

First record of the Mediterranean mussel *Mytilus galloprovincialis* (Bivalvia, Mytilidae) in Brazil

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Abstract. The genus *Mytilus* comprises a large number of bivalve mollusk species distributed throughout the world and many of these species are considered invasive. In South America, many introductions of species of this genus have already taken place, including reports of hybridization between them. Now, the occurrence of the Mediterranean mussel *Mytilus galloprovincialis* is reported for the first time from the Brazilian coast. Several specimens of this mytilid were found in a shellfish growing areas in Florianópolis and Palhoça, Santa Catarina State, Brazil. Morphological analysis of the shells and molecular analysis through sequencing of the cytochrome oxidase subunit 1 (COI) confirmed the taxonomic identification. The species is known for its great invasive potential and can become a major environmental problem for seafood business and coastal communities, as it can compete and even hybridize with local species.

Key-Words. Mediterranean mussel; *Mytilus galloprovincialis*; Brazilian coast; First record; Santa Catarina.

INTRODUCTION

The Mytilidae *Mytilus galloprovincialis* (Lamarck, 1819) was originally described for the Mediterranean Sea, and later introduced in areas far from its region of origin. In the northern hemisphere, its presence has been confirmed in California (McDonald & Koehn, 1988), Japan (Wilkins *et al.*, 1983), Hong Kong (Lee & Morton, 1985), China and Korea (McDonald *et al.*, 1991). In the southern hemisphere, it occurs in South Africa (Grant & Cherry, 1985) and is widely distributed throughout Australasia (McDonald *et al.*, 1991). Its previous absence in South America was, for a long time, considered intriguing given the long history of trade between this continent and the countries bordering the Mediterranean (Seed, 1992). Later, then, the species began to be cited for South America, in Chile, Argentina and Uruguay (Seed, 1992; Daguin & Borsa, 2000; Astorga *et al.*, 2015;

GISD, 2019). It is a species with high capacity of invasion and with potential for competition and hybridization with native species (Lowe *et al.*, 2000; Branch & Steffani, 2004).

Species of the genus *Mytilus* are usually known as blue mussels. Its species mostly occur in temperate and cold regions of the world, and the different species have similar shells, so much that they are considered subspecies of the European *M. edulis* Linnaeus, 1758 by some authors. The shell similarity precludes easy detection of non-indigenous congener species (Astorga *et al.*, 2015). In a South American example, a region that supposedly must have only the native *M. platensis* d'Orbigny, 1842, other two non-indigenous species were detected, *M. edulis* and *M. galloprovincialis*, in the Strait of Magellan (Oyarzún *et al.*, 2016).

The aim of this work is to report for the first time the occurrence of the exotic species *M. galloprovincialis* in Brazilian coast.

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MATERIAL AND METHODS

Study area and sampling

Individuals of *M. galloprovincialis* (Fig. 1) were found associated with the cultures of the Mytilidae *Perna perna* (Linnaeus, 1758) from September 2016 to September 2018 in South Bay and in North Bay, between Santa Catarina Island (municipality of Florianópolis) and the continent (municipality of Palhoça), state of Santa Catarina, southern region of Brazil (27°40'S, 48°35'W) (Fig. 2). Seeds and adults were observed adhered to the *P. perna* planting structures during the maricultural management. The specimens (n = 50) were collected in six points along the South Bay and one point in the North Bay and they are deposited in the malacological collection of the Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil (MZUSP 143542; MZUSP 143543) and in the malacological collection of the Laboratório de Ecologia Aplicada e Bioinvasões da Universidade Federal do Paraná, Pontal do Paraná, Brazil (LEBIO 543).

Morphological and molecular identification

Shell characters were used for initial identification (Poppe & Goto, 1993; Sanjuan *et al.*, 1994; Innes & Bates, 1999; FAO, 2019) and the molecular analysis was performed through approximately a 650 bp sequence of the cytochrome oxidase subunit 1 (COI). DNA from adductor muscle of three samples was extract using the EZ-DNA kit (Biological Industries). The amplification was carried out in 25 µl PCR with final concentrations of 2.5x buffer, 3 mM of MgCl₂, 0.4 µM of dNTP, 0.1 pmol of each HCO and LCO primer (Folmer *et al.*, 1994), 0.1 U of Taq Polymerase and 50 ng of DNA template. Sequencing was performed with BigDye® kit (Applied Biosystems) according to the manufacturer protocol. The sequencing product was purified with Sephadex G-50 (GE Healthcare) and sequenced with an ABI 3130 automatic sequencer (Applied Biosystems). The sequence was deposited in GenBank under the accession number MN615419.

Molecular identification was performed with three approaches. First, the barcode identification of each individual was performed in the BoldSystems v3 (Ratnasingham & Hebert, 2007). Then, the barcode identification was confirmed by taxa similarity and phylogenetic analysis under Neighbor Joining (NJ) and Bayesian Inference (BI) approaches, respectively. Sequences of *M. galloprovincialis* and of close related species (*i.e.*, *Mytilus californianus* Conrad, 1837, *M. chilensis*, *M. coruscus* Gould, 1861, *M. edulis*, *M. platensis*, *M. trossulus* Gould, 1850) accessed from GenBank (Table 1) were used as reference sequences. Sequences of *P. perna* were used to root the tree. The sequences were aligned using the online tool Guidance2 (Sela *et al.*, 2015) with MAFFT version 7.123b algorithm (Katoh & Standley, 2013). The substitution model (TrN93 + G, Tamura & Nei, 1993) was selected with jModeltest 2.1.10 (Darriba *et al.*, 2012). The NJ tree was constructed with Mega 7.0.26 (Kumar *et al.*, 2016), and its robustness was assessed using 1,000 bootstrap replicates. The BI tree was constructed in Beast 1.8 (Drummond *et al.*, 2012) with three independent runs of 50 million MCMC steps sampled each 5,000 trees (10% of burn-in).

Table 1. Species and the GenBank accession number of the sequences used as reference for the molecular identification of the focal individual.

Species	GenBank accession numbers
<i>M. californianus</i>	KF931702.1, KF931724.1, KF931745.1
<i>M. chilensis</i>	KR066667.1, KR066668.1, KR066669.1, KR066670.1, KR066677.1, KR066678.1, KR066679.1, KR066680.1, KR066687.1, KR066756.1, KR066764.1
<i>M. coruscus</i>	KC139326.1, KF154239.1, KM197995.1, MG214428.1
<i>M. edulis</i>	AF241969.1, AY130053.1, EU915572.1, JF825556.1
<i>M. galloprovincialis</i>	AB076943.1, AF241997.1, AY130054.1, AY130055.1, AY130056.1, AY130057.1, AY130058.1, AY130059.1, AY130060.1, AY497292.2, DQ917605.1, KP052906.1, KP052907.1, KP052908.1, KP052909.1, KP052910.1, KP052911.1, KP052912.1, KP052913.1, KP052914.1, KP052915.1, KP052916.1, KP052917.1, KP052918.1, KP052919.1, KP052920.1, KP052921.1, KP052922.1, KP052923.1, KP052924.1, KP052925.1, KP052926.1, KP052927.1
<i>M. platensis</i>	KY454035.1, KY454034.1
<i>M. trossulus</i>	AY130061.1, KF932117.1, KF932152.1, KF932224.1, KF932255.1, MF544912.1
<i>P. perna</i>	DQ351427.1, DQ351453.1, DQ351475.1

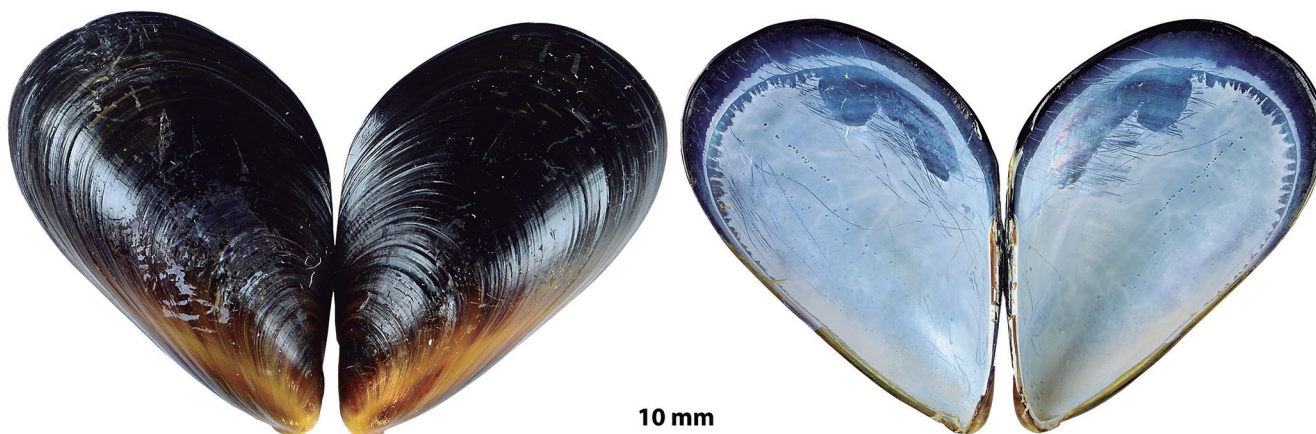


Figure 1. Shells of *Mytilus galloprovincialis*. Lot LEBIO 543. Shell length = 60 mm.

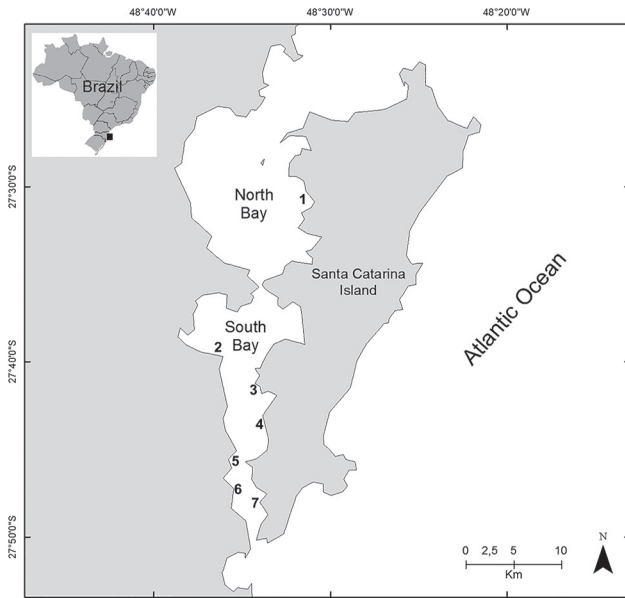


Figure 2. Sites of *Perna perna* planting where *Mytilus galloprovincialis* was found. (1) Santo Antônio de Lisboa, Florianópolis; (2) Ponta do Tomé, Palhoça; (3) Tapera, Florianópolis; (4) Costeira do Ribeirão, Florianópolis; (5) Ponta do Cedro, Palhoça; (6) Pedras Altas, Palhoça; (7) Caieira da Barra do Sul, Florianópolis.

RESULTS

Specimens were found associated to the cultivation of *P. perna* mussels during the management of cleaning and harvesting of bivalve production for commercialization. The first individuals were observed during spring 2016, but it was in spring 2017 that they began to cause concern among farmers, with higher density.

An internal small subumbonal white fold and a very dark color easily differentiate *Mytilus* spp. from the more common and planting cultivated *P. perna*. As the native species *M. platensis* has its northern boundary in Santa Catarina-Paraná region (Rios, 2009), a differentiation with *M. galloprovincialis* was necessary. Normally, *M. platensis* is smaller (up to 30 mm), the valves are more elliptical, with ventral edge straight or convex (Rios, 2009). On the other hand, *M. galloprovincialis* easily reached 40 mm, and usually has a more pointed anterior, umbonal region, with the ventral valves edge concave (FAO, 2019). The length of the individuals collected ranged from 45 mm to 83 mm.

The molecular identification was coincident with the morphological identification of *M. galloprovincialis*. The

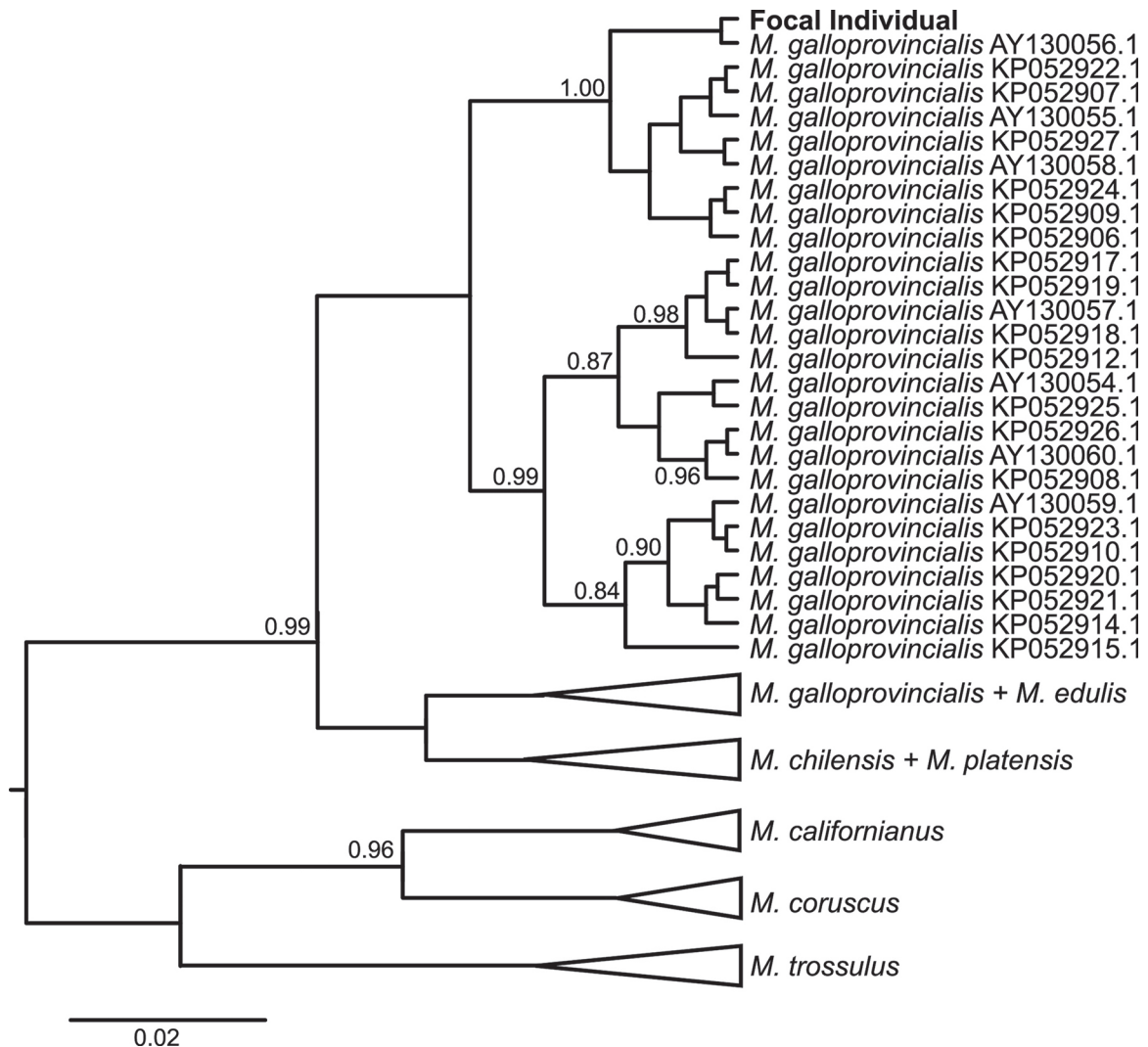


Figure 3. Genetic identification of the focal individual by phylogenetic analysis under Bayesian Inference (BI) based on COI mitochondrial gene. Only posterior probabilities higher than 0.75 are shown. Branch lengths represent units of substitutions per site.

Brazilian samples presented between 99.5 and 97.6% of similarity with sequences of *M. galloprovincialis* available in the BoldSystems v3 database. Similarly, the BI (Fig. 3) and NJ (Fig. 4) trees assigned the Brazilian sample in the monophyletic groups of *M. galloprovincialis* with high branch support. These results indicate the occurrence of this invasive species in Brazilian coast.

In consecutive observations made during the spring of 2018, out of a total of 26 mussel ropes with an individual average weight of 15 kg, occupying approximately 13 linear meters of cultivation, the average total weight was 366 kg of *P. perna* clean and ready for sale and 8.8 kg of *M. galloprovincialis*, or 2.4% of current production.

DISCUSSION

The interaction between *P. perna* and *M. galloprovincialis* has already been reported to the African coast, where the two species live together (Bownes & McQuaid, 2006). However, hybridization cases such as those that occur on the Chilean coast can cause impact to other species (Valenzuela et al., 2016), as is the case in Venezuela with the introduction of another Mytilidae species [*Perna viridis* (Linnaeus, 1758)] in the early 1990s, and which

has been competing strongly with the most abundant Mytilidae in the region (*P. perna*) (Rylander et al., 1996). Recently *P. viridis* was also introduced in Guanabara Bay, Rio de Janeiro, Brazil (Messano et al., 2019), which makes this scenario of frequent introductions of alien species and possible hybridizations increasingly worrying.

The introduction vector of *M. galloprovincialis* in Brazil is unknown, but Castro et al. (2017) indicate that ballast water, biofouling and aquaculture are the most common vectors of species introduction and propagation in the Atlantic Ocean.

The Santa Catarina region is today the largest producer of edible mollusks in Brazil, with 95% of all production in the country (Suplicy et al., 2017). The results of this work show a 2.4% proportion of *M. galloprovincialis* in the cultivated areas studied. This is already a major economic impact because it reduces *P. perna* production. However, further studies should be conducted to identify other possible impacts, for only time will show whether this new introduction will become more of a cultivation possibility or more of a competitor of space and food with local species, compromising the production of *P. perna*.

In the last two centuries there has been an unprecedented increase in human influence on species exchange, leading to a homogenization of flora and fauna

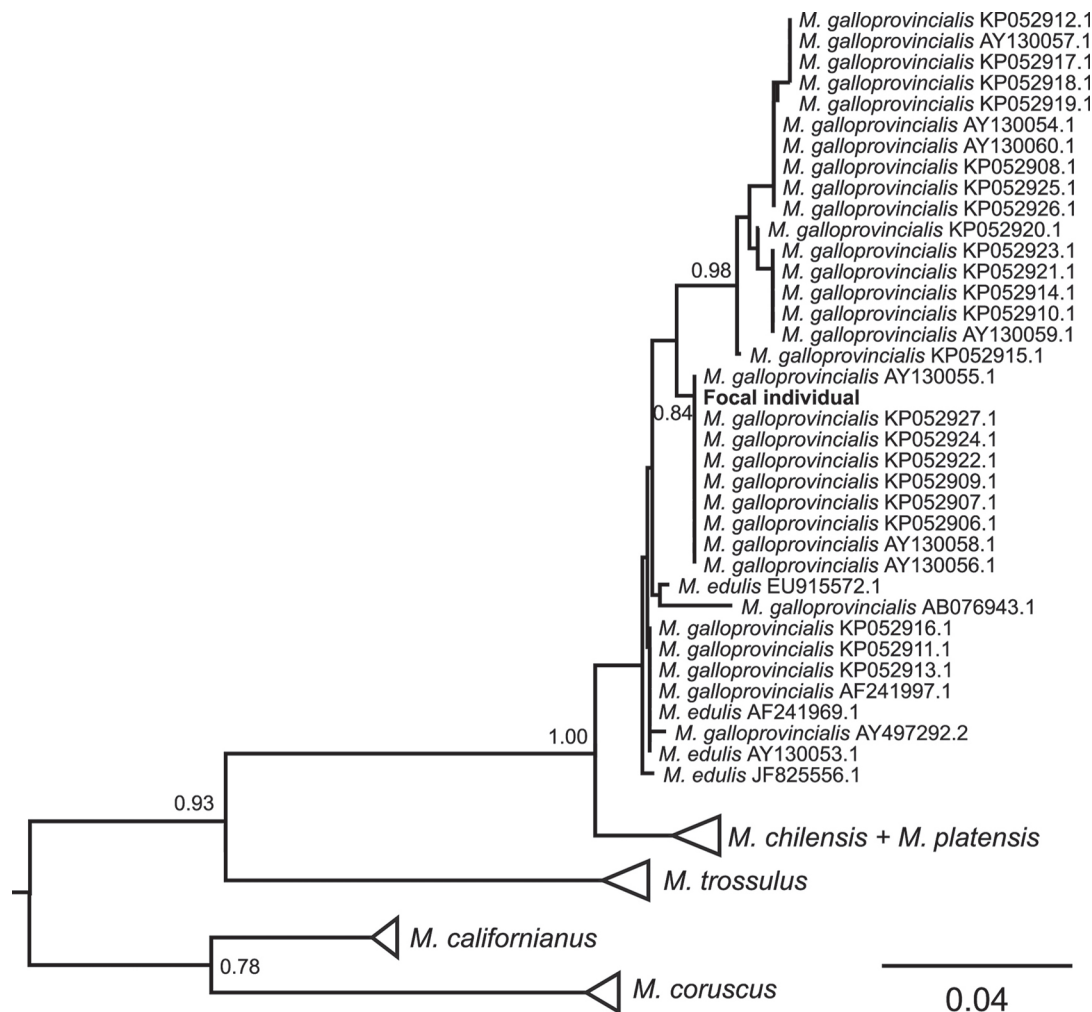


Figure 4. Genetic identification of the focal individual by similarity analysis under Neighbor-Joining (NJ) based on COI mitochondrial gene. Only bootstraps higher than 0.75 are shown. Branch lengths represent units of distance.

and redefining species occurrence limits, affecting ecosystem functioning, human and animal health and economy (Seebens *et al.*, 2017). This is particularly challenging in a megabiodiverse country like Brazil.

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