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

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

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6
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13
14 **ABSTRACT**

15 The taxonomy of the freshwater shrimps *Caridina* (Atyidae) is very complex and confused
16 mostly because the morphological characters that have traditionally been used for species
17 delimitation and identification are highly plastic. There is thus a need for an integrative
18 approach of their taxonomy. A total of 42 specimens belonging to either *Caridina buehleri*
19 Roux, 1934 or *Caridina gueryi* Marquet, Keith & Kalfatak, 2009 were examined. We
20 combined here morphological data from 12 specimens from the whole distribution range of
21 the species, including type specimens with 16S mtDNA analyses from 7 freshly sampled
22 specimens, in order to verify the specific status of *Caridina buehleri*, from Papua New-
23 Guinea, Central Sulawesi, Western Samoa, Solomon Islands, and *C. gueryi* from Vanuatu.
24 The comparison of 24 morphological showed that morphological variations of character traits,
25 between *C. gueryi* and *C. buehleri*, are indeed widely overlapping and that no morphological
26 feature can effectively split specimens into two clear groups. Molecular characters
27 corroborated these results, as specimens from both groups were only separated by 2% p-
28 distance, a genetic distance that is coherent with their potentially high dispersal abilities. We
29 thus consider *C. gueryi* as a junior synonym of *C. buehleri*. Finally, *C. buehleri* is
30 characterized mainly by a styliform and characteristically indented rostrum and a long
31 stylocerite. Detailed re-descriptions of the type specimens of *C. buehleri* and *C. gueryi* are
32 given as well as their geographical and ecological distribution.

33
34 **Key words:** Freshwater shrimp, Indo-Pacific, Integrative taxonomy, 16S.

35

36 INTRODUCTION

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

41 The freshwater shrimp *Caridina* H. Milne-Edwards, 1837, comprising 298 species
42 (WoRMS database as of Oct. 2016) and mostly present in the Indo-Pacific region, is the most
43 diversified genus of the family Atyidae (De Grave *et al*, 2015) and an important ecological
44 component in the tropical streams (Covich *et al*, 1999; Pringle *et al*, 1993). Their high
45 diversity combined with the lack of informative morphological characters has led to a
46 confused taxonomy (Richard & Clark, 2009). Indeed, until recently, their taxonomy was
47 mainly based on morphological characters. Some have been proven highly variable within a
48 single species (e.g. rostrum shape and indentation or coloration) and so taxonomically non-
49 informative, making it difficult to identify boundaries among species (von Rintelen & Cai,
50 2009). There is thus a need for an integrative and standardized approach to improve the
51 systematic of the group, focusing on informative morphological features and using molecular
52 characters (Page *et al*, 2005; Page & Hughes, 2011). To illustrate this problem, we here
53 focused on two species that are morphologically very close: *Caridina buehleri* Roux, 1934
54 and *Caridina gueryi* Marquet, Keith & Kalfatak, 2009.

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

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

66 As we collected more and more specimens from different localities, we started to raise
67 doubts regarding the validity of *C. gueryi*, as the specimens identified as such fitted well with
68 the description of *C. buehleri*. Consequently, we here examined type specimens of these two

69 species and combined morphological data with a 16S mtDNA analysis in order to clarify their
70 respective status.

71

72 **MATERIALS AND METHODS**

73

74 **Abbreviations for Museums.** Muséum national d'Histoire naturelle, Paris: MNHN;
75 Naturhistorisches Museum Basel, Basel: NMB; Museum für Naturkunde, Berlin: ZMB.

76

77 **Abbreviations for morphological analyses.** The following abbreviations are used in the
78 present text: CL, carapace length (mm): measured from the post-orbital margin to the
79 posterior margin of the carapace; TL, total length (mm): measured from the tip of the rostrum
80 to the posterior margin of the telson; P1: first pereopod; P2: second pereopod; P3: third
81 pereopod; P5: fifth pereopod; PII: first pleopod.

82

83 **Molecular analyses**

84 **DNA extraction, amplification and sequencing.** DNA was extracted from abdominal tissues
85 using the semi-automatic Eppendorf ep-Motion 5075 robot. Fragments of the mitochondrial
86 16S rRNA (~ 520 bp) were amplified using the primers 16Sa-L
87 (CGCCTGTTTATCAAAAACAT) and 16Sb-H2 (CTCCGGTTTGAAGTCAAGATCA)
88 (Palumbi, 1996). DNA amplification was performed in 25µl PCR reactions, containing
89 approximately 3 ng of template DNA, 2.5 mM MgCL₂, 0.26 mM of each nucleotide, 0.3 µM
90 of each primer, 5% DMSO, 1 ng of BSA and 1.5 units of QBIOTAQ polymerase
91 (MPBiomedicals). Amplification products were generated by an initial denaturation step of 4
92 min at 94°C followed by 30 cycles of denaturation at 94°C for 30s, annealing at 51°C for 60 s
93 and extension at 72°C for 60 s. PCR products were sequenced using same primers and in both
94 directions to insure the accuracy of base calls. Chromatograms were edited using BioEdit
95 Sequence Alignment Editor Version 7.0.4.1 (Hall, 1999). All sequences were deposited in
96 GenBank (GenBank Accession N°s: [XXXXXXXXXX](#), Table 1). Sequences for the genera
97 *Caridina* and *Paratya* published by Page *et al.*, (T.J. Page, von Rintelen, & Hughes, 2007)
98 were included in our analysis, *Paratya* sequences being used as outgroup.

99

100 (Table 1 here)

101

102 **Sequence and phylogenetic analyses.**

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

105 Since non-coding markers can be difficult to align, two datasets were produced; a first
106 one was obtained by analyzing our alignment through GBLOCKS (Talavera & Castresana,
107 2007) in order to remove poorly aligned portions of sequences, and a second by leaving the
108 sequences as they were after the alignment.

109 Using Bayesian information criterion in PartitionFinder (Lanfear *et al.*, 2012) we
110 retained the GTR + G + I model for both datasets. Best-scoring ML trees were estimated for
111 each dataset using RAxML HPC2 v.8.2.4 (Stamatakis, 2006) on Teragrid v.7.2.7,
112 implemented in the Cyber Infrastructure for phylogenetic Research (CIPRES) portal v.3.1.
113 (Miller, Pfeiffer, & Schwartz, 2010) (<https://www.phylo.org/>). One hundred independent
114 searches, each starting from distinct random trees, were conducted. Robustness of the nodes
115 was assessed using non-parametric bootstrapping (Felsenstein, 1985) with 1,000 bootstrap
116 replicates. Bayesian trees were calculated using MrBayes software (Huelsenbeck & Ronquist,
117 2001) with the previously determined model, running for 5,000,000 generations, a sampling
118 frequency of 1,000 and a burn in of 25%. Support for nodes was determined using posterior
119 probabilities (PP) calculated by MrBayes. We considered a group to be ‘moderately
120 supported’ if it had a bootstrap support value (B) between 75 and 89% and posterior
121 probability (PP) between 0.95 and 0.97, and ‘highly supported’ when $B \geq 90\%$ and $PP \geq 0.98$.

122

123 ***Morphological comparisons.*** The rostrum, the general cephalothorax, the pereopods 1, 2, 3
124 and 5 and the abdomen were observed using a stereoscopic microscope. The proportions of
125 the various joints of the appendages were measured using microphotographs and the
126 AnalySIS Works software (Olympus).

127 The examinations were made on the type specimens of *C. buehleri* and *C. gueryi*, and
128 on a total of 12 specimens grouped by locality (6 from Sulawesi, 2 from Kolombangara and 4
129 from Malekula). The ranges were then compared side by side and a global range of all the
130 specimens was given.

131 Drawings were made using the “Digital Inking” method (Coleman 2003; Coleman
132 2006) by tracing vectorial paths on high resolution photographs using Adobe Illustrator (CS6)
133 and a WACOM MPTZ-1230 graphic tablet.

134 SEM micrographs were obtained from ethanol preserved specimens, some treated with
135 10% bleach for a night in order to remove the setae.

136

137 **RESULTS**

138

139 **Molecular results.**

140 A 16S gene fragment was successfully sequenced for seven specimens (Table 1). Sequences
141 included 490 bp, containing 10 variable sites. After alignment with 87 atyid sequences (83
142 *Caridina* and 4 *Paratya* as outgroup) from Page *et al.* (T.J. Page et al., 2007), the 16S datasets
143 of 94 sequences of 513 bp included 193 variable sites, of which 174 were phylogenetically
144 informative. After removal of ambiguous blocks, 374 bp of sequence remained to be used in
145 phylogenetic analyses (73% of the original 513 positions), of which 131 bp were variable and
146 112 bp phylogenetically informative.

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

151 In each of the BI and ML analysis, the consensus tree showed *C. buehleri* and *C.*
152 *gueryi* as a monophyletic and highly supported group (PP=0.99; B=97%), which was well
153 differentiated from all other *Caridina* analyzed (p-distance to the closest relative: 12.3%), and
154 appeared as belonging to the *C. weberi* species complex. Within this monophyletic group,
155 sequences from *C. buehleri* and *C. gueryi* were clustered in 2 well supported genetic groups;
156 the first one (PP=0.94; B=100%) including 2 specimens identified as *C. buehleri* from
157 Indonesia, and the second one (PP=0.99; B=100%) including 3 specimens identified as *C.*
158 *gueryi* from Vanuatu, 1 specimen identified as *C. buehleri* from Indonesia and 1 specimen
159 identified as *C. buehleri* too, from Solomon Islands. Clade 1 is 2% (p-distance) distant from
160 Clade 2.

161

162 (Figure 1 here)

163

164 **Taxonomy**

165

Atyidae De Haan, 1849

166

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

167

***Caridina buehleri* Roux, 1934**

168

(Figure 2)

169

170

(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, Kolobangara
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).

203 *Caridina gueryi* IU-MNHN-2015-23 Marquet, Keith & Kalfatak, 2009 Vanuatu, Malakula
204 Island, Brenwe river, 16°07.849'S 167°17.342'E; coll. Kalfatak, Keith & Lord, 20-11-2008,
205 det. Marquet : 4 ovigerous females (CL 5–6 mm).

206

207 **Diagnosis.** Carapace smooth, glabrous, with sharp antennal spine placed at lower orbital
208 angle. Pterygostomial margin blunt. Rostrum long, 0.5–1.4 of CL, styliform, reaching to the
209 end of scaphocerite, curved upwards, distal margin unarmed without apical teeth, armed with
210 8 to 16 teeth on basal part of dorsal margin, 1–4 of them situated on carapace behind orbital
211 margin, ventral margin with 2–7 teeth. Stylocerite sharp, long, reaching to about half of
212 second segment of peduncle.

213 First pereopod stout, chela about 1.9–2.7 times as long as wide, dactylus 1.3–2.9
214 times as long as wide, 0.4–0.8 length of palm; tips of both fingers with prominent claws,
215 carpus 1.2–1.7 times as long as wide.

216 Second pereopod more slender and longer than P1; chela 2.2–3.2 times as long as
217 wide, dactylus 3.0–4.8 times as long as wide, 1.0–1.6 times length of palm, tips of fingers
218 rounded, carpus slender 4.4–7.0 times as long as wide.

219 Third pereopod slender, dactylus 2.7–4.0 times as long as wide, terminal spiniform
220 setae included) with 5–8 spiniform setae on flexor margin in addition to the terminal one;
221 propodus 8.0–14.0 times as long as wide, 3.0–5.3 times as long as dactylus.

222 Fifth pereopod with dactylus 3.3–5.6 as long as wide with 26–36 spiniform setae on
223 flexor margin; propodus 9.4–20.5 times as long as wide, 3.8–6.2 as long as dactylus.

224 Endopod of first pleopod with well developed appendix interna in male specimens;
225 Appendix masculina on second pleopod reaching to about 0.6 times length of endopod;
226 appendix interna reaching about 2/3 of appendix masculina. Telson ending in broadly convex
227 margin bearing a median projection; distal margin with 5–10 long plumose setae flanked by
228 one pair of stout spiniform setae. Uropodal diaeresis with 16–20 short cuspidate teeth. Pre-
229 anal carina high, without a spine. Eggs size: 0.36–0.49 x 0.23–0.32 mm.

230

231 **Distribution.** *C. buehleri* is known from Indonesia (Sulawesi), Papua New Guinea (New
232 Ireland), Vanuatu (Santo, Malekula), Western Samoa (Upolu) and Solomon islands
233 (Kolobangara) (Figure 3).

234

235 (Figure 3 here)

236

237 **Habitat.** All specimens were collected in a typical brackish water environment (brackish
238 water pools or lower part of rivers, near the estuary). The species is rarely found over the
239 superior limit of the tide-influenced zone, and was not found in the true freshwater zone.

240

241 **Live colour pattern.** This species shows a rather specific pattern of seven white stripes (one
242 on each somite and one on the cephalothorax), upon a varying colour that tends to match the
243 environment, ranging from bright yellow to black, with reddish dots (Figure 4).

244

245 (Figure 4 here)

246

247 **Morphological comparison**

248 A total of 24 variables were obtained for each group and were compared in Table 2. It appears
249 that all of the variations of the measures are globally overlapping, and no significant
250 differences were observed.

251

252 (Table 2 here)

253

254 **DISCUSSION**

255

256 ***Caridina gueryi*, a junior synonym of *C. buehleri*.**

257 Genetic results showed that specimens attributed to both of the two species clustered in the
258 same clade, and that the two groups were too close (2% p-distance) to support the hypothesis
259 of separate species, and thus suggesting that one of the two species names has to be
260 synonymized. After morphological examination of both the type specimens and the
261 populations from the known distribution of the two species (Sulawesi, Vanuatu and Solomon
262 Islands), we found that no morphological feature could split our specimens in two clear
263 groups. Through the integrative taxonomy approach applied here, we regard both populations
264 as belonging to *C. buehleri*, since no morphological or ecological difference was observed
265 between them. Therefore, our results suggested that *C. gueryi* Marquet, Keith & Kalfatak,
266 2009 should be treated as a junior synonym of *C. buehleri* Roux, 1934.

267

268 **Diagnostic morphological characters of *C. buehleri***

269 From our morphological observations we found 4 very specific characters that are diagnostic
270 of this species and allow to separate it from all other *Caridina* species: 1) a long styliform

271 rostrum, reaching the end of scaphocerite, distodorsal margin of the rostrum unarmed without
272 apical teeth, with 8–16 teeth on basal part of dorsal margin, 1–4 of them situated on carapace
273 behind orbital margin and 3–6 teeth on ventral margin; 2) the stylocerite is long, reaching at
274 least about half of the second segment of antennular peduncle; 3) the first pereopod is stout,
275 with a very short dactylus and both fingers of chela with prominent distal claws; 4) a high
276 pre-anal carina with an indistinct tooth. According to Richard & Clark (2014), the number of
277 teeth and their placement on the rostrum (character 1) remain an important identification
278 character, in contrast to its length, which has been shown to be greatly plastic in atyid shrimps
279 (Jugovic *et al.*, 2010). Indeed, we found that the disposition of the teeth on the rostrum of *C.*
280 *buehleri* is quite characteristic (without an apical dorsal tooth and few ventral teeth) and
281 cannot be confused with any other species of the genus. The long stylocerite reaching to the
282 second segment of the antennular peduncle (character 2) is also found in *C. serratirostris* De
283 Man, 1892, *C. celebensis* De Man, 1892, *C. lobocensis* Cai, Choy & Ng, 2009 and in all
284 members of the *Caridina serrata* species group (Cai & Ng, 1999; Klotz & Rintelen, 2014).
285 However, these species can be easily differentiated from *C. buehleri* by the rostrum
286 denticulations and the proportions of the carpus of the first pereopod for *C. serratirostris* and
287 *C. celebensis*, the spines of the telson, the shape of the male first pleopod and the size of the
288 eggs for species of the *C. serrata* group (Klotz *et al.* 2007) and the setae of the fifth pereopod
289 for *C. lobocensis*. The chelae of the first pereopods exhibit a distinctive claw on both fingers
290 (character 3). This character was not reported by Roux (1934) in the original description of *C.*
291 *buehleri* nor in *C. gueryi*. It might thus be more widely distributed in this genus than it
292 appears, as it is quite difficult to see, hidden in the setae. According to Klotz *et al.* (2007), *C.*
293 *buehleri* shows a high pre-anal carina with an indistinct tooth (character 4). Marquet *et al.*
294 (2009) have reported a spine on this structure in *C. gueryi*. After re-examination of the
295 holotype of *C. gueryi*, it appears that there is actually no tooth on the pre-anal carina and that
296 the carina is similar to the one that *C. buehleri* possesses.

297

298 **Perspectives on the taxonomy of *Caridina*: *C. buehleri*, part of the *C. weberi* complex?**

299 Our single-gene phylogenetic analysis (Figure 1) corroborated the statement of Roux (1934),
300 identifying *C. buehleri* as closely related to *C. weberi* De Man, 1892 and *C. multidentata*
301 Stimpson, 1860, and more generally to species traditionally included in the *C. typus* group
302 (*sensu* Bouvier, 1925). This whole group includes species with rather stout bodies, short
303 antennular peduncle, short sixth abdominal segment, a high pre-anal carina and a high number
304 of spinules on the uropodial diaeresis.

305 In the present work (Figure 1), this group was genetically supported by BI (PP = 0.99),
306 thus suggesting the existence of a *C. weberi* species complex in the same way that there exist
307 species complexes for the groups *C. nilotica* (Karge & Klotz, 2008), *C. serrata* (Cai & Ng,
308 1999); (Klotz & von Rintelen, 2014), and *C. gracilirostris* (Cai & Ng, 2007). Indeed our
309 sequences clustered with *C. cf. weberi* from Moorea in both datasets, but also with *C. weberi*
310 *longicarpus* from New Caledonia, *C. multidentata* from Taiwan, *C. multidentata* from
311 Madagascar and *Caridina cf. weberi papuana* from Solomon Islands, in the BI analyses.
312 *Caridina sumatrensis* from Indonesia and *Caridina aff. weberi* from Papua New Guinea are
313 not supported in this group, but due to the low resolution of the node that bring them together
314 with the “*C. weberi* complex”, some further analyses are needed. *Caridina weberi* from
315 Micronesia and *Caridina aff. weberi* from Vanuatu are far from this group in the tree and are
316 most likely a misidentification. Multi-locus phylogenetic approach combined with
317 morphological and ecological data is highly needed to clarify the taxonomy of *Caridina*.

318

319 *New insights on the biology of C. buehleri*

320 *Caridina buehleri* exhibits a stout first pereopod with both fingers showing a prominent claw
321 (Figure 5). These features are also found in other species of *Caridina* such as *C. typus* (Klotz,
322 pers. com.). Clawed fingers might prove advantageous in feeding on discrete particles, as
323 shown in *Limnocaridina* species (Fryer, 2006) or small invertebrates, like oligochaetes or
324 juvenile insect or larvae, while species without clawed finger tips are primarily feeding on
325 detritus or single-celled periphyton (Klotz *et al.* 2007). This morphological feature could be
326 linked to a predatory behaviour by scrapping fixed invertebrates that evolved as an adaptation
327 to the strong competition in the part of the rivers where *C. buehleri* lives.

328

329 (Figure 5 here)

330

331 It is interesting to note that *C. buehleri* is a species inhabiting brackish water
332 environment, and that ovigerous females carry small and numerous eggs, indicative of a
333 prolonged larval development. A wide distribution range could therefore be expected (von
334 Rintelen *et al.*, 2008) rather than a scattered distribution as known until today. Further
335 fieldworks are necessary to collect more specimens and get a better picture of the extent of the
336 distribution range of *C. buehleri*, whose identification should be facilitated by this study.

337

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348 operated under a permit granted to Philippe Bouchet (MNHN) by the Environment Unit of the
349 Government of Vanuatu. During the expedition, the scientists operated under the “Forests,
350 Mountains, Rivers” theme coordinated by Bruno Corbara, Philippe Keith and Jérôme
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366

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450 **Legends to figures**

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

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

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458 Figure 3: A, C, D = *Caridina buehleri* from Kolobangara, Solomon Islands (Photo P. Keith)
459 respectively specimen CA1519, and 2 not sequenced specimens and; B = *Caridina gueryi*
460 from Santo, Vanuatu (Photo P. Keith), not sequenced; E = *Caridina buehleri* from Sulawesi,
461 Indonesia (Photo H. Evers), not sequenced.

462

463 Figure 4: Molecular phylogeny obtained from the full dataset showing the position of
464 *Caridina buehleri* (16S). Numbers above branches are the Bayesian posterior probabilities;
465 numbers under branches are the bootstrap from the Maximum Likelihood analysis. Dark grey
466 shading indicates the genetic “*C. weberi* species complex” and light grey shading indicates
467 taxa morphologically close to the “*C. weberi* species complex”. Locality codes: AUS =
468 Australia; FRP = French Polynesia; HKG = Hong Kong; HOW = Lord Howe Island; IND =
469 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.

482

483 Table 1:

484

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

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	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

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	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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