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# *Characidium serrano* Buckup & Reis, 1997 (Characiformes, Crenuchidae): new insights into its phylogenetic relationships and distribution

## *Characidium serrano* Buckup & Reis, 1997 (Characiformes, Crenuchidae): nuevos conocimientos sobre sus relaciones filogenéticas y distribución

Wilson S. Serra[1,2*], Fabrizio Scarabino[2,1], Ezequiel Méttola[3], Martín Miguel Montes[4], Guillermo Terán[3], Melisa Moncada[4], Germán Sanguinetti[5,8], Alejandro Duarte[6], Matías García[7,8]

[1] Sección Ictiología, Dpto. de Zoología, Museo Nacional de Historia Natural (MNHN), Miguelete 1825, Montevideo, Uruguay.
[2] Centro Universitario Regional del Este (CURE) – Sede Rocha, Ruta 15 y Ruta 9, Rocha, Uruguay.
[3] Fundación Miguel Lillo – Unidad Ejecutora Lillo FML-UEL-CONICET, (4000), Miguel Lillo 251, San Miguel de Tucumán PC 4000, Tucumán, Argentina.
[4] Centro de Estudios Parasitológicos y Vectores (CEPAVE), Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional de La Plata (CCT, CONICET-UNLP), La Plata, Buenos Aires, Argentina.
[5] Baltazar Brum 445, Canelones, Canelones, Uruguay.
[6] Departamento de Acuicultura y Aguas Continentales, Dirección Nacional de Recursos Acuáticos (DINARA), Montevideo, Uruguay.
[7] Teniente Rinaldi 4870 EE, Montevideo, Uruguay.
[8] Autóctonos de Uruguay (ADU), https://www.instagram.com/autoctonosdeuruguay/
* e-mail: <serraelbicho@gmail.com>

## Abstract

*Characidium* is the most diverse genus within the family Crenuchidae, with a total of 88 valid species, although there is evidence that it is probably a polyphyletic group. Recent expeditions to northern Uruguay revealed the first records of *Characidium serrano* Buckup & Reis, 1997, extending its distribution in about 300 km to the south and allowing to consider it as an endangered species for Uruguay, considering its restricted distribution in the country. Different phylogenetic reconstruc-

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tions and comparisons based on COI (Model-based analysis, Parsimony analysis and Evolutionary Divergence between sequences) of new sequences with those previously published indicate: 1) a closer phylogenetic relationship of *C. serrano* with *C. clistenesi* or alternatively with (*C. rachovii* + *C. orientale*) than with *C. pterostictum* and 2) non-coincident topologies that in all cases suggest the non-monophyly of *Characidium*. The analyzed sequences of the genus *Characidium* formed 9 main stables groups that are obtained in each of the analyzes carried out, which were also recovered in studies previously performed by other authors, but with general topologies not coincident and poorly supported in their basal nodes.

**Keywords:** Characidiinae, Cuareim River basin, COI marker, conservation priority.

## Resumen

*Characidium* es el género más diverso dentro de la familia Crenuchidae, con un total de 88 especies válidas, aunque existen evidencias de que probablemente sea un grupo polifilético. Recientes expediciones al norte de Uruguay revelaron los primeros registros de *Characidium serrano* Buckup & Reis, 1997, extendiendo su distribución en unos 300 km hacia el sur y permitiendo considerarla como una especie en peligro de extinción para Uruguay, considerando su restringida distribución en el país. Las diferentes reconstrucciones filogenéticas y comparaciones basadas en COI (Análisis basado en modelos, Análisis de parsimonia y Divergencia evolutiva entre secuencias) de nuevas secuencias con las previamente publicadas indican: 1) una mayor afinidad filogenética de *C. serrano* con *C. clistenesi* o alternativamente con (*C. rachovii* + *C. orientale*) que con *C. pterostictum* y 2) topologías no coincidentes que en todos los casos sugieren la no-monofilia de *Characidium*. Las secuencias analizadas del género *Characidium* formaron 9 grupos estables principales que se obtienen en cada uno de los análisis realizados, los cuales también fueron recuperados en estudios previos realizados por otros autores, pero con topologías generales no coincidentes y pobremente soportadas en sus nodos basales.

**Palabras clave:** Characidiinae, cuenca del Río Cuareim, marcador COI, prioridad para la conservación.

## INTRODUCTION

*Characidium* is the most diverse genus within the family Crenuchidae, with a total of 88 valid species described (Fricke, Eschmeyer, Van der Laan, 2024; Toledo-Piza et al., 2024; Stabile, Reis, Frota, da Graça, Oliveira, 2024; Zanata, Oliveira, Oliveira-Silva, 2024). Its distribution extends from Panama to Uruguay and La Plata basin in northeastern Argentina (Zanata, Oliveira-Silva, Ohara, 2023; Fricke et al., 2024; Toledo-Piza et al., 2024). Phylogenetic hypothesis based on morphological data recovered this genus as monophyletic and supported by a single synapomorphy, the presence of a basicaudal spot, which is not present in all species (Buckup, 1993a). Although molecular approaches have recently begun to be used as part of the delimitation and description of new species (e.g. Malanski et al., 2019; Serrano et al., 2019; Agudelo-Zamora, Tavera, Murillo, Ortega-Lara, 2020; Oliveira-Silva, Batalha-Filho, Camelier, Zanata, 2024; Stabile et al., 2024; Zanata et al., 2024), the monophyly of the group has still not been tested using genetic sequences, and there is evidence that it is probably polyphyletic (Oliveira et al., 2011; Zanata et al., 2023; Oliveira-Silva et al., 2024).

For Uruguay, six species of this genus have been reported: *Characidium occidentale* Buckup & Reis, 1997, *Characidium orientale* Buckup & Reis, 1997, *Characidium pterostictum* Gomes, 1947, *Characidium rachovii* Regan, 1913, *Characidium tenue* (Cope, 1894) and *Characidium* aff. *zebra* Eigenmann, 1909 (Litz and Koerber, 2014; Serra et al., 2014; Loureiro, González-Bergonzoni, Teixeira de Melo, 2023).

*Characidium serrano* Buckup & Reis, 1997 was described based on specimens from Rio Grande do Sul and Santa Catarina (Brazil), and later recorded for the province of Misiones in Argentina (Buckup and Reis, 1997; Casciotta, Almirón, Doubnerová, Piálek, Říčan, 2015). Although in the studies describing the species the authors gave no hypothesis about its phylogenetic relationships with other species, subsequent phylogenetic analyses based on molecular characters (COI and Cyt-b sequences) and cytological comparisons (karyotype), found that it is closely related to *Characidium pterostictum* (Scacchetti et al., 2015; Agudelo-Zamora et al., 2020; Oliveira-Silva et al., 2024).

In the present work we report the presence of a seventh species of the genus for the Uruguayan territory, *Characidium serrano* Buckup & Reis, 1997, identified through morphology and molecular characters (COI), and comment its phylogenetic relationships. Additionally, we incorporated into the analyzes sequences of *C. orientale* and *C. tenue*, the last one type species of the genus *Chorimycterus* Cope, 1894 (junior synonym of *Characidium*), and discussed some points to consider for future research.

## MATERIALS AND METHODS

Specimens were collected with hand nets and euthanized by overdose in eugenol solution. Some specimens were fixed in 10% formaline solution and preserved in 70% ethanol, and others directly preserved in 95° ethanol for molecular analyses. Samples are housed in the ichthyologic collection of Museo Nacional de Historia Natural (MHNM), Montevideo, Uruguay. Measurements and counts taken according to Buckup (1993b) and Melo and Oyakawa (2015). Measurements were recorded to the nearest milimeter as straight-line distances, taken with a digital caliper under a stereomicroscope. Identification was based on Buckup and Reis (1997) and comparisons with collection specimens (Appendix). Institutional abbreviations are as listed at http://www.asih.org/resources. The distribution map was based on records from scientific collections and the following references: Buckup and Reis (1997), Casciotta et al. (2015) and speciesLink network (2024).

Total genomic DNA from specimens of *C. serrano* and *C. tenue* (Table 1) was extracted from muscle tissue preserved in cold 96% ethanol, utilizing PURO-Genomic DNA produced by PB-L Productos Bio-Logicos® S.A. following the manufacturer's protocol. Amplification was carried out using Polymerase Chain Reaction (PCR) on an Eppendorf Mastercycler thermal cycler with forward primer Fish F1 (5' - TCAACCAACCACAAAGACATTGGCAC -3′) and reverse primer Fish R1 (5' - TAGACTTCTGGGTGGCCAAAGAATCA -3') (Ward, Zemlak, Innes, Last, Hebert, 2005). The PCR reactions were executed according to the protocols described by Souza-Shibatta et al. (2018). Both strands of the PCR products were sequenced using an ABI 3730XL sequencer by Macrogen Inc. (Korea). Sequences were aligned and double-checked by eye using the platform MEGA v.11 (Tamura, Stecher, Kumar, 2021). New sequences were deposited in GenBank (Table 1), and sequences from other species were downloaded from GenBank and BOLD v.4 (Table 2).

## Model-based analysis

The best partitioning scheme and substitution model for the DNA partition were chosen based on the Bayesian information criterion (BIC; Schwarz, 1978) using the 'greedy' search strategy in Partition Finder v.1.1.1 (Lanfear, Calcott, Kainer, Mayer, Stamatakis, 2014). The optimal nucleotide substitution models were SYM+I+G, HKY and GTR+G for the first, second, and third codon positions of the COI sequences, respectively. Phylogenetic reconstruction was conducted using Bayesian inference (BI) in MrBayes

**Table 1**. Voucher information from the sequences generated for the present work.

**Tabla 1.** Información de los ejemplares de referencia de las secuencias generadas para el presente trabajo.

| Species | Museum sample | Locality | GenBank accession number |
|---|---|---|---|
| *Characidium serrano* | MHNM 6056 | A° de la Invernada (30°49′06″S – 56°00′49″W), Artigas, Uruguay | PQ790678 |
| | CIT-FML 104 | A° Melo (27°25′02″S – 54°42′07″W), Misiones, Argentina | PQ790675 |
| | CIT-FML 112 | A° Melo (27°25′02″S – 54°42′07″W), Misiones, Argentina | PQ790676 |
| | CIT-FML 651 | Balneario El Bonito (27°27′17″S – 54°55′43″W), Misiones, Argentina | PQ790677 |
| *Characidium orientale* | MHNM 5985 | A° India Muerta (34°05′52″S – 54°15′52″W), Rocha, Uruguay | PQ790679 |
| *Characidium rachovii* | MHNM 4721-1 | Cañada San Germán (33°36′54″S – 54°36′05″W), Lavalleja, Uruguay | PQ790681 |
| *Characidium tenue* | MHNM 5081 | Río Uruguay, Nuevo Berlín (32°59′21″S – 58°03′54″W), Río Negro, Uruguay | PQ790680 |

**Table 2.** List of taxa considered for the phylogenetic analyses, compiled from GenBank and BOLD System.

**Tabla 2.** Lista de los taxa considerados para los análisis filogenéticos, compilados de GenBank y BOLD System.

| Species | GenBank accession number |
|---|---|
| *Characidium alipioi* | MH667868, MH667874, MH667901 |
| *Characidium bahiense* | OQ998794, OQ998795, OQ998796 |
| *Characidium bimaculatum* | KY348899, KY348900 |
| *Characidium cacah* | OQ998801 |
| *Characidium caucanum* | MT946170, MT946171, MT946172 |
| *Characidium chancoense* | MT946176 |
| *Characidium chupa* | FBCH106-21, FBCH113-21, FBCH163-21 (BOLDSystem ID) |
| *Characidium clistenesi* | OQ998803 |
| *Characidium* cf. *crandellii* | PQ141390, PQ141391 |
| *Characidium cricarense* | MH667899, MH667900, MH667902 |
| *Characidium deludens* | PP725101, PP725102, PP725103 |
| *Characidium dule* | MT946179, MT946180, MT946181 |
| *Characidium etheostoma* | MK861668, MK861669, MK861670 |
| *Characidium fasciatum* | MK464031 |
| *Characidium gomesi* | GU701425, GU701426, GU701427 |
| *Characidium heirmostigmata* | OP586610, OP586615, OP586616 |
| *Characidium helmeri* | PP725111, PP725112, PP725113 |
| *Characidium interruptum* | PQ141395, PQ141396 |
| *Characidium itarare* | OQ749887, OQ749888 |
| *Characidium kamakan* | PP725114, PP725115, PP725116 |
| *Characidium lagosantense* | HM405083, HM906012 |
| *Characidium lanei* | KF914697, KF914702, MG825016 |
| *Characidium lauroi* | KM229366 |
| *Characidium litorale* | MH667873 |
| *Characidium marshi* | MG936835, MG936836, MG936837 |
| *Characidium nana* | PQ141400, PQ141401, PQ141402 |
| *Characidium oiticicai* | GU701447, GU701448, GU701449 |
| *Characidium pellucidum* | MZ051026, MZ051108, MZ051140 |
| *Characidium phoxocephalum* | MT946182, MT946183 |
| *Characidium pterostictum* | KF914700, KF914708, KF914709, KF914710, MH667871 |
| *Characidium* cf. *purpuratum* | MT946184 |
| *Characidium rachovii* | JX111702, KU288854, KU289033 |
| *Characidium samurai* | PP725117, PP725118 |
| *Characidium sanctjohanni* | MT946188, MT946189 |
| *Characidium satoi* | OQ998802, PP725119, PP725120 |
| *Characidium schubarti* | GU701439, GU701440, GU701442 |
| *Characidium* cf. *steindachneri* | MT946198 |
| *Characidium "serrano"* | KM229364 |
| *Characidium tapuia* | PQ141411, PQ141412 |
| *Characidium tatama* | MT946199, MT946200 |
| *Characidium timbuiense* | KM229365, MH667892 |
| *Characidium travassosi* | OP586614, OP586617, OP586618 |
| *Characidium varii* | PQ141408, PQ141409, PQ141410 |
| *Characidium vidali* | MG825007, MH667875 |
| *Characidium xanthopterum* | GU701835, GU701836, GU701837 |
| *Characidium zebra* | MZ050992, MZ051379, MZ051462, MZ051565, MZ051607 |
| *Leptocharacidium* sp. | MZ051603 |
| *Melanocharacidium blennioides* | MZ051471 |
| *Melanocharacidium dispilomma* | MZ051881 |
| *Microcharacidium eleotrioides* | MZ051949 |
| *Poecilocharax weitzmani* | ON063044 |

v.3.2.3 (Ronquist et al., 2012). Phylogenetic trees were generated using two parallel analyses of Metropoliscoupled Markov chain Monte Carlo (MCMC) for 20 million generations each to estimate the posterior probability (PP) distribution. Topologies were sampled every 1000 generations, and the average standard deviation of split frequencies was maintained below 0.01, as recommended by Ronquist et al. (2012). The robustness of the clades was evaluated using Bayesian PP, with PP > 0.95 considered strongly supported. A majority consensus tree with branch lengths was constructed.

The maximum Likelihood (ML) analysis was performed in IQtree web version (Trifinopoulos, Nguyen, von Haeseler, Minh, 2016), with confidence intervals for the edges of the ML tree using fast bootstrapping based on 10000 repetitions (Hoang, Chernomor, Von Haeseler, Minh, Vinh, 2018). The TIM2+F+I+G4 model was identified as the best-fitting model for COI sequences (chosen according to BIC) and was used to infer the phylogenetic hypothesis (Chernomor, von Haeseler, Minh, 2016; Kalyaanamoorthy, Minh, Wong, von Haeseler, Jermiin, 2017).

### Cladistic phylogenetic analysis under maximum parsimony

The phylogenetic analysis under Maximum Parsimony (MP) principle was done in TNT software (Goloboff, Farris, Nixon, 2008) under extended implied weighting, with a concavity of K=3 and K=12 (Goloboff, 2014). Tree searches were conducted using the default parameters of the New Technology search methods in the TNT program. Clade support was estimated using symmetric resampling (10000 replicates, with 10 additional sequences, saving up to 10 trees each), expressed as GC values (groups present/contradicted) (Goloboff et al., 2003).

### Evolutionary divergence between sequences

Estimates of evolutionary divergence between sequences (D), representing the number of base substitutions per site, were calculated using the Maximum Composite Likelihood model (Tamura, Nei, Kumar, 2004) in MEGA v.11 (Tamura et al., 2021). Codon positions included in the analysis were 1st, 2nd, 3rd, and noncoding regions.

## RESULTS

### ***Characidium serrano*** Buckup & Reis, 1997

URUGUAY: Artigas: MHNM 6056, 1 ex., 37.4 mm SL, fixed in 95° ethanol, Paso del Tractor, Arroyo de la Invernada (30°49'06.4"S 56°00'49.6"W), col. W.S. Serra, F. Scarabino, G. Tabares, N. Pauer, G. Sanguinetti, G. Núñez,

M. García & M. Pérez, 20/II/2023; MHNM 6585, 2 ex., 40.5-44.0 mm SL, fixed in 95° ethanol, Paso del Tractor, Arroyo de la Invernada (30°49'06.4"S 56°00'49.6"W), col. W.S. Serra, G. Sanguinetti & A. Duarte, 22-24/I/2024; MHNM 6589, 4 ex., 39.6-44.9 mm SL, fixed in 10% formaline solution, Paso del Tractor, Arroyo de La Invernada (30°49'06.4"S 56°00'49.6"W), col. W.S. Serra, G. Sanguinetti & A. Duarte, 22-24/I/2024.

According to literature *C. serrano* differs from congeneric species, except *Characidium papachibe* Peixoto & Wosiacki, 2013 and *Characidium heirmostigmata* da Graça & Pavanelli, 2008, by the presence of oblique vertical bands centered on lateral line and not reaching the dorsum, independent of the eight or nine dorsal transverse bars. It can be distinguished from *C. papachibe* by the presence of dark spots on caudal fin (vs. absence), by the presence of four or five scales above and four or five below the lateral line (vs. three above and three below the lateral line), and by the number of scales around the caudal peduncle (12 vs. 10). Additionally, it differs from *C. heirmostigmata* by its narrower body width (8.7 to 11.1% SL vs. 12.2 to 15.7%) (Buckup and Reis, 1997; da Graça and Pavanelli, 2008; Peixoto and Wosiacki, 2013).

The color pattern, measurements and counts of the examined specimens (Figure 1) coincide with those reported for the species (Tables 3 and 4), with minimal differences (<3%): prepelvic distance 46.6-50.2% SL vs. 46.8-51.4% SL in the original description (OD) plus Casciotta et al. (2015) (C); preanal distance 70.6-76.7% SL vs. 71.8-78.0% SL OD+C; anal-apex distance 89.2-94.9% SL vs. 90.7-96.2% SL OD; head length 20.9-23.9% SL vs. 21.4-26.3% SL OD+C; snout length 20.9-24.3% HL vs. 23.4-27.0% HL OD+C; snout-maxillary tip 23.2-28.8% HL vs. 22.1-28.0% HL OD+C; anterior nares-eye 6.2-10.0% HL vs. 8.4-11.8% HL OD+C; cheek depth 5.4-7.9 % HL vs. 7.2-10.9% HL OD; orbital diameter 27.8-32.8% HL vs. 27.0-32.0% HL OD+C; unbranched pectoral fin rays II-III vs III OD; oblique bands 5-12 vs. 10-14 OD.

## Distribution

The previously known distribution of *C. serrano* extends through the upper and middle Uruguay River basin in Rio Grande do Sul and Santa Catarina (Brazil), and Misiones (Argentina) (Buckup and Reis, 1997; Casciotta et al., 2015; Fricke et al., 2024). The new locality here reported extends its distribution in about 300 km to the south, and represent the first record for Uruguay (Figure 2).

## Ecological and conservations aspects

All specimens recorded from Uruguay are from Paso del Tractor in Arroyo de la Invernada, Artigas Department, northern Uruguay (Figure 2). The

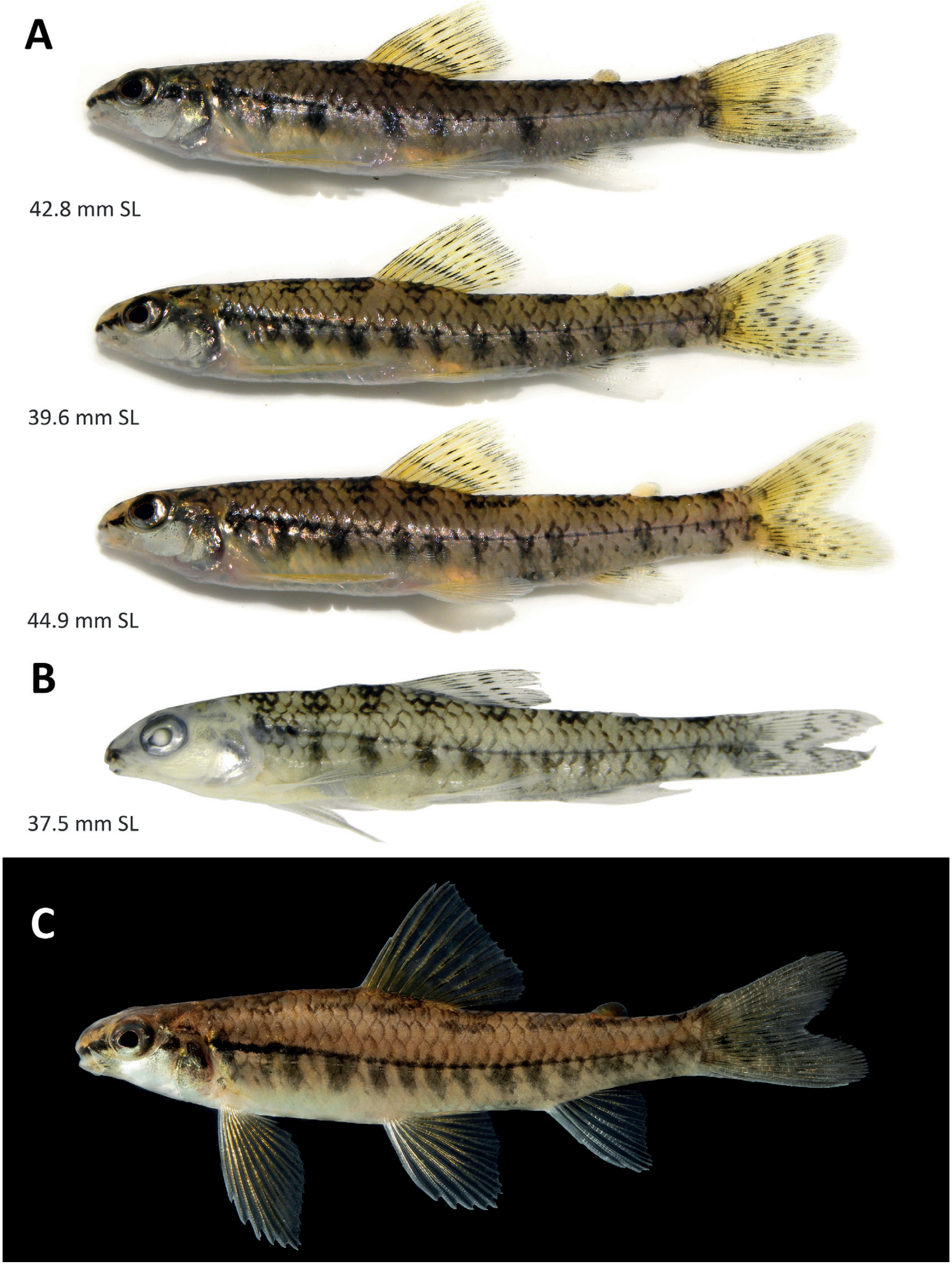

**Figure 1.** *Characidium serrano*: A – (MHNM 6589) Uruguayan specimens before fixation; B – (MHNM 6056) Uruguayan specimen sequenced; C – (CI FML 8106) live specimen from Argentina.

**Figura 1.** *Characidium serrano*: A – (MHNM 6589) especímenes uruguayos antes de ser fijados; B – (MHNM 6056) espécimen uruguayo secuenciado; C – (CI FML 8106) espécimen vivo proveniente de Argentina.

**Table 3.** Morphometric data of specimens analized. Total and standard length is expressed in mm; measurements (numbered 3–21) are percentage of standard length; subunits of head measurements (numbered 22–28) are percentage of head length. Characters with * according to Melo & Oyakawa (2015), all the other according to Buckup (1993b). Specimen 1 = MHNM 6056; 2 and 3 = MHNM 6585; 4, 5, 6 and 7 = MHNM 6589.

**Tabla 3.** Datos morfométricos de los especímenes analizados. La longitud total y estándar se expresa en mm; las mediciones (numeradas 3–21) se presentan como porcentajes de la longitud estándar; las mediciones de las subunidades de la cabeza (numeradas 22–28) se presentan como como porcentajes de la longitud de la cabeza. Caracteres con * son considerados de acuerdo a Melo y Oyakawa (2015), todos los otros de acuerdo a Buckup (1993b). Espécimen 1 = MHNM 6056; 2 y 3 = MHNM 6585; 4, 5, 6 y 7 = MHNM 6589.

|  | Character | Specimen |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|
|  |  | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 1 | Total length* | 45.1 | 54.1 | 49.6 | 55.3 | 49.2 | 53.6 | 54.5 |
| 2 | Standard length | 37.5 | 44.0 | 40.5 | 44.9 | 39.6 | 42.8 | 43.9 |
| 3 | Prepectoral distance | 24.4 | 22.7 | 23.3 | 22.9 | 24.4 | 24.4 | 22.4 |
| 4 | Pectoral-fin height* | 21.4 | 21.8 | 24.0 | 20.4 | 23.0 | 25.0 | 23.5 |
| 5 | Predorsal distance | 43.7 | 44.9 | 46.0 | 42.8 | 46.3 | 46.6 | 44.1 |
| 6 | Dorsal-fin height* | 21.1 | 18.7 | 20.5 | 19.6 | 20.1 | 17.8 | 20.3 |
| 7 | Dorsal-fin base* | 15.2 | 14.9 | 14.9 | 14.8 | 15.9 | 15.2 | 14.5 |
| 8 | Prepelvic distance | 46.6 | 48.1 | 47.2 | 47.2 | 50.2 | 50.1 | 47.7 |
| 9 | Pelvic-fin height* | 17.6 | 14.9 | 16.4 | 14.8 | 16.5 | 17.8 | 15.7 |
| 10 | Preanal distance | 70.6 | 73.8 | 72.8 | 73.1 | 76.7 | 76.3 | 72.9 |
| 11 | Anal-apex distance | 89.2 | 91.3 | 91.1 | 90.8 | 94.0 | 94.9 | 92.1 |
| 12 | Anus to anal-fin distance* | 12.1 | 12.2 | 11.5 | 11.9 | 13.2 | 12.1 | 10.7 |
| 13 | Anal-fin height* | 16.4 | 15.2 | 16.4 | 13.9 | 16.7 | 16.4 | 16.2 |
| 14 | Anal-fin base* | 8.0 | 7.9 | 7.0 | 7.5 | 9.7 | 8.3 | 7.7 |
| 15 | Adipose-fin height* | 5.0 | 5.4 | 5.9 | 4.9 | 5.4 | 5.9 | 5.0 |
| 16 | Peduncle length* | 20.8 | 20.7 | 20.3 | 19.4 | 16.8 | 17.2 | 19.8 |
| 17 | Body width | 9.0 | 9.0 | 9.3 | 9.6 | 9.9 | 10.3 | 9.3 |
| 18 | Body depth at dorsal origin | 18.7 | 18.3 | 18.5 | 19.0 | 17.7 | 19.0 | 17.9 |
| 19 | Body depth at anal origin | 14.0 | 14.2 | 13.8 | 14.2 | 13.4 | 14.5 | 14.0 |
| 20 | Body depth at caudal peduncle | 9.7 | 9.7 | 9.5 | 9.7 | 9.6 | 9.9 | 9.7 |
| 21 | Head length | 22.6 | 20.9 | 23.1 | 22.3 | 23.7 | 23.9 | 22.0 |
| 22 | Snout length | 23.6 | 24.3 | 20.9 | 23.6 | 22.6 | 22.8 | 22.3 |
| 23 | Snout-maxillary tip | 25.9 | 25.8 | 23.2 | 23.8 | 25.1 | 28.8 | 23.3 |
| 24 | Anterior nares-eye | 10.0 | 8.5 | 9.4 | 9.6 | 9.4 | 9.2 | 6.2 |
| 25 | Posterior nares-eye | 3.5 | 3.6 | 3.4 | 3.0 | 3.5 | 3.6 | 3.0 |
| 26 | Cheek depth | 6.0 | 7.5 | 5.4 | 7.9 | 7.3 | 5.6 | 7.9 |
| 27 | Orbital diameter | 32.3 | 32.8 | 29.6 | 28.0 | 27.8 | 29.4 | 29.6 |
| 28 | Interorbital distance | 16.0 | 17.0 | 13.9 | 14.1 | 13.8 | 15.6 | 17.0 |

collection site has clear water and rocky bottom, with scarce submerged vegetation (Figure 3). Other 41 fish species were recorded in the same locality: *Leporinus amae* Godoy, 1980 (Anostomidae), *Andromakhe saguazu* (Casciotta, Almirón & Azpelicueta, 2003), *Ectrepopterus uruguayensis* (Fowler, 1943), *Hyphessobrycon meridionalis* Ringuelet, Miquelarena & Menni, 1978, *Oligosarcus jenynsii* (Günther, 1864), *Psalidodon togoi* (Miquelarena & López, 2006), *Psalidodon rutilus* (Jenyns, 1842), *Psalidodon* sp.1, *Psalidodon* sp.2 (Acestrorhamphidae), *Charax stenopterus* (Cope, 1894) (Characidae), *Bryconamericus iheringii* (Boulenger, 1887), *Bryconamericus poi* (Almirón, Casciotta, Azpelicueta & Cione, 2001), *Diapoma terofali* (Géry, 1964), *Diapoma urugua-*

**Table 4.** Counts of specimens analized. Specimen 1 = MHNM 6056; 2 and 3 = MHNM 6585; 4, 5, 6 and 7 = MHNM 6589.

**Tabla 4.** Conteos de los especímenes analizados. Espécimen 1 = MHNM 6056; 2 y 3 = MHNM 6585; 4, 5, 6 y 7 = MHNM 6589.

| | Character | Specimen | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 1 | Lateral series of scales | 37 | 36 | 36 | 37 | 37 | 36 | 36 |
| 2 | Scales above lateral line | 5 | 4 | 4 | 4 | 5 | 4 | 5 |
| 3 | Scales below lateral line | 5 | 5 | 5 | 4 | 5 | 5 | 4 |
| 4 | Scales around caudal peduncle | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
| 5 | Scales in pre-dorsal series | 11 | 11 | 10 | 10 | 12 | 10 | 11 |
| 6 | Scales between anus and anal fin origin | 5 | 5 | 4 | 5 | 6 | 6 | 7 |
| 7 | Pectoral-fin rays | II-8-I | III-7-I | III-7-I | II-9 | III-8 | III-8 | III-7 |
| 8 | Pelvic-fin rays | I-7-I | I-7-I | I-6-I | I-7-I | I-7-I | I-7 | I-8 |
| 9 | Anal-fin rays | II-6 | II-6-I | II-7 | II-6-I | II-7 | II-7 | II-7 |
| 10 | Caudal-fin rays | I-9-8-I | I-9-8-I | I-9-8-I | I-9-8-I | I-9-8-I | I-9-8-I | I-9-8-I |
| 11 | Dorsal-fin rays | II-9 | II-9 | II-9 | II-9 | II-9 | II-9 | II-9 |
| 12 | Oblique lateral bands | 11 | 9 | 10 | 10 | 12 | 5 | 11 |

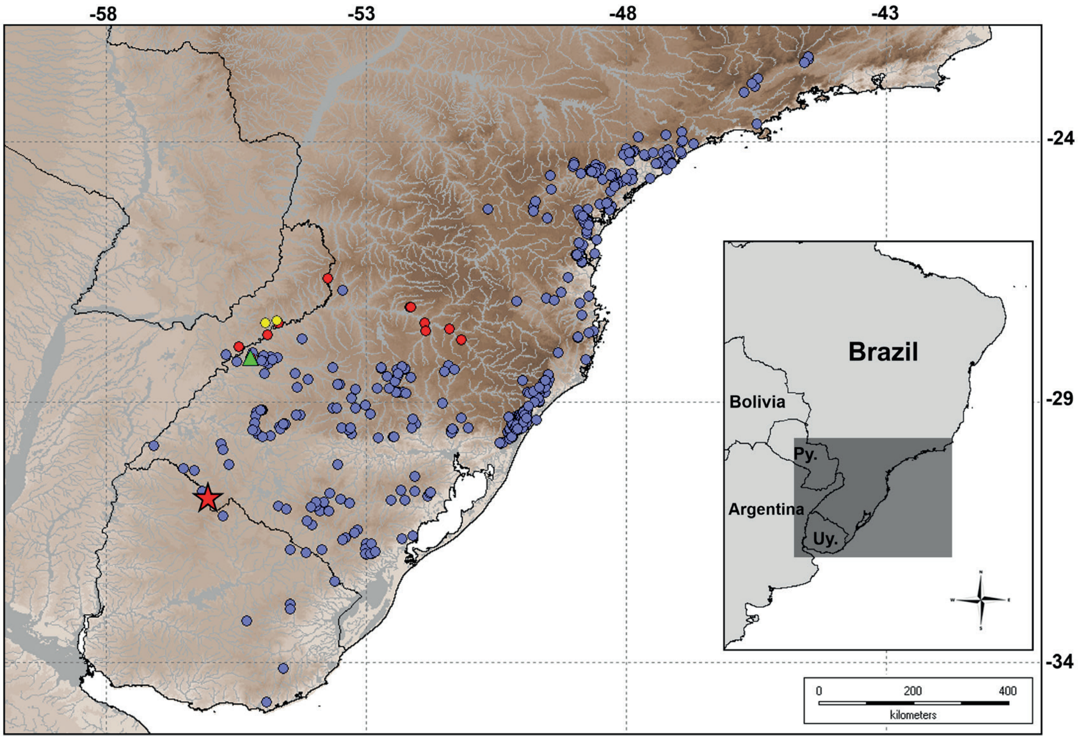

**Figure 2.** Distribution map of *Characidium serrano* and *C. pterostictum*: red dots = previous records of *C. serrano*; yellow dots = *C. serrano* analyzed from Argentina; red star = record from Uruguay; green triangle = specimen sequenced by Scacchetti et al. (2015); blue dots = records of *C. pterostictum*. Py. = Paraguay; Uy. = Uruguay.

**Figura 2.** Mapa de distribución de *Characidium serrano* y *C. pterostictum*: círculos rojos = reportes previos de *C. serrano*; círculos amarillos = *C. serrano* analizados de Argentina; estrella roja = registro de Uruguay; triángulo verde = espécimen secuenciado por Scacchetti et al. (2015); círculos azules = reportes de *C. pterostictum*. Py. = Paraguay; Uy. = Uruguay.

**Figure 3.** Paso del Tractor, Arroyo de la Invernada (30°49’06”S 56°00’49”W) (Artigas, Uruguay).

**Figura 3.** Paso del Tractor, Arroyo de la Invernada (30°49’06”S 56°00’49”W) (Artigas, Uruguay).

*yense* (Messner, 1962), *Pseudocorynopoma doriae* Perugia, 1891 (Stevardiidae), *Characidium rachovii* Regan, 1913, *Characidium* sp. aff. *zebra* Eigenmann, 1909 (Crenuchidae), *Steindachnerina biornata* (Braga & Azpelicueta, 1987) (Curimatidae), *Hoplias lacerdae* Miranda Ribeiro, 1908 (Erythrinidae), *Australoheros scitulus* (Říčan & Kullander, 2003), *Crenicichla celidochilus* Casciotta, 1987, *Crenicichla minuano* Lucena & Kullander, 1992, *Crenicichla missioneira* Lucena & Kullander, 1992, *Crenicichla scottii* (Eigenmann, 1907), *Crenicichla tendybaguassu* Lucena & Kullander, 1992, *Gymnogeophagus pseudolabiatus* Malabarba, Malabarba & Reis, 2015, *Gymnogeophagus* sp., *Saxatilia lepidota* (Heckel, 1840) (Cichlidae), *Trachelyopterus teaguei* (Devincenzi, 1942) (Auchenipteridae), *Ancistrus* sp., *Hisonotus charrua* Almirón, Azpelicueta, Casciotta & Litz, 2006, *Hisonotus ringueleti* Aquino, Schaefer & Miquelarena, 2001, *Rineloricaria isaaci* Rodriguez & Miquelarena, 2008, *Rineloricaria* spp. (Loricariidae), *Heptapterus mustelinus* (Valenciennes, 1835), *Rhamdella longiuscula* Lucena & da Silva, 1991, *Rhamdia* cf. *quelen* (Quoy & Gaimard, 1824) (Heptapteridae), *Brachyhypopomus bombilla* Loureiro & Silva, 2006 (Hypopomidae), *Eigenmannia trilineata* López & Castello, 1966, *Eigenmannia virescens* (Valenciennes, 1836) (Sternopygidae), and *Synbranchus* sp. (Synbranchidae).

According to the criteria defined by Soutullo et al. (2013), we indicate that *C. serrano* must be considered a priority for conservation and an Endangered Species for the species list of Sistema Nacional de Áreas Protegidas (SNAP) of Uruguay: its global distribution occupies less than 200.000 km$^2$ (Criteria 1) and its occurrence area represents less than 10% of the national territory (Criteria 4).

## Molecular analysis and phylogenetic relationships

The final COI alignment was 654 bp long and consisted of 124 terminals with 48 species of *Characidium* (≈54% of the described species), including the newly sequenced specimens, and five other species of Crenuchidae as outgroups.

The different phylogenetic reconstructions resulted in non-coincident topologies, and in all cases *Characidium* is obtained as para or polyphyletic (Figures 4-6). The analyzed species of the genus *Characidium* formed nine main groups that are obtained in each of the analyzes carried out:

"*Characidium* cf. *crandellii*" clade, composed solely by *Characidium* cf. *crandellii* Steindachner, 1915.

"*Characidium*" clade, composed by *Characidium dule* Agudelo-Zamora, Tavera, Murillo & Ortega-Lara, 2020, *Characidium marshi* Breder, 1925, *Characidium gomesi* Travassos, 1956, *Characidium purpuratum* Steindachner, 1882, *Characidium tatama* Agudelo-Zamora, Tavera, Murillo & Ortega-Lara, 2020 and its type species, *Characidium fasciatum* Reinhardt, 1867.

"*Jobertina*" clade, composed by *Characidium helmeri* Zanata, Sarmento-Soares & Martins-Pinheiro, 2015, *Characidium lanei* Travassos, 1967, *Characidium lauroi* Travassos, 1949, *Characidium oiticicai* Travassos, 1967, *Characidium pterostictum* Gomes, 1947, *Characidium schubarti* Travassos, 1955, *Characidium travassosi* Melo, Buckup & Oyakawa, 2016, *Characidium timbuiense* Travassos, 1946, *Characidium vidali* Travassos, 1967 and its type species, *Characidium interruptum* Pellegrin, 1909.

"*Characidium alipioi*" clade, composed by *Characidium alipioi* Travassos, 1955, *Characidium cricarense* Malanski, Sarmento-Soares, Silva-Malanski, Lopes, Ingenito & Buckup, 2019 and *Characidium kamakan* Zanata & Camelier, 2015.

"*Characidium caucanum*" clade, composed by *Characidium caucanum* Eigenmann 1912, *Characidium chancoense* Agudelo-Zamora, Ortega-Lara & Taphorn, 2020, *Characidium chupa* Schultz, 1944 and *Characidium phoxocephalum* Eigenmann, 1912.

"*Characidium pellucidum*" clade, composed by *Characidium pellucidum* Eigenmann, 1909 and *Characidium sanctjohanni* Dahl, 1960.

"*Poecilosomatops*" clade, composed by *Characidium nana* Mendonça & Netto-Ferreira 2015, *Characidium steindachneri* Cope, 1878, *Characidium tapuia* Zanata, Ramos & Oliveira-Silva, 2018, *Characidium zebra* Eigenmann, 1909 and its type species, *Characidium etheostoma* Cope, 1872.

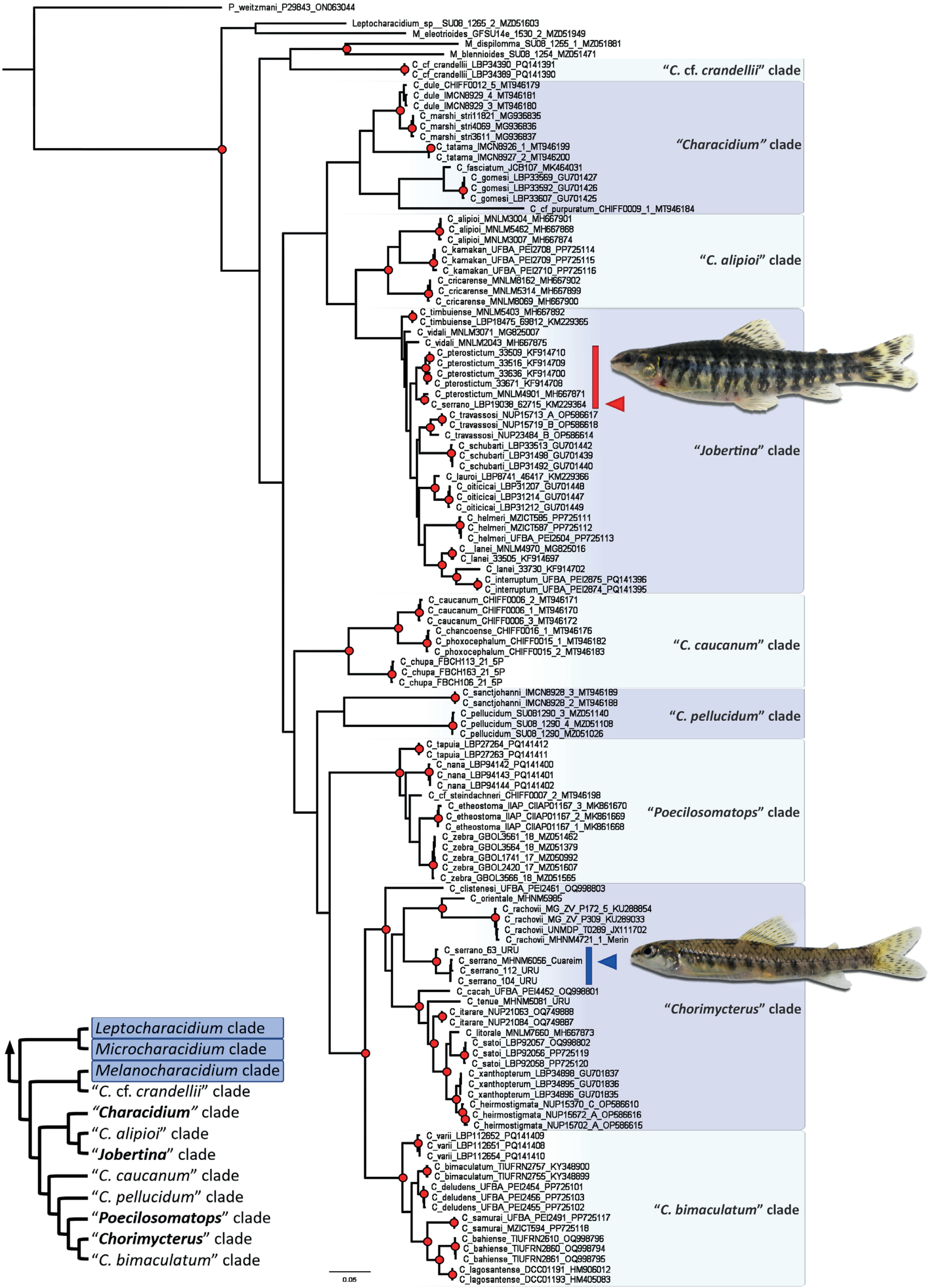

**Figure 4.** Bayesian phylogenetic tree of *Characidium* obtained from COI data.

Red circles on nodes represent posterior probability higher than 95%. Blue bar = *C. serrano*; blue arrow = Uruguayan specimen of *C. serrano* sequenced (MHNM 6056). Red bar = *C. pterostictum*; red arrow = "*C. serrano*" from Arroio Canoinha, Brazil (KM229364). Bottom left, general topology considering the major clades. Top right = unpreserved specimen of *C. pterostictum* from Uruguay; down right = Uruguayan specimen of *C. serrano* (MHNM 6589).

**Figura 4.** Árbol filogenético Bayesiano de *Characidium* obtenido a partir de secuencias COI. Los círculos rojos sobre los nodos representan una probabilidad posterior mayor a 95%. Barra azul = *C. serrano*; flecha azul = espécimen uruguayo secuenciado de *C. serrano* (MHNM 6056). Barra roja = *C. pterostictum*; fleche roja = "*C. serrano*" proveniente del Arroio Canoinha, Brasil (KM229364). Abajo a la izquierda, topología general considerando los clados principales. Arriba a la derecha = espécimen no preservado de *C. pterostictum* proveniente de Uruguay; abajo a la izquierda = espécimen uruguayo de *C. serrano* (MHNM 6589).

"*Chorimycterus*" clade, composed by *Characidium cacah* Zanata, Ribeiro, Araújo-Porto, Pessali & Oliveira-Silva, 2020, *Characidium clistenesi* Melo & Espíndola, 2016, *Characidium heirmostigmata* da Graça & Pavanelli, 2008, *Characidium itarare* Stabile, Reis, Frota, da Graça & Oliveira, 2024, *Characidium litorale* Leitão & Buckup, 2014, *Characidium orientale* Buckup & Reis, 1997, *Characidium rachovii* Regan, 1913, *Characidium satoi* Melo & Oyakawa, 2015, *Characidium serrano* Buckup & Reis, 1997, *Characidium xanthopterum* Silveira, Langeani, da Graça, Pavanelli & Buckup, 2008 and its type species, *Characidium tenue* (Cope, 1894).

"*Characidium bimaculatum*" clade, composed by *Characidium bahiense* Almeida, 1971, *Characidium bimaculatum* Fowler, 1941, *Characidium deludens* Zanata & Camelier, 2015, *Characidium lagosantense* Travassos, 1947, *Characidium samurai* Zanata & Camelier, 2014 and *Characidium varii* Zanata, Oliveira & Oliveira-Silva, 2024.

## Model-based analysis

The topology of the phylogenetic tree the Characidiinae, obtained through Bayesian Inference (Figure 4), reveals *Characidium* as a paraphyletic group, with several of its nine major clades well supported. The "*C.* cf. *crandellii*" clade was recovered as the sister group to the genus *Melanocharacidium*, and together they form a sister group to the remaining eight clades. These clades are arranged into two major groups: one comprising the ("*Jobertina*" clade + "*C. alipioi*" clade) + "*Characidium*" clade, and the other consisting of the "*C. caucanum*" clade + ("*C. pellucidum*" clade + ("*Poecilosomatops*" clade + ("*Chorimycterus*" clade + "*C. bimaculatum*" clade))). Most of the basal nodes present low support.

The topology of the phylogenetic tree generated using the Maximum Likelihood approach (Figure 5) also recoverd *Characidium* as a paraphyletic genus. The "*C.* cf. *crandellii*" clade was recovered as the sister group to a clade containing (*Leptocharacidium* + *Microcharacidium*), which is sister of

**Figure 5.** Maximum Likelihood phylogenetic tree of *Characidium* obtained from COI data.

Grey and black circles on nodes represent bootstrap supports higher than 75% and 95% respectively. Blue bar = *C. serrano*; blue arrow = Uruguayan specimen of *C. serrano* sequenced (MHNM 6056). Red bar = *C. pterostictum*; red arrow = "*C. serrano*" from Arroio Canoinha, Brazil (KM229364). Bottom left, general topology considering the major clades.

**Figura 5.** Árbol filogenético de Máxima Verosimilitud de *Characidium* obtenido a partir de secuencias COI. Círculos grices y negros sobre los nodos representan soportes de *bootstrap* mayores a 75% y 95% respectivamente. Barra azul = *C. serrano*; flecha azul = espécimen uruguayo de *C. serrano* secuenciado (MHNM 6056). Barra roja = *C. pterostictum*; flecha roja = "*C. serrano*" proveniente de Arroio Canoinha, Brasil (KM229364). Abajo a la izquierda, topología general considerando los clados principales.

the other eight *Characidium* clades. The topology of this latter section of the tree was recovered as follows: the "*C. caucanum*" clade + ((("*Jobertina*" clade + "*C. alipioi*" clade) + "*Characidium*") + ("*Poecilosomatops*" clade + ("*C. pellucidum*" clade + ("*Chorimycterus*" clade + "*C. bimaculatum*" clade)))). Most of the basal nodes present low support too.

Sequences obtained from *C. serrano* specimens collected in Argentina and Uruguay formed a well-supported monophyletic clade, which is sister to (*C. rachovii* + *C. orientale*) in both phylogenetic analysis.

## Parsimony analysis

The results yielded two trees rooted in *Poecilocharax weitzmani* under extended implied weighting (K=3, fit=136.86086, length=1803; K=12, fit=69.22522, length=1777) (Figure 6). The tree topologies differ with concavities K=3 and K=12. The genus *Characidium* was not recovered as monophyletic under implied weighting with different K values or equal weighting. Most of the basal nodes in both results present low support (GC values ≤ 29).

*Melanocharacidium* and *Leptocharacidium* are the taxa that disrupts *Characidium* monophyly under K=3 condition. "*C. pellucidum*" clade was recovered as sister group of the genera *Melanocharacidium*, *Leptocharacidium* and the other eight clades of *Characidium*. Topology of this section of the tree was recovered as follow: (("*Jobertina*" clade + "*C. alipioi*" clade) + "*Characidium*") + (((*Leptocharacidium* + "*C.* cf. *crandellii*" clade) + *Melanocharacidium*) + "*C. caucanum*" clade) + ("*Chorimycterus*" clade + ("*Poecilosomatops*" clade + "*C. bimaculatum*" clade)).

Under K=12 condition *Microcharacidium* and *Leptocharacidium* are the taxa that disrupts *Characidium* monophyly. A clade grouping *Leptocharacidium* plus "*C.* cf. *crandellii*" clade was recovered as sister of *Microcharacidium* + the other eight clades of *Characidium*. Topology of this section of the tree was recovered as follow: "*C. caucanum*" clade + ((("*Jobertina*" clade + "*C. alipioi*" clade) + "*Characidium*") + ("*C. pellucidum*" clade + ("*Poecilosomatops*" clade + ("*Chorimycterus*" clade + "*C. bimaculatum*" clade)))).

The sequences obtained from specimens of *C. serrano* from Argentina and Uruguay form a well-supported monophyletic clade, sister to *C. clistenesi* under K=3 and K=12 conditions.

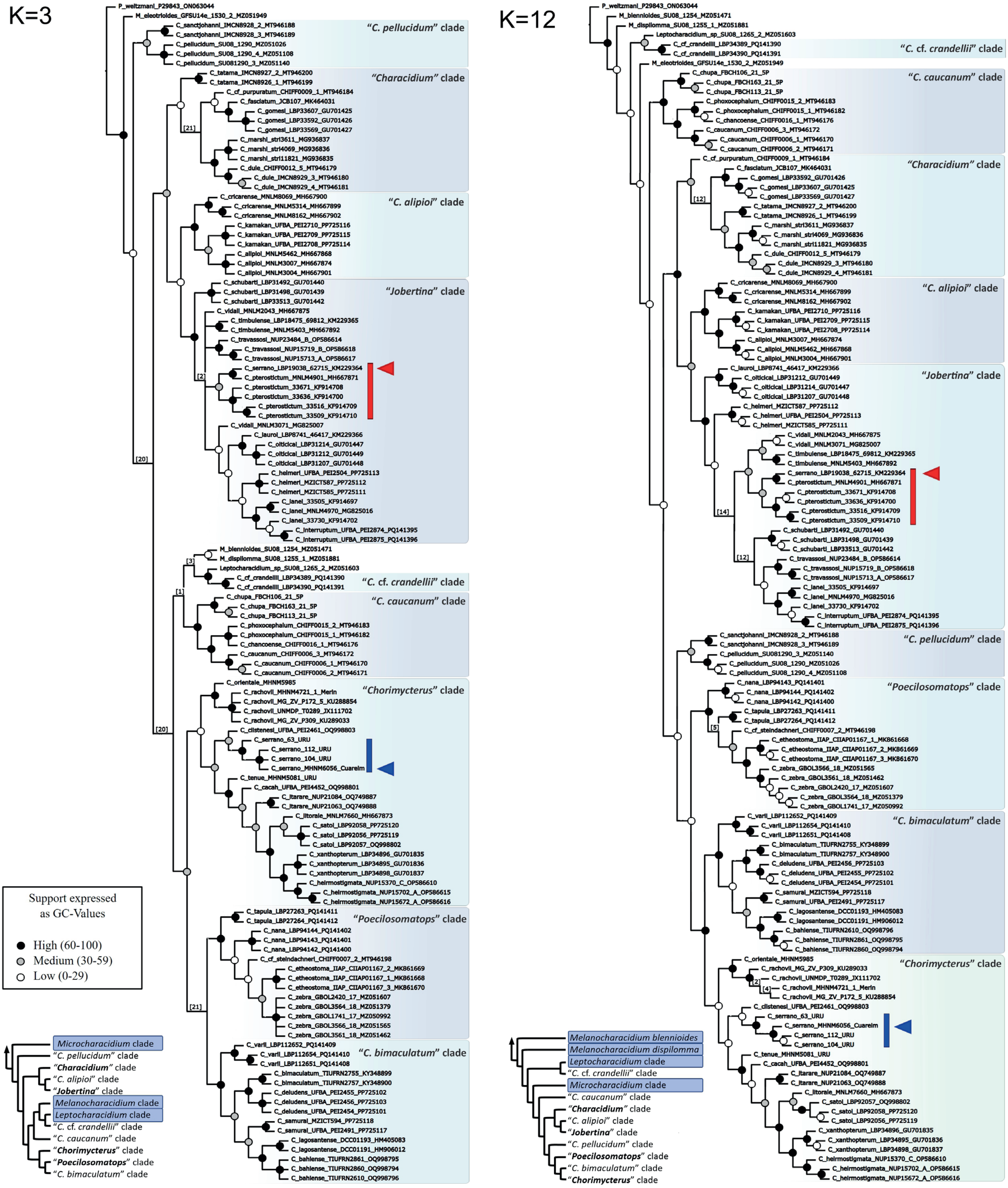

**Figure 6.** Phylogenetic tree under parsimony and extended implied weighting (K=3, fit=136.86086, length=1803; K=12, fit=69.22522, length=1777) of *Characidium* obtained from COI data.

The circles represent the GC values and support grades. Blue bar = *C. serrano*; blue arrow = Uruguayan specimen of *C. serrano* sequenced (MHNM 6056). Red bar = *C. pterostictum*; red arrow = "*C. serrano*" from Arroio Canoinha, Brazil (KM229364). Bottom left, general topology considering the major clades.

**Figura 6.** Árboles filogenéticos bajo parsimonia usando pesos implicados extendidos (K=3, ajuste=136.86086, largo=1803; K=12, ajuste=69.22522, largo=1777) de *Characidium* obtenido a partir de secuencias COI. Los círculos representan los valores GC y grados de soporte. Barra azul = *C. serrano*; flecha azul = espécimen uruguayo de *C. serrano* secuenciado (MHNM 6056). Barra roja = *C. pterostictum*; flecha roja = "*C. serrano*" proveniente de Arroio Canoinha, Brasil (KM229364). Abajo a la izquierda, topología general considerando los clados principales.

## Evolutionary divergence between sequences

The Estimates of evolutionary divergence between sequences (D) (Table 5), obtained from the comparison between the sequence of *C. serrano* from middle Uruguay River basin in Uruguay and Argentina gave relatively low values (D≤0.0202), while the values obtained in the comparison of these sequences and that from the same basin in Brazil (KM229364 from Scacchetti et al., 2015) were high (D=0.1531-0.1757). The sequences that presented the lowest values of divergence with respect to *C. serrano* from Brazil were those of *C. pterostictum*, with values that ranged between D = 0.0046 and 0.0219.

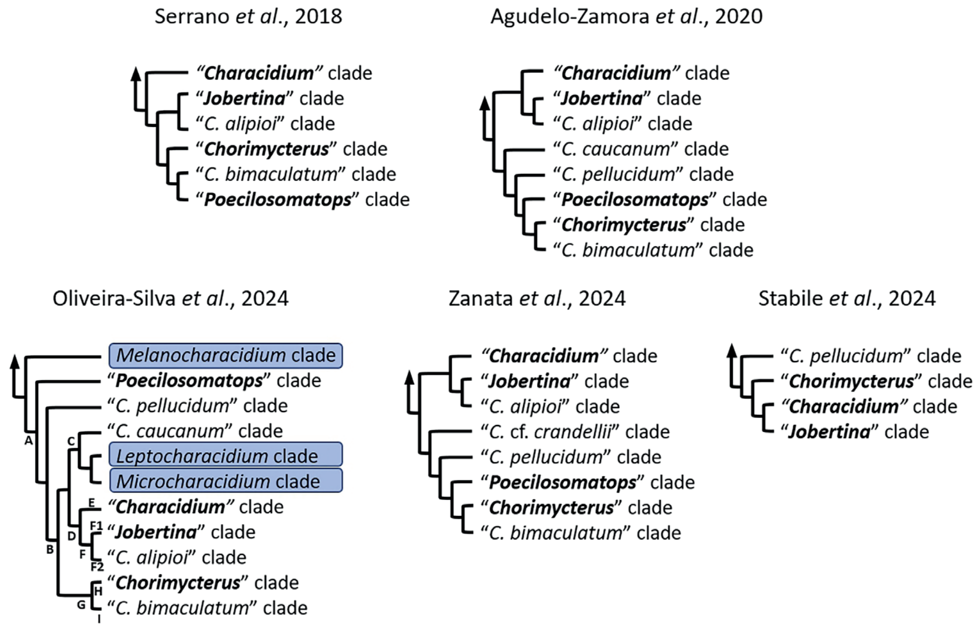

**Figure 7.** Schematic reinterpretation of previous phylogenies according the groups discussed in the present work.

**Figura 7.** Reinterpretación esquemática de filogenias previas de acuerdo a los grupos discutidos en este trabajo.

**Table 5**. Estimates of evolutionary divergence between sequences (D). Divergence values between sequences of the specimen identified as *C. serrano* from Uruguay and other specimens are highlighted in bold. ARG = Misiones, Argentina; CAN = Arroio Canoinha, Rio Grande do Sul, Brazil; CUA = Río Cuareim, Uruguay; SEB = southeastern Brazil; TRM = Tramandaí, Rio Grande do Sul, Brazil.

**Tabla 5**. Estimativo de la divergencia evolutiva entre secuencias (D). Valores de divergencia entre la secuencia del espécimen identificado como *C. serrano* de Uruguay y las de otros especímenes se remarcan en negrita. ARG = Misiones, Argentina; CAN = Arroio Canoinha, Rio Grande do Sul, Brasil; CUA = Río Cuareim, Uruguay; SEB = sureste de Brazil; TRM = Tramandaí, Rio Grande do Sul, Brasil.

| | Specimen | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *C. pterostictum* KF914710 SEB | 0.0000 | | | | | | | | | |
| 2 | *C. pterostictum* KF914709 SEB | 0.0000 | 0.0000 | | | | | | | | |
| 3 | *C. pterostictum* KF914700 SEB | 0.0072 | 0.0072 | 0.0000 | | | | | | | |
| 4 | *C. pterostictum* KF914708 SEB | 0.0036 | 0.0036 | 0.0018 | 0.0000 | | | | | | |
| 5 | *C. pterostictum* MH667871 TRM | 0.0237 | 0.0238 | 0.0238 | 0.0201 | 0.0000 | | | | | |
| 6 | *C. "serrano"* KM229364 CAN | 0.0219 | 0.0219 | 0.0219 | 0.0183 | 0.0046 | 0.0000 | | | | |
| 7 | *C. serrano* PQ790678 CUA | **0.1616** | **0.1600** | **0.1644** | **0.1579** | **0.1736** | **0.1756** | 0.0000 | | | |
| 8 | *C. serrano* PQ790677 ARG | 0.1518 | 0.1501 | 0.1545 | 0.1505 | 0.1504 | 0.1531 | **0.0193** | 0.0000 | | |
| 9 | *C. serrano* PQ790676 ARG | 0.1613 | 0.1597 | 0.1640 | 0.1576 | 0.1728 | 0.1757 | **0.0016** | 0.0202 | 0.0000 | |
| 10 | *C. serrano* PQ790675 ARG | 0.1613 | 0.1597 | 0.1640 | 0.1576 | 0.1708 | 0.1737 | **0.0000** | 0.0187 | 0.0015 | 0.0000 |

# DISCUSSION

## *Characidium serrano* and its systematic position

When describing *C. serrano*, Buckup and Reis (1997) commented that they could not point out its relationship with other species of the genus. Although they admit that it superficially resembles *C. pterostictum* in terms of its color pattern, they also point out that *C. serrano* lacks the area free of scales in the pectoral region that is characteristic of *C. pterostictum* and related species. However, based on molecular characters (COI and Cyt-b), Scacchetti et al. (2015) found that *C. serrano* would be closely related to *C. pterostictum*, which they reaffirm with karyotypic evidence. This result was also obtained by Agudelo-Zamora et al. (2020) and Oliveira-Silva et al. (2024) in their phylogenetic reconstructions of the genus based on sequence analysis of the COI marker. Surprisingly, the results obtained in our analyses (Figures 4-6) do not coincide with the previously mentioned studies. The phylogenetic reconstructions obtained based on the analysis of the COI gene sequenced from individuals from Argentina and Uruguay, indicate a closer phylogenetic affinity with *C. clistenesi* or alternatively with (*C. rachovii* + *C. orientale*) within the "*Chorimycterus*" clade, a group containing species with scales in the pectoral region, than with *C. pterostictum* or the "*C. serrano*" sequence (KM229364) obtained by Scacchetti and collaborators and here recovered in the "*Jobertina*" clade, a group containing species with a scaleless area in the pectoral.

The estimated evolutionary divergence was found to be high (D = 0.1531 to 0.1757) between the individuals identified as *C. serrano* in this study and the specimen from the Middle Uruguay River basin in Brazil (KM229364 from Scacchetti et al., 2015) (Table 5). This significant divergence suggests that they are not conspecific. Based on this discrepancy, we conclude that the *C. serrano* sequence used in previous studies likely belongs to a *C. pterostictum* specimen. This is supported by the fact that in the area where that specimen was obtained, *C. pterostictum* is ubiquitously reported (Figure 2) (Buckup and Reis, 1997; Scacchetti et al., 2015, speciesLink network, 2024). The low estimated evolutionary divergence between this sequence (KM229364) and those of the rest of the *C. pterostictum* analyzed, between 0.0046 (sequence from a specimen from Rio Tramandaí, Rio Grande do Sul, Brazil) and 0.0219 (sequences from specimen from SE of Brazil) (Table 5), reinforces this idea. An estimated evolutionary divergence of around 0.020 or less is usually considered evidence of conspecificity for the COI marker (Pereira, Hanner, Foresti, Oliveira, 2013).

Studies carried out during the last decade have demonstrated the importance of the Cuareim River as an area of high diversity of freshwater fish species in Uruguay (Zarucki et al., 2010; Serra, Duarte, Burres, Loureiro, 2011; Serra et al., 2013, Brazeiro et al., 2015). The presence of *C. serrano* in the upper basin of the Cuareim River, almost 300 km further south of the nearest known locality, underscores the importance of this area for consideration in future conservation efforts.

## *Characidium* phylogeny

The most recent and comprehensive phylogenetic analyses of *Characidium* have covered slightly more than 50% of the recognized species and have relied on mitochondrial markers (COI), primarily aiming to delimit and recognize molecular operational taxonomic units (MOTUs) (Agudelo-Zamora et al., 2020; Oliveira-Silva et al., 2024). In contrast, the analysis conducted in the present study (Figures 4–6) also utilized the COI marker but incorporated representative sequences for all recognized species of the genus available in GenBank and the BOLD System v.4.

In this work we recognize nine lineages, which were also recovered in studies previously carried out by other authors (Serrano et al., 2018; Agudelo-Zamora et al., 2020; Oliveira-Silva et al., 2024; Stabile et al., 2024; Zanata et al., 2024), in which despite being biased towards a few species, lacking external groups to *Characidium* and/or present different methodological approaches to analysis, they recover the same units (Figure 7). However, the topologies obtained both in our work and in previous publications are not coincident and poorly supported in their basal nodes.

Coincidentally with Oliveira-Silva et al. (2024) we find topologies that suggests *Characidium* as non-monophyletic, where both *Leptocharacidium* and *Microcharacidium* or *Melanocharacidium* appear nested within the genus in different clades, depending on the analysis performed. The aforementioned authors also discuss a series of clades named from “A” to “I”, several of which are equivalent to those obtained in our analyses: E clade = “*Chara-*

*cidium*” clade, F1 clade = “*Jobertina*” clade, F2 clade = “*C. alipioi*” clade, D clade = (“*Characidium*” clade + “*Jobertina*” clade + “*C. alipioi*” clade), H clade = “*Chorimycterus*” clade, and I clade = “*C. bimaculatum*” clade.

Despite the great differences between the topologies of the trees obtained both in our work and in previous works, one of the groupings that is repeated in all cases is “*Characidium*”+“*Jobertina*”+“*C. alipioi*” clades, which brings together the species with reduction of scales in the pectoral region and/or isthmus. The exception is given by the *C. dule* + *C. marshi* clade nested within the “*Characidium*” clade, where both species present complete scaling.

Finally, regarding the phylogeny based on morphological characters carried out by Buckup (1993a), although there seem to be some coincidences between the clades obtained in his work and those obtained by us, further comparisons are not possible due to its limited taxon sampling with only 16 *Characidium* species, since we were only able to analyze sequences from 10 of those species.

While a comprehensive taxonomic revision would require a significant increase in taxon sampling and an integrated analysis of both morphological data and genetic sequences from additional genes, the current information provides an initial framework for identifying emerging patterns. At present, COI sequences are available for four of the nine genera within Characidiinae. We excluded the *Ammocryptocharax* sequence in GenBank (KM229369), which likely corresponds to *C. xanthopterum* since their estimated evolutionary divergence was found to be low ($D < 0.0016$ compared with GU701835, GU701836 and GU701837). Similarly, COI sequences are available for four of the seven nominotypic species from genera or subgenera synonymized with *Characidium*, including *Characidium tenue*, the type species of the genus *Chorimycterus*, which is incorporated in this study.

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## PARTICIPATION

WSSA, FS, EM, MMM and GT contributed equally to the idealization, analysis, and writing. MM, GS, AD and MG contributed to the idealization, laboratory and the fieldwork logistics.

## CONFLICTS OF INTEREST

Authors declare no conflict of interest.

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## APPENDIX

### Comparative material

*Characidium orientale*: URUGUAY: Rocha: MHNM 5985, 1 ex., 38.3 mm SL, Paso Santiago, A° India Muerta (34°05'52"S 54°15'52"W), col. W.S. Serra, M. Caligari, G. Sanguinetti, G. Núñez, M. García, E. Caligari & L. Ortiz, 26/XI/2022. Treinta y Tres: MHNM 3704, 4 ex., 36.8-50.5 mm SL, Paso Sabino (32°52'52"S 54°27'24"W), Quebrada de los Cuervos, col. W.S. Serra & M. Loureiro, 25/XI/2014.

*Characidium pterostictum*: URUGUAY: Artigas: MHNM 4806, 1 ex, 65.0 mm SL, Rincón de Pacheco (30°41'53"S 56°09'00"W), Río Cuareim, col. Wilson. S. Serra, Germán Sanguinetti, Matías García, Mauricio Caligari, Gastón Núñez & Gabriel Sosa, 31/X/2021-1/XI/2021; MHNM 5062, 2 ex., 42.7-48.9 mm SL, Rincón de Pacheco, laguna (30°42'03"S 56°09'14"W), Río Cuareim, col. W.S. Serra, Germán Sanguinetti & Matías García, 26-27/II/2022. Cerro Largo: MHNM 2184, 39 ex., 31.5-55.7 mm SL, Puntas Sierra de Aceguá (ca. 31°53'S 54°09'W), Estancia San Pedro, col. A. Ximénez, 31/III/1974; MHNM 4367, 16 ex., 38.3-57.2 mm SL, Paso Sarandí (32°26'25"S 53°36'46"W), Arroyo Sarandí de Barceló, col. Wilson S. Serra, Giovanni Furtado & Andrés Balao, 1/

IV/2018; MHNM 4488 1 ex., 39.2 mm SL, Paso Sarandí (32°26'25"S 53°36'46"W), Arroyo Sarandí de Barceló, col. Wilson S. Serra, Giovanni Furtado & Andrés Balao, 1/IV/2018; MHNM 6221, 1 ex., 44.4 mm SL, Paso Sarandí (32°26'25"S 53°36'46"W), Arroyo Sarandí de Barceló, col. W.S. Serra, M. Caligari, G. Sanguinetti, G. Núñez, M. García, E. Caligari & L. Ortiz, 28/XI/2022. Florida: MHNM 6117, 1 ex., 35.3 mm SL, Río Yí (33°11'54"S 55°18'14"W), col. W.S. Serra, C. Clavijo & N. Ríos, 11/I/2018. Maldonado: MHNM 4736, 1 ex., 34.9 mm SL, Aº Sarandí Grande, Salamanca (34°06'12"S 54°36'10"W), col. Wilson. S. Serra, Germán Sanguinetti & Matías García, 2/X/2021; MHNM 4762, 3 ex., 29.1-36.8 mm SL, Aº Sarandí Grande, Salamanca (34°06'12"S 54°36'10"W), col. Wilson. S. Serra, Germán Sanguinetti & Matías García, 2/X/2021; MHNM 5111, 1 ex., 41.5 mm SL, Cañada de Chaves, Ruta 9 km 137 (34°45'49"S 54°55'35"W), col. W.S. Serra, N. Ríos & S. Rodríguez, 3/IV/2022. Rivera: MHNM 5567, 1 ex., 47.4 mm SL, Río Tacuarembó (31°10'39"S 55°45'45"W), col. Skuk & Burmidad, 17/IV/1981. Treinta y Tres: MHNM 3700, 3 ex., 46.6-49.9 mm SL, Paso Sabino (32°52'52"S 54°27'24"W), Quebrada de los Cuervos, col. W.S. Serra, M. Loureiro, 25/XI/2014; MHNM 3714, 1 ex., 37.1 mm SL, Paso Polo (32°58'04"S 54°28'11"W), Quebrada de los Cuervos, col. W.S. Serra, M. Loureiro, 24/XI/2014.

*Characidium rachovii*: URUGUAY: Artigas: MHNM 5665, 1 ex., 38.8 mm SL, Rincón de Pacheco (30°41'46"S 56°09'04"W), canales paralelos al río Cuareim, col. W.S. Serra, G. Sanguinetti, R. Bentancor, J. Maciel, & Michel Reboledo, 8/X/2022; MHNM 6590, 2 ex., 30.6-33.4 mm SL, Paso del Tractor, A° de la Invernada (30°49'06"S 56°00'49"W), col. W.S. Serra, G. Sanguinetti & A. Duarte, 22-24/I/2024. Colonia: MHNM 5391, 1 ex., 38.3 mm SL, Paso Arballo (34°19'08"S 57°20'14"W), Arroyo Colla, col. Santiago Rodríguez, Alejandra Bauzada, Dana Bentancour & Dyamela Moreira, 29/V/2022. Florida: MHNM 6456, 1 ex., 41.6 mm SL, Arroyo Aguas Sucias (33°22'57"S 55°35'45"W), Sarandí del Yí, col. W.S. Serra, G. Sanguinetti & M. García, 19/VI/2022. Lavalleja: MHNM 4721, 2 ex., 31.5-35.7 mm SL, cañada San Germán (33°36'54"S 54°36'05"W), ramal del Aº Molles sobre Ruta 8, col. Wilson. S. Serra, Germán Sanguinetti & Matías García, 3/X/2021. Salto: MHNM 4512, 6 ex., 22.5-35.9 mm SL, Base de DINARA (31°04'18"S 57°51'32"W), Villa Constitución, col. W.S. Serra, 22-26/IX/2017.

*Characidium serrano*: ARGENTINA: Misiones: CI-FML 8105 (CIT-FML 104 and CIT-FML 112), 7 ex., 34.8-52.9 mm SL, A° Melo (27°25'02"S 54°42'07"W), col. G. Aguilera, J. M. Mirande, G. Terán, M. Benitez, D. Baldo, J. M. Ferro and F. Alonso ; CI-FML 8106 (CIT-FML 651), 24 ex., 32.3-50.5 mm SL, Balneario El Bonito (27°27'17"S 54°55'43"W), col. J.M. Mirande, G. Aguilera, A. Méndez-López, M. Benitez & G.E. Terán.

*Characidium tenue*: URUGUAY: Paysandú: MHNM 3562, 1 ex., 45.0 mm SL, Cañada de las Piedras (32°05’34”S 57°55’46”W), d. Río Queguay, col. W.S. Serra, M. Zarucki, M. Loureiro & D. Arrieta, 20/III/2014. Río Negro: MHNM 5081, 1 ex., 51.5 mm SL, Río Uruguay, Nuevo Berlín (32°59’21”S 58°03’54”W), campaña CARU “Programa de Conservación de la Fauna Íctica y los Recursos Pesqueros del Río Uruguay”, 19/III/2022. Rocha: MHNM 6147, 1 ex., 43.4 mm SL, canal en Ruta 19 al sur de Ruta 15 (33°31’39”S 53°54’19”W), col. W.S. Serra, G. Sanguinetti & G. Núñez, 25/VI/2023; MHNM 6664, 1 ex., 40.2 mm SL, canal en Ruta 19 al sur de Ruta 15 (33°31’39”S 53°54’19”W), col. W.S. Serra, G. Sanguinetti & G. Núñez, 25/VI/2023.

*Characidium* aff. *zebra*: URUGUAY: Artigas: MHNM 5785, 1 ex., 34.2 mm SL, Bella Unión, Puerto Pedregullo (30°11’23”S 57°36’52”W), campaña CARU “Programa de Conservación de la Fauna Íctica y los Recursos Pesqueros del Río Uruguay”, 22/IV/2017; MHNM 6495, 1 ex., 35.3 mm SL, Bella Unión, Puerto Pedregullo (30°11’23”S 57°36’52”W), campaña CARU “Programa de Conservación de la Fauna Íctica y los Recursos Pesqueros del Río Uruguay”, 18/X/2014.