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

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**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).
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.

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,
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, Pantanal 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 µl 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 to 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.
| 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 KJ006966.7 | Nassarius olivaceus MG682442.1 |
| Nassarius acuminatus KY100533.1 | Nassarius fraudulentus KY451309.1 | Nassarius olomea KY451343.1 |
| Nassarius acuminatus KY451253.1 | Nassarius flettorum KY451304.1 | Nassarius oneratus KY451344.1 |
| Nassarius arcticus KY100534.1 | Nassarius gautheriacus KY451305.1 | Nassarius pagodaux FM999173.1 |
| Nassarius arcticus KY451254.1 | Nassarius glans KI754006.1 | Nassarius papillosus KY451347.1 |
| Nassarius agapetus KY451255.1 | Nassarius glans KY451308.1 | Nassarius pernicus LC167813.1 |
| Nassarius albescens KY499727.1 | Nassarius globosus KY451306.1 | Nassarius poupin KY451351.1 |
| Nassarius algadus KY100535.1 | Nassarius globosus KY451309.1 | Nassarius pulus JQ975355.1 |
| Nassarius algidus KY100536.1 | Nassarius graniferus KY451311.1 | Nassarius pulus KY100627.1 |
| Nassarius algidus GU393388.1 | Nassarius haldemani KY451313.1 | Nassarius pulus KY451353.1 |
| Nassarius algidus GU393389.1 | Nassarius heptacius JQ975462.1 | Nassarius radons KG70054.1 |
| Nassarius antracina KY451259.1 | Nassarius heptacius FJ660664.1 | Nassarius radons KG70058.1 |
| Nassarius anulata KY451340.1 | Nassarius heptacius FJ660664.1 | Nassarius reeveanus KY451355.1 |
| Nassarius anulata KY451260.1 | Nassarius hisiae KY451314.1 | Nassarius reticulatus EF571446.1 |
| Nassarius arcur KY451261.1 | Nassarius idyllus KY451345.1 | Nassarius semissulcatus KY451360.1 |
| Nassarius babylonicus KY451261.1 | Nassarius incrustatus KT988325.1 | Nassarius sinars JX591508.1 |
| Nassarius barnesi KY451262.1 | Nassarius incrassatus KU714729.1 | Nassarius sinars KY100645.1 |
| Nassarius bellulus KY451263.1 | Nassarius hornudas KY451240.1 | Nassarius samiae KY451359.1 |
| Nassarius bicollis KY451264.1 | Nassarius houbriki KF700035.1 | Nassarius semplicatus JQ975563.1 |
| Nassarius bimaculatus KY451265.1 | Nassarius houbriki KF700031.1 | Nassarius semplicatus JQ975564.1 |
| Nassarius boauchet KY451266.1 | Nassarius idyllus KY451345.1 | Nassarius semplicatus JQ975564.1 |
| Nassarius callospira KY451267.1 | Nassarius incrassatus EF636006.1 | Nassarius semissulcatus KY451360.1 |
| Nassarius camelus KY451268.1 | Nassarius incrassatus EF636006.1 | Nassarius semissulcatus KY451360.1 |
| Nassarius cancillatus KY451279.1 | Nassarius incrassatus MG934953.1 | Nassarius sinars KG70058.1 |
| Nassarius cannamomeus KY451280.1 | Nassarius interteratus KY451316.1 | Nassarius siquiporensis JQ975552.1 |
| Nassarius cancillas KY451283.1 | Nassarius nius KI451317.1 | Nassarius siquiporensis KG250021.1 |
| Nassarius cancillas KY451364.1 | Nassarius javanus KY451318.1 | Nassarius sinars KG700645.1 |
| Nassarius canosus JQ975565.1 | Nassarius javanus KY451318.1 | Nassarius sinars KG700645.1 |
| Nassarius conoidalis KY451284.1 | Nassarius kraussianus EF636006.1 | Nassarius sinars KG700645.1 |
| Nassarius corone KY451287.1 | Nassarius kraussianus KY451322.1 | Nassarius sinars KG700645.1 |
| Nassarius crementus KY451288.1 | Nassarius laberdei KY451323.1 | Nassarius succinctus KG250021.1 |
| Nassarius crementus KY451330.1 | Nassarius leptoscrinus KY451324.1 | Nassarius succinctus KG250021.1 |
| Nassarius cremolatius KY451289.1 | Nassarius limneniformis KY451325.1 | Nassarius succinctus JQ975501.1 |
| Nassarius caviarii JQ975524.1 | Nassarius lvacesens JQ975514.1 | Nassarius succinctus JQ975501.1 |
| Nassarius diji KY451293.1 | Nassarius lvacesens KY100592.1 | Nassarius suflatus KT290129.1 |
| Nassarius discans KY451294.1 | Nassarius lochi KY451326.1 | Nassarius suflatus GU393387.1 |
| Nassarius distortus KY451295.1 | Nassarius lunatus KY451327.1 | Nassarius suflatus KG700689.1 |
| Nassarius distorsus JQ975553.1 | Nassarius margarinller KY451329.1 | Nassarius suflatus KG700691.1 |
| Nassarius distorsus JQ975554.1 | Nassarius martinez KF700050.1 | Nassarius suflatus KG700691.1 |
| Nassarius dorosus KY451296.1 | Nassarius mendoz KY869671.1 | Nassarius tabescens KY451394.1 |
| Nassarius exilissus KY451297.1 | Nassarius moolenberki KY451332.1 | Nassarius tre tessatus KY100695.1 |
| Nassarius euglyptus KX519090.1 | Nassarius multistatus KY451333.1 | Nassarius thaci KX519070.1 |
| Nassarius euglyptus KY100558.1 | Nassarius multipunctatus KY451334.1 | Nassarius thachi KG451395.1 |
| Nassarius euglyptus KY451299.1 | Nassarius mirus KY451241.1 | Nassarius thachi KG451395.1 |
| Nassarius extimus KY451300.1 | Nassarius mitinus GU270625.1 | Nassarius vanuatuensis KE700626.1 |
| Nassarius finestri KY451301.1 | Nassarius mitinus MG634857.1 | Nassarius velvetosus KE700641.1 |
| Nassarius festivus JQ975431.1 | Nassarius madfer KY100593.1 | Nassarius velvetosus KE700641.1 |
| Nassarius festivus AB642676.1 | Nassarius madfer KY100621.1 | Nassarius venustas KY451400.1 |
| Nassarius filosus KY451302.1 | Nassarius nagachi KY451338.1 | Nassarius vibex KU905198.1 |
| Nassarius fozeolatus KT100587.1 | Nassarius novazelandiae KY451339.1 | Nassarius vibens KY451403.1 |
| Nassarius fozeolatus MH346209.1 | Nassarius ocellatus KY451341.1 | Nassarius vibens KY451403.1 |
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/m², in sympathy 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/m² 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 Emboguacu 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 species having the same niche. As previously only *N. vibex* occurred 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.

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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.