A PRECAUTIONARY TALE WHEN DESCRIBING SPECIES IN A WORLD OF INVADERS: MORPHOLOGY, COLORATION AND GENETICS DEMONSTRATE THAT *LYSMATA RAULI* IS NOT A NEW SPECIES ENDEMIC TO BRAZIL BUT A JUNIOR SYNONYM OF THE INDO-PACIFIC *L. VITTATA*

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ABSTRACT

The objective of this study was to investigate morphological variation in traits of systematic relevance and the phylogenetic position, ecology, and reproductive biology of the shrimp *Lysmata rauli* Laubenheimer and Rhyne, 2010 (Caridea: Hippolytidae), described based only on a single specimen collected in Salvador, Bahia, Brazil. We analyzed a total of 89 specimens from Camamu Bay, Bahia (n = 88) and from São Vicente estuary, São Paulo (n = 1). Considerable morphological variation was detected in the rostral spine series, number of segments on the carpus and merus of pereiopod 2, number of spiniform setae on the ventrolateral margin of merus and on the ventral margin of propodus of pereiopods 3-5. Importantly, *L. rauli* can be distinguished neither using morphology, nor coloration from the Indo-Pacific *L. vittata* (Stimpson, 1860). Furthermore, molecular phylogenetic analyses (using the 16S mt DNA fragment) did not reveal any considerable genetic dissimilarities between *L. rauli* and *L. vittata*. Thus, our results clearly indicate that *L. rauli* is not a new species but a junior synonym of *L. vittata*. The high density observed within the structures of oyster farming indicates that the invasive *L. vittata* lives in "crowds" in Brazil. The studied population was composed of males, hermaphrodites, and transitional individuals (having characteristics of males and hermaphrodites). The above information suggests that *L. rauli* is a protandric simultaneous hermaphrodite, as it has been observed in all species of *Lysmata* that have been investigated. *Lysmata vittata* has invaded the southwestern Atlantic and is present in Bahia, Rio de Janeiro and São Paulo, Brazil.

KEY WORDS: alien species, hermaphroditism, *Lysmata*, morphology, population structure

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INTRODUCTION

The description of a new species implies testing the hypothesis that a single, i.e., a holotype, or a set of specimens under study represents a sample of a natural entity that is different from all other natural entities, i.e., a species, already known to scientists (see Winston, 1998). Ideally, this process involves the gathering of comprehensive information (morphological traits, color, physiology, and genetic markers) that will permit differentiating, unambiguously, the specimens under study from all of the remaining congeneric species living both in sympatry and allopatry. Certainly, this is a complex and time-consuming process that might further be complicated, among others, by intrinsic trait variability of the natural entities, by the existence of cryptic and/or sibling species complexes (Knowlton, 1986, 1993), and the presence of hitherto unrecognized exotic species in non-native geographical areas (see Ruiz et al., 1999; Tavares & Mendonça Jr., 2004; Tavares, 2011). Unfortunately, in many cases, not all types of evidence, e.g., genetic, are readily available to the investigator. In various other instances, the comparison between the specimens under study and allopatric congeners is logistically challenging or simply neglected. In a world in which bioinvasions are increasingly witnessed (Ruiz et al., 1999), it has become particularly important to compare type specimens of purportedly new species with those of allopatric congeners. In the most extreme case, when the comparison of specimens under study with those of allopatric species is ignored, the specimens used for the formal description of a new species might, in reality, pertain to an invasive species previously unnoticed in the region. In this study, we focused on exploring whether or not specimens described as a new species with limited material actually pertain to a hitherto unnoticed invasive species of caridean shrimp of the genus *Lysmata*.

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Species of *Lysmata* display a considerable diversity of life habits, mating systems, social behaviors, symbiotic relationships, and color (Baeza et al., 2009b). This genus currently comprises 42 species worldwide (Chace, 1997; Okuno and Fiedler, 2010; Anker and Cox, 2011; De Grave and Fransen, 2011). Some species that are not conspicuous in terms of color live in large aggregations among rocks in temperate localities (Bauer and Holt, 1998). Other species live in small groups and, on occasion, can be found living in the same refuge with toadfishes (Baeza et al., 2009a) or moray eels (Limbaugh et al., 1961). Other species have also adopted a strictly symbiotic lifestyle with tube sponges and have been shown to be socially monogamous (Baeza, 2010b). Remarkably, some of these monogamous species display striking color patterns and apparently provide cleaning services to fishes (Limbaugh et al., 1961; Bruce, 1983; Fiedler, 1998; Baeza, 2009).

Lysmata is also recognized for its unusual sexual system: protandric simultaneous hermaphroditism (Bauer and Holt, 1998; Fiedler, 1998; Baeza, 2009). In sequentially simultaneous hermaphroditic species, juveniles first mature as functional males (or male phase [MP] individuals), and later become functional simultaneous hermaphrodites (or hermaphrodite phase [SH] individuals) capable of reproducing either in the male and female role (Bauer and Holt, 1998; d'Udekem d'Acoz, 2000; Bauer and Newman, 2004; Baeza et al., 2007; Baeza, 2008; Baeza and Anker, 2008; Anker et al., 2009). Protandric simultaneous hermaphroditism is believed to be a fixed trait in *Lysmata* (Baeza, 2009; Onaga et al., 2012). Nonetheless, new descriptions of the natural history and sexual behavior of more shrimp are needed to test the notion of lack of variability in gender expression within *Lysmata*.

In the southwestern Atlantic (Brazil and Argentina), a total of six species of *Lysmata* has been reported: *L. moorei* (Rathbun, 1901), *L. intermedia* (Kingsley, 1878), *Lysmata grabhami* (Gordon, 1935) and *L. bahia* Rhyne and Lin, 2006, *L. ankeri* Rhyne and Lin, 2006, and *L. rauli* Laubenheimer and Rhyne, 2010 (Christoffersen, 1998; Rhyne and Lin, 2006; Laubenheimer and Rhyne, 2010). Rhyne and Lin (2006) revised the complex *L. wurdemanni*, redescribing this species and describing four new species within the complex (see above). The previous records of *L. wurdemanni* from the Brazilian coast (Chace, 1972; Williams, 1984; Christoffersen, 1998) were attributed to *L. ankeri* and *L. bahia*.

Lysmata rauli was the last species described for the region (Laubenheimer and Rhyne, 2010). The description was based only on a single specimen collected in Salvador, Bahia, Brazil. Beyond the type locality, *L. rauli* is also known by anecdotal reports from Cabo Frio, Rio de Janeiro, Brazil (Laubenheimer and Rhyne, 2010). No biological information for *L. rauli* was provided other than a putatively hermaphroditic condition of the holotype. Thus, whether *L. rauli* is a protandric simultaneous hermaphroditic species, as reported before for every species of the genus whose sexual system has been studied (see Baeza, 2009), needs confirmation. Also, Laubenheimer and Rhyne (2010) compared *L. rauli* with the rest of the species from the southwestern Atlantic. However, no comparison of the holotype

with material or descriptions of species from other biogeographic regions was conducted. Previous studies on the systematics of *Lysmata* have demonstrated the relevance of such pan-geographic comparisons; the closest extant relative of *L. hochi* Baeza and Anker, 2008 from the greater Caribbean is, both morphologically and genetically, *L. kuekenthali* (De Man, 1902) from the Indo-Pacific (Baeza and Anker, 2008; Baeza et al., 2009a; Baeza, 2010a). Furthermore, the southwestern Atlantic is known to harbor several exotic crustaceans (Tavares and Mendonça Jr., 2004; Pachelle et al., 2011; Tavares, 2011), including three caridean shrimps, e.g., *Athanas dimorphus* Ortmann, 1894 – Pachelle et al. (2011); *A. nitescens* (Leach, 1813) – Almeida et al. (in press); *Palaemon macrodactylus* Rathbun, 1902 – Spivak et al. (2006). Thus, at present, a hypothesis stating that *L. rauli* is a synonym of a second alien species of *Lysmata* that has been introduced to the southwestern Atlantic cannot be refuted. Indeed, a preliminary comparison between the description of *L. rauli* suggests that the holotype is very similar to *L. vittata* from the Indo-Pacific.

The aim of this study was to explore the species status and phylogenetic position of *L. rauli* using both morphological and molecular markers. We investigated intra-specific morphological variation and compared newly collected specimens with other species from the region as well as from other biogeographical regions. We also determined the sexual system of *L. rauli*. We used dissections and anatomical observations to test if this species is a protandric simultaneous hermaphrodite. We provide additional information on its ecology and reproductive biology. Lastly, we report a range extension for this species on the Brazilian coast.

MATERIALS AND METHODS

Sampling of *Lysmata rauli*

Individuals of *L. rauli* were collected between August 2010 and July 2011 from lantern-nets used for farming the oyster *Crassostrea rhizophorae* (Guilding, 1828) in Camamu Bay (13°56′04″S, 039°01′08″W), Bahia, northeastern Brazil. Sampling was conducted onboard a small boat, from which the permanently submerged lantern-nets were raised and overturned into plastic trays on the boat. Shrimps were found inside and outside of the lantern-nets among the bio-fouling that included various species of algae, sponges and cnidarians. After their study, specimens retrieved from this locality were deposited at the Crustacean Collection of the Universidade Estadual de Santa Cruz, Ilhéus, Bahia, Brazil (sample codes UESC 1451-1455) and at the Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil (MZUSP 24485).

An additional specimen of *L. rauli* was collected during October 2009 from São Vicente estuary (23°58′41″S, 46°24′88″W), São Paulo, southeastern Brazil. This specimen was retrieved from the bottom of the bay during daytime using an otter trawl net (mesh size 20 mm and 18 mm at the cod end) trawled by a small boat. After its study, this specimen was deposited at the Crustacean Collection of the Departmento de Biologia (CCDB 3925), Faculdade de Filosofia Ciências e Letras de Ribeirão Preto (FFCLRP), Universidade de São Paulo (USP), Brazil. At this study site, salinity, temperature, and water transparency were recorded with a manual refractometer (Atago SMill), a thermometer and Secchi disk, respectively.

Several specimens were photographed in order to record the color pattern after anesthetization on ice and before being fixed in 70% ethanol.

Morphological Variation and Comparison with Allopatric Congeners

First, the following characters were checked for variation in a total of 48 specimens collected in Bahia State and one specimen collected in São Paulo State: dorsal and ventral dentition of the rostrum, number of segments on the carpus and merus of the second pereiopod, and the number of spiniform setae on the ventro-lateral margin of the merus and on the ventral margin of the propodus of pereiopods 3-5. Next, we compared *L. rauli* to *L.*

vittata taking into account character variability. The above, considering that preliminary analyses suggested that *L. rauli* closely resembled *L. vittata*, a widespread species from the Indo-Pacific that has also been reported as possibly exotic, e.g., in New Zealand (Ahyong, 2010).

Phylogenetic Position of *Lysmata rauli*

We were interested in the phylogenetic position of *L. rauli* within the morpho-variable clade of peppermint shrimp (Baeza et al., 2009a, b). Furthermore, we tested the hypothesis that *L. rauli* was a genetically dissimilar entity from *L. vittata*. Therefore, we construct a molecular phylogeny using the 16S DNA fragment. Tissue extraction, PCR amplification with specific primers, product cleanup, and sequencing were conducted as described in Baeza et al. (2009a, b) and Baeza (2010a). A total of 32 sequences: two sequences from two specimens of *L. rauli*, two sequences from two specimens of *L. vittata*, and 29 sequences from other 28 species pertaining to the Neotropical, cosmopolitan, cleaner, and morpho-variable clades of peppermint shrimp were included in the present phylogenetic analysis. Other 2 sequences from the species *Merguia rhizophorae* (Rathbun, 1900) and *M. oligodon* (De Man, 1888) were included as out-groups during the phylogenetic analyses (see Table 1 in Baeza, 2010a for Genebank accession numbers). Alignment of the set of sequences was conducted in MUSCLE as implemented in MEGA 5 (Tamura et al., 2011). The aligned sequences of the 16S gene fragment did contain various indels and was ambiguous. Thus, we identified positions that were highly divergent and poorly aligned in the 16S gene segment using the software GBlocks v0.91b (Castresana, 2000) and we omitted them from the analyses. After highly divergent positions were pruned, the 16S consisted of 402 bp (53% of a total of 750 original positions). Selection of an optimal model of base substitution was conducted with jModelTest 0.1.1 (Posada, 2008). The optimal model found by jModelTest (selected by the AIC_c) was a TPM3uf + I + G evolutionary model ($-\ln L = 2815.6927$). The calculated parameters were as follow: assumed nucleotide frequencies A = 0.3209, C = 0.1429, G = 0.1951, T = 0.3410; substitution rate matrix with A-C substitution = 0.2597, A-G = 5.5114, A-T = 1.0, C-G = 0.2597, C-T = 5.5114, G-T = 1.0; rates for variable sites assumed to follow a gamma distribution (G) with shape parameter = 0.6510 and proportion of invariable sites I = 0.4720. This model was implemented in MrBayes (for Bayesian Inference analysis) and Treefinder (for maximum likelihood analysis – Gangolf et al. 2004). In MrBayes, 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 to estimate posterior probabilities. The robustness of the ML tree topologies was assessed by bootstrap resampling of the observed data 1000 times.

Lastly, we tested if the different specimens of *L. rauli* and *L. vittata* segregated and formed different species-specific monophyletic clades (see results for details). For this purpose, constrained trees (in which the monophyly of *L. rauli* and *L. vittata* specimens were 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 of both *L. rauli* and *L. vittata* (unconstrained versus constrained trees) according to the criteria of Kass and Raftery (1995). Bayes factors compare the total harmonic mean of the marginal likelihood of unconstrained versus monophyly-constrained models. The higher the value of the Bayes factor statistic implies stronger support against the monophyly of a particular group (Kass and Raftery, 1995). Specifically, a value for the test statistic $2 \log_e(B_{10})$ between 0 and 2 indicates no evidence against H_0; values from 2 to 6 indicate positive evidence against H_0; values from 6 to 10 indicate strong evidence against H_0; and values > 10 indicate very strong evidence against H_0 (Kass and Raftery, 1995).

Sexual System of *Lysmata rauli*

To examine the sexual system of *L. rauli*, observations on external morphology were made on a total of 88 specimens extracted from lantern-nets. First, the carapace length (CL) of each shrimp was measured with a digital caliper (precision = 0.01 mm) from the postorbital margin to the posterior margin of the carapace. Next, we recorded in all shrimps the presence/absence of cincinnuli (coupling hooks) on the endopods of the first pleopods, of appendices masculinae on the endopods of the second pleopods, and of brooded embryos on the pleopods. A particular set of the characters above permitted the identification of MPs and SHs. Shrimps were considered MPs by the presence of coupling hooks on the endopods of the first pleopods and appendices masculinae on the endopods of the second

pleopods. Shrimps were considered SHs by the absence of coupling hooks on the endopod of first pleopods and the absence of appendices masculinae on the endopods of the second pleopods (Bauer and Holt, 1998; Baeza et al., 2008; Baeza, 2010a, b). Lastly, the anatomy of the reproductive system was examined in six individuals brought alive to the laboratory: 3 small individuals, presumably males (all with CL = 6.3 mm) and 3 ovigerous individuals, presumably hermaphrodites (CL = 8.1, 8.5 and 9.3 mm). The specimens were dissected for gonads observation and the presence of ovarian and testicular tissues was examined under the microscope. Lastly, differences in the CL between sexual phases (see results) were tested using a t-test (Sokal and Rohlf, 1995).

RESULTS

Morphological Variation and Comparison with Allopatric Species

In *L. rauli*, the rostral dentition varied considerably (Table 1, Fig. 1). Most of the specimens analyzed possessed 7 dorsal and 4 ventral teeth (n = 22). Seven specimens have 7 dorsal and 3 ventral teeth, 6 have 6 dorsal and 3 ventral, 4 have 8 dorsal and 4 ventral, 4 have 6 dorsal and 4 ventral, 3 have 7 dorsal and 5 ventral and only 2 specimens have 8 dorsal and 5 ventral. The number of dorsal teeth placed posteriorly to the postorbital margin varied from 2 to 4. The majority of the individuals (n = 36) exhibit 3 teeth posteriorly to the orbits, but individuals with 2 (n = 11) and 4 (n = 1) were observed. The number of meral segments on pereiopod 2 (P2) varied from 5 to 9. The number of carpal segments on the second pereiopod (P2) varied from 15 to 19. Twelve and 7 individuals presented difference of one segment on meral and carpus segments between right and left P2, respectively. The number of spiniform setae on the ventrolateral margin of merus of pereiopods 3-5 (P3-5) varied between 3-6, 2-5, and 1-2 on P3, P4 and P5, respectively. Less than one-third of the individuals exhibit differences of one seta between right and left P3-5. The number of spiniform setae on the ventral margin of P3-5 varied between 5-9, 4-7, and 2-7, on P3, P4 and P5, respectively. Similarly to the meri, approximately one-third of the individuals exhibit differences of one seta between right and left P3-5. Variation found in material from São Paulo is according those described above (Fig. 1).

Considering its overall morphology, *L. rauli* fits well into the cosmopolitan clade of peppermint shrimp (*sensu* Baeza, 2010a) and particularly resembles *L. kuekenthali* and *L. vittata*, both from the Indo-West Pacific, and *L. hochi*, from the Caribbean (Table 2). However, *L. rauli* differs from *L. hochi* by the lower number of segments on carpus of the second pereiopod (15-19 in *L. rauli* vs. 21-24 in *L. hochi*) and by the presence of a developed pterygostomial tooth (absent in *L. hochi*). In turn, *L. rauli* differs from *L. kuekenthali* by the number and disposition of the rostral teeth, and the number of segments comprising the rudimentary accessory branch on the dorsal antennular flagellum (single-segmented in *L. rauli* vs. two-segmented in *L. kuekenthali*). Comparison of our material with descriptions of *L. vittata* provided by Bruce (1986) and Chace (1997) demonstrates considerable overlap on almost all characters analyzed (Table 2). The length of the rostrum seems to be shorter in our material compared to that in *L. vittata*. However, this possible difference should be considered with caution, considering the existence of variation in morphology and rostrum length found in other *Lysmata* spp. Another possible difference between *L. rauli* and *L. vittata* would lie on the accessory branch

Table 1.   Variability in characters of systematic relevance in the shrimp *Lysmata rauli* Laubenheimer and Rhyne, 2010.

Character	Mean	SD	Range
Number of dorsal rostral spines	6.92	0.58	6–8
Number of ventral rostral spines	3.83	0.60	3–5
Number of segments on P2 merus (left side)	7.06	0.48	6–8
Number of segments on P2 merus (right side)	6.90	1.21	5–9
Number of carpal segments on P2 merus (left side)	16.08	0.79	15–19
Number of carpal segments on P2 merus (right side)	16.33	0.93	15–19
Number of spiniform setae on P3 merus (left side)	3.55	1.21	3–5
Number of spiniform setae on P3 merus (right side)	3.83	1.06	3–6
Number of spiniform setae on P4 merus (left side)	3.44	1.34	2–5
Number of spiniform setae on P4 merus (right side)	3.46	1.22	2–5
Number of spiniform setae on P5 merus (left side)	1.81	0.79	1–3
Number of spiniform setae on P5 merus (right side)	1.88	0.82	1–3
Number of spiniform setae on P3 propodus (left side)	5.47	2.01	5–9
Number of spiniform setae on P3 propodus (right side)	5.71	1.58	5–8
Number of spiniform setae on P4 propodus (left side)	5.17	2.04	5–7
Number of spiniform setae on P4 propodus (rigth side)	5.33	1.72	5–7
Number of spiniform setae on P5 propodus (left side)	4.33	1.80	4–6
Number of spiniform setae on P5 propodus (right side)	4.71	1.53	4–7

on the dorsal antennular flagellum (absent in *L. vittata*, see Chace, 1997, vs. rudimentary in *L. rauli*, see Laubenheimer and Rhyne, 2010). However, this character, not taken into account on previous *Lysmata* descriptions is actually present and unguiform in shape in *L. vittata* (Sammy De Grave, pers. communication). Laubenheimer and Rhyne (2010) refer it as "not well developed" or "rudimentary" in *L. rauli*. In our material, the accessory branch is one-segmented and unguiform as in *L. vittata*.

The color pattern also differs between *L. rauli* and *L. hochi* and *L. kuekenthali*. The color of the later two species is very similar; the carapace has a complex pattern of longitudinal, oblique, and transverse reddish bands and patches, the post-rostral carina is red, the pleon has broad transverse bands, the telson and uropods are mostly red, the antennular and antennal peduncles are reddish with white; and the pereiopods are reddish with white around articulations (Kemp, 1914; Kubo, 1951; Baeza and Anker, 2008). Nonetheless, *L. rauli* and *L. vittata* cannot be differentiated by their color pattern (Fig. 2).

Kemp's (1914) described in detail the color of *L. vittata* as follows: "*The whole animal is practically transparent with narrow longitudinal stripes and streaks on the carapace and abdomen. At the anterior end of the first abdominal somite there is a complete transverse band and another is distinct at the anterior end of the fourth somite. The latter stops half way down on either side where it meets the uppermost of the three complete longitudinal stripes of the abdomen. There*
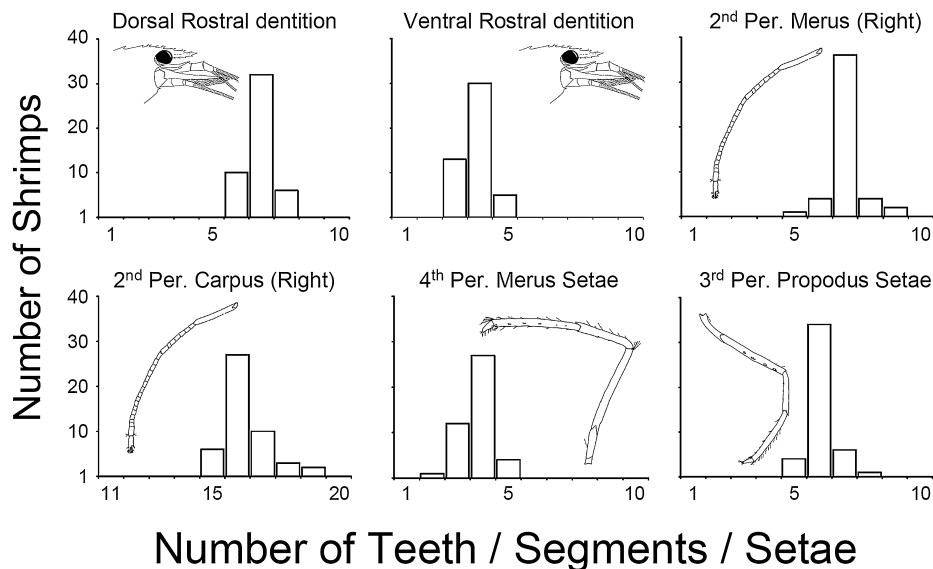

Fig. 1.   Variability in selected characters of systematic relevance in the shrimp *Lysmata rauli* Laubenheimer and Rhyne, 2010.

Table 2.   Characters of systematic relevance and color pattern of the shrimp *Lysmata rauli* Laubenheimer and Rhyne, 2010 and morphologically similar species.

Character	*Lysmata rauli* (present study)	*Lysmata vittata* (according to Bruce, 1986; Chace, 1997)	*Lysmata kuekenthali* (according to Kubo, 1951; Chace, 1997)	*Lysmata hochi* (according to Baeza and Anker, 2008)
Number of dorsal rostral teeth	6–8	4–8	4–5	5
Number of dorsal rostral teeth posterior to the orbit	2–4	2–3	1	2
Number of ventral rostral teeth	3–5	1–5	1–3	1–3
Rostrum length	Reaching 1/2 of the second segment of the antennular peduncle	Reaching slightly beyond end of the second segment of the antennular peduncle	Almost reaching the end of the second segment of the antennular peduncle	Reaching slightly beyond end of the first segment of the antennular peduncle
Pterygostomial tooth	Present	Present	Very small when present	Absent
Accessory branch of dorsal antennular flagellum	With one unguiform segment (see comments on the text)	With one unguiform segment (see comments on the text)	With 2 free segments	With 1 unguiform segment
Number of meral segments on pereiopod 2	5–9	9	Not reported	15–21
Number of carpal segments on pereiopod 2	15–19	15–31	17–21	21–24
Number of spiniform setae on ventral margin of merus/propodus of pereiopod 3	3–6 on merus; 5–9 on propodus	5 on merus; 6 on propodus	1–3 on merus	2–4 on merus
Number of spiniform setae on ventral margin of merus/propodus of pereiopod 4	2–5 on merus; 4–7 on propodus	5 on merus	Not reported	Not reported
Number of spiniform setae on ventral margin of merus/propodus of pereiopod 5	1–2 on merus; 2–7 on propodus	1 on merus; 4 on propodus	Not reported	2 on merus
Color pattern	Body semi-transparent, with longitudinal, oblique and transverse red bands	Body semi-transparent with narrow longitudinal red bands	Body with narrow longitudinal red bands	Body semi-transparent; carapace with a complex pattern of longitudinal, oblique and transversal bands and patches; pleon with red broad transverse bands; telson and uropods mostly red

are other short longitudinal streaks on the carapace and abdomen, those on the anterior portion of the former being oblique. There is a median red stripe on the telson and on each inner uropod. The thoracic appendages are clear red and the eggs light green." After careful examination of our specimens and the photographs available in Laubenheimer and Rhyne (2010), we have not been able to find any significant difference between the color pattern of *L. rauli* and *L. vittata* (Fig. 2).

Phylogenetic Position of *Lysmata rauli*

We obtained nucleotide sequences of a section of the 16S gene from two specimens of *L. rauli* (Genbank accession numbers JX912539 and JX912540). The two sequences were a close match (Tamura Nei distance = 0.006). The phylogenetic analyses confirmed that *L. rauli* pertains to the morpho-variable clade, and thus, that is closely related to *L. hochi*, *L. kuekenthali*, and *L. vittata* (Fig. 3). The two specimens of *L. rauli* did not segregate together in a
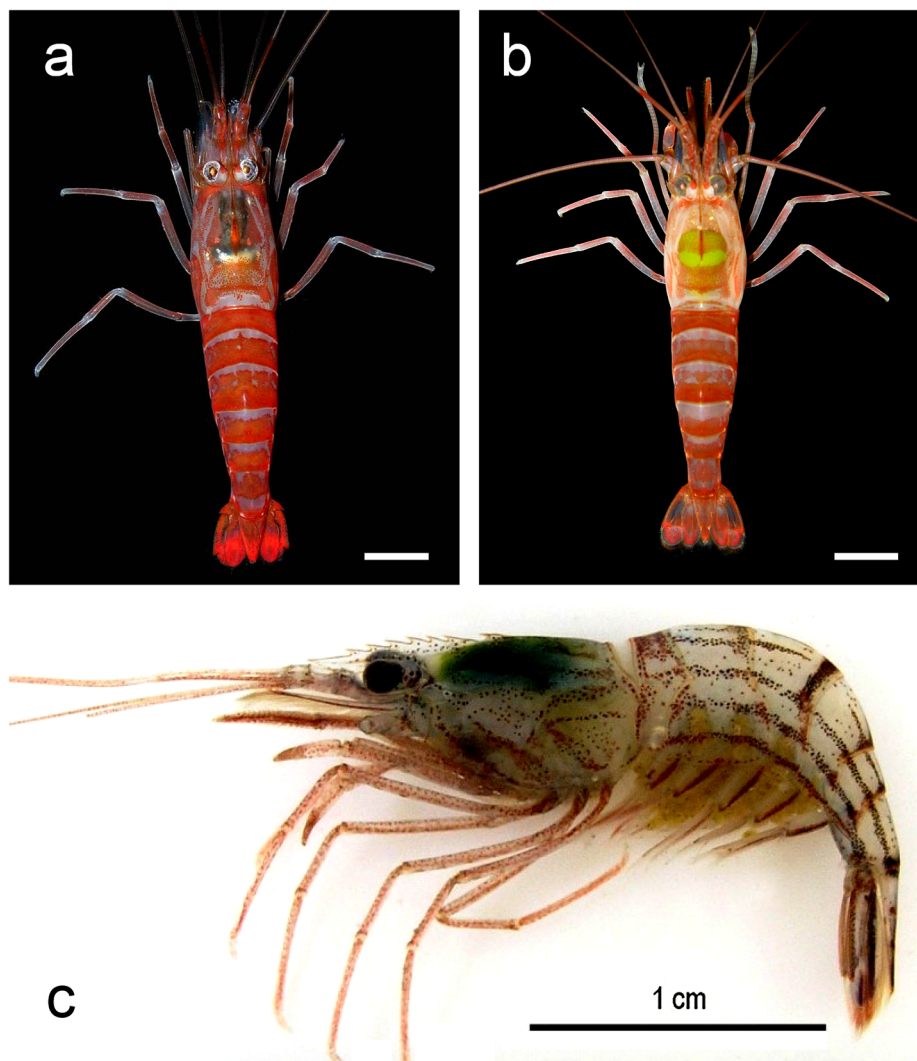
Fig. 2.   Color patterns of *Lysmata*. A, *L. hochi* (dorsal view); B, *L. kuekenthali* (dorsal view); C, *L. rauli* (lateral view). Specimen of *L. rauli* is a ovigerous hermaphrodite from São Vicente estuary, São Paulo, Brazil. Photographs by Arthur Anker (a), J. Antonio Baeza (b), and S. M. Simões (c). This figure is published in colour in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/1937240x.

single clade but clustered together with two other sequences from specimens of *L. vittata*. More importantly, the Bayes factor analysis revealed no support for the monophyly of specimens pertaining to *L. rauli* and *L. vittata*. Comparison of the unconstrained tree (harmonic mean = −4421.66) versus the tree wherein specimens of *L. rauli* and of *L. vittata* were imposed as different monophyletic clades, indicated strong support for the unconstrained tree (harmonic mean = −4213.58, $2 \ln(B_{10}) = 10.68$).

Sexual System of *Lysmata rauli*

A total of 19 out of the 88 shrimp analyzed were classified as males considering their external morphology. All of these male shrimp had gonopores on the coxae of the last pair of pereiopods, cincinulli (coupling hooks) on the endopods of the first pair of pleopods, and appendices masculinae on the endopods of the second pair of pleopods (Fig. 4). Long spines on the distal portion of the appendices masculinae were observed in all these males. Lastly, dissections of the gonads from small shrimps (N = 3) considered males ac-

cording to their external morphology demonstrated the presence of ovotestes with ovarian tissue on the anterior region and testicular tissue on the posterior region (Fig. 4). The ovarian portion of the ovotestes was poorly developed and translucent, containing small colorless oöcytes, whereas the testicular portion was well developed (Fig. 4). In all these three shrimp, two pairs of genital ducts, i.e., oviducts connected to the female portion and vasa deferentia connected to the male portion were observed in these three males (Fig. 4).

A total of 57 out of the 88 shrimp analyzed were classified as hermaphrodites considering their external morphology. All these shrimp were characterized by the absence of cincinnuli and appendix masculina on the endopods of the first and second pleopods, respectively (Fig. 4). Importantly, all these shrimp had gonopores on the coxae of the fifth pereiopods. Also, mature green ovaries were observed through the carapace in all these shrimps (see Fig. 2c). Dissections of the gonads from large shrimps (N = 3) considered hermaphrodites according to their external morphology
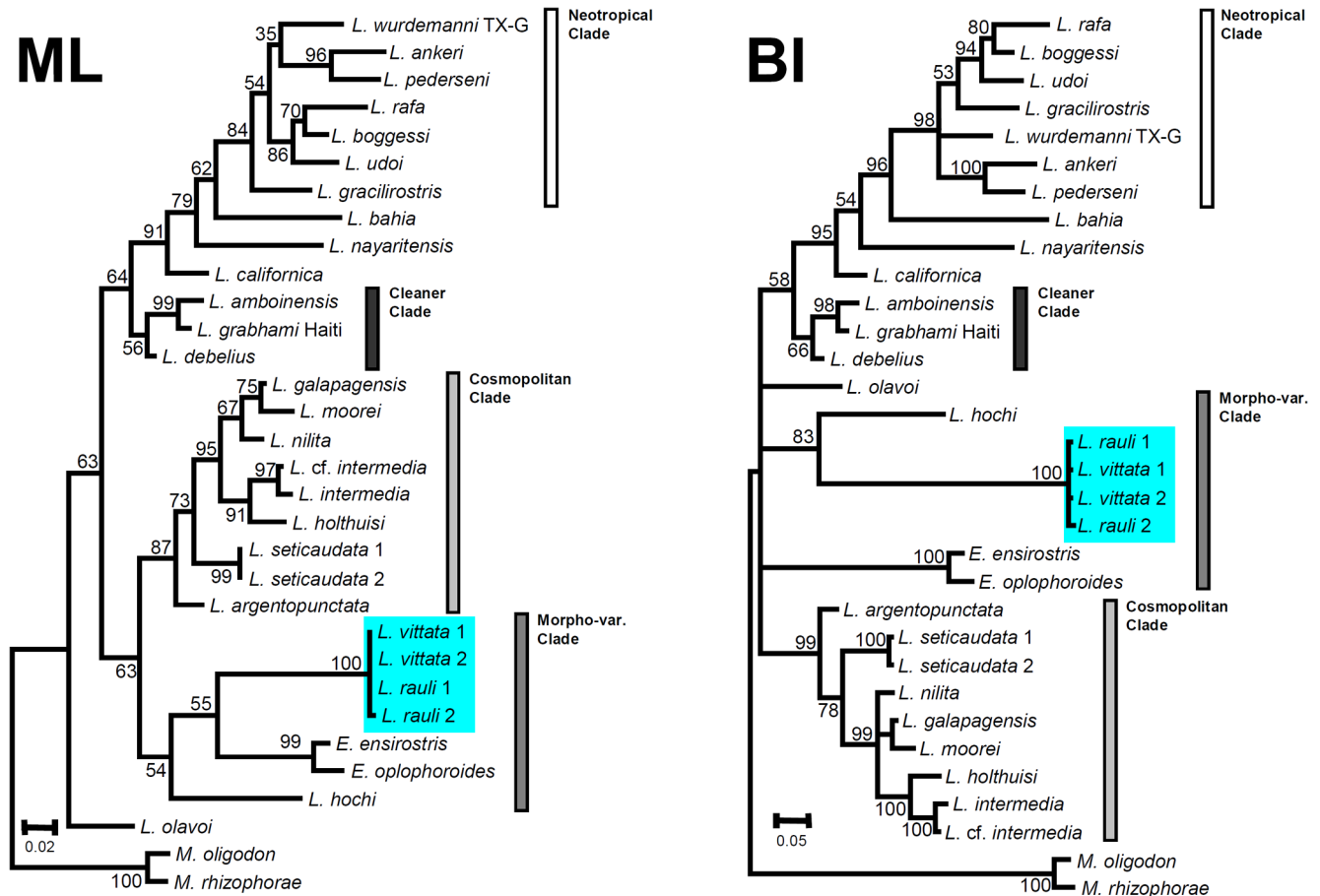
Fig. 3.   Phylogenetic tree obtained from ML analysis (ML) and BI analysis (BI) of the partial 16S rRNA gene for the genus *Lysmata*, including *L. rauli*. Numbers above or below the branches represent the bootstrap values obtained from ML and the posterior probabilities from the BI analysis (ML/BI). This figure is published in colour in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/1937240x.

demonstrated the presence of ovotestes with ovarian tissue on the anterior region and testicular tissue on the posterior region. The ovarian portion of the ovotestes was developed and contained large green vitellogenic oöcytes. Also, the testicular portion was developed in all these shrimp.

Lastly, a total of 7 out of the 88 shrimp analyzed were classified as "transitional" individuals considering their external morphology. All these transitional shrimps had both male external traits, i.e., cincinnuli in the first pleopods and well-developed appendices masculinae in the endopods of the second pleopods, and female external traits, i.e., ovotestes with maturing oöcytes, noticed after dissection.

Males measured from 4.2 to 8.5 mm in CL size, hermaphrodites from 5.4 to 11.2 mm and the transitional specimens from 5.5 to 8.7 mm. The average body size of hermaphrodites (8.5 mm $\pm$ 0.97 mm) was larger than that of males (5.4 $\pm$ 0.58) (t-test: P = 0.0016) and transitional (6.7 $\pm$ 0.87) (t-test: P = 0.017). However, although males and transitional differed in mean size, this difference was not statistically significant (P > 0.05) (Fig. 5).

Ecological Notes on *Lysmata rauli*

The fouling where the shrimp were sampled was composed mainly by algae, sponges, tunicates, the snowflake octocoral *Carijoa riisei* (Duchassaing and Michelotti, 1860) (Antho-

zoa; Clavulariidae), and other unidentified colonial cnidarians. The oyster farming is approximately 100 m far away from the mangrove and has a maximum depth of 8 m. Salinity at the oyster farm site ranged from 32 to 35 p.s.u., temperature from 21 to 28°C, and water transparency from 1.7 to 2.2 m.

DISCUSSION

Is *Lysmata rauli* a Valid Species?

In the description of *L. rauli*, information on this species morphology, biology, and ecology was limited by having only a single specimen: the holotype (Laubenheimer and Rhyne, 2010). Herein, we have studied morphological variation in *L. rauli* using a much larger sample size and we have found that *L. rauli* shows considerable variation in characters of systematic importance. The number of rostral teeth, the number of articles on the carpus and merus of the second pereiopod, and the number of spiniform setae were the most variable characters. The presence of morphological variation has already been noticed in other congeneric shrimp (Rhyne and Lin, 2006; Anker et al., 2009; Baeza et al., 2009a). Knowledge on the limits of morphological variation is useful for species recognition especially in taxonomically com-
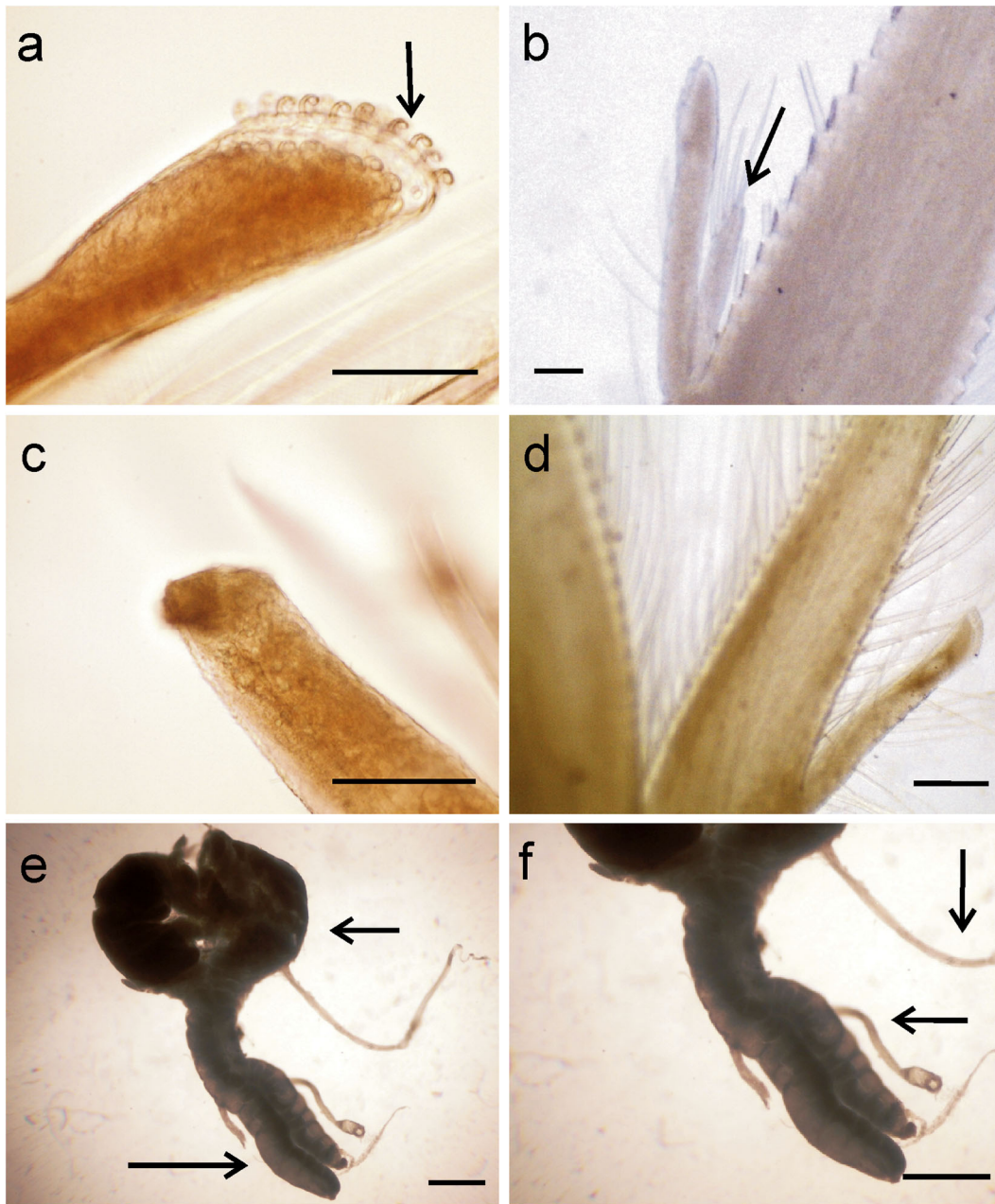
Fig. 4. Attributed specimens to *Lysmata rauli*, external and internal reproductive characters. a, endopod of male pleopod 1 showing cincinnuli (arrow); b, endopod of male pleopod 2 showing the appendix masculina (arrow); c, endopod of hermaphrodite pleopod 1 showing no cincinnuli; d, endopod of hermaphrodite pleopod 1 showing no appendix masculina; e, oötestes of a male showing testicular (longer arrow) and ovarian (shorter arrow) portions; f, detail of a male ootestes showing vas deferens (longer arrow) and oviducts (shorter arrow). Photographs by G. O. Soledade. This figure is published in colour in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/1937240x.

plicated genus like *Lysmata* that contains species complexes (Rhyne and Lin, 2006; Anker et al., 2009).

Laubenheimer and Rhyne (2010) stated that *L. rauli* could be easily distinguished from any other species of the western Atlantic by the presence of a well-developed pterygostomial tooth, rudimentary accessory branch of dorsal antennular flagellum, and reduced number of carpal segments on the second pereiopod. At least some of these characters are shared with species of *Lysmata* from other geographical regions, such as *L. kuekenthali* and *L. vittata*, both from the Indo-West Pacific. Moreover, the western Atlantic *L. hochi* also exhibits morphological similarity with *L. rauli*. All these species present some degree of overlap in the variation of some morphological characters such as the number of dorsal and ventral rostral spines, the number of segments on merus and carpus of pereiopod 2, or in the number of spiniform setae on ambulatory legs (Table 2).

As pointed out above, *L. rauli* can be differentiated from *L. kuekenthali* and *L. hochi* taking into account morphology only. Nonetheless, the overlap in the morphological
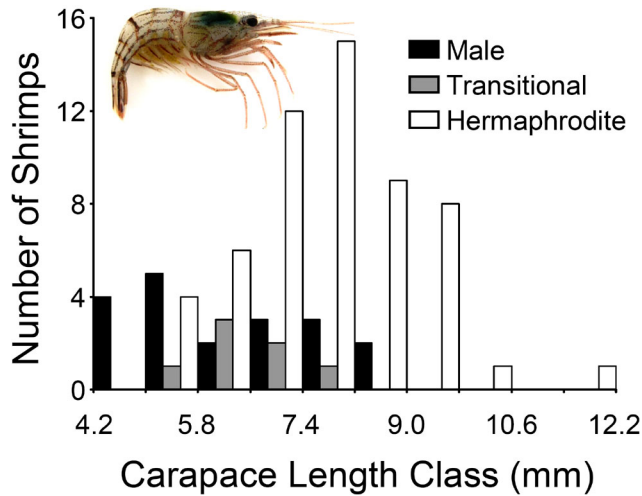
Fig. 5. Distribution of number of individuals in each size-class of male, transitional and hermaphrodite, of individuals attributed to *Lysmata rauli* collected from long-lines used for aquaculture of the oyster *Crassostrea rhizophorae* at Grande Island, Camamu Bay, Bahia, Brazil, from November 2010 to August 2011. This figure is published in colour in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/1937240x.

characters examined prevents an unambiguous differentiation between *L. rauli* and *L. vittata*. *Lysmata vittata* was described from Hong Kong based on the holotype only (Stimpson, 1860). Description of their junior synonyms *Nauticaris unirecedens* Spence Bate, 1888 and *Hippolysmata durbanensis* Stebbing, 1921 (see Chace, 1997; De Grave and Fransen, 2011) were also based only on the holotypes (Spence Bate, 1888; Stebbing, 1921). Bruce (1986) redescribed *L. vittata* based upon a single ovigerous individual from Hong Kong. Chace (1997) provided a species diagnosis pointing out, in litt. that "*As currently conceived,* L. vittata *seems to be quite variable, especially in regard to the rostral formula and the number of articles in the carpus of the second pereiopod.*" However, he did not report any additional material. Other records of the species were also based on few individuals (Kemp, 1914; Kubo, 1951; Ahyong, 2010). Thus, the morphological variation typically found in other species of *Lysmata* apparently has not been reported in *L. vittata* due to the fact that previous morphological accounts were based on the examination of a few specimens available by those authors. On the other hand, the small differences observed between the Brazilian material (*L. rauli*) and the Indo-Pacific species (*L. vittata*) (Table 2) can be considered within the range of variation of *L. vittata*, conforms that *L. rauli* is not a valid entity and is therefore a junior synonym of *L. vittata*.

Importantly, this study found no genetic segregation among specimens of *L. rauli* and *L. vittata*. Quite the opposite, the two sequences of *L. rauli* clustered together with the two other sequences from specimens of *L. vittata*. Lastly, the Bayes factor analysis revealed no support for the monophyly of specimens pertaining to *L. rauli* and *L. vittata*. Therefore, we must conclude again that *L. rauli* is conspecific with *L. vittata*. Also, our findings suggest the need for the taxonomic revision of the *L. vittata* 'species complex' which includes various other species, i.e., *Nauticaris unirecedens* and *Hippolysmata durbanensis* –

(see Chace, 1997; De Grave and Fransen, 2011), that might well be junior synonyms of *L. vittata*. Molecular 'barcoding' analyses using 16S and COI mitochondrial markers will be most useful to reveal cryptic, sibling and hitherto unrecognized alien species in this group.

Sexual System and Ecology of *Lysmata vittata* in Brazil

Observations on the external sexual morphology, internal anatomy, and population size-frequency distribution of *L. vittata* strongly indicate that this species is a protandric simultaneous hermaphrodite. First, shrimps with external characteristics of "pure" females were absent among the smallest body size classes (juveniles), ruling out gonochorism (separate sexes) as the sexual system of *L. vittata*. Second, the studied population was composed of Males (MPs) and simultaneous hermaphrodites (SHs, and not pure females) and males were, on average, smaller than SHs. This size-frequency distribution of the sex phases agrees with expectations for protandric simultaneous hermaphrodites (Bauer and Holt, 1998; d'Udekem d'Acoz, 2000; Bauer and Newman, 2004; Baeza et al., 2007; Baeza, 2008, Baeza and Anker, 2008; Anker et al., 2009; Braga et al., 2009; Baeza et al., 2010; Nunes et al., 2010; Onaga et al., 2012). Lastly, several transitional individuals having traits of both males and simultaneous hermaphrodites, e.g., male gonopores and ovotestes with mature oöcytes, respectively, were observed during dissections. Altogether, the information above demonstrates that *L. vittata* is a protandric simultaneous hermaphrodite; shrimp invariably start their benthic life as males that later in life become "transitional" individuals when losing the appendices masculinae and developing female characters, e.g., maturing ovaries. During the next molt, "transitional" individuals become simultaneous hermaphrodites and remain so for the rest of their lives. Importantly though, experimental studies in the laboratory (Baeza et al., 2009a) are needed in order to determine the functionality, i.e., the capability of reproducing both as male and female at the same time, of simultaneous hermaphrodites in *L. vittata*.

Protandric simultaneous hermaphroditism (PSH) has been confirmed to date in all species of the genus *Lysmata* that have been investigated (Baeza et al., 2009a and references therein). Thus, this finding of PSH in *L. vittata* supports the notion that this sexual system is conserved within the genus *Lysmata* (Baeza 2009). PSH is also a trait observed in the closely related genus *Exhippolysmata* (Baeza et al., 2009b, 2010; Braga et al., 2009; Nunes et al., 2010) and in the barbouriid (*sensu* De Grave and Fransen, 2011) shrimp *Parhippolyte misticia* (Clark, 1989) (Onaga et al., 2012). Molecular phylogeny studies have suggested a paraphyletic relationship between *Lysmata* and *Exhippolysmata* and the inclusion of the latter among *Lysmata*-species (Baeza et al., 2009b). The position of *P. misticia* in relation to the clade comprised of *Lysmata* + *Exhippolysmata* is unsettled (see Onaga et al., 2012). Phylogenetic studies including representatives of *Lysmata*, *Exhippolysmata*, *Parhippolyte*, and other closely related genera of hippolytid and barbouriid shrimp (*Barbouria*, *Calliasmata*, *Ligur*, *Merguia*) are needed to reveal the number of times and ecological conditions that have favored PSH in shrimp from the infraorder Caridea.

Shrimp from the genus *Lysmata* are quite diverse in terms of ecology and behavior (Limbaugh et al., 1961; Rhyne and Anker, 2007; Anker et al., 2009; Baeza et al., 2009a). Species of this genus are classified into two main ecological categories: "crowd" and "cleaner" (Bauer, 2000, but see Baeza et al., 2007). "Crowd" species live freely in crevices and among rocks as dense aggregations and develop no obligatory symbiotic interactions with other organisms. Likewise, "cleaner" species live in pairs and generally establish cleaning symbiotic interactions with fishes (Bauer, 2000). During this study, *L. vittata* was found comprising the fouling community in long lines for cultivation of oysters in a tropical environment (Bahia, Brazil). The abundance of this species was always higher in long lines with a more developed and dense fouling community, and especially when the octocoral *Carijoa riisei* was present and abundant in these communities. *Lysmata vittata* was observed in most samples aggregated and in groups of ranging from 9 to 40 individuals. Thus, *L. vittata* apparently fits among the "crowd" group of species given its high abundance and the apparent absence of symbiotic interactions with other organisms comprising the fouling community in which these shrimps are found. Nonetheless, potential benefits, e.g., protection against predators, that *L. vittata* might obtain from *C. riisei* remain to be addressed. A second feature that suggests the inclusion of the species in this category is its coloration. The body coloration of "crowd" species is semi-translucent, with reddish longitudinal and transverse bands running along the body. The same basic color pattern was herein observed in *L. vittata*. By contrast, "cleaner" species feature contrasting bright colors, usually yellow or red (Bauer, 2000; Rhyne and Lin, 2006). Additional detailed studies on the ecology (and sexual system) of representatives from the genera *Lysmata* are warranted, as they are relevant for elucidating the fascinating evolutionary history of gender expression in the infraorder Caridea.

Conclusions

Here, we have investigated morphological variation in traits of systematic importance and the phylogenetic position, ecology and reproductive biology of the shrimp *L. rauli*, described based only on a single specimen collected in Salvador, Bahia, Brazil. We detected considerable morphological variation in the studied species and we were not capable of distinguishing *L. rauli* from the Indo-Pacific congeneric *L. vittata*. Importantly, molecular phylogenetic analyses (using the 16S mt DNA fragment) revealed genetic similarities between *L. rauli* and *L. vittata*. Altogether, the information above clearly indicates that *L. rauli* is not a valid species but a junior synonym of *L. vittata*. Moreover, our results indicate that *L. vittata* has invaded successfully the southwestern Atlantic and is present in Bahia, Rio de Janeiro, and São Paulo, Brazil. Possible mechanisms of *L. vittata* introduction to the Brazilian coast include ballast water from cargo ships, bio-fouling on ship hulls, and direct importing and release (aquarium trade). The first two mechanisms above have been suggested before as introduction pathways for other alien crustaceans present in Brazil (Tavares and Mendonça Jr., 2004; Pachelle et al., 2011; Tavares, 2011; Almeida et al., in press). In a world in which invasive species are pervasive, it is imperative that one compare specimens of putatively "new species" not only with sympatric congeners, but also with allopatric congeneric species. Only this kind of approach will assure a robust test of the hypothesis stating that a putative holotype does not represent a synonym of an alien species introduced to a particular geographical zone. Such an approach will also help with the early detection of exotic species and the implementation of rapid and efficient plans for their control and eradication (Tavares, 2011).

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