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# Shrimps of genus *Lysmata* Risso, 1816 (Caridea: Lysmatidae) from Queimada Grande Island region, southeastern Brazil

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## ABSTRACT

The objective of this study is to report the first occurrences of three species from the genus *Lysmata* Risso, 1816 in an area of the Queimada Grande Island (QGI), a conservation unit on the southern coast of São Paulo, southeastern Brazil. The specimens were sampled manually in August 2018 from a small rocky formation from the QGI region. The species *Lysmata bahia* Rhyne and Lin, 2006, *Lysmata unicoloris* Holthuis and Maruin, 1952, and *Lysmata vittata* (Stimpson, 1860) are reported, of which the last two are not native to the Western Atlantic waters. The present study reports *L. unicoloris* for the first time on the coast of São Paulo State. The records informed herein not only provide more information about biodiversity, but can also help in the development of management plans and conservation.

## KEYWORDS

Aquarium trade, Decapoda, Geographical distribution, Peppermint shrimp, Western Atlantic

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## INTRODUCTION

The genus *Lysmata* Risso, 1816, which belongs to the family Lysmatidae Dana, 1852b, is composed of widely distributed species with distinct behavior (social and sexual) and life habits in different marine environments (Bauer, 2000; Rhyne and Lin, 2006; Baeza, 2009; Soledade *et al.*, 2013). Currently, the genus includes 47 species distributed worldwide (Pachelle *et al.*, 2020), with 18 reported in the Atlantic (Chace, 1972; Rhyne and Lin, 2006; Baeza and Anker, 2008; Anker and Cox, 2011; De Grave and Franssen, 2011; Rhyne *et al.*, 2012; Soledade *et al.*, 2013; Gan and Li, 2016; Pachelle *et al.*, 2016; Prakash and Baeza, 2017; Wang and Sha, 2018; De Grave and Anker, 2018; González-Ortegón *et al.*, 2020) and 11 of these on the Brazilian coast in which three of them, *Lysmata lipkei* Okuno and Fiedler, 2010, *Lysmata unicoloris* Holthuis and Maruin, 1952, and *Lysmata vittata* (Stimpson, 1860), are exotic (Pachelle *et al.*, 2020).

The genus *Lysmata* has species that are highly valuable in the ornamental aquarium industry (Calado *et al.*, 2003; Baeza and Behringer, 2017; Giraldes *et al.*, 2018; González-Ortegón *et al.*, 2020) because they help control pests (anemones of the genus *Aiptasia* Gosse, 1858) in aquariums, and are visually appealing due to their vivid color patterns (Karplus, 2014; Giraldes *et al.*, 2018). Due to the popularity of *Lysmata* in the aquarium trade, the demand for them has grown among marketed marine invertebrates (Wabnitz *et al.*, 2003; Calado *et al.*, 2007; Rhyne *et al.*, 2017). Therefore, areas where *Lysmata* species occur are targeted for the commercialization of these organisms, including the southeastern coast of Brazil.

Queimada Grande Island (QGI) is part of an Area of Relevant Ecological Interest (AREI) encompassing Queimada Grande and Queimada Pequena Islands; a “sustainable use” marine protected area on the southern coast of São Paulo State, Brazil (Pivetta *et al.*, 2012). Additionally, QGI is part of the Marine Environmental Protected Area “Litoral Centro”, and this area has different types of zones, according to the zoning protocol — version 18/12/2018 — measuring the level of permission for human activity (APAMLC, 2018). Queimada Pequena Island is inside a Zone of Special Protection (ZSP), forbidding any human activity in a radius of 1 km from the island coast. QGI is inside

a Zone for Low-Scale Uses (ZLSU) within a radius of 3 km from the island coast and a Zone of Intensive Use (ZUI) beyond this 3 km, allowing some fisheries activities. Studies on the fauna of the subtidal region in QGI have already been reported, including on reef fish (Pivetta *et al.*, 2012) and coral reefs (Pereira-Filho *et al.*, 2019). Despite this, the biodiversity of crustaceans has not yet been studied in this area. Thus, the aim of the present study is to register the occurrence of *Lysmata bahia* Rhyne and Lin, 2006, *L. unicoloris*, and *L. vittata* at Queimada Grande Island, using an integrative approach (morphological and molecular analysis). In addition, we expanded the knowledge about the distribution of two invasive species in the Western Atlantic, with a relevant record of these species in a conservation unit area.

## MATERIAL AND METHODS

### Sampling of *Lysmata* shrimps

Sampling was accomplished by artisanal collectors who catch ornamental marine organisms. The shrimps were removed from areas near the Queimadas Area of Relevant Ecological Interest (AREI) (Fig. 1) by free divers who collected the shrimps using a hand net from a small rocky formation with biogenic substrate at 6 to 12 m deep (C.H.A. Marques, artisanal fisherman, personal communication). The collection information and the shrimps were voluntarily transferred to one of us (AC-B) to be used in the present study. The sampled specimens were individually stored in plastic bags, kept in insulated boxes with ice and transported to the laboratory. Individuals were identified according to an identification key (Pachelle *et al.*, 2020) and deposited in the carcinological collection of the Museum of Zoology at the University of São Paulo (MZUSP).

### Morphology analysis

Each shrimp was measured and sexed. The carapace length (CL, the distance from the posterior orbital margin to the posterior margin of the carapace) was measured with a Vernier caliper (accuracy 0.01 mm). Due to protandric hermaphroditism reported in many species of the genus *Lysmata* (see Bauer, 2006; Alves *et al.*, 2019), individuals were simply divided into ovigerous (ov.) and non-ovigerous (non-ov.)

specimens. For identification shrimps were examined using a Leica MZ12S stereomicroscope equipped with a camera lucida. The following morphological characters were analyzed: [1] armature of the rostrum, [2] presence or absence of sharp tooth on pterygostomial margin, [3] number of free articles on accessory ramus of lateral antennular flagellum, [4] reach of rostrum in relation to distal margin of third article of antennular peduncle, [5] reach of stylocerite in relation to row of spiniform setae on dorsal surface of first article of antennular peduncle, [6] length of

carpus of first pereopod in relation to height in lateral view, [7] number of subdivisions on carpus of second pereopod, [8] length of merus of second pereopod in relation to ischium length, [9] number of fused articles on lateral antennular flagellum before bifurcation with accessory ramus, [10] number of subdivisions on merus of second pereopod, and [11] intraorbital process form. These characters were used because of their importance in the previous identification of *Lysmata* species (Rhyne and Lin, 2006; Giraldez *et al.*, 2018; Pachelles *et al.*, 2020).



**Figure 1.** Sampling region in the Area of Relevant Ecological Interest (AREI) Queimada Grande and Queimada Pequena Islands and the Marine Environmental Protected Area “Litoral Centro” in the Zone of Special Protection (Queimada Pequena Island) and Zone for Low-Scale Use (Queimada Grande Island), southeastern Brazil.

### Molecular analysis of *Lysmata shrimps*

Molecular analysis was based on the 16S rRNA mitochondrial DNA segment following the protocols provided by Schubart *et al.* (2000). The analysis was carried out to genetically compare the specimens received, with 27 species of *Lysmata* and *Exhippolysmata* Stebbing, 1915 (Tab. 1).

Total genomic DNA was extracted from abdominal muscle tissue using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) following the manufacturer's protocol. For amplification of the 16S gene segment, we used primers 16 L2 (5'-TGCCTGTTTATCAAAAACAT-3') and 1472 (5'-AGATAGAAACCAACCTGG-3') (Schubart *et al.*, 2000; 2002). PCR reactions were performed in a Veriti® Thermal Cycler (Applied Biosystems,

Foster City, CA, USA) and included 12.5 mL of PCR Buffer Go Taq Green (Promega, Madison, WI, USA), 1.0 mL of each primer, 8.5 mL of Milli-Q water (Milipore, Billerica, MA, USA) and 2 mL of each sample at a final volume of 25 mL. PCR cycles proceeded as follows: initial denaturation at 95 °C for 5 min; 40 cycles of 95 °C for 45 sec, 52 °C for 45 sec, and 72 °C for 1 min; and final extension at 72 °C for 5 min. Negative controls (sterile water) were used in all reactions. The results were checked on a 1.5 % agarose gel stained with GelRed. The PCR products were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega) and sequenced on an ABI 3500 Genetic Analyzer (Applied Biosystems) in the Bioinformatics and Evolutionary Biology Laboratory in the Genetics Department at the Federal University of Pernambuco, Brazil.

**Table 1.** Details of specimens and sequences used in the phylogenetic analyses in this study. The new sequences obtained in this study are highlighted. CCDB = Crustacean Collection of Biology Department of FFCLRP, University of São Paulo; IEO-CDCAD = Decapoda and Stomatopoda Crustacean Collection of Spanish Institute of Oceanography in Cadiz; CNCR = National Crustacean Collection of the Universidad Nacional Autónoma de México; LCP/UFSC = Laboratory of Crustaceans and Plankton Collection of Santa Catarina Federal University; MNRJ = Crustacean Collection of National Museum of Rio de Janeiro; MZUSP = Zoological Collection of Museum of Zoology of the University of São Paulo; RMNH = Naturalis Biodiversity Center (formerly Rijksmuseum van Natuurlijke Historie); SMF = Collection of the Senckenberg Natural History Museum; UMML = Collection of The Marine Laboratory, University of Miami.

Species	Catalogue Number	Sampling locality	GenBank 16S
<i>Lysmata amboinensis</i> De Man, 1888	UMML 32.9451	Philippines	EU861488
<i>Lysmata ankeri</i> Rhyne and Lin, 2006	CCDB 4715	Ubatuba, São Paulo, Brazil	KU312981
<i>Lysmata ankeri</i> Rhyne and Lin, 2006	UMML 32.9452	USA	EU861501
<i>Lysmata argentopunctata</i> Wicksten, 2000	CNCR 20998	Mexico	GQ227814
<i>Lysmata bahia</i> Rhyne and Lin, 2006	UMML 32.9453	Panama	EU861503
<i>Lysmata bahia</i> Rhyne and Lin, 2006	MZUSP 37511	Sergipe, Brazil	MH102006
<i>Lysmata bahia</i> Rhyne and Lin, 2006	MZUSP 37511	Sergipe, Brazil	MH102007
<i>Lysmata bahia</i> Rhyne and Lin, 2006	MZUSP 41122	Peruíbe, São Paulo, Brazil	MT459816
<i>Lysmata bahia</i> Rhyne and Lin, 2006	MZUSP 41122	Peruíbe, São Paulo, Brazil	MT459817
<i>Lysmata boggessi</i> Rhyne and Lin, 2006	UMML 32.9454	USA	EU861505
<i>Lysmata californica</i> (Stimpson, 1866)	UMML 32.9455	USA	EU861498
<i>Lysmata debelius</i> Bruce, 1983	UMML 32.9456	Philippines	EU861492
<i>Lysmata galapagensis</i> Wicksten, 2000	UMML 32.9457	Panama	EU861480
<i>Lysmata grabhami</i> (Gordon, 1935)	UMML 32.9459	Haiti	EU861489
<i>Lysmata gracilirostris</i> Wicksten, 2000	UMML 32.9458	Panama	EU861502
<i>Lysmata hochi</i> Baeza and Anker, 2008	UMML 32.9460	USA	EU861507
<i>Lysmata holthuisi</i> Anker, Baeza and De Grave, 2009	UMML 32.9466	Panama	EU861483
<i>Lysmata intermedia</i> (Kingsley, 1878)	UMML 32.9461	Panama	EU861484
<i>Lysmata intermedia</i> (Kingsley, 1878)	Not vouchered	USA	HQ315580
<i>Lysmata intermedia</i> (Kingsley, 1878)	MZUSP 37512	Sergipe, Brazil	MH102008
<i>Lysmata lipkei</i> Okuno and Fiedler, 2010	Not vouchered	Okinawa, Japan	HQ315574

Table 1. Cont.

Species	Catalogue Number	Sampling locality	GenBank 16S
<i>Lysmata lipkei</i> Okuno and Fiedler, 2010	MZUSP 37513	Sergipe, Brazil	MH102009
<i>Lysmata lipkei</i> Okuno and Fiedler, 2010	MZUSP 37513	Sergipe, Brazil	MH102010
<i>Lysmata moorei</i> (Rathbun, 1901)	UMML 32.9462	Panama	EU861481
<i>Lysmata nayaritensis</i> Wicksten, 2000	UMML 32.9463	Panama	EU861506
<i>Lysmata nilita</i> Dohrn and Holthuis, 1950	SMF 32005	Italy	EU861482
<i>Lysmata olavo</i> Fransen, 1991	SMF 32006	Portugal	EU861494
<i>Lysmata pedersenii</i> Rhyne and Lin, 2006	UMML 32.9464	Belize	EU861504
<i>Lysmata rafa</i> Rhyne and Anker, 2007	UMML 32.9465	Haiti	EU861495
<i>Lysmata seticaudata</i> (Risso, 1816)	UMML 32.9614	Portugal	GQ227827
<i>Lysmata udoi</i> Baeza <i>et al.</i> , 2009	UMML 32.9603	Venezuela	GQ227815
<i>Lysmata unicoloris</i> Holthuis and Maurin, 1952	MNRJ 27976	Florianópolis, Santa Catarina, Brazil	MH142085
<i>Lysmata unicoloris</i> Holthuis and Maurin, 1952	LCP/UFSC-106	Florianópolis, Santa Catarina, Brazil	MH142086
<i>Lysmata unicoloris</i> Holthuis and Maurin, 1952	RMNH D.7812	Casablanca, Morocco	MT002801
<i>Lysmata unicoloris</i> Holthuis and Maurin, 1952	IEO-CDCAD18/2464	Cadiz, Spain	MN294751
<i>Lysmata unicoloris</i> Holthuis and Maurin, 1952	MZUSP 41123	Peruíbe, São Paulo, Brazil	MT459814
<i>Lysmata unicoloris</i> Holthuis and Maurin, 1952	MZUSP 41123	Peruíbe, São Paulo, Brazil	MT459815
<i>Lysmata vittata</i> (Stimpson, 1860)	RMNH D 35616	Thailand	GQ227828
<i>Lysmata vittata</i> (Stimpson, 1860)	RMNH D 35616	Thailand	GQ227829
<i>Lysmata vittata</i> (Stimpson, 1860)	Not vouchered	Bahia, Brazil	JX912539
<i>Lysmata vittata</i> (Stimpson, 1860)	Not vouchered	Bahia, Brazil	JX912540
<i>Lysmata vittata</i> (Stimpson, 1860)	MZUSP 37509	Sergipe, Brazil	MH102011
<i>Lysmata vittata</i> (Stimpson, 1860)	MZUSP 37509	Sergipe, Brazil	MH102012
<i>Lysmata vittata</i> (Stimpson, 1860)	MZUSP 41124	Peruíbe, São Paulo, Brazil	MT459818
<i>Lysmata wurdemanni</i> (Gibbes, 1850)	CCDB 5510	Ubatuba, São Paulo, Brazil	KU312984
<i>Lysmata wurdemanni</i> (Gibbes, 1850)	UMML 32.9468	USA	EU861500
<i>Exhippolysmata ensirostris</i> (Kemp, 1914)	UMML 32.9602	China	GQ227819
<i>Exhippolysmata oplophoroides</i> (Holthuis, 1948)	UMML 32.9469	Ubatuba, São Paulo, Brazil	EU861510

Forward and reverse sequences were aligned to obtain the consensus sequence in BioEdit v.7.0.5 (Hall, 1999). Consensus sequences alignment was performed using ClustalW (Thompson *et al.*, 1994) enforcement in BioEdit v.7.0.5 (Hall, 1999). The selection of an optimal model of base substitution, based on AICc and conducted in MEGA 7 (see Kumar *et al.*, 2016), identified an HKY + G + I evolutionary model (-lnL = -3914.411). Phylogenetic analysis was estimated via the Maximum Likelihood (ML) method in MEGA 7. The robustness of the ML tree topologies was assessed by bootstrap resampling of the observed data 1000 times. To verify intra- and interspecific genetic divergence among the studied sequences, a matrix of genetic divergence was calculated in MEGA 7 based on the p distance (Kumar *et al.*, 2016).

## RESULTS

In the present study, five individuals of *Lysmata* were collected from areas around Queimada Grande Island, with two *L. bahia*, two *L. unicoloris*, and one *L. vittata*.

### Systematics

#### Order Decapoda Latreille, 1802

#### Infraorder Caridea Dana, 1852a

#### Family Lysmatidae Dana, 1852b

#### *Lysmata* Risso, 1816

#### *Lysmata bahia* Rhyne and Lin, 2006

*Lysmata bahia* Rhyne and Lin, 2006: 191, figs. 16–18, pls. 1F, 2; Barros-Alves *et al.*, 2015: 3, figs. 1C,

3; Pachelles *et al.*, 2016: 16, tab. 1; Pachelles *et al.*, 2020: 60, figs. 3–4.

*Hyppolysmata* (*Hippolysmata*) *wurdemanni*: Fausto Filho, 1970: 56; Coelho and Ramos, 1972: 153 (*partim?*) [not *L. wurdemanni* (Gibbes, 1850), see Rhyne and Lin, 2006]

*Lysmata wurdemanni*: Chace, 1972: 129 (remarks on São Paulo material); Coelho *et al.*, 2006: 121, tab. 3 (*partim?*) [not *L. wurdemanni* (Gibbes, 1850)]

*Material examined.* 1 ov. specimen (10.86 mm CL), 1 non-ov. specimen (10.26 mm CL), MZUSP 41122, Queimada Grande Island, Peruíbe, São Paulo State, Brazil, collector Marques, C.H.A. (artisanal fisherman), 01.viii.2018.

*First record for São Paulo.* Santos harbor (Rhyne and Lin, 2006).

*Distribution.* Western Atlantic: Panama and Brazil (Ceará, Sergipe, Bahia, Rio de Janeiro, São Paulo) (Rhyne and Lin, 2006; Barros-Alves *et al.*, 2015; Pachelles *et al.*, 2016; Terossi *et al.*, 2018; Pachelles *et al.*, 2020; present study).

*Remarks.* *Lysmata bahia* was first reported from Brazil by Fausto Filho (1970) as *Hippolysmata wurdemanni* Gibbes, 1850, followed by the first occurrence in São Paulo as *Lysmata wurdemanni* (Gibbes, 1850) by Chace (1972), which were later considered paratypes of *L. bahia* by Rhyne and Lin (2006). It is also one of the three species that belongs to the *L. wurdemanni* complex in Brazil, along with *Lysmata ankeri* Rhyne and Lin, 2006 and *L. wurdemanni* (see Pachelles *et al.*, 2020). The analyzed specimens presented all the diagnostic characteristics of *L. bahia*, according to Pachelles *et al.* (2020). The two specimens presented long stylocerites, almost reaching the distal margin of the first article of the antennular peduncle, which is a characteristic that differentiates *L. bahia* from *L. ankeri* and *L. wurdemanni* (which have a comparatively shorter stylocerite) (Pachelles *et al.*, 2020). However, the two specimens presented the carpus of the first pereopod about 4 times longer than tall, instead of 4.5 times, as indicated by Pachelles *et al.* (2020).

### ***Lysmata unicoloris* Holthuis and Maurin, 1952**

*Lysmata unicoloris* Holthuis and Maurin, 1952: 198, figs. 1–2; Lagardère, 1971: 99, figs. 232–235; González-Ortégón *et al.*, 2020: 26, figs. 1–4.

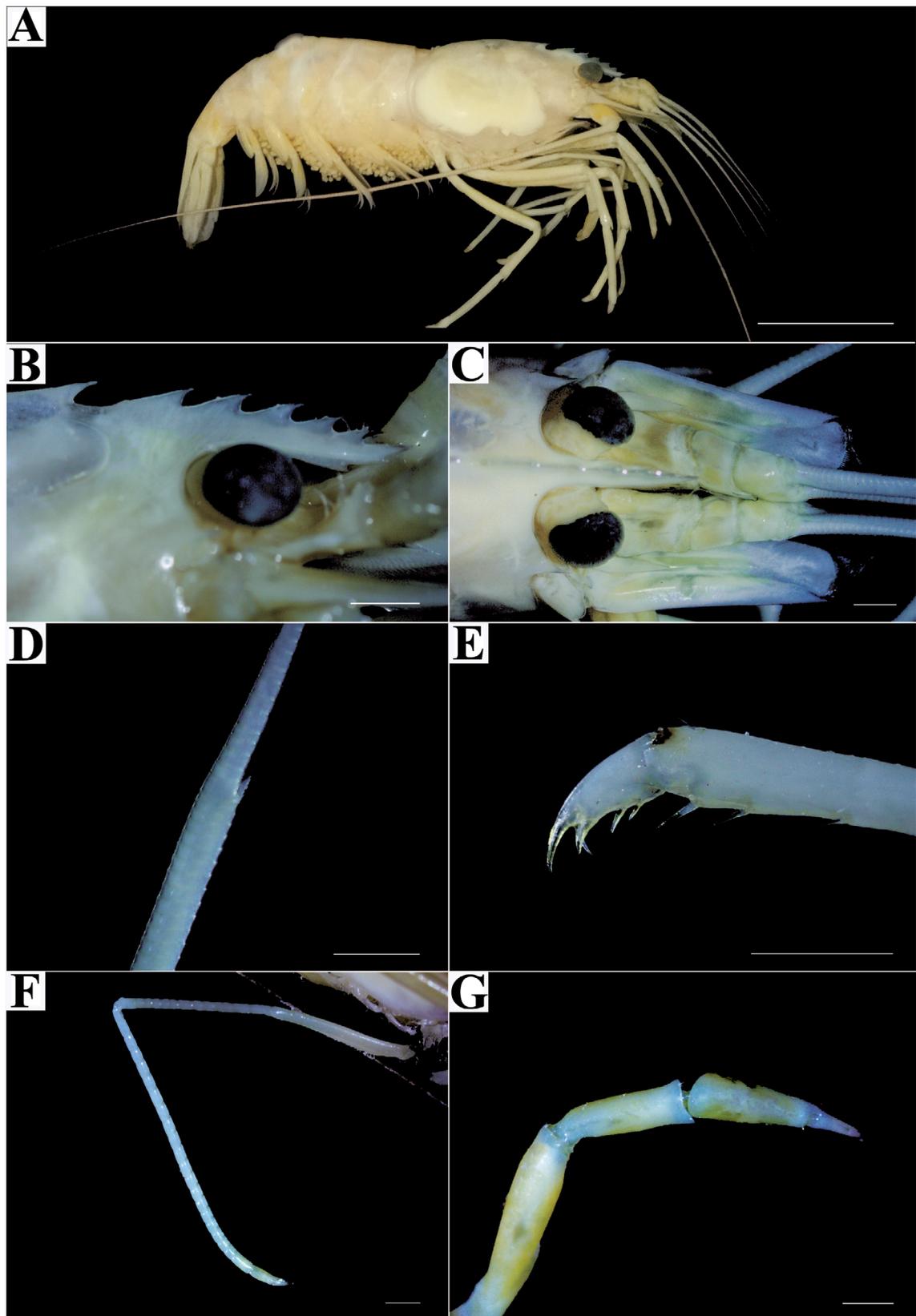
*Lysmata arvoredensis* Giraldes, Macedo, Brandão, Baeza and Freire, 2018: 5, figs 1–4F.

*Material examined.* 2 ov. specimens (10.09, 10.12 mm CL), MZUSP 41123 (Fig. 1), Queimada Grande Island, Peruíbe, São Paulo State, Brazil, collector Marques, C.H.A. (artisanal fisherman), 01.viii.2018.

*First record for São Paulo.* Present study.

*Distribution.* Eastern Atlantic: from Morocco to Congo; Gulf of Cadiz (between Spain and Portugal). Western Atlantic: Brazil (Santa Catarina [as *L. arvoredensis*] and São Paulo) (Holthuis and Maurin, 1952; Lagardère, 1971; Giraldes *et al.*, 2018; González-Ortégón *et al.*, 2020; present study).

*Remarks.* *Lysmata unicoloris* was recently classified as a senior synonym of *L. arvoredensis* (see González-Ortégón *et al.*, 2020). The record of *L. unicoloris* in São Paulo mentioned by Pachelles *et al.* (2020) was made via photographic register from a mussel farm in 2012, but was not tracked and remained an uncertain record. Therefore, the present study offers the first documented record of the occurrence of this species on the coast of São Paulo State, based on morphological and molecular analysis, and is the northern limit for the species in Western Atlantic waters. The occurrence of *L. unicoloris* in Brazilian waters may be associated with the introduction of non-native species by anthropogenic influence related to the marine aquarium trade (González-Ortégón *et al.*, 2020). Both specimens presented all diagnostic characteristics and expected morphological variation for *L. unicoloris*, according to Pachelles *et al.* (2020) (Fig. 2). The species differs from *L. bahia* by the proportion of the length and height of the carpus of the first pereopod (longer in *L. bahia*); by the number of subdivisions in the carpus of the second pereopod (22–24 versus 29–32 in *L. bahia*); and the length of the free portion of the accessory ramus of the antennular flagellum (shorter in *L. unicoloris*).



**Figure 2.** *Lysmata unicoloris* Holthuis and Maurin, 1952. **A**, Specimen collected in the area near to the border of the protected area of Queimada Grande Island, lateral view; **B**, lateral view, rostrum details and absence of pterogostomial spine; **C**, dorsal view, reach of rostrum in relation to distal margin of third article of antennular peduncle; **D**, dorsal antennular flagellum with trace of accessory branch; **E**, dactyl of pereopod 3, lateral view; **F**, second pereopod, lateral view, number of subdivisions on carpus; **G**, first pereopod, lateral view, length of carpus in relation to height. Scale bars, **A** = 1 cm; **B–F** = 1 mm. Photographs by R. C. Santos.

***Lysmata vittata* (Stimpson, 1860)**

*Hippolysmata vittata* Stimpson, 1860: 26.

*Nauticaris unirecedens* Spence Bate, 1888: 608, pl. 110, fig. 1

*Hippolysmata vittata* var. *subtilis* Thallwitz, 1891: 22.

*Lysmata vittata*: Bruce, 1990: 601, figs. 23–28; Soledade *et al.*, 2013: 67; Alves *et al.*, 2018: figs. 1B, 3; Terossi *et al.*, 2018: 83.

*Hippolysmata durbanensis* Stebbing, 1921: 20, pl. 5.

*Lysmata rauli* Laubenheimer and Rhyne, 2010: 299, figs. 1–3; Soledade *et al.* 2013: 67, fig. 2C.

**Material examined.** 1 non-ov. specimen (7.02 mm CL), MZUSP 41124, Queimada Grande Island, Peruíbe, São Paulo State, Brazil, collector Marques, C.H.A. (artisanal fisherman), 01.viii.2018.

**First record for São Paulo.** São Vicent Estuary (Soledade *et al.*, 2013).

**Distribution.** Indo-West Pacific: from Mozambique to Hong Kong; Philippines, Japan, Russia, Australia, and New Zealand. Mediterranean Sea. Western Atlantic: Brazil (Sergipe, Bahia, Rio de Janeiro, São Paulo) (Barnard, 1955; Chace, 1997; Ah Yong, 2010; Laubenheimer and Rhyne, 2010; Marin *et al.*, 2012; Soledade *et al.*, 2013; Abdelsalam, 2018; Alves *et al.*, 2018; Pachelles *et al.*, 2018; Terossi *et al.*, 2018; Present study).

**Remarks.** It is the first non-native species of *Lysmata* reported on the Brazilian coast (Soledade *et al.*, 2013), followed by *L. lipkei* (see Pachelles *et al.*, 2016). Recently recognized as a senior synonym of *L. rauli* (see Laubenheimer and Rhyne, 2010) and due to its wide distribution and diverse color patterns may be considered a species complex (Marin *et al.* 2012; Soledade *et al.*, 2013; Anker and De Grave 2016; Pachelles *et al.*, 2018; 2020). The analyzed specimen was very damaged, with several broken appendages. However, despite the damage the identification by morphological characteristics was possible, since the rostrum and some appendages were still intact. Thus, it was possible to verify: [1] the presence of a sharp tooth on the pterygostomial margin; [2]

seven dorsal and three ventral teeth on the rostrum; [3] a single short free article in the accessory ramus of the antennular flagellum; [4] stylocerite longer, overreaching distal margin of cornea; [5] intraorbital process of carapace longer than wide in lateral view; and [6] first pereopod carpus short, about three times as long as wide in lateral view. These characteristics can be used as morphological evidence, allowing the analyzed specimen to be identified according to the key proposed by Pachelles *et al.* (2020). The proposition of *L. vittata* as a senior synonym of *L. rauli* may be carefully checked using more extensive material and through morphological and molecular analysis because of the possibility of *L. vittata* being a species complex (Marin *et al.*, 2012; Soledade *et al.*, 2013; Terossi *et al.*, 2018; Pachelles *et al.*, 2018; 2020).

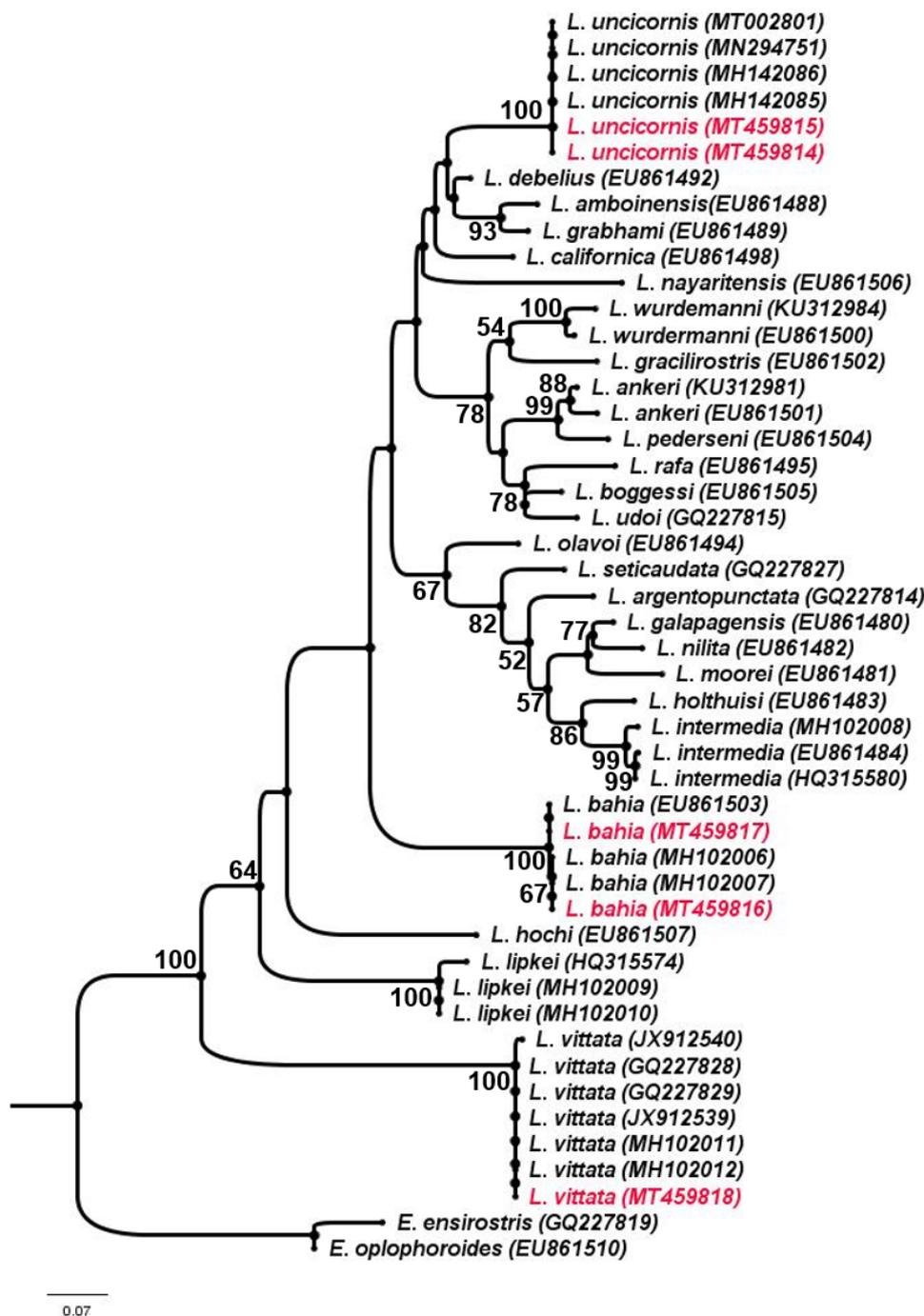
**Molecular analysis**

The two sequences obtained for *L. bahia* from QGI (Brazil) matched with the sequence from a *L. bahia* specimen from Sergipe State (Brazil) and Panama (Fig. 3).

The genetic divergence among these three *L. bahia* specimens varied from 0 to 0.004 (p distance). The two sequences obtained for *L. unicolornis* specimens from QGI (Brazil) matched with the sequences of *L. unicolornis* from Florianópolis, Santa Catarina State, Brazil (Fig. 3). There were no differences among the four sequences of this species (p distance = 0). The sequence of *L. vittata* from Ilha da Queimada Grande matched with the sequences of *L. vittata* from Thailand and Sergipe and Bahia States (Brazil) (Fig. 3). The genetic divergence among the five *L. vittata* specimens varied from 0 to 0.007 (p distance).

**DISCUSSION**

Through morphological and molecular analyses, the present study confirms the first record of three species from the genus *Lysmata* in areas surrounding the Queimada Grande Island, southern coast of São Paulo State, of which two species are non-native to the Western Atlantic. Integrative techniques, such as DNA (16S) and morphological characters, contributed and complemented the identification process of *Lysmata*, which had already proven to be arduous due to ambiguous morphological characteristics, and



**Figure 3.** Phylogenetic tree of genus *Lysmata* (Risso, 1816), including *Lysmata bahia* Rhyne and Lin, 2006, *Lysmata uncicornis* Holthuis and Maurin, 1952, and *Lysmata vittata* (Stimpson, 1860) from Queimada Grande Island, Brazil (indicated in red), constructed via Maximum Likelihood analysis of the 16S DNA gene. Numbers are support values for 1000 bootstraps; values < 50 % were not included.

consequently, by the presence of species complexes (Rhyne and Lin, 2006; Baeza, 2010; Soledade *et al.*, 2013; Baeza and Behringer, 2017; Wang and Sha, 2018; González-Ortegón *et al.*, 2020; Pachelle *et al.*, 2020).

*Lysmata bahia* is widely distributed on the Brazilian coast since its description by Rhyne and Lin (2006). The present record is the southern-most limit of this

species occurrence in the Western Atlantic, after the occurrence record in Santos Bay, São Paulo State (Rhyne and Lin, 2006; Alves *et al.*, 2018; Terossi *et al.*, 2018). Regarding the non-native species found in the QGI area, *L. vittata* has already been well established in Brazilian waters (Soledade *et al.*, 2013; Alves *et al.*, 2018; 2019), while there have been few

occurrence records for *L. unicolornis* on the coast of Brazil (Giraldes *et al.*, 2018; González-Ortegón *et al.*, 2020; Pachelles *et al.*, 2020).

Our results confirm the presence of *L. unicolornis* on the coast of São Paulo State. This species was previously recorded in São Paulo by Pachelles *et al.* (2020), however, this record could not be confirmed because the specimen was lost. Thus, the present study extends the range of *L. unicolornis* in the Western Atlantic (from 27°25' S to 24°29' S of latitude) (Giraldes *et al.*, 2018, González-Ortegón *et al.*, 2020; Pachelles *et al.*, 2020). *Lysmata unicolornis* is the sixth species of *Lysmata* to be confirmed on the São Paulo State coast, along with *L. ankeri*, *L. bahia*, *Lysmata intermedia* (Kingsley, 1878), *L. vittata*, and *L. wurdemanni*, and is the second non-native species to be recorded there (Soledade *et al.*, 2013; Pachelles *et al.*, 2016; Barros-Alves *et al.*, 2016; Terossi *et al.*, 2018; Pachelles *et al.*, 2020).

The dispersal vector responsible for the presence of exotic *Lysmata* species on the São Paulo coast is still uncertain, nevertheless, ballast water transportation, as previously registered for other Decapoda crustaceans, as well as the high demand of *Lysmata* specimens for the marine aquarium trade are the most likely causes (Mantelatto and Dias, 1999; Calado, 2009; Pachelles *et al.*, 2011; Tavares, 2011; Soledade *et al.*, 2013; Barros-Alves *et al.*, 2016). The increased demand for these shrimps may result in deregulated exploitation, as well as inappropriate trade, which can lead to the introduction of exotic species, as is the case for *L. lipkei*, *L. unicolornis*, and *L. vittata* on the Brazilian coast, which are originally from Indo-West Pacific (Alves *et al.*, 2018).

The introduction of non-native species by the ornamental aquarium trade needs to be explored (González-Ortegón *et al.*, 2020; Alves *et al.*, 2021). According to Mainka and Howard (2010), the occurrence of invasive species is currently one of the largest causes of biodiversity loss on the planet along with climate change. The ecological consequences of this introduction can be direct, with the loss of native species, or indirect, through the transmission of pathogens (Rodríguez and Suárez, 2001; Tavares, 2003; Almeida *et al.*, 2012).

The present study is the first to report crustacean species in areas near to Queimada Grande Island

AREI, but is limited to the genus *Lysmata*. We highlight the importance of carrying out new studies that use new sampling techniques in this area in order to more effectively determine the local diversity in different substrates, as well as mechanisms that may evaluate the process of non-native species introduction and how it occurs; such as molecular analysis. This information could facilitate management and conservation measures that decrease the possible impacts that invasive species have on native species in this conservation unit.

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