UNIVERSIDADE
ESTADUAL DE LONDRINA

HUMBERTO DE LIMA FILHO

ANÁLISE FILOGENÉTICA DO GÊNERO *MEGALEPORINUS* RAMIREZ *ET AL.*, 2016 (CHARACIFORMES: ANOSTOMIDAE).

Londrina
2024

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Ficha de identificação da obra elaborada pelo autor, através do Programa de Geração Automática do Sistema de Bibliotecas da UEL

Lima Filho, Humberto.

Análise filogenética do gênero Megaleporinus Ramirez et al., 2016 (Characiformes, Anostomidae) / Humberto Lima Filho. - Londrina, 2024.
91 f. : il.

Orientador: José Luis Olivan Birindelli.
Dissertação (Mestrado em Ciências Biológicas) - Universidade Estadual de Londrina, Centro de Ciências Biológicas, Programa de Pós-Graduação em Ciências Biológicas, 2024.
Inclui bibliografia.

1. Região Neotropical - Tese. 2. Ictiofauna - Tese. 3. Evidência Total - Tese. 4. Registro Fóssil - Tese. I. Luis Olivan Birindelli, José . II. Universidade Estadual de Londrina. Centro de Ciências Biológicas. Programa de Pós-Graduação em Ciências Biológicas. III. Título.

CDU 59

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Londrina, 26 de fevereiro de 2024

AGRADECIMENTOS

Ao Prof. Dr. José Birindelli, meu orientador, que teve paciência e confiança no meu trabalho, e que se fez presente em todos os momentos de dúvida e dificuldade.

À Dra. Lenice Souza Shibatta, por sua ajuda no sequenciamento e obtenção das amostras para as análises moleculares que são tão importantes nesse trabalho.

Aos meus companheiros de laboratório, pelas conversas frutíferas e pontos de vista que, por diversas vezes, foram de grande ajuda na elaboração deste trabalho.

Sou muito grato às instituições nacionais como o NUPELIA da Universidade Estadual de Maringá e a Universidade de São Paulo, e também às internacionais como a Academia de Ciências Naturais da Filadelfia, ao Museu da Universidade de Auburn, ao Museu Field de História Natural e a Universidade da Flórida pela contribuição na forma de empréstimos de material biológico para o estudo da morfologia.

À minha mãe Andresa Andrelino e irmã Jordana Andrelino de Lima por estar sempre comigo na maior parte da minha jornada acadêmica e por me apoiar mesmo sem saber com o que eu trabalho exatamente. E meu tio Paulo José Andrelino (Tio Paulinho) por ser meu modelo e inspiração para fazer da pesquisa a minha profissão.

Por fim, mas não menos importante, agradeço a Gabriela Marins C. de Oliveira por seu companheirismo e amor que me serviram de apoio em tempos de tristeza e inspiração para meus momentos de alegria. Te amo Gabi!

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior -Brazil (CAPES) - Finance Code 001

“Como podemos estar à luz de uma descoberta
e não agir?”
- John Hammond

Lima, HF & Birindelli, JLO. Análise filogenética do gênero *Megaleporinus* Ramirez *et al.*, 2016 (Characiformes: Anostomidae). 2024. 84 f. Dissertação (Mestrado em Ciências Biológicas) – Universidade Estadual de Londrina, Londrina, 2024.

RESUMO GERAL

A diversidade de peixes neotropicais é ainda subestimada e as relações filogenéticas entre suas espécies ainda são pouco conhecidas. Os Characiformes integram cerca de 40% das espécies de peixes dulcícolas da América do Sul e possuem os Anostomidae como membros da sua segunda família mais diversa. Em 2016, o gênero *Megaleporinus* foi descrito para abrigar espécies de Anostomidae anteriormente alocadas em *Hypomasticus* e *Leporinus*. As relações entre as espécies de *Megaleporinus* só haviam sido investigadas com base em dados moleculares. Há suspeitas de que esse gênero seja mais diverso do que se conhece e que possivelmente abrigue a única espécie extinta de Anostomidae: *Leporinus scalabrinii*†. Este trabalho tem como objetivo investigar a evolução e classificação das espécies de *Megaleporinus*. Para isso, foram propostos 70 caracteres morfológicos, que foram combinados com sequências de cinco genes, dois deles mitocondriais e três nucleares. Os resultados indicam que *Megaleporinus* teria se originado a cerca de 18 milhões de anos atrás e seria composto por *M. muyscorum*, a única espécie trans-Andina, como grupo irmão de todas as congêneres, e por dois clados. O primeiro clado é formado por *M. macrocephalus*, *M. trifasciatus* e por duas espécies não descritas, uma proveniente do rio Orinoco e outra do Tocantins. O outro clado seria formado pelas espécies *M. conirostris*, *M. reinhardti, M. brinco*, *M. garmani*, *M. gaiero*, *M. piavussu*, *M. obtusidens*, *M. elongatus* e *Leporinus scalabrinii*†, espécie extinta há cerca de 6 milhões de anos, que deveria ser transferida a *Megaleporinus*. Nossos resultados ainda mostraram que há uma série de características morfológicas que permitem a descrição e o reconhecimento das duas espécies novas de *Megaleporinus* relacionadas à *M. trifasciatus* e *M. macrocephalus*.

Palavras-chave: Região-Neotropical; Ictiofauna; Evidência-Total; Registro-fóssil; Taxonomia.

Lima, HF & Birindelli, JLO. Phylogenetic Analysis of the genus Megaleporinus Ramirez *et al.*, 2016 (Characiformes: Anostomidae). 2024. 84 pp. Dissertation (Master's degree in Biological Sciences) – Universidade Estadual de Londrina, Londrina, 2024.

GENERAL ABSTRACT

The diversity of neotropical fish is still underestimated, and the phylogenetic relationships among their species are still poorly understood. Characiformes comprise about 40% of the freshwater fish species in South America and have Anostomidae as their second most diverse family. In 2016, the genus *Megaleporinus* was described to accommodate species of Anostomidae previously classified under the *Hypomasticus* and *Leporinus* genera. The relationships among *Megaleporinus* species had only been investigated based on molecular data. There are suspicions that this genus is more diverse than currently known and may harbor the only extinct species of Anostomidae: *Leporinus scalabrinii*†. This study aims to investigate the evolution and classification of *Megaleporinus* species. For this purpose, 70 morphological characters were proposed, combined with sequences of five genes, two mitochondrial and three nuclear. The results indicate that *Megaleporinus* possibly originated about 18 million years ago, and comprises *M. muyscorum*, the only trans-Andean species, as the sister group of all congeners, and two clades. The first clade consists of *M. macrocephalus, M. trifasciatus*, and two undescribed species, one from the Orinoco river and another from the Tocantins river. The other clade would be formed by *M. conirostris, M. reinhardti, M. brinco*, *M. garmani*, *M. gaiero, M. piavussu, M. obtusidens*, *M. elongatus* and *Leporinus scalabrinii*†, an extinct species about 6 million years old, which should be transferred to *Megaleporinus*. Our results also showed a series of morphological characteristics that allow the description and recognition of the two new species of *Megaleporinus* related to *M. trifasciatus* and *M. macrocephalus*.

Key-words: Neotropical-Region; Ichthyofauna; Total-Evidence; Fossil-Record; Taxonomy.

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ANSP	Academy of Natural Sciences of Philadelphia
AUM	Auburn University Museum
CS	Cleared and Stained
FMNH	Field Museum of Natural History
NUPELIA	Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura
MZUEL	Museu de Zoologia da Universidade Estadual de Londrina
MZUSP	Museu de Zoologia da Universidade de São Paulo
NI	Neotropical Ichthyology
SK	Dry Skeleton
TAO	Teleost Anatomy and Ontology
UEL	Universidade Estadual de Londrina
UEM	Universidade Estadual de Maringá
UF	University of Florida

SUMMARY

APRESENTAÇÃO

O presente estudo está configurado conforme as normas exigidas pela revista NI (Neotropical Ichthyology), às quais podem ser encontradas em https://www.ni.bio.br/

INTRODUCTION

The South American ichthyofauna was formed more than 100 Mya in Gondwana (Albert *et al.,* 2011). The Neotropical region was the only one to endure most of the mass extinctions, as well as the biotic and climatic variations of the Cenozoic era (Reis *et al.,* 2016). This historic of ecological resistance contributed to the vast Biodiversity found in the Neotropics, especially in freshwater environments (Tisseuil *et al.,* 2013). On the other hand, the human occupation of South America is causing massive impacts on the environment by means of excessive human harvesting principally of large-bodied fish species (as the *Megaleporinus* species), for example, causing Biodiversity loss and extinction (Olden *et al*., 2007; Tedesco *et al.,* 2017).

The South American fish fauna is megadiverse, with approximately 6.500 described species and an estimative of 9.500 when total diversity is revealed (Reis *et al.,* 2016; Birindelli & Sidlauskas, 2018). Therefore, about 3.000 species of freshwater fish have yet to be discovered! Neotropical fishes represent 27% of the world's fish fauna, encompassing 20 orders, 69 families, and 739 genera (Reis *et al.,* 2016). Approximately 40% of this diversity is represented by the Characiformes, currently classified in 24 families, most of them exclusively Neotropical (Fricke *et al.,* 2023). Characiformes are widely distributed in the American continent, ranging from Mexico to Argentina and Africa, where the Alestidae, and Hepsetidae represent the African fish fauna. Evidence exists of a much wider distribution of Characiformes in the past, based on fossil record in the Europe and Arabian Peninsula (Gayet, 1981; Monod & Gaudant, 1998; Otero & Gayet, 2001; Malabarba & Malabarba 2010; Toledo-Piza *et al.*, 2024).

Anostomidae is the second most diverse family of Characiformes and possesses a wide distribution in South America, from the north of Colombia to Argentina (Sidlauskas & Birindelli, 2017). The family includes 16 genera and 145 valid species (Toledo-Piza *et al.*, 2024). The main features that characterize Anostomidae are: three or four large incisiform teeth on each premaxilla and dentary; maxilla small and toothless; complete lateral-line with 33 to 100 perforated scales; adipose fin present; dorsal, anal and pelvic fins relatively short (with less than 20 branched rays each); complete infraorbital series (i.e., with supraorbital); opercular opening united to isthmus, and pharyngeal teeth present and generally bearing two cusps per tooth (Sidlauskas & Vari, 2008; Baumgartner *et al.,* 2012; Ramirez*et al*., 2016). Most species are herbivorous and medium sized (around 150 mm standard length). Some species (especially those of *Leporinus* and *Megaleporinus*) possess economic value and are important resources in aquaculture and commercial and sport fishing (Zeinad & Prado, 2012; Lizarro & Arteaga, 2021). Other species, as those of *Abramites, Anostomus*, and *Leporinus*, play important roles in the Aquarium trade, where they are known as "headstanders" for their habit of swimming with their head down.

Anostomidae was proposed by Regan (1911) and initially encompassed species now recognized in Anostomidae, Chilodontidae, Curimatidae, and Prochilodontidae (Fricke *et al.,* 2023). Years later, Roberts (1973) hypothesized a close evolutionary relationship between Anostomidae and Prochilodontidae. After a decade, Vari (1983) suggested that Anostomidae was more related to Chilodontidae than to Prochilodontidae and designated the superfamily Anostomoidea to include Anostomidae, Chilodontidae, Curimatidae and Prochilodontidae. With the advance of molecular biology, Vari's hypothesis was tested and corroborated (*e.g.,* Melo *et al.,* 2022).

Recently, by integrating molecular and morphological data using traditional and new methods, our planet's biodiversity is being reevaluated, leading to the discovery of new species and new hypotheses of phylogenetic relationships that improve their biological classification. One such effort resulted in the description of *Megaleporinus*, a genus composed of previously allocated species in *Leporinus* and *Hypomasticus* (Ramirez *et al*., 2016; Figure 1). *Megaleporinus* was proposed based on phylogenetic analyses using molecular markers, cytogenetic features and diagnosed based on morphological characteristics (Ramirez *et al*., 2016). *Megaleporinus* is characterized by having only three unicuspidal teeth on each premaxilla and dentary (except for *M. garmani,* which has four teeth on the dentary); one to four dark rounded midlateral blotches on the body; 32 to 45 perforated scales on the lateral line; and a ZZ/ZW sexual chromosome system (Ramirez *et al*., 2016). The genus includes the largest species of its family, some reaching up to 500 mm in total length and weighing about 5 kg, hence its generic name. Most species of *Megaleporinus* migrate upstream during the reproductive season (Godoy, 1975; Ramirez *et al*., 2016). Most species of the genus are part of the local fishery resource in Brazil and neighbor countries, whereas a few species (*M. obtusidens*, *M. macrocephalus*) are farmed and exploited as a food resource (Zeinad & Prado, 2012; Lizarro & Arteaga, 2021).

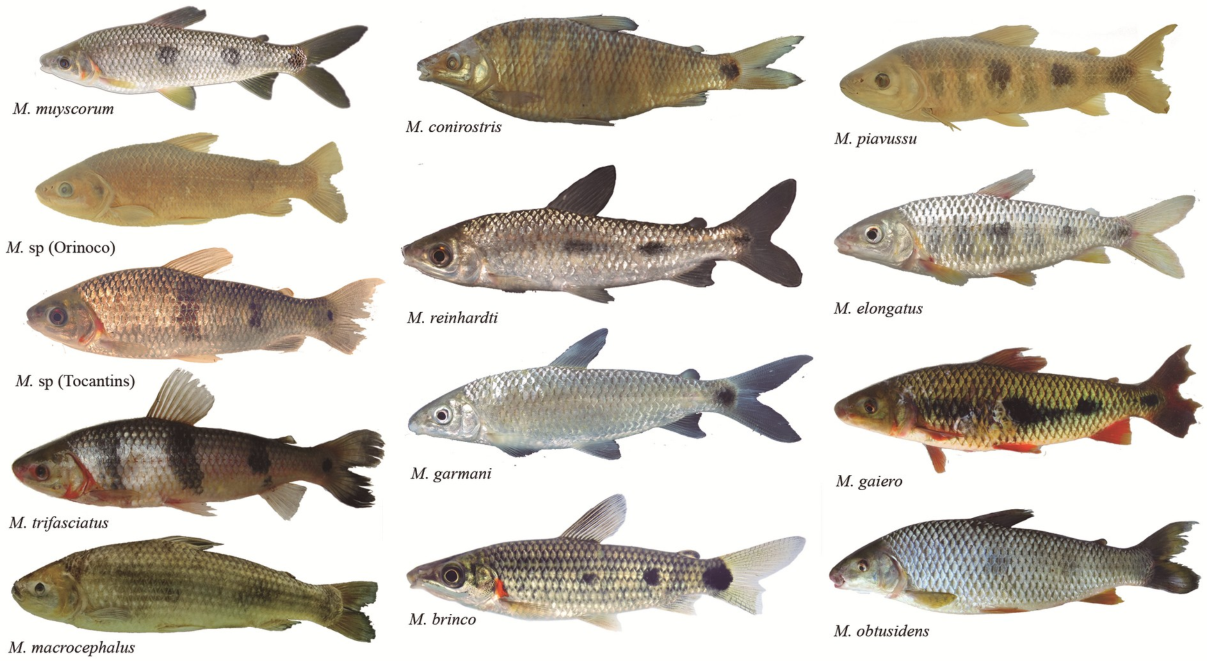


Figure 1. Specimens representing all valid species of *Megaleporinus*.

However, despite their commercial value, wide distribution, and large sizes, our knowledge of the morphology, diversity, and phylogenetic relationships of the species of *Megaleporinus* is still incomplete (Birindelli & Sidlauskas, 2017). The only comprehensive phylogenetic analysis focusing on Anostomidae used only morphological characters and did not include a single species of *Megaleporinus* (Sidlauskas & Vari, 2008; Figure 2a). On the other hand, the phylogenetic relationships of the species of *Megaleporinus* were studied based exclusively on molecular data (Ramirez *et al*., 2016); Figure 2b).

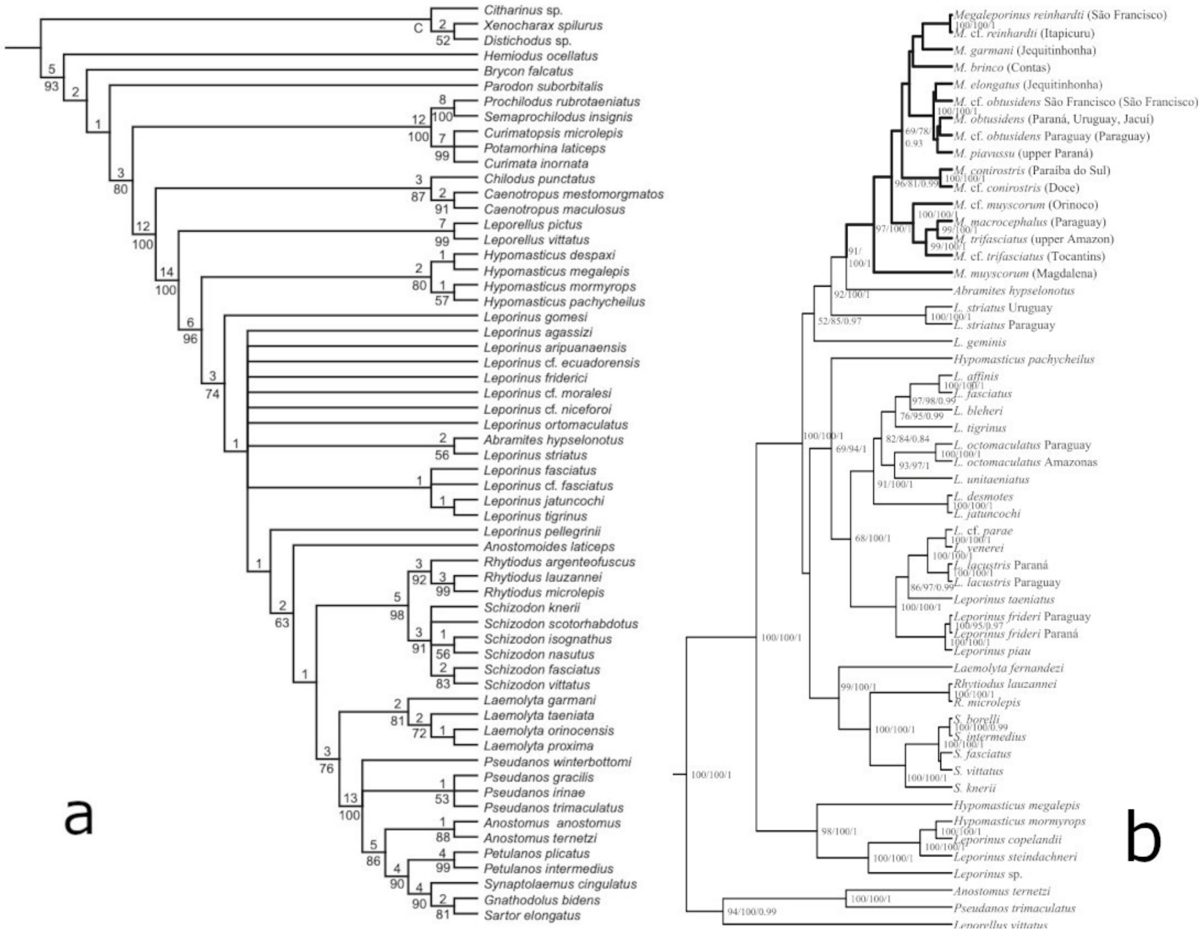


Figure 2. (**a**) Phylogenetic relationships of Anostomidae based on 123 morphological characters sed in a maximum parsimonious analysis by Sidlauskas & Vari (2008); (**b**) phylogenetic relationships among species of *Megaleporinus* based on a Bayesian-Inference analysis using five genetic markers (COI, Cytb, RAG1, RAG2, Myh6).

In addition, *Leporinus scalabrinii*† (Ameghino, 1898), the only known extinct species of Anostomidae, is considered closely related to *Abramites* (Bogan *et al.*, 2012) and to *Megaleporinus*, but its exact phylogenetic position in that clade requires further investigation (Ramirez *et al.*, 2016). Therefore, this study aims to fill the knowledge gap on the phylogeny, classification and diversification of species in *Megaleporinus* by studying morphological and molecular data.

MATERIAL & METHODS

TAXA SELECTION

We included all eleven valid species of *Megaleporinus* (Toledo-Piza *et al.*, 2024): *Megaleporinus brinco, M. conirostris, M. elongatus, M. gaiero, M. garmani, M. macrocephalus, M. muyscorum, M. obtusidens, M. piavussu, M. reinhardti,* and *M. trifasciatus*. In addition, we included two putatively new (undescribed) species, that are molecularly divergent (Ramirez *et al.*, 2017): one from the Tocantins-Araguaia basin and another from the Orinoco basin, herein referred to as *Megaleporinus* sp. (Tocantins) and *Megaleporinus* sp. (Orinoco) respectively. Furthermore, for morphological and molecular comparisons, we included: *Abramites hypselonotus, Anostomus anostomus, Hypomasticus megalepis, Hypomasticus mormyrops, Laemolyta taeniata, Leporellus vittatus, Leporinus amblyrhynchus, L. fasciatus, L. friderici, L. scalabrinii*†, *L. striatus, Pseudanos trimaculatus,* and *Schizodon intermedius* (see table 1). The Anostomidae is composed of three main groups/clades (Sidlauskas *et al.*, 2021): the first composed exclusively of *Leporellus*, the second composed of the Anostominae (sensu Winterbottom, 1980, and Sidlauskas & Vari, 2008), and the third including the remaining genera. *Leporellus* is represented herein by its type species: *L. vittatus*. Anostominae is represented in our study by two species, *Anostomus anostomus* and *Pseudanos trimaculatus*. The former is the type species of the type genus of the family, and the latter represents the subfamily's typical morphology, according to Winterbottom (1980). *Abramites* is a genus represented by two very similar species (Vari & Raredon, 1991), and the genus is considered the sister of *Megaleporinus* (Ramirez *et al.,* 2017). *Insperanos* putatively represent an ancient lineage within the remaining Anostomidae (Sidlauskas *et al.*, 2021) and is represented by its monotypic species: *I. nattereri*. *Hypomasticus megalepis* and *H. mormyrops* were selected as representants of *Hypomasticus*, a genus recently defined and redefined by Sidlauskas & Vari (2008) and Birindelli *et al.* (2020). *Leporinus* is a polyphyletic and diverse genus represented by approximately 80 valid species (Ramirez *et al.*, 2015; Toledo-Piza *et al.*, 2024) and is herein represented by four species: *L. fasciatus*, its type species; *L. friderici*, a widespread and common species; *L. amblyrhynchus*, a species that has only three teeth on each premaxilla and dentary, a feature known otherwise to characterize *Megaleporinus*; and *L. striatus*, a species considered to be closely related to *Abramites* plus *Megaleporinus* (Ramirez, Birindelli & Galetti, 2016). *Laemolyta taeniata* and *Schizodon intermedius* are included to represent a monophyletic group that also encompass *Rhytiodus* (Sidlauskas & Vari, 2008; Sidlauskas *et al.*, 2021). Finally, *L. scalabrinii*† is included because of its potential close relation to *Megaleporinus* (Ramirez *et al.*, 2016).

MOLECULAR DATA

Two mitochondrial (COI and CytB) and three nuclear (RAG1, RAG2 and MyH6) markers were selected due to the great quantity of data already available in the GenBank and Bold System databases (Table 1) for the loci in other anostomid species. Additional sequences of some taxa were obtained by extracting DNA using the MagMAX® kit and following the manufacturer's instructions (Applied Biosystems). For the polymerase reaction chain (PCR), we used the same primers as suggested by Ramirez, Galetti & Birindelli (2016), and the GoTaq Green Master Mix® (Promega) and FastPol HF DNA Pollymerase® (Cellco) kits, also following the manufacturer's instructions. The amplified samples were washed using 0.5µl of ExoSap IT® (Prodimol Biotecnologia S.A), and the sequencing reaction was made following the manufacturer's instructions of the Big Dye Terminator® v3.1 kit (Applied Biosystems). Sequencing used an ABI Prism 35000 XL (Applied Biosystems). Finally, the sequences were edited in Mega v.11 (Tamura, Stecher & Kumar, 2021) and aligned using the MUSCLE algorithm (Edgar, 2004). To prevent any problem caused by erroneous identification of genetic sequences from the databases, we performed previous phylogenetic analyses for each marker, using Maximum Likelihood in Mega, v. 11 (and compared each tree with the topology obtained from previous molecular phylogenetic studies). For this, the best model for nucleotide evolution was calculated for each marker individually also in Mega, v. 11. The different markers matrices were concatenated on Mesquite v.3.7 to export on ".nex" for further uses.

For molecular data, we include a single terminal per species, preferably identical specimens for all genetic markers. In fact, only *Anostomus anostomus* and *Laemolyta taeniata* did not have all these markers for the same specimen, so we combined sequences from different sources into their single terminals (see Table 1).

Table 1. List of molecular data used in the analysis.

Utilized material for molecular analysis								
Species	Basin	Museum voucher	COI	CytB	Myh6	RAG1	RAG2	Reference
Megaleporinus brinco	Contas	MZUSP 118670	KU134850	KU134868	KU134886.1	KU134904.1	KU134922.1	Ramirez *et al.*, 2017
Megaleporinus conirostris	Paraíba do Sul	-	KU134852.1	KU134870.1	KU134888	KU134906.1	KU134924.1	Ramirez *et al*., 2017
Megaleporinus elongatus	Jequitinhonha	MCNIP 0375	KU134853.1	KU134871.1	KU134889	KU134907	KU134925.1	Ramirez *et al*., 2017
Megaleporinus gaiero	Contas	-	MN731315.1	MN725061	MN725063	MN725065.1	MN725067.1	Birindelli *et al.*, 2020
Megaleporinus garmani	Jequitinhonha	MCNIP 0021	KU134855.1	KU134873	KU134891	KU134909.1	KU134927	Ramirez *et al.*, 2017
egaleporinus macrocephalu	Paraguay	MZUSP 118667	KU134856.1	KU134874	KU134892.1	KU134910.1	KU134928	Ramirez *et al*., 2017
Megaleporinus muyscorum	Magdalena	ICNMHN 19074	KU134857.1	KU1344875.1	KU134893.1	KU134911.1	KU134929.1	Ramirez *et al*., 2017
Megaleporinus sp. (Orinoco)	Orinoco	-	KU134851.1	KU134869.1	KU134887.1	KU134905.1	KU134923.1	Ramirez *et al.*, 2017
Megaleporinus obtusidens	Paraná	MZUSP 113982	KU134858.1	KU134876.1	KU134894.1	KU134912.1	KU134930.1	Ramirez *et al.*, 2017
Megaleporinus piavussu	Paraná	MZUSP 113981	KF568991	KF569034	KF569163.1	KF569077.1	KF569120.1	Ramirez *et al.*, 2017
Megaleporinus reinhardti	São Francisco	MCP 44776	KF568992	KF569035	KF569164.1	KF569078.1	KF569121.1	Ramirez *et al*., 2017
Megaleporinus trifasciatus	Amazonas	MUSM 47351	KU134865	KU134883.1	KU134901.1	KU134919	KU134937.1	Ramirez *et al*., 2017
Megaleporinus sp. (Tocantin	Tocantins-Araguaia	GEPEMA 5095	KF568998	KF569041	KF569170.1	KF569084	KF569127.1	Ramirez *et al*., 2017
Abramites hypselonotus	Madeira	-	KF568968	KF569011	KF569140	KF569054	KF569097	Ramirez *et al.*, 2016
Anostomus anostomus	Unknown	AMCC 102188	AMCC098-06	-	-	-	-	Ratnasingham & Herbert, 2007
Anostomus anostomus	Unknown	AMNH 233421	-	AY791352	-	-	-	Calcagnotto, Schaefer & DeSalle, 2005
Hypomasticus megalepis	Uatumã	MZUEL 10200	KX020571	KX020574	KX020577	KX020580	KX020583	Ramirez *et al.*, 2017
Hypomasticus mormyrops	Paraíba do Sul	MZUEL 08022	KX020572	KX020575	KX020578	KX020581	KX020584	Ramirez *et al.*, 2017
Laemolyta taeniata	Amazonas	MZUEL 14859	Voucher	-	-	-	-	The Authors
Laemolyta taeniata	Amazonas	UFRO 8004	-	KP864708	KP864720	KP864714	KP864726	Ramirez & Galetti, 2015
Leporellus vittatus	Paraguay	MZUSP 113987	KF568980	KF569023	KF569152	KF509066	KF569159	Ramirez *et al.*, 2016
Leporinus amblyrhynchus	Paraná	LBP 3917	EU185543	EU183046	-	-	-	Santos *et al.* (Unpublished)
Leporinus fasciatus	Amazonas	UFRO 13343	KF568981	KF569024	KF569153	KF569067	KF569110	Ramirez *et al.*, 2016
Leporinus friderici	Paraná	MZUSP 113983	KF568982	KF569025	KF569154	KF569068	KF569111	Ramirez *et al.*, 2016
Leporinus striatus	Paraguay	MZUSP 113986	KF568995	KF569038	KF569167	KF569081	KF569124	Ramirez *et al.*, 2016
Pseudanos trimaculatus	Amazonas	UFRO-I 14970	KF569003	KF569046	KF569175	KF569089	KF569132	Ramirez *et al.*, 2016
Schizodon intermedius	Paraná	MZUSP 113995	KF569008	KF569051	KF569180	KF569094	KF569137	Ramirez *et al.*, 2016

MORPHOLOGICAL DATA

For characters that vary ontogenetically, we considered the state only in adult specimens those with standard length (SL) equal to or greater than the size at the first maturity provided by Nakatani *et al.* (2001). In cases where information on the first maturation was unavailable, we used the SL listed by Nakatani *et al.* (2001) for the closest congener.

For external features, including coloration, we examined specimens preserved in 70% alcohol or based our characters on information provided in the species descriptions (especially for live coloration). For the bones and cartilaginous structures, we used cleared and stained specimens (CS) prepared according to the protocol proposed by Taylor & Van Dyke (1985), or specimens prepared as dry skeletons (SK), using the method described by Bemis *et al.* (2004), for specimens larger than 150 mm SL. Once prepared, the individuals were dissected using the Ridewood protocol (Bemis *et al.,* 2004). The osteological nomenclature used in this study followed Weitzman (1962) with modifications suggested in Sidlauskas & Vari (2008), with terms updated accordingly to the Teleost Anatomy Ontology (TAO) from Dahdul *et al.* (2010).

The illustrations were prepared via photographs taken by a three-megapixel camera attached to a LEICA DFC295 stereomicroscope or (especially for individuals larger than 150 mm SL) a NIKON D5600. After being photographed, images were edited to enhance quality and to highlight the nomenclature of the structures.

The morphological matrix included characters described by Winterbottom (1980), Vari (1983), and Sidlauskas & Vari (2008) that were verified in the specimens examined during this study (see Comparative Material) and re-interpreted when needed. In addition, new characters were proposed. Apomorphic characters of ingroup species (i.e., species of *Megaleporinus*) were included, whereas those that proved autapomorphic for outgroup species were not utilized or excluded. Delimitation of characters and character states followed the principles discussed by Sereno (2007). Due to the impossibility of treating multistate characters as ordered in Bayesian Analyses, multistate morphological characters were treated as unordered. A commented and illustrated list of morphological characters is included in the Results. The matrix was edited in Mesquite v. 3.7 (Maddison & Maddison, 2023) and exported in ".nex" and ".tnt" formats.

Examined specimens belong to the following collections: MZUEL, MZUSP, FMNH, NUPELIA (from UEM), AUM, UF, and ANSP (see list of acronyms for fish collections in Sabaj, 2020). Specimens not cataloged at the MZUEL were obtained through loans.

Comparative Material:

Anostomus anostomus, (Linnaeus 1758): MZUEL20134; N= 2; 35,1-42,27mm SL; Amazonas; MZUEL20447; N=2; 46,3-83,92mm SL; Kuyuwini; MZUSP85153; N=1; C&S; Negro;

Abramites hypselonotus (Günther 1868): MZUEL01389; N=1; 57,07mm SL; Miranda; MZUEL20135; N=2; 59,04-61,46mm SL; Orinoco; MZUEL05425; 72,5-107,94mm SL; Branco; MZUEL13323; N=1; 108,52mm SL; Paraná; MZUSP48123; N=1; C&S; Solimões;

Hypomasticus megalepis (Günther 1863): MZUEL10200; N=7; 36,37-105,48mm SL; Pitinga;

Hypomasticus mormyrops (Günther 1863): MZUEL15901; N=5; 87,92-174,4mm SL; Doce; MZUSP110150; N=1; SK; Paraíba do Sul;

Insperanos nattereri (Steindachner 1976): MZUEL20021; N=1; 102,71mm SL; Xingu; MZUSP5429; N=1; C&S; Trombetas;

Laemolyta taeniata (Kner, 1958): MZUEL10218; N=6; 153,3-178,8mm SL; Uatumã; MZUEL14859; N=3; 166,55-176,32mm SL; Negro; MZUEL10218; N=1; SK; Uatumã;

Leporellus vittatus (Eigenmann 1922): MZUEL00401; N=1; 36,68mm SL; Tibagi; MZUEL13998; N=2; 63,8-71,75mm SL; Miranda; MZUEL12263; N=2; 75,93-90,99mm SL; Miranda; MZUEL 15107; N=1; 57,03mm SL; Paranapanema; MZUEL01910; N=1; 107,92mm SL; Paranapanema; MZUEL16410; N=1; 167,55mm SL; Paranapanema; MZUEL05353; N=3; 160,96-189,16mm SL; Paranapanema; MZUEL16947; N=1; SK; Paraná;

Leporinus amblyrhynchus Garavello & Britski 1987: MZUEL16433; N=13; 92,35-174,8mm SL; Jequitaí; MZUEL04479; N=10; 125,41-156,94mm SL; Taquara; MZUEL16949; N=2; 159,56-180,75mm SL; Tibagi; MZUEL14553; N=1; 196,45mm SL; Tibagi; MZUEL22260; N=2; SK; Paranapanema;

Leporinus fasciatus (Bloch 1794): MZUEL10187; N=1; 201,34mm SL; Uatumã; MZUEL11547; N=2; 112,5-116,26mm SL; Pitinga; MZUEL10196; N=1; SK; Pitinga;

Leporinus friderici (Bloch 1794): MZUEL20561; N=1; 122,07mm SL; Pirarara; MZUEL20562; N=1; 149,73mm SL; Pirarara; MZUEL22168; N=2; 52,92-139,45mm SL; Miranda; MZUEL08467; N=1; SK; Paraguay;

Leporinus striatus Kner 1858: MZUEL22224; N=36; 24,51-77,36mm SL; Miranda; MZUEL09520; N=1; 125,18mm SL; Cinzas; MZUSP10633; N=5; SK=5; Paraná;

Megaleporinus brinco (Birindelli, Britski & Garavello 2013): MZUEL18590; N=2; 90,31-145,86mm SL; Contas; MZUEL18635; N=2; 30,04-116,23mm SL; Contas; MZUEL17832; N=3; SK=3; Contas; MZUSP110617; N=3; SK=1; Contas; MZUSP111261; N=1; SK; Contas;

Megaleporinus conirostris (Steindachner 1875): MZUEL17713; N=1; 253,91mm SL; Paraibuna; MZUSP87872; N=1; C&S; Mucuri;

Megaleporinus elongatus (Valenciennes 1850): MZUEL10686; N=1; 28,58mm SL; Jequitinhonha; MZUEL16472; N=1; 161,4mm SL; Pardo; MZUEL16472; N=2; SK=2; Pardo; MZUSP106810; N=1; SK; Jequitinhonha; MZUSP112107; N=1; SK; Pardo;

Megaleporinus gaiero Birindelli, Britski & Ramirez 2020: MZUEL18000; N=1; 301,15mm SL; Contas MZUSP111130; N=1; SK; Contas;

Megaleporinus garmani (Borodin, 1929): MZUEL16479; N=1; 93,5mm SL; Pardo; MZUEL18631; N=1; 97,71mm SL; Jequitinhonha; MZUSP86036; N=1; C&S; Jequitinhonha;

Megaleporinus macrocephalus (Garavello & Britski 1988): MZUEL10223; N=13; 43,6-63,57mm SL; Acquired from pisciculture (São Paulo); MZUEL09456; N=1; 125,6mm SL; Cinzas; MZUEL01362; N=1; 135,47mm SL; Tibagi; MZUEL20404; N=1; SK; Paranapanema; MZUEL15431; N=1; SK; Acquired from pisciculture; MZUSP89501; N=1; Paraguay;

Megaleporinus muyscorum (Steindachner, 1900): AUM35394; N=2; C&S=1; 98,67-118,42mm SL; Magdalena; FMNH76379; N=4; 240,28-256,24mm SL; Magdalena; MZUEL20453; N=1; 211,78mm SL; Magdalena;

Megaleporinus aff. *muyscorum* (Steindachner, 1900): UF78141; N=8; 99.98-184,84mm SL; Orinoco; MZUEL20454; N=1; 124,87mm SL; Orinoco; ANSP206311; N=1; SK; Apure; ANSP206986; N=1; SK; Apure;

Megaleporinus obtusidens (Valenciennes 1837): MZUEL09503; N=2; 84,45-85,67mm SL; Paranapanema; MZUEL06981; N=7; 73,91-88,48mm SL; Tibagi; MZUEL18370; N=4; 155,04-185,8mm SL; Uruguay; MZUEL11264; N=3; 200-242,2mm SL; Miranda; MZUEL20604; N=1; SK=1; Paraná; MZUEL22579; N=1; SK; Paraná; MZUEL16470; N=1; SK; São Francisco; MZUSP105067; N=1; SK; Acquired from a market in city of São Paulo;

Megaleporinus piavussu (Britski, Birindelli & Garavello 2012): MZUEL00449; N=1; 102,99mm SL; Paraná; MZUEL00448; N=1; 118, 92mm SL; Paraná; MZUEL04245; N=1; 93,18mm SL; Paraná; MZUSP25334; N=1; C&S; Grande;

Megaleporinus reinhardti (Lütken 1875): MZUEL07058; N=1; 118; 56mm SL; São Francisco; MZUSP110169; N=2; SK; São Francisco;

Megaleporinus trifasciatus (Steindachner 1876): MZUEL13868; N=4; SK; Amazonas; NUP019745; N=1; 410,2mm SL; Amazonas;

Megaleporinus aff. *trifasciatus* (Steindachner 1876): NUP24138; N=1; 183,64mm SL; Araguaia; MZUEL20700; N=2; C&S=1; 126,8-160,47mm SL; Araguaia;

Pseudanos trimaculatus (Kner 1858): MZUEL21578; N=1; 169,99mm SL; Jari; MZUEL14694; N=1; 140,79mm SL; Negro; MZUSP103509; N=1; C&S; Jari;

Schizodon intermedius Garavello & Britski 1990: MZUEL00483; N=2; 113,92-116,86mm SL; Tibagi; MZUEL1092; N=3; 103,8-112,78mm SL; Tibagi; MZUEL10237; N=4; SK=4; Tibagi;

PHYLOGENETIC ANALYSES

The two previously described matrices (molecular and morphological) were imported into Beauti v2. 7. 6, and used with the following guidelines: "Optimized Relaxed clock model" (linked to all partitions) that allow the existence of independent rates of molecular evolution in different branches (Drummond *&* Rambaut, 2007) and the Birth-Death Model (as a tree prior, also linked to all partitions), admitting that, in any point in the analysis timeline, each lineage can be extinct or suffer from speciation independently.

The best model for nucleotide evolution, for each molecular sequence, were given by the bModelTest, with mutation rate and NamedExtended based on empirical rates (Bouckaert & Drummond, 2017), with morphological characters using Lewis MK model via MM package (Lewis, 2001).

All other parameters were set as default. For time calibration, we applied a most recent common ancestor constraint on the node, including all terminal taxa, except for *Leporellus vittatus*, *Anostomus anostomus*, and *Pseudanos trimaculatus*. We based the age of this clade on Sidlauskas *et al.* (2021)´s estimate of 38.9 million years for that clade. For this prior, we applied a log-normal distribution and a monophyletic constraint with an offset of 34.4, M of 1, and Sigma of 1.0, with a median of 38.9. with raw age of 6 million years (mya), following the estimate provided by Bogan *et al.* (2012).

The ".xml" file bearing the parametrization of the analysis was imported into Beast v2. 7. 6, with instructions to include 100 million generations, sampling every 10 thousand generations. The Tracer v1.7.2 program ensured that the Effective Sample Size (ESS) exceeded 200 samples. After that, a maximum clade credibility tree was constructed using the Tree Annotator v2.7.6, using a burn-in rate of 10%, as Sidlauskas et al. (2021) used. Finally, the phylogenetic tree was edited using the FigTree v1.4.4 program (Rambaut, 2007).

As a sensitivity analyses, to understand the contribution of each of the phylogenetic data (morphological and molecular), additional separate analyses were performed. The analysis, including the molecular data (all five partitions), were performed via BEAST v2. 7. 6, following all parameters described above for the total evidence analysis. In addition, a maximum parsimony analysis exclusive to the morphological matrix was performed in TNT, v. 1.6 (Goloboff & Moraes, 2023), using the traditional search, with 20 thousand replicas and setting *Leporellus vittatus* as the outgroup and applying TBR (Tree Branching Recombination) was performed. Both strict and majority consensus were calculated from the most parsimonious recovered trees.

RESULTS

CHARACTER LIST

The following list includes 70 characters describing observed variation in external and internal morphology, including coloration patterns, scale counting and osteology (e.g., neurocranium bones), as well as a single character related to the sexual-chromosome system described by Galetti *et al.* (1981, 1991 & 1995), Molina *et al.* (1998), Venere *et al.* (2004) and Ramirez, Birindelli & Galetti (2016).

Coloration

1. Coloration, conspicuous vertical bands encircling the body, in adults: 0. absent, 1. present (Sidlauskas & Vari, 2008: #116) (CI = 0.500; RI = 0).

Several authors used distinct patterns of body coloration to describe and classify species of Anostomidae (Géry, 1978; Garavello, 1979;). Most species of Anostomidae have dark bands on the body when larvae and juveniles become inconspicuous in adults (Santos, 1980, 1983; Nakatani *et al.*, 2001; Birindelli & Britski, 2013). Nevertheless, in a few cases, the dark bands are persistent in adults (Géry, 1978; Garavello, 1979; Britski & Birindelli, 2016). In our study, *Abramites hypselonotus* and *Leporinus fasciatus* have dark bands formed by epidermal pigments encircling the body that are persistent in both preserved and alive adults (Vari & Williams, 1987; Britski & Birindelli, 2016). All *Megaleporinus* show the alternative state of this character. In fact, most *Megaleporinus* exhibit inconspicuous dark bands, persistent in adults, that inconspicuous pattern is easily observed in preserved specimens due to the loss of guanina that is present in most live *Megaleporinus.* However, these dark bands do not encircle the body, a condition treated in a distinct character.

2. Coloration, inconspicuous vertical bands (not fragmented, nor encircling the body) persistent in adults: 0. absent, 1. present. (CI = 1.000; RI = 1.000).

As noticed by (Britski *et al.*, 2012), *Megaleporinus obtusidens* and *M. piavussu* have eight dark vertical bands on the body persistent in adults, formed primarily on dermal layers (and not encircling the body) and overlapped by a more conspicuous dark pigmentation on epidermal layer, latter usually forming blotches. These bands are rarely seen in alive specimens of the aforementioned species due to the presence of guanine in the scales, but frequently observed in alcohol preserved specimens. They are also entirely or almost completely faded in other congeners and most anostomids, both alive and preserved. In the few cases in which the bands persist in adults (*e.g.*, *Leporinus friderici* and *Megaleporinus conirostris*), these are fragmented vertically and/or longitudinally (Figure 3). The alternative state of this character is present in all outgroups in the present study.

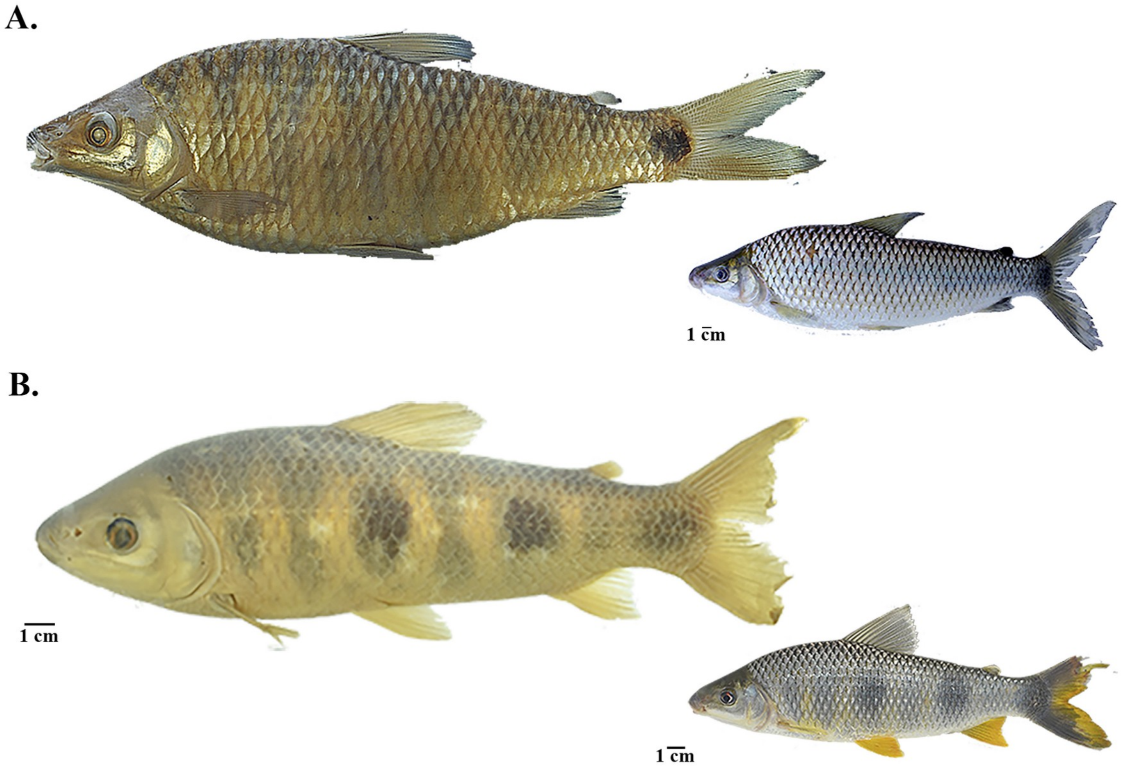


Figure 3. Photos in lateral view of **A.** *Megaleporinus conirostris*, preserved (left) and live (right) specimens; and **B.** *Megaleporinus obtusidens*, preserved (left) and live (right) specimens. Notice the inconspicuous vertical bands formed by dermal pigmentation, persistent in adults of both species but vertically fragmented in *M. conirostris* (A).

3. Coloration, two dark midlateral vertically-elongated blotches on body, one below dorsal fin and one above anal fin: 0. absent; 1. present (Sidlauskas & Vari, 2008: #117) (CI = 0.500; RI = 0.500).

There are dark vertical blotches, with both dorsal and ventral extremities gradually faded and not well delimited. In fact, there are vertically elongated blotches on the lateral surface of the body in *Megaleporinus trifasciatus, M.* sp. (Tocantins), and *Schizodon intermedius*, that are visible in both preserved and alive specimens. *Leporinus maculatus* has alternating thick and thin dark vertical bands that form saddles, with counterparts united dorsally. This pattern is herein considered not homologous with the vertically elongated blotch in *Megaleporinus trifasciatus*.

4. Coloration, dark midlateral vertically elongated blotch on anterior portion of body: 0. absent, 1. present (CI = 0.500; RI = 0).

Megaleporinus trifasciatus and *Schizodon intermedius* have a conspicuous dark vertically-elongated blotch on the anterior portion of the body, between the opercle and the vertical through the dorsal-fin origin. On the other hand, *Megaleporinus* sp. (Tocantins), which is very similar to *M. trifasciatus* in many morphological aspects (*i.e.*, scale counts and teeth morphology), differs from the latter by lacking such dark pigmentation. All other examined species also lack a dark vertical band on the anterior portion of the body.

5. Coloration, dark midlateral rounded blotch below the dorsal-fin base: 0. absent, 1. present (Sidlauskas & Vari, 2008: #118, modified) (CI = 0.200; RI = 0.600).

Some species of Anostomidae, including most of the *Megaleporinus*, have dark, rounded midlateral blotches that appear in a variety of sizes along the middle and posterior portions of the body (*e.g.*, *Hypomasticus megalepis*, *Pseudanos trimaculatus,* and *Megaleporinus obtusidens*) (Steindachner, 1875; Garavello, 1979; Ramirez *et al.*, 2017). Among studied species, a rounded dark blotch below the dorsal-fin base is present in *Megaleporinus gaiero*, *M. macrocephalus*, *M. muyscorum, M. obtusidens*, *M. piavussu*, *M. reihardti* and *M.* sp. (Orinoco), and *Hypomasticus megalepis*, *H. mormyrops*, *Leporinus friderici,* and *Pseudanos trimaculatus*. The blotch can be difficult to visualize in a few individuals of *M. muyscorum, M. obtusidens,* and *M.* sp. (Orinoco) due to lousy conservation of the specimens (Garavello, 1979: 298). *Megaleporinus brinco* is codded as polymorphic because although the dark blotch is present, it is smaller than its congeners and sometimes absent in larger individuals independently of their preservation. Sidlauskas & Vari (2008: #117) used a single character for the presence of the dark midlateral blotches, despite their position on the body. However, we consider each blotch in separate characters, depending on their position on the body. That option allowed us to consider the presence of the dark blotch on the caudal peduncle independent from the one below the dorsal-fin base.

6. Coloration, blurring of dark midlateral blotch below dorsal fin, in large adults (> 300mm SL): 0. absent, 1. present (autapomorphy).

The first dark blotch (below the dorsal fin) in larger specimens of *Megaleporinus gaiero* presents blurred delimitations that sometimes leads the blotch to being confluent dark blotch above the anal fin.

7. Coloration, dark midlateral rounded blotch above anal-fin base: 0. absent, 1. present (Sidlauskas & Vari, 2008: #118, modified) (CI = 0.250; RI = 0.720).

Among the studied species, the dark midlateral rounded blotch above the anal-fin base is present in the outgroup taxa *Hypomasticus megalepis, H. mormyrops,* and *Leporinus friderici*, and in ingroup taxa *Megaleporinus brinco*, *M. elongatus, M. gaiero*, *M. macrocephalus*, *M. muyscorum*, *M. obtusidens*, *M. piavussu*, *M. reinhardti* and *M.* sp. (Orinoco), independently of their preservation.

8. Coloration, dark midlateral blotch on caudal peduncle, persistent in adults: 0. absent, 1. present (Sidlauskas & Vari, 2008: #118, modified) (CI = 0.500; RI = 0.870).

There is a relatively large (approximately as large as the eye diameter) dark spot (term used to distinguish it from the poorly delimited-round pigmentation, previously named blotch) on the caudal peduncle in most Anostomidae, including most *Leporinus* and *Megaleporinus* (Garavello, 1979; Winterbottom, 1985; Sidlauskas & Vari, 2008; Ramirez *et al.*, 2016; Birindelli et *al,* 2016). Furthermore, a small (smaller than eye diameter) dark spot can vary slightly in position relative to its proximity to the caudal-fin base in the caudal peduncle area (i.e., *Megaleporinus conirostris* and *M. reinhardti*). However, we do not consider those variations as phylogenetically informative since they seem too small and continuous, and we decided to treat only the occurrence of the dark spot on the caudal peduncle as a character. That said, all the species of *Megaleporinus* have the well-delimited spot on the caudal peduncle, just like in the *Hypomasticus,* observed in this study*,* and *Leporinus friderici, L. maculatus* and *Pseudanos trimaculatus* that represents our outgroup.

9. Coloration, size of dark midlateral blotch on caudal peduncle, persistent in adults, relative to pupil: 0. greater, 1. smaller (Sidlauskas & Vari, 2008: #118, modified) (CI = 1.000; RI = 1.000).

Megaleporinus trifasciatus, *M.* sp. (Tocantins), and *M.* sp. (Orinoco) have a small (slightly smaller than pupil) dark blotch in the caudal peduncle. In contrast, other species have relatively large (usually as large as the eye size) blotch. The condition described here for *M. trifasciatus* is similar to that in *Schizodon fasciatus*. *Anostomus anostomus, Abramites* *hypselonotus*, and *Leporinus striatus* have no rounded blotch on the caudal peduncle and are assigned as inapplicable (-) herein.

10. Coloration, dark midlateral stripe in adults: 0. absent, 1. present (Winterbottom, 1890; Sidlauskas & Vari, 2008; Birindelli & Britski, 2013) (CI = 0.200; RI = 0).

Some species of Anostomidae possess a continuous dark midlateral stripe, extended from opercle to caudal peduncle, covering the lateral-line scale row. The species that present this condition in our study are *Anostomus anostomus, Laemolyta taeniata*, *Leporellus vittatus, Leporinus amblyrhynchus,* and *L. striatus*. In a few large specimens of *Megaleporinus gaiero,* the dark midlateral blotches are extended anteriorly and posteriorly, forming a weakly delimited midlateral stripe below the lateral line, a unique feature that is somewhat similar but clearly not homologous with the well-delimited dark midlateral stripe present in the aforementioned taxa. Therefore, *M. gaiero* is coded with character state 0.

11. Coloration, longitudinal stripes dorsal and ventral to lateral-line scale row: 0. absent, 1. present (Winterbottom, 1980: 55-56; Sidlauskas & Vari, 2008: #121) (CI = 0.500; RI = 0).

Among the studied species, only *Anostomus anostomus* and *Leporinus striatus* present a set of dark longitudinal stripes above and below the lateral-line scale row.

12. Coloration, longitudinal lines between scale rows: 0. absent, 1. present (CI = 0.500; RI = 0).

Unlike the longitudinal stripes that the previous character describes, the pigmentation of these lines is not present in the scales but in the skin below them in *Megaleporinus brinco* and *M. macrocephalus* (see also Ramirez *et al.*, 2017). These lines are visible between the dorsal and ventral margins of the scales on both preservation states of the specimens, but more easily in alcohol preserved ones. The above species represent this character's presence (state 1) in our study. A similar coloration pattern was described for *Schizodon trivittatus* (Garavello *et al.*, 2021) and Prochilodontidae (Castro & Vari, 2004).

13. Coloration, dark spots at the center of scales: 0. absent, 1. present (Winterbottom, 1980: 54; Sidlauskas & Vari, 2008: #122) (CI = 0.500; RI = 0).

In some Anostomidae, small dark spots are present at the center of each scale on the body. The presence of these spots was herein confirmed in *Leporellus vittatus* and *Pseudanos* *trimaculatus.* A similar characteristic was observed in *Megaleporinus brinco*. However, the spots differ from those present in *Leporellus* and *Pseudanos* by their distribution on the body, shape and size, indicating their lack of homology. In addition, the dark spots on the scales of *M. brinco* are only present in some (and not all) of the larger individuals. Therefore, *M. brinco* is coded with state 0.

14. Coloration, dark pigment at the anterior border of scales in adults: 0. absent, 1. present (CI = 0.500; RI = 0).

A conspicuous dark pigment at the anterior margin of each scale along the whole body is present in *Megaleporinus conirostris* and *M. gaiero*.

15. Coloration, red pigmentation immediately dorsal to pectoral-fin origin: 0. absent, 1. present (Autapomorphy).

Megaleporinus brinco has red pigmentation immediately dorsal to pectoral-fin origin. This pigment fades away with preservation; thus, it is only visible in live specimens (Birindelli *et al.*, 2013).

16. Coloration, red pigmentation covering ventral portion of opercular series: 0. absent, 1. present (CI = 1.000; RI = 1.000).

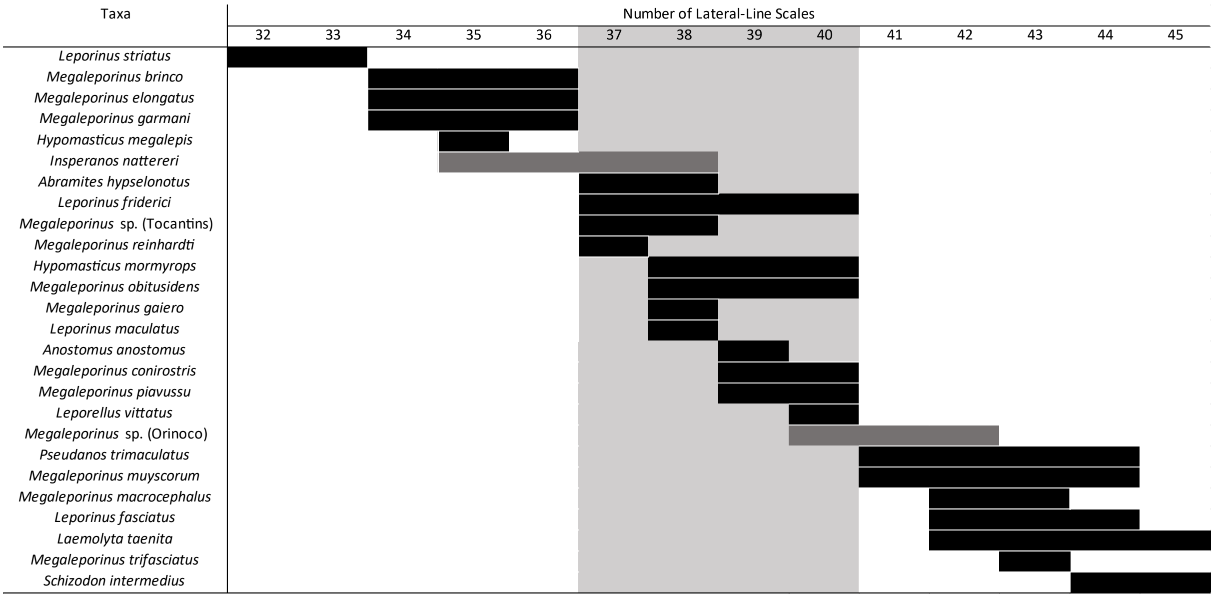
M. trifasciatus and *M.* sp. (Tocantins) possess red pigmentation covering the opercular series' ventral portion and membrane. As the red blotch of *M. brinco*, the red pigmentation in *M. trifasciatus* and *M.* sp. (Tocantins) fades away with ethanol preservation.

Scales

17. Scales, number of lateral-line scales: 0. 32 to 36; 1. 37 to 40, 2. 41 to 45 (Sidlauskas & Vari, 2008: #115) (CI = 0.200; RI = 0.200).

The number of perforated scales in the lateral line varies among different species of Anostomidae. *Hypomasticus megalepis,* some individuals of *Insperanos nattereri*, *Leporinus striatus, Megaleporinus brinco, M. elongatus,* and *M. garmani* are examples of the character state 0 and represent the anostomids with fewest scales. Most species in the present study are coded with state 1, including: *Anostomus anostomus, Abramites hypselonotus*, *Hypomasticus* *mormyrops*, some individuals of *Insperanos nattereri*, *Leporellus vittatus*, *Leporinus amblyrhynchus, Leporinus friderici*, *Leporinus maculatus*, *Megaleporinus conirostris*, *M. gaiero*, *M. obtusidens*, *M. piavussu*, *M. reinhardti, Megaleporinus* sp. (Tocantins) and some individuals of *Megaleporinus* sp. (Orinoco). On the other hand, a few species have more than 40 scales (character state 2), including: *Laemolyta taeniata*, *Leporinus fasciatus*, *Pseudanos trimaculatus*, *Schizodon intermedius*, *Megaleporinus macrocephalus*, *M. muyscorum,* some individuals of *Megaleporinus* sp. (Orinoco) and *M. trifasciatus. Insperanos nattereri* and *Megaleporinus* sp. (Orinoco) were coded as polymorphic for varying scales, corresponding to this character's two states (state 0 and 1; state 1 and 2, respectively). The character states were delimited to ensure the minimum number of polymorphic species, and threated as unordered as all the other characters (Table 2).

Table 2. Elaboration of the three states of character 17 (divided in the columns by color), ensuring the minimum number of polymorphic taxa.



Taxa	Number of Lateral-Line Scales													
	32	33	34	35	36	37	38	39	40	41	42	43	44	45
Leporinus striatus	■	■												
Megaleporinus brinco			■	■	■									
Megaleporinus elongatus			■	■	■									
Megaleporinus garmani			■	■	■									
Hypomasticus megalepis				■										
Insperanos nattereri				▨	▨	▨	▨							
Abramites hypselonotus						■	■							
Leporinus friderici						■	■	■	■					
Megaleporinus sp. (Tocantins)						■	■							
Megaleporinus reinhardti						■								
Hypomasticus mormyrops							■	■	■					
Megaleporinus obitusidens							■	■	■					
Megaleporinus gaiero							■							
Leporinus maculatus							■							
Anostomus anostomus								■						
Megaleporinus conirostris								■	■					
Megaleporinus piavussu								■	■					
Leporellus vittatus									■					
Megaleporinus sp. (Orinoco)									▨	▨	▨			
Pseudanos trimaculatus										■	■	■	■	
Megaleporinus muyscorum										■	■	■	■	
Megaleporinus macrocephalus											■	■		
Leporinus fasciatus											■	■	■	
Laemolyta taenita											■	■	■	■
Megaleporinus trifasciatus												■		
Schizodon intermedius													■	■

18. Scales, number of scale rows around the caudal peduncle: 0. 12; 1. 14; 2. 16 (CI = 0.280; RI = 0.440).

Most Anostomidae have 16 scales Around the caudal peduncle (Garavello, 1979; Winterbottom, 1980). However, some species have only 12 scale rows around the caudal peduncle, including *Megaleporinus brinco*, *M. conirostris,* and *M. garmani,* and the outgroup taxa *Hypomasticus mormyrops* and *Leporinus amblyrhynchus*. In rare cases, including *Abramites* and *Leporinus desmotes,* 14 scale rows encircle the caudal peduncle (Burns *et al.,* 2017; Vari & Williams, 1987).

19. Scales, number of scale rows between lateral line and dorsal-fin origin: 0. four or five; 1. six or seven (CI = 0.200; RI = 0.330).

Anostomus anostomus, Hypomasticus megalepis, *H. mormyrops*, *Laemolyta taeniata, Leporinus amblyrhynchus, L. friderici, L. maculatus, L. striatus, Pseudanos trimaculatus, Schizodon intermedius, Megaleporinus brinco, M. conirostris, M. elongatus, M. gaiero, M. garmani, M. muyscorum, M. reinhardti* and *Megaleporinus* sp. (Tocantins) present less than six horizontal rows of scales, here assigned as state 0. At the same time, *Abramites hypselonotus*, *Leporellus vittatus*, *Leporinus fasciatus*, *Megaleporinus macrocephalus*, *M. obtusidens,* and *M. trifasciatus* possess more than five scale rows between dorsal fin and lateral line scale row. *Insperanos nattereri* and *Megaleporinus* sp. (Orinoco) were assigned as polymorphic (0/1). The character states were delimited to minimize the number of polymorphic species (Table 3).

20. Scales, number of scale rows between lateral line and pelvic-fin origin: 0. four or five; 1. six or seven (CI = 0.250; RI = 0).

The presence of less than six ventral scale rows is observed in *Anostomus anostomus, Hypomasticus megalepis, H. mormyrops, Insperanos nattereri, Laemolyta taeniata, Leporellus vittatus, Leporinus amblyrhynchus, L. friderici, L. maculatus, L. striatus, Pseudanos trimaculatus, Schizodon intermedius, Megaleporinus brinco, M. conirostris, M. elongatus, M. gaiero, M. garmani, M. macrocephalus, M. reinhardti, M. trifasciatus* and *Megaleporinus* sp. (Tocantins). Meanwhile, *Abramites hypselonotus, Leporinus fasciatus,* and *Megaleporinus* sp. (Orinoco) present six or more scale rows below the lateral line (state 1). *Megaleporinus muyscorum* and *M. obtusidens* were considered polymorphic to this character (0/1). The character states were delimited to minimize the number of polymorphic species (Table 4).

Table 3. Elaboration of the two states of character 19, ensuring the minimum number of polymorphic taxa.

Number of scale rows between lateral line and dorsal-fin origin				
Taxa	4	5	6	7
Insperanos nattereri	■			
Megaleporinus brinco	■			
Schizodon intermedius	■	■		
Megaleporinus conirostris	■	■		
Megaleporinus gaiero	■	■		
Hypomasticus megalepis	■	■		
Abramites hypselonotus		■		
Hypomasticus mormyrops		■		
Leporellus vittatus		■		
Leporinus striatus		■		
Pseudanos trimaculatus		■		
Megaleporinus elongatus		■		
Megaleporinus garmani		■		
Megaleporinus macrocephalus		■		
Megaleporinus trifasciatus		■		
Megaleporinus sp. (Orinoco)		■		
Laemolyta taenita		▨	▨	
Megaleporinus obitusidens		▨	▨	
Megaleporinus reinhardti			■	
Megaleporinus muyscorum			■	
Leporinus fasciatus			■	
Megaleporinus sp. (Tocantins)			■	
Leporinus friderici			■	■

Table 4. Elaboration of the two states of character 20, ensuring the minimum number of polymorphic taxa.

Number of scale rows between lateral line and pelvic-fin origin				
Taxa	4	5	6	7
Insperanos nattereri	■			
Megaleporinus conirostris	■			
Hypomasticus megalepis	■			
Leporinus striatus	■	■		
Schizodon intermedius	■	■		
Megaleporinus brinco	■	■		
Megaleporinus gaiero	■	■		
Leporellus vittatus	■	■		
Laemolyta taenita		■		
Abramites hypselonotus		■		
Hypomasticus mormyrops		■		
Leporinus fasciatus		■		
Pseudanos trimaculatus		■		
Megaleporinus elongatus		■		
Megaleporinus garmani		■		
Megaleporinus macrocephalus		■		
Megaleporinus muyscorum		■		
Megaleporinus trifasciatus		■		
Megaleporinus sp. (Tocantins)		■		
Megaleporinus sp. (Orinoco)		▨	▨	
Megaleporinus reinhardti			■	
Leporinus friderici			■	
Megaleporinus obitusidens			■	

Dentition

21. Dentition, Premaxilla, number of teeth: 0. three; 1. Four (Sidlauskas & Vari, 2008: #31) (CI = 0.250; RI = 0.660).

Most Anostomidae possess four teeth in the premaxilla (Winterbottom, 1980; Sidlauskas &

Vari, 2008; Britski & Birindelli, 2008). On the other hand, *Abramites hypselonotus, Hypomasticus megalepis, Leporinus striatus,* and all species of *Megaleporinus* have only three teeth on the premaxilla (Vari & Williams, 1987; Gery *et al.*, 1988; Birindelli *et al.*, 2013; Ramirez, Birindelli & Galetti, 2016).

22. Dentition, premaxilla, cutting edge of teeth: 0. blunt (unicuspidal tooth); 1. multicuspid (Sidlauskas & Vari, 2008: #32, #41, modified) (CI = 1.000; RI = 1.000).

The form of the crown of the symphyseal teeth in Anostomidae varies greatly, from incisiform unicuspid that resembles human inferior incisors to multicuspid chisel-like teeth with two to four cusps (cusps distinctly separated by notches). Most anostomids, including all *Megaleporinus*, have incisiform teeth with blunt cutting edge. In these cases, the shape of the cutting edge varies through development, with juveniles having two or three cusps, whereas larger specimens have teeth with blunt cutting edges (Scharcansky & Lucena, 2008; Birindelli *et al.*, 2013). This feature also varies seasonally as wear generates blunter, straight-cutting edges (Sidlauskas & Vari, 2008). Our study found multicuspid teeth in *Anostomus anostomus, Pseudanos trimaculatus, Laemolyta taeniata,* and *Schizodon intermedius*.

23. Dentition, premaxilla, number of cusps on teeth: 0. three; 1. four (Sidlauskas & Vari, 2008. #32, #33, #41, modified) (CI = 1.000; RI = 1.000).

Ansotomus anostomus and *Pseudanos trimaculatus* have three cusps, whereas *Schizodon intermedius* and *Laemolyta taeniata* have four cusps on their premaxillary teeth. This character was codded as inapplicable (-) for all other species, as they lack multicuspid premaxillary teeth.

24. Dentition, premaxilla, marginal ridge on lingual surface of teeth, in adults: 0. rudimentary, 1. distinct (Sidlauskas & Vari, 2008: #34, modified) (CI = 0.330; RI = 0.770).

In some Anostomidae, the premaxillary teeth are chisel-like or shovel-like, with labial surface convex and lingual surface concave and bearing rudimentary marginal ridges (Figure 4). This condition is present in *Anostomus anostomus*, *Abramites hypselonotus, Hypomasticus*, *Insperanos nattereri*, *Laemolyta taeniata*, *Leporellus vittatus*, *Leporinus striatus*, *Pseudanos trimaculatus*, and *Schizodon intermedius*. However, in *Megaleporinus* and *Leporinus*, the premaxillary teeth are incisiform with a convex labial surface and concave lingual surface bearing marginal ridges (teeth resembling a human´s inferior canine) (Figure 5). In these taxa, the ridges' development varies ontogenetically, with juveniles tending to have less prominent ridges (i.e., shovel-like teeth) and adults having prominent ridges. Ridges are also gradually more prominent in lateral teeth. Nevertheless, there are also variations in the development of the ridges when examined species are compared (see next character). Herein, we considered the presence of distinct marginal ridges on the lingual surface of all premaxillary teeth instead of just in the second and third (as in Sidlauskas & Vari, 2008) because all of the examined species that bear distinct marginal ridges on the symphyseal tooth, also have them of the second and third premaxillary teeth.

25. Dentition, premaxilla, development of marginal ridge on lingual surface of teeth, in adults: 0. weakly developed, 1. well-developed (Sidlauskas & Vari, 2008: #34, modified) (CI = 0.500; RI = 0.750).

The marginal ridges of the lingual surface of the premaxillary teeth are weakly developed in some species, including *Megaleporinus brinco*, *M. conirostris*, *M. elongatus*, *M. gaiero,* and *Laemolyta taeniata*. In these, the ridges do not form prominent folds that bend towards each other, leaving a continuous path in the lingual surface of teeth from base to cutting edge. However, in other taxa, including *Megaleporinus obtusidens*, *M. macrocephalus*, *M. reinhardti*, *M. trifasciatus*, *M.* sp. (Orinoco), *M. muyscorum*, *M.* sp (Tocantins) and *M. piavussu*, *Leporinus amblyrhynchus*, *L. fasciatus*, *L. friderici*, *L. maculatus*, the ridges are prominent folds that bend towards each other forming a lingual fossa near the cutting edge of the teeth (Figure 6).

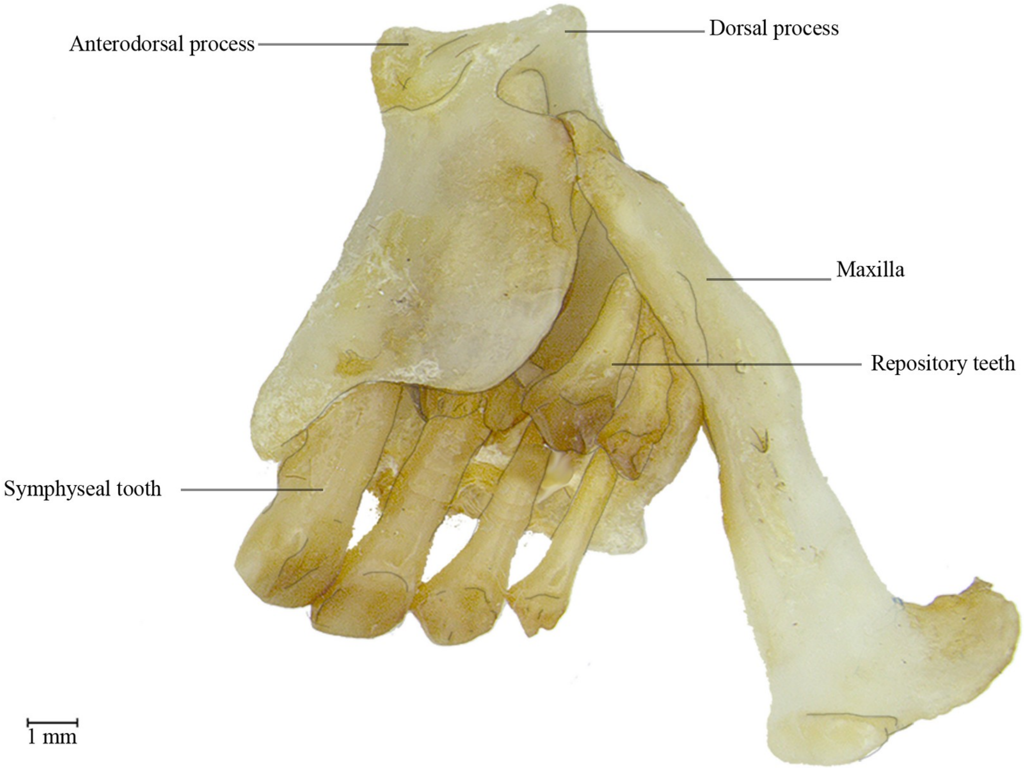


Figure 4. Medial view of the upper jaw of *Insperanos nattereri* (MZUSP 110595).

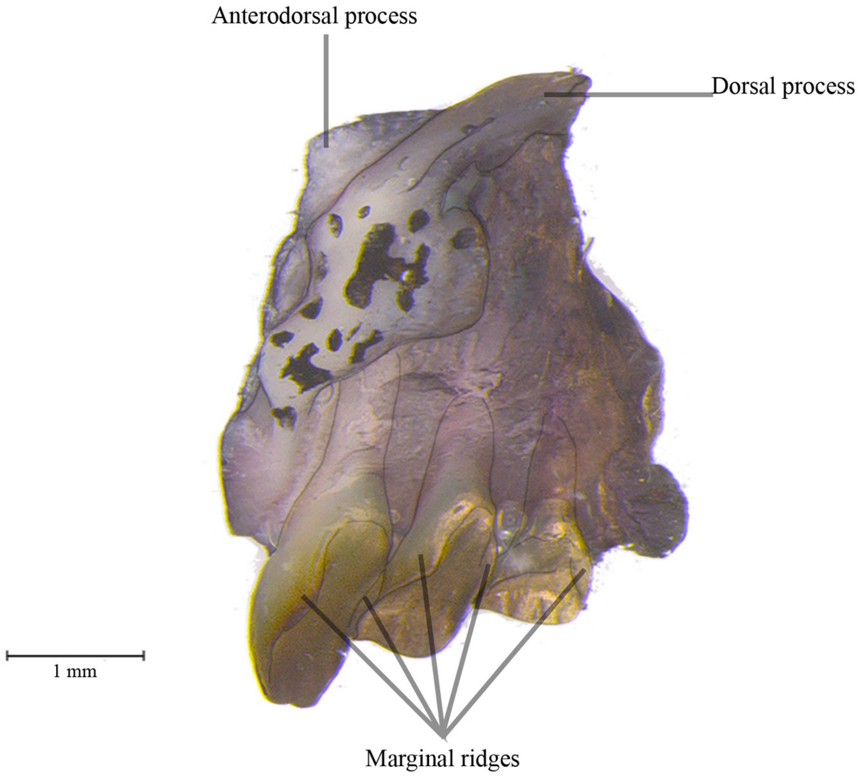


Figure 5. Medial view of the premaxilla of *Megaleporinus conirostris* (MZUSP 87872).

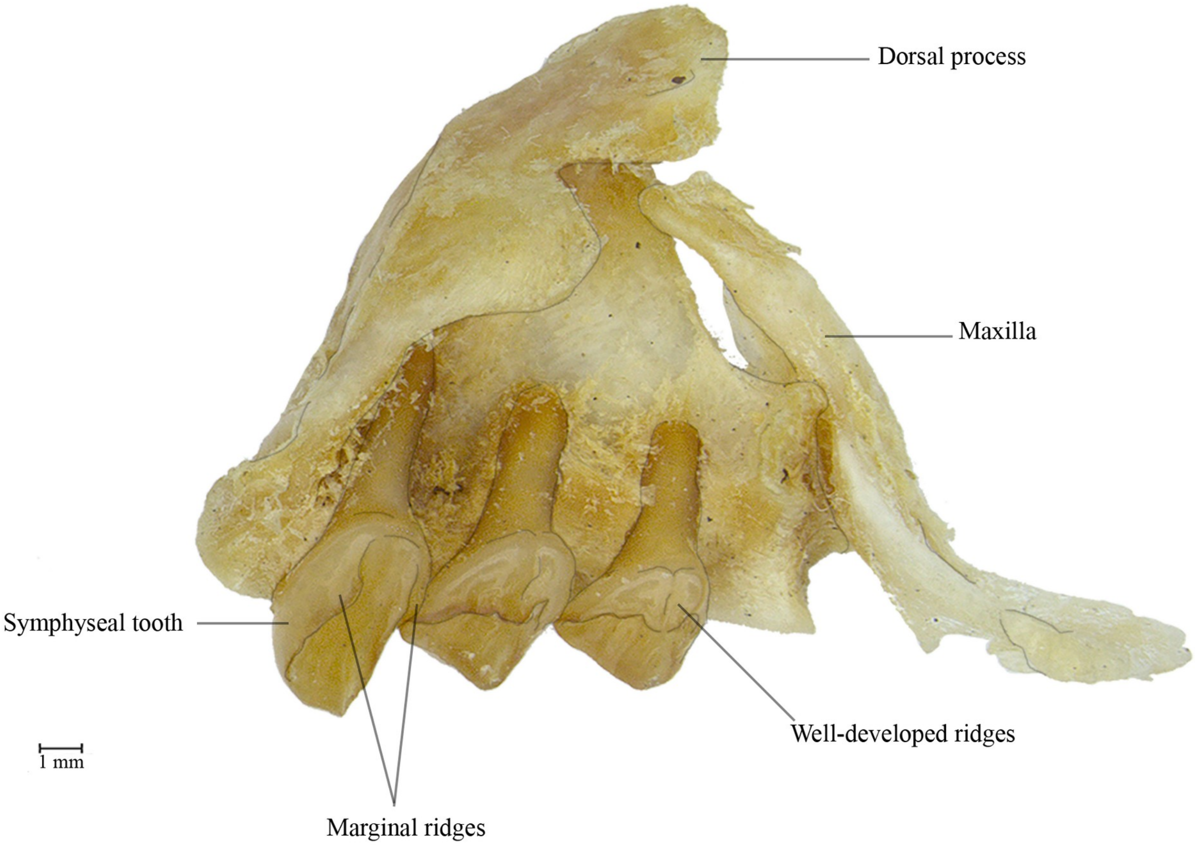


Figure 6. Medial view of the upper jaw of *Megaleporinus macrocephalus* (MZUSP 89501).

26. Dentition, Dentary, number of teeth: 0. three, 1. four. (Sidlauskas & Vari, 2008: #36) (CI = 0.330; RI = 0.830).

Anostomidae species present small variations in their dentary formulae (Winterbottom, 1980; Géry, 1988; Sidlauskas *et al.*, 2021). In our study, all the outgroup species possess four teeth in the dentary (state 1), while species of *Megaleporinus* have three (state 0), except *Megaleporinus garmani*. Noteworthy, a rare aberrant specimen of *Megaleporinus elongatus* (MZUEL 16472) possesses four teeth in the dentary bone on both sides. Three sympatric and conspecific specimens (same lot) possess three dentary teeth. This case was interpreted as an aberrant individual; thus, the species was coded as having only three dentary teeth, following the redescription of the species (Britski *et al.*, 2012).

27. Dentition, Dentary, notch at cutting edge: 0. absent, 1. present. (Sidlauskas & Vari, 2008: #39, #40, modified) (CI = 0.500; RI = 0).

Abramites hypselonotus and Schizodon intermedius have large, distinct notches along the cutting edge of the dentary teeth. According to Sidlauskas and Vari

(2008), this condition is also present in *Gnathodolus* and *Rhytidous*. The notch is present in the first and second dentary teeth in *Abramites hypselonotus*, whereas in all four teeth in *Schizodon intermedius*. Sidlauskas & Vari (2008) proposed two distinct characters for the presence of a distinct notch in the dentary teeth; the first (#39) referred only to the symphyseal tooth, whereas the second (#40) to the second and third teeth.

28. Dentition, dentary, cutting edge of teeth: 0. blunt (unicuspidal tooth); 1. multicuspid (Sidlauskas & Vari, 2008: #32, #37, #41, modified) (CI = 0.500; RI = 0.500).

The dentary teeth maintain the same aspect of the cutting edge as the premaxillary teeth in many anostomids, thus keeping almost the same distribution of states observed in character 22 herein, except for *Laemolyta taeniata* possesses blunt teeth in its dentary, in counterpart with its multicuspid teeth in premaxilla.

29. Dentition, dentary, number of cusps on teeth: 0. three; 1. four (Sidlauskas & Vari, 2008. #32, #33, #41, #42 and #43, modified) (Synapomorphy).

The cuspidation of the teeth varies among the studied multicuspid anostomids. *Anostomus anostomus* has three cusps in all premaxillary teeth, but two on the first and second dentary teeth, three in the third, and four in the fourth. The species was coded with state 0, as most of its teeth had three cusps. A similar situation occurs in *Pseudanos trimaculatus*, and *Schizodon intermedius* maintains four cusps, as do its premaxillary teeth. Other species, including *Laemolyta taeniata*, are coded as inapplicable for this character since they possess unicuspid teeth in the dentary bone.

Upper Jaw

30. Upper jaw, premaxilla, orientation relative to longitudinal body axis: 0. oblique, 1. longitudinal, 2. transversal (Sidlauskas & Vari, 2008: #46) (CI = 0.660; RI = 0.900).

The orientation of the premaxilla reflects the position of the mouth opening. In most Anostomidae, the premaxilla is oblique with teeth directed anteroventrally and mouth subterminal (sub-inferior in some cases). However, in species with upturned mouth, the premaxilla is almost longitudinal relative to the body axis, with teeth directed almost completely anterior (or completely anterior in some cases, such as in *Gnathodolus* and

Sartor). In our study, this condition was observed in *Anostomus* and *Pseudanos*. On the other hand, in species with downturned mouth, including *Hypomasticus mormyrops*, *H. megalepis*, and also in *Megaleporinus garmani*, M. *conirostris* and *M. brinco*, the premaxilla is transversal, with teeth slightly directed posteriorly.

31. Upper jaw, premaxilla, direction of the ascending process: 0. dorsal, 1. posterior (CI = 0.330; RI = 0.800).

The ascending process of the premaxilla is large in all Anostomidae (Vari, 1983; Sidlauskas & Vari, 2008) and usually directed dorsally, accompanying the general orientation of the entire premaxilla. However, in *Hypomasticus mormyrops*, *H. megalepis*, *Leporinus amblyrhynchus*, *Megaleporinus gaiero*, *M. brinco*, *M. obtusidens*, *M. piavussu*, *M. elongatus*, *M. conirostris,* and *M. garmani*, the ascending process is distinctly bent and directed posteriorly.

32. Upper jaw, premaxilla, size of the anterodorsal process, relative to crown of lateralmost teeth: 0. larger; 1. smaller (Sidlauskas & Vari, 2008: #47, modified) (CI = 0.200; RI = 0.200).

The premaxillary has two sites of attachment with the mesethmoid, both on the medial (anterior) face, one more dorsal and attached to the lateral process of the mesethmoid, and one more ventral and attached to the ventral process of the mesethmoid. *Leporellus*, *Insperanos,* and *Hypomasticus mormyrops* have a distinctively large anterodorsal process in the premaxilla. This condition was previously observed for *Hypomasticus mormyrops* and *H. pachycheilus* by Sidlauskas & Vari (2008). Herein, we confirm this condition for *Insperanos nattereri*, *Leporellus* (Figures 4 and 7, respectively), *Megaleporinus conirostris*, and *M. garmani* (Figure 5). The remaining taxa possess this process in rudimentary forms, smaller than their lateralmost tooth of the premaxilla (figure 6).

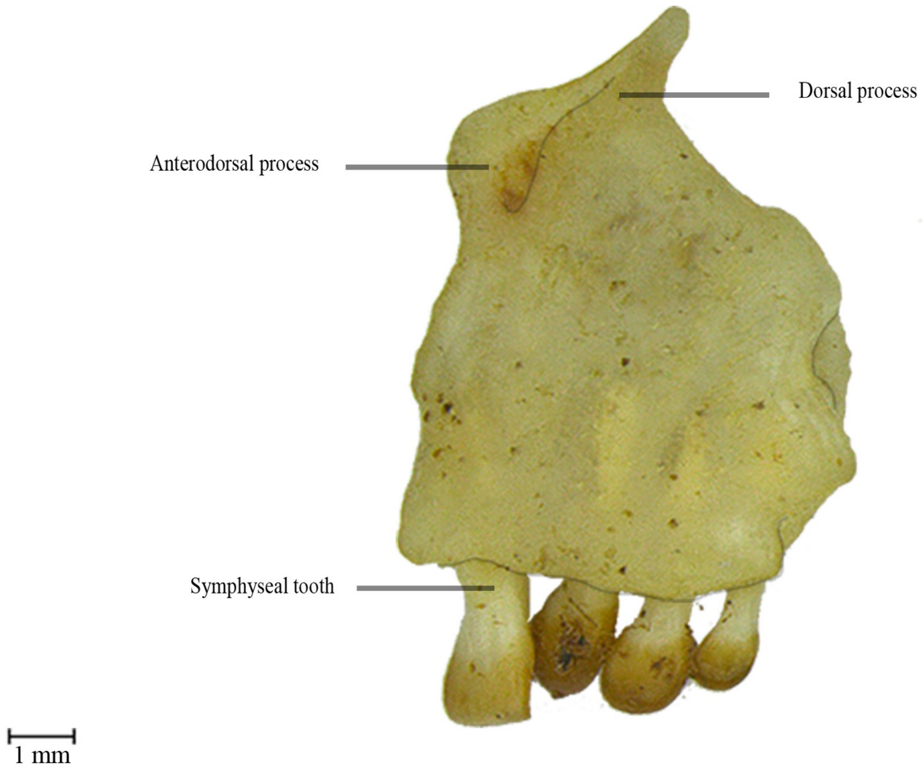


Figure 7. Lateral view of the premaxilla of *Leporellus vittatus* (MZUSP106332).

33. Upper jaw, maxilla, direction of ventral end, relative to dorsal end: 0. anterior; 1. vertical; 2. posterior. (Sidlauskas & Vari, 2008: #48) (CI = 0.500; RI = 1.000).

In the upturned mouth Anostomidae (represented in our study by *Anostomus* and *Pseudanos*), the ventral end of the maxilla is directed anteriorly. However, in most Anostomidae, the ventral end of the maxilla is directed ventrally. On the other hand, in a few downturned mouth species, including *Hypomasticus megalepis*, *M. mormyrops* and in *Megaleporinus brinco*, *M. conirostris*, and *M. garmani*, the maxilla is sickle-shaped and, its ventral end, directed posteriorly.

34. Upper jaw, maxilla, length of the ventral portion, relative to the dorsal portion: 0. longer, 1. shorter. (Sidlauskas & Vari, 2008: #49, modified) (CI = 0.500; RI = 0).

The maxilla limit between the maxilla's dorsal and ventral portions is the primordial ligament's attachment site, where this portion of the bone is thinner (strangled). In most Anostomidae, the ventral portion of the maxilla is shorter than the dorsal portion. However, in

Leporellus and *Megaleporinus brinco*, the ventral portion is distinctly longer than the dorsal portion.

Lower jaw

35. Lower jaw, anguloarticular, size of tip of ascending process, width relative to its length: 0. small; 1. large. (Sidlauskas and Vari, 2008: #55) (CI = 0.500; RI = 0.800).

The tip of the ascending process of the anguloarticular is narrow, with the width decreasing dorsally and, at the tip, much smaller than the length. However, in *Hypomasticus megalepis, H. mormyrops*, *Schizodon* and *Megaleporinus brinco*, *M. conirostris*, and *M. garmani*, the tip of the ascending process is expanded laterally with its width much larger than its length.

36. Lower jaw, anguloarticular, groove on tip of ascending process: 0. absent, 1. present. (Sidlauskas and Vari, 2008: #56) (CI = 1.000; RI = 1.000).

In *Hypomasticus mormyrops, Megaleporinus brinco, M. conirostris,* and *M. garmani,* there is a groove on the dorsal face of the tip of the ascending process of the anguloarticular, where the tendon of the *pars stegalis* of the *adductor mandibulae* passes before inserting into the posterior face of the maxilla. This groove was reported in *Schizodon nasutus* by Sidlauskas & Vari (2008). The presence of the groove is probably a modification related to the downturned mouth and the consequent rearrangement of the jaws.

37. Lower jaw, dentary, middle portion of ventral margin: 0. convex (rounded); 1. straight. (Sidlauskas & Vari, 2008: #58) (CI = 0.500; RI = 0.750).

The middle portion of the ventral margin of the dentary is straight in all *Megaleporinus* and most Anostomidae. However, it is convex (rounded) in *Leporellus, Anostomus, Pseudanos, Schizodon*, and *Laemolyta*.

Suspensorium

38. Suspensorium, autopalatine, distinct process: 0. absent; 1. present. (Sidlauskas & Vari, 2008; #67) (CI = 0.500; RI = 0.500).

The presence of a distinct process in the autopalatine is characteristic of most Anostomidae, with exceptions including *Anostomus, Pseudanos,* and *Insperanos* (Winterbottom, 1980; Sidlauskas & Vari, 2008; Sidlauskas *et al.*, 2021). This feature was extensively discussed by Sidlauskas *et al.* (2021).

39. Suspensorium, autopalatine, orientation of process: 0. lateral, 1. anterior. (Sidlauskas & Vari, 2008: #68) (CI = 0.500; RI = 0.830).

The autopalatine is a thick bone with a distinct lateral process in most Anostomidae. However, it is a slender bone with a process oriented anteriorly in *Leporellus*, *Hypomasticus, Abramites*, and *Megaleporinus brinco, M. conirostris, M. garmani*. Unfortunately, this character could not be confirmed in large specimens of *M. conirostris*, as the only available specimen for dissection is small (MZUSP87872, 107.4 mm SL). This character was coded as inapplicable for *Anostomus, Pseudanos,* and *Insperanos* as these taxa lack the process of the autopalatine.

40. Suspensorium, palatine arch, orientation of ectopterygoid: 0. vertical; 1. inclined (Sidlauskas & Vari, 2008: #72, modified) (CI = 1.000; RI = 1.000).

All species of *Megaleporinus* have an ectopterygoid oriented vertically, with anterodorsal margin and anteroventral margin, more or less aligned vertically. The same condition occurs in most outgroup taxa. However, in *Anostomus* and *Pseudanos,* the ectopterygoid has an inclined orientation, with the anteroventral margin much more anterior than the anterodorsal margin. Sidlauskas & Vari (2008) described three states for this character (anterodorsally inclined, vertical, and posterodorsally inclined). However, the positioning described by the authors as anterodorsal and vertical were interpreted as small variations of the same state. Since the anterior margin of the ectopterygoid bends in both states, the positioning of the dorsal and ventral margins do not vary significantly, and the degree of this bending is gradual and could not be quantified herein.

41. Suspensorium, palatine arch, overlapping of quadrate by ventral expansion of entopterygoid: 0. present; 1. absent. (Sidlauskas & Vari, 2008. #75) (CI = 0.500; RI = 0.500).

Some Anostomidae, such as *Abramites, Hypomasticus, Leporellus, Leporinus, Schizodon* (Sidlauskas & Vari, 2008), and *Megaleporinus,* possess a triangular process on the ventral portion of entopterygoid that overlaps the dorsal part of quadrate and creates a tight suture in the medial view of palatine arch (state 0). Nevertheless, *Anostomus anostomus*, *Laemolyta taeniata,* and *Pseudanos trimaculatus* possess a poorly developed process and do not exhibit the contact described above in state 1.

42. Suspensorium, palatine arch, size of lateral shelf of quadrate, relative to symplectic: 0. moderate, posterior end falling short beyond posterior end of symplectic; 1. small, posterior end reaching approximately midpoint of symplectic, 2. large, posterior end distinctly exceeding end of symplectic (Sidlauskas & Vari, 2008: #77) (CI = 0.400; RI = 0.810).

Species of Anostomidae possess a teardrop-shaped shelf on the quadrate that serves as an attachment area for the ventral portion of the *adductor mandibulae* muscle (Winterbottom, 1980; Vari, 1983). Nevertheless, the size of the quadrate shelf varies among the family. It is small in Anostominae, including *Anostomus* and *Pseudanos*, and symplectic reaches approximately the symplectic midpoint. On the other hand, in *Abramites, Insperanos, Laemolyta, Leporellus, Leporinus amblyrhynchus*, *Schizodon intermedius*, and in *Megaleporinus brinco* and *M. garmani*, the quadrate shelf is moderate in size, slightly passing the vertical through the posterior end of the symplectic. In all other species of *Megaleporinus*, as well as in *Hypomasticus megalepis, M. mormyrops*, *Leporinus fasciatus, L. friderici, L. striatus*, and the quadrate shelf is relatively large, posteriorly reaching way beyond the posterior limit of the symplectic, and instead surpassing the anterior margin of the hyomandibula. The codification of this character is slightly distinct from that of Sidlauskas & Vari (2008), which could be due, at least in some cases, to the examination of specimens of distinct sizes (i.e., larger specimens were examined herein).

43. Suspensorium, palatine arch, longitudinal crest on dorsal margin of metapterygoid: 0. absent; 1. present. (Sidlauskas & Vari, 1980: #81, modified) (CI = 0.500; RI = 0.660).

In *Leporellus* and Anostominae (including *Anostomus ternetzi* and *Pseudanos trimaculatus*), the dorsal margin of the metapterygoid is gently bent medially. This condition was described by Winterbottom (1980: 50) as a medial slope. Two species of Megaleporinus (*M. brinco* and *M. garmani*) exhibit a similar condition (and this character was coded as 0 for them here). All other examined species of Anostomidae have a distinct longitudinal crest in the dorsal margin of the metapterygoid that gives a groove shape to the dorsal face of the metapterygoid. Sidlauskas & Vari (2008) described this character as the faceting of the metapterygoid.

44. Suspensorium, symplectic, overlapping between symplectic and metapterygoid: 0. absent; 1. present (CI = 0.500; RI = 0.830).

In some Anostomidae, including *Anostomus, Abramites, Insperanos, Laemolyta, Leporellus, Leporinus amblyrhynchus,* and *Pseudanos,* the dorsal margin of the symplectic is completely separated or only contacts the ventral margin of the metapterygoid. On the other hand, in *Hypomasticus, Leporinus* (except *L. amblyrhynchus*)*, Megaleporinus,* and *Schizodon*, the dorsal margin of the symplectic is expanded and medially overlaps the ventral margin of the metapterygoid.

Opercular series

45. Opercular series, opercle, concavity on posterodorsal margin of opercle: 0. present; 1. absent. (Winterbottom, 1980; Sidlauskas & Vari 2008: #90) (CI = 1.000; RI = 1.000).

The presence of a concavity on the posterodorsal margin of the opercle was noticed in *Anostomus, Petulanos, Gnathodolus, Sartor,* and *Synaptolaemus* (Winterbottom, 1980: 51), as well as in most Anostomidae (Sidlauskas & Vari, 2008). In our study, the only species possessing an opercle with a continuously rounded (convex) posterodorsal margin were those of *Laemolyta* and Schizodon, corroborating earlier observations of Sidlauskas & Vari (2008). The examined specimens of *Pseudanos* had a small but distinct concavity on the posterodorsal margin of the opercle.

Gill arches

46. Gill arches, first basibranchial, ossification: 0. absent; 1. present. (Sidlauskas & Vari 2008: #92) (CI = 0.330; RI = 0).

As observed by Vari (1983: 14) and corroborated by Sidlauskas & Vari (2008), the first basibranchial is not ossified in some Anostomidae, including *Abramites, Pseudanos,* and *Schizodon*. However, the ossification of the first basibranchial is a common condition in species of *Megaleporinus* and the rest of the genera of our outgroup.

47. Gill arches, Upper pharyngeal tooth-plate, number of tooth cusps: 0. one, 1. two to four (Sidlauskas & Vari, 2008: #95, modified) (CI = 0.500; RI = 0).

The teeth on the upper pharyngeal tooth plate in most Anostomidae have a single cusp. The teeth are laterally compressed and with sickle-like distal edges. In Leporellus, teeth are more or less conical, contrary to all other Anostomidae. On the other hand, teeth have three or four cups, gradually smaller posteriorly (*i.e.*, *Rhytiodus* and *Schizodon*). *Pseudanos trimaculatus* is the only species with two cusps on its pharyngeal tooth-plate. The number of cusps in the upper pharyngeal tooth plate and fifth ceratobranchial teeth are the same in each species observed herein. Therefore, there is no need for the elaboration of a similar character to the fifth ceratobranchial.

48. Gill arches, fifth ceratobranchial, number of rows of teeth: 0. one or two; 1. three to five (Sidlauskas & Vari, 2008: #96) (CI = 0.250; RI = 0).

Several species of Anostomidae, such as *Abramites, Laemolyta, Leporinus, Pseudanos, Hypomasticus megalepis*, *Insperanos nattereri,* and *Megaleporinus*, possess two complete rows of teeth on the ceratobranchial bone. Alternatively, *Anostomus anostomus*, *Hypomasticus mormyrops, Leporellus vittatus,* and *Schizodon intermedius* possess more than two rows of teeth (state 1), and the species of *Leporellus* present three complete rows of teeth. In contrast, *Hypomasticus mormyrops* and *Schizodon intermedius* possess five rows.

Circum orbital bones

49. Circum-orbital bones, supraorbital, shape of anterior portion: 0. plate-like; 1. rod-like. (Sidlauskas & Vari, 2008: #3) (CI = 0.330; RI = 0.600).

Most Anostomidae have the anterior portion of the supraorbital in a plate-like, with the entire bone exhibiting a rhomboid shape. However, the anterior portion of the supraorbital is thin and rod-like in *Hypomasticus, Insperanos, Megaleporinus brinco, M. gaiero,* and *M. garmani*.

50. Circum-orbital bones, antorbital, bony expansion: 0. absent; 1. present. (Sidlauskas & Vari, 2008: #1) (CI = 1.000; RI = 1.000).

In most species of Anostomidae, the antorbital is a relatively wide plate-like bone. However, in *Leporellus vittatus,* the antorbital is slender, lacking bony expansions.

51. Circum-orbital bones, antorbital, anterior portion: 0. absent, 1. present. (Sidlauskas & Vari, 2008: #2, modified) (CI = 0.500; RI = 0.660).

Anostomus, Leporellus, Insperanos, and *Pseudanos* have only the posterior portion of the antorbital, which is inclined and straight, extending anteriorly from the midpoint of the first infraorbital. All other examined taxa have an anterior portion of the antorbital extended from the anterior to the midpoint of the first infraorbital. In all these taxa, the anterorbital is sickle-shaped, with a concave dorsal margin. Sidlauskas & Vari (2008) illustrated this character when discussing their character for positioning the anterior margin of the antorbital relative to the first infraorbital, also included herein.

52. Circum-orbital bones, antorbital, position of anterior margin relative to anterodorsal pore of sensory canal of first infraorbital: 0. posterior; 1. anterior. (Sidlauskas & Vari, 2008: #2, modified) (CI = 0.500; RI = 0).

The anterior margin of the antorbital is anterior to the opening of the anterodorsal sensory canal in most Anostomidae. However, it is posterior in *Megaleporinus brinco* and *Hypomasticus pachycheilus*. This character was coded as inapplicable for species lacking the anterior portion of the antorbital (*Anostomus, Leporellus, Insperanos,* and *Pseudanos*). In these taxa, the anterior margin of the antorbital is posterior to the anteriormost opening of the sensory canal of the first infraorbital. However, we herein interpret this condition as due to the absence of the anterior portion of the antorbital and, thus, as not homologous with the condition observed in *M. brinco*. Interestingly, *Leporinus pachycheilus* has a condition very similar to that of *M. brinco* and *H. mormyrops*, which was interpreted by Sidlauskas & Vari (2008) as homologous with that observed in *Leporellus, Anostomus,* and *Pseudanos*.

53. Circum-orbital bones, first infraorbital, length of sensory canal, relative to height: 0. smaller; 1. larger (Sidlauskas & Vari, 2008: #4, modified) (CI = 0.330; RI = 0.660).

The sensory canal of the first infraorbital is typically longer than its height. However, in *Abramites hypselonotus, Leporellus vittatus, Insperanos nattereri, Leporinus amblyrhynchus, L. friderici,* and in the Anostominae (including *Anostomus anostomus*, *A. ternetzi,* and *Pseudanos trimaculatus*), the height of the sensory canal of the first infraorbital is larger than its length. This character is similar to what was considered in character four by Sidlauskas & Vari (2008). However, the delimitation of the character states and the coding of the two characters' states are slightly different in relation to the geometry of the canals and, consequently, the positioning of the pores.

54. Circum-orbital bones, first infraorbital, additional ventral pore on sensory canal: 0. absent; 1. present. (Sidlauskas & Vari, 2008: #5, modified) (CI = 0.500; RI = 0.500).

The first infraorbital sensory canal usually has only three openings (i.e., pores), including one posterior where the canal is continued to the second infraorbital and two anterior ones, one anterodorsal and another anteroventral. However, in some taxa, including *Laemolyta taeniata*, *Megaleporinus brinco*, and M. garmani, there is a ventral opening between the anteroventral and posterior pores. The sensory canal of the first infraorbital has one dorsal pore that is posterior to the anterodorsal pore only in *Anostomus ternetzi* and other Anostominae (see Sidlauskas & Vari, 2008: fig. 13). This dorsal opening is treated herein as distinct from the additional ventral pore described above for several Anostomidae. An additional ventral pore was observed in the sensory canal of the first infraorbital only on the right side of one specimen of *Leporinus striatus* (MZUEL 10633) out of five dry skeletons. The same situation was described by Sidlauskas & Vari (2008: 84), and, as them, we coded this species as 0, considering this specimen´s condition as aberrant.

55. Circum-orbital bones, second infraorbital, length, relative to maximum depth: 0. less than two times; 1. more than two times. (Sidlauskas & Vari, 2008: #7, modified) (CI = 1.000; RI = 1.000).

Among examined species, only *Laemolyta taeniata* and *Schizodon intermedius* have a relatively elongate and slender second infraorbital, with a width more than twice its depth. According to Sidlauskas & Vari (2008), other species of *Schizodon* and *Laemolyta* also have a slender second infraorbital due to the absence or poor development of a dorsal bony extension.

56. Circum-orbital bones, second infraorbital, number of intermediate pores along sensory canal: 0. one; 1. two. (Sidlauskas & Vari, 2008: #8) (CI = 0.250; RI = 0).

All species of *Megaleporinus* possess a single intermediate pore in the sensory canal of the second infraorbital. The same condition is present in *Anostomus anostomus, Abramites*, *Hypomasticus mormyrops, Laemolyta taeniata, Leporinus friderici,* and *Leporinus striatus*. On the other hand, all other examined species, including *Leporellus vittatus, Hypomasticus megalepis, Leporinus amblyrhynchus,* and *L. fasciatus,* have two intermediate pores in the sensory canal of the second infraorbital. *Insperanos nattereri* is polymorphic for this character, according to Sidlauskas *et al.* (2021: #12).

57. Circum-orbital bones, size of fifth infraorbital, relative to fourth infraorbital: 0. approximately equal; 1. distinctly smaller. (Sidlauskas & Vari, 2008: #10, modified) (CI = 0.250; RI = 0).

The fifth infraorbital is distinctly smaller than the fourth and sixth infraorbital in some species of Anostomidae, including those of *Anostomus, Abramites, Insperanos nattereri*, *Laemolyta, Leporinus amblyrhynchus, L. friderici, L. fasciatus, Pseudanos trimaculatus, Schizodon intermedius* and most of *Megaleporinus* species. On the other hand, it is similar in size, especially in height, to the adjacent infraorbitals (fourth and sixth) in the remaining examined species, including *Hypomasticus mormyrops, Leporellus vittatus, L. striatus,* and *Megaleporinus garmani*. As Sidlauskas & Vari (2008: 86) noted, the latter is the most common condition observed in Characiformes. *Hypomasticus megalepis* possess their fourth and fifth infraorbitals fused. Due to this, *H. megalepis* is considered inapplicable for this character.

58. Circum-orbital bones, sixth infraorbital, number of pores: 0. two; 1. three (Sidlauskas & Vari, 2008: #12) (CI = 0.160; RI = 0).

The sixth infraorbital has a tripartite sensory canal with three pores in most Anostomidae. However, in *Anostomus anostomus, Hypomasticus mormyrops, Insperanos nattereri, Laemolyta taeniata, Megaleporinus elongatus,* and *M. muyscorum*, the sensory canal of the sixth infraorbital is straight and bear only two pores. This condition was described for Anostominae by Winterbottom (1980: fig 30 to 32). *M. brinco* is coded as polymorphic herein since three investigated individuals (MZUEL17832 (SK), MZUSP111261 (SK), and MZUSP110617 (SK)) possess two pores only on one side of their bodies.

59. Circum-orbital bones, sixth infraorbital, overlapping of anteroventral process of sphenotic: 0. present; 1. absent. (Sidlauskas & Vari, 2008: #13) (CI = 0.200; RI = 0.500).

Most Anostomidae have the anteroventral portion of the sixth infraorbital slightly expanded so that it does not overlap nor surpass the sphenotic process´s anteroventral edge. On the other hand, the sixth infraorbital overlaps the sphenotic process in *Megaleporinus*, *Ansotomus anostomus, Abramites hypselonotus,* and *Leporellus vittatus*. *Hypomasticus mormyrops* was coded as polymorphic, as noticed by Sidlauskas & Vari (2008) and confirmed in our observations. *Leporinus striatus* was herein coded for state 1. Of the five (SK) specimens of the latter, only one (MZUSP106333) possesses the sixth infraorbital surpassing the edge of the sphenotic process, but only on the right side of the head. Vari & Sidlauskas (2008) had previously coded the later species as polymorphic for this character.

Neurocranium

60. Neurocranium, mesethmoid, width of mesethmoid, relative to its length: 0. greater; 1. smaller. (Sidlauskas & Vari 2008: #19) (CI = 0.330; RI = 0.710).

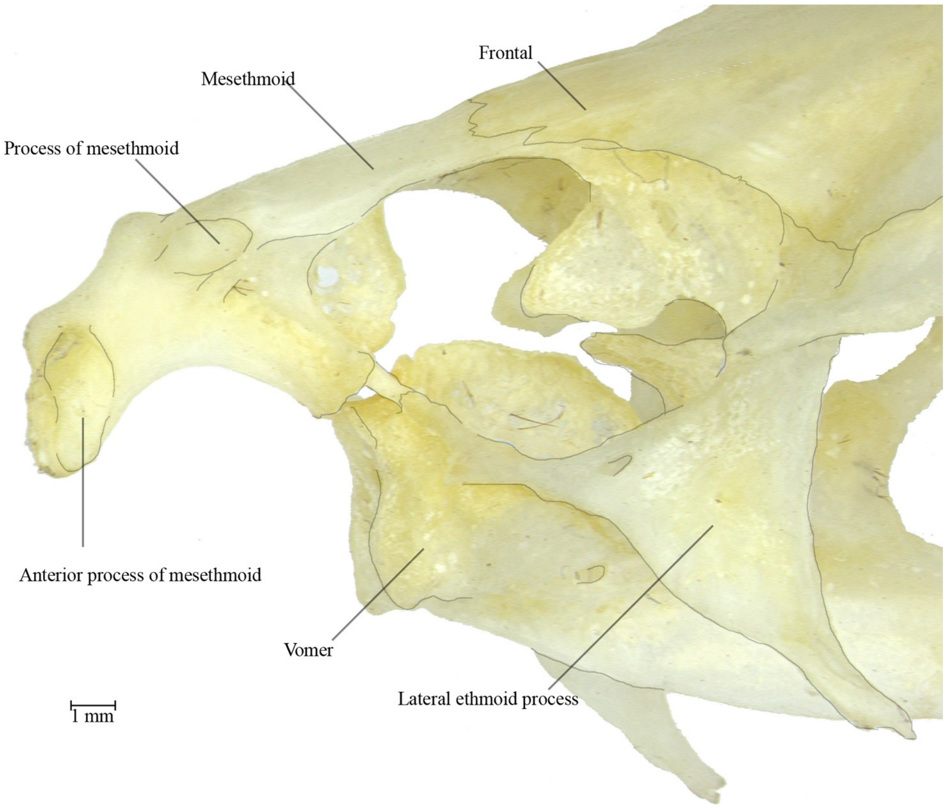
Several species of *Megaleporinus, including M. brinco, M. conirostris, M. elongatus, M. garmani, M. gaiero, M. reinhardti,* and some specimens of *M. obtusidens* have a narrow mesethmoid. The same condition occurs in outgroup *Insperanos nattereri, Hypomasticus mormyrops,* and *Leporellus vittatus* (Sidlauskas & Vari (2008: fig 21). However, *Megaleporinus macrocephalus, M. muyscorum, M. piavussu, M.* sp. (Orinoco)*, M. trifasciatus* and some specimens of *M. obtusidens*, have mesethmoid wide, mainly shaped as an equilateral triangle (as described for Anostominae by Winterbottom, 1980: 51). Outgroup species *Abramites hypselonotus*, *Pseudanos trimaculatus*, also possess wide mesethmoid. The states of this character are arbitrary, as the shape and length of the mesethmoid varies continuously among examined species. For example, in *Schizodon intermedius*, the maximum width of the mesethmoid is one and a half times larger than its length (measured from its posterior midpoint to the anteriormost portion, including the anterior process). On the other side of the spectrum, in *Megaleporinus brinco*, the mesethmoid's width is only around half its length. Several species have somewhat intermediate conditions, including *Insperanos nattereri* and *Megaleporinus reinhardti*, which have mesethmoids slightly longer than wide. Herein, we followed the state definition suggested by Sidlauskas & Vari (2008: 93), acknowledging that the shape of the mesethmoid is indeed an important phylogenetic characteristic among Anostomidae that could be further explored in future studies.

61. Neurocranium, mesethmoid, length of anterior portion, relative to width at lateral process: 0. distinctly longer (approximately two times longer than width of mesethmoid at lateral process). 1. approximately equal. (Sidlauskas & Vari, 2008: #15, modified) (CI = 1.000; RI = 1.000).

The mesethmoid in Anostomidae has an anterior tip that is much slenderer than the rest of the mesethmoid, and it is where the ascending process of the premaxilla articulates. In *Leporellus* and *Insperanos*, the anterior tip of the mesethmoid is distinctly longer when compared to other Anostomidae. The length of the anterior tip is slightly more than two times the width of the mesethmoid at the lateral process, whereas it is much shorter in the remaining Anostomidae. The elongation of the anterior tip of the mesethmoid was described as a strong ventrally-directed hook by Sidlauskas & Vari (2008: 90) for *Leporellus* and *Hypomasticus* and *Insperanos* (Sidlauskas *et al.*, 2021: fig 4). Herein, we considered that the elongation of the mesethmoid is only present in *Leporellus* and *Insperanos*. In contrast, in *Hypomasticus,* there is a distal process (also present in *Insperanos*) that can impact the general shape of the anterior tip of the mesethmoid, making it resemble a hook, as in *Leporellus*.

62. Neurocranium, mesethmoid, distal lateral process: 0. absent, 1. present. (Sidlauskas & Vari, 2008: #16, modified) (CI = 0.500; RI = 0.500).

The anterior tip of the mesethmoid is uniformly slender in most Anostomidae. However, in *Hypomasticus megalepis, H. mormyrops*, and *Insperanos*, there is a distal lateral process along the anterior tip of the mesethmoid. This process was described by Sidlauskas & Vari (2008: 91) for *Hypomasticus* but not for *Insperanos* (Sidlauskas *et al.*, 2021). The process seems to be absent in small specimens of *Insperanos nattereri* (Sidlauskas *et al.*, 2021: fig. 5), but it is present in the two large dry skeletons (MZUSP 110595, 233-242 mm SL, Figure 8).


Frontal
Mesethmoid
Process of mesethmoid
Anterior process of mesethmoid
Vomer
Lateral ethmoid process
1 mm

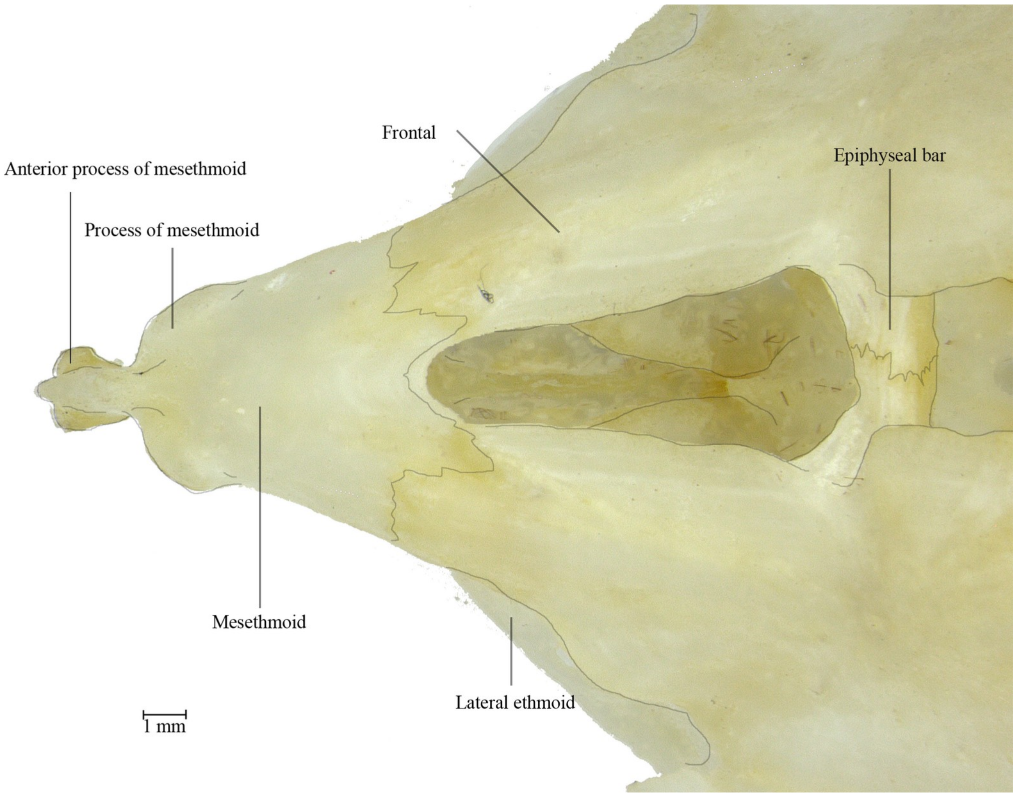

Frontal
Anterior process of mesethmoid
Epiphyseal bar
Process of mesethmoid
Mesethmoid
Lateral ethmoid
1 mm

Figure 8. Lateral (upper) and dorsal (lower) view of the anterior portion of neurocranium of *Insperanos nattereri* (MZUSP110595).

63. Neurocranium, lateral ethmoid, ridge on posterior margin of ventral portion of lateral ethmoid, 0. absent; 1. present. (Sidlauskas & Vari, 2008: 25, modified) (CI = 0.100; RI = 0.180).

The ventral face of the wing of the lateral ethmoid is flat in most Anostomidae. Because of this morphology, the insertion of the ectopterygoid-lateral ligament on the bone seems to be ventral (Sidlauskas & Vari, 2008: fig 23-25). On the other hand, the presence of a ridge on the posterior edge of the wing in the ventral face of the lateral ethmoid in *Anostomus anostomus, Abramites hypselonotus, Leporinus striatus,* and *Pseudanos trimaculatus* cause the face to bend anteriorly, making the insertion ectopterygoid-lateral ligament appear to be anterior, as illustrated in *Petulanos plicatus* in Sidlauskas & Vari (2008: fig 26).

64. Neurocranium, lateral ethmoid, lateral face of lateral ethmoid forming the cephalic shield, 0. absent; 1. present (CI = 0.330; RI = 0).

Most anostomids have their frontal bone positioned above the dorsal portion of the lateral ethmoid wing, on dorsal view. However, in *Megaleporinus brinco,* the lateral ethmoid contacts most of the distal lateral limit of the frontal and do not appear to be below the bone, in dorsal view, as in the alternative state, causing the lateral ethmoid bone also to compose the cranial shield in this species.

65. Neurocranium, lateral ethmoid, process directed anteriorly towards posterolateral border of mesethmoid: 0. absent; 1. present. (Sidlauskas & Vari, 2008: #27) (CI = 0.110; RI = 0.110).

In some Anostomidae, including *Abramites hypselonotus, Hypomasticus mormyrops, Laemolyta taeniata, Leporinus amblyrhynchus, L. friderici, Megaleporinus brinco, M. conirostris, M. elongatus, M. garmani, M. muyscorum, M. obtusidens, M. reinhardti, M. sp. (Tocantins), Pseudanos trimaculatus* and *Schizodon intermedius*, there is a process on the lateral ethmoid that is anteriorly-directed towards the posterolateral border of the mesethmoid, forming a bony bridge between the two bones (Figure 9; Sidlauskas & Vari, fig. 20). In one side of the head of a specimen of *Megaleporinus brinco* (MZUEL17832) the process contacts the two bones. In contrast, it is not on the other side of the specimen. In one of the large dry skeletons of *M. obtusidens*, the process is slightly shorter on one of the sides of the head. Due to the variation, we decided not to consider the contact as a character or character state but simply the presence of the process.

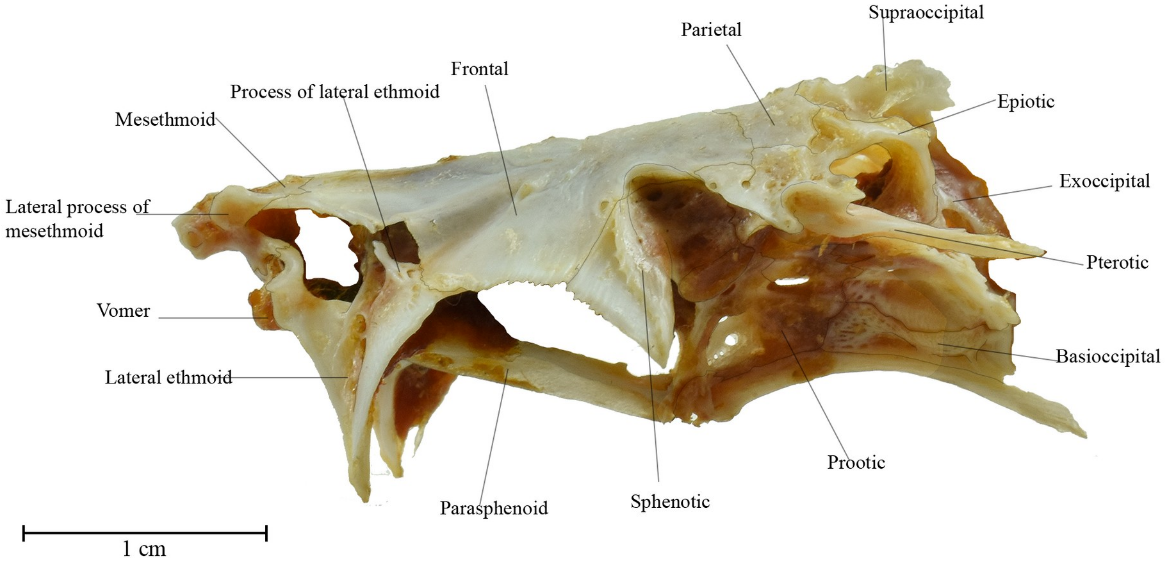


Figure 9. Neurocranium of *Schizodon intermedius* (MZUEL11252) in lateral view.

66. Neurocranium, cranial fontanel, aperture: 0. completely opened along entire length; 1. partially or completely closed. (Sidlauskas & Vari, 2008: #28) (CI = 0.500; RI = 0).

The frontal-parietal fontanel is completely opened along the entire length, from mesethmoid to parietals in most Anostomidae (Winterbottom, 1980; Sidlauskas & Vari, 2008). However, it is partially closed in *Pseudanos trimaculatus* (Winterbottom, 1980: fig. 53) and completely closed in *Abramites* (Vari & Williams, 1987; Sidlauskas & Vari, 2008). The fontanel is also partially closed in a few specimens of Leporinus friderici. However, it is coded as bearer of state 0 herein.

67. Neurocranium, cranial fontanel, inclusion of mesethmoid: 0. not included; 1. included. (Autapomorphy)

In some Anostomidae as *Hypomasticus mormyrops, Leporellus vittatus,* *Leporinus friderici, L. striatus, Megaleporinus conirostris, M. gaiero, M. macrocephalus M. muyscorum, M. obtusidens, M. reinhardti, M. trifasciatus, M.* sp (Orinoco), *M.* sp. (Tocantins)*, Pseudanos trimaculatus* and *Schizodon intermedius*, the cranial fontanel is composed only by parietal and frontal bones (Figs. 9 and 10), and close right before the frontal-mesethmoid suture. Alternatively, species such as *Anostomus anostomus, Hypomasticus megalepis, Insperanos nattereri, Laemolyta taeniata, Leporinus amblyrhynchus, L. fasciatus, Megaleporinus brinco, M. elongatus, M. garmani,* and *M. piavussu*, possess a fontanel that is composed, in its anteriormost extremity, by the mesethmoid (Fig. 11). That character is inapplicable for *Abramites hypselonotus*, due to the absence of a cranial fontanel.

68. Neurocranium, frontal, additional contact of contralateral bones: 0. absent; 1. present (CI = 0.200; RI = 0.600).

In some cases, there is an extra ossification on the fontanel, besides the epiphyseal bar, it is a contact of frontal bones that seals a portion of the rift that is present (state 1) in *M. gaiero* and *M*. sp. (Tocantins) (Figure 13) but absent in all other species of the study. *Abramites hypselonotus* and *Pseudanos trimaculatus* are coded as inapplicable (-) for this character due to the closure of their fontanel (character 60).

69. Neurocranium, frontal, position of contact of contralateral bones: 0. proximal to epiphyseal bar; 1. proximal to mesethmoid (CI = 0.500; RI = 0.910).

The contact between the contralateral bones of the frontal that interrupts the cranial fontanel in *Megaleporinus gaiero* is anterior to the epiphyseal bar (Fig. 12) in the medial portion of the frontal. On the other hand, *Megaleporinus* sp. (Tocantins) (Figure 13) has this contact on the anteriormost portion of the frontal bone, posterior to the suture between the frontal and mesethmoid.

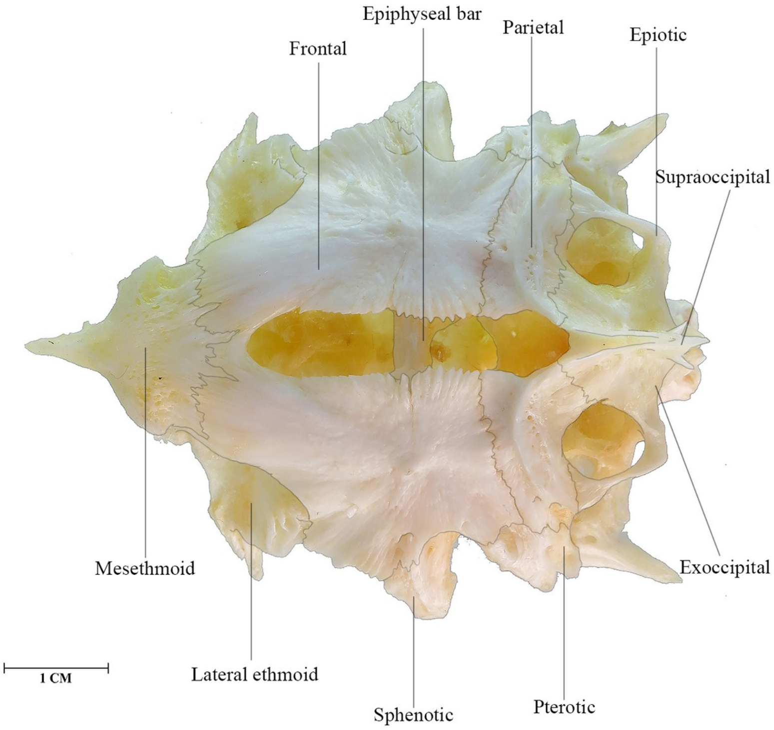


Figure 10. Dorsal view of the neurocranium of *Megaleporinus macrocephalus* (MZUEL20404).

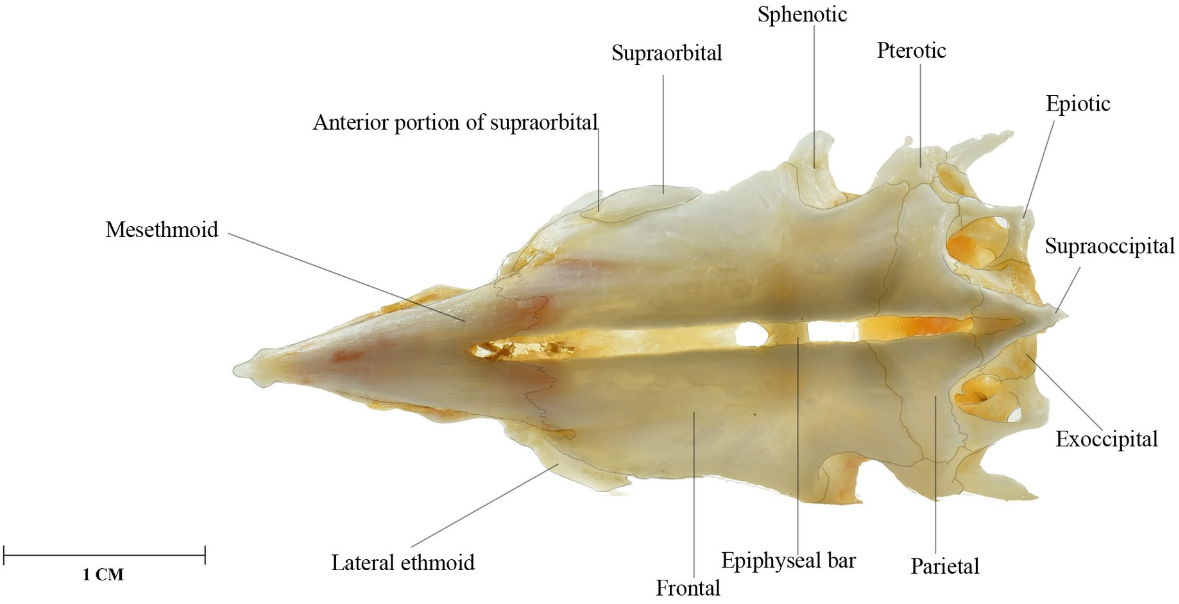


Figure 11. Dorsal view of the neurocranium of *Megaleporinus brinco* (MZUEL17832).

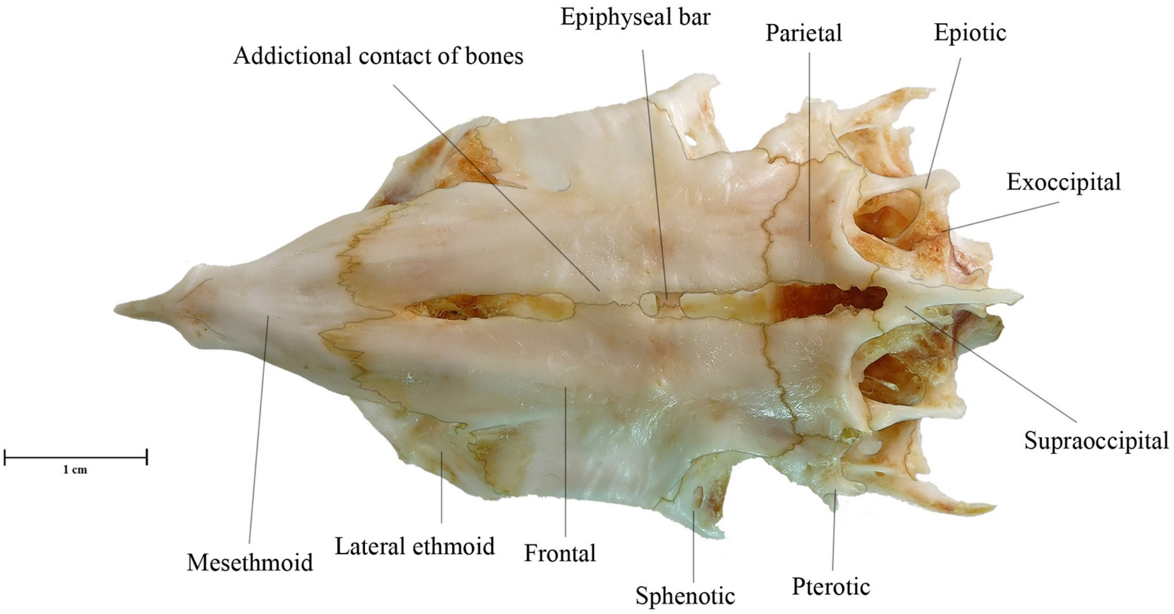


Figure 12. Dorsal view of the neurocranium of *Megaleporinus gaiero* (MZUSP111130).

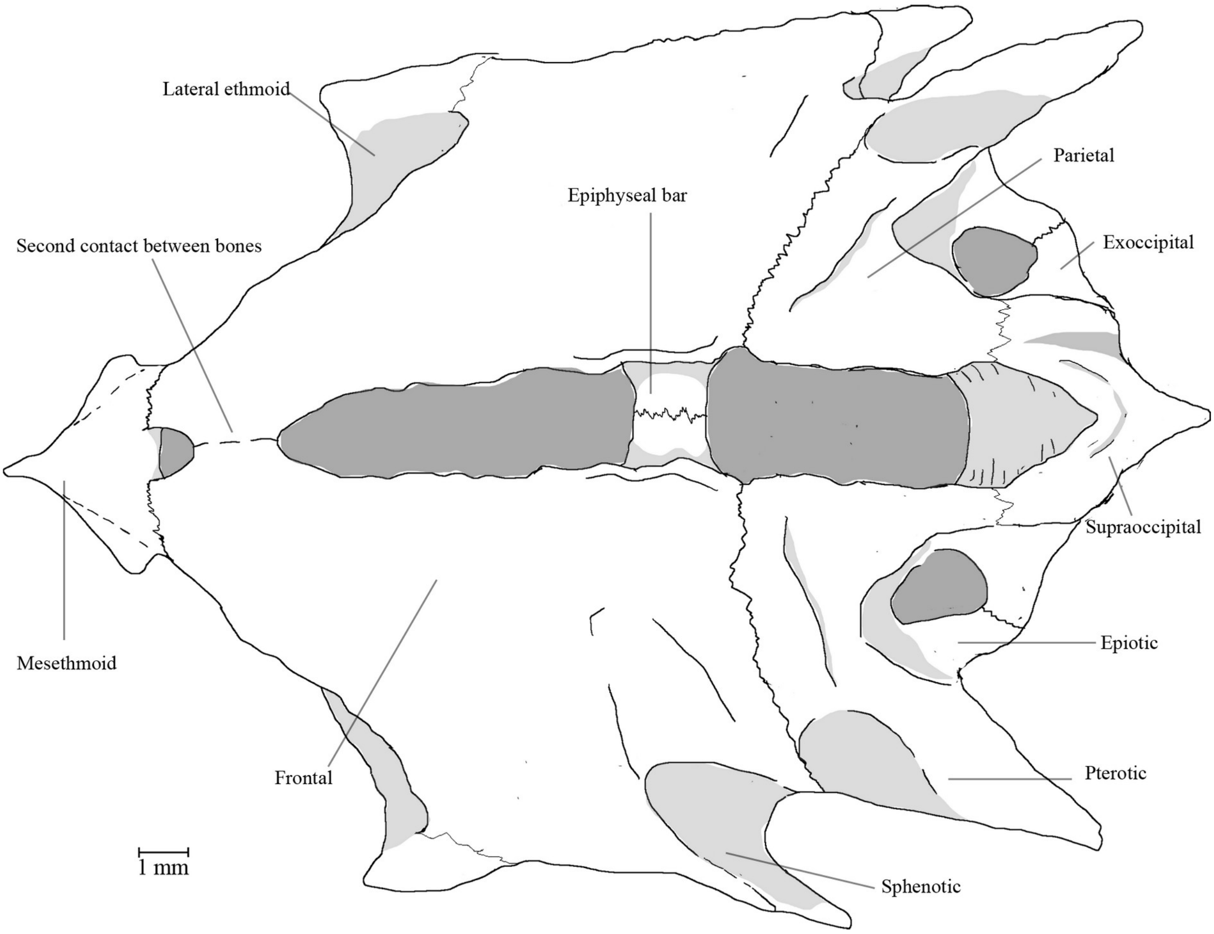


Figure 13. Representation of neurocranium of *Megaleporinus* sp. (Tocantins) (MZUEL20700) in dorsal view.

Caudal Fin

70. Caudal fin, urostyle, angle formed between urostyle and parhypural: 0. approximately 90 degrees; 1. distinctly less than 90 degrees (CI = 0.330; RI = 0.330).

The angle of the notochord flexion is measured by tracing a horizontal line in the center of the last caudal vertebrae to its end and a line from the tip of the urostyle to the center of the same vertebrae. The greater the opening of this angle, the bigger the caudal fin's contact surface. The differences in the sizes of caudal fins can be related to the locomotor habits of the different species (Breda *et al.,* 2005). *Anostomus anostomus, Hypomasticus, Laemolyta taeniata, Leporellus vittatus, Leporinus amblyrhynchus, L. fasciatus, L. friderici, L. striatus, Pseudanos trimaculatus, Megaleporinus brinco, M. conirostris, M. elongatus,* and *M. muyscorum* possess closest angles between urostyle and parhypural that gives the caudal fin a slender aspect. Alternatively, *Abramites, Insperanos nattereri, Schizodon intermedius, M.*

gaiero, M. macrocephalus, M. obtusidens, M. piavussu, M. reinhardti, M. trifasciatus, M. sp. (Orinoco) and *M.* sp. (Tocantins), have the open most angles and therefore, greater caudal-fin area (state 0).

Sexual Chromosome system

71. Sexual chromosome system, possession of sex chromosome system. 0. absent; 1. present (Synapomorphy) (CI = 1.000; RI = 1.000).

Many Anostomidae species do not possess a sexual chromosomic system, except for *Megaleporinus,* which possesses the ZZ/ZW system, and *Leporinus geminis* which possess a different system. (The aforementioned chromosomes in *Megaleporinus* are defined as a synapomorphy of the genus by Ramirez, Birindelli & Galetti (2016).

CHARACTER APPLICATION IN *LEPORINUS SCALABRINII*†

The species is represented by one fossilized individual that, despite being partially covered by rock matrix and dorso-ventrally compressed due to the preservation, possesses its jaws and teeth well preserved, and infraorbital series, suspensorium and dorsal portions of neurocranium visible (Fig. 14 and 15; Bogan *et al.*, 2012). Therefore, we describe the characters listed above that apply to that species.

Leporinus scalabrinii† possess three blunt teeth on the premaxilla (character 21, state 0; character 22, state 0) and three on the dentary, also blunt (character 26, state 0; character 28, state 0).

Its premaxillary bones are still connected to the anterior process of mesethmoid and are oriented in an oblique position relative to the horizontal main axis of the neurocranium (character 30, state 0). Furthermore, the ventral end of the premaxilla is ventrally aligned (character 33, state 1), as seen in most anostomids with terminal mouths in our study. The premaxilla in *L. scalabrinii*† also possesses an ascending process on the premaxilla that is bent posteriorly, similar to that of *Hypomasticus*, *Leporinus amblyrhynchus,* and some species of *Megaleporinus* (character 31, state 1). The premaxillary bone in *L. scalabrinii*† also has an anterodorsal process that is larger than the crown of its third tooth, similar to *Leporellus*, *Insperanos, Hypomasticus mormyrops, Megaleporinus conirostris* and *M. garmani*. (character

32, state 0). The rock matrix partially covers the posterior extremity of the dentary bone. However, it is visible that the middle portion of the ventral margin of the dentary does not present any concavity and is, therefore, considered straight (character 37, state 1).

The opercular series of *L. scalabrinii*† is fragmented due to the compression of the preservation; however, it is possible to observe that the posterodorsal margin of the opercle does not present any sort of concavity, similar to *Laemolyta* and *Schizodon* herein (character 45, state 1).

Besides the compression of the neurocranium, some osteological characteristics can be observed (Bogan *et al.,* 2012) and coded herein. The mesethmoid bone is well preserved and possesses a shape that is somehow similar to that in *Megaleporinus macrocephalus, Schizodon intermedius,* and other species that present the width of the mesethmoid, in the posterior limit, larger than its length (character 61, state 0), and width on the lateral process approximately equal to the length of the anterior process of mesethmoid (see character 62, state 1). Furthermore, *L. scalabrinii*† does not possess the mesethmoid distal-lateral process observed in *Hypomasticus* and *Insperanos* (character 63, state 0). The rock matrix partially covers the lateral ethmoid on the species. However, it is possible to observe a small knob projecting from the bone, where a process that contacts the posterolateral border of mesethmoid exists in many anostomids. Nevertheless, the knob is not as observed in the bearers of this character. Therefore, the process in *L. scalabrinii*† is interpreted as absent herein (character 64, state 0).

The fontanel seems to be closed also in the extinct species *Leporinus scalabrinii*† (Bogan *et al.*, 2012). However, the entire skull of the only specimen known of the latter is dorso-ventrally compressed due to preservation (Bogan *et al.*, 2012), which could have affected the opening of the fontanel. Given that this character is supposed to be important for understanding the relationships between *L. scalabrinii*† and other anostomids due to the positioning of the few species that possess closed or partially closed fontanels within Anostomidae, and their positions relative to *Megaleporinus* (*Abramites* and *Pseudanos trimaculatus* respectively), we performed two phylogenetic analyses considering the fontanel as closed in one and open in the other (character 65).

The participation of the mesethmoid bone in the cranial fontanel also varies among the two analyses (character 66). When the cranial fontanel is considered absent, this character becomes inapplicable (-) for the same reasons as in *Abramites hypselonotus*. Alternatively, even when the cranial fontanel is considered present, it does not seem to surpass the frontal-mesethmoid suture. Therefore, the species is coded as a representative of the state 0.

The results showed that the fossil's phylogenetic position is the same despite the coding of these characters. Therefore, the fontanel of *Leporinus scalabrinii*† is still considered closed herein, as Bogan et al. (2012) suggested.

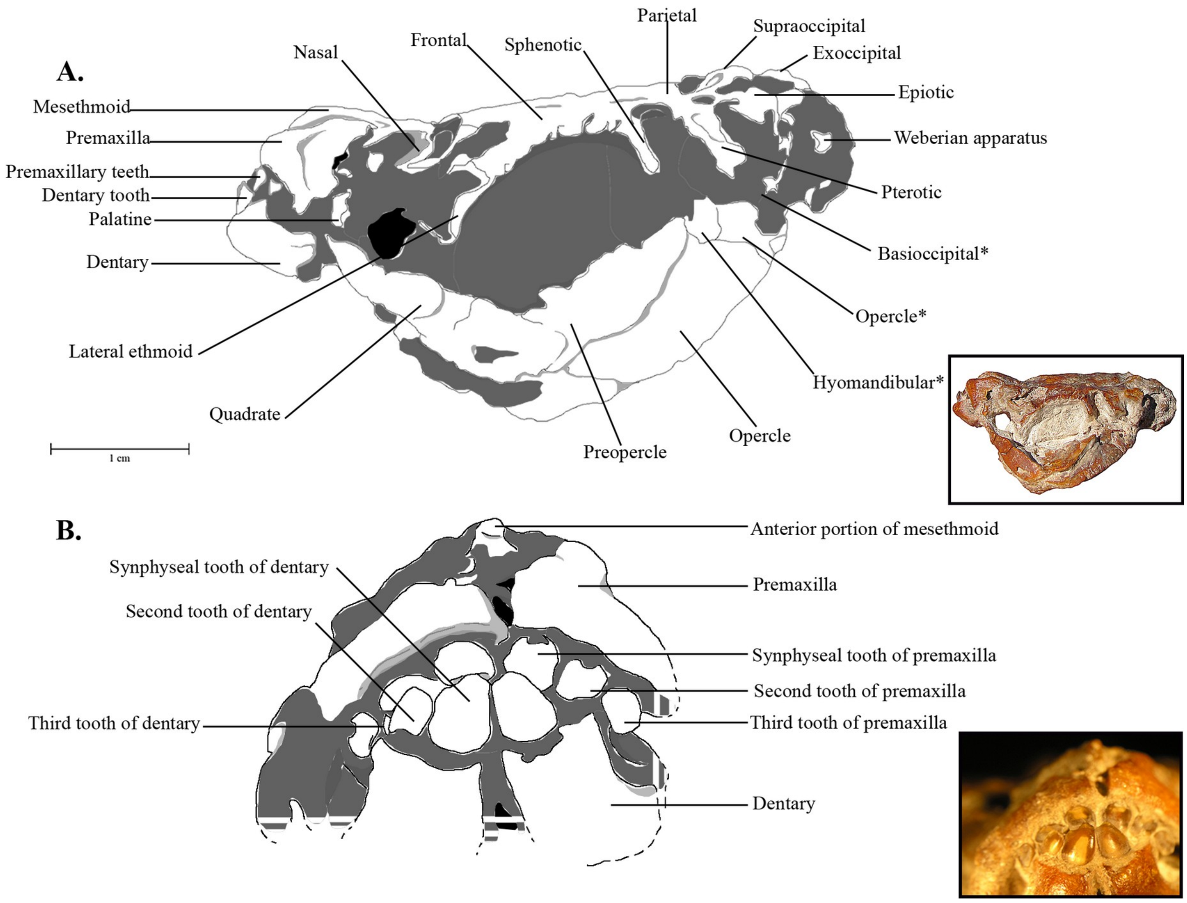


Figure 14. A. Representation of the neurocranium of *Leporinus scalabrinii*† in lateral view; **B**. Representation of the jaw in frontal view. The dark gray areas represent the portions covered by a rock matrix. Asterisks indicate morphological structures tentatively identified by Bogan *et al.* (2012).

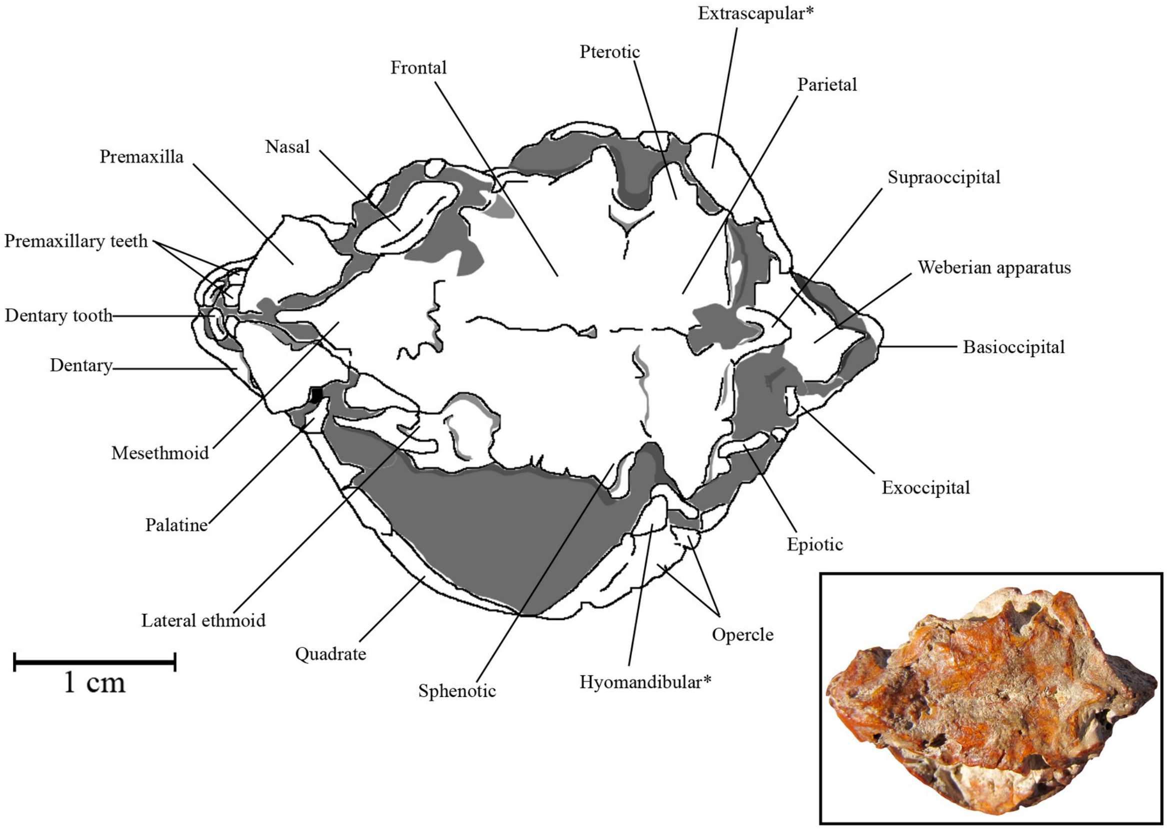


Figure 15. Representation of the neurocranium of *Leporinus scalabrinii* in dorsal view. The dark gray areas represent the portions covered by a rock matrix. Asterisks indicate morphological structures identified by Bogan *et al.* (2012).

UNUTILIZED CHARACTERS

As previously mentioned, some characters that could not be correctly interpreted nor modified are discriminated as unutilized and discussed below. Characters used by Winterbottom (1980) and Sidlauskas & Vari (2008) that include morphological conditions present in taxa that were not included in our analyses (see taxa selection) were not included in this list.

1. Circum-orbital series, sixth infraorbital, posterior extent of sixth infraorbital and fusion with suprapreopercle: 0. Sixth infraorbital not posteriorly expanded and entirely separate from suprapreopercle: 1. Sixth infraorbital posteriorly expanded and fused with suprapreopercle (Sidlauskas & Vari, 2008: #14).

The feature mentioned in state 1 could not be observed in the examined cleaned and stained specimens of *Anostomus anostomus* and *Pseudanos trimaculatus*. According to Sidlauskas & Vari (2008), this condition seems to be a synapomorphy of the representatives of Anostominae. In any case, removing this character did not affect the topology of the trees obtained herein.

2. Neurocranium, mesethmoid, the orientation of ventral process of mesethmoid contacting anterior limit of vomer: 0. Ventral process of mesethmoid runs vertically or nearly so, anterior portion of vomer positioned directly ventral to the ventral process of mesethmoid; 1. Ventral process of mesethmoid posteroventrally aligned; 2. The ventral process of the mesethmoid is horizontally or slightly posterodorsally aligned, vomer directly posterior to the ventral portion of the mesethmoid (Sidlauskas & Vari, 2008: #17).

Due to its gradual nature, the orientation described in states 0 and 1 could not be separated herein. We tried to modify this character, but, as a result, state 2 was interpreted as an autapomorphy of *Hypomasticus mormyrops*.

3. Neurocranium, vomer, presence or absence of pentagonal ventral extension of vomer: 0. Ventral surface of the main body of vomer flat; 1. Ventral surface of the main body of the vomer with a pentagonal raised area medial to articulations with left and right palatines in ventral view (Sidlauskas & Vari, 2008: #21)

The apomorphic state of this character (state 1) is considered a synapomorphy of the Anostominae by Sidlauskas & Vari (2008). However, this condition is not confirmed in the cleared and stained specimens of *Anostomus* and *Pseudanos*.

4. Neurocranium, vomer, form of articulation of vomer with palatine: 0. articular facets of vomer with palatines angled toward each other anteriorly; 1. articular facets of vomer with palatines parallel and widely separated; 2. articular facets of vomer with palatines parallel and narrowly separated (Sidlauskas & Vari, 2008: #22).

We could not distinctly see any clear difference among the three states of this character due to the continuous variation in the shape of the articular faces of the vomer among the examined species.

5. Form of posterior lamina of dentary: 0. dentary teeth with large, well-developed posterior lamina; 1. dentary teeth either with small, poorly developed posterior lamina or posterior lamina absent. (Sidlauskas & Vari, 2008: #38)

The authors consider the dentary teeth to be composed of two parts: the main body and a thinner portion extending posterolaterally (the so-called lamina). Herein, all examined specimens exhibit the dentary teeth incisiform, bearing a small, continuous posterolateral extension that makes each tooth asymmetrical. There is no apparent limit between the main body of the teeth and the suggested lamina.

6. Presence or absence of cusps on posterior lobes of dentary teeth: 0. Posterior loes of

dentary teeth absent, or if posterior lobes present, no distinct cusp present on posterior lobes of tooth; 1. Posterior lobes of dentary teeth with one or more distinct cusps. (Sidlauskas & Vari, 2008: #44).

Although this character was not reported to vary in any taxa of our study, we did not use it herein mostly because we do not consider the existence of the lamina (previous character).

7. Form of insertion of anterolateral flange of maxilla on flat ventral portion of maxilla: (0) anterolateral flange of maxilla merges with ventral portion of maxilla on lateral face of ventral portion; (1) anterolateral flange of maxilla merges with ventral portion of maxilla on anterior edge of ventral portion (Sidlauskas & Vari, 2008: #50)

The apomorphic state of this character (state 1) is considered a synapomorphy of the Anostominae by Sidlauskas & Vari (2008). However, this condition is not confirmed in the cleared and stained specimens of *Anostomus* and *Pseudanos*.

8. Gill arches, upper pharyngeal tooth-plate, number of tooth rows: 0. one or two rows; 1. three or more rows. (Sidlauskas & Vari, 2008: #94).

The teeth on the upper pharyngeal tooth plate are not neatly aligned in most specimens, and therefore, it is impossible to determine with precision the number of tooth rows on the bone. This condition was already noticed by Sidlauskas & Vari (2008). Nevertheless, the teeth are more or less aligned, making it possible to estimate the number of rows. In addition, the number of teeth is related to the number of rows. Among Anostomidae, only *Schizodon* and *Rhytiodus* have more than 25 teeth arranged in more or less four rows on the pharyngeal tooth plate (Sidlauskas & Vari, 2008). This condition was herein confirmed. On the other hand, all other Anostomidae, including all *Megaleporinus*, have 25 or fewer teeth arranged in somewhat two rows.

9. Presence or absence of supraneural dorsal to Weberian apparatus: (0) supraneural absent; (1) supraneural present (Sidlauskas & Vari, 2008: #106).

Since most of the specimens investigated were prepared as dry skeletons due to their large sizes, this character could not be adequately observed. Therefore, we choose not to use this character herein to avoid misinterpretations, but we highlight the need to observe this character in future studies about anostomids.

ANALYSIS OF MORPHOLOGICAL DATA

The parsimonious analysis based exclusively on the morphological characters resulted in 98 trees with 195 steps, a CI of 0.370, and an RI of 0.570. A strict consensus tree was calculated, resulting in a largely polytomic tree (Figure 16). A majority-consensus tree was calculated, resulting in a mostly resolved phylogenetic tree (Figure 17).

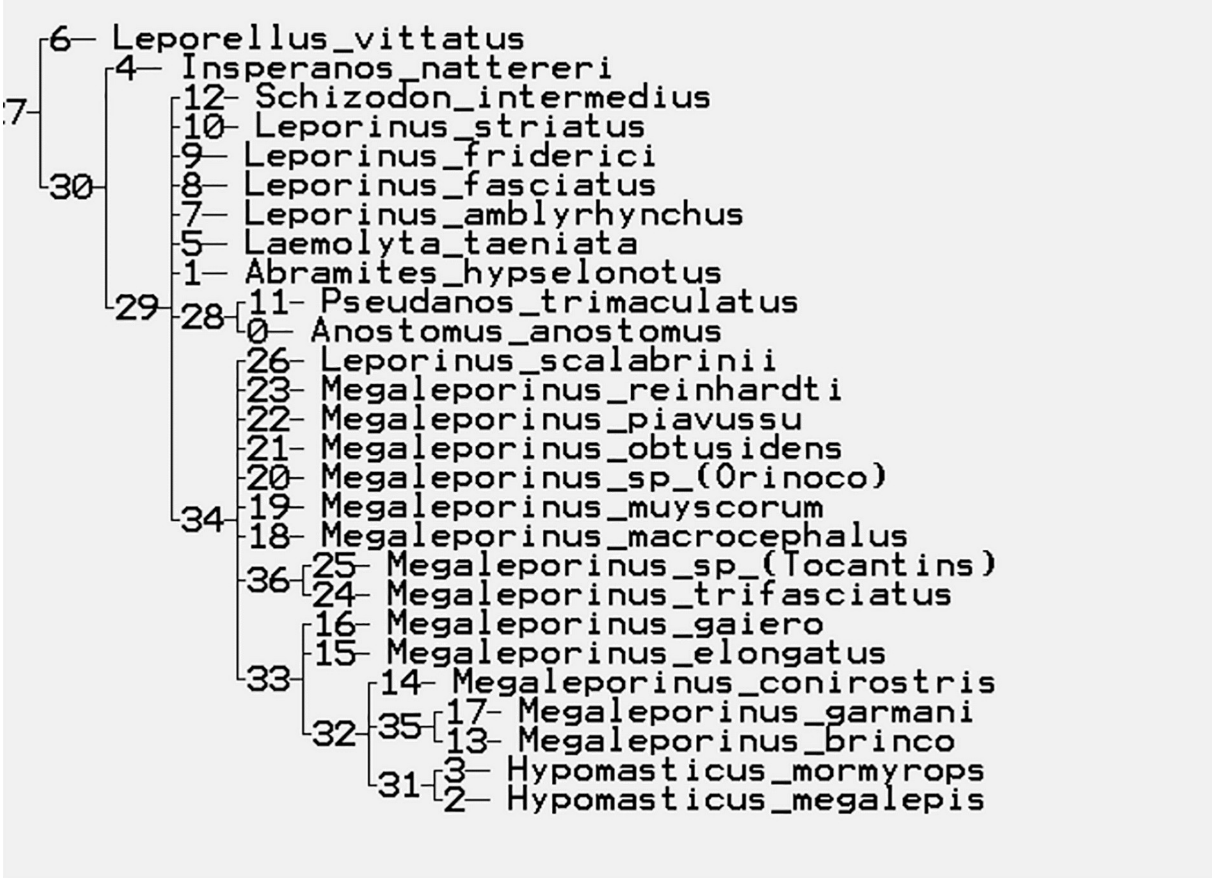


Figure 16. Phylogenetic relationships among *Megaleporinus* inferred exclusively based on 70 morphological characters, using a maximum parsimonious analysis. Strict Consensus tree obtained through 98 trees. The numbers on the nodes represent the percentage of consensus from the analysis.

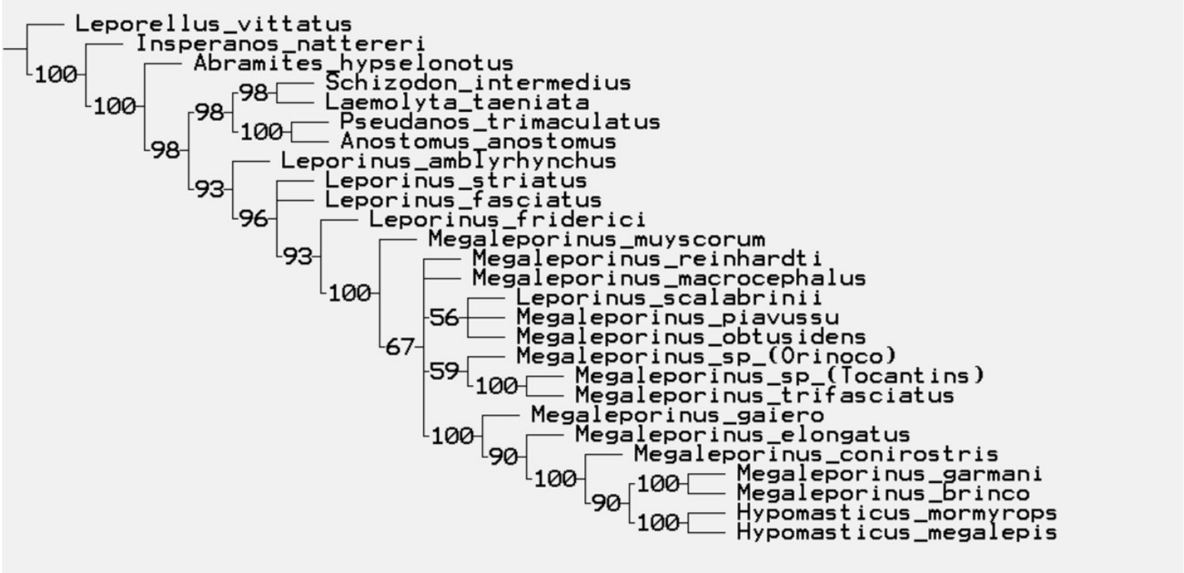


Figure 17. Phylogenetic relationships among *Megaleporinus* inferred exclusively based on 70 morphological characters, using a maximum parsimonious analysis. Consensus tree obtained via majority rule through 98 trees. The numbers on the nodes represent the percentage of consensus from the analysis.

All character-state changes that support each node of the consensus tree (obtained using the majority-rule) are listed below:

Node 1 (*Anostominae, Schizodon, Laemolyta, Leporinus, Megaleporinus,* and *Hypomasticus*): #20- Four or five scale rows between lateral line and pelvic-fin origin. Present in *Leporellus vittatus* and *Insperanos* and reversed in node 1 (except *Megaleporinus piavussu, M.* sp. (Orinoco) and *Leporinus striatus*).

Node 2 (*Anostominae, Schizodon* and *Laemolyta*): #22- Multicuspid cutting edges of premaxillary teeth; #28- Multicuspid teeth on dentary, reversed in *Laemolyta taeniata*; #37- Middle portion of ventral margin of dentary, convex in *Leporellus vittatus* and reversed in node 2); #41- overlapping of quadrate by ventral expansion of entopterygoid absent, except in *Schizodon intermedius* which present the plesiomorphic state.

Node 3 (*Anostomus anostomus* and *Pseudanos trimaculatus*): #23 Three cusps on premaxillary teeth; #28 Multicuspid dentary teeth, also present in *Schizodon intermedius*; #29- Three cusps on dentary teeth; #30- Premaxilla longitudinally orientated in relation to the longitudinal body axis; #33- Ventral end of maxilla anteriorly directed; #38- Distinct process present in the autopalatine, independently present in *Insperanos nattereri*; #40- Inclined ectopterygoid; #42- Small lateral shelf relative to the symplectic; #51- Anterior portion of the antorbital bone absent in *Leporellus vittatus* and *Insperanos nattereri*, and reversed in node 3; #70- Ridge on posterior margin of ventral portion of lateral ethmoid, also present in

Leporinus striatus and *Abramites*.

Node 4 (*Schizodon* and *Laemolyta*): #23- Four cusps on premaxillary teeth; #45- Concavity on the posterodorsal margin of opercle; #53- Length of the sensory canal of first infraorbital smaller than its height. Also present in node 5 (except *Leporinus friderici*); #55- second infraorbital length more than two times bigger than its maximum depth.

Node 5 (*Megaleporinus, Hypomasticus,* and *Leporinus*): #24- Distinct marginal ridge on the lingual surface of teeth in adults, it is reversed to its plesiomorphic state in node 11 and *L. striatus*; #25- Well-developed marginal ridge on the lingual surface of teeth in adults, except in *M. gaiero, M. elongatus* and node 9. Inapplicable for node 9 and *L. striatus*; #42- Large lateral shelf of the quadrate, relative to symplectic, except in *L. amblyrhynchus* and reversed in node 10; #44- Overlapping between symplectic and metapterygoid, also present in *Schizodon intermedius*.

Node 6 *Megaleporinus*: #5- Dark midlateral rounded blotch below the dorsal-fin base, except in *M. trifasciatus, M. Tocantins, M. elongatus,* and node 9 (except node 11 and *M. brinco* which is polymorphic for this character), and appears independently in *Pseudanos trimaculatus*; #7- Dark midlateral rounded blotch above anal-fin base, except in *M. conirostris, M. garmani*, *M. trifasciatus* and *M.* sp. (Tocantins), the character appears independently in *Leporinus friderici*; #8- Dark midlateral blotch on caudal peduncle persistent in adults, independently present in *Pseudanos trimaculatus*; #9- Great dark midlateral blotch on caudal peduncle persistent in adults, relative to pupil, except node 8 and independently present in *Leporinus friderici* and *Pseudanos trimaculatus*; #25- Well- developed marginal ridge on the lingual surface of teeth in adults, except in *M. elongatus, M. gaiero* and node 9. Inapplicable to node 11 and *L. striatus*; #26- Three teeth on dentary, except *M. garmani* and node 11; #59- Overlapping of anteroventral process of sphenotic by the sixth infraorbital, also present in *Anostomus anostomus, Abramites hypselonotus* and *Leporellus vittatus*, and polymorphic in *Hypomasticus mormyrops*; #68 Angle formed between urostyle and parhypural with approximately 90 degrees, except in *M. elongatus* and node 9. This state is also the same in *Abramites hypselonotus* and *Insperanos nattereri*; #69- Possession of a sex chromosome system, absent in node 11.

Node 7 (*Megaleporinus piavussu, M. obtusidens,* and *Leporinus scalabrinii*): #2- vertical bands formed by dermal pigmentation (not fragmented, nor encircling the body) persistent in adults. However, it is valid to comment that *L. scalabrinii*† does not possess any coloration conserved during the preservation process, and the relation among these species is supported principally by the next commented character and the consequence of the organization of other characters in the *Megaleporinus* genera; #31- Ascending process of the premaxilla posteriorly directed. Also present in *Megaleporinus elongatus, M. gaiero,* node 9, and *Leporinus amblyrhynchus*.

Node 8 (*Megaleporinus trifasciatus*, *M.* sp. (Tocantins)): #3- Two dark midlateral vertically-elongated blotches on the body, one below the dorsal fin and one above the anal fin. This character is also independently present in *Schizodon intermedius*; #5- Dark midlateral rounded blotch below the dorsal-fin base that is also absent in *M. conirostris, M. elongatus, M. garmani* and polymorphic in *M. brinco*; #9- Size of dark midlateral blotch on caudal peduncle persistent in adults, smaller than the pupil, also present in *M.* sp (Orinoco); #16- Red pigmentation covering ventral portion of opercular series.

Node 9 (*M. conirostris* and (*M. garmani* and *M. brinco* + *Hypomasticus*): #18- 12 scale rows around the caudal peduncle, except in *M. megalepis*. This state independently appears in *Leporinus amblyrhynchus*; #30- Oblique premaxilla, relative to longitudinal body axis; #31- Posterior ascending process of the premaxilla, also present in *M. elongatus, M. gaiero,* node 7 and *Leporinus amblyrhynchus*; #33- Ventral end of the maxilla posteriorly positioned; #35- size of tip of anguloarticular ascending process wider than longer. This character is also confirmed in *Schizodon intermedius* independently; #36- Groove on the tip of the ascending process of anguloarticular; #39- Laterally oriented process of autopalatine, also present in *Abramites hypselonotus* and *Leporellus vittatus*; #49- Rod-like shape of the anterior portion of the supraorbital, except in *M. conirostris*. This character is also confirmed in *M. gaiero* and *Insperanos nattereri*; #60- The width of the mesethmoid is smaller than its length, also smaller in *M. elongatus, M. gaiero, Leporellus vittatus,* and *Insperanos nattereri*, and reversed in *H. megalepis*. This character is polymorphic in *M. obtusidens*); #68- Angle formed between urostyle and parhypural distinctly less than 90 degrees. This character is a plesiomorphy shared by most outgroup species (except *Abramites, Insperanos,* and *Schizodon*) and reversed in *M. elongatus* and node 9.

Node 10 (*Megaleporinus garmani* and *M. brinco*): #42- Small size of lateral shelf of quadrate, relative to symplectic. This plesiomorphy is present in most species of the outgroup (except node 3, *Leporinus fasciatus, L. friderici,* and *L. striatus*) and recovered in this node; #43- longitudinal crest on the dorsal margin of metapterygoid. The only two nodes not possessing this feature are node 3 and node 10; #54- Additional ventral pore on the sensory canal of first infraorbital, also present in *Laemolyta taeniata.*

Node 11 *Hypomasticus*: #5- Dark midlateral rounded blotch below the dorsal-fin base. It is also present in most *Megaleporinus* species (except node 8, *M. conirostris*, *M. elongatus,* and *M. garmani*) and *Pseudanos trimaculatus*. This character is also polymorphic in *M. brinco*); #59- Overlapping of the anteroventral process of sphenotic by the sixth infraorbital. This character is polymorphic in *H. mormyrops* and a shared apomorphy among *Leporinus*, node 2 (except *Anostomus anostomus*) and *Insperanos nattereri*; #62- Distal lateral process of mesethmoid, also observed in *Insperanos nattereri*.

ANALYSIS OF MOLECULAR DATA

The Bayesian-Inference analysis of the molecular data exclusively resulted in a tree that included *Leporellus* and the Anostominae as sister to remaining genera (Figure 18). The other clade was composed by *Hypomasticus* as sister group of the rest of the anostomids, which divide into a group formed by other groups composed by *Schizodon* plus *Laemolyta* [as sister group of *Leporinus* (except *L. striatus*)] and *L. striatus* plus *Abramites hypselonotus* (as sister group of *Megaleporinus*). As expected, these results match those of Ramirez, Birindelli & Galetti (2016), who used many of the same molecular sequences.

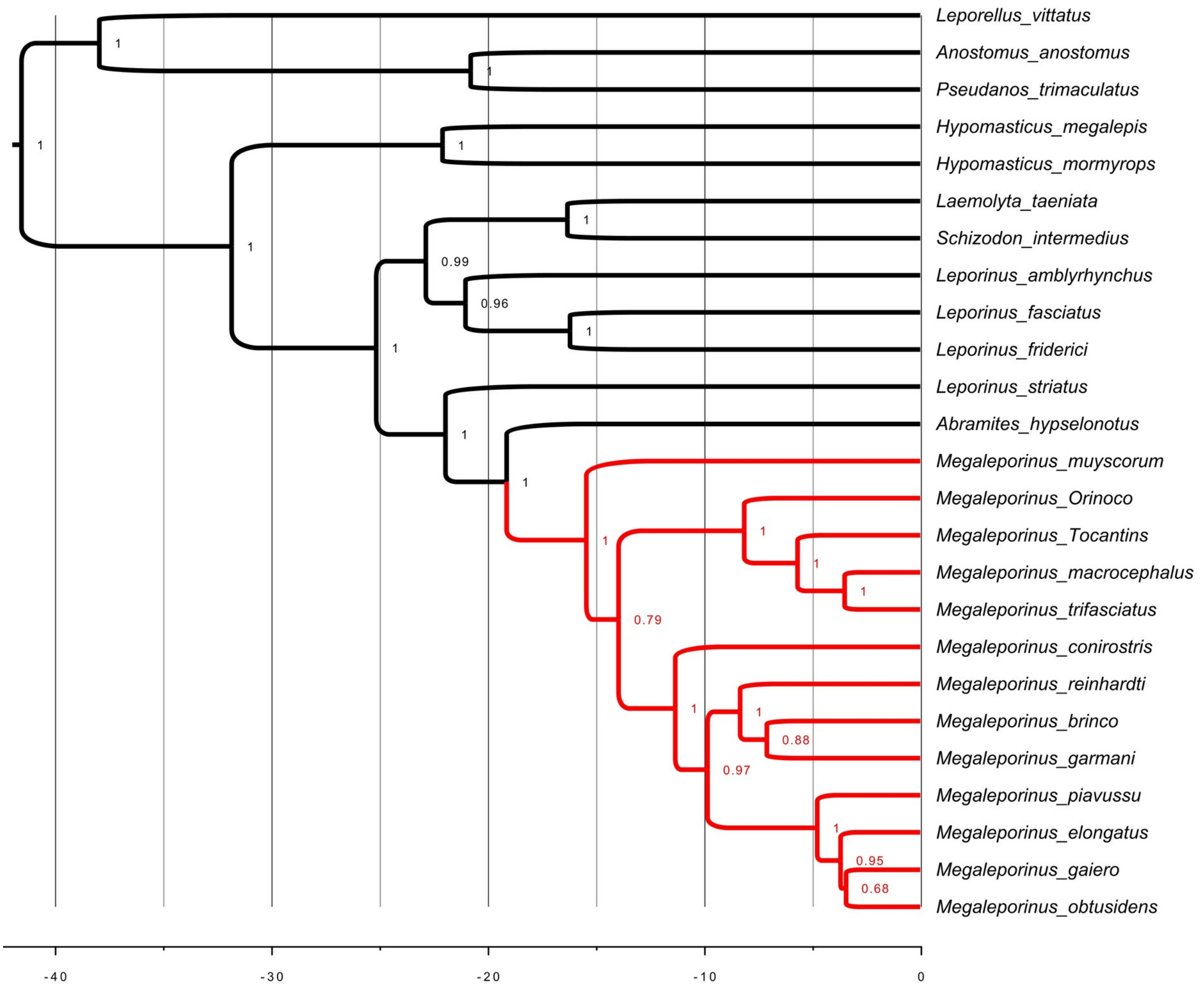


Figure 18. Phylogenetic relationships among *Megaleporinus*, based exclusively on molecular data and using Bayesian inference analysis. The *Megaleporinus* genus is highlighted in red; the scale below represents the age in million years.

TOTAL EVIDENCE ANALYSIS

The Bayesian inference analysis of the combined morphological and molecular data resulted in 1000 trees summarized in a single tree (Figure 19). All character-state changes that support each node of the consensus tree (obtained using the majority-rule) are listed below:

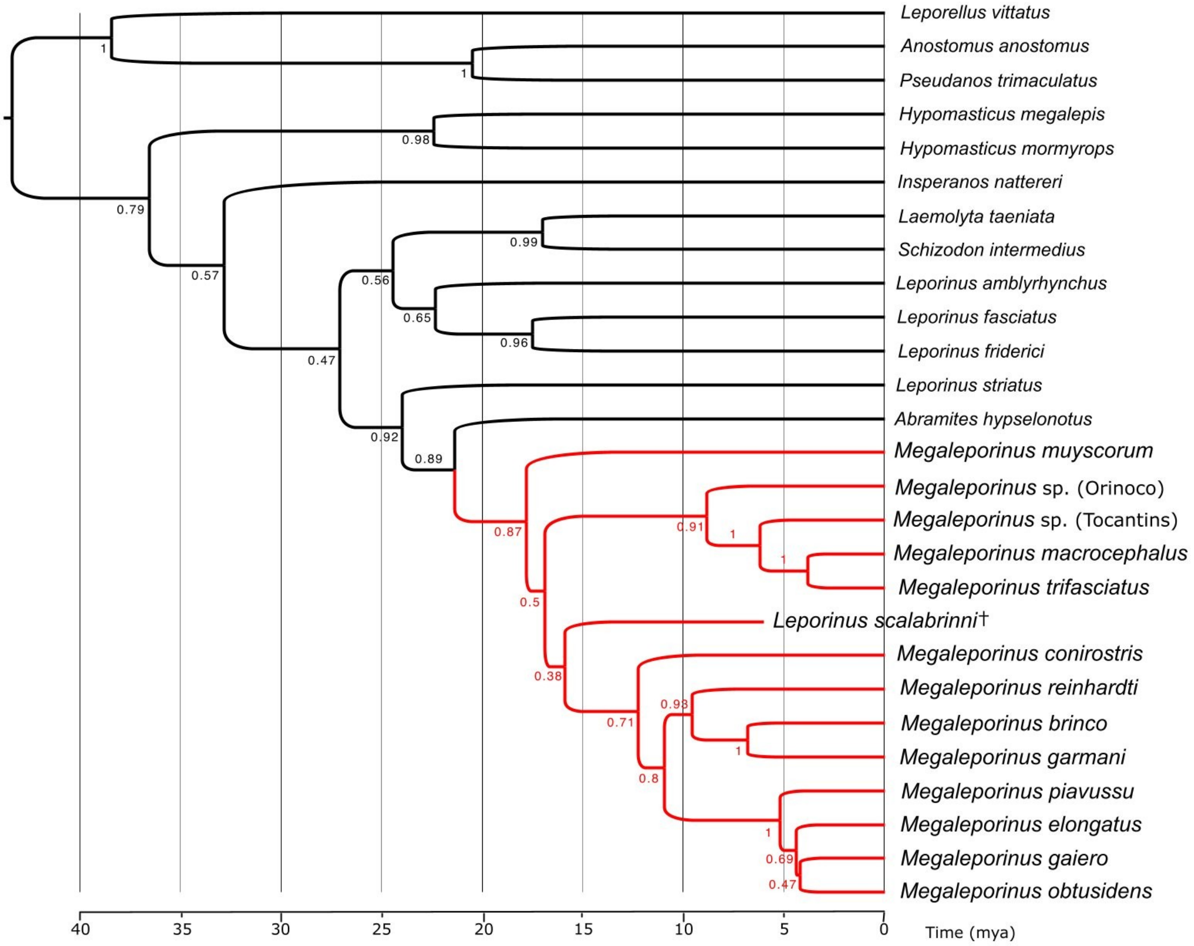


Figure 19. Phylogenetic relationships among *Megaleporinus* inferred based on 70 morphological characters and molecular data derived from five genetic markers (COI, Cytb, RAG1, RAG2, Myh6) and a calibrated Bayesian-Inference analysis. The posterior probability is indicated at each node.

Node 1 (*Leporellus, Anostomus,* and *Pseudanos*): #13- Dark spots at the center of scales. *Anostomus anostomus* features a secondary loss in this character; #37- Convex middle portion of the ventral margin of dentary. This character is reversed in node 4 species; #44- No overlap between symplectic and metapterygoid. This character is also present in *Insperanos nattereri* and reappears independently in *Laemolyta taeniata, Leporinus amblyrhynchus,* and *Abramites hypselonotus*; #51- The anterior portion of the antorbital is also absent in *Insperanos nattereri*; #53- Larger length of the sensory canal, relative to its height. That state is also similar in *Insperanos nattereri* and reversed in *Leporinus amblyrhynchus, L. friderici,* and *Abramites hypselonotus*.

Node 2 (*Anostomus* and *Pseudanos*): #22- Multicuspid teeth on premaxilla. This character also appears independently in node 4; #23- Three cusps on premaxillary teeth; #28- Multicuspid dentary teeth. This character also appears independently in *Schizodon intermedius*; #29- Three cusps on dentary teeth; #30- Longitudinal premaxilla, relative to longitudinal body axis; #33- Ventral end of maxilla anteriorly directed; #38- Absence of the distinct process of autopalatine; #40- Inclined ectopterygoid; #41- Overlapping of quadrate by ventral expansion of entopterygoid. This character appears independently in *Laemolyta taeniata*; #42- Small lateral shelf of quadrate, relative to symplectic; #43- Longitudinal crest on the dorsal margin of metapterygoid. This character is also independently present on node 8; #70- Ridge on the posterior margin of the ventral portion of the lateral ethmoid. It is also present in *Abramites* and *Leporinus striatus*.

Node 3 (*Hypomasticus*): #5- Dark midlateral rounded blotch below the dorsal-fin base. This character is also present in *Megaleporinus, L. friderici,* and *Pseudanos trimaculatus*; #7- Dark midlateral rounded blotch above the anal-fin base. This character also appears in *Megaleporinus* and independently in *Leporinus friderici*; #8- Dark midlateral blotch on caudal peduncle persistent in adults. This also appears independently in *Hypomasticus, Leporinus friderici,* and *Pseudanos trimaculatus*; #30- Transversal premaxilla, relative to the longitudinal body axis. Also, the same state was observed in node 8 and *M. conirostris*, independently; #31- Posterior ascending process of the premaxilla. This character state is also independently present in node 7 (except *M. reinhardti*) and *Leporinus amblyrhynchus*; #33- Ventral end of maxilla posteriorly directed. This character is also present in node 8 and *M. conirostris*; #35- Larger tip of ascending process, width relative to length. Also, independently present in *M. conirostris*, node 8 and *Schizodon intermedius*; #36- Groove on the tip of ascending process. This is also observed in node 8 and *M. conirostris*; #39- Process of autopalatine laterally oriented. This character is a plesiomorphy in *Leporellus* and *Abramites hypselonotus*, *M. conirostris,* and node 8; #42- Large lateral shelf of quadrate, relative to symplectic. This character is independently present in node 5, *Leporinus striatus,* and node 6 (except node 8); #49- Rod-like anterior portion of supraorbital. This character appears independently in node 8 and *M. gaiero*; #62- Distal lateral process on mesethmoid. This structure is also present in *Insperanos nattereri*.

Node 4 (*Schizodon* and *Laemolyta*): #17- 41 to 45 lateral-line scales. This is also a synapomorphy of *Megaleporinus trifasciatus* and *M. macrocephalus*, polymorphic in *M.* sp. (Orinoco), and appears independently in *Leporinus fasciatus, M. muyscorum Pseudanos trimaculatus*; #22- Multicuspid teeth on premaxilla. This character also appears independently in node 2; #23- Four cusps on premaxillary teeth; #37- Convex middle portion of the ventral margin of dentary. This character is a plesiomorphy present in node 1 species; #45- Concavity on the posterodorsal margin of opercle; #55- length more than two times the size of the maximum depth of second infraorbital.

Node 5 (*Leporinus friderici* and *L. fasciatus*): #24- Distinct marginal ridge on the lingual surface of teeth in adults. This character is also similar to node 6.

Node 6 (*Megaleporinus*): #5- Dark midlateral rounded blotch below the dorsal-fin base. That character presents secondary losses in *M. conirostris, M. elongatus, M. garmani, M. trifasciatus,* and *M.* sp. (Tocantins) and is also present in *Hypomasticus, Leporinus friderici,* and *Pseudanos trimaculatus*; #7- Dark midlateral rounded blotch above anal-fin base. This character presents secondary losses in *M. conirostris, M. garmani, M. trifasciatus,* and *M.* sp. (Tocantins) and is present in *Hypomasticus* and independently in *Leporinus friderici*; #8- Dark midlateral blotch on caudal peduncle persistent in adults. Also, independently present in *Hypomasticus*, *Leporinus friderici,* and *Pseudanos trimaculatus*; #21- three teeth on premaxilla. This character is also shared by the nearby taxa *Abramites hypselonotus* and *Leporinus striatus* and appears independently in *Hypomasticus megalepis*; #24- Distinct marginal ridge on the lingual surface of teeth in adults. This character is also present in node 5 and *Leporinus amblyrhynchus*; #25- Marginal ridge on the lingual surface of teeth well-developed in adults. This character is also present in node 5 and *Leporinus amblyrhynchus*; however, it is modified in node 8, *M. conirostris, M. elongatus,* and *M. gaiero*; #26- Three dentary teeth. This character is reversed to its plesiomorphic state in *M. garmani*; #59- Overlapping of the anteroventral process of sphenotic by the sixth infraorbital. This character is a regression of the plesiomorphy in *Leporellus, Anostomus,* and *Abramites hypselonotus*. This character is also polymorphic in *Hypomasticus mormyrops*; #69- Possession of sexual chromosome system.

Node 7 (*Leporinus scalabrinii, Megaleporinus conirostris, M. reinhardti, M. garmani, M. brinco, M. piavussu, M. elongatus, M. gaiero* and *M. obtusidens*): #31-

Posterior ascending process of premaxilla. This character reverses in *M. reinhardti* and is also independently present in node 3 and *Leporinus amblyrhynchus*; #60 Smaller mesethmoid, relative to its length. Although this plesiomorphy is not shared by *Leporinus scalabrinii*, *M. reinhardti,* and *M. piavussu*, it is present in most taxa in the node and *Hypomasticus mormyrops, Insperanos nattereri,* and *Leporellus*.

Node 8 (*Megaleporinus garmani* and *M. brinco*): #17- 32- 36 lateral-line scales. This character also appears independently in *Hypomasticus megalepis, Leporinus striatus,* and *M. elongatus* herein; #18- 12 scale rows around the caudal peduncle. This character seems independent in *Hypomasticus mormyrops, Leporinus amblyrhynchus,* and *M. piavussu*; #25- Marginal ridge on the lingual surface of teeth weakly developed in adults. This character appears more times in the genera, in *M. conirostris, M. elongatus,* and *M. gaiero*, and independently in *Laemolyta taeniata*; #30 Transversal premaxilla, relative to the longitudinal body axis. Also, the same state was observed in *M. conirostris* and node 3, independently; #33- Ventral end of maxilla posteriorly directed. This character is also present in node 4 and *M. conirostris*; #35- Larger tip of ascending process, width relative to length. Also, independently present in node 3, *M. conirostris* and *Schizodon intermedius*; #36- Groove on the tip of ascending process. This is also observed in *M. conirostris* and node 3; #39- Process of autopalatine laterally oriented. This character is a plesiomorphy present in *Leporellus* and also in *Abramites hypselonotus*, *M. conirostris,* and node 3; #42- Moderate size of lateral shelf of quadrate, relative to symplectic. This state is a reversion to the plesiomorphic state present in *Leporellus,* node 4, *Leporinus amblyrhynchus,* and *Abramites hypselonotus*; #43- Longitudinal crest on the dorsal margin of metapterygoid. This character is also independently present on node 2; #49- Rod-like anterior portion of supraorbital. This character appears independently in node 3 and *M. gaiero*; #54- Additional ventral pore on the sensory canal of first infraorbital. It also appears independently in *Laemolyta taeniata*.

Node 9 (*Megaleporinus* sp. (Tocantins), *M. trifasciatus,* and *M. macrocephalus*): #9- Small dark midlateral blotch on caudal peduncle persistent in adults, relative to the pupil. This character presents a regression in *M. macrocephalus;* however, it is shared between this node and *M.* sp. (Orinoco); #16- Red pigmentation covering the ventral portion of the opercular series. *Megaleporinus macrocephalus* features a secondary loss in this character; #17- 41 to 45 lateral-line scales. Whereas this character is polymorphic (states 1 and 2) in *M.* sp. (Orinoco), it is not the same in *M.* sp. (Tocantins). This character also appears independently in node 4, *Leporinus fasciatus, Megaleporinus muyscorum,* and *Pseudanos* *trimaculatus*; #19- Six or seven scale rows between lateral line and dorsal-fin origin. That character behaves like #17, which means it is polymorphic in *M.* sp. (Orinoco) and that *M.* sp. (Tocantins) does not share the character. It is also a character of *Leporellus* that reverses in *Abramites hypselonotus, Leporinus fasciatus, M. obtusidens,* and *M. piavussu*, and polymorphic in *Insperanos nattereri*.

DISCUSSION

A total of 70 morphological characters were employed herein, with most showing different states among species of *Megaleporinus*. Several of these characters (16 out of 70) are related to coloration, a feature that was noticed to vary greatly in the Anostomidae (Géry, 1977; Garavello, 1979), both ontogenetically (Santos, 1980; Machado-Evangelista *et al.*, 2015), and phylogenetically (Sidlauskas & Vari, 2008). However, these characters present high indices of homoplasy, according to the consistency and retention indexes.

On the other hand, several morphologically complex structures, such as the caudal fin skeleton or the branchial arches, showed no, or minimal, variation among examined species. This pattern was previously observed for other species of Anostomidae by other authors (Winterbottom, 1980; Sidlauskas & Vari, 2008).

The morphological data alone did not corroborate the monophyly of *Megaleporinus* for including *Hypomasticus* deeply nested within the former. Several morphological characters are shared between the down-turned mouth species of *Megaleporinus* (i.e., *M. garmani, M. brinco,* and *M. conirostris*) and the two species of *Hypomasticus* included in our study (*H. mormyrops*, and *H. megalepis*). *Hypomasticus* was created by Borodin (1929) to encompass species with down-turned mouths, such as *H. mormyrops*, and later expanded by Géry (1977) to include other anostomids with down-turned mouths, such as *H. megalepis*. The genus was considered artificial by Garavello (1979) and subsequent authors (Garavello & Britski, 2003) until revalidated by Sidlauskas & Vari (2008), who proposed its diagnosis based on several morphological characters related to the down-turned mouth (e.g., the relative position of the maxilla, the relative position of premaxilla). Birindelli *et al.* (2020) later expanded *Hypomasticus* based on molecular data to include species with subterminal mouths, such as *H. copelandi*, indicating that the mouth position (and related features) was homoplastic among anostomids. In our analyses, this scenario is corroborated. There seem to be several morphological features that are convergent in *M. garmani*, *M. brinco*, and *M. conirostris* and down-turned mouth species of *Hypomasticus*. These features include many characters related to the positioning of upper and lower jaws, such as the orientation of the premaxilla relative to the longitudinal body axis (state 2), the direction of the ventral end of the maxilla relative to the dorsal end, the possession of a groove on tip of the ascending process of dentary and orientation of process of the autopalatine (characters 30, 33, 36 and 39 herein, respectively) that were found to be a synapomorphy for *Hypomasticus* in Sidlauskas & Vari (2008: #46, #48, #56 and #68 respectively), since no species of *Megaleporinus* were included. The distant phylogenetic relations between *Hypomasticus* and *Megaleporinus garmani*, *M. brinco,* and *M. conirostris* are well supported by molecular data (Ramirez *et al*., 2016; Ramirez *et al.*, 2017; Birindelli *et al.*, 2020).

The relationships among species of *Megaleporinus* obtained herein based on molecular data is almost identical to those previously obtained elsewhere (Ramirez *et al*., 2016; Birindelli *et al.*, 2020). The only difference that appears in the total evidence analysis were the position of *M. piavussu* and the sister-group relationships of *M. gaiero*, which were inverted in relation to the molecular inferences of previous authors. These relationships were also obtained in the analyses using molecular data only. On the other hand, the analysis using only morphological data, a close relationship among *M. brinco*, *M. conirostris,* and *M. garmani* was obtained based on about nine characters (25, 30, 31, 32, 33, 35, 36, 39, and 60) mostly related to the mouth positioning. But these are highly homoplastic characters and are also present in the representants of *Hypomasticus* herein. Besides the morphology alone do not contribute to the monophyly hypothesis in *Megaleporinus,* the total evidence analysis shows a different result, which corroborates the hypothesis proposed by Ramirez *et al*. (2016; 2017).

The total evidence corroborates the position of *M. muyscorum* as the sister taxon for the rest of *Megaleporinus*, and its relationship among *M.* sp. (Orinoco), *M.* sp. (Tocantins), *M. trifasciatus* and *M. macrocephalus* hypothesized by Ramirez *et al*. (2016). However, the strictly morphologic tree did not recover *M. macrocephalus* as sister of *M. trifasciatus*. Rather, it grouped *M. trifasciatus* with *M.* sp. (Tocantins) due to their similarities in coloration and neurocranium morphology.

Another taxa group topology herein corroborated is that composed by *M. piavussu, M. obtusidens, M. elongatus,* and *M. gaiero,* described by Birindelli *et al.* (2020). On the other hand, this topology is unclear in the morphology-only analysis, confirming that the dentition and mouth positioning evolve rapidly and plastically and therefore converge frequently among Anostomidae in general (Sidlauskas & Vari, 2008).

Besides, the morphological data does not indicate *M. reinhardti* as the sister group of *M. brinco* and *M. garmani* as concluded by the total evidence analysis, the relation among these last two species, which often varies among works from sister taxa (and sister group from *M. reinhardti*) to closed related taxa (with *M. brinco* as the sister of *M. garmani* and *M. reinhardti* group) (Ramirez *et al.*, 2016; 2017; Birindelli *et al.*, 2020, respectively). Therefore, due to the consensus molecular-only (Fig 18) and total-evidence analysis (Fig. 19), *Megaleporinus brinco* is considered sister taxa of *M. garmani*, and *M. reinhardti* is sister to this group. So, the morphological similarities of *M. reinhardti* in relation to *M.*

macrocephalus, *M. trifasciatus* and *M.* sp. (Tocantins) pointed by the morphology only analysis (Fig 17) are most likely convergences.

The age of *Megaleporinus* was estimated at approximately 12 million years by Ramirez *et al*., (2016), due to their decision to use the uplifting of the Andea Cordillera (approximately 9mya) as a calibration point between the split of *M. muyscorum* and congeners. The former is endemic to the Magdalena River basin, a drainage that flows directly to the Caribbean Sea and is isolated from the Cis-Andean drainage by the northmost portion of the Andes. The last uplift of the Andes is considered to have taken place during the Quechua Phase, in the late Neogene period, between 8 and 9 m.y.a. Nevertheless, the use of a generic geological event, despite informative, does not offer much precision in determining the age of the investigated taxa if compared to the fossil record (Pfiffner & Gonzales, 2013; Ribeiro, 2006).

Instead of setting the calibration of the tree in this biogeographical event, we set our tree calibration in the clade composed of *Leporellus* plus Anostominae, which was considered by Sidlauskas *et al.* (2021) to be approximately 38 million years ago. Our analysis also considered *Leporinus scalabrinii*† as a tip-dated terminal, set according to its estimated age (6 m.y.a). Using a combination of two calibration sets strengthens the age estimation for *Megaleporinus*. Doing so, our results show that *Megaleporinus* is much older than previously considered and diversified approximately 18 million years ago (Figure 17).

Our results strongly suggest that *Leporinus scalabrinii*† (Bogan *et al*., 2012) should be transferred to *Megaleporinus*, because of its morphologic similarity with the genus, and its placement inside *Megaleporinus* by the total evidence analysis. In addition to that, the locality where the fossil was discovered is also comprehended in the occurring area of *Megaleporinus* aff. *obtusidens* (Bogan *et al*., 2012; Ramirez *et al*., 2017).

Our study also gathered morphological features that could be used to diagnose the undescribed species *M*. sp. (Tocantins) and *M*. sp. (Orinoco), corroborating the hypotheses of Ramirez *et al*. (2017) that considered these two as new species.

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APPENDIX

CHARACTER MATRIX

Table A1: Phylogenetic character matrix. Character numbers correspond to the Results section. Missing data are indicated by “?”; Inaplicable characters are represented by “-”; Polymorphisms are indicated by both states separated by “&” (example: “0&1”).

Taxa/Character	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**#9**	**#10**	**#11**	**#12**	**#13**	**#14**	**#15**	**#16**	**#17**	**#18**	**#19**	**#20**	**#21**	**#22**	**#23**	**#24**	**#25**
Anostomus anostomus	0	0	0	0	0	0	0	0	-	1	1	0	0	0	0	0	1	2	0	0	1	1	0	0	-
Abramites hypselonotus	1	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	1	1	1	1	0	0	-	0	-
Hypomasticus megalepis	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0	-	0	-
Hypomasticus mormyrops	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	-	0	-
Insperanos nattereri	0	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0&1	2	0&1	0	1	0	-	0	-
Laemolyta taeniata	0	0	0	0	0	0	0	0	-	1	0	0	0	0	0	0	2	2	0	0	1	1	1	0	0
Leporellus vittatus	0	0	0	0	0	0	0	0	-	1	0	0	1	0	0	0	1	2	1	0	1	0	-	0	-
Leporinus amblyrhynchus	0	0	0	0	0	0	0	0	-	1	0	0	0	0	0	0	1	0	0	0	1	0	-	1	1
Leporinus fasciatus	1	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	2	2	1	1	1	0	-	1	1
Leporinus friderici	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	1	2	0	0	1	0	-	1	1
Leporinus scalabrinii †	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	-	0	0	?	?	-
Leporinus striatus	0	0	0	0	0	0	0	0	-	1	1	0	0	0	0	0	0	2	0	0	0	0	-	0	-
Megaleporinus brinco	0	0	0	0	0&1	0	1	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	-	1	0
Megaleporinus conirostris	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	-	1	0
Megaleporinus elongatus	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0	-	1	0
Megaleporinus gaiero	0	0	0	0	1	1	1	1	0	0	0	0	0	1	0	0	1	2	0	0	0	0	-	1	0
Megaleporinus garmani	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	1	0
Megaleporinus macrocephalus	0	0	0	0	1	0	1	1	0	0	0	1	0	0	0	0	2	2	1	0	0	0	-	1	1
Megaleporinus muyscorum	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	2	2	0	0&1	0	0	-	1	1
Megaleporinus sp (Orinoco)	0	0	0	0	1	0	1	1	1	0	0	0	0	0	0	0	1&2	2	0&1	1	0	0	-	1	1
Megaleporinus obtusidens	0	1	0	0	1	0	1	1	0	0	0	0	0	0	0	0	1	2	1	0&1	0	0	-	1	1
Megaleporinus piavussu	0	1	0	0	1	0	1	1	0	0	0	0	0	0	0	0	1	2	1	1	0	0	-	1	1
Megaleporinus reinhardti	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	1	2	0	0	0	0	-	1	1
Megaleporinus trifasciatus	0	0	1	1	0	0	0	1	1	0	0	0	0	0	0	1	2	2	1	0	0	0	-	1	1
Megaleporinus sp (Tocantins)	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	1	1	2	0	0	0	0	-	1	1
Pseudanos trimaculatus	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	2	2	0	0	1	1	0	0	-
Schizodon intermedius	0	0	1	1	0	0	0	0	-	0	0	0	0	0	0	0	2	2	0	0	1	1	1	0	-

Table A1: Continued

Taxa/Character	#26	#27	#28	#29	#30	#31	#32	#33	#34	#35	#36	#37	#38	#39	#40	#41	#42	#43	#44	#45	#46	#47	#48	#49	#50
Anostomus anostomus	1	0	1	0	1	0	1	0	1	0	0	0	0	-	1	1	1	0	0	0	1	0	1	0	1
Abramites hypselonotus	1	1	0	-	0	0	1	1	1	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	1
Hypomasticus megalepis	1	0	0	-	2	1	1	2	1	1	1	1	1	0	0	0	2	1	1	0	1	0	0	1	1
Hypomasticus mormyrops	1	0	0	-	2	1	0	2	1	1	1	1	1	0	0	0	2	1	1	0	1	0	1	1	1
Insperanos nattereri	1	0	0	-	0	0	0	1	1	0	0	1	0	-	0	0	0	1	0	0	1	0	0	1	0
Laemolyta taeniata	1	0	0	-	0	0	1	1	1	0	0	0	1	1	0	1	0	1	0	1	1	0	0	0	1
Leporellus vittatus	1	0	0	-	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	1	0	1	0	0
Leporinus amblyrhynchus	1	0	0	-	0	1	1	1	1	0	0	1	1	1	0	0	0	1	0	0	1	0	0	0	1
Leporinus fasciatus	1	0	0	-	0	0	1	1	1	0	0	1	1	1	0	0	2	1	1	0	1	0	0	0	1
Leporinus friderici	1	0	0	-	0	0	1	1	1	0	0	1	1	1	0	0	2	1	1	0	1	0	0	0	1
Leporinus scalabrinii †	0	?	0	?	0	1	0	1	?	?	?	1	?	?	?	?	?	?	?	0	?	?	?	?	?
Leporinus striatus	1	0	0	-	0	0	1	1	1	0	0	1	1	1	0	0	2	1	1	0	1	0	0	0	1
Megaleporinus brinco	0	0	0	-	2	1	1	2	0	1	1	1	1	0	0	0	0	0	1	0	1	0	0	1	1
Megaleporinus conirostris	0	0	0	-	2	1	0	2	1	1	1	1	1	0	0	0	2	1	1	0	1	0	0	0	1
Megaleporinus elongatus	0	0	0	-	0	1	1	1	1	0	0	1	1	1	0	0	2	1	1	0	1	0	0	0	1
Megaleporinus gaiero	0	0	0	-	0	1	1	1	1	0	0	1	1	1	0	0	2	1	1	0	1	0	0	1	1
Megaleporinus garmani	1	0	0	-	2	1	0	2	1	1	1	1	1	0	0	0	0	0	1	0	1	0	0	1	1
Megaleporinus macrocephalus	0	0	0	-	0	0	1	1	1	0	0	1	1	1	0	0	2	1	1	0	1	0	0	0	1
Megaleporinus muyscorum	0	0	0	-	0	0	1	1	1	0	0	1	1	1	0	0	2	1	1	0	1	0	0	0	1
Megaleporinus sp (Orinoco)	0	0	0	-	0	0	1	1	1	0	0	1	1	1	0	0	2	1	1	0	1	0	0	0	1
Megaleporinus obtusidens	0	0	0	-	0	1	1	1	1	0	0	1	1	1	0	0	2	1	1	0	1	0	0	0	1
Megaleporinus piavussu	0	0	0	-	0	1	1	1	1	0	0	1	1	1	0	0	2	1	1	0	1	0	0	0	1
Megaleporinus reinhardti	0	0	0	-	0	0	1	1	1	0	0	1	1	1	0	0	2	1	1	0	1	0	0	0	1
Megaleporinus trifasciatus	0	0	0	-	0	0	1	1	1	0	0	1	1	1	0	0	2	1	1	0	1	0	0	0	1
Megaleporinus sp (Tocantins)	0	0	0	-	0	0	1	1	1	0	0	1	1	1	0	0	2	1	1	0	1	0	0	0	1
Pseudanos trimaculatus	1	0	1	0	1	0	1	0	1	0	0	0	0	-	1	1	1	0	0	0	0	1	0	0	1
Schizodon intermedius	1	1	1	1	0	0	1	1	1	1	0	0	1	1	0	0	0	1	1	1	0	1	1	0	1

Taxa/Character	#51	#52	#53	#54	#55	#56	#57	#58	#59	#60	#61	#62	#63	#64	#65	#66	#67	#68	#69	#70	#71
Anostomus anostomus	0	-	1	0	0	0	1	0	0	0	1	0	0	0	1	0	-	1	0	1	0
Abramites hypselonotus	1	1	1	0	0	0	1	1	0	0	1	0	1	1	-	0	-	0	0	1	0
Hypomasticus megalepis	1	1	0	0	0	1	-	1	1	0	1	1	0	0	1	0	-	1	0	0	0
Hypomasticus mormyrops	1	0	0	0	0	0	0	0	0&1	1	1	1	1	0	0	0	-	1	0	0	0
Insperanos nattereri	0	-	1	0	0	0&1	1	0	1	1	0	1	0	0	1	0	-	0	0	0	0
Laemolyta taeniata	1	1	0	1	1	0	1	0	1	0	1	0	1	0	1	0	-	1	0	0	0
Leporellus vittatus	0	-	1	0	0	1	0	1	0	1	0	0	0	0	0	0	-	1	0	0	0
Leporinus amblyrhynchus	1	1	1	0	0	1	1	1	1	0	1	0	1	0	1	0	-	1	0	0	0
Leporinus fasciatus	1	1	0	0	0	1	1	1	1	0	1	0	0	0	1	0	-	1	0	0	0
Leporinus friderici	1	1	1	0	0	0	1	1	1	0	1	0	1	0	0	0	-	1	0	0	0
Leporinus scalabrinii †	?	?	?	?	?	?	?	?	?	0	1	0	0	1	0	?	?	?	?	?	?
Leporinus striatus	1	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	-	1	0	1	0
Megaleporinus brinco	1	0	0	1	0	0	1	0&1	0	1	1	0	1	0	1	0	-	1	1	0	1
Megaleporinus conirostris	1	1	0	0	0	0	1	1	0	1	1	0	1	0	0	0	-	1	1	0	0
Megaleporinus elongatus	1	1	0	0	0	0	1	0	0	1	1	0	1	0	1	0	-	1	1	0	0
Megaleporinus gaiero	1	1	0	0	0	0	1	1	0	1	1	0	0	0	0	1	0	0	1	0	0
Megaleporinus garmani	1	1	0	1	0	0	0	1	0	1	1	0	1	0	1	0	-	1	1	0	0
Megaleporinus macrocephalus	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	-	0	1	0	0
Megaleporinus muyscorum	1	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0	-	1	1	0	0
Megaleporinus sp (Orinoco)	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	-	0	1	0	0
Megaleporinus obtusidens	1	1	0	0	0	0	1	1	0	0&1	1	0	1	0	0	0	-	0	1	0	0
Megaleporinus piavussu	1	1	0	0	0	0	1	1	0	0	1	0	0	0	1	0	-	0	1	0	0
Megaleporinus reinhardti	1	1	0	0	0	0	1	1	0	0	1	0	1	0	0	0	-	0	1	0	0
Megaleporinus trifasciatus	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	-	0	1	0	0
Megaleporinus sp (Tocantins)	1	1	0	0	0	0	1	1	0	0	1	0	1	0	0	1	1	0	1	0	0
Pseudanos trimaculatus	0	-	1	0	0	0	1	1	1	0	1	0	1	1	0	0	-	1	0	1	0
Schizodon intermedius	1	1	0	0	1	0	1	1	1	0	1	0	1	0	0	0	-	0	0	0	0

Table A1: Continued