



Unveiling fish diversity in the Parque Nacional dos Lençóis Maranhenses and adjacent areas through DNA barcoding, Northeastern Brazil

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Abstract

Accurate species identification is a challenge in megadiverse regions, where morphology alone can lead to overestimation or underestimation of biodiversity. In this context, the incorporation of molecular approaches, such as DNA barcoding, has been useful in accurately estimating regional biodiversity, revealing cryptic diversity, and enabling identification by non-specialists. This study generated a molecular database of fishes for Parque Nacional dos Lençóis Maranhenses and adjacent areas, based on sequences of the mitochondrial cytochrome c oxidase subunit I (COI) gene. Haplotype analyses were conducted using Maximum likelihood (ML), and the delimitation of Operational Taxonomic Units (OTUs) combined the following methods: Kimura 2-parameter model (K2P), Assemble Species by Automatic Partitioning (ASAP) and Poisson Tree Processes (PTP) methods, using $K2P \geq 2\%$ to resolve cases of incongruence between ASAP and PTP. A total of 59 species were recorded, distributed across 51 genera, 29 families, and 13 orders. In total, 258 sequences were analyzed (Two hundred and fifty-three generated in this study and five additional from GenBank), corresponding to the 59 species. K2P genetic distances revealed a clear separation between intraspecific and interspecific variation, with maximum and minimum values of 1.78% and 3.63%, respectively, resulting in a barcoding gap of 1.85% without overlap, demonstrating the high efficiency of the COI marker in species discrimination. Five new records for the area were identified, including two non-native species. The integration of molecular and morphological data enabled a revision of the ichthyofauna, including nomenclatural updates and the correction of identifications. These findings are essential for conservation strategies.

Keywords COI · Maranhão · Molecular identification · Non-native species · Reference library

Introduction

South America is home to the greatest diversity of described freshwater fish in the world, accounting for more than 36% of all known species, totaling about 5,150 species (Dagosta and de Pinna 2019). Of these, 3,148 species occur in Brazil (ICMBio 2018), representing more than 61% of all freshwater fish species in the South American continent. It is estimated that between 34 and 42% of this diversity remains undescribed (Reis et al. 2016), highlighting the need for increasingly robust studies to reduce these knowledge gaps. In this context, the high and still taxonomically complex diversity of Neotropical freshwater fishes is evidenced by

inconsistencies between morphological and molecular identifications, the occurrence of cryptic diversity, species complexes, and unresolved lineages (e.g. Santana et al. 2019; Brito et al. 2021; Guimarães et al. 2022; Escobar-Camacho et al. 2024; Freitas et al. 2024; Nunes et al. 2024), highlighting the importance of molecular data and approaches, such as DNA barcoding, molecular species delimitation methods, and environmental DNA, especially when used in an integrative framework (i.e. integrative taxonomy), to improve species identification accuracy, resolve taxonomic inconsistencies and improve taxonomic resolution, and enhance biodiversity assessments.

The state of Maranhão harbors a remarkable diversity of freshwater fishes (e.g. Soares 2005; Barros et al. 2011; Piorski et al. 2017; Brito et al. 2019b, 2020; Guimarães et al. 2020b; Vieira et al. 2023; Limeira-Filho et al. 2023),

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comprising more than 285 freshwater fish species (Koerber et al. 2023). This species diversity is distributed across seven state hydrographic systems [Mearim, Itapecuru, Munim, Turiaçu, Maracaçumé, Preguiças, and Peria], in addition to three basins under federal jurisdiction that Maranhão shares with other states [Parnaíba, to the east; Tocantins, to the south; and Gurupi, to the west], as well as other isolated smaller coastal hydrographic systems [state hydrographic systems of the “Ilhas Maranhenses” and the Western Coastal region] (NuGeo 2016). These hydrographic systems are located in a biogeographically complex transitional region between major Neotropical basins, where historical processes such as river capture and coastal dynamics have promoted connectivity among adjacent basins, shaping patterns of species distribution and regional endemism (Lundberg et al. 1998; Hubert and Renno 2006; Dagosta and de Pinna 2017; Abreu et al. 2019). Ichthyofaunal inventories conducted in these hydrographic systems (e.g. Soares 2005; Barros et al. 2011; Piorski et al. 2017; Brito et al. 2019b, 2020; Guimarães et al. 2020b; Vieira et al. 2023; Limeira-Filho et al. 2023) reveal a highly diverse ichthyofauna for the state, with several species shared among different river systems. However, most of these studies are based exclusively on morphological analyses, which limit the detection of cryptic lineages. The exceptions are the fish DNA barcoding studies by Nascimento et al. (2016), conducted in the Itapecuru River basin, and Silva et al. (2026), conducted in the Mearim River basin. Despite these studies, molecular databases for fishes in Maranhão remain scarce, considering the number of river basins present in the state.

Situated within a biogeographically complex ecotonal zone, Maranhão lies at the intersection of the Amazon, Caatinga, and Cerrado biomes (Spinelli-Araujo et al. 2016; Silva et al. 2016; Silva-Junior et al. 2022). This transitional setting promotes the coexistence of faunal elements from distinct biogeographic domains, resulting in a highly heterogeneous ichthyofauna with overlapping distributions across different drainage systems. Such complexity, combined with historical connectivity among basins, poses significant challenges for accurately understanding species distributions based solely on traditional taxonomic approaches. In this context, recent integrative studies focusing on specific taxonomic groups have revealed species complexes and previously unrecognized diversity within the Neotropical ichthyofauna (e.g. Guimarães et al. 2018a, 2019, 2020a; Brito et al. 2019a, 2021; Aguiar et al. 2022; Nascimento et al. 2023, 2024; Limeira-Filho et al. 2024; Lustosa-Costa et al. 2024; Guimarães et al. 2025). These works indicate that fish species diversity may be substantially underestimated in Maranhão.

In this context, the need for complementary approaches that enable more accurate species delimitation and a more robust understanding of diversity patterns becomes evident.

Therefore, DNA barcoding has become a widely used tool since its proposal by Hebert et al. (2003). This approach is based on the analysis of a fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene, which functions as a standardized marker characterized by low intraspecific variation and high interspecific divergence, thereby allowing reliable species discrimination. Over the years, DNA barcoding has played a crucial role in the development of reference databases (Díaz et al. 2016; Bagley et al. 2019; Mejía-Estrada et al. 2022); identification of species nurseries (Becker et al. 2015; Lima et al. 2020, 2024; Depintor et al. 2025; Costa et al. 2025); monitoring and tracking of species (Rodrigues et al. 2016; Almerón-Souza et al. 2018; Camacho-Oliveira et al. 2020; Santana et al. 2023; Kaifu et al. 2025); detection of non-native species (Sales et al. 2018; Monroe et al. 2023; Seemani et al. 2024); analysis of feeding habits through stomach content examination (Côté et al. 2013; Jo et al. 2015; Dahl et al. 2017); identification of species complexes and resolution of taxonomic issues (Melo et al. 2016; Vicente et al. 2020; Brito et al. 2021; Guimarães et al. 2022; Almeida et al. 2024); and description of new species (e.g. Zanata et al. 2024; Dutra et al. 2024; Silva et al. 2025).

The Parque Nacional dos Lençóis Maranhenses (PNLM) represents one of the most distinctive tropical coastal landscapes in the world and was recognised as a UNESCO World Natural Heritage Site in 2024 (UNESCO, 2024). Located within an ecotonal zone between the Amazon, Cerrado, and Caatinga biomes, the park comprises a dynamic mosaic of dunes, *restingas*, freshwater interdunal lagoons, mangroves, and estuarine systems, whose formation results from the interaction of wind, precipitation, and sedimentary processes (Amaral et al. 2019; Santos et al. 2019). The interdunal lagoons exhibit strong hydrological seasonality, playing a central role in structuring environmental conditions and local biological communities (Moschini-Carlos et al. 2008; Van Damme and Dumont 2010). This high environmental heterogeneity is associated with elevated biological diversity and the organisation of distinct communities across different compartments of the park, including restinga flora, ichthyofauna, reptiles, among other groups, reinforcing its importance as a priority area for biodiversity research and conservation at regional and global scales (Van Damme and Dumont 2010; Miranda et al. 2012; Rodrigues et al. 2019; Brito et al. 2019b; Cruz et al. 2023). Therefore, this study aims to establish a molecular reference database for the ichthyofauna of the PNLM and adjacent areas using the mitochondrial cytochrome c oxidase subunit I (COI) gene, compare different species delimitation methods, and provide a reference library to support molecular identification and conservation efforts.

Materials and methods

Study area and sampling procedures

The study was conducted at the Parque Nacional dos Lençóis Maranhenses (PNLM) and adjacent areas (Fig. 1). The PNLM was established by Decree No. 86,060 of 2 June 1981 and is located on the eastern coast of the state of Maranhão, northeastern Brazil, within an ecotonal *restingas* region between the Amazon, Caatinga, and Cerrado biomes. The protected area covers approximately 155,000 ha, partially distributed across the municipalities of Barreirinhas, Santo Amaro do Maranhão, and Primeira Cruz. The climate is equatorial, characterised by two well-defined seasons throughout the year: a rainy season from March to June and a dry season from July to February (IBAMA 2002; Amaral et al. 2019; Rodrigues et al. 2019). The PNLM comprises a variety of freshwater bodies, including streams, lagoons, and predominantly interdunal lagoons. Sampling was conducted between 2016 and 2024, including 34 collecting sites within the PNLM and adjacent areas (Figs. 1 and 2; and Online Resource 1), under authorizations from the Chico Mendes Institute for Biodiversity Conservation – ICMBio (License numbers 51540, 54949, 57787, 64415, and 73267). After collection, specimens were subsequently euthanized by immersion in an eugenol anesthetic solution until opercular movements ceased (Fujimoto et al. 2015). Immediately after euthanasia, specimens were fixed according to their intended use: individuals designated for molecular analyses were preserved in absolute ethanol, whereas

those intended for morphological examination were fixed in 10% formalin, following the procedures described by Souza and Auricchio (2002).

All examined material was deposited in the following ichthyological collections: Coleção Ictiológica do Centro de Ciências Agrárias e Ambientais (CICCAA), Universidade Federal do Maranhão (UFMA), Chapadinha, MA, and the Coleção de Peixes do Laboratório de Biologia de Peixes (LBP), Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP), Botucatu, Brazil. The mitochondrial COI gene sequences generated in this study are available in Online Resource 2. In addition, five COI sequences available in GenBank, from Monroe et al. (2023), were included in the analyses, available in Online Resource 3.

Taxonomic classification and morphological identification of the specimens

The specimens were identified based on specialized taxonomic literature for each species and/or through consultation with taxonomic specialists. The spelling of species and genus names, authorship and year of publication, species taxonomic status, and geographical distribution followed Fricke et al. (2026b). We followed Fricke et al. (2026a) for taxonomic classification. The species lists published by Piorski et al. (2017), Brito et al. (2019b, 2020), and Monroe et al. (2023) have been updated herein with respect to the taxonomic classification and identification of species.

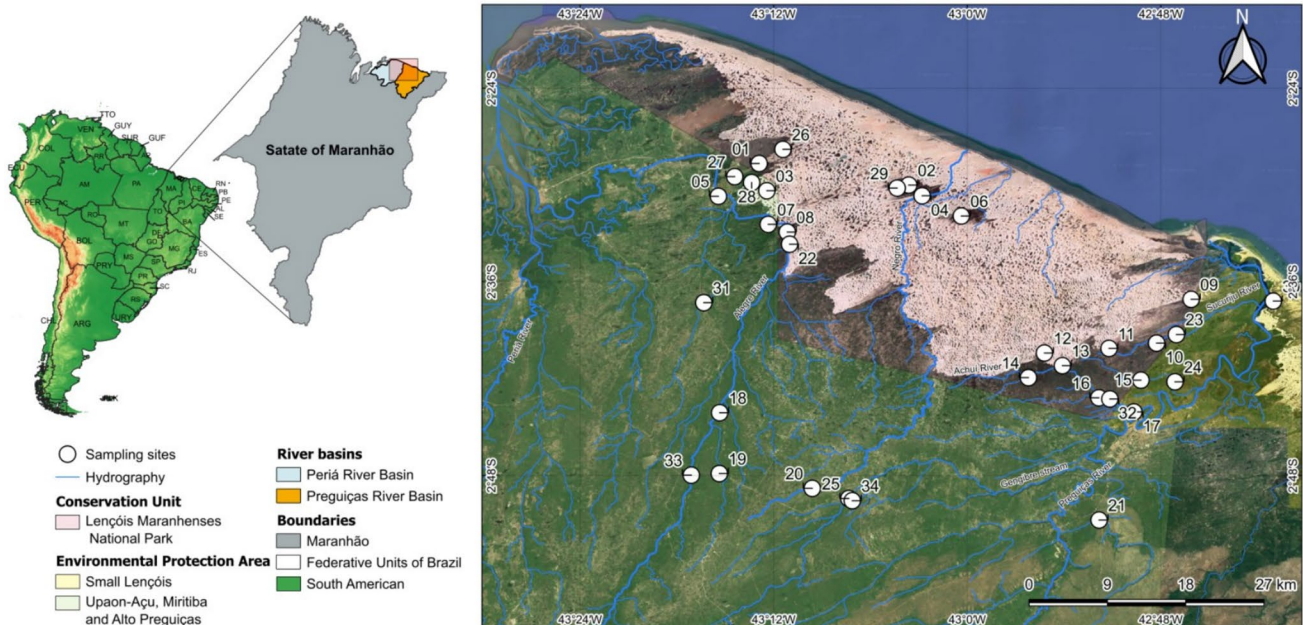


Fig. 1 Sampling sites in Parque Nacional dos Lençóis Maranhenses (PNLM) and adjacent areas



Fig. 2 Selected sampling sites within the study area: S03 – Andorinhas Lagoon; S04 – Negro River; S07 – Alegre River; S13 – Achuí River; and S22 – Alegre River Lagoon

Extraction, amplification, and sequencing

DNA was extracted from muscle tissue fragments preserved in 95% ethanol using the commercial Wizard® Genomic DNA Purification Kit (Promega), following the manufacturer's instructions. In addition, a saline extraction protocol adapted from Miller et al. (1988) was also employed. The mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified by polymerase chain reaction (PCR) using different primer pairs (Table 1).

PCR reactions were carried out in a final volume of 12.5 µL containing 1.25 µL of 10× custom PCR buffer, 0.50 µL of MgCl₂ (50 mM), 0.50 µL of dNTPs (2 mM), 0.25 µL of each primer (10 µM), 0.20 µL of PHT Taq DNA polymerase (5 U µL⁻¹), 2 µL of template DNA (10–80 ng µL⁻¹), and 7.55 µL of Milli-Q water. The amplification protocol consisted of an initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 30 s, primer annealing at 52 °C for 45 s, and extension at 68 °C for 1 min, with a final extension step at 68 °C for 7 min. Samples that failed to amplify under these conditions were subjected to a temperature-gradient PCR with three annealing stages: 5 cycles at 50 °C for 45 s, 10 cycles at 52 °C for 45 s, and 15 cycles at 54 °C for 45 s. PCR products were visualized on 1% agarose gels and purified using ExoSAP-IT® (USB Corporation), following the manufacturer instructions. Sanger sequencing (Sanger

Table 1 Primers used in the study

PRIMER	Nº Ref
FishF1—5'TCAACCAACCACAAAGACATTGGCAC3'	1
FishR1—5'TAGACTTCTGGGTGGCCAAAGAATCA3'	
FishF2—5'TCGACTAATCATAAAGATATCGGCAC3'	1
FishR2—5'ACTTCAGGGTGACCGAAGAATCAGAA3'	
FishF6—5'TGTAAAACGACGGCCAGTACYAAYCAC AAAGAYATTGGCA3'	2
FishR7—5'CAGGAAACAGCTATGACCTARACTTCT GGRTGDCCRAGAAYCA3'	
LCO1490—5'GGTCAACAAATCATAAAGATATTGG3'	3
HCO2198—5'TAACTTCAGGGTGACCAAAAAATCA3'	

The numbers presented in the table refer to the bibliographic sources of the primers: 1 – Ward et al. (2005); 2 – Jennings et al. (2019); and 3 – Hebert et al. (2003).

et al. 1977) was performed using purified PCR products and the BigDye™ Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems). Sequencing reactions were purified by precipitation in a solution of 100% ice-cold absolute ethanol, 3 M sodium acetate, and 1 mg/mL glycogen, and subsequently sequenced on an ABI 3500 Genetic Analyzer (Applied Biosystems) at Instituto de Biotecnologia (IBTEC), Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP), Botucatu, Brazil.

Data analysis

The sequences were assembled from forward and reverse reads using the Geneious® software (Kearse et al. 2012). Multiple alignment was performed using Multiple Sequence Alignment—MAFFT (Kato et al. 2002), applying the default parameters available on the web server (<https://www.ebi.ac.uk/jdispatcher/msa/mafft>). Nucleotide variation, substitution saturation, and the best evolutionary model were estimated using MEGA12 (Kumar et al. 2024). A maximum likelihood (ML) analysis (haplotype analysis) was conducted in IQ-TREE, using the complete sequence database, with 1,000 ultrafast bootstrap replicates (Trifinopoulos et al. 2016) and the GTR + G substitution model, on the high-performance server equipped with multiple CPUs, each comprising multiple cores at the, at the Laboratório de Biologia e Genética de Peixes (LBGP). The phylogenetic tree was visualized in FigTree v1.4.4 (Rambaut 2018) and edited in Inkscape v1.4.2 (Inkscape 2024).

COI genetic distances were estimated using the Kimura 2-parameter (K2P) model (Kimura 1980), based on both the complete sequence dataset (Online Resource 4) and the reduced matrix (Online Resource 5). The reduced matrix (including only one individual per haplotypes) was visualized using DAMBE (Xia 2013) and generated in the HIV Sequence Database (2025) (<https://www.hiv.lanl.gov/content/sequence/elimdupesv2/elimdupes.html>). The K2P analyses were performed using MEGA 12 (Tamura et al. 2024). We considered a COI cutoff of 2% as sufficient to discriminate species, since this threshold is commonly inferred by species delimitation among Neotropical fish (Jacobina et al. 2018). A barcoding gap analysis was performed in the R environment (R Core Team 2024), using the ggplot2 package (Wickham 2016), based on the K2P genetic distance matrix. Distances were initially organized into intraspecific and interspecific pairs, and null genetic distance values (K2P=0) were removed to avoid bias associated with identical sequences. The distributions of intraspecific and interspecific distances were visualized using superimposed histograms, allowing inspection of the separation between the two datasets. The barcoding gap was estimated as the difference between the largest observed intraspecific distance and the smallest interspecific distance, considering only interspecific comparisons with K2P values $\geq 2\%$.

Subsequently, two other species delimitation methods were performed, using the same reduced matrix mentioned above (Online Resource 5). The first species delimitation method, which relies on genetic distance, was the Assemble Species by Automatic Partitioning (ASAP) (Puillandre et al. 2021), which was performed in ASAPy 1.0 (<https://itaxotools.org/download.html>). The partition with the lowest ASAP score was chosen, as it represents the most

parsimonious hypothesis of the analysis. The second one was a Coalescent-based species delimitation approach, the Poisson tree processes (PTP) (Zhang et al. 2013), using a ML phylogenetic tree based on the reduced matrix (Online Resource 5) as input on the web server (<https://species.h-its.org/>). The PTP outgroup was *Prochilodus lacustris* LBPV107039. In addition, maximum likelihood (ML) analyses were conducted separately using the same parameters for each tree, considering both the complete dataset and the following groups individually: Characiformes, Siluriformes, Cichliformes, Gymnotiformes, Cyprinodontiformes, the less representative orders, and the non-native species. The independent trees were generated using the same parameters as the overall tree (Online Resource 6) to provide a clearer visualization of the groups.

ASAP is a distance-based method that identifies species boundaries by detecting significant gaps between the largest intraspecific and the smallest interspecific genetic distances (Puillandre et al. 2021). PTP is a coalescent-based method that infers species boundaries by modelling speciation and coalescent events as distinct Poisson processes. This approach detects the number of substitutions between haplotypes and assumes that greater molecular variability is expected between species than within species (Zhang et al. 2013). An advantage of this method is that it does not require ultrametric calibration, unlike other coalescent approaches, thereby avoiding potential errors and computationally intensive procedures (Zhang et al. 2013). These two approaches were employed here because they are based on distinct assumptions and are therefore complementary. ASAP relies on genetic distances, whereas PTP uses the number of substitutions between haplotypes to infer species boundaries under a coalescent framework. The combined application of different molecular species delimitation methods, especially those based on different assumptions, is widely used in recent fish DNA barcoding studies (e.g. Berbel-Filho et al. 2018; Lima et al. 2024; Silva et al. 2026), as it provides more robust species delimitation by integrating complementary analytical approaches.

Results

The study generated 253 new sequences for the mitochondrial COI gene. Voucher numbers and GenBank accession codes are provided in Online Resource 2, corresponding to 59 species with material available for molecular analysis, collected in PNLM and adjacent areas across the Preguiças and Periaí river basins (Fig. 1, Online Resource 1). In addition to these sequences, five COI sequences available in GenBank from Monroe et al. (2023) were included, resulting in a total of 258 sequences used in the ML analyses (Online Resource 3).

The analysis of K2P genetic distances revealed a clear separation between intraspecific and interspecific distributions. Intraspecific distances showed low values, with a maximum of 1.78%, while interspecific distances started at 3.63%. Between these values, a barcoding gap of 1.85% was identified, indicating an absence of overlap between the distributions. This pattern indicates high efficiency of the molecular marker used in separating the analyzed species (Fig. 3).

Previous studies registered 60 fish species for PNLM and adjacent areas (Piorski et al. 2017; Brito et al. 2019b, 2020; Monroe et al. 2023; Aguiar-Santos and Meneses 2025); of these 60 species, 11 were not recorded in the present study. The present study recorded 59 fish species in the study area. Some of these previously reported species had their taxonomic identifications revised or updated here (Table 2). The sampled taxa are distributed in 59 species, 51 genera, 29 families, and 13 orders (Fig. 4). Species registered by Brito et al. (2020), with material deposited in CICCAA, were re-examined in the present study and compared with the material analyzed herein.

The ASAP and PTP methods showed few incongruences. The ASAP method delimited 54 species, whereas the PTP method delimited 60 species. Incongruences were observed in the following taxa: *Nannostomus beckfordi* Günther, 1872; *P. parnahybae*; *Pimelodella* sp.; *E. cacuria*; *E. pavulagem*; *O. niloticus*; and hybrids of *O. aureus* × *O. niloticus* and *O. mossambicus* × *O. niloticus* (Figs. 5, 6, 7, 8, and 11). In cases where the results of the two species delimitation methods differed, K2P genetic distance was used as the criterion for defining OTUs: sequences possessing a COI genetic distance $\geq 2\%$ are considered as different OTUs if they were recovered as such by at least one of the other two species delimitation methods (ASP or PTP), regardless of the results from the other method (Figs. 5, 6, 7, 8, and 11; Online Resource 4). Thus, 59 species were recognized for the PNLM and adjacent areas (Online Resource 6, 7). The maximum likelihood (ML) analyses conducted separately for Characiformes, Siluriformes, Cichliformes, Gymnotiformes,

Cyprinodontiformes, the least representative orders, and non-native species, with the plotted K2P, PTP, and ASAP results are shown in Figs. 5, 6, 7, 8, 9, 10, and 11.

Characiformes was the order with larger species number in the sampled dataset, encompassing 22 species and 120 newly generated sequences (Fig. 5). This was followed by Siluriformes, with nine species and 25 sequences (Fig. 6); Cichliformes, with nine species and 45 sequences (Fig. 7); Gymnotiformes, with six species and 26 sequences (Fig. 8); and Cyprinodontiformes, with four species and 19 sequences (Fig. 9). The remaining orders, less representative in the sampling, totaled nine species and 23 sequences (Fig. 10). This pattern of predominance of Characiformes and Siluriformes is consistent with the typical composition of the Neotropical ichthyofauna, recognized for its high diversity in these orders. All the data used to conduct the molecular analyses is available in the Online Resource 8.

Our study reported five first records for the state of Maranhão: *Eigenmannia pavulagem*, *Hasemania* cf. *diastatos*, *Ramirezella pyrophthalma*, and the non-natives *Betta splendens* Regan, 1910 and a hybrid lineage *Oreochromis aureus* × *O. niloticus* (Online Resource 7). In this case, this represents the first record of the genus *Hasemania* Ellis, 1911 for the state.

Discussion

The importance of databases for conservation and molecular identification

For a long time, identification was based exclusively on morphological characteristics, however, with the establishment of molecular methods and, especially, with the spread of DNA barcoding, molecular data have become widely used in species identification within the framework of integrative taxonomy (Köblmüller 2023). The development of molecular databases is essential for understanding biodiversity, as it provides reference libraries that enable more accurate taxonomic identification (Hebert et al. 2003; Ratnasingham and Hebert 2007; Oliveira et al. 2024a). Moreover, these databases allow the detection of cryptic diversity and taxonomic incongruences (Ratnasingham and Hebert 2013), and support modern DNA-based biomonitoring approaches, such as metabarcoding and eDNA, whose effectiveness depends on the quality and comprehensiveness of these reference libraries (Weigand et al. 2019; Fueyo et al. 2024).

The creation of reference libraries provides an additional tool for species identification (e.g. Andersen et al. 2019; Weigand et al. 2019), often revealing cryptic lineages (e.g. Pires et al. 2021; Ribolli et al. 2021; Nunes et al. 2024) and potentially leading to the description of new taxa (e.g.

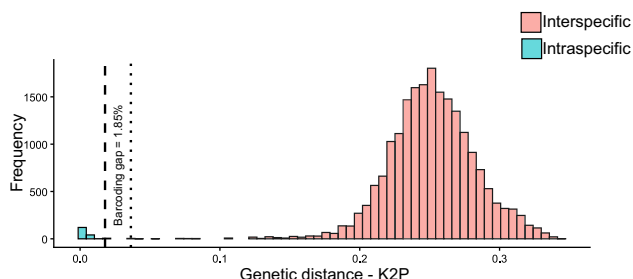


Fig. 3 Distribution of intraspecific and interspecific Kimura-2-parameter (K2P) genetic distances. Maximum intraspecific = 1.78% and minimum interspecific = 3.63%

Table 2 Updated species identifications for the Parque Nacional dos Lençóis Maranhenses (PNLM) and adjacent areas, including the revision of names that have undergone taxonomic changes

Original identification	Nº Ref	Revised identification proposed here
<i>Astyanax</i> cf. <i>lacustris</i>	2,3	<i>Astyanax</i> cf. <i>bimaculatus</i>
<i>Bryconops</i> cf. <i>affinis</i>	2,3	<i>Bryconops</i> cf. <i>melanurus</i>
<i>Cichla monoculus</i> Spix & Agassiz, 1831	4	<i>Cichla kelberi</i> Kullander & Ferreira, 2006
<i>Eigenmannia virescens</i> (Valenciennes, 1836)	2,3,5	<i>Eigenmannia cacuria</i> Dutra, Ramos & Menezes, 2022 <i>Eigenmannia pavulagem</i> Peixoto, Dutra & Wosiacki, 2015 <i>Eigenmannia robsoni</i> Dutra, Ramos & Menezes, 2022
<i>Hemigrammus</i> sp.1, sp.2, sp.3	2,3	<i>Holopristis</i> cf. <i>ocellifera</i> <i>Hemigrammus</i> sp. <i>Hasemania</i> cf. <i>diastatos</i>
<i>Hoplias malabaricus</i> (Bloch, 1794)	1,2,3,5	<i>Hoplias cazumba</i> Abreu, Pedroza, Oyakawa, Melo, Tchaicka & Piorski, 2025
<i>Hoplias</i> cf. <i>malabaricus</i>		
<i>Hypostomus johnii</i> (Steindachner, 1877)	2,3,5	<i>Hypostomus cari</i> Lustosa-Costa, Ramos, Zawadzki, Jacobina & Lima, 2024
<i>Hypostomus</i> sp.		
<i>Leporinus</i> aff. <i>friderici</i>	2,3,5	<i>Leporinus piau</i> Fowler, 1941
<i>Leporinus</i> cf. <i>friderici</i>		
<i>Loricaria parnahybae</i> Steindachner, 1907	2,3,5	<i>Loricaria catirina</i> Serejo-Saraira, Piorski, Ottoni, Sabaj & Lima, 2026
<i>Loricaria</i> cf. <i>parnahybae</i>		
<i>Lycengraulis batesii</i> (Günther, 1868)	2,3,5	<i>Anchovia surinamensis</i> (Bleeker, 1865) <i>Pterengraulis atherinoides</i> (Linnaeus, 1766)
<i>Melanorivulus</i> cf. <i>parnaibensis</i>	2,3	<i>Melanorivulus parnaibensis</i> (Costa, 2003)
<i>Moenkhausia</i> sp.	5	<i>Ramirezella pyrophthalma</i> (Costa, 1994)
<i>Moenkhausia cotinho</i> Eigenmann, 1908	2,3	
<i>Moenkhausia oligolepis</i> (Günther, 1864)	2,3	<i>Bario oligolepis</i> (Günther, 1864)
<i>Moenkhausia</i> sp.	2,3	<i>Hemigrammus ora</i> Zarske, Le Bail & Géry, 2006
<i>Oreochromis</i> sp.	2,3,5	<i>O. aureus</i> (Steindachner, 1864) x <i>O. niloticus</i> (Linnaeus, 1758) <i>O. mossambicus</i> x <i>O. niloticus</i> <i>O. niloticus</i>
<i>Oreochromis mossambicus</i> (Peters, 1852)	4	<i>O. mossambicus</i> x <i>O. niloticus</i>
<i>Pimelodella parnahybae</i> Fowler, 1941	2,3,5	<i>Pimelodella parnahybae</i> <i>Pimelodella</i> sp.
<i>Poptella compressa</i> (Günther, 1864)	2,3	<i>Poptella</i> sp.

The numbers presented correspond to the studies that previously recorded the species: 1 – Abreu et al. (2025); 2 – Brito et al. (2019b); 3– Brito et al. (2020); 4 – Monroe et al. (2023); 5 – Piorski et al. (2017).

Guimarães et al. 2018a, 2019; Brito et al. 2019b; Lustosa-Costa et al. 2022; Dopazo et al. 2025). These databases are also essential for environmental monitoring, particularly through metabarcoding approaches (e.g. Mendoza et al. 2015; Xiong et al. 2022; Zhao et al. 2023; Deng et al. 2024; Costa et al. 2025), as they provide a reliable reference framework. Expanding these databases in regions with limited information is therefore a strategic mechanism for biodiversity conservation, enabling accurate species identification, assessment of genetic diversity, and rapid detection of changes in biological communities (Gostel and Kress 2022). In this way, the development of robust reference. Consequently, these databases emerges as an important constitute a critical scientific and technological and scientific foundation for conservation, enabling reliable

estimates of local biodiversity, particularly especially when sequences are linked to voucher specimens deposited in collections and identified by specialists. Furthermore, these databases facilitate ecological monitoring, support the formulation of conservation policies, and enable the early detection of changes in biological composition (Vuataz et al. 2024). Moreover, these reference libraries support molecular identification by non-specialists.

Accordingly, the development of comprehensive reference libraries across different taxa and regions is essential (e.g. Bagley et al. 2019; Morinière et al. 2019; Zangl et al. 2020; Dinča et al. 2021; Geiger et al. 2021; Janzen et al. 2022), as accurate species identification and delimitation represent the first step in any biological study. The construction of robust reference databases facilitates ecological monitoring,

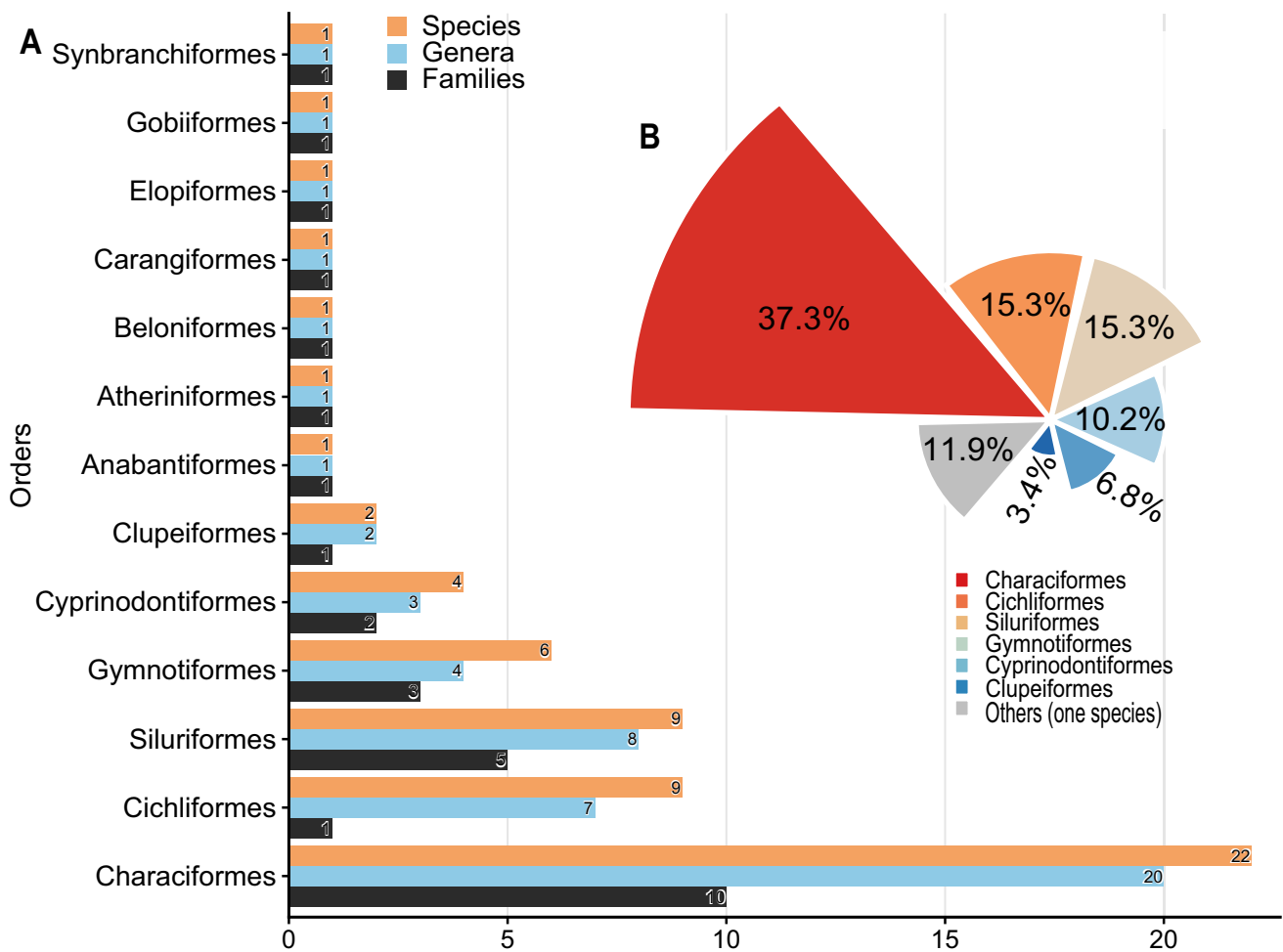


Fig. 4 Taxonomic composition of fish species from the Parque Nacional dos Lençóis Maranhenses (PNLM) and adjacent areas. Number of families, genera, and species recorded for each fish

order (A). Relative contribution (%) of species richness by order, with rare orders (represented by a single species) grouped as others (B)

supports conservation policies, and enables early detection of changes in biological composition (Vuataz et al. 2024). Maintaining up-to-date databases increases identification accuracy and improves environmental quality assessments (Vuataz et al. 2024). Thus, molecular libraries such as BOLD Systems (<https://boldsystems.org/>) and GenBank (<https://www.ncbi.nlm.nih.gov/genbank/about/>) not only accelerate taxonomic research but also provide essential infrastructure for the strategic conservation of biodiversity.

The efficiency of identification using DNA barcoding

In biodiverse regions, imprecise species identification can lead to either overestimation or underestimation of species richness, as well as increased taxonomic confusion (Ely et al. 2017). Studies employing DNA barcoding methodology in different river basins in Brazil have revealed previously unrecognized and hidden diversity (e.g. Carvalho et al. 2011; Pereira et al. 2011, 2013;

Henriques et al. 2015; Gomes et al. 2015; Freitas et al. 2024; Nunes et al. 2024). Therefore, the incorporation of molecular approaches, such as DNA barcoding, has demonstrated to be useful in accurately estimating regional biodiversity, as well as revealing cryptic diversity (cryptic species), particularly in contexts where morphological and taxonomic identification is problematic or poorly resolved. Such limitations may arise from high intraspecific variation, including polymorphism, sexual dimorphism, and ontogenetic variation, or from the absence of clear diagnostic characters among species and cryptic lineages (e.g. Pereira et al. 2013; Ramirez et al. 2017). In these scenarios, identification errors may result from both overestimation and underestimation of diversity. On the one hand, morphological variation among populations of the same species may lead to their erroneous interpretation as distinct taxa, despite representing a single species from a molecular perspective (Reiss et al. 2012; Ornelas-García et al. 2024). On the other hand, the opposite may also

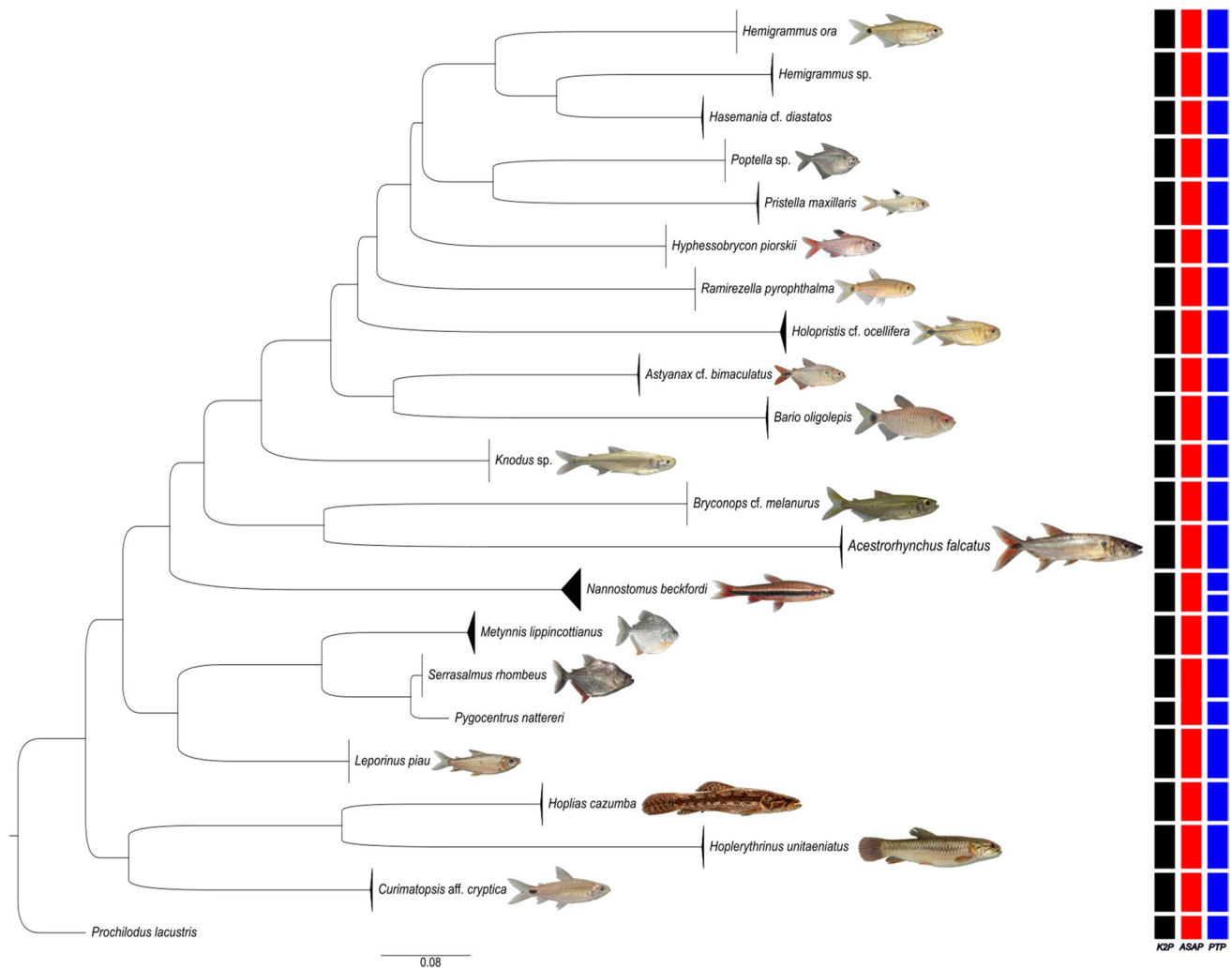


Fig. 5 Maximum likelihood tree of the order Characiformes from the Parque Nacional dos Lençóis Maranhenses (PNLM) and adjacent areas

occur: genetically distinct lineages may exhibit little or no morphological differentiation, thus characterizing cryptic species (Hending 2024). Therefore, morphological variation, or its absence, is not always reliably captured by exclusively morphological approaches.

In this context, the construction of a fish molecular reference library based on the COI gene for PNLM and adjacent areas (Online Resource 2) enabled a more precise estimate of local biodiversity, as well as assisting in the identification of fish specimens. Previous studies had recorded 60 species for the region (Piorski et al. 2017; Brito et al. 2019b, 2020; Monroe et al. 2023; Aguiar-Santos and Meneses 2025). However, the incorporation of molecular analyses based on the developed dataset revealed inconsistencies in these surveys, indicating that the diversity of some groups had been overestimated, while in other cases it had been underestimated.

Nomenclatural and identification update

Our results show that the diversity of some genera has been underestimated in previous studies (e.g. *Eigenmannia* Jordan & Evermann, 1896, with three instead of one species previously recorded from PNLM and adjacent areas), while other genera had their diversities overestimated (e.g. *Bryconops* Kner, 1858, with one instead of two species, although two morphotypes are recognized) (other examples can be found in Table 2), and are discussed below.

Bruto et al. (2019b, 2020) recognized two morphotypes of *Bryconops*, previously assigned to *B. cf. affinis* and *B. cf. melanurus*. However, the species delimitation analyses employed herein recovered a single lineage for both (Fig. 5), indicating that the purported diagnostic characters between them are, in fact, intraspecific polymorphic traits. This pattern is consistent with evidence that colouration

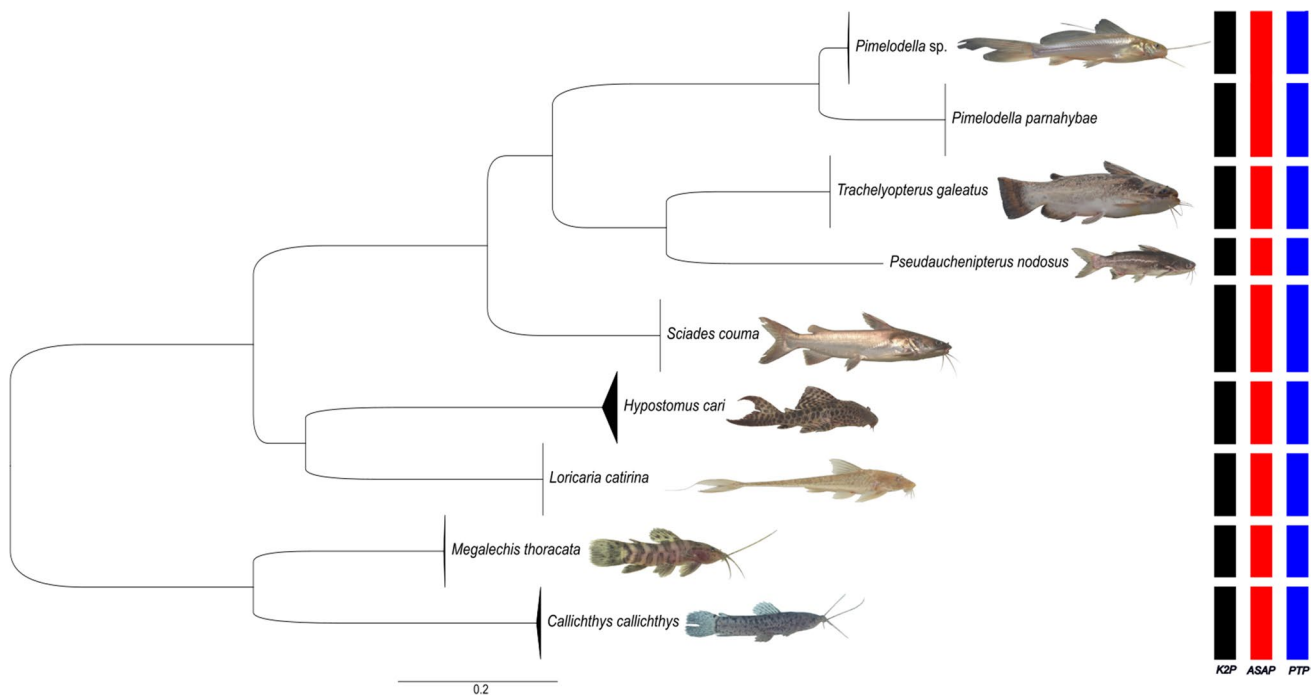


Fig. 6 Maximum likelihood tree of the order Siluriformes from the Parque Nacional dos Lençóis Maranhenses (PNLM) and adjacent areas

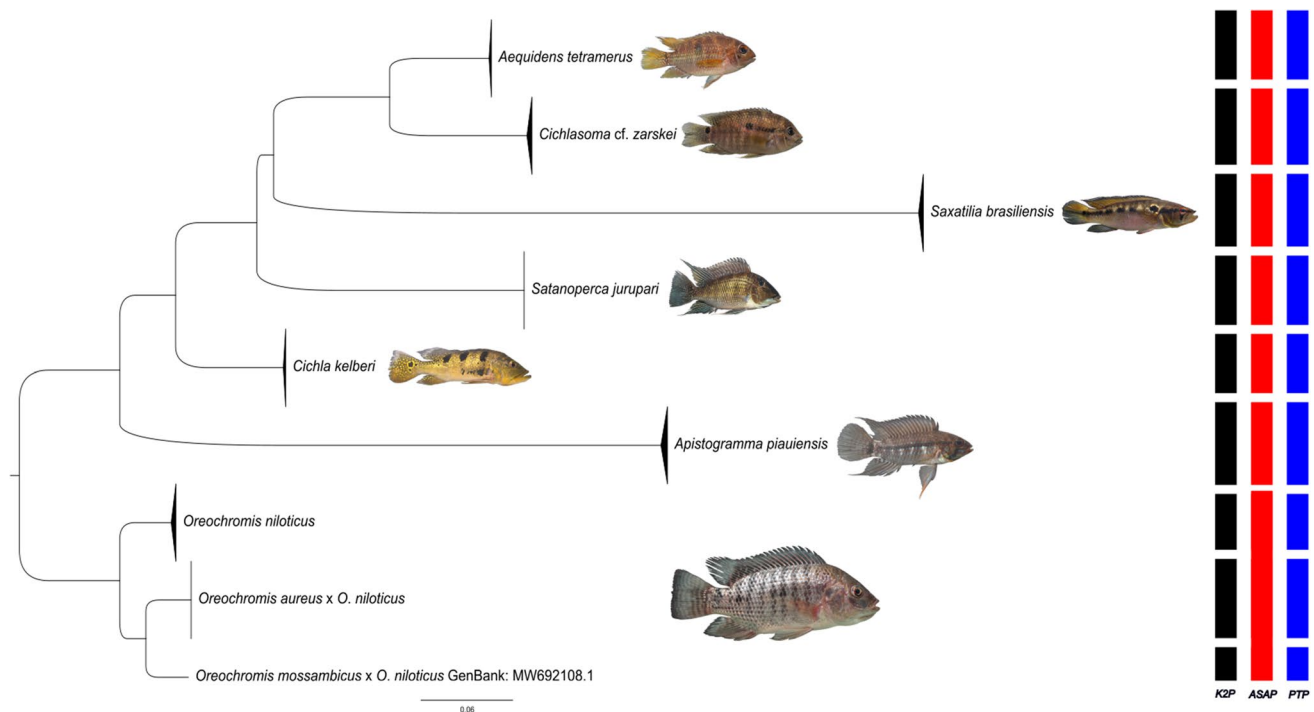


Fig. 7 Maximum likelihood tree of the order Cichliformes from the Parque Nacional dos Lençóis Maranhenses (PNLM) and adjacent areas

characters in *Bryconops* are variable and subject to convergence or environmental plasticity, thereby limiting their diagnostic value (Gonçalves et al. 2024).

Considering also the morphological overlap between *B. melanurus* (Bloch, 1794) and *B. affinis* (Günther, 1864), both described from the Guiana Shield (Guyana and Suriname)

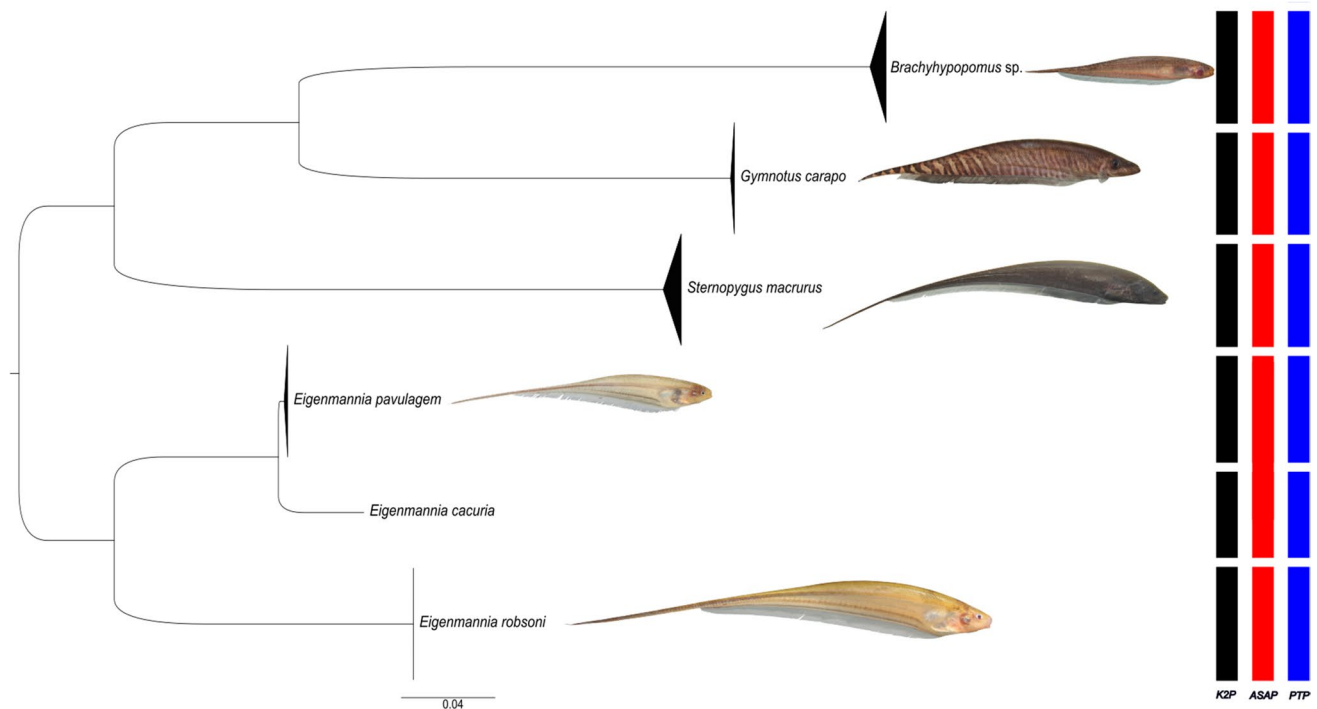


Fig. 8 Maximum likelihood tree of the order Gymnotiformes from the Parque Nacional dos Lençóis Maranhenses (PNLM) and adjacent areas

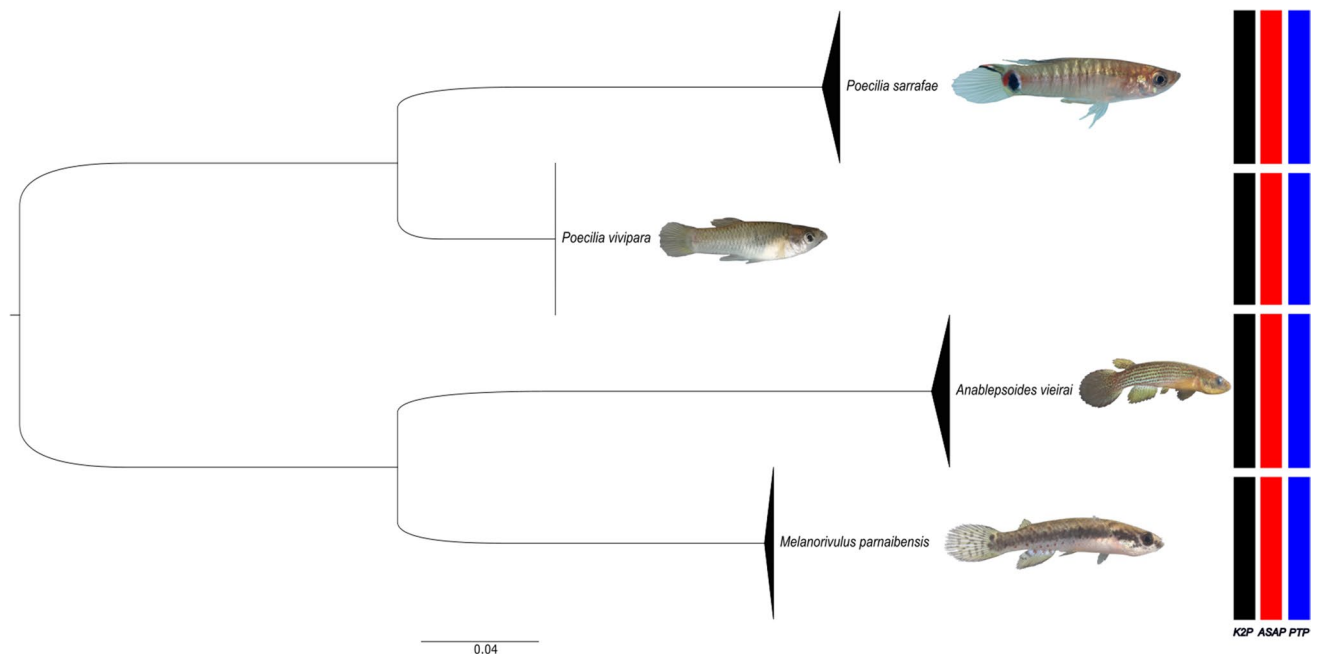


Fig. 9 Maximum likelihood tree of the order Cyprinodontiformes from the Parque Nacional dos Lençóis Maranhenses (PNLM) and adjacent areas

in geographically proximate regions, the distinction between these taxa becomes weakly supported. Both share a silvery body, a similar pigmentation pattern on the caudal fin (with conspicuous markings at the basal portion), cephalic

morphology and body proportions that largely overlap, as well as meristic characters and fin positioning that are virtually identical. In this context, we opted to identify the

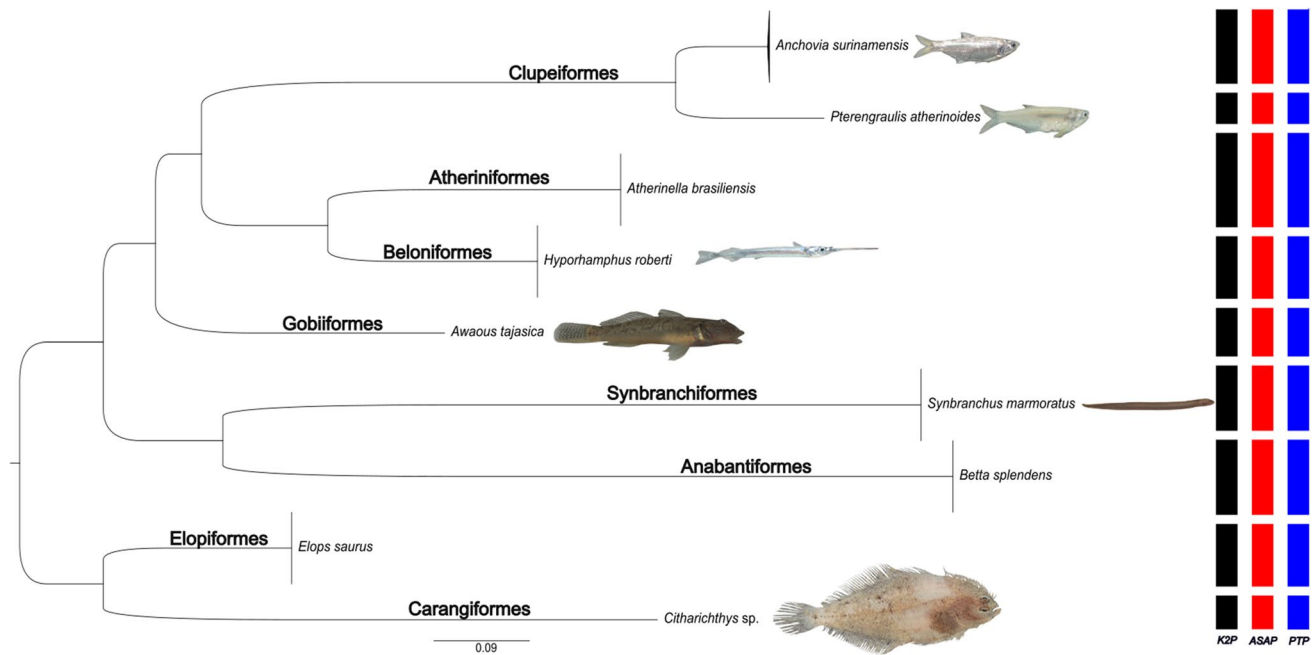


Fig. 10 Maximum likelihood tree of the least representative orders of the Parque Nacional dos Lençóis Maranhenses (PNLM) and adjacent areas

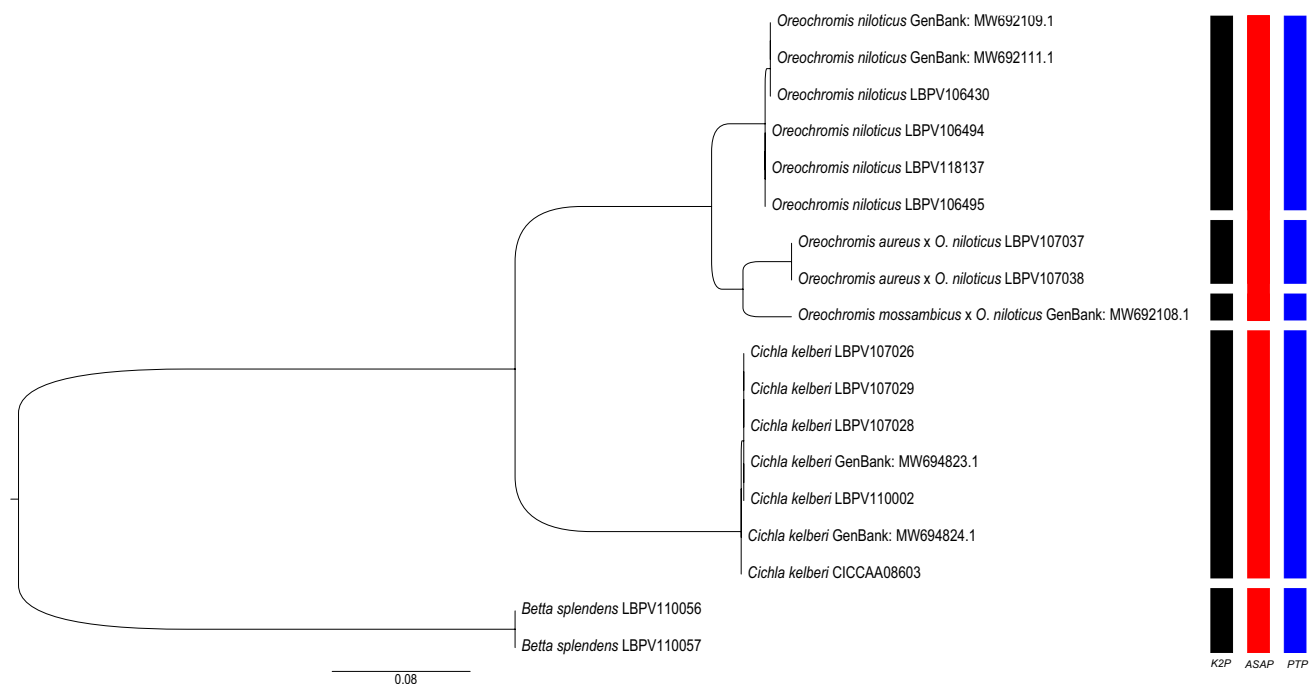


Fig. 11 Non-native species present in the Parque Nacional dos Lençóis Maranhenses (PNLM) and adjacent areas

specimens as *B. cf. melanurus*, as *B. melanurus* represents the oldest available valid name associated with this lineage.

Previous studies recorded the occurrence of three species of *Hemigrammus* Gill, 1858 and three species of *Moenkhausia* Eigenmann, 1903. However, the phylogenomic

study conducted by Melo et al. (2024) restructured the diversity attributed to these genera, as several species previously assigned to *Hemigrammus* and *Moenkhausia* were reallocated to other revalidated genera (Fig. 4; Table 2). In addition to the nomenclatural changes in

Characidae, errors were also detected in the identification of *Moenkhausia* sp. and *M. cotinho*. *Moenkhausia* sp. of Brito et al. (2019b, 2020). Instead it is a *H. ora*, which can be identified by the absence of maxillary teeth; short maxilla; humeral spot restricted to the region above the lateral line; narrow lateral stripe; rounded caudal spot; scales on the caudal fin covering about one-third of the dorsal lobe and half to two-thirds of the lower lobe; 21–24 branched rays on the anal fin; 32–33 scales on the lateral line; and 10–15 perforated scales on the lateral line (Zarske et al. 2006; Jerep et al. 2011). Similarly, the species identified by Piorski et al. (2017) as *Moenkhausia* sp., and by Brito et al. (2019b, 2020) as *M. cotinho* corresponds to *R. pyrophthalma*, previously in *Moenkhausia*, but reclassified by Melo et al. (2024). *R. pyrophthalma* is distinguished from *M. cotinho* by having an incomplete lateral line vs. complete (except in some populations from Guyana, not molecularly tested); 27–28 scales in the lateral series vs. 27–34; one tooth on the maxilla vs. two; and 32 vertebrae vs. 24–25 (Costa 1994; Mathubara and Toledo-Piza 2020).

The species that Brito et al. (2019b, 2020) identified as members of *Hemigrammus* were here reidentified or had their taxonomic status updated (see Fig. 4, Table 2, Online Resources 6, 7). Furthermore, the taxon previously identified as *Astyanax* cf. *lacustris* corresponds to *A.* cf. *bimaculatus* based on Oliveira et al. (2020b). Similarly, specimens previously identified as *Hoplias malabaricus* or *H.* cf. *malabaricus*, (Piorski et al. 2017; Brito et al. 2019b, 2020; Abreu et al. 2025) are *H. cazumba*, as indicated by comparison with topotypes of the latter species (Vieira et al. 2023). Finally, the taxon identified by Piorski et al. (2017) and Brito et al. (2019b, 2020) as *Leporinus* aff. *friderici* and *L.* cf. *friderici* corresponds to *L. piau* based on Nascimento et al. (2023; 2024).

The taxa previously identified as *Hypostomus johnii*, *Hypostomus* sp., *Loricaria parnahybae* and *L.* cf. *parnahybae* correspond to *H. cari* (Lustosa-Costa et al. 2024) and *L. catirina* (Serejo-Saraira et al. 2026), meaning that the diversity of these genera in PNLM and adjacent areas was overestimated. Conversely, only part of the specimens previously identified as *Pimelodella parnahybae* correspond, in fact, to that species; the remaining specimens represent a possible undescribed species identified herein as *Pimelodella* sp. As for the genus *Eigenmannia*, previous works recorded only the occurrence of *E. virescens*; however, the analyses carried out in this study indicated the presence of three species in the study area: *E. pavulagem*; *E. cacuria*; and *E. robsoni* (Fig. 8). In addition, the record of *Melanorivulus* cf. *parnaibensis* corresponds to *M. parnaibensis*, since our material fits the diagnostic features of the species original description.

Two nominal species of *Cichla* Bloch & Schneider, 1801 had been reported in previous studies on the ichthyofauna

of PNLM and adjacent areas, *C. monoculus* and *C. kelberi* (Brito et al. 2020; Monroe et al. 2023). Specimens collected in this study are phenotypically closest to *C. kelberi*, according to Kullander and Ferreira 2006 (this species differs from *C. monoculus* mainly by having the fins extensively covered with spots vs. spotless), in agreement with Brito et al. (2020). In addition, our molecular analyses indicate that our *C. kelberi* sequences clustered with sequences previously identified as *C. monoculus* by Monroe et al. (2023), available in GenBank (Online Resource 3), suggesting a misidentification of *C. monoculus* in the region (Fig. 7). Additionally, the records attributed by Piorski et al. 2017 and Brito et al. 2020) to *Lycengraulis batesii* correspond to *Anchovia surinamensis* and *Pterengraulis atherinoides*. It should be noted that, although our results are based on molecular data, some taxa may present diversity not yet detected. This is the case, for example, of genus *Hypostomus* Lacepède 1803, in which the genetic distance between congeneric species tends to be less than 2% (Pereira et al. 2013; Anjos et al. 2020; De Queiroz et al. 2020), with 1.2% being the average value estimated for sister species of the genus (De Queiroz et al. 2020). The present study is taking into account a genetic distance $\geq 2\%$ K2P to consider delimiting the taxa, therefore, we considered in this work the sequences of *Hypostomus* as a single OTU.

Brito et al. (2019b, 2020) reported the occurrence of *Brachychalcinus parnaibae* Reis, 1989 in the study area, a representative of the subfamily Stethaprioninae; however, this record was based on a single specimen collected in 2000, and no additional records of this genus have been reported since then. During expeditions conducted between 2016 and 2024, no specimens of *B. parnaibae* were collected. However, the only representative currently being recorded belonging to this subfamily is a possible new species of the genus *Poptella* Eigenmann, 1908. Similarly, *Pygocentrus nattereri* Kner, 1858 was collected only once and has not been recorded again in the PNLM or its surrounding areas. However, this species is considered native to several river basins near the PNLM, such as the Mearim, Munim, and Parnaíba river basins (e.g. Ramos et al. 2014; Guimarães et al. 2020b; Vieira et al. 2023). Therefore, it is likely rare within the PNLM and adjacent areas, yet it is still regarded as a native species.

Barcoding gap

The presence of a well-defined barcoding gap, with a clear separation between intraspecific and interspecific distances, is considered one of the main criteria for the effectiveness of DNA barcoding in species delimitation (Hebert et al. 2003; Meyer and Paulay 2005). In the present study, the maximum intraspecific distance (1.78%) is significantly lower than the minimum interspecific distance (3.63%),

resulting in a barcoding gap of 1.85% (Fig. 3). This pattern suggests that the analyzed native species represent genetically well-defined lineages, with low sharing of haplotype variation; however, much of the Neotropical diversity arose approximately between 2 to 23 million of years ago, characterizing recent radiations, which results in some morphologically similar and genetically close species (Hoorn et al. 2010, 2022; Pereira et al. 2013; Albert et al. 2018; Cassemiro et al. 2023; Tagliacollo et al. 2024). Similar results have been reported for Neotropical fish, in which the mitochondrial marker COI frequently exhibits high discriminatory capacity when there is a sufficiently long evolutionary history and an absence of recent hybridization (Ward et al. 2005; Ardura et al. 2010; Pereira et al. 2011, 2013; Castro Paz et al. 2014; Díaz et al. 2016; Rossini et al. 2016; Guimarães et al. 2018b; Santana et al. 2023). Thus, the data corroborate the robustness of DNA barcoding for species identification and reinforce its applicability in taxonomic and biodiversity studies.

New records for Maranhão

Among the newly recorded species, *Eigenmannia pavulagem*, *Hasemanina* cf. *diastatos*, and *Ramirezella pyrophthalma* are native to the PNLM and adjacent areas. Characterized by incomplete lateral line; absence of humeral mark; a dark-brown, oval mark spanning from the distal portion of the caudal peduncle to the caudal-fin base; presence of only four infraorbitals (personal observation); 6–7 total pelvic-fin rays; hooks absent from all fins in males; 15–18 branched anal-fin rays; and anal fin falcate in males, *H. diastatos* was originally described from waterbodies draining the Serra Geral de Goiás and emptying in the upper Tocantins and São Francisco basins, distant nearly 1,000 km from the Lençóis Maranhenses. No records of this species are known between the two regions. A possible difference between the specimens collected in the Lençóis Maranhenses and typical specimens of *H. diastatos* is the degree of elongation of the dorsal fin: mature males of *H. diastatos* have the dorsal fin typically longer than that of females (30.2–39.7%SL vs. 24.7–31.3) and nearly reaching or even surpassing the adipose fin (Dagosta et al. 2014, Figs. 1, 3); in all specimens from the Lençóis Maranhenses, the dorsal fin falls short from the adipose fin and there seems to be no significant length difference between the fins of males and females. *Ramirezella pyrophthalma* was described from the Araguaia River drainage, State of Mato Grosso, central Brazil (Costa 1994), about 1,600 km from the Lençóis Maranhenses area. Nonetheless, the *R. pyrophthalma* specimens from the Lençóis Maranhenses agree morphologically in every aspect with its original description. *E. pavulagem* was described from small tributaries of the Capim and Guamá rivers, State of Pará, northern Brazil (Peixoto et al. 2015), about 500

km from the Lençóis Maranhenses area. Nonetheless, the *E. pavulagem* specimens from the Lençóis Maranhenses agree morphologically in every aspect with its original description. In addition, two non-native fish species are herein first record for Maranhão: *Betta splendens*, which occur naturally in the Mekong River basin in Thailand and Cambodia, Southeastern Asia (Fricke et al. 2026b), and a hybrid lineage *Oreochromis aureus* x *O. niloticus*. It is important to note that species of the genus *Oreochromis* Günther, 1889 occur naturally on the African continent (Fricke et al. 2026b).

Non-native species and mechanism of introduction

The PNLM and its adjacent areas have records of six non-native species: *Oreochromis niloticus* by Monroe et al. (2023); *O. mossambicus* by Monroe et al. (2023); *Oreochromis* sp. by Piorski et al. (2017), Brito et al. (2019b, 2020); *Cichla kelberi* by Brito et al. (2020), and *C. monoculus* by Monroe et al. (2023); and *Salminus brasiliensis* (Cuvier, 1816) by Aguiar-Santo and Meneses (2025). However, the report of *C. monoculus* represents a misidentification, as mentioned above (Nomenclatural and identification update section). Similarly, the record of *O. mossambicus* is also erroneous, since it corresponds to a hybrid lineage resulting between *O. mossambicus* x *O. niloticus*, rather than a true record of *O. mossambicus*. In addition to these records, the present study adds the occurrence of *Betta splendens* and another hybrid lineage of *O. aureus* x *O. niloticus* (Fig. 11). The presence of *S. brasiliensis* recorded by Aguiar-Santo and Meneses (2025) was not included in our study, since we do not have tissues samples for DNA extraction. However, we consider this record valid based on morphology. Therefore, the following non-native fish species occur in the PNLM and in adjacent areas: *C. kelberi*, *S. brasiliensis*, *B. splendens*, *O. niloticus*, and two hybrid lineages of *Oreochromis* (*O. mossambicus* x *O. niloticus*, and *O. aureus* x *O. niloticus*) (Fig. 11).

These results highlight the need to monitoring and control species that have already been introduced, as well as to develop strategies to prevent new species introductions. The introduction of non-native species can cause direct or indirect ecological changes in invaded ecosystems, leading, in some cases, to the decline and even extinction of native species through harmful interactions such as predation, competition, hybridization, and parasitism, as well as potentially accelerating biotic homogenization processes (Rahel 2002; Clavero and García-Berthou 2005; Gozlan et al. 2010; Cucherousset and Olden 2011; Rocha et al. 2023). Additionally, non-native species can modify environmental conditions and compromise ecosystem functions (Rocha et al. 2023).

Biological reserves and conservation units are crucial for sustaining native biodiversity and ecosystem services (Casarim et al. 2020; Carneiro et al. 2024). In this sense, the introduction of species into protected areas compromises the ecological balance and can transform environments intended for conservation into systems marked by harmful biological interactions. Thus, it becomes necessary for management plans to incorporate clear guidelines to prevent the introduction of species, considering that their presence in protected areas represents a direct risk to ecological integrity and the maintenance of native communities (Doria et al. 2021; Rocha et al. 2023).

Non-native species have been recorded from the Parque Nacional dos Lençóis Maranhenses and adjacent areas since the 2000 s, with the occurrence of the genus *Oreochromis*. The other non-native fish were only recorded to the PNLM and adjacent areas more recently (e.g. Brito et al. 2020; Monroe et al. 2023; Aguiar-Santos and Meneses 2025; and present study). The risk of introductions is heightened in touristic regions, particularly when sport fishing is implemented (Anderson et al. 2015; Azevedo-Santos et al. 2015), in addition to already known routes such as aquaculture and aquarium trade (Azevedo-Santos et al. 2015; Doria et al. 2021; Rocha et al. 2023). Therefore, it is essential to adopt strategies for the removal and control of these organisms, since they can alter fundamental ecological processes and reduce native diversity (Carneiro et al. 2024).

Conclusions

Previous studies recorded 60 fish species in PNLM and adjacent areas (Piorski et al. 2017; Brito et al. 2019b, 2020; Monroe et al. 2023; Aguiar-Santos and Meneses 2025), and some of these previously recorded species had their identifications revised in the present study. In addition to the species examined herein (59 species), there are further records for PNLM and adjacent areas that were not sampled in this study [e.g. *Achirus achirus* (Linnaeus, 1758), *Anableps anableps* (Linnaeus, 1758), *Apteronotus albifrons* (Linnaeus, 1766), *Brachychalcinus parnaibae* Reis, 1989, *Centropomus undecimalis* (Bloch, 1792), *Eucinostomus argenteus* Baird & Girard, 1855, *Megalops atlanticus* Valenciennes, 1847, *Mugil curema* Valenciennes, 1836, *Polydactylus virginicus* (Linnaeus, 1758), and *Serrapinnus* sp., *Steindachnerina notonota* (Miranda Ribeiro, 1937)]. Consequently, this study expands the known ichthyofaunal diversity of the PNLM and its surrounding areas to a total of 70 species.

The COI molecular database provided here for the PNLM and adjacent areas provides a robust reference base and tool for fish molecular identification, as well as for applications

in integrative taxonomy, monitoring, and conservation. The well-defined barcoding gap of 1.85% and the clear separation between intraspecific and interspecific distances demonstrate the high discriminatory capacity of COI in the area, strengthening the reliability of identifications and recognized OTUs. The analyses also highlighted that exclusive reliance on morphology can lead to under- and overestimation of species richness, reinforcing the role of DNA barcoding in detecting hidden diversity and in correcting previous records, including nomenclatural updates and identification corrections across different groups. Moreover, an important aspect to be highlighted is that molecular databases (libraries) are consolidated tools enabling reliable estimates of local biodiversity, as well as molecular identification particularly when sequences are linked to voucher material deposited in collections and identified by specialists. Additionally, this study reports five new records for Maranhão. *Eigenmannia pavulagem*, *Hasemanian cf. diastatos*, *Ramirezella pyrophthalma*, and the non-natives *Betta splendens* and a hybrid lineage *Oreochromis aureus* x *O. niloticus*, thereby increasing knowledge of regional biodiversity.

The record of non-native species and hybrid lineages in the studied region indicates anthropogenic pressure and an increased risk of introductions (especially due to tourism, sport fishing, aquaculture, and aquarium trade), highlighting the need for control and removal actions, prevention of new introductions, and the inclusion of specific guidelines in management plans. In light of this, the results obtained expand knowledge of regional biodiversity and provide direct support for the management and conservation of fish fauna in the PNLM. Additionally, it is important to emphasise the importance of molecular approaches in detecting hidden lineages of non-native species. In the case of tilapias, biologists and environmental monitoring agents often record only a single species. However, as demonstrated here, tilapias are not always introduced from a single source lineage and may comprise multiple lineages, including hybrid forms. In this context, molecular approaches are essential for detecting "hidden" tilapia lineages and for demonstrating that introductions may originate from different sources. Furthermore, it is noteworthy that tilapias (including both hybrid and non-hybrid lineages) exhibit distinct biological and physiological characteristics, which may negatively affect ecosystems in different ways and confer varying degrees of resistance to environmental factors.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11756-026-02217-1>.

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Authors contributions Felipe P. Ottoni, Cláudio Oliveira and Lucas O. Vieira were responsible for the conception and design of the study, including the definition of objectives, methodological approach, and overall research framework. Biological material was collected by Felipe P. Ottoni, Cláudio Oliveira, Lucas O. Vieira, Mariana Kuranaka, Rafael F. Oliveira, Erick C. Guimarães, and Pâmella S. Brito. The screening of biological material was conducted by James R. Garcia-Ayala, Jefferson L. Crispim, Felipe P. Ottoni, and Lucas O. Vieira. Taxonomic identification of the material was performed by Lucas O. Vieira, James R. Garcia-Ayala, Gabriel C. Deprá, Felipe P. Ottoni, Erick C. Guimarães, Pâmella S. Brito, Jefferson L. Crispim, and Rafael F. Oliveira. Laboratory work, including all bench procedures and the generation of mitochondrial cytochrome c oxidase subunit I (COI) gene sequences, was carried out by Felipe P. Ottoni, Lucas O. Vieira and Mariana Kuranaka. Molecular data analyses were performed by Lucas O. Vieira and Jefferson L. Crispim. All authors contributed to manuscript writing, critically revised the content, and approved the final version of the manuscript.

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Data availability All data generated in this study are available in the supplementary material files (Online Resource 1—8). All DNA sequences generated in this study are available in GenBank.

Declarations

Ethical approval Not applicable.

Conflict of interest The authors declare no competing interests.

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