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The spreading of the invasive bivalve *Mytilopsis leucophaeata* (Dreissenidae) into estuaries of Rio de Janeiro, Brazil

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Abstract: An extensive field survey was conducted to evaluate the spreading of the invasive estuarine bivalve *Mytilopsis leucophaeata* into the metropolitan region of Rio de Janeiro (Brazil), in which the single invaded site so far recorded is Rodrigo de Freitas Lagoon. A novel record is made to the Marapendi coastal lagoon, where *M. leucophaeata* mainly colonizes mangrove roots. A reef composed of druses above the unconsolidated substratum is recorded for the first time for this species. The fauna associated to agglomerates of *M. leucophaeata* was composed of eight taxa: polychaetes *Alitta succinea* and *Polydora* sp., amphipods *Melita mangrovi* and *Elasmopus* sp., barnacles *Amphibalanus eburneus* and *A. improvisus*, the crab *Eurypanopeus* cf. *dissimilis* and the gastropod *Heleobia* spp. Based on COI sequences, the haplotype diversity of *M. leucophaeata* invasion in Marapendi Lagoon is high, like that previously recorded in Rodrigo de Freitas Lagoon. The expansion of *M. leucophaeata* demands a continuous investigation, including the knowledge of its impacts on the biota.

Key words: Biological invasion, COI, dark false mussel, Marapendi Lagoon, non-native species.

INTRODUCTION

Mytilopsis leucophaeata (Conrad, 1831) is an estuarine bivalve native from the Gulf of Mexico and possibly part of the eastern U.S.A., although invasive in many regions around the globe, especially in Europe, causing a series of negative economic and ecological effects (Verween et al. 2010, Kennedy 2011). In Brazil, this species is only known from the city of Rio de Janeiro (southeastern Brazil), regarding the recent finding that the record from the city of Recife (northeastern Brazil) is related to another species (Fernandes et al. 2018), probably *Mytilopsis sallei* (Récluz, 1849).

Based on published data, the invasion of *M. leucophaeata* in Rio de Janeiro is restricted to the Rodrigo de Freitas coastal lagoon (Rizzo et al. 2014, Fernandes et al. 2018), where it forms large clusters in rocky/woody substrata and others, such as styrofoam, plastic packaging, tires and buoys (Rizzo et al. 2014). *Mytilopsis leucophaeata* is believed to not colonize mangrove roots in Rodrigo de Freitas Lagoon (Rizzo et al. 2014), contrary to some native sites, where it may form dense aggregates in roots of *Rhizophora mangle* L. (Odum & Heald 1972, Pérez-Sarabia et al. 2012, Ruiz & López-Portillo 2014, Hernández et al. 2015).

Medium or small-scale dispersion of *M. leucophaeata* may occur through the incrustation of specimens on the hull of boats or other vectors. In the metropolitan region of Rio de Janeiro, several estuaries are found near Rodrigo de Freitas Lagoon, increasing the propagation risks of this biological invasion through man-made vectors or even larval dispersal (Rizzo et al. 2014, Fernandes et al. 2018). The present study aimed to ascertain whether or not *M. leucophaeata* reached other estuaries in the metropolitan region of Rio de Janeiro, after an extensive field survey.

MATERIALS AND METHODS

Study area

The coastal lagoons of the metropolitan region of Rio de Janeiro were formed during the Quaternary after sea-level fluctuations that isolated small marine bays, resulting in lentic and brackish water bodies due to the discharge of rivers (Bohrer 2012). The lagoon system of Barra da Tijuca and Jacarepaguá is composed of three main waterbodies (Figure 1a), of which Tijuca Lagoon (4.8 km^2) is connected to Jacarepaguá Lagoon (3.7 km^2) and Marapendi Lagoon (3.5 km^2), and an artificial channel allows the entrance of marine waters (Rebelo 2016). Tijuca and Jacarepaguá lagoons currently have high levels of urbanization, with intense sewage discharge, cyanobacteria blooms and siltation (Gomes et al. 2009, Bohrer 2012, INEA 2017). The salinity in Jacarepaguá Lagoon usually varies from 0–10 ppt (Gomes et al. 2009), in spite of rare marine intrusions that may rise it to

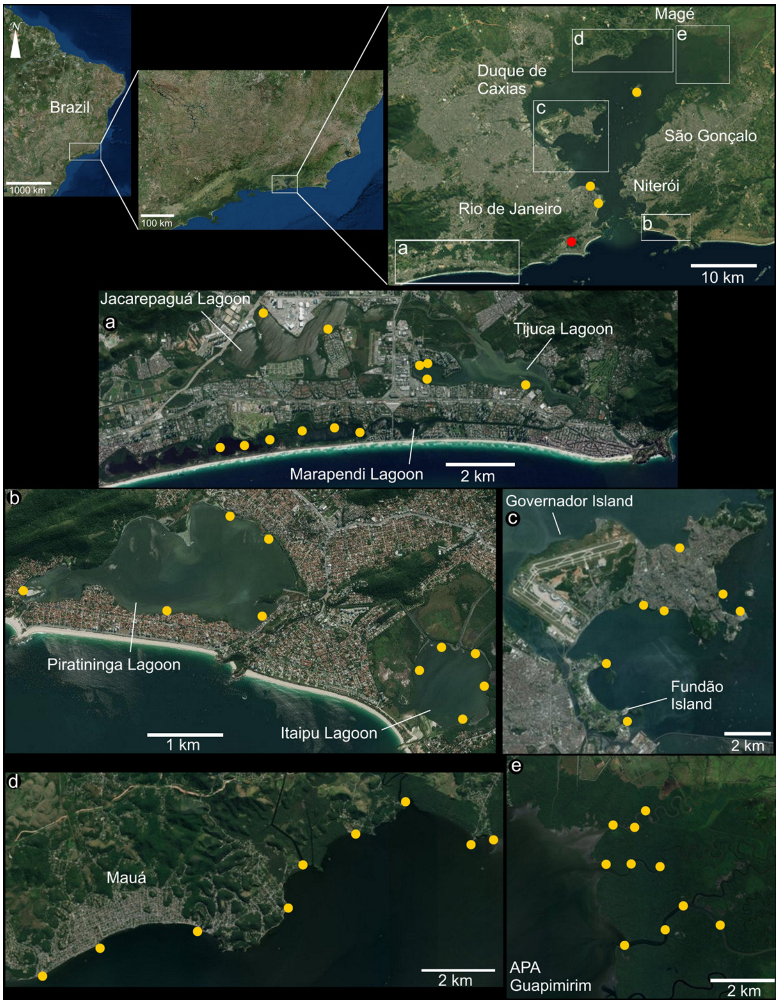

Figure 1. Investigated sites (yellow dots) in the metropolitan region of Rio de Janeiro. The red dot indicates the previously known invasive population of *Mytilopsis leucophaeata* in Rodrigo de Freitas Lagoon. Map adapted from ArcGIS®.

14 ppt (Carvalho & Silva 2017), whereas Tijuca Lagoon shows salinities of 5.5–29 ppt (Soares 1999). Marapendi Lagoon was a freshwater body before the creation of an artificial channel connecting it to Tijuca Lagoon during the 1950's, which allowed the entrance of marine waters and the formation of mangroves, in addition to some underground percolation of seawater (Carvalheira 1993, Poian 2013). A wide salinity gradient is present, with freshwater in its distal portion and up to 29–32 ppt towards the connection with Tijuca Lagoon (Carvalheira 1993, Lacerda & Gonçalves 2001). The most intense sewage discharge is observed in its distal portion, although somewhat reduced in the rest of Marapendi Lagoon after the recent implantation of a sewage treatment system (Poian 2013).

Two coastal lagoons situated in the city of Niterói were surveyed (Figure 1b), Itaipu Lagoon (2.0 km^2) and Piratininga Lagoon (2.9 km^2). Both have access to the sea owing to artificial channels, which considerably raised their salinity levels: Itaipu Lagoon currently shows 25–33 ppt, whereas Piratininga Lagoon had an increase from 3–21 ppt before 2008 to 20–32 ppt nowadays (Lacerda & Gonçalves 2001, Cerda et al. 2013, Mendes & Soares-Gomes 2013, Prestrelo & Monteiro-Neto 2016). Itaipu Lagoon shows considerable siltation, whereas Piratininga Lagoon receives high loads of domestic sewage, in addition to siltation, massive benthic algal growth and anoxic events (Bohrer 2012, Mendes & Soares-Gomes 2013, INEA 2017). The lagoons are interconnected due to the creation of an artificial channel in the 1940's decade.

The Guanabara Bay margin comprises some of the most populous municipalities of Rio de Janeiro state. Proper treatment of domestic sewage or industrial waste is lacking in many sites, resulting in degraded water conditions in most extension of the bay, in addition to the presence of large harbors and oil pollution (Meniconi et al. 2012, Fistarol et al. 2015, INEA 2017). Regions close to the mouth of the bay and the central channel possess near-marine salinities and moderate pollution, whereas the end of the bay may show reduced salinities due to river discharges, as low as 8.2–9.9 ppt during low tide events in summer; however, most areas of the bay have salinities higher than 26 ppt (Mayr et al. 1989, Kjerfve et al. 1997, Paranhos et al. 1998). The investigated sites in Guanabara Bay encompass Governador Island and Fundão Island (Figure 1c), which lie close to the most polluted area of the bay (Fistarol et al. 2015). Sites surveyed in the municipality of Magé (Figure 1d), at the end of the bay, have varied degrees of anthropic influence. The conservation unit 'APA Guapimirim' (Figure 1e) concentrates ~60% of the freshwater discharge into Guanabara Bay, presenting large areas of preserved mangroves and events of salinity intrusion in estuary zones (Melo et al. 2014).

Field and laboratory procedures

Field surveys were conducted in search of possible settlement areas of *M. leucophaeata*, mainly consolidated substrata (natural or artificial) in estuaries or coastal lagoons (Figure 1), demanding a careful visual inspection; empty shells were also searched in the near sediment. Environmental data such as water temperature, pH, salinity, dissolved oxygen and chlorophyll were measured in the sampling days through a multi-parameter sonde YSI-6920-V2 (Table I). When present, aggregates of the invasive bivalve and its associated fauna were sampled with plastic bags. Local fishermen and port authorities were asked about the presence of the invasive bivalve.

At laboratory, the largest specimens of *M. leucophaeata* were measured with a digital caliper rule, and shell features were analysed through a stereomicroscope. The associated fauna to *M. leucophaeata* was sorted, identified and stored in scientific collections; their identification was confirmed by taxonomists (see 'Acknowledgments'). Most colored photographs were conducted on a Zeiss Axio Cam ICc5 camera coupled to a Zeiss Discovery. V20 stereomicroscope.

To explore the genetic diversity of the new *M. leucophaeata* invasion, 33 specimens were DNA-sequenced targeting the mitochondrial COI gene, following the same procedures as in Fernandes et al. (2018). DNA extraction was based on a salting-out procedure and amplification performed with universal primers LCO1490/HCO2198 (Folmer et al. 1994). PCR products were purified and sequenced by Macrogen Inc. (Seoul, South Korea) for both directions. Additional sequences of *M. leucophaeata* and other dreissenids were retrieved from GenBank, resulting in a final alignment of 570 bp (Table II). A maximum-likelihood tree was computed in PhyML 3.0 (Guindon et al. 2010), following parameters used by Fernandes et al. (2018). The same alignment of 570 bp was used to compare the haplotype and nucleotide diversity between the former invasion in Rio de Janeiro (Rodrigo de Freitas Lagoon) and the new one, which was estimated with Arlequin 3.5 (Excoffier & Lischer 2010).

Acronyms: (MNRJ) Museu Nacional do Rio de Janeiro, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; (UERJ) Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil; (UFF) Universidade Federal Fluminense, Niterói, Brazil.

Table I. Environmental factors measured in visited sites. TDS = Total Dissolved Solids. ORP = Oxidation Reduction Potential. ODO = Optical Dissolved Oxygen.

	Marapendi Lagoon (06/vii/2017)	**Governador Is. / Fundão Is. (09/xi/2017)**	**Tijuca Lagoon (05/vii/2018)**	**Jacarepaguá Lagoon (05/vii/2018)**	**Itaipu Lagoon (09/vii/2018)**	**Piratininga Lagoon (09/vii/2018)**	**Magé (18/vii/2018)**
Temperature (°C)	20.8–21.1	23.2–25.4	24.9–25.9	24.7	23.9–25.4	22.3–25.4	23.7–26.4
Conductivity (mS/cm)	15.9–23.1	46.7–52.0	24.8–30.2	11.0	37.9–50.3	48.3–49.8	41.2–50.8
TDS	11.2–16.2	31.3–34.1	16.2–19.3	7.2	24.5–33.4	32.1–33.1	26.6–33.1
Salinity (ppt)	10.2–15.2	31.3–34.5	15.1–18.4	6.3	23.9–33.8	32.3–33.5	26.1–33.5
pH	7.44–8.16	8.0–8.7	7.8–8.3	7.2	7.7–8.0	7.9–8.5	7.1–8.2
pHm	-73.2–-33.8	-103.5 – -67.0	-81.7– -53.5	-21.9	-66.3 – -49.9	-95.9 – -61.5	-74.7 – -13.5
ORP	-29.1 – +165.6	+113.7 – +139.3	-184.1 – +121.5	+137.3	+114.4 – +176.8	+97.5 – +116.7	+36.5 – +162.4
ODO (%)	82.7–148.7	96.3–295.0	3.6–83.1	40.5	82.9–159.3	54.9–231.3	48.4–238.9
ODO (mg/L)	6.7–12.2	6.8–20.1	0.3–6.3	3.25	5.9–11.1	3.9–15.8	3.3–16.6
Chlorophyll	27.1–220.7	24.1–244.3	61.6–211.0	90.1	18.8–117.6	17.5–21.3	71.9–442.4

Table II. Sequences used in the present study; those from Marapendi Lagoon are new, and the remaining were retrieved from GenBank.

Species	Individuals	Locality	GenBank sequences
Mytilopsis leucophaeta	33	Marapendi Lagoon, Rio de Janeiro (Brazil)	MK268707.1– MK268739.1
Mytilopsis psisleucophaeata	31	Rodrigo de Freitas Lagoon, Rio de Janeiro (Brazil)	MF139836.1 – MF139866.1
Mytilopsis psisleucophaeata	1	New York (U.S.A.)	U47649.1
Mytilopsis psisleucophaeata	2	Maryland (U.S.A.)	KU905904.1; KU906056.1
Mytilopsis psisleucophaeata	1	Germany	HM100254.1
Mytilopsis psisleucophaeata	1	Caspian Sea (Iran)	HM100257.1
Mytilopsis cf. *sallei*	3	Recife (Brazil)	MF139892.1– MF139894.1
Mytilopsis cf. *sallei*	1	Southern China	EU780672.1
Dreissena polymorpha	1	–	AF474404.1

RESULTS

The single locality observed to contain a new invasion of *Mytilopsis leucophaeata* is Marapendi Lagoon (Figures 1 and 2). No other sites had any trace of this bivalve, despite the existence of several suitable places, and local fishermen were unaware about its presence. In Marapendi Lagoon, the maximum shell length obtained for *M. leucophaeata* was 26.1 mm. Larges hells are often clearer (sometimes whitish-green), possibly due to the loss of periostracum and of the typical zigzag pattern of younger specimens (Figure 3a-c). Shell outline and apophysis morphology (Figure 3d) fit previous descriptions of this species (Marelli & Gray 1983, Rizzo et al. 2014, Fernandes et al. 2018).

The colonization of *M. leucophaeata* in Marapendi Lagoon is mainly on mangrove roots, forming dense aggregates (Figure 2b-e), but it was also found in artificial hard substrata such as piers. An interesting gradient of colonization occurs between these bivalves and barnacles, i.e., *Amphibalanus eburneus* (Gould, 1841) and *Amphibalanus improvisus* (Darwin, 1854). In higher salinities (in the sampling day, >18 ppt), towards the connection with Tijuca Lagoon, barnacles predominate in mangrove roots or artificial substrata. In medium salinities (~13–18 ppt) there is a continuous dispute for empty spaces between bivalves and barnacles (Figure 3g-i), whereas *M. leucophaeata* predominates in reduced salinities (<13 ppt). We address that the sampling day was after a sequence of rainy events, possibly having reduced usual salinity levels.

An intriguing discovery in Marapendi Lagoon is the formation of *M. leucophaeata* druses in a moderately wide area (about 150 m^2) above an unconsolidated substratum of sand/clay (Figure 2f-h). This ‘reef’ is air-exposed in some points, and the maximum water depth is not greater than half meter above the dense aggregates, hampering the passage of boats in this site. Nucleus of the aggregates of *M. leucophaeata* are usually empty shells of the bivalve *Anomalocardia flexuosa* (Linnaeus, 1767) or even mangrove seeds, *Casuarina* fruits and other tiny hard substrata (Figure 3e-f), enabling the overgrowing of other specimens after the colonization of this nucleus, leading to the formation of compact boulders (druses) and thereafter the reef.

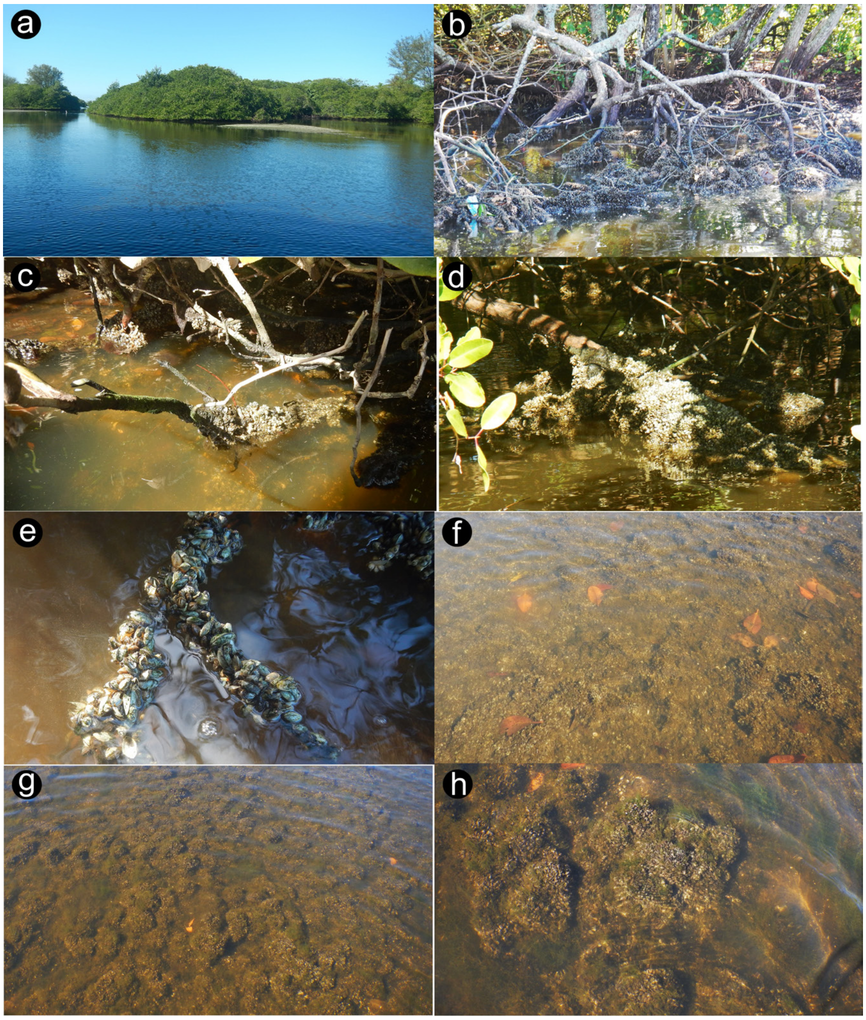

Figure 2. **The invasion of *Mytilopsis leucophaeata* in Marapendi Lagoon, Rio de Janeiro. (a-e) Specimens attached to mangrove roots. (f-h) Druses forming a reef over the unconsolidated substratum.**

The fauna associated to aggregates of *M. leucophaeata* was particularly noticed by high densities of the polychaete *Alitta succinea* (Leuckart, 1847), both in mangrove roots or in the reef (Figure 4a-c). Shell-damaging polychaetes of *Polydora* sp. were solely present in reef samples (Figure 4d), being probably benefited by the abundance of *Mytilopsis* shells. The amphipods *Elasmopus* sp. and *Melita mangrovi* Oliveira, 1953 were common in samples from mangrove roots (Figure 4e-f). The deposit-feeding gastropod *Heleobia* spp. had high densities especially in reef samples (Figure 4g-h). One individual of the mud crab *Eurypanopeus* cf. *dissimilis* (Benedict & Rathbun, 1891) was sampled (Figure 4i-j)

The 33 sequences of *M. leucophaeata* from Marapendi Lagoon clustered within the clade from Rio de Janeiro (Fernandes et al. 2018) and were set apart from the single haplotype from the Northern Hemisphere (Figure 5). Ten haplotypes were found in Marapendi Lagoon, of which five are shared with Rodrigo de Freitas Lagoon and five are exclusive from Marapendi, in addition to five exclusive from Rodrigo de Freitas (Figure 5). Haplotype and nucleotide diversities are similar between both invasive populations in Rio de Janeiro (Marapendi Lagoon: $h = 0.865 \pm 0.028$, $\pi = 0.0024 \pm 0.0017$; Rodrigo de Freitas Lagoon: $h = 0.837 \pm 0.048$, $\pi = 0.0026 \pm 0.0018$).

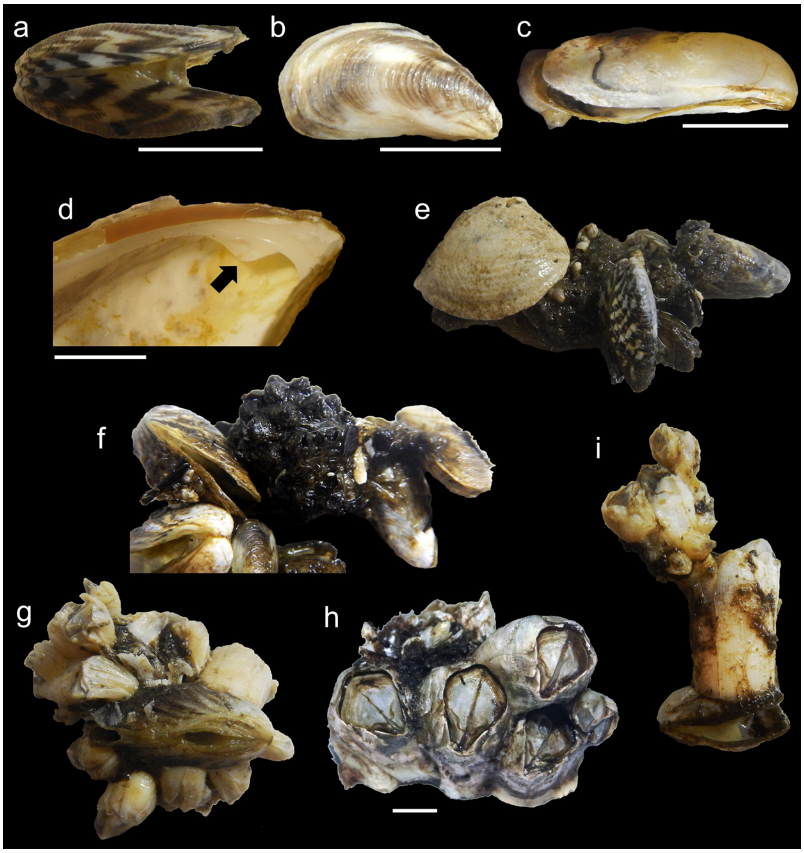

Figure 3. *Mytilopsis leucophaeata* and its associated fauna in Marapendi Lagoon - 1. **a-f** Specimens of *M. leucophaeata*, sampled freely (**a-c** MNRJ 29523) or adhered to empty shells of *Anomalocardia flexuosa* or *Casuarina* fruits (**e-f** MNRJ 29524); **d** illustrates the apophysis, indicated by a black arrow. **g-i** Associated barnacles (*Amphibalanus* spp.), UFF. Scale bars: **a-c**, **h**, 1 cm; **d**, 3 mm.

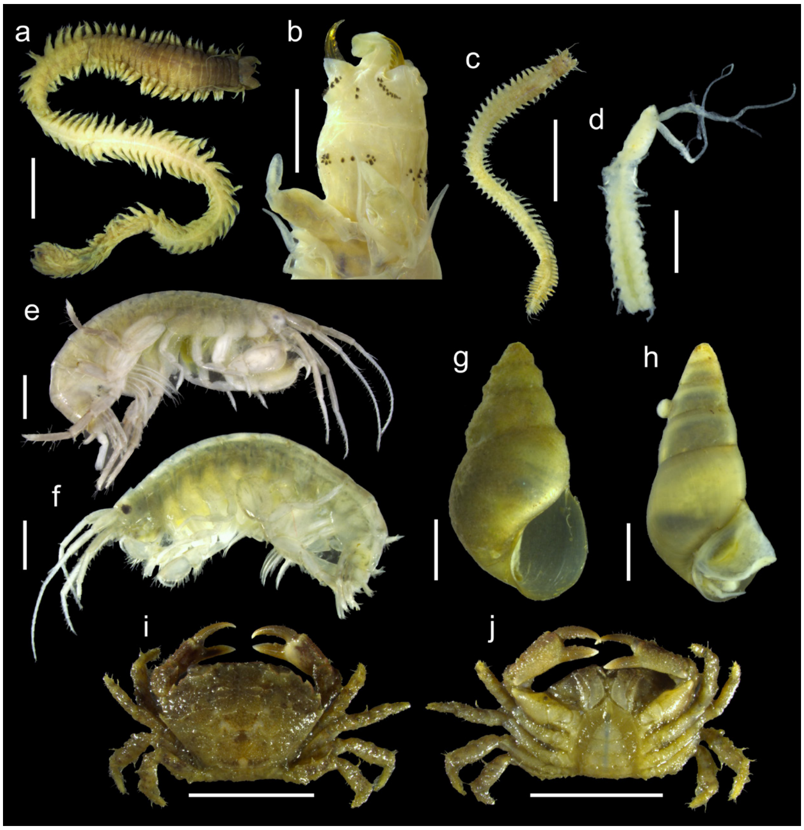

Figure 4. *Mytilopsis leucophaeata* and its associated fauna in Marapendi Lagoon - 2. **a-c** *Alitta succinea*, UERJ. **d** *Polydora* sp., UERJ. **e** *Elasmopus* sp., MNRJ. **f** *Melita mangrovi*, MNRJ. **g-h** *Heleobia* spp., MNRJ 29526 (**g**), MNRJ 29527 (**h**). **i-j** *Eurypanopeus* cf. *dissimilis*, MNRJ 28871. Scale bars: **a**, **c**, 5 mm; **b**, **d-h**, 1 mm; **i-j**, 1 cm.

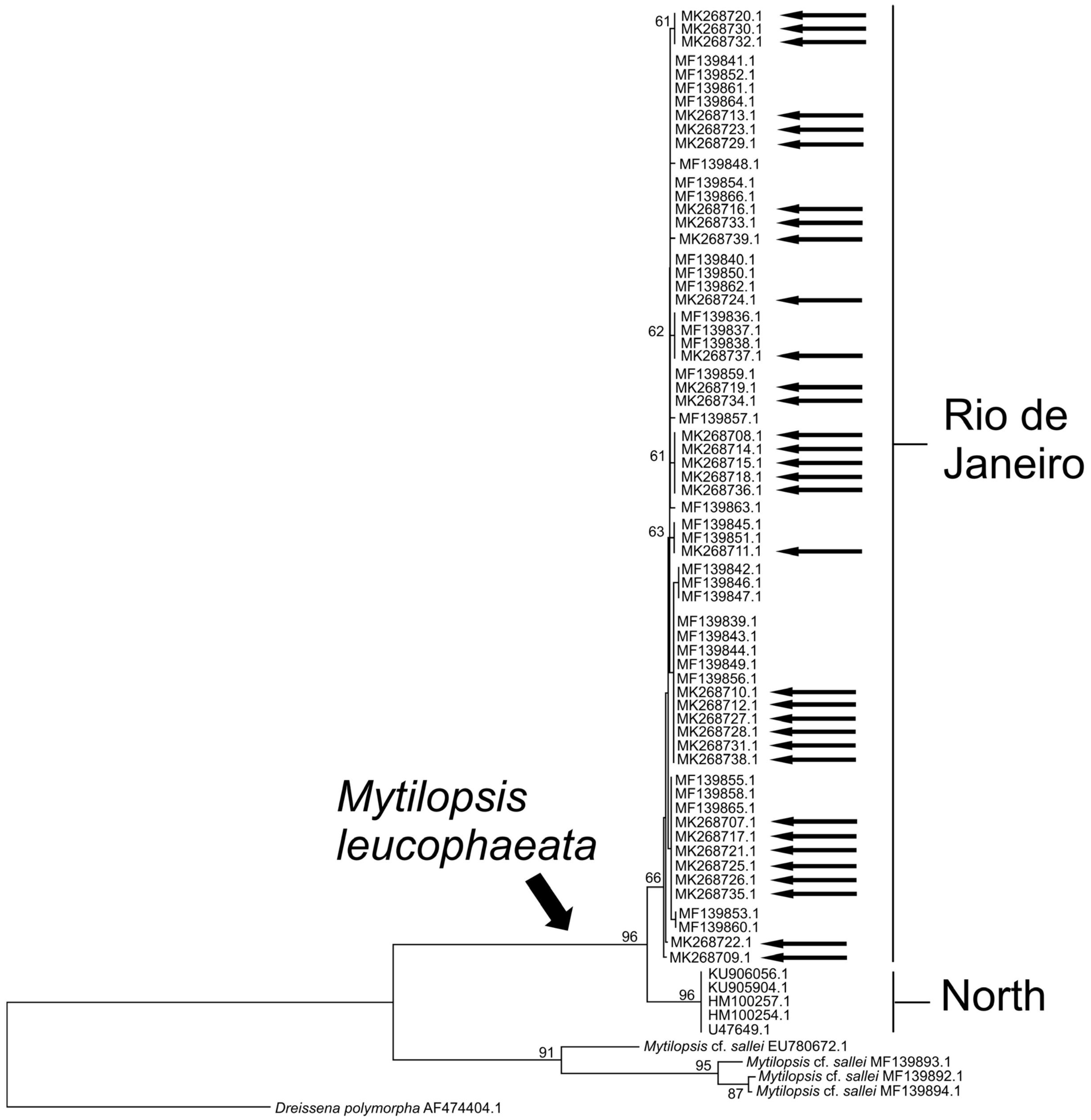

Figure 5. **Maximum-likelihood tree based on COI sequences, rooted in *D. polymorpha*. The population of *M. leucophaeata* named 'Rio de Janeiro' comprises records from Marapendi (indicated by black arrows) and Rodrigo de Freitas lagoons, whereas 'North' comprises sequences from northeastern U.S.A., Germany and Caspian Sea. Indices of bootstrap (%) displayed only for clades recovered with more than 60% of support.**

DISCUSSION

The two sites invaded by *Mytilopsis leucophaeata* in the metropolitan region of Rio de Janeiro, i.e., Rodrigo de Freitas and Marapendi lagoons, share some common features, although they are not directly connected. Firstly, they show salinity levels proper for the survivorship and reproduction of *M. leucophaeata*, usually 13–18 ppt in Rodrigo de Freitas Lagoon (Fernandes et al. 2018) and a broad gradient in Marapendi Lagoon, where the bivalve seems to prevail in salinity below 18 ppt. The water pollution in both coastal lagoons, although considerable, is far below the scenario seen in Tijuca and Jacarepaguá lagoons, where salinity levels are actually proper for the establishment of the invasive bivalve (a gradient of 0–29 ppt), but perhaps the organic pollution is excessive (Gomes et al. 2009, Bohrer 2012, INEA 2017). Elevated organic pollution is also seen in Piratininga Lagoon and some visited sites in Guanabara Bay (INEA 2017), but the near-marine salinity in these localities and in Itaipu Lagoon is certainly a main inhibitor against the spreading of *M. leucophaeata*. The preserved estuaries in 'APA Guapimirim' constitute an exception, with moderately clean and brackish waters not yet colonized by *M. leucophaeata*. This bivalve is present in Marapendi Lagoon at least since 2015 (M. Moscatelli, personal communication); fishermen boats are commonly observed therein during high tide, probably accidentally serving as vectors to bivalves adhered to their hulls.

We are unaware of the existence of *M. leucophaeata* druses growing over soft substrata prior to the present study. A low abundance of individuals (up to 8/0.1 m^2) of *M. leucophaeata* was observed in soft sediment at Chesapeake Bay, U.S.A. (Jordan & Sutton 1984), and eight live specimens were found in a sand-clay bottom in the Azov Sea, Russia (Zhulidov et al. 2015). The unprecedented formation of *M. leucophaeata* druses in Marapendi Lagoon enabled the formation of a new hard and tridimensional habitat, like a reef, to be colonized by species from nearby sites and possibly increase their population densities. Similarly, other bivalves known by their invasive potential, such as *Limnoperna fortunei* Dunker, 1857 and *Dreissena polymorpha* (Pallas, 1771), also may colonize soft substrata in varied densities when the preferred hard substrata are not available (Karatayev et al. 2010, Correa et al. 2015, Dzierzynska-Bialonczyk et al. 2018). The nucleus of *M. leucophaeata* druses in Marapendi Lagoon seems to be always formed by hard structures, such as empty shells of native species, and the byssus of pioneer individuals are not merely glued in sand grains.

Mangrove roots in Marapendi Lagoon are usually densely covered by *M. leucophaeata*, and temporal studies would be interesting to check the gradient of colonization *vs*. salinity between this bivalve and barnacles. Similarly, recent field surveys conducted by the authors in Rodrigo de Freitas Lagoon revealed that *M. leucophaeata* is also established therein in some mangrove roots (contrary to Rizzo et al. 2014), especially those deeply submerged, possibly owing to the current scarcity of other available hard surfaces without aggregates of these bivalves. At least for *D. polymorpha*, large agglomerates are usually avoided by young individuals when another hard structure is available (Dzierzynska-Bialonczyk et al. 2018). Following this scenario, perhaps the colonization of mangrove roots is not preferential for *M. leucophaeata* when other hard structures (such as some artificial ones) are abundant and free of conspecifics, which is not the case in Marapendi Lagoon, with few human-made structures in the water. Artificial substrates may also provide greater distance between the bivalve and the soft bottom, reducing sedimentation over it and enabling filter feeding (Brzana et al. 2017).

The fauna associated to *M. leucophaeata* druses in Marapendi Lagoon is similar to that obtained from infaunal samples in this same coastal lagoon some decades ago (Carvalheira 1993), in spite of considerable differences in sampling effort. Gastropods *Heleobia* spp. [commonly referred in southeastern Brazil as *Heleobia australis* (d'Orbigny, 1835) – although a problematic name (I. Gonçalves, personal communication)] and polychaetes *Polydora* sp. [identified as *Polydora websteri* Hartman, 1943 in Carvalheira (1993)] were sampled in great numbers in both studies. Polychaetes *Laeonereis culveri* (Webster, 1879) were most often sampled in Carvalheira (1993) than *Alitta succinea*, which was the single nereidid sampled in the present study. The high abundance of *A. succinea* is an indicator of local organic pollution, tolerating episodes of hypoxia; this species is mainly detritivorous, also feeding on animal or plant material, occupying several types of substrata and regarded as euryhaline and eurythermal (Pardo & Dauer 2003, Gillet et al. 2011, Villalobos-Guerrero 2012).

Because the study of Carvalheira (1993) was restricted to infaunal samples, she rarely sampled amphipods *Melita mangrovi*, which were very common in samples from mangrove roots together with *Elasmopus* sp. in the present study. No records in literature or museum collections indicate the existence of any native bivalve with a niche similar to that of *M. leucophaeata* in Marapendi Lagoon, i.e., occupying hard substrata, such as the estuarine mussel *Brachidontes darwinianus* (d'Orbigny, 1842).

Molecular analyses presented herein were based on the gene COI and confirmed the identity of *M. leucophaeata* into Marapendi Lagoon. The number of shared haplotypes between the former site and Rodrigo de Freitas Lagoon suggests a common genetic pool in Rio de Janeiro, in spite of unique haplotypes for each lagoon; (which might reflect an inadequate sampling effort). The similar haplotype diversities between Rodrigo de Freitas and Marapendi lagoons show a lack of any apparent genetic bottleneck between them.

The continuous investigation about range expansion of invasive species is necessary, especially if complemented by other studies such as population dynamics and genetic variability of the introduced populations. However, more rigor on the inspection of vectors of long or small distances to avoid the dispersal of *Mytilopsis* specimens is an urgent need (Rizzo et al. 2014, Fernandes et al. 2018), requiring a partnership with public organs in order to achieve substantial results. Focusing on the metropolitan area of Rio de Janeiro, the assessment of benthic fauna in estuaries not yet invaded by *M. leucophaeata*, such as those in 'APA Guapimirim' and Tijuca-Jacarepaguá lagoon complex, would be desirable prior to a possible arrival of this unwanted guest.

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MRF designed the project, performed the molecular studies and wrote the initial manuscript. MRF, ICM and CHSC conducted the field works, sorted and identified the associated fauna. FS provided supplies and equipment for the molecular studies, and assisted in the molecular analyses. All authors contributed to the improvement of the final manuscript.