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DIVERSIDADE E FILOGENIA DAS ESPÉCIES DE *LEPORELLUS* LÜTKEN, 1875 (CHARACIFORMES: ANOSTOMIDAE)

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Tese de Doutorado apresentada à Banca de Defesa do Programa de Pós-Graduação em Ciências Biológicas, Universidade Estadual de Londrina, como requisito para a obtenção do título de Doutor em Ciências Biológicas.

Orientador: Prof. Dr. José Luís O. Birindelli

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Londrina, 06 de setembro de 2023.

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RESUMO

Estima-se que o número de espécies de peixes dulcícolas da América do Sul seja cerca de 50% maior do que o conhecido atualmente. Por outro lado, ações humanas diretas, como o desmatamento, ou indiretas, como o aquecimento global, têm causado um acentuado declínio na Biodiversidade do planeta. Desta forma, esforços que visem aumentar o conhecimento sobre a diversidade de peixes neotropicais são urgentes. Entre as famílias de Characiformes, Anostomidae é a segunda mais diversa, com 16 gêneros e pouco mais de 150 espécies válidas. O gênero *Leporellus* possui uma posição filogenética de destaque para o conhecimento sobre a evolução das espécies da família, sendo considerado próximo à base de Anostomidae. Indivíduos de *Leporellus* foram coletados em todas as grandes drenagens Sul-Americanas, desde a Colômbia à Argentina. Apesar disso, a diversidade e filogenia das espécies do gênero são pouco conhecidas. O gênero abriga seis espécies nominais, mas não há nenhuma informação disponível que permita o reconhecimento e a identificação delas. Além disso, há dados preliminares e não publicados que indicam a presença de espécies novas. O presente trabalho teve como objetivo investigar a diversidade e filogenia das espécies de *Leporellus*. Para isso, foram utilizados dados morfométricos, merísticos, e moleculares, incluindo sequências dos genes mitocondriais COI e Cytb e nucleares RAG1, RAG2 e Myh6. Esta tese foi dividida em três capítulos. O primeiro trata sobre a descrição de uma espécie nova do rio Aripuanã, um tributário do Rio Madeira, que apresenta apenas 12 séries de escamas ao redor do pedúnculo caudal (vs. 16). O segundo aborda a descrição de uma segunda espécie nova, esta endêmica das partes altas da bacia do rio Paraguai, que possui apenas três barras verticais escuras sobre os raios da nadadeira caudal (vs. 5 a 7). O último capítulo traz uma investigação sobre a diversidade e a filogenia das espécies do gênero utilizando dados morfológicos e moleculares. Os resultados desta tese apontam para a existência de pelo menos sete espécies de *Leporellus*, duas delas descritas aqui. Além disso, a diversidade de espécies ainda é subestimada, especialmente para algumas regiões, como a Amazônia ocidental. Dentre as sete espécies, apenas duas delas podem ser identificadas por características externas diagnósticas, sendo as demais cinco consideradas crípticas. Comentários sobre a distribuição geográfica das espécies são feitos sob a luz da filogenia obtida.

Palavras-chave: Peixes; Filogenia; Taxonomia; Morfologia; Sistemática.

Narezzi, Nick Tramontina. **Diversity and Phylogeny of species of *Leporellus* Lutken, 1875 (Characiformes: Anostomidae),**, 2023. 103p. PhD Dissertation in Biological Sciences – Universidade Estadual de Londrina, Londrina, 2023.

ABSTRACT

The number of species of freshwater fishes in South America is estimated to be 50% larger than what is currently known. On the other hand, direct human actions, such as deforestation, or indirect, such as global climate change, have caused a severe decline in the planet Biodiversity. Therefore, efforts aimed at increasing knowledge about the diversity of Neotropical fish are urgent. Among families of Characiformes, Anostomidae is the second most diverse with 16 genera and over 150 valid species. The genus *Leporellus* has a prominent phylogenetic position for knowledge about the evolution of species of the family, being considered close to the base of the Anostomidae. Individuals of *Leporellus* were collected in all large South-American drainages, from Colombia to Argentina. Despite of that, the diversity and phylogeny of species of the genus are poorly known. The genus currently includes six nominal species, but there is no information available that allows the recognition and identification of them. Furthermore, preliminary unpublished data indicate the presence of new species. The present study aimed to investigate the diversity and phylogeny of species of *Leporellus*. For that, morphometric, meristic, and molecular data were used, including sequences of mitochondrial genes COI and Cytb, and nuclear genes RAG1, RAG2 and Myh6. This PhD dissertation is divided into three chapters. The first includes the description of a new species from the Aripuanã river, Madeira river basin, which has only 12 scale rows around the caudal peduncle (vs. 16). The second includes the description of a new species endemic to the higher lands of the Paraguay river basin, that possesses only three dark stripe on the caudal-fin rays (vs. 5 or 7). The third chapter investigates the diversity and phylogeny of the species of genus using morphological and molecular data. The results of this dissertation indicate to the existence of at least seven species within *Leporellus*, two of them described herein. In addition, the diversity is yet underestimated, especially for some areas, such as the Western Amazon. Among the seven species, external diagnostic features can identify only two of them, the remaining five being considered cryptic. Comments on the geographical distribution of the species are made in light of the phylogeny obtained.

Key words: Fishes; Phylogeny; Taxonomy; Morphology; Systematics.

SUMÁRIO

INTRODUÇÃO GERAL

O conhecimento sobre sistemática é essencial para os estudos das mais variadas áreas da biologia (Carvalho *et al.*, 2005). Além disso, a iminente perda de biodiversidade causada pela alteração dos ambientes naturais torna urgente estudos que visem o conhecimento das espécies. A fauna de peixes dulcícolas da América do Sul é a mais diversa do planeta, com aproximadamente 6.500 espécies descritas atualmente, e mais cerca de 2.500 para serem descritas (Birindelli & Sidlauskas, 2018). Por outro lado, as atividades humanas diretas, como o desmatamento, ou indiretas, como o aquecimento global, tem causado um declínio no Biodiversidade do planeta. Desta forma, aumentar o conhecimento sobre a diversidade de peixes neotropicais é necessário e urgente.

Os Characiformes constituem a maior ordem de peixes da região Neotropical e, entre as famílias com maior diversidade do grupo, está Anostomidae, que abriga cerca de 150 espécies de piaus, aracus e piaparas (Sidlauskas & Birindelli, 2017). A família Anostomidae compreende 16 gêneros (Ramirez *et al.*, 2016), incluindo *Leporellus*, um gênero amplamente distribuído e de taxonomia pouco conhecida. O gênero *Leporellus* foi descrito por Lütken, 1875 com base em um único exemplar coletado por ele no rio Cipó, um tributário do rio São Francisco, e identificado como *L.* pictus* (Kner, 1858). Lütken (1875) citou três características que diagnosticam *Leporellus* de *Leporinus* (e dos demais anostomídeos): narinas anterior e posterior imediatamente próximas, escamas recobrindo os raios da nadadeira caudal e membrana branquial não unida no istmo. Enquanto a primeira característica está ausente em todos os anostomídeos (inclusive em *Leporellus*), as duas últimas são usadas ainda hoje para diagnosticar o gênero e o tornam facilmente reconhecível dentre os demais gêneros da família (Sidlauskas & Vari, 2012).

Leporellus é amplamente distribuído na América do Sul, desde a Argentina até a Colômbia, ocorrendo nas bacias dos rios Amazonas, Essequibo, Magdalena, Orenoco, Paraná, Paraguai, São Francisco, e Uruguai.

Trabalhos com foco em maturação e morfologia gonadal de peixes ao longo do ano, quando possuem indivíduos de *Leporellus* em suas amostragens, apontam que tais exemplares apresentam atividade reprodutiva sempre presente nos períodos de cheia (Rezende *et al.* 1996, Arantes *et al.* 2017).

Zuanon (1999) descreveu detalhadamente a preferência de *Leporellus* por ambientes de água lóticas, com sua ocorrência sendo majoritariamente associada a corredeiras fortes ou de média intensidade. O substrato observado como sítio de forrageio é composto por rochas grandes, seixos e areia (Zuanon, 1999), onde busca o principalmente por insetos, o principal (acima de 80%) item de sua dieta (Tomazzelli & Bessa, 2009; Hahn *et al.,* 2002)

Além da descrição da preferência de *Leporellus* por águas correntes intensas, e dos principais substratos presentes, Zuanon (1999) fez apontamentos a respeito da morfologia das nadadeiras pélvicas e peitorais, como adaptações à vida em águas rápidas, e sobre os lábios espessados que se mostram ligados ao método de forrageio, que implica em "investidas bruscas" contra os seixos e areia, no ato de revirar o fundo à procura de alimento.

A posição filogenética do gênero *Leporellus* é extremamente importante para o conhecimento da evolução da família, porque ele é considerado grupo irmão de todos os demais Anostomidae com base em dados morfológicos (Sidlauskas & Vari, 2008) ou como pertencente ao clado grupo irmão dos demais (Ramirez *et al.*, 2016) com base em caracteres moleculares.

Por outro lado, a taxonomia das espécies de *Leporellus* ainda permanece sendo pouco conhecida. O gênero possui seis espécies nominais, sendo a mais antiga *Leporellus vittatus*, descrita por Valenciennes (1850) com base em exemplares coletados no rio Amazonas. Kner (1855) descreveu *L. pictus* a partir de exemplares coletados na bacia do Alto rio Paraná. Eigenmann (1922) descreveu três espécies: *L. retropinnis,* do rio Piracicaba (bacia do Alto Paraná), *L. sexdentatus* da bacia do rio Magdalena, uma drenagem transandina da Colômbia, e *L. timbore*, com base em exemplares do rio das Velhas (bacia do rio São Francisco). E finalmente, *L. cartledgei* foi descrita por Fowler (1941) com base em exemplares do rio São Francisco. Atualmente não é conhecida qualquer característica morfológica, externa ou interna, que permita o reconhecimento de mais de uma espécie válida de *Leporellus* (Sidlauskas & Vari, 2012). Por este motivo, a grande maioria dos trabalhos de inventário de ictiofauna, independente da região amostrada, identificam exemplares de *Leporellus* como *L. vittatus*, a espécie mais antiga do gênero (e.g., Britski *et al.*, 2007; Sidlauskas & Vari, 2012; Ota *et al.*, 2018).

O presente trabalho teve como objetivo investigar a diversidade e filogenia das espécies de *Leporellus*, reavaliando todas as espécies nominais e descrevendo as espécies novas descobertas durante o estudo. Para isso, exemplares depositados em diferentes coleções científicas foram examinados, incluindo todos os exemplares tipo de todas as espécies nominais. Foram utilizados dados morfométricos relacionados ao formato do corpo, dados merísticos, consistindo na contagem dos raios das nadadeiras, dentes das maxilas, e escamas no corpo, e dados moleculares, incluindo as sequências de dois genes mitocondriais (COI e Cytb) e três genes nucleares (RAG1, RAG2, e Myh6). Análises de Componentes Principais foram feitas para condensar a informação obtida através dos dados referentes a variação no formato do corpo das espécies de *Leporellus*. Análises de delimitação de espécies (ASAP, PTP) e da distância genética sobre utilizadas para delimitar as MOTUs (*Molecular Taxonomic Units*) em *Leporellus* com base na sequência do DNA Barcode (gene COI). A combinação dos cinco marcadores moleculares foi utilizada em análises de Máxima Verossimilhança e de Inferência Bayesiana para proposição de hipóteses sobre as relações filogenéticas entre as espécies de *Leporellus*. Os resultados da dissertação foram organizados em três capítulos, cada um deles representando uma publicação independente.

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* Sempre que uma espécie for escrita com o gênero abreviado, ela pertence a *Leporellus* e a abreviação "*L.*" significa "*Leporellus*". Ou seja, os demais gêneros citados no texto, incluindo "*Leporinus*", sempre estarão escrito por extenso.

CAPÍTULO 1

O primeiro capítulo consiste na descrição de uma espécie nova de *Leporellus* aparentemente endêmica do alto rio Aripuanã, um tributário do Rio Madeira, acima das cachoeiras de Dardanelos e Andorinhas. Este capítulo foi submetido à Zootaxa, seguindo suas regras de publicação, explicitadas em https://www.mapress.com/zt/about/submissions.

A new species of *Leporellus* (Characiformes, Anostomidae) from the Aripuanã river, Amazon basin, Brazil

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Running title: New species of *Leporellus* from Brazil

Key-words: Biodiversity; Ostariophysi; Neotropical; Anostomoidea

Anostomidae is the second most diverse family of Characiformes and encompasses many widespread taxa currently in urgent need of taxonomic revision. *Leporellus* is one of the most intriguing taxonomic puzzle in Anostomidae, including *L. vittatus* currently considered widespread and the senior synonym of up to six nominal species. A new, easily diagnosable congener was recently discovered in the Ariapunã river, a tributary of the Madeira river in the Brazilian Amazon, and is herein described. The new species is allocated in *Leporellus* based on its scaled caudal fin, downturned mouth with labial folds, four incisiform teeth with a blunt cutting edge on each premaxillary and dentary, a dark median stripe on the dorsal fin, and five longitudinal stripes on the caudal fin. The new species is diagnosed from congeners (including the type specimens of all nominal species) by possessing 12 scale rows around the caudal peduncle (vs. 16). The new species is apparently endemic to the upper portion of the Aripuanã river, above the Dardanelos and Andorinha falls. Comments on the taxonomy of the species of *Leporellus* are provided.

Key-words: Anostomoidea, Neotropical, Phylogeny, Systematics, Taxonomy

The number of new species of Characiformes discovered yearly is one of the highest among Vertebrates, perhaps only comparable to that of the other ostariophysan orders, Cypriniformes and Siluriformes (Fricke *et al.*, 2023). In fact, many areas remain unexplored in the Neotropics, and far we are from understanding the existent diversity of world freshwater fishes (Birindelli & Sidlauskas, 2018). The Anostomidae is the second most diverse family of Characiformes, a megadiverse order of freshwater fishes distributed in Africa, and Central and South America (Melo *et al.*, 2022). With 16 genera and around 150 species, our understanding of the diversity and evolution of anostomid fishes has been expanded recently (Sidlauskas & Vari, 2008; Ramirez *et al.*, 2017; Sidlauskas *et al.*, 2021). However, several groups still need attention, including a few cases for which our knowledge has remained unchanged over the last hundred years or so (Sidlauskas & Vari, 2012).

Lütken (1875) described *Leporellus* to allocate *Leporinus pictus* Kner, 1855, whose type locality is in the upper Paraná basin. Lütken (1875: 129) based his comments on specimens he collected in the São Francisco river near Lagoa Santa, Minas Gerais state, Brazil. In the same contribution, he considered *L. vittatus* Valenciennes, with the type locality in the Amazon, possibly a synonym of Kner's *L. pictus*. Similar comments considering both species possibly synonymies were made by subsequent authors (e.g., Steindachner, 1880; Garavello & Britski, 2003) and paved the taxonomic confusion regarding species of *Leporellus*. Lütken's (1875) generic diagnosis included the scaled caudal fin, a feature still today only known to be exclusive of *Leporellus* among Anostomidae. Four other nominal species are currently considered junior synonyms of either *L. pictus* or *L. vittatus* (Garavello & Britski, 2003; Fricke *et al.*, 2023), including *L. cartledgei* Fowler (type locality in the São Francisco river), *L. retropinnis* (Eigenmann) (type locality in the upper Paraná), *L. sexdentatus* (Eigenmann) (type locality in the Cauca river, Magdalena basin, Colombia), and *L. timbore* (Eigenmann) (type locality in the São Francisco river). Specimens of *Leporellus* occur in the Amazon (including most of the Amazonas-river tributaries), Orinoco, Essequibo, Magdalena, São Francisco, Paraguay, and Paraná drainages (Sidlauskas & Birindelli, 2017). The validity and distribution of the nominal species of *Leporellus* are currently uncertain (Sidlauskas & Vari, 2012; Sidlauskas & Birindelli, 2017).

During expeditions in the Aripuanã river, a tributary of the Madeira river in Brazil, a new species of Anostomidae was discovered. The species is consistently included in *Leporellus* and easily diagnosed from congeners. The present contribution aim to describe it, diagnose it among species of the family, and discuss its putative endemicity. Furthermore, the conservation status of the new species was evaluated based on the IUCN criteria (IUCN, 2019). Finally, comments on the taxonomy of the nominal species of *Leporellus* are provided.

MATERIALS AND METHODS

The material was obtained from scientific collections and included alcohol-preserved specimens, except when indicated by CS, which were Cleared and Stained according to Taylor and Van Dyke (1985). Institutional abbreviations follow Sabaj (2020). Counts and measurements were taken according to Winterbottom (1980). Geographical distribution was illustrated using QGiz v.3.26.

All measurements were taken point to point on the left side of specimens with digital calipers and recorded to 0.1 mm. Standard length (SL) is expressed in mm, and all other measurements are expressed as percents of SL, except subunits of the head, which are expressed as percents of head length. Meristic data are given in the description, with the frequency of each count provided in parentheses after the respective count and an asterisk indicating counts of holotype. Lateral-line scale counts included pored scales extending onto the base of median caudal-fin rays; counts of longitudinal scale rows above the lateral line exclude the lateral-line scale row and the middorsal scale row; and counts of longitudinal scale rows below the lateral line exclude the lateral-line scale row.

RESULTS

Leporellus sp.n. (Aripuanã), new species

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Figs. 1–3; Table 1

Fig 1. *Leporellus* sp.n. (Aripuanã), holotype, MZUSP 101048, 136.5 mm SL, Brazil, Mato Grosso, Aripuanã, Aripuanã river, tributary of Madeira river, immediately above the Dardanelos falls, 10°12’33”S 59°27’39”W.

Holotype.— MZUSP 101048, alc, 136.5 mm SL, Rio Aripuanã, immediately above the Dardanelos falls, 10°12'33" S 59°27'39" W, C.M.C. Leite, N.E. Silva & F. Rosa, 4 Jul 2004.

Paratypes.—All from Brazil, Mato Grosso state, Aripuanã municipality. INPA 11003, 1 alc, 115.8 mm SL, Igarapé Genipapo, tributary of rio Aripuanã, approximately 70 km above Dardanelos falls, 10°10'00”S 59°27’34”W, INPA team, 14 Nov 1976. INPA 50878, 2 alc, 84.1-86.6 mm SL, rio Aripuanã, immediately below the UHE Dardanelos complex, and immediately above the Dardanelos falls, 10°09’52”S 59°27’23”W, N. Faustino Jr., J.A. Zuanon, 16 Nov 2006. MZUEL ?? [ex.MZUSP100499], 1 alc, 133.0 mm SL, rio Aripuanã at Lago dos Patos, above the Dardanelos falls, 10°12’33”S 59°27’39”W, F.A. Machado, C.M.C. Leite, N.E. Silva & F. Rosa, 14 Dec 2003. MZUSP ??? [ex. 101048], 1 alc [dissected], 177.3 mm SL, collected with holotype. MZUSP 100499, 2 alc, 92.4-93.3 mm SL, collected with

MZUEL ??. MZUSP 100517, 1, 103.0 mm SL, Rio Aripuanã, immediately above the Dardanelos falls, 10°12'33"S 59°27'39"W, F.A. Machado, C.M.C. Leite, N.E. Silva & F. Rosa, 14 Dec 2003. MZUSP 101061, 1 alc, 97. 6 mm SL, Rio Aripuanã, immediately above the Dardanelos falls, 10°12'33"S 59°27'39"W, C.M.C. Leite, N.E. Silva & F. Rosa, 7 Jan 2005. ZUEC 9471, 1 alc, ?? mm SL, rio Aripuanã, 2 km above Dardanelos/Andorinhas falls, 4 Jul 2004. ZUEC 9473, 1 alc, ?? mm SL, rio Aripuanã, immediately above the Dardanelos/Andorinhas falls, 4 Jul 2004. ZUEC 9184, ?? mm SL, rio Aripuanã at Cachoeira de Andorinhas, 1 Oct 2004.

Diagnosis.— *Leporellus* sp.n. (Aripuanã) is distinguished from all other Anostomidae, except congeners, by having the caudal-fin rays extensively covered by scales (Fig. 2) (vs. caudal-fin rays not covered by scales except for the base), a dark stripe on median portion of dorsal-fin rays (vs. stripe absent), five dark longitudinal stripe on caudal fin (Fig. 2) (vs. stripes absent), and labial folds present (Fig. 3) (vs. absent). *Leporellus* sp.n. (Aripuanã) is distinguished from all congeners by having 12 scale rows around the caudal peduncle (*v.* 16).

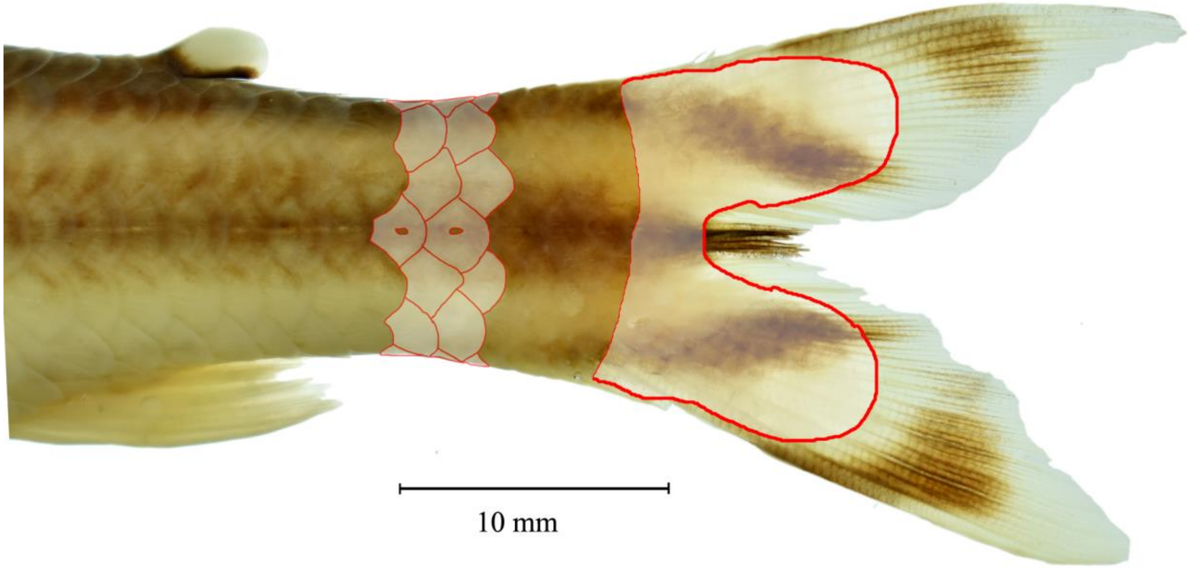

Fig. 2. Caudal peduncle and fin of *Leporellus* sp.n. (Aripuanã), holotype, MZUSP 101048, 136.5 mm SL, showing the 12 scale rows around the caudal peduncle and the scales coverage of the caudal-fin lobes.

Description.—Morphometric data summarized in Table 1. Medium size relative to congeners. Largest examined specimen 182.3 mm SL. Head and body elongate and moderately compressed. Dorsal profile distinctly convex from snout tip to vertical through nares, gently convex from latter point to dorsal-fin origin, more or less straight along dorsal-fin base, straight from dorsal-fin terminus to adipose-fin origin, and slightly concave from adipose-fin origin to anteriormost dorsal caudal-fin procurrent ray. Ventral profile concave from lower jaw tip to posterior margin of opercle, gently convex from latter point to anal-fin origin, distinctly convex along anal-fin base, distinctly concave from last anal-fin ray base to anteriormost ventral caudal-fin procurrent ray. Greatest body depth at dorsal-fin origin.

Table 1. Morphometric data for *Leporellus* sp.n. (Aripuanã). Mean includes the holotype.

	Holotype	n	Mean	Min-Max			SD
Standard length (mm)	136.5	9	115. 7	85.6	-	182.3	
Percentages of standard length							
Lower jaw to anal-fin origin	71.9	9	72.7	71.5	-	73.7	0.8
Lower jaw to adipose-fin origin	79.7	9	81.0	79.6	-	83.2	1.2
Lower jaw to dorsal-fin origin	44.5	9	45.3	41.8	-	47.1	1.6
Lower jaw to pelvic-fin origin	47.4	9	49.0	45.1	-	52.1	2.3
Dorsal-fin origin to caudal-fin origin	56.2	9	56.0	52.3	-	60.5	2.2
Dorsal-fin origin to adipose-fin origin	37.3	9	37.1	33.7	-	40.9	2.1
Caudal peduncle length	20.0	9	18.3	16.3	-	20.0	1.3
Caudal peduncle depth	9.5	9	9.6	9.1	-	10.2	0.4
Body depth	23.0	9	23.3	22.1	-	24.5	0.9
Body width	13.6	9	13.6	12.9	-	14. 5	0.6
Head depth	20.0	9	20.9	19.2	-	22.0	1.0
Head length	24.1	9	26.1	24.1	-	28.4	1.6
Percentages of head length							
Preopercle length	76.9	9	75.7	72.9	-	78.2	1.5
Snout length	39.6	9	38.2	35.4	-	43.5	2.7
Preopercle depth	76.4	9	72. 6	69.6	-	76.4	2.5
Snout depth	55.7	9	51. 8	46.7	-	57.4!	3.6
Eye diameter	24.1	9	23.9	19.0	-	26.6	2.6
Bony interorbital	40.0	9	36.8	30.1	-	40.0	2.9

Mouth subinferior, cleft longitudinally aligned with longitudinal between opercle ventral margin and infraorbital series ventral margin in specimens around 150 mm SL. Upper and lower lips hypertrophied, forming folds (herein named labial folds) (Fig. 3). Snout rounded, anteriorly blunt. Premaxillary bone with four teeth arranged side-by-side and gently decreasing in size from symphyseal tooth. Dentary bone with four teeth arranged side by side and gently decreasing in size laterally. Teeth incisiform, compressed, with blunt distal (biting) margin, and medial face curved hollow without raised ridge.

Fig. 3. Head of *Leporellus* sp.n. (Aripuanã), paratype, MZUSP ?? [ex101048], 182.3 mm SL, showing the labial fold formed by the hypertrophied lips.

Lateral line complete, 42* (6) or 43 (3) perforated scales, extending from supracleithrum to base of median caudal-fin rays. Horizontal scale rows between dorsal-fin origin and lateral line 4 (1) or 5* (8). Horizontal scale rows between lateral line and pelvic-fin origin 4* (9). Scale rows around caudal peduncle 12* (9). Caudal-fin lobes extensively covered by small scales, about ¾ the size of body scales, and covering ¾ of fin lobes.

Dorsal fin ii,10* (9). Dorsal-fin origin slightly anterior to middle of standard length and slightly anterior to vertical through pelvic-fin origin; its distal margin straight or gently concave or convex. Adipose fin small, teardrop shaped, origin approximately at vertical through base of last anal-fin branched ray. Pectoral fin i,15* (9); tip extending more than half distance between pectoral- and pelvic-fin origins; distal margin rounded. Pelvic fin i,8* (9);

tip extending approximately half distance between pelvic- and anal-fin origins, with distal margin slightly convex. Anal fin ii,9* (9); origin slightly anterior to vertical between terminal of dorsal-fin base and anteriormost dorsal procurrent caudal-fin ray, when adpressed fin not reaching base of caudal-fin rays; distal margin straight or slightly concave. Principal caudal-fin rays i,8,9,i* (9). Caudal fin forked, lobes rounded, upper lobe slightly more acute than lower lobe.

Color in alcohol.—Head and body ground color light brown and countershaded. Trunk with two to four dark stripes, dorsalmost near middorsal portion of body, second dorsalmost stripe between lateral line and middorsal portion of body, third stripe on midlateral covering lateral-line scale series, fourth between lateral line and ventral portion of body. First and second stripes indistinct (i.e., united) in some specimens, fourth stripe absent in some specimens. Body lateral scales, especially dorsal to lateral line and on anterior portion of body with a dark spot at center. Head with small dark blotches, especially on dorsal portion where blotches might be connected, forming vermiculations. Head ventral surfaces and body pale to cream. Dorsal fin with two dark stripes, one at base, and another, more conspicuous between half and distal one-fourth of rays; stripe at base sometimes absent or inconspicuous. Adipose fin with central dark spot. Caudal fin with five dark stripes, dorsalmost stripe at two-third of upper lobe, second stripe at dorsal caudal-peduncle limit and continuous with dark body stripe, one midlateral and continuous with midlateral body dark stripe, third at ventral limit of caudal peduncle, and fourth at two-thirds on lower lobe. Pectoral, pelvic, and anal fins generally hyaline.

Distribution.— *Leporellus* sp.n. (Aripuanã) is only known from the Aripuanã river immediately above and below the Dardanelos and Andorinhas falls, at Aripuanã municipality, Mato Grosso state, Brazil (Fig. 4).

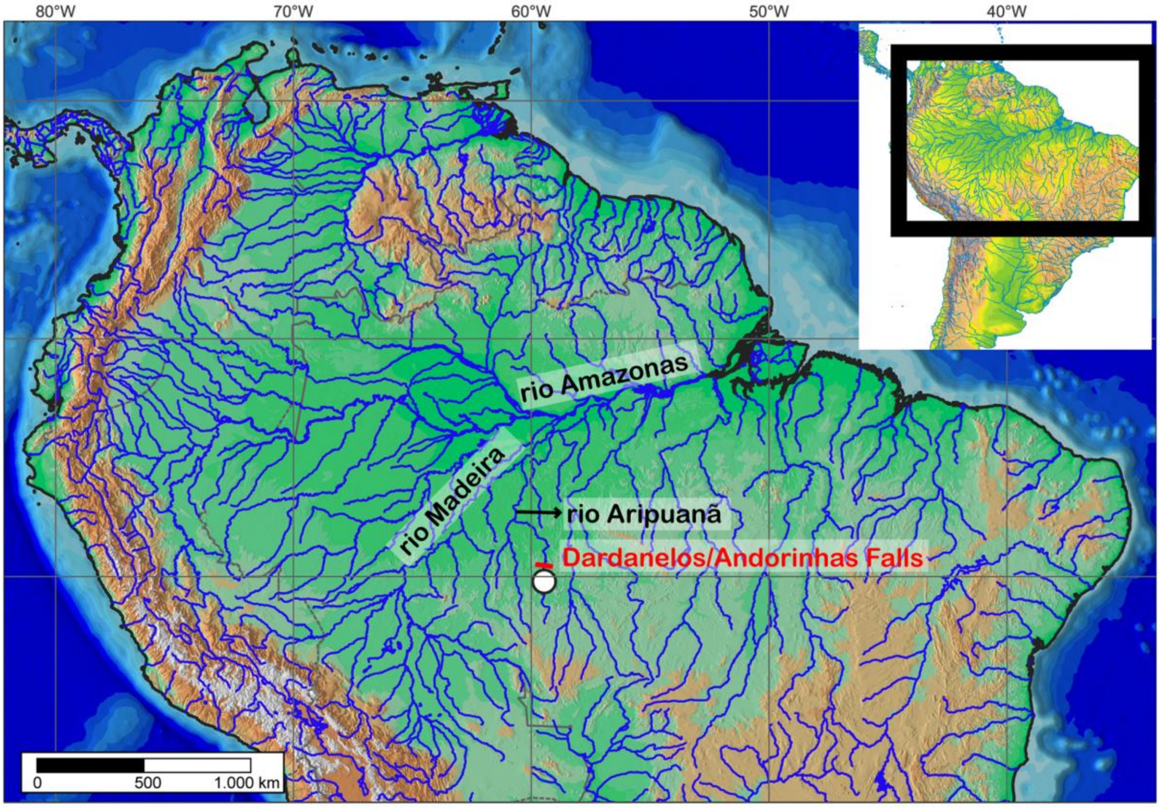

Fig. 4. Geographic distribution of *Leporellus* sp.n. (Aripuanã) displayed on the north of South America.

Conservation status — *Leporellus* sp.n. (Aripuanã) is apparently endemic to the Aripuanã river basin, above the Dardanelos and Andorinha falls. The only known records include the area close to Aripuanã city immediately above the c.135 m falls of Dardanelos and Andorinhas and a site approximately 70 km upstream. Species of *Leporellus* are known to be associated with rapids with rocky bedrooms and fast-flowing water. The occurrence of the species in the upper portions of the Aripuanã basin is uncertain as the area is largely unexplored. The drainage is calculated at approximately 50.000 km^2, including many protected areas, like the Parque do Aripuanã and several Indigenous lands. This area is still covered mainly by intact Amazonian rainforests. Therefore, the species is herein considered a Least Concern, according to the IUCN Criteria (IUCN, 2019), due to its putative occurrence in a relatively large (>20.000 km^2) and well-preserved area in the Brazilian Amazon. However, the recent expansion of land use, especially for agricultural practices near two large roads that run north to the city of Aripuanã (MT-183, MT-420), has likely continually impacted the distribution and abundance of the species. Similarly, the impact of constructing the UHE Dardanelos, a hydroelectric dam built in 2010, is possibly negative. Future evaluations are recommended to elucidate the species distribution and how the growing land use in the drainage can impact its populations.

Etymology.—The species epithet sp.n. (Aripuanã) is the Latin word for waterfall, in reference to its type locality, the Dardanelos and Andorinha waterfalls in the rio Aripuanã.

DISCUSSION

Leporellus sp.n. (Aripuanã) is the first species of its genus described after 1941. The validity and distribution of the species of *Leporellus* are uncertain and need further investigation (Sidlauskas & Vari, 2012). Some nominal species were described for the same river drainage and possibly represent a single valid species, such as *L. pictus* and *L. retropinnis*, both described for the upper Paraná. Other species, including *L. vittatus*, are considered widespread in drainages that are completely unconnected today, such as the Paraguay and São Francisco (Garavello & Britski, 2003). Furthermore, recent DNA Barcode data indicate at least three BINs clusters within *Leporellus* (BoldSystem.org), suggesting that the diversity might be underestimated. The discovery reported here strengthens that hypothesis.

Garavello & Britski (2003) considered three valid species of *Leporellus*: *L. pictus* (including *L. cartledgei* and *L. timbore* as junior synonymies), *L. retropinnis*, and *L. vittatus* (including *L. sexdentatus* as a junior synonym). The information was copied in subsequent catalogs (Fricke *et al.*, 2023). Unfortunately, there is no information to diagnose any of the aforementioned nominal species (Sidlauskas & Vari, 2012). Garavello & Britski (2003) doubted the validity of all these nominal species, considering they could be junior synonymies of *Leporellus vittatus*. However, based on the DNA Barcode data recently uploaded and available through the Bold System (BoldSystem.org), it is likely that there are at least three species in *Leporellus*, one in the Magdalena basin in Colombia, another in the Amazon basin in Brazil, and a third one in the Paraná basin also in Brazil. Herein, we tentatively identified specimens of the Paraná-Paraguay and São Francisco basins as *L. pictus*, specimens from the Amazon, Essequibo, and Orinoco basins as *L. vittatus*, and specimens from the Magdalena river basin in Colombia as *L. sexdentatus*. Our putative identification is based on the DNA Barcode data and the type localities of the nominal species. However, it is noteworthy that these identifications are tentative, and more data is necessary to test the validity and distribution of the species of *Leporellus*.

Despite the taxonomic confusion regarding *Leporellus* species, *L.* sp.n. (Aripuanã) is easily diagnosed from all congeners by having 12 scale rows around the caudal peduncle (vs. 16). The presence of 16 scale rows around the caudal peduncle was confirmed in the type specimens of all nominal species of *Leporellus* as well as in specimens collected in distinct river basins in South America including the Amazon, Essequibo, Orinoco, Paraná, Paraguay, São Francisco, and Magdalena. The number of scale rows around the caudal peduncle is an important feature for species diagnosis in fish in general (Menezes *et al.*, 2010; Armbruster, 2012), including Characiformes (Dagosta *et al.*, 2022; Silva *et al.*, 2020). That characteristic is especially important for the taxonomy of species of Anostomidae. Several examples of closely-related or similar-looking species that are only distinguished based on the number of scale rows around the caudal peduncle exist, including *Leporinus granti* and *L. nijsseni* (Garavello, 1990), *L. arcus* and *L. tepui* (Birindelli *et al.*, 2019), *L. multimaculatus* and *L. torrenticola* (Birindelli *et al.*, 2016), *L. desmotes* and *L. jatuncochi* (Burns *et al.*, 2017), *L. trimaculatus* and *L. friderici* (Garavello & Santos, 1992), *Hypomasticus pachycheilus* and *H. julii* (Santos *et al.*, 1996), *Sartor respectus* and congeners (Santos & Jegú, 1987).

The Dardanelos and Andorinhas falls might have acted as a geographical barrier that led to the speciation of *Leporellus* sp.n. (Aripuanã). Inventories of small tributaries above and below the Dardanelos/Andorinhas falls (Silva *et al.*, 2019) also concluded that the falls acted as a geographic barrier isolating the fish fauna above the falls. In fact, there are more than 13 species of fish currently known to be endemic to the upper Aripuanã river (i.e., above the Dardanelos/Andorinhas falls) (Kullander, 1995; Britski & Akama, 2011; Deprá *et al.*, 2014), including (but limited to) *Hemigrammus silimoni* Britski & Lima; *Geophagus mirabilis* Deprá, Kullander, Pavanelli & Graça, *Utiaritichthys longidorsalis* Jégu, de Moraes & Santos, *Trachycorystes menezesi* Britski & Akama. *Leporellus* sp.n. (Aripuanã) might be another species endemic to the upper Aripuanã river.

MATERIAL EXAMINED

Leporellus pictus**: Paraná basin:** FMNH 55714 (holotype of *Leporellus retropinnis*), MZUEL 386 (17 alc), MZUEL 387 (1 alc), MZUEL 388 (1 alc), MZUEL 401 (2 alc), MZUEL 657 (3 alc), MZUEL 1381 (1 alc), MZUEL 1910 (1 alc), MZUEL 3317 (4 alc), MZUEL 4694 (1 alc), MZUEL 5353 (3 alc), MZUEL 8815 (1 alc), MZUEL 9505 (1 alc), MZUEL 15107 (1 alc), MZUEL 16410 (1 alc), MZUEL 16411 (7 alc), MZUSP 1649 (2 alc), MZUSP 20690 (11 alc), MZUSP 20701 (6 alc), MZUSP 20731 (1 alc), MZUSP 20743 (2 alc), MZUSP 20751 (5 alc), MZUSP 21623 (2 alc), MZUSP 35681 (1 alc), MZUSP 42899 (1 alc), MZUSP 42900 (1 alc), MZUSP 42902 (5 alc), MZUSP 42903 (1 alc), MZUSP 42904 (1 alc), MZUSP 42905 (1 alc), MZUSP 42906 (1 alc), MZUSP 42907 (1 alc), MZUSP 42909 (1 alc), MZUSP 42911 (11 alc), MZUSP 42913 (2 alc), MZUSP 42916 (1 alc), MZUSP 42917 (1 alc), MZUSP 42918 (1 alc), MZUSP 42921 (1 alc), MZUSP 51458 (2 alc), MZUSP 58408 (2 alc), MZUSP 63676 (6 alc), MZUSP 108082 (1 alc), MZUSP 1102018 (2 alc), NMW 81315 (holotype of *Leporellus pictus*), NUP 1902 (1 alc), NUP 1967 (1alc), NUP 2675 (1 alc), NUP 4211 (1 alc), NUP 8868 (1 alc), NUP 9178 (2 alc), NUP 9201 (1 alc). **São Francisco basin:** ANSP 69522 (holotype of *Leporellus cartledge*), ANSP 171806 (1 alc), FMNH 55715 (holotype of *Leporellus timbore*), MCP 16732 (2 alc), MCP 16750 (4 alc), MZUEL 16409 (1 alc), MZUEL 16424 (1 alc), MZUSP 50739 (1 alc), MZUSP 54658 (1 alc), MZUSP 73734 (1 alc), MZUSP 74742 (1 alc), MZUSP 94649 (1 alc), MZUSP 94676 (1 alc), MZUSP 98749 (1 alc).

Leporellus sexdentatus**: Magdalena basin:** ANSP 128896 (1 alc), MCZ 35804 (1 alc). Type specimen of *Leporellus sexdentatus* not found at NMW (Fricke *et al.*, 2023).

Leporellus vittatus**: Amazon basin:** IEPA 3200 (1 alc), INPA 14047 (1 alc), INPA 32478 (1 alc), INPA 45190 (1 alc), MCP 14401 (1 alc), MCP 40330 (1 alc), MCP 42675 (1 alc), MCP 43675 (1 alc), MNHN A9802 (holotype of *Leporellus vittatus*), MCZ 19389 (5 alc), MZUSP

4989 (1 alc), MZUSP 40930 (1 alc), MZUSP 58225 (1 alc), MZUSP 84018 (1 alc), MZUSP 89023 (1 alc), MZUSP 89024 (1 alc), MZUSP 89807 (5 alc), MZUSP 91910 (2 alc), MZUSP 94872 (2 alc), MZUSP 95537 (7 alc), MZUSP 98136 (3 alc), MZUSP 103465 (1 alc), MZUSP 104476 (2 alc), MZUSP 106039 (1 alc), NUP 8391 (1 alc). **Essequibo basin:** ANSP 39715 (1 alc), ANSP 180299 (1 alc). **Orinoco basin:** ANSP 159372 (19 alc), ANSP 198986 (1 alc), ANSP 192064 (1 alc), MCZ 32158 (1 alc), MZUSP 97809 (1 alc).

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CAPÍTULO 2

O segundo capítulo consiste na descrição de uma espécie nova de *Leporellus* distribuída da bacia do rio Paraguai. Este capítulo ainda será submetido à Papéis Avulsos de Zoologia, e já foi formatado seguindo as normas de publicação do periódico, explicitadas em https://www.revistas.usp.br/paz/about/submissions .

A new species of *Leporellus* (Characiformes, Anostomidae) from the upper Paraguay basin, Brazil

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Running title: New species of *Leporellus* from upper Paraguay

Key-words: Biodiversity; Ostariophysi; Neotropical; Anostomoidea

Anostomidae is the second most diverse family of Characiformes and still encompasses many still undescribed species. *Leporellus* is one of the most interesting genus within Anostomidae as it represents an ancient widespread lineage of South-American fishes. Here, a new easily diagnosable congener is described from the upper Paraguay basin in Brazil. The new species is diagnosed based on unique caudal-fin coloration, including only three dark longitudinal stripes on the caudal fin (vs. five). The new species is allocated in *Leporellus* based on its scaled caudal fin, downturned mouth with labial folds, four incisiform teeth with a blunt cutting edge on each premaxillary and dentary, a dark median stripe on the dorsal fin, and dark longitudinal stripes on the caudal fin. The validity of the new species was investigated and corroborated through species delimitation analyses based on DNA Barcode. On the other hand, comparisons of the body shape, morphometric and meristic data showed not to distinguish the new species, *Leporellus pictus*, and *L. vittatus*. The new species is apparently endemic to the upper Paraguay river basin, in Brazil.

Key-words: Anostomoidea, Neotropical, Phylogeny, Systematics, Taxonomy

The diversity of fishes in tropical areas is still underestimated and it still constitutes one of the most fascinating subjects in Comparative *Biology* (Lévêque, 1997; de Pinna, 2005). Nine thousand species are estimated for the complete diversity of fishes in South America (Reis *et al.*, 2016; Birindelli & Sidlauskas, 2018), meaning that there is still 30% to be discovered and/or described. Approximately 40% of that diversity is of Characiformes (Lowe McConnel, 1999), which includes Anostomidae as the second most diverse family (Fricke *et al.*, 2023).

Anostomidae was proposed by Günther, 1864 to encompass species previously described in *Anostomus*, *Leporinus* and *Rhytiodus*. The known diversity of the family grew from 24 valid species in 1864, to 75 in the half of the last century (Myers, 1950), to around 130 in beginning of the 21st century (Garavello & Britski, 2003), and has recently surpassed the 150 valid species (Fricke *et al.*, 2023). Still, much more species are been discovered and described every year (Sidlauskas & Birindelli, 2017), especially from largely unexplored areas in South America (Birindelli *et al.*, 2019; Narezzi & Birindelli, in press).

Leporellus is the easiest genera of Anostomidae to diagnose as it is unique in possessing scaled caudal fin, downturned mouth with labial folds, four incisiform teeth with a blunt cutting edge on each premaxillary and dentary, a dark median stripe on the dorsal fin, and dark longitudinal stripes on the caudal fin (Sidlauskas & Vari, 2008; Sidlauskas & Birindelli, 2017). On the other hand, the validity and distribution of the nominal species of *Leporellus* are yet not completely understood (Sidlauskas & Vari, 2012; Sidlauskas & Birindelli, 2017). *Leporellus* was described by Lütken (1875) to allocate *Leporinus pictus* Kner, 1855, a species distributed in the upper Paraná and São Francisco basins in Brazil (Narezzi & Birindelli, in press). The genus encompasses today at least three other valid species: *L. vittatus* (Valenciennes, 1850), distributed in the Amazon and Orinoco, *L. sexdentatus* (Eigenmann, 1922), distributed in the Magdalena drainage in Colombia, and the recently described species from the Madeira river in Brazil (Narezzi & Birindelli, in press).

Leporellus was recognized as belonging to its own subfamily by Eigenmann (1910) and subsequent authors (Fowler, 1939; Géry, 1972). The first phylogenetic analysis, based on morphological characters, corroborated this classification, recovering *Leporellus* as sister to all other anostomids (Sidlauskas & Vari, 2008). Later phylogenetic analyses, based on molecular markers, recovered *Leporellus* as a distinct lineage close to the tree base, but not sister to remaining anostomids; *Leporellus* was rather recovered as sister to Anostominae, forming a clade sister to all other anostomids (Ramirez *et al.*, 2016; Mirande, 2018 Betancur *et al.*, 2018; Melo *et al.*, 2021; Sidlauskas *et al.*, 2021). Nevertheless, the phylogenetic position of *Leporellus* is key in understanding the evolution of the entire family.

The present contribution aims to describe a new species of *Leporellus*, testing its validity by comparisons to congeners using morphological and molecular analyses. The new species is diagnosed, described, illustrated, and its geographical distribution documented.

MATERIALS AND METHODS

The material was obtained from scientific collections and included alcohol-preserved specimens, except when indicated by CS, which were Cleared and Stained according to Taylor and Van Dyke (1985). Institutional abbreviations follow Sabaj (2020). Geographical distribution was illustrated using QGiz v.3.26. Counts and measurements were taken according to Winterbottom (1980), expect for considering the anterior portion of the specimens the snout tip, instead of the lower jaw tip. All measurements were taken point to point on the left side of specimens with digital calipers and recorded to 0.1 mm. Standard length (SL) is expressed in mm, and all other measurements are expressed as percentages of SL, except subunits of the head, which are expressed as percentages of head length. Meristic data are given in the description, with the frequency of each count provided in parentheses after the respective count and an asterisk indicating counts of holotype. Lateral-line scale counts included pored scales extending onto the base of median caudal-fin rays; counts of longitudinal scale rows above the lateral line exclude the lateral-line scale row and the middorsal scale row; and counts of longitudinal scale rows below the lateral line exclude the lateral-line scale row. Last dorsal and anal fin rays branched at base and counted as a single element. Morphometric data were transferred to Past v.4 (Hammer *et al.*, 2001), where it was logarithimized and used in a variance-covariance Principal Component Analysis disregarding groups.

Genomic material (DNA) was isolated from muscle tissue using the Wizard Kit (Promega). GoTaq Master Mix Kit (Promega) was used to amplify the region of interest, following the manufacturer's recommendations. For this study, a ±640 bp segment of the mitochondrial gene Cytochrome Oxidase I (COI), called DNA Barcode (Herbert *et al.*, 2003), was amplified and sequenced using Fish F1 and Fish R1 primers (Ward *et al.*, 2005). Additional sequences of the new species and congeners were obtained online at the GenBank website (https://www.ncbi.nlm.nih.gov/genbank/) and at the Bold System (http://boldsystems.org/). A complete list of tissue vouchers used in the molecular analyses is available in Table 1. The sequences were edited in the MEGA v.11 program (Kumar *et al.*, 2018) and aligned using the MUSCLE v3.8.4 algorithm (Edgar, 2004) implemented in MEGA. In the same software, a search was performed to obtain the best nucleotide model to our data, which was then used to calculate a genetic distance matrix between and within species, and also the species tree. Phylogenetic relationships were investigated through an analysis of Maximum-Likelihood, also in MEGA, using 1000 bootstrap replica to calculate tree support. Resulting tree was edited in FigTree, v.1.4.4. Two models were used for species delimitation analyses: Poisson Tree Processes (PTP) model to infer putative species boundaries on a given phylogenetic input tree (Puillandre *et al.*, 2021), and Assemble Species by Automatic Partitioning – ASAP, that infer the number of species in a given aligned molecular matrix using the genetic distance between each sample (Puillandre *et al.*, 2021). For PTP, the maximum likelihood tree generated in MEGA was uploaded to the website (https://species.h-its.org/ptp/.) For ASAP, a file with aligned sequences was used on the online server (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html), with the K80 distance used as the model and other parameters used in default.

Table 1. Voucher data for sequences of the DNA Barcode used in the molecular-based analyses.

Species	Tissue Voucher	Accession number	Locality
Leporellus sp.n.	MZUSP 113987-L070	KF568980	Paraguay river, Brazil
Leporellus sp.n.	MZUEL 22204-117	unregistred	Paraguay river, Brazil
Leporellus sp.n.	MZUEL 22204-228	unregistred	Paraguay river, Brazil
Leporellus sp.n.	MZUEL 22204-229	unregistred	Paraguay river, Brazil
Leporellus pictus	MZUEL 22707	unregistred	Mogi-Guaçu river, Brazil
Leporellus pictus	MZUEL 22701-1	unregistred	Mogi-Guaçu river, Brazil
Leporellus pictus	MZUEL 22701-2	unregistred	Mogi-Guaçu river, Brazil
Leporellus pictus	MZUEL 16947-1	unregistred	Paranapanema river, Brazil
Leporellus pictus	MZUEL 16947-2	unregistred	Paranapanema river, Brazil
Leporellus pictus	MZUEL 16947-3	unregistred	Paranapanema river, Brazil
Leporellus pictus	LBPV-11256	JN988978	Paranapanema river, Brazil
Leporellus pictus	LBPV-11255	GU701640	Paranapanema river, Brazil
Leporellus pictus	LBPV-11254	GU701641	Paranapanema river, Brazil
Leporellus pictus	LBPV-31749	GU701943	Paranapanema river, Brazil
Leporellus pictus		CAPV843-17	Paranapanema river, Brazil
Leporellus pictus		CAPV271-17	Paranapanema river, Brazil
Leporellus pictus		CAPV565-17	Paranapanema river, Brazil
Leporellus sexdentatus	7596-2609	CIUA1052-21	Cauca river, Colombia
Leporellus vittatus	MZUEL 21742	unregistred	Negro river, Brazil

Results

Systematic account

Leporellus sp.n. (Paraguay)**, new species**

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Figs. 1–5; Table 2

Fig 1. *Leporellus* sp.n., NUP ??, 125.1 mm SL, holotype, in lateral view.

Holotype.— NUP ??, 125.1 mm SL, Mato Grosso, rio Manso, tributary of rio Paraguay, 14°52’22”S 55°46’28”W, 19 Apr 2001, Nupelia.

Paratypes.—All from Brazil. MZUEL 12263, 2 alc, 79.5-85.5 mm SL, Mato Grosso do Sul, Corumbá, rio Miranda at Base de Estudos do Pantanal (BEP-UFMS), 19°34’35”S 57°01’04”W, 5 Sep 2002, Oscar A. Shibatta, Edson Santana & UEL undergraduate students. MZUEL 13998, 2 alc, 68.7-79.6 mm SL, Mato Grosso do Sul, Corumbá, rio Miranda at Base de Estudos do Pantanal (BEP-UFMS), 19°34’35”S 57°01’04”W, 19 Aug 2015, Fernando C. Jerep, Edson Santana & UEL undergraduate students. MZUEL 22204, 2 alc (tissue vouchers), 17.1-33.8 mm SL, Mato Grosso do Sul, Corumbá, rio Miranda at Base de Estudos do Pantanal (BEP-UFMS), 19°34’35”S 57°01’04”W, 19 May 2022, José L.O. Birindelli, Edson Santana & UEL undergraduate students. MZUEL 23055, 1 alc (tissue voucher), 80.3 mm SL, Mato Grosso do Sul, Corumbá, rio Miranda at Base de Estudos do Pantanal (BEP-UFMS), 19°36’42”S 57°02’06”W, 30 Apr 2023, José L. O. Birindelli, Edson Santana & UEL undergraduate students. MZUEL 23228, 2 alc, 119.1-127.8 mm SL, 1 CS, 110.8 mm SL, collected with holotype. MZUSP 59672, 1 alc, 61.2 mm SL, Mato Grosso do Sul, Corumbá, rio Vermelho, triburaty of rio Miranda, at Fazenda Xaroes, 19°37’13”S 56°55’42”W, 5 Sep 1998, Francisco A. Machado, & Barry Chernoff. MZUSP 78788, 2 alc, 109.7-115.6 mm SL, Mato Grosso, Reserva do Cabaçal, rio Cabaçal, below the waterfall, 15°09’31”S 58°20’36”W, 5 Mar 2002, Francisco A. Machado, Flávio C.T. Lima, G. Borges & Nakagaki. MZUSP 78872, 1 alc, 108.9 mm SL, Mato Grosso, Diamantino, rio Paraguay, below PCH Diamantino, 14°28’32”S 56°23’32”W, 10 Mar 2002, Francisco A. Machado, Flácio F.T. Lima & G. Borges. MZUSP 82061, 1 alc, unmeasured, Mato Grosso, Jauru, rio Jauru, at PCH Jauru, 15°17’04”S 58°42’23”W, 17 Jan 2002, Keve de Silimon. MZUSP 89972, 1 alc, 115.0 mm SL, Mato Grosso, Tangará da Serra, rio Sepotuba, below Salto das Núvens waterfall, 14°47’15”S 57°44’20”W, Heraldo A. Britski, Otávio Froehlich & F. Marques. MZUSP 99271, 3 alc, 99.0-117.7 mm SL, Mato Grosso, Nortelância, ribeirão Santana, below PCH Arrosensal, 14°19’08”S 56°44’50”W, Francisco A. Machado, Flávio Lima, Célia Leite & Silva *et al.* MZUSP 113987, 1 alc (Tissue voucher), 92.8 mm SL, Mato Grosso, Tangará da

Serra, rio Sepotuba, below Salto das Núvens waterfall, 14°47’15”S 57°44’20”W, 4 Mar 2012, Jorge Ramirez. NUP 2164, 7 alc, 103.6-128.3 mm SL, collected with holotype. NUP 3364, 3 alc, 77.5-144.1 mm SL, Mato Grosso, Nobres, córrego Cancela, tributary of rio Cuiabá, 14°43’14”S 56°19’44”W, 15 Jan 2003, Nupelia. NUP 7834, 2 alc, 91.6-173.2 mm SL, Mato Grosso, Alto Araguaia, rio Ariranha, tributary of rio Taquari, 17°38’39”S 53°35’32”W, 8 May 2009, Nupelia. NUP 9422, 1 alc, 125.6 mm SL, Mato Grosso, Nobres, córrego Cancela, tributary of rio Cuiabá, 14°42’30”S 56°15’51”W, 21 Jan 2001, Nupelia. NUP 9423, 1 alc, 102.7 mm SL, Mato Grosso, Rosário do Oeste, rio Manso, 14°52’22”S 55°46’28”W, 17 Apr 2003, Nupelia. NUP 9424, 1 alc, 66.7 mm SL.

Diagnosis — *Leporellus* sp.n. is distinguished from all other Anostomidae, except congeners, by having the caudal-fin rays extensively covered by scales (Fig. 2) (vs. caudal-fin rays not covered by scales except for the base), a dark stripe on median portion of dorsal-fin rays (vs. stripe absent), five dark longitudinal stripe on caudal fin (Fig. 2) (vs. stripes absent), and labial folds present (Fig. 3) (vs. absent). *Leporellus* sp.n. is distinguished from all congeners by having three dark longitudinal stripes on the caudal fin (*v.* five).

Fig. 2. Head in dorsal (top), lateral (middle), and ventral (bottom) views of *Leporellus* sp.n., NUP ??, 125.1 mm SL, holotype.

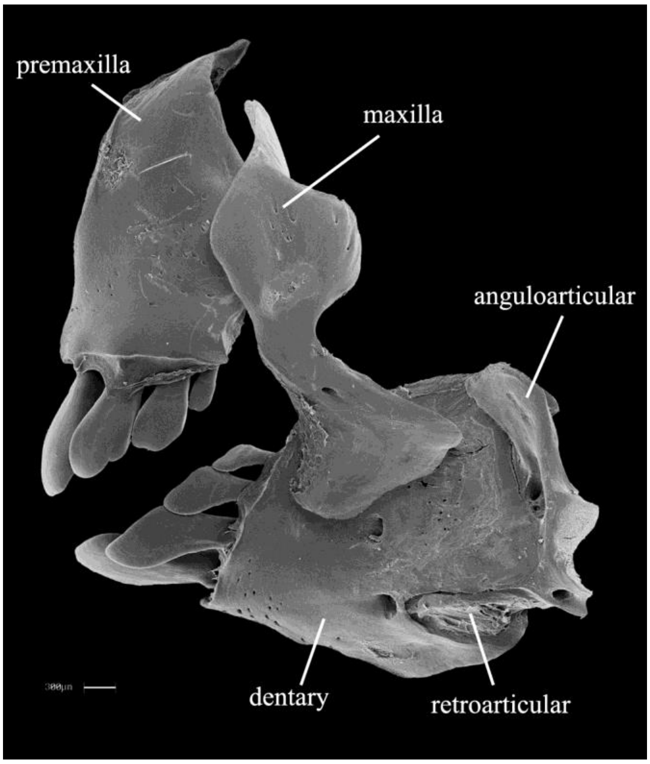

Fig. 3. Jaws and teeth of *Leporellus* sp.n., MZUEL 23228, 110.8 mm SL, paratype, in lateral (left) and median (right) views.

Description.—Morphometric data summarized in Table 2. Medium size relative to congeners. Largest examined specimen 144.1 mm SL. Head and body elongate and moderately compressed (Fig. 1). Dorsal profile distinctly convex from snout tip to vertical through nares, gently convex from latter point to dorsal-fin origin, straight along dorsal-fin base, and from dorsal-fin terminus to adipose-fin origin, and slightly concave from adipose-fin origin to anteriormost dorsal caudal-fin procurrent ray. Ventral profile gently convex from lower jaw tip to posterior margin of opercle, convex from latter point to anal-fin origin, distinctly convex from anal-fin origin to anteriormost ventral caudal-fin procurrent ray. Greatest body depth immediately anterior to dorsal-fin origin.

Tab. 2. Morphometric data for *Leporellus* sp.n.. Mean includes the holotype. SD means Standard Deviation.

	Holotype	**n**	**Mean**	**Min**	-	**Max**	**SD**
Standard length	125.10	16	104.22	61.23	-	129.04	
		Percentage of SL					
Snout tip to anal-fin origin	73.62	16	72.80	70.11	-	77.48	1.93
Snout tip to adipose-fin origin	83.66	16	82.26	79.00	-	87.78	2.71
Snout tip to dorsal-fin origin	46.91	16	44.99	42.14	-	47.89	1.79
Snout tip to pelvic-fin origin	50.22	16	48.85	46.70	-	51.82	1.73
Dorsal-fin origin to caudal-fin origin	55.96	16	55.97	48.46	-	59.07	2.82
Dorsal-fin origin to adipose-fin origin	39.78	16	38.97	35.27	-	44.02	2.77
Caudal-peduncle length	18.56	16	17.39	10.97	-	20.26	3.27
Caudal-peduncle depth	10.83	16	18.67	10.83	-	23.46	4.55
Body depth at dorsal-fin origin	25.48	16	25.79	23.41	-	29.41	1.42
Body width at dorsal-fin origin	12.60	16	13.48	12.25	-	15.62	1.04
Head length	24.55	16	21.35	18.27	-	24.90	2.54
Head depth	19.22	16	19.15	17.47	-	20.38	0.83
		Percentage of HL					
Preopercle length	74.89	6	75.58	73.54	-	78.14	1.86
Preopercle depth	69.36	6	72.83	69.19	-	76.98	3.10
Snout length	42.98	6	40.90	39.23	-	42.98	1.34
Snout depth	53.57	6	54.03	51.20	-	56.42	2.16
Eye diameter	22.53	6	24.90	22.53	-	27.42	1.58
Bony interorbital	33.54	6	35.65	33.54	-	37.62	1.53

Mouth subinferior, cleft longitudinally aligned with longitudinal through infraorbital series ventral margin in specimens around 100 mm SL. Upper and lower lips hypertrophied, forming labial folds (Fig. 2). Snout rounded, anteriorly blunt. Premaxillary bone with four teeth arranged side-by-side and gently decreasing in size from symphyseal tooth. Sympheseal premaxiilary teeth compressed (without lateral ridge) and with blunt distal (cutting) margin; second, third, and fourth premaxillary teeth compressed and with three slightly distinct rounded cusps, medial one largest (Fig. 3). Dentary bone with four teeth arranged side by side and gently decreasing in size laterally. All dentary teeth compressed (without lateral ridge) and with blunt distal margin (Fig. 3).

Lateral line complete, 42 (3), 43* (12), or 44 (1) perforated scales, extending from supracleithrum to base of median caudal-fin rays. Horizontal scale rows between dorsal-fin origin and lateral line 5* (6). Horizontal scale rows between lateral line and pelvic-fin origin 6* (6). Predorsal scales 10 (1), 11* (9), or 12 (1). Scale rows around caudal peduncle 16* (15). Caudal-fin lobes extensively covered by small scales, about ¾ the size of body scales, and covering ¾ of fin lobes (Fig. 4).

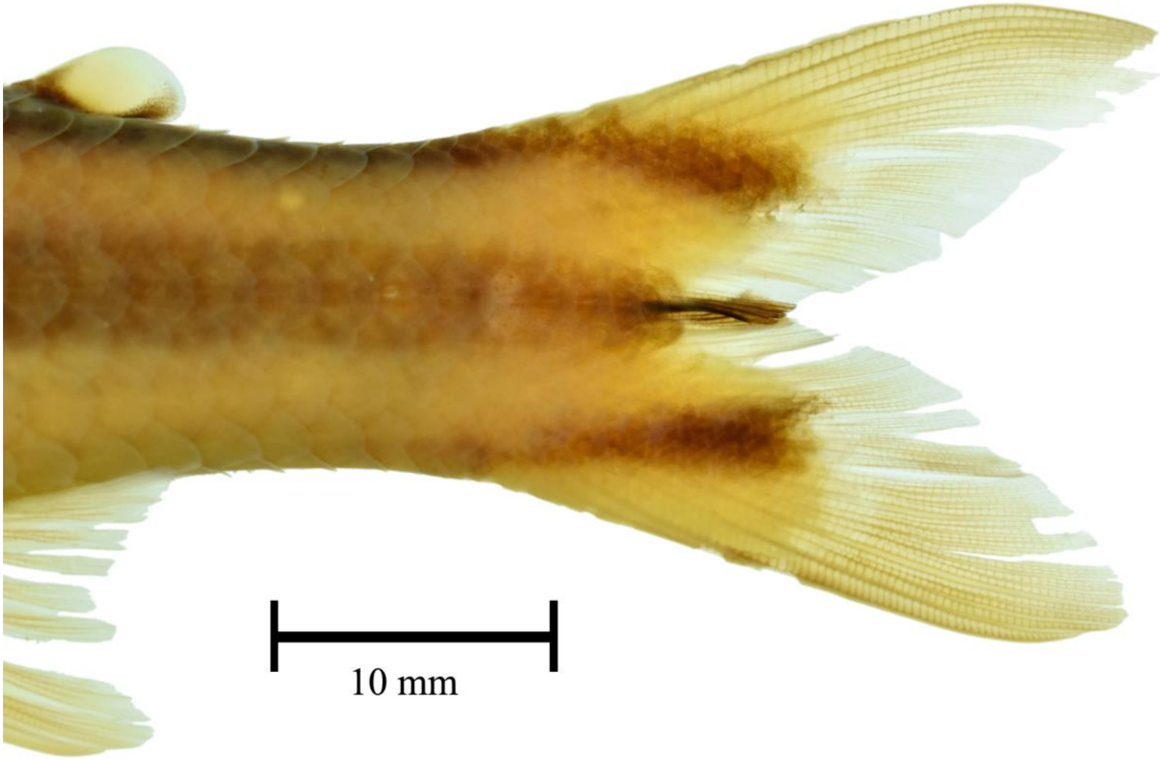

Fig. 4. Caudal peduncle and fin of *Leporellus* sp.n. (Paraguay), NUP ??, 125.1 mm SL, holotype, in lateral view.

Dorsal fin ii,10* (6). Dorsal-fin origin slightly anterior to middle of standard length and slightly anterior to vertical through pelvic-fin origin; its distal margin straight or gently concave. Adipose fin small, teardrop shaped, origin approximately at vertical through base of last anal-fin branched ray. Pectoral fin i,15 (3), i,16* (3); tip extending more than half distance between pectoral- and pelvic-fin origins; distal margin rounded. Pelvic fin i,8* (6); tip extending approximately half distance between pelvic- and anal-fin origins, with distal margin slightly convex. Anal fin ii,8* (6); origin slightly anterior to vertical between terminal of dorsal-fin base and anteriormost dorsal procurrent caudal-fin ray, when adpressed fin not reaching base of caudal-fin rays; distal margin concave. Principal caudal-fin rays i,8,9,i* (6). Caudal fin forked, lobes rounded, upper lobe slightly more acute than lower lobe.

Color in alcohol.—Head and body ground color light brown and countershaded (Fig. 1). Trunk with two to four dark stripes, dorsalmost near middorsal portion of body, second dorsalmost stripe between lateral line and middorsal portion of body, third stripe on midlateral covering lateral-line scale series, fourth between lateral line and ventral portion of body. First and second stripes indistinct (i.e., united) in some specimens, fourth stripe absent in some specimens. Body lateral scales, especially dorsal to lateral line and on anterior portion of body with a dark spot at center. Head with small dark blotches, especially on dorsal portion where blotches might be connected, forming vermiculations (Fig. 2). Head ventral surfaces and body pale to cream. Dorsal fin with one large dark stripe between half and distal one-fourth of rays. Adipose fin with dark margin. Caudal fin with three dark stripes, dorsalmost stripe continuous to dorsal limit of caudal peduncle and continuous with dark body stripe, one midlateral and continuous with midlateral body dark stripe, third at ventral limit of caudal peduncle. Pectoral, pelvic, and anal fins generally hyaline. Juveniles (of around 20 to 30 mm SL) with trunk possessing a dark midlateral stripe from snout tip to median caudal-fin rays; and a dark inconspicuous longitudinal stripe from head to dorsal portion of caudal peduncle and continuous on base of caudal fin rays, forming dorsalmost caudal-fin dark stripe (Fig. 5). Dorsal fin of juveniles with a large dark blotch on distal half, dark pigment on first pelvic-fin rays, and three dark longitudinal stripes on the caudal fin (arranged as in adults). Larvae (around 10 mm SL) with a dark midlateral stripe with cromatophores scattered on entire lateral face of caudal peduncle, and stripe extended onto lower jaw; fins hyaline (Fig. 5).

Fig. 5. Juvenile and larva of *Leporellus* sp.n., MZUEL 22204, 17.1-33.8 mm SL.

Distribution.—*Leporellus* sp.n. is known from tributaries of the Paraguay river, mostly in the upper portion of the basin, in the states of Mato Grosso and Mato Grosso do Sul, in Brazil (Fig. 6).

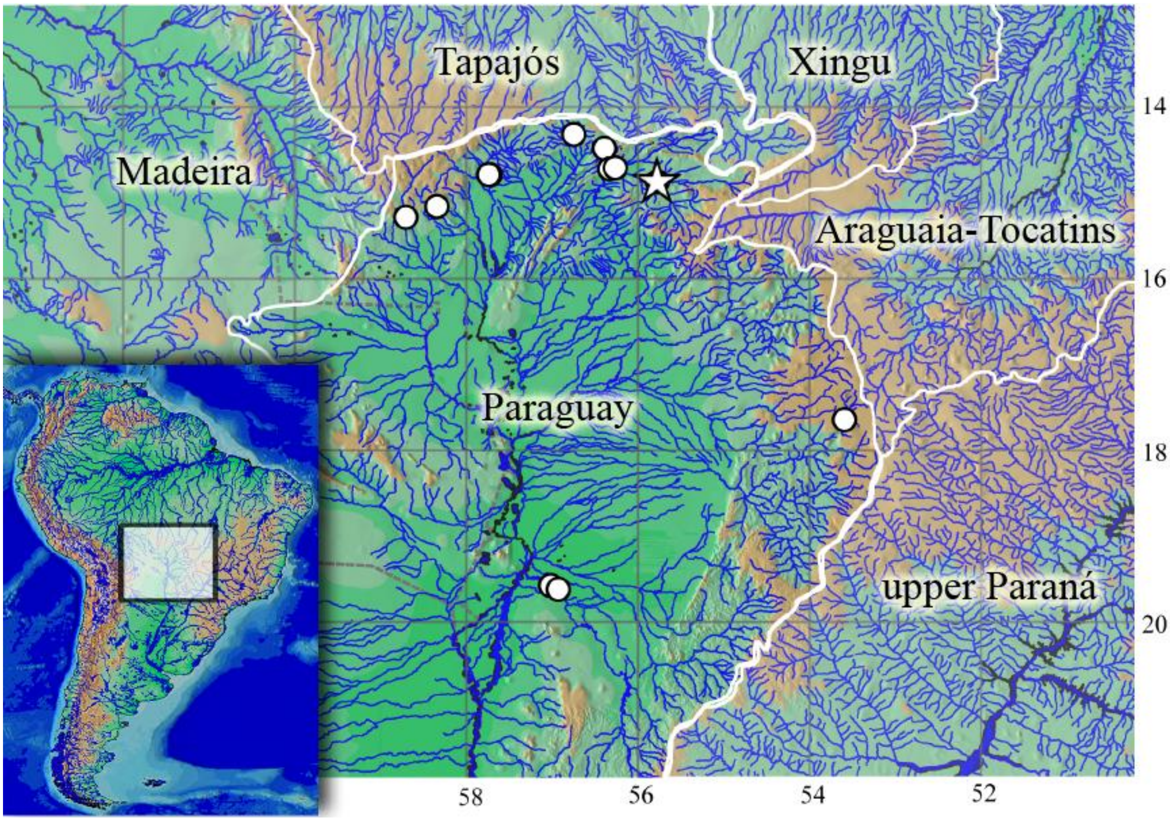

Fig. 6. Geographic distribution of *Leporellus* sp.n.. Star represents the type locality.

Conservation status.—*Leporellus* sp.n. is apparently endemic to the upper Paraguay basin, where it is widespread. All species of *Leporellus* are known to prefer fast-flowing habitat (Sidlauskas & Birindelli, 2017). Freshwater environments such as these are potential areas for constructions of dams for hydroelectric proposes. For example, the type locality of the new species was altered by the construction of the UHE de Manso, where specimens of *Leporellus* were likely much more common and abundant. Despite the population reduction estimated by the construction of the aforementioned dam, and others in other rivers of the upper Paraguay basin, the species is widespread and occur in areas that are currently largely preserved. Therefore, no potential negative impact is expected to place *L.* sp.n. under threat in the near future. The species is herein considered a Least Concern, according to the IUCN Criteria (IUCN, 2019).

Etymology.— to be included before submission.

Comparative Analyses

Morphometric analysis.— Due to the paucity of specimens of *Leporellus sexdentatus* in fish collections, the species could not be included in the morphometric analysis. In the principal component analysis (PCA), PC1 explained 97.32% of the varicance, whereas PC2 0.79%, and PC3 0.48%. PC1 is known to be influenced by body size, and therefore were not used in the plotted graphic. The PCA, using PC2 and PC3, shows a large overlap between all three samples, including *Leporellus pictus* (from upper Paraná), *L. vittatus* (from the Amazon basin), and the new species (Fig. 7). The morphometric variables that showed the highest and lowest values, and therefore most influenced PC2: head length, preopercle length, snout length, and caudal peduncle length (Table 3). The variables that most influenced PC3: dorsal-fin origin to caudal-fin origin, caudal peduncle length, head depth, body width, and eye diameter.

All meristic variables showed great overlap between samples. The lateral line scale count varied from 42 to 44 in all samples, with mode of 43. Similarly, all samples had five (rarely six) scale rows between the dorsal-fin origin and the lateral line, and five between lateral line and pelvic-fin origin. The number of scale rows around caudal peduncle was also 16 in all samples. The number of fin rays were also constant in all samples, with pectoral fin showing the great variation, ranging from 14 to 16 branched rays. The morphometric data and its analysis showed that there is no difference in the body shape, as well as in the number of scale and fin-rays in specimens of *Leporellus* from distinct species and drainages, including *Leporellus pictus* (from upper Paraná), *L. sexdentatus* (from Magdalena), and *L. vittatus* (from Amazonas).

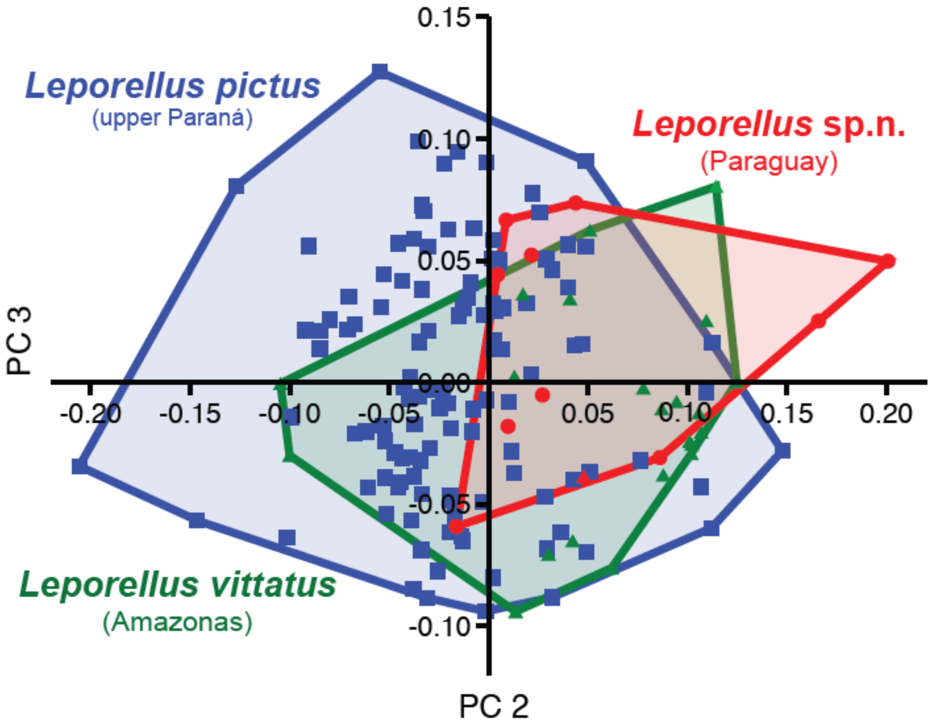

Fig. 7. Principal Component Analysis on morphometric variables comparing the body shape of *Leporellus* sp.n., *L. pictus* and *L. vittatus*.

Table 3. Loading values of Principal Component Analysis for each morphometric variable in PC2 and 3.

Morphometric variables	**PC 2**	**PC 3**
Standard length	-0.064839	0.10972
Snout tip to anal-fin origin	-0.007937	0.048397
Snout tip to adipose-fin origin	-0.056661	0.050633
Snout tip to dorsal-fin origin	0.026029	0.056413
Snout tip to pelvic-fin origin	0.053339	0.016846
Dorsal-fin origin to caudal-fin origin	-0.29506	0.21582
Dorsal-fin origin to adipose-fin origin	-0.30426	0.046856
Caudal peduncle length	-0.27678	0.57455
Head length	0.34011	0.070963
Preopercle length	0.35801	0.10615
Snout length	0.57107	-0.011434
Body depth	-0.2409	-0.19804
Caudal peduncle depth	-0.084036	-0.045775
Head depth	0.0010666	-0.20912
Preopercle depth	0.11335	-0.10895
Snout depth	0.15403	-0.11167
Body width	-0.21924	-0.57445
Eye diameter	0.11108	0.32809
Bony interorbital	-0.026876	-0.19299

Genetic distance. A total of 681 nucleotide base pairs of the COI gene were obtained for the 19 samples, which included 89 variable and 39 parsimony-informative sites. The best model for nucleotide evolution obtained was Kimura-2-parameters, which was applied into the genetic distance estimative and the phylogenetic analysis. The overall mean genetic distance was calculated as 3.82% (±0.51) for all samples. The genetic distance was lower between samples of *Leporellus pictus* from the Paranapanema and the Grande rivers, and higher between *Leporinus sexdentatus* (from Magdalena), and *L. pictus* (from the Grande river) (Table 4). The genetic distance within samples was very low, ranging from 0.000% in the new species to 0.005 in *L. pictus* (from the Grande river). The genetic distance between the new species and congeners ranged from 6.1% (for *L. pictus*) to 7.24% (for L. vittatus) and 9.12% (for *L. sexdentatus*). The genetic distance between *Leporellus* sp.n. and congeners indicate the validity and distinction of the new species relative to congeners. The minimal genetic distance values between species herein obtained (6.10%) are slightly higher than the ones found in similar studies focused on Anostomidae. For example, the genetic distance ranged from 0.92 to 5.76% between species of *Laemolyta* (Ramirez & Galetti Jr., 2015), between 0.67 and 15.31% for species of *Megaleporinus* (Ramirez *et al.*, 2017), between 0.67 and 6.48% for species of *Schizodon* (Ramirez *et al.*, 2020). The genetic distances between species of *Hypomasticus* ranged from 2.6 to 19.6% (Birindelli *et al.*, 2020), values much closer to the ones herein observed for species of *Leporellus*. Both the latter and *Hypomasticus* are old lineages in Anostomidae, especially compared to *Schizodon*, *Laemolyta* and *Megaleporinus*, what could explain their higher values of genetic distance between congeners.

Table 4. Genetic distances between samples in red and within samples in bold (diagonal), with standard error estimates shown in blue. Analysis conducted using Kimura-2-parameters model and uniform rates. Values in percentage.

	Paranapanema	**Grande**	**Paraguay**	**Amazonas**	**Magdalena**
Paranapanema (n=10)	**0.001**	0.29	1.09	1.25	1.56
Grande (n=3)	0.59	**0.005**	1.13	1.30	1.62
Paraguay (n=4)	6.10	6.53	**0.000**	1.27	1.43
Amazonas (n=1)	7.35	8.01	7.24	**n/c**	1.53
Magdalena (n=1)	10.19	10.97	9.12	9.76	**n/c**

Species delimitation analyses. The two methods for species delimitation based on COI gene sequences generated the same results (Fig. 8), both recovering MOTUs (Molecular Operational Taxonomic Units). ASAP found one partition with the lowest asap-score (1.0). In both analyses, samples of *L. pictus* from the Grande and Paranapenema rivers were recovered in the same MOTU. All other valid species were recovered in its own MOTU, including samples of the new species. In the phylogenetic analysis, the new species were recovered as sister to *Leporinus pictus*.

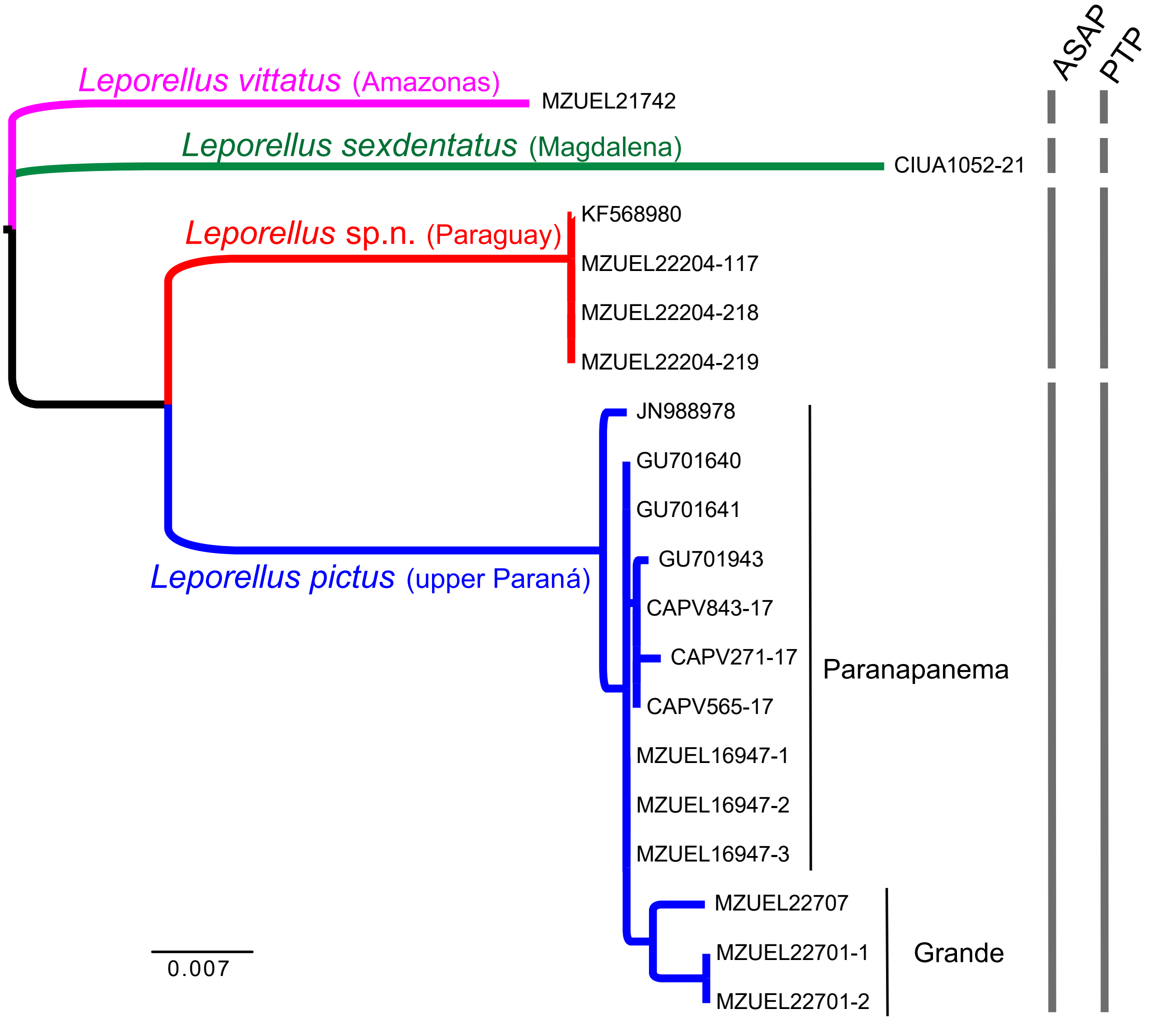

Fig. 8. Species tree of *Leporellus* sp.n., *L. pictus*, *L. sexdentatus*, and *L. vittatus*, base on Barcode sequences and using a Maximum Likelihood analysis with K2P model. Results of species delimitation analyses in the right margin.

DISCUSSION

This is the second species of *Leporellus* described recently. Unfortunately, tissues of *L.* sp.n. (Aripuanã) were not available for study. The latter, however, is easily distinguished from the new species by having 12 scale rows around the caudal peduncle (vs. 16), four scale rows between lateral line and dorsal-fin origin (vs. five), and three scale rows between lateral line and pelvic-fin origin (vs. five). In addition, *L.* sp.n. (Aripuanã) exhibit five dark longitudinal stripes on the caudal fin (vs. three). Both species are easily distinguished from congeners (*Leporellus pictus*, *L. vittatus* and *L. sexdentatus*). Nevertheless, the validity, diagnoses and distribution of these three species still uncertain and need further investigation (Sidlauskas & Vari, 2012). The molecular data available online (BoldSystem.org) and also resulted from our analyses suggests that *L. sexdentatus*, *L. vittatus* and *L. pictus* are distinct species. Further investigation, using additional samples and methods, are necessary to test the validity of these species, as well as to investigate the presence of cryptic species.

Despite the taxonomic confusion among *Leporellus* species, *Leporellus* sp.n. is easily diagnosed from all congeners by having only three dark longitudinal stripes on the caudal fin (vs. five). The presence of five dark longitudinal stripes on the caudal fin was confirmed in the type specimens of all nominal species of *Leporellus* as well as in specimens collected in distinct river basins in South America including the Amazon, Essequibo, Orinoco, Paraná, São Francisco, and Magdalena. That characteristic (the presence of longitudinal stripes of the caudal fin) is especially important for the taxonomy of species of Anostomidae, as it is unique to species of *Leporellus*. All examined specimens from the Paraguay basin had three dark longitudinal stripes on the caudal. On the other hand, almost all examined specimens from outside the caudal fin had five dark longitudinal stripes on the caudal fin. Nevertheless, a few examined specimens from outside the Paraguay basin had only three dark longitudinal stripes on the caudal fin. These included MZUEL 8815 (68.1 mm SL) and INPA 14047 (c.80 mm SL). The presence of this coloration in the latter specimens is herein interpreted as a consequence of inadequate preservation (light exposure). However, it is possible that specimens collected outside the Paraguay basin possessing only three dark stripes do exist and, if so, this is an anomalous rare feature. In any case, this does not invalidate the usefulness of this feature to diagnose the new species, nor its validity that was additionally supported by molecular data.

The north, east and south border of the Paraguay basin in located in the high lands of the Brazilian Crystalline shield formed by Precambrian rocks (Assine *et al.*, 2015) with altitude ranging from 1.000 to 200 meters. In this area and close to it, rivers tend to have rapids and waterfalls and drain a steep slope. On the other hand, in the Pantanal depression, due to the small slope (altitude ranging from 200 to 80 meters), rivers tend to have slow flowing waters, sinuous channels and marginal lagoons. The geographical distribution of *Leporellus* sp.n. is a result of its association to fast-flowing rivers that drains the high lands of the border of the Pantanal depression.

The discovery of two new species of *Leporellus* (*L.* sp.n. (Aripuanã) [Narezzi & Birindelli, in press] and *Leporellus* sp.n.) makes one wonder how great the diversity of this fish group really is. The morphological uniqueness and old age of this lineage of Anostomidae are additional factors that transform this puzzle in one of the most interesting in the Systematics of the family.

MATERIAL EXAMINED

***Leporellus pictus*: upper Paraná basin:** FMNH 55714 (holotype of *Leporellus retropinnis*), MZUEL 386 (17 alc), MZUEL 387 (1 alc), MZUEL 388 (1 alc), MZUEL 401 (2 alc), MZUEL 657 (3 alc), MZUEL 1381 (1 alc), MZUEL 1910 (1 alc), MZUEL 3317 (4 alc), MZUEL 4694 (1 alc), MZUEL 5353 (3 alc), MZUEL 8815 (1 alc), MZUEL 9505 (1 alc), MZUEL 15107 (1 alc), MZUEL 16410 (1 alc), MZUEL 16411 (7 alc), MZUSP 1649 (2 alc), MZUSP 20690 (11 alc), MZUSP 20701 (6 alc), MZUSP 20731 (1 alc), MZUSP 20743 (2 alc), MZUSP 20751 (5 alc), MZUSP 21623 (2 alc), MZUSP 35681 (1 alc), MZUSP 42899 (1 alc), MZUSP 42900 (1 alc), MZUSP 42902 (5 alc), MZUSP 42903 (1 alc), MZUSP 42904 (1 alc), MZUSP 42905 (1 alc), MZUSP 42906 (1 alc), MZUSP 42907 (1 alc), MZUSP 42909 (1 alc), MZUSP 42911 (11 alc), MZUSP 42913 (2 alc), MZUSP 42916 (1 alc), MZUSP 42917 (1 alc), MZUSP 42918 (1 alc), MZUSP 42921 (1 alc), MZUSP 51458 (2 alc), MZUSP 58408 (2 alc), MZUSP 63676 (6 alc), MZUSP 108082 (1 alc), MZUSP 1102018 (2 alc), NMW 81315 (holotype of *Leporellus pictus*), NUP 1902 (1 alc), NUP 1967 (1alc), NUP 2675 (1 alc), NUP 4211 (1 alc), NUP 8868 (1 alc), NUP 9178 (2 alc), NUP 9201 (1 alc).

***Leporellus sexdentatus*: Magdalena basin:** ANSP 128896 (1 alc), MCZ 35804 (1 alc). Type specimen of *Leporellus sexdentatus* not found at NMW (Fricke *et al.*, 2023).

***Leporellus vittatus*: Amazon basin:** IEPA 3200 (1 alc), INPA 14047 (1 alc), INPA 32478 (1 alc), INPA 45190 (1 alc), MCP 14401 (1 alc), MCP 40330 (1 alc), MCP 42675 (1 alc), MCP 43675 (1 alc), MNHN A9802 (holotype of *Leporellus vittatus*), MCZ 19389 (5 alc), MZUSP 4989 (1 alc), MZUSP 40930 (1 alc), MZUSP 58225 (1 alc), MZUSP 84018 (1 alc), MZUSP 89023 (1 alc), MZUSP 89024 (1 alc), MZUSP 89807

(5 alc), MZUSP 91910 (2 alc), MZUSP 94872 (2 alc), MZUSP 95537 (7 alc), MZUSP 98136 (3 alc), MZUSP 103465 (1 alc), MZUSP 104476 (2 alc), MZUSP 106039 (1 alc), NUP 8391 (1 alc).

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CAPÍTULO 3

O terceiro capítulo traz uma investigação sobre a diversidade, filogenia e biogeografia das espécies de *Leporellus*. Este capítulo está em preparação, ainda não foi enviado para as correções dos co-autores, e deverá ser submetido à *Molecular Phylogenetics and Evolution*, e já está formatado conforme as regras de publicação da revista, explicitadas em https://www.elsevier.com/journals/molecular-phylogenetics-and-evolution/1055-7903/guide-for-authors .

Hidden diversity and phylogeny of the species of *Leporellus*, an ancient lineage of the Anostomidae (Characiformes)

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Running title: Diversity and Phylogeny of *Leporellus*

The diversity and evolution of Neotropical fishes are yet far from understood. *Leporellus* is one of the most interesting genus within Anostomidae (Characiformes) as it represents an ancient widespread lineage of South-American fishes. Two new species of *Leporellus* were recently discovered, and still many doubts remains on the diversity and evolution of the genus. Here, the diversity and phylogeny of species of *Leporellus* are investigated integrating morphology and molecules. Species were delimited using morphometric, meristic, external morphological data, and coloration, as well as DNA Barcode. Their phylogeny was hypothesized using two mitochondrial and three nuclear markers and applying fossil ages to estimate date of diversification. The results indicated that there are at least seven distinct species within *Leporellus*, including *L. vittatus* (type species, from eastern Amazon, and Orinoco), *L. pictus* (upper Paraná), *L. timbore* (São Francisco), *L.* sp.n. (Aripuanã) (upper Aripuanã), *L. sexdentatus* (Magdalena), and two undescribed species, one from the Paraguay basin (previously recognized by Narezzi, Souza & Birindelli), and a putative new species from western Amazon. Of the seven species of *Leporellus*, only two are distinguishable based on scale count and color pattern: *L.* sp.n. (Aripuanã), and *L.* sp.n. (Paraguay). All other five are externally indistinct, and thus herein considered cryptic species with allopatric distributions. The biogeography of the species of *Leporellus* is discussed based on time-calibrated phylogeny.

Key-words: Anostomoidea, Neotropical, Phylogeny, Systematics, Taxonomy

Nine thousand species are estimated for the complete diversity of fishes in South America (Reis *et al.*, 2016), meaning that there is still 30% to be discovered and/or described (Birindelli & Sidlauskas, 2018). Approximately 40% of that diversity is of Characiformes (Lowe McConnel, 1999), which includes Anostomidae as the second most diverse family (Fricke *et al.*, 2023). The known diversity of the family grew from 24 valid species in 1864, to 75 in the half of the last century (Myers, 1950), to around 130 in beginning of the 21st century (Garavello & Britski, 2003), and has recently surpassed the 150 valid species (Fricke *et al.*, 2023). Still, much more species are been discovered and described every year (Sidlauskas & Birindelli, 2017; Birindelli *et al.*, 2020a,b), especially from largely unexplored areas in South America (Birindelli *et al.*, 2019; Narezzi & Birindelli, in press).

Leporellus is the sole genus of Leporellinae, one of the subfamilies of Anostomidae, and encompass colorful medium-size fishes (up to 250 mm SL) inhabiting preferencably fast-flowing waters such as those of rapids (Sidlauskas & Birindelli, 2017) (Figure 1). This genus is easily distinguished by possessing at least three exclusive features among Anostomidae: small scales covering approximately two-thirds of caudal fin lobes, labial folds (sensu Narezzzi & Birindelli, in press), and dark longitudinal stripes on caudal fin. On the other hand, the diagnoses of species of *Leporellus* are far from trivial, what contributed to the complex and fuzzy taxonomic history of the genus. In fact, up to now the validity and distribution of most nominal species of *Leporellus* are completely unclear and under debate (Sidlauskas & Birindelli, 2017; Narezzi & Birindelli, in press).

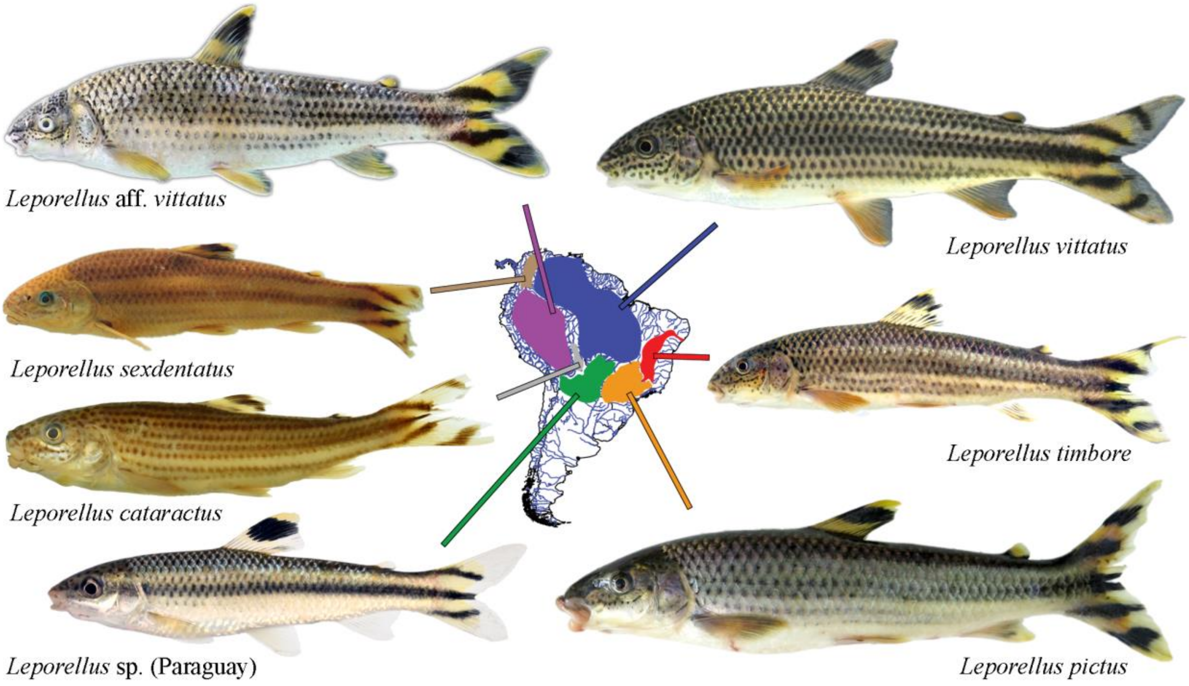

Fig 1. Diversity and geographical distribution of species of *Leporellus* that are herein considered valid.

Leporellus was described by Lütken (1875) to allocate *Leporinus pictus* Kner, 1855, a species described based on specimens collected in the upper Paraná basin, and presumed by Lütken (1875) to also occur in the Rio das Velhas (a tributary of the São Francisco river). In the same contribution, the latter author considered, that Valenciennes's *L. vittatus*, whose type locality lies in the Amazon basin, was likely a synonym of Kner's *L. pictus*. Similar comments were made by subsequent authors (Steindachner, 1880; Eigenmann, 1922; Garavello & Britski, 2003), generating confusion and doubts regarding these names. Eigenmann (1922) had a distinct interpretation on Lütken's contribution. He considered that Lütken (1875) did not identify the specimens he collected in the Rio das Velhas as *Leporinus pictus* Kner 1855, but rather provided a new species name for his specimen. If so, he would have erroneously used a preoccupied species name (*Leporinus pictus* Kner 1855). Eigenmann (1922: 117) then provided a replacement name (*Leporellus timbore*) for Lütken's specimen collected in the Rio das Velhas. Interestingly, a similar name (*Salmo timbure*) was previously applied by Naterrer in Kner (1859) for specimens from the upper Paraná. Timboré (timburê, or similar corruptions) is a word with indigenous origin (Tupi) and the vernacular name that local people in some parts of Brazil use to recognize specimens of *Leporellus*. The name is considered a *nomen dubium* (Fricke *et al.*, 2023), or a *species inquirienda* (Garavello & Britski, 2003). In any case, it is an objective junior synonym of *Leporellus pictus* (Kner, 1858), as it was likely based on the same type specimen (NMW 81315).

Leporinodus retropinnis was described by Eigenmann, 1922 based on specimens from the upper Paraná. Although its type locality is close to that of *Leporellus pictus*, the species was recently considered valid by Garavello & Britski (2003), and Fricke *et al.,* (2023). In the same contribution, Eigenmann (1922) described *Leporinodus* as a new genus to include *L. retropinnis*, and also *L. sexdentatus*. The latter was named by him based on a description provided by Steindachner (1880) of a 200 mm long specimen collected in the Magdalena river basin in Colombia, and that presumably had only three teeth on each premaxillary and dentary. *Leporellus sexdentatus* was considered synonym of *L. vittatus* by Garavello & Britski (2003), and Fricke *et al.* (2023). *Leporinodus* was considered a synonym of *Leporellus* by Taphoorn (1992), and subsequent authors (Garavello & Britski, 2003; Fricke *et al.*, 2023).

Apparently unaware of species previously described by Lütken (1875) for the São Francisco basin, Fowler (1941) described *Leporellus cartledgei* based on specimens in the lower São Francisco river in Pernambuco state, Brazil. This species was later considered a synonym of *L. pictus* (Garavello & Britski, 2003; López *et al.*, 2003; Mirande & Koerber, 2015).

The diagnosis, distribution, and distinction of all these nominal species remain unclear today (Sidlauskas & Vari, 2012; Sidlauskas & Birindelli, 2017). Many authors simply identify specimens of *Leporellus* as *L. vittatus*, the oldest available name, despite the locality of the specimen (e.g., Langeani *et al.*, 2007).

Recently, two new species of *Leporellus* were discovered. *Leporellus* sp.n. (Aripuanã) was diagnosed from congeners by the few number of scales on body (Narezzi & Birindelli, in press). The species is putatively restricted to the upper Aripuanã river, above the Dardanelos and Andorinhas falls. Narezzi *et al.* (in prep.) recognized *Leporellus* sp.n. (Paraguay) based on the specimens from the surrounding highlands draining to the Paraguay basin. The species is unique in having only three dark longitudinal stripes on the caudal fin (vs. five or seven). The discovery of these new species led one to wonder how diverse *Leporellus* can really be, once investigated using modern tools.

Leporellus was recognized as belonging to its own subfamily by Eigenmann (1910) and subsequent authors (Fowler, 1939; Géry, 1972). The first phylogenetic analysis including *Leporellus* was based on morphological characters and corroborated this classification, recovering *Leporellus* as sister to all other anostomids (Sidlauskas & Vari, 2008). Later phylogenetic analyses, based on molecular markers, recovered *Leporellus* as a distinct lineage close to the tree base, but not sister to remaining anostomids. *Leporellus* was rather recovered as sister to Anostominae, forming a clade sister to all other anostomids (Ramirez *et al.*, 2016; Mirande, 2018 Betancur-R *et al.*, 2019; Melo *et al.*, 2021; Sidlauskas *et al.*, 2021). Be that as it may, the phylogenetic position of *Leporellus* is key in understanding the evolution of the entire family. No attempt so far has investigated the phylogenetic relationships within the genus.

The present contribution aim to investigate the diversity of species of *Leporellus*, and their phylogenetic relationships, combining morphological and molecular data, using distinct approaches, methods and to provide the first comprehensive view on the diversity and evolution of species of *Leporellus*.

MATERIALS AND METHODS

Diversity analyses based on DNA Barcode. Sequences of a ±640 bp fragment of the Cytochrome Oxydase I (COI), also known as DNA Barcode (Herbert *et al.*, 2003), were used in the analyses to investigate the diversity within *Leporellus*. Genomic material (DNA) was isolated from muscle tissue using the Wizard Kit (Promega). GoTaq Master Mix Kit (Promega) was used to amplify the region of interest, following the manufacturer's recommendations. DNA Barcode was amplified and sequenced using Fish F1 and Fish R1 primers (Ward *et al.*, 2005). Additional sequences of were obtained online at the GenBank website and at the Bold System. A complete list of tissue vouchers used in the molecular analyses is available in Table 1. The sequences were edited in the MEGA v.11 program (Kumar *et al.*, 2018) and aligned using the MUSCLE v3.8.4 algorithm (Edgar, 2004) implemented in MEGA. In the same software, a search was performed to obtain the best nucleotide model to our data, which was then used to calculate a genetic distance matrix between and within species, and also the species tree. Phylogenetic relationships were investigated through an analysis of Maximum-Likelihood, also in MEGA, using 1,000 bootstrap replica to calculate tree support. Resulting tree was edited in FigTree, v.1.4.4. Two models were used for species delimitation analyses: Poisson Tree Processes (PTP) model to infer putative species boundaries on a given phylogenetic input tree (Puillandre *et al.*, 2021), and Assemble Species by Automatic Partitioning – ASAP, that infer the number of species in a given aligned molecular matrix using the genetic distance between each sample (Puillandre *et al.*, 2021). For PTP, the maximum likelihood tree generated in MEGA was uploaded to the website (https://species.h-its.org/ptp/.) For ASAP, a file with aligned sequences was used on the online server (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html), with the K80 distance used as the model and other parameters used in default.

Tab. 1. List of tissue vouchers used in the DNA Barcode based analyses.

Species	Tissue Voucher	Locality	Accession number
Leporellus sp.n. (Paraguay)	L0066	Paraguay river, Brazil	unregistred
Leporellus sp.n. (Paraguay)	L0067	Paraguay river, Brazil	unregistred
Leporellus sp.n. (Paraguay)	L0071	Paraguay river, Brazil	unregistred
Leporellus sp.n. (Paraguay)	MZUEL 22204-117	Paraguay river, Brazil	unregistred
Leporellus sp.n. (Paraguay)	MZUEL 22204-228	Paraguay river, Brazil	unregistred
Leporellus sp.n. (Paraguay)	MZUEL 22204-229	Paraguay river, Brazil	unregistred
Leporellus sp.n. (Paraguay)	MZUSP 113987-L070	Paraguay river, Brazil	KF568980
Leporellus pictus	L0879	Mogi-Guaçu river, Brazil	unregistred
Leporellus pictus	L0881	Mogi-Guaçu river, Brazil	unregistred
Leporellus pictus	MZUEL 22701-1	Mogi-Guaçu river, Brazil	unregistred
Leporellus pictus	MZUEL 22701-2	Mogi-Guaçu river, Brazil	unregistred
Leporellus pictus	MZUEL 22707	Mogi-Guaçu river, Brazil	unregistred
Leporellus pictus	MZUEL 16947-1	Paranapanema river, Brazil	unregistred
Leporellus pictus	MZUEL 16947-2	Paranapanema river, Brazil	unregistred
Leporellus pictus	MZUEL 16947-3	Paranapanema river, Brazil	unregistred
Leporellus pictus	LBPV-11256	Paranapanema river, Brazil	JN988978
Leporellus pictus	LBPV-11255	Paranapanema river, Brazil	GU701640
Leporellus pictus	LBPV-11254	Paranapanema river, Brazil	GU701641
Leporellus pictus	LBPV-31749	Paranapanema river, Brazil	GU701943
Leporellus pictus		Paranapanema river, Brazil	CAPV843-17
Leporellus pictus		Paranapanema river, Brazil	CAPV271-17
Leporellus pictus		Paranapanema river, Brazil	CAPV565-17
Leporellus pictus		Paranapanema river, Brazil	unregistred
Leporellus sexdentatus	7596-2609	Cauca river, Colombia	CIUA1052-21
Leporellus timbore	L0929	São Francisco river, Brazil	unregistred
Leporellus timbore	MZUEL 16409	São Francisco river, Brazil	unregistred
Leporellus timbore	MZUEL 16424	São Francisco river, Brazil	unregistred
Leporellus aff. *vittatus*	L0489	Madeira river, Brazil	unregistred
Leporellus aff. *vittatus*	LSMFF000856	Ucayali river, Peru	unregistred
Leporellus aff. *vittatus*	ANSP 182218-P6295	Amazonas river, Peru	unregistred
Leporellus vittatus	L0550	Araguaia river, Brazil	unregistred
Leporellus vittatus	MZUEL 21742	Negro river, Brazil	unregistred
Leporellus vittatus	AUM 54212_T09913	Orinoco river, Venezuela	KX086795

Phylogenetic relationships and dating. Five molecular markers, including two mitochondrial ones (COI, and Cytb), and three nuclear markers (RAG1, RAG2, and Myh6), were used to investigate the phylogenetic relationships of the species of *Leporellus*. All of these markers proved to be successful in recent studies focused on the phylogenetic relationships of Anostomidae (Ramirez & Galetti, 2015; Ramirez *et al.,* 2016, 2017, 2020; Birindelli *et al.*, 2020a,b; Burns *et al.*, 2017; Sidlauskas *et al.*, 2021), as well as closely related characiform families (Melo *et al.,* 2014, 2016, 2018). Genomic material (DNA) was isolated from muscle tissue using the Wizard Kit (Promega). GoTaq Master Mix Kit

(Promega) was used to amplify the region of interest, following the manufacturer's recommendations. RAG1 (±1,500 bp), RAG2 (±1,100 bp), Myh6 (±750 bp), and Cytb (±1,100 bp) were amplified and sequenced using primers described by Ramirez & Galetti (2015). Genetic sequences from outgroup taxa were obtained in GenBank (Table 2). Four of the six valid species of *Leporellus* (according to our own results on the diversity investigation using DNA Barcode) were included in the analyses. *Leporellus* sp.n. (Aripuanã) and *L. sexdentatus* were not included due to lack of data available and tissues for sequencing. Likewise, the Outgroup taxa included representatives of all genera of Anostomidae, whose data is available in GenBank for at least two of the chosen genetic markers. Including taxa with only one of the markers (e.g., COI) showed inconsistent results. Outgroup taxa included one species of each of the following genera: *Abramites*, *Anostomus*, *Anostomoides*, *Gnathodolus*, *Hypomasticus*, *Laemolyta*, *Megaleporinus*, *Petulanos*, *Pseudanos*, *Rhytiodus*, and *Schizodon*. Three species of *Leporinus* (including its type species, *L. fasciatus*) were included, in order to better sample this species-rich, diverse and paraphyletic genus. *Insperanos*, *Sartor*, and *Synaptolaemus* were not included in the analyses because genetic data is not available for these genera yet. The tree was rooted in *Caenotropus mestomorgmatus*, a Chilodontidae.

Tab. 2. List of tissue vouchers used in the Phylogenetic relationships analyses.

Species	Tissue voucher	Accession number				
		COI	**Cytb**	**RAG1**	**RAG2**	**Myh6**
Abramites hypselonotus	UFRO-I 8234	KF568968	KF569011	KF569054	KF569097	KF569140
Anostomoides atrianalis	FMNH 123875	MT427906	MK214000	MW929620	MK208235	MK208226
Anostomus ternetzi	MZUSP 113996	KF568970	KF569013	KF569056	KF569099	KF569142
Gnathodolus bidens	AUM 53991		MK213997		MK208232	MK208229
Hypomasticus mormyrops	MZUEL 8022	KX020572	KX020575	KX020581	KX020584	KX020578
Laemolyta garmani	MUSM 47361	KP864686	KP864704	KP864710	KP864722	KP864716
***Leporellus* sp.n. (Paraguay)**	MZUSP 113987-L070	KF568980	KF569023	KF569066	KF569109	KF569152
Leporellus pictus	MZUEL 22701-1	unregistred	unregistred	unregistred	unregistred	
Leporellus timbore	MZUEL 16409	unregistred	unregistred	unregistred	unregistred	
Leporellus* aff. *vittatus	ANSP 182218-P6295	unregistred	unregistred	unregistred	unregistred	
Leporellus vittatus	AUM 54212-T09913	KX086795	KX086801	KX086972	KX086987	
Leporinus fasciatus	UFRO-I 3343	KF568981	KF569024	KF569067	KF569110	KF569153
Leporinus friderici	MZUSP 113983	KF568982	KF569025	KF569068	KF569111	KF569154
Leporinus striatus	MZUSP 113986	KF568995	KF569038	KF569081	KF569124	KF569167
Megaleporinus obtusidens	MCP 25476	KU134859	KU134877	KU134913	KU134931	KU134895
Petulanos plicatus	AUM 44717		MK214003		MK208240	MK208224
Pseudanos trimaculatus	UFRO-I 14970	KF569003	KF569046	KF569089	KF569132	KF569175
Rhytiodus microlepis	UFRO-I 18647	KF569004	KF569047	KF569090	KF569133	KF569176
Schizodon fasciatus	MZUSP 113989	KF569007	KF569050	KF569093	KF569136	KF569179
Caenotropus mestomorgmatos (Chilodontidae)	ANSP 180516	KF562412	KF562442	KF562490	KX086991	KF562466

Two distinct phylogenetic analyses were performed. A Maximum Likelihood analysis was performed in MEGA, using General Time Reversible G+I (Nei & Kumar, 2000), and bootstrap of 1,000 replica. In addition, a Bayesian Inference analysis was performed in Beast, v. 2.6.7 (Bouckaert *et al.*, 2014). For this, the best nucleotide evolution models for each marker was selected using bModelTest package (Bouckaert & Drummond, 2017). Trees and molecular clock was link for all markers, and Birth-Death prior and a relaxed Log-Normal clock were chosed. All remaining parameters were left in default. Tree was calibrated using a MRCA prior set to all terminal taxa, except *Caenotropus mestomorgmatus*, using a Log-Normal distribution, offset of 26.63, and Sigma of 1.03. This prior set was based on the fossil teeth of Anostomidae that is dated from with age estimated to range from 27.1 to 41.4 mya (considering 5 and 95%HP). This same prior was used in Sidlauskas *et al.* (2021). A second MRCA prior was set to all Anostomidae except *Leporellus* and the Anostominae, based on the fossil of the extinct *Leporinus scalabrinni*, estimated from 6 mya. The prior was set through a Log-Normal distribution, offset of 6.0 and Sigma of 1.56, given the date estimated to range from 6.21 to 41.4 (considering 5 and 95%HP).

Morphological investigation of cryptic species. The material was obtained from scientific collections and included alcohol-preserved specimens. Institutional acronyms follow Sabaj (2020). The identifications of specimens included in the morphometric and meristic analyses were based on the results based on DNA Barcode. The latter suggested the recognition of two distinct species within the Amazon and Orinoco basins, one in the West portion of the Amazon, and another in the East portion of the Amazon. Furthermore, specimens from the Araguaia, Tocantins, Xingu, Negro, Jari and Orinoco river basins were tentatively identified as *Leporellus vittatus*, whereas specimens from the upper Amazon river basin, and the Madeira river basin were identified as *Leporellus* aff. *vittatus* (excluding those from the Aripuanã river above the Dardanelos and Andorinhas falls, which belong to *L.* sp.n. (Aripuanã), and can be easily distinguished by the presence of 12 series of scales around the caudal peduncle). Morphometric data were taken according to Winterbottom (1980), except for using the snout tip instead of the tip of the lower jaw as the anteriormost site for measurements. Data were taken with aid of a digital caliper with 0,01 mm precision, and transferred to Past, v.4 (Hammer *et al.*, 2001), where analyzed as a varicance-covariance matrix. Data were logarithmized and used in a Principal Component Analysis, disregarding groups, and with graphic results showed with convex hulls plotted in PC2 vs. PC3, as PC1 is know to be influenced by body size. Morphometric data was also summarized in Table 3, where all measurements were included as mean, minimum and maximum values. Number of samples and standard deviation was calculated to show sample size and variation. Meristic data was taken according to Birindelli & Britski (2013) and showed in Table 4, where counts were included as mode, minimum and maximum. Also in this case, number of samples was included to show sample size and variation. Lateral-line scale counts included pored scales extending onto the base of median caudal-fin rays; counts of longitudinal scale rows above the lateral line exclude the lateral-line scale row and the middorsal scale row; and counts of longitudinal scale rows below the lateral line exclude the lateral-line scale row. Last dorsal and anal fin rays branched at base and counted as a single element.

Results

Diversity of species of *Leporellus*. A total of 748 nucleotide base pairs of the COI gene were obtained for the 32 samples (when aligned), which included 119 variable and 88 parsimony-informative sites. The best model for nucleotide evolution obtained was K2+G, which was applied into the genetic distance estimative and the phylogenetic analysis. The overall mean genetic distance was calculated as 5.29% (±0.64) for all samples. The genetic distances (DNA Barcode) varied from 0,41% between specimens of *Leporellus pictus* from the Paranapanema and the Mogi-Guaçu rivers (both localities inside the upper Paraná basin), and 11.91% between *L. pictus* (samples from Mogi-Guaçu), and *L. sexdentatus* (Magdalena basin) (Table 3). The closest species, with genetic distance of 3.25% were *L. pictus* (upper Paraná) and *L. timbore* (São Francisco) (Fig. 2). On the other hand, genetic distances within each sample ranged from 2.16% in *L. vittatus* to 0.00% in *Leporellus* sp.n. (Paraguay). Most divergent species was *L. sexdentatus*, with genetic distances ranging between 9.98% (*L.* sp.n. Paraguay) and 11.91% *(L. pictus* Mogi-Guaçu).

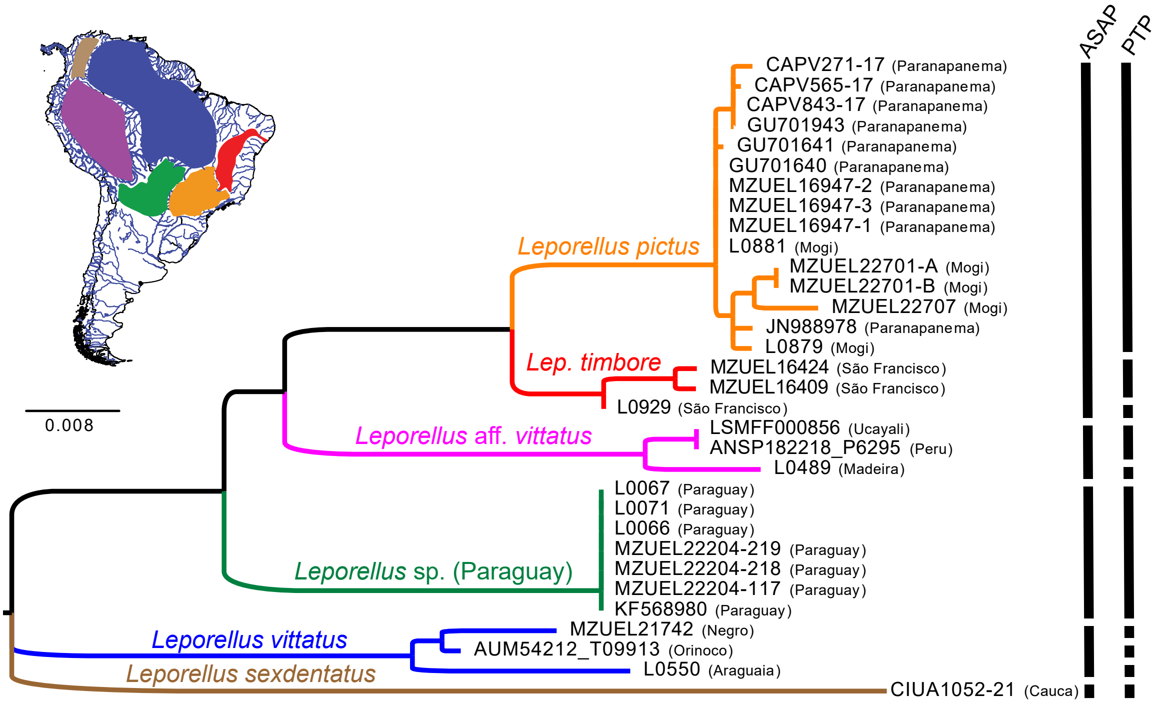

Fig. 2. Evolutionary divergence of species of *Leporellus* based on DNA Barcode (COI) derived from a Maximum Likelihood (ML) analysis using HKY G+I model. In the right side, results of two analysis of species delimitation: ASAP (based on the sequence matrix), and PTP (based on the ML tree). *Leporellus* sp.n. (Aripuanã) was not included due to lack of available tissue and genetic sequence.

The two species delimitation analyses had slightly distinct results (Fig. 2). ASAP score was 1.50 and results in the recognition of five Molecular Taxonomic Units (MOTUs), including *Leporellus pictus* and *L. timbore* in the same MOTU, whereas all other species was recognized as distinct. PTP recognized 10 MOTUs, with each specimen of *L. vittatus* recognized as a distinct MOTU. In addition, one specimen of each *L.* aff. *vittatus* and of *L. timbore* were recognized in their own MOTUs. Even though, no sample of *Leporellus* sp.n. (Aripuanã) was available for genetic study, the species was recognized as valid and distinct from congeners based on the unique presence of fewer rows of scales on the body (Tab. 3), confirming the diagnosis of the species (Narezzi & Birindelli, in press).

Tab. 3. Genetic distances (%) between (below diagonal, with standard error estimates above diagonal) and within (diagonal in bold) samples of *Leporellus*, including all species recognizd in this study, except for *L.* sp.n. Aripuanã, due to lack of tissue samples and sequences.

	1	**2**	**3**	**4**	**5**	**6**	**7**
1. ***L. pictus*** (Paranapanema, n=10)	**0,11**	0,20	0,80	1,21	1,35	1,27	1,91
2. ***L. pictus* (**Mogi-Guaçu n=5)	0,41	**0,49**	0,80	1,25	1,40	1,37	1,97
3. ***L. timbore*** (São Francisco, n=3)	3,25	3,39	**0,65**	1,19	1,52	1,24	1,94
4. ***L.* sp.n. (Paraguay)** (Paraguay, n=7)	6,54	6,83	6,42	**0,00**	1,32	1,22	1,74
5. ***L. vittatus*** (East Amazon/Orinoco, n=3)	8,14	8,65	9,49	7,93	**2,16**	1,51	1,65
6. ***L.* aff. *vittatus*** (West Amazon, n=3)	7,22	7,88	6,87	6,93	10,32	**0,99**	1,83
7. ***L. sexdentatus* (**Magdalena, n=1)	11,28	11,91	11,83	9,98	10,06	11,37	**n/c**

Phylogenetic relationships and speciation dating. In the concatenate matrix, a total of 5.045 nucleotide base pair (bp) were obtained for the 20 terminal taxa. Of these, a total of 748 bp was from COI, which included 248 variable and 208 parsimony-informative sites for 19 taxa; 1,005 bp from CytB, including 444 variable and 362 parsimony-informative sites for 20 taxa; 772 bp from Myh6, including 108 variable and 43 parsimony-informative sites for 16 taxa; 1,483 bp from RAG1, including 265 variable and 117 parsimony-informative sites for 18 taxa; and 1,037 bp from RAG2, including 835 variable and 99 parsimony-informative sites for 20 taxa.

The Maximum likelihood analyses based on HKY G+I model (the best model according to a search on MEGA) corroborated the monophyly of Anostomidae (Fig. 3), as well as the monophyly its three subfamilies: Anostominae, Leporelinae, and Leporininae. The analysis recovered Anostominae and Leporellinae as sister, and *Leporinus fasciatus* and *L. friderici* closer to the *Anostomoides* clade, than to the *Megaleporinus* clade. The Bayesian Inference analysis also recovered the monophyly of the Anostomidae and its three subfamilies. However, it considered Leporellinae as closer to Leporininae, than to Anostomidae. Similarly, it recovered *Leporinus fasciatus* and *L. friderici* as sister to the *Megaleporinus* clade, instead of to the *Anostomoides* clade.

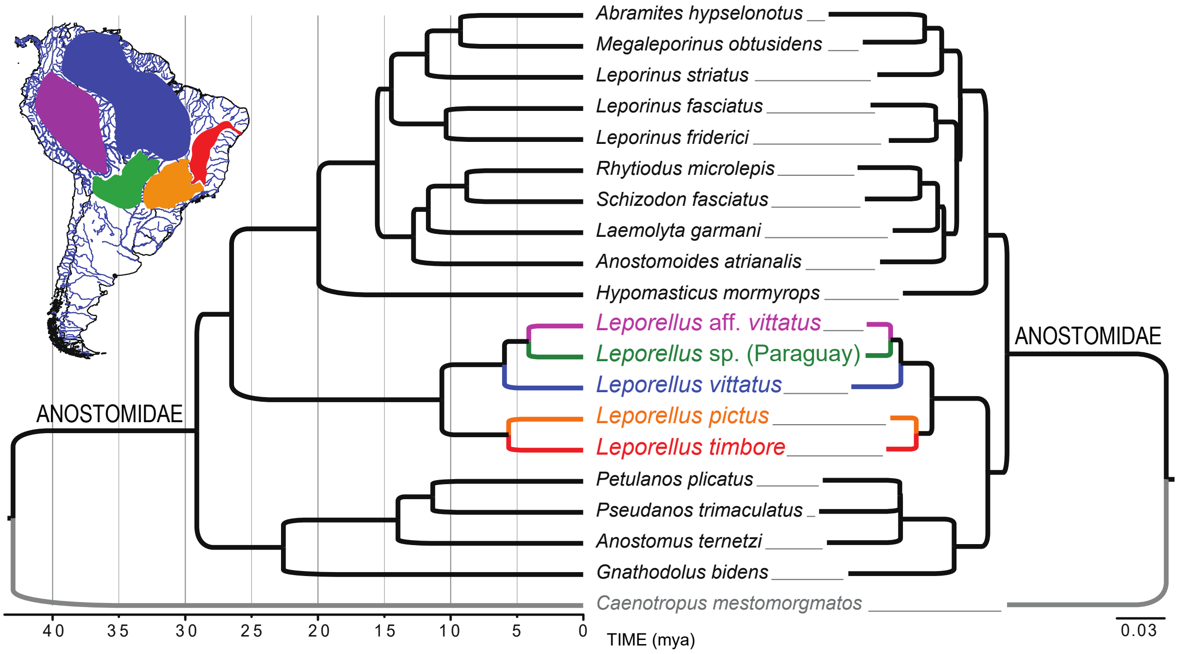

Fig. 3. Cladogram of phylogenetic relationships between species of *Leporellus*, and outgroup including most genera of Anostomidae, plus one species of Chilodontidae (*Caenotropus mestomorgmatus*) using two mitochondrial (COI, CytB), and three nuclear (RAG1, RAG2, Myh6) markers. *Leporellus* sp.n. (Aripuanã) and *L. sexdentatus* were not included due to lack of available tissue and genetic sequence. Dated Bayesian Inference tree on the right side, and Maximum Likelihood tree on left side.

Both analyses recovered the same relationships between species of *Leporellus*, with *L. pictus* and *L. timbore* as sister, and *L. vittatus*, *L.* sp.n. (Paraguay), and *L.* aff. *vittatus* forming another clade. In addition, Anostomidae was recognized as having approximately 29 million years, and *Leporellus* as around 26 million years. Nevertheless, the diversification of species of *Leporellus* was estimated to have happen between 11 and 4 million years ago.

Morphological variation in species of *Leporellus*. Meristic data corroborated recent findings that resulted in the description of *Leporellus* sp.n. (Aripuanã) by Narezzi & Birindelli (in press). The latter has only three or four scale rows between lateral line and dorsal-fin and anal-fin origins, and just 12 scale rows around caudal peduncle, whereas all other congener has five or six between lateral line and dorsal-fin and anal-fin origins, and 16 scale rows around caudal peduncle (Tabs. 4, 5). All other meristic data are virtually identical in all species of *Leporellus* recognized in this study (Tabs. 4, 5). Similarly, all 19 morphometric variables greatly overlapped in the species of *Leporellus* (Tabs. 6, 7). The Principal Component Analysis resulted in a large overlapping morphospace that encompass all species of *Leporellus* (Fig. 4). PC1 explained 97.671% of all variation in our data, whereas PC2 explained 0.738% and PC3 0.379%. Remaining components explained less than 3% each and 1.42% in total (Tab. 7). The morphometric variables that best explained the variation in PC2 were: snout length, head length, dorsal-fin to adipose-fin origin, and caudal peduncle length (Tab. 8). In PC3, the morphometric variables that best explained the variation were: caudal peduncle length, and body width at dorsal-fin origin.

Tab. 4. Meristic data for all species of *Leporellus* recognized in this study.

	Leporellus vittatus				***Leporellus pictus***				***Leporellus timbore***			
	N	**Mode**	**Min**	**Max**	**N**	**Mode**	**Min**	**Max**	**N**	**Mode**	**Min**	**Max**
Lateral-line scales	29	43	43	44	117	43	42	44	9	43	42	43
Scale rows around caudal peduncle	29	16	16	16	117	16	16	16	9	16	16	16
Scale rows above lateral line	29	6	6	6	117	6	5	6	9	6	6	6
Scale rows below lateral line	29	5	5	5	116	5	5	5	9	5	5	5
Predorsal scales	29	12	11	13	115	12	11	14	9	12	12	14
Dorsal-fin unbranched rays	29	2	2	2	117	2	2	2	9	2	2	2
Dorsal-fin branched rays	29	10	10	10	117	10	10	10	9	10	10	10
Pectoral-fin branched rays	29	16	16	16	117	16	16	16	9	16	16	16
Pelvic-fin branched rays	29	9	9	9	117	9	9	9	9	9	9	9
Anal-fin unbranched rays	29	3	3	3	117	3	3	3	9	3	3	3
Anal-fin branched rays	29	9	9	9	117	9	9	9	9	9	9	9
Upper caudal-fin branched rays	29	9	9	9	117	9	9	9	9	9	9	9
Lower caudal-fin branched rays	29	8	8	8	117	8	8	8	9	8	8	8

Tab. 5. Meristic data for all species of *Leporellus* recognized in this study.

	***Leporellus* sp.n. (Aripuanã)**				***Leporellus* sp.n. (Paraguay)**				***Leporellus* aff. *vittatus***				***Leporellus sexdentatus***			
	N	**Mode**	**Min**	**Max**	**N**	**Mode**	**Min**	**Max**	**N**	**Mode**	**Min**	**Max**	**N**	**Mode**	**Min**	**Max**
Lateral-line scales	9	42	42	43	37	43	42	44	2	#N/A	43	44	3	#N/A	43	45
Scale rows around caudal peduncle	**9**	**12**	**12**	**12**	38	16	16	16	2	16	16	16	3	16	16	16
Scale rows above lateral line	**9**	**4**	**4**	**4**	38	6	6	6	2	6	6	6	3	5	5	6
Scale rows below lateral line	**9**	**4**	**3**	**4**	38	5	5	5	2	5	5	5	3	5	4	5
Predorsal scales	9	11	10	12	38	12	10	15	2	#N/A	12	13	3	12	12	13
Dorsal-fin unbranched rays	9	2	2	2	38	2	2	2	2	2	2	2	3	2	2	2
Dorsal-fin branched rays	9	10	10	10	38	10	10	10	2	10	10	10	3	10	10	10
Pectoral-fin branched rays	9	15	15	15	38	16	15	16	2	16	16	16	3	14	14	14
Pelvic-fin branched rays	9	9	8	9	38	9	8	9	2	9	9	9	3	8	8	8
Anal-fin unbranched rays	9	3	3	3	38	3	3	3	2	3	3	3	3	3	3	3
Anal-fin branched rays	9	9	8	9	38	9	9	9	2	9	9	9	3	9	9	9
Upper caudal-fin branched rays	9	9	9	9	38	9	9	9	2	9	9	9	3	9	9	9
Lower caudal-fin branched rays	9	8	8	8	38	8	8	8	2	8	8	8	3	8	8	8

Tab. 6. Morphometric data for *Leporellus vittatus*, *L. pictus*, and *L. timbore*.

	Leporellus vittatus					***Leporellus pictus***					***Leporellus timbore***				
	N	**Mean**	**Min**	**Max**	**SD**	**N**	**Mean**	**Min**	**Max**	**SD**	**N**	**Mean**	**Min**	**Max**	**SD**
Standard length	29	116,39	28,10	238,70	48,97	117	127,97	30,13	247,74	39,97	9	111,13	28,57	206,65	69,04
Percentages of Standard Length															
Snout tip to anal-fin origin	29	71,34	67,51	74,92	1,42	117	71,56	66,90	81,30	2,07	9	72,19	69,41	75,35	1,77
Snout tip to adipose-fin origin	29	81,75	79,33	84,45	1,03	117	81,61	77,43	86,87	1,66	9	81,37	79,31	83,58	1,42
Snout tip to dorsal-fin origin	29	44,36	42,21	47,30	1,06	117	43,54	39,86	47,20	1,43	9	44,67	42,34	48,79	1,93
Snout tip to pelvic-fin origin	29	49,06	44,86	52,78	1,54	116	48,17	44,63	73,05	2,87	9	49,37	45,71	53,66	2,40
Dorsal-fin origin to caudal-fin origin	29	56,56	52,41	59,56	1,32	117	56,98	42,93	93,43	4,57	9	56,04	35,86	57,85	1,67
Dorsal-fin origin to adipose-fin origin	29	38,32	35,20	42,43	1,39	117	39,31	35,42	81,14	4,16	9	37,73	35,86	40,73	1,63
Body depth at dorsal-fin origin	29	23,44	21,29	27,76	1,64	116	23,84	19,18	27,79	1,66	9	23,08	20,69	24,97	1,40
Body width at dorsal-fin origin	29	14,10	9,89	17,55	1,66	117	13,82	8,99	18,08	1,74	9	13,41	10,47	16,27	1,61
Caudal peduncle length	29	18,84	16,07	21,21	1,24	117	19,29	14,01	24,82	1,73	9	18,20	15,17	22,15	2,25
Caudal peduncle depth	29	11,19	10,36	12,07	0,43	117	10,92	9,81	12,09	0,40	9	10,67	10,05	11,69	0,55
Head length	29	25,59	21,12	30,00	1,92	117	23,23	20,41	28,29	1,41	9	25,53	23,05	28,64	1,77
Percentages of Head Length															
Head depth	29	82,48	72,20	97,20	5,77	117	84,82	70,35	99,71	5,86	9	77,70	69,78	83,00	4,87
Preopercle length	29	76,05	72,24	78,61	1,53	117	76,82	72,95	83,16	2,00	9	77,00	74,82	79,28	1,31
Preopercle depth	29	73,84	64,95	83,68	4,27	117	75,69	65,07	89,27	4,26	9	71,68	65,21	74,75	3,26
Snout length	29	37,84	25,03	43,12	3,52	117	37,58	31,84	64,88	4,01	9	37,53	32,31	44,50	4,16
Snout depth	29	52,79	41,40	62,20	4,30	117	54,95	42,54	64,88	4,01	9	50,94	40,17	57,78	5,98
Eye diameter	29	24,58	19,15	36,06	3,36	117	24,29	17,85	30,98	2,70	9	25,67	18,76	32,43	5,41
Bony interorbital	29	37,84	22,61	44,17	4,80	117	40,23	34,66	47,00	2,36	9	38,92	35,57	44,65	3,20

Tab. 7. Morphometric data for *Leporellus* sp.n. (Aripuanã), *Leporellus* sp.n. (Paraguay), *L.* aff. *vittatus*, and *L. sexdentatus*.

	***Leporellus* sp.n. (Aripuanã)**					***Leporellus* sp.n. (Paraguay)**					***Leporellus* aff. *vittatus***					***Leporellus sexdentatus***				
	N	**Mean**	**Min**	**Max**	**SD**	**N**	**Mean**	**Min**	**Max**	**SD**	**N**	**Mean**	**Min**	**Max**	**SD**	**N**	**Mean**	**Min**	**Max**	**SD**
Standard length	9	115,67	85,62	182,30	30,69	38	110,90	61,23	173,20	23,92	2	151,09	138,67	163,50	17,56	3	166,00	128,00	206,00	39,04
Percentages of Standard Length																				
Snout tip to anal-fin origin	9	72,69	71,51	73,72	0,77	38	71,25	68,11	76,13	1,79	2	71,51	71,23	71,78	0,39	3	72,81	70,73	74,27	1,85
Snout tip to adipose-fin origin	9	81,04	79,61	83,16	1,15	38	80,82	78,34	86,24	1,48	2	81,37	81,08	81,66	0,41	3	82,58	80,79	83,59	1,55
Snout tip to dorsal-fin origin	9	45,28	41,77	47,13	1,63	38	43,28	40,43	46,63	1,42	2	43,07	42,28	43,85	1,11	3	45,47	43,05	48,06	2,51
Snout tip to pelvic-fin origin	9	48,97	45,12	52,11	2,26	38	47,67	45,19	51,82	1,78	2	46,18	45,80	46,56	0,54	3	48,50	47,07	48,44	1,46
Dorsal-fin origin to caudal-fin origin	9	55,98	52,27	60,45	2,25	38	57,16	48,46	60,89	2,41	2	58,49	58,13	58,85	0,51	3	55,92	54,37	57,13	1,41
Dorsal-fin origin to adipose-fin origin	9	37,13	33,74	40,90	2,13	38	38,18	32,68	41,98	1,92	2	39,61	38,70	40,52	1,29	3	39,10	38,19	39,84	0,41
Body depth at dorsal-fin origin	9	23,26	22,10	24,54	0,90	38	23,57	19,56	26,60	1,88	2	23,75	22,85	24,65	1,27	3	26,38	25,00	27,44	1,25
Body width at dorsal-fin origin	9	13,61	12,87	14,45	0,55	38	13,59	11,24	15,58	1,03	2	14,13	13,76	14,51	0,53	0	-	-	-	-
Caudal peduncle length	9	18,26	16,27	20,03	1,32	38	19,77	13,72	22,64	1,85	2	19,53	19,01	20,05	0,74	3	17,64	16,41	19,27	1,47
Caudal peduncle depth	9	9,61	9,08	10,18	0,36	38	10,34	9,37	11,19	0,39	2	10,52	10,31	10,73	0,30	3	11,53	11,36	11,72	0,18
Head length	9	26,08	24,09	28,37	1,64	38	24,35	21,56	29,41	1,59	2	22,12	20,67	23,58	2,06	3	23,59	21,88	25,73	1,96
Percentages of Head Length																				
Head depth	9	80,43	73,26	88,42	5,19	37	81,12	65,80	96,28	7,53	2	94,11	90,56	97,66	5,02	3	83,50	79,25	85,71	3,68
Preopercle length	9	75,72	72,86	78,16	1,46	38	75,93	73,34	79,46	1,64	2	75,80	74,63	76,97	1,66	3	72,90	71,43	73,68	1,59

Preopercle depth	9	72,56	69,59	76,40	2,45	38	72,69	61,39	82,03	4,79	2	82,98	82,54	83,43	0,63	3	72,67	71,32	75,00	2,03
Snout length	9	38,21	35,43	43,52	2,71	38	37,53	32,94	45,16	2,68	2	36,38	35,90	36,86	0,68	3	36,64	34,21	40,00	3,00
Snout depth	9	51,78	46,68	57,40	3,63	38	51,37	40,73	59,85	4,04	2	57,40	56,56	58,24	1,19	3	49,11	44,29	56,60	6,58
Eye diameter	9	23,86	18,95	26,64	2,61	38	23,68	19,27	29,76	2,30	2	25,51	23,94	27,08	2,22	3	19,53	17,17	21,43	2,17
Bony interorbital	9	36,82	30,10	39,99	2,89	38	37,65	31,66	43,48	2,81	2	45,28	44,05	46,51	1,74	0	-	-	-	-

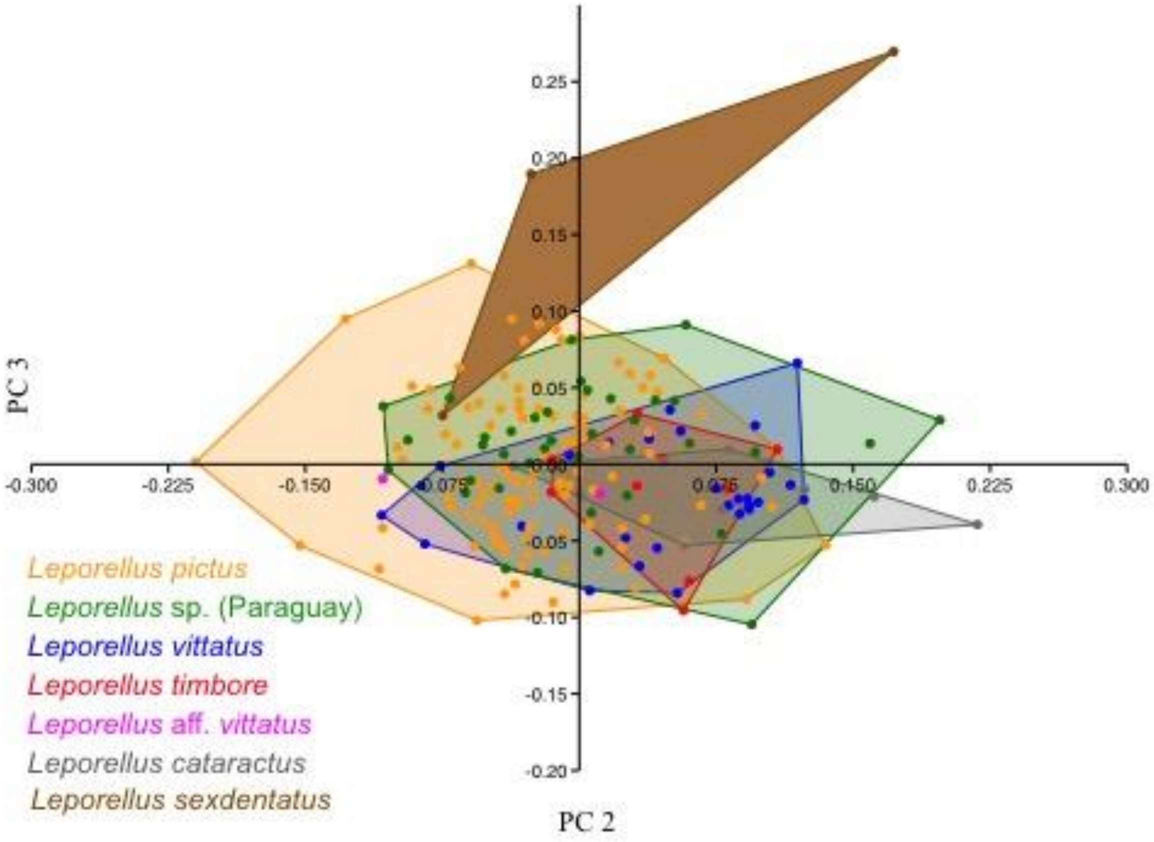

Fig. 4. Principal Component Analysis of 19 morphometric variables assessed for species of *Leporellus*, showing a great overlap in the body shape of the species.

Tab. 8. Eigenvalues and % of variance for each of the principal components in the PCA.

PC	Eigenvalue	% variance
1	0,547	97,459
2	0,004	0,755
3	0,002	0,434
4	0,002	0,312
5	0,001	0,261
6	0,001	0,184
7	0,001	0,141
8	0,001	0,131
9	0,000	0,082
10	0,000	0,060
11	0,000	0,042
12	0,000	0,036
13	0,000	0,034
14	0,000	0,027
15	8,51E-05	0,015
16	5,65E-05	0,010
17	5,10E-05	0,009
18	2,69E-05	0,005
19	1,90E-05	0,003

Tab. 9. Values for each of the morphometric variable in the Principal Components 2 and 3.

Measurements	PC 2	PC 3
Standard length	-0,066	0,106
Snout tip to anal-fin origin	-0,006	0,059
Snout tip to adipose-fin origin	-0,056	0,072
Snout tip to dorsal-fin origin	0,048	0,082
Snout tip to pelvic-fin origin	0,059	0,035
Dorsal-fin origin to caudal-fin origin	-0,253	0,193
Dorsal-fin origin to adipose-fin origin	-0,304	0,114
Caudal peduncle length	-0,201	0,031
Head length	-0,298	-0,716
Preopercle length	-0,289	0,472
Snout length	-0,141	0,066
Body depth at dorsal-fin origin	0,335	0,050
Caudal peduncle depth	0,008	-0,081
Head depth	0,352	0,059
Preopercle depth	0,126	-0,056
Snout depth	0,545	-0,008
Body width at dorsal-fin origin	0,178	-0,092
Eye diameter	0,132	0,079
Bony interorbital	-0,018	-0,388

DISCUSSION

The genetic divergence in the DNA Barcode for species of *Leporellus* is relatively larger than those found in other studies on Anostomidae. Studying species of *Laemolyta*, Ramirez & Galetti (2015) found distances ranging from 0.92% to 5.59% between species. Similar results were obtained between species of *Schizodon* (Ramirez *et al.*, 2020), with sister species with as low as 1.0% divergence (between *S. intermedius* and *S. borelli*). On the other hand, similar results to those obtained herein for *Leporellus* was found between species of *Hypomasticus* (Birindelli *et al.*, 2020a), with closely related species diverging 7.6% (*H. despaxi* and *H. megalepis*). Similar to *Hypomasticus, Leporellus* is an old lineage of Anostomidae and species diversification in such cases could havee happened much earlier than that in more recent lineages such as in *Schizodon* or *Laemolyta*. In any case, the DNA Barcode data, either though the genetic divergence estimates, or though the species delimitation analyses, are strong evidence for the recognition of at least six distinct species within *Leporellus*.

Furthermore, the genetic distances within two samples (or MOTUs) are relatively large, as is the case of *Leporellus vittatus* and *L.* aff. *vittatus* (2,16% and 0,99% respectively). The two species are recognized herein as distinct, although only one has a nominal species name. Both had widespread distribution in the tropical South America, and only three samples were obtained from each of those species (or MOTUs). Based on our results, there is little doubt that these represent distinct species. However, the complexity and wideness of the Amazon and Orinoco basins makes one wonder how many species could be recognized if samples were large in this area. Further investigations, including more samples and better geographical coverage is still necessary for a better understanding of the diversity of *Leporellus* in the Amazon, and Orinoco basins. Here, we are tentatively identified the eastern species using Valencinnes´s name *Leporellus vittatus*, which was based on two specimens collected by Deville and Castelnau in 1847 and with the poorly-precise location of L´Amazone´. One of the specimens is lost (Fricke *et al.*, 2023). Devile and Castelnau traveled in the Amazon basin from the upper Ucayali river at Urubamba, Peru, in May of 1846, to the Marañon river in Nauta, Peru, where they arrived in November 1846; from there they went downstream on the Marañon (Solimões/Amazonas) river entering Brazil in January of 1847; and reaching Belém in March 1847 (Papavero, 1971). Therefore, the type of *Leporellus vittatus* could be both of the species herein recognized for the Amazon basin, but more likely the eastern species, as Devile and Castelnau spend more time in the eastern portion of the Amazon basin in 1847. Ancient DNA analysis of the type specimen could help to solve this puzzle.

The long and complex taxonomic history encompassing the species of *Leporellus* is at least partially a reflection of the outstanding similarity and lack of distinguishing features between species. The morphometric and meristic analyses evidenced that five of seven species of *Leporellus* are externally identical, although bearing a significant genetic variation. In fact, the species of *Leporellus* are independently evolving lineages for millions of years. *Leporellus* sp.n. (Paraguay) and *L.* aff. *vittatus* are separately by around four millions years, whereas the clade composed of *L. pictus* and *L. timbore* is separated from the clade including *L. vittatus*, *L.* aff. *vittatus*, and *Leporellus* sp.n. (Paraguay) by slightly more than 10 million years. And yet three of these four species are completely indistinguishable based on external morphological features, being perfect examples of cryptic species. That term is used for two or more species that were erroneously identified as a single one due to paucity of morphological differences (Hebert *et al.*, 2004). The discovery of cryptic species improves our understanding of Biodiversity, and has implications on Conservation (Bickford *et al.*, 2007). The advances of Molecular Systematics have allowed us to detect cryptic species (Jörger & Schördl, 2013), and to improve our knowledge on Biodiversity.

The age for diversification of species of *Leporellus* is consistent with their widespread geographical distribution, as well as to the presence of one species (*L. sexdentatus*) in the Magdalena river basin. The latter drainage is a transandine river system that was isolated from the Amazon and Orinoco basins at approximately 10 mya (Lundberg, 1998; Hoorn *et al.*, 2010). There are only two other species of Anostomidae occurring in Magdalena river basin: *Abramites hypselonotus*, and *Megaleporinus muyscorum*. The age of diversification of these two genera were estimated of occurring also approximately at 10 mya (Ramirez *et al.*, 2016). Unfortunately, genetic sequences of *L. sexdentatus* were not available and the species could not be included in the phylogenetic analyses preformed herein.

According to our hypothesis (Fig. 3), the first speciation of species of *Leporellus*, occurred between two clade, first with two species that inhabit the upper Paraná and the São Francisco basins, and another with three species distributed in the Amazon, Orinoco, and Paraguay basins. The upper Paraná is considered a biogeographic unit (Géry, 1969; Langeani *et al.*, 2007; Abell *et al.*, 2008), that is isolated from the lower Paraná by Sete Quedas, a complex of falls that was submerged by the construction of the Itaipu dam in the 1970s. Past riverine connections are known to have occurred between the headwaters of the upper Paraná and the São Francisco multiples times in recent times (Britski *et al.*, 1988; Britto & Castro, 2002; Vilardo *et al.*, 2023), and explain the sister group relationships *Leporellus pictus* and *L. timbore*.

In the other branch of the tree lie *Leporellus vittatus*, and a clade composed of *L.* aff. *vittatus* and *Leporellus* sp.n. (Paraguay). The split that led to the base of this tree branch is associated to the formation of the Pebas Lake, around 12 to 18 million years ago (Hoorn *et al.*, 2010). By that time, the western portion of South America drained a low land area surrounding by the Andes in the west, and the high land of the South American crystalline shield in the east (Lundberg, 1998; Hoorn, *et al.*, 2010). In fact, during the middle Miocene (around 15 mya), the eastern limit of the Pebas Lake was formed by the Purus Arch, until its break up in the late Miocene led to the reconfiguration of the system and the course change of the Amazon river (Albert *et al.*, 2018). The sister-group relationship between *Leporellus* sp.n. (Paraguay) and *L.* aff. *vittatus* is likely due to recent past riverine connections between the Paraguay and the Madeira basins (Assine *et al.*, 2015). Both of these basins mainly occupy the low land areas adjacent to the Andes. The connections between both drainages were responsible for the dispersal of several lineages of fish from the West Amazon to the Paraguay basin, especially in the last 5 million years (Roxo *et al.*, 2014; Fontenelle *et al.*, 2021; Melo *et al.*, 2021).

The combination of distinct sources of data (morphometrics, meristics, coloration, and molecular data) was imperative for the recognition of seven distinct species of *Leporellus*, and also to understand that five of them were hidden over a similar external appearance. Understanding this diversity allowed us to propose new hypotheses on the species phylogeny, and use this to study their biogeography. Although this was a step forward in our knowledge, further investigations are still necessary to fully comprehend the diversity and evolution of the species of *Leporellus* in the Amazon basin.

MATERIAL EXAMINED

***Leporellus* sp.n. (Aripuanã): (upper Aripuanã river):** MZUSP 101048 (1 alc) 136.5 mm SL (holotype), INPA 11003 (1 alc) 115.8 mm SL, INPA 50878 (2 alc) 84.1-86.6 mm SL, MZUEL ??? [ex. MZUSP 100499] (1 alc) 133.0 mm SL, MZUSP ??? [ex. MZUSP 101048] (1 alc) 177.3 mm SL, MZUSP 100499 (2 alc) 92.4-93.3 mm SL, MZUSP 100517 (1 alc) 103.0 mm SL, MZUSP 101061 (1 alc) 97.6 mm SL.

***Leporellus pictus*: upper Paraná basin:** FMNH 55714 (1 alc) 210.0 mm SL (holotype of *Leporinodus retropinnis* Eigenmann), MZUEL 386 (17 alc) 110.4- 176.1 mm SL, MZUEL 387 (1 alc) 224.9 mm SL, MZUEL 388 (1 alc) 167.9 mm SL, MZUEL 401 (2 alc) 31.9-39.6 mm SL, MZUEL 657 (3 alc) 30.1-36.8 mm SL, MZUEL 1381 (1 alc) 98.5 mm SL, MZUEL 1910 (1 alc) 107.7 mm SL, MZUEL 3317 (4 alc) 123.4-170.4 mm SL, MZUEL 4694 (1 alc) 150.1 mm SL, MZUEL 5353 (3 alc) 163.5-200.5 mm SL, MZUEL 8815 (1 alc) 68.1 mm SL, MZUEL 9505 (1 alc) 115.7 mm SL, MZUEL 15107 (1 alc) 60.0 mm SL, MZUEL 16410 (1 alc) 181.8 mm SL, MZUEL 16411 (7 alc) 135.2- 180.6 mm SL, MZUSP 1649 (2 alc) 106.1-106.5 mm SL, MZUSP 20690 (11 alc) 88.7- 177.5 mm SL, MZUSP 20701 (6 alc) 108.7- 205.9 mm SL, MZUSP 20731 (1 alc) 132.5 mm SL, MZUSP 20743 (2 alc) 97.5-113.9 mm SL, MZUSP 20751 (5 alc) 89.5- 125.7 mm SL, MZUSP 21623 (2 alc) 126.7-133.7 mm SL, MZUSP 35681 (1 alc) 169.4 mm SL, MZUSP 42899 (1 alc) 148.9 mm SL, MZUSP 42900 (1 alc) 45.0 mm SL, MZUSP 42902 (5 alc) 110.4-115.9 mm SL, MZUSP 42903 (1 alc) 148.4 mm SL, MZUSP 42904 (1 alc) 151.9 mm SL, MZUSP 42905 (1 alc) 105.7 mm SL, MZUSP 42906 (1 alc) 105.4 mm SL, MZUSP 42907 (1 alc) 131.3 mm SL, MZUSP 42909 (1 alc) 117.3

mm SL, MZUSP 42911 (1 alc) 117.9 mm SL, MZUSP 42913 (2 alc) 122.4-124.1 mm SL, MZUSP 42916 (1 alc) 135.4 mm SL, MZUSP 42917 (1 alc) 145.7 mm SL, MZUSP 42918 (1 alc) 247.7 mm SL, MZUSP 42921 (1 alc) 209.5 mm SL, MZUSP 51458 (2 alc) 129.0-217.8 mm SL, MZUSP 58408 (2 alc) 75.6-98.6 mm SL, MZUSP 63676 (6 alc) 78.0-97.5 mm SL, MZUSP 108082 (1 alc) 179.4 mm SL, MZUSP 110218 (2 alc) 135.5-136.3 mm SL, NMW 81315 (1 alc) 151.4 mm SL (holotype of *Leporinus pictus* Kner), NUP 1902 (1 alc) 149.7 mm SL, NUP 1967 (1 alc) 142.3 mm SL, NUP 2675 (1 alc) 99.4 mm SL, NUP 4211 (1 alc) 154.0 mm SL, NUP 8868 (1 alc) 121.5 mm SL, NUP 9178 (2 alc) 138.2-153.5 mm SL, NUP 9201 (1 alc) 156.9 mm SL.

***Leporellus sexdentatus*: Magdalena basin:** ANSP 128896 (2 alc) 164.0-206.0 mm SL, MCZ 35804 (1 alc) 128.0 mm SL. Type specimen of *Leporellus sexdentatus* not found at NMW (Fricke *et al.*, 2023).

***Leporellus timbore*: São Francisco basin:** ANSP 69522 (1 alc) 71.4 mm SL (holotype of *Leporellus cartledgei* Fowler), FMNH 55175 (1 alc) 60.0 mm SL (holotype *Leporellus timbore* Eigenmann), MZUEL 16409 (1 alc) 142.6 mm SL, MZUEL 16424 (1 alc) 206.7 mm SL, MZUSP 050739 (1 alc) 182.7 mm SL, MZUSP 054658 (1 alc) 101.3 mm SL, MZUSP 073734 (1 alc) 177.5 mm SL, MZUSP 074742 (1 alc) 28.6 mm SL, MZUSP 094649 (1 alc) 40.6 mm SL, MZUSP 094676 (1 alc) 33.6 mm SL, MZUSP 098749 (1 alc) 86.8 mm SL.

***Leporellus vittatus*: Eastern Amazon and Orinoco (including Xingu, Araguaia, Jari, Negro, and Orinoco):** IEPA 3200 (1 alc), INPA 14047 (1 alc), INPA 32478 (1 alc), INPA 45190 (1 alc), MCP 14401 (1 alc), MCP 40330 (1 alc), MCP 42675 (1 alc), MCP 43675 (1 alc), MNHN A9802 (1 alc) 165.0 mm SL (holotype of *Leporinus vittatus* Valenciennes), MCZ 19389 (5 alc), MZUSP 4989 (1 alc) 28.9 mm SL, MZUSP 40930 (1 alc) 98.4 mm SL, MZUSP 58225 (1 alc) 106.7 mm SL, MZUSP 84018 (1 alc) 74.7 mm SL, MZUSP 89023 (1 alc) 148.5 mm SL, MZUSP 89024 (1 alc) 112.2 mm SL, MZUSP 89807 (5 alc) 86.5-125.2 mm SL, MZUSP 91910 (2 alc) 172.2-202.2 mm SL, MZUSP 94872 (2 alc) 60.0-206.6 mm SL, MZUSP 95537 (7 alc) 86.0-163.5 mm SL, MZUSP 98136 (3 alc) 76.2-130.6 mm SL, MZUSP 103465 (1 alc) 139.8 mm SL, MZUSP 104476 (2 alc), MZUSP 106039 (1 alc) 158.6 mm SL, NUP 8391 (1 alc) 238.7 mm SL.

Leporellus **aff.** ***vittatus*****: Western Amazon (including Madeira, and Ucayali):** MZUSP 104476 (2 alc) 138.7- 163.5 mm SL.

Leporellus **sp.n. (Paraguay): Paraguay basin:** NUP ???, 125.1 mm SL (holotype), MZUEL 12263 (2 alc) 79.5-85.5 mm SL, MZUEL 13998 (2 alc) 68.7-79.6 mm SL, MZUEL 22204 (2 alc) 17.1-33.8 mm SL, MZUEL 23055 (1 alc) 80.3 mm SL, MZUEL 23228 (2 alc) 119.1-127.8 mm SL (1 CS), 110.8 mm SL, MZUSP 59672 (1 alc) 61.2 mm SL, MZUSP 78788 (2 alc) 109.7-115.6 mm SL, MZUSP 78872 (1 alc) 108.9 mm SL, MZUSP 89972 (1 alc) 115.0 mm SL, MZUSP 99271 (3 alc) 99.0-117.7 mm SL, MZUSP 113987 (1 alc) 92.8 mm SL, NUP 2164 (7 alc) 103.6-128.3 mm SL, NUP 3364 (3 alc) 77.5-144.1 mm SL, NUP 7834 (2 alc) 91.6-173.2 mm SL, NUP 9422 (1 alc) 125.6 mm SL, NUP 9423 (1 alc) 102.7 mm SL, NUP 9424 (1 alc) 66.7 mm SL.

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CONCLUSÕES GERAIS

Leporellus sp. (Aripuanã) [Narezzi & Birindelli, *in press*] e *Leporellus* sp. (Paraguai) foram as duas primeiras espécies do gênero a serem descritas desde 1941. A descoberta destas duas espécies revela a presença de uma diversidade morfológica ainda desconhecida para o gênero. Além disso, estas espécies destacam-se por serem isoladas geográficamente de congêneres, sendo que a primeira delas ocorre apenas no rio Aripuanã acima das cachoeiras de Dardanelos e Andorinhas, e a segunda noas porções altas da bacia do rio Paraguai.

A combinação de fontes distintas de dados (morfometria, merística, coloração e dados moleculares) foi imprescindível para o reconhecimento de sete espécies distintas de *Leporellus*, e também para entender que cinco delas são crípticas, ou seja, estavam escondidas sob uma aparência externa semelhante. A compreensão dessa diversidade permitiu propor uma hipótese sobre a filogenia das espécies, e utilizá-la para discutir a biogeografia do grupo. A linhagem que deu origem às espécies de *Leporellus* é antiga (cerca de 26 milhões de anos), mas a diversificação das espécies é bem mais recente (cerca de 11 milhões de anos).

Embora este tenha sido um avanço em nosso conhecimento, novas investigações ainda são necessárias para compreender plenamente a diversidade e evolução das espécies de *Leporellus.*

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