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Research Article

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Museum specimens reveal a rare new characid fish genus, helping to refine the interrelationships of the Probolodini (Characidae: Stethaprioninae)

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Two new characid fish species are described from the upper rio Tocantins basin, Chapada dos Veadeiros, Goiás State, Brazil. Both species were discovered among specimens in museum collections. Relationships of taxa were evaluated in a more comprehensive phylogenetic analysis utilizing the largest dataset available of molecular and morphological data for the family Characidae. The two species were recovered as sister species and described in a new genus, closely related to *Erythrocharax* and *Phycocharax*. *Dinotopterygium* gen. nov. is distinguished from all other characid genera by the unique combination of ten synapomorphies, including the unique anal-fin morphology with a small number of branched anal-fin rays (13-16). *Dinotopterygium uniodon* sp. nov. and *D. diodon* sp. nov. differ by the number of tooth series in the premaxillary bone (one or two) and number of tooth cusps (7 or 5). The additional phenotypic variation for a taxonomically informative character within the Characidae through the discovery of new forms has helped to refine the interrelationship of the tribe Probolodini (Characidae: Stethaprioninae). The discovery of these new and possibly critically endangered species emphasizes the importance of museum collections for understanding biodiversity past and present.

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Key words: Biodiversity, Characidae, *Dinotopterygium*, museum collections, phylogeny

Introduction

Museum collections are a valuable source of information for understanding biodiversity in our changing world (Meineke et al., 2019; Shaffer et al., 1998; Suarez & Tsutsui, 2004). The new taxa described here were discovered in museum collections, in a peculiar story. The first specimens were collected in 1989 from near the municipality of Cavalcante, Goiás State, Brazil, and were found at MZUSP fish collection by one of us (LRM) while gathering material for the revision of the Cheirodontinae subfamily of Characidae. Although the specimens were registered in the collection as *Cheirodon* sp. due to the presence of a single series of multicuspid teeth in the premaxilla, they did not fit in *Cheirodon* or even in the Cheirodontinae definitions latter proposed by Malabarba (1998). The peculiar anal fin of males deeply convex precluded its recognition as related to other characid genera, remaining the new species undescribed for a long time.

Due to the availability of a few specimens (15 and all males), an expedition to the locality of one of the species was planned, but although we have had success in collecting several new characid species in the surrounding areas (Bertaco et al., 2010; 2011a, 2011b; Bertaco

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& Carvalho, 2010; Carvalho et al., 2010) we failed to collect additional material of this characid, except for a single young specimen. Further search for specimens from near the collecting locality in fish collections had an unexpected result; we found at Museu de Ciências Naturais (MCN) a second new species bearing equal modifications in the anal fin of males, collected in the same river drainage (rio Paranã drainage, upper rio Tocantins) but surprisingly bearing two tooth series in the premaxilla. Like the first species, it showed no clear affinity to any other characid genera.

Knowledge on the phylogenetic relationships of the Characidae has improved considerably in almost two decades (Mirande, 2009; 2019; Ohara et al., 2017; Terán et al., 2020), after the listing of about 60% of its species and most of its genera as *incertae sedis* (*sensu* Lima et al., 2003). Four main clades are currently recognized in the family: three of these were initially hypothesized by Javonillo et al. (2010) (clades A, B, and C) and latter supported in subsequent works that further recognized a separate fourth characid clade (Spintherobolinae) (Mirande, 2010, 2019; Oliveira et al., 2011; Terán et al., 2020). The advances in the comprehension of the evolutionary history of these major clades, however, show different levels of resolution among the included taxa (Mirande, 2019). Clade A (Stevardiinae) and clade C (Aphyoditeinae, Aphyocharacinae, Characinae, Cheirodontinae, Exodontinae) *sensu* Mirande (2019) are mostly congruent with previous studies regarding their composition and internal relationships, based on previous phylogenetic analyses defining subgroups among their components (Lucena & Menezes, 1998; Malabarba, 1998; Mariguela et al., 2013; Mattox & Toledo-Piza, 2012; Mirande, 2019; Tagliacollo et al., 2012; Thomaz et al., 2015).

Due to the great diversity and complexity found in clade B (i.e. Stethaprioninae *sensu* Mirande, 2019), however, a stable classification has not yet been achieved. The Stethaprioninae is the most diverse subfamily of Characidae (Mirande, 2019) and its internal relationships and classification varies as a result of the limited available information (Terán et al., 2020) and a high degree of homoplasy among described characters. Most of the genera in clade B are not defined by synapomorphies and some are hypothesized to be polyphyletic, especially in those with many species. All of these problems make it difficult to discover the relationships of new taxa to the current clade B genera and species. Here we describe and discuss the relationships of two new species of characid fishes that putatively belong to Stethaprioninae *sensu* Mirande (2019). The two taxa are proposed as sister species, but the rare and alternative presence of one or two tooth series in the premaxilla in each species, a character that has been used since Eigenmann (1915) to diagnose characids at the subfamily level, make them of particular interest in discussing synapomorphies among characid clades.

Material and methods

Morphological data

Counts and measurements follow Fink and Weitzman (1974), except for head depth (vertically measured at supraoccipital tip) and number of longitudinal series of scales below lateral line, counted from pelvic-fin insertion. All measurements are presented as percentages of standard length (SL), except for head measurements presented as percentages of head length (HL). The frequency of each count is provided in parentheses given after the respective count and the counts of the holotypes are marked with an asterisk. Vertebrae, including the four vertebrae of the Weberian apparatus and the last compound vertebra, and supraneurals were counted in specimens cleared and stained (c&s) according to Taylor & Van Dyke (1985). Scanning electronic micrographs (SEM) were made on jaws removed from c&s specimens. Institutional abbreviations include American Museum of Natural History, New York (AMNH), Academy of Natural Sciences of Drexel University, Philadelphia (ANSP), Field Museum of Natural History, Chicago (FMNH), Royal Ontario Museum, Department of Natural History, Toronto, Ontario, Canada (ROM), Museu de Ciências Naturais, Secretaria do Meio Ambiente e Infraestrutura (formerly Fundação Zoobotânica do Rio Grande do Sul), Porto Alegre (MCN), Museo de Ciencias Naturales de la UNELLEZ, Portuguesa, Venezuela (MCNG), Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre (MCP), Museu de Zoologia da Universidade de São Paulo, São Paulo (MZUSP), and Departamento de Zoologia, Universidade Federal do Rio Grande do Sul, Porto Alegre (UFRGS).

Molecular data

Molecular data from two specimens of one species were included into the dataset provided by Terán et al. (2020). Tissue samples were preserved in 99% ethanol at −18 °C. DNA extraction from tissues followed a modified CTAB protocol (Doyle & Doyle, 1987). Polymerase chain reaction (PCR) was used to amplify one mitochondrial (*Cox1*) and two nuclear (*CytB*, *16S*) markers (Appendix 1). PCR products were checked by electrophoresis in agarose gel, purified using ExoSap (Exonuclease I and Shrimp Alkaline Phosphatase GE Healthcare®, Piscataway, NJ, USA) and sequenced by

Macrogen Inc (Seoul, South Korea). Sequences were aligned with all available information for each marker in Terán et al. (2020) using the MUSCLE algorithm embedded in the MEGA software (Kumar et al., 2018) under default parameters. Sequences for each marker were visually inspected and, then, manually edited to fit into the existing dataset (*CytB* [990 pb]; *Cox1* [651 pb]; *16S* [547 pb]).

Phylogenetic analysis

Phylogenetic relationships of the new taxa are proposed by including it in the largest dataset available for the Characidae (Mirande, 2009; 2010, 2019; Mirande et al., 2013; Ohara et al., 2017; Terán et al., 2020) and assessed by parsimony using TNT software (Goloboff et al., 2008). Five new characters were created and coded following Sereno's (2007) proposition and added to the matrix summarized by Terán et al. (2020) in order to compare the diagnostic features of the new taxa with a broad sample of species in Characidae (n = 70) (Appendix S3).

As proposed in previous studies, the analysis was also performed under extended implied weighting to handle missing information for molecular characters (Goloboff, 2014). Five weighting schemes (Mirande, 2019) were defined to explore the most parsimonious trees among distinct approaches on character homoplasy for non-ribosomal sequences: SEP - each character weighted according to its own homoplasy; COD - sequences divided in sets of three contiguous sites (codons) and each character weighted by the average homoplasy of its set; GRO - sequences divided in sets of 30 contiguous sites (ten codons) and each character weighted by the average homoplasy of its set; BLK - each character weighted by the average homoplasy of entire data partition (markers); POS - sets formed by codon positions for each partition and each character weighted according to its position (Mirande, 2019; Terán et al., 2020). Nine weighting strengths (K-values, the concavity constant) (Goloboff, 1993) were combined to the five weighting schemes thus totalizing 45 analytical conditions.

Searches for each analytical condition were performed under Wagner trees and TBR with help of parsimony ratchet (Nixon, 1999), sectorial searches, tree drifting, and tree fusing (Goloboff, 1999). Searches were carried out until the best provisionally fit for each condition reached three times. All trees were subsequently refined using the most parsimonious trees from all searches as the source for rounds of tree fusing for each analytical condition, with the addition of sectorial searches when trees were improved by the fusing. Successive refinements were performed until the most parsimonious trees under all conditions remained stable (Terán et al., 2020).

The most parsimonious trees for all analytical conditions were ranked between 0 and 100 according to its degree of optimality: higher values indicate trees with best fit and, thus, the elected overall most parsimonious one(s) (Terán et al., 2020). Support was calculated through symmetric resampling (300 replicates, probability of change 0.33) using sectorial searches and tree fusing (Goloboff, 1999). Results are expressed as differences of frequencies "Group present/Contradicted" (GC-values) (Goloboff et al., 2003).

Comparative material provided in Appendix S1. The new taxa coding and molecular sequences for one species is provided in Appendix S2. The new characters coding for 70 taxa is provided in Appendix S3. All TNT files are provided in Appendix S4.

Results

Phylogenetic analysis

The monophyly of the new genus (GC = 92) was obtained from the strict consensus of most parsimonious trees with equal weighting procedure (76910 steps, CI = 0.105, RI = 0.590). The new genus seems to be related to the monotypic *Erythrocharax altipinnis* as sister group but with low support (GC = 2), sharing a combination of morphological synapomorphies (35:0, 275:1, 341:0, 404:0). The clade formed by *E. altipinnis* and the new genus remained stable in all trees as sister group of the monotypic *Phycocharax rasbora* under the combination of 12 synapomorphies (29:0, 62:1, 92:0, 117:1, 123:1, 129:1, 170:0, 193:1, 292:1, 303:0, 467:1, 522:1). The recovery of the clade [*P. rasbora* [*E. altipinnis* [*Dinotopterygium*]]], was also observed using the extended implied weighting procedure as it appears in the resulting tree based on the metacriterion (i.e., SEP68) (Fig. 1, Appendix S5).

Dinotopterygium gen. nov.

Zoobank ID. 6167DE39-E361-4E9C-996A-875D640345B1

Type species. *Dinotopterygium uniodon* sp. nov., by original designation.

Diagnosis. *Dinotopterygium* has an unusual body shape with the short anal-fin base associated with few branched anal-fin rays (13-16) (Fig. 2); shape of the ventral profile of body along anal-fin base and by the shape of the distal border of anal fin, both strongly convex in males (*vs*. usually straight or concave), and the

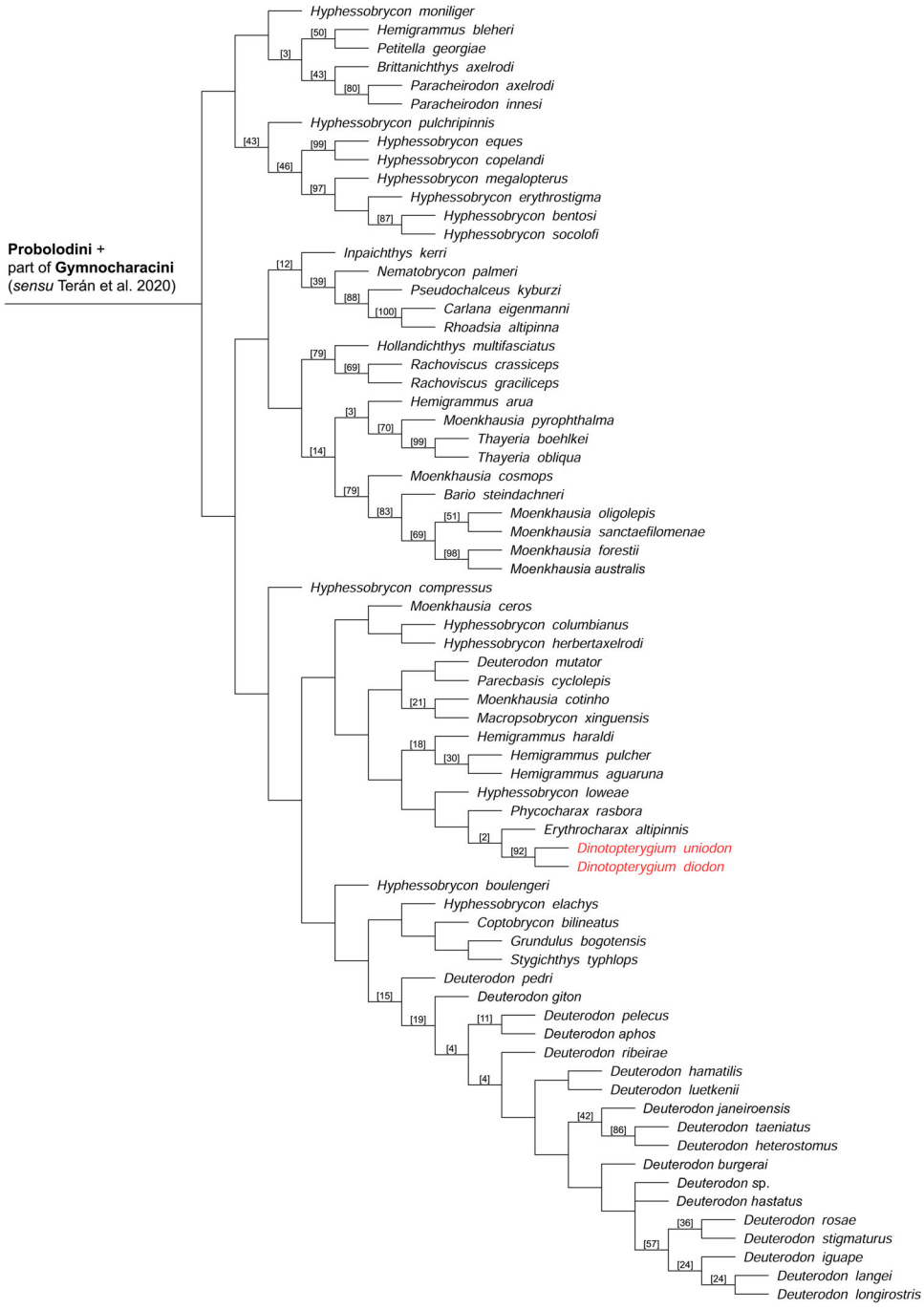

Fig. 1. Phylogenetic relationships of the two new species (red) within Probolodini tribe plus part of Gymnocharacini (*sensu* Terán et al., 2020). The final hypothesis is the consensus tree of 12 most parsimonious trees (SEP; K = 68; Fit = 1152.13528; Length = 69485 steps) based on the combination of both parsimony and extended implied weighting methods.

relative posterior position of the pelvic fins near mid body length and nearly below the origin of the dorsal fin (*vs*. anterior to mid body length and to dorsal-fin origin). Additionally, the segments of the anal fin rays are sagitally expanded and squarish in males, and the proximal radials are longer than anal fin rays.

Synapomorphies of *Dinotopterygium*

1. Anterior paired projections of parasphenoid absent (character 43, state 0); 2. Ventral extent of the third infraorbital reaching the horizontal arm of the preopercle (character 88, state 0); 3. Position of coronomeckelian

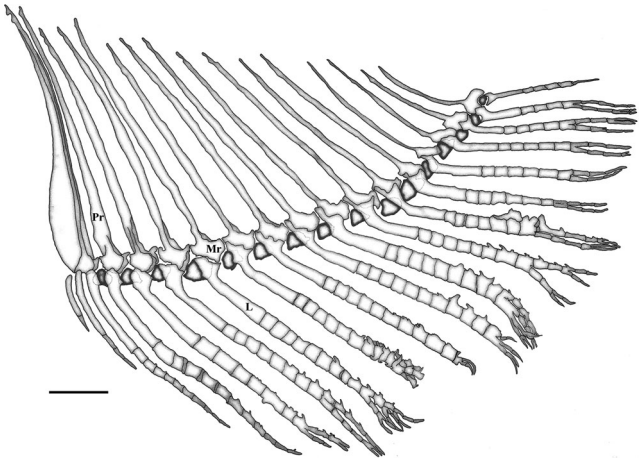

Fig. 2. Anal fin of an adult male *Dinotopterygium diodon* sp. nov. (MCN 13430, paratype, 37.2 mm SL). L, lepidotrichia; Pr, proximal radial; Mr, medial radial.

Fig. 3. *Dinotopterygium uniodon* sp. nov., (A) MZUSP 40358, holotype, 40.23 mm SL, male; (B) MZUSP 40358, paratype, 29.4 mm SL, female.

situated mainly dorsal to Meckelian cartilage (character 159, state 1); 4. Anterior margin of the basihyal expanded, with two-thirds or more of its length (character 260, state 1); 5. Ventral exit of laterosensory canal of supracleithrum medially positioned, covered by posterior lamella of supracleithrum (character 337, state 0); 6. Seven or more supraneurals (character 394, state 1); 7. Number of branched anal-fin rays not surpassing 17 (character 419, state 0); 8. Bony hooks on fin rays present in adult males (character 440, state 1); 9. Circuli on posterior field of scales present (character 456, state 0); 10. Insertion of the mandibular accessory tendon positioned anterior to Meckelian cartilage (character 475, state 1).

Etymology. *Dinotopterygium*, from the Greek *dinotos*, meaning rounded and *pterygium*, meaning fin, as reference to the shape of the anal fin of males that is convex in its distal border.

Dinotopterygium uniodon Frainer, Carvalho, Bertaco & Malabarba sp. nov.

(Fig. 3)

Zoobank ID. 2B674F60-2163-4A01-8E3D-40A7FB0A60E4

Holotype. MZUSP 125875, 36.1 mm SL, male, Brazil, Goiás State, municipality of Cavalcante, tributary of córrego Ave Maria at 14 km north of Cavalcante, upper rio Tocantins basin, 13°40'42"S 47°28'22"W, (Fig. 4) 6 Jan 1989, J. C. Oliveira & W. J. E. M. Costa.

Paratypes. All from Brazil, Goiás State, municipality of Cavalcante. MCN 13426, 12 (5 males, 24.5-38.1 mm SL; 5 females, 32.5-34.6 mm SL; 2 juveniles, 22.9-24.5 mm SL), tributary of córrego Criminoso at km 12 of the road GO-241, 13°47'17"S 47°22'16"W, 6 Nov 1996, W. R. Koch & K. M. Grosser. MZUSP 40358, 6 (2 males, 25.0-40.2 mm SL; 3 females, 23.8-43.1 mm SL; 1 c&s female, 28.7 mm SL; 1 juvenile, 21.3 mm SL), collected with the holotype. MZUSP 40360, 8 (3 males, 32.9-41.5 mm SL, 1 c&s male, 29.6 mm SL; 4 juveniles, 32.9-40.7 mm SL), tributary of córrego de Pedra at 1 km north of Cavalcante, 13°45'31"S 47°27'19"W, 5 Jan 1989, J. C. Oliveira & W. J. E. M. Costa. UFRGS 11921 (male, 31.7 mm SL), same locality as MZUSP 40360, 25 May 2008, T. P. Carvalho & F. C. Jerep.

Diagnosis. *Dinotopterygium uniodon* is distinguished from its sister species, *D. diodon*, by the presence of one tooth series in the premaxilla (*vs*. two), number of cusps of maxillary teeth (heptacuspidate *vs*. pentacuspidate), and by teeth bearing the central cusp clearly larger than remaining tooth cusps (*vs*. all teeth bearing all cusps nearly equal in size in the maxilla and premaxilla and central cusp slightly larger than remaining cusps in dentary).

Description. Morphometric data in Table 1. Body compressed and elongate; greatest body depth anterior to dorsal and pelvic fins. Dorsal profile convex from snout tip to dorsal-fin origin; dorsoventrally slanted along dorsal-fin base and irregularly straight from that point to caudal fin. Ventral body profile convex from lower jaw to pelvic-fin origin, and slightly concave to anal-fin origin. Body profile along anal-fin base nearly straight in

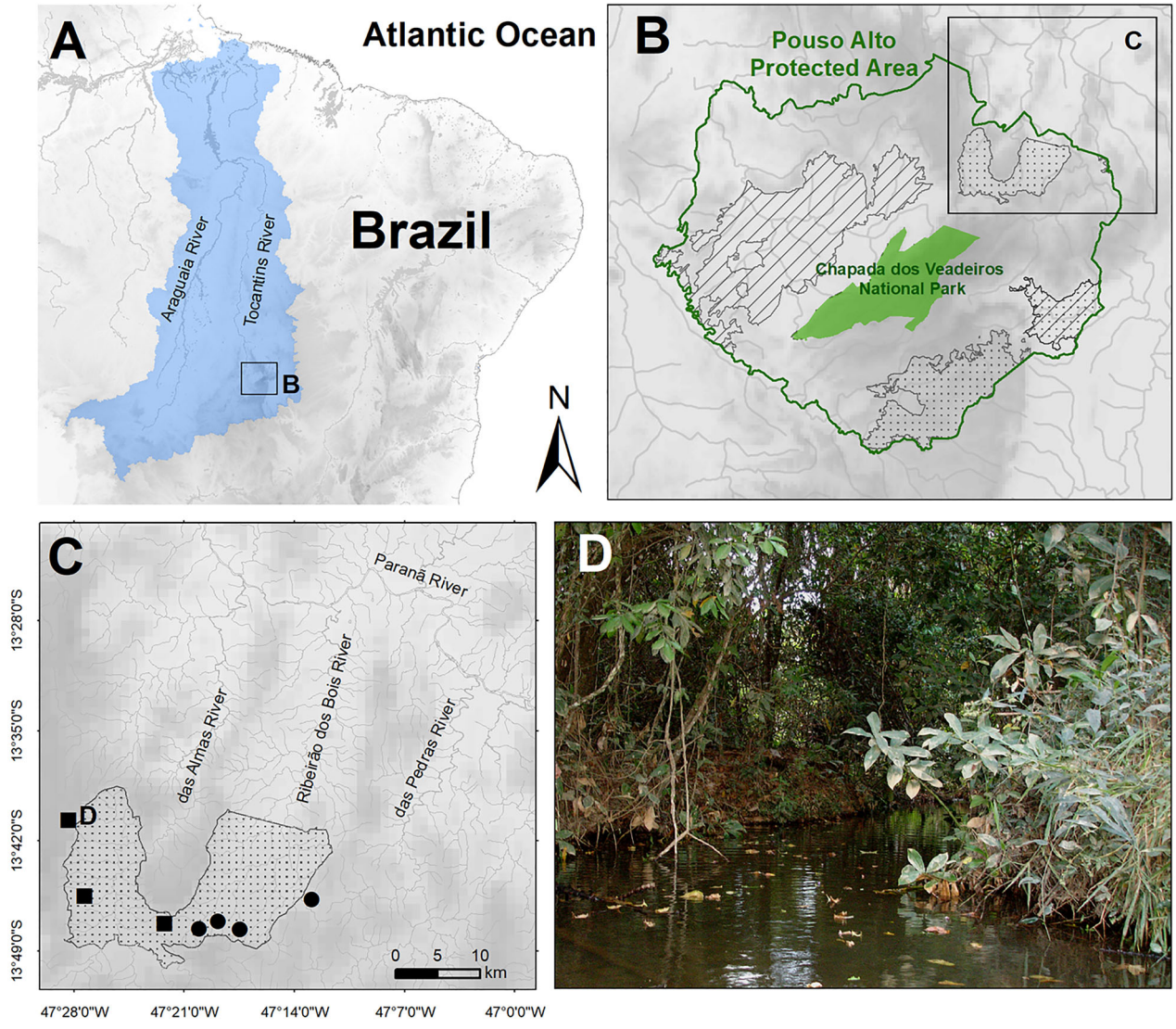

Fig. 4. The new taxa are described for the (A) upper rio Tocantins basin, which is part of the so-called Araguaia-Tocantins basin (blue). Both species are restricted to few tributaries of the rio Paranã within the (B) multiple use Pouso Alto Protected Area, specifically in (C) an intense agricultural activities regime (dotted area). Squares and circles represent the distribution of *D. uniodon* sp. nov. and *D. diodon* sp. nov., respectively. (D) Type locality of *Dinotopterygium uniodon* sp. nov.: tributary of córrego Ave Maria at 14 km north of Cavalcante Municipality (13°40'42"S 47°28'22"W), upper rio Tocantins basin, Goiás State, Brazil. Photo: Fernando R. Carvalho.

females and clearly convex in males. Ventral margin of the caudal peduncle nearly straight.

Mouth terminal, lower jaw and upper jaw nearly equal. Maxilla short and not extending beyond the posterior border of infraorbital 2 and aligned at an angle of approximately 50 degrees relative to the longitudinal body axis. Maxilla slightly widened anteroposteriorly with anterior border convex; posterior tip reaching a vertical beyond anterior border of pupil. Eyes larger than snout length.

Premaxilla with one tooth row with five* (13) or six (3) teeth hepta- to nonacuspidate, usually nonacuspidate. Maxilla with two* (26) or three (1) teeth, usually heptacuspidate. Four anteriormost dentary teeth bearing nine cusps, followed by 1-4 (15; 2*) teeth gradually smaller with seven, five and three cusps, in a single row. All cusps nearly equal in size in premaxillary and maxillary teeth; central cusp slightly larger than other cusps in dentary teeth. Premaxillary and maxillary teeth strongly flattened and slightly pedunculate (Fig. 5).

Dorsal-fin rays ii,8(2), iii,8(1) ii,9*(24), or iii,9(1); first unbranched ray approximately half length of second ray. Dorsal-fin origin located slightly posterior to middle of SL and nearly vertical through pelvic-fin origin.

Table 1. Morphometric data of *Dinotopterygium uniodon* and *D. diodon*. Range includes the holotype. H, holotype; SD, standard deviation; m, males; f, females.

	D. uniodon					***D. diodon***				
		Paratypes					**Paratypes**			
	H	**N**	**Range**	**Mean**	**SD**	**H**	**N**	**Range**	**Mean**	**SD**
Standard length (mm)	36.1	28	21.3-43.1	33.5	–	46.4	89	22.3-46.5	33.8	–
Percents of Standard length										
Predorsal distance	53.7	28	49.5-55.6	53.1	1.3	51.1	89	50.0-57.4	53.5	1.4
Prepelvic distance	50.8	28	44.8-55.8	49.9	1.9	49.6	89	48.6-56.9	52.6	1.6
Prepectoral distance	25.4	28	23.9-28.5	25.5	1.1	25.3	89	24.9-30.0	27.3	1.5
Preanal distance	67.9	28	64.1-68.1	66.8	1.5	65.7	89	61.7-70.9	66.9	1.9
Depth at dorsal-fin origin	30.2	28	28.3-36.3	31.4	2.1	32.3	89	28.7-35.8	32.4	1.5
Caudal peduncle depth	11.0	28	8.9-12.4	11.0	0.7	11.5	89	9.6-12.9	11.3	0.7
Caudal peduncle length	16.6	28	16.3-24.9	19.0	1.8	18.8	89	15.1-22.3	18.8	1.7
Dorsal-fin base	13.2	28	10.0-16.4	12.8	1.3	12.7	89	10.1-15.2	12.8	1.2
Anal-fin base (m)	19.7	15	15.9-24.0	19.7	1.7	20.3	44	16.9-23.9	20.3	1.5
Anal-fin base (f)	–	10	16.0-18.6	17.5	0.8	–	45	14.6-20.5	18.0	1.4
Dorsal-fin length	24.4	28	19.2-25.7	22.6	1.7	22.4	89	18.3-26.4	22.6	1.9
Pelvic-fin length (m)	16.5	15	13.5-17.7	15.7	1.2	14.5	44	12.9-16.7	15.0	0.9
Pelvic-fin length (f)	–	10	13.7-16.0	14.8	0.8	–	45	12.5-16.2	14.7	0.9
Pectoral-fin length	19.8	28	14.0-20.8	18.4	1.6	17.8	89	13.9-21.4	18.0	1.8
Head length	25.2	28	23.3-28.1	25.5	1.2	25.2	89	24.5-30.9	27.5	1.3
Percents of Head length										
Head depth	88.3	28	77.6-99.5	87.4	5.6	93.1	89	79.2-101.6	88.8	4.4
Snout length	22.9	28	18.4-26.3	22.7	1.8	18.4	89	17.5-28.0	23.2	1.9
Upper jaw length	38.3	28	22.1-43.1	36.8	3.9	38.8	89	31.5-49.5	40.9	2.9
Orbital diameter	31.6	28	27.7-39.1	33.0	2.7	29.9	89	29.2-37.1	33.2	1.7
Interorbital width	27.1	28	27.1-36.2	31.3	2.3	35.3	89	28.3-40.7	32.5	2.4

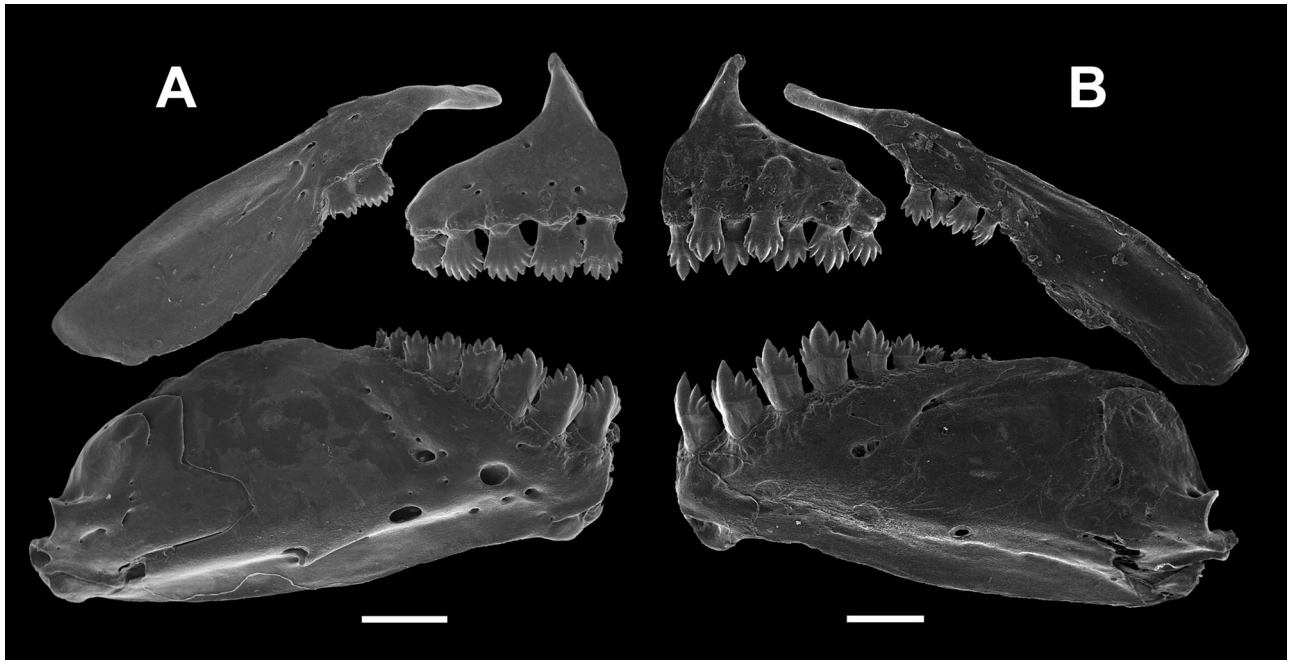

Fig. 5. Medial view of right and left-hand side premaxilla, maxilla and dentary of (A) *Dinotopterygium uniodon* sp. nov. (MZUSP 40358, paratype, 29.4 mm SL, female); and (B) *D. diodon* (MCN 13430, paratype, 37.2 mm SL, male, respectively, showing the polymorphism in the premaxillary teeth. Scale bar: 500 µm.

Distal margin of dorsal fin slightly convex. Adipose-fin located at or slightly posterior to vertical through insertion of last anal-fin ray. Anal-fin rays iii,14(1), iii,15(4), iv,13(3), iv,14(13), iv,15*(6), or v,15(1). Anal-fin origin clearly posterior to vertical through base of last dorsal-fin ray in both males and females. Last unbranched and

anterior 10-12 anal-fin rays sagitally expanded and bearing retrorse hooks in posterolateral border, along distal half-length of fin rays. Pectoral-fin rays i,9(2), i,10*(18), i,11(7), or ii,10(1); not reaching pelvic-fin origin. Pelvic-fin rays i,6(1), i,6, i(10), i,7*(16), or i,8(1). Pelvic-fin origin located near the middle of SL. Pelvic fin larger in males; tip of longest ray not reaching anal-fin origin in both sexes. Caudal fin forked, lobes similar in size, with 19(28) principal rays, with scales solely on the base. Dorsal procurrent rays 4-8(20), ventral procurrent rays 4-7(19).

Scales cycloid, moderately large. Lateral line incomplete, perforated scales 8(1), 9(6), 10(5), 11(4), 12*(7), 13(2), or 14(1). Longitudinal scale series including pored scales 33(17), 34*(9), or 35(1). Scale rows between dorsal-fin origin and lateral line 5*(27); scale rows between lateral line and pelvic-fin origin 3*(4), 4(22) or 5(1). Predorsal scales 11*(11) or 12(17), arranged in regular series. Scale rows around caudal peduncle 11(6), 12*(20), or 13(1). Axillary scale on pelvic fin origin extending over one or two longitudinal scale series. Scale sheath along anal-fin base 4(2), 5(14), 6*(9) or 7(2) scales in single series, extending to a base of sixth to tenth branched rays.

Precaudal vertebrae 16(2), caudal vertebrae 17(2), total vertebrae 33(2); first pterygiophore of dorsal fin located between 14th and 15th precaudal vertebra; first pterygiophore of anal fin located between third and fifth caudal vertebra. Supraneurals 7(2). Frontals contacting each other anteriorly to fontanel, that extends from that point to supraoccipital. Supraorbital absent. Third infraorbital largest; fourth infraorbital reduced allowing a partial contact between infraorbitals 3 and 5. Anterior ceratohyal bearing 3 branchiostegal rays and posterior ceratohyal one. Gill rakers 6 on epibranchial, 1 on cartilage connecting epibranchial and ceratobranchial, 9 on ceratobranchial 10, and 2 on hypobranchial in two c&s specimens.

Colour in alcohol. Specimens are deeply discolored by the long time of preservation. Dorsal and dorsolateral portions of head and body dark brown. Apparently a very faint vertical humeral spot is visible in holotype extending over four or five scale series. Midlateral body stripe extending from the humeral spot to caudal peduncle. Midlateral black line present along the junction of dorsal and ventral myotomes. A very faint longitudinal spot is visible in the posterior termination of the caudal peduncle. Fins lacking distinctive marks, except for the black pigmentation along middle caudal-fin rays.

Colour in life. Unknown.

Sexual dimorphism. As described for the genus. Males and females also slightly differ in anal-fin base and pelvic-fin lengths (Table 1). Gill glands (*sensu* Burns & Weitzman, 1996) were not found on the first gill arch on both males and females. Males exhibited variable shades of red irregularly scattered at the base of the anal and caudal fins and its respective interradial membranes.

Distribution. *Dinotopterygium uniodon* is known from tributaries of the rio das Almas, rio Paranã drainage, upper rio Tocantins basin, Chapada dos Veadeiros, Goiás State, Brazil (Fig. 4).

Conservation status. The new taxon seems to be restricted to a small tributary of the rio Paranã which surrounds the Chapada dos Veadeiros National Park, central Brazil. The few records are within the multiple use Pouso Alto Protected Area (Área de Proteção Ambiental do Pouso Alto), specifically at a destined portion for intense agricultural activities regime (Fig. 4). Since the use of pesticides and man-induced burnings for soil management are commonly known for the region, the agricultural activities represent the main threat for the new and restricted taxon. These intense activities cover most of the upper portion of the tributary including many streams that the species could potentially occur. Thus, the use of pesticides might affect a considerably large portion of the species distribution as it flows along the whole tributary until the rio das Almas. The species is known from solely three sites in the Cerrado biome, municipality of Cavalcante, GO, in the area with intense deforestation (http://semcerrado.org.br/eng/). The Extent of Occurrence (EOO) is 38.209 Km^2 and the Area of Occupancy (AOO) is 12 Km^2. The specimens were collected in 1989, 1996 and 2008, with only one in 2008. Additional efforts in type locality did not find the species. Additional efforts were done in last 10 years in this area, but no more specimens were found. The region suffers intense pressure from deforestation and burning for agriculture, with evident decline in the quality of habitat due to the use and occupation of the soil. Therefore, the species might be categorized as Critically Endangered (CR) by criteria B1 2ab(i,iii) following IUCN's criteria (IUCN Standards & Petitions Committee, 2019).

Etymology. The name *uniodon* from the Greek *uni* meaning one and *odon* meaning tooth refers to the single series of teeth in the premaxilla. A name in apposition.

Remarks. In our analyses the following character states were found to be autapomorphies of *Dinotopterygium uniodon*: eight or fewer dentary teeth on outer row (character 198, state 0); main portion of fourth basibranchial ossified (character 276, state 1).

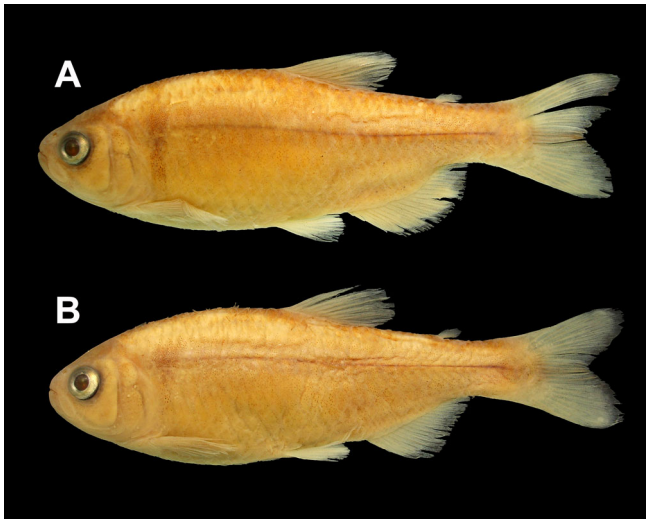

Fig. 6. *Dinotopterygium diodon* sp. nov., (A) MCN 18936, holotype, 46.4 mm SL, male; (B) MCN 13437, paratype, 46.5 mm SL, female.

Dinotopterygium diodon Frainer, Carvalho, Bertaco & Malabarba sp. nov.

(Figs. 1, 5, 6, 7)

Zoobank ID. D79DBDB4-ED08-47C1-A118-3E5D40D30BAD

Representative sequences. *CytB*, GenBank accession number OK143443; *COI*, GenBank accession number OK143442; and *16S*, GenBank accession number OK143444.

Holotype. MCN 18936, 46.4 mm SL, male, Brazil, Goiás State, municipality of Teresina de Goiás, córrego Tereza at km 2 of the road GO-241, upper rio Tocantins basin, 13°47'39.6"S 47°17'29.1"W, 6 Nov 1996, W. R. Koch & K. M. Grosser.

Paratypes. All from Brazil, Goiás State. MCN 13437, 46.5 mm SL, female, collected with the holotype. MCN 13430, 20 (6 males, 28.9-46.4 mm SL, 1 c&s male, 37.2 mm SL; 14 females, 25.2-46.5 mm SL, 1 c&s female, 31.0 mm SL), MCN 13436, 1 female, 35.8 mm SL, MCN 13429, 11 (5 males, 33.6-39.0 mm SL, 6 females, 31.8-37.3 mm SL), córrego Poções at km 7 of the road GO-241, Teresina de Goiás, 13°47'08.4"S 47°19'08.5"W, 6 Nov 1996, W. R. Koch & K. M. Grosser. MCN 13142, 5 (3 males, 35.7-38.6 mm SL; 2 females, 29.4-29.7 mm SL), córrego Dois Irmãos, GO-241, Cavalcante, 13°47'43.8"S 47°19'56.8"W, 2 Aug 1996, W. R. Koch et al. LBP 19062, 30 (13 males, 31.4-38.8 mm SL; 17 females, 22.34-36.69 mm SL), córrego Tereza, rio Tocantins drainage, Teresina de Goiás, 13°47'39.6"S 47°17'29.1"W, 16 Aug 2014, C. Oliveira, M. Taylor, B. Melo & G. Costa Silva. MZUSP 113683, 20 (16 males, 31.6-40.8 mm SL, 4 females, 28.6-39.5 mm SL), same locality as LBP 19062, 25 May 2008. MZUSP 114399, 1 female, 35.6 mm SL (+ tissue sample) Teresina de Goiás; unnamed creek, rio Tocantins drainage, 13°45'44.1''S 47°12'54''W, O. T. Oyakawa, A. M. Zanata, P. Camelier, M. Melo.

Diagnosis. *Dinotopterygium diodon* is distinguished from *D. uniodon* by the presence of two tooth series in the premaxilla (*vs*. one); number of cusps of maxillary teeth (pentacuspidate *vs*. heptacuspidate); teeth bearing all cusps nearly equal in size in the maxilla and premaxilla; and central cusp slightly larger than remaining cusps in dentary (*vs.* all teeth bearing the central cusp clearly larger than remaining tooth cusps).

Description. Morphometric data summarized in Table 1. Body compressed and elongate; greatest body depth anterior to dorsal and pelvic fins. Dorsal profile convex from snout tip to dorsal-fin origin; nearly straight and dorsoventrally slanted along dorsal-fin base, and straight from dorsal fin to adipose fin. Ventral body profile smoothly convex from lower jaw to pelvic-fin origin, and slightly concave from pelvic fin to anal-fin origin. Body profile along anal-fin base nearly straight in females and clearly convex in males. Dorsal and ventral margins of the caudal peduncle slightly concave.

Mouth terminal, lower jaw and upper jaw nearly equal. Maxilla short extending to half-length of infraorbital 2 and aligned at angle of approximately 45 degrees relative to longitudinal body axis. Maxilla slightly widened anteroposteriorly; posterior tip reaching a vertical slightly posterior to anterior border of pupil. Eyes slightly larger than snout length.

Premaxilla with two tooth rows: outer row with two (15) or three* (7) pentacuspid teeth; inner row with five* (22) teeth with 5 to 7* cusps, usually heptacuspidate. Maxilla with two (2), three* (18) or four (2) teeth, usually pentacuspidate. Five* anteriormost dentary teeth larger, heptacuspid, followed by five teeth smaller and pentacuspid, in a single row. Central cusp in all teeth larger than other cusps. Premaxillary and maxillary teeth flattened and slightly pedunculate (Fig. 5).

Dorsal-fin rays ii,9*(20; ii,8 in one specimen); first unbranched ray approximately half length of second ray. Dorsal-fin origin located slightly posterior to middle of SL and nearly at vertical through pelvic-fin origin. Distal margin of dorsal fin convex. Adipose-fin located

approximately at vertical through insertion of last anal-fin ray. Anal-fin rays iv,14(3), iv,15*(16), or iv,16(2). Anal-fin origin nearly at vertical through base of last dorsal-fin ray in males and posterior to that point in females. Last unbranched and anterior 7-11 anal-fin rays sagitally expanded and bearing hooks in posterolateral border, along distal half-length of fin ray. Pectoral-fin rays i,9(1), i,10*(14), or i,11 (6); not reaching pelvic-fin origin. Pelvic-fin rays i,5(2), i,7*(19), or i,8(1). Pelvic-fin origin located near middle of SL or slightly posterior to that point. Pelvic fin larger in males; tip of longest ray reaching close to anal-fin origin in males and remaining distant from that fin in females. Caudal fin forked, lobes similar in size, with 19(20) principal rays, with scales solely on the base. Dorsal procurrent rays 4-7(20; 5*), ventral procurrent rays 4-7(20; 5*).

Scales cycloid, moderately large. Lateral line incomplete, perforated scales 9(2), 10(4), 11*(6), 12(7), 14(2), or 17(1). Longitudinal scale series including pored scales 33(14) or 34*(8). Scale rows between dorsal-fin origin and lateral line 5*(22); scale rows between lateral line and pelvic-fin origin 4*(22). Predorsal scales 12(2), 13(13), or 14*(6), arranged in regular series. Scale rows around caudal peduncle 13(9), 14*(7) or 15(5). Axillary scale of pelvic fin extending over one or two longitudinal scale series. Scale sheath along anal-fin base 6(2), 7(3), 8(1), 9(10), 10(2), 11*(2), or 12(1) scales in single series, extending to base of 9-12 branched rays.

Precaudal vertebrae 17(2), caudal vertebrae 18(2), total vertebrae 35(2); first pterygiophore of dorsal fin located between 14th and 16th precaudal vertebra; first pterygiophore of anal fin located between second and fourth caudal vertebra. Supraneurals 7(2). Frontals contacting each other anteriorly to fontanel, that extends from that point to supraoccipital. Supraorbital absent. Third infraorbital largest; fourth infraorbital reduced or absent. Anterior ceratohyal bearing 3 branchiostegal rays and posterior ceratohyal one. Gill rakers 6 on epibranchial, 1 on cartilage connecting epibranchial and ceratobranchial, 10 on ceratobranchial 10, and 2 on hypobranchial counted in two c&s specimens.

Colour in alcohol. Specimens collected in 1996 are discolored by the long time of preservation. Dorsal and dorsolateral portions of head and body dark brown. A faint vertical humeral spot is visible in the holotype (male) forming a vertical rectangular stripe, darker dorsally and extending ventrally close to the pectoral fin. Humeral spot in female triangular, more expanded horizontally in its dorsal portion and not extended close to pectoral fin. Midlateral body stripe extending from the humeral spot to caudal peduncle. Midlateral black line present along the junction of dorsal and ventral myotomes. A faint triangular caudal spot is visible in the female but it is not clearly discernible in the holotype. Fins lacking distinctive marks, except for the light black pigmentation along middle caudal-fin rays, visible in the holotype.

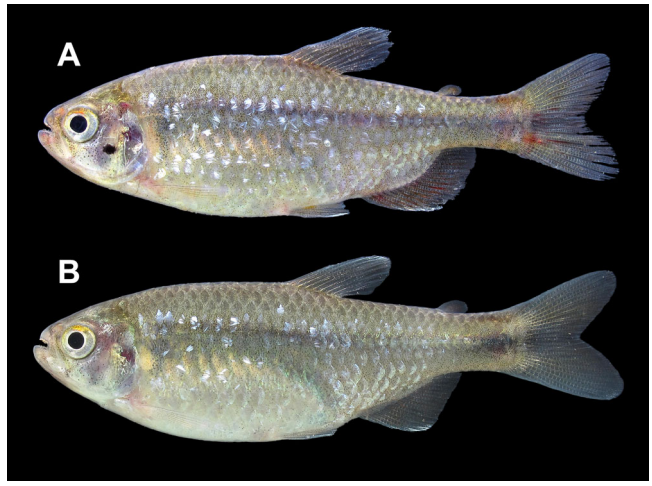

Fig. 7. *Dinotopterygium diodon* sp. nov., (A) MZUSP 113683, paratype, 40.79 mm SL, male; (B) MZUSP 113683, 36.26 mm SL, female just after collection. The circular black spot on the infraorbital 3 in (A) does not represents the general coloration in males. Photo: Fernando Dagosta.

Colour in life. Males more colorful than females presenting variable shades of red irregularly scattered at the base of the dorsal, anal and caudal fins, and at the interradial membranes of pectoral, pelvic, adipose, anal, and caudal fins (Fig. 7). Except by this sexual dimorphism, the whole fins exhibited light yellow pigmentation to hyaline. Dorsal portion of the body light silver. Inconspicuous humeral spot vertically oriented formed by chromatophores concentration just posterior to the opercular opening. Upper portion of the eye yellow to red in males and yellow to light red in females. Head of males light red blotches at anterior region of the dentary, premaxilla and nasal bones.

Sexual dimorphism. As described for the genus. Gill glands (*sensu* Burns & Weitzman, 1996) were not found on the first gill arch on both males and females. Males slightly more colorful than females with red chromatophores at the base of the dorsal, caudal and anal fins (Fig. 7). In addition, few branched rays in the caudal and anal fin also exhibited red chromatophores with more intensity in the anal fin.

Distribution. *Dinotopterygium diodon* is known from a very restricted range in the rio Paranã drainage, upper rio Tocantins basin, Chapada dos Veadeiros, Goiás State, Brazil (Fig. 4).

Conservation status. Same conditions described for *D. uniodon*. The Extent of Occurrence (EOO) is 6.294 km^2 and the Area of Occupancy (AOO) is 16 km^2 for *Dinotopterygium diodon*. Thus, the species might be categorized as Critically Endangered (CR) by criteria B1 2ab(i,iii) following IUCN's criteria (IUCN Standards & Petitions Committee, 2019).

Etymology. The name *diodon* from the Greek *di*, meaning two, and *odon*, meaning tooth refers to the double series of teeth in the premaxilla. A name in apposition.

Remarks. In our analyses the following character states were found to be autapomorphies of *Dinotopterygium diodon*: two rows of premaxillary teeth (character 170, state 1); Neural pedicle of the third vertebra articulating synchondrally with neural complex (character 310, state 0).

Discussion

The new taxa share a unique combination of synapomorphies previously unknown in any other genus of Characidae (Eigenmann, 1915; Eigenmann & Myers, 1929; Gery, 1977; Mirande, 2010; Terán et al., 2020). The low support precluded us to include the new taxa in an existing genus (e.g., *Erythrocharax*, GC = 2) and thus the new species have been housed in a new genus. The features shared by the two new species of *Dinotopterygium* include the multicuspid teeth, and clear sexual dimorphism observed in body coloration and anal-fin morphology in adult males. Successive fusions between the posteroventral margin of proximal radials and the anterodorsal margin of medial radials might imply slight inclination of the last pterygiophores thus shaping the base of the fin (i.e., convex).

Adult males of *Erythrocharax altipinnis*, which were recovered as the sister group of the new taxa in most searches and in the most parsimonious tree (Fig. 1), exhibit elongated anal-fin rays compared to females (Netto-Ferreira et al., 2013), which visually change the typical convex profile found in the anal-fin of most characids to a straight distal margin. *Phycocharax rasbora*, recovered as the sister group of [*Erythrocharax* Netto-Ferreira, Birindelli, de Sousa, Mariguela & Oliveira + *Dinotopterygium* (new)], also exhibits anal-fin dimorphism and convex anal-fin base in adult males (Ohara et al., 2017).

The number of teeth rows in the premaxilla has been used in the traditional classification of Characidae (with few exceptions, such as *Paracheirodon*) to define groups at subfamily level (*e.g.* Cheirodontinae) (Malabarba, 1998). However, Ohara et al. (2017) noted this plasticity in mouth morphology in the group currently included in Probolodini (i.e., comparing *P. rasbora* – single row – and *Hemigrammus* species – two rows). Additionally, species from the so-called "*Phycocharax* clade" (*sensu* Ohara et al., 2017) exhibited similar mouth morphology compared to the new taxa, including the presence of distally expanded teeth with five or more cusps at premaxillary, maxillary and dentary bones.

The addition of new taxa (i.e., the discovery of new forms in nature) showing this particular polymorphism between two sister species within the Characidae seems to have slightly changed the interrelationships of the Stethaprioninae (Terán et al., 2020) as some clades previously proposed have fluctuated between Stethaprionini, Probolodini, and Gymnocharacini tribes in our analyses. This is the case of the *Makunaima* group from the Probolodini and a well-supported *Moenkhausia* group from the Gymnocharacini, which moved to Stethaprionini (*sensu* Terán et al., 2020) and seems to be closely related to each other (Fig. 1). Additionally, other well supported groups from the Gymnocharacini have been moved into the Probolodini (*sensu* Terán et al., 2020), while part of the Probolodini was recovered as the sister group of both tribes. The second most parsimonious tree (i.e., GRO30), on the other hand, moved part of the Probolodini species into the Gymnocharacini in a similar arrangement found in the final tree (i.e., SEP68, Appendix S5).

Further comments on the classification and taxonomy of the Stethaprioninae, as well as on the exact position of the new taxa, should be taken with caution. The low support or its absence for the recovered groups precluded us from formally revising the subfamily-level classification as it remains labile to further data inclusion. However, the stability observed among some species included in Probolodini gave us insights on its potential position in current classifications, as well as on the evolutionary history of the highly polymorphic character in mouth morphology.

Although presenting no support, a group formed by some species from the Probolodini (*sensu* Terán et al., 2020) remained stable in both high scoring trees and included the new taxa. This group included *Moenkhausia ceros*, *Hyphessobrycon herbertaxelrodi*, *H. columbianus*, *Parecbasis cyclolepis*, *Macropsobrycon xinguensis*, *Moenkhausia cotinho*, *Deuterodon mutator*, *H. loweae*, *Phycocharax rasbora*, and *Erythrocharax altipinnis*. Additionally, the well supported lineage formed by *Hemigrammus haraldi* [*Hemigrammus aguaruna* + *Hemigrammus pulcher*] (hereafter, *Hemigrammus* clade) seems to have close relationships to the new taxa as both high scoring trees recovered this clade as the sister group

formed of (minimally) *Phycocharax rasbora* and *Dinotopterygium*. Thus, as long as the new taxa exhibit a sister group relationships to *E. altipinnis* and the clade formed by these species to *P. rasbora*, both sharing a single tooth series in the premaxilla, the polymorphism observed in the mouth morphology of the new taxa is most parsimoniously interpreted as a character reversion from one to two rows in the premaxilla. This is plausible given *Hyphessobrycon loweae* and *Hemigrammus* clade species are known for the second condition (Fig. 1). In this way, we selected *Dinotopterygium uniodon* as the type species for the genus.

Laland et al. (2015) proposed that independent acquired features or repeated evolution (i.e., homoplasies, in our cladistic point of view) may be due to convergent selection and/or developmental bias in isolated lineages. Phenotypic plasticity was already discussed as the main source of variation in Cichlidae mainly because of the timing of formation of mouth structures. Thus, it would contribute to differences in speciation rate and degree of endemism in this group (Meyer, 1987). Although character transformation/reversion (i.e., character $123-1 \rightarrow 0$) is known to be due to merging the external tooth row with the inner row of the premaxilla during ontogeny of *Bryconamericus lethostigmus*, Stevardiinae clade (Hirschmann et al., 2017), we did not perceive any variation in this respect. However, there was an exception of one young male (31.7 mm SL) presenting the second tooth row that was collected at the type locality of *D. uniodon*. Thus, it might be possible that the great phenotypic plasticity observed in the new taxa reflects a unique character transformation in this small clade of Probolodini characids.

Herein, we demonstrated how phylogenetic hypotheses of highly diverse groups (e.g., Characidae) may change when adding new information. The instability in tree topology, based on both morphology and molecules, compared to previous studies (Mirande, 2019; Terán et al., 2020) might reflect the informativeness of key characters for these three tribes within Stethaprioninae (i.e., Stethaprionini, Probolodini and Gymnocharacini). The Characidae tree of life seems to depend on further sampling at unresolved clades, including the Probolodini, as discussed herein. We recommend the two species of *Dinotopterygium* be categorized as critically endangered (CR) and it reflects the actual scenario of the Cerrado biome (Latrubesse et al., 2019). Additionally, both new lineages were discovered in historical scientific collections, which illustrates the importance of the institutions that house specimens for the biodiversity interpretation and classification, as well as conservation (Shaffer et al., 1998). However, such institutions are constantly vulnerable to funding reductions and potential closures. Many important scientific institutions in Brazil are currently under threat, including the fish collection of the Museu de Ciências Naturais (MCN), Fundação Zoobotânica, where *D. diodon* was discovered. This study highlights the importance of scientific collections in allowing us to continually assess and refine our understanding of organismal diversity and conservation needs in a rapidly changing world.

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Disclosure statement

No potential conflict of interest was reported by the autor(s).

Supplementary material

Supplementary material for this article can be accessed here: http://dx.doi.org/10.1080/14772000.2021.1986167.

Appendix S1. List of examined specimens.

Appendix S2. New taxa coding for morphological characters, and molecular sequences generated in this study (*D. diodon*; *CytB* [990 bp], *COI* [651 bp] and *16S* [547 bp]).

Appendix S3. Five new characters coding for 70 species.

Appendix S4. TNT files: the analyzed dataset (dinotopterygium.tnt); the resulting list with points for the most parsimonious trees (metacriterion.txt); the resulting 45 trees obtained from the combination of the five weighting schemes and nine weighting strengths, including the final hypothesis (i.e., SEP68), and the consensus of the most parsimonious trees under equal weights (equal.tree). All trees are in parenthetical TNT format.

Appendix S5. Final hypothesis (i.e., SEP68_GC.svg) and the consensus of the most parsimonious trees under equal weights (i.e., equal_GC.svg) in .svg format including branches supports (GC values).

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