A new cryptic species of *Hyphessobrycon* Durbin, 1908 (Characiformes, Characidae) from the Eastern Amazon, revealed by integrative taxonomy

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Abstract

*Hyphessobrycon caru* sp. nov. is described based on five different and independent methods of species delimitation, making the hypothesis of this new species supported by an integrative taxonomy perspective. This new species has a restricted distribution, occurring just in the upper Pindaré river drainage, Mearim river basin, Brazil. It is a member of the rosy tetra clade, which is characterized mainly by the presence of a dark brown or black blotch on dorsal fin and absence of a midlateral stripe on the body. *Hyphessobrycon caru* sp. nov. is distinguished from the members of this clade mainly by the shape of its humeral spot, possessing few irregular inconspicuous vertically arranged chromatophores in the humeral region, or sometimes a very thin and inconspicuous humeral spot, and other characters related to teeth count, and color pattern. The phylogenetic position of the new species within the rosy tetra clade was based on molecular phylogenetic analysis using sequences of the mitochondrial gene cytochrome oxidase subunit 1. In addition, a new clade (here termed *Hyphessobrycon micropterus* clade) within the rosy tetra clade is proposed based on molecular data, comprising *H. caru* sp. nov., *H. micropterus*, *H. piorskii*, and *H. simulatus*, and with *H. caru* sp. nov. and *H. piorskii* recovered as sister species. Our results suggest cryptic speciation in the rosy tetra clade and, more specifically, in the *H. micropterus* clade. We recommend the use of integrative taxonomy for future taxonomic revisions and species descriptions when dealing with species complexes and groups containing possible cryptic species.

Key Words

bPTP, DNA barcoding, rosy tetra clade, species complex, Stethapioninæ
Introduction

**Hyphessobrycon** Durbin, 1908 is a species-rich characoid genus comprising about 160 valid species (Fricke et al. 2019). It is widely distributed along the river basins of the Neotropical region, from southern Mexico to the La Plata River basin in northeastern Argentina (Carvalho and Malabarba 2015; Garcia-Alzate et al. 2017; Guimarães et al. 2018). The genus was first proposed as a subgenus of *Hemigrammus* Gill, 1858 by Durbin in Eigenmann (1908), differing from the latter only by the absence of scales covering the caudal-fin. *Hyphessobrycon* was reviewed by Eigenmann (1918, 1921) in a work which still constitutes the most comprehensive revisionary studies on the genus. The large number of species included within *Hyphessobrycon* and the poor knowledge of the alpha and beta-taxonomy of species and species groups are among the major challenges for a more comprehensive taxonomic study and phylogenetic analyses of the genus. It is widely known that *Hyphessobrycon* does not constitute a monophyletic group (Weitzman and Palmer 1997a; Mirande 2010, 2018; Oliveira et al. 2011; Carvalho and Malabarba 2015; Carvalho et al. 2017; Moreira and Lima 2017; Betancur-R. et al. 2018; Guimarães et al. 2018). Nevertheless, groups of species have been proposed based primarily on similarities of color pattern and other external features (e.g. Weitzman and Palmer 1997a; Garcia-Alzate et al. 2008; Moreira and Lima 2017). Some of them are probably merely artificial operational assemblages to aid species identification, whereas others represent potential monophyletic groups, delimited by exclusive character states (e.g. Castro-Paz et al. 2014; Carvalho and Malabarba 2015; Guimarães et al. 2018).

Several genetic studies focusing on characid fishes, such as *Astyanax* Baird & Girard, 1854 (e.g. Ornelas-García et al. 2008), *Caenotropus* Günther, 1864 (e.g. Melo et al. 2014), *Chilodus* Müller & Troschel, 1844 (e.g. Melo et al. 2014), *Curimatopsis* Steindachner, 1876 (e.g. Melo et al. 2016a), *Gymnocybium* Eigenmann, 1908 (e.g. Benezine et al. 2015), *Hyphessobrycon* (e.g. Castro-Paz et al. 2014, Guimarães et al. 2018), *Piabina* Reinhardt, 1867 (e.g. Pereira et al. 2011), *Prochilodus* Agassiz, 1829 (e.g. Melo et al. 2016b), *Nannostomus* Günther, 1872 (e.g. Benzaque et al. 2015) and *Tetragonopterus* Bleeker, 1863 (e.g Melo et al. 2016c) have evidenced that some species may exhibit large discontinuities in their geographic distribution patterns, with high genetic divergences, but little morphological variability among geographically isolated lineages. These results suggest that these groups may represent species complexes or cryptic species, that is, they might even including morphologically quite similar or undistinguishable species that are hidden and erroneously classified (Brown et al. 1995; Bickford et al. 2006; Adams et al. 2014; Souza et al. 2018). Studies relying solely on morphology may be inadequate in recognizing species within groups including cryptic species (Guimarães et al. 2018). Integrative studies, using more than one criteria, such as character-based, tree-based, genetic distance and coalescent-based approaches, especially including molecular data, are useful and powerful for the recognition of hidden and/or possible new species in such species complexes (Sytsma and Schaal 1985; Bickford et al. 2006; Goldstein and Desalle 2010; Pal abdominal 2010; Adams et al. 2014; Costa-Silva et al. 2015; Souza et al. 2018; Ottoni et al. 2019).

In this context of integrative taxonomy, the present study aims to investigate the diversity within the rosy tetra clade sensu Weitzman and Palmer (1997a). This clade comprises around 30 species, including some species of *Hyphessobrycon* and other allied species, that are appreciated as aquarium fishes due to their attractive color patterns (e.g. Weitzman and Palmer 1997a, 1997b, 1997c, 1997d; Zarske 2008; Hein 2009; Guimarães et al. 2018).

This group has had its composition and name changed over the last decades, and a detailed taxonomic history is presented by Weitzman and Palmer (1997a). Two previous papers (e.g. Castro-Paz et al. 2014; Guimarães et al. 2018) applied molecular approaches to investigate the diversity of rosy tetra clade, and they suggested that its taxonomic resolution should be better investigated as it could include cryptic species or valid species which may have been synonymized. A new species of *Hyphessobrycon* and member of the rosy tetra clade is described from the upper Pindaré river drainage, Mearim river basin, a coastal river basin of the Eastern Amazon region, Brazil, based on both morphology and molecular data. Furthermore, a new clade, within the rosy tetra clade, is proposed based on the phylogenetic tree topology presented.

Materials and methods

**Taxa sampling, specimens collection, and preservation**

Individuals collected for this study were euthanized with a buffered solution of MS-222 at a concentration of 250 mg L⁻¹ for a period of 10 min or more until opercular movements completely ceased. Specimens selected for morphological analysis were fixed in formalin and left for 10 days, after which they were preserved in 70% ethanol. Molecular data were obtained from specimens that were euthanized, fixed, and preserved in absolute ethanol.

Specimens for morphological analysis are listed in type and comparative material lists. Specimens for molecular approaches are listed in Table 1. We also retrieved sequences from other species of *Hyphessobrycon* and allied species for a comparative analysis from the Barcode of Life Database (BOLD) and the National Center for Biotechnology Information (NCBI) databases (Table 1).
Table 1. List of species, specimens and their respective catalogue numbers, Region/state/country, and BOLD Systems and GenBank sequence accession numbers. Sequences available in the current study are in Bold.

| N°  | Species                      | Catalogue number | Region/state/country                      | Accession no. |
|-----|------------------------------|------------------|------------------------------------------|---------------|
| 1   | Hyphessobrycon erythrostigma | INPA 37681-HERY1 | Tabatinga/Amazonas/Brazil                | HY0076-13     |
| 2   | Hyphessobrycon erythrostigma | INPA 37681-HERY10| Tabatinga/Amazonas/Brazil               | HY0077-13     |
| 3   | Hyphessobrycon erythrostigma | INPA 37681-HERY2 | Tabatinga/Amazonas/Brazil               | HY0078-13     |
| 4   | Hyphessobrycon pyrrhonotus   | INPA 37672-TR010 | Santa Isabel do Rio Negro/Amazonas/Brazil| HY0040-13     |
| 5   | Hyphessobrycon pyrrhonotus   | INPA 37672-TR011 | Santa Isabel do Rio Negro/Amazonas/Brazil| HY0041-13     |
| 6   | Hyphessobrycon pyrrhonotus   | –                | Barcelos/Amazonas/Brazil                | HY157-13      |
| 7   | Hyphessobrycon pyrrhonotus   | –                | Barcelos/Amazonas/Brazil                | HY158-13      |
| 8   | Hyphessobrycon socoloti      | INPA 39530-6152  | Barcelos/Amazonas/Brazil                | HY131-13      |
| 9   | Hyphessobrycon socoloti      | INPA 39530-6155  | Barcelos/Amazonas/Brazil                | HY134-13      |
| 10  | Hyphessobrycon socoloti      | INPA 39530-6178  | Barcelos/Amazonas/Brazil                | HY135-13      |
| 11  | Hyphessobrycon socoloti      | INPA 39530-BCR8  | Barcelos/Amazonas/Brazil                | HY148-13      |
| 12  | Hyphessobrycon copelandi     | INPA 37683-TU1   | Tabatinga/Amazonas/Brazil               | HY0094-13     |
| 13  | Hyphessobrycon copelandi     | INPA 37683-TU2   | Tabatinga/Amazonas/Brazil               | HY0095-13     |
| 14  | Hyphessobrycon copelandi     | INPA 37683-TU3   | Tabatinga/Amazonas/Brazil               | HY0096-13     |
| 15  | Hyphessobrycon compressus    | CINV-NEC7411     | Flores Magon/Campeche/México            | FYP0054-10    |
| 16  | Hyphessobrycon compressus    | ECOCH            | Hativa/Brasile/Belize                   | MX765-15      |
| 17  | Hyphessobrycon compressus    | ECOCH            | Hativa/Brasile/Belize                   | MX766-15      |
| 18  | Hyphessobrycon compressus    | ECOCH            | Hativa/Brasile/Belize                   | MX767-15      |
| 19  | Hyphessobrycon bentosi       | INPA 37684-5939  | Barcelos/Amazonas/Brazil                | HY0097-13     |
| 20  | Hyphessobrycon bentosi       | INPA 37684-5940  | Barcelos/Amazonas/Brazil                | HY0098-13     |
| 21  | Hyphessobrycon bentosi       | INPA 39527-BA1   | –                                        | HY111-13      |
| 22  | Hyphessobrycon bentosi       | INPA 39527-BA2   | –                                        | HY111-13      |
| 23  | Hyphessobrycon bentosi       | CICCAA02349      | Santarém/Pará/Brazil                    | MK240339      |
| 24  | Hyphessobrycon bentosi       | CICCAA02350      | Santarém/Pará/Brazil                    | MK240340      |
| 25  | Hyphessobrycon bentosi       | CICCAA02351      | Santarém/Pará/Brazil                    | MK240341      |
| 26  | Hyphessobrycon simulatus     | MHNG 2743.087    | Pisiemoengo/Commewijne/Suriname         | GBOL761-15    |
| 27  | Hyphessobrycon simulatus     | MHNG 2743.087    | Pisiemoengo/Commewijne/Suriname         | GBOL762-15    |
| 28  | Hyphessobrycon simulatus     | MHNG 2735.007    | Sinnamary/Cayenne/French Guiana         | GBOL771-17    |
| 29  | Hyphessobrycon simulatus     | MHNG 2757.080    | Kourou/Cayenne/French Guiana            | GBOL3296-18   |
| 30  | Hyphessobrycon simulatus     | MHNG 2759.026    | Kaw/Cayenne/French Guiana               | GBOL3300-18   |
| 31  | Hyphessobrycon simulatus     | MHNG 2759.026    | Kaw/Cayenne/French Guiana               | GBOL3301-18   |
| 32  | Hyphessobrycon simulatus     | MHNG 2759.035    | Régina/ Cayenne/French Guiana           | GBOL3302-18   |
| 33  | Hyphessobrycon cf. sweglesi  | INPA 37686-JAR3  | São Gabriel da Cachoeira/Amazonas/Brazil| HY0026-13     |
| 34  | Hyphessobrycon cf. sweglesi  | INPA 37686-JAR4  | São Gabriel da Cachoeira/Amazonas/Brazil| HY0027-13     |
| 35  | Hyphessobrycon cf. sweglesi  | INPA 37686-JAR5  | São Gabriel da Cachoeira/Amazonas/Brazil| HY0028-13     |
| 36  | Hyphessobrycon micropterus   | –                | Várzea da Palma/Minas Gerais/Brazil     | BS1287-10     |
| 37  | Hyphessobrycon micropterus   | –                | Várzea da Palma/Minas Gerais/Brazil     | BS1288-10     |
| 38  | Hyphessobrycon micropterus   | –                | Várzea da Palma/Minas Gerais/Brazil     | BS1289-10     |
| 39  | Hyphessobrycon micropterus   | –                | Várzea da Palma/Minas Gerais/Brazil     | BS1290-10     |
| 40  | Hyphessobrycon piorskii      | CICC1A007251     | Chapadinhia/Maranhão/Brazil             | MFS67596      |
| 41  | Hyphessobrycon piorskii      | CICC1A007261     | Chapadinhia/Maranhão/Brazil             | MFS67597      |
| 42  | Hyphessobrycon piorskii      | CICC1A006501     | Barreirinhas/Maranhão/Brazil            | MG791915      |
| 43  | Hyphessobrycon piorskii      | CICC1A006511     | Barreirinhas/Maranhão/Brazil            | MG791914      |
| 44  | Hyphessobrycon piorskii      | CICC1A021641     | Codi/Maranhão/Brazil                    | MK240337      |
| 45  | Hyphessobrycon piorskii      | CICC1A021644     | Codi/Maranhão/Brazil                    | MK240338      |
| 46  | Hyphessobrycon caru           | CICC1A007481     | Buriticupu/Maranhão/Brazil              | MHS38230      |
| 47  | Hyphessobrycon caru           | CICC1A007491     | Buriticupu/Maranhão/Brazil              | MHS38231      |
| 48  | Hyphessobrycon caru           | CICC1A023001     | Buriticupu/Maranhão/Brazil              | MHS38232      |
| 49  | Hyphessobrycon caru           | CICC1A023011     | Buriticupu/Maranhão/Brazil              | MHS38233      |
| 50  | Pristella maxillaris          | –                | –                                        | KUS6898-1      |
| 51  | Pristella maxillaris          | –                | –                                        | KUS6898-11     |
| 52  | Pristella maxillaris          | –                | –                                        | TZZAA025-06    |
| 53  | Pristella maxillaris          | –                | –                                        | TZZAA127-06    |
| 54  | Moenkhausia hemigrammatoides  | INPA 38532-PR1   | Guyana                                   | HY0102-13     |
| 55  | Moenkhausia hemigrammatoides  | INPA 38532-PR2   | Guyana                                   | HY0103-13     |
| 56  | Moenkhausia hemigrammatoides  | INPA 38532-PR3   | Guyana                                   | HY0103-13     |
| 57  | Hyphessobrycon panamensis     | STRI-05303       | Cocle/Panama                            | BSF760-07     |
| 58  | Hyphessobrycon flammeus       | LBPV-40464       | Biritiba-Mirim/São Paulo/Brazil          | FUPR088-09    |
Morphological analysis

Measurements and counts were made according to Fink and Weitzman (1974), with exception of the scale rows below lateral line, which were counted to the insertion of pelvic-fin. Vertical scale rows between the dorsal-fin origin and lateral line do not include the scale of the median predorsal series situated just anterior to the first dorsal-fin ray. Counts of supraneurals, vertebrae, procurent caudal-fin rays, unbranched dorsal and anal-fin rays, branchiostegal rays, gill-rakers, premaxillary, maxillary, and dentary teeth were taken only from cleared and stained paratypes (C&S), prepared according to Taylor and Van Dyke (1985). The four modified vertebrae that constitute the Weberian apparatus were not included in the vertebrae counts and the fused PU1 + U1 was considered as a single element. Osteological nomenclature follows Weitzman (1962). Institutional abbreviations follow Fricke and Eschmeyer (2019), with addition of LIOP.UFAM Coleção Ictiológica do Laboratório de Ictiologia e Ordenamento Pesqueiro do Vale do Rio Madeira da Universidade Federal do Amazonas.

DNA extraction, amplification, and sequencing

DNA was extracted from fin clips using Wizard Genomic DNA Purification kit (Promega) according to the manufacturer’s protocol. Fragments of the cytochrome c oxidase subunit 1 gene (hereafter COI) from mitochondrial DNA were amplified, using the universal primers designed by Ward et al. (2005) for fish. Polymerase chain reactions (PCR) comprised a total volume of 15µl containing 1× Polymerase buffer, 1.5 mM MgCl₂, 200 µM dNTP, 0.2 uM of each primer, 1U of Taq Polymerase (Invitrogen), 100 ng of DNA template, and ultrapure water. The PCR cycles were as follows: 2 min at 94 °C, followed by 35 cycles of 94 °C for 30s, 54 °C for 30s, and 72 °C for 1 min, and 10 min at 72 °C. Amplicons were purified using Illustra GFX PCR DNA and Gel Purification Kit (GE Healthcare Systems) and sequenced using the forward primer by an outsourced sequencing service (Applied Biosystems).

Data partition, evolution models, and alignment

The dataset included the following gene: COI (680 Base pairs, BP). Sequences were aligned using ClustalW (Chenna et al. 2003). The DNA sequences were translated into amino acids residues to test for the absence of premature stop codons or indels using the program MEGA 7 (Kumar et al. 2016). In the alignment, gaps were coded with a dash (−) and missing data with a question mark (?), but during analyses, both were treated as missing data. Measure Substitution Saturation tests were performed in DAMBE5 (Xia 2013) according to the algorithm proposed by Xia et al. (2003). The best-fit evolutionary model (GTR+G) was calculated, using the corrected Akaike Information Criterion (AICc) determined by the jModelTest 2.1.7 (Darriba et al. 2012).

Species concept, species delimitation, and diagnoses

The unified species concept is herein adopted by expressing the conceptual definition shared by all traditional species concepts, “species are (segments of) separately evolving metapopulation lineages”, disentangling operational criterion elements to delimit taxa from species concepts (de Queiroz 2005, 2007). According to this concept, species are treated as hypothetical units and could be tested by the application of distinct criteria (species delimitation methods) (de Queiroz 2005, 2007). It allows for any criteria to separately provide evidence about species limits and identities, independently from other criteria (de Queiroz 2005, 2007). However, evidence corroborated from multiple operational criteria is considered to produce stronger support for hypotheses of lineage separation (de Queiroz 2007; Goldstein and Desalle 2010), a practice called “integrative taxonomy” (Dayrat 2005; Goldstein and Desalle 2010; Padial et al. 2010).

Five distinct and independent operational criteria for species delimitation, based on morphological and molecular data, were implemented here: Population Aggregation Analysis (Davis and Nixon 1992) (hereafter PAA); DNA barcoding, as proposed by Hebert et al. (2003a, 2003b, 2004a, 2004b) (hereafter DBC); a tree-based method as proposed by Wiens and Penkrot (2002) (hereafter WP, following Sites and Marshall 2003); a character-based DNA barcoding as proposed by Desalle et al. (2005) (hereafter CBB); and a coalescent species delimitation method termed the Bayesian implementation of the Poisson tree processes (hereafter bPTP, following Zhang et al. 2013). All species delimitation methods here adopted, except PAA, were performed on cytochrome c oxidase subunit 1 (COI) sequences, as it is a mitochondrial gene with fast evolutionary rate, suitable for single locus species delimitation approaches (Avise 2000).

Population aggregation analysis (PAA)

The PAA (Davis and Nixon 1992) is a character-based method, in which species are delimited by unique combination of morphological character states occurring in one or more populations (Costa et al. 2014). The morphological data was based on both examined material and literature (e.g. Steindachner 1882; Meek 1904; Eigenmann, 1908; Durbin 1909; Eigenmann 1915; Ahl 1937; Fowler 1943; Géry 1960, 1961, 1964, 1977; Géry and Uj 1987; Burgess 1993; Planquette et al. 1996; Weitzman and...
Traditional DNA barcoding (DBC) and Phylogenetic analysis

We used the Kimura-2-parameters model (K2P) (Kimura 1980) to estimate the pairwise genetic distances between species in MEGA 7 software (Kumar et al. 2016). We used DnaSP v. 6 (Rozas et al. 2003) to estimate the number of variable sites and haplotypes. A Bayesian inference-based phylogenetic (BI) tree was estimated in MrBayes (Huelsenbeck and Ronquist 2001) plugin in Geneious 9.0.5 to reconstruct the evolutionary relationships among terminals using General Time Reversible (GTR+G) as evolutionary model. Bayesian tree inference was based in a chain length of 10 million, a burn-in length of 500,000 generations subsampling trees every 10,000 generations. We used a sequence of Hyphessobrycon flammneus Myers, 1924 as outgroup.

Wiens and Penkrot analysis (WP)

WP is based on the direct inspection of haplotype trees generated from the phylogenetic analysis having as terminals at least two individuals (haplotypes) of each focal species. In this method, the term “exclusive” is used instead of monophyletic, as the term monophyly is considered inapplicable below the species level (Wiens and Penkrot 2002). Clustered haplotypes with concordant geographic distribution forming mutual and well supported clades (exclusive lineages) are considered strong evidence for species discrimination (absence of gene flow with other lineages). When haplotypes from the same locality fail to cluster together, there is potential evidence for gene flow with other populations (Wiens and Penkrot 2002). Statistical support for clades is assessed by the posterior probability, considered as significant at values about 0.95 or higher (Alfaro and Holder 2006). When only one haplotype (specimen) from one putative population was available, the species delimitation was based on the exclusivity of the sister clade of this single haplotype, supported by significant values, allowing us to perform the test in populations with only one haplotype (Wiens and Penkrot 2002). In addition, the method allows recognition of nonexclusive lineages as species since their sister clades are exclusive and supported by significant values (Wiens and Penkrot 2002).

Character-based DNA barcoding (CBB)

The CBB is similar to the population aggregation analysis proposed by Davis and Nixon (1992), but directed to nucleotides as an alternative method for diagnosing taxa through DNA barcodes, as the original method is based on subjective cut-off distance measures to species designation (Hebert et al. 2003a, 2003b, 2004a, 2004b). This method delimits species based on a unique combination of nucleotides within a site shared by individuals of the same population or group of populations. In addition, species were diagnosed by nucleotide substitutions following Costa et al. (2014). Optimization of nucleotide substitutions among lineages of the Hyphessobrycon micropterus clade were obtained from the Maximum Parsimony topology, using TNT 1.5 (Goloboff and Catalano 2016). Maximum Parsimony analysis (MP) was obtained with the following parameters: traditional search, tree bisection reconnection branch swapping (TBR), 1 random seed, setting random taxon-addition replicates to 1,000, multi-trees in effect, collapsing branches of zero length, characters equally weighted, and 10,000 trees saved per replication. MP tree branch support was given by bootstrap analysis (Felsenstein 1985), using a heuristic search with 1,000 replicates and the same settings used in the MP search, saving a maximum of 1,000 trees in each random taxon-addition replicate. The analysis was rooted on Hyphessobrycon flammneus Myers, 1924. Each nucleotide substitution is represented by its relative numeric position determined through sequence alignment with the complete mitochondrial genome of Astyanax paranae Eigenmann 1914 (KX609386.1:5503-7062 – mitochondrion complete genome), followed by the specific nucleotide substitution in parentheses. The results of this analysis are presented in Suppl. material 1: Box 1 and molecular diagnosis section.

Bayesian implementation of the poisson tree processes (bPTP)

The bPTP is a coalescent phylogeny-based species delimitation method aimed at delimiting species based on single locus molecular data (Zhang et al. 2013). An advantage of bPTP is that it does not need an ultrametric calibration like other coalescent approaches, avoiding errors and computer intensive processes (Zhang et al. 2013). The method relies on the number of substitutions between haplotypes and assumes that more molecular variability is expected between species than within a species (Zhang et al. 2013). In our analysis the dataset was reduced to include only unique haplotypes from the species of the H. micropterus clade. Outgroups were restricted to Hyphessobrycon ben-tosii Durbin, 1908 and Hyphessobrycon copeandi Durbin 1908. Sequences were aligned using ClustalW (Chenna et al. 2003). The best-fit evolutionary model (GTR+G) for the reduced dataset was calculated using the corrected Akaike Information Criterion (AICc) determined by the jModelTest 2.1.7 (Darriba et al. 2012). The input phylogenetic tree was performed in MrBayes 3.2.6 (Ronquist et al. 2012), with the following parameters: independent runs of two Markov chain Monte Carlo (MCMC) runs of four chains each for 3 million generations and sampling frequency of 1,000. The bPTP analysis was performed in the Exelixis Lab’s web server http://species.h-its.org/ptp/, following the default parameters except for a 20% burn in.
Results

*Hyphessobrycon caru* sp. nov.

http://zoobank.org/3BC35EBB-E138-4E24-A06E-DF985F015ED5

Figures 1, 2a; Table 2

**Holotype.** CICCAA 02286, 22.2 mm SL, Brazil, Maranhão state, Buriticupu municipality, Buritizinho river, Pindaré river drainage, Meirim river basin, 04°22'52"S, 46°30'35"W, 24 Jan. 2017, Guimarães E. C., Brito P. S.

**Paratypes.** All from Brazil, Maranhão state: CICCAA 00706, 37, 15.9–25.4 mm SL; CICCAA 0709, 12 C&S, 15.1–20.6 mm SL; LIOP UFAM 1009, 1, 16.2 mm SL collected with holotype. CICCAA00708, 2, 19.9–21.6 mm SL, Buriticupu municipality, Buritizinho river, Pindaré river drainage, Meirim river basin, 04°19'45"S, 46°29'46"W, 24 Jan. 2017, Guimarães E. C., Brito P. S. CICCAA00708, 2, 19.9–21.6 mm SL, Buriticupu municipality, Buritizinho river, Pindaré river drainage, Meirim river basin, 04°19'45"S, 46°29'46"W, 24 Jan. 2017, Guimarães E. C., Brito P. S. CICCAA 00708, 2, 19.9–21.6 mm SL, Buriticupu municipality, Buritizinho river, Pindaré river drainage, Meirim river basin, 04°19'45"S, 46°29'46"W, 24 Jan. 2017, Guimarães E. C., Brito P. S. CICCAA 00708, 2, 19.9–21.6 mm SL, Buriticupu municipality, Buritizinho river, Pindaré river drainage, Meirim river basin, 04°19'45"S, 46°29'46"W, 24 Jan. 2017, Guimarães E. C., Brito P. S.

**Diagnosis (PAA).** The new species *Hyphessobrycon caru* sp. nov. differs from most of its congeners, except members of the rosy tetra clade, by the presence of a dark brown or black blotch on dorsal-fin (vs absence) and absence of a midlateral stripe on the body (vs presence). The new species differs from most of its congeners in the rosy tetra clade by possessing few irregular inconspicuous vertically arranged chromatophores in the humeral region, or sometimes a very thin and inconspicuous humeral spot (Fig. 2a) (vs inconspicuously vertically elongated humeral spot in *H. hasemani* Fowler, 1913, *H. pior斯基* Guimarães, De Brito, Feitosa, Carvalho-Costa, Ottoni, 2018 (Fig. 2b); approximately rounded humeral spot in *H. erythrostigma* (Fowler, 1943), *H. jackrobertsi* Zarske, 2014, *H. minor* Durbin, 1909, *H. pando* Hein, 2009, *H. paeckei* Zarske, 2014, *H. pyrrhonot us* Burgess, 1993, *H. roseus* (Géry, 1960), *H. socolofi* Weitzman, 1977, and *H. sweglesi* (Géry, 1961) (Fig. 2c); humeral spot horizontally or posteriorly elongated in *H. epicharis* Weitzman & Palmer, 1997, *H. khardinae* Zarske, 2008, and *H. werneri* Géry & Uj, 1987 (Fig. 2d); conspicuous humeral spot at least on males in *H. copelandi* Durbin, 1908, *H. eques* (Steindacher, 1882), *H. haraldschultzi* Travassos, 1960, *H. micropterus* (Eigenmann, 1915), *H. megalopterus* (Eigenmann, 1915), *H. similanus* (Géry, 1960) and *H. takasei* Géry, 1964 (Fig. 2e); and absence of humeral spot in *H. compressus* (Meek, 1904), *H. dorsalis* Zarske, 2014, *H. georgettae* Géry, 1961, *H. pulchripinnis* Ahl, 1937, and *H. rosaceus* Durbin, 1909 (Fig. 2f).

Furthermore, the new species differs from *H. bentosi* Durbin, 1908, *H. erythrostigma*, *H. pyrrhonotus*, *H. rosaceus*, and *H. socolofi* by presenting only one tooth in the outer row of premaxillary, and this unique tooth just slightly displaced from inner row [vs two or more teeth, displaced from the inner row]; from *H. hasemani* and *H. micropterus* by the dorsal-fin spot located approximately at the middle of the fin’s depth, not reaching its tip (vs spot located approximately at the middle of the fin’s depth, reaching its tip in adults); from *H. hasemani* by presenting tri to unicusp teeth in the inner row of premaxillary and dentary (vs tricuspid or pentacuspid teeth); from *H. pior斯基* by having the anal-fin profile usually nearly straight (vs anal-fin profile usually falcate). In addition, *H. caru* sp. nov. is easily distinguished from *Pristella maxillaris* (Ulrey, 1894), *Moenkhausia hemigrammoides* Géry, 1965, and *Hemigrammus unilineatus* (Gill, 1858) by the absence of a black oblique stripe or band on the anterior portion of the anal-fin (Fig. 1) (vs presence).

**Description.** Morphometric data of holotype and paratypes are presented in Table 2. Body small (with maximum SL of 25.4 mm), compressed, moderately deep, greatest body depth slightly anterior to dorsal-fin base. Lateral body profile straight and downward directed from the end of dorsal-fin to adipose-fin, straight or slightly convex between later point and origin of dorsal most prominent caudal-fin ray. Dorsal profile of head convex from upper lip to vertical through eye; predorsal profile of body roughly straight, dorsal-fin base slightly convex, posteroventrally inclined; ventral profile of head convex from lower jaw to pelvic-fin origin. Ventral profile of body straight or slightly convex from pelvic-fin origin to anal-fin origin; straight and posterodorsally slanted along anal-fin base; and slightly concave on caudal peduncle. Jaws equal, mouth terminal, anterovelar end of dentary protruding. Maxilla reaching vertical to anterior margin of pupil.

**Table 2.** Morphometric data (N = 45) of *Hyphessobrycon caru* sp. nov. SD: Standard deviation.

|                     | Holotype | Paratypes | Mean | SD |
|---------------------|----------|-----------|------|----|
| **Standard length** | 22.2     | 14.8–25.4 | 18.9 | –  |
| **Percentages of standard length** |          |           |      |    |
| Depth at dorsal-fin origin | 37.3     | 33.1–38.5 | 35.2 | 1.1|
| Snout to dorsal-fin origin | 53.7     | 49.4–55.0 | 51.7 | 1.2|
| Snout to pectoral-fin origin | 29.5     | 28.2–32.3 | 29.9 | 1.0|
| Snout to pelvic-fin origin | 46.0     | 43.6–48.8 | 45.6 | 1.0|
| Snout to anal-fin origin | 62.5     | 58.5–64.0 | 61.0 | 1.3|
| Caudal peduncle depth | 12.3     | 8.5–12.3  | 10.3 | 0.8|
| Caudal peduncle length | 11.7     | 9.5–12.7  | 11.2 | 0.8|
| Pectoral-fin length | 23.2     | 16.5–23.7 | 19.6 | 1.9|
| Pelvic-fin length | 20.6     | 14.1–20.5 | 17.4 | 1.4|
| Dorsal-fin base length | 15.2     | 12.9–15.7 | 14.3 | 0.8|
| Dorsal-fin height | 32.2     | 27.9–34.1 | 30.8 | 1.5|
| Anal-fin base length | 32.4     | 26.4–32.7 | 29.6 | 1.3|
| Eye to dorsal-fin origin | 37.5     | 34.5–38.8 | 37.3 | 0.9|
| Dorsal-fin origin to caudal-fin base | 55.1     | 50.6–56.1 | 53.4 | 1.1|
| Head length | 29.8     | 27.4–31.1 | 29.3 | 1.0|
| **Percentages of head length** |          |           |      |    |
| Horizontal eye diameter | 39.2     | 35.4–43.6 | 39.2 | 1.7|
| Snout length | 24.4     | 17.3–24.3 | 21.5 | 1.8|
| Least interorbital width | 29.1     | 22.4–30.7 | 27.2 | 1.8|
| Upper jaw length | 37.8     | 33.1–42.5 | 37.4 | 2.1|
Figure 1. *Hyphessobrycon caru* sp. nov., CICCAA 02286, holotype, 22.2 mm SL; Brazil: Maranhão state: Buritizinho River, Pindaré river drainage, Mearim river basin.

Figure 2. Humeral spot of: a *Hyphessobrycon caru*, holotype, CICCAA 02286 b *H. piorski*, holotype, CICCAA 00695 c *H. pyrrhonotus*, holotype, MZUSP 45714 d *H. werneri*, holotype, MZUSP 42365 e *H. eques*, CICCAA 00300 f *H. compressus*, paratype, MHNG 2181.076.
Premaxillary teeth in two rows. Outer row with one unicuspid or tricuspid tooth, just slightly displaced from inner row; inner row with 6(5), 7(6), or 8(1) tricuspid teeth and one unicuspid tooth. Maxilla with 3(2) tricuspid teeth and two unicuspid teeth, 4(3) tricuspid teeth and two unicuspid teeth or 5(7) tricuspid teeth. Dentary with five (10) or six (1) larger tricuspid teeth followed by one smaller tricuspid teeth 5(2), 6(2), 7(3), and 8(5) smaller unicuspid teeth (Fig. 3).

Scales cycloid, three to eight radii strongly marked, circuli well-marked anteriorly, weakly marked posteriorly; lateral line incompletely pored, with 5(1), 6(2), 7(24), 8(14), or 9(4) perforated scales. Longitudinal scales series including lateral-line scales 31(1), 32(7), 33(14), 34(13), 35(3), or 36(7). Longitudinal scales rows between dorsal-fin origin and lateral line 5(3), 6(32), or 7(10). Horizontal scale rows between lateral line and pelvic-fin origin 4(43) or 5(2). Scales in median series between tip of supraoccipital spine and dorsal-fin origin 10(9), 11(12), 12(21), or 13(3). Cirrumpeduncular scales 11(6), 12(35), 13(2), or 14(2).

Dorsal-fin origin at midbody. Base of last dorsal-fin ray at vertical through first third of anal-fin. Dorsal-fin rays ii + 9(48), iii + 9(5), ii + 10(4). First dorsal-fin pterygiophore main body located behind neural spine of 4th vertebrae. Adipose-fin present. Anal-fin origin aligned with vertical line through middle of dorsal-fin, between 6th and 8th dorsal-fin rays base. Anteriormost anal-fin pterygiophore inserting posterior to haemal spine of 11th vertebrae. First anal-fin ray in vertical through the middle of dorsal-fin (with about 7th or 8th ray base). Anal-fin iii + 22(10) or iii + 23(47); anal-fin origin aligned with vertical line through middle of dorsal-fin (between base of 6th and 8th dorsal-fin rays); Anal-fin profile nearly straight; Anal-fin rays with a sexually dimorphic pattern, which is absent in females, described below. Pectoral-fin rays 12(57) total rays. Tip of pectoral-fin surpassing pelvic-fin base. Pelvic-fin rays 8(57) total rays, surpassing anal-fin origin. Pelvic-fin rays with a sexually dimorphic pattern, which are absent in females, described below. Caudal-fin forked, upper and lower lobes similar in size. Principal caudal-fin rays 11+10(50) or 10+9(7); dorsal procurent rays 8(2), 9(8) or 11(2) and ventral procurent rays 7(4) or 8(8).

Branchiostegal rays 4(12). First gill arch with 1(11), 2(1) hypobranchial, 11(1), 12(10), or 13(1) ceratobranchial, 1(12) on cartilage between ceratobranchial and epibranchial, and 5(1) or 6(1) epibranchial gill-rakers. Supraneurals 3(2), 4(9), or 5(1). Total vertebrae 28(2) or 29(10).
Colours in alcohol. Ground coloration light yellowish brown. Humeral region with few irregular inconspicuous vertically arranged chromatophores, sometimes very thin and inconspicuous humeral spot. Flank with chromatophores homogeneously scattered, more concentrated on posterior region to humeral spot, posterior region of dorsal-fin base origin and below mid-portion of trunk, between anal-fin origin and caudal peduncle. Ventral region lacking dark-brown chromatophores. Dark-brown chromatophores present on head and more concentrated on dorsal portion, becoming sparser on cheek and preopercular regions.

Dorsal-fin ground coloration hyaline, with conspicuous black or dark-brown spot located on anterior portion of fin, reaching about 6th ray, approximately between one-half to two-thirds of fin depth. Anal and caudal-fins hyaline. Caudal-fin with a darker, usually dark brown, posterior margin and on its base. Adipose-fin hyaline to light brown, with dark-brown or black chromatophores more concentrated on its dorsal portion, depending on the specimen preservation state. Pectoral and pelvic-fins hyaline; pelvic-fin with variable amounts of dark-brown pigmentation remaining depending on the specimen preservation state.

Sexual dimorphism. Mature males with small bone hooks on anal and pelvic-fin rays. Bone hooks absent on females. Anal-fin presenting bone hooks from 3rd, 4th, or 5th rays to the last ray. Number of hooks variable, increasing from the first to the last rays. Pelvic-fin presenting 2nd, 3rd, 4th, or 5th rays with 5, 6, or 7 smaller hooks.

Etymology. The specific epithet honors the term “Caru”. Caru is the name of an area (about 70,000 ha) inhabited by Brazilian native tribes from the ethnicities Guajá and Guajajara. People from this area use the Tupi language and have suffered consequences of European colonization and are under threat due to the pressure for exploration of the protected territory.

Geographic distribution. Hyphessobrycon caru sp. nov. has a restricted geographic distribution, being known only from the upper Pindaré river drainage, Mearim river basin, in the state of Maranhão, northeastern Brazil (Fig. 4). This species was never collected in the lower portions of this river drainage during 8 years of field trips conducted by EG and PB, including about 15 expeditions.

Molecular diagnosis (CBB). Hyphessobrycon caru sp. nov. belongs to the H. micropterus clade possessing 20 synapomorphic nucleotide substitutions: COI 73 (C→T), COI 88 (T→C), COI 217 (C→T), COI 1274 (C→T), COI 298 (C→T), COI 334 (C→G), COI 338 (T→C), COI 370 (A→G), COI 418 (A→G), COI 433 (C→T), COI 439 (C→A), COI...
Figure 5. Phylogenetic tree based on Bayesian Inference (BI). Numbers above branches are posterior probability values. Posterior probability value supporting the *Hyphessobrycon micropterus* clade is indicated in green (haplotypes marked with a green bar); posterior probability value supporting the *H. cura* sp. nov. lineage under WP method is indicated in red (haplotypes marked with a red bar); and the other species (lineages) under WP method, within this clade, are indicated in black. **b** Strict consensus phylogenetic tree based on Maximum Parsimony (MP), obtained from the 38 most parsimonious trees, in which 587 characters were constant, 20 variable but parsimony-uninformative, and 248 parsimony-informative (total length 833, consistency index 0.489, retention index 0.901). The image is focusing on the *Hyphessobrycon micropterus* clade. Numbers above branch are bootstrap values and letters below branches correspond to nucleotide substitutions, listed in Suppl. material 1: Box 1, corresponding to the CBB method. Green circle indicating *Hyphessobrycon micropterus* clade, red circle *H. cura* sp. nov., blue circles the other congeners within the clade, and black circle the clade *H. cura* sp. nov. + *H. pioriskii*.
Figure 6. Species delimitation tree generated by the Bayesian Poisson Tree Processes (bPTP) model, using a fragment of the mitochondrial gene COI. The blue lines indicate branching processes among species, while red lines indicate branching processes within species.

DBC. COI sequences support the existence of a new species of *Hyphessobrycon* inhabiting the Pindaré river basin in Maranhão state. After trimming, the final alignment yielded 680 base pairs with 159 polymorphic sites and 26 haplotypes. Average genetic distances were 18.3%, with the highest values between *H. epicharis* and *H. erythrostigma* (23.4%), while the lowest value (0.7%) was between *H. pyrrhonotus* and *H. erythrostigma* (Table 3). *Hyphessobrycon caru* sp. nov. is divergent on average 17.0% from the other taxa, with a minimum distance of 3.6% to *H. piorcki* and a maximum of 21.8% to *Pristella maxillaris* (Table 3).

WP and CBB. Both phylogenetic analysis based on BI and MP supported a clade comprising *H. caru* sp. nov., *H. micropterus*, *H. piorcki*, and *H. simulatus*, hereafter termed *Hyphessobrycon micropterus* clade, with maximum posterior probability value and 99% bootstrap value in BI and MP, respectively. *Hyphessobrycon caru* sp. nov. formed a single exclusive lineage with maximum posterior probability value (posterior probability = 1) and 99% bootstrap value in BI and MP, respectively.
These species delimitation analysis (WP and CBB) have identical results, delimiting four species within the *Hyphessobrycon micropterus* clade: *H. caru* sp. nov., *H. micropterus*, *H. pior斯基*, and *H. simulatus* (Fig. 5a, b). The nucleotide substitutions supporting these four lineages within the *H. micropterus* clade, and the nucleotide substitutions supporting this clade are presented in Figure 5b and Suppl. material 1: Box 1. The combination of nucleotide substitutions diagnosing *H. caru* sp. nov. are presented in the molecular diagnosis section.

**bPTP.** This species delimitation analysis also indicates four lineages (species) within the *Hyphessobrycon micropterus* clade: *H. caru* sp.n., *H. micropterus*, *H. piorски*, and *H. simulatus* (Fig. 6). This outcome was similar to the aforementioned results. The species included as out-group (H. bentosi and H. copelandi) were also supported as independent lineages.

**Discussion**

Currently molecular techniques are frequently useful for solve species complexes and discover cryptic species (e.g. Bickford et al. 2006; Costa and Amorim 2011; Pereira et al. 2011; Adams et al. 2014; Costa-Silva 2015; Costa et al. 2012, 2014, 2017; Amorim 2018; Ottoni et al. 2011; Adams et al. 2014; Costa and Amorim 2011; Pereira et al. 2011; Castro-Paz et al. 2014; Melo et al. 2014, 2016a; Benzaquen et al. 2015; Benine et al. 2015; Ottoni et al. 2019). DNA techniques can help to uncover morphological hidden diversity (Bickford et al. 2006; Adams et al. 2014), delimiting a putative population or group of populations as an independent lineage (species), and, subsequently, through a more meticulous analysis of morphological features, morphological differences between cryptic species can be found.

The large number of the described *Hyphessobrycon* species (about 160 spp.), with new species described every year, reveal an astonishing diversity within the genus. During the past 10 years, about 50 new species have been described (Fricke et al. 2019). However, historically *Hyphessobrycon* species have been described only on the basis of morphological features, including differences in the pigmentation patterns and teeth numbers and morphology, using few individuals per species (e.g. Steindachner 1882; Eigenmann 1915; Zarske 2008, 2014; Bragança et al. 2015). Recently, DNA barcoding in characid fishes has been used to discriminate species, identify new ones, and reveal that it is not always possible to differentiate species based solely on their morphology (Ornelas-Garcia et al. 2008; Pereira et al. 2011; Castro-Paz et al. 2014; Melo et al. 2014, 2016a; Benine et al. 2015).

Our results suggest a cryptic speciation in the rosy tetra clade, more specifically in a new clade here defined, the *Hyphessobrycon micropterus* clade, including *H. caru* sp. nov., *H. micropterus*, *H. piorски*, and *H. simulatus*, so far only known from the Pindaré, Itapecuru, Munim, Preguicas, and São Francisco river drainages of Brazil and the coastal river basins of French Guiana and Suriname (Guimarães et al. 2018; Brito et al. 2019; Fricke et al. 2019; this study). The clade proposed here is supported by high node support values (maximum posterior probability value and 99% of bootstrap value in BI and MP, respectively). In addition, this clade was corroborated by 20 synapomorphic nucleotide substitutions (Fig. 5; Suppl. material 1: Box 1).

### Table 3. Kimura-2 parameters pairwise genetic distances among species.

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *H. erythrostigma* | | | | | | | | | | | | | | |
| 2 | *H. pyrrhodonius* | 0.007 | | | | | | | | | | | | | |
| 3 | *H. socolofi* | 0.037 | 0.035 | | | | | | | | | | | | |
| 4 | *H. simulatus* | 0.192 | 0.194 | 0.179 | | | | | | | | | | | |
| 5 | *H. micropterus* | 0.205 | 0.202 | 0.198 | 0.041 | | | | | | | | | | |
| 6 | *H. piorски* | 0.188 | 0.185 | 0.178 | 0.063 | 0.064 | | | | | | | | | |
| 7 | *H. caru* | 0.206 | 0.210 | 0.203 | 0.062 | 0.057 | 0.036 | | | | | | | | |
| 8 | *H. equus* | 0.175 | 0.178 | 0.163 | 0.154 | 0.157 | 0.160 | 0.160 | | | | | | | | |
| 9 | *H. copelandi* | 0.192 | 0.189 | 0.186 | 0.170 | 0.176 | 0.158 | 0.168 | 0.102 | | | | | | |
| 10 | *H. equus* | 0.234 | 0.230 | 0.220 | 0.170 | 0.175 | 0.189 | 0.190 | 0.187 | 0.197 | | | | | |
| 11 | *H. bentosi* | 0.106 | 0.103 | 0.112 | 0.197 | 0.194 | 0.205 | 0.204 | 0.195 | 0.220 | 0.219 | | | | |
| 12 | *H. certa* | 0.210 | 0.207 | 0.197 | 0.182 | 0.187 | 0.201 | 0.209 | 0.191 | 0.197 | 0.030 | 0.222 | | | |
| 13 | *P. maxillaris* | 0.225 | 0.232 | 0.206 | 0.201 | 0.211 | 0.220 | 0.218 | 0.194 | 0.213 | 0.180 | 0.202 | 0.183 | | |
| 14 | *M. hemigrammoides* | 0.219 | 0.219 | 0.209 | 0.179 | 0.185 | 0.178 | 0.179 | 0.193 | 0.196 | 0.211 | 0.221 | 0.199 | 0.166 | | |
| 15 | *H. compressus* | 0.214 | 0.218 | 0.215 | 0.202 | 0.208 | 0.198 | 0.208 | 0.198 | 0.201 | 0.212 | 0.212 | 0.203 | 0.215 | | |
| 16 | *H. panamensis* | 0.210 | 0.213 | 0.209 | 0.202 | 0.199 | 0.179 | 0.187 | 0.215 | 0.229 | 0.221 | 0.213 | 0.218 | 0.204 | 0.208 | 0.145 | |
Hyphessobrycon caru sp. nov. is herein described within the Hyphessobrycon micropterus clade based on five different and independent methods of species delimitation (PAA, DBC, WP, CBB and bPTP), characterized by different criteria and assumptions. Hyphessobrycon caru sp. nov. is distinguished from all its congeners by a combination of unambiguous morphological character states [see Diagnosis (PAA)]. In our Bayesian phylogenetic analysis (Fig. 5A), haplotypes of H. caru sp. nov. formed a single exclusive clade with maximum posterior probability value (posterior probability = 1) (WP). Furthermore, the COI average genetic distance of H. caru sp. nov. when compared with the other taxa herein analyzed was 19.6% and its minimum COI genetic distance was 3.6% to H. pioriskii (DBC). Considering this value, the threshold of H. caru sp. nov. would be greater than that inferred by delimitations among Neotropical fish species (2% according to Pereira et al. 2011). Moreover, H. caru sp. nov. was also molecularly diagnosed by six synapomorphic nucleotide substitutions (Fig. 5b; Suppl. material 1: Box 1), as well as, by a combination of other nucleotide substitutions (see CBB - molecular diagnosis), and corroborated by a bPTP analysis. Thus, it makes the hypothesis of this new species stronger from an integrative taxonomy perspective (see Dayrat 2005; de Queiroz 2007; Goldstein and DesALLE 2010; Palial et al. 2010). Therefore, we recommend the use of integrative taxonomy for future taxonomic revisions and species descriptions when dealing with species complexes and groups containing possible cryptic species.

Comparative material

Hyphessobrycon amandae: UFRJ 1557, 5 spcms, Jussara municipality, Goiás state, Brazil. H. bentosi: MCZ 20842, 1 spcm (Syntype), Obidos municipality, Pará state, Brazil. H. bifasciatus: UFRJ 0068, 6 spcms, Marataizes and Guarapari municipality, Esprírito Santo state, Brazil. H. compressus: BMNH 1905.12.6.4-5, 2 spcms (Paratypes), Oaxaca state, Mexico. H. copelandi: CAS 42683, 1 spcm (Syntype); MCZ 20771, 1 spcm (Syntype), Tabatinga municipality, Amazonas state, Brazil. H. eques: CICCAA 00715, 4 spcms (C&S); CICCAA 00710, 51 spcms, Tombos municipality Carangola river, Minas Gerais state, Brazil. H. erythrostigma: ANSP 70208, 1 spcm (Holotype), Peru and Brazil. H. epichiris: FMNH100609, 1 spcm (Paratype), Baria river, Amazonas, Venezuela. H. haraldschultzi: CICCAA 00873, 20 spcms, Ilha do Bananal municipality, Javáes river, Tocantins state, Brazil. H. hasemani: ANSP 39230, 1 spcm (Holotype), Guajamarim municipality, Madeira river, Rondônia state, Brazil. H. micropterus: FMNH 57916, 1 spcm (Holotype), São Francisco river at Lagoa de Porto, Minas Gerais state, Brazil. H. pioriskii: CICCAA 00695, 1 spcm (Holotype); CICCAA 00430, 15 spcms (Paratype); CICCAA 00431, 21 spcms (Paratype); CICCAA 00696, 15 spcms (Paratype); CICCAA 00697, 16 spcms (C&S) (Paratype); CICCAA 00698, 6 spcms, 1 spcm (C&S) (Paratype); CICCAA 00750, 9 spcms (Paratype); CICCAA01654, 1 spcm (Paratype); CPUFMA 171664, 15 spcms (Paratype); UFRJ 11553, 6 spcms (Paratype), stream at the Anapurus municipality, Munim river, Maranhão state, Brazil. CICCAA 00089, 1 spcm (C&S) (Paratype); CICCAA 00881, 1 spcm (Paratype); CICCAA 01563, 1 spcm (Paratype); stream at Mata de Itamaacoa, Chapadinha municipality, Munim river, Maranhão state, Brazil. CICCAA 01382, 5 spcms (Paratype); CICCAA 02088, 12 (C&S) spcms (Paratype), stream at Mata Fome, Barreirinhas municipality, Preguiças river, Maranhão state, Brazil. H. pyrrhonotus: MZUSP 45714, 1 spcm (Holotype), Ererê river, Brazil. H. rosaceus: FMNH 52791, 1 spcm (Holotype), Gluck Island, Essequibo River, Guyana. H. werner: MZUSP 42365, 1 spcm (Holotype), Santa Maria do Pará and São Miguel de Guama municipality, Guama river, Pará state, Brazil. CICCAA 00751, 1 spcm, Paragominas municipality, Cândido river, Pará state, Brazil. H. socolofi: MZUSP 13181, 1 spcm (Holotype), Barcelos municipality, Negro river, Amazonas state, Brazil.

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References

Adams M, Raadik TA, Burridge CP, Georges A (2014) Global biodiversity assessment and hyper-cryptic species complexes: more than one species of elephant in the room? Systematic Biology 63: 518–533. https://doi.org/10.1093/sysbio/syu017

Ahl E (1937) Über einen neuen südamerikanischen Characiniden der Gattung Hyphessobrycon. Zoologischer Anzeiger 120 (9/10): 235–236.
Alfaro ME, Holder MT (2006) The posterior and the prior in Bayesian phylogenetics. Annual Review of Ecology, Evolution, and Systematics 37: 19–42. https://doi.org/10.1146/annurev.ecolsys.37.091305.110021

Amorim FP (2018) Jenynsia lineata species complex, revision and new species description (Cyprinodontiformes: Anablepidae). Journal of Fish Biology 92(5): 1312–1332. https://doi.org/10.1111/jfb.13587

Avise JC (2000) Phylogeography: the history on formation of species. University Press, Cambridge, 447 pp.

Benine RC, Melo BF, Castro RMC, Oliveira C (2015) Taxonomic re- vision and molecular phylogeny of Gymnocorymbus Eigenmann, 1908 (Teleosti, Characiformes, Characidae). Zootaxa 3956(1): 1–28. https://doi.org/10.11646/zootaxa.3956.1.1

Benzaquem DC, Oliveira C, da Silva Batista J, Zuanon J, Porto JIR (2015) DNA Barcoding in Pencilfishes (Lebiasinidae: Nannostomus) Reveals Crypto Diversity across the Brazilian Amazon. PLoS One 10(2): e0112217. https://doi.org/10.1371/journal.pone.0112217

Betancur-R R, Arcila D, Vare RP, Hughes LC, Oliveira C, Sabaj MH, Orti G (2018) Phylogenetic incongruence, hypothesis testing, and taxonomic sampling: The monophyly of characiform fishes. Evolution 72(3): 329–345. https://doi.org/10.1111/evo.13649

Bickford D, Lohman DJ, Soliti NS, NG PKL, Meir R, Winker K, Ingram KK, Das I (2006) Cryptic species as a window on diversity and conservation. Trends in Ecology and Evolution 22(3): 148–155. https://doi.org/10.1016/j.tree.2006.11.004

Burgess WE (1993) Hyphessobrycon pyrrhonotus, a new species of bleeding heart tetra (Teleostei: Characidae) from the Rio Erere, Brazil. Tropical Fish Hobbyist 42(1): 156–160.

Bragança PHN, Ottoni FP, Rangel-Pereira FS (2015) Hyphessobrycon ellisei, a new species from northeastern Brazil (Teleostei: Characidae). Ichthyological Exploration of Freshwaters 26(3): 255–262. http://pfeil-verlag.de/wp-content/uploads/2016/07/ief26_3_07.pdf

Brito PS, Guimarães EC, Ferreira, BRA, Ottoni, FP, Piorcio NM (2019) Freshwater fishes of the Parque Nacional dos Lençóis Maranhenses and adjacent areas. Biota Neotropica. 19(3): e20180660. https://doi.org/10.1590/1676-0611-bn-2018-0660

Brown JK, Frohlich DR, Rosell RC (1995) The sweetpotato or silverleaf whiteflies: biotypes of Bemisia tabaci or a species complex? Annual Review of Entomology 40: 511–534. https://doi.org/10.1146/annurev.ento.40.1.511

Carvalho FR, Malabarba LR (2015) Redescription and osteology of Hyphessobrycon compressus (Teleostei, Characiformes) (Characidae: Haemulidae). Ichthyological Exploration of Freshwaters 26(3): 255–262. http://pfeil-verlag.de/wp-content/uploads/2016/07/ief26_3_07.pdf

Carvalho FR, Cabeceira FG, Carvalho LN (2017) New species of Hyphessobrycon (Ostariophysi, Characiformes). Journal of Fish Biology 91(3): 750–763. https://doi.org/10.1111/jfb.13362

Castro-Faz FP, Batista JS, Porto JIR (2014) DNA Barcodes of Rosy Tetras and Allied Species (Characiformes: Characidae: Hyphessobrycon) from the Brazilian Amazon Basin. PLoS ONE 9(5): e98603. https://doi.org/10.1371/journal.pone.0098603

Costa WJEM, Amorim PF (2011) A new annual killifish species of the Hypsolebias flavidus complex from the São Francisco River basin, Brazilian Caatinga (Cyprinodontiformes: Rivulidae). Vertebrate Zoology 61(1): 99–104.

Costa WJEM, Amorim PF, Mattos JLO (2012) Species delimitation in annual killifishes from the Brazilian Caatinga, the Hypsolebias flavidus complex (Cyprinodontiformes: Rivulidae): implications for taxonomy and conservation. Systematics and Biodiversity 10: 71–91. https://doi.org/10.1080/14772000.2012.664177

Costa WJEM, Amorim PF, Araújo GN (2014) Species limits and DNA barcodes in Nematobrycon, a genus of seasonal killifishes threatened with extinction from the Atlantic Forest of southeastern-Brazil, with description of a new species (Teleostei: Rivulidae). Ichthyological Exploration of Freshwaters 24(3): 225–236.

Costa WJEM, Cheffe MM, Amorim PF (2017) Two new seasonal killifishes of the Austrolebias adolfi group from the Lagoa dos Patos basin, southern Brazil (Cyprinodontiformes: Aplocheilidae). Vertebrate Zoology 67(2): 139–149.

Costa-Silva GJ, Rodrigues MS, Roxo FF, Foresti F, Oliveira C (2015) Using different methods to access the difficult task of delimiting species in a complex neotropical hyperdiverse group. PLoS One 10: e0135075. https://doi.org/10.1371/journal.pone.0135075

Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG, Thompson JD (2003) Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Research 31: 3967–3970. https://doi.org/10.1093/nar/gkg500

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9(8): 772. https://doi.org/10.1038/nmeth.2109

Davis JJ, Nixon KC (1992) Populations, genetic variation, and the delimitation of phylogenetic species. Systematic Biology 41(4): 421–435. https://doi.org/10.1093/sysbio/41.4.421

Dayrat B (2005) Towards integrative taxonomy. Biological Journal of the Linnean Society 85(3): 407–415. https://doi.org/10.1111/j.1095-8312.2005.00503.x

de Queiroz K (2005) Different species problems and their resolution. BioEssays 27(12): 1263–1269. https://doi.org/10.1002/bies.20325

de Queiroz K (2007) Species concepts and species delimitation. Systematic Biology 56(6): 879–886. https://doi.org/10.1080/10635150701701083

Desalle R, Egan MG, Siddall M (2005) The unholly trinity: taxonomy, species delimitation and DNA barcoding. Philophical Transactions of the Royal Society B 360: 1905–1916. https://doi.org/10.1098/rstb.2005.1722

Durbin ML (1909) Reports on the expedition to British Guiana of the Indiana University and the Carnegie Museum, 1908. Report No. 2. A new genus and twelve new species of tetragonopterid characins. Annals of the Carnegie Museum 6(1): 55–72.

Eigenmann CH (1908) Preliminary descriptions of new genera and species of Tetragonopteridae characins. Bulletin of the Museum of Comparative Zoology 52: 91–106.

Eigenmann CH (1915) The Cheirodontinae, a subfamily of minute characid fishes of South America. Memoirs of the Carnegie Museum 7(1): 1–99. https://doi.org/10.5962/bhl.title.46579

Eigenmann CH (1918) The American Characidae (Part 2). Memoirs of the Museum of Comparative Zoology 43: 101–208.

Eigenmann CH (1921) The American Characidae (Part 3). Memoirs of Museum of Comparative Zoology 43: 209–310.

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x
Fink W, Weitzman S (1974) The so called Cheirodontin fishes of Central America with descriptions of two new species (Pisces: Characidae). Smithsonian Contributions to Zoology 172: 1–45. https://doi.org/10.5479/si.00810282.172

Fowler HW (1943) Description of a new South American characin referred to Hemigrammus. The Fish Culturist 22(5): 33–34.

Fricke R, Eschmeyer WN, van der Laan R (2019) Catalog of Fishes: Genera, Species, References. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp [Electronic version accessed 22/04/2019]

Fricke R, Eschmeyer WN (2019) Guide to fish collections. http://researcharchive.calacademy.org/research/ichthyology/catalog/collections.asp [Electronic version accessed 22/04/2019]

Garcia-Alzate CA, Román-Valencia C, Taphorn DC (2017) A new species of Hyphessobrycon (Characiformes, Characidae) from the upper Guaviare River, Orinoco River Basin, Colombia. ZooKeys 668: 123–138. https://doi.org/10.3897/zookeys.668.11489

Géry J (1960) Contributions to the study of the characid fishes, No. 6. New Chariodontinae from French Guiana. Senckenbergiana Biologica 41(1/2): 15–39.

Géry J (1961) Three new South-American Characids: Knodus savannensis, Hyphessobrycon herbertaxelrodi and Megalamphodus sweglesi, with a review of some Hyphessobrycon-group of species. Tropical Fish Hobbyist 9(9): 26–46.

Géry J (1964) Two new tetras from the Lower Amazon Basin. Tropical Fish Hobbyist 12(7): 13–15, 59–60.

Géry J (1977) Characoids of the world. TFH-publications, Neptune City Inc.

Géry J, Uj A (1987) Ein neuer tetra (Characoidae, Characidae, Tetraragopterinae) aus dem unteren Amazonasgebiet: Hyphessobrycon werneri n. sp. Aquarien und Terrarien-Zeitschrift 40(12): 546–550.

Goldstein PZ, Desalle R (2010) Integrating DNA barcode data and taxonomic practice: determination, discovery, and description. BioEssays 33(3): 135–147. https://doi.org/10.1002/bies.201000036

Goloboff P, Catalano S (2016) TNT version 1.5, including a full implementation of phylogenetic morphometrics. Cladistics 32: 221–238. https://doi.org/10.1111/cla.121160

Guimarães EC, De Brito PS, Feitosa LM, Carvalho-Costa LF, Ottoni FP (2018) A new species of Hyphessobrycon Durbin from northeastern Brazil: evidence from morphological data and DNA barcoding (Characiformes, Characidae). ZooKeys 765: 79–101. https://doi.org/10.3897/zookeys.765.23157

Hebert PDN, Cywinska A, Ball SL, de Ward JR (2003a) Biological identifications through DNA barcodes. Proceedings of the Royal Society B 270(S152): 313–321. https://doi.org/10.1098/rspb.2002.2218

Hebert PDN, Ratnasingham S, de Ward JR (2003b) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society B 270(1): 96–99. https://doi.org/10.1098/rspb.2003.0025

Hebert PDN, Cutter MY, Zemlak TS, Francis CM (2004b) Identification of birds through DNA barcodes. PLoS Biology 2(10): e312. https://doi.org/10.1371/journal.pbio.0020312

Hein G (2009) Hyphessobrycon pando sp. nov., a new rosy tetra from northern Bolivia (Teleostei, Characiformes, Characidae). Bulletin of Fish Biology 10(1/2): 1–10.

Hueslenbeck JP, Ronquist F (2001) Mr. Bayes: Bayesian inference of phylogenetic trees. Bioinformatics 17(8):754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Kekkonen M, Hebert PDN (2014) DNA barcode-based delineation of putative species: efficient start for taxonomic workflows. Molecular Ecology Resources 14(4): 706–715. https://doi.org/10.1111/1755-0998.12233

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16(2): 111–120. https://doi.org/10.1007/BF01731581

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870–1874. https://doi.org/10.1093/molbev/msw054

Lima FCT, Pires THS, Ohara WM, Jerpe FC, Carvalho FR, Marinho MMF, Zuanon J (2013) Characidae. In: Queiroz LJ, Torrente-Vilara G, Ohara WM, Pires THS, Zuanon J, Dória CRC (Orgs) Peixos do rio Madeira. Dialetro Latin American Documentary, São Paulo: 213–395.

MeeK SE (1904) The fresh-water fishes of Mexico north of the isthmus of Tehuantepec. Field Columbian Museum, Zoological Series 5: 1–17. https://doi.org/10.5962/bhl.title.2229

Melo BF, Sidlauskas BL, Hoekzema K, Vari RP, Oliveira C (2014) The first molecular phylogeny of Chilodontidae (Teleostei: Ostariophysi: Characiformes) reveals cryptic biodiversity and taxonomic uncertainty. Molecular Phylogenetics and Evolution 70: 286–295. https://doi.org/10.1016/j.ympev.2013.09.025

Melo BF, Ochoa LE, Vari RP, Oliveira C (2016a) Cryptic species in the Neotropical fish genus (Teleostei, Characiformes). Zoologica Scripta 45: 650–658. https://doi.org/10.1111/zsc.12178

Melo BF, Sidlauskas BL, Hoekzema K, Frable BW, Vari RP, Oliveira C (2016b) Molecular phylogenetics of the Neotropical fish family Prochilodontidae (Teleostei: Characiformes). Molecular Phylogenetics and Evolution 102: 189–201. https://doi.org/10.1016/j.ympev.2016.05.037

Melo BF, Benine RC, Silva GSC, Avelino GS, Oliveira C (2016c) Molecular phylogeny of the Neotropical fish genus Tetragonopterus (Teleostei: Characiformes). Molecular Phylogenetics and Evolution 94: 709–717. https://doi.org/10.1016/j.ympev.2015.10.022

Mirande M (2010) Phylogeny of the family Characidae (Teleostei, Characiformes). Cladistics 1–19.

Mirande JM (2018) Morphology, molecules and the phylogeny of the Neotropical fish family Prochilodontidae (Teleostei: Characiformes). Cladistics 33: 187–199. https://doi.org/10.1111/cla.12235

Moreira CR, Lima FCT (2017) Two new Hyphessobrycon (Characiformes: Characidae) species from Central Amazon basin, Brazil. Zoosystema 39(1): 123–134. https://doi.org/10.1590/0164-6205.zootaxa.4318.1.5

Oliveira C, Avelino GS, Abe KT, Mariugela TC, Benine RC, Orti G, Vari RP, Castro RMC (2011) Phylogenetic relationships within the speciose family Characidae (Teleostei: Ostariophysi: Characiformes) based on multilocus analysis and extensive ingroup sampling. BMC Evolutionary Biology 11: 275. https://doi.org/10.1186/1471-2148-11-275
Ornelas-Garcia CP, Dominguez-Dominguez O, Doadrio I (2008) Evolutionary history of the fish genus Astyanax Baird & Girard (1854) (Actinopterygii, Characidae) in Mesoamerica reveals multiple morphological homoplasies. BMC Evolutionary Biology 8: 340. https://doi.org/10.1186/1471-2148-8-340

Ottoni FP, Mattos JLO, Katz AM, Bragança PHN (2019) Phylogeny and species delimitation based on molecular approaches on the species of the Astraloheros aurein group (Teleostei, Cichlidae), with biogeographic comments. Zoosystematics and Evolution 95(1): 49–64. https://doi.org/10.3897/zse.95.31658

Padial JM, Miralles A, De la Riva I, Vences M (2010) The integrative future of taxonomy. Frontiers in Zoology 7: 16. https://doi.org/10.1186/1742-9994-7-16

Pereira LHG, Pazian MF, Hanner R, Foresti F, Oliveira C (2011) DNA barcoding reveals hidden diversity in the Neotropical freshwater fish Piabina argentea (Characiformes: Characidae) from the Upper Paraná basin of Brazil. Mitochondrial DNA 22(1): 87–96. https://doi.org/10.3109/19401736.2011.588213

Planquette P, Keith P, Le Bail PY (1996) Atlas des Poissons d’Eau Douce de Guyane Tome 1. Collection du Patrimoine Naturel, vol. 22. Paris, IEGB – M.N.H.N., INRA, CSP, Ministère de l’Environnement.

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Roxo FF, Oliveira C, Zawadzki CH (2012) Three new species of Neoplecostomus (Teleostei: Siluriformes: Loricariidae) from the Upper Rio Paraná basin of southeastern Brazil. Zootaxa 3233: 1–21.

Rozas J, Sánchez JC, Messegue X, Rozas R (2003) DnaSP, DNA polymorphism analyses by the 506 coalescent and other methods. Bioinformatics 19: 2496–2497. https://doi.org/10.1093/bioinformatics/btg359

Sites JW, Marshall JC (2003) Delimiting species: A Renaissance issue in systematic biology. Trends in Ecology and Evolution 18: 462–470. https://doi.org/10.1016/S0169-5347(03)00184-8

Souza CS, Costa-Silva GJ, Roxo FF, Foresti F, Oliveira C (2018) Genetic and Morphological Analyses Demonstrate That Schizolecos guntheri (Siluriformes: Loricariidae) Is Likely to Be a Species Complex. Frontiers in Genetics 9: 69. https://doi.org/10.3389/fgene.2018.00069

Sysma KJ, Schaal BA (1985) Genetic variation, differentiation, and evolution in a species complex of tropical shrimps based on isozymic data. Evolution 39: 582–593. https://doi.org/10.1111/j.1558-5646.1985.tb00396.x

Steindachner F (1882) Beitragz zur Kenntnnis der Flussfische Südamerikas (IV). Anzeiger der Akademie der Wissenschaften in Wien, Mathematisch-Naturwissenschaftliche Klasse 19(19): 175–180.

Taylor W, Van Dyke G (1985) Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. Cybium 9: 107–119.

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

Weitzman SH (1962) The osteology of Brycon meeki, a generalized characid fish, with an osteological definition of the family. Stanford Ichthyological Bulletin 8(1): 3–77.

Weitzman SH, Palmer L (1997a) A new species of Hyphessobrycon (Teleostei: Characidae) from the Neblina region of Venezuela and Brazil, with comments on the putative ‘rosy tetra clade’. Ichthyological Exploration of Freshwaters 7(3): 209–242.

Weitzman SH, Palmer L (1997b) The Rosy Tetra, Hyphessobrycon rosaceus, its identification and history as an aquarium fish. Tropical Fish Hobbyist 45(11): 158–168.

Weitzman SH, Palmer L (1997c) The common Serpa Tetra of Aquarists identified as Hyphessobrycon eques (Steindachner, 1882). Tropical Fish Hobbyist 45(9): 140–150.

Weitzman SH, Palmer L (1997d) The Sicklefin or Roberts’ Tetra Identified as Hyphessobrycon bentosi, Tropical Fish Hobbyist 46(2): 150–159.

Wiens JJ, Penkrot TA (2002) Delimiting species using DNA and Morphological variation and discordant limits in spiny lizards (Sceloporus). Systematic biology 51(1): 69–91. https://doi.org/10.1080/106351502753475880

Xia XY (2013) Dambos: a comprehensive software package for data analysis in molecular biology and evolution. Molecular Biology and Evolution 30: 1720–1728. https://doi.org/10.1093/molbev/ms3064

Xia XY, Xie Z, Salemi M, Chen L, Wang Y (2003) An index of substitution saturation and its application. Molecular Phylogenetics and Evolution 26: 1–7. https://doi.org/10.1016/S1055-7903(02)00326-3

Zarske A (2008) Hyphessobrycon bharinhae sp. nov. – ein neuer Blutsalmler aus Brasilien (Teleostei: Characiformes: Characidae). Vertebrate Zoology 58(1): 5–13.

Zarske A (2014) Zur Systematik einiger Blutsalmler oder “Rosy Tetras” (Teleostei: Ostariophysi: Characidae). Vertebrate Zoology 64(2): 139–167.

Zhang J, Kapli P, Pavlidis P, Stamatakis A (2013) A general species delimitation approach for phylogenetic placements. Bioinformatics 29: 2869–2876. https://doi.org/10.1093/bioinformatics/btt499

Supplementary material 1

Box 1

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Data type: DOCX file

Explanation note: List of nucleotide substitutions (synapomorphies and autapomorphies) from each lineage (species) and some crucial points of the cladogram of the Fig. 5B.

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