UNIVERSIDADE ESTADUAL DO MARANHÃO
PROGRAMA DE PÓS-GRADUAÇÃO EM BIODIVERSIDADE
E BIOTECNOLOGIA REDE BIONORTE

# SISTEMÁTICA DE *LEPORINUS* (CHARACIFORMES, ANOSTOMIDAE) A PARTIR DE FERRAMENTAS MOLECULARES E MORFOLÓGICA

MARIA HISTELLE SOUSA DO NASCIMENTO

**Caxias - MA**
**JUNHO/2024**

MARIA HISTELLE SOUSA DO NASCIMENTO

# SISTEMÁTICA DE *LEPORINUS* (CHARACIFORMES, ANOSTOMIDAE) A PARTIR DE FERRAMENTAS MOLECULARES E MORFOLÓGICA

Tese de doutorado apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Biodiversidade e Biotecnologia da Rede BIONORTE, na Universidade Estadual do Maranhão-UEMA, como requisito para a obtenção do Título de Doutor em Biodiversidade e Biotecnologia.

Orientador(a): Prof. Dra. Maria Claudene Barros
Coorientador (a): Prof. Dr. Elmary da Costa Fraga

**Caxias/MA**
**JUNHO /2024**

N244s Nascimento, Maria Histelle Sousa do

Sistemática de leporinus (characiformes, anostomidae) a partir de ferramentas moleculares e morfológica / Maria Histelle Sousa do Nascimento.__Caxias: Campus Caxias, 2024.

89f.

Tese (Doutorado) – Universidade Estadual do Maranhão – Campus Caxias, Curso de Pós-Graduação em Biodiversidade e Biotecnologia Rede Bionorte.

Orientador: Profª. Dra. Maria Claudene Barros.

Co-orientadora: Prof. Dr. Elmary da Costa Fraga.

1. Biodiversidade. 2. Espécie - Delimitação. 3. Identificação integrativa. 4. Piau. I. Título.

CDU 597.551.3

Elaborada pelo bibliotecário Wilberth Santos Raiol CRB 13/608

MARIA HISTELLE SOUSA DO NASCIMENTO

# SISTEMÁTICA DE *LEPORINUS* (CHARACIFORMES, ANOSTOMIDAE) A PARTIR DE FERRAMENTAS MOLECULARES E MORFOLÓGICA

Tese de doutorado apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Biodiversidade e Biotecnologia - Rede BIONORTE, na Universidade Estadual do Maranhão, como requisito para a obtenção do Título de Doutor em Biodiversidade e Biotecnologia.

Aprovada em 12 / 04 / 2024

**Banca examinadora**

Documento assinado digitalmente
MARIA CLAUDENE BARROS
Data: 05/06/2024 17:58:52-0300
Verifique em https://validar.iti.gov.br

Prof. Dra. Maria Claudene Barros (Orientador)
Universidade Estadual do Maranhão

Documento assinado digitalmente
JULIANA ARARIPE GOMES DA SILVA
Data: 12/06/2024 11:31:05-0300
Verifique em https://validar.iti.gov.br

Profa. Dra. Juliana Araripe Gomes
Universidade Federal do Pará

JoanaRobalo

Profa. Dra. Joana Isabel Espírito Robalo
Instituto Universitário-ISPA

Documento assinado digitalmente
LIGIA TCHAICKA
Data: 12/06/2024 08:21:39-0300
Verifique em https://validar.iti.gov.br

Profa. Dra. Ligia Tchaicka
Universidade Estadual do Maranhão

Documento assinado digitalmente
PEDRO MANOEL GALETTI JUNIOR
Data: 07/06/2024 10:36:43-0300
Verifique em https://validar.iti.gov.br

Prof. Dr. Pedro Manoel Galleti Junior
Universidade Federal de São Carlos

## TERMO DE AUTORIZAÇÃO PARA PUBLICAÇÃO

Eu, Maria Histelle Sousa do Nascimento, (X) autorizo ( ) não autorizo a publicação da versão final aprovada de minha Tese de Doutorado intitulada "**Sistemática de *Leporinus* (Characiformes, Anostomidae) a partir de ferramentas moleculares e morfológica**" no Portal do Programa de Pós-Graduação em Biodiversidade e Biotecnologia – Rede BIONORTE (PPG-BIONORTE), bem como no repositório de Teses da CAPES ou junto à biblioteca da Instituição Certificadora.

Local/Data: Caxias-MA, 05 de junho de 2024

gov.br
Documento assinado digitalmente
**MARIA HISTELLE SOUSA DO NASCIMENTO**
Data: 05/06/2024 17:46:08-0300
Verifique em https://validar.iti.gov.br

(Nome Completo do Discente)

CPF: 602.422.983-62
RG: 029714252005-8

## DEDICATÓRIA

À minha família, que acreditou nos meus sonhos e nas oportunidades... papai e mamãe (Sr. Francisco e Sra. Nazaré), aos meus irmãos, minhas irmãs, sobrinhos e sobrinhas, cunhados (as) e aos meus saudosos avós: Joaquim e Benedita, José e Maria Gonçalves (*in memorian*).

Por todos os sacrifícios e ensinamentos meu MUITO OBRIGADA!

# AGRADECIMENTOS

À DEUS por me permitir vivenciar esse momento;

À Universidade Estadual do Maranhão pela minha formação acadêmica (graduação e mestrado) e por mais uma vez abrigar meu sonho de fazer um doutorado e por todos os serviços prestados;

À Fundação de Amparo à Pesquisa e Desenvolvimento Científico e Tecnológico do Maranhão, Banco do Nordeste e Coordenação de Aperfeiçoamento de Pessoal do Ensino Superior pelos financiamentos incentivando a pesquisa cientifica no Estado do Maranhão;

Ao Instituto Federal do Maranhão por colaborar com viabilização do cumprimento das disciplinas, congressos e coletas;

À Profª. Dra. Claudene Barros por toda demonstração de confiança e pela orientação durante todo desenvolvimento do trabalho. Deixo aqui registrado meu respeito, minha imensa admiração e gratidão;

Ao Prof. Dr. Elmary Fraga pelo incentivo aos estudos dos peixes do Maranhão desde a iniciação cientifica. Obrigada pelo apoio e coorientação;

A todos os professores do Programa em Biodiversidade e Biotecnologia – REDE BIONORTE pelos ensinamentos durante o curso;

A toda equipe do complexo GENBIMOL, onde está situado os laboratórios de Genética e de Biologia Molecular da UEMA, Campus Caxias: Andrelina Alves, Samira Brito, Jordânia Leticia, Amanda Caroline, Eline Santos, Elidy França, Roseane Oliveira, Bruna Gonçalves, Luana Pereira, Marcelo Almeida, Renato Correia, Wilson Gaída Dalton Prado, Daniel Limeira, Ana Priscila, Walna Pires, Aglay Morgana, Paulo Rubens, Alessandra Vidigal, Aryel Morais, Cleison Luis, Amanda Cristiny, Marcelo Alves, Marxo Morais, Naruna Aritana e Ayra Vitória pela parceria, ensinamentos, compreensão e colaborações nos momentos de alegria e principalmente nos momentos tensos. Meu muito obrigada!

À Jordânia Leticia pela ajuda para aprender os métodos de delimitação e nas diversas etapas da pesquisa que envolveram todas as temidas análises de bioinformática. Assim como à Ana Priscila e ao Marcelo Almeida por todo apoio na escrita, pela companhia até altas horas no laboratório para realização dos processos moleculares.

Ao meu dueto de pós-graduandas favorito, Andrelina Alves e Samira Brito. Obrigada por todos os momentos de parceria no laboratório e fora dele, e principalmente aquele que vocês sabem do qual eu me refiro, vocês foram essenciais - até quando não disseram nada - para que eu não desistisse. Muitos artigos qualis A para vocês.

À minha GRAAANDE família pelo amor, cuidado, atenção, compreensão, financiamento, renúncias, sacrifícios, ajuda com as coletas, por tudo e mais um pouco em especial a mamãe e ao papai, OBRIGADA!

## EPÍGRAFE

“Costumávamos pensar que o nosso futuro estava nas estrelas. Agora sabemos que está nos nossos genes”

(James Watson)

# RESUMO

O gênero *Leporinus* inclui cerca de 80 espécies válidas, agrupando o maior número de espécies dentro da ordem Characiformes e sendo o mais diversificado dentro da família Anostomidae. As espécies encontram-se amplamente distribuídas em bacias hidrográficas da região Neotropical, porém é fato a dificuldade na compreensão da biodiversidade do gênero *Leporinus*, isto é, do limite específico, pois os padrões morfológicos são bastante similares entre as espécies deste gênero, o mesmo ocorrendo com as características fenotípicas observadas em indivíduos juvenis e adultos. Neste estudo utilizou-se sequências do genoma mitocondrial e nuclear e dados morfológicos para identificar e fornecer as relações de parentescos das espécies de *Leporinus* contribuindo assim com informações que deverão ser usadas em plano de manejo e conservação das espécies. O estudo integrativo usando dados morfológicos e moleculares possibilitou o registro de *Megaleporinus macrocephalus* nas bacias hidrográficas maranhenses, representando uma importante extensão da sua distribuição que hoje é confirmada na bacia do rio Itapecuru e Mearim. A análise de 157 sequencias de *Leporinus* para gene COI das bacias dos rios Itapecuru, Mearim, Turiacu, Pericumã, Periá, Preguicas, Parnaíba e Tocantins identificou *L. maculatus, L. unitaeniatus, L. affinis* e *L. piau* (do rio Parnaíba) como agrupamentos distintos corroborados por métodos de delimitação das espécies, os quais revelaram a presença de quatro MOTUs distintas correspondendo a cada uma destas espécies. O método bPTP restringiu *L. venerei* a uma única MOTU apontando para a ocorrência pela primeira vez desta espécie em rios do Maranhão. Ainda foi evidenciado a separação de *L.* cf. *friderici* em dois clados e a subsequente formação de diferentes MOTUs, sendo, portanto, consistente com a polifilia desta espécie indicando a existência de diversidade críptica, bem como a configuração de *L.* cf. *friderici* e *L. piau* em dois clados suportando a conclusão de que os espécimes de *L. piau* do Maranhão foram identificados erroneamente. Na investigação da diversidade críptica em *L. piau* das bacias hidrográficas do Maranhão e Piauí, usando marcadores mitocondriais e nuclear foram observados dois grupos bem suportados, em que os espécimes dos rios Itapecuru, Mearim, Turiaçu e Pericumã foram atribuídos a um clado sem nome, representando uma diversificação relativamente antiga e provavelmente escondendo uma diversidade críptica. O segundo grupo incluiu os espécimes dos rios Parnaíba e Mearim, denominado *L.* cf. *friderici sensu stricto*, um grupo que se diversificou relativamente recente e mais estreitamente relacionado a *L. friderici* da localidade tipo. Os resultados confirmaram a existência de um cenário complexo de diversidade críptica no gênero *Leporinus* e as inconsistências taxonômicas encontradas neste grupo de peixes.

**Palavras-chaves:** Biodiversidade; Delimitação de espécie; Identificação integrativa; Piau.

# ABSTRACT

The genus *Leporinus* includes around 80 valid species, grouping the largest number of species within the Characiformes order and being the most diverse within the Anostomidae family. The species are widely distributed in river basins in the Neotropical region, but it is a fact that it is difficult to understand the biodiversity of the genus *Leporinus*, i.e. the specific limit, because the morphological patterns are very similar between the species of this genus, as are the phenotypic characteristics observed in juvenile and adult individuals. This study used mitochondrial and nuclear genome sequences and morphological data to identify and provide the kinship relationships of the *Leporinus* species, thus contributing information that should be used in the species' management and conservation plans. The integrative study using morphological and molecular data made it possible to record *Megaleporinus macrocephalus* in the Maranhão river basins, representing an important extension of its distribution, which is now confirmed in the Itapecuru and Mearim river basins. The analysis of 157 *Leporinus* COI gene sequences from the Itapecuru, Mearim, Turiacu, Pericumã, Periá, Preguicas, Parnaíba and Tocantins river basins identified *L. maculatus*, *L. unitaeniatus*, *L. affinis* and *L. piau* (from the Parnaíba river) as distinct groupings corroborated by species delimitation methods, which revealed the presence of four distinct MOTUs corresponding to each of these species. The bPTP method restricted *L. venerei* to a single MOTU, pointing to the first occurrence of this species in rivers in Maranhão. There was also evidence of the separation of *L.* cf. *friderici* into two clades and the subsequent formation of different MOTUs, which is therefore consistent with the polyphyly of this species indicating the existence of cryptic diversity, as well as the configuration of *L.* cf. *friderici* and *L. piau* into two clades supporting the conclusion that the specimens of *L. piau* from Maranhão were misidentified. In the investigation of cryptic diversity in *L. piau* from the Maranhão and Piauí river basins, using mitochondrial and nuclear markers, two well-supported groups were observed, in which specimens from the Itapecuru, Mearim, Turiaçu and Pericumã rivers were assigned to an unnamed clade, representing relatively ancient diversification and probably hiding cryptic diversity. The second group included specimens from the Parnaíba and Mearim rivers, called *L.* cf. *friderici* sensu stricto, a group that diversified relatively recently and is more closely related to *L. friderici* from the type locality. The results confirmed the existence of a complex scenario of cryptic diversity in the Leporinus genus and the taxonomic inconsistencies found in this group of fish.

**Keywords:** Biodiversity; Fish; Integrative identification; Piau; Species delimitation.

# APRESENTAÇÃO

A tese está estruturada em uma introdução geral, referencial teórico e dois capítulos, onde no primeiro apresenta-se o artigo publicado revista PeerJ intituilado “The DNA barcode reveals cryptic diversity and a new record for the genus *Leporinus* (Characiformes, Anostomidae) in the hydrographic basins of central northern Brazil”. O segundo capítulo refere-se ao artigo publicado na revista Journal of Fish Biology com título “Exploring Hidden Diversity: Molecular Insights into the *Leporinus* Species of the Rivers of the Brazilian States of Maranhão and Piauí”. Na seção apêndice encontra-se artigo de coautoria publicado na revista Brazilian Journal of Biology com título “New records of the occurrence of *Megaleporinus macrocephalus* (Garavello and Britski, 1988) (Characiformes, Anostomidae) from the basins of the Itapecuru and Mearim rivers in Maranhão, northeastern Brazil”.

Esta tese foi desenvolvida no complexo GENBIMOL que compreende os laboratórios de Genética e de Biologia Molecular da Universidade Estadual do Maranhão, campus Caxias. A pesquisa com os peixes do gênero *Leporinus* é resultante de projetos fomentados pelo Banco do Nordeste do Brasil S/A (aviso: ETENE/FUNDECI 01/2004) e Fundação de Amparo à Pesquisa e o Desenvolvimento Científico e Tecnológico do Maranhão - FAPEMA (Editais: Nº 30/2010; Nº 001/2013; Nº 004/2018; Nº 002/2018; Nº 002/2022). Teve aprovação da Comissão de Controle para Tratamento Ético de Animais, Universidade Estadual do Maranhão (Protocolo Nº 47/2022) e Comissão de Ética no Uso de Animais do Instituto Nacional de Pesquisas da Amazônia (Nº 006/2021), SEI 01280.000116/2021-45. As coletas foram autorizadas pelo Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis e Sistema de Autorização e Informação em Biodiversidade por meio de licenças (IBAMA 02012.004159/2006; ICMBio/MMA 42119-1/2013; ICMBio/MMA 46367-1/2015; ICMBio/MMA 83138-1/2022 e ICMBio/MMA 73790-6/2022).

# LISTA DE FIGURAS E TABELAS

## LISTA DE FIGURAS REVISÃO BIBLIOGRÁFICA

## LISTA DE FIGURAS E TABELAS DO CAPÍTULO I

## LISTA DE FIGURAS E TABELAS DO CAPÍTULO II

rRNA 16S, Cyt *b*, and TROP *b* genes. The presence of n/c in the results denotes cases in which it was not possible to estimate evolutionary distances

# SUMÁRIO

# 1 INTRODUÇÃO GERAL

Os peixes de água doce da região Neotropical apresentam uma significativa diversidade ultrapassando 8.000 espécies, com destaque para bacia amazônica que compreende cerca de 2.400 espécies de água doce conhecidas. No entanto, existem muitas incertezas taxonômicas levando a uma diversidade subestimada (PEREIRA *et al*., 2013; REIS *et al*., 2016; JÉZÉQUEL *et al*., 2020). Dentre os peixes de água doce neotropicais, a ordem Characiformes representa mais de 30% das espécies conhecidas e Anostomidae é uma das famílias mais ricas em espécies, ocorrendo em todas as grandes bacias hidrográficas da América do Sul (REIS *et al*., 2003).

A familia Anostomidae compreende 16 gêneros e aproximadamente 147 espécies válidas encontrando-se amplamente distribuída desde o Sul da América Central até o Norte da Argentina (SANTOS, JEGU e LIMA 1996; NELSON, 2006; BUCKUP *et al*., 2007; ESCHMEYER e FONG, 2014; RAMIREZ *et al*., 2016; BRITSKI e BIRINDELLI, 2019; RAMIREZ *et al*., 2020; FRICKE *et al*., 2024). Na última década esta família tem sido objeto de interesse de muitos estudos, resultando na criação de dois novos gêneros e na descrição de 11 espécies (FRICKE *et al.,* 2024). Apesar das recorrentes revisões taxonômicas em peixes da família Anostomidae na América do Sul, esta família ainda permanece com o *status* de um grupo muito complexo (VARI e WILLIAMS, 1987; SIDLAUSKAS e SANTOS, 2005; MAUTARI e MENEZES, 2006; BRITSKI e BIRINDELLI, 2008; SIDLAUSKAS *et al*., 2011; BRITSKI *et al.,* 2012; BIRINDELLI *et al*., 2012; BIRINDELLI e BRITSKI, 2013; BURNS *et al*., 2014; ASSEGA e BIRINDELLI, 2019).

Dentre os gêneros da família Anostomidae, *Leporinus* é o mais especioso com cerca de 80 espécies nominais válidas (FRICKE *et al*., 2024; TOLEDO-PIZA *et al*., 2024). Segundo Géry (1977), constitui um dos gêneros que agrupa maior número de espécies dentro da ordem Characiformes, ocorrendo desde a América Central ao Sul da América do Sul. As espécies de *Leporinus* possuem uma grande diversidade em forma, posição da boca, número de dentes e padrão de colorido. Britski e Garavello (1978) dividiu o gênero em três categorias considerando o padrão de colorido: barras transversais, manchas e linhas longitudinais.

O monofiletismo do gênero é controverso, resultando em várias tentativas de subdividi-lo em outros gêneros ou subgêneros (BRITSKI e BIRINDELLI, 2008; SIDLAUSKAS *et al*., 2011; BRITSKI *et al*., 2012; BIRINDELLI e BRITSKI, 2013; BURNS *et al.,* 2014; RAMIREZ *et al.,* 2016; RAMIREZ *et al*., 2017a), no entanto, sem grandes sucessos. Do ponto de vista citogenético, *Leporinus* tem sido bastante estudado (GALETTI JR *et al*., 1981; GALETTI JR *et al*., 1991; GALETTI JR *et al*., 1995; KOEHLER *et al*., 1997a, 1997b), as espécies apresentam um número de cromossomo conservado 2n=54 e a presença de apenas um par de cromossomos com regiões organizadoras de nucléolos (NORs), embora para seis espécies até então classificadas dentro deste gênero, *Leporinus conirostris*, *Leporinus piavussu, Leporinus macrocephalus, Leporinus obtusidens, Leporinus reinhardti* e *Leporinus trifasciatus* a existência de um sistema de cromossomos sexuais ZZ/ZW descrito as diferenciavam das demais espécies. Galetti Jr et al. (1995) propuseram que a presença do sistema ZW em seis espécies de *Leporinus*, configuraria uma sinapomorfia e que essas espécies formariam um grupo monofilético, hipótese esta que é corroborada por caracteres morfológicos. Ramirez et al. (2017a) usando dados moleculares descreveu um novo gênero denominado *Megaleporinus*, no qual realocou espécies de dois gêneros de Anostomidae, *Leporinus* e *Hypomasticus,* com cromossomos sexuais ZZ/ZW constituindo um grupo monofilético.

Atualmente para as espécies que compões o gênero *Leporinus* verifica-se divergências entre dados moleculares e morfológicos, com baixos índices de divergência genética entre espécies, sugerindo assim a presença de espécies crípticas, e possivelmente por isso, inúmeras espécies vêm sendo designadas como *Leporinus* sp. Estudos realizados por Fraga et al. (2014) e Nascimento et al. (2016) em rios maranhenses evidenciaram uma diferenciação genética em *Leporinus piau* Fowler, 1941 apontando para ocorrência de no mínimo duas linhagens, pois foi revelado um alto índices de similaridade genética entre *L. piau* e *Leporinus* cf. *friderici*, advertindo para a necessidade do uso de outras ferramentas para discriminar as espécies.

Dentre os diversos acordos firmados na Conferência das Nações Unidas sobre Meio Ambiente e Desenvolvimento, ocorrida no Rio de Janeiro e assinada por 168 países, incluindo o Brasil, está a importância da conservação da biodiversidade (SECRETARIAT OF THE CONVENTION ON BIOLOGICAL DIVERSITY, 1992). Essa preocupação de associar estudos sobre ictiofauna com a conservação da biodiversidade vai ao encontro da Agenda 2030 para o desenvolvimento sustentável, publicada pela Organização das Nações Unidas (ONU) em 2015 e que tem como 15º Objetivo de Desenvolvimento Sustentável (ODS 15): “Proteger, restaurar e promover o uso sustentável dos ecossistemas terrestres, gerir de forma sustentável as florestas, combater a desertificação, travar e reverter a degradação dos solos e travar a perda da biodiversidade”. Dentre as 12 metas do ODS 15, a meta 15.1 estabelece que, “Até 2020, assegurar a conservação, recuperação e uso sustentável de ecossistemas terrestres e de água doce interiores e seus serviços, em especial florestas, zonas úmidas, montanhas e terras áridas, em conformidade com as obrigações decorrentes dos acordos internacionais”, e a meta 15.5 “Tomar medidas urgentes e significativas para reduzir a degradação de habitat naturais, deter a perda de biodiversidade e, até 2020, proteger e evitar a extinção de espécies ameaçadas” (NAÇÕES UNIDAS BRASIL, 2023). Embora o prazo para o estabelecimento de tais metas tenha expirado, os esforços para frear a perda da biodiversidade são permanentes e em constante atualização. No entanto, a conservação para vários grupos existente só será efetivamente bem-sucedida quando questões taxonômicas de grupos problemáticos, como *Leporinus*, forem esclarecidas.

O grande número de espécies no gênero *Leporinus*, reflete uma alta complexidade em relação a identificação e descrição de novas espécies, uma vez que a plasticidade fenotípica (principalmente do indivíduo juvenil para o adulto) e os caracteres utilizados na identificação morfológica de espécies pode levar a identificações errôneas. Deste modo, uma alternativa a catalogação é a utilização de dados morfológicos e citogenéticos atrelados a genética molecular, com ênfase nas relações filogenéticas usando marcadores mitocondriais e nucleares para que as topologias obtidas reflitam a história evolutiva das espécies, e ainda, a identificação molecular via DNA barcode.

As contribuições geradas neste estudo são de extrema relevância, pois, fornecerão informações sobre diversidade das espécies de *Leporinus* em importantes bacias hidrográficas maranhenses. Neste sentido, as hipóteses norteadoras consistiram em corroborar a parafilia do grupo, pois há evidências que o gênero *Leporinus* constitua um grupo parafilético. Ainda hipotetizamos que, os resultados obtidos forneçam códigos de barras de DNA específico para cada espécie, ocorrendo o *barcoding gap* entre as distâncias intraespecíficas e interespecífica, bem como sinalizem a ocorrência de novos registros de espécies para as bacias hidrográficas maranhenses, uma vez que estudos com *Leporinus* pra essa região ainda são escassos.

A presente pesquisa tem como objetivo geral utilizar sequências dos genomas mitocondrial, nuclear e dados morfológicos para identificar e fornecer as relações de parentescos das espécies de *Leporinus* e assim buscar solucionar as incertezas taxonômicas para este gênero contribuindo com informações que deverão ser usadas em plano de manejo e conservação das espécies. Ainda, utilizando ferramentas de taxonomia integrativa avaliar a diversidade de espécies objetivando (i) Diferenciar a partir de dados morfológicos as espécies de *Leporinus*; (ii) Identificar as espécies do gênero *Leporinus* baseado em DNA *barcode* e (iii) Reconstruir as relações filogenéticas do gênero *Leporinus* com base nos genes mitocondriais e nucleares.

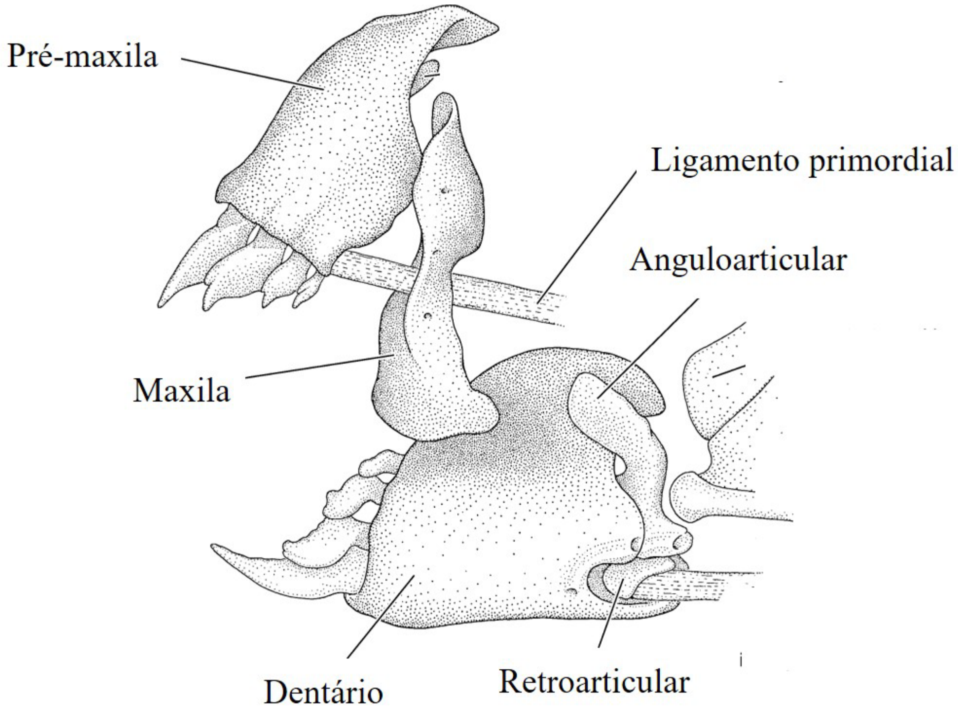
# 2 REVISÃO BIBLIOGRÁFICA

## 2.1 FAMÍLIA ANOSTOMIDAE

Os peixes da família Anostomidae são facilmente reconhecíveis pelo corpo fusiforme, variando de 10 cm (espécies pequenas dos sistemas do Rio Orinoco e a Amazônia) a 80 cm (espécies grandes dos tributários dos rios Paraná e do Pantanal) com 3 ou 4 dentes grandes no pré-maxilar e dentário (maxilar sem dentes), escamas grandes e pouco numerosas (32 a 46 escamas perfuradas na linha lateral na maioria das espécies), nadadeira anal curta com menos de 10 raios ramificados (GÉRY, 1977; GARAVELLO e BRITSKI, 2003). Apresentam grande variação na posição da boca, como também, no padrão de colorido, composto desde manchas redondas no flanco a faixas longitudinais, ou ainda barras verticais (GARAVELLO e BRITSKI, 1987). Essas duas características são fortemente influenciadas pela ontogenia sendo possível encontrar formas diferenciadas de posição da boca e padrão de coloração durante o desenvolvimento de uma mesma espécie (GARAVELLO, 1979; BIRINDELLI e BRITSKI, 2009).

Os primeiros anostomideos foram descritos por Linnaeus (LINNAEUS, 1758) e incluídos em um grupo denominado Anostomina. Com o passar dos anos e a ocorrência de revisões taxonômicas, Regan (1911) propôs a criação da família Anostomidae, na qual incluiu grupos que conhecemos como Anostomidae, Chilodontidae, Curimatidae e Prochilodontidae. Atualmente, a família Anostomidae é representada por 147 espécies válidas que estão distribuídas em 16 gêneros (FRICKE *et al*., 2024).

Dentre os gêneros de Anostomidae*, Leporinus*, foi descrito por Agassiz (SPIX e AGASSIZ, 1829) e nomeado em referência aos fortes dentes incisivos de seus representantes (Figura 1) com *Leporinus novemfasciatus* Spix e Agassiz, 1829 como a primeira espécie do gênero, que mais tarde foi sinonimizada como *Leporinus fasciatus* (BLOCH,1794*)*. Britski (1976), ao examinar a coleção de peixes do Instituto Nacional de Pesquisas da Amazônia (INPA) coletadas no rio Ariapuana, encontrou espécimes incomuns de Anostomidae, cuja parte inferior da boca acabou por ser um carater muito diferente de outras espécies de *Leporinus*. A referida espécie foi descrita pelo mesmo autor em 1976 como *Leporinus pachychelius*, que para a época era classificada dentro do subgênero *Hypomasticus* (BORODIN, 1929), 40 anos mais tarde *Hypomasticus* adquiriu o status de gênero (SIDLAUSKAS e VARI, 2008).



Fonte: Imagem adaptada de Sidlauskas e Vari (2008).

Figura 1. Vista lateral dos maxilares superior e inferior esquerdos de *Leporinus fasciatus* USNM 225991, 87,8 mm SL dissecado.

*Leporinus* é considerado o gênero com maior riqueza dentre os anostomideos, compreendendo 80 espécies nominais válidas (FRICKE *et al*., 2024; TOLEDO-PIZA *et al*., 2024). Seus representantes apresentam uma grande variabilidade de caracteres fenotípicos, variando desde tamanho, posição da boca e padrão de coloração. Segundo Birindelli e Britski (2013), o padrão de cor pode ser usado para subdividir as espécies de *Leporinus* em três grupos: o primeiro grupo formado por aquelas que possuem entre seis e 14 bandas transversais no corpo, o segundo com uma ou várias manchas escuras no corpo e o terceiro grupo inclui aquelas espécies que apresentam uma ou mais listras longitudinais escuras como ilustra a figura 2. Contudo, estudos moleculares demonstraram que esses padrões na cor não representam grupos monofiléticos (RAMIREZ *et al*., 2016; RAMIREZ *et al*., 2017a).

No longo histórico de consideráveis revisões taxonômicas, nas quais os anostomideos foram submetidos, as tentativas de elucidar as classificações foram muito importantes para se seguir uma linha de estudos, tanto para a família Anostomidae quanto para os gêneros. As principais revisões no nível de gênero ocorreram em *Abramites* (VARI e WILLIAMS, 1987), *Laemolyta* (MAUTARI e MENEZES, 2006), *Pseudanos* (SIDLAUSKAS e SANTOS, 2005; BIRINDELLI *et al*., 2012), *Anostomoides* (ASSEGA e BIRINDELLI, 2019) e *Leporinus* (BRITSKI e BIRINDELLI, 2008; SIDLAUSKAS *et al*., 2011; BRITSKI *et al*., 2012; BIRINDELLI e BRITSKI, 2013; BURNS *et al.,* 2014; RAMIREZ *et al.,* 2016).

Fonte: Fotos de A-Q por P. Venere e J. Ramirez; R-T por J. Birindelli e H. Britski; U-V por J. Birindelli, H. Britski e J. Garavello.

Figura 2. Espécies de *Leporinus* segundo subdivisão por padrão de coloração. A-D: Espécies com seis a 14 bandas transversais no corpo; E-N: Padrão de coloração com uma ou várias manchas escuras no corpo; O-V: Espécies com uma ou mais listras longitudinais escuras. **A**: *Leporinus octofasciatus* Steindachner 1915; **B**: *Leporinus desmotes* Fowler 1914 ; **C**: *Leporinus fasciatus* (BLOCH, 1794) ; **D**: *Leporinus reticulatus* Britski e Garavello 1993; **E**: *Leporinus venerei* Britski e Birindelli 2008*;* **F**: *Leporinus lacustris* Amaral Campos 1945*;* **G**: *Leporinus* cf. *parae;* **H**: *Leporinus* sp. 4, **I**: *Leporinus* cf. *friderici*; **J**: *L.* sp.; **K**: *Leporinus* sp.; **L**: *Leporinus geminis* Garavello e Santos 2009; **M**: *Leporinus* cf. *niceforoi*; **N**: *Leporinus amazonicus* Santos e Zuanon 2008; **O**: *Leporinus unitaeniatus* Garavello e Santos 2009; **P**: *Leporinus agassizi* Steindachner 1876; **Q**: *Leporinus brunneus* Myers 1950; **R**: *Leporinus microphysus* Birindelli e Britski 2013; **S**: *Leporinus tristriatus* Birindelli e Britski 2013; **T**: *Leporinus striatus* Kner 1858; **U**: *Leporinus melanopleura* Günther 1864; **V**: *Leporinus melanopleurodes* Birindelli, Britski e Garavello 2013.

## 2.2 GÊNERO *LEPORINUS*

As diversas tentativas de subdividir gênero *Leporinus* em novos gêneros ou subgêneros não tem sido uma tarefa muito exitosa (GARAVELLO e BRITSKI, 2003). Por exemplo, Fowler (1914) descreveu o subgênero *Myocharax* baseado na espécie *Leporinus desmotes* Fowler,1914 da Guiana. O subgênero *Leporinops* foi descrito a partir de *Leporinus moralesi* Fowler, 1942 como espécie tipo, no qual também foi incluído a espécie *Leporinus jamesi* Garman, 1929 (GÉRY, 1960). No entanto, a partir de dados osteológicos, Sidlauskas e

Vari (2008) não encontraram evidências para reconhecer *Myocharax* e *Leporinops* como grupos válidos.

O subgênero *Hypomasticus* (BORODIN, 1929) foi descrito pelo caráter peculiar da boca, a qual é totalmente voltada para baixo e com dentes não serrilhados. *Hypomasticus mormyrops* (STEINDACHNER, 1875) foi designado como espécie tipo e *Hypomasticus garmani* (BORODIN, 1929) foi incluído nesse subgênero (BORODIN, 1929), no entanto esta última espécie atualmente está classificada dentro do novo gênero *Megaleporinus* Ramirez, Birindelli e Galleti, 2017 (RAMIREZ *et al*., 2017a). Quando Sidlauskas e Vari (2008) elevaram *Hypomasticus* ao status de gênero incluíram no mesmo cinco espécies até então classificadas dentro de *Leporinus*, a saber: *Hypomasticus despaxi* (PUYO, 1943), *Hypomasticus julii* (SANTOS, JÉGU e LIMA, 1996), *Hypomasticus megalepis* (GÜNTHER, 1863), *Hypomasticus pachycheilus* (BRITSKI, 1976) e *Hypomasticus thayeri* (BORODIN, 1929). Na última década, duas novas espécies foram descritas para o gênero *Hypomasticus (*BIRINDELLI *et al*., 2013; BIRINDELLI *et al*., 2020), no entanto, os autores reforçam que os limites do gênero ainda são incertos, sendo impossível alocar com precisão algumas espécies dentro de *Leporinus* ou de *Hypomasticus* com base no conhecimento atual (BIRINDELLI e BRITSKI, 2009).

Estudos filogenéticos a partir de abordagens morfológicas e moleculares caracterizaram *Leporinus* como um gênero parafilético (SIDLAUSKAS e VARI, 2008; RAMIREZ *et al*., 2016). Sidlauskas e Vari, (2008) estabeleceram a reconstrução filogenética com 152 variáveis osteológicas confirmando Anostomidae como um grupo monofilético, mas não o gênero *Leporinus*. O que foi corroborado mais tarde por Ramires et al. (2016), usando marcadores moleculares nucleares e mitocondriais que ao tentarem provar a monofilia de *Leporinus*, tiveram a hipótese rejeitada, confirmando a parafilia do grupo.

O gênero *Megaleporinus* foi descrito por Ramirez et al. (2017a) para incluir 16 linhagens, correspondendo a 10 espécies nominais, previamente reconhecidas em *Leporinu*s e *Hypomasticus*, e como tentativa de recuperação da monofilia de *Leporinus* (RAMIREZ *et al*., 2017a). A descrição desse novo gênero foi suportada por dados citogenéticos, moleculares e morfológicos, caracterizando-se por ter um sistema único de cromossomos sexuais ZZ/ZW (GALETTI JR *et al*., 1995), enquanto a maioria das espécies de *Leporinus* conhecidas pela citogenética não possuem estes cromossomos sexuais (GALETTI JR *et al*., 1981, 1991). A monofilia do gênero *Megaleporinus* ainda é bem suportada por marcadores mitocondriais e nucleares, que o identificaram como o grupo irmão de *Abramites* (RAMIREZ *et al*., 2017a). Quanto à morfologia caracteriza-se por suas espécies apresentarem um tamanho relativamente grande, três dentes unicúspides em cada osso pré-maxilar e dentário e um padrão de cor composto por uma a quatro manchas mediolaterais variando de um a quatro (RAMIREZ *et al.*, 2017a).

Nos últimos anos, estudos moleculares mostraram que existe uma diversidade oculta ao nível de espécie dentro da família Anostomidae, como é o caso dos gêneros *Laemolyta* (RAMIREZ e GALETTI JR, 2015), *Megaleporinus* (RAMIREZ *et al.*, 2017b) e *Leporinus* (SILVA-SANTOS *et al.*, 2018). No estudo de Silva-Santos et al. (2018) a partir de dados moleculares foi sugerido que a espécie *Leporinus friderici* Block (1794) representaria um complexo de espécies, do qual apenas *L. friderici* dos rios do Suriname e Guiana Francesa deve ser reconhecido como espécie-tipo (SIDLAUSKAS e VARI, 2012) e aos demais morfotipo ocorrendo dentro do Escudo Brasileiro foi recomendado a adoção da nomenclatura provisória de *L.* cf. *friderici* (SILVA-SANTOS *et al.,* 2018). Essa diversidade pode representar uma situação típica de especiação recente formando grupos estritamente relacionados revelando incertezas taxonômicas típicas (RAMIREZ *et al.*, 2017b).

A identificação de espécies de peixes neotropicais avançou significativamente com o estabelecimento de abordagens moleculares (PEREIRA *et al.*, 2011, 2013; ROSSINI *et al.*, 2016; MACHADO *et al.*, 2018) e análises integrativas de dados morfológicos e moleculares (MELO *et al.*, 2016; RAMIREZ *et al.*, 2016; SILVA *et al.*, 2016; CALEGARI *et al.*, 2019). Essas abordagens têm sido úteis na resolução de incertezas taxonômicas (BRITSKI *et al.,* 2019), na detecção de diversidade oculta (CARVALHO *et al.*, 2012; PIRES et al., 2017; RAMIRREZ *et al.*, 2017a), bem como na descoberta de novas espécies e linhagens crípticas (GOMES *et al.,* 2015; RAMIREZ e GALETTI JR, 2015; MELO *et al.*, 2016; RAMIREZ *et al.,* 2016; PUGEDO *et al.*, 2016; SILVA-SANTOS *et al.*, 2018). Porém, estudos utilizando dados moleculares ou integrando morfologia e molecular em anostomideos ainda são incipientes, visto a grande problemática taxonômica evidenciada do grupo, o cenário é ainda mais preocupante quando volta-se para os anostomideos de bacias do Maranhão (FRAGA *et al.*, 2014; NASCIMENTO *et al.*, 2016).

Atualmente, para o Maranhão são descritas as ocorrências das espécies: *Leporinus affinis* (GÜNTHER, 1864) para bacia do Rio Itapecuru (ABREU *et al.*, 2019); *L. friderici* distribuídos nas bacias dos rios Itapecuru, Mearim, Maracaçumé, Munim, Periá e Parnaíba (PIORSKI *et al.*, 1998; SOARES, 2005; RAMOS *et al.*, 2014; MELO *et al.*, 2016; ABREU *et al.*, 2019; BRITO *et al.*, 2019; BRITO *et al.*, 2020; GUIMARÃES *et al.*, 2021a, 2021b, 2021c; NASCIMENTO *et al.*, 2023); *L. piau* nas bacias dos rios Itapecuru, Mearim, Turiaçu e Parnaíba (BARROS *et al.*, 2011; RAMOS *et al.*, 2014; RIBEIRO *et al.*, 2014; FRAGA *et al.*, 2014; NASCIMENTO *et al.*, 2016; ASSEGA e BIRINDELLI, 2019; ABREU *et al.*, 2019 NASCIMENTO *et al.*, 2023); bem como a ocorrência de *Megaleporinus macrocephalus*

(GARAVELLO e BRITSKI, 1988) para as bacias dos rios Itapecuru e Mearim (GUIMARÃES *et al*., 2020c; ALMEIDA *et al*., 2021).

Em um cenário em que a identificação baseada em morfologia, por si só, não é suficiente, o uso de informações moleculares pode ser útil para delimitar espécies e indicar a potencial diversidade oculta (COSTA-SILVA *et al*., 2015; SILVA-SANTOS *et al*., 2018), bem como para a construção de filogenias robustas como observado nos estudos de Oliveira *et al.* (2011) e Ramirez *et al.* (2016; 2017b). Portanto, estudos que integram diferentes abordagens, como o apresentado aqui, possibilitam uma melhor resolução das complexidades taxonômicas permitindo o reconhecimento de espécies de importância pesqueira sob um nome comum, informação esta muito importante em tempos em que muitas espécies estão se extinguindo rapidamente.

## 2.3 MARCADORES MOLECULARES

Abordagens utilizando marcadores moleculares têm sido cada vez mais adotadas em estudos de peixes do gênero *Leporinus*, tanto a partir da análise de marcadores mitocondriais, quanto nucleares ou ambas combinadas (CHIARI; SODRÉ 1999; MARTINS *et al*., 2003; CALCAGNOTTO *et al*., 2005; SIDLAUSKAS; VARI, 2012; FRAGA *et al*., 2014; RAMIREZ; GALETTI JR, 2015; RAMIREZ *et al*., 2016; NASCIMENTO *et al*., 2016; SILVA-SANTOS et al., 2018).

O genoma animal está distribuído no núcleo e nas mitocôndrias. O genoma nuclear apresenta uma extrema complexidade organizacional e um tamanho de ordem de bilhões de pares de bases nitrogenadas, enquanto o genoma mitocondrial apresenta alguns poucos genes codificados por algumas dezenas de pares de bases. A maior parte das investigações com informações de DNA em animais utiliza genes de genoma mitocondrial e isso se deve ao fato de que esse genoma apresenta uma série de particularidades importantes, como sua herança quase que exclusivamente materna e sua presença nos organismos em número haplóide, o que torna raro os eventos de recombinação (MEYER, 1993; ROCHA-OLIVARES *et al*., 1999; LOVEJOY e DE ARAUJO, 2000; TSIGENOPOULOS e BERREBI, 2000).

Entre os marcadores moleculares do DNA mitocondrial destaca-se o gene Citocromo Oxidase Subunidade I (COI) bastante utilizados nas investigações moleculares. Em 2004 foi proposta a utilização de um fragmento deste gene como um sistema de identificação global (HERBET *et al*., 2004; HUBERT *et al*., 2008; WARD *et al.,* 2008) popularizado mundialmente como técnica de DNA *barcoding* ou simplesmente, “código de barras de DNA”. O gene COI é uma subunidade do complexo Citocromo Oxidase, que faz parte da cadeia transportadora de elétrons e tem sido utilizado com o objetivo de identificar espécies, possibilitar o estudo comparativo entre espécimes de posição taxonômica desconhecida, aumentar a descoberta de novas espécies e como ferramenta auxiliar em estudos filogenéticos e catalogação da biodiversidade, constituindo uma ferramenta poderosa nos estudos de peixes neotropicais (ROSA e PAIVA, 2009).

Outro gene bastante utilizado em investigações moleculares é o rRNA 16S, um gene estrutural que faz parte da grande subunidade ribossomal do DNA mitocondrial, assim como o rRNA 12S (ROMANO e PALUMBI, 1996). As sequencias do rRNA 16S são razoavelmente conservadas, e tem se mostrado como um bom marcador na diferenciação de peixes, como também em estudos comparativos intergenéricos e interespecíficos (SANTOS *et al*., 2003; CALCAGNOTTO *et al*., 2005; FRAGA *et al*., 2007; FRAGA *et al*., 2014). O uso desse gene tem sido utilizado com sucesso em estudos filogenéticos de diversos grupos de vertebrados, como em peixes, pois existe uma considerável variação em algumas espécies (CALCAGNOTTO *et al*., 2005).

A exemplo dos genes COI e rRNA 16S, o gene Citocromo *b* (Cyt *b*) também é um gene mitocondrial amplamente utilizado em estudos sistemáticos para resolver divergências em muitos níveis taxonômicos. A despeito da sua ampla utilização, provavelmente constitui o gene mitocondrial mais conhecido no que diz respeito a estrutura e função do seu produto protéico (ESPOSTI *et al*., 1993). O gene Cyt *b* contém posições de códons de evolução lenta e rápida, assim como regiões mais conservadoras e outras mais variáveis, o que reflete na sua utilização em estudos com grupos que apresentam conflitos taxonômicos e em investigações filogenéticas (MEYER e WILSON, 1990; IRWIN *et al*., 1991; NORMARK *et al*., 1991; CANTATORE *et al*, 1994; LYDEARD e ROE, 1997; KUMAZAWA e NISHIDA, 2000; RAMIREZ *et al*., 2016; SILVA-SANTOS *et al*; 2018); assim como em investigações com táxons que apresentam níveis de divergência recente (STURMBAUER e MEYER, 1992; ROCHA-OLIVARES *et al*., 1999; KIRCHMAN *et al*., 2000; LOVEJOY e DE ARAUJO, 2000).

O uso de genes nucleares na elaboração de filogenias é bastante útil quando o tempo de divergência dos grupos é elevado, uma vez que esses genes aprestam taxas evolutivas mais lentas (os genes são mais conservados), quando comparados aos mitocondriais, no entanto por apresentar extrema complexidade organizacional e um tamanho da ordem de bilhões de pares de bases pode refletir diretamente na baixa adesão nos estudos, quando comparado ao número daqueles que utilizam genes mitocondriais (STEPIEN e KOCHER, 1997; ORTÍ, 1997). Segundo Slowinski e Page (1999), em estudos moleculares o ideal seria combinar dados obtidos de DNA mitocondrial e nuclear, pois as histórias dos genes são independentes.

O gene nuclear α-tropomiosina (TROP) é responsável pela produção da proteína Tropomiosina que em conjunto a Troponina medeiam a regulação da contração dos músculos esqueléticos por meio dos íons $Ca^{2+}$ (ALBERTS, *et al*. 2006). Alguns estudos filogenéticos têm sido realizados mostrando a utilidade e eficácia deste gene em diversos grupos de peixes (ORTÍ e MEYER, 1996; LOVEJOY e COLLETE, 2001; HARDMAN, 2004; NEAR *et al*., 2004; MOYER *et al*., 2004; QUENOUILLE *et al*., 2004; CALCAGNOTTO *et al*. 2005).

## 2.4 REDE HIDROLÓGICA DO ESTADO DO MARANHÃO

O Maranhão, apesar de conter uma importante rede hidrológica localizada em uma área de transição entre o semiárido nordestino e a floresta amazônica, tem sua fauna piscícola pouco estudada. Os estudos realizados com os peixes maranhenses são em sua maioria inventários, sendo ainda escassos estudos sobre sistemática, variações geográficas e biogeografia integrandos dados moleculares aos morfológicos, principalmente para os grupos de ampla distribuição, como os *Leporinus*.

O Núcleo Geoambiental da Universidade Estadual do Maranhão - NuGeo/UEMA, em 2006, delimitou no estado do Maranhão 12 bacias hidrográficas. Divisão semelhante a aquela definida por meio do Decreto Estadual nº 27.845/2011, do Governo do Maranhão, em que no seu Art. 5º estabelece a divisão do estado em 12 bacias hidrográficas. Sendo assim, o Estado do Maranhão é constituído de nove bacias hidrográficas de domínio estadual e três de domínio federal. As bacias de domínio estadual são representadas pelos Sistemas hidrográficos estaduais das Ilhas Maranhenses e do Litoral Ocidental, bem como as bacias do Mearim, Itapecuru, Munim, Turiaçú, Maracaçumé, Preguiças e Periá. De domino federal tem-se as bacias do Parnaíba, a leste; do Tocantins, a sudoeste; e Gurupi, a noroeste.

Entre as bacias de domínio estadual, também designada por bacias genuinamente maranhenses, enfocaremos nas bacias hidrográficas dos rios Mearim, Itapecuru, Pericumã, Turiaçu, Preguiças e Periá. Para as bacias de domínio federal destacaremos as bacias hidrográficas dos rios Parnaíba e Tocantins (Figura 3).

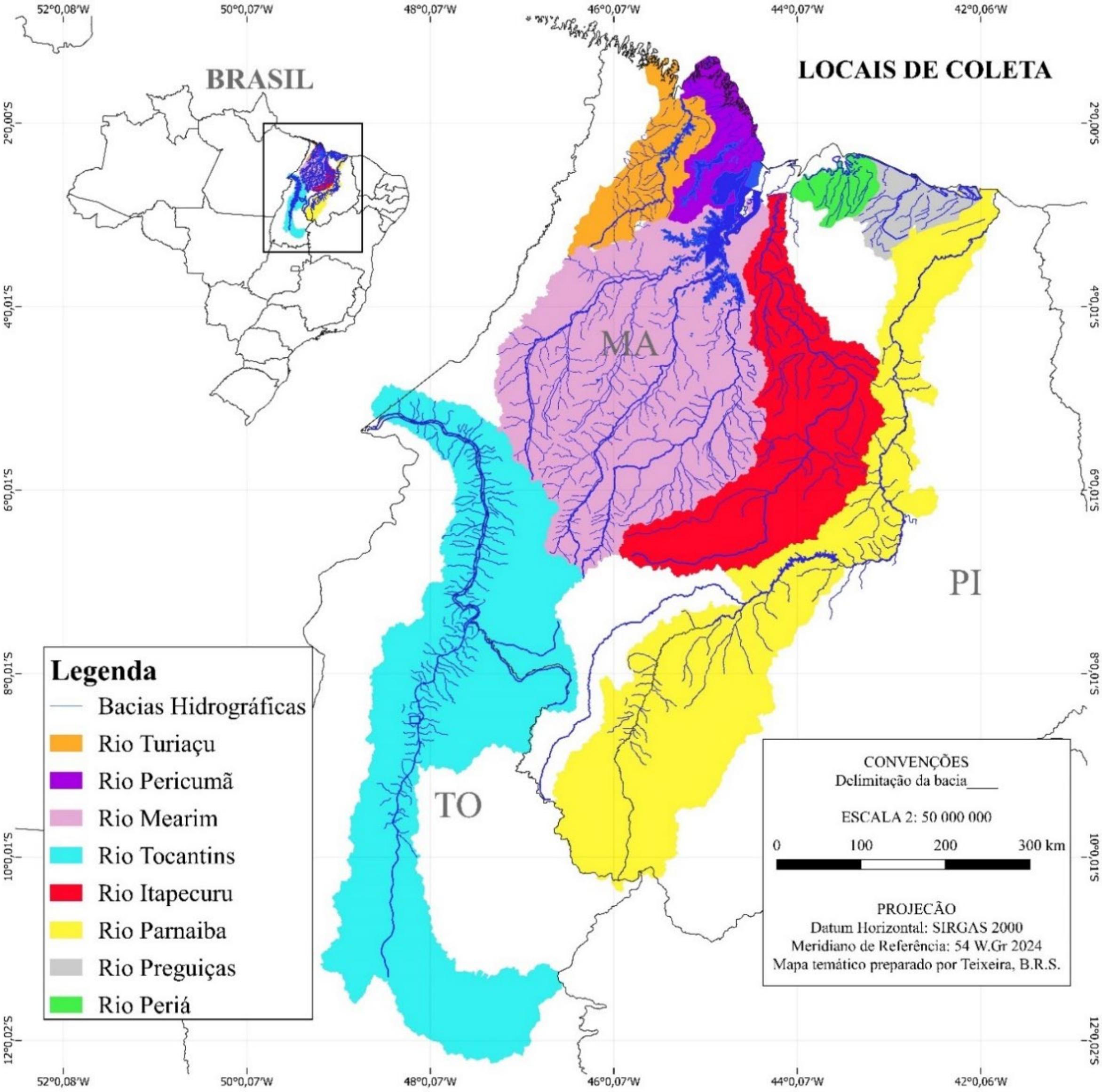


Figura 3. Mapa evidenciando as bacias hidrográficas dos rios Itapecuru, Mearim, Periá, Preguiças, Turiaçu, Pericumã (Maranhão), Tocantins (Tocantins) e Parnaíba (Piauí), locais de coleta das amostras de *Leporinus*.

A bacia do rio Mearim possui uma área de aproximadamente 96.000 Km$^2$ e nasce na confluência das serras Negra, Menina e Cruzeiras desembocando na Baia de São Marcos, na altura da ilha do Caranguejo (STELLA, 2011). Esta bacia tem o rio Pindaré como principal afluente, nasce nas elevações que formam o divisor entre as bacias Mearim e Tocantins, com aproximadamente 686 Km$^2$, sendo navegável no trecho compreendido entre a sua foz no Km 41 do rio Mearim até a foz do rio Buriticupu no Km 456 (STELLA, 2011).

A bacia do rio Itapecuru apresenta uma área de 52.972,1 Km$^2$ extensão, que percorre desde sua nascente, nas serras da Crueira, Itapecuru e Alpercatas até sua desembocadura na baia do Arraial, a sul da ilha de São Luís/MA, caracterizando-se como a segunda maior bacia hidrográfica do estado do Maranhão (DE ALCÂNTARA, 2004).

Os rios Itapecuru, Mearim e Pindaré, formam um grupo de bacias com topografia similar desaguando no golfão maranhense (Mearim/Pindaré na Baía de São Marcos e Itapecuru na Baía de São José), sendo que em seus trechos inferiores, exibem padrão diferenciado com formação de meandros e lagos exclusivo na bacia do Mearim. A evolução geomorfológica do Golfão Maranhense resultou de flutuações marinhas durante o Plioceno e Pleistoceno delineando o padrão atual de desembocadura dos rios da região central do Maranhão (AB'SÁBER, 1960).

A bacia do rio Pericumã está localizada na Microrregião da Baixada Maranhense é uma das bacias hidrográficas secundárias do Estado do Maranhão, com aproximadamente 10.800 km², sua nascente está situada na Lagoa da Traíra e deságua na baía de Cumã, entre os municípios de Guimarães e Alcântara (PINHEIRO, 2000; BRASIL, 2006).

A bacia hidrográfica do rio Turiaçu possui uma área de 14.149,87 km², representando cerca de 4,26% da área do Estado, cujo leito principal é o rio Turiaçu. Sua nascente está localizada nas vertentes da Serra do Tiracambu, a partir desse ponto percorre 720 km de extensão em direção à baía de Turiaçu, entre os municípios de Turiaçu e Bacuri. Durante este percurso, o rio Turiaçu recebe a contribuição dos rios Paraná e Caxias pela margem esquerda e, inúmeros igarapés pela margem direita (MARANHÃO, 2016; MARTINS e OLIVEIRA, 2011).

As bacias dos rios Preguiças e Periá situadas na porção Nordeste do Estado, pertencem à região dos Lençóis Maranhenses e são pouco amostradas em termos de sua ictiofauna, apresentando pouca ou nenhuma informação, principalmente no que diz respeito aos aspectos genéticos (BRITO *et al.*, 2019). A bacia Hidrográfica do Rio Preguiças possui uma área total de 6.750,91 km$^2$ e constituída por três rios: o rio Preguiças, que é o rio principal e tem a maior extensão; o rio Negro e o rio Cangatã. O rio Preguiças, também chamado de rio Grande, nasce no município de Santana do Maranhão percorrendo quase 135 km de extensão até chegar à sua foz, no Oceano Atlântico no município de Barreirinhas (MARANHÃO, 2002). A bacia hidrográfica do rio Periá apresenta a menor área hidrográfica do Estado com um total de 5.395,37 km², representando cerca de 1,62% da área total do Maranhão. Limitando-se com as bacias do Munim, Preguiças e com o Oceano Atlântico, e tem como seus principais afluentes os rios Bacaba e Ribeira.

A bacia hidrográfica do rio Parnaíba possui uma área de 342.988 Km$^2$, nasce nos contrafortes da Chapada das Mangabeiras, sul do Maranhão, percorrendo cerca de 1450 km até sua desembocadura no Oceano Atlântico (STELLA, 2011). Esta bacia se consagra como a segunda maior bacia hidrográfica do nordeste brasileiro com ampla diversidade ictiofaunística, tem regime perene e drena cerca de 275 municípios localizados nos estados do Piauí, Ceará e

Maranhão (ROSA *et al*., 2003; RAMOS *et al*., 2014; MINISTÉRIO DO MEIO AMBIENTE, 2006).

A bacia do rio Tocantins faz parte da região hidrográfica do Tocantins-Araguaia que cobre uma área de 921.921 km$^2$ e abrange parte dos estados do Maranhão (3,8%), Tocantins (34,2%), Pará (20,8%), Goiás (26,8%), Mato Grosso (14,3%) e Distrito Federal (0,1%), o equivalente a aproximadamente 10,8% do território brasileiro (MINISTÉRIO DO MEIO AMBIENTE, 2006). Esta região hidrográfica possui configuração alongada no sentido longitudinal, seguindo as diretrizes dos dois importantes eixos fluviais – os rios Tocantins e Araguaia – unindo-se no extremo setentrional da bacia, formando o baixo Tocantins, que desemboca no rio Pará, pertencente ao estuário do rio Amazonas (MARTINS e OLIVEIRA, 2011). O rio Tocantins, por sua vez, tem sua nascente no planalto de Goiás, sendo formado pelos rios das Almas e Maranhão, e possui a extensão total aproximada de 1.960 km até a sua foz no Oceano Atlântico (AGÊNCIA NACIONAL DE ÁGUAS, 2013).

# 3 RESULTADOS

## 3.1 CAPÍTULO I

Artigo publicado na revista PeerJ: **THE DNA BARCODE REVEALS CRYPTIC DIVERSITY AND A NEW RECORD FOR THE GENUS *LEPORINUS* (CHARACIFORMES, ANOSTOMIDAE) IN THE HYDROGRAPHIC BASINS OF CENTRAL NORTHERN BRAZIL**

# The DNA barcode reveals cryptic diversity and a new record for the genus *Leporinus* (Characiformes, Anostomidae) in the hydrographic basins of central northern Brazil

Maria Histelle Sousa Nascimento[1,2], Deborah Gaído Aragão[1],
Jordânia Leticia Nascimento Silva[1], Renato Correia Lima[3],
José Luis Olivan Birindelli[4], Elmary Costa Fraga[1] and Maria Claudene Barros[1,2]

[1] Department of Chemistry and Biology, Maranhão State University, Caxias, Maranhão, Brazil
[2] Graduate Network Program in the Biodiversity and Biotechnology of Legal Amazonia, Biological Sciences Institute, Belem, Pará, Brazil
[3] Graduate Program in Genetics, Conservation, and Evolutionary Biology, National Amazonian Research Institute, Manaus, Amazonas, Brazil
[4] Department of Animal and Plant Biology, Londrina State University, Londrina, Paraná, Brazil

## ABSTRACT

*Leporinus* is one of the most speciose genera of the order Characiformes, with 81 valid species distributed throughout much of Central and South America. The considerable diversity of this genus has generated extensive debate on its classification and internal arrangement. In the present study, we investigated the species diversity of the genus *Leporinus* in central northern Brazil, and conclude that six valid species—*Leporinus maculatus*, *Leporinus unitaeniatus*, *Leporinus affinis*, *Leporinus venerei*, *Leporinus* cf. *friderici*, and *Leporinus piau*—are found in the hydrographic basins of the Brazilian states of Maranhão, Piauí, and Tocantins. We analyzed 182 sequences of the Cytochrome Oxidase subunit I gene, of which, 157 were obtained from *Leporinus* specimens collected from the basins of the Itapecuru, Mearim, Turiaçu, Pericumã, Periá, Preguiças, Parnaíba, and Tocantins rivers. The species delimitation analyses, based on the ABGD, ASAP, mPTP, bPTP, and GMYC methods, revealed the presence of four distinct molecular operational taxonomic units (MOTUs), identified as *L. maculatus*, *L. unitaeniatus*, *L. affinis*, and *L. piau* (from the Parnaíba River). The bPTP method restricted *L. venerei* to a single MOTU, and confirmed the occurrence of this species in the rivers of Maranhão for the first time. The separation of *L.* cf. *friderici* into two clades and the subsequent formation of different operational taxonomic units was consistent with polyphyly in this species, which indicates the existence of cryptic diversity. The arrangement of *L.* cf. *friderici* and *L. piau* in two different clades supports the conclusion that the *L. piau* specimens from Maranhão were misidentified, based on their morphological traits, reflecting the taxonomic inconsistencies that exist among morphologically similar species. Overall, then, the species delimitation methods employed in the present study indicated the presence of six MOTUs—*L. maculatus*, *L. unitaenitus*, *L. affinis*, *L.* cf. *friderici*, *L. venerei*, and *L. piau*. In the case of two other MOTUs identified in the present study, one (*L. venerei*) is a new record for the state of

Submitted 28 November 2022
Accepted 15 March 2023
Published 25 May 2023

Corresponding author
Maria Claudene Barros,
mbdene@yahoo.com.br

Academic editor
Jörg Oehlmann

Additional Information and Declarations can be found on page 15

DOI **10.7717/peerj.15184**

**How to cite this article** Nascimento MHS, Aragão DGí, Silva JLN, Lima RC, Birindelli JLO, Fraga EC, Barros MC. 2023. The DNA barcode reveals cryptic diversity and a new record for the genus *Leporinus* (Characiformes, Anostomidae) in the hydrographic basins of central northern Brazil. *PeerJ* **11**:e15184 http://doi.org/10.7717/peerj.15184

Maranhão, and we believe that the other represents a population of *L. piau* from the basin of the Parnaíba River.

**Subjects** Aquaculture, Fisheries and Fish Science, Genetics, Molecular Biology, Zoology, Freshwater Biology
**Keywords** Molecular identification, Freshwater fish, Leporinus, Neotropical biodiversity, Systematics

# INTRODUCTION

The family Anostomidae is a prominent group of Neotropical fish that includes 15 genera and approximately 151 valid species (*Ramirez et al., 2016*; *Britski & Birindelli, 2019*; *Ramirez et al., 2020*). The most speciose genus is *Leporinus*, which has approximately 81 valid nominal species (*Fricke, Eschmeyer & Laan, 2021*). *Géry (1977)* concluded that *Leporinus* is one of the most diverse genera of the order Characiformes, which is distributed between Central America and southern South America.

The considerable diversity found in the genus *Leporinus* has led to numerous attempts to classify its species and determine its internal arrangement. A number of studies have proposed subdivisions based on the position of the mouth, and the shape and arrangement of the teeth (*Borodin, 1929*; *Myers, 1950*; *Garavello, 1979*). *Britski & Garavello (1978)* divided the genus into three groups based on coloration patterns, that is, banding, spots, and longitudinal lines, although these proposals have been contradicted by more comprehensive studies, such as those of *Sidlauskas & Vari (2008)* and *Ramirez et al. (2016)*. In their cytogenetic study, *Galetti, Lima & Venere (1995)* confirmed the existence of a well-defined ZZ/ZW sex chromosome system in six *Leporinus* species. These authors proposed that the presence of the ZW system represents a synapomorphy, and that the six species with this system form a monophyletic group. This conclusion is reinforced by morphological traits, such as coloration patterns, relatively large body sizes, and the number of teeth, as confirmed by *Ramirez et al. (2016)*, which led to the allocation of this group to a new genus, *Megaleporinus*, by *Ramirez, Birindelli & Galetti (2017)*.

Using osteological markers, *Sidlauskas & Vari (2008)* evaluated the phylogenetic relationships of the anostomids, and concluded that this family is monophyletic, although they were unable to confirm the monophyly of the genus *Leporinus*. *Ramirez et al. (2016)* used nuclear and mitochondrial molecular markers to confirm the paraphyly of the genus *Leporinus*, and concluded that the recuperation of the monophyly of the group would depend on further taxonomic reviews, including the creation of new genera and the description of new species.

Traditional taxonomic approaches have been essential for the delimitation of anastomid species based on morphological traits, although this does not necessarily resolve some natural groups, given that morphologically similar species may be assigned to the same nominal taxon (*Bickford et al., 2007*). Deciphering and defining cryptic diversity accurately is fundamental to the understanding of the ecological, biogeographic, and evolutionary patterns of a group of organisms, in addition to its other biological features (*Kress et al., 2015*).

*Hebert et al. (2003)* proposed the use of a DNA barcode, based on a standard sequence of the mitochondrial Cytochrome Oxidase subunit I (COI) gene, as the basis for a global species identification system. This approach has been widely-used for the identification of species and the resolution of cryptic diversity within genera and, in particular, in species complexes. A species complex consists of a group of closely-related taxa that have typically undergone recent speciation, which means that their taxonomic differences are still incipient, as observed in the case of the *Leporinus* cf. *friderici* species complex, in which *Silva-Santos et al. (2018)* confirmed the presence of eight distinct Molecular Operational Taxonomic Units (MOTUs) arranged in three clades.

*Leporinus* is not only one of the most diverse fish genera, but its species also play an important ecological role in many freshwater ecosystems, as well as having considerable economic and social importance for local fisheries. Given this, we compiled a dataset of the mitochondrial COI gene of 182,179 *Leporinus* specimens, which included specimens from the hydrographic basins of the Brazilian state of Maranhão to verify the potential intrageneric diversity of this genus, *i.e.*, the presence of different putative species for the study region.

Here we present the diversity of *Leporinus* from hydrographic basins of central northern Brazil. We used integrative taxonomy tools to assess the species diversity of *Leporinus* based on (i) morphological identification from external characters, (ii) morphological identification from dentary characters, and (iii) molecular identification from COI gene fragment.

## MATERIAL AND METHODS

### Sampling

The present study was based on the analysis of a total of 185 sequences, of which 182 were of *Leporinus* species, with the other three representing the outgroup. The vast majority (157) of these 182 *Leporinus* sequences were collected during the present study, being extracted from specimens collected from basins in the Brazilian states of Maranhão (Itapecuru, Mearim, Turiaçu, Pericumã, and Periá rivers), Piauí (Parnaíba River), and Tocantins, that is, the Tocantins River (Fig. 1 and Table S1). The other 25 sequences were obtained from GenBank (Table S2).

The samples from the rivers of Maranhão, Piaui and Tocantins were obtained during extensive fieldwork, which has been ongoing since 2006. This research was authorized by the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA) through license 02012.004159/2006, and licenses ICMBio/MMA 42119-1/2013, ICMBio/MMA 46367-1/2015, ICMBio/MMA 83138-1/2022, ICMBio/MMA 73790-6/2022 issued by the Chico Mendes Institute for Biodiversity Conservation.

After collection, the specimens were taken to the Genetics and Molecular Biology Laboratory (GENBIMOL) of the Advanced Studies Center of Maranhão State University (CESC/UEMA), where they photographed and registered using a coding system. Samples

**Figure 1** **Sample localities.** Each data point indicates the location where Leporinus samples were collected.

Full-size DOI: 10.7717/peerj.15184/fig-1

of muscle tissue were extracted from the specimens for the genetic analyses. The specimens were then fixed em 10% formaldehyde and conserved in 70% alcohol, before being sent to the Museum of Zoology at Londrina State University (MZUEL) in Londrina, Paraná, Brazil, for morphological identification and cataloguing. The study of wild animals was approved by the Regulatory Committee for the Ethical Treatment of Animals of Maranhão State University (protocol 47/2022) and by the Committee for the Ethical Use of Animals of the National Institute for Amazonian Research, registered under protocol number 006/2021, SEI 01280.000116/2021-45.

The total DNA was extracted using the Wizard Genomic DNA Purification kit from Promega, following the maker's instructions. The genomic region was isolated and amplified by Polymerase Chain Reaction (PCR), using the universal primers COI FishF1 5′-TCAACCAACCACAAAGACATTGCCAC-3′ and COI FishR1 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′, described by *Ward et al. (2005)*. The samples were sequenced by the *Sanger, Nicklen & Coulson (1977)* method, using the Big Dye kit in an ABI Prism™ 3500 automatic sequencer (Applied Biosystems, EUA).

The sequences were aligned and edited in the Clustal W (*Thompson, Higgins & Gibson, 1994*) application of the Bioedit 7.2.5 program (*Hall, 1999*). All newly generated sequences (175) were deposited in GenBank under accession numbers OP781850–OP781884, OP782222–OP782283, OP782350–OP7882375 and OP782385–OP782418 (Table S1). The haplotypes were delineated in DnaSP 5.1 (*Librado & Rozas, 2009*). The mean genetic distances and the Maximum Likelihood (ML) tree were obtained in MEGA X (*Kumar et al., 2018*), using the Kimura 2-Parameter and Hasegawa-Kishino-Yano (HKY) models, respectively, with the trees being reconstructed using 1,000 bootstrap replicates.

The optimum evolutionary model for the construction of the Bayesian Inference (BI) and Maximum Likelihood (ML) trees was generated in JModelTest2 (*Darriba et al., 2012*), which is available at CIPRES Science Gateway v3.3 (*Miller, Pfeiffer & Schwartz, 2010*), using the Hasegawa-Kishino-Yano (HKY+G+I) algorithm. The BI tree was generated in BEAST v.1.10.4 (*Drummond et al., 2012*; *Suchard et al., 2018*), using the relaxed lognormal clock (*Drummond et al., 2006*) and the birth-death speciation model (*Gernhard, 2008*).

This analysis was based on 40,000,000 generations with the log files being verified in Tracer v1.6 (*Rambaut et al., 2014*) to evaluate convergence and the most adequate burn-in, with the convergence being considered adequate when the Effective Sample Size (ESS) was over 200. The trees generated in BEAST were summarized in TreeAnnotator v.10.4 (*Suchard et al., 2018*) to obtain the consensus tree, which was then visualized and edited in Fig Tree v1.4.2 (*Rambaut, 2014*) and the Inkscape image editing system. Clades with a bootstrap percentage of at least 85% or posterior probability of at least 0.95 were considered to be well supported.

The delimitation analyses of the MOTUs of the COI gene were run using the following models: the Automatic Barcode Gap Discovery (ABGD), Assemble Species by Automatic Partitioning (ASAP), Poisson Tree Process (PTP), and the Generalized Mixed Yule Coalescent (GMYC) model. The ABGD test (*Puillandre et al., 2012*) was run in https://bioinfo.mnhn.fr/abi/public/abgd/ using the dataset of aligned sequences, while the ASAP test (*Puillandre, Brouillet & Achaz, 2020*) was implemented in https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html using the matrix of genetic distances, extracted using MEGA X, as the input. The PTP (*Zhang et al., 2013*) was run on the web server https://species.h-its.org/. In this case, the input was the Maximum Likelihood phylogenetic tree produced in RaxML v.8.29 (*Stamatakis, 2014*), which is available in the CIPRES Science Gateway v3.3 (*Miller, Pfeiffer & Schwartz, 2010*). The GMYC (*Fujisawa & Barraclough, 2013*) was based on the ultrametric consensus tree constructed in BEAST v1.10.1, which was processed in the Ape (*Paradis & Schliep, 2019*), Splits (*Ezard, Fujisawa & Barraclough, 2009*), Paran (*Dinno, 2009*), and Mass (*Venables & Ripley, 2002*) packages available in the R *v.* 4.1.0 software (*R Core Team, 2021*).

## RESULTS

The present study focused on 182 sequences of the COI gene of *Leporinus*, each consisting of 620 base pairs (bps). The phylogenetic trees generated by the ML and BI analyses were highly congruent and well-supported at both the intra- and interspecific levels (Figs. 2–4), except in the case of *Leporinus piau*, which grouped with either *Leporinus* cf. *friderici* or *Leporinus venerei*. The ABGD analysis delimited 12 MOTUs, while the ASAP defined 15, the mPTP and bPTP each delimited nine, and the GMYC, six MOTUs (Fig. 2).

The results of the five delimitation methods applied in the present study had three species in common—*L. maculatus*, *L. unitaeniatus*, and *L. affinis*—as well as differentiating two specimens (PALEP01 and PALEP09) from the basin of the Parnaíba River in a distinct molecular taxonomic unit, which indicates the occurrence of a fourth species, which we believe to be *L. piau*.

In the present study, the five delimitation methods had three species in common—*L. maculatus*, *L. unitaeniatus*, and *L. affinis*—and differentiated specimens from the basin of the Parnaíba River in a distinct molecular taxonomic unit, which is more basal than the other *Leporinus* species, and groups with the the *Megaleporinus* species that were previously assigned to *Leporinus*.

Clade VI (Fig. 3) was strongly supported, and includes *L. venerei*, *L. lacustris*, *L. piau*, and *L.* cf. *friderici*, with *L. piau* occurring in Maranhão, in the Mearim, Itapecuru, Pericumã, Turiaçu, Preguiças and Periá basins. In this case, the clade was formed by *L. venerei* from the Tocantins basin, *L. lacustris* from the basin of the Paraná River, *L. piau* from the Jaguaribe, Itapecuru, Mearim, Pericumã, Turiaçu, Preguiças and Periá basins, and *L.* cf. *friderici* from the Amazon and Mearim basins, which all share a single molecular taxonomic unit. Only the bPTP analysis separated *L. venerei* from *L. lacustris*, *L. piau*, and *L.* cf. *friderici*, which together formed a single MOTU in the ABGD, ASAP, mPTP, and GMYC models (Fig. 2).

The BI and ML analyses identified the formation of subclades within clade VI (Figs. 2 and 3), in which the *L. piau* from Maranhão, in the Itapecuru, Turiaçu, Mearim, and Periá basins, grouped with *L. venerei* from the Tocantins basin, with genetic distances ranging from only 0.16% to 1.54% (Table S3). Other *L. lacustris* and *L. piau* subclades were identified in the Jaguaribe basin, where the genetic distances ranged from 0.0% to 3.5% (Table S3). The *L. piau* subclade from Maranhão, found in the Itapecuru, Mearim, Pericumã, and Turiaçu basins, grouped with *L.* cf. *friderici* from the Mearim (Maranhão) and Amazon basins (Amazonas state), with genetic distances of between 0.16% and 5.88% (Table S3). All three groups were supported by significant posterior probability (BI) and bootstrap(ML) values (Figs. 2 and 3).

*Leporinus* cf. *friderici*, whose type locality is the basin of the Tampok River in French Guiana, formed a group together with *L. piau* from the basins of the São Francisco, Amazon, and Mearim rivers, an arrangement found in both the species delimitation models and the BI and ML trees. In the ABGD, ASAP mPTP, and bPTP delimitation models, however, *L.* cf. *friderici* was differentiated in its own operational unit (Fig. 2).

The genetic distance matrix derived from the molecular taxonomic units revealed relatively high values for both the intra- and inter-MOTU distances. The highest mean intra-MOTU distance was 5.9%, in *L. maculatus*, while the lowest mean was 0.4%, in *L. unitaeniatus*, whereas the mean inter-MOTU distances ranged from 7.8% to 17.4% The MOTUs formed by *L. venerei*, *L. lacustris*, *L. piau*, and *L.* cf. *friderici* were separated by a mean genetic distance of 2.2% (Table 1). In this context, it is important to note the genetic

Figure 2 **Bayesian Inference tree showing the arrangement of the MOTUs of the Leporinus species analyzed in the present study.** This arrangement was obtained using the ABGD, ASAP, mPTP, bPTP, and GMYC species delimitation approaches for the analysis of the mitochondrial COI gene, based on the Hasegawa-Kishino-Yano (HKY+G+I) algorithm, generated in BEAST. The species delimitated by the specific estimates are shown by the vertical bars, with the color representing the current status of the species. The blue bars correspond to valid species, while the gray bars indicate the species delimited diûerently from the current classification.

Full-size DOI: 10.7717/peerj.15184/fig-2

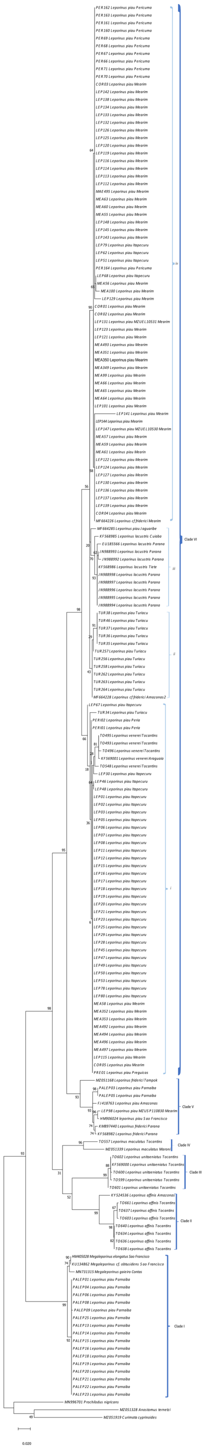


**Figure 3 Maximum Likelihood tree of the Leporinus species.** Maximum Likelihood tree showing the arrangement of the Leporinus species based on the analysis of 185 samples of the mitochondrial COI gene using the Hasegawa-Kishino Yano (HKY+G+I) algorithm, generated in MEGA X. The node support, that is, is given by the Bayesian posterior probability/ML bootstrap values, respectively. Each clade and its subdivisions (when present) are demarcated by the brackets. The Roman numerals in upper case represent the clades, while those in lower case indicate the subclades.

Full-size DOI: 10.7717/peerj.15184/fig-3

distance of 7.8% between *L. piau* (MOTU 1) and *L.* cf. *friderici* (MOTU 2), which may be the result of an error in the identification of the species of one of the groups.

Given the levels of congruence identified in the different delimitation analyses applied in the present study, the ASAP method appeared to be the most effective interpretation, in biological terms, of the dataset considered here, given that it identified 10 MOTUs, which distinguished four of the seven nominal species, including *L. venerei*, in a distinct MOTU. This confirmed the occurrence of this species the Itapecuru, Mearim, Turiaçu, Preguiças and Periá basins, which constitutes the first record of *L. venerei* in the Brazilian state of Maranhão.

## DISCUSSION

An adequate taxonomic assessment is fundamental for the success of many types of biological research, and DNA data have provided additional insights for the resolution of taxonomic questions in many groups of organisms, including elements of the megadiverse Neotropical fish fauna, such as the anostomids. The COI barcode proved to be an extremely valuable tool for the identification and separation of the species assessed in the present study, based on the analysis of genetic distances and species delimitation, which identified evidence of the potential presence of more than one taxon in some nominal species.

In many previous studies of DNA barcoding and molecular diversity, the number of species or lineages delimited by the analysis has tended to exceed the number of nominal taxa or even the morphospecies analyzed (*Carvalho et al., 2018*). A similar tendency was observed here, in addition to the opposite pattern, given that, in some species delimitation analyses, more than one valid species was allocated to the same MOTU, as in the case of *L. venerei*, *L. lacustris*, *L.* cf. *friderici*, and *L. piau*.

In the present study, the *L. venerei*, *L. lacustris*, *L.* cf. *friderici*, and *L. piau* specimens were assigned to a single molecular taxonomic unit by the ABGD, ASAP, mPTP, and GMYC methods, reflecting their similar morphological characteristics, such as their coloration pattern, dental formula, and meristic parameters (Table 2), although the intra-MOTU analyses revealed mean genetic distances of 2.7% (Table 1), ranging from 0.0% to 6.11% (Table S3). This is consistent with the current classification of the valid nominal species (BI and ML analyses: Figs. 2–4; Table 1). All the species of clade VI shared the same morphological pattern, which is considered to be diagnostic of *L. friderici*, such as the number of spots along the lateral line (1–3) and the 4/4 dental formula, except for *L. venerei*, which has four teeth in the pre-maxilla and three in the dentary row. *Leporinus lacustris* and *L. venerei* are highly similar morphologically, given their relatively tall body, terminal mouth, long, dark anal fin, and three spots on the lateral line (*Britski & Birindelli, 2008*; *Silva-Santos et al., 2018*). *Leporinus* piau presents the D-type coloration pattern described by *Garavello (1979)*, which consists of three well-defined black spots on the lateral line, and four teeth in both rows, with a dental formula of 4/4. The *L. venerei*, *L. lacustris*, *L.* cf. *friderici*, and *L. piau* MOTU was subdivided into three subclades (*i*, *ii*, *iii* and *iv*—Fig. 3). Subclade *i* includes *L. piau* from Maranhão and *L. venerei* from the Tocantins basin, while subclade *ii* has *L.* cf. *friderici* from the Amazon and *L. piau* from the Turiaçu

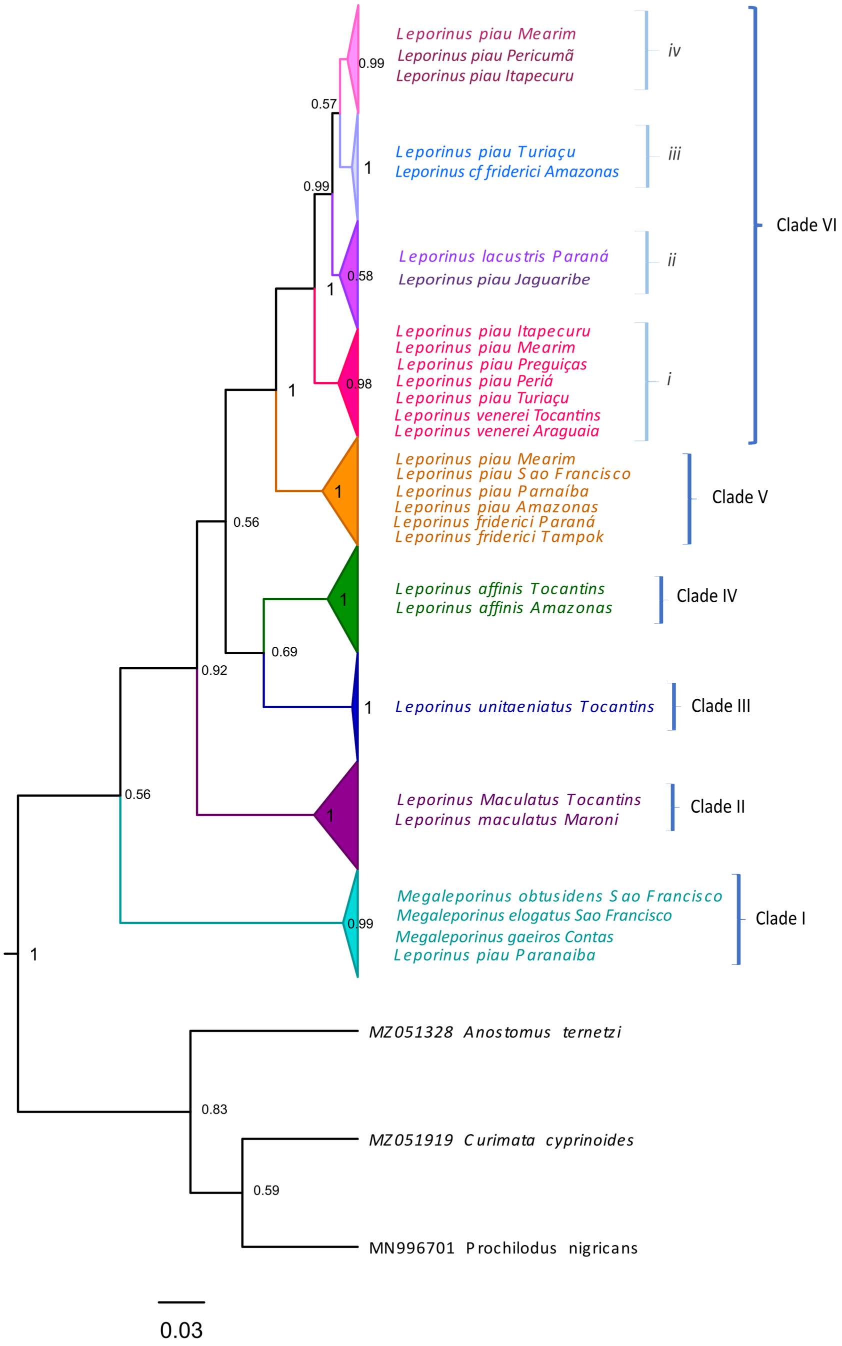


**Figure 4** **Collapsed Bayesian inference tree of the MOTUs of the Leporinus species.** Collapsed Bayesian inference tree showing the arrangement of the MOTUs of the Leporinus species based on 185 samples of the mitochondrial COI gene analyzed using the Hasegawa-Kishino-Yano (HKY+G+I) algorithm, applied in BEAST. The groups were defined by observing the congruence between the MOTUs generated in the species delimitation analyses based on the ABGD, ASAP, mPTP, bPTP, and GMYC methods.

Full-size DOI: 10.7717/peerj.15184/fig-4

**Table 1** **Mean genetic distance of the Leporinus and Megaleporinus.** Mean genetic distance, based on the Kimura 2-Parameter algorithm, generated by MEGA X for the MOTUs defined by the ABGD, ASAP, mPTP, bPTP, and GMYC analyses.

| MOTU | Genetic Distance | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| 1. *Leporinus lacustris* + *Leporinus venerei* + *Leporinus piau* + *Leporinus* cf *friderici* | **2.7** | | | | | |
| 2. *Leporinus piau* + *Leporinus friderici* | 7.91 | **2.2** | | | | |
| 3. *Leporinus maculatus* | 10.89 | 10.58 | **5.9** | | | |
| 4. *Leporinus affinis* | 12.61 | 11.26 | 11.05 | **1.8** | | |
| 5. *Leporinus unitaeniatus* | 13.04 | 13.49 | 11.97 | 11.27 | **0.4** | |
| 6. *Leporinus piau* | 15.49 | 16.24 | 15.64 | 17.45 | 14.53 | **0.5** |

basins. Subclade *iii* groups *L. lacustris* and *L. piau* from the Jaguaribe basin, and subclade *iv* groups *L. piau* from Maranhão and *L.* cf. *friderici* from the Mearim. The composition of subclade *i* (*L. piau* and *L. venerei*—Fig. 3), together with the diagnostic morphological features of the species, indicates that the specimens from the basins of Maranhão identified as *L. piau* may in fact be *L. venerei*, which would be the first record of this species from this Brazilian state.

The *L. venerei*, *L. lacustris*, *L.* cf. *friderici*, and *L. piau* MOTU was subdivided into three subclades (*i*, *ii*, *iii* and *iv*—Fig. 3). Subclade *i* includes *L. piau* from Maranhão and *L. venerei* from the Tocantins basin, while subclade *ii* has *L.* cf. *friderici* from the Amazon and *L. piau* from the Turiaçu basins. Subclade *iii* groups *L. lacustris* and *L. piau* from the Jaguaribe basin, and subclade *iv* groups *L. piau* from Maranhão and *L.* cf. *friderici* from the Mearim. The composition of subclade *i* (*L. piau* and *L. venerei*—Fig. 3), together with the diagnostic morphological features of the species, indicates that the specimens from the basins of Maranhão identified as *L. piau* may in fact be *L. venerei*, which would be the first record of this species from this Brazilian state.

One other clade, formed by *L. friderici* from French Guiana and Paraná with *L. piau* from the Mearim, São Francisco, Parnaíba, and Amazon basins, is also well supported (Fig. 3). This raises two important points: (1) the clear polyphyly of *L. friderici* and *L. piau*, which, in the latter case implies a possible error of identification based on the type specimen, and (2) the existence of cryptic diversity in the genus *Leporinus*, in particular in *L. friderici*. *Silva-Santos et al. (2018)* concluded that the samples identified morphologically as *L. friderici* are in fact a polyphyletic group, given that the specimens collected from the basins of the Brazilian Shield are different from those of *L. friderici* from the type locality. The polyphyly of *L. cf. friderici* was also confirmed in the present study, which is consistent with *Silva-Santos et al. (2018)*, in which a species complex is revealed by the genetic differentiation of the populations present in distinct hydrographic basins. In this case, individuals identified consistently as *L. cf. friderici* may not in fact be conspecific with *L. friderici* from the type locality, that is, they represent different species. *Ramirez, Birindelli & Galetti (2017)* confirmed the presence of cryptic diversity in this taxon, which may represent a typical scenario of recent diversification, when closely-related taxa may be

**Table 2 Meristic traits of the adult *Leporinus* species.** Meristic traits of the adult *Leporinus* used to identify the samples analyzed in the present study, following *Garavello (1979)*, *Garavello (1989)*, *Garavello & Santos (2007)*, *Britski, Sato & Rosa (1984)*, and *Britski & Birindelli (2008)*.

| Species | Body | Coloration | Number of scales around the peduncle | Dental formula | Number of scales in the lateral line | Fin coloration |
|---|---|---|---|---|---|---|
| *L. affinis* Günther (1864) | Pre-dorsal somewhat convex; dorsal inclined slightly between the dorsal and adipose fins, and concave between the adipose and caudal fins. | Body yellowish, with 7 dark transversal stripes on the body and 3–4 on the head. | 16 | 4/4 | * | Peitoral and pelvic fins light yellow; all other fins hyaline. |
| *L. friderici* Bloch (1794) | Body tall and robust; large, with Standard Length (SL) of *ca.* 40 cm; body height 26–30% of SL, head length 27–29% of SL; mouth terminal. | Body browny chestnut, with 2–4 dark spots, rounded or oval, on the lateral line. | 16 | 4/4 | 38 to 40 | Anal fin dark gray; all other fins yellowish-gray. |
| *L. lacustris* Campos (1945) | Body elongated, with a maximum standard length of 20 cm; mouth terminal; incisors truncated. | 2–3 dark, rounded, mediolateral spots on the dorsal fin, the first larger and more conspicuous. | 16 | 4/4 | 33 to 36 | All fins yellowish, except the adipose and anal fins, que which are darkened. |
| *L. maculatus* Müller and Troschel (1844) | Body small, with Standard Length (SL) of *ca.* 10 cm; body height 22–26% of SL, head length 23–25% of SL; mouth subterminal. | Body with 4 black, rounded spots connected by 3 transversal stripes, which cross the lateral line. | 16 | 4/4 | 39 to 40 | * |
| *L. piau* | Body relatively tall. | Body with 3 black spots on the flank, which are elongated horizontally. | 16 | 4/4 | 35 to 37 | * |
| *L. unitaeniatus* *Garavello & Santos (2007)* | Body elongated and fusiform; small, with maximum standard length of 12 cm; relatively low body (23% of the standard length); mouth subterminal. | Body yellowish, with a conspicuous black longitudinal streak running along the lateral line; 11–13 dark chestnut transversal stripes separated from the lateral line by two rows of scales. | 16 | 4/4 | 40 to 44 | Hyaline. |

(*continued on next page*)

Table 2 (*continued*)

| Species | Body | Coloration | Number of scales around the peduncle | Dental formula | Number of scales in the lateral line | Fin coloration |
|---|---|---|---|---|---|---|
| *L. venerei* *Britski & Birindelli (2008)* | Body tall; mouth terminal; anal fin long and dark. | 3 small, dark spots on the lateral line, of which, the last 2, in particular, the last, are typically faded. | 16 | 4/3 | 36 to 37 | * |

**Notes.**
*Data not available.

poorly-distinguished morphologically, creating predictable taxonomic uncertainties, such as those observed in the populations of *L. friderici*.

In the present study, the relationship found among *L. piau*, *L. friderici*, and *L.* cf. *friderici* (Figs. 2–4) alludes to a possible taxonomic inconsistency derived from *Fowler*'s (*1941*) description of *L. piau*, as well as the geographic origin of the specimen analyzed in the present study, which was from the São Francisco basin. *Fowler (1941)* described *Leporinus piau* based on a type specimen from the Salgado River in the Jaguaribe basin of the Brazilian state of Ceará, but included a paratype from the Jatobá River, in the São Francisco basin, which led to the subsequent identification of most *Leporinus* specimens from the São Francisco River as *L. piau* (*Garavello & Britski, 2003*; *Carvalho et al., 2011*). However, *Silva-Santos et al. (2018)*, who analyzed nuclear and mitochondrial genetic markers, including COI, noted that the *Leporinus* specimens from the São Francisco basin represent a species distinct from *L. piau* from the type locality in the Jaguaribe basin. Clearly, *Fowler*'s (*1941*) inclusion of a paratype from a distinct hydrographic basin have contributed fundamentally to the taxonomic uncertainties surrounding *L. piau*.

In the present study, *L. maculatus*, *L.unitaeniatus*, and *L. affinis* are valid nominal species, which presented considerable congruence between the traditional and molecular taxonomies. These three species constitute distinct MOTUs, which reflect their arrangement in different clades (BI and ML analyses: Figs. 2–4). All these species present easily distinguished diagnostic traits, such as the numerous spots dotting the body of *L. maculatus*, the single longitudinal stripe of *L. unitaeniatus*, and the lateral bands with no subdivisions observed in *L. affinis* (*Britski & Garavello, 2005*; *Sidlauskas & Vari, 2012*).

The samples from the Parnaíba basin identified here as *L. piau* and defined as a single MOTU by all the species delimitation models were grouped in a single clade with a genetic distance of 0.5%. These samples were delimited clearly as a more basal species separate from all the others, with evidence that they had been wrongly identified, and are in fact representatives of the genus *Megaleporinus*. This genus was described recently by *Ramirez, Birindelli & Galetti (2017)*, based on a combined morphological, chromosomal, and molecular approach, which assigned the large-bodied *Leporinus* to a monophyletic clade, which was denominated *Megaleporinus*. In the present study, these samples were delimited clearly as a single, basal species well separated from all the others, although a more detailed analysis would be necessary to better determine their taxonomic status.

The samples from the basins of Maranhão and Piauí, together with those from the Tocantins River collected for the present study revealed the cryptic diversity found in *Leporinus*, given that the specimens from the basins of the Itapecuru, Mearim, Pericumã, Turiaçu, Periá, and Preguiças rivers in Maranhão, and the Parnaíba River in Piauí were identified as *L. piau* based on their morphological traits. The study of the DNA barcode and the analytical tools employed here confirmed that *L. friderici* likely constitutes a polyphyletic species complex, leading to the frequent misidentification of specimens as *L. piau*. It will only be possible to resolve this scenario definitively with a systematic re-evaluation of the specimens collected from the hydrographic basins of the states of Maranhão and Piauí.

In the specific case of subclade *i* (Fig. 3; Tables S3-S4), which groups *L. piau* from Maranhão with *L. venerei* from the Tocantins basin, the most parsimonious interpretation of the results of this analysis, together with the diagnostic traits of the two species, would be to consider them to be a single taxon, that is, *L. venerei*. This would thus be the first record of *L. venerei* from the basins of the Itapecuru, Mearim, Turiaçu, and Periá rivers, in the state of Maranhão.

Prior to the present study, three *Leporinus* species were considered to be present in the hydrographic basins of the Brazilian state of Maranhão—*L. affinis*, in the Itapecuru basin(*Abreu et al., 2019*), *L. friderici* in the Itapecuru, Mearim, Maracaçumé, Munim, Periá, and Parnaíba basins (*Piorski et al., 1998*; *Soares, 2005*; *Ramos, Ramos & Ramos, 2014*; *Melo et al., 2016*; *Abreu et al., 2019*; *Brito et al., 2019*; *Brito et al., 2020*; *Guimarães et al., 2021a*; *Guimarães et al., 2021b*; *Guimarães et al., 2021c*), and *L. piau* in the basins of the Itapecuru, Mearim, Turiaçu, and Parnaíba rivers (*Barros, Fraga & Birindelli, 2011*; *Ramos, Ramos & Ramos, 2014*; *Ribeiro et al., 2014*; *Assega & Birindelli, 2019*; *Abreu et al., 2019*). Based on analyses of molecular data, however, *Fraga et al. (2014)* and *Nascimento et al. (2016)* found evidence of two distinct lineages in the *L. piau* group from the Itapecuru basin, while in the present study, *L. piau* was assigned to three different clades, being associated strongly with *L.* cf. *friderici* in two clades and with *L. venerei* in one. This leads us to conclude that *L. piau* is, in fact, absent from the basins of Maranhão, which are instead populated by *L.* cf. *friderici* and *L. venerei*, with the latter being recorded in Maranhão for the first time. This restricts *L. piau* to the basin of the Parnaíba River.

## CONCLUSIONS

The molecular analyses presented here, including the different species delimitation approaches, identified the presence of four *Leporinus* species in the hydrographic basins of central northern Brazil—*L. maculatus, L. unitaenitus, L. affinis*, and *L. venerei*. However, the species delimitation analyses also assigned *L.* cf. *friderici* and *L. piau* to two different molecular operational units, which leads us to believe that an additional species, morphologically indistinguishable from *L.* cf. *friderici*, may be present. The analyses also revealed a distinct group of two of the specimens, which indicates emphatically the presence of *L. piau* in the basin of the Parnaíba River, which indicates the presence of a total of six nominal species in the hydrographic basins of central northern Brazil. The confirmation of the presence of *L. venerei* in the Itapecuru, Mearim, Turiaçu, Preguiças and Periá basins represents a new record for the state of Maranhão, amplifying the known distribution of this species in Brazil.

## ADDITIONAL INFORMATION AND DECLARATIONS

### Funding

This work was supported by the Fundação de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão and Banco do Nordeste do Brazil. The Fundação de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão and Banco do Nordeste do Brazil had a role in the study design, data collection and analysis.

### Grant Disclosures

The following grant information was disclosed by the authors:
Fundação de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão.
Banco do Nordeste do Brazil. The Fundação de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão.
Banco do Nordeste do Brazil.

### Competing Interests

The authors declare there are no competing interests.

### Author Contributions

- Maria Histelle Sousa Nascimento conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Deborah Gaído Aragão performed the experiments, prepared figures and/or tables, and approved the final draft.
- Jordânia Leticia Nascimento Silva performed the experiments, prepared figures and/or tables, and approved the final draft.
- Renato Correia Lima performed the experiments, prepared figures and/or tables, and approved the final draft.
- José Luis Olivan Birindelli performed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Elmary Costa Fraga conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Maria Claudene Barros conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.

### Animal Ethics

The following information was supplied relating to ethical approvals (*i.e.*, approving body and any reference numbers):

Committee for the Ethical Treatment of Animals of Maranhão State University (protocol 47/2022) and by the Committee for the Ethical Use of Animals of the National Institute for

Amazonian Research, registered under protocol number 006/2021, SEI 01280.000116/2021-45.

## Data Availability

The following information was supplied regarding data availability:

The data is available at NCBI GenBank: OP781850 to OP781884, OP782222 to OP782283, OP782350 to OP7882375 and OP782385 to OP782418.

## Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.15184#supplemental-information.

# REFERENCES

**Abreu JMS, Craig JM, Albert JS, Piorski NM. 2019.** Historical biogeography of fishes from coastal basins of Maranhão State, northeastern Brazil. *Neotropical Ichthyology* **17**(**2**):e180156 DOI 10.1590/1982-0224-20180156.

**Ardura A, Linde AR, Moreira JC, Garcia-Vazquez E. 2010.** DNA barcoding for conservation and management of Amazonian commercial fish. *Biological Conservation* **143**:1438–1443 DOI 10.1016/j.biocon.2010.03.019.

**Assega FY, Birindelli JLO. 2019.** Taxonomic revision of the genus Anostomoides (Characiformes: Anostomidae). *Zootaxa* **4646**(**1**):124–144 DOI 10.11646/ZOOTAXA.4646.1.7.

**Barros MC, Fraga EC, Birindelli JLO. 2011.** Fishes from the Itapecuru River basin, State of Maranhão, Northeastern Brazil. *Brazilian Journal of Biology* **71**(**2**):375–380 DOI 10.1590/S1519-69842011000300006.

**Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I. 2007.** Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution* **22**:148–155 DOI 10.1016/j.tree.2006.11.004.

**Birindelli JLO, Britski HA, Ramirez JL. 2020.** A new endangered species of Megaleporinus (Characiformes: Anostomidae) from the Rio de Contas basin, eastern Brazil. *Journal of Fish Biology* **96**(**6**):1349–1359 DOI 10.1111/jfb.14299.

**Borodin NA. 1929.** Notes on some species and subspecies of the genus *Leporinus* Spix. *Memoirs of the Museum of Comparative Zoölogy* **50**:269–290.

**Brito OS, Guimarães EC, Ferreira BRA, Ottoni FP, Piorski NM. 2019.** Freshwater fishes of the Parque Nacional dos Lençóis Maranhenses and adjacent areas. *Biota Neotropica* **19**(**3**):e20180660 DOI 10.1590/1676-0611-BN-2018-0660.

**Brito OS, Guimarães EC, Ferreira BRA, Santo SJP, Amaral YT, Ottoni FP. 2020.** Updated and supplementary data on Brito et al. (2019): Freshwater fishes of the PN dos Lençóis Maranhenses and adjacent areas. *Ichthyological Contributions of Pecescriollos* **73**:1–17.

**Britski HA, Birindelli JLO. 2008.** Description of a new species of the genus Leporinus Spix (Characiformes: Anostomidae) from the rio Araguaia, Brazil, with comments on the taxonomy and distribution of L. parae and L. lacustris. *Neotropical Ichthyology* **6**:45–51 DOI 10.1590/S1679-62252008000100005.

**Britski HA, Birindelli JL. 2019.** Description of a new species of *Leporinus* (Characiformes: Anostomidae) from the Rio Tapajós basin, Brazil. *Zootaxa* **4603(1)**:183–191 DOI 10.11646/zootaxa.4603.1.10.

**Britski HA, Garavello JC. 1978.** Sobre *Leporinus octofasciatus* steindachner da bacia do Paraná (Pisces, Anostomidae). *Papéis Avulsos de Zoologia* **31(16)**:237–250.

**Britski HA, Garavello JC. 2005.** Uma nova espécie de Leporinus Agassiz, 1829, da Bacia Amazônica (Ostariophysi: Characiformes: Anostomidae). *Comunicações do Museu de ciências e Tecnologia da PUCRS: Série Zoologia* **18(2)**:75–83.

**Britski HA, Sato Y, Rosa ABS. 1984.** *Manual de identificação de peixes da Região de Três Marias.* Brasília: Câmara dos Deputados/CODEVASF, 143.

**Burns MD, Chatfield M, Birindelli JLO, Sidlauskas BL. 2017.** Systematic assessment of the Leporinus desmotes species complex, with a description of two new species. *Neotropical Ichthyology* **15(2)**:e160166 DOI 10.1590/1982-0224-20160166.

**Carvalho APC, Collins RA, Martínez JG, Farias IP, Hrbek T. 2018.** From shallow to deep divergences: mixed messages from Amazon Basin cichlids. *Hydrobiologia* **832**:317–329 DOI 10.1007/s10750-018-3790-x.

**Carvalho DC, Oliveira DA, Pompeu PS, Leal CG, Oliveira C, Hanne R. 2011.** Deep barcode divergence in Brazilian freshwater fishes: the case of the Sao Francisco River basin. *Mitochondrial DNA* **22(Supp 1)**:80–86 DOI 10.3109/19401736.2011.588214.

**Darriba D, Taboada GL, Doallo R, Posada D. 2012.** JModelTest2: more models, new heuristics and parallel computing. *Nature Methods* **9**:772.

**Dinno A. 2009.** Exploring the sensitivity of horn's parallel analysis to the distributional form of simulated data. *Multivariate Behavioral Research* **44(3)**:362–388 DOI 10.1080/00273170902938969.

**Drummond AJ, Simon YW, Phillips MJ, Rambaut A. 2006.** Relaxed phylogenetics and dating with confidence. *PLOS Biology* **4(5)**:e88 DOI 10.1371/journal.pbio.0040088.

**Drummond AJ, Suchard AM, Xie D, Rambaut A. 2012.** Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29(8)**:1969–1973 DOI 10.1093/molbev/mss075.

**Ezard T, Fujisawa T, Barraclough TG. 2009.** SPLITS: SPecies' LImits by Threshold Statistics. R package version 1.0-18/r45. *Available at http://R-Forge.R-project.org/projects/splits/*.

**Fowler HW. 1941.** Academy of natural sciences a collection of fresh-water fishes obtained in Eastern Brazil by Dr. Rodolpho Von Ihering. *Proceedings of the Academy of Natural Sciences of Philadelphia* **93**:123–199.

**Fraga E, Silva LMM, Schneider H, Sampaio I, Barros MC. 2014.** Variabilidade genética em populações naturais de Leporinus piau (Anostomidae, Characiformes) da bacia do Rio Itapecuru. *Revista Trópica* **8(2)**:28–40.

**Frantine-Silva W, Sofia SH, Orsi ML, Almeida FS. 2015.** DNA barcoding of freshwater ichthyoplankton in the Neotropics as a tool for ecological monitoring. *Molecular Ecology Resources* **15(5)**:1226–1237 DOI 10.1111/1755-0998.12385.

**Fricke R, Eschmeyer W, Laan RVanDer. 2021.** CAS - Eschmeyer's Catalog of Fishes - Species by Family. 2021. *Available at https://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp* (accessed 13 September 2021).

**Fujisawa T, Barraclough TG. 2013.** Delimiting species using single-locus data and the generalized mixed yule coalescent (GMYC) approach: a revised method and evaluation on simulated datasets. *Systematic Biology* **62**:707–724 DOI 10.1093/sysbio/syt033.

**Galetti Jr PM, Lima NRW, Venere PC. 1995.** A monophyletic ZW sex chromosome system in *Leporinus* (Anostomidae, Characiformes). *Cytologia* **60**:375–382 DOI 10.1508/cytologia.60.375.

**Garavello JC. 1979.** *Revisão taxonômica do gênero Leporinus Spix, 1829 (ostariophysi, Anostomidae) Tese de Doutorado*. São Paulo: Universidade de São Paulo..

**Garavello JC. 1989.** Leporinus microphthalmus sp.n. Bacia do Rio Paranaiba, Alto Paraná (Pisces, Anostomidae). *Revista Brasileira de Biologia* **49(2)**:497–501.

**Garavello JC, Britski HA. 2003.** Family Anostomidae. REIS and others, RE. Checklist of the Freshwater Fishes of South and Central America. Porto Alegre, Editora da Pontifícia Universidade Católica do rio Grande do Sul. 71–84.

**Garavello JC, Santos GM. 2007.** Two news species of Leporinus Agassiz, 1829 from Araguaia-Tocantins system, Amazon basin, Brazil (Ostariophysi, Anostomidae). *Brazilian Journal of Biology* **69(1)**:109–116.

**Gernhard T. 2008.** The conditioned reconstruted process. *Journal of Theoretical Biology* **253(4)**:769–778 DOI 10.1016/j.jtbi.2008.04.005.

**Géry J. 1977.** *Characoids of the world.* Neptune City: T.F.H. Publications.

**Guimarães EC, Brito OS, Oliveira RF, Aguiar RG, Ottoni FP, Guimarães KLA, Santos JP, Rodrigues LRR. 2021a.** *Peixes do rio Pindaré e suas potencialidades ornamentais.* São Luís: IMESC.

**Guimarães EC, Brito OS, Santos JP, Oliveira FR, FP Ottoni. 2021c.** Supplementary material to Guimarães others (2020): Peixes, Fauna de vertebrados ao longo da Estrada de Ferro Carajás. *Ichthyological Contributions of PecesCriollos* **1**:1–10.

**Guimarães EC, Oliveira RF, Brito OS, Vieira LO, Santos JP, Oliveira ES, Aguiar RG, Katz AM, Lopes DF, Nunes JLS, Ottoni FP. 2021b.** *Biodiversidade, potencialidades ornamentais e guia ilustrado dos peixes da Mata Itamacaoca município de Chapadinha-MA.* São Luis: IMESC.

**Hall TA. 1999.** BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**:95–98.

**Hebert PDN, Cywinska A, Ball SL, De Waard JR. 2003.** Biological 58 identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences* **270(1512)**:313–321 DOI 10.1098/rspb.2002.2218.

**Kress WJ, García-Robledo C, Uriarte M, Erickson DL. 2015.** DNA barcodes for ecology, evolution, and conservation. *Trends in Ecology and Evolution* **30**:25–35 DOI 10.1016/j.tree.2014.10.008.

**Kumar S, Stecher G, Li M, Knyar C, Tamura K. 2018.** MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* **35**:1547–1549 DOI 10.1093/molbev/msy096.

**Librado P, Rozas J. 2009.** DNAsp v5: a software for comprehensive analyses of DNA polymorphism data. *Bioinformatics* **25**:1451–1452 DOI 10.1093/bioinformatics/btp187.

**Melo FAG, Buckup PA, Ramos TPA, Souza AKN, Silva CMA, Costa TC, Torres AR. 2016.** Fish fauna of the lower course of the Parnaíba river, northeastern Brazil. *Boletim do Museu de Biologia* **38(4)**:363–400.

**Miller MA, Pfeiffer W, Schwartz T. 2010.** Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the gateway computing environments workshop*. 1–8.

**Myers GS. 1950.** Studies on South American fresh-water fishes. II. The genera of Anostominae Characids. *Stanford Ichthyological Bulletin* **3**:184–198.

**Nascimento MHS, Almeida MS, Veira MNS, Limeira Filho D, Lima RC, Barros MC, Fraga EC. 2016.** DNA barcoding reveals high levels of genetic diversity in the fishes of the Itapecuru Basin in Maranhão, Brazil. *Genetics and Molecular Research* **15(3)**:1–11 DOI 10.4238/GMR.15038476.

**Papa Y, Bail PYLe, Covain R. 2021.** Genetic landscape clustering of a large DNA barcoding data set reveals shared patterns of genetic divergence among freshwater fishes of the Maroni Basin. *Molecular Ecology Resources* **21(6)**:2109–2124 DOI 10.1111/1755-0998.13402.

**Paradis E, Schliep K. 2019.** Ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* **35**:526–528 DOI 10.1093/bioinformatics/bty633.

**Pereira LH, Hanner R, Foresti F, Oliveira C. 2013.** Can DNA barcoding accurately discriminate megadiverse Neotropical freshwater fish fauna? *BMC Genomic Data* **14**:20 DOI 10.1186/1471-2156-14-20.

**Piorski NM, Castro ACL, Pereira LG, Muniz MEL. 1998.** Ictiofauna do trecho inferior do Rio Itapecuru, nordeste do Brasil. *Boletim do Laboratório de Hidrobiologia* **11**:15–24.

**Puillandre N, Brouillet S, Achaz G. 2020.** ASAP: Assemble Species by Automatic Partitioning. *Molecular Ecology Resources* **21**:609–620.

**Puillandre N, Lambert A, Brouillet S, Achaz G. 2012.** ABGD, Automatic barcode gap discovery for primary species delimitation. *Molecular Ecology* **21(8)**:1864–1877 DOI 10.1111/j.1365-294X.2011.05239.x.

**R Core Team. 2021.** R: A language and environment for statistical computing. Version 4.1.0. Vienna: R Foundation for Statistical Computing. *Available at https://www.r-project.org*.

**Rambaut A. 2014.** FigTree v1.4.2. Institute of Evolutionary Biology, University of Edinburgh. *Available at http://tree.bio.ed.ac.uk/software/figtree/*.

**Rambaut A, Suchard MA, Xie D, Drummond AJ. 2014.** Tracer v1.6. *Available at http://tree.bio.ed.ac.uk/software/tracer*.

**Ramirez JL, Birindelli JLO, Galetti PM. 2017.** Um novo gênero de Anostomidae (Ostariophysi: Characiformes): diversidade, filogenia e biogeografia com base em dados citogenéticos, moleculares e morfológicos. *Molecular Phylogenetic Evolution* **107**:308–323 DOI 10.1016/j.ympev.2016.11.012.

**Ramirez JL, Carvalho-Costa LF, Venere PC, Carvalho DC, Troy WP, Galetti PM. 2016.** Testing monophyly of the freshwater fish Leporinus (Characiformes, Anostomidae) through molecular analysis. *Journal of Fish Biology* **88(3)**:1204–1214 DOI 10.1111/jfb.12906.

**Ramirez JL, Santos CA, Machado CB, Oliveira AK, Garavello JC, Britski HA, Galetti PM. 2020.** Molecular phylogeny and species delimitation of the genus *Schizodon* (Characiformes, Anostomidae). *Molecular Phylogenetics and Evolution* **153**:106959 DOI 10.1016/j.ympev.2020.106959.

**Ramos TPA, Ramos RTC, Ramos SAQA. 2014.** Ichthyofauna of the Parnaíba river basin, northeastern Brazil. *Biota Neotropical* **14(1)**:1–8 DOI 10.1590/S1676-06020140039.

**Ribeiro MFR, Piorski NM, Almeida ZS, Nunes JLS. 2014.** Fish aggregating known as moita, an artesanal fishing technique performed in the Munim River, State of Maranhão, Brazil. *Boletim Instituto de Pesca* **40(4)**:677–682.

**Sanger F, Nicklen S, Coulson AR. 1977.** DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences of the United States of America* **74(12)**:5463–5467 DOI 10.1073/pnas.74.12.5463.

**Sidlauskas BL, Vari RP. 2008.** Phylogenetic relationships within the South American fish family Anostomidae (Teleostei, Ostariophysi, Characiformes). *Zoological Journal of the Linnean Society* **154(1)**:70–210 DOI 10.1111/j.1096-3642.2008.00407.x.

**Sidlauskas BL, Vari RP. 2012.** Diversity and distribution of anostomoid fishes (Teleostei: Characiformes) throughout the Guianas. *Cybium* **36(1)**:71–103.

**Silva-Santos R, Ramirez JL, Freitas PD, Galetti Jr PM, Freitas PD. 2018.** Molecular evidences of a hidden complex scenario in *Leporinus cf. friderici*. *Frontiers in Genetics* **9**:47 DOI 10.3389/fgene.2018.00047.

**Soares EC. 2005.** *Peixes do Mearim.* São Luís: Instituto Geia.

**Stamatakis A. 2014.** RAxML Version 8: a tool for phylogenetic analysis and postanalysis of large phylogenies. *Bioinformatics* **30(9)**:1312–1313 DOI 10.1093/bioinformatics/btu033.

**Suchard MA, Lemey P, Baele G, Ayres DL, Drummond AJ, Rambaut A. 2018.** Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evolution* **4(1)**:vey016 DOI 10.1093/ve/vey016.

**Thompson JD, Higgins DG, Gibson TJ. 1994.** CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**:4673–4680 DOI 10.1093/nar/22.22.4673.

**Venables WN, Ripley BD. 2002.** *Modern Applied Statistics with S*. Fourth edition. New York: Springer. *Available at https://www.stats.ox.ac.uk/pub/MASS4/*.

**Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN. 2005.** DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences* **360**(**1462**):1847–1857 DOI 10.1098/rstb.2005.1716.

**Zhang J, Kapli P, Pavlidis P, Stamatakis A. 2013.** A general species delimitation method with applications to phylogenetic placements. *Bioinformatics* **29**(**22**):2869–2876 DOI 10.1093/bioinformatics/btt499.

## 3.2 CAPÍTULO II

Artigo publicado na revista Journal of Fish Biology**: EXPLORING HIDDEN DIVERSITY: MOLECULAR INSIGHTS INTO THE *LEPORINUS* SPECIES OF THE RIVERS OF THE BRAZILIAN STATES OF MARANHÃO AND PIAUÍ**

Received: 24 September 2023 | Revised: 10 March 2024 | Accepted: 27 March 2024
DOI: 10.1111/jfb.15753

REGULAR ARTICLE

JOURNAL OF FISH BIOLOGY

# Exploring hidden diversity: Molecular insights into the *Leporinus* species of the rivers of the Brazilian states of Maranhão and Piauí

**Maria Histelle Sousa Nascimento**[1,2,3] | **José Luis Olivan Birindelli**[4] | **Elmary Fraga**[3] | **Maria Claudene Barros**[2,3]

[1]Departamento de Desenvolvimento e Ensino, Instituto Federal de Educação, Ciência e Tecnologia do Maranhão, Caxias, Brazil

[2]Rede de Biodiversidade e Biotecnologia da Amazônia Legal, Universidade Federal do Maranhão, São Luis, Brazil

[3]Departamento de Química e Biologia, Universidade Estadual do Maranhão, Caxias, Brazil

[4]Departamento de Biologia Animal e Vegetal, Universidade Estadual de Londrina, Londrina, Paraná, Brazil

**Correspondence**
Maria Histelle Sousa Nascimento, State University of Maranhão, Departamento de Química e Biologia, Praça Duque de Caxias, S/N, Morro do Alecrim, Caxias, Maranhão, Brazil.
Email: histelle.nascimento@ifma.edu.br

**Funding information**
Fundação de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão; Universidade Estadual do Maranhão

## Abstract

The present study delved into the world of hidden diversity by examining specimens identified as *Leporinus piau* from the river basins of the northern Brazilian states of Maranhão and Piauí. Using genetic analyses that combined data from three mitochondrial markers and one nuclear marker, the study identified two well-supported groups, reinforcing the findings of previous publications. The first group, found in samples from the Itapecuru, Mearim, Turiaçu, and Pericumã basins, in Maranhão, appears to represent a relatively ancient diversification and the possibility of concealed cryptic diversity. The second group, comprising specimens from the Parnaíba (Piauí) and Mearim (Maranhão) basins, appears to have resulted from a more recent process of diversification and has a close relationship with *Leporinus friderici* from the type locality. Our findings not only confirm the existence of a complex scenario of cryptic diversity in the genus *Leporinus* from the study basins but also underscore the taxonomic inconsistencies within this group of fish. This study offers a comprehensive analysis of the species diversity of the Maranhão and Piauí basins, which are critical regions for the conservation of Amazonian fish, providing valuable insights for the sustainable management and conservation of these fish.

KEYWORDS
cryptic diversity, morphologically similar, species delimitation methods, taxonomic inconsistencies

## 1 | INTRODUCTION

The fish genus *Leporinus* Agassiz 1829 (Spix & Agassiz, 1829) of the family Anostomidae is considered to be one of the most speciose genera of the order Characiformes, which is distributed between Central America and southern South America, and the Amazonian basin holds most of this variability; the isolated coastal rivers of the Guianas, the São Francisco basin, and other coastal rivers of northeastern Brazil are less represented (Garavello & Britski, 2003; Santos & Zuanon, 2008). The species of this genus present a close anatomical similarity, which infers a widely distributed natural group (Garavello, 1979). Morphological and molecular studies indicate that this genus is not monophyletic (Ramirez et al., 2016; Sidlauskas & Vari, 2008), which highlights the need for a thorough taxonomic review of the species of this group. For example, in a study that integrated morphological, chromosomal, and molecular data, Ramirez, Birindelli, Carvalho, et al. (2017) described a new genus, *Megaleporinus* (Ramirez et al., 2017), which assigns the large-bodied *Leporinus* species to a distinct, monophyletic clade.

*Leporinus friderici* (Bloch, 1794) is one of the most amply distributed species of the genus and has been found in the majority of the hydrographic basins of South America (Garavello et al., 1992). Given its broad distribution, many different studies have investigated the characteristics of this species over the years and have found considerable variation in the morphological and genetic patterns of the different *L. friderici* populations (Garavello et al., 1992; Géry et al., 1987; Nascimento et al., 2023; Renno et al., 1990; Renno et al., 1991; Sidlauskas & Vari, 2012; Silva-Santos et al., 2018). The sum of this evidence indicates that the *L. friderici* morphotype does in fact represent a species complex, with only the *L. friderici* specimens from the rivers of Suriname and French Guiana being recognized as members of the type species (Sidlauskas & Vari, 2012). This led to the application of the provisional nomenclature *Leporinus cf. friderici* to designate the specimens that deviate from the type species (Silva-Santos et al., 2018).

In their molecular study, Silva-Santos et al. (2018) identified two principal scenarios within the *L. cf. friderici* complex. The first scenario is an *L. cf. friderici* sensu stricto group or clade, which may in fact represent a true species complex, formed by recent diversification in the basins of the Brazilian Shield. This would have resulted in genetic structuring throughout this hydrographic network, which is probably related to the geomorphological processes that established the present-day hydrographic layout. The authors nevertheless conclude that the taxonomic status of these populations is still unclear and requires further, more detailed research. The second scenario identified by Silva-Santos et al. (2018) refers to an unnamed group or clade, which encompasses the relatively ancient diversification of this taxon, and includes as-yet undescribed cryptic species currently designated *L. cf. friderici* (Figure 1a).

Nascimento et al. (2023) recently published a rearrangement of the taxa, in which the *L. friderici/L cf. friderici* and *Leporinus piau* Fowler (1941) populations of the basins of the Itapecuru, Mearim, Pericumã, and Parnaíba rivers form two distinct groups—one group that includes *L. friderici* from the basin of the Tampok River and *L. piau* from the basins of the Mearim and Parnaíba rivers, and a second group that includes *L. piau* from the basins of Maranhão, together with *L. cf. friderici* from the Amazon basin (Figure 1b). The presence of *L. piau* in both groups indicates that the specimens of this species collected in central Maranhão and Piauí were identified erroneously, based on their morphological characteristics, which reflects the taxonomic inconsistencies that persist among the morphologically similar species of this genus. A relatively high level of genetic divergence, of up to 10.7%, was also found between these two groups.

The present study tested two hypotheses based on the analysis of mitochondrial and nuclear DNA sequences: (i) the *L cf. friderici/L. friderici* and *L. piau* group identified by Nascimento et al. (2023) is in fact the *L. friderici* sensu stricto clade proposed by Silva-Santos et al. (2018), and (ii) the second group of *L. cf. friderici* and *L. piau* corresponds to the unnamed clade.

Given the clear taxonomic instability of *Leporinus*, we sampled the species *L. piau* in five basins, namely those of the Itapecuru, Mearim, Pericumã, and Turiaçu rivers in the Brazilian state of Maranhão and the Parnaíba basin in the state of Piauí. The study revealed the same taxonomic scenario in Maranhão as that found in South America as a whole by Silva-Santos et al. (2018). This similarity indicates that

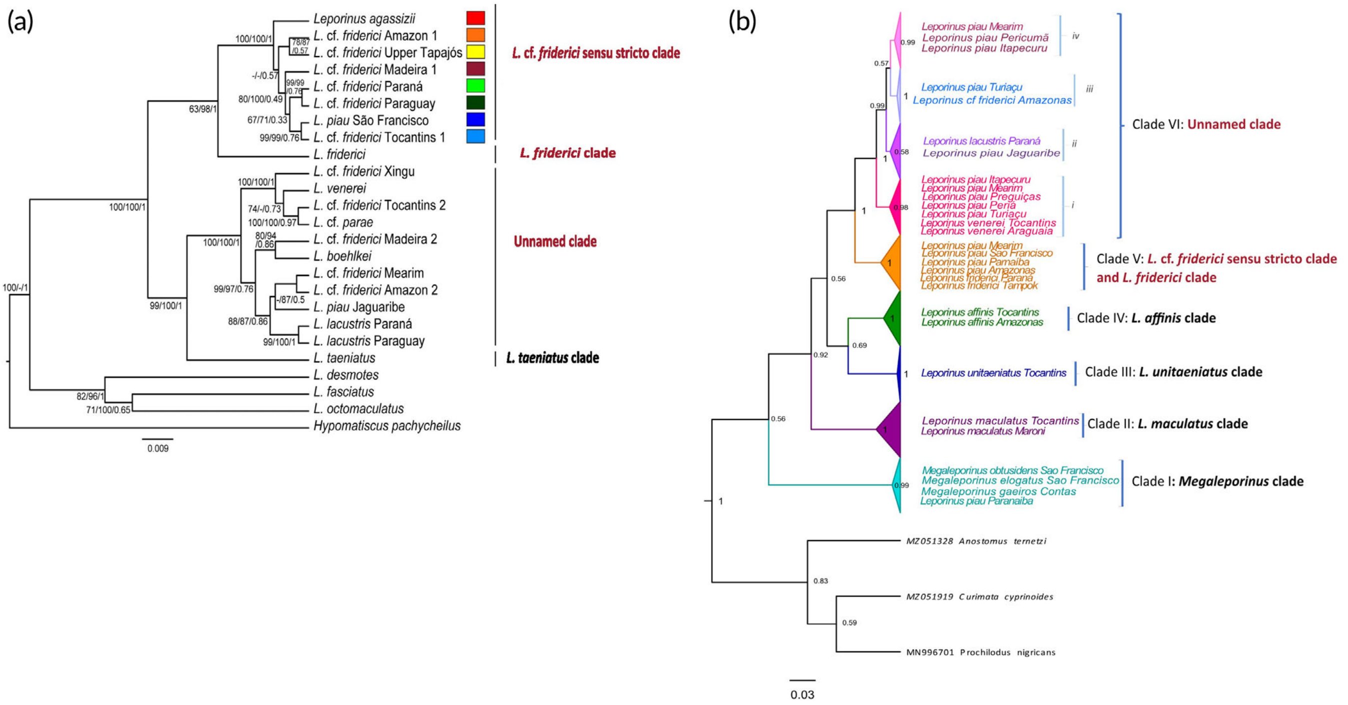


**FIGURE 1** (a) Species tree, showing the phylogenetic relationships of *Leporinus cf. friderici*, adapted from Silva-Santos et al. (2018). (b) Collapsed tree showing the arrangement of the MOTUs (molecular operational taxonomic units) of the *Leporinus piau* and *L. cf. friderici* species, adapted from Nascimento et al. (2023).

Maranhão is an ideal geographic model for the analysis of the genetic diversity of this genus.

## 2 | MATERIALS AND METHODS

A total of 77 *L. piau* specimens were collected from the Itapecuru, Mearim, Turiaçu, Pericumã, and Parnaíba rivers (Figure 2; Table S1). Twenty-seven sequences were obtained from GenBank, namely 25 of *Leporinus* species and 2 species of other characiform genera, which were included as the out-group (Table S2). The collection of the specimens analysed in the present study was authorized by IBAMA (license 02012.004159/2006) and SISBIO (licenses 42119-2/2013, 46367-1/2014, 83138-1/2022). The experimental procedures were approved by the Maranhão State University (UEMA) Committee for the Ethical Treatment of Animals (protocol 47/2022).

Once collected, the specimens were taken to the Molecular Biology and Genetics Laboratory (GENBIMOL) at the UEMA Caxias campus, where they were photographed and each identified with an individual code. Samples of muscle tissue were extracted from these specimens for genetic analyses. The specimens were fixed in 10% formaldehyde and preserved in 70% alcohol. The prepared specimens were deposited in the Zoology Museum of Londrina State University (MZUEL) in Paraná, Brazil, for cataloging by specialists.

### 2.1 | Molecular analyses

The DNA of the samples was extracted, amplified, and sequenced in the GENBIMOL. The total DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega). The partial gene sequences were isolated and amplified by PCR using specific primers for the 16S rRNA (ribosomal RNA) (Palumbi et al., 1991) and cytochrome c oxidase subunit I (COI) genes (Ward et al., 2005), as well as the L14725L (Smith & Patton, 1993) and MVZ16 (Santos et al., 2003) primers for the cytochrome b (cyt *b*) gene, and the TROPF (Calcagnotto et al., 2005) and TROPR (Santos, 2007) primers for the α-Tropomyosin (TROP) gene (Table S3). The TROPF primers are located in the region of intron 3 and the TROPR primers in exon 5 (Table S3). The COI sequences used in this study are the same as those obtained by Nascimento et al. (2023).

The PCRs were run in a final volume of 25 μL containing 4 μL of deoxynucleotide triphosphate (1.25 M), 2.5 μL of 10× buffer, 0.5 μL of $MgCl_2$ (25 mM), 0.25 μL of the forward and reverse primers at 200 nM,

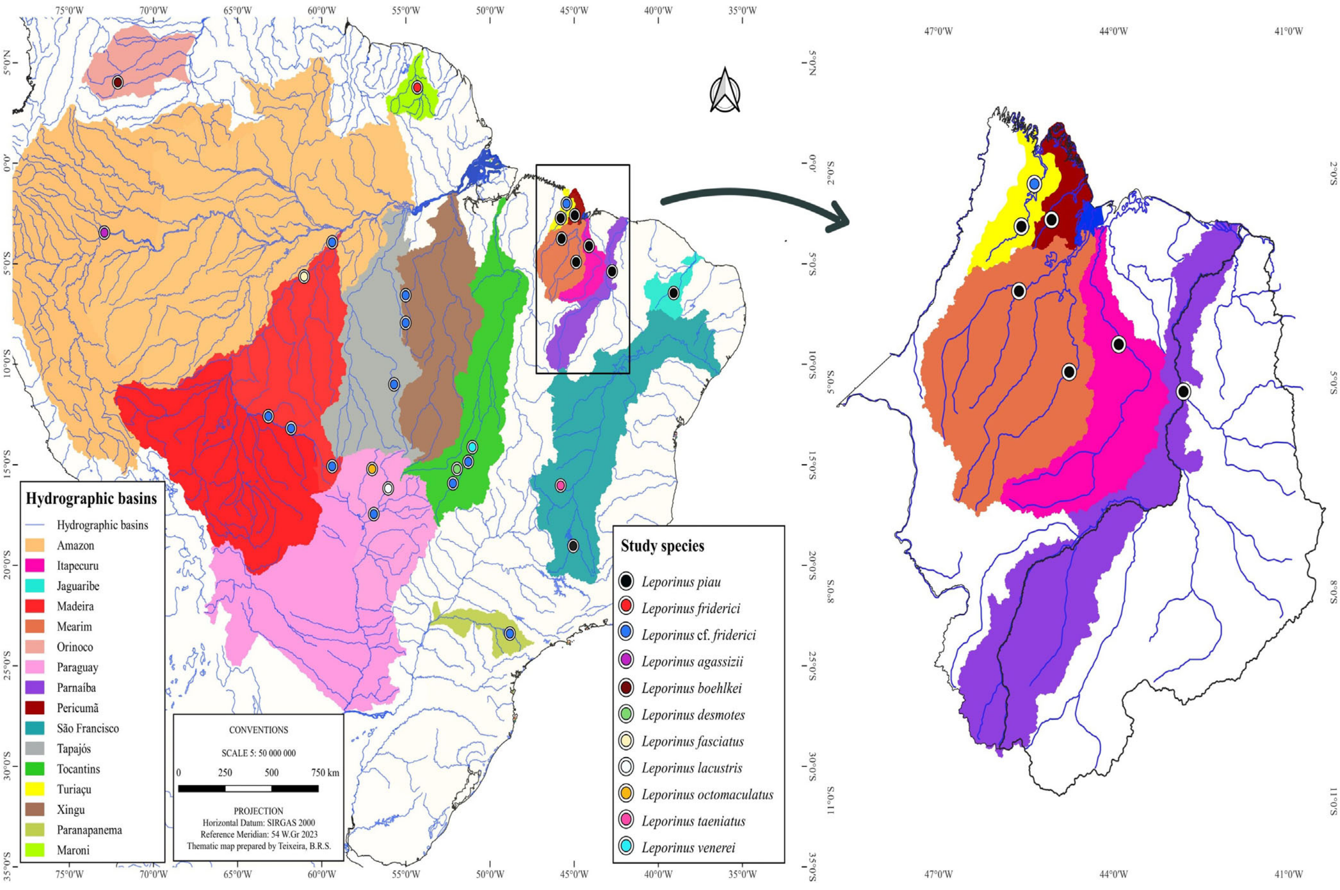


**FIGURE 2** *Leporinus* specimens analysed in the present study. The dots (localities) are color coded by species, and each hydrographic basin included in the present study is highlighted using a different color.

1 μL of the DNA sample at 50 ng, and 0.2 μL of 0.02 U/μL of Taq DNA polymerase. The amplification cycle began with an initial denaturation for 5 min at 95°C; a cycle of denaturation, annealing, and extension specific to each pair of primers (Table S3); and a final extension of 5 min at 72°C. The positive PCR products were purified using an Exo-Sap IT Kit and sequenced using the Sanger et al. (1977) method using the BigDye Terminator 3.1 Kit, with the products being processed in an automated ABI 3500 capillary system (Life Technologies).

## 2.2 | Genetic data analyses

The sequences were aligned and edited using the CLUSTAL-W package (Thompson et al., 1994) of Bioedit 7.2.5 (Hall, 1999), where the gaps, insertions, and deletions were identified and corrected manually. The haplotypes were delineated using DnaSP 5.1 (Librado & Rozas, 2009), based on the sequences obtained from the basins of Maranhão and Piauí, with an unrooted haplotype network being compiled in NETWORK 10.2 using the median-joining network algorithm (Bandelt et al., 1999). The mitochondrial and nuclear gene sequences were concatenated using SeaView 4 (Gouy et al., 2010) for phylogenetic analysis.

The maximum likelihood (ML) and Bayesian inference (BI) phylogenetic trees were constructed through the partitioning of the dataset in PartitionFinder2 (Lanfear et al., 2017). The ML analyses were run in RAxML, version 8.2.10 (Stamatakis, 2014), with 1000 rapid bootstrap replicates and the best scores being determined using the GTRGAMMA model, run in the CIPRES Science Gateway (Miller et al., 2010).

The partitioned BI analyses were run in BEAST, version1.10.4 (Drummond et al., 2012; Suchard et al., 2018), using the GTR + I, K81UF + G, and TIM + I + G nucleotide substitution models, which were selected following the Bayesian criterion in JModeltest 2 (Darriba et al., 2012) for the 16S rRNA (GTR + I), COI (K81UF + G), cyt *b* (K81UF + G), and TROP (TIM + I + G) genes. A relaxed clock with lognormal distribution and the birth–death speciation process were applied to all the partitions. This analysis was based on 150 million generations, with the parameters logged every 5000 generations and the log files being verified in Tracer version 1.6 (Rambaut et al., 2014) to evaluate the convergence and the most adequate interval for the burn-in, with the first 10% of the trees discarded as burn-in. Convergence was considered to be adequate when the parameters presented an effective sample size of at least 200. The consensus tree was generated in BEAST, summarized in TreeAnnotator version 10.4 (Suchard et al., 2018), and visualized and edited in FigTree version 1.4.2 (Rambaut, 2014).

The mean genetic distances between the clades identified in the phylogenetic trees were obtained based on p-distance model using MEGA 11 (Tamura et al., 2021).

## 2.3 | Species delimitation analyses

Species delimitation based on the molecular data was run in two steps, with no out-group. In the first step, exploration or candidate species discovery methods were used without attributing a priori information on the existence of potential species. Three distinct approaches were used here—the automatic barcode gap discovery (ABGD) for the data on a single gene (rRNA 16S, COI, cyt *b*, or TROP, see Supporting Information S1), the Poisson tree process (PTP), and the generalized mixed Yule coalescent (GMYC) approach for the concatenated mitochondrial sequences (rRNA 16S + COI + cyt *b*). The second step was based on the Bayesian phylogenetics and phylogeography (BPP) species validation test, using the concatenated mitochondrial and nuclear data (rRNA16S + COI + cyt *b* + TROP).

The ABGD test (Puillandre et al., 2012) was run in an online tool (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) using the dataset of the aligned sequences with the default settings, P (prior limit of intraspecific diversity) ranging between 0.001 and 0.1; X (gap widths) = 1 using the K80 (Kimura) and JC69 (Jukes-Cantor) models. The PTP (Zhang et al., 2013) was run on the web server https://species.h-its.org/. In this case, the input was the ML phylogenetic tree produced in RaxML, version 8.29 (Stamatakis, 2014), which is available in the CIPRES Science Gateway, version 3.3 (Miller et al., 2010). The GMYC (Fujisawa & Barraclough, 2013) was based on the ultrametric consensus tree built in BEAST, version 1.8, which was submitted to the Ape (Paradis & Schliep, 2019), Splits (Ezard & Purvis, 2009), Paran (Dinno & Dinno, 2018), and Mass (Venables & Ripley, 2002) packages available in R, version 4.1.0 (Venables & Smith, 2021).

A Bayesian multilocus species delimitation analysis was conducted using the BPP programme, version 4.7.0 (Rannala & Yang, 2003; Yang, 2015; Yang & Rannala, 2010). The BPP is a Bayesian Markov chain Monte Carlo (MCMC) method for analysing DNA sequence alignments under the multispecies coalescent (MSC) model, which can accurately assign individuals at the species level without setting determined distance thresholds in advance and thus provides significant improvements for species delimitation analyses (Yang, 2015; Yang & Rannala, 2017).

The population size ($\theta$) and divergence times ($\tau$) were attributed to the gamma priors $\theta \sim G\ (2, 2000)$ and $\theta \sim G\ (2, 1000)$, respectively. We also investigated the influence of different gamma priors on the posterior probabilities, although the results were robust in terms of the attribution of the priors.

The number of species and the allocation of individuals to the different species were determined by analysis A11 of the BPP. We also ran analysis A01, which encompasses the heterogeneity of the gene trees and uses the nearest neighbor interchange or subtree pruning and regrafting algorithms to infer the topology of the species tree based on coalescence. In comparison with more traditional phylogenetic analysis, the MSC models the coalescence process along the branches, in both the extant and ancestral species. This method can thus be considered to provide an incomplete ordination of the lineages, due to the ancestral polymorphism and the conflicts between the gene and species trees.

Following the A11 and A01 analyses, we ran the A00 analysis to outline the posterior theta distribution. Each rjMCMC was run for 100,000 generations, with a burn-in of 8000 generations and a sampling frequency of 2. In this analysis, the posterior probabilities of at

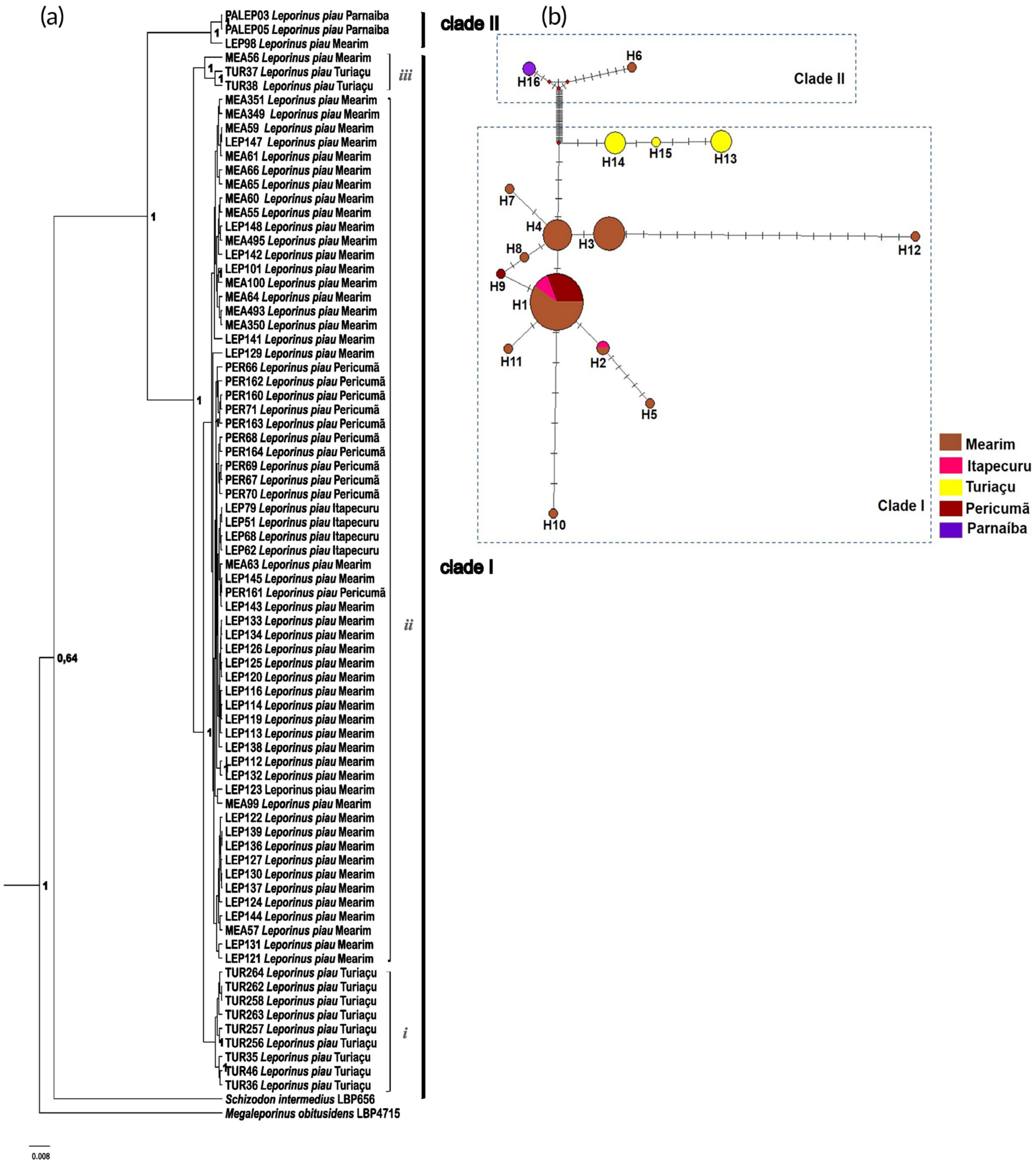


**FIGURE 3** (a) Bayesian Inference phylogenetic tree for the *Leporinus* specimens from the hydrographic basins of the Brazilian states of Maranhão and Piauí. This tree was generated using the four concatenated molecular markers sequenced in the present study (COI, rRNA 16S, cyt *b*, and TROP). (b) Haplotype network derived from the *Leporinus* sequences. Each circle represents a haplotype, whose size is proportional to its frequency, whereas the bars on the lines that connect the haplotypes indicate the number of mutations that separate them. The red circles indicate haplotypes that are missing or were not sampled. The collecting localities are color coded, as shown in the legend.

least 0.95 were considered to be strongly supported, those between 0.90 and 0.95 were considered to have moderate support, and those <0.90 were classified as weakly supported.

The A00 analysis provides an estimate of the parameters of the MSC model (species delimitation = 0, species tree = 0), which generates the posterior distribution of the divergence times ($\theta$) and population sizes ($\tau$) of the species in the MSC model when the phylogeny of the species is fixed, with the parameters being a product of the time and mutation rates. The A01 analysis estimates the species tree (species delimitation = 0, species tree = 1) based on the assumption that the attribution and delimitation of the species are fixed. Based on the MSC model, the A01 analysis considers the polymorphism of the ancestral species and the conflicts between the species tree and the genetic trees. The A11 analysis provides a combined delimitation of the species and estimates the species trees (species delimitation = 1, species tree = 1), with the algorithm assessing different species delimitation models and phylogenies.

**TABLE 1** Mean inter- and intra-clade genetic distances in *Leporinus*.

**(a)**

**% Genetic distance**

| **Clade** | **Clade I** | **Clade II** |
|---|---|---|
| Clade I | **0.68** | |
| Clade II | 5.36 | **0.55** |

**(b)**

**% Genetic distance**

| | | | | **Unnamed** | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Clade** | | ***Leporinus cf. friderici senso stricto*** | ***L. friderici*** | ***i*** | ***ii*** | ***iii*** | ***iv*** | ***v*** | ***vi*** |
| *L. cf. friderici senso stricto* | | **2.17** | | | | | | | |
| *L. friderici* | | 4.97 | **n/c** | | | | | | |
| Unnamed | *i* | 7.11 | 6.78 | **2.48** | | | | | |
| | *ii* | 6.85 | 6.98 | 3.63 | **2.00** | | | | |
| | *iii* | 7.19 | 6.98 | 3.18 | 3.82 | **n/c** | | | |
| | *iv* | 7.33 | 7.06 | 2.95 | 3.16 | 2.41 | **n/c** | | |
| | *v* | 6.70 | 7.35 | 3.15 | 2.95 | 2.39 | 1.98 | **0.31** | |
| | *vi* | 6.75 | 7.44 | 3.20 | 3.26 | 2.74 | 1.90 | 1.17 | **0.39** |

*Note*: (a) Genetic distances between and within (bold values) the *Leporinus* clades from the basins of Maranhão and Piauí based on the concatenated sequences of the COI, rRNA 16S, cyt *b*, and TROP genes; (b) genetic distances between the *Leporinus* clades from Maranhão and Piauí, and the GenBank sequences, based on the concatenated sequences of the COI, rRNA 16S, cyt *b*, and TROP genes. The presence of n/c in the results denotes cases in which it was not possible to estimate evolutionary distances.

Abbreviation: COI, cytochrome c oxidase subunit I.

## 3 | RESULTS

The ML and BI trees generated from the multilocus sequences obtained from the Maranhão and Piaui basins reveal two extremely well-supported groups (posterior probability = 1, bootstrap = 100), identified here as clades I and II (Figure 3a; Figure S1), which correspond to Nascimento et al.'s (2023) clades VI and V, respectively. Clade I grouped the *L. piau* specimens from the basins of the Maranhão river, whereas clade II included those from the basins of the Parnaíba and Mearim rivers (Figure 3a). Three subgroups can be observed within clade I; however, the first includes specimens only from the Turiaçu basin; the second comprises specimens from the Mearim, Itapecuru, and Pericumã basins; and the third includes specimens from the Turiaçu and Mearim basins. The haplotype network (Figure 3b) further supported the differentiation of clades I and II, as well as the separation of the Turiaçu group and the differentiation of haplotype H12 from the Mearim basin, which may represent a distinct group. The mean genetic divergence between clades I and II was 5.36% (Table 1a).

When the GenBank *Leporinus* sequences were added to the analyses, the BI and ML trees revealed that the samples collected in the present study encompassed four distinct subclades differentiated by the colouration of the branches (Figure 4) and distributed in two superclades. The first group that includes our samples is the *L. cf. friderici* clade from the Amazon, Madeira, Tapajós, Tocantins, Paraná, and Paraguay basins, together with *L. piau* from the Parnaíba (PALEP03 and 05), Mearim (LEP98), and São Francisco basins, on the branches highlighted in blue (Figure 4) with a mean distance of 2.17%. We adopted the nomenclature of Silva-Santos et al. (2018) for this group, *L. cf. friderici* sensu stricto (Figure 4; Figure S2) and associated with this group; we have a clade formed only by *L. friderici* from the basins of Suriname (Figure 4; Figure S2), which was designated as the *L. friderici* clade, following Silva-Santos et al. (2018). The mean genetic divergence between the *L. friderici* and *L. cf. friderici* sensu stricto clades was 4.97% (Table 1b).

The clade highlighted in green includes *L. piau* from the Turiaçu (TUR37 and 38) and Mearim (MEA56) basins, which was grouped with a posterior probability of 0.88 with *Leporinus venerei* Britski and Birindelli (2008) from the Tocantins basin, *L. cf. friderici* from the Tocantins and Xingu basins, and *Leporinus trimaculatus* Garavello and Santos (1992) from the Amazon basin (Figure 4), with a mean divergence of 2%. The samples from Maranhão form a subclade here, revealing their presumed differentiation from the other species. The majority of the *L. piau* samples from the Turiaçu basin was grouped with *L. cf. friderici* from the Amazon and Madeira basins, with a posterior probability of 1, on the branches in orange (Figure 4). The fourth clade (magenta) was formed by the remaining *L. piau* samples from the basins of Maranhão (Mearim, Itapecuru, and Pericumã) obtained in the present study, together with the GenBank *L. cf. friderici* sequence from the Mearim basin (Figure 4), with a mean genetic divergence of 0.39% (Table 1b).

All these clades, with the exception of the *L. cf. friderici* sensu stricto clade and *L. friderici* from the type locality, form an unnamed

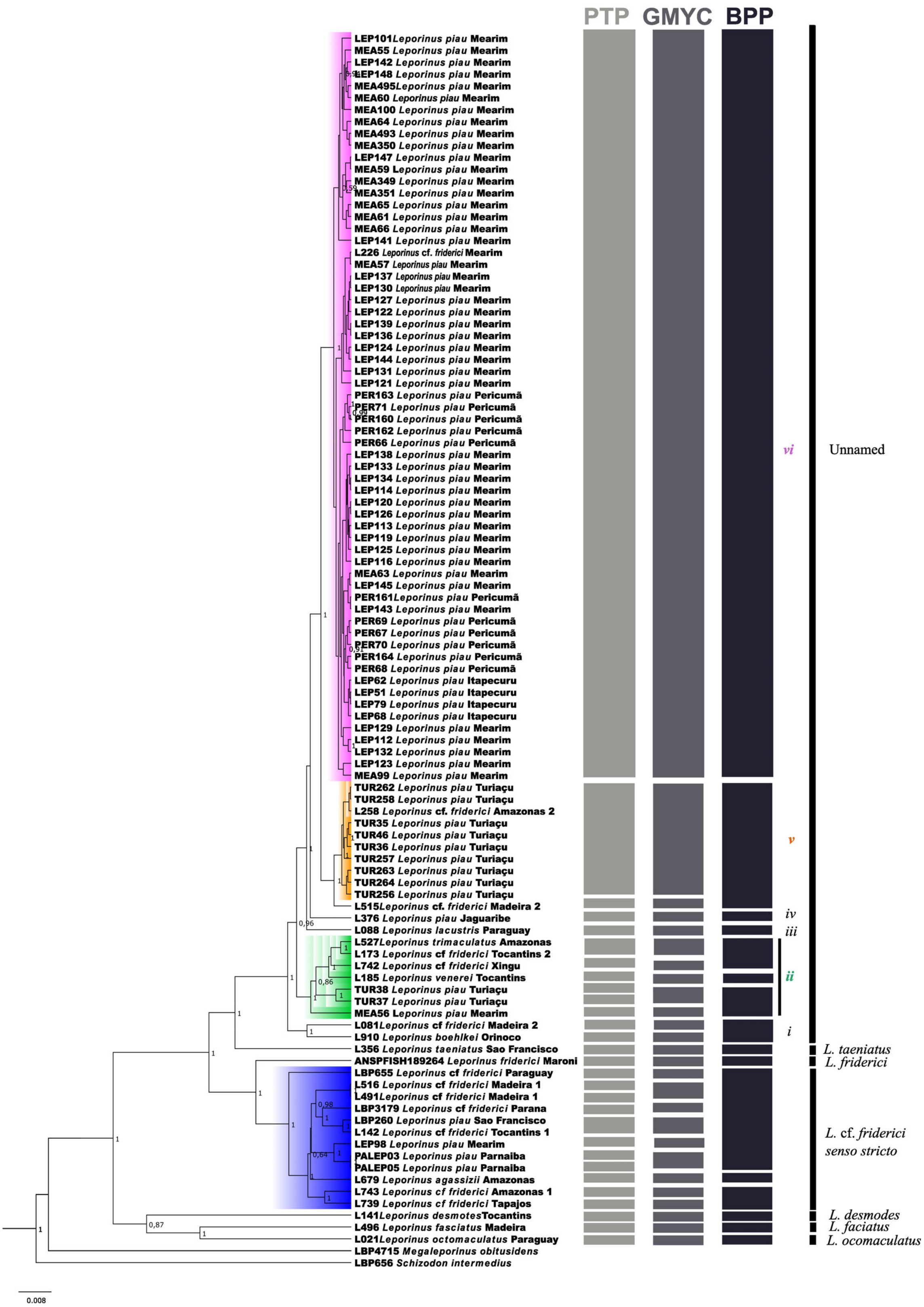


**FIGURE 4** Bayesian Inference phylogenetic tree for the *Leporinus* specimens from the basins of the Brazilian states of Maranhão and Piauí, together with the GenBank sequences from other basins, based on the concatenated sequences of the mitochondrial and nuclear genes. The numbers above the branches are the posterior probabilities for the Bayesian Inference. The species identified using the different species delimitation methods are shown by color-coded bars.

superclade, following the nomenclature adopted by Silva-Santos et al. (2018), together with *L. piau* from the Jaguaribe basin, *Leporinus lacustris* Amaral Campos (1945) from the Paraguay basin, and *Leporinus boehlkei* Garavello (1988) from the basin of the Orinoco River, with absolute support, that is, a posterior probability of 1. To facilitate the understanding of this configuration, all the unnamed groups of this superclade have been designated a lowercase Roman numeral, from *i* to *vi* (Figure 4).

The genetic divergence between these six groups was high, ranging from 1.17% to 3.82% (Table 1b).

The species delimitation analyses used here produced similar arrangements (Figure 4; Supporting Information S1). The exploration and candidate species discovery methods employed here (Figure 4), using only the mitochondrial data, revealed 29 (PTP) and 26 (GMYC) molecular operational taxonomic units (MOTU). The results of both these analyses made biological sense in the context of our dataset, given that the valid nominal species were allocated to distinct MOTUs (Figure 4).

In the candidate species validation approach, using both mitochondrial and nuclear data, the BPP separated the samples in 16 MOTUs, once again, with the valid nominal species being allocated to distinct MOTUs and the *L. cf. friderici* sensu stricto clade being defined as a single MOTU. In addition, group *ii* of the unnamed superclade is divided into three MOTUs: the first corresponds to the samples from the Turiaçu (TUR37 and 38) and Mearim (MEA56) basins, the second contains only *L. venerei*, and the third includes *L. cf. friderici* from the Tocantins and Xingu basins together with *L. trimaculatus* from the Amazon basin (Figure 4). This separation in distinct MOTUs is consistent with the differentiation of the samples from Maranhão, in terms of both their formation of a subgroup and the mean genetic divergence of 2% observed among the samples of group *ii* (Table 1b).

For the unnamed clade, once again, the distance-based species delimitation methods were the most effective for the distribution of the valid nominal taxa in distinct MOTUs *L. venerei*, *L. trimaculatus*, *L. boehlkei*, *L. lacustris*, and *L. piau* from the type locality.

## 4 | DISCUSSION

The correct identification of species is an essential prerequisite for any biological research and in particular for successful conservation practices (Li et al., 2018). Traditional taxonomic approaches depend on the interpretation of morphological characteristics and rely fundamentally on the knowledge of specialists in the fields of taxonomy and systematics (Bingpeng et al., 2018; Nelson et al., 2016; Xu et al., 2021). However, individual fish may vary enormously in their body shape and size, number of rays and scales, and colouration (Strauss & Bond, 1990; Zhang & Hanner, 2012), whereas some natural groups may present little noticeable morphological variation, leading to their inclusion in a single nominal taxon (Bickford et al., 2007). In addition, sister species may often share morphological traits, leading to identification errors in taxonomic studies (Li et al., 2018). These inherent limitations of the traditional taxonomic systems reinforce the need for a new, more reliable approach to the identification of fish species, in which DNA sequences provide a more objective and reliable tool for the delimitation of species, when used systematically in conjunction with morphological traits (Steinke et al., 2009).

Species with an ample geographic distribution and a long history of taxonomic controversy, such as those of the genus *Leporinus*, may often conceal cryptic diversity, given that the identification of species based on morphological traits may not contemplate the full intraspecific variation in the taxon, especially considering that the morphological characteristics of an organism may not necessarily be altered by the speciation process, especially in the case of recent events (Fraga et al., 2014; Garavello et al., 1992; Géry et al., 1987; Nascimento et al., 2023; Ramirez et al., 2016; Renno et al., 1990; Renno et al., 1991; Sidlauskas & Vari, 2012; Silva-Santos et al., 2018). The reliable identification of cryptic diversity has profound implications for the advancement of our understanding of species and their ecological and biogeographic features, as well as evolutionary patterns, through the integrated analysis of morphological and molecular data, which has proven to be the most effective approach (Kress et al., 2015).

In the present study, the set of specimens identified initially as *L. piau*, which occur in the basins of Maranhão and Piauí, were grouped together with *L. cf. friderici*, in a well-supported arrangement, rather than with *L. friderici*, in either the sensu stricto or the unnamed clade (Figure 4), which indicates that they are genetically distinct from the *L. friderici* from Suriname (Maroni basin). This is consistent with the study of Sidlauskas and Vari (2012), who found that the *L. friderici* populations of Suriname and French Guiana are genetically distant from the Brazilian populations, which also supports the provisional identification of the Brazilian specimens as *L. cf. friderici*. As observed by Silva-Santos et al. (2018), *L. friderici* from Suriname formed a clade distinct from those of the other specimens (Figure 4), which reinforces the hypothesis presented here, that is, that the *L. cf. friderici* and *L. piau* specimens collected here are not conspecific with *L. friderici* from the type locality, which further reinforces the conclusion that *L. piau* from Maranhão and Piauí is nominally *L. cf. friderici*, or at least part of the specimens, given that the other part makes up the unnamed clade and are subdivided into three groups (groups *ii*, *v*, and *vi* of the unnamed superclade; Figure 4).

The sum of the evidence thus indicates that the *L. piau* denomination was adopted erroneously for the samples from the Maranhão and Piauí basins, given that the morphology of *L. piau* is consistent with that of *L. friderici*, which is increasingly being considered to be a species complex (Nascimento et al., 2023; Ramirez et al., 2016; Sidlauskas & Vari, 2012; Silva-Santos et al., 2018). The results of the present study indicate the presence of two groups, one formed by the specimens of the Mearim and Parnaíba basins, and the other by the *L. cf. friderici* specimens from the other basins that represent the *L. cf. friderici* clade (sensu stricto), given that, as found by Silva-Santos et al. (2018), this clade is also associated with the *L. friderici* clade from the type locality. The samples of these two clades share a number of morphological traits, such as the presence of three dark, elongated spots along the lateral line, a 4/4 dental formula, and certain meristic parameters (Britski & Birindelli, 2008; Garavello & Britski, 1987; Silva-

Santos et al., 2018). These common characteristics have generated persistent inconsistencies in the taxonomy of this group, which has led to the constant revision and reevaluation of the fish species of the genus *Leporinus*.

The second group matched with the unnamed clade identified by Silva-Santos et al. (2018), which is made up of a number of different *Leporinus* species, including *L. piau* from the type locality (Jaguaribe), which indicates that this species may occur in the basins of Maranhão; its more basal position in comparison with the *L. piau* from the Turiaçu, Pericumã, Mearim, and Itapecuru basins means that this arrangement provides an excellent baseline for a more detailed investigation of the possible differentiation of the populations from the different basins, especially as the samples from the Mearim, Pericumã, and Itapecuru basins share more genetic similarities than those of *L. piau* from the Turiaçu basin, whose biodiversity has, historically, been seen as having greater affinities with that of the Amazon basin (Abreu et al., 2019).

The majority of the samples identified as *L. piau* in the unnamed clade (Figure 4), together with the *L. cf. friderici* specimens from the Mearim, Tocantins, Turiaçu, Xingu, and Madeira basins, and *L. piau* from the Jaguaribe basin indicate a relatively ancient diversification process. This is consistent with the findings of Silva-Santos et al. (2018), who grouped these taxa with four other nominal species, *L. boehlkei*, *L. trimaculatus*, *L. lacustris*, and *L. venerei*. These authors nevertheless alert that this group may still contain cryptic species identified as *L. cf. friderici*. In addition to corroborating the findings of Silva-Santos et al. (2018), the results of the present study show that the specimens that make up the unnamed clade form subgroups in the basins of Maranhão, as seen in the case of subgroup *i* in Figure 3a. The formation of these subgroups is likely the result of the size of the sample employed here, which emphasizes the need for a sample of adequate size to ensure the most conclusive analysis of the data.

The *L. cf. friderici* sensu stricto clade encompassed specimens of *L. cf. friderici* from different basins, together with *L. piau* from the Mearim and Parnaíba basins, which reinforces the hypothesis that the *L. piau* samples from this group are, in fact, *L. cf. friderici* (Figure 3). Silva-Santos et al. (2018) obtained similar results, concluding that this group represents a typical scenario of recent diversification from the Suriname population of *L. friderici* (the type species), which appears as the basal group, forming a strictly related group that is nevertheless composed of potentially cryptic species, revealing the taxonomic uncertainties that are inherent to this type of diversification process (Ramirez, Birindelli, Carvalho, et al., 2017).

One of the principal taxonomic problems of Brazilian *Leporinus* is related to the general lack of morphological differentiation among the species, as observed in the case of *L. piau* and *L. friderici*, which became even more problematic when Fowler (1941) selected the Salgado River, in the Jaguaribe basin, as the type locality of *L. piau*, and included a paratype from the Jatobá River, in the basin of the São Francisco River, which favored the recurrent collection of records of *L. piau* that were genetically distinct from *L. piau* from the type locality. Given their significant genetic differences in comparison with *L. piau* from the type locality and the fact that their morphotype aligns with that of *L. cf. friderici*, the specimens from the São Francisco basin identified as *L. piau* are, in fact, a different species, which has been designated *L. cf. friderici* (Carvalho et al., 2011; Garavello & Britski, 2003; Ramirez et al., 2016; Silva-Santos et al., 2018).

The complex scenario reported by Silva-Santos et al. (2018) in their analysis of *L. cf. friderici* specimens from nine basins that originate on the Brazilian Shield (the Mearim, Amazon, Madeira, Tapajós, Xingu, Tocantins, São Francisco, Paraguay, and Paraná rivers), which differentiated two distinct groups, was also observed here in the hydrographic basins of Maranhão (Itapecuru, Mearim, Pericumã, and Turiaçu) and Piauí (Parnaíba). The sharing of genetic stocks between basins was also apparent, as in the case of the Itapecuru, Mearim, and Pericumã basins, which shared haplotypes, although the Turiaçu basin had distinct and unique haplotypes. The samples from the Parnaíba River in Piauí were also different from those of the rivers of Maranhão, once again, with a unique haplotype (Figure 3b).

The sharing of haplotypes among the Maranhão basins has been confirmed in previous studies, as well as the greater distance of these fish in comparison with those from the Parnaíba basin, in Piauí (Carvalho-Costa et al., 2011; Hubert & Renno, 2006). In Maranhão, this close relationship is found primarily in the fish of the order Characiformes, which form an isolated and divergent clade, with a considerable number of endemic species, in comparison with the fish of other orders (Hubert & Renno, 2006). This interaction may have been supported by the temporary connections arising during past marine regressions, which linked rivers through their estuaries, favoring dispersal between basins that had previously been isolated (Abreu et al., 2019; Vail & Hardenbol, 1979). By contrast, the subsequent increase in sea level likely fragmented populations in the basins that had previously been interlinked, supporting allopatric differentiation (Cardoso & Montoya-Burgos, 2009), as observed in the present study.

The group of samples that includes the Turiaçu basin and rivers of the Amazon basin, which had significant support (Figure 4), can be accounted for by the occurrence of dispersal across the coast during periods of marine regression. In a study of the fish of the genus *Pseudoplatystoma* Bleeker, 1862, Carvalho-Costa et al. (2011) found that the specimens from the Turiaçu basin were more closely related, in evolutionary terms, to those of the Amazon basin, in comparison with the other basins of Maranhão, and concluded that the Turiaçu population originated more recently. In a study of the biogeography of the coastal basins of Maranhão, Abreu et al. (2019) included sequences from Turiaçu, which invariably formed a separate clade that was normally closer to the Amazonian fish than those of Maranhão, apparently reflecting vicariant events (Hubert et al., 2007).

## 5 | CONCLUSIONS

The molecular delimitation methods applied in the present study showed that the *Leporinus* specimens from the basins of the Parnaíba and Mearim rivers that formed the *L. cf. friderici* sensu stricto clade should be designated as the *L. cf. friderici* morphotype. The *L. piau* specimens from the Itapecuru, Mearim, Turiaçu, and Pericumã rivers that were assigned to the unnamed clade should be designated provisionally as *L. cf. piau*. This is because the cryptic diversity of this clade does not exclude the presence of *L. piau* from the basins of Maranhão, given that the *L. piau* from the type locality is also in this clade. This supports the conclusion that the *L. piau* may be included in the species of the unnamed clade under the denomination of *L. cf. friderici*.

The results of the present study of the Maranhão and Piauí basins confirmed the hypothesis that the *L cf. friderici/L. piau* group that we designated here corresponds to *L. cf. friderici* sensu stricto and that the *L. cf. friderici* and *L. piau* groups correspond to the unnamed group. In summary, our analyses substantiate the existence of a complex scenario of cryptic diversity within the *Leporinus* genus, underscore the taxonomic inconsistencies within this fish group, and highlight the potential of the hydrographic basins of Maranhão to act as a geographic model for the study of this genus.

## AUTHOR CONTRIBUTIONS

Data generation: Maria Histelle Sousa Nascimento, Elmary Fraga, José Luis Olivan Birindelli, and Maria Claudene Barros; data analysis: Maria Histelle Sousa Nascimento, Elmary Fraga, José Luis Olivan Birindelli, and Maria Claudene Barros; manuscript preparation: Maria Histelle Sousa Nascimento, José Luis Olivan Birindelli, Elmary Fraga, and Maria Claudene Barros; writing, reviewing, and editing: Maria Histelle Sousa Nascimento, Elmary Fraga, and Maria Claudene Barros; supervision: Maria Claudene Barros; funding acquisition: Elmary Fraga and Maria Claudene Barros.

## ACKNOWLEDGMENTS

We are grateful to the FAPEMA, which financially supported the present study, and the GENBIMOL Laboratory on the Caxias campus of the Maranhão State University for its support during all the different stages of the study.

## ORCID

*Maria Histelle Sousa Nascimento* https://orcid.org/0000-0002-4332-4289

## REFERENCES

Abreu, J. M. S., Craig, J. M., Albert, J. S., & Piorski, N. M. (2019). Historical biogeography of fishes from coastal basins of Maranhão state, northeastern Brazil. *Neotropical Ichthyology*, *17*, e180156. https://doi.org/10.1590/1982-0224-20180156

Amaral Campos, A. (1945). Novo gênero e novas espécies de Caracídios do baixo amazonas. *Papeis Avulsos Zoologia*, *7*, 217–220.

Bandelt, H. J., Forster, P., & Röhl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, *16*, 37–48. https://doi.org/10.1093/oxfordjournals.molbev.a026036

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., Ingram, K. K., & Das, I. (2007). Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution*, *22*, 148–155. https://doi.org/10.1016/j.tree.2006.11.004

Bingpeng, X., Heshan, L., Zhilan, Z., Chunguang, W., Yanguo, W., & Jianjun, W. (2018). DNA barcoding for identification of fish species in the Taiwan Strait. *PLoS One*, *13*, e0198109. https://doi.org/10.1371/journal.pone.0198109

Bleeker, P. (1862). *Atlas ichthiologique des indes Orientales Neerlandais*, *Tome II Siluroides*, *Chacoides et Heterobranchoides*. J. Smith E Gide, Amsterdam: The Netherlands.

Bloch, M. E. (1794). *Naturgeschichte der ausländischen Fische* (Vol. 8). Ichthyologie, ou Histire naturelle, générale et particulière des poissons.

Britski, H. A., & Birindelli, J. L. (2008). Description of a new species of the genus *Leporinus* Spix (Characiformes: Anostomidae) from the rio Araguaia, Brazil, with comments on the taxonomy and distribution of *L. parae* and *L. lacustris*. *Neotropical Ichthyology*, *6*, 45–51. https://doi.org/10.1590/S1679-62252008000100005

Britski, H. A., & Garavello, J. C. (1993). Descrição de duas espécies novas de *Leporinus* da bacia do Tapajós (Pisces, Characiformes). *Comunicacoes Do Museu de Ciencias da PUCRS*, *Serie Zoologia*, *6*, 29–40.

Calcagnotto, D., Schaefer, A. S., & Desalle, R. (2005). Relationship among characiform fishes inferred from analysis of nuclear and mitochondrial gene sequences. *Molecular Phylogenetics and Evolution*, *36*, 135–153. https://doi.org/10.1016/j.ympev.2005.01.004

Cardoso, Y. P., & Montoya-Burgos, J. I. (2009). Unexpected diversity in the catfish *Pseudancistrus brevispinis* reveals dispersal routes in a neotropical center of endemism: The Guyanas region. *Molecular Ecology*, *18*, 947–964. https://doi.org/10.1111/j.1365-294X.2008.04068.x

Carvalho, D. C., Oliveira, D. A., Pompeu, P. S., Leal, C. G., Oliveira, C., & Hanne, R. (2011). Deep barcode divergence in Brazilian freshwater fishes: The case of the Sao Francisco River basin. *Mitochondrial DNA. Part A*, *DNA Mapping*, *Sequencing*, *and Analysis*, *22*, 80–86. https://doi.org/10.3109/19401736.2011.588214

Carvalho-Costa, L. F., Piorski, N. M., Willis, S. C., Galetti, P. M., Jr., & Ortí, G. (2011). Molecular systematics of the neotropical shovelnose catfish genus *Pseudoplatystoma* Bleeker 1862 based on nuclear and mtDNA markers. *Molecular Phylogenetics and Evolution*, *59*, 177–194. https://doi.org/10.1016/j.ympev.2011.02.005

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods*, *9*, 772. https://doi.org/10.1038/nmeth.2109

Dinno, A., & Dinno, M. A. (2018). Package 'paran'. http://bioconductor.statistik.tu-dortmund.de/cran/web/packages/paran/paran.pdf

Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, *29*, 1969–1973. https://doi.org/10.1093/molbev/mss075

Ezard, T. H. G., & Purvis, A. (2009). PaleoPhylo: Free software to draw paleobiological phylogenies. *Paleobiology*, *35*, 460–464. https://doi.org/10.1666/0094-8373-35.3.460

Fowler, H. W. (1914). Peixes do rio Rupununi, Guiana Inglesa. *Proceedings of the Academy of Natural Sciences of Philadelphia*, *66*, 229–284. https://www.jstor.org/stable/4063634

Fowler, H. W. (1941). Academy of natural sciences a collection of freshwater fishes obtained in eastern Brazil by Dr. Rodolpho Von Ihering. *Proceedings of the Academy of Natural Sciences of Philadelphia*, *93*, 123–336. https://www.jstor.org/stable/4064332

Fraga, E. C., Silva, L. M. M., Schneider, H., Sampaio, I., & Barros, M. C. (2014). Variabilidade Genética em populações naturais de *Leporinus piau* (Anostomidae, Characiformes) da bacia do Rio Itapecuru. *Revista Brasileira de Ciências Agrárias*, *09*, 28–40. https://core.ac.uk/download/pdf/233140326.pdf

Fujisawa, T., & Barraclough, T. G. (2013). Delimiting species using single-locus data and the generalized mixed yule coalescent (GMYC) approach: A revised method and evaluation on simulated datasets. *Systematic Biology*, *62*, 707–724. https://doi.org/10.1093/sysbio/syt033

Garavello, J. C. (1979). *Revisão taxonômica do gênero Leporinus SPIX*, *1829 (Ostariophysi*, *Anostomidae)*. Doctoral thesis. Universidade de São Paulo.

Garavello, J. C. (1988). A new species of the genus *Leporinus* Spix from the Rio meta, Colombia, South America (Pisces, Ostariophysi, Anostomidae). *Proceedings of the Academy of Natural Sciences of Philadelphia*, *140*, 143–149. https://www.jstor.org/stable/4064939

Garavello, J. C., & Britski, H. A. (1987). Duas novas especies de *Leporinus* Spix, 1829, da bacia do alto Parana (Teleostei, Anostomidae). *Comunicações Do Museu de Ciências da PUCRS*, *44*, 153–165. https://repositorio.usp.br/item/000768508

Garavello, J. C., & Britski, H. A. (2003). Family Anostomidae (Headstanders). In I. Reis, E. Roberto, S. O. Kullander, F. Jr, & J. Carl (Eds.), *Check list of the freshwater fishes of south and Central America* (pp. 71–84). Edipucrs.

Garavello, J. C., Dos Reis, S. F., & Strauss, R. E. (1992). Geographic variation in *Leporinus friderici* (Bloch) (Pisces: Ostariophysi: Anostomidae) from the Paraná-Paraguay and Amazon River basins. *Zoologica Scripta*, *21*, 197–200. https://doi.org/10.1111/j.1463-6409.1992.tb00320.x

Garavello, J. C., & Santos, G. M. (1992). *Leporinus trimaculatus*, a new species from Amazonia, Brazil, and redescription of the sympatric *Leporinus aripuanaensis* (Pisces, Characiformes, Anostomidae). *Bulletin Zoologisch Museum*, *13*, 109–117.

Géry, J., Mahnert, V., & Dlouhy, C. (1987). Poissons Characoides non Characidae du Paraguay (Pisces, Ostariophysi). *Revue Suisse de Zoologie; Annales de la Société Zoologique Suisse et du Muséum d'histoire Naturelle de Genève*, *94*, 357–464. https://doi.org/10.5962/bhl.part.79720

Gouy, M., Guindon, S., & Gascuel, O. (2010). SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution*, *27*, 221–224. https://doi.org/10.1093/molbev/msp259

Hall, T. A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symposium Series*, *41*, 95–98. https://d1wqtxts1xzle7.cloudfront.net/29520866/1999hall1-libre.pdf

Hubert, N., Duponchelle, F., Nuñez, J., Garcia-Davila, C., Paugy, D., & Renno, J. F. (2007). Phylogeography of the piranha genera *Serrasalmus* and *Pygocentrus*: Implications for the diversification of the neotropical ichthyofauna. *Molecular Ecology*, *16*, 2115–2136. https://doi.org/10.1111/j.1365-294X.2007.03267.x

Hubert, N., & Renno, J. F. (2006). Historical biogeography of south American freshwater fishes. *Journal of Biogeography*, *33*, 1414–1436. https://doi.org/10.1111/j.1365-2699.2006.01518.x

Kress, W. J., García-Robledo, C., Uriarte, M., & Erickson, D. L. (2015). DNA barcodes for ecology, evolution, and conservation. *Trends in Ecology and Evolution*, *30*, 25–35. https://doi.org/10.1016/j.tree.2014.10.008

Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T., & Calcott, B. (2017). PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*, *34*, 772–773. https://doi.org/10.1093/molbev/msw260

Li, Y., Zhang, L., Song, P., Zhang, R., Wang, L., & Lin, L. (2018). Fish diversity and molecular taxonomy in the Prydz Bay during the 29th CHINARE. *Acta Oceanologica Sinica*, *37*, 15–20. https://doi.org/10.1007/s13131-018-1228-y

Librado, P., & Rozas, J. (2009). DNAsp v5: A software for comprehensive analyses of DNA polymorphism data. *Bioinformatics*, *25*, 1451–1452. https://doi.org/10.1093/bioinformatics/btp187

Lütken, C. F. (1875). Characinae novae Brasiliae centralis a clarissimo J. Reinhardt in provincia Minas-Geraes circa oppidulum Lagoa Santa in lacu ejusdem nominis, flumine Rio das Velhas et rivulis affluentibus collectae, secundum characteres essentiales breviter descriptae. *Oversigl over Det Kongelige Danske Videnskabernes Selskabs Forhandlinger*, *3*, 127–143.

Miller, M. A., Pfeiffer, W., & Schwartz, T. (2010). Creating the CIPRES science gateway for inference of large phylogenetic trees. In *In: 2010 gateway computing environments workshop (GCE)* (pp. 1–8). IEEE. https://doi.org/10.1109/GCE.2010.5676129

Nascimento, M. H. S., Aragão, D. G., Silva, J. L. N., Lima, R. C., Birindelli, J. L. O., Fraga, E. C., & Barros, M. C. (2023). The DNA barcode reveals cryptic diversity and a new record for the genus *Leporinus* (Characiformes, Anostomidae) in the hydrographic basins of central northern Brazil. *PeerJ*, *11*, e15184. https://doi.org/10.7717/peerj.15184

Nelson, J. S., Grande, T. C., & Wilson, M. V. (2016). *Fishes of the world*. John Wiley and Sons.

Palumbi, S. R., Martin, A., Romano, S., McMillan, W. O., Stice, L., & Grabowski, G. (1991). *The simple fool's guide to PCR, version 2.0*. University of Hawaii.

Paradis, E., & Schliep, K. (2019). Ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, *35*, 526–528. https://doi.org/10.1093/bioinformatics/bty633

Puillandre, N., Lambert, A., Brouillet, S., & Achaz, G. (2012). ABGD, automatic barcode gap discovery for primary species delimitation. *Molecular Ecology*, *21*, 1864–1877. https://doi.org/10.1111/j.1365-294X.2011.05239.x

Rambaut, A. (2014). FigTree v1.4.2. Available from: http://tree.bio.ed.ac.uk/software/figtree.

Rambaut, A., Suchard, M. A., Xie, D., & Drummond, A. J. (2014). Tracer v1.6. Available from: Http.Beast. Bio. Ed. ac. UK/tracer.

Ramirez, J. L., Birindelli, J. L. O., Carvalho, D. C., Affonso, P. R., Venere, P. C., Ortega, H., Carrillo-Avila, M., Rodríguez-Pulido, J. A., & Galetti, P. M. (2017). Revealing hidden diversity of the underestimated neotropical ichthyofauna: DNA barcoding in the recently described genus *Megaleporinus* (Characiformes: Anostomidae). *Frontiers in Genetics*, *8*, 1–11. https://doi.org/10.3389/fgene.2017.00149

Ramirez, J. L., Birindelli, J. L. O., & Galetti, P. M. (2017). A new genus of Anostomidae (Ostariophysi: Characiformes): Diversity, phylogeny and biogeography based on cytogenetic, molecular and morphological data. *Molecular Phylogenetics and Evolution*, *107*, 308–323. https://doi.org/10.1016/j.ympev.2016.11.012

Ramirez, J. L., Carvalho-Costa, L. F., Venere, P. C., Carvalho, D. C., Troy, W. P., & Galetti, P. M. (2016). Testing monophyly of the freshwater fish *Leporinus* (Characiformes, Anostomidae) through molecular analysis. *Journal of Fish Biology*, *88*, 1204–1214. https://doi.org/10.1111/jfb.12906

Rannala, B., & Yang, Z. (2003). Bayes estimation of species divergence times and ancestral population sizes using DNA sequences from multiple loci. *Genetics*, *164*, 1645–1656. https://doi.org/10.1093/genetics/164.4.1645

Renno, J. E., Machordom, A., Blanquer, A., & Boursot, P. (1991). Polymorphism of mitochondrial genes in populations of *Leporinus friderici* (Bloch, 1794): Intraspecific structure and zoogeography of the neotropical fish. *Genetica*, *84*, 137–142. https://doi.org/10.1007/BF00116554

Renno, J. F., Berreb, P., Boujard, T., & Guyomard, R. (1990). Intraspecific genetic differentiation of *Leporinus friderici* (Anostomidae, Pisces) in French Guiana and Brazil: A genetic approach to the refuge theory. *Journal of Fish Biology*, *36*, 85–95. https://doi.org/10.1111/j.1095-8649.1990.tb03522.x

Sanger, F., Nicklen, S., & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences of the United States of America*, *74*, 5463–5467. https://doi.org/10.1073/pnas.74.12.5463

Santos, G. M., & Zuanon, J. (2008). *Leporinus amazonicus*, a new anostomid species from the Amazon lowlands, Brazil (Osteichthyes: Characiformes). *Zootaxa*, *1815*, 35–42. https://doi.org/10.11646/zootaxa.1815.1.3

Santos, G. S. A. (2007). *Análise filogenética das espécies de Leporinus (Ostariophysi: Characiformes: Anostomidae) das Bacias do Prata e São Francisco* (Masters thesis). Available from UNESP Library https://www2.ibb.unesp.br/posgrad/teses/zoologia_me_2007_gleisy_santos.pdf

Santos, S., Schneider, H., & Sampaio, I. (2003). Genetic differentiation of *Macrodon ancylodon* (Sciaenidae, Perciformes) populations in Atlantic coastal waters of South America as revealed by mtDNA analysis. *Genetics and Molecular Biology*, *26*, 151–161. https://doi.org/10.1590/S1415-47572003000200008

Sidlauskas, B. L., & Vari, R. P. (2008). Phylogenetic relationships within the south American fish family Anostomidae (Teleostei, Ostariophysi, Characiformes). *Zoological Journal of the Linnean Society*, *154*, 70–210. https://doi.org/10.1111/j.1096-3642.2008.00407.x

Sidlauskas, B. L., & Vari, R. P. (2012). Diversity and distribution of anostomoid fishes (Teleostei: Characiformes) throughout the Guianas. *Cybium*, *36*, 71–103.

Silva-Santos, R., Ramirez, J. L., Freitas, P. D., Galetti, P. M., Jr., & Freitas, P. D. (2018). Molecular evidences of a hidden complex scenario in *Leporinus* cf. *friderici*. *Frontiers in Genetics*, *9*, 1–9. https://doi.org/10.3389/fgene.2018.00047

Smith, M. F., & Patton, J. L. (1993). The diversification of south American murid rodents: Evidence from mitochondrial DNA sequence data for the Akodontine tribe. *Biological Journal of the Linnean Society*, *50*, 149–177. https://doi.org/10.1111/j.1095-8312.1993.tb00924.x

Spix, J. B., & Agassiz, L. (1829). *Selecta genera et species piscium: quos in itinere per Brasiliam annis MDCCCXVII-MDCCCXX jussu et auspiciis Maximiliani Josephi I*. Wolf.

Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post analysis of large phylogenies. *Bioinformatics*, *30*, 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Steindachner, F. (1876). Ichthyologische Beiträge (V). Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften. *Mathematisch-Naturwissenschaftliche Classe*, *74*, 49–240.

Steinke, D., Zemlak, T. S., Boutillier, J. A., & Hebert, P. D. (2009). DNA barcoding of Pacific Canada's fishes. *Marine Biology*, *156*, 2641–2647. https://doi.org/10.1007/s00227-009-1284-0

Strauss, R. E., & Bond, C. E. (1990). Taxonomic methods: Morphology. In P. Moyle & C. Schreck (Eds.), *Methods for fish biology* (pp. 109–140). American Fisheries Society.

Suchard, M. A., Lemey, P., Baele, G., Ayres, D. L., Drummond, A. J., & Rambaut, A. (2018). Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evolution*, *4*, 1–5. https://doi.org/10.1093/ve/vey016

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, *38*, 3022–3027. https://doi.org/10.1093/molbev/msab120

Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, *22*, 4673–4680. https://doi.org/10.1093/nar/22.22.4673

Vail, P. R., & Hardenbol, J. (1979). Sea-level changes during the tertiary. *Oceanus*, *22*, 71–77.

Valenciennes, A. (1837). Poissons [plates]. In *In: d'Orbigny, A. Voyage dans l'Amérique méridionale* (pp. 1–16). Pitois-Levrault.

Venables, W. N., & Ripley, B. D. (2002). *Modern applied statistics with S*. Springer.

Venables, W. N., & Smith, D. M. (2021). The R Core team. R version 4.1.0 patched. R. A programming environment for data analysis and graphics. Vienna: The Foundation for Statistical Computing 144. https://www.R-project.org

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. N. (2005). DNA barcoding Australia's fish species. Philosophical transactions of the Royal Society of London. *Series B, Biological Sciences*, *360*, 1847–1857. https://doi.org/10.1098/rstb.2005.1716

Xu, L., Wang, X., Van Damme, K., Huang, D., Li, Y., Wang, L., Ning, J., & Du, F. (2021). Assessment of fish diversity in the South China Sea using DNA taxonomy. *Fisheries Research*, *233*, 105771. https://doi.org/10.1016/j.fishres.2020.105771

Yang, Z. (2015). The BPP program for species tree estimation and species delimitation. *Current Zoology*, *61*, 854–865. https://doi.org/10.1093/czoolo/61.5.854

Yang, Z., & Rannala, B. (2010). Bayesian species delimitation using multilocus sequence data. *Proceedings of the National Academy of Sciences*, *107*, 9264–9269. https://doi.org/10.1073/pnas.0913022107

Yang, Z., & Rannala, B. (2017). Bayesian species identification under the multispecies coalescent provides significant improvements to DNA barcoding analyses. *Molecular Ecology*, *26*, 3028–3036. https://doi.org/10.1111/mec.14093

Zhang, J., & Hanner, R. (2012). Molecular approach to the identification of fish in the South China Sea. *PLoS One*, *7*, e30621. https://doi.org/10.1371/journal.pone.0030621

Zhang, J., Kapli, P., Pavlidis, P., & Stamatakis, A. (2013). A general species delimitation method with applications to phylogenetic placements. *Bioinformatics*, *29*, 2869–2876. https://doi.org/10.1093/bioinformatics/btt499

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Nascimento, M. H. S., Birindelli, J. L. O., Fraga, E., & Barros, M. C. (2024). Exploring hidden diversity: Molecular insights into the *Leporinus* species of the rivers of the Brazilian states of Maranhão and Piauí. *Journal of Fish Biology*, 1–12. https://doi.org/10.1111/jfb.15753

# 4 DISCUSSÃO INTEGRADORA

Estudos de identificação e filogenéticos empregando sequências parciais ou totais do DNA em peixes são uma realidade bastante atual, o que pode ser confirmado pelo grande número de trabalhos recentemente publicados com essa abordagem (PEREIRA *et al.*, 2011; CARVALHO *et al.*, 2012; PEREIRA *et al.*, 2013; GOMES *et al.,* 2015; RAMIREZ e GALETTI JR, 2015; MELO B *et al.*, 2016; MELO F *et al.*, 2016; PUGEDO *et al.*, 2016; RAMIREZ *et al.*, 2016; ROSSINI *et al.*, 2016; SILVA *et al.*, 2016; PIRES *et al.*, 2017; RAMIRREZ *et al.*, 2017a; MACHADO *et al.*, 2018; SILVA-SANTOS *et al.*, 2018; CALEGARI *et al.*, 2019; BRITSKI *et al.,* 2019; RAMIREZ *et al.*, 2020; GARAVELLO *et al.*, 2021; LIMEIRA-FILHO *et al.*, 2023; entre outros). O uso de genes nucleares é bastante útil quando o tempo de divergência dos grupos é antigo, uma vez que esses genes evoluem muito mais lentamente do que os genes mitocondriais. Quando juntos, estudos com genes mitocondriais e nucleares, apresentam grande poder de resolução de problemas enfrentados pela taxonomia tradicional, como na separação de espécies crípticas e identificação de espécies com grande plasticidade fenotípica (STEPIEN e KOCHER, 1997; ORTÍ e KOCHER, 1997; HEBERT, *et al.,* 2003).

Os dados apresentados nos dois capítulos e apêndices constituíram uma ferramenta útil para identificar e separar a maioria das espécies de anostomideos aqui apresentadas. Por exemplo, o uso de dados morfológicos integrados a informações moleculares possibilitou a identificação de *M. macrocephalus* permitindo a inferência de que este estudo constituiu o primeiro registro desta espécie para as bacias do Mearim e Itapecuru, ampliando a distribuição geográfica desta espécie para a região Nordeste, uma vez que dados da literatura apontavam sua ocorrência para as bacias das regiões Sul, Sudeste e Centro-Oeste do Brasil (GARAVELLO e BRITSKI, 1988;ALVES *et al.*, 2007; GRAÇA e PAVANELLI 2007; LANGEANI *et al.*, 2007; GOMES *et al.*, 2015; BERTACO *et al.*, 2016). Por tartar-se de uma espécie de peixe comercialmente importante (SOARES *et al.*, 2000; ZEINAD e PRADO, 2012), a ocorrência *M. macrocephalus* nas bacias do Mearim e Itapecuru é provavelmente resultante da introdução de espécies exóticas na piscultura maranhense, onde espécimes escapam dos tanques, por transbordamento, para lagos e rios durante a estação chuvosa (ver artigo em apêndice).

A Convenção sobre a Diversidade Biológica (CDB), Decisão VI-23, (SECRETARIAT OF THE CONVENTION ON BIOLOGICAL DIVERSITY, 2005) define espécies exóticas como: “uma espécie, subespécie ou táxon de classificação inferior que ocorre fora da sua área de distribuição natural passada ou presente; inclui qualquer parte, como gametas, sementes, ovos ou propágulos que possam sobreviver e reproduzir-se subsequentemente”. A CDB também define espécie exótica invasora como “uma espécie exótica cuja introdução e/ou dispersão ameaça a diversidade biológica”; e ainda conceitua introdução de espécies como: “movimento por ação humana, direta ou indireta, de uma espécie exótica para fora de sua área de distribuição natural (passada ou presente). Esse movimento pode ocorrer dentro de um país ou entre países ou áreas além da jurisdição nacional”. Assim, a introdução de *M. macrocephalus* nos bacias dos rios Itapecuru e Mearim, por escape de indivíduos de criações, deve ser melhor monitorada, uma vez que populações viavéis sejam estabelecidas nos rios maranhenses, esta espécie poderá representar um problema a ictiofauna nativa. Estudos apontam a introdução de espécies exóticas como um dos principais motivos de perda de biodiversidade mundial, causando alterações nos processos e interações ecológicas, como predação, e ainda competição por alimento, espaços e sítios de reprodução, resultando na diminuição da riqueza da ictiofauna nativa e a homogeneização da biota aquática (LEÃO *et al*., 2011; LATINI *et al*., 2016).

A análise do código de barras de DNA nos deu indícios por meio de análises de distância genética e métodos de delimitação de espécies, da ocorrência de mais de uma espécie de *Leporinus* dentro de espécies nominais válidas e subdividas em MOTUs distintas, apoiada pela distância genética, bem como a ampliação da distribuição geográfica de uma espécie nominal para bacias maranhenses.

Em relação ao limiar de diferenciação intraespecífico recomendado na literatura, para a gênero *Leporinus* de bacias hidrográficas do Maranhão e Piauí podemos inferir que o uso de 2% não é adequado, uma vez que esse percentual não foi suficiente para separar as espécies entre bacias diferentes. No entanto, pode ser aplicado quando for necessário avaliar algumas espécies provenientes de mesma bacia. O valor tradicional de limiar na separação espécies de peixes continentais, mediante uso do código de barras de DNA ou DNA *barcoding* (2%) deve ser feito com bastante cautela, uma vez que algumas espécies possuem mínimas interespecíficas menores a esse valor. Como alternativa, Hebert et al. (2003) propõe a adoção de 10 vezes o valor da média intraespecífica, para grupos que possuem valores de distância mínima interespecíficas inferiores a esse limiar. Ainda foi proposto como limiar de delimitação, o menor valor entre duas espécies taxonomicamente válidas (FEITOSA, SANTOS e BIRINDELLI, 2011). Esses resultados demonstram que o uso de valores “fechados” como limiar para separar espécies podem levar a informações subestimadas acerca da biodiversidade, representando assim um risco para o reconhecimento de espécies que não se enquadrem nesse perfil.

Nas análises moleculares empregadas no presente estudo, incluindo as diferentes abordagens de delimitação de espécies de locus único (COI) foi identificada a presença de quatro espécies de *Leporinus*, sendo elas: *L. maculatus, L. unitaenitus, L. affinis* e *L. venerei.*

A confirmação da presença de *L. venerei* nas bacias do Itapecuru, Mearim, Turiaçu, Preguiças e Periá representa um novo registro para o estado do Maranhão e ampliou a distribuição conhecida desta espécie no Brasil. Esses dados já eram sinalizados em estudos anteriores quando Fraga et al. (2014) e Nascimento et al. (2016) evidenciaram a formação de dois subclado em *L. piau* do Maranhão, sugerindo tratar-se de linhagens distintas, resultado este que o presente estudo revelou como agrupamentos que na verdade correspondem as espécies *L. venerei e L.* cf. *friderici*.

A problemática taxonômica envolvendo *L. piau, L* cf. *friderici* e *L. venerei* está diretamente relacionada a elevada semelhança dos caracteres morfológicos diagnósticos que estas espécies compartilham, dificultando o reconhecimento de *L. venerei* nos rios maranhenses ao longo dos anos, no entanto através de dados moleculares gerados em nosso estudo foi possível apontar para ocorrência de *L. venerei* em quatro bacias maranhenses representando o primeiro registro desta espécie  para o estado do Maranhão.

O compartilhamento de agrupamentos e MOTUs entre *L. piau* e *L.* cf. *friderici*, bem como a evidência de elevadas distancias genéticas dentro das amostras que compõem esses dois táxons, tanto nas análises de locus único como multilocus, reforçam que tanto *L. piau* das bacias do Maranhão e Piauí, quanto *L.* cf. *friderici* de diferentes bacias brasileiras não constituem duas entidades taxonômicas, ambas possivelmente tratam-se um complexo de espécie, como já havia sido observado para *L.* cf *friderici* (RAMIREZ, BIRINDELLI e GALETTI JR, 2017; SILVA-SANTOS *et al*., 2018).

Para *L. piau* do Maranhão, no entanto, pode-se notar uma maior similaridade entre bacias que apresentam semelhantes característica hidrológicas resultantes dos processos biogeográficos responsáveis pelos padrões de dispersão e interação entre a biota pretérita, como é o caso das amostras proveniente das bacias do Mearim, Itapecuru e Pericumã. Tais bacias apresentam pequenas conexões entre si através de lagoas marginais e pequenos rios permitindo fluxo gênico entre as populações dessas drenagens (PIORSKI, 2010; ABREU, 2013). Essas três bacias (Mearim, Itapecuru e Pericumã) estão situadas na Região Hidrográfica Atlântico Nordeste Oriental (RHANO), e dentro dessa região Dagosta e Pinna (2017) reconhece três áreas de endemismo, onde Mearim/Itapecuru aparecem como área endêmica única, o que favorece a maior similaridade genética entre os peixes dessas duas bacias. Por outro lado, a formação de um subagrupamento para *L. piau* da bacia do Turiaçu junto a *L. piau* da bacia amazônica reforça as observações de Abreu (2020) de que as espécies de peixes do Turiaçu são mais proximamente relacionadas aos peixes da bacia amazônica que as bacias maranhenses. Essa proximidade com os peixes amazônicos pode ser derivada de eventos de vicariância e o isolamento e diferenciação da ictiofauna Turiaçu pode ter resultado de reconstruções de paleodrenagens, bem como pelo surgimento da Serra do Tiracambu (COSTA *et al.,* 1996; SOARES *et al.,* 2011).

# 5 CONCLUSÃO

Os resultados obtidos neste estudo demonstram a ocorrência e separação de *L. maculatus, L. unitaenitus, L. affinis* e *L. venerei* em táxons distintos. Além de diferencia-las, conseguimos registrar pela primeira vez a ocorrência de *L. venerei* em quatro bacias maranhenses: Itapecuru, Mearim, Periá e Preguiças. Dessa forma, adicionar dados genéticos ao estudo de *Leporinus* pode garantir que a identificação das espécies seja mais precisa.

O registro de *Megaleporinus macrocephalus,* uma espécie exótica, nas bacias hidrográficas maranhenses, representa uma extensão da sua distribuição que hoje é confirmada na bacia do rio Itapecuru e Mearim, evidenciando para a necessidade do monitoramento dos mecanismos de interações estabelecidas entre *M. macrocephalus* e as espécies nativas, melhorarando a capacidade de previsão sobre os impactos e consequências que a presença desta espécie nos rios maranhenses pode provocar, para assim estabelecer medidas preventivas de controle.

Os marcadores mitocondriais e nucleares evidenciam a separação de espécimes de *L. piau* das bacias dos rios Parnaíba e Mearim que formaram o clado *L.* cf. *friderici* sensu stricto, sendo assim devem ser denominados como o morfotipo *L.* cf. *friderici*, e que provavelmente tais espécimes foram identificados erroneamente, tornando claro a importância do uso de dados moleculares aliados a taxonomia tradicional.

Os espécimes de *L. piau* dos rios Itapecuru, Mearim, Turiaçu e Pericumã que foram atribuídos ao clado "sem nome" devem ser denominados provisoriamente como *L.* cf. *piau*, pois a diversidade críptica observada neste clado não exclui categoricamente a presença de *L. piau* das bacias do Maranhão, uma vez que o *L. piau* da bacia do Rio Jaguaribe também compõe este agrupamento.

Os espécimes de *L. piau* do Maranhão que constituíram o clado "sem nome" tanto para os estudos de locus único quanto multilocus revelam diferenciação genética entre as bacias, com evidente separação da bacia do Turiaçu em clado mais geneticamente relacionado com espécimes da bacia Amazônica, bem como mostra uma relação mais estreita entre os *Leporinus* do Mearim e Itapecuru.

A partir dos resultados obtidos podemos inferir que os diferentes agrupamentos de *L.* cf. *friderici*/*L. piau* formados nas análises de locus único e multilocus confirmam a hipótese de que ocorre os dois agrupamentos denominados respectivamente de *L.* cf. *friderici* sensu stricto e "sem nome" refletindo um cenário complexo na diversidade de *Leporinus* que ocorrem nos rios do estado do Maranhão e Piauí, o que evidencia o potencial das bacias hidrográficas maranhenses como modelo geográfico para o estudo do gênero.

## REFERÊNCIAS BIBLIOGRÁFICAS

ABREU, João Marcelo da Silva. **Explorando o desconhecido: Influencias paleogeográficas na distribuição dos peixes de água doce do Maranhão**. 2020. Tese (Doutorado em Rede da Biodiversidade e Biotecnologia da Amazonia Legal) – Universidade Federal do Maranhão, São Luis, 2020.

ABREU, João Marcelo da Silva. **Variação geográfica em *Schizodon dissimilis* (Garman, 1890) e diversidade genética e filogeográfica do grupo *Schizodon fasciatus* sensu lato (Characiformes: Anostomidae)**. 2013. Dissertação (Mestrado em Zoologia) - Universidade Federal do Pará, Museu Paraense Emílio Goeldi, Belém, 2013.

ABREU, J.M.S.; CRAIG, J.M.; ALBERT, J.S.; PIORSKI, N.M. Historical biogeography of fishes from coastal basins of Maranhão State, northeastern Brazil. **Neotropical Ichthyology,** v. 17, n. 2, e180156, 2019. https://doi.org/10.1590/1982-0224-20180156

AB'SABER, A. N. Posição das superfícies aplainadas no planalto brasileiro. **Notícia Geomorfológica**, v. 3, n. 5, p. 52-54, 1960. Disponível em: https://biblio.fflch.usp.br/AbSaber_AN_1346021_PosicaoDasSuperficies.pdf. Acesso em: 01 maio 2024.

AGÊNCIA NACIONAL DE ÁGUAS. **Conjuntura dos Recursos Hídricos no Brasil.** 2013. Disponível em: www.arquivos.ana.gov.br/institucional/spr/conjuntura/webSite_relatorioConjuntura/projeto/index.html, Acessado em: 10/10/2023.

ALBERTS, B., BRAY, D., HOPKIN, K., JOHNSON, A. LEWIS, J.; RAFF, M.; *et al.* **Fundamentos da biologia celular**. 2 ed., Artmed. Porto Alegre. 740 pp. 2006.

ALMEIDA, M.S.; MORAES, P.S.S.; NASCIMENTO, M.H.S.; BIRINDELLI, J.L.O.; ASSEGA, F.M.; BARROS, M.C. *et al.* New records of the occurrence of *Megaleporinus macrocephalus* (Garavello; Britski, 1988) (Characiformes, Anostomidae) from the basins of the Itapecuru and Mearim rivers in Maranhão, Northeastern Brazil. **Brazilian Journal of Biology,** v. 82, e232868, 2021. https://doi.org/10.1590/1519-6984.232868

ALVES, C.B.M.; VIEIRA, F.; MAGALHÃES, A.L.B.; BRITO, M.F.G. Impacts of nonnative fish species in Minas Gerais, Brazil: present situation and prospects. In: BERT, T. M. (ed.). **Ecological and genetic implications of aquaculture activities**. V. 6, Editora: Springer Press, Dordrecht, 291-314 p. 2007.

ASSEGA, F.M.; BIRINDELLI, J.L.O. Taxonomic revision of the genus *Anostomoides* (Characiformes: Anostomidae). **Zootaxa**, v. 4646, n. 1, p. 124–144, 2019. https://doi.org/10.11646/zootaxa.4646.1.7

BARROS, M.C.; FRAGA, E.C.; BIRINDELLI, J.L.O. Fishes from the Itapecuru River basin, State of Maranhão, Northeastern Brazil. **Brazilian Journal of Biology**, v. 71, n. 2, p. 375-380, 2011.https://doi.org/10.1590/S1519-69842011000300006

BERTACO, V.A.; FERRER, J.; CARVALHO, F.R.; MALABARBA, L.R. Inventory of the freshwater fishes from a densely collected area in South America – a case study of the current knowledge of Neotropical fish diversity. **Zootaxa**, v. 4138, n. 3, p. 401-440, 2016. https://doi.org/10.11646/zootaxa.4138.3.1

BIRINDELLI, J.L.O; BRITSKI, H.A. New species of the genus *Leporinus* Agassiz (Characiformes: Anostomidae) from the rio Curuá, rio Xingu basin, Serra do Cachimbo, Brazil, with comments on Leporinus reticulatus. **Neotropical Ichthyology**, v. *7*, n. 1, p. 1-9, 2009. https://doi.org/10.1590/S1679-62252009000100001

BIRINDELLI, J.L.O.; BRITSKI, H.A. Two new species of *Leporinus* (Characiformes: Anostomidae) from the Brazilian Amazon, and redescription of *Leporinus striatus* Kner 1858. **Journal of Fish Biology**, v. 83, n. 5, p. 1128–1160, 2013. https://doi.org/10.1111/jfb.12206

BIRINDELLI, J.L.O.; BRITSKI, H.A.; LIMA, F.C.T. New species of *Leporinus* from the Rio Tapajós Basin, Brazil, and redescription of *L. moralesi* (Characiformes: Anostomidae). **Copeia**, v. 2013, n. *2*, p. 238–247, 2013. https://doi.org/10.1643/CI-12-081

BIRINDELLI, J.L.O.; BRITSKI, H.A.; RAMIREZ, J.L. A new endangered species of *Megaleporinus* (Characiformes: Anostomidae) from the Rio de Contas basin, Eastern Brazil. **Journal of Fish Biology**, v. 96, n. 6, p. 1349–1359, 2020. https://doi.org/10.1111/jfb.14299

BIRINDELLI, J.L.O; LIMA, F.C.T.; BRITSKI, H.A. New species of *Pseudanos* Winterbottom, 1980 (Characiformes: Anostomidae), with notes on the taxonomy of *P. gracilis* and *P. trimaculatus*. **Zootaxa**, v. 3425, n. 1, p. 55–68, 2012.

BLOCH, M.E. **Naturgeschichte der ausländischen Fische**. vol. 8. Berlin, Schlesinger, 1794. p. 396.

BORODIN, N.A. Notes on some species and subespecies of the genus *Leporinus* Spix. **Memoirs of the Museum of Comparative Zoology**, v. 50, n. 3, p. 269-290, 1929.

BRITO, P.S.; GUIMARÃES, E.C.; FERREIRA, B.R.A.; OTTONI, F.P.; PIORSKI, N.M. Freshwater fishes of the Parque Nacional dos Lençóis Maranhenses and adjacent areas. **Biota Neotropica**, v. 19, n. 3, e20180660, 2019. https://doi.org/10.1590/1676-0611-BN-2018-0660

BRITO, P.S.; GUIMARÃES, E.C.; FERREIRA, B.R.A.; SANTO, S.J.P.; AMARAL, Y.T.; OTTONI, F.P. Updated and supplementary data on Brito et al. (2020): Freshwater Fishes of the Parque Nacional dos Lençóis Maranhenses and adjacent areas. **Ichthyological Contributions of Pecescriollos,** v. 73, n. 1, p. 1-17, 2020. https://doi.org/10.1590/1676-0611-bn-2018-0660

BRITSKI, H.A. Sobre uma nova espécie *Leporinus* da Amazônia. **Acta Amazonica**, v. 6, n. 4, p. 87–89, 1976.

BRITSKI, H.A.; BIRINDELLI, J.L.O. Description of a new species of the genus *Leporinus* Spix (Characiformes: Anostomidae) from the rio Araguaia, Brazil, with comments on the taxonomy and distribution of *L. parae* and *L. lacustris*. **Neotropical Ichthyology**, v. 6, n. 1, p. 45–51, 2008. https://doi.org/10.1590/S1679-62252008000100005

BRITSKI, H.A.; BIRINDELLI, J.L.O. Description of a new species of *Leporinus* (Characiformes: Anostomidae) from the Rio Tapajós basin, Brazil. **Zootaxa**, v. 4603, n. 1, p. 183, 2019. http://doi:10.11646/zootaxa.4603.1.10

BRITSKI, H.A.; BIRINDELLI, J.L.O.; GARAVELLO, J.C. A new species of *Leporinus* agassiz, 1829 from the upper Rio Paraná basin (Characiformes, Anostomidae) with redescription of *L. elongatus* Valenciennes, 1850 and *L. obtusidens* (Valenciennes, 1837).

**Papeis Avulsos de Zoologia**, v. 52, n. 37, p. 441–475, 2012. https://doi.org/10.1590/s0031-10492012021700001

BRITSKI, H.A.; GARAVELLO, J.C. Sobre *Leporinus octofasciatus* Steindachner da bacia do Paraná (Pisces, Anostomidae). **Papéis Avulsos de Zoologia**, v. 31, n. 16, p. 237–250, 1978.

BRITSKI, H.A.; MELO, B.F.; VARI, R.P.; OLIVEIRA, C. Revalidation and redescription of Steindachnerina nigrotaenia and redescription of S. *insculpta* (Characiformes: Curimatidae). **Neotropical Ichthyology**, v. 17, e180076, 2019. https://doi.org/10.1590/1982-0224-20180076

BUCKUP, P.A.; MENEZES, N.A.; GHAZZI, M.S. **Catálogo das espécies de peixes de água doce do Brasil.** Rio de Janeiro, Museu Nacional, 2007.

BURNS, M.D.; FRABLE, B.W.; SIDLAUSKAS, B.L. A New Species of *Leporinus* (Characiformes: Anostomidae), from the Orinoco Basin, Venezuela. **Copeia**, v. 2014, n. 2, p. 206–214, 2014. https://doi.org/http://dx.doi.org/10.1643/CI-13-071

CALCAGNOTTO, D.; SCHAEFER, S.A.; DESALLE, R. Relationships among Characiform fishes inferred from analysis of nuclear and mitochondrial gene sequences. **Molecular phylogenetics and evolution**, v. 36, n. 1, p. 135-153, 2005. https://doi.org/10.1016/j.ympev.2005.01.004

CALEGARI, B.B.; VARI, R.P.; REIS, R.E. Phylogenetic systematics of the driftwood catfishes (Siluriformes: Auchenipteridae): a combined morphological and molecular analysis. **Zoological Journal of the Linnean Society**, v. 187, n. 3, p. 661-773, 2019.

CANTATORE, P.; ROBERTI, M.; PESOLE, G.; LUDOVICO, A.; MILELLA, F.; GADALETA, M.N.; *et al*. Evolutionary analysis of cytochrome b sequences in some Perciformes: evidence for a slower rate of evolution than in mammals. **Journal of Molecular Evolution**, v. 39, p. 589-597, 1994.

CARVALHO, D.C.; OLIVEIRA, D.A.A.; BEHEREGARAY, L.B.; TORRES, R.A. Hidden genetic diversity and distinct evolutionarily significant units in an commercially important Neotropical apex predator, the catfish Pseudoplatystoma corruscans. **Conservation Genetics**, v. 13, p. 1671-1675, 2012. https://doi.org/10.1007/s10592-012-0402-6

CHIARI, L.; SODRÉ, L.M.K. Genetic variability in five species of Anostomidae (Ostariophysi-Characiformes). **Genetics and Molecular Biology**, v. 22, p. 517-523, 1999. https://doi.org/10.1590/S1415-47571999000400009

COSTA, J.B.S.; BEMERGUY, R.L.; HASUI, Y.; DA SILVA BORGES, M.; JÚNIOR, C. R.P.F.; BEZERRA, P.É.L.; *et al*. Neotectônica da região amazônica: aspectos tectônicos, geomorfológicos e deposicionais. **Geonomos**, v. 4, n. 2, p. 23-44, 1996. https://doi.org/10.18285/geonomos.v4i2.199

COSTA-SILVA, G.J.; RODRIGUEZ, M.S.; ROXO, F.F.; FORESTI, F.; OLIVEIRA, C. Using different methods to access the difficult task of delimiting species in a complex Neotropical hyperdiverse group. **PLoS One** v. 10, n. 9, e0135075, 2015. https://doi.org/10.1371/journal.pone.0135075

DAGOSTA, F.C.; PINNA, M.D. Biogeography of Amazonian fishes: deconstructing river basins as biogeographic units. **Neotropical Ichthyology**, v. 15, n. 3, e170034, 2017. https://doi.org/10.1590/1982-0224-20170034

DE ALCÂNTARA, E. H. Caracterização da bacia hidrográfica do rio Itapecuru, Maranhão-Brasil. **Caminhos de Geografia**, v. 7, n. 11, p. 97-113, 2004.

ESCHMEYER, W.N.; FONG, J.D. Catalog of fishes. Online Version. Disponível em: http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp#Anostomidae. Acesso em: 06/05/2014.

ESPOSTI, D.M.; DE VRIES, S.; CRIMI, M.; GHELLI, A.; PATARNELLO, T.; MEYER, A. Mitochondrial cytochrome b: evolution and structure of the protein. **Biochimica et Biophysica Acta (BBA)-Bioenergetics**, v. 1143, n. 3, p. 243-271, 1993. https://doi.org/10.1016/0005-2728(93)90197-N

FEITOSA, F.D.S.; DOS SANTOS, G.M.; BIRINDELLI, J.L. *Leporinus britskii*: a new anostomid species from the Tapajós and Jari drainages, Brazil (Characiformes: Anostomidae). **Zootaxa**, v. 3120, n. 1, p. 55-62, 2011. https://doi.org/10.11646/ZOOTAXA.3120.1.4

FOWLER, H.W. Fishes from the Rupununi River, British Guiana. **Proceedings of the Academy of Natural Sciences, Philadelphia**, v. 66, p. 229–284, 1914.

FOWLER, H.W. Academy of Natural Sciences A Collection of Fresh-Water Fishes Obtained in Eastern Brazil by Dr. Rodolpho Von Ihering. **Proceedings of the Academy of Natural Sciences of Philadelphia**, v. 93, p. 123–199, 1941.

FOWLER, H.W. Los peces del Peru. Catálogo sistemático das peças que habitam em águas peruanas. **Boletín del Museo de Historia Natural Javier Prado**, v. 6, p. 206–222, 1942.

FRAGA, E.; SCHNEIDER, H.; NIRCHIO, M.; SANTA-BRIGIDA, E.; RODRIGUES-FILHO, L.F.; SAMPAIO, I. Molecular phylogenetic analyses of mullets (Mugilidae, Mugiliformes) based on two mitochondrial genes. **Journal of Applied Ichthyology**, v. 23, n. 5, p. 598-604, 2007. https://doi.org/10.1111/j.1439-0426.2007.00911.x

FRAGA, E.; SILVA, L.M.M.; SCHNEIDER, H.; SAMPAIO, I.; BARROS, M.C. Variabilidade genética em populações naturais de *Leporinus piau* (Anostomidae, Characiformes) da bacia do Rio Itapecuru. **Revista Trópica**, p.28-40, v.8, n. 2, 2014.

FRICKE, R.; ESCHMEYER, W.N.; FONG, J.D. **Eschmeyer's Catalog of Fishes: Genera/Species by Family/Subfamily**. Disponivel em: http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp, Acesso em: 29/03/2024

GALLETTI JR., P.M.; FORESTI, F.; BERTOLLO, L.A.C.; MOREIRA FILHO, O. Karyotypic similarity in three genera (*Leporinus*, *Leporellus* and *Schizodon*) of the family Anostomidae (Pisces-Teleostei). **Revista Brasileira de Genética**, v. 4, n. 1, p. 11-15, 1981.

GALLETTI JR, P.M.; MESTRINER, C.A.; VENERE, P.C.; FORESTI, F. Heterochromatin and karyotype reorganization in fish of the family Anostomidae (Characiformes). **Cytogenetic Cell Genetic,** v. 56, n. 1, p. 116-121, 1991.

GALETTI JR, P.M.; LIMA, N.R.W.; VENERE, P.C. A Monophyletic ZW Sex Chromosome System in *Leporinus* (Anostomidae, Characiformes). **Cytologia**, v. 60, p. 375–382, 1995.

GARAVELLO, Júlio Cesar. **Revisão taxonômica do gênero *Leporinus* Spix, 1829 (ostariophysi, Anostomidae).** 1979. Tese (Doutorado) - Universidade de São Paulo, São Paulo, 1979.

GARAVELLO, J.C.; BRITSKI, H.A. **Duas novas espécies do gênero *Leporinus* Spix, 1829, da bacia do alto Paraná (Teleostei, Anostomidae).** Com. Mus. Ciênc. PUCRS. Porto Alegre, n. 44, p. 153-165, 1987.

GARAVELLO, J.C.; BRITSKI, H.Á. *Leporinus macrocephalus* sp. n. da bacia do rio Paraguai (Ostariophysi, Anostomidae). **Naturalia**, São Paulo, v. 13, p. 67-74, 1988.

GARAVELLO, J.C.; BRITSKI, H.A. Family Anostomidae. In: REIS, R.E.; KULLANDER, S.O.; FERRARIS JR, C.J. (Eds.). **Check list of the freshwater fishes of South and Central America.** Porto Alegre: EDIPUCRS, 2003. p.p. 71–84.

GARAVELLO, J.C., RAMIREZ, J.L., OLIVEIRA, A.K.D., BRITSKI, H.A., BIRINDELLI, J.L.O.; GALETTI JR, P. M. (2021). Integrative taxonomy reveals a new species of Neotropical headstanding fish in genus *Schizodon* (Characiformes: Anostomidae). **Neotropical Ichthyology**, v. 19, p. e210016, 2021. https://doi.org/10.1590/1982-0224-2021-0016

GÉRY, J. Contributions a l'étude des poissons characoides (Nº7) Validité de *Leporinus* despaxi Puyo et du sous-genre *Hypomasticus* Borodin**. Bulletin Du Muséum National d'Histoire Naturelle**, v. 32, n. 3, p. 222-229, 1960.

GÉRY, J**. Characoids of the world.** T.F.H. Publications, Neptune City, New Jersey, 1977.

GOMES, L.C.; PESSALI, T.C.; SALES, N.G.; POMPEU, P.S.; CARVALHO, D.C. Integrative taxonomy detects cryptic and overlooked fish species in a neotropical river basin. **Genetica**, v. 143, p. 581-588, 2015. https://doi.org/10.1007/s10709-015-9856-z

GRAÇA, W.J.; PAVANELLI, C.S. **Peixes da planície de inundação do Rio Paraná**. Maringá: EDUEM, p. 241, 2007.

GUIMARÃES, E.C.; BRITO, P.S.; OTTONI, F.P. Peixes. In: DORNAS, R.A.P.; ROLIM, S.G. (eds.): Fauna de vertebrados do entorno da estrada de ferro Carajás. **Editora Rupestre**, Belo Horizonte. p. 32-51, 2020c.

GUIMARÃES, E.C.; BRITO, P.S.; OLIVEIRA, R.F.; AGUIAR, R.G.; OTTONI, F.P.; GUIMARÃES, K.L.A. *et al.* Peixes do rio Pindaré e suas potencialidades ornamentais. **Instituto Maranhense de Estudos Socioeconômicos e Cartográficos**, São Luís. 2021a.

GUIMARÃES, E.C.; OLIVEIRA, R.F.; BRITO, P.S.; VIEIRA, L.O.; SANTOS, J.P.; OLIVEIRA, E.S. *et al.* Biodiversidade, potencialidades ornamentais e guia ilustrado dos peixes da Mata Itamacaoca município de Chapadinha-MA. **Instituto Maranhense de Estudos Socioeconômicos e Cartográficos**, São Luís, Brazil, v. 45, 2021b.

GUIMARÃES, E.C.; BRITO, P.S.; SANTOS, J.P.; OLIVEIRA, F.R.; OTTONI, F.P. Supplementary material to Guimarães *et al*. (2020): Peixes. Fauna de vertebrados ao longo da Estrada de Ferro Carajás. **Ichthyological Contributions of PecesCriollos**, v. 1, p. 1-10, 2021c.

GÜNTHER, A. XXXV.—Third account of new species of snakes in the collection of the British Museum. **Annals and Magazine of Natural History**, v. 12, n. 71, p. 348-365, 1863.

GÜNTHER, A. Catalogue of the fishes in the British Museum. Catalogue of the Physostomi, containing the families Siluridae, Characinidae, Haplochitonidae, Sternoptychidae, Scopelidae, Stomiatidae in the collection of the British Museum. **Catologue of Fishes**, v. 5, p. 1-455, 1864.

HARDMAN, M. The phylogenetic relationships among Noturus catfishes (Siluriformes: Ictaluridae) as inferred from mitochondrial gene cytochrome b and nuclear recombination activating gene 2. **Molecular phylogenetics and evolution**, v. 30, n. 2, p. 395-408, 2004. https://doi.org/10.1016/S1055-7903(03)00203-3

HEBERT, P.D.N.; CYWINSKA, A.; BALL, S.L.; DEWAARD, J.R. Biological identifications through DNA barcodes. **Proceedings of the Royal Society of London. Series B: Biological Sciences**, v. 270, n. 1512, p. 313-321, 2003. https://doi.org/10.1098/rspb.2002.2218

HUBERT, N.; HANNER, R.; HOLM, E.; MANDRAK, N.E.; TAYLOR, E.; BURRIDGE, M.; *et al*. Identifying canadian freshwater fishes trough DNA barcodes. **PLoS one**, v. 3, n. 6, p. e2490, 2008. https://doi.org/10.1371/journal.pone.0002490

IRWIN, D.M.; KOCHER, T.D.; WILSON, A.C. Evolution of the cytochrome b gene of mammals. **Journal of molecular evolution**, v. 32, p. 128-144, 1991.

JÉZÉQUEL, C.; TEDESCO, P.A.; BIGORNE, R.; MALDONADO-OCAMPO, J.A.; ORTEGA, H.; HIDALGO, M. *et al.* A database of freshwater fish species of the Amazon Basin. **Scientific Data**, v. 7, n. 1, p. 1-9, 2020. https://doi.org/10.1038/s41597-020-0436-4

KIRCHMAN, J.J.; WHITTINGHAM, L.A.; SHELDON, F.H. Relationships among cave swallow populations (Petrochelidon fulva) determined by comparisons of microsatellite and cytochrome b data. **Molecular Phylogenetics and Evolution**, v. 14, n. 1, p. 107-121, 2000. https://doi.org/10.1006/mpev.1999.0681

KOEHLER, MR.; DEHM, D.; GUTTENBACH, M.; NANDA, I.; HAAF, T.; MOLINA, WF.; *et al*. Cytogenetics of the genus *Leporinus* (Pisces, Anostomidae). 1. Karyotype analysis, heterochromatin distribution and sex chromosomes. **Chromosome Research** 5, p. 12–22. 1997a. https://doi.org/10.1023/A:1018485217391

KOEHLER, M.R.; HAAF, T.; GUTTENBACH, M.; SCHARTL, M.; SCHMID, M. Cytogenetic of the genus *Leporinus* (Pisces, Anostomidae). II. Molecular cytogenetics, organization and evolutionary conservation of a chromosomespecific satellite DNA from *Leporinus obtusidens*. **Chromosome Research**, v. 5, p. 325-331. 1997b. https://doi.org/10.1023/B:CHRO.0000038763.52875.48

KUMAZAWA, Y.; NISHIDA, M. Molecular phylogeny of osteoglossoids: a new model for Gondwanian origin and plate tectonic transportation of the Asian arowana. **Molecular Biology and Evolution**, v. 17, n. 12, p. 1869-1878, 2000. https://doi.org/10.1093/oxfordjournals.molbev.a026288

LANGEANI, F.; CASTRO, R.M.C.; OYAKAWA, O.T.; SHIBATTA, O.A.; PAVANELLI, C.S.; CASATTI, L. Diversidade da ictiofauna do Alto Rio Paraná: composição atual e perspectivas futuras. **Biota Neotropica**, v. 7, n. 3, p. 1-18. 2007.

LATINI, A.O.; OPORTO, L.T.; LIMA-JÚNIOR, D.P.; RESENDE, D.C.; LATINI, R.O. Peixes. In: LATINI, A.O.; RESENDE, D.C. (Eds.). **Espécies exóticas invasoras de águas continentais no Brasil**. Brasília, Ministério do Meio Ambiente. p. 295-581. 2016.

LEÃO, T.C.C.; ALMEIDA, W.R.; DECHOUM, M.; ZILLER, S.R. **Espécies exóticas invasoras no Nordeste do Brasil: contextualização, manejo e políticas públicas**. Recife, Cepan. 2011.

LIMEIRA-FILHO, D., MORAES, P.S.S., ALMEIDA, M.S., SILVA, A.C.C., SILVA, J.L. N., FARIAS-RODRIGUES, L.; *et al*. Fish diversity in the rivers that drain the Baixada Maranhense and the Mearim basin in northeastern Brazil. **Brazilian Journal of Biology**, v. 83, e273525, 2023.https://doi.org/10.1590/1519-6984.273525

LINNAEUS, C. **Systema Naturae per regna tria naturae, secundum classes, ordines,genera, species, cum characteribus, differentiis, synonymis, locis**. 10 ed. Edito duodecima, reformata. Laurentius Salvius, Holmiae, 1758. p. 824. https://doi.org/10.5962/bhl.title.157601

LOVEJOY, N.R.; DE ARAÚJO, M.L.G. Molecular systematics, biogeography and population structure of Neotropical freshwater needlefishes of the genus *Potamorrhaphis*. **Molecular Ecology**, v. 9, n. 3, p. 259-268, 2000. https://doi.org/10.1046/j.1365-294x.2000.00845.x

LOVEJOY, N.R.; COLLETTE, B.B. Phylogenetic relationships of New World needlefishes (Teleostei: Belonidae) and the biogeography of transitions between marine and freshwater habitats. **Copeia**, v. 2001, n. 2, p. 324-338, 2001. https://doi.org/10.1643/0045-8511(2001)001[0324:PRONWN]2.0.CO;2

LYDEARD, C.; ROE, K.J. The phylogenetic utility of the mitochondrial cytochrome b gene for inferring relationships among actinopterygian fishes. **Molecular systematics of fishes**, v. 1, p. 285-303, 1997.

MACHADO, V. N.; COLLINS, R. A.; OTA, R. P.; ANDRADE, M. C.; FARIAS, I. P.; HRBEK, T. One thousand DNA barcodes of piranhas and pacus reveal geographic structure and unrecognised diversity in the Amazon. **Scientific Reports**, v. 8, n. 1, p. 8387, 2018. https://doi.org/10.1038/s41598-018-26550-x

MARANHÃO. Governo do Estado do Maranhão. **Atlas do Maranhão**. Gerência de Planejamento e Desenvolvimento Econômico/Laboratório de Geoprocessamento - UEMA. São Luís: GEPLAN, 2002.

MARANHÃO. Governo do Estado do Maranhão**. Bacias hidrográficas: Subsídios para o planejamento e a gestão territorial.** São Luis, 2011.

MARTINS, C.; WASKO, A.P.; OLIVEIRA, C.; FORESTI, F. Mitochondrial DNA variation in wild populations of Leporinus elongatus from the Paraná River basin. **Genetics and Molecular Biology**, v. 26, p. 33-38, 2003. https://doi.org/10.1590/S1415-47572003000100006

MARTINS, M. B.; DE OLIVEIRA, T. G. **Amazônia maranhense: diversidade e conservação**. Belém: Museu Paraense Emílio Goeldi. 2011.

MAUTARI, K.C.; MENEZES, N.A. Revision of the South American freshwater fish genus Laemolyta Cope, 1872 (Ostariophysi: Characiformes: Anostomidae). **Neotropical Ichthyology**, v.4, n. 1, p. 27–44, 2006. https://doi.org/10.1590/S1679-62252006000100002

MELO, B.F.; OCHOA, L.E.; VARI, R.P.; OLIVEIRA, C. Cryptic species in the Neotropical fish genus Curimatopsis (Teleostei, Characiformes). **Zoologica Scripta**, v. 45, n. 6, p. 650-658, 2016. https://doi.org/10.1111/zsc.12178

MELO, F.A.G.; BUCKUP, P.A.; RAMOS, T.P.A.; SOUZA, A.K.N.; SILVA, C.M.A.; COSTA, T.C. *et al*. Fish fauna of the lower course of the Parnaíba river, northeastern Brazil. **Boletim do Museu de Biologia,** v. 38, n. 4, p. 363-400, 2016.

MEYER, A.; WILSON, A.C. Origin of tetrapods inferred from their mitochondrial DNA affiliation to lungfish. **Journal of Molecular Evolution**, v. 31, p. 359-364, 1990.

MEYER, A. Evolution of mitochondrial DNA in fishes. In: Hochachka, Mommsen (eds) **Biochemistry and molecular biology of fishes, vol. 2**. Elsevier, Amsterdam p. 1-38, 1993.

MINISTÉRIO DO MEIO AMBIENTE. **Caderno da Região Hidrográfica do Parnaíba. Brasília: Ministério do Meio Ambiente, Secretaria de Recursos Hídricos**. Brasília: MMA, 184 pp, 2006.

MINISTÉRIO DO MEIO AMBIENTE. **Caderno da Região Hidrográfica do Tocantins-Araguaia, Secretaria de Recursos Hídricos**. Brasília: MMA, 132 p., 2006.

MOYER, G.R.; BURR, B.M.; KRAJEWSKI, C. Phylogenetic relationships of thorny catfishes (Siluriformes: Doradidae) inferred from molecular and morphological data. **Zoological Journal of the Linnean Society**, v. 140, n. 4, p. 551-575, 2004. https://doi.org/10.1111/j.1096-3642.2004.00114.x

NAÇÕES UNIDAS BRASIL. **Objetivos de Desenvolvimento Sustentável**. Disponível em: https://brasil.un.org/pt-br/sdgs, Acesso em: 03/08/2023.

NASCIMENTO, M.H.S.; ALMEIDA, M.S.; VEIRA, M.N.S.; LIMEIRA FILHO, D.; LIMA, R.C.; BARROS, M.C. *et al*. DNA barcoding reveals high levels of genetic diversity in the fishes of the Itapecuru Basin in Maranhão, Brazil. **Genetics and Molecular Research**, v. 15, n. 3, p. 1-11, 2016. http://dx.doi.org/10.4238/gmr.15038476

NASCIMENTO, M.H.S., ARAGÃO, D.G., SILVA, J.L.N., LIMA, R.C., BIRINDELLI, J.L. O., FRAGA, E.C. *et al.* The DNA barcode reveals cryptic diversity and a new record for the genus Leporinus (Characiformes, Anostomidae) in the hydrographic basins of central northern Brazil. **PeerJ**, v. 11, p. e15184, 2023. https://doi.org/10.7717/peerj.15184

NEAR, T.J.; BOLNICK, D.I.; WAINWRIGHT, P.C. Investigating phylogenetic relationships of sunfishes and black basses (Actinopterygii: Centrarchidae) using DNA sequences from mitochondrial and nuclear genes. **Molecular Phylogenetics and Evolution**, v. 32, n. 1, p. 344-357, 2004. https://doi.org/10.1016/j.ympev.2003.12.010

NELSON, J.S. **Fishes of the World.** 4. ed. New Jersey: John Wiley & Sons, 2006.

NORMARK, B.B.; MCCUNE, A.R.; HARRISON, R.G. Phylogenetic relationships of neopterygian fishes, inferred from mitochondrial DNA sequences. **Molecular biology and evolution**, v. 8, n. 6, p. 819-834, 1991. https://doi.org/10.1093/oxfordjournals.molbev.a040685

OLIVEIRA, C.; AVELINO, G.S.A.; ABE, K.T.; MARIGUELA, T.C.; BENINE, R.C.; ORTÍ, G. *et al*. Phylogenetic relationships within the speciose family Characidae (Teleostei: Ostariophysi: Characiformes) based on multilocus analysis and extensive ingroup sampling. **BMC Evolutionary Biology**, v. 11, n.1, p. 1-25, 2011. https://doi.org/10.1186/1471-2148-11-275

ORTI, G.; MEYER, A. Molecular evolution of ependymin and the phylogenetic resolution of early divergences among euteleost fishes. **Molecular Biology and Evolution**, v. 13, n. 4, p. 556-573, 1996. https://doi.org/10.1093/oxfordjournals.molbev.a025616

ORTÍ, G.; KOCHER, T. D. Radiation of characiform fishes: evidence from mitochondrial and nuclear DNA sequences. In: KOCHER, T. D.; STEPIEN, C. A. (Eds.), **Molecular systematics of fishes**. San Diego, CA: Academic Press. 1997. p.p. 219-243.

PEREIRA, L.H.G.; MAIA, G.M.G.; HANNER, R.; FORESTI, F.; OLIVEIRA, C. DNA barcodes discriminate freshwater fishes from the Paraíba do sul River Basin, São Paulo, Brazil. **Mitochondrial DNA**, v. 22, n. 1, p. 71–79, 2011. https://doi.org/10.3109/19401736.2010.532213

PEREIRA, L.H.G.; HANNER, R.; FORESTI, F.; OLIVEIRA, C. Can DNA barcoding accurately discriminate megadiverse Neotropical freshwater fish fauna? **BMC genetics**, v. 14, n. 1, p. 1-14, 2013. https://doi.org/10.1186/1471-2156-14-20

PINHEIRO, C.U.B. **Estudo etnobiológicos: Baixada Maranhense**. Relatório final – Universidade Federal do Maranhão / DEOLI. São Luís, 2000-2003.

PIORSKI, N.M.; CASTRO, A.C.L.; PEREIRA, L.G.; MUNIZ, M.E.L. Ictiofauna do trecho inferior do Rio Itapecuru, Nordeste do Brasil. **Boletim do Laboratório de Hidrobiologia**, v. 11, n. 1, p. 15-24, 1998.

PIORSKI, Nivaldo Magalhães. **Diversidade genética e filogeografia das espécies *Hoplias malabaricus* (Bloch, 1794) e *Prochilodus lacustris* Steindachner, 1907 no Nordeste do Brasil**. 2010. Tese (Doutorado em Genética e Evolução) - Universidade de São Carlos, São Paulo, 2010.

PIRES, A.A.; RAMIREZ, J.L.; GALETTI JR, P.M.; TRÓY, W.P.; FREITAS, P.D. Molecular analysis reveals hidden diversity in Zungaro (Siluriformes: Pimelodidade): a genus of giant South American catfish. **Genetica**, v. 145, p. 335-340, 2017. https://doi.org/10.1007/s10709-017-9968-8

PUGEDO, M.L.; DE ANDRADE NETO, F.R.; PESSALI, T.C.; BIRINDELLI, J.L.O.; CARVALHO, D.C. Integrative taxonomy supports new candidate fish species in a poorly studied neotropical region: the Jequitinhonha River Basin. **Genetica**, v. 144, n. 3, p. 341-349, 2016. https://doi.org/10.1007/s10709-016-9903-4

PUYO, J. 1943. Nouveaux poissons d'eau douce de la Guyane francaise**. Bulletin de la Société d'Histoire Naturelle de Toulouse**, v. 78, n. 3, p.141-149, 1943.

QUENOUILLE, B.; BERMINGHAM, E.; PLANES, S. Molecular systematics of the damselfishes (Teleostei: Pomacentridae): Bayesian phylogenetic analyses of mitochondrial and nuclear DNA sequences. **Molecular Phylogenetics and Evolution**, v. 31, n. 1, p. 66-88, 2004. https://doi.org/10.1016/S1055-7903(03)00278-1

RAMIREZ, J.L.; GALETTI JR, P.M. DNA barcode and evolutionary relationship within Laemolyta Cope 1872 (Characiformes: Anostomidae) through molecular analyses. **Molecular Phylogenetics and Evolution**, v. 93, p. 77-82, 2015. https://doi.org/10.1016/j.ympev.2015.07.021

RAMIREZ, J.L.; CARVALHO-COSTA, L.F.; VENERE, P.C.; CARVALHO, D.C.; TROY, W.P.; GALETTI JR, P.M. Testing monophyly of the freshwater fish *Leporinus* (Characiformes, Anostomidae) through molecular analysis. **Journal of Fish Biology,** v. 88, n. 3, p. 1204–1214, 2016.https://doi.org/10.1111/jfb.12906

RAMIREZ, J.L.; BIRINDELLI, J.L.O.; GALETTI JR, P.M. A new genus of Anostomidae (Ostariophysi: Characiformes): Diversity, phylogeny and biogeography based on cytogenetic, molecular and morphological data. **Molecular Phylogenetics and Evolution**, v.107, n. 1, p. 308–323, 2017a.https://doi.org/10.1016/j.ympev.2016.11.012

RAMIREZ, J.L.; BIRINDELLI, J.L.; CARVALHO, D.C.; AFFONSO, P.; VENERE, P.C.; ORTEGA, H.; *et al*. Revealing hidden diversity of the underestimated neotropical ichthyofauna: DNA barcoding in the recently described genus *Megaleporinus* (characiformes: Anostomidae). **Frontiers in Genetics**, v. 8, n. 1, p. 1–11, 2017b. https://doi.org/10.3389/fgene.2017.00149

RAMIREZ, J.L.; SANTOS, C.A.; MACHADO, C.B.; OLIVEIRA, A.K.; GARAVELLO, J.C.; BRITSKI, H.A. *et al*. Molecular phylogeny and species delimitation of the genus *Schizodon* (Characiformes, Anostomidae). **Molecular Phylogenetics and Evolution**, v. 153, n. 106959, p. 1-14, 2020. http://doi:10.1016/j.ympev.2020.106959

RAMOS, T.P.A.; RAMOS, R.T.C.; RAMOS, S.A.Q.A. Ichthyofauna of the Parnaíba river basin, northeastern Brazil. **Biota Neotropica**, v. 14, n. 1, p. 1-8, 2014. https://doi.org/10.1590/S1676-06020140039

REGAN, C.T. II. — The classification of the teleostean fishes of the order Ostariophysi.— *1* . Cyprinoidea. **Annals and Magazine of Natural History**, v. 8, n. 43, p. 37-41, 1911. https://doi.org/10.1080/00222931108692993

REIS, R.E.; KULLANDER, S.O.; FERRARIS JR, C.J. **Check list of the freshwater fishes of South and Central America**. Porto Alegre: EDIPUCRS, 2003, p. 742.

REIS, R.E.; ALBERT, J.S.; DI DARIO, F.; MINCARONE, M. M.; PETRY, P.; ROCHA, L.A. Fish biodiversity and conservation in South America. **Journal of Fish Biology**, v. 89, n. 1, p. 12–47. 2016. https://doi.org/10.1111/jfb.13016

RIBEIRO, M.F.R.; PIORSKI, N. M.; ALMEIDA, Z.S.; NUNES, J.L.S. Fish aggregating known as moita, an artesanal fishing technique performed in the Munim River, State of Maranhão, Brazil. **Boletim Instituto de Pesca**, v. 40, n. 4, p. 677-682, 2014.

ROCHA-OLIVARES, A.; ROSENBLATT, R.H.; VETTER, R.D. Molecular Evolution, Systematics, and Zoogeography of the Rockfish Subgenus *Sebastomus* (Sebastes, Scorpaenidae) Based on Mitochondrial Cytochromeband Control Region Sequences. **Molecular Phylogenetics and Evolution**, v. 11, n. 3, p. 441-458, 1999. https://doi.org/10.1006/mpev.1998.0585

ROMANO, S.L.; PALUMBI, S.R. Evolution of scleractinian corals inferred from molecular systematics. **Science**, v. 271, n. 5249, p. 640-642, 1996. https://doi.org/10.1126/science.271.5249.640

ROSA, R. S.; MENEZES, N. A.; BRITSKI, H. A.; COSTA, W. J. E.; GROTH, F. **Diversidade, padrões de distribuição e conservação dos peixes da caatinga**. p.135-180. In: I.R. LEAL, M. TABARELLI; J.M.C. DA SILVA (ed.). Ecologia e Conservação da Caatinga. Recife: Editora Universitária da Universidade Federal de Pernambuco. 822 pp, 2003.

ROSSINI, B.C.; OLIVEIRA, C.A.M.; DE MELO, F.A.G.; DE ARAÚJO BERTACO, V.; DE ASTARLOA, J.M.D.; ROSSO, J.J.; *et al.* Highlighting Astyanax species diversity through DNA Barcoding. **PLoS ONE**, v. 11, n. 12, e0167203, 2016. https://doi.org/10.1371/journal.pone.0167203

SANTOS, G.M.; JÉGU, M.; LIMA, Á.C. Novas ocorrências de Leporinus pachycheilus Britsky, 1976 e descrição de uma especie nova do mesmo grupo na amazonia brasileira (Osteichthyes, Anostomidae). **Acta Amazonica**, v. 26, n. 4, p. 265–280, 1996. https://doi.org/10.1590/1809-43921996264280

SANTOS, S.; SCHNEIDER, H.; SAMPAIO, I. Genetic differentiation of *Macrodon ancylodon* (Sciaenidae, Perciformes) populations in Atlantic coastal waters of South America as revealed by mtDNA analysis. **Genetics and Molecular Biology**, v. 26, p. 151-161, 2003. https://doi.org/10.1590/S1415-47572003000200008

SECRETARIAT OF THE CONVENTION ON BIOLOGICAL DIVERSITY. **Convention on biological diversity**. Rio de Janeiro: United Nations Environment Programme, 1992.

SECRETARIAT OF THE CONVENTION ON BIOLOGICAL DIVERSITY. **Handbook of the convention on biological diversity including its Cartagena protocol on biosafety**. 3. ed. Canada: Convention on biological diversity, 2005.

SIDLAUSKAS, B.L.; SANTOS, G.M. Pseudanos winterbottomi: A New Anostomine Species (Teleostei: Characiformes: Anostomidae) from Venezuela and Brazil, and Comments on Its Phylogenetic Relationships. **Copeia**, v. 2005, n. 1, p. 109–123, 2005. https://doi.org/10.1643/CI-04-100R2

SIDLAUSKAS, B.L.; VARI, R.P. Phylogenetic relationships within the South American fish family Anostomidae (Teleostei, Ostariophysi, Characiformes). **Zoological Journal of the Linnean Society**, v. 154, n. 1, p. 70–210, 2008.https://doi.org/10.1111/j.1096-3642.2008.00407.x

SIDLAUSKAS, B. L.; MOL, J. H.; VARI, R.P. Dealing with allometry in linear and geometric morphometrics: A taxonomic case study in the Leporinus cylindriformis group (Characiformes: Anostomidae) with description of a new species from Suriname. **Zoological Journal of the**

**Linnean Society**, v. 162, n. 1, p. 103–130, 2011. https://doi.org/10.1111/j.1096-3642.2010.00677.x

SIDLAUSKAS, B. L.; VARI, R. P. Diversity and distribution of anostomoid fishes (Teleostei: Characiformes) throughout the Guianas. **Cíbio**. v. 36, n. 1, p. 71-103, 2012.

SILVA, G. S. C.; MELO, B. F.; OLIVEIRA, C.; BENINE, R. C. Revision of the South American genus Tetragonopterus Cuvier, 1816 (Teleostei: Characidae) with description of four new species. **Zootaxa**, v. 4200, n. 1, p. 1-46, 2016.

SILVA-SANTOS, R.; RAMIREZ, J. L.; GALETTI JR, P. M.; FREITAS, P. D. Molecular Evidences of a Hidden Complex Scenario in *Leporinus* cf. *friderici*. **Frontiers in Genetics**, v. 9, p. 47, 2018. https://doi.org/10.3389/fgene.2018.00047

SLOWINSKI, J.B., PAGE, R.D.M. How should species phylogenies be inferred from sequence data?. **Systematic Biology**, v. 48, n. 4, p. 814-825, 1999.

SOARES, C.M.; HAYASHI, C.; FURUYA, V.R.B.; FURUYA, W.M.; GALDIOLI, E.M. Substituição parcial e total da proteína do farelo de soja pela proteína do farelo de canola na alimentaçãoo de alevinos de Piavuçu (*Leporinus macrocephalus*). **Revista Brasileira de Zootecnia**, v. 29, n. 1, p. 15-22, 2000.

SOARES, E.C. **Peixes do Mearim**. Instituto Geia, São Luís. 2005.

SOARES JÚNIOR, A. V.; HASUI, Y.; COSTA, J. B. S.; MACHADO, F. B. Evolução do rifteamento e paleogeografia da margem atlântica equatorial do Brasil: Triássico ao Holoceno. **Geociências**, v. 30, n. 4, p. 669-692, 2011.

SPIX, J. B.; AGASSIZ, L. Selecta genera et species Piscium quos in itinere per Brazilian annis 1817–1820; Peracto et pigendus curavit Dr. J.B. de Spix. **Digessit descripsit et observationibus anatomicis illustravit Dr. L. Agassiz. [Memoriae J.B. de Spix]. Munich: Typis C. Wolf**, 1829.

STEINDACHNER, F. Die Süsswasserfische des südöstlichen Brasilien (II). **Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften. Mathematisch-Naturwissenschaftliche Classe**, v. 70, n. 28, p. 499-538, 1875.

STELLA, A. **Síntese do diagnóstico, matriz do plano e contribuições do processo de consulta pública para elaboração do PPCD-MA**. Secretaria de Estado do Meio Ambiente. Brasília, Distrito Federal, 2011.

STEPIEN, C. A.; KOCHER, T.D. Molecules and morphology in studies of fish **evolution**. In: KOCHER, T.D.; STEPIEN, C.A. (Eds.), **Molecular systematics of fishes**. San Diego, CA: Academic Press. 1997. p.p. 1-11.

STURMBAUER, C.; MEYER, A. Genetic divergence, speciation and morphological stasis in a lineage of African cichlid fishes. **Nature**, v. 358, n. 6387, p. 578-581, 1992.

TOLEDO-PIZA, M.; BAENA, E.G.; DAGOSTA, F.C.P.; MENEZES, N.A.; ÂNDRADE, M.; BENINE, R.C. *et al*. Checklist of the species of the Order Characiformes (Teleostei: Ostariophysi). **Neotropical Ichthyology,** v. 22, n. 1, p. e230086, 2024. https://doi.org/10.1590/1982-0224-2023-0086

TSIGENOPOULOS, C.S.; BERREBI, P. Molecular phylogeny of North Mediterranean freshwater barbs (genus Barbus: Cyprinidae) inferred from cytochrome b sequences: biogeographic and systematic implications. **Molecular Phylogenetics and Evolution**, v. 14, n. 2, p. 165-179, 2000. https://doi.org/10.1006/mpev.1999.0702

VARI, R.P.; WILLIAMS, A.M. Headstanders of the neotropical Anostomid genus *Abramites* (Pisces: Characiformes: Anostomidae). **Proceedings of the Biological Society of Washington**, v. 100, n. 1, p. 89-103,1987.

WARD, R. D.; COSTA, F. O.; HOLMES, B. H.; STEINKE, D. DNA barcoding of shared fish species from the North Atlantic and Australasia: minimal divergence for most taxa, but *Zeus faber* and *Lepidopus caudatus* each probably constitute two species. **Aquatic Biology**, v. 3, n. 1, p. 71-78, 2008. https://doi.org/10.3354/ab00068

ZEINAD, A.K.; PRADO, R.A. Peixes Fluviais do Brasil - espécies esportivas. São Paulo: Editora: **Pescaventura**, 360p. 2012.

# APÊNDICE

Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

# New records of the occurrence of *Megaleporinus macrocephalus* (Garavello & Britski, 1988) (Characiformes, Anostomidae) from the basins of the Itapecuru and Mearim rivers in Maranhão, Northeastern Brazil

## Novos registros da ocorrência de *Megaleporinus macrocephalus* (Garavello & Britski, 1988) (Characiformes, Anostomidae) nas bacias dos rios Itapecuru e Mearim no Maranhão, Nordeste, Brasil

M. S. Almeida[a]*, P. S. S. Moraes[b], M. H. S. Nascimento[c], J. L. O. Birindelli[d], F. M. Assega[d], M. C. Barros[b] and E. C. Fraga[a]

[a]Universidade Estadual do Maranhão – UEMA, Departamento de Química e Biologia, Programa de Pós-Graduação em Recursos Aquáticos e Pesca, São Luís, MA, Brasil

[b]Universidade Estadual do Maranhão – UEMA, Laboratório de Genética e Biologia Molecular – GENBIMOL, Caxias, MA, Brasil

[c]Universidade Estadual do Maranhão – UEMA, Centro de Ciências Agrárias – CCA, Programa de Mestrado em Ciência Animal – CCMA, São Luís, MA, Brasil

[d]Universidade Estadual de Londrina, Departamento de Biologia Animal e Vegetal, Londrina, PR, Brasil

### Abstract

The “piaussu”, *Megaleporinus macrocephalus* is an anostomatid fish species native to the basin of the Paraguay River, in the Pantanal biome of western Brazil. However, this species has now been recorded in a number of other drainages, including those of the upper Paraná, Uruguay, Jacuí, Doce, Mucuri, and Paraíba do Sulrivers. This study presents two new records of the occurrence of *M. macrocephalus*, in the basins of the Itapecuru and Mearim rivers in the state of Maranhão, in the Brazilian Northeast. The piaussu is a large-bodied fish of commercial interest that is widely raised on fish farms, and its occurrence in the Itapecuru and Mearim rivers is likely the result of individuals escaping from fish tanks when they overflow during the rainy season. Morphological analyses and sequences of the Cytochrome Oxidase Subunit I (COI) gene confirmed the taxonomic identification of the specimens as *M. macrocephalus*. The COI sequences were 99.66% similar to those of *M. macrocephalus* deposited in the BOLDSystems database. These records extend the known distribution of *M. macrocephalus* to the basins of the Itapecuru and Mearim rivers in the Brazilian Northeast, highlighting a new case of introduction of exotic fish species into Brazilian river basins.

**Keywords:** freshwater, Ichthyofauna, taxonomy, biological invasion, COI.

### Resumo

*Megaleporinus macrocephalus* é uma espécie de peixe anostomatídeo nativa da bacia do rio Paraguai, no bioma Pantanal do oeste do Brasil. No entanto, essa espécie já foi registrada em várias outras drenagens, incluindo as dos rios Alto Paraná, Uruguai, Jacuí, Doce, Mucuri e Paraíba do Sul. Este estudo apresenta dois novos registros da ocorrência de *M. macrocephalus*, nas bacias dos rios Itapecuru e Mearim, no estado do Maranhão, no nordeste brasileiro. O piaussu é um peixe de grande porte, de interesse comercial, amplamente criado em pisciculturas, e sua ocorrência nos rios Itapecuru e Mearim é provavelmente o resultado de indivíduos que escapam dos tanques quando transbordam durante a estação chuvosa. Análises morfológicas e sequências do gene da subunidade I do citocromo oxidase (COI) confirmaram a identificação taxonômica dos espécimes como *M. macrocephalus*. As sequências de COI foram 99,66% semelhantes às de *M. macrocephalus* depositadas no banco de dados BOLDSystems. Esses registros estendem a conhecida distribuição de *M. macrocephalus* às bacias dos rios Itapecuru e Mearim, no nordeste brasileiro, destacando um novo caso de introdução de espécies exóticas de peixes nas bacias hidrográficas brasileiras.

**Palavras-chave:** água doce, Ictiofauna, taxonomia, invasão biológica, COI.

---

*e-mail: almeidasp101@gmail.com

Received: January 9, 2020 – Accepted: June 15, 2020

## 1. Introduction

The family Anostomidae is currently composed of 149 valid species, distribuided in 15 genera (Fricke et al., 2020). Species of the family are endemicthe Neotropical region, and a widely distributed from north of the Colombia to La Plata River, in Argentina (Garavello and Britski, 2003). The greatest diversity of anostomatids is found in the Amazon basin, where 91 species have been recorded to date (Dagosta and Pinna, 2019). The genus *Leporinus* Agassiz, 1829, is the most species-rich of Anostomidae (Burns et al., 2017).

Garavello and Britski (1988) described *Leporinus macrocephalus* based on 15 specimens collected in the Paraguay River basin in Brazil. The species was recently transferred to *Megaleporinus*, a genus described in 2017 by Ramirez et al. (2017), based on morphological, molecular, and cytogenetic data, including a unique system of ZZ/ZW sex chromosomes.

The fish of the genus *Megaleporinus* are relatively large in size (adult standard length typically over 35 cm), and are diagnosed among anostomids by having the following combination of exclusive features: premaxilla with three unicuspid teeth; dentary with three unicuspid teeth; and body with one to four dark midlateral blotches (Ramirez et al., 2017).

*Megaleporinus macrocephalus* (Garavello and Britski, 1988), known in Brazil as the“piaussu”, “piavuçu” or “piau-açu”, is an omnivorous fish that feeds on small fruit and seeds, as well as small fish and crabs (Peruca et al., 2000; Navarro et al., 2006). *M. macrocephalus* is distributed in the Paraguay River in Argentina, Brazil, Bolivia and Paraguay (Garavello and Britski, 1988; Britski et al., 1999; Fricke, 2019). However, individuals escaped from fish farms have colonized a number of other South American basins, including that of the upper Paraná River (Graça and Pavanelli, 2007; Langeani et al., 2007), the Jacuí and Uruguay Rivers in the Brazilian state of Rio Grande do Sul (Bertaco et al., 2016), the Mucuri River (Gomes et al., 2015), and the Doce and Paraíba do Sul rivers (Alves et al., 2007). The piaussu is commercially important for sports fishing (Zeinad and Prado, 2012) and is widely raised on fish farms to supply markets in southern and central Brazil (Soares et al., 2000; IBGE, 2014).

The present study provides the first report of the occurrence of *M. macrocephalus* in northeastern Brazil, more specifically in the Itapecuru and Mearim rivers, in the state of Maranhão. The Mearim is the state’s largest hydrographic basin, and plays an important role in the economy and subsistence of the region’s riverside populations (Maranhão, 2011). These records are the first evidence of the occurrence of *M. macrocephalus* in the Itapecuru and Mearim Rivers, extending the limits of the known geographic distribution of the species.

## 2. Material and Methods

Specimens of *M. macrocephalus* were collected in the basins of the Itapecuru and Mearim rivers, in the state of Maranhão, Northeastern Brazil (Figure 1). The Mearim

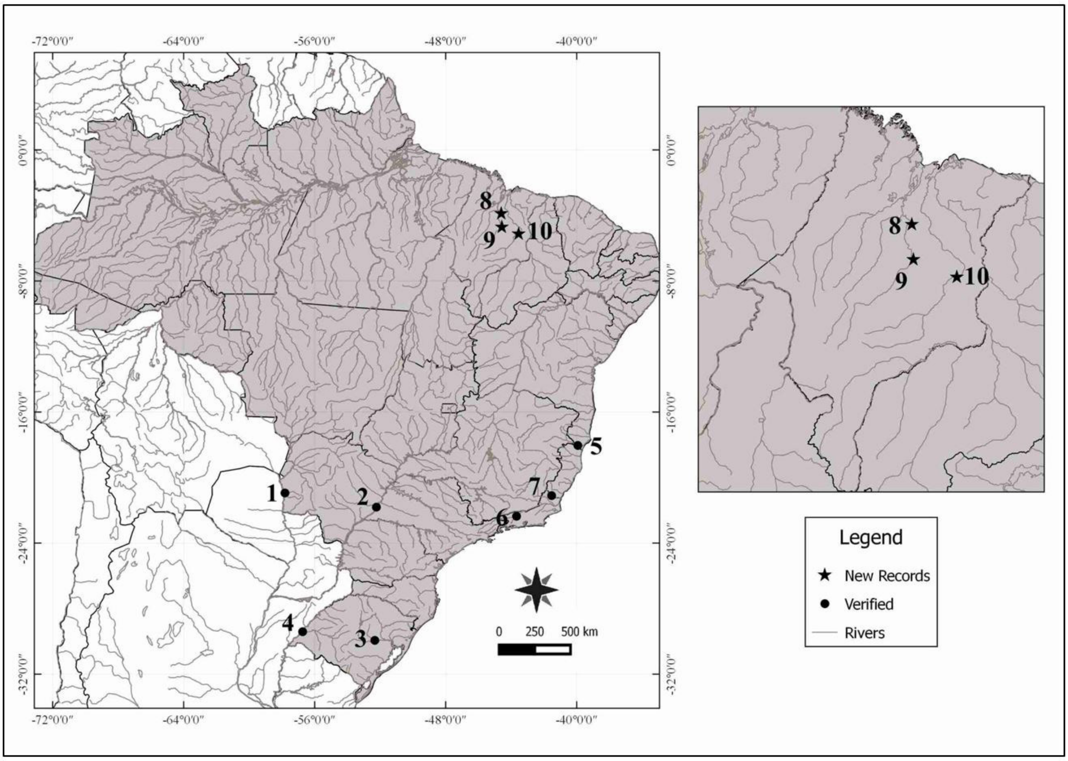


**Figure 1.** Geographic distribution of *Megaleporinus macrocephalus* in Brazil. The area of the new registrations for the Itapecuru rivers in the municipality of Pé da Serra and Mearim in the locality Laje dos Currais, in São Mateus, and in the city of Pedreiras in the Northeast Region.

River has a total extension of 832.18 km, and has a number of tributaries, in particular the Pindaré River, which joins the Mearim only 20 km from the mouth of the Grajaú River, a second important tributary of the Mearim River. The Itapecuru River is located wholly within the state of Maranhão, and has three distinct stretches – the upper river (from the source to the municipality of Colinas), the middle river (from Colinas to the municipality of Caxias), and the lower Itapecuru, from Caxias to the mouth, in São José Bay (Alcântara, 2004). The Itapecuru River, which is 852.71 km long, has eight major tributaries on its right margin, in particular the Pirapemas and Itapecuruzinho Rivers, and the Seco, do Ouro, Gameleira, Cachimbo, and Guariba streams (UEMA, 2016).

The specimens were collected using gill, drag, and cast nets. The voucher specimens were taken to the Laboratory of Genetics and Molecular Biology (GENBIMOL) at the Center for Higher Studies at Maranhão State University (CESC/UEMA), fixed in 10% formalin, and conserved in 70% alcohol. The prepared specimens were sent to the Zoology Museum at the State University of Londrina (MZUEL), Paraná, Brazil, for morphological identification and cataloguing (voucher MZUEL: 15357 – Mearim river) e (voucher MZUEL: 20221 – Itapecuru river).

The collection of specimens was authorized by the Brazilian Federal Institute for the Environment and Renewable Natural Resources (IBAMA) through licence number 02012.004159/2006, and ICMBio/MMA licence 42119-2, from the Chico Mendes Institute for Biodiversity Conservation.

Measurements were taken following Britski and Garavello (1978) and Birindelli et al. (2013). Morphometric data were obtained with a digital caliper with accuracy of 0.01 mm. Counts of scales and rays were taken according to Birindelli et al. (2013).

Samples of muscle tissue were extracted from the specimens for the genetic analyses. The total DNA was extracted using the Promega Wizard Genomic DNA purification kit, following the manufacturer's instructions. The Cytochrome Oxidase Subunit I (COI) was then amplified by Polymerase Chain Reaction, using the universal primers COI FishF1: 5" -TCAACCAACCACAAAGACATTGCCAC – 3"; and COI FishR1: 5" - TAGACTTCTGGGTGGCCAAAGAATCA – 3", as described by Ward et al. (2005). The samples were sequenced by Sanger et al. (1977) dideoxyterminal method, using a Big Dye kit in an ABI Prism™ 3500 automatic sequencer (Applied Biosystems, USA). The sequences were edited and aligned in BIOEDIT 7.0 (Hall, 1999), and plotted in the BOLD Systems v4 platform (BOLD, 2020) to evaluate their similarity with existing sequences (Ratnasingham and Hebert, 2007).

## 3. Results

On the Mearim River, one *M. macrocephalus* specimen was collected on the lower course of the river at Laje dos Currais, in the municipality of São Mateus, during the flood season, while two specimen was obtained on the middle course of the river in the town of Pedreiras during the dry (low water) season. The *M. macrocephalus* specimen was collected at the locality of Pé da Serra in the municipality of Caxias during the dry season.

The four *M. macrocephalus* specimens (Table 1) present a characteristic coloration pattern, with a darker superior half of the body, three large black spots on each flank, and dark lines between the rows of scales (Ramirez et al., 2017). The body is robust, and the mouth terminal, with three teeth in the pre-maxillary bone and three teeth in

**Table 1.** Morphological data of *Megaleporinus macrocephalus*.

| Characters | MZUEL 20221 | MZUEL 15357 |
|---|---|---|
| | **Itapecuru** | **Mearim** |
| Standard Length (mm) | 387.1 | 175.2 |
| **Percentages of standard length** | | |
| Predorsal distance | 41.10 | 43.83 |
| Dorsal-fin origin to adipose-fin origin | 41.37 | 37.76 |
| Prepelvic distance | 43.40 | 47.62 |
| Body depth | 28.14 | 28.06 |
| Caudal-peduncle length | 11.41 | 12.50 |
| Caudal-peduncle depth | 16.93 | 15.45 |
| Anal-fin lobe length | 10.59 | 10.04 |
| Head length | 22.32 | 27.26 |
| **Percentages of head length** | | |
| Preopercle length | 75.81 | 64.68 |
| Snout length | 35.78 | 29.45 |
| Head depth | 96.92 | 85.41 |
| Eye diameter | 13.33 | 23.51 |
| Bony interorbital | 58.88 | 54.53 |
| **Meristics** | | |
| Lateral-line scales | 43 | 42 |
| Scale rows between lateral line and dorsal-fin origin | 7 | 7 |
| Scale rows between lateral line and anal-fin origin | 6 | 6 |
| Scale rows around caudal peduncle | 16 | 16 |
| Predorsal scales | 13 | 13 |
| Dorsal-fin unbranched rays | 2 | 2 |
| Dorsal-fin branched rays | 10 | 10 |
| Pectoral-fin branched rays | 14 | 15 |
| Pelvic-fin branched rays | 8 | 8 |
| Anal-fin unbranched rays | 3 | 3 |
| Anal-fin branched rays | 8 | 8 |
| Upper caudal-fin branched rays | 9 | 9 |
| Upper caudal-fin branched rays | 8 | 8 |
| Teeth on left premaxilla | 3 | 3 |
| Teeth on left dentary | 3 | 3 |

**Figure 2.** *Megaleporinus macrocephalus*, MZUEL 20221, 387.10 mm SL.

**Table 2.** Similarity percentage obtained by comparing the sequences of the COI gene on the BOLDSystems platform (The Barcode of Life Data System) for the *M. macrocephalus* species from the Itapecuru and Mearim rivers, Maranhão, Brazil, identified morphologically and which were confirmed with the molecular data.

| Identificação | | | |
|---|---|---|---|
| **Código** | **N** | **Taxonômica** | **Molecular: Bold** |
| MEA 831 | 1 | *M. macrocephalus* | *M. macrocephalus* (99. 66%) |
| MEA 835 | 1 | *M. macrocephalus* | *M. macrocephalus* (99. 66%) |
| MEA 836 | 1 | *M. macrocephalus* | *M. macrocephalus* (99. 66%) |
| LEP 158 | 1 | *M. macrocephalus* | *M. macrocephalus* (99. 66%) |

the dentary bone (Figure 2). The specimens have 42–43 scales in the lateral line, seven rows of scales between the lateral line and the origin of the dorsal fin, six rows of scales between the lateral line and the origin of the pelvic fin, and 16 rows of scales around the caudal peduncle. These characteristics, together with the morphometry of the specimens, are fully consistent with the descriptions of *M. macrocephalus* published Garavello and Britski (1988) and Britski et al. (1999), and leave no doubt with regard to the identity of the taxon.

Four COI sequences were obtained, one from the specimen collected from the Itapecuru River, and three from the specimens obtained from the basin of the Mearim River. Comparisons with the sequences available in the BOLDSystems database confirmed the morphological identification of the specimens. The COI sequences obtained from the specimens collected in the present study were more than 99% similar to that of *M. macrocephalus* from the basin of the Paraná River (Table 2). This value is well within the 2% threshold for the identification of fish species by DNA barcoding (Ward et al., 2009; April et al., 2011; Carvalho et al., 2011; Mabragaña et al., 2011; Castro Paz et al., 2014).

## 4. Discussion

No records of *M. macrocephalus* species were obtained during previous studies in the Itapecuru and Mearim rivers (Soares, 2005; Barros et al., 2011; Fraga et al., 2012; Nascimento et al., 2016; Abreu et al., 2019). It seems likely that the species was exhaust accidentally during the rainy season, when the tanks of local fish farms overflow and become connected with the tributaries of the two basins. The species *M. macrocephalus* is used in fish pisciculture, larger and has economic importance for fishing in some regions, in particular in the Pantanal Mato-grossense (Navarro et al., 2007). *M. macrocephalus* is well-suited to fish pisciculture, which has led to its accidental introduction into a number of different Brazilian river basins (Godinho and Godinho, 2003; Barbosa and Soares, 2009; Vieira, 2010).

It is interesting to note that *M. macrocephalus* has not been recorded in recent surveys of other, nearby basins, in the Brazilian Northeast, including the Munim and Parnaíba in Maranhão/Piauí (Matavelli et al., 2015), the Parnaíba in Maranhão/Piauí (Ramos et al., 2014), and the São Francisco, in Bahia (Carvalho et al., 2011).

The records of *M. macrocephalus* presented here represent an important extension of the known distribution of the species, whose occurrence is now confirmed in the Itapecuru and Mearim rivers of Maranhão, in the Brazilian Northeast. It is nevertheless premature to confirm that *M. macrocephalus* has established viable populations in these rivers, and what effects its presence may have on the resident species. However, the introduction or transfer of exotic fish species into a river basin, whether intentional or otherwise, almost always has negative implications for the resident native species (Pelicice et al., 2014), and any invasion of this type requires carefully-planned management strategies to guarantee the long-term integrity of the local biota.

## 5. Conclusion

The combined analysis of morphological and molecular analysis confirmed the occurrence of *M. macrocephalus* in the Itapecuru and Mearim Rivers, state of Maranhão.

This substantially amplifies the known distribution of this species to the Brazilian Northeast.

## Acknowledgements

We are grateful to the Maranhão State Foundation for Technology and Scientific Research (FAPEMA). We would also like to thank CNPq for conceding a research stipend tp JLOB (302872/2018-3).

## References

ABREU, J.M.S., CRAIG, J.M., ALBERT, J.S. and PIORSKI, N.M., 2019. Historical biogeography of fishes from coastal basins of Maranhão State, northeastern Brazil. *Neotropical Ichthyology*, vol. 17, no. 2, pp. e180156. http://dx.doi.org/10.1590/1982-0224-20180156.

ALCÂNTARA, E.H., 2004. Caracterização da bacia hidrográfica do Rio Itapecuru, Maranhão. *Caminhos de Geografia*, vol. 7, no. 11, pp. 97-113.

ALVES, C.B.M., VIEIRA, F., MAGALHÃES, A.L.B. and BRITO, M.F.G., 2007. Impacts of nonnative fish species in Minas Gerais, Brazil: present situation and prospects. In: T.M. BERT, ed. *Ecological and genetic implications of aquaculture activities.* Dordrecht: Springer, pp. 291-314. http://dx.doi.org/10.1007/978-1-4020-6148-6_16.

APRIL, J., MAYDEN, R.L., HANNER, R.H. and BERNATCHEZ, L., 2011. Genetic calibration of species diversity among North America's freshwater fishes. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 26, pp. 10602-10607. http://dx.doi.org/10.1073/pnas.1016437108. PMid:21670289.

BARBOSA, J.M. and SOARES, E.C., 2009. Perfil da ictiofauna da bacia do São Francisco: estudo preliminar. *Revista Brasileira de Engenharia de Pesca*, vol. 4, no. 1. http://dx.doi.org/10.18817/repesca.v4il.140.

BARCODE OF LIFE DATA SYSTEMS – BOLD [online], 2020 [viewed 17 May 2020]. Available from: http://www.boldsystems.org

BARROS, M.C., FRAGA, E.C. and BIRINDELLI, J.L.O., 2011. Fishes from the Itapecuru river basin, state of Maranhão, northeast Brazil. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 71, no. 2, pp. 375-380. http://dx.doi.org/10.1590/S1519-69842011000300006. PMid:21755154.

BERTACO, V.A., FERRER, J., CARVALHO, F.R. and MALABARBA, L.R., 2016. Inventory of the freshwater fishes from a densely collected area in South America: a case study of the current knowledge of Neotropical fish diversity. *Zootaxa*, vol. 4138, no. 3, pp. 401-440. http://dx.doi.org/10.11646/zootaxa.4138.3.1. PMid:27470773.

BIRINDELLI, J.L.O., BRITSKI, H.A. and GARAVELLO, J.C., 2013. Two new species of *Leporinus Agassiz* (Characiformes: Anostomidae) from eastern basins of Brazil, and redescription of *L. melanopleura* Günther. *Neotropical Ichthyology*, vol. 11, no. 1, pp. 9-23. http://dx.doi.org/10.1590/S1679-62252013000100002.

BRITSKI, H.A. and GARAVELLO, J.C., 1978. Sobre *Leporinus octofasciatus Steindachner* da bacia do Paraná (Pisces, Anostomidae). *Papéis Avulsos de Zoologia*, vol. 31, pp. 237-250. http://dx.doi.org/10.1590/S1679-62252013000100003.

BRITSKI, H.A., SILIMON, K.Z.S. and LOPES, B.S., 1999. *Peixes do Pantanal: manual de identificação*. Corumbá: Embrapa-SPI, Brasília /Embrapa-CPAP, 184 pp.

BURNS, M.D., CHATFIELD, M., BIRINDELLI, J.L.O. and SIDLAUSKAS, B.L., 2017. Systematic assessment of the *Leporinus desmotes* species complex, with a description of two new species. *Neotropical Ichthyology*, vol. 15, no. 2. http://dx.doi.org/10.1590/1982-0224-20160166.

CARVALHO, D.C., OLIVEIRA, D.A.A., POMPEU, O.S., LEAL, C.G., OLIVEIRA, C. and HANNER, R., 2011. Deep barcode divergence in Brazilian fresh water fishes: the case of the São Francisco river basin. *Mitochondrial DNA*, vol. 22, suppl. 1, pp. 80-86. http://dx.doi.org/10.3109/19401736.2011.588214. PMid:21699373.

CASTRO PAZ, F.P., BATISTA, J.D.S. and PORTO, J.I.R., 2014. DNA barcodes of rosy tetras andallied species (Characiformes: Characidae: *Hyphessobrycon*) from the Brazilian Amazon basin. *PLoS One*, vol. 9, no. 5, pp. 1-8. http://dx.doi.org/10.1371/journal.pone.0098603.

DAGOSTA, F.C.P. and PINNA, M.C.C., 2019. The fishes of the Amazon: distribution and biogeographical patterns, with a comprehensive list of species. *Bulletin of the American Museum of Natural History*, vol. 431, no. 1, pp. 1-163. http://dx.doi.org/10.1206/0003-0090.431.1.1.

FRAGA, E.C., BIRINDELLI, J.L.O., AZEVEDO, C.A.S. and BARROS, M.C., 2012. A Ictiofauna da Área de Proteção Ambiental Municipal do Inhamum, Caxias/MA. In: M.C. BARROS, ed. *Biodiversidade na Área de Proteção Ambiental Municipal do Inhamum*. São Luís: UEMA, pp. 107-116.

FRICKE, R., ESCHMEYER, W.N. and FONG, J.D., 2020 [viewed 17 May 2020]. *Eschmeyer's catalog of fishes: species by family/subfamily* [online]. Available from: http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp

FRICKE, S.N., 2019. Introduction: indigenous futurisms in the hyperpresent now. *World Art*, vol. 9, no. 2, pp. 107-121. http://dx.doi.org/10.1080/21500894.2019.1627674.

GARAVELLO, J.C. and BRITSKI, H.A. 2003. Family Anostomidae. In: R. E. REIS, S. O. KULLANDER and C. J. FERRARIS JÚNIOR, eds. *Check List of the Freshwater Fishes of South and Central America*. Porto Alegre: EDIPUCRS, pp. 71–84.

GARAVELLO, J.C. and BRITSKI, H.A., 1988. *Leporinus macrocephalus* sp. da bacia do rio Paraguai (Ostariophysi, Anostomidae). *Naturalia*, vol. 13, pp. 67-74.

GODINHO, H.P. and GODINHO, A.L., 2003. Águas, peixes e pescadores do São Francisco das Minas Gerais. Belo Horizonte: PUC Minas, pp. 133-148.

GOMES, L.C., PESSALI, T.C., SALES, N.G., POMPEU, P.S. and CARVALHO, D.C., 2015. Integrative Taxonomy detects cryptic and overlooked fish species in a Neotropical river basin. *Genetica*, vol. 143, no. 5, pp. 581-588. http://dx.doi.org/10.1007/s10709-015-9856-z. PMid:26142058.

GRAÇA, W.J. and PAVANELLI, C.S., 2007. *Peixes da planície de inundação do Rio Paraná*. Maringá: EDUEM, 241 p.

HALL, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, vol. 41, pp. 95-98.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA – IBGE, 2014. *Produção da pecuária municipal*. Rio de Janeiro: IBGE.

LANGEANI, F., CASTRO, R.M.C., OYAKAWA, O.T., SHIBATTA, O.A., PAVANELLI, C.S. and CASATTI, L., 2007. Diversidade da ictiofauna do Alto Rio Paraná: composição atual e perspectivas futuras. *Biota Neotropica*, vol. 7, no. 3, pp. 1-18. http://dx.doi.org/10.1590/S1676-06032007000300020.

MABRAGAÑA, E., DÍAZ DE ASTARLOA, J.M., HANNER, R., ZHANG, J. and GONZÁLEZ CASTRO, M., 2011. DNA Barcoding Identifies Argentine Fishes from Marine and Brackish Waters. *PLoS One*, vol. 6, no. 12, pp. e28655. http://dx.doi.org/10.1371/journal.pone.0028655. PMid:22174860.

MARANHÃO. Governo do Estado do Maranhão, 2011. *Bacias hidrográficas: subsídios para o planejamento e a gestão territorial*. São Luís: Universidade Estadual do Maranhão/Núcleo Geoambiental.

MATAVELLI, R., CAMPOS, A.M., VALE, J., PIORSKI, N.M. and POMPEU, P.S., 2015. Ichthyofauna sampled with tadpoles in northeastern Maranhão state, Brazil. *Check List*, vol. 11, no. 1, pp. 1550. http://dx.doi.org/10.15560/11.1.1550.

NASCIMENTO, M.H.S., ALMEIDA, M.S., VEIRA, M.N.S., LIMEIRA FILHO, D., LIMA, R.C., BARROS, M.C. and FRAGA, E.C., 2016. DNA barcoding reveals high levels of genetic diversity in the fishes of the Itapecuru Basin in Maranhão, Brazil. *Genetics and Molecular Research*, vol. 15, no. 3, pp. 1-11. http://dx.doi.org/10.4238/gmr.15038476. PMid:27706636.

NAVARRO, R.D., LANNA, E.A.T., DONZELE, J.L., MATTA, S.L.P. and SOUZA, M.A., 2007. Níveis de energia digestível da dieta sobre o desempenho de piauçu (*Leporinus Macrocephalus*) em fase pós-larval. *Acta Scientiarum*, vol. 29, no. 1, pp. 109-114. http://dx.doi.org/10.4025/actascianimsci.v29i1.266.

NAVARRO, R.D., MATTA, S.L.P. and LANNA, E.A.T., 2006. Níveis de energia digestível na dieta do piauçu (*Leporinus macrocephalus*) no desenvolvimento testicular em estágio pós-larval. *Zootecnia Tropical*, vol. 24, no. 2, pp. 153-163.

PELICICE, F.M., VITULE, J.R.S., LIMA JUNIOR, D.P., ORSI, M.L. and AGOSTINHO, A.A., 2014. A serious new threat to brazilian freshwater ecosystems: the naturalization of nonnative fish by decree. *Conservation Letters*, vol. 7, no. 1, pp. 55-60. http://dx.doi.org/10.1111/conl.12029.

PERUCA, A.P.S., TEIXEIRA, I. and BENNEMANN, S.T., 2000. Frugivoria em jovens de três espécies de peixe do gênero *Leporinus*. In: *Anais do XXIII Congresso Brasileiro de Zoologia*, 2000, Cuiabá. Cuiabá: Sociedade Brasileira de Zoologia, 381 p.

RAMIREZ, J.L., BIRINDELLI, J.L.O. and GALETTI JUNIOR, P.M., 2017. A new genus of Anostomidae (Ostariophysi: Characiformes): diversity, phylogeny and biogeography based on cytogenetic, molecular and morphological data. *Molecular Phylogenetics and Evolution*, vol. 107, pp. 308-323. http://dx.doi.org/10.1016/j.ympev.2016.11.012. PMid:27894994.

RAMOS, T.P.A., RAMOS, R.T.C. and RAMOS, S.A.Q.A., 2014. Ichthyofauna of the Parnaíba river basin, northeastern Brazil. *Biota Neotropica*, vol. 14, no. 1, pp. e20130039. http://dx.doi.org/10.1590/S1676-06020140039.

RATNASINGHAM, S. and HEBERT, P.D.N., 2007. BOLD: the Barcode of Life Data System (www.barcodinglife.org). *Molecular Ecology Notes*, vol. 7, no. 3, pp. 355-364. http://dx.doi.org/10.1111/j.1471-8286.2007.01678.x. PMid:18784790.

SANGER, F., NICKLEN, S. and COULSON, A.R., 1977. DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 74, no. 12, pp. 5463-5467. http://dx.doi.org/10.1073/pnas.74.12.5463. PMid:271968.

SOARES, C.M., HAYASHI, C., FURUYA, V.R.B., FURUYA, W.M. and GALDIOLI, E.M., 2000. Substituição parcial e total da proteína do farelo de soja pela proteína do farelo de canola na alimentação de alevinos de Piavuçu (*Leporinus macrocephalus*). *Revista Brasileira de Zootecnia*, vol. 29, no. 1, pp. 15-22. http://dx.doi.org/10.1590/S1516-35982000000100003.

SOARES, E.C., 2005. *Peixes do Mearim.* 10. ed. São Luis: Editor Instituto Geia, vol. 10, 143 p.

UNIVERSIDADE ESTADUAL DO MARANHÃO – UEMA. Centro de Ciências Agrárias. Núcleo Geoambiental, 2016. *Bacias hidrográficas e climatologia no Maranhão*. São Luís: UEMA, 165 p.

VIEIRA, F., 2010. Distribuição, impactos ambientais e conservação da fauna de peixes da bacia do rio Doce. *MG.Biota*, vol. 2, pp. 5-22.

WARD, R.D., HANNER, R. and HEBERT, P.D.N., 2009. The campaign to DNA barcode all fishes. *Journal of Fish Biology*, vol. 74, no. 2, pp. 329-356. http://dx.doi.org/10.1111/j.1095-8649.2008.02080.x. PMid:20735564.

WARD, R.D., ZEMLAK, T.S., INNES, B.H., LAST, P.R. and HEBERT, P.D.N., 2005. DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, vol. 360, no. 1462, pp. 1847-1857. http://dx.doi.org/10.1098/rstb.2005.1716. PMid:16214743.

ZEINAD, A.K. and PRADO, R.A., 2012. *Peixes fluviais do Brasil espécies esportivas*. São Paulo: Pescaventura, 360 p.