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Caridina malanda, a new species of freshwater shrimp (Crustacea: Decapoda: Atyidae) from the Wet Tropics World Heritage area, north–eastern Queensland, Australia

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Abstract

Integrated molecular and morphological studies of newly collected and curated specimens of the genus *Caridina* from the Atherton Tablelands, Wet Tropics World Heritage Area in north–eastern Queensland, Australia indicated the presence of an undescribed species belonging to the *Caridina zebra* Short 1993 complex. This species is somewhat intermediate, although distinct on the basis of molecular data and morphology, from two known sympatric species, *Caridina zebra* and *C. confusa* Choy & Marshall 1997, and an allopatric species, *C. spinula* Choy & Marshall 1997, from the Cape York Peninsula, about 500 km north. It is described here as a new species, *C. malanda* **sp. nov.**, and compared with similar congeners. A key for the identification of the species, as well as notes on its distribution, ecology, and conservation, are provided.

Keywords: Atyidae, *Caridina zebra* complex, Atherton Tablelands

Introduction

Currently, three species of the *Caridina zebra* complex (Crustacea: Decapoda: Atyidae) are known from Australia, namely *C. zebra* Short 1993, *C. confusa* Choy & Marshall 1997, and *C. spinula* Choy & Marshall 1997. *C. spinula* is known only from the Kulla (McIlwraith Range) National Park in the Cape York Peninsula, northern Queensland, while *C. confusa* and *C. zebra* are known only from sites about 500 km south, on the Atherton Tablelands in the Wet Tropics World Heritage Area (Fig. 1). A recent survey of *Caridina* from anthropogenically disturbed streams in the Atherton Tablelands in north–eastern Queensland suggested the presence of another species that had some substantial morphological, genetic, and ecological differences from all species described to date. This same undescribed taxon was recently included in a comprehensive molecular phylogenetic study (de Mazancourt *et al.* 2019) as *C.* sp. Malanda. They found that it was a phylogenetically distinct taxon, and formed a strong clade with *C. zebra*, with *C. confusa* as a sister taxon to them both, also in a strong clade. This new species co-exists with *C. confusa* and, to a lesser extent, with *C. zebra* in some places, although the habitats in which they occur are quite different.

Material and methods

The materials examined were collected in 2016 using a scoop net and preserved in absolute alcohol before being transferred to 70–80% ethanol for long term storage. The collection locality details for the specimens are given below. Morphological analyses and measurements were carried out separately on all specimens from several localities and followed Choy & Marshall (1997). Measurements were taken using an Olympus SZX7 dissecting microscope

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with Olympus DP26 digital camera, and Olympus CellSens Entry v1.18 software calibrated using an ISSCO OPtek 10 mm stage micrometer. Measurements: CL, postorbital carapace length, measured from the postorbital margin to the posterior median margin of the carapace; RL, rostrum length, measured from the postorbital margin to the tip of the rostrum; CD, maximum depth of the carapace; SL, total length of the animal taken from the tip of the rostrum to the tip of the telson; TL, total length of the animal, taken from the tip of the rostrum to the tip of the uropod; D, depth; L, length.

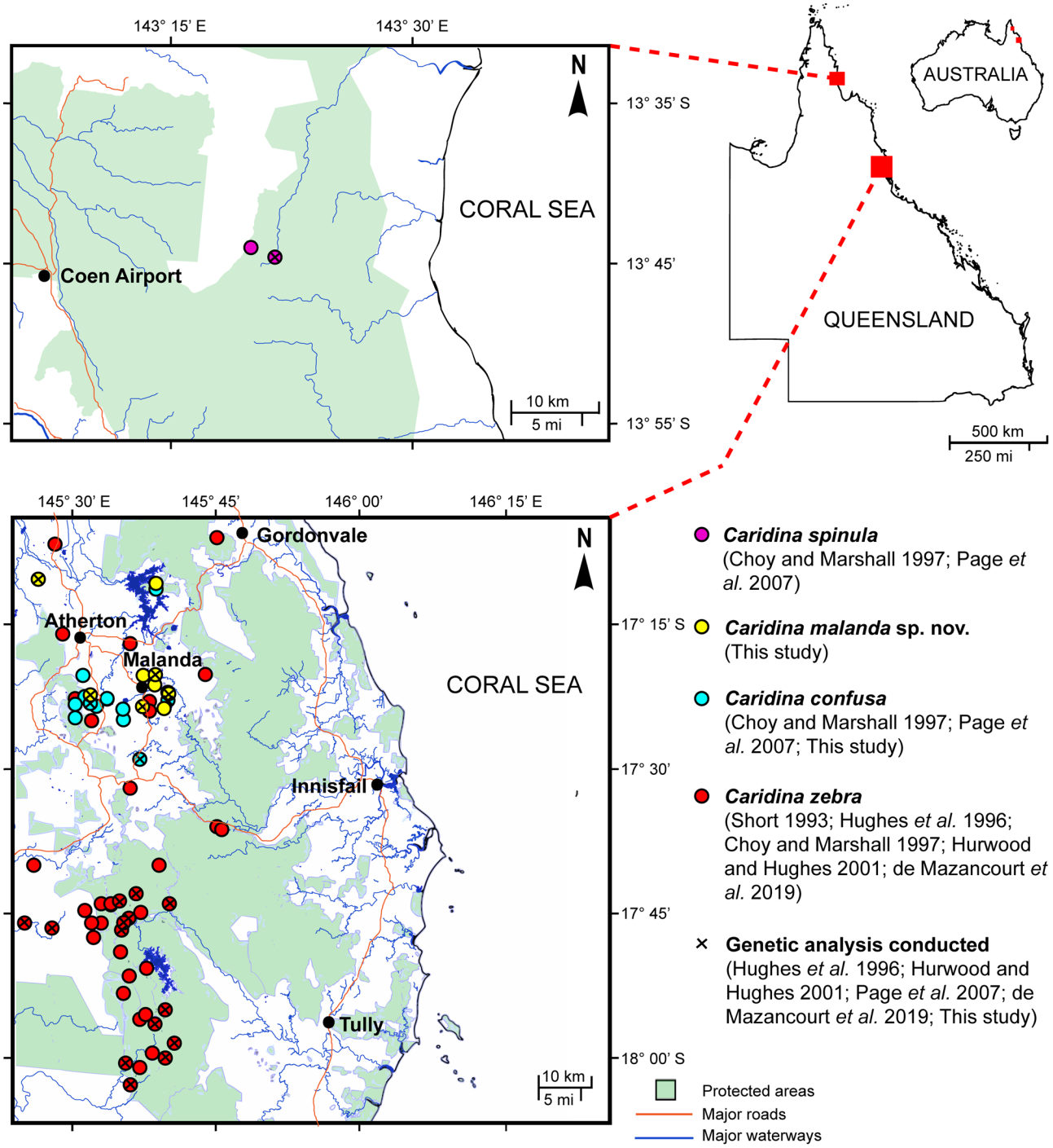

FIGURE 1. Literature records of *Caridina* from the *C. zebra* complex from the Wet Tropics of north–eastern Queensland, Australia. Light green shading indicates national parks, forest reserves, and protected areas (Collaborative Australian Protected Areas database [CAPAD], 2010, https://data.gov.au/data/dataset/1f420717-e600-4ff0-8c04-90122bfa04a7, accessed 2019, May 7).

The holotype and some paratypes were deposited in the Queensland Museum, Brisbane, Australia. Additional paratypes were deposited in the Australian Museum, Sydney, Australia. Some of the molecular sequenced paratypes have been deposited in the Muséum national d'Histoire naturelle, Paris, France. All registration numbers are provided below. For the molecular analyses, the current study generated new molecular data from eight specimens of *C. malanda* **sp. nov.** from five sites, and included published data (de Mazancourt *et al*. 2019) from a further two specimens (CA1736, CA1737) of the same taxon from two sites (Table 1).

TABLE 1. Specimen information of *Caridina* included in molecular analyses, with relevant GenBank accession numbers. All sequences are new to this paper unless otherwise specified; * = de Mazancourt *et al.* (2019), # = Page *et al.* (2007). Reg. No. = registration number for specimen deposited with the Muséum national d'Histoire naturelle, Paris, France.

Species	Site (Catchment)	Reg. No.	Spec. No.	COI	16S
C. malanda	Site 30, Ithaca River (Johnstone)	MNHN-IU-2014-20766	CA1735	MK883793	MK880178
C. malanda	Site 30, Ithaca River (Johnstone)	MNHN-IU-2014-20767	CA1736	MK190071*	MK189911*
C. malanda	Site 23, Short Creek (Johnstone)	MNHN-IU-2014-20768	CA1737	MK190072*	MK189912*
C. malanda	Site 23, Short Creek (Johnstone)	MNHN-IU-2014-20769	CA1738	MK883796	MK880181
C. malanda	Site 22, Brodie Creek (Johnstone)	MNHN-IU-2014-20770	CA1739	MK883797	MK880182
C. malanda	Site 22, Brodie Creek (Johnstone)	MNHN-IU-2014-20771	CA1740	MK883798	MK880183
C. malanda	Site 24, Malanda Creek (Johnstone)	MNHN-IU-2014-20772	CA1741	MK883799	MK880184
C. malanda	Site 24, Malanda Creek (Johnstone)	MNHN-IU-2014-20773	CA1742	MK883800	MK880185
C. malanda	Site 20, Barney Springs (Barron)	MNHN-IU-2014-20774	CA1749	MK883801	MK880186
C. malanda	Site 20, Barney Springs (Barron)	MNHN-IU-2014-20775	CA1750	Mk883802	MK880187
C. zebra	Tributary of Tully River (Tully)	MNHN-IU-2014-20776	CA1731	MK190069*	MK189909*
C. confusa	Thiara Creek (Johnstone)	MNHN-IU-2014-20777	CA1727	MK190067*	MK189907*
C. spinula	McIlwraith Range (Lockhart)	-	GUCCI1	-	DQ478527#
C. typus	New Caledonia	MNHN-IU-2014-20778	CA1568	MK190052*	MK189893*

Molecular Methods. The current study added molecular data from eight specimens of *C. malanda* **sp. nov.** from five sites for two mitochondrial fragments (16S, COI) (see Table 1) for new mitochondrial analyses. Fragments were sequenced as per de Mazancourt *et al*. (2019). GenBank sequences of *C. zebra*, *C. confusa*, *C. typus* (16S, COI; de Mazancourt *et al*. 2019), and *C. spinula* (16S; Page *et al*. 2007) were downloaded as outgroups to add to the new analyses (Table 1).

Sequences of each gene fragment (16S, COI) were aligned and analysed in MEGA version 6 (Tamura *et al*. 2013) using the general methods of DNA barcoding (Costa *et al*. 2007). The levels of genetic divergence for 16S and COI among and within the taxa were calculated using a Kimura 2-parameter distance model. The two fragments were then combined for a Bayesian phylogenetic analysis with MrBayes version 3.2 (Huelsenbeck & Ronquist 2001; parameters: 3 million generations, trees sampled every 1000 cycles, 25% burn in, two runs of four chains

heated to 0.2) using the most appropriate substitution model (lowest Bayesian Information Criterion score) as chosen with Mega for each fragment.

Taxonomy

Caridina malanda sp. nov.

(Figs. 1, 2, 3, 4, 5; Tables 1, 2)

Caridina sp. Malanda (de Mazancourt *et al*. 2019)

Holotype. Adult female, Site 24, Malanda Creek, Johnstone River catchment (17°20'13"S, 145°38'37"E), 10 June 2016, deposited QM (Queensland Museum), registration number W29455.

Paratypes. Adult male, same data as holotype, deposited AM (Australian Museum), registration number P.103594. 2 specimens, same data as holotype, deposited Muséum national d'Histoire naturelle, Paris, France, registration numbers MNHN-IU-2014-20772 and MNHN-IU-2014-20773. Other material: 1 female, same data as holotype, deposited AM, P.103594; Site 21, unknown creek, Malanda (17°23'12"S, 145°31'52"E), 2 specimens deposited QM W29460; Site 20, Barney Springs, Rocky Creek, Barron River catchment (17°10'41"S, 145°27'22"E), 10 June 2016, 5 specimens, and 2 specimens deposited MNHN-IU-2014-20774 and MNHN-IU-2014-20775. No specimens have been found downstream of this location (i.e. in Rocky Creek) despite multiple attempts over several years; Site 22, Brodie Creek, Glen Alynn, Johnstone River catchment (17°22'12"S, 145°40'00"E), 10 June 2016, 12 specimens deposited AM, P.103600; Site 23, Short Creek, Johnstone River catchment (17°22'53"S, 145°39'55"E), 10 June 2016, 1 male deposited QM W29456, 1 female deposited QM W29457, 4 specimens deposited AM P.103595, 2 specimens deposited MNHN-IU-2014-20768 and MNHN-IU-2014-20769, also caught *C. confusa* here, 28 specimens of which were deposited AM P.103601; Site 30, Ithaca River, Clarks Track, Johnstone River catchment, (17°23'32"S, 145°37'17"E), 12 June 2016, 6 small specimens deposited AM P.103599, 2 specimens deposited MNHN-IU-2014-20766 and MNHN-IU-2014-20767; Site 32, Johnstone River at Glen Alynn Road, Johnstone River catchment, (17°21'17"S, 145°38'33"E), 12 June 2016, 5 specimens deposited AM P.103596, and 1 ovigerous female deposited QM W29458; Site 33, Wallace Crossing, Johnstone River catchment, (17°23'44"S, 145°39'31"E), 12 June 2016, 5 specimens deposited AM P.103597; Site 34, Johnstone River off Barrine Lake Road, Johnstone River catchment, (17°20'18"S, 145°37'21"E), 12 June 2016, 5 specimens deposited AM P.103598; Site 48, unnamed creek near Boar Pocket and Tinaroo, Barron River catchment (17°10'15"S, 145°38'14"E), 20 June 2016, 2 specimens deposited QM W29459, also caught *C. confusa* here. All collections were made by B. Mos with Bob Kroll or Mykala P. Mos under Queensland Fisheries Collection Permit No. 184726. Video of collection locales by B. Mos: Site 24, Malanda Creek, for holotype: https://youtu.be/ue_HlLQCqcY; Site 20, Barney Springs, for paratypes: https://youtu.be/TJ7XT6RGGBE.

Description. Small, delicate animals; ovigerous female size from Johnson River at Glen Alynn Road (QM W29458): 3.42 mm CL, 1.70 mm RL, 17.8 mm SL, 19.8 mm TL. Adult male size from Malanda Creek (AM P.103594): 3.94 mm CL, 1.08 mm RL, 15.2 mm SL, 16.0 mm TL.

Carapace sub cylindrical, glaborous, 0.7–0.8 times deep as long (post orbital carapace length, CL), generally deeper in mature females; rostrum 0.3–0.4 CL, straight or curving downwards, slightly dorsoventrally flattened, reaching near to end of basal segment, or to just beyond it, lateral carina may be prominent, no tooth dorsally and ventrally; antennal spine fused, inferior orbital angle; pterygostomian margin obtuse, subrectangular. Eyes well developed, anterior end reaching just beyond half to tip of the basal segment of antennular peduncle and well before the tip of stylocerite. Eye ball diameter about 0.3–0.5 RL and 0.11–0.15 CL. Antennular peduncle slender, 0.5–0.7 CL; basal segment half length of the antennular peduncle, second segment 1.2 times as long as third one; stylocerite reaching 0.7 times length of basal segment. Scaphocerite 0.5–0.6 CL, extending beyond first antennular peduncle, 2.1–2.5 times as long as wide, antennal spine at about 0.8 times length of scaphocerite, reaching tip of antennular peduncle; antennal peduncle reaching about half of second segment of antennular peduncle and about one third to tip of scaphocerite.

Mouthparts similar to *C. zebra* and *C. confusa*. Third maxilliped reaching to just beyond tip of antennular peduncle, with exopod, reaching to before middle of penultimate endopod segment, ultimate endopod segment length

about equal to penultimate segment length, ending in a prominent claw and some posterior claw-like spines, behind which are tufts of setae. Epipodites on first four pereiopods.

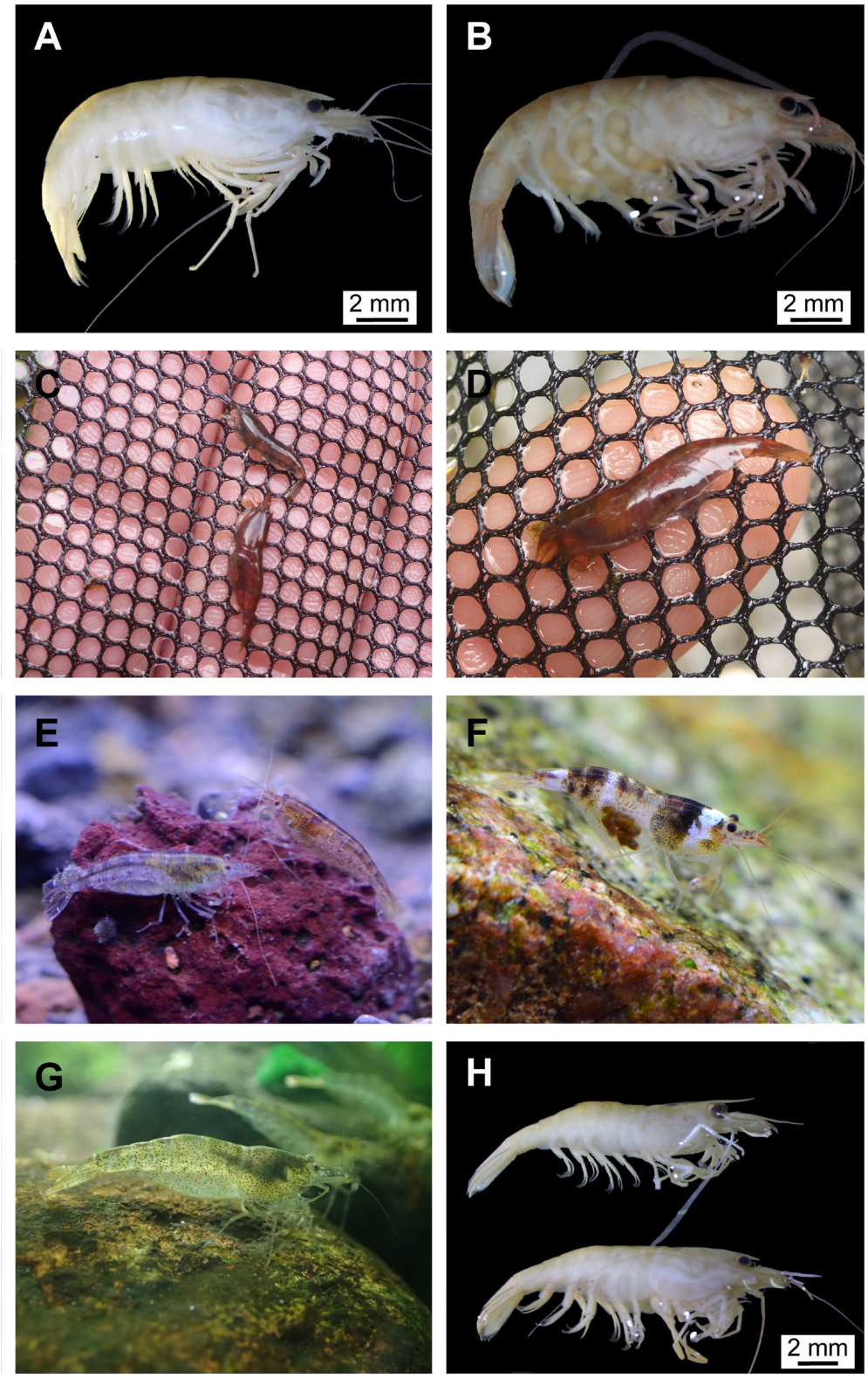

FIGURE 2. *Caridina malanda* **sp. nov.** *(A)* Paratype, preserved in ethanol, Site 23, Short Creek, deposited in QM (Queensland Museum, Australia), registration number W29457; *(B)* Ovigerous female, preserved in ethanol, Site 32, Johnstone River, deposited QM W29458; *(C, D)* Colouration on capture, Site 24, Malanda Creek; *(E)* Live colouration in aquaria, Site 20, Barney Springs; *(F) Caridina zebra* Short 1993; *(G) Caridina confusa* Choy & Marshall 1997; *(H)* Top. *C. confusa*, preserved in ethanol. Bottom. *C. malanda* **sp. nov.** preserved in ethanol; both specimens collected at Site 23, Short Creek. Photos *A*, *B*, *H* by S. Choy; *C*–*G* by B. Mos.

TABLE 2. Morphometric ratios (range) of pereiopods of *Caridina malanda* **sp. nov.** Abbreviations: CL = postorbital carapace length, RL = rostrum length, AtL = antennular peduncle length, 2AbD = 2nd abdominal segment depth, 6AbL = 6th abdominal segment length, TL = telson length, Dt = dactylus, P = propodus, Cp = carpus, M = merus, D = depth, L = length, W = width. 1, 2, 3, and 5 refer to the corresponding pereiopods.

Attribute	Value
CD/CL	0.65–0.85
RL/CL	0.27–0.42
AtL/CL	0.48–0.68
2AbD/CL	0.72–1.13
6AbL/CL	0.41–0.53
6AbL/D	1.36–1.65
TL/CL	0.55–0.70
Dt1L/Dt1W	3.0–4.0
P1L/P1W	2.0–2.5
Cp1L/Cp1W	1.9–2.5
M1L/M1W	1.7–2.7
Dt1L/P1L	0.5–0.6
Cp1L/P1L	0.6–0.9
M1L/P1L	0.8–1.1
Dt2L/Dt2W	4.5–5.1
P2L/P2W	2.6–3.2
Cp2L/Cp2W	5.0–6.0
M2L/M2W	4.7–5.7
Dt2L/P2L	0.6–0.7
Cp2L/P2L	1.1–1.5
M2L/P2L	0.7–1.4
P3L/P3/W	8.5–9.1
Cp3L/Cp3W	3.8–4.2
M3L/M3W	4.7–6.9
Cp3L/P3L	0.5–0.7
M3L/P3L	0.9–1.2
P5L/P5W	9.5–13.2
Cp5L/Cp5W	4.7–5.6
M5L/M5W	6.3–6.6
Cp5L/P5L	0.5–0.7
M5L/P5L	0.7–1.0

First pereiopod short, reaching tip of basal segment of antennular peduncle; chela 2.0–2.5 times as long as wide, ending in a tuft of long setae; movable finger (dactylus) slightly longer than palm of chela and about 0.55–0.60 times propodus length and 3–4 times as long as wide; carpus excavated strongly anteriorly, shorter (0.65–0.94 times) than chela, 1.9–2.5 times as long as wide; merus 1.7–2.7 times as long as wide, slightly longer than carpus; ischium length about 0.6 times merus length, about 2.1 times as long as wide.

Second pereiopod reaching beyond antennular peduncle; chela 2.6–3.2 times as long as wide, ending in a tuft of long setae; movable finger (dactylus) 1.3–1.8 times as long as palm, about 0.65 times propodus length and 4.5–5.1 times as long as wide; carpus 1.1–1.5 times longer than chela, 5–6 times as long as wide, broader anteriorly; merus just shorter than carpus, 4.7–5.7 times as long as wide; ischium length about 0.6 times merus length, 2.8–3.5 times as long as wide.

Third pereiopod long, robust, reaching tip of antennular peduncle; dactylus ending in a strong claw with some

setate behind which are 4–6 spines strongly curved inwards and decreasing in size posteriorly, no sexual dimorphism, 0.10–0.25 times as long as propodus, 2.0–3.3 times as long as wide (terminal claw excluded); propodus 1.5–2.1 times as long as carpus, about 8.5–9.1 times as long as broad, inner anterior with a large apical spine and 11 smaller spines along the posterior margin; carpus 0.49–0.68 as long as propodus, 3.8–4.2 times as long as wide, a few strong spines around anterior apex and about 5 smaller ones along the front outer margin; merus 0.95–1.21 times as long as propodus, 4.7–6.9 times as long as wide; ischium 0.25–0.35 times as long as propodus and 1.5–2.1 times as long as wide.

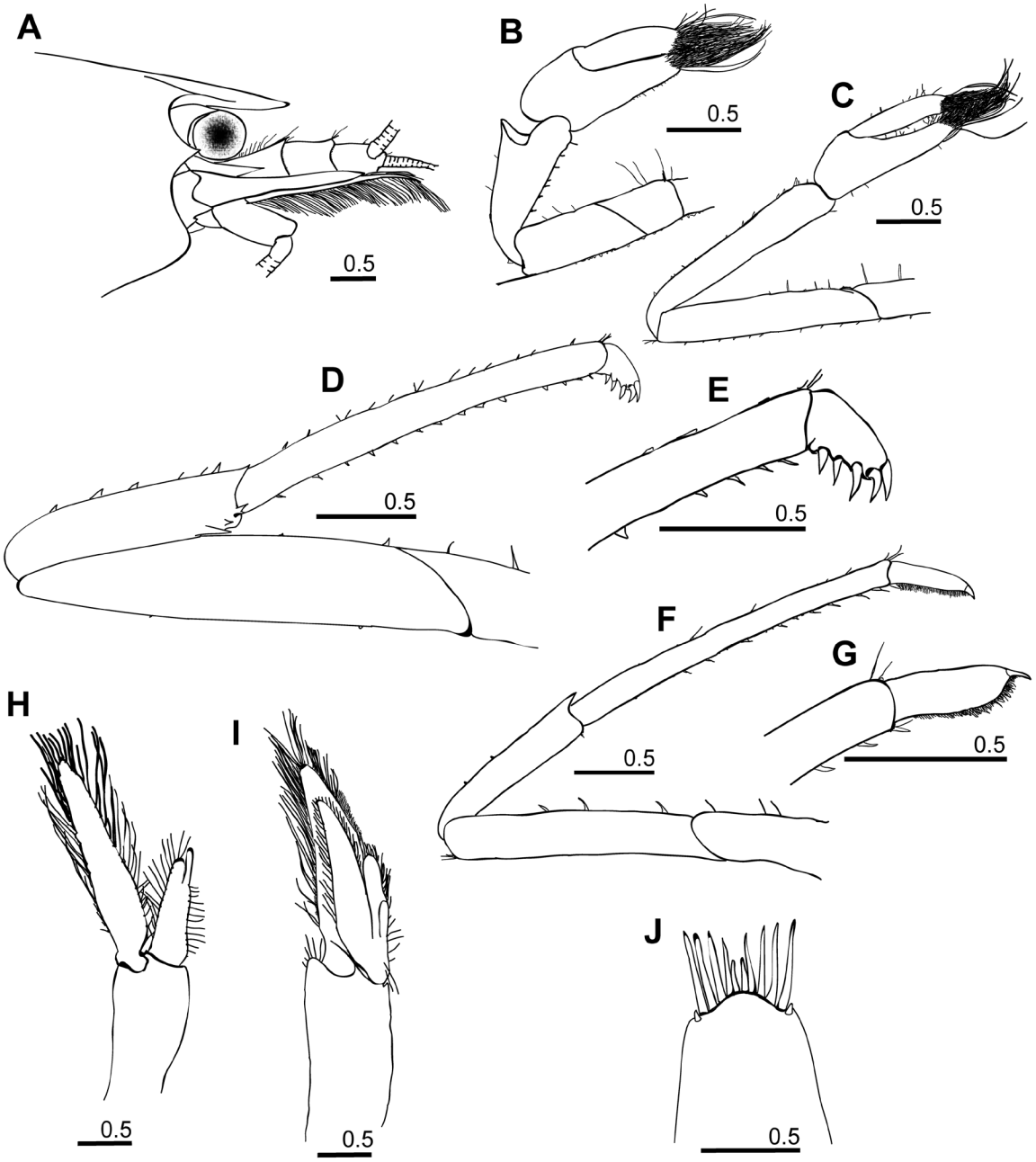

FIGURE 3. *Caridina malanda* **sp. nov.** *(A)* anterior region of cephalothorax; *(B)* first pereiopod; *(C)* second pereiopod; *(D)* third pereiopod; *(E)* dactylus of third pereiopod; *(F)* fifth pereiopod; *(G)* dactylus of fifth pereiopod; *(H)* first male pleopod; *(I)* second male pleopod; *(J)* posterior margin of telson. Scale bars: in mm. Drawings based on *A*: holotype, female, site 24, Malanda Creek, deposited QM (Queensland Museum), registration number W29455. *B* and *C*: female, site 23, Short Creek, deposited QM W29457. *D* and *E*: large male, site 30, Ithaca River, deposited AM (Australian Museum), registration number P.103599. Note: the dactylus of the third pereiopod is not sexually dimorphic. *F*: female, site 24, Malanda Creek, deposited AM P.103594. *G*: male, site 23, Short Creek, QM W29456. *H* and *I*: male, site 22, Brodie Creek, deposited AM P.103600. *J*: female specimen from site 32, Johnstone River, deposited AM P.103596.

Fifth pereiopod relatively long and slender, reaching to tip of antennular peduncle; dactylus curved inner medially, ending in a strong claw with about 50 fine setae posterior to that along the posterior margin decreasing in size posteriorly; 0.22–0.27 times as long as propodus, 2.8–3.9 times as long as wide (terminal claw excluded); propodus 1.5–2.1 times as long as carpus, about 9.5–13.2 times as long as broad, about 12 spines along the posterior margin; carpus 4.7–5.6 times as long as wide, a few large spines around the anterior apex and a smaller one about midway on the posterior margin; merus 0.76–0.95 times as long as propodus, 6.3–6.6 times as long as wide; posterior margin with three large spines placed equidistantly; ischium 0.25–0.40 times as long as propodus and 2.3–2.8 times as long as wide.

Second abdominal segment (pleomere) 0.7–0.9 L/CL, deeper in mature females than males and 1.0–1.2 CL in ovigerous females; sixth pleomere 0.40–0.50 L/CL and 1.3–1.7 L/D, shorter than telson. Telson 0.55–0.70 CL, 2.4 times as long as anterior width, 4.8 times as long as posterior width; not terminating in posteromedian projection or spine; 4–6 pairs of dorsal spinules, situated on distal two-third of telson length, 1 pair of dorsolateral spines near distal end; 4–5 pairs of spines on distal margin, lateral pair longer than subequal to intermedian pairs, innermost pair short and thin; preanal carina lacking spine. Length of uropodial endopodite 0.70–0.75 CL, length of uropodial exopodite 0.75–0.85 CL, uropodite length to diaeresis tip 0.58–0.76 CL, uropodal diaeresis with 17–21 spinules.

Endopod of male first pleopod extending to half length of exopod, elongate, subrectangular, 2.0–2.5 times as long as broad, with a prominent appendix interna near distal end of endopod. Appendix masculina of male second pleopod slender, reaching to 0.5 to 0.7 the length of endopod, inner and distal surface densely lined with long spines; appendix interna at basal 0.3 of appendix masculina, extending to distal 0.3 of appendix masculina.

Egg size, embryo without eyes: 0.6–0.74 mm wide, 0.8–1.1 mm long. Number of eggs carried per female: 50–55.

Colour pattern in life. Live colouration is typically translucent to solid black, brown, red, or dark blue, with black spots (Fig. 1). *C. malanda* **sp. nov.** from Site 20 (Barney Springs) are typically translucent to solid red or blue, but are occasionally translucent brown. Colouration is temporally variable, with stressed individuals becoming pale. In some locations and at some times, colour can be associated with sex (e.g. blue for females, red for males at Barney Springs) or size (e.g. small individuals < 15 mm TL are red, large individuals > 15 mm TL are blue or black at Site 24 (Malanda Creek). *C. malanda* **sp. nov.** does not display a striped pattern as commonly seen for *C. zebra* (Fig. 2), although this does not differentiate the species as *C. zebra* may not display stripes either. Video of live specimens from the *C. zebra* complex: *C. malanda* **sp. nov.**: https://youtu.be/WWmBvhem_uY; *C. zebra*: https://youtu.be/uyufUARxbcw; *C. confusa*: https://youtu.be/PwOefPJr9lU.

Molecular results. Specimens of *C. malanda* **sp. nov.** formed a strong clade in the Bayesian phylogenetic analysis (Bayesian posterior probability 1.00). This clade contained two strongly supported (0.97, 1.00) intraspecific clades that were delineated by the two different catchments (Fig. 4). The sequences from the four sites from the Johnstone River catchment (Brodie Creek, Ithaca River, Malanda Creek, Short Creek) were largely indistinguishable from each other, but were distinct from the sequences from the site in the Barron River catchment (Barney Springs), with a COI genetic distance of 1.73% (0.24% 16S). Within the *C. zebra* complex, the genetic distances among different species ranged between 13.29–17.79% for COI (no *C. spinula*), and 4.36–8.49% for 16S.

The best-fit models of molecular evolution for the molecular datasets were Hasegawa-Kishino-Yano with a gamma shape parameter for the 16S dataset, and Tamura 3-parameter with a gamma shape parameter and an estimated fraction of invariant sites and an estimated fraction of invariant sites for the 3' COI dataset. While the topology of the Bayesian analysis (Bayesian arithmetric mean = –3055.48) clearly supports *C. malanda* **sp. nov.** as a distinct taxon, it is unclear which species may be its sister, as all of the members of the *C. zebra* complex form a polytomy here (Fig. 4).

Etymology. The specific name is derived from the type locality, Malanda Creek, which reportedly is an Australian Aboriginal name of unknown language and dialect, with a possible connotation "little stream with big stones" (Queensland Government 2019). It is used as a noun in apposition.

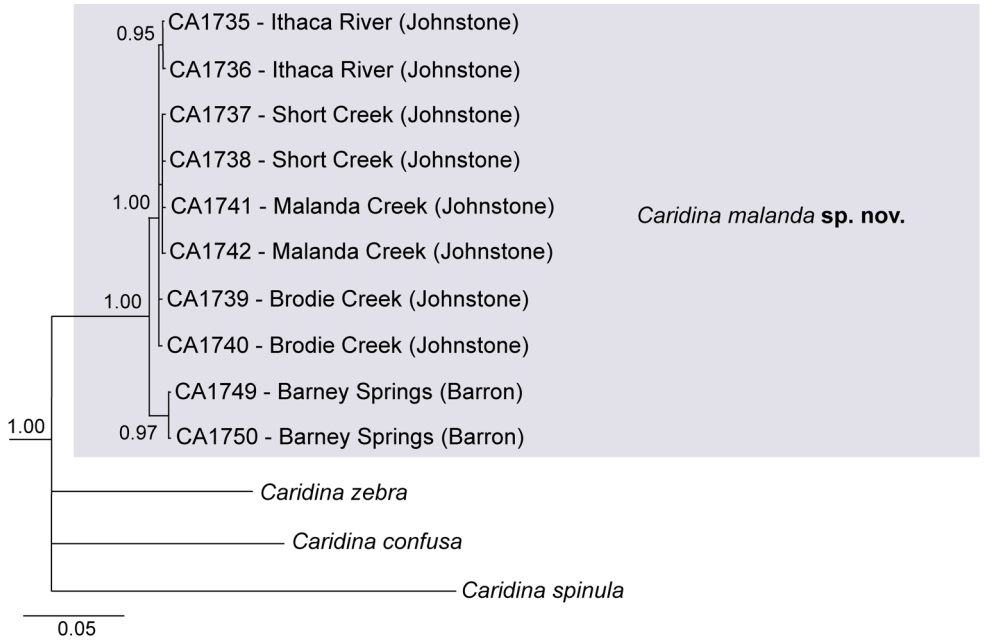

FIGURE 4. Bayesian majority rule consensus topology of *Caridina malanda* **sp. nov.** and species from the *Caridina zebra* complex using combined 16S rDNA and COI dataset with Bayesian posterior probability values. Nodes with probability values < 0.50 have been collapsed. Specimen information of *Caridina* included in molecular analyses, with relevant GenBank accession numbers and collection locations, are listed in Table 1.

Key. The four species from the ***Caridina zebra*** **complex** can be identified using the following key:

1. Straight to sigmoid shaped rostrum; rostrum long, extending beyond tip of second segment of antennular peduncle, >0.4 times post orbital carapace length (CL); stylocerite long, >0.4 times CL; scaphocerite long, >0.9 times CL; sixth abdominal segment long, >0.5 times CL . . . . . *C. confusa*
- Straight to downward curved rostrum; rostrum short, not reaching tip of second segment of antennular peduncle, <0.4 times CL; stylocerite short, <0.4 times CL; scaphocerite long, <0.9 times CL; sixth abdominal segment short, <0.5 times 2
2. Rostrum short, not extending beyond tip of first segment of antennular peduncle; eggs large (>0.8 mm wide and >1.3 mm long) and few (<25) . . . . . *C. spinula*
- Rostrum relatively long, extending beyond tip of first segment of antennular peduncle; eggs relatively small (<0.8 mm wide and <1.3mm long) and numerous (>25) 3
3. Stylocerite short, never to tip of first segment of antennular peduncle, <0.28 CL; sixth abdominal segment elongate, L/W >1.3; telson long >0.55 CL; posterior telsonic margin never with a median spine . . . . . *C. malanda* **sp. nov.**
- Stylocerite long, to tip of first segment of antennular peduncle, >0.28 CL; sixth abdominal segment short, L/W <1.3; telson short, <0.55 CL, posterior telsonic margin may have a median spine . . . . . *C. zebra*

It is emphasised that individual morphometric and meristic characters can be highly variable, especially for samples collected from a wide geographic area or from streams with different environmental conditions, and so a combination of characters and information should be used to confirm the identity of specimens or species. Keys are only meant to be the first step and other characters should be ascertained. Where possible, molecular data should also be used to verify an identification made using morphological characteristics, as ontogeny and environmental factors often lead to morphological plasticity (de Mazancourt *et al*. 2017, 2018; Yasser *et al*. 2018; Purushothaman *et al*. 2019).

Remarks. *C. malanda* **sp. nov.** has a relatively short rostrum and so it is somewhat similar to *C. zebra*, a bit longer than in *C. spinula***,** and much shorter than in *C. confusa.* The stylocerite, the length of the first antennular peducle, the scaphocerite, and the sixth abdominal somite are all generally shorter than in *C. spinula* and *C. confusa* but slightly longer than in *C. zebra*. The telson in *C. malanda* **sp. nov.** is longer than in *C. zebra* and *C. spinula* but shorter than in *C. confusa*. Some of the ratios of appendage segments are also different (Table 2; Choy & Marshall 1997: Tables 1, 2). So while *C. malanda* **sp. nov.** looks superficially like *C. zebra* and *C. spinula*, it is geographically separated from *C. spinula* (which occurs in a small area in the eastern Cape York region, almost 500 km to the north–west), and is found in a different habitat to *C. zebra*, and has different live colouration (Fig. 2), a shorter

antennular peduncle, a shorter stylocerite, and a shorter sixth abdominal segment than *C. zebra*. It also does not have a median spine on the telsonic margin. While *C. malanda* **sp. nov.** co-exists with *C. confusa*, the latter is a much more slender animal with relatively long and pronounced rostrum (Fig. 2H), and their behaviour is distinctive. In locations where they co-occur, *C. confusa* is found on the substrate, often in the open, whereas *C. malanda* **sp. nov.** is only found hidden among riparian or aquatic vegetation. Overall, while the morphological differences among the four species within the *Caridina zebra* complex are relatively subtle, the molecular data of de Mazancourt *et al*. (2019) and the current study clearly show significant differences between *C. malanda* **sp. nov.** and the other *Caridina* species, highlighting the interactive relationship between morphology and molecules (Page *et al.* 2005).

Phylogenetic relationships. The intraspecific divergence between specimens of *C. malanda* from the Johnstone and Barron Catchments (~2% at COI) probably reflects the isolation enforced by catchment boundaries, and is in line with many similar phylogeographic breaks within Australian freshwater species (Page & Hughes 2014). At a higher taxonomic level, the molecular divergence between *C. malanda* **sp. nov.** and other members of the *C. zebra* complex (~15% COI) probably reflects a species-level divergence. While certainly not a definite test of species status, these data imply that the four taxa of the *C. zebra* complex are probably distinct species given their COI distances are within the average ranges between decapod species within a genus (17.16%, Costa *et al.* 2007; 15.49%, Matzen da Silva *et al.* 2011). This was confirmed in findings of de Mazancourt *et al.* (2019), which used a much larger dataset (seven mitochondrial and two nuclear genes) and also recovered *C. malanda, C. zebra,* and *C. confusa* as clearly distinct taxa.

At higher phylogenetic levels, the analyses in this study were undecided on the sister taxon of *C. malanda* **sp. nov.**. However, de Mazancourt *et al.* (2019) recovered *C. zebra* as the sister taxon of *C. malanda* **sp. nov.** with *C. confusa* sister to them both with strong support. The place of *C. spinula* is unclear as it was not included in their analyses, but it was recovered as sister to a clade of *C. zebra* and *C. confusa* in Page *et al.* (2007). This was achieved with only one gene (16S), and so is a preliminary, if potentially accurate, finding.

Choy & Marshall (1997) included *C. confusa*, *C. spinula*, and *C. zebra* as part of the *C. typus* H. Milne Edwards 1837 species-group, characterised by a short, dorsally unarmed rostrum. However, this study, Page *et al.* (2007), and de Mazancourt *et al.* (2019) indicate that these species do not form a natural clade with *C. typus*, based on its morphology (e.g. somewhat laterally flattened and ventrally toothed rostrum, angular posterior telsonic margin, and small eggs) and molecular data. Hence, all are now included within the *C. zebra* complex given their close morphological and genetic similarity.

Within the *C. zebra* complex, the types of streams, habitats, and environmental conditions within which each of the species occurs are somewhat uniform and so not too much morphological variation (except for live colour) is discernible within each species. Some of the variations in morphology previously reported for *C. zebra* and *C. confusa* might be due to the presence of *C. malanda* **sp. nov.**, and possibly other cryptic species, in samples where specimens were collected over a large area and lumped together. In other atyids, intraspecific variations have been reported between populations occupying different parts of the river system (e.g. lower, low gradient and upper, high gradient reaches) or between different river, island, or country locations (de Mazancourt *et al*. 2017; 2018; Yassar *et al*. 2019). Given that such variations are known, it is recommended that all collections should strive to get a good size/age and sex series from different localities, with specimens from different locales preserved, labelled, and stored separately, so that appropriate analyses can be performed.

Ecology, distribution, and conservation. All specimens of *C. malanda* **sp. nov.** were caught from riparian marginal vegetation, among tree roots along the bank edge, or among aquatic vegetation and leaf litter; sometimes in spring pools and slow water. They were caught alongside other shrimps (*C. confusa*, *Paratya* sp., and *Macrobrachium* spp.), crayfish (likely *Euastacus* spp.), invasive guppies (*Poecilia* sp.), and gudgeons (*Mogurnda* spp.). Water quality range: water temperature 19.3–21°C, pH 5.88–6.83, salinity 0.01–0.03 ppt, conductivity 27.7–58.1 µS/cm, TDS 16–33. At Barney Springs, the water quality was slightly different: water temperature 24.1 °C, pH 6.72, salinity 0.11 ppt, conductivity 221 µS/cm, TDS 120.

C. malanda **sp. nov.**, *C. confusa*, *C. spinula*, and *C. zebra* are all endemic to north–eastern Queensland (Fig. 1) and have a restricted distribution of less than 500 km^2 each and a total area of about 6000 km^2, and so likely speciated there (Page *et al.* 2007). While *C. spinula* and *C. zebra* generally occur within protected rainforest ecosystems, *C. malanda* **sp. nov.** and *C. confusa* generally occur in streams surrounded by disturbed rainforest and by open grassland used for cattle grazing and other forms of agriculture (Fig. 1). These two species may be adapted to living in these relatively disturbed areas but likely have a much greater risk of extinction due to human activities within

their restricted range (e.g. disturbance to habitats, changes to water flow regimes, pollution, introduced species, etc.). Regular monitoring may be necessary to ensure populations are sustained in the face of further anthropogenic disturbances and climate change. In addition, species from the *C. zebra* complex are collected, reared, and traded in private and commercial aquarium industries, albeit in low numbers. This can have certain advantages in reducing the risk of extinction if populations can be maintained in captivity long term. However, over-collection is a key threat for atyids and the ornamental trade may increase the potential for genetic contamination and translocations, highlighting the need for appropriate management (De Grave *et al*. 2015).

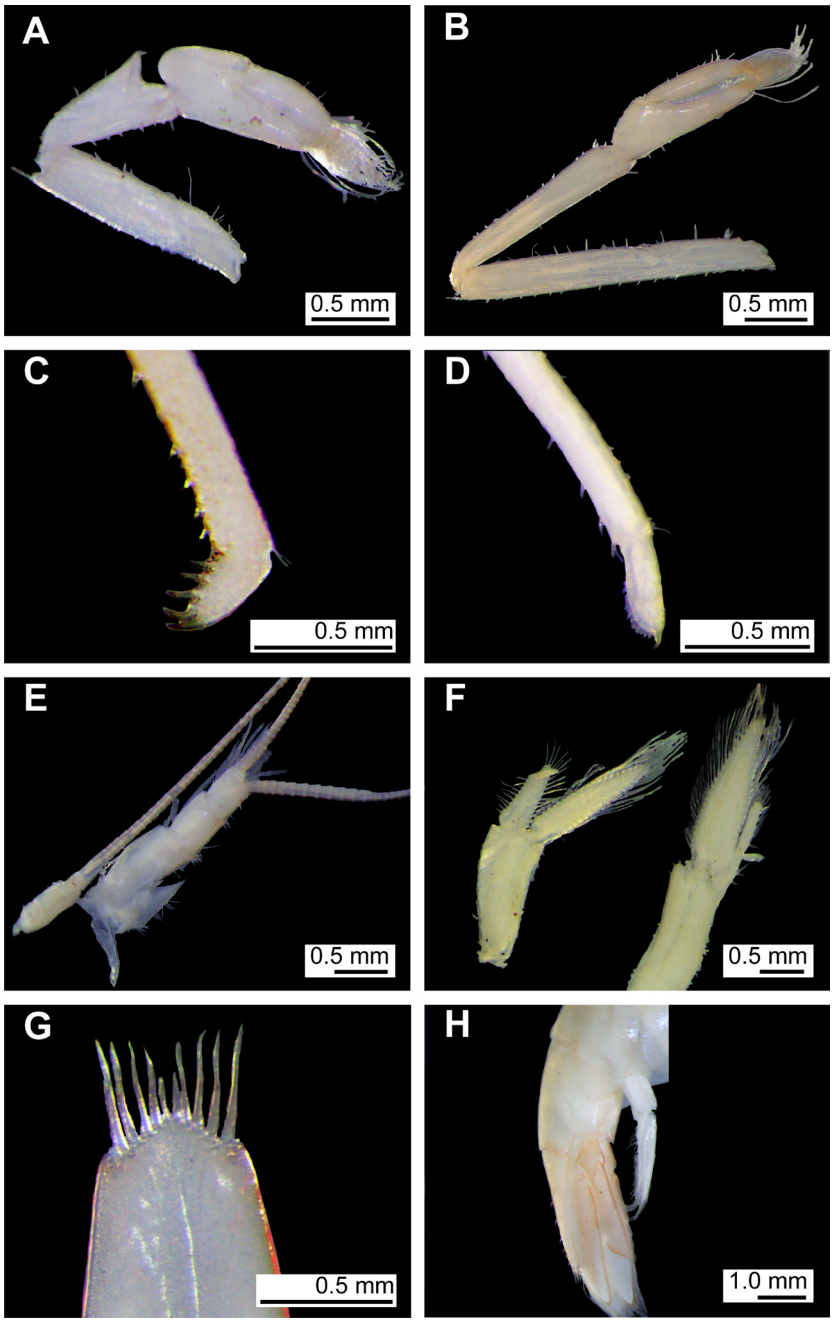

FIGURE. 5. *Caridina malanda* **sp. nov.** *(A)* first pereiopod; *(B)* second pereiopod; *(C)* dactylus of third pereiopod; *(D)* dactylus of fifth pereiopod; *(E)* Left. antennal flagellum. Right. antennule with stylocerite; *(F)* Left. first male pleopod. Right. second male pleopod; *(G)* posterior margin of telson; *(H)* lateral view of posterior of abdomen, showing telson, uropods and fifth pleopod. Photos: *A*, *B*, *E* are female specimen from site 23, Short Creek, deposited QM (Queensland Museum), registration number W29457; *C* is large male specimen from site 30, Ithaca River, deposited AM (Australian Museum), registration number P.103599. *D* is male specimen from site 23, Short Creek, deposited QM W29456. *F* is male specimen from site 22, Brodie Creek, deposited AM P.103600. *G* is female specimen from site 32, Johnstone River, deposited AM P.103596; *H* is holotype, female specimen, site 24, Malanda Creek, deposited QM W29455. Photos by S. Choy and B. Mos.

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Statement of Author Contribution

Concieved the project: SC, BM, VdM. Collected specimens and ecological data: BM. Performed morphological analysis: SC, BM. Performed phylogenetic analysis: TP, VdM. Wrote the manuscript. SC, BM, TP. Edited the manuscript. TP, VdM.

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