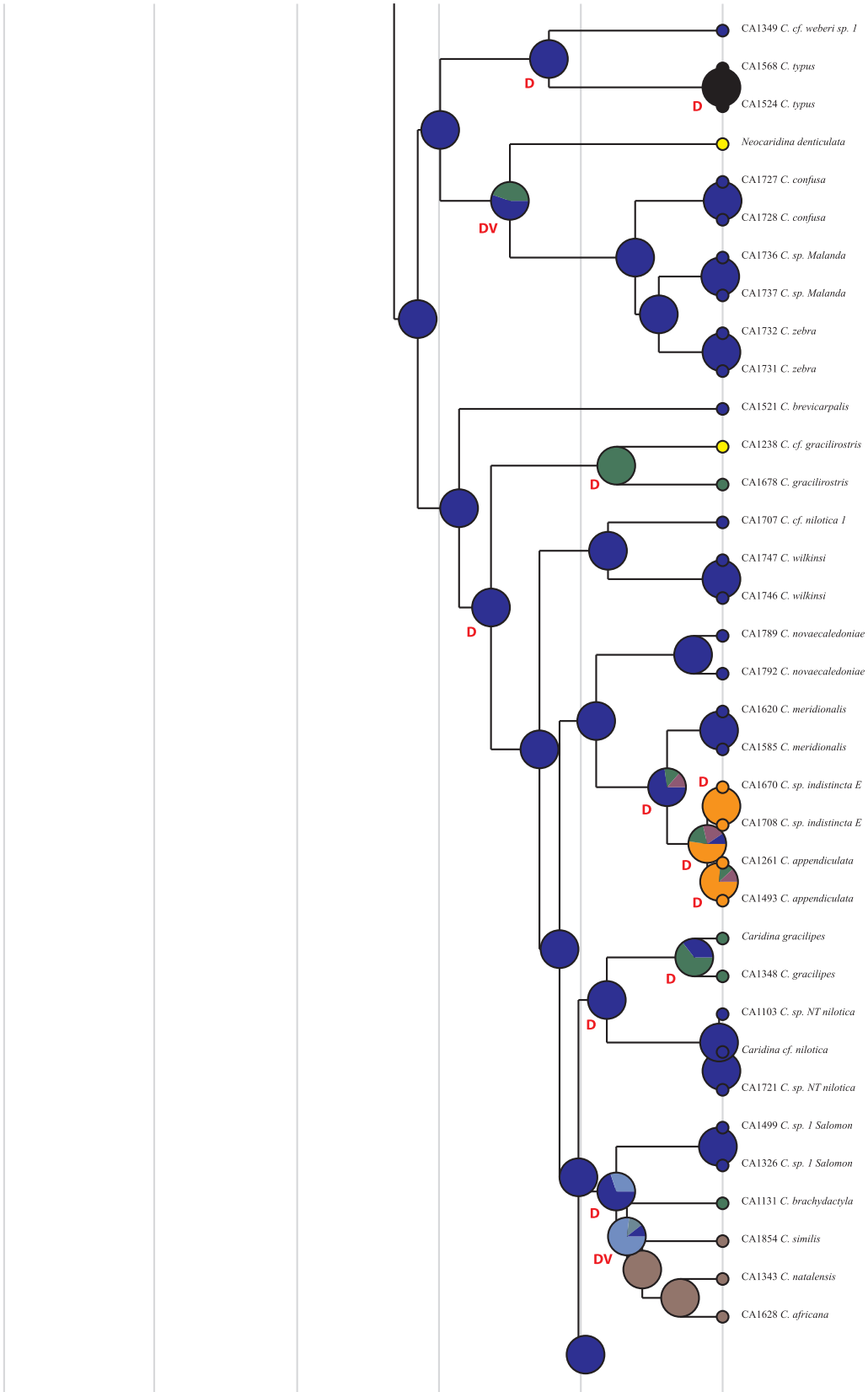


Fig. 5. (*continued*)

calibration points. Additionally, time-estimates could be over-estimated due to high mutation rates in the *Typhlatya* group (see the branch length of *H. rubra* on Fig. 2). Likewise differences between the results of this study and Davis et al. (2018), which proposed the origin of Atyidae during the Early Jurassic without rapid diversification and an equal rate of speciation throughout the tree, may be explained by differences in methodology and underlying assumptions. Combining trees from different sources as used by Davis et al. (2018), in which original branch

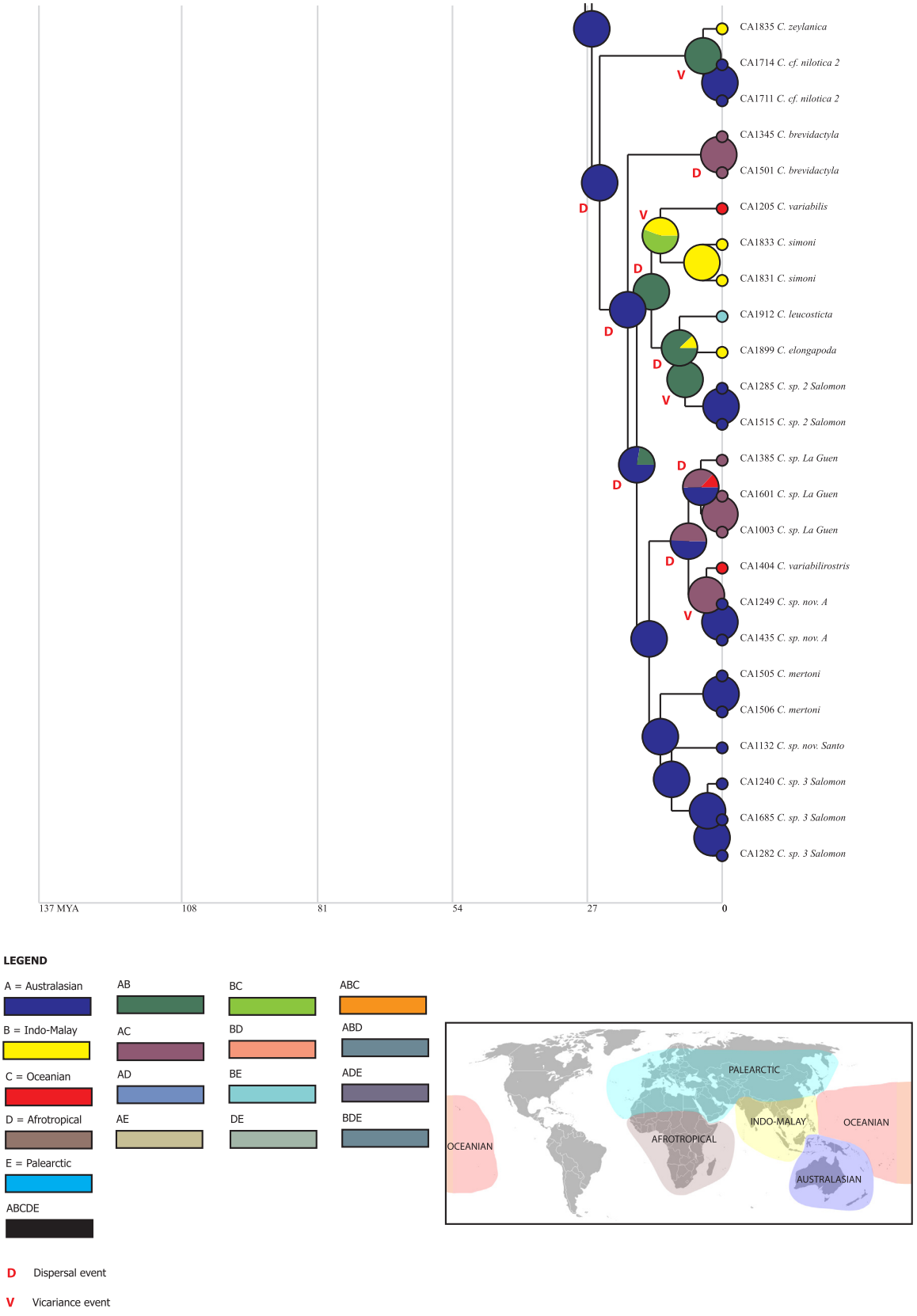


Fig. 5. (*continued*)

lengths are lost and recalculated, can lead to over-estimation of time-estimates (Bryant et al., 2004).

4.3. Historical biogeography and character mapping

For the geographic coding, we first tried to use freshwater ecoregions (Abell et al., 2008) but the great number of distinct regions made the analysis unreadable. As most caridean species are postulated to have colonized their range via marine larval dispersal, we tried to test marine biogeographic provinces based on plankton communities and currents (Longhurst, 1998). This approach was easier to read due to the fewer number of coded provinces, but could not highlight endemic taxa. Given the difficulties in our approaches, no biogeographic hypotheses can be proposed for the deeper nodes of the phylogeny due to a strong bias of sampling towards Melanesia, however, some of the terminal nodes can be explained by their geographical distribution. For example, nodes 6 and 7 clearly originate in Australia (Fig. 4A), which supports the hypothesis that this continent was colonised in several events (Page et al., 2007a,b). Node 8, containing all the New Caledonian endemic species is likely to have originated there (33% likelihood, Fig. 4B). Node 9, composed only of species living in the Indian Ocean region, would likely have originated there. It is interesting to note, however, that an Indonesian species, *C. brachydactyla* is sister to the clade delineated at Node 9, supporting the hypothesis of a colonization of the Indian Ocean region from Indonesia. The historical biogeography analysis was made on a shorter list of areas based on terrestrial biogeographical realms. It confirmed the results obtained for the marine and freshwater bioregions in showing no deep geographic structuration of the phylogeny and still with a bias of sampling towards Melanesia (here included among others in the Australasian realm). It further showed that dispersal is frequent in the evolutionary history of *Caridina*, suggesting that amphidromy may be the ancestral character in this genus that facilitated such behaviour. Within-region cladogenesis is more common than vicariance divergence across terrestrial biogeographical realms, even in deeper nodes, and the vicariance events inferred could hardly be explained by geological events such as continental drift, since most of the species are found on islands, but could be linked to changes in oceanic currents that no longer carry larvae from one island to another (see Bernardes et al. 2017). It is important to note that the Indo-Malay region was not sampled as extensively as other regions in our study. The Indo-Malay region is a biodiversity hotspot for many taxa, including freshwater shrimps (De Grave et al. 2015). Given this, it is possible that a significant proportion of species may be missing from our analyses, which could have biased our results. Whilst we included species from this region representing a broad range of geographical and ecological types, further samples are required to better understand the phylogeny and historical biogeography of the shrimp from the Indo-Malay region.

Our analyses provide new insights into the habitat and potential ecology of the common ancestors of the genus *Caridina*. According to the analyses, the common ancestors of the *C. nilotica* and *C. weberi* complexes seem to have lived at low altitudes (67% of chances in the estuary or lower course, Fig. 4C), on gravel substrate (Fig. 4D) in a lentic environment (Fig. 4E) and among vegetation (Fig. 4F). Differences in the ecology of shrimps from complexes other than '*C. nilotica*' and '*C. weberi*' used here might provide further explanation for the separation of complexes that have been identified using genetics (this study) and morphology (Cai & Ng, 1999; 2007; Klotz & von Rintelen, 2014). For example, in the lower reaches of rivers, there often is strong predation pressure (from Eleotridae, Kuhliidae, Anguillidae) but an abundance of food available, whereas in the higher course, there are almost no predators (except Anguillidae and Palaemonidae) but a strong competition for food with other atyids (namely the filter-feeding *Atya*-like species).

4.4. Differences between C. nilotica and C. weberi complexes

The analyses highlighted the genetic reality of some species complexes that were established using morphological characteristics, namely *C. weberi* and *C. nilotica* complexes on which this study focused. Both of these complexes were well separated from other taxa and well supported in the phylogeny regardless of the method used to differentiate them. As pointed out by previous authors (Page et al., 2007a,b, von Rintelen et al., 2008, 2012), genus *Caridina* appears polyphyletic, with genera *Elephantis* Castelin et al., (2013) and *Neocaridina* Kubo (1938) nested in the middle of the *Caridina* species.

Our time calibration showed that *C. weberi* complex is older than *C. nilotica*. Ancestral states reconstruction and character mapping analyses showed that the separation of the clades was due to habitat at first (including altitude, habitat, and hydrology) and then geography was responsible for the diversification within the complexes. This was not a surprising result given environment has a profound influence on the morphology of atyid species (Jugovic et al., 2010; de Mazancourt et al., 2017b). For example, as altitude decreases, along with changes in associated environmental factors (current flow, substrate type, etc.), the length of the rostrum and the segments of the legs tend to increase; a phenomenon known as the "Pinocchio-Shrimp effect" (de Mazancourt et al., 2017b). This effect is visible in both species complexes studied here. *C. weberi* complex displays robust morphology, with usually a short rostrum and stout legs, associated with their affinities to mostly lotic environment in high altitudes among boulders with no vegetation (Fig. 4). In contrast, *C. nilotica* complex species tend to display a slender morphology, with a long rostrum and long legs associated with their affinity to lentic environments on the lower course with gravel bed and the presence of aquatic vegetation (Fig. 4). It is important to note, however, some species belonging to these complexes have adapted to different habitats than their ancestors, and this is associated with differences in their morphology. For example, species in *C. nilotica* complex living in the higher course, like *C. mertoni*, *C. variabilis* or *C. variabilirostris* (species of the node 5), often show a shorter rostrum (de Mazancourt et al., 2018a,b). Conversely, species of the *C. weberi* complex that are adapted to lower altitudes, like *C. buehleri* (species of node 3), can possess a longer rostrum than other species of this complex (de Mazancourt et al., 2017a). Likewise, *C. cf. weberi* sp. *1* from the Solomon Islands, living in environments close to those of species of *C. weberi* complex exhibit a close morphology, which led to this species being included as part of the complex. *C. cf. jeani* that was thought to be close to *C. typus* because of its unarmed rostrum (Cai, 2010) falls within the *C. weberi* complex in our analysis. Our results highlight the need for careful morphological examination to identify discriminating characters and prevent misidentifications.

4.5. Implications for conservation and management of Caridina

Our genetic analyses provide strong evidence for the existence of multiple valid and undescribed species within the *C. nilotica* and *C. weberi* species complexes. Currently, both '*C. nilotica*' and '*C. weberi*' are classified as "Least Concern" on the IUCN Red List (De Grave, 2013a; Cai et al., 2013). These classifications will need to be reassessed to account for differences in the vulnerability of the species identified in this study that are clustered within the two complexes. For example, our analysis indicated the presence of three undescribed species within the *C. nilotica* complex that are likely to be restricted to the Solomon Islands and/or nearby islands where freshwater biota are impacted by habitat destruction and to a lesser extent invasive species (Polhemus et al., 2008). Species with restricted distributions are generally more vulnerable to extinction compared to widely dispersed species (Purvis et al., 2000). Similarly, our genetic data indicates the range of some species reported as widespread (e.g. *Caridina longirostris*) and in turn classified as "Least Concern" (De Grave, 2013b), may be smaller than currently thought due to difficulties in distinguishing among specimens

with very similar morphology (de Mazancourt et al., 2018c). Our genetic analysis indicated specimens from Australia fitting the morphological description of *Caridina longirostris* were two different species, *C. gracilipes* and *C.* sp. Solomon 3. Given this, it is likely that the risk of extinction for many shrimp populations within the *'C. nilotica'* and *'C. weberi'* species complexes is greater than previously thought. This is concerning given one third of caridean shrimps are already classed as 'Threatened' or 'Near Threatened' (De Grave et al., 2015). Until full taxonomic assessments can be made, managers and conservationists should use a 'precautionary approach' in managing and protecting populations that may be closely related and have similar morphology to other wide-spread species.

5. Concluding remarks

This study reconstructing the phylogeny of genus *Caridina* provided a well-resolved and supported phylogenetic tree for the first time, with clades clearly identified that allowed us to propose novel evolutionary hypotheses. Our results highlight how inclusion of species complexes in phylogenies can provide new insights into evolutionary relationships and propose new avenues for distinguishing closely related species, as well as providing information crucial to conservation and management initiatives. To expand this work and improve the taxonomy of the whole genus, some species positions will need to be clarified (e.g. *C. typus*), which could be obtained with a larger dataset of complete mitochondrial genomes. The inclusion of comparatively unexplored species complexes in our phylogeny, such as Sulawesi, African, Chinese landlocked groups, *C. gracilirostris* complex, or the species close to *C. serratirostris/C. celebensis*, would likely add substantial information on the evolution of this group. Finally, the evolutionary hypotheses proposed here can be improved by refining time calibrations, but this is reliant on the discovery of new fossils or the improvement of existing techniques that do not rely on fossils.

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Appendix A. Supplementary material

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Résumé

Les cours d'eau des îles tropicales abritent des organismes qui ont développé un cycle de vie diadrome, partagé entre une phase adulte en eau douce et une phase larvaire marine : l'amphidromie. Parmi ces organismes, dans la zone Indo-Pacifique, on trouve les crevettes du genre *Caridina* H. Milne Edwards, 1837. Avec plus de 300 espèces décrites, il s'agit du genre le plus diversifié de l'infra-ordre des Caridea, avec une systématique extrêmement confuse et compliquée. Au sein de ce genre, deux complexes d'espèces sont particulièrement bien représentés dans les systèmes insulaires de l'Indo-Pacifique, le complexe *Caridina nilotica* et le complexe *C. weberi*. Grâce au développement de nouvelles techniques de séquençage de nouvelles méthodes de taxonomie dite intégrative sont apparues, permettant de résoudre une partie des problèmes taxonomiques de ces groupes. L'objectif de la thèse était d'appliquer une approche de taxonomie intégrative aux espèces des complexes *C. nilotica* et *C. weberi* afin de clarifier leur systématique et, de fait, mieux appréhender leur biologie et fournir les outils aux gestionnaires pour mettre en place une meilleure conservation de ces espèces et de leurs milieux. Après avoir montré que certains caractères morphologiques traditionnellement utilisés pour décrire les espèces étaient influencés par l'environnement et donc fortement variables, l'étude de taxonomie intégrative a été conduite sur 92 espèces, permettant d'obtenir 1682 séquences auxquelles s'ajoutent 32 génomes mitochondriaux complets et 97 partiels, mettant en évidence 43 espèces nouvelles, certaines décrites au cours de la thèse. Les relations phylogénétiques entre les espèces des deux complexes ont été reconstruites à partir d'un grand jeu de données moléculaires, permettant de montrer que les complexes sont des groupes monophylétiques avec des différences en terme d'habitats occupés. Enfin, la faisabilité de l'étude sclérochronologique de l'amphidromie chez une espèce du complexe *C. weberi* (*C. multidentata*) a été testée sur la cuticule du pédoncule oculaire, avec une étude de l'ultrastructure de la cuticule, décrite pour la première fois chez cette espèce.

Abstract

Rivers of tropical islands harbor organisms that have developped a diadromous lifecycle, shared between a freshwater adult phase and a marine larval phase: amphidromy. Among these organisms, in the Indo-Pacific area are found shrimps of the genus *Caridina* H. Milne Edwards, 1837. With more than 300 described species it is the most speciose genus of the infra-order Caridea, with a most confused and complicated taxonomy. Within this genus, two species complexes are particularly well-represented in insular systems of the Indo-Pacific, the *C. nilotica* complex and the *C. weberi* complex. Thanks to the development of new sequencing techniques, new methods of integrative taxonomy appeared, allowing to resolve part of the taxonomic complexity of these taxa. The aim of the thesis was to apply an integrative taxonomy approach to species belonging to *C. nilotica* and *C. weberi* complexes in order to clarify their taxonomy and have a better understanding of their biology and provide tools to managers for establishing a better conservation of these species and their environments. After showing that some morphological characters traditionally used to describe species were influenced by the environment and so, highly variable, the integrative taxonomy was led on 92 species, allowing to obtain 1,682 sequences to which are added 32 complete and 97 partial mitochondrial genomes, highlighting 43 new species, some of them described during the thesis. Phylogenetic relationships among the species of the two complexes were reconstructed from a large molecular dataset, allowing to show that the complexes are monophyletic groups, with habitat differences. Finally, the feasibility of a sclerochronological study of amphidromy in a species of the *C. weberi* complex (*C. multidentata*) was tested on the eyestalk cuticle, with a study of the ultrastructure of the cuticle, described for the first time in this species.