



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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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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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 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 were 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.

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 (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 single species (e.g. rostrum shape and indentation or coloration) and so taxonomically non-informative, making it difficult to identify boundaries among species (von 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 (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 Kolombangara 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.

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 pereopod; P2: second pereopod; P3: third pereopod; P5: fifth pereopod; PII: first pleopod.

Molecular analyses

DNA extraction, amplification and sequencing. 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 (CTCCGGTTTGAAGTCAGATCA) (Palumbi, 1996). DNA amplification was performed in 25 µl 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 (MPBiochemicals). 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: [XXXXXXXXXX](#), Table 1). Sequences for the genera *Caridina* and *Paratya* published by Page *et al.*, (T.J. Page, von Rintelen, & Hughes, 2007) were included in our analysis, *Paratya* sequences being used as outgroup.

(Table 1 here)

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, Pfeiffer, & Schwartz, 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 $B \geq 90\%$ and $PP \geq 0.98$.

Morphological comparisons. The rostrum, the general cephalothorax, 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 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 Kolombangara 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 MPTZ-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.

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 4 *Paratya* as outgroup) from Page *et al.* (T.J. 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 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 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 1 here)

Taxonomy

Atyidae De Haan, 1849

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

***Caridina buehleri* Roux, 1934**

(Figure 2)

(Figure 2 here)

171

172 *Caridina buehleri* Roux, 1934: 219, Figs 1–5 [type locality: Bimoun, New Ireland west coast,

173 Papua New Guinea].

174 *Caridina gueryi* Marquet, Keith & Kalfatak, 2009:159–166, Figs 1–3 [type locality: unnamed

175 river (15.29590S; 167.16E), Santo, Vanuatu].

176

177 **Material examined**

178 **Type material:** *Caridina buehleri* Roux, 1934. Holotype NMB 822 Papua New Guinea, New

179 Ireland, Bimoun, one non ovigerous female, CL 7 mm.

180 *Caridina gueryi* Marquet, Keith & Kalfatak, 2009. Vanuatu, Santo island Holotype: MNHN-

181 IU-2015-1768, ovigerous female, CL 6.4 mm, unnamed river, 17°50' 7S, 145° 31W, 22-07-

182 2003, coll. Keith & Marquet; Allotype: MNHN-IU-2015-1769, 16-236, male, TL 14.5 mm,

183 CL 3.2 mm 22-07-2003, coll. Keith & Marquet; Paratypes: MNHN-IU-2015-1770, 12

184 females, MNHN-IU-2015-1771, one ovigerous female TL 23.1 mm, Mantatas river 23-07-

185 2003 coll. Keith Marquet, MNHN-Na 16-239, one ovigerous female TL 18.9 mm Sarataka

186 river, 25-07-2003 coll. Keith & Marquet.

187 **Other material:** *Caridina buehleri* Roux, 1934. ZMB 29002 Indonesia Central Sulawesi,

188 Luwuk Peninsula, freshwater spring in Malontong, West of Ampana, brackish water pool;

189 0°53.125'S 121°31.371'E (field code 70-05); coll. M. Glaubrecht, T. von Rintelen & K.

190 Zitzler, 27-05-2005, det. Klotz/Karge. 1 male (CL 2.9 mm), 1 female (CL 3.9 mm) and 3

191 ovigerous females (CL 4.3–6.4mm). IU-MNHN-2015-17 Solomon Islands, Kolombangara

192 Island, Vagé river; 08°05.112'S 156°59.867'E; coll. P. Keith, C. Lord & G. Marquet, 10-11-

193 2015, det. Marquet : 3 ovigerous females (CL 3.7 mm; 3.2 mm; 3.5 mm). IU-MNHN-2015-18

194 Liva river; 08°03.859'S 157°10.684'E; 11-11-2015, det. Marquet: 3 ovigerous females (CL

195 3.5 mm; 3.8 mm; 4.2 mm). IU-MNHN-2015-19 Jack Harbour river; 08°03.085'S

196 157°10.945'E; 11-11-2015, det. Marquet: 3 ovigerous females (CL 4.4 mm; 3.4 mm; 4.3 mm)

197 and 1 non-ovigerous female (CL 4 mm). IU-MNHN-2015-20 Pipiro river; 07°53.083'S

198 157°08.195'E; 13-11-2015, det. Marquet: 2 ovigerous females (CL 3.8 mm; 4.2 mm). IU-

199 MNHN-2015-21 Vanga I river; 07°55.088'S 156°57.624'E; 18-11-2015, det. Marquet. 2

200 ovigerous females (CL 4.4 mm; 4.6 mm). IU-MNHN-2015-22 Vanga II river; 07°54.825'S

201 156°57.762'E; 18-11-2015, det. Marquet: 4 ovigerous females (CL 4.1 mm; 3.2 mm; 4 mm;

202 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).

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 pereopod 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 pereopod 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 pereopod 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 pereopod 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 (Kolombangara) (Figure 3).

(Figure 3 here)

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 4).

(Figure 4 here)

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.

(Table 2 here)

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 pereopod 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 (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 pereopod 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 pereopod for *C. lobocensis*. The chelae of the first pereopods 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.* (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 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 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, 2008), *C. serrata* (Cai & Ng, 1999); (Klotz & von Rintelen, 2014), and *C. gracilirostris* (Cai & Ng, 2007). 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 pereopod with both fingers showing a prominent claw (Figure 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.

(Figure 5 here)

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 (von 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.

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REFERENCES

- Bouvier, E.-L. (1925) Recherches sur la Morphologie, les variations et la Distribution systématique des Crevettes d'eau douce de la famille des Atyidés. *Encyclopédie*, 4, 1–370.

371 Cai, Y. & Ng, P. K. L. (1999) A revision of the *Caridina serrata* species group, with
 372 descriptions of five new species (Crustacea: Decapoda: Caridea: Atyidae). *Journal of*
 373 *Natural History*, 33, 1603-1638.

374 Cai, Y. & Ng, P. K. L. (2007) A revision of the *Caridina gracilirostris* De Man, 1892, species
 375 group, with descriptions of two new taxa (Decapoda; Caridea; Atyidae). *Journal of*
 376 *Natural History*, 41, 1585-1602.

377 Covich, A. P., Palmer, M. A. & Cowl, T. A. (1999) The role of benthic invertebrate species
 378 in freshwater ecosystems. *BioScience*, 49, 119-128.

379 De Grave, S., Smith, K. G., Adeler, N. A., Allen, D. J., Alvarez, F., Anker, A., et al. (2015)
 380 Dead Shrimp Blues: A Global Assessment of Extinction Risk in Freshwater
 381 Shrimps (Crustacea: Decapoda: Caridea). *PLoS ONE*, 10, e0120198.
 382 doi:10.1371/journal.pone.0120198

383 Edgar, R. C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high
 384 throughput. *Nucleic Acids Research*, 32, 1792.

385 Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap.
 386 *Evolution*, 39, 783-791.

387 Fryer, G. (2006) Evolution in ancient lakes: radiation of Tanganyikan atyid prawns and
 388 speciation of pelagic cichlid fishes in Lake Malawi. *Hydrobiologia*, 568, 131-142.
 389 10.1007/s10750-006-0322-x

390 Huelsenbeck, J. P. & Ronquist, F. (2001) MrBayes: a program for the Bayesian inference of
 391 phylogeny. *Bioinformatics*, 17, 754-755.

392 Jugovic, J., Prevorcnik, S., Aljancic, G. & Sket, B. (2010) The atyid shrimp (Crustacea:
 393 Decapoda: Atyidae) rostrum: phylogeny versus adaptation, taxonomy versus trophic
 394 ecology. *Journal of Natural History*, 44, 2509-2533. 10.1080/00222933.2010.502258

395 Karge, A. & Klotz, W. (2008) *Süßwassergarnelen aus aller Welt*. Etlingen: Dähne Verlag.

396 Klotz, W. & von Rintelen, T. (2014) To “bee” or not to be—on some ornamental shrimp from
 397 Guangdong Province, Southern China and Hong Kong SAR, with descriptions of three
 398 new species. *Zootaxa*, 3889, 151-184.

399 Lanfear, R., Calcott, B., Ho, S. Y. W. & Guindon, S. (2012) PartitionFinder: combined
 400 selection of partitioning schemes and substitution models for phylogenetic analyses.
 401 *Molecular Biology and Evolution*, 29, 1695-1701.
 402 <http://dx.doi.org/10.1093/molbev/mss020>

- Marquet, G., Keith, P. & Kalfatak, D. (2009) *Caridina gueryi*, a new species of freshwater shrimp (Decapoda, Atyidae) from Santo island, Vanuatu. *Crustaceana*, 82, 159-166.
10.1163/156854008x367179
- Miller, M. A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *In: Proceedings of the Gateway Computing Environments Workshop (GCE)*, New Orleans, pp. 1 - 8.
- Page, T., Choy, S. & Hughes, J. (2005) The taxonomic feedback loop: symbiosis of morphology and molecules. *Biology Letters*, 1, 139-142.
- Page, T. J. & Hughes, J. M. (2011) Neither molecular nor morphological data have all the answers; with an example from *Macrobrachium* (Decapoda: Palaemonidae) from Australia. *Zootaxa*, 2874, 65-68.
- Page, T. J., von Rintelen, K. & Hughes, J. M. (2007) An island in the stream: Australia's place in the cosmopolitan world of Indo-West Pacific freshwater shrimp (Decapoda: Atyidae: *Caridina*). *Molecular Phylogenetics and Evolution*, 43, 645-659.
- Palumbi, S. R. (1996) Nucleic acids II: the polymerase chain reaction. *In: D. M. Hillis, B. K. Mable & C. Moritz (Eds), Molecular systematics*. Sinauer Associates, Sunderland, USA, pp. 205-247.
- Pringle, C. M., Blake, G. A., Covich, A. P., Buzby, K. M. & Finley, A. (1993) Effects of omnivorous shrimp in a montane tropical stream: sediment removal, disturbance of sessile invertebrates and enhancement of understory algal biomass. *Oecologia*, 93, 1-11.
- Richard, J. & Clark, P. F. (2009) African *Caridina* (Crustacea: Decapoda: Caridea: Atyidae): redescrptions of *C. africana* Kingsley, 1882, *C. togoensis* Hilgendorf, 1893, *C. natalensis* Bouvier, 1925 and *C. roubaudi* Bouvier, 1925 with descriptions of 14 new species. *Zootaxa*, 1995, 1-75.
- Richard, J. & Clark, P. F. (2014) *Caridina simoni* Bouvier, 1904 (Crustacea: Decapoda: Caridea: Atyoidea: Atyidae) and the synonymy by Johnson, 1963. *Zootaxa*, 3841, 301-338. <http://dx.doi.org/10.11646/zootaxa.3841.3.1>
- von Rintelen, K., Karge, A. & Klotz, W. (2008) News from a small island - first record of a freshwater shrimp (Decapoda, Atyidae, *Caridina*) from Peleng, Banggai Islands, Indonesia. *Journal of Natural History*, 42, 2243-2256.
- von Rintelen, K. & Cai, Y. (2009) Radiation of endemic species flocks in ancient lakes: systematic revision of the freshwater shrimp *Caridina* H. Milne Edwards, 1837

(Crustacea: Decapoda: Atyidae) from the ancient lakes of Sulawesi, Indonesia, with the description of eight new species. *Raffles Bulletin of Zoology*, 57, 343-452.

Roux, J. (1934) Notes de carcinologie mélanésienne. I. Décapodes d'eau douce de l'Archipel Bismarck et des îles de l'Amirauté. *Revue Suisse de Zoologie*, 41, 217-229.

Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, 22, 2688-2690.

Talavera, G. & Castresana, J. (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology*, 56, 564-577.

Tamura, K., Stetcher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*, 30, 2725-2729. doi:10.1093/molbev/mst197

Legends to figures

Figure 1: *Caridina buehleri*, holotype female (cl 7 mm): a. cephalothorax; b. first pereopod; c. tips of fingers of first pereopod; d. second pereopod; e. fifth pereopod; f. dactylus of fifth pereopod. *Caridina gueryi* holotype female (cl 6.4 mm) g. third pereopod; h. dactylus of third pereopod; i. preanal carina.

Figure 2: Collecting sites of *Caridina buehleri* in Pacific Ocean.

Figure 3: 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.

Figure 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;

470 OKI = Okinawa; PHI = Philippines; PNG = Papua New Guinea; SAF = South Africa; SOL =
471 Solomon Islands; TAI = Taiwan; VAN = Vanuatu; VTN = Vietnam.

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473 Figure 5: A = SEM micrograph of a first pereopod of *Caridina buehleri*. B = Optical
474 microscope photograph of a first pereopod of *Caridina buehleri*. C = SEM micrograph of a
475 first pereopod of *Caridina buehleri* after bleach treatment. D = Optical microscope
476 photograph of a first pereopod of *Caridina buehleri* after bleach treatment.

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478 Table 1: GenBank accession numbers for the obtained sequences.

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480 Table 2: Comparison of the type specimens of *C. buehleri* and *C. gueryi* with specimens from
481 Sulawesi, Kolobangara and Malekula.

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483 Table 1:

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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

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487 Table 2:
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	Rostrum times length of cl	Dorsal teeth (Ventral teeth)	Unarmed distal portion of dorsal margin / armed portion	Teeth placed on carapace
<i>C. buehleri</i> Holotype cl 7 mm	1.3	15 (5)	0.5	4
<i>C. gueryi</i> 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 <i>et al.</i> , 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
<i>C. buehleri</i> Holotype cl 7 mm	2.1	2.2	1.6	0.7
<i>C. gueryi</i> 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 <i>et al.</i> , 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
<i>C. buehleri</i> Holotype cl 7mm	4.0	2.7	4.4	1.4
<i>C. gueryi</i> 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 <i>et al.</i> , 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

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	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
<i>C. buehleri</i> Holotype cl 7 mm (Roux, 1934)	Missing (3.3)	Missing (8.0)	Missing (3.0)	Missing (8)
<i>C. gueryi</i> 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 <i>et al.</i> , 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

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	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
<i>C. buehleri</i> Holotype cl 7 mm	3.6	9.4	3.8	30
<i>C. gueryi</i> Holotype cl 6.4 mm (Marquet <i>et al.</i> , 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 <i>et al.</i> , 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

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	Uropodial diaeresis spinules	Egg size	Preanal carina	Appendix interna on P11
<i>C. buehleri</i> Holotype cl 7 mm	16 (left) – 17 (right)	Non ovigerous female	High, with no spine	NA
<i>C. gueryi</i> Holotype cl 6.4 mm (Marquet <i>et al.</i> , 2009)	18	Male (0.48 x 0.28)	High, with no spine	Well developed
Sulawesi specimens N = 6 (Klotz <i>et al.</i> , 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

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