Papéis Avulsos de Zoologia
Museu de Zoologia da Universidade de São Paulo

ARTICLE

Nassarius foveolatus (Gastropoda, Nassariidae), a new record of an exotic species in Brazil

Marcos de Vasconcellos Gernet[1,6]; Carlos Eduardo Belz[1,7]; Rafael Antunes Baggio[2]; Carlos João Birckolz[1,8]; Elizângela da Veiga Santos[1,3]; Luiz Ricardo L. Simone[4,9]; Daniel Abbate[4,10] & Rafael Metri[5]

[1] Universidade Federal do Paraná (UFPR), Centro de Estudos do Mar (CEM), Laboratório de Ecologia Aplicada e Bioinvasões (LEBIO). Pontal do Paraná, PR, Brasil.
[2] Universidade Federal do Paraná (UFPR), Departamento de Zoologia (DZOO), Laboratório de Ecologia Molecular e Parasitologia Evolutiva (LEMPE). Curitiba, PR, Brasil. ORCID: http://orcid.org/0000-0001-8307-1426. E-mail: rbaggio@ufpr.br
[3] Universidade Federal do Paraná (UFPR), Setor Litoral. Matinhos, PR, Brasil. ORCID: http://orcid.org/0000-0001-9834-8379. E-mail: lizveiga.ga@gmail.com
[4] Universidade de São Paulo (USP), Museu de Zoologia (MZUSP). São Paulo, SP, Brasil.
[5] Universidade Estadual do Paraná (UNESPAR). Paranaguá, PR, Brasil. ORCID: http://orcid.org/0000-0002-1502-0720. E-mail: rmetri@gmail.com
[6] ORCID: http://orcid.org/0000-0001-5116-5719. E-mail: lmvgernet@gmail.com (corresponding author)
[7] ORCID: http://orcid.org/0000-0002-2381-8185. E-mail: belzoceanos@gmail.com
[8] ORCID: http://orcid.org/0000-0002-7896-1018. E-mail: carlosbirc@gmail.com
[9] ORCID: http://orcid.org/0000-0002-1397-9823. E-mail: lrsimone@usp.br
[10] ORCID: http://orcid.org/0000-0002-6460-9224. E-mail: danielabbate@usp.br

Abstract. Exotic species are those that occur in an area beyond their natural limit and they are considered invasive when they cause harm to the economy, environment, or human health. In coastal environments, ballast water and inlays on the hull and other parts of vessels are the main ways of introducing invasive aquatic alien species. *Nassarius foveolatus* (Dunker, 1847) is native from the Central and East Indian Ocean to the East China Sea. The first specimens (empty shells) of *N. foveolatus* were collected manually on November 11, 2017 on the Rocio footbridge, located in the Paranaguá Estuarine Complex, on the coast of the State of Paraná, southern Brazil. Posteriorly, live specimens were collected in other localities of this bay. It is already possible to infer that the specimens of *N. foveolatus* occur together with the native specimens of *N. vibex* (Say, 1822), having the same niche. As previously only *N. vibex* existed in that place, at least a displacement of this native species has been occurred. However, certainly future ecological studies may confirm this displacement and additional consequences to the local ecosystem, as nassariids can be predators and scavengers. Control procedures should be also greatly implemented.

Key-Words. Invasive; Exotic; Mollusca; *Nassarius foveolatus;* Paraná coast.

INTRODUCTION

Exotic, non-native or introduced species are those that occur in an area beyond their natural limit and they are considered invasive when they cause harm to the economy, environment, or human health (Carlton, 1996). Bioinvasions, *i.e.,* the introduction of alien species into different ecosystems, are the major causes of biodiversity loss in the world, causing damage to local species and to the functioning of ecosystems (Stachowicz *et al.,* 1999; Silva & Barros, 2011).

In coastal environments, ballast water (with sediments) and inlays on the hull and other parts of vessels are the main ways of introducing invasive aquatic alien species (Ferreira *et al.,* 2004). Ballast water introduces aquatic organisms that are harmful to the environmental balance (including bacteria and viruses), both in marine and freshwater ecosystems, degrading important commercial activities such as those associated with fishing (Souza *et al.,* 2009).

Nassariidae is a family of almost exclusively marine detritivores snails inhabiting bottoms of unconsolidated substrates and, to a lesser extent, rocky shores in tropical waters, with greater abundance between 0 and 300 m deep (Nekhaev, 2014). This group belongs to the neogastropod superfamily Buccinoidea and consists of more than 400 species (Brown, 1982; Galindo *et al.,* 2016) divided into 18 extant genera, of which *Buccinanops* d'Orbigny, 1841 and *Nassarius* Duméril, 1805, are the largest genera in the family currently represented in the Brazilian coast (Rios, 2009; Rosenberg, 2009). In Brazilian waters, nine species of *Nassarius* have been recorded (Abbate & Cavallari, 2013).

Pap. Avulsos Zool., 2019; v.59: e20195955
http://doi.org/10.11606/1807-0205/2019.59.55
http://www.revistas.usp.br/paz
http://www.scielo.br/paz
Edited by: Marcelo Veronesi Fukuda
Received: 19/07/2019
Accepted: 07/10/2019
Published: 31/10/2019

ISSN On-Line: 1807-0205
ISSN Printed: 0031-1049
ISNI: 0000-0004-0384-1825

The typical shells of the family Nassariidae are characterized as being small to medium, with short and curved siphonal canal, conical protoconch, and horny operculum (Rios, 2009). It presents a high shell spire, oval shape with or without shoulder in the opening. The surface of the shell may be smooth or may exhibit axial and spiral sculptures (Abbate & Cavallari, 2013; Rios, 2009). Most nassariids have a well-developed callus. It has a rich fossil record dated since the Late Cretaceous (Taylor *et al.,* 1980; Van Dingenen *et al.,* 2015; Galindo *et al.,* 2016).

The genus *Nassarius* and its close relatives tend to be restricted to muddy environments, though a few species shelter among loose rocks and sand. Ecologically, most species of *Nassarius* are thought to be facultative scavengers inhabiting inter- to subtidal shallow marine environments. As scavengers, they are important in maintaining the balance of ecological systems, especially for the benthic community (Cernohorsky, 1984). Their taxonomy, especially the status of the alleged genera and subgenera, is still far from a resolution and the distribution of most species is poorly understood (Cavallari & Abbate, 2013; Galindo *et al.,* 2016). Compared to other gastropods, *Nassarius* snails have a relatively long planktonic larval phase, leading to a high level of dispersal capacity by marine currents (Tallmark, 1980). They can also be transported by ballast water and oysters farming to other environments and, by surviving, consequently becoming exotic species (*e.g.,* Carlton, 1992; Bachelet *et al.,* 2004; Townsend *et al.,* 2010; Fofonoff *et al.,* 2018; Goka, 2019).

Nassarius foveolatus (Dunker, 1847) is native from Central and East Indian Ocean to East China Sea, including countries as Mauritius, Pakistan, India, Myanmar, Thailand, Singapore, Malaysia and China (Cernohorsky, 1984; Subba Rao *et al.,* 1992; Raut *et al.,* 2005; Rosenberg, 2006; Robba *et al.,* 2007; Tan & Woo, 2010; Zhang & Yang, 2010; Mahapatro *et al.,* 2018). It presents a small, oval, slightly striated shell with furrowed suture, columella with slight callosity, denticulate external lip, and long and narrow feet.

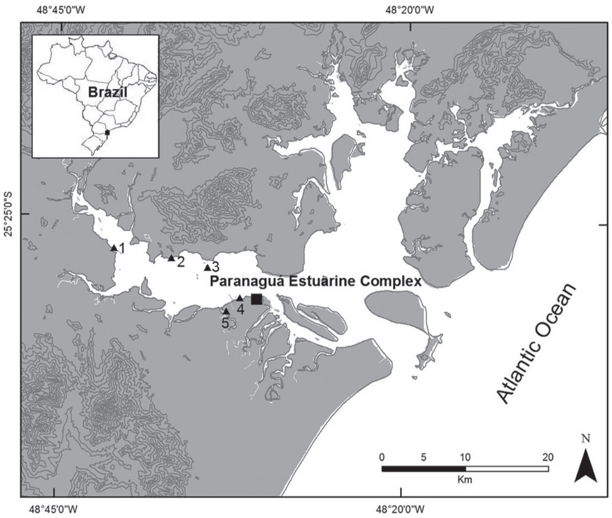

Figure 1. Paranaguá Estuarine Complex and sites where *Nassarius foveolatus* was found. Black square = Port of Paranaguá; 1 = Ponta da Pita; 2 = Europinha; 3 = Lamin Island; 4 = Rocio; 5 = Emboguaçu River.

The aim of this study is to report the first occurrence of the exotic species *N. foveolatus* in Brazil, confirmed both by morphological and molecular approaches. A discussion on its possible via of transportation and environmental consequences is also included.

MATERIAL AND METHODS

The first specimens (empty shells) of an unknown *Nassarius* species were collected manually on November 11, 2017, on the Rocio footbridge (25°30.236'S 48°31.891'W), located in the Paranaguá Estuarine Complex, on the coast of the state of Paraná, southern Brazil (Fig. 1). Subsequently, on December 17, 2017, live specimens were collected in the same locality. The material collected was deposited in the molluscan collections of the Museu de Zoologia da Universidade de São Paulo,

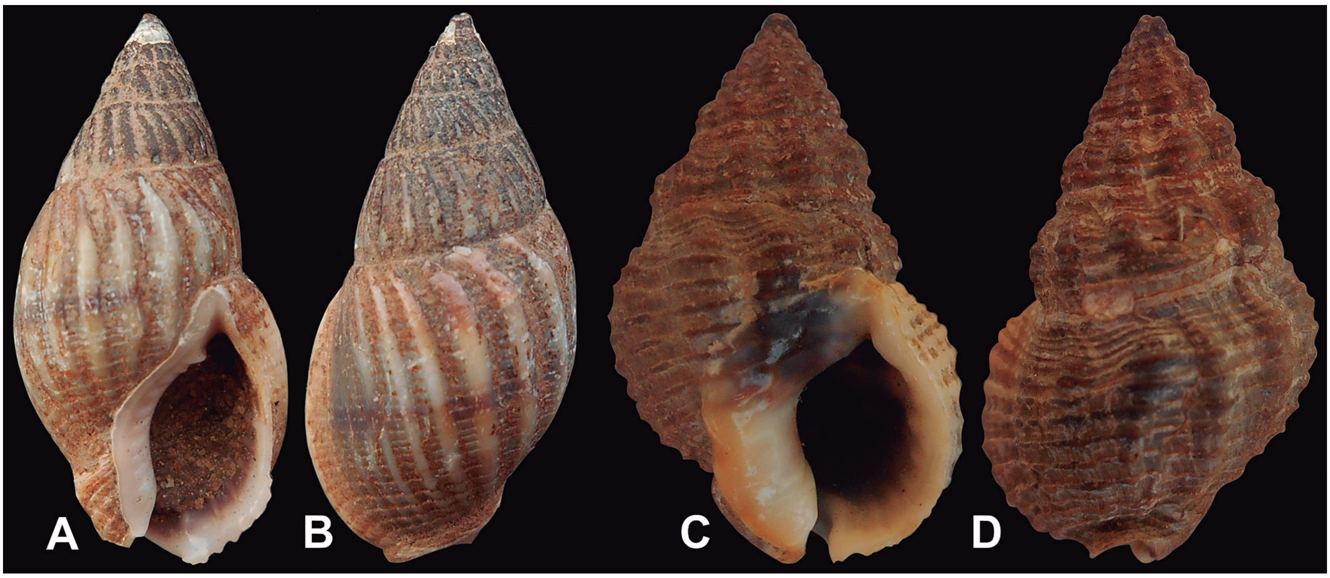

Figure 2. Shells of *Nassarius* found in Paranaguá Estuarine Complex. H: Shell height; D: shell greatest diameter. (A) Ventral view of *N. foveolatus;* (B) Dorsal view of *N. foveolatus;* (A-B) LEBIO 577 #2; H = 17.2 mm; D = 9.4 mm. (C) Ventral view of *N. vibex;* (D) Dorsal view of *N. vibex;* (C-D) LEBIO 347; H = 18.6 mm; D = 14.7 mm.

São Paulo, Brazil (MZUSP 136955, 3 spm.; MZUSP 143545, 11 spm.; MZUSP 143546, 20 spm.; MZUSP 143617, 14 spm.; MZUSP 143618, 16 spm.) and at the Laboratório de Ecologia Aplicada e Bioinvasões, Pontal do Paraná, Brazil (LEBIO 577, 9 spm.; LEBIO 578, 6 spm.).

The morphological identification was done according to Cernohorsky (1984). To confirm the morphological identification, molecular identification was performed using the DNA Barcode method (Hebert *et al.,* 2003). Firstly, the genomic DNA was extracted using the EZ-DNA kit (Biological Industries) from muscle. Then, a fragment of 650 bp of the cytochrome oxidase subunit 1 (COI) was sequenced. The amplification comprises a PCR with 25 ul final concentrations of 2.5x buffer, 3 mM of MgCl2, 0.4 uM of dNTP, 0.1 pmol of each HCO and LCO (Folmer *et al.,* 1994), 0.1 U of Taq Polymerase and 50 ng of DNA template. PCR product was purified using PEG 8000 (Amresco Inc., Cleveland, OH, USA). The sequencing reactions were performed with BigDye® kit (Applied Biosystems) according the manufacturer protocol and was purified with Sephadex G-50 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). The final product was sequenced in an ABI 3130 Genetic Analyzer (Applied Biosystems).

The identification was confirmed though Neighbor-Joining (NJ) and Bayesian (BI) trees. Sequences of *Nassarius* spp. accessed from GenBank (see species and accession number in Table 1) were used as reference sequences and *Antillophos* sp. (GU393391.1) was used as outgroup. Sequences were aligned using the Muscle Algorithm (Edgar, 2004) in Geneious 2019.1.1. The NJ was constructed in Geneious, using the Tamura-Nei evolution model (Tamura & Nei, 1993) and 10 thousand bootstraps. The BI tree was constructed in the Beast 1.8 (Drummond *et al.,* 2012), with three independent runs of 100 million MCMC generations sampled each 10,000 trees, 10% of burn-in and substitution model indicated by jModeltest 2.1.10 (Darriba *et al.,* 2012).

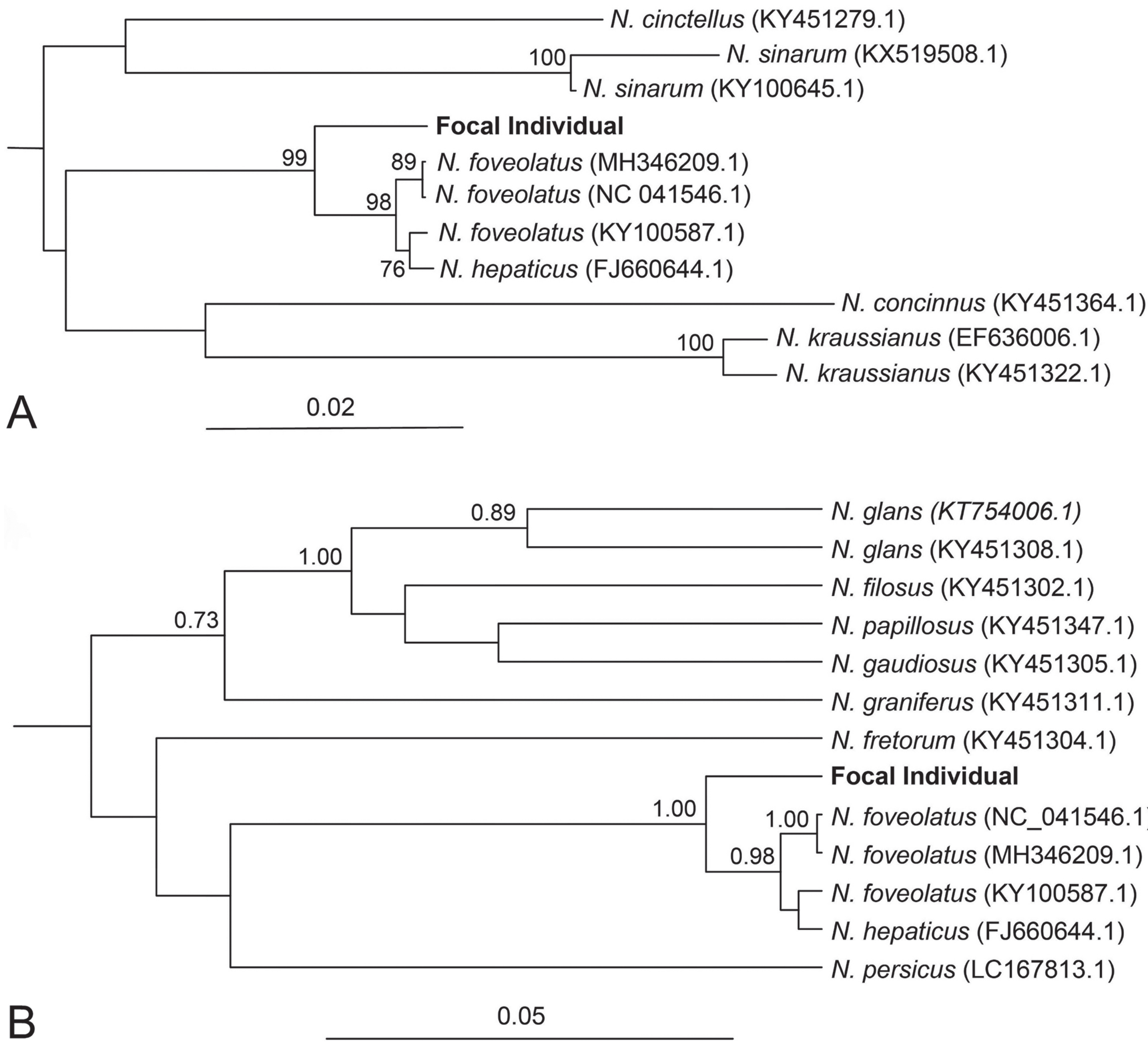

Figure 3. Summarized genetic identification of the focal individual through (A) Neighbor-Joining and (B) Bayesian trees of the COI fragment. Only bootstrap values higher than 70% (A) and posterior probabilities higher than 0.70 (B) are shown.

Table 1. Species of *Nassarius* accessed from GenBank and accession numbers.

Species	Accession number	Species	Accession number	Species	Accession number
Antillophos sp.	GU393391.1	*Nassarius foveolatus*	NC041546.1	*Nassarius olivaceus*	KY451342.1
Nassarius acuminatus	GU393380.1	*Nassarius fraterculus*	KX069667.1	*Nassarius olivaceus*	MG682443.1
Nassarius acuminatus	KY100533.1	*Nassarius fraudulentus*	KY451303.1	*Nassarius olomea*	KY451343.1
Nassarius acuminatus	KY451253.1	*Nassarius fretorum*	KY451304.1	*Nassarius oneratus*	KY451344.1
Nassarius acuticostus	KY100534.1	*Nassarius gaudiosus*	KY451305.1	*Nassarius pagodus*	FM999173.1
Nassarius acuticostus	KY451254.1	*Nassarius glans*	KT754006.1	*Nassarius papillosus*	KY451347.1
Nassarius agapetus	KY451255.1	*Nassarius glans*	KY451308.1	*Nassarius persicus*	LC167813.1
Nassarius albescens	KY499727.1	*Nassarius globosus*	KY451306.1	*Nassarius poupini*	KY451351.1
Nassarius algidus	KY100535.1	*Nassarius globosus*	KY451309.1	*Nassarius pullus*	JQ975555.1
Nassarius algidus	KY100536.1	*Nassarius graniferus*	KY451311.1	*Nassarius pullus*	KY100627.1
Nassarius algidus	GU393388.1	*Nassarius haldemani*	KY451313.1	*Nassarius pullus*	KY451353.1
Nassarius algidus	GU393389.1	*Nassarius hepaticus*	JQ975462.1	*Nassarius radians*	KC970054.1
Nassarius arcularia	KY451259.1	*Nassarius hepaticus*	FJ660667.1	*Nassarius radians*	KC970058.1
Nassarius arcularia	KY451340.1	*Nassarius hepaticus*	FJ660644.1	*Nassarius reeveanus*	KY451355.1
Nassarius arcus	KY451260.1	*Nassarius herosae*	KY451314.1	*Nassarius reticulatus*	EF571446.1
Nassarius babylonicus	KY451261.1	*Nassarius hirasei*	KY100588.1	*Nassarius reticulatus*	KR084688.1
Nassarius barsdelli	KY451262.1	*Nassarius hirasei*	KY100589.1	*Nassarius rufus*	KY451358.1
Nassarius bellulus	KY451263.1	*Nassarius horridus*	KY451240.1	*Nassarius samiae*	KY451359.1
Nassarius bicallosus	KY451264.1	*Nassarius houbricki*	KC970035.1	*Nassarius semiplicatus*	JQ975563.1
Nassarius bimaculosus	KY451265.1	*Nassarius houbricki*	KC970031.1	*Nassarius semiplicatus*	JQ975564.1
Nassarius boucheti	KY451266.1	*Nassarius idyllius*	KY451345.1	*Nassarius semisulcatus*	KY451360.1
Nassarius callospira	KY451267.1	*Nassarius incrassatus*	KT988325.1	*Nassarius sinarus*	KX519508.1
Nassarius camelus	KY451268.1	*Nassarius incrassatus*	KU714729.1	*Nassarius sinarus*	KY100645.1
Nassarius cinctellus	KY451279.1	*Nassarius incrassatus*	MG934953.1	*Nassarius sinusigerus*	KY451363.1
Nassarius cinnamomeus	KY451280.1	*Nassarius interliratus*	KY451316.1	*Nassarius siquijorensis*	JQ975552.1
Nassarius concinnus	KY451283.1	*Nassarius irus*	KY451317.1	*Nassarius siquijorensis*	KP253020.1
Nassarius concinnus	KY451364.1	*Nassarius javanus*	KY451318.1	*Nassarius splendidulus*	KY451365.1
Nassarius conoidalis	JQ975565.1	*Nassarius kooli*	KY451321.1	*Nassarius stigmarius*	KY451392.1
Nassarius conoidalis	KY451284.1	*Nassarius kraussianus*	EF636006.1	*Nassarius subspinosus*	KY451243.1
Nassarius coronatus	KY451287.1	*Nassarius kraussianus*	KY451322.1	*Nassarius subtranslucidus*	KY451393.1
Nassarius crematus	KY451288.1	*Nassarius labordei*	KY451323.1	*Nassarius succinctus*	KP253021.1
Nassarius crematus	KY451330.1	*Nassarius leptospirus*	KY451324.1	*Nassarius succinctus*	JQ975500.1
Nassarius crenoliratus	KY451289.1	*Nassarius limnaeiformis*	KY451325.1	*Nassarius succinctus*	JQ975501.1
Nassarius cuvierii	JQ950234.1	*Nassarius livescens*	JQ975514.1	*Nassarius succinctus*	KY100688.1
Nassarius dijki	KY451293.1	*Nassarius livescens*	KY100592.1	*Nassarius sufflatus*	KT290129.1
Nassarius disparilis	KY451294.1	*Nassarius lochi*	KY451326.1	*Nassarius sufflatus*	GU393387.1
Nassarius distortus	KY451295.1	*Nassarius luridus*	KY451327.1	*Nassarius sufflatus*	KY100689.1
Nassarius dorsatus	JQ975553.1	*Nassarius margaritifer*	KY451329.1	*Nassarius sufflatus*	KY100691.1
Nassarius dorsatus	JQ975554.1	*Nassarius martinezi*	KC970050.1	*Nassarius sufflatus*	KY100694.1
Nassarius dorsuosus	KY451296.1	*Nassarius mendicus*	KX069671.1	*Nassarius tabescens*	KY451394.1
Nassarius ecstilbus	KY451297.1	*Nassarius moolenbeeki*	KY451332.1	*Nassarius teretiusculus*	KY100695.1
Nassarius euglyptus	KX519509.1	*Nassarius multicostatus*	KY451333.1	*Nassarius thachi*	KX519507.1
Nassarius euglyptus	KY100558.1	*Nassarius multipunctatus*	KY451334.1	*Nassarius thachi*	KY451395.1
Nassarius euglyptus	KY451299.1	*Nassarius nigrus*	KY451241.1	*Nassarius vanpeli*	KY451399.1
Nassarius eximius	KY451300.1	*Nassarius nitidus*	GU270625.1	*Nassarius vanuatuensis*	KC970062.1
Nassarius fenistratus	KY451301.1	*Nassarius nitidus*	MG934857.1	*Nassarius velvetosus*	KC970064.1
Nassarius festivus	JQ975431.1	*Nassarius nodifer*	KY100593.1	*Nassarius velvetosus*	KC970063.1
Nassarius festivus	AB642676.1	*Nassarius nodifer*	KY100621.1	*Nassarius venustus*	KY451400.1
Nassarius filosus	KY451302.1	*Nassarius noguchii*	KY451338.1	*Nassarius vibex*	KU905981.1
Nassarius foveolatus	KY100587.1	*Nassarius novaezelandiae*	KY451339.1	*Nassarius vitiensis*	KY451403.1
Nassarius foveolatus	MH346209.1	*Nassarius ocellatus*	KY451341.1		

RESULTS AND DISCUSSION

As referred above, the specimens of *N. foveolatus* (Fig. 2A-B) were initially collected in Rocio, Paranaguá, Paraná. This locality is adjacent to Dom Pedro II Port (Port of Paranaguá), one of the largest in Brazil, which suggests that the species may have been introduced through ballast water. On the site, there is a small patch of mangrove bordered by a tidal flat with marshes. During low tides the plain is exposed, keeping some pools of water. Inside and around these pools, active live specimens were found at densities of up to 11 individuals/m^2, in sympatry with *N. vibex* (Say, 1822) (Fig. 2C-D). The native *N. vibex* can be easily distinguished by being smaller, with the peristome callus much more developed in the adult specimens, by the thicker outer lip, and by the spire sculpture of strong axial ribs.

For the Bay of Bengal, in Odisha state, India, Ghosh *et al.* (2017) found a density of up to 11.8 individuals/m^2 of *N. foveolatus,* suggesting that the density of this species in the Paranaguá Estuarine Complex is already reaching the same levels as those observed in its natural environment. On June 5, 2019, living individuals of *N. foveolatus* were found in the Emboguaçu River, in the Lamin Island, in Europinha (Vista Bela), and also in Ponta da Pita, all localities in the Paranaguá Estuarine Complex (Fig. 1).

The genetic identification for *N. foveolatus* suggested both by NJ (Fig. 3A) and BI (Fig. 3B) trees is highly supported. Interestingly, a sequence deposited as *N. hepaticus* (Pulteney, 1799) (FJ660644.1) in GenBank was also assigned to this group. However, since the other sequences of this species were assigned in a distant clade with *N. nodifer* (Powys, 1835) (see Appendices session for the entire phylogeny), this sequence probably represents an error of identification (as both species have similar shells) or even introgression between *N. hepaticus* and *N. foveolatus,* process already proposed for other *Nassarius* species (for details, see Pu *et al.,* 2017).

Despite the inference of ballast water as responsible for the artificial dispersal of *N. foveolatus* in Paranaguá Estuarine Complex, the confirmation of this via of dispersal must be verified by additional studies. These could serve as alert on the species worldwide. The event can be the tip of the iceberg, representing a wide range of invasive species in the region (*e.g.,* Rocha & Kremer, 2005; Neves & Rocha, 2008; Lopes, 2009; Altvater & Coutinho, 2015).

Based only on the collection experience, it is already possible to infer that the specimens of *N. foveolatus* occur side by side with the native specimens of *N. vibex,* having the same niche. As previously only *N. vibex* existed in that place (Rios, 2009; Absher *et al.,* 2015), at least a displacement of the native species has occurred. However, more ecological studies may certainly confirm this displacement and additional consequences in the local ecosystem, as nassariids can be predators and scavengers. Control procedures should be also greatly implemented.

ACKNOWLEDGEMENTS

We thank Esther Valentina da Veiga de Vasconcellos Gernet who collected the first specimens of *N. foveolatus* on the coast of Paraná state, two anonymous reviewers for their valuable comments in the manuscript, and Desyrêe Mathiew Teixeira Delgado for her help to edit Fig. 2.

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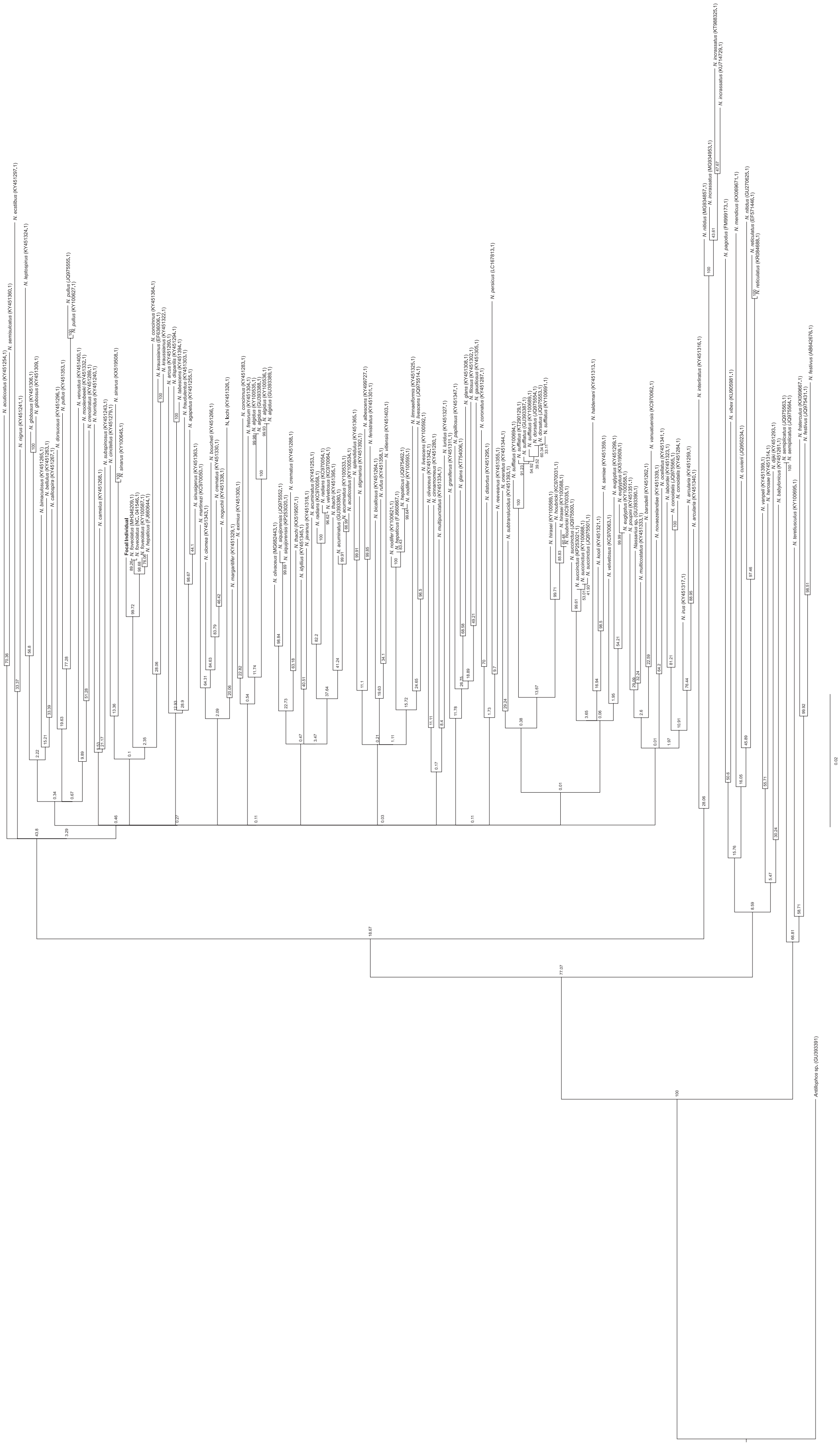
APPENDIX 1

Neighbor-Joining tree of the COI gene with all *Nassarius* sequences analyzed for genetic identification of the focal individual. Numbers in the nodes represent bootstrap values.



APPENDIX 2

Bayesian tree of the COI gene with all *Nassarius* sequences analyzed for genetic identification of the focal individual. Numbers in the nodes represent posterior probabilities.

Published with the financial support of the "Programa de Apoio às Publicações Científicas Periódicas da USP"