





Distribution of invasive bivalves in Paraguay: filling the gaps in the heart of South America

Distribuição de bivalves invasores no Paraguai: preenchendo as lacunas no coração da América do Sul

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Cite as: Miyahira, I.C. et al. Distribution of invasive bivalves in Paraguay: filling the gaps in the heart of South America. *Acta Limnologica Brasiliensia*, 2024, vol. 36, e2

Abstract: La Plata Basin is the second largest hydrographic system in South America but the most impacted by invasive species of molluscs in the continent. Paraguay is a land-surrounded South American country with scarce information about malacological fauna. To fill some of these gaps, a large field expedition was conducted to analyze the distribution of invasive freshwater bivalves in Paraguay. The field survey encompassed 41 sampling stations in seven Departments of Paraguay. The mussels were searched using bare hands and feet. All stations were georeferenced and some environmental parameters were measured. Results indicated the presence of three invasive species of freshwater bivalves in Paraguay: *Corbicula fluminea*, *C. largillierti*, and *Limnoperna fortunei*. The Asiatic clam *Corbicula fluminea* was the most widespread invasive species, covering main rivers and small tributaries in the four freshwater ecoregions of Paraguay. The two remaining invasive species were more restricted. *Corbicula largillierti* were found at the lower section of the Paraná River, and *Limnoperna fortunei* inhabits the main river canals of the Paraguay River and the lower section of the Paraná River. The invasive species were found in three protected areas of Paraguay and can represent a threat to the mostly unknown fauna of native mussels. The invasive bivalves are widespread in Paraguay. Mitigation measurements must be taken to avoid the spread of these species and more drawbacks over native fauna.

Keywords: South America; La Plata Basin; Paraguay; *Limnoperna*; *Corbicula*.



Resumo: A bacia do Plata é a segunda maior da América do Sul, mas a mais impactada pelas espécies invasoras de moluscos nesse continente. O Paraguai é uma país cercado por terra com pouca informação sobre a sua malacofauna. Com o objetivo de preencher algumas destas lacunas, uma expedição de campo foi organizada a fim de analisar a distribuição dos bivalves invasores no Paraguai. Foram realizadas 41 estações de coleta em sete departamentos do Paraguai. A busca pelos bivalves foi feita utilizando pés e mãos. Todos os pontos foram georreferenciados e algumas variáveis ambientais foram medidas. Os resultados indicam a presença de três espécies de bivalves invasores no Paraguai: *Corbicula fluminea*, *C. largillierti* e *Limnoperna fortunei*. O berbigão asiático *Corbicula fluminea* foi a espécie com a distribuição mais ampla, cobrindo os principais rios e tributários, em quatro ecoregiões de água doce do Paraguai. As outras duas espécies apresentaram uma distribuição mais restrita. *Corbicula largillierti* foi encontrado no Baixo rio Paraná, enquanto *Limnoperna fortunei* foi encontrado na calha principal do rio Paraguai e no Baixo rio Paraná. As espécies invasoras foram encontradas em três unidades de conservação e pode representar a ameaça para a pouco conhecida fauna de bivalves nativos do Paraguai. As espécies de bivalves invasores estão amplamente distribuídas no Paraguai. Medidas de mitigação são necessárias para evitar que estas espécies se dispersem ainda mais, provocando mais efeitos negativos sobre as espécies nativas.

Palavras-chave: América do Sul; Bacia do Río de La Plata; Paraguai; *Limnoperna*; *Corbicula*.

Paraguay is a landlocked country with a central position in South America, and two main rivers of the La Plata Basin run through the country: Paraná and Paraguay rivers. This is the second-largest basin in South America and harbors an important molluscan fauna (Strong et al., 2008; Pereira et al., 2014; Cuzzo et al., 2020). A total of 16 freshwater species of molluscs were reported as non-native from South America by Darrigran et al. (2020). These authors reported the presence of seven non-native species in the La Plata Basin, from which were highlighted *Corbicula fluminea* (Müller, 1774), the Asiatic clam; and *Limnoperna fortunei* (Dunker, 1857), the golden mussel. *Corbicula* species arrived in South America in the 1970s and *Limnoperna fortunei* some years later (around the 1990s), both are widespread now (Darrigran et al., 2020; Miyahira et al., 2020; Hermes-Silva et al., 2021). Invasive species can promote modification of environmental and biological aspects of the invaded habitat (e.g., Darrigran & Damborenea, 2011; Sousa et al., 2014; Boltovskoy & Correa, 2015), affecting not only native molluscs, but also other taxonomic groups. Understanding the distribution of invasive species is the first step to proposing strategies to mitigate the negative impacts.

Main reports of molluscs from Paraguay were done several years ago by Schade (1965) and Quintana (1982) and were before the arrival of invasive species in the country. The Paraguayan territory is a gap in the malacological information of South America. *Corbicula* spp. and *L. fortunei* were first recorded in the Paraguay River during the 1990s (Callil & Mansur, 2002; Oliveira et al., 2006), and there are few records of invasive bivalves

in the country, most of them from areas shared with Argentina or Brazil (Peso et al., 2011; Oliveira et al., 2015; Hermes-Silva et al., 2021). A comprehensive revision of the golden mussel invasion in La Plata Basin was done recently by Oliveira et al. (2015) and Ludwig et al. (2021), however, with scarce records from Paraguay and several occurrences upstream (Brazil) and downstream (Brazil, Argentina and Uruguay). Thus, there are records upstream and downstream to Paraguay, but any in Paraguayan area. Moreover, the understanding of the invasion process of bivalves in the La Plata Basin was probably biased due this lack of information.

The main goal of this study was to present an updated distribution of invasive bivalves in Paraguay, relying on new records and museum data, relating this to freshwater and terrestrial ecoregions, as also presenting physical and environmental variables (pH, conductivity, TDS). The occurrence of invasive species in protected areas are discussed, and comments on the possible impacts of invasive on native species.

The records of invasive bivalves came from a recent field expedition conducted in Paraguay (September 2021). The field surveys encompassed 41 collecting stations, in seven Departments (Concepción, Guairá, Misiones, Ñeembucú, Paraguari, Presidente Hayes and San Pedro) and the Capital District (Asunción), where bivalves were searched using bare hands and feet. Almost all surveyed sites were never sampled before for freshwater mussels. At all collecting stations, georeferenced coordinates were obtained using a GPS Garmin 64s; and conductivity, pH and TDS (Total Dissolved Solids) using a Hanna HI98129

Multiparameter. Following Brasil (1997), the Paraguay River can be divided into three parts, Upper Paraguay from the headwaters to Jauru River; Middle Paraguay from Cáceres (state of Mato Grosso, Brazil) to Apa River (border of Brazil and Paraguay); and Lower Paraguay from Apa River to the mouth at Paraná River (border of Paraguay and Argentina). All collecting stations at Paraguay River Basin were carried out at the lower part of the basin but covered an important portion of Paraguay, including tributaries from the western and eastern regions of the country. There is scarce information about native freshwater mussels of Paraguay, but based in this piece of information, the sampled area can be pointed as a high diversity area (Schade, 1965; Quintana, 1982). The data from field surveys were complemented with a review from the collection of the Museo Nacional de Historia Natural del Paraguay.

The study was carried out with the authorization of the Ministerio del Ambiente y Desarrollo Sostenible of Paraguay (Permission number 028/2021) and all the specimens obtained were deposited at the Invertebrates Collection of the Museo Nacional de Historia Natural del Paraguay under field trip code PY-21-01 to PY-21-40.

Paraguay is divided into four freshwater ecoregions (Abell et al., 2008) and these were used for analyzing the invasive species distribution. The Paraguay River divides the country on a north-south axis, at the West, one large ecoregion (Chaco) that drain a huge dry area, and in the East, a more humid area with perennial rivers (Paraguay, Lower Paraná and Upper Paraná ecoregions) (Abell et al., 2008;

Koerber et al., 2017). We also analyzed the distribution of bivalves in terrestrial ecoregions (Olson et al., 2001), which retain some information about the nearby environment. There are five main terrestrial ecoregions in Paraguay: Cerrado, Dry Chaco, Upper Paraná Atlantic Forest, Pantanal and Humid Chaco. There are also small portions of the Paraná flooded savanna and Southern Cone Mesopotamian savanna that was considered along with the surrounding Humid Chaco in our analysis. The terrestrial ecoregions were also evaluated because add different division of Paraguayan territory that bring environmental information that cannot be perceived at freshwater ecoregions analysis. The georeferenced data of protected areas in Paraguay were obtained from World Database on Protected Areas (WDPA, 2021). The percentage of invasive species occurrences was calculated with a 1km buffer around each occurrence to discount any inaccuracy in the GPS measurements.

Three non-native invasive bivalves species were found in Paraguay, two species of Asiatic clams, *Corbicula fluminea* and *C. largillierti*; and the golden mussel, *Limnoperna fortunei* (Figure 1). The invasive species occurred in 33 localities (21 from field survey and 12 from museum collection), and in 20 localities no invasive bivalves were found (Figure 2A-C). Only *C. fluminea* was found at the museum survey, the other two species were found at field survey.

Corbicula fluminea was the species with wider distribution, occurring in 33 localities and all four freshwater ecoregions of Paraguay (Chaco, Paraguay, Upper Paraná and Lower Paraná).

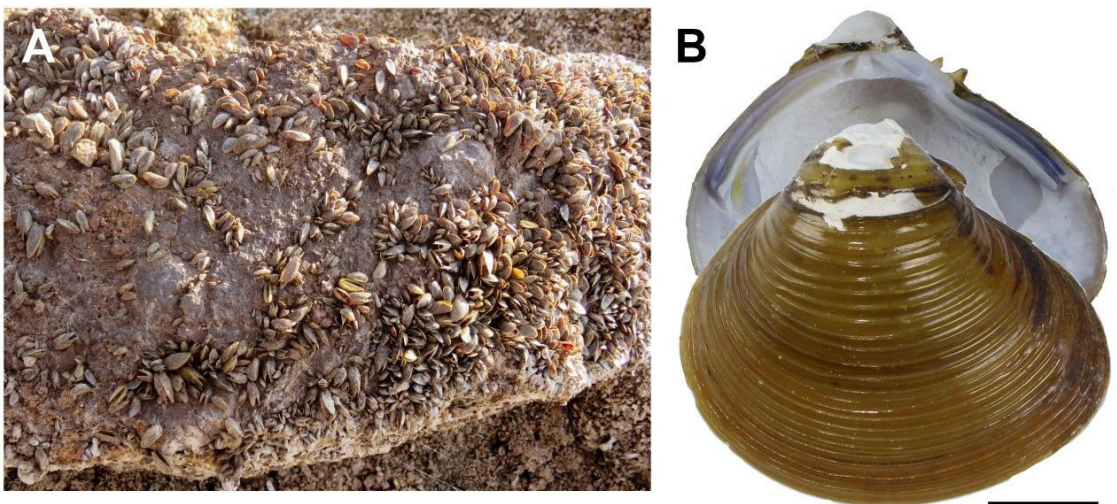


Figure 1 . Invasive bivalves in Paraguay. A – Clusters of *Limnoperna fortunei* in an exposed rock. B – Specimen of *Corbicula fluminea* collected in Paraguay. Scale bar = 1cm.

The other Asiatic clam, *C. largillierti*, occurred in one locality in the Lower Paraná ecoregion. *Limnoperna fortunei* occurred in six localities in three freshwater ecoregions (Chaco, Lower Paraná and Paraguay) (Figure 2A). The invasive bivalves occurred together six times, always with the combination of *Corbicula fluminea* and *Limnoperna fortunei* in sympatry, except for one locality where the three invasive bivalves occurred together (Table 1).

Considering terrestrial ecoregions of Paraguay, there were 31 records of invasive bivalves at Humid Chaco (24 of *Corbicula fluminea*, one of *C. largillierti* and six of *Limnoperna fortunei*), eight at Upper Paraná Atlantic forests (*C. fluminea*) and one at Cerrado (*C. fluminea*). The field survey did not include areas of Pantanal and Dry Chaco ecoregions, however, we revised local museum specimens and found no records from these

regions at Museo Nacional de Historia Natural de Paraguay (Figure 2B).

Invasive bivalves occurred in three protected areas of Paraguay. In the protected area Carrizales del Paraná National Park the three invasive bivalves (*Corbicula fluminea*, *C. largillierti* and *Limnoperna fortunei*) were recorded in two sites (south of Paraguay; coordinates in Table 1); in the Ecological Reserve Banco San Miguel y Bahía de Asunción (on the banks of Paraguay River in Asunción; -25.271149, -57.618595), and in the Managed Resources Reserve Lago Ypacaraí y el sistema de humedales adyacentes were found the species *C. fluminea* in two sites (near Asunción; -25.153036, -57.382539 and -25.252658, -57.341042) (Figure 2C).

Corbicula fluminea was more flexible in environmental preferences, occurring in waters with a lower pH, and also with higher conductivity and TDS (Table 2).

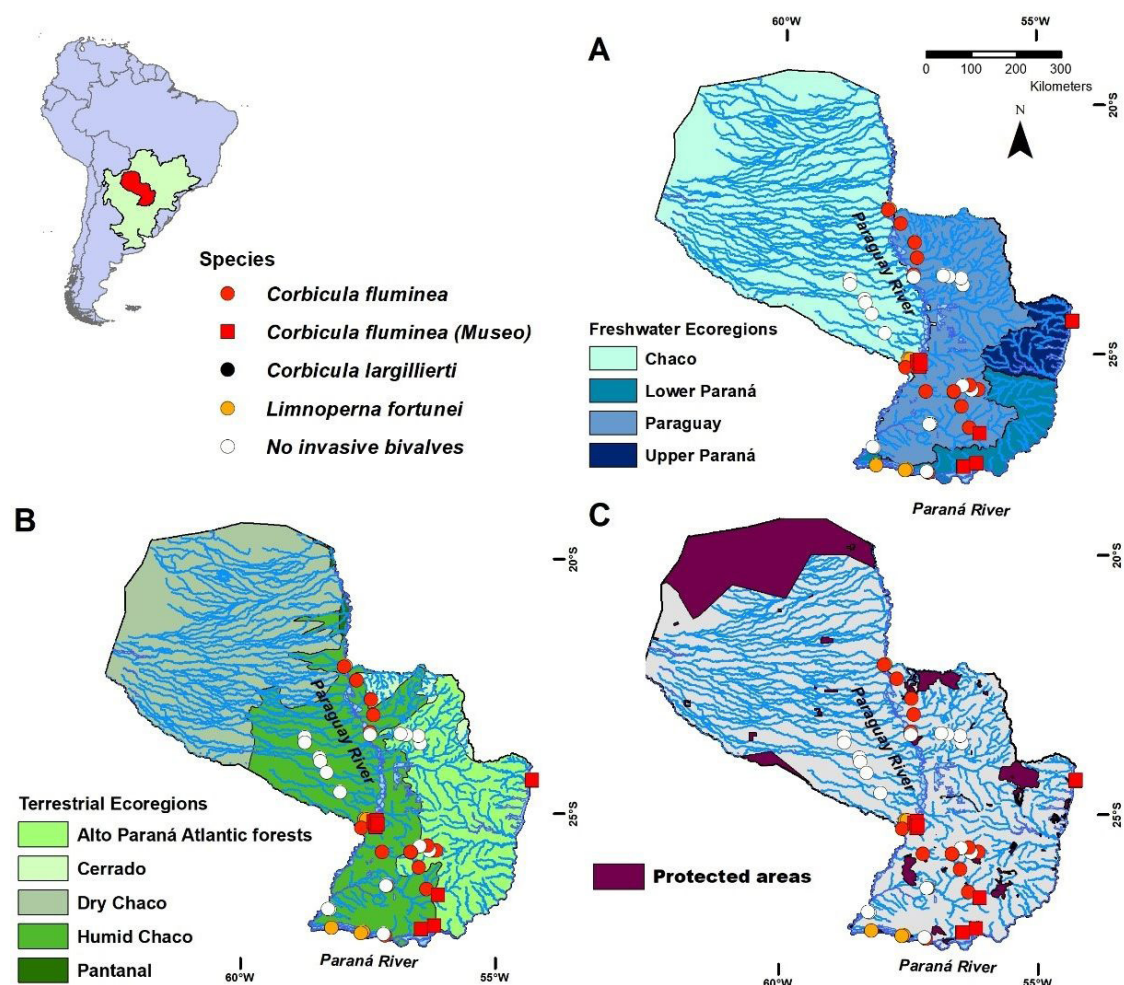


Figure 2. Distribution of invasive bivalves in Paraguay. The circles are the records from sampled sites and the squares the records from the Museo Nacional de Historia Natural del Paraguay. A – Freshwater ecoregions, B – Terrestrial ecoregions, C – Protected areas.

Previous data only included some scattered records in the rivers shared with nearby countries (mainly Brazil and Argentina), with the contribution of this manuscript the distribution of invasive bivalves in Paraguay is now more detailed, with new records inside the country, including large rivers and small tributaries. Most of the records were to the east of Paraguay River, except by two sites in Confuso River near to the mouth of this river in Paraguay River. The gap in invasive species distribution in Paraguay is not a real absence of these species, but a lack of sampling effort. The works done before in Paraguay were isolated efforts (Schade, 1965; Quintana, 1982) and was never established continuous research in malacology at Paraguay. Therefore, the recent records of invasive species at Paraguay were done at the borders (e.g. Oliveira et al., 2015). Our records indicate that invasive bivalves were also widespread in this part of the La Plata Basin which corresponds to the lower portion of the Paraguay River.

The occurrence of invasive species was concentrated at the east of the Paraguay River. The large freshwater ecoregion of Chaco dominates the area west of the Paraguay River, and despite the sampling effort in our survey was concentrated at south (Humid Chaco), is probably that this ecoregion is really poor in bivalve occurrences. We raise this hypothesis considering that the collecting stations done by us at the south portion of the freshwater ecoregion of Chaco returned no invasive bivalves (Figure 2A-B). The north portion of Chaco (which corresponds to the terrestrial ecoregion of Dry Chaco) was an area not sampled by us, however, data from the literature indicates that the environmental conditions became even harsher (Lemenkova, 2021) and suggests to us

that should be unsuitable for most invasive bivalves. Although can be possible to find some species of Sphaeriidae that resist extreme dry climates (e.g., Vila et al., 2020), this is not usual for invasive bivalves (i.e. *Corbicula* and *Limnoperna*) and large native freshwater mussels (Unionida). Guareschi & Wood (2020) showed a massive die-off of *C. fluminea* in water temperatures higher than 25°C. *Limnoperna fortunei* can survive in higher temperatures than *C. fluminea* (Oliveira et al., 2015), although extreme water level variation during the year as occurs at Chaco is also a limitation. Annual variation in water level associated with water acidification at Pantanal was suggested as a limitation to the establishment of large populations of golden mussel in this region (Pessotto & Nogueira, 2018). Therefore, the combination of dry climates and extreme water variation makes almost half of Paraguayan territory unsuitable for freshwater bivalves (native and invasive).

The species of *Corbicula* were widely distributed in South America, and in Paraguay the situation is similar. These species, especially *C. fluminea*, occur virtually in all basins of South America (e.g., Darrigran et al., 2020; Miyahira et al., 2020). *Corbicula fluminea* occurred from small to large rivers in Paraguay, suggesting some flexibility in environmental requirements. There is also a wide range of environmental variables (Table 2) in the sites where this species occurs. However, more information is needed to test the relationship of the occurrence of *C. fluminea* with environmental variables in Paraguay. *Corbicula fluminea* was the only invasive species to occur in interior basins. *Corbicula largillierti* presented a restricted distribution in Paraguay, and this species is usually displaced by *C. fluminea* in invaded sites (Reshaid et al., 2017).

Table 1. Species of invasive bivalves found occurring in sympatry in Paraguay.

Coordinates	Site description	Invasive bivalves
-27.322861, -57.587125	Laguna in Carrizales del Paraná National Park	<i>C. fluminea</i> , <i>C. largillierti</i> , <i>L. fortunei</i>
-27.334721, -57.629748	Next to city of Cerrito in Carrizales del Paraná National Park	<i>C. fluminea</i> , <i>L. fortunei</i>
-27.234586, -58.208522	Paraná River, Itá Corá	<i>C. fluminea</i> , <i>L. fortunei</i>
-22.092381, -57.937253	Apa River, San Lázaro	<i>C. fluminea</i> , <i>L. fortunei</i>
-25.096648, -57.559497	Confuso River, Villa Hayes	<i>C. fluminea</i> , <i>L. fortunei</i>
-25.101533, -57.519476	Paraguay River, Villa Hayes	<i>C. fluminea</i> , <i>L. fortunei</i>

Table 2. Environmental variables range in the site with invasive bivalves in Paraguay, except for *C. largillierti* which occurred in one area.

	pH	Conductivity (µS)	TDS (ppm)
<i>Corbicula fluminea</i>	6.89-9.38	36-1353	18-677
<i>Corbicula largillierti</i>	8.05	50	25
<i>Limnoperna fortunei</i>	7.70-9.38	50-188	25-94

Thus, a narrow distribution is expected for *C. largillierti* compared to *C. fluminea*. Noteworthy, is that the Confuso River recorded dead shells of *C. fluminea* and *L. fortunei* at really high conductivity values, above 4000 μ S, probably unappropriated for these species. Shells of other native and more sensible mussels were found at the same collecting station. Despite the *Corbicula fluminea* can survive in estuarine conditions (higher salinity and conductivity than freshwaters), the Confuso River can sporadically receive water from the Pilcomayo River becoming an almost salt river, a type of river that is common in the Chaco ecoregion (Karatayev et al., 2007; Facetti- Masulli et al., 2009; Ferreira-Rodríguez & Pardo, 2016; Crespo et al., 2017). Those bivalves (invasive and native) probably colonize the Confuso River seasonally from the Paraguay River, during the period of floods, and were extirpated during dry periods of higher influence of the Pilcomayo River. Our collecting survey was conducted during a period of low level of the Paraguay River waters. As we considered that these species do not truly occur under these conditions, these values were not considered in Table 2. Darrigran et al. (2011) already proposed the Pilcomayo River as a salinity barrier for the dispersion of *L. fortunei* to the Andean tributaries of La Plata Basin.

In Paraguay, the golden mussel *Limnoperna fortunei* was restricted to the main channel of large rivers (Paraguay and Paraná) and nearby areas. However, not only on the borders with Brazil and Argentina but also on the Paraguay River inside the country. *Limnoperna fortunei* is usually restricted to large rivers and rarely attains small tributaries (Barbosa et al., 2016). The faster dispersion of the golden mussel throughout the La Plata Basin is usually linked to commercial navigation (Pessotto & Nogueira, 2018). This can also explain the distribution observed in Paraguay (i.e. in large rivers with more ships), although a dispersion in large tributaries of the Paraguay River can be possible, as, in Aquidabán and Tebicuary rivers, the golden mussel has not achieved those basins yet. In our study, *L. fortunei* was found in higher pH values than in previous studies (Pessotto & Nogueira, 2018), but with lower values of conductivity. Despite being less flexible to environmental conditions than *Corbicula fluminea*; *L. fortunei* also presented a wide range of tolerance to different conditions.

The presence of invasive bivalves in protected areas is a silent threat in Paraguay. The occurrence

of the two most invasive bivalves of South America (*Corbicula fluminea* and *Limnoperna fortunei*) in three protected areas of Paraguay is an indication that these areas are not fully efficient to protect the freshwater fauna. The invasive bivalves affect not only their native counterparts but may also affect the whole environment, affecting transparency and plankton dynamics (e.g., Boltovskoy et al., 2009; Darrigran & Damborenea, 2011; Sousa et al., 2014; Ferreira-Rodríguez et al., 2018). Protected areas are usually developed based on the needs of plants and terrestrial vertebrate animals (Fagundes et al., 2016; Frederico et al., 2018), and freshwater species usually have different requirements. Thus, the existing protected areas are not always suitable for freshwater animals and environments. Our surveys were not directed to protected areas, and probably the invasive bivalves occur in other areas of Paraguay. The management plans of those areas must be updated to consider the presence of invasive species and develop strategies to mitigate their impacts on native fauna. Moreover, the occurrence of invasive bivalves in sympatry (Table 1) can potentialize their negative effects.

Detailed distribution is important to establish proper strategies to deal with invasive species and for the conservation of native species. Several species of native mussels occur in Paraguay and Paraná basins (Cuezco et al., 2020), although the information is still limited to old records (Schade, 1965; Quintana, 1982). Recent efforts done in the state of Mato Grosso, upstream of Paraguay, showed a rich and ecologically important fauna of native freshwater mussels (e.g., Colle & Callil, 2012; Olivera-Hyde et al., 2020; Santos et al., 2020, 2021). Pereira et al. (2014) considered the Paraguay Basin at Mato Grosso as of high diversity, but the Paraguayan portion as of medium richness. However, it is probably an artifact of low sampling effort as were observed for invasive species, but further investigation is needed to reveal the real diversity of native freshwater mussels in Paraguay.

Acknowledgments

Our gratitude goes to Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ E-26/201.347/2021) for the grant to ICM; to Agencia Nacional de Investigación e Innovación (ANII), Programa de Desarrollo de las Ciencias Básicas (PEDECIBA), and Mohamed bin Zayed species conservation fund (MBZ 202524562) for the financial support for CC.

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Received: 13 June 2023

Accepted: 24 November 2023

Associate Editor: André Andrian Padial