Phylogenetic relationships and description of two new species of *Diapoma* (Characidae: Stevardiinae) from the La Plata River basin

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Herein we describe two new species of *Diapoma*, one from the Negro River, a tributary of the Uruguay River in Brazil and Uruguay, and one from the Iguaçu River, in Brazil and Argentina. The new species from the Negro River basin is distinguished from its congeners by the following combination of characters: a black narrow and conspicuous line restricted to the body horizontal septum, incomplete lateral line, tricuspid teeth in the inner series of the premaxilla, and a lower body depth at vertical through the dorsal-fin origin (29.3–32.8% SL in males and 27.7–33.3% SL in females). The new species from the Iguaçu River basin is distinguished from its congeners by the following combination of characters: a discontinuous lateral line, adipose fin hyaline, longer anal-fin base (26.5–32.4% SL), and a longitudinal black stripe along the median region of caudal-fin rays. Additionally, we updated the molecular phylogeny of the genus, including new sequences from these two new species and *Diapoma thauma*. An identification key for species of *Diapoma* is presented, modified from previous study.

Keywords: Diapomini, Gill gland, Identification key, Iguaçu River, Negro River.

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Neste trabalho descrevemos duas espécies novas de *Diapoma*, uma do rio Negro, afluente do rio Uruguai, Brasil e Uruguai, e a segunda do rio Iguaçu, Brasil e Argentina. A espécie nova do rio Negro é diagnosticada de suas congêneres pela combinação das seguintes características: uma linha preta, estreita e conspícua restrita ao septo horizontal do corpo, linha lateral incompleta, dentes da série interna da pré-maxila tricuspidados, e baixa altura do corpo na vertical que passa pela origem da nadadeira dorsal (29,3–32,8% SL em machos e 27,7–33,3% SL em fêmeas). A espécie nova do rio Iguaçu é diagnosticada de suas congêneres pela combinação das seguintes características: linha lateral descontínua, nadadeira adiposa não pigmentada de preto, base da nadadeira anal longa (26,5–32,4% SL) e raios médios da nadadeira caudal com uma linha preta longitudinal. Adicionalmente, atualizamos a filogenia molecular do gênero, incluindo novas sequências destas duas espécies novas e de *Diapoma thauma*. Uma chave de identificação para as espécies de *Diapoma* é apresentada, modificada de estudo anterior.

Palavras-chave: Chave de identificação, Diapomini, Glândula branquial, Rio Iguaçu, Rio Negro.

INTRODUCTION

Diapoma was proposed in a brief description of its type species, *Diapoma speculiferum* Cope, 1894. Among features described by Cope (1894a) were the metallic colored and posteriorly elongated opercle; the dorsal-fin origin located posterior to the pelvic-fin origin; the lateral line interrupted; the border of the anal fin concave, and the presence of a curved patch of scales extending on the inferior lobe of the caudal fin (illustrated in Cope, 1894b, plate V, 4, p.110). Eigenmann (1909 in an identification key, 1910) classified *Diapoma* on its monotypic subfamily Diapominae and later (Eigenmann, 1914) included the genus in the subfamily Glandulocaudinae with eight other genera characterized by their highlighted sexual dimorphism. Adult males of the species assigned to the Glandulocaudinae bear modified caudal-fin scales putatively associated with hypertrophied glandular tissue (Weitzman, Fink, 1985). However, the homology of this character has been questioned since the modified caudal organs could be formed by different scales and located on different parts of the caudal fin (Weitzman *et al.* in Weitzman, Fink, 1985; Weitzman *et al.*, 1988, 2005; Weitzman, Menezes, 1998; Menezes, Weitzman, 2009).

A subgroup of glandulocaudines, composed by *Acrobrycon* Eigenmann & Pearson, 1924, *Diapoma*, and *Planaltina* Böhlke, 1954, was included in the tribe Diapomini by several authors (*e.g.*, Weitzman *et al.*, 1988; Menezes, Weitzman, 1990; Burns *et al.*, 1995; Weitzman, Menezes, 1998; Menezes *et al.*, 2003). Two putative synapomorphies support this tribe: the presence of three or more series of modified scales on the caudal fin immediately ventral to the lateral-line series, which are not associated with hypertrophied soft tissues, and its presence in both males and females (not only in mature males as the condition observed in other glandulocaudines). Additional information on this group indicates that spermatic cells of species in Diapomini have a distinct nucleus

shape from other Glandulocaudinae (*i.e.*, *Planaltina* with sperm nuclei as nearly spherical, and *Diapoma* and *Acrobrycon* with moderately elongate sperm nuclei, contrasting to strongly elongated sperm nuclei in other glandulocaudines; Burns *et al.*, 1995).

Later, Malabarba, Weitzman (2003) proposed that all genera in Glandulocaudinae plus 19 genera previously referred to Tetragonopterinae and the new genus *Cyanocharax* Malabarba & Weitzman, 2003 constitute a monophyletic group in Characidae, named therein as Clade A. This clade was proposed based on two putative morphological synapomorphies: dorsal fin with two unbranched and eight branched rays and four teeth in the inner series of the premaxilla. No exclusive features were found to diagnose *Cyanocharax*, and the genus was proposed based on the combination of seven characters.

Morphological, molecular, and combined phylogenetic approaches corroborated the monophyly of the Clade A, which later received a subfamily rank name with the resurrection of Stevardiinae Gill, 1858 (*e.g.*, Mirande, 2009, 2010; Javonillo *et al.*, 2010; Oliveira *et al.*, 2011; Casciotta *et al.*, 2012; Thomaz *et al.*, 2015; Mirande, 2019; Betancur *et al.*, 2019; Vanegas-Ríos *et al.*, 2020; Ferreira *et al.*, 2021). Early molecular analyses pointed *Cyanocharax* as a paraphyletic group containing species of *Diapoma* (Javonillo *et al.*, 2010; Casciotta *et al.*, 2012). Later, species comprehensive evaluations of the phylogeny of Stevardiinae proposed *Cyanocharax* as a junior synonym of *Diapoma* (Thomaz *et al.*, 2015; Mirande, 2019; Ferreira *et al.*, 2021), including a new combination for *Diapoma guarani* (Mahnert & Géry, 1987) that was originally described in *Hyphessobrycon* Durbin, 1908. In these analyses, Diapomini was expanded to include *Diapoma* and several other genera within Stevardiinae (*e.g.*, *Attonitus* Vari & Ortega, 2000, *Bryconacidnus* Myers, 1929, *Bryconadenos* Weitzman Menezes, Evers & Burns, 2005, *Bryconamericus* Eigenmann, 1907, *Ceratobranchia* Eigenmann, 1914, *Diapoma*, *Knodus* Eigenmann, 1911; *Monotocheirodon* Eigenmann & Pearson, 1924; *Odontostoechus* Gomes, 1947, *Lepidocharax* Ferreira, Menezes & Quágio-Grassiotto, 2011, *Planaltina*, *Nantis* Mirande, Aguilera & Azpelicueta, 2006, *Piabarchus* Myers, 1928, *Piabina* Reinhardt, 1867, *Phallobrycon* Menezes, Ferreira & Netto-Ferreira, 2009, and *Rhinobrycon* Myers, 1944; in Thomaz *et al.*, 2015; Deprá *et al.*, 2018; Mirande, 2019; Ferreira *et al.*, 2021). *Diapoma* was no further found as closely related to *Acrobrycon* and *Planaltina*, the former being reallocated to Hemibryconini Géry, 1966 (Thomaz *et al.*, 2015) and the latter to Creagrutini (Mirande, 2019).

Diapoma has fourteen valid species (Fricke *et al.*, 2022) distributed in southeastern South America. Most species are endemic to a single basin, and few are widespread. Six species are known from the Uruguay River basin: *D. alegretense* (Malabarba & Weitzman, 2003), *D. guarani*, *D. lepiclastum* (Malabarba, Weitzman & Casciotta, 2003), *D. pyrrhopteryx* Menezes & Weitzman, 2011, *D. terofali* (Géry, 1964), and *D. uruguayense* (Messner, 1962); three occur in the lower Paraná River basin: *D. guarani*, *D. obi* (Casciotta, Almirón, Piálek & Rícan, 2012), and *D. nandi* Vanegas-Ríos, Azpelicueta & Malabarba, 2018; four in the Laguna dos Patos basin: *D. dicropotamicus* (Malabarba & Weitzman, 2003), *D. speculiferum*, *D. thauma* Menezes & Weitzman, 2011, and *D. tipiaia* (Malabarba & Weitzman, 2003); one in the coastal drainages of South Brazil: *D. itaimbe* (Malabarba & Weitzman, 2003); and one, *D. alburnum* (Hensel, 1870), is widely distributed in Uruguay and Laguna dos Patos River basins and coastal drainages of southern Brazil. Additionally, the species *Hyphessobrycon procerus* Mahnert & Géry, 1987 and *H. wajat* Almirón & Casciotta, 1999, from the Paraná River, were also suggested as

Stevardiinae (Malabarba, Weitzman, 2003; Carvalho, Langeani, 2013) and potentially belong to *Diapoma* (Vanegas-Ríos *et al.*, 2018; Mirande, 2019), but no changes were proposed in the classification of these species.

Here we describe two new species of *Diapoma*, one from the Negro River basin, a tributary of the lower Uruguay River basin, and another from the Iguaçú River basin, both tributaries of the La Plata River system. In this paper, we also reevaluate the phylogenetic relationships of *Diapoma* based on molecular data, including these two new species and the newly sequenced *D. thauma*.

MATERIAL AND METHODS

Taxonomic description. Measurements and counts were taken according to Fink, Weitzman (1974), with the modifications proposed by Malabarba, Kindel (1995). Measurements and counts were taken on the left side of specimens whenever possible, using a digital caliper with the precision of 0.1 mm and under a stereomicroscope. All measurements other than standard length (SL) are expressed as percentages of SL, and subunits of the head are recorded as percentages of head length (HL). Meristic characters are given in the description, followed by the number of specimens examined with each count in parentheses (differences in the number of specimens in a given count are due to specimens missing scales); an asterisk indicates holotype. Unbranched fin rays, pterygiophores, vertebrae, supraneurals, and procurrent caudal-fin rays counts were taken from cleared and stained (c&s) specimens, prepared according to the method of Taylor, Van Dyke (1985). Vertebrae counts include the four elements of the Weberian apparatus; the fused preural centrum one plus ural centrum (PU1+U1) was counted as a single element. Photographs of teeth and jaws were taken from c&s dissected specimens using a Nikon AZ100 stereomicroscope. The specimens studied are deposited in the following institutions: Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Ciudad Autónoma de Buenos Aires (MACN–ict); Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre (MCP); Muséum d'Histoire Naturelle, Genève (MHNG); Museo de La Plata, Buenos Aires (MLP); Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura, Universidade Estadual de Maringá, Maringá (NUP); Departamento de Zoologia, Universidade Federal do Rio Grande do Sul, Porto Alegre (UFRGS), and Faculdad de Ciencias de la Universidad de la Republica, Montevideo (ZVCP). An identification key for species of *Diapoma* is presented, modified from Vanegas-Ríos *et al.* (2018) to include the new species described herein. Specimens not used for counts and measurements were treated as non-types.

Molecular phylogenetic analysis. To investigate phylogenetic relationships among species of *Diapoma* using molecular data, DNA was extracted using the CTAB method (Doyle, Doyle, 1987) with a fragment of 2-5 mg from each specimen. Four molecular markers were amplified with primers already described in the literature; two mitochondrial genes: 16S (Palumbi, 1996: 16Sa–L and 16Sb–H) and COI (Melo *et al.*, 2011: L6252–Asn and H7271–COXI); and two nuclear: ptchd1 (Thomaz *et al.*, 2015: ptr_ca34F, ptr_ca607R; ptr_ca60F, and ptr_ca624R) and RAG2 (Oliveira *et al.*,

2011: 164F, RAG2-R6, 176R, and RAG2Ri). The PCR reactions have a total volume of 20μL with the follow concentrations: 1μL de extracted DNA, 13,8μL de H_2O, 2μL of 10x buffer (10mM Tris–HCl+15mM $MgCl_2$), 2μL of dNTPs (2mM), 0,6μL of $MgCl_2$ (50mM), 0,2μL of each primer (10mM), 0,2μL of Taq DNA polimerase (5U). We used the same PCR condition of each primer from the original description. PCR products were checked by electrophoresis in agarose gel.

PCR products were purified using EXOSAP (Exonuclease I and Shrimp Alkaline Phosphatase GE Healthcare, Piscataway, USA) before sending to sequencing. The PCR products were sent to Macrogen, Inc (Seoul, South Korea) or ACTGene (Porto Alegre, Brazil). Sequences were obtained with chromatograms (.ab format). The contiguous sequences of the gene segments were created by assembling DNA strands (forward and reverse) using default settings of program Geneious 7.1.3.0. A total of nine new sequences were submitted to GenBank and can be located under the accession numbers MZ558466–MZ558474 (Tab. **S1**).

Diapoma nandi, *Hyphessobrycon procerus*, and *H. wajat* were not included in the molecular phylogeny because of the lack of ethanol fixed specimens for DNA extraction. For phylogenetic estimation, newly sequenced data were concatenated with sequences from GenBank database: *Diapoma obi* from Casciotta *et al.* (2012); species of Stevardiinae representing outgroup genera plus *Diapoma* species from Thomaz *et al.* (2015) (see below about treatment of sequences from GenBank). Previous analyses showed that the genera *Piabina*, *Piabarchus*, and *Bryconamericus* are closely related to *Diapoma*, therefore we used species belonging to these genera as outgroups to *Diapoma* (Thomaz *et al.*, 2015; Mirande, 2019; Ferreira *et al.*, 2021). All sequences used are also listed with the accession number of GenBank in the Tab. **S1**.

We used the program Geneious to align sequences using MUSCLE (Edgar, 2004) with default settings and build a concatenated dataset for individual specimens (three mitochondrial: 12S, 16S, COI; four nuclear: MYH6, ptchd1, RAG1, RAG2). Phylogenetic analysis using the concatenated dataset was conducted using Bayesian Inference. To evaluate the best nucleotide substitution models and partition configuration, we use PartitionFinder 2.1.1 (Lanfear *et al.*, 2012) with data blocks defined a priori according to the commonly used structural and functional criteria of the genes, that resulted into 16 different blocks (same criteria as Thomaz *et al.*, 2015). To penalize model parameters, we use BIC under the greedy search algorithm. Bayesian Inference analysis was performed using MrBayes 3.2.6 (Ronquist *et al.*, 2012) in CIPRES Portal (Miller *et al.*, 2010) with 50 million MCMC iterations, printing one tree per 5,000 generations. Posterior and parameter stabilization were examined on Tracer 1.7 (Rambaut *et al.*, 2018), considering values above 200 ESS. We summarized trees using TreeAnnotator 1.8.4 (Rambaut, Drummond, 2013), with 10% of burn-in and the maximum clade credibility criteria. We also evaluate individual gene trees using the same substitution models and methods as for the concatenated analysis. Phylogenetic trees were edited with FigTree 1.4.2 (Rambaut, 2014).

Species Trees analysis were conducted in BEAST 2.6.2 (Bouckaert *et al.*, 2014) using the StarBEAST template. We group individual specimens' sequences into species according to morphological diagnostic traits. All partitions were treated with linked clock models, and we selected a StrictClockRate with a mean (M) of 0.001 and a standard deviation (S) of 0.1, following the tutorial (www.cs.rice.edu/~ogilvie/oeb125/2019/03/27/clock-

priors.html). Multi-species coalescence prior was set to linear with constant root; tree prior set to Yule model with uniform distribution (1/X). Markov chain Monte Carlo (MCMC) ran for 40 million generations with sampling every 50.000 generations. We checked stabilization (ESS > 200) in Tracer for posterior and prior parameters values. Species Tree was summarized in TreeAnnotator using the maximum clade credibility tree function and a burn-in of 10%.

Treatment of sequences from GenBank. Since we gathered sequences from Genbank for our analysis, we followed a list of sequences removed by Mirande (2019: appendix S8), which used a similarity criterion with BLAST search to remove putative contaminated sequences. Sequences removed following Mirande (2019) are: 16S, *Bryconamericus exodon* [KF209736, UFRGS 13571, TEC 1693]; COI, *B. exodon* [KF210060, UFRGS 13571, TEC 1693], *Diapoma lepiclastum* [KF210133, UFRGS 15020, TEC1801A]; RAG2, *B. iheringii* [KF211036, UFRGS 12473, TEC1281], *D. terofali* [KF211124, UFRGS 12891, TEC392]).

After this first treatment of sequences, we analyzed each gene tree, using all sequences of Stevardiinae from Thomaz *et al.* (2015) available in GenBank and a Bayesian inference analysis (see above), and found additional sequences presenting a highly incongruent phylogenetic relationships. Therefore, we removed ten sequences for our phylogenetic analyses for both the concatenated and the Species Tree framework. Sequences removed were: 12S, *Piabina argentea* [KF209674, UFRGS 12888, TEC1021A]; COI, *Diapoma guarani* [KF210246, UFRGS 12647, TEC1379A]; ptch1, *Bryconamericus exodon* [KF210589, UFRGS 13571, TEC 1693], *B. patriciae* [KF210602, UFRGS 12894, TEC716]; RAG2, *D. itaimbe* [KF211114, UFRGS 12651, TEC1303; KF211115, UFRGS 12717, TEC1383], *D. uruguayense* [KF211119, UFRGS 10962, TEC393; KF211120, UFRGS 11644, TEC457], *B. lethostigmus* [KF211233, MCP 23595; KF211234, UFRGS 12537, TEC1239]. To show the discrepancy that was observed here, we attached each gene tree with all sequences of Stevardiinae tree in the Fig. **S2**.

RESULTS

Diapoma pampeana, new species

urn:lsid:zoobank.org:act:4B4370D0-0662-4B0B-9D40-6AA1AC0DE842

(Figs. 1–4; Tab. 1)

Diapoma sp. n. —Bertaco *et al.*, 2016:413 (listed, UFRGS 12642, Uruguay River drainage).

Holotype. UFRGS 28705, male, 29.6 mm SL, Brazil, Rio Grande do Sul, Bagé, road between Aceguá and Bagé, BR-153, Cinco Saltos creek, affluent of Negro River, 31°36'53"S 54°08'42"W, 29 Mar 2006, L. R. Malabarba & students.

Paratypes. All from the Negro River basin. **Brazil, Rio Grande do Sul State:** MCP

16400, 17, 24.5–32.1 mm SL (2 males, 26.4–26.5 mm SL, 13 females, 24.5–32.1 mm SL, 1 male c&s, 30.0 mm SL, 1 female c&s, 28.9 mm SL), Bagé, Negro River, road *ca*. 14 km from Bagé, 31°28'37"S 54°08'20"W, 9 Dec 1992, P. H. Wimberger, R. E. Reis & J. F. Pezzi. UFRGS 8429, 28, 17.0–28.1 mm SL (1 male, 25.7 mm SL, 27 juveniles/females 17.0–28.1 mm SL), Bagé, road between Aceguá and Bagé, Negro River, 31°28'37"S 54°08'20"W, 29 Mar 2006, L. R. Malabarba & students. UFRGS 8464, 93, 17.6–33.6 mm SL (2 males, 27.6–28.8 mm SL, 89 juveniles/females 17.6–33.6 mm SL), 2 c&s, 30.8–31.9 mm SL, same locality and collector as holotype. UFRGS 12642, 16, 26.9–31.8 mm SL, TEC 1377, Bagé, BR-153 between Bagé and Aceguá, Negro River, 31°28'37"S 54°08'19"W, 3 Mar 2005, M. A. Azevedo, J. Ferrer, L. R. Malabarba & C. Oliveira. UFRGS 27318, 6, 24.3–29.7 mm SL, Aceguá, stream affluent of Negro River, 31°30'42.12"S 54°05'46.85"W, 12 Apr 2019, M. Camana, P. M. Ito, M. Souza & F. Collar. UFRGS 27336, 1, 27.8 SL, Aceguá, stream affluent of Negro River at Linha do Silêncio, 31°39'24.41"S 54°19'03.35"W, 15 Apr 2019, M. Camana, P. M. Ito, M. Souza & F. Collar. **Uruguay.** UFRGS 8119, 7, 24.7–33.5 mm SL (1 male 33.5 mm SL, 6 females 24.7–33.0 mm SL, Melo, Departamento de Cerro Largo, small stream at Route 26, *ca*. 59 km from Melo, between Sauce Creek and Fraile Muerto Creek, 32°17'39"S 54°44'59"W, 28 May 2005, L. R. Malabarba, V. A. Bertaco, P. Lehmann & F. Cantera. UFRGS 8120, 9, females 23.0–29.0 mm SL, Departamento de Tacuarembó, Tacuarembó River, at Route 26, Villa Ansina, 31°58'33"S 55°28'13"W, 28 May 2005, L. R. Malabarba, V. A. Bertaco, P. Lehmann & F. Cantera. UFRGS 8121, 1, female 24.3 mm SL, Departamento de Rivera, Negro River, Mazangano Bridge at Route 44, 32°06'33"S 54°40'08.6"W, 27 May 2005, L. R. Malabarba, V. A. Bertaco, P. Lehmann & F. Cantera. UFRGS 8122, 123, 17.7–32.0 mm SL (13 males 22.9–32.0 mm SL, 110 juveniles/females 17.7–31.1 mm SL), 2 c&s, 30.4–31.5 mm SL, Departamento de Rivera, lateral puddles and Corrales Creek, affluent of Tacuarembó River, Route 27, 31°23'26"S 55°15'14"W, 27 May 2005, L. R. Malabarba, V. A. Bertaco, P. Lehmann & F. Cantera. UFRGS 8123, 245, 17.3–29.7 mm SL (3 males 27.7–29.3 mm SL, 242 juveniles/females 17.3–29.7 mm SL), Departamento de Tacuarembó, Caraguatá creek, tributary to Tacuarembó River, Route 26, Las Toscas, 32°09'29"S 55°01'27"W, 28 May 2005, L. R. Malabarba, V. A. Bertaco, P. Lehmann & F. Cantera. ZVCP 15417, 21, 19.7–32.2 mm SL (6 males 25.0–28.9 mm SL, 15 juveniles/females 19.7–32.2 mm SL), Departamento de Rivera, lateral puddles and Corrales Creek, affluent of Tacuarembó River, Route 27, 31°23'26"S 55°15'14"W, 27 May 2005, L. R. Malabarba, V. A. Bertaco, P. Lehmann & F. Cantera. ZVCP 15418, 15, 24.3–27.9 mm SL (3 males 26.1–26.5 mm SL, 12 juveniles/females 24.3–27.9 mm SL), Departamento de Tacuarembó, Caraguatá creek, tributary to Tacuarembó River, Route 26, Las Toscas, 32°09'29"S 55°01'27"W, 28 May 2005, L. R. Malabarba, V. A. Bertaco, P. Lehmann & F. Cantera.

Diagnosis. *Diapoma pampeana* can be distinguished from its congeners by the presence of a narrow and conspicuous black line along horizontal septum, never forming a wide lateral stripe (*vs*. lateral dark stripe thick at horizontal septum, usually covering one row of scales at vertical through anal-fin origin); by having a longitudinal black stripe extending posteriorly on middle caudal-fin rays (*vs*. lack of a black stripe on middle caudal-fin rays in *D. alegretense* and *D. uruguayense*); by the presence of a small black blotch, restricted on the base of the middle caudal-fin rays (*vs*. presence of a large black

FIGURE 1 | Holotype of *Diapoma pampeana*, male, UFRGS 28705, 29.6 mm SL, Brazil, Rio Grande do Sul, Bagé, Sanga Cinco Saltos, affluent of Negro River, BR-153, between Aceguá and Bagé.

blotch extending from the caudal peduncle to the base of most branched caudal-fin rays in *D. guarani*). *Diapoma pampeana* can be differentiated from *D. alegretense*, *D. guarani*, *D. lepiclastum*, *D. obi*, *D. tipiaia*, and *D. uruguayense* by having distal border of the anal fin concave in males (*vs.* convex in males of *D. alegretense*, *D. tipiaia*, and *D. uruguayense*; and nearly straight in *D. guarani*, *D. lepiclastum*, and *D. obi*). It can be distinguished from *D. alegretense*, *D. lepiclastum* and *D. uruguayense* by the number of scales forming sheath along anal-fin base (5–12 *vs.* 12–18 in *D. alegretense*, 13–20 in *D. lepiclastum*, and 20–28 in *D. uruguayense*); and from *D. uruguayense* by the number of branched anal-fin rays (21–25 *vs.* and 29–35). *Diapoma pampeana* can be distinguished from *D. nandi*, *D. obi* and *D. potamohadros* by the smaller number of vertebrae (34–35 *vs.* 36–37 in *D. nandi*, 36 in *D. potamohadros*, and 37 in *D. obi*); and can be differentiated from *D. nandi* and *D. obi* by the lower body depth at dorsal-fin origin (27.7–33.3% SL *vs.* 32.4–38.8% SL in *D. nandi* and 34.5–40.8% SL in *D. obi*). *Diapoma pampeana* is distinguished from *D. alburnum*, *D. dicropotamicus* and *D. itaimbe* by the presence of an incomplete lateral line (*vs.* usually complete in *D. alburnum*, *D. dicropotamicus* and *D. itaimbe*). *Diapoma pampeana* can be distinguished from *D. pyrrhopteryx*, *D. speculiferum*, *D. terofali*, and *D. thauma* by the absence of modified scales in the caudal fin (*vs.* presence), and additionally, from *D. pyrrhopteryx* and *D. speculiferum* by the lack of posterior elongation of the opercle and subopercle bones (*vs.* presence).

Description. Morphometric data are given in Tab. 1. Largest specimen 33.6 mm SL. Body laterally compressed, maximum depth at vertical through dorsal-fin origin or at posterior tip of pelvic-fin rays when adnate to body. Dorsal head profile slightly convex or straight, dorsal body profile slightly concave at supraoccipital and straight to slightly convex from tip of supraoccipital spine to dorsal-fin origin, posteroventrally straight from terminus of dorsal fin to adipose-fin origin. Dorsal profile of caudal peduncle somewhat straight to slightly concave. Ventral body profile convex from tip of lower jaw to pelvic-fin origin, straight between pelvic- and anal-fin origins, and posterodorsally slightly straight from this point to caudal peduncle. Ventral profile of caudal peduncle nearly straight. Head with anterior region rounded. Anterior and posterior nostrils rounded, separated by skin fold; posterior nostril opening larger, twice size of anterior nostril.

Mouth terminal, anterior tip of premaxilla slit at the horizontal through upper half of eye. Premaxilla with two rows of teeth (Fig. 2). Outer row with two (2), three (15), or four* (18) teeth; usually conical (27) but sometimes tricuspid (8). Inner row with four* (34) or rarely five (1) tricuspid teeth. Maxilla toothless (1) or with one (10), two (11), three (11), or four* (2) conical teeth (Fig. 2). Posterior tip of maxilla reaching vertical through anterior margin of eye, but not surpassing. Dentary with six (1), seven (2), eight (8), nine (15), 10 (7), 11 (1), or 14* (1) teeth; four anterior-most teeth large and tricuspid, followed by conical teeth (Fig. 2). Third anterior tooth with same size, not aligned and more ventrally located than other teeth of dentary. First gill arch with four (1), six (7), seven* (18), eight (6), or nine (3) gill rakers on epibranchial, 13* (13), 14 (14), or 15 (8) on ceratobranchial.

Dorsal-fin rays ii (35), seven (2), eight* (32), or nine (1). Nine pterygiophores in dorsal fin (4 c&s). Dorsal-fin origin at vertical through slightly anterior to anal-fin origin, often reaching vertical through tip of pelvic-fin rays. Adipose-fin origin at vertical through origin of posteriormost two to three anal-fin rays. Anal-fin rays iv* (10), v (22), or vi (3), 21 (3), 22* (12), 23 (11), 24 (7), or 25 (2). Twenty-two (1), 23 (2), or 24 (1) pterygiophores in anal fin (4 c&s). Anal-fin origin at posterior half of body, posterior to vertical through dorsal-fin origin. Pectoral-fin rays i (35), seven (4), eight* (27), or nine (4). Pectoral-fin inserted immediately after the opercle, posterior tip surpassing pelvic-fin origin, usually reaching the half of elongated scale covering pelvic-fin origin. Pelvic-fin rays i, six* (35). Caudal fin forked with 10/9 principal rays.

FIGURE 2 | Left premaxilla, maxilla and dentary of *Diapoma pampeana*, UFRGS 8464, 31.9 mm SL, paratype.

Scales cycloid, almost all with the same size and form. Lateral line incomplete, with five (2), six (5), seven* (11), eight (14), or nine (3) anterior pored scales and a total of 32 (1), 33 (2), 34 (8), 35* (9), 36 (12), or 37 (3). Terminal lateral–line tube absent on caudal–fin interradial membrane. Predorsal scales 10 (1), 11 (3), 12* (17), 13 (12), or 14 (2), forming with irregular row of scales. Five* (14) or six (21) longitudinal scale rows between dorsal–fin origin and lateral line. Four (18) or five* (17) longitudinal scale rows between lateral line and pelvic–fin origin. Circumpeduncular scales 11 (1), 12 (10), 13* (12), 14 (11), or 15 (1). One row of scales forming sheath along anal–fin base (eight individuals less than five scales probably lost their scales) five* (3), six (2), seven (3), eight (1), nine (10), 10 (3), 11 (3), or 12 (2) scales. Caudal–fin lower lobe covered by a set of four or five large unmodified scales, not extending beyond anterior one–third of caudal–fin rays. Total number of vertebrae 34 (1), or 35 (3) (4 c&s); 16 (4) precaudal and 18 (1), or 19 (3) caudal.

Coloration in alcohol. Ground color pale yellowish in preserved specimens. Dorsal and dorsolateral portions of body with small black dark chromatophores. Abdominal region lacking pigment. Scattered black dark chromatophores above anal fin. Chromatophores

TABLE 1 | Morphometric data for holotype and 34 paratypes of *Diapoma pampeana*.

	Holotype	Males (N = 12)			Females and juveniles (N = 22)		
		Range	Mean	SD	Range	Mean	SD
Standard length (mm)	29.6	25.1–33.3	28.8		25.9–33.6	29.6	–
Percentages of standard length							
Head length	25.2	21.4–25.2	23.0	1.0	21.3–24.7	23.1	0.1
Depth at dorsal-fin origin	31.8	29.7–33.7	31.6	1.2	27.7–32.6	30.6	1.2
Snout to dorsal-fin origin	51.5	48.9–54.5	52.0	1.8	50.8–56.2	53.2	1.6
Snout to pectoral-fin origin	25.8	23.2–26.1	25.0	0.9	23.7–27.5	25.2	0.9
Snout to pelvic-fin origin	45.8	41.9–46.4	44.6	1.4	42.5–47.0	45.1	1.1
Snout to anal-fin origin	59.2	53.9–59.0	57.4	1.5	53.8–60.6	58.7	1.4
Distance between dorsal- and adipose-fin origins	37.7	34.9–39.9	37.0	1.3	33.0–39.5	37.2	1.5
Dorsal- fin origin to caudal-fin base	49.9	46.2–54.8	49.5	2.4	45.9–51.6	48.8	1.8
Dorsal-fin length	25.2	23.2–26.7	25.1	1.0	22.7–26.3	24.2	1.1
Dorsal-fin base length	14.0	10.1–13.7	11.9	1.0	10.4–12.7	11.5	0.8
Pectoral-fin length	24.8	21.9–25.8	23.7	1.0	23.7–27.5	25.2	0.9
Pelvic-fin length	14.4	12.0–15.6	14.1	1.0	12.2–15.7	13.7	0.9
Anal-fin base-length	35.8	32.5–36.1	34.8	1.1	32.1–36.6	34.2	1.4
Caudal peduncle depth	9.2	8.2–10.0	9.1	0.6	7.8–9.7	8.9	0.5
Caudal peduncle length	11.5	9.6–12.4	11.3	0.8	8.9–13.4	11.2	1.1
Percentages of head length							
Snout length	17.3	16.3–21.7	19.5	1.6	16.9–22.1	19.1	1.6
Horizontal eye length	43.0	39.5–45.0	43.3	1.8	40.3–46.8	43.6	1.6
Postorbital head length	40.8	36.2–41.6	38.7	1.6	35.6–43.1	39.4	2.1
Least interorbital width	29.8	29.3–34.5	31.9	1.6	28.6–38.7	32.5	2.0
Upper jaw length	34.5	34.6–39.1	36.6	1.5	32.4–38.6	35.3	1.9
Dentary length	36.5	36.7–42.2	39.2	1.8	36.5–41.1	38.8	1.5

delineating scale margins above horizontal septum and forming a chevron pattern below and above thin midlateral line. Head darkly pigmented dorsally. Black chromatophores around border of orbit. Opercle with black scattered chromatophores, mostly on its dorsal portion. Infraorbitals pale yellowish (somewhat silvery in some specimens), with scattered black chromatophores. Humeral blotch vertically elongate, covering at least four series of scales, with diffuse black chromatophores in some specimens. Black midlateral line, more diffuse at the humeral area forming a conspicuous narrow line from this region to caudal-fin base. Small caudal peduncle blotch with scattered brown chromatophores, concentrated from middle region of caudal peduncle to interradialis muscles, and faintly through interradial membranes of middle caudal-fin rays. Dorsal fin with chromatophores concentrated on interradial membranes proximally, and hyaline on distal tips. Adipose fin with few scattered black chromatophores. Anal fin dusky, with black chromatophores more concentrated on interradial membranes than on rays; distal border of fin darkly pigmented. Pectoral and pelvic fins mostly hyaline with black chromatophores on interradial membranes and rays. Coloration just after fixation in formalin (Fig. 3) with similar pattern described above, but with dorsal, anal and caudal fins reddish-orange.

Sexual dimorphism. Males have tiny hooks on the distal portion of the anterior three or four branched rays of the anal fin (more clearly visualized in c&s specimens). We observed gill glands on the ten anteriormost filaments of the first branchial arch in sexually dimorphic males, and absence of gill glands on juveniles and females (up to 25 mm SL). No conspicuous morphometric differences between males and females (Tab. 1).


FIGURE 3 | *Diapoma pampeana*, paratypes, UFRGS 8123, **A.** Male, 27.2 mm SL, **B.** Female, 26.0 mm SL, Caraguatá creek, tributary of the Tacuarembó River, Ruta 26, Las Toscas, Tacuarembó, Uruguay. Photograph taken just after fixation in formalin.

Geographical distribution. *Diapoma pampeana* is apparently endemic to the Negro River basin, a tributary to the Uruguay River, in Brazil and Uruguay countries (Fig. 4).

Ecological notes. *Diapoma pampeana* occurs in sympatry with the congeners *D. terofali*, *D. uruguayense*, and *D. alburnum*.

Etymology. The name of the new species is an allusion to the Pampa, a peculiar biome distributed along lowlands of Rio Grande do Sul State, Brazil, Uruguay, and Argentina countries. The new species is apparently endemic to this biome.

Conservation status. There are no clear specific threats detected for this species in the Negro River basin. Based on collecting sites of *Diapoma pampeana*, we estimate the extent of occurrence (EOO) to be 6,509.329 km² and for area of occupancy (AOO) applying a 2 km² area for each locality, to be *ca.* 32.000 km², which are beyond the minimum limits defined for International Union for Conservation of Nature (IUCN) for threatened categories under the criteria B (B1: EOO < 5,000 km²; B2: AOO < 500 km²). Therefore, this species can be classified as Least Concern (LC) according to IUCN categories and criteria (IUCN Standards and Petitions Subcommittee, 2019).
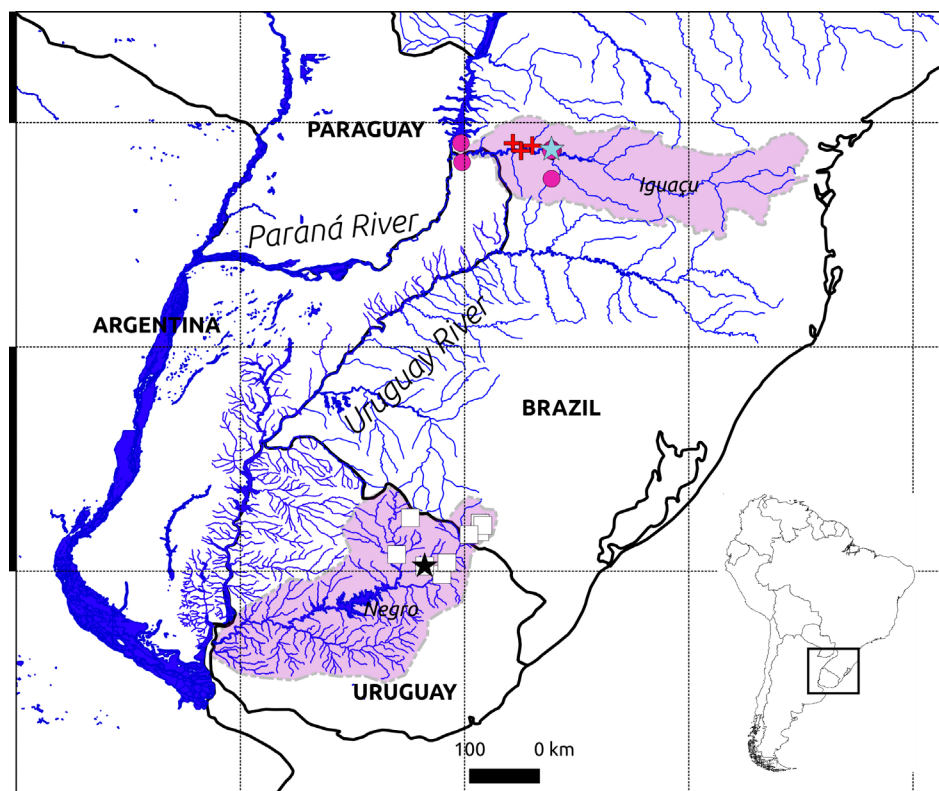
FIGURE 4 | Map of part of southern Brazil, Paraguay, Uruguay, and east Argentina showing the geographic distributions of *Diapoma pampeana* (white squares) and *D. potamohadros* (paratypes with pink circles, and non-types with plus sign in red). Stars indicate type localities.

Diapoma potamohadros, new species

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(Figs. 5–7; Tab. 2)

Bryconamericus sp. 2. —Russo *et al.*, 2004:176–179 (diet analysis; tab. 2 fig. 2B, photo from NUP 719; fig. 4B1, head in lateral view; fig. 4B2, premaxillary teeth arrangement in ventral view; Salto Caxias Reservoir, Capitão Leônidas Marques, Paraná, Brazil).

Bryconamericus sp. C. —Baumgartner *et al.*, 2006:2 (checklist, Reservatório Salto Osório, Quedas do Iguaçu, Paraná, Brazil; referred as the same species listed by Russo *et al.*, 2004).

Cyanocharax aff. *alburnus*. —Baumgartner *et al.*, 2012:96 (description, table with measurements and counts, photo of vouchers NUP 2461 and NUP 4123, Reservatório Caxias, Capitão Leônidas Marques, Quedas do Iguaçu, Paraná, Brazil). —Delariva *et al.*, 2013:894–899 (diet; tabs. 1–5, NUP 6620 and NUP 7248, Reservatório Caxias, Capitão Leônidas Marques, Quedas do Iguaçu, Paraná, Brazil).

Cyanocharax sp. Iguaçu. —Thomaz *et al.*, 2015:16 (phylogenetic relationships; voucher UFRGS 27622).

Diapoma aff. *alburnus*. —Deprá *et al.*, 2018:20 (listed in comparative material; voucher NUP 11174).

Diapoma sp. —Mezzaroba *et al.*, 2021:6 (listed for Iguaçu River, NUP 6620, same voucher used in Delariva *et al.*, 2013).

Holotype. UFRGS 28700, male, 48.6 mm SL, Iguaçu River, Salto Osório Reservoir, Quedas do Iguaçú municipality, Paraná State, Brazil, 25°30'49"S 53°00'04"W, 17 Sep 2005, GERPEL (Grupo de Pesquisas em Recursos Pesqueiros e Limnologia).

Paratypes. All from the Iguaçu River basin. **Argentina, Misiones Province:** MACN-ict 10364, 7, 31.3–41.7 mm SL (2 males, 37.5–37.9 mm, 5 females, 31.3–41.7 mm SL), Iguazu Falls National Park, above Iguazu falls, Ñandú Chico stream, near 25°43'16.9"S 54°25'37.3"W, 16 Oct 1975. MLP 11343, 1 female, 43.5 mm SL, Deseado stream, 25°40'15.3"S 53°56'1.8"W, 29 Apr 2010, J. Casciotta & A. Almirón. **Brazil, Paraná State:** MCP 41353, 13, 44.1–56.4 mm SL (6 males, 46.9–51.4 mm SL, 7 females, 44.1–56.4 mm SL), 2 c&s, 1 male, 51.4 mm SL, 1 female, 50.7 mm SL, collected with holotype. MCP 41541, 53, 39.5–52.0 mm SL (30 males, 39.5–50.7 mm SL, 23 females, 42.2–52.0 mm SL), Salto Osório Reservoir, Quedas do Iguaçú municipality, 25°30'49"S 53°00'04"W, Mar 2007, GERPEL. MCP 41542, 56, 37.2–53.0 mm SL (24 males, 39.5–49.0 mm SL, 32 females, 37.2–53.0 mm SL), Salto Osório Reservoir, Quedas do Iguaçú municipality, 25°30'49"S 53°00'04"W, Nov 2005, GERPEL. NUP 22687, 9, 40.8–53.4 mm SL, Quedas do Iguaçu municipality, Salto Osório Reservoir, 25°32'05"S 52°59'09"W, 1 Nov 2006, GERPEL. UFRGS 27622, 27, 37.5–53.2 mm SL, TEC 1827 (23 males, 37.5–53.0 mm SL, 4 females, 43.6–53.2 SL), Salto Osório Reservoir, Quedas do Iguaçu municipality, 25°32'05"S 53°00'33"W, 21 Nov 2010, C. S. Pavanelli.

Non-types. All from Brazil, Paraná State, Iguaçu River basin. NUP 7245, 259, 14.0–60.0 mm SL, Três Barras do Paraná municipality, Adelaide River, 25°27'18"S 53°18'26"W, 1 Aug 2009, Nupelia. Capitão Leônidas Marques municipality, Salto Caxias Reservoir: NUP 719, 52, 44.4–56.5 mm SL, 25°32'12"S 53°29'11"W, 13 Aug 1997, Nupelia. NUP 2461, 76, 28.3–71.2 mm SL, 25°32'12"S 53°29'11"W, 18 Jan 2000, Nupelia. NUP 6620, 11, 52.0–61.0 mm SL, 25°32'12"S 53°29'11"W, 1 Aug 2005, Nupelia. NUP 7247, 24, 54.0–60.0 mm SL, 25°32'12"S 53°29'11"W, 1 Jul 2000, Nupelia. NUP 7248, 16, 55.0–62.0 mm SL, 25°32'12"S 53°29'11"W, 1 Sep 2000, Nupelia. Quedas do Iguaçu municipality, Salto Osório Reservoir: NUP 4123, 75, 20.7–41.0 mm SL, 25°30'49"S 53°00'04"W, 17 Sep 2005, Gerpel. NUP 4129, 1, 27.0 mm SL, 25°30'49"S 53°00'04"W, 17 Sep 2005, GERPEL. NUP 4335, 23, 26.5–33.2 mm SL, 23°30'48"S 53°00'04"W, 1 Feb 2005, GERPEL. NUP 4336, 6, 42.2–53.9 mm SL, 23°30'48"S 53°00'04"W, 1 Dec 2004, GERPEL. NUP 6229, 57, 24.5–56.4 mm SL, 25°30'49"S 53°00'04"W, 27 Jan 2008, GERPEL. NUP 11161, 8, 53.1–53.9 mm SL, 25°32'05"S 52°59'09"W, 1 Nov 2006, GERPEL. NUP 11162, 7, 50.3–55.1 mm SL, 25°32'05"S 52°59'09"W, 1 Jul 2007, GERPEL. NUP 11163, 7, 46.6–55.8 mm SL, 25°32'05"S 52°59'09"W, 15 Jan 2007, GERPEL. NUP 11164, 13, 46.2–57.1 mm SL, 25°32'05"S 52°59'09"W, 1 May 2007, GERPEL. NUP 11169, 1, 51.1 mm SL, 25°32'05"S 52°59'09"W, 1 Jul 2008, GERPEL. NUP 11174, 44, 48.6–57.0 mm SL, 25°32'05"S 52°59'09"W, 1 Jul 2008, GERPEL.

Diagnosis. *Diapoma potamohadros* can be distinguished from *D. alburnum*, *D. dicropotamicus*, and *D. itaimbe* by having discontinuous perforation of lateral line scales (*vs.* complete lateral line); and can be also distinguished from these species, except from *D. alburnum*, by having an unpigmented adipose fin (*vs.* darkly pigmented adipose fin in *D. dicropotamicus* and *D. itaimbe*). *Diapoma potamohadros* can be distinguished from *D. pyrrhopteryx*, *D. speculiferum*, *D. terofali*, and *D. thauma* by the absence of modified scales in the caudal fin (*vs.* presence); and can be distinguished from *D. pyrrhopteryx* and *D. speculiferum* by the lack of a posterior elongation of opercular bones (*vs.* opercle and subopercle posteriorly extended). *Diapoma potamohadros* can be distinguished by the distal border of the anal fin concave in males (*vs.* convex in males of *D. alegretense*, *D. tipiaia*, and *D. uruguayense*, and nearly straight in males of *D. guarani*, *D. lepiclastum*, and *D. obi*); by the lower number of scales forming the anal-fin sheath (5–12 *vs.* 12–18 in *D.*

FIGURE 5 | Holotype of *Diapoma potamohadros*, UFRGS 28700, male, 48.6 mm SL, Brazil, Paraná, Quedas do Iguaçu, Salto Osório Reservoir.

alegretense, 13–20 in *D. lepiclastum*, and 20–28 in *D. uruguayense*); and by the number of branched anal-fin rays (20–26 *vs.* 29–35 in *D. uruguayense*). *Diapoma potamohadros* can be distinguished from *D. nandi* and *D. obi* by a lower body depth (27.6–31.9% SL *vs.* 32.4–38.8% SL in *D. nandi*; 35.5–44.1% SL in *D. obi*). The new species is distinguished from *D. tipiaia* and *D. pampeana* by the presence of five or more cusps on the teeth in the inner series of premaxilla (*vs.* tricuspid teeth). *Diapoma potamohadros* can be further distinguished from *D. alegretense* and *D. uruguayense* by having a longitudinal black stripe extending posteriorly on the middle caudal-fin rays (*vs.* black stripe on middle caudal-fin rays absent); and from *D. guarani* by absence of a conspicuous black blotch on the caudal-fin base (*vs.* presence).

Description. Morphometric data are given in Tab. 2. Largest specimen 56.4 mm SL. Body laterally compressed, maximum depth at vertical through dorsal-fin origin or at posterior tip of pelvic-fin rays when adnate to body. Dorsal head profile slightly convex or straight, dorsal body profile slightly convex from tip of supraoccipital spine to dorsal-fin origin, straight from this point to adipose-fin origin. Dorsal profile of caudal peduncle somewhat straight to slightly concave. Ventral body profile convex from tip of lower jaw to pelvic-fin origin, straight between pelvic and anal-fin origins, straight or slightly convex from this point to caudal peduncle. Ventral profile of caudal peduncle straight to slightly concave. Head with anterior region rounded. Anterior and posterior nostrils rounded, separated by skin fold from anterior nostril; posterior nostril opening larger, with double size than anterior nostril.

Mouth terminal or slightly superior, anterior tip of premaxilla slit at horizontal through 1/3 depth of eye. Premaxilla with two rows of teeth (Fig. 6). Outer row with two (1), three (9), four* (18), or five (7) tricuspid teeth. Inner row with four (16) or five* (19) teeth; usually penta- to hexacuspid (rarely heptacuspid), with centralmost cuspid slightly larger than others. Maxilla with two (7), three* (10), four (14), five (3), or six (1) teeth (Fig. 6); usually tricuspid (30) and rarely penta- or hexacuspid (4). Posterior tip of maxilla reaching vertical through anterior margin of eye. Dentary with six (3), seven* (9), eight (8), nine (5), 10 (6), 11 (1), or 12 (3) teeth; four anterior-most teeth large and pentacuspid, followed by conical, bi- or tricuspid teeth (Fig. 6). Second anteriormost tooth slightly displaced ventrally in comparison with contiguous, similar sized teeth of dentary. First gill arch with six (11), seven* (14), or eight (9) gill rakers on epibranchial, 10 (1), 11 (9), 12* (16), or 13 (8) on ceratobranchial.

Dorsal-fin rays ii, eight* (35). Nine pterygiophores in dorsal fin (2 c&s). Dorsal-fin origin at vertical slightly anterior to anal-fin origin, often reaching posterior tip of pelvic-fin rays. Adipose-fin origin at vertical crossing posteriormost two to three anal-fin rays. Anal-fin rays iii (9), iv* (19), or v (7), 20 (1), 21 (1) 22 (8), 23 (11), 24* (10), 25 (3), or 26 (1). Twenty-seven pterygiophores in anal fin (2 c&s). Anal-fin origin at posterior half of body, usually posterior to vertical through dorsal-fin origin. Pectoral-fin rays i (35), eight (1), nine* (13), 10 (15), or 11 (6). Pectoral fin inserted immediately after the opercle, posterior tip at vertical surpassing pelvic-fin origin, usually reaching the half of elongated scale covering pelvic-fin origin. Pelvic-fin rays i,6* (35). Pelvic-fin origin at vertical between 9th or 12th scale of lateral line. Caudal fin forked with 10/9 principal rays (2 c&s).

Scales cycloid, almost all with the same size and form, except for anal-fin base with

FIGURE 6 | Right premaxilla, maxilla and dentary of *Diapoma potamohadros*, UFRGS 41353, 51.4 mm SL, paratype.

elongated scales. Most of specimens (28) with discontinuous lateral line, with tubed scales interspersed by non–tubed scales; five specimens with incomplete lateral line with canal of lateral line present until 11 to 19th scales, and two specimens with lateral line scales completely tubed. Absence of lateral-line canal on caudal-fin interradial membrane. Total number of scales in longitudinal series 35 (2), 37 (7), 38* (11), 39 (12), 40 (2), or 41 (1). Predorsal scales 12 (8), 13* (15), 14 (11), or 16 (1). Five (8) or six* (27) scale rows between dorsal-fin origin and row of lateral line. Three (5), four (5), or five* (24) scale rows between lateral line and pelvic-fin origin. Circumpeduncular scales 14* (20) or 15 (14). One row of scales forming sheath along anal-fin base with five (1), six (3), seven (4), eight (8), nine* (7), 10 (6), or 12 (1) scales. Scales along anal-fin base cover at least to 11th anal-fin branched ray. Caudal-fin lower lobe covered by a set of four or five large unmodified scales, not extending beyond anterior one-third of caudal-fin rays. Total number of vertebrae 36, 16 precaudal and 20 caudal (2 c&s).

Coloration in alcohol. Ground color pale or yellowish in preserved specimens. Black chromatophores delineating scale margins on latero-dorsal portion of body, forming a feeble chevron pattern on the lateral of body above anal-fin base. Black chromatophores concentrated along mid-dorsal region of head and anterior tip of snout and lower jaw. Infraorbitals and opercle bright silver. Black humeral blotch vertically elongated. Black and large midlateral stripe, more diffuse anteriorly near humeral blotch, wide and conspicuous from vertical through dorsal fin to caudal peduncle, extending posteriorly to proximal portion of middle caudal-fin rays. Caudal fin with scattered black chromatophores along dorsal, ventral, and posterior portion. Dorsal fin with diffuse black chromatophores on

TABLE 2 | Morphometric data for holotype and 34 paratypes of *Diapoma potamohadros*.

	Holotype	Males (N = 18)			Females and juveniles (N = 16)		
		Range	Mean	SD	Range	Mean	SD
Standard Length (mm)	48.6	37.5–52.9	47.9		31.3–56.4	45.7	–
Percentages of standard length							
Head length	22.7	20.4–22.9	21.3	1.8	20.3–24.6	22.6	2.3
Depth at dorsal-fin origin	29.1	28.6–31.9	29.8	0.9	27.6–30.7	29.0	1.0
Snout to dorsal-fin origin	52.0	51.0–55.9	52.6	1.4	50.8–57.01	54.1	2.0
Snout to pectoral-fin origin	23.8	21.7–27.5	23.4	1.4	22.1–27.8	24.4	1.8
Snout to pelvic-fin origin	45.1	43.2–46.2	44.6	0.9	42.7–47.8	42.4	1.5
Snout to anal-fin origin	59.2	59.5–63.4	61.2	1.2	59.6–64.9	61.9	1.6
Distance between dorsal- and adipose-fin origins	38.4	32.6–39.3	36.8	1.8	31.0–38.3	35.7	2.1
Dorsal -fin origin to caudal-fin base	50.2	46.6–52.6	50.3	1.4	45.3–52.3	48.9	2.1
Dorsal-fin length	22.6	20.7–24.1	22.2	1.0	20.4–23.9	22.2	1.1
Dorsal-fin base length	11.8	10.2–12.5	11.5	0.6	8.9–12.0	11.1	0.9
Pectoral-fin length	22.1	20.0–21.9	20.9	0.6	20.5–22.5	21.4	0.6
Pelvic-fin length	14.1	12.5–14.9	13.6	0.6	12.6–14.6	13.7	0.6
Anal-fin base-length	30.3	28.1–31.4	29.5	0.9	26.5–32.4	29.2	1.6
Caudal peduncle depth	9.9	9.2–10.9	9.7	0.5	8.9–11.6	10.1	0.7
Caudal peduncle length	11.8	11.5–14.1	12.8	0.7	11.1–13.4	12.6	0.7
Percentages of head length							
Snout length	20.8	19.7–26.8	22.0	1.8	19.0–26.9	22.6	2.3
Horizontal eye length	42.3	39.6–44.6	42.6	1.2	39.4–45.3	42.5	1.7
Postorbital head length	37.7	33.0–40.8	36.8	1.8	34.2–38.3	36.1	1.1
Least interorbital width	33.8	33.2–37.3	35.1	1.2	31.3–39.1	35.0	2.0
Upper jaw length	37,5	37.5–42.2	39.5	1.5	38.3–43.0	40.2	1.4
Dentary length	39.1	38.5–46.2	40.7	1.9	39.1–45.4	41.5	1.9

interradial membranes. Adipose fin hyaline with few scattered black chromatophores. Anal fin dusky, with scattered black chromatophores on interradial membranes; distal region more darkly pigmented. First unbranched pectoral–fin ray pigmented with black chromatophores, other rays hyaline. Pelvic fin hyaline.

Sexual dimorphism. The presence of bony hooks was only observed in two adult males from the Ñandú Chico stream (Fig. 7A; MACN–ict 10364, male, 37.8 mm SL). The distal border of the anal fin is concave in both males and females, lacking the sexual dimorphism observed in other species of the genus (see Diagnosis). All pelvic–fin rays of males (except the last one) have several pairs (usually one pair per segment) of short slender hooks extended mainly on the segmented region of each ray but also present on the unsegmented area of the middle rays. The pelvic-fin hooks are oriented anterolaterally on the inner border of each ray and its branches. The distal half of the anal–fin rays of males bears extremely tiny hooks that are arranged in one pair per each segment from the last unbranched ray until the 9th branched ray and are oriented nearly anteriorly or laterally on the posterior border of the rays. Gill glands were found in approximately the six to 10 anteriormost filaments of the lower branch of the

first branchial arch. The gill glands were not observed on females and in all unsexed examined specimens up to 25 mm SL.

Geographical distribution. *Diapoma potamohadros* is known from the lower Iguaçu River basin, a tributary to the Paraná River, downstream from the Salto Santiago Reservoir to the Iguaçu falls (Fig. 4).

Ecological notes. According to a previous study (Russo *et al.*, 2004, tab. 3), this new species of *Diapoma* consumes mainly allochthonous resources, such as adults of Diptera (60% in April, flood period), macrophytes (79.4% in August, flood period), Hymenoptera (70.2% in September 54.8% in November and 58.8% in December) and adult Ephemeroptera (67.5% in May and 44.3% in October).

Etymology. We named the species *Diapoma potamohadros* in reference of Iguaçu River (Gr.: *potamo* = river; *hadros* = big, bigger), a noun in apposition of "Iguaçu" ("igua" = river, "açu" = big in tupi–guarani, Brazilian indigenous language).

Conservation status. The specimens were collected in a restricted area that is under influence of the reservoirs of Salto Osório, Salto Caxias and some tributaries upstream the Iguazu falls within the Iguazu Falls National Park in Argentina. Based on collecting sites, we estimate the EOO to be 5,197.778 km² and AOO to be 343.000 km², which
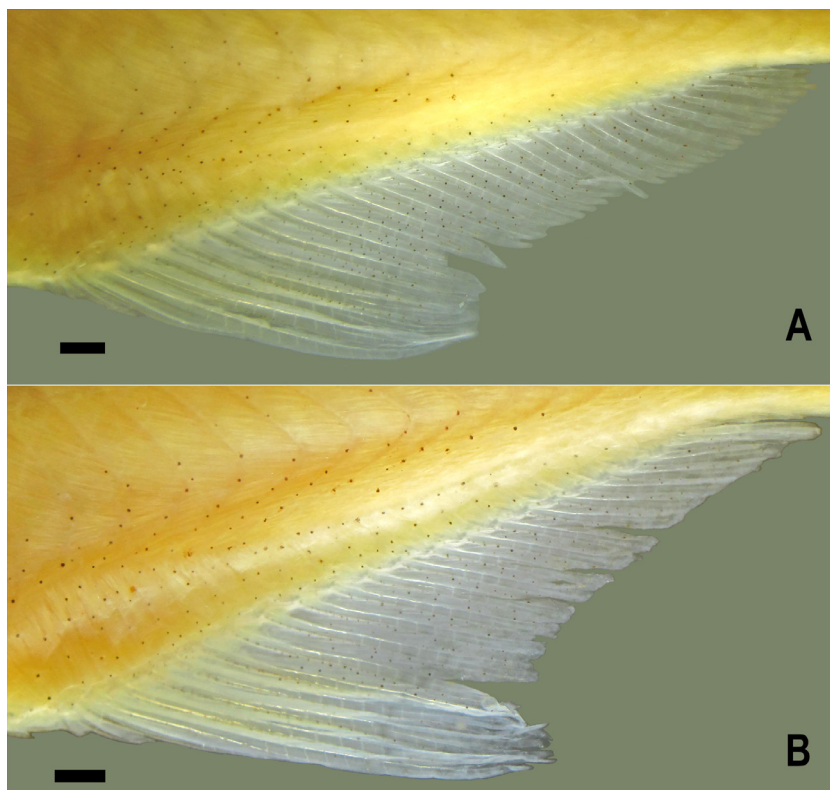
FIGURE 7 | Anal fin of *Diapoma potamohadros*, MACN-ict 10364, **A.** Male, 37.8 mm SL; **B.** Female, 37.6 mm SL. Lateral view, left side. Scale bars = 1 mm.

can be classified as Vulnerable (VU) with criteria B(B1: EOO < 20.000 km², B2: AOO < 2.000 km²). However, no specific threats were detected for the entire distribution of this species, and its population seems to be abundance in the nature, based in total of specimens collected and registered in the scientific collections. Therefore, this species can be classified as Least Concern (LC) according to IUCN categories and criteria (IUCN Standards and Petitions Subcommittee, 2019).

Key to species of *Diapoma* (Modified from Vanegas–Ríos *et al.*, 2018)

1a. Presence of modified scales on lower caudal-fin lobe, slightly more pronounced in adult males, forming a pocket-shaped structure 2

1b. Absence of modified scales on lower caudal-fin lobe, caudal scales at base never forming a pocket-shaped structure 3

2a. Opercle and subopercle unmodified, not posteriorly prolonged......................... 4

2b. Opercle and subopercle modified, posteriorly prolonged.................................... 5

3a. Complete lateral line 6

3b. Incomplete lateral line or discontinuous lateral line.. 8

4a. 11–13 gill rakers on lower limb of first gill arch ....*D. thauma*

4b. 15–18 gill rakers on lower limb of fist gill arch...*D. terofali*

5a. Snout length 21.3–24.5% HL; maxillary teeth pentacuspid; live specimens with intense red pigmentation on some portions of all fins except pectoral fin ....*D. pyrrhopteryx*

5b. Snout length 17.0–21.4% HL; maxillary teeth tricuspid (rarely with more cusps); no red coloration on any fin in live specimens..*D. speculiferum*

6a. Anal fin unpigmented, without distinctive marks; adipose fin not pigmented in preserved mature males and females; snout to pelvic-fin origin 48.1–52.6% SL ....*D. alburnum*

6b. Anal fin pigmented, with distal tip of anterior lobe unpigmented; adipose fin dark in preserved mature males and females; snout to pelvic-fin origin 40.4–46.6% SL 7

7a. Number of scale rows between dorsal and pelvic-fin origins 11–13 ......*D. itaimbe*

7b. Number of scale rows between dorsal and pelvic-fin origins 9–11 ....*D. dicropotamicus*

8a. Body without well-defined, wide lateral band of chromatophores, only narrow lateral stripe present (usually dark)..................................*D. pampeana*

8b. Body with variedly developed, wide lateral band of intense chromatophores (usually dark or silvery), lateral stripe never reduced to narrow line 9

9a. In adult males, anal-fin distal margin strongly convex 10

9b. In adult males, anal-fin distal margin somewhat concave, nearly straight or slightly convex 11

10a. Anal-fin sheath consisting of 20–28 aligned scales, covering three-quarters or entire length of anal-fin base (usually reaching 22nd branched ray); 29–35 branched anal-fin rays (usually 29–33) ....*D. uruguayense*

10b. Anal-fin sheath consisting of 23–30 aligned scales reaching no more than half length of anal-fin base (usually extending to 12th or 18th branched ray); 23–30 branched anal-fin rays (usually 25–27) .... *D. alegretense*

11a. Humeral blotch diffuse or absent; horizontal eye length 31.5–37.0% HL (mean = 35.4% HL)..................................*D. tipiaia*

11b. Humeral blotch always discernible; horizontal eye length 36.4–48.5% HL (mean = 41.4% HL).. 12

12a. Distance between snout to anal-fin origin 52.1–58.8% SL .... *D. lepiclastum*

12b. Distance between snout to anal-fin origin 59.3–66.4% SL 13

13a. First three (often four) dentary teeth tetra or pentacuspid in adults; middle and distal portions of interradial membranes of posterior branched dorsal-fin rays hyaline or, when faintly dusky, being similarly pigmented in both sexes; discontinuous lateral line 14

13b. First three (usually four) dentary teeth tricuspid in adults; middle and distal portions of interradial membranes of posterior branched dorsal-fin rays dusky, intensely darker in adult males than in females or juveniles; incomplete lateral line 15

14a. Body depth at dorsal-fin origin 27.6–31.9% SL; distance between snout to anal-fin origin 50.8–57.0% SL.. *D. potamohadros*

14b. Body depth at dorsal-fin origin 35.5–44.1% SL; distance between snout to anal-fin origin 57.1–61.5% SL...*D. obi*

15a. Presence of a large dark round blotch on middle region of caudal fin, more noticeable in males; urogenital region darkly pigmented in females; 22–25 gill rakers on first gill arch (mode = 23, 8–9 + 14–17); maximum known body size 30.5 mm SL... *D. guarani*

15b. Absence of a large dark blotch on middle region of caudal fin, with caudal spot much more concentrated on peduncle (or partially on interradialis muscles) than on middle caudal-fin rays (rarely faintly scattered dark chromatophores reaching midpoint of ray); urogenital region unpigmented in females; 18–21 gill rakers on first gill arch (mode = 20, 6–8 + 12–14); maximum known body size 59.1 mm SL ......*D. nandi*

Phylogenetic analysis. The phylogenetic analysis using molecular data includes 25 species (55 specimens' samples), of which 15 correspond to *Diapoma* (37 specimens sampled) (see Tab. **S1**). The concatenated alignment contained 5,039 sites, representing seven markers. The best partition scheme found using PartitionFinder suggests a single partition for 12S and 16S; and 15 partitions representing each codon position of the five genes (COI, MYH6, PTR, RAG1, RAG2; Tab. 3). The topology using the concatenated dataset is present in Fig. 8, and the species trees analysis is illustrated in Fig. 9. For each gene tree representing the seven genes examined with all sequences available in GenBank are available in the Fig. **S2**.

The genus *Diapoma* is recovered as a monophyletic group in both the concatenated analysis (CT) and in the Species Tree (ST) analysis (PP = 1). The genus is divided into two well supported clades, one composed of (*D. alburnum* (*D. dicropotamicus*, *D. itaimbe*)) with high support (PP =1 CT and ST) which is sister-group to a clade composed of all

other examined species of the genus, including the type species of the genus *Cyanocharax* (*D. uruguayense*) plus the type species of the genus *Diapoma* (*D. speculiferum*) (PP = 0.95 CT and PP = 0.99 for ST). A large monophyletic group, herein named *Diapoma uruguayense* clade, has a low support (PP = 0.72 CT; PP = 0.87 ST), being composed of (*D. thauma* (*D. alegretense* (*D. uruguayense, D. lepiclastum*))) and is sister group to the *D. speculiferum* clade.

The *Diapoma speculiferum* clade (PP = 0.86 CT; PP = 0.73 ST) is composed by eight species and includes the two new species proposed herein. *Diapoma potamohadros* is closely related to *D. tipiaia,* with a low support (PP = 0.62 CT; PP = 0.67 ST), and *D. pampeana* is highly supported (PP = 1 CT; PP = 0.97 ST) as the sister-group to the clade (*D. obi* and *D. guarani*). These two clades formed the sister-group of the well-supported clade (PP = 0.99 CT; PP = 1 ST) (*D. speculiferum* (*D. pyrrhopteryx, D. terofali*)).
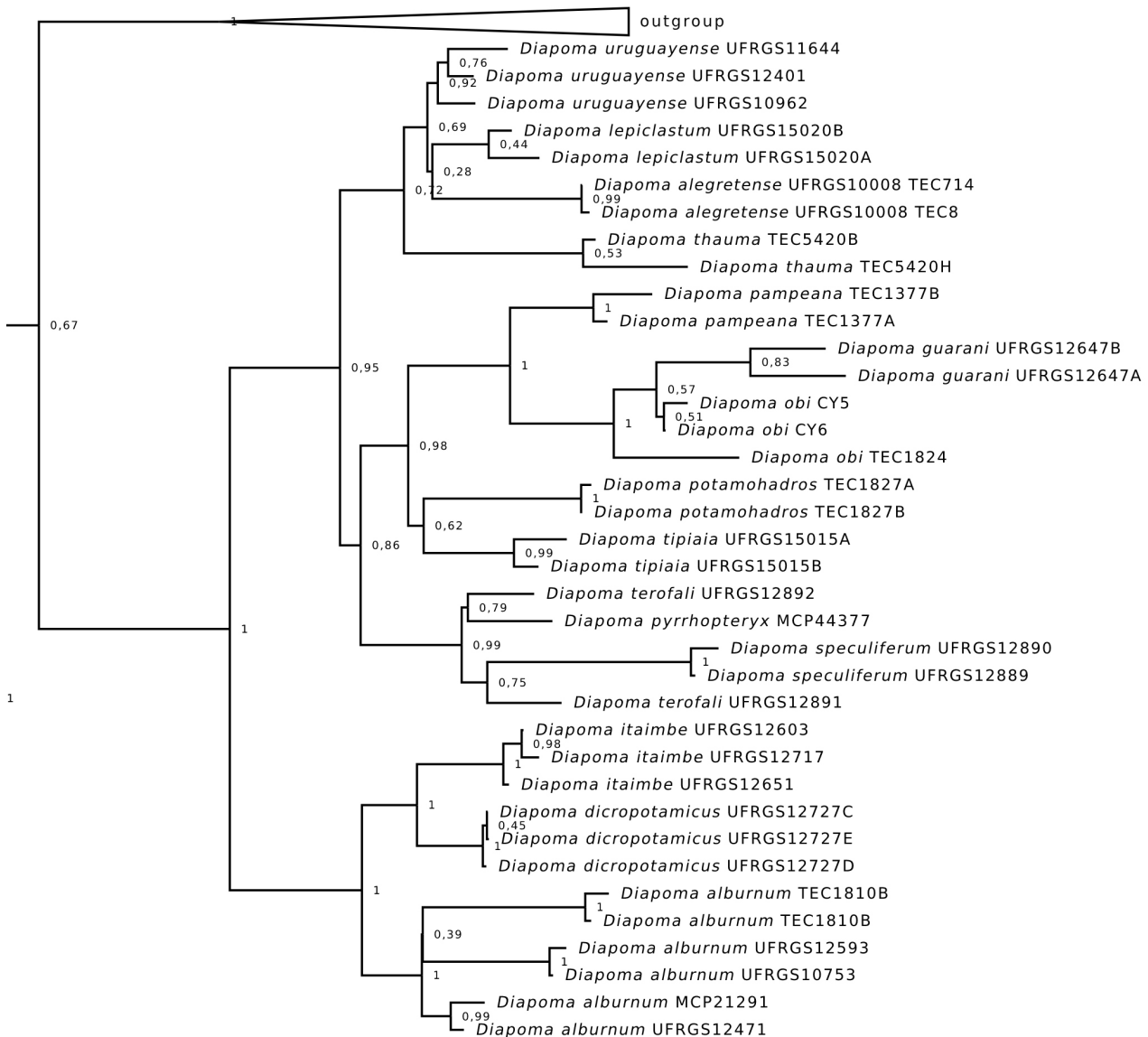
FIGURE 8 | Bayesian tree showing the phylogenetic relationships within *Diapoma* inferred by the concatenated dataset (12S, 16S, COI, MYH6, ptchd1, RAG1, RAG2, total of 5067pb).
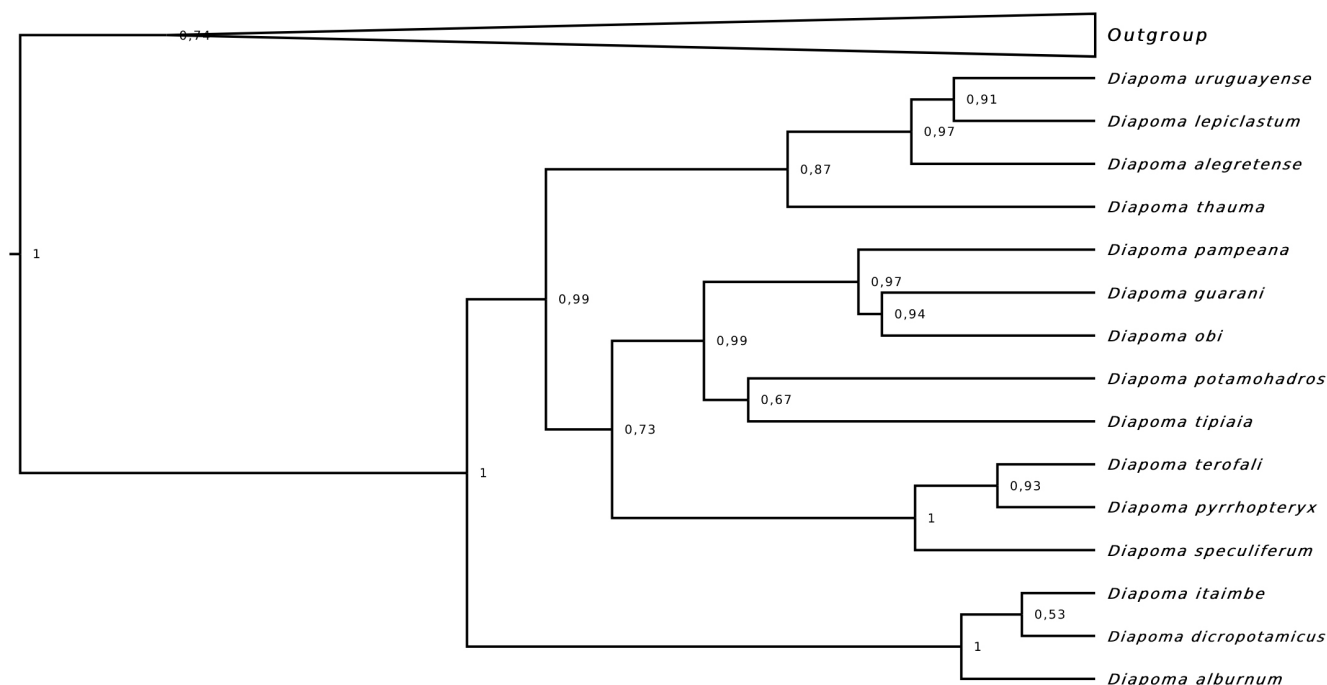
FIGURE 9 | Bayesian species tree of *Diapoma* obtained from multilocus sequences (12S, 16S, COI, MYH6, ptchd1, RAG1, RAG2, total of 5067pb).

TABLE 3 | Best-fit model and partition scheme.

Partitioning scheme	Best Model	Subset Partitions	Subset Sites
1	HKY+I+G	RAG2_codon1, 12S16S	4295-5067\3 1-1026
2	GTR+I+G	COI_codon1	1027-1747\3
3	K80+I	RAG1_codon2, COI_codon2	2934-4294\3 1028-1747\3
4	F81	COI_codon3	1029-1747\3
5	F81+I	RAG2_codon3, RAG2_codon2, RAG1_codon1, MYH6_codon1	4297-5067\3 4296-5067\3 2933-4294\3 1748-2368\3
6	F81	PTR_codon1, PTR_codon2, MYH6_codon2	2369-2932\3 2370-2932\3 1749-2368\3
7	K80+G	MYH6_codon3, RAG1_codon3	1750-2368\3 2935-4294\3
8	F81	PTR_codon3	2371-2932\3

DISCUSSION

Species comprehensive morphological and molecular analyses have held significant advances for understanding the phylogenetic relationship of neotropical freshwater fishes, being crucial for the establishment of the taxonomy of species–rich groups like Characidae (Javonillo *et al.*, 2010; Oliveira *et al.*, 2011; Tagliacollo *et al.*, 2012; Netto-Ferreira *et al.*, 2013; Benine *et al.*, 2015; Thomaz *et al.*, 2015; Melo *et al.*, 2016; Mirande, 2019; Terán *et al.*, 2020; Vanegas-Ríos *et al.*, 2020; Ferreira *et al.*, 2021). This steady progress is imperative on recent classification schemes of this family. Characters that were previously considered unique and essential to classify a species in a given group (*e.g.*, presence of modified scales in the caudal fin, presence of insemination, elongation

of the opercle, completeness of the lateral line) have shown to be highly homoplastic and vary among closely related species. Menezes, Weitzman (2011) have proposed the interrupted lateral line to distinguish *Diapoma* from *Acrobrycon*. We found this character polymorphic in the genus, grouping species with complete and incomplete lateral lines, as well as two species (*D. potamohadros* and *D. dicropotamicus*) presenting intraspecific variation with complete, discontinuous or incomplete lateral line among examined specimens (see discussion in Marinho *et al.*, 2021), suggesting this feature as highly labile and not diagnostic of *Diapoma*.

Another feature often used as a generic or suprageneric level diagnostic character in Characidae is the presence of modified scales in the caudal fin. This feature, more specifically the presence of a caudal organ nearly equivalent in males and females with the dorsal border of the pouch formed by 4 to 8 scales, is present in four species of *Diapoma*: *D. terofali*, *D. thauma*, *D. speculiferum* and *D. pyrrhopteryx* (*Diapoma sensu* Menezes, Weitzman, 2011). The clade (*D. speculiferum* (*D. terofali* and *D. pyrrhopteryx*)) corroborates previous phylogenetic studies (*e.g.*, Mirande, 2019). In our analyses, *Diapoma thauma* appeared as closely related to the clade composed by *D. alegretense*, *D. lepiclastum*, and *D. uruguayense*, species that lack a caudal organ. Although branch support was not strong, this result refutes the hypothesis that *D. thauma* shares a most recent common ancestor with *D. terofali*, *D. speculiferum* and *D. pyrrhopteryx* (as proposed by Menezes, Weitzman, 2011). Given the phylogenetic relationships presented here, the modified caudal scales in the caudal fin of *D. thauma* are likely non-homologous and have evolved independently in the lineage formed by *D. terofali*, *D. speculiferum*, and *D. pyrrhopteryx*, and in the lineage given rise to *D. thauma*.

Another feature found in some *Diapoma* species is the presence of gill glands in males (coded as absent in *D. speculiferum* and *D. terofali* in Mirande, 2010, 2019: chs. 352 or 511). Firstly, described by Burns, Weitzman (1996), the gill glands are formed by fusion of the anterior gill filaments of the first branchial arch (sometimes along all filaments of the first branchial arch; *e.g.*, Cheirodontinae – Oliveira *et al.*, 2012). Those gill glands in *Diapoma* are similar to that described in other characid groups (see Burns, Weitzman, 1996; Menezes *et al.*, 2003; Oliveira *et al.*, 2012; Terán *et al.*, 2015). The absence of gill glands (either confirmed by observations of the external morphology of the gill filaments or by histological procedures) is reported in *D. nandi*, *D. obi*, *D. pyrrhopteryx*, *D. speculiferum*, *D. terofali* (Bushmann *et al.*, 2002; Menezes, Weitzman, 2011; Vanegas-Ríos *et al.*, 2018, 2020: ch. 413), *D. alegretense* and *D. uruguayense*, whereas it is present in all others remaining species examined of the genus.

The paraphyly of *D. obi* and the low support of the clade formed by *D. guarani* and *D. obi* (PP = 0.45, CT; Fig. 8) must be further investigated. Data includes mitochondrial sequences available from Casciotta *et al.* (2012), and mitochondrial and nuclear sequences from Thomaz *et al.* (2015), but no vouchers were available from both sources for morphological comparison. The clade composed of *D. alegretense*, *D. lepiclastum*, and *D. uruguayense* has been consistently found in morphological and some molecular analyses (*e.g.*, Malabarba, Weitzman, 2003; Casciotta *et al.*, 2012), but not in others (*e.g.*, Thomaz *et al.*, 2015; Mirande, 2019). The high number of scales sheath covering the basal portion of anal-fin rays (12–20 scales), previously proposed as a synapomorphy grouping these three species (Malabarba, Weitzman, 2003), is congruent with our tree topology.

In this work, we weren't able to compare DNA data of two species currently in the genus *Hyphessobrycon* (*i.e.*, *H. wajat* and *H. procerus*) that were suggested to be related to *Diapoma* based on putative shared apomorphic features (*e.g.*, i,6 pelvic-fin rays) (Vanegas-Ríos *et al.*, 2018; Mirande, 2019). Given the possibility that these two species, currently in *Hyphessobrycon*, may be allocated in the future to *Diapoma*, we also provided herein a diagnosis of the two new species from *H. procerus* and *H. wajat*. The longitudinal line coloration differs *D. pampeana* from *H. procerus* (narrow line extended along its entire length *vs.* wide stripe, especially more developed on the posterior half of body); and the pigmentation of the middle caudal-fin rays distinguishes *D. pampeana* and *D. potamohadros* from *H. wajat* (absence of any defined caudal blotch or with middles rays slightly pigmented, as an extension of the chromatophores of the caudal peduncle blotch *vs.* dark large blotch covering widely the caudal-fin base and part of the middle caudal-fin rays). *Diapoma potamohadros* can be further differentiated from *H. procerus* by the distances between the dorsal- and adipose-fin origin (31.0–39.3% SL *vs.* 29.5–30.2% SL) and between the snout and the dorsal-fin origin (50.8–57.0% SL *vs.* 57.2–59.6% SL), and the number of vertebrae (36 *vs.* 37).

Diapoma pampeana is the seventh species of *Diapoma* known to occur in the Uruguay River basin, together with *D. alburnum*, *D. alegretense*, *D. guarani*, *D. lepiclastum*, *D. pyrrhopteryx*, *D. terofali*, and *D. uruguayense*. This distribution contrasts with *Diapoma potamohadros* which represents the first and unique species of the genus described from the Iguaçu River, being absent from previous lists of fish species from the Iguaçu River (*e.g.*, Ingenito *et al.*, 2004; Casciotta *et al.*, 2016; Larentis *et al.*, 2016). The Iguaçu River is known for its high endemism of fishes (Zawadzki *et al.*, 1999).

Comparative material examined. *Diapoma alburnum*: **Brazil: Rio Grande do Sul:** UFRGS 13309, 11, 33.4–56.0 mm SL. UFRGS 22130, 98, 23.0–54.4 mm SL. UFRGS 26430, 48, 39.4–48.7 mm SL. UFRGS 27018, 2, 43.1–44.8 mm SL. UFRGS 27019, 59, 22.9–42.7 mm SL. *Diapoma alegretense*: **Brazil: Rio Grande do Sul:** UFRGS 21203, 128, 18.6–40.0 mm SL. UFRGS 21204, 62, 18.4–39.1 mm SL. UFRGS 21215, 19, 22.8–38.6 mm SL. *Diapoma dicropotamicus*: **Brazil: Rio Grande do Sul:** UFRGS 9193, 32, 26.4–44.9 mm SL. *Diapoma guarani:* **Brazil: Rio Grande do Sul:** UFRGS 8480, 63, 18.4–35.5 mm SL, 5 c&s. UFRGS 12647 (TEC 1379), 15, 27.6–37.6 mm SL. **Paraguay: Alto Paraná:** MHNG 2370.013, 6, 22.5–28.2 mm SL, 1 c&s 28.2 mm SL). *Diapoma itaimbe*: **Brazil: Rio Grande do Sul:** UFRGS 12651 (TEC 1383), 14, 25.8–43.7 mm SL. *Diapoma lepiclastum:* **Brazil: Santa Catarina:** UFRGS 10917, 42, 23.3–41.0 mm SL. **Rio Grande do Sul:** UFRGS 15020 (TEC 1801), 28, 22.2–29.3 mm SL. *Diapoma nandi:* **Argentina: Misiones Province:** MLP 11311, 26, paratypes, 30.1–46.8 mm SL, 2 c&s, 37.6–43.6 mm SL. *Diapoma obi*: **Argentina: Misiones:** AI 282, 1 c&s, paratypes, 58.7 mm SL. AI 284, 2, paratypes, 52.2–57.4 mm SL. MACN-ict 9557, 9, paratypes, 41.0–52.7 mm SL. MACN-ict 9558, 3, paratype, 38.1–46.6 mm SL. MACN-ict 9559, 3, paratypes, 48.4–56.7 mm SL. MACN-ict 9560, holotype, 53.3 mm SL. *Diapoma pyrrhopteryx*: **Brazil: Rio Grande do Sul:** UFRGS 15738, 1, 59.0 mm SL. *Diapoma speculiferum*: **Brazil: Rio Grande do Sul:** UFRGS 12889 (TEC 1069), 1, 35.1 mm SL. UFRGS 12890 (TEC 1084), 1, 32.9 mm SL. *Diapoma terofali:* **Brazil: Rio Grande do Sul:** UFRGS 27293, 3 46.3–55.1 mm SL. **Uruguay: Rivera:** UFRGS 7227, 283, 19.1–45.6 mm SL. *Diapoma thauma:* **Brazil: Rio Grande do Sul:** UFRGS 8948, 39, 15.0–41.2 mm SL. UFRGS 20044 (TEC 5420), 29, 29.8–45.0 mm SL. *Diapoma tipiaia*: **Brazil: Rio Grande do Sul:** UFRGS 15015 (TEC 1796), 8, 13.8–20.2 mm SL. *Diapoma uruguayense*: **Brazil: Rio Grande do Sul:** UFRGS 10962 (TEC393),

22.7–34.6 mm SL. UFRGS 11644 (TEC457), 2 (only one available), 33.3 mm SL. UFRGS 12401 (TEC 592), 10, 24.1–42.4 mm SL. *Hyphessobrycon procerus*: **Paraguay:** MHNG 2385.069, 5, paratypes, 22.2–29.3 mm SL. *Hyphessobrycon wajat*: **Argentina, Corrientes Province:** MLP 9321, holotype, 26.8 mm SL. MLP 9322, 5, paratypes, 27.5–30.3 mm SL.

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