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**ECOSYSTEMS**

# Diversity of non-marine mollusks in the southernmost Paranaense forest of the world

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**Abstract:** The Natural Reserve of Punta Lara (RNPL) is a protected natural area that includes Paranaense forest southernmost in the world. This area is surrounded by a densely populated and touristic sector. The objective of this work was to estimate the richness, diversity and equity of the RNPL mollusks (aquatic and terrestrial) and to establish the relationship of their species in aquatic environments. One sampling per year was carried out between 2013 and 2019. There were 32 species recorded (six are non-native species): 23 gastropods (14 freshwater, nine terrestrial) and nine bivalves. Three species were recorded in all sampling years, while six only on one occasion. The land snail genus *Drepanostomella* is reported for the first time in that region, and five freshwater species are reported for the first time for the RNPL. The similarity analysis of the freshwater environment separated the coastal environments from the internal environments. The environments with the highest specific richness were in internal sites of the RNPL, while the least diverse was the coast of the Río de la Plata, where the invasive species *Limnoperna fortunei* predominates. It is recommended to increase the conservation efforts of the different environments of the RNPL continuously threatened by urbanization.

**Key words:** Bivalvia, density, diversity, Gastropoda, native, non-native.

## INTRODUCTION

The Natural Reserve of Punta Lara (RNPL 34° 47'33" S, 57° 59'91" W) comprises approximately 6,000 ha of riparian environments, including the coast, the gallery forest, the spinal, the flooded grasslands, and the pasturelands (Cabrera & Dawson 1944). It houses the relict Galery Forest (= Marginal Forest) of the southernmost Paranaense forest in the world and therefore many of the species present have their southern limit of geographic distribution in the neotropics in this place (Núñez Bustos et al. 2013). In addition to having the southernmost Paranaense forest in the world, the RNPL has been declared an Important Area for the Conservation of Birds, Valuable Pasture Area, and Core Area of the Pereyra Iraola Biosphere Reserve by the MaB program of UNESCO (Roesler & Agostini 2012). The weather is temperate-warm, with an average annual rainfall of 1,000 mm and an average annual temperature of 18° C. In winter, there are usually mild frosts. It is located on the coast of the Río de la Plata River within the three important cities as La Plata, Ensenada and Berazategui (Figure 1). Its location makes it a remnant of important fauna and flora to be conserved. According to Bertonatti & Corcuera (2000), the RNPL is in one of the areas with the highest levels of environmental degradation, and at the same time, it is affected by the most densely populated area of the country ("Gran Buenos Aires" with 25% of the inhabitants of Argentina,

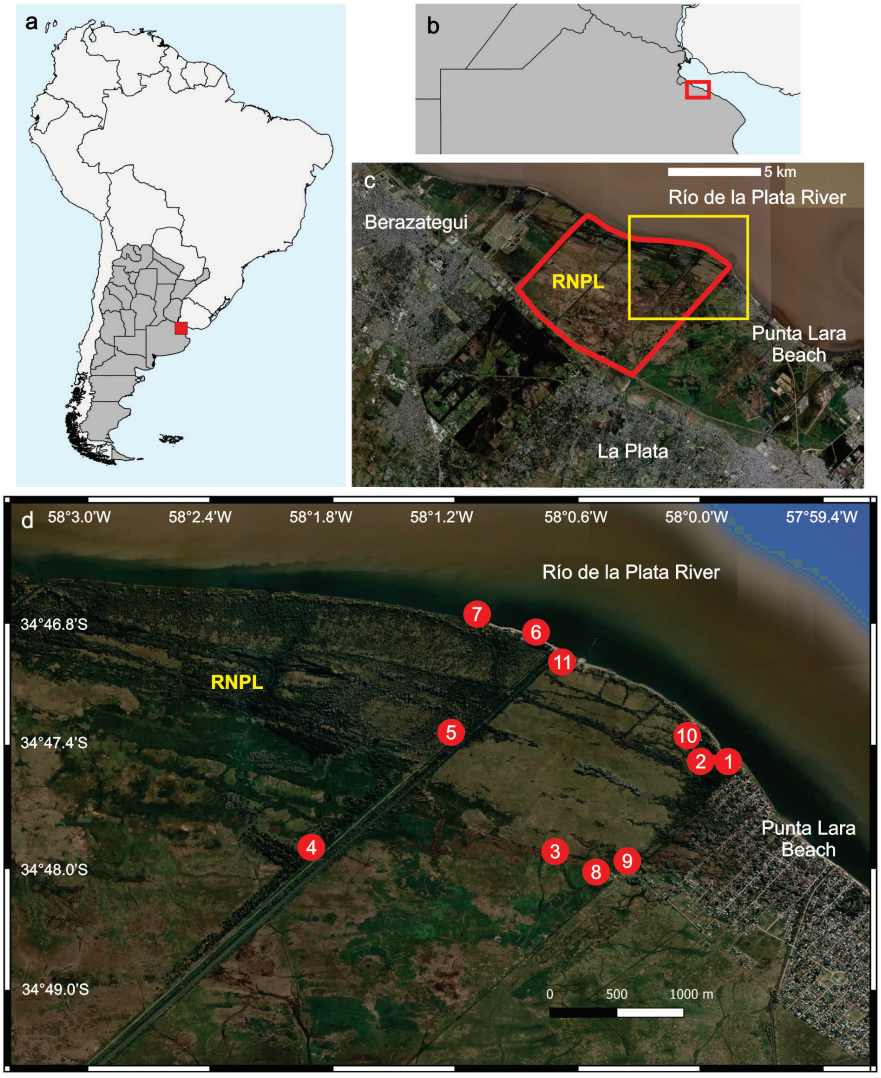


**Figure 1.** Natural Reserve of Punta Lara (RNPL). a: Location of the RNPL (red square) within South America. b: Northeast of the province of Buenos Aires with details of where the RNPL is located (red square). c: Limits of the RNPL (red polygon) surrounded by urban centers and the coast of the Río de la Plata. The yellow square indicates the sampled area of the RNPL. d: Detail of the sites sampled in the RNPL. Sites: 1- Gallery Forest; 2- Wetland in Gallery Forest; 3- Lagoon-grassland of Pampa grass; 4- Grassland of Irises; 5- Stream in the Gallery Forest; 6- Río de la Plata River Coast; 7- Riparian Scrub / Coastal reed bed; 8- Wetland in field ("El Coronillo" Bridge); 9- "El Coronillo" entrance channel; 10- Under the "El Burrito" Trail; 11- Villa Elisa Channel mouth.

9,916,715 in 2010[1]). In summary, the RNLP shelters the relict of the southernmost Paranaense forest in the world, where the flora and fauna are threatened by the anthropic expansion and by the introduction of non-native species.

Due to its geographical location, its different environments favor a high diversity in both the known flora and fauna, such as vertebrates and a group of arthropods (e.g., Giudice et al. 2011, Herrera & Torres Robles 2012, Roesler & Agostini 2012, Cazorla et al. 2018). Despite carrying out inventories, catalogs, and other studies based on the aforementioned, the mollusks only have specific studies in the RNPL, which refer specifically to mentions of some species, some ecological study or on the impact of invasive species (e.g., Ituarte 1982, Miquel 1984, César et al. 2000, Darrigran & Lagreca 2005, Gutiérrez Gregoric 2008, Rumi et al. 2009). Yet, none encompasses the entire RNPL.

From the malacological point of view, more precisely in terms of freshwater gastropods, the RNPL is in the malacological province "Bajo Paraná - Río de la Plata". This province has a richness of 38 species and is one of the most diverse in Argentina (Núñez et al. 2010) with a

[1] https://www.ign.gob.ar/NuestrasActividades/Geografia/DatosArgentina/Poblacion2

particular malacofauna, since it is influenced by the malacological provinces of the Paraná Medio and Río Uruguay and, therefore, it presents both mixed and its own characteristics. To Strong et al. (2008) is one of the world hotspots for freshwater gastropods. Of the 11 families registered in Argentina, nine are present in this malacological province. However, currently, there is little research carried out on mollusks present in the protected areas of the region (= Province of Buenos Aires). The most relevant studies carried out are those of the Martín García Island (34°10'57" S, 58°14'57" W) (Rumi et al. 1996, César et al. 2012), in the Multiple Use Natural Reserve Guillermo Enrique Hudson (34°51'23" S, 58°13'54" W) (Díaz & Martín 2013) and in the Municipal Reserve "Quilmeña Gallery Forest" (34°42'09" S, 58°14'36" W) (Daglio et al. 2014). In terms of land gastropods, the RNPL is located within the Flooded Pampa sub-ecoregion (Dos Santos et al. 2021), with 10 species of land gastropods present in the region. The studies of land gastropods around the three cities (La Plata, Ensenada, and Berisso) are practically null. Studies were recently carried out in commercial garden shops in the City of La Plata (34°55'00" S, 57°57'00" W) and surrounding areas, where eight species of land slugs were identified (Gutiérrez Gregoric et al. 2020). This study presents an updated list of species of Mollusca and their association with different environments (aquatic and terrestrial) from the Natural Reserve of Punta Lara, Argentina.

## MATERIALS AND METHODS

### Sampling and Sites

The samplings (seven) were carried out in autumn from 2013 to 2019 in the framework of field trips with the students of the Malacology course (FCNyM-UNLP). For this purpose, different sampling techniques were carried out according to the environment and type of organism following the methodology described by Gutiérrez Gregoric & Núñez (2010).

Eleven sites were sampled, which included terrestrial environments (Gallery Forest) and freshwater environments that were divided into internal and external water bodies. The latter is linked to the Río de la Plata (Figures 1 and 2). The characteristics of the vegetation of each environment are described in Herrera & Torres Robles (2012).

Selected sites:

1. *Gallery forest* bordering the Las Cañas stream (34°47'28" S, 57°59'55" W). In this terrestrial environment, quantitative samples were taken, establishing an area of 5 m x 5 m where a 1 m x 1 m grid was placed for litter collection. The samples were sieved with different mesh openings (2, 1 and 0.5cm), to be later analyzed under a binocular stereoscopic microscope.

2. *Wetland in Gallery Forest* (34°47'28" S, 57°59'56" W). It is a freshwater environment within forest surroundings, which corresponds with the limits of the internal marshlands of the RNPL. The vegetation is scarce, and there are mainly submerged fallen leaves. Specimens were collected with a 1 m x 1 m grid (same for site 1) and a perforated dipper, 17 cm in diameter, and 1 mm of the grid. The number of perforated dippers passed was counted to estimate the density (the same procedure used in the other sites).

3. *Lagoon-grassland of Pampa grass* (34°47'56" S, 58°00'41" W). Lagoon with defined edges, rather shallow (50 cm to 1 m) that usually dries up in times of scarce rainfall (Quirós 2004). It corresponds to an old wetland with floating vegetation (*Spirodela intermedia; Lemna* spp.) and rooted vegetation (*Ludwigia peploides; Myriophyllum aquaticum*) in the center of the lagoon and on the coast (*Solanum glaucophyllum; Scirpus giganteus*). Specimens


**Figure 2. Images of some environments of the RNPL. Sites: a: Gallery Forest; b: Wetland in Gallery Forest; c: Lagoon-grassland of Pampa grass; d: Grassland of Irises; e: Stream in the Gallery Forest; f: Río de la Plata River Coast; g: Riparian Scrub / Coastal reed bed; h: Wetland in field ("El Coronillo" Bridge); i: "El Coronillo" entrance channel.**

were collected with a perforated dipper (same as site 2).

4. *Grassland of Irises* (34°47'55" S, 58°01'52" W). Water bodies of shallow depth, near roads. The vegetation has a predominance of yellow irises (*Iris pseudacorus*) and to a lesser extent water lettuces (*Pistia stratiotes*). Specimens were collected with a perforated dipper (same as site 2).

5. *Stream in the Gallery Forest* (34°47'21" S, 58°01'13" W). Small water course (2m wide), without vegetation and with abundant fallen leaves at the bottom. Specimens were collected with a perforated dipper (same as site 2).

6. *Río de la Plata River Coast* (34°46'52" S, 58°00'48" W). A freshwater environment with a mixture between natural (sandy beach) and artificial (coastal wall and broken masonry). Without vegetation, exposed to the tides of the Río de la Plata, with two highs and lows during the day. Epifaunal specimens were collected with a perforated dipper in the channels over the Río de la Plata. Plastic samplers of 30 cm in diameter, which were 5 cm buried, were used to search for infaunal organisms on the sand. In the artificial substrates, 10 x 10 cm squares were used and the specimens, according to their way of life, were collected with a spatula, brushes, or clamps.

7. *Riparian Scrub / Coastal reed bed* (34°46'46" S, 58°01'05" W). A coastal environment with abundant reed bed and, at low tides, small bodies of water at its base, also exposed to the tides of the Río de la Plata. Predominant vegetation: *Schoenoplectus californicus*. The collection of specimens in the bodies of water and the sand was carried out in the same way as in site 6.

8. *Wetland in field ("El Coronillo" Bridge)* (34°48'00" S, 58°00'30" W). Internal lentic environment, which flows into the grassland. Abundant vegetation, mainly *Spirodela intermedia, Lemna spp* and *Ludwigia peploides*. Specimens were collected with a perforated dipper (same as site 2).

9. *"El Coronillo" entrance channel* (34°47'59" S, 58°00'22" W). Artificial channel with submerged vegetation (*Elodea* sp., *Myriophyllum*

sp.). Specimens were collected with a perforated dipper (same as site 2).

10. *Under the "El Burrito" Trail* (34°47'22" S, 58°00'02" W). It is an aquatic environment within jungle surroundings. There is scarce aquatic vegetation, there are mainly submerged fallen leaves. Specimens were collected with a perforated dipper (same as site 2).

11. *Villa Elisa Channel mouth* (34°46'59" S, 58°00'41" W). Connection of the Villa Elisa channel with the Río de la Plata River. Sandy beach, with isolated reed beds. The collection of specimens in the bodies of water and the sand was carried out in the same way as in site 6.

### Preparation and identification of specimens

Adult specimens were first relaxed in menthol for 12 h, then immersed in hot water (70°C), and finally stored in 96% (v/v) aqueous ethanol or fixed in modified Raillet-Henry (R-H) solution for freshwater animals—93% (v/v) distilled water, 2% (v/v) glacial acetic acid, 5% (v/v) formaldehyde, and 6 g sodium chloride per liter (Paraense 1976). For the taxonomic identification, the appropriated literature was used for Gastropods (land and freshwater) and Bivalves. The collections from the La Plata Museum (MLP) and Argentinian Museum of Natural Sciences (MACN) were reviewed, as well as the bibliography to account for the historical records of the RNPL. For the search of published works, the following search engines were used: CONICET Digital, Biodiversity Heritage Library, Google Scholar, SEDICI, Latin Index, Scielo, PubMed, among others; using as keywords: Punta Lara, Gastropoda, Bivalvia, Mollusca, specific names, generic names, families and combinations thereof.

The material was deposited in the Malacological Collection of the Museo de La Plata with the numbering MLP-Ma 15249 to 15397.

### Statistical analyzes

Terrestrial and aquatic environments were analyzed separately. To evaluate the assemblies of mollusks, the following items were calculated: taxonomic richness (S), density (ind/m²), diversity through the Shannon-Wiener index (H), dominance through the Simpson index (D) and equity through the Pielou index (J'). The expected total number of taxa in the area was evaluated using the Chao 1 and first-order Jackknife (Jack 1) richness calculation models through using the EstimateS 9.1.0 software (Colwell & Elsensohn 2014).

To compare the composition of the different freshwater sampling sites, the Pearson similarity index was used, by using the UPGMA algorithm (Magurran 1998). The UPGMA analysis was also used to see the closest relationships between species, yet the species that were only recorded in a single environment were not used in it. To get an insight into environmental variability, a principal component analysis (PCA) was performed.

## RESULTS

Throughout seven years of sampling, 32 species of mollusks were identified in the RNPL, 23 gastropods (nine terrestrial, 14 freshwaters) and nine bivalves (Figures 3, 4 and 5, Table I), out of which six are non-native species (four gastropods -three terrestrial and one freshwater- and two bivalves). Three species were recorded in all the years along the different environments considered and all of them from freshwater environments: *Pomacea canaliculata* (Lamarck, 1822), *Limnoperna fortunei* (Dunker, 1857) and *Corbicula fluminea* (Müller, 1774) (Table I). Several species had low frequencies of appearance (only one appearance) such as *Erodona mactroides* Bosc, 1801 (only empty valves, maybe subfossil), *Antillorbis nordestensis* (Lucena,

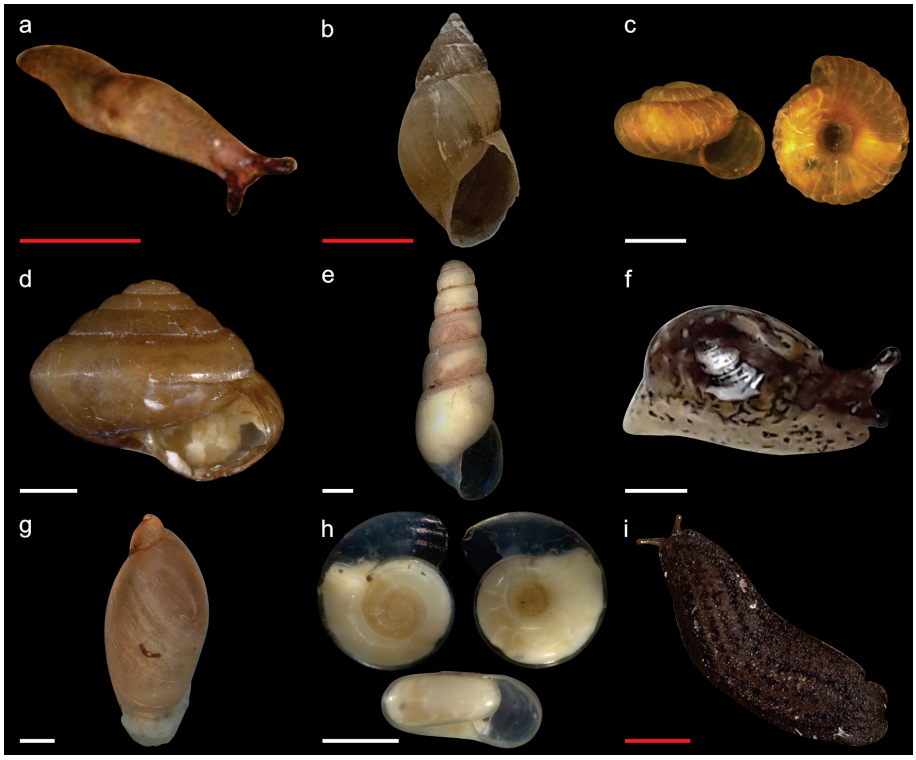


**Figure 3. Land gastropods recorded in the RNPL. a: *Deroceras leave*; b: *Bulimulus bonariensis*; c: *Paralaoma servilis*; d: *Habroconus* sp.; e: *Allopeas* aff. *gracile*; f: *Omalonyx unguis*; g: *Succinea meridionalis*; h: *Drepanostomella* sp.; i: *Belocaulus angustipes*. Scale: a-b, i: 1 cm (red bar); c-h: 1 mm (white bar).**

1954), *Hebetancylus moricandi* (d'Orbigny, 1837), *Omalonix unguis* (d'Orbigny, 1836), *Succinea meridionalis* d'Orbigny, 1837 and *Belocaulus angustipes* (Heynemann, 1885) (= *Angustipes difficilis* (Colosi, 1921)) (Supplementary Material - Table SIa).

In terms of land gastropods species, the representatives of the Subulinidae family (*Allopeas* (= *Lamellaxis*) aff. *gracile* (Hutton, 1834)) were the ones that appeared the most in the records of the seven years of sampling, registering in five of the seven years, and with a total average of 5,4 ind/m$^2$ (Table SIa). Out of the nine land gastropods species recorded, only two were not recorded in the Gallery Forest (Site 1), and both belong to the Succineidae family. *Succinea meridionalis* was recorded on the coast of the Río de la Plata (Site 6), while *Omalonix unguis* in the "El Coronillo" Entrance channel (Site 9) (Table I and Table SIa).

As for the freshwater environments, *Pomacea canaliculata* was the species that was recorded in the largest number of sites (nine out of 10). The higher density (713 ind/m$^2$) was present in site 7 in 2019 (Table SIa), because of a reproductive event, in which newly hatched specimens were recorded. In terms of densities, *Limnoperna fortunei* was the species with the highest density, with an average of 64,030 ind/m$^2$ (DS 13,178) at site 6 (Table SIa), due to its reproductive/adaptive capacity and the availability of hard substrates. There was a total density of 32,074 ind/m$^2$ for the whole RNPL (Table II). The next in terms of higher densities are two species of *Heleobia* (*H. parchappii* (d'Orbigny, 1835) and *H. piscium* (d'Orbigny, 1835)) and *Drepanotrema cimex* (Moricand, 1837), which presented average total densities varying between 65 and 78 ind/m$^2$ (Table II). In 2019, at site 8, *Drepanotrema cimex*, with 2,000 ind/m$^2$, presented the highest density peak excluding *Limnoperna fortunei* (Table SIa). The lowest density corresponds to *Antillorbis nordestensis* with only 0.2 ind/m$^2$ (Table II and Table SIa).

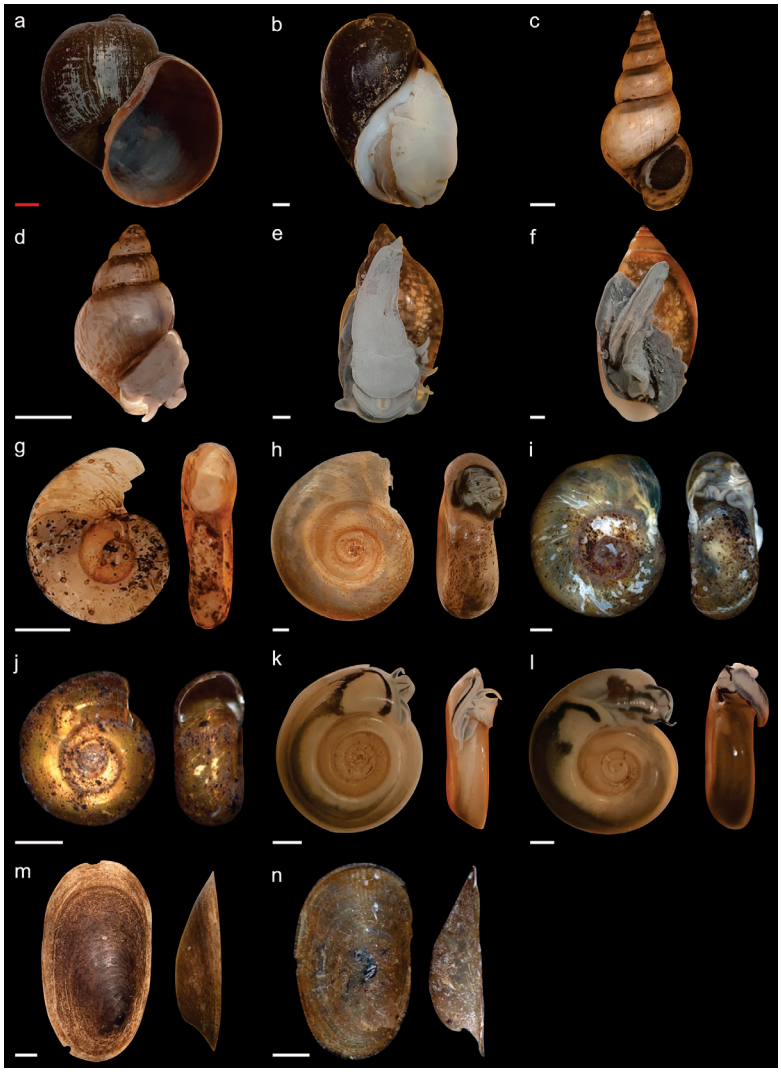


**Figure 4. Freshwater gastropods recorded in the RNPL. a: *Pomacea canaliculata*; b: *Chilina fluminea*; c: *Heleobia parchappii*; d: *Heleobia piscium*; e: *Physa acuta*; f: *Stenophysa marmorata*; g: *Antillorbis nordestensis*; h: *Biomphalaria peregrina*; i: *Biomphalaria straminea*; j: *Drepanotrema anatinum*; k: *Drepanotrema cimex*; l: *Drepanotrema lucidum*; m: *Hebetancylus moricandi*; n: *Uncancylus concentricus*. Scale: a: 1 cm (red bar); b-n: 1 mm (white bar).**

The highest richness was observed in site 7 (Riparian Scrub / Coastal reed bed) with 14 species, whereas the lowest was in site 11 (Villa-Elisa Channel) with four species (Table III). Regarding diversity, the site with the greatest diversity was site 4 (Grassland of Irises) with an H of 1.61, while the site with the least diversity was site 6 (Coast of the Río de la Plata), with an H of 0.02. In terms of the Simpson (D) and Pielou (J') indexes, site 6 (Coast of the Río de la Plata) showed values that indicate low equality between the registered density (D=0.99, J=0.004) (Table III). The richness indicators showed similar values and were very close to what was observed (Chao1=33 species, Jack1=38 species).

The similarity analysis disaggregated the coastal environments (sites 6, 7 and 11) from the internal environments (Figure 6). Within the internal environments, those related to jungle environments (sites 5 and 10), both with seven and five species shared were disaggregated from the rest (Figure 6). Site 2 (low in Gallery Forest) and Site 3 (Lagoon-Pampa grass) also have a great similarity, which is a result of the fact that both sites are the limits of the grasslands. In the same way, the analysis of the main components (Figure 7, Table SIb) also

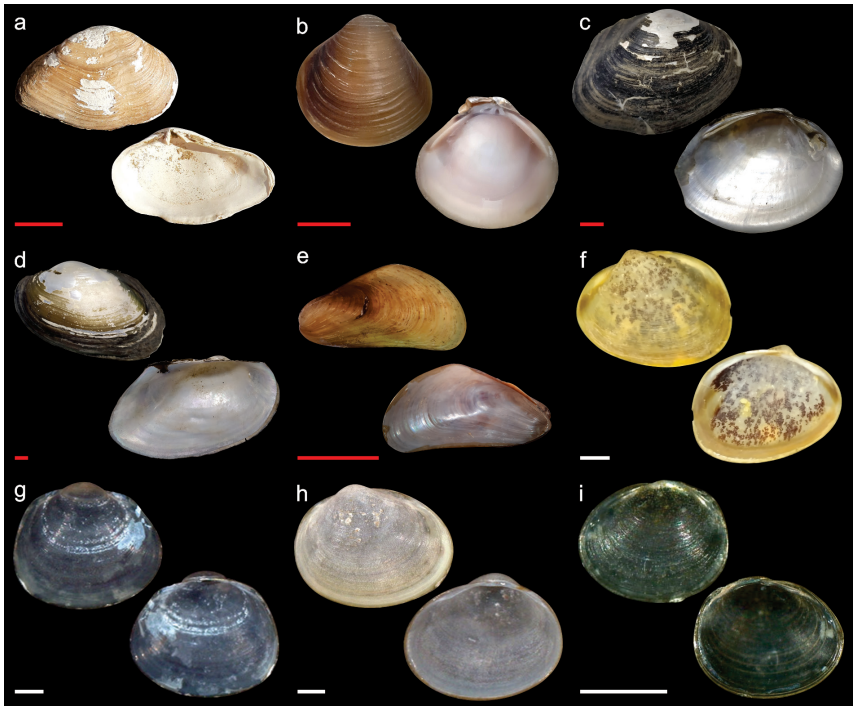


**Figure 5. Bivalves recorded in the RNPL. a: *Erodona mactroides*; b: *Corbicula fluminea*; c: *Rhipidodonta burroughiana*; d: *Anodontites trapesialis*; e: *Limnoperna fortunei*; f: *Eupera platensis*; g: *Musculium argentinum*; h: *Pisidium sterkianum*; i: *Pisidium vile*. Scale: a-e: 1 cm (red bar); f-i: 1 mm (white bar).**

disaggregated the coastal environments from the internal ones, as it may be observed in the first main component -CP1- (37% contribution). The species *Hebetancylus moricandi, Chilina fluminea* (Maton, 1809), *Corbicula fluminea, Anodontites trapesialis* (Lamarck, 1839) and *Riphidodonta burroughiana* (Lea, 1834) were the ones that contribute more to the coastal environments (positive values on the axis), and which were only found in those environments. Meanwhile, in CP2 (17% explanation), *Eupera platensis* Doello-Jurado, 1921 and *Heleobia piscium* were the species that contributed the most on the negative axis, observing in this axis a disintegration of the interior environments of the jungle from the rest of the environments (Figure 7, Table SIb).

## DISCUSSION AND CONCLUSION

The collections made during these seven years on the RNPL yielded many species, which, although it is lower than what has been historically recorded for the Río de la Plata and its surroundings, allowed us to collect updated information for mollusks. It should be noted that the historical records of mollusks cover data from the late 1890s to the mid-1990s, of which many species have not been recorded again. It is in this period that the Río de la Plata basin was strongly impacted by being first the recipient of a livestock/agricultural area, to later capture the impact of an industrialized and urbanized area. At this point, Linares et al. (2023) by pointing out that the past is never dead. Legacy effects of past anthropogenic land use and cover may also affect the structure and functioning of current lotic ecosystems. This may support the reason for a decrease in the biodiversity of the mollusks in the Basin. The historical studies, without certainty of collection dates, in the Coastline of the Río de la Plata and adjoining water bodies, mention 25 species of gastropods and 32 of bivalves (César et al. 2000). An exhaustive sampling of the coastal malacofauna of the Río de la Plata has been carried out between 1985 and 1988, and it has proven the existence of only 25 species of mollusks (eight

**Table I. Species per year recorded in the Reserve Natural of Punta Lara. *: non-native species.**

| | | Family | Species | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 |
|---|---|---|---|---|---|---|---|---|---|---|
| Gastropoda | Land | Agriolimacidae | *Deroceras laeve* (Müller, 1774)* | | | x | | x | | x |
| | | Bulimulidae | *Bulimulus bonariensis* (Rafinesque, 1833) | x | | | x | | | x |
| | | Punctidae | *Paralaoma servilis* (Shuttleworth, 1852)* | x | | x | | | x | x |
| | | Euconulidae | *Habroconus* sp. | | | x | | x | x | x |
| | | Subulinidae | *Allopeas* aff. *gracile** (Hutton, 1834) | | x | x | x | x | x | |
| | | Succineidae | *Omalonyx unguis* (d'Orbigny, 1836) | | | | | | | x |
| | | | *Succinea meridionalis* d'Orbigny, 1837 | | | x | | | | |
| | | Scolodontidae | *Drepanostomella* sp. | | | x | x | | | x |
| | | Veronicellidae | *Belocaulus angustipes* (Heynemann, 1885) | | | | | x | | |
| | Freshwater | Ampullaridae | *Pomacea canaliculata* (Lamarck, 1822) | x | x | x | x | x | x | x |
| | | Chilinidae | *Chilina fluminea* (Maton, 1809) | x | x | x | | x | x | x |
| | | Cochliopidae | *Heleobia parchappii* (d'Orbigny, 1835) | | | | | x | | x |
| | | | *Heleobia piscium* (d'Orbigny, 1835) | x | x | x | | x | x | x |
| | | Physidae | *Physa acuta* Draparnaud, 1805* | x | x | x | x | | x | x |
| | | | *Stenophysa marmorata* (Guilding, 1828) | | x | x | x | | | x |
| | | Planorbidae | *Antillorbis nordestensis* (Lucena, 1954) | | | | x | | | |
| | | | *Biomphalaria peregrina* (d'Orbigny, 1835) | x | x | x | | | | |
| | | | *Biomphalaria straminea* (Dunker, 1848) | x | x | | | x | x | x |
| | | | *Drepanotrema anatinum* (d'Orbigny, 1835) | | | | | | x | x |
| | | | *Drepanotrema cimex* (Moricand, 1837) | x | x | x | x | | x | x |
| | | | *Drepanotrema lucidum* (Pfeiffer, 1839) | x | x | x | x | | x | x |
| | | | *Hebetancylus moricandi* (d'Orbigny, 1837) | | x | | | | | |
| | | | *Uncancylus concentricus* (d'Orbigny, 1835) | x | x | x | x | x | x | x |
| Bivalvia | | Corbulidae | *Erodona mactroides* Bosc, 1801 | | | | | x | | |
| | | Cyrenidae | *Corbicula fluminea* (Müller, 1774)* | x | x | x | x | x | x | x |
| | | Hyriidae | *Rhipidodonta burroughiana* (Lea, 1834) | | | | x | x | x | |
| | | Mycetopodidae | *Anodontites trapesialis* (Lamarck, 1839) | | | | x | x | x | |
| | | Mytilidae | *Limnoperna fortunei* (Dunker, 1857)* | x | x | x | x | x | x | x |
| | | Sphaeriidae | *Eupera platensis* Doello-Jurado, 1921 | | | x | | | x | x |
| | | | *Musculium argentinum* (d'Orbigny, 1835) | | | x | x | | x | x |
| | | | *Pisidium sterkianum* Pilsbry, 1897 | | x | | | x | | x |
| | | | *Pisidium vile* Pilsbry, 1897 | | | x | | | x | x |
| Richness | | | | 13 | 15 | 19 | 15 | 16 | 19 | 23 |

bivalves and 17 gastropods) (Darrigran & Lagreca 2005). Undoubtedly, another factor to consider in the risk of global biodiversity decline is due to climate change and it is expected that, for example, freshwater ecosystems will be one of the most impacted (Steeves et al. 2018).

In the studies that concern the protected areas in the region for freshwater mollusks, Rumi et al. (1996), Martín & Negrete (2006), and César et al. (2012) mention 27 species of mollusks (22 gastropods and five bivalves) for the Isla Martín García Multiple Uses Reserve (IMG, 34°10'57" S, 58°14'57" W). Whereas, Díaz & Martín (2013) mention nine species (five gastropods and four bivalves) for the Hudson reserve and Daglio et al. (2014) mention 13 mollusks (nine gastropods and four bivalves) for the "Quilmes" reserve. For the RNPL, according to databases from museums and publications (Mussel project, Núñez et al. 2010, MLP, MACN, Torres et al. 2018), 15 species of freshwater gastropods and 16 bivalves were recorded. On the contrary, in the present work, lower values are recorded for the RNPL (14 gastropods and nine bivalves). Although in the different protected areas the sampling effort may have been different, the number of species of freshwater gastropods turned out to be lower than the IMG, and higher than that of the other reserves mentioned before. This decrease in the number of species with the IMG maybe since this last reserve has a greater influence of the Uruguay River, where there are species that do not reach the coast of the Río de la Plata, such as those of *Potamolithus* (with 3 species in the IMG), *Pomacea megastoma*, and some species of *Drepanotrema* and *Biomphalaria*, which are better represented in the Argentine Mesopotamia.

**Table II. Average total density ($\bar{x}$= ind/m²) by freshwater species in the seven years of sampling ordered from highest to lowest. SD: Standard Deviation.**

| Species | $\bar{x}$ | DS |
|---|---|---|
| *Limnoperna fortunei* | 32074.8 | 34200.2 |
| *Heleobia parchappii* | 77.6 | 211.5 |
| *Heleobia piscium* | 69.4 | 122.1 |
| *Drepanotrema cimex* | 65.4 | 337.1 |
| *Physa acuta* | 35.6 | 42.8 |
| *Corbicula fluminea* | 33.9 | 45.3 |
| *Pomacea canaliculata* | 33.0 | 116.3 |
| *Biomphalaria straminea* | 27.9 | 66.4 |
| *Chilina fluminea* | 21.2 | 50.8 |
| *Stenophysa marmorata* | 19.3 | 45.6 |
| *Pisidium sterkianum* | 17.4 | 18.4 |
| *Uncancylus concentricus* | 16.6 | 52.3 |
| *Drepanotrema lucidum* | 7.1 | 17.7 |
| *Pisidium vile* | 5.5 | 11.8 |
| *Eupera platensis* | 5.2 | 11.8 |
| *Drepanotrema anatinum* | 3.0 | 3.6 |
| *Musculium argentinum* | 2.2 | 4.5 |
| *Biomphalaria peregrina* | 1.8 | 479.7 |
| *Hebetancylus moricandi* | 1.0 | 2.9 |
| *Rhipidodonta burrughiana* | 0.7 | 1.1 |
| *Anodontites trapesialis* | 0.7 | 1.5 |
| *Erodona mactroides* | 0.6 | 1.3 |
| *Antillorbis nordestensis* | 0.2 | 0.4 |

From the freshwater gastropods, five are cited for the first time from the RNPL and its surroundings: *Antillorbis nordestensis*, *Biomphalaria straminea* (Dunker, 1848), *Drepanotrema anatinum* (d'Orbigny, 1835), *D. cimex* and *D. lucidum* (Pfeiffer, 1839), being the closest records in the locality of Berisso (approximately 18 km SW of the RNPL) for the five species. *Biomphalaria straminea* is of sanitary interest since it is a natural host of *Schistosoma mansoni* in South America (although this parasite is not currently found in Argentina) (Rumi & Núñez 2013). From the species previously cited for the RNPL, we did not record: *Asolene platae* (Maton, 1811), *Pomacea scalaris* (d'Orbigny, 1835) and *Galba viator* (d'Orbigny, 1835). The three species have not been collected in areas surrounding the RNPL during the last years (D.E. Gutiérrez Gregoric, pers. obs).

**Table III. Parameters analyzed for each environment of the RNPL in the total number of years sampled. Richness (S), Density (ind/m$^2$), Simpson's index (D), Shannon's index (H), Pielou's equity (J').**

| | S | Density | H | D | J' |
|---|---|---|---|---|---|
| 1- Gallery Forest | 7 | 71 | 1.38 | 0.34 | 0.49 |
| 2- Wetland in gallery forest | 6 | 208 | 0.41 | 0.82 | 0.16 |
| 3- Lagoon-grassland of Pampa grass | 8 | 411 | 1.51 | 0.26 | 0.50 |
| 4- Grassland of Irises | 11 | 583 | 1.61 | 0.25 | 0.47 |
| 5- Stream in the Gallery Forest | 7 | 101 | 1.05 | 0.52 | 0.38 |
| 6- Río de la Plata River Coast | 13 | 385.388 | 0.02 | 0.99 | 0.01 |
| 7- Riparian Scrub / Coastal reedbed | 14 | 2.372 | 1.60 | 0.24 | 0.42 |
| 8- Wetland in field ("El Coronillo" Bridge) | 9 | 2.316 | 0.49 | 0.81 | 0.15 |
| 9- "El Coronillo" entrance channel | 13 | 1.773 | 1.50 | 0.29 | 0.40 |
| 10- Under the "El Burrito" Trail | 7 | 685 | 1.45 | 0.32 | 0.52 |
| 11- Villa Elisa Channel, estuary. | 4 | 57 | 0.59 | 0.72 | 0.30 |

Eight species of the bivalves out of the sixteen mentioned in the publications in the Punta Lara Beach (34°47'33" S, 57°59'91" W) have been found. *Rhipidodonta burroughiana* has been found after 100 years (the last record in the databases was from 1920) in that town. This species may have more recent records, yet they are not reflected in the scientific collections or publications. This species has been recently mentioned in the province of Entre Ríos (Cao 2021). Out of the species which have been mentioned, but not collected here, the majority has records of more than 50 years for the RNPL and the Punta Lara Beach, such as *Anodontites patagonica* (Lamarck, 1819) (last record -LR- 1950); *Diplodon parallelopipedon* (Lea, 1834) (LR 1912); *Mycetopoda legumen* (Martens, 1888) (LR 1960); *Rhipidodonta variabilis* (Maton, 1809) (LR 1931) and *Rhipidodonta charruana* (d'Orbigny, 1835) (LR 1913). Others have records from 40 years ago, such as *Neocorbicula* (= *Cyanocyclas*) *limosa* (Maton, 1809) (which could be displaced by *Corbicula fluminea*), and *Corbicula largillierti* (Philippi, 1844) (exotic species with last records taken in the 1980s). Regarding this last species, Reshaid et al. (2017) have already mentioned its absence in the shores of the Río de la Plata. According to Gama et al. (2017), climate change is generally related to the dispersal of invasive bivalves (e.g. *Corbicula fluminea*). These authors observe the expansion of *C. fluminea* in new river basins, in future climate scenarios, where the impacted area would double, and they would be adapted for the presence of non-native species. The changes in the coastal environment, with the construction of walls, in addition to the recreational and commercial activity on the coast of the Río de la Plata, as well as the presence of the invasive bivalve *Limnoperna fortunei* (as observed in site 6, where the Simpson and Pielou indices were the lowest), coincide with the decrease in the number of several species of bivalves, especially naiads (Unionida) (Torres et al. 2018) and the genus *Neocorbicula*. Both the presence of non-native species with an invasive nature, as well as the environmental deterioration caused by tourism and urbanization and the consequent contamination of the area have led several of these species to be found on the list of threatened and priority species for their conservation in Uruguay and Brazil (Scarabino & Clavijo 2009, Clavijo & Scarabino 2013, Machado et al. 2018).

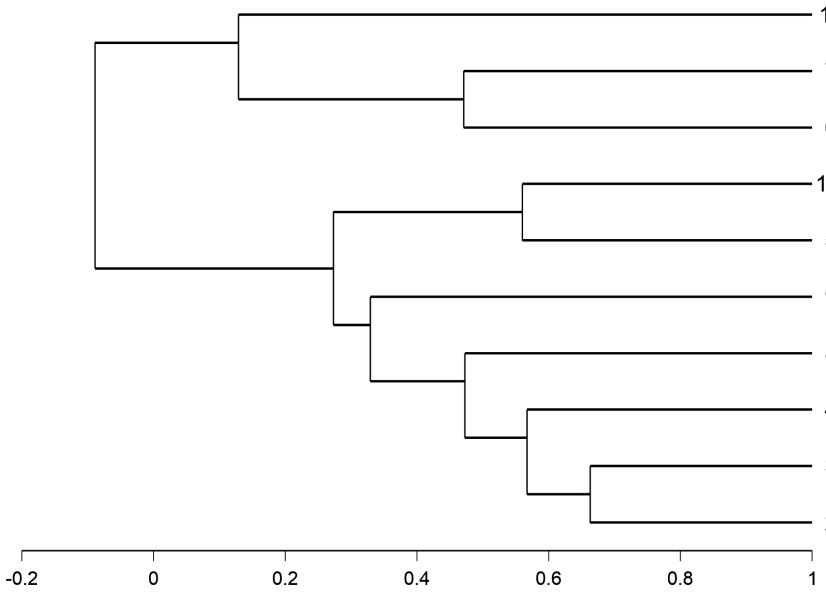


**Figure 6. Similarity dendrogram groups the RNPL freshwater sampling sites based on the presence/absence of the taxa. Sites: 2- Wetland in Gallery Forest; 3- Lagoon-grassland of Pampa grass; 4- Grassland of Irises; 5- Stream in the Gallery Forest; 6: Río de la Plata River Coast; 7- Riparian Scrub / Coastal reed bed; 8- Wetland in field ("El Coronillo" Bridge); 9- "El Coronillo" entrance channel; 10- Under the "El Burrito" Trail; 11- Villa Elisa Channel mouth.**

About terrestrial gastropods, the knowledge for the studied area is practically null. There is material in the Museums (many undetermined) and some isolated works on a particular entity, yet there is no work that refers to the terrestrial gastropods of the area. Recently, Dos Santos et al. (2021) have updated the list of terrestrial species for Argentina, mentioning the presence of ten species in the province of Buenos Aires, more specifically at the Flooded Pampa sub-ecoregion, where the RNPL is located. Of those ten species, six were counted for the RNPL according to the review of the museums and bibliography. Our results have shown the presence of ten species (two coincide with the records published by Dos Santos et al. (2021): *Belocaulus angustipes* and *Bulimulus bonariensis* (Rafinesque, 1833). We have not found the species *Bulimulus vesicalis* (Pfeiffer, 1853) that was cited in the RNPL by Ringuelet (1962) and Miquel (1991), nor the species *Phyllocaulis variegatus* (Semper 1885). However, the first was found in the Municipal Natural Reserve of Vicente López (34°29'30" S, 58°28'46" W) in 2014 (Virgillito et al. 2016) and the second was recently found in a garden shop in the city of La Plata (Gutiérrez Gregoric et al. 2020). *Paralaoma servilis* (Shuttleworth, 1852) has been recorded in our sampling, but it has been previously cited for the Punta Lara (without specifying if it is the RNPL or the beach cited as *Radiodiscus pilsbryi* Hylton Scott (1957), currently synonymous with *P. servilis*), without a collection date (Miquel et al. 2007). This last species is considered non-native in Argentina (Darrigran et al. 2020), and with recent records in urban environments in the region Miquel & Santin (2021).

Within the new records of terrestrial gastropods, the presence of two non-native species stands out: *Allopeas* aff. *gracile*, which is cited for the city of La Plata. It also stands out the presence of *Deroceras laeve*, (Müller, 1774) which is a slug that was recently cited for the Punta Lara Beach in garden shops (Gutiérrez Gregoric et al. 2020) and a private house in the locality of San Isidro (34°28'17" S, 58°30'28" W) (Miquel & Santin 2021).

Additionally, in the present work, the presence of the terrestrial mollusk of the genus *Habroconus* in the region has been confirmed. Although in the MLP (MLP-Ma 8976), a specimen identified as *Habroconus mayi* (Baker, 1914) is deposited without a collection date, for the Punta Lara (without specifying if it is the RNPL

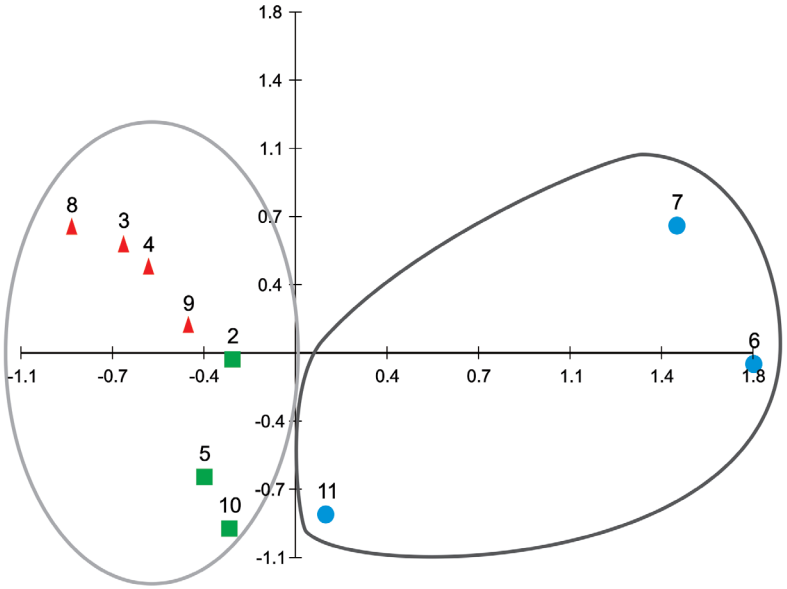


**Figure 7. Principal component analysis (PCA), using the presence/absence matrix of freshwater species by the environment. Abscissa: CP1. Ordinate: CP2. Dark gray line: coastal environments; Light gray line: internal environments. Red triangles: grassland environments; green squares: Gallery Forest environments, light blue circles: coastal environments. Sites: 2- Wetland in Gallery Forest; 3- Lagoon-grassland of Pampa grass; 4- Grassland of Irises; 5- Stream in the Gallery Forest; 6: Río de la Plata River Coast; 7- Riparian Scrub / Coastal reed bed; 8- Wetland in field ("El Coronillo" Bridge); 9- "El Coronillo" entrance channel; 10- Under the "El Burrito" Trail; 11- Villa Elisa Channel mouth.**

or the beach), and the only one of the region (Yet, it is for certain that it dates back to 1950). However, this species is present in Brazil, with no records in other countries (Simone 2006, de Lima et al. 2021), so this identification should be doubtful, as defined by Hylton Scott (1984). In Argentina, *Habroconus* was mentioned in the northwest and northeast (Dos Santos et al. 2021). In addition, we cite for the first time, for the province of the region, the terrestrial genus *Drepanostomella* Bourguignat, 1890. This genus is present in Argentina with three species in the provinces of Salta and Tucumán (Dos Santos et al. 2021). However, there are specimens deposited in the MLP identified as *Drepanostomella* sp., which are similar to the specimens registered here. From the specimens of the *Drepanostomella* genus found here, only one specimen could be obtained while alive, and it has been preserved in ethanol. The species of these two genera need a systematic review to define new characters for their identification.

Our collections of terrestrial gastropods have only been limited to an area of the Gallery Forest, and, thus, we have registered the presence of seven species. In the environments most closely related to bodies of water, we found other two species of terrestrial gastropods belonging to the Succinidae, *Omalonix unguis* and *Succinea meridionalis*, which are linked to water hyacinth ("camalotes") and other vegetation that flows down the Paraná River. Surely, new collections of terrestrial mollusks in other environments of the RNPL and surrounding areas, especially in micro-mollusks, will increase the number of species. Recently, Miquel & Santin (2021) have recorded the presence of at least ten species of gastropods at the garden of a private house in the locality of San Isidro. Among these species, one is a new species for science, three were first records for Argentina (without determining their species) and the other six species are non-native. Although these non-native species were cited for Argentina, they were not from that location in particular. This demonstrates the difficulty in determining these micro-mollusks, together with the lack of studies in the area.

Although there are several of the species that present scarce records or very low densities, we have not found endemism for the RNPL, but with the number of species found, it is most likely that there is. Latin America and the Caribbean is

a region that is particularly vulnerable to the threats of climate change. This is due, among other reasons, to the richness in biodiversity and the endemic species it harbors (Botero 2015). As it has been mentioned, the RNPL is surrounded by cities and it constitutes a relict for the conservation of biodiversity. Our results have proven different connections between species in the coastal versus internal environments, even within the internal ones, and those of jungle environments as opposed to the rest. We recommend increasing the conservation efforts of the different environments of the RNPL that are constantly threatended by the advance of urbanization, as also recommended by Roesler et al. (2012). Thus, the coastal environment of the RNPL has been affected in the last four decades by the constant contributions of hydrocarbons, e.g. the spill of 1,000 tons of oil in January 1999 in the Río de la Plata (Colombo et al. 2005); the construction of the wall and coastal streets; constructions; fishermen housing; different touristic resources.

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## SUPPLEMENTARY MATERIAL

**Tables SIa, b.**

**How to cite**

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## Author contributions

GD and DEGG conceived the main idea. MdL and DEGG helped with sampling; analyzed the data and wrote the manuscript. GD helped with the revision of the drafts and contributed to the discussion of the results.