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ARTICLE

First record of the exotic *Indothais lacera* (Gastropoda, Muricidae) in Brazil

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Abstract. Bioinvasions are one of the main causes of the decline of native biodiversity. *Indothais lacera* (Born, 1778) is a carnivorous gastropod, native to the Indo-Pacific. We present the first records of *I. lacera* on the Brazilian coast and the first record of this invasive species in the Western Atlantic. The specimens were found in two locations in the Baía de Todos os Santos, Bahia state, northeast of Brazil. Live specimens were collected in the Itapagipe Peninsula, in Ribeira, Salvador, and dry samples (empty shells) were collected in the extreme north of Itaparica Island. Molecular analyses indicate that there was more than one event of introduction of *I. lacera* in the bay and alert to the potential impacts that this species can have on the benthic community in the region. We suspect that this exotic species is affecting a native population of *Thaisella,* which was previously locally abundant but has since become rare.

Keywords. Bioinvasions; Coastal ecosystems; Exotic species; Rapaninae.

INTRODUCTION

Bioinvasions have become a growing concern in recent years due to the potential threat that the introduction of exotic species poses to native biodiversity. In environments profoundly altered by humans, bioinvasions can lead to a substantial change in the structure and composition of native communities, causing significant changes in natural ecosystems (Ruiz *et al.,* 1997; Byers, 2002).

In estuaries, bays and other coastal environments, the inadvertent introduction of exotic species, mediated by anthropogenic actions, is mainly related to activities of socio-economic interest, such as maritime transport and aquaculture. As a result, transfer via ballast water in ships has acted as the main vehicle in the dispersion of bioinvaders across coastal environments (Wonham *et al.,* 2001; Silva & Barros, 2011). For mollusks, in particular, Brazil has not been an exception, and several recent discoveries have been made in coastal marine environments, including gastropods, bivalves and a chiton (Simone & Gonçalves, 2006; Breves-Ramos *et al.,* 2010; Rocha *et al.,* 2013; Oliveira *et al.,* 2017; Spotorno-Oliveira *et al.,* 2018, 2020; Amaral *et al.,* 2019; Gernet *et al.,* 2019; Belz *et al.,* 2020; Jardim & Pedro, 2021).

In a study dealing with Western Atlantic estuarine muricids, focusing on the genus *Thaisella* Clench, 1947 (Rapaninae), an anomalous population has been found in the region of Baía de Todos os Santos, Bahia state, Brazil. The shells have a tall spire with highly shouldered, even carinated whorls, with peripheric tubercles in the form of spirally elongated blades. These characters are unusual in *Thaisella,* but are found in the related Indo-Pacific genus *Indothais* Claremont, Vermeij, Williams & Reid, 2013. Both *Thaisella* and *Indothais* share the estuarine habitat and a well-developed thread flanking the umbilical area of the shell. In the genus *Indothais,* the type species *I. lacera* (Born, 1778) has already been recorded outside its native range, namely in the Eastern Mediterranean (Gofas & Zenetos, 2003). This shallow-water muricid is native to the Indo-Pacific, occurring from the Arabian Peninsula to Southeast Asia (Rosenberg, 2006; Kumar *et al.,* 2015; Kantharajan *et al.,* 2017; Niamaimandi *et al.,* 2017; Zhong *et al.,* 2017; Abu-Zied & Bantan, 2018; Jeeva *et al.,* 2018; Mahapatro *et al.,* 2018; Al-Asif *et al.,* 2020; Sultana *et al.,* 2021). It has a biconical shell, with strong spiral cords, whorls often bearing strong triangular tubercles, aperture ovate and a broad and apparent pseudoumbilicus (Kumar *et al.,* 2017).

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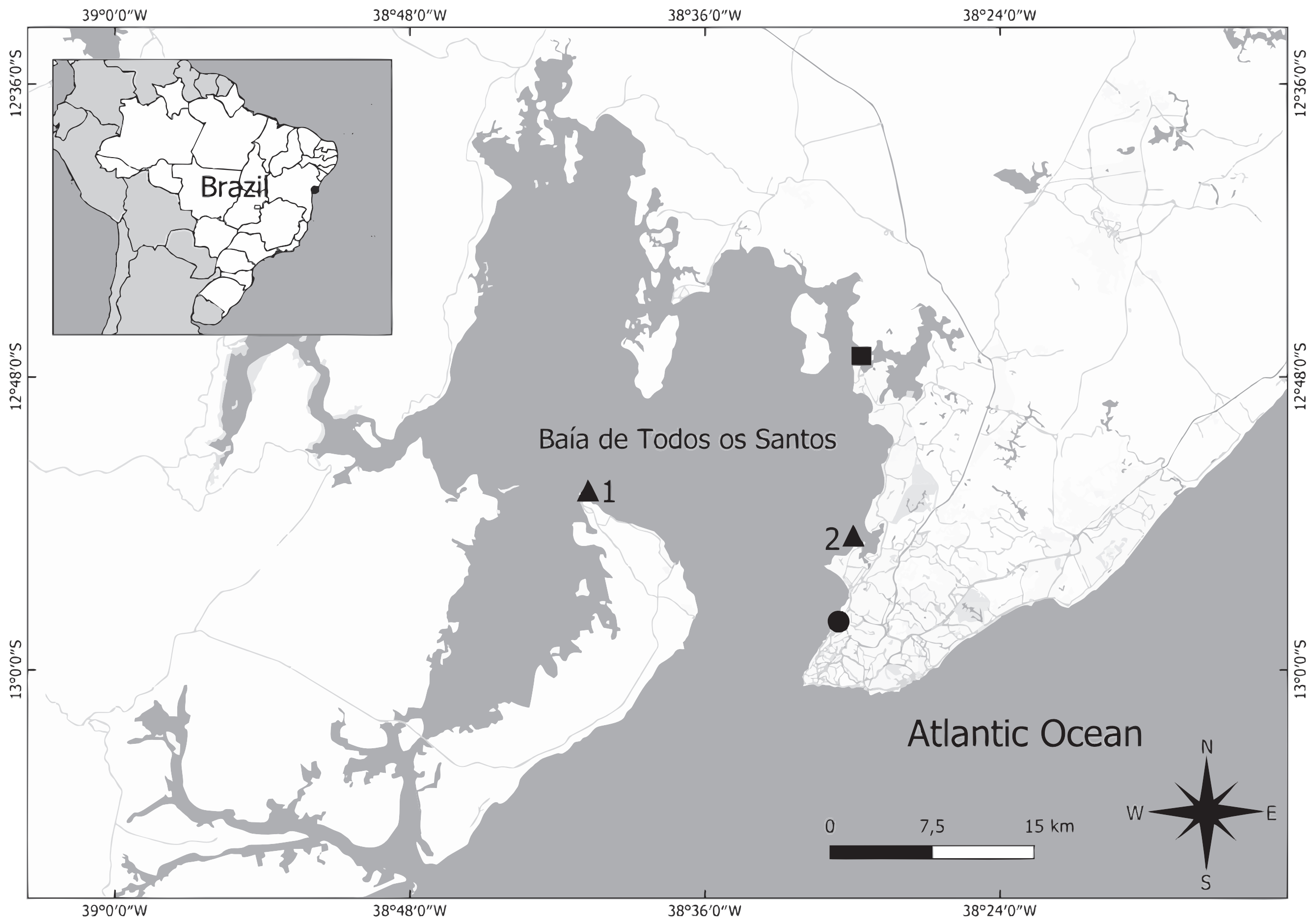

Figure 1. Baía de Todos os Santos and sites where *Indothais lacera* was found: 1 = Itaparica Island; 2 = Itapagipe Peninsula. The other symbols indicate the major ports in the region: square = Port of Aratu; circle = Port of Salvador.

The conchological and molecular analysis of specimens from the anomalous population from Baía de Todos os Santos demonstrates that they actually belong to *I. lacera*. Thus, a new exotic species is reported here from the Brazilian coast, being likewise the first report from the Western Atlantic. We also discuss its possible mode of transportation and the potential implications of its introduction to Brazilian coastal ecosystems.

MATERIAL AND METHODS

The specimens in this study were manually collected in two locations in Baía de Todos os Santos, Bahia state, Brazil (Fig. 1). The dry samples (empty shells) (Fig. 2A-B) were collected at Praia do Forte (12°52′43.11″S, 38°41′02.09″W), located at the northern end of Itaparica Island (Fig. 1) and the live specimens (Fig. 2C-D) were collected north of

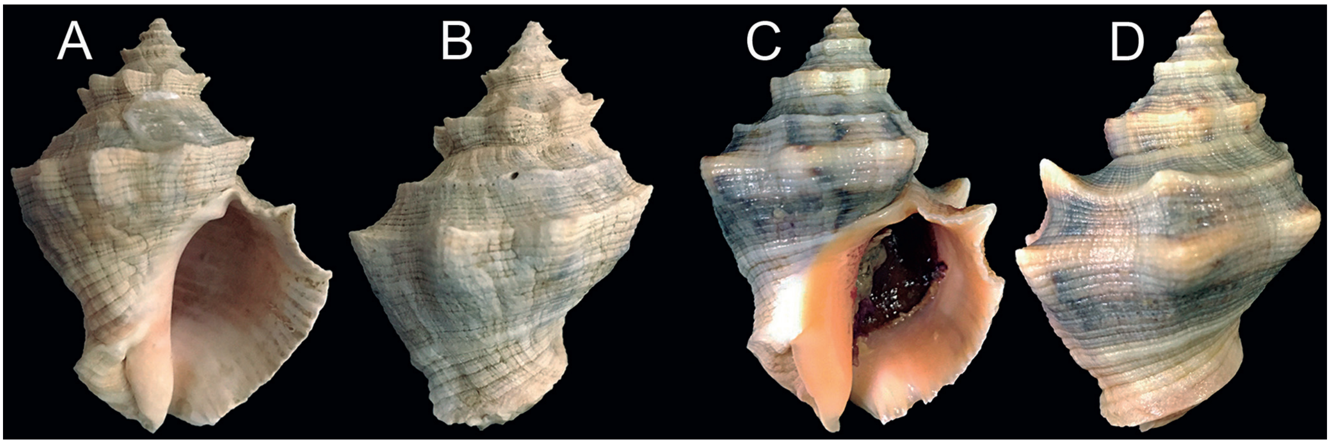

Figure 2. *Indothais lacera* from Baía de Todos os Santos. H = Shell height; D = shell greatest diameter. (A-B) *I. lacera* from Itaparica Island, MZUSP 136190. (A) Ventral view; (B) Dorsal view; H = 39.78 mm; D = 30.40 mm. (C-D) *I. lacera* from Itapagipe Peninsula, MZUSP 149013. (C) Ventral view; (D) Dorsal view; H = 44.18 mm; D = 32.46 mm.

Praia da Penha (also called Caribe da Ribeira) (Fig. 1) on the Itapagipe Peninsula, in Ribeira, Salvador (12°54′35.32″S, 38°29′50.62″W). The material was deposited in the mollusc collection of the Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil (MZUSP 136190, 4 shells.; MZUSP 136193, 5 spm.; MZUSP 149013 6 spm.).

DNA extraction, amplification and sequencing

Selected ethanol-preserved specimens (three from lot MZSP136193 and two from lot MZSP149013) had a small section of their foot clipped for DNA extraction. The extraction was conducted following the standard protocol of the QIAGEN DNEasy® Blood & Tissue Kit, repeating the final step to increase the yield.

Two mitochondrial markers were targeted for this study: the circa 650 bp long barcoding fragment of the mitochondrial COI (cytochrome c oxidase subunit I) gene, using the primers LCO and HCO of Folmer *et al.* (1994); and the circa 450 bp long 16S rRNA (large subunit rRNA) gene, using the primers 16SarL and 16SbrH of Simon *et al.* (1994). The protocol for PCR amplification for both markers was: initial denaturation at 96°C (3 min); 35 cycles of denaturation at 95°C (30 s), annealing at 48°C (1 min), and extension at 72°C (2 min); final extension at 72°C (5 min). The success of the PCR was assessed via agarose gel electrophoresis and the PCR products were cleaned using ExoSAP-IT™ (Affymetrix Inc.) according to the manufacturer's protocol. The samples were sent to Massey Genome Service (Massey University, Palmerston North, New Zealand) for Sanger sequencing. The resulting sequences were quality-proofed and assembled in Geneious Prime (v.2020.2.2, Biomatters Ltd.), and the consensus sequences were uploaded to GenBank (*see* Table 1 for registration numbers).

Phylogenetic analysis

To confirm the identity of the Brazilian specimens, a phylogenetic analysis was conducted including GenBank sequences of the species of interest (*Indothais lacera*), as well as seven further *Indothais* spp. and four other species belonging to related Rapaninae genera (Table 1). The Muricinae species *Murex pecten* [Lightfoot], 1786 was used as an outgroup.

The alignment of the sequences of each marker was done in Geneious Prime using the MUSCLE plugin (Edgar, 2004) with default settings (*i.e.,* optimized for accuracy). The alignments were visually proofed for inconsistencies and then run through Gblocks (Castresana, 2000; Talavera & Castresana, 2007) with the least restrictive settings, to

Table 1. List of species used in the phylogenetic analysis (including our specimens), with GenBank registration numbers of the COI and 16S markers, and provenance data of the specimens sequenced.

Species	COI	16S	Locality
Indothais blanfordi (Melvill, 1893)	KF906133	—	India
Indothais gradata (Jonas, 1846)	HE584333	HE584241	Malaysia, Johor
Indothais gradata (Jonas, 1846)	GU188228	GU188127	China, Guangxi
Indothais gradata (Jonas, 1846)	MT896361	MT896855	Brunei, Brunei Bay
Indothais javanica (Philippi, 1848)	HE584334	HE584242	China, Hong Kong
Indothais javanica (Philippi, 1848)	GU188230	GU188128	China, Hainan Island
Indothais javanica (Philippi, 1848)	MN389133	—	China, Hainan Island
Indothais lacera (Born, 1778)	MG099702	MG099702	China, Guangxi
Indothais lacera (Born, 1778)	FR695723	HE584243	Malaysia, Tanjong Laboh
Indothais lacera (Born, 1778)	MN703084	—	Bangladesh
Indothais lacera (Born, 1778)	MN703085	—	Bangladesh
Indothais lacera (Born, 1778)	HE584335	—	Malaysia, Penang
Indothais lacera (Born, 1778)	GU188253	GU188147	China, Jiangsu
Indothais lacera (Born, 1778)	ON004198	ON004193	Brazil, Bahia
Indothais lacera (Born, 1778)	ON004199	ON004194	Brazil, Bahia
Indothais lacera (Born, 1778)	—	ON004195	Brazil, Bahia
Indothais lacera (Born, 1778)	ON004200	ON004196	Brazil, Bahia
Indothais lacera (Born, 1778)	ON004201	ON004197	Brazil, Bahia
Indothais malayensis (Tan & Sigurdsson, 1996)	HE587941	HE587943	Malaysia, Johor
Indothais rufotincta (Tan & Sigurdsson, 1996)	HE584336	HE584244	Malaysia, Melaka
Indothais rufotincta (Tan & Sigurdsson, 1996)	MT896377	MT896871	Brunei, Brunei Bay
Indothais sacellum (Gmelin, 1791)	HE584337	HE584245	Oman
Indothais sacellum (Gmelin, 1791)	MN389136	—	China, Hainan Island
Indothais scalaris (Schubert & Wagner, 1829)	HE584338	HE584246	UAE, Khor Fakkan
Indothais wutingi (Tan, 1997)	HE584337	HE584247	Australia, Northern Territory
Murex pecten [Lightfoot], 1786	GU575382	FN651903	Philippines, Luzon
Rapana bezoar (Linnaeus, 1767)	FN677438	FN677421	Japan, Tosa Bay
Thais nodosa (Linnaeus, 1758)	EU391579	FN677425	Ghana, Matrakni Point
Thaisella chocolata (Duclos, 1832)	FR695724	HE584306	Peru, Lomas
Thaisella kiosquiformis (Duclos, 1832)	HE584387	HE584307	Peru, Tumbes

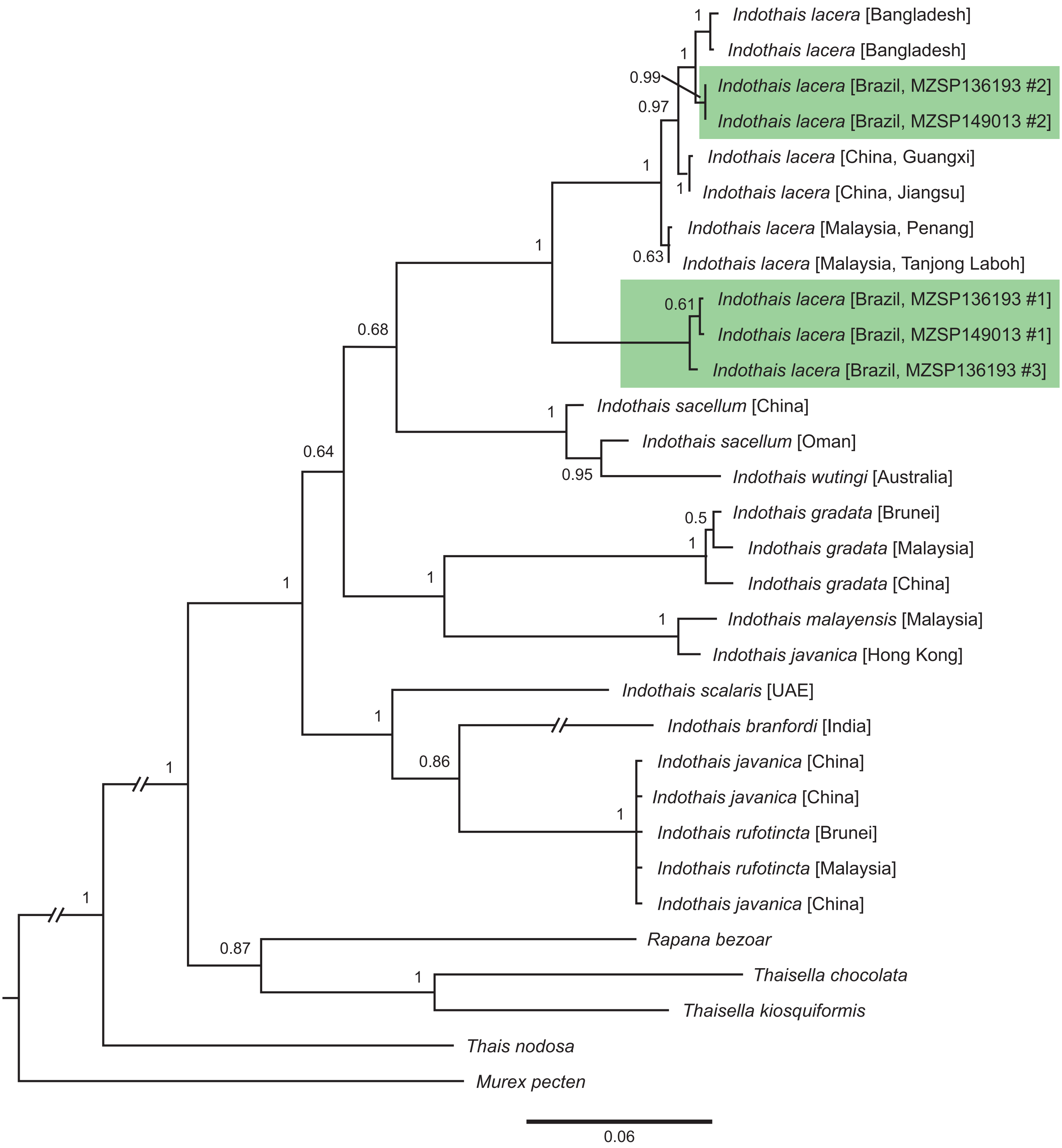

Figure 3. Bayesian inference phylogenetic tree based on C01 and 16S, with the specimens collected in Brazil highlighted. Posterior probabilities are shown on nodes; scale bar is substitutions per site.

eliminate poorly-aligned or data-deficient positions that could interfere with the analysis. The resulting COI and 16S alignments were then concatenated for the phylogenetic analysis.

The Bayesian inference phylogenetic analysis was done using MrBayes (v.3.2.7, Ronquist *et al.,* 2012) via the CIPRES Science Gateway (v.3.3, Miller *et al.,* 2015). Two concurrent analyses were run, each with 4 Markov chains of 30 million generations (the first 20% of generations were discarded as 'burn-in'), the default priors, nst = 6, rates = invgamma, temperature parameter = 0.1, sampling every 1,000 generations, and with substitution model parameters unlinked across the two markers. MCMC convergence was assessed by the standard deviation of split frequencies (< 0.01) and the potential scale reduction factor (PSRF ~ 1.0), as well as by observing the trace plots in Geneious (Ronquist *et al.,* 2009).

RESULTS

The specimens of *Indothais* from the Brazilian coast exhibit shell features consistent with those of *I. lacera,* which are: a biconical shell with strong spiral cords, the presence of a ridge on the sutural ramp, whorls provided of strong triangular tubercles, aperture ovate and a broad and apparent pseudoumbilicus (Tan & Sigurdsson, 1996; Kumar *et al.,* 2017).

In total, 31 terminal taxa were used in the molecular phylogenetic analysis, including the outgroup (Table 1). After the exclusion of poorly-aligned or data-deficient positions with Gblocks, the resulting COI sequences were 654 bp long, and 16S were 445 bp long, resulting in concatenated sequences 1099 bp long. The Bayesian analysis obtained a well-resolved tree, with mostly well-supported nodes throughout (posterior probabilities, PP ≥ 0.95; Fig. 3).

The sequences of *I. lacera* are all gathered in a single clade with full support (PP = 1), which includes all specimens collected on the Brazilian coast (Fig. 3). Three of the Brazilian specimens form a strongly-supported (PP = 1) monophyletic taxon that is sister to all other *I. lacera*. The other two specimens form another strongly-supported (PP = 0.99) monophyletic branch that is sister to a clade formed by two specimens of *I. lacera* from Bangladesh (PP = 1). This confirms the identity of the Brazilian specimens as *I. lacera* and also indicates that the population of this species on the Brazilian coast could have more than one origin, *i.e.,* it was introduced more than once, from different localities.

Another result of note, albeit unrelated to the present question, is that the specimens of *I. javanica* from China are potentially misidentified specimens of *I. rufotincta* (Fig. 3). The description and diagnostic features of *I. javanica* have been flagged as potentially problematic in other recent studies that made use of both morphological features and molecular data (Marshall & Taha, 2021).

DISCUSSION

Baía de Todos os Santos is the second largest coastal bay in Brazil, covering a maximum area of 1,233 km² (Cirano & Lessa, 2007). That bay is located in the northeast region of the country, close to the city of Salvador, the capital of the state of Bahia, and has ten large port terminals (Hatje & Andrade, 2009), which means intense maritime activities in the region. Judging by the high port activity in the bay, it is expected that the dispersion of *I. lacera* to Brazil has occurred through inter-oceanic travel. This can take place either through ballast water (the species has a long planktonic larval stage) or biofouling (*i.e.,* as an epibiont on ship hulls).

Considering that the Brazilian specimens of *I. lacera* form two distinct clades in the resulting phylogenetic tree (Fig. 3), it can be surmised that there was more than one introduction event of this species in Brazil, from at least two different localities. One of these localities is the region around Bangladesh, given the small genetic distance between them (Fig. 3). The remaining specimens from Brazil display a greater genetic distance from other sequenced specimens of *I. lacera* from China and Malaysia (Fig. 3). Given the absence of sequenced specimens from additional localities, it is presently not possible to narrow down the locality of origin for the second introduction of *I. lacera* in Brazil. Nevertheless, given that each branch of Brazilian *I. lacera* on the tree contains specimens of the two localities sampled, it can be assumed that the populations from each introduction event have spread throughout the bay.

Rapaninae species are known to be generalist predators of other molluscs, exerting great influence on the structure of faunal communities (Vermeij & Carlson, 2000). Consequently, this invasion may pose a potential risk to native mollusc assemblages in the region. Although rapanines are widely known to inhabit rocky shores and reef ecosystems, the broad hyaline tolerance of *I. lacera* (Claremont *et al.,* 2013) may also signify a risk to estuarine ecosystems. This fact may be related to the apparent disappearance of the native species *Thaisella mariae* (Morretes, 1954) on Itaparica Island (NCP, unpublished data), although it is impossible to ascertain this at present. *Thaisella mariae* is a muricid endemic to Brazil that occurs in estuarine areas, with a northern limit in Bahia state (Itaparica) and a southern limit in Paraná state (Rios, 2009). This species has a fusiform shell, relatively smaller in size compared to the invasive species (about 30 mm), characterized by sharp nodules that form fissured needles (although quite variable), a wide umbilical area surrounded by a thick keel, and by the presence of 3 to 4 bands of colour ranging from bluish-purple to brown inside the outer lip. However, in recent collections (2018) carried out in the region, no evidence of its occurrence was found. As specimens of *T. mariae* were relatively common in those areas, it is possible to speculate that the native species may have been displaced and locally extinct by the Indo-Pacific exotic species (*T. mariae* apparently occupies the same niche as *I. lacera*).

Although the real impact of this invader in Baía de Todos os Santos is only speculative for the moment, its introduction (from at least two source populations) is a reminder that there are still few efforts dedicated to the prevention of marine bioinvaders in Brazilian ports (*e.g.,* Gernet *et al.,* 2019; Spotorno-Oliveira *et al.,* 2020). Considering the ecological importance of molluscs within the marine benthic fauna, a significant change in the species composition of mollusc assemblages could have cascading effects on the entire benthic community, especially considering a fauna that already suffers from the impacts generated by port activity (Hatje & Andrade, 2009). Considering that bays and other coastal regions with intense port activity are frequent sites of marine invasions (Ruiz *et al.,* 1999), stricter and more optimized methods of controlling and preventing bioinvasions in those areas are urgently needed (Darrigran *et al.,* 2020).

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