A new species of the *Hoplias malabaricus* species complex (Characiformes: Erythrinidae) from the La Plata River basin

by

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© SFI Received: 1 Nov. 2015 Accepted: 12 Sep. 2016 Editor: H. Persat

Key words

Erythrinidae Hoplias malabaricus Hoplias misionera n. sp. Argentina La Plata River Basin New species Abstract. - Hoplias malabaricus is one of the 12 valid species in the genus Hoplias, the most diverse genus among the Erythrinidae. During the last decades, cytogenetic studies had shown that this species represents a well-populated species complex. The new species of the Hoplias malabaricus group is described from the Uruguay, Paraná and Paraguay Rivers, in Argentina and Brazil. The Y-shaped configuration of the medial margins of dentaries easily separates Hoplias misionera n. sp. from all the remainder species of Hoplias (parallel-shaped in H. lacerdae group and H. aimara and V-shaped in H. malabaricus group). Hoplias misionera n. sp. can be further distinguished from *H. malabaricus* by the total number of dorsal (14-16 vs 14) and pectoral (12-14 vs 11) fin rays and the number of scales in lateral line (40-43 vs 38-39). Hoplias misionera n. sp. differs from H. microlepis in unbranched (2-4 vs 2) dorsal-fin rays, the number of scales along lateral line (40-43 vs 43-47) and a lower number of scales around the caudal peduncle (20 vs 22-24, usually 24). Hoplias misionera n. sp. differs from *H. teres* by number of scales in lateral line (40-43 vs 38), number of total (14-16 vs 13) and unbranched (2-4 vs 3) dorsal-fin rays and pectoral (12-14 vs 13-15) fin rays, total vertebrae count (39-40 vs 42), larger dorsal-fin base (16.7-20.9 vs 16.2-17.6% SL) and body depth (20.6-25.4 vs 17-20.6% SL). Finally, Hoplias misionera n. sp. can be distinguished from the recently described *H. mbigua* by a distinctly shorter snout length (20.4-24.7 vs 25.2-28.6% HL) and lower pre-nasal distance (12.5-16.2 vs 15.2-18.4% HL). *Hoplias misionera* further differs from H. mbigua by dorsal profile of head markedly straight vs dorsal profile of head markedly concave; lower jaw with either brown bands, dots or blotches vs always five distinctive transversally brown bands; infraorbital 5 lacking pores in laterosensory canal vs infraorbital 5 with one pore, last vertical series of scales on caudal peduncle forming marked curve vs forming a relatively straight line. Finally, colour pattern also contributes to the discrimination between *H. misionera* and *H. mbigua*.

Résumé. – Une nouvelle espèce du complexe *Hoplias malabaricus* (Characiformes : Erythrinidae) décrite du bassin du Río de la Plata, Argentine.

Hoplias malabaricus est l'une des 12 espèces valides du genre Hoplias, genre le plus diversifié parmi les Erythrinidae. Au cours des dernières décennies, les études cytogénétiques ont montré que cette espèce représente un riche complexe d'espèces. La nouvelle espèce du groupe Hoplias malabaricus est décrite des bassins de l'Uruguay, du Paraguay et du Paraná, en Argentine et au Brésil. La configuration en forme de Y de la symphyse des dentaires sépare facilement *Hoplias misionera* n. sp. de toutes les autres espèces d'*Hoplias* (parallèle dans le groupe *H. lacerdae* et chez *H. aimara* et en forme de V dans le groupe *H. malabaricus*). *Hoplias misionera* n. sp. peut être aussi distingué de *H. malabaricus* par le nombre total de rayons dorsaux (14-16 vs 14) et pectoraux (12-14 vs 11) et le nombre d'écailles de la ligne latérale (40-43 vs 38-39). *Hoplias misionera* n. sp. diffère de *H. microlepis* par le nombre de rayons non ramifiés de la nageoire dorsale (2-4 vs 2), le nombre d'écailles le long de la ligne latérale (40-43 vs 43-47) et le nombre inférieur d'écailles autour du pédoncule caudal (20 vs 22-24, généralement 24). Hoplias misionera n. sp. diffère de H. teres par le nombre d'écailles de la ligne latérale (40-43 vs 38), le nombre total de rayons aux nageoires dorsale (14-16 vs 13) et pectorales (12 - 14 vs 13-15), le nombre de rayons non ramifiés à la nageoire dorsale (2-4 vs 3), le nombre total de vertèbres (39-40 vs 42), la plus grande base de la nageoire dorsale (16,7 à 20,9 contre 16,2 à 17,6% SL) et la hauteur du corps (20,6 à 25,4 contre 17 à 20,6% SL). Enfin, *H. misionera* n. sp. peut être distingué de l'espèce récemment décrite *Hoplias mbigua* par un museau nettement plus court (20,4 à 24,7 contre 25,2 à 28,6% HL), et une distance pré-nasale plus petite (12,5 à 16,2 contre 15,2 à 18,4% HL). Hoplias misionera n. sp. diffère aussi de Hoplias mbigua : par un profil dorsal de la tête nettement droit contre un profil nettement concave ; par une mâchoire inférieure avec soit des bandes brunes, des points ou des taches contre une mâchoire présentant toujours cinq bandes brunes transversales bien distinctes ; par l'absence de pore sur le canal latéro-sensoriel du 5^e infraorbital contre la présence d'un pore ; par la dernière série verticale des écailles sur le pédoncule caudal formant une courbe marquée vs une ligne relativement droite. Enfin, le patron de coloration contribue également à la discrimination entre H. misionera n. sp. et H. mbigua.

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During the last decades, genetics has shown that several emblematic species from the freshwater fish fauna of South America (Torres et al., 2005; de Carvalho et al., 2011; Rosso et al., 2012), hidden during centuries, a not easily anticipated cryptic diversity. Among them, Hoplias malabaricus (Bloch, 1794) represents a well-populated species complex (Bertollo et al., 2000). Hoplias malabaricus is one of the 12 valid species in the genus Hoplias (Eschmeyer et al., 2016), the most diverse genus among the Erythrinidae, which also harbour the wolf fishes of genera Erythrinus and Hoplerythrinus (Oyakawa, 2003). Species of Hoplias may be classified following morphological characters into three different species groups (Oyakawa, 1990). One of these groups, Hoplias aimara (Valenciennes, 1847), has been revised and proved to be monotypic after the synonymization of Hoplias macrophtalmus (Pellegrin, 1907) (Mattox et al., 2006). The Hoplias lacerdae group encompasses five species: Hoplias lacerdae Miranda Ribeiro, 1908, Hoplias australis Oyakawa & Mattox, 2009, Hoplias brasiliensis (Spix, 1829), Hoplias intermedius (Günther, 1864) and Hoplias curupira Oyakawa & Mattox, 2009. A revision of Hoplias lacerdae was also performed (Oyakawa and Mattox, 2009). The third group, the Hoplias malabaricus group, contains the homonymous species together with Hoplias microlepis (Günther, 1864), Hoplias mbigua Azpelicueta, Benítez, Aichino & Mendez, 2015 and Hoplias teres (Valenciennes, 1847) but still lacks a formal revision. As an initial attempt to address the taxonomic complexity of the H. malabaricus species group, Mattox et al. (2014) presented a redescription of H. microlepis, designated lectotype and paralectotypes, and estimated the geographic distribution of the species. The Hoplias *malabaricus* group can be distinguished as those species with medial margins of dentary abruptly converging toward the mandibular symphysis forming an inverted V in ventral view, four pores of latero-sensory system on each branch of the dentary and tooth plates present on the basihial and basibranchials (Oyakawa, 1990). Recent results evidenced a strong geographic structure in the genetic identity at the species level in H. malabaricus from South America (Rosso et al., 2012; Ferreira Marques et al., 2013). The aim of this paper is to describe a new species of the *H*. malabaricus group from the Uruguay, Paraná and Paraguay Rivers, in Argentina and Brazil.

MATERIALS AND METHODS

A total of 18 individuals of *Hoplias misionera* were examined in order to characterize this new species. Meristic data were obtained by visual inspection and corroborated under microscope when necessary. Linear morphometric measurements were taken with a digital caliper to the nearest 0.1 mm. Measurements and counts were made on the left side of the body following Fink and Weitzman (1974) and Mattox *et al.* (2006). We additionally performed vertebral counts on radiographed specimens including the anterior four vertebrae of the Weberian apparatus. Institution abbreviations are as follows: UNMDP: Instituto de Investigaciones Marinas y Costeras, Mar del Plata, Argentine; CI-FML: Colección Ictiológica Fundación Miguel Lillo, Tucumán, Argentine; LBV: Laboratório de Biologia e Genética de Peixes, São Paulo, Brazil; MNHN: Muséum national d'Histoire naturelle, Paris, France; ZMB: Museum für Naturkunde, Berlin, Germany; MHNN: Muséum d'Histoire Naturelle de Neuchâtel, Switzerland; BMNH: Natural History Museum, United Kingdom.

In order to further discriminate and characterize the genetic identity of this new species, a small piece of white muscle was excised from 10 individuals to obtain their BAR-CODE sequences. 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 fish (Ivanova et al., 2007). This approach has proved to be effective in Neotropical marine (Mabragaña et al., 2011, 2012) and freshwater (Rosso et al., 2012) fish fauna. The BARCODE sequence of the holotype is presented as part of the description. The BARCODE sequences of the three paratypes from Brazil were gathered from the public portal of Barcode of Life Database (BOLD) (Ratnasingham and Hebert, 2007). Comparative distance-based analysis including other Hoplias cf. malabaricus from lower Plata basin was also performed. A neighbour-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). For each specimen, we report the corresponding Barcode Index Number (BIN) assigned by BOLD. BIN analysis clusters barcode sequences to create Operational Taxonomic Units that closely reflect species groupings (Ratnasingham and Hebert, 2013).

RESULTS

Hoplias misionera n. sp. (Fig. 1; Tab. I)

Hoplias malabaricus - Pereira et al., 2013

Holotype. – Argentine: Misiones. Stream tributary of the Acaraguá River (27°27'44.63"S; 54°57'5.58"W), Uruguay River Basin. Villa Bonita, Municipio de Campo Ramón, Oberá: UNMDP 574, 1 ex., 164 mm SL, Rosso, Mabragaña, Avigliano and Schenone coll., 07 Feb. 2011.

Paratypes. – Argentine: Misiones. Stream tributary of the Acaraguá River, Uruguay River Basin (27°27'44.63"S; 54°57'5.58"W): UNMDP 3320, 1 ex., 174 mm SL; UNMDP



Figure 2. – Configuration of the medial margins of the dentary in selected specimens of *Hoplias*. A: Parallel-shaped in *Hoplias lacerdae*, UNMDP 594, 163 mm SL, Ramos stream, Misiones, Argentina; B: V-shaped in *Hoplias cf malabaricus*, UNMDP 504, 159 mm SL, Rio Rojas, Ascención, Buenos Aires, Argentina; C: Y-shaped in *Hoplias misionera* n. sp., holotype, UNMDP 574, 164 mm SL, stream tributary of the Acaraguá River, Misiones, Argentina. Scale bars = 2 cm.

3391, 1 ex., 149 mm SL; UNMDP 3392, 1 ex., 104 mm SL, Rosso, Avigliano and Schenone coll., 10 Feb. 2014.

Formosa. Paraguay River, Laguna Oca: UNMDP 1950, 1 ex., 49 mm SL; UNMDP 1951, 1 ex., 50 mm SL, Rosso, Mabragaña, Avigliano and Schenone coll., 12 Apr. 2012; UNMDP 1868, 1 ex., 40 mm SL, Rosso, Mabragaña, Avigliano and Schenone coll., 09 Apr. 2012; Riacho Saladillo: UNMDP 3321, 1 ex., 142 mm SL; UNMDP 3322, 1 ex., 148 mm SL, Rosso, Mabragaña, del Rosso, Avigliano and Schenone coll., 08 Feb. 2014; Riacho Salado: UNMDP 3327, 1 ex., 171 mm SL; UNMDP 3328, 1 ex., 146 mm SL; UNMDP 3329, 1 ex., 134 mm SL, Rosso, Mabragaña, del Rosso, Avigliano and Schenone coll., 09 Feb. 2014; Riacho Mbiguá: UNMDP 3371, 1 ex., 154 mm SL; UNMDP 3376, 1 ex., 165 mm SL, Rosso, Mabragaña, del Rosso, Avigliano and Schenone coll., 08 Feb. 2014.

Chaco. Small interior lake in an island of Paraná River: UNMDP 1983, 1 ex., 75 mm SL, Rosso, Mabragaña, Avigliano and Schenone coll., 07 Apr. 2012.

Brazil: Sao Paulo. Lagoa Marginal, Paraná River: LBV 32184-32186, 3 ex., 77-155 mm SL, L.H.G. Pereira, F.F. Roxo, J.M. Henriques, R. Devidé and V. Paes, coll., 03 Jul. 2008.

Diagnosis

The Y-shaped configuration of the medial margin of dentaries easily separates *Hoplias misionera* n. sp. from all the remainder species of *Hoplias* (parallel-shaped in both *Hoplias lacerdae* group and *Hoplias aimara* and V-shaped in *Hoplias malabaricus* group; Fig. 2).

Hoplias misionera n. sp. can also be distinguished from species in the Hoplias malabaricus group by additional characters other than medial margin of dentaries. The higher number of total dorsal (14-16 vs 14) and pectoral (12-14 vs 11) fin rays and the number of scales in lateral line (40-43 vs 38-39) distinguishes Hoplias misionera n. sp. from Hoplias malabaricus. Hoplias misionera n. sp. differs from H. microlepis in unbranched (2-4 vs 2) dorsal-fin rays, the number of scales along lateral line (40-43 vs 43-47) and by a lower number of scales around the caudal peduncle (20 vs 22-24, usually 24). Hoplias misionera n. sp. differs from *H. teres* by number of scales in lateral line (40-43 vs 38), number of total dorsal-fin rays (14-16 vs 13) and (2-4 vs 3) unbranched dorsal-fin rays and pectoral-fin rays (12-14 vs 13-15), total vertebrae count (39-40 vs 42), larger dorsal-fin base (16.7-20.9 vs 16.2-17.6% SL) and body depth (20.6-25.4 vs 17-20.6% SL). Hoplias misionera n.

Table I. – Morphometric data of *Hoplias misionera* n. sp. Standard length in mm; values 1-14 are percentages of the standard length and values 15-22 are percentages of head length. SD = standard deviation.

	Holotype	Count	Paratypes			
			Mean	Minimum	Maximum	SD
Standard length	164.00	18	124.78	39.22	174.00	45.79
1. Body depth	24.21	18	23.36	20.60	25.46	1.32
2. Head length	30.61	18	32.71	30.61	34.57	1.10
3. Pectoral fin length	19.76	18	18.47	15.71	20.92	1.21
4. Pelvic fin length	19.15	18	20.11	18.51	22.44	1.16
5. Anal fin length	19.15	18	19.46	17.29	21.37	1.04
6. Dorsal fin length	33.23	14	33.24	30.68	34.77	1.39
7. Dorsal fin base length	19.45	18	18.80	16.72	20.92	1.21
8. Anal fin base length	8.96	18	9.12	7.11	10.04	0.72
9. Prepectoral distance	28.96	18	30.78	28.96	34.31	1.71
10. Prepelvic distance	53.41	18	54.96	51.93	58.08	1.50
11. Predorsal distance	50	18	49.24	46.88	51.83	1.30
12. Preanal distance	79.45	18	80.30	77.53	82.05	1.32
13. Caudal peduncle depth	14.02	18	13.76	12.03	14.85	0.70
14. Caudal peduncle length	10.37	18	11.41	9.98	13.99	1.09
15. Head depth	48.80	18	48.75	44.34	53.13	2.11
16. Snout length	22.91	18	22.99	20.47	24.72	1.30
17. Snout width	23.90	18	23.14	20.30	26.41	1.54
18. Snout depth	19.92	18	18.99	17.01	22.67	1.42
19. Pre nasal distance	15.94	18	14.43	12.52	16.20	1.14
20. Orbital diameter	18.73	18	19.77	15.66	27.41	3.69
21. Interorbital width	28.49	18	25.81	21.87	28.82	2.16
22. Upper jaw length	54.78	18	52.93	42.36	56.21	3.33

sp. can be distinguished from the recently described Hoplias mbigua by a distinctly shorter snout length (20.4-24.7 vs 25.2-28.6% HL) and lower pre-nasal distance (12.5-16.2 vs 15.2-18.4% HL). Hoplias misionera further differs from Hoplias mbigua by dorsal profile of head markedly straight vs dorsal profile of head markedly concave (Fig. 3); lower jaw with either brown bands, dots or blotches vs always five distinctive transversally brown bands; infraorbital 5 lacking pores in laterosensory canal vs infraorbital 5 with one pore, last vertical series of scales on caudal peduncle forming a marked curve vs forming a relatively straight line (Fig. 3); total vertebrae count 39-40 vs 42; first epibranchial with 10-11 vs 12-14 gill rakers. Finally, color pattern also contributes to the discrimination between H. misionera and H. mbigua. H. misionera presents a paler background colour and at least 8 lateral posterior-oriented chevron blotches, irregularly spaced with decreasing separation between them as blotches proceed backwards. In H. mbigua flanks display a conspicuous dark longitudinal band along perforated line scales, covering approximately half of the series immediately above and below lateral line; also, most specimens with a light band below dark band (Fig. 3).

Description

Morphometric data are summarized in table I. Body subcylindrical. Greatest body depth at the vertical through the origin of dorsal fin. Anterior profile of head angular in lateral view. Dorsal profile of head markedly straight, only slightly convex in small specimens. Dorsal margin of orbit slightly reaching dorsal profile of head but much closer in small specimens. Dorsal profile of body slightly convex from postoccipital region to dorsal-fin origin; then posteroventrally inclined along the entire dorsal-fin base and finally almost straight but slightly inclined until the origin of dorsalmost procurrent caudal-fin ray. Ventral profile of body slightly convex to pelvicfin origin, then almost straight from the latter point to anal-fin origin and finally marked concave to origin of ventralmost procurrent caudal-fin ray. Medial margins of contralateral dentaries converging to midline and then running parallel in a characteristic Y-shaped (Fig. 2). Extension of the parallel section as well as the degree of closeness of margins may vary slightly Upper jaw slightly shorter than lower jaw. Distal portion of maxilla straight. Upper and lower lips slightly fleshy with short skin projections cover-

ing externally the entire length of larger caniniform teeth. Nostrils situated along horizontal through ventral half of the orbit. Only anterior nostril tubular with a fleshy skin covering its whole opening. Posterior nostril equidistant to the anterior nostril and the anterior margin of orbit. Infraorbitals 3 and 4 excluded from the orbital margin. Teeth caniniform in both jaws. A single premaxillary tooth row. First two premaxillary teeth large and caniniform, then four-five very small teeth followed by other two large canines. Extreme canines in this series the largest. Then, one-two small teeth almost in contact with extremely small first maxillary tooth. Maxilla with 30-49 teeth, first five increasing progressively in size. Dentary external series composed of three to five small teeth followed by two larger canines, then other series of four-six small teeth and finally ten-sixteeen teeth arranged in a repetitive series of one large and one-two small conic teeth. Internal series of dentary beginning immediately posterior or slightly anterior to last conical tooth of external row and formed of approximately 15 very small teeth. Accessory ectopterygoids and ectopterygoids with small conical teeth along their ventrolateral margins and several much smaller viliform teeth over their ventromedial surfaces. Accessory ectopterygoids not fragmented, anteriorsly expanded and bearing 12-15 conical teeth along their ventrolateral margins. Total dorsal-fin rays 14-16 (ii-12 n = 2; iii-11 n = 1; ii-13 n = 5, iii-12 n = 8, iii-13 n = 1; iv-12 n = 1). Dorsal fin located well anteriorly to midbody, its origin one scale anterior to the vertical through the pelvic fin origin. Tip of longest rays of depressed dorsal fin extending (two lateral lines scales length) beyond vertical through anal-fin origin. Total anal-fin rays 10 (ii-8 n = 18). Total pectoral-fin rays 12-14 (i-11 n = 5, i-12 n = 5, i-13 n = 8). Tip of pectoral fin separated from pelvic-fin origin by only two scales. Total pelvicfin rays 8 (i-7 n = 18). Tip of pelvic fin separated from vertical through anus by only two scales. Total caudal-fin rays 17-18 (i-15-i n = 17, i-16-i n = 1). Predorsal scales (15-16; only one with 18) in an irregular series, decreasing in sizes backwards. Last vertical series of scales on caudal peduncle forming a marked curve. Lateral line complete with 40-43 perforated scales. Perforated scales with a single tubular-like canal not covering total scale length. Longitudinal series of scales between dorsal fin origin and lateral line 5-6; between lateral line and pelvic fin origin 4-5.5. Longitudinal series of scales around caudal peduncle, invariable 20. First epibranchial with 10-11 plate-like denticulated gill rakers. One laminar gill raker on cartilage. First ceratobranchial with five-six more elongated rakers and 12-15 plate-like denticulated gill rakers. Laterosensory canal along ventral surface of dentary with four pores; one specimen (UNMDP 3392) with four pores on the left margin and five on the right margin. A single laterosensory canal along infraorbitals bearing 10-11 pores. Four pores with small ventral branches (two in infraorbital 2; one in infraorbital 3; one in infraorbital 4) and one pore with a small dorsally oriented branch in infraorbital 6. Infraorbital 5 lacking pores. Laterosensory pores on dorsal surface of head disposed as follows: two pores on nasals, four pores on frontals, the anteriormost close to the orbit followed posteriorly by three pores laterally arranged. Two pores on the pterotic bones and two pores on the extra-scapular bones. Sometimes one extra-scapular pore displaced to the suprapreopercle bones. One pore in the posterior end of the symphysis between parietal bones. Six laterosensory pores in the preopercle. Total vertebrae count 39-40 (n = 3).

Colour in alcohol

Specimens as small as 40 mm of SL display a conspicuous dark, wide midlateral band over a brown background. Both, dorsal and ventral margins of this band ornamented with regularly spaced lights dots. Below this band, the ventral flank scattered with small irregular light spots and stripes. In larger specimens (over 50 mm of SL) these light marks larger, fused each other and, beyond midlateral dark band losses wideness. In both cases, a clear stripe evident below the dark band in the head, from the contact between the orbit margin and the 5th infraorbital throughout the entire length of the fourth infraorbital. Lateral blotches, that will be the outstanding colour feature in larger specimens, incipiently visible. Individuals over 80 mm already reach the final coloration. A paler background colour and at least eight lateral posterior-oriented chevron blotches, irregularly spaced with decreasing separation between them as blotches proceed backwards. The midlateral band as well as most striking coloration features of smaller individuals, completely lost. Instead, larger specimens with a dark circular spot in the dorsal extreme of caudal fin-base rays. Ventral surface of body pale-yellowish. All fins light brown with dark spots on rays and interradial membrane forming pattern of irregular dark stripes. This pattern more conspicuous in larger individuals.

Distribution

Hoplias misionera n. sp. is known from several localities of northeastern Argentina in the Uruguay, Paraná and Paraguay River basins and one locality in the upper Parana River, in Brazil (Fig. 4). The type locality in Argentina is a small lotic stream (27°27'44.63"S; 54°57'5.58"W) tributary of the Acaraguá River system, in the Uruguay River basin. As most mountain streams in Misiones province, bottom is dominated by basalt rock due to the geological nature of the soils in this region. This stream is originated by a great number of little wellsprings, which drain the excess of water from the central hills. Stream width varies between 2 to 5 m, depending on the characteristic of the margins. Riparian native vegetation is dominated by native rain forest trees and several woody debris and other natural occurring lodges are a common feature.

Etymology

The specific epithet *misionera* is named in reference to the Argentine province containing the type locality and also because small streams in Misiones province commonly host several species of *Hoplias*. Indeed, Misiones province also contains the type locality of the recently described *Hoplias mbigua* (Azpelicueta *et al.*, 2015). Moreover, several specimens used in the original description of *Hoplias australis* (Oyakawa and Mattox, 2009) were collected in this province.

Barcode Sequence

The mtDNA COI Barcode profile (652 bp) of the holotype is reported herein as an aspect of the type description: CCTGTATCTAGTATTTGGTGCCTGAGCCGGAATAGTTGGTACAGCTC CAGCCTTCTAATCCGAGCAGAGCTAAGCCAACCCGGGGGCA TACTTGGCGATGACCAGATTTATAATGTTATCGTTACTGCACA GCCTTCGTAATAATTTTCTTCATAGTAATGCCTATTATAATC GGGGATTTGGAAACTGACTTGTTCCCCTCATGATTGGAGCACCTG CATAGCCTTCCCGCGAATAAATAACATAAGTTTCTGGCTTC TCCCCCCTCATTACTTCTCCTACTAGCCTCCTCCGGCGTAGAA CAGGGGTAGGTACAGGTTGAACTGTTTACCCCCCTCTAGCC



Figure 3. – Comparison between *Hoplias mbigua*, CI-FML 6764, 248 mm SL (above) and *Hoplias misionera* n sp., UNMDP 3328, 146 mm SL (below), showing differences. A: Body colouration; B: Dorsal profile of head; C: Last vertical series of scales on the base of the caudal-fin rays. Scale bars = 5 cm.

GAAACCTTGCACATGCAGGGGGCCTCTGTTGACCTAGCAATTTT TCTCTTCATCTTGCAGGGGGTCTCCTCAATTTTAGGAGCTA TAATTTTATTACAACAATTATTAACATAAAACCCCCCTGCCAT TCACAATATCAAACCCCCCTTATTTGTTTGAGCTATTTTAA CACAGCCGTTCTTCTTCTCCTCCCCCCCGTTCTTGCTGCC GAATCACAATACTTTTAACAGACCGAAACCTTAACACACCT TCTTTGACCCCGCAGGAGGGGGGGGAGATCCCATTCTTTATCAACATCTA

The nucleotide composition was A (157), G (114), C (178) and T (203). The K2P genetic distances within *Hop*-

lias misionera averaged 0.3% (0-0.77%). All specimens of *H. misionera* received the BIN AAB1732 from BOLD and differed by 5.61% from the nearest neighbour species. The K2P/NJ tree together with the BIN algorithm unambiguously discriminated among *H. misionera* and the remaining two clusters of the *H. malabaricus* species complex from lower Plata basin (Fig. 5).





Comparative material examined

Hoplias malabaricus. South America, probably Suriname (not "Tranquebar"): lectotype ZMB 3515, 1 ex., 167 mm SL; paralecto-type ZMB 33059, 1 ex., 69 mm SL, photos and X-rays.

Hoplias cf. *malabaricus*. Las Nutrias stream, Luján River Basin, Buenos Aires: UNMDP 1247, 1 ex., 45 mm SL; UNMDP 1248, 1 ex., 43 mm SL; UNMDP 1249, 1 ex., 55 mm SL, J.J. Rosso coll., 02 May 2011; Salto Grande Reservoir, Entre Ríos: UNMDP 1279, 1 ex., 240 mm SL, Rosso and Mabragaña coll., 12 Sep. 2011; Paraná-Guazú River, Delta of Paraná River, Entre Ríos: UNMDP 1370, 1 ex., 309 mm SL; UNMDP 1371, 1 ex., 265 mm SL, Rosso, Mabragaña, Avigliano and Schenone coll., 07 Oct. 2011; Bergara stream, Entre Rios: UNMDP 1595, 1 ex., 98 mm SL, Rosso and Mabragaña coll., 09 Sep. 2011; El Pescado Lake, Paraná River floodplain, Victoria, Entre Ríos: UNMDP 2452, 1 ex., 202 mm



SL; UNMDP 2453, 1 ex., 203 mm SL, Rosso and Mabragaña coll., 11 Nov. 2012; Nogoyá stream, Paraná River Basin, Entre Ríos: UNMDP 2565, 1 ex., 134 mm SL, Rosso and Mabragaña coll., 10 Nov. 2012; Ayuí stream, Uruguay River Basin, Entre Ríos: UNMDP 2616, 1 ex., 116 mm SL, Rosso and Mabragaña coll., 14 Nov. 2012; Rojas River, Paraná Basin, Ascensión, Buenos Aires: UNMDP 492, 1 ex., 410 mm SL; UNMDP 502, 1 ex., 170 mm SL; UNMDP 503, 1 ex., 145 mm SL; UNMDP 504, 1 ex., 159 mm SL, Rosso, Villamil, González-Castro and Mabragaña coll., 10 Dec. 2010.

Hoplias mbigua. Parana River in Nemesio Parma, Dep. Capital, Misiones province, Argentine: holotype, CI-FML 6763, 1 ex., 224 mm SL, D. Aichino, M. Azpelicueta, D, Méndez, I. Rodriguez coll., Nov. 2005; CI-FML 6764, 2 ex., 224-248 mm SL, paratyypes, collected with the holotype.

Hoplias teres. Lake Maracaibo, Venezuela: syntypes MNHN-4377_1, 1 ex., 121 mm SL; MNHN-4377_2, 1 ex., 116 mm SL.

Hoplias microlepis. Rio Chagres, Panamá, lectotype, BMNH 1860.1.26.221, 1 ex., photograph; paralectotype BMNH 1860.1.26.222, 1 ex., photograph; Llano Sucio River, Atlantic Drainage, Panamá: LBV-18503, 1 ex., 215 mm SL, C Oliveira, RG Reina, C Vega, S Perez coll., 14 Jul. 2005.

Erythrinus macrodon. Lake Almada, province of Bahia, and Rio São Francisco, Brazil: holotype: MHNN 0773, 1 ex., photos and X-rays.

Macrodon tareira. Brazil and French Guiana: syntypes: MNHN 4409, 1 ex., 108 mm SL; MNHN 4421, 3 ex., 175-237 mm SL; MNHN A-9746, 1 ex., 93,4 mm SL; MNHN A-9747, 1 ex., 183 mm SL; MNHN A-9748, 1 ex., 245 mm SL.

Hoplias lacerdae. Ramos stream, Uruguay River Basin, Oberá, Misiones: UNMDP 570, 1 ex., 346 mm SL; UNMDP 571, 1 ex., 350 mm SL; UNMDP 594, 1 ex., 163 mm SL, Rosso, Mabragaña, Avigliano and Schenone coll., 05 Feb. 2011; Yabotí River, Uruguay River Basin, Misiones: UNMDP 2725, 1 ex., 192 mm SL; UNMDP 2735, 1 ex., 244 mm SL, Rosso and Díaz de Astarloa coll., 18 Nov. 2012.

Hoplias australis. Ramos stream, Uruguay River Basin, Oberá, Misiones: UNMDP 1991, 1 ex., 43.9 mm SL, Rosso, Avigliano and Schenone coll., 06 Apr. 2012; Oveja Negra stream, Yabotí River Basin, Misiones: UNMDP 2721, 1 ex., 271 mm SL; UNMDP 2722, 1 ex., 220 mm SL; UNMDP 2723, 1 ex., 171 mm SL; UNMDP 2724, 1 ex., 166 mm SL, Rosso and Díaz de Astarloa coll., 18 Nov. 2012.

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

Hoplias curupira. Rio Amazonas/Rio Tapajós, Itaituba, Brazil: LBV 67349, 1 ex., 153 mm SL, R. Britzke coll., 11 Jun. 2012.

Hoplias intermedius. San Francisco River, Gararu, Brazil: LBV 48702, 1 ex., 231 mm SL, Mehanna and Milano coll., 21 Nov. 2010.

REMARKS

The Hoplias malabaricus species complex is composed by at least seven different putative species as revealed by cytogenetics (Bertollo et al., 2000) and mitochondrial markers (Rosso et al., 2012; Ferreira Marques et al., 2013). This paper elucidated taxonomic features of one of these putative species. Since the original description of Bloch (1794) seven nominal species were synonymized with H. malabaricus (Oyakawa, 2003). Among all these species, non types are known for Synodus palustris Bloch & Schneider, 1801 and Synodus tareira Bloch & Schneider, 1801 whereas Macrodon auritus Valenciennes, 1847 and Esox tararira Larrañaga, 1923 represent nomen dubium (Oyakawa & Mattox, 2009). Therefore, there are three nominal species that need to be contrasted with Hoplias misionera n. sp. Revision of the original description of Macrodon ferox Gill, 1858 together with syntypes of *M. tareira* and photographs and X-rays of Erythrinus macrodon Spix & Agassiz, 1829, provided additional evidence supporting that H. misionera n. sp. represents a new different species. For instance, H. misionera n. sp. may be easily differentiated from *E. macrodon* by the number of scales in lateral line (40-43 vs 40), total anal-fin rays (10 vs 12) and Y-shaped vs V-shaped medial margin of dentaries; from *M. tareira* by the longitudinal series of scales between dorsal-fin origin and lateral line (5-6 vs 5-7), total anal-fin rays (invariable 10 vs 10-12), number of scales in lateral line (40-43 vs 35-40) and Y-shaped vs V-shaped medial margin of dentaries. Hoplias misionera may further be differentiated from *M. ferox* by total pectoral (12-14 vs 10) and anal (10 vs11) fin rays, longitudinal series of scales between dorsal-fin origin and pelvic-fin origin (9-11.5 vs 12) and number of scales in lateral line (40-43 vs "about" 40).

Hoplias misionera n. sp. displayed a marked curved arrangement of the last vertical series of scales on the base of the caudal-fin rays. This character, that was firstly proposed by Rodrigues Pacheco (2004) and also considered by Bifi (2013) in respective unpublished post-graduates thesis, could be informative in the *H. malabaricus* species group. Indeed, considering this character, *H. misionera* n. sp. with a marked curved arrangement of these scales, may be differentiated from *H. malabaricus*, *H. mbigua*, *H. teres* and *H. microlepis* which present a straight disposition of the last vertical series of scales on the base of caudal-fin rays.

Hoplias misionera n. sp. can be further distinguished from species in the *H. lacerdae* group by several characters. For instance, *H. misionera* n. sp. is distinguished from *H. curupira* in the number of scales along lateral line (40-43 vs 34-39) and the number and arrangement of pores of the laterosensory system along the ventral surface of dentary (4 single pores vs 4 patches of pores), and from *H. australis* and *H. lacerdae* in the number of pores of the laterosensory system along the ventral surface of dentary (4 vs always 5 and ROSSO ET AL.

6-8 respectively). *Hoplias misionera* n. sp. is distinguished from *H. aimara* by the lack of a vertically elongate dark spot on the opercular membrane (vs dark spot present) and the presence (vs absence) of accessory ectopterygoids. *Hoplias misionera* n. sp. can be further distinguished from *H. brasiliensis* and *H. intermedius* by the pores of the laterosensory system along ventral surface of dentary (4 vs 4-6 pores).

Acknowledgements. - This research was partially funded by Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina IBOL grants and PIP 11220130100339 CO), Agencia Nacional de Promoción Científica y Tecnológica (PICT-2014-0665), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata (15/E619 EXA669/14) and "Bosques Nativos Argentinos para la Biodiversidad". We particularly wish to mention the honourable work of the president of the Foundation, Mr. Horacio Juan Schenone. The authors also wish to thank the Ministerio de Ecología v Recursos Naturales Renovables de la Provincia de Misiones and Ministerio de Producción y Ambiente de la Provincia de Formosa for logistical support. Professor José Osinalde from Entre Ríos Province managed field research permission. Matías Armanazqui helped to contact local authorities of Victoria, Entre Ríos Province, which greatly facilitated field sampling during research trip to this city. We are also greatly indebted to Julia Pusterla and family, Franco del Rosso, Javier Unizony, Gastón Ramos and Eusebio Soto for their invaluable collaboration in the field. JJR acknowledges the financial support from the International Development Research Centre (IDRC) of Canada. MG-C, EM, EA, NS, JJR, MSD and JMDA acknowledge the support provided by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) of Argentina. JMDA is much indebted to Dr. Peter Bartsch (Museum für Naturkunde Leibniz Institute for Evolution and Biodiversity Science, Berlin) for giving assistance and technical support in recent visit to the ZMB and for providing photographs and X-rays of the type specimens of Hoplias malabaricus and Dr. Celia Bueno (Muséum d'Histoire Naturelle Neuchâtel) for providing photographs and X-rays of the type specimen of Erythrinus macrodon. Also, JMDA extends his appreciation to Muséum national d'Histoire naturelle. Paris (MNHN) for financial assistance that provided opportunities to conduct research in its respective fish collections and libraries. Pr. Guy Duhamel, Director of the Département Milieux et Peuplements Aquatiques, MNHN, provided laboratory space and equipment. Patrice Pruvost, curator manager of fishes at the fish collection of the MNHN, and Romain Causse kindly assisted the junior author while he was in Paris. The technical assistance of Claude Ferrara, Zora Gabsi, Lina Duque-Vélez and Aurélie Laurent in supplying the material examined of Hoplias spp. is greatly appreciated. Authors are indebted to Mr. James Maclaine from the Natural History Museum of United Kingdom for providing photographs of the lectotype and paralectotype of *H. microlepis*. Authors are greatly indebted to Prof. Dr. Claudio Oliveira for material examined in the Fish Collection of the Laboratório de Biologia e Genética de Peixes of UNESP, Botucatu, São Paulo, Brazil and to Dr. Gastón Aguilera for support provided during revision of the holotype and paratypes of Hoplias mbigua in Fish Collection of the Fundación Miguel Lillo in Tucumán, Argentine. Authors are very grateful to Dr. Carlos Capiel and his troop at the Instituto Radiológico of Mar del Plata, for technical assistance in obtaining X-rays of Hoplias misionera. Nalani Schnell gently assisted authors in obtaining X-rays of Hoplias teres and Macrodon tareira at the MNHN in Paris. P. Béarez (Sorbonne Université, Muséum) kindly commented and translated the abstract.

REFERENCES

- AZPELICUETA M. de las M., BENÍTEZ M.F., AICHINO D.R. & MENDEZ C.M.D., 2015. - A new species of the genus *Hoplias* (Characiformes, Erythrinidae), a tararira from the lower Paraná River, in Misiones, Argentina. *Acta Zool. Lilloana*, 59(1-2): 71-82.
- BERTOLLO L.A.C., BORN G.G., DERGAM J.A., FENOCCHIO A.S. & MOREIRA-FILHO O., 2000. - A biodiversity approach in the Neotropical fish *Hoplias malabaricus*. Karyotypic survey, geographic distribution of cytotypes and cytotaxonomic considerations. *Chromosome Res.*, 8: 603-613.
- BIFI A.G., 2013. Revisão taxonômica das espécies do grupo Hoplias malabaricus (Bloch, 1794) (Characiformes: Erythrinidae) da bacia do rio da Prata. Ph.D Thesis, 51 p. Univ. Estadual de Maringá, Paraná, Brazil. http://nou-rau.uem.br/nou-rau/ document/?code = vtls000205331 (28 Oct. 2014).
- DE CARVALHO D.C., OLIVEIRA D.A.A., POMPEU P.S., GON-TIJO-LEAL C., OLIVEIRA C. & HANNER R., 2011. - Deep barcode divergence in Brazilian freshwater fishes: the case of the Sao Francisco River basin. *Mitochondr. DNA*, 22: 80-86.
- ESCHMEYER W.N., FRICKE R. & VAN DER LAAN R., 2016. -Catalog of Fishes: genera, species and references. (http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp). Electronic version accessed 10 Jun. 2016.
- FERREIRA MARQUES D., DOS SANTOS F. & DA SILVA S., 2013. - Cytogenetic and DNA barcoding reveals high divergence within the trahira, *Hoplias malabaricus* (Characiformes: Erythrinidae) from the lower Amazon River. *Neotrop. Ichthyol.*, 11: 459-466.
- FINK W.L. & WEITZMAN S.H., 1974. The so-called cheirodontin fishes of Central America with descriptions of two new species (Pisces: Characidae). *Smithson. Contr. Zool.*, 172: 1-46.
- IVANOVA N.V., DEWAARD J.R. & HEBERT P.D.N., 2006. An inexpensive, automation friendly protocol for recovering highquality DNA. *Mol. Ecol. Notes*, 6: 998-1002.
- IVANOVA N.V., ZEMLAK T.S., HANNER R.H. & HEBERT P.D.N., 2007. - Universal primer cocktails for fish DNA barcoding. *Mol. Ecol. Notes*, 7: 544-548.
- KIMURA M., 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol., 16: 111-120.
- MABRAGAÑA E., DÍAZ DE ASTARLOA J.M., HANNER R., ZHANG J. & GONZÁLEZ CASTRO M., 2011. - DNA Barcoding identifies Argentine fishes from marine and brackish waters. *PLoS ONE*, 6: e28655.
- MABRAGAÑA E., DELPIANI S.M., BLASINA G.E., GONZÁLEZ-CASTRO M., ROSSO J.J. & DÍAZ DE ASTAR-LOA J.M., 2012. - Sardinella aurita (Clupeidae) in Mar Chiquita coastal lagoon: morphological and DNA barcoding identification approaches. Cybium, 36: 397-398.
- MATTOX G.M.T., TOLEDO-PIZA M. & OYAKAWA O.T., 2006. - Taxonomic study of *Hoplias aimara* (Valenciennes, 1846) and *Hoplias macrophthalmus* (Pellegrin, 1907) (Ostariophysi, Characiformes, Erythrinidae). *Copeia*, 2006(3): 516-528.
- MATTOX G.M.T., BIFI A.G. & OYAKAWA O.T., 2014. Taxonomic study of *Hoplias microlepis* (Günther, 1864), a trans-Andean species of trahiras (Ostariophysi: Characiformes: Erythrinidae). *Neotrop. Ichthyol.*, 12: 343-352.
- OYAKAWA O.T., 1990. Revisão sistemática das espécies do gênero *Hoplias* (grupo *lacerdae*) da Amazônia brasileira e região leste do Brasil (Teleostei: Erythrinidae). MS.c. Thesis, 114 p. Univ. de São Paulo, São Paulo, Brazil.

- OYAKAWA O.T., 2003. Family Erythrinidae. *In*: Check-List of the Freshwater Fishes of South and Central America (Reis R.E., Kullander S.O. & Ferraris C.J., eds), pp 238-240. Porto Alegre: Edipucrs.
- OYAKAWA O.T. & MATTOX G.M.T., 2009. Revision of the Neotropical trahiras of the *Hoplias lacerdae* species-group (Ostariophysi: Characiformes: Erythrinidae) with descriptions of two new species. *Neotrop. Ichthyol.*, 7: 117-140.
- PEREIRA L.H.G., HANNER R., FORESTI F. & OLIVEIRA C., 2013. - Can DNA barcoding accurately discriminate megadiverse Neotropical freshwater fish fauna. *BMC Genet.*, 1-14. http://www.biomedcentral.com/1471-2156/14/20.
- RATNASINGHAM S. & HEBERT P.D.N., 2007. BOLD: the Barcode of Life Data System (www.barcodinglife.org). *Mol. Ecol. Notes*, 7: 355-364.
- RATNASINGHAM S. & HEBERT P.D.N., 2013 DNA-based registry for all animal species: the Barcode Index Number (BIN) system. *PLoS ONE*, 8: 8e66213.

- 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. Unpublished Mg. Sc. Thesis, 107 p. Univ. Federal de São Carlos, Brazil.
- ROSSO J.J. MABRAGAÑA E., GONZALEZ CASTRO M. & DIAZ DE ASTARLOA J.M., 2012. DNA barcoding Neotropical fishes: recent advances from the Pampa Plain, Argentina. *Mol. Ecol. Resour.*, 12: 999-1011.
- TAMURA K., PETERSON D., PETERSON N., STECHER G., NEI M. & KUMAR S., 2011. - MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.*, 28: 2731-2739.
- TORRES R.A., ROPER J.J., FORESTI F. & OLIVEIRA C., 2005. - Surprising genomic diversity in the Neotropical fish Synbranchus marmoratus (Teleostei: Synbranchidae): how many species? Neotrop. Ichthyol., 3: 277-284.