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DIVERSIDADE E ADAPTAÇÃO DE CICLÍDEOS NÃO NATIVOS EM BACIAS HIDROGRÁFICAS DO BRASIL

THERCIA GONÇALVES RIBEIRO MONROE

São Luís – MA
2024

THÉRCIA GONÇALVES RIBEIRO MONROE

DIVERSIDADE E ADAPTAÇÃO DE CICLÍDEOS NÃO NATIVOS EM BACIAS HIDROGRÁFICAS DO BRASIL

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, como requisito para a obtenção do Título de Doutor em Biodiversidade e Biotecnologia.

Orientador (a): Profª. Dra. Lígia Tchaicka

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“*A Ciência nunca resolve um problema sem criar pelo menos outros dez*”

George Bernard Shaw

MONROE, Thércia Gonçalves Ribeiro. **Diversidade e Adaptação de Ciclídeos não nativos em Bacias Hidrográficas do Brasil**. 2024. 195f. Tese (Doutorado em Biodiversidade e Biotecnologia – REDE BIONORTE) – Universidade Estadual do Maranhão, São Luís, 2024.

RESUMO

As invasões biológicas constituem uma das principais causas de perturbações ambientais, seja por competição de recursos, predação ou introdução de patógenos. No Brasil, um dos grupos mais introduzidos no território nacional são os Ciclídeos, especialmente dos gêneros *Oreochromis* e *Cichla*. Os estudos dos impactos dessas invasões estão crescendo conforme os impactos são vistos. Nesse sentido, o objetivo deste trabalho foi identificar as espécies de peixes dulcícolas não nativas no Maranhão e investigar os padrões de ocorrência e evolução do tucunaré invasor nessas bacias hidrográficas. Para tal, obtivemos 100 amostras de tilápia (*Oreochromis* spp.) e 118 amostras de tucunarés (*Cichla* spp.) de diferentes regiões hidrográficas: Bacia do Atlântico Nordeste Ocidental (Penalva/MA e Viana /MA, Parnaíba (Lençóis Maranhenses/MA e Balsas/MA) e Paraná (Ilha Solteira/SP, Aporé/GO, Santa Terezinha de Itaipu/PR) a fim de ampliar os resultados. Os espécimes foram coletados com auxílio de redes de espera, tarrafa e anzol, no período de 2016 a 2023. Os exemplares foram identificados morfologicamente, etiquetados, fotografados e o tecido muscular armazenado em álcool 99,9% para as análises genéticas. O DNA genômico total foi extraído, quantificado e enviado para genotipagem por sequenciamento illumina para obtenção de reads e identificação de polimorfismos de único nucleotídeo e análise de diversidade. Foram separados 56 indivíduos dentre todos os amostrados, que foram selecionados por critérios de preservação do espécime e qualidade das fotografias para as análises morfométricas. Registramos então a ocorrência e o estabelecimento de *Cichla monoculus*, *Cichla kelberi*, *Oreochromis niloticus* e *Oreochromis mossambicus* nas bacias hidrográficas do Maranhão e nas populações de Ilha Solteira, Aporé, Paraná os táxons *Cichla piquiti* e *Cichla kelberi*. As associações da morfometria geométrica, morfologia e sequenciamento de alto rendimento mostraram variações significativas nas populações não nativas, indicando diferenciação e variação em relação às populações nativas, além de ocorrência de hibridização das espécies *C. piquiti* e *C. kelberi*.

Palavras-chave: Ciclídeos; Bioinvasão; SNPs; Morfologia.

MONROE, Thércia Gonçalves Ribeiro. **Diversity and Adaptation of Non-native Cichlids in Brazilian River Basins.** 2024. 195 p. Thesis (Doctorate in Biodiversity and Biotechnology – REDE BIONORTE) – Maranhão State University, São Luís, 2024.

ABSTRACT

Biological invasions constitute one of the main causes of environmental disturbances, whether due to resource competition, predation or introduction of pathogens. In Brazil, one of the groups most introduced into the national territory are the Cichlids, especially the genera *Oreochromis* and *Cichla*. Studies of the impacts of these invasions are growing as the impacts are seen. In this sense, the objective of this work was to identify non-native freshwater fish species in Maranhão and investigate the patterns of occurrence and evolution of the invasive peacock bass in these river basins. To this end, we obtained 100 samples of tilapia (*Oreochromis* spp.) and 118 samples of peacock bass (*Cichla* spp.) from different hydrographic regions: Western Northeast Atlantic Basin (Penalva/MA and Viana/MA, Parnaíba (Lençóis Maranhenses/MA and Balsas /MA) and Paraná (Ilha Solteira/SP, Aporé/GO, Santa Terezinha de Itaipu/PR) in order to expand the results. The specimens were collected with the aid of gillnets, nets and hooks, from 2016 to 2023. The specimens were morphologically identified, labeled, photographed and the muscle tissue was stored in 99.9% alcohol for genetic analysis. Total genomic DNA was extracted, quantified and sent for genotyping by illumina sequencing to obtain reads and identify polymorphisms of single nucleotide and diversity analysis. 56 individuals were separated from all those sampled, which were selected based on criteria of specimen preservation and quality of photographs for morphometric analyses. We then recorded the occurrence and establishment of *Cichla monoculus, Cichla kelberi, Oreochromis niloticus* and *Oreochromis mossambicus* in the Maranhão river basins and in the populations of Ilha Solteira, Aporé, Paraná, the taxa *Cichla piquiti* and *Cichla kelberi*. The associations of geometric morphometry, morphology and high-throughput sequencing showed significant variations in non-native populations, indicating differentiation and variation in relation to native populations, in addition to the occurrence of hybridization of the species *C. piquiti* and *C. kelberi.*

Keywords: Cichlids; Bioinvasion; SNPs; Morphology.

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1 INTRODUÇÃO

Um táxon que ocorre fora de sua área de distribuição natural passada ou presente; incluindo qualquer parte do organismo, como gametas, sementes, ovos ou propágulos que possam sobreviver e posteriormente reproduzir-se são caracterizados como espécies não nativas (Mack *et al.*, 2000; Ziller, 2001). Após serem transportadas para fora de sua área nativa, introduzidas em um novo local, estabelecem populações autossustentáveis por sobrevivência e reprodução, se espalham para novos locais e têm impactos ecológicos e/ou econômicos negativos no ecossistema receptor podem ser consideradas espécies invasoras (Olden *et al.*, 2021)

As invasões biológicas constituem uma das principais ameaças à conservação da biodiversidade no mundo (Pyšek *et al.*, 2020). De acordo com Heringer *et al.* (2021) um custo de aproximadamente 102,5 bilhões de dólares entre 1975 e 2020 foi atribuído às consequências das invasões biológicas. Cerca de 90% do gasto concentrou-se no Brasil, Argentina e Colômbia.

Esse contexto se torna relevante quando consideramos a ictiofauna neotropical como a mais diversa taxonômica e funcional do globo (Toussaint *et al.*, 2016), com aproximadamente 35% dessa diversidade ameaçada de extinção (IUCN, 2017). As águas continentais constituem um ambiente que sofre diretamente com as ameaças à biodiversidade, de acordo com Turak *et al.* (2017) as taxas de perda de diversidade são maiores em comparação a ambientes terrestres.

No Brasil, diversas espécies de peixes foram introduzidas (Frehse *et al.*, 2016). A exemplo de Ciclídeos africanos (Forneck *et al.*, 2016) que estão entre as espécies mais cultivadas na aquicultura. E os ciclídeos amazônicos, predadores ativos que têm sido translocados pela pesca esportiva (Fugi *et al.*, 2008) para várias regiões do mundo.

Compreender a dinâmica das invasões biológicas é fundamental para tomada de decisão e conservação da bioidiversidade. O estudo da variação da forma dos indivíduos é um dos aspectos cruciais para compreensão da evolução dos organismos, principalmente em cenários de invasão biológica. A morfometria geométrica permite avaliar os mecanismos que estejam relacionados às mudanças evolutivas.

Porém, associar metodologias que permitam vislumbrar um panorama geral dessas invasões é fundamental para obtenção de informações mais detalhadas. E nesse sentido, o sequenciamento de nova geração contribui significativamente para o conhecimento do status das invasões biológicas. Diante do exposto, esse trabalho é de fundamental importância para o conhecimento do status da biodiversidade de peixes na região amazônica, além de possibilitar o entendimento do processo de bioinvasão e evolução de ciclídeos em ecótonos e seus desdobramentos.

Dessa forma, propomos que os diferentes ambientes invadidos por ciclídeos tenham distintas forças evolutivas atuando de maneira que haja diferenciação entre essas populações. Incluindo variáveis como o tempo de introdução, número de matrizes, nível de impactos ambientais associados entre outros.

1.1 OBJETIVO GERAL

Investigar os padrões de ocorrência e evolução de ciclídeos invasores em Bacias Hidrográficas Brasileiras

1.2 OBJETIVOS ESPECÍFICOS

- Identificar as espécies de ciclídeos introduzidas nas bacias hidrográficas do Maranhão;
- Identificar a origem das introduções de peixes no Maranhão;
- Verificar diferenças morfológicas e genéticas (intra e interespecíficas) nas espécies das diferentes populações introduzidas;
- Comparar os padrões de evolução das populações introduzidas no Paraná, Goiás, São Paulo, Maranhão com as populações nativas do Pará e Tocantins;
- Fornecer informações para o monitoramento das espécies nas áreas protegidas do Estado;
- Comparar a diversidade de peixes nativos nas áreas protegidas do Maranhão com a presença de espécies não nativas

2 REVISÃO DE LITERATURA

2.1 CICLÍDEOS INVASORES

Os ciclídeos são peixes de água doce e ocasionalmente de água salobra que ocorrem na América Central e do Sul, Índias Ocidentais, África, Madagascar, Israel, Síria, Índia costeira e Sri Lanka (Nelson *et al.*, 2016). Constituem a família com maior riqueza de espécies de água doce do mundo (Kullander, 2003), com 253 gêneros válidos (Frick; Eschmeyer; Fong, 2020). Apresentando também um alto grau de endemismo associado à formação de tribos (Salzburguer; Meyer, 2004).

Dentre os ciclídeos, se destacam como invasores, os representantes dos gêneros: *Cichla* (Schneider, 1801), endêmico da região amazônica com 16 espécies descritas (15 por Kullander e Ferreira, 2006 e 1 por Sabaj *et al.*, 2020) e o gênero mais rico em número de espécies: *Oreochromis* Günther 1889, com 32 espécies encontradas em toda a África Subsaariana, bem como na bacia do Nilo e no Oriente Médio (Trewavas, 1983). Esses ciclídeos são invasores em diversos países, pois características inerentes a essas espécies são particularmente propícias às invasões biológicas a exemplo, a plasticidade fenotípica, resistência ambiental e altas taxas reprodutivas.

Para o gênero *Oreochromis*, das 44 espécies e subespécies nativas, destaca-se *Oreochromis niloticus* (Linnaeus 1758), *Oreochromis mossambicus* (Peters 1852), *Oreochromis aureus* (Steindachner 1864) com registros na região neotropical. De acordo com Dunz e Schliewen (2013), a tribo Oreochromini é composta pelas linhagens de criação bucal anteriormente atribuídas à tribo tilapiine: *Alcolapia, Danakilia, IranoCichla, Oreochromis, Sarotherodon, Tristramella*, juntamente com quatro gêneros derivados de *Sarotherodon* endêmicos da cratera camaronesa Lago Barombi Mbo (*Konia, Myaka, Pungu, Stomatepia*).

As espécies desse gênero possuem hábito alimentar oportunista, com ampla base alimentar. Apresentam um alto grau de eurialinidade, rápida reprodução, resistentes a patógenos e condições abióticas adversas (Philippart; Ruwet, 1982, Ulotu *et al.*, 2023)

Pela sua morfologia menos especializada, os Oreochromini são altamente adaptáveis a habitats ecológicos diversos e presumivelmente menos propensos à extinção (Klett; Meyer, 2002). Embora aproximadamente 70 espécies recebam a denominação de “tilápia”, somente *Oreochromis niloticus, Oreochromis mossambicus, Oreochromis aureus, Oreochromis urolepis e Oreochromis hornorum* têm grande importância na piscicultura mundial. O sabor suave da carne, padrão de coloração do corpo, a reprodução em cativeiro sem necessidade de indução hormonal, o cuidado parental e a tolerância a diferentes condições ambientais tornam as tilápias ideais para a aquicultura (Watanabe *et al.*, 2002).

Eles foram introduzidos em mais de 90 países em todo o mundo, com uma distribuição global perdendo apenas para a carpa comum (FAO, 2020). A contribuição das tilápias para a aquicultura global e a produção pesqueira de captura aumentou dramaticamente nas últimas três décadas, e a tilápia do Nilo, *Oreochromis niloticus* (L.), é responsável por grande parte desse aumento, superando os 6,2 milhões de toneladas em 2021 (FAO, 2022).

No Brasil, em 1952, alguns exemplares de *Coptodon rendalli* (Boulenger 1897) foram trazidos do Congo Belga para o cultivo. Contudo, a criação não apresentou resultados satisfatórios. Na tentativa de melhorar a produção por área, em 1971 foram trazidas outras espécies por técnicos do DNOCS (Departamento Nacional de Obras Contra Seca) de Fortaleza – Ceará, algumas do gênero *Oreochromis*, que se caracterizam por desovar em ninhos e as fêmeas incubarem seus ovos e larvas na boca (Pinto, 1988). E introduziu-se nos tanques da região Nordeste *Oreochromis niloticus* (Embrapa, 2007; Leão *et al.*, 2011) e em 1981 uma linhagem modificada de *Oreochromis mossambicus*, a tilápia vermelha.

O outro grupo, formado pelos tucunarés (*Cichla* spp.), são uma linhagem basal dentro dos cliclideos neotropicais, de origem compatível aos eventos de deriva genética associados à fragmentação da Godwana (aproximadamente há 250 m.a.), pertencendo ao agrupamento: *Retroculus* (*Cichla* + *Astronotus*+ *Geophagini* + *CreniCichla*). Ocorrem nas bacias dos rios Amazonas, Tocantins, Orinoco e nas pequenas drenagens das Guianas (Kullander; Ferreira, 2006).

As 16 espécies descritas possuem o cariótipo 2n=48 (Brinn; Porto; Feldberg, 2004) e estão entre os maiores predadores de topo das águas brasileiras. São sazonalmente monogâmicas e apresentam cuidado parental extensivo (Winemiller, 2001).

Kullander e Ferreira (2006) revisaram FAo gênero *Cichla* e propuseram 15 espécies morfologicamente válidas: *Cichla piquiti*, *Cichla intermedia*, *Cichla ocellaris*, *Cichla nigromaculata*, *Cichla monoculus*, *Cichla kelberi*, *Cichla pleizona*, *Cichla orinocencis*, *Cichla minianae*, *Cichla thyrous*, *Cichla jariina*, *Cichla pinima*, *Cichla vazzoleri*, *Cichla temensis, Cichla melaniae*. E recentemente outra espécie foi descrita (*Cichla cataractae* Sabaj *et al.* 2020)

No entanto, não há um consenso acerca da taxonomia do grupo, visto que Willis *et al.* (2012) reformulou o gênero com base em dados moleculares. Os autores validaram 8 espécies: *Cichla piquiti, C. intermedia, C. ocellaris, C. orinocensis, C. mirianae, C. melaniae, C temensis, C pinima* apoiados no conceito politípico de espécie. Aspectos estes que tornam complexos os panoramas de identificação, estudos ecológicos, reprodutivos, de distribuição e hibridização nesses táxons.

Os tucunarés têm sido introduzidos em vários ambientes fora de sua ocorrência natural (Zaret; Paine,1973). No entanto, não há um controle a respeito de quais estoques são utilizados, nem quais espécies são introduzidas, na região nordeste, por exemplo, foram introduzidos no final da década de 1940 pelo governo com o objetivo de instalar pisciculturas (Fontenele, 1948; Fontenele; Peixoto, 1979).

Posteriormente, o táxon também se estabeleceu bem no sudeste do Brasil (Alves *et al.*, 2007), embora não se saiba se essas populações foram estabelecidas por animais que escaparam de fazendas de aquicultura ou foram deliberadamente soltos. Atualmente, têm-se o registro total de 373 ocorrências, sendo 88 nativas nas bacias amazônica e Tocantins-araguaia e 285 registros não nativos no mundo (Franco *et al.*, 2022)

Importante ressaltar que esse grupo apresenta questões bem peculiares relatadas na literatura, a exemplo de hibridização interespecífica (Andrade *et al.*, 2001; Oliveira *et al.*, 2006), hibridação e introgressão em ambientes invadidos (Diamante *et al.*, 2020), policromatismo em ambiente nativo (Pelicice *et al.*, 2022; Nobrega *et al.*, 2023), alta diversidade genética em populações invasoras (Khaleel *et al.*, 2023).

2.2 A AMEAÇA DA INVASÃO BIOLÓGICA EM ÁREAS PROTEGIDAS

As áreas protegidas são essenciais para a manutenção da diversidade biológica em seus variados níveis. De acordo com Pelicice *et al.* (2017), as ameaças à biodiversidade íctica neotropical são intensificadas com políticas públicas cada vez mais insustentáveis. Aliadas a alterações hidrológicas, introdução de espécies não nativas, destruição de habitats e poluição, os prejuízos à diversidade em ambientes aquáticos estão em progresso.

No Brasil foram registradas 144 espécies exóticas invasoras em unidades de conservação federais (Sampaio; Schimidt, 2014). E o Sistema Nacional de Unidades de Conservação proíbe a introdução de espécies não nativas, visto que o objetivo das áreas protegidas está em manter a biodiversidade, os processos ecológicos e evolutivos (MMA, 2000)

Contudo, diante das consequências das invasões biológicas, é preocupante que áreas protegidas por razões citadas acima estejam ameaçadas e suscetíveis a alterações como extinção de espécies nativas e espécies endêmicas e ameaçadas. Thresher, Smith e Cutajar (2019) afirmam que a invasão de *Oreochromis niloticus* no lago Kutubu, sítio Ramsar, trouxe impactos como diminuição de diversidade e consequentes danos ecológicos.

Espécies invasoras modificaram substancialmente a ictiofauna do rio Paranapanema; quando associado às condições lênticas alteradas causadas pelo represamento, indicando que os peixes nativos do rio estão cada vez mais ameaçados (Garcia *et al.*, 2018)

O estado do Maranhão, localizado na região nordeste do país, é um dos maiores estados do Brasil, com área aproximada de 332 mil km² (Batistella *et al.*, 2014). Também é considerado um mosaico dos biomas Amazônia (35%), Caatinga (1%) e Cerrado (64%). Cerca de 19% do território está protegido por unidades de conservação, embora menos de 5% sejam de proteção integral inseridos no bioma amazônico.

No estado estão delimitadas 40 unidades de conservação nas seguintes categorias: Reserva de Patrimônio (12), Área de Proteção Ambiental (10), Parque nacional ou estadual (9), Reserva extrativista (08), Reserva Biológica (01) (MMA, 2022)

Uma importante unidade de conservação é a Baixada Maranhense, um Sítio Ramsar, área natural de zona úmida de significância internacional para a ecologia, zoologia, limnologia e hidrologia, possui aproximadamente 1.775.035 hectares e está totalmente inserida no bioma Amazônia, Costeiro e Marinho. Compreende uma área rica em biodiversidade por incorporar uma complexa interface de ecossistemas, incluindo manguezais, babaçuais, campos abertos e inundáveis, estuários, lagos e matas ciliares. Esse mosaico de fisionomias e sua extensão na paisagem tornam uma unidade de conservação importante, pois permite a ocorrência de processos ecológicos de grande escala, além de possuir áreas de manguezais que funcionam como regulador local dos estoques pesqueiros. Nos estuários, os manguezais ocorrem penetrando os igarapés, por entre os campos, até onde existe influência das marés. A região é drenada pelos rios Mearim, Pindaré, Grajaú, Pericumã e afluentes e tem como ecossistema característico os babaçuais ou cocais (MMA, 2003)

Outra UC de destaque no Estado, é o Parque Nacional dos lençóis Maranhenses (PNLM), criado pelo Decreto Federal Nº 86.060 de 02/06/1981, que apresenta uma área de 155.000ha e é composto por um extenso campo de dunas com lagoas temporárias e perenes, apresentando morfodinâmica diferente de outros locais da costa brasileira. A hidrografia do PNLM é caracterizada pela presença de rios, córregos, lagos, lagoas e lagunas. Lago de Santo Amaro, Travosa, Rio Grande, Rio Negro, Lagoa da Esperança, Lagoa da Betânia (MMA, 2003)

Ambientes contrastantes são fonte para seleção natural divergente e consequentemente, especiação (Nosil, 2012), para tal, as áreas protegidas amostradas neste trabalho apresentam variações ambientais e níveis de proteção à diversidade locais diferenciadas. Diante disso, as invasões biológicas podem ter consequências distintas e desdobramentos únicos para as biotas locais, na rota evolutiva das espécies nativas e invasoras.

2.3 METODOLOGIAS PARA O ESTUDO DE ESPÉCIES INVASORAS

Os peixes são um grupo de particular interesse para investigar pressão de seleção associada à variações fenotípicas intraespecíficas, considerando variações de salinidade (Dennenmoser *et al.*, 2017) e fluxo da água (Mise *et al.*, 2015), por exemplo que trazem alterações no fenótipo e gerando variabilidade.

Inicialmente, as variações morfológicas são as primeiras a serem identificadas, pela facilidade de visualização. No entanto, os ciclídeos são um grupo taxonomicamente complexo, gerando incertezas na utilização de caracteres morfológicos. Outra forma de observar padrões e variações fenotípicas é por meio da morfometria geométrica, que é capaz de descrever e localizar regiões de mudanças na forma e, sobretudo de reconstruir graficamente estas diferenças, através de marcos anatômicos (Bookstein, 1991; Cordeiro-Estrela; Fornel, 2015).

Esta técnica propicia a interpretação e comparação dos padrões de variação de caracteres quantitativos e entre parâmetros biológicos subjacentes às relações morfológicas entre indivíduos, na discriminação entre grupos intraespecíficos ou populações geográficas ou mesmo diferenciação entre sexos (Viana *et al.*, 2006; Nunes, 2008; Franssen, 2011; Araújo *et al.*, 2012; Fabrin *et al.*, 2014; Reiss; Grothues, 2015), principalmente quando os caracteres morfológicos são semelhantes, como nos ciclídeos

Em peixes, diversos estudos utilizam a morfometria geométrica associada à variabilidade intraespecífica e interespecífica (Lazarotto *et al.*, 2017; Azevedo; Junior; Molina, 2009), para seleção de características de interesse comercial (Bopo *et al.*, 2018) entre outros. A morfometria geométrica tem suas bases na visualização de conjunto de dados obtidos de marcos anatômicos (coordenadas cartesianas). Esses marcos anatômicos assumem uma homologia entre si e uma cobertura adequada da morfologia (Zelditch;Swiderski;Sheets, 2012)

Obter os dados para morfometria geométrica utilizando imagens digitalizadas do tipo fotografias, e posterior digitalização dos marcos anatômicos em softwares (Monteiro; Reis, 1999) e através dessa metodologia trabalhamos com a morfometria geométrica em duas dimensões. Esses marcos anatômicos, após serem ligados compõem a forma do organismo, ou seja, toda a informação mantida após efeitos de rotação, posição e escala são removidos (Kendall, 1977). Essa metodologia é conhecida como sobreposição de Procrustes (Zelditch; Swiderski; Sheets, 2012)

O estudo da genética, evolução e interações de espécies invasoras e nativas em áreas invadidas oferece uma oportunidade única para estudar processos em genética populacional e a capacidade de expansão de uma espécie.

Nesse sentido, geneticamente, as invasões biológicas são explicadas pelo efeito fundador, onde as populações podem ser originadas por uma pequena amostra de uma população original (Mayr; 1942; Ridley, 2006). O consequente aumento populacional, a tendência das espécies é apresentarem populações endogâmicas, por excesso de endocruzamentos e/ou autofertilização devido ao isolamento, fenômeno denominado efeito do gargalo genético (Nei; Maruyama; Chakraborty, 1975).

Dentre os impactos na biodiversidade causados pelas invasões destacam-se as invasões crípticas, que consistem na introdução e fixação de alelos de não nativos em populações nativas (Geller *et al.*, 1997). Este evento, ao longo de várias gerações, pode suprimir ou extinguir as variações alélicas nativas (Saltonstall, 2001).

Com o advento do sequenciamento massivo em paralelo, conhecido como sequenciamento de nova geração (NGS), que iniciou em 2005 com o lançamento da plataforma 454 Life Sciences, da companhia Roche, novas possibilidades através dessa ferramenta estão sendo utilizadas para resolver as inter-relações de táxons problemáticos (Smith *et al.*, 2014; Ochoa *et al.*, 2020), elucidando questões populacionais de espécies.

O sequenciamento por genotipagem (GBS) é uma abordagem de genotipagem simples e repetível que permite um alto nível de multiplexação de amostras (De Donato *et al.*, 2013), tornando possível sequenciar apenas uma fração do genoma, omitindo elementos repetitivos do genoma (Narum *et al.*, 2013). Ao contrário de outros métodos com representação reduzida do genoma, o GBS tem menos etapas e um procedimento mais simples (Elshire *et al.*, 2011). A preparação da biblioteca pode ser realizada com pequenas quantidades de DNA inicial, como 100 ng, e pode ser realizada com altos níveis de multiplexação, tornando possível descobrir e genotipar dezenas de milhares de polimorfismos de nucleotídeo único (SNPs) em uma única execução e vários indivíduos (Sonah *et al.*, 2013).

Os SNPs são polimorfismos estáveis e usualmente bi-alélicos (Mullikin *et al.*, 2000), encontrados em regiões codificantes e não codificadoras (Bruneaux *et al.*, 2013; Stölting *et al.*, 2013), homogeneamente distribuídos ao longo do genoma em alta densidades (Ching *et al.*, 2002). São considerados os marcadores moleculares mais aptos à genotipagem de automação e capazes de revelar polimorfismos ocultos não detectados em outros marcadores moleculares (Liu; Cordes, 2004).

Nos peixes, os SNPs são encontrados aproximadamente a cada 100 pb (Vera *et al.*, 2013), o que os torna ideais para genômica comparativa (Bouza *et al.*, 2012, Cheng *et al.*, 2021; Ciezarek *et al.*, 2022), genômica evolutiva (Layton *et al.*, 2021), mapeamento fino de genes associados a características produtivas (Rajesh *et al.*, 2018; Mastrochirico *et al.*, 2020), triagem genômica em populações para conservação e manejo (Albaina *et al.*, 2013; Glaubitz *et al.*, 2003;

Kuhner *et al.*, 2000; Vilas *et al.*, 2015) e hibridização e impacto de invasões biológicas (Bers *et al.*, 2010).

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3 CAPÍTULO I

Inventory reveals non-native species and variation in spatial-temporal dynamics of fish community in a Brazilian protected area

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Inventory reveals non-native species and variation in spatial-temporal dynamics of fish community in a Brazilian protected area

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Abstract

The increase in the number of Brazilian protected areas has been progressive and, although it is essential for the conservation of biodiversity, it is important to monitor and properly manage these areas, as they present several cases of biological invasions. The Lençóis Maranhenses constitute the peculiar delta of the Americas and are under the consequences of the bioinvasion of tilapias and peacock bass. Collections were carried out in the Lençóis Maranhenses National Park from March/2016 to November/2020, with the aid of gill nets and cast nets. The species were identified with the help of specialized literature and a historical comparison with previous works was carried out. Cytochrome oxidase subunit I was sequenced to confirm identification of non-native species. We recorded the expansion of the occurrence of *Oreochromis niloticus*, and the first record of the species *Oreochromis mossambicus* and *Cichla monoculus.* A total of 31 species belonging to eight orders, eighteen families and twenty-nine genera were identified, indicating a lag in the diversity of species found in relation to previous studies. After 20 years of the first record of invasive fish, there is an expansion of bioinvasion and new cases that indicate a lack of monitoring and containment measures for the species, indicating the fragility of conservation in the area

Keywords: National Park; Cichlidae; Neotropical freshwater fish; Species richness.

Resumo

O aumento do número de áreas protegidas brasileiras tem sido progressivo e, embora seja essencial para a conservação da biodiversidade, é importante o monitoramento e o manejo adequado dessas áreas, já que apresentam diversos casos de invasões biológicas. Os Lençóis Maranhenses constituem o peculiar delta das Américas e estão sob as consequências da bioinvasão de tilápias e tucunarés. Foram realizadas coletas no Parque Nacional dos Lençóis Maranhenses no período de março/2016 a novembro/2020, com o auxílio de redes de emalhe e tarrafas. As especies foram identificadas com o auxilio de literatura especializada e uma comparacao historica com trabalhos anteriores foi realizada. O Citocromo oxidase subunidade I foi sequenciado para confirmar a identificação das espécies não nativas. Registramos a expansão da ocorrência de *Oreochromis niloticus*, e o primeiro registro das espécies *Oreochromis mossambicus* e *Cichla monoculus*. Um total de 31 espécies pertencentes a oito ordens, dezoito famílias e vinte e nove gêneros foram identificadas, indicando uma defasagem na diversidade de espécies encontradas em relação a estudos anteriores. Após 20 anos do primeiro registro de peixes invasores, constata-se a expansão da bioinvasão e novos casos que assinalam ausência de monitoramento e de medidas de contenção para as espécies indicando a fragilidade na conservação da área

Palavras-chave: Parque Nacional; Cichlidae; Peixes neotropicais, Riqueza de espécies

Introduction

Maintaining ecosystem services is the global strategic plan for biodiversity until 2050 (Convention on Biological Diversity, 2019). And to achieve this goal, indirect and direct factors of biodiversity loss and its consequences for humanity must be considered, in addition to the concern with the effectiveness of protected areas, which are essential for the maintenance of species, populations and ecosystems (UNEP- WCMC and IUCN, 2018).

However, the global panorama shows that the fulfillment of these objectives is still far from what was proposed (Azevedo-Santos et al. 2019; Mormul et al. 2022). In Brazil, despite advances related to the creation of protected areas, preventing biological invasion has become a main challenge, and around $104.33 billion was spent due to damage and loss caused by trespassers in the period 1984 to 2019 (Adelino et al. 2021). Biological invasion is known to cause the loss of 50% of the world's native fish species, and 11 non-native fish species are registered in the protected areas of the Brazilian federation (Sampaio and Schimidt, 2013).

As in the whole globe, several fish species have been introduced in Brazil (Frehse et al. 2016, Ziller et al. 2020, Bueno et al. 2021), such as African Cichlids (Forneck et al. 2016), which are among the most cultivated species in aquaculture and active predators that have been translocated by sport fishing (Fugi et al. 2008) in the country. Among these cichlids, we highlight the representatives of the genera: *Cichla* (Schneider, 1801), endemic to the Amazon region with 15 described species (Kullander and Ferreira, 2006) and *Oreochromis* Gunther with 32 native African species.

In this context, we highlight the Lençóis Maranhenses National Park, created by decree No. 86060 (June 2, 1981), in the Lençóis region with an area of 155,000 hectares (Ministry of the

Environment, 2003). The Management Plan of PNLM presents data on freshwater fish fauna, such as the list of species. However, these data refer to studies conducted in the year 1999 reporting the occurrence of *Oreochromis* sp. as a non-native species (Ministry of the Environment, 2003). Additionally, Brito et al. (2019) increased the occurrence of other native species and reinforced the occurrence of *Oreochromis* sp. in the region.

Therefore, this paper provides a current list of freshwater fish species in the Lençóis Maranhenses National Park, as well as the first record of *Cichla monoculus* and *Oreochromis mossambicus*, adding the spatial expansion of the occurrence of *Oreochromis niloticus*. In addition to a historical comparison with data on richness and spatial dynamics, contributing to updating the record of native and alien species and subsidizing actions for the management and conservation of species in the region.

Methods

Study area

The state of Maranhão, located on the North Equatorial Coast of Brazil, corresponds to an ecotone between the Amazon, Cerrado and Caatinga biomes. It houses the Lençóis Maranhenses National Park (PNLM), an international tourist destination peculiar to the delta of the Americas, crucial for global biodiversity (Figure 1).

The region has a megathermal climate, very hot and ranging from humid to sub-humid, with annual precipitation of 1,600.00 to 1,800.00 mm and temperatures ranging from 26 C to 38 C (Ministry of Environment, 2003)

The Maranhão sheets are made up of areas of free and fixed dunes, in addition to being a mosaic of ecosystems such as mangroves, cerrado and restinga (Ministry of the Enviroment, 2003). Several water bodies are present in the park, rivers, creeks, streams, lakes, ponds, lagoons, supplied by the Periá and Preguiças rivers (Silva, 2008)

With an area of 155,000 ha, located between the municipalities of Primeira Cruz and Barreirinhas (Brazilian Institute for the Environment, 1989), the Lençóis Maranhenses National Park moves around 150,000 tourists per year (Chico Mendes Institute for Biodiversity Conservation, 2020) and presents conflicts such as artisanal fishing versus industrial fishing, mangrove cutting, extractivism, hunting, rally, irregular occupation and public use activities as bathing, camping, hiking, boat trips, surfing and windsurfing (Ministry of the Environment, 2003).

The limnological characteristics were obtained from the park management plan (Ministry of the Environment, 2003)

Sampling

All collections for this study were done with authorization from the Brazilian Institute of the Environment and Non-Renewable Natural Resources (SISBIO-Number 53224-1). The collections were taken from March/2016, March and July/2017, July/2019 and November/2020 in twenty sampled points among rivers, creek, lakes, ponds and lagoons (Figure 2), in the four campaigns distributed in dry and rainy seasons (Supplementary data)

Figure 2. Heterogeneous sampled points in Lençóis Maranhenses. A and B= Pond in Paulino Neves, sandy substrate; C= Tutóia, Delta das Américas, sandy substrate; D= Lago de Santo Amaro, muddy substrate; E= Prainha do amor, Barreirinhas, muddy substrate; F= Tamacão Creek, Tutóia, muddy substrate

Captures were developed through passive fishing using gillnets with spacing of 10 and 20 mm between nodes, during the day, twilight and night, with an average permanence time of 4 hours and review every two hours. It was then followed by active fishing using gear such as trawl nets with spacing between opposing nodes of 1.5mm, cast nets with 50mm between nodes and sieves with an average effort of 15 minutes per gear, adapted from Magnusson et al. (2005)

The identifications were carried out from the specialized literature for each group: Fowler (1954); Mago-Leccia (1994), Reis et al., (1997), Chernoff & Machado-Allison (2005), Piorski et al., (2017) and taxonomic review articles. The vouchers of identified fishes were deposited in the Tissue and DNA Collection of Maranhenses Fauna (CoFauMA) of the State University of Maranhão, Brazil. The identification of specimens obtained in this work followed the classification of Fricke, Eschmeyer and Van der Laan (2022).

Data analysis

For historical verification of diversity, we accessed published data from the work carried out in the 2000s for the Management Plan of the Lençóis Maranhenses National Park (Ministério do Meio Ambiente, 2003) and from the work carried out in 2017 (Brito et al. 2019). Considering the same sampling points, we noted the collection methodologies in order to compare the species richness, as well as the absence/presence of native and non-native species recorded over the last 20 years.

Molecular procedures

For non-native species, total genomic DNA was extracted from individuals by the salting out method based on proteinase K digestion, followed by sodium chloride extraction and ethanol precipitation (Aljanabi and Martinez, 1997). A fragment (635 bp) of the mitochondrial cytochrome oxidase I subunit (COI) locus was amplified from six specimens (MW694823-MW694824 and MW692108 to MW692111)

The fragment was amplified using two pairs of universal primers FishF1 and FishR1, described by Ward et al. (2005). For amplification, 0.4 µl of DNA, 0.1 µl of each primer, 1.0 µl of buffer

(10X), 0.4 µl of MgCl2 (50 nM), 1.6 µl of dNTP, 0 were used in each sample., 1 µl of Taq DNA polymerase and 6.3 µl of ultrapure water to complete the reaction.

The PCR reaction followed an initial denaturation at 94°C for 5 min followed by 35 cycles of 1 min of denaturation at 94°C, 30s of hybridization at 56°C and 1 min of extension at 72°C, in addition to a final extension of 7 min at 72ºC. Amplified fragments were purified using the Wizad/Promega Purification Kit following the manufacturer's protocol and recommendations. The samples sequencing by the company ACTGene Analytical Moleculars Ltda. (Biotechnology Center, UFRGS, Porto Alegre, RS) using the automatic sequencer ABI-PRISM 3100 Genetic Analyzer armed with 50 cm capillaries.

COI sequences were visually checked and manually corrected. The statistical method chosen for the phylogenetic analyzes and tree assembly was neighbor-joining (K2P) which is recommended as a standard methodology. Additionally, the identification tool available in the Barcode of Life Data System was used, considering as an identification criterion, the similarity above 98% within the same species.

Results

In this work, we obtained a total of 1010 specimens collected in the Lençóis Maranhenses National Park, distributed in 08 orders, 18 families, 29 genera and 31 species (Supplementary data)

The most representative Orders were Characiformes (n=12), Cichliformes (n=8) and Siluriformes (n=5), followed by the orders Clupeiformes, Mugiliformes, Pleuronectiformes and Synbranchiformes, Gymnotiformes (n=1).

In our records, the most abundant species were *Oreochromis niloticus* (Linnaeus, 1758) (n= 148), *Bryconops* sp. and *Astyanax* sp. (n= 139). We also highlight that 16.4% of the total captured correspond to the occurrence of non-native species: *Oreochromis niloticus, Oreochromis mossambicus* (Peters, 1852) and *Cichla monoculus* Spix & Agassiz 1831 (Figure 3)

Figure 3. Non-native specimens collected in Lençóis Maranhenses National Park: A= *Oreochromis niloticus*; B= *Oreochromis mossambicus*; C= *Cichla monoculus*

The identification of these taxa was confirmed by the similarity of the COI gene sequences with the data available in GenBank. The COI gene cluster for the species found, as proposed by Herbert et al. (2003), presents a similarity superior to 98% (Figure 4).

Figure 4. Phenogram generated by the MEGA X program for the COI gene sequences, by the neighbor joining method with 1000 replicates to support the clusters, demonstrating the identification of *Cichla monoculus, Oreochromis niloticus* and *Oreochromis mossambicus*

We also observed that in the Lençóis Maranhenses National Park *Oreochromis* spp. was found in 94.11% of the sampled points and in all campaigns.

As for the record of *Cichla monoculus*, it initially occurred in a lotic environment of the Preguiça River near the mouth in 2016, however, the occurrence in lentic environments was also recorded in the following years (Supplementary data)

In our comparison of the distribution and occurrence of invasive species in two distinct moments: the occasion of the elaboration of the management plan, in 1999 and the collections carried out in this work, we verified the spatial expansion of *Oreochromis niloticus* in the Park and in the adjacent areas (Figure 5)

Figure 5. Distribution of allochthonous species A= *Oreochromis* spp. and B= *Cichla* spp. collected in Lençóis Maranhenses National Park recorded in 1999 and in this work

In addition to the increase in the number of non-native species, we found a gap in native fish species in Lençóis Maranhenses, as can be seen in the comparison of species captured in the work of the Ministry of the Environment (2003) and Brito et al. (2019) (Figure 6)

Figure 6. Graph of the historical comparison of the species registrated of freshwater fish collected in the Maranhão wetlands by the authors Ministry of Environment (2003), Brito et al. (2019) and this work

According to the Ministry of the Environment (2003), in six expeditions in 2000, carried out preferably during the day with trawls and sieves, 43 species of fish were captured, with the first record of Oreochromis sp. While in the work by Brito et al. (2019) 49 species were collected with two trawl nets (20 m long, 2.5 m high, 10 mm mesh; and 4 m long, 2 m high, 5 mm mesh), cast nets (2 m high, 15 mm mesh), gill nets of various mesh sizes (15, 25, 35, 45 and 55 mm) and dip nets (5 and 10 mm mesh) in three expeditions, also indicating the occurrence of *Oreochromis* sp.

Five families registered in 2000, with the representatives *Awaous tajasica* (Lichtenstein 1822), *Polydactylus virginicus* (Linnaeus 1758), *Lycengraulis batesii* (Günther 1868), *Mugil curema* Valenciennes, 1836 e *Eucinostomus argenteus* Baird & Girard 1855, have a marine habit and were not found in this survey.

Other species such as: *Brachychalcinus parnaibae* Reis, 1989, *Hyphessobrycon piorskii* Guimarães, Brito, Feitosa, Carvalho-Costa & Ottoni 2018, *Hemigrammus* spp., *Serrapinus* sp., *Steindachnerina notonota* (Miranda Ribeiro, 1937), *Aequidens tetramerus* (Heckel, 1840), *Poecilia vivipara* Bloch & Schneider, 1801, *Poecilia sarrafae* Bragança & Costa, 2011, *Melanorivulus* cf. *parnaibensis* , *Anablepsoides vieirai* Nielsen 2016, *Apteronotus albifrons* (Linnaeus, 1766), *Brachyhypopomus* sp., *Megalechis thoracata* (Valenciennes,1840) and *Callichthys callichthys* (Linnaeus, 1758) were not recorded in our work.

On the other hand, the Cichliformes had an increase in representativeness, due to the capture of representatives such as *Oreochromis mossambicus* and *Cichla monoculus*.

Discussion

The representativeness pattern of the taxa recorded in this study, despite being consistent with what was expected for semi-arid areas of the Neotropical region (Reis et al. 2016) and similar to the patterns of the next ones: Parnaíba, Itapecuru and Tocantins (Melo et al. 2016; Barbosa et al. al. 2017; Ramos et al. 2016), reveals the presence of three non-native species: *Oreochromis niloticus, Oreochromis mossambicus* and *Cichla monoculus.*

Oreochromis niloticus was introduced in Brazil in 1953 in lakes in the Northeast region (Oliveira et al. 2007, Leão et al. 2011) and *Oreochromis mossambicus,* the red tilapia, a modified strain was introduced in 1981 (Oliveira et al. 2007). In 1940, *Cichla monoculus*, *Cichla kelberi* Kullander & Ferreira 2006, *Cichla piquiti* Kullander & Ferreira 2006 and *Cichla temensis* Humboldt 1821 were introduced to control invasive species and sport fishing in the northeast region with the first record by Peixoto (1954).

The introduction of tilapia in the PNLM was recorded in 1999, at the time of the rapid ecological assessment for the management plan, while peacock bass is probably recent, as there is no record in surveys of the local ichthyofauna (Ministry of the Environment, 2003; Brito et al. 2019).

In the park region, the main factors for the occurrence and expansion of these species were fish farming and sport fishing. And the success of these introductions is due to the increase and intensity of human activity acting as propagation pressure for these bioinvasions, corroborating with other cases cited by Lima Jr et al. (2018), Latini et al. (2016) and Magalhães et al. (2017). The high propagation pressures are attributed to the size of the propagule in the case of tilapia and the frequency in the case of peacock bass, making the environment more unstable and susceptible to new invasions (Ricciardi et al. 2007)

Due to the ecological characteristics of the environments in the Lençóis Maranhenses region, such as the presence of lakes, rivers and small bodies of water (Ministry of the Environment, 2003), the occurrence of these species is favored and, associated with phenotypic, feeding and reproductive plasticity, culminates in the establishment of these organisms, such as Kovalenko et al. (2010) and Diamante et al. (2017) report for other regions.

In our temporal analysis, we verified a variation in the number of native and non-native species recorded. An increase in non-native species and a reduction in the occurrence of native species. Like species that were cited in historical data (Brito, et al. 2019, MMA, 2003) *Hemigrammus* spp. and *Hyphessobrycon* spp. and were not recorded in our expeditions. Both have opportunistic habits, being omnivores with a preference for insects, microcrustaceans and filamentous algae (Barreto et al. 2018), a factor that may be associated with competition with *O. niloticus*.

A scenario similar to that reported by Attayde et al. (2007), in which Nile tilapia causes a reduction in the abundance of certain planktonic microcrustaceans, an increase in the biomass of nanoplanktonic algae and a reduction in water transparency. These effects of Nile tilapia can negatively affect the recruitment of other fish species that feed essentially on zooplankton in the juvenile stage and are visually oriented to locate and capture their prey (Attayde et al. 2007). Furthermore, the presence of tucunaré may be associated with the non-registration of these species, since this invader hunts and devours whole prey (Ellis et al. 2018, Sales et al. 2018, Barjer et al. 2019, Santos et al. 2019). Also considering that most of the species not recorded in this study are small Characids and Cyprinids, corroborating Pelicice and Agostinho (2009) when they found a reduction in small fish, mainly Characiformes, such as *Hemigrammus* spp. and *Hyphessobrycon* spp. in environments invaded by peacock bass.

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negatively affect the recruitment of other fish species that feed essentially on zooplankton in the juvenile stage and are visually oriented to locate and capture their prey (Attayde et al. 2007). Another bias to be considered is the impact of climate change such as the implications of increased temperature and its effect on reducing the oxygen content of aquatic systems (Jenny et al. 2016, Blaszczak et al. 2019), compromising the species most sensitive to abiotic changes. The climate is also associated with the increase, loss or changes in areas suitable for various species of fish, being a factor that can directly interfere with the maintenance of aquatic biota (Heino et al., 2009) impacting the increase in organic matter and pollution (MacNeil et al. 2004) can generate differential effects in bioinvasions (Dickey et al. 2021).

Additionally, one should take into account the growing impact of tourism in the region, which in 2022 was visited by 367,000 people (Ministry of the Enviroiment, 2023), the construction of roads and facilitation of access contributes to transport of species and increased impact on the local ichthyofauna due to effects in synergy with the aspects mentioned above due to the complexity of the study in this scenario.

Given the potential and environmental dynamics of Lençóis Maranhenses, it is necessary to establish monitoring of populations of *Oreochromis niloticus, O. mossambicus* and *Cichla monoculus* and together with native species, mainly associated with the endemicities of *Anablepsoides vieirai* Nielsen 2016, *Apistogramma piauienses* Kullander 1980, *Hyphessobrycon worstskii* Guimarães, Brito, Feitosa, Carvalho-Costa and Ottoni 2018, *Hypostomus johnii* (Steindachner, 1877), *Poecilia sarrafae* Bragança e Costa 2011 and *Pimelodella parnahybae* Fowler, 1941 registered by Brito et al. (2019) in the region.

Considering the management plan as a tool to protect the protected area, the information has not been sufficient to contain the advance of non-native species (Monroe et al. 2021). This fact contributes to intensifying the impacts on the park's biodiversity, which, although it stands out for having more severe restrictions and controls, should bring greater visibility to policies and practices in this protected area (Pressey et al. 2015).

Mainly in a territory highlighted by areas of endemism and biodiversity hotspots, with approximately 20% of its ichthyofauna endemic (Abell et al. 2008), it is necessary to advance in relation to the maintenance of this diversity, given the lack of studies, with data considered underestimated for the area by some authors (Dagosta and de Pinna, 2017; Brito et al. 2019). We also indicate environmental education actions for the local and tourist population, in order to sensitize social actors to the consequences of these invasions. In addition to constant vigilance to avoid the release and exchange of species between the park and adjacent areas, considering that Brazilian legislation establishes the need for a routine of eradication, containment, control and monitoring of invasions

Acknowledgments

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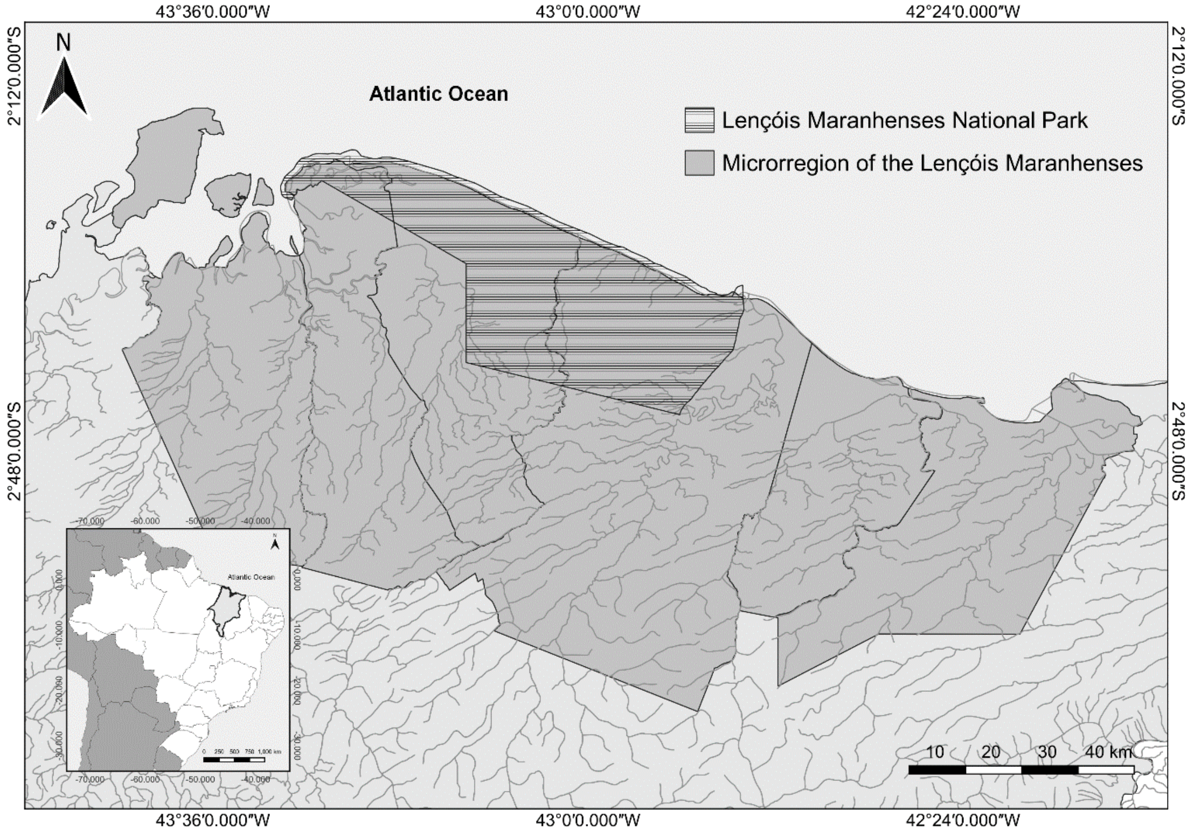
Figure 1. Map of the location of the Lençóis Maranhenses National Park and region on the equatorial coast of Brazil



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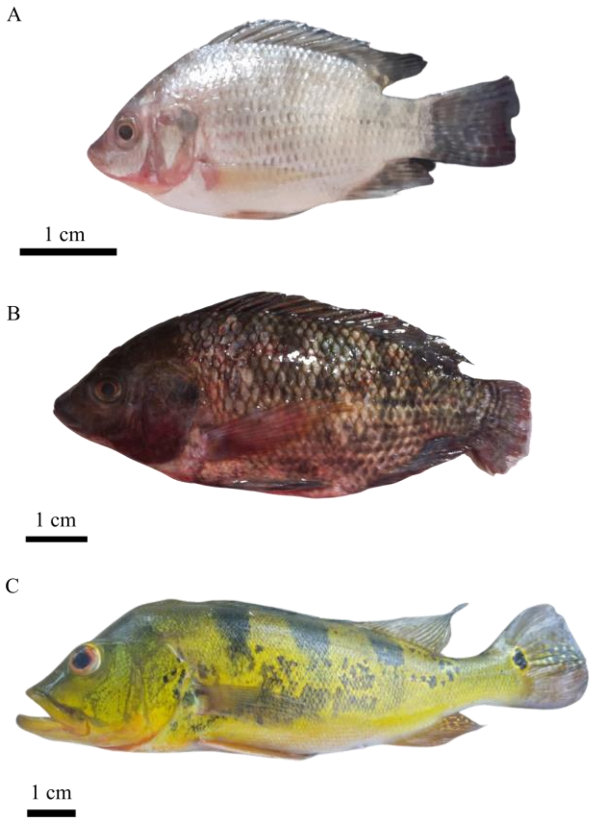

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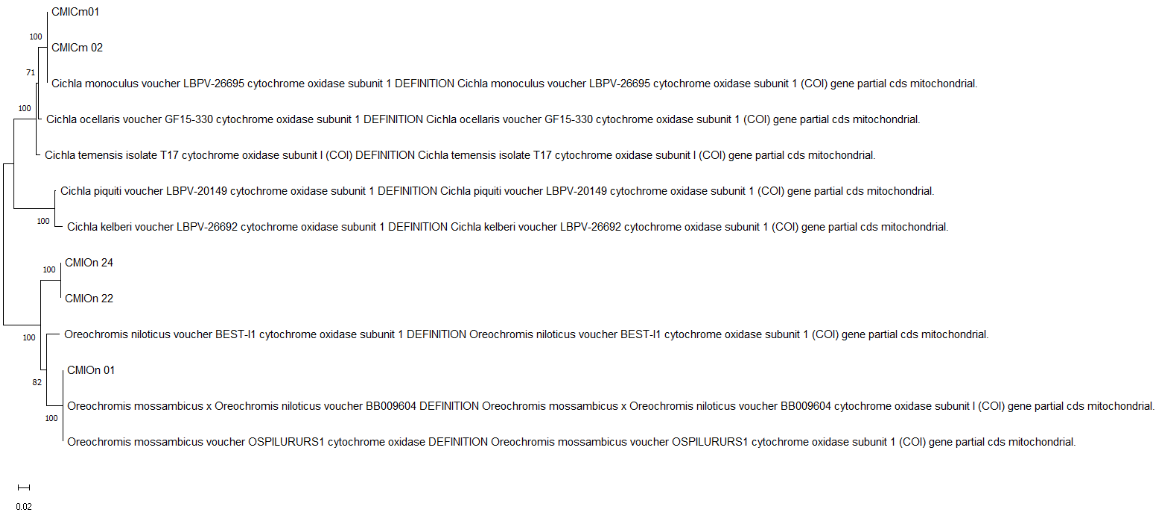

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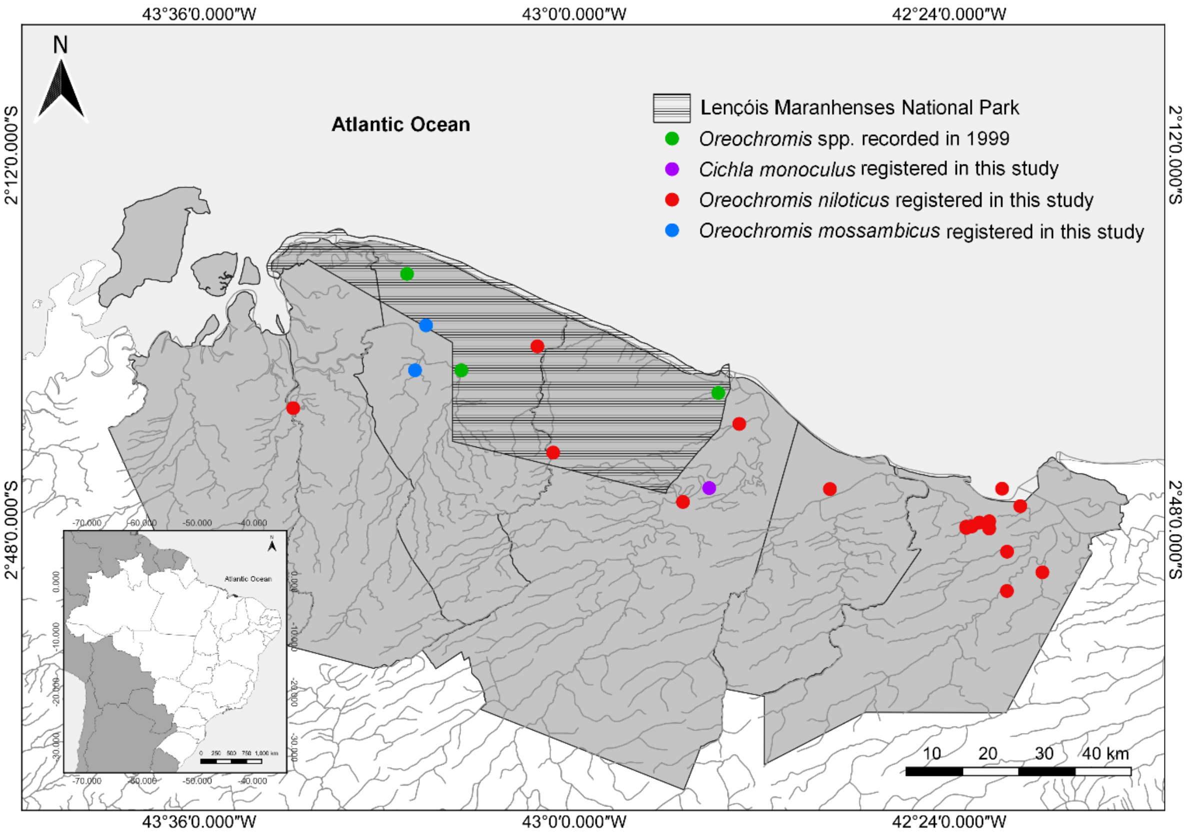

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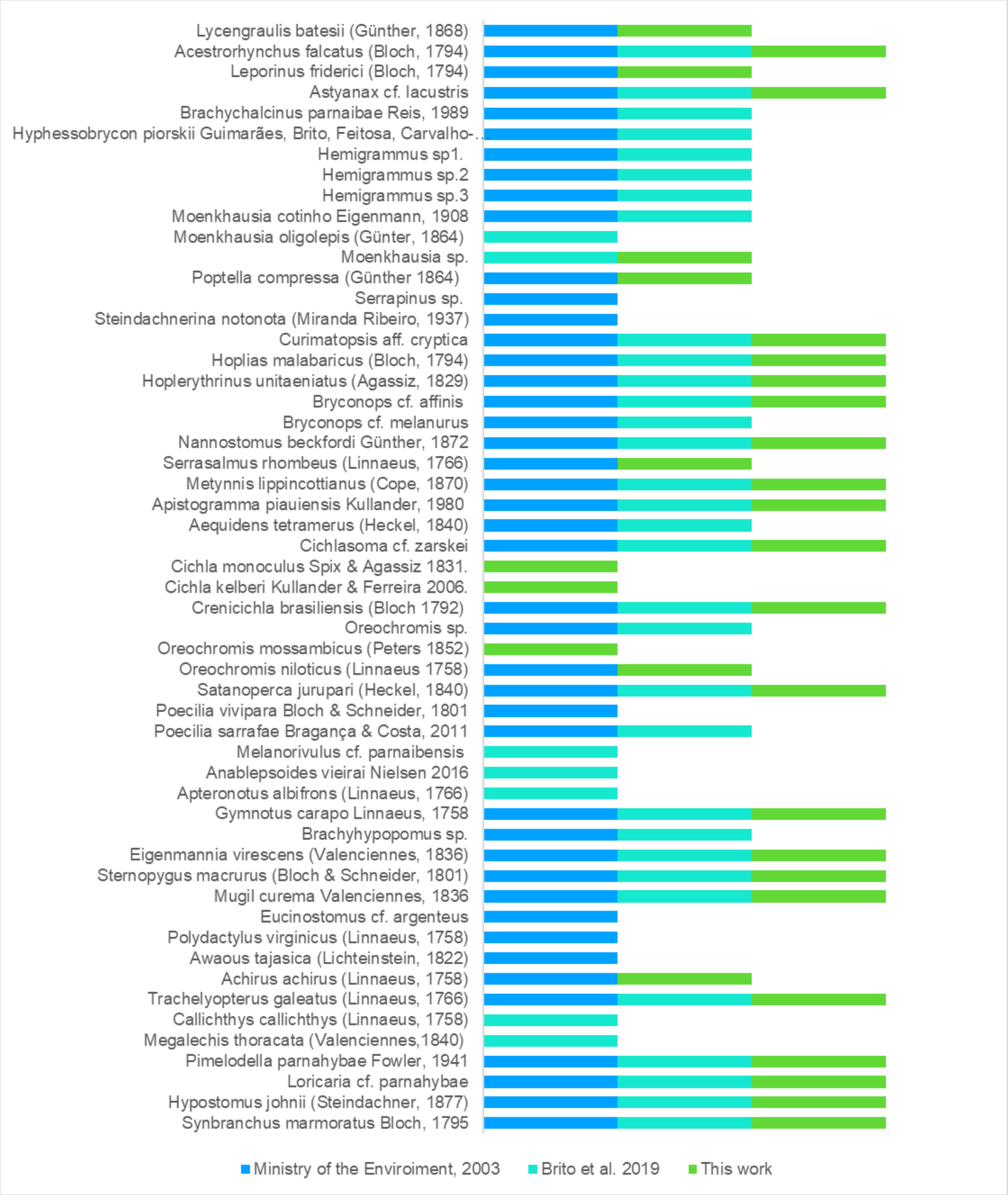

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Authors’ Contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Thércia Goncalves Ribeiro Monroe, Selma Patrícia Diniz Cantanhêde, Nivia Sandiele de Melo Sousa, Natanael Bezerra Monroe and Lígia Tchaicka. The first draft of the manuscript was written by Thércia Goncalves Ribeiro Monroe and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors have no conflicts of interest to declare that are relevant to the content of this article

Ethics and Permits

All research pertaining to this article did not require any research permit(s).

Sequence data that support the findings of this study have been deposited in GenBank with the MW694823-MW694824 and MW692108-MW692111 accession codes

4 CAPÍTULO II

Ichthyofauna composition and bioinvasion in the Baixada Maranhense environmental protection area, a Ramsar site in Brazil

Scientia Plena (A4)

Status: Submetido

Ichthyofauna composition and bioinvasion in the Baixada maranhense environmental protection area, a ramsar site in Brazil

Diversidade e estrutura em área protegida invadida por ciclídeos

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The Baixada Maranhense is considered a wetland for the conservation of biodiversity and an economically important region for fishing activities, with a set of floodable fields supplied by the Pindaré and Mearim rivers, which are home to a diversity of fish essential to the region's population. In this context, we describe the richness and diversity of the ichthyofauna of five lakes in the Baixada Maranhão environmental protection area, in addition to recording non-native species. The collections were carried out in 2014 and 2015, with the aid of gill nets measuring 20,30,40,50,60 and 80 mm between opposite nodes and the specimens were identified based on specialized literature. We characterize diversity based on Hill numbers calculated in the R program (4.1.3). Specimens belonging to fifty fish species distributed across ten orders, twenty-six families and fifty genera were captured. Of these, four non-native species were identified: *Cichla kelberi*, *Cichla monoculus*, *Oreochromis mossambicus* and *Oreochromis niloticus*. The least diverse lakes were the smallest, with the greatest fishing pressure and which showed a dominance of non-native species.
Keywords: Diversity; Wetland; Freshwater fish; Bioinvasion

A Baixada Maranhense é considerada uma zona úmida para a conservação da biodiversidade e uma região importante economicamente na atividade pesqueira, com um conjunto de campos alagáveis abastecidos pelos rios Pindaré e Mearim, dentre outros, que abrigam uma diversidade de peixes essenciais para a população da região. Nesse contexto, nós descrevemos a riqueza e diversidade da ictiofauna de cinco lagos na área de proteção ambiental da Baixada maranhense, além de registrar espécies não nativas. As coletas foram realizadas nos anos de 2014 e 2015, com auxílio de redes de emalhe de 20,30,40,50,60 e 80 mm entre nós opostos e os espécimes foram identificados com base em literatura especializada. Caracterizamos a diversidade com base nos números de Hill calculados no programa R (4.1.3). Foram capturadas espécimes pertencentes a cinquenta espécies ícticas distribuídas em dez ordens, vinte e seis famílias e cinquenta gêneros. Destas, quatro espécies não nativas foram identificadas: *Cichla kelberi, Cichla monoculus, Oreochromis mossambicus* e *Oreochromis niloticus*. Os lagos menos diversos foram os menos extensos, com maior pressão de pesca e que apresentaram dominância de espécies não nativas.
Palavras-chave: Diversidade; Zona úmida; Peixes dulcícolas; Bioinvasão

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1. INTRODUCTION

Anthropogenic actions have been reported as one of the main factors impacting biodiversity and ecosystem balance [1,2,3], and concern about the exploitation and depletion of natural resources is at the core of conservation efforts [4,5,6]. Aquatic ecosystems serve as biological models for population studies, and the fragility of these environments [7], in conjunction with a anthropogenic impacts, underscores the importance of developing studies focused on ichthyological biodiversity [8].

The neotropical region stands out for its diversity, owing to its history of biogeographic processes. Currently, there are approximately 5160 described fish species in the South American continent [9]. The Amazon basin particularly distinguishes itself in this context [10], housing around 2,700 species, representing approximately 15% of all freshwater species [11,12]. However, the relationship between existing diversity and knowledge of species distribution and occurrence in Brazilian basins is tenuous and disproportionate, this is linked to the lack of taxonomic studies at the national, regional, and local levels [13,14,15,16].

The Baixada Maranhense, located in the Eastern Amazon, is a Ramsar site with highly threatened conservation targets (State Decree No. 11,900), It is known for its water and fishery potential, where the balance between sustainability and the conservation of fish species has been a constant challenge [17]. The region is supplied with a set of lakes bathed by the Pindaré and Mearim rivers in a cycle of floods that connect them, in addition its flooded fields witness common practices such as rice cultivation, buffalo farming, and fish farming [18,19]

Human actions have been reported as one of the main factors impacting biodiversity and ecosystem balance [1, 2, 3], and concern about the exploitation and depletion of natural resources is at the core of conservation efforts [4,5,6].

Aquatic ecosystems are biological models used for population studies, and the fragility of these environments [7], related with a lack of knowledge about existing diversity and anthropogenic impacts, underscores the importance of developing studies focused on ichthyic biodiversity [5].

Considering that these flood and drought dynamics are the main ecological driving force shaping evolutionary and adaptive processes in the Amazon [20,21], it is crucial to understand the impacts and processes occurring in the region.

Thus, this study identified freshwater fish species in five lakes within the Pindaré-Mearim lacustrine system, examining their composition, diversity, and distribution in relation to environmental factors. It also documented the occurrence of non-native species of the *Cichla* and *Oreochromis* genera, providing information for an internationally significant protected area.

2. MATERIALS AND METHODS

2.1 Study area

This study was carried out in five lakes, in the three zones of the lake system formed by the rivers Pindaré and Mearim, located in the State of Maranhão, Eastern Amazon. In the pre-confluence zone (lakes Cajari and Viana), confluence zone (lake Aquiri) and post confluence zone (lakes Itans and Coqueiro), according figure 1

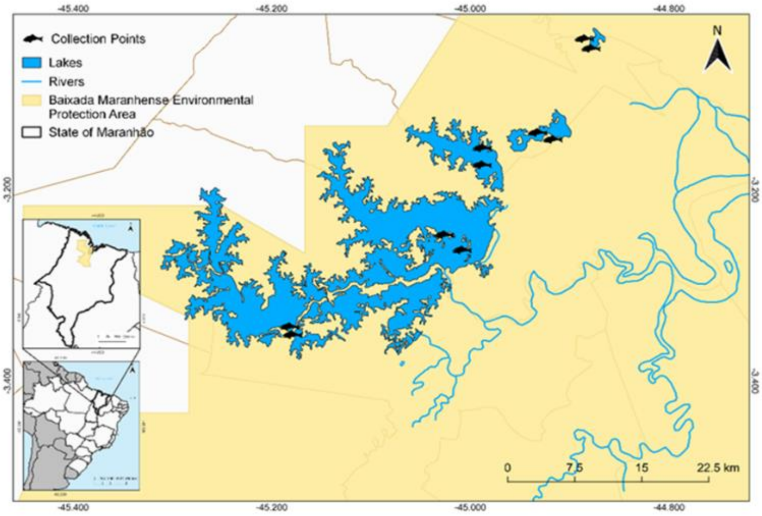

Figure 3. 1 Map of the location of collection points for the lakes sampled in the Baixada *Maranhense wetland.*

The dynamics of the natural fields of the Baixada Maranhense, according to Costa Neto [22], involve two cycles of energy exchange that cover two different periods, according to the season of the year. During the rainy season (January to June), the rivers and perennial lakes overflow, flooding the grasslands and transforming them into extensive shallow lakes. And during the dry season (July to December), the fields remain dry, which promotes the growth of vegetation consisting mainly of Gramineae and Cyperaceae [23].

The lakes Cajari and Viana are located at the pre-confluence of the Pindaré and Mearim rivers, according to Franco [18], the lacustrine indentation of Cajari covers an area of 283.1 km², this considerable size allows large and deep lakes to stand out, such as Lake Cajari [24]. The region of this lake receives the two most important pulses of surface flooding, originating from the lacustrine systems of the Monção region and the tributary rivers of Lake Formoso.

As for Lake Viana, it has an extension of 255.2 km² and is formed by floodplain lakes through the flooding of the waters of the Pindaré River, a tributary on the left bank of the Mearim River [18,19]. The lacustrine region of Viana is composed of the lakes Maracu, Viana, and Maracassumé, without defined divisions by geographic accidents. During the rainy season, they form a single liquid mass, but in droughts, the divisions become apparent.

The region of this lake receives the two most important surface flood pulses, the lake systems of the Monção region and the tributary rivers of Lake Formoso. The Viana lake is 255.2km² in length and is formed by floodplain lakes, through the flooding of the waters of the Pindaré river, a tributary of the left bank of the Mearim river [18,19]. The lake region of Viana is formed by the Maracu, Viana and Maracassumé lakes, without divisions defined by geographical features. During the rainy season they form a single liquid mass, but during droughts the divisions become apparent.

The Aquiri lake recess is located in the Pindaré-Mearim post-confluence zone, interfering with the estuarine area of the Mearim river (approximately a straight distance of 18 km) [18]. The recess of Lake Coqueiro has contiguous plains and forms an immense mouth through which flood pulses are conducted. Lake Itans is part of a set of lakes juxtaposed between the floodplains of the Belém and Coqueiro recesses and the large floodplain of the Mearim river, functioning as a distribution axis, releasing the water surplus to other recesses [18].

2.2 Sampling and identification

The monthly collections were carried out from August 2014 to June 2015, in Lakes Cajari (pre-confluence), Aquiri and Viana (confluence), Coqueiro and Itans (post-confluence) of the Pindaré-Mearim lake system. For each lake, lentic and lotic collection points were plotted, obtaining a sample number of 4,956 specimens, under license No. 32643 SISBIO.

At each sampling point, abiotic parameters such as water temperature (TºC), precipitation, pH, and Dissolved Oxygen (mg/I) were measured. Nets of different lengths and meshes were used in bodies of lentic water, for example, nets 100 to 150 m long with meshes between opposite nodes varying from 2 cm to 5 cm. In lotic environments, 50m meshes with meshes between opposite nodes varying from 2 cm to 5 cm. In general, the meshes remained in the water for a period of 4 to 8 hours, between twilight periods in order to sample nocturnal and diurnal fauna.

For taxonomic identification, specialized bibliography was used for each group. The ichthyological material was deposited in the Maranhão Fauna DNA and Tissue Collection (CoFauMA) at the State University of Maranhão. The taxonomic classification, name, author, year of publication, validity, distribution of each species was checked in Van der Laan, Fricke and Eschmeyer [26].

2.3 Species composition

To characterize the species diversity of the lakes studied, we used three integrated rarefaction/extrapolation curves based on the first three Hill numbers (matrix ii). Hill numbers are a mathematically unified family of diversity indices (differing only by one exponent, q) that incorporate relative abundance and species richness [27]. This method is based on the multinomial probability distribution of Hill numbers: species richness ($q = 0$), exponential Shannon entropy (which we refer to as Shannon diversity, $q = 1$), and inverse Simpson concentration (a which we refer to as Simpson diversity, $q = 2$). Shannon diversity weights species in proportion to their frequency, whereas Simpson diversity places more weight on the frequencies of abundant species and discounts rare species.

We estimated species richness and diversity at each site using rarefaction curves and extrapolation of Hill numbers to incidence data, according to the procedures and functions proposed by Chao et al. [27] and using the "iNEXT.Sam" and "plot.iNEXT" functions from the iNEXT package version 2.0.9 [28] of R 4.3.1 [29]. This analysis, based on incidence data, allows the systematic comparison of different sets in time or space, given the dardization of the sample size [27]. The data were plotted with a 95% confidence interval obtained using the bootstrap method, to ensure the robustness of comparisons between different samples [27].

3. RESULTS

Fifty-four (54) species of fish were identified, distributed in ten (10) orders (Myliobatiformes, Elopiformes, Clupeiformes, Characiformes, Gymnotiformes, Siluriformes, Synbranchiformes, Carangiformes, Cichliformes, Achanturiformes), twenty-six (26) families and forty and nine (49) genera, according to Van der Laan, Fricke and Eschmeyer [26]. (Table 1).

Table 3. 1 Table of fish species collected in the environmental protection area of the Maranhão, to the classification of Van der Laan, Fricke and Eschmeyer (2023)

Myliobatiformes	Potamotrygonidae	*Potamotrygon motoro* (Müller & Henle 1841)
Elopiformes	Megalopidae	*Megalops atlanticus* Valenciennes 1847
Clupeiformes	Engraulidae	*Pterengraulis atherinoides* (Linnaeus 1766)
		Cetengraulis edentulus (Cuvier 1829)
		Lycengraulis batesii (Günther 1868)
Characiformes	Erythrinidae	*Hoplerythrinus unitaeniatus* (Spix & Agassiz 1829).
		Hoplias malabaricus (Bloch 1794)
	Cynodontidae	*Cynodon gibbus* (Spix & Agassiz 1829)
	Serrasalmidae	*Metynnis lippincottianus* (Cope 1870)
		Pygocentrus nattereri Kner 1858
		Serrasalmus rhombeus (Linnaeus 1766)
	Hemiodontidae	*Hemiodus parnaguae* Eigenmann & Henn 1916
	Anostomidae	*Leporinus friderici* (Bloch 1794)
		Schizodon dissimilis (Garman 1890)
	Curimatidae	*Curimata macrops* Eigenmann & Eigenmann 1889.
		Psectrogaster rhomboides (Eigenmann & Eigenmann, 1889)
	Prochilodontidae	*Prochilodus lacustris* Steindachner 1907

		Prochilodus nigricans Spix & Agassiz 1829.
	Thriportheidae	*Triportheus signatus* (Garman 1890)
	Acestrorhynchidae	*Acestrorhynchus lacustris* (Lütken, 1875)
	Characidae	*Roeboides margareteae* Lucena 2003
Gymnotiformes	Sternopygidae	*Sternopygus macrurus* (Bloch & Schneider 1801)
	Gymnotidae	*Gymnotus carapo* Linnaeus 1758
	Rhamphichthyidae	*Rhamphichthys atlanticus* Triques 1999
Siluriformes	Callichthyidae	*Callichthys callichthys* (Linnaeus 1758)
		Hoplosternum littorale (Hancock 1828)
	Loricariidae	*Loricaria cataphracta* Linnaeus 1758
		Loricariichthys sp.
		Pterygoplichthys parnaibae (Weber, 1991)
		Rineloricaria sp.
	Auchenipteridae	*Ageneiosus ucayalensis* Castelnau 1855
		Auchenipterus menezesi Ferraris & Vari 1999
		Hypostomus cf. *plecostomus*
		Trachelyopterus galeatus (Linnaeus 1766)
	Doradidae	*Hassar affinis* Steidachner, 1881
		Platydoras brachylecis Piorski, Garavello, Arce H. & Sabaj Pérez, 2008
	Heptapteridae	*Pimelodella parnahybae* Fowler, 1941
		Pimelodella cristata (Muller & Troschel, 1849)
	Pimelodidae	*Hemisorubim platyrhynchos* (Valenciennes 1840)
		Pimelodus blochii Valenciennes 1840
		Pimelodus ornatus Kner 1858
		Pseudoplatystoma punctifer (Castelnau 1855)
		Sorubim lima (Bloch & Schneider 1801)
Synbranchiformes	Synbranchidae	*Synbranchus marmoratus* Bloch 1795
Carangiformes	Achiridae	*Achirus lineatus* (Linnaeus 1758)

Cichliformes	Cichlidae	*Cichla monoculus* Spix & Agassiz 1831
		Cichla kelberi Kullander & Ferreira 2006
		Cichlasoma zarskei Ottoni 2011
		CreniCichla brasiliensis (Bloch 1792)
		Geophagus parnaibae Staeck & Schindler 2006
		Satanoperca jurupari (Heckel 1840)
		Oreochromis niloticus (Linnaeus 1758)
		Oreochromis mossambicus (Peters 1852)
Achanturiformes	Sciaenidae	*Plagioscion squamosissimus* (Heckel 1840)

The composition of fish assemblages in the lakes presented 4956 specimens and the most abundant orders were Characiformes (47.09%), Siluriformes (23.82%) and Cichliformes (17.12%), respectively. As for families, Erythrinidae (18.12%), Cichlidae (17.12%) and Doradidae (10.06%) stand out (Figure 2).

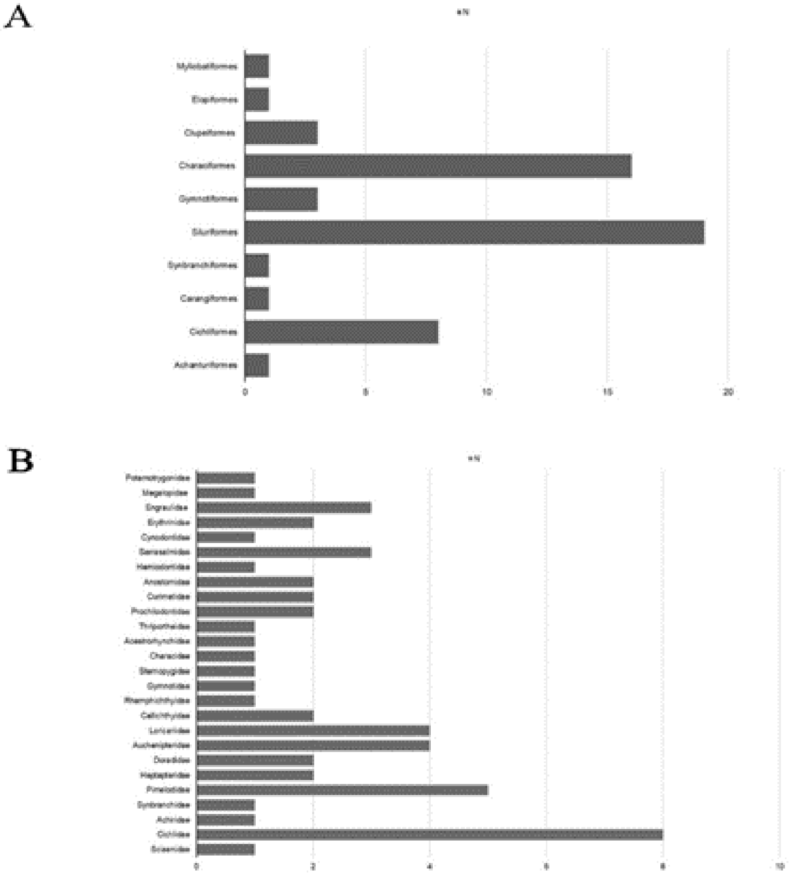

Figure 3. 2 Graphs representing the ichthyofauna of the Baixada *Maranhense lakes with emphasis on A- the orders Siluriformes, Characiformes and Cichlifomes with the highest number of species and B- the families Cichlidae, Pimelodidae, Auchenipteridae and Locariidae with the highest number of spcies.*

Regarding species, the most abundant were *Hoplias malabaricus* (n=1026), *Cichlasoma zarskei* (n= 694), *Curimata macrops* (n=354). The greatest richness was recorded in the dry period (50 species) compared to the rainy period (47 species). During the dry season, the highest absolute abundance values were recorded (n=2845), with greater dominance of individuals belonging to the orders Characiformes (n=1296), Cichliformes (n=647) and Gymnotiformes (n=27) and in the rainy season, lower abundance (n=1718), with dominance of the orders Characiformes (n=1019), Clupeiformes (n=219) and Gymnotiformes (n= 53).

Regarding frequency of occurrence, the constant species were *Hoplias malabaricus*, and *Pygocentrus nattereri* (58%), *Plagioscion squamosissimus* (54%), *Schizodon dissimilis* (52%) and *Serrasalmus rhombeus* (50%). The moderate ones were *Geophagus surinamensis* (46%) and *Triportheus signatus* (40%), *Curimata macrops* and *Loricaria cataphracta* (38%). The less constant species were *Pterengraulis athrerinoides* and *Loricariichthys* sp. (28%), *Hoplerythrinus unitaeniatus*, *Ageneisus ucayalensis* and *Cichla monoculus* (26%) and the rare species were *Potamotrygon motoro*, *Lycengraulis* sp., *Hoplosternum littorale*, *Pimelodus ornatus*, *Mugil* sp., *Achirus lineatus* and *Oreochromis niloticus* (2%).

In relation to taxonomic diversity, we can highlight in descending order the lakes Viana, Cajari and Aquiri, distinct from the lakes Coqueiro and Itans. The hill number q=0 indicates that Lake Viana presented greater taxonomic richness, grouped with Lake Cajari. Although the Hill number q=1 point greater diversity in Lake Cajari.

Highlighting here, a different pattern found between the grouping of pre-confluence and confluence lakes of the SLPM, and the post-confluence lakes. This pattern repeats itself when comparing the hydrological cycle of floods and droughts in general. (Figure 3)

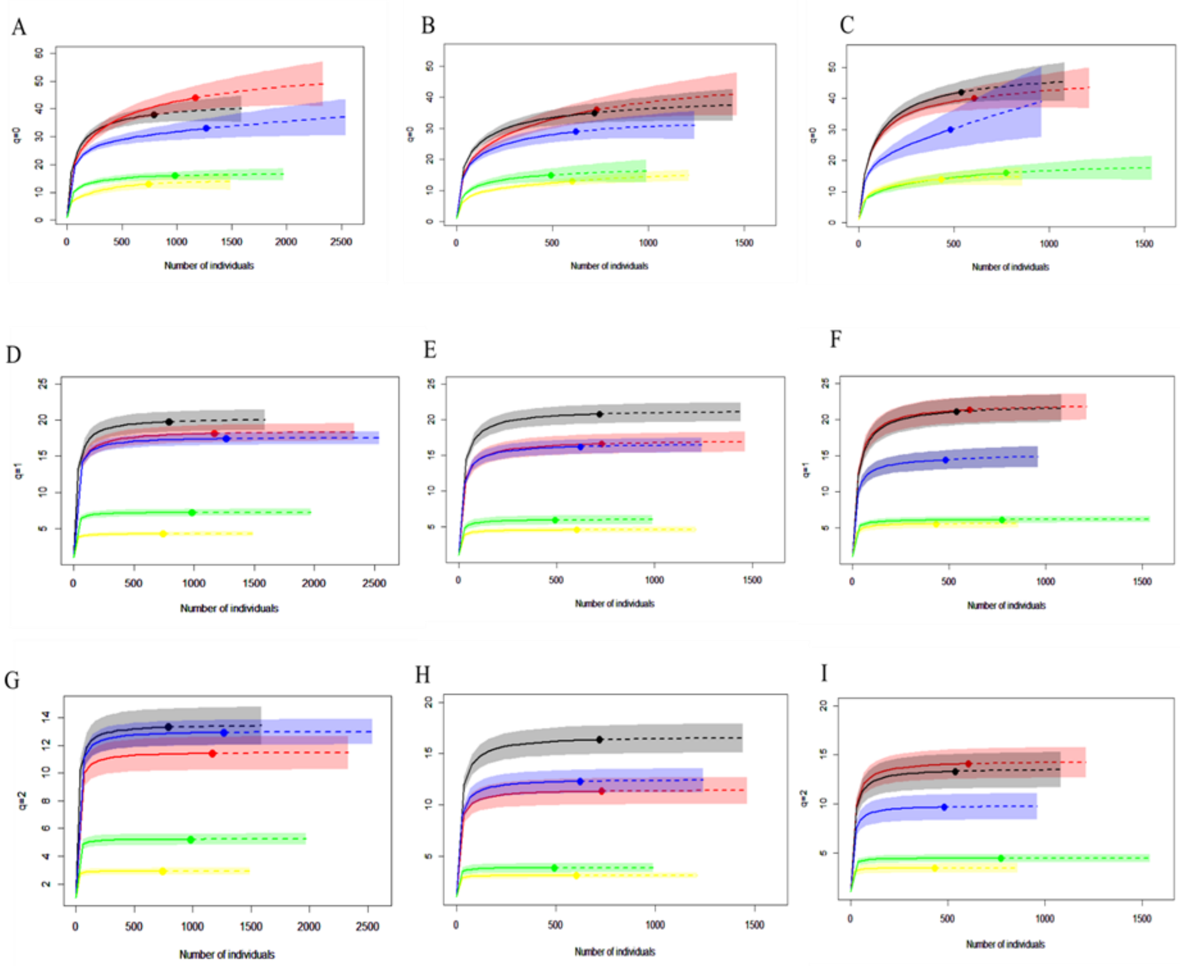

Figure 3. 3 Plots of rarefation (solid lines) and extrapolations (dashed lines), and 95% confidence intervals (shaded areas) for fishes diversity in lakes Cajari (black), Viana (red),

Aquiri (blue), Coqueiro (yellow) and Itans (green) of first three Hill numbers for species diversity: species richness (q=0) A - All campaigns B- Rain C- Dry, Shannon diversity (q=1) D- All campaigns E- Rain F- Dry and Simpson diversity (q=2), G- All campaigns H- Rain I- Dry

For abiotic parameters, the lakes presented O.D values that varied from 4.53 to 6.43 and pH with averages ranging from 4.7 to 6.8. The variation occurred from 18.02 mm in the dry period to 242.34 mm in the rainy period and the temperature varied from 31.4 to 35.18 in the sampling period. There was no variation in variation and temperature parameters among the lakes sampled.

Also noteworthy is the presence of non-native species at the Baixada Maranhense Ramsar Síte, namely, two species of the genus *Cichla* Bloch & Schneider 1801, *Cichla monoculus* Spix & Agassiz 1831 and *C. kelberi* Kullander & Ferreira 2006 and two species of tilapia, *Oreochromis niloticus* and *Oreochromis mossambicus*

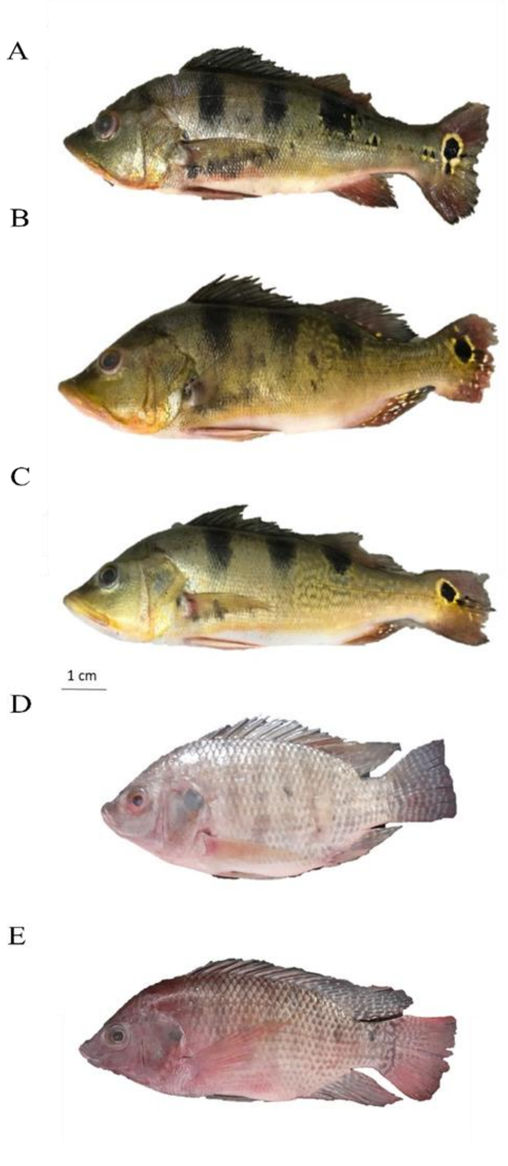

Figure 4. 1 Non-native species collected in the Lakes of the Ramsar Site of Baixada *Maranhense: A- Cichla monoculus, B and C- Cichla kelberi, D- Oreochromis niloticus, E- Oreochromis mossambicus*

4. DISCUSSION

Our results demonstrate that the lakes studied here can be considered as possible shelter, feeding and reproduction areas for fish from the Pindaré-Mearim lacustrine system, since around 40% of the species recorded for this river basin [31] were found in this inventory.

As these are specific areas that suffer periods of disconnection from the main rivers, a lower species richness is expected than that recorded for the entire hydrographic basin, for example, which includes different environments such as rivers, streams and lakes. Abreu et al. [32] recorded 100 species for the basin, while Guimarães et al. [33] recorded 100 species only for the Pindaré river, one of the main rivers that make up this hydrographic system. In the most recent survey, this number was increased to 136 species [31]

The richness of ichthyofauna in the Pindaré-Mearim lake system can be considered low in comparison to Amazonian inventories, such as that carried out by Guimarães et al. [33], with 101 species identified. This difference must be considered that the local scope of the inventory includes environments of rivers, streams and lakes, distributed across 28 sampling points, while here we consider a limited region of six lakes.

In this way, we can consider other works carried out in Amazonian lakes that obtained a variation in richness compatible with the data found here. Like Gomes et al. (2016) who identified an average of 79 to 26 species in thirteen Amazonian lakes, García-Silva [35] who identified 54 species in Lake Papucu, Amazonas, and Freitas et al. [36] who identified 55 species in the five lakes of the Solimões River.

We also highlight that this compatibility refers to floodplain lakes in the central Amazon, such as the Comada, Praia, Tracajá lakes (Yamamoto, 2004) in contrast to the black water lakes that presented a high diversity (Farias et al. 2018)

Regarding the representation of this diversity, the orders Siluriformes, Characiformes and Cichliformes stand out, which is expected for the Neotropical region, as well as for the Amazon according to Polaz et al. [39], Reis et al. [8] among others. For the Amazon region, the three main groups that concentrate the region's species are recent: Siluriformes, Characiformes and Cichliformes [40,41] corroborated by García- Silva [35]. Surveys carried out in Maranhão also corroborate this result (42, 43, 44, 31,30].

Another important aspect to be addressed is that this region is extremely complex, presenting diverse environments, such as estuaries, lakes, rivers and floodplains, with high diversity and fishing productivity that provides food for the region's population (Ibañez et al. [24] and in lakes for commercial use, Siluriformes have a greater representation than in lakes for preservation and management, according Freitas et al. [37]

It is important to highlight that this pattern changes throughout the lakes' flood pulse, in the dry period we can highlight Curimatidae (n=), and), while in the flood period we highlight the families Serrasalmidae (n= 197), Engraulidae (n = 177) and Auchenipterity (n= 172).

In relation to the most numerous families, we can highlight the Cichlidae (n=1346), Erythrinidae (n=1168) and Serrasalmidae (n=722). This pattern, although it does not agree with what is expected for the Neotropical region, can be justified by the geomorphology of lakes which, similar to African lakes, present a high diversity of cichlids, also considering that the richness of cichlids is positively associated with quality environmental

We also recorded the invasive species *Cichla monoculus*, *Cichla kelberi*, *Oreochromis niloticus* and *Oreochromis mossambicus* highlighting the importance of carrying out studies that evaluate in detail the impact of these introductions on the local ecosystem as they are highlighted by Almeida-Funo, Pinheiro & Monteles [45] as tensors environmental issues in Baixada Maranhense, in addition to the dams, the introduction and proliferation of the Malaysian giant shrimp (*Macrobrachium rosembergii*).

The occurrence of exotic species of the genus *Cichla* is recorded in our data, and, due to the complexity of the group, it showed discordant inferences between the identification techniques used. The peacock bass is a problematic case for morphological identification, since the species have a reasonable variation and there are morphotypes within these species [46]. Kullander et al. [47] states that many authors describe these morphotypes as species or subspecies. For a taxon introduced in most Brazilian basins, this difficulty in identification sets precedents for management actions to be applied incorrectly, due to the fact that, in some cases, it is not the introduction of just one species, but as we inferred for the Baixada Maranhense, two species.

In reference to the group's problems, Willis et al. [48] consider five species of peacock bass to be valid: *Cichla monoculus, Cichla ocellaris, Cichla temensis, Cichla intermedia* and *Cichla orinocensis,* however Kullander & Ferreira [48] revised the genus *Cichla* and further validated nine new species, among them: *Cichla kelberi and Cichla piquiti*. For the latter authors, *Cichla kelberi* was mistakenly identified as *Cichla monoculus* and *Cichla ocellaris*, due to their morphological similarities. Mourão [46] genetically characterizes the species *Cichla kelberi* and *Cichla piquiti*, using the PCR-RFLP technique, in addition to comparing the morphological identifications using the COI gene and RAG1. Morphological and genetic differences support species differentiation.

The peacock bass specimens collected in the study area presented three distinct morphotypes, one of which was identified as *Cichla monoculus* by morphological analysis. However, molecular identification found that despite the phenotypic variation, the specimens belong to two species: *Cichla monoculus* and *Cichla kelberi*.

The occurrence of these exotic species is justified by the increasing number of fish farms in the collection municipalities, also verified by Piorski et al. [14] who previously recorded *Cichla ocellaris* for the Pindaré River. Leão et al. [50] carried out a survey of species introduced in the Brazilian Northeast, indicating *Cichla ocellaris* and *Cichla monoculus* as species used in fish farming.

Yellow peacock bass (*Cichla monoculus* and *Cichla kelberi*) are predators and, like most invasive species, resistant to environmental variations [47,48]. Agostinho et al. [4], when studying the impact of introducing *Cichla kelberi* into the Paranapanema River, they found a reduction in the diversity of native fish. Leão et al. [50] consider the introduction of peacock bass and tilapia to be the most serious cases of species introduction in northeastern Brazil. In Minas Gerais, around 50% of the richness of native species was documented 10 years after the introduction of peacock bass [53,54]. A worrying fact, as in Baixada Maranhense there is no record of the diversity and richness prior to the introduction of these species.

Another invasive species recorded for the area was *Oreochromis mossambicus* and *Oreochromis niloticus*. Piorski et al [14], despite recording *Oreochromis*, did not define the species of occurrence for this genus. In the inventory for the Mearim and Pindaré rivers carried out by

In 1953, Brazil imported specimens of *Oreochromis rendalli* for fish farming in the State of São Paulo, with the government's encouragement of aquaculture, *Oreochromis niloticus* was introduced into tanks in the Northeast region [55] and in 1981 a strain modified from *Oreochromis mossambicus*, the red tilapia.

The species found in the lowland lakes is *Oreochromis mossambicus*, bringing direct implications to the dynamics of the lakes, since in the flood cycle they can be interconnected

5. CONCLUSION

The development of this study is necessary for numerous reasons, such as knowledge of biodi-versity and preservation, mainly because fishing is the main economic activity in the area. In addition to the exploitation of several species being evident over the years, the intensification of human activities such as rice cultivation and buffalo breeding, and consequent environmental degradation, introduction of non-native species, some sensitive species tend to disappear before they are even known. In this way, this work contributes to management prognoses, studies of reproductive biology, food, fishing, production chain, among others.

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5 CAPÍTULO III

Invisible dynamics: exploring the genetic diversity of peacock bass in Brazil

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Dinâmicas invisíveis: explorando a diversidade genética de tucunarés no Brasil

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Dinámica invisible: explorando la diversidad genética del pavón en Brasil

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RESUMO

No Brasil, a introdução de espécies não nativas em ambientes dulcícolas, como os tucunarés (Cichla spp.), foi impulsionada pela aquicultura e pesca esportiva. Esses peixes amazônicos têm plasticidade fenotípica e resistência que facilitam sua invasão e estabelecimento em novos habitats. Devido à complexidade taxonômica dos ciclídeos, estudos sobre variação intraespecífica são essenciais para mitigar os impactos das bioinvasões. Este trabalho teve como objetivo analisar a diversidade genética dos tucunarés, avaliando como as populações invasoras se diversificaram ao longo do tempo e entre diferentes regiões no Brasil. As amostras foram provenientes de diferentes populações invasoras no Maranhão, São Paulo, Paraná e Goiás. O DNA foi extraído e amplificados os genes 16S e COX1. Foram coletados 97 espécimes do gênero Cichla Schneider,1801 identificadas como Cichla monoculus Agassiz, 1831 (n=12); Cichla kelberi Kullander e Ferreira, 2006 (n=75) e Cichla piquiti (Kullander e Ferreira, 2006) (n=10). Foram identificados 16 haplótipos de 16S (445pb), com valores de diversidade haplotípica e diversidade nucleotídica de Hd: 0.830 e Pi: 0.03777, respectivamente. Os resultados demonstraram proximidade das populações invasoras do Maranhão e de São Paulo, com compartilhamento de haplótipos entre Parque Nacional dos Lençóis Maranhenses, Baixada Maranhense e Ilha Solteira. Reforçamos assim a fragilidade das delimitações e definições de espécie no grupo, com base apenas em dados morfológicos. Avaliando o conjunto de dados morfológicos e genéticos encontramos variações importantes intraespecíficas, apesar de esperar homogeneidade das populações, por conta do efeito fundador e do gargalo de garrafa comuns em populações invasoras. Isso reforça o potencial adaptativo desses ciclídeos.

Palavras-chave: Bioinvasão, Diversidade Genética,Tucunaré, Brasil

ABSTRACT

In Brazil, the introduction of non-native species into freshwater environments, such as peacock bass (Cichla spp.), was driven by aquaculture and sport fishing. These Amazonian fish possess phenotypic plasticity and resilience that facilitate their invasion and establishment in new habitats. Due to the taxonomic complexity of cichlids, studies on intraspecific variation are essential to mitigate the impacts of biological invasions. This study aimed to analyze the genetic diversity of peacock bass, assessing how invasive populations have diversified over time and across different regions in Brazil. Samples were collected from different invasive populations in Maranhão, São Paulo, Paraná, and Goiás. DNA was extracted and the 16S and COX1 genes were amplified. Ninety-seven specimens of the genus Cichla Schneider, 1801 were identified as Cichla monoculus Agassiz, 1831 (n=12); Cichla kelberi Kullander and Ferreira, 2006 (n=75); and Cichla piquiti (Kullander and Ferreira, 2006) (n=10). Sixteen 16S haplotypes (445bp) were identified, with haplotype diversity and nucleotide diversity values of Hd: 0.830 and Pi: 0.03777, respectively. The results showed proximity between the invasive populations from Maranhão and São Paulo, with haplotype sharing among Parque Nacional dos Lençóis Maranhenses, Baixada Maranhense, and Ilha Solteira. This highlights the fragility of species delimitation and definitions within the group, based solely on morphological data. By evaluating the set of morphological and genetic data, we found significant intraspecific variations, despite expecting population homogeneity due to the founder effect and bottleneck commonly observed in invasive populations. This underscores the adaptive potential of these cichlids.

Keywords: Bioinvasion, Genetics diversity, Peacock basses, Brazil

RESUMEN

En Brasil, la introducción de especies no nativas en ambientes de agua dulce, como los tucunarés (Cichla spp.), fue impulsada por la acuicultura y la pesca deportiva. Estos peces amazónicos poseen plasticidad fenotípica y resistencia que facilitan su invasión y establecimiento en nuevos hábitats. Debido a la complejidad taxonómica de los cíclidos, los estudios sobre la variación intraespecífica son esenciales para mitigar los impactos de las invasiones biológicas. Este estudio tuvo como objetivo analizar la diversidad genética de los tucunarés, evaluando cómo las poblaciones invasoras se han diversificado a lo largo del tiempo y en diferentes regiones de Brasil. Se recolectaron muestras de diferentes poblaciones invasoras en Maranhão, São Paulo, Paraná y Goiás. Se extrajo ADN y se amplificaron los genes 16S y COX1. Se identificaron noventa y siete especímenes del género Cichla Schneider, 1801 como Cichla monoculus Agassiz, 1831 (n=12); Cichla kelberi Kullander y Ferreira, 2006 (n=75); y Cichla piquiti (Kullander y Ferreira, 2006) (n=10). Se identificaron dieciséis haplotipos de 16S (445pb), con valores de diversidad haplotípica y diversidad nucleotídica de Hd: 0.830 y Pi: 0.03777, respectivamente. Los resultados mostraron proximidad entre las poblaciones invasoras de Maranhão y São Paulo, con intercambio de haplotipos entre el Parque Nacional dos Lençóis Maranhenses, Baixada Maranhense e Ilha Solteira. Esto resalta la fragilidad de las delimitaciones y definiciones de especies dentro del grupo, basadas únicamente en datos morfológicos. Al evaluar el conjunto de datos morfológicos y genéticos, encontramos variaciones intraespecíficas significativas, a pesar de esperar homogeneidad en las poblaciones debido al efecto fundador y el cuello de botella comúnmente observados en poblaciones invasoras. Esto subraya el potencial adaptativo de estos cíclidos.

Palabras clave: Bioinvasión, Diversidad Genética, Tucunaré, Brasil

1 INTRODUCTION

The introduction of non-native species into freshwater environments in Brazil was caused, among other factors, by aquaculture and sport fishing (Gubiani *et al.*, 2018). Tucunarés (*Cichla* spp.) are a group of Amazonian fish that have been widely translocated in Brazil. In this case, sport fishing and inadequate farming in hatcheries represent anthropogenic pressures that have caused the bioinvasion of these organisms in different environments (Fugi *et al.*, 2008).

These fish, which belong to the diverse group of cichlids (Nelson *et al.*, 2016), display characteristics that facilitate their success in the process of invasion and establishment in areas outside their natural distribution. These include phenotypic plasticity and environmental resistance (Williams *et al.*, 1998; Bedarf *et al.*, 2001).

In light of these characteristics, an in-depth study of the peacock bass and its intraspecific variation in invaded environments is of paramount importance in order to mitigate the impacts of bioinvasion on native biota. As a result of phenotypic plasticity, the invasive population is capable of persisting and reproducing in diverse habitats and under varying pressures (Waddington, 1975).

Consequently, a holistic evaluation of morphology and genetics facilitates more accurate differentiation between groups of organisms. Morphological variations are initially identified due to their ease of visualisation. However, cichlids are a taxonomically complex group, which introduces uncertainty regarding the use of morphological characters. To gain a more comprehensive understanding, genetic approaches are an invaluable tool. The utilisation of mitochondrial genes (COX1 and 16S), which exhibit a more rapid evolutionary rate (Avise, 1994), facilitates the acquisition of data pertaining to variability, particularly within the context of invasion, where signals are slower.

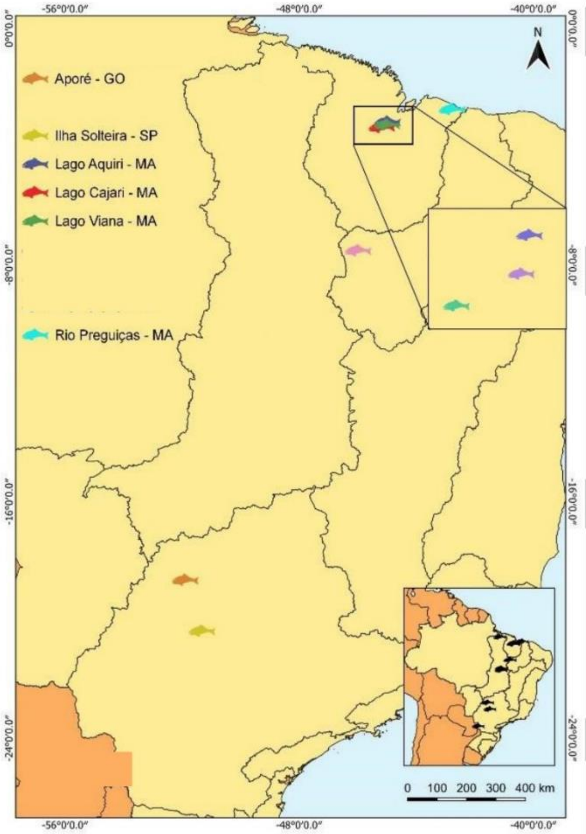
The aim of this study is to make a comparison between the invasive populations of Cichla spp. located in distinct geographical áreas with particular focus on the exami- nation of morphological and genetic characteristics.

2METHODS

2.1 SAMPLING

Samples were obtained from a range of invasive populations across several hydrographic basins of the Western Northeast Atlantic. These included the Baixada Maran- hense (MA), Parnaíba (MA) (Lençóis Maranhenses National Park), Paraná (Ilha Solteira/SP, Aporé/GO), as illustrated in Figure 1.

Figure 1. Map of the hydrography of South America with the location of the sampled populations of *Cichla* spp.



Source: Authors

The specimens were procured from 2014 to 2022 via the utilisation of gillnets (with a mesh size of 10 and 20 mm between opposite nodes), cast nets, and hooks. Morphological identification was conducted in accordance with the methodology outlined by Kullander and Ferreira (2006).

2.2 GENETIC DIVERSITY

The genetic material was extracted using the WIZARD/PROMEGA extraction kit, in accordance with the instructions provided by the manufacturer. The PCR producto obtained with the 16S primer had an approximate length of 600 base pairs (bp), while the COI region product had an approximate length of 700 bp after the PCR reaction. The 16S PCR reaction involved an initial denaturation at 94 ºC for 3 min, followed by 30 cycles of 1 min of denaturation at 94 ºC, 1 min of annealing at 55 ºC and 3 min of extension at 72 ºC. Furthermore, a final extension of seven minutes at 72°C was conducted for the COI. The initial denaturation was performed at 94°C for five minutes, followed by 35 cycles of one minute of denaturation at 94°C, 30 seconds of annealing at 56°C, and one minute of extension at 72°C. Additionally, a final extension of seven minutes at 72°C was conducted. The sequencing of the samples was conducted at ACTGene Análises Moleculares Ltda. (Centro de Biotecnologia, UFRGS, Porto Alegre, RS) using the ABI- PRISM 3100 Genetic Analyzer automated sequencer, equipped with 50 cm capillaries. The sequences obtained in this study for the 16S region and COI region will be submitted to the GenBank database for archiving.

The chromatograms were subjected to a rigorous examination and the sequences were aligned using the ClustalW software (Thompson *et al.*, 1997), which was implemented in the MEGAX program. The chromatograms were examined and the sequenceswere aligned using the ClustalW software (Thompson *et al.*, 1997), which was implemented in the MEGAX program. Phylogenetic trees were constructed using the MEGAX software, with Geophagus brasiliensis employed as the outgroup in all analyses.

In order to incorporate the maximum amount of information, the 16S and COI regions were analysed separately.

3. RESULTS AND DISCUSSION

The samples of the genus Cichla Schneider, 1801 were identified as Cichla mo- noculus Agassiz, 1831 Cichla kelberi Kullander and Ferreira, 2006 and Cichla piquiti (Kullander and Ferreira, 2006) as illustrated in Figure 2

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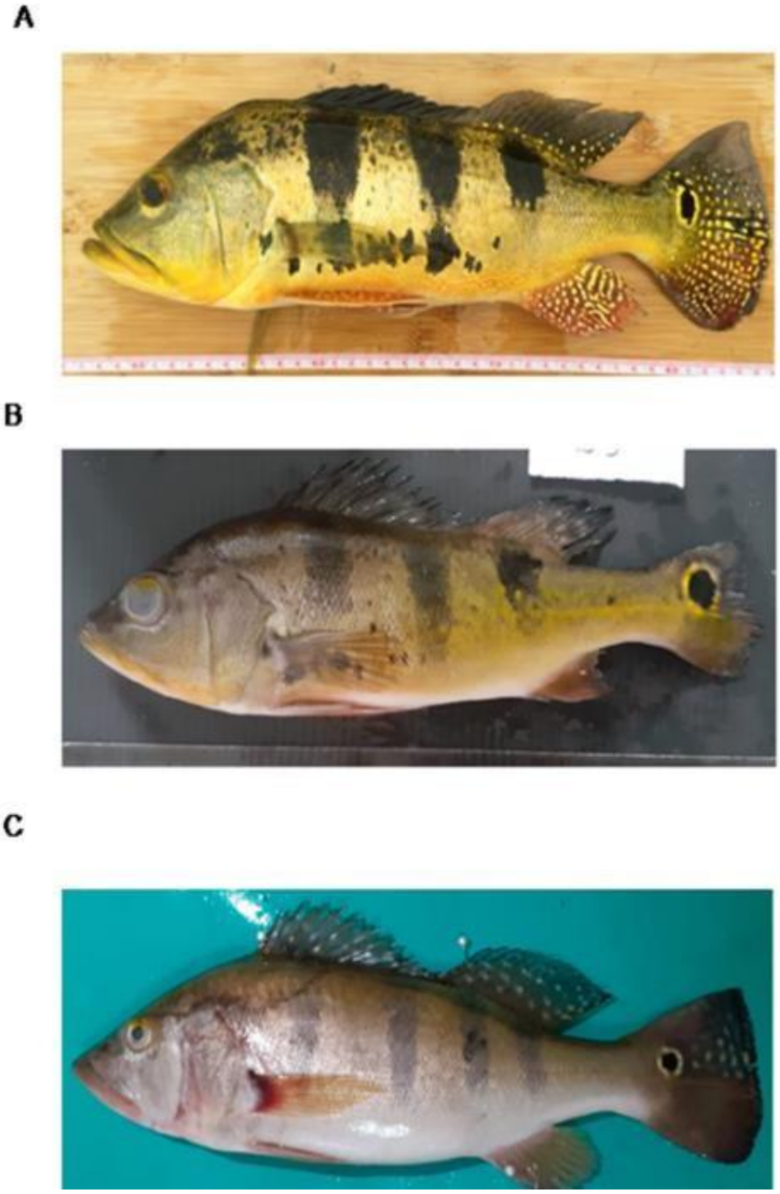
Figure 2. Species of *Cichla kelberi* collected in Ilha Solteira/SP (A), *Cichla monoculus*

collected in theMaranhão lowlands/MA (B) and *Cichla piquiti* collected in Ilha Solteira/SP (C) translocated from the Amazon basin

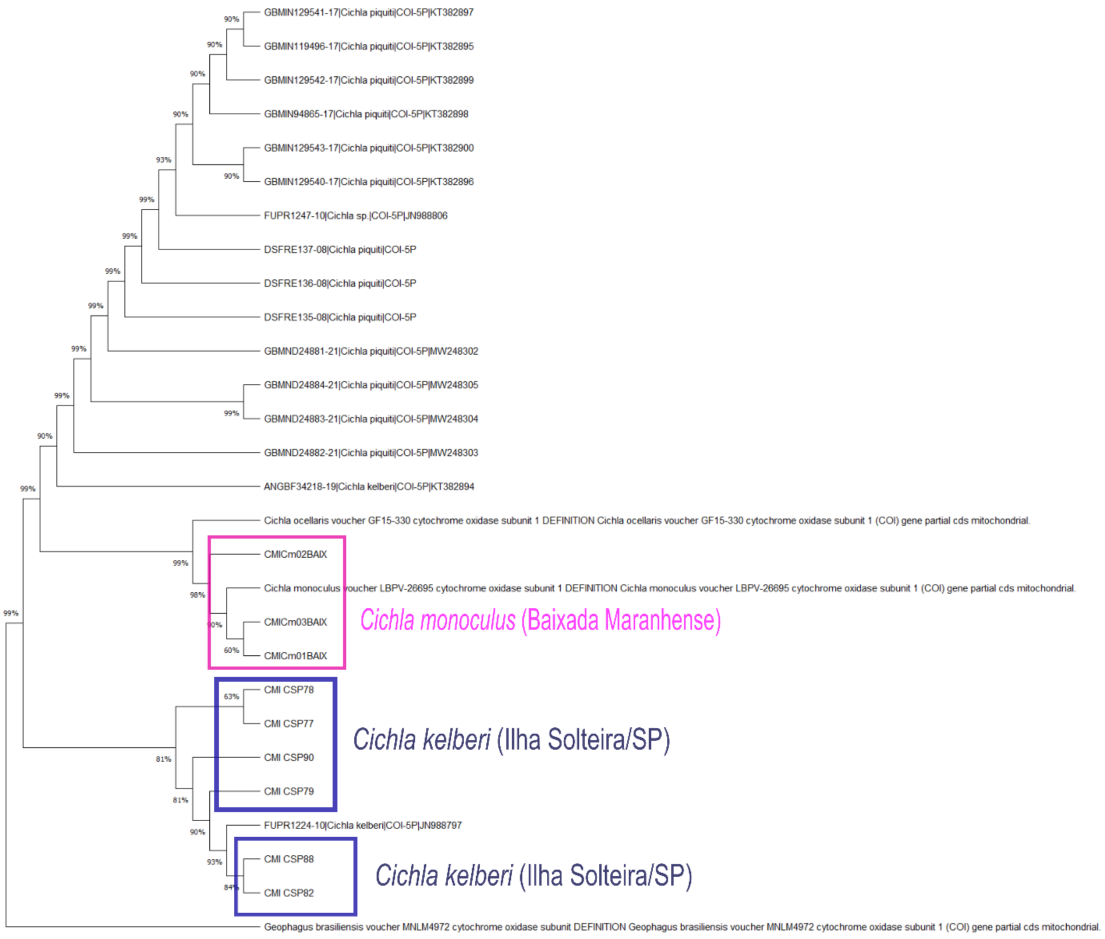


Source: Authors

Mitochondrial DNA from the COX1 region was amplified from 12 samples whose morphology showed unidentifiable features. And for the 16S gene, 28 samples were am- plified from samples that showed morphological variation

After editing and alignment of the DNA sequences, 502 base pairs (bp) were ob-tained for the COX1 region with 158 variable sites, of which 94 were informative in the parsimony analysis. The topology and confidence of the maximum parsimony branches are shown in Figure 3.

Figure 3. Maximum Neighborhood phylogenetic tree of mitochondrial sequences from the COI region of Cichla



Source: Authors

Genetic distances based on the K2P model of the COX1 gene between samples from Baixada ranged from 0.0000 to 0.00277, while samples from São Paulo showed a variation of 0.000 to 0.847, the same found between populations.

Our analysis confirms the data obtained from the BOLDSYSTEMS platform, in dicating the separation of the C. monoculus clade with organisms collected in Baixada Maranhão, being a sister group to C. ocellaris, forming a significant evolutionary unit according to Willis et al. (2017).

As posited by Kullander and Ferreira (2006), the morphological discrepancies between C. monoculus and C. kelberi, and C. ocellaris are primarily manifested in the continuity or lack thereof of the lateral line, the presence or absence of an occipital bar, and the absence or presence of vertical bars. In contrast, C. monoculus and C. kelberi exhibit notable differences in the number of scales present in the lateral and median line, as well as in the number and distribution of spots on the caudal, pelvic, and anal fins.

In this context, the use of genes with a higher rate of variation may prove more sensitive to changes in cases of bioinvasion. The 16S (445 bp) analysis revealed that 16 haplotypes were identified among the 29 specimens of invasive peacock bass that were sampled. The values of haplotypic diversity and nucleotide diversity for the set of invasive populations were Hd: 0.830 and Pi: 0.03777, respectively.

In contrast, several authors have reported low rates of genetic variability in introduced populations of Cichla (Marques et al., 2016). However, Diamante et al. (2017) identified four haplotypes in eight specimens when using the COI gene for Cichla introduced into the São João River Basin, which suggests that invasive populations may in fact be diverse. Furthermore, Mueller et al. (2017) also indicated high variability in nuclear genes.

The analysis of the 16S gene indicated a close genetic relationship between the invasive populations of Maranhão and São Paulo, with the presence of shared haplotypes between Barreirinhas, Viana and Ilha Solteira. The sharing of haplotypes constitutes a robust indication that the same matrix was employed in the invasion, even in the initial analysis.
It is noteworthy that despite the organism collected in Goiás being identified as *C.piquiti*, the grouping with the organisms from Ilha Solteira *(C. kelberi*) exhibited a high bootstrap value for the 16S gene (89%) and a low value for COI (63%).

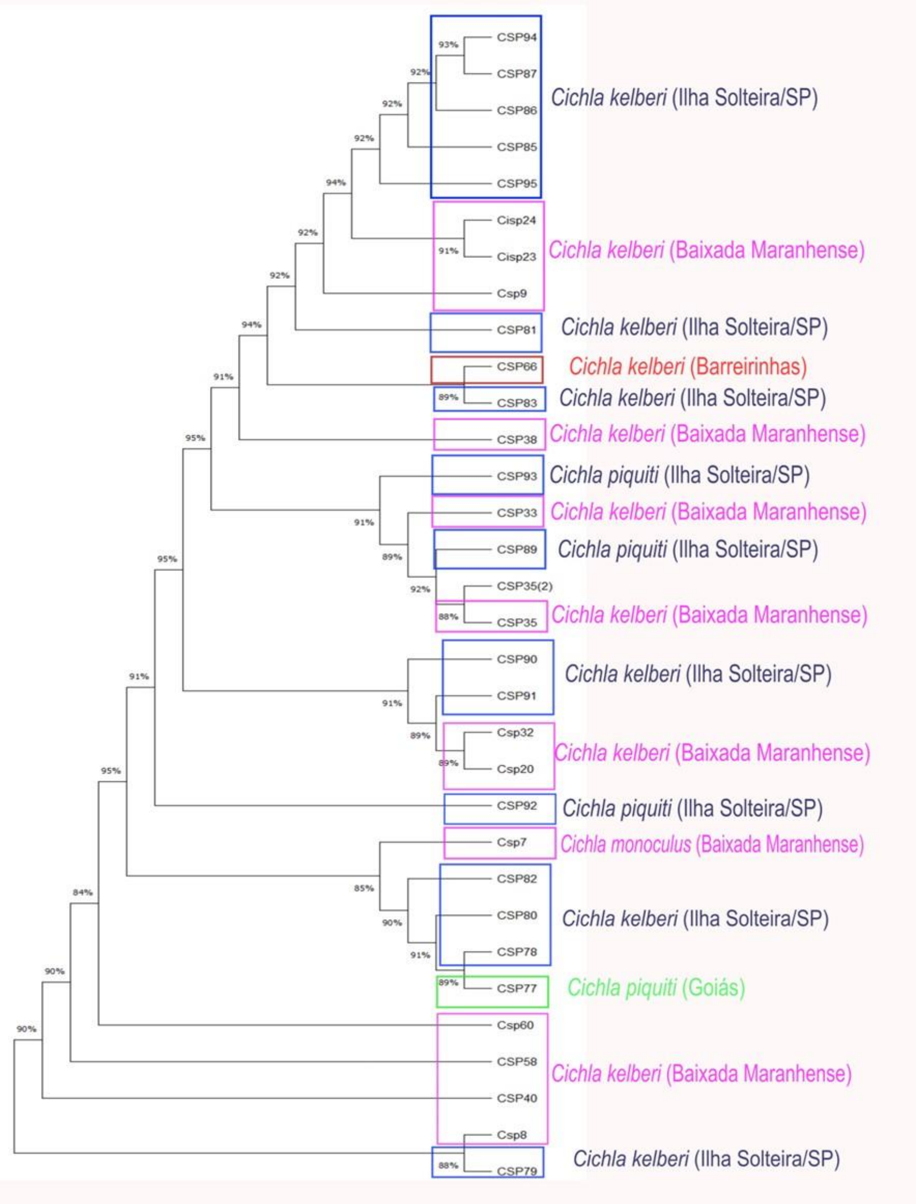
The specimen inquestion lacks the vertical bars 1a and 2a, as well as the occipital bar. Additionally, the body is more elongated. This serves to reinforce the fragility of the delimitations and definitions of species in the group, based on morphological and molecular data (Figure 4).

Figure 4. Specimens collected in Aporé/GO (CMICsp77) of *Cichla piquiti* and in Ilha Solteira (CMICsp 78) of *Cichla kelberi*.

Source: Authors

A phenogram of the sampled peacock bass 16S gene haplotypes showing the above relationships is shown in Figure 5. The high bootstrap value (confidence) of the analyses can also be observed.

Figure 5. Evolutionary history was inferred using the Neighbor-Joining method [1]. The ideal tree is shown.



Source: Authors

The data set was evaluated, and it was found that there were significant intraspe- cific variations, despite the expectation of homogeneity among the populations. This was due to the founder effect and bottleneck, which are common occurrences in invasive populations. This serves to reinforce the adaptive potential of these cichlids.

5 CONCLUSION

In view of the above, morphologic analysis demonstrated the presence of intras- pecific variation related to environments, even in those that are more closely related or connected. These findings are accompanied by molecular data. A diversity of intriguing forms and haplotypes were identified. Given that these cichlids are recent species, we advise that the selection pressures in these populations be investigated through the use of extensive data, such as next-generation sequencing methodologies.

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5 CAPÍTULO IV

Insights revealed by SNPs in non- native *Cichla* populations in Brazil

Biological Invasion (A1)

Status: Submetido

Insights revealed by SNPs in non- native *Cichla* populations in Brazil

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Abstract

Peacock bass are cichlids native to the Amazon basin and transferred to several other Brazilian basins. The group presents a problematic taxonomy due to several factors, including phenotypic plasticity and introgression. In non-native environments, these species have caused environmental disturbances such as the loss of local biodiversity. In this work, we propose to identify the species introduced in the state of Maranhão, as well as verify the diversity and status of these populations, comparing them with native and non-native populations from other river basins. For this, we obtained a number of 119 individuals analyzed through morphology, geometric morphometry and genotyping by high-throughput sequencing. We identified three non-native species *Cichla monoculus*, *Cichla kelberi* and *Cichla piquiti*. We found greater diversity in the native populations of Pará and Tocantins, although the non-native populations are differentiated from the original ones. We also noted the presence of possible hybrids in non-native populations where *C. kelberi* and *C. piquiti* co-occur. In addition to endorsing the mixture of phenotypic characteristics of these species.

Keywords: SNPs; *Cichla* spp.; Morphology; Freshwater fish

Summary

Os tucunarés são ciclideos nativos da bacia amazônica e transladados para diversas outras bacias brasileiras. O grupo apresenta uma taxonomia problemática por diversos fatores, incluindo plasticidade fenotípica e introgressão. Nos ambientes não nativos, essas espécies têm causado distúrbios ambientais a exemplo da perda de biodiversidade local. Neste trabalho, nos propomos a identificar as espécies introduzidas no estado do Maranhão, bem como verificar a diversidade e status dessas populações, comparando com as populações nativas e não nativas de outras bacias hidrográficas. Para isso, obtivemos um número de 119 indivíduos analisados através da morfologia, morfometria geométrica e genotipagem por sequenciamento de alto rendimento. Identificamos três espécies não nativas *Cichla monoculus*, *Cichla kelberi* e *Cichla piquiti*. Verificamos maior diversidade nas populações nativas do Pará e Tocantins, embora as populações não nativas estejam diferenciadas das originárias. Constatamos também a presença de possíveis híbridos nas populações não nativas em que coocorrem de *C. kelberi* e *C. piquiti*. Além de endossar a mistura de características fenotípicas dessas espécies.

Palavras-chave: SNPs; *Cichla* spp.; Morfologia.; Peixes dulcícolas

Introduction

In Brazil, the introduction of non-native species into freshwater environments is most commonly caused by factors such as aquaculture, sport fishing, aquarium farming, among others. And in this context, *Cichla* spp., peacock bass, an Amazonian group widely translocated in the country. And in that case, sport fishing and inappropriate cultivation in farms are anthropogenic pressures that caused the bioinvasion of these organisms in different environments (Fugi et al. 2008)

Belonging to the diverse group of Cichlids (Nelson et al. 2016), these fish have characteristics that provide success in the process of invasion and establishment in areas outside their natural distribution, such as phenotypic plasticity and environmental resistance (Williams et al. 1998; Bedarf et al. 2001)

This taxon presents problematic species delimitation (Willis et al. 2007, 2012, 2013), with diverse morphotypes and introgression even in more distant species (Willis et al. 2012). The most common species in invaded environments are *Cichla kelberi* and *Cichla piquiti*, which are naturally sympatric in the Tocantins-Araguaia basin, and *Cichla monoculus*, native to the Amazon basin.

Due to morphological variations, the study of peacock bass in invaded environments is essential to mitigate the impacts of bioinvasion on native biota. Since the phenotypic plasticity of the invasive population allows reproduction in different habitats and pressures, thus being able to undergo phenotypic modifications (Waddington 1975)

In this sense, a comprehensive assessment of morphology, morphometry and genetics allows for better discrimination of these populations. Initially, morphological variations are the first to be identified, due to their ease of visualization. However, cichlids are a taxonomically complex group, generating uncertainty in the use of morphological characters.

Another way of observing phenotypic patterns and variations is through geometric morphometrics, which is capable of describing and locating regions of changes in shape and, above all, of graphically reconstructing these differences, through anatomical landmarks (Bookstein 1991, Cordeiro-Estrela and Fornel 2015). Associated with the use of SNP-type markers, obtained through illumina high-throughput sequencing, which allows access to single nucleotide polymorphisms throughout the genome.

Therefore, we propose the use of morphology, morphometry and SNPs to verify the intra and interpopulation variability of native and invasive *Cichla kelberi* and *Cichla piquiti* in Brazil, as well as the existence or not of structuring and introgression in these populations.

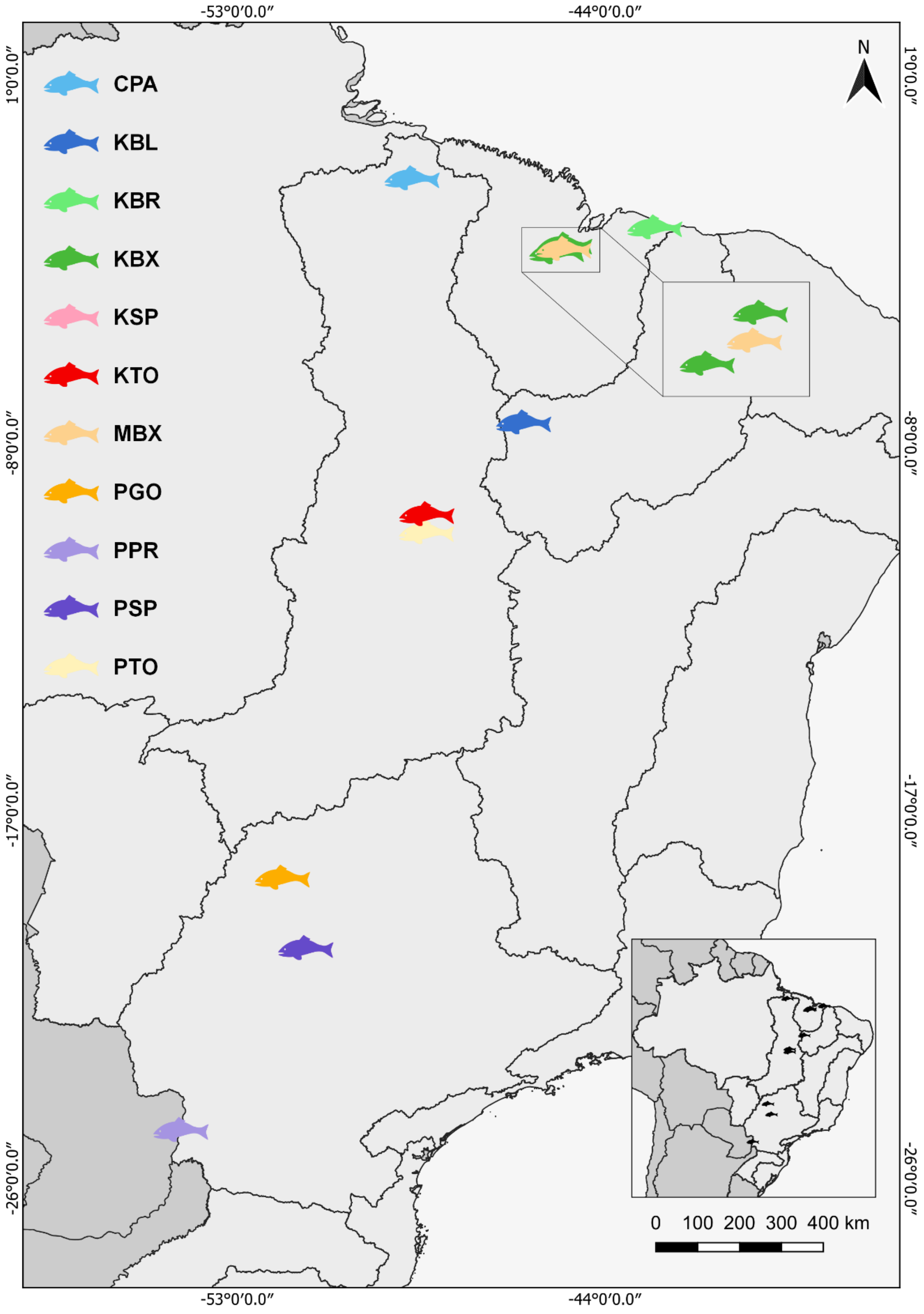
Methods

Study area and sampling

The samples came from different non-native and native populations in the following hydrographic regions, according to Abell et al. (2008): Amazon Estuary and Coastal Drainages (Baixada maranhense, Maranhão/ Non-native), Tocantins/ Araguaia (Lajeado and Palmas, Tocantins/Native; Vila do Conde, Pará /Native), Parnaíba

(Balsas and Lençóis Maranhenses National Park /Non-native), Upper Paraná (Ilha Solteira, São Paulo/Non-native; Aporé, Goiás/Non-native), Lower Paraná (Santa Terezinha de Itaipu, Paraná/Non-native) (Figure 1)

Fig. 1 Collection points for specimens belonging to the native and invasive species *Cichla kelberi, Cichla monoculus* and *Cichla piquiti* in Brazilian hydrographic regions, plotted in the QGIS 3.32.0 software.



All collections for this study were done with authorization from the Brazilian Institute of the Environment and Non-Renewable Natural Resources (SISBIO-Number 53224-1). The collections were taken from 2014 to 2022. Captures were developed through passive fishing using gillnets with spacing of 10 and 20 mm between nodes, nets and hooks.

Individuals of *Cichla* spp. collected from native and non-native populations, including the specimens used in morphological, morphometric and SNP analyses, are included in the supplementary material (Appendix D)

Morphology and Morphometry

Morphological identification was carried out based on specialized literature for the genus *Cichla*: Kullander and Ferreira (2006), and 56 individuals were separated from all those sampled, which were selected based on criteria of specimen preservation and quality of photographs. The specimens were photographed individually using a digital camera, and then the photos were standardized using the Adobe Photoshop program, image resolution of 800x600 pixels. Each anatomical landmark was transformed into Cartesian coordinates using the R program (4.3.2) with the StereoMorph package using 20 landmarks (Adapted from Luz 2014).

The anatomical landmarks were located in the following regions: 1- Anterior skull region; 2- Posterior end of the cranial cavity; 3- Posterior region of the mandible bone; 4- Notch above the operculum; 5-Extremity of the operculum; 6- Upper insertion of the pectoral fin; 7- Lower insertion of the pectoral fin; 8- Anterior insertion of the dorsal fin; 9- Beginning of the second dorsal fin; 10- Posterior insertion of the dorsal fin; 11- Upper median region of the caudal peduncle; 12- Lower median region of the caudal peduncle; 13- Posterior insertion of the anal fin; 14- Anterior insertion of the anal fin; 15- point parallel to the beginning of the second dorsal fin; 16- Anterior insertion of the pelvic fin; 17- Upper extremity of the eye; 18- Lower extremity of the eye; 19- Left end of the eye; 20- Right end of the eye.

The coordinates were superimposed using the Generalized Procrustes Analysis (Rohlf and Fatia 1990) using the R program with the geomorph package. This method calculates a consensus configuration (Procrustes least squares mean) based on the reference coordinate of all specimens (Bookstein 1991), generating a matrix W, containing the partial deformation scores.

Canonical variable analyzes were carried out based on the definitions of groups: Species and Locality in order to generate new axes that reduce variance within groups and maximize between groups (Webster and Sheets 2010). Discriminant Function analyzes were performed to assess the degree of separation.

Genotyping by Sequencing

Genomic DNA was extracted using the commercial Genomic DNA Isolation kit (Wizard®), following the manufacturer's recommendations. The products from 34 specimens were quantified on a Qubit fluorimeter (Invitrogen™) and purity was assessed on a Nanodrop spectrophotometer (ThermoFisher). After quantification, the samples were used for library construction and were normalized on a plate to a concentration of 5ng/ul.

The Genotyping by Sequencing (GBS) library was constructed using the method described by Elshire et al. (2011). The DNA from the samples was digested by the restriction enzyme *Pstl* and subsequently lyophilized in vacuum. Each sample received an adapter with indexing sequences (barcodes). These barcodes were ligated to specific cut ends generated by the *Pstl* enzyme through the T4 DNA Ligase enzyme.

After adding the barcodes, the samples were pooled and proceeded to purification with magnetic beads. The restriction fragments generated were then amplified and the constructed library generated fragments between

200-450bp. These products were purified again with magnetic beads and quality was assessed on the TapeStation equipment (Agilent) with the High Sensitivity DNA kit.

The final library was quantified in real-time PCR with the KAPA Biosystems Quantification Kit (Illumina) diluted to 10nM. The pool was sequenced in a flowcell of the NextSeq 2000 Illumina equipment with the P2V2 Kit (100 cycles-Single Read with single reads of 110bp).

The initial quality control of the reads was carried out using the FastQC 3 program (www.bioinformatics.babraham.a*C*.uk/projects/), which checks for noise and sequencing problems, through the QC report provided by the sequencer. The software shows a graph with an overview of the range of quality values in all bases at each position in the FastQ file, those sequences that had a score above 20 were selected.

We later used the Stacks version 2.66 “process_radtags” script (Catchen et al. 2013, Rochette et al. 2019) to trim all reads to 82 bases pairs and discard reads with low quality scores, using the default settings. for window size (0.15x read length) and base quality Threshold (30 in Phred score).

Given the absence of a reference genome for any of the species in this study, we constructed a reference catalog of all loci using a “denovo assembly” approach in Stacks version 2.66. To determine the best parameters for building the catalog, we followed the approach recommended by Rivera-Coclón and Catchen (2021).

The first module, ustacks, was used to merge reads at putative loci for each individual. For this module, we allow up to four mismatches between tags (-M 4) and a minimum depth of three identical reads to construct a locus (-m 3). Next, a catalog of loci was created using the cstacks module using all samples (n =33) allowing four mismatches (-n 4) between them. Once this stage was completed, the tsv2bam module was used, which transposed the data by orienting it by locus, instead of by samples. Then, gstacks was used to identify SNPs within the population for each locus, so that each individual was genotyped at each identified SNP. And finally, the populations module was finally used to obtain the initial SNP dataset, genotyping in no less than 80% of individuals, in at least eleven populations (-r 0.90, -p 11). Furthermore, the --write_single_snp flag was used to not obtain more than one SNP per locus.

To better filter the SNPs found, the VCFTools program (Danecek et al. 2011) was used, where we selected only the SNPs that were common to all individuals (--max-missing-count 0), in which each allele should appear in at least 2 distinct reads (--mac 2), without insertions or deletions (--remove-indels) and that the locus is bi-allelic (--min-alleles 2 –max-alleles 2).)

Genomic data analyzes were performed in the R program (4.3.2) R Core Team (2023), using the vcfR package (Knaus and Grünwald 2017). For each location, the following were calculated: the average number of alleles per locus (Na), the expected Heterozygosity (HE), observed Heterozygosity (HO) and the deviation from the Hardy-Weinberg Equilibrium, using the GENEPOP package (Rousset 2008), in R and ARLEQUIN v3.5.2.2 (Excofier et al. 2010). Population structure analyzes were performed using the total SNPs datasets and, in order to analyze genetic differentiation between populations, pairwise FST, AMOVA (analysis of molecular variance) and inbreeding coefficient values were calculated. (Fis), also through the ARLEQUIN program. Cluster analysis and graphical representation of the genetic structure were performed by Discriminant Analysis of Principal Components (DAPC) (Jombart and Ahmed 2011), using the R statistical package. Scatterplots were performed using the ADEGENET package (Jombart and Ahmed 2011) for total datasets.

The number of principal components retained was 30 and the number of genetic clusters using the Bayesian Information Criterion (BIC) were 16 and 20 discriminant functions were retained for the total data sets. The R package was used to group individuals into populations, based on the total data set. Cluster numbers (K) were evaluated from 1 to the number of locations (8). Ten independent runs for each K were implemented with a burn-in period of 50,000 iterations, followed by 100,000 Monte Carlo Markov Chains (MCMC) replications. An admixture model and correlated allele frequencies were assumed.

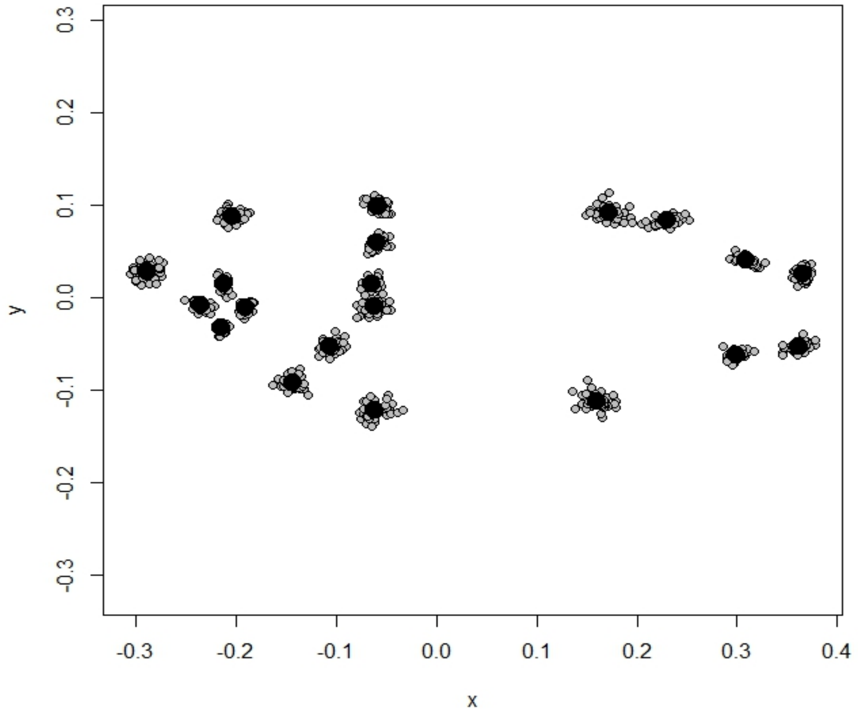
Results

The 118 samples of the genus *Cichla* Schneider, 1801 were identified as *Cichla monoculus* Agassiz, 1831; *Cichla kelberi* Kullander and Ferreira, 2006 and *Cichla piquiti* (Kullander and Ferreira, 2006) according to figure 2.

Fig. 2 Species of *Cichla monoculus* collected in Baixada Maranhense/MA (A) and *Cichla piquiti* collected in Ilha Solteira/SP (B) *Cichla kelberi* collected in Ilha Solteira/SP (C) translocated from the Amazon and Tocantins-Araguaia basins

After filtering the images by quality and integrity of the specimens, we selected photographs, referring to 56 individuals that were included in the morphometric analysis. After Procrustes superimposition and exclusion of outiliers, 50 individuals remained, with the general shape as shown in figure 3

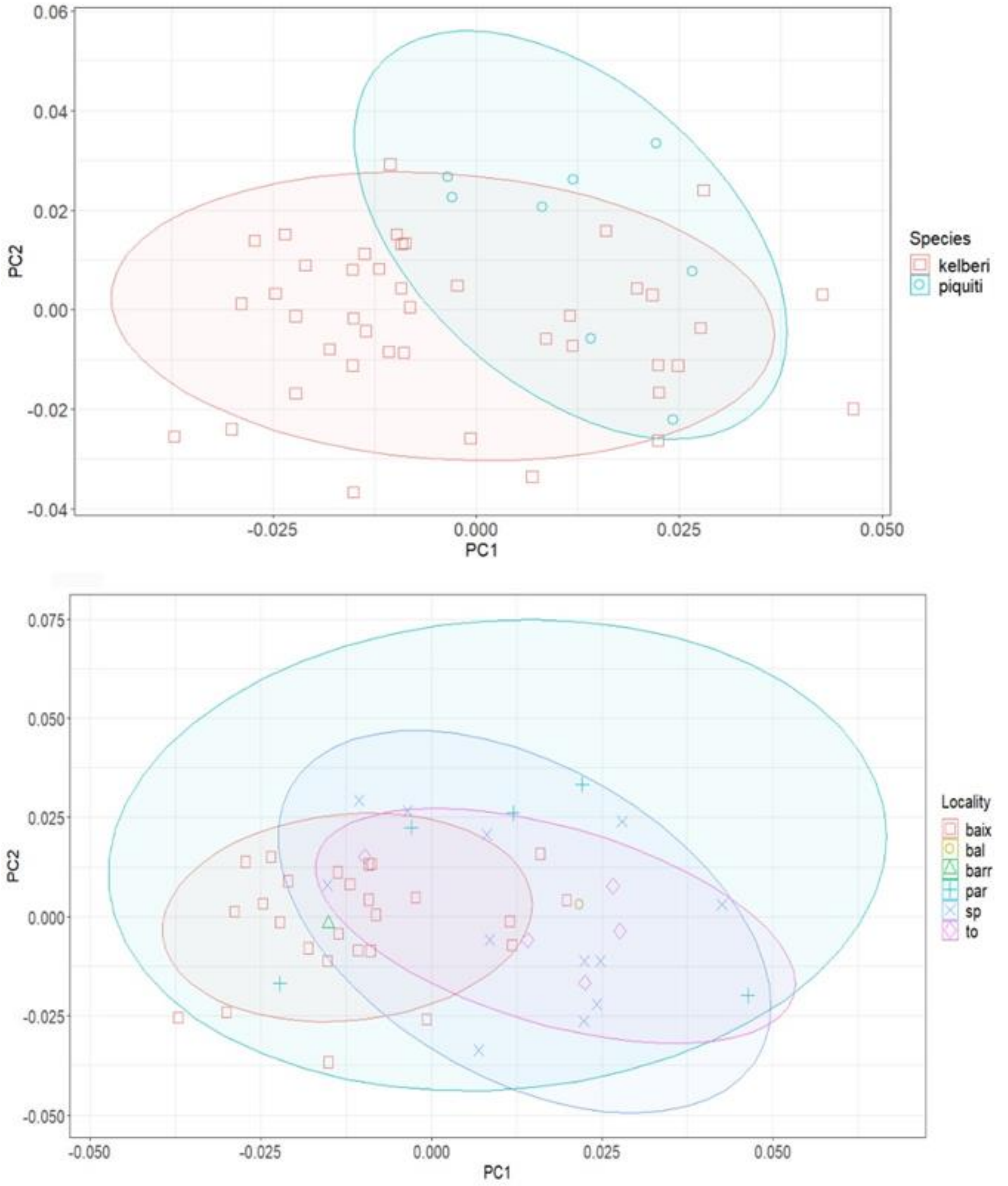
Fig. 3 Generalized Procrustes Analysis of the 50 individuals included after filtering, generated by the geomorph and StereoMorph packages in the R program (4.3.2)



Principal component analysis (PCA) showed that the first two principal components explained 47% of the variance (Appendix A). The classifiers associated with the PCA were species (*C. kelberi* and *C. piquiti*) and locality (low - Lagos Viana and Penalva/MA; bal- Rio Balsas/MA; barr- Rio Preguiças/MA; par- Itaipu/PR; sp- Ilha Solteira/SP; to- Lajeado and Palmas/TO).

In the graph produced, we see that there is no clear differentiation for either of the two classifiers, although the grouping by species was clearer with main component 1, as shown in figure 4

Fig. 4 Analysis of Principal Components of specimens of *Cichla kelberi* and *Cichla piquiti* from native and invasive populations according to species and locality classifiers. PCA with ellipses, 95% confidence for species and locality, respectively



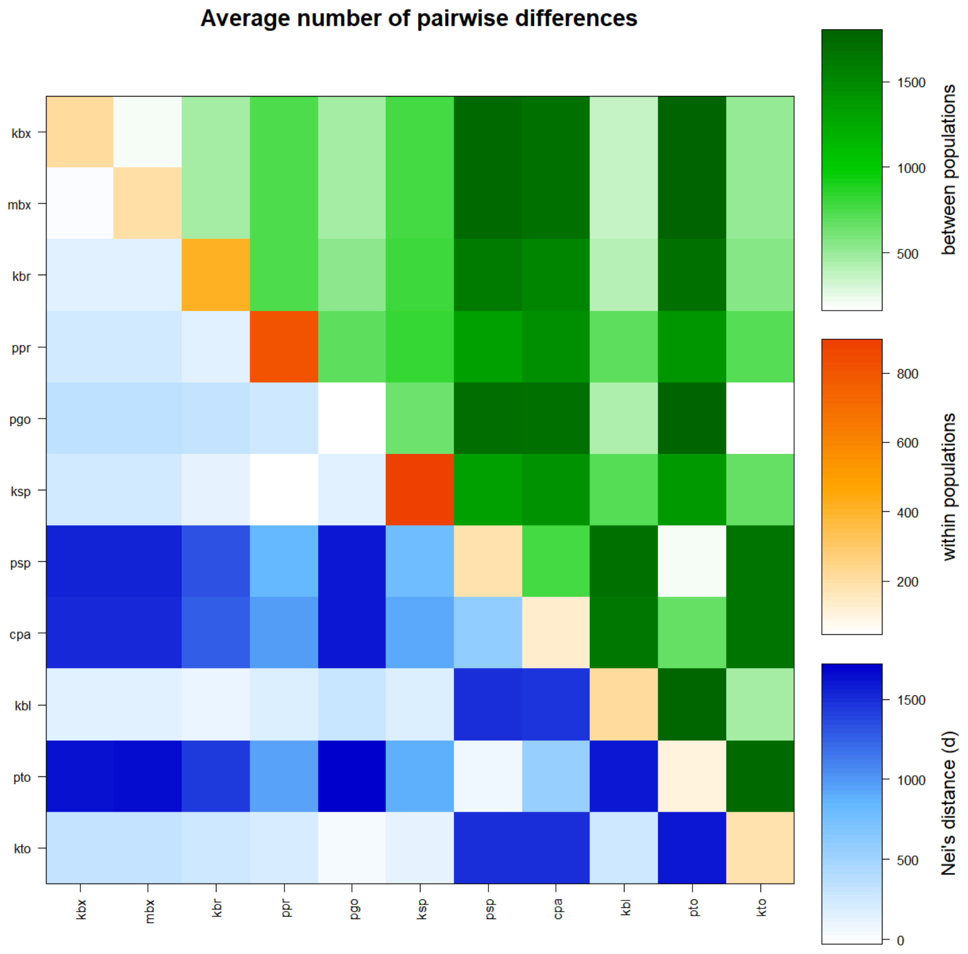
For high-throughput sequencing analyses, a total of 523,594,315 raw reads were retrieved from the platform. After applying the initial quality filters and sequence trimming, 259,813,156 reads were obtained. Using Stacks (2.66), a catalog of 689,261 loci was built using the cstacks module and, after executing the populations module, 674,670 loci were recovered. After filtering by VCFTools, 33 individuals, 2,853 haplotypes and 9,975 SNPs were retained through the filters.

First, we verified the results from the native populations of Tocantins and Pará separately, and later associated with non-native populations. Native species were grouped into different clusters, so that populations of different species were considered. Here we will only represent the associated results, as there was no distinction between the results.

Regarding diversity, we found that the nucleotide diversity of the populations ranged from 0.015 to 0.314. The lowest values were found in the populations of *C. piquiti* from Goiás, *C. piquiti* from São Paulo and Baixada maranhense. While the highest values refer to the populations of Barreirinhas, Paraná and *C. kelberi* in São Paulo.

As for diversity statistics, in the pairwise analysis, we found that Fst values varied from 0.12 to 0.88. The highest Fst values were between the psp and pgo, kbl and psp, pto and kbl, psp and mbx populations. While the lowest values were recorded between ksp and ppr, ksp and pgo, kbl and ppr, kbl and ksp, kbx and mbx (Figure 5)

Fig. 5 Fst values calculated pairwise, between populations, within populations and Nei's distance by Arlequin 3.5 based on SNPs from genotyping by high-throughput sequencing on the Ilumina platform. *$p<0.05$



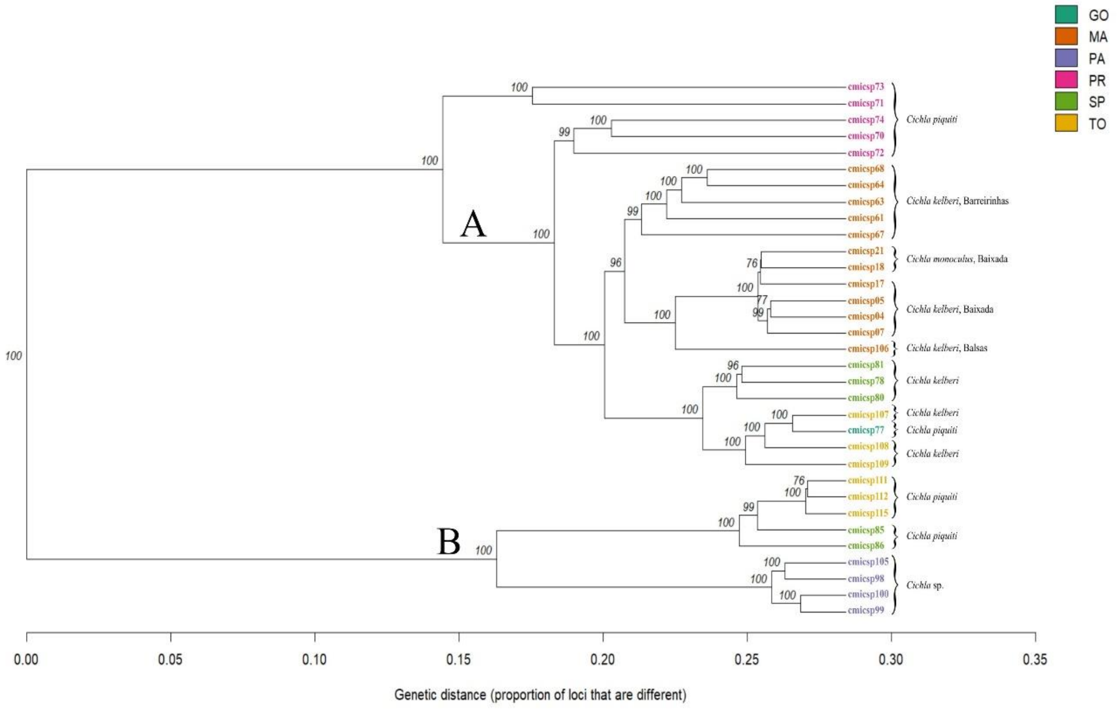
With the Molecular Variance analysis, the variation between the groups was 64.82%, demonstrating that the populations are differentiated from each other and between individuals there was a variation of approximately 30%. However, variation between individuals within populations did not show significant variation.

In relation to private alleles, we highlight the native population of Pará, the population of barreirinhas (MA) and the two native populations of Tocantins with the highest values, including the highest number of polymorphic sites, including ppr and ksp (Suplementary Table)

When comparing the Fis of the sampled populations, we found that the level of homozygosity in native populations varies from 0.001 to 0.02 while in non-native populations we have a variation from -0.006 to 0.50.

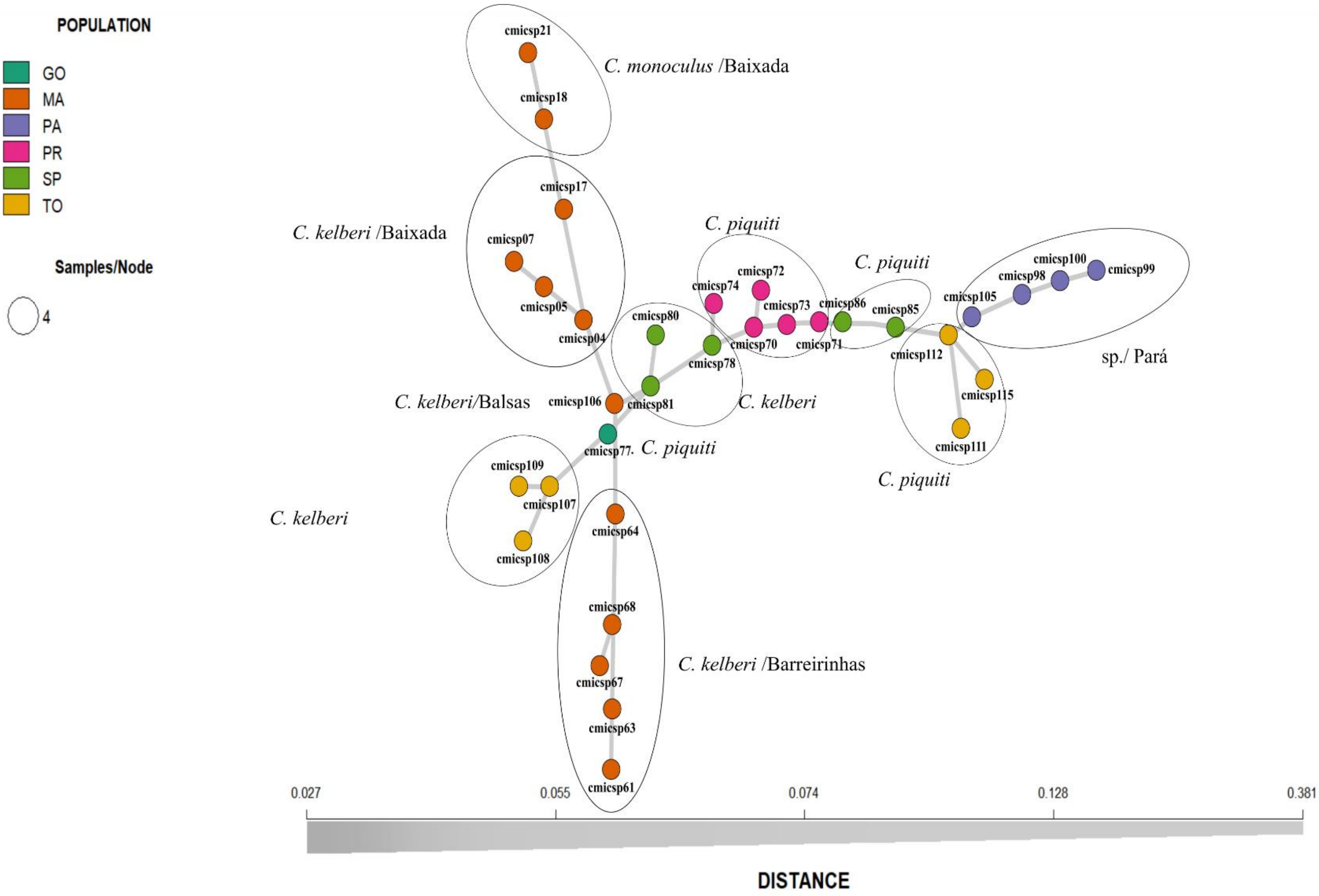
To represent the genetic parentage of the sampled individuals, a distance tree was produced and it can be observed that there was no exclusive grouping by location or by species. It verified the formation of two main clusters, in which the samples are not grouped by species and a cluster with part of the individuals from Paraná. Cluster A includes individuals from São Paulo, Balsas, Barreirinhas, Baixada, Tocantins, Goiás and Paraná of the species *C. kelberi* and *C. piquiti*, while cluster B includes samples from Tocantins, São Paulo and Pará belonging to the species *C. kelberi, C. piquiti* and *Cichla* sp. (Figure 6)

Fig. 6 Genetic distance tree, based on the UPGMA method with 1000 bootstrap replicates, showing the formation of two main clusters: A- including non-native populations from Maranhão, Paraná, Goiás, São Paulo and the native population of *C. kelberi* from Tocantins and B- including the native populations of Tocantins and Pará and a non-native individual from São Paulo



Another way to evaluate the population structure of our sample was carried out through the minimum coverage network, in which multilocus genotypes are grouped by distance between them, as shown in figure 7

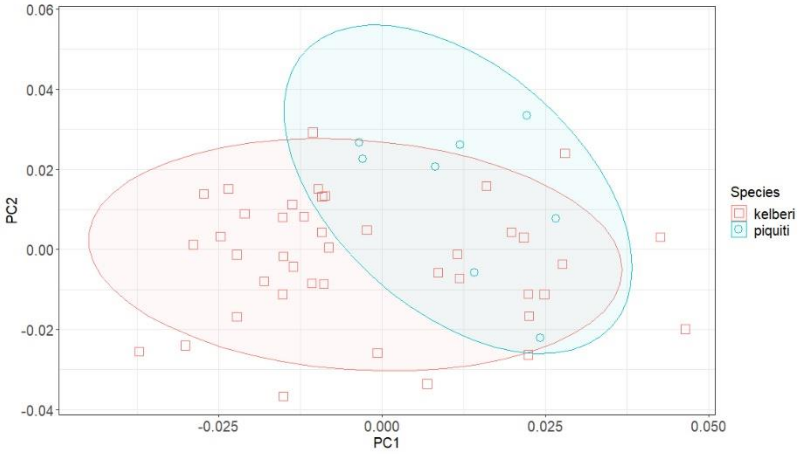
Fig. 7 Minimum coverage network with multilocus genotypes grouped by the distance between them. (*Cichla* spp. /PA -cpa; *Cichla kelberi*/Balsas-kbl; *Cichla kelberi*/Barreirinhas-kbr; *Cichla kelberi*/Baixada-kbx; *Cichla kelberi*/SP-ksp; *Cichla kelberi*/TO- kto; *Cichla monoculus*/Baixada-mbx ; *Cichla piquiti*/GO-pgo; *Cichla piquiti*/PR-ppr, *Cichla piquiti*/SP-psp; *Cichla piquiti*/TO- pto).

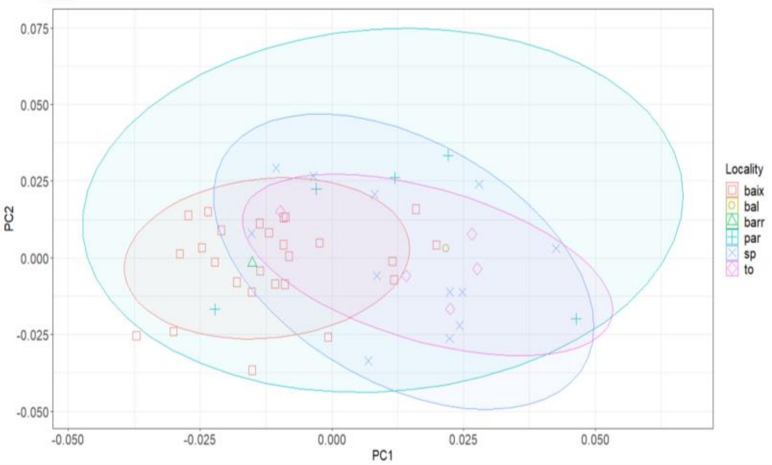


Just like the distance tree, the coverage network demonstrated that there is no clear differentiation for species in non-native populations, while for locality there appears to be a distance in population and proximity between individuals. And to verify these signs, a PCA was analyzed, which identified the first three components to present the highest percentage of variance explaining the variation found.

As for the groupings, four groups were formed in the two rounds (PCA1 and PCA2; PCA1 and PCA3). The first group refers to all individuals from Barcarena (PA), the second group brings together individuals collected in Maranhão (Baixada and Barreirinhas), the third includes individuals from the population of Paraná (Santa Terezinha de Itaipu) and the fourth refers to specimens collected in São Paulo (Ilha Solteira), as shown in figure 8.

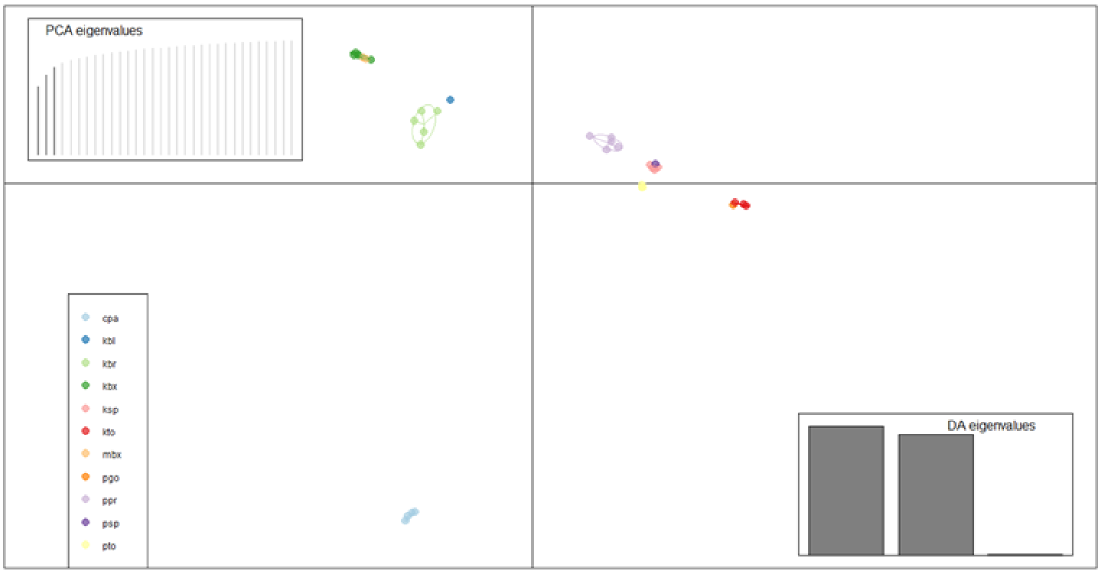
Fig.8 PCA analysis (A) PCA1 and PCA2, (B) PCA1 and PCA3 using 9,975 SNPs, with all sequenced individuals of the species *Cichla monoculus*, *Cichla kelberi* and *Cichla piquiti*. The populations (*Cichla* spp. /PA -cpa; *Cichla kelberi*/Balsas-kbl; *Cichla kelberi*/Barreirinhas-kbr; *Cichla kelberi*/Baixada-kbx; *Cichla kelberi*/SP-ksp; *Cichla kelberi*/TO- kto; *Cichla monoculus*/Baixada -mbx; *Cichla piquiti*/GO-pgo; *Cichla piquiti*/PR-ppr, *Cichla piquiti*/SP-psp; *Cichla piquiti*/TO- pto).





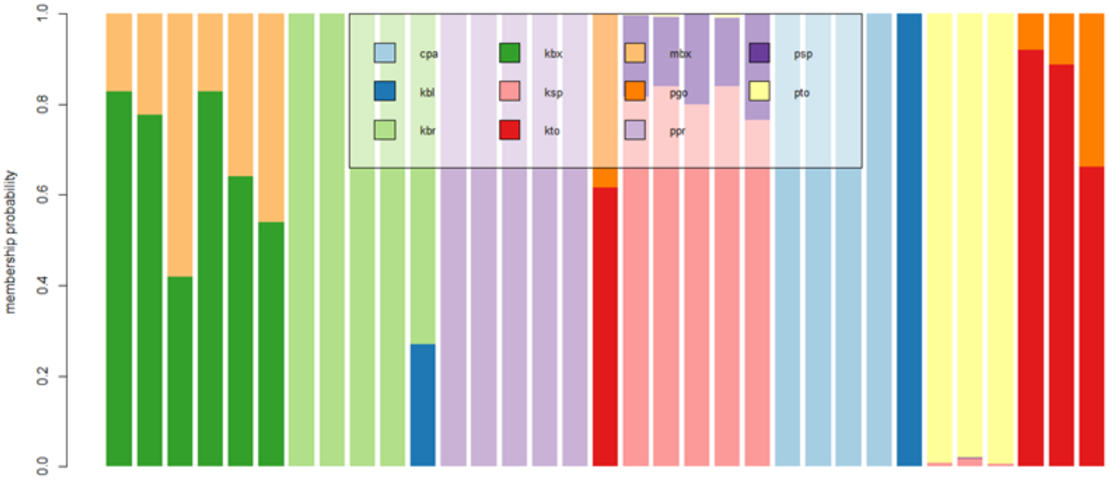
DPCA maximized the variances between the sampled populations, thus obtaining the allocation of samples in each population, suggesting mixing in the populations of *C. kelberi* and *C. monoculus* from Baixada, *C. kelberi* and *C. piquiti* from São Paulo, *C. piquiti* from Goiás and *C. kelberi* from Tocantins (Figure 9)

Fig.9 DAPC dispersion plot of individuals based on principal component analysis, indicating mixing in the populations of *C. kelberi* and *C. monoculus* from Baixada, *C. piquiti* and *C. kelberi* from São Paulo, *C. piquiti* from Goiás and *C. kelberi* from Tocantins



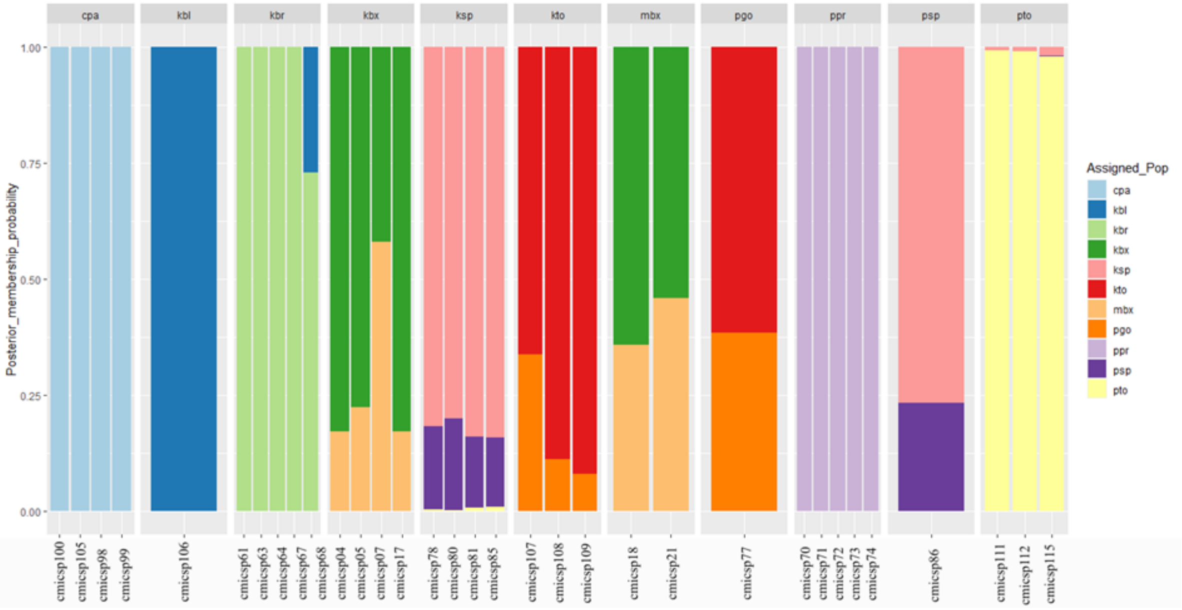
To illustrate the individual's probability of belonging to the population, the complete mebership probability plot was generated, where each sample is a compartment on the x-axis, and the assigned probability of belonging to the population is shown as a stacked bar graph with populations shown in colors, as shown in figure 10

Fig.10 Population structure of *Cichla* spp in native and invaded environments in Brazil. Each bar represents an individual and the colors represent the probability of membership of an individual in the population (cpa, *Cichla* spp.- Pará; kbl, *C. kelberi*- Balsas; kbr, *C. kelberi*-Barreirinhas; kbx, *C. kelberi*-Baixada ; ksp, *C. kelberi*- São Paulo; kto, *C. kelberi*-Tocantins; mbx, *C. monoculus*- Baixada; pgo, *C. piquiti*- Goiás; ppr, *C. piquiti*- Paraná; pto, *C. piquiti*-Tocantins)



By contrasting the attributions of probability of belonging to the population with their original populations, it was verified that the individuals of peacock bass collected in Barcarena/PA were correctly allocated and present a group separate from the others, just like the individual from Balsas, Paraná, Tocantins (*C. piquiti*). The other populations present probabilities of mixing between individuals (Figure 11)

Fig.11 Graph of the contrast between the attribution of probability of association of the population with the origin of the individuals' belonging (cpa, *Cichla* spp.- Pará; kbl, *C. kelberi*- Balsas; kbr, *C. kelberi*-Barreirinhas; kbx, *C. kelberi*- Baixada; ksp, *C. kelberi*- São Paulo; kto, *C. kelberi*-Tocantins; mbx, *C. monoculus*- Baixada; pgo, *C. piquiti*- Goiás; ppr, *C. piquiti*- Paraná; pto, *C. piquiti*-Tocantins)



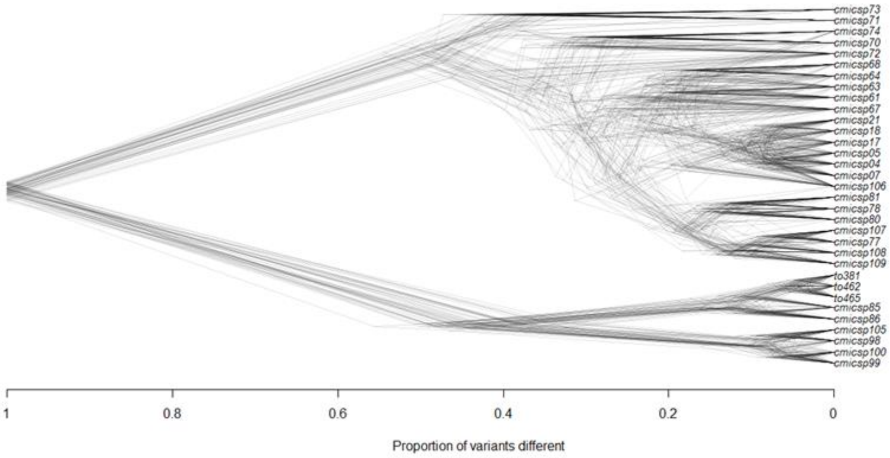
The individual CMICsp68 was collected in Barreirinhas/MA and identified as *C. kelberi*, however it presented less than 75% probability of belonging to this group and more than 25% probability of belonging to the kbl group (*C. kelberi*/ Balsas). On the other hand, individuals from the lowlands have more balanced probabilities of belonging to the species *C. kelberi* and *C. monoculus*.

The invasive populations in São Paulo also present mixed probabilities between species, the individual CMICsp86 presents more than 75% of belonging to the ksp group (*C. kelberi* SP), as well as individuals 78, 80, 81 and 85 present an average of 20% probability for the psp group (*C. piquiti*/SP).

In native populations, no mixed allocations were observed between the species *C. kelberi* and *C. piquiti*, although interestingly individuals from the pto group (*C. piquiti*/TO) have a percentage of allocation to the ksp group (*C. kelberi*/SP). While individuals from the kto group have a percentage ranging from 10 to 40% allocated to the pgo group (*C. piquiti*/GO).

The densiTree reconstruction shows an overall topology very similar to the initial consensus tree: we see 8/9 main clades, and each of the clades is composed of samples from different populations, indicating low geographic population structure. Additionally, we observed how multiple subsets can result in similar patterns to the overall data (Figure 12)

Fig. 12 DensiTree of SNP data from collected native and non-native specimens. The topology corroborates the general data and other analyses, indicating low geographic population structure.



Discussion

Our analyzes demonstrate that the invasive populations have low intrapopulation structure, as expected, since the origin of these invasions comes primarily from native populations, which are sympatric and endemic to the Tocantins-Araguaia basin (Kullander and Ferreira 2006).

The pattern found in native populations (Pará- *Cichla* spp. and Tocantins- *C. kelberi*) suggests species more closely related to each other, agreeing with the findings of Willis et al. (2012), in their clade grouping. Although we did not identify the species collected in Pará, as all individuals were juveniles, we consider here the possibility that they belong to the species *Cichla pinima*, for two factors, the first, due to the record in the collected area, in Barcarena two species have registered occurrence, *C monoculus* and *C. pinima*. Then, the resolute separation of individuals of the species *C. kelberi* and proximity to *C. piquiti*.

In the population structure, we found that native populations presented lower inbreeding coefficient values in relation to non-native populations, indicating greater proximity to Hardy-Weinberg equilibrium and corroborating (Hartl and Clark 1997). Oliveira et al. (2014) also found lower Fis values for native *Cichla* populations compared to introduced ones, using microsatellite data.

While non-native populations, in general, showed a high degree of inbreeding, which is expected due to the founder effect. However, the ppr and mbx populations presented negative values, indicating a high number of heterozygotes. In these populations, a mixture of the species *Cichla kelberi* and *C. monoculus* was observed in mbx, suggesting the possibility of backcrossing of these distinct individuals being responsible for the high number of heterozygotes.

However, in ppr, we have part of the individuals as a differentiated group of *C. kelberi*, possibly from an undocumented invasion, which appears to have a negative Fis due to the high percentage of polymorphic loci, since the number of private alleles is not so significant, corroborating the data from Nobrega et al. (2023)

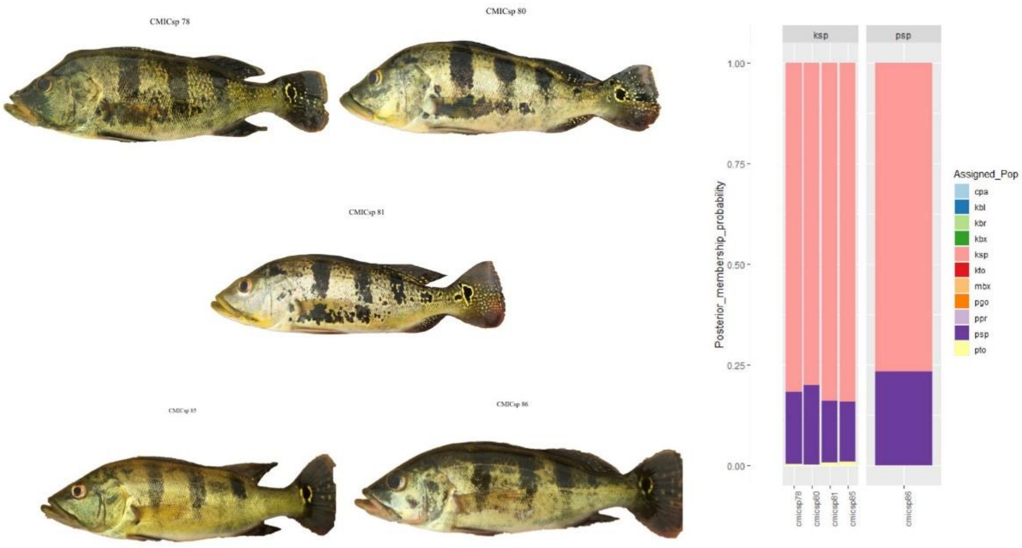
In relation to the genetic differentiation index, we have the least differentiated populations ppr and ksp, pgo and ksp, kbl and ppr, kbl and ksp, mbx and kbx, in ascending order. The kbx and mbx populations follow the other results, showing less differentiation, as they are closer species and because the individuals present a more homogeneous mixture of taxa.

For the individuals collected in São Paulo, belonging to the species *C. kelberi* and *C. piquiti*, we found a pattern of hybridization. Our data demonstrate this pattern, with some peculiar reservations due to the more comprehensive methodology and collections, similar to a study that evaluated hybridization in invasive populations of *C. piquiti* and *C. kelberi* in the Lajes reservoir, verifying the existence of hybrids of these species, with morphological characteristics of both parents (Nobrega et al. 2023).

Our individuals presented intermediate characteristics, which prove our initial hypothesis, that the variation in the collected specimens was not solely attributed to the phenotypic plasticity of a single species, corroborating the proposal by Willis et al. (2012).

Figure 13 shows the morphological patterns of the analyzed and sequenced specimens from the non-native populations of São Paulo, as well as the probability of belonging to the proposed grouping.

Fig.13 Morphotypes of non-native *Cichla kelberi* and *Cichla piquiti*, collected in the Ilha Solteira (SP).



On the other hand, crossing between genetically distinct populations can increase their genetic diversity by introducing alleles previously non-existent in a given species, alleviating the founder effect, generating new genotypes and increasing levels of heterozygosity (Lee 2002, Zalapa et al . 2010). A possible result of higher levels of heterozygosity is heterosis (hybrid vigor) (Birchler et al. 2006), which is the increase in biological and behavioral processes such as growth, fecundity, survival and aggressiveness, of hybrid individuals, when compared to parental individuals (Wohlfarth 1993; Rahman et al. 1995).

Furthermore, new genotypes and phenotypes, as well as intermediate characteristics of hybrids, may allow them to occupy niches not previously enjoyed by parental species (Selz and Seehausen 2019).

However, we found that there is dominance of *Cichla kelberi* in invasive populations, even when they occur in sympatry with *C. piquiti*. This aspect was addressed by Andrade and Pelicice (2022), when they verified the opposite pattern in native populations. This demonstrates that under the conditions of new environments, the greater swimming power and body size associated with a longer reproductive period are not sufficient for the dominance of *C. piquiti*.

Corroborating the larger sampling of *C. kelberi* in this study, since capturing *C. piquiti* required greater effort. In the population of the Baixada maranhense, for example, where *C. kelberi* and *C. monoculus* co-occur, the same pattern repeats itself.

Although our data demonstrate that the genetic mixture of these taxa was preponderant in all individuals collected. This is due to the fact that *C. monoculus* is a taxon derived from *C. kelberi*, which is dominant in these environments, associated with the niche partition that occurs naturally between the species (Andrade and Pelicice 2022)

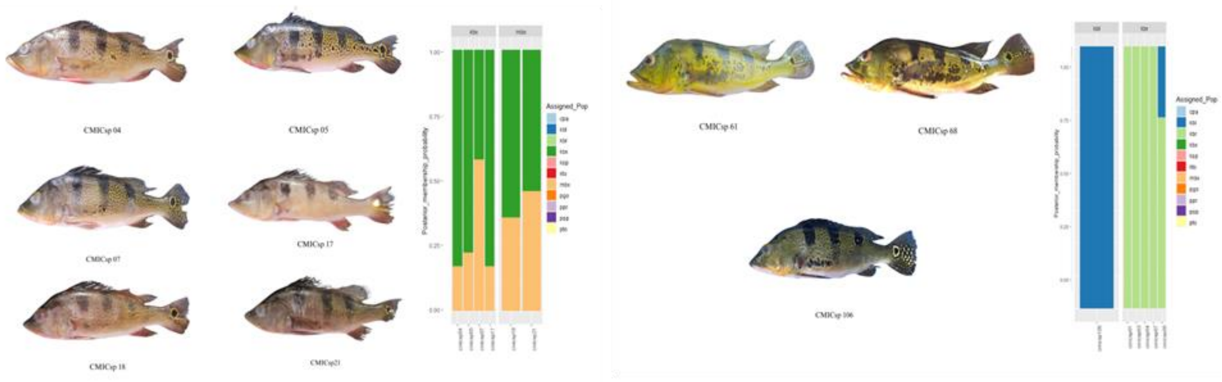
A study carried out in lakes in the lowlands of Maranhão concluded that *C. monoculus* presents a positive allometry for the weight-length relationship, spawning period during the rainy season and sexual maturation size of 16.8 cm (Carvalho et al. 2021). In São Paulo, a study of the reproduction of *C. piquiti* demonstrated the occurrence of two peaks, one in March and the other considerably higher in the months of November and December (Costa et al. 2008)

Unlike what occurs in the natural environment, where *C. kelberi* has its reproductive peak between the months of September and December while *C. piquiti* has its reproductive peak between the months of January and July (Andrade and Pelicice 2022), thus contributing as a barrier reproductive, which in the case of peacock bass, appears to be prezygotic due to the number of equal chromosomes among others as a shared ancestral character.

Mixing in populations from Baixada maranhense was also expected, as studies based on multilocus data (Willis et al. 2012) have proposed that the species *C. monoculus*, *C. kelberi* and *C.* nigromaculata should be synonymized with *C.* ocellaris. However, the species *C. piquiti* and *C. kelberi* also showed a considerable degree of mixing in the São Paulo population. These species are considered more distant, and even in cases of evaluating introgression between *C. monoculus* and *C. kelberi*, the external group used is *Cichla piquiti* (Diamante et al. 2017)

Figure 14 shows the morphological patterns of the analyzed and sequenced specimens from the non-native populations of Maranhão, as well as the probability of belonging to the proposed grouping.

Fig.14 Morphotypes of non-native *Cichla kelberi* and *Cichla monoculus*, collected in the Maranhão river basins, on the left *C. monoculus* and *C. kelberi* from Baixada Maranhense and on the right *Cichla kelberi* from Barreirinhas and Balsas



The pattern found in these populations indicates that Baixada Maranhense presents individuals with mixed characteristics of the species *C. monoculus* and *C. kelberi*, in addition to the proportions of belonging to the groups being more balanced. While in the Lençóis Maranhenses and Balsas National Park, despite different morphotypes, only *C. kelberi* was found. This finding can be explained by some factors, such as the time of introduction being longer in the Baixada (around 13 years), while Lençóis records a more recent occurrence (Monroe et al. 2023).

The species *C. monoculus* and *C. kelberi* are more closely related than *C. kelberi* and *C. piquiti*, for example, elucidating the more balanced mixture in individuals. Although in Lençóis, we have a population with different morphotypes, the group is more homogeneous, probably due to the recent introduction and a single taxon.

Our coverage network exemplifies the argument proposed by Willis et al. (2012) in synonymizing the species *C. kelberi* and *C. monoculus*, and the shared characteristics are probably plesiomorphic, for example, all species have mouth and head morphology appropriate for piscivory (Stiassny 1987).

Another suspicion confirmed in our results is that non-native populations are differentiated from native populations (Suplementary data S2), regardless of the time these populations were founded, such as Balsas, Barreirinhas and Baixada, which constitute more recent populations than in Paraná, São Paulo and Goiás. For the populations of São Paulo and Goiás, interestingly we found interspecific similarities with native individuals.

Cichla species as discrete entities have been increasingly questioned. A conflict between evolutionary significantly units and described species is still under discussion (Willis et al. 2012, Willis et al. 2017)

The separation of the *Cichla* genera into two clades by Willis et al. (2012) states that among species, despite there being deep coalescence in mitochondrial DNA lineages, there is little evidence of reproductive isolation even among the most divergent lineages, which allows us to understand that, despite non-native populations being differentiated from native ones and other populations, is not sufficient to designate new species.

According to Kullander and Ferreira (2006), the morphological differences between (*C. monoculus* + *C. kelberi*) and *C. ocellaris* are concentrated in the continuity or not of the lateral line, presence/absence of occipital bar and absence/presence of vertical bars. *C. monoculus* and *C. kelberi* concentrate differences in the number of scales on the lateral and median line, spots on the caudal, pelvic and anal fins. *C. piquiti* is more distinct, as it has pre-spots and five vertical bars.

Although meristic characters are taxonomically valid and widely used, including by Kullander and Ferreira (2006) in the review of the genus *Cichla*, it is necessary to highlight that the presence of an occipital bar, vertical bars, spots on the fins anal and caudal as well as scales on the lateral and median lines of the fish presented overlap in the individuals collected.

The body shape in the genus *Cichla* is a character with a high degree of conservation (Kullander and Ferreira 2006), but our data demonstrate that changes in shape are related to the environment. This aspect is not only associated with shape, reflected in some genes, such as the visual system (Escobar- Camacho et al. 2019).

Sá et al. (2004) attributed differences in the number of scales of a species of Astyanax in two streams in the São Francisco basin to salinity, dissolved oxygen and water temperature. In addition to changes in the color pattern, which is associated with territorial behavior, spawning and sexual maturation of cichlids (Barlow 2000)

Furthermore, the time of introduction of these invaders is different, introductions in the south and southeast date back to 1950 (Oliveira et al 1986) and 1980, while the Baixada maranhense register these invaders after the year 2010, since Almeida-Funo et al. (2010) only record the introduction of Malaysian shrimp (*Macrobrachium rosembergii*) as an environmental tensor.

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DISCUSSÃO INTEGRADORA

A partir de nossas hipóteses e métodos aplicados neste trabalho de tese para investigação do status da bioinvasão ocasionada pela introdução de espécies não nativas do gênero *Cichla* spp. e *Oreochromis* spp. pudemos verificar que as bacias hidrográficas do Maranhão apresentam pelo menos uma espécie de cada gênero supracitado. Além de verificarmos que na Baixada maranhense o estabelecimento de *Cichla monoculus* e *Cichla kelberi*, associado à presença de *Oreochromis niloticus* e *Oreochromis mossambicus* influenciam diretamente na diversidade dos lagos interconectados da Amazônia maranhense. Aspecto este que já está agravado pela presença de barramentos na região, mudança nos ciclos hidrológicos, aumento da pressão de pesca e qualidade ambiental.

Outro ambiente protegido, no estado, que apresenta essas espécies não nativas é o Parque Nacional dos Lençóis Maranhenses, onde pode-se verificar que após a introdução dessas espécies houve redução da diversidade alfa, baseado nos estudos pretéritos realizados no local. Quanto à diversidade, ocorrência e padrões de evolução de tucunarés não nativos (*Cichla kelberi, Cichla monoculus e Cichla piquiti*), foi possível apresentar um panorama das populações de diferentes regiões do Brasil e comparar com as populações nativas do Tocantis. Além do grupo externo nativo do Pará.

Verificamos que as populações não nativas estão diferenciadas das populações nativas, e apesar de apresentarem menor diversidade genética estão estabelecidas e apresentando comportamentos reprodutivos com mecanismos que possibilitem a permanência e ampliação nos mais diversos habitats. A exemplo de possíveis híbridos de *C. piquiti* e *C. kelberi* nas populações de Ilha Solteira, diferenciação de *Cichla monoculus* do grupo *C. kelberi* na Baixada maranhense proporcionando aumento da diversidade.

Recomendamos o estudo de diversidade e monitoramento das populações nativas, além de medidas protetivas de encontro ao aumento das pressões de propágulo nessas invasões. Fiscalização e garantia do cumprimento da legislação quanto a proibição e punição em casos de introdução de novos indivíduos à essas populações.

Estudos posteriores que investiguem patógenos, fusão invasora, reprodução, ecologia e seleção natural devem ser estimulados e financiados para uma tomada de decisão gestão mais eficientes.

CONCLUSÃO

- Identificou-se, nas bacias hidrográficas do Maranhão, duas espécies de Tucunarés: *Cichla kelberi* e *Cichla* monoculus, e duas espécies de tilápia*: Oreochromis niloticus e Oreochromis mossambicus*;
- A origem das espécies introduzidas corresponde à Bacia do Tocantins-Araguaia para os tucunarés e para as tilápias, as bacias do rio Nilo e do sudeste da África;
- Nas bacias hidrográficas do Maranhão, a diversidade de espécies nativas tem diminuído em contraste com as espécies não nativas;
- Verificou-se diferenciação genética e morfológica das populações de tucunarés nativos e não nativos, embora não suficientes para designar novas espécies;
- A diversidade genética foi menor nas populações de tucunarés não nativos em comparação com as populações nativas;
- Indícios de hibridização de grupos distintos de tucunarés introduzidos nas áreas estudadas podem ter sido gerados por mecanismos de mudanças no período reprodutivo, com consequente retrocruzamento tanto em áreas protegidas quanto em áreas impactadas;

APÊNDICES

APÊNDICE A - Material Suplementar elaborado para o manuscrito "Inventory reveals non-native species and variation in spatial-temporal dynamics of fish community in a Brazilian protected area", publicado no Brazilian Journal of Biology (A3)

Table S1. Details of surveys conducted in 2016/2020 in Lençóis Maranhenses National Park: location name and coordinates, habitat features, presence of non native species and habitat features.

Site no	Municipality	Locality	Latitude	Longitude	Habitat Characteristics	Presence of *Oreochromis niloticus* (Yes/No)	Presence of *Oreochromis mossambicus* (Yes/No)	Presence of *Cichla monoculus* (Yes/No)
1	Primeira Cruz	Cassó lagoon	-2.964.056	-43.254.972	Vegetation on banks; sandy substrate, clean water, lentic	Yes	No	No
2	Humberto de Campos	Periá river	-2.605.083	-43.450.389	Vegetation on the banks; sandy substrate, lotic	Yes	Yes	No
3	Santo Amaro	Lake Santo Amaro	-2.472444	-43.237.278	Vegetation on the banks; muddy substrate, lentic	Yes	Yes	No
4	Tutóia	Passagem river	-2.868	-42.251.333	Vegetation on the banks; muddy substrate and turbid water, lotic	Yes	No	No
5	Tutóia	Lagoinha	-2.786167	-42.336417	Vegetation on the banks; sandy substrate, lentic	Yes	No	No
6	Tutóia	Tamancão stream	-2.794361	-42.364194	Vegetation on the banks; muddy substrate and turbid water, lotic	Yes	No	No
7	Tutóia	Passagem dos bois stream	-2.79675	-42.373111	Vegetation on the banks; muddy substrate and clear water, lotic	Yes	No	No
8	Barreirinhas	Prainha do amor	-2.79475	-42.824333	Sandy soil; with vegetation on the banks, lentic	Yes	No	No

9	Santo Amaro	Queimada dos britos/ Negro river	-2.506.111	-43.059194	Vegetation on the banks; muddy substrate and turbid water, lotic	Yes	No	No
10	Santo Amaro	Baixa grande	-2.761889	-42.286583	Sandy soil; with vegetation on the banks, lentic	Yes	No	No
11	Santo Amaro	Betânia	-2.773306	-42.838361	Vegetation on the banks; muddy substrate and turbid water, lotic	Yes	Yes	No
11	Santo Amaro	Betânia	-2.773306	-42.838361	Vegetation on the banks; muddy substrate and turbid water, lotic	Yes	Yes	No
12	Barreirinhas	Boa esperança lagoon	-2.676139	-42.033972	Vegetation on the banks; muddy substrate and turbid water	Yes	No	No
13	Barreirinhas	Sucuruju river	-2.630278	-42.73625	Vegetation on the banks; muddy substrate and turbid water, lotic	Yes	No	No
14	Barreirinhas	São Domingos/ Preguiça river	-2.755194	-42.826389	Vegetation on the banks; sandy substrate and turbid water, lotic	Yes	No	Yes
15	Tutóia	Dendê stream	-2.797.722	-42.336.417	Vegetation on the banks; sandy substrate and clear water, lotic	Yes	No	No
16	Tutóia	Cana Brava	-2.733.972	-42.315.806	Vegetation on the banks; sandy substrate and clear water, lotic	Yes	No	No
17	Tutóia	Bom gosto	-2.834.778	-42.307.889	Vegetation on the banks; sandy substrate and clear water, lotic	Yes	No	No
18	Tutóia	Barro duro	-2.897.722	-42.308.139	Vegetation on the banks; muddy	Yes	No	No

					substrate and turbid water			
19	Tutóia	Delta	-2.734.583	-42.591.194	Small pond and sandy sediment	Yes	No	No
20	Tutóia	Santa Rosa do Jardim	-2.788.167	-4.235.225	Clear and crystalline water; sandy substrate	Yes	No	No

APÊNDICE A (Cont.)

Table S2. Comparison of species richness between this study, Brasil (2003) and Brito et al. (2019).

Fish species recorded for the Lençóis Maranhenses National Park	Ministry of the Environment (2003)	Brito et al. (2019)	This work
Classe ACTINOPETRYGII			
Ordem CLUPEIFORMES			
Família Engraulidae			
Lycengraulis batesii (Günther, 1868)	**X**		**X**
Ordem CHARACIFORMES			
Família Acestrorhynchidae			
Acestrorhynchus falcatus (Bloch, 1794)	**X**	**X**	**X**
Família Anostomidae			
Leporinus friderici (Bloch, 1794)	**X**		**X**
Família Characidae			
Astyanax cf. lacustris	**X**	**X**	**X**
Brachychalcinus parnaibae Reis, 1989	**X**	**X**	
Hyphessobrycon piorskii Guimarães, Brito, Feitosa, Carvalho-Costa & Ottoni 2018	**X**	**X**	
Hemigrammus sp1.	**X**		
Hemigrammus sp.2	**X**		
Hemigrammus sp.3			
Moenkhausia cotinho Eigenmann, 1908	**X**	**X**	
Moenkhausia oligolepis (Günter, 1864)		**X**	
Moenkhausia sp.		**X**	**X**
Poptella compressa (Günther 1864)	**X**		**X**
Serrapinus sp.	**X**		
Família Curimatidae			
Steindachnerina notonota (Miranda Ribeiro, 1937)	**X**		
Curimatopsis aff. cryptica	**X**	**X**	**X**
Família Erythrinidae			

Hoplias malabaricus (Bloch, 1794)	**X**	**X**	**X**
Hoplerythrinus unitaeniatus (Agassiz, 1829)	**X**	**X**	**X**
Família Iguanodectidae			
Bryconops cf. affinis	**X**	**X**	**X**
Bryconops cf. melanurus	**X**	**X**	
Família Lebiasinidae			
Nannostomus beckfordi Günther, 1872	**X**	**X**	**X**
Família Serrasalmidae			
Serrasalmus rhombeus (Linnaeus, 1766)	**X**		**X**
Metynnis lippincottianus (Cope, 1870)	**X**	**X**	**X**
Ordem CICHILIFORMES			
Família Cichlidae			
Apistogramma piauiensis Kullander, 1980	**X**	**X**	**X**
Aequidens tetramerus (Heckel, 1840)	**X**	**X**	
Cichlasoma cf. zarskei	**X**	**X**	**X**
Cichla monoculus Spix & Agassiz 1831.			**X**
Cichla kelberi Kullander & Ferreira 2006.			**X**
Crenicichla brasiliensis (Bloch 1792)	**X**	**X**	**X**
Oreochromis sp.	**X**	**X**	
Oreochromis mossambicus (Peters 1852)			**X**
Oreochromis niloticus (Linnaeus 1758)	**X**		**X**
Satanoperca jurupari (Heckel, 1840)	**X**	**X**	**X**
Ordem CYPRINODONTIFORMES			
Família Poeciliidae			
Poecilia vivipara Bloch & Schneider, 1801	**X**		
Poecilia sarrafae Bragança & Costa, 2011	**X**	**X**	
Família Rivulidae			
Melanorivulus cf. parnaibensis		**X**	
Anablepsoides vieirai Nielsen 2016		**X**	
Ordem GYMNOTIFORMES			
Família Apteronotidae			

Apteronotus albifrons (Linnaeus, 1766)		**X**	
Família Gymnotidae			
Gymnotus carapo Linnaeus, 1758	**X**	**X**	**X**
Família Hypopomidae			
Brachyhypopomus sp.	**X**	**X**	
Família Sternopygidae			
Eigenmannia virescens (Valenciennes, 1836)	**X**	**X**	**X**
Sternopygus macrurus (Bloch & Schneider, 1801)	**X**	**X**	**X**
Ordem MUGILIFORMES			
Família Mugilidae			
Mugil curema Valenciennes, 1836	**X**	**X**	**X**
Ordem PERCIFORMES			
Família Gerreidae			
Eucinostomus cf. argenteus	**X**		
Família Polynemidae			
Polydactylus virginicus (Linnaeus, 1758)	**X**		
Família GOBIIFORMES			
Ordem GOBIIDAE			
Awaous tajasica (Lichteinstein, 1822)	**X**		
Ordem PLEURONECTIFORMES			
Família Achiridae			
*Achirus achiru*s (Linnaeus, 1758)	**X**		**X**
Ordem SILURIFORMES			
Família Auchenipteridae			
Trachelyopterus galeatus (Linnaeus, 1766)	**X**	**X**	**X**
Família Callichthyidae			
Callichthys callichthys (Linnaeus, 1758)		**X**	
Megalechis thoracata (Valenciennes,1840)		**X**	
Família Heptapteridae			
Pimelodella parnahybae Fowler, 1941	**X**	**X**	**X**
Família Loricariidae			

Loricaria cf. parnahybae	**X**	**X**	**X**
Hypostomus johnii (Steindachner, 1877)	**X**	**X**	**X**
Ordem SYNBRANCHIFORMES			
Família Synbranchidae			
Synbranchus marmoratus Bloch, 1795	**X**	**X**	**X**

APÊNDICE A (Cont.)

Table S3. Details of collection campains conducted in 2016/2020 in Lençóis Maranhenses region: Campain and date of capture.

Site no	Municipality	Locality	Latitude	Longitude	Habitat Characteristics	Collection campaign
1	Primeira Cruz	Cassó lagoon	-2.964.056	-43.254.972	Vegetation on banks; sandy substrate, clean water, lentic	10/03/2016
2	Humberto de Campos	Periá river	-2.605.083	-43.450.389	Vegetation on the banks; sandy substrate, lotic	11/03/2016
3	Santo Amaro	Lake Santo Amaro	-2.472444	-43.237.278	Vegetation on the banks; muddy substrate, lentic	15/03/2017
4	Tutóia	Passagem river	-2.868	-42.251.333	Vegetation on the banks; muddy substrate and turbid water, lotic	06/07/2017
0	Tutóia	Lagoinha	-2.786167	-42.336417	Vegetation on the banks; sandy substrate, lentic	06/07/2017
6	Tutóia	Tamancão stream	-2.794361	-42.364194	Vegetation on the banks; muddy substrate and turbid water, lotic	07/07/2017
7	Tutóia	Passagem dos bois stream	-2.79675	-42.373111	Vegetation on the banks; muddy substrate and clear water, lotic	07/07/2017
8	Barreirinhas	Prainha do amor	-2.79475	-42.824333	Sandy soil; with vegetation on the banks, lentic	08/07/2017
9	Santo Amaro	Queimada dos britos/ Negro river	-2.506.111	-43.059194	Vegetation on the banks; muddy substrate and turbid water, lotic	16/03/2017
10	Santo Amaro	Baixa grande	-2.761889	-42.286583	Sandy soil; with vegetation on the banks, lentic	16/03/2017
11	Santo Amaro	Betânia	-2.773306	-42.838361	Vegetation on the banks; muddy substrate and turbid water, lotic	16/03/2017
12	Barreirinhas	Boa esperança lagoon	-2.676139	-42.033972	Vegetation on the banks; muddy substrate and turbid water	09/07/2017

13	Barreirinhas	Sucuruju river	-2.630278	-42.73625	Vegetation on the banks; muddy substrate and turbid water, lotic	09/07/2017
14	Barreirinhas	São Domingos/ Preguiça river	-2.755194	-42.826389	Vegetation on the banks; sandy substrate and turbid water, lotic	09/07/2017
15	Tutóia	Dendê stream	-2.797.722	-42.336.417	Vegetation on the banks; sandy substrate and clear water, lotic	
16	Tutóia	Cana Brava	-2.733.972	-42.315.806	Vegetation on the banks; sandy substrate and clear water, lotic	
17	Tutóia	Bom gosto	-2.834.778	-42.307.889	Vegetation on the banks; sandy substrate and clear water, lotic	
18	Tutóia	Barro duro	-2.897.722	-42.308.139	Vegetation on the banks; muddy substrate and turbid water	
19	Tutóia	Delta	-2.734.583	-42.591.194	Small pond and sandy sediment	06/07/2017
20	Tutóia	Santa Rosa do Jardim	-2.788.167	-4.235.225	Clear and crystalline water; sandy substrate	07/07/2017

APÊNDICE B- Material Suplementar elaborado para o manuscrito “Ichthyofauna composition and bioinvasion in the Baixada maranhense environmental protection area, a ramsar site in Brazil”, submetido em Scientia Plena (A4)

S1- Table of species of Baixada Maranhense Monroe et al.

ORDER	FAMILY	SPECIE	VIANA	CAJARI	AQUIRI	COQUEIRO	ITANS	Total
Myliobatiformes	Potamotrygonidae	*Potamotrygon motoro* (Müller & Henle 1841)	3	1				4
Elopiformes	Megalopidae	*Megalops atlanticus* Valenciennes 1847	3					3
Clupeiformes	Engraulidae	*Pterengraulis atherinoides* (Linnaeus 1766)	40	27				67
		Cetengraulis edentulus (Cuvier 1829)	266	83				349
		Lycengraulis batesii (Günther 1868)	3					3
Characiformes	Erythrinidae	*Hoplerythrinus unitaeniatus* (Spix & Agassiz 1829)	4		9	119	8	140
		Hoplias malabaricus (Bloch 1794)	37	17	88	526	358	1026
	Cynodontidae	*Cynodon gibbus* (Spix & Agassiz 1829)	62	8	1			71
	Serrasalmidae	*Metynnis lippincottianus* (Cope 1870)	7	49	44	33	33	166
		Pygocentrus nattereri Kner 1858	104	33	96	40	65	338
		Serrasalmus rhombeus (Linnaeus 1766)	27	28	52	9	29	145
	Hemiodontidae	*Hemiodus parnaguae* Eigenmann & Henn 1916			5	17		22
	Anostomidae	*Leporinus friderici* (Bloch 1794)	9	3	19		4	35
		Schizodon dissimilis (Garman 1890)	86	33	46	5	36	206
	Curimatidae	*Curimata macrops* Eigenmann & Eigenmann 1889.	38	68	230			336

		Psectrogaster rhomboides (Eigenmann & Eigenmann, 1889)	8	34	23		89	154
	Prochilodontidae	*Prochilodus lacustris* Steindachner 1907	22	29	33			84
		Prochilodus nigricans Spix & Agassiz 1829.						
	Thriportheidae	*Triportheus signatus* (Garman 1890)	105	17	12		4	138
	Acestrorhynchidae	*Acestrorhynchus lacustris* (Lütken, 1875)		6	21	2	3	32
	Characidae	*Roeboides margareteae* Lucena 2003	5	19	1			25
Gymnotiformes	Sternopygidae	*Sternopygus macrurus* (Bloch & Schneider 1801)	1	8				9
	Gymnotidae	*Gymnotus carapo* Linnaeus 1758	8	11	3			22
	Rhamphichthyidae	*Rhamphichthys atlanticus* Triques 1999	2	18	1		1	22
Siluriformes	Callichthyidae	*Callichthys callichthys* (Linnaeus 1758)	2					2
		Hoplosternum littorale (Hancock 1828)	5					5
	Loricariidae	*Loricaria cataphracta* Linnaeus 1758	35	38	31		6	110
		Loricariichthys sp.	56	36	8			100
		Pterygoplichthys parnaibae (Weber, 1991)	4		5			9
		Rineloricaria sp.		1				1
	Auchenipteridae	*Ageneiosus ucayalensis* Castelnau 1855	50	3	2			55
		Auchenipterus menezesi Ferraris & Vari 1999	87	64				151
		Hypostomus cf. *plecostomus*	10	19	2			31

		Trachelyopterus galeatus (Linnaeus 1766)	4	18	13	31	58	124
	Doradidae	*Hassar affinis* Steidachner, 1881	43	158	196			397
		Platydoras brachylecis Piorski, Garavello, Arce H. & Sabaj Pérez, 2008	39	55	39		6	139
	Heptapteridae	*Pimelodella parnahybae* Fowler, 1941	1	3				4
		Pimelodella cristata (Muller & Troschel, 1849)	2	24				26
	Pimelodidae	*Hemisorubim platyrhynchos* (Valenciennes 1840)						
		Pimelodus blochii Valenciennes 1840	2	6	9			17
		Pimelodus ornatus Kner 1858		6				6
		Pseudoplatystoma punctifer (Castelnau 1855)	7	4	1			12
		Sorubim lima (Bloch & Schneider 1801)	2	2	3			7
Synbranchiformes	Synbranchidae	*Synbranchus marmoratus* Bloch 1795						
Carangiformes	Achiridae	*Achirus lineatus* (Linnaeus 1758)	1					1
Cichliformes	Cichlidae	*Cichla monoculus* Spix & Agassiz 1831	3	7	23	2	25	60
		Cichla kelberi Kullander & Ferreira 2006						
		Cichlasoma zarskei Ottoni 2011	1	120	163	410		694
		Crenicichla brasiliensis (Bloch 1792)				5		5
		Geophagus parnaibae Staeck & Schindler 2006	14	49	83	2	118	266
		Satanoperca jurupari (Heckel 1840)						
		Oreochromis niloticus (Linnaeus 1758)				1	6	7

		Oreochromis mossambicus (Peters 1852)						
Achanturiformes	Sciaenidae	*Plagioscion squamosissimus* (Heckel 1840)	72	110	48			230

APÊNDICE C - Material Suplementar elaborado para o manuscrito “**Insights revealed by SNPs in non- native *Cichla* populations in Brazil”,** submetido no periódico Biological Invasion

S1- Data from individuals of the genus *Cichla* collected from native and non-native populations in Brazil, with morphological identification and used in geometric morphometry methodologies, COI, 16S genes and SNPs.

ID	Locality	Population	Native/ Non native	Morphology	Morphometry	SNPs
CMI Cso01	Viana	kbx	Non native	kelberi	x	
CMI Csp 02	Itans	mbx	Non native	monoculus	x	
CMI Csp 03	Viana	kbx	Non native	kelberi	x	x
CMI Csp 04	Viana	kbx	Non native	kelberi	x	x
CMI Csp 5	Viana	kbx	Non native	kelberi	x	x
CMI Csp 6	Viana	mbx	Non native	monoculus		
CMI Csp 7	Viana	kbx	Non native	kelberi	x	x
CMI Csp 8	Viana	kbx	Non native	kelberi		
CMI Csp 9	Viana	kbx	Non native	kelberi		
CMI Csp 10	Viana	mbx	Non native	monoculus	x	
CMI Csp 11	Viana	mbx	Non native	monoculus		
CMI Csp 12	Viana	mbx	Non native	monoculus		
CMI Csp 14	Viana	mbx	Non native	monoculus	x	
CMI Csp 15	Viana	mbx	Non native	monoculus		
CMI Csp 16	Viana	mbx	Non native	monoculus		
CMI Csp 17	Penalva	kbx	Non native	kelberi	x	x
CMI Csp 18	Penalva	mbx	Non native	monoculus	x	x
CMI Csp 19	Penalva	kbx	Non native	kelberi	x	
CMI Csp 20	Penalva	kbx	Non native	kelberi	x	
CMI Csp 21	Penalva	mbx	Non native	monoculus	x	x

CMI Csp 22	Penalva	kbx	Non native	Kelberi	x	
CMI Csp 23	Penalva	kbx	Non native	Kelberi	x	
CMI Csp 24	Penalva	kbx	Non native	Kelberi	x	
CMI Csp 25	Penalva	kbx	Non native	Kelberi	x	
CMI Csp 27	Penalva	kbx	Non native	Kelberi		
CMI Csp 28	Penalva	kbx	Non native	Kelberi		
CMI Csp 29	Penalva	kbx	Non native	Kelberi		
CMI Csp 30	Penalva	kbx	Non native	Kelberi		
CMI Csp 31	Penalva	kbx	Non native	Kelberi		
CMI Csp 32	Penalva	kbx	Non native	Kelberi		
CMI Csp 33	Penalva	kbx	Non native	Kelberi		
CMI Csp 35	Penalva	kbx	Non native	Kelberi		
CMI Csp 38	Penalva	kbx	Non native	Kelberi		
CMI Csp 40	Penalva	kbx	Non native	Kelberi		
CMI Csp 58	Penalva	kbx	Non native	Kelberi		
CMI Csp 60	Penalva	kbx	Non native	Kelberi		
CMI Csp 61	Barreirinhas	kbr	Non native	Kelberi		x
CMICsp 62	Barreirinhas	kbr	Non native	Kelberi		
CMI Csp 63	Barreirinhas	kbr	Non native	Kelberi		x
CMI Csp 64	Barreirinhas	kbr	Non native	Kelberi		x
CMI Csp 66	Barreirinhas	kbr	Non native	Kelberi		
CMI Csp 67	Barreirinhas	kbr	Non native	Kelberi		x
CMI Csp 68	Barreirinhas	kbr	Non native	Kelberi	x	x
CMI Csp 77	Aporé/GO	pgo	Non native	Piquiti		x
CMI Csp 78	Ilha Soteira/SP	ksp	Non native	Kelberi		x
CMI Csp 79	Ilha Soteira/SP	ksp	Non native	Kelberi		
CMI Csp 80	Ilha Soteira/SP	ksp	Non native	Kelberi	x	x
CMI Csp 81	Ilha Soteira/SP	ksp	Non native	Kelberi		x
CMI Csp 82	Ilha Soteira/SP	ksp	Non native	Kelberi	x	x
CMI Csp 83	Ilha Soteira/SP	ksp	Non native	Kelberi	x	
CMI Csp 84	Ilha Soteira/SP	ksp	Non native	Kelberi	x	

CMI Csp 85	Ilha Soteira/SP	ksp	Non native	Kelberi	x	x
CMI Csp 86	Ilha Soteira/SP	ksp	Non native	Piquiti	x	x
CMI Csp 87	Ilha Soteira/SP	psp	Non native	Kelberi		
CMI Csp 88	Ilha Soteira/SP	ksp	Non native	Kelberi		
CMI Csp 89	Ilha Soteira/SP	ksp	Non native	Piquiti		

		psp	Non native		x
CMI Csp 90	Ilha Soteira/SP			Piquiti	

CMI Csp 91	Ilha Soteira/SP	psp	Non native	Piquiti	x	
CMI Csp 92	Kelberi	ksp	Non native	Kelberi		
CMI Csp 93	Kelberi	ksp	Non native	Kelberi		
CMI Csp 94	Kelberi	ksp	Non native	Kelberi		
CMI Csp 95	Kelberi	ksp	Non native	Kelberi	x	
CMI Csp 70	Paraná	ppr	Non native	Piquiti		x
CMI Csp 71	Paraná	ppr	Non native	Piquiti	x	x
CMI Csp 72	Paraná	ppr	Non native	Piquiti	x	x
CMI Csp 73	Paraná	ppr	Non native	Piquiti		x
CMI Csp 74	Paraná	ppr	Non native	Piquiti	x	
CMICsp 96	Minas Gerais	kmg	Non native	Kelberi		
CMICsp 97	Para	cpa	Native	Juv.		
CMICsp 98	Para	cpa	Native	Juv.		
CMICsp 99	Para	cpa	Native	Juv.		x
CMICsp 100	Para	cpa	Native	Juv.		x
CMICsp 101	Para	cpa	Native	Juv.		
CMICsp 102	Para	cpa	Native	Juv.		
CMICsp 103	Para	cpa	Native	Juv.		
CMICsp 104	Para	cpa	Native	Juv.		
CMICsp 105	Para	cpa	Native	Juv.		x
CMICsp 106	Balsas	kbl	Non native	Kelberi	x	x
CMICsp 107	Tocantins	kto	Native	Kelberi		x
CMICsp 108	Tocantins	kto	Native	Kelberi		x
CMICsp 109	Tocantins	kto	Native	Kelberi		x
CMICsp 110	Tocantins	pto	Native	Piquiti	x	
CMICsp 111	Tocantins	pto	Native	Piquiti	x	x
CMICsp 112	Tocantins	pto	Native	Piquiti	x	x
CMICsp 113	Tocantins	pto	Native	piquiti	x	
CMICsp 115	Tocantins	pto	Native	piquiti		x
CMICsp 116	Tocantins	pto	Native	Piquiti	x	
CMICsp 117	Tocantins	pto	Native	Piquiti	x	

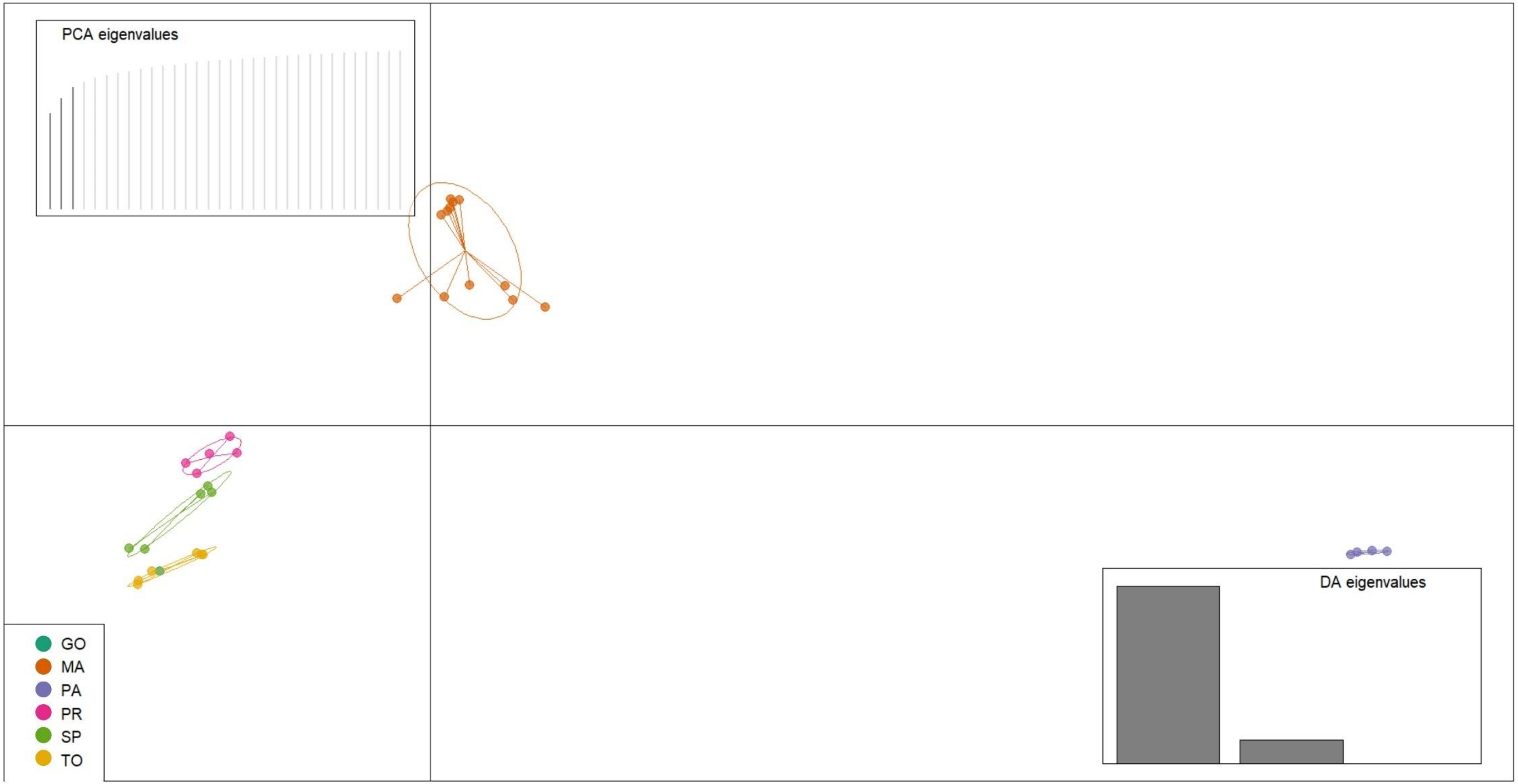
CMICsp 118	Tocantins	pto	Native	Piquiti	x

APÊNDICE C – Cont.

S2- PCA analysis (A) PCA1 and PCA2 using 9,975 SNPs, with all sequenced individuals of the species *Cichla monoculus, Cichla kelberi* and *Cichla piquiti.*

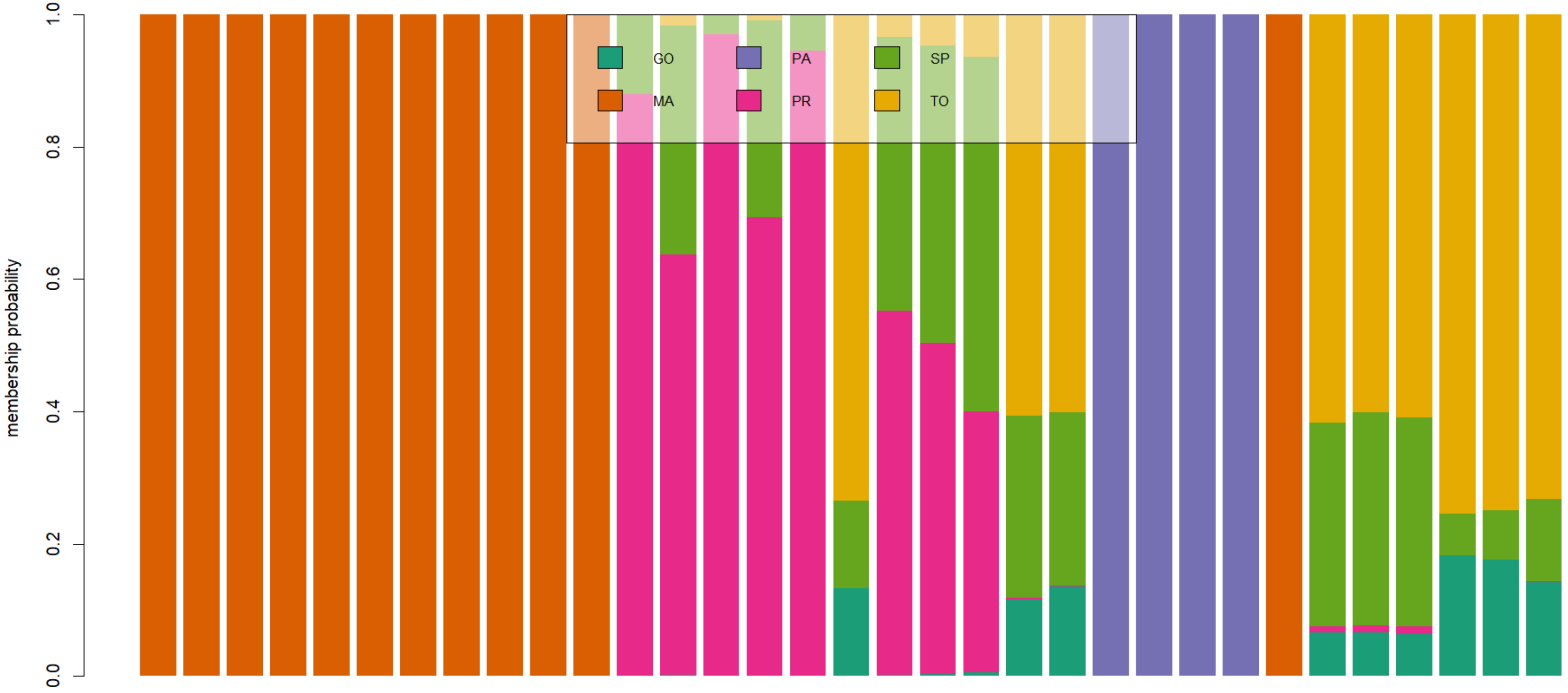
APÊNDICE C – Cont.

S2 - DAPC dispersion plot of individuals based on principal component analysis, indicating indicating differentiation of populations of *Cichla* spp.



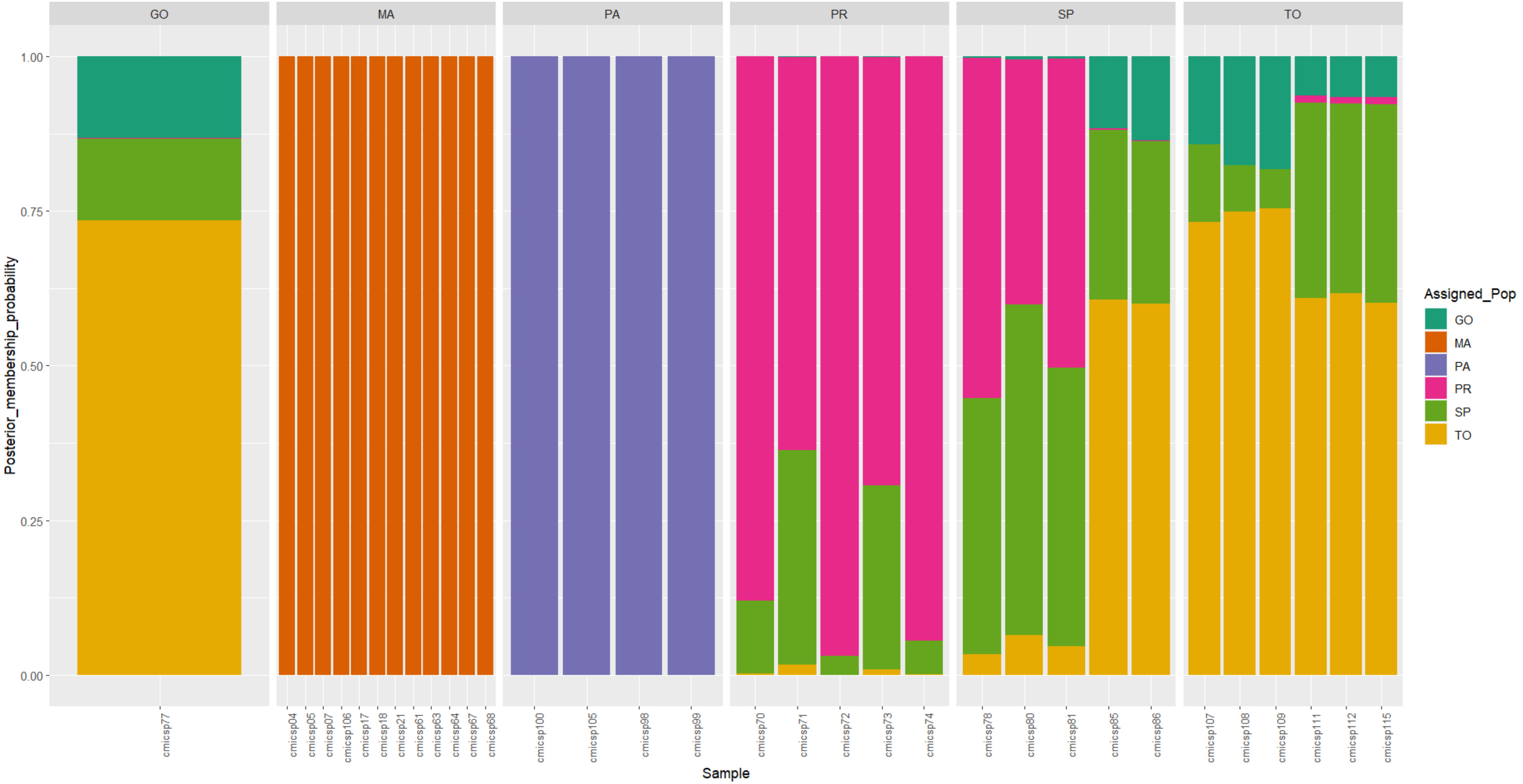
APÊNDICE C – Cont.

S3- Population structure of Cichla spp in native and invaded environments in Brazil. Each bar represents an individual and the colors represent the probability of membership of an individual in the population

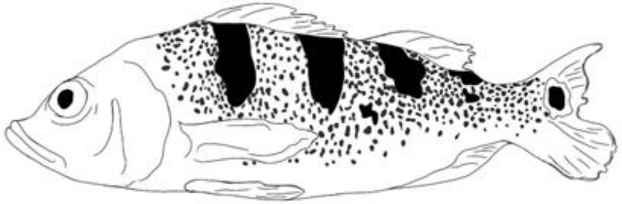


APÊNDICE C – Cont.

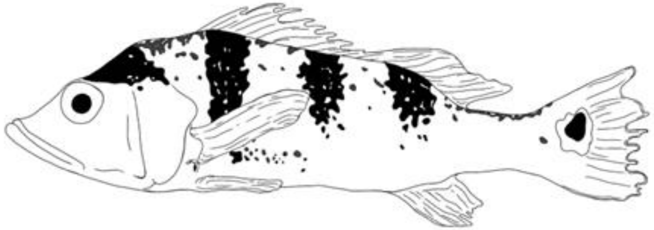
S4- Graph of the contrast between the attribution of probability of association of the population with the origin of the individuals' belonging



APÊNDICE D- Representations of Cichla morphotypes in native and non-native populations, indicating a mixture of species characteristics



CMICsp 04



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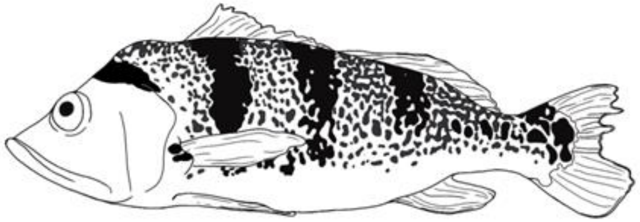

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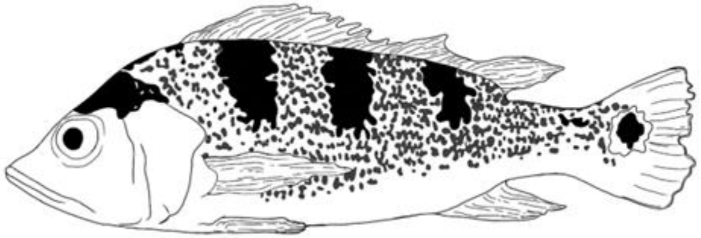

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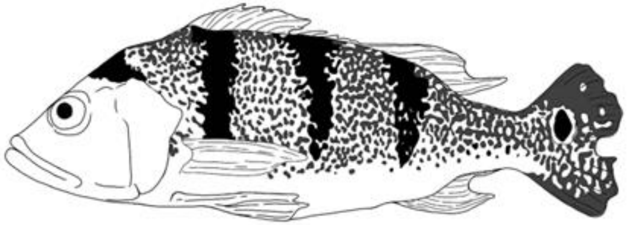

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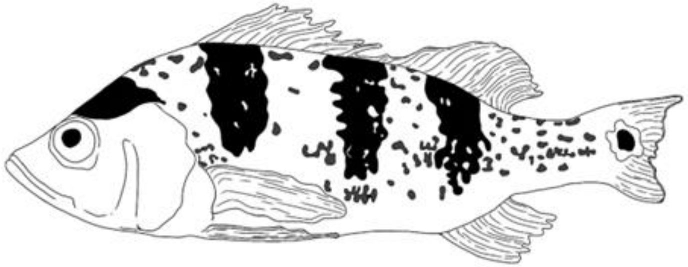

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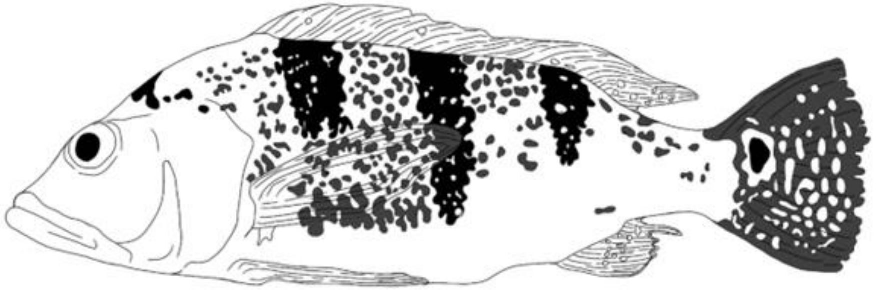
APÊNDICE D- Cont.

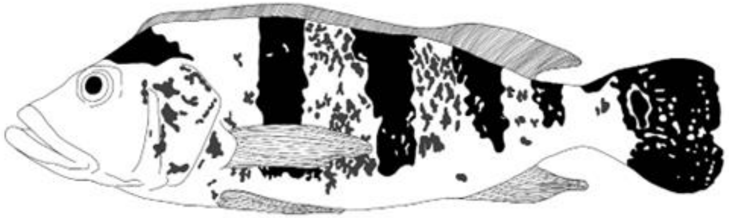
CMICsp 61

CMICsp 68



CMICsp 106

APÊNDICE D- Cont.



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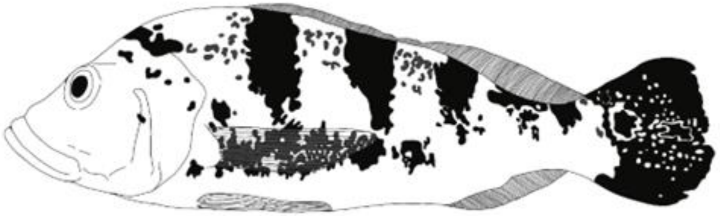
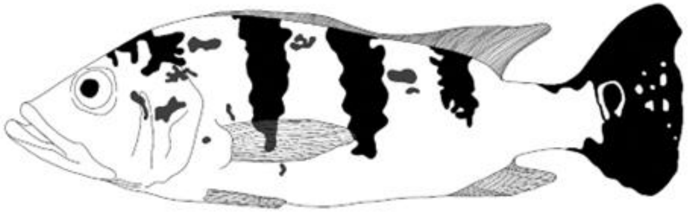


CMICsp 80

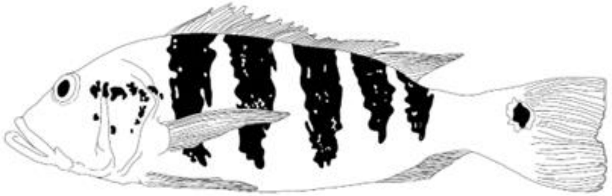
CMICsp 81



CMICsp 83

CMICsp 85

APÊNDICE D- Cont.



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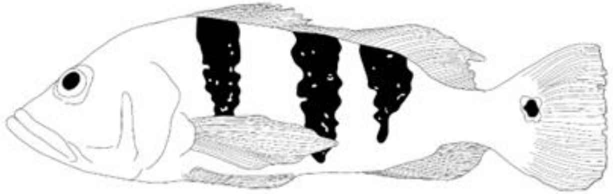

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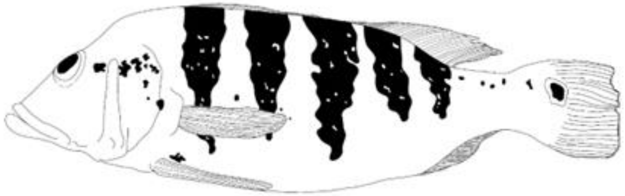

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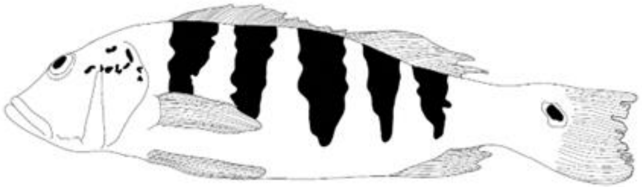
CMICsp 108



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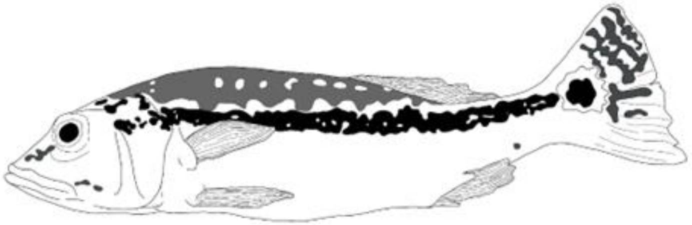
CMICsp 109

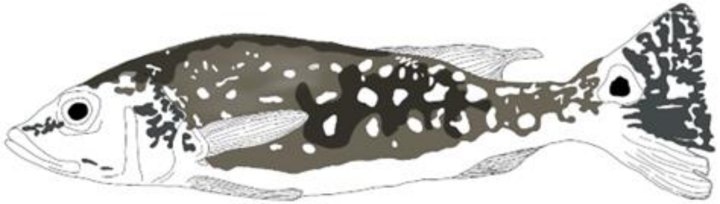
APÊNDICE D- Cont.



CMICsp 98

CMICsp 99

CMICsp 100



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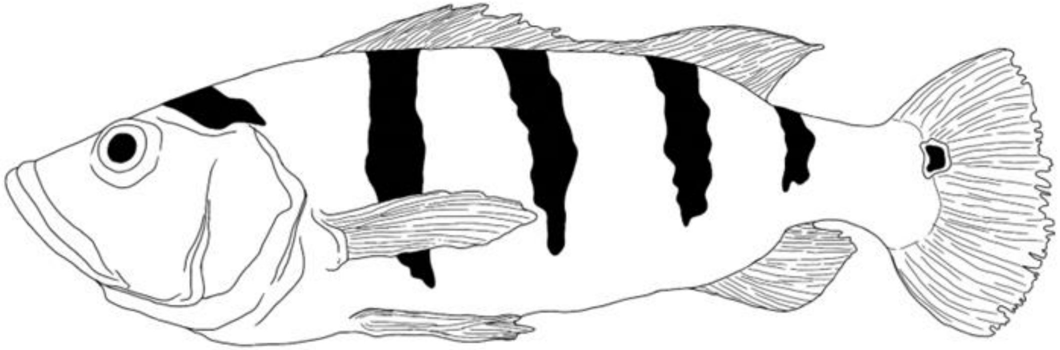

CMICsp 77

ANEXOS

ANEXO A – Artigo: “Inventory reveals non-native species and variation in spatial-temporal dynamics of fish community in a Brazilian protected area”, publicado no Brazilian Journal of Biology (A3)

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Original Article

Inventory reveals non-native species and variation in spatial-temporal dynamics of fish community in a Brazilian protected area

Inventário revela espécies não nativas e variação na dinâmica espaço-temporal da comunidade de peixes em uma área protegida brasileira

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Abstract

The increase in the number of Brazilian protected areas has been progressive and, although it is essential for the conservation of biodiversity, it is important to monitor and properly manage these areas, as they present several cases of biological invasions. The Lençóis Maranhenses constitute the peculiar delta of the Americas and are under the consequences of the bioinvasion of tilapias and peacock bass. Collections were carried out in the Lençóis Maranhenses National Park from March/2016 to November/2020, with the aid of gill nets and cast nets. The species were identified with the help of specialized literature and a historical comparison with previous works was carried out. Cytochrome oxidase subunit I was sequenced to confirm identification of non-native species. We recorded the expansion of the occurrence of *Oreochromis niloticus*, and the first record of the species *Oreochromis mossambicus* and *Cichla monoculus*. A total of 31 species belonging to eight orders, eighteen families and twenty-nine genera were identified, indicating a lag in the diversity of species found in relation to previous studies. After 20 years of the first record of invasive fish, there is an expansion of bioinvasion and new cases that indicate a lack of monitoring and containment measures for the species, indicating the fragility of conservation in the area

Keywords: National Park, Cichlidae, neotropical freshwater fish, species richness.

Resumo

O aumento do número de áreas protegidas brasileiras tem sido progressivo e, embora seja essencial para a conservação da biodiversidade, é importante o monitoramento e o manejo adequado dessas áreas, já que apresentam diversos casos de invasões biológicas. Os Lençóis Maranhenses constituem o peculiar delta das Américas e estão sob as consequências da bioinvasão de tilápias e tucunarés. Foram realizadas coletas no Parque Nacional dos Lençóis Maranhenses no período de março/2016 a novembro/2020, com o auxílio de redes de emalhe e tarrafas. As especies foram identificadas com o auxilio de literatura especializada e uma comparacao historica com trabalhos anteriores foi realizada. O Citocromo oxidase subunidade I foi sequenciado para confirmar a identificação das espécies não nativas. Registramos a expansão da ocorrência de *Oreochromis niloticus*, e o primeiro registro das espécies *Oreochromis mossambicus* e *Cichla monoculus*. Um total de 31 espécies pertencentes a oito ordens, dezoito famílias e vinte e nove gêneros foram identificadas, indicando uma defasagem na diversidade de espécies encontradas em relação a estudos anteriores. Após 20 anos do primeiro registro de peixes invasores, constata-se a expansão da bioinvasão e novos casos que assinalam ausência de monitoramento e de medidas de contenção para as espécies indicando a fragilidade na conservação da área

Palavras-chave: Parque Nacional, Cichlidae, peixes neotropicais, riqueza de espécies.

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Received: April 4, 2023 – Accepted: September 22, 2023

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ANEXO B – Normas do Brazilian Journal of Biology

Finalidade e normas gerais

O Brazilian Journal of Biology publica resultados de pesquisa original em qualquer ramo das ciências biológicas. Estará sendo estimulada a publicação de trabalhos nas áreas de biologia celular, sistemática, ecologia (auto-ecologia e sinecologia) e biologia evolutiva.

A revista publica somente artigos em inglês. Artigos de revisões de temas gerais também serão publicados desde que previamente propostos e aprovados pela Comissão Editorial.

Informações Gerais:

Os originais deverão ser enviados à Comissão Editorial e estar de acordo com as Instruções aos Autores, trabalhos que não se enquadrem nesses moldes serão imediatamente devolvidos ao(s) autor(es) para reformulação.

Os trabalhos que estejam de acordo com as Instruções aos Autores, serão enviados aos assessores científicos, indicados pela Comissão Editorial. Em cada caso, o parecer será transmitido anonimamente aos autores. Em caso de recomendação desfavorável por parte de um assessor, poderá ser solicitada a opinião de um outro. Os trabalhos serão publicados na ordem de aceitação pela Comissão Editorial, e não de seu recebimento.

Os artigos aceitos para a publicação se tornam propriedade da revista.

Preparação de originais

O trabalho a ser considerado para publicação deve obedecer às seguintes recomendações gerais:

Ser digitado em espaço duplo, fonte Times New Roman, tamanho da fonte 12, parágrafo justificado.

O título deve dar uma idéia precisa do conteúdo e ser o mais curto possível. Um título abreviado deve ser fornecido para impressão nas cabeças de página.

Nomes dos autores - As indicações Júnior, Filho, Neto, Sobrinho etc. devem ser sempre antecedidas por um hífen. Exemplo: J. Pereira-Neto. Usar também hífen para nomes compostos (exemplos: C. Azevedo-Ramos, M. L. López-Rulf). Os nomes dos autores devem constar sempre na sua ordem correta, sem inversões. Não usar nunca, como autor ou co-autor nomes como Pereira-Neto J. Usar *e, y, and, et* em vez de & para ligar o último co-autor aos antecedentes.

TODOS OS AUTORES DEVERÃO INFORMAR O ORCID, INCLUÍ-LOS NO ARQUIVO DO ARTIGO.

Os trabalhos devem ser redigidos de forma concisa, com a exatidão e a clareza necessárias para sua fiel compreensão. Sua redação deve ser definitiva a fim de evitar modificações nas provas de conteúdo e final. Os trabalhos (incluindo ilustração e tabelas) devem ser submetidos através da interface de administração do sistema "Submission da SciELO" cujo endereço **www.scielo.br/bjb** **(SUBMISSÃO - ONLINE).**

Serão considerados para publicação apenas os artigos redigidos em inglês.

O Abstract e o Resumo deverão constar no início do trabalho e iniciar com o título traduzido para o idioma correspondente, devem conter as mesmas informações e sempre sumariar resultados e conclusões.

Em linhas gerais, as diferentes partes dos artigos devem ter a seguinte seriação:

1ª página - Título do trabalho. Nome(s) do(s) autor(es). ORCID dos autorees. Instituição ou instituições, com endereço. Indicação do número de figuras existentes no trabalho. Palavras-chave em português e inglês (no máximo 5). Título abreviado para cabeça das páginas. Rodapé: nome do autor correspondente e endereço atual com e-mail.

2ª página e seguintes - Abstract (sem título). Resumo: em português (com título); Introdução, Material e Métodos, Resultados, Discussão, Agradecimentos.

Em separado - Referências, Legendas das figuras, Tabelas e Figuras.

As seguintes informações devem acompanhar todas as espécies citadas no artigo:

- Para zoologia, o nome do autor e da data de publicação da descrição original deve ser dada a primeira vez que a espécie é citada nos trabalhos;
- Para botânica e ecologia, somente o nome do autor que fez a descrição deve ser dada a primeira vez que a espécie é citada nos trabalhos.

O trabalho deverá ter, *no máximo*, 25 páginas, incluindo tabelas e figuras, em caso de Notes and Comments limitar-se a 4 páginas.

A seriação dos itens de Introducão e Agradecimentos só se aplicam, obviamente, a trabalhos capazes de adotá-la. Os demais artigos (como os de Sistemática) devem ser redigidos de acordo com critérios geralmente aceitos na área.

Referências Bibliográficas:

1. Citação no texto: Use o nome e o ano de publicação: Reis (1980); (Reis, 1980); (Zaluar and Rocha, 2000); Zaluar and Rocha (2000). Se houver mais de dois autores, usar "et al."

2. Citações na lista de referências devem estar em conformidade com a norma ***ISO 690/2010***.

No texto, será usado o sistema autor-ano para citações bibliográficas (estritamente o necessário), utilizando-se "***and***" no caso de 2 autores. As referências, digitadas em folha separada, devem constar em ordem alfabética. Nas referências de artigos de periódicos deverão conter nome(s) e iniciais do(s) autor(es), ano, título por extenso, nome da revista (por extenso e em itálico), volume, número, primeira e última páginas. Referências de livros e monografias deverão também incluir a editora e, conforme citação, referir o capítulo do livro. Deve(m) também ser referido(s) nome(s) do(s) organizador(es) da coletânea. Exemplos:

Livro:
LOMINADZE, D.G., 1981. *Cyclotron waves in plasma*. 2nd ed. Oxford: Pergamon Press. 206 p. International series in natural philosophy, no. 3.

Capítulo de livro:
WRIGLEY, E.A., 1968. Parish registers and the historian. In: D. J. STEEL, ed. *National index of parish registers*. London: Society of Genealogists, pp. 15-167.

Artigo de periódico:
CYRINO, J.E. and MULVANEY, D.R., 1999. Mitogenic activity of fetal bovine serum, fish fry extract, insulin-like growth factor-I, and fibroblast growth factor on brown bullhead catfish cells--BB line. *Revista Brasileira de Biologia = Brazilian Journal of Biology*, vol. 59, no. 3, pp. 517-525. http://dx.doi.org/10.1590/S0034-71081999000300017. PMid: 10765463.

Dissertação ou tese:
LIMA, P.R.S., 2004. *Dinâmica populacional da Serra Scomberomorus brasiliensis (Osteichthyes; Scombridae), no litoral ocidental do Maranhã-Brasil*. Recife: Universidade Federal Rural de Pernambuco, 45 p. Dissertação de Mestrado em Recursos Pesqueiros e Aquicultura.

Trabalho apresentado em evento:
RANDALL, D.J., HUNG, C.Y. and POON, W.L., 2004. Response of aquatic vertebrates to hypoxia. In: *Proceedings of the Eighth International Symposium on Fish Physiology*, *Toxicology and Water Quality*, October 12-14, Chongqing, China. Athens, Georgia, USA: EPA, 2006, pp. 1-10.

Referência disponível online:
AGÊNCIA NACIONAL DE ÁGUAS – ANA, 2013 [viewed 4 February 2013]. *Hidro Web: Sistema de Informações hidrológicas* [online]. Available from: http://hidroweb.ana.gov.br/

A Revista publicará um Índice inteiramente em inglês, para uso das revistas internacionais de referência.

As provas serão enviadas aos autores para uma revisão final (restrita a erros e composição) e deverão ser devolvidas imediatamente. As provas que não forem devolvidas no tempo solicitado - 2 dias - terão sua publicação postergada para uma próxima oportunidade, dependendo de espaço.

Material Ilustrativo - Os autores deverão limitar as tabelas e as figuras (ambas numeradas em arábicos) ao estritamente necessário. No texto do manuscrito, o autor indicará os locais onde elas deverão ser intercaladas.

As tabelas deverão ter seu próprio título e, em rodapé, as demais informações explicativas. Símbolos e abreviaturas devem ser definidos no texto principal e/ou legendas.

Na preparação do material ilustrativo e das tabelas, deve-se ter em mente o tamanho da página útil da REVISTA (22 cm x 15,0 cm); (coluna: 7 cm) e a idéia de conservar o sentido vertical. Desenhos e fotografias exageradamente grandes poderão perder muito em nitidez quando forem reduzidos às dimensões da página útil. As pranchas deverão ter no máximo 30 cm de altura por 25 cm de largura e incluir barra(s) de calibração.

As ilustrações devem ser agrupadas, sempre que possível. A Comissão Editorial reserva-se o direito de dispor esse material do modo mais econômico, sem prejudicar sua apresentação.

Recomendações Finais: Antes de remeter seu trabalho, preparado de acordo com as instruções anteriores, deve o autor relê-lo cuidadosamente, dando atenção aos seguintes itens: ORCID dos autores, título reduzido para cabeçalho, indicar o autor correspondente com o respectivo e-mail, correção gramatical, correção datilográfica (apenas uma leitura sílaba por sílaba a garantirá), correspondência entre os trabalhos citados no texto e os referidos na bibliografia, tabelas e figuras em arábicos, correspondência entre os números de tabelas e figuras citadas no texto e os referidos em cada um e posição correta das legendas.

ANEXO C- Comprovante de submissão da Revista Scientia Plena

Outlook

[SP] Agradecimento pela submissão

De Carlos Alexandre Borges Garcia via Revista SCIENTIA PLENA <pen-bounces@emnuvens.com.br>
Data Sáb, 02/03/2024 17:19
Para Thércia Monroe <tgrmonroe@outlook.com>

Prezado(a) Thércia Monroe,

Agradecemos a submissão do seu manuscrito "Composição da Ictiofauna e Bioinvasão no sitio Ramsar da Baixada Maranhense: Diversidade e estrutura em área protegida invadida por ciclídeos" para a revista Scientia Plena. Através da interface de administração do sistema, utilizado para a submissão, será possível acompanhar o progresso da avaliação do documento dentro do processo editorial, bastando logar no sistema disponível em:

URL do Manuscrito: https://www.scientiaplena.org.br/sp/authorDashboard/submission/7789
Login: tgrm

Em caso de dúvidas, entre em contato através da página de submissão na revista ("Discussão da pré avaliação" ou "Discussão da avaliação"). Pedimos a atenção em acompanhar a comunicação entre editor-autor via email, checando regularmente o *spam*. Informamos que a submissão será analisada previamente no atendimento ao foco e escopo da revista, pelo sistema antiplágio CrossCheck e na adequação às normas disponibilizadas em www.scientiaplena.org.br/sp/about/submissions#onlineSubmissions, podendo ser arquivada em caso de inadequação/não atendimento.

Agradecemos mais uma vez considerar nossa revista como meio de transmitir ao público seu trabalho.

Scientia Plena

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Scientia Plena http://www.scientiaplena.org.br/ojs/index.php/sp

unsubscribe

ANEXO D- Normas da Revista Scientia Plena

Diretrizes para Autores

A revista Scientia Plena aceita submissões de artigos originais e inéditos em Português, Inglês ou Espanhol. Os artigos devem ser redigidos e submetidos por pesquisadores vinculados a instituições de ensino e/ou de pesquisa nacionais ou internacionais.

A submissão deve estar em formato ".doc" (limite de 2MB). ATENÇÃO: a partir de março de 2021, há um novo modelo disponível como artigo-exemplo:

https://www.scientiaplena.org.br/public/journals/1/modeloScientiaPlena.docx

Submissões que se apresentarem fora das normas da revista serão arquivadas. Recomendamos atenção na adequação do texto à essas normas, principalmente no que diz respeito ao estilo de citação (tipo Vancouver) e padronização das referências bibliográficas. Não serão consideradas citações de trabalhos acadêmicos (dissertações, teses, monografias, TCCs) e/ou apresentados em eventos científicos.

No ato da submissão do arquivo o autor deve obrigatoriamente:

1) Indicar a área do conhecimento, de acordo com a lista a seguir, e uma subárea, de preenchimento livre (campo Metadados).

Áreas do conhecimento:

Ciências Agrárias - Ciências Biológicas - Ciências da Saúde - Ciências Exatas e da Terra - Ciências Humanas, Letras e Artes - Ciências Sociais Aplicadas - Engenharias e Computação - Multidisciplinar

2) Cadastrar o nome completo de todos os autores, bem como sua afiliação institucional, no campo Metadados de acordo com a ordem de autoria apresentada no trabalho. Não será permitida a alteração da ordem, inclusão e/ou exclusão de autor(es) após o início do processo de avaliação.

3) Indicar três nomes de avaliadores (nome completo, email e afiliação institucional) no campo "Comentários para o Editor". Os avaliadores indicados devem ser pesquisadores de reconhecida

competência no tema do trabalho e que não tenham participado do desenvolvimento do artigo submetido. Não indicar avaliadores da mesma instituição de origem do(s) autore(s) da submissão, visando evitar conflito de interesses. Editores da revista Scientia Plena não deverão ser indicados para a avaliação.

Um tutorial para auxiliar no processo de submissão pode ser obtido no link: "Tutorial para Submissão de manuscrito"

ATENÇÃO: Trabalhos que utilizaram seres humanos como objeto de estudo ou realizaram experimentação animal devem indicar no texto do manuscrito o número da aprovação do projeto pelos respectivos Comitês de Ética. Estudos que envolvem a aplicação de questionários devem informar a utilização do “Termo de consentimento livre e esclarecido”. Estudos com captura e/ou coleta de grupos biológicos devem indicar o número da licença de autorização para atividades com finalidade científica (IBAMA, SISBIO ou órgão estadual/municipal).

Taxa de publicação

Se o manuscrito for aceito para publicação, será cobrada uma taxa de publicação visando auxiliar na manutenção do custo do serviço de editoração, cadastro do DOI e marcação do texto em XML.

A taxa de publicação é fixada em função do enquadramento do autor da submissão do manuscrito.

Valor da taxa de publicação:

- Autor correspondente associado da ASCi: R$ 100,00

- Autor correspondente não associado a ASCi: R$ 200,00

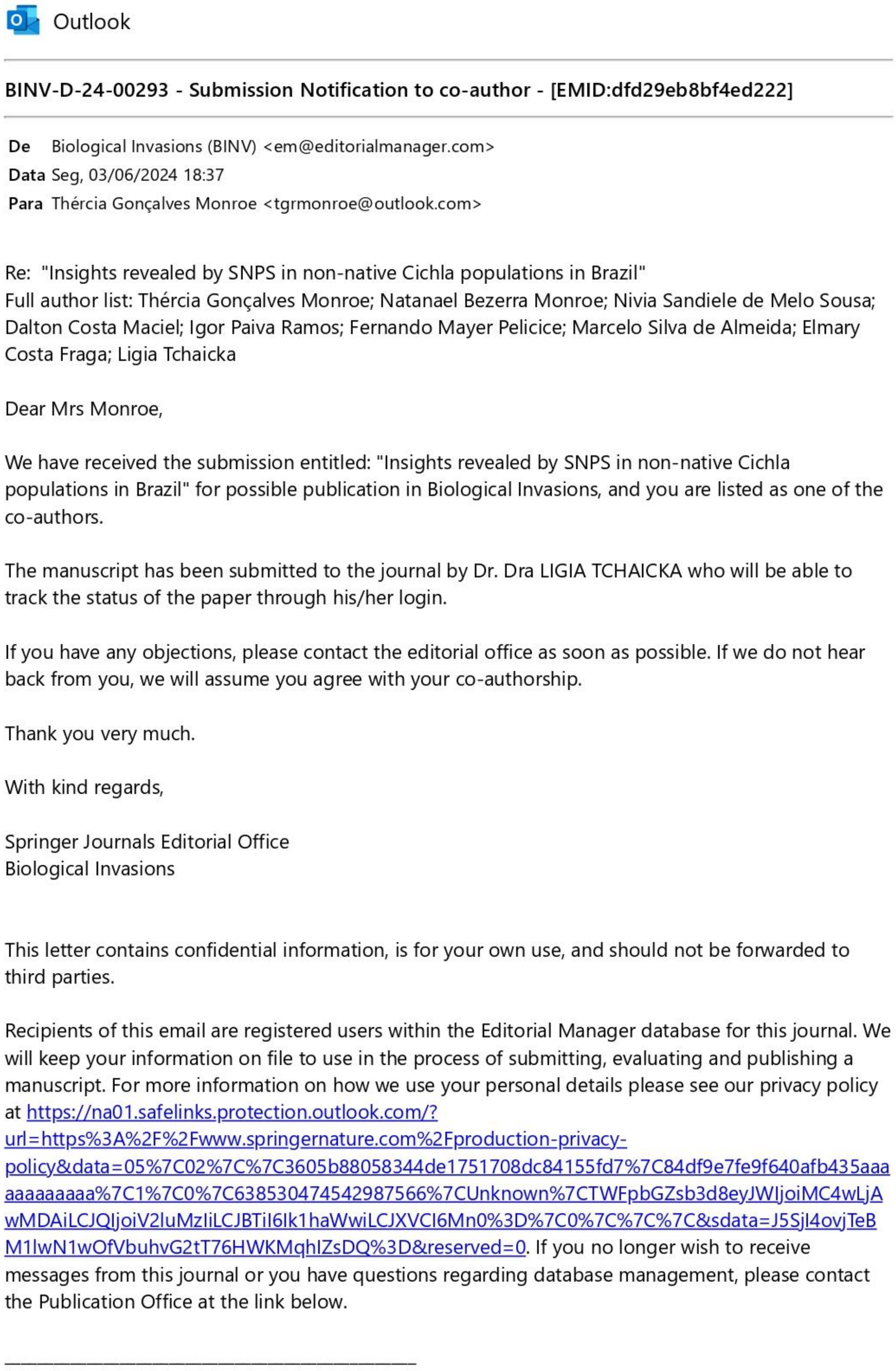
Desconto para autores que colaboraram como revisores da Scientia Plena nos últimos 12 meses: 50% do valor correspondente à categoria.

Informações sobre o pagamento serão fornecidas junto com a decisão editorial de aceite. A editoração do artigo somente iniciará após identificada a efetiva realização do pagamento. Se o pagamento não ocorrer no prazo de até 60 dias após a decisão final de aceite de sua publicação na Scientia Plena, o manuscrito será arquivado.

Declaração de Direito Autoral
Autores que publicam nesta revista concordam com os seguintes termos:

Autores mantém os direitos autorais e concedem à revista o direito de primeira publicação, com o trabalho simultaneamente licenciado sob a Licença Creative Commons Attribution que permite o compartilhamento do trabalho com reconhecimento da autoria e publicação inicial nesta revista.
Autores têm autorização para assumir contratos adicionais separadamente, para distribuição não-exclusiva da versão do trabalho publicada nesta revista (ex.: publicar em repositório institucional ou como capítulo de livro), com reconhecimento de autoria e publicação inicial nesta revista.
Autores têm permissão e são estimulados a publicar e distribuir seu trabalho online (ex.: em repositórios institucionais ou na sua página pessoal) a qualquer ponto antes ou durante o processo editorial, já que isso pode gerar alterações produtivas, bem como aumentar o impacto e a citação do trabalho publicado.
Política de Privacidade
Os nomes e endereços informados nesta revista serão usados exclusivamente para os serviços prestados por esta publicação, não sendo disponibilizados para outras finalidades ou a terceiros.

ANEXO E- Comprovante de submissão Biological Invasion



BINV-D-24-00293 - Submission Notification to co-author - [EMID:dfd29eb8bf4ed222]

De Biological Invasions (BINV) <em@editorialmanager.com>
Data Seg, 03/06/2024 18:37
Para Thércia Gonçalves Monroe <tgrmonroe@outlook.com>

Re: "Insights revealed by SNPS in non-native Cichla populations in Brazil"
Full author list: Thércia Gonçalves Monroe; Natanael Bezerra Monroe; Nivia Sandiele de Melo Sousa; Dalton Costa Maciel; Igor Paiva Ramos; Fernando Mayer Pelicice; Marcelo Silva de Almeida; Elmary Costa Fraga; Ligia Tchaicka

Dear Mrs Monroe,

We have received the submission entitled: "Insights revealed by SNPS in non-native Cichla populations in Brazil" for possible publication in Biological Invasions, and you are listed as one of the co-authors.

The manuscript has been submitted to the journal by Dr. Dra LIGIA TCHAICKA who will be able to track the status of the paper through his/her login.

If you have any objections, please contact the editorial office as soon as possible. If we do not hear back from you, we will assume you agree with your co-authorship.

Thank you very much.

With kind regards,

Springer Journals Editorial Office
Biological Invasions

This letter contains confidential information, is for your own use, and should not be forwarded to third parties.

Recipients of this email are registered users within the Editorial Manager database for this journal. We will keep your information on file to use in the process of submitting, evaluating and publishing a manuscript. For more information on how we use your personal details please see our privacy policy at https://na01.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.springernature.com%2Fproduction-privacy-policy&data=05%7C02%7C%7C3605b88058344de1751708dc84155fd7%7C84df9e7fe9f640afb435aaaaaaaaaaaa%7C1%7C0%7C638530474542987566%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C0%7C%7C%7C&sdata=J5SjI4ovjTeBM1lwN1wOfVbuhvG2tT76HWKMqhIZsDQ%3D&reserved=0. If you no longer wish to receive messages from this journal or you have questions regarding database management, please contact the Publication Office at the link below.

ANEXO F- Normas Biological Invasion

Instructions for Authors

Paper Categories

Biological Invasions publishes research and synthesis papers on patterns and processes of biological invasions in terrestrial, freshwater, and marine (including brackish) ecosystems. Also of interest are scholarly papers on management and policy issues as they relate to conservation programs and the global amelioration or control of invasions. The journal will consider proposals for special issues resulting from conferences or workshops on invasions.

Paper categories

ORIGINAL PAPERS

Novel empirical and theoretical research on topics in invasion biology, such as ecological consequences of invasions (including changes in community and ecosystem structure and processes), factors that influence transport, establishment, and spread of invasions, mechanisms that control the abundance and distribution of invasive species, biogeography, genetics of invaders (as genetics casts light on processes and pathways of invasions), dispersal vectors, evolutionary consequences of invasions in both historical and geological time, innovative management techniques, and analytical syntheses and overviews of invasive biotas. Authors must, in their cover letters, explain how the reported research is novel and exciting.

PERSPECTIVES, PARADIGMS, AND SYNTHESES

Overviews of policies on invasion management; perspectives on invasions and paradigms of invasion biology; syntheses of literature reports. Prospective authors should contact the Editor-in-Chief about suitability of proposed topics.

REVIEWS

Synthetic, timely reviews of topics in invasion biology for which there is a substantial literature. Prospective authors must contact the Editor-in-Chief about suitability of proposed review topic.

INVASION NOTES

Short reports (10 manuscript pages including cover page, less than 25 references, figures, and tables) of new and particularly noteworthy invasions, important changes in status or range of existing invasions, novel and promising techniques for managing particular invasions, evidence on an invasion pathway of particular interest, and the like. A note simply reporting a new species in a new location would not merit publication as an Invasion Note. Invasion Notes are not full research papers and must have (a) an abstract of one short paragraph, (b) a short introductory paragraph explaining the context of the note, (c) the reported information, and (d) a brief discussion of the significance of the note.

ELTON REVIEW

The Elton Review series in Biological Invasions is named in recognition of Charles Elton, whose 1958 monograph, The Ecology of Invasions by Animals and Plants, called attention to the scope and impacts of invasions. These invited in-depth reviews are written by leading scientists engaged in exciting, groundbreaking research, and we encourage a personal perspective with provocative discussion. Although relevant older work should be discussed, an Elton Review should be less an exercise in literature documentation and rather a forum for synthesizing and presenting ideas to advance the field. A typical Elton Review is 6000 - 8000 words long, with up to 150 references and 8 figures. Prospective authors should contact the Editor-in-Chief about suitability of proposed topics. Examples can be found on the journal homepage

FLASHPOINTS

Flashpoints are invited paired contributions published together on substantial disputes, knowledge gaps, or controversies over invasion biology, management, or policy. Authors may propose a topic and, if accepted, the editors will invite an appropriate respondent. These short articles (1500 words or less, maximum of 25 citations) are intended to generate significant dialogue among researchers, managers, and policy makers and to shed light on specific or interdisciplinary aspects of biological invasions. Authors may submit short rebuttals to the original articles of no more than 300 words long. Rebuttals will be published simultaneously with no further comment from the authors. Prospective authors should contact the Editor-in-Chief about suitability of proposed topics. Examples can be found on the journal homepage

ALIEN FLORAS AND FAUNAS

This section includes papers that provide information on complete alien floras or faunas of large regions, such as countries, with clearly described criteria used to assess the species' status as casual, naturalized, or invasive. The papers typically contain information on the structure of the studied alien flora or fauna, in terms of geographic origin, life histories, invaded habitat, time of introduction or introduction pathways, accompanied by an analysis of basic patterns of alien species richness, diversity, or impacts, as well as factors underlying variation in these characteristics. Full species lists with relevant available information must be published with the paper as electronic supplementary material. Examples can be found on the journal homepage

BOOK REVIEWS

To be solicited by the Editor-in-Chief.

Journal Homepage

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Additional instructions
Paper Length

Papers must be concise and well written. While there are no specific page or word limits for any paper category except Invasion Notes, as a guide the average original paper contains approximately 8,000 words. Longer papers may be considered if the information justifies the length.

General Instructions

1) Biological Invasions is read by specialists in invasion biology. Introductory material pointing to the general importance of invasions is unnecessary and inappropriate.

2) Authors must submit, with their manuscripts, names and e-mail addresses of 4 unbiased, expert potential referees who have not previously read the manuscript. Authors may submit names of potential referees that they request not be used and may also request a particular handling editor.

Author Cover Letter

Upon submission authors should include the cover letter at the beginning of their manuscript.

Acknowledging reviewers

Authors are encouraged to thank / acknowledge the reviewers in their papers.

Second Abstract in native language

Authors are allowed to include a second abstract in their native language if this is preferred.

Species Names

For scientific names, which should be in italics, genus should be capitalized and species should be lower case. However, common names should not be italicized and should be lower case unless the common name includes a proper name. For example: "green crab" or "Japanese knotweed."

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Authorship Guidelines
Authorship credit should be based on:

1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data;

AND

2) drafting the article or revising it critically for important intellectual content;

AND

3) final approval of the version to be submitted for publication.

All of these conditions should be met by all authors.

Acquisition of funding, collection of data, or general supervision of the research group alone does not constitute authorship.

All contributors who do not meet the criteria for authorship should be listed in an acknowledgments section.

All authors must agree on the sequence of authors listed before submitting the article.

All authors must agree to designate one author as the corresponding author for the submission. It is the responsibility of the corresponding author to dialogue with the co-authors during the peer-reviewing and proofing stages and to also act on their behalf.

If the article is accepted for publication, after acceptance, no changes in authorship, the order of authors, or designation of the corresponding author will be permitted.

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Manuscript Submission

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

Permissions

Authors wishing to include figures, tables, or text passages that have already been published elsewhere are required to obtain permission from the copyright owner(s) for both the print and online format and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to originate from the authors.

Online Submission

Please follow the hyperlink “Submit manuscript” and upload all of your manuscript files following the instructions given on the screen.

Source Files

Please ensure you provide all relevant editable source files at every submission and revision. Failing to submit a complete set of editable source files will result in your article not being considered for review. For your manuscript text please always submit in common word processing formats such as .docx or LaTeX.

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Title Page

Please make sure your title page contains the following information.

Title

The title should be concise and informative.

Author information

The name(s) of the author(s)
The affiliation(s) of the author(s), i.e. institution, (department), city, (state), country
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This result was later contradicted by Becker and Seligman (1996).

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Book chapter

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ANEXO G- Artigo: “Importance of biodiversity in conservation planning of Brazilian National Parks”, publicado na Research, Society and Development.

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Importance of fish biodiversity in conservation planning of Brazilian National Parks

Importância da biodiversidade de peixes no planejamento da conservação dos Parques Nacionais Brasileiros

Importancia de la biodiversidad de peces en la planificación de la conservación de los Parques Nacionales Brasileños

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Abstract
Protected areas are essential for the maintenance of biodiversity. In Brazil, national parks encompass one of the most important portions of this area (26.864.003,74ha) that needs to be adequately managed to achieve conservation. Aiming to understand how the ichthyofauna data is included in management plans of Brazilian National Parks, we review 55 Brazilian national parks management plans to compare how data of freshwater fish fauna are included in these documents. The data evaluated from management plans were Hydrography, ichthyofauna list, participation of professionals trained in rapid ecological assessment and species richness, endangered and invasive species. This information was used to categorize the management plans through two sets of assessing: quality of rapid ecological assessment and coverage of species diversity. The categorization results were assumed as an indicator of the potential for biodiversity conservation of the management plans. We compare obtained results between biomes. We were able to understand that many of the Brazilian National Parks do not have management plans, and among the plans that are prepared, the lack of essential information compromises their potential for the conservation of biodiversity. We highlight the need to improve management plans for the Caatinga parks and expand the analysis of invasive species for all biomes.

Keywords: Conservation; Management; Protected areas; Freshwater fishes.

Resumo
As áreas protegidas são essenciais para a manutenção da biodiversidade. No Brasil, os parques nacionais abrangem uma das partes mais importantes dessas áreas (26.864.003,74 ha) que precisam ser manejadas de forma adequada para se alcançar a conservação. Para compreender como os dados a respeito da ictiofauna são abordados nos planos de manejo de parques nacionais brasileiros e o potencial dessas informações para a conservação, revisamos 55 planos de manejo. As variáveis utilizadas foram: plano de manejo disponível, hidrografia, dados da ictiofauna de água doce, profissionais treinados em avaliação ecológica rápida e riqueza de espécies. Além disso, dados sobre espécies ameaçadas e invasoras, usando uma matriz binária (ausência / presença). Comparamos nossas classificações com dados disponíveis na literatura especializada para cada bioma brasileiro. Pudemos apreender que muitos dos Parques Nacionais brasileiros ainda não possuem planos de manejo, e entre os planos já elaborados a falta de informações essenciais compromete seu potencial para a conservação da biodiversidade. Destacamos a necessidade de melhoria dos planos de manejo para os parques da Caatinga e a ampliação da análise de espécies invasoras para todos os biomas.

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Scientific articles must be longer than 5 pages.

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