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Molecular methods confirm the presence of the alien mussel *Perna viridis* Linnaeus, 1758 (Bivalvia, Mytilidae) in Southern Brazil

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Abstract

The mussel *Perna viridis*, commonly known as the green mussel, is native from the Indo-Pacific region and has been introduced in various sites around the globe. In Brazil, the species has already been recorded in Rio de Janeiro and Ceará states. With the aim of assessing the presence of mussels in the southern region of the country, 14 individuals were collected in the Paranaguá Estuarine Complex, Paraná. The mussels were found attached at a depth of 2 meters on the artificial structure of Ponta do Poço Marina. The DNA was extracted using a commercial kit, the COI gene was amplified through PCR by the primers forward dgLCO-1490 and reverse dgHCO-2198, sequenced by the Sanger method, assembled in CLC Genomics Workbench, and the species was identified through the BOLD Systems. The phylogenetic tree was built on MEGA11 using 28 sequences from three species within the genus *Perna*. Therefore, the present study represents the first to confirm the occurrence of the exotic species *Perna viridis* in Brazil through molecular identification and characterizes the third Brazilian state where the mollusk has been recorded. This indicates that Brazilian coastline provides optimal environmental conditions for the establishment and development of the *P. viridis*.

Keywords. Alien species, green mussel, marine species, Paraná, molecular analysis

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Introduction

Alien species are defined as those found outside their natural distribution area and that have the potential to spread in this new area, where they could not occur without anthropic introduction (Pyšek et al. 2020). When they harm the economy, environment or human health, these species are classified as “invasive” (IUCN 2021). The mytilids are one of the main invasive alien groups recognized for causing several impacts. Their success stems from their capability in adapting to new environments, becoming well-established and dominant in coastal areas (Micklem et al. 2016; Santos et al. 2023).

Once established, invasive mytilids demonstrate increased tolerance to environmental stress in comparison to taxonomically related native species (Lenz et al. 2011), consequently modifying ecosystem functioning and structure through competition, altering food chains and nutrient cycling (Pyšek et al. 2020). These species also affect human well-being by harming water systems in industrial complexes and marine transport, as well as causing human health problems through food poisoning. This is because their filter-feeding behavior concentrates heavy metals and other contaminants (Freire and Marafon 2018; Siriwardena 2022; Turbelin et al. 2022).

In Brazil, the occurrence of nine invasive species of marine/estuarine bivalves is documented, with seven recorded in the southern region of the country: the oysters *Isognomon bicolor* (C. B. Adams, 1845), *Crassostrea virginica* (Gmelin, 1791), *Saccostrea cucullata* (Born, 1778) and *Crassostrea gigas* (Thunberg, 1793); and the mussels *Mytilus galloprovincialis* Lamarck, 1819, *Leiosolenus aristatus* (Dillwyn, 1817) and *Perna perna* (Linnaeus, 1758) (Moura-Britto and Patrocínio 2005; Freire and Marafon 2018; Messano et al. 2019; Amaral et al. 2020; Belz et al. 2020; Teixeira and Creed 2020; Agudo-Padrón 2022; Stanski et al. 2022).

In relation to the species of mussels, the blue mussel *M. galloprovincialis* is an intertidal species, native to the Mediterranean and the Atlantic coasts of Europe and North Africa. This mollusk is widely distributed, occupying shores on every continent except Antarctica (McQuaid et al., 2015; Zardi et al., 2018). The small bivalve *L. aristatus* is an endolithic and wood-boring species that often causes damage and deformities in other mollusks. This mussel is native to the Caribbean Sea and having been identified in the Atlantic, Mediterranean, Caribbean, and Pacific

regions (Simone & Golçalves, 2006; Soriano & Salgado, 2019). Concerning the brown mussel *P. perna*, which is widely distributed around the world, and along the Brazilian coast it is particularly abundant from Espírito Santo to Santa Catarina, there has been extensive analysis and technical discussion about the species' origin in the country. According to Pierri et al. (2016), the presence of the species in Brazilian territory predates colonization in 1500, suggesting that *P. perna* is a native species. However, as demonstrated by Silva et al. (2018), the occurrence of the species along the Brazilian coast is attributed to invasion during the slave trade between Africa and Brazil from the 16th to the 19th centuries. Despite its origin, the species is naturalized in South America, with multiple introductions occurring over the past 500 years (Oliveira et al., 2017).

Regarding the mussel *Perna viridis*, although the presence of the species has not yet been confirmed by molecular methods in Brazil, based on specimens sampled in May 2018, Messano et al. (2019) reported the first occurrence in the Southwest Atlantic. The bivalve was found attached to experimental plates installed to test anti-fouling systems at Guanabara Bay, Rio de Janeiro, on the southeastern Brazilian coast. Recently, Soares et al. (2022) reported the species settled near the port of Mucuripe (Fortaleza, Ceará), located in northeast Brazil, also in settlement plates. Subsequently, Santos et al. (2023) reported *P. viridis* in lantern nets within the Marine Extractive Reserve, from Arraial do Cabo (Rio de Janeiro). It is suggested that all these introductions of the mussel on the Brazilian coast were accidental, probably via fouling or ballast water (Santos et al. 2023).

The mollusk bivalve *Perna viridis* (Linnaeus, 1758), synonymous with *Mytilus viridis* Linnaeus, 1758, *Mytilus smaragdinus* Gmelin, 1791 and *Mytilus opalus* Lamarck, 1819, (MolluscaBase 2024), inhabits the midlittoral and sublittoral zones of marine and estuarine ecosystems, where it is usually found forming dense colonies attached to a variety of structures or substrates via byssus threads (Rajagopal et al. 2006). Over the years, it has been reported in polluted and anthropogenic areas (Rajagopal et al. 1997; Micklem et al. 2016), but also in pristine and protected systems (Gracia and Rangel-Buitrago 2020; Santos et al. 2023).

The species is native to the Indo-Pacific region, extending from the Persian Gulf to the Philippines, and in adjacent areas, such as China and Japan (Baker et al. 2007). The mussel *P. viridis* has been introduced in different regions around the globe, mainly through ship hull fouling,

and its occurrence has been documented in the Northwest and Northeast Pacific, Oceania, South Africa, Gulf of Mexico, Caribbean, and Northwest Atlantic (Santos et al. 2023). In the early 1990s, the first records of the species introduction in South America were made in Trinidad and Tobago and, subsequently, it dispersed to Jamaica, Venezuela, Colombia, and the United States by the end of the decade (Baker et al. 2007). In the introduced areas, this species can cause significant negative impacts, including competition for space leading to displacement of native species and alterations in community structure. Additionally, there is a risk of transmitting diseases and parasites to local species, as well as harming other aquaculture species, such as crabs and mussels, by obstructing traps and cultivation bags (Gracia et al. 2011).

The exceptional invasive skills of *P. viridis* is primarily attributed to their physiology, characterized by a high rate of dispersion, phenotypic plasticity, rapid growth and recruitment, the competence to detach and re-attach with byssus, and the capacity to form dense clusters that alter the native ecosystem (Gobin et al. 2013). In addition to these characteristics, one of the main reasons for the competitive superiority of *P. viridis* is its high resistance to environmental stress. A study conducted by Lenz et al. (2011), comparing *Brachidontes exustus*, a native species from Caribbean, with the related non-indigenous species *P. viridis*, concluded that under stressful conditions, the non-native species exhibits a wider tolerance range. The mussel *P. viridis* tolerates a high level of particulate matter, a temperature range of 15°C to 32.5°C, and can even survive at a temperature of 39°C for about 200 minutes (Rajagopal et al. 2006).

Although *P. viridis* is recognized in the literature as a green-shelled mussel, with the color being one of the taxonomic characteristics used for its identification, it is not a reliable character. Micklem et al. (2016) concluded that specimens genetically identified as the variants of indigenous *P. perna* in South Africa displayed a wide range of shell colors, including blue-green. This finding indicates that the designations *P. viridis* as ‘green mussel’ and *P. perna* as ‘brown mussel’ are unsuitable for discerning these species. The only morphological differences found to separate the taxa in the study were the poorly developed mantle papillae and the wavy pallial line in *P. viridis*. Thus, the exclusive reliance on morphological characteristics can present challenges in identification. In certain cases, species are indistinguishable based on morphology alone, therefore, the use of DNA assumes significant relevance as a tool to confirm the species identification (Smith et al. 2022). In this context, this study aimed to confirm the presence of a new non-indigenous

mussel using molecular methods in southern Brazil with the purpose of compiling information about the species' expansion in the South Atlantic Ocean.

Methods

The subtropical Paranaguá Bay Estuarine Complex, located in the extreme east of Paraná State, spans an area of 612 km², making it the primary estuarine system in the state (Lana et al. 2001). The region, bordered by mangroves, marshes and extensive tidal flats, exhibits seasonal variation patterns of water circulation and stratification (Knoppers et al. 1987; Egres et al. 2012; Moreira-González et al. 2020). The estuary has an average depth of 5.4 meters and water residence time of 3.49 days (Lana et al., 2001). The salinity varies from 14.6 to 30.0 and temperatures range between 16.9 and 29.3°C. This area experiences a significant freshwater input, especially during the summer (rainy season, December to May), when freshwater discharge is five times higher than in winter (dry season, June to November) (Mantovanelli et al., 2004). The region holds considerable ecological and economic importance for the entire southern region (Bumbeer and Rocha, 2016), where numerous nautical structures, including marinas and ports, are situated. Notably, the Port Complex of Paraná represents the largest grain port in Latin America and the second largest public port in Brazil (Menem et al. 2019; Portos do Paraná 2023).

To confirm the species identification, on February 15, 2023, fourteen individuals possibly belonging to the species *P. viridis*, were sampled at a depth of 2 meters on the artificial structures of Ponta do Poço Marina Club (25°32'55"S and 48°23'19"W) (Figure 1). In the sampling area, some of the collected individuals were forming agglomerates (clusters), while others were individually dispersed. For species evaluation, the entire specimens, including shells and tissues, were carefully detached from the artificial substrata using a metal spatula, placed in plastic containers, and transported on ice to the laboratory, where they were stored in the freezer.

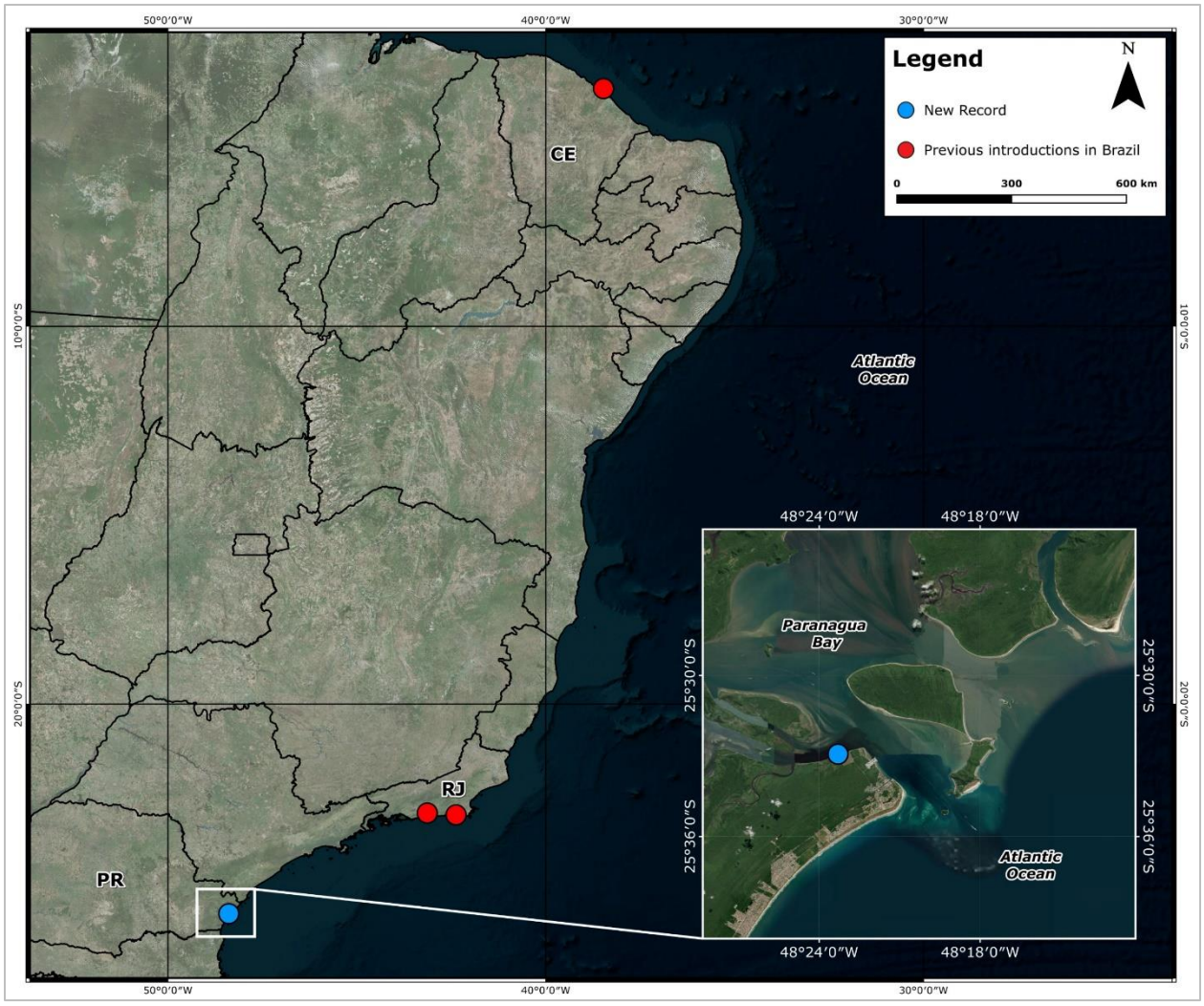

Figure 1. The range expansion of the Asian green mussel *Perna viridis* in Brazil. Red dots indicate areas where the mussel has already been recorded, and a blue dot marks the location of the new record in the Paranaguá Bay Estuarine Complex, southern Brazil. CE, Ceará. RJ, Rio de Janeiro. PR, Paraná.

The extraction of genomic DNA from the mussel samples was carried out using the commercial DNeasy Blood & Tissue kit (Qiagen, Germany). Subsequently, the DNA was quantified via spectrophotometry on the NanoQuant Infinite M200 Pro equipment (Tecan, Switzerland). The DNA was then used as a template for PCR amplification of the gene encoding for cytochrome oxidase subunit I (COI). For this, the primer forward dgLCO-1490 (5´-TAAACTTCAGGGTGACCAAARAAYCA-3´) and reverse dgHCO-2198 (5´-GGTCAACAAATCATAAAGAYATYGG-3´) were used, according to Meyer (2003). The 25 µl

PCR reaction consisted of: 1x SuperFi™ II Buffer (ThermoFisher Scientific), 0.2mM dNTP, 0.5mM of each primer, 1U Invitrogen™ Platinum™ SuperFi™ II DNA Polymerase (ThermoFisher Scientific), 50ng genomic DNA, and the final volume was adjusted with ultrapure water. The PCR reaction was conducted on the thermocycler (Veriti®, Applied Biosystems®), with the program: 94°C for 2min, 35 cycles of 94°C for 15sec, 58°C for 30sec, and 72°C for 30sec, with a final extension of 72°C for 2.5min. To confirm the amplification, the PCR products were evaluated through agarose gel electrophoresis (1%) and compared with the molecular marker DNA (λ Hind III, Invitrogen). The fragments were sequenced by the GoGenetics company, in both directions using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA), with the mentioned primers and the product evaluated on the ABI 3500x Genetic Analyzer (Applied Biosystems, USA) as described by the manufacturer.

The sequencing results were processed using the bioinformatics package CLC Genomics Workbench v. 6.5.2 (QIAGEN, Denmark). Initially, the sequences obtained in both directions were subjected to a step of eliminating low-quality segments (<Q30) using the Trim tool. Then, the shared regions between the two sequences were recognized and overlapped in order to obtain a single continuous segment of the COI gene, using the DNA Assemble tool. Finally, the obtained segment was then analyzed on the Barcode of Life Data System - BOLD Systems v.4 (RATNASINGHAM; HEBERT, 2007) portal in order to carry out the first molecular identification step. The sequence was compared to those available in the Species Level Barcode Records database. In this database, every COI barcode record with a species-level identification and a minimum sequence length of 500bp is included.

The second molecular identification of the mussel was done through a comparative analysis of its COI sequence and that of other specimens within the same genus, including the same species. This was done by searching for published COI sequences in the NCBI nucleotide bank that also had the location in which they were sampled from (Table 1). Then, their sequences were aligned using the ClustalW algorithm embedded in MEGA11 software. The Maximum Likelihood phylogenetic tree was then built in the same software, with sequences from *P. perna*, *P. viridis* and *P. canaliculus*, using the Hasegawa-Kishino-Yano model (HASEGAWA; KISHINO; YANO, 1985), and using *Aulacomya atra* as an outgroup. The test was made with the bootstrap method, using 1000 replications.

Table 1. All sequences used to build the phylogenetic tree, organized by species name, location from where it was collected, its GenBank Accession, its nucleotide length, and references. *P. viridis, Perna viridis; P. perna, Perna Perna; P. canaliculus, Perna canaliculus.*

Species	Location	Genbank Acession	Nucleotide length	Reference
P.viridis	Parana-Brazil	PP702447.1	608	-
P.viridis	Kochin-India	JN179068.1	650	(GILG et al., 2013)
P.viridis	Kochin-India	JN179072.1	650	(GILG et al., 2013)
P.viridis	Hong Kong	GQ497818.1	650	(GILG et al., 2013)
P.viridis	Hong Kong	JN179047.1	650	(GILG et al., 2013)
P.viridis	Singapore	HQ197379.1	650	(GILG et al., 2013)
P.viridis	Singapore	JN179049.1	650	(GILG et al., 2013)
P.viridis	Chennai - India	DQ917612.1	617	(WOOD et al., 2007)
P.viridis	Southern India	DQ917586.1	617	(WOOD et al., 2007)
P.viridis	Philippines	DQ917599.1	617	(WOOD et al., 2007)
P.viridis	Thailand	DQ917590.1	617	(WOOD et al., 2007)
P. viridis	Vietnam	DQ917584.1	617	(WOOD et al., 2007)
P. perna	Luanda-Angola	KC692001.1	614	(CUNHA et al., 2014)
P. perna	Punta D'Ouro-Mozambique	KC692009.1	614	(CUNHA et al., 2014)
P. perna	Swakopmund-Namibia	KC692005.1	614	(CUNHA et al., 2014)
P. perna	Muscat-Oman	KC692013.1	614	(CUNHA et al., 2014)
P. perna	Gans Bay-South Africa	KC691990.1	614	(CUNHA et al., 2014)
P. perna	Bizerte-Tunisia	KC691986.1	614	(CUNHA et al., 2014)
P. perna	Africa	DQ917618.1	617	(WOOD et al., 2007)
P. perna	Venezuela	DQ917588.1	617	(WOOD et al., 2007)
P. perna	Santa Catarina-Brazil	DQ917594.1	617	(WOOD et al., 2007)
P. perna	Sao Paulo-Brazil	DQ917592.1	617	(WOOD et al., 2007)
P. canaliculus	Houhora-New Zealand	DQ917607.1	617	(WOOD et al., 2007)
P. canaliculus	Castlepoint-New Zealand	DQ917613.1	617	(WOOD et al., 2007)
P. canaliculus	Gore Bay-New Zealand	DQ917608.1	617	(WOOD et al., 2007)
P. canaliculus	Fiordland-New Zealand	DQ917609.1	617	(WOOD et al., 2007)
P. canaliculus	Nelson-New Zealand	HG005373.1	706	(POCHON et al., 2013)
Aulacomya atra	Wellington-New Zealand	DQ917614.1	620	(WOOD et al., 2007)

Results

Through the comparison of the mitochondrial gene COI from the mussel collected in Paranaguá Bay Estuarine Complex (*Perna viridis*, GenBank: PP702447.1), with sequences from all three species within the *Perna* genus, obtained from NCBI's nucleotide bank, it has been confirmed that the collected specimen corresponds to the exotic mussel *Perna viridis* (Linnaeus, 1758) (Figure 2 e Figure 3).

New records. BRAZIL – **Paraná** • Paranaguá Estuarine Complex, Ponta do Poço Marina Club; 25°32'55"S, 48°23'19"W; 2 meters deep; 15.II.2023; E.I. Xavier leg.; artificial substrata; 14 spec.; metal spatula; UNIVALI collection (mol.PV1/PR).

Identification. Green or bluish-green external color of shells; poorly developed mantle papillae; wavy S-shaped posterior pallial line; downward-pointing beak.

Figure 2. Left shell of *Perna viridis* sequenced for COI, collected in Paranaguá Estuarine Complex, Paraná, Brazil.

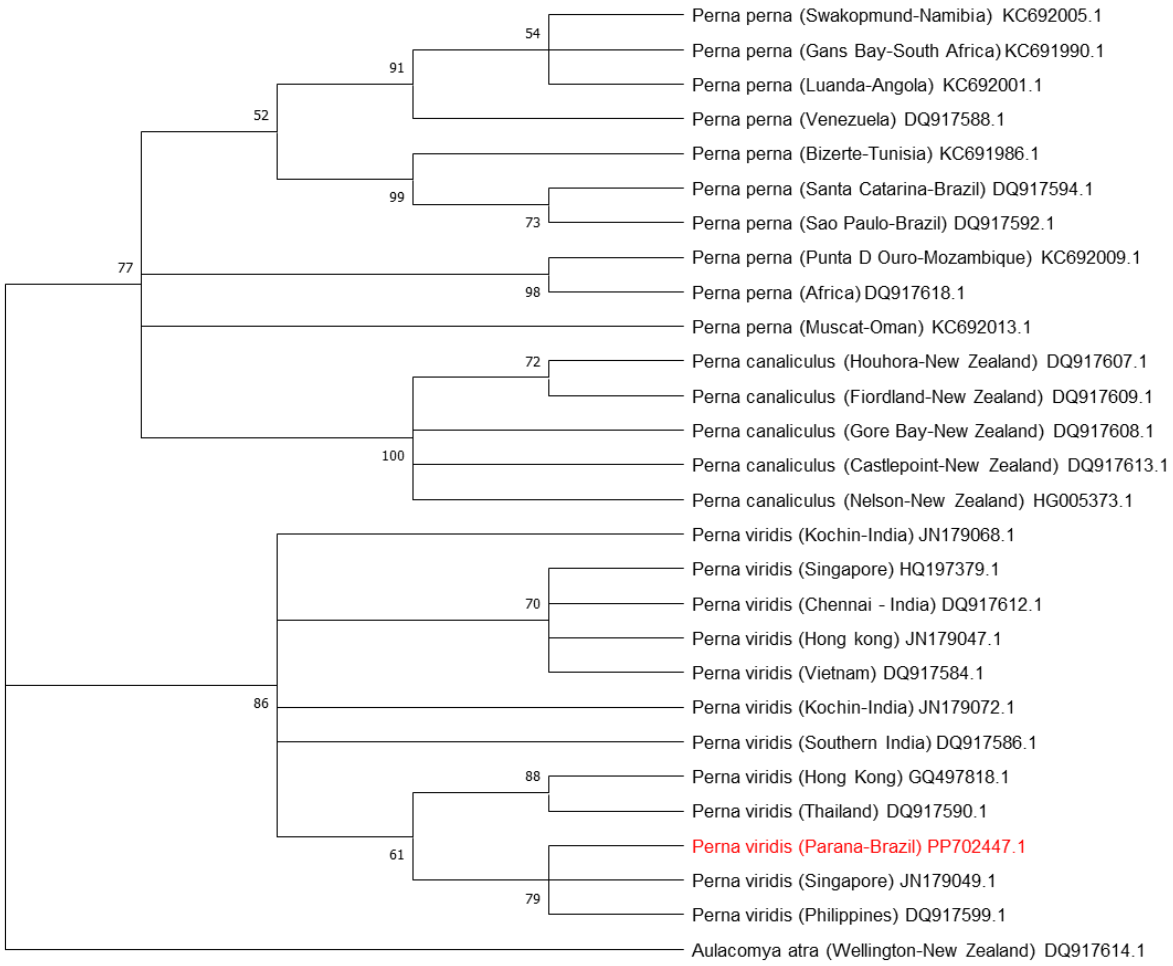

Figure 3. Maximum Likelihood phylogenetic tree (HKY+G+I) of various species within the genus Perna. Each node represents a different specimen's COI sequence, with its GenBank Accession and the location from where it was sampled from. Each branch has a label that represents its bootstrap percentage. Branches with less than 50% bootstrap value were condensed. This report's specimen is highlighted in red.

Discussion

Based on the amplified COI gene, the present study is the first to confirm the occurrence of the exotic species *Perna viridis* in Brazil through molecular identification and represents the third Brazilian state where the presence of the mussel was recorded, expanding its range approximately 600 km south of the first recorded instance five years ago. The mussel has already been identified in the northeastern and southeastern regions of the Brazil (Messano et al. 2019; Soares et al. 2022;

Santos et al. 2023), and now its occurrence has been documented in the southern region, indicating that the country's coast presents optimal environmental conditions for the establishment and growth of the mussel. According to Siddall (1980), *P. viridis* has the capacity to disperse beyond its native range through step-wise larval dispersal or island hopping. However, due to the absence of rafting skills, members of the Mytilidae require vectors to move over long distances (Gracia and Rangel-Buitrago 2020). Due to these characteristics, ship ballast water and hull fouling have been identified as the main sources of the species' introduction (Bumbeer and Rocha 2016; Siriwardena 2022; Santos et al. 2023; Castro et al. 2023).

The activities associated with port operations represent one of the most significant impacts on coastal environments worldwide, leading to alteration of aquatic ecosystems and the increasing presence of non-indigenous species (Madon et al. 2023). Regardless of the Paranaguá Estuarine Complex being considered a preserved coastal environment (UNESCO 2014), the region is exposed to numerous anthropogenic impacts, including port activities that may facilitate the invasion of species. This trend was as noted in previous investigations in the area, which have indicated that the port acts as an entry point for non-indigenous species. Neves et al. (2007) identified in a marina near Paranaguá Port the presence of four introduced species and another 30 cryptogenic species. In the study conducted by Bumbeer and Rocha (2016), which compared the Paranaguá Estuarine Complex and the adjacent open coast, the results evidenced that percentage of non-indigenous species was more pronounced in the estuary. Additionally, Rocha and Kremer (2005) confirm that the area is susceptible to non-indigenous species invasion.

Therefore, considering the potential for bioinvasion in the Paranaguá Estuarine Complex, primarily due to port activity, associated with the dispersal characteristics of *P. viridis*, and the distances between records of the species' occurrence in Brazil, the most likely means of mussel introduction in the study area is accidental — via ballast water or ship hull fouling — rather than natural dispersal.

Despite extensive discussions surrounding invasive species, this issue is globally acknowledged as the second largest threat impacting biodiversity, and its negative consequences are generally irreversible (Loehle and Eschenbach 2012; Latini and Resende 2016; Stanski et al. 2022). Therefore, following the proposal of Pyšek et al. (2020), it is suggested to implement an

integrated national invasive alien species surveillance program on the Brazilian coast, as well as to continue monitoring along the southwest Atlantic coastline to verify the range expansion of *P. viridis*. Future steps should include advancing the discussion about the recruitment and growth of *P. viridis* populations, as well as interactions and impacts on native species in places where their occurrence has been confirmed. This is important because the influence of alien species varies notably across species, regions, and ecosystems (Blackburn et al. 2014).

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Authors' Contributions

Conceptualization: MCB. Validation: FLD. Investigation: MCB, NJRC, YOL, TDS Resources: FLD. Writing - Original draft: MCB, NJRC, YOL, TDS. Writing - Review and Editing: MCB, NJRC, YOL. Supervision: MCB. Project administration: MCB. Funding Acquisition: FLD.

Conflicts of interest

The authors confirm that there are no conflicts of interest.

References

Agudo-Padrón AI (2022) Updated and referenced inventory of invasing exotic molluscs that occur in the state of Santa Catarina/SC, central southern Brazil region. BJAER 5 (4): 3710–3727. https://doi.org/10.34188/bjaerv5n4-022

Amaral VS, Simone LRL, Tâmega FTS, Barbieri E, Calazans SH, Coutinho R, Spotorno-Oliveira P (2020) New records of the non-indigenous oyster *Saccostrea cucullata* (Bivalvia: Ostreidae) from the southeast and south Brazilian coast. Regional Studies in Marine Science 33: 1–6. https://doi.org/10.1016/j.rsma.2019.100924

Belz CE, Simone LRL, Silveira N Jr, Baggio RA, Gernet MV, Birckolz CJ (2020) First record of the Mediterranean mussel *Mytilus galloprovincialis* (Bivalvia, Mytilidae) in Brazil. Papéis Avulsos de Zoologia 60: e20206007. https://doi.org/10.11606/1807-0205/2020.60.07

Blackburn TM, Essl F, Evans T, Hulme PE, Jeschke JM, Kühn I, Kumschick S, Markova Z, Mrugała A, Nentwig W, Pergl J, Pyšek P, Rabitsch W, Ricciardi A, Richardson DM, Sendek A, Vilà M, Wilson JRU, Winter M, Genovesi P, Bacher S (2014) A unified classification of alien species based on the magnitude of their environmental impacts. PLOS Biology 12 (5): 1–11. https://doi.org/10.1371/journal.pbio.1001850

Bumbeer JA, Rocha RM (2016) Invading the natural substrate: a case study in South Brazil. Zoologia 33 (1): 7. https://doi.org/10.1590/S1984-4689zool-20150211

Castro MCT, Scapolatempore, MP, Rangel-Pereira, FS (2023) Biofouling in-water cleaning and scrubbers wash waters, potential sources of marine pollution: the Brazilian case. Environmental Monitoring and Assessment 195 (12): 1551. https://doi.org/10.1007/s10661-023-12123-7

Cunha RL, Nicastro KR, Costa J, McQuaid CD, Serrao EA, Zardi GI (2014). Wider sampling reveals a non-sister relationship for geographically contiguous lineages of a marine mussel. Ecology and Evolution 4(11): 2070-2081. https://doi.org/10.1002/ece3.1033

Egres AG, Martins CC, de Oliveira VM, Lana PC (2012) Effects of an experimental in situ diesel oil spill on the benthic community of unvegetated tidal flats in a subtropical estuary (Paranaguá Bay, Brazil). Mar Pollut Bull 64 (12): 2681–2691. https://doi.org/10.1016/j.marpolbul.2012.10.007

Freire CG, Marafon AT (2018) Invasive mollusc species in Brazilian aquatic ecosystems and their impact on the environment. InterfacEHS 13 (1): 2–16

Gilg MR, Johnson EG, Gobin J, Matthew BB, Ortolaza, A. I. (2013). Population genetics of introduced and native populations of the green mussel, Perna viridis: determining patterns of introduction. Biological Invasions 15: 459-472. https://doi.org/10.1007/s10530-012-0301-2

Gmelin JF (1791) Vermes. In: Gmelin JF (Ed.) Caroli a linnaei systema naturae per regna tria naturae, Lipsiae Ed. 13. 1 (6): 3021-3910.

Gobin J, Agard J, Madera J, Mohammed A (2013) The Asian green mussel *Perna viridis* (Linnaeus 1758): 20 years after its introduction in Trinidad and Tobago. Open Journal of Marine Science 3 (2): 62–65. https://doi.org/10.4236/ojms.2013.32007

Gracia A, Medellín-Mora J, Gil-Agudelo DL, Puentes V (2011) Guía de las especies introducidas marinas y costeras de Colombia. INVEMAR. Ministerio de Ambiente y Desarrollo Sostenible. 64-66.

Gracia A, Rangel-Buitrago N (2020) The invasive species *Perna viridis* (Linnaeus, 1758 - Bivalvia: Mytilidae) on artificial substrates: A baseline assessment for the Colombian Caribbean Sea. Marine Pollution Bulletin 152: 110926, https://doi.org/10.1016/j.marpolbul.2020.110926

Hasegawa M, Kishino H, Yano TA (1985). Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of molecular evolution 22: 160-174. https://doi.org/10.1007/BF02101694

IUCN (International Union for Conservation of Nature) (2021) Invasive alien species and climate change. https://www.iucn.org/resources/issues-brief/invasive-alien-species-and-climate-change. Accessed on: 2023-6-9

Jukes-Browne AJ (1905) A review of the genera of the family Mytilidae. https://www.biodiversitylibrary.org/partpdf/50916. Accessed on: 2023-12-10

Knoppers BA, Brandini FP, Thamm CA (1987) Ecological studies in the Bay of Paranaguá: II - some physical and chemical characteristics. Nerítica 2 (1): 1–36.

Lana PC, Marone E, Lopes RM, Machado EC (2001) The Subtropical Estuarine Complex of Paranaguá Bay, Brazil. In: Seeliger U, Kjerfve B (Eds.) Coastal Marine Ecosystems of Latin America. Springer, Berlin, Heidelberg 131-145. https://doi.org/10.1007/978-3-662-04482-7_11

Lamarck JBM de (1819) Histoire naturelle des animaux sans vertèbres. Paris 6(1): vi, 343 pp.

Latini AO, Resende DC (2016) Capítulo 1 – introdução. In: Latini AO, Resende DC, Pombo VB, Coradin L (Eds.) Série biodiversidade, Espécies exóticas invasoras de águas continentais no Brasil. Ministério do Meio Ambiente, Brasília, Brasil 11–20.

Lenz M, Gama BAP, Gerner NV, Gobin J, Gröner F, Harry A, Jenkins SR, Kraufvelin P, Mummelthei C, Sareyka J, Xavier EA, Wahl M (2011) Non-native marine invertebrates are more tolerant towards environmental stress than taxonomically related native species: results from a globally replicated study. Environmental Research 111 (7): 943–952. https://doi.org/10.1016/j.envres.2011.05.001

Linnaeus C (1758) Systema Naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Ed. 10(1): 824 pp.

Loehle C, Eschenbach W (2012) Historical bird and terrestrial mammal extinction rates and causes. Diversity and Distributions 18(1):84–91. https://doi.org/10.1111/j.1472-4642.2011.00856.x

Madon B, David R, Torralba A, Jung A, Marengo M, Thomas H (2023) A review of biodiversity research in ports: Let's not overlook everyday nature! Ocean & Coastal Management 242: 106623. https://doi.org/10.1016/j.ocecoaman.2023.106623

McQuaid CD, Porri F, Nicastro KR, Zardi GI. (2015). Simple, scale-dependent patterns emerge from very complex effects: an example from the intertidal mussels Mytilus galloprovincialis and Perna perna. Oceanography and Marine Biology – an Annual Review 53: 127–156. doi:10.1201/B18733-5

Menem IR, Serafim AB, Chiarelli JR (2019) A importância do complexo portuário de Paranaguá para a economia graneleira brasileira. Orbis Latina 9(2): 208–222

Messano LVR, Gonçalves JEA, Messano HF, Campos SHC, Coutinho R (2019) First report of the Asian green mussel *Perna viridis* (Linnaeus, 1758) in Rio de Janeiro, Brazil: a new record for the southern Atlantic Ocean. BioInvasions Records 8 (3): 653–660. https://doi.org/10.3391/bir.2019.8.3.22

Meyer CP (2003) Molecular systematics of cowries (Gastropoda: Cypraeidae) and diversification patterns in the tropics. Biological Journal of the Linnean Society 79: 401–459. https://doi.org/10.1046/j.1095-8312.2003.00197.x

Micklem JM, Griffiths CL, Ntuli N, Mwale M (2016) The invasive Asian green mussel *Perna viridis* in South Africa: all that is green is not viridis. African Journal of Marine Science 38 (2): 207–215. https://doi.org/10.2989/1814232X.2016.1180323

MolluscaBase (2024). *Perna viridis* (Linnaeus, 1758). World Register of Marine Species https://www.marinespecies.org/aphia.php?p=taxdetails&id=367822. Accessed on: 2024-04-05

Mantovanelli A, Marone E, Silva ET, Lautert LF, Klingenfuss MS, Prata VP, Angulo RJ. (2004). Combined tidal velocity and duration asymmetries as a determinant of water transport and residual flow in Paranaguá Bay estuary. Estuarine, Coastal and Shelf Science 59(4): 523-537. https://doi.org/10.1016/j.ecss.2003.09.001

Moreira-González AR, Brustolin MC, Mafra LL Jr. (2020) Composition and abundance of benthic microalgae from the Estuarine Complex of Paranaguá Bay (southern Brazil) with special emphasis on toxic species. Ocean and Coastal Research 68: 1–22. https://doi.org/10.1590/S2675-28242020068276

Moura-Britto M, Patrocínio DNM (2005) A fauna de espécies exóticas no Paraná: contexto nacional e situação atual. In: Campos JB, Tossulino MGP, Müller CRC (Eds.) Unidades de conservação: ações para valorização da biodiversidade. Instituto Ambiental do Paraná, Curitiba, Brasil, 53–94.

Neves CS, Rocha RM, Pitombo FB, Roper JJ (2007) Use of artificial substrata by introduced and cryptogenic marine species in Paranaguá Bay, southern Brazil. Biofouling 23 (5): 319-330. https://doi.org/10.1080/08927010701399174

Pierri BDS, Fossari TD, Magalhães ARM (2016) O mexilhão *Perna perna* no Brasil: nativo ou exótico? Arquivo Brasileiro de Medicina Veterinária e Zootecnia 68 (2): 404–414. https://doi.org/10.1590/1678-4162-8534

Pochon X, Bott NJ, Smith KF, Wood SA (2013). Evaluating detection limits of next-generation sequencing for the surveillance and monitoring of international marine pests. PloS one 8(9): e73935. https://doi.org/10.1371/journal.pone.0073935

Portos do Paraná (2023) FAQ Perguntas Frequentes. https://www.portosdoparana.pr.gov.br/Pagina/FAQ-Perguntas-Frequentes. Accessed on: 2023-12-10

Pyšek P, Hulme PE, Simberloff D, Bacher S, Blackburn TM, Carlton JT, Dawson W, Essl F, Foxcroft LC, Genovesi P, Jeschke JM, Kühn I. Liebhold AM, Mandrak NE, Meyerson LA, Pauchard A, Pergl J, Roy HE, Seebens H, van Kleunen M, Vilà M. Wingfield MJ. Richardson DM (2020) Scientists' warning on invasive alien species. Biological Reviews 95: 1511–1534. https://doi.org/10.1111/brv.12627

Rajagopal S, Nair KVK, Van der Velde G, Jenner HA (1997) Seasonal settlement and succession of fouling communities in Kalpakkam, east coast of India. Netherland Journal of Aquatic Ecology 30 (4): 309 –325. https://doi.org/10.1007/BF02085874

Rajagopal S, Venugopalan VP, Van der Velde G, Jenner HA (2006) Greening of the coasts: a review of the *Perna viridis* success story. Aquatic Ecology 40: 273-297. https://doi.org/10.1007/s10452-006-9032-8

Ratnasingham S, Hebert PD (2007). BOLD: The Barcode of Life Data System (http://www. barcodinglife. org). Molecular ecology notes 7(3): 355-364. https://doi.org/10.1111/j.1471-8286.2007.01678.x

Rocha RM, Kremer LP (2005) Introduced ascidians in Paranaguá Bay, Paraná, southern Brazil. Revista Brasileira de Zoologia 22 (4): 1170–1184. https://doi.org/10.1590/S0101-81752005000400052

Santos HS, Bertollo JC, Creed JC (2023) Range extension of the Asian green mussel *Perna viridis* (Linnaeus, 1758) into a Marine Extractive Reserve in Brazil. BioInvasions Records 12 (1): 208–222. https://doi.org/10.3391/bir.2023.12.1.17

Siddall SE (1980) A clarification of the genus *Perna* (Mytilidae). Bulletin of Marine Science 30 (4): 858-870

Simone LRL, Gonçalves EP (2006). Anatomical study on Myoforceps aristatus, an invasive boring bivalve in SE Brazilian coast (Mytilidae). Papéis Avulsos de Zoologia, 46: 57-65. https://doi.org/10.1590/S0031-10492006000600001

Silva EP, Souza RC, Lima TA, Fernandes FC, Macario KD, Netto BM, Alves EQ, Carvalho C, Aguilera O, Duarte MR (2018) Zooarchaeological evidence that the brown mussel (*Perna perna*) is a bioinvader of coastal Brazil. The Holocene 28 (11): 1771–1780. https://doi.org/10.1177/0959683618788670

Siriwardena S (2022) CABI Compendium: *Perna viridis* (Asian green mussel). https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.70090. Accessed on: 2023-6-6

Smith CH, Robertson CR, Randklev CR (2022) DNA barcoding identifies a novel population of the imperiled Trinity Pigtoe, Fusconaia chunii (Lea, 1862) (Bivalvia, Unionidae), in the San Jacinto River drainage in Texas. Check List 18(6): 1255-1262. https://doi.org/10.15560/18.6.1255

Soares MO, Xavier FRL, Dias NM, Silva MQM, Lima JP, Barroso CX, Vieira LM, Paiva SV, Matthews-Cascon H, Bezerra LEA, Oliveira-Filho RR, Salani S, Bandeira EVP (2022) Alien hotspot: benthic marine species introduced in the brazilian semiarid coast. Marine Pollution Bulletin 174: 1–7. https://doi.org/10.1016/j.marpolbul.2021.113250

Soriano JL, Salgado SQ (2019). Primera cita de Leiosolenus aristatus (Dillwyn, 1817)(Bivalvia: Mytilidae) para las costas catalanas. Spira, 7: 93-95.

Stanski G, Boos H, Pinheiro MAA (2022) Animais marinhos exóticos invasores no Sul do Brasil. Revista CEPSUL Biodiversidade e Conservação Marinha 11: 1–11. https://doi.org/10.37002/revistacepsul.vol11.2336e2022002

Teixeira LM, Creed JC (2020) A decade on: an updated assessment of the status of marine non-indigenous species in Brazil. Aquatic Invasions 15 (1): 30–43. https://doi.org/10.3391/ai.2020.15.1.03

Turbelin AJ, Diagne C, Hudgins EJ, Moodley D, Kourantidou M, Novoa A, Haubrock PJ, Bernery C, Gozlan RE, Francis RA, Courchamp F (2022) Introduction pathways of economically costly invasive alien species. Biological Invasions 24 (7): 2061–2079. https://doi.org/10.1007/s10530-022-02796-5

UNESCO (2014) Atlantic Forest South-East Reserves. United Nations Educational, Scientific and Cultural Organization. http://whc.unesco.org/en/list/893. Accessed on: 2023-11-4

Zardi GI, McQuaid CD, Jacinto R, Lourenço CR, Serrão EA, Nicastro, KR (2018). Re-assessing the origins of the invasive mussel Mytilus galloprovincialis in southern Africa. Marine and Freshwater Research, 69(4): 607-613. doi.org/10.1071/MF17132

Wood AR, Apte S, MacAvoy ES, Gardner JP (2007) A molecular phylogeny of the marine mussel genus Perna (Bivalvia: Mytilidae) based on nuclear (ITS1&2) and mitochondrial (COI) DNA sequences. Molecular Phylogenetics and Evolution, 44(2): 685-698. https://doi.org/10.1016/j.ympev.2006.12.019

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