

Molecular phylogeny of shrimps from the genus *Lysmata* (Caridea: Hippolytidae): the evolutionary origins of protandric simultaneous hermaphroditism and social monogamy

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Received 9 May 2008; accepted for publication 20 June 2008

Shrimps from the genus *Lysmata* are known because of their wide diversity of lifestyles, mating systems, symbiotic partnerships, and conspicuous coloration. They can occur in crowds (large aggregations), in small groups, or as socially monogamous pairs. Shrimps from this genus are rare, if not unique among crustaceans, because of their unusual sexual system. To date, the sexual system of all species investigated comprises a protandric simultaneous hermaphroditism: shrimps initially mature and reproduce as males and later in life turn into functional simultaneous hermaphrodites. The evolutionary relationships of the species within the genus are unsettled. A molecular phylogeny of the group may shed light on the evolutionary origins of the peculiar sexual and social systems of these shrimps and help resolve standing taxonomic questions long overdue. Using a 647-bp alignment of the 16S rRNA mitochondrial DNA, we examined the phylogenetic relationship of 21 species of shrimps from the genus *Lysmata* from several biogeographical regions; the Atlantic, Pacific, and Indo-Pacific. The resulting phylogeny indicates that the genus is paraphyletic and includes the genus *Exhippolysmata*. The constituent species are subdivided into three well supported clades: one group exclusively composed of neotropical species; a second clade comprising the Indo-Pacific and Atlantic symbiotic fish cleaner shrimps; and a third clade including tropical and temperate species from the Atlantic and Pacific. The molecular phylogeny presented here does not support a historical contingency hypothesis, previously proposed to explain the origins of protandric simultaneous hermaphroditism within the genus. Furthermore, the present study shows that monogamous pair-living is restricted to one monophyletic group of shrimps and therefore probably evolved only once. © 2009 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2009, **96**, 415–424.

ADDITIONAL KEYWORDS: 16s rRNA – hermaphrodite – mitochondrial DNA.

INTRODUCTION

Shrimps from the genus *Lysmata* Risso, 1816 demonstrate a wide diversity of lifestyles, mating systems, symbiotic partnerships, and coloration. The 36 described species (Chace, 1997; Rhyne & Lin, 2006; Baeza & Anker, 2008; Rhyne & Anker, 2008) inhabit

shallow or deep warm temperate and tropical rocky and coral reefs around the world. Some species live in crowds (aggregations), others in small groups, whereas some species are socially monogamous (pair-living) [e.g. *Lysmata grabhami* (Gordon, 1935); Wirtz, 1997]. Several species with a somewhat drab coloration dwell freely among rocks in temperate localities, whereas other more colourful species inhabit tropical sponges (*Lysmata pedersenii* Rhyne & Lin, 2006;

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Rhyne & Lin, 2006). Other strikingly brilliant species clean fishes [*Lysmata amboinensis* (De Man, 1888); Limbaugh, Pederson & Chace, 1961; Fiedler, 1998]. This behavioural and ecological diversity has attracted the attention of systematists, evolutionary biologists, and behavioural ecologists (Bauer & Holt, 1998; Baeza, 2006; Baeza & Anker, 2008). Ongoing studies use various species from this genus as model systems to test sex allocation and sexual selection theories and the effect of environmental conditions in explaining phenotypic plasticity (Baeza & Bauer, 2004; Baeza, 2006, 2007a, b). Due to their diverse socioecology, shrimps from the genus *Lysmata* represent ideal candidates to explore the role of ecological conditions in explaining evolutionary innovations in the marine environment.

In addition to their assorted lifestyles, shrimps from the genus *Lysmata* are unusual among crustaceans for their enigmatic sexual system. All species studied to date are protandric simultaneous hermaphrodites (PSH) [*L. grabhami*: Wirtz, 1997; *L. amboinensis*: Fiedler, 1998; *Lysmata wurdemanni* (Gibbes, 1850): Bauer & Holt, 1998; *Lysmata seticaudata* Risso, 1816 and *Lysmata nilita* Dohrn & Holthuis, 1950: d'Udekem d'Acoz, 2003; *Lysmata californica* (Stimpson, 1866): Bauer & Newman, 2004; *Lysmata hochi* Baeza & Anker, 2008; Baeza & Anker, 2008; *Lysmata bahia* Rhyne & Lin, 2006 and *Lysmata intermedia* (Kingsley, 1879): Baeza, 2008a; *Lysmata nayaritensis* Wicksten, 2000: Baeza, Reitz & Collin, 2008; *Lysmata boggessi* Rhyne & Lin, 2006 and *Lysmata galapaguensis* Wicksten, 2000: J.A. Baeza, unpubl. observ.]. This unusual sexual system appears to be a trait shared by all species of the genus (Baeza, 2008a).

In shrimps with PSH, juveniles develop to males ('male-phase'; Bauer, 2000). The gonads of these males are ovotestes, with well developed testes but undeveloped ovaries, and these shrimps reproduce solely as males (Bauer & Holt, 1998; Bauer & Newman, 2004; Baeza & Anker, 2008; Baeza *et al.*, 2008). Later in life, males mature to functional simultaneous hermaphrodites capable of reproducing both as male and female. These functional simultaneous hermaphrodites have been termed female-phase individuals or simultaneous hermaphrodite phase individuals by Bauer (2000) and Baeza (2006), respectively. Because most studies employ the term 'simultaneous hermaphrodite' or some variation thereof (Lin & Zhang, 2001; Baeza, 2006, 2007a, b, c; Calado & Dinis, 2007), the terms males and hermaphrodites are used hereafter to describe the two sexual phases.

Protandric simultaneous hermaphroditism appears to be shared by the related genus *Exhippolysmata* Stebbing, 1915 (Kagwade, 1982; Braga *et al.*, 2007; Laubenheimer & Rhyne, 2008). Other than in the

Crustacea, PSH has been demonstrated for a few other marine invertebrates (i.e. the polychaete *Ophryotrocha diadema*: Premoli & Sella, 1995; the land snail *Achatina fulica*: Tomiyama, 1996; the tunicate *Pyura chilensis*: Manríquez & Castilla, 2005; the barnacle *Chelonibia patula*: Crisp, 1983). However, PSH might be more common (Baeza, 2006) than originally reported among both invertebrates and vertebrates (Ghiselin, 1974; Policansky, 1982; Crisp, 1983).

Several interesting questions of evolutionary significance can be raised about the genus *Lysmata*, which has been the focus of an increasing number of studies focusing on systematics (Rhyne & Lin, 2006; Baeza & Anker, 2008; Rhyne & Anker, 2008), behavioural ecology (Baeza & Bauer, 2004; Baeza, 2006, 2007a, b, 2008b; Bauer, 2006), and reproductive biology (Fiedler, 1998; Bauer & Holt, 1998; Bauer & Newman, 2004; Baeza, 2006, 2008a; Baeza *et al.*, 2008). The evolutionary relationships among *Lysmata* spp. are currently unknown because no phylogenies, neither morphological nor molecular, have been published. This lack of phylogenetic knowledge is constraining an understanding of the evolution of sexual and social systems, sex allocation patterns, and cleaning behaviours, among other topics, in marine shrimps.

First, a molecular phylogeny of the *Lysmata* should help resolve the historical origins of the rare, if not unique sexual system from this genus. Although we now know that the variety of lifestyles of *Lysmata* is greater than originally recognized (Baeza, 2008a; Baeza & Anker, 2008; Baeza *et al.*, 2008), an emerging dichotomy in social organization and ecology was noticed in the initial studies. Some species were noticed to live in crowds (aggregations), whereas other specialized fish cleaners are socially monogamous (living in pairs) and live in symbiosis with sea anemones (Bauer, 2006). Currently, it is not known whether the symbiotic socially monogamous condition evolved once or various times independently within the genus. Based on this initial dichotomy, Bauer (2006) suggested that PSH evolved in the tropics from an ancestral protandric species of *Lysmata* that became a specialized fish cleaner. Restricted mobility of individuals due to their association with the host, and hence a reduced probability of encountering mating partners, would have favoured PSH (Bauer, 2000). Under such a scenario, the crowd warm-temperate species that do not exhibit specialized cleaning behaviours would have evolved from tropical species with specialized cleaning behaviours and more complex mating systems (social monogamy) (Bauer, 2006).

A phylogeny should also help answer other long-standing controversies about various systematic questions. In the present study, we have specifically focused in addressing the phylogenetic position of the

Table 1. *Lyсмata* species and other hippolytid shrimps used for the phylogeny reconstruction

Species	Collection site (year)	CN/GenBank
<i>Lyсмata amboinensis</i> (De Man, 1888), Java	Aquarium store, Singapore (2000)	SMF 32281/EU861487
<i>Lyсмata amboinensis</i> , Philippines	Aquarium store, FL, USA (2006)	UMML 32.9451/EU861488
<i>Lyсмata ankeri</i> Rhyne & Lin, 2006	SMEE, Fort Pierce (2006)	UMML 32.9452/EU861501
<i>Lyсмata bahia</i> Rhyne & Lin, 2006	Bocas del Toro, Panama (2006)	UMML 32.9453/EU861503
<i>Lyсмata boggești</i> Rhyne & Lin, 2006	St Petersburg, FL, USA (2006)	UMML 32.9454/EU861505
<i>Lyсмata californica</i> (Stimpson, 1866)	La Jolla, CA, USA (2006)	UMML 32.9455/EU861498
<i>Lyсмata debelius</i> Bruce, 1983, Indo-Pacific	Aquarium store, LA, USA (2001)	SMF 32009/EU861491
<i>Lyсмata debelius</i> , Java	Aquarium store, Singapore (1999)	SMF 32280/EU861493
<i>Lyсмata debelius</i> , Philippines	Aquarium store, FL, USA (2006)	UMML32.9456/EU861492
<i>Lyсмata galapaguensis</i> Wicksten, 2000	Islas Secas, Panama (2007)	UMML32.9457/EU861480
<i>Lyсмata gracilirostris</i> Wicksten, 2000	Venao, Panama (2006)	UMML32.9458/EU861502
<i>Lyсмata grabhami</i> (Gordon, 1935), Haiti	Aquarium store, FL, USA (2006)	UMML32.9459/EU861489
<i>Lyсмata grabhami</i> , Madeira	Madeira, Portugal (2001) (Ricardo Calado)	SMF 32007/EU861490
<i>Lyсмata hochi</i> Baeza and Anker, 2008	Long Key, FL (2007)	UMML32.9460/EU861507
<i>Lyсмata intermedia</i> (Kingsley, 1879)	Bocas del Toro, Panama (2007)	UMML32.9461/EU861484
<i>Lyсмata moorei</i> (Rathbun, 1901)	Galeta, Panama (2007)	UMML32.9462/EU861481
<i>Lyсмata nayaritensis</i> Wicksten, 2000	Chumical, Panama (2007)	UMML32.9463/EU861506
<i>Lyсмata nilita</i> Dohrn and Holthuis, 1950	Giglio, Italy (2000) (Cédric d'Udekem d'Acoz)	SMF32005/EU861482
<i>Lyсмata olavoï</i> Fransen, 1991	Azores, Portugal (1999) (Cédric d'Udekem d'Acoz)	SMF 32006/EU861494
<i>Lyсмata pedersenï</i> Rhyne & Lin, 2006	Carrie Bow, Belize (2007)	UMML 32.9464/EU861504
<i>Lyсмata rafa</i> Rhyne & Anker, 2008	Aquarium Store, FL, USA	UMML 32.9465/EU861495
<i>Lyсмata seticaudata</i> (Risso, 1816)	Corsica, France (2003)	SMF 32004/EU861485
<i>Lyсмata seticaudata</i>	Cabo Raso, Cascais, Portugal (Cédric d'Udekem d'Acoz)	SMF 32003/EU861486
<i>Lyсмata cf. trisetacea</i> (Heller, 1861)	Chumical, Panama (2007)	UMML 32.9466/EU 861483
<i>Lyсмata wurdemanni</i> (Gibbes, 1850), TX	Port Aransas, TX, USA (2000)	SMF 32008/EU861496
<i>Lyсмata wurdemanni</i> , West FL	St Petersburg, FL, USA (2006)	UMML 32.9467/EU861497
<i>Lyсмata wurdemanni</i> , East FL	Fort Pierce, FL, USA (2006)	UMML 32.9468/EU861500
<i>Exhippolysmata ophloroides</i> (Holthuis, 1948)	Ubatuba Bay, Brazil (2006)	UMML 32.9469/EU861510
<i>Heptacarpus palpator</i> (Owen, 1939)	La Jolla, CA, USA (2001)	SMF 32282/EU861509
<i>Hippolyte inermis</i> Leach, 1815	Venice Lagoon, Italy (1997) (Cédric d'Udekem d'Acoz)	SMF 32283/EU861511
<i>Hippolyte williamsi</i> Schmitt, 1924	Puerto Aldea, Chile (2007)	UMML 32.9470/EU861512
<i>Merguia rhizophorae</i> (Rathbun, 1900)	Bocas del Toro, Panama (2007)	UMML 32.9471/EU861508
<i>Tozeuma carolinense</i> Kingsley, 1878	St Petersburg, FL (2007)	UMML 32.9472/EU861513

The sites of collection, dates, museum catalogue number (CN: UMML, University of Miami Marine Laboratories, Rosenthal School of Marine Science, University of Miami, SMF Senckenberg Museum Frankfurt, Germany) and the Genbank accession numbers (GenBank) are shown for each species.

genus *Exhippolysmata*, the only other known caridean genus with PSH (Braga *et al.*, 2007; Laubenhimer & Rhyne, 2008), with respect to members of the genus *Lyсмata*.

We present a molecular phylogeny of the genus *Lyсмata* based on the large subunit 16S mitochondrial rRNA gene upon examination of 20 available species from the genus plus outgroups. The significance of the phylogeny for answering the questions posed above is discussed.

MATERIAL AND METHODS

A total of 28 specimens from 21 species of shrimps from the genus *Lyсмata* and *Exhippolysmata ophloroides* were included in the present study (Table 1). One specimen each of *Merguia rhizophorae*, *Heptacarpus palpator*, *Tozeuma carolinense*, *Hippolyte williamsi*, and *Hippolyte inermis* was also included as the outgroup during the phylogenetic analyses. Most shrimp species were collected between

2006 and 2007 from different localities in Bocas del Toro and Islas Secas and other localities close to Naos Island (Panama), Florida and Texas (USA). Four species (*Lysemata ankeri*, *Lysemata debelius*, *L. amboinensis* and *L. grabhami*) were either purchased from aquarium stores in Fort Pierce (FL, USA) or donated from the Smithsonian Marine Ecosystems Exhibit (Fort Pierce, FL, USA). The remaining species were from colleague donations (see Acknowledgements). Immediately after collection or purchasing, specimens were preserved in 95–99% ethanol. In the laboratory, the different species were identified as previously reported by Bruce (1983), Rhyne & Lin (2006), Baeza & Anker (2008), Rhyne & Anker (2008) and using the keys of Chace (1972, 1997) and Wicksten (2000).

Total genomic DNA was extracted from abdominal muscle tissue using the Qiagen DNeasy Blood and Tissue Kit following the manufacturer's protocol. The polymerase chain reaction (PCR) was used to amplify an approximately 550-bp region (excluding primers) of the 16S rRNA with the primers 16L2 (5'-TGCCTGTTTATCAAAAACAT-3'), and 1472 (5'-AGATAGAAACCAACCTGG-3') (Schubart, Neigel & Felder, 2000; Schubart, Cuesta & Felder, 2002). Standard PCR 25- μ L reactions [2.5 μ L of 10 \times Taq buffer, 2 μ L of 50 mM MgCl₂, 2.5 μ L of 10 mM dNTPs, 2.5 μ L each of the two primers (10 mM), 0.625 U Taq, 1.25 μ L of 20 mM BSI and 8.625 μ L double distilled water] were performed on a Peltier Thermal Cycler (DYAD) under the conditions: initial denaturation at 96 °C for 4 min followed by 40 cycles of 94 °C for 45 s, 48–52 °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 sequenced with the ABI Big Dye Terminator Mix (Applied Biosystems) at the Laboratory of Analytical Biology of the National Museum of Natural History (Washington, DC), which is equipped with an ABI Prism 3730xl Genetic Analyser (Applied Biosystems). All sequences were confirmed by sequencing both strands and a consensus sequence for the two strands was obtained using the software SEQUENCHER, version 4.5 (Gene Codes Corp.). A smaller number of sequences were obtained at the laboratories of the University of Regensburg using the same primers and PCR conditions and otherwise the protocol outlined in Schubart *et al.* (2002).

The final set of consensus sequences was aligned with the integrated CLUSTALW and corrected manually with BIOEDIT, version 7 (Hall, 1999) and then exported to PAUP* (Swofford, 2002) and MrBayes (Huelsenbeck, 2000). First, the dataset was analysed with MODELTEST, version 3.7 (Posada & Crandall,

1998) in PAUP*, which compares different models of DNA substitution in a hierarchical hypothesis-testing framework to select a base substitution model that best fits the data. The optimal model found by MODELTEST (selected with hierarchical likelihood ratio tests) was a TVM+I+G evolutionary model ($-\ln L = 6189.1997$). The calculated parameters were: assumed nucleotide frequencies A = 0.3441, G = 0.1839, T = 0.3796, C = 0.0924; substitution rate matrix with A–C substitution = 1.0, A–G = 6.4199, A–T = 1.0, C–G = 1.0, C–T = 9.3030, G–T = 1.0; rates for variable sites assumed to follow a gamma distribution (G) with shape parameter = 0.3711 and a proportion of invariable sites (I) = 0.2193.

Phylogenetic analyses conducted herein were maximum parsimony (MP) and maximum likelihood (ML) (in PAUP*) and Bayesian inference (BI; in MrBayes). MP analysis was performed as a heuristic search with a starting tree obtained via stepwise addition, random addition of sequences, random replicates, and tree-bisection-reconnection branch swapping. For ML, the specifications were the same as in MP. However, branch swapping was performed in the starting tree and all other parameters used were those of the default option in PAUP*. For BI, we used unique random starting trees in the Metropolis-coupled Markov Monte Carlo Chain (MCMC) (Huelsenbeck, 2000). During a first preliminary run, convergence was achieved after 5000 generations at a likelihood value of -6222.45 . A final analysis was performed for 6 000 000 generations. Every 100th tree was sampled from the MCMC analysis obtaining a total of 60 000 trees. From the preliminary run we determined a burn-in period of 10 000 generations, calculating a consensus tree with the 50% majority rule for the last 59 900 sampled trees. We assessed the robustness of the MP and ML tree topologies by bootstrap reiterations of the data 2000 and 100 times, respectively and reconstructing trees using each resampled data set (Felsenstein, 1985). Support for nodes in the BI tree topology was obtained by posterior probability values that represent the frequency with which each clade occurred within the collection of trees provided by the analysis.

RESULTS

A total of 647 homologous alignment positions were used during the present phylogenetic analysis and 257 of these were found to be parsimony informative positions. It is noteworthy that the species *L. bahia* had an insertion of 71 bp in the middle of the 16S fragment and not shared by any other species. All phylogenetic trees obtained with the different inference methods (MP, ML, and BI) resulted in the same general topology (Fig. 1). Considering our pool of out-

group species, belonging to three different genera within the Hippolytidae, the genus *Lyсмata* plus *Exhippolymata ophophoroides* represent a monophyletic clade and *Lyсмata olavoi* is the most basally positioned species within this clade, which is supported by a high posterior probability obtained from the BI analysis. Bootstrap support from the ML and MP was in general lower. The overall tree topology suggests that the genus *Lyсмata* can be divided into three main clades plus a number of unresolved species. One clade, hereafter named 'Tropical American', is composed almost entirely by species from the Caribbean. Within this clade, the only species from outside the Caribbean is *Lyсмata gracilirostris* from the eastern tropical Pacific. The basal position of this species within the clade is not well supported. Also, two pairs of species are well supported as sister species: *L. pedersenii*–*L. ankeri* and *L. boggessi*–*Lyсмata rafa*. Interestingly, the specimens of *L. wurdemanni* collected from distant localities from the northern Gulf of Mexico (Texas and western Florida) are more closely related to each other than to the specimen collected from the east coast of Florida. Consequently, the latter specimen holds a basal position within this species.

The second so-called 'Cosmopolitan' clade is composed of six species, two from the Mediterranean (*L. seticaudata* and *L. nilita*), two from the Atlantic (*Lyсмata moorei* and *L. intermedia*) and two from the tropical eastern Pacific (*L. galapaguensis* and *Lyсмata cf. trisetacea*). Within this clade, the status of *L. intermedia* and *L. cf. trisetacea* as a pair of trans-isthmian sister species is well supported and may be used for molecular clock calibrations in the future.

The third group, hereafter named 'Cleaner' clade, is composed by the fish-cleaning shrimps *L. debelius*, *L. amboinensis* and *L. grabhami*. In the tree, the phylogenetic positions of *L. bahia*, *L. californica* and *L. nayaritensis* are not well supported by any of our phylogenetic analyses. Most interesting is that the Caribbean *L. hochi* and the Brazilian *Exhippolymata ophophoroides* form a monophyletic clade in all reconstruction methods, which is highly supported by BI. However, the position of this clade among the other representatives of *Lyсмata* is not well supported.

Interestingly, all of the species whose sexual system have been examined (all of them feature PSH) belong to all three natural clades here revealed (Fig. 1). The distributions of the different socioecologies (social monogamy versus crowds) and lifestyles (symbiosis versus free-living) were not interspersed in the tree (Fig. 2). Social monogamy was restricted to the 'Cleaner' clade, whereas crowd species were found in other clades either more or less derived than the 'Cleaner' clade. Some species recently reported as living in small groups with local abundances much

lower than that reported for crowd species (*L. bahia*, *L. hochi*, and *L. intermedia*; Baeza *et al.*, 2008) are also present in two natural groups other than the 'Cleaner' clade (Fig. 2). In addition, symbiotic species are almost invariably restricted to the 'Cleaner' clade. The only exception is *L. pedersenii* from the 'American-Tropical' clade known from tube sponges in the Caribbean.

DISCUSSION

We present, for the first time, a molecular phylogeny of shrimps from the genus *Lyсмata* based on a segment of the 16S rRNA mitochondrial gene. Although not all of the 36 species described for the genus could be included, our analyses with three different phylogenetic reconstruction methods support the monophyly of this genus together with the genus *Exhippolymata*. Therefore, the genus *Lyсмata* is currently paraphyletic. Below, we discuss our findings with respect to the evolutionary origins of PSH and the different social structures observed in this genus.

EVOLUTIONARY ORIGINS OF PSH AND LIFESTYLES IN *LYSMATA*

In our molecular phylogeny, the symbiotic fish cleaner shrimps *L. amboinensis*, *L. grabhami*, and *L. debelius* cluster together within a well-supported clade. This 'Cleaner' clade holds a derived phylogenetic position with respect to the 'Cosmopolitan' clade and *L. olavoi*. By contrast to the 'Cleaner' clade, composed solely by socially monogamous species, several species in the 'Cosmopolitan' clade as well as in the other natural clades live in crowds and do not engage in any symbiotic partnership with sessile macroinvertebrates (*L. galapaguensis*, *L. cf. trisetacea*; J. A. Baeza, unpubl. data). Furthermore, all of the species belonging to the 'Cosmopolitan' and 'American Tropical' clades whose sexual system have been examined, were found to be protandric simultaneous hermaphrodites (Rhyne & Lin, 2006; Baeza, 2008a; J. A. Baeza, unpubl. data). Indeed, it appears that PSH represents a conserved trait within the genus *Lyсмata* (Baeza, 2008a) and *Exhippolymata* (Kagwade, 1982; Braga *et al.*, 2007). The fact that a basally positioned group of species pertaining to the 'Cosmopolitan' clade features PSH and most commonly lives freely in the intertidal as aggregations (crowds) does not support Bauer's (2000) view about the historical origins of the sexual system. According to the historical contingency hypothesis of Bauer (2000), PSH originated in an ancestral protandric species of *Lyсмata* that became a symbiotic specialized fish cleaner. The crowd warm-temperate species that do not exhibit specialized cleaning behaviours would have invariably evolved from tropical

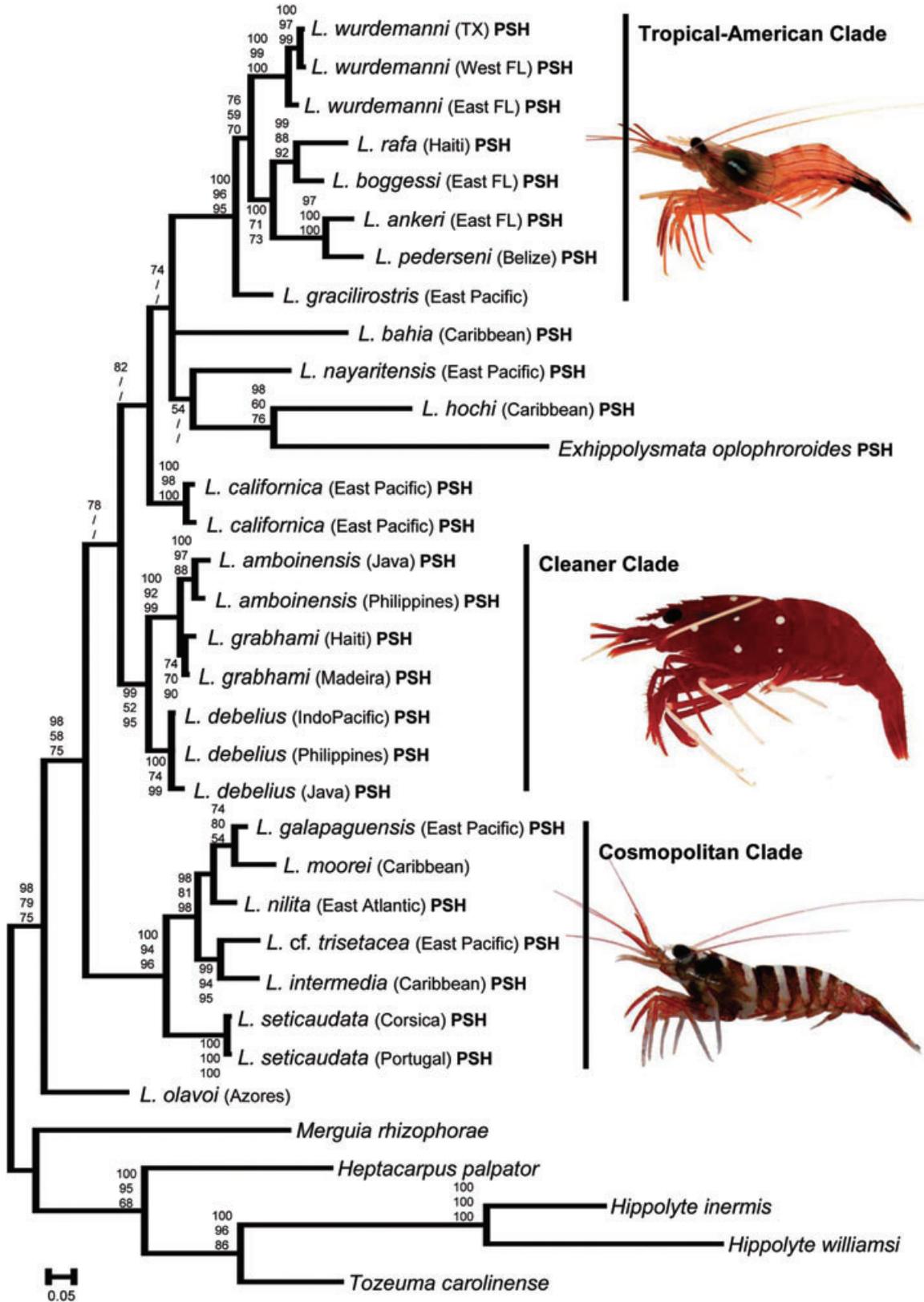


Figure 1. Phylogenetic tree obtained from Bayesian inference (BI) analysis of the partial 16S rRNA gene for shrimps from the genus *Lyсмata*, and other selected taxa from the family Hippolytidae. Numbers above or below the branches represent the posterior probabilities from the BI analysis and bootstrap values obtained either from maximum likelihood (ML) or maximum parsimony (MP) analyses in PAUP* (BI/ML/MP). The general topology of the trees obtained from MP and ML analyses was the same. PSH indicates that protandric simultaneous hermaphroditism has been confirmed as the sexual system of a particular species (for details, see text). The images of the shrimps (from top to bottom) represent *Lyсмata boggeѕsi*, *Lyсмata debelius*, and *Lyсмata galapaguensis*.

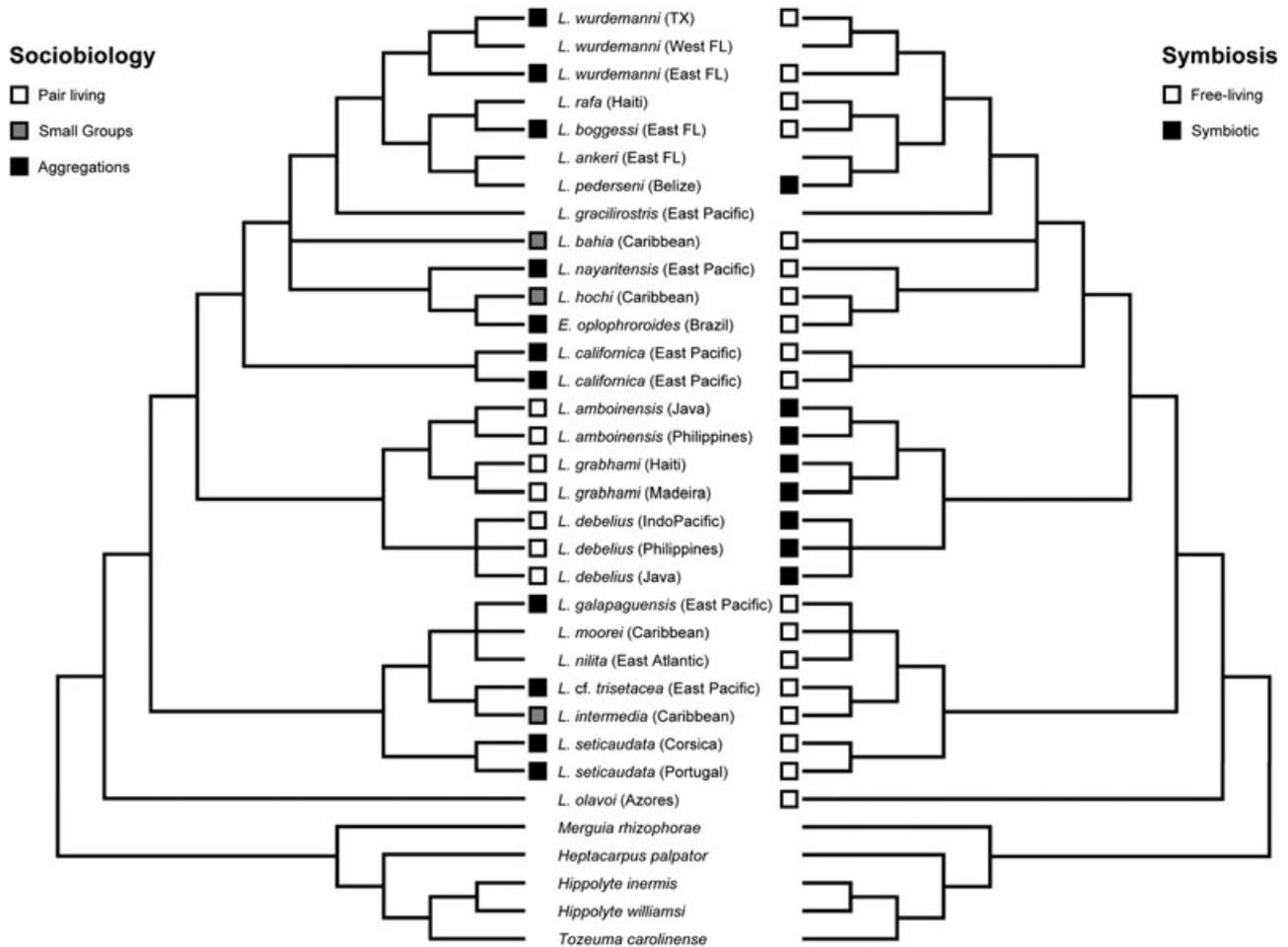


Figure 2. Sociobiology and lifestyle in shrimps from the genus *Lyсмata*. On the left, the white, gray and black squares represent the socioecology (social monogamy, small groups and crowds, respectively) of each species. On the right, the white and black squares represent the adoption of a symbiotic or free-living lifestyle by a particular species, respectively. The absence of a square indicates that the socioecology or lifestyle of a particular species is not known. In the text, ‘social monogamy’ and ‘crowds’ are occasionally used as terms for ‘pair-living’ and ‘aggregations’, respectively.

species with specialized cleaning behaviours and more complex mating systems (social monogamy) (Bauer, 2006). Thus, we a priori expected that all of the species with a free-living life style and not featuring specialized cleaning behaviours (originally termed as ‘crowd’ species; Bauer, 2000), but shown as belonging to the ‘Cosmopolitan’ and ‘Tropical-American’ clades in the present study, would have clustered in one or more clades invariably separated on their own branch from

the ‘Cleaner’ socially monogamous clade. Recent studies have demonstrated that shrimps from the genus *Lyсмata* feature a diversity of lifestyles, wider than initially recognized (Baeza, 2008a; Baeza & Anker, 2008; Baeza *et al.*, 2008). Further detailed studies on the lifestyle and sexual system of the species within this genus and others related (*Lyсмatella*, *Mimocaris*, *Exhippolyсmata*) and the development of a new more robust phylogeny with additional molecular

markers should help us elucidate the actual historical origins of PSH among shrimps in the near future.

The fish cleaner shrimps *L. amboinensis*, *L. grabhami*, and *L. debelius* clustered together within a well supported clade that is derived compared to the 'Cosmopolitan' clade and *L. olavoi*. The information above suggests that social monogamy and the adoption of a symbiotic lifestyle (e.g. associated with sea anemones) are derived conditions within the genus and that these traits evolved once in the ancestor of the 'Cleaner' clade. A second independent origin of a symbiotic lifestyle occurred in the 'Tropical American' clade because *L. pedersenii* is known only from tube sponges (Rhyne & Lin, 2006; J. A. Baeza, unpubl. data). Unfortunately, the host use pattern and population distribution of this species in their sponge host is presently not known. Most importantly, for the 'Cleaner' clade, the origins of pair living and the adoption of a symbiotic lifestyle appear to be linked in some manner with the origins of cleaning behaviour; all three traits are present in the 'Cleaner' clade but absent in the remaining natural clades, except for *L. pedersenii*. However, the reasons for the association among these three traits are not evident at first sight. Social monogamy is known to occur in several other free-living and symbiotic crustaceans, including shrimps (Baeza, 1999, 2008b; Baeza & Thiel, 2007). Various hypotheses have been proposed to explain the origins and adaptive value of social monogamy ('mate guarding' hypothesis: Parker, 1970; Grafen & Ridley, 1983; 'territorial cooperation' hypothesis: Wickler & Seibt, 1981; 'environmental constraints' hypothesis: Baeza & Thiel, 2007). Because food obtained from client fish might be the most important food source for these cleaner shrimps, and a cleaning station therefore would be a highly valuable resource (as suggested for cleaner fish; Sikkel, Cheney & Côté, 2004), it might be possible that a pair of shrimps will be more successful in establishing and defending a cleaning spot (i.e. a territory) than a single individual. The benefits derived from the shared defence of a cleaning station might be driving territoriality and social monogamy in these shrimps once cleaning behaviour has evolved. Alternatively, cleaner shrimps might live in pairs but not necessarily in long-term monogamy; shrimps might be moving rather frequently among cleaning stations shifting mating partners serially during their lifetime, as suggested for other socially monogamous shrimp *Hymenocera picta* (see Wickler & Seibt, 1981). The adaptive value of social monogamy for *Lysmata* cleaner shrimps needs to be clarified. Shrimps in the 'Cleaner' clade might be interesting examples with which to experimentally test hypotheses about social monogamy and the adoption of symbiotic lifestyles in the marine environment.

Interestingly, although social monogamy in the Cleaner clade appears to represent a conserved trait, the lifestyle in the other clades is variable, with closely related species occurring as small groups or aggregations within the same clade. Some species of *Lysmata* inhabit environments in which persistence might be difficult. For example, *L. pedersenii* occur at very low frequencies in tube sponges that harbour a diverse assemblage of fish and other crustaceans (Rhyne & Lin, 2006; JAB, unpublished observations). Similarly, *L. rafa* occur at low frequency in an environment where predation pressure seems to be high (Rhyne & Anker, 2008). PSH in the genus *Lysmata* might represent a key innovation favouring the radiation of shrimps into environments in which it might be difficult to persist for species with separate sexes.

SYSTEMATICS OF THE GENUS *LYSMATA*

The apparently paraphyletic status of the genus *Lysmata* is caused by the position of *E. oplophoroides*, from Brazil, that clusters together with the recently described *L. hochi* from the Caribbean. Although the position of these species among the other representatives of *Lysmata* is not clear, their relatedness was supported by all tree construction methods. Inclusion of a shorter unpublished sequence of a second species of *Exhippolysmata* made available by Dr. Xinzhen Li (Institute of Oceanology, Chinese Academy of Sciences, China), confirmed the position of a second member of this genus next to *L. hochi* (not shown). The differences in morphology between these two genera are obvious. Shrimps from the genus *Exhippolysmata* are characterized by slender legs, an extremely long rostrum (longer than the carapace), and a dorsal basal crest of teeth (Holthuis, 1948). In turn, in *L. hochi*, as well as in other *Lysmata* spp., the pereopods are comparatively robust, the rostrum is short (usually much shorter than the carapace), and the dorsal teeth of the carapace are equidistantly spaced, never forming a crest (Holthuis, 1948; Baeza & Anker, 2008). This degree of morphological differentiation between closely related species may not be uncommon within the genus. Because the topology of our phylogenetic trees does not support the position of the genus *Exhippolysmata* as a natural sister clade to *Lysmata*, the latter might represent a derived clade of *Lysmata* shrimps that colonized deeper waters, with the presence of a long rostrum and slender long pereopods being common among several deep water shrimp taxa (Bauer, 2004). Because this relationship was supported by all reconstruction methods, regardless whether based on parsimony, likelihood, or distances (results of simpler distance models not shown), it appears highly unlikely that the clustering of *E. oplophoroides* and *L. hochi* into a single clade is due

to long-branch attraction. Future phylogenies including more species from both genera as well as other related ones (i.e. *Lyssmatella*, *Merhippolyte*, *Parhippolyte*, *Mimocaris*), and with more and independently segregating genetic markers, are necessary to confirm the actual phylogenetic position of *Exhippolyasmata*.

ACKNOWLEDGEMENTS

J.A.B. thanks Bill Hoffman from the Smithsonian Marine Ecosystems Exhibit (SMEE), Valerie Paul, Raphael Ritson-Williams, Koty Sharp, and Sherry Reed from the Smithsonian Marine Station at Fort Pierce (SMSFP); ORA at Harbor Branch Oceanographic Institution (HBOI); Darryl Felder from the University of Louisiana at Lafayette (ULL); and Arthur Anker, Rachel Collin, Arcadio Ortiz, and Maricela Salazar from the Smithsonian Tropical Research Institute (STRI), Panama, for their help during the different steps of specimens collection, fixation, transportation and sequencing. Arcadio Ortiz from STRI gently worked out local collecting permits for shrimps in Kunayala, Panama. Drs Cédric d'Udekem d'Acoz, Fernando Mantelatto, and Ricardo Calado kindly made available some species from their collections. J.A.B. thanks Dr Rachel Collin for inviting him to participate on the research cruise to Islas Secas, Isla de la Coiba, and other islands off the tropical eastern Pacific onboard of the R/V Urraca, STRI, Panama, where various shrimp species were collected. Many thanks to Jeff Hunt and Lee Weigt at the Laboratory of Analytical Biology (NMNH, Washington, DC) for their logistical support. Dr. Xinzheng Li (Institute of Oceanology, Chinese Academy of Sciences, China) kindly made available an unpublished sequence of a species of *Exhippolyasmata*. J.A.B. thanks the support from a STRI Marine Postdoctoral Fellowship and a SMSFP Postdoctoral Fellowship. C.D.S. and P.Z. thank Jürgen Heinze for making available facilities at the Department of Biology of the University of Regensburg to carry out parts of this study. This is contribution number 122 of the ULL Laboratory for Crustacean Research. This is contribution number 753 of the Smithsonian Marine Station of Fort Pierce, Florida.

REFERENCES

- Baeza JA. 1999.** Indicators of monogamy in the commensal crab *Pinnixa transversalis* (Milne Edwards & Lucas) (Decapoda: Brachyura: Pinnotheridae): population distribution, male–female association, and sexual dimorphism. *Revista de Biología Marina y Oceanografía, Valparaíso* **34**: 303–313.
- Baeza JA. 2006.** Testing three models on the adaptive significance of protandric simultaneous hermaphroditism in a marine shrimp. *Evolution* **59**: 1840–1850.
- Baeza JA. 2007a.** Sex allocation in a simultaneously hermaphroditic marine shrimp. *Evolution* **61**: 2360–2373.
- Baeza JA. 2007b.** Male mating opportunities affect sex allocation in a protandric-simultaneous hermaphroditic shrimp. *Behavioral Ecology and Sociobiology* **61**: 365–370.
- Baeza JA. 2007c.** No effect of group size on sex allocation in a protandric-simultaneous hermaphroditic shrimp. *Journal of the Marine Biological Association of the United Kingdom* **87**: 1169–1174.
- Baeza JA. 2008a.** Protandric simultaneous hermaphroditism in the shrimps *Lyssmata bahia* and *L. intermedia*. *Invertebrate Biology* **127**: 181–188.
- Baeza JA. 2008b.** Social monogamy in the shrimp *Pontonia margarita*, a symbiont of *Pinctada mazatlanica*, in the tropical eastern Pacific coast. *Marine Biology* **153**: 387–395.
- Baeza JA, Anker A. 2008.** *Lyssmata hochi* n. sp., a new species of hermaphroditic shrimp from the southern Caribbean. *Journal of Crustacean Biology* **28**: 148–155.
- Baeza JA, Bauer RT. 2004.** Experimental test of socially mediated sex change in a protandric simultaneous hermaphroditic, the marine shrimp *Lyssmata wurdemanni* (Caridea: Hippolytidae). *Behavioral Ecology and Sociobiology* **55**: 544–550.
- Baeza JA, Reitz R, Collin R. 2008.** Protandric simultaneous hermaphroditism and sex ratio in the shrimp *Lyssmata nayaritensis*. *Journal of Natural History* **41**: 2843–2850.
- Baeza JA, Thiel M. 2007.** The mating system of symbiotic crustaceans: a conceptual model based on optimality and ecological constraints (Chapter 12). In: Duffy JE, Thiel M, eds. *Evolutionary ecology of social and sexual systems: crustaceans as model organisms*. New York, NY: Oxford University Press, 250–267.
- Bauer RT. 2000.** Simultaneous hermaphroditism in caridean shrimps: a unique and puzzling sexual system in the Decapoda. *Journal of Crustacean Biology* **20** (Spec. No. 2): 116–128.
- Bauer RT. 2004.** *Remarkable shrimps: natural history and adaptations of the carideans*. Norman, OK: University of Oklahoma Press.
- Bauer RT. 2006.** Same sexual system but variable sociobiology: evolution of protandric simultaneous hermaphroditism in *Lyssmata* shrimps. *Integrative and Comparative Biology* **46**: 430–438.
- Bauer RT, Holt GJ. 1998.** Simultaneous hermaphroditism in the marine shrimp *Lyssmata wurdemanni* (Caridea: Hippolytidae): an undescribed sexual system in the decapod Crustacea. *Marine Biology* **132**: 223–235.
- Bauer RT, Newman WA. 2004.** Protandric simultaneous hermaphroditism in the marine shrimp *Lyssmata californica* (Caridea: Hippolytidae). *Journal of Crustacean Biology* **24**: 131–139.
- Braga AA, Lopez-Greco LS, Santos DC, Fransozo A. 2007.** *Exhippolyasmata oplophoroides* (Alpheoidea, Hippolytidae): a new species of protandric simultaneous hermaphroditic caridean shrimp. *Proceedings of the Crustacean Society Mid-Year Meeting*, Coquimbo, Chile: 78.
- Bruce AJ. 1983.** *Lyssmata debelius*, new species, a new

- hippolytid shrimp from the Philippines. *Revue Francaise d'Aquariologie et Herpetologie* **4**: 115–120.
- Calado R, Dinis MT. 2007.** Minimization of precocious sexual phase change during culture of juvenile ornamental shrimps *Lysmata seticaudata* (Decapoda: Hippolytidae). *Aquaculture* **269**: 299–305.
- Chace FA Jr. 1972.** The shrimps of the Smithsonian-Bredin Caribbean Expeditions with a summary of the West Indian shallow-water species. *Smithsonian Contributions to Zoology* **98**: 1–179.
- Chace FA Jr. 1997.** The caridean shrimps (Crustacea: Decapoda) of the Albatross Philippine Expedition, 1907–1910, Part 7: Families Atyidae, Eugonatonotidae, Rhynchocinetidae, Bathypalaemonellidae, Processidae, and Hippolytidae. *Smithsonian Contributions to Zoology* **587**: 1–106.
- Crisp DJ. 1983.** *Chelonobia patula* (Ranzani), a pointer to the evolution of the complementary male. *Marine Biology Letters* **4**: 281–294.
- Felsenstein J. 1985.** Phylogenies and the comparative method. *American Naturalist* **125**: 1–15.
- Fiedler GC. 1998.** Functional, simultaneous hermaphroditism in female-phase *Lysmata amboinensis* (Decapoda: Caridea: Hippolytidae). *Pacific Science* **52**: 161–169.
- Ghiselin MT. 1974.** *The economy of nature and the evolution of sex*. Berkeley, CA: University of California Press.
- Grafen A, Ridley M. 1983.** A model of mate guarding. *Journal of Theoretical Biology* **102**: 549–567.
- Hall TA. 1999.** BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95–98.
- Holthuis LB. 1948.** Note on some Crustacea Decapoda Natantia from Suriname. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, Series C, Biological and Medical Sciences* **51**: 1104–1113.
- Huelsenbeck JP. 2000.** *MrBayes: Bayesian Inferences of Phylogeny (software)*. New York, NY: University of Rochester.
- Kagwade PV. 1982.** The hermaphrodite prawn *Hippolysmata ensirostris* Kemp. *Indian Journal of Fisheries* **28**: 189–194.
- Laubenheimer H, Rhyne AL. 2008.** Experimental confirmation of protandric simultaneous hermaphroditism in a Caridean shrimp outside of the genus *Lysmata*. *Journal of the Marine Biological Association of the United Kingdom* **88**: 301–305.
- Limbaugh C, Pederson H, Chace FA Jr. 1961.** Shrimps that clean fishes. *Bulletin of Marine Science* **11**: 237–257.
- Lin J, Zhang D. 2001.** Reproduction in a simultaneous hermaphroditic shrimp, *Lysmata wurdemanni*: any two will do? *Marine Biology* **139**: 919–922.
- Manríquez PH, Castilla JC. 2005.** Self-fertilization as an alternative mode of reproduction in the solitary tunicate *Pyura chilensis*. *Marine Ecology Progress Series* **305**: 113–125.
- Parker GA. 1970.** Sperm competition and its evolutionary consequences in the insects. *Biological Reviews* **45**: 525–567.
- Policansky D. 1982.** Sex change in plants and animals. *Annual Review of Ecology and Systematics* **13**: 417–495.
- Posada D, Crandall KA. 1998.** Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**: 817–818.
- Premoli MC, Sella G. 1995.** Sex economy in benthic polychaetes. *Ethology, Ecology and Evolution* **7**: 27–48.
- Rhyne AL, Anker A. 2008.** *Lysmata rafa*, a new species of peppermint shrimp (Crustacea, Caridea, Hippolytidae) from the subtropical western Atlantic. *Helgoländer Marine Research* **61**: 291–296.
- Rhyne AL, Lin J. 2006.** A western Atlantic peppermint shrimp complex: redescription of *Lysmata wurdemanni*, description of four new species, and remarks on *Lysmata rathbunae* (Crustacea: Decapoda: Hippolytidae). *Bulletin of Marine Science* **79**: 166–204.
- Schubart CD, Cuesta JA, Felder DL. 2002.** Glyptograpsidae, a new brachyuran family from Central America: larval and adult morphology, and a molecular phylogeny of the Grapsoidea. *Journal of Crustacean Biology* **22**: 28–44.
- Schubart CD, Neigel JE, Felder DL. 2000.** Use of the mitochondrial 16S rRNA gene for phylogenetic and population studies of Crustacea. *Crustacean Issues* **12**: 817–830.
- Sikkel P, Cheney KL, Côté IM. 2004.** In situ evidence for ectoparasites as the proximate cause of cleaning interactions in reef fish. *Animal Behaviour* **68**: 241–247.
- Swofford DL. 2002.** *PAUP*: phylogenetic analysis using parsimony (and other methods), version 4.010b*. Sunderland, MA: Sinauer Associates.
- Tomiyama K. 1996.** Mate-choice criteria in a protandrous simultaneously hermaphroditic land snail *Achatina fulica* (Férussac) (Stylommatophora: Achatinidae). *Journal of Molluscan Studies* **62**: 101–111.
- d'Udekem d'Acoz C. 2003.** *Lysmata seticaudata* (Risso, 1816) and *L. nilita* Dohrn & Holthuis, 1950 are protandrous simultaneous hermaphrodites (Decapoda, Caridea, Hippolytidae). *Crustaceana* **75**: 1149–1152.
- Wickler W, Seibt U. 1981.** Monogamy in Crustacea and man. *Zeitschrift für Tierpsychologie* **57**: 215–234.
- Wicksten MK. 2000.** The species of *Lysmata* (Caridea: Hippolytidae) from the eastern Pacific ocean. *Amphipacifica* **2**: 3–22.
- Wirtz P. 1997.** Crustaceans symbionts of the sea anemone *Telmatactis cricoides* at Madeira and the Canary Islands. *Journal of Zoology* **242**: 799–811.