



Molecular systematics of peppermint and cleaner shrimps: phylogeny and taxonomy of the genera *Lysmata* and *Exhippolysmata* (Crustacea: Caridea: Hippolytidae)

J. ANTONIO BAEZA^{1,2,3*}

¹Smithsonian Tropical Research Institute, Apartado Postal 0843-03092, Balboa, Ancón, Republic of Panama

²Smithsonian Marine Station at Fort Pierce, 701 Seaway Drive, Fort Pierce, Florida 34949, USA

³Departamento de Biología Marina, Facultad de Ciencias del Mar, Universidad Católica del Norte, Larrondo 1281, Coquimbo, Chile

Received 31 December 2008; accepted for publication 22 May 2009

Shrimps from the ecologically diverse genera *Lysmata* and *Exhippolysmata* are rare among marine invertebrates because they are protandric simultaneous hermaphrodites: shrimps initially mature and reproduce solely as males, and later in life become functional simultaneous hermaphrodites. Considerable progress on the reproductive ecology of members from these two genera has been achieved during the last decade. However, several outstanding issues of systematic nature remain to be addressed. Here, a molecular phylogeny of these two genera was used to examine the overall evolutionary relationship within and between species and genera, and to answer various questions related to the systematic status of several species. The present phylogenetic analysis, including 53 sequences and 26 species of *Lysmata* and *Exhippolysmata*, indicates that semiterrestrial shrimps from the genus *Merguia* represent the sister group to a second natural clade composed by shrimps from the genera *Lysmata* and *Exhippolysmata*. Also, the phylogenetic analysis confirmed that the genus *Lysmata* is paraphyletic, and includes the genus *Exhippolysmata*, as noted in a preliminary study. The tree partially supports the separation of species with or without a developed accessory branch into two different genera or subgenera (i.e. *Lysmata* and *Hippolysmata* having a well-developed accessory branch, or not, respectively). The genetic distance between the cleaner shrimps *Lysmata amboinensis* and *Lysmata grabhami* was smaller than has been observed between other sister species. On the other hand, the topology of the tree indicates that these two entities are reciprocally monophyletic. Thus, this latter result, together with minor but constant differences in the colour pattern reported for these two entities, indicates that there is no reason to stop treating them as different valid species. This study enabled the long overdue resolution of standing taxonomic questions in shrimps from the genera *Lysmata* and *Exhippolysmata*. In the future, this phylogeny will help to reveal the conditions favouring the origins of several behavioural and morphological novelties in these unique shrimps.

© 2010 The Linnean Society of London, *Zoological Journal of the Linnean Society*, 2010, **160**, 254–265.
doi: 10.1111/j.1096-3642.2009.00605.x

ADDITIONAL KEYWORDS: *Exhippolysmata* – hermaphrodite – Hippolytidae – *Lysmata* – *Merguia*.

INTRODUCTION

Shrimps from the genera *Lysmata* Risso, 1816 and *Exhippolysmata* Stebbing, 1915 are unique among crustaceans because of their enigmatic sexual system.

All species studied so far are protandric simultaneous hermaphrodites, in which individuals consistently mature and reproduce initially as males, and then later in life become functional simultaneous hermaphrodites (Kagwade, 1982; Bauer, 2000, 2006; Braga *et al.*, 2009). Recent studies suggest that this unusual sex allocation pattern is a trait fixed within

*E-mail: baezaa@si.edu

each genera (Baeza, 2009, and references therein). Other than in shrimps, protandric simultaneous hermaphroditism has independently evolved a few times, and has been experimentally demonstrated in the polychaete worm *Ophryotrocha diadema* (Lorenzi *et al.*, 2005), the land snail *Achatina fulica* (Tomiyama, 1996), and the tunicate *Pyura chilensis* (Manríquez & Castilla, 2005). The adaptive value of this unusual sex allocation pattern is currently being studied in various species (Bauer, 2000, 2006; Lorenzi *et al.*, 2005; Baeza, 2006, 2007a, b, c).

In addition to their sexual system, shrimps from the genus *Lysmata* are particularly peculiar among other caridean shrimp genera because of their wide diversity of lifestyles. For instance, many species are not conspicuous in terms of coloration, occur in dense aggregations, and dwell freely among rocks or sea-grass blades, both at rocky intertidal and/or subtidal temperate and subtropical zones (e.g. *Lysmata wurdemanni*, Baldwin & Bauer, 2003; *Lysmata seticaudata*, d'Udekem d'Acoz, 2003; *Lysmata californica*, Bauer & Newman, 2004; *Lysmata nayaritensis*, Bauer, Reitz & Collin, 2008). Other species live in small groups and might develop symbiotic associations with sessile macroinvertebrates (i.e. *Lysmata ankeri* and *Lysmata pedersenii* inhabiting sea anemones and sponges, respectively; Baeza, 2009). Finally, a few species have a striking coloration, live as socially monogamous pairs, and provide cleaning services to fishes (e.g. the red blood shrimps *Lysmata splendida* and *Lysmata debelius*, and the skunk and/or lady scarlet shrimps *Lysmata amboinensis* and *Lysmata grabhami*; Limbaugh, Pederson & Chace, 1961; Bruce, 1983; Fiedler, 1998; Baeza, 2009). Because of this lifestyle diversity, shrimps from the genus *Lysmata* and *Exhippolysmata* have captured the attention of behavioural ecologists, and are currently being used to explore the importance of the environment in favouring particular behavioural and morphological innovations (Baeza & Bauer, 2004; Baeza, 2006, 2007a, b, c, 2008, 2009).

The natural history of shrimp from the genus *Exhippolysmata* is much less well known. The only species in which the biology has been studied is conspicuously red (but not as striking as the red blood shrimp *L. debelius*), and is reported to form large aggregations in relatively deep (> 10 m) soft-bottom subtropical environments (*Exhippolysmata oplophoroides*; Braga, 2006; Braga *et al.*, 2009).

Most recently, a phylogenetic framework was used to understand the evolutionary origins of the unusual sex allocation pattern featured by members of the genera *Lysmata* and *Exhippolysmata* (Baeza, 2009; Baeza *et al.*, 2009). Early studies suggested that protandric simultaneous hermaphroditism in *Lysmata* evolved in the tropics, from an ancestral,

strictly protandric hermaphroditic species that adopted fish-cleaning behaviour (Bauer, 2000, 2006). The reduced probability of encountering mating partners resulting from their obligatory association with macroinvertebrates would have favoured this sexual system (Bauer, 2000, 2006). Recent studies have provided partial support for this 'historical contingency' hypothesis (Baeza, 2009; Baeza *et al.*, 2009). Ancestral character state reconstruction indicated that the most common recent ancestor of *Lysmata* was most likely to have been socially monogamous. However, the ancestral lifestyle was equally likely to be free-living or symbiotic (Baeza, 2009). Further studies are needed to define the conditions that might have given rise to this sex allocation pattern in *Lysmata* and *Exhippolysmata*.

The first molecular phylogeny published for the genera *Lysmata* and *Exhippolysmata* also helped to resolve one of several long-lasting and overdue systematic questions (Baeza *et al.*, 2009). The phylogenetic analysis revealed that the genus *Lysmata* is paraphyletic, and includes the genus *Exhippolysmata*, the only other known caridean shrimps featuring protandric simultaneous hermaphroditism (Kagwade, 1982; Laubenheimer & Rhyne, 2008; Braga *et al.*, 2009). This finding needs confirmation. A new phylogeny should help resolve other outstanding systematic controversies. First, a new phylogeny should help evaluate Christoffersen's (1987) proposal, which placed the species of *Lysmata*, *Exhippolysmata*, and *Lysmatella* in their own family (i.e. the Lysmatidae Dana, 1852), separate from the family Hippolytidae that contains, among others, the genera *Hippolyte*, *Tozeuma*, and *Merhippolyte*. Second, the status of a formerly used genus, *Hippolysmata* Stimpson, 1860, which would include species of *Lysmata* without a developed accessory branch of the outer (upper) antennular flagellum (Chace, 1972, 1997), needs to be clarified. Finally, a third unresolved question is whether the Indo-Pacific *L. amboinensis* (De Man, 1888) and the Atlantic *L. grabhami* (Gordon, 1935) are separate species or constitute a single pantropical species (see Hayashi, 1975 vs. Chace, 1997).

In the present study, a molecular phylogeny of the genus *Lysmata* and *Exhippolysmata* is generated, and its significance for answering the taxonomic questions posed above is discussed. Specifically, I formally tested for: (1) the natural segregation of the species within the group in *Lysmata* and *Hippolysmata*, according to the presence or absence of a well-developed accessory branch; (2) the natural separation of *Lysmata s.l.* (*Lysmata* + *Hippolysmata*) and *Exhippolysmata*, according to the presence or absence of a dorsal abdominal spine, rostral crest of teeth, and long rostrum; and (3) the monophyly of the Lysmatidae. It is predicted that a molecular phylogeny of

the species included within the three genera should segregate the species into well-supported genus-specific monophyletic clades. Similarly, if the *Lysmata* is a naturally valid clade, then a molecular phylogeny should segregate species of *Lysmata* and *Exhippolysmata* from species pertaining to other Hippolytidae genera. I formally examined the predictions above using Bayesian hypothesis testing. This study differs from that of Baeza *et al.* (2009) because a new molecular phylogeny of the genera *Lysmata* and *Exhippolysmata* is proposed here, and is used to address the overall evolutionary relationship within and between genera, and to answer various questions (1, 2, and 3 above) related to the systematic status of several species and clades. All these systematic issues were not addressed by Baeza *et al.* (2009).

MATERIAL AND METHODS

A total of 53 sequences, including 42 specimens from 24 species of shrimps from the genus *Lysmata*, and two species from the genus *Exhippolysmata*, were included in the present phylogenetic analysis (Table 1). One specimen each of the Hippolytidae species *Merguia rhizophorae*, *Merguia oligodon*, *Hep-tacarpus palpator*, *Tozeuma carolinense*, *Hippolyte williamsi*, and *Hippolyte inermis*, as well as specimens from five species of the family Palaemonidae, were included as out-groups during the phylogenetic analyses. Thirty-three of the sequences were generated and used previously to explore the conditions explaining the origins of protandric simultaneous hermaphroditism (Baeza *et al.*, 2009). Most shrimp species were collected between 2006 and 2008 from different localities in Belize, Panama, Venezuela, and the USA (California, Florida, and Texas). Immediately after collection, specimens were preserved in 95–99% ethanol. The different species were identified using Bruce (1983), Rhyne & Lin (2006), Baeza & Anker (2008), Rhyne & Anker (2007), and the keys of Chace (1972, 1997) and Wicksten (2000).

Total genomic DNA was extracted from pleopods or 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; Baeza *et al.*, 2009). 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 deoxynucleotide triphosphates (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 fol-

lowing conditions: initial denaturation at 96 °C for 4 min, followed by 40 cycles of 94 °C for 45 s, 48–57 °C (depending on the species) for 1 min, and 72 °C for 1 min, followed by a 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 were 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, USA), which is equipped with an ABI Prism 3730xl Genetic Analyser (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 Sequencher 4.5 (Gene Codes Corporation).

The final set of consensus sequences was aligned with the integrated ClustalW, corrected manually with MEGA 4.1 (Kumar *et al.*, 2008), and then exported to PAUP* (Swofford, 2002) and MrBayes (Huelsenbeck, 2000). First, the dataset was analysed with Modeltest 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 by both the hierarchical likelihood ratio tests and Akaike's information criterion, AIC) was a TVM+I+G evolutionary model ($-\ln L = 8965.8066$). The calculated parameters were as follows: assumed nucleotide frequencies, A = 0.3451, C = 0.0969, G = 0.1616, and T = 0.3964; substitution rate matrix with A \rightarrow C substitution = 0.6573, A \rightarrow G = 4.5475, A \rightarrow T = 0.6640, C \rightarrow G = 0.6446, C \rightarrow T = 4.5475, and G \rightarrow T = 1.0; rates for variable sites assumed to follow a gamma distribution (G) with shape parameter $\alpha = 0.4811$ and a proportion of invariable sites (I) = 0.1765.

The phylogenetic analyses conducted herein were the maximum parsimony (MP) and maximum likelihood (ML) in PAUP*, minimum evolution (ME) in MEGA 4.1, and Bayesian inference (BI) in MrBayes. The first two analyses were performed as a heuristic search with a starting tree obtained via stepwise addition, random addition of sequences, random replicates, and tree bisection and reconnection (TBR) branch swapping. All other parameters used were those of the default option in PAUP*. For BI, unique random starting trees were used in the Metropolis-coupled Markov Monte Carlo Chain (MCMC) (Huelsenbeck, 2000). The analysis was performed for 6 000 000 generations. 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.

Table 1. *Lysmata* species and other caridean shrimps used for the phylogeny reconstruction

Species	Collection site, date	Catalogue number	GenBank
<i>Lysmata amboinensis</i> (De Man, 1888), Java	Aqua. Store, Singapore, 2000	SMF 32281	EU861487
<i>Lysmata amboinensis</i> , Philippines	Aqua. Store, FL, USA, 2006	UMML 32.9451	EU861488
<i>Lysmata ankeri</i> Rhyne & Lin, 2006	SMEE, Fort Pierce, 2006	UMML 32.9452	EU861501
<i>Lysmata argentopunctata</i> , Wicksten, 2000	Cabo San Lucas, Mexico, 1997	CNCR 20998	GQ227814
<i>Lysmata bahia</i> Rhyne & Lin, 2006	Bocas del Toro, Panama, 2006	UMML 32.9453	EU861503
<i>Lysmata boggei</i> Rhyne & Lin, 2006	NA	MLP120	DQ079719
<i>Lysmata boggei</i>	St Petersburg, FL, USA, 2006	UMML 32.9454	EU861505
<i>Lysmata californica</i> 1 (Stimpson, 1866)	La Jolla, CA, USA, 2006	UMML 32.9455	EU861498
<i>Lysmata californica</i> 2	La Jolla, CA, USA, 2006	UMML 32.9622	EU861499
<i>Lysmata debelius</i> Bruce, 1983, Indo-Pacific	Aqua. Store, LA, USA, 2001	SMF 32009	EU861491
<i>Lysmata debelius</i> , Java	Aqua. Store, Singapore, 1999	SMF 32280	EU861493
<i>Lysmata debelius</i> , Philippines	Aqua. Store, FL, USA, 2006	UMML 32.9456	EU861492
<i>Lysmata debelius</i>	NA	MLP121	DQ079718
<i>Lysmata galapagensis</i> Schmitt, 1924	Islas Secas, Panama, 2007	UMML 32.9457	EU861480
<i>Lysmata gracilirostris</i> Wicksten, 2000	Venao, Panama, 2006	UMML 32.9458	EU861502
<i>Lysmata grabhami</i> (Gordon, 1935), Haiti	Aqua. Store, FL, USA, 2006	UMML 32.9459	EU861489
<i>Lysmata grabhami</i> , Madeira	Madeira, Portugal, 2001	SMF 32007	EU861490
<i>Lysmata hochi</i> Baeza & Anker, 2008	Long Key, FL, 2007	UMML 32.9460	EU861507
<i>Lysmata holthuisi</i> Anker, de Grave & Baeza, 2009	Chumical, Panama, 2007	UMML 32.9466	EU 861483
<i>Lysmata intermedia</i> (Kingsley, 1879)	Bocas del Toro, Panama, 2007	UMML 32.9461	EU861484
<i>Lysmata moorei</i> (Rathbun, 1901)	Galeta, Panama, 2007	UMML 32.9462	EU861481
<i>Lysmata moorei</i> , FL Keys	Key Largo, Florida, 2008	UMML 32.9605	GQ227825
<i>Lysmata nayaritensis</i> Wicksten, 2000	Chumical, Panama, 2007	UMML 32.9463	EU861506
<i>Lysmata</i> sp. nov. cf. boggei	Cubagua, Venezuela, 2008	UMML 32.9603	GQ227815
<i>Lysmata</i> sp. nov. cf. boggei	Cubagua, Venezuela, 2008	UMML 32.9601	GQ227816
<i>Lysmata</i> sp. nov. cf. intermedia	Carrie Bow Key, Belize, 2008	UMML 32.9606	GQ227817
<i>Lysmata nilita</i> Dohrn & Holthuis, 1950	Giglio, Italy, 2000	SMF32005	EU861482
<i>Lysmata olavoi</i> Franssen, 1991	Azores, Portugal, 1999	SMF 32006	EU861494
<i>Lysmata pedersenii</i> Rhyne & Lin, 2006	Carrie Bow, Belize, 2007	UMML 32.9464	EU861504
<i>Lysmata rafa</i> Rhyne & Anker, 2008	Aquarium Store, FL, USA	UMML 32.9465	EU861495
<i>Lysmata seticaudata</i> (Risso, 1816)	Corsica, France, 2003	SMF 32004	EU861485
<i>Lysmata seticaudata</i> 1	Cabo Raso, Cascais, Portugal	SMF 32003	EU861486
<i>Lysmata seticaudata</i> 2	Portugal, 2007	UMML 32.9614	GQ227827
<i>Lysmata vittata</i> 1 (Stimpson, 1860)	Thailand, 1982	RMNH D 35616	GQ227829
<i>Lysmata vittata</i> 2	Thailand, 1982	RMNH D 35616	GQ227828
<i>Lysmata wurdemanni</i> (Gibbes, 1850), PA-TX	Port Aransas, TX, USA, 2000	SMF 32008	EU861496
<i>Lysmata wurdemanni</i> , G-TX	Galveston, TX, USA, 2007	UMML 32.9607	GQ227830
<i>Lysmata wurdemanni</i> , West FL	St Petersburg, FL, USA, 2006	UMML 32.9467	EU861497
<i>Lysmata wurdemanni</i> , East FL	Fort Pierce, FL, USA, 2006	UMML 32.9468	EU861500
<i>Ascidonia miserabilis</i> (Holthuis, 1951)	Cubagua, Venezuela, 2008	UMML 32.9611	GQ227824
<i>Exhippolysmata oplophoroides</i> (Holthuis, 1948)	Ubatuba Bay, Brazil, 2006	UMML 32.9469	EU861510
<i>Exhippolysmata ensirostris</i> (Kemp, 1914)	Quindao, China, 2007	UMML 32.9602	GQ227819
<i>Heptacarpus palpator</i> (Owen, 1939)	La Jolla, CA, USA, 2001	SMF 32282	EU861509
<i>Hippolyte inermis</i> Leach, 1815	Venice Lagoon, Italy, 1997	SMF 32283	EU861511
<i>Hippolyte williamsi</i> Schmitt, 1924	Puerto Aldea, Chile, 2007	UMML 32.9470	EU861512
<i>Leander tenuicornis</i> (Say, 1818)	Fort Pierce, FL, 2007	UMML 32.9608	GQ227821
<i>Merguia oligodon</i> (De Man, 1888)	Africa.	OUMNH.ZC.2002-04-01	GQ227826
<i>Merguia rhizophorae</i> (Rathbun 1900)	Bocas del Toro, Panama, 2007	UMML 32.9471	EU861508
<i>Merhippolyte ancistrota</i> Crosnier & Forest, 1973	Off Mauritania, 1988	RMNH D 46291	GQ227822
<i>Palaemon floridanus</i> Chace, 1942	Fort Pierce, FL, 2007	UMML 32.9620	GQ227820
<i>Pontonia domestica</i> Gibbes, 1850	Cubagua, Venezuela, 2008	UMML 32.9621	GQ227818
<i>Pontonia mexicana</i> Guérin-Méneville, 1856	Cubagua, Venezuela, 2008	UMML 32.9609	GQ227823
<i>Tozeuma carolinense</i> Kingsley, 1878	St. Petersburg, FL, 2007	UMML 32.9472	EU861513

The sites of collection (NA: not available from the literature), dates, museum catalogue number (CN: UMML, University of Miami Marine Laboratories, Rosenthal School of Marine Science, University of Miami; SMF Senckenberg Museum Frankfurt, Germany; MLP, Monte L. Bean Life Science Museum, Brigham Young University, Provo, Utah; CNCR, Colección de Crustáceos, Instituto de Biología, Departamento de Zoología, Universidad Nacional Autónoma de México, México; RMNH, National Museum of Natural History/Naturalis, Leiden, the Netherlands), and the GenBank accession numbers (GenBank) are shown for each species.

The robustness of the MP, ML, and ME tree topologies was assessed by bootstrap reiterations of the observed data 2000, 80, and 1000 times, respectively, and by reconstructing trees using each resampled data set. Support for nodes in the BI tree topology was obtained by posterior probability.

I tested if the different species of the genera *Hippolysmata* and *Lysmata* s.s., and *Lysmata* s.l. (*Hippolysmata* + *Lysmata*), and *Exhippolysmata* segregated and formed different genus-specific monophyletic clades. For this purpose, constrained trees (in which the monophyly of particular genera was enforced) were obtained in MrBayes with the command *constraint*. MCMC searches were run and the harmonic mean of tree-likelihood values was 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 (unconstrained vs. constrained trees) according to the criteria of Kass & Raftery (1995). The validity of the Lysmatidae *sensu* Christoffersen (1987) as a valid natural clade was tested as above, considering species from the genera *Lysmata* s.l. (*Hippolysmata* + *Lysmata*) and *Exhippolysmata*, as pertaining to the Lysmatidae, and species from the genera *Heptacarpus*, *Hippolyte*, *Merguia*, *Merhippolyte*, and *Tozeuma*, as comprising the Hippolytidae s.s.

RESULTS

During the present phylogenetic analysis, a total of 328 positions were found to be parsimony informative out of 744 homologous alignment positions. Noticeably, the two specimens of *Lysmata vittata* as well as the single specimen of *Lysmata bahia* had an insertion of 99 bp in the middle of the 16S sequenced fragment. Sequencing of a second and third specimen of *L. bahia* and *L. vittata*, respectively, confirmed the presence of this insertion (sequences not included in the present analysis). All phylogenetic trees obtained with the different inference methods (MP, ML, ME, and BI) resulted in somewhat similar general topologies (Figs 1, 2). Considering the pool of species used herein, including seven different genera within the Hippolytidae, the phylogenetic analyses support the genus *Merguia* as the sister group of a second natural group composed by shrimps from the genera *Lysmata* and *Exhippolysmata*. The monophyly of these two later genera is also well supported by a high posterior probability obtained from the BI analysis, and by bootstrap support from the ML and ME analyses. The bootstrap support from the MP was, in general, lower. Within the monophyletic clade comprised by species of *Lysmata* and *Exhippolysmata*, the position of *Lysmata olavoï* as the most basally placed species is

supported by the BI analysis, but not by the ML, MP, and ME analyses. Overall, the tree topology indicates that this clade can be divided into different main subclades, with three of them having been previously recognized.

The first monophyletic clade, called 'Tropical American', is composed of seven species of peppermint shrimps, six species from the Caribbean and/or the Gulf of Mexico, and one species from the Pacific (Figs 1, 2). This clade is well supported by all phylogenetic analyses (Figs 1, 2). Within this clade, the basal position of *Lysmata gracilirostris* from the Pacific is well supported by the BI and ML analyses, but not by the MP and ME analyses. The species *L. pedersenii* and *L. ankeri* are well supported as sister taxa by all analyses excepting MP. Although the ML, MP, and ME analyses do not completely resolve the position of three species in the tree (*L. bahia*, *L. nayaritensis* and *L. californica*), the BI analysis suggest that these latter species are closely related to members of the 'Tropical American' clade.

The second monophyletic group, previously named the 'Cleaner' clade, is composed by colourful fish-cleaning species that associate with macroinvertebrates. Within this clade, the basal position of the red blood shrimp *L. debelius* is well supported by BI and MP analyses, but not by ML and ME analyses. Also, all phylogenetic analyses support the skunk shrimps *L. amboinensis* and *L. grabhami* as sister taxa within this group (Figs 1, 2).

The third group, called the 'Cosmopolitan' clade, is composed of eight species, two from the Mediterranean (*L. seticaudata* and *Lysmata nilita*), three from the western Atlantic (*Lysmata moorei*, *Lysmata intermedia*, and *Lysmata* sp. nov. cf. *intermedia*), and three from the tropical eastern Pacific (*Lysmata galapagensis*, *Lysmata argentopunctata*, and *Lysmata holthuisi*). Within this clade, one pair of species (*L. intermedia* and *Lysmata* sp. nov. cf. *intermedia*) are well supported as sister taxa by all phylogenetic analyses.

A fourth group, here named 'Morphovvariable', was retrieved from all phylogenetic analyses (Figs 1, 2). However, support (moderate) for the monophyly of this group was obtained only from BI analysis. This putatively monophyletic group is composed of the Caribbean *Lysmata hochi*, the Indo-Pacific *L. vittata*, and two shrimps from the genus *Exhippolysmata* (*E. oplophoroides*, from the western Atlantic, and *Exhippolysmata ensirostris* from the Indo-Pacific) that are well supported as sister taxa by all phylogenetic analyses (Figs 1, 2).

The pairwise genetic distances based on the TVM + I + G model of nucleotide substitution indicate that the four pairs of non-cleaner sister shrimp species (well supported by the ML and BI analyses)

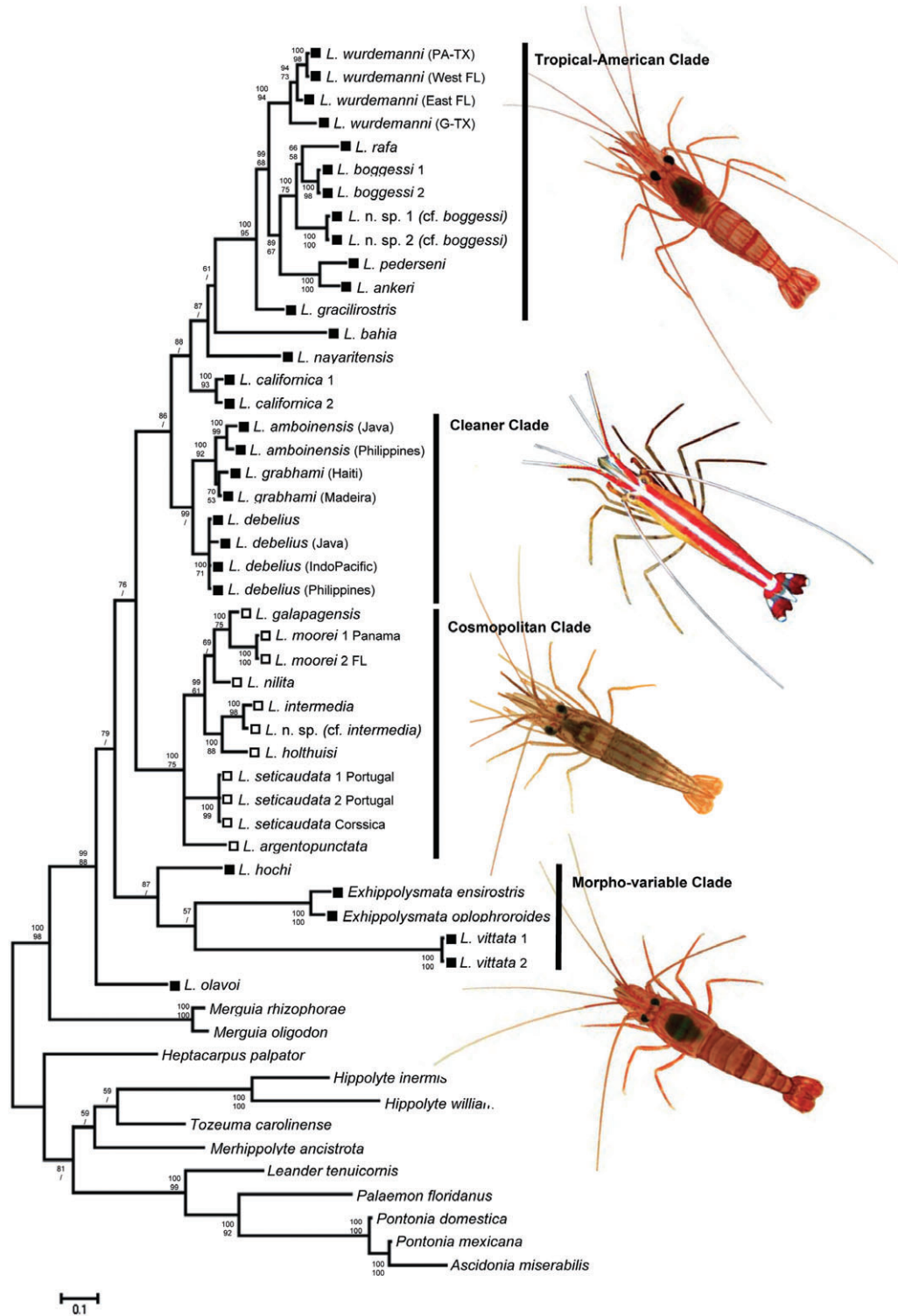


Figure 1. Phylogenetic tree obtained from Bayesian inference (BI) analysis of the partial 16S rRNA gene for shrimps from the genus *Lysmata*, and other selected taxa from the Caridea. Numbers above or below the branches represent the posterior probabilities from the BI analysis and bootstrap values obtained from maximum likelihood (ML) in PAUP* (BI/ML). The white and black squares represent the presence or absence, respectively, of a developed accessory branch in each species. The images of the shrimps (from top to bottom) represent *Lysmata wurdemanni*, *Lysmata grabhami*, *Lysmata intermedia*, and *Lysmata hochi*.

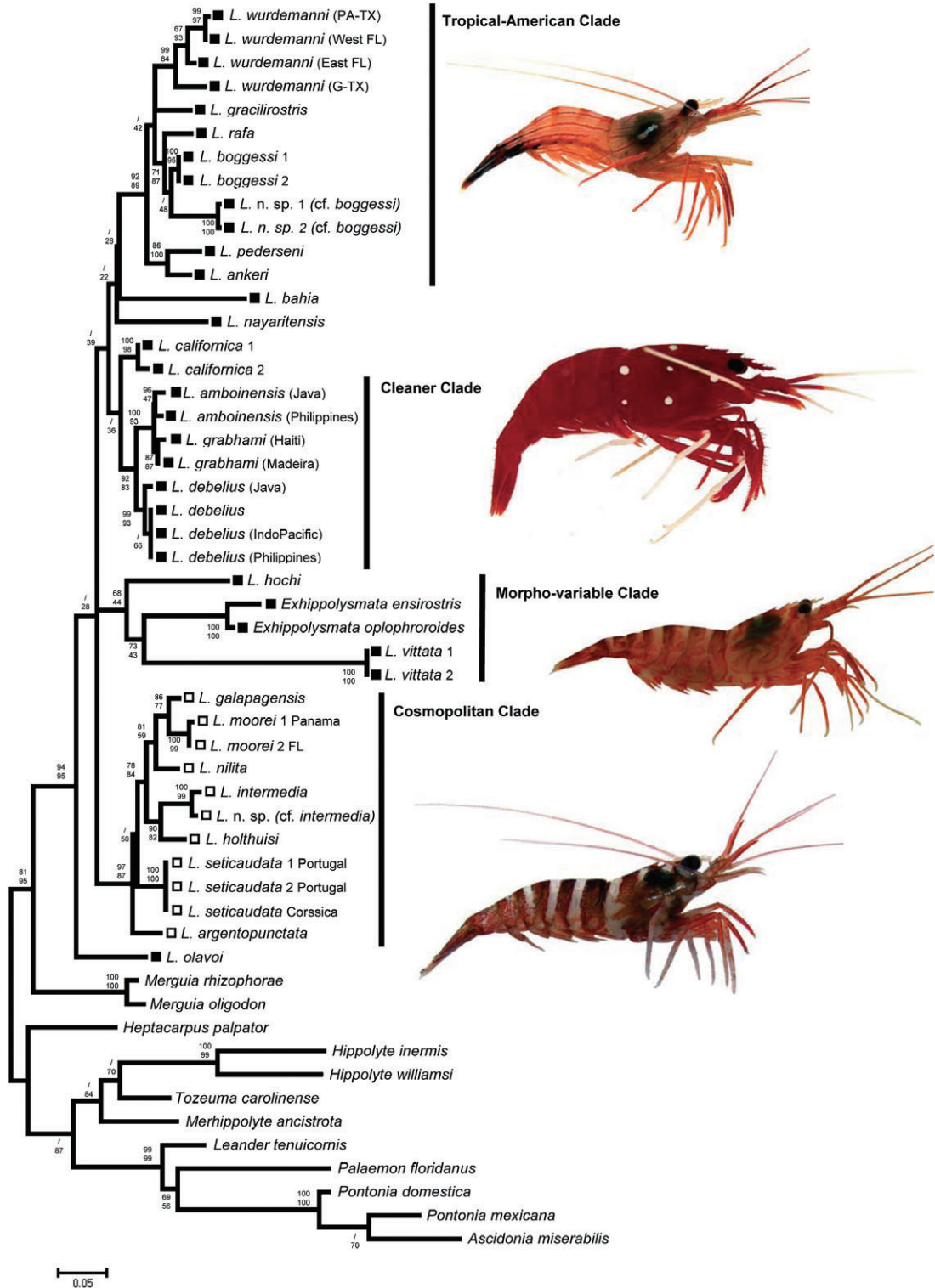


Figure 2. Phylogenetic tree obtained from minimum evolution (ME) analysis of the partial 16S rRNA gene for shrimps from the genus *Lysmata*, and other selected taxa from the Caridea. Numbers above or below the branches represent the bootstrap values obtained from maximum parsimony (MP) and ME analyses in PAUP* and MEGA 4.4 (MP/ME). The white and black squares represent the presence or absence, respectively, of a developed accessory branch in each species. The images of the shrimps (from top to bottom) represent *Lysmata wurdemanni*, *Lysmata debelius*, *Lysmata hochi*, and *Lysmata galapagensis*.

Table 2. Bayes-factor testing of phylogenetic hypotheses

Hypotheses	Harmonic mean	$2 \log_e(B_{10})$	Evidence
Unconstrained tree	-10964.39		
Monophyletic <i>Lysmata s.s.</i> vs. <i>Hippolysmata</i>	-10791.83	10.301	Very strong against constrained tree
Monophyletic <i>Lysmata s.l.</i> vs. <i>Exhippolysmata</i>	-10776.16	10.475	Very strong against constrained tree
Monophyletic Lysmatidae vs. Hippolytidae	-10428.97	12.567	Very strong against constrained tree

The different hypotheses on the monophyly of particular groups of shrimp are ordered based on the degree of evidence against the constrained tree. The higher the value of $2 \log_e(B_{10})$ statistic implies stronger support against the monophyly of a particular group.

had values ranging from 0.07071 (*L. ankeri* vs. *L. pedersenii*) to 0.0871 (*Lysmata* sp. nov. cf. *intermedia* vs. *L. intermedia*). The genetic distances between the species of cleaner shrimps *L. grabhami* and *L. amboinensis* (0.0318–0.0501) are smaller than the distances calculated for the different pairs of non-cleaner sister shrimp species above, and are similar to the distances calculated for the different specimens of *L. wurdemanni* (0.00185–0.06657).

In the phylogenetic tree, species with a rudimentary accessory branch (i.e. composed by a single segment or no more than three segments) included the basally positioned *L. olavoii* and also clustered together into the ‘Tropical American’, ‘Cleaner’ and ‘Morphovvariable’ clades. The latter clade includes the type species of *Hippolysmata* (*L. vittata*). On the other hand, a developed accessory branch was consistently present in one of the three main natural groups of species (the ‘Cosmopolitan’ clade) that includes the type species of *Lysmata* (*L. seticaudata*) (Figs 1, 2).

Bayesian-factor analyses revealed strong support for the unconstrained tree, instead of alternative trees in which the monophyly of *Lysmata s.l.*, *Lysmata s.s.*, *Hippolysmata*, or *Exhippolysmata* was forced (Table 2). The absence of support for the separation of *Lysmata s.l.* into *Lysmata* and *Hippolysmata* (according to the presence or absence of a well-developed antennal accessory branch, respectively) occurred because the clade composed of species with a developed accessory branch have a derived position compared with the basally positioned *L. olavoii*, a species that does not have a well-developed accessory branch. Similarly, the absence of support for a monophyletic clade of *Exhippolysmata* was a result of the basal position of *L. olavoii*. Lastly, Bayes factors provided strong support against the Lysmatidae because all analyses revealed the genus *Merguia* as being sister to a clade comprised of *Lysmata* and *Exhippolysmata*, and because this genus did not group together with other members of the Hippolytidae.

DISCUSSION

The present study helped resolve several outstanding but long overdue questions of systematic nature with respect to the genera *Lysmata s.l.* and *Exhippolysmata*. Albeit the collection of genera from the diverse family Hippolytidae is incomplete, this analysis provides evidence for the existence of a natural clade that includes species from the genera *Lysmata*, *Exhippolysmata*, and *Merguia*. Indeed, bootstrap support values from ML, MP, and ME, and posterior probabilities from BI, strongly suggest that the genus *Merguia* is the sister group of a second natural clade composed by the genera *Lysmata* and *Exhippolysmata*. Interestingly, the ecology of shrimps from the genus *Merguia* differs widely from that of *Lysmata*. Both *M. oligodon* from Africa and *M. rhizophorae* from the Caribbean are nocturnal semiterrestrial shrimps that dwell among mangrove roots (Abele, 1970; Vannini & Oluoch, 1993; Gillikin, de Grave & Tack, 2001; J.A. Baeza, pers. observ.) In contrast, shrimps from the genera *Lysmata* and *Exhippolysmata* are invariably marine based (Baeza, 2009, and references therein). Research on the ecology of *Merguia* is warranted, as it might provide insights into the evolution of protandric simultaneous hermaphroditism in caridean shrimps.

The existence of a natural clade composed by members from the genera *Lysmata* and *Exhippolysmata*, as indicated by tree topologies and support/bootstrap values, speak in favour of the family Lysmatidae *sensu* Christoffersen (1987). Christoffersen proposed that the genera *Mimocaris*, *Calliasmata*, *Lysmata*, and *Exhippolysmata* represented a natural group because of two shared characters: dorsal flagellum of the first antenna biramous, with the shorter ramus consisting of at least one free article; infraorbital angle of carapace depressed and inconspicuous, lying below antennal tooth. On the other hand, the Bayesian factors analysis conducted herein did not support the Lysmatidae as a monophyletic clade. Nevertheless, this lack of support is

explained because of the newly resolved position of *Merguia*, which did not group together with other members of the Hippolytidae (e.g. *Tozeuma* and *Hippolyte*), but was instead closely related to the genera *Lysmata* and *Exhippolysmata*. Thus, the present results suggest that the genus *Merguia* needs to be considered as a monogeneric family, as proposed before by Christoffersen (1990), in order to make the system phylogenetically sound. In general, sequences from the related genera *Lysmatella* and *Calliasmata*, as well as other members from the family Hippolytidae are needed to confirm whether the Lysmatidae *sensu* Christoffersen (1987) represents a valid natural clade.

The present phylogenetic and Bayesian factor analyses also confirmed the notion that *Lysmata* is paraphyletic because of the position of *E. oplophoroides* and *E. ensirostris* that clustered together with *L. hochi* and *L. vittata* (Baeza *et al.*, 2009). It must be noted that only the BI analysis moderately supported the relatedness and basal position of the above four species. On the other hand, dissections of *L. hochi* and *E. oplophoroides* have revealed that the sperm cells of these two species are packed inside small ovoid capsules, a character not shared by any other species of *Lysmata* in which the gonads and/or vas deference have been examined (Baeza & Anker, 2008; Braga *et al.*, 2009; J.A. Baeza, unpubl. data) This finding argues in favour of the genus *Exhippolysmata* plus *L. hochi* and *L. vittata* as a natural clade. As noted earlier, the differences among the species of *Lysmata* and *Exhippolysmata* are considerable (rostrum much longer and legs much more slender in *Exhippolysmata* compared with *Lysmata*; Baeza *et al.*, 2009). Overall, morphological differences as prominent as those observed between *L. hochi* and *L. vittata* vs. *Exhippolysmata* are also evident when comparing *L. rafa*, *L. boggeysi*, and *Lysmata* sp. nov. cf. *boggeysi*, a trio of closely related species pertaining to the 'Tropical American' clade. Among other characters, *L. rafa* features a curved and longer rostrum, and has much longer pereopods than *L. boggeysi*, and *Lysmata* sp. nov. cf. *boggeysi* (Rhyne & Lin, 2006; Rhyne & Anker, 2007; J.A. Baeza, pers. observ.). This degree of morphological differentiation between closely related species is not unusual within clades (Rhyne & Lin, 2006), and the conditions favouring such dissimilarities deserve further attention.

The different phylogenetic trees partially support the separation of species with or without a developed accessory branch into two different genera or subgenera (i.e. *Lysmata* and *Hippolysmata* having a well-developed accessory branch, or not, respectively), as was initially proposed by Stimpson (1860). In the analysis, species with a rudimentary accessory

branch (i.e. composed by a single segment, or by no more than three segments) included the basally positioned *L. olavoii*, and also clustered together into the 'Tropical American' and 'Cleaner' clades. On the other hand, a developed accessory branch was consistently present in only one of the four main natural groups of species (the 'Cosmopolitan' clade) that includes the type species of *Lysmata* (*L. seticaudata*). The distribution of this character in the tree suggests that the development of an accessory branch is a useful character with systematic and phylogenetic information; the character is a synapomorphy for *Lysmata* s.s. On the other hand, the absence of a well-developed accessory branch represents a plesiomorphic state, with no use in defining a second genus within the *Lysmata* s.l. containing all species with a rudimentary accessory branch. This explains why Bayesian factor analyses provided strong support against the grouping of *Lysmata* s.s. as a natural clade. Examination of several species at the Smithsonian National Museum of Natural History (NMNH), Washington D.C., indicates that the length of the accessory branch varies remarkably within the genus, from species having a single segmented rudimentary accessory branch (i.e. *L. hochi*; Baeza & Anker, 2008) to others with a highly developed accessory branch composed of many articles (i.e. *L. galapagensis*; Wicksten, 2000). A revision of the genus using not only molecular but also morphological and larval characters is necessary in order to make the system phylogenetically sound.

The comparisons of genetic distances of the species *L. amboinensis* and *L. grabhami* were smaller than those observed between other sister species from the genus. One possible explanation for this genetic similarity between specimens from these two cleaner shrimp species is that they actually pertain to a single pantropical species, as proposed by Hayashi (1975), who found only minor morphological differences between the two entities. On the other hand, the results indicate that the two species are reciprocally monophyletic. Chace (1997) noticed minor but apparently constant differences in colour pattern between these two species (Fig. 3) (also, shown in Debelius, 2001). Until further evidence is obtained from more variable markers and additional specimens from a wider geographical range, there is no reason to stop treating *L. grabhami* and *L. amboinensis* as different valid species. In addition to more sampling, and the use of more variable molecular markers, studies examining interbreeding between *L. grabhami* and *L. amboinensis* should help resolve the systematic status of these species in the near future.

The major differences in tree topology among the different methods of phylogenetic inference used here were: (1) the basal position of *L. gracilirostris* within the 'Neotropical' clade, well supported by the BI and

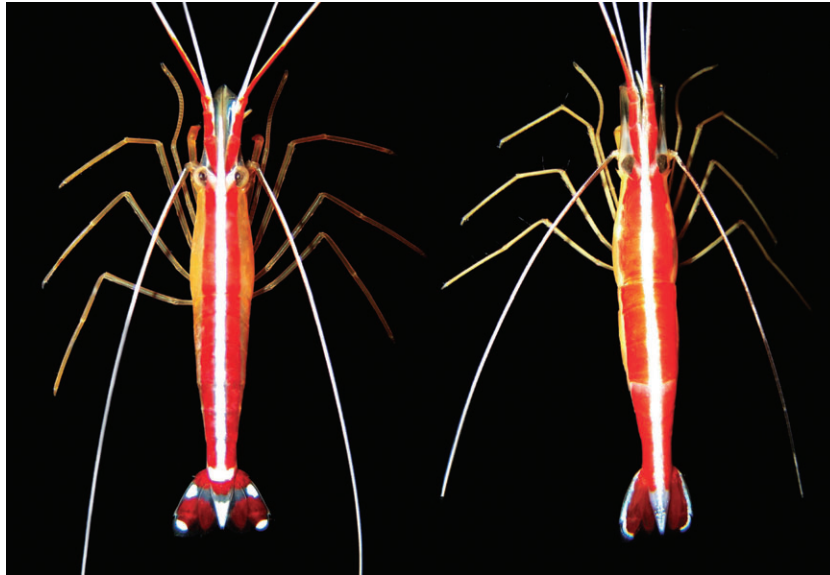


Figure 3. The habitus and colour pattern (dorsal view) of the shrimps *Lysmata amboinensis* (left) and *Lysmata grabhami* (right).

ML analyses, but not by the MP and ME analyses; (2) the position of *L. californica*, pertaining to the 'Neotropical' clade according to BI analysis, but related to the 'Cleaner' clade according to MP analysis; and (3) the basal position and relatedness of species comprising the 'Morphovvariable' group within the *Lysmata* + *Exhippolysmata* clade, well supported by BI analysis, but not by the ML, MP, and ME analyses. The observed differences among methods (in particular, between MP and ML + BI) are expected, given that all these different methodologies employ dissimilar optimality criteria, and rest upon differing assumptions. For instance, MP does not use a likelihood function, and does not rely on an explicit model of character evolution, in contrast to ML and BI (Kolaczkowski & Thornton, 2004). In general, each of the different phylogenetic methods used here has its own set of advantages and disadvantages; they should be used to complement molecular phylogenetic reconstruction, as exemplified by Kolaczkowski & Thornton's (2004, and references therein) study.

Overall, shrimps from the genera *Lysmata*, *Exhippolysmata*, *Merguia*, and, most probably, *Calliasmata* and *Lysmatella* represent a unique natural clade, with a wide diversity of lifestyles (Abele, 1970; Vannini & Oluoch, 1993; Gillikin *et al.*, 2001; Baeza & Anker, 2008; Baeza, 2009). Thus, this natural clade represents an opportunity to investigate the effect of the environment in determining behavioural, physiological, and morphological novelties, as well as the conditions favouring them in the marine environment. The present molecular phylogeny will help in the near future to test ideas about the evolution of

particular behavioral traits (e.g. sexual and social systems, sex allocation patterns, fish cleaning habits) in these shrimps.

ACKNOWLEDGEMENTS

Many thanks to Bill Hoffman from the Smithsonian Marine Ecosystems Exhibit (SMEE), Valerie Paul, Raphael Ritson-Williams, and Julie Piraino from the Smithsonian Marine Station at Fort Pierce (SMSFP), Narissa Bax from New Zealand, Luis Ignacio Vilchis and William A. Newman from the Scripps Institution of Oceanography (SIO), University of California at San Diego, Juan Bolaños, Jesus Enrique Hernandez, and Regulo Lopez from the Universidad de Oriente (UDO), Boca del Rio, Venezuela, Adriane A. Braga from the Universidade Estadual Paulista (UNESP), Botucatu, Brazil, Arthur Anker from the University of Florida, Gainesville, USA, Carla Piantonni from the National Museum of Natural History, Washington D.C., USA, Arcadio Ortiz from the Smithsonian Tropical Research Institute (STRI), Panama, Nancy Voss from the University of Miami and Fernando Alvarez from the Universidad Nacional Autónoma de Mexico (UNAM), Mexico City, for their help during the different steps of specimen collection and loans. My sincere appreciation goes to Juan Bolaños for inviting me to dictate a course on the Behaviour of Marine Invertebrates at Isla Margarita, where a great portion of this manuscript was written. Especial thanks to Jeff Hunt and Lee Weigt at the Laboratory of Analytical Biology, NMNH, Washington D.C. for their logistical support. JAB thanks the support from

a STRI Marine Postdoctoral Fellowship and a SMSFP Postdoctoral Fellowship. This study was partial funded by a National Geographic Research Grant from the National Geographic Society, USA. Raymond T. Bauer (University of Louisiana at Lafayette, USA) and Christoph Schubart (Regensburg Universitat, Germany) helped improve preliminary versions of this manuscript. The comments of two anonymous reviewers substantially improved this manuscript. This is contribution number 790 of the Smithsonian Marine Station at Fort Pierce.

REFERENCES

- Abele LG. 1970.** Semi-terrestrial shrimp (*Merguia rhizophorae*). *Nature* **226**: 661–662.
- 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. 2008.** Protandric simultaneous hermaphroditism in the shrimps *Lysmata bahia* and *L. intermedia*. *Invertebrate Biology* **127**: 181–188.
- Baeza JA. 2009.** Protandric simultaneous hermaphroditism is a conserved trait in *Lysmata* (Caridea: Hippolytidae): implications for the evolution of hermaphroditism in the genus. *Smithsonian Contributions to Marine Science* **38**: in press.
- Baeza JA, Anker A. 2008.** *Lysmata 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 hermaphrodite, the marine shrimp *Lysmata 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 *Lysmata nayaritensis*. *Journal of Natural History* **41**: 2843–2850.
- Baeza JA, Schubart CD, Zillner P, Fuentes S, Bauer RT. 2009.** Molecular phylogeny of shrimps from the genus *Lysmata* (Caridea: Hippolytidae): the evolutionary origins of pair-living, protandric simultaneous hermaphroditism and species status. *Biological Journal of the Linnean Society* **96**: 415–424.
- Baldwin AP, Bauer RT. 2003.** Growth, survivorship, life span, and sex change in the hermaphroditic shrimp *Lysmata wurdemanni* (Decapoda: Caridea: Hippolytidae). *Marine Biology* **143**: 157–166.
- 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. 2006.** Same sexual system but variable sociobiology: evolution of protandric simultaneous hermaphroditism in *Lysmata* shrimps. *Integrative and Comparative Biology* **46**: 430–438.
- Bauer RT, Newman WA. 2004.** Protandric simultaneous hermaphroditism in the marine shrimp *Lysmata californica* (Caridea: Hippolytidae). *Journal of Crustacean Biology* **24**: 131–139.
- Braga AA. 2006.** *Biology and Ecology of the spiny shrimp Exhippolysmata oplophoroides (Holthuis, 1948) (Caridea: Alpheoidea: Hippolytidae) at the Ubatuba Region, North of the Sao Paulo Littoral*. Unpublished D. Phil. Thesis, Universidade Estadual Paulista.
- Braga AA, López-Greco LS, Santos DC, Fransozo A. 2009.** Morphological evidence for protandric simultaneous hermaphroditism in the caridean shrimp *Exhippolysmata oplophoroides*. *Journal of Crustacean Biology* **29**: 34–41.
- Bruce AJ. 1983.** *Lysmata debelius*, new species, a new hippolytid shrimp from the Philippines. *Revue Francaise d'Aquariologie et Herpetologie* **4**: 115–120.
- 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.
- Christoffersen ML. 1987.** Phylogenetic relationships of hippolytid genera, with an assignment of new families for the Crangonoidea and Alpheoidea (Crustacea, Decapoda, Caridea). *Cladistics* **3**: 348–362.
- Christoffersen ML. 1990.** A new superfamily classification of the Caridea (Crustacea: Pleocyemata) based on phylogenetic pattern. *Journal of Zoological Systematics and Evolutionary Research* **28**: 94–106.
- Debelius H. 2001.** *Crustacea guide of the world*. Unterwasserarchiv, Frankfurt: IKAN.
- Fiedler GC. 1998.** Functional, simultaneous hermaphroditism in female-phase *Lysmata amboinensis* (Decapoda: Caridea: Hippolytidae). *Pacific Science* **52**: 161–169.
- Gillikin DP, de Grave S, Tack JF. 2001.** The occurrence of the semi-terrestrial shrimp *Merguia oligodon* (De Man, 1888) in *Neosarmatium smithi* H. Milne Edwards, 1853 burrows in kenyan mangroves. *Crustaceana* **74**: 505–507.
- Hayashi KI. 1975.** *Hippolysmata grabhami* Gordon, a synonym of *Lysmata amboinensis* (De Man) (Decapoda, Caridea, Hippolytidae). *Publications of the Seto Marine Laboratory* **12**: 285–296.
- Huelsenbeck JP. 2000.** *MrBayes: bayesian inferences of phylogeny (software)*. New York: University of Rochester.
- Kagwade PV. 1982.** The hermaphroditic prawn *Hippolysmata ensirostris* Kemp. *Indian Journal of Fisheries* **28**: 189–194.

- Kass RE, Raftery AE. 1995.** Bayes factors. *Journal of the American Statistical Association* **90**: 773–795.
- Kolaczowski B, Thornton JW. 2004.** Performance of maximum parsimony and likelihood phylogenetics when evolution is heterogeneous. *Nature* **431**: 980–984.
- Kumar S, Dudley J, Nei M, Tamura K. 2008.** MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. *Brief Bioinformatics* **9**: 299–306.
- 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.
- Lorenzi MC, Sella G, Schleicherová D, Ramella L. 2005.** Outcrossing hermaphroditic polychaete worms adjust their sex allocation to social conditions. *Journal of Evolutionary Biology* **18**: 1341–1347.
- 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.
- Posada D, Crandall KA. 1998.** Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**: 817–818.
- Rhyne AL, Anker A. 2007.** *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, Neigel JE, Felder DL. 2000.** Use of the mitochondrial 16S rRNA gene for phylogenetic and population studies of Crustacea. *Crustacean Issues* **12**: 817–830.
- Stimpson W. 1860.** Prodromus descriptionis animalium evertebratorum, quae in Expeditione ad Oceanum Pacificum Septentrionalem, a Republica Federata missa, C. Ringgold et J. Rodgers Ducibus, observavit et descripsit. *Proceedings of the Academy of Natural Sciences of Philadelphia* **1860**: 22–48.
- 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.
- Vannini M, Oluoch A. 1993.** Notes on *Merguia oligodon* (De Man, 1888) the Indo-Pacific semi-terrestrial shrimp (Hippolytidae Natantia). *Tropical Zoology* **6**: 281–286.
- Wicksten MK. 2000.** The species of *Lysmata* (Caridea: Hippolytidae) from the eastern Pacific ocean. *Amphipacifica* **2**: 3–22.