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Removing stripes from the zebra: Two new species of *Characidium* (Characiformes: Crenuchidae) from the Paraná and Uruguay River basins

Quitando las rayas a la cebra: dos nuevas especies de *Characidium* (Characiformes: Crenuchidae) de las cuencas de los ríos Paraná y Uruguay

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Abstract

An integrative taxonomic analysis based on morphological and molecular data reveals the presence of two new species of *Characidium* from the Paraná and Uruguay River basins. These new taxa were historically identified as *Characidium* cf. *zebra* or *Characidium fasciatum*. However, biogeographic, morphological, and molecular evidence indicates that they more closely related to *C. borellii* than to the species of the *C. zebra* complex. Both species are distinguished from their congeners by a unique combination of characters: a completely scaled isthmus, a complete lateral line, 14 scales around the caudal peduncle, two rows of dentary teeth, mostly hyaline fins, regular dark transverse bars on the body, the presence of the parietal branch of the supraorbital canal, and specific osteological traits. The new species are distinguished from each other by their pectoral-fin ray counts (13–14 vs. 12 or fewer), coloration patterns (a diffuse vs. a well-defined longitudinal stripe), and internal anatomical features, specifically the number of pseudotympanum openings and vertebral counts. Additionally, we provide data regarding their phylogenetic position, sexual dimorphism, morphological variation, and conservation status. These findings highlight a previously unrecognized diversity of *Characidium* within the lower La Plata basin.

Keywords: Characidiinae; cryptic species; La Plata basin; integrative taxonomy; Neotropical.

Resumen

Un análisis taxonómico integrativo basado en datos morfológicos y moleculares revela la presencia de dos nuevas especies de *Characidium* de las cuencas de los ríos Paraná y Uruguay. Estos nuevos taxa fueron identificados históricamente de manera indistinta como *Characidium* cf. *zebra* o *Characidium fasciatum*. Sin embargo, la evidencia biogeográfica, morfológica y molecular indica están más estrechamente emparentadas con *Characidium borellii* que con las especies del complejo *C. zebra*. Ambas especies se distinguen de sus congéneres por una combinación única de caracteres: istmo completamente escamado, línea lateral completa, 14 escamas alrededor del pedúnculo caudal, dos hileras de dientes del dentario, aletas mayoritariamente hialinas, barras transversales oscuras regulares en el cuerpo, la presencia de la rama parietal del canal supraorbital y por caracteres osteológicos específicos. Ambas especies se distinguen entre sí por el número de radios de la aleta pectoral (13–14 vs. 12 o menos), patrones de coloración (una banda longitudinal difusa vs. una bien definida) y características anatómicas internas, específicamente el número de aberturas del pseudotímpano y el conteo de vértebras. Además, aportamos datos sobre su posición filogenética, dimorfismo sexual, variación morfológica y estado de conservación. Estos hallazgos resaltan una diversidad de *Characidium* previamente no reconocida dentro de la cuenca baja del Plata.

Palabras clave: Characidiinae; especies crípticas; cuenca del Plata; taxonomía integrativa; Neotropical.

INTRODUCTION

The Neotropical genus *Characidium* Reinhardt comprises small benthic fishes widely distributed throughout South America, locally known as "tritolos" or "maripositas" in Argentina and Uruguay (Liotta et al., 2006; Serra et al., 2014; Almirón, Casciotta, Ciotek, Giorgis, 2015). With 93 currently recognized species (Fricke, Eschmeyer, Van der Laan, 2026; Oliveira-Silva et al., 2025; Zanata et al., 2025), the genus extends from southern Panama to central Argentina, occupying a broad range of river systems across tropical and subtropical regions. Buckup (1993a, b) recovered *Characidium* as a monophyletic clade and diagnosed it by the presence of a dark spot near the base of the middle caudal-fin rays, a character state reversed in some lineages.

The La Plata River basin, one of South America's major hydrological systems, extends across Argentina, Bolivia, Brazil, Paraguay, and Uruguay. The basin is divided into three major sub-basins: the Paraná River (which includes the Iguazu and Salado rivers), the Paraguay River (with the Pilcomayo and Bermejo as its main western tributaries), and the Uruguay River (Pasquini & Depetris, 2006). Currently, 25 valid species of *Characidium* have been recorded in La Plata basin (Méttola et al., 2025). Within this system, there are seven freshwater ecoregions: Lower Paraná, Upper Paraná, Lower Uruguay, Upper Uruguay, Iguassu (or Iguazu), Chaco, and Upper Paraguay (*sensu* Abell et al., 2008).

In the Lower Paraná ecoregion, five valid species are currently recognized: *Characidium borellii* Boulenger; *Characidium etzeli* Zarske & Gery; *Characidium lilloi* Terán et al.; *Characidium pterostictum* Gomes; and *Characidium rachovii* Regan. In addition to these, there are several unidentified populations usually referred to as *Characidium* cf. *zebra* Eigenmann (Almirón et al., 2015; Casciotta, Almirón, Doubnerová, Piálek, Rican, 2015; Mirande & Koerber, 2020; Méttola et al., 2025; Terán et al., 2026).

Regarding the Upper Paraná ecoregion, 11 valid species are recognized: *Characidium fasciatum* Reinhardt; *Characidium gomesi* Travassos; *Characidium heirmostigmata* Graça & Pavanelli; *Characidium itarare* Stabile et al.; *Characidium lagosantense* Travassos; *Characidium oiticicai* Travassos; *Characidium onca* Melo, Brito Ribeiro & Lima; *Characidium schubarti* Travassos; *Characidium stigmosum* Melo & Buckup; *Characidium urucum* Uzeda et al.; *Characidium xanthopterum* Silveira, Langeani, Graça, Pavanelli & Buckup (Graça & Pavanelli, 2007; Langeani et al., 2007; Ota, Deprá, da Graca, Pavanelli, 2018; Dagosta et al., 2024).

Five species inhabit in the Lower Uruguay ecoregion: *C. occidentale* Buckup & Reis; *C. pterostictum*; *C. rachovii*; *Characidium serrano* Buckup & Reis; and *Characidium tenue* Cope (Buckup & Reis, 1997; Serra et al., 2014; Bogan, Meluso, Bauni, Cardoso, 2015; Casciotta et al., 2015; Loureiro, Gonzáles-Bergonzoni, Teixeira de Melo, 2023); and two valid species in the Upper Uruguay basin: *C. serrano*; and *C. vestigipinne* Buckup & Hahn (Buckup & Hahn, 2000; Serra et al., 2025).

In addition, all the aforementioned ecoregions contain unidentified populations usually referred to as *Characidium* sp., *Characidium* cf. *zebra* or *Characidium* aff. *zebra* (Buckup & Reis, 1997; Silveira, Langeani, Graca, Pavanelii & Buckup, 2008).

In his taxonomic review, Buckup (1992) restricted *Characidium fasciatum* to the São Francisco and upper Paraná drainages and classifying specimens previously identified as *C. fasciatum* from other regions to *C.* cf. *zebra*. The species *Characidium zebra* was described by Eigenmann (1909) from the Maripicru Creek, a tributary of the Ireng River in the upper Branco River basin, on the Guiana Shield but, after Buckup (1992), specimens identified as *Characidium* cf. *zebra* are distributed in many Neotropical basins, including La Plata system. Historically, specimens of *Characidium* from the La Plata basin have been inconsistently referred to as *C.* cf. *zebra*, *C.* aff. *zebra*, or *C. fasciatum* (e.g., Liotta, 2006; Serra et al., 2014; Cancino & Aguilera, 2016; Terán, Jarduli, Alonso, Mirande, Shibatta, 2016; Almirón et al., 2015; Casciotta et al., 2016; Alonso et al., 2024). Thus, the wide distribution of *Characidium* cf. *zebra* in this region is an indicative of poor taxonomic knowledge of the group and the presence of species complexes involving cryptic diversity. Indeed, for example, populations previously identified as *C.* cf. *zebra* from the Bermejo and Juramento River basins were analyzed and re-identified as *C. borellii* (Méttola et al., 2025), while populations from other basins assigned to *C.* cf. *zebra* have been found to form distinct clades (Agudelo-Zamora et al. 2020b; Méttola et al., 2025).

In this study, we conduct an anatomical and genetic analysis to delimit and recognized the species traditionally addressed as *Characidium* cf. *zebra*, *C.* aff. *zebra*, or *C. fasciatum* from the lower and middle Paraná and lower Uruguay River basins, resulting in the description of two new species.

MATERIAL AND METHODS

Fish sampling

Fish were collected using hand nets and electrofishing, then euthanized by immersion in an anesthetic solution (0.1% 2-phenoxyethanol). Also, specimens from scientific collections were utilized.

For genetic analysis, small tissue samples were taken from fresh specimens and immediately preserved in absolute ethanol. These tissue samples were deposited in the ichthyological tissue collections of LGE and CIT-FML for future reference. All procedures followed the ethical guidelines and animal welfare regulations established by the Comité Nacional de Ética en la Ciencia y Tecnología, Argentina.

Institution names follow Sabaj (2025).

Morphological analyses

Measurements were taken as straight-line distances using a caliper to the nearest 0.1 mm. Morphometric and meristic data were taken following Buckup (1993a) with modifications of Melo and Oyakawa (2015), Zanata, Oliveira-Silva and Ohara (2023), and additional measurements from Méttola et al. (2025). Sex identification was based on the presence of secondary sexual characters (Teixeira & Melo, 2020) and gonadal examination.

Specimens were cleared and stained (cs) following the protocol of Taylor and Van Dyke (1985). In the morphological description, the counts for holotypes are marked with an asterisk (*), and the number of specimens examined for each meristic character is given in parentheses.

Material is deposited in the ichthyological collection of the following institutions: Fundación de Historia Natural Félix de Azara (CFA-IC), Universidad Maimónides, Buenos Aires, Argentina; Fundación Miguel Lillo (CI-FML), Tucumán, Argentina; Instituto de Bio- y Geociencias del NOA, Salta, Argentina (IBIGEO); Laboratorio de Genética Evolutiva (LGE), Instituto de Biología Subtropical, Misiones, Argentina; Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN-Ict), Buenos Aires, Argentina; Museo Nacional de Historia Natural (MHNM), Montevideo, Uruguay; and Facultad de Ciencias (ZVC-P), Universidad de la República, Montevideo, Uruguay.

Statistical analyses

A total of 73 specimens were analyzed. Thirty-six linear morphometric variables were measured to the nearest 0.1 mm, including the total length (TL), standard length (SL) and head length (HL).

Morphometric variation among populations was analyzed using multivariate methods based on raw linear measurements. All variables were log-transformed to stabilize variances and linearize allometric relationships. To account for size-related variation, each variable was regressed against standard length (log–log), and the residuals were used as size-corrected shape variables. Pairwise correlations among variables were examined and highly collinear variables were excluded prior to analysis. The resulting dataset was standardized (z-scores), assumed to approximate a correlation matrix structure, and subjected to Principal Component Analysis (PCA) in PAST (Hammer & Harper, 2001) to explore patterns of morphological variation. Missing values (less than 0.6% of the dataset) were estimated using iterative multivariate imputation based on PCA. The broken-stick model was used as a criterion to determine the number of principal components retained for interpretation.

Differences among populations were tested using a multivariate, permutation-based approach (PERMANOVA) on the size-corrected variables with Bonferroni-adjusted p-values for multiple comparisons. To evaluate potential morphometric differentiation between Paraná and Uruguay basin populations of *Characidium* n. sp., specimens were analyzed within the same PCA framework and compared visually across retained principal components.

DNA protocols

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-μl reaction mixture consisting of 25 μl of PB-L master mix (PB-L Productos Bio-Lógicos, Quilmes, Argentina), 0.4 μM of each forward and reverse primer, and 4 μl 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., 1991) 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.

Species delimitation and phylogenetic analyses

We constructed a matrix using genetic sequences from the *Cytochrome Oxidase Subunit I* (COI) and *Cytochrome B* (CytB) markers incorporating newly generated sequences (Table S1), additional to those used in Méttola et al. (2025) and Oliveira-Silva et al. (2025) (Table S2). Sequence alignment was performed using Muscle (Edgar, 2004), checking codon positions with Aliview (Larsson, 2014). Genetic distances of the COI marker were computed in MEGA v11 (Kumar, Stecher, Li, Knyaz, Tamura, 2018) using the Kimura 2-parameter (K2P) model, with all substitution types included (d: Transitions + Transversions), pairwise deletion of missing data, and uniform rates among sites.

Parsimony analyses.— Phylogenetic analyses were performed under parsimony by combining both markers (COI + CytB). Analyses were done in TNT (Windows version 1.6) (Goloboff, Farris, Nixon, 2008) under extended implied weighting (Goloboff, 2014) following the criteria of Terán et al. (2020) and Mirande (2023) to select the final hypothesis among the most parsimonious trees obtained under different parameters. A range of concavity constants (K = 3-24) was explored to assess sensitivity of tree topology to differential down-weighting of homoplastic characters. Under implied weighting, lower K values impose stronger penalization to homoplastic transformations (Goloboff, 1993). Alternative character weighting and partitioning schemes were evaluated (SEP, COD, GRO, BLK, POS) (details in Mirande, 2017). For the COI dataset, results are reported under the SEP scheme with K = 16. For the concatenated mitochondrial dataset (COI + CytB), results are presented under SEP with K = 12. The SEP scheme weights each character independently according to own homoplasy. Node support was assessed using GC (group support; frequency difference) values obtained through symmetric resampling (Groups present/Contradicted) (Goloboff et al., 2003). Strict consensus trees were calculated when multiple most-parsimonious trees (MPTs) were recovered.

Maximum Likelihood analyses.— Partitioned Maximum Likelihood (ML) analyses were conducted in IQtree v3 (Trifinopoulos, Nguyen, von Haeseler, Minh, 2016). The preferred nucleotide substitution model for each codon position according to Bayesian Information Criterion (BIC) was selected using ModelFinder (Chernomor, Von Haeseler, Minh, 2016; Kalyaanamoorthy, Minh, Wong, Von Haeseler, Jermiin, 2017). For COI, the selected models were: TNe+I+G4 (first codon position), F81+F+R2 (second), TN+F+G4 (third). For cytB: TPM3u+F+I+G4 (first codon position), HKY+F+I (second), TN+F+G4 (third). Branch support was assessed using SH-aLRT and ultrafast bootstrap (UFBoot) with 10,000 replicates each. Nodes were considered strongly supported when SH-aLRT ≥ 80 and UFBoot ≥ 95% (Hoang, Chernomor, Von Haeseler, Minh, Vinh, 2018).

For both analyses, *Hoplias malabaricus* was used as an outgroup in phylogenetic trees.

Nomenclatural acts

This contribution follows the requirements of the International Code of Zoological Nomenclature, and the new names contained herein are available from the electronic publication of this article. All the nomenclatural acts it contains have been registered in Zoobank. The ZooBank LSIDs (Life Science Identifiers) can be resolved, and the associated information viewed through any standard web browser by appending the LSID to the prefix "http://zoobank.org/". The LSID for this publication is: urn:lsid:zoobank.org:pub:DFBFAFA5-FAC9-44BA-82BE-F81C5156D023

RESULTS

Characidium koerberi, new species

urn:lsid:zoobank.org:act:C7E48D1C-EAE6-411DBDB1-793DB898F1E2
(Fig. 1, 2, 3; Table 1)

Characidium cf. *zebra* (non Eigenmann, 1909): Miquelarena, Protogino, Filiberto, Lopéz, 2002:77 (listed)

Holotype.— CI-FML 8298, 67.8 mm SL, Argentina, Misiones, middle Paraná River basin, Tabay stream, 26°59'58.5"S 55°10'41.1"W, G. Aguilera, M.F. Benitez, M.A. Cortés Hernández, V.E. Méttola & G.E. Terán, March 2025.

Paratypes. Argentina: Misiones.— LGEP 1556, 2, 71.8–75.0 mm SL, collected with the holotype; LGEP 1680, 2, 64.9–66.5 mm SL, Gruta India, 26°45'32.26"S 54°55'24.63"W, G. Aguilera, F. Alonso, M.F. Benitez, M. Mirande, & G.E. Terán, November 2016; CI-FML 7458, 1, 38.6 mm SL, Puerto Maní, 27°6'22.92"S 55°31'20.42"W, M. Mirande, G.E. Terán & G. Aguilera, November 2008; CI-FML 8299 (CIT-FML 153), 3, 49.7–73.8 mm SL, Tabay stream, 27°0'0.00"S 55°9'60.00"W, G. Aguilera, F. Alonso, M.F. Benitez, M. Mirande, & G.E. Terán, November 2016; CI-FML 8300



Figure 1. *Characidium koerberi* CI-FML 8298. Holotype, female, 67.8 mm SL. Tabay stream, Misiones, Argentina, Paraná River basin. Scale bar = 10 mm.

Figura 1. *Characidium koerberi* CI-FML 8298. Holotipo, hembra, 67,8 mm SL. Arroyo Tabay, Misiones, Argentina, cuenca del río Paraná. Barra de escala = 10 mm.

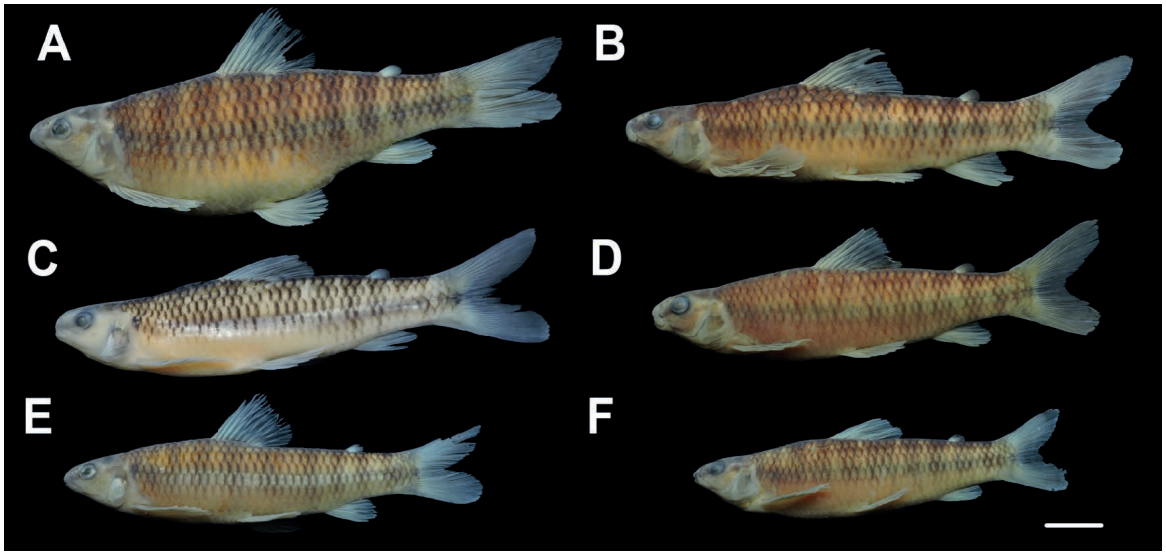


Figure 2. Paratypes of *Characidium koerberi*, all from middle Paraná basin, Misiones, Argentina. A. CI-FML 8299. Gravid female, 73.8 mm SL. Tabay stream. B. CI-FML 8300. Female, 70.6 mm SL. Gruta India. C. CI-FML 8247. Male, 63.9 mm SL. Itaembe stream. D. CI-FML 8300. Male, 62.6 mm SL. Gruta India. E. CI-FML 8299. Male, 58.1 mm SL. Tabay stream. F. CI-FML 8300. Male, 54.1 mm SL. Gruta India. Scale bar = 10 mm..

Figura 2. Paratipos de *Characidium koerberi*, todos de la cuenca media del Paraná, Misiones, Argentina. A. CI-FML 8299. Hembra grávida, 73,8 mm SL. Arroyo Tabay. B. CI-FML 8300. Hembra, 70,6 mm SL. Gruta India. C. CI-FML 8247. Macho, 63,9 mm SL. Arroyo Itaembe. D. CI-FML 8300. Macho, 62,6 mm SL. Gruta India. E. CI-FML 8299. Macho, 58,1 mm SL. Arroyo Tabay. F. CI-FML 8300. Macho, 54,1 mm SL. Gruta India. Barra de escala = 10 mm.

(CIT-FML 132), 12 (2 cs), 43.5–74.1 mm SL, Gruta India, 26°45'32.26"S 54°55'24.63"W, G. Aguilera, F. Alonso, M.F. Benitez, M. Mirande, & G.E. Terán, November 2016; CI-FML 8247, 1, 63.9 mm SL, Itaembe stream, 27°27'20.8"S 56°03'02.3"W, G. Aguilera, M.F. Benitez, M.A. Cortés Hernández, V.E. Méttola & G.E. Terán, March 2025; IBIGEO-I 527, 2, 60.7–69.7 mm SL, Gruta India, 26°45'32.26"S 54°55'24.63"W, G. Aguilera, F. Alonso, M.F. Benitez, M. Mirande, & G.E. Terán, November 2016. MHNM 7814, 1, 71.9 mm SL, Gruta India, 26°45'32.26"S 54°55'24.63"W, G. Aguilera, F. Alonso, M.F. Benitez, M. Mirande, & G.E. Terán, November 2016. **Corrientes:** IBIGEO-I 526, 2, 31.1–32.6 mm SL, Itá-Ibaté beach, 27°25'20.38"S 57°20'1.20"W, G. Aguilera, G.E. Terán, F. Ruíz Díaz & B. Bugeau, December 2018; CI-FML 7672, 4, 31.2–34.1 mm SL, Itá-Ibaté beach, 27°25'20.38"S 57°20'1.20"W, G. Aguilera, G.E. Terán, F. Ruíz Díaz & B. Bugeau, December 2018; CI-FML 7673, 2, 32.5–39.0 mm SL, Ituzaingó beach, 27°34'41.32"S 56°40'59.53"W, M. Mirande, G.E. Terán & G. Aguilera, December 2018. CI-FML 7675, 1, 38.1 mm SL, Toropi stream, 28°35'3.33"S 59°3'46.22"W, G. Aguilera, G. Terán, F. Ruíz Díaz & B. Bugeau, 02 December 2018. CFA-IC 1082, 1, 36.1 mm SL, Riacho el Carrizal, ca. 28°28'52"S 59°02'17"W, Bella Vista, 1989.



Figure 3. Live specimens of *Characidium koerberi* (LGEP 1556) from Tabay stream, Misiones, Argentina, Paraná River basin. A. Female, 75.1 mm SL. B. Female, 71.8 mm SL. Scale bar = 10 mm.

Figura 3. Ejemplares vivos de *Characidium koerberi* (LGEP 1556) del arroyo Tabay, Misiones, Argentina, cuenca del río Paraná. A. Hembra, 75,1 mm SL. B. Hembra, 71,8 mm SL. Barra de escala = 10 mm.

Diagnosis.— The isthmus covered with scales (*vs*. unscaled) and the presence of 14 scales around the caudal peduncle (*vs*. 12 or fewer) distinguish *Characidium koerberi* n.sp. from all congeners except *Characidium bimaculatum* Fowler, *C. borellii*, *Characidium chupa* Schultz, *Characidium deludens* Zanata & Camelier, *Characidium etheostoma* Cope, *Characidium geryi* (Zarske), *Characidium interruptum* Pellegrin, *Characidium mariposita* n.sp., *Characidium onca* Melo, Brito, Ribeiro & Lima, *Characidium pumarinri* Teixeira & Melo, *Characidium sanctjohanni* Dahl, *Characidium samurai* Melo & Oyakawa, *Characidium satoi* Melo & Oyakawa, *Characidium steindachneri* Cope, *C. stigmosum*, *Characidium varii* Zanata, Oliveira & Oliveira-Silva, *C. xanthopterum*, and *C. zebra*. It differs from *C. pumarinri* by having a basicaudal spot near the base of middle caudal-fin rays (*vs.* absent); from *C. etheostoma*, *C. geryi*, *C. mariposita* n.sp., *C. sanctjohanni*, *C. xanthopterum* and *C. zebra* by having 13 or 14 total pectoral-fin rays (*vs*. 12 or fewer rays); from *C. varii* by having fins mostly hyaline (*vs*. having dark stripes); from *C. onca* and *C. stigmosum* by lacking dark dots on flanks (*vs*. small dots in *C. onca*, and dots superimposed on the transverse bars in *C. stigmosum*); from *C. satoi* and *C. deludens* by having regular transversal bars (*vs*. irregular); from *C. steindachneri* by having 3–4 scales between the anus and the anal fin (*vs*.

Table 1. Summary of morphometric data for *Characidium koerberi* and *C. mariposita*. Asterisks indicate additional variables.

Tabla 1. Tabla morfométrica resumida de *Characidium koerberi* y *C. mariposita*. Variables adicionales con asterisco.

Measurements	*Characidium koerberi* n. sp.					*Characidium mariposita* n. sp.				
	Holotype	Min.	Max.	Mean	SD	Holotype	Min.	Max.	Mean	SD
Total length	84.4	40.0	90.2	66.2	17.8	57.2	31.1	57.2	44.6	6.3
Standard length	67.8	31.1	75.0	51.1	15.7	45.3	24.2	45.3	34.9	5.3
Head length	14.9	8.2	16.8	12.6	3.0	10.6	6.7	11.0	8.7	1.1
Percentages of SL										
Head length (%)	22.0	21.1	27.9	23.8	1.7	23.3	22.6	28.9	25.1	1.4
Prepectoral distance	20.7	19.9	27.2	23.6	2.1	22.2	20.7	29.2	24.2	1.7
Pectoral fin length*	26.7	17.3	31.3	26.5	2.6	24.6	20.1	26.7	24.2	1.6
Predorsal distance	45.3	41.7	48.0	44.4	1.3	45.8	41.3	49.9	46.3	1.8
Dorsal fin height	20.0	15.2	23.3	19.5	2.1	22.0	19.5	25.6	22.6	1.8
Dorsal fin base	15.2	13.5	16.6	14.9	0.7	15.3	13.6	17.6	15.3	0.9
Prepelvic distance	51.1	48.7	53.4	51.9	1.1	52.3	47.9	54.9	51.3	1.4
Pelvic fin length*	20.5	18.3	23.6	20.8	1.3	22.4	19.2	25.9	21.9	1.7
Preanal distance	77.0	75.1	80.4	77.3	1.2	77.2	72.8	81.3	75.6	1.5
Anal apex distance	95.3	91.3	98.5	94.6	1.5	95.0	88.5	98.6	93.9	2.0
Anus to anal fin distance	9.0	7.7	10.9	8.9	0.8	5.1	4.5	7.5	6.4	0.7
Anal fin length*	19.2	15.8	20.5	17.9	1.3	18.6	15.4	21.7	18.4	1.4
Anal fin base	7.9	5.6	8.0	7.3	0.5	7.4	5.6	8.8	7.4	0.6
Adipose fin height	5.7	4.4	7.6	5.9	0.7	5.3	4.3	6.8	5.7	0.6
Peduncle length	19.1	16.4	19.1	17.9	0.7	19.2	16.4	21.9	19.3	1.1
Body depth at dorsal fin origin	25.1	15.5	27.1	22.0	3.0	27.2	18.0	28.6	24.8	1.9
Body depth at anal fin origin	19.4	11.3	19.4	16.7	1.9	16.9	14.0	18.3	16.9	0.8
Body depth at caudal peduncle	12.2	10.2	12.7	11.5	0.6	11.8	10.3	12.4	11.4	0.5
Body width	12.8	9.7	13.3	11.6	1.0	9.5	7.7	10.7	9.3	0.7
Percentages of HL										
Snout length	27.8	22.0	28.5	25.6	1.8	22.9	18.8	25.6	22.3	1.6
Snout maxillary tip	23.0	19.5	24.7	22.6	1.2	20.4	19.4	23.7	21.4	1.1
Anterior naris orbit	10.7	8.7	13.1	10.6	1.1	10.6	6.9	12.8	10.4	1.4
Posterior naris orbit	5.3	3.8	6.4	5.2	0.7	4.6	4.1	7.5	5.6	0.9
Cheek	14.1	6.5	14.1	10.4	1.9	7.9	6.0	11.3	8.3	1.3
Orbital diameter	23.5	21.6	30.6	25.2	3.0	28.5	26.5	35.6	30.0	2.3
Interorbital diameter	20.4	14.4	20.4	16.8	1.7	19.2	14.9	23.9	17.7	1.8

6–8); from *C. bimaculatum* by having 36–38 scales in longitudinal line (*vs.* 35 or fewer); and from *C. chupa*, *C. samurai*, and *C. zebra* by having a black longitudinal stripe almost absent in adults (*vs.* conspicuous longitudinal stripe along entire flank).

The new species differs from *Characidium bahiense* Almeida, *C. interruptum*, *Characidium laterale* (Boulenger), *Characidium mirim* Netto-Ferreira, Birindelli & Buckup, *Characidium nupelia* da Graça, Pavanelli & Buckup, *C. stigmosum*, and *Characidium xavante* da Graça, Pavanelli & Buckup by having a complete lateral line (*vs*. incomplete); from *C. barbosai* by the presence of a preorbital stripe (*vs*. absent); from *C. borellii*, *C. deludens*, *C. etzeli*, *C. lagosantense*, *Characidium litorale* Leitão & Buckup, *C. satoi*, and *C. xanthopterum* by having two rows of teeth on dentary (*vs*. one row); from *C. bimaculatum* by presenting a parietal branch of supraorbital laterosensory canal (*vs*. absent); from *C. mariposita* n.sp. by having one

pseudotympanum opening bordered by fifth and sixth vertebral ribs (*vs*. two openings separated by fifth vertebral rib), and by having 35 or 36 total vertebrae (*vs*. 33 or 34); and from *C. zebra* by absence of secondary transversal bars on flanks (*vs*. presence), by supracleithrum with scaled-shaped projection (*vs*. absent, Buckup, 1993b) and irregularly-shaped ischiatic process (*vs*. cylindrical, Melo, 2001).

Additionally, males of *Characidium koerberi* n.sp. are distinguished from males of *C. bimaculatum* and *C. pumarinri* by the absence of dimorphic fin coloration (*vs*. proximal third of dorsal fin dark in *C. bimaculatum*, and darker fins, except caudal-fin, in *C. pumarinri*); from males of *C. borellii*, *C. deludens*, *Characidium orientale* Buckup & Reis, *C. rachovii*, and *C. vestigipinne* by shorter pelvic fin, not reaching anal-fin origin (*vs*. reaching or slightly surpassing it); from males of *C. clistenesi* Melo & Espíndola, *C. mirim*, *C. satoi*, *C. stigmosum*, and *C. xanthopterum* by having bony hooks of spine type *sensu* Teixeira & Melo (2020) (*vs*. ridge type); and from males of *C. bahiense*, *C. borellii*, *C. nupelia*, and *C. xavante* by having spiny hooks only on pelvic fins (*vs*. present on pelvic and pectoral fins).

Description.— Morphometric data in Table 1, additional data in Table S3. Largest specimen examined reaching 74.1 mm SL. Body elongated. Dorsal profile in lateral view, moderately convex from tip of snout to dorsal fin, slightly convex from this point to adipose fin, and then straight to caudal fin. Ventral profile in lateral view slightly convex from snout to pelvic fins. Body in transverse section thickest at dorsal fin origin. Caudal peduncle height short.

Snout rounded in lateral view, tip below horizontal plane passing through ventral eye margin. Mouth subterminal. Orbit oval, positioned laterodorsally. Two nasal openings; anterior one with full dermal flap, posterior with flap on anterior margin only.

Squamation. Body covered with cycloid scales lacking *circuli* on posterior field. Isthmus completely scaled. Longitudinal scales rows: 36(6), 37*(15), 38(11), 39(1), perforated scales along lateral line. Transverse scale rows above lateral line: 4*(33). Additional scale row below predorsal row, counted anterior to first scale at dorsal-fin origin: 0(21), 1(2), 2*(6), 3(2), 6(1), 8(1) scales. Transverse scale rows below lateral line: 5*(33). Predorsal scale rows: 9(12), 10*(18), 11(3). Preadipose scale rows: 9*(17), 10(13), 11(3). Scales between adipose and caudal fin: 5(1), 6*(19), 7(12). Scale rows between pectoral- and pelvic-fin origins: 10(7), 11*(22), 12(2), 13(1), 14(1). Preanal scale rows: 4(15), 5*(16), 6(1), 7(1). Scale row between anus and anal-fin origin: 3*(7), 4(24), 5(2). Scales between anal and caudal-fin origin: 6*(18), 7(15). Circumpeduncular scale rows: 14*(33).

Fins. Pectoral fin not reaching pelvic-fin origin; rays iii,9,i*(20); iii,10,i(13). Pelvic fin not reaching anal-fin origin; rays i,6,i(1); i,7,i*(31); i,8,i(1). Dorsal fin truncated, base narrow; rays ii,8(2); ii,9*(31). Anal-fin base narrow; rays ii,6(11); ii,7*(22). A small anterior unbranched ray

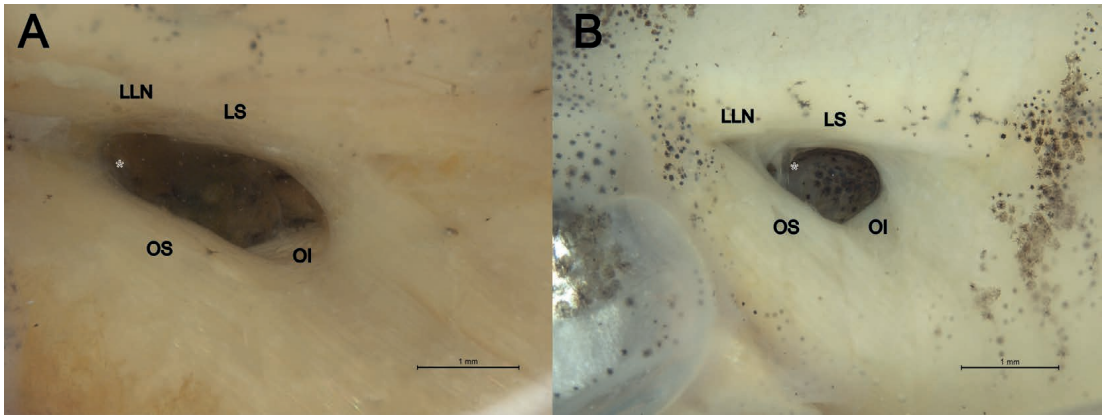


Figure 4. A. Pseudotympanum of *Characidium koerberi* CI-FML 8300. Paratype, female, 62.6 mm SL, from Gruta India, Misiones, Argentina, Paraná River basin. B. Pseudotympanum of *Characidium mariposita* n. sp. CI-FML 8305. Male, 30.4 mm SL, from Aguapey stream, Corrientes, Argentina, Uruguay River basin. Overlying skin, adipose tissue and lateral line nerve broken. LNN, lateral line nerve; OS, *obliquus superioris*; oi, *obliquus inferioris*; LS, *lateralis superficialis*. Asterisk corresponds to the fifth vertebrae associated with pleural ribs.

Figura 4. A. Pseudotímpano de *Characidium koerberi* CI-FML 8300. Paratipo, hembra, 62,6 mm SL, de Gruta India, Misiones, Argentina, cuenca del río Paraná. B. Pseudotímpano de *Characidium mariposita* n. sp. CI-FML 8305. Macho, 30,4 mm SL, del arroyo Aguapey, Corrientes, Argentina, cuenca del río Uruguay. Piel suprayacente, tejido adiposo y nervio de la línea lateral rotos. LNN, nervio de la línea lateral; OS, oblicuo superior; oi, oblicuo inferior; LS, lateral superficial. El asterisco corresponde a la quinta vértebra asociada con costillas pleurales.

present on dorsal and anal fins, observable only in cleared and stained (cs) specimens. Adipose fin well developed. Caudal fin usually with upper lobe slightly longer; rays i,8,8,i(1); i,9,7,i(1); i,9,8,i*(31).

Pseudotympanum present as muscular hiatus, oval in shape, aligned with anterior chamber of swimbladder, posterior to humeral spot. Single horizontally elongated oval opening between fifth and sixth vertebral ribs (Fig. 4A). Swimbladder reduced, composed of two chambers; anterior chamber larger and wider than posterior chamber.

Jaws and branchial apparatus. Premaxilla bearing 5(2), 6*(14), 7(15), 8(1) tricuspid teeth, decreasing in size posteriorly (Fig. 5A). Dentary with two rows, outer series with 5(2), 6(9), 7*(16), 8(5) tricuspid teeth, decreasing posteriorly, inner row composed of minute unicuspid teeth (Fig. 5B). Small replacement teeth present on dentary and premaxilla. Maxilla edentulous. Ectopterygoid elongated, with one row of 6(2 cs), 10(2 cs) minute conical teeth. Mesopterygoid and endopterygoid edentulous. Branchiostegal rays: 5(4 cs). One row of gill rakers on first gill arch: hypobranchial 1(2 cs), 2(2 cs); ceratobranchial 5(4 cs); epibranchial 6(1 cs), 7(3 cs).

Cephalic laterosensory system and girdles. Parietal branch of supraorbital canal present, extending to parietal. Cranial fontanel anteriorly limited by frontals. Infraorbitals 1–6 present, infraorbitals 4–6 reduced to tubular canal. Supracleithrum bearing scale-like projection on posterodorsal mar-

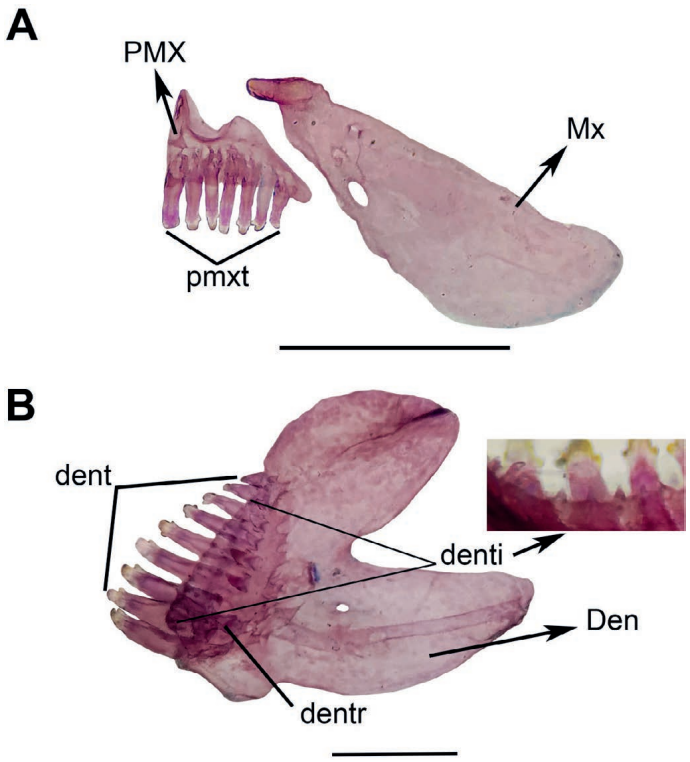


Figure 5. A. Right upper jaw in medial view of *Characidium koerberi* (CI-FML 8300) **B.** Right dentary in medial view of *Characidium koerberi* (CI-FML 8300). Abbreviations: Den, dentary; dent, dentary teeth; dentr, replacement dentary teeth; denti, inner row of dentary teeth; Mx, maxillary; PMX, premaxillary; pmxt, premaxillary teeth. Scale bar = 1 mm.

Figura 5. A. Vista medial del maxilar superior derecho de *Characidium koerberi* (CI-FML 8300). **B.** Vista medial del dentario derecho de *Characidium koerberi* (CI-FML 8300). Abreviaturas: Den, dentario; dent, dientes del dentario; dentr, dientes de reemplazo del dentario; denti, hilera interna de dientes del dentario; Mx, maxilar; PMX, premaxilar; pmxt, dientes premaxilares. Barra de escala = 1 mm.

gin. Poscleithra 1–3 present. Ischiatic process of pelvic bone irregular in shape.

Vertebral column and caudal skeleton. Total vertebrae: 35(2 cs), 36(2 cs). First four anterior vertebrae incorporated into Weberian apparatus, followed by 31–32 free vertebrae. Hypurals: 6(4 cs). Epurals: 3(4 cs). Uroneurals one pair (4 cs). Upper procurrent caudal-fin rays: 9(2 cs), 10(2 cs). Lower procurrent caudal-fin rays: 8(4 cs).

Coloration of preserved specimens.— Body and head brownish, darker dorsally. Black preorbital stripe with diffuse margins, postorbital stripe absent or short with diffuse margins. Black to silvery longitudinal stripe from operculum to caudal-fin base thin with diffuse edges, almost absent in adult specimens. Dark brown transverse bars across the body: 8(7), 9(17), 10*(7), 11(1) each wider than one scale row, regular in shape, not confluent in dorsal view, extending beyond midlateral stripe. Humeral black blotch present, vertically elongated. Small whitish spot anterior to humeral blotch.

Caudal peduncular blotch usually rounded with diffuse margins, more conspicuous in small specimens and faint or absent in adults. Basicaudal black spot present, less conspicuous in adults. Fins mostly hyaline, pectoral fin with few dark black chromatophores on dorsal margin of first four to five rays, and dorsal fin with two horizontal dark stripes. Distal portion of adipose fin with black pigmentation. Scales darker in posterior margin, dark brown pigmentation almost entirely covering scales on transverse bars and midlateral stripe. Scales between isthmus and anal-fin origin unpigmented. Small black spot anterior to dorsal-fin origin, thin black stripe on the middle of pre-dorsal scale row (Fig. 1, dorsal view).

Coloration in life.— Background color of body and head dark-brownish. Same overall pattern as color of preserved specimens except for yellowish fins and more intense black pigmentation on transverse bars, longitudinal stripe, preorbital stripe, postorbital stripe, humeral blotch, and basicaudal spot (Fig. 3).

Sexual dimorphism.— Males (7 specimens out of 33) with bony hooks (spine type *sensu* Teixeira & Melo, 2020) on pelvic-fin rays, located on distal half of first four or five unbranched rays. Smallest male with hooks in pelvic fins measuring 48.2 mm SL. Hooks on paired fins absent in females.

Distribution.— Middle and lower Paraná basin in Argentina (Fig. 6). Recorded from Corrientes and Misiones provinces in Argentina.

Ecological notes.— The Tabay Stream basin extends 192 km from its headwaters at Campo Viera to its mouth on the Paraná River at Jardín América and Puerto Leoni. The stream bed (at the sampling site) is composed mainly of basaltic bedrock, with alternating sections of waterfalls and pools along its course. At the type locality, the stream is bordered by remnants of the Paranaense riparian forest, and its left margin degraded by the presence of a camping site (Terán et al., 2017). *Characidium koerberi* occurs in sympatry with *C. mariposita* n.sp. in middle Paraná drainages.

Conservation status.— Despite having a known Extent of Occurrence (EOO) of about 16,300 km^2, *Characidium koerberi* is known from multiple tributaries of the middle Paraná River, including areas adjacent to provincial or municipal protected areas in Misiones. No evidence of severe fragmentation, extreme fluctuations, or continuing decline has been documented. Based on current knowledge, the species is assessed as Least Concern (LC) according to IUCN Red List Categories and Criteria.

Etymology.— The specific name *koerberi* honors the ichthyologist Stefan Koerber, whose long-standing contributions to ichthyological systematics, taxonomic databases "*https://pecescriollos.de/*", and nomenclatural taxonomic rigor advanced the understanding, nomenclatural stability, and distribution knowledge of South American fish taxonomy.

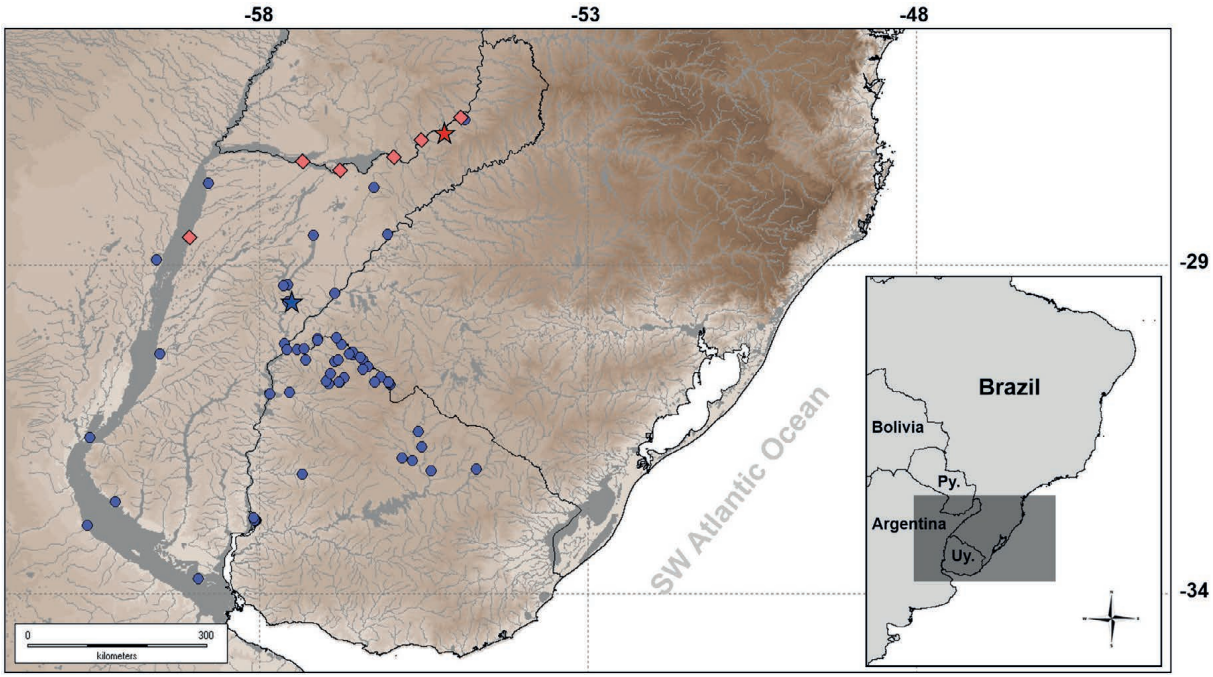


Figure 6. Geographical distribution of *Characidium koerberi* (red spots) and *Characidium mariposita* (blue spots) Paraná basin. Red star: type locality of *Characidium koerberi* in Tabay stream, Misiones, Argentina. Blue star: type localitiy of *Characidium mariposita* in Miriñay, Corrientes, Argentina.

Figura 6. Distribución geográfica de *Characidium koerberi* (puntos rojos) y *Characidium mariposita* (puntos azules) en la cuenca del Paraná. Estrella roja: localidad tipo de *Characidium koerberi* en el arroyo Tabay, Misiones, Argentina. Estrella azul: localidad tipo de *Characidium mariposita* en Miriñay, Corrientes, Argentina.

Characidium mariposita, **new species**

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(Fig. 7, 8; Table 1)

Characidium fasciatum (non Reinhardt, 1867): Liotta, 2006:74-75 (listed).

Characidium zebra (non Eigenmann, 1909): Almirón et al., 2015:75 (listed); Díaz et al., 2016:7 (barcoding); Dománico, Arrieta, Colautti, 2021:4605 (listed); Loureiro et al., 2023:31-32 (listed).

Characidium aff. *zebra* (non Eigenmann, 1909): Serra et al., 2014:39 (listed); Paullier, Bessonart, Brum, Loureiro, 2019:68 (listed); Serra et al., 2025:28 (comparative material); Méttola et al., 2025:19 (comparative material).

Characidium cf. *zebra* (non Eigenmann, 1909): Mirande & Koerber, 2020:12 (listed).

Holotype.— CI-FML 8301, 45.3 mm SL, Argentina, Corrientes, lower Uruguay River basin, Miriñay stream lower part, route 123, under the bridge, 29°33'42.0"S 57°30'36.6"W, F. Ruíz Díaz, G.E. Terán, G. Aguilera, J.M. Mirande & B. Bugeau, December 2021.

Paratypes.— Argentina: Corrientes: CI-FML 8302 (CIT-FML 424, 425, 426), 8 (1cs), 28.9–41.42 mm SL, collected with holotype; CI-FML 8303, 2, 36.0–31.9 mm SL, puddles in Miriñay River basin, 29º17'42.0"S 57º33'49.0"W, G. Aguilera, G.E. Terán, J.M. Mirande, B. Bugeau, December 2021; CI-FML 8304, 4, 23.2–33.6 mm SL, unnamed stream, 29°18'38.7"S 57°37'32.3"W, G. Aguilera, G.E. Terán, J.M. Mirande & B. Bugeau, December 2021; CI-FML 8305, 11, 29.6–45.3 mm SL, Aguapey River, road 120, 27°49'22.4"S 56°15'25.7"W, G. Aguilera, J.M. Mirande & G.E. Terán, September 2019; CI-FML 7680 (CIT-FML 342), 5 (1cs), 26.8-37.2 mm SL, Pehuajo stream, on route 12, 27°44'56.05"S 58°46'2.25"W, G. Aguilera, B. Bugeau, F. Ruíz Díaz & G.E. Terán, December 2018; MACN-Ict 7068, 20, 18.1–34.0 mm SL, Esteros de Santa Lucía, ca. 28°07'44"S 58°08'33"W, col. H. Castello, August 1974. CFA-IC 1046, 1, 28.9 mm SL, Mota stream at road 14, 30°28'52.4"S 57°59'12.2"W, Mocoretá, J.R. Casciotta, April 1990; CFA-IC 1802, 1, 29.6 mm SL, Ayuí Grande stream at route 40, Mercedes, 28°57'10.0"S 57°39'19.4"W, F. Brancolini, A. Brancolini & M.C. Nouzaret, October 9, 2008. IBIGEO-I 528, 5, 23.5–32.6 mm SL, Aguapey River, road 120, 27°49'22.4"S 56°15'25.7"W, G. Aguilera, J.M. Mirande & G.E. Terán, September 2019. **Entre Ríos:** MACN-Ict 10495, 1, 32.6 mm SL, La Ensenada stream, Camping Municipal, ca. 32°03'01"S 60°38'20"W, L. Ciotek & P. Giorgis, March 2006; MACN-Ict



Figure 7. *Characidium mariposita* CI-FML 8301. Holotype, male, 45.3 mm SL; Miriñay, Corrientes, Argentina, Uruguay River basin. Scale bar = 10 mm.

Figura 7. *Characidium mariposita* CI-FML 8301. Holotipo, macho, 45,3 mm SL; Miriñay, Corrientes, Argentina, cuenca del río Uruguay. Barra de escala = 10 mm.

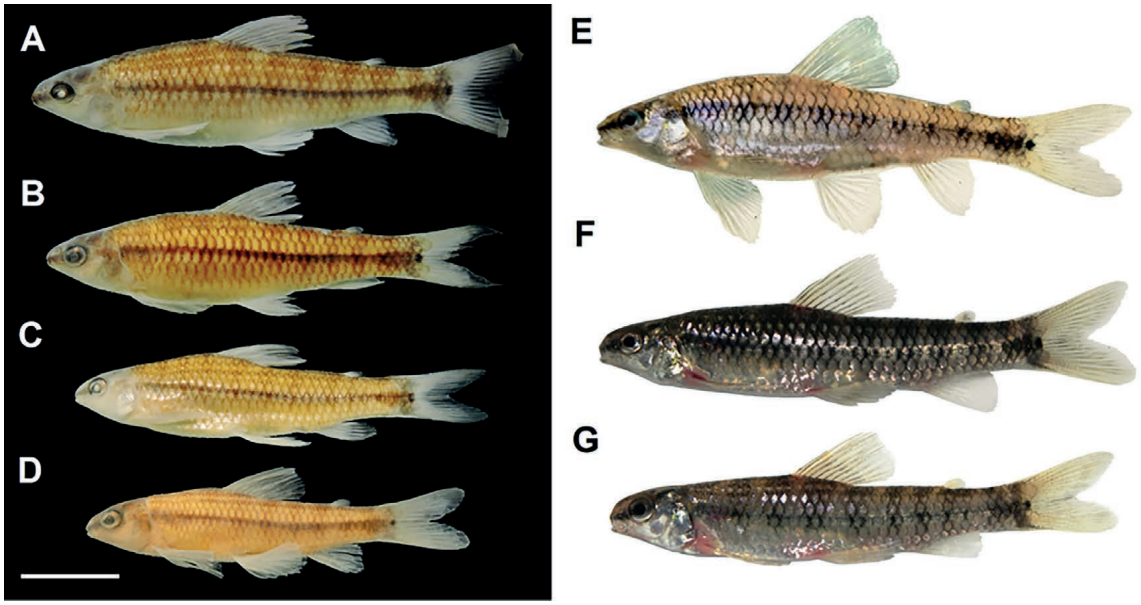


Figure 8. Paratypes of *Characidium mariposita*. **A.** CI-FML 8302. Female, 41.4 mm SL, Miriñay, Corrientes, Argentina, Uruguay River basin. **B.** CI-FML 7680. Female, 37.2 mm SL, Pehuajo stream, Corrientes, Argentina, Paraná River basin. **C.** CI-FML 8302. Male, 33.0 mm SL, Miriñay, Corrientes, Argentina, Uruguay River basin. **D.** CI-FML 7680. Male, 30.3 mm SL, Pehuajo stream, Corrientes, Argentina, Paraná River basin. **E.** CI-FML 7680. Live specimen, female, 37.2 mm SL, Pehuajo stream, Corrientes, Argentina, Paraná River basin. **F.** MHNM 6587. Live specimen, male, 42.2 mm SL, Paso del Tractor, Invernada stream, Artigas, Uruguay, Uruguay River basin. **G.** MHNM 7396. Live specimen, male, 37.4 mm SL, Bella Unión, Puerto Pedregullo, Artigas, Uruguay, Uruguay River basin. Scale bar = 10 mm.

Figura 8. Paratipos de *Characidium mariposita*. **A.** CI-FML 8302. Hembra, 41,4 mm SL, Miriñay, Corrientes, Argentina, cuenca del río Uruguay. **B.** CI-FML 7680. Hembra, 37,2 mm SL, arroyo Pehuajo, Corrientes, Argentina, cuenca del río Paraná. **C.** CI-FML 8302. Macho, 33,0 mm SL, Miriñay, Corrientes, Argentina, cuenca del río Uruguay. **D.** CI-FML 7680. Macho, 30,3 mm SL, arroyo Pehuajo, Corrientes, Argentina, cuenca del río Paraná. **E.** CI-FML 7680. Ejemplar vivo, hembra, 37,2 mm SL, arroyo Pehuajo, Corrientes, Argentina, cuenca del río Paraná. **F.** MHNM 6587. Ejemplar vivo, macho, 42,2 mm SL, Paso del Tractor, arroyo Invernada, Artigas, Uruguay, cuenca del río Uruguay. **G.** MHNM 7396. Ejemplar vivo, macho, 37,4 mm SL, Bella Unión, Puerto Pedregullo, Artigas, Uruguay, cuenca del río Uruguay. Barra de escala = 10 mm.

10887, 1, 35.4 mm SL, Victoria, ca. 32°37'11"S 60°10'26"W, G. López & C. Bentos, May 1991. **Misiones:** LGEP 1681, 1, 32.6 mm SL, Gruta India, 26°45'32.26"S 54°55'24.63"W, G. Aguilera, F. Alonso, M.F. Benitez, J.M. Mirande, & G.E. Terán, November 2016. LGEP 1682, 3, 34.8–39.8 mm SL, Gruta India, 26°45'32.26"S 54°55'24.63"W, G. Aguilera, F. Alonso, M.F. Benitez, J.M. Mirande, & G.E. Terán, November 2016. CI-FML 8306 (CIT-FML 131, 133), 7 (2 cs), 31.8–42.7 mm SL, Gruta India, 26°45'32.26"S 54°55'24.63"W, G. Aguilera, F. Alonso, M.F. Benitez, J.M. Mirande, & G.E. Terán, November 2016. IBIGEO-I 529, 3, 35.5–36.7 mm SL, Gruta India, 26°45'32.26"S 54°55'24.63"W, G. Aguilera, F. Alonso, M.F. Benitez, J.M. Mirande, & G.E. Terán, 13 November 2016. **Santa Fe**: CI-FML 8308, 2

(2cs), 34.1–34.5 mm SL, San Jose del Rincón-Capital, 31°36'S 60°35'W, Fernandez, Scrocchi & Lavilla, December 1991. CI-FML 8309 (CIT-FML 794), 2, 32.1–35.8 mm SL, Rosario, 32°57'27"S 60°38'22"W, G.E. Terán, F. Alonso, P. Calviño, September 2014; MACN-Ict 7065, 53, 16.2–34.0 mm SL, Colastiné River, ca. 31°37'58"S 60°35'41"W, Colastiné, H. Castello, July 1975; MACN-Ict 7066, 17, 19.8–31.5 mm SL, Tapialito stream at route 11, 28°53'30.1"S 59°33'05.0"W, Ehrlich, May 1975. **Uruguay: Artigas:** MHNM 3759, 1, 33.1 mm SL, Paso Ferrayen, Cuareim River, 30°25'57"S 56°25'25"W, W.S. Serra, M. Loureiro, F. Teixeira de Mello, C. Clavijo, S. Villars, D. Olsson, N. Zaldúa & F. Stábile, September 2015; MHNM 3778, 3, 23.0–28.3 mm SL, Paso de la Cruz, 30°16'19"S 57°19'12"W, Cuareim River, W.S. Serra, M. Loureiro, F. Teixeira de Mello, S. Villars, D. Olsson, N. Zaldúa & F. Stábile, September 2015; MHNM 4390, 5, 24.0-40.8 mm SL, Cañada del Medio, 30°20'43"S 56°38'00"W, March 2007; MHNM 5785, 1, 34.2 mm SL, Bella Unión, Puerto Pedregullo, 30°11'23"S 57°36'52"W, CARU survey, April 2017; MHNM 6587, 1, 42.2 mm SL, Paso del Tractor, Invernada stream, 30°49'06"S 56°00'49"W, W.S. Serra, G. Sanguinetti & A. Duarte, January 2024; MHNM 6674, 4, 22.8–25.5 mm SL, Rincón de Pacheco, 30°42'03"S 56°09'14"W, Cuareim River, W.S. Serra, G. Sanguinetti & A. Duarte, January 2024; MHNM 7216, 1, 31.9 mm SL, Lemos stream, 30°19'44"S 56°34'24"W, March 2007; MHNM 7380, 2, 41.0–52.5 mm SL, Catalán Grande stream, Paso Urumbeba, 30°32'35"S 56°20'27"W, M. García & G. Sanguinetti, November 2024; MHNM 7381, 1, 39.2 mm SL, Cuaró stream and route 4, Paso Campamento, 30°46'59"S 56°46'47"W, M. García & G. Sanguinetti, November 2024; MHNM 7395, 1, 28.5 mm SL, Uruguay River, costanera de Bella Unión, 30°16'44"S 57°34'35"W, G. Sanguinetti, March 2025; MHNM 7396, 1, 37.4 mm SL, Bella Unión, Puerto Pedregullo, 30°11'23"S 57°36'52"W, CARU survey, April 2025; MHNM 7713, 1, 28.6 mm SL, Paso de la Cruz, 30°16'19"S 57°19'12"W, Cuareim River, W.S. Serra, M. Loureiro, F. Teixeira de Mello, S. Villars, D. Olsson, N. Zaldúa & F. Stábile, September 2015; ZVC-P 8004, 3, 43.0–47.0 mm SL, Cuareim River, Paso de la Cruz, 30°16'12"S 57°19'09"W, M. Loureiro, F. Teixeira, I. González & F. Quintans, February 2006; ZVC-P 8007, 6, 40.6–56.5 mm SL, Cuaró Grande stream and route 4, 30°46'54"S 56°46'50"W, M. Loureiro, F. Teixeira, I. González & F. Quintans, February 2006; ZVC-P 10175, 5, 41.7–52.1 mm SL, Lemos stream, 30°21'16"S 56°34'38"W, M. Loureiro, F. Teixeira, I. González & F. Quintans, August 2006; ZVC-P 10206, 6, 42.0–50.0 mm SL, Cañada del Medio, 30°20'41"S 56°37'56"W, M. Loureiro, F. Teixeira, I. González & F. Quintans, August 2006; ZVC-P 12581, 2, 40.8–41.6 mm SL, cauce abandonado del Rio Cuareim, Paso de León, 30°08'05"S 57°06'17"W, A. Duarte, W.S. Serra, M. Loureiro & L. Ziegler, December 2012; ZVC-P 12632, 9, 40.2–54.1 mm SL, Cuareim River, Laguna Salamanca, 30°46'36"S 56°02'16"W, A. Duarte, W.S. Serra, M. Loureiro & L. Ziegler, December 2012; ZVC-P 13765, 12, 41.7–54.1 mm SL, Catalán Grande stream, Paso Santiño, 30°46'26"S 56°14'36"W, M.

Zarucki, W.S. Serra & M. Loureiro, March 2016. **Salto:** MHNM 7657, 1, 44.4 mm SL, Arapey River and ex. Road 3, 30°57'14.42"S 57°45'0.54"W, CARU survey, October 2025. **Tacuarembó:** MHNM 7711, 1, 29.6 mm SL, Ansina, Río Tacuarembó, 31°52'34.4"S 55°28'15.0"W, M. García & G. Sanguinetti, September 2024; ZVC-P 14651, 1, 39.6 mm SL, Tacuarembó Chico River, Paso de los Novillos, 31°57'57"S 55°40'30"W, E. Burress, A. Duarte, D. Hernández, M. Loureiro & W.S. Serra, December 2013.

Non-type material.— **Argentina: Corrientes:** CI-FML 5175, 46, 22.9–29 mm SL, Guayquiraró, Paraná River near Esquina, 30°20'30.36"S 59°30'54.74"W, J.M. Mirande, M. Azpelicueta, J. Lundberg & M. Sabaj, April 2005; CI-FML 8310, 15, 31.4–38.2 mm SL, Iberá lagoon, 28°32'25"S 57°10'49"W, M.M. Montes, J.R. Casciotta & A. Almirón, November 2015; CI-FML 8311, 20, 19.35–45.3 mm SL, Aguapey River, road 120, 27°49'22.4"S 56°15'25.7"W, G. Aguilera, J.M. Mirande & G.E. Terán, September 2019; CI-FML 8312, 10, 26.7–45.5 mm SL, Yurupe stream, "camino vecinal", 29º33'28.2"S 57º34'16.0"W, G. Aguilera, J.M. Mirande & G.E. Terán, September 2019; CI-FML 8313, 25, 25.1-37.5 mm SL, Itacui stream, 28°31'54.47"S 56° 3'10.84"W, G. Aguilera, J.M. Mirande & G.E. Terán, September 2019; CI-FML 8314, 29, 24.0–37.8 mm SL, Guaviravi stream, 29°25'24.43"S 56°51'11.78"W, G. Aguilera, J.M. Mirande & G.E. Terán, September 2019. **Entre Ríos:** MACN-Ict 11139, 1, 38.0 mm SL, Feliciano stream, L. Braga, August 1974; MACN-Ict 11197, 3, 24.9–40.6 mm SL, Paraná Guazú River, July 1977. **Misiones:** CI-FML 8307, 14, 31.8–42.7 mm SL, Gruta India, 26°45'32.26"S 54°55'24.63"W, G. Aguilera, F. Alonso, M.F. Benitez, J.M. Mirande, & G.E. Terán, November 2016. **Santa Fe**: CI-FML 1739, 12, 13.6–34.7 mm SL, San Jose del Rincón-Capital, 31º 36' S 60º 35' W, Fernández, Scrocchi & Lavilla, December 1991. **Uruguay: Artigas:** MHNM 5566, 2, 21.9–25.8 mm SL, marginal lagoon, Paso de León, 30°08'12"S 57°06'14", W.S. Serra, M. Zarucki, M. Loureiro & D. Arrieta, March 2014; ZVC-P 2500, 4, 32.7–39.5 mm SL, Los Molles stream, Estancia Yuquery, 32 km NO from Artigas city, 30°12'24"S 56°44'31"W, Excursion of the Faculty of Humanities and Science, February 1970; ZVC-P 2931, 3, 40.0–50.2 mm SL, Invernada stream, 30°47'29"S 56°01'16"W, Exc. Lab. Zool., February 1954; ZVC-P 7985, 1, 56.3 mm SL, Cuaró Chico stream y route 4, 30°42'40"S 56°42'42"W, M. Loureiro, F. Teixeira, I. González & F. Quintans, February 2006; ZVC-P 7993, 4, 38.7–48.2 mm SL, Cuareim River al NW de Paso Ramos, 30°06'21"S 56°49'10"W, M. Loureiro, F. Teixeira, I. González & F. Quintans, February 2006; ZVC-P 7999, 8, 39.8–54.7 mm SL, Cuareim River, Paypaso, 30°16'43"S 57°25'00"W, M. Loureiro, F. Teixeira, I. González & F. Quintans, February 2006; ZVC-P 8001, 4, 43.8–51.1 mm SL, Cuareim River, Paso de León, 30°06'50"S 57°06'56"W, M. Loureiro, F. Teixeira, I. González & F. Quintans, February 2006; ZVC-P 9906, 4, 43.8–51.1 mm SL, Cuareim River, Paso de León, 30°06'50"S 57°06'56"W, M. Loureiro, F. Teixeira, I. González & F. Quintans, February 2006; ZVC-P

9901, 1, 39.6 mm SL, Cañada Mataojo, 30°47'46"S 56°56'52"W, M. Loureiro, F. Teixeira, I. González & F. Quintans, August 2006; ZVC-P 9977, 2, 26.4–38.8 mm SL, Cañada Mataojo, 30°46'04"S 56°59'13"W, M. Loureiro, F. Teixeira, I. González & F. Quintans, August 2006; ZVC-P 10021, 1, 51.2 mm SL, tributary of Cañada Honda, 30°27'47"S 56°51'24"W, M. Loureiro, F. Teixeira, I. González & F. Quintans, August 2006; ZVC-P 10040, 1, 47.6 mm SL, Cañada de Brum, 30°35'16"S 56°25'14"W, M. Loureiro, F. Teixeira, I. González & F. Quintans, August 2006; ZVC-P 10195, 1, 50.1 mm SL, Lemos stream, 30°19'37"S 56°34'31"W, M. Loureiro, F. Teixeira, I. González & F. Quintans, August 2006; ZVC-P 11944, 2, 39.1–45.3 mm SL, Cuareim River, Artigas City, 30°23'44"S 56°27'21"W, M. Loureiro, 2010; ZVC-P 13499, 2, 46.2–51.3 mm SL, Cuareim River, Salamanca lake, 30°46'36"S 56°02'16"W, M. Loureiro, E. Burress, A. Duarte & W.S. Serra, November 2010; ZVC-P 13812, 1, 36.7 mm SL, Paso Ferrayen, Cuareim River, 30°25'57"S 56°25'25"W, M. Zarucki, W.S. Serra & M. Loureiro, March 2016; ZVC-P 13833, 6, 34.0–46.7 mm SL, Yucutujá stream and route 30, 30°26'15"S 57°17'46"W, M. Zarucki, W.S. Serra & M. Loureiro, March 2016; ZVC-P 13870, 4, 37.7–53.4 mm SL, Tres Cruce stream and route 30, 30°26'19"S 56°48'13"W, M. Zarucki, W.S. Serra & M. Loureiro, March 2016; ZVC-P 13894, 2, 35.1–43.1 mm SL, Cuaró stream, Paso Artola, 30°38'30"S 56°54'56"W, M. Zarucki, W.S. Serra & M. Loureiro, March 2016. **Paysandú:** ZVC-P 13985, 2, 38.5–40.3 mm SL, Bañado Figari, 32°10'26"S 57°20'55"W, S. Paullier, M. Loureiro, J. Bessonart, D. Díaz, P. Maissonave & E. Brum, April 2017. **Río Negro:** ZVC-P 4675, 1, 37.1 mm SL, Isleta stream behind San Javier cementery, 32°40'30"S 58°07'59"W, M. Loureiro, D. García, F. Teixeira de Mello & A. D'Anatro, April 2002; ZVC-P 8025, 1, 38.3 mm SL, Esteros de Farrapos, mouth of Román Grande stream, 32°50'12"S 58°05'15"W, M. Loureiro, A. D'Anatro, F. Teixeira de Mello & E. Charbonier, May 2002; ZVC-P 14080, 12, 29.0–36.2 mm SL, Isleta stream behind San Javier cementery, 32°40'30"S 58°07'59"W, M. Loureiro, J. Bessonart & D. Díaz, February 2018; ZVC-P 14204, 1, 29.2 mm SL, on the way to Puerto Viejo, San Javier, 32°38'33"S 58°08'40"W, N. Gobel, G. Laufer & M. Carabbio, 25 February 2018; ZVC-P 14992, 3, 37.8–43.5 mm SL, Puerto Viejo, San Javier, 32°38'33"S 58°08'40"W, M. Loureiro, F. Teixeira de Mello, I. González & A. D`Anatro, November 2007. **Rivera**: ZVC-P 2820, 2, 33.4–34.3 mm SL, puddle near to Arroyo Cuñapirú, 31°45'40"S 55°31'21"W, Excursion of the Faculty of Humanities and Sciences, March 1969; ZVC-P 3543, 1, 32.0 mm SL, puddle near to Cuñapirú stream, 31°45'40"S 55°31'21"W, Excursion of the Faculty of Humanities and Sciences, February 1969; ZVC-P 5097, 1, 33.8 mm SL, Cuñapirú stream near to Paso Piedras, 31°31'44"S 55°35'01"W, February 1969; ZVC-P 15732**,** 1, 42.6 mm SL, marginal lagoon in Paso Mazangano, Negro River and route 44, 32°06'17"S 54°41'36"W, M. Loureiro, F. Cortondo, N. Vidal, C. Clavijo & S. Wlodek, October 2023. **Salto**: MHNM 2859, 1, 40.9 mm SL, Espinillar stream, Establecimiento "El Espinillar", ANCAP, L. H. Amato and G.

Acosta y Lara, June 1987; ZVC-P 15410, 3, 37.0–38.8 mm SL, wetlands of Termas del Arapey, 30°56'18"S 57°32'00"W, M. Loureiro, S. Paullier & V Pinelli, March 2021. **Tacuarembó**: ZVC-P 3027, 1, 40.9 mm SL, Yaguarí stream, Paso del Sauce, 32°07'53"S 55°23'24"W, Carlos Ríos, October 1970; ZVC-P 11911, 2, 35.8–48.1 mm SL, Sauce stream, 31°55'56"S 55°49'52"W, F. Teixeira.

Diagnosis.— *Characidium mariposita* is distinguished from all congeners except *C. bahiense*, *C. bimaculatum*, *C. clistenesi*, *C. fleurdelis*, *C. laterale*, *C. marshi* Breder, *C. nana*, *C. nupelia*, and *C. xavante* by having an enlarged conspicuous peduncular blotch (*vs.* absent or diffuse). From *C. bahiense*, *C. fleurdelis*, *C. laterale*, *C. nana*, *C. nupelia*, and *C. xavante* by presence of complete lateral line (*vs.* incomplete); from *C. clistenesi* and *C. marshi* by presence of circumpeduncular 14 scales (*vs.* 12); and from *C. bimaculatum* by having a parietal branch of supraorbital canal present, reaching the parietal (*vs*. absent).

The new species differs from *C. bahiense*, *C. bimaculatum*, *C. laterale*, *C. mirim*, *C. nupelia*, C. *stigmosum*, and *C. xavante* by having a parietal branch of supraorbital present (*vs*. absent); from *C. barbosai* by the presence of preorbital stripe (*vs*. absent); from *C. borellii*, *C. deludens*, *C. etzeli*, *C. lagosantense*, *C. litorale*, *C. satoi*, and *C. xanthopterum* by having two rows teeth on dentary (*vs*. one row); from *C. borellii* and *C. koerberi* n.sp. by having iii,6,i, iii-7,i or iii-8,i pectoral fin rays (*vs*. iii-9,i or iii-10,i) (Fig. S4), two pseudotympanum openings bordered by fifth and sixth vertebral ribs (*vs*. one opening), and by having 33 or 34 total vertebrae (*vs*. 35 or 36); from *C. deludens* by having regular transverse bars (*vs*. irregular); from *C. etzeli* by having 14 circumpeduncular scales (*vs*. 12); and from *C. zebra* by having shorter anus to anal-fin distance (4.5–7.5% SL) (*vs*. more than 8% SL).

Additionally, *Characidium mariposita* differs on sexually dimorphic characters from males of *C. bimaculatum* and *C. pumanrinri* by having a stripe of melanophores on the middle of the dorsal fin (*vs*. proximal third dark in *C. bimaculatum*, and entirely dark in *C. pumarinri*); from *C. koerberi* n.sp. by a longer pelvic fin, reaching anal-fin origin (*vs*. not reaching in males); from *C. clistenesi*, *C. mirim*, *C. satoi*, *C. stigmosum*, and *C. xanthopterum* by having bony hooks of spine type (*sensu* Teixeira & Melo, 2020) (*vs.* ridge type); and from *C. bahiense*, *C. borellii*, *C. nupelia*, and *C. xavante* by having spiny hooks only on pelvic fins (*vs*. present on pelvic and pectoral fins).

Description.— Morphometric data in Table 1, additional data by basin in Table S5. Small-size species of *Characidium*, reaching 48.7 mm SL. Body elongated. Dorsal profile convex from snout to dorsal fin, slightly convex to adipose-fin origin, slightly concave to caudal-fin base. Ventral profile convex from snout to pelvic-fin origin. Body deepest at dorsal-fin origin; caudal peduncle short and shallow.

Snout pointed, tip slightly above to ventral margin of eye. Mouth terminal. Orbit oval, positioned laterodorsally. Two nasal openings, anterior nostril with full dermal flap, posterior nostril flap absent or with flap on anterior margin only.

Squamation. Scales cycloid, *circuli* absent. Isthmus fully scaled. Longitudinal scale row: 32(3), 33(4), 34*(23), 35(54), 36(21), 37(2), perforated by lateral line. Transverse scale rows above lateral line: 4* (64). Scales on additional scale row below predorsal row, counted anterior to first scale at dorsal-fin origin: 0(28), 1(3), 2(7), 3(9), 4(1), 5(4), 6(3), 8(2), 9 (2), 10(2), 13(1), 14(1). Transverse scale rows below lateral line: 5*(64). Pre-dorsal scale row: 8(5), 9(25), 10*(32), 11(2). Preadipose scale rows: 7*(4), 8(19), 9(36), 10(5). Scale row between adipose-fin base and caudal-fin origin: 4(1), 5*(8), 6(37), 7(16). Scale row between pectoral- and pelvic-fin origins: 8(2), 9*(19), 10(35), 11(7). Pre-anal scale rows: 4(15), 5*(45), 6(4). Scale rows between anus and anal-fin origin: 2*(32), 3(32). Scale rows between anal-fin origin and caudal-fin origin: 5(4), 6*(27), 7(22), 8(8), 9(1). Circumpeduncular scale rows: 13(6), 14*(48).

Fins. Pectoral fin short, not reaching pelvic-fin origin; rays: iii,6,i(5), iii,7,i*(58), iii,8,i(43). Pelvic fin reaching anal-fin origin in adult males; rays: i,6,i(3), i,7,i*(59), i,8,i(2). Dorsal fin with narrow base; rays: iii,8(1), ii,9*(60), iii,9(1), ii,10(2). Anal fin with narrow base; rays: ii,6(37); iii,6(1); ii,7*(36). Adipose fin well developed. Caudal fin with lobes equal; rays: i,8,8,i(1), i,9,8,i*(61).

Pseudotympanum present as muscular hiatus, rounded, aligned with anterior chamber of swimbladder, posterior to humeral spot. Two openings delimited by fifth and sixth vertebral ribs (Fig. 4B). Swimbladder well developed, with two chambers, posterior chamber larger than anterior.

Jaws and branchial apparatus. Premaxilla small, bearing 6(16), 7*(26), 8(17), 9(1) tricuspid teeth, decreasing in size posteriorly (Fig. 9A). Dentary with two rows of teeth; outer teeth with 5(2), 6(12), 7(24), 8(15), 9*(9) tricuspid teeth, decreasing posteriorly, inner row of teeth minute and unicuspid (Fig. 9B). Replacement teeth present on dentary and premaxilla. Maxilla edentulous. Ectopterygoid elongated, with one row of 7(1cs), 8(1cs), 9(3cs), 10(1cs) minute conical teeth. Mesopterygoid and endopterygoid edentulous. Branchiostegal rays: 4(2 cs), 5(4 cs). One row of gill rakers on first gill arch: hypobranchial 2(4 cs), 3(2 cs), ceratabronchial 5(6 cs), epibranchial 5(2 cs), 6(3 cs), 7(1 cs).

Cephalic laterosensory system and girdles. Parietal branch of supraorbital canal present, extending to parietal. Cranial fontanel anteriorly limited by frontals. Infraorbital 1–3 present; infraorbitals 4–6 reduced to tubular canal. Pelvic bone distally rounded, ischiatic process irregular in shape. Small anterior unbranched ray on dorsal and anal fins.

Vertebral column and caudal skeleton. Total vertebrae: 33(3 cs), 34(3 cs). First four anterior vertebrae incorporated into Weberian apparatus, followed by 29–30 free vertebrae. Hypurals: 6(6 cs). Epural bones: 2(3 cs),

3(3 cs). Uroneural one pair (6 cs) pair. Upper procurrent caudal-fin rays: 7(2 cs), 8(3 cs), 9(1 cs). Lower procurrent caudal-fin rays: 6(3 cs), 7(3 cs).

Coloration of preserved specimens.— Body brownish, darker dorsally, fading to whitish on ventral region. Dark midlateral stripe from posterior margin of opercle to caudal-fin base, with well-defined margins, narrower than one scale row, increasing in width posteriorly (Fig. 8A-D). Dark brown regular transverse bars across trunk: 7(1), 8(12), 9*(28), 10(13), 11(1), 12(1), 13(2), 14(1), wide and crossing midlateral stripe. In some adult males, transverse bars faint and diffuse, not crossing midlateral stripe. In juveniles, transverse bars usually thinner and more numerous (>11). Black preorbital stripe conspicuous. Black postorbital stripe short, incomplete, faint in some specimens. Transverse bars well separated, not confluent. Humeral

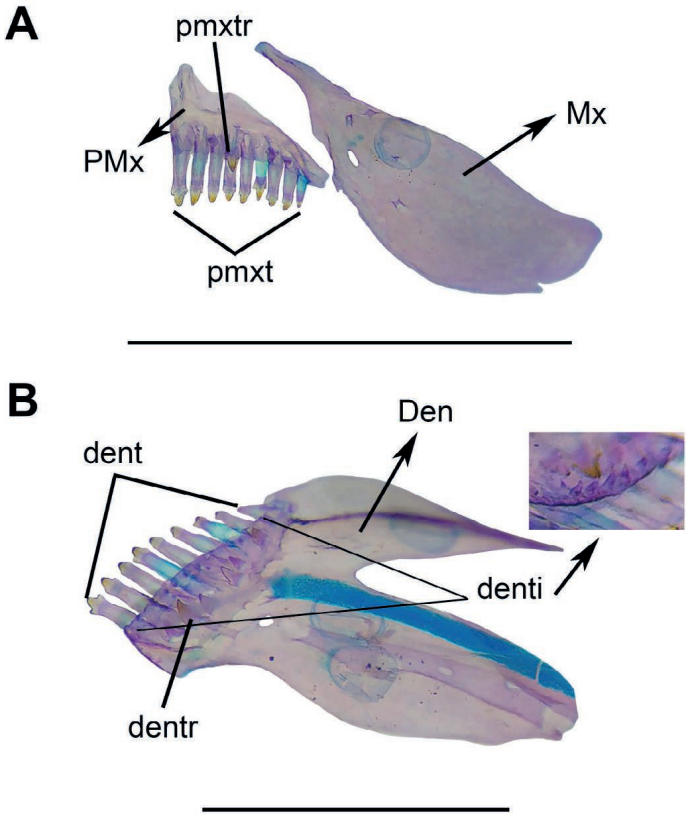


Figure 9. A. Right upper jaw in medial view of *Characidium mariposita* (CI-FML 8302). **B.** Right dentary in medial view of *Characidium mariposita* (CI-FML 8302). Abbreviations: Den, dentary; dent, dentary teeth; dentr, replacement dentary teeth; denti, inner row of dentary teeth; Mx, maxillary; PMx, premaxillary; pmxt, premaxillary teeth; pmxtr, replacement premaxillary teeth. Scale bar = 1 mm.

Figura 9. A. Vista medial del maxilar superior derecho de *Characidium mariposita* (CI-FML 8302). **B.** Vista medial del dentario derecho de *Characidium mariposita* (CI-FML 8302). Abreviaturas: Den, dentario; dent, dientes del dentario; dentr, dientes de reemplazo del dentario; denti, hilera interna de dientes del dentario; Mx, maxilar; PMx, premaxilar; pmxt, dientes premaxilares; pmxtr, dientes premaxilares de reemplazo. Escala = 1 mm.

blotch vertically elongated. Peduncular blotch black, very conspicuous, horizontally elongated, fused with posterior portion of midlateral stripe. Basicaudal spot small and rounded. Fins mostly hyaline; dorsal fin with few dark-brown chromatophores in larger specimens, usually forming single dark stripe along middle portion of rays. Black spot anterior to dorsal-fin origin. Melanophores present on anterior unbranched rays of pectoral and pelvic fins.

Coloration in life.— Dorsal region silvery to light brown, ventral region yellowish to whitish. Black midlateral stripe extending from snout to caudal-fin base. Black transverse bars (7–14) present along trunk, crossing midlateral stripe. Fins mostly hyaline, dorsal fin with scattered dark-brown chromatophores in some specimens. Small dark rounded spots near opercular region and caudal-fin base. Small whitish spot anterior to humeral spot. Peduncular blotch elongated and conspicuous (Fig. 8E-F).

Sexual dimorphism.— Bony hooks (spine type) present on pelvic fins of males (14 out of 40), occurring in specimens from both, Uruguay and Paraná basins, located on distal half of first four unbranched pelvic-fin rays. Smallest male bearing pelvic-fin hooks 30.0 mm SL. Bony hooks absent on paired fins of females. In some males, pelvic fins longer, reaching or slightly surpassing anal-fin origin (Fig. 8D) and transverse bars fainter than in females.

Geographic distribution.— The geographic distribution encompasses the lower and middle Paraná River basin and lower Uruguay River basin, in Argentina and Uruguay (Fig. 6). In Argentina the species has been recorded in the provinces of Santa Fé, Buenos Aires, Corrientes, Entre Ríos and Misiones. In Uruguay, it has been recorded from the departments of Artigas, Paysandú, Río Negro, Rivera, Salto, and Tacuarembó.

Ecological notes.— *Characidium mariposita* has been recorded in sympatry with *C. koerberi, C. pterostictum,* and *C. rachovii* in the Paraná basin, and with *C. tenue, C. serrano, C. pterostictum* and *C. rachovii* in the Uruguay basin. This species is typically found among macrophytes in shallow marginal habitats of streams, which usually have sandy substrates with varying organic detritus content. Specimens typically rest on the substrate supported by the pectoral fin. Its diet consists primarily of aquatic insect larvae and nymphs, including Chironomidae, Trichoptera, Ephemeroptera, and Hemiptera, followed by small gastropods and a low proportion of algal material (Almirón et al., 2015).

Conservation status.— *Characidium mariposita* is widespread and frequently recorded in tributaries of lower Paraná and Uruguay basins, with an Extent of Occurrence (EOO) of about 322,600 km^2, and has been recorded within or adjacent to at least five protected areas: National Parks “Pre-Delta” (Entre Ríos, Argentina) and “Iberá” (Corrientes, Argentina), and the

protected areas of "Esteros de Farrapos" (Río Negro, Uruguay), "Montes del Queguay" (Paysandú, Uruguay) and "Rincón de Franquía" (Artigas, Uruguay). No evidence of severe fragmentation, continuing decline, or extreme fluctuations has been documented. Based on current knowledge, the species is assessed as Least Concern (LC) following the IUCN Red List Categories and Criteria.

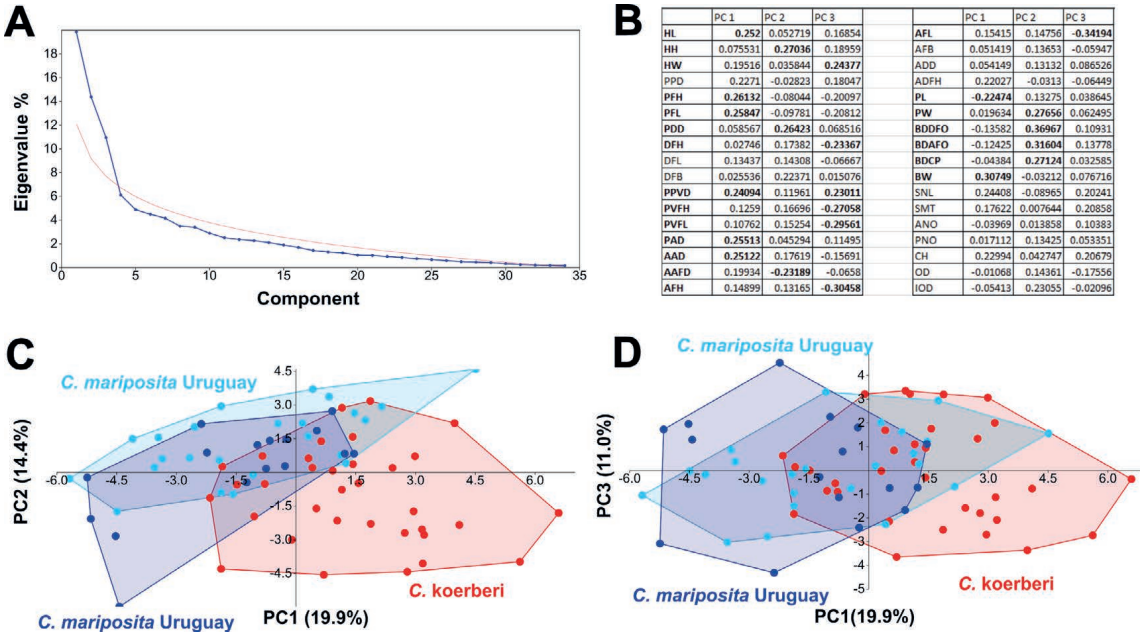
Etymology.— The specific name *mariposita* is the vernacular name used by people in Argentina and Uruguay for species of *Characidium* and is treated as a noun in apposition.

Statistical analyses

According to the broken stick method, the selection resulted in the first three components (Fig. 10A): PC1 (19.9%), PC2 (14.4%), and PC3 (11.0%), which together accounted for 45.3% of the variability. The measurements with the highest loadings for PC1 were: body width (0.31), pectoral-fin height (0.26), pectoral-fin length (0.26), preanal distance (0.26), anal-apex distance (0.25), head length (0.25), prepelvic distance (0.24), and peduncle length (-0.22). Measurements that weighed the most for PC2 were: body depth at dorsal-fin origin (0.37), body depth at anal-fin origin (0.32), peduncle width (0.28), body depth at caudal peduncle (0.27), head height (0.27), predorsal distance (0.26), and anus to anal-fin distance (-0.23). Measurements that weighed the most for PC3 were: anal-fin length (-0.34), anal fin height (-0.30), pelvic-fin length (-0.29), pelvic-fin height (-0.27), head width (0.24), dorsal-fin height (-0.23), and prepelvic distance (0.23). The measurements loadings are shown in Fig. 10B. The PCA analysis shows significant morphological differences between *Characidium koerberi* and *C. mariposita*. Both species groups partially overlapped in the plot of PC1 *vs*. PC2 (Fig. 10C), and mostly overlapped in PC1 *vs*. PC3 (Fig. 10D). Between *C. mariposita* from distinct basins (Paraná *vs.* Uruguay), both groups overlapped by three PCs, to the multivariate PERMANOVA test did not show differences between populations ($p = 0.1257$). None of the measurements show high values of Pearson correlation coefficient ($r < 0.80$).

Characidium koerberi and *C. mariposita* exhibit consistent morphometric differences. Specimens of *C. koerberi* are generally larger (SL), with longer pectoral fins, wider body, shorter peduncle length, and a longer anus to anal fin distance. In contrast, *C. mariposita* shows a deeper body, and proportionally shorter pectoral fins, indicating a more compact body shape. All measurement ranges partially overlap, except for the anus to anal-fin distance (5.1–7.5% SL in *C. mariposita vs*. 7.7–10.4% SL in *C. koerberi*) (Table 1).

In addition, reproductive males of *C. koerberi* (48.2–62.6 mm SL) further differ from those of *C. borellii* (43.5–69.2 mm SL) by: a shorter pelvic fin (19.3–21.5% SL *vs.* 21.7–27.7% SL), and longer anus to anal-fin distance (8.4–10.4% SL *vs.* 4.5–7.8% SL).



	PC 1	PC 2	PC 3
HL	**0.252**	0.052719	0.16854
HH	0.075531	**0.27036**	0.18959
HW	0.19516	0.035844	**0.24377**
PPD	0.2271	-0.02823	0.18047
PFH	**0.26132**	-0.08044	-0.20097
PFL	**0.25847**	-0.09781	-0.20812
PDD	0.058567	**0.26423**	0.068516
DFH	0.02746	0.17382	**-0.23367**
DFL	0.13437	0.14308	-0.06667
DFB	0.025536	0.22371	0.015076
PPVD	**0.24094**	0.11961	**0.23011**
PVFH	0.1259	0.16696	**-0.27058**
PVFL	0.10762	0.15254	**-0.29561**
PAD	**0.25513**	0.045294	0.11495
AAD	**0.25122**	0.17619	-0.15691
AAFD	0.19934	**-0.23189**	-0.0658
AFH	0.14899	0.13165	**-0.30458**

	PC 1	PC 2	PC 3
AFL	0.15415	0.14756	**-0.34194**
AFB	0.051419	0.13653	-0.05947
ADD	0.054149	0.13132	0.086526
ADFH	0.22027	-0.0313	-0.06449
PL	**-0.22474**	0.13275	0.038645
PW	0.019634	**0.27656**	0.062495
BDDFO	-0.13582	**0.36967**	0.10931
BDAFO	-0.12425	**0.31604**	0.13778
BDCP	-0.04384	**0.27124**	0.032585
BW	**0.30749**	-0.03212	0.076716
SNL	0.24408	-0.08965	0.20241
SMT	0.17622	0.007644	0.20858
ANO	-0.03969	0.013858	0.10383
PNO	0.017112	0.13425	0.053351
CH	0.22994	0.042747	0.20679
OD	-0.01068	0.14361	-0.17556
IOD	-0.05413	0.23055	-0.02096

Figure 10. Principal Component Analysis (PCA) of morphometric variables for *Characidium koerberi* (red) and *C. mariposita* (blue). **A.** Assessment of informative components: the broken stick model (red line) indicates two significant components. **B.** Variable loadings for relevant components; highest loadings are highlighted in bold. **C.** Scatterplot of PC1 *vs*. PC2 showing distinct separation between species without overlap. **D.** Scatterplot of PC1 *vs*. PC3 showing partial overlap between species.
Note that populations of *C. mariposita* from the Paraná (blue) and Uruguay (sky blue) River basins overlap completely. Axis values are expressed in eigenvalue scale.

Figura 10. Análisis de Componentes Principales (ACP) de variables morfométricas para *Characidium koerberi* (rojo) y *C. mariposita* (azul). A. Evaluación de componentes informativos: el modelo de varilla quebrada (línea roja) indica dos componentes significativos. B. Cargas de las variables para los componentes relevantes; las cargas más altas se resaltan en negrita. C. Diagrama de dispersión de PC1 vs. PC2 que muestra una clara separación entre especies sin solapamiento. D. Diagrama de dispersión de PC1 vs. PC3 que muestra un solapamiento parcial entre especies.
Nótese que las poblaciones de *C. mariposita* de las cuencas de los ríos Paraná (azul) y Uruguay (azul claro) se solapan completamente. Los valores de los ejes se expresan en escala de autovalores.

Species delimitation and phylogenetic analysis

The COI and CytB sequence alignments comprised 654 bp and 1,221 bp, respectively. Pairwise genetic divergences were calculated for COI using the Kimura 2-parameter (K2) model (Table 2). Mean intraspecific COI divergence was 0.8% for *Characidium mariposita* and 0.2% for *C. koerberi.* The closest taxon to *C. mariposita* was *C. koerberi* (1.5%), followed by *C. deludens* (1.6%), *C. bimaculatum* (2.0%), *C.* cf. *zebra* São Francisco (2.4%) and *Characidium* "sp.1 Iguazu" (2.4%). Taxa exhibiting external similarity in overall appearance (*e.g. C. borellii* and *C.* cf. *zebra* from Maroni basin in Guiana Shield) showed substantially higher COI divergence (4.4% and 14.6%, respectively).

Parsimony.— The COI dataset yielded nine most parsimonious trees. The strict consensus tree under K = 16 (length = 2,651; fit = 67.32884) recovered both *Characidium koerberi* and *C. mariposita* as monophyletic groups with high GC support (72 and 95, respectively) (Fig. 11, S6.A).

The concatenated mitochondrial dataset (COI + CytB) produced a single most-parsimonious tree under K = 12 (length = 3,942; fit = 292.37823). Both *Characidium koerberi* and *C. mariposita* were recovered within the "*Nanognathus*" clade (GC = 91), and as sister lineages with relatively good support (GC = 53) (Fig. S6.B). The clade including *C. bimaculatum*, *C. deludens*, *C.* cf. *zebra* São Francisco, and the new species was strongly supported (GC = 91); however, relationships among these taxa were unresolved in the strict consensus.

Maximum likelihood analysis.— For the COI dataset, the ML tree had a log-likelihood of -10,874.1226 (Fig. S6.C). For the concatenated mitochondrial dataset (COI + CytB), the ML tree had a log-likelihood = -16,690.6553 (Fig. S6.D).

In both datasets, *Characidium koerberi* and *C. mariposita* were recovered as reciprocally monophyletic lineages with strong support. The overall topology of the ML trees was congruent with that obtained under parsimony at deeper nodes (Fig. 11). The "*Nanognathus*" clade was recovered with strong support (SH-aLRT ≥ 80%; UFBoot ≥ 95%). Within this clade, the placement of *C. borellii* and *C. varii* varied among analyses and was supported by lower support values (Fig. S6).

The nine clades of *Characidium* proposed by Serra et al. (2025) were recovered in both analyses (ML and parsimony) with varying levels of support. Most clades defined by Oliveira Silva et al. (2024; 2025) were recovered, except clades C, I and I1 which were not consistently resolved as monophyletic (Fig. S7).

DISCUSSION

Reassessment of the *Characidium zebra* complex in the La Plata basin

Populations of *Characidium* from the Paraná and Uruguay River basins have historically been identified as *C. zebra*, *C.* cf. *zebra*, or *C. fasciatum* (Liotta, 2006; Serra et al., 2014; Almirón et al., 2015). This taxonomic uncertainty reflects the long-standing recognition of *Characidium zebra* as a morphologically variable and geographically widespread species complex extending from Colombia to Uruguay and Argentina (Buckup, 1992, 1993b, 1997; Leitão & Buckup, 2014). The broad circumscription of *C. zebra*— including a fully-scaled isthmus, lightly-colored body, hyaline fins, complete lateral line, and complete set of pectoral girdle bones— likely masked the presence of independently evolving lineages within the La Plata basin.

Table 2. Pairwise genetic distances under K2 model for *Characidium* species of the "*Nanognathus*" clade and *C.* cf. *zebra* taxa. New species, *C. koerberi* and *C. mariposita*, are in bold.

Tabla 2. Distancias genéticas por pares bajo el modelo K2 para especies de *Characidium* del clado "*Nanognathus*" y taxones de *C.* cf. *zebra*. Las nuevas especies, *C. koerberi* y *C. mariposita*, están en negrita.

Species	1	2	3	4	5	6	7	8	9	10	11	12	Intraspecific distance
1. *C. mariposita*													0.8
2. *C. koerberi*	1.5												0.2
3. *C. deludens*	1.6	1.1											0
4. *C. bimaculatum*	2.0	1.1	1.0										0.2
5. *C.* cf. *zebra* Sao Francisco	2.4	1.5	1.5	1.6									0.1
6. *C.* sp1 Iguazu	2.4	1.5	2.0	2.0	2.5								n/c
7. *C. varii*	4.6	3.5	3.9	3.9	4.5	4.3							0.2
8. *C. borellii*	5.4	4.4	4.6	4.9	5.4	4.7	6.0						0.6
9. *C. lagosantense*	5.8	5.5	5.4	5.7	5.9	5.6	6.8	7.2					0
10. *C. bahiense*	6.0	5.7	5.7	6.0	5.5	5.5	6.3	6.0	3.2				0.1
11. *C. samurai*	6.4	5.9	5.7	6.1	6.0	5.6	7.1	7.5	5.8	5.8			0
12. *C.* cf. *zebra* Tocantins	12.7	11.9	11.1	12.0	11.9	11.2	11.6	11.2	12.6	12.5	12.9		0
13. *C.* cf. *zebra* Maroni (Guiana Shield)	15.4	14.6	14.2	14.8	14.5	15.3	14.8	15.8	16.3	15.2	14.9	15.5	0.1

Our results show that material previously assigned to *Characidium* cf. *zebra*, *C.* aff. *zebra* or *C. fasciatum* from lower and middle Paraná and lower Uruguay basins comprises at least two distinct evolutionary lineages, herein described as *C. koerberi* and *C. mariposita*. These results further clarify that *C. zebra sensu stricto* does not occur in the lower Paraná-Uruguay system.

Integrative evidence supporting species boundaries

Species delimitation in this study is supported by congruent morphological, morphometric, and mitochondrial evidence. *Characidium koerberi*, *C. mariposita*, and *C. borellii* share a suite of characters consistent with close morphological similarity, including a scaled isthmus, complete lateral line, 14 scales around the caudal peduncle, fewer than five scales between anus and anal-fin origin, bony hooks (spine type *sensu* Teixeira & Melo, 2020) on pelvic fins of males, and the presence of parietal branch of supraorbital. They also share a coloration pattern of 9–11 regular, wide, dark transverse bars crossing midlateral stripe and a peduncular blotch. This blotch is often masked in adults of *C. borellii* and *C. koerberi* by longitudinal stripe or posteriormost transversal bar, though it remains more evident in smaller specimens. Furthermore, they lack both distinctive fin pigmentation and secondary transverse bars on the flanks.

Despite these shared features, *Characidium koerberi* and *C. mariposita* are diagnosable based on discrete morphological and morphometric traits. Statistical analyses revealed differences in the pectoral-fin length, body width, anus to anal-fin distance, peduncle length, and body depth. The

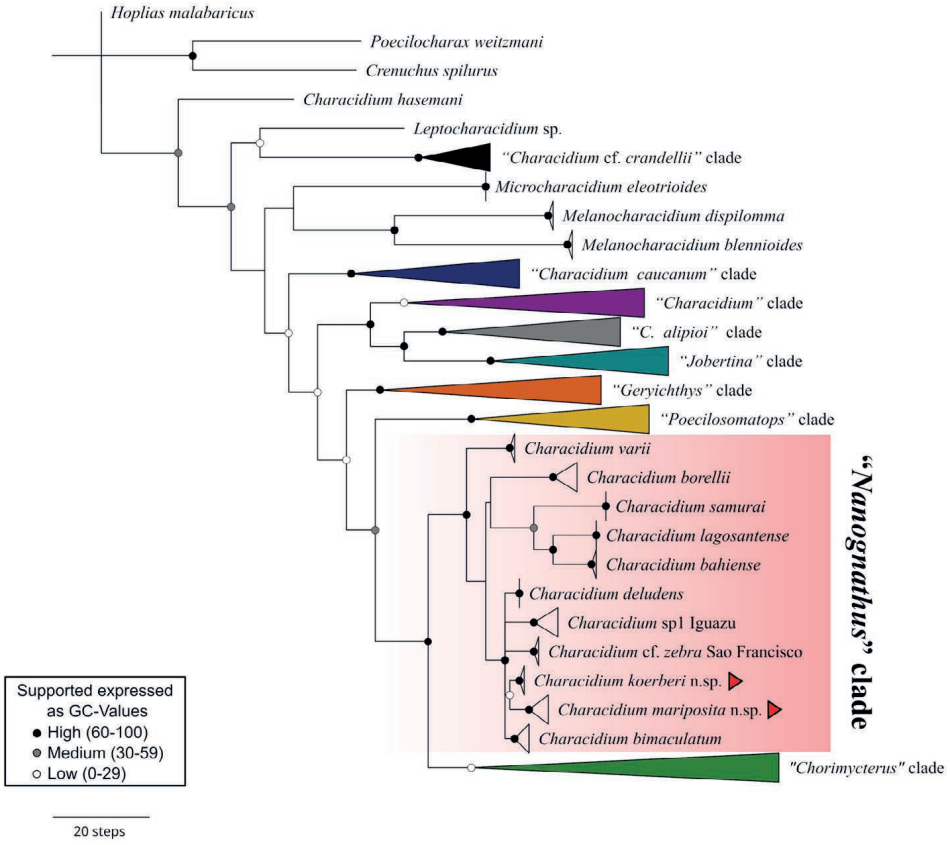


Figure 11. Consensus tree of COI marker under parsimony, extended implied weighting (K reference = 16, length = 2,651, fit = 67.39483). GC supporting values generated from 10,000 replicates. Red triangle: *Characidium koerberi* n.sp. and *Characidium mariposita* n.sp. Scale bar: number of steps.

Figura 11. Árbol de consenso de marcador COI bajo parsimonia, pesos implicados extendidos (K de referencia = 16, longitud = 2.254, ajuste = 67,39483). Valores de soporte de GC generados a partir de 10.000 réplicas. Triángulo rojo: *Characidium koerberi* n.sp. y *Characidium mariposita* n.sp. Barra de escala: número de pasos.

anus to anal-fin distance is particularly informative, with non-overlapping ranges between species (5.1–7.5% SL in *C. mariposita vs*. 7.7–10.4% SL in *C. koerberi*). This character, often used in combination with the number of scales between the anus and the anal-fin origin, has proven diagnostic in several congeners (Melo, 2001; Melo & Oyakawa, 2015; Malanski et al., 2019; Oliveira-Silva, Santos, Lopes, Zanata, 2022). Another useful character is the size of the swimbladder (Agudelo-Zamora, Ortega-Lara & Taphorn, 2020a; Agudelo-Zamora et al., 2020b). In *C. koerberi* the posterior chamber is smaller than the anterior chamber, whereas in *C. mariposita*, it is larger.

Although *C. mariposita* exhibits a larger orbital diameter, this likely reflects negative allometry associated with its relatively small body size rather than a discrete structural modification. Longer pelvic fins were observed only in males of *C. mariposita* and represent a sexual dimorphic character. Fin elongation measured at the distal point of the longest

branched fin rays proved informative for distinguishing species and sexes, (e.g. pelvic-fin length as a sexual dimorphic trait present in *C. borellii* and *C. rachovii* (Teixeira & Melo, 2020; Méttola et al., 2025) and pectoral fin length as morphometric trait present in *C. fleurdelis* (Zanata, Oliveira-Silva & Ohara, 2023) in contrast, pectoral- and pelvic-fin height measured at the distal point of the longest unbranched fin ray were not diagnostic.

Phylogenetic placement and relationships within the "*Nanognathus*" clade

The "*Nanognathus*" clade corresponds to one of the nine lineages recognized by Serra et al. (2025) and other authors (Agudelo-Zamora et al., 2020; Zanata, Oliveira, Oliveira-Silva, 2024; Oliveira-Silva et al., 2024, 2025; Méttola et al., 2025) using mitochondrial evidence. Species delimitation analyses consistently recover *C. koerberi* and *C. mariposita* with *C. bahiense*, *C. borellii*, *C. bimaculatum*, *C. deludens*, *C. lagosantense*, *C. samurai*, and *C. varii*. The genetic divergence between species of this clade is lower than 7.5%, being *C. borellii*, *C. koerberi*, and *C. mariposita* the most similar to each other, congruently with their morphological features. Despite the close phylogenetic proximity, *C. mariposita* and *C. koerberi* differ from *C. borellii* in key characters. Both new species possess two rows of dentary teeth (*vs*. a single row in *C. borellii*) and differ in pectoral-fin ray counts, with *C. mariposita* exhibiting iii,8,i or fewer, whereas *C. borellii* and *C. koerberi* have iii,9,i or iii,10,i (S3). Mitochondrial divergence under COI further distinguishes *C. borellii* from *C. koerberi* (4.4%) and *C. mariposita* (5.4%). The new species differ from other members of "*Nanognathus*" clade by having two rows of teeth on the dentary (vs. one in *C. bahiense*, *C. deludens*, *C. lagosantense*, and *C. varii*), a thin or almost absent longitudinal stripe (vs. broad, wider than one scale in *C. samurai*), and a parietal branch of the supraorbital canal (vs. absent in *C. bimaculatum*).

Low mitochondrial divergence and morphological differentiation

Interestingly, low mitochondrial divergence between *Characidium koerberi* n.sp. and *C. mariposita* n.sp. is relatively low (≤ 2%), particularly when compared to divergences involving *C. bimaculatum* and *C. deludens.* However, mitochondrial divergence does not necessarily imply conspecificity. *Characidium* is one of the genera where a strict 2% cutoff can obscure real diversity, as low mitochondrial divergence may reflect recent radiation events or mitochondrial introgression; therefore such cases must be resolved through an integrative taxonomic approach (Pereira et al., 2013; Oliveira-Silva et al., 2024). Similar cases of low COI divergence (< 1%) occur between *C. bimaculatum* and *C. deludens*, two species that remain

taxonomically valid based on clear morphological differentiation (Oliveira-Silva et al., 2024).

Morphological evidence clearly rejects close affinity of new species with *C. bimaculatum* and *C. deludens*. *Characidium bimaculatum* forms part of a species complex within the C4 clade with *C. bahiense*, *C. xavante*, and *C. nupelia* (Melo & Oyakawa, 2015; Melo & Espindola, 2016) characterized by a short, deep body, 12–18 regular transverse bars, and large peduncular blotch. Although *C. koerberi* and *C. mariposita* share the presence of a peduncular blotch with *C. bimaculatum* (less evident in adult *C. koerberi*), they lack the defining synapomorphies of the C4 clade (Netto-Ferreira, Birindelli, Buckup, 2013; Mendonça & Netto-Ferreira, 2015). Specifically, the new species exhibit a parietal branch of the supraorbital canal (*vs.* absent), two dentary teeth rows (*vs*. one), and 7–11 transverse bars in adults (*vs.* 12 or more). *Characidium deludens* shares the absence of inner row of teeth on dentary with species from the C4 clade, it does not appear to fit into any defined morphological group (Zanata & Camelier, 2015). The new species are clearly distinguished from *C. deludens* by having regular transverse bars on dorsum (vs. irregular blotches).

The combination of morphological diagnosability and reciprocal mitochondrial monophyly supports the recognition of *C. koerberi* and *C. mariposita* as distinct evolutionary lineages despite relatively shallow genetic divergence. The fact that both species coexist in part of their distribution suggests also that they are consistent with reproductive isolation.

Biogeographic implications within the La Plata basin

Characidium mariposita appears to be the most common and widespread species of *Characidium* in the lower portions of the Paraná and Uruguay River basins, with no significant meristic or morphometric differences found between populations from these drainages. In contrast, *C. koerberi* seems to be restricted to the Paraná River basin, particularly its middle portion. This distributional pattern may reflect historical connectivity and subsequent lineage divergence within the La Plata system.

Specimens previously reported as *C. zebra* from Pre-Delta National Park (Almirón et al., 2015), *C.* aff. *zebra* from the Negro River (Serra et al., 2014) and Queguay River (Paullier et al., 2019) correspond to *C. mariposita*. The recognition of these taxa underscores the need for continued integrative reassessment of populations historically assigned to *C. fasciatum*, *C.* cf. *zebra* or *C.* aff. *zebra* across the La Plata basin, additional undescribed lineages may be present.

Comparative material examined.— Comparative material is listed in Méttola et al. (2025) and Terán et al. (2026) with additional comparative material listed below. *Characidium bimaculatum*: Brazil: MHNM 3490, 1,

25.4 mm SL, Estação Ecológica de Añaeba, Ceará, G. Cannella, October 1980. *Characidium etzeli*: MTD F 22650 (photographs of holotype, 55.9 mm SL): Paraguay, 4 kilometers from Bella Vista, about 50 kilometers northeast of Encarnación towards the Rio Paraná. MTD F 22651-22652 (photographs of paratypes, 2, 52.7-54.8 mm SL). Collected with the holotype. *Characidium heinianum*: ZSM 29465-29469 (photographs of paratypes, 5, 16.4-17.5 mm SL): Bolivia, Departamento de Beni, Río Ipurupuru, tributary to Río Mamoré, near crossing with road to San Ramón, about 73 km north of Trinidad, 14°12'538"S 64°56'268"W, Zarske, Hein, Tonoki & Zapata, June 2000. *Characidium laterale*: Brazil: MHNM 7192, 1, 20.2 mm SL, Ruta Transpantaneira, km110, in a bay near the IBDF Research Base, Pantanal, Poconé, L.H. Amato & G. Skuk, February to March 1986. *Characidium schindleri*: ZSM 29470 (photographs of holotype, 66.8 mm SL): Bolivia, left tributary to Palmar, km 144, Schindler & Forster, October 1953. ZSM 29471 (photographs of paratype, 61.8 mm SL): Bolivia, Yungas de Palmar, km 144, 620 m, Schindler & Forster, October 1953. ZSM 29472 (photographs of paratype, 52.5 mm SL): Bolivia, Yungas de Palmar, km 144, Schindler & Forster, October 1953. *Characidium zebra*: Guyana, Maripicru Creek, a branch of the Ireng River; FMNH 53547, (photographs of holotype, 52.5 mm SL). FMNH 52766 (photographs and radiograph of paratypes, 4, 30–34 mm SL). Brazil, Roraima; MZUSP 117743, 5, 30.8-39.3 mm SL: Bonfim, Rio Arraias, afluente do rio Tacutu, bacia do rio Branco, Datovo, Carvalho, February 2014. MZUSP 117946, 1, 33.57 mm SL: Boa Vista, Rio Cauame, proximo a desembocadura do Igarape Au-Au, bacia do rio Branco, Datovo, Carvalho, February 2014.

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PARTICIPATION

VEM, GET, WSS, GA, MMM, AF, and JMM contributed equally to the idealization, analysis, and writing.
VEM, GET, GA, AF, MFB, and FRD contributed to the idealization, laboratory and the fieldwork logistics.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

LITERATURE CITED

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SUPPLEMENTARY MATERIAL

S1: https://www.lillo.org.ar/journals/index.php/acta-zoologica-lilloana/article/view/2374/2177
S2: https://www.lillo.org.ar/journals/index.php/acta-zoologica-lilloana/article/view/2374/2178
S3: https://www.lillo.org.ar/journals/index.php/acta-zoologica-lilloana/article/view/2374/2179
S4: https://www.lillo.org.ar/journals/index.php/acta-zoologica-lilloana/article/view/2374/2180
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