A new species of *Hoplias malabaricus* species complex (Characiformes: Erythrinidae) from the Crepori River, Amazon basin, Brazil

Karen Larissa Auzier Guimarães[1,2,*], Juan José Rosso[3,4,*], Mariano González-Castro[3,4], Mendelsohn Fujiie Belém Souza[2], Juan Martín Díaz de Astarloa[3,4] and Luís Reginaldo Ribeiro Rodrigues[1,2]

[1]Programa de Pós-graduação em Biodiversidade e Biotecnologia (Rede BIONORTE – Polo Pará), Universidade Federal do Oeste do Pará, Instituto de Saúde Coletiva, Campus Tapajós, Rua Vera Paz SN, CEP68040-255, Santarém, Pará, Brazil.

[2]Laboratório de Genética e Biodiversidade (LGBio), Universidade Federal do Oeste do Pará, Instituto de Ciências da Educação, Campus Tapajós, Rua Vera Paz SN, CEP68040-255, Santarém, Pará, Brazil.

[3]Grupo de Biotaxonomía Morfológica y Molecular de Peces, Instituto de Investigaciones Marinas y Costeras (IIMyC), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Mar del Plata, Argentina.

[4]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina.

E-mail:
karen.guimaraes.bio@gmail.com (KLAG)
plurosso@yahoo.com.ar (JJR) (corresponding author)
gocastro@mdp.edu.ar (MGC)
mendelsohn2008@gmail.com (MFBS)
astarloa@mdp.edu.ar (JMDA)
luisreginaldo.ufpa@hotmail.com (LRRR)

Orcid number:
KLAG – 0000-0002-3699-7629
JJR – 0000-0001-6730-9385
MGC – 0000-0003-2403-3168
MFBS – 0000-0003-0591-3303
JMDA – 0000-0002-9250-1771
LRRR – 0000-0003-2849-0382

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Abstract

A new species belonging to *Hoplias malabaricus* complex from the Amazon basin, Brazil, is described. The new species is characterized by 15-16 predorsal scales, 37-39 lateral-line scales, 5 scales from dorsal fin to lateral line, 38-39 vertebrae, iii-iv, 7-8 anal-fin rays, ii-iv,12-15 caudal-fin rays, last vertical series of scales on the base of caudal-fin rays forming a straight line, 6-7 dark bands in anal fin and no distinctive dark bands or blotches on flanks. The new species is also distinguished from other congeners of *H. malabaricus* species-group by means of landmark-based morphometrics and DNA barcoding, *Cytochrome c Oxidase I* gene. An identification key to species of the *H. malabaricus* species complex is provided.

Key Words: Integrative Taxonomy, Cryptic diversity, *Hoplias malabaricus*, Tapajós basin, Trahira.

Introduction

Freshwater environments harbor more than half of fish species in the world (Fricke *et al*., 2021). In South America, the freshwater fish diversity accounts for more to 5000 species formally described, but it is estimated that the actual diversity may achieve twice that number (Reis *et al*., 2016). The Amazon, Orinoco and La Plata rivers drainages are among the largest hydrographic basins of South America and retain over 20% of the global freshwater fish diversity (Reis *et al*., 2016). The Amazon basin alone is home to 2716 freshwater fish species (Dagosta and De Pinna, 2019), accounting for 13% of the global diversity in this group of vertebrates (Reis *et al*., 2016; Fricke *et al*., 2021). The majority of Amazonian fish groups belong to the series Otophysi, with predominance of Characiformes and Siluriformes Orders (Dagosta and De Pinna, 2019).

Despite the Neotropical ichthyofauna has been investigated for centuries, the diversity of species continues to increase rapidly, which indicates that the current knowledge still underestimates the real diversity (Nelson *et al*., 2016). The complexity of evolutionary relationships between groups of fishes can generate scenarios of taxonomic uncertainty, especially in taxa with wide distributions, such as *Pimelodus blochii* (Ribeiro and Lucena, 2006; Rocha, 2006), *Rhamdia quelen* (Martinez *et al*., 2011; Ríos *et al*., 2017; Usso *et al*., 2018), *Synbranchus marmoratus* (Kullander, 2003), and *Hoplias* spp. (Oyakawa, 2003; Oyakawa and Mattox, 2009; Cardoso *et al*., 2018).

The family Erythrinidae (Characiformes) includes 19 valid species and three genera, *Erythrinus* Scopoli 1777, *Hoplerythrinus* Gill 1896 and *Hoplias* Gill 1903 (Oyakawa, 2003; Fricke *et al*., 2021). Three species groups within *Hoplias* can be recognized based on morphology: *H. lacerdae* group, *H. malabaricus* group and the monotypic *H. aimara* (Oyakawa, 1990; Oyakawa and Mattox, 2009). The

species *Hoplias microcephalus* (Agassiz 1829) and *Hoplias patana* (Valenciennes 1847) are not associated with these morphological groups.

Hoplias malabaricus (Bloch, 1794), distinguished from congeners by a characteristic rough tongue and V-shaped margins of medial contralateral dentaries converging towards the symphysis in ventral view of head (Oyakawa, 1990; Oyakawa and Mattox, 2009), revealed to hide a species complex that was firstly evidenced by karyotypic variation (Bertollo *et al*., 2000; Blanco *et al*., 2009; Santos *et al*., 2016). Molecular data appended evidence supporting the *H. malabaricus* complex (Dergam *et al*., 1998; Rosso *et al*., 2012; Marques *et al*., 2013; Pereira *et al*., 2013; Jacobina *et al*., 2018). Currently, *H. malabaricus* group comprises six valid species: *H. malabaricus*, *Hoplias microlepis* (Günther, 1864), *Hoplias teres* (Valenciennes, 1847), *Hoplias mbigua* Azpelicueta, Benítez, Aichino & Mendez 2015, *Hoplias misionera* Rosso, Mabragaña, González-Castro, Delpiani, Avigliano, Schenone & Díaz de Astarloa 2016 and *Hoplias argentinensis* Rosso, González-Castro, Bogan, Cardoso, Mabragaña, Delpiani & Díaz de Astarloa 2018.

Although the *H. malabaricus* species complex has long been recognized from cytogenetic and molecular evidence, new species of this complex have been formally described only recently: *H. mbigua* (Azpelicueta *et al*., 2015), *H. misionera* (Rosso *et al*., 2016) and *H. argentinensis* (Rosso *et al*., 2018). Species of the *H. malabaricus* complex inhabit most of the freshwater ecosystems present in South America (Oyakawa, 2003; Dagosta and De Pinna, 2019). Nevertheless, the advance of taxonomic knowledge on this group is confined to the La Plata River basin, with the description of the above-mentioned species. This leaves a large portion of South America, where species of the *H. malabaricus* complex are common and abundant, with a strong uncertainty in taxonomic resolution.

The adoption of molecular tools, such as DNA barcoding, allied with traditional morphology and other markers provides increased power to investigate taxonomic boundaries among species, in a way that is commonly referred to as "integrative taxonomy" (Dayrat, 2005). It has proven to be effective for clarifying cryptic species hidden in species complex and is becoming usual in taxonomic fish studies (*e.g*. Melo *et al*., 2016; Rosso *et al*, 2016, 2018; Guimarães *et al*., 2019, Faria *et al*., 2021). Through DNA barcoding, at least 15 new candidate species are expected in the *H. malabaricus* complex (Cardoso *et al*., 2018).

In this study, we describe a new cryptic species belonging to the *Hoplias malabaricus* species complex from the Crepori River drainage, in the upper Tapajós River basin. The new species is recognized by means of an integrative taxonomic approach, combining genetic, morphometric and morphological traits, coupled to an extensive revision of all the type-material available for *H. malabaricus* species complex.

Materials and Methods

Ethics statement and specimen preservation

Collections of samples were authorized by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) (Permit nº 32653-3). The fish were anesthetized and euthanized by immersion in clove oil solution until the full stop of opercular beat in accordance to the guidelines by the Animal Use Ethics Committee (CEUA) of the Universidade Federal do Oeste do Pará (CEUA/UFOPA #09003/2016).

Voucher specimens (n=17) were fixed in 10% formalin for 72h, rinsed with tap water and preserved in 70% ethanol. The specimens were stored at Fish Collection of the Instituto de Ciências e Tecnologia das Águas of the Universidade Federal do Oeste do Pará (UFOPA) and Instituto de Investigaciones Marinas y Costeras (IIMyC-UNMDP-CONICET).

Morphological analysis

Traditional measurements and counts were made on the left side of the body following Fink and Weitzman (1974) in *strict sensu* with further modifications incorporated by Mattox *et al*. (2006) and Rosso *et al*. (2018). A total of 35 counts (14 for pores, 7 for scales, 5 for teeth and fin-rays, 3 for gill rakers and 1 for bands in anal fin) were obtained by either visual or microscope inspection. Linear body measurements (standard length and 22 additional measurements; see table 1) were taken with a digital caliper to the nearest 0.01 mm. Vertebrae counts, including the anterior four vertebrae of the Weberian apparatus, were performed on radiographed specimens. Counts of the holotype are denoted by an asterisk.

Examined material are deposited in the following Institutions: BMNH: Natural History Museum, London, U.K.; CFA-IC: Fundación de História Natural Félix de Azara, Universidad Maimónides, Buenos Aires, Argentina; CI-FML: Fundación Miguel Lillo, San Miguel de Tucumán, Argentina; INPA: Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil; LBP: Laboratório de Biologia e Genética de Peixes, Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de Botucatu, São Paulo, Brazil; LGEP: Laboratorio de Genética Evolutiva-Peces, Posadas, Argentina; MHNN: Museum d'Histoire Naturelle de Neuchâtel, Neuchâtel, Switzerland; MLP: Museo de La Plata, Instituto de Limnologia, La Plata, Argentina; MNHN: Muséum National d'Histoire Naturelle, Systématique et Évolution, Laboratoire d'Ichthyologie Générale et Appliquée, Paris, France; UFOPA: Universidade Federal do Oeste do Pará, Coleção Ictiológica, Instituto de Ciências e Tecnologia das Águas, Santarém, Brazil; UNMDP: Instituto de Investigaciones Marinas y Costeras, Universidad Nacional de Mar del Plata,

Argentina; ZMB: Museumfür Naturkunde, Leibniz-Institutfür Evolutionsund Biodiversitätsforschung, Berlin, Germany; Institutional abbreviations follow Fricke & Eschmeyer (2021).

Morphometric analysis

In order to characterize body shape variation among different species within the *H. malabaricus* complex, a geometric morphometric approach based on interlandmarks distances (Ild) was conducted according to González-Castro *et al*. (2016) and González-Castro and Ghasemzadeh (2016). Twenty-three morphometric variables were taken as Ild over the left side of the specimens, employing a digital calliper (0.05-mm precision). These variables were based on 11 anatomical landmarks obtained by a truss network following the protocol designed in Rosso *et al*. (2018) (Figure 1). Additional comparative morphometric data of the recently described species *H. mbigua* (n=8), *H. misionera* (n=21) and *H. argentinensis* (n=19), obtained from Rosso *et al*. (2018) were included in order to compare and characterize the new species under description. Six new specimens (CFA-IC 45600; CFA-IC 10451 (2); CFA-IC 34; CFA-IC 3083; CFA-IC 1783) of *H. mbigua* (a total of n= 14) were measured for this study.

The morphometric characters were organized according to the *H. malabaricus* species group. A normalization technique to scale the data that exhibit an allometric growth was used according to Lleonart *et al*. (2000). The standard length (SL) was employed as the independent variable and the remaining Ild were considered as dependent variables. For this work, SL0 represents a reference value (170 mm) to which all individuals were reduced (or amplified). This transformation scales the data that exhibit allometric growth (Lleonart *et al*., 2000). After transformation, a new matrix was constructed containing the corrected matrices for each species, and a Principal Component Analysis (PCA) was performed using MULTIVARIADO software (Salomón *et al*., 2004). The principal components scores (PCs) obtained were submitted to cross-validated discriminant analysis (DA) using SPSS v.13.0, in order to build a predictive model of group membership based on observed characteristics of each case.

Molecular data

Prior to preservation of specimens, we collected tissue samples from epaxial muscle in most specimens (n=15) composing the type material (see supplementary table for details) that were preserved in absolute ethanol and frozen at -20°C until the molecular analysis. Genetic analyses were conducted using the *Cytochrome c oxidase subunit I* (COI) gene as a molecular marker. Genomic DNA was extracted from the holotype and 14 paratypes using the Salting out protocol (Aljanabi and

Martinez, 1997) adapted by Vitorino *et al*. (2015). The fragment of COI was amplified by polymerase chain reaction (PCR) using FishF1 and FishR1 primers (Ward *et al*., 2005). The reactions were performed according to Guimarães *et al*. (2018). The DNA barcoding sequences were obtained through the capillary sequencing by di-desoxiterminal reaction (Sanger method) using ABI PRISM BigDye Terminator V.3 Cycle Sequencing kit and the genetic analyzer ABI3500 (Applied Biosystems).

The sequences were aligned using the ClustalW Algorithm (Thompson *et al*., 1994) implemented in the software Bioedit (Hall, 1999). All sequences and specimen metadata were assembled to Barcode of Life Database Systems (BOLD) - http://www.boldsystems.org (Ratnasingham and Hebert, 2007), linked to the Project - "Amazonian Trahiras" (AMTRA). In order to enrich the molecular database with comparative material, we downloaded from BOLD supplementary sequences of the following taxa: *H. argentinensis* (n=15), *H. malabaricus* (n=20), *H. mbigua* (n=15), *H. microlepis* (n=7), *H. misionera* (n=15) and *H. lacerdae* (n=2) (S1).

Molecular species delimitation

To identify the species based on DNA barcoding sequences we applied four species delimitation approaches: 1) Barcode Index Number (BIN) (Ratnasingham and Hebert, 2013); 2) Automatic Barcode Gap Discovery (ABGD) (Puillandre *et al*., 2012); 3) Assemble Species by Automatic Partitioning (ASAP) (Puillandre *et al*., 2021) and 4) Generalized Mixed Yule Coalescent (GMYC) (Pons *et al*., 2006; Fujisawa and Barraclough, 2013).

The BIN is an online tool implemented in the BOLD System workbench (www.boldsystems.org) that uses the algorithm RESL (Refined Single Linkage Analysis) to find clusters of DNA barcodes from entry data and the BOLD archived library (Ratnasingham and Hebert, 2013). This procedure assigns a tag (BIN identification) for the clusters recovered and assumed as an Operational Taxonomic Unit (OTU) that can be considered putative species based on a maximum intra-cluster distance threshold. The maximum intra-cluster distance is prefixed to 2.2%, if a new sequence diverges from any existing cluster in more than twice the threshold (>4.4%) it is assigned as the founder of a new cluster (Ratnasingham and Hebert, 2013).

We used the ABGD method to explore the existence of discontinuities within the pairwise distance distributions plots (barcoding gap) as an evidence of species boundary (Puillandre *et al*., 2012). This analysis was implemented with the ABGD online platform - bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html, starting with a distance matrix generated with MEGA X (Kumar *et al*., 2018) and setting parameters for K80 model, intraspecific divergence min. 0.001, max. 0.1 and barcoding gap as default (X=1.5).

The ASAP method is used to build species partitions from single-locus sequence alignments and forms a hierarchical clustering and merging sequences into "groups" that are successively further merged until all sequences form a single group. The probability and barcode gap width are combined into a single asap-score that is used to rank the partitions (Puillandre *et al*., 2021). For this method we used the web-interface https://bioinfo.mnhn.fr/abi/public/asap.

For the GMYC method we removed the repeated haplotypes and constructed an ultrametric tree processed in BEAST v1.8.0 (Drummond and Rambaut, 2007), based on: HKY evolution model, Gamma distribution (G) + proportion of invariable sites (I) chosen with jModelTest (Posada, 2008), molecular clock lognormal relaxed and Yule process speciation, with 100 million MCMC iterations and sampled at each 1000 iterations with 10% burn-in. The convergence and stability were checked with the software Tracer v.1.7.1 and retained for Effective Sample Sizes (ESS) > 200 (Drummond and Rambaut, 2007). The resulting trees were combined with TreeAnotator v1.8.0 and saved as a Newick file (Drummond and Rambaut, 2007). To test the branching events for speciation and coalescence null hypothesis we used the packages Splits (Species Limits by Threshold Statistics) (Ezard *et al*., 2017) and Ape (Analyses of Phylogenetics and Evolution) (Paradis and Schliep, 2018), with the single threshold model implemented in R 3.4.0 statistical software (R Core Team, 2014).

As additional evidence for species delimitation, genetic divergences among species were estimated. A neighbor-joining (NJ) tree of Kimura two-parameter (K2P) distances (Kimura, 1980) was created using the software MEGA X (Kumar *et al*., 2018), that was configured using FigTree v.1.2.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

Hoplias auri, new species

385F9898-0094-4C04-99A7-A80379DD4CBB

(Figure 2, Table 1)

Hoplias malabaricus (non Bloch 1794). Silva (2017); Guimarães (2020): 44-45, 51 (Brazil, Pará, Crepori River; supported by distinct molecular methods as a putative new undescribed species in the *H. malabaricus* complex).

Holotype. UFOPA - I 1353, 229 mm SL, Brazil, Pará State, Tapajós basin: Crepori River, Itaituba region, Creporizão District: Lago do Sr. Pena, 6°50'1.30"S, 56°50'50.90"W, L. Rodrigues, J. Santos, C. Silva, M. Brito, 21 July 2016.

P**aratypes.** All from Brazil, Pará State, Tapajós basin: Crepori River, Itaituba region, Creporizão Village: Lago do Sr. Pena, 6°50'1.30"S, 56°50'50.90"W, UFOPA - I 1353, 3, 159-202 mm SL, L. Rodrigues, J. Santos, C. Silva, M. Brito, 20-21 July 2016, UNMDP 5206, 1, 163 mm SL, L. Rodrigues, J. Santos, C. Silva, M. Brito, 20 July 2016, Lago Creporizão, 6°49'11.56"S, 56°51'4.52"W, L. Rodrigues, J. Santos, C. Silva, M. Brito, 21 July 2016, UFOPA - I 1355, 2, 153-154 mm SL, Igarapé da Sra. Maria Brito, 6°49'12.50"S, 56°50'52.80"W, L. Rodrigues, J. Santos, C. Silva, M. Brito, 20 July 2016: UFOPA - I 1354, 8, 138-321 mm SL, UNMDP 5204, 1, 245 mm SL, UNMDP 5205, 1, 194 mm SL, Igarapé Creporizão, 6°49'09''S, 56°51'31''W, C. Duarte, 30 April 2008: INPA 32732, 2, 30.82-44.28 mm SL.

Allocation to species group. The *Hoplias auri* belongs to the *H. malabaricus* species-group based on (1) morphological characteristics such as: medial margins of dentaries converging in a "V" or "Y" shape towards the mandibular symphysis; presence of tooth plates on the basihial and basibranchial bones, four pores along the dentary latero-sensory system, presence of the accessory ectopterygoid bone and the absence of an oval dark spot in the opercular membrane and (2) closest molecular phylogenetic affinity with *H. malabaricus* species-group (3.0 – 8.3% interspecific distances).

Diagnosis. *Hoplias auri* is distinguished from other species of the *H. malabaricus* group by the following combination of characters: 15-16 predorsal scales, 37-39 lateral-line scales, 5 scales from dorsal fin to lateral line, 38-39 vertebrae, iii-iv unbranched anal-fin rays, 12-15 branched caudal-fin rays, last vertical series of scales on the base of caudal-fin rays forming a straight line and 6-7 dark bands in anal fin.

The number of predorsal scales (15-16) distinguishes *H. auri* from *H. argentinensis* (17-19), *H. microlepis* (17-19) and *H. teres* (18 in one paratype). The number of lateral-line scales (37-39) and vertebrae (38-39) distinguishes *H. auri* from *H. argentinensis* (41-44 and 42-43 respectively), *H. teres* (40-41 and 42 respectively), *H. microlepis* (43-46 and 42-43 respectively) and *H. mbigua* (41-44 and 42 respectively). *Hoplias auri* differs from *H. misionera* in the last vertical series of scales on the base of caudal-fin rays forming a straight line (vs. a curved line) and body depth (16.23-22.95, mean 20 vs. 20.6-26.47, mean 23.74). Body depth also helps to discriminate *H. auri* from *H. argentinensis* (21.7-25.59, mean 23.34). *Hoplias auri* differs from *H. mbigua* by dorsal profile of head straight or slightly convex (vs. concave), pre-pectoral (27.91-32.89, mean 30.28 vs. 25.8-28.9, mean 27.4) and pre-pelvic (53.14-58.81, mean 55.49 vs. 46.3-54.7, mean 51.7) lengths. A longer (snout length 23.38-27.31 vs. 21.95-23.07) and narrow (snout width 21.43-24.94 vs. 29.45-29.49) snout discriminates *H. auri* from

H. teres. *Hoplias auri* is most similar to *H. malabaricus* differing by snout width (21.43-24.94, mean 23.07 vs. 24.51-31.03, mean 27.95), number of vertebrae (38-39 vs. 39-41), unbranched anal (iii-iv vs. ii) and branched (Figure 3) caudal (12-15 vs. 14-15) fin-rays counts. *Hoplias auri* can be also characterized by color patterns. The conspicuous dark longitudinal band commonly observed in *H. mbigua* and *H. microlepis* is lacking (Figure 4). Bands in anal fin discriminate from *H. mbigua* (4-5), *H. misionera* (3-5), *H. microlepis* (5-6) and *H. auri* (6 n=6 or 7 n=10) (Figure 5a-d).

Description. Morphometric data are summarized in Table 1. Body cylindrical. Dorsal profile of head straight or slightly convex. Anterior profile of head angular to slightly rounded in lateral view. Greatest body depth at dorsal-fin origin. Medial margins of contralateral dentaries converging to midline forming a V or Y-shaped angle. Lower and upper lips fleshy. Anterior nostril with incomplete tubular skin flap covering to entire opening. Posterior nostril without fleshy flap and equidistant to anterior nostril and anterior bony margin of orbit. Infraorbitals 3 and 4 completely excluded from orbital ring or infraorbital 4 tip reaching the orbit (n=4; 154, 159, 163 and 212 mm SL). Teeth caniniform in both jaws. Single premaxillary tooth row with 8 (2), 9* (10) or 10 (4) teeth. First two medial teeth large, followed by 3 to 4 smaller teeth, and other two large canines. First and last canines in this series are the largest. One or two small teeth posterior to the last largest canine and almost in contact with the small first maxillary tooth. Maxillary row with 4 to 6 first teeth increasing progressively in size followed by 35-44 small teeth. Dentary external series with one symphyseal tooth followed by 2 or 3 smaller teeth and another tooth as large as symphyseal tooth followed by the largest dentary canine. Posteriorly, a series of 4-6 small teeth followed by 5-10 teeth arranged in a repetitive series of one large and one or two small conic teeth. Internal series of dentary with 15 very small conical teeth immediately posterior or slightly anterior to the last conical tooth of the external row. Accessory ectopterygoid not fragmented, bearing 10 (2) or 11 (5) conical teeth along its ventrolateral margin.

Dorsal-fin origin placed at midbody 2-3 scales anterior to vertical through pelvic fin origin. Dorsal-fin rays ii-11 (1), ii-12 (4) or iii-11* (11). Tip of longest ray of depressed dorsal fin extending at or slightly beyond vertical through anus. Anal-fin rays iii-7* (3), iii-8 (8) or iv-7 (5). Pectoral-fin rays i-12* (9) or i, 13 (7). Pelvic-fin rays i-7* (16). Total caudal-fin rays 16 (ii-12-ii (2); i-13-ii (1); i-14-i (1)) or 17 (ii-13-ii (1); ii-14-i* (5); i-15-i (5)). The tip of pectoral fin separated from pelvic-fin origin by 4-6 scales. Tip of pelvic fin separated from vertical through anus by 2-5 scales. Predorsal scales 15 (9) or 16* (7) in irregular series.

Lateral line with 37* (5), 38 (7) or 39 (4) perforated scales, with 1 (1) or 2* (15) unperforated scales beneath the opercle membrane. Longitudinal series of scales between dorsal-fin origin and lateral line 5; longitudinal series of scales between lateral line and pelvic-fin origin 4*-5. Longitudinal series of

scales around caudal peduncle 20. First epibranchial with 9*-11 gill rakers. The first one in this series is somewhat elongated. One elongated angular raker. Ceratobranchial with 5 elongated and 10 plate-like denticulate rakers. Latero-sensory canal along ventral surface of dentary with four (three specimens with 5 pores on one dentary) pores. Latero-sensory canal with 6 pores in preopercle. Latero-sensory canal along infraorbitals (Figure 6) with 10 (4), 11 (7), 12* (3) or 13 (2) pores. Infraorbital 1: 2-4 pores, infraorbital 2: 2-3 pores, infraorbital 3: 1-2 pores, infraorbital 4: 1 pore, infraorbital 5 lacking pores and infraorbital 6 with 2-4 pores. Latero-sensory system of dorsal surface of head (Figure 7) with 10-11* pores. Nasal bone: 2 pores, frontal bone: 4 pores, pterotic bone: 1-2* pores. One pore between parietal bones, on the posterior end of suture. The following combination of pores (Figure 8) in supraopercle: extra-escapular bones: 0-2* (13) or 1-1 (3). Total vertebrae 38-39*.

Color in alcohol. Background coloration brown and light brown, darker at dorsum. Ventral surface white or pale-yellowish. Scales on dorsal half of body with scattered dark brown melanophores. Scales on ventral half of body with a dark vertical blotch on their left margins, sometimes expanding and covering the half surface of scales; more noticeably in paler individuals. Lateral chevron-shaped blotches, common in *Hoplias malabaricus* species group, inconspicuous, hardly visible in some specimens. Some specimens with heads completely marbled, others with marbled snouts. Four dark stripes radiating posteriorly from eye along infraorbital 2, 3, 6 and between infraorbitals 4 and 5. The latter is the shortest. A wide dark posterodorsally blotch along the joint between opercle and subopercle bones. In those specimens where chevron lateral blotches are more discernible (less than 200 mm SL), the dark elliptical or slightly rounded spot on dorsal part of caudal peduncle fusing with the dorsal eroventrally branch of the last chevron lateral blotch. Latero-ventral surface of dentaries with transverse brown bands or blotches. These bands are sometimes visible on maxilla and lips. All fins light cream to light brown, with dark spots on rays and interradial membranes. Spots in anal fin larger, forming six or seven dark bands.

Etymology. The specific epithet "*auri*" is used as a noun, comes from the Latin (*auri* = gold) and refers to locality that is in an area for gold mineral extraction.

Distribution and habitat. *Hoplias auri* has a restricted distribution in the Crepori River and tributaries (Figure 9). The Crepori River is a medium sized tributary of the Tapajós River in the Amazon Basin with a drainage area of roughly 50.000 km^2 and a mean discharge of 700 m^3/s (Telmer *et al*., 2006). Its headwater is located in the central portion of Tapajós basin, on the border between the municipalities of Itaituba and Jacareacanga and flows northward to meet the Marupá River, where the Creporizão village is located, and continues until it meets the Tapajós River, roughly 250 km south of

Itaituba Municipality. The main channel and tributaries of the Crepori River presented muddy waters due to the intensive gold mining on their banks. Particularly, *H. auri* was collected in three sites at the Creporizão village. Site 1, Lago do Sr. Pena (Figure 10a), is an artificial lake resulting from a stream impoundment, situated aside of a landing strip, where the marginal vegetation was suppressed and a narrow strip of riparian vegetation has been preserved along the course of the stream. This lake has clear waters and is used for recreation and possibly fish farming. During the fieldwork in this site, we recorded small catfish (*Pimelodella* sp.), jejú (*Hoplerythrinus unitaeniatus*), piau (*Leporinus* sp.), acará (*Aequidens* sp.) and the exotic species tilapia (*Oreochromis niloticus*). Site 2, Igarapé da Sra. Maria Brito (Figure 10b), is a severely disturbed water body that flows into the center of the village. This area is characterized by a stripe of flooded lowland covered by shrub vegetation and aquatic macrophytes. Water is turbid due to suspended particles that are loaded from the surroundings. Such surroundings function as residence dumping areas, so loads of residuals and contaminants may be carried to the water body. In this site we observed many tiny fish of the family Characidae (piabas). Site 3, Lago Creporizão (Figure 10c) is a marginal lagoon with a round shape that resulted from an abandoned digging. It has sporadic connection with the river main channel during the high water periods. The water is turbid and grasses, aquatic plants and small trees are present in the riparian vegetation. All the sampling sites present reduced riparian vegetation, with slow-flowing waters and substrates composed of alluvial, sandy and clayey sediments.

Conservation issues. The aquatic environments in the type locality and surroundings in the Crepori River have been disturbed by gold mining, logging and cattle breeding. Consequently, the Crepori River was altered due to silting, channel deviation and water contamination, which in turn, can lead to harmful effects on fish assemblage. The Tapajós River basin has a long history of aquatic contamination by mercury, linked to artisanal gold extraction, and the mercury bioaccumulation in its fish species is well-documented (see: Akagi *et al*., 1995; Nevado *et al*., 2010; Sampaio da Silva *et al*., 2013; Castilhos *et al*., 2015). A preliminary assessment of mercury exposure showed that *H. auri* had levels of mercury up to the legal limits stated by the World Health Organization (WHO) and that was much higher than levels observed in *H. malabaricus* from Santarém, at lower Tapajós River (Silva, 2017). Additionally, significant increase of DNA lesions observed in *H. auri* is likely caused by mercury contamination (Silva, 2017). Contamination of freshwater ecosystems by heavy metals is widely known to cause biological damage to fish (Porto *et al*., 2005; Mela *et al*., 2007; Vicari *et al*., 2012). *Hoplias auri* inhabits waters classified as unsuitable for ichthyofauna (ICMBio, 2009) due to a high level of anthropic pressure and environmental degradation. *Hoplias auri* is only known from four locations in the Crepori River, with an Extent of Occurrence (EOO) of 0.882 km^2 and Area of

Occupancy (AOO) of 8.000 km^2. Considering the small EOO/AOO and the increase in threats that may put *H. auri* populations at risk, we recommend to be considered Critically Endangered (CR) in agreement with the B1ab(iii) + B2ab(iii) IUCN's criteria [International Union for Conservation of Nature (IUCN), 2020]. Further studies along the Crepori river basin are recommended to more clearly define the geographic distribution and conservation status of *H. auri*.

Morphometric analysis

The 23 normalized interlandmark distances, which were analyzed by PCA of the correlation matrix, produced six eigenvalues greater than one (6.24745; 4.95827; 2.23676; 1.56763; 1.51916 and 1.17899). The first three PCs explained more than 58% of the variance in the data. Only correlations (between variables and components) higher than 0.5 were taken as significant (Table 2). PCA based on IlD allowed graphic segregation of the four species groups analyzed. The multivariate ordination provided by the first two principal components allowed graphic segregation of *H. auri*, *H. argentinensis*, *H. misionera* and *H. mbigua*, almost without overlapping between them (Figure 11). *Hoplias auri* was basically located in the second quadrant (Figure 11), being characterized by the higher loadings for the 1-2, 1-3, 1-4, 2-3 and 2-4 ILDs and 3-4, 4-5 (in minor way). These variables represent the head shape (Table 2) and in particular 1-2 can be correlated to the snout, denoting *H. auri* possesses a long snout when compared with *H. mbigua* and *H. argentinensis*. Moreover, *H. auri* was defined by the lowest loadings for the 3-6, 4-6, 5-6 (variables that represents the relationship between the posterior part of the head and the origin of the dorsal fin) but also lowest values for 5-8, 6-7, 7-8 IlDs (belonging to the third box-truss and related to the dorsal-fin base and origins of pectoral and ventral fins) (Figure 1). Conversely, *H. argentinensis* was located in the fourth quadrant, being characterized by the highest loadings for the 3-6, 4-6 and 5-6 IlDs, higher loadings for 5-8, 6-7 and 7-8 and the lowest values for 1-2, 1-3, 1-4, 2-3, and 2-4 IlDs (Figure 11). *Hoplias misionera* basically plotted on the first quadrant and showed the highest loadings for the 3-4 and 4-5 IlDs and higher loadings for the 1-2, 1-3, 1-4, 2-3, and 2-4, IlDs. At last, *H. mbigua* showed the highest loadings for the 8-10 IlDs (which represents the distance between the insertion of the dorsal fin and the first dorsal caudal fin ray insertion) (PC1-PC3, data not shown). This species also showed the lowest values for the 3-4 and 4-5 IlDs and lower values for 1-2, 1-3, 2-3, 1-4, 2-4 but also 5-6, 5-8, 6-7 and 7-8 (Figure 11).

The data corresponding to the 23 PCs of the PCA were employed to perform the Discriminant Analysis. DA for the 70 individuals of *Hoplias* produced three significant canonical discrimination functions, where the first two explained 86.4% of the total variance in the data, (Wilks'- lambda =

0.003, P < 0.0001). Four groups were defined, and their centroids and individuals were completely separated on both the 1st and 2nd discriminant functions (Figure 12). The DA correctly classified 100% of the *Hoplias* individuals according to the species groups defined a priori, whereas the cross-validated analysis correctly classified 95.7 of the fish according to their body shape (Table 3). Accordingly, group misclassifications were scarce, with a highest rate of 6.25% of *H. auri* misclassified as *H. mbigua* (Table 3). Four groups were clearly defined, according to those defined a priori, and their centroids and individuals were separated both on the first and second discriminant functions (Figure 12).

Molecular species delimitation

The DNA barcode sequences resulted in an alignment of 651 nucleotides, after to be trimmed to remove the residuals in the tips. No indels or stop codons along the reads were observed. The nucleotide base composition was T (30.3%), C (28.2%), A (23.2%), G (18.3%). The BIN analysis revealed that all the individuals collected from the Crepori River were clustered in a single clade and established a new BIN (ADL3159), which is described herein as the new species, *H. auri*. This BIN was closer to *H. malabaricus* species-group than to *H. lacerdae* and its nearest-neighbor (NN) was *H. malabaricus* (ABZ3047) with genetic distance of 3.0% followed by *H. mbigua* (ACO5223) distantly 3.2% (Table 4). *Hoplias auri* was most divergent (7.7%) from *H. argentinensis* (AAZ3734) within the *H. malabaricus* species-group and far distant (15.0%) from *H. lacerdae* (ABW2258). The mean intraspecific genetic distance of *H. auri* was 0.001%. The specific status of the BIN ADL3159 – *H. auri* was also demonstrated with evidence from barcoding gap analysis (ABGD, ASAP) and from coalescence simulation (GMYC) (Figure 13).

The mtDNA COI barcode profile of the holotype of *H. auri* is deposited in BOLD Systems (Process ID AMTRA115-18) and was allocated to the BIN: BOLD:ADL3159.

Discussion

Integrative taxonomy has contributed in recent years to unravel and describe a large number of cryptic species of fishes (*e.g*. Melo *et al*., 2016, Allen *et al*., 2016, Guimarães *et al*., 2019; Faria *et al*., 2021). *Hoplias auri* is formally described herein and has been previously reported as *H. malabaricus* (Silva, 2017) and assumed as a putative undescribed new species from the *H. malabaricus* species-group, based on DNA barcoding distances (Guimarães, 2020). Herein, a combination of

morphological, morphometric, meristic, molecular and morphogeometric evidences supports the evolutionary independence of *H. auri* as a single specific lineage.

The mean interspecific COI divergence of *H. auri* to others members of *H. malabaricus* species-group varied from 3.0 to 7.7%, which is in agreement with previous divergence values observed among *H. malabaricus* species-group members (Cardoso *et al*., 2018). The distances between *H. auri* and its NN *H. malabaricus* (3.0%) and *H. mbigua* (3.2%) are the smallest in the species pairs of the *H. malabaricus* group. Conversely, NN distances values of 9.08% and 5.61% separated *H. auri* from *H. argentinensis* (Rosso *et al*., 2018) and *H. misionera* (Rosso *et al*., 2016). Altogether, the clustering of all individuals of the new species in a single BIN (ADL3159) and the monophyly of this cluster supported with barcoding gap (ABGD, ASAP) and Bayesian inference of the evolutionary lineage history (GMYC) are strong molecular evidences to the distinctiveness of *H. auri*. With the available molecular evidence, it could be assumed that speciation in the *H. malabaricus* complex may be driven under DNA barcoding divergences below to 3.5%.

Traditional morphometrics are very conservative among species of the *H. malabaricus* complex where only few informative measures can be outlined (Rosso *et al*., 2016; 2018). Indeed, no discrete ranges of traditional morphometrics were able to discriminate *H. auri* from known species of this group. Conversely, the application of Box-Truss protocols based on anatomical landmarks has been crucial to solve taxonomic conflicts on fishes, at the morphotypes, populations and species levels (González-Castro *et al*., 2008, 2012, 2016; Díaz de Astarloa *et al*., 2011; González-Castro and Díaz de Astarloa, 2017). Particularly, this morphogeometric approach has proved to be effective regarding the characterization of the shape and discrimination among several species of *Hoplias* (Rosso *et al*., 2018). In this scenario, *H. auri* was characterized by having a long snout (1-2 IlD) and head (1-2, 1-3, 2-3, 1-4, 2-4 and 3-4 IlDs), dorsal fin closer to the head (lower loadings of 3-6, 4-6, 5-6 IlDs) and slender body at its mid-part (Box-Truss III; lower loadings of 5-6, 5-8, 6-7, 7.8 IlDs).

The cumulative knowledge about taxonomy of the *H. malabaricus* group during the last years allows revising some characters proposed in original diagnoses. The Y-shaped disposition of medial margins of dentaries was formerly claimed to be present solely in *H. misionera* (Rosso *et al*., 2016). Later, revision of specimens of this species from different basins from those belonging to the type description (Guimaraes *et al*., 2021) allowed recognizing that this character was variable where some specimens of *H. misionera* also presented the characteristic V-shaped disposition of the *H. malabaricus* group. Here, a new species of the *H. malabaricus* group is presented with its specimens presenting either a V-shaped or Y-shaped disposition. These results demonstrate that the disposition of medial margins of dentaries lacks taxonomic value other than discriminating species of the *H. malabaricus* group (V or Y-shaped) from *H. lacerdae* group (parallel) as early proposed (Oyakawa,

1990). Similarly, the conspicuous brown bands observed in dentaries of *H. mbigua* (Azpelicueta *et al*., 2015) can be also found in some specimens of *H. microlepis*, *H. misionera* and *H. auri*.

Overall, taxonomic identification of species from the *H. malabaricus* group can be firstly approached by the disposition of the last series of vertical scales on the caudal-fin base. This character discriminates *H. misionera*, with a marked curve disposition of these scales, from all the remaining species. These species with a nearly straight disposition of these scales can be further discriminated by means of meristic counts in two groups: the low count group and the high count group (Rosso *et al*., 2018). Interestingly, *H. misionera* that occupied a basal position in the COI-based phylogeny of this group (Cardoso *et al*., 2018) covers almost the entire range of lateral line scales (38-43) of both groups (37-40 in low count and 40-46 in high count). For details see the key provided.

Key to the species of the *Hoplias malabaricus* group

1a Last vertical series of scales on caudal-fin base curved….....*Hoplias misionera*
1b Last vertical series of scales on caudal-fin base nearly straight…….....2

2a Thirty-seven to 40 scales on the lateral line, 38–41 vertebrae (low count group *sensu* Rosso *et al*., 2018)……....……....…….....….3
2b Forty to 46 scales on the lateral line, 42–43 vertebrae (high count group *sensu* Rosso *et al*., 2018) ……....……....…….4

3a Snout width 21.71-24.93, mean 23.31, iii-iv unbranched anal fin rays, ii-iv, 12-15 caudal-fin rays and 38-39 vertebrae..…….……*H. auri* sp. nov.
3b Snout width 24.51-31.03, mean 27.95, ii unbranched anal fin rays, ii,14-15 caudal-fin rays and 39–41 vertebrae……..……....*H. malabaricus*

4a Twenty-two to 24 scales around caudal peduncle……..…….……...… *H. microlepis*
4b Twenty scales around caudal peduncle ……..…….……..……..5

5a Five distinctive transverse brown bands in the lower jaw, dorsal profile of head concave.....*H. mbigua*
5b No distinctive transverse brown bands in the lower jaw, dorsal profile of head straight..…..6

6a Forty-one to 44 scales on the lateral line, snout width less than 25 % of head length.....*H. argentinensis*
6b Forty or 41 scales on the lateral line, snout width more than 29 % of head length.....*H. teres*

Comparative material

Hoplias aimara: French Guiana: MNHN A-9968 (dry mount), 1, 770 mm SL, holotype; Cayenne.

Hoplias argentinensis: All from Argentina. Holotype: UNMDP 4417, 302 mm SL. Santa Fe Province: Río Paraná Basin: Río Coronda, 31°50.1'S 60°51.48'W; J. J. Rosso, E. Mabragaña & M. González-Castro, 3 Dec 2015. Paratypes: Buenos Aires Province: UNMDP 492, 1, 410 mm SL;

UNMDP 502, 1, 170 mm SL; UNMDP 503, 1, 145 mm SL; and UNMDP 504, 1, 159 mm SL; Ascensión: Río Paraná Basin: Río Rojas; J. J. Rosso *et al*., 10 Dec 2010. — CFA-IC 3825, 1, 215 mm SL; Junín: Laguna Gómez; J. R. Miranda *et al*., 30 Sep 2014. — CFA-IC 4364, 2, 125–188 mm SL; Río de La Plata, Punta Indio wetlands; S. Bogan, 22 Mar 2015. — CFA-IC 4355, 1, 140 mm SL; Río Paraná Basin, Cañada Arias; S. Bogan, 21 Mar 2015. — CFA-IC 1741, 1, 138 mm SL; Arroyo El Destino; L. Protogino *et al*., 19 Jan 2007. — CFA-IC 2452, 1, 105 mm SL; Río Paraná; J. M. Meluso & S. Bogan, 4 Feb 2013. — CFA-IC 4665, 1, 315 mm SL; Río de La Plata; J. Meluso *et al*., 6 Jul 2015. — MLP 6586, 1, 202 mm SL; Punta Lara; M. Galván & E. Martín, 27 Jun 1960. Entre Ríos Province: UNMDP 1279, 1, 240 mm SL; Embalse Salto Grande; J. J. Rosso & E. Mabragaña, 12 Sep 2011. — UNMDP 1370, 1, 309 mm SL and UNMDP 1371, 1, 265 mm SL; Río Paraná-Guazú, Delta of Río Paraná; J. J. Rosso *et al*., 7 Oct 2011. — UNMDP 1595, 1, 98 mm SL; Arroyo Bergara; J. J. Rosso & E. Mabragaña, 9 Sep 2011. — UNMDP 2452, 1, 202 mm SL and UNMDP 2453, 1, 203 mm SL; Río Paraná basin: Laguna El Pescado: J. J. Rosso & E. Mabragaña, 11 Nov 2012. — UNMDP 2565, 1, 134 mm SL; Río Paraná Basin: Arroyo Nogoyá; J. J. Rosso & E. Mabragaña, 10 Nov 2012. — UNMDP 2616, 1, 116 mm SL; Río Uruguay Basin: Arroyo Ayuí; J. J. Rosso & E. Mabragaña, 14 Nov 2012. — CFA-IC-3480, 1, 116 mm SL; Arroyo Urquiza; A. Miquelarena *et al*., 18 Nov 2005. — CFA-IC 5812, 1, 190 mm SL; Arroyo El Tigre; H. López *et al*., 17 Aug 2010. Santa Fé Province: Río Paraná Basin: UNMDP 3867, 1, 177 mm SL; Arroyo Leyes; J. J. Rosso *et al*., 25 Apr 2015. — UNMDP 4416, 1, 342 mm SL; UNMDP 4423, 1, 239 mm SL; UNMDP 4425, 1, 203 mm SL; UNMDP 4426, 1, 206 mm SL; UNMDP 4427, 1, 314 mm SL; and UNMDP 4428, 1, 351 mm SL; Río Coronda: collected with the holotype. — CFA-IC-3976, 1, 220 mm SL; Río Carcaraña; Y. P. Cardoso *et al*., 24 Nov 2014. Córdoba Province: MLP 11302, 1, 87 mm SL; Río Primero, before Capilla de los Remedios; 24 Jul 1939. Santiago del Estero Province: — CFA-IC 5537, 1, 134 mm SL; Embalse Tacañitas, J. Montoya-Burgos *et al*., 8 Nov 2015. — CFA-IC 5519, 2, 124–165 mm SL; Río Dulce; J. Montoya-Burgos *et al*., 8 Nov 2015. Tucumán Province: — CFA-IC 5655, 1, 90 mm SL; Río Vipos; J. Montoya-Burgos *et al*., 11 Nov 2015. Misiones Province: Río Uruguay Basin: CFA-IC-4414, 1, 165 mm SL; Arroyo Dorado; S. Bogan & J. M. Meluso, 4 May 2015. — UNMDP 4837, 1, 176 mm SL; Arroyo Fortaleza; J. J. Rosso *et al*., 8 Mar 2017.

Hoplias australis: Argentina: Misiones: UNMDP 1991, 1, 43.9 mm SL; Río Uruguay Basin: Arroyo Ramos. — UNMDP 2721, 1, 271 mm SL; UNMDP 2722, 1, 220 mm SL; UNMDP 2723, 1, 171 mm SL; and UNMDP 2724, 1, 166 mm SL; Río Yabotí Basin: Arroyo Oveja Negra.

Hoplias curupira: Brazil: Pará State: LBP 67349, 1, 153 mm SL; Itaituba: Rio Tapajós.

Hoplias intermedius: Brazil: Sergipe State: LBP 48702, 1, 231 mm SL; Gararu: Rio São Francisco.

Hoplias lacerdae: Argentina: Misiones: Río Uruguay Basin: UNMDP 570, 1, 346 mm SL; UNMDP 571, 1, 350 mm SL; and UNMDP 594, 1, 163 mm SL; Arroyo Ramos. — UNMDP 2725, 1, 192 mm SL and UNMDP 2735, 1, 244 mm SL; Río Yabotí.

Hoplias malabaricus: MHNN 773, 1, holotype of *Erythrinus macrodon*; Brazil: Bahia: Lake Almada; photograph and x-rays. — MNHN 4409, 1, 108 mm SL; MNHN 4421, 3, 175-237 mm SL; MNHN A-9746, 1, 93 mm SL; MNHN A-9747, 1, 183 mm SL; and MNHN A-9748, 1, 245 mm SL; syntypes of *Macrodon tareira*; Brazil and French Guiana.— USNM 1112, 1, 111 mm SL, syntype of *Macrodon ferox*; Trinidad Island; photographs and x-rays. — ZMB 3515, 1, 167 mm SL; lectotype; South America, probably Suriname. — ZMB 33059, 1, 69 mm SL; paralectotype; South America, probably Suriname.

Hoplias cf. *malabaricus*: Brazil: Pará State: Tapajós basin: INPA 32735, 1, 168 mm SL, Jacareacanga: Rio Marupá, C. Duarte, 07 May 2008; INPA 32646, 1, 58.8 mm SL, Jacareacanga: Rio Pacu, C. Duarte, 07 Aug 2008; INPA 7001, 1, 240 mm SL, Itaituba: Rio Jamanxim, L. Rapp Py-Daniel & J. Zuanon, 23 Oct, 1991; INPA 34256, 2, 64.14-88.63, Rurópolis: Rio Cupari, F. Ribeiro & W. Pedroza, 18 Sep 2009; Amazonas State: INPA 26158, 2, 62.6-122 mm SL, Rio Barati, L. Rapp Py-Daniel *et al*., 03 Jul 2006.

Hoplias mbigua: CI-FML 6763, 1, 224 mm SL, holotype; Argentina: Misiones: Río Paraná, Nemesio Parma. – CI-FML 6764, 2, 224–248 mm SL; collected with the holotype. — LGE-P 314, 1, 237 mm SL and LGE-P 435, 1, 154 mm SL; Río Paraná, Garupá. — LGE-P 316, 1, 229 mm SL; Río Paraná, mouth of Arroyo Yabebiry. — LGE-P 317, 1, 302 mm SL; Río Paraná, Toma de Agua Eriday.

Hoplias microlepis: BMNH 1864.1.26.221, 1, 278 mm SL, lectotype; Panamá: Río Chagres. — BMNH 1864.1.26.222, 1, 225 mm SL and BMNH 1864.1.26.309, 1, 176 mm SL, paralectotypes; Panamá: Río Chagres. — BMNH.1860.6.16.128, 1, 293 mm SL; and BMNH.1860.6.16.154, 1, 124 mm SL, paralectotypes; Ecuador. — LBP 18503, 1, 215 mm SL; Panamá: Atlantic Drainage: Río Llano Sucio.

Hoplias misionera: Argentina: — UNMDP 574, 1, 164 mm SL, holotype; Misiones: Río Uruguay Basin: stream tributary to Río Acaraguá. — UNMDP 1868, 1, 40 mm SL; UNMDP 1950, 1, 49 mm SL; and UNMDP 1951, 1, 50 mm SL; Formosa: Río Paraguay: Laguna Oca. — UNMDP 1983, 1, 75 mm SL; Chaco: Río Paraná. — UNMDP 3320, 1, 174 mm SL; UNMDP 3391, 1, 149 mm SL; and UNMDP 3392, 1, 104 mm SL; same locality as holotype. — UNMDP 3321, 1, 142 mm SL; and UNMDP 3322, 1, 148 mm SL; Formosa: Río Paraguay: Riacho Saladillo. — UNMDP 3327, 1, 171 mm SL; UNMDP 3328, 1, 146 mm SL; and UNMDP 3329, 1, 134 mm SL; Formosa: Río Paraguay: Riacho Salado. — UNMDP 3371, 1, 154 mm SL; and UNMDP 3376, 1, 165 mm SL; Formosa: Río Paraguay: Riacho Mbiguá. BRAZIL: LBP 32184–32186, 3, 77-155 mm SL; São Paulo: marginal lagoon: Paraná River.

Hoplias teres: MNHN-4377-1, 1, 121 mm SL and MNHN-4377-2, 1, 116 mm SL, syntypes; Venezuela: Lago Maracaibo.

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Supporting Information

S1. Detailed information of DNA barcoding sequences

Contributions

KLAG: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Museum specimens examination, Project administration, Software, Validation, Visualization, Writing-original draft, Writing-review and editing.

JJR: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Museum specimens examination, Resources, Supervision, Validation, Visualization, Writing-original draft, Writing-review and editing.

MGC: Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Writing-original draft, Writing-review and editing.

MFBS: Investigation, Formal analysis, Methodology, Writing-review and editing.

LRRR: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing-original draft, Writing-review and editing.

JMDA: Investigation, Museum specimens examination, Resources, Supervision, Validation, Writing-original draft, Writing-review and editing.

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Figure Legends

Figure 1. Box truss showing interlandmarks distances based on 11 homologous anatomical landmarks collected in the specimens of the four different species of *Hoplias* analyzed. Numbers of box truss in Roman numerals

Figure 2. *Hoplias auri*, UFOPA-I 1353, holotype, 229 mm SL; Brazil: Pará State, Tapajós basin, Crepori River. Scale bar = 1 cm

Figure 3. Caudal rays for *Hoplias auri*: UNMDP 5205, 194 mm SL (a); UFOPA-I 1353, 159 mm SL (b); UFOPA-I 1353, holotype, 229 mm SL (c). Illustrations by: Tauanny Lima. Photos by: Juan J. Rosso

Figure 4. Color patterns in species of the *Hoplias malabaricus* species-group. (a) *H. auri*, UFOPA - I 1353, holotype, 229 mm SL; (b) *H. misionera*, UNMDP 3865, 187 mm SL; (c) *H. argentinensis*, UNMDP 3867, 177 mm SL; (d) *H. mbigua*, UNMDP 4966, 282 mm SL. Scale bars = 1 cm

Figure 5. Bands in anal fin from *Hoplias mbigua* CI-FML 6763 holotype, 223 mm SL (a), *Hoplias misionera* UNMDP 3672, 199 mm SL (b), *Hoplias microlepis* BMNH 1864.1.26.309 paralectotype, 176 mm SL (c) and *Hoplias auri* UNMDP 5204, 245 mm SL (d). Scale bars = 1 cm

Figure 6. Schematic figure depicting latero-sensory canal along infraorbitals of *Hoplias auri* (UFOPA-I 1355 154 mm SL). infraorbital 1 (a); infraorbitals 2-4 (b); infraorbitals 5-6 (c). Arrows point to the location of pores in the corresponding infraorbital bones. Illustration by: Tauanny Lima. Photos by: Juan J. Rosso

Figure 7. Latero-sensory system of dorsal surface of head of *Hoplias auri* (UFOPA-I 1353, 159 mm SL): nasal bone (a); frontal bone (b); pterotic, extra-escapular and supraopercle bones (c); parietal bone (d). Arrows point to the location of pores in the corresponding bones. Illustration by: Tauanny Lima. Photos by: Juan J. Rosso

Figure 8. Combination of pores in pterotic: supraopercle: extra-escapular bones of *Hoplias auri*. UNMDP 5205, 194 mm SL, pterotic 1: extra-escapular 2: supraopercle 0

(a); UFOPA - I 1355, 153 mm SL, pterotic 2: extra-escapular 2: supraopercle 0 (b); UFOPA - I 1354, 212 mm SL, pterotic 2: extra-escapular 1: supraopercle 1 (c). Arrows point to the location of pores in the corresponding bones. Illustrations by: Tauanny Lima. Photos by: Juan J. Rosso

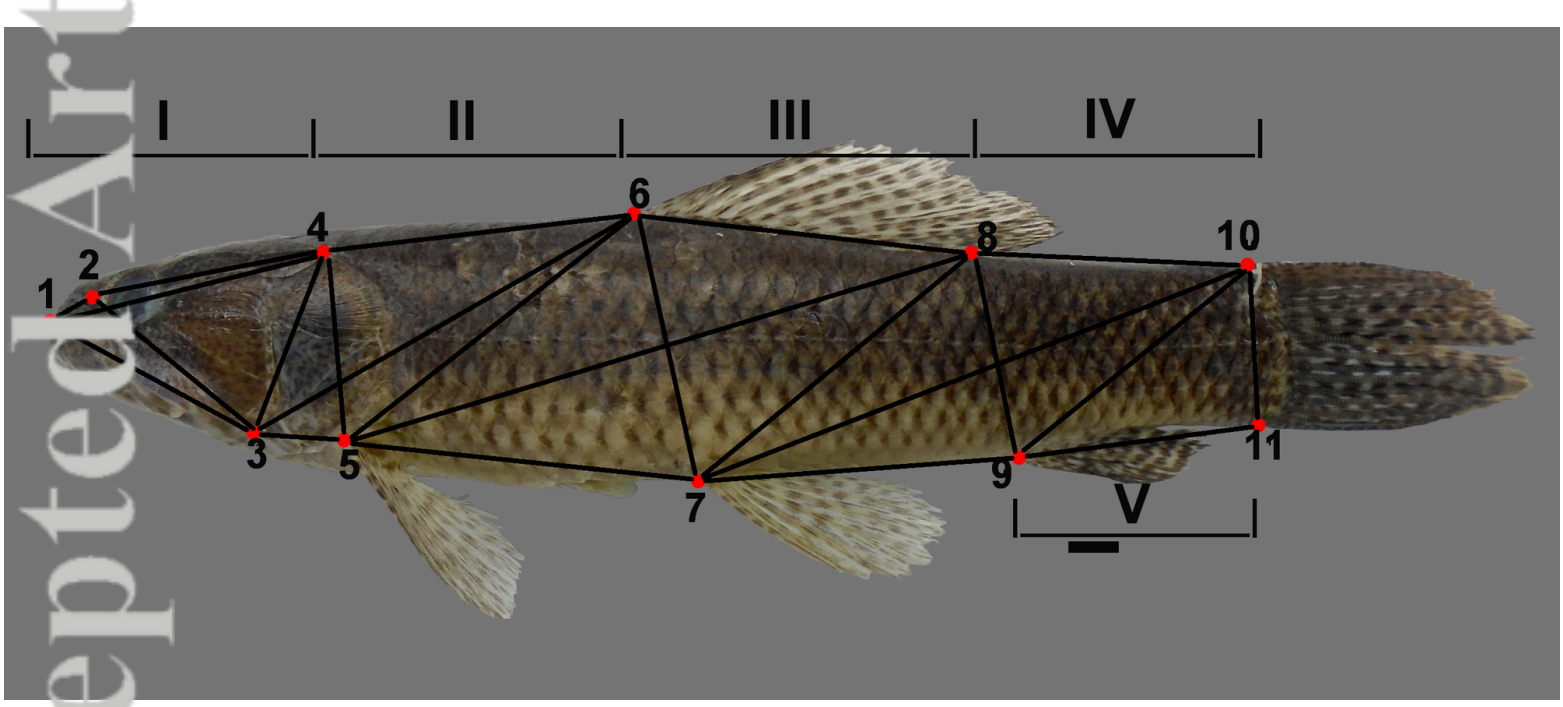
Figure 9. Map of Tapajós River basin and adjoining areas, showing the Crepori River basin, type locality and collection sites of *Hoplias auri* (orange circles indicate paratypes localities; star indicates type locality).

Figure 10. Collecting sites at the Creporizão Village: Lago do Sr. Pena (a), Igarapé da Sra. Maria Brito (b), Lago Creporizão (c).

Figure 11. a) Score plot of first (PC1) and second (PC2) components of a principal component analysis on 23 interlandmarks distances taken on four species of *Hoplias malabaricus* species complex: *H. auri* (yellow triangles) ; *H. argentinensis* (light-blue triangles); *H. mbigua* (blue diamonds); *H. misionera* (pink squares). b) Correlation between interlandmarks distances and the first two principals components.

Figure 12. Discriminant Analysis of the four species of *Hoplias malabaricus* species complex: *H. auri* (yellow triangles) ; *H. argentinensis* (light-blue triangles); *Hoplias mbigua* (blue diamonds); *H. misionera* (pink squares). Black squares indicates group centroid location.

Figure 13. Neighbor-joining tree of *Hoplias auri* and related species of the *H. malabaricus* group. Gray color indicates the new species. The lateral bar indicates the partitions of species delimitation delimited through BIN, ABGD, ASAP and GMYC analysis. Values in branches indicate the bootstrap values.



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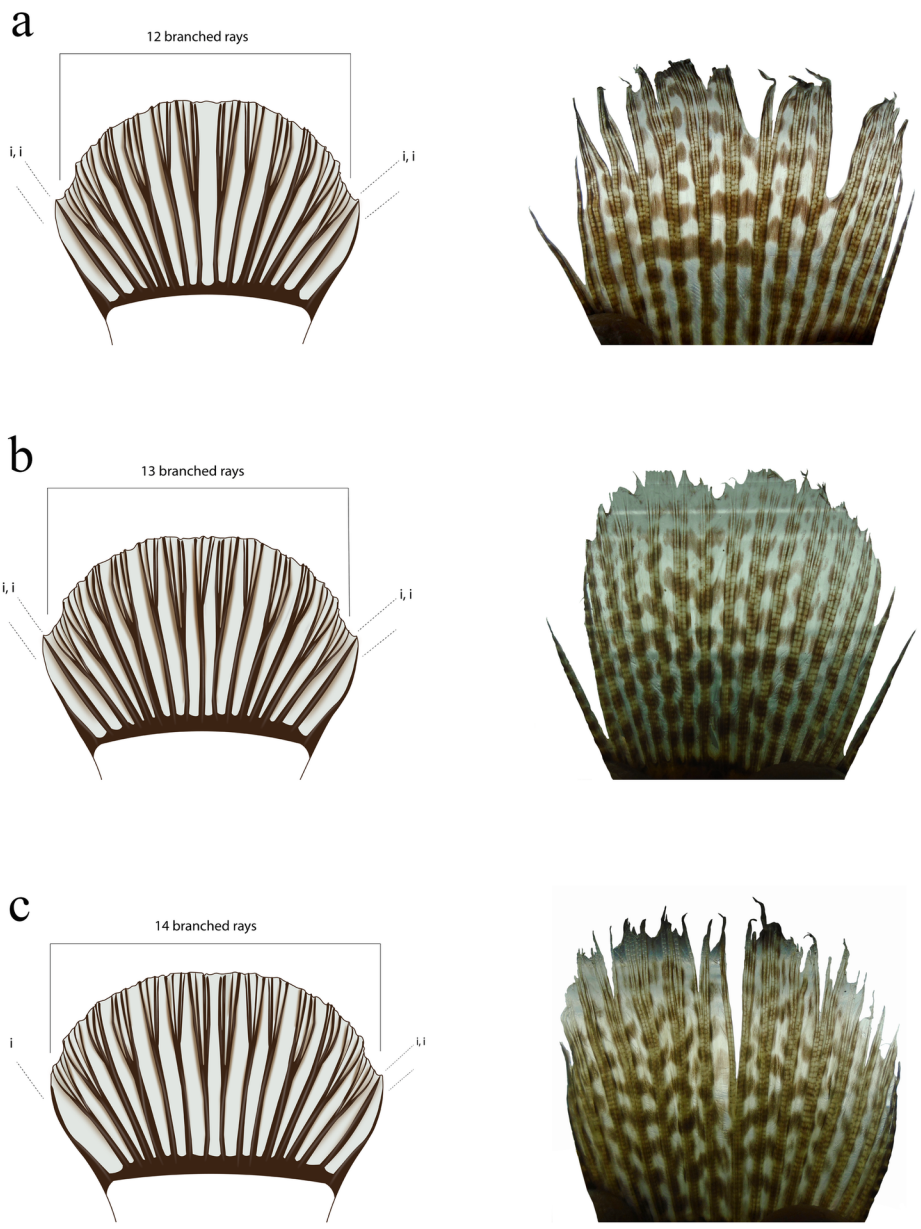

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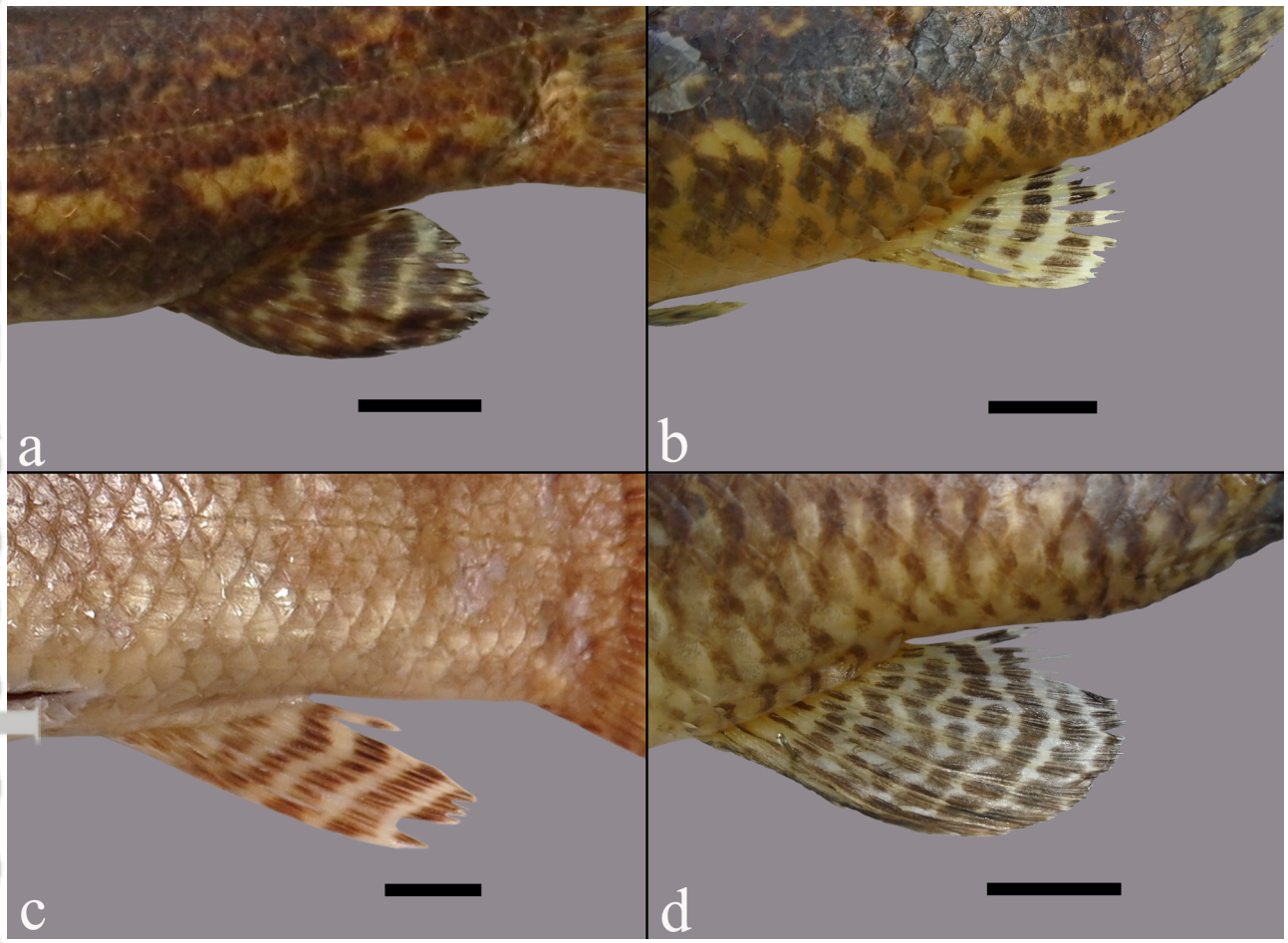

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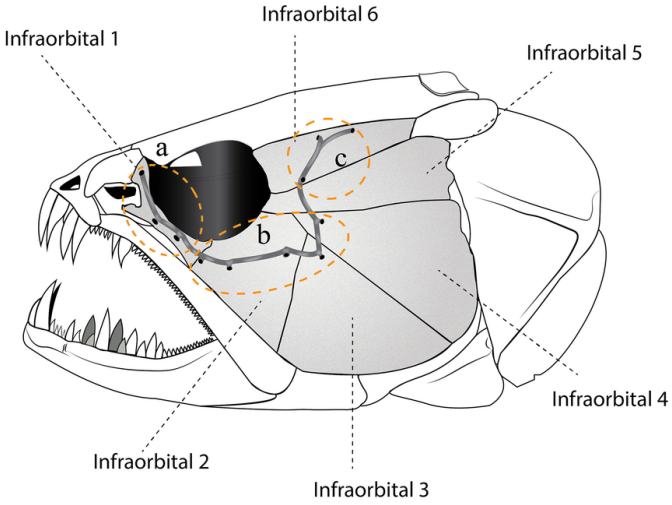

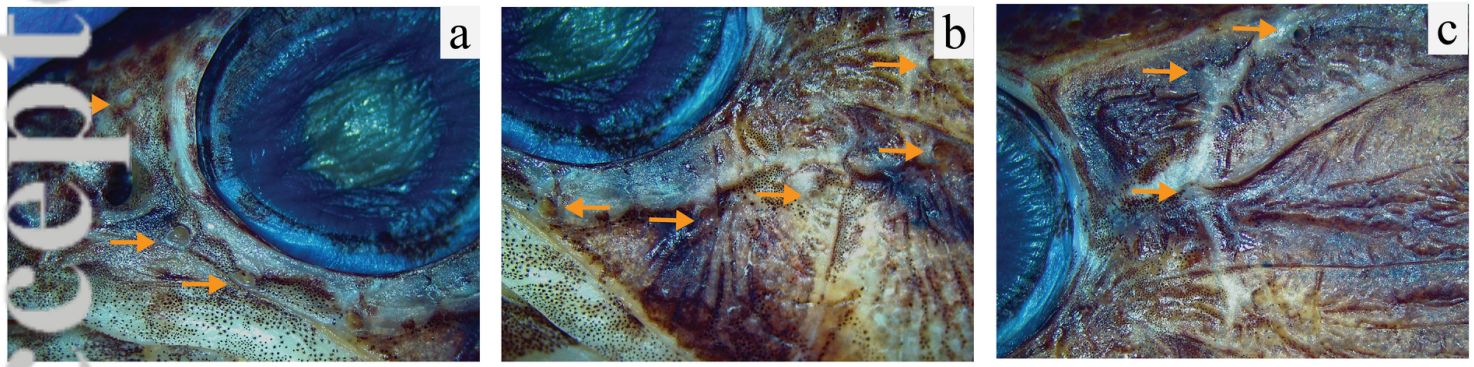

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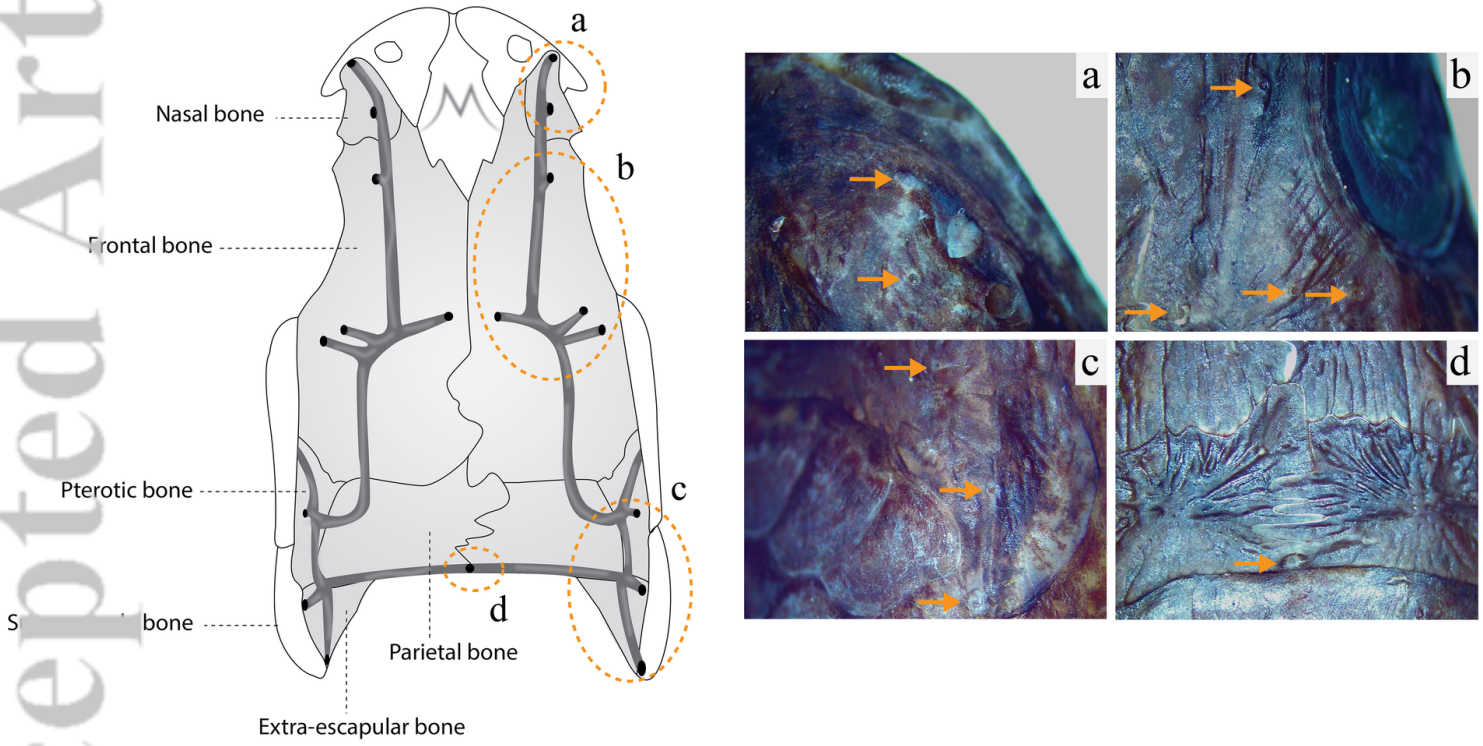

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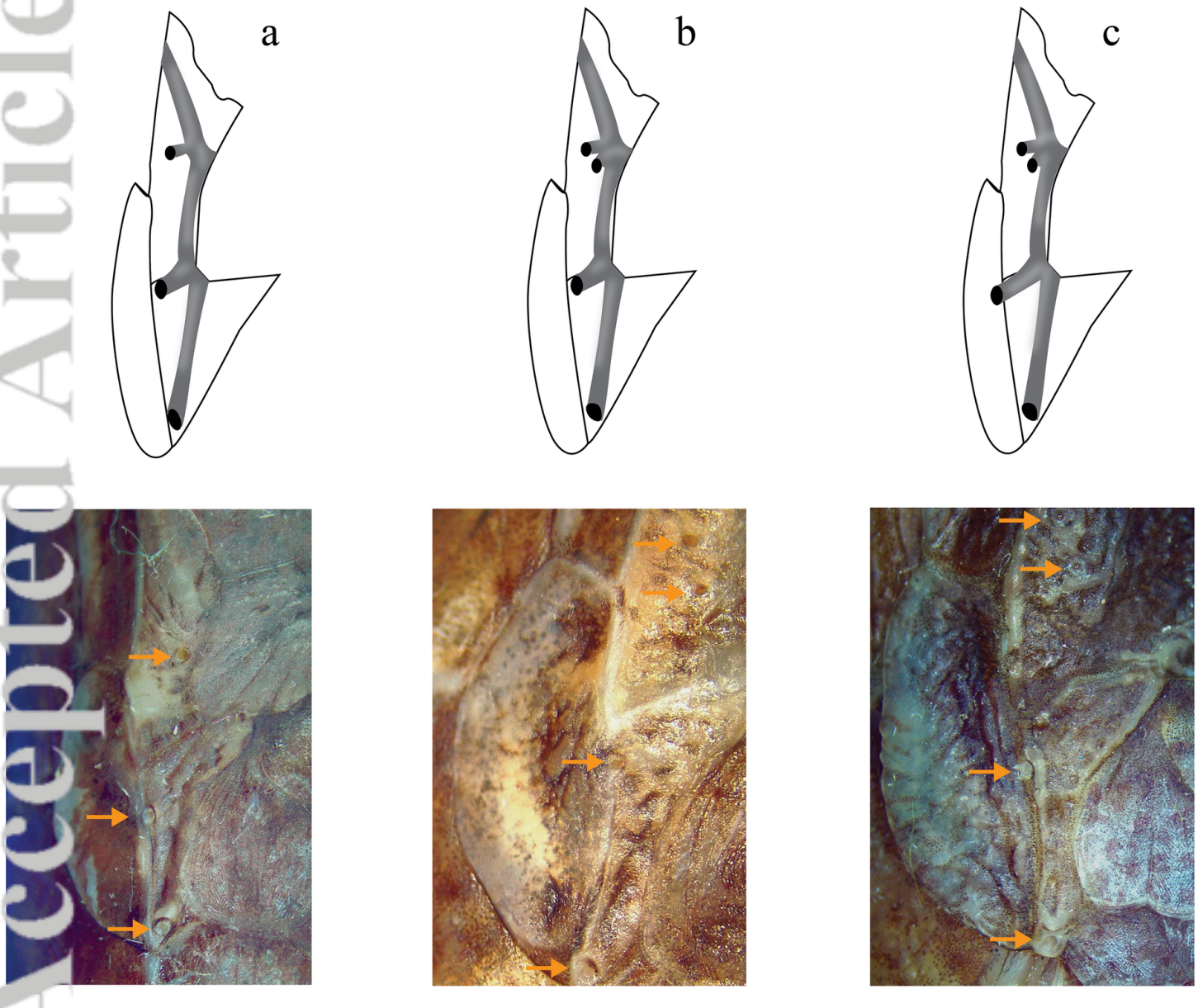

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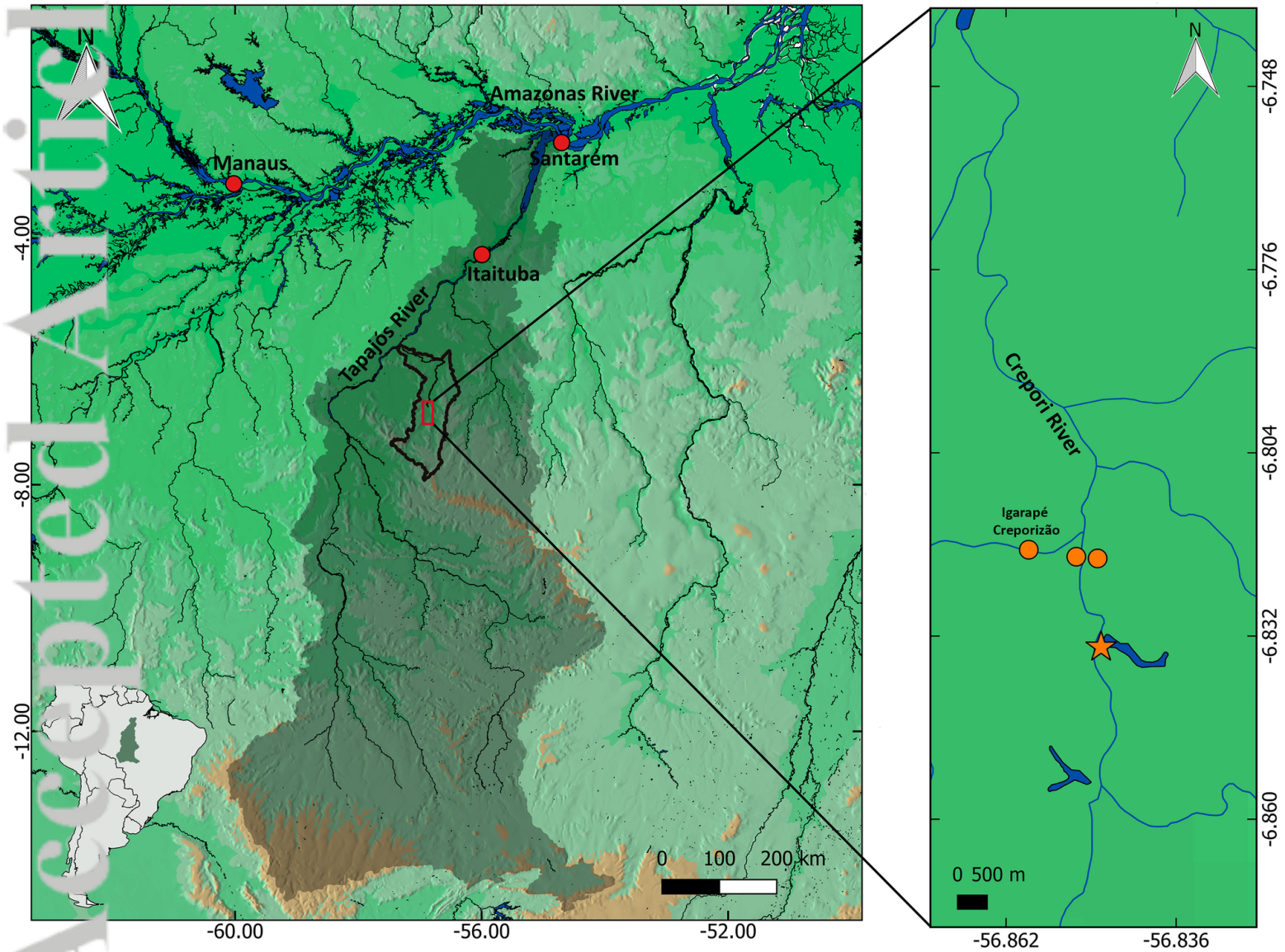

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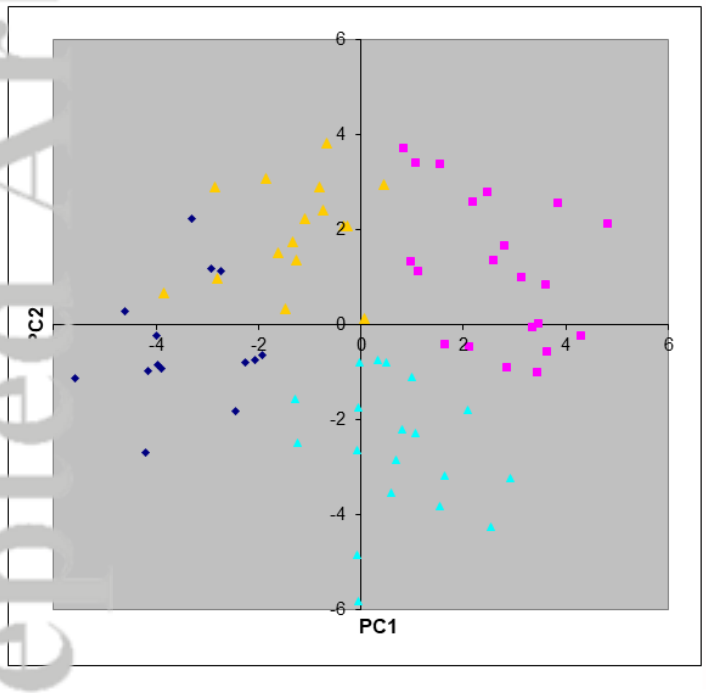

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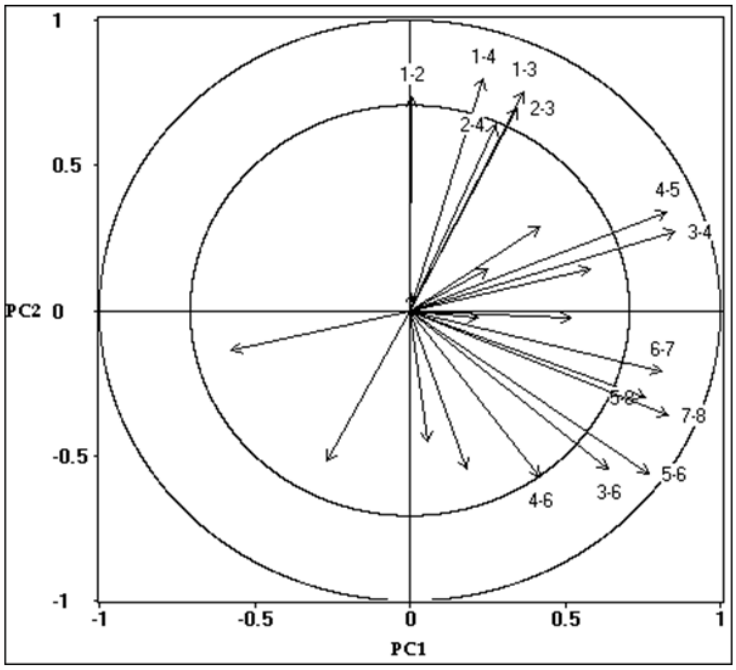

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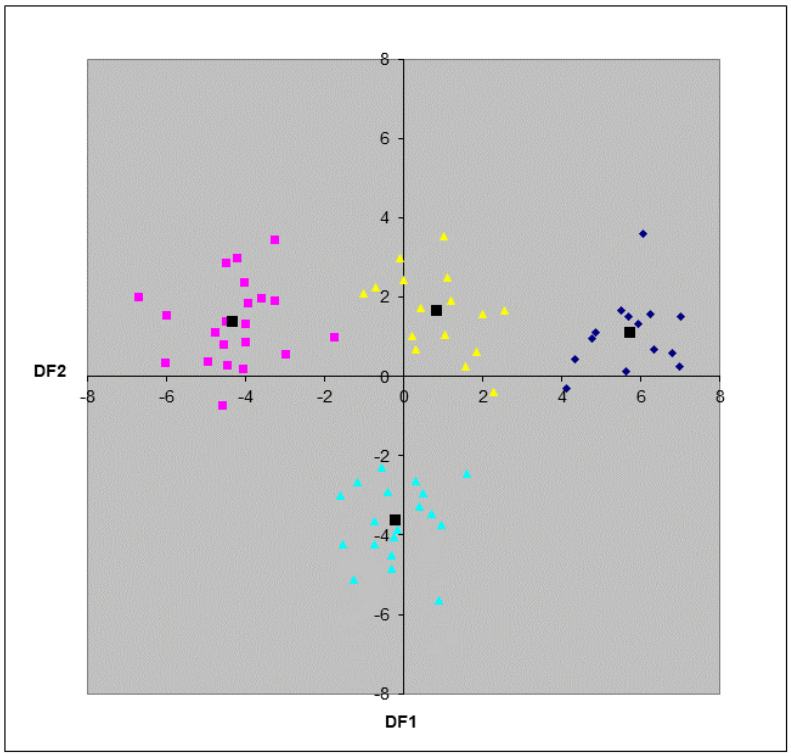

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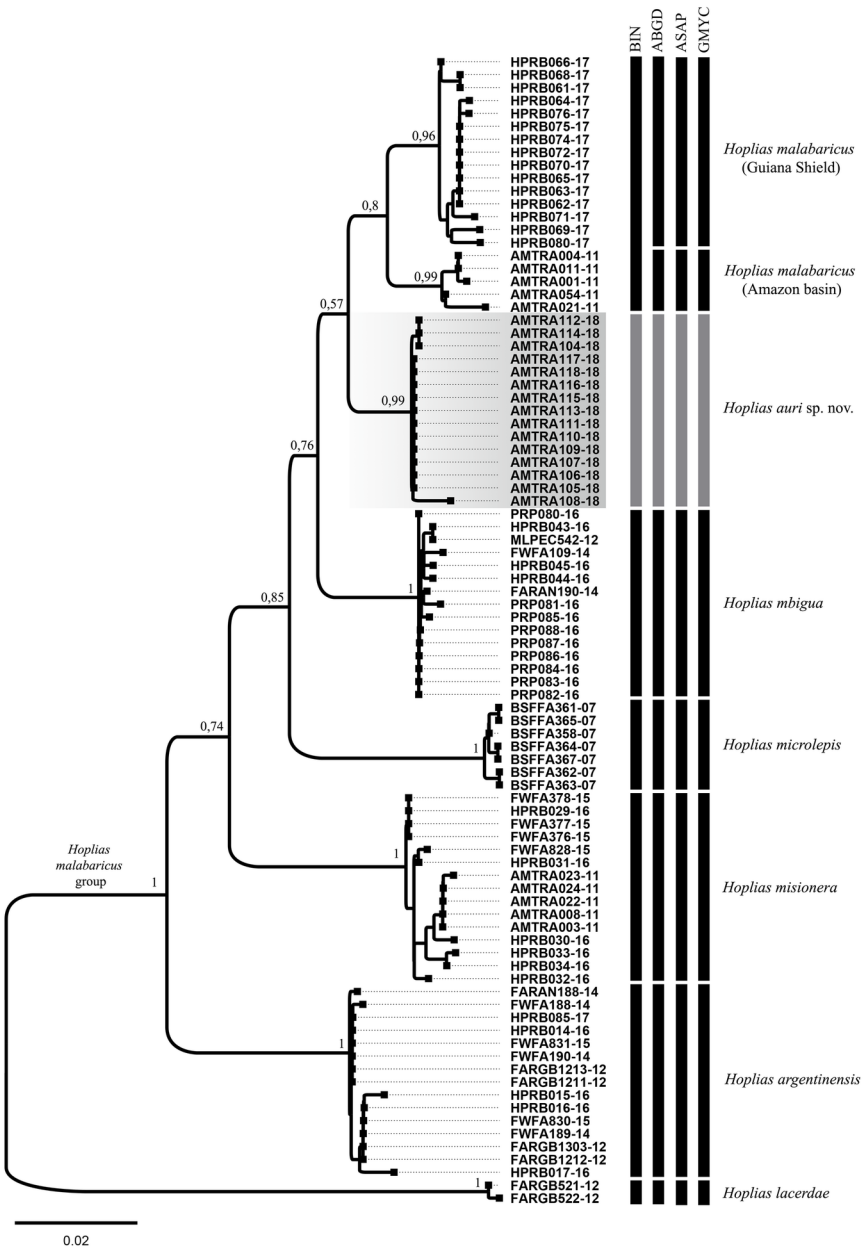

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Table 1. Morphometric data of *Hoplias auri*, new species. Standard length in mm; values 1-14 in percents of standard length; values 15-22 in percents of head length. SD, standard deviation; n, number of specimens; Range includes the holotype.

	H. auri (n=18)				
	Holotype	Mean	Min	Max	SD
	Standard Length	229	181.23	30.82	272	63.93
1	Body depth	20.26	20.0	16.23	22.96	1.61
2	Head length	30.35	31.0	28.43	34.1	1.34
3	Pectoral-fin length	17.69	16.85	15.85	19.01	0.84
4	Pelvic-fin length	19.65	18.96	17.94	20.52	0.82
5	Anal-fin length	17.77	18.13	16.55	20.26	1.02
6	Dorsal-fin length	35.2	31.39	28.01	35.2	1.90
7	Dorsal-fin base	18.73	17.45	15.07	19.49	1.11
8	Anal-fin base	9.08	8.45	7.59	9.48	0.56
9	Pre-pectoral length	29.3	30.28	27.92	32.89	1.10
10	Pre-pelvic length	53.71	55.49	53.14	58.82	1.52
11	Pre-dorsal length	47.12	46.95	43.03	52.19	2.6
12	Pre-anal length	79.83	79.94	75.15	82.11	2.11
13	Caudal peduncle depth	12.79	13.10	11.55	14.3	0.78
14	Caudal peduncle length	13.76	13.45	11.56	14.97	0.90
15	Head Depth	51.37	49.53	47.81	51.57	1.30
16	Snout length	27.19	25.32	23.38	27.31	1.22
17	Snout width	24.6	23.07	21.43	24.94	1.13
18	Snout depth	22.88	19.74	17.38	22.88	1.69
19	Pre-nasal length	16.26	15.12	12.94	17.79	1.18
20	Orbital diameter	16.83	17.28	13.94	20.84	1.94
21	Interorbital width	28.49	26.58	23.88	30.28	2.22
22	Upper jaw length	54.39	52.20	48.33	54.64	1.73

Table 2. Factor loadings and proportions of variance explained by first three principal components (PC1, PC2 and PC3) of a principal component analysis (PCA) conducted on 23 morphometric variables of four species of *Hoplias malabaricus* species-group. Most important variables are represented in bold.

	PC1	PC2	PC3
1-2	0.01	**0.73**	0.29
1-3	0.37	**0.75**	-0.33
1-4	0.23	**0.80**	0.13
2-3	0.35	**0.69**	-0.37
3-4	**0.86**	0.27	0.11
3-5	0.01	0.06	**0.72**
3-6	**0.64**	**-0.55**	0.41
4-5	**0.83**	0.34	0.00
5-6	**0.77**	**-0.56**	0.09
5-7	0.22	-0.02	0.07
5-8	**0.76**	-0.30	0.05
6-7	**0.81**	-0.21	-0.13
7-8	**0.83**	-0.36	-0.14
7-9	-0.27	**-0.52**	-0.03
7-10	0.18	**-0.55**	**-0.63**
8-9	0.06	-0.45	-0.02
9-10	**0.52**	-0.02	-0.47
9-11	0.25	0.14	-0.33
10-11	**0.58**	0.15	-0.11
2-4	0.28	**0.64**	0.29
4-6	0.42	**-0.57**	0.35
6-8	0.42	0.29	-0.09
8-10	**-0.58**	-0.14	-0.44
% of variance	27.1	21.6	9.7
Cummulative variance	27.1	48.7	58.4

Table 3. Cross-validated discriminant analysis on scores of a principal component analysis (PCA) of 23 morphometric variables in four species of *Hoplias malabaricus* species-group.

			Predicted Group Membership				Total
			H. mbigua	*H. misionera*	*H. argentinensis*	*H. auri*	
Original	**Count**	*H. mbigua*	14	0	0	0	14
		H. misionera	0	21	0	0	21
		H. argentinensis	0	0	19	0	19
		H. auri	0	0	0	16	16
	%	*H. mbigua*	100.0	0	0	0	100.0
		H. misionera	0	100.0	0	0	100.0
		H. argentinensis	0	0	100.0	0	100.0
		H. auri	0	0	0	100.0	100.0
Cross-validated	**Count**	*H. mbigua*	14	0	0	0	14
		H. misionera	0	20	0	1	21
		H. argentinensis	0	0	18	1	19
		H. auri	0	1	0	15	16
	%	*H. mbigua*	100.0	0	0	0	100.0
		H. misionera	0	95.2	0	4.8	100.0
		H. argentinensis	0	0	94.7	5.3	100.0
		H. auri	0	6.3	0	93.8	100.0

a. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

b. 100.0% of original grouped cases correctly classified.

c. 95.7% of cross-validated grouped cases correctly classified.

Table 4. Mean genetic distances of the Kimura two-parameter (K2P) model between *Hoplias* clusters.

Species	*H. auri*	*H. misionera*	*H. argentinensis*	*H. malabaricus*	*H. mbigua*	*H. microlepis*
H. auri						
H. misionera	0.067					
H. argentinensis	0.077	0.079				
H. malabaricus	0.030	0.069	0.074			
H. mbigua	0.032	0.068	0.080	0.043		
H. microlepis	0.057	0.083	0.070	0.058	0.052	
H. lacerdae	0.150	0.139	0.138	0.146	0.157	0.159