



Integrative study of *Characidium borellii* (Characiformes: Crenuchidae): phylogenetic relationships and geographic distribution

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The genus *Characidium* comprises a diverse group of small Neotropical freshwater fishes. Among the 89 valid species, eight occur in Argentina. *Characidium borellii*, originally described based on material from the río Salí basin, show morphological similarities to specimens from nearby basins, often identified as *Characidium* cf. *zebra*. We redescribe *C. borellii*, based on detailed morphological and molecular analyses of specimens collected from the Salí, Bermejo, and Juramento-Salado basins. *Characidium borellii* can be distinguished from its congeners by an isthmus completely covered with scales, 14 scale rows around the caudal peduncle, a single row of teeth on the dentary, 13 or 14 pectoral-fin rays, a complete lateral line with 36–38 scales, fins mostly hyaline, regular transversal bars on the body, and the presence of hooks on the pelvic and pectoral fins. The distribution of *C. borellii* is here extended to include the Bermejo and Juramento-Salado basins. Additional data are provided regarding its phylogenetic position and sexual dimorphism.

Keywords: Biogeography, Characidiinae, Freshwater fish, Phylogeny, Taxonomy.

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El género *Characidium* comprende un grupo diverso de pequeños peces de agua dulce neotropicales. Entre las 89 especies, ocho se encuentran en Argentina. *Characidium borellii*, originalmente descrita en base a material de la cuenca del río Salí, tiene morfología similar con ejemplares de cuencas cercanas, que a menudo se identifican como *Characidium* cf. *zebra*. Se presenta una redescrición de *C. borellii* basado en análisis morfológicos y moleculares detallados de ejemplares recolectados en las cuencas de los ríos Salí, Bermejo y Juramento-Salado. *Characidium borellii* se puede distinguir de sus congéneres por istmo completamente cubierto de escamas, 14 escamas alrededor del pedúnculo caudal, una sola hilera de dientes en el dentario, 13 o 14 radios en la aletas pectoral, línea lateral completa con 36–38 escamas, aletas mayormente hialinas, barras transversales regulares en el cuerpo, y presencia de ganchos en las aletas pélvicas y pectorales. Se amplía su distribución a las cuencas de los ríos Bermejo y Juramento-Salado. Se aportan además nuevos datos sobre su posición filogenética y dimorfismo sexual.

Palabras clave: Biogeografía, Characidiinae, Filogenia, Peces de agua Dulce, Taxonomía.

INTRODUCTION

The genus *Characidium* Reinhardt, 1867 (Characiformes: Crenuchidae) is a group of Neotropical freshwater fishes commonly referred to as “tritolos” and “mariposas” in Argentina and Uruguay (Serra *et al.*, 2014; Almiron *et al.*, 2015; Loureiro *et al.*, 2023). It currently includes 89 valid species (Fricke *et al.*, 2025) and is geographically restricted to tropical South America and southern Panama. The genus was defined by Buckup (1993a,b) as a monophyletic group based on a single synapomorphy: a black spot near the base of the middle caudal-fin rays, although this character has been lost in some species within the genus.

The río de La Plata basin, one of major hydrographic systems in South, extends across Argentina, Bolivia, Brazil, Paraguay, and Uruguay. It includes three major basins: the río Paraná (including the Iguacu and Salado rivers), the Paraguay (with the río Bermejo and Pilcomayo as western tributaries) and the río Uruguay (Pasquini, Depetris, 2007). Currently, 24 species of *Characidium* have been recorded in the La Plata basin, including: *C. borellii* (Boulenger, 1895); *C. chicoi* Graça, Ota & Domingues, 2019; *C. dumonti* Stabile, Reis, Oliveria & Graça, 2025; *C. etzeli* Zarske & Géry, 2001; *C. fasciatum* Reinhardt, 1867; *C. gomesi* Travassos, 1956; *C. heirmostigmata* Graça & Pavanelli, 2008; *C. itarare* Stabile, Reis, Frota, Graça & Oliveira, 2024; *C. lagosantense* Travassos, 1947; *C. laterale* (Boulenger, 1895); *C. nupelia* Graça, Pavanelli & Buckup, 2008; *C. occidentale* Buckup & Reis, 1997; *C. oiticica* Travassos, 1967; *C. onca* Melo, Brito Ribeiro & Lima, 2021; *C. pterostictum* Gomes, 1947; *C. rachovii* (Regan, 1913); *C. schubarti* Travassos, 1955; *C. serrano* Buckup & Reis, 1997; *C. stigmatosum* Melo & Buckup, 2002; *C. tenue* (Cope, 1894); *C. travassosi* Melo, Buckup & Oyakawa, 2016; *C. vestigipinne* Buckup & Hahn, 2000; *C. xanthopteron* Silveira, Langeani, Graça, Pavanelli & Buckup, 2008; *C. cf. zebra* Eigenmann, 1909 (Buckup, Reis, 1997; Casciotta *et al.*, 2015; Dagosta *et al.*, 2024;

Fricke *et al.*, 2025). Among these, eight species have been recorded for Argentina: *C. borellii*, *C. heirmostigmata*, *C. occidentale*, *C. pterostictum*, *C. rachovii*, *C. serrano*, *C. tenue*, and *C. cf. zebra* (Mirande, Koerber, 2020).

Among these species, *C. borellii* appears to be endemic to the río Salí-Dulce, an endorheic basin in northwestern Argentina (Butí, Cancino, 2005; Mirande, Aguilera, 2009). Initially described as part of the genus *Nanognathus* by Boulenger (1895), the species was later reassigned to *Characidium* by Géry *et al.* (2001) and Zarske, Géry (2001), who also provided a supplementary description and clarified its type-locality as San Pablo, Tucumán, Argentina.

The morphology of *C. borellii* closely resembles that of *C. zebra* (Boulenger, 1895; Eigenmann, 1909; Buckup, 1992; Buckup, Reis, 1997; Zarske, Géry, 2001) a species described by Eigenmann (1909) from Maripicru Creek, a tributary of the río Branco basin in the Guiana Shield. Due to the limited number of diagnostic characters, many specimens distributed across South America basins have been identified as *C. cf. zebra*, forming a species complex (Buckup, 1992). This taxonomic ambiguity also occurs in northwestern Argentina, where specimens from basins adjacent to the río Salí basin, such as the Bermejo and Juramento-Salado basins have traditionally been identified as *C. cf. zebra* (Liotta, 2005; Cancino, Aguilera, 2016; Terán *et al.*, 2016; Alonso *et al.*, 2025).

In this study, we perform an integrative analysis of *Characidium* populations from northwestern Argentina. We present a revised redescription of *C. borellii* that allows for its unequivocal distinction from congeneric species. Additionally, we assess the taxonomic status of specimens from adjacent basins based on morphological and meristic data and discuss their phylogenetic placement using molecular and morphological evidence.

MATERIAL AND METHODS

Morphological analyses. Morphometric measurements were obtained as straight-line distances using a caliper to the nearest 0.1 mm. Morphological and meristic data followed Buckup (1993a), with modifications from Melo, Oyakawa (2015). Additional variables measured included: 1) number of scales between the anus and the anal-fin origin, 2) number of scales between the anus and the caudal fin, 3) number of scales between the adipose fin and the caudal fin, 4) number of scales between the pectoral and pelvic fins, 5) lengths of the dorsal, anal, pectoral and pelvic fins, measured to the distal tips of longest branched ray, 6) peduncle width measured at the vertical through the lowest caudal-peduncle depth, 7) head height and width measured at the posterior region of the orbit. A summary of these measurements is presented in Tab. 1, with additional data provided in Tab. S1. Selected specimens were cleared and stained (cs) following the protocol of Taylor, Van Dyke (1985). Specimens are deposited in the ichthyological collection of Fundación Miguel Lillo (CI-FML) and Instituto de Bio y Geociencias del Noroeste Argentino (IBIGEO-I).

Diagnostic characters were compared with the original description of *Characidium* species and related taxonomic works (Buckup, 1992; Taphorn, 1990; Buckup, Reis, 1997; Taphorn *et al.*, 2006; Netto-Ferreira *et al.*, 2013; Leitão, Buckup, 2014; Melo, Espíndola, 2016; Agudelo-Zamora *et al.*, 2020; Flausino *et al.*, 2020; Armbruster *et al.*, 2021). Sex identification was based on the presence of secondary sexual characteristics (Teixeira, Melo, 2021) and on gonadal examination.

TABLE 1 | Morphometric summary table of *Characidium borellii*. Morphometric data from holotype of *C. borellii* were obtained from Géry *et al.* (2001).

Variables		Holotype	N	Min-Max	Mean	SD	K
Total length	TL (mm)		83	32.4–84.5	61.3	11.1	-
Standard length	SL (mm)	67.3	84	25.5–70.0	50.1	9.2	-
Head length	HL (mm)	14.2	84	6.9–17.4	11.5	2.1	-
Percentages of standard length							
Head length (%)	HL%	21.1	84	19.7–26.9	23.1	1.4	0.9
Pre-pectoral distance	PPD	20.1	84	17.3–27.9	22.3	1.6	0.9
Pectoral-fin height	PFH		84	15.3–23.1	19.9	1.5	0.8
Pre-dorsal distance	PDD	45.5	83	40.5–49.3	45.9	1.5	1.1
Dorsal-fin height	DFH	17.7	84	13.8–23.0	18.4	1.6	0.8
Dorsal-fin base	DFB		84	11.4–15.4	14.1	0.8	1.0
Pre-pelvic distance	PPVD	52.1	76	46.7–54.7	51.6	1.6	1.1
Pelvic-fin height	PVPH		84	9.1–16.2	12.6	1.2	0.8
Pre-anal distance	PAD		76	71.2–80.4	76.5	1.5	1.0
Anal-apex distance	AAD		76	88.9–97.5	94.0	1.7	1.0
Anus to anal fin distance	AAFD		84	3.8–9.9	6.5	1.2	0.9
Anal-fin height	AFH	12.9	70	12.0–17.1	14.3	1.1	0.8
Anal-fin base	AFB		84	6.5–10.4	8.0	0.7	1.2
Adipose-fin height	ADFH		84	3.3–6.8	5.6	0.6	0.9
Peduncle length	PL	16.6	83	15.5–19.2	17.4	1.0	1.0
Body depth at dorsal-fin origin	BDDFO	24.2	76	17.4–25.2	21.5	1.7	1.3
Body depth at anal-fin origin	BDAFO		76	12.9–19.9	15.9	1.4	1.2
Body depth at caudal peduncle	BDCP	11.9	82	9.0–12.8	11.6	0.6	1.1
Body width	BW		84	9.3–13.2	11.2	0.9	1.2
Percentages of head length							
Snout length	SNL	25.4	84	20.7–29.6	24.2	1.8	1.1
Snout-maxillary tip	SMT		83	20.3–26.6	23.5	1.2	0.9
Anterior-naris orbit	ANO		84	7.2–12.0	9.7	0.9	0.9
Posterior-naris orbit	PNO		84	2.9–6.3	4.4	0.7	1.0
Cheek	CH		83	5.9–14.5	9.9	1.6	1.3
Orbital diameter	OD	19.9	84	19.7–30.9	25.0	2.4	0.5
Interorbital distance	IOD	18.3	84	11.3–20.8	15.8	1.7	1.0

Statistical analyses. Occurrence localities for *Characidium borellii* were grouped into three major geographic basins: Salí, Bermejo, and Juramento-Salado. Specimens from the arheic río Urueña and río Horcones basins are included in the Juramento-Salado group, as both connected with the río Salado during rainy seasons (Tenchini, 1984). In total, 84 specimens were analyzed (36 specimens from the Salí basin, 33 from the Bermejo basin and the 15 from the Juramento-Salado basin), using 36 morphometric variables, including the standard length (SL) and head length (HL). Allometric coefficient (K) were calculated for each measurement using an ordinary least square (OLS) regression line of base 10 log transformed data (Huxley, 1993; Klingenberg, 1996; Kilmer, Rodriguez, 2017), with SL as the reference. Measurements were transformed into percentages of SL and HL to mitigate size effects and missing values were imputed using mean value substitution. To compare populations, a Principal Component Analysis (PCA)

was conducted using the PAST software package (Hammer *et al.*, 2001). Significant components were determined using the broken-stick model (Frontier, 1976) and scree plot analysis (Cattell, 1966). Eigenvalues were calculated from the covariance matrix of the morphometric variables. Sexual dimorphism features and intraspecific variation were assessed using both original and transformed measurements. Statistical differences between populations and groups were evaluated using a PERMANOVA test (based on the Euclidean similarity index). Differences among major variables were tested using the Kruskal-Wallis test, followed by a Mann-Whitney pairwise test with Bonferroni correction. Statistical significance was set at $p < 0.05$.

DNA extraction. Total genomic DNA was extracted from muscle tissue samples, preserved in cold 96% ethanol. Extractions were performed using the PURO-Genomic DNA kit (PB-L Productos Bio-Lógicos® S.A.), following the manufacturer's protocol. The mitochondrial *Cytochrome Oxidase Subunit I* (COI) and the *Cytochrome b* (cytB) genes were amplified using Polymerase Chain Reaction (PCR) on an Eppendorf Mastercycler thermal cycler (Hamburg, Germany) in a 50- μ l reaction mixture consisting of 25 μ l of PB-L master mix (PB-L Productos Bio-Lógicos, Quilmes, Argentina), 0.4 μ M of each forward and reverse primer and 4 μ l of the template DNA. The partial COI gene was amplified with the primers Fish F1 (5'-TCA ACC AAC CAC AAA GAC ATT GGC AC-3') and Fish R1 (5'-AGA CTT CTG GGT GGC CAA AGA ATC A-3') (Ward *et al.*, 2005), according to Souza-Shibatta *et al.* (2018). The fragment of cytB was amplified with the forward primer CB3-H (GGC AAA TAG GAA RTA TCA TTC) and reverse primer Gludg-L (TGA CTT GAA RAA CCA YCG TTG) (Palumbi *et al.*, 2002) following thermocycling conditions as described by Loureiro *et al.* (2018). Sequencing was performed bidirectionally using an ABI 3730XL sequencer (Macrogen Inc. Korea), to ensure sequence accuracy.

Molecular analyses. Molecular data included sequences of the *Cytochrome Oxidase Subunit I* (COI) and *Cytochrome b* (cytB) markers. New sequences (Tab. S2A), were compared alongside sequences mainly from Oliveira-Silva *et al.* (2024) and Serra *et al.* (2025), and other sequences available in GenBank (Tab. S2B). Sequences were aligned using MUSCLE (Edgar, 2004), via MEGA (Kumar *et al.*, 2018), which was also used to calculate genetic distances for the COI marker under the Kimura 2-parameter (K2) substitution model, chosen for its standard use in DNA barcoding (Díaz *et al.*, 2016). Phylogenetic analyses were conducted using two approaches: (1) Parsimony Analysis: conducted in TNT (v. 1.6 Goloboff *et al.*, 2008) under extended implied weighting exploring a broad range of concavity (K) values (3–27) (Goloboff, 2014); clade support was estimated using symmetric resampling (10,000 replicates), with GC values ("Group present/Contradicted") reported (Goloboff *et al.*, 2003); separate analyses were run for COI alone and the combined COI + cytB dataset. (2) Maximum Likelihood (ML) analysis: performed using IQ-TREE (Trifinopoulos *et al.*, 2016). Edge confidence intervals were calculated using fast bootstrapping with 10,000 repetitions (Hoang *et al.*, 2018). The TIM2+F+I+G4 model was identified as the best-fit for COI (Kalyaanamoorthy *et al.*, 2017), was used for tree inference. For both methods, *Hoplias malabaricus* (Bloch, 1794) was used as the root. In cases of incongruent species

delimitation (e.g., *Characidium bimaculatum* Fowler, 1941; *C. bahiense* Almeida, 1971; *C. fasciatum*, and *C. gomesi*), only sequences corresponding to type-locality ecoregions or basins were included.

Collection's acronyms: CI-FML, Colección Ictiológica Fundación Miguel Lillo, San Miguel de Tucumán; FMNH, Field Museum of Natural History, Chicago; IBIGEO, Instituto de Bio y Geociencias del NOA, Salta; IAvH-P, Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Colección de Peces, Villa de Leyva; MG-ZV-P, Museo Provincial de Ciencias Naturales “Dr. Ángel Gallardo”, Rosario; MZUSP, Museu de Zoologia, Universidade de São Paulo, São Paulo; and MZUT, Dipartimento di Biologia Animale e dell’Uomo, Museo Zoologico, Università di Torino, Torino.

RESULTS

Characidium borellii (Boulenger, 1895)

(Figs. 1–5; Tab. 1)

Nanognathus borellii Boulenger, 1895:3 (original description; type-locality: San Pablo, Tucuman Province, Argentina).

Characidium borellii (Boulenger, 1895). —Buckup, 1993b:101 (generic location to *Characidium*). —Géry *et al.*, 2001:27 (correction on type-locality). —Zarske, Gery, 2001:238 (comparative material). —Mirande, 2009:579 (phylogeny). —Mirande, 2010:466 (phylogeny). —Mirande, Koerber, 2015:27 (listed). —Alonso *et al.*, 2015:274 (anatomical study) —Saravia *et al.*, 2023:333 (listed)..

Characidium cf. *zebra* (non Eigenmann, 1909). —Butí, Cancino, 2005:26 (listed). —Mirande, Aguilera, 2009:178 (listed). —Cancino, Aguilera, 2016:43 (listed). —Terán *et al.*, 2016:199 (mention). —Alonso *et al.*, 2025:18 (mention). —Cortés Hernández *et al.*, 2025:10 (listed).

Characidium fasciatum (non Reinhardt, 1867). —Ringuelet *et al.*, 1967:188 (listed). —Butí, Miquelarena, 1995:26 (listed). —Liotta, 2005:88 (listed).

Diagnosis. *Characidium borellii* can be distinguished from all congeners, except *C. bahiense*, *C. barbosai* Flausino, Lima, Machado & Melo, 2020; *C. bimaculatum*; *C. brevirostre* Pellegrin, 1909; *C. cacah* Zanata, Ribeiro, Araújo-Porto, Pessali & Oliveira-Silva, 2020; *C. caucanum* Eigenmann, 1912; *C. chancoense* Agudelo-Zamora, Ortega-Lara & Taphorn, 2020; *C. chicoi*; *C. chupa* Schultz, 1944; *C. clistenesi* Melo & Espíndola, 2016; *C. dumonti*; *C. deludens* Zanata & Camelier, 2015; *C. etheostoma* Cope, 1872; *C. etzeli*; *C. fleurdelis* Zanata, Oliveira-Silva & Ohara, 2023; *C. geryi* (Zarske, 1997); *C. heiniamum* Zarske & Géry, 2001; *C. heirmostigmata*; *C. interruptum* Pellegrin, 1909; *C. itarare*; *C. lagosantense*; *C. laterale*; *C. litorale* Leitão & Buckup, 2014; *C. longum* Taphorn, Montaña & Buckup, 2006; *C. marshi* Breder, 1925; *C. mirim* Netto-Ferreira, Birindellii & Buckup, 2013; *C. nana* Mendonça & Netto-Ferreira, 2015; *C. nupelia*; *C. occidentale*; *C. onca*; *C. orientale* Buckup & Reis, 1997; *C. papachibe* Peixoto & Wosiacki, 2013; *C. pellucidum* Eigenmann, 1909; *C. phoxocephalum* Eigenmann, 1909; *C. pteroides* Eigenmann, 1909; *C. pumarini* Teixeira & Melo, 2021; *C. rachovii*; *C. sancti johanni* Dahl, 1960; *C. samurai* Zanata & Camelier, 2014; *C. satoi* Melo & Oyakawa, 2015; *C. schindleri* Zarske & Géry, 2001; *C. serrano*; *C. steindachneri* Cope, 1878; *C. sterbai* (Zarske, 1997); *C. stigmatosum*;



FIGURE 1 | Specimen of *Characidium borellii*, male, CI FML 8183, 53.6 mm SL, from río Romano, Tucumán, Argentina, in río Salí basin. **A.** Lateral view. **B.** Dorsal view. **C.** Ventral view. Scale bar = 1 cm.

C. tapuia Zanata, Ramos & Oliveira-Silva, 2018; *C. tenue*; *C. varii* Zanata, Oliveira & Oliveira-Silva, 2024; *C. vestigipinne*; *C. xanthopteron*; *C. xavante* Graça, Pavanelli & Buckup, 2008; and *C. zebra* by having an isthmus covered with scales (*vs.* unscaled). It differs from *C. bahiense*, *C. barbosai*, *C. brevirostre*, *C. cacah*, *C. caucanum*, *C. chancoense*, *C. chicoi*, *C. clistenesi*, *C. dule*, *C. etzeli*, *C. fleurdelis*, *C. heiniamum*, *C. heirmostigmata*, *C. itarare*, *C. lagsantense*, *C. laterale*, *C. litorale*, *C. longum*, *C. marshi*, *C. mirim*, *C. nana*, *C. nupelia*, *C. occidentale*, *C. orientale*, *C. papachibe*, *C. pellucidum*, *C. phoxocephalum*, *C. pteroides*, *C. rachovii*, *C. schinderei*, *C. serrano*, *C. tapuia*, *C. tenue*, *C. vestigipinne*, and *C. xavante* by having 14 scales around the caudal peduncle (*vs.* 12 or fewer scales); from *C. chupa*, *C. onca*, *C. pumarini*, *C. samurai* and *C. zebra* by having one row of dentary teeth (*vs.* two rows, inner teeth present); from *C. etheostoma*, *C. geryi*, *C. sterbai* and *C. sanctijohanni* by having 13 or 14 pectoral-fin rays (*vs.* 11 or fewer rays); from *C. interruptum* by having a complete lateral line (*vs.* incomplete); from *C. varii* by having fins mostly hyaline (*vs.* dark stripes present on fins); from *C. stigmatosum* by lacking dots on body (*vs.* dots present); and from *C. satoi* and *C. deludens* by having regular transversal bars (*vs.* irregular). Additionally, *Characidium borellii* can be distinguished from congeners, except *C. bahiense*, *C. barbosai*, *C. bimaculatum*, *C. clistenesi*, *C. deludens*, *C. lagsantense*, *C. laterale*, *C. litorale*, *C. mirim*, *C. nupelia*, *C. satoi*, *C. stigmatosum*, *C.*



FIGURE 2 | Specimens of *Characidium borellii* from río Salí (A–F), río Juramento (G) and río Bermejo (H) basins. **A.** Male from río Los Sosa, Tucumán, Argentina (IBIGEO-I 524, 50.9 mm SL). **B.** Female specimen from río Sucio, Tucumán, Argentina (CI FML 8003, 58.1 mm SL). **C.** Male from río Lules (CI FML 8181, 45.8 mm SL). **D.** Female from río Lules, Tucumán, Argentina (CI FML 8181, 44.5 mm SL). **E.** Hyperpigmented male from río Las Tunas, Catamarca, Argentina (CI FML 8005, 63.5 mm SL). **F.** Gravid female from río Las Tunas, Catamarca, Argentina (CI FML 8005, 60.5 mm SL). **G.** Female specimen from río Guanaco, Salta, Argentina (CI FML 8014, 57.4 mm SL). **H.** Female specimen from río Popayan, National Park “El Rey”, Salta, Argentina (CI FML 8012, 46.9 mm SL). Scale bar = 1 cm.

xanthoptertum, and *C. xavante*, by possessing bony processes (hooks) on pelvic fins. It is further distinguished from *C. bahiense*, *C. bimaculatum*, *C. clistenesi*, *C. lagosantense*, *C. laterale*, *C. mirim*, *C. nupelia*, *C. stigmosum*, and *C. xavante* by having a parietal branch of the supraorbital canal (*vs.* absent); from *C. litorale* by lacking secondary bars (*vs.* thin dark secondary bars present); from *C. satoi* and *C. xanthoptertum* by having conspicuous transversal bars (*vs.* absent in breeding males of *C. satoi* and adults of *C. xanthoptertum*); from *C. barbosai* by having a preorbital stripe (*vs.* absent); and from *C. deludens* by having hooks on pectoral fins of males (*vs.* hooks absent).

Description. Morphometric data summarized in Tab. 1, full dataset by basin in Tab. S1. Largest specimen 70.0 mm SL; smallest 25.5 mm SL. Body elongated. Dorsal profile moderately convex to dorsal-fin origin, slightly convex to adipose fin, straight to caudal fin. Ventral profile slightly convex from snout to pelvic fins. Body deepest at dorsal fin

origin. Caudal peduncle depth smaller than body depth at anal-fin origin (Fig. 1A). Head length equal to or slightly greater than pre-pectoral distance. Snout rounded in lateral view, tip below of ventral eye margin level. Mouth subterminal. Maxillary not or barely reaching anterior orbit margin. Orbit oval, laterodorsally positioned; orbital diameter equal to or slightly longer than snout length. Two nasal openings, well separated; anterior with full dermal flap, posterior with flap on anterior margin only.

Cycloid scales lacking *circuli*. Isthmus completely scaled, except for one (Fig. 1C). Longitudinal scales: 35(2), 36(16), 37(34), 38(28), 39(2); all perforated by lateral line. Transversal scales: 4/5(56), 5/5(18). Pre-dorsal series: 7(1), 9(10), 10(35), 11(27), 12(11). Pre-adipose series: 6(1), 8(8), 9(26), 10(40), 11(8) (Fig. 1B). Pre-anal series: 4(2), 5(60), 6(22). Scales between adipose and caudal fin: 4(2), 5(10), 6(50), 7(17), 8(1), 9(1). Scales between anus and anal-fin origin: 2(18), 3(56), 4(10). Scales between anal and caudal-fin origin: 5(9), 6(45), 7(26), 8(1). Scales between pectoral and pelvic fins: 11(18), 12(39), 13(4). Scales around caudal peduncle: 14(84) (Fig. 1A).

Pectoral fin well-developed; surpassing vertical through dorsal-fin origin; rays: iii,9,i(38); iii,10,i(45); iii,11,i(1). Pelvic fin well-developed, barely reaching anal-fin origin in males; rays: i,6,i(6); i,7,i(74); i,8,i(3). Dorsal fin truncated, narrow base; rays: ii,8(3); ii,9(79); ii,10(2). Anal fin with narrow base; rays: ii,6(2); ii,7(70); ii,8(12). Small unbranched anterior rays in dorsal and anal fins, visible in cs specimens. Adipose fin well-developed. Caudal fin with lower lobe slightly longer; rays: i,8,7,i(2); i,8,8,i(2); i,9,8,i(80).

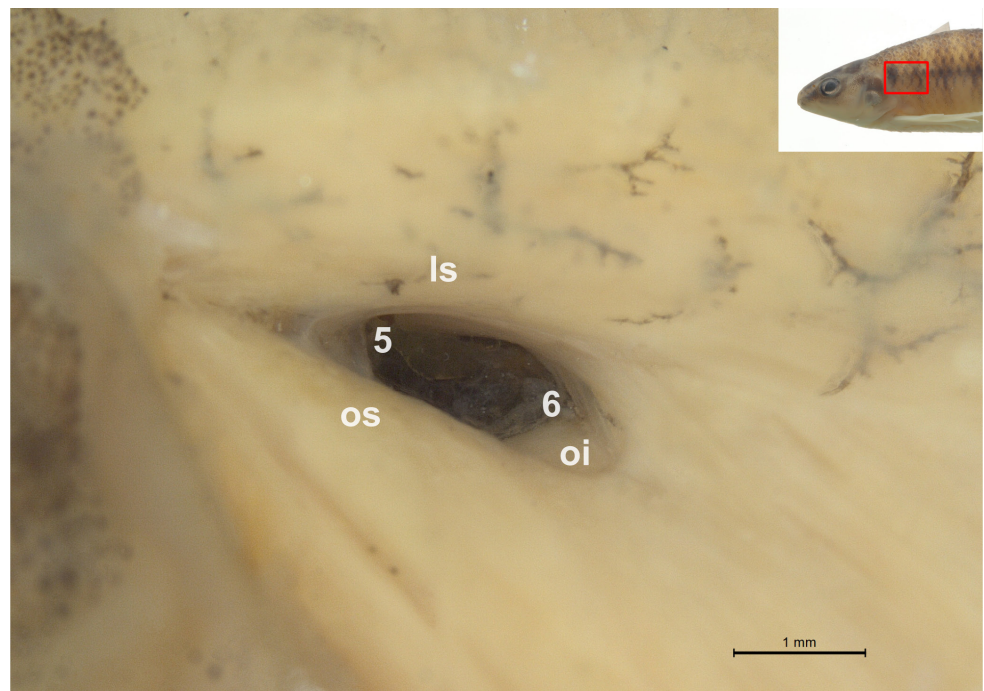


FIGURE 3 | Pseudotympanum of *Characidium borellii*, male, CL FML 8013, 60.0 mm SL, from National Park “El Rey”, Salta, Argentina. Overlying skin, adipose tissue and lateral line nerve removed. os, *obliquus superioris*; oi, *obliquus inferioris*; ls, *lateralis superficialis*. Numbers correspond to vertebrae associated with pleural ribs.

Pseudotympanum present as muscular hiatus between *obliquus superioris* and *obliquus inferioris*, aligned with swimbladder, posterior to humeral spot. Oval shaped opening between ribs of fifth and sixth vertebrae (Fig. 3). Swimbladder reduced, with two chambers; anterior chamber wider than posterior, both of equal length.

Premaxillary with 6(2 cs) or 7(7 cs) tricuspid teeth, decreasing in size posteriorly. Dentary with a one row of 6(2 cs) or 7(7 cs) tricuspid teeth, decreasing posteriorly. Replacement teeth present on dentary and premaxillary (Figs. 4A, B). Maxillary lacking teeth. Ectopterygoid elongated, bearing 7(4 cs), 8(2 cs) and 10(2 cs) minute conical teeth. Mesopterygoid and endopterygoid lacking teeth. Branchiostegal rays: 5(6 cs). Gill rakers: hypobranchial 1(3 cs); ceratobranchial 6(6 cs); epibranchial 6(1 cs), 7(1 cs), 8(1 cs).

Parietal branch of supraorbital canal present, reaching parietal. Cranial fontanel present bordered anteriorly by the frontals. Infraorbitals 1–6 present, infraorbitals 4–6 reduced to a canal. Pelvic bone distally rounded; ischiatic process irregular (Fig. 4C). Total vertebrae: 35(2 cs), 36(3 cs). Four anterior vertebrae fused in Weberian apparatus,

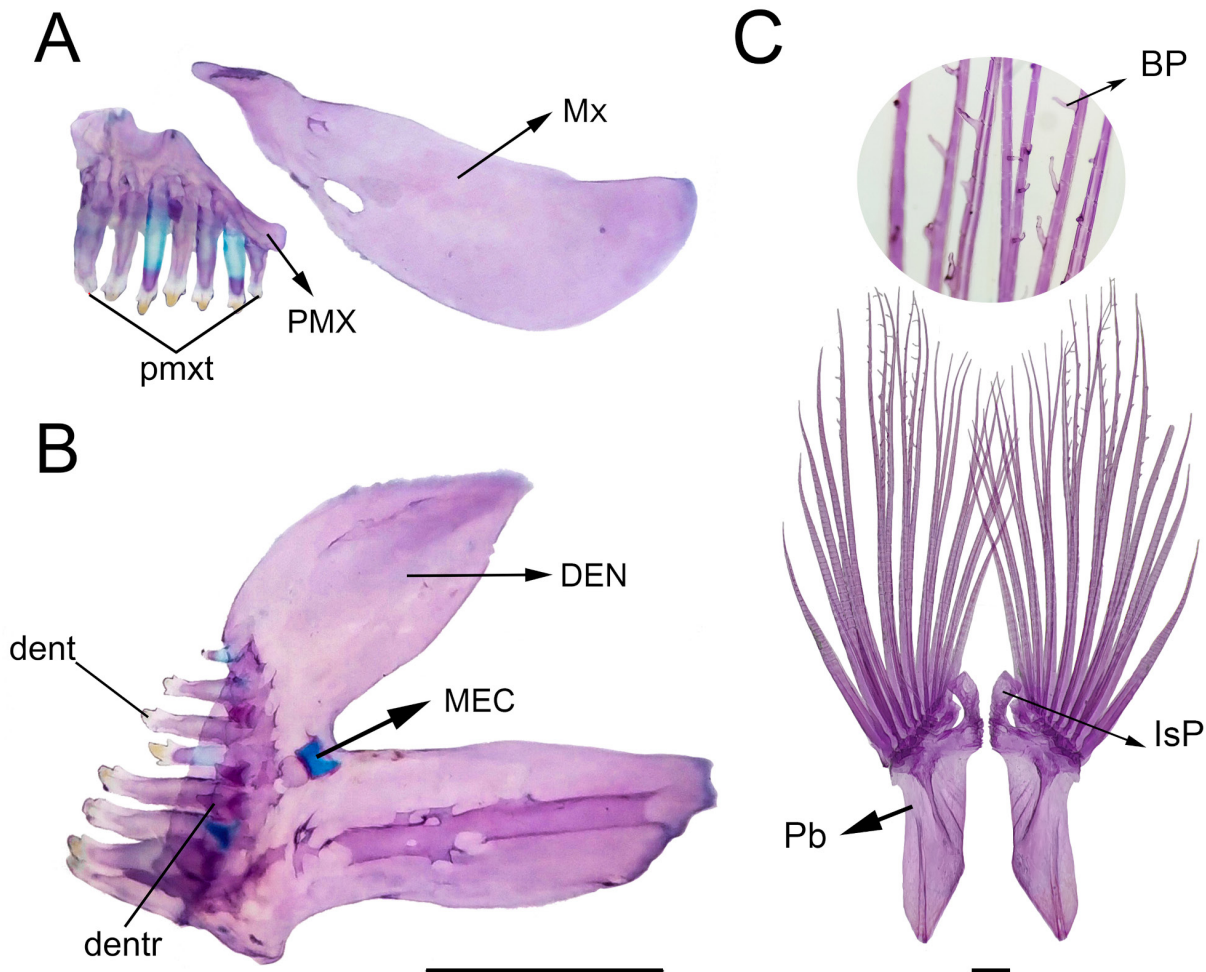


FIGURE 4 | **A.** Right upper jaw in medial view of *Characidium borellii* (CI FML 3865). **B.** Right dentary in medial view of *C. borellii* (CI FML 3865). **C.** Pelvic girdle and fins in ventral view of a male *C. borellii* (CI FML 3865) with a detail of bony processes. Abbreviations: BP, bony processes; Den, dentary; dent, dentary teeth; dentr, replacement dentary teeth; IsP: ischiatic process; MEC, Meckel cartilage; MX, maxillary; Pb, pelvic bone; PMX, premaxillary; pmxt, premaxillary teeth. Scale bars = 1 mm.

32 free vertebrae. Precaudal vertebrae: 15(5 cs). Caudal vertebrae: 16(1 cs), 17(4 cs). Hypural bones: 6(5 cs). Epural bones: 2(2 cs), 3(2 cs). Pair of uroneurals: 1(1 cs). Upper procurrent rays: 8(2 cs), 9(4 cs). Lower procurrent rays: 7(5 cs), 8(1 cs).

Coloration in alcohol. Body and head yellowish, darker dorsally, ventrally whitish. Dark pre-orbital stripe present, oblique, from snout tip to anterior eye margin, stripe poorly defined. Dark post-orbital stripe present with poorly defined edges. Area around nares lacking melanophores, Opercle mostly dark, with quadrangular blotch in some specimens. Dark longitudinal stripe along mid-body, from opercle to caudal fin, edges diffuse, narrower than one scale depth anteriorly, widening posteriorly. Dark transversal bars across body: 7(3), 8(8), 9(38), 10(18), 11(6), or 12(2), wider than scale, regular in shape, surpassing midlateral stripe. Dark stripe from dorsal fin origin to head along mid-dorsal scales. Thin dark stripe between dorsal and adipose fins. Humeral blotch present, slightly vertically elongated. Basicaudal spot present, more conspicuous in small specimens. Peduncular blotch present, circular or oval; more diffuse in larger specimens. Fins mostly hyaline. Few dark-brown chromatophores between rays in the middle dorsal fin. Dark melanophores on posterior margin of first three to five rays of pectoral and pelvic fins.

Coloration in life. Dorsal body silver or light brown, ventral region yellowish or whitish. Dark or silver longitudinal stripe, diffuse anteriorly, from snout to caudal-fin base. Dark transversal bars (9–11) from anterior to posterior region. Fins mostly hyaline, paired fins yellowish, with dark-brown pigmentation on posterior margin of first pectoral and pelvic fin rays (Fig. 5). Small, dark, rounded spots near operculum and caudal-fin base. Peduncular blotch visible or diffuse.



FIGURE 5 | Life specimens of *Characidium borellii*, CI FML 8183, from río Salí basin, Tucumán, Argentina. **A.** Female, 54.9 mm SL. **B.** Male, 57.3 mm SL.

Sexual dimorphism. Mature males of *Characidium borellii* can be distinguished from mature females and juveniles by the presence of bony processes (hooks) on pelvic and pectoral fins, and by pelvic-fin length. Hooks are consistently present on the pelvic fins of males from all basins examined but are rarely found on pectoral fins. When present, these hooks are located on the dorsal and distal half of the first four unbranched rays of the pair fins (Fig. 4C). These bony processes are classified as “spine type” according to classification proposed by Teixeira, Melo (2021), exhibiting a cylindrical cross-section along their entire length. Females typically lack hooks on their pair fins, one female was observed with three small hooks on the pelvic fins. Pelvic-fin length is sexually dimorphic. In males, the pelvic fin reaches or slightly surpasses the anal-fin origin, whereas in females, it does not reach the anal-fin origin (Fig. S4). One hyperpigmented male from río Tunas, characterized by a darker body and well-developed pelvic and pectoral hooks, was examined (Fig. 2E). No differences in counts were observed between males and females. However, males generally have a longer head and pelvic fin, as well as greater body depth at dorsal-fin origin, compared to females.

Historical remarks. Ringuelet *et al.* (1967) erroneously cited *Characidium borellii* for San Pablo, Paraguay, and *C. fasciatum* for Argentina. Géry *et al.* (2001) later clarified the type-locality of *C. borellii* as San Pablo, Tucumán, Argentina. Buckup (1992) determined that many specimens were incorrectly identified as *C. fasciatum*, leading to the species exclusion from checklists of freshwater fishes in Argentina (Mirande, Koerber, 2020). Subsequently, specimens previously identified as *C. fasciatum* were re-identified as *C. borellii* from Salí basin or *C. cf. zebra* in several other basins in Argentina (Butí, Cancino, 2005; Liotta, 2005; Almirón *et al.*, 2015; Cancino, Aguilera, 2016; Casciotta *et al.*, 2016; Saravia *et al.*, 2023).

Statistical analysis. Allometry. The allometric coefficients (K) for *Characidium borellii* indicated a range of negative and positive allometry, with some variables displaying isometry. Measurements exhibiting the greatest positive allometry were peduncle width (K = 1.3), body depth at the dorsal-fin origin (K = 1.3) and cheek (K = 1.3). Measurements with the greatest negative allometry included pelvic-fin height (K = 0.8), dorsal-fin height (K = 0.8), pelvic-fin length (K = 0.8), anal-fin height (K = 0.8) and orbital diameter (K = 0.5). Orbital diameter exhibited strong negative allometry with a K value of 0.5.

Principal Component Analysis. A consensus of scree plot and broken stick methods, resulted in the selection of four relevant components: PC1 (65.9%), PC2 (7.1%), PC3 (4.0%) and PC4 (3.4%), which together accounted for 80.4% of the variability (Fig. 6A; Tab. S3A). The plot of the first two components, PC1 *vs.* PC2 (Fig. 6C), explained 73% of the variability, whereas PC2 *vs.* PC4 (Fig. 7D) explained 7.4%. Both plots suggested no significant differences in overall morphology among specimens from Salí, Bermejo, and Juramento-Salado basins. The loadings of measurements are presented in Fig. 6B and Tab. S3B.

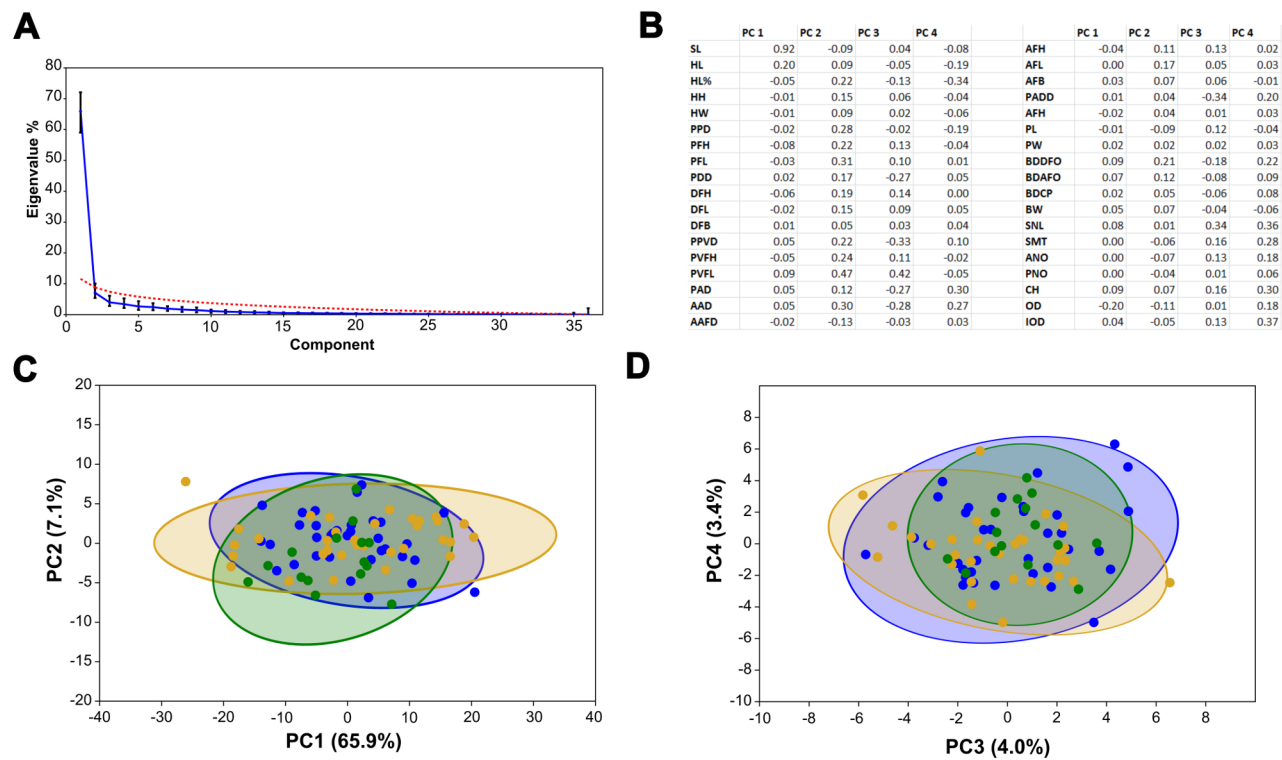


FIGURE 6 | Principal component analysis (PCA) of morphometric variables for specimens of *Characidium borellii* from the Salí (blue), Bermejo (yellow/orange) and Juramento-Salado (green) basins. **A.** The curves start to flatten out after the five-six components (scree plot method - blue line) and to two-three components (broken stick model - red line). **B.** Measurement loadings for relevant components **C.** PC1 vs. PC2, showing no significant differences between the specimens of the different basins. **D.** PC3 vs. PC4, showing no significant differences between the specimens of the different basins.

Intraspecific variability. The PERMANOVA test did not detect statistically significant morphological differences among specimens of *Characidium borellii* from the Bermejo, Juramento-Salado and Salí basins.

Sexual dimorphism. The PERMANOVA test revealed significant statistical morphological differences among males, females, and juveniles. The SIMPER test indicated that the following variables contributed cumulatively to over 80% of the observed dissimilarity: standard length, orbital diameter, pelvic-fin length, head length, snout length, body depth at dorsal-fin origin, anal apex distance, check, dorsal-fin height, and interorbital distance (Tab. S3C). Kruskal-Wallis and pairwise Mann-Whitney tests identified significant differences in several variables: pelvic-fin length (males > females), orbital diameter (juveniles > males and females), head length (males > females), anal apex distance (juveniles < males and females), check (juvenile < males and females), body depth at the dorsal-fin origin (males > females), and dorsal-fin height (juveniles > males and females).

Geographical distribution. *Characidium borellii* is distributed across the upper basins of the río Salí-Dulce, Bermejo, and río Juramento-Salado in Northwestern Argentina (NOA). This species has been recorded in the provinces of Tucumán, Catamarca, Salta, Jujuy, and Santiago del Estero (Fig. 7). In the Salí and Juramento-Salado basins, *C. borellii* occurs sympatrically with *C. rachovii*.

Molecular analysis. The final COI and cytB alignments were 654 and 747 base pairs (bp), respectively.

Parsimony analysis. Under parsimony, the consensus tree (K reference = 12, length = 2130, fit = 79.82753) of 15 most parsimonious trees (MPTs) using the COI marker (Fig. S5A) recovered *C. borellii* from the Salí, Bermejo, and Juramento-Salado basins as a highly supported monophyletic group (GC value = 99). This clade was sister to a group comprising *C. bahiense*, *C. lagosantense*, and *C. samurai* (Fig. 8). Specimens of *C. cf. zebra* from Chocó region and Maroni basin (near to *C. zebra* distribution) formed

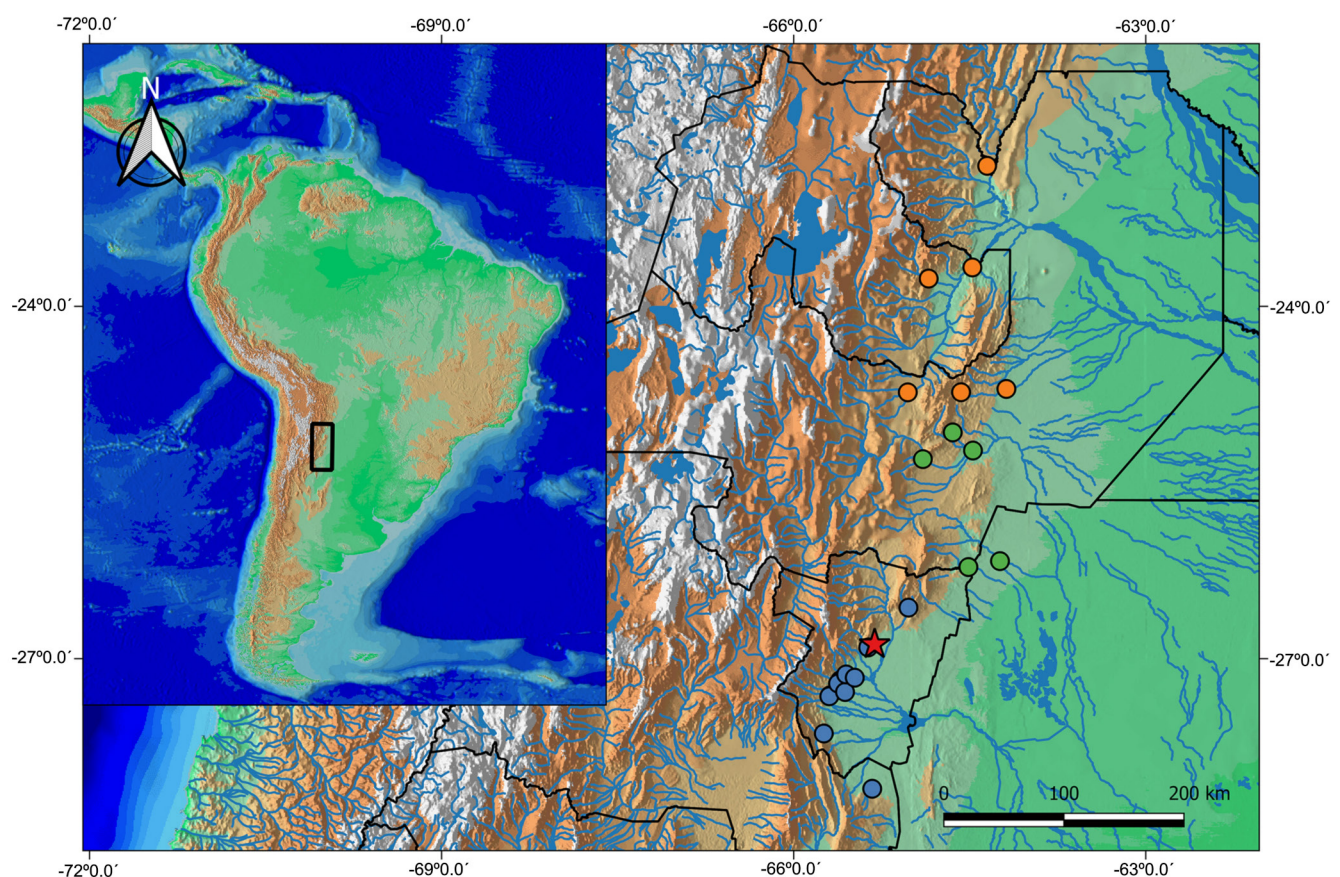


FIGURE 7 | Distribution map of *Characidium borellii* in Northwestern Argentina. Blue spots represent specimens from the río Salí basin, green spots from the río Juramento-Salado basin and orange spots from the río Bermejo basin. The red star indicates the type-locality: San Pablo, Tucumán, Argentina.

a highly supported monophyletic clade (GC value = 100), more closely related to *C. etheostoma*, *C. nana*, *C. cf. steindachneri*, and *C. cf. tapuia* than to *C. borellii*. Sequences of *C. rachovii* from río Salí basin and *C. pterostictum* from río Paraná basin clustered with their respective conspecifics, supporting their identification. A single MPT from combined COI and cytB (K reference = 12, length = 2608, fit = 91.14823) revealed a topology consistent with the COI-only analysis (Fig. 9A).

Maximum Likelihood analysis. The phylogenetic tree (TIM2+F+I+G4 model, Log-likelihood = -9310.2156, BIC = 19891.1597) exhibited a topology similar to parsimony (Fig. S5B). Specimens of *Characidium borellii* from the Salí, Bermejo, and Juramento-Salado basins formed a monophyletic clade with high statistical support (bootstrap value $\geq 95\%$) (Fig. 9B). *Characidium borellii* was found to be more closely

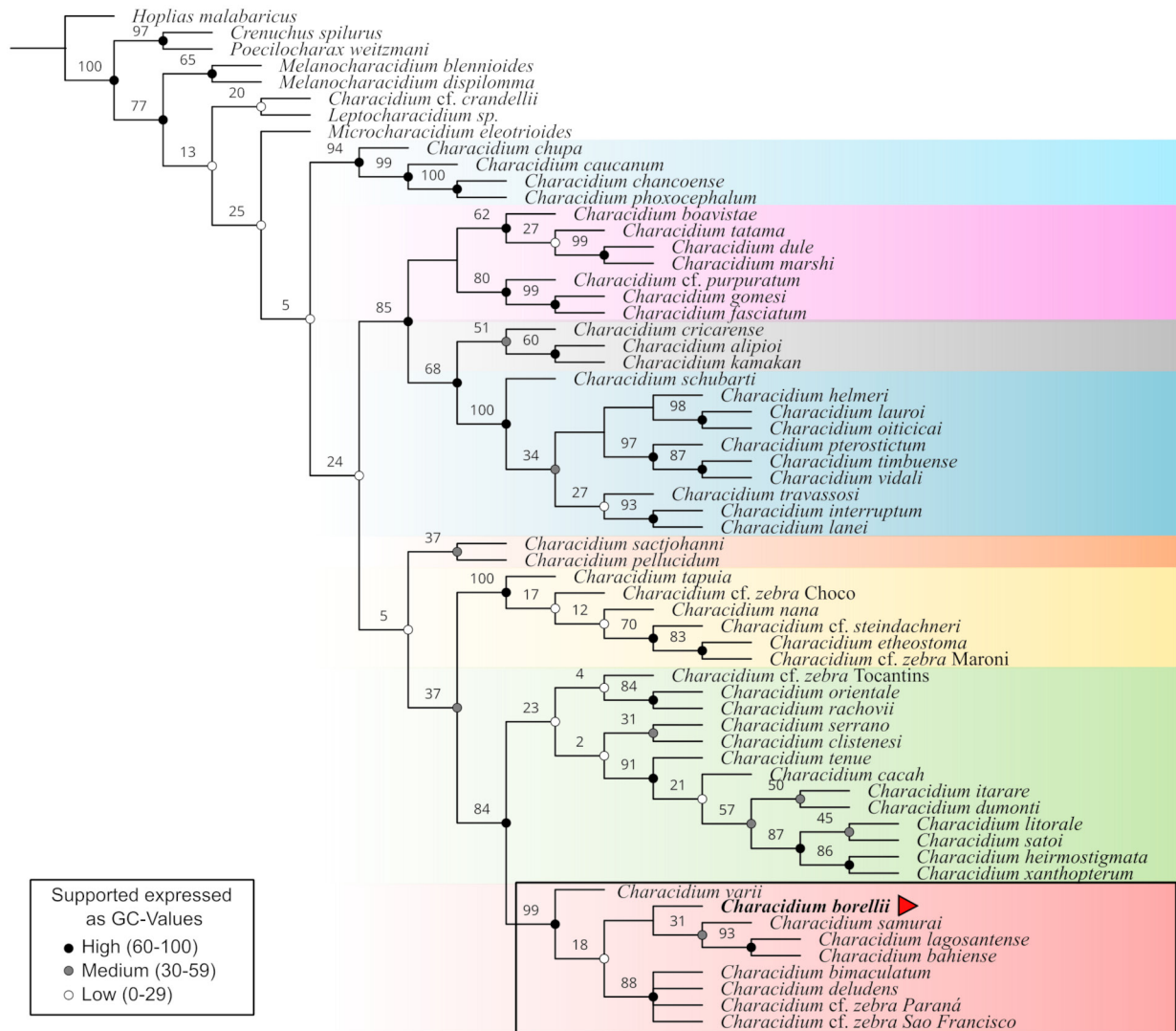


FIGURE 8 | COI + cytB markers under extended implied weighting (K reference = 12, length = 2608, fit = 91.14823), GC supporting values generated from 10,000 replicates.

related to *C. samurai*, *C. lagoonense*, and *C. bahiense* than to taxa in the *C. zebra* complex. Seven of the nine main clades mentioned by Serra *et al.* (2025) were recovered across all analyses: “*Characidium* cf. *crandellii*”, “*Characidium*”, “*Characidium alipioi*”, “*Characidium caucanum*”, “*Poecilosomatops*”, “*Chorimycteris*”, and “*Characidium bimaculatum*”. The “*Chorimycteris*” and “*Characidium pellucidum*” clades were not recovered by parsimony under low K values ($K = 3$). Topologies among analyses were not entirely congruent, and most major clades showed low support. *Characidium borellii* was placed within the “*C. bimaculatum*” clade, which is hereby renamed the “*Nanognathus*” clade to reflect the historical placement of *C. borellii* as the first and only species originally described within the genus *Nanognathus* (Fig. 9A).

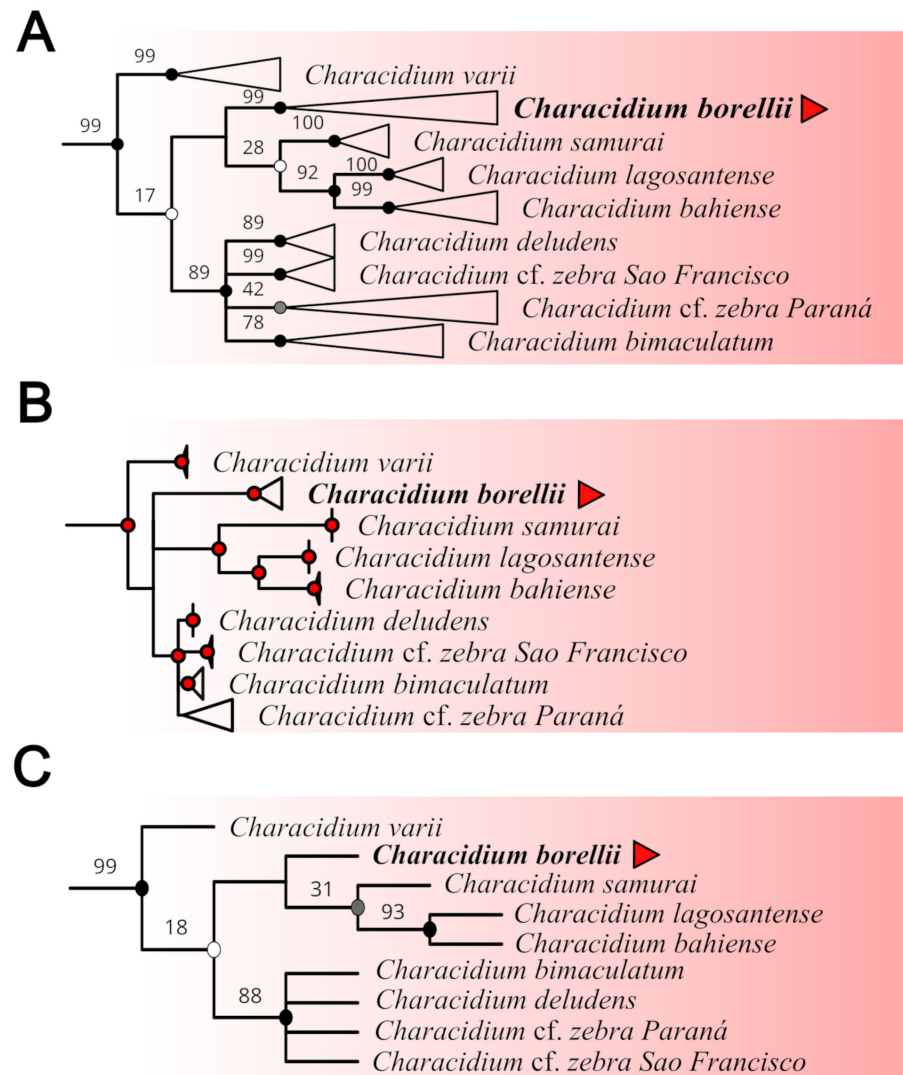


FIGURE 9 | Clade I or “*Nanognathus*”. **A.** Clade obtained using COI marker under extended weighting, parsimony ($K = 12$). **B.** Clade obtained using COI marker under maximum likelihood (ML). **C.** Clade obtained using COI and cytB under extended weighting, parsimony ($K = 12$). Black dots, high support ($\geq 95\%$); grey dots, medium support (60–100 GC); white dots, low support (0–29 GC), red dots, high statistical support ($\geq 95\%$); red triangle, sequences of *Characidium borellii*.

Genetic distances. The between-group mean distances using the COI marker showed over 1.6% divergence between *C. borellii* and other species of *Characidium*, exceeding the maximum intraspecific distance (Tab. 2). Species within “*Nanognathus*” clade, such as *C. bahiense*, *C. bimaculatum*, *C. cf. zebra* (from Paraná and São Francisco), *C. deludens*, *C. lagoonantense*, *C. samurai*, and *C. varii*, exhibited interspecific distances ranging from 5.0% to 8.5% compared to *C. borellii*. Greater divergences with *Characidium borellii* were observed for morphologically distinct species, such as: *C. fasciatum* (23.1%, a species with unscaled isthmus) and *C. rachovii* (16.0%, a sympatric species of *C. borellii*). Sequences of *C. cf. zebra* species showed genetic distances ranging from 5.6% to 19.2%. The intraspecific genetic distance within *C. borellii* was less than 0.6%. *Characidium rachovii* and *C. pterostictum* showed intraspecific distances of 0.3% and 1.6%, respectively.

Material examined. All from Argentina, river basins: Salí: MZUT P 2563, 1, 67.3 mm SL, holotype, (photographs in Zarske, Géry, 2001). CI FML 3865, 103, 42.9–53.6 mm SL. CI FML 7822, 1, 43.3 mm SL. CI FML 7862, 2, 51.0–52.6 mm SL. CI FML 7835, 4, 33.75–45.62 mm SL. CI FML 7924, 6, 41.9–54.6 mm SL. CI FML 8003, 3, 58.1–70.0 mm SL. CI FML 8004, 1, 57.0 mm SL. CI FML 8005, 4, 54.9–63.5 mm SL. CI FML 8179, 3, 37.6–55.8 mm SL. CI FML 8180, 4, 37.5–49.3 mm SL. CI FML 8181, 8, 36.4–45.6 mm SL. CI FML 8182, 3, 47.2–53.6 mm SL. CI FML 8183, 3, 54.9–57.3 mm SL. IBIGEO-I 524, 4, 35.8–50.9 mm SL. Bermejo: CI FML 8006, 1, 63.2 mm SL. CI FML 8007, 22, 69.1–36.2 mm SL. CI FML 8008, 1, 52.7 mm SL. CI FML 8009, 8, 66.9–46.9 mm SL. CI FML 8010, 3, 25.9–41.7 mm SL. CI FML 8011, 45, 33.1–55.7 mm SL. CI FML 8012, 20, 32.7–54.0 mm SL. CI FML 8013, 33, 43.8–60.3 mm SL. CI FML 8015, 1, 46.7 mm SL. CI FML 8184, 1, 47.5 mm SL. CI FML 8185, 1, 49.7 mm SL.

TABLE 2 | Interspecific and intraspecific genetic distances of *Characidium* species under K2 substitution model.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Intraspecific
1 <i>C. borellii</i>	-															0.6
2 <i>C. deludens</i>	5.0	-														0.0
3 <i>C. bimaculatum</i>	5.4	1.2	-													0.1
4 <i>C. cf. zebra</i> Paraná	5.6	1.3	1.6	-												1.1
5 <i>C. cf. zebra</i> Sao Francisco	5.7	1.4	1.5	1.8	-											0.1
6 <i>C. varii</i>	6.3	4.5	4.4	4.7	4.8	-										0.0
7 <i>C. bahiense</i>	6.6	6.2	6.6	6.5	6.3	6.9	-									0.2
8 <i>C. lagoonantense</i>	8.0	5.6	6.0	5.9	5.9	7.5	3.5	-								0.0
9 <i>C. samurai</i>	8.5	6.5	7.0	6.7	6.4	8.3	6.7	6.5	-							0.0
10 <i>C. cf. zebra</i> Tocantins	12.8	13.0	14.2	14.0	14.2	13.6	14.6	14.7	15.3	-						0.0
11 <i>C. rachovii</i>	16.0	14.1	14.5	14.2	13.6	14.4	12.8	13.3	14.1	12.6	-					0.3
12 <i>C. cf. zebra</i> Choco	17.7	15.8	16.5	16.9	16.1	15.9	18.4	17.3	15.5	17.9	19.7	-				0.0
13 <i>C. cf. zebra</i> Maroni	19.2	17.1	17.9	18.4	18.1	17.8	18.8	19.0	17.8	18.7	19.2	5.5	-			0.1
14 <i>C. pterostictum</i>	20.3	20.0	21.2	20.0	20.3	17.5	19.1	18.3	19.6	20.4	19.8	15.9	15.6	-		1.6
15 <i>C. fasciatum</i>	23.1	21.8	22.7	21.4	21.9	20.4	20.4	18.8	19.2	19.5	20.0	19.2	20.3	15.1	-	0.0
16 <i>P. weitzmani</i>	40.6	37.6	37.7	37.6	37.7	36.7	34.5	33.7	34.1	31.6	34.7	35.9	36.6	31.2	32.0	n/c

CI FML 8186, 1, 32.9 mm SL. CI FML 8192, 20, 45.1–62.5 mm SL. CI FML 8193, 2, 53.4–58.8 mm SL. IBIGEO-I 520, 10, 44.5–51.3 mm SL. IBIGEO-I 521, 12, 27.3–46.9 mm SL. IBIGEO-I 522, 4, 48.1–54.6 mm SL. IBIGEO-I 523, 5, 38.7–63.2 mm SL. Juramento: CI FML 632, 1, 50.3 mm SL. CI FML 6177, 2, 43.2–44.5 mm SL. CI FML 8014, 2, 57.4–54.2 mm. CI FML 8015, 2, 44.4–48.4 SL. Horcones: CI FML 8016, 1, 35.2 mm SL. Urueña: CI FML 8017, 7, 38.1–53.1 mm SL.

DISCUSSION

Historically, limited information has been available regarding the morphology, phylogenetic relationships and distribution of *C. borellii*, with only a brief redescription by Géry *et al.* (2001). Several characters described by these authors differ from the findings of this study, including iii,9,i or iii,10,i pectoral-fin rays (*vs.* iii,9 rays), tricuspid teeth in the dentary and premaxillary (*vs.* conical teeth), and confirmation of a single row of teeth in the dentary. Additional features with phylogenetic potential, such as hooks on the pelvic and pectoral fins of males (Mirande, 2010; Teixeira, Melo, 2021); swimbladder morphology (Agudelo-Zamora *et al.*, 2020) and pseudotympanum anatomy (Zanata, Camelier, 2014), are also provided.

Secondary sexual characteristics including bony process on the pelvic, also observed by Mirande (2010), and pectoral fins, and longer pelvic fin in males, are recorded in *C. borellii*. These dimorphic characters are also found in several other species of *Characidium* (Teixeira, Melo, 2021): bony processes on paired fins in *C. bahiense*, *C. barbosai*, *C. bimaculatum*, *C. clistenesi*, *C. deludens* (only on pelvic-fin rays), *C. lagsantense*, *C. laterale*, *C. litorale*, *C. mirim*, *C. nupelia*, *C. satoi*, *C. stigmosum*, *C. xanthopterum*, *C. xavante*; and longer pelvic fins in males of *C. deludens*, *C. kamakan* Zanata & Camelier, 2015, *C. rachovii*, *C. orientale*, and *C. vestigipinne*. The combination of having both characters appears to be unique in *C. borellii*.

The genus *Characidium* is diagnosed by the presence of a black basicaudal spot (Buckup, 1993b), a character also present in *C. borellii*, despite some reversions within the genus. Buckup (1993b) recovered three major clades within *Characidium*: C1, C4, and C5. Clade C1 (C2+C3) comprises species (*e.g.*, *C. fasciatum* and *C. gomesi*) characterized by an unscaled isthmus, reduced or absent poscleithrum 1, and reduction or absence of the cranial fontanel. Clade C4 includes species (*e.g.*, *C. rachovii*, *C. occidentale*, and *C. laterale*) defined by three synapomorphies (Netto-Ferreira *et al.*, 2013; Mendonça, Netto-Ferreira, 2015): absence of the parietal branch of the supraorbital canal, more than 12 transversal bars, and absence of inner row of dentary teeth. Clade C5 includes species with thin transversal bars. *Characidium borellii* only shares the absence of inner teeth with species in C4 and does not clearly conform to any of these morphological clades.

Most recent phylogenetic analyses using mitochondrial markers (COI) recognize different lineages or clades (Oliveira-Silva *et al.*, 2024; Serra *et al.*, 2025) but with distinct relationships among them. According to these clades, we recovered *C. borellii* in the “*Nanognathus*” clade (= “*C. bimaculatum*” clade from Serra *et al.*, 2025 or CI clade from Oliveira-Silva *et al.*, 2024), grouped with *C. bahiense*, *C. bimaculatum*, *C. cf. zebra* (from Paraná and São Francisco basins), *C. deludens*, *C. lagsantense*, *C. samurai*, and *C. varii*. Genetic distances are consistent with the close phylogenetic relationships among these

species. Combining the morphology data, *C. borellii* appears most closely related to *C. bimaculatum* and *C. deludens*, sharing several features: the presence of hooks on the pelvic fins, a muscular hiatus of the pseudotympanum formed by a single aperture, absence of an inner row of dentary teeth (in *C. deludens*), and the presence of parietal branch of supraorbital canal (in *C. deludens*). A combined phylogenetic analysis integrating both morphological and molecular data with a broader range of species is needed to confirm these relationships.

Specimens previously identified as *Characidium* cf. *zebra* or *C. fasciatum* from Sali, Bermejo and Juramento-Salado basins (Liotta, 2005; Mirande, Aguilera, 2009; Cancino, Aguilera, 2016; Terán *et al.*, 2016; Alonso *et al.*, 2025) were misidentified and correspond to *C. borellii*. The molecular and morphological data showed no significant differences among these populations, with high morphological variation attributed to sexual dimorphism or allometry. Therefore, *Characidium borellii* represents a valid species within the genus *Characidium*, with a distribution restricted to Northwestern Argentina.

Comparative material examined. Comparative material is listed in Serra *et al.* (2025), additional comparative material: *Characidium pterostictum*: **Argentina**: CI FML 8189, 11, 26.6–54.9 mm SL. CI FML 8190, 31, 33.5–63.1. CI FML 8130, 6, 34.5–52.5. *Characidium rachovii*: CI FML 5372, 1, 28.2 mm SL. CI FML 8018, 8, 36.4–25.4 mm SL. CI FML 8191, 5, 18.2–21.5 mm SL. MG-ZV-P 2870 (ex-INALI 832), 8, 32.7–34.9 mm SL. *Characidium serrano*: CI FML 8105, 7, 32.7–52.5 mm SL. CI-FML 8106, 24, 31.1–47.9 mm SL. *Characidium tenue*: CI FML 8187, 1, 29.9 mm SL. CL FML 8188, 1, 29.1 mm SL. *Characidium* aff. *zebra*: CI FML 1739, 14, 13.6–34.7 mm SL. CI FML 5175, 47, 22.9–29 mm SL. CI FML 7680, 5, 26.7–37.2 mm SL. **Brazil**: *Characidium alipioi*: MZUSP 109873, 1, 78.6 mm SL. MZUSP 79427, 25, 70.0–86.2 mm SL. *Characidium bimaculatum*: MZUSP 68959, 3 (1cs), 20.2–25.3 mm SL. *Characidium deludens*: MZUSP 115009, 1, 48.15 mm SL, holotype. MZUSP 115010, 9, 27.01–40.95 mm SL, paratypes. *Characidium fasciatum*: MZUSP 37067, 2, 42.4–48.8 mm SL. MZUSP 85981, 13, 21.4–35.6 mm SL. MZUSP 85995, 32 (1cs), 22.6–26.3 mm SL. *Characidium gomesi*: MZUSP 110032, 3, 31.1 mm SL. MZUSP 110447, 6, 32.9–59.3 mm SL. MZUSP 88349, 6, 31.2–38.7 mm SL. *Characidium lagosantense*: MZUSP 73708, 2, 19.3–23.0 mm SL. *Characidium lanei*: MZUSP 114844, 11, 28.9–46.3 mm SL. MZUSP 120519, 1, 41.8 mm SL. *Characidium* cf. *lanei*: CI FML 2133, 2, 50.2–67.0 mm SL. *Characidium lauroi*: MZUSP 110359, 5, 30.8–45.0 mm SL. MZUSP 124033, 12, 37.4–49.1 mm SL. *Characidium litorale*: MZUSP 129926, 2, 18.8–25.0 mm SL. MZUSP 129946, 1, 34.3 mm SL. *Characidium nana*: MZUSP 125919, 10, 15.8–20.7 mm SL. *Characidium nupelia*: MZUSP 87743, 1, 28.96 mm SL, holotype. MZUSP 87742, 20, 21.82–28.95 mm SL, paratypes. *Characidium oiticicai*: MZUSP 110245, 14, 23.2–55.7 mm SL. MZUSP 87716, 3, 34.0–44.8 mm SL. *Characidium onca*: MZUSP 125807, 1, 39.7 mm SL, holotype. MZUSP 125803, 6 (2 cs), 26.8–30.5 mm SL, paratypes. *Characidium schubarti*: MZUSP 71023, 3, 34.2–42.7 mm SL. *Characidium stigmatosum*: MZUSP 40804, 1, 27.9–35.5 mm SL, paratype. *Characidium travassosi*: MZUSP 85940, 1, 33.7 mm SL, holotype. MZUSP 85938, 6 (1 cs), 32.6–33.3 mm SL, paratypes. *Characidium xanthopterum*: MZUSP 41454, 20, 21.6–31.3 mm SL. MZUSP 117743, 5, 30.8–39.3 mm SL. MZUSP 117946, 1, 33.6 mm SL. *Characidium boavistae*: **Colombia**: IAvH-P-11801, 19, 38.1–49.8 mm SL. IAvH-P-21619, 3, 29.8–38.5 mm SL. IAvH-P-23640, 8, 33.9–44.1 mm SL. IAvH-P-29935, 1, 40.9 mm SL. IAvH-P-9760, 16, 37.5–49.4 mm SL. IAvH-P-9767, 26, 31.0–46.4 mm SL. *Characidium caucanum*: IAvH-P-1520, 1, 43.8 mm SL. *Characidium crandellii*: IAvH-P-20573, 3, 38.7–41.7 mm SL. *Characidium dule*: IAvH-P-13334, 42, 28.2–42.1 mm SL. *Characidium phoxocephalum*: IAvH-P-8432, 1, 53.9 mm SL. IAvH-P-8434, 1, 89.7 mm SL. *Melanocharacidium dispilomma*: IAvH-P-12885, 27, 21.2–31.6 mm SL. IAvH-P-26305, 6, 27.5–33.8 mm SL. *Characidium zebra*: **Guyana**: FMNH 53547, 52.5 mm SL, holotype (photographs). FMNH 52766, 4, 30–34 mm SL, paratypes (photographs and radiograph).

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ETHICAL STATEMENT

All procedures followed the ethical guidelines and animal welfare regulations established by the Comité Nacional de Ética en la Ciencia y Tecnología, Argentina. The material used in this work comes from specimens deposited in museum collections.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article and in the supplementary material of this article.

COMPETING INTERESTS

The authors declare no competing interests.

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