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VICTOR AUGUSTO DE QUEIROZ BATISTA

**REDESCRIÇÃO DE *Psalidodon jequitinhonhae* (STEINDACHNER, 1877),
OSTARIOPHYSI: CHARACIDAE**

PORTO ALEGRE
2022

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OSTARIOPHYSI: CHARACIDAE**

Dissertação apresentada ao Programa de Pós-Graduação em Biologia Animal, Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como requisito parcial à obtenção do título de Mestre em Biologia Animal.

Área de concentração: Biologia Comparada

Orientador: Prof. Dr. Luiz Roberto Malabarba

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Histórico

A região Neotropical é detentora da maior diversidade de peixes do mundo. As estimativas do número de espécies de água doce variam, entre 6.000 (REIS et al., 2003), 7.000 (ALBERT, REIS, 2011) e 8.000 espécies (SCHAEFER, 1998), chegando a mais de 9.100, quando incluem-se também as espécies marinhas costeiras, o que representa cerca de 27% da riqueza de espécies do mundo todo (REIS et al., 2016). Considerando o número de espécies descritas até 2016, a América do Sul apresentava mais de 5.160 espécies de água doce, ou um terço de todas as espécies de peixes de água doce do mundo, alocadas em mais de 739 gêneros e 69 famílias (REIS et al., 2016).

Dentro dessa grande diversidade neotropical, a Ordem Characiformes chama a atenção, com mais de 1.700 espécies (REIS, 2013), distribuídas ao sul da América do Norte e ao longo das Américas Central e do Sul e na África (REIS et al., 2003; CALCAGNOTTO et al., 2005). Habitam desde rios e córregos lóticos e com corredeiras, até remansos lênticos das planícies de inundação das terras baixas (OLIVEIRA et al., 2011). Estão alocados em 24 famílias, das quais 20 ocorrem na região neotropical: Acestrorhynchidae, Anostomidae, Bryconidae, Chalceidae, Characidae, Chilodontidae, Crenuchidae, Curimatidae, Ctenoluciidae, Cynodontidae, Erythrinidae, Gasteropelecidae, Hemiodontidae, Iguanodectidae, Lebiasinidae, Parodontidae, Prochilodontidae, Serrasalminidae, Tarumaniidae, Triportheidae (MIRANDE, 2010; DE PINNA et al., 2017; FRICKE et al., 2022).

Por sua vez, Characidae é a quarta família de peixes em número de riqueza de espécies descritas, e a primeira em Characiformes, com cerca de 142 gêneros e 1.244 espécies válidas, sendo 221 destas descritas nos últimos dez anos (2013-2022) (MIRANDE, 2010; FRICKE et al., 2022). É dividida em 8 subfamílias: Aphyocharacinae, Characinae, Cheirodontinae, Exodontinae, Spintherobolinae, Stethaprioninae, Stevardiinae e Tetragonopterinae) (FRICKE et al., 2022). Characidae possui uma ampla variedade de formas e tamanho corporal, com a

maioria de pequeno porte (menor que 8 cm de comprimento padrão), porém variando de tamanho desde de espécies miniaturas a tamanhos médio (NELSON, 2006; JAVONILLO et al., 2010, OLIVEIRA et al., 2011, MIRANDE, 2018). Ocupam habitats que na maioria dos outros continentes são habitados por outros grupos dominantes, como os Cypriniformes (MIRANDE, 2018). Os caracídeos possuem funções ecológicas importantes, são fontes de grande biomassa, exploram diferentes nichos ecológicos, muitos são mantidos como peixes ornamentais e vendidos no mercado de aquários, e representam uma alta diversidade taxonômica em nível de espécie (JAVONILLO et al., 2010; OLIVEIRA et al., 2011; MIRANDE, 2018).

Apesar da importância de Characidae e a sua grande diversidade taxonômica, as relações filogenéticas dentro da família e entre as subfamílias não eram completamente esclarecidas e não havia consenso sobre a monofilia de Characidae até o início deste século (GÉRY, 1972; WEITZMAN, MALABARBA, 1998; CALCAGNOTTO et al., 2005). Devido à ausência de sinapomorfias dentro da família Characidae, oitenta e oito gêneros, incluindo 620 espécies, foram considerados como *incertae sedis* por Lima et al. (2003). Entre estas espécies, muitas eram alocadas em gêneros com um grande número de espécies e amplamente distribuídos, taxonomicamente problemáticos, e possivelmente não monofiléticos, como *Astyanax* Baird & Girard, 1854, *Bryconamericus* Eigenmann, 1907, *Hemigrammus* Gill, 1858, *Hyphessobrycon* Durbin, 1908, *Moenkhausia* Eigenmann, 1903 (LIMA et al., 2003). Ainda, muitos gêneros eram monotípicos ou contendo apenas duas ou três espécies, como *Axelrodia* Géry, 1965 e *Psalidodon* Eigenmann, 1911 (LIMA et al., 2003).

Diversos trabalhos propuseram a monofilia de algumas subfamílias ou gêneros e corroboraram com um melhor entendimento e esclarecimento sobre as relações filogenéticas entre as espécies de Characidae (MALABARBA, 1998; LUCENA, LUCENA; 2002; MALABARBA, WEITZMAN, 2003; CALCAGNOTTO et al., 2005; MIRANDE, 2009,

2010, 2018; JAVONILLO et al., 2010; OLIVEIRA et al., 2011; DE PINNA et al., 2017; SILVA, 2017). Alguns destes trabalhos (MALABARBA, WEITZMAN, 2003; MIRANDE, 2010, 2018; OLIVEIRA et al., 2011) ajudaram a elucidar as relações filogenéticas de Characidae e a recuperar três clados: A (Stevardiinae), B e C. O clado C é o mais rico em espécies dentre os três (FRICKE et al., 2022) e o que também possui as relações filogenéticas internas mais pobremente resolvidas, como a presença de vários gêneros não monofiléticos. Dentre estes, destacam-se os gêneros *Jupiaba* Zanata, 1997 e *Astyanax* (MIRANDE, 2010; OLIVEIRA et al., 2011).

Astyanax foi descrito como um gênero monotípico, com uma diagnose curta e pouco detalhada, com a espécie *Astyanax argentatus* (Baird & Girard, 1854) endêmica do Rio Nueces, no Texas, EUA. Eigenmann (1917, 1921) foi o primeiro autor a extensivamente revisar o gênero, à época, contendo 74 espécies válidas, e propôs um conjunto de estados de caracteres morfológicos não exclusivos para definir o gênero, os quais são: “premaxillary with two series of teeth, the first series with several teeth; mandible with strong teeth in front, abruptly minute conical ones on the sides or more gradually very small ones on the sides, without conical teeth in a second series; teeth of second series of premaxillary equal or graduated, usually five in number, four in a few species; crowns of premaxillary and mandibular teeth usually ridged and denticulate; maxillary with a few or no (0-10) teeth; caudal naked; lateral line complete; gill-rakers setiform; no predorsal spine. Form usually slender, depth more than two in the length” (EIGENMANN, 1921). Entretanto, a maioria desses caracteres está amplamente distribuída entre outras espécies de caracídeos (EIGENMANN, 1917, 1921; OLIVEIRA et al., 2011; PAVANELLI, OLIVEIRA, 2009; MIRANDE, 2010, 2018; SILVA, 2016; TERÁN et al., 2020).

Astyanax foi considerado o gênero mais rico em número de espécies da família Characidae (MIRANDE, 2010; OLIVEIRA et al., 2011), com mais de 170 espécies válidas

(FRICKE et al., 2019). Possuía ampla distribuição geográfica, ocorrendo pelas Américas do Norte, Central e Sul, com a ocorrência mais setentrional, com *Astyanax argentatus* no Texas, EUA, e mais meridional na Patagônia Argentina, com *Astyanax pampa* (Casciotta, Almirón & Azpelicueta 2005), ocupando as regiões Neártica e Neotropical (CASCIOTTA et al., 2005).

Diversos estudos consideraram *Astyanax* um gênero taxonomicamente problemático e complexo, devido à alta similaridade morfológica e conservativa entre as espécies, as quais são detectáveis muitas vezes apenas com estudos osteológicos, e à ausência de caracteres diagnósticos únicos para diagnosticar as espécies do gênero, sendo necessário, portanto, a combinação de caracteres não-exclusivos para a diagnose das espécies (MELO, 2001; LUCENA et al., 2003; MIRANDE, 2007; VARI, CASTRO, 2007; ZANATA & CAMELIER, 2009; MARINHO et al., 2015; SILVA et al., 2019, TERÁN et al., 2020). Eigenmann (1917, 1921) já sugeria a não monofilia do grupo. Estudos filogenéticos após os trabalhos de Eigenmann reforçaram a não-monofilia do gênero, tanto em hipóteses usando caracteres morfológicos (MIRANDE, 2010; SCHMITTER-SOTO, 2016; SILVA, 2017;) quanto para dados moleculares (CALCAGNOTTO et al., 2005; ORNELAS-GARCÍA et al., 2008; JAVONILLO et al., 2010; OLIVEIRA et al., 2011; SILVA, 2017;) ou evidência total (MIRANDE, 2018; TERÁN et al., 2020).

Porém, por cerca de quase 100 anos, os trabalhos de descrição de espécies do gênero seguiam a diagnose proposta por Eigenmann (1917,1921) para *Astyanax*, apesar de já terem sido propostas hipóteses para a não-monofilia do gênero e a indicação de grupos de espécies do gênero serem mais próximos filogeneticamente a outros gêneros que propriamente a *Astyanax* (ORNELAS-GARCÍA et al., 2008; MARINHO et al., 2015; SILVA, 2017; MIRANDE, 2009, 2018). Schmitter-Soto (2016), no trabalho de filogenia morfológica de 34 espécies de *Astyanax*, endêmicas/restritas das Américas do Norte e Central, usando 52 caracteres morfológicos, dos quais 51 são osteológicos e um de coloração, não recuperou a

monofilia do grupo. Ornelas-García et al. (2008), por sua vez, na filogenia molecular, usando três genes mitocondriais (CYTB, COI, 16S) e um nuclear (RAG1), analisaram 10 espécies de *Astyanax* das regiões do Panamá até a fronteira México- EUA - Américas do Norte e Central - também não recuperaram a monofilia do gênero e seus resultados sugeriram o reconhecimento de diversas espécies novas dentro do clado *Astyanax*. Ambos os trabalhos analisaram uma quantidade pequena de espécies de *Astyanax* e com ocorrência restrita, dada a riqueza e a ampla distribuição das espécies de *Astyanax*.

Contudo, após Eigenmann, Terán et al. (2020) foram os únicos a analisar amplamente o gênero. 98 espécies foram analisadas, com um total de 520 caracteres morfológicos e nove marcadores moleculares (12S, 16S, ATP6, COI, CYTB, MYH6, PTCHD1, RAG1 e RAG2). Eles propuseram a filogenia do gênero, redefinindo-o com uma nova combinação de caracteres sinapomórficos para *Astyanax*, que ficou restrito a espécies distribuídas nas regiões das Américas do Norte e Central. As espécies classificadas até então como *Astyanax*, passaram a ser classificadas em oito gêneros: (a) *Tetragonopterus* Cuvier, 1816; (b) *Eretmobrycon* Fink, 1976; (c) *Makunaima* Terán, Benitez & Mirande, 2020; (d) *Deuterodon* Eigenmann, 1907; (e) *Jupiaba* Zanata, 1997; (f) *Astyanax* Baird & Girard, 1854; (g) *Andromakhe* Terán, Benitez & Mirande, 2020; (h) *Psalidodon* Eigenmann, 1911.

Psalidodon, considerado um gênero *incertae sedis* (LIMA et al., 2003; MIRANDE, 2009), e posteriormente sinônimo-júnior de *Astyanax* (PAVANELLI, OLIVEIRA, 2009), foi ressuscitado e validado, apresentando um suporte moderado para a monofilia do grupo (TERÁN et al., 2020). Com a redefinição, *Psalidodon* passou a ser o gênero mais rico em número de espécies dentro da subfamília Stethaprioninae, tribo Gymnocharacini, com 33 espécies válidas - 32 redefinidas por Terán et al. (2020), mais *Psalidodon rioparanaibanus* Alves, Oliveira, Pasa & Kavalco, 2020, espécie descrita recentemente (ALVES et al., 2020; TERÁN et al., 2020) e o gênero restringiu-se à América do Sul. Das 32 espécies redefinidas

por Terán et al. (2020), muitas pertenciam aos grupos *A. scabripinnis* (Jenyns, 1842; sensu Bertaco & Lucena, 2006), *A. fasciatus* (Cuvier, 1819) e *A. eigenmanniorum* (Cope, 1894).

Psalidodon jequitinhonhae foi uma dentre as espécies transferidas para *Psalidodon* sensu Terán (2020). Descrita originalmente como *Tetragonopterus rutilus jequitinhonhae* Steindachner, 1877, como uma variação morfológica de *Tetragonopterus rutilus* Jenyns, 1842, onde os espécimes da bacia do Rio Jequitinhonha apresentaram uma forma corporal notavelmente alongada em comparação às populações de *T. rutilus* das bacias dos rios Paraná, Paraíba do Sul, Velhas e Doce (STEINDACHNER, 1877). No entanto, *T. rutilus* foi descrita a partir de um único exemplar coletado por Darwin, na bacia do rio Paraná, na Argentina. Enquanto *T. rutilus jequitinhonhae* foi descrito com base em 14 (quatorze) espécimes coletados na bacia do rio Jequitinhonha, Sudeste do Brasil (JENYNS, 1842; DARWIN, 1860; STEINDACHNER, 1877).

Embora *T. rutilus jequitinhonhae* tenha sido descrita na segunda metade do século XIX, há 145 anos (1877-2022), e durante esse período, houve muitos trabalhos de redescrição de espécies de ‘*Astyanax*’ (por exemplo, EIGENMANN, 1917, 1921; MELO, 2001; PAVANELLI, OLIVEIRA, 2009; BERTACO, LUCENA, 2010; MARINHO et al., 2015; SILVA et al., 2019), nenhum destes trabalhos analisou ou incluiu *T. rutilus jequitinhonhae*. Ainda, para filogenias propostas do gênero (ORNELAS-GARCÍA et al., 2008; SCHMITTER-SOTO, 2016; TERÁN et al., 2020), o único trabalho a incluir a espécie foi Terán et al. (2020). Nas análises da espécie destes autores, entretanto, foram analisados somente caracteres morfológicos em apenas dois exemplares diafanizados e corados e não foram produzidas sequências para os marcadores moleculares.

Desta maneira, o objetivo deste trabalho é redescrever a espécie *Psalidodon jequitinhonhae*, obtendo sequências do marcador molecular Citocromo Oxidase subunidade I (COI) a partir dos sítipos para a comparação com amostras recentes e combinando dados

morfológicos, merísticos e moleculares.

Objetivo geral:

1. Delimitar e descrever a espécie *Psalidodon jequitinhonhae* com base em dados morfológicos e moleculares.

Objetivos específicos:

- 2.1. Redescrever a espécie *Psalidodon jequitinhonhae* para propor caracteres morfológicos que diagnosticam a espécie;
- 2.2. Sequenciar o COI do sítipo e comparar com sequências modernas, visando à identificação de amostras recentes de *Psalidodon jequitinhonhae*;
- 2.3. Propor o DNA *barcoding* da espécie através da sequência do gene mitocondrial COI;
- 2.4. Redescrever a espécie com base nos sítipos e em espécimes modernos.

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**INTEGRATIVE TAXONOMY: ANCIENT DNA COMBINED WITH MORPHOLOGY
TO REDESCRIBE THE CHARACID FISH *Psalidodon jequitinhonhae*
(STEINDACHNER, 1877) (OSTARIOPHYSI: CHARACIFORMES)**

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Resumo

Psalidodon jequitinhonhae (Steindachner, 1877) é uma espécie de peixe caracídeo, conhecida popularmente como lambari ou piaba e endêmica da bacia do rio Jequitinhonha, Sudeste do Brasil. Foi descrita originalmente como uma subespécie de *Tetragonopterus rutilus* Jenyns, 1842, a partir da análise de quatorze exemplares coletados no rio Jequitinhonha. Após revisões taxonômicas e sistemáticas da família, a espécie foi realocada em 1917 no gênero *Astyanax* Baird & Girard 1854 na subfamília Tetragonopterinae e em 2020 no gênero *Psalidodon* Eigenmann 1911, subfamília Stethaprioninae. Porém, em nenhuma dessas revisões, *P. jequitinhonhae* foi descrita morfologicamente. A obtenção de sequências do gene COI geradas a partir dos sítipos e comparadas a sequências obtidas em novas amostras e no genbank permitiram o reconhecimento dos espécimes pertencentes à espécie e sua separação de outras espécies disponíveis no gênero. Tanto os dados morfológicos quanto os moleculares convergiram e corroboraram para a validade da espécie e a sua redescrção.

Palavras-chave: Bacias costeiras, Delimitação de espécies, Lectótipo, Rio Jequitinhonha.

Abstract

Psalidodon jequitinhonhae (Steindachner, 1877) is a species of characid fish, popularly known as lambari or piaba and endemic to the Jequitinhonha River Basin, Southeast Brazil. It was originally described as a subspecies of *Tetragonopterus rutilus* Jenyns, 1842, based on the analysis of fourteen specimens collected in the Jequitinhonha River. After taxonomic and systematic reviews of the family, the species was relocated in 1917 in the genus *Astyanax* Baird & Girard 1854 in the subfamily Tetragonopterinae and in 2020 in the genus *Psalidodon* Eigenmann 1911, subfamily Stethaprioninae. However, in none of these reviews, *P. jequitinhonhae* was morphologically described. Sequences of the COI gene generated from the syntypes and compared to sequences obtained from new samples and from the genbank allowed the recognition of specimens belonging to the species and its separation from other species available in the genus. Both morphological and molecular data converged and corroborated the validity of the species and its redescription.

Keywords: Coastal basins, Jequitinhonha River, Lectotype, Species delimitation.

1. Introduction

Tetragonopterus rutilus Jenyns, 1842 was described from a single specimen collected by Darwin in the Paraná River basin, Argentina. Steindachner (1877) expanded the description of *T. rutilus*, after examining specimens housed at Naturhistorisches Museum Wien, Austria, from Paraná River, near Montevideo, Uruguay; Paraíba do Sul River and its tributaries in the mountains at Rio de Janeiro, Brazil; Doce, Jequitinhonha and Velhas Rivers, Brazil; and Xamapa [current Xalapa], Mexico. Steindachner (1877) synonymized the following species to *T. rutilus*: *Tetragonopterus aeneus* Hensel, 1870 [= *Astyanax henseli* Melo & Buckup, 2006]; *Tetragonopterus taeniatus* Jenyns, 1842 [= *Deuterodon taeniatus*]; *Chalceus fasciatus* Cuvier, 1819 [= *Psalidodon fasciatus*]; *Tetragonopterus microstoma* Günther, 1864 [= *Species inquirenda*] and *Tetragonopterus cuvieri* Lütken, 1875 [= synonym of *Psalidodon fasciatus*] (Lima et al., 2003; Melo & Buckup, 2006; Silva et al., 2019; Terán et al., 2020).

Among the specimens analyzed of *T. rutilus*, Steindachner (1877) observed morphological differences related to each river basin. In particular, he noticed that the specimens from the Jequitinhonha River basin had a remarkably elongated body shape compared to the other specimens from Paraíba do Sul and Doce rivers. Therefore, (Steindachner, 1877: Plate II, fig. 3) designated them as *Tetragonopterus rutilus* var. *Jequitinhonha* in an illustration (Fig. 1).

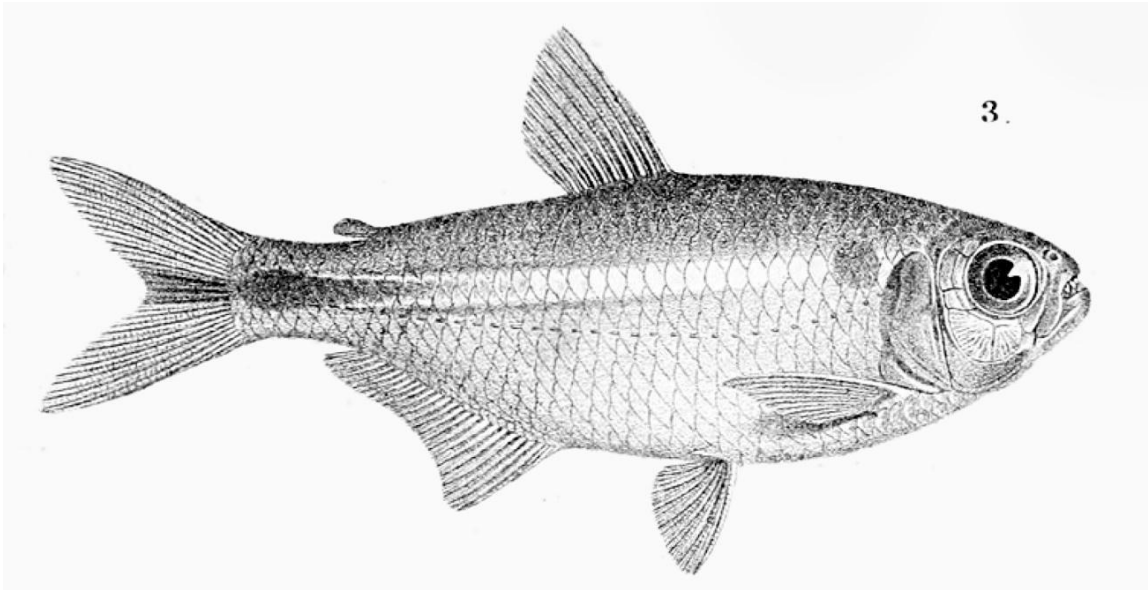


Figure 1. *Tetragonopterus rutilus* var. Jequitinhonha as illustrated by Steindachner (1877: Plate II, fig. 3).

Later, Eigenmann (1921) proposed *T. rutilus*, as well as *T. aeneus* Günther and *T. aeneus* Hensel, as junior-synonyms of *Astyanax fasciatus* Cuvier (1819). Eigenmann (1921, 1927) believed that part of the specimens identified and described by Steindachner (1877) as *T. rutilus* actually belonged to other species, such as *A. jenynsii*, *A. scabripinnis* and *A. taeniatus* (Eigenmann, 1921; 1927). Furthermore, Eigenmann (1921) presented a table with the numbers of anal-fin rays and scales along the lateral line for '*A. fasciatus*' and its varieties/subspecies.

Eigenmann (1921:304) presented a new combination and briefly described the species of Steindachner as *Astyanax fasciatus jequitinhonhae* (Fig. 2), listing specimens examined from the Arassuahy [current Araçuaí] and Jequitinhonha rivers, both belonging to the Jequitinhonha River basin. Eigenmann further referred ten specimens from Doce River basin and two specimens from São Matheos as possibly belonging to this “variety” [sic], but mentioned they were in really bad conditions and differed from *T. rutilus jequitinhonhae* in the increased number of gill rakers.

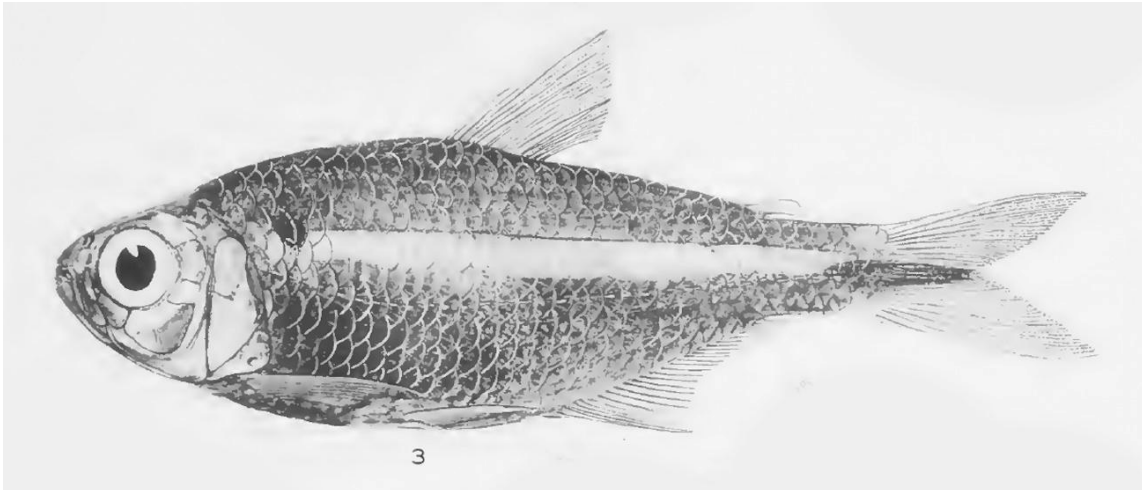


Figure 2. *Astyanax fasciatus jequitinhonhae* (Steindachner) illustrated by Eigenmann (1921: Plate 50, fig. 3; MCZ 20901; 83 mm; Rio Jequitinhonha, Brazil).

Melo (2005) restricted *A. fasciatus* to the São Francisco River basin and Melo & Buckup (2006) validated *A. rutilus*, occurring in the Paraná River basin, thus referring *Astyanax jequitinhonhae* as a valid species, and not a subspecies of *A. fasciatus* or *A. rutilus*. No further information has been furnished, however, to diagnose *A. jequitinhonhae*, Melo (2005) listed eleven specimens as comparative material examined from the São Miguel River, a tributary of the Jequitinhonha River (MNRJ 11576, 59.3–78.3 mm SL). Triques et al. (2003) compared morphological characters of *A. fasciatus jequitinhonhae* with *Astyanax turmalinensis* Triques, Vono & Caiafa 2003, but based exclusively on data from literature, not examining additional specimens.

In a comprehensive phylogenetic analysis of *Astyanax*, involving both morphological and molecular data, Terán et al. (2020) propose a new combination, *Psalidodon jequitinhonhae* (Steindachner, 1877). *Psalidodon* Eigenmann, 1911 was initially distinguished from *Astyanax* by absence of lips (vs. lips present); premaxilla with a single series of teeth (vs. two series); presence of relatively compressed teeth on the premaxillary and dentary bones (vs. relatively robust teeth) (Eigenmann, 1911, 1917, 1921; Pavanelli & Oliveira, 2009). Nevertheless, after the examination of specimens of *Psalidodon* and *Astyanax* from the Iguaçu

River basin, Pavanelli & Oliveira (2009) considered these characters as variable, and *Psalidodon* as a junior-synonym of *Astyanax* (Pavanelli & Oliveira, 2009). Terán et al. (2020), however, rearranged *Astyanax* species into eight different genera. *Psalidodon* was resurrected, and the monophyly of the genus was obtained from four molecular and two morphological synapomorphies: the presence of five or more cusps on teeth both from the outer premaxillary row and the maxilla. *Psalidodon* was originally described as monotypic, with only *Psalidodon gymnodontus* Eigenmann, 1911, an endemic species from the Iguaçu River basin, in Southern Brazil, but now contains 33 species.

Although the work by Terán et al. (2020) redefined the relationship of *Psalidodon jequitinhonhae*, a detailed description and diagnosis of the species was not presented. Only two cleared and stained specimens from Upper Jequitinhonha River basin were analyzed for osteological studies, lacking molecular markers. So, the main objective of this work is to redescribe the *Psalidodon jequitinhonhae* species, using integrative approaches: morphological analysis and sequencing ancient DNA (aDNA) obtained from syntypes and newly collected specimens, to propose morphological characters and the DNA *barcoding* to diagnose the species.

2. Materials and Methods

2.1. Morphology

All measurements and counts followed Fink & Weitzman (1974), with the exception of the number of scale rows below the lateral line, which were counted from the scale row ventral to the lateral line to the scale row nearest to the origin of the first pelvic-fin ray following (Silva et al., 2017, 2019a). Measurements were taken point to point, with an electronic caliper, on the left side of the specimens whenever possible. All measurements were converted to percentages of standard length (SL), except the subunits of the head, which are

expressed in percentages of head length (HL). In the description, counts are followed by the frequency in parentheses.

2.2. DNA extraction and sequencing for museum specimens

A fragment of 2mg muscle tissue was removed, as invasively as possible to avoid unnecessary damage to specimens, from the right side of the body of one of the *Tetragonopterus rutilus jequitinhonhae* syntypes (NMW57760:2) for aDNA analysis. Extractions were conducted in a dedicated area free from DNA and PCR products (amplicons) at the molecular biology facilities of the National Museum of Natural History (Smithsonian Institution, Washington DC, USA). Amplifications (PCR reactions) were conducted in a clean room of an aDNA laboratory in a totally different facility: ARCHGEN, at UFRGS, Porto Alegre, Brazil. Extraction, amplification and purification procedures, conditions and protocols follow Silva et al. (2019a, b).

Sequencing was performed on ACTgene (Porto Alegre, Brazil). Strands (forward and reverse) of each sequence fragment were independently aligned using Clustal W in MEGA 6.0 software (Tamura et al., 2013). The p-distance between the ancient sequence and modern ones, was estimated using the default conditions (d: Transitions. Transversions; uniform rates; Pairwise deletion; three codon positions selected) of the MEGA 6.0 software (Tamura et al., 2013).

2.3. DNA extraction and sequencing for modern specimens

DNA was extracted from muscle, gill filaments, or liver tissue, according to a modified CTAB protocol (Doyle & Doyle, 1987). COI gene was amplified with primers cocktail FishF1t1 and FishR1t1 (Ivanova et al., 2007), in PCR reactions performed at 20 uL total volume: 10.3 mL of H₂O, 2 mL of 10 reaction buffer (Platinum®Taq), 0.6 mL of MgCl₂ (50

mM), 2 mL of dNTPs (2 mM), 2 mL of each primer (2 mM), 0.1 mL (5 U) of Platinum® Taq (Invitrogen), and 1 mL of template DNA. The PCR conditions were: an initial DNA denaturation at 94° C for 3 min, followed by 35 cycles at 94° C for 30 s, at 52° C for 40 s, and at 72° C for 1 min, and a final extension at 72° C for 10 min.

PCR products were checked by electrophoresis in 1% agarose gel, purified using QIAGEN® QIAquick PCR Purification Kit according to the manufacturer protocol and sequencing was performed by Macrogen Inc, Seoul, South Korea and by ACTgene at Porto Alegre, RS, Brazil. Sequences were aligned using Clustal W in MEGA 6.0 software (Tamura et al., 2013) and alignments were visually inspected for any obvious misalignments and then corrected. Genetic distances among specimens were calculated using p-distance in MEGA 6.0, in order to demonstrate the relationships between specimens.

All work involving modern DNA was performed at the Molecular Laboratory of the Zoology Department (UFRGS, Porto Alegre, RS, Brazil). Vouchers, locality information, and GenBank accession numbers are summarized in Supplementary Table 1.

2.4. Phylogenetic analysis

A Neighbor-joining tree was performed at Mega 6.0, using two evolution models: p-distance and Kimura 2-parameter (Kimura, 1980), to estimate the relationship between the sequences. The p-distance between the ancient sequence and modern ones was estimated using the default conditions (Kimura 2-parameter model; d: Transitions + Transversions; uniform rates; Pairwise deletion; three codon positions selected).

2.5. Literature consulted

Morphological data used to the diagnosis from most congeners were consulted according to the following articles: for *P. anisitsi* (Eigenmann, 1907) from Eigenmann &

Ogle, 1907; for *P. bifasciatus* (Garavello & Sampaio, 2010) from Garavello & Sampaio, 2010; for *P. bockmanni* (Vari & Castro, 2007) from Vari & Castro, 2007; for *P. chico* (Casciotta & Almirón, 2004) from Casciotta & Almirón, 2004; for *P. correntinus* (Holmberg, 1891) and *P. pelegri* (Eigenmann, 1907) from Mirande et al. 2006b; *P. dissensus* (Lucena & Thofehn, 2013) and *P. xiru* (Lucena, Castro & Bertaco, 2013) from Lucena, Castro & Bertaco, 2013; for *P. eigenmanniorum* (Cope, 1894) from Bertaco & Lucena, 2010 and Malabarba et al. 2013; for *P. endy* (Mirande, Aguilera & Azpelicueta, 2006) from Mirande et al., 2006a; for *P. erythropterus* (Holmberg, 1891) from Soneira et al. 2010; for *P. gymnodontus* (Eigenmann, 1911) from Pavanelli & Oliveira, 2009; for *P. gymnogenys* (Eigenmann, 1911) from Oliveira et al. 2019; for *P. hermosus* (Miquelarena, Protogino & López, 2005) from Miquelarena et al. 2005b; for *P. ita* (Almirón, Azpelicueta & Casciotta, 2002) from Almirón et al. 2002; for *P. leonidas* (Azpelicueta, Casciotta & Almirón, 2002) and *P. troya* (Azpelicueta, Casciotta & Almirón, 2002) from Azpelicueta et al. 2002b; for *P. marionae* (Eigenmann, 1911) from Eigenmann, 1911; for *P. ojiara* (Azpelicueta & Garcia, 2000) from Azpelicueta & Garcia, 2000; for *P. pampa* (Casciotta, Almirón & Azpelicueta, 2005) from Casciotta et al. 2005; for *P. parahybae* (Eigenmann, 1908) from Melo, 2001; for *P. powelli* (Terán, Butí & Mirande, 2017) from Terán et al. 2017; *P. puka* (Mirande, Aguilera & Azpelicueta, 2007) from Mirande et al. 2007; for *P. pynandi* (Casciotta, Almirón, Bechara, Roux & Ruíz Díaz, 2003) from Casciotta et al. 2003; for *P. rioparanaibanus* Alves, Oliveira, Pasa & Kavalco, 2020 from Alves et al. 2003; for *P. schubarti* (Britski, 1964) from Britski, 1964; for *P. tumbayaensis* (Miquelarena & Menni, 2005) from Miquelarena & Menni, 2005; for *P. xavante* (Garutti & Venere, 2009) from Garutti & Venere, 2009.

2.6. Institutional abbreviation

Colección Ictiológica de la Fundación Miguel Lillo, Argentina (CI-FML); Fundación

Felix de Azara, Buenos Aires (CFA-IC); Laboratório de Biologia e Genética de Peixes, Botucatu (LBP); Laboratório de Ictiologia, Departamento de Zoologia, Universidade Federal do Rio Grande do Sul, Porto Alegre (UFRGS); Laboratorio de Genética Evolutiva-Peces, Posadas, Argentina (LGEP); Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre (MCP); Muséum National d'Histoire naturelle, Paris, France (MNHN); Museu de Zoologia João Moojen, Universidade Federal de Viçosa, Viçosa (MZUFV); Natural History Museum, London (BMNH); Naturhistorisches Museum, Wien (NMW); Universidad Nacional de Mar del Plata, Funes, Argentina (UNMDP).

3. Results

3.1. Systematics

3.1.1 The identity of *Psalidodon jequitinhonhae*

Since the morphology of the syntypes of *Tetragonopterus rutilus jequitinhonhae* were not enough for a reliable identification, we searched for accessing the DNA of the syntypes to compare with recently collected materials. Using ancient DNA techniques (see Material and Methods), a sequence of 254 bp fragment of COI was extracted and amplified. This small sequence was compared with characid specimens from Atlantic coastal rivers in Brazil and species of *Psalidodon* and the closely related genera *Astyanax*, *Andromakhe*, and *Deuterodon*. There were no sequences available in Genbank for comparison with the following species: *P. anisitsi*, *P. correntinus*, *P. ita*, *P. ojiara*, *P. pynandi*, *P. rioparanaibanus*. The lowest p-distance value, 0.03, between the *T. jequitinhonhae* syntype sequence (NWM 57760:2) was found with the specimens registered in Genbank as *Astyanax fasciatus jequitinhonhae*, which were also from the Jequitinhonha River (LBP 8311). Same findings are illustrated by the neighbor joining trees based on the Kimura 2-parameter (Fig. 3) and p-distance (Fig. 4). Also, the morphological information of these modern specimens match with that obtained from the

syntypes.

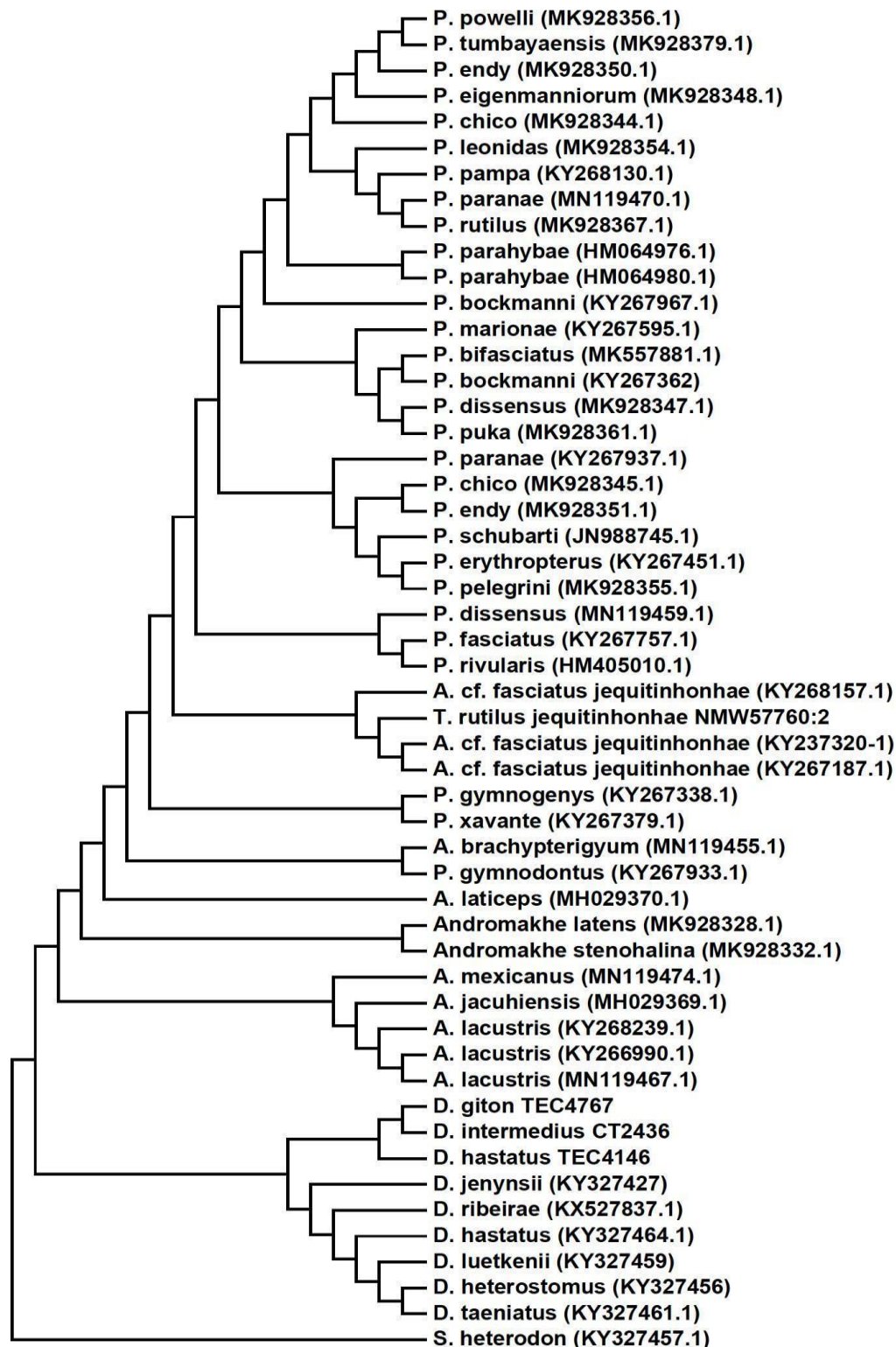


Figure 3. Neighbor-joining tree based on COI gene Kimura 2-parameters among *Tetragnopterus rutilus jequitinhonhae* lectotype (NWM 577:2) and species of the Stethaprioninae genera *Psalidodon*, *Astyanax*, *Andromakhe* and *Deuterodon*. Rooted in *Serrapinnus heterodon*.

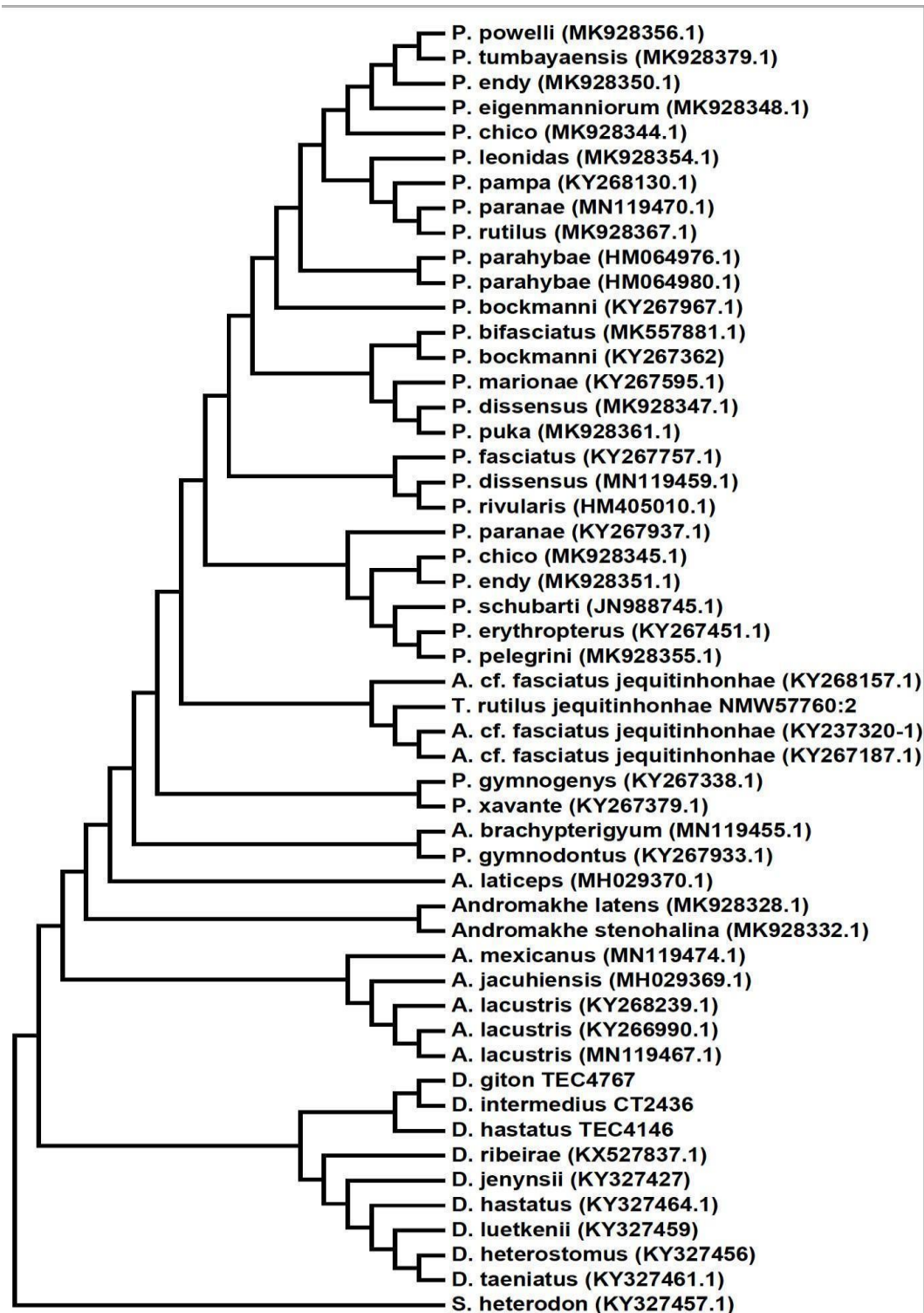


Figure 4. Neighbor-joining tree based on COI gene p-distance among *Tetragonopterus rutilus jequitinhonhae* lectotype (NWM 577:2) and species of the Stethaptrioninae genera *Psalidodon*, *Astyanax*, *Andromakhe* and *Deuterodon*. Rooted in *Serrapinnus heterodon*.

3.1.2. Redescription of *Psalidodon jequitinhonhae* (Steindachner, 1877)

Psalidodon jequitinhonhae (Steindachner, 1877)

(Figs. 1, 2, 5, 6)

Tetragonopterus rutilus jequitinhonhae Steindachner, 1877:693 [135], Pl. 2 (fig. 3). On p. 135 of separate. Name given in caption on p. 693 for the plate.

Astyanax fasciatus jequitinhonhae. –Eigenmann, 1921 (description, Jequitinhonha and Arassuahy [Araçuaí] rivers, Jequitinhonha River Basin). –Fowler, 1948:48 (checklist). –Triques et al., 2003:149 (listed and compared to *Astyanax turmalinensis*).

Astyanax fasciatus. –Lima et al., 2003:109 (listed as provisional synonym).

Astyanax jequitinhonhae. –Melo, 2005 (listed as a valid species and not as a subspecies; specimens examined MNRJ 11576, from rio São Miguel, tributary of Jequitinhonha River, Jequitinhonha, MG, Brazil). –Salgado, 2021 (listed as a valid species and compared to *Astyanax pardensis*).

Psalidodon jequitinhonhae. –Terán et al., 2020:10 (phylogenetic relationships).

Examined material. *Tetragonopterus rutilus jequitinhonhae*

Lectotype (by present designation): NMW 57760. 2, 67.52 mm SL, Brazil, Jequitinhonha River Basin. **Lectotype COI sequence:** GenBank accession number XXXXXXXX.

Paralectotypes: Brazil, Jequitinhonha River Basin: **NMW 57759**, 5, 54.59-67.25 mm SL, **NMW 57760**, 3, 65.2-74.01 mm SL, **NMW 57761**, 6, 66.49-74.72 mm SL.

Non-type specimens: Brazil, Minas Gerais State, Jequitinhonha River Basin: LBP 8311, 9 of 43, 47.8-83.26 mm SL, Jequitinhonha River, Itaobim, Minas Gerais, Brazil, 16°30'36"S 41°20'02"W, 15 May 2009, C. Oliveira, G. J. C. Silva, F. F. Roxo & T. N. A. Pereira.

Diagnosis. *Psalidodon jequitinhonhae* can be distinguished from most congeners by the

presence of one humeral spot (vs. two), except *Psalidodon correntinus* (Holmberg, 1891), *Psalidodon erythropterus* (Holmberg, 1891), *Psalidodon hermosus* (Miquelarena, Protogino & López, 2005), *Psalidodon ita* (Almirón, Azpelicueta & Casciotta, 2002), *Psalidodon parahybae* (Eigenmann, 1908), *Psalidodon pelegriini* (Eigenmann, 1907), *Psalidodon rioparanaibanus* Alves, Oliveira, Pasa & Kavalco, 2020. And by the humeral spot conspicuous, vertically elongated (vs. a small and diffuse rounded humeral spot in *P. correntinus*; vs. Y-shaped humeral spot in *P. hermosus*; vs. horizontal humeral spot in *P. pelegriini*). From *P. ita*, *P. jequitinhonhae* is distinguishable by four anteriormost dentary teeth large with five cusps, followed by a small tricuspidate tooth and six conical teeth (vs. dentary teeth decreasing in size anteroposteriorly). The number of scales in lateral line 36-37 and anal-fin rays iii-iv,20-25, respectively, distinguish *P. jequitinhonhae* from *P. erythropterus* (vs. 47-54 and iii-v,38-45), *P. parahybae* (30-40 and iii-iv,24-31) and *P. rioparanaibanus* (33-36 and 16-19).

Description. Morphometric data provided in Table 1. Body compressed and slightly elongated, with highest body depth at vertical through dorsal-fin origin. Dorsal profile of head nearly straight from upper lip to tip of supraoccipital spine, sometimes slightly concave at supraoccipital region. Dorsal body profile more convex posteriorly to tip of supraoccipital and slightly convex from that point to dorsal-fin base; straight and posteroventrally slanted along dorsal-fin base and slightly convex between dorsal and adipose fins. Ventral head profile slightly convex from anterior tip of dentary to isthmus. Ventral body profile smoothly convex from isthmus to origin of last anal-fin ray. Caudal peduncle concave along both dorsal and ventral margins.



Figure 5. Lectotype of *Tetragonopterus rutilus jequitinhonhae* (NMW 57760:2), 67.52 mm SL.



Figure 6. *Psalidodon jequitinhonhae*. Modern specimens from Jequitinhonha River basin LBP 8311: (A) male, 60 mm SL, (B) female, 49 mm SL.

Head small, head length nearly one-fourth of SL. Snout length smaller than eye diameter. Snout profile almost straight from margin of upper lip to vertical through anterior nostrils. Mouth terminal. Maxilla almost vertically positioned; posteriormost margins positioned in a vertical through anterior border of eye. Anteroventral border of maxilla convex and posterodorsal border concave. Infraorbital series complete with third infraorbital reaching preopercle along ventral and posterior margins. Fourth infraorbital more developed dorsoventrally than longitudinally.

Premaxillary teeth in two rows; outer row with 4(10) teeth bearing three cusps, with central cusp longer. Inner row with 5(10) teeth bearing five or more, frequently seven cusps, with last tooth smaller. Symphyseal premaxillary tooth of inner series asymmetrical, with one or two shorter cusps on medial side near symphysis, followed by one highest cusp and another two or three shorter cusps on lateral side of tooth. Maxilla with 1(10) tooth with three cusps, central cusp highest. Four anteriormost dentary teeth larger, with five cusps, followed by a smaller tricuspidate tooth and six conical teeth.

Dorsal-fin rays ii, 9(14). First unbranched ray approximately one-half length of second unbranched ray. Distal margin of dorsal fin straight. Dorsal-fin origin approximately at middle of standard length (SL) and posterior to vertical through pelvic-fin origin. Adipose fin approximately at vertical through last anal-fin rays insertion. Anal-fin rays iii(7)-iv(7), 20(1), 21(2), 22(1), 23(4), 24(1), 25(5). Anal-fin distal border concave; anterior-most rays longer than others. Anal-fin origin approximately at vertical through base of last dorsal-fin ray. Pectoral-fin rays i(14), 12(12), 13(2). Pectoral-fin tip reaching pelvic-fin insertion in males and not reaching in females. Pelvic-fin rays i(14), 7(14). Pelvic-fin origin anterior to vertical line through dorsal-fin origin. Pelvic-fin tip reaching anal-fin origin in males and not reaching in females. Caudal-fin forked with 19(5) principal rays, lobes similar in size.

Lateral line slightly curved ventrally in anteriormost portion, below miosepta, and then

nearly straight through caudal fin; completely pored, with 36(4) or 37(8) perforated scales. Horizontal scale rows between dorsal-fin origin and lateral line 5(2) or 6(10). Horizontal scale rows between lateral line and pelvic-fin origin 4(4) or 5(8). Pre-dorsal scales 10(1), 11(1), 12(2) arranged in regular series. Scale rows around caudal peduncle 13(1), 14(12), 15(1). Scale sheath along anal-fin base formed by ten to fourteen scales in single series and covering base of anteriormost rays.

Coloration in alcohol (Figs. 5, 6). Dorsal and dorsolateral portions of head light gray, lacking chromatophores. Infraorbitals, preopercle and opercular bones silvery, lacking chromatophores. Lips and snout unpigmented. Dorsal and dorsolateral portion of body light brown. Scales covering and ventral to lateral band silvery and unpigmented. Black and wide midlateral band visible under two longitudinal series of scales located dorsally to lateral line scale series, extending from two scales after humeral spot to middle caudal-fin rays. Brownish humeral spot, small, vertically elongate, two to three scales wide and two scales above lateral line. All fins mostly unpigmented, except for the middle caudal fin rays with a conspicuous black stripe.

Sexual dimorphism. Bony hooks were observed on pelvic-, pectoral and anal fins, only in males. In the anal fin, the bony hooks are elongate, one per segment of each lepidotrichia, more numerous from the last unbranched ray to the eighth or ninth branched rays, and smaller and less numerous on the branched portion of remaining anal-fin rays, with the number decreasing posteriorly to the anal fin; the hooks are nearly straight with a rounded base and distal end directed laterodorsally nearly parallel to ray axis. On the pelvic fin, the bony hooks are elongated, one per segment and positioned ventrally; the hooks are nearly straight with a rounded base and distal end directed to the fin ray base end nearly parallel to ray axis. Pectoral fin with fewer, smaller and short bony hooks near the tip of anal-fin rays.

Geographical distribution. (Fig. 7). Examined specimens are from the Jequitinhonha River

and have not been found so far in other river drainages.

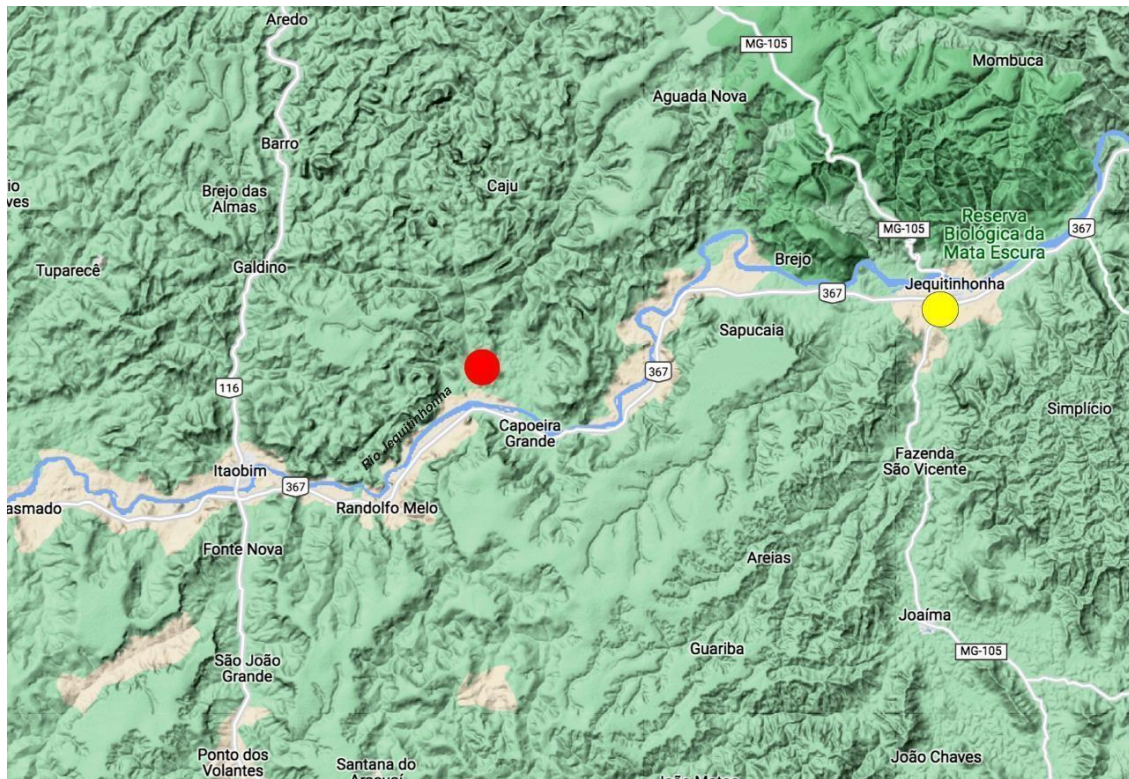


Figure 7. Collecting site of *Psalidodon jequitinhonhae* (red circle) LBP 8311, Jequitinhonha River, Itaobim, Minas Gerais, Brazil, 16°30'36"S 41°20'02"W.

4. Discussion

The analysis of syntypes and recently collected specimens allowed us to redescribe *Psalidodon jequitinhonhae* and designate a lectotype. No additional lots of *P. jequitinhonhae* were found from the Rio de Janeiro State rivers and from the Doce river in Minas Gerais in the analysis of different lots from the MCP, MZUFV and UFRGS fish collections (see comparative material examined).

Psalidodon jequitinhonhae is endemic to the Jequitinhonha River basin, however, the analysis of more material is necessary to delimit the distribution and determine the real conservation status of the species. Although the Jequitinhonha River is the most important natural resource in the region, according to IBGE (1997) and IEPHA (2014) several human

activities have contributed to the degradation of the basin and silting, which dates back to the 18th century with the extraction and trade of gold and diamonds, to the present day with deforestation, replaced by eucalyptus monocultures, pastures and agricultural crops, mining, extensive livestock, family farming, forestry, coffee growing, tourism, and agro-industry.

Weitzman et al. (1986) already pointed out the lack of studies on the ichthyofauna of the Jequitinhonha River basin. Although over the years there has been an increase in works describing or reviewing species in the basin (see Godinho et al., 1999; Triques et al., 2003, 2004; Oyakawa & Mattox. 2009; Carvalho et al., 2014; Teixeira et al., 2020), there is still a lack of data and information on the species in the basin (Weitzman et al., 1986; Andrade Neto, 2010; Triques et al., 2003). Two species of *Astyanax* (*sensu* Eigenmann 1921) were described for the basin: *Astyanax brevirohinus* Eigenmann, 1908 and *Astyanax turmalinensis* Triques, Vono & Caiafa, 2003, however no later work has included them in a phylogenetic analysis, as well as there are no genetic sequences deposited of these two species in the GENBANK and BOLD databases.

According to Triques et al. (2003) and Zanata & Camelier (2009) *Astyanax brevirohinus* has 28 anal-fins rays, the presence of an oval humeral spot shaped and five teeth in both outer and inner rows of premaxillary, 35 or more lateral-lines scales, presence of three, four or five teeth in maxillary. While *Astyanax turmalinensis* has ii-iii, 18-21 anal-fin rays; 34-36 lateral-lines scales, one or two teeth on maxillary, dentary with four large anterior teeth and four small posterior ones, outer and inner rows of premaxillary with four teeth, presence of two humeral spot, the first one conspicuous and vertically-elongated (Triques et al., 2003). Thus, *Psalidodon jequitinhonhae* can be distinguished from *A. brevirohinus* and *A. turmalinensis*, respectively, by the presence of a humeral spot vertically-elongated (vs. one oval humeral spot, presence of two humeral spots); 23-29 total anal-fin rays (vs. 28 in *A. brevirohinus* and 20-25 in *A. turmalinensis*). From *A. brevirohinus*, *P. jequitinhonhae* can be

distinguished by one maxillary tooth (vs. three to five). And from *A. turmalinensis*, *P. jequitinhonhae* can be distinguished by the presence of five teeth in outer series of premaxillary (vs. the presence of four teeth); by four anteriormost dentary teeth larger, followed by a small tooth and six teeth smaller (vs. four larger teeth, followed by four smaller).

Recently, in a study involving several researchers, Silva et al. (2020) published a checklist of freshwater fish species of the Bahia state. From 15 species of *Astyanax* (*sensu* Eigenmann 1921) listed: *Astyanax* aff. *bimaculatus* (Linnaeus, 1758); *Astyanax brucutu* Zanata, Lima, di Dario & Gerhard, 2017; *Astyanax burgerai* Zanata & Camelier, 2009; *Astyanax epiagos* Zanata & Camelier, 2008; *Astyanax fasciatus* (Cuvier, 1819); *Astyanax hamatilis* Camelier & Zanata, 2014; *Astyanax jacobinae* Zanata & Camelier, 2008; *Astyanax lacustris* (Lütken, 1875); *Astyanax lorien* Zanata, Burger & Camelier, 2018; *Astyanax pelecus* Bertaco & Lucena, 2006; *Astyanax rivularis* (Lütken, 1875); *Astyanax rupestris* Zanata, Burger & Camelier, 2018; *Astyanax sincora* Burger, Carvalho & Zanata, 2019; *Astyanax varii* Zanata, Burger, Vita & Camelier, 2019; *Astyanax vermilion* Zanata & Camelier, 2009, there are no records of the occurrence of any of them in the Jequitinhonha river basin. In addition, *Astyanax bahiensis* (Steindachner, 1877) and *Astyanax novae* (Eigenmann, 1911) are reported to occur in drainages of Bahia state, but there are no records of the occurrence of these species in the Jequitinhonha river basin (Godinho et al., 1999; Silva et al., 2020; Frick et al., 2022).

A neighbor-joining tree with two models is presented, Kimura 2-parameter (Fig. 5) and p-distance (Fig. 6), both based on COI gene, supports the hypothesis that the lectotype NMW57760.2 is more closely related to sequences from *P. jequitinhonhae* (LBP 8311). The short fragment of COI from the lectotype (254 pb) was enough to determine the relationship between specimens of *P. jequitinhonhae* from different sources and sufficiently informative in distinguishing it from other species of *Psalidodon* and related genera. The use of

mini-barcode has been proposed as an alternative use, when it is not possible to obtain the entire gene fragment, due to the natural DNA degradation (Boyer et al., 2012; Silva et al., 2017, 2019a). We have demonstrated that the short region obtained from the lectotype NMW57760.2 of *P. jequitinhonhae* contains enough interspecific variability to ensure accurate specimen identification (Boyer et al., 2012; Silva et al., 2017).

Terán et al. (2020) redefined *Psalidodon* including *P. jequitinhonhae*. Even though our analysis is limited to COI gene and does not intend to propose a phylogeny for *Psalidodon*, it is remarkable that this species grouped along with other species of the genus, and not to *Astyanax*, *Andromakhe* or *Deuterodon*. Our analysis also indicates the generic position of *Astyanax brachypterygium* and *A. laticeps* must be reviewed, once they grouped together with *Psalidodon*, instead of *Astyanax*. Some species do not seem to be taxonomically well resolved, as is the case of *P. bockmanni*, *P. chico*, *P. dissensus*, *P. endy*, *P. paranae*, which were recovered in more than one clade. This may have been due to the erroneous identification of specimens at the lowest taxonomic level. Although Terán et al. (2020) have contributed to elucidating the relationships between *Psalidodon*, many species remain unresolved, requiring redescriptions, further studies on the morphology, molecular, and distribution of the species.

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Table 1. Morphometric and meristic data of holotype of *Tetragonopterus rutilus* (BMNH 1917.7.14.14), syntypes of *Tetragonopterus rutilus jequitinhonhae* (NMW 57759, NMW 57760, NMW 57761) and *Psalidodon jequitinhonhae* from Jequitinhonha river basin. SD= standard deviation.

Character	<i>T. rutilus</i>		<i>T. rutilus jequitinhonhae</i>				<i>Psalidodon jequitinhonhae</i>				
		n	Low	High	Mean	SD	n	Low	High	Mean	SD
Standard length (mm)	83.59	14	54.33	75.60	66.87	-	9	47.9	83.26	60.38	-
Percents of Standard Length											
Predorsal distance	51.17	8	50.2	56.8	52.0	2.13	9	48.1	51.9	50.0	1.52
Prepelvic distance	49.13	8	44.2	48.4	46.2	1.41	9	44.9	48.8	46.7	1.36
Prepectoral distance	-	8	23.7	26.5	24.9	0.84	9	24.0	28.2	25.7	1.15
Preanal distance	-	8	62.2	65.3	64.1	1.07	9	61.9	65.7	63.3	1.39
Depth at dorsal-fin origin	38.24	8	32.9	35.8	34.4	1.14	9	27.9	37.0	29.4	9.95
Caudal peduncle depth	10.99	7	11.1	12.0	11.5	0.31	9	10.1	12.0	11.1	0.71
Caudal peduncle length	13.75	7	12.4	13.7	12.9	0.45	9	8.20	10.5	8.90	0.69
Anal-fin base	30.13	7	28.5	32.0	30.2	1.33	9	26.6	31.8	29.2	1.75
Dorsal fin length	25.88	7	25.5	27.9	26.8	0.87	9	22.9	27.6	25.6	1.50
Pelvic fin length (m)	16.79	3	17.5	20.4	18.8	1.49	9	15.8	19.3	17.3	1.04
Pelvic fin length (f)	-	4	17.1	18.0	17.6	0.41	-	-	-	-	-
Pectoral fin length	21.73	7	21.9	25.0	23.2	1.04	9	20.5	23.8	22.2	1.06
Head length	23.47	14	24.9	27.1	25.9	0.58	9	23.7	27.0	24.8	0.95
Percents of Head Length											
Snout length	25.12	7	20.7	21.8	21.3	0.41	9	27.8	31.0	29.5	1.00
Upper jaw length	42.30	7	39.2	42.3	40.7	1.26	9	39.3	44.5	42.2	1.64
Orbital diameter	33.02	14	38.8	42.4	41.1	1.06	9	33.3	37.2	35.2	1.07
Interorbital width	33.58	7	30.7	33.1	32.0	0.89	9	31.2	34.0	32.8	0.89

Comparative material examined. In addition, the following specimens were examined for this study. All listed specimens are alcohol-preserved material.

Brazil, Rio de Janeiro State. *Astyanax aff. jenynsii*: UFRGS 18913, 6, 63.83-76.57 mm SL, Visconde de Imbé/Trajano de Moraes, Macabu river basin, 22°04'16"S 42°08'42"W, 11 Jan 2014, P. C. Silva, U. Santos, A. Hirschmann, A. Thomaz & T. P. Carvalho. *Astyanax lacustris*: UFRGS 18910, 1, 63.02 mm SL, Macaé, Macaé river basin, 22°13'38"S 41°41'07"W, 11 Jan 2014, P. C. Silva, U. Santos, A. Hirschmann, A. Thomaz & T. P. Carvalho. UFRGS 19337, 10, 63.61-85.77 mm SL, Aduelas stream, Macaé river basin, Conceição de Macabu, 22°11'53.91"S 41°50'30.76"W, 26 May 2014, P. C. Silva & F. Di Dario. UFRGS 27168, 11, 69.18-105.13 mm SL, Bom Jardim, Grande river basin, 22°09'26"S 42°24'57"W, 03 Jan 2019, P. M. M. Ito. *Deuterodon intermedius*: UFRGS 10257, 3 of 62, 43.16-52.78 mm SL, Escola Municipal Adalberto de Mesquita, distrito de Ypiranga, Macacu, Macacu river, 22°38'11.6"S 42°42'42.3"W, 15 May 2004, C. E. Lopes, R. Pazza & K. F. Kavalco. UFRGS 10259, 4 of 52, 41.64-47.97 mm SL, Cachoeiras de Macacu, Macacu river, 22°29'06"S 42°39'40"W, 15 May 2004, C. E. Lopes, R. Pazza & K. F. Kavalco. UFRGS 18810, 1, 33.45 mm SL, Japeri, São Pedro river, São João basin, 22°36'32"S 43°34'08"W, 08 Jan 2014, P. C. Silva, U. Santos, A. Hirschmann, A. Thomaz & T. P. Carvalho. UFRGS 19336, 3 of 99, 37.95-42.94 mm SL, Sossego farm, Córrego das Aduelas, Conceição de Macabu, Macaé basin, 22°11'53.91"S 41°50'30.76"W, 26 May 2014, P. C. Silva & F. Di Dario. UFRGS 19341, 3 of 95, 50.86-56.41 mm SL, Sossego farm, Córrego das Aduelas, Conceição de Macabu, Macaé basin, 22°11'56.1"S 41°50'23.9"W, 26 May 2014, P. C. Silva & F. Di Dario. UFRGS 20035, 1 of 2, 45.32 mm SL, Silva Jardim, São João river basin, 22°32'45"S 42°27'55"W, 10 Jan 2014, P. C. Silva, U. Santos, A. Hirschmann, A. Thomaz & T. P. Carvalho. UFRGS 20038, 3 of 13, 42.83-49.65 mm SL, Silva Jardim, Pirineus river, São João basin, 22°30'26"S 42°29'12.3"W, 10 Jan 2014, P. C. Silva, U. Santos, A. Hirschmann, A.

Thomaz & T. P. Carvalho. UFRGS 22042, 4 of 11, 24.57-47.41 mm SL, Silva Jardim, Pirineus river, São João basin, 22°30'26"S 42°29'12.3"W, 10 Jan 2014, P. C. Silva, U. Santos, A. Hirschmann, A. Thomaz & T. P. Carvalho. ***Deuterodon hastatus***: UFRGS 10258, 4 of 10, 28.48-40.58 mm SL, Santana de Japuíba, Cachoeiras de Macacu, Macacu river basin, 22°33'39"S 42°40'53"W, 15 May 2004 C. E. Lopes, R. Pazza & K. F. Kavalco. UFRGS 18806, 2 of 63, 50.02-57.50 mm SL, Japeri, São Pedro river, 22°36'32"S 43°34'08"W, 08 Jan 2014, P. C. Silva, U. Santos, A. Hirschmann, A. Thomaz & T. P. Carvalho. UFRGS 18849, 6 of 19, 35.08-53.27 mm SL, Guapimirim, Guapimirim river basin, 22°31'04"S 42°53'55"W, 10 Jan 2014, P. C. Silva, U. Santos, A. Hirschmann, A. Thomaz & T. P. Carvalho. UFRGS 18859, 2, 42.53-51.64 mm SL, Guapimirim, Guapimirim river basin, 22°31'04"S 42°53'55"W, 10 Jan 2014, P. C. Silva, U. Santos, A. Hirschmann, A. Thomaz & T. P. Carvalho. UFRGS 18904, 3 of 9, 25.78-38.03 mm SL, Macaé, Macaé river basin, 22°13'38"S 41°41'07"W, 11 Jan 2014, P. C. Silva, U. Santos, A. Hirschmann, A. Thomaz & T. P. Carvalho. UFRGS 18906, 1, 41.91 mm SL, Macaé, Macaé river basin, 22°13'38"S 41°41'07"W, 11 Jan 2014, P. C. Silva, U. Santos, A. Hirschmann, A. Thomaz & T. P. Carvalho. UFRGS 19340, 3 of 17, 25.78-35.63 mm SL, Conceição de Macabu, Macaé river basin, 22°11'53.91"S 41°50'30.76"W, 19 May 2014, P. C. Silva & F. Di Dario. UFRGS 20036, 2 of 3, 24.49-39.21 mm SL, Silva Jardim, São João river basin, 22°32'45"S 42°27'55"W, 10 Jan 2014, P. C. Silva, U. Santos, A. Hirschmann, A. Thomaz & T. P. Carvalho. UFRGS 20039, 4 of 9, 31.02-37.13 mm SL, Silva Jardim, São João river basin, 22°30'26"S 42°29'12.3"W, 10 Jan 2014, P. C. Silva, U. Santos, A. Hirschmann, A. Thomaz & T. P. Carvalho. ***Deuterodon taeniatus***: UFRGS 18870, 1, 66.78 mm SL, Silva Jardim, São João river basin, 22°34'58"S 42°34'29"W, 10 Jan 2014, P. C. Silva, U. Santos, A. Hirschmann, A. Thomaz & T. P. Carvalho. UFRGS 18884, 6 of 24, 46.45-56.35 mm SL, Silva Jardim, São João river basin, 22°30'26"S 42°29'12.3"W, 10 Jan 2014, P. C. Silva, U. Santos, A. Hirschmann, A. Thomaz & T. P.

Carvalho. UFRGS 18888, 2 of 7, 47.14-52.52 mm SL, Silva Jardim, São João river basin, 22°32'45"S 42°27'55"W, 10 Jan 2014, P. C. Silva, U. Santos, A. Hirschmann, A. Thomaz & T. P. Carvalho. UFRGS 18917, 1, 95.22 mm SL, São Sebastião do Alto, Grande river, 22°01'9.5"S 42°07'51.5"W, 11 Jan 2014, P. C. Silva, U. Santos, A. Hirschmann, A. Thomaz & T. P. Carvalho. UFRGS 19335, 6 of 61, 31.61-40.17 mm SL, Conceição do Macabu, Aduelas stream, Macaé river basin, 22°11'53.91"S 41°50'30.76"W, 26 May 2014, P. C. Silva & F. Di Dario. UFRGS 19342, 3 of 17, 42.46-63.28 mm SL, Conceição do Macabu, Aduelas stream, Macaé river basin, 22°11'56.1"S 41°50'23.9"W, 26 May 2014, P. C. Silva & F. Di Dario.

Brazil, Minas Gerais State. *Deuterodon giton*: MZUFV 3461, 1, 73.8 mm SL, Lagoa da Funarbe, UFV, Doce river basin, Viçosa, Minas Gerais, 20°46'16"S 42°52'27"W, 20 Jun 2004, E. S. Eler, L. C. Paiva & G. A. S. Miranda. MZUFV 4120, 2, 51.67-53.88 mm SL, Córrego Latão, Turvo river, Doce river basin, Coimbra, Minas Gerais, 20°49'01"S 42°49'57"W, 09 May 2013, Melo, Lima & J. Dergam. MZUFV 4459, 8, 46.25-51.96 mm SL, Doce river basin, Catas Altas, Minas Gerais, 20°04'07"S 43°24'49"W, 14 Jun 2012, Ferreira, Reis, J. Dergam & Cunha.

Psalidodon sp. Brazil, Minas Gerais State, Doce river basin: MZUFV 2574, 6 of 13, 72.59-79.22 mm SL, Prainha, Santana river, Doce river drainage, Canaã, Minas Gerais, Brazil, 20°36'18"S 42°32'30"W, 23 Dez 1997, J. L. Pontes & C. Rocha. MZUFV 3972, 1, 88.43 mm SL, Preto river, Itambé do Mato Dentro, Minas Gerais, Brazil, 19°23'43"S 43°21'44"W, 19 Set 2011, U. Santos & E. Valgas. MZUFV 5006, 1, 88.67 mm SL, Carmo river, Barra Longa, Minas Gerais, Brazil, 20°16'52"S 43°02'28"W, F. Ferreira & F. Machado. MZUFV 5297, 4 of 7, 68.02-86.17 mm SL, Sete Cachoeiras, upper Santo Antônio river, Ferros, Minas Gerais, Brazil, 19°13'58"S 43°01'20"W, 21 April 2012, J. Dergam.

Brazil, Minas Gerais State, Paraíba do Sul basin: MZUFV 5262, 3, 75.20-92.61 mm SL, Ribeirão Espírito Santo, Paraíbuna river, Juiz de Fora, Minas Gerais, Brazil, 22°04'51"S

43°08'56"W. MZUFV 5671, 5 of 10, 68.36-87.61 mm SL, Glória river, Muriaé, Minas Gerais, Brazil, 21°07'44"S 42°22'13"W, 15 Dez 2015, Raul Vert Ambiental.

7. Supplementary material

Supplementary Table 1. List of species used in molecular phylogenies. It contains the voucher, tissue code, location, basin, GenBank identification, and references to sequences downloaded and used from the databases.

Species	Voucher	Tissue	Location	Drainage/Basin	GenBank	Reference
<i>Psalidodon bifasciatus</i>	UFRGS 26258	TEC 8960	-	Piquiri river, upper Paraná river basin	MK557881	Neves et al., 2020
<i>Psalidodon bockmanni</i>	-	LBPV 59950	Rio Paranaíba, Minas Gerais, Brazil	Tributary of Parnaíba river, Paraná basin	KY267967	Rossini et al., 2016
	-	LBPV 59951	Rio Paranaíba, Minas Gerais, Brazil	Tributary of Parnaíba river, Paraná basin	KY267362	Rossini et al., 2016
<i>Psalidodon chico</i>	CI-FML 6111	FML 5897	Salta, Argentina	Unnamed lagoon close to Itau river	MK928344	Terán et al., 2020
	CI-FML 5363	B43	Salta, Argentina	Blanco river, Bermejo river basin	MK928345	Terán et al., 2020
<i>Psalidodon dissensus</i>	MNHN-I 4008	TUC 74 (2)	Paysandú, Uruguay	Guaviyú stream, Uruguay River basin	MK928347	Terán et al., 2020
	UFRGS 16521	TEC 3225C	Rio Grande do Sul, Brazil	Laguna dos Patos	MN119459	Wendt et al., 2019
<i>Psalidodon eigenmanniorum</i>	CI-FML 7328	FML 300	Catamarca, Argentina	Lower Sucuma River	MK928348	Terán et al., 2020
<i>Psalidodon endy</i>	CI-FML 5362	B47	Salta, Argentina	El Oculito stream, Bermejo River basin	MK928350	Terán et al., 2020
	CI-FML 6112	ex 5898	Salta, Argentina	Unnamed lagoon close to Itau river.	MK928351	Terán et al., 2020
<i>Psalidodon erythropterus</i>	UNMDP-T 557	-	Buenos Aires, Argentina	Paraná river	KY267451	Rossini et al., 2016
<i>Psalidodon fasciatus</i>	-	LBPV 48984	Pirapora, Minas Gerais, Brazil	Velhas river, São Francisco river basin	KY267757	Rossini et al., 2016
<i>Psalidodon gymnodontus</i>	-	LBPV 69181	Porto Barreiro, Paraná, Brazil	Iguaçu river, Paraná basin	KY267933	Rossini et al., 2016
<i>Psalidodon gymnogenys</i>	-	LBPV 69188	São Jorge d'Oeste, Paraná, Brazil	Iguaçu river, Paraná basin	KY267338	Rossini et al., 2016
<i>Psalidodon jequitinhonhae</i>	LBP 8311	LBPV 38393	Itaobim, Minas Gerais, Brazil	Jequitinhonha river basin	KY267320	Rossini et al., 2016
	LBP 8311	LBPV 38395	Itaobim, Minas Gerais, Brazil	Jequitinhonha river basin	KY267187	Rossini et al., 2016
	LBP 8311	LBPV 38396	Itaobim, Minas Gerais, Brazil	Jequitinhonha river basin	KY267961	Rossini et al., 2016
	LBP 8311	LBPV 38397	Itaobim, Minas Gerais, Brazil	Jequitinhonha river basin	KY268157	Rossini et al., 2016

Species	Voucher	Tissue	Location	Drainage/Basin	GenBank	Reference
<i>Psalidodon leonidas</i>	LGEP 703	LGEP 703	Misiones, Argentina	Uruguai, Uruzú stream, Paraná river basin	MK928354	Terán et al., 2020
<i>Psalidodon marionae</i>	-	LBPV 28314	Cuiabá, Mato Grosso, Brazil	Coxipó-açu river, Paraguai river basin	KY267595	Rossini et al. 2016; Terán et al., 2020
<i>Psalidodon pampa</i>	-	LBPV 21014	Rio Grande, Rio Grande do Sul, Brazil	Unnamed stream, Atlântico	KY268130	Rossini et al., 2016
<i>Psalidodon parahybae</i>	LBP 7706	LBP 36323	Paraibuna, São Paulo, Brazil	Paraibuna, Paraíba do Sul river basin	HM064980	Pereira et al., 2011
	LBP 6469	LBP 29090	Jacareí, São Paulo, Brazil	Paraíba do Sul river basin	HM064976	Pereira et al., 2011
<i>Psalidodon paranae</i>	UFRGS 15071	TEC 1855	São Paulo, Brazil	Upper Paraná river basin	MN119470	Wendt et al., 2019
	-	LBPV 45841	Miranda, Mato Grosso do Sul, Brazil	Abobral river, Paraguai river basin	KY267937	Rossini et al., 2016
<i>Psalidodon pelegriini</i>	CFA-IC 3147	ARII 555	Formosa, Argentina	Paraguay river basin	MK928355	Terán et al., 2020
<i>Psalidodon powelli</i>	CI-FML 7327	FML 291	Catamarca, Argentina	Lower Sucuma river	MK928356	Terán et al., 2020
<i>Psalidodon puka</i>	CI-FML 7125	FML 062	Simoca, Tucumán, Argentina	Los Perez stream, Salí river basin	MK928361	Terán et al., 2020
<i>Psalidodon rivularis</i>	-	DCC 42099	Pedro Leopoldo, Minas Gerais, Brazil	Velhas river, São Francisco river basin	HM405010	de Carvalho et al., 2011
<i>Psalidodon rutilus</i>	CI-FML 7329	FML 069	Gualeduay, Entre Rios, Argentina	Paraná river basin	MK928367	Terán et al., 2020
<i>Psalidodon schubarti</i>	-	LBPV 31723	Botucatu, São Paulo, Brazil	Paraná river basin	JN988745	Pereira et al., 2013
<i>Psalidodon troya</i>	CI-FML 7332	YCO-1	Misiones, Argentina	unnamed stream, Paraná river basin	MK928373	Terán et al., 2020
<i>Psalidodon tumbayaensis</i>	CFA-IC 5236	FHN 2044	Tumbaya, Jujuy, Argentina	Artificial canal, Bermejo river basin	MK928379	Terán et al., 2020
<i>Psalidodon xavante</i>	-	LBPV 12556	Alto Araguaia, Mato Grosso, Brazil	Sapo stream, Araguaia river basin	KY267379	Rossini et al., 2016
<i>Tetragonopterus rutilus jequitinhonhae</i>	NMW 57760	2	-	Jequitinhonhae river basin	-	This study
<i>Andromakhe latens</i>	CI-FML 7392	A5	Salta, Argentina	El Oculito stream, Bermejo river basin	MK928328	Terán et al., 2020
<i>Andromakhe</i>	CI-FML 7331	0046	Victoria-Rosário,	Paraná river basin	MK928332	Terán et al.,

Species	Voucher	Tissue	Location	Drainage/Basin	GenBank	Reference
<i>stenohalina</i>			Entre Rios, Argentina			2020
<i>Astyanax brachpterygum</i>	UFRGS 21849	TEC6844	Rio Grande do Sul, Brazil	Pelotas river, Laguna dos Patos	MN119455	Wendt et al., 2019
<i>Astyanax jacuhiensis</i>	UFRGS 19151	TEC4869 B	Três Forquilhas, Rio Grande do Sul, Brazil	Três Forquilhas river	-	Silva et al., 2017
<i>Astyanax jenynsii</i>	UFRGS 18917	TEC 4272	São Sebastião do Sul, São Paulo, Brazil	Grande river	-	Silva et al., 2017
<i>Astyanax lacustris</i>	UFRGS 19033	TEC4783	Pedro Canário, Espírito Santo, Brazil	Engano river	-	Silva et al., 2017
	-	LBPV 38390	Itaobim, Minas Gerais, Brazil	Jequitinhonha river basin	KY266990	Rossini et al., 2016
	-	LBPV 38392	Itaobim, Minas Gerais, Brazil	Jequitinhonha river basin	KY268239	Rossini et al., 2016
<i>Astyanax laticeps</i>	UFRGS 18251	TEC3748 A	Maquiné, Rio Grande do Sul, Brazil	Maquiné river	-	Silva et al., 2017
<i>Astyanax mexicanus</i>	UFRGS 23111	TEC7407	México	-	-	Silva et al., 2017
<i>Deuterodon intermedius</i>	MZUFV 4458	CT 2436	-	Doce river basin	-	Silva et al., 2017
<i>Deuterodon giton</i>	UFRGS 18952	TEC 4767	Santa Maria de Jetibá, Espírito Santo, Brazil	Santa Maria da Vitória river	-	Silva et al., 2017
<i>Deuterodon hastatus</i>	UFRGS 18904	TEC 4527	Macaé, Rio de Janeiro, Brazil	Macaé river basin	KY327464	Silva et al., 2017
	UFRGS 18526	TEC 4146	Peruíbe, São Paulo, Brazil	Batatal river	-	Silva et al., 2017
<i>Deuterodon heterostomus</i>	UFRGS 18758	TEC 4184	-	Paraíbuna river, Paraíba do Sul river basin	KY327456	Silva et al., 2017
<i>Deuterodon luetkenii</i>	UFRGS 19226	TEC 4921	-	Mostardas river	KY327459	Silva et al., 2017
<i>Deuterodon ribeirae</i>	UFRGS 18647	TEC 3953	Garuva, Santa Catarina, Brazil	Guaratuba river	-	Silva et al., 2017
<i>Deuterodon taeniatus</i>	UFRGS 19342	TEC 5000	Conceição de Macabu, Rio de Janeiro, Brazil	Aduelas stream, Macaé river basin	KY327461	Silva et al., 2017
<i>Serrapinnus heterodon</i>	UFRGS 22004	TEC 6956	-	Doce river basin	KY327457	Silva et al., 2017

8. Conclusão

Este trabalho apresenta a redescrição da espécie de peixe caracídeo *Psalidodon jequitinhonhae*, combinando dados morfológicos, merísticos e moleculares, endêmica de uma região ainda pouco estudada e com uma lacuna de informações sobre a ictiofauna local. Por tanto, buscamos propor caracteres morfológicos que diagnosticam a espécie, o DNA *barcode* e designamos um lectótipo. Dessa forma, pretendemos contribuir com a diagnose mais acurada da espécie e suas relações filogenéticas com as demais espécies de *Psalidodon*, a fim de auxiliar o conhecimento e preencher as lacunas de informação da ictiofauna das bacias estudadas.