Ichthyological Exploration of Freshwaters

An international journal for field-orientated ichthyology

Verlag Dr. Friedrich Pfeil · München

Ichthyological Exploration of Freshwaters/**IEF-1076**/pp. 1–18 Published 2 August 2018
LSID: http://zoobank.org/urn:lsid:zoobank.org:pub:D86F1F17-D310-4D18-A8BB-94C0FA70759E
DOI: http://doi.org/10.23788/IEF-1076

Integrative taxonomy reveals a new species of the *Hoplias malabaricus* species complex (Teleostei: Erythrinidae)

Juan J. Rosso*,**, Mariano González-Castro*,****, Sergio Bogan**, Yamila P. Cardoso***,****, Ezequiel Mabragaña*,****, Matías Delpiani*,**** and Juan M. Díaz de Astarloa*,****

By means of an integrative taxonomic approach, a new species of the *H. malabaricus* group from the Río de La Plata basin is described. *Hoplias argentinensis*, new species, is characterized by: 17–19 predorsal scales, 42–43 vertebrae, 20 scales around caudal peduncle, no distinctive brown bands in lower jaw, last series of vertical scales on caudal fin nearly straight, 41–44 lateral-line scales and snout width (18.4–24.9 % of SL). *Hoplias argentinensis* is discriminated from all other species of the *H. malabaricus* group by means of traditional characters, landmarks-based morphometrics and genetics. A key to species of the *Hoplias malabaricus* group is provided.

Introduction

With more than 5100 species, Neotropical freshwater fishes represent about one-third of all freshwater fishes worldwide, albeit some estimates regard a final diversity between 8000 and 9000 species (Reis et al., 2016). Wolf fishes of the family Erythrinidae are predator fishes that inhabit a large array of aquatic ecosystems throughout South America and comprise three genera: *Erythrinus*, *Hoplerythrinus* and *Hoplias* (Oyakawa, 2003). *Hoplias*, the most species-rich genus within Erythinid fishes, comprises 13 valid species (Eschmeyer et al., 2017). Species of *Hoplias* can be morphologically discriminated into three different groups by means of the arrangement of medial margins and number of latero-sensory pores of dentaries and the presence of tooth plates on basihyal and basibranchial bones (Oyakawa, 1990; Oyakawa & Mattox, 2009; Mattox et al., 2006). One of these groups, the *Hoplias malabaricus* species group, includes *Hoplias malabaricus*, which

* 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, CONICET, Funes 3350, (7600) Mar del Plata, Argentina. JJR (corresponding author). E-mail: plurosso@yahoo.com.ar
** Fundación de Historia Natural "Félix de Azara". Departamento de Ciencias Naturales y Antropología. Universidad Maimónides. Hidalgo 775, piso 7. 1405-BDB Buenos Aires, Argentina
*** Laboratorio de Sistemática y Biología Evolutiva (LASBE), Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Paseo del Bosque S/N, B1900FWA, La Plata, Buenos Aires, Argentina
**** Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Godoy Cruz 2290, C1425FQB, Buenos Aires, Argentina

Table 1. Collecting site and identification of specimens of *Hoplias malabaricus* species complex in landmark-based analysis on 23 morphometric variables. Institutional abbreviations: CFA-IC, Fundación de Historia Natural Félix de Azara, Universidad Maimónides, Buenos Aires; LGE-P, Laboratorio de Genética Evolutiva, Instituto de Biología Subtropical, Universidad Nacional de Misiones, Posadas; UNMDP, Instituto de Investigaciones Marinas y Costeras, Universidad Nacional de Mar del Plata, Mar del Plata.

Catalog number	Voucher number	Basin	Locality	Latitude	Longitude
H. argentinensis					
CFA-IC 5537	AR15-678	Salado-Juramento	Embalse Tacañitas	28°37.3'S	62°36.6'W
UNMDP 1370	UNMDP 1370	Paraná	Paraná Delta	34°02.3'S	58°35.6'W
UNMDP 2565	UNMDP 2565	Paraná	Arroyo Nogoyá	32°51.1'S	59°51.7'W
UNMDP 503	UNMDP 503	Paraná	Río Rojas	34°04.9'S	61°00.0'W
UNMDP 504	UNMDP 504	Paraná	Río Rojas	34°04.9'S	61°00.0'W
CFA-IC-4355	FHN-645	Paraná	Arroyo Cañada Arias	34°40.1'S	59°08.2'W
CFA-IC 4665	R-07	La Plata	Río de La Plata	34°24.9'S	58°28.9'W
CFA-IC-3825	YC13-425	Salado del Sur	Laguna Gómez	34°40.2'S	61°01.2'W
CFA-IC-4364	FHN-673	La Plata	Bañados de Punta Indio	35°36.1'S	57°24.9'W
CFA-IC-5519	AR15-903	Salí-Dulce	Río Saladillo	28°53.4'S	63°17.4'W
CFA-IC-5519	R-01	Salí-Dulce	Río Saladillo	28°53.4'S	63°17.4'W
UNMDP 1279	UNMDP 1279	Uruguay	Embalse Salto Grande	30°56.7'S	58°01.2'W
UNMDP 2452	UNMDP 2452	Paraná	Laguna El Pescado	32°39.3'S	60°09.4'W
UNMDP 2453	UNMDP 2453	Paraná	Laguna El Pescado	32°39.3'S	60°09.4'W
UNMDP 2616	UNMDP 2616	Uruguay	Arroyo Ayuí	31°16.4'S	58°00.3'W
UNMDP 4423	UNMDP 4423	Paraná	Río Coronda	31°50.1'S	60°51.5'W
UNMDP 4425	UNMDP 4425	Paraná	Río Coronda	31°50.1'S	60°51.5'W
UNMDP 4427	UNMDP 4427	Paraná	Río Coronda	31°50.1'S	60°51.5'W
UNMDP 502	UNMDP 502	Paraná	Arroyo 4 de noviembre	34°10.3'S	60°54.1'W
H. mbigua					
CFA-IC 10451	R-03	Paraguay	Río Pilcomayo	25°12.9'S	58°00.0'W
CFA-IC 10451	R-04	Paraguay	Río Pilcomayo	25°12.9'S	58°00.0'W
CFA-IC-034		Paraná	Río Apipé Grande	27°30.3'S	56°54.1'W
CFA-IC-3083	AR11-488	Paraguay	Río Paraguay	26°05.3'S	57°59.8'W
LGE-P 435	LGE-P 435	Paraná	Río Paraná Medio	27°27.5'S	55°48.6'W
LGE-P 314	LGE-P 314	Paraná	Río Paraná Medio	27°27.5'S	55°48.6'W
LGE-P 316	LGE-P 316	Paraná	Arroyo Yabebiry	27°17.6'S	55°33.5'W
LGE-P 317	LGE-P 317	Paraná	Río Paraná Medio	27°29.1'S	56°40.5'W
H. misionera					
UNMDP 3321	UNMDP 3321	Paraguay	Riacho Saladillo	26°26.6'S	58°23.9'W
UNMDP 3322	UNMDP 3322	Paraguay	Riacho Saladillo	26°26.6'S	58°23.9'W
UNMDP 3327	UNMDP 3327	Paraguay	Riacho Salado	26°28.9'S	58°18.6'W
UNMDP 3328	UNMDP 3328	Paraguay	Riacho Salado	26°28.9'S	58°18.6'W
UNMDP 3329	UNMDP 3329	Paraguay	Riacho Salado	26°28.9'S	58°18.6'W
UNMDP 3371	UNMDP 3371	Paraguay	Riacho Mbiguá	26°32.7'S	58°30.7'W
UNMDP 3376	UNMDP 3376	Paraguay	Riacho Mbiguá	26°32.7'S	58°30.7'W
UNMDP 3391	UNMDP 3391	Uruguay	Arroyo sin nombre	27°27.8'S	54°57.1'W
UNMDP 3612	UNMDP 3612	Paraguay	Bañado RN 81	25°59.4'S	58°25.7'W
UNMDP 3613	UNMDP 3613	Paraguay	Bañado RN 81	25°59.4'S	58°25.7'W
UNMDP 3646	UNMDP 3646	Paraguay	Riacho Pingo Chico	24°49.6'S	59°31.8'W
UNMDP 3647	UNMDP 3647	Paraguay	Riacho Pingo Chico	24°49.6'S	59°31.8'W
UNMDP 3650	UNMDP 3650	Paraguay	Riacho Malvado	25°09.1'S	59°40.7'W
UNMDP 3651	UNMDP 3651	Paraguay	Riacho Malvado	25°09.1'S	59°40.7'W
UNMDP 3655	UNMDP 3655	Paraguay	Estero Poí	25°03.6'S	58°09.3'W
UNMDP 3672	UNMDP 3672	Paraguay	Riacho San Hilario	26°14.8'S	58°16.4'W
UNMDP 3703	UNMDP 3703	Paraná	Arroyo Potrero	31°30.6'S	60°28.2'W
UNMDP 3865	UNMDP 3865	Paraná	Arroyo Leyes	31°29.6'S	60°26.7'W
UNMDP 3970	UNMDP 3970	Paraná	Río Colastiné	31°37.4'S	60°35.1'W
UNMDP 4054	UNMDP 4054	Paraná	Laguna Iberá	28°31.4'S	57°11.0'W
UNMDP 4424	UNMDP 4424	Paraná	Río Coronda	31°50.1'S	60°51.5'W

has long been regarded as one of the most complex problems in the taxonomy of Neotropical fishes. Indeed, both cytogenetic (e. g. Bertollo et al., 1986, 2000; Scavone, 1994; Cioffi et al., 2009; Blanco et al., 2010; Grassi et al., 2017) and molecular evidence (e. g. Rosso et al., 2012; Marques et al., 2013; Pereira et al., 2013) clearly suggest that the name *Hoplias malabaricus* refers to a complex of species. The taxonomic discrimination between species of this group began to be solved lately, with the formal descriptions of *H. mbigua* and *H. misionera* (Azpelicueta et al., 2015; Rosso et al., 2016). Nevertheless, an accurate discrimination of all species within the *Hoplias malabaricus* species complex is needed since these species play a central role in recreational, subsistence and commercial fisheries (Goulding, 1980; Iwaszkiw & Firpo Lacoste, 2011; García-Vásquez et al., 2014).

The aim of this paper is the description of a new species of the *H. malabaricus* species complex by means of an integrative approach (genetics, meristics, traditional and landmark-based morphometrics).

Material and methods

Traditional measurements and counts were made on the left side of the body following Fink & Weitzman (1974) and Mattox et al. (2006) except the number of pre-dorsal scales which did not include the modified scale just before the insertion of the dorsal fin and number of scales of the lateral line system which did not include the first one or two unperforated scales beneath the opercle membrane. Vertebral counts were taken from radiographs and included the anterior four vertebrae of the Weberian apparatus. Counts of the holotype are indicated with asterisks. Institutional abbreviations are as follows: BMNH: Natural History Museum, London; CFA-IC: Fundación de Historia Natural Félix de Azara, Universidad Maimónides, Buenos Aires; CI-FML: Colección Ictiológica Fundación Miguel Lillo, San Miguel de Tucumán; LBP: Laboratório de Genética de Peixes, Universidade Estadual Paulista "Júlio de Mesquita Filho", Botucatu; LGE-P: Laboratório de Genética Evolutiva, Instituto de Biología Subtropical, Universidad Nacional de Misiones, Posadas; MHNN: Muséum d'Histoire naturelle de Neuchâtel; MLP: Museo de La Plata, La Plata; MNHN: Muséum national d'Histoire naturelle, Paris; UNMDP: Instituto de Investigaciones Marinas y Costeras, Universidad Nacional de Mar del Plata, Mar del Plata; ZMB: Zoologisches Museum, Humboldt-Universität zu Berlin, Berlin.

In order to characterize the body shape of species of the *H. malabaricus* complex, a landmarks-based morphometric analysis was conducted. Twenty-three morphometric variables were taken as interlandmark distances over the left side of 44 specimens (Table 1) using a digital caliper. These variables were based on 11 landmarks (Fig. 1, Table 2) obtained by truss networks (Bookstein et al., 1985). Landmarks were defined on the basis of external anatomy and are homologous among the species (Fig. 1). Five box-trusses were obtained. The first four were composed by four landmarks each one with its corresponding six interlandmarks distances and the fifth by three landmarks (9, 10 and 11) and consequently three interlandmarks distances (Table 2). Statistical and mathematical procedures for analysis of interlandmark distances (ILD) followed González-Castro et al. (2012, 2016). Quadrants in the graphic interpretation of the scores of principal components were numbered anticlockwise (Brannan et al., 1999; González-Castro & Díaz de Astarloa, 2017). Prior to analysis, the normalization technique of Lleonart et al. (2000) was used in order to scale the data with allometric growth. Standard length (SL) represented the independent variable, whereas the remaining 23 morphometric characters represented the dependent variables. In this normalization, SL_0 represented a reference value of 170 mm to which all individuals were reduced or amplified (Lombarte & Lleonart, 1993).

Samples of muscle tissue were collected from the holotype and 24 paratypes and preserved in 99.5 % ethanol for genetic analysis. DNA extraction, polymerase chain reaction (PCR) and sequencing of the COI gene were performed according to standard DNA barcoding protocols (Ivanova et al., 2006) and primer cocktails developed for fishes (Ivanova et al., 2007). In the present study, the mtDNA COI barcode profile of the holotype is reported as an aspect of the type description. The molecular approach aimed to further discriminate and characterize the genetic identity of this new species in relation to recently described species of the lower Río de La Plata basin and *H. malabaricus*. Therefore, the COI sequences of *H. mbigua* (n = 5), *H. misionera* (n = 10) and *H. malabaricus* (n = 25) were gathered from different projects ("*Hoplias* of South America, Barcoding des Poissons des Guyanes and *Hoplias*

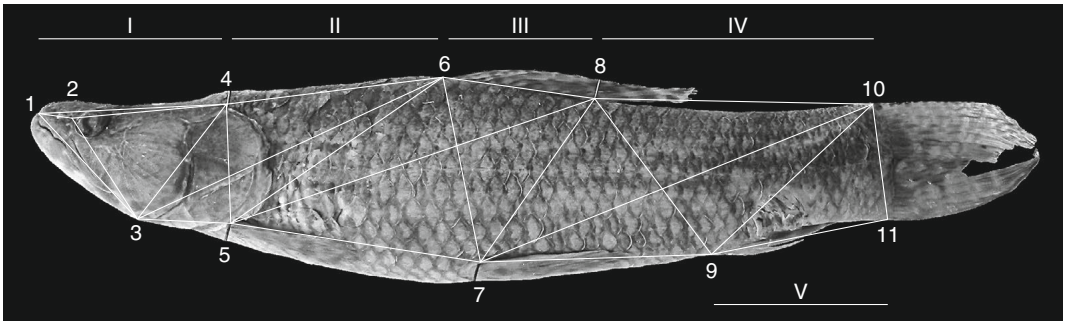

Fig. 1. Box truss showing interlandmarks distances based on 11 homologous anatomical landmarks collected in 44 specimens of different species of *Hoplias*. Numbers of box truss in Roman numerals.

Table 2. Landmarks and interlandmark distances (ILD) obtained by truss network taken from 44 specimens of three different species of the *Hoplias malabaricus* group (*H. argentinensis, H. misionera, H. mbigua*). Interlandmark distances defined five box-trusses which are listed in last column.

Landmarks	Landmark description	ILDs	box-trusses
1	Tip of snout	1–2; 1–3; 1–4	I
2	Posterior nostril	2–3; 2–4	I
3	Anteriormost extreme of interopercle	3–4	I
4	Posterodorsal end of opercle	3–4; 4–5; 4–6	II
5	Pectoral-fin origin	3–5; 5–6	II
6	Dorsal-fin origin	3–6; 5–6	II and III
7	Pelvic-fin origin	5–7; 6–7; 7–8	III
8	Insertion of last dorsal-fin ray	5–8; 6–8; 7–8	III and IV
9	Anal-fin origin	7–9; 8–9; 9–10	IV
10	Insertion of uppermost caudal-fin ray	7–10; 8–10	IV
11	Insertion of lowermost caudal-fin ray	9–10; 10–11; 9–11	V

malabaricus species complex") in the Barcode of Life Database (BOLD) (Ratnasingham & Hebert, 2007). Four additional sequences of the new species herein described from the Pampa Plain (referred to as *H. malabaricus* in Rosso et al., 2012) were also incorporated from BOLD. After alignment (Edgar, 2004) a final set of 69 sequences (Table 3) was subjected to distance analysis. The Barcode Index Number (BIN) was reported for each sequence. BIN analysis clusters barcode sequences to create Operational Taxonomic Units that closely reflect species groupings (Ratnasingham & Hebert, 2013). Genetic divergences among BINs were estimated. A neighbor-joining (NJ) tree of K2P distances (Kimura, 1980) was created using the software MEGA version 5.0 to provide a graphic representation of genetic divergences (Tamura et al., 2011).

Results

Molecular analysis. The mtDNA COI Barcode profile (652 bp) of the holotype was:

CCTATATCTAGTATTTGGTGCCTGAGCC
GGGATAGTTGGTACAGCTCTCAGCCTTCTA
ATCCGAGCGGAGCTAAGCCAACCCGGGGC
ATTACTTGGCGATGACCAGATTTACAATGT
TATCGTTACTGCACATGCCTTCGTGATAATT
TTCTTCATAGTAATACCTATTATAATCGGGG
GGTTTGGGAATTGACTTGTTCCCCTCATGAT
CGGGGCACCTGACATGGCTTTCCCGCGAAT
AAATAACATAAGTTTCTGACTTCTTCCCCCC
TCATTACTTCTCCTGCTGGCTTCCTCTGGCG
TAGAGGCGGGGGTTGGAACAGGTTGAACT
GTTTACCCCCCTCTCGCCGGAAACCTTGCA
CATGCAGGAGCCTCAGTTGACCTGGCAATC
TTTTCTCTCCACCTTGCAGGGGTCTCCTCAA
TTTTAGGAGCCATTAATTTTATTACAACAAT
TATTAACATAAAACCTCCTGCCATTTCACA
GTACCAAACCCCCCTATTTGTTTGAGCTATT

TTAATTACAGCCGTCCTTCTTCTCCTCTCCC
TCCCCGTTCTTGCTGCCGGGATTACAATACT
TCTAACAGACCGAAACCTTAACACCACCT
TCTTCGACCCTGCGGGGGGAGGAGACCCC
ATTCTCTACCAACATCTA.

The nucleotide composition of COI sequences of the new species (herein recognized) was A (23.2 %), G (18.3 %), C (28.1 %) and T (30.3 %). The K2P genetic distances within the new species averaged 0.25 % (0–1.03 %). All specimens of the new species received the BIN AAZ3734 and differed by 9.08 % from its nearest neighbors (HPRB069 and HPRB079). The K2P/NJ tree together with the BIN algorithm unambiguously discriminated the new species from *H. malabaricus* sensu stricto and the remaining two valid species of the *H. malabaricus* species complex from the lower Río de La Plata basin, *H. misionera* and *H. mbigua* (Fig. 2).

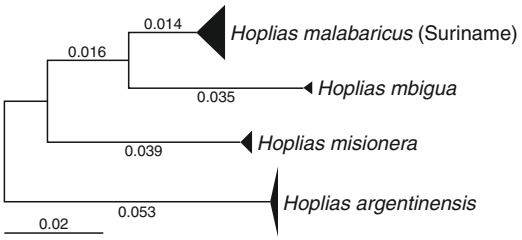

Fig. 2. The K2P/NJ tree of BARCODE sequences from *Hoplias argentinensis* and related species of *H. malabaricus* group.

Morphometric analysis based on ILD. The 23 normalized interlandmark distances were analyzed by a principal components analysis (PCA) of the correlation matrix, which produced six eigenvalues greater than one. The first two principal components explained 51.4 % of the variance in the data (Table 4). The multivariate ordination provided by the first two principal components allowed graphic segregation of an undescribed species, *H. misionera* and *H. mbigua*, without overlapping between them (Fig. 3). The undescribed species was mainly located in the second quadrant (Fig. 3), being characterized by ILD loadings higher in the positive end of the second axis and less evidently in the negative end of the first axis. Accordingly, this species can be characterized (Table 4) by large values for the 3–6, 4–6, 5–6 interlandmark distances (which are part of the second box-truss, and represents the relationship between the posterior part of the head and the origin of the dorsal fin). As well, its shape is defined by large values (Table 4) for 5–8, 6–7 and 7–8 variables that represent the height and shape of the portion of the body comprised in the third box-truss, which is related to the dorsal-fin base and origins of pectoral and ventral fins. The specimens of *H. misionera* were restricted to the first and fourth quadrants being confined to the positive end of the first axis (Fig. 3). Hence, *H. misionera* can be characterized by high values for interlandmarks describing head shape (1–2, 1–3, 1–4, 2–3, 2–4, 3–4, 4–5). Its shape is also defined by large values for 5–8, 6–7 and 7–8 interlandmark distances defining a high body at dorsal-fin base (box-truss III). At last, *H. mbigua* specimens were restricted to the third quadrant being characterized by ILD loadings higher for negative ends of both axes (Fig. 3). In consequence, this species is characterized by large values for 1–2, 1–3, 1–4, 2–3 variables (indicating an elongated snout and head) and high loadings for the variables 7–9, 7–10, 8–10 (which indicates an elongated body posteriorly to dorsal and pelvic fins).

Data of all principal components where employed to perform a discriminant analysis (DA), which yielded two significant canonical discriminant functions. The first one explained 75.8 % of the total variance of the data (Wilks' lambda $=0.007$ $p<0.0001$). The DA correctly classified 100 % of the *Hoplias* individuals according to the three groups defined a priori, whereas the cross-validated analysis correctly classified 91.7 % of the fishes according to their body shape. Group

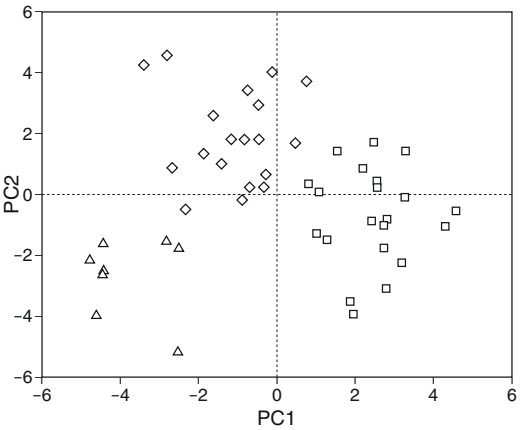

Fig. 3. Score plot of first (PC1) and second (PC2) components of a principal component analysis on 23 interlandmarks distances taken on three species of *Hoplias malabaricus* species complex: *H. argentinensis* (◇); *Hoplias mbigua* (△); *H. misionera* (□).

Table 3. Collecting site, molecular and voucher identification of specimens of *Hoplias malabaricus* species complex in DNA Barcoding analysis. BIN: Barcode Index Number. BOLD ID: Identification code of each sequence in the Barcode of Life Database.

BOLD ID	Determined as	Voucher number	Basin	Locality
FARAN190-14	*Hoplias mbigua*	UNMDP 2919	La Plata River	Arroyo Piquieri
FWFA109-14	*Hoplias mbigua*	UNMDP 1807	La Plata River	Río Paraguay
HPRB043-16	*Hoplias mbigua*	AR11-488	La Plata River	Río Paraguay
HPRB044-16	*Hoplias mbigua*	FHN-1236	La Plata River	Arroyo Yabebiry
HPRB045-16	*Hoplias mbigua*	FHN-1166	La Plata River	Arroyo Garupá
FARGB336-11	*Hoplias argentinensis*	UNMDP 504	La Plata River	Rio Rojas
FARGB334-11	*Hoplias argentinensis*	UNMDP 502	La Plata River	Arroyo 4 de noviembre
FARGB335-11	*Hoplias argentinensis*	UNMDP 503	La Plata River	Río Rojas
FARGB324-11	*Hoplias argentinensis*	UNMDP 492	La Plata River	Arroyo Santa Cruz
FARAN188-14	*Hoplias argentinensis*	UNMDP 2616	La Plata River	Arroyo Ayuí
FARGB1303-12	*Hoplias argentinensis*	UNMDP 1370	La Plata River	Paraná Delta
FARGB1304-12	*Hoplias argentinensis*	UNMDP 1371	La Plata River	Paraná Delta
FWFA1105-15	*Hoplias argentinensis*	UNMDP 1279	La Plata River	Embalse Salto Grande
FWFA1534-16	*Hoplias argentinensis*	UNMDP 4416	La Plata River	Río Coronda
FWFA1535-16	*Hoplias argentinensis*	UNMDP 4417	La Plata River	Río Coronda
FWFA1541-16	*Hoplias argentinensis*	UNMDP 4423	La Plata River	Río Coronda
FWFA1543-16	*Hoplias argentinensis*	UNMDP 4425	La Plata River	Río Coronda
FWFA1544-16	*Hoplias argentinensis*	UNMDP 4426	La Plata River	Río Coronda
FWFA1545-16	*Hoplias argentinensis*	UNMDP 4427	La Plata River	Río Coronda
FWFA1546-16	*Hoplias argentinensis*	UNMDP 4428	La Plata River	Río Coronda
FWFA188-14	*Hoplias argentinensis*	UNMDP 2452	La Plata River	Laguna El Pescado
FWFA189-14	*Hoplias argentinensis*	UNMDP 2453	La Plata River	Laguna El Pescado
FWFA190-14	*Hoplias argentinensis*	UNMDP 2565	La Plata River	Arroyo Nogoyá
FWFA830-15	*Hoplias argentinensis*	UNMDP 3867	La Plata River	Arroyo Leyes
FWFA899-15	*Hoplias argentinensis*	UNMDP 1595	La Plata River	Arroyo Bergara
HPRB011-16	*Hoplias argentinensis*	FHN-673	La Plata River	Bañados de Punta Indio
HPRB014-16	*Hoplias argentinensis*	FHN-643	La Plata River	Arroyo Cañada Arias
HPRB015-16	*Hoplias argentinensis*	FHN-924	La Plata River	Arroyo Dorado
HPRB016-16	*Hoplias argentinensis*	YC13-583	La Plata River	Río Carcaraña
HPRB017-16	*Hoplias argentinensis*	YC13-425	La Plata River	Laguna Gómez
HPRB085-17	*Hoplias argentinensis*	FHN-690	La Plata River	Bañados de Punta Indio
HPRB087-17	*Hoplias argentinensis*	AR15678	La Plata River	Embalse Tacañitas
HPRB090-17	*Hoplias argentinensis*	AR15903	Sali-Dulce River	Río Dulce
HPRB092-17	*Hoplias argentinensis*	AR151172	Sali-Dulce River	Río Vipos
HPRB060-17	*Hoplias malabaricus*	GFSU141307	Guiana Shield Basin	Crique Passoura
HPRB061-17	*Hoplias malabaricus*	GFSU141354	Guiana Shield Basin	Marais de Kaw
HPRB062-17	*Hoplias malabaricus*	SUGF12109	Guiana Shield Basin	Crique Canceler
HPRB063-17	*Hoplias malabaricus*	SU08967	Guiana Shield Basin	Mapana Creek
HPRB064-17	*Hoplias malabaricus*	GFSU12e162	Guiana Shield Basin	Crique Anjanwoye
HPRB065-17	*Hoplias malabaricus*	SU081123	Guiana Shield Basin	Mapana Creek
HPRB066-17	*Hoplias malabaricus*	GFSU141327	Guiana Shield Basin	Crique Soumourou
HPRB067-17	*Hoplias malabaricus*	SU01077	Guiana Shield Basin	Mindrinetti kreek
HPRB068-17	*Hoplias malabaricus*	GFSU141353	Guiana Shield Basin	Marais de Kaw
HPRB069-17	*Hoplias malabaricus*	GFSU14591	Guiana Shield Basin	Krebibato Creek
HPRB070-17	*Hoplias malabaricus*	GFSU141239	Guiana Shield Basin	Spari Creek
HPRB071-17	*Hoplias malabaricus*	SUGF12356	Guiana Shield Basin	Rivière Kilo
HPRB072-17	*Hoplias malabaricus*	GFSU141238	Guiana Shield Basin	Spari Creek
HPRB073-17	*Hoplias malabaricus*	SU08555	Guiana Shield Basin	Crique WaWapsi
HPRB074-17	*Hoplias malabaricus*	GFSU141308	Guiana Shield Basin	Crique Passoura
HPRB075-17	*Hoplias malabaricus*	GFSU14592	Guiana Shield Basin	Krebibato Creek
HPRB076-17	*Hoplias malabaricus*	SUGF12135	Guiana Shield Basin	Rivière Organabo
HPRB077-17	*Hoplias malabaricus*	SU08023	Guiana Shield Basin	Marshall Creek
HPRB078-17	*Hoplias malabaricus*	GFSU14589	Guiana Shield Basin	Krebibato Creek

Latitude	Longitude	BIN
26°15.8'S	58°10.1'W	BOLD:ACO5223
26°15.8'S	58°10.1'W	BOLD:ACO5223
26°12.2'S	58°08.7'W	BOLD:ACO5223
27°17.1'S	55°31.9'W	BOLD:ACO5223
27°28.9'S	55°46.9'W	BOLD:ACO5223
34°04.2'S	60°57.2'W	BOLD:AAZ3734
34°10.2'S	60°54.1'W	BOLD:AAZ3734
34°04.9'S	61°00.0'W	BOLD:AAZ3734
34°04.2'S	61°02.6'W	BOLD:AAZ3734
31°16.4'S	58°00.3'W	BOLD:AAZ3734
34°01.5'S	58°29.5'W	BOLD:AAZ3734
34°01.5'S	58°29.5'W	BOLD:AAZ3734
30°56.2'S	58°03.2'W	BOLD:AAZ3734
31°50.1'S	60°51.5'W	BOLD:AAZ3734
31°50.1'S	60°51.5'W	BOLD:AAZ3734
31°50.1'S	60°51.5'W	BOLD:AAZ3734
31°50.1'S	60°51.5'W	BOLD:AAZ3734
31°50.1'S	60°51.5'W	BOLD:AAZ3734
31°50.1'S	60°51.5'W	BOLD:AAZ3734
31°50.1'S	60°51.5'W	BOLD:AAZ3734
32°39.3'S	60°09.4'W	BOLD:AAZ3734
32°39.3'S	60°09.4'W	BOLD:AAZ3734
32°51.1'S	59°51.7'W	BOLD:AAZ3734
31°29.6'S	60°26.7'W	BOLD:AAZ3734
32°16.4'S	59°03.7'W	BOLD:AAZ3734
35°36.1'S	57°00.4'W	BOLD:AAZ3734
34°39.5'S	59°08.1'W	BOLD:AAZ3734
27°22.5'S	54°26.3'W	BOLD:AAZ3734
33°01.5'S	61°47.1'W	BOLD:AAZ3734
34°40.2'S	61°01.2'W	BOLD:AAZ3734
35°36.1'S	57°24.6'W	BOLD:AAZ3734
28°37.2'S	62°36.6'W	BOLD:AAZ3734
28°53.4'S	63°16.1'W	BOLD:AAZ3734
26°28.9'S	65°19.9'W	BOLD:AAZ3734
5°09.1'N	52°41.4'W	BOLD:ABZ3047
4°29.8'N	52°03.1'W	BOLD:ABZ3047
5°25.7'N	53°02.3'W	BOLD:ABZ3047
5°18.9'N	54°49.9'W	BOLD:ABZ3047
4°10.9'N	55°25.9'W	BOLD:ABZ3047
5°18.9'N	54°49.9'W	BOLD:ABZ3047
5°07.5'N	52°13.9'W	BOLD:ABZ3047
5°07.1'N	55°16.9'W	BOLD:ABZ3047
4°29.8'N	52°03.1'W	BOLD:ABZ3047
4°25.7'N	55°45.8'W	BOLD:ABZ3047
5°13.9'N	55°48.2'W	BOLD:ABZ3047
4°27.0'N	57°09.8'W	BOLD:ABZ3047
5°13.9'N	55°48.2'W	BOLD:ABZ3047
3°10.7'N	55°25.1'W	BOLD:ABZ3047
5°09.1'N	52°41.4'W	BOLD:ABZ3047
4°25.7'N	55°45.8'W	BOLD:ABZ3047
5°32.9'N	53°28.1'W	BOLD:ABZ3047
5°14.6'N	55°06.1'W	BOLD:ABZ3047
4°25.7'N	55°45.8'W	BOLD:ABZ3047

Table 4. Factor loadings and proportions of variance explained by first two principal components (PC1 and PC2) of a principal component analysis (PCA) carried out on 23 morphometric variables of three species of *Hoplias malabaricus* species complex. Most important variables are represented in bold.

	PC1	PC2
1–2	0.38	**−0.63**
1–3	**0.62**	**−0.64**
1–4	**0.69**	**−0.51**
2–3	**0.66**	**−0.57**
3–4	**0.91**	0.07
3–5	0.12	0.18
3–6	0.25	**0.81**
4–5	**0.90**	0.02
5–6	0.40	**0.87**
5–7	0.17	0.46
5–8	0.53	**0.69**
6–7	**0.66**	**0.44**
7–8	**0.68**	**0.56**
7–9	**−0.51**	0.11
7–10	−0.24	0.38
8–9	−0.11	0.37
9–10	0.36	0.25
9–11	0.19	−0.17
10–11	0.58	−0.04
2–4	**0.70**	−0.24
4–6	−0.03	**0.79**
6–8	0.57	−0.18
8–10	**−0.72**	−0.06
% of variance	29.00	22.40
Cumulative variance	29.00	51.40

Table 5. Cross-validated discriminant analysis on scores of a principal component analysis (PCA) of 23 morphometric variables in three species of *Hoplias malabaricus* species complex. Species-code: Harg, *Hoplias argentinensis*, new species; Hmis, *H. misionera*; Hmbi, *H. mbigua*.

Groups	Predicted group membership (%)			
	Harg (n = 19)	Hmis (n = 21)	Hmbi (n = 8)	Total
Harg	89.5	5.3	5.3	100
Hmis	9.5	90.5	0	100
Hmbi	0	0	100	100

Note: 100 % of original grouped cases correctly classified

91.7 % of cross-validated grouped cases correctly classified.

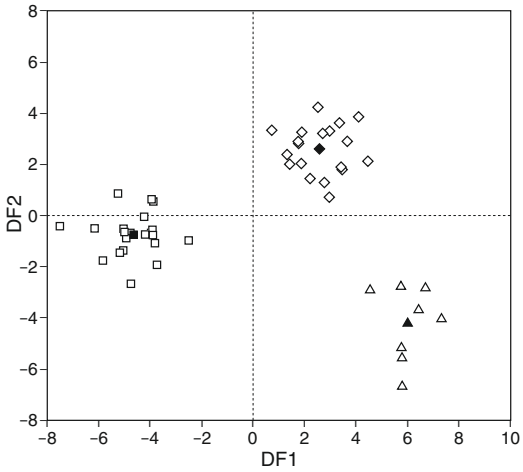

Fig. 4. Score plot of first two discrimant functions (DF1 and DF2) from a discriminant analysis performed on all principal component scores of a principal component analysis (PCA) on 23 interlandmarks distances taken on three species of *Hoplias malabaricus* species complex: *H. argentinensis* (◇); *Hoplias mbigua* (△); *H. misionera* (□). Filled symbols indicate the centroids.

misclassifications were scarce, with a highest rate of 9.5 % of the new species misclassified as *H. misionera* (Table 5). The DA clearly segregated the three groups (intrepreted as species) being their centroids and individuals separated both on the first and second discriminant functions (Fig. 4). There was no overlap for the centroids/specimens (Fig. 4).

Hoplias argentinensis, new species
(Fig. 5, Table 6)

Hoplias malabaricus. – McDonagh, 1934 [record, Laguna El Carpincho, Buenos Aires]; Ringuelet, 1942 [record, Laguna Chascomús, Buenos Aires]; Dománico et al., 1993 [age and growth], 1998 [age and growth]; Rosso et al., 2012 [genetics]; Díaz et al., 2016 [in part, genetics].

Hoplias aff. *malabaricus*. – Balboni et al., 2011 [age and growth].

Hoplias cf. *malabaricus*. – Rosso et al., 2016 [genetics].

Holotype. UNMDP 4417, 302 mm SL; Argentina: Santa Fe Province: Río Paraná basin: Río Coronda, 31°50.1 'S 60°51.5 'W; J. J. Rosso, E. Mabragaña & M. González-Castro, 3 Dec 2015.

Paratypes. All from Argentina (from South to North): 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

Table 3. (continued).

BOLD ID	Determined as	Voucher number	Basin	Locality
HPRB079-17	*Hoplias malabaricus*	GFSU14933	Guiana Shield Basin	Latambo Creek
HPRB080-17	*Hoplias malabaricus*	SUGF12425	Guiana Shield Basin	Cascade Moi Moi
HPRB081-17	*Hoplias malabaricus*	SU01076	Guiana Shield Basin	Mindrinetti kreek
HPRB082-17	*Hoplias malabaricus*	SU08557	Guiana Shield Basin	Crique Wawapsi
HPRB083-17	*Hoplias malabaricus*	SU08558	Guiana Shield Basin	Crique Wawapsi
HPRB084-17	*Hoplias malabaricus*	GFSU14945	Guiana Shield Basin	Latambo Creek
FARGB525-12	*Hoplias misionera*	UNMDP 574	La Plata River	Arroyo sin nombre
FWFA170-14	*Hoplias misionera*	UNMDP 1868	La Plata River	Río Paraguay
FWFA185-14	*Hoplias misionera*	UNMDP 1950	La Plata River	Río Paraguay
FWFA187-14	*Hoplias misionera*	UNMDP 1983	La Plata River	Río Paraná
FWFA494-15	*Hoplias misionera*	UNMDP 3321	La Plata River	Riacho Saladillo
FWFA495-15	*Hoplias misionera*	UNMDP 3322	La Plata River	Riacho Saladillo
FWFA496-15	*Hoplias misionera*	UNMDP 3328	La Plata River	Riacho Salado
FWFA497-15	*Hoplias misionera*	UNMDP 3329	La Plata River	Riacho Salado
FWFA500-15	*Hoplias misionera*	UNMDP 3371	La Plata River	Riacho Mbiguá
FWFA501-15	*Hoplias misionera*	UNMDP 3376	La Plata River	Riacho Mbiguá

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 Fe 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.

Latitude	Longitude	BIN
4°17.4'N	55°48.2'W	BOLD:ABZ3047
4°25.3'N	57°11.4'W	BOLD:ABZ3047
5°07.1'N	55°16.9'W	BOLD:ABZ3047
3°10.7'N	55°25.1'W	BOLD:ABZ3047
3°10.7'N	55°25.1'W	BOLD:ABZ3047
4°17.4'N	55°48.2'W	BOLD:ABZ3047
27°27.8'S	54°57.1'W	BOLD:AAB1732
26°15.8'S	58°10.1'W	BOLD:AAB1732
26°15.8'S	58°10.1'W	BOLD:AAB1732
27°47.5'S	58°49.4'W	BOLD:AAB1732
26°26.6'S	58°23.9'W	BOLD:AAB1732
26°26.6'S	58°23.9'W	BOLD:AAB1732
26°28.9'S	58°18.6'W	BOLD:AAB1732
26°28.9'S	58°18.6'W	BOLD:AAB1732
26°32.7'S	58°30.7'W	BOLD:AAB1732
26°32.7'S	58°30.7'W	BOLD:AAB1732

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.

Diagnosis. The medial margins of contralateral dentaries converging abruptly to mandibular symphysis in ventral view, four latero-sensory pores in dentary and the presence of tooth plates on basihyal and basibranchial bones diagnose this new species as a member of the *H. malabaricus* group. The number of predorsal scales (17–19) and vertebrae (42–43) distinguishes *Hoplias argentinensis* from *H. misionera* (15–17 scales and 39–40 vertebrae) and *H. malabaricus* (15–16 scales and 39–41 vertebrae). The number of lateral-line scales (41–44) distinguishes *H. argentinensis* from *H. malabaricus* (37–40). *Hoplias argentinensis* differs from *H. misionera* in the last vertical series of scales on the base of caudal-fin rays (forming a straight line vs. a marked curved line, respectively), in the number of gill rakers on first epibranchial (11–13 vs. 10–11, respectively) and dorsal-fin rays (13–14 vs. 14–16, respectively). Color pattern of lower jaw distinguishes *Hoplias argentinensis* (brown bands absent) from *H. mbigua* (five transverse brown bands). The number of scales around caudal peduncle distinguishes *H. argentinensis* from *H. microlepis* (20 vs. 22–24, respectively). *Hoplias argentinensis* can be further distinguished from *H. teres* by the snout depth (16.6–23.2 vs. 24.4–26.6 % HL, respectively) and width (18.4–24.9 vs. 29.4–29.5 % HL, respectively). Moreover, *Hoplias argentinensis* is further characterized by a shorter head, larger predorsal distance and higher body at dorsal-fin base (see detailed comments in the discussion).

Description. Proportions of morphometric features in Table 6. Body notably subcylindrical. Anterior profile of head angular in lateral view. Dorsal profile of head markedly straight. Lateral margin of skull markedly concave at orbit. Dorsal profile of body convex from postoccipital region to dorsal-fin origin; posteroventrally inclined along dorsal-fin base; conspicuously straight to origin of dorsalmost procurrent caudal-fin ray. Ventral profile of body convex to anal-fin origin, concave to origin of ventralmost procurrent caudal-fin ray. Greatest body depth at vertical

Fig. 5. *Hoplias argentinensis*, UNMDP 4417, holotype, 302 mm SL; Argentina: Río Coronda, Río Paraná basin.

through origin of dorsal fin. Medial margins of contralateral dentaries converging abruptly to midline forming inverted V in ventral view. Upper jaw shorter than lower jaw, more so in specimens larger than 250 mm SL. Lip fleshy. Lower lip notably fleshier than upper lip. Anterior nostril with incomplete tubular skin flap covering 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. Teeth caniniform in both jaws. Largest canines covered by skin fold. Single premaxillary tooth row with 9 (4), 10* (26) or 11 (2) teeth. First two medial teeth large, followed by four to five smaller teeth, and other two large canines. First and last canines in this series largest. One or two small teeth posterior to last largest canine and almost in contact with small first maxillary tooth. Maxillary teeth 30 (1), 31 (1), 33 (1), 36 (7), 37 (4), 38 (3), 39 (3), 40 (2), 41 (1), 42* (4) 44 (2) or 46 (1); first five or six increasing progressively in size. Dentary external series with three or four small teeth followed by two large canines. Posteriorly, a series of four or five small teeth followed by 10–15 teeth arranged in repetitive series of one large and one or two small conic

Table 6. Morphometric data of *Hoplias argentinensis*, 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; min, minimum; max, maximum. Range includes the holotype.

		Holotype	n	mean	min	max	SD
	Standard Length	302	38	200.4	90	410	–
1	Body depth	23.5	38	23.3	21.7	25.6	0.92
2	Head length	30.8	38	30.6	28.7	33.9	1.15
3	Pectoral-fin length	17.2	38	17.3	15.8	19.7	1.15
4	Pelvic-fin length	18.7	38	18.6	16.2	20.9	1.20
5	Anal-fin length	18.9	38	18.4	16.3	21.1	1.23
6	Dorsal-fin length	31.7	38	30.2	27.8	32.8	1.21
7	Dorsal-fin base length	18.5	38	17.8	16.0	20.2	0.91
8	Anal-fin base length	8.4	38	9.3	7.9	11.0	0.79
9	Pre-pectoral length	30.4	38	28.8	26.9	32.1	1.38
10	Pre-pelvic length	57.7	38	54.3	51.8	57.7	1.50
11	Pre-dorsal length	50.6	38	50.1	47.3	52.6	1.41
12	Pre-anal length	83.1	38	81.1	77.9	86.5	2.02
13	Caudal-peduncle depth	13.7	38	13.7	11.8	14.9	0.63
14	Caudal-peduncle length	12.5	38	12.7	10.9	14.5	0.89
15	Head depth	49	38	49	41	56	3.08
16	Snout length	24	38	24	21	27	1.46
17	Snout width	22	38	23	18	25	1.51
18	Snout depth	22	38	20	17	23	1.28
19	Pre-nasal distance	16	38	15	12	18	1.50
20	Orbital diameter	13	38	17	12	22	2.52
21	Interorbital width	28	38	27	22	30	1.76
22	Upper jaw length	54	38	52	48	57	2.28

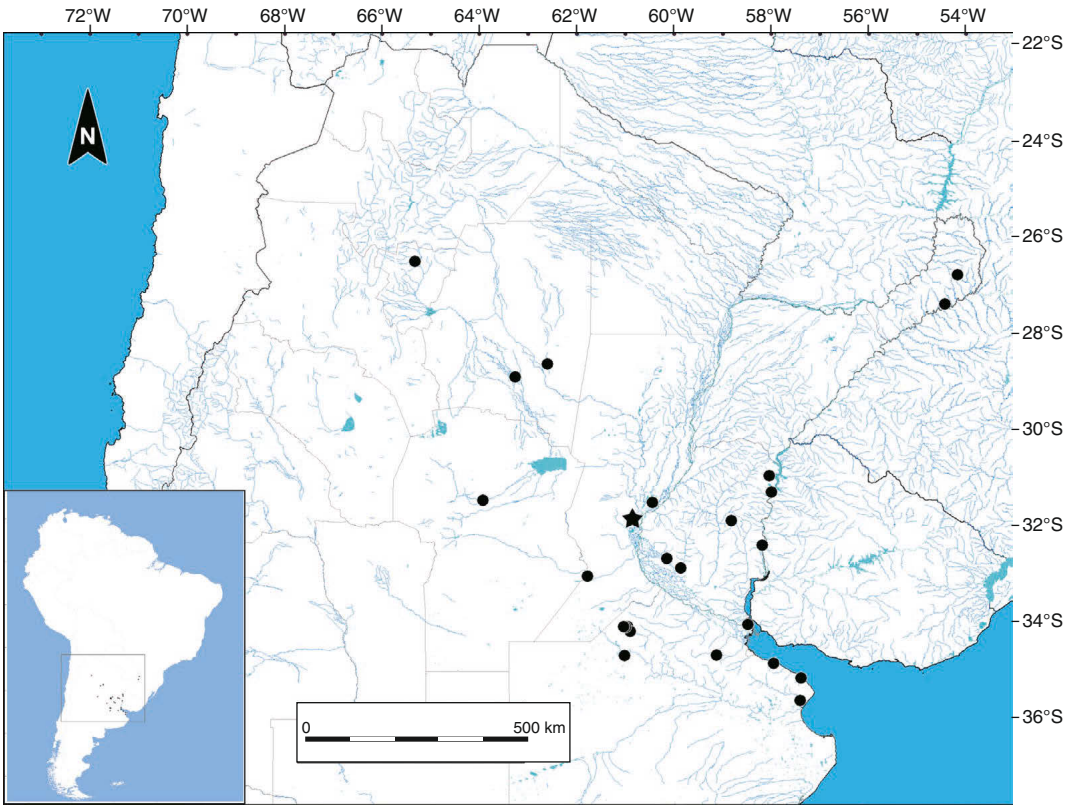

Fig. 6. Known distribution of *Hoplias argentinensis* in Río de La Plata basin. ★: type locality.

teeth. Internal series of dentary (about 15–17 very small teeth) beginning immediately posterior or slightly anterior to last conical tooth of external row. Accessory ectopterygoid not fragmented, bearing 10*(7), 11(6), 12(8) or 13(3) conical teeth along its ventrolateral margin. Bony tooth plates on basihyal and basibranchials displayed in two different arrangements: two lateral rows (n=13*) or a central large plate formed by several smaller plates (n=4). Dorsal-fin rays ii,11(3); ii,12*(30) or iii,11(3). Dorsal-fin origin placed at mid-body, one or two scales anterior to vertical through pelvic fin origin. Tip of longest ray of depressed dorsal fin extending slightly beyond vertical through anal-fin origin. Anal-fin rays ii,7(3) or ii,8*(34). Pectoral-fin rays i,11*(2) i,12(10); i,13(12) or i,14(12). Tip of pectoral fin separated from pelvic-fin origin by three to six scales. Total pelvic-fin rays i,7*(35) or i,8(2). Tip of pelvic fin separated from vertical through anus by one to five scales. Caudal-fin rays i,14,i(2) or i,15,i*(35). Predorsal scales 17*(12), 18(15) or 19(7) in irregular series. Lateral line with 41(13), 42(15), 43*(8) or 44(2) perforated scales, plus one or two unperforated scales anteriorly and located beneath opercle membrane. Longitudinal series of scales between dorsal-fin origin and lateral line 5.5(4) or 6*(34); between lateral line and pelvic-fin origin 5*(30), 5.5(2) or 6(6). Longitudinal series of scales around caudal peduncle, invariably 20. Last vertical series of scales around caudal peduncle nearly straight. First epibranchial with 11(1), 12*(3) or 13(2) plate-like denticulate gill rakers. One raker on cartilage. Ceratobranchial with five elongated rakers followed by 11(1), 12*(3), 13(1) or 14(1) small plate-like rakers. Latero-sensory canal along ventral surface of dentary with four pores (n=38). Six laterosensory pores in preopercle (n=38). Latero-sensory canal along infraorbitals with 10*(7), 11(21), 12(8) 13(1) or 14(1) pores. Three (27), 4(10) or 5(1) pores in infraorbital 1. Two (1) or 3(37) pores in infraorbital 2. One (37) or 2(1) pores in both infraorbitals 3 and 4. Two (9), 3(27), 4(1) or 6(1) pores in infraorbital 6. Infraorbital 5 lacking

pores. Eleven (33) or 12 (5) pores in laterosensory system of dorsal surface of head. Two pores on nasal (38), 4 (36) or 5 (2) pores on frontal; anteriormost at mid-length of orbital diameter, followed posteriorly by three pores laterally arranged. One pore (38) on posterior end of symphysis between parietal bones. Two pores (38) on pterotic bone. Supraopercle and extra-scapular bones with following combination of pores: 1:1*(20); 0:2(15); 2:0(1); or 0:3(1). Total vertebrae 42(2) or 43(1). Supraopercle large, contacting anteriorly with posterodorsal margin of infraorbital 5 and posterior margin of infraorbital 6.

Color in alcohol. Dorsal half of body dark brown becoming lighter towards ventral half. Light cream blotches irregularly spaced along just below lateral line and confined to posterior half of body in larger specimens. Ventral surface of body light cream. Lateral posteriorly-oriented chevron blotches, irregularly spaced with distance between subsequent blotches gradually decreasing towards caudal peduncle. Blotches less conspicuous in larger specimens. Specimens smaller than 160 mm SL with distinctively dark midlateral band extending along entire length of lateral line. Light stripe between midlateral band and dark dorsal surface. In larger specimens band is largely inconspicuous. Dark brown roundish spot on dorsal half of caudal peduncle close to base of uppermost procurrent rays. Three dark bands in infraorbital region extending radially from orbit to distal margin of infraorbitals 2, 3 and 6. Patch of dark brown pigmentation covering entire mid-length of opercular bone, sometimes forming distinct band reaching posterior border of orbit. Last vertical series of scales on base of caudal-fin rays dark-pigmented distally, sometimes forming thin dark vertical line. Fin rays light cream to light brown with alternating series of dark pigmented blotches and lighter areas. Blotches in dorsal, anal and pelvic fins extending over entire length of fin rays. In specimens larger than 160 mm SL, spots of anal fin larger and fused together forming five-seven dark stripes.

Distribution. *Hoplias argentinensis* is currently only known from Argentina. Specimens of *H. argentinensis* were collected over a wide geographic range of the lower Río de La Plata basin, including several localities of lower Río Paraná and Río Uruguay as well as streams, rivers and shallow lakes of the Pampa Plain (Table 3, Fig. 6). Specimens of *H. argentinensis* were also collected in two tributaries of the middle portion of Río Uruguay in Misiones as well as in the Río Sali-Dulce basin, in Tucumán and Santiago del Estero provinces. The type locality is situated within the Río Coronda drainage. The Río Coronda heads at the confluence of the Río Salado and Río Santa Fe and then runs as a braided channel of the Río Paraná at a distance ranging from 5 to 15 km from the main channel. The Río Coronda finally tributes its waters to the Río Paraná approximately 100 km downstream from its headwaters.

Unpublished results and ongoing field sampling programs show that *Hoplias argentinensis* is sympatric with *H. misionera* in the lower Río Paraná whereas *H. mbigua* is restricted to the lower Río Paraguay and middle Río Paraná. Moreover, in a comprehensive ichthyological survey of the lower Río Paraguay during four consecutive summers (2012–2015) only *H. mbigua* and *H. misionera* were recorded. This biogeographic scenario shows that *H. misionera* is sympatric with *H. mbigua* and *H. argentinensis* whereas *H. argentinensis* and *H. mbigua* do not live in sympatry.

Etymology. The specific name *argentinensis* is named in reference to Argentina.

Discussion

Overall, *Hoplias argentinensis* is unambiguously discriminated from all other species of the *H. malabaricus* group by means of traditional meristic and morphometric characters, landmarks-based morphometrics and genetic characters. Due to similarity in body shape, the species of the *Hoplias malabaricus* species-group are poorly distinguishable by traditional morphometric characters. Instead, species of this complex can be discriminated by means of landmarks-based morphometrics.

Meristic characters such as the number of predorsal and lateral-line scales as well as vertebral counts are also informative (see diagnosis and key to the species). Indeed, *H. argentinensis* is distinguished from *H. malabaricus* by greater counts in vertebrae, lateral-line and predorsal scales. Species of the *H. malabaricus* group can be easily separated in two discrete groups according to the number of vertebrae. One group with 39–41 vertebrae (low-count group) is composed by *H. misionera* and *H. malabaricus*; whereas a high-count group (42–43 vertebrae) is composed

by *H. mbigua*, *H. teres*, *H. microlepis* and *H. argentinensis* (Table 7).

Previous morphometric studies on *Hoplias* were limited to single species (e. g. Rodrigues Pacheco, 2004; Aguirre et al., 2013). Contrarily, in this paper, the landmarks-based morphometric analysis discriminated *H. argentinensis* from other species of the *H. malabaricus* complex. The body shape of *H. argentinensis* is characterized by a relatively short head and a deep body at the level of the second and third box-trusses. Additionally, the higher loadings for the 3–6, 4–6, 5–6 interlandmark distances strongly suggested that the dorsal fin originates more distant to the head with respect to other species. This is in agreement with the higher count of pre-dorsal scales in this species. Specimens of *H. misionera* were characterized by a deep body at the second and third box-trusses. They also presented positive loadings for all the variables related to the head shape, thus indicating that *H. misionera* is characterized by a larger head. Similarly, *H. mbigua* showed high values for distances defining the shape of head, also with higher loadings of 7–9, 7–10 and 8–10 variables, suggesting a relatively elongated body posterior to the dorsal fin (a long-tailed fish). Overall, *H. argentinensis* is a deep-bodied trahira with a short head and a large pre-dorsal region, whereas *H. mbigua* and *H. misionera* present larger heads, being the former also characterized by a longer tail and the latter by a deeper body.

The COI sequence composition of *H. argentinensis* received a private BIN (AAZ3734) and clearly contributed to discriminate this new species from all the species of the *H. malabaricus* group with known COI sequence composition (Rosso et al., 2016). Moreover, genetics allowed discriminating the new species from topotypes of the type species of the genus, *H. malabaricus*. In addition to a private BIN, an outstanding large genetic divergence (more than 9 %) separates *H. argentinensis* from its nearest neighbor. This result is congruent with the average congeneric distance for freshwater fishes by means of DNA barcoding, estimated to be approximately 8 % (Ward et al., 2009).

Three nominal species, *H. malabaricus*, *H. microlepis* and *H. teres*, have long been considered as valid species-names within the *H. malabaricus* species group. Nevertheless, only *H. microlepis* has been revisited since its original description when Mattox et al. (2014) presented a redescription of *H. microlepis*, designated a lectotype, and estimated the geographic distribution of the species. *Hoplias malabaricus* proved to be a species complex based on cytogenetic (Bertollo et al., 1986, 2000; Scavone, 1994; Cioffi et al., 2009; Blanco et al., 2010; Grassi et al., 2017) and molecular evidence (Rosso et al., 2012; Marques et al., 2013; Pereira et al., 2013). Recently two additional species of the *H. malabaricus* species complex (*H. mbigua* and *H. misionera*) were recognized and described (Azpelicueta et al., 2015; Rosso et al., 2016). Such taxonomic, molecular and cytogenetic results largely improved our knowledge about the diversity of the *H. malabaricus* species complex. However, large areas of South America are not fully sampled and the real diversity of species within this group may be considerably larger than currently known. In this respect, information about geographic variation of taxonomic characters of *H. malabaricus* sensu stricto will be of great relevance for further recognition and discrimination of taxonomic entities within this species complex.

Key to the species of the *Hoplias malabaricus* group

1 – Last vertical series of scales on caudal-fin base curved. Most specimens with a Y-shaped disposition of medial margins of contralateral dentaries (Figs. 7–8). ..... *Hoplias misionera*

– Last vertical series of scales on caudal-fin base nearly straight. Most specimens with a V-shaped disposition of medial margins of contralateral dentaries (Figs. 7–8). 2

2 – Thirty-seven to 40 scales on the lateral line, 15–16 pre-dorsal scales, 39–41 vertebrae (Table 7). ..... *H. malabaricus*

– Forty to 46 scales on the lateral line, 15–19 pre-dorsal scales, 42–43 vertebrae (Table 7). 3

3 – Five distinctive transverse brown bands in the lower jaw. 4

Fig. 7. Medial margin of contralateral dentaries in species of the *Hoplias malabaricus* group. **a,** Y-shaped contralateral dentaries, *H. misionera*, UNMDP 3865, 188 mm SL; **b,** V-shaped contralateral dentaries, *H. argentinensis*, UNMDP 1279, 240 mm SL. Scale bars: 20 mm.

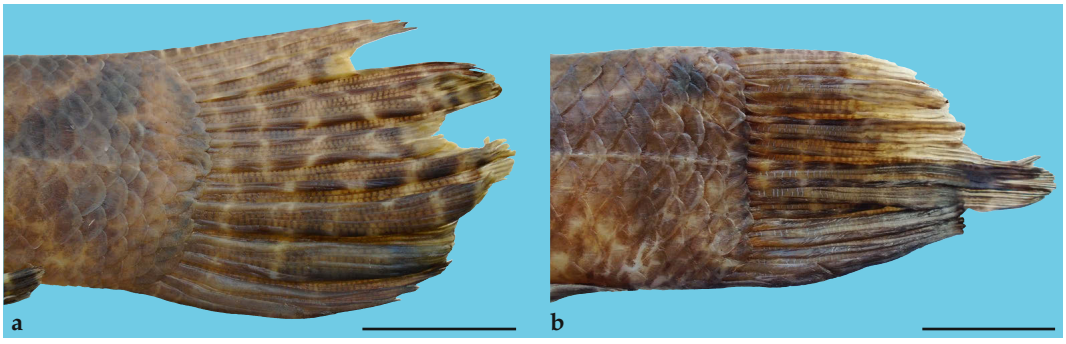

Fig. 8. Pattern of last vertical series of scales on base of caudal-fin rays in species of the *Hoplias malabaricus* group. **a,** Vertical series of scales on caudal fin curved, *H. misionera*, UNMDP 3865, 188 mm SL; **b,** vertical series of scales on caudal fin nearly straight, *H. argentinensis*, UNMDP 1279, 240 mm SL. Scale bars: 20 mm.

Table 7. Discrimination of species in *Hoplias malabaricus* group by the number of vertebrae.

Species/Vertebrae	39	40	41	42	43
Hoplias misionera	x	x			
Hoplias malabaricus	x	x	x		
Hoplias mbigua				x	
Hoplias teres				x	
Hoplias microlepis				x	x
Hoplias argentinensis				x	x

– Color pattern of lower jaw distinct from above. 5

4 – Twenty-two to 24 scales around caudal peduncle, 17–19 pre-dorsal scales, 43–46 scales on the lateral line, dorsal profile of head straight (Fig. 9). ..... *H. microlepis*

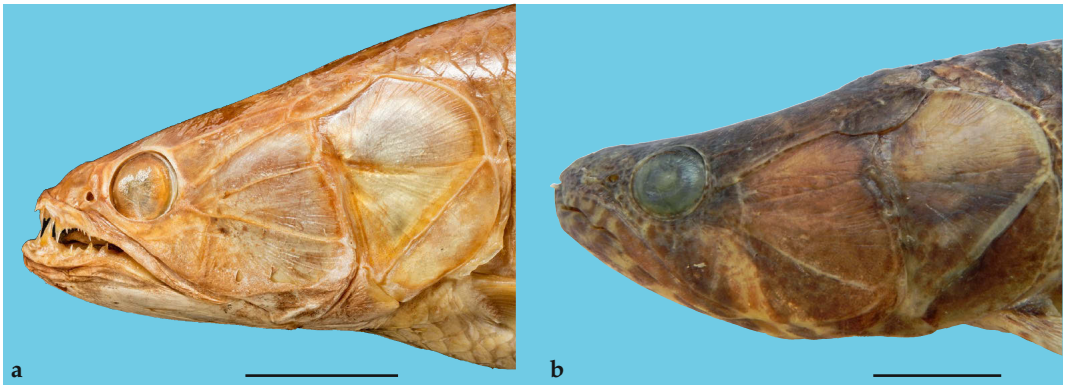

Fig. 9. Dorsal profile of head in species of the *H. malabaricus* group. **a,** Dorsal profile straight, *H. microlepis*, BMNH 1864.1.26.222, 225 mm SL; **b,** dorsal profile concave, *H. mbigua*, CI-FML 6764, 248 mm SL. Scale bars: 20 mm.

– Twenty scales around caudal peduncle, 15–17 pre-dorsal scales, 42–44 scales on the lateral line, dorsal profile of head concave (Fig. 9).
..... *H. mbigua*

5 – Forty-one to 44 scales on the lateral line, snout width less than 25 % of head length.
..... *H. argentinensis*, new species

– 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.

H. 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.

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

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

H. 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 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: Rio Paraná.

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

Acknowledgements

This research was partially funded by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina: IBOL grants and PIP 11220130100339 CO), Agencia Nacional de Promoción Científica y Tecnológica (PICT-2014-0665, JMDA and PICT2014-0580, YC) and Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata (15/E619 EXA669/14). JJR acknowledges the financial support from the International Development Research Centre (IDRC) of Canada. YC, MG-C, EM, JJR, MSD and JMDA acknowledge the support provided by CONICET. SB is much indebted to Fundación Félix de Azara for financial and technical support. Ministerio de Ecología y Recursos Naturales Renovables of Misiones granted field permissions and Federico Castia assisted during field trips to the Estación Biológica "Marcio Ayres". Raphael Covain (Museum of Natural History of Geneva) gently shared the sequences of *H. malabaricus* from the Guianas. JMDA is indebted to Peter Bartsch (ZMB) for assistance and technical support in a recent visit to the ZMB and for providing photographs and x-rays of the type specimens of *Hoplias malabaricus* and Celia Bueno (MHNN) for providing photographs and x-rays of the type specimen of *Erythrinus macrodon*. Also, JMDA extends his appreciation to Guy Duhamel, Patrice Pruvost, Romain Causse, Claude Ferrara and Zora Gabsi (MNHN). JJR and JMDA are indebted to James Maclaine (BMNH) for technical support in a recent visit and providing photographs of the lectotype and paralectotype of *H. microlepis*. JJR extends his appreciation to Sandra Raredon (USNM) for providing photographs and x-rays of the holotype of *Macrodon ferox*. Authors are greatly indebted to Gastón Aguilera (CI-FML) for support provided during revision of the holotype and paratypes of *Hoplias mbigua*. Diego Nadalín and Hugo López kindly received the visit of the first author when examining specimens in the Museo de La Plata. Authors also acknowledge the contribution of Mauricio Benítez in providing additional paratypes of *Hoplias mbigua*. Authors are very grateful to Carlos Capiel and his team for technical assistance in obtaining x-rays of *Hoplias argentinensis*. Nalani Schenell (MNHN) gently assisted authors in obtaining x-rays of *Hoplias teres* and *Macrodon tareira*. The map of distribution (Fig. 6) was performed by Jorge Liotta (Museo de Ciencias Naturales "P. Antonio Scasso") following the standards from the database of geographic distribution of freshwater fishes of Argentina: http://www.pecesargentina.com.ar.

Literature cited

Aguirre, W. E., V. R. Shervette, R. Navarrete, P. Calle & S. Agorastos. 2013. Morphological and genetic divergence of *Hoplias microlepis* (Characiformes: Erythrinidae) in rivers and artificial impoundments of western Ecuador. Copeia, 2013: 312–323.

Azpelicueta, M. M., M. F. Benítez, D. R. Aichino & C. M. D. Mendez. 2015. A new species of the genus *Hoplias* (Characiformes, Erythrinidae), a tararira from the lower Paraná River, in Misiones, Argentina. Acta Zoológica Lilloana, 59: 71–82.

Balboni, L., D. C. Colautti & C. R. M. Baigún. 2011. Biology of growth of *Hoplias malabaricus* (Bloch, 1794) in a shallow pampean lake (Argentina). Neotropical Ichthyology, 9: 437–444.

Brannan, D. A., M. F. Esplen & J. J. Gray. 1999. Geometry. Cambridge University Press, Cambridge, 497 pp.

Bertollo, L. A. C., O. Moreira-Filho & P. M. Galetti. 1986. Cytogenetics and taxonomy: considerations based on chromosome studies of freshwater fish. Journal of Fish Biology, 28: 153–159.

Bertollo, L. A. C., G. G. Born, J. A. Dergam, A. S. Fenocchio & O. Moreira-Filho. 2000. A biodiversity approach in the Neotropical fish *Hoplias malabaricus*. Karyotypic survey, geographic distribution of cytotypes and cytotaxonomic considerations. Chromosome Research, 8: 603–613.

Blanco, D. R., R. L. Lui, L. A. C. Bertollo, D. Diniz & O. Moreira-Filho. 2010. Characterization of invasive fish species in a river transposition region: evolutionary chromosome studies in the genus *Hoplias* (Characiformes, Erythrinidae). Reviews in Fish Biology and Fisheries, 20: 1–8.

Bookstein, F. B., R. Chernoff, J. Elder, Jr. Humpries, G. R. Smith & R. E. Strauss. 1985. Morphometrics in evolutionary biology. Academy of Natural Sciences of Philadelphia Special Publications, 15: 1–277.

Cioffi, M. B., C. Martins, L. Centofante, U. Jacobina & L. A. C. Bertollo. 2009. Chromosomal variability among allopatric populations of Erythrinidae fish *Hoplias malabaricus*: mapping of three classes of repetitive DNAs. Cytogenetic and Genome Research, 125: 132–141.

Díaz, J., G. V. Villanova, F. Brancolini, F. Del Pazo, V. M. Posner, A. Grimberg & S. E. Arranz. 2016. First DNA barcode reference library for the identification of South American freshwater fish from the lower Paraná River. PLoS One, 11: 1–20.

Dománico, A. A. 1998. Edad y crecimiento de *Hoplias malabaricus malabaricus* (Bloch, 1794) (Teleostei, Erythrinidae) en la laguna de San Miguel del Monte (Argentina) y comparación con otros ambientes lénticos pampásicos. Revista del Museo Argentino de Ciencias Naturales, 7: 31–41.

Dománico, A. A., R. Delfino & L. Freyre. 1993. Edad y crecimiento de *Hoplias malabaricus malabaricus* (Bloch, 1794) (Teleostei, Erythrinidae) en la laguna

de Lobos (Argentina). Iheringia, Série Zoológica, 74: 141–149.
Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research, 32: 1792–1797.
Eschmeyer, W. N., R. Fricke & R. van der Laan. 2017. Catalog of Fishes: genera, species and references. Available from: http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp (accessed 2 August 2017).
Fink, W. L & S. H. Weitzman. 1974. The so-called cheirodontin fishes of Central America with descriptions of two new species (Pisces: Characidae). Smithsonian Contributions to Zoology, 172: 1–46.
García-Vásquez, A. R., G. Vargas, H. Sanchez-Ribeiro, R. A. Ismiño-Orbe, C. Nolorbe-Payahua, C. R. García-Dávila & J. S. Tello-Martín. 2014. Recursos pesqueros y pesca en los ríos Curaray, Arabela y Napo (Amazonía peruana). Folia Amazónica, 23: 95–104.
González-Castro, M., A. L. Ibáñez, S. Heras, M. I. Roldán & M.B. Cousseau. 2012. Assessment of lineal versus landmarks- based morphometry for discriminating species of Mugilidae (Actinopterygii). Zoological Studies, 51: 1515–1528.
González-Castro, M., J. J. Rosso, E. Mabragaña & J. M. Díaz de Astarloa. 2016. Surfing among species, populations and morphotypes: inferring boundaries between two species of new world silversides (Atherinopsidae). Comptes Rendus Biologies, 399: 10–29.
González-Castro, M. & J. M. Díaz de Astarloa. 2017. Metodologías morfométricas aplicadas a la taxonomía integrativa de peces. Pp. 163–181 in: V. Abdala et al. (eds.), Morfología de vertebrados: conceptos, métodos y grupos de investigación en Argentina. EUDEM, Mar del Plata.
Goulding, M. 1980. The fishes and the forest: explorations in Amazonian natural history. University of California Press, Berkeley, 280 pp.
Grassi, D. J., A. C. Swarça, J. A. Dergam, M. C. Pastori & A. S. Fenocchio. 2017. Cytogenetic characterization of *Hoplias malabaricus* (Bloch, 1794) from the Ctalamochita River (Córdoba, Argentina): first evidence for southernmost populations of this species complex and comments on its biogeography. Comparative Cytogenetics, 11: 15–28.
Ivanova, N. V., J. R. Dewaard & P. D. N. Hebert. 2006. An inexpensive, automation friendly protocol for recovering high-quality DNA. Molecular Ecology Notes, 6: 998–1002.
Ivanova, N. V., T. S. Zemlak, R. H. Hanner & P. D. N. Hebert. 2007. Universal primer cocktails for fish DNA barcoding. Molecular Ecology Notes, 7: 544–548.
Iwaszkiw, J. M. & F. Firpo Lacoste. 2011. La pesca artesanal en la Cuenca del Plata (Argentina) y sus implicancias en la conservación de la biodiversidad. Revista del Museo Argentino de Ciencias Naturales, 13: 21–25.
Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, 16: 111–120.
Lleonart, J., J. Salat & G. J. Torres. 2000. Removing allometric effects of body size in morphological analysis. Journal of Theoretical Biology, 205: 85–93.
Lombarte, A. & J. Lleonart. 1993. Otolith size changes related with body growth, habitat depth and temperature. Environmental Biology of Fishes, 37: 297–306.
Mac Donagh, E. 1934. Nuevos conceptos sobre distribución geográfica de los peces argentinos basados en expediciones del Museo de La Plata. Revista del Museo de La Plata, 34: 21–170.
Marques, D. F., F. A. Santos, S. S. Silva, I. Sampaio & L. R. R. Rodrigues. 2013. Cytogenetic and DNA barcoding reveals high divergence within the trahira, *Hoplias malabaricus* (Characiformes: Erythrinidae) from the lower Amazon River. Neotropical Ichthyology, 11: 459–466.
Mattox, G. M. T., M. Toledo-Piza & O. T. Oyakawa. 2006. Taxonomic study of *Hoplias aimara* Valenciennes, (1846) and *Hoplias macrophthalmus* (Pellegrin, 1907) (Ostariophysi, Characiformes, Erythrinidae). Copeia, 2006: 516–528.
Mattox, G. M. T., A. G. Bifi & O. T. Oyakawa. 2014. Taxonomic study of *Hoplias microlepis* (Günther, 1864), a trans-Andean species of trahiras (Ostariophysi: Characiformes: Erythrinidae). Neotropical Ichthyology, 12: 343–352.
Oyakawa, O. T. & G. M. T. Mattox. 2009. Revision of the Neotropical trahiras of the *Hoplias lacerdae* species-group (Ostariophysi: Characiformes: Erythrinidae) with descriptions of two new species. Neotropical Ichthyology, 7: 117–140.
Oyakawa, O. T. 1990. Revisão sistemática das espécies do gênero *Hoplias* (grupo *lacerdae*) da Amazônia brasileira e região leste do Brasil (Teleostei: Erythrinidae). M.Sc. Thesis. Universidade de São Paulo, 200 pp.
— 2003. Family Erythrinidae. Pp. 238–240 in: R. E. Reis et al. (eds.), Check list of the freshwater fishes of South and Central America. Edipucrs, Porto Alegre.
Pereira, L. H. G., R. Hanner, F. Foresti & C. Oliveira. 2013. Can DNA barcoding accurately discriminate megadiverse Neotropical freshwater fish fauna? BMC Genetics, 14: 1–14.
Ratnasingham, S. & P. D. N. Hebert. 2007. BOLD: the barcode of life data system (www.barcodinglife.org). Molecular Ecology Notes, 7: 355–364.
Ratnasingham, S. & P. D. N. Hebert. 2013. DNA-based registry for all animal species: the barcode index number (BIN) system. PLoS ONE, 8: 8e66213.
Reis, R. E., J. S. Albert, F. Di Dario, M. M. Mincarone, P. Petry & L. A. Rocha. 2016. Fish biodiversity and conservation in South America. Journal of Fish Biology, 89: 12–47.

Ringuelet, R. A. 1942. Ecología alimenticia del pejerrey (*Odontesthes bonariensis*) con notas limnológicas sobre la laguna Chascomús. Revista del Museo de La Plata, Nueva Sección, Sección Zoología, 2: 427–461.

Rodrigues Pacheco, M. 2004. Estudo morfológico de duas populações de *Hoplias* grupo *malabaricus* (Characiformes: Erythrinidae) procedentes da bacia do Rio Grande, Estado de São Paulo. M.Sc. Thesis. Universidade Federal de São Carlos, 107 pp.

Rosso, J. J., E. Mabragaña, M. González-Castro & J. M. Díaz de Astarloa. 2012. DNA barcoding Neotropical fishes: recent advances from the Pampa Plain, Argentina. Molecular Ecology Resources, 12: 999–1011.

Rosso, J. J., E. Mabragaña, M. González-Castro, M. S. Delpiani, E. Avigliano, N. F. Schenone & J. M. Díaz de Astarloa. 2016. A new species of the *Hoplias malabaricus* species complex (Characiformes: Erythrinidae) from the La Plata River basin. Cybium, 40: 199–208.

Scavone, M. D. P. 1994. Sympatric occurrence of two karyotypic forms of *Hoplias malabaricus* (Pisces, Erythrinidae). Cytobios, 80: 223–227.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei & S. Kumar. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular and Biological Evolution, 28: 2731–2739.

Ward, R. D., R. Hanner & P. D. N. Hebert. 2009. The campaign to DNA barcode all fishes, FISH-BOL. Journal of Fish Biology, 74: 329–356.

Received 29 December 2017
Revised 19 January 2018
Accepted 31 May 2018