Distribution of freshwater fish from the Southern Neotropics reveals three new areas of endemism and show diffuse limits among freshwater ecoregions

Correspondence:
Marcelo Loureiro
mapy@fcien.edu.uy

José Bessonart[1], Marcelo Loureiro[1], José Carlos Guerrero[2] and Claudia Szumik[3]

Submitted December 29, 2020
Accepted June 7, 2021
by Marcos Mirande
Epub Dec 01, 2021

Ecoregions and areas of endemism are central concepts in biogeography. Based on collection records and the Endemic Analyses implemented with NDM/VNDM method we analyzed fish areas of endemism in the junction of three freshwater ecoregions related to the Rio de la Plata estuary (Lower Parana, Lower Uruguay, Laguna dos Patos) in Southern Neotropical region. Using two grid cell sizes, results obtained showed the same general patterns. Areas of endemism recovered were mainly associated either to Lower Uruguay or to Laguna dos Patos. In both ecoregions nested areas of endemism were identified within larger patterns of endemism. Noteworthy, one area recovered occurred across Lower Uruguay and Laguna dos Patos limits. Our results also suggest a revision of the Lower Uruguay and Lower Parana ecoregion limits, and highlight the relevance of the Rio de la Plata estuary as a barrier and corridor for freshwater fishes in the area.

Keywords: Endemisms, Freshwater Fish, Laguna dos Patos, Lower Parana, Lower Uruguay.

Online version ISSN 1982-0224
Print version ISSN 1679-6225

Neotrop. Ichthyol.
vol. 19, no. 4, Maringá 2021

1 Laboratorio de Zoología de Vertebrados, Departamento de Ecología y Evolución, Facultad de Ciencias (UDELAR), Iguá 4225, 11400, Montevideo, Uruguay. (JB) 1aguaraguazu@gmail.com, (ML) mapy@fcien.edu.uy (corresponding author).

2 Laboratorio de Desarrollo Sustentable y Gestión Ambiental del Territorio, Instituto de Ecología y Ciencias Ambientales, Facultad de Ciencias (UDELAR), Iguá 4225, 11400, Montevideo, Uruguay. jguerrero@fcien.edu.uy.

3 Unidad Ejecutora Lillo, Consejo Nacional de Investigaciones Científicas y Técnicas, Fundación Miguel Lillo, Miguel Lillo 251, 4000, S.M. de Tucumán, Argentina. szu.claudia@gmail.com.

Las ecorregiones y áreas de endemismo son conceptos centrales en biogeografía. Basados en registros de colecciones y el análisis de endemismo implementado en NDM/VNDM, analizamos áreas de endemismo de peces en el sur de la región Neotropical, en la conjunción de tres ecorregiones de agua dulce relacionadas al estuario del Río de la Plata (Bajo Paraná, Bajo Uruguay, Laguna de los Patos). Usando dos tamaños de grilla, los resultados obtenidos mostraron los mismos patrones generales. Las áreas de endemismo obtenidas estuvieron principalmente asociadas tanto a la ecorregión Bajo Uruguay como a la Laguna de los Patos. En los dos casos, fueron identificadas áreas de endemismo anidadas dentro de los grandes patrones de endemismo. Además, se destacó un área que atravesó los límites de ambas ecorregiones. Nuestros resultados también sugieren que es necesaria una revisión de los límites entre Bajo Uruguay y Bajo Paraná y resaltan la relevancia del estuario del Río de la Plata como barrera y corredor para los peces de agua dulce de esta región.

Palabras clave: Bajo Paraná, Bajo Uruguay, Endemismos, Laguna de los Patos, Peces de agua dulce.

INTRODUCTION

Distribution of organisms is the result of their ecological and evolutionary history, acting complementarily to generate biogeographic patterns (Wiens, Donoghue, 2004). Areas of endemism are among the most evident biogeographic patterns and one of the main focuses in biogeographic research and debates (Platnick, 1991; Harold, Mooi, 1994; Hovenkamp, 1997; Szumik *et al*., 2019). Areas of endemism are defined based on the congruent distribution of at least two taxa that do not occur anywhere else (Platnick, 1991; Linder, 2001), which are presumed to have originated by the same historical and/or ecological factors (Morrone, Crisci, 1995). This concept also leads to consider areas of endemism as biologically relevant from the conservation point of view (Moritz, 2002; Richardson, Whittaker, 2010). Areas of endemism occur in a nested pattern (Morrone, 2008), where smaller areas are included in larger ones, which emphasize their importance in conservation policies, since large areas are the focus of international agencies while smaller ones have to or should be the focus of conservation policies at the national levels.

Ecoregions, originally created for silviculture purposes (Loucks, 1962), are relatively large and finite areas, smaller than a biome, where environmental conditions and species assemblages are relatively homogeneous compared to the heterogeneity that occurs in a larger area (Olson *et al*., 2001). Abell *et al*. (2008) defined a freshwater ecoregion as a large area that includes one or more freshwater system, with a natural and distinctive assemblage of communities and species. Therefore, ecoregions are spatial units that contain within and also compose an area of endemism per se.

Freshwater fishes are a suitable group to study areas of endemism and ecoregions due to their relatively lower dispersal capabilities conditioned by the dendritic systems of the hydrographic basins (Moyle *et al*., 2003). In particular the Neotropical ichthyofauna, that is one of the richest biogeographic regions with 20% of total fish diversity of the world (Lévêque *et al*., 2007) and numerous endemic families (Malabarba, Malabarba, 2020). The high Neotropical fish diversity is mainly associated to tropical ecoregions of the so called Amazon-Orinoco-Guiana core, whereas higher endemicity levels are associated to the peripheral ecoregions (Albert *et al*., 2011, 2020). Among these, ecoregions associated to La Plata basin present relatively high levels of diversity while Atlantic coastal ecoregions present higher levels of endemicity (Albert *et al*., 2011, 2020).

According to Abell *et al*. (2008), there are three ecoregions associated with the Rio de la Plata estuary: "Lower Parana" (LP–345), "Lower Uruguay" (LU–332), and "Laguna dos Patos" (P–334) (Fig. 1). Whereas the Paraná and Uruguay rivers outflow directly to Rio de la Plata, the boundary between LP and P ecoregions corresponds to the external limit of the estuary, an imaginary line that joins Punta del Este (Uruguay) and Punta Rasa (Argentina); estuary that acts as a cyclic barrier to freshwater species. In a short time scale, depending on the discharge of the Paraná and Uruguay rivers, the freshwater-saltwater front moves seasonally in a W-E direction (Framiñan, Brown, 1996). At larger time scales, depending on the sea levels fluctuations related to glacial cycles, marine transgressions may have generated strong barriers to freshwater species dispersal through the Miocene, in extreme events such as the formation of Paranean Sea (Lundberg *et al*., 1998), to more recent and subtle events in the Holocene (Martínez, Rojas, 2013). On the other hand, marine regressions associated to the Last Glacial Maximum, with extreme events such as a lowering of 149 m in sea level (Lambeck *et al*., 2003), may have provoked a strong retraction of the Rio de la Plata estuary with the concomitant effect that the Paraná River emptied close to the Atlantic Ocean (Violante, Parker, 2004; Ayup-Zouain, 2006). This could have opened a corridor for dispersion of freshwater organisms between the Paraná and Uruguay rivers and Atlantic coastal drainages, such as the Laguna dos Patos ecoregion. The presence of some species typical of LP and LU ecoregions in the small Atlantic coastal drainages in Uruguay (Loureiro, García, 2006; Loureiro *et al*., 2016a), considered as part of the Laguna dos Patos ecoregion, supports this hypothesis, and also cast a doubt over the nature of current ecoregions limits. The phylogeographic structure of *Cnesterodon decemmaculatus* (Jenyns, 1842), a Poecilid widely distributed in the area, also showed this pattern (Ramos-Fregonezi *et al*., 2017).

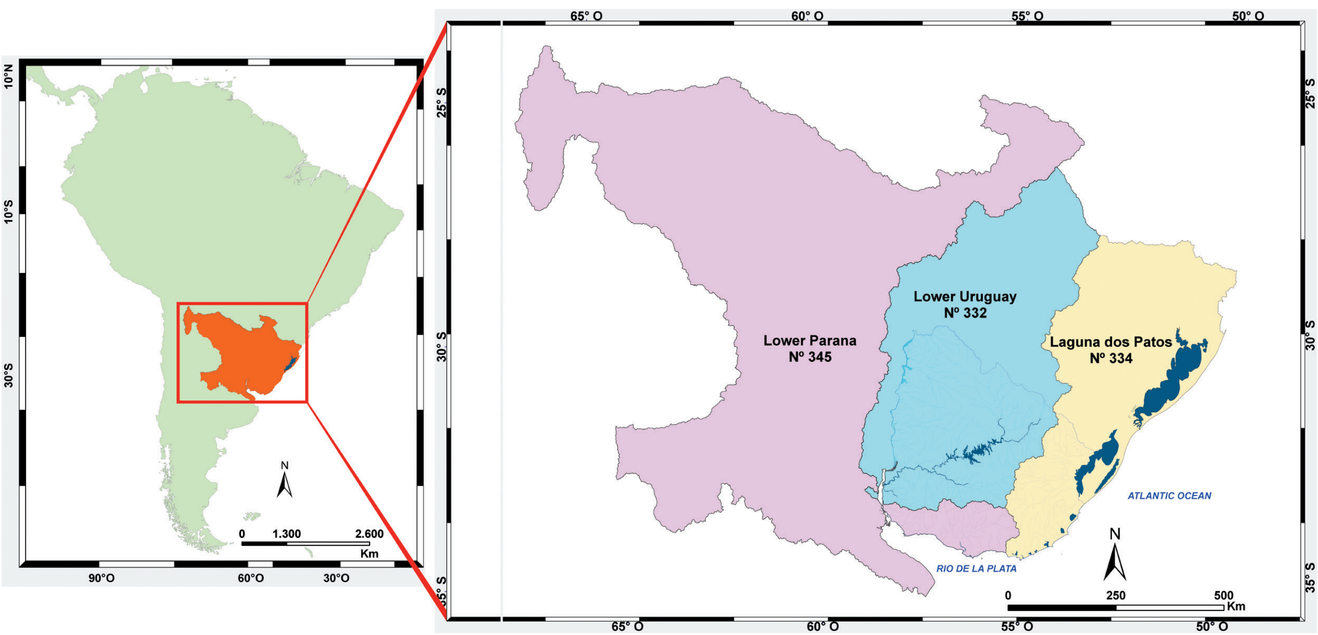

FIGURE 1 | Freshwater Ecoregions (Abell *et al*., 2008) associated to the Rio de la Plata estuary.

Within this biogeographic context, we took advantage of a dense sampling effort made in the territory of Uruguay and the databases of the national collections, built along more than 50 years (Loureiro *et al*., 2016b), to address questions about areas of endemism, and their relation to freshwater ecoregions and their limits. The territory of Uruguay presents more than 250 fish species (Loureiro *et al*., 2013), distributed in the convergence of the three freshwater ecoregions mentioned. Our working hypothesis was that the low vagility of freshwater fishes favors that the historical processes that generate endemic areas are related to the basins limits. The prediction is that in case of recovering areas of endemism they should coincide with basin limits and by extension with the ecoregion's limits.

MATERIAL AND METHODS

The study area comprehends the continental territory of Uruguay, between parallels 30ºS and 35ºS, and meridians 53ºW and 58.5ºW. In this area, LU is represented by the eastern tributaries of middle and low Uruguay River basin, LP by northern tributaries of Rio de la Plata estuary, and P by small coastal Atlantic Ocean drainages and southern Laguna Merín basin (Fig. 1). Fish records were obtained from the National Fish collection of Facultad de Ciencias, Montevideo (ZVCP). From a total of approximately 14,000 records, we filtered those taxa with problematic taxonomy and redundant records. The final matrix consisted of 4,335 geo-referenced records that correspond to 165 species, 76 genera, 30 families, and 9 orders (Tab. **S1**, Fig. **S2**; https://doi.org/10.6084/m9.figshare.14535561.v1). Information on species distributions and endemicity in the region were obtained from: Ferraris (2003), Lima *et al*. (2003), López *et al*. (2005), Malabarba *et al*. (2012), Almirón *et al*. (2015), Bertaco *et al*. (2016), Loureiro *et al*. (2016a, 2018), Turcati *et al*. (2018). The species names were checked according to Fricke *et al.* (2021).

Analysis of areas of endemism were carried out using NDM/VNDM (Goloboff, 2016), and was performed using two grid cells, 45 x 45 km and 59 x 59 km respectively. When numerous areas were obtained, a loose consensus rule was performed (see Aagesen *et al*., 2013). The procedure of NDM/VNDM to identify areas of endemism is based on the evaluation of the congruence of the distribution ranges of species (Szumik, Goloboff, 2004). The adjustment of the species to the area is quantitatively measured through the Endemicity Index (EI), whose values vary between 0 and 1, where 1 indicates a perfect congruence of the species distribution to the area evaluated (Casagranda *et al*., 2009).

RESULTS

Analyses with 59 x 59 km grid cell size resulted in 40 preliminary Areas of Endemism that after applying a tight consensus of 50 % (Aagesen *et al*., 2013) were reduced to 22 Consensus Areas (CA, Tab. 1; Fig. **S3**) whose EI values ranged between 2.3 and 11.8. The results are presented according to correspondent ecoregions.

TABLE 1 | Consensus Areas of Endemism (CA) obtained with a 59 X 59 Km grid cells, and their correspondent Endemic Indexes (EI), diagnostic species (DS), freshwater systems included (FS) and ecoregion occupied (ER); species endemic to Lower Uruguay (black bold); species endemic to Laguna dos Patos (*); species present in upper Negro, Tacuarí and Yaguarón rivers (**).

CA	EI	DS	FS	ER
0	10.3–10.5	*Ageneiosus militaris*, *Aphyocharax anisitsi*, *Apistogramma commbrae*, *Auchenipterus osteomystax*, ***Austrolebias alexandri***, *A. bellottii*, *A. nigripinnis*, *Corydoras undulatus*, *Cynopotamus argenteus*, *Cyphocharax platanus*, *Galeocharax humeralis*, *Gymnogeophagus australis*, *Hoplosternum littorale*, *Leporinus striatus*, *Lepthoplosternum pectorale*, *Odontostilbe pequira*, *Pimelodus absconditus*, *Parastegophilus maculatus*, *Rineloricaria parva*	Uruguay River basin	LU
3	5.5–5.8	*Acestrorhynchus pantaneiro*, *Aphyocharax anisitsi*, *Apistogramma commbrae*, *Corydoras undulatus*, *Cynopotamus argenteus*, *Eigenmannia trilineata*, *Hoplosternum littorale*, *Lepthoplosternum pectorale*, *Odontostilbe pequira*, *Steindachnerina brevipinna*	Uruguay River basin	LU
9	8.5–8.8	*Aphyocharax anisitsi*, ***Austrolebias alexandri***, *A. bellottii*, *A. nigripinnis*, *Corydoras undulatus*, *Cynopotamus argenteus*, *Cyphocharax platanus*, *Gymnogeophagus australis*, *Hoplosternum littorale*, *Leporinus striatus*, *Odontostilbe pequira*, *Pimelodus absconditus*, *Parastegophilus maculatus*, *Rineloricaria parva*, *Serrapinnus calliurus*	Uruguay River basin	LU
2	4.5–4.7	*Apareiodon affinis*, ***Bryconamericus poi***, ***Crenicichla minuano***, ***C. missioneira***, ***Diapoma uruguayense***, *Gymnogeophagus meridionalis*, *Bryconamericus straminneus*, *Pimelodella gracilis*, ***Trachelyopterus teaguei***	Uruguay River basin	LU
6	4.3–4.6	***Bryconamericus poi***, *Catathyridium* sp., *Characidium* aff. *zebra*, ***Crenicichla missioneira***, ***Hemiancistrus* cf. *votouro***, *Jenynsia onca*, ***Rhamdella longiuscula***, ***Trachelyopterus teaguei***	Uruguay River basin	LU
8	4.3–4.5	*Apareiodon affinis*, ***Bryconamericus poi***, *B. straminneus*, ***Crenicichla minuano***, ***C. missioneira***, ***Diapoma uruguayense***, *Gymnogeophagus meridionalis*, *Pimelodella gracilis*, ***Trachelyopterus teaguei***	Uruguay River basin	LU
13	4.2–4.4	*Apareiodon affinis*, *Catathyridium sp*, *Characidium* aff. *zebra*, ***Crenicichla missioneira***, *Cyphocharax saladensis*, ***Diapoma uruguayense***, *Loricariichthys melanocheilus*, *Pimelodella gracilis*	Uruguay River basin	LU
17	2.7–3.0	*Eigenmannia virescens*, ***Hypostomus roseopunctatus***, *H. uruguayensis*, *Odontesthes perugiae*	Uruguay and Negro rivers	LU
10	3.8–4.0	*Acestrorhynchus pantaneiro*, *Aphyocharax anisitsi*, *Apistogramma commbrae*, *Characidium* aff. *zebra*, *Steindachnerina brevipinna*, *Trachelyopterus albicrux*	Uruguay, Cuareim and upper Negro rivers	LU
11	4.6–4.9	*Astyanax lacustris*, *Gymnogeophagus balzanii*, *Hyphessobrycon eques*, *Leporinus lacustris*, *Moenkhausia* cf. *dichroura*, *Serrapinnus kriegi*	Middle Uruguay and Lower Cuareim rivers	LU
16	5.6–5.9	***Gymnogeophagus* aff. *setequedas***, ***G. peliochelynion***, ***G. pseudolabiatus***, ***Hemiancistrus* cf. *votouro***, ***Hisonotus ringueleti***, *Hyphessobrycon eques*, ***Leporinus amae***, *Leporinus lacustris*, *Moenkhausia* cf. *dichroura*, *Schizodon nasutus*, *Serrapinnus kriegi*	Middle Uruguay, and Cuareim and Arapey basins	LU
1	4.3–4.5	***Gymnogeophagus* aff. *setequedas***, ***G. peliochelynion***, ***G. pseudolabiatus***, ***Hemiancistrus* cf. *votouro***, ***Hisonotus ringueleti***, ***Leporinus amae***, *Moenkhausia* cf. *dichroura*, *Schizodon nasutus*	Cuareim and Arapey basins	LU
4	1.9–2.2	***Gymnogeophagus peliochelynion***, ***Hemiancistrus* cf. *votouro***, *Odontostilbe* sp.	Cuareim, Arapey, and Queguay basins	LU
5	4.6–4.9	***Ancistrus taunayi***, *Bryconamericus straminneus*, *Crenicichla scottii*, *Diapoma terofali*, ***Ectrepopterus uruguayensis***, ***Gymnogeophagus mekinos***, ***G. terrapurpura***, *Hoplias lacerdae*, *Hypostomus aspilogaster*	Uruguay basin and Rio de la Plata affluents	LU-LP
12	5.8–6.0	***Ancistrus taunayi***, *Bryconamericus straminneus*, *Cichlasoma dimerus*, *Diapoma terofali*, *Gymnogeophagus meridionalis*, *Heterocheirodon yatai*, *Iheringichthys labrosus*, *Pachyurus bonariensis*, *Paraloricaria vetula*, *Parapimelodus valenciennis*, *Pimelodus maculatus*	Uruguay basin and Rio de la Plata affluents	LU-LP
15	3.6–3.9	***Ancistrus taunayi***, *Diapoma terofali*, ***Ectrepopterus uruguayensis***, *Heterocheirodon yatai*, *Hoplias lacerdae*, *Pimelodus maculatus*	Uruguay basin and Rio de la Plata affluents	LU-LP
20	3.9–4.1	*Austrolebias nigripinnis*, *Cyphocharax platanus*, *Luciopimelodus pati*, *Pygocentrus nattereri*, *Salminus brasiliensis*, *Serrasalmus maculatus*	Uruguay River and Rio de la Plata affluents	LU-LP
14	2.3–2.5	*Hyphessobrycon boulengeri*, *Jenynsia lineata*, *Platanichthys platana*	Lower Uruguay River, Rio de la Plata affluents, and Laguna Merín basin	LU-LP-P

TABLE 1 | (Continued)

CA	EI	DS	FS	ER
18	2.3–2.5	*Characidium tenue, Diapoma alburnum, Hypostomus aspilogaster*	Uruguay River basin, Rio de la Plata affluents, Laguna Merín basin	LU-LP-P
7	11.5–11.8	*Ancistrus brevipinnis**, *Austrolebias charrua**, *A. cheradophilus**, *A. gymnoventris**, *A. luteoflammulatus**, *A. reicherti**, *A. wolterstorffi**, *Characidium orientale**, *Crenicichla punctata**, *Cynopoecilus melanotaenia**, *Diapoma speculiferum**, *Geophagus iporangensis, Gymnogeophagus gymnogenys**, *G. labiatus, Hemiancistrus punctulatus, Heterocheirodon jacuiensis**, *Hisonotus armatus**, *H. taimensis**, *Mimagoniates inequalis, Oligosarcus robustus, Otocinclus flexilis**, *Otothyris rostrata, Pimelodus pintado**, *Rhamdella eriarcha**, *Rineloricaria strigilata**	Laguna Merín basin and Atlantic coastal drainages	P
21	7.7–7.9	*Austrolebias charrua**, *A. cheradophilus**, *A. gymnoventris**, *A. luteoflammulatus**, *A. prognathus**, *A. viarius**, *A. wolterstorffi**, *Cynopoecilus melanotaenia**, *Heptapterus sympterygium, Otothyris rostrata, Pimelodus pintado**	Southern Laguna Merín basin and Atlantic coastal drainages	P
19	5.5–5.7	*Austrolebias juanlangi***, *A. quirogai***, *A. reicherti**, *Cichlasoma portalegrense**, *Diapoma speculiferum**, *Geophagus iporangensis, Heterocheirodon jacuiensis**, *Hisonotus armatus**, *Oligosarcus robustus, Pimelodus pintado**	Northern Laguna Merín basin and upper Negro River	P-LU

Lower Uruguay (LU) ecoregion. Thirteen CA included grid cells exclusively from LU and showed eight different patterns (Tab. 1; Fig. **S3**):

1. Uruguay River and lower sections of its main effluents; diagnostic taxa included species typical of large rivers and effluents, and adjacent permanent and seasonal wetlands; one endemic is to LU, the rest are also found in LP (Tab. 1, CA 0, 3, 9; Fig. 2A).
2. Uruguay and Negro rivers; diagnostic taxa included species typical of large rivers; one is endemic to LU (Tab. 1, CA 17).
3. Low Uruguay River basin; diagnostic taxa included species typical of large rivers and species from smaller streams; seven are endemic to this ecoregion, and the rest are also found in LP (Tab. 1, CA 2, 6, 8, 13; Figs. 2B,C).
4. Uruguay, Cuareim, and upper Negro rivers; diagnostic taxa included species typical of rivers and adjacent wetlands; all of them are also present in LP (Tab. 1, CA 10).
5. Middle Uruguay River and lower Cuareim River basin; diagnostic taxa included species typical of streams and adjacent wetlands; one is endemic to LU and P (Tab. 1, CA 11; Fig. 3A).
6. Middle Uruguay, Cuareim, and Arapey river basins; diagnostic taxa included species typical of medium to small size streams and adjacent wetlands; six are endemic to LU and two are endemic to the Cuareim River basin (Tab. 1, CA 16).
7. Cuareim and Arapey river basins; diagnostic taxa included species typical of medium and small size streams; six are endemic to LU and two are endemic to the Cuareim River basin (Tab. 1, CA 1).
8. Cuareim, Arapey, and Queguay river basins; diagnostic taxa included species typical of middle and small streams; two are endemic of LU (Tab. 1, CA 4; Fig. 3B).

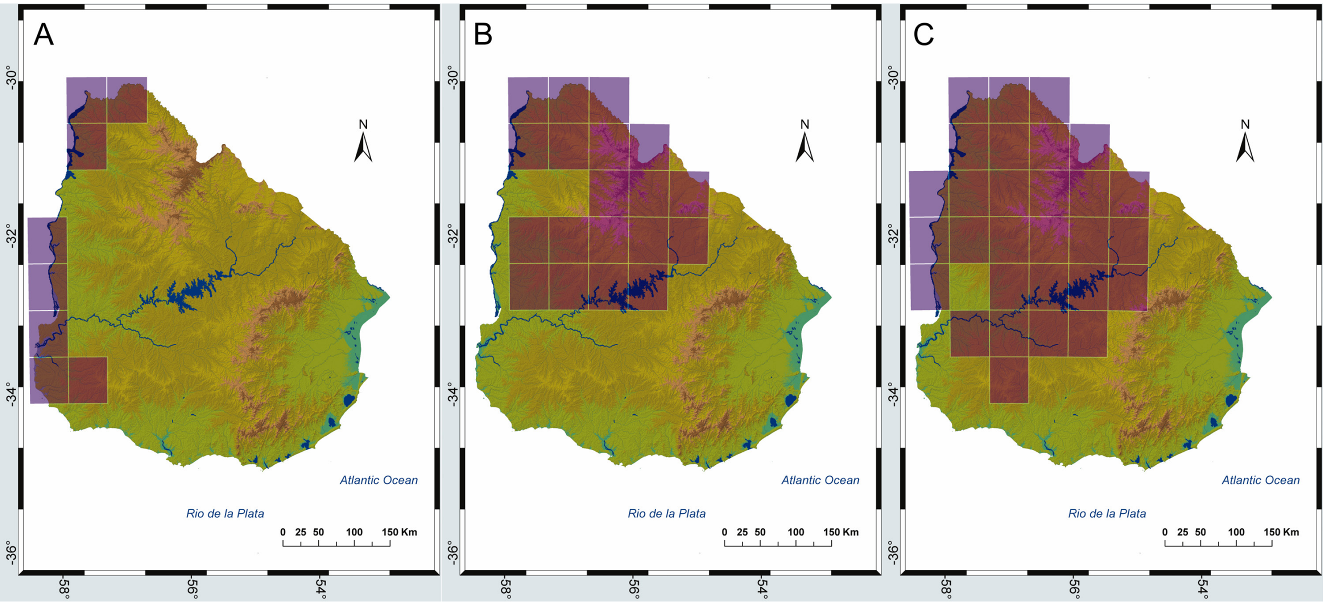

FIGURE 2 | Consensus Areas of Endemism obtained with the 59 x 59 km grid cell, representative of the Lower Uruguay ecoregion; **A**. CA 0; **B**. CA 6; **C**. CA 2.

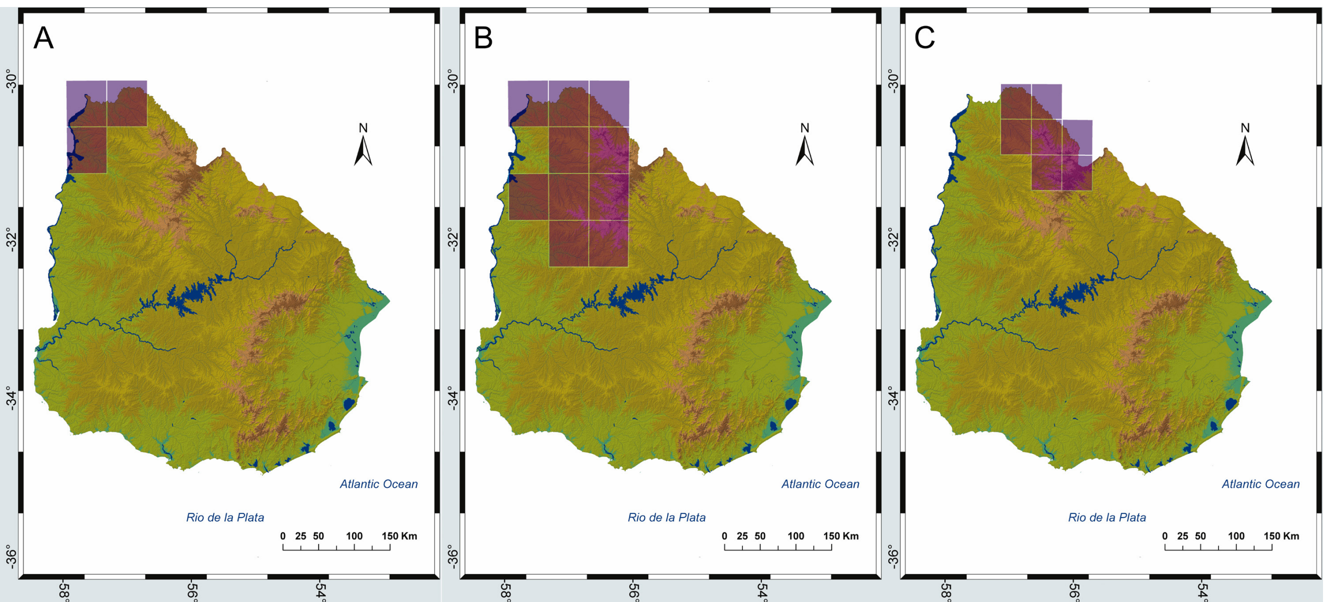

FIGURE 3 | Consensus Areas of Endemism representative of the Lower Uruguay ecoregion; **A**. CA 11 (59 x 59 km grid cell); **B**. CA 4 (59 x 59 km grid cell); **C**. CA 8 (45 x 45 km grid cell).

Lower Uruguay (LU) – Lower Parana (LP) ecoregions. Four CA included grid cells from LU and LP ecoregions (Tab. 1) and showed two different patterns:

1. All correspondent basins; diagnostic taxa included species of large and medium size streams, and adjacent permanent and seasonal wetlands; three are endemic to LU (Tab. 1, CA 5, 12, 15; Fig. 4A).

2. Lower sections effluents; diagnostic taxa included species typical of large rivers and inner estuary (including large migratory species) and adjacent seasonal wetlands; all are present in lower La Plata basin (Tab. 1, CA 20; Fig. 4B).

Lower Uruguay (LU) – Lower Parana (LP) – Patos (P). Two CA included grid cells from the three ecoregions (Tab. 1).

1. Lower Uruguay River, coastal Rio de la Plata effluents, and the whole Laguna Merín basin; diagnostic taxa included two widespread euryhaline species and one commonly found in wetlands of Patos and lower La Plata basin (Tab. 1, CA 14; Fig. 4C).

2. Most of the grid cells; diagnostic taxa included species typical of large and small streams; all of them are endemic to the LU and P (Tab. 1, CA 18).

Laguna dos Patos (P) ecoregion. Two CA included grid cells from P (Tab. 1).
1. The whole Laguna Merín basin and Atlantic coastal drainages; diagnostic taxa included species both from streams, and permanent and seasonal wetlands; at least 19 species are endemic to P (Tab. 1, CA 7; Fig. 5A).

2. Southern Laguna Merín basin (Cebollatí river basin) and Atlantic coastal lagoons; most diagnostic taxa included species typical of permanent and seasonal wetlands; all are endemic to P and three to southern Laguna Merín basin (Tab. 1, CA 21; Fig. 5B).

Patos (P) – Lower Uruguay (LU) ecoregions. Northern Laguna Merín basin and upper Negro River basin; diagnostic taxa included species from streams and species from permanent and temporary wetlands; most species are endemic of P and two annual fish species are distributed in upper Tacuarí (P), Yaguarón (P), and Negro river (LU) basins (Tab. 1, CA 19; Fig. 6A).

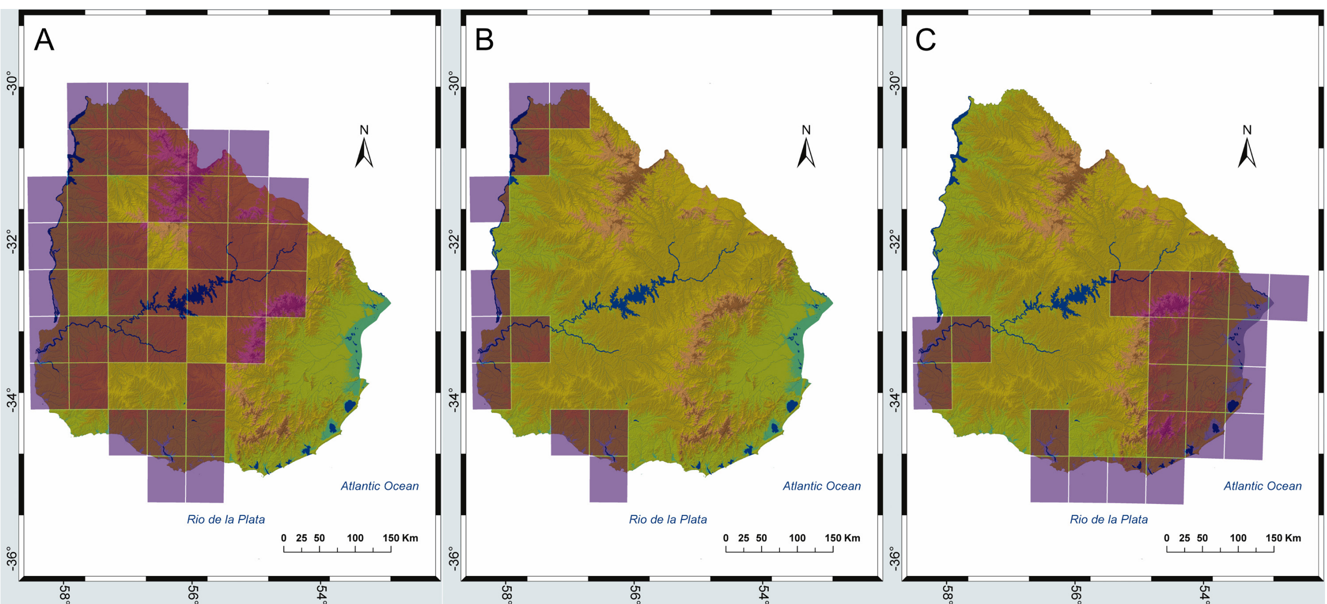

FIGURE 4 | Consensus Areas of Endemism obtained with the 59 x 59 km grid cell which included more than one ecoregion; **A**. CA 12 (LU + LP); **B**. CA 20 (LU + LP); **C**. CA 14 (LU + LP + P).

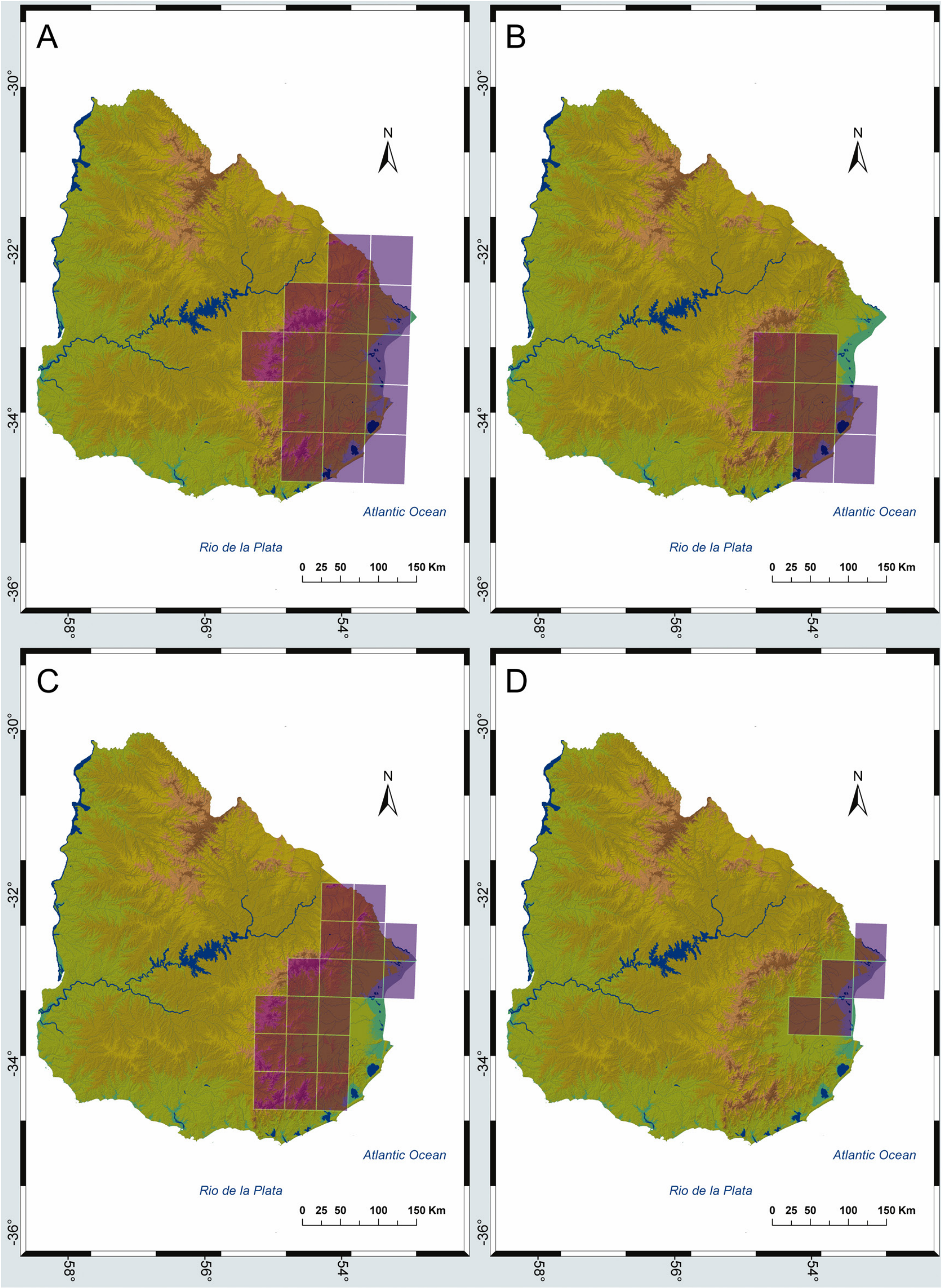

FIGURE 5 | Consensus Areas of Endemism representative of the Laguna dos Patos ecoregion; **A**. CA 7 (59 x 59 km grid cell); **B**. (59 x 59 km grid cell); **C**. CA 10 (45 x 45 km grid cell); **D**. CA 12 (45 km x 45 grid cell).

Analyses with 45 x 45 km grid cell identified 30 preliminary areas of endemism, which, after applying a loose consensus of 50% were reduced to 19 CA (Tab. 2; Fig. **S4**) with EI values varied between 1.9 and 9.3. Most areas and diagnostic taxa recovered were highly coincident and redundant with those obtained with the 59 km grid cells (Tabs. 1–2). For this reason, only areas obtained exclusively with 45 km grid cell will be fully described.

Lower Uruguay (LU) ecoregion. Eight CA included cells from LU. One corresponded exclusively to the Cuareim river basin; diagnostic taxa included species typical of streams; all of them are endemic to LU, two endemic to the Cuareim River basin (Tab. 2, CA 8; Fig. 3C).

Laguna dos Patos (P) ecoregion. Seven CA included grid cells from LP. One corresponded to most of the Laguna Merín basin; diagnostic taxa included species only found in rivers and streams, most of them are endemic to P (Tab. 2, CA 10; Fig. 5C). One corresponded to northern Laguna Merín basin; diagnostic taxa included species both typical of streams, and permanent and seasonal wetlands; all are endemic to P, one endemic to this section of Laguna Merín basin (Tab. 2, CA 12; Fig. 5D).

Laguna dos Patos – Lower Uruguay (P – LU) ecoregions. One CA recovered included grid cells both from the upper Negro (LU), Tacuarí (P), Yaguarón (P) river basins; diagnostic taxa are two annual species inhabitants of seasonal wetlands; both endemic of this area (Tab. 2, CA 14; Fig. 6B).

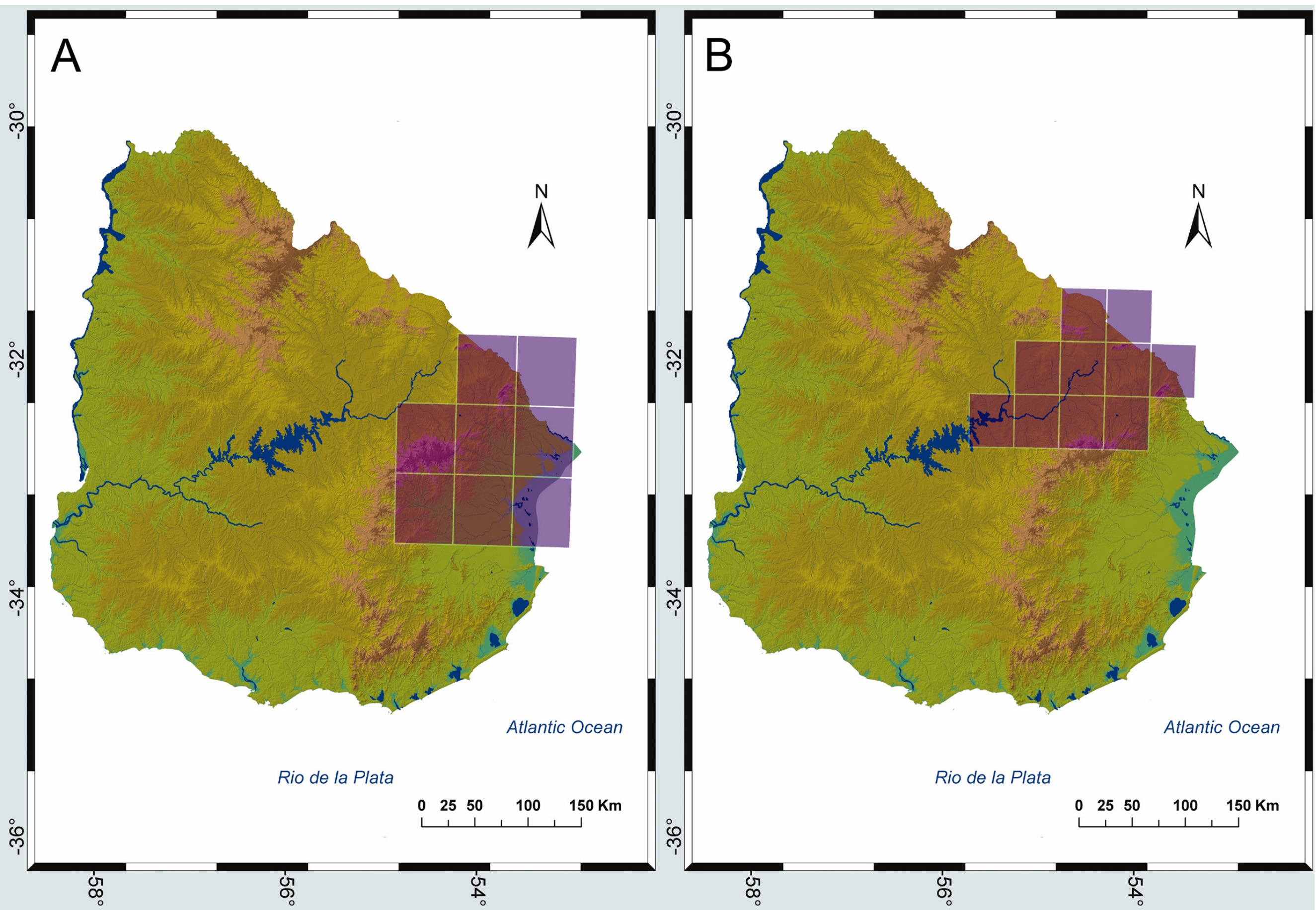

FIGURE 6 | Consensus Areas of Endemism that included Laguna dos Patos and Lower Uruguay ecoregions grid cells; **A**. CA 19 (59 x 59 km grid cell); **B**. CA 14 (45 x 45 km grid cell).

TABLE 2 | Consensus Areas of Endemism (CA) obtained with a 45 x 45 km grid cells, and their correspondent Endemic Indexes (EI), diagnostic species (DS), freshwater systems included (FS) and ecoregion occupied (ER); species endemic to Lower Uruguay (**black bold**); species endemic to Laguna dos Patos (*); species present in upper Negro, Tacuarí and Yaguarón rivers (**).

CA	EI	DS	FS	ER
4	5.4–5.7	*Ageneiosus militaris, Auchenipterus osteomystax,* ***Austrolebias alexandri****, A. bellottii, Corydoras undulatus, Cynopotamus argenteus, Gymnogeophagus australis, Leporinus striatus, Lepthoplosternum pectorale, Odontostilbe pequira, Pimelodus absconditus, Parastegophilus maculatus, Rineloricaria parva*	Uruguay River and lower affluents	LU
9	4.5–4.7	*Austrolebias bellottii, Cynopotamus argenteus, Hoplosternum littorale, Leporinus striatus, Odontostilbe pequira, Parastegophilusmaculatus*	Uruguay River and lower affluents	LU
17	5.0–5.3	*Ageneiosus militaris, Aphyocharax anisitsi, Auchenipterus osteomystax, Austrolebias bellottii, Cynopotamus argenteus, Gymnogeophagus australis, Leporinus striatus, Lepthoplosternum pectorale, Odontostilbe pequira, Pimelodus absconditus, Parastegophilus maculatus*	Uruguay River and lower affluents	LU
18	3.9–4.1	*Ageneiosus militaris, Apistogramma commbrae, Auchenipterus osteomystax, Galeocharax humeralis, Gymnogeophagus australis, Lepthoplosternum pectorale, Pimelodus absconditus*	Uruguay River and lower affluents	LU
5	2.5–2.7	*Acestrorhynchus pantaneiro, Steindachnerina brevipinna, Trachelyopterus albicrux*	Uruguay and Cuareim rivers	LU
7	4.8–5.0	***Gymnogeophagus* aff. *setequedas*, *G. pseudolabiatus*, *Hisonotus ringueleti***, *Hyphessobrycon eques, Leporinus lacustris, Moenkhausia* cf. *dichroura, Schizodon nasutus, Serrapinnuskriegi*	Middle Uruguay and Cuareim rivers	LU
16	4.1–4.4	*Astyanax lacustris*, *Corydoras hastatus*, *Gymnogeophagus balzanii*, *Hyphessobrycon eques*, *Leporinus lacustris*, *Serrapinnus kriegi*	Middle Uruguay and Lower Cuareim rivers	LU
8	2.6–2.8	***Gymnogeophagus* aff. *setequedas***, ***G. pseudolabiatus***, ***Hisonotus ringueleti***, ***Leporinus amae***	Cuareim basin	LU
2	2.8–3.1	*Austrolebias nigripinnis, Cyphocharax platanus, Luciopimelodus pati, Pygocentrus nattereri, Serrasalmus maculatus*	Uruguay River and Rio de la Plata affluents	LU-LP
6	2.6–2.9	*Megaleporinus obtusidens, Luciopimelodus pati, Salminus brasiliensis, Serrasalmus maculatus*	Uruguay River and Rio de la Plata affluents	LU-LP
11	2.4–2.6	***Ancistrus taunayi***, *Diapoma terofali*, ***Ectrepopterus uruguayensis***	Uruguay and Rio de la Plata basins	LU-LP
0	8.8–9.3	*Ancistrus brevipinnis*, Austrolebias charrua*, A. cheradophilus*, A. luteoflammulatus*, A. wolterstorffi*, Characidium orientale*, Crenicichla punctata*, Cynopoecilus melanotaenia*, Diapoma speculiferum*, Gymnogeophagus gymnogenys*, G. labiatus, Hemiancistrus* cf. *punctulatus, Hisonotus armatus*, H. taimensis*, Mimagoniates inequalis, Oligosarcus robustus, Otocinclus flexilis*, Pimelodus pintado*, Rhamdella eriarcha*, Rineloricaria strigilata**	Laguna Merín basin and Atlantic coastal drainages	P
10	7.8–8.2	*Ancistrus brevipinnis*, Characidium orientale*, Crenicichla punctata*, Diapoma speculiferum*, Gymnogeophagus gymnogenys*, G. labiatus, Heterocheirodon jacuiensis*, Hisonotus armatus*, Mimagoniates inequalis, Oligosarcus robustus, Otocinclus flexilis*, Rhamdella eriarcha*, Rineloricaria strigilata**	Laguna Merín basin and Atlantic coastal drainages	P
13	8.7–8.9	*Austrolebias charrua*, A. cheradophilus*, A. gymnoventris*, A. luteoflammulatus*, A. wolterstorffi*, Characidium orientale*, Crenicichla punctata*, Cynopoecilus melanotaenia*, Diapoma speculiferum*, Gymnogeophagus gymnogenys*, G. labiatus, Hisonotus taimensis*, Mimagoniates inequalis, Otocinclus flexilis*, Otothyris rostrata, Pimelodus pintado**	Laguna Merín basin and Atlantic coastal drainages	P
1	5.2–5.5	*Austrolebias charrua*, A. gymnoventris*, A. luteoflammulatus*, A. prognathus*, A. viarius*, A. wolterstorffi*, Cynopoecilus melanotaenia*, Heptapterus sympterygium, Otothyris rostrata*	Southern Laguna Merín basin and Atlantic coastal drainages	P
15	1.9–2.2	*Austrolebias* aff. *quirogai*, A. gymnoventris*, A. viarius*, Otothyris rostrata*	Southern Laguna Merín basin and Atlantic coastal drainages	P
3	4.2–4.5	*Austrolebias charrua*, A. cheradophilus*, A. wolterstorffi*, Geophagus iporangensis, Hisonotus armatus*, H. taimensis*, Mimagoniates inequalis, Otocinclus flexilis*, Pimelodus pintado**	Lower Laguna Merín basin	P
12	2.7–2.9	*Austrolebias reicherti*, Cichlasoma portalegrense*, Geophagus iporangensis, Hisonotus armatus*, Pimelodus pintado**	Northern Laguna Merín basin	P
14	1.9–2.2	*Austrolebias juanlangi**, A. quirogai***	Upper Negro and Yaguarón and Tacuarí rivers	P-LU

DISCUSSION

Some of the results of this study support the prediction of the hypothesis; most AE recovered were included within Abell *et al.* (2008) ecoregions limits, either in the Lower Uruguay or in the Laguna dos Patos ecoregions. However, some areas obtained challenged this view and occur across ecoregions and basins boundaries, either completely or partially. This implies the existence of historical processes that may have connected ecoregions and/or common ecological factors. This also suggests that the current ecoregion limits should be revised.

An important point to keep in mind is that due to the nature of our data base, restricted to political limits, not all areas recovered are necessarily AE according to Platnick´s (1991) definition, since most species that diagnosed them have wider distribution ranges. Nonetheless, the results obtained here highlight the usefulness of the method to recover AE at low spatial scales and to discover distribution patterns that also could be interpreted beyond the concept of AE, for example the identification of different assemblages in a community ecology context. This was evident with the results obtained in Lower Uruguay, where the analyses discriminated areas diagnosed by species typical from large rivers (59 km CA 0, 3, 9) from those of the tributaries (59 km CA 2, 6, 8), which suggest the action of ecological filters, physical (river flow, slope), geological, or biological (trophic web size). This was also observed in the discrimination of areas diagnosed by stream species (45 km CA 10) from areas diagnosed by permanent and seasonal wetlands species (45 km CA 1) in the Patos ecoregion, where filters seem to be more associated to the hydrological cycle.

Our analyses also showed that the indicator species of the Lower Uruguay ecoregion, at least in the area analyzed here, are almost exclusively distributed in the effluents (59 km CA 2, 6, 8, 13), and not in the Uruguay River main channel and adjacent wetlands, inhabited by species also common in Lower Parana (López *et al*., 2005; Almirón *et al*., 2015). The distribution of some freshwater decapods (Collins *et al*., 2011), is congruent with our findings and suggest that common historical and ecological factor are acting on the freshwater communities.

Within the Lower Uruguay ecoregion we also found nested areas that correspond to the limit between the lower and the Middle Uruguay River basin (59 km CA 1, 16; 45 km CA 7, 8, 16), which is defined by the Salto falls (Zaniboni Filho, Schulz, 2003), currently flooded by the Salto dam. One of them was diagnosed by four species widely distributed in wetlands or vegetated coastal habitats of most of La Plata basin (45 km CA 16), which are not found in the Uruguay River southern to the Salto Falls (Miquelarena *et al*., 2008; Serra *et al*., 2018). Noteworthy, this pattern is shared with *Aegla singularis* Ringuelet, 1948, a species of freshwater decapod endemic to the Upper and Middle Uruguay River basin (Tumini *et al*., 2019).

The other area suggests that the Cuareim river basin could be considered as an AE at a global scale (45 km CA 8). Although there is evidence that *Gymnogeophagus pseudolabiatus* Malabarba, Malabarba & Reis, 2015, is also distributed in Rio Grande do Sul, it is restricted to this basin (Malabarba *et al*., 2015). The recent description of the trichomycterid *Scleronema teiniagua* Ferrer & Malabarba, 2020 (not included in our analyses), endemic to the Cuareim River basin (Ferrer, Malabarba, 2020), supports this statement and highlights the importance of generating conservation strategies to protect its freshwater environments. Which historical and/or ecological factors may have generated this basin as an Area of Endemism are unknown.

Lower Parana, which in our analysis was represented by the small basins from the northern bank of the Rio de la Plata estuary, was only recovered as an AE together with grid cells from Lower Uruguay (59 km CA 5, 12, 15, 20). Similar to some areas found in Lower Uruguay, one pattern involved grid cells associated to the Uruguay River main channel or adjacent coastal areas and Rio de la Plata coast (59 km CA 20). This area was diagnosed by highly mobile and migratory species, commonly found in large rivers of the La Plata basin (Zaniboni Filho, Schulz, 2003; Almirón *et al*., 2015; Serra *et al*., 2019), and one annual fish species, that is commonly found in wetlands associated to the floodplains of both ecoregions (Costa, 2006). In spite of not being included as diagnostic in our analyses, the distribution of the annual fish *Austrolebias bellottii* (Steindchaner, 1881), widely distributed in Lower Parana and Lower Uruguay (García *et al*., 2012) coincides with this pattern.

However, the AE that included the Rio de la Plata effluent basins and the whole Lower Uruguay, was defined by species that are widely distributed in most effluents of the Uruguay river basin and absent from the rest of Lower Parana ecoregion. *Ancistrus taunayi* Miranda Ribeiro, 1918, is considered endemic of the Lower Uruguay ecoregion (Bertaco *et al*., 2016), *Ectrepopterus uruguayensis* (Fowler, 1943) is restricted to Lower Uruguay and Rio de la Plata effluents (Malabarba *et al*., 2012), and *Gymnogeophagus terrapurpura* Loureiro, Zarucki, Malabarba & González-Bergonzoni, 2016 and *Crenicichla scotti* (Eigenmann, 1907) are restricted to the Lower Uruguay, Rio de la Plata northern effluents, and some Atlantic coastal drainages (Loureiro *et al*., 2016a).

The more remarkable characteristic of the Rio de la Plata drainages is its lower species richness, where species commonly found in the rest of the area analyzed are absent or rare. Some of them are indeed widely distributed in the rest of Lower Uruguay and Laguna dos Patos ecoregions (Bertaco *et al*., 2016). Whereas others are relatively common in different environments of Lower Uruguay (Reis *et al*., 1990; Reis, Pereira, 2000; Almirón *et al*., 2007; Mariguela *et al*., 2013; Burress *et al*., 2018; Bono *et al*., 2019).

The Rio de la Plata estuary receives the discharges from the Paraná and Uruguay rivers, with the consequent arrival of many species typical from Lower Parana, that occasionally end up in the lower section of the Uruguay River and in Rio de la Plata estuary (García *et al*., 2010). Also, the most common large and medium size migratory species of the whole La Plata basin can be found in the coast of the estuary and in the mouth of streams and rivers, though with no evidence that they remain or breed there. On the other hand, Rio de la Plata shares exclusively two endemic species with Lower Uruguay (*G. terrapurpura* and *E. uruguayensis*) and none with Lower Parana. According to Bertora *et al*. (2018) and Paracampo *et al.* (2015), coastal drainages in the southern bank of Rio de la Plata have also reduced species richness. However, their species composition lack the endemic species of Lower Uruguay. These findings supports the idea that the estuary acts as a barrier to some freshwater species, influenced by the sea levels variations that have been occurred from the late Miocene to the Holocene (Lundberg *et al*., 1998; Martínez, del Río, 2005; Brea, Zucol, 2011; Martínez, Rojas, 2013). In this scenario, estuarine and marine environments may have reached the Lower Paraná, Uruguay, and Negro rivers creating a barrier against dispersion and colonization. The small area of the basins that flow to Rio de la Plata is also a possible cause of their low species richness.

These results suggest a reevaluation of the northern Rio de la Plata estuary basins as part of the Lower Parana ecoregion and a consideration to be included in Lower Uruguay. Furthermore, the physical landscape (geologic and geomorphologic) of Rio de la Plata drainages is more similar to adjacent Lower Uruguay than to Lower Parana (Brea, Zucol, 2011). Additionally, hydrological analyses have shown that the northern coast of Rio de la Plata is more influenced by the Uruguay River discharge than by the Parana River discharge, which mainly moves along the southern Rio de la Plata along the Argentinean coast (Piedra-Cueva, Fossati, 2007). Our results also suggest that the eastern limit of this area goes beyond the current external limit of the estuary and highlights the importance of the cyclic nature of this barrier to freshwater fishes.

Isolation is one of the main processes that favors endemicity (Dias *et al*., 2014) and Laguna dos Patos and other Atlantic coastal ecoregions high endemicity levels among Neotropical freshwater ecoregions (Albert *et al.,* 2011) and the nested areas we found in this analysis, are a good example of this. In this context, strong patterns of endemism were recovered in Laguna Merín southwestern basin (Laguna dos Patos) (59 km CA 7; 45 km CA 0, 10, 13), which were diagnosed by different combinations of up to 21 species widely distributed in the ecoregion (Costa, 2006; Bertaco *et al*., 2016; Loureiro *et al*., 2018). However, our results also indicate a nested area of endemism in the southern half of Laguna Merín basin and Atlantic coastal basins (59 km CA 21; 45 km CA 1). Diagnostic species include four threatened annual fish (Loureiro, Bessonart, 2017; Rosa, Lima, 2008): *Austrolebias cheradophilus* (Vaz-Ferreira, Sierra de Soriano & Scaglia de Paulete, 1965) (northern limit Yaguarón river basin), *A. luteoflammulatus* (Vaz-Ferreira, Sierra de Soriano & Scaglia de Paulete, 1965) (northern limit Tacuarí river basin), *A. viarius* (Vaz-Ferreira, Sierra de Soriano & Scaglia de Paulete, 1965) and *A. prognathus* (Amato, 1986) (northern limit Cebollatí River basin) Although three of these species are also recorded in Rio Grande do Sul (Volcan *et al*., 2010; Lanes *et al*., 2014), these records fall within the grid cells used in this study. These findings suggest that the southern Laguna Merín basin can be considered an AE at global scale. This AE includes large wetlands associated to the southern Laguna Merín and Atlantic coastal lagoons basins, currently isolated from Laguna Merín, which support the hypothesis that they may have been connected among each other at low sea level periods.

Our analyses also recovered one AE that trespasses the limits of two ecoregions, the Lower Uruguay and Laguna dos Patos (45 km CA 14). This area involves the upper Negro River basin (LU) and the upper Yaguarón and Tacuarí rivers (P), a flat area with large wetlands and a smooth basin divide. Diagnostic annual fish species are also present also in Rio Grande do Sul (Brazil) (Volcan *et al*., 2011, 2014), however these records are still included in the grid cells defined by our analysis. This distribution pattern was mentioned by Loureiro *et al*. (2011), and included three other annual fish species that present a wider distribution. Annual fishes are supposed to present low vagility and thus restricted distribution. The hypothesis to explain their presence in two different basin (and ecoregions) involves geomorphologic events such as river drainage rearrangements (Loureiro *et al*., 2011), and indicates that this area may have functioned as biogeographic corridor as previously suggested (Loureiro *et al*., 2016c, 2018). These results are supported by the phylogeographic pattern of *Cnesterodon decemmaculatus* (Ramos-Fregonezi *et al*., 2017; Souza *et al*., 2020).

In this article we analyzed the distribution patterns of freshwater fishes within the political limits of Uruguay, an area of confluence of three freshwater ecoregions. In spite of this limitation, our results corroborated the identity of two of them, Lower Uruguay and Laguna dos Patos, and also challenged the pertinence of considering the northern Rio de la Plata affluents as part of the Lower Parana ecoregion. Through the results and analysis of existing literature we showed that the limits of the areas obtained are influenced by a mixture of historical and ecological factors. In this context, these results agree with Dagosta, de Pinna (2018) suggestion to be cautious to take for granted hydrographic basins as biogeographic units.

This article supports what Simó *et al.* (2014) pointed out about Uruguayan territory as a biogeographic crossroads for various taxonomic groups. We also showed the utility of the AE not only to recover areas if endemism in freshwater fishes but also to identify different groupings of species associated to different habitats. In this sense AE identified three areas that may be considered as areas of endemism at a global scale that have to be considered in global, regional, and local conservation plans.

ACKNOWLEDGMENTS

We thank Dolores Casagranda, Carolina Correa, Raquel Gandolfo, Salvador Arias, and Pablo Goloboff for her kind help during JB stay in Tucumán; to all fish collectors that contributed to build national collections in Uruguay. We also thank the anonymous reviewers that improved the quality of the manuscript. ML and JCG belong to Sistema Nacional de Investigadores (SNI) of the Agencia Nacional de Investigación e Innovación (ANII) and Programa de Desarrollo de las Ciencias Básicas (PEDECIBA).

REFERENCES

- **Aagesen L, Szumik C, Goloboff P.** Consensus in the search for areas of endemism. J Biogeogr. 2013; 40(11):2011–16. https://doi.org/10.1111/jbi.12172
- **Abell R, Thieme ML, Revenga C, Bryer M, Kottelat M, Bogutskaya N *et al*.** Freshwater ecoregions of the World: a new map of biogeographic units for freshwater biodiversity conservation. BioScience. 2008; 58(5):403–14. https://doi.org/10.1641/B580507
- **Albert JS, Petry P, Reis RE.** Major biogeographic and phylogenetic patterns. In: Albert JS, Reis RE, editors. Historical biogeography of Neotropical freshwater fishes. Berkeley, Los Angeles, London: University of California Press; 2011. p.21–57. http://dx.doi.org/10.1525/california/9780520268685.003.0002
- **Albert JS, Tagliacollo VA, Dagosta F.** Diversification of Neotropical freshwater fishes. Annu Rev Ecol Evol Syst. 2020; 51:27–53. https://dx.doi.org/10.1146/annurev-ecolsys-011620-031032
- **Almirón AE, Casciotta JRC, Bechara J, Ruíz Díaz F, Bruno C, D'Ambrosio S *et al.*** *Imparfinis mishky* (Siluriformes, Heptapteridae) a new species from the ríos Paraná and Uruguay basins in Argentina. Rev Suisse Zool. 2007; 114(4):817–24. https://doi.org/10.5962/bhl.part.80416
- **Almirón AE, Casciotta JRC, Ciotek L, Giorgis P.** Guía de los peces del Parque Nacional Pre-Delta. Ciudad Autónoma de Buenos Aires, Argentina: Administración de Parques Nacionales; 2015. Available from: https://sib.gob.ar/archivos/bfa004429.pdf

- **Ayup-Zouain R.** Evolución paleogeográfica y dispersión de los sedimentos del río de la Plata. In: Menafra R, Rodríguez-Gallego L, Scarabino F, Conde D, editors. Bases para la conservación y manejo de la costa uruguaya. Montevideo: Vida Silvestre Uruguay; 2006. p.1–08.
- **Bertaco VA, Ferrer J, Carvalho FR, Malabarba LM.** Inventory of the freshwater fishes from a densely collected area in South America – a case study of the current knowledge of Neotropical fish diversity. Zootaxa. 2016; 4138(3):401–40. http://dx.doi.org/10.11646/zootaxa.4138.3.1
- **Bertora A, Grosman F, Sanzano P, Rosso JJ.** Fish fauna from the Langueyú basin, Argentina: a prairie stream in a heavily modified landscape. Check List. 2018; 14(2):461–70. http://dx.doi.org/10.15560/14.2.461
- **Bono A, Tencatt LFC, Alonso F, Lehmann-A P.** Redescription of *Corydoras undulatus* Regan, 1912 (Siluriformes: Callichthyidae), with comments on the identity of *Corydoras latus* Pearson, 1924. PLoS ONE. 2019; 14(1):e0211352. https://doi.org/10.1371/journal.pone.0211352
- **Brea M, Zucol AF.** Paraná-Paraguay basin: geology and paleoenvironments. In: Albert JS, Reis RE, editors. Historical biogeography of Neotropical freshwater fishes. Berkeley, Los Angeles, London: University California Press; 2011. p.69–87.
- **Burress ED, Alda F, Duarte A, Loureiro M, Armbruster JW, Chakrabarty P.** Phylogenomics of pike cichlids (Cichlidae: *Crenicichla*): the rapid ecological speciation of an incipient species flock. J Evol Biol. 2018; 31(1):14–30. https://doi.org/10.1111/jeb.13196
- **Casagranda MD, Roig-Juñent S, Szumik C.** Endemismo a diferentes escalas espaciales: un ejemplo con Carabidae (Coleoptera: Insecta) de América del Sur austral. Rev Chil Hist Nat. 2009; 82(1):17–42. http://dx.doi.org/10.4067/S0716-078X2009000100002
- **Collins PA, Giri F, Williner V.** Biogeography of the freshwater decapods in the La Plata basin, South America. J Crustac Biol. 2011; 31(1):179–91. http://dx.doi.org/10.1651/10-3306.1
- **Costa WJEM.** The South American annual killifish genus *Austrolebias* (Teleostei: Cyprinodontiformes: Rivulidae): phylogenetic relationships, descriptive morphology and taxonomic revision. Zootaxa. 2006; 1213(1):1–162. http://dx.doi.org/10.11646/zootaxa.1067.1.1
- **Dagosta FCP, de Pinna M.** A history of the biogeography of Amazonian fishes. Neotrop Ichthyol. 2018; 16(3):e180023. https://dx.doi.org/10.1590/1982-0224-20180023
- **Dias M, Oberdorff T, Hugueny B, Leprieur F, Jezequel C, Cornu JF *et al*.** Global imprint of historical connectivity on freshwater fish biodiversity. Ecol Lett. 2014; 17(9):1130–40. https://doi.org/10.1111/ele.12319
- **Ferraris CJ Jr.** Family Auchenipteridae (Driftwood catfishes). In: Reis RE, Kullander SO, Ferraris CJ Jr, organizers. Checklist of the freshwater fishes of South and Central America. Porto Alegre: Edipucrs; 2003. p.470–82.
- **Ferrer J, Malabarba LR.** Systematic revision of the Neotropical catfish genus *Scleronema* (Siluriformes: Trichomycteridae), with descriptions of six new species from Pampa grasslands. Neotrop Ichthyol. 2020; 18(2):e190081. https://doi.org/10.1590/1982-0224-2019-0081
- **Framiñan MB, Brown OB.** Study of the Rio de la Plata turbidity front, Part 1: spatial and temporal distribution. Cont Shelf Res. 1996; 16(10):1259–82. https://doi.org/10.1016/0278-4343(95)00071-2
- **Fricke R, Eschmeyer WN, Fong JD.** Eschmeyer's catalog of fishes: species by family/subfamily [Internet]. San Francisco: California; 2021. Available from: https://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp
- **García G, Gutiérrez V, Vergara J, Calviño P, Duarte A, Loureiro M.** Patterns of population differentiation in annual killifishes from the Paraná-Uruguay-La Plata basin: the role of vicariance and dispersal. J Biogeogr. 2012; 39(9):1707–19. http://dx.doi.org/10.1111/j.1365-2699.2012.02722.x
- **Garcia ML, Jaureguizar AJ, Protogino LC.** From fresh water to the slope: fish community ecology in the Río de la Plata and the sea beyond. Lat Am J Aquat Res. 2010; 38(1):81–94. http://dx.doi.org/10.3856/vol38-issue1-fulltext-8

- **Goloboff P.** NDM/VNDM, Programs for identification of areas of endemism. Program and documentation, version 3; 2016. Available from: http://www.lillo.org.ar/phylogeny/endemism/
- **Harold AS, Mooi RD.** Areas of endemism: definition and recognition criteria. Syst Biol. 1994; 43(2):261–66. http://dx.doi.org/10.1093/sysbio/43.2.261
- **Hovenkamp P.** Vicariance events, not areas, should be used in biogeographical analysis. Cladistics. 1997; 13(1–2):67–79. https://doi.org/10.1006/clad.1997.0032
- **Lanés LEK, Gonçalves ÂC, Volcan MV.** Discovery of endangered annual killifish *Austrolebias cheradophilus* (Aplocheiloidei: Rivulidae) in Brazil, with comments on habitat, population structure and conservation status. Neotrop Ichthyol. 2014; 12(1):117–24. https://doi.org/10.1590/S1679-62252014000100012
- **Lévêque C, Oberdorff T, Paugy D, Stiassny MLJ, Tedesco PA.** Global diversity of fish (Pisces) in freshwater. In: Balian EV, Lévêque C, Segers H, Martens K, editors. Freshwater animal diversity assessment. Dordrecht: Springer; 2007. p.545–67. (Developments in Hydrobiology; vol 198). http://dx.doi.org/10.1007/978-1-4020-8259-7_53
- **Lima FCT, Malabarba LR, Buckup PA, Pezzi da Silva JF, Vari RP, Harold A *et al.*** Genera *Incertae Sedis* in Characidae. In: Reis RE, Kullander SO, Ferraris Jr., CJ, organizers. Checklist of the freshwater fishes of South and Central America. Porto Alegre: Edipucrs; 2003. p.106–68.
- **Linder P.** On areas of endemism, with an example from the African Restionaceae. Syst Biol. 2001; 50(6):892–912. https://doi.org/10.1080/106351501753462867
- **López Hl, Miquelarena AM, Ponte Gómez J.** Biodiversidad y distribución de la ictiofauna mesopotámica. In: Aceñolaza FG, editor. Temas de la biodiversidad del litoral fluvial argentino II. Tucumán: Magna Publicaciones; 2005. p.311–54. (Miscelánea; Nº 14). Available from: http://www.insugeo.org.ar/publicaciones/docs/misc-14-01.pdf
- **Loucks OL.** A forest classification for the maritime provinces. Proc N S Inst Sci. 1962; 25(2):86–167. Available from: http://hdl.handle.net/10222/13605
- **Loureiro M, Bessonart J.** *Austrolebias viarius* (errata version published in 2018). The IUCN Red List of Threatened Species 2017:e.T187245A124132630. https://dx.doi.org/10.2305/IUCN.UK.2017-3.RLTS.T187245A1825294.en
- **Loureiro M, Duarte A, Zarucki M.** A new species of *Austrolebias* Costa (Cyprinodontiformes: Rivulidae) from northeastern Uruguay, with comments on distribution patterns. Neotrop Ichthyol. 2011; 9(2):335–42. https://doi.org/10.1590/S1679-62252011000200010
- **Loureiro M, García G.** Transgresiones y regresiones marinas en la costa atlántica y lagunas costeras del Uruguay: efectos sobre los peces continentales. In: Menara R, Rodríguez-Gallego, Scarabino F, Conde D, editors. Bases para la conservación y el manejo de la costa uruguaya. Montevideo: Vida Silvestre Uruguay; 2006. p545–55.
- **Loureiro M, Zarucki M, Malabarba LR, González-Bergonzoni I.** A new species of *Gymnogeophagus* Miranda Ribeiro from Uruguay (Teleostei: Cichliformes). Neotrop Ichthyol. 2016a; 14(1):e150082. https://doi.org/10.1590/1982-0224-20150082
- **Loureiro M, Serra WS, Scarabino F.** Colecciones Ictiológicas del Uruguay: pasado y presente. In: Del Moral Flores LF, Ramírez Villalobos AJ, Martínez Pérez JA, González Acosta AF, Franco López J, coordinators. Colecciones ictiológicas de Latinoamérica. Tlalnepantla: Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México; 2016b. p.400–14.
- **Loureiro M, Borthagaray A, Hernández D, Duarte A, Pinelli V, Arim M.** *Austrolebias* in space: scaling from ponds to biogeographical regions. In: Berois N, García G, Sá RO, editors. Annual fishes: life history strategy, diversity, and evolution. Boca Ratón: CRC Press; 2016c. p.111–32.
- **Loureiro M, Zarucki M, González I, Vidal N, Fabiano G.** Peces continentales. In: Soutullo A, Clavijo C, Martínez-Lanfranco JA, editors. Especies prioritarias para la conservación en Uruguay: Vertebrados, moluscos continentales y plantas vasculares. Montevideo: SNAP/DINAMA/MVOTMA y DICYT/MEC; 2013. p.91–112. Available from: http://vidasilvestre.org.uy/wp-content/uploads/2013/12/Especies-prioritarias-para-la-conservacion-en-Uruguay.pdf

- **Loureiro M, Sá RO, Serra WS, Alonso F, Nielsen D, Calviño P *et al.*** Review of the family Rivulidae (Cyprinodontiformes, Aplocheiloidei) and a molecular and morphological phylogeny of the annual fish genus *Austrolebias* Costa, 1998. Neotrop Ichthyol. 2018; 16(3):e180007. https://doi.org/10.1590/1982-0224-20180007
- **Lundberg JG, Marshall JG, Guerrero J, Horton B, Malabarba MC, Wesslingh F.** The stage for neotropical fish diversification: a history of tropical South American rivers. In: Malabarba LR, Reis RE, Vari RP, Lucena ZMS, Lucena CAS, editors. Phylogeny and classification of Neotropical fishes. Porto Alegre: Edipucrs; 1998. p.13–48.
- **Malabarba LR, Bertaco VA, Carvalho FR, Litz TO.** Revalidation of the genus *Ectrepopterus* Fowler (Teleostei: Characiformes), with the redescription of its type species, *E. uruguayensis*. Zootaxa. 2012; 3204(1):47–60. http://dx.doi.org/10.11646/zootaxa.3204.1.5
- **Malabarba LR, Malabarba MC, Reis RE.** Descriptions of five new species of the Neotropical cichlid genus *Gymnogeophagus* Miranda Ribeiro, 1918 (Teleostei: Cichliformes) from the rio Uruguay drainage. Neotrop Ichthyol. 2015; 13(4):637–62. https://doi.org/10.1590/1982-0224-20140188
- **Malabarba LR, Malabarba MC.** Phylogeny and classification of Neotropical fish. In: Baldissetotto B, Urbinati E, Cyrino J, editors. Biology and physiology of freshwater Neotropical fish. Cambridge: Academic Press; 2020. p.1–19.
- **Mariguela TC, Alexandrou MA, Foresti F, Oliveira C.** Historical biogeography and cryptic diversity in the Callichthyinae (Siluriformes, Callichthyidae). J Zool Syst Evol Res. 2013; 51(4):308–15. http://dx.doi.org/10.1111/jzs.12029
- **Martínez SA, del Río CJ.** Las ingresiones marinas del Neógeno en el sur de Entre Ríos (Argentina) y Litoral Oeste de Uruguay y su contenido malacológico. In: Aceñolaza FG, editor. Temas de la biodiversidad del litoral fluvial argentino II. Tucumán: Magna Publicaciones; 2005. p.13–26. (Miscelánea; Nº 14). Available from: http://www.insugeo.org.ar/publicaciones/docs/misc-14-01.pdf
- **Martínez S, Rojas A.** Relative sea level during the Holocene in Uruguay. Palaeogeogr Palaeoclimatol Palaeoecol. 2013; 374:123–31. https://doi.org/10.1016/j.palaeo.2013.01.010
- **Miquelarena AM, Mantinián JE, López HL.** Peces de la Mesopotamia Argentina (Characiformes: Characidae: Cheirodontinae). In: Aceñolaza FG, editor. Temas de la biodiversidad del litoral III. Tucumán: INSUGEO; 2008. p.51–90. (Miscelánea INSUGEO; vol 17; No 2). Available from: http://www.insugeo.org.ar/publicaciones/docs/misc-17-2-01.pdf
- **Moritz C.** Strategies to protect biological diversity and the evolutionary processes that sustain it. Syst Biol. 2002; 51(2):238–54. https://doi.org/10.1080/10635150252899752
- **Morrone JJ.** Endemism. In: Jørgensen SE, Fath BD, editors. Encyclopedia of ecology. Amsterdam, Boston: Elsevier; 2008. p.1254–59.
- **Morrone JJ, Crisci JV.** Historical biogeography: introduction to methods. Annu Rev Ecol Syst. 1995; 26:373–401. http://dx.doi.org/10.1146/annurev.es.26.110195.002105
- **Moyle PB, Crain PK, Whitener K, Mount JF.** Alien fishes in natural streams: fish distribution, assemblage structure, and conservation in the Cosumnes River, California, USA. Environ Biol Fishes. 2003; 68:143–62. https://doi.org/10.1023/B:EBFI.0000003846.54826.a6
- **Olson D, Dinerstein E, Wikramanayake E, Burgess N, Powell G, Underwood E *et al.*** Terrestrial ecoregions of the world: a new map of life on Earth. BioScience. 2001; 51(11):933–38. https://doi.org/10.1641/0006-3568(2001)051[0933:TEOTWA]2.0.CO;2
- **Paracampo A, García I, Mugni H, Marrochi N, Carriquiriborde P, Bonetto C.** Fish assemblage of a Pampasic stream (Buenos Aires, Argentina): temporal variations and relationships with environmental variables. Stud Neotrop Fauna Environ. 2015; 50(3):145–53. https://doi.org/10.1080/01650521.2015.1065658
- **Piedra-Cueva I, Fossati M.** Residual currents and corridor of flow in the Rio de la Plata. Appl Math Model. 2007; 31(3):564–77. http://dx.doi.org/10.1016/j.apm.2005.11.033
- **Platnick N.** On areas of endemism. (Commentary). In: Ladiges PY, Humphries CJ, Martinelli LW. Austral Biogeography. Melbourne: CSIRO; 1991. p.1–12.

- **Ramos-Fregonezi A, Malabarba LR, Fagundes NJ.** Population genetic structure of *Cnesterodon decemmaculatus* (Poeciliidae): a freshwater look at the Pampa biome in Southern South America. FrontGenet. 2017; 8:214. https://doi.org/10.3389/fgene.2017.00214
- **Reis RE, Pereira EH.** Three new species of the loricariid catfish genus *Loricariichthys* (Teleostei: Siluriformes) from southern South America. Copeia. 2000; (4):1029–47. http://dx.doi.org/10.1643/0045-8511(2000)000[1029:TNSOTL]2.0.CO;2
- **Reis RE, Weber C, Malabarba LR.** Review of the genus *Hypostomus* Lacepede, 1803 in Southern Brazil, with descriptions of three new species (Pisces, Siluriformes, Loricariidae). Rev Suisse Zool. 1990; 97(1):729–66. http://dx.doi.org/10.5962/bhl.part.79760
- **Richardson DM, Whittaker RJ.** Conservation biogeography – foundations, concepts and challenges. Divers Distrib. 2010; 16(3):313–20. https://doi.org/10.1111/j.1472-4642.2010.00660.x
- **Rosa RS, Lima FCT.** Os peixes brasileiros ameaçados de extinção. In: Machado ABM, Drummond GM, Paglia AP, editors. Livro vermelho da fauna brasileira ameaçada de extinção. Brasília, Belo Horizonte: Ministério do Meio Ambiente, Fundação Biodiversitas; 2008. p.9–285. Available from: https://www.icmbio.gov.br/portal/images/stories/biodiversidade/fauna-brasileira/livro-vermelho/volumeII/Peixes.pdf
- **Serra WS, Loureiro M, Clavijo C, Alonso F, Scarabino F, Rios N.** Peces del bajo Rio Uruguay: especies destacadas. Paysandú: CARU; 2019.
- **Serra WS, Scarabino F, Paullier S.** First record of *Serrapinnus kriegi* (Schindler, 1937) and confirmed presence of *S. calliurus* (Boulenger, 1900) for Uruguay (Characiformes: Characidae). Ichthyological Contributions of PecesCriollos. 2018; 59:1–06. Available from: https://usercontent.one/wp/pecescriollos.de/wp-content/uploads/2020/12/ICP-59-Serra-et-al-2018-First-record-of-Serrapinnus-kriegi-from-Uruguay.pdf
- **Simó M, Guerrero JC, Giuliani L, Castellano I, Acosta L.** A predictive modeling approach to test distributional uniformity of Uruguayan harvestmen (Arachnida: Opiliones). Zool Stud. 2014; 53:50. http://dx.doi.org/10.1186/s40555-014-0050-2
- **Souza MS, Thomaz AT, Fagundes NJ.** River capture or ancestral polymorphism: an empirical genetic test in a freshwater fish using approximate Bayesian computation. Biol J Linnean Soc. 2020; 131(3):575–84. https://doi.org/10.1093/biolinnean/blaa140
- **Szumik CA, Goloboff PA.** Areas of endemism: an improved optimality criterion. Syst Biol. 2004; 53(6):968–77. https://doi.org/10.1080/10635150490888859
- **Szumik CA, Pereyra VV, Casagranda MD.** Areas of endemism: to overlap or not to overlap, that is the question. Cladistics. 2019; 35(2):198–229. https://doi.org/10.1111/cla.12343
- **Tumini G, Giri F, Williner V, Collins PA, Morrone JJ.** Selecting and ranking areas for conservation of *Aegla* (Crustacea: Decapoda: Anomura) in southern South America integrating biogeography, phylogeny and assessments of extinction risk. Aquat Conserv. 2019; 29(5):693–705. https://doi.org/10.1002/aqc.3098
- **Turcati A, Serra WS, Malabarba LR.** A new mouth brooder species of *Gymnogeophagus* with hypertrophied lips (Cichliformes: Cichlidae). Neotrop Ichthyol. 2018; 16(4):e180118. https://doi.org/10.1590/1982-0224-20180118
- **Violante RA, Parker G.** The post-last glacial maximum transgression in the de la Plata River and adjacent inner continental shelf, Argentina. Quat Int. 2004; 114(1):167–81. https://doi.org/10.1016/S1040-6182(03)00036-3
- **Volcan MV, Gonçalves ÂC, Lanés LEK.** Distribution, habitat and conservation status of two threatened annual fishes (Rivulidae) from southern Brazil. Endanger Species Res. 2011; 13:79–85. http://dx.doi.org/10.3354/esr00316
- **Volcan MV, Gonçalves ÂC, Lanés LEK.** *Austrolebias quirogai* (Actinopterygii: Cyprinodontiformes: Rivulidae) in Brazil: occurrence, population parameters, habitat characteristics, and conservation status. Acta Ichthyol Piscat. 2014; 44(1):37–44. http://dx.doi.org/10.3750/AIP2014.44.1.05

- **Volcan MV, Lanés LEK, Cheffe MM.** Distribuição e conservação de peixes anuais (Cyprinodontiformes: Rivulidae) no município do Chuí, sul do Brasil. Biotemas. 2010; 23(4):51–58. http://dx.doi.org/10.5007/2175-7925.2010v23n4p51
- **Wiens JJ, Donoghue MJ.** Historical biogeography, ecology and species richness. Trends Ecol Evol. 2004; 19(12):639–44. https://doi.org/10.1016/j.tree.2004.09.011
- **Zaniboni Filho E, Schulz UH.** Migratory fishes of the Uruguay River. In: Carolsfeld J, Harvey B, Ross C, Baer A, editors. Migratory fishes of South America: biology, fisheries and conservation status. Ottawa: World Fisheries Trust, World Bank, IDRC; 2003. p.157–94.

AUTHORS' CONTRIBUTION

José Bessonart: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Writing-original draft, Writing-review and editing.

Marcelo Loureiro: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing-original draft, Writing-review and editing.

José Carlos Guerrero: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Supervision, Writing-original draft, Writing-review and editing.

Claudia Szumik: Conceptualization, Formal analysis, Investigation, Methodology, Writing-original draft, Writing-review and editing.

ETHICAL STATEMENT

Not applicable.

COMPETING INTERESTS

The authors declare no competing interests.

HOW TO CITE THIS ARTICLE

- **Bessonart J, Loureiro M, Guerrero JC, Szumik C.** Distribution of freshwater fish from the Southern Neotropics reveals three new areas of endemism and show diffuse limits among freshwater ecoregions. Neotrop Ichthyol. 2021; 19(4):e200153. https://doi.org/10.1590/1982-0224-2020-0153

Neotropical Ichthyology

OPEN ACCESS

SBI Official Journal of the Sociedade Brasileira de Ictiologia