



***Limnoperna fortunei* - Updating the geographic distribution in the Brazilian watersheds and mapping the regional occurrence in the Upper Uruguay River basin**

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Abstract: *Limnoperna fortunei* is an invasive alien species (IAS) that cause serious ecological and economic problems in Brazilian freshwater environments. Due to its high dispersion capacity and the lack of new records in peer-reviewed journals we carried out an extensive survey to update the distribution of *L. fortunei* in the Brazilian hydrographic basins. We also performed a detailed investigation of its distribution in the Upper Uruguay River basin using a molecular method. We presented new records, showing the invasion in new basins and a wide distribution in the basins previously infested. Additionally, we confirmed that the Upper Uruguay River is fully colonized by the golden mussel, being distributed in the lentic, lotic, and transitional lotic/lentic environments presented in this region. This update is an important tool for the implementation of guidelines and the development of safety protocols and sanitary barriers to avoid the dispersion of this IAS to new environments..

Keywords: Biological invasion; dispersion; freshwater; golden mussel; bivalves.

***Limnoperna fortunei* - Atualização da distribuição geográfica nas bacias hidrográficas brasileiras e mapeamento da ocorrência regional na bacia do Alto Rio Uruguai**

Resumo: *Limnoperna fortunei* é uma espécie exótica invasora que causa sérios problemas ecológicos e econômicos em ambientes de água doce do Brasil. Devido à sua elevada capacidade de dispersão e à falta de novos registros em publicações científicas, o objetivo deste estudo foi realizar uma extensa pesquisa para entender e alertar sobre o atual cenário de distribuição de *L. fortunei* nas bacias hidrográficas brasileiras. Também realizamos uma investigação mais detalhada sobre a distribuição da espécie na bacia do Alto Rio Uruguai, utilizando um método molecular. Apresentamos novos registros de ocorrência da espécie, mostrando a invasão em novas bacias e uma ampla distribuição nas bacias anteriormente infestadas. Além disso, confirmamos que o Alto Rio Uruguai está totalmente colonizado pelo mexilhão-dourado, estando distribuído pelos ambientes lênticos, lóticos e de transição existentes na região. Esta atualização se mostra como uma importante ferramenta para a implementação de diretrizes e o desenvolvimento de protocolos de segurança e barreiras sanitárias para evitar a dispersão desta espécie invasora em novos ambientes.

Palavras-chave: Invasões biológicas; dispersão; água doce; mexilhão-dourado; bivalves.

Introduction

Limnoperna fortunei, known as golden mussel, is a freshwater bivalve considered an invasive alien species (IAS) in South America with great potential for dispersion that causes substantial impacts on community structure and ecosystems function, and also generate substantial economic damages (Darrigran et al. 2020).

IAS are defined as species living outside of their natural geographical range due to human actions that can maintain a self-sustainable population and cause environmental or socio-economic impact (Turbelin et al. 2017). The dispersion of the IAS increased mainly due to rapid technological advances and globalization observed during the last decades (Karatayev et al. 2007, Darrigran et al. 2020). These biological invasions accelerate biodiversity loss, and compromise the supporting, provisioning, regulating, and cultural services (Vilà & Hulme 2017).

Native from China and Southeast Asia, *L. fortunei* is currently dispersed in Hong Kong, Taiwan, Japan, and South America, where it was first registered in 1991 in the Río de La Plata estuary (Pastorino et al. 1993). Since then, it has spread throughout the continent (Argentina, Bolivia, Paraguay, Uruguay, and Brazil) (Fusaro et al. 2020).

The *L. fortunei* dispersion success is mainly related to some biological attributes of the species, such as free-living planktonic larvae, sessile byssate adult (Boltovskoy 2015), and high fecundity (Callil et al. 2012). Besides, the golden mussel can survive in extreme environmental conditions and highly polluted waters (Karatayev et al. 2007), tolerate long exposures to air (Andrade et al. 2020), low calcium concentrations, and wide water temperature variations (Karatayev et al. 2007).

In many Brazilian rivers, heavy traffic of boats, mainly for fishing, occurs with no control measures against golden mussel spread (i.e., not preventing the spread associated with hull fouling, live fishing baits, or even water inside the boat and engine). Also, ships with ballast water navigate in some Brazilian watersheds. Hence, we hypothesize that the spread of the *L. fortunei* is much more extensive than it is known.

The combination of golden mussel high dispersion capacity with the lack of records in peer-reviewed journals showing new occurrences of golden mussels in Brazilian watersheds motivated us to update *L. fortunei* distribution in the Brazilian territory.

Therefore, the objective of the present study was to update the scenario of distribution of *L. fortunei* in Brazilian hydrographic basins through bibliographic survey and by point data. We also intend to present a more comprehensive investigation of the golden mussel distribution in the Upper Uruguay River basin, giving particular attention to tributaries, transitional stretches, bays, and the main body of all five hydroelectric power plants reservoirs located in this area.

Material and Methods

To update the distribution of the golden mussel among the Brazilian river basins, we performed an extensive survey of new publications in Medline, Web of Science, Scopus, ERIC, CSA, Biological Abstracts, Scielo, and Google Scholar databases, searching for scientific papers, books, thesis, and abstracts, published between 2016 and 2020, which have the terms “*Limnoperna fortunei* AND Brazil” or “*Limnoperna fortunei* AND Brasil”. This search updates the data published by Hydroelectric Invasive Species Bioengineering Center (CBEIH), which presents golden mussel distribution until 2016 (CBEIH 2020). Approximately 250 publications were found in the survey, but only 60 of them addressed *L. fortunei* samplings.

Also, we create a network of professionals associated to the hydroelectric power plants (HPP) located in different Brazilian watersheds, that includes generation and environment staffs, engineers, consultants, and researchers, to report the presence of the golden mussel in each HPP and, when known, the year of the invasion.

The HPP studied were selected by hydrographic basin, excluding those with registered presence of the golden mussel, and selecting those positioned in the upper, middle or lower stretch of the rivers, for which no presence was cited in the literature in the analyzed period.

A total of 66 HPP was selected, distributed in the Southeast Atlantic Basin ($n=13$; Aimorés, Baguari, Candonga, Guilman Amorim, Ilha dos Pombos, Mascarenhas, Nilo Peçanha, Paraibuna, Pereira Passos, Porto Estrela, Sá Carvalho, Salto Grande, Simplicio), in the Parana River Basin ($n=11$; Amador Aguiar I and II, Camargos, Funil, Itumbiara, Itutinga, Jaguará, Miranda, Nova Ponte, Piraju, Salto Santiago), in the Amazon Basin ($n=11$; Balbina, Belo Monte, Cachoeira Caldeirão, Colíder, Curuá-Una, Dardanelos, Jirau, Santo Antônio, São Manoel, Sinop, Teles Pires), in the San Francisco River Basin ($n=10$; Itaparica, Moxotó, Paulo Afonso I, II, III and IV, Queimado, Retiro Baixo, Três Marias, Xingó), in the Araguaia-Tocantins River Basin ($n=7$; Cana Brava, Estreito, Lajeado, Peixe Angical, São Salvador, Serra da Mesa, Tucuruí), in the South Atlantic Basin ($n=6$; 14 de Julho, Castro Alves, Itaúba, Jacuí, Monte Claro, Passo Real), in the Paraguay River Basin ($n=3$; Jauru, Manso, Ponte de Pedra), in the East Atlantic Basin ($n=3$; Irapé, Itapebi, Santa Clara), in the Parnaíba River Basin ($n=1$; Boa Esperança), and in the Uruguay River Basin ($n=1$; Passo Fundo).

In September 2018, we also performed a field survey in Campos Novos, Barra Grande, Machadinho, Itá, and Foz do Chapecó reservoirs, all located in the Upper Uruguay River basin, to evaluate the presence of the golden mussel through a molecular method.

In each reservoir, we investigated ten sites distributed in the main reservoir body and in the surrounding areas, which include tributaries, transitional stretches, and bays, totalizing 50 sampling sites. We filtered water from these sites in a plankton net (53µm), collected just below the surface with a motor pump (Tschá et al. 2012). Two replicates, separated by a distance of approximately 30 m, were performed at each site, and the 200 l filtered in each replicate were mixed in one bottle (400 l of filtered water). Samples were fixed with 95% ethanol (1:4 water:ethanol proportion) and kept on ice until arriving at the laboratory, in which the storage was made at -20°C until processing.

Plankton samples were filtered again in a 100-micron nylon mesh to remove large particles that could impair DNA extraction and subsequently filtered in smooth membranes (0.22 µm) using a vacuum pump. Total DNA was extracted with the PureLink™ Microbiome DNA Purification Kit (Invitrogen™). The DNA was quantified in NanoDrop Lite (Thermo Scientific) and standardized at a concentration of 10 ng/µl using ultra-pure water.

Polymerase Chain Reactions (PCR) were adapted from Pie et al. (2006) and Boeger et al. (2007), using universal 18S-1100R (5'-GATCGTCTTTCGAACCTCTG-3') and 18S-7F (5'-GATCGTCTTTCGAACCTCTG-3'), and specific primers Limno-COIR1 (5'-TCCAACCAGTCCCTACTCCACCCTCTA-3') and Limno-COIF1 (5'-TTTAGAGTTAGCACGTCCTGGTAGGTT-3').

We carried out the PCR in 25 µL mixes containing 1.5 mM of MgCl₂, 0.5 mM of dNTPs, 1X reaction buffer, 1U of Taq platinum (Invitrogen), 2.0 mM of each specific primers (Limno-COIR1 and

Limno-COIF1), 0.2 mM of each universal primers (18S), 0.5 ng/μL of BSA and 0.8 ng/μL of DNA. Standard cycling parameters were adapted from Pie et al. (2006), with initial denaturation at 94 °C for 4 min, followed by 35 cycles of 94 °C for 30 s, annealing temperature of 59 °C for 30 s, extension at 72 °C for 60 s, followed by a final elongation step at 70 °C for 3 min. We visualized the PCR products on 1.5% agarose. All negative PCR amplifications were tested twice to confirm the absence of amplification.

Results

We identified three new records of *L. fortunei* in the 60 publications available between 2016 and 2020, one of them in a new invaded basin (Online Resource 1). This record was published in 2019 and referred to 10 specimens of *L. fortunei* collected in October 2010 in the bay-estuary complex of Santos, São Vicente, and Bertioga Channel, in the Southeast Atlantic Basin (Senske et al. 2019). The other records refer to basins where *L. fortunei* was already present, such as the San Francisco River Basin, were

L. fortunei was collected in the lower stretch of the river (Melo 2018), and the Paraná River Basin, in the Salto Santiago HPP (Borges et al. 2017).

From our network, we had feedback from 44 collaborators, 15 of them notifying new records of *L. fortunei* and 29 indicating the absence of the invasive species (Online Resource 2). Additionally, three new records from not selected plants were also notified, expanding the network to 69 HPP and 18 new records.

Two other new records were registered in a river stretch with no HPP, both in the north and east channels of the water transposition system of the San Francisco River, which enabled the invasion of *L. fortunei* in the Eastern Northeast Atlantic Basin.

These 23 new records (3 from publications and 20 from collaborators) were plotted in Figure 1, complementing the information related to golden mussel distribution in Brazilian watersheds previously available (CBEIH 2020).

The PCR analysis showed bands in 42 of the 50 collected samples, which confirmed the presence of *L. fortunei* in the plankton and revealed that it is widely distributed in the five reservoirs of the Upper Uruguay River (Figure 2).



Figure 1. Distribution of the golden mussel *Limnoperna fortunei* in South America, updating the distribution in the Brazilian watershed. Dots represent the presence of *L. fortunei*. Data until 2016 are from CBEIH (2020). Double strokes represent the Hydroelectric Power Plants selected. SFRIP means São Francisco River Integration Project

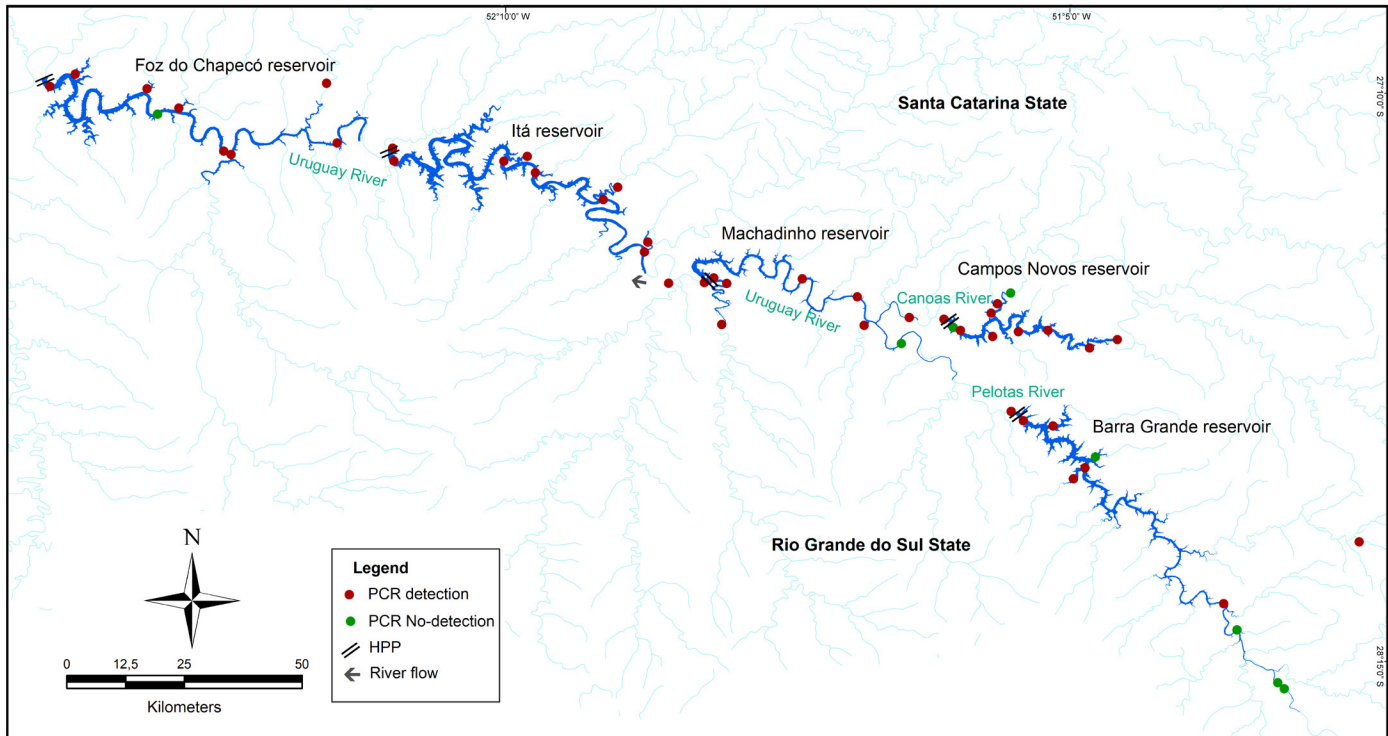


Figure 2. Distribution of the golden mussel *Limnoperna fortunei* in the Upper Uruguay River Basin. Red dots: presence of *L. fortunei*. Green dots: absence of *L. fortunei*

Discussion

Brazil has twelve hydrographic basins, and in five of which (Uruguay, South Atlantic, Paraná, Paraguay, and San Francisco) the invasion by *L. fortunei* had already been reported in the CBEIH mapping 2016 (CBEIH 2020).

The present survey identified two new watersheds invaded by the species: the Southeast Atlantic Basin and the Eastern Northeast Atlantic Basin. Thus, only five Brazilian hydrographic basins (Eastern Atlantic Basin, Araguaia-Tocantins, Parnaíba, Amazon, and Western North Atlantic) are still free of this invasive species.

The new records here identified showed that in the last four years (2016-2020), *L. fortunei* was more widely distributed in basins previously infested, in some cases reaching their upper stretches and in others dispersing downstream with the current.

The biological traits of the golden mussel allow a wide dispersion in the environment invaded by the species (Boltovskoy 2015, Giglio et al. 2016), and are remarkably efficient in downstream colonization. The presence of a planktonic larval phase in the life cycle of the species, which can last from 10 to 20 days depending on the water temperature (Cataldo et al. 2005), permits the dispersion over long stretches in rivers with currents.

However, as highlighted by Boltovskoy (2015), the natural geographic barriers end up being insurmountable for the natural dispersion of the golden mussel. Therefore, human activity acts as the primary vector of the species dispersion between basins (Boltovskoy 2015), either through the transit of boats (commercial or recreational), aquaculture (Oliveira et al. 2015) or even through the construction of water transposition systems between basins. In this way, human action ends up being responsible for taking the species to new watersheds and enabling the invasion of the species upstream in the watersheds it is already present (known as the dispersion jumps) (Oliveira et al. 2015).

The invasion of *L. fortunei* in the São Francisco River basin was recorded in 2015 by Barbosa et al. (2016) in the middle stretch of the basin in Sobradinho HPP, and also in the inlet of the north axis of the irrigation channels (near the municipality of Cabrobó, PE) of the São Francisco River Integration Project (SFRIP). In the next year (2016) the species dispersed to other reservoirs located in this region (Itaparica, Moxotó, Paulo Afonso I, II, III, IV, and Xingó), and around 2018 through the two channels of the transposition system (north and east), reaching a new hydrographic basin, the Eastern Northeast Atlantic Basin. As a result, golden mussel dispersion throughout the northeastern region of the country seems to be a matter of time, considering that the SFRIP irrigation channels reach four states in this region (Pernambuco, Paraíba, Rio Grande do Norte, and Ceará).

Despite this, the upper stretch of the São Francisco basin remains free from *L. fortunei* invasion, as confirmed by the absence of golden mussel records above the Três Marias dam, even with its proximity to environments with a high abundance of golden mussel for several years, such as the Grande and Paraíba rivers (Parana River Basin). In these rivers, an intense sport and commercial fishing activity and possible transit of boats between basins are registered (Oliveira et al. 2015).

The sampling carried out in the Upper Uruguay River region in 2018 confirmed the prognosis presented by Oliveira et al. (2015), who suggested, from some records and personal observations, that the golden mussel would fully colonize the Uruguay River in a few years. The species is now present in more than 80% of the sampled environments in the Uruguay River, spread in lentic, lotic, transitional lotic/lentic environments and in tributaries of that river. This is possibly the dispersion condition of golden mussels in places where the species is already registered.

In general, we can say that the lack of connectivity between the basins and the low navigability in many river stretches has helped in decelerating the dispersion of *L. fortunei* within the Brazilian territory. In rivers that are

barely navigable or do not have commercial navigation, the golden mussel ends up not dispersing as intensely. Pessotto & Nogueira (2018) did not find larvae of this species in the upper stretches of the Grande and Paranaíba rivers in samples carried out in 2010, although low larval densities were already recorded in the lower stretches of these rivers in 2006 (Campos et al. 2012).

Based on the updated map of areas invaded by *L. fortunei*, managers can concentrate efforts to implement safety protocols or sanitary barriers to avoid the dispersion of the golden mussel to new areas. Aside from the need to monitor the dispersion of the golden mussel, through the standardization of a monitoring protocol, it is essential to implement sanitary controls and authorities inspections between basins, because only them can prevent the last five Brazilian basins from being colonized by this invasive alien species.

Supplementary Material

The following online material is available for this article:

Online Resource 1 - List of the 60 publications found between 2016 and 2020 addressing *L. fortunei* samplings (new records are in bold).

Online Resource 2 - Summary of the information obtained from the network of collaborators, indicating the presence or absence of the golden mussel and year of the observation. (NI means ‘Not Informed’; NA means that the contact was ‘Not Answered’).

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Author Contributions

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Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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