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DNA extracted from museum specimens of the 19th century provides a taxonomic resolution on the identity of the characid fish *Psalidodon jequitinhonhae* (Ostariophysi: Characiformes)

Correspondence:
Victor de Queiroz
dequeirozvictor@gmail.com

Victor de Queiroz[1,2], Priscilla Caroline Silva[1], Maria Claudia Malabarba[1], Lee Weigt[3], Jorge Abdala Dergam[2] and Luiz R. Malabarba[1]

Psalidodon jequitinhonhae was originally proposed as a variety of *Tetragonopterus rutilus*, based on the analysis of 14 specimens from the Jequitinhonha River, Brazil. In 1910 it was relocated in *Astyanax*, as *A. fasciatus jequitinhonhae* in Tetragonopterinae and in 2020 in *Psalidodon*, as Stethaprioninae member. However, in none of these revisions, *P. jequitinhonhae* was morphologically redescribed. A short sequence of the Cytochrome c oxidase subunit I (COI) gene obtained from one of the syntypes is compared to sequences obtained from new samples, allowing the recognition of the species and its morphological redescription based on new specimens. Both morphological and molecular data converged and corroborated *P. jequitinhonhae* as a valid species, occurring in the Jequitinhonha and Pardo river basins in Brazil. The syntype that provided the analyzed COI sequence is referred to as the lectotype by present designation.

Keywords: Historical DNA, Jequitinhonha River, Mini-barcode, Museomics, Pardo River.

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1 Programa de Pós-Graduação em Biologia Animal, Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, 91501-970 Porto Alegre, RS, Brazil. (VQ) dequeirozvictor@gmail.com (corresponding author), (PCS) pricarola@gmail.com, (MCM) claudia.malabarba@ufrgs.br, (LRM) malabarb@ufrgs.br.

2 Laboratório de Sistemática Molecular - Beagle, Departamento de Biologia Animal, Universidade Federal de Viçosa, Av. P.H. Rolfs, s/ n, Anexo CCB II, 36570-900 Viçosa, MG, Brazil. (JAD) jdergam@gmail.com.

3 National Museum of Natural History, Smithsonian Institution, 10th and Constitution Ave, NW, Washington, DC 20560, USA. (LW) weigtl@si.edu.

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Psalidodon jequitinhonhae foi proposta originalmente como uma variedade de *Tetragonopterus rutilus*, a partir da análise de 14 exemplares coletados no rio Jequitinhonha, Brasil. Em 1910 foi realocada em *Astyanax*, como *A. fasciatus jequitinhonhae* em Tetragonopterinae e em 2020 em *Psalidodon*, como membro de Stethaprioninae. Em nenhuma dessas revisões, no entanto, *P. jequitinhonhae* foi redescrita morfologicamente. A obtenção de uma sequência curta do gene COI a partir de um dos síntipos, comparada a sequências obtidas em novas amostras, permitiu o reconhecimento da espécie e a sua redescrição morfológica com base em novos espécimes. Tanto os dados morfológicos quanto os moleculares convergiram e corroboraram *P. jequitinhonhae* como uma espécie válida, ocorrendo nas bacias dos rios Jequitinhonha e Pardo no Brasil. O síntipo que forneceu a sequência de COI analisada é designado como lectótipo.

Palavras-chave: DNA histórico, Mini-barcode, Museômica, Rio Jequitinhonha, Rio Pardo.

INTRODUCTION

The species name referred herein was first proposed as *Tetragonopterus rutilus* var. *Jequitinhonhae* [sic] in an illustration (Steindachner, 1877:683, plate II, fig. 3), referring to specimens from the Jequitinhonha River identified as *Tetragonopterus rutilus* Jenyns, 1842 by Steindachner and housed in the Naturhistorisches Museum, Vienna. Besides the illustration (Fig. 1A) and the reference to examined specimens, latter referred as syntypes (NMW 57759–61: 5, 3, 6; Fricke *et al*., 2023), Steindachner (1877:577–78) described his new "variety" by the strikingly elongated body shape, when compared to other populations of *T. rutilus* from the Paraíba and Doce rivers, but also stated that since they did not differ on fin rays and scales count, they would not be described as a separate species from *T. rutilus*.

Tetragonopterus rutilus was previously described from a single specimen collected by Charles Darwin in the Paraná River basin, Argentina (Jenyns, 1842). Steindachner (1877), however, largely expanded the diagnosis and distribution of the species, including specimens from Uruguay, Brazil, and Mexico. In his inclusive concept, Steindachner (1877) listed some species as junior synonyms of *T. rutilus*, that were latter revalidated or even transferred to other genera by subsequent authors (Lima *et al*., 2003; Melo, Buckup, 2006; Silva *et al*., 2019a; Terán *et al*., 2020), leaving the taxonomic status of *Tetragonopterus rutilus jequitinhonhae* uncertain.

Eigenmann (1910:433) listed *T. rutilus* as a junior synonym of *Astyanax fasciatus* (Cuvier, 1819), and the 'variety' of Steindachner in a new combination as a subspecies, *A. fasciatus jequitinhonhae*. Later, Eigenmann (1921:304) briefly diagnosed the subspecies and illustrated (Fig. 1B) a new specimen (Eigenmann, 1921: plate 50, fig. 3; MCZ 20901), listing specimens examined from the Arassuahy [current Araçuaí Municipality, Minas Gerais State] and Jequitinhonha rivers, both belonging to the Jequitinhonha River basin. Eigenmann (1921) further referred ten specimens from Doce River basin and two specimens from "São Matheos" [current São Mateus Municipality, Espírito Santo State] as possibly belonging to this "variety" [sic], but mentioned they were in really

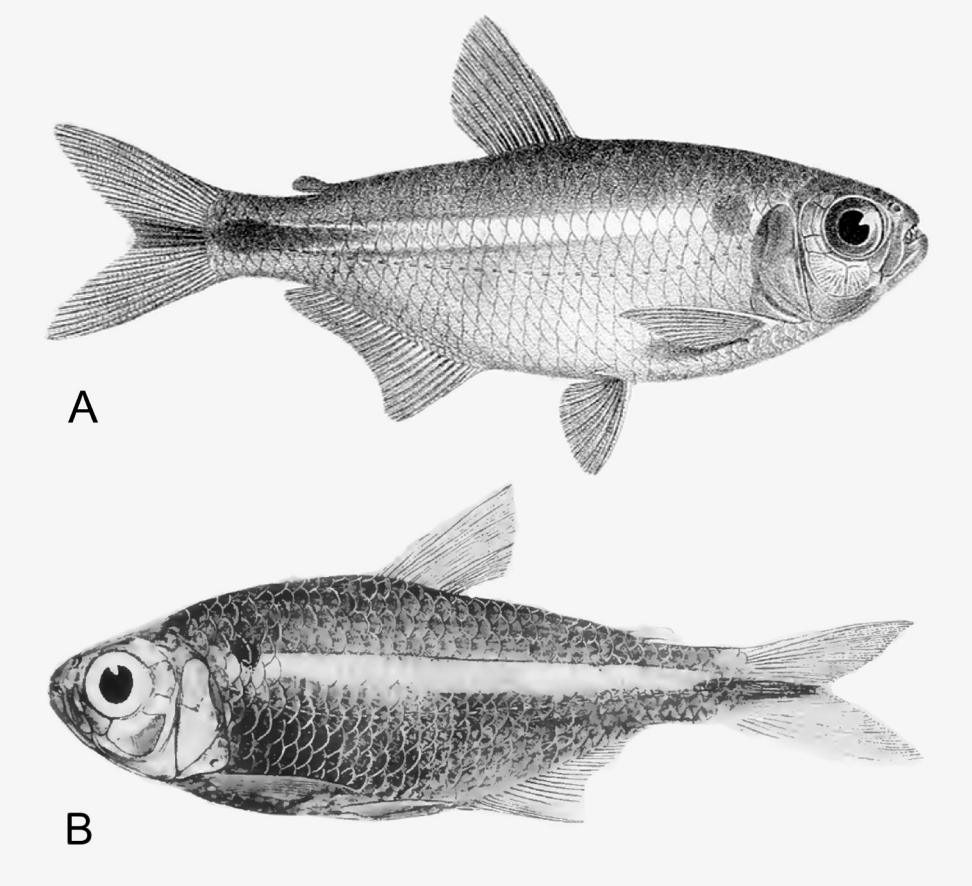

FIGURE 1 | **A.** *Tetragonopterus rutilus* var. *Jequitinhonhae* [sic] as illustrated by Steindachner (1877: plate II, fig. 3). **B.** *Astyanax fasciatus jequitinhonhae* as illustrated by Eigenmann (1921: plate 50, fig. 3; MCZ 20901; 83 mm; Jequitinhonha Rive, Brazil.

bad conditions and differed from "*A. jequitinhonhae*" in the increased number of gill rakers. Eigenmann (1921) believed that part of the specimens identified and described by Steindachner (1877) as *T. rutilus* actually belonged to other species, such as *A. jenynsii* (Steindachner, 1877), *A. scabripinnis* (Jenyns, 1842), and *A. taeniatus* (Jenyns, 1842) (currently in *Deuterodon* Eigenmann, 1907), and presented a table with the numbers of anal-fin rays and scales along the lateral line for '*A. fasciatus*' and its varieties/subspecies.

In an unpublished thesis, Melo (2005) proposed *Astyanax jequitinhonhae* as a valid species, and not a subspecies of *A. fasciatus* or *A. rutilus*, but, once unpublished, this was not a valid nomenclatural act. Following results from Melo (2005), Melo, Buckup (2006) restricted *A. fasciatus* to the São Francisco River basin (later corroborated by Gavazzoni *et al*., 2023) and revalidated *A. rutilus*, occurring in the Paraná River basin, but not mentioning *A. jequitinhonhae*.

Rossini *et al*. (2016) presented a comprehensive comparative analysis of 64 nominal species then referred to *Astyanax* Baird & Girard, 1854 using a barcode segment of the 5' region of the mitochondrial Cytochrome c oxidase subunit I (COI) gene. One of these species was provisionally identified as *Astyanax* cf. *jequitinhonhae*, and includes COI sequences of four specimens from Itaobim Municipality, Minas Gerais State, collected in the Jequitinhonha River (LBPV 38393, 38395, 38396, and 38397).

Silva *et al*. (2019b) reported the successful extraction of DNA from syntypes of *T. rutilus jequitinhonhae* and compared to sequences from samples of *A. fasciatus* from São Francisco River and *A.* aff. *fasciatus* from Rio Grande do Sul, Brazil, but did not go further in diagnosing the species.

Terán *et al*. (2020) proposed a new combination, *Psalidodon jequitinhonhae* (Steindachner, 1877) based on the examination of specimens from Brazil, Itamarandiba Municipality, Minas Gerais State, Jequitinhonha River basin (CI-FML 7126; ex-MZUEL 7244). Although Terán *et al*. (2020) redefined the relationship of *P. jequitinhonhae*, a detailed redescription and diagnosis of the species was not presented. Only two cleared and stained specimens from upper Jequitinhonha River basin were cited by Terán *et al*. (2020).

Analysis of DNA content of museum collections, or museomics, has transformed museums into huge and valuable warehouses for DNA-based studies in a broad range of biodiversity topics (Graves, Braun, 1992; Gilbert *et al*., 2005; Fong *et al*., 2023). In the last decades, the combination of museum specimens with DNA sequencing has proven to be a successful powerful methodology to extract ancient DNA (aDNA) from samples recovered from field after natural death, such as mammoths and cave bears (Orlando *et al*., 2002; Gilbert *et al*., 2008). More recently distinguished from aDNA, historical DNA (hDNA) is the one extracted from formalin-fixed or ethanol-fixed museum specimens that were collected over the past few hundred years, such as type specimens of a given species (Silva *et al*., 2017, 2019; Billerman, Walsh, 2019; Fong *et al*., 2023).

On this work, we compare hDNA data generated from more than 140-year-old syntypes (Silva *et al*., 2019b) of the characid *Tetragonopterus rutilus jequitinhonhae* to molecular data of *Astyanax* cf. *jequitinhonhae* from Rossini *et al*. (2016), and from newly collected specimens. The morphological examination of the corresponding vouchers allowed us to diagnose and describe the morphology of *Psalidodon jequitinhonhae*. All information so far available in the literature is based on short morphological comparisons to other species (Steindachner, 1877; Eigenmann, 1921), provisional identifications (Rossini *et al*., 2016) or incomplete molecular or morphological data (Silva *et al*., 2019b; Terán *et al*., 2020).

MATERIAL AND METHODS

DNA extraction and sequencing for museum specimens. All procedures involved in obtaining and amplifying hDNA were performed following the established sterilization guidelines (Cooper, Poinar, 2000; Gilbert *et al*., 2005; Fulton, 2012; Silva *et al*., 2017, 2019a,b), to discard any possibility of contamination. For our hDNA analysis, a gill arch fragment of approximately 2 mg was removed, less invasively as possible to avoid unnecessary damage to specimens, from the right side of the body (see Silva *et al*., 2019b) of the *Tetragonopterus rutilus jequitinhonhae* syntypes: NMW57759, NMW57760:1, NMW57760:2. Extractions were conducted in a dedicated area free from DNA and PCR products (amplicons) at the molecular biology facilities at Museum Support Center (MSC) of the National Museum of Natural History, Smithsonian Institution, Washington DC, USA (NMNH-SI). Amplifications (PCR reactions) were conducted in a clean room of an hDNA laboratory at MSC, using specially designed set of primers. For more details about extraction, primer design, amplification and purification procedures, conditions and protocols for type specimens see Silva *et al*. (2019b).

PCR products obtained from museum specimens were sequenced on Laboratories of Analytical Biology (LAB) at NMNH-SI. Strands (forward and reverse) of each sequence fragment were independently aligned using MUSCLE in MEGA11 software (Tamura *et al*., 2021). The p-distance between the historical sequence and modern ones, was estimated using the default conditions (d: Transitions. Transversions; uniform rates; Pairwise deletion; three codon positions selected) of the MEGA 11 software (Tamura *et al*., 2021). Neighbor joining topology was constructed using default conditions for barcode analysis (Hebert *et al*., 2003) and using p-distance model.

DNA extraction and sequencing for modern specimens. DNA was extracted 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 H20, 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 11 software (Tamura *et al*., 2021) and alignments were visually inspected for any obvious misalignments and then corrected. Sequences of modern specimens were trimmed to the same length of the hDNA sequence before all analyses. Genetic distances among specimens were calculated using p-distance in MEGA 11, in order to demonstrate the relationships between specimens.

All work involving modern DNA was performed at the Laboratório Molecular, Departamento de Zoologia, Universidade Federal do Rio Grande do Sul, Porto Alegre. Vouchers, locality information, and GenBank accession numbers are summarized in Tab. **S1**.

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. 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). Counts of vertebrae, supraneurals, pterygiophores, and gill-rakers were taken from cleared and stained (c&s) specimens prepared according to Taylor, Van Dyke (1985). Vertebral counts included the four vertebrae of the Weberian apparatus, and the terminal centrum counted as a single element. Morphological and meristic data from syntypes of *Tetragonopterus rutilus jequitinhonhae* were taken by PCS while visiting to Naturhistorisches Museum, Wien. In the description, counts are followed by the frequency in parentheses, and the lectotype counts are marked with an asterisk. The diagnosis was prepared by examining specimens listed as comparative material and literature data available in Britski (1964), Azpelicueta,

García (2000), Almirón *et al*. (2002), Azpelicueta *et al*. (2002), Casciotta, Almirón (2004), Casciotta *et al*. (2003, 2005), Miquelarena, Menni (2005), Miquelarena *et al*. (2005), Mirande *et al*. (2006, 2007), Vari, Castro (2007), Garutti, Venere (2009), Garavello, Sampaio (2010), Lucena *et al*. (2013), Terán *et al*. (2017), and Alves *et al*. (2020).

Institutional abbreviations. CI-FML. Ichthyological Collection of Fundación Miguel Lillo, Tucumán; DZUFMG, Departamento de Zoologia, Universidade Federal de Minas Gerais, Belo Horizonte; LBP and LBPV, fish and tissue collections, respectively, Laboratório de Biologia de Peixes, Universidade Estadual Paulista "Júlio de Mesquita Filho", Botucatu; MCP, Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre; MZUEL, Museu de Zoologia da Universidade Estadual de Londrina, Londrina; MZUFV Museu de Zoologia João Moojen, Universidade Federal de Viçosa, Viçosa; NMW, Naturhistorisches Museum, Wien; UFRGS, Departamento de Zoologia, Universidade Federal do Rio Grande do Sul, Porto Alegre.

RESULTS

The identity of *Psalidodon jequitinhonhae*. Historical DNA (hDNA) obtained from museum specimens is an additional and valuable tool to support accurate identifications based on type specimens. This is the case of the syntypes of *Tetragonopterus rutilus jequitinhonhae*. We succeed to extract DNA from the three syntypes of *Tetragonopterus rutilus jequitinhonhae* (see Silva *et al*., 2019b), but only the amplification of the syntype NMW57760.2 (Fig. 2) generated a viable sequence fragment (COI-2) with 218 base pairs.

A first BLAST search for this small sequence in the Genbank, indicated the greatest similarity (97.80%) with sequences identified as *Psalidodon* cf. *fasciatus jequitinhonhae* (tissues LBPV 38393, 38395, 38396 and 38397, belonging to vouchers lot LBP 8311), the same specimens from Rossini *et al*. (2016). In addition, new sequences obtained from materials recently collected in the Jequitinhonha River (the river basin referred as the type locality; MZUFV 3167 and 3582) and Pardo River drainages (MZUFV 3422) were analyzed together with the historical sequence. The syntype sequence clustered together with sequences of recently collected specimens of *P. jequitinhonhae* under neighbor joining analyses, but the syntype showed the longest branch among terminals in this

FIGURE 2 | Lectotype of *Tetragonopterus rutilus jequitinhonhae*, NMW 57760:2, 67.52 mm SL.

clade (Fig. **S2**). Given that result, an exhaustive analysis of the sequences was made and showed that most of the sites that differ in the syntype sequence presented double pics in the chromatograms. The different bases on those cases were degenerated and were not considered in a second analysis. Furthermore, 21 bp at the beginning of the old sequence were discarded, due to the poor quality observed in the chromatograms. The final edited sequence had a length of 197 bp. This resulted in a higher similarity of the sequence of the syntype with the new sequences of *P. jequitinhonhae*, with a p-distance shorter than 2%. Also, the syntype clustered again inside the "*P. jequitinhonhae* group" under Neighbor Joining analysis (Fig. 3) and the branch length resulted shorter than on the previous analysis without sequence edition.

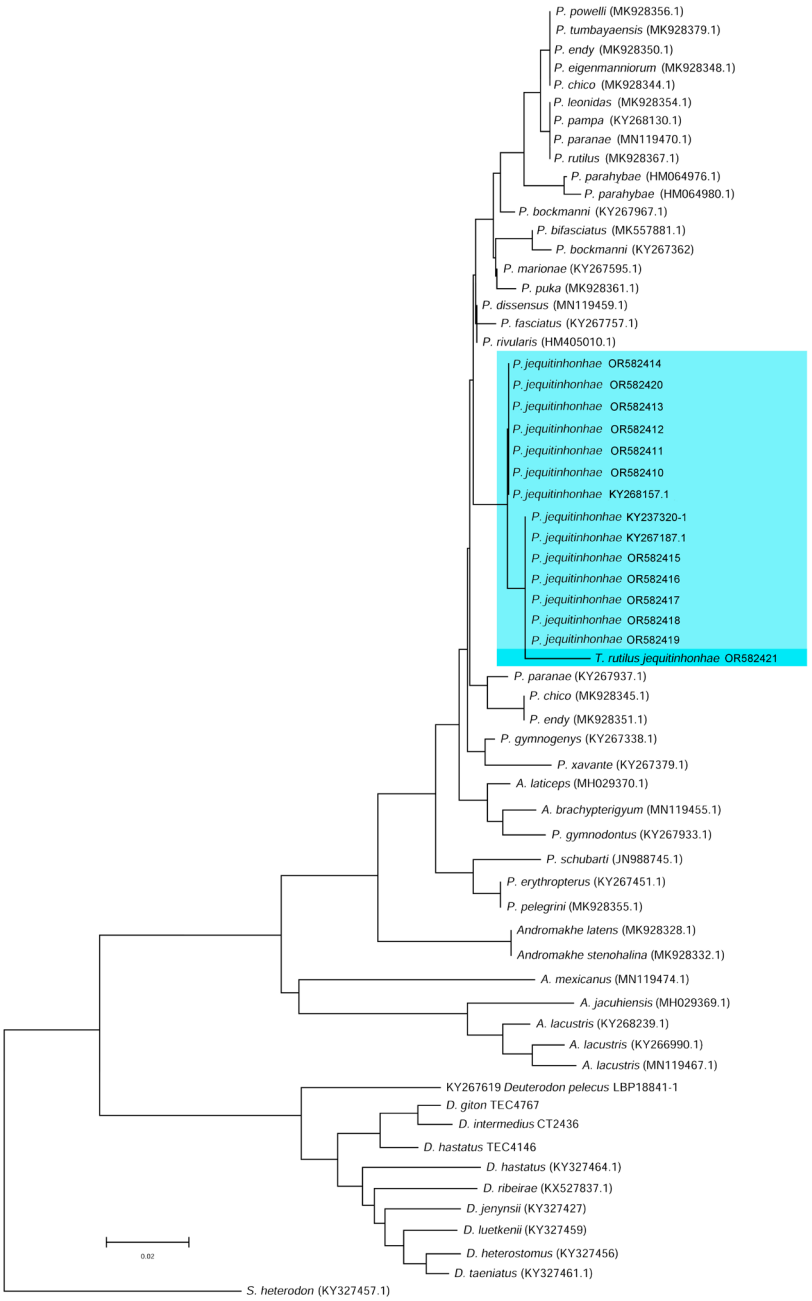

FIGURE 3 | Neighbor-joining tree based on p-distance model generated with a sequence fragment of the gene COI with 197 base pairs. The tree includes the lectotype of *Tetragonopterus rutilus jequitinhonhae* (NWM 57760:2) (dark blue) and species of the stethaprionine genera *Psalidodon*, *Astyanax*, *Andromakhe*, and *Deuterodon*. Sequences obtained from modern specimens of *P. jequitinhonhae* in light blue. Rooted in *Serrapinnus heterodon*.

Concerning to morphology, the examination of these new specimens from LBP and MZUFV demonstrated that their meristic and measurement data match with those obtained from the syntypes (Tab. 1), allowing the identification of newly collected specimens from the Jequitinhonha River drainage and a more comprehensive redescription of this species.

TABLE 1 | Morphometric and meristic data of the lectotype (NMW 57760:2) and paralectotypes of *Tetragonopterus rutilus jequitinhonhae* (NMW 57759, 5, 54.3–68.4 mm SL; NMW 57760, 2, 65.2–74.1 mm SL; NMW 57761, 6, 66.2–75.6 mm SL) and non-type specimens of *Psalidodon jequitinhonhae* from Jequitinhonha (N = 51) and Pardo river basins (N = 20). SD = standard deviation.

	Lectotype		Paralectotypes			Jequitinhonha River			Pardo River		
		N	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
Standard length (mm)	67.5	13	54.3–75.6	66.8	–	27.2–113.1	60.6	–	46.6–80.9	63.3	–
Percents of standard length											
Predorsal distance	50.2	8	50.2–56.8	52.0	2.1	46.2–55.9	50.3	2.1	49.7–54.1	51.8	1.3
Prepelvic distance	45.8	8	44.2–48.4	46.2	1.4	44.5–51.0	47.8	1.7	47.3–51.8	49.1	1.3
Prepectoral distance	24.8	8	23.7–26.5	24.9	0.8	23.8–31.0	26.9	1.8	24.6–29.9	27.1	1.3
Preanal distance	65.0	8	62.2–65.3	64.1	1.1	59.7–71.5	64.4	2.1	62.1–69.7	65.7	1.9
Depth at dorsal-fin origin	33.9	8	32.9–35.8	34.4	1.1	28.6–37.0	32.9	1.7	34.3–42.3	37.6	1.9
Caudal peduncle depth	11.3	7	11.1–12.0	11.5	0.3	8.5–11.6	10.3	0.7	10.0–12.4	10.8	0.6
Caudal peduncle length	12.9	7	12.4–13.7	12.9	0.4	9.6–14.3	12.1	1.1	10.7–13.3	11.9	0.7
Anal-fin base	30.2	7	28.5–32.0	30.2	1.3	23.6–30.5	26.7	1.7	25.2–30.5	28.1	1.7
Dorsal fin length	24.7	7	25.5–27.9	26.8	0.9	22.3–29.1	25.0	1.6	24.0–28.1	25.5	1.1
Pelvic fin length (m)	–	3	17.5–20.4	18.8	1.5	15.5–19.4	16.7	1.0	–	–	–
Pelvic fin length (f)	17.5	4	17.1–18.0	17.6	0.4	14.1–17.7	15.6	0.9	15.1–17.4	16.3	0.6
Pectoral fin length	21.6	7	21.9–25.0	23.2	1.0	19.6–26.1	21.7	1.3	20.0–23.2	21.9	0.9
Head length	26.3	13	24.9–27.1	25.9	0.6	20.4–28.7	25.2	1.7	24.6–27.3	25.9	0.8
Percents of head length											
Snout length	16.0	7	20.7–21.8	21.3	0.4	15.6–23.4	19.5	1.7	17.7–21.7	19.6	1.2
Upper jaw length	40.8	7	39.2–42.3	40.7	1.3	33.3–47.1	40.1	2.3	36.8–43.9	41.1	1.8
Orbital diameter	38.5	13	38.8–42.4	41.1	1.1	32.5–48.5	38.6	3.7	32.3–38.5	35.6	2.0
Interorbital width	–	7	30.7–33.1	32.0	0.9	28.3–47.4	34.5	4.0	30.8–38.0	34.6	2.3

Psalidodon jequitinhonhae (Steindachner, 1877)

(Figs. 2, 4–6)

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

Tetragonopterus rutilus jequitinhonhae. —Eigenmann, Eigenmann, 1891:52 (checklist).

Astyanax fasciatus jequitinhonhae. —Eigenmann, 1910:433 (checklist). —Eigenmann, 1921:304 (description, Jequitinhonha and Arassuahy [Araçuaí] rivers, Jequitinhonha River basin; plate 50, fig. 3 – photo). —Fowler, 1948:48 (checklist, fig. 38 – drawing based on Eigenmann's photo; distribution Bahia). —Triques *et al*., 2003:149 (listed and compared to *Astyanax turmalinensis* Triques, Vono & Caiafa, 2003).

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

Astyanax jequitinhonhae. —Melo, 2005 (unpublished thesis, new combination not available according to the ICZN; listed as a valid species and not as a subspecies).

Astyanax cf. *jequitinhonhae*. —Rossini *et al*., 2016:4 (barcode segment of the 5' region of the mitochondrial COI gene of four specimens from Itaobim, MG, in the Jequitinhonha River, compared to other species of *Astyanax*).

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

Astyanax aff. *jequitinhonhae*. —Silva-Santos *et al*., 2023:7 (molecular comparison to *Astyanax* species from the upper Paraguaçu River basin), tab. S2 (sequences downloaded from BOLD system; same sequences of Rossini *et al*., 2016).

FIGURE 4 | *Psalidodon jequitinhonhae*. Specimens from Jequitinhonha River, Itaobim Municipality, Minas Gerais State, Brazil, 16°30'36"S 41°20'02"W. LBP 8311, male, 57.7 mm SL, female, 57.4 mm SL.

Diagnosis. The presence of a single humeral spot diagnoses *P. jequitinhonhae* from *P. bifasciatus* (Garavello & Sampaio, 2010), *P. bockmanni* (Vari & Castro, 2007), *P. chico* (Casciotta & Almirón, 2004), *P. dissensus* (Lucena & Thofehrn, 2013), *P. eigenmanniorum* (Cope, 1894), *P. gymnodontus* Eigenmann, 1911, *P. leonidas* (Azpelicueta, Casciotta & Almirón, 2002), *P. ojiara* (Azpelicueta & Garcia, 2000), *P. pampa* (Casciotta, Almirón & Azpelicueta, 2005), *P. powelli* (Terán, Butí & Mirande, 2017), *P. pynandi* (Casciotta, Almirón, Bechara, Roux & Ruíz Díaz, 2003), *P. rivularis* (Lütken, 1875), *P. troya* (Azpelicueta, Casciotta & Almirón, 2002), *P. xavante* (Garutti & Venere, 2009), and *P. xiru* (Lucena, Castro & Bertaco, 2013) that present two humeral spots. The number of branched anal-fin rays, 21–25, differs *P. jequitinhonhae* from *P. pellegrini* (Eigenmann, 1907), *P. erythropterus* (Holmberg, 1891), *P. correntinus* (Holmberg, 1891), and *P. schubarti* (Britski, 1964) that have 27 or more branched anal-fin rays, and from *P. pampa*, *P. paranae* (Eigenmann, 1914), and *P. rioparanaibanus* Alves, Oliveira, Pasa & Kavalco, 2020 that have up to 18 branched anal-fin rays. The number of perforated lateral line scales, 34–37, differs *P. jequitinhonhae* from *P. erythropterus*, *P. pellegrini*, *P. gymnogenys* (Eigenmann, 1911), *P. rutilus*, and *P. correntinus* that have 39 or more perforated lateral line scales and from *Psalidodon parahybae* (Eigenmann, 1908) that has 37–40 perforated scales. The presence of 5 or 6 scales between the lateral line and dorsal fin and of 4 or 5 scales between lateral line and pelvic fin distinguish *P. jequitinhonhae* from *P. correntinus*, *P. hermosus* (Miquelarena, Protogino & López, 2005), *P. marionae* (Eigenmann, 1911), and *Psalidodon endy* (Mirande, Aguilera & Azpelicueta, 2006) that presents 7 or 8 scales between the lateral line and dorsal fin and of 6 or 7 scales between lateral line and pelvic fin. The presence of four anterior large penta- do heptacupspidate teeth, followed by an intermediary size tricuspidate tooth and eighth smaller tricuspid or conical teeth differs *P. jequitinhonhae* from *P. ita* (Almirón, Azpelicueta & Casciotta, 2002) and *P. puka* (Mirande, Aguilera & Azpelicueta, 2007) that shows a series of 7 or more teeth gradually decreasing in size posteriorly. The light pigmentation of body scales distinguishes *P. jequitinhonhae* from *P. tumbayaensis* (Miquelarena & Menni, 2005) that shows a distinctive reticulated color pattern on body scales. The fully pored lateral line series distinguish *P. jequitinhonhae* from *P. anisitsi* (Eigenmann, 1907) with 8 to 25 perforated scales. The lack of an elongated dorsal fin in males distinguish *P. jequitinhonhae* from *P. fasciatus*.

Description. Morphometric data in Tab. 1. Body compressed and elongated, with highest body depth at vertical through dorsal-fin origin. Dorsal profile of head smoothly convex or nearly straight from upper lip to tip of supraoccipital spine, slightly concave at supraoccipital in some specimens. Dorsal body profile slightly convex from tip of supraoccipital to dorsal-fin base; straight and posteroventrally slanted along dorsal-fin base and slightly convex between dorsal and adipose fins. Ventral profile smoothly convex from anterior tip of dentary to pelvic-fin insertion, and nearly straight from that point to anal-fin origin. Ventral body profile nearly straight along anal-fin base. Caudal peduncle nearly straight along both dorsal and ventral margins.

Head small, head length nearly one-fourth of SL. Snout length smaller than eye diameter. Mouth terminal. Maxilla almost vertically positioned; posteriormost margin positioned nearly in a vertical through anterior border of pupil with mouth closed. Anteroventral border of maxilla convex and posterodorsal border concave. Infraorbital series complete, third infraorbital leaving naked area posteroventrally, not contacting preopercle.

Premaxillary teeth in two rows; outer row with 4(49) teeth with three cusps, with central cusp longer. Inner row with 5(49) teeth; symphyseal tooth 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; second to fourth teeth bearing five to seven cusps, usually five cusps; last tooth abruptly smaller with three to five cusps. Maxilla with 1(49) tooth, rarely 2(4) teeth, with three to five cusps, central cusp highest. Four anteriormost dentary teeth larger, with five cusps (43), rarely seven cusps (3), followed by an intermediary tricuspidate tooth (23) and a series of usually eight smaller tricuspid or conical teeth (Fig. 5).

Dorsal-fin rays ii,9*(70). 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 slightly posterior to vertical through pelvic-fin origin. Adipose fin approximately at vertical through last anal-fin rays insertion. Anal-fin rays iii(44), iv*(34), 21(7), 22(18), 23*(14), 24(17), 25(22). Anal-fin distal border concave; anteriormost branched rays longer forming anterior lobe. Anal-fin origin approximately at vertical through base of last dorsal-fin ray. Pectoral-fin rays i,12(28), 13(23), 14(6). Pectoral-fin tip reaching pelvic-fin insertion in males and not reaching in females. Pelvic-fin rays i,7(72). 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(69) principal rays, lobes similar in size.

FIGURE 5 | *Psalidodon jequitinhonhae*, LBP 8311, 55.9 mm SL. To the left: lateral view of maxillary, premaxillary and dentary teeth. To the right: medial view of premaxillary and dentary teeth.

Lateral line slightly curved ventrally in abdomen, and then nearly straight through caudal fin; completely pored, with 34(2), 35(8), 36(47), 37*(21) scales. Horizontal scale rows between dorsal-fin origin and lateral line 5(9) or 6*(75). Horizontal scale rows between lateral line and pelvic-fin origin 4(22) or 5*(61). Pre-dorsal scales 10(7), 11(28), 12(29) arranged in regular series. Scale rows around caudal peduncle 12(17), 13(29), 14(19). Scale sheath along anal-fin base formed by eight to fourteen scales in single series and covering base of anteriormost rays.

Supraneurals 5(2), dorsal pterygiophores 11(2), anal pterygiophores 21(1) or 22(1). Total vertebrae 36(1) or 37(1): precaudal vertebrae 15(1) or 16(1) and caudal vertebrae 19(1) or 21(1). Upper branch gill-rakers 9+1(1), lower branch 12(1). First dorsal-fin pterygiophore inserted posterior to neural spine of eighth (1) or nineth (1) vertebra, first anal-fin pterygiophore inserted posterior to haemal spine of 13th(1) or 14th(1) vertebra.

Coloration in alcohol. Dorsal and dorsolateral portions of head light gray. Infraorbitals, preopercle and opercle silvery, lacking chromatophores. Dorsal and dorsolateral portion of body light brown, with scattered black chromatophores not forming distinctive marks. Lateral of body with a wide conspicuous silvery lateral band partially covering two or three longitudinal series of scales, located dorsally to lateral line scale series on belly and over lateral line scale series on caudal peduncle. In specimens lacking guanine, the lateral band forms a wide black stripe laterally on body and continuous to black pigmented middle caudal-fin rays. Brownish humeral blotch, small, vertically elongate, two to three scales wide and extending two scales above lateral line. All fins mostly unpigmented, except for the middle caudal fin rays with a conspicuous black stripe (Fig. 4).

Coloration in life. Dorsal and dorsolateral portions of head and body olive brown. Scales above lateral band with scattered black chromatophores not forming distinctive marks. Infraorbitals, preopercle and opercle white silvery, showing sparse black chromatophores in the area posterior to eye. Lateral of body with a wide conspicuous olive green bright longitudinal band partially covering two or three longitudinal series of scales, located dorsally to lateral line scale series on belly and bordering lateral line scale series on caudal peduncle. A black horizontally elongated blotch on caudal peduncle, distant from upper and lower borders of caudal peduncle, continuing on caudal-fin base and middle caudal-fin rays. Humeral blotch black, in two longitudinal series of scales immediately above the lateral line, two scales wide. Lateral of body below lateral band white. Pectoral, pelvic and anal fins mostly hyalines. Dorsal and adipose fins beige. Caudal fin yellowish proximally and light reddish distally, with a conspicuous black stripe on middle caudal fin rays (Fig. 6).

Sexual dimorphism. Bony hooks were observed on pelvic-, pectoral- and anal-fin rays, 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; 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 fin rays (MZUEL 10809).

Geographical distribution. *Psalidodon jequitinhonhae* is currently known from the Jequitinhonha and Pardo river basins, two coastal drainages, in the States of Minas Gerais and Bahia, southeastern and northeastern Brazil (Fig. 7).

Conservation status. The Extent of Occurrence (EOO) and Area of Occupancy (AOO), applying a 2 km² area for each locality of *Psalidodon jequitinhonhae* were estimated, according to the collection sites of the analyzed specimens. *Psalidodon jequitinhonhae* has, EOO to 8,274.050 km² and AOO of 40.000 km². These values are beyond the minimum limits defined by the International Union for Conservation of Nature (IUCN) for threatened categories, under the criteria B (B1: EOO < 5,000 km²; B2: AOO < 500 km²). Thereby, *P. jequitinhonhae* can be classified as Least Concern (LC), according to IUCN categories and criteria (IUCN Standards and Petitions Subcommittee, 2022).

FIGURE 6 | *Psalidodon jequitinhonhae*. Two live specimens from Fanado River, tributary of Araçuaí River, Barragem das Almas, Minas Novas Municipality, Minas Gerais State, Brazil, Jequitinhonha River basin, 17°14'16.1"S 42°35'25.9"W. MZUEL 16425, not measured. Photo: José L. O. Birindelli.

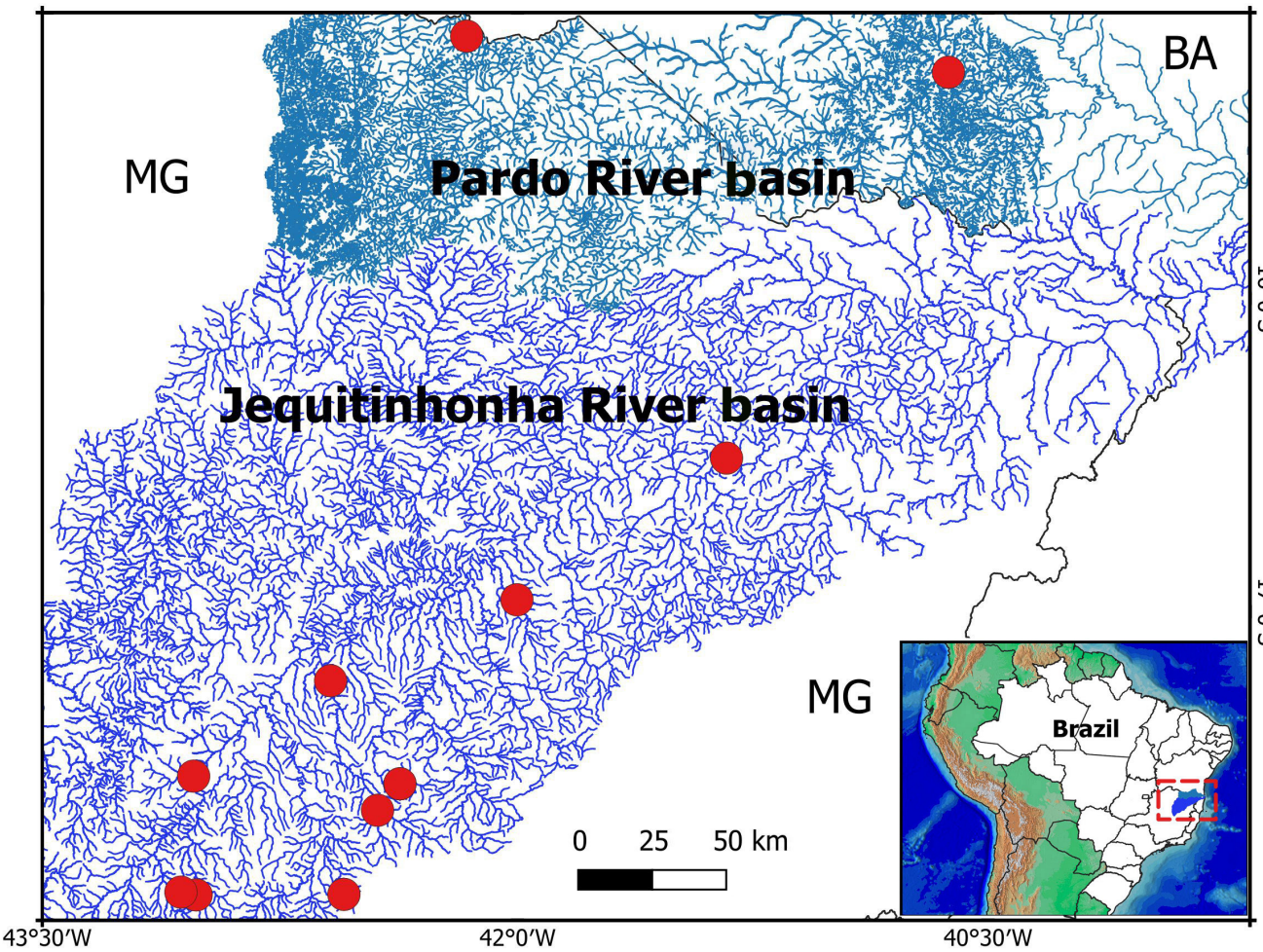

FIGURE 7 | Distribution map of *Psalidodon jequitinhonhae*, encompassing its occurrence in the Jequitinhonha and Pardo river basins, Minas Gerais and Bahia States, Brazil.

Material examined. All from Brazil. *Psalidodon jequitinhonhae*: Lectotype of *Tetragonopterus rutilus jequitinhonhae* by present designation (Fig. 2): NMW 57760:2, 67.5 mm SL, rio Jequitinhonha, 1874. Paralectotypes: NMW 57759, 5, 54.3–68.4 mm SL. NMW 57760, 2, 65.2–74.1 mm SL. NMW 57761, 6, 66.2–75.6 mm SL, same data as the lectotype. **Minas Gerais State, Jequitinhonha River basin.** LBP 8311, 14, 35.6–74.4 mm SL, 2 c&s 47.4–55.9 mm SL of 43, rio Jequitinhonha, Itaobim, 16°30'36"S 41°20'02"W, 15 May 2009, C. Oliveira, G. J. C. Silva, F. F. Roxo & T. N. A. Pereira. MZUEL 10809, 6 of 12 measured (ms), 70.3–78.1 mm SL, rio Fanado, tributary of rio Araçuaí, Capelinha, 17°38'01"S 42°26'48"W, 21 Jun 2014, F. Andrade-Neto, T. Barroso & I. G. Prado. MZUEL 10810, 3 of 7 ms, 30.5–60.7 mm SL, córrego Varão, tributary of rio Araçuaí, Capelinha, 17°32'57"S 42°22'31"W, 1 July 2014, F. Andrade-Neto, T. Barroso & I. G. Prado. MZUEL 10812, 2 of 9 ms, 42.8–76.4 mm SL, rio Fanado, tributary of rio Araçuaí, Capelinha, 17°32'57"S 42°22'31"W, 21 Jun 2014, F. Andrade-Neto, T. Barroso & I. G. Prado. MZUEL 12166, 4 of 10 ms, 72.5–77.5 mm SL, tributary of rio Araçuaí, Turmalina, 17°54'07"S 42°33'14"W, 21 Jun 2014, F.

Andrade-Neto, T. Barroso & I. G. Prado. MZUEL 16425, 13, rio Fanado, tributary of rio Araçuaí, Barragem das Almas, Minas Novas, 17°14'16.1"S 42°35'25.9"W, 5 Jul 2016, J. Birindelli, F. Jerep, E. Santana & R. Nascimento. MZUEL 17997, 3 of 9 ms, 75.7–82.7 mm SL, rio Fanado, tributary of rio Araçuaí, on the bridge to Turmalina, Minas Novas, 17°13'13"S 42°35'47"W, 5 Jul 2016, J. Birindelli, F. Jerep, E. Santana & R. Nascimento. MZUEL 7197, 3 of 4 ms, 68.2–74.0 mm SL, rio Soledade, Carbonita, 17°31'29"S 43°01'59"W, 14 Sep 2012, F. Andrade-Neto, T. Barroso & I. G. Prado. MZUEL 7217, 3 of 14 ms, 27.2–41.6 mm SL, rio Jequitinhonha, road between Itamarandiba and Senador Modestino, Itamarandiba, 17°54'13"S 43°01'30"W, 16 Sep 2012, F. Andrade-Neto, T. Barroso & I. G. Prado. MZUEL 7223, 4 of 24 ms, 29.1–31.9 mm SL, rio Jequitinhonha, road between Itamarandiba and Senador Modestino, Itamarandiba, 17°53'41"S 43°04'25"W, 16 Sep 2012, F. Andrade-Neto, T. Barroso & I. G. Prado. UFRGS 29499 15 ms, 42.0–62.1 mm SL (formerly MZUFV 3167), Calhauzinho Dam, rio Araçuaí, Araçuaí, 16°57'43"S 42°0'8"W, 19 Dez 2001, A. A. Oliveira. MZUFV 3582, 3, 76.4–113.1 mm SL, rio Setúbal [precise locality not specified], rio Araçuaí basin, J. A. Dergam & A. A. Oliveira. MZUFV 4193, 6 ms, 44.96–84.13 mm SL, rio Jequitinhonha [precise locality not specified], 17 Aug 2007, F. P. Rezende. **Pardo River basin:** MZUFV 3422, 6 of 14 ms, 70.7–80.9 mm SL, rio Pardo [precise locality not specified], 17 Mar 2004, A. A. Oliveira. MCP 17936, 8 of 33 ms, 47.5–62.3 mm SL, rio Pardo on the road bridge from Itambé to Tomba, about 3 km south of Itambé, Itambé, Bahia, 15°16'44"S 40°37'35"W, 22 Jan 1995, R. E. Reis, J. F. P. da Silva, W. G. Saul & E. H. L. Pereira. MCP 40142, 6 of 10 ms, 46.7–74.1 mm SL, rio São João, tributary of rio Pardo, São João do Paraíso, Minas Gerais, 15°09'50"S 42°09'45"W, 26 Apr 2006, J. Dergam & A. A. Oliveira.

DISCUSSION

The use of DNA obtained from museum and herbarium specimens is a growing field in molecular biology. It provides new insights into the history of organisms (Raxworthy, Smith, 2021), as well as allows the resolution of taxonomic and phylogenetic uncertainties (Silva *et al*., 2017, 2019a,b; Goulding *et al*., 2021; Sullivan *et al*., 2022). We successfully obtained an identification of *Psalidodon jequitinhonhae* based on a sequence (197 bp) of the gene COI. This fragment, although short, is located in a variable region of the gene and is suitably informative in distinguishing it from other species of *Psalidodon* Eigenmann, 1911 and related genera. Similar results with the same fragment allowed the identification of the lectotype of *Deuterodon pedri* Eigenmann, 1908 (based on a sequence of 179 base pairs; Silva *et al*., 2017) and of the lectotype of *Deuterodon taeniatus* (based on a sequence of 186 base pairs; Silva *et al*., 2019a). In all three cases, museum specimens were fixed in alcohol and are preserved in museums since the 19th century. So, albeit hDNA may not provide long DNA barcode sequences, the use of mini-barcode is an alternative tool when it is not possible to obtain a large gene fragment due to DNA degradation (Meusnier *et al*., 2008; Shokralla *et al*., 2011; Boyer *et al*., 2012; Silva *et al*., 2017, 2019a; Govender *et al*., 2019). In our case, neighbor-joining trees generated with p-distance (Fig. 3) and Kimura 2-parameter (Fig. **S2**), both based on COI gene, cluster one of the syntypes within sequences extracted from specimens collected in the Jequitinhonha and Pardo basins. This syntype (Fig. 2) is designated herein as a lectotype.

Although DNA extraction worked on the three historic specimens, the amplification process was possible only in one of those. It can be explained due to the age of the samples, collected in the nineteenth century. The damage to the DNA accrues over time and can make the DNA unable to serve as a template for PCR (Höss *et al*., 1996) or very hard to amplify. In our case, the relatively high number of degenerated bases in the lectotype recovered sequence is also a reflection of the natural damage of the time under the DNA. The observed changes had not affected the translation. The modification in bases can be explained by oxidative damage due to ionizing radiation that generates free radicals from water molecules resulting in modified bases (Höss *et al*., 1996). Most variant positions (50%) in the lectotype sequence were on the third base of the codon (pyrimidines). According to Dabney *et al*. (2013) the historical DNA extracted is invariably degraded to a small average size by processes that at least partly involve depurination, containing large amounts of deaminated cytosine residues that are accumulated toward the ends of the molecules.

We failed to find specimens that fits on *Psalidodon jequitinhonhae* in the rio Doce drainage considering morphological or molecular data, and so we question Eigenmann (1921) hypothesis that the specimens he examined with an increased number of gill rakers would possibly belong to this "variety" [sic]. Such a hypothesis may be later tested through the examination of the specimens cited by Eigenmann (1921) and comparison to the more extended description given herein. The morphological analysis of specimens from DZUFMG, MCP, MZUFV, MZUEL, and UFRGS fish collections (see Comparative material examined), as well as DNA sequences available from river drainages geographically located close to the Jequitinhonha and Pardo rivers (Doce and Mucuri rivers and small Atlantic coastal drainages) did not allow the identification of additional lots of *P. jequitinhonhae*. This means that actual records of *P. jequitinhonhae* are restricted only to Jequitinhonha and Pardo river basins. The lack of studies on the ichthyofauna of the Jequitinhonha River basin pointed out by Weitzman *et al*. (1986) and the still limited knowledge on its diversity (Sales *et al*., 2021) may have contributed to the long permanence of uncertainty identity of *P. jequitinhonhae*.

Comparative material examined. In addition to the material listed by Silva *et al*. (2017, 2019a), the following specimens were examined for this study. **Brazil: Jequitinhonha River basin:** *Astyanax turmalinensis*: DZUFMG 2796, 12 of 22, 29.6–42.7 mm SL, rio Preto, rio Araçuaí subbasin, Parque Estadual do Rio Preto, São Gonçalo do Rio Preto, Minas Gerais, 18°06'46"S 43°20'26"W, 2005, G. Santos & C. Leal. *Astyanax brevirhinus*: MZUEL 10757, 10 of 18, 28.5–64.9 mm SL, arroio Manoel Luiz, rio Fanado, Capelinha, Minas Gerais, 17°38'21"S 42°25'49"W, 28 Jun 2014, F. A. Neto, T. Barroso & I. G. Prado. *Psalidodon* sp.: DZUFMG 2790, 10 of 84, 28.7–44.9 mm SL, rio Preto, rio Araçuaí subbasin, Parque Estadual do Rio Preto, São Gonçalo do Rio Preto, Minas Gerais, 18°06'46"S 43°20'26"W, 2005, G. Santos & C. Leal. MZUEL 7188, 3 of 5, 34.8–39.6 mm SL, rio Soledade, Carbonita, Minas Gerais, 17°32'07"S 43°02'50"W, 14 Sep 2012, F. A. Neto, T. Barroso & I. G. Prado. **Pardo River basin:** *Deuterodon pelecus* (Bertaco & Lucena, 2006): MCP 37570, holotype, 59.4 mm SL, rio Pardo on BR-116 road bridge, Cândido Sales, Bahia, 15°30'49"S 41°14'11"W, 21 Jan 1995, J. C. Garavello, S. A. Schaefer, A. S. Santos, J. P. da Silva, E. H. L. Pereira, R. E. Reis & W. G. Saul. MCP 17919, 7 paratype, 26.9–64.7 mm SL, same data as the holotype. **Mucuri River basin:** *Psalidodon* sp.: MZUFV 5067, 15, 52.9–78.9 mm SL, rio Mucuri, Carlos Chagas, Minas Gerais, U. Santos, P. C. Silva & N. M. Travenzoli. **Doce River basin.** *Deuterodon giton* (Eigenmann, 1908): MZUFV 4459, 8, 46.3–51.9 mm SL, rio Doce, Catas Altas, Minas Gerais, 20°04'07"S 43°24'49"W, 14 Jun 2012, J. Dergam *et*

al. *Psalidodon* sp.: MZUFV 2574, 6 of 13, 72.6–79.2 mm SL, Prainha, rio Santana, rio Doce drainage, Canaã, Minas Gerais, Brazil, 20°36'18"S 42°32'30"W, 23 Dez 1997, J. L. Pontes & C. Rocha. MZUFV 5297, 4 of 7, 68.0–86.2 mm SL, Sete Cachoeiras, upper rio Santo Antônio, Ferros, Minas Gerais, Brazil, 19°13'58"S 43°01'20"W, 21 Apr 2012, J. Dergam. **Paraíba do Sul River basin.** *Psalidodon* sp.: MZUFV 5262, 3, 75.2–92.6 mm SL, Ribeirão Espírito Santo, rio Paraíbuna, Juiz de Fora, Minas Gerais, 22°04'51"S 43°08'56"W. MZUFV 5671, 5 of 10, 68.4–87.6 mm SL, rio Glória, Muriaé, Minas Gerais, 21°07'44"S 42°22'13"W, 15 Dez 2015, Raul Vert Ambiental. **Macabu River basin.** *Astyanax* aff. *jenynsii*: UFRGS 18913, 6, 63.8–76.6 mm SL, Visconde de Imbé/Trajano de Moraes, rio Macabu, 22°04'16"S 42°08'42"W, 11 Jan 2014, P. C. Silva, U. Santos, A. Hirschmann, A. Thomaz & T. P. Carvalho. **Macaé River basin.** *Astyanax lacustris* (Lütken, 1875): UFRGS 19337, 10, 63.6–85.8 mm SL, arroio Aduelas, Conceição de Macabu, 22°11'53.91"S 41°50'30.76"W, 26 May 2014, P. C. Silva, F. Di Dario. **Macacu River basin.** *Deuterodon intermedius* (Eigenmann, 1908): UFRGS 10257, 3 of 62, 43.2–52.8 mm SL, Escola Municipal Adalberto de Mesquita, distrito de Ypiranga, Macacu, rio Macacu, 22°38'11.6"S 42°42'42.3"W, 15 May 2004, C. E. Lopes, R. Pazza & K. F. Kavalco. *Deuterodon hastatus* (Myers, 1928): UFRGS 10258, 4 of 10, 28.5–40.6 mm SL, Santana de Japuíba, Cachoeiras de Macacu, 22°33'39"S 42°40'53"W, 15 May 2004, C. E. Lopes, R. Pazza & K. F. Kavalco. **São João River basin.** *Deuterodon taeniatus*: UFRGS 18884, 6 of 24, 46.4–56.3 mm SL, Silva Jardim, rio São João, 22°30'26"S 42°29'12.3"W, 10 Jan 2014, P. C. Silva, U. Santos, A. Hirschmann, A. Thomaz & T. P. Carvalho.

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AUTHORS' CONTRIBUTION

Victor de Queiroz: Formal analysis, Investigation, Writing-original draft.

Priscilla Caroline Silva: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Writing-original draft, Writing-review and editing.

Maria Claudia Malabarba: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Writing-original draft, Writing-review and editing.

Lee Weigt: Funding acquisition, Resources, Validation, Writing-review and editing.

Jorge Abdala Dergam: Data curation, Funding acquisition, Resources.

Luiz R. Malabarba: Conceptualization, Data curation, Project administration, Resources, Supervision, Validation, Writing-original draft, Writing-review and editing.

ETHICAL STATEMENT

All examined specimens belong to fish collections.

COMPETING INTERESTS

The author declares no competing interests.

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