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Molecular phylogeny of hinge-beak shrimps (Decapoda: Caridea: *Rhynchocinetes* and *Cinetorhynchus*) and allies: a formal test of familiar and generic monophyly using a multilocus phylogeny

J. ANTONIO BAEZA[1,2,3]*, RAYMOND T. BAUER[4], JUNJI OKUNO[5] and MARTIN THIEL[3,6]

[1]*Department of Biological Sciences, Clemson University, 132 Long Hall, Clemson, South Carolina 29634, USA*
[2]*Smithsonian Marine Station at Fort Pierce, 701 Seaway Drive, Fort Pierce, Florida 34949, USA*
[3]*Departamento de Biología Marina, Facultad de Ciencias del Mar, Universidad Católica del Norte, Larrondo 1281, Coquimbo, Chile*
[4]*Department of Biology, University of Louisiana at Lafayette, P.O. Box 42451, Lafayette, Louisiana, USA*
[5]*Coastal Branch of Natural History Museum and Institute, Chiba, 123 Yoshio, Katsuura, Chiba, 299-5242, Japan*
[6]*Centro de Estudios Avanzados en Zonas Aridas CEAZA, Coquimbo, Chile*

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The Rhynchocinetidae ('hinge-beak' shrimps) is a family of marine caridean decapods with considerable variation in sexual dimorphism, male weaponry, mating tactics, and sexual systems. Thus, this group is an excellent model with which to analyse the evolution of these important characteristics, which are of interest not only in shrimps specifically but also in animal taxa in general. Yet, there exists no phylogenetic hypothesis, either molecular or morphological, for this taxon against which to test either the evolution of behavioural traits within the Rhynchocinetidae or its genealogical relationships with other caridean taxa. In this study, we tested (1) hypotheses on the phylogenetic relationships of rhynchocinetid shrimps, and (2) the efficacy of different (one-, two-, and three-phase) methods to generate a reliable phylogeny. Total genomic DNA was extracted from tissue samples taken from 17 species of Rhynchocinetidae and five other species currently or previously assigned to the same superfamily (Nematocarcinoidea); six species from other superfamilies were used as outgroups. Sequences from two nuclear genes (*H3* and *Enolase*) and one mitochondrial gene (*12S*) were used to construct phylogenies. One-phase phylogenetic analyses (SATé-II) and classical two- and three-phase phylogenetic analyses were employed, using both maximum likelihood and Bayesian inference methods. Both a two-gene data set (*H3* and *Enolase*) and a three-gene data set (*H3*, *Enolase*, *12S*) were utilized to explore the relationships amongst the targeted species. These analyses showed that the superfamily Nematocarcinoidea, as currently accepted, is polyphyletic. Furthermore, the two major clades recognized by the SATé-II analysis are clearly concordant with the genera *Rhynchocinetes* and *Cinetorhynchus*, which are currently recognized in the morphological-based classification (implicit phylogeny) as composing the family Rhynchocinetidae. The SATé-II method is considered superior to the other phylogenetic analyses employed, which failed to recognize these two major clades. Studies using more genes and a more complete species data set are needed to test yet unresolved inter- and intrafamilial systematic and evolutionary questions about this remarkable clade of caridean shrimps.

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*Corresponding author. E-mail: baeza.antonio@gmail.com

INTRODUCTION

Within the infraorder Caridea Dana, 1852, one of the most species-rich and diverse decapod crustacean clades (De Grave *et al*., 2009), the shrimp genera *Cinetorhynchus* Holthuis, 1995, and *Rhynchocinetes* H. Milne Edwards, 1837, exhibit remarkable ecological and behavioural diversity (Bauer, 2004) (Figs 1, 2). In various species the males are larger than females and have enlarged chelipeds, which they use during intrasexual competition for receptive females (Correa *et al*., 2003; Thiel, Chak & Dumont, 2010) (Fig. 1A). Different male morphotypes (i.e. dominant 'robustus' and sneaker 'typus') have been described for at least three species, *Rhynchocinetes typus* H. Milne Edwards, 1837 (see Correa *et al*., 2003), *Rhynchocinetes brucei* Okuno, 1994 (see Thiel *et al*., 2010), and *Rhynchocinetes durbanensis* Gordon, 1936 (S. Prakash & T. Subramoniam, pers. comm.). Dominant 'robustus' males monopolize females during mating interactions, whereas smaller 'typus' male morphotypes with less developed chelipeds sneak mating opportunities when receptive females are released during combats amongst dominant 'robustus' males (Thiel & Hinojosa, 2003). At present, species from the genus *Rhynchocinetes* are the only marine shrimps in which alternative mating tactics have been reported. Nonetheless, it is likely that these alternative mating strategies are found in other species within the family Rhynchocinetidae Ortmann, 1890, in which dominant males have well-developed weapons.

Interestingly, a recent study revealed the absence of dominant males and the presence of protandric hermaphroditism in one species, *Rhynchocinetes uritai* Kubo, 1942 (see Bauer & Thiel, 2011). Limited observations on mating in *R. uritai* indicated a 'pure searching' or promiscuous mating system in which males do not defend or fight over females, before a very brief copulation. This shows that there is a high variability of sexual and mating systems within the family, and phylogenetic studies are needed to confirm whether these systems have evolved multiple times independently or not. Furthermore, phylogenetic studies will help to understand the environmental conditions favouring and/or constraining different sexual and mating systems.

In several species from the related genus *Cinetorhynchus* large males also have strongly developed chelipeds, albeit of different morphology than in the genus *Rhynchocinetes* (see Okuno, 1994, 1997, 2009; Okuno & Tachikawa, 1997). This leads to several interesting questions of evolutionary significance regarding the genera *Cinetorhynchus* and *Rhynchocinetes*, which have been the focus of an increasing number of studies dealing with systematics (Okuno, 1994, 1997, 2009; Okuno & Tachikawa, 1997; Holthuis, 1995; Chace, 1997), fertilization dynamics and developmental biology (Barros, Dupré & Viveros, 1986; Bustamante *et al*., 2001; Dupré, Flores & Palma, 2008; Dupré & Barros, 2011), population and community ecology (Caillaux & Stotz, 2003; Ory *et al*., 2012), and behavioural and evolutionary ecology (Correa *et al*., 2003; van Son & Thiel, 2006; Dennenmoser & Thiel, 2007, 2008; Thiel *et al*., 2010; Bauer & Thiel, 2011). Unfortunately, no phylogenies, either morphological or molecular, have been published thus far. This lack of phylogenetic knowledge is limiting our understanding of e.g. the evolution of sexual and social systems, amongst other topics, in these shrimps as well as in other marine invertebrates.

A phylogeny should also help to answer long-standing controversies about various systematic questions. For instance, a phylogeny would help to evaluate Yaldwyn's (1960) versus Holthuis's (1993) and Okuno's (1997) proposals regarding the number of genera constituting the family Rhynchocinetidae. Yaldwyn (1960) proposed that the species of *Rhynchocinetes*, *Lipkius* Yaldwyn, 1960, and *Eugonatonotus* Schmitt, 1926, comprised the family Rhynchocinetidae. Later, however, Holthuis (1995) and Okuno (1997) suggested that only the species of *Rhynchocinetes* (and *Cinetorhynchus*, see below) should be placed in the Rhynchocinetidae separately from the genus *Lipkius*. This latter genus was included, amongst others, in the family Nematocarcinidae Smith, 1884. In turn, the genus *Eugonatonotus* was placed in its own family, the Eugonatonotidae Chace, 1937.

Furthermore, the status of the genus *Cinetorhynchus*, which would include species of *Rhynchocinetes* that have three teeth at the median carina of the carapace, an indistinct rostral articulation, no supraorbital spine, and one spine at the posterolateral margin of fifth abdominal somite, needs to be clarified (Fig. 1B–H; Okuno, 1997, 2009). Holthuis (1995) erected the subgenus *Cinetorhynchus* that was then raised to generic status by Okuno (1997). Lastly, a third, more comprehensive, unresolved question is whether or not the superfamily Nematocarcinoidea Smith, 1884, constitutes a natural entity. Based on morphological similarity of adult individuals, the superfamily Nematocarcinoidea currently contains the families Nematocarcinidae, Eugonatonotidae, and Xiphocarididae Ortmann, 1895, in addition to the family Rhynchocinetidae (see Martin & Davis, 2001; De Grave & Fransen, 2011). Nonetheless, recent molecular phylogenetic analyses do not support this grouping as natural as the species from the different families comprising this superfamily do not cluster together into a single well-supported monophyletic clade (Bracken, De Grave & Felder, 2009; Li *et al*., 2011). Table 1 shows a more detailed view of the historical taxonomical ordering within the family Rhynchocinetidae and between this and other closely related families. Certainly, studies on the phylogenetics of caridean shrimps are needed to improve

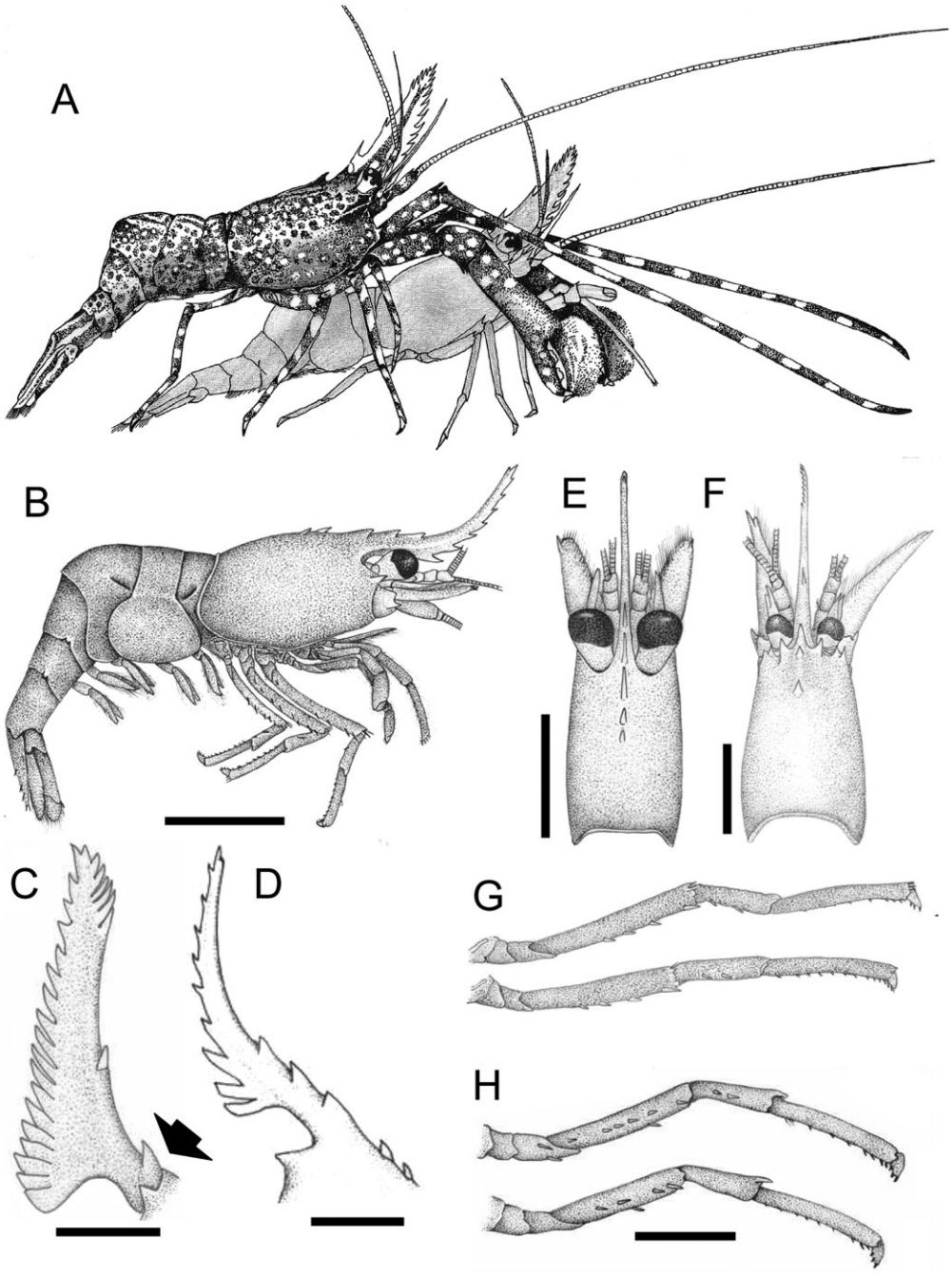

Figure 1. Some morphological characters of shrimps from the genera *Rhynchocinetes* and *Cinetorhynchus*. A, 'cage' position during mating in the shrimp *Rhynchocinetes typus*, the only species of marine caridean shrimp for which alternative mating tactics have been demonstrated so far. Notice the well-developed third maxillipeds and chelipeds characteristic of the 'robustus' male morphotype. B, habitus (view of the entire animal) of the hinged-beak shrimp genus *Cinetorhynchus*. C, lateral view of the rostrum of *R. typus*. Notice the articulation (arrow) of the rostrum with the remainder of the carapace. D, lateral view of the rostrum of *Cinetorhynchus rigens*. Notice the indistinct articulation between the carapace and the rostrum (compared with *Rhynchocinetes*). E, dorsal view of the carapace in *C. rigens*. Notice the three teeth at the median carina of the carapace and the absence of a supraorbital spine. F, dorsal view of the carapace in *R. typus*. Notice the two acute teeth at the median carina of the carapace and the supraorbital spine. G, lateral view of the fourth and fifth pereopods of *R. typus*. Notice the presence of only one row of meral spines on these pereopods. H, lateral view of the fourth and fifth pereopods of *C. rigens*. Notice the presence of two rows of meral spines. A from Correa *et al*. (2003); B–H from de Melo (2007).

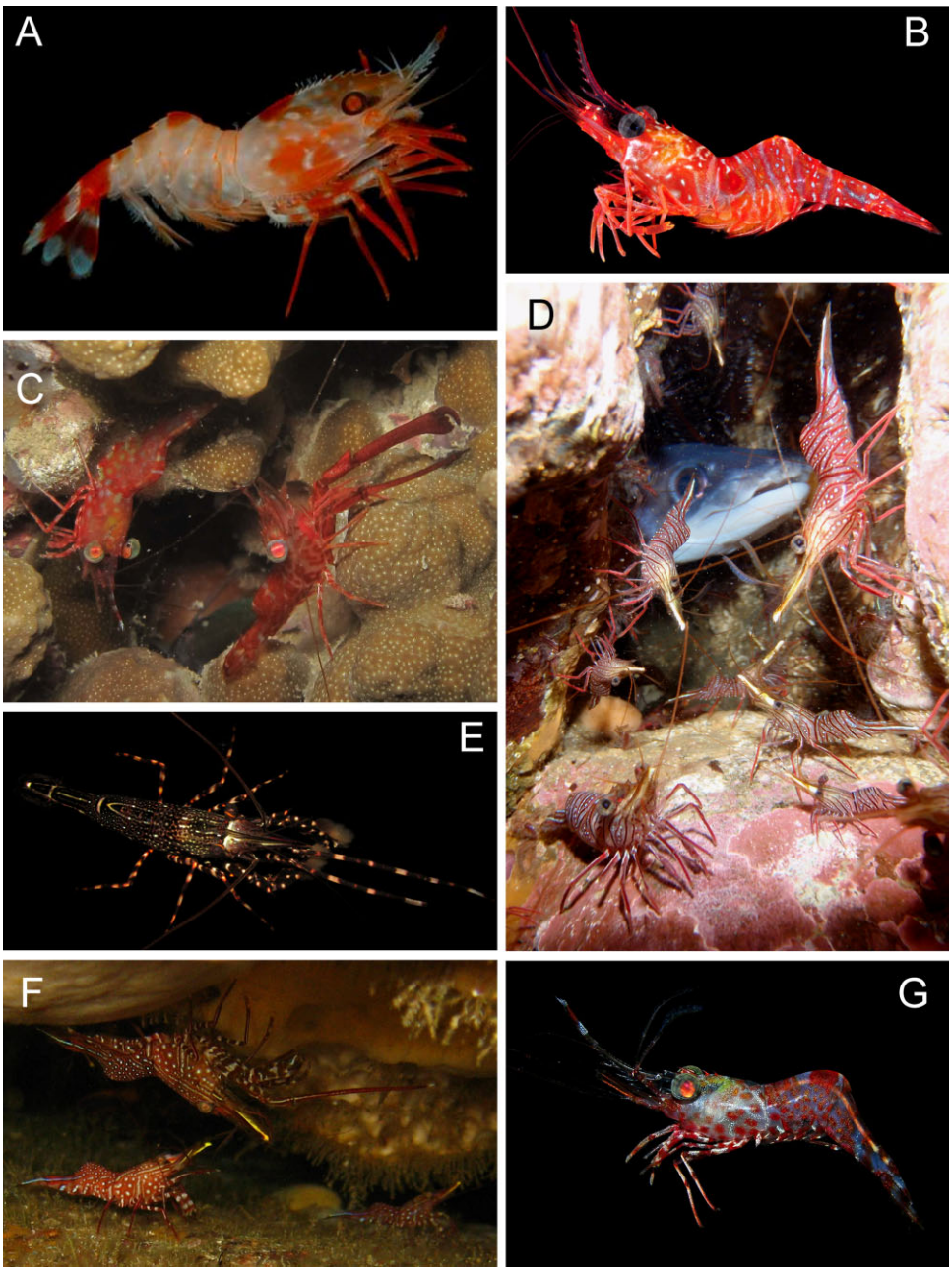

Figure 2. Habitus and morphological diversity of hinged-beak shrimps from the genera *Rhynchocinetes* and *Cinetorhynchus* and allied species in the superfamily Nematocarcinoidea. A, lateral view of *Eugonatonotus crassus* (Eugonatonotidae) (photo credit: Charles Bump, SERT). B, lateral view of *Cinetorhynchus* cf. *maningi* (photo credit: Arthur Anker). C, pair of *Cinetorhynchus hendersoni in situ* (photo credit: Nicolas Ory). Notice the male on the right with extremely elongated pereopods. D, large aggregation of *Rhynchocinetes uritai* in Japan (photo credit: Martin Thiel). E, dorsal view of a 'robustus' male of *Rhynchocinetes typus* (photo credit: Ivan Hinojosa). Notice the elongated third maxillipeds and the dense setae in the chelipeds. F, small aggregation of *Rhynchocinetes serratus* (photo credit: Ivan Hinojosa). In the male perched on the roof of the crevice, notice the elongated third maxillipeds and the absence of dense setae on the chelipeds. G, lateral view of *Cinetorhynchus* cf. *rigens* (photo credit: Arthur Anker).

Table 1. Different hypotheses of phylogenetic relationships between species within the family Rhynchocinetidae and related caridean taxa proposed during recent decades. All of the hypotheses are based on morphological characters

Holthuis (1955)
Superfamily Oplophoroida Alcock, 1901
Family Nematocarcinidae Smith, 1884
Nematocarcinus A. Milne-Edwards, 1881
Family Atyidae Dana, 1852
Xiphocaris von Martens, 1872
Superfamily Bresilioida Holthuis, 1955
Family Eugonatonotidae Chace, 1937
Eugonatonotus Schmitt, 1926
Family Rhynchocinetidae Ortmann, 1890
Rhynchocinetes H. Milne Edwards, 1837
Yaldwyn (1960)
Superfamily Bresilioida Holthuis, 1955
Family Rhynchocinetidae Ortmann, 1890
Rhynchocinetes H. Milne Edwards, 1837
Lipkius Yaldwyn, 1960
Eugonatonotus Schmitt, 1926
Thompson (1966, 1967)
Superfamily Bresilioidea Calman, 1896
Family Nematocarcinidae Smith, 1884
Nematocarcinus A. Milne-Edwards, 1881
Lipkius Yaldwyn, 1960
Superfamily undefined
Family Rhynchocinetidae Ortmann, 1890
Rhynchocinetes H. Milne Edwards, 1837
Superfamily Oplophoroidea
Family Eugonatonotidae Chace, 1937
Eugonatonotus Schmitt, 1926
Christoffersen (1990)
Superfamily Eugonatonotoidea Chace, 1937
Family Eugonatonotidae Chace, 1937
Eugonatonotus Schmitt, 1926
Superfamily Palaeomonoidea Rafinesque, 1815
Family Rhynchocinetidae Ortmann, 1890
Rhynchocinetes H. Milne Edwards, 1837
Superfamily Nematocarcinoidea Smith, 1884
Family Nematocarcinidae, Smith, 1884
Lipkius Yaldwyn 1960
Chace (1992)
Superfamily Nematocarcinoidea Smith, 1884
Family Eugonatonotidae Chace, 1937
Family Nematocarcinidae, Smith, 1884
Family Rhynchocinetidae Ortmann, 1890
Family Xiphocarididae Ortmann, 1895
Holthuis (1995)
Family Rhynchocinetidae Ortmann, 1890
Rhynchocinetes H. Milne Edwards, 1837
Subgenus *Rhynchocinetes* H. Milne Edwards, 1837
Subgenus *Cinetorhynchus* Holthuis, 1995
Okuno (1997)
Family Rhynchocinetidae Ortmann, 1890
Rhynchocinetes H. Milne Edwards, 1837
Cinetorhynchus Holthuis, 1995
Martin & Davis (2001)
Superfamily Nematocarcinoidea Smith, 1884
Family Eugonatonotidae Chace, 1937
Family Nematocarcinidae, Smith, 1884
Family Rhynchocinetidae Ortmann, 1890
Family Xiphocarididae Ortmann, 1895
De Grave & Fransen (2011)
Superfamily Nematocarcinoidea Smith, 1884
Family Eugonatonotidae Chace, 1937
Eugonatonotus Schmitt, 1926
Family Nematocarcinidae, Smith, 1884
Lipkius Yaldwyn, 1960
Nematocarcinus A. Milne-Edwards, 1881
Family Rhynchocinetidae Ortmann, 1890
Cinetorhynchus Holthuis, 1995
Rhynchocinetes H. Milne Edwards, 1837
Family Xiphocarididae Ortmann, 1895
Xiphocaris von Martens, 1872

our understanding of morphological and life history evolution within this crustacean clade.

In this study, we were particularly interested in elucidating the phylogenetic relationships amongst shrimps from the genera *Cinetorhynchus* and *Rhynchocinetes* as well as other related genera comprising the superfamily Nematocarcinoidea. Disentangling the phylogenetic relationships amongst the species above is the first step to understanding the evolution of impressive reproductive behaviours (i.e. alternative mating tactics) and lability of sexual systems in caridean shrimps. Furthermore, in this study we used caridean shrimps from the genera *Cinetorhynchus* and *Rhynchocinetes* as a model to explore the effect of different methods of phylogenetic inference on the accuracy and robustness of the reconstructed phylogenies. Specifically, the second main goal of this study was to use the 'one-phase' simultaneous alignment and tree estimation (SATé-II) method for the simultaneous generation of multiple alignments and phylogenetic trees and exploring any dissimilarity between this new 'one-phase' method and other 'classical' two- and three-step phylogenetic inference methods (see below).

Of paramount importance to all evolutionary studies that profit from molecular phylogenies is the accuracy and robustness of the reconstructed phylogenies. Inferences from evolutionary studies might be seriously flawed when phylogenies are either inaccurate or imprecise. Importantly, the occurrence of low-quality alignments of molecular sequence data sets is one of the various causes of systematic error that can mislead phylogenetic methods (Thompson, Plewniak & Poch, 1999; Edgar, 2004a, b) and that have received considerable attention during recent years (Fleissner, Metzler & von Haeseler, 2005; Redelings & Suchard, 2005; Suchard & Redelings, 2006; Novák *et al*., 2008; Liu *et al*., 2009a, b, 2011; Varón, Vinh & Wheeler, 2010). The precise and accurate alignment of a set of related sequences is usually complex, time consuming, and even 'idiosyncratic', in particular when the set of sequences studied are highly divergent and/or include high rates of insertions and deletions (i.e. indels; Liu *et al*., 2009a). Furthermore, automated alignments might require additional manual realignment. The latter is error-prone because of limitations in the alignment software and constraints in our own cognitive abilities. Manual realignment also introduces unspecified realignment criteria that are usually not reported and thus, are impossible to replicate by future studies (but see Anker & Baeza, 2012). The problem of aligning complicated sequences has been intensively studied during recent decades, resulting in considerable improvements in sequence alignment algorithms (Edgar, 2004a, b; Katoh & Standley, 2013). Still, alignment estimation is difficult when the studied sequences have

many substitutions and indels, and thus, phylogenetic reconstruction may still be inaccurate (Liu *et al*., 2009b).

Considering the above, phylogeny estimations from molecular sequences currently include two or more phases: (1) alignment estimation (that may be inaccurate) and (2) phylogenetic inference (Liu *et al*., 2009b). The accuracy and robustness of reconstructed phylogenies may be compromised if the base alignment is inaccurate. Additionally, when the aligned sequences contain hard-to-align DNA regions, unreliable information might be obtained. Thus, a third step [in between (1) and (2) above] is to identify and eliminate highly divergent and poorly aligned gene segments and omit them from phylogenetic analyses (Castresana, 2000). Although this additional step has been shown to improve the accuracy of phylogenetic estimation (see Castresana, 2000; Talavera & Castresana, 2007), it also discards phylogenetically useful information contained in the hard-to-align regions (Dessimoz & Gil, 2010). Furthermore, such regions are typically aligned differently by different programs, and phylogenies estimated based on these different alignments can differ considerably (Mugridge *et al*., 2000; Wong, Suchard & Huelsenbeck, 2008).

An alternative philosophy/technique to solve the problems of alignment inaccuracy and phylogenetic inference is to estimate trees directly from unaligned sequences (Fleissner *et al*., 2005; Redelings & Suchard, 2005; Suchard & Redelings, 2006; Novák *et al*., 2008; Liu *et al*., 2009a, b, 2011; Varón *et al*., 2010). Current methods that simultaneously estimate sequence alignments and phylogenetic trees are classified into two categories: (1) nonparametric methods that resemble maximum parsimony (i.e. POY and POY* – Varón *et al*., 2010), and (2) methods based on parametric statistical models of sequence evolution that include substitution and indel events (Fleissner *et al*., 2005; Redelings & Suchard, 2005; Suchard & Redelings, 2006; Novák *et al*., 2008; Liu *et al*., 2009a, b, 2011). Unfortunately, methods that resemble maximum parsimony suffer from substantial limitations, e.g. the computational burden is extraordinary and POY and POY* have not produced trees more accurate than those of the best two-phase methods (Ogden & Rosenberg, 2005; see also Liu *et al*., 2009a, b, 2011). Methods based on parametric statistical models of sequence evolution include, amongst a few others, BAli-Phy (Redelings & Suchard, 2005; Suchard & Redelings, 2006), StatAlign (Novák *et al*., 2008), ALIFRITZ (Fleissner *et al*., 2005), and SATé-II (Liu *et al*., 2009a, 2011). Recent studies have shown that the computational burden of some of these methods is also substantial (Liu *et al*., 2009a, 2011). Perhaps more importantly, amongst the different methods above, recent simulation and empirical studies have shown that only SATé-II, a maximum likelihood method that treats gaps as missing data, outperforms one-phase and other two-phase (parametric and parsimony-like) methods and is capable of coestimating accurate trees and alignments in relatively short periods of time (Liu *et al*., 2011).

In the present study, a molecular phylogeny of the genera *Cinetorhynchus* and *Rhynchocinetes* was generated using classical two- and three-phase methods and the most recent one-phase maximum likelihood SATé-II method for the simultaneous generation of multiple alignments and phylogenetic trees. We compared the different methods above to explore putative inaccuracies inherent to two- and three-phase methods. Considering the superior performance of the SATé-II method, any dissimilarity in tree inference amongst the latter and classical two- and three-phase methods is expected to reveal inaccuracies inherent to these two- and three-phase methods. We then used the resulting phylogenetic inferences to answer the taxonomic questions posed above. Specifically, we formally tested hypotheses on (1) the monophyly of the superfamily Nematocarcinoidea, (2) the monophyly of the family Rhynchocinetidae, and (3) the natural segregation of the species within family Rhynchocinetidae in the genera *Cinetorhynchus* and *Rhynchocinetes*. It was predicted that a molecular phylogeny of the species included within the different genera and families above should segregate the species into well-supported, clade-specific monophyletic clades. For instance, if the family Rhynchocinetidae is a naturally valid clade, then, a molecular phylogeny should segregate species of *Cinetorhynchus* and *Rhynchocinetes* from species pertaining to other nematocarcinoid genera. We formally examined the predictions above using Bayesian hypothesis testing.

MATERIAL AND METHODS

Taxon sampling, ingroups, and outgroup terminals

A total of 12 species in the genera *Rhynchocinetes* (seven species) and *Cinetorhynchus* (five species) were included as ingroup terminals in the molecular analyses. Five other species, *Eugonatonotus chacei* Chan & Yu, 1991, *Lipkius holthuisi* Yaldwyn, 1960, *Nematocarcinus gracilis* Spence Bate, 1888, *Nematocarcinus tenuipes* Spence Bate, 1888, and *Nematocarcinus* aff. *combensis* Burukovsky, 2000, were also included in the analysis to have representatives of three out of four recognized families (i.e. Eugonatonotidae, Nematocarcinidae, Rhynchocinetidae, and Xiphocarididae) comprising the superfamily Nematocarcinoidea. Lastly, six species from four different families, namely Alpheidae Rafinesque, 1815 (two species from two different genera), Lysmatidae Dana, 1852, Pasiphaeidae Dana, 1852 (two species from one

genus), and Oplophoridae Dana, 1852, were used as outgroup terminals. Most shrimp species were collected by the authors in the field in Chile, Japan, Hong Kong, and Hawaii and Florida (USA), or were obtained from various institutions and colleagues (see Acknowledgements). Immediately after collection, specimens were preserved in 95–99% ethanol. The different species were identified using Kubo (1942), Nomura & Hayashi (1992), Okuno & Takeda (1992), Okuno (1994, 1997), Okuno & Tachikawa, 1997 and the keys of Chace (1972, 1975, 1984, 1997) and Okuno & Tachikawa (1997). For further details of voucher specimens and GenBank accession information, see Table 2.

Altogether, the set of species above was used to reveal the relationship within the family Rhynchocinetidae and amongst the families Rhynchocinetidae, Nematocarcinidae, and Eugonatonotidae, all of the above belonging, according to Martin & Davis (2001), to the superfamily Nematocarcinoidea. We also tested for the main hypotheses of monophyly of the genera *Rhynchocinetes* and *Cinetorhynchus* in the family Rhynchocinetidae (see sections 'Data sets for phylogenetic analyses' and 'Hypothesis testing of monophyletic clades with three-phase phylogenetic inference'). In total, 70 sequences were generated and 15 other sequences were retrieved from GenBank (Table 2).

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from pleopods or abdominal muscle tissue using the QIAGEN DNeasy Blood and Tissue Kit following the manufacturer's protocol. PCR was used to amplify target regions of two nuclear genes [Histone *H3* (328 bp – Colgan *et al*., 1998) and *Enolase* (373 bp – Tsang *et al*., 2011)] and one mitochondrial gene [*12S* ribosomal RNA, abbreviated *12S* (355–382 bp – Mokady *et al*. 1994)]. For amplification of the *12S*, *H3*, and *Enolase* gene segments, we used the primers 12Sf (5′-GAA ACC AGG ATT AGA TAC CC-3′) and 12S1R (5′-AGC GAC GGG CGA TAT GTA C-3′) (Mokady *et al*., 1994 modified from Kocher *et al*., 1989), H3AF (5′-ATG GCT CGT ACC AAG CAG ACV GC-3′) and H3AR (5′-ATA TCC TTR GGC ATR ATR GTG AC-3′) (Colgan *et al*., 1998), and EA2 (5′-AGT TGG CTA TGC AGG ART TYA TGA T-3′) and ES2 (5′-ACC TGG TCG AAT GGR TCY TC-3′) (Tsang *et al*., 2011), respectively.

Standard PCR 25-µl reactions [2.5 µl of 10× Taq buffer, 2 µl of 50 mM $MgCl_2$, 2.5 µl of 10 mM deoxyribonucleotide triphosphates, 2.5 µl each of the two primers (10 mM), 0.625 U Taq, 1.25 µl of 20 mM bovine serum albumin and 8.625 µl double-distilled water] were performed on a Peltier Thermal Cycler (DYAD) under the following conditions: initial denaturation at 95 °C for 5 min followed by 40 cycles of 95 °C for 45 s, 52–57 °C (depending on the species) for 1 min, and 72 °C for 1 min, followed by chain extension at 72 °C for 10 min. PCR products were purified with ExoSapIT (a mixture of exonuclease and shrimp alkali phosphatase, Amersham Pharmacia) and then sent for sequencing with the ABI Big Dye Terminator Mix (Applied Biosystems) to the Laboratory of Analytical Biology of the National Museum of Natural History (LAB – NMNH, Maryland), which is equipped with an ABI Prism 3730xl Genetic Analyzer (Applied Biosystems automated sequencer). All sequences were confirmed by sequencing both strands and a consensus sequence for the two strands was obtained using the software SEQUENCER 4.5 (Gene Codes Corp.) or ALIGNER (CodonCode Corp.).

Data sets for phylogenetic analyses

We used two different sets of sequences (data sets) to infer the relationship within and amongst the different families, genera, and species targeted during this study (see section 'Taxon sampling, ingroups, and outgroup terminals'). The first data set consisted of species for which sequences from the two different nuclear gene fragments (i.e. *Enolase* and *H3*, see section 'Phylogenetic analyses') were available (see Table 2). With this first data set, we tested hypotheses on natural entities (= monophyletic clades) at the superfamily and family level as nuclear genes (compared with mitochondrial genes) are expected to have greater phylogenetic information and power to reveal relationships at the suprafamiliar level (Tsang *et al*., 2011; Li *et al*., 2011; Anker & Baeza, 2012; Baeza, 2013; Baeza & Fuentes, 2013). The first data set comprised a total of 17 species pertaining to the families Rhynchocinetidae, Eugonatonotidae, and Nematocarcinidae, and six species from other caridean families used as outgroup terminals (for details see Table 2).

The second data set consisted of species for which sequences from two nuclear genes (i.e. *Enolase* and *H3*) plus an additional mitochondrial gene (i.e. *12S*) were available (see Table 2). We did not obtain *12S* sequences from a few species we collected. Additionally, a few sequences from the *12S* gene fragment were not available at GenBank from species from which we retrieved *H3* and *Enolase* sequences from the same source (for details see Table 2). With this second data set, we tested hypotheses on natural entities at and below the family level as the addition of the *12S* gene fragment is expected to increase phylogenetic informativeness of the data set to reveal relationships at the infrafamiliar level (Baeza & Fuentes, 2013). The second data set comprised a total of 12 species pertaining to the family Rhynchocinetidae *sensu* Holthuis (1995) and five species from other caridean families used as outgroup terminals (Table 2).

Table 2. Species of the families Rhynchocinetidae, Eugonatonotidae, Nematocarcinidae, and other caridean shrimps used for the molecular phylogenetic reconstruction. GenBank accession numbers (GenBank) are shown for each species. See Discussion for a newly proposed classification scheme

Taxon	Locality	Voucher	GenBank *12S*	GenBank *H3*	GenBank *Enolase*
Rhynchocinetidae					
Cinetorhynchus hendersoni	Lizard Island, Australia	FLMNH UF 20206	KM051057	KM051017	KM051038
Cinetorhynchus hendersoni A	Hawaii, USA	–	KM051058	KM051018	KM051079
Cinetorhynchus hendersoni 'stout' 17	Hawaii, USA	ULLZ 15519	KM051059	KM051019	KM051039
Cinetorhynchus hendersoni 'stout' 19	Hawaii, USA	ULLZ 15521	KM051060	KM051020	KM051040
Cinetorhynchus hendersoni 'slender' 6	Hawaii, USA	ULLZ 15520	KM051061	KM051021	KM051041
Cinetorhynchus hendersoni 'slender' 10	Hawaii. USA	ULLZ 15518	KM051062	KM051022	KM051042
Cinetorhynchus erythrostictus	Okinawa, Japan	ULLZ 15522	KM051063	KM051023	KM051043
Cinetorhynchus reticulatus	Okinawa, Japan	ULLZ 15523	KM051064	KM051024	KM051044
Cinetorhynchus cf. *rigens*	South Caicos, Turk and Caicos	CMNH-ZC 02472	KM051065	KM051025	–
Cinetorhynchus striatus	Okinawa, Japan	FLMNH UF 7191	KM051066	KM051026	–
Cinetorhynchus striatus 2	Okinawa, Japan	ULLZ 15525	KM051067	KM051027	KM051045
Cinetorhynchus striatus 3	Okinawa, Japan	ULLZ 15340	KM051068	KM051028	KM051046
Rhynchocinetes australis	Tazmania, Australia	CU.CC.2014-06-0001	KM051069	KM051029	KM051047
Rhynchocinetes balssi	Juan Fernandez, Chile	CU.CC.2014-06-0003	KM051070	KM051030	KM051048
Rhynchocinetes balssi	New Zealand	NIWA 46549	–	–	KM051049
Rhynchocinetes brucei	Hong Kong, China	CU.CC.2014-06-0004	KM051071	KM051031	KM051050
Rhynchocinetes conspiciocellus	Okinawa, Japan	ULLZ 15524	KM051072	KM051032	KM051051
Rhynchocinetes durbanensis	Okinawa, Japan	ULLZ 15341	KM051073	KM051033	KM051052
Rhynchocinetes typus	Coquimbo, Chile	CU.CC.2014-06-0002	KM051074	KM051034	KM051053
Rhynchocinetes uritai	Shimoda, Japan	ULLZ 15342	KM051075	KM051035	KM051054
Eugonatonotidae					
Eugonatonotus chacei	Taiwan, Republic (Rep.) of China	NTOU M00876	DQ642858	JF346310	JF346274
Nematocarcinidae					
Nematocarcinus gracilis	Taiwan, Rep. of China	NTOU M00725	–	JF346307	JF346271
Nematocarcinus aff. *combensis*	Taiwan, Rep. of China	NTOU M00724	–	JF346305	JF346269
Nematocarcinus tenuipes	Taiwan, Rep. of China	NTOU M01003	–	JF346316	JF346280
Lipkius holthuisi 1	New Zealand	NIWA 635981-1	KM051076	KM051036	KM051055
Lipkius holthuisi 2	New Zealand	NIWA 635981-2	–	KM051037	KM051056
Outgroups					
Alpheidae					
Betaeus lilianae	Mar del Plata, Argentina	OUMNH.ZC.2012-01-0035	KM051077	JX010770	KF178881
Nennalpheus sibogae	Mayotte, south-west Indian Ocean	FLMNH UF 13629	KM051078	JX010775	KF178883
Lysmatidae					
Lysmata wurdemanni, G-TX	Texas, USA	UMML 32.9607	KC962182	KF178854	KF178876
Oplophoridae					
Systellaspis pellucida	Taiwan, Rep. of China	NTOU M01001	–	JF346319	JF346283
Pasiphaeidae					
Pasiphaea japonica	Taiwan, Rep. of China	NTOU M00999	–	JF346331	JF346295
Pasiphaea levicarinata	Taiwan, Rep. of China	NTOU M01000	–	JF346332	JF346296

Deposition institution abbreviations: CMNH-ZC, Chiba Museum of Natural History Zoological Collection (Natural History Museum and Institute, Chiba, Japan); CU.CC, Clemson University Crustacean Collection (Clemson University, Clemson, South Carolina, USA); FLMNH, Florida Museum of Natural History (University of Florida, Gainesville, Florida, USA); NIWA, National Institute of Water and Atmospheric Research (New Zealand); NTOU, National Taiwan Ocean University (Keelung, Taiwan); ULLZ, University of Louisiana at Lafayette Zoological Collection (University of Louisiana, Lafayette, LA, USA); UMML, University of Miami Marine Laboratories (University of Miami, Rosenthiel School of Marine Science, Miami, Florida, USA). G-TX = Galveston, *H3*, Histone; Texas. – = not available

Phylogenetic analyses

We used one-phase phylogenetic analyses in SATé-II and classical two- and three-phase phylogenetic analyses to explore the relationship amongst the targeted species with the two different data sets. The superior performance of SATé-II compared with two- and three-phase phylogenetic analyses has been previously documented (Liu *et al*., 2009b, 2011). However, we cannot discard the possibility that any putative difference observed amongst one-, two-, and three-phase analyses is because, at least partially, of (1) the effect of differences in sequence alignments resulting from using different alignment software packages [e.g. MAFFT v. 7 (Katoh & Standley, 2013) used by SATé-II vs. other software such as MUSCLE (Edgar, 2004a, b) regularly employed during two- and three-phase analyses]; (2) the effect of extracting poorly aligned sequence positions with the software GBlocks v. 0.91b (Castresana, 2000) when needed; and/or (3) the effect of the intrinsic algorithm used by the different software products for one-phase and two- and three-phase phylogenetic inference. In this study, we used a series of particular workflows (see below) that included the use of two different alignment software packages (MUSCLE and MAFFT) and the omission or not of highly divergent and poorly aligned positions [i.e. using GBlocks v. 0.91b (Castresana, 2000), see below] when present in the different studied gene segments and data sets to determine whether or not any putative difference observed in phylogenetic trees produced by one-, two-, and three-phase analyses were explained by (1), (2), and/or (3) above. Comparison of tree topography and clade robustness amongst phylogenetic trees retrieved from the different workflows detailed below permitted us to infer whether the superior qualities of SATé-II or other conditions can explain differences between the results. For instance, a monophyletic clade that is well supported in SATé-II but not by two- and three-phase phylogenetic analyses implies that alignments and/or different strategies for the extraction of poorly aligned sequence positions does not account for the observed differences amongst phylogenetic software packages (see below). Thus, any difference between one-phase SATé-II analysis and the other two- and three-phase phylogenetic analyses would be because of the superiority of the former methodology.

Simultaneous estimation of alignments and phylogenetic trees

First, we simultaneously estimated alignments and phylogenetic trees using SATé-II (Liu *et al*., 2009b, 2011) and the two different data sets [i.e. the two nuclear genes data set (hereafter 'two-gene data set') and two nuclear plus one mitochondrial gene data set (hereafter 'three-gene data set')]. SATé-II is a novel and highly accurate method for simultaneously estimating alignments and phylogenetic trees that offers dramatic improvements over two-phase methods and other one-phase methods [i.e. POY (Varón *et al*., 2010), SATé-I (Liu *et al*., 2009b, 2011), BAli-Phy (Redelings & Suchard, 2005; Suchard & Redelings, 2006), StatAlign (Novák *et al*., 2008), and ALIFRITZ (Fleissner *et al*., 2005)] for data sets that are difficult to align, including the partial *12S* gene fragment used in this study (see below).

During the SATé-II analysis, MAFFT 6.717 was used as the aligner (Katoh & Standley, 2013), MUSCLE (Edgar, 2004a, b) was used as the merger, and maximum likelihood trees during each iteration (see below) were created with FastTree 2.1.4 under the general time-reversible + Gamma20 substitution model. We used the SATé-II fast algorithm, the centroid procedure to find the edge that should be broken to create subproblems (the largest total number of taxa in the full data set that will be aligned during iterations), and we conducted a total of 1000 iterations (equivalent to more than 24 h of exploration) to find the 'best' phylogenetic tree. All other settings were those automatically determined by SATé-II (Liu *et al*., 2011). Lastly, we used the 'Extra RAxML Search' postprocessing option to request SATé-II to conduct a final RAxML (Stamatakis, 2006) search on the alignment returned by the SATé-II fast algorithm.

Two-phase phylogenetic analyses

During two-phase phylogenetic analyses, alignment of each set of sequences pertaining to each data set (i.e. the two-gene and three-gene data sets) was conducted using two different software packages; MAFFT v. 7 (Katoh & Standley, 2013) and multiple sequence comparison by log-expectation in MUSCLE (Edgar, 2004a, b) as implemented in MEGA5 (Tamura *et al*., 2011). Next, each gene fragment, either aligned with MUSCLE or with MAFFT, was analysed with the software jModelTest 2 (Darriba *et al*., 2012), which compares different models of DNA substitution in a hierarchical hypothesis-testing framework to select a base substitution model that best fits the data. For the three gene fragments and two different alignment strategies, the optimal models found by jModelTest 2 [selected with the corrected Akaike information criterion (AIC_c)] are shown in Tables 3 and 4. These models were implemented in MrBayes (Huelsenbeck & Ronquist, 2001) for Bayesian inference (BI) analysis and TREEFINDER (Gangolf, von Haeseler & Strimmer, 2004) for maximum likelihood (ML) analysis of the different data sets (see section 'Maximum likelihood and Bayesian inference phylogenetic analyses').

Three-phase phylogenetic analyses

Three-phase analyses comprised sequence alignment using MUSCLE (Edgar, 2004a, b) and MAFFT (Katoh

Table 3. Nuclear markers including informative sites and maximum likelihood models selected through the Akaike information criterion as implemented in jModelTest2. The base frequencies, rate matrix, gamma shape parameter, and proportion of invariable sites resulting from jModelTest2 are shown

	Gene fragment	
	H3	*Enolase*
Total sites	328	373
Informative sites	81	120
Model	GTR + G	TIM2 + G
Base frequency		
%A	0.2152	0.2567
%C	0.3110	0.2218
%G	0.2464	0.2312
%T	0.2274	0.2904
Rate matrix		
[A-C]	1.5943	2.5417
[A-G]	7.2541	6.2893
[A-T]	5.4063	2.5417
[C-G]	1.1857	1.0000
[C-T]	13.0972	13.4010
[G-T]	1.0000	1.000
Shape parameter	0.1670	0.3010
Invariable sites	–	–

GTR + G, general time-reversible + Gamma; *H3*, Histone; TIM2 + G, transitional model 2 + Gamma.

& Standley, 2013) and then omission of positions that were highly divergent and poorly aligned if needed. Importantly, the alignment of the *H3* and *Enolase* gene fragments had no indels and were unambiguous. However, the aligned sequences of the *12S* gene fragment did contain several indels. Therefore, positions that were highly divergent and poorly aligned in the *12S* gene segment were identified in the software GBlocks v. 0.91b, omitting them from the analyses. After highly divergent positions were pruned, the *12S* data set consisted of 344 and 315 bp when the sequences were aligned with MUSCLE and MAFFT, respectively. After GBlocks, each gene fragment, either aligned with MUSCLE or with MAFFT, was analysed with the software jModelTest 2 (Darriba *et al*., 2012). Lastly, the optimal models found by jModelTest 2 (selected with the AIC_c, see Table 4) for each gene fragment were implemented in MrBayes (Huelsenbeck & Ronquist, 2001) for BI analysis and TREEFINDER (Gangolf *et al*., 2004) for ML analysis of the different data sets.

Maximum likelihood and Bayesian inference phylogenetic analyses

Different 'total evidence' analyses (Grant & Kluge, 2003) were conducted during this study using the two different data sets (i.e. the two-gene and the three-gene data sets) as well as two- and three-phase phylogenetic analyses. Total evidence analyses enhance the detection of real phylogenetic groups if there is no or minimal heterogeneity amongst different (e.g. *H3*, *Enolase*, and *12S*) data sets (de Queiroz, Donohue & Kim, 1995). Preliminary phylogenetic analyses using only one gene fragment at a time demonstrated minimal heterogeneity. Thus, a 'total evidence' analysis has the ability to more accurately reflect phylogenetic relationships in this study (see de Queiroz *et al*., 1995). Total evidence analyses have been used before to infer the phylogeny of many other clades of marine and terrestrial vertebrates and invertebrates, including marine shrimps (Duffy, Morrison & Ríos, 2000; Anker & Baeza, 2012; Baeza, 2013; Baeza & Fuentes, 2013).

The first set of 'total evidence' analyses using the first data set (*Enolase* and *H3*) comprised a total of 701 bp, 31 sequences, and 17 species pertaining to the families Rhynchocinetidae, Eugonatonotidae, and Nematocarcinidae and six species from other caridean families used as outgroup terminals (for details see Table 2). This first data set was partitioned into two different segments, each with a different model of evolution. Missing data were designated as a '?' in the alignment. All the parameters used for the ML analysis were those of the default option in TREEFINDER. For BI, unique random starting trees were used in the Metropolis-coupled Markov Monte Carlo Chain (MCMC) analysis (see Huelsenbeck & Ronquist, 2001; Ronquist *et al*., 2012). The analysis was performed for 6 000 000 generations. Visual analysis of log-likelihood scores against generation time indicated that the log-likelihood values reached a stable equilibrium before the 100 000th generation. Thus, a burn-in of 1000 samples was conducted, every 100th tree was sampled from the MCMC analysis obtaining a total of 60 000 trees, and a consensus tree with the 50% majority rule was calculated for the last 59 900 sampled trees. The robustness of the ML tree topology was assessed by bootstrap reiterations of the observed data 2000 times and reconstructing trees using each resampled data set. Support for nodes in the BI tree topology was obtained by posterior probability.

The second set of 'total evidence' analyses performed on the second data set (*Enolase* + *H3* + *12S*) comprised a total of 12 species pertaining to the family Rhynchocinetidae *sensu* Holthuis (1995) and five species from other caridean families used as outgroup terminals (for details see Table 2). In this second set of 'total evidence' analyses, the two nuclear alignments (*Enolase* and *H3*) and the aligned mitochondrial gene fragment (*12S*) were concatenated into a single data set consisting of between 1016 and 1045 bp (depending on the alignment tool used; Table 4). The data set was partitioned into three different segments, each with

Table 4. Mitochondrial marker including informative sites and maximum likelihood (ML) models selected through the Akaike information criterion as implemented in jModelTest2. The base frequencies, rate matrix, gamma shape parameter, and proportion of invariable sites resulting from jModelTest2 are shown

	Two-phase analyses, aligned with		Three-phase analyses, aligned with	
	MUSCLE	MAFFT	MUSCLE	MAFFT
Total sites	430	415	344	315
Model	TrN + G	TrN + G	TrN + G	TrN + G
Base frequency				
%A	0.3602	0.3619	0.3666	0.3473
%C	0.0988	0.0968	0.1003	0.1003
%G	0.1754	0.1778	0.1880	0.1891
%T	0.3655	0.3636	0.3452	0.3633
Rate matrix				
[A-C]	1.0000	1.0000	1.0000	1.0000
[A-G]	4.7268	4.5214	4.2789	4.6773
[A-T]	1.0000	1.0000	1.0000	1.0000
[C-G]	1.0000	1.0000	1.0000	1.0000
[C-T]	7.3364	7.9251	7.7649	8.8296
[G-T]	1.0000	1.0000	1.0000	1.0000
Shape parameter	0.4790	0.4440	0.4620	0.4170
Invariable sites	–	–	–	–

TrN + G, Tamura-Nei + Gamma.

a different model of evolution. Missing data were designated as a '?' in the alignment. Gaps were treated as a fifth state in the analysis. All the parameters used for the ML analysis were those of the default option in TREEFINDER. For BI, unique random starting trees were used in the Metropolis-coupled MCMC analysis (see Huelsenbeck & Ronquist, 2001; Ronquist *et al*., 2012). The analysis was performed for 6 000 000 generations. Visual analysis of log-likelihood scores against generation time indicated that the log-likelihood values reached a stable equilibrium before the 100 000th generation. Thus, a burn-in of 1000 samples was conducted, every 100th tree was sampled from the MCMC analysis obtaining a total of 60 000 trees, and a consensus tree with the 50% majority rule was calculated for the last 59 900 sampled trees. The robustness of the ML tree topology wasassessed by bootstrap reiterations of the observed data 2000 times and reconstructing trees using each resampled data set. Support for nodes in the BI tree topology was obtained by posterior probability.

Hypothesis testing of monophyletic clades with three-phase phylogenetic inference

Five different hypotheses were examined in this study: (1) the monophyly of the superfamily Nematocarcinoidea; (2) the monophyly of the family Rhynchocinetidae (*Rhynchocinetes* + *Cinetorhynchus*) *sensu* Holthuis (1995); (3) the monophyly of the family Rhynchocinetidae (*Rhynchocinetes* + *Cinetorhynchus* + *Lipkius* + *Eugonatonotus*) *sensu* Yaldwyn (1960); (4) the monophyly of the genus *Rhynchocinetes*; and (5) the monophyly of the genus *Cinetorhynchus*. The first three hypotheses were tested using the first data set, which included 23 species for which two nuclear genes but no mitochondrial gene were available. The last two hypotheses were tested using the second data set, for which both nuclear and mitochondrial sequences were available.

Monophyly was inferred when all the specimens from the different families or genera within a purportedly natural clade segregated and formed different clade-specific monophyletic clades. To test the validity of the different genera as natural clades, constrained trees (in which the monophyly of a particular clade was enforced) were obtained in MrBayes with the command 'constraint'. MCMC searches were run and the harmonic mean of tree-likelihood values were obtained by sampling the post burn-in, posterior distribution as above. Next, Bayes factors were used to evaluate whether or not there was evidence against monophyly of the different families (unconstrained vs. constrained trees) according to the criteria of Kass & Raftery (1995). Bayes factors compare the total harmonic mean of the marginal likelihood of unconstrained vs. monophyly-constrained models. Higher values of the Bayes factor statistic imply stronger support against the monophyly of a particular group (Kass & Raftery, 1995).

Specifically, a value for the test statistic $2\log_e(B_{10})$ between 0 and 2 indicates no evidence against H_0; values from 2 to 6 indicate positive evidence against H_0; values from 6 to 10 indicate strong evidence against H_0; and values > 10 indicate very strong evidence against H_0 (Kass & Raftery, 1995; Nylander *et al*., 2004).

Importantly, the first three hypotheses above were tested using the BI phylogenetic tree resulting from total evidence three-phase analyses. Nonetheless, the results from the testing of hypotheses 4 and 5 above must be considered with caution because the two- and three-phase analyses did not result in the same tree topology produced by the one-phase SATé-II analysis that is superior to two- and three-phase phylogenetic analyses. At present, hypothesis testing using Bayes factors is not available in software packages that conduct one-phase phylogenetic analyses.

RESULTS

One-phase SATé-II and two-phase analyses using two nuclear gene fragments

The first molecular data set analysed comprised a total of 701 characters, 201 of them parsimony informative, for a total of 31 terminals, including a total of 17 species pertaining to the families Rhynchocinetidae, Eugonatonotidae, and Nematocarcinidae, and six species from other caridean families used as outgroup terminals (for details see Table 2). All molecular phylogenetic trees obtained with the one-phase SATé-II method and the two-phase approaches that used different inference methods (ML and BI) resulted in the same general topology (Fig. 3). This similarity in tree topologies and clade robustness was expected considering that the alignments of the *H3* and *Enolase* gene fragments had no indels and were unambiguous. The above also implies that there was no need to apply the three-phase method to this first data set.

For the pool of taxa used herein, the molecular analyses did not support the monophyletic status of the superfamily Nematocarcinoidea *sensu* Martin & Davis (2001) (also, see De Grave & Fransen, 2011) and the family Rhynchocinetidae *sensu* Yaldwyn (1960) but the same analyses did support the monophyletic status of the family Rhynchocinetidae *sensu* Holthuis (1995). The one-phase SATé-II ML tree topology and the two two-phase ML and BI tree topologies demonstrated that species did not cluster together according to superfamily (Nematocarcinoidea and others) and formed a single, well-supported, monophyletic clade, as expected according to traditional systematics based on adult morphology. Both in the ML (SATé-II and TREEFINDER) and BI trees, *Eugonatonotus chacei* was sister to a clade including species of *Cinetorhynchus*, *Rhynchocinetes*, *Lipkius*, and *Nematocarcinus* (at present, all belonging to the superfamily Nematocarcinoidea – Martin & Davis, 2001; De Grave & Fransen, 2011) but also containing species from the genera *Systellaspis* Spence Bate, 1888 (superfamily Oplophoroidea Dana, 1852) and *Pasiphaea* Savigny, 1816 (superfamily Pasiphaeoidea Dana, 1852). The two specimens of *Lipkius* and the three species of *Nematocarcinus* segregated according to genus and formed a monophyletic clade. Nonetheless, their monophyletic status and sister relationship was poorly supported by ML (in SATé-II and TREEFINDER) and BI analyses. In addition, *Lipkius* + *Nematocarcinus* were sister to a clade including species of *Cinetorhynchus* and *Rhynchocinetes* but also containing *Systellaspis* (superfamily Oplophoroidea). Thus, the family Rhynchocinetidae *sensu* Yaldwyn (1960) is not monophyletic because of the position of *Lipkius holthuisi*, which did not cluster together with other members from the genera *Rhynchocinetes* and *Cinetorhynchus*. In all phylogenetic analyses, the species from the genera *Rhynchocinetes* and *Cinetorhynchus* clustered together and formed a well-supported monophyletic clade. Importantly however, the species from the two genera above did not segregate according to genus in the two-phase analyses (TREEFINDER and MrBayes). However, in the one-phase SATé-II ML analysis, the species from the two genera did segregate according to genus and formed well-supported, genus-specific monophyletic clades (see sections 'One-phase SATé-II analysis using nuclear and mitochondrial gene fragments' and 'Two- and three-phase analyses using nuclear and mitochondrial gene fragments'; Fig. 3).

In agreement with the above results, the Bayes factor analyses (conducted in MrBayes) revealed no support for the monophyly of the superfamily Nematocarcinoidea and the family Rhynchocinetidae *sensu* Yaldwyn (1960). Comparison of the unconstrained tree vs. the trees wherein Nematocarcinoidea and Rhynchocinetidae *sensu* Yaldwyn (1960) were imposed as monophyletic clades indicated support for the unconstrained trees [Nematocarcinoidea: $2\ln(B_{10}) = 15.51$; Rhynchocinetidae *sensu* Yaldwyn (1960): $2\ln(B_{10}) = 12.80$; Table 5].

One-phase SATé-II analysis using nuclear and mitochondrial gene fragments

The second data set (the three-gene data set of two nuclear genes plus one mitochondrial gene) used for the SATé-II analysis comprised a total of 26 terminals, including a total of 12 species pertaining to the families Rhynchocinetidae *sensu* Holthuis (1995) and five species from other caridean families used as outgroup terminals.

For the pool of taxa used herein, the SATé-II analysis supported the monophyletic status of the family

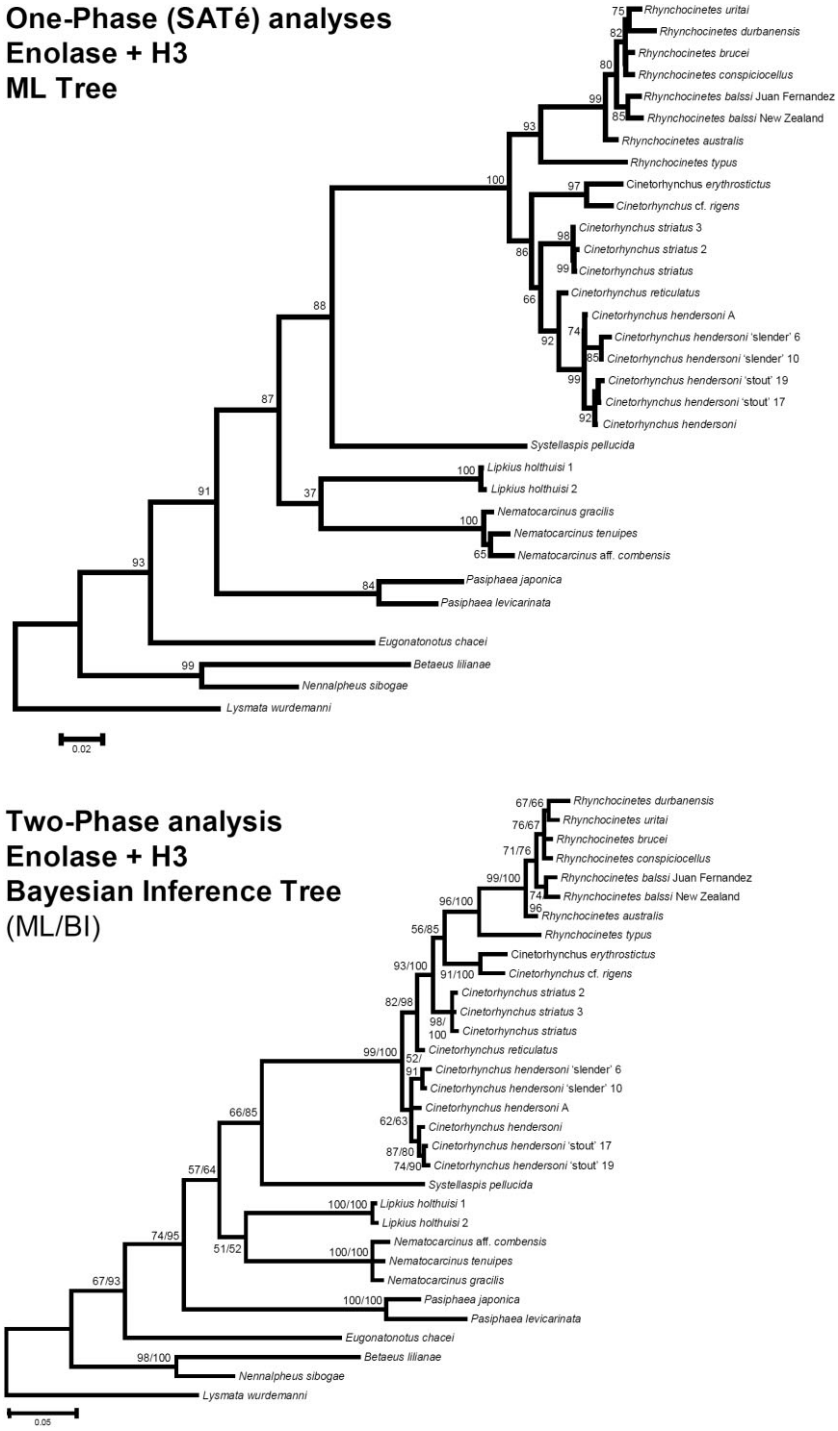

Figure 3. A, one-phase simultaneous alignment and tree estimation (SATé-II) analysis of maximum likelihood (ML) for representatives of the superfamily Nematocarcinoidea using two nuclear genes. B, two-phase phylogenetic analysis of Bayesian inference (BI) using two nuclear genes for representatives of the superfamily Nematocarcinoidea. The two phylogenetic trees resulted from the combined analysis of Histone (*H3*) and *Enolase* gene fragments of *Rhynchocinetes* (seven taxa and eight terminals), *Cinetorhynchus* (five taxa and 12 terminals), *Lipkius* (one taxon and two terminals), *Nematocarcinus* (three taxa), *Eugonatonotus* (one taxon), and outgroups. In (B), the general topology of the trees obtained from two-phase ML and BI analyses was the same. In (A), the numbers above or below the branches represent the bootstrap values obtained from the ML analysis in SATé-II. In (B), numbers above or below the branches represent the posterior probabilities from the BI analysis in MrBayes and bootstrap values obtained from the ML analyses in TREEFINDER (ML/BI).

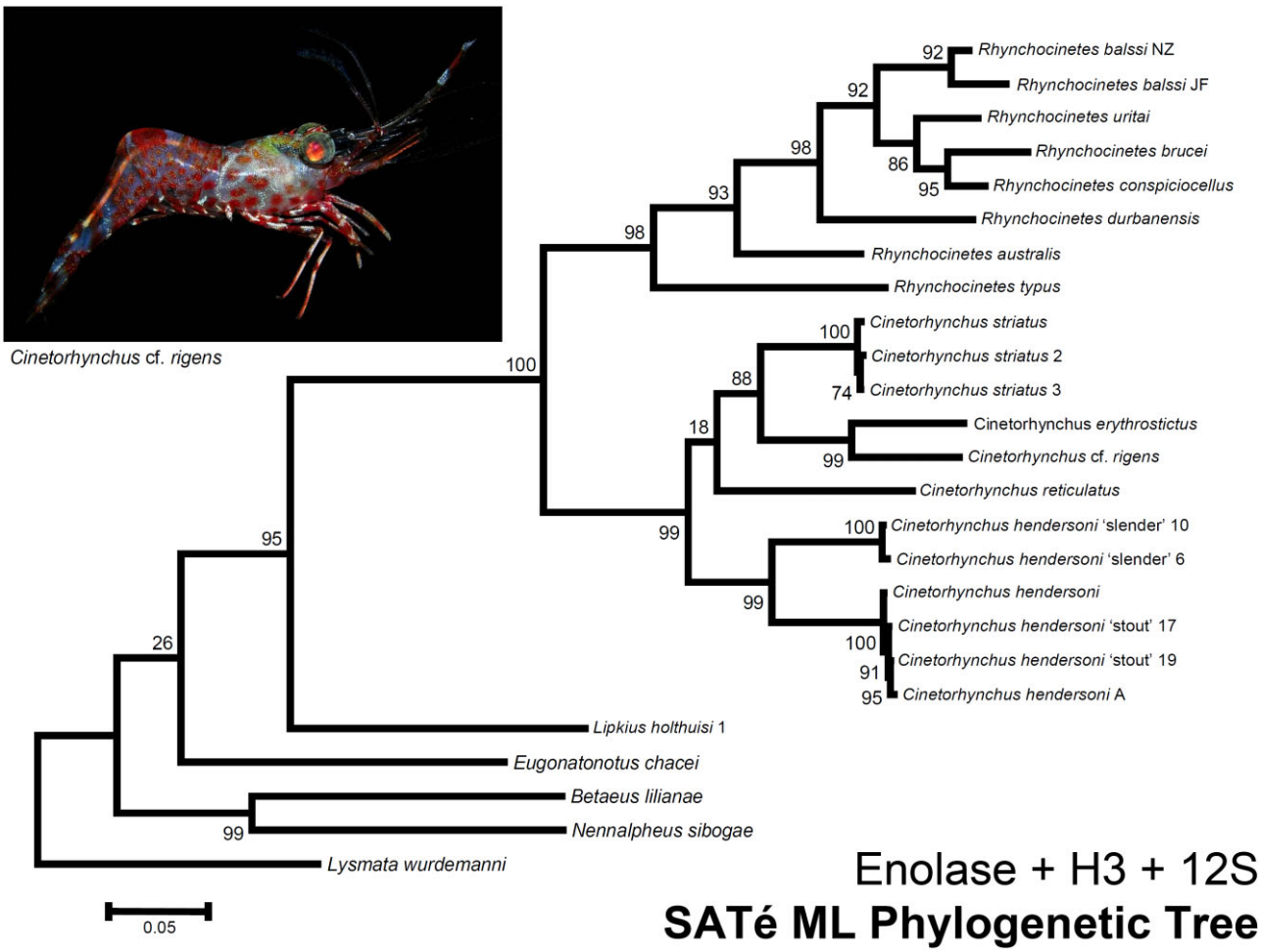

Figure 4. One-phase simultaneous alignment and tree estimation (SATé-II) analysis of maximum likelihood (ML) for representatives of the family Rhynchocinetidae using two nuclear genes. The phylogenetic tree resulted from the combined analysis of *12S*, Histone (*H3*), and *Enolase* gene fragments of *Rhynchocinetes* (seven taxa and eight terminals), *Cinetorhynchus* (five taxa and 12 terminals), *Lipkius* (one taxon and two terminals), *Eugonatonotus* (one taxon), and outgroups. The numbers above or below the branches represent the bootstrap values obtained from the ML analyses in SATé-II.

Rhynchocinetidae *sensu* Holthuis (1995) (Fig. 4). The ML and BI tree topologies demonstrated that species of *Cinetorhynchus* and *Rhynchocinetes* did cluster together and formed a single, well-supported, monophyletic clade, as expected according to Holthuis's (1995) and Okuno's (1997) views on systematics based on adult morphology. Importantly, within this monophyletic clade [the Rhynchocinetidae *sensu* Holthuis (1995)], the different species of *Cinetorhynchus* and *Rhynchocinetes* did segregate according to genera and formed two well-supported monophyletic clades, as expected according to Okuno's (1997) systematic view based on adult morphology (Fig. 4).

Within the genus *Cinetorhynchus*, the analysis placed the different specimens of *Cinetorhynchus hendersoni* (Kemp, 1925) as a sister group to the remaining species of *Cinetorhynchus*. Within this *C. hendersoni* clade, the two specimens of *C. hendersoni* 'slender' and the three specimens of *C. hendersoni* 'stout' (all 'slender' and 'stout' specimens collected in Hawaii, USA) plus a fourth specimen of *C. hendersoni* collected from Lizard Island, Australia, but with similar 'stout' morphology, segregated according to morphotype and formed two well-supported monophyletic clades. The divergence within specimens of *C. hendersoni* 'slender' and within specimens of *C. hendersoni* 'stout' was low (p-distance: *C. hendersoni* 'slender' = 0.002; *C. hendersoni* 'stout' = 0.001–0.003, mean ± SD = 0.0018 ± 0.0009) and much lower than that calculated between specimens of *C. hendersoni* 'stout' and 'slender' (p-distance: 0.066–0.094, mean ± SD = 0.0748 ± 0.0117).

Table 5. Bayes factor testing of phylogenetic hypotheses. The different hypotheses on the monophyly of particular groups of shrimp are ordered based on the degree of evidence against the unconstrained tree. The higher the value of the 2 $\log_e(B_{10})$ statistic the stronger the support against the monophyly of a particular group

Hypotheses	Harmonic mean	2 $\log_e(B_{10})$	Evidence
Unconstrained tree (*H3* + *Enolase* data set)	–6583.08		
Monophyletic Nematocarcinoidea	–4248.59	15.91	Very strong against constrained tree
Monophyletic Rhynchocinetidae *sensu* Yaldwyn (1960)	–5979.88	12.80	Very strong against constrained tree
Monophyletic Rhynchocinetidae *sensu* Holthuis (1995)	–	–	Supported by unconstrained tree
Unconstrained tree (*H3* + *Enolase* + *12S* data set)	–9942.99		
Monophyletic *Rhynchocinetes*	–	–	Supported by unconstrained tree
Monophyletic *Cinetorhynchus*	–9287.76	12.97	Very strong against constrained tree

H3, Histone.

Within the genus *Cinetorhynchus*, the position of *Cinetorhynchus reticulatus* Okuno, 1997, was not well resolved. The tree topology in Figure 4 suggests that *Cinetorhynchus erythrostictus* Okuno, 1997, and *Cinetorhynchus* cf. *rigens* (Gordon, 1936) are sister taxa and their geminate status is well supported by the ML bootstrap value. The three specimens of *Cinetorhynchus striatus* (Nomura & Hayashi, 1992) clustered together, forming a well-supported monophyletic clade, and the SATé-II tree topology suggests that *C. striatus* is sister to the clade comprised of *C. erythrostictus* and *C.* cf. *rigens*.

Within the genus *Rhynchocinetes*, *R. typus* is sister to all remaining species of *Rhynchocinetes* and *Rhynchocinetes australis* Hale, 1941, is sister to all remaining species of *Rhynchocinetes* but *R. typus*. Furthermore, *R. durbanensis* Gordon, 1936, is sister to all remaining species of *Rhynchocinetes* but *R. typus* and *R. australis*. Additionally, *Rhynchocinetes conspiciocellus* Okuno & Takeda, 1992, and *R. brucei* Okuno, 1994 are sister taxa and their geminate status is well supported by the SATé-II analysis. Lastly, *Rhynchocinetes balssi* Gordon, 1936, from New Zealand and *R. balssi* from the Juan Fernandez archipelago clustered together and their monophyletic status was well supported by the ML bootstrap value. Importantly however, the genetic distance between *R. balssi* from New Zealand and from the Juan Fernandez archipelago was relatively large (p-distance = 0.011) and comparable to that calculated for the geminate pair of species *C. erythrostictus* – *C.* cf. *rigens* (p-distance = 0.079) and greater than those genetic distances calculated for the different specimens of *R. striatus* (p-distances = 0.001–0.003). The above suggests the existence of more than one species currently classified as *R. balssi* in the temperate/subtropical South Pacific or the presence of strong genetic structuring within *R. balssi*.

TWO- AND THREE-PHASE ANALYSES USING NUCLEAR AND MITOCHONDRIAL GENE FRAGMENTS

All two- and three-phase molecular phylogenetic trees obtained with the different alignment tools (MAFFT and MUSCLE) and inference methods (ML and BI) resulted in the same general topology (Figs 5, 6). Additionally, all two- and three-phase molecular phylogenetic analyses resulted in trees somewhat similar to those previously obtained with SATé-II and two-phase phylogenetic analyses using only nuclear gene fragments. For instance, for the pool of taxa used herein, all the molecular analyses again supported the monophyletic status of the family Rhynchocinetidae *sensu* Holthuis (1995) and Okuno (1997). Nonetheless, the main difference between previous one-phase SATé-II ML analyses and the present two- and three-phase analyses using nuclear and mitochondrial molecular markers is that within the Rhynchocinetidae *sensu* Holthuis (1995), the present two- and three-phase analyses using nuclear and mitochondrial molecular markers did not support the genera *Cinetorhynchus* and *Rhynchocinetes* as natural entities (= monophyletic clades); species of *Cinetorhynchus* and *Rhynchocinetes* did not segregate completely according to genus.

All two- and three-phase analyses placed the specimens of *C. hendersoni* as a sister group to the remaining species of *Cinetorhynchus* and *Rhynchocinetes*. Within this basal clade, the two specimens of *C. hendersoni* 'slender' and the three specimens of *C. hendersoni* 'stout' plus a fourth specimen collected from Lizard Island, Queensland, Australia, but with similar 'stout' morphology, segregated according to morphotype and formed two well-supported monophyletic clades, similar to as mentioned above for the One-phase SATé-II analysis.

The three specimens of *C. striatus* clustered together and formed a well-supported monophyletic clade.

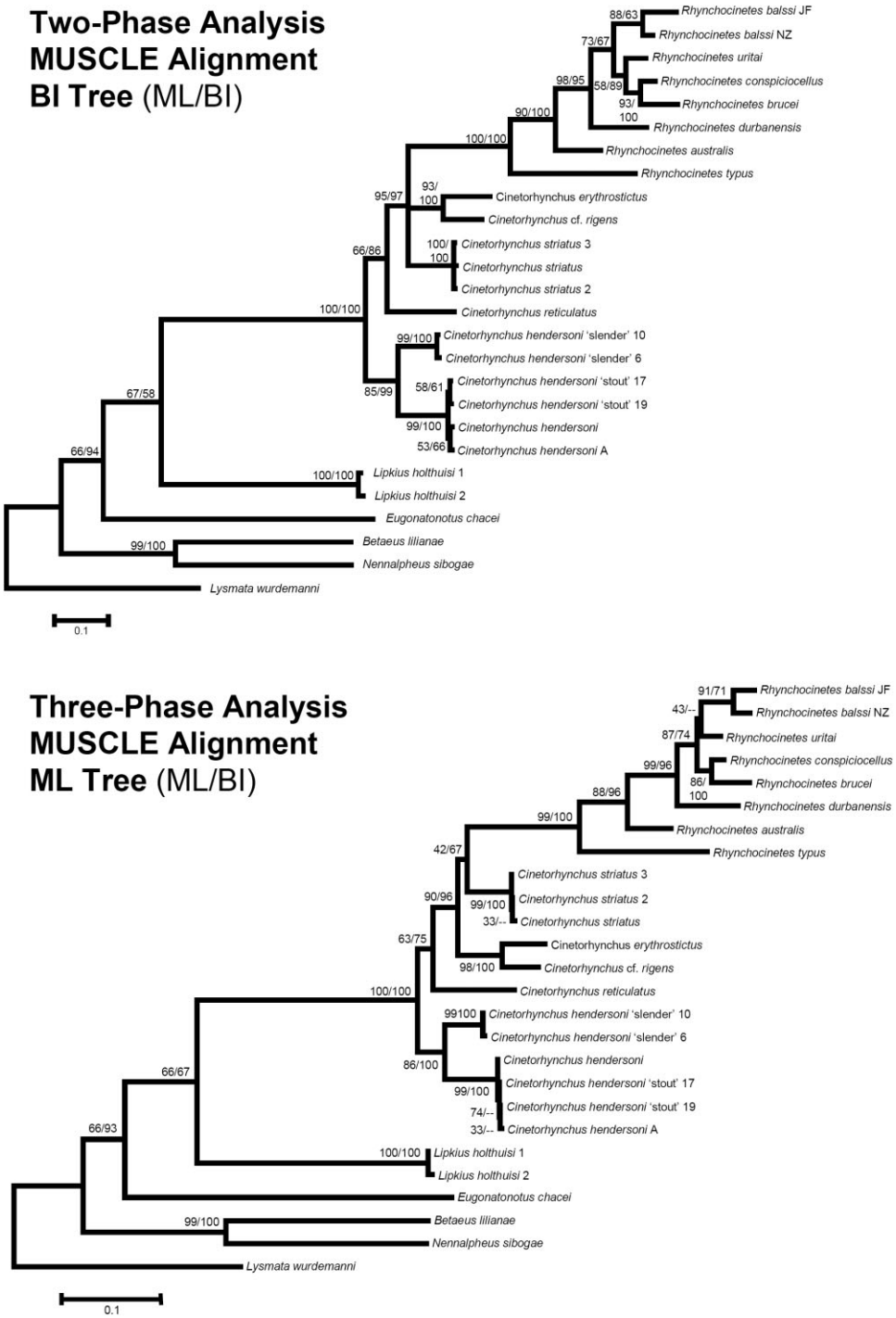

Figure 5. Two-phase (above) and three-phase (below) phylogenetic analyses of maximum likelihood (ML) and Bayesian inference (BI) for representatives of the family Rhynchocinetidae using three genes. The software MUSCLE was used for sequence alignment The two phylogenetic trees resulted from the combined analysis of *12S*, Histone (*H3*), and *Enolase* gene fragments of *Rhynchocinetes* (seven taxa and eight terminals), *Cinetorhynchus* (five taxa and 12 terminals), *Lipkius* (one taxon and two terminals), *Eugonatonotus* (one taxon) and outgroups. The general topology of the trees obtained from two-phase and three-phase ML and BI analyses was the same. The numbers above or below the branches represent the posterior probabilities from the BI analysis in MrBayes and bootstrap values obtained from ML analyses in TREEFINDER (ML/BI).

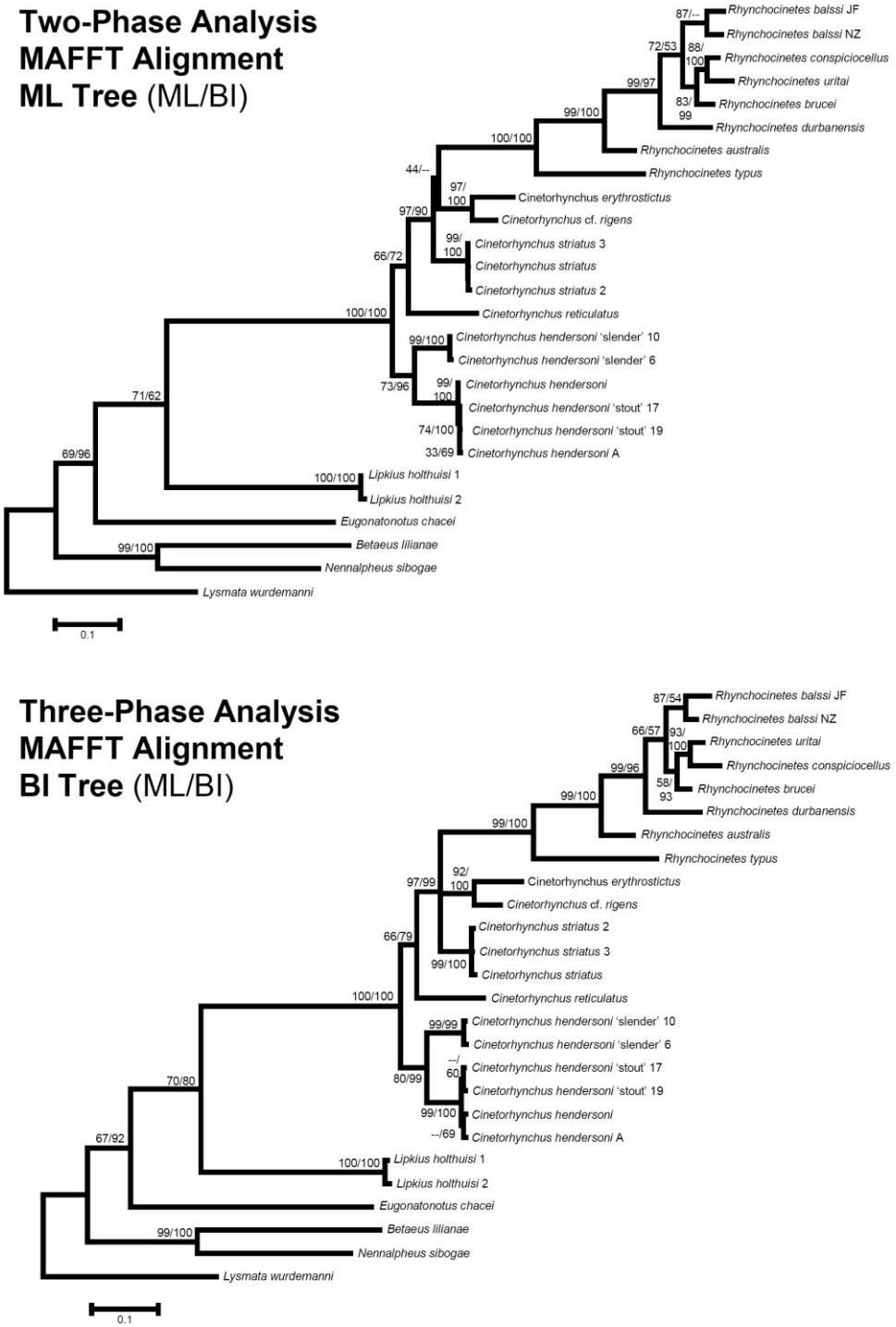

Figure 6. Two-phase (above) and three-phase (below) phylogenetic analyses of maximum likelihood (ML) and Bayesian inference (BI) for representatives of the family Rhynchocinetidae using three genes. The software MAFFT was used for sequence alignment The two phylogenetic trees resulted from the combined analysis of *12S*, Histone (*H3*), and *Enolase* gene fragments of *Rhynchocinetes* (seven taxa and eight terminals), *Cinetorhynchus* (five taxa and 12 terminals), *Lipkius* (one taxon and two terminals), *Eugonatonotus* (one taxon) and outgroups. The general topology of the trees obtained from two-phase and three-phase ML and BI analyses was the same. The numbers above or below the branches represent the posterior probabilities from the BI analysis in MrBayes and bootstrap values obtained from ML analyses in TREEFINDER (ML/BI).

In addition, *C. erythrostictus* and *C.* cf. *rigens* are sister taxa and their geminate status is well supported by the ML and BI analyses. However, the positions of *C. reticulatus* and of the monophyletic clades comprised of the three specimens of *C. striatus* and of *C. erythrostictus* + *C.* cf. *rigens* were not well resolved in the retrieved trees but did cluster together with other species of *Cinetorhynchus*.

The monophyly of *Rhynchocinetes* (seven species) is well supported by a high posterior probability obtained from the BI analysis and bootstrap support values from all two- and three-phase ML analyses. Within this clade, the relationship amongst between the different species was the same as indicated by previous SATé-II analyses and two-phase analyses based only on two nuclear fragments (compare Fig. 4 with Figs 5 and 6).

The Bayes factor analyses (conducted in MrBayes) revealed no support for the monophyly of the family Rhynchocinetidae *sensu* Yaldwyn (1960) (Table 5), in agreement with the results above from the two- and three-phase analyses. Comparison of the unconstrained tree vs. the trees wherein the Rhynchocinetidae *sensu* Yaldwyn (1960) were imposed as monophyletic clades, indicated support for the unconstrained trees [Rhynchocinetidae *sensu* Yaldwyn (1960): $2\ln(B_{10}) = 15.79$; Table 5]. The Bayes factor analyses also revealed no support for the monophyly of the genus *Cinetorhynchus* [$2\ln(B_{10}) = 12.97$].

DISCUSSION

The phylogenetic trees of the superfamily Nematocarcinoidea, and in particular, of the family Rhynchocinetidae and other related genera of caridean shrimps that resulted from multiple one-, two-, and three-phase phylogenetic analyses that used two or three molecular marker [one mitochondrial gene (*12S*) and two nuclear genes (*H3* and *Enolase*)], are sufficiently robust for the first tentative examination of key but unresolved systematic questions in these shrimp lineages. Below, we discuss how our findings help in resolving current systematic problems in this remarkable clade of shrimps. We also highlight the importance of using one-phase over two- and three-phase phylogenetic analyses and the putative problems that might arise when using tools (e.g. Bayes factors) that so far rely upon multi-phase analyses.

The superfamily Nematocarcinoidea is not a natural entity

In the species-rich and diverse infraorder Caridea, the systematic relationships amongst genera, families, and superfamilies are unsettled (Chace, 1992, 1997; Martin & Davis, 2001; Bracken *et al*., 2009; De Grave *et al*., 2009; De Grave & Fransen, 2011; Li *et al*., 2011; Baeza, 2013; see also Table 1). In particular, the superfamily Nematocarcinoidea has historically been considered a monophyletic clade including a total of four families (i.e. the marine Eugonatonotidae, Nematocarcinidae, Rhynchocinetidae, and the freshwater Xiphocarididae) and nine genera (Chace, 1992; Holthuis, 1993; Martin & Davis, 2001; De Grave *et al*., 2009; De Grave & Fransen, 2011). Traits that define the Nematocarcinoidea include, amongst others, (1) the mandible with a subtruncate molar process that also has a transversely ridged grinding surface; (2) the first maxilla with the distal endite not usually large; (3) the second maxilla with the distal endite mesially bilobate bearing a palp that is not vestigial; (4) the first maxilliped with an endite not unusually large bearing an exopod with a lash and distinct caridean lobe; (5) the slender, pereopod-like third maxilliped, neither broad nor operculate, that bears an exopod; (6) the first pereopod stouter than the second pereopod; (7) chelate first and second pereopods; (8) the second pereopod with an entire carpus; and (9) pereopods one to three with strap-like epipods (Chace, 1992).

Specimens from three families and five currently recognized genera were included in the present study. Based on cladistic analysis of morphological characters, Christoffersen (1990) concluded that the Nematocarcinoidea was polyphyletic and resurrected various superfamilies to contain the different families and genera within the group. One of these superfamilies, the Eugonatonotoidea, included the monotypic family Eugonatonotoidae and the genus *Eugonatonotus*. The Nematocarcinoidea was redefined to include two genera in the family Nematocarcinidae: *Nematocarcinus* and *Lipkius* (see Christoffersen, 1990). The genus *Lipkius*, originally placed in the family Rhynchocinetidae by Yaldwyn (1960), was transferred to this family Nematocarcinidae by Christoffersen (1990). Lastly, the family Rhynchocinetidae was grouped with the family Palaemonidae into the superfamily Palaemonoidea. Christoffersen's proposal was rejected by most taxonomists during recent decades, including the most recent taxonomic arrangements of the Palaemonoidea used by De Grave *et al*. (2009) and De Grave & Fransen (2011).

The results from this study partially support Christoffersen's (1990) phylogenetic hypothesis based on morphological traits. In disagreement with Chace (1992), Holthuis (1993), De Grave *et al*. (2009), and De Grave & Fransen (2011), the one-phase SATé-II and two-phase phylogenetic analyses using two nuclear genes showed that the specimens from the families Eugonatonotidae, Nematocarcinidae, and Rhynchocinetidae did not cluster together and did not form a single, well-supported monophyletic clade. Furthermore, the Bayes factor analyses (conducted in MrBayes and using the two-phase Bayesian inference phylogenetic

analysis as a framework) revealed no support for the monophyly of the superfamily Nematocarcinoidea. These Bayes factor analyses are considered robust herein given that all molecular phylogenetic trees obtained with the one-phase SATé-II method and the two-phase approaches that used different inference methods (ML and BI) resulted in the same general topology. Altogether, the above information implies that the Nematocarcinoidea [*sensu* Holthuis, (1993), Chace (1992), and Martin & Davis (2001)] is polyphyletic as suggested by Christoffersen (1990).

Also in agreement with Christoffersen's (1990) ideas, the specimen of *Eugonatonotus* did not form a well-supported monophyletic clade with members of the Nematocarcinidae and Rhynchocinetidae in any of the reconstructions. Thus, shrimps from the genus *Eugonatonotus* represent a natural entity deserving elevation to the superfamily and family level, namely the Eugonatonotoidea and Eugonatonotidae, respectively, as suggested by Christoffersen (1990) (see proposed taxonomic rearrangement below). Importantly, the topologies of the different one- and two-phase phylogenetic trees further suggest that the genera *Lipkius* and *Nematocarcinus* do pertain to the Nematocarcinidae *sensu* Christoffersen (1990), in disagreement with the currently accepted taxonomic arrangement in the Caridea (Holthuis, 1993; Chace, 1997; Burukovsky, 2005; De Grave *et al*., 2009; De Grave & Fransen, 2011). The two specimens of *Lipkius* and the three species of *Nematocarcinus* segregated according to genus and formed a monophyletic clade. Nonetheless, their monophyletic status and sister relationship was poorly supported by ML and BI analyses. Certainly, future studies including additional representatives from the different families above and molecular markers will help to decipher the systematics of nematocarcinid shrimps.

Overall, the present phylogenetic findings are in line with those of Bracken *et al*. (2009), Li *et al*. (2011), and Baeza (2013), who questioned the validity of the superfamily arrangement within the Caridea and monophyly of several families. The results from this study and those of Bracken *et al*. (2009) and Li *et al*. (2011) point to the notion that the Nematocarcinoidea is not a natural clade within the Caridea.

The family Rhynchocinetidae *sensu* Yaldwyn (1960) is not a natural entity

Yaldwyn (1960) proposed that the species of *Rhynchocinetes*, *Lipkius*, and *Eugonatonotus* comprised the family Rhynchocinetidae. Bowman & Abele (1982) then established the superfamily Rhynchocinetoidea, comprising three families, Bresiliidae Calman, 1896, Eugonatonotidae, and Rhynchocinetidae. These authors provided no explanation for the establishment of the Rhynchocinetoidea and their proposal has been rejected by most taxonomists in recent decades. Later, Christoffersen (1990) transferred the genus *Lipkius* to the family Nematocarcinidae (see above). Most recently, Holthuis (1995) and Okuno (1997) suggested that only the species of *Rhynchocinetes* (and *Cinetorhynchus*) should be placed in the Rhynchocinetidae, separately from the genus *Lipkius*. This latter genus was included, amongst others, in the family Nematocarcinidae. In turn, the genus *Eugonatonotus* was placed in its own family, the Eugonatonotidae. Traits that define the Rhynchocinetidae *sensu* Holthuis (1995) and Okuno (1997) include, amongst others, a rostrum that is usually incompletely fused with the remainder of the carapace that has two or three teeth on the median carina, no supraorbital spine, and one spine at the posterolateral margin of the fifth abdominal somite (Okuno, 1997).

The various analyses used in this study do not support either Yaldwyn's (1960) grouping of the genera *Rhynchocinetes*, *Lipkius*, and *Eugonatonotus* in a single clade, or monophyly of his Rhynchocinetidae. Notably, the genera *Rhynchocinetes* and *Cinetorhynchus* clustered together, forming a single, well-supported monophyletic clade. Altogether, the above information implies that the Rhynchocinetidae represent a natural clade, as suggested by Okuno (1997). Our results are also in line with those of Li *et al*. (2011), who found no support for the monophyly of Rhynchocinetidae *sensu* Yaldwyn (1960) using five different nuclear gene fragments but a smaller number of representatives from this family than in the present study.

The genera *Rhynchocinetes* and *Cinetorhynchus* are natural entities: the relevance of one-phase phylogenetic analyses

Within the monophyletic Rhynchocinetidae *sensu* Holthuis (1995), the one-phase SATé-II analysis demonstrated that the different species of *Cinetorhynchus* and *Rhynchocinetes* segregated according to genera and formed two well-supported monophyletic clades, in agreement with Okuno's (1997) systematic views based on adult morphology.

Holthuis (1995) divided the genus *Rhynchocinetes* into two subgenera based on distinctive morphological characters. The subgenus *Rhynchocinetes* comprised species bearing two acute teeth on the median carina of the carapace behind a distinct rostral articulation, a supraorbital spine, and no spine on the posterolateral margins of the fourth and fifth abdominal somites. In turn, the subgenus *Cinetorhynchus* included species bearing three teeth on the median carina of the carapace, an indistinct articulation between the carapace and the rostrum, no supraorbital spine, and

one spine on the posterolateral margin of the fifth abdominal somite (Holthuis, 1995; Okuno, 1997). Okuno (1997) elevated the two genera above to the generic level and found other significant differences between these two genera: *Cinetorhynchus* has two rows of spines on the ischia and meri of the third to fifth pereiopods. By contrast, *Rhynchocinetes* exhibits a single row of spines on the ischia and meri of the third to fifth pereiopods (see Okuno, 1997: table 1). Importantly, Okuno (1997) found no intermediate species between these two genera and our one-phase SATé-II analysis clearly confirmed this split within the family Rhynchocinetidae using molecular mitochondrial and nuclear gene fragments.

Remarkably, all two- and three-phase phylogenetic analyses using different alignment algorithms and different strategies for the extraction of poorly aligned sequence positions failed to recognize the *Rhynchocinetes–Cinetorhynchus* split within the Rhynchocinetidae *sensu* Holthuis (1995) and Okuno (1997). The well-supported split in SATé-II but its absence in two- and three-phase phylogenetic analyses is herein interpreted as evidence for the superiority of the one-phase SATé-II methodology for phylogenetic inference; the effect of differences in sequence alignments resulting from using different alignment software packages and/or the effect of extracting (or not) poorly aligned sequence positions do not account for the observed differences amongst the one-phase SATé-II and the other two- and three-phase phylogenetic analyses. Instead, the observed differences between the one-phase and two- and three-phase phylogenetic methods can be attributed to the superiority of the SATé-II methodology (see Liu *et al*., 2009a, b, 2011). Overall, this study illustrates the extent of systematic uncertainty that might be caused if two- and three-phase analyses are used during phylogenetic inference with sets of sequences that are difficult to align (i.e. *12S*). This study also illustrates the importance of using one-phase methods for phylogenetic inference. If only the classical two- and/or three-phase methods of phylogenetic inference (and Bayesian hypothesis testing based on these two- and/or three-phase methods) had been used in this study, our phylogenetic analyses would have been flawed as we would have failed to recognize a systematic split, i.e. *Rhynchocinetes–Cinetorhynchus*, that is well supported by morphological traits of adult individuals (Holthuis, 1995; Okuno, 1997). In other words, the *Rhynchocinetes* and *Cinetorhynchus* species studied here would have clustered together and not formed well-supported monophyletic clades as in the one-phase method, incorrectly indicating the absence of morphological homologies and disparities within species from the two different monophyletic clades. We argue in favour of future studies using state-of-the-art one-phase phylogenetic analyses to disentangle the phylogenetic relationships in caridean shrimps and other groups of marine invertebrates. In particular, reviews of previous phylogenetic inferences that may well be robust but not necessarily accurate when using two- and three-phase phylogenetic analyses may be needed using one-phase phylogenetic methods.

The results of this study are based on 50% of the known species of the *Rhynchocinetes–Cinetorhynchus* species complex (De Grave & Fransen, 2011). The lack of a total data set of species is a common problem in phylogenetic studies, and all such studies should be considered somewhat preliminary until the data set is complete. However, our results serve as the first independent test of detailed views on the phylogeny of this group based until now strictly on morphological data. We argue in favour of additional studies using more genes and more complete data sets in order to test outstanding systematic and evolutionary questions about this remarkable clade of caridean shrimps.

Proposal of a phylogenetic rearrangement

Taking into account the discussion above and recent molecular phylogenetic analyses focused on other caridean representatives (i.e. *Xiphocaris*: Page *et al*., 2008; Bracken *et al*., 2009: various caridean families: Bracken *et al*., 2009; Li *et al*., 2011), we tentatively propose the following taxonomic rearrangement within the Infraorder Caridea Dana, 1852:

Eugonatonotus Schmitt, 1926
Family Eugonatonotidae Chace, 1937
Superfamily Eugonatonotoidea Chace, 1937

Nematocarcinus A. Milne-Edwards, 1881
Family Nematocarcinidae Smith, 1884
Superfamily Nematocarcinoidea Smith, 1884

Cinetorhynchus Holthuis, 1995
Rhynchocinetes H. Milne Edwards, 1837
Family Rhynchocinetidae Ortmann, 1890
Superfamily Rhynchocinetoidea Ortmann, 1890

Xiphocaris von Martens, 1872
Family Xiphocarididae Ortmann, 1895
Superfamily Atyoidea De Haan, 1849

Lipkius Yaldwyn, 1960
Family Lipkiidae Burukovsky, 2012
Superfamily incertae sedis

For traits defining the new Family Lipkiidae, please, see Burukovsky (2012).

Implications for the evolution of mating and sexual systems in the Rhynchocinetidae

Although relatively few species have been studied in any detail, it is apparent that there is considerable

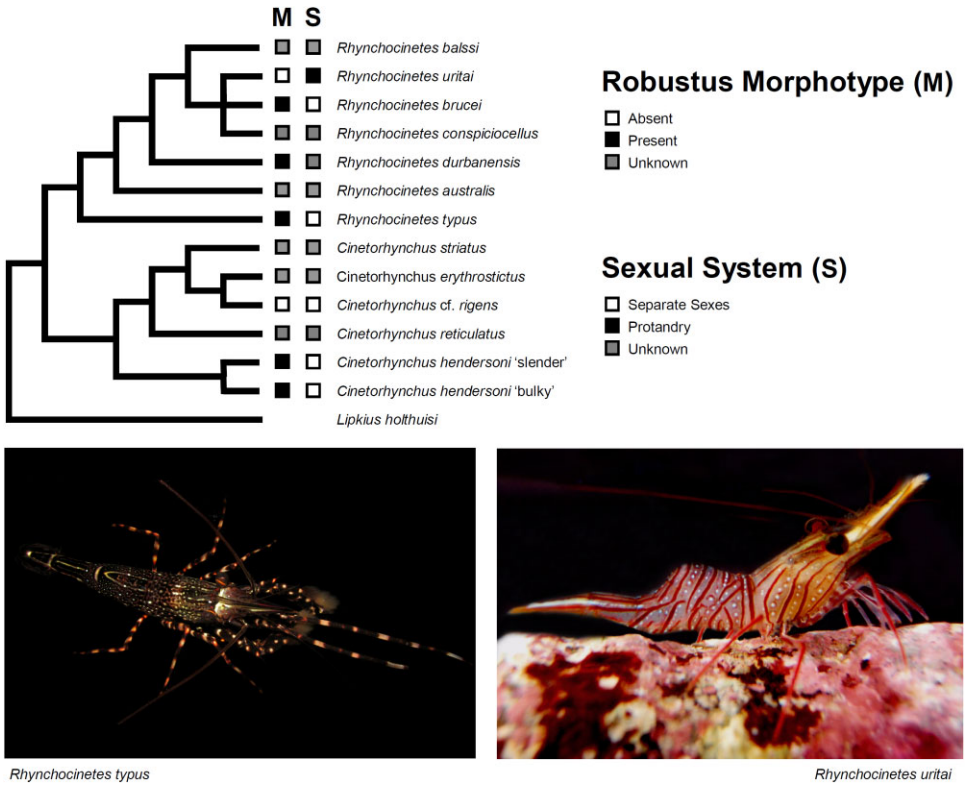

Figure 7. Presence/absence of the 'robustus' morphotype and sexual systems of *Rhynchocinetes* and *Cinetorhynchus* shrimps synthesized on the tree resulting from the one-phase SATé-II analysis of maximum likelihood. Robustus morphotype: presence (black squares), absence (white squares), unknown (grey squares). Sexual system: separate sexes (white squares), protandry (black squares), unknown (grey squares). The photographs show a 'robustus' male morphotype of *Rhynchocinetes typus* (left, bottom) and a male specimen of the protandric *Cinetorhynchus uritai* (right, bottom). Males in the latter species exhibit poorly developed chelipeds and maxillipeds in comparison with 'robustus' males of species of *Rhynchocinetes*. For further details see text. Photographic credits: M. Thiel (*C. uritai*), I. Hinojosa (*Rhynchocinetes typus*).

variation in sexual dimorphism and mating systems amongst species of the Rhynchocinetidae (Correa *et al*., 2003 and subsequent papers on *Rhynchocinetes typus*; Thiel *et al*., 2010 on *R. brucei*; Bauer & Thiel, 2011 on *R. uritai*; Bauer *et al*., in press, on two species from the genus *Cinetorhynchus*; Okuno, 1997 for *Cinetorhynchus* spp.; J. Okuno, pers. observ. on *Rhynchocinetes* and *Cinetorhynchus* spp.). Figure 7 shows an updated but preliminary review of the presence of 'robustus' male morphotypes and sexual systems in the family. Current information indicates two extremes: species with some males of large body size and hypertrophied weapons (first chelipeds and, in some, third maxillipeds) such as *R. typus* (see Correa *et al*., 2003) and *R. brucei* (see Thiel *et al*., 2010). In such species, males display and fight to monopolize matings with females. In two *Cinetorhynchus* species from Hawaii, there is similar sexual dimorphism in size, weaponry, and injuries suggestive of fighting amongst males, presumably over access to females (Bauer *et al*., in press). The other extreme may be represented by *R. uritai*, in which males are smaller than females without sexual dimorphism in weaponry, with limited observations indicating a promiscuous 'pure searching' mating strategy (Correa & Thiel, 2003).

The phylogeny presented in our results is not sufficiently complete (12 of 25 rhynchocinetid species were included in this study) to make a definitive statement on the course of evolution of mating systems and sexual dimorphism in this family. Indeed, the information on mating behaviour, including the presence/absence of males exhibiting large body size and hypertrophied weapons, is limited in this family (Fig. 7). However, the results presented here indicate that in *Rhynchocinetes*, *R. typus*, with large male size and weaponry, and a mate-guarding mating system, is most basal. Yet, the two categories of sexual dimorphism/mating system are scattered amongst the remaining *Rhynchocinetes* species (Fig. 7). In *Cinetorhynchus*, there is no indication at present about which category of sexual dimorphism/mating system is basal or derived (Fig. 7).

Although there are many caridean shrimp species with large males and mate-guarding mating systems, many more caridean species are composed of populations with small males and larger females without sexual dimorphism in weaponry (Bauer, 2004). Such species invariably have a pure search mating strategy. The general occurrence amongst carideans of such sexual dimorphism and mating strategy suggested to Bauer (2004) that these characteristics are ancestral whereas the other extreme is derived. Our results do not yet allow us to accept or reject this hypothesis in the Rhynchocinetidae, and, in order to do so, it is essential to obtain information on these characteristics in as many rhynchocinetid species as possible in order to include a majority if not all species in a phylogenetic analysis.

Another important species trait that varies in the Rhynchocinetidae is the sexual system (gonochory vs. hermaphroditism; see Introduction). Although the majority of caridean species are gonochoric (separate sexes), some species have some sort of sequential (protandric) hermaphroditism in which individuals first develop as males and then later change sex to female (Bauer, 2001; Bauer & Conner, 2011). In a few hippolytid genera, particularly *Lysmata* Risso, 1816, the male-phase individual changes into a simultaneous hermaphrodite although with an overall female phenotype (Bauer & Holt, 1998; Baeza *et al*., 2009, 2010; Onaga *et al*., 2012). The distribution of hermaphroditic species in the Caridea is scattered and somewhat bewildering, with no apparent pattern (Bauer & Conner, 2011). As the sexual system of more and more species is carefully examined, more hermaphroditic species are being discovered (e.g. Baeza, 2010; Baeza & Piantoni, 2010). Thus, it is not surprising that protandry might appear in a rhynchocinetid species comprising small males and larger females (*R. uritai*, Bauer & Thiel, 2011; Fig. 7). Other species whose population structure and reproductive characters have been carefully examined are all gonochoric species with large males and hypertrophied chelipeds (*R. typus*, Correa & Thiel, 2003; *R. brucei*, Thiel *et al*., 2010; two *Cinetorhynchus* species from Hawaii, Bauer *et al*., in press; Fig. 7). As over half of rhynchocinetid species are likely to have a population structure similar to that in *R. uritai*, the discovery of other protandric rhynchocinetids would not be surprising (see Bauer, 2004). Thus, as with mating systems/sexual dimorphism, sexual systems, when known, can be mapped onto a more complete phylogeny in order to follow the course of their evolution within the family. Once the evolutionary history of sexual and/or mating systems is known for the majority of the species within the Rhynchocinetidae, hypotheses on the selective pressures responsible can be made and tested.

Outlook

This study has shed light on the phylogenetic relationships of hinge-beak shrimps (*Rhynchocinetes* and *Cinetorhynchus*) and formally tested key but unresolved systematic questions in shrimps pertaining to the ecologically diverse and species-rich infraorder Caridea (Bracken *et al*., 2009; De Grave *et al*., 2009; De Grave & Fransen, 2011; Liu *et al*., 2011; Baeza, 2013). Additional studies on the morphometrics, behaviour, and sexual system of *Rhynchocinetes*, *Cinetorhynchus*, and related genera as well as new, multilocus phylogenetic hypotheses in this clade are needed in order to reveal the number of times and ecological conditions that have favoured different sexual and social, including mating, systems in the infraorder Caridea.

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