The complex study of complexes: The first well-supported phylogeny of two species complexes within genus *Caridina* (Decapoda: Caridea: Atyidae) sheds light on evolution, biogeography, and habitat

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► **To cite this version:**

Valentin de Mazancourt, Werner Klotz, Gerard Marquet, Benjamin Mos, D. Christopher Rogers, et al.. The complex study of complexes: The first well-supported phylogeny of two species complexes within genus *Caridina* (Decapoda: Caridea: Atyidae) sheds light on evolution, biogeography, and habitat. *Molecular Phylogenetics and Evolution*, 2019, 131, pp.164-180. 10.1016/j.ympev.2018.11.002 . hal-02171186

HAL Id: hal-02171186

<https://hal.science/hal-02171186>

Submitted on 2 Jul 2019

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The complex study of complexes: The first well-supported phylogeny of two species complexes within genus *Caridina* (Decapoda: Caridea: Atyidae) sheds light on evolution, biogeography, and habitat.

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Abstract

Atyid shrimps, a key component of tropical freshwater ecosystems, face multiple anthropogenic threats and thus need special attention. With more than 300 described species, the genus *Caridina* is the most speciose of all the Caridea infra-order. *Caridina* spp. occupy diverse habitats in tropical freshwaters of the Indo-West Pacific region. Several species complexes have been recognized, based on common morphological features, but little is known about how well these morphological characteristics align with phylogenetic characteristics. Furthermore, no phylogeny of the genus *Caridina* published so far has provided well-resolved and supported relationships among different species, thus impeding the possibility of proposing evolutionary hypotheses. In this study we used next generation sequencing (NGS) to provide new insights into the phylogenetic relationships among the genus *Caridina*, focusing on two complexes: ‘*Caridina nilotica*’ and ‘*Caridina weberi*’. We collected 92 specimens belonging to these two groups from most of their known geographical range, representing 50 species, for which we sequenced seven mitochondrial genes and two nuclear markers using ion torrent NGS. We performed a phylogenetic analysis, which yielded the first well-

supported tree for the genus *Caridina*. On this tree were mapped the geographic ranges and the habitats used by the different species, and a time calibration was tested. We found the driving factors that most likely account for separation of clades are differences in habitat and to a lesser extent geography. This work provides new insights into the taxonomy of this group and identifies opportunities for further studies in order to fill knowledge gaps that currently impede the management and conservation of atyid species.

Keywords: Amphidromy; Freshwater shrimp; Indo-Pacific; Mitochondrial genome; Molecular systematics; Taxonomy

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

1. Introduction

Atyidae is the most diversified decapod crustacean family in freshwaters worldwide, comprising nearly 500 species and showing a large variety of feeding and breeding strategies in diverse habitats. The genus *Caridina* H. Milne Edwards, 1837, with around 300 described species, is the most speciose of the Atyidae and also the most diversified genus of shrimps (De Grave et al., 2015). Currently, around one third of all known freshwater shrimp species are threatened or near-threatened as a result of human activity, primarily habitat destruction (De Grave et al., 2015). Shrimp play important ecological roles in their natural habitats (e.g. nutrient cycling) and are important prey species for a variety of other organisms (Covich et al., 1999; Crowl et al., 2001). Freshwater shrimps are also an important food source for humans, especially in the Indo-Pacific where they often support the livelihoods of the poorest communities through fishing or aquaculture (Russi et al., 2013). Declines in the abundance or extinctions of shrimp species could therefore have important implications for food security and the ecology of freshwater and brackish habitats, with the potential to hamper ecosystem services.

One obstacle to addressing the problem of rapid species loss is that conservationists cannot properly monitor species or implement conservation plans without good taxonomic information. There is considerable confusion surrounding the taxonomy of the genus *Caridina* that has, for example,

61 hampered efforts to assess the impacts of wild harvest for the aquarium trade (Klotz & von
62 Rintelen, 2014). Confusion surrounding the taxonomy of the genus has been, in part, due to natural
63 variability in traditionally retained characters that have been used to separate species (de
64 Mazancourt et al., 2017b). Additionally, some specimens have been grouped in complexes based on
65 common morphological features, but little work has been done to differentiate among closely
66 related species. For example, Bouvier (1925), following the previous attempts of De Man (1892)
67 and Ortmann (1894), separated the 50 known species at that time into five species groups: *Caridina*
68 *nilotica*, *C. laevis*, *C. africana*, *C. brevirostris*, and *C. typus*. As more species were described,
69 species groups were modified, abandoned, or combined and new groups were created, including the
70 *C. serrata* complex (Cai & Ng, 1999; Klotz & von Rintelen, 2014), *C. gracilirostris* complex (Cai
71 & Ng, 2007) and *C. weberi* complex (de Mazancourt et al., 2017a). Two of these, *C. nilotica*
72 complex and *C. weberi* complex, are well represented in insular systems of the Indo-West Pacific
73 region thanks to the great number of amphidromous representatives that allowed them to colonize
74 new habitats quickly. *C. nilotica* complex exists in tropical freshwaters from Africa to Melanesia
75 and Micronesia, whereas *C. weberi* complex representatives can be found further eastward in
76 Central Pacific Islands (Keith et al., 2013). Few species from the *C. nilotica* or *C. weberi* complexes
77 have been separated and described since the complexes were established.

78 To overcome the taxonomic complexity of the family Atyidae, molecular data is being increasingly
79 used to uncover phylogenetic relationships (Page et al., 2007a,b; von Rintelen et al., 2007; 2012).
80 Published phylogenies including species of *Caridina* have been predominantly built from a single
81 locus (von Rintelen et al., 2008), although phylogenies built using two or three loci have become
82 available in recent years (von Rintelen et al., 2012; Bossuyt et al., 2004; Page et al., 2007a,b; Cook
83 et al., 2011; Klotz & von Rintelen, 2014). Despite this, no phylogeny of the genus *Caridina*
84 published so far has provided well-resolved and supported relationships among different species,
85 impeding the possibility of proposing evolutionary hypotheses. For example, the most
86 comprehensive phylogeny of Atyidae built from one mitochondrial and two nuclear markers was
87 unfortunately unable to provide reliable dating and biogeographic evolution at the generic level
88 (von Rintelen et al. 2012). Greater understanding of the phylogenetic relationships and evolution of
89 *Caridina* requires the combination of morphological and/or ecological data with DNA sequencing
90 to improve the accuracy and reliability of species delineations (von Rintelen et al., 2008; von
91 Rintelen & Cai, 2009; Klotz & von Rintelen, 2014; de Mazancourt et al., 2017a; 2018a,b).

92 The aim of this study was to (i) provide the first well-resolved partial phylogeny of *Caridina*
93 focusing on two species complexes, *C. nilotica* and *C. weberi*, using next generation sequencing

94 methods (NGS), and (ii) combine our DNA sequencing with ecological, habitat, and
95 biogeographical data to better understand the evolutionary and phylogenetic relationships among
96 *Caridina*. Large datasets obtained using NGS often provide better resolution of the phylogenetic
97 relationships than analyses with single or few markers (Hillis, 1996). Using data from seven
98 mitochondrial and two nuclear markers, we constructed a phylogenetic tree that provides novel
99 insights into the evolution of this group and the family Atyidae. The implications of our phylogeny
100 for the management of caridean shrimp are also discussed.

101 **2. Material and methods**

102 **2.1 Specimens sampling**

103 We obtained specimens of *Caridina* from most of its range thanks to numerous expeditions made
104 by two of the authors (GM & PK), from the collections held at the Muséum national d'Histoire
105 naturelle (Paris, France), as well as specimens obtained from a network of naturalists and scientists
106 worldwide. A total of 92 specimens of atyid shrimps (89 *Caridina*, 2 *Paratya* and 1 *Elephantis*)
107 representing 50 species (48 *Caridina*, 1 *Paratya* and 1 *Elephantis*) were collected from different
108 localities of the Indo-West Pacific region (Fig. 1, Table 1) mostly by electro-fishing or by hand net
109 scooping in waterways. Specimens were then preserved in absolute ethanol and stored prior to
110 morphological examination and tissue sampling. Identifications were made following keys provided
111 by relevant publications focusing on the different prospected areas, comparisons with type
112 specimens, and by DNA barcoding (see de Mazancourt et al., 2017a; 2017b; 2018a,b).

113 (Figure 1 here)

114 **2.2 DNA extraction, amplification and sequencing**

115 DNA was extracted from abdominal tissue using the semi-automatic Eppendorf ep-Motion 5075
116 robot. Fragments of mitochondrial genome (MGF)(~3,200 bp, containing COII, ATP8, ATP6,
117 COIII and ND3), 16S rRNA (~500 bp), COI (~640 bp), nuclear 28S rRNA (~1,090 bp) and nuclear
118 Histone 3 (H3)(328 bp) were amplified by PCR using primers and amplification protocols detailed
119 in Table 2. DNA amplifications were performed in 20 µl PCR reactions, containing approximately
120 3 ng of template DNA, 2.5 mM MgCl₂, 0.26 mM of each nucleotide, 0.3 µM of each primer, 5%
121 DMSO, 1 ng of BSA and 1.5 units of QBIOTAQ polymerase (MPBiochemicals) for nuclear
122 markers, 16S and COI or LongAmp Taq Polymerase (BioLabs) for MGF. PCR products were
123 sequenced using an Ion-Torrent PGM platform following the method developed by Hinsinger et al.
124 (2015). Contigs obtained after sequencing were assembled iteratively by mapping them to

125 previously Sanger-sequenced or assembled sequences and each gene of the MGF was annotated and
126 extracted separately in Geneious v.8 software (<http://www.geneious.com/>; Kearse *et al.* 2012).

127 All sequences were deposited in GenBank (Table 1).

128 (Table 1 and Table 2 here)

129 **2.3 Phylogenetic analyses**

130 Six sequences retrieved from GenBank for species that had a mitochondrial genome and nuclear
131 markers published, were included with sequences obtained from specimens (Table 1).

132 *Macrobrachium bullatum* (Palaemonidae) from Australia was used as an extra-family outgroup and
133 *Paratya australiensis* from Australia, *P. caledonica* from New Caledonia, and *Halocaridina rubra*
134 from Hawaii were used as intra-family, extra-genus outgroups. Sequences from *Caridina* cf.
135 *nilotica*, *C. gracilipes* and *Neocaridina denticulata* were added as comparison. The origin and
136 identification of the GenBank sequences could not be ascertained, but including them allowed us to
137 analyse all the atyid mitochondrial genomes published at that time and confirm their identification.

138 Alignments of the genes and gene fragments were conducted using Muscle algorithm (Edgar, 2004)
139 implemented in MEGA7 software (Kumar et al., 2016). Fast Neighbor-Joining analyses were
140 performed on each alignment and the trees obtained were compared by eye. As the trees obtained
141 from the different mitochondrial and nuclear markers were consistent, we concatenated all the
142 alignments. In total, 98 specimens were included in the concatenated analysis, all of them having
143 the MGF, 96 a 16S sequence, 90 a COI sequence, 85 a 28S sequence, and 85 a H3 sequence (Table
144 1).

145 The best substitution model was calculated independently for each marker of the concatenated
146 alignment and each codon position for protein-coding genes (Table 3) using Bayesian Information
147 Criterion in PartitionFinder (Lanfear et al., 2012). Best-scoring ML trees were estimated using
148 RAxML HPC2 v.8.2.10 (Stamatakis, 2014) and best-scoring Bayesian Inference trees were
149 estimated using MrBayes v.3.2.6 (Ronquist and Huelsenbeck, 2003), both methods implemented in
150 the Cyber Infrastructure for Phylogenetic Research (CIPRES) portal v.3.1. (Miller et al. 2010)
151 (<https://www.phylo.org/>) with the previously determined models applied to their respective subset,
152 with two independent searches running for 10,000,000 generations, a sampling frequency of 2,000
153 and a burn in of 6% determined by checking trace files in the Tracer program of the BEAST
154 package (Drummond et al., 2012). Convergence was tested by comparing the two runs in Tracer.
155 Support for nodes for Bayesian analysis was determined using posterior probabilities (PP)

156 calculated by MrBayes. One hundred independent searches, each starting from distinct random
157 trees, were conducted. Robustness of the nodes for ML analysis was assessed using non-parametric
158 bootstrapping (Felsenstein, 1985) with 1,000 bootstrap replicates (B). We considered a node to be
159 moderately supported with PP between 0.9 and 0.95 and B between 80 and 95. Below these values,
160 we considered the node to be unsupported. At values above this range, we considered the node to be
161 highly supported.

162 (Table 3 here)

163 **2.4 Time-calibrated tree**

164 The concatenated dataset was used in BEAST v.1.8.3 (Drummond et al., 2012) to obtain a time-
165 calibrated phylogenetic tree constraining the topology obtained in Bayesian Inference. The fossil
166 shrimps *Delclosia roselli* (Via, 1971) and *D. martinelli* Rabadá, 1993 from Early Cretaceous
167 freshwater deposits (127 ± 2 MYA [million years ago]) in Spain were used as the calibration point,
168 i.e. stem of the Atyidae family. Two separate analyses (to assess the results reproducibility) ran for
169 50,000,000 generations, sampling every 1,000 generations, with a burn-in of 2,000,000, under Yule
170 process speciation, and using an uncorrelated relaxed molecular clock (because evolutionary rates
171 appear to vary depending on the branches in the phylogenetic analyses) with a lognormal
172 distribution, and a lognormal distribution around the calibration point. Priors for the calibration
173 point were: mean = 0.01; standard deviation = 2.0; offset = 127.0. The other parameters were set to
174 default. Convergence was assessed by comparing the two runs in Tracer. The trees were summed
175 using the TreeAnnotator program from the Beast package in a maximum clade credibility tree.

176 **2.5 Character mapping on the tree**

177 Six different characters were mapped against the Bayesian tree obtained previously, reduced to one
178 specimen per species, and the most parsimonious ancestral character states were reconstructed using
179 Mesquite v.3.4 software (Maddison & Maddison, 2018) using a taxa-character matrix (55 taxa for 6
180 characters tested) (Supplementary material). Two biogeographic codings were tested, one with 31
181 freshwater ecoregions adapted from Abell et al. (2008) and one with 9 marine geographic provinces
182 adapted from Longhurst (1998). The preferred habitat of the species was also tested with the
183 Altitude coded in 5 characters: Estuary, Lower course, Middle course, Higher course and
184 Headwater stream, the Substrate Type coded in 5 characters: Sand, Gravel, Pebbles, Boulders and
185 Mud, the Hydrology coded in 2 characters: Lotic and Lentic, and the affinity with vegetation coded
186 in 2 characters: Not in Plants and In Plants.

187 2.6 Historical biogeography analysis

188 A biogeographical analysis was performed on trees obtained from the time calibration using
189 different programs implemented in RASP (Yu et al. 2015). To each taxa were assigned
190 biogeographical ranges based on the terrestrial biogeographical realms recognised by the WWF
191 (Global 200, see Olson et al. 2001): Australasian, Indo-Malay, Oceanian, Afrotropical and
192 Palearctic (see Supplementary material). A BioGeoBEARS analysis determined the best fit model
193 to our data. Using Akaike Information Criterion, the model retained was Dispersal-Extinction-
194 Cladogenesis (DEC) (Ree et al. 2008). Using Lagrange algorithm implemented in RASP, with a
195 dispersal constraints matrix set to default, ancestral areas were reconstructed on the Bayesian tree.

196 3. Results

197 3.1 Phylogenetic analysis

198 A total of 9 sequences alignments were used in the analysis: 16S (546 bp); COI (620 bp); COII (674
199 bp); ATP8 (150 bp); ATP6 (676 bp); COIII (789 bp); ND3 (357 bp); 28S (1284 bp) and H3 (327
200 bp). Trees obtained in ML and Bayesian analyses showed no inconsistencies; only the Bayesian tree
201 is presented here with ML bootstrap values plotted (Fig. 2). Overall support for the tree was high.
202 Most of the deep and the shallowest nodes were well supported in both methods, and only a few
203 intermediate nodes showed low support (PP < 0.9, B < 80). Atyid sequences were separated in two
204 highly supported clades. The first clade comprised species of *Paratya* and *Halocaridina rubra*, and
205 the other clade included all of the other species. In this second clade, a first dichotomy excluded
206 *Caridina sp. WA4* from Australia from all others. Within this clade emerged a strongly supported
207 clade containing almost all the species of the *C. weberi* complex (node 1) and dispatched further
208 into two highly supported clades. The tree was further divided into clades with low support; one
209 clade comprised *Neocaridina denticulata*, *C. confusa*, *C. zebra* and *C. sp. Malanda*, another with *C.*
210 *cf. weberi sp. 1* and *C. typus*, and a third with all the remaining species. Subsequent dichotomies
211 separated *C. brevicarpalis*, *C. gracilirostris* and *C. cf. gracilirostris*, and all species belonging to
212 the *C. nilotica* complex in a highly supported clade, respectively.

213 (Figure 2 here)

214 3.2 Time calibration

215 The time calibration gave an origin date for the split of the two most basal atyid clades ranging
216 from 140 to 127 MYA (Mean = 128.01), following the calibration given by the fossils *Delclosia*
217 spp. (Fig. 3). The origin of *Caridina* group was estimated to have occurred between 95 and 52

218 MYA (Mean = 72.68). The last common ancestor between *C. nilotica* and *C. weberi* complexes
219 existed around 82 to 44 MYA (Mean = 62.5). The origin of *C. weberi* complex (Fig. 3, Node 1) was
220 dated to between 62 to 32 MYA (Mean = 45.68), whilst the origin of *C. nilotica* complex (Fig. 3,
221 Node 2) was estimated to have occurred more recently, between 47 to 27 MYA (Mean = 44.09). A
222 rapid radiation of the *C. nilotica* complex seems to have occurred around 27 MYA.

223 (Figure 3 here)

224 3.3 Character mapping

225 The primary factor driving evolution in the character mapping analyses appeared to be habitat, with
226 some secondary influence of geography (Fig. 4A and 4B). There were clear correlations between
227 habitat-related characters and the phylogeny, especially for deeper nodes. For example, following
228 ancestral states reconstructions, node 1 (Fig. 2 and 4) corresponding to *C. weberi* complex, was
229 likely to have originated in the higher course (Fig. 4C), on gravel substrate (Fig. 4D), in a lotic
230 environment (Fig. 4E), and with no vegetation (Fig. 4F). In contrast, node 2 corresponding to *C.*
231 *nilotica* complex appeared to have originated in the lower course (Fig. 4C), on gravel substrate (Fig.
232 4D), in a lentic environment (Fig. 4E), and among vegetation (Fig. 4F). Some characters may have
233 changed or reverted in more shallow nodes. For example, nodes 3 and 4, both belonging to *C.*
234 *weberi* complex, differed from their ancestor in having originated on boulder substrate (Fig. 4D).
235 Node 3 further differed in showing affinities to lentic environments (Fig. 4E). For node 5, members
236 of *C. nilotica* complex showed affinities with the higher course (Fig. 4C), with the absence of
237 vegetation (Fig. 4F), and several of its subclades were correlated with a boulder substrate (Fig. 4D).

238 (Figure 4 here)

239 3.4 Historical biogeography analysis

240 To study more thoroughly the historical biogeography of the species, a statistical reconstruction of
241 ancestral areas was performed (Fig. 5). The Australasian realm appeared to be the ancestral range of
242 most of the clades. Over the tree, the analysis found divergences within biogeographic realm more
243 common than vicariance events. Dispersal is frequent, while only one extinction event was found
244 for the species close to *C. multidentata*.

245 (Figure 5 here)

246 4. Discussion

247 4.1 A large dataset for robust analyses

248 This study presents the first well resolved and well supported phylogenetic tree for the genus
249 *Caridina* thanks to the large molecular dataset obtained by next generation sequencing (NGS) and
250 the number of species included that have been well determined. Indeed, with nine different
251 molecular markers from both mitochondrial and nuclear genomes, totalling around 5,420 bp for 98
252 specimens (of which 92 were newly sequenced), this is the most ambitious study made on this taxon
253 to date. Obtaining such a large dataset was made possible by the increasingly affordable NGS
254 technologies, namely the ion-torrent sequencing coupled with multiplexing. This method,
255 developed by Hinsinger et al. (2015), allowed us to get fragments of the mitochondrial genome
256 (MGF) for up to 40 specimens in a single sequencing, for prices comparable to regular Sanger
257 sequencings. Furthermore, thanks to our network of researchers from all around the world and the
258 numerous field trips made by the Muséum national d'Histoire naturelle, we could use a great
259 number of specimens from many different localities, some of them with a poorly studied freshwater
260 carcinofauna until now, such as the Solomon Islands or Micronesia. As expected with a dataset this
261 large, the overall support of the tree was high, regardless of the reconstructing method used. This
262 allowed us to have, for the first time, a clear representation of the phylogenetic relationships among
263 the species of *Caridina*, showing that species complexes defined previously using morphological
264 resemblances were monophyletic.

265 4.2 Time calibration

266 Until now, few studies have proposed dated phylogenies of atyid shrimps (Botello et al., 2013;
267 Jurado-Rivera et al., 2017; Davis et al., 2018) and only two have discussed *Caridina* (von Rintelen
268 et al., 2012; Bernardes et al., 2017). This can be explained by the lack of fossils attributed to this
269 family. Caridean shrimps are quite rare in the fossil record (52 species according to Schweitzer et
270 al., 2010) and even scarcer in freshwater deposits. Five atyid species belonging to three genera exist
271 in the fossil record, *Delclosia martinelli* (Via, 1971), *D. roselli* Rabadá, 1990, *Atyoida roxoi*
272 Beurlen, 1950, *A. tremembeensis* Beurlen, 1950, and *Caridina nitida* A. Milne Edwards, 1879. A
273 fourth species (and genus), currently *incertae sedis* could be added to this list, *Homelys minor*
274 Meyer, 1862, that Ortmann (1894) proposed as an Atyidae because of the small size of the
275 specimens and the freshwater environment. It is doubtful that extant genera could be represented in
276 the fossil record, the two species of fossil *Atyoida* Randall, 1840 described by Beurlen (1950) being
277 separated by almost 100 million years. These fossils were identified as atyids because they resemble
278 modern day *Potimirim* Holthuis, 1954 (known as *Atyoida* at the time of Beurlen) that live today in
279 the same area. Additionally, *Caridina* was the default name of small freshwater shrimps at the time
280 of A. Milne Edwards (see *Caridina desmarestii* (Millet, 1831) (Joly, 1843, A. Milne Edwards,

1879) now *Atyaephyra desmarestii* (Millet, 1831)). Regardless, because of their small size (3cm at most), morphological characters as demonstrated by Rabadá (1990), and age (oldest fossil atyid record), the two species of *Delclosia*, *D. roselli* (Via, 1971) and *D. martinelli* (Rabadá, 1990) from the Lower Cretaceous of Spain remain the most convincing fossil atyid, which is why we retained *Delclosia* as our calibration point.

Only one calibration point was used in our analysis given the limited availability of suitable fossils. Multiple calibration points can improve the reliability of molecular timing analyses (Lukoschek et al., 2012; Hipsley & Müller, 2014). Despite this, we consider our results to be reliable because they are consistent with geographical events. For example, the clade containing all New Caledonian endemic species is posterior to the re-emergence of the island after its complete submersion, 37 MYA (million years ago) (Grandcolas et al., 2008). Similarly, *C. variabilirostris*, a species endemic to Pohnpei Island, Micronesia appeared to have split from its sister-species between 10 and 3 MYA (Mean = 6.82 MYA) in our analysis, congruent with the apparition of the island, between 3–8.6 MYA (Craig et al., 2001). Furthermore, if the fossils we used for the calibration point were excluded from the Atyidae, the molecular clock would still give us a congruent timing. Substitution rates are sometimes used to calibrate a tree where no fossils exist or a geological event cannot be used. For *Caridina*, Bernardes et al. (2017), following Hurwood & Hughes (2001) who were following Knowlton et al. (1993), a substitution rate of 1.1–1.3% per MYR (million years) in COI was retained as originally calculated for alpheid shrimps based on the closing of the Isthmus of Panama. If we suppose this rate to be constant in caridean shrimps, our data agrees somewhat for intra-specific divergences. For example, the two sequences of *C. simoni* are separated by 5.1% divergence (K2P) in COI, corresponding to 3.97 MYA in the calibrated tree, which gives a rate of 1.28% per MYR. For *C. novaecaledoniae*, a COI divergence of 6% between the two sequences, corresponding to 5.68 MYA, gives a substitution rate of 1.06% per MYR.

An arising of the Atyidae in the Early Cretaceous agrees with most studies that have examined the origin of the family. For example, four out of six analyses by von Rintelen et al. (2012) suggested a Cretaceous origin of the family and a Paleogene radiation. Conversely, Jurado-Rivera et al. (2017) suggest a Permo-Triassic origin of the Atyidae, but only included fossils that are phylogenetically outside of the family as calibration points. Additionally, time-estimates could be over-estimated due to high mutation rates in the *Typhlatya* group (see the branch length of *H. rubra* on Fig. 2). Likewise differences between the results of this study and Davis et al. (2018), which proposed the origin of Atyidae during the Early Jurassic without rapid diversification and an equal rate of speciation throughout the tree, may be explained by differences in methodology and underlying

assumptions. Combining trees from different sources as used by Davis et al. (2018), in which original branch lengths are lost and recalculated, can lead to over-estimation of time-estimates (Bryant et al., 2004).

4.3 Historical biogeography and character mapping

For the geographic coding, we first tried to use freshwater ecoregions (Abell et al., 2008) but the great number of distinct regions made the analysis unreadable. As most caridean species are postulated to have colonized their range via marine larval dispersal, we tried to test marine biogeographic provinces based on plankton communities and currents (Longhurst, 1998). This approach was easier to read due to the fewer number of coded provinces, but could not highlight endemic taxa. Given the difficulties in our approaches, no biogeographic hypotheses can be proposed for the deeper nodes of the phylogeny due to a strong bias of sampling towards Melanesia, however, some of the terminal nodes can be explained by their geographical distribution. For example, nodes 6 and 7 clearly originate in Australia (Fig. 4A), which supports the hypothesis that this continent was colonised in several events (Page et al. 2007a,b). Node 8, containing all the New Caledonian endemic species is likely to have originated there (33% likelihood, Fig. 4B). Node 9, composed only of species living in the Indian Ocean region, would likely have originated there. It is interesting to note, however, that an Indonesian species, *C. brachydactyla* is sister to the clade delineated at Node 9, supporting the hypothesis of a colonization of the Indian Ocean region from Indonesia. The historical biogeography analysis was made on a shorter list of areas based on terrestrial biogeographical realms. It confirmed the results obtained for the marine and freshwater bioregions in showing no deep geographic structuration of the phylogeny and still with a bias of sampling towards Melanesia (here included among others in the Australasian realm). It further showed that dispersal is frequent in the evolutionary history of *Caridina*, suggesting that amphidromy may be the ancestral character in this genus that facilitated such behaviour. Within-region cladogenesis is more common than vicariance divergence across terrestrial biogeographical realms, even in deeper nodes, and the vicariance events inferred could hardly be explained by geological events such as continental drift, since most of the species are found on islands, but could be linked to changes in oceanic currents that no longer carry larvae from one island to another (see Bernardes et al. 2017). It is important to note that the Indo-Malay region was not sampled as extensively as other regions in our study. The Indo-Malay region is a biodiversity hotspot for many taxa, including freshwater shrimps (De Grave et al. 2015). Given this, it is possible that a significant proportion of species may be missing from our analyses, which could have biased our results. Whilst we included species from this region representing a broad range of geographical and

347 ecological types, further samples are required to better understand the phylogeny and historical
348 biogeography of the shrimp from the Indo-Malay region.

349 Our analyses provide new insights into the habitat and potential ecology of the common ancestors
350 of the genus *Caridina*. According to the analyses, the common ancestors of the *C. nilotica* and *C.*
351 *weberi* complexes seem to have lived at low altitudes (67% of chances in the estuary or lower
352 course, Fig. 4C), on gravel substrate (Fig. 4D) in a lentic environment (Fig. 4E) and among
353 vegetation (Fig. 4F). Differences in the ecology of shrimps from complexes other than ‘*C. nilotica*’
354 and ‘*C. weberi*’ used here might provide further explanation for the separation of complexes that
355 have been identified using genetics (this study) and morphology (Cai & Ng, 1999; 2007; Klotz &
356 von Rintelen, 2014). For example, in the lower reaches of rivers, there often is strong predation
357 pressure (from Eleotridae, Kuhliidae, Anguillidae) but an abundance of food available, whereas in
358 the higher course, there are almost no predators (except Anguillidae and Palaemonidae) but a strong
359 competition for food with other atyids (namely the filter-feeding *Atya*-like species).

360 **4.4 Differences between *C. nilotica* and *C. weberi* complexes**

361 The analyses highlighted the genetic reality of some species complexes that were established using
362 morphological characteristics, namely *C. weberi* and *C. nilotica* complexes on which this study
363 focused. Both of these complexes were well separated from other taxa and well supported in the
364 phylogeny regardless of the method used to differentiate them. As pointed out by previous authors
365 (Page et al., 2007a,b, von Rintelen et al., 2008, 2012), genus *Caridina* appears polyphyletic, with
366 genera *Elephantis* Castelin, Marquet & Klotz, 2013 and *Neocaridina* Kubo, 1938 nested in the
367 middle of the *Caridina* species.

368 Our time calibration showed that *C. weberi* complex is older than *C. nilotica*. Ancestral states
369 reconstruction and character mapping analyses showed that the separation of the clades was due to
370 habitat at first (including altitude, habitat, and hydrology) and then geography was responsible for
371 the diversification within the complexes. This was not a surprising result given environment has a
372 profound influence on the morphology of atyid species (Jugovic et al., 2010; de Mazancourt et al.,
373 2017b). For example, as altitude decreases, along with changes in associated environmental factors
374 (current flow, substrate type, etc.), the length of the rostrum and the segments of the legs tend to
375 increase; a phenomenon known as the “Pinocchio-Shrimp effect” (de Mazancourt et al., 2017b).
376 This effect is visible in both species complexes studied here. *C. weberi* complex displays robust
377 morphology, with usually a short rostrum and stout legs, associated with their affinities to mostly
378 lotic environment in high altitudes among boulders with no vegetation (Fig. 4). In contrast, *C.*

379 *nilotica* complex species tend to display a slender morphology, with a long rostrum and long legs
380 associated with their affinity to lentic environments on the lower course with gravel bed and the
381 presence of aquatic vegetation (Fig. 4). It is important to note, however, some species belonging to
382 these complexes have adapted to different habitats than their ancestors, and this is associated with
383 differences in their morphology. For example, species in *C. nilotica* complex living in the higher
384 course, like *C. mertoni*, *C. variabilis* or *C. variabilirostris* (species of the node 5), often show a
385 shorter rostrum (de Mazancourt et al., 2018a,b). Conversely, species of the *C. weberi* complex that
386 are adapted to lower altitudes, like *C. buehleri* (species of node 3), can possess a longer rostrum
387 than other species of this complex (de Mazancourt et al., 2017a). Likewise, *C. cf. weberi* sp. 1 from
388 the Solomon Islands, living in environments close to those of species of *C. weberi* complex exhibit
389 a close morphology, which led to this species being included as part of the complex. *C. cf. jeani* that
390 was thought to be close to *C. typus* because of its unarmed rostrum (Cai, 2010) falls within the *C.*
391 *weberi* complex in our analysis. Our results highlight the need for careful morphological
392 examination to identify discriminating characters and prevent misidentifications.

393 **4.5 Implications for conservation and management of *Caridina***

394 Our genetic analyses provide strong evidence for the existence of multiple valid and undescribed
395 species within the *C. nilotica* and *C. weberi* species complexes. Currently, both '*C. nilotica*' and '*C.*
396 *weberi*' are classified as "Least Concern" on the IUCN Red List (IUCN, 2018a,b). These
397 classifications will need to be reassessed to account for differences in the vulnerability of the
398 species identified in this study that are clustered within the two complexes. For example, our
399 analysis indicated the presence of three undescribed species within the *C. nilotica* complex that are
400 likely to be restricted to the Solomon Islands and/or nearby islands where freshwater biota are
401 impacted by habitat destruction and to a lesser extent invasive species (Polhemus et al., 2008).
402 Species with restricted distributions are generally more vulnerable to extinction compared to widely
403 dispersed species (Purvis et al., 2000). Similarly, our genetic data indicates the range of some
404 species reported as widespread (e.g. *Caridina longirostris*) and in turn classified as "Least Concern"
405 (IUCN, 2018c), may be smaller than currently thought due to difficulties in distinguishing among
406 specimens with very similar morphology (de Mazancourt et al., in press). Our genetic analysis
407 indicated specimens from Australia fitting the morphological description of *Caridina longirostris*
408 were two different species, *C. gracilipes* and *C. sp. Solomon 3*. Given this, it is likely that the risk
409 of extinction for many shrimp populations within the '*C. nilotica*' and '*C. weberi*' species
410 complexes is greater than previously thought. This is concerning given one third of caridean
411 shrimps are already classed as 'Threatened' or 'Near Threatened' (De Grave et al., 2015). Until full

412 taxonomic assessments can be made, managers and conservationists should use a ‘precautionary
413 approach’ in managing and protecting populations that may be closely related and have similar
414 morphology to other wide-spread species.

415 **5. Concluding remarks**

416 This study reconstructing the phylogeny of genus *Caridina* provided a well-resolved and supported
417 phylogenetic tree for the first time, with clades clearly identified that allowed us to propose novel
418 evolutionary hypotheses. Our results highlight how inclusion of species complexes in phylogenies
419 can provide new insights into evolutionary relationships and propose new avenues for
420 distinguishing closely related species, as well as providing information crucial to conservation and
421 management initiatives. To expand this work and improve the taxonomy of the whole genus, some
422 species positions will need to be clarified (e.g. *C. typus*), which could be obtained with a larger
423 dataset of complete mitochondrial genomes. The inclusion of comparatively unexplored species
424 complexes in our phylogeny, such as Sulawesi, African, Chinese landlocked groups, *C.*
425 *gracilirostris* complex, or the species close to *C. serratirostris*/*C. celebensis*, would likely add
426 substantial information on the evolution of this group. Finally, the evolutionary hypotheses
427 proposed here can be improved by refining time calibrations, but this is reliant on the discovery of
428 new fossils or the improvement of existing techniques that do not rely on fossils.

429

430 **Acknowledgments**

431 The authors thank Dave Wilson (Aquagreen), Robert Kroll, Mykala P. Mos, and Symon Dworjanyn
432 for assistance in the collection of specimens from Australia, Alexandra Holland for sending
433 specimens from South Africa, Clara Lord, David Boseto, Brendan Ebner, and Philippe Gerbeaux,
434 for their help collecting specimens in the Solomon Islands. The study was made possible by a grant
435 given to the French Ichthyological Society in the context of the ‘Critical Ecosystem Partnership Fund
436 (CEPF)’ (Melanesia hotspot). The Critical Ecosystem Partnership Fund is a joint initiative of
437 l’Agence Française de Développement, Conservation International, the Global Environment
438 Facility, the Government of Japan, the MacArthur Foundation and the World Bank. A fundamental
439 goal is to ensure civil society is engaged in biodiversity conservation. We would like to
440 acknowledge the customary landowners and tribes of Mount Maetambe to Kolobangara River
441 Watershed, the Lauru Land Conference of Tribal Community, Choiseul Province, villages and
442 tribes of Hunda-Kena, Jack Harbour, Poitete, Lodumoe and Vanga who welcomed us on
443 Kolobangara Island and Vella Lavella, KIBCA and ESSI, and the Solomon Islands Government for

444 the support and facilitation of the legal process that have allowed the expedition team to conduct the
445 scientific research. Joel Delafenêtre, Clementine Flouhr, and Pascal Tiberghien for their help
446 collecting specimens in New Caledonia. The work was supported by a convention between OEIL
447 and South Province of New Caledonia. We would like to thank Jean-Jérôme Cassan (North
448 Province) and Damien Grima (South Province) for providing us the research permit. Special thanks
449 to Florence and Jean-Pierre Cabée, Marie and Claude Hugon for their hospitality. Thanks to the
450 OEIL staff (Fabien Albouy, Adrien Bertaud, Deiphine Bossy and Matthieu Juncker (Director). "*Our*
451 *Planet Reviewed*" - New Caledonia expedition (2016-2019) is a joint project of MNHN and
452 Conservatoire d'Espaces Naturels (CEN) de Nouvelle-Calédonie. It is/was funded mainly by the
453 Gouvernement de la Nouvelle-Calédonie, Province Sud and Province Nord, and Office des Postes
454 et Télécommunications (OPT) and in-kind support from AirCalin and Toyota Nouvelle-Calédonie.
455 The expedition operated under permits issued by the Direction de l'Environnement (DENV) of
456 Province Sud and Direction du Développement Economique et de l'Environnement (DDEE) of
457 Province Nord. *Our Planet Reviewed / La Planète Revisitée* is a global initiative founded in 2007 by
458 Muséum National d'Histoire Naturelle (MNHN) and Pro-Natura International (PNI). Brian Lynch,
459 Magalie Castelin, Laura Taillebois and Philippe Gerbeaux for their help collecting specimens in
460 Palau and Micronesia. This work was supported by the National Museum of Natural History of
461 Paris, the AIMARA Association, and the French Ichthyological Society. We would like to thank the
462 Ministry of Natural Resources, Environment, and Tourism Koror, Republic of Palau, and in
463 particular Helena Rebelkuul from the Bureau of Marine Resources, and Kashgar Rengulbai (and his
464 assistant Gwen) from the Bureau of Agriculture (BOAGRI), for their help with processing the
465 research permits. Thanks to the Conservation Society of Pohnpei and particularly Eugene Joseph
466 (Director), Francesca Sohl Obispo, Relio Lengsi and Iakop loans, and the Office of Fisheries and
467 Aquaculture. DAF (Direction de l'agriculture et de la forêt) and ARDA (Association Réunionaise de
468 Développement de l'Aquaculture) for their help collecting specimens in Mayotte. Ahmed Abdou
469 for giving us specimens from Moheli Island, Comoros. The DIAMSOI team (The IH-SM from
470 Tuléar with Dr Man-Wai Rabenevanana and the MNHN from Paris with E. Feunteun and T.
471 Robinet) and the ARDA team (P. Bosc, H. Grondin and P. Valade) for their help collecting
472 specimens in Madagascar. We are grateful to Joe Aride from Madagascar National Parks (MNP-
473 ANGAP) and to the manager of Masoala National Parc at Maroantsetra. Lastly, we want to thank
474 all the Responsible Chiefs of the areas concerned for their kind permission, and the villages and
475 communities who have always heartily received us and helped us in our prospecting of rivers. This
476 research was financed by the Institut Français de la Biodiversité (IFB) for the DIAMSOI program
477 (2007-2010). The Office of the Prime Minister of Rarotonga and in particular Teumere Koteka-

478 Heather, D. Charlie and M. Mokoroa for the research permit in the Cook Islands. We are also
 479 grateful to G. MacCormack for the invaluable help he has provided us on the field. We thank also
 480 the Te Ipukara Society. The Samoan Ministry of Natural Resources Environment and Meteorology
 481 (MNRE), Conservation International (CI), The Japan International Cooperation Agency (JICA), the
 482 University of The South Pacific (USP) and the International Union for Conservation of Nature
 483 (IUCN). J. Atherton (CI) and N. Doherty (MNRE) played a major role in the mapping, coordination
 484 and logistic arrangements. With his presence in the field we are very happy to thank P. Gerbeaux.
 485 Lengguru 2010 expedition and The Environment Unit of Vanuatu, especially J. White, R. Nari, and
 486 Ernest Banni, for their interest and concerns for the conservation of flora and fauna native to
 487 Vanuatu, Mr Alsen Obed and Mr Glen Alo, Fisheries Office in Santo; Mr Kenery Alvea,
 488 Environment Officer, Sanma Province, Ms. Rollenas Tavue, Vathe Office, and all people of
 489 Vanuatu along the rivers prospected for their kindness. Minoru Saito for kindly sending us
 490 specimens from Japan. National Park Service for funding DCR's trip to Guam. We are especially
 491 grateful to Brent Tibbatts of the Guam Department of Agriculture, Division of Aquatic and Wildlife
 492 Resources for permits, help in the field and advice on good collection locations.

493 BM was supported by a postdoctoral fellowship from AgriFutures Australia.

494 Thanks to the Service de Systématique Moléculaire of the MNHN and its staff for allowing us to
 495 perform the molecular work.

496

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721

721 **Tables and Figures captions:**

722

723 Table 1: List of the specimens included in the phylogeny

724

725 Table 2: List of the primers used to amplify the different markers

726

727 Table 3: Substitution models retained by PartitionFinder for each partition of the dataset

728

729 Figure 1: Map of the sampling localities of the specimens used in the study

730

731 Figure 2: Bayesian Inference Phylogram obtained from the concatenated dataset showing the
732 position of the two species complexes and the type species of the genus, *Caridina typus*. Numbers
733 above branches indicate Bayesian posterior probabilities (PP); numbers under branches indicate
734 Maximum Likelihood bootstrap values (B). Support values under 0.8 in PP or 80% in B are not
735 shown. Nodes highlighted with a colored circle are discussed. Nodes with a red circle correspond to
736 the “*C. weberi*” and “*C. nilotica*” complexes.

737

738 Figure 3: Time calibrated Bayesian tree obtained with Beast software. Blue bars indicate confidence
739 intervals; numbers indicate mean date of the node. The red dot indicates the calibration point.

740

741 Figure 4: Character mapping on the Bayesian tree obtained in Fig. 2. A: Geography coded
742 following Longhurst’s (1998) marine provinces; B: Geography coded following Abell et al.’s
743 (2008) freshwater ecoregions; C: Altitude; D: Substrate type; E: Hydrology; F: Habitat

744

745 Figure 5: Ancestral areas reconstruction based on the time calibration consensus tree.

Figure 1

Figure 2

Figure 3

Figure 4

Geography 1 (Longhurst, 1998)

Geography 2 (Abell et al., 2008)

Altitude

Substrate type

Hydrology

Habitat

Figure 5

LEGEND

A = Australasian

B = Indo-Malay

C = Oceanian

D = Afrotropical

E = Palearctic

ABCDE

AB

AC

AD

AE

BC

BD

BE

DE

ABC

ABD

ADE

BDE

D Dispersal event

V Vicariance event

E Extinction event

Table 1

Species	DNA voucher	Country	16S	COI	COII	ATP8	ATP6	COIII	ND3	28S	H3
<i>C. africana</i>	CA1628	South Africa	MK189899	MK190058	MK189648	MK189557	MK190240	MK190149	MK189819	MK189980	MK189729
<i>C. appendiculata</i>	CA1670	Australia	MH497534	MK190059	MK189649	MK189558	MK190241	MK190150	MK189820	MK189981	MK189730
	CA1708		MK189903	MK190063	MK189653	MK189562	MK190245	MK190154	MK189824	MK189985	MK189733
	CA1493	Solomon Islands	MH497524	MK190038	MK189624	MK189533	MK190216	MK190125	MK189795	MK189956	MK189711
	CA1261	Vanuatu	MH497510	MK190018	MK189601	MK189510	MK190193	MK190102	MK189772	MK189935	MK189689
<i>C. brachydactyla</i>	CA1131	Indonesia	MH497502	MK190011	MK189594	MK189503	MK190186	MK190095	MK189765	MK189930	MK189682
<i>C. brevicarpalis</i>	CA1521	Solomon Islands	MK189887	MK190049	MK189636	MK189545	MK190228	MK190137	MK189807	MK189968	MK189719
<i>C. brevidactyla</i>	CA1345	Solomon Islands	MH497518	MK190024	MK189607	MK189516	MK190199	MK190108	MK189778	MK189941	MK189694
	CA1501		MK189881	MK190040	MK189626	MK189535	MK190218	MK190127	MK189797	MK189958	
<i>C. buehleri</i>	CA1519	Solomon Islands	KY350244	MK190047	MK189634	MK189543	MK190226	MK190135	MK189805	MK189966	MK189717
	CA1520		MK189886	MK190048	MK189635	MK189544	MK190227	MK190136	MK189806	MK189967	MK189718
<i>C. cf. gracilirostris</i>	CA1238	Indonesia	MK189858	MK190015	MK189598	MK189507	MK190190	MK190099	MK189769	MK189932	MK189686
<i>C. cf. jeani</i>	CA1415	Samoa	MK189877		MK189621	MK189530	MK190213	MK190122	MK189792	MK189953	MK189708
	CA1416		MK189878		MK189622	MK189531	MK190214	MK190123	MK189793	MK189954	MK189709
	CA1370	Vanuatu	MK189867	MK190028	MK189611	MK189520	MK190203	MK190112	MK189782	MK189945	MK189698
	CA1371		MK189868	MK190029	MK189612	MK189521	MK190204	MK190113	MK189783	MK189946	MK189699
	CA1377		MK189871	MK190032	MK189615	MK189524	MK190207	MK190116	MK189786	MK189949	MK189702

<i>C. cf. multidentata</i>	CA1053	Madagascar	MK189853		MK189591	MK189500	MK190183	MK190092	MK189762		
<i>C. cf. nilotica 1</i>	CA1707	Australia	MK189902	MK190062	MK189652	MK189561	MK190244	MK190153	MK189823	MK189984	MK189732
<i>C. cf. nilotica 2</i>	CA1711	Australia	MK189904	MK190064	MK189654	MK189563	MK190246	MK190155	MK189825	MK189986	MK189734
	CA1714		MK189905	MK190065	MK189655	MK189564	MK190247	MK190156	MK189826	MK189987	MK189735
<i>C. cf. vitiensis</i>	CA1385	Fiji	MK189872	MK190033	MK189616	MK189525	MK190208	MK190117	MK189787	MK189950	MK189703
	CA1003	New Caledonia	MK189851	MK190007	MK189589	MK189498	MK190181	MK190090	MK189760	MK189928	MK189680
	CA1601		MK189896	MK190055	MK189645	MK189554	MK190237	MK190146	MK189816	MK189977	MK189726
<i>C. cf. weberi Pohnpei</i>	CA1388	Micronesia	MK189873		MK189617	MK189526	MK190209	MK190118	MK189788	MK189951	MK189704
	CA1390		MK189874	MK190034	MK189618	MK189527	MK190210	MK190119	MK189789		MK189705
<i>C. cf. weberi sp. 1</i>	CA1349	Solomon Islands	MK189865	MK190026	MK189609	MK189518	MK190201	MK190110	MK189780	MK189943	MK189696
<i>C. cf. weberi sp. 2</i>	CA1516	Solomon Islands	MK189885	MK190046	MK189633	MK189542	MK190225	MK190134	MK189804	MK189965	MK189716
<i>C. cf. weberi sp. 3</i>	CA1048	Cook Islands	MK189852	MK190008	MK189590	MK189499	MK190182	MK190091	MK189761		
	CA1410	Samoa	MK189876	MK190036	MK189620	MK189529	MK190212	MK190121	MK189791	MK189952	MK189707
	CA1508	Solomon Islands	MK189882	MK190043	MK189629	MK189538	MK190221	MK190130	MK189800	MK189961	MK189714
<i>C. cf. weberi sp. 4</i>	CA1509	Solomon Islands	MK189883		MK189630	MK189539	MK190222	MK190131	MK189801	MK189962	MK189715
	CA1510			MK190044	MK189631	MK189540	MK190223	MK190132	MK189802	MK189963	
<i>C. cf. weberi sp. 5</i>	CA1909	Solomon Islands	MK189927	MK190088	MK189678	MK189587	MK190270	MK190179	MK189849	MK190005	MK189758

<i>C. cf. weberi</i> sp. 6	CA1902	Solomon Islands	MK189925	MK190086	MK189676	MK189585	MK190268	MK190177	MK189847		MK189756
	CA1903		MK189926	MK190087	MK189677	MK189586	MK190269	MK190178	MK189848		MK189757
<i>C. confusa</i>	CA1727	Australia	MK189907	MK190067	MK189657	MK189566	MK190249	MK190158	MK189828	MK189988	MK189737
	CA1728		MK189908	MK190068	MK189658	MK189567	MK190250	MK190159	MK189829	MK189989	MK189738
<i>C. elongapoda</i>	CA1899	Malaysia	MK189924	MK190085	MK189675	MK189584	MK190267	MK190176	MK189846	MK190004	MK189755
<i>C. gracilipes</i>	CA1348	Indonesia	MH497520	MK190025	MK189608	MK189517	MK190200	MK190109	MK189779	MK189942	MK189695
		?	NC_024751	NC_024751	NC_024751	NC_024751	NC_024751	NC_024751	NC_024751	NC_024751	FN995565 FN995481
<i>C. gracilirostris</i>	CA1678	Australia	MK189900	MK190060	MK189650	MK189559	MK190242	MK190151	MK189821	MK189982	MK189731
<i>C. leucosticta</i>	CA1912	Japan	MH497556	MK190089	MK189679	MK189588	MK190271	MK190180	MK189850	MK190006	MK189759
<i>C. lobocensis</i>	CA1056	Palau	MK189854	MK190009	MK189592	MK189501	MK190184	MK190093	MK189763	MK189929	MK189681
	CA1554		MK189891		MK189640	MK189549	MK190232	MK190141	MK189811	MK189972	
<i>C. longicarpus</i>	CA1557	New Caledonia	MK189892	MK190051	MK189641	MK189550	MK190233	MK190142	MK189812	MK189973	MK189723
	CA1599		MK189895	MK190054	MK189644	MK189553	MK190236	MK190145	MK189815	MK189976	MK189725
	CA1611		MK189897	MK190056	MK189646	MK189555	MK190238	MK190147	MK189817	MK189978	MK189727
<i>C. macrodentata</i>	CA1374	Vanuatu	MK189784	MK190030	MK189613	MK189522	MK190205	MK190114	MK189784	MK189947	MK189700
	CA1375		MK189785	MK190031	MK189614	MK189523	MK190206	MK190115	MK189785	MK189948	MK189701
<i>C. meridionalis</i>	CA1585	New Caledonia	MK189814	MK190053	MK189643	MK189552	MK190235	MK190144	MK189814	MK189975	
	CA1620		MK189818	MK190057	MK189647	MK189556	MK190239	MK190148	MK189818	MK189979	MK189728
<i>C. mertoni</i>	CA1505	Solomon Islands	MG707141	MK190041	MK189627	MK189536	MK190219	MK190128	MK189798	MK189959	MK189713
	CA1506		MG707142	MK190042	MK189628	MK189537	MK190220	MK190129	MK189799	MK189960	

<i>C. multidentata</i>	CA1545	Aquarium trade (Taiwan)	MK189809		MK189638	MK189547	MK190230	MK190139	MK189809	MK189970	MK189721
	CA1546		MK189810		MK189639	MK189548	MK190231	MK190140	MK189810	MK189971	MK189722
<i>C. natalensis</i>	CA1343	Comoros	MH644416	MK190023	MK189606	MK189515	MK190198	MK190107	MK189777	MK189940	MK189693
<i>C. novaecaledoniae</i>	CA1789	New Caledonia	MK189838	MK190077	MK189667	MK189576	MK190259	MK190168	MK189838	MK189998	MK189747
	CA1792		MK189839	MK190078	MK189668	MK189577	MK190260	MK190169	MK189839	MK189999	MK189748
<i>C. papuana</i>	CA1286	Solomon Islands	MK189775	MK190021	MK189604	MK189513	MK190196	MK190105	MK189775	MK189938	MK189691
	CA1361		MK189781	MK190027	MK189610	MK189519	MK190202	MK190111	MK189781	MK189944	MK189697
<i>C. similis</i>	CA1854	Seychelles	MK189845	MK190084	MK189674	MK189583	MK190266	MK190175	MK189845	MK190003	MK189754
<i>C. simoni</i>	CA1831	Sri Lanka	MK189842	MK190081	MK189671	MK189580	MK190263	MK190172	MK189842	MK190000	MK189751
	CA1833		MK189843	MK190082	MK189672	MK189581	MK190264	MK190173	MK189843	MK190001	MK189752
<i>C. sp. 1 Solomon</i>	CA1326	Solomon Islands	MK189776	MK190022	MK189605	MK189514	MK190197	MK190106	MK189776	MK189939	MK189692
	CA1499		MK189796	MK190039	MK189625	MK189534	MK190217	MK190126	MK189796	MK189957	MK189712
<i>C. sp. 2 Solomon</i>	CA1285	Solomon Islands	MK189774	MK190020	MK189603	MK189512	MK190195	MK190104	MK189774	MK189937	
	CA1515		MK189803	MK190045	MK189632	MK189541	MK190224	MK190133	MK189803	MK189964	
<i>C. sp. 3 Solomon</i>	CA1685	Australia	MK189822	MK190061	MK189651	MK189560	MK190243	MK190152	MK189822	MK189983	
	CA1240	Indonesia	MK189770	MK190016	MK189599	MK189508	MK190191	MK190100	MK189770	MK189933	MK189687
	CA1282	Solomon Islands	MK189773	MK190019	MK189602	MK189511	MK190194	MK190103	MK189773	MK189936	MK189690
<i>C. sp. Malanda</i>	CA1736	Australia	MK189832	MK190071	MK189661	MK189570	MK190253	MK190162	MK189832	MK189992	MK189741

	CA1737		MK189833	MK190072	MK189662	MK189571	MK190254	MK190163	MK189833	MK189993	MK189742
<i>C. sp. NT nilotica</i>	CA1721	Australia	MK189827	MK190066	MK189656	MK189565	MK190248	MK190157	MK189827		MK189736
		Australia	NC_030219	NC_030219	NC_030219	NC_030219	NC_030219	NC_030219	NC_030219		
	CA1103	Indonesia	MK189764	MK190010	MK189593	MK189502	MK190185	MK190094	MK189764		
<i>C. sp. Santo</i>	CA1132	Vanuatu	MK189766	MK190012	MK189595	MK189504	MK190187	MK190096	MK189766		MK189683
<i>C. sp. WA4</i>	CA1743	Australia		MK190073	MK189663	MK189572	MK190255	MK190164	MK189834	MK189994	MK189743
	CA1744		MK189835	MK190074	MK189664	MK189573	MK190256	MK190165	MK189835	MK189995	MK189744
<i>C. typus</i>	CA1568	New Caledonia	MK189813	MK190052	MK189642	MK189551	MK190234	MK190143	MK189813	MK189974	MK189724
	CA1524	Solomon Islands	MK189808	MK190050	MK189637	MK189546	MK190229	MK190138	MK189808	MK189969	MK189720
<i>C. variabilirostris</i>	CA1404	Micronesia	MK189790	MK190035	MK189619	MK189528	MK190211	MK190120	MK189790		MK189706
	CA1249	Vanuatu	MK189771	MK190017	MK189600	MK189509	MK190192	MK190101	MK189771	MK189934	MK189688
	CA1435		MK189794	MK190037	MK189623	MK189532	MK190215	MK190124	MK189794	MK189955	MK189710
<i>C. variabilis</i>	CA1205	Palau	MK189768	MK190014	MK189597	MK189506	MK190189	MK190098	MK189768	MK189931	MK189685
<i>C. wilkinsi</i>	CA1746	Australia	MK189836	MK190075	MK189665	MK189574	MK190257	MK190166	MK189836	MK189996	MK189745
	CA1747		MK189837	MK190076	MK189666	MK189575	MK190258	MK190167	MK189837	MK189997	MK189746
<i>C. zebra</i>	CA1731	Australia	MK189830	MK190069	MK189659	MK189568	MK190251	MK190160	MK189830	MK189990	MK189739
	CA1732		MK189831	MK190070	MK189660	MK189569	MK190252	MK190161	MK189831	MK189991	MK189740
<i>C. zeylanica</i>	CA1835	Sri Lanka	MK189844	MK190083	MK189673	MK189582	MK190265	MK190174	MK189844	MK190002	MK189753
<i>Elephantis jaggeri</i>	CA1160	Madagascar	KF468795	MK190013	MK189596	MK189505	MK190188	MK190097	MK189767		MK189684
<i>Halocaridina rubra</i>		Hawaii	NC_008413	NC_008413	NC_008413	NC_008413	NC_008413	NC_008413	NC_008413	FN995585	FN995500

<i>Macrobrachium bullatum</i>	Australia	NC_027602	NC_027602	NC_027602	NC_027602	NC_027602	NC_027602	NC_027602	AY374147	
<i>Neocaridina denticulata</i>	China	NC_023823	NC_023823	NC_023823	NC_023823	NC_023823	NC_023823	NC_023823	FN995598	FN995513
<i>Paratya australiensis</i>	Australia	NC_027603	NC_027603	NC_027603	NC_027603	NC_027603	NC_027603	NC_027603	FN995610	FN995525
<i>Paratya caledonica</i>	CA1800	New Caledonia	MK189840	MK190079	MK189669	MK189578	MK190261	MK190170	MK189840	MK189749
	CA1801		MK189841	MK190080	MK189670	MK189579	MK190262	MK190171	MK189841	MK189750

Table 2

Marker	Primers	Sequence	PCR protocol	Reference
COI (~640 bp)	LCOI1490mod HCO2198mod	TGTCAACTAATCATAAAGATATTGG GGGTGTCCAAAAAACCAAAATAA	Initial denaturation 94°C for 4 min, 45 cycles of 94°C for 30s, 48°C for 30s, 72°C for 40s, final extension 72°C for 7min.	Modified from Folmer et al., 1994
16S rRNA (~500 bp)	16Sar-Lmod 16Sbmod	TACTTCTGCCTGTTTATCAAAAA GGTCTGAACTCAAATCATGTAAA	Initial denaturation 94°C for 4 min, 45 cycles of 94°C for 30s, 55°C for 30s, 72°C for 40s, final extension 72°C for 7min.	Modified from Palumbi, 1996
Mitochondrial genome fragment (COII, ATP8, ATP6, COIII, ND3) (~3,200 bp)	tRNA- Leu1540F tRNA- Glu4760R	AAAATGGCAGATCATTGCATAG ACCTTTAGTTTTACAATGAAAATG	Initial denaturation 94°C for 30s, 60 cycles of 94°C for 20s, 50°C for 30s, 65°C for 15min, final extension 65°C for 15min.	This study

28S rRNA (~1,090 bp)	28S-F-Cru 28S-R-Met	ACCCGCCTAATTTAAGCATAT GCTATCCTGAGGGAAACTTC	Initial denaturation 94°C for 3min, 35 cycles of 94°C for 30s, 60°C to 52°C (-1°C/cycle) for 30s, 72°C for 2min, final extension 72°C for 5min.	von Rintelen et al., 2012
Histone 3 (328 bp)	H3F H3R	ATGGCTCGTACCAAGCAGACVGC ATATCCTTRGGCATRATRGTGAC	Initial denaturation 94°C for 3min, 35 cycles of 94°C for 30s, 50°C for 60s, 72°C for 60s, final extension 72°C for 5min.	Colgan et al., 2000

Table 3

Partition	Substitution Model	Partition Finder subset
16S	HKY+I+G	1
COI position 1	SYM+I+G	2
COI position 2	F81+I	3
COI position 3	GTR+G	4
COII position 1	SYM+I+G	5
COII position 2	GTR+I+G	6
COII position 3	GTR+I+G	7
ATP8 position 1	HKY+I+G	1
ATP8 position 2	GTR+G	8
ATP8 position 3	GTR+I+G	7
ATP6 position 1	GTR+I+G	9
ATP6 position 2	SYM+G	10
ATP6 position 3	GTR+I+G	9
COIII position 1	SYM+I+G	11
COIII position 2	GTR+I+G	6
COIII position 3	GTR+I+G	7
ND3 position 1	SYM+I+G	5
ND3 position 2	GTR+G	8
ND3 position 3	GTR+I+G	7
28S	GTR+I+G	12
H3 position 1	K80+I	13
H3 position 2	K80+I	13
H3 position 3	HKY+G	14