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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%.

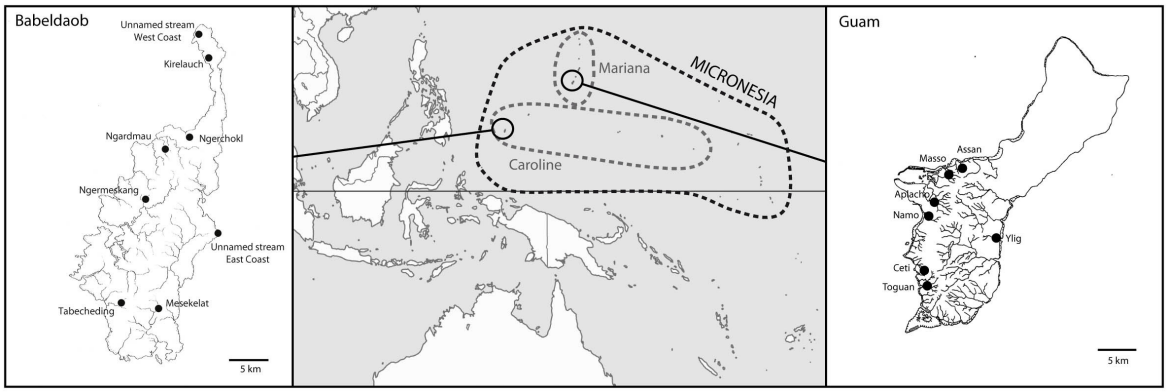

FIGURE 1. Rivers and sites surveyed in Babeldaob and Guam (black dots: prospected sites)

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.

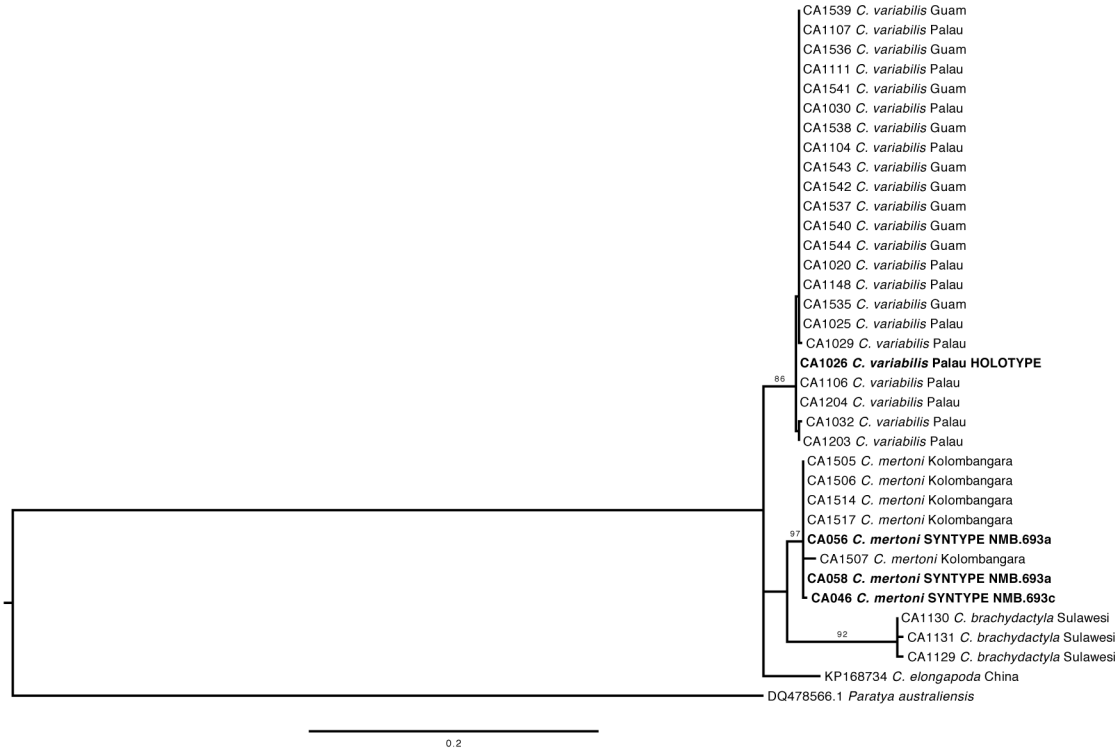

FIGURE 2. Maximum likelihood phylogenetic tree (16S mtDNA) of the specimens. CAXXXX numbers are lab IDs of the specimens.

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.

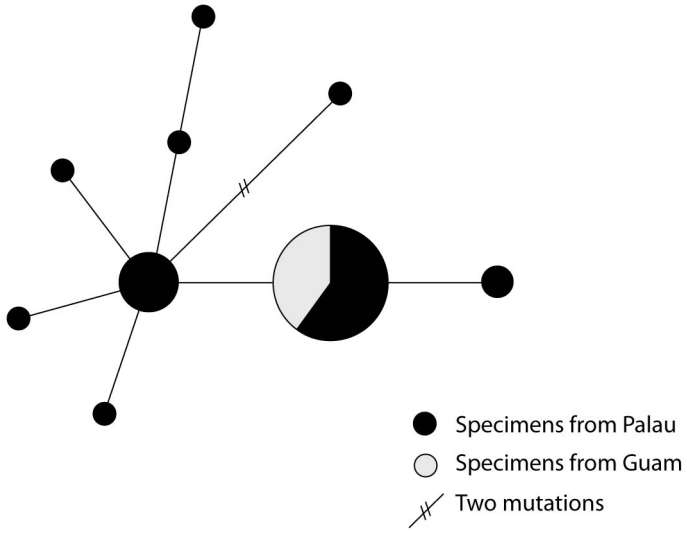

FIGURE 3. Haplotypes network of the specimens sequenced.

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.

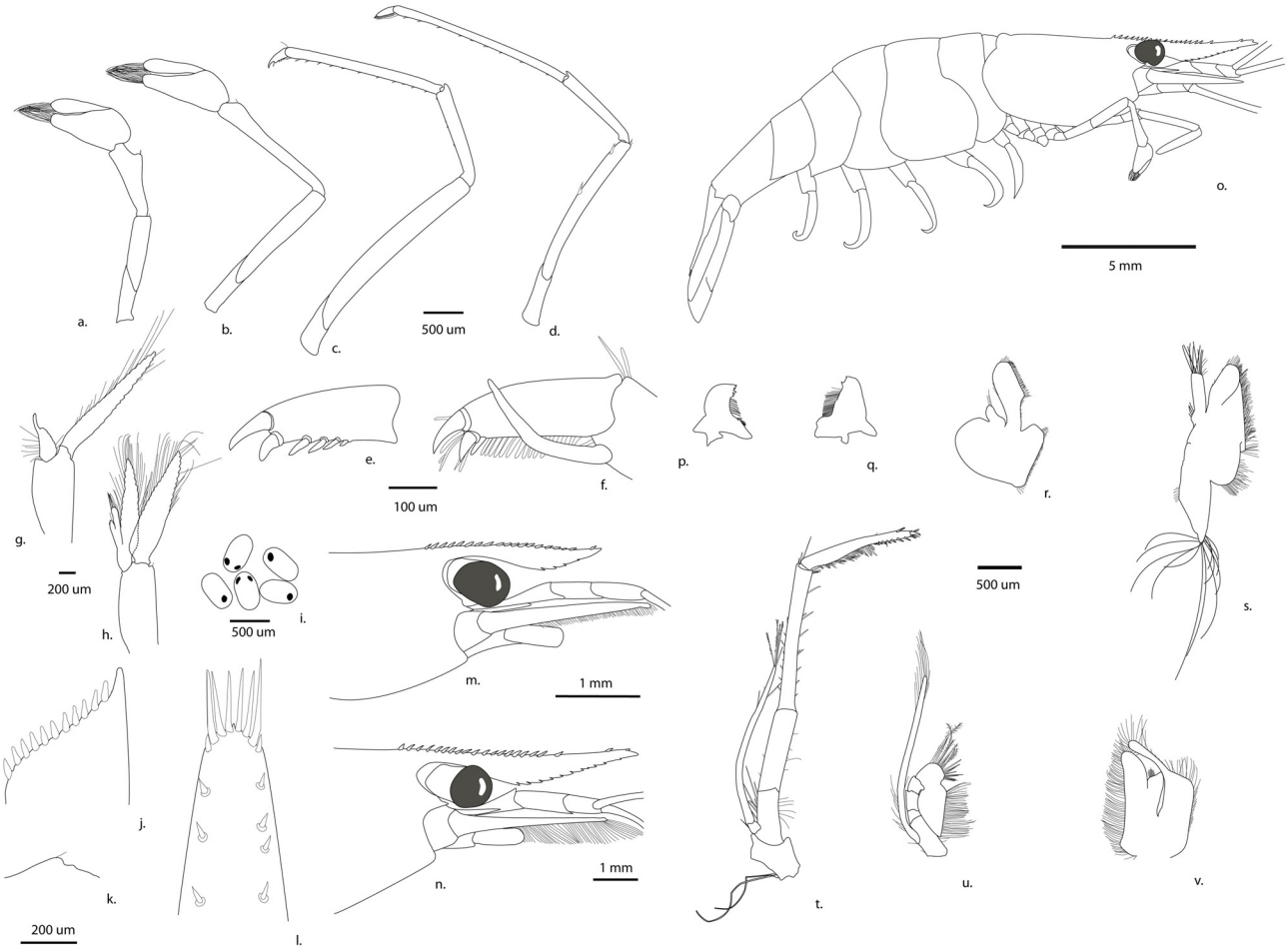

FIGURE 4. a. First pereiopod; b. Second pereiopod 2; c. Third pereiopod; d. Fifth pereiopod; e. Dactylus of third pereiopod; f. Dactylus of fifth pereiopod; g. Male first pleopod; h. Male second pleopod; i. Eggs; j. Uropodial diaeresis; k. Pre-anal carina; l. Telson; m., n. Cephalothorax; o. General 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.

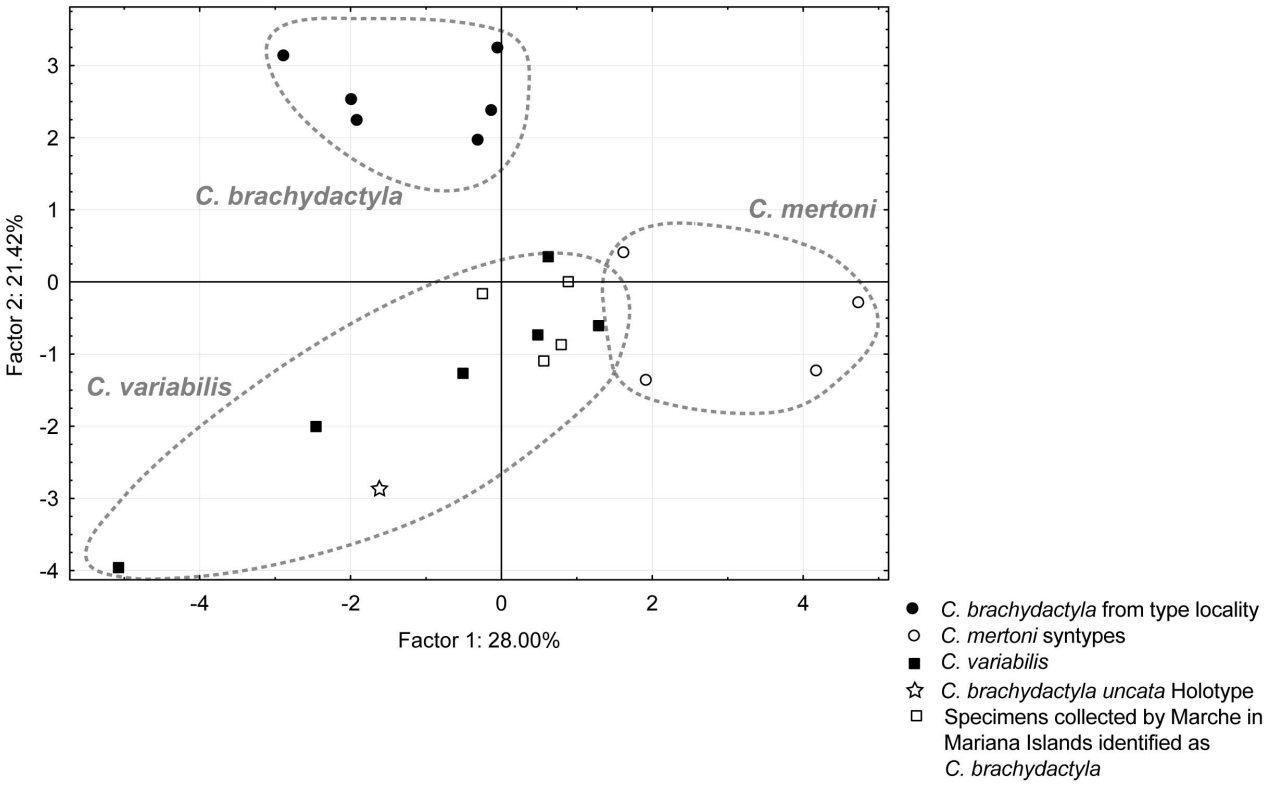

FIGURE 5. Scatterplot of the specimens projected on a plane defined by the first two factors of the CPA.

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.

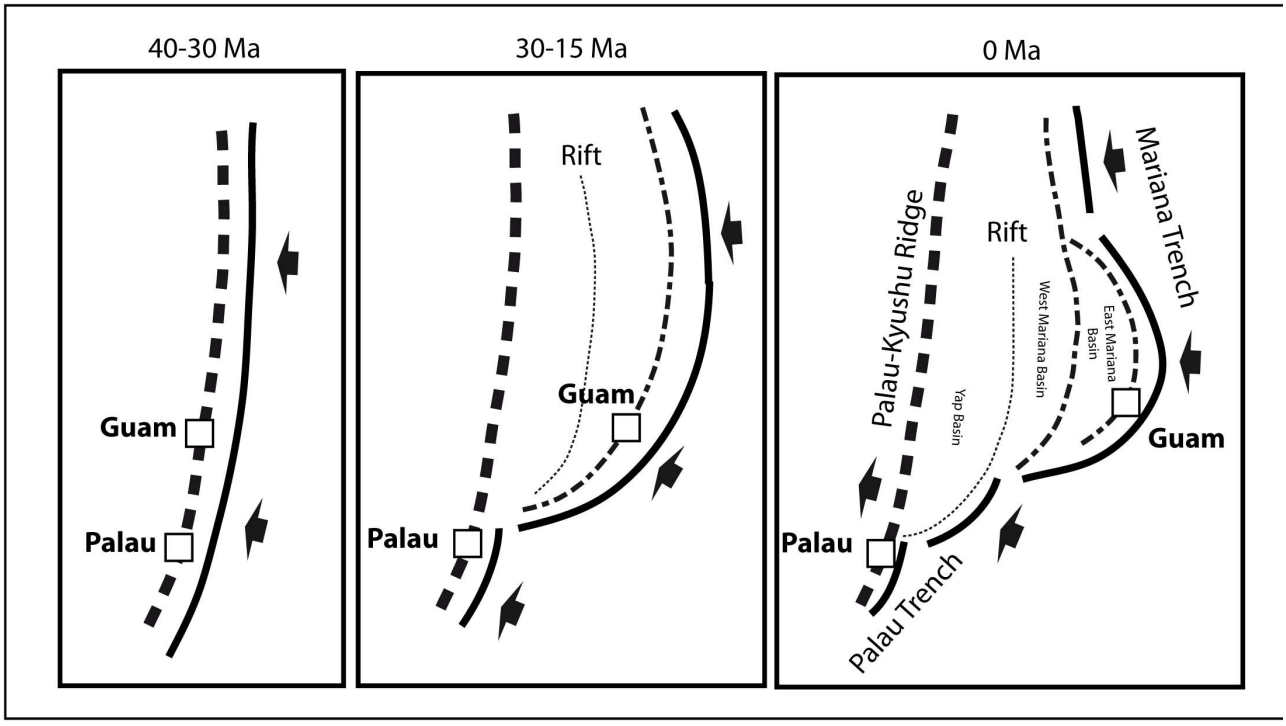

FIGURE 6. Schematic representation of the geological history of Micronesia (redrawn from Kobayashi *et al.*, 2004).

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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