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Distinct evolutionary lineages of *Hoplisoma carlae* (Siluriformes: Callichthyidae) due to dispersal barriers

Linajes evolutivos distintos de *Hoplisoma carlae* (Siluriformes: Callichthyidae) debido a barreras de dispersión

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Abstract

The freshwater systems of South America have been strongly shaped by natural barriers and landscape fragmentation, driving high levels of endemism and diversification among Neotropical fishes. In this context, the subfamily Corydoradinae, particularly the genus *Hoplisoma*, offers an ideal model to investigate how geographic isolation has influenced evolutionary processes. This study focuses on *Hoplisoma carlae*, a species endemic to the Iguazú River basin, a region characterized by major dispersal barriers such as waterfalls, rapids, mountain ranges, and dams. We conducted an integrated analysis combining mitochondrial DNA (COI) sequences and morphological data from populations of *H. carlae* from Argentina and Brazil. Phylogenetic and population structure analyses revealed three well-differentiated groups corresponding to (1) the Urugua-í stream, (2) the upstream Iguazú River ("upstream A"), and

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(3) downstream sections of the Iguazú (including specimens from near the type locality of *H. carlae*) ("upstream B"). These groups are likely undergoing recent divergence driven by geographic isolation. The divergence time of *H. carlae* coincides with the formation of the Iguazu Falls. Our results suggest that natural barriers, including mountain ranges like the Sierra de la Victoria and historical waterfalls, along with recent anthropogenic barriers such as the Urugua-í dam, have contributed to population fragmentation and ongoing lineage divergence in *H. carlae*. These findings highlight the importance of preserving genetic diversity in fragmented freshwater ecosystems.

Keywords: Genetic divergence, Iguazú Waterfalls, isolation, population structure, Sierra de la Victoria, Urugua-í Stream.

Resumen

Los sistemas de agua dulce de Sudamérica han sido fuertemente moldeados por barreras naturales y la fragmentación del paisaje, lo que ha impulsado altos niveles de endemismo y diversificación entre los peces neotropicales. En este contexto, la subfamilia Corydoradinae, y en particular el género *Hoplisoma*, constituye un modelo ideal para investigar cómo el aislamiento geográfico ha influido en los procesos evolutivos. Este estudio se centra en *Hoplisoma carlae*, una especie endémica de la cuenca del río Iguazú, una región caracterizada por importantes barreras de dispersión, como cascadas, rápidos, cordilleras y represas. Realizamos un análisis integrado que combina secuencias de ADN mitocondrial (COI) y datos morfológicos de poblaciones de *H. carlae* de Argentina y Brasil. Los análisis filogenéticos y de estructura poblacional revelaron tres grupos claramente diferenciados, correspondientes a: (1) el arroyo Urugua-í, (2) el tramo superior del río Iguazú ("upstream A") y (3) los tramos inferiores del Iguazú (incluyendo ejemplares cercanos a la localidad tipo de *H. carlae*) ("upstream B"). Es probable que estos grupos estén experimentando una divergencia reciente impulsada por el aislamiento geográfico. El tiempo de divergencia estimado para *H. carlae* coincide con la formación de las Cataratas del Iguazú. Nuestros resultados sugieren que barreras naturales —como la Sierra de la Victoria y las cascadas históricas—, junto con barreras antropogénicas recientes, como la represa del Urugua-í, han contribuido a la fragmentación poblacional y a la divergencia en curso de linajes en *H. carlae*. Estos hallazgos subrayan la importancia de preservar la diversidad genética en ecosistemas de agua dulce fragmentados.

Palabras clave: Aislamiento, arroyo Urugua-í, Cataratas del Iguazú, divergencia genética, estructura poblacional, Sierra de la Victoria.

INTRODUCTION

Riverine populations are forecasted to be particularly vulnerable to fragmentation due to their dendritic structure, which may be exacerbated by unidirectional migration (Fagan, 2002, 2005). Natural barriers, such as waterfalls, rapids, and mountain ranges, often restrict dispersal and promote genetic differentiation among populations (Wofford et al., 2005). Anthropogenic structures, such as dams, can have more complex effects: while they frequently fragment habitats and accentuate isolation, artificial channels built to facilitate reproductive migration may occasionally connect populations that were historically isolated (Wu et al., 2019). These factors play a critical role in shaping the genetic structure and evolutionary trajectories of freshwater organisms, especially in species-rich regions like the Neotropics (Agostinho et al., 2008, 2016). The freshwater systems of South America, especially those of the La Plata Basin, illustrate this dynamic. The Iguazu River, a major tributary of the Paraná River, flows through a highly fragmented landscape characterized by spectacular waterfalls, steep canyons, and rugged topography (Stevaux and Latrubesse, 2010). The most emblematic feature of this region is the Iguazu Falls, a system of enormous cascades that not only constitutes a significant ecological landmark but also acts as a major biogeographic barrier, isolating aquatic populations and promoting diversification. The Iguazu River hosts a high number of endemic species and a great diversity of fish species (Baumgartner et al., 2012; Delariva et al., 2018; Ranuccii Pini et al., 2021). However, the Iguazu Falls are not the only waterfalls in this region; numerous mountain ranges, such as the Sierra de la Victoria (Rolón and Chebez, 1998), contribute to the region's complex hydrographic network by creating local watersheds and restricting aquatic connectivity.

The dynamic biogeographic history and fragmentation of South American freshwater systems have played a crucial role in shaping the diversity of fish groups (Montoya-Burgos, 2003) and the subfamily Corydoradinae, one of the most species-rich groups of Neotropical catfishes, currently comprises over 220 valid species (Fricke et al., 2025). These small-bodied fishes exhibit a body shape ranging from laterally compressed to moderately depressed and possess spines on the pectoral fins, cryptic and aposematic color patterns, and the repeated evolution of Müllerian co-mimicries (Alexandrou et al., 2011). They inhabit freshwater systems predominantly in tropical South America, including rivers, streams, and floodplain habitats, with a few species extending into subtropical and high-altitude (Alonso et al., 2025a). Many Corydoradinae display schooling behaviour and benthic habits (Paxton, 1997), and they are notable for their diverse coloration and morphological variation, features that have contributed to their popularity in the aquarium trade.

Historically, the majority of Corydoradinae species were classified under the genus *Corydoras* Lacépède, 1803, which for decades was considered one of the most species-rich genera among Siluriformes. However, phylogenetic studies have revealed that *Corydoras sensu lato* is paraphyletic, as demonstrated by phylogenetic hypothesis generated by morphological (Britto, 2003; Reis, 1998), molecular (Alexandrou et al., 2011; Dias et al., 2025; Marburger et al., 2018), and combined data (Alonso et al., 2025b). Dias et al. (2025) proposed the resurrection of four genera previously synonymized with *Corydoras*: *Brochis* Cope 1871, *Gastrodermus* Cope 1878, *Hoplisoma* Swainson 1838, and *Osteogaster* Cope 1894. Later Alonso et al., (2025b) erected the genus *Urkumayu* Alonso, Terán, Aguilera, Montes, Serra Alanís, Calviño, Vera-Alcaraz, Cardoso, Koerber and Mirande (2025) to include a separate lineage composed of three species from andean northwestern La Plata basin. This taxonomic revision aimed to restore the monophyly of *Corydoras* while recognizing distinct evolutionary lineages within the subfamily. Following this new reclassification, *Hoplisoma* emerged as the most species-rich genus within Corydoradinae, encompassing nearly 90 valid species (Dias et al., 2025; Alonso et al., 2023). These fishes inhabit temperate and tropical freshwater systems across central and South America, making them a key group for studying biogeographic patterns and evolutionary processes in Neotropical aquatic ecosystems.

Despite recent taxonomic advances, the assessment of diversity within *Hoplisoma*, particularly in the La Plata Basin, remains challenging due to ongoing species discoveries and name changes across taxonomic levels (Alonso et al., 2025b). For instance, da Rocha et al. (2022) integrated mitochondrial, nuclear, cytogenetic, and morphological data to examine the delimitation of *Hoplisoma carlae* (Nijssen and Isbrücker, 1983) and an undescribed species, *Hoplisoma* sp., both from the tributaries of the Iguazú River, situated upstream and downstream of Iguazú Falls. Their results revealed distinct mitochondrial (COI) and nuclear (RAG1) haplotypes for the two taxa, along with consistent morphometric differences, suggesting the absence of gene flow and ongoing lineage divergence, suggesting distinct evolutionary units undergoing recent speciation. However, their study did not include specimens from or nearby the type locality of *H. carlae*, a small creek, left bank tributary of Río Iguazu, 50 kilometers from Puerto Iguazu, Paraná basin, Misiones Province, Argentina (Nijseen and Isbrucker, 1983) or any populations from Argentinean tributaries, limiting our understanding of the species' full genetic and morphological variability. To address these knowledge gaps, the present study expands upon the work of da Rocha et al. (2022) by conducting an integrated morphometric and mitochondrial genetic analysis of *Hoplisoma carlae* (Figure 1), incorporating samples from near the type locality of *H. carlae* and additional populations from Argentinean tributaries such as Urugua-í Stream (Figure 2).

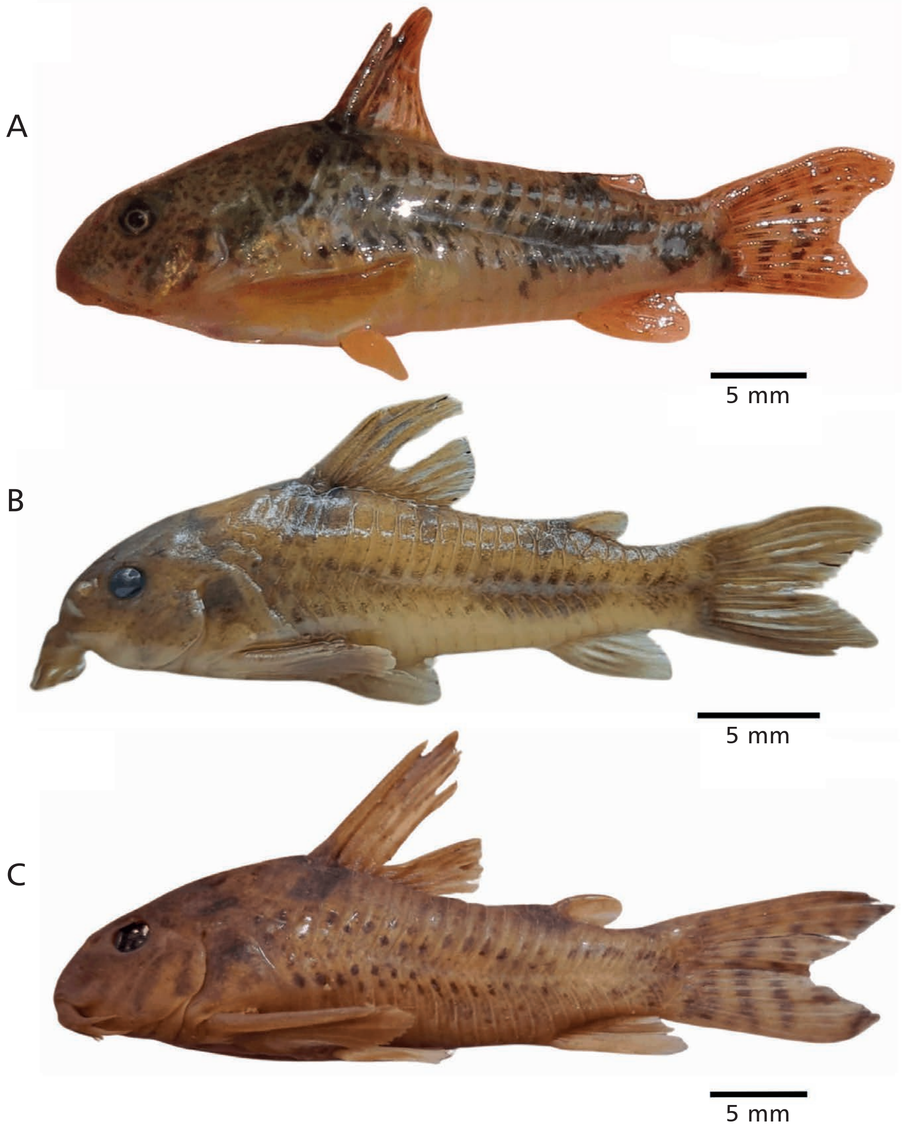

Figure 1. *Hoplisoma carlae*. A) *H. carlae* "Urugua-í" in vivo collected in Tirica Stream. B) *H. carlae* "Urugua-í" (CFA-IC/5494) collected in Tirica Stream. C) *H. carlae* "upstream B" (MACN-IC/12629) collected in Deseado Chico Stream. All of them from Misiones Province, Argentina.

Figura 1. *Hoplisoma carlae*. A) *H. carlae* "Urugua-í" in vivo colectado en el arroyo Tirica. B) *H. carlae* "Urugua-í" (CFA-IC/5494) colectado en el arroyo Tirica. C) *H. carlae* "upstream B" (MACN-IC/12629) colectado en el arroyo Deseado Chico. Todos colectados en la Provincia de Misiones, Argentina.

By combining morphometric and molecular dataset, this research aims to clarify the populations studied putatively assigned to *H. carlae a priori*, assess patterns of genetic differentiation, and contribute to a deeper understanding of the diversification within *Hoplisoma* in the La Plata Basin—a biogeographically complex and ecologically significant Neotropical region.

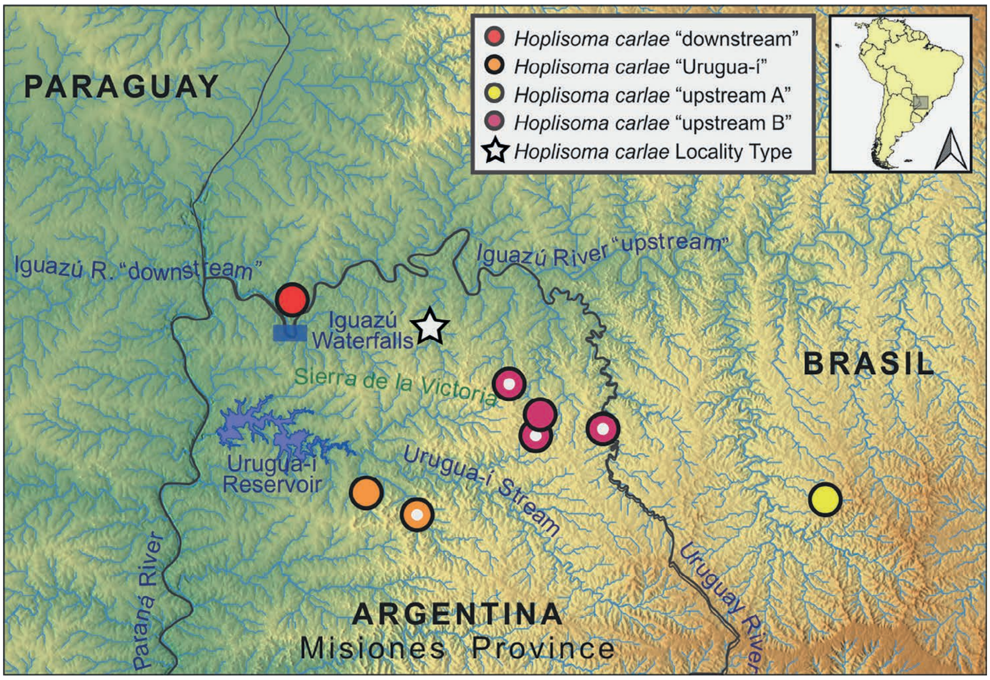

Figure 2. Study area for *Hoplisoma carlae* including the four sampling areas. The circles with a smaller white circle in the centre represent individuals for which only morphological measurements were taken.

Figure 2. Área de estudio para *Hoplisoma carlae* incluyendo los 4 sitios de muestreo. Los círculos con un pequeño circulo blanco en su interior representa individuos que fueron únicamente medidos morfométicamente.

MATERIALS AND METHODS

Sampling

In Argentina, fish were collected with the permission of the local authorities. The study was approved by the National Council of Scientific and Technical Research of Argentina (exp. 7879/14) and it is a requirement of this institution to follow the guidelines of its "Comité de Ética" (https://www.conicet.gov.ar/wp-content/uploads/OCR-RD-20050701-1047.pdf) and its biological sampling guide (https://proyectosinv.conicet.gov.ar/solicitud-colecta-cientifica/). Also, fish handling during sampling was performed following guidelines of the UFAW Handbook on the Care and Management of Laboratory Animals (http://www.ufaw.org.uk). Fish were anesthetized and killed using water containing a lethal dose of eugenol (clove oil).

According to the original description of *H. carlae* (Nijssen and Isbrucker, 1983), its type locality is a stream on a left-bank tributary of the Río Iguazú, located approximately 50 km from Puerto Iguazú along National Route 101, on a left-bank tributary of the Río Iguazú that lies upstream of Iguazú Falls (Figure 2). Fishes were caught with seine nets or trawl nets. The specimens collected were identified using the original descriptions and updated taxonomic literature (Nijssen and Isbrucker, 1983, da Rocha et al., 2022).

Voucher specimens were deposited in the fish collections of the Fundación de Historia Natural “Félix de Azara” Buenos Aires (CFA-IC) and Museo Argentino de Ciencias Naturales “Bernardino Rivadavia” (MACN-IC) (Table S1).

Molecular data

For the molecular analyses, three specimens from the Iguazú River near the type locality “upstream B” and six individuals “Urugua-í” from the Urugua-í Stream basin were used (Figure 2). Additionally, sequences available in GenBank (from da Rocha et al., 2022) were included in the analyses, corresponding to *H. carlae* “upstream A” and “downstream” of the Iguazú waterfalls (See details in Table S1). For the morphometric analyses, 14 individuals of *H. carlae* “upstream B” and five individuals of *H. carlae* “Urugua-í” were used (Table S2).

Tissue samples were stored in 98% ethanol, and DNA was extracted using the salt extraction protocol (Aljanabi and Martinez, 1997). A total of 31 sequences of *Hoplisoma carlae* were analysed, where 22 were obtained from GenBank (Table S1). A 612-base pair (bp) of the mitochondrial cytochrome c oxidase I gene (COI) was amplified by polymerase chain reaction (PCR) from each fish sample. The following primers were used for COI amplification: SILCOI-D and SILCOI-R (Jardim de Queiroz et al., 2020). The amplifications were performed in a final volume of 25 μl containing 1X Green GoTaq Reaction Buffer, 0.2 mM dNTP mix, 0.5 μM of each primer, 1.25 U GoTaq DNA polymerase (Promega, Madison, WI, USA), and 50–100 ng of DNA template. The amplification protocol consisted of 94°C for 1 min; 35 cycles of 94°C for 30 s, 52–56° C for 30 s, and 72°C for 1 min; and a final extension of 72°C for 10 min. PCR products were visualized in a 1% agarose gel. Purification and sequencing, in one direction, were performed by the company Macrogen, Inc. (Seoul, South Korea). The sequences were aligned using BioEdit 7.1.3.0 (Hall, 1999). All new sequences were deposited in Genbank (Table S1).

Phylogenetic reconstruction and haplotype network

To test the monophyly of the *Hoplisoma carlae* populations studied, a phylogenetic reconstruction was performed with maximum likelihood (ML) using MEGA 7.0.26 (Kumar et al., 2016). The branch support values of the nodes were obtained using 1,000 bootstrap pseudoreplicates (Felsenstein, 1985). In the ML analysis, the optimal nucleotide substitution model (HKY+G+I) was selected according to the Bayesian information criterion (BIC) as implemented by JModelTest 2.1.10 (Darriba et al., 2015). We also graphed the minimum spanning network of the COI haplotypes using PopART 1.7 (Leigh and Bryant, 2015).

Population genetic analyses

To assess the role of geographical distance on genetic structure, we used the Mantel test (Mantel, 1967). We built a matrix of genetic distance by localities and a matrix of geographical distance. However, the geographical distance between localities in a riverscape is poorly estimated when calculated based on the geographical coordinates of the localities (the distance separating two localities is generally not a straight line). Thus, geographical distances following the course of the rivers were calculated with Google Earth Pro. Each distance between pairs of localities was estimated in kilometres, with which a matrix was made.

Morphometric analyses

Morphometric analyses were conducted to assess variation in overall body shape to assess if there are significant differences between different populations. Morphometric character measurements were performed using a digital caliper (Table S2). The measurements used are the same as those used by da Rocha et al. (2022). To accommodate the effect of the size of the measured specimens, proportions were calculated using the measurement of each variable (mm) in relation to the standard length (SL- for measurements referring to the post cephalic portion of the body) and in relation to the head length (HL- measurements referring to the head). Prior to any analysis, all these measurements were transformed to logarithmic scales. Then, this dataset was used to do a Principal Components Analysis (PCA). The PCA was conducted on percentage-transformed morphometric variables to account for size variation with the ade4 package in R (Dray and Dufour, 2007; R Core Team, 2025).

RESULTS

Molecular phylogenetic tree

The mitochondrial COI gene sequences were effective in discriminating *Hoplisoma carlae* from other species within the genus. All *H. carlae* populations formed a monophyletic group (Figure 3A). In the haplotype network (Figure 3B), five distinct haplotypes were observed, with each population represented by its corresponding haplotype, except for the *H. carlae* “downstream” population, which exhibited two haplotypes. Individuals of *H. carlae* from “Urugua-í” genetically differ from a *H. carlae* “upstream B” by eight mutations, while individuals *H. carlae* from “downstream” only differ by one mutation from *H. carlae* “upstream B”. The individuals *H. carlae* from “upstream A” also differ by eight mutations from *H. carlae* “upstream B”.

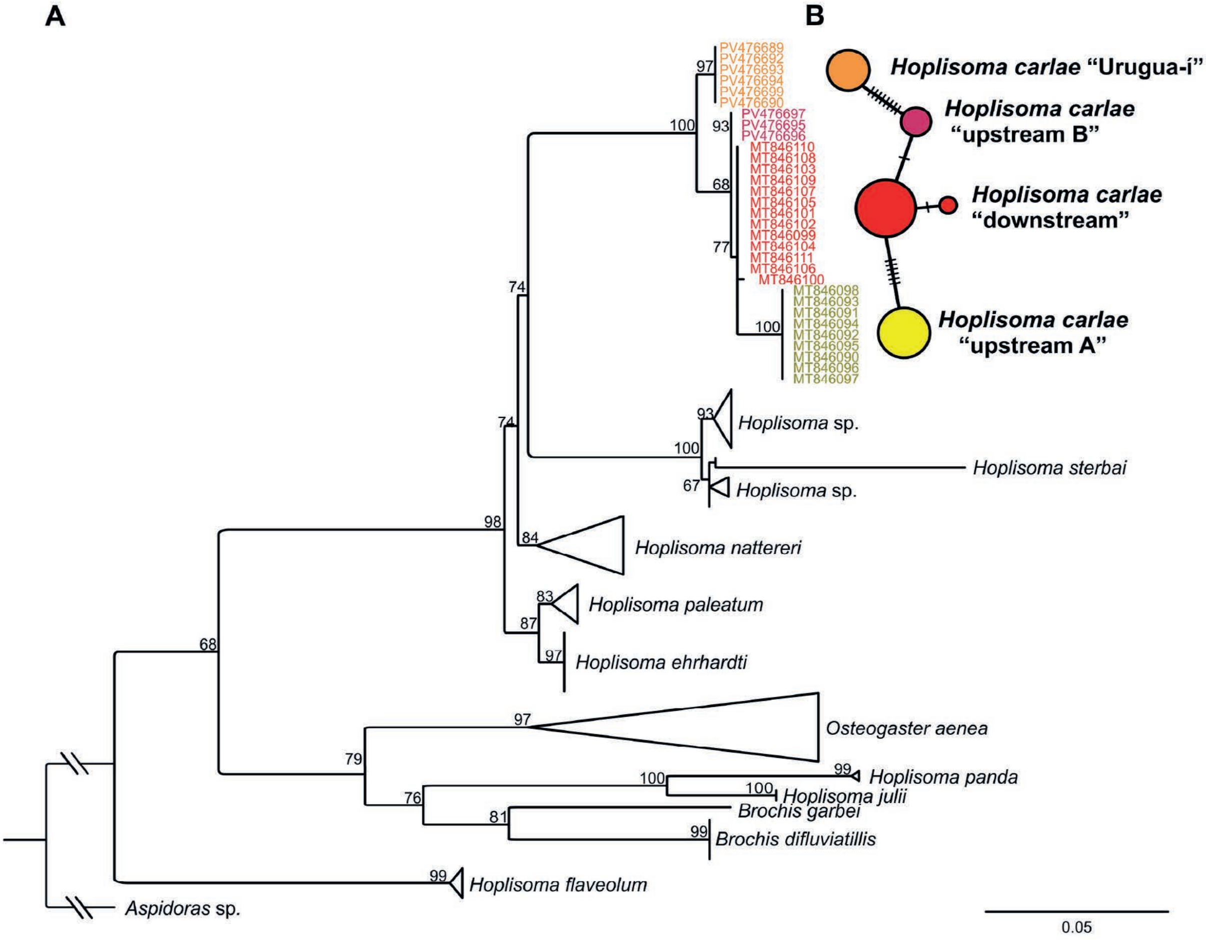

Figure 3. Phylogenetic ML tree and haplotype network for Corydoradinae. A) Maximum-likelihood tree of *Hoplisoma* based on 612 nucleotides of the mitochondrial gene COI. Bootstrap values are shown above the branches. Values below 60 are not shown. B) Haplotype network coloured by sampling sites.

Figure 3. Árbol filogenético de ML y red de haplotipos para Corydoradinae. A) Árbol de Maximum-likelihood para *Hoplisoma* basado en 612 nucleótidos del gen mitocondrial COI. Valores de Bootstrap valores son mostrados encima de las ramas. Valores por debajo de 60 no son mostrados. B) Red de haplotipos coloreada según los sitios de muestreo.

Regarding genetic distances, the smallest percentage was observed between individuals from near the type locality and those from Iguazú "downstream" (0.23%), while the greatest distance was found between "Urugua-í" and Iguazú "upstream A" (2.34%) (Table S3). The Mantel test results were not significant ($p = 0.172$), suggesting that the populations are not subject to isolation by distance.

Morphometric analyses

No differences were found in the variables to differentiate individuals of *H. carlae* “upstream B” from those of *H. carlae* “Urugua-í” (Table 1, Figure 1). All measurements showed very similar values. Regarding the principal component analysis (PCA), the first two axes exhibited eigenvalues >1 and were therefore retained for interpretation, together explaining PC1= 23.18% and PC1= 15.02% of the total variance, respectively (Figure 4A). Individuals of *H. carlae* “Urugua-í” were predominantly aligned along the positive side of the first axis, while most individuals of *H. carlae* “upstream B” were aligned along the negative side of the same axis, although some overlap was observed on the positive side with individuals of *H. carlae* “Urugua-í” (Figure 4A). The positive side of the first axis was associated with higher values of 1) Standard length, 16) Maximum width of the cleitrrum, 19) Head height, and 23) Internarial distance (Figure 4B), whereas the negative side of the first axis was related to the remaining characters.

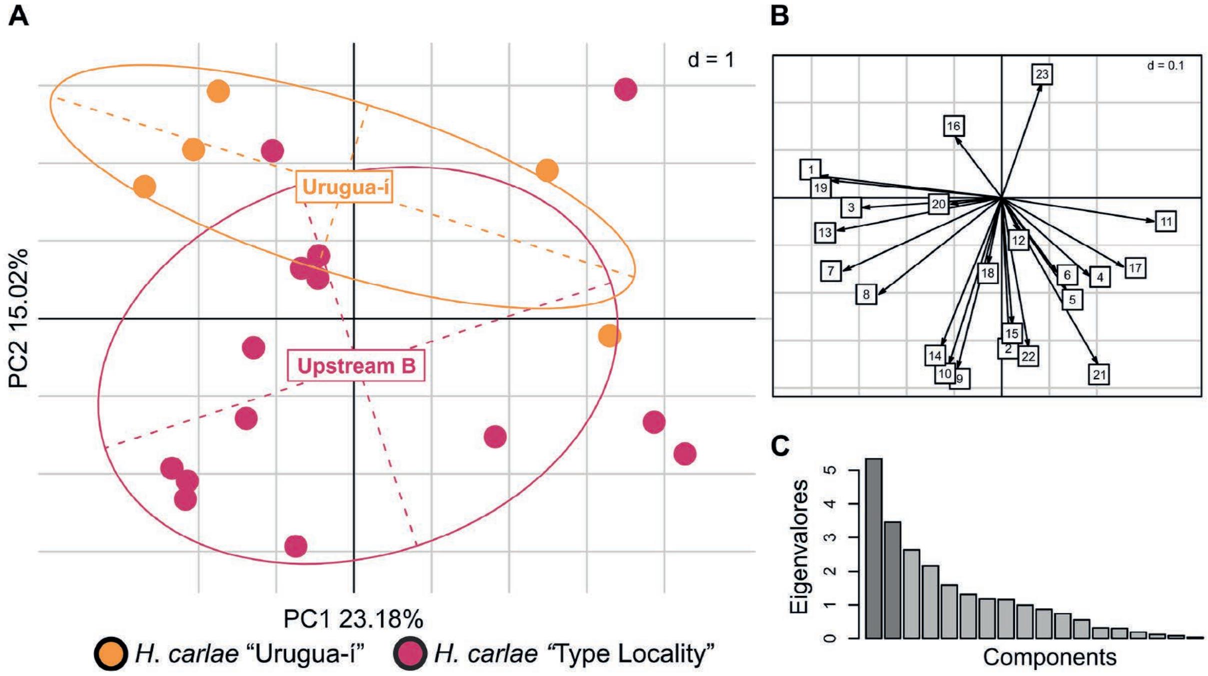

Figure 4. Principal Component Analysis of morphometric data table of *Hoplisoma carlae* “upstream B” and *H. carlae* from “Urugua-í”. A) Projection of individuals’ scores onto the first factorial plane of PCA, axis 1 horizontal and axis 2 vertical; B) Correlation of the variables labelled as in Table 1; C) Eigenvalues.

Figure 4. Análisis de Componentes Principales de la tabla de datos morfométricos de *Hoplisoma carlae* “upstream B” y *H. carlae* “Urugua-í”. A) Proyección de las puntuaciones de los individuos sobre el primer plano factorial del PCA, eje 1 (horizontal) y eje 2 (vertical); B) Correlación de las variables, identificadas según la Tabla 1; C) Eigenvalues.

Table 1. Morphometric measurements of the specimens of *Hoplisoma carlae* "upstream B" and *H. carlae* "Urugua-í", and morphometric proportions in relation to the standard length (SL) and the head length (HL).

Tabla 1. Medidas morfométricas de los ejemplares de *Hoplisoma carlae* "upstreamB" y *H. carlae* "Urugua-í", y proporciones morfométricas en relación con la longitud estándar (SL) y la longitud de la cabeza (HL).

Parameter	*Hoplisoma carlae* "upstream B"			*Hoplisoma carlae* "Urugua-í"		
	Minimum	Maximum	Average	Minimum	Maximum	Average
1. Standard length (mm)	**24.72**	**41.44**	**37.0**	31.45	43.78	38.434
% standard length						
2. Thorax length	13.40	22.74	20.58	15.98	24.30	18.68
3. Abdomen length	21.63	37.43	32.53	24.64	37.67	32.29
4. Body height at the origin of the dorsal fin	21.01	35.54	31.74	26.85	34.73	31.09
5. Predorsal distance	31.38	50.05	45.78	37.86	48.86	44.38
6. Prepelvic distance	29.25	51.51	46.94	35.91	48.78	43.44
7. Preanal distance	48.10	85.94	76.85	59.63	85.18	74.62
8. Preadipose distance	52.69	90.07	79.37	63.75	90.73	78.69
9. Dorsal-fin spine length	15.40	32.09	25.24	16.47	25.78	21.01
10. Pectoral-fin spine length	13.83	28.06	24.37	18.55	24.22	21.60
11. Caudal peduncle height	9.99	14.69	13.24	11.73	14.36	13.21
12. Adipose-fin spine lenght	6.10	10.83	9.25	7.75	11.53	9.53
13. Distance between the end of the base of the dorsal-fin and the origin of the spine of the adipose-fin	11.64	25.55	20.16	15.72	27.37	22.18
14. Dorsal-fin base length	13.86	23.88	20.07	13.71	21.13	18.50
15. Anal-fin base length	6.54	14.56	10.79	7.88	11.60	9.92
16. Maximum width of the cleiter	16.85	28.90	25.63	17.69	29.79	25.41
17. Head length (mm)	9.22	16.03	14.31	12.89	16.04	14.61
% head length						
18. Maxillary barbell length	19.07	54.42	39.02	26.83	47.91	40.08
19. Head height	50.51	89.22	76.21	65.49	85.20	77.11
20. Interorbital distance	19.56	33.67	29.03	24.84	34.97	30.81
21. Horizontal orbit diameter	13.90	23.40	20.09	17.18	21.08	18.96
22. Snout length	23.12	43.11	36.70	31.34	40.10	35.89
23. Internareal distance	10.06	17.95	15.91	12.93	18.48	16.07

DISCUSSION

Although our sample sizes are limited, the observed patterns provide preliminary evidence of lineage differentiation in populations *a priori* determined as *Hoplisoma carlae*, with important biogeographic and conservation implications. Our results revealed three clear populations in *H. carlae*, divided into: (1) Urugua-í Stream, (2) near type locality "upstream B" and "downstream" of the Iguazú Falls, and (3) "upstream A" section of the Iguazú Falls. These groups are likely undergoing a recent process of diversification driven by significant dispersal barriers, such as waterfalls and rapids of the Iguazú River and its tributaries, including the Urugua-í Stream, as well as the presence of mountain ranges in the region. Morphometric data between the populations from the Urugua-í Stream and near the type locality do not show clear variation that differentiate each group. Therefore, despite the genetic differences, there is no reliable way to distinguish these populations morphometrically.

In this study, all pairwise comparisons among *Hoplisoma carlae* populations showed genetic distances between 0.23% (Iguazú "upstream B" vs Iguazú "downstream") and 2.34% ("Urugua-í" population vs Iguazú "upstream A"). These last two populations with the highest genetic distance value are the most distant in the haplotype network and are the ones with the greatest geographical distances following the course of the rivers. Our findings are consistent with those of da Rocha et al. (2022), who, using mitochondrial (COI) and nuclear (RAG1) markers along with morphometric analyses, identified distinct evolutionary lineages between *H. carlae* "upstream A" and *H. carlae* "downstream" (referred to as *Hoplisoma* sp. in their work). In our study, sequences from near the type locality of *H. carlae* "upstream B" differ by only one mutation from those of the "downstream" individuals, despite being geographically separated by the big Iguazú Falls. This suggests that the waterfalls have not acted as a barrier between these two populations, as has been previously studied in other regions (Ebner et al., 2021; Kano et al., 2012; Torrente-Vilara et al., 2011). Or that the "downstream" population is a newly established population (since it is easy to "fall down the waterfall," but not to climb up). Conversely, individuals from the type locality are separated by several mutations from those inhabiting the Urugua-í Stream. This genetic differentiation suggests limited dispersal between these two populations, likely driven by the absence of hydrological connectivity imposed by the Sierra de la Victoria (Figure 2), which acts as a significant topographic barrier in the region. These two populations face multiple obstacles to their free dispersal: the Urugua-í Stream dam (which replaced the historic "Salto del Urugua-í"), the Iguazú Falls, and the absence of water bodies that directly connect the Urugua-í Stream with the type locality due to the Sierra de la Victoria.

The Sierra de la Victoria, located in northeastern Misiones Province, Argentina, is part of the Misiones Mountain System and extends approximately 70 km south of Iguazú National Park. This mountain range, with its dome-shaped ridges, acts as a local watershed divide. Streams originating from its central axis flow northward into the Iguazú River and eastward into the San Antonio River, which marks part of the Argentina–Brazil border (Frenguelli, 1946). To the south and southwest, waters drain into the Urugua-í Stream, a tributary of the Upper Paraná River (Rolón and Chebez, 1998). Due to the region's basaltic and sandstone geology, many watercourses descend in waterfalls and rapids, forming steep canyons and gorges that act as natural dispersal barriers for freshwater species (Frenguelli, 1946). These physical features could contribute to both historical and recent patterns of diversification among regional fish lineages as the structure and the diversity of the *H. carlae*. Today, many geomorphological barriers, such as rapids and waterfalls, that may have contributed to population fragmentation (Maack, 2012), are now submerged due to the construction of dams (Baumgartner et al., 2012; Souza-Shibatta et al., 2018). A key example for this study is the Urugua-í stream dam, inaugurated in 1991, which is the largest reservoir in the province of Misiones, Argentina, with a surface area of almost 90 km^2 (Gómez, 2015). Before its construction, the Urugua-í waterfall acted as a natural barrier that prevented the free dispersal of fish from the Paraná River (Menni, 2004; Ringuelet, 1975). Although the dam is a recent barrier, it is known that the impact of such constructions influences the current configuration of populations, threatening genetic diversity and the survival of species (Souza-Shibatta et al., 2018).

Notably, the Iguazú Falls, formed during the Pleistocene (~1.5–2.0 Mya) through active headward erosion (Stevaux and Latrubesse, 2010), coincide temporally with the divergence times estimated for *H. carlae* lineages in the study of (da Rocha et al., 2022). The falls have historically isolated the ichthyofauna of the Iguazú River (Zawadzki et al., 1999), leading to remarkably high levels of endemism—estimated at approximately 61% (Abell et al., 2008; Hales and Petry, 2015; Baumgartner et al., 2012). This pattern is exemplified by killifishes *Argolebias adrianae* and *Argolebias guarani*, which occur on opposite sides of the Iguazú Falls and represent a striking case of allopatric divergence driven by this barrier (Alonso et al., 2024). In recognition of its ecological distinctiveness, the Iguazú River basin has been classified as an ecoregion separate from the rest of the Paraná basin (Abell et al., 2008).

Similar patterns of diversification linked to geomorphological barriers have been documented in other fish taxa within the La Plata Basin, such as *Oligosarcus*, where the formation of the Iguazú and Sete Quedas waterfalls during the Pliocene and Pleistocene fostered high levels of endemism (Baumgartner et al., 2012; Langeani et al., 2007; Wendt et al., 2019). These examples reinforce the central role that physical barriers and landscape evolution have played in the diversification of Neotropical ichthyofauna.

In summary, the genetic and morphological differentiation observed among populations of *Hoplisoma carlae* is likely driven by a combination of ancient and more recent geographic barriers, including waterfalls, rapids, mountain ranges, and dams. These features continue to shape the structure of regional biodiversity, underscoring the need for further studies focused on analysing and preserving the genetic diversity of freshwater species in this ecologically complex and evolutionarily significant region. In this context, our findings indicate that the three lineages of *H. carlae*—Urugua-í Stream, near type locality plus downstream, and upstream Iguazú—should be regarded as distinct management units, since they represent independent evolutionary trajectories shaped by geographic barriers. Considering them as separate units is crucial to preserve the full spectrum of genetic diversity and adaptive potential within the species. By integrating evolutionary insights with conservation planning, this study contributes both to the understanding of diversification processes in the Iguazú system and to the development of strategies aimed at safeguarding its unique freshwater biodiversity.

Table S1 (1 of 2). Information for each sequence of *Hoplisoma carlae* analysed. The rows highlighted in light blue correspond to individuals sequenced for this study.

Tabla S1 (1 de 2). Información de cada secuencia de *Hoplisoma carlae* analizada. Las filas resaltadas en azul claro corresponden a los individuos secuenciados para este estudio.

Genbank Number	Sequenced by	Collection Code	Code	Specie	Locality Name	Locality Number	Basin	Country	Province	Locality	Latitude	Longitude
PV476689	This work	CFA-IC-5494	FHN-2797	*Hoplisoma carlae*	Urugua-i	1	Urugua-í Basin	Argentina	Misiones	Brazo del arroyo Tirica	-25,98858345	-54,28870119
PV476690	This work	CFA-IC-5494	FHN-2798	*Hoplisoma carlae*	Urugua-i	1	Urugua-í Basin	Argentina	Misiones	Brazo del arroyo Tirica	-25,98858345	-54,28870119
PV476691	This work	CFA-IC-5494	FHN-2799	*Hoplisoma carlae*	Urugua-i	1	Urugua-í Basin	Argentina	Misiones	Brazo del arroyo Tirica	-25,98858345	-54,28870119
PV476692	This work	CFA-IC-5494	FHN-2800	*Hoplisoma carlae*	Urugua-i	1	Urugua-í Basin	Argentina	Misiones	Brazo del arroyo Tirica	-25,98858345	-54,28870119
PV476693	This work	CFA-IC-5494	FHN-2801	*Hoplisoma carlae*	Urugua-i	1	Urugua-í Basin	Argentina	Misiones	Brazo del arroyo Tirica	-25,98858345	-54,28870119
PV476694	This work	CFA-IC-5491	FHN-2837	*Hoplisoma carlae*	Urugua-i	1	Urugua-í Basin	Argentina	Misiones	Uruguai	-25,98858345	-54,28870119
PV476695	This work	CFA-IC-9114	FHN-4023	*Hoplisoma carlae*	Iguazu upstream B	2	Iguazu Basin	Argentina	Misiones	Reserva Natural Karadya	-25,83251642	-53,97885024
PV476696	This work	CFA-IC-9145	FHN-4047	*Hoplisoma carlae*	Iguazu upstream B	2	Iguazu Basin	Argentina	Misiones	Arroyo Los Tatetos	-25,83251642	-53,97885024
PV476697	This work	CFA-IC-9145	FHN-4048	*Hoplisoma carlae*	Iguazu upstream B	2	Iguazu Basin	Argentina	Misiones	Arroyo Los Tatetos	-25,83251642	-53,97885024
MT846111	da Rocha et al. 2022			*Hoplisoma carlae*	Iguazu downstream	3	Iguazu Basin	Brazil	Paraná	Arroyo pozo negro	-25,6126389	-54,43075
MT846110	da Rocha et al. 2022			*Hoplisoma carlae*	Iguazu downstream	3	Iguazu Basin	Brazil	Paraná	Arroyo pozo negro	-25,6126389	-54,43075
MT846109	da Rocha et al. 2022			*Hoplisoma carlae*	Iguazu downstream	3	Iguazu Basin	Brazil	Paraná	Arroyo pozo negro	-25,6126389	-54,43075
MT846108	da Rocha et al. 2022			*Hoplisoma carlae*	Iguazu downstream	3	Iguazu Basin	Brazil	Paraná	Arroyo pozo negro	-25,6126389	-54,43075
MT846107	da Rocha et al. 2022			*Hoplisoma carlae*	Iguazu downstream	3	Iguazu Basin	Brazil	Paraná	Arroyo pozo negro	-25,6126389	-54,43075
MT846106	da Rocha et al. 2022			*Hoplisoma carlae*	Iguazu downstream	3	Iguazu Basin	Brazil	Paraná	Arroyo pozo negro	-25,6126389	-54,43075
MT846105	da Rocha et al. 2022			*Hoplisoma carlae*	Iguazu downstream	3	Iguazu Basin	Brazil	Paraná	Arroyo pozo negro	-25,6126389	-54,43075

Table S1 (2 of 2). Information for each sequence of *Hoplisoma carlae* analysed. The rows highlighted in light blue correspond to individuals sequenced for this study.

Tabla S1 (2 de 2). Información de cada secuencia de *Hoplisoma carlae* analizada. Las filas resaltadas en azul claro corresponden a los individuos secuenciados para este estudio.

Genbank Number	Sequenced by	Collection Code	Code	Specie	Locality Name	Locality Number	Basin	Country	Province	Locality	Latitude	Longitude
MT846104	da Rocha et al. 2022			*Hoplisoma carlae*	Iguazu downstream	3	Iguazu Basin	Brazil	Paraná	Arroyo pozo negro	-25,6126389	-54,43075
MT846103	da Rocha et al. 2022			*Hoplisoma carlae*	Iguazu downstream	3	Iguazu Basin	Brazil	Paraná	Arroyo pozo negro	-25,6126389	-54,43075
MT846102	da Rocha et al. 2022			*Hoplisoma carlae*	Iguazu downstream	3	Iguazu Basin	Brazil	Paraná	Arroyo pozo negro	-25,6126389	-54,43075
MT846101	da Rocha et al. 2022			*Hoplisoma carlae*	Iguazu downstream	3	Iguazu Basin	Brazil	Paraná	Arroyo pozo negro	-25,6126389	-54,43075
MT846099	da Rocha et al. 2022			*Hoplisoma carlae*	Iguazu downstream	3	Iguazu Basin	Brazil	Paraná	Arroyo pozo negro	-25,6126389	-54,43075
MT846100	da Rocha et al. 2022			*Hoplisoma carlae*	Iguazu downstream	3	Iguazu Basin	Brazil	Paraná	Arroyo pozo negro	-25,6126389	-54,43075
MT846098	da Rocha et al. 2022			*Hoplisoma carlae*	Iguazu upstream A	4	Iguazu Basin	Brazil	Paraná	Rio Florido	-26,0090556	-53,43075
MT846097	da Rocha et al. 2022			*Hoplisoma carlae*	Iguazu upstream A	4	Iguazu Basin	Brazil	Paraná	Rio Florido	-26,0090556	-53,43075
MT846096	da Rocha et al. 2022			*Hoplisoma carlae*	Iguazu upstream A	4	Iguazu Basin	Brazil	Paraná	Rio Florido	-26,0090556	-53,43075
MT846095	da Rocha et al. 2022			*Hoplisoma carlae*	Iguazu upstream A	4	Iguazu Basin	Brazil	Paraná	Rio Florido	-26,0090556	-53,43075
MT846094	da Rocha et al. 2022			*Hoplisoma carlae*	Iguazu upstream A	4	Iguazu Basin	Brazil	Paraná	Rio Florido	-26,0090556	-53,43075
MT846093	da Rocha et al. 2022			*Hoplisoma carlae*	Iguazu upstream A	4	Iguazu Basin	Brazil	Paraná	Rio Florido	-26,0090556	-53,43075
MT846092	da Rocha et al. 2022			*Hoplisoma carlae*	Iguazu upstream A	4	Iguazu Basin	Brazil	Paraná	Rio Florido	-26,0090556	-53,43075
MT846091	da Rocha et al. 2022			*Hoplisoma carlae*	Iguazu upstream A	4	Iguazu Basin	Brazil	Paraná	Rio Florido	-26,0090556	-53,43075
MT846090	da Rocha et al. 2022			*Hoplisoma carlae*	Iguazu upstream A	4	Iguazu Basin	Brazil	Paraná	Rio Florido	-26,0090556	-53,43075

Table S2. Information for each *Hoplisoma carlae* with morphometric measurements.

Tabla S2. Información de cada *Hoplisoma carlae* con medidas morfométricas.

Locality Name	Collection Code	Voucher	Collector	Date	Locality	Latitude	Longitude
Iguazu upstream B	MACN-IC/12629	Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”	Casciotta Jorge	26/02/2002	Argentina: Misiones, Arroyo Deseado Chico	-25,7888056	-54,029167
Iguazu upstream B	MACN-IC/12629	Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”	Casciotta Jorge	26/02/2002	Argentina: Misiones, Arroyo Deseado Chico	-25,7888056	-54,029167
Iguazu upstream B	MACN-IC/12629	Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”	Casciotta Jorge	26/02/2002	Argentina: Misiones, Arroyo Deseado Chico	-25,7888056	-54,029167
Iguazu upstream B	MACN-IC/12629	Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”	Casciotta Jorge	26/02/2002	Argentina: Misiones, Arroyo Deseado Chico	-25,7888056	-54,029167
Iguazu upstream B	MACN-IC/12629	Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”	Casciotta Jorge	26/02/2002	Argentina: Misiones, Arroyo Deseado Chico	-25,7888056	-54,029167
Iguazu upstream B	CFA-IC/9126	Fundación de Historia Natural Félix de Azara	Bogan Sergio, Meluso Juan	24/01/2019	Argentina: Misiones, arroyo Tateto	-25,8594417	-53,981583
Iguazu upstream B	CFA-IC/9126	Fundación de Historia Natural Félix de Azara	Bogan Sergio, Meluso Juan	24/01/2019	Argentina: Misiones, arroyo Tateto	-25,8594417	-53,981583
Iguazu upstream B	CFA-IC/9126	Fundación de Historia Natural Félix de Azara	Bogan Sergio, Meluso Juan	24/01/2019	Argentina: Misiones, arroyo Tateto	-25,8594417	-53,981583
Iguazu upstream B	CFA-IC/9114	Fundación de Historia Natural Félix de Azara	Bogan Sergio, Meluso Juan	22/10/2019	Argentina: Misiones, Reserva Karadya	-25,83251642	-53,978851
Iguazu upstream B	CFA-IC/9145	Fundación de Historia Natural Félix de Azara	Bogan Sergio, Meluso Juan	25/01/2019	Argentina: Misiones, arroyo Tateto	-25,8594417	-53,981583
Iguazu upstream B	CFA-IC/9145	Fundación de Historia Natural Félix de Azara	Bogan Sergio, Meluso Juan	25/01/2019	Argentina: Misiones, arroyo Tateto	-25,8594417	-53,981583
Iguazu upstream B	CFA-IC/9096	Fundación de Historia Natural Félix de Azara	Bogan Sergio, Meluso Juan	02/01/2019	Argentina: Misiones, Arroyo Los Chanchos, Parque Provincial Horacio Foerster	-25,8472167	-53,842483
Iguazu upstream B	CFA-IC/9096	Fundación de Historia Natural Félix de Azara	Bogan Sergio, Meluso Juan	02/01/2019	Argentina: Misiones, Arroyo Los Chanchos, Parque Provincial Horacio Foerster	-25,8472167	-53,842483
Iguazu upstream B	CFA-IC/9096	Fundación de Historia Natural Félix de Azara	Bogan Sergio, Meluso Juan	02/01/2019	Argentina: Misiones, Arroyo Los Chanchos, Parque Provincial Horacio Foerster	-25,8472167	-53,842483
Uruguai	CFA-IC/5494	Fundación de Historia Natural Félix de Azara	Bogan Sergio, Meluso Juan	15/11/2015	Argentina: Misiones, Arroyo Tirica	-25,988783	-54,289283
Uruguai	CFA-IC/11527	Fundación de Historia Natural Félix de Azara	R. Filiberto	05/01/2001	Argentina: Misiones, Arroyo Tirica	-25,988783	-54,289283
Uruguai	CFA-IC/11528	Fundación de Historia Natural Félix de Azara	R. Filiberto	05/01/2001	Argentina: Misiones, Arroyo Tirica	-25,988783	-54,289283
Uruguai	CFA-IC/11529	Fundación de Historia Natural Félix de Azara	R. Filiberto	05/01/2001	Argentina: Misiones, Arroyo Tirica	-25,988783	-54,289283
Uruguai	CFA-IC/5491	Fundación de Historia Natural Félix de Azara	Bogan Sergio, Meluso Juan	15/11/2015	Argentina: Misiones, Arroyo Falso	-26,005667	-54,22175

Table S3. Average genetic distances (K2P) for *Hoplisoma carlae* populations, expressed as percentages.

Tabla S3. Distancias genéticas promedio (K2P) para las poblaciones de *Hoplisoma carlae*, expresadas como porcentajes.

Population	Urugua-í	Iguazu up B	Iguazu down	Iguazu up A
Urugua-í	0			
Iguazu up B	1,27 %	0		
Iguazu down	1,50 %	0,23 %	0	
Iguazu up A	2,34 %	1,27 %	1,17 %	0

SUPPORTING INFORMATION

All the data used in the article is available within it and the supplementary files (Tables S1, S2, and S3).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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