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Hypostomus formosae, a new catfish species from the Paraguay River Basin with redescription of *H. boulengeri* (Siluriformes: Loricariidae)

Yamila P. Cardoso*, Florencia Brancolini, Ariel Paracampo**, Marta Lizarralde***, Raphael Covain**** and Juan I. Montoya-Burgos*******

Hypostomus formosae, new species, is described from the Paraguay River Basin and *H. boulengeri* is redescribed. Morphological and molecular analyses show that these two species belong to the '*H. plecostomus* species group'. *Hypostomus formosae* can be distinguished from *H. boulengeri* by having the tip of the snout completely covered with small plates (vs. naked snout tip) and fewer premaxillary and dentary teeth (13–28 vs. 16–32, and 10–25 vs. 15–31, respectively). The molecular phylogenetic analysis indicates that the sister species of *H. formosae* is *H. plecostomus* from the Amazon and the Guyanas, highlighting past inter-basin ichthyofauna exchanges.

Introduction

The Paraguay River is the main tributary of the Parana River. Despite its great extension and importance, knowledge about its ichthyofauna is still limited. The Paraguay River hosts 14 species of *Hypostomus* La Cepède, 1803: *H. cochliodon* Kner, 1854, *H. ternetzi* (Boulenger, 1895), *H. borellii* (Boulenger, 1897), *H. boulengeri* (Eigenmann & Kennedy, 1903), *H. latirostris* (Regan, 1904), *H. variostictus* (Miranda Ribeiro, 1912), *H. latifrons* Weber, 1986, *H. piratatu* Weber, 1986, *H. mutucae* Knaack, 1999, *H. peckoltoides* Zawadzki et al., 2010, *H. commersoni* Valenciennes, 1836, *H. robinii* Valenciennes, 1840, *H. paranensis* Weyenbergh, 1877 and *H. regani* (Ihering, 1905) (Liotta, 2005; Zawadzki et al., 2010).

Weber (1985, 1986, 1987) provided a regional

* Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Instituto de Investigaciones Biotecnológicas-Instituto Tecnológico Chascomús (IIB-INTECH), Av. Intendente Marino Km 8200 CC 164 (7130) Chascomús, Buenos Aires, Argentina (corresponding author). E-mail: yamilapcardoso@gmail.com
** Instituto de Limnología Dr. Raúl A. Ringuelet-CONICET-CCT-La Plata-UNLP, Boulevar 120 y 62, La Plata, Argentina.
*** Centro Austral de Investigaciones Científicas, CADIC – CONICET, Av. Houssay 200. 9410 Ushuaia, Tierra del Fuego, Argentina.
**** Muséum d'Histoire naturelle de la Ville de Genève, Département d'herpétologie et d'ichtyologie, Route de Malagnou 1, 1208 Genève, Switzerland.
***** Départment de Génétique et Evolution, Université de Genève, 30 quai Ernest Ansermet, 1211 Genève 4, Switzerland.

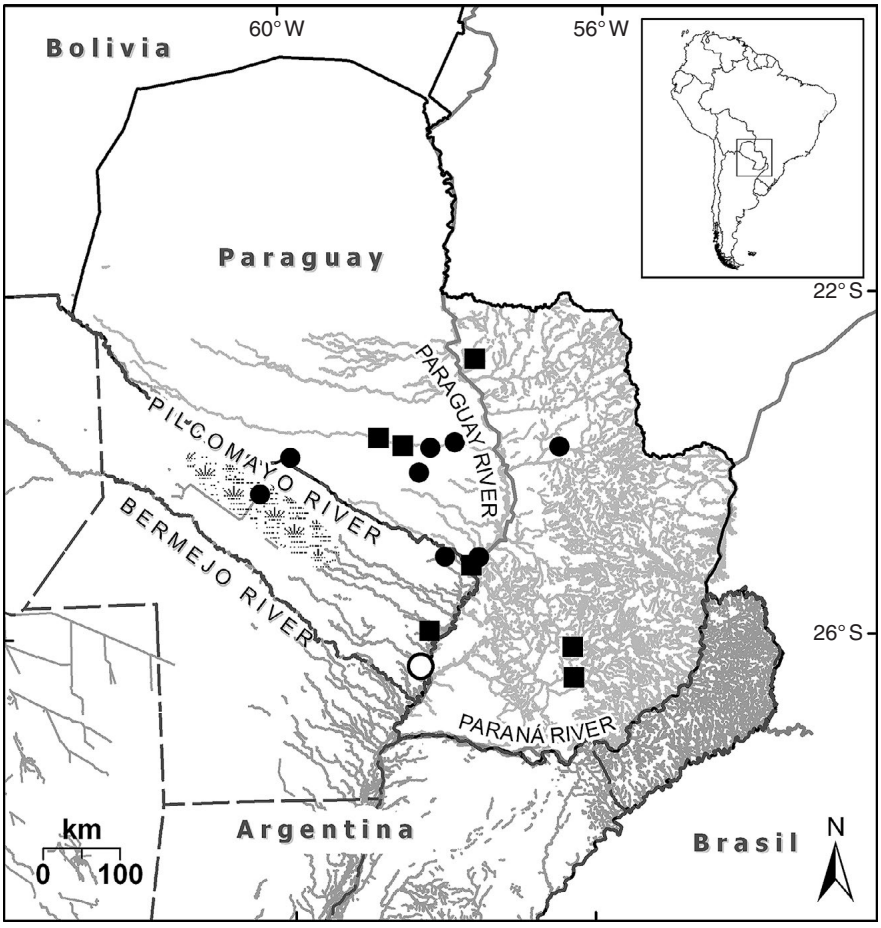

Fig. 1. Map showing the collection localities of *Hypostomus formosae* (●; ○, type locality) and *H. boulengeri* (■).

revision of *Hypostomus* from Paraguayan Rivers. In 1986, he reviewed *H. boulengeri* and described *H. piratatu* and *H. latifrons*. He also showed that the specimens from the Paraguay Basin previously identified as *H. plecostomus* (Linnaeus, 1758) are in fact *H. boulengeri*. More recently, Weber et al. (2012) examined and clarified the identity of *H. plecostomus* based on morphometric and allozyme analyses.

From a biogeographical point of view, the Paraguay River cannot be separated from the middle and Lower Parana River. In particular, the lower section reaches the middle Parana River and there is no evidence of physical barrier to fish fauna exchanges. Thus, it is expected that the fish species inhabiting the middle-lower Parana River could also be present in the Lower Paraguay River and vice versa. Therefore, the *Hypostomus* species that inhabit the middle-lower Parana River could also be present in the Paraguay River, although their presence has not been reported to date. The species of *Hypostomus* that have been listed in the middle-lower Parana River are: *H. alatus* Castelnau, 1855, *H. hermanni* and *H. paulinus* (Ihering, 1905), *H. albopunctatus* (Regan, 1908), *H. luteomaculatus* (Devincenzi, 1942), *H. microstomus* Weber, 1987 and *H. uruguayensis* Reis et al., 1990 (López et al. 2005; Liotta, 2005; Cardoso et al. 2012).

Based on new material collected in Argentinean tributaries of the Paraguay River and on the examination of specimens belonging to several institutional collections (Fig. 1), we describe here a new species of *Hypostomus* from Paraguay River Basin and provide a redescription of *H. boulengeri*.

Fig. 2. *Hypostomus formosae*, MACN Ict 9720, holotype, 177 mm SL; Argentina: Paraguay River drainage**.** Dorsal, lateral, and ventral views. (Photographs by Yamila P. Cardoso).

Material and methods

Fishes were caught using cast and hand nets. Tissue samples for genetic studies were preserved in 96 % ethanol and stored at –20 °C. Voucher specimens were fixed in 4 % formalin for morphological studies. We examinated specimens in the following collections: MHNG, Muséum d'Histoire naturelle, Genève; CFA-IC, Fundación Azara, Buenos Aires; MACN, Museo Argentino de Ciencias Naturales, Buenos Aires; and MFA-ZV-I, Museo Provincial de Ciencias Naturales F. Ameghino, Santa Fe. Measurements were taken point to point with digital calipers to the nearest 0.1 mm. Measurements and counts of bilaterally symmetrical features were taken from the left side of the body whenever possible; if a feature was missing or broken on the left side, it was exam-

Table 1. Morphometric and meristic data for holotype and 25 paratypes of *Hypostomus formosae* and 25 specimens of *H. boulengeri*. Values of holotype included in range, mean and SD.

	H. formosae			*H. boulengeri*	
	holotype	range	mean ± SD	range	mean ± SD
Standard length (mm)	177	64–249		54–191	
Percent of standard length					
Predorsal length [D]	35	35–44	40.0 ± 2.3	36.5–43	39.9 ± 1.6
Head length [E]	31	31–36	32.5 ± 1.1	31–38	34.4 ± 1.9
Cleithral width [F]	31	31–39	34.2 ± 2.4	27–34	30.8± 1.6
Dorsal-fin spine length [K]	35	27–40	34.8 ± 3.3	26–38	33.1± 3.1
Dorsal-fin base length [L]	30	28–34	31.0 ± 1.4	27–33	29.5 ± 1.6
Inter-dorsal length [M]	18	12–20	16.8 ± 2.2	16–21	18.8 ±1.5
Thoracic length [N]	24	22–27	24.4 ± 1.2	21–28	24.4 ± 1.7
Pectoral-fin spine length [O]	34	30–36	32.5 ± 1.5	28–34	30.9 ± 1.4
Abdominal length [P]	24	19–30	22.0 ± 2.1	19–24	21.4 ± 1.3
Pelvic-fin spine length [Q]	27	21–29	25.5 ± 2.0	21–28	25.9 ± 1.8
Caudal-peduncle length [R]	31	29–34	30.9 ± 1.6	29–36	32.8 ± 2.2
Caudal-peduncle depth [S]	11	10–13	11.0 ± 0.6	9–11	10.1 ± 0.7
Adipose-fin spine length [T]	12	4–12	8.7 ± 1.8	8–14	9.5 ± 1.5
Upper caudal-fin ray length [U]	34	25–52	34.1 ± 6.1	25–43	34.1 ± 4.2
Lower caudal-fin ray length [V]	27	20–52	35.3 ± 8.9	26–46	36.2 ± 6.0
Percent of head length					
Head depth [G]	65	54–91	64.5 ± 7.9	54–79	62.2 ± 5.0
Snout length [H]	60	52–63	59.2 ± 2.5	54–61	58.1 ± 1.7
Orbital diameter [I]	13	10–18	12.7 ± 2.0	13–20	16.1 ± 2.1
Interorbital width [J]	45	39–47	43.6 ± 1.8	38–45	42.1 ± 1.9
Maxillary barbel length [barb]	18	9–19	13.9 ± 2.7	7–16	11.9 ± 2.4
Mandibulary ramus length [RM]	11	10–13	11.4 ± 0.9	9–16	12.8 ± 1.7
Counts			mode		mode
Median plates series [Lateral]	25	25–26	25	25–26	25
Dorsal plates below dorsal-fin base [baseD]	8	8–9	8	7–9	8
Plates between dorsal and adipose fin [baseD.Adi]	6	6–8	6	6–8	7
Plates between adipose and caudal fin [baseAdi.Cau]	4	4–6	5	4–6	5
Plates between anal and caudal fin [Postanal]	11	11–14	12	13–15	13
Plates adipose-fin base [baseAdi]	3	3–4	3	3–5	3
Plates anal-fin base [baseAnal]	2	2–3	2	2–3	2
Supraoccipital plates [Supraocci]	1	1–2	1	1–2	1
Left premaxillary teeth [PremaxIzq]	13	13–27	20	17–32	25
Right premaxillary teeth [PremaxDer]	14	14–28	21	16–32	26
Left dentary teeth [DentIzq]	10	10–25	20	15–33	26
Right dentary teeth [DentDer]	14	13–25	20	17–31	26
Multi-state character					
Naked zone in the snout [Zone A]	No	No		Yes	
Snout completely covered with plates [Zone B]	Yes	Yes		No	

ined on the right side. Methods for counts and measurements follow Weber (1985, 1986). Body plate counts and nomenclature follow Oyakawa et al. (2005). We also counted the plates along the base of the anal and adipose fins.

We performed two morphometric analyses. The first was based on 93 specimens belonging to 11 different species of the '*H. plecostomus* group', and including 21 continuous morphometric and 12 discrete meristic variables (Table 1, with abbreviations in squared brackets). This dataset was submitted to a Principal Components Analysis (PCA). The second analysis was based on 25 specimens of *H. boulengeri* and 26 specimens of *H. formosae*, and included 21 continuous morphometric variables, 12 discrete meristic variables and one multi-state character. This dataset was submitted to a Hill-Smith Analyses (HSA; Hill & Smith, 1976) that allows to perform a PCA mixing quantitative and qualitative data. Prior to the analyses, all measurements were standardized by the SL and log transformed to control for size effect and linearize the allometric growth. The HSA and PCA were performed with the ade4 1.4-14 (Dray & Dufour, 2007) and ade4tkgui 0.2-5 (Thioulouse & Dray, 2007) packages in R 2.10.1 (R Development Core team, 2009).

For the molecular analysis, total DNA was extracted using the salt-extraction protocol (Aljanabi & Martinez, 1997). The PCR amplification of the Control Region (D-loop) of the mitochondrial DNA was performed as in Cardoso et al. (2011). The PCR products were purified and sequenced by MACROGEN (Korea). New DNA sequences of *H. boulengeri* and of the new species were deposited in GenBank (accession number: JX290092 to JX290099). The editing of the sequences, the alignment and the phylogenetic methods used were as in Cardoso et al. (2012). For the phylogenetic tree reconstruction, we used sequences of different *Hypostomus* species deposited in GenBank (AJ318344; AJ318347–AJ318358; AJ318369–AJ318379). See also Appendix in Montoya-Burgos et al. (2002) for taxonomic and collections details. In addition, nine species of Loricariidae were used as outgroup, as in Montoya-Burgos (2003).

Hypostomus formosae, new species
(Fig. 2)

Holotype. MACN Ict 9720, 1, 177.0 mm SL; Argentina: Formosa Province: Saladillo Stream: tributary of Paraguay River, 26°26'28"S 58°24'03"W; Y. Cardoso, A. Paracampo, J. Montoya-Burgos & C. Rivera, 22 Nov 2011.

Paratypes. MACN Ict 9721, 1, 170 mm SL; same data of holotype. – MACN Ict 9722, 4, 74.2–141 mm SL; Argentina: Formosa Province: Bañado La Estrella: Pilcomayo River, 24°24'32"S 60°20'02"W; Y. Cardoso et al., 24 Nov 2011. – CFA-IC 11972 (ex-ILPLA 1972), 2, 64.1–74 mm SL; Argentina: Formosa Province: Pilcomayo River Basin: Porteño Stream: close to Veraí Lagoon, 25°10'32"S 57°58'11"W; C. Baigún et al., 21 Feb 2007 – MHNG 2251.43, 2, 178.9–184.2 mm SL; Paraguay: Aguaray-Guazu River and Estero Patino; C. Dlouhy. – MHNG 2251.44, 1, 249.1 mm SL; Paraguay: Aguaray-Guazu River and Estero Patino; C. Dlouhy. – MHNG 2251.41, 1, 197.1 mm SL; Paraguay: Monte Lindo River; C. Dlouhy. – MHNG 2251.40, 4, 149.6–211.6 mm SL; Paraguay, Monte Lindo River; C. Dlouhy. – MHNG 2754.043, 2, 198.6–240.3 mm SL; Paraguay: Central: Bahía de Asuncion: Paraguay River; S. Fisher-Muller et al. – MHNG 2677.005, 1, 178.8 mm SL; Paraguay: Central: Condominio Surubi and route Asuncion-Limpio: San Francisco Stream: branch of Paraguay River; C. Dlouhy. – MHNG2600.050, 3, 80.3–125.0 mm SL; Paraguay: Boqueron: channel derivation of Pilcomayo River: Ea La Dorada; C. Dlouhy. – MHNG 2358.63, 1, 75.2 mm SL; Paraguay: Route to Transchaco: km 180; C. Dlouhy. – MHNG 2676.097, 3, 130.4–208.4 mm SL; Paraguay: Central: Condominio Surubi and route Asuncion-Limpio: San Francisco Stream: branch of Paraguay River; C. Dlouhy.

Diagnosis. *Hypostomus formosae* is distinguished from the species of the *Hypostomus cochliodon* group by having bicuspid teeth (vs. spoon-shaped unicuspid teeth). The color pattern of *H. formosae* (dark roundish dots on a lighter background) differentiates this species from species that have dorsum dark grey with numerous creamy dots. *Hypostomus formosae* is distinguished from the rest of its congeners by the combination of high values for abdominal length (19.1–30.3 % SL), pectoral-fin spine length (30.4–35.5 % SL), head

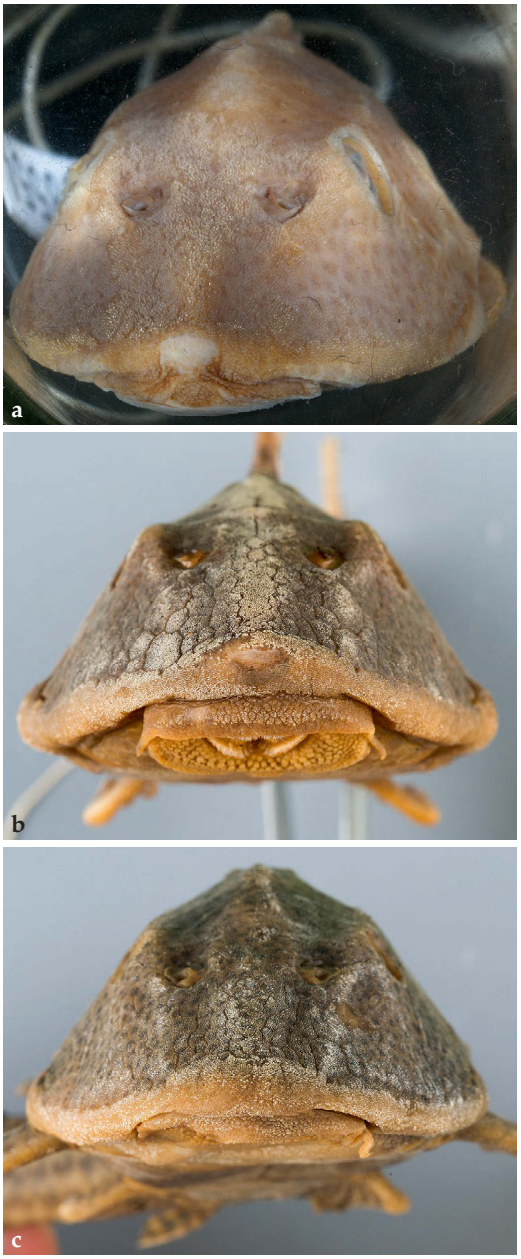

Fig. 3. Detail of tip of snout in: **a,** *Hypostomus boulengeri*, CAS 58554, holotype, 78 mm SL; **b,** *H. boulengeri*, MHNG 2519.23, 151.4 mm SL; **c,** *H. formosae*, MHNG 2251.40, 149.5 mm SL. (Photographs: a, California Academy of Sciences, San Francisco; b–c, Muséum d'histoire naturelle, Genève).

depth (54–90.5 % HL), caudal-peduncle depth (9.7–12.5 % SL), cleithral width (31.1–39.3 % SL) and dorsal-fin spine length (26.8–40.5 % SL), and low values for caudal-peduncle length (28.5–34.8 % SL), inter-dorsal length (12.0–20.1 % SL), median plate series (25–26), number of plates between dorsal and adipose fin (6–8), number of plates between anal and caudal fin (11–14). Also, *H. formosae* can be distinguished from *H. piratatu* by the shape of teeth (short vs. long crown) and from *H. boulengeri* by having the tip of the snout completely covered with small plates (vs. naked snout tip) (Fig. 3) and fewer premaxillary and dentary teeth (13–28 vs. 16–32, and 10–25 vs. 15–31, respectively).

Description. Morphometric and meristic data given in Table 1. Overall view of body shape in Figures 2–4. Dorsal profile straight from snout tip to inter-orbital area, somewhat convex from interorbital area to dorsal-fin origin, and gently descending from this point to end of caudal peduncle. Ventral profile almost straight from snout tip to caudal fin. Dorsal plates between end of dorsal-fin and adipose-fin spine flattened. Body width at cleithral region greater than head depth. Head covered dorsally with plates. Noticeable keel originating laterally to nare, passing through supra-orbital, and extending to throughout dorsal-fin base. Mouth rounded, ventral surface covered with numerous small papillae decreasing in size posteriorly. Premaxillary teeth 13–28, dentary teeth 10–25. Snout tip completely covered with small plates. Dorsal-fin base naked. Body with four well-developed keels along flanks. Dorsal-most keel situated on dorsal series plates. Keel on mid-dorsal series of plates interrupted at end of dorsal-fin base. Keels on median series of plates and mid-ventral originating immediately posterior to cleithrum, more conspicuous on anterior half of trunk. Caudal peduncle laterally compressed, roughly ovoid in cross section.

Abdomen completely covered with minute platelets in specimens larger than 140 mm SL, with exception of small areas around pectoral- and pelvic-fin insertions and at urogenital opening. Dorsal plates 21, mid-dorsal plates 24 (one specimen with 25), median plates 25–26, mid-ventral plates 25, ventral plates 21. Pre-dorsal plates 3, plates below dorsal fin 8–9, plates between adipose fin and caudal fin 4–6, plates between anal fin and caudal fin 11–14. Dorsal-fin: rays II,7, origin at vertical

Fig. 4. Lateral views of life specimens of: **a,** *Hypostomus formosae*, MACN Ict 9722, 82.8 mm SL; Argentina: Formosa Province: Bañado La Estrella; **b,** *H. boulengeri*, MACN Ict 9723, 57.8 mm SL; Argentina: Paraguay River: Formosa City, right side, reverse. (Photographs by Yamila P. Cardoso).

through midpoint between pectoral and pelvic fins, or slightly posterior to that point. Dorsal-fin margin straight. Adipose-fin spine curved inward. Pectoral-fin: rays I,6; posterior border straight. Pectoral-fin spine slightly curved inward, covered with weakly developed odontodes, slightly more developed on its distal portion in specimens larger than 140 mm SL. Pectoral-fin tip reaching half pelvic-fin spine length. Pelvic-fin: rays i,5; posterior border slightly curved. Pelvic-fin spine just surpassing anal-fin origin when depressed. Anal-fin: rays i,4; tip reaching seventh plate after its origin. Anal-fin rays progressively increasing in size, third branched ray usually longest. Caudal-fin: rays i,14,i; margin slightly curved with inferior lobe longer than superior one.

Coloration. In alcohol: Overall ground colour of body and fins light grey/brown, lighter on ventral surface. Dorsal surface of head, body, and fins entirely covered by numerous conspicuous black dots, smaller and closer on head. Ventral surface variable: with evident dark dots, subtle dark dots or without dots. All fins with dots regularly arranged in rows along rays.

Alive: based on observations and pictures of MACN Ict 9720, MACN Ict 9721, MACN Ict 9722 and CFA-IC 11972: same as in alcohol, but stronger contrast between background and dots (Fig. 4a).

Distribution. *Hypostomus formosae* is known from the Paraguay River Basin in Argentina and Paraguay.

Ecological notes. Habitat description based on collecting localities of MACN Ict 9720, 9721, 9722 and CFA IC-11972. The specimens were obtained recently at three sites in the Paraguay River Basin: La Estrella, Saladillo Stream and Riacho Porteño, Argentina. The bottom of these streams was mainly composed of sandstone boulders with patches of sand and pebbles. *Hypostomus formosae* was found in well oxygenated waters (5.9–6.6 $mg \cdot l^{-1}$) with moderate current. Water turbidity was 51.1–98.1 N.T.U., conductivity 67–660 $\mu S \cdot cm^{-1}$, and the pH 6.4–7.3. We do not have habitat information for the others specimens.

Etymology. The species is named after the Formosa Province, Argentina. A noun in genitive.

Hypostomus boulengeri (Eigenmann & Kennedy, 1903)

(Fig. 5)

Plecostomus boulengeri Eigenmann & Kennedy, 1903.

Plecostomus guacari (non La Cepède, 1803): Regan, 1904.

Plecostomus plecostomus (non Linnaeus, 1758): Eigenmann et al., 1907.

Material examined. MACN Ict 9723, 16, 54.2–75.3 mm SL; Argentina: Formosa Province: Paraguay River: Formosa City, 26°12'05"S 58°08'44"W. – MHNG 2251.050, 2, 153.7–190.9 mm SL; Paraguay: Caazapa: Pirapo River. – MHNG 2251.051, 1, 122.1 mm SL; Paraguay: Caazapa: Yta-y Stream. – MHNG 2251.054, 1, 92.85 mm SL; Paraguay: Ovie Stream: Paso Ybucu: Tagatya-guazu Stream.– MHNG 2251.55, 1, 92.4 mm SL; MHNG 2251.53, 2, 115.4–170.7 mm SL; MHNG 2251.57, 2, 115.2– 133.4 mm SL; Paraguay: Ovie Stream: Paso Ybucu Stream. – MHNG 2519.23, 4, 151.45– 178.8 mm SL; Paraguay, Central, Bahía de Asuncion, Paraguay River. – MHNG 2543.51/55/56/59/62, 5, 112.6–148.1 mm SL; Paraguay: Santa Sofia. – MHNG 2251.45, 1, 85.38 mm SL; MHNG 2251.46, 1, 125.07 mm SL; Paraguay: Monte Lindo River: Negro River.

Diagnosis. *Hypostomus boulengeri* is distinguished from species of the *Hypostomus cochliodon* species group by having bicuspid teeth (vs. unicuspid spoon-shaped teeth). The color pattern of *H. boulengeri* (dark roundish dots on a lighter background) differenciates this species from species that have dorsum dark grey/brown covered by numerous rounded creamy dots. *Hypostomus boulengeri* is distinguished from the rest of its congeners, with the exception of *H. piratatu* and *H. formosae*, by the combination of high values for mandibulary ramus length (9–16 % HL), orbital diameter (13–20 % HL), upper caudal-fin ray length (24.9–42.8 % SL) and lower values for caudal-fin ray length (26.1–46.4 % SL), and by having 25–26 plates in the median plates series. *Hypostomus boulengeri* can be distinguished from *H. piratatu* by the shape of teeth (short vs. long crown) and from *H. formosae* by having the snout tip with a naked zone (vs. completely covered with minute plates) (Fig. 3) and more premaxillar and dentary teeth (16–33 vs. 13–28 and 15–33 vs. 10–25, respectively).

Description. Morphometric and meristic data given in Table 1. Overall view of body shape in Figures 3–5. Dorsal profile slightly straight from snout tip to inter-orbital area. Dorsal plates between end of dorsal-fin and adipose-fin spine flattened. Body width at cleithral region greater than head depth. Head covered dorsally with plates, except for naked area on snout tip. Mouth rounded, lower lip not reaching transversal through gill openings, ventral surface covered with numerous small papillae. Premaxillary teeth 17–32, dentary teeth 15–33. Median plates series 25–26. Number of plates along dorsal fin base 7–9, plates between adipose fin and caudal fin 4–6, plates between anal fin and caudal fin 13–15. Dorsal-fin: rays II,7; margin straight. Adipose-fin spine curved inward. Pectoral-fin: rays I,6; posterior border straight. Pectoral-fin spine slightly curved inward. Pelvic-fin: rays i,5; posterior border slightly curved. Pelvic-fin spine just surpassing anal-fin origin when depressed. Anal-fin: rays i,4; tip reaching seventh plate after its origin. Anal-fin rays progressively increasing in size, third branched ray usually longest. Caudal-fin rays i,14,i.

Coloration. In alcohol: Overall ground colour of body and fins light brown, lighter on ventral surface. Dorsal surface of head, body, and fins entirely covered by numerous dark patches, smaller and closer on head and less clear in the caudal peduncle. Ventral surface variable: with evident dark dots, subtle dark dots or without dots.

Alive: based on MACN Ict 9723: same as coloration in alcohol with the exception of overall ground colour of body and fins grey/brown (Fig. 4b).

Distribution. *Hypostomus boulengeri* is known from the Paraguay River Basin in Argentina, Brazil and Paraguay.

Ecological notes. Based on the collecting locality of sample MACN Ict 9723. These specimens were found in the margin of the large Paraguay River. The bottom of the river is made of sand and pebbles. The surface of the water was covered by vegetation. The specimens were found in well oxygenated waters (4.97 $mg \cdot l^{-1}$) with slow current. Water turbidity was 193 N.T.U. Conductivity was 163.3 $\mu S \cdot cm^{-1}$. The pH was 6.9. We do not have ecological information for the others specimens.

Morphometric analyses. Our first morphological dataset, containing species of the *H. plecostomus* group showed structure on the first two axes of PCA (Fig. 6b). The species were mostly aligned along the first axis (Fig. 6a). On the negative side of axis 1, *H. formosae* corresponded to high values for abdominal length, pectoral-fin spine length, head depth, caudal-peduncle depth and interorbital width (Fig. 6c). On the positive side of axis 1, *H. dlouhyi* is characterized by high values for plates between dorsal and adipose fins, median plate series, plates between anal and caudal fins, caudal-peduncle length and inter-dorsal length. Morphological data for *H. formosae* and *H. boulengeri* were mainly structured on the first two axes of HSA (Fig. 7c). The first axis split the two species, while the second aligned the specimens of both species according to their size (Fig. 7a). On the positive side of axis 1, *H. boulengeri* corresponded to high values for premaxillary and dentary teeth, plates between anal and caudal fins, plates adipose-fin base, inter-dorsal length, caudal-peduncle length and the presence of a naked zone in the snout (Fig. 7b). The small specimens of both species located on the negative side of axis 2 (Fig. 7a), corresponded to high values for upper and lower caudal-fin ray length, pelvic-fin spine length, head length and predorsal length (Fig. 7b).

Phylogenetic analyses. A molecular phylogenetic approach was used to corroborate the species status of *H. formosae*, and also to place it within the evolutionary tree of the genus. The mitochondrial D-loop sequences were obtained for four individuals of *H. formosae* and three of *H. boulengeri* recently collected in the Paraguay River Basin. The sequence alignment of the mitochondrial D-loop region comprised 592 positions and 81 sequences of different species of *Hypostomus*. The evolutionary relationships within the genus are the same as the one found in Montoya-Burgos (2003) and Cardoso et al. (2012). All species of *Hypostomus* form a monophyletic group named Clade D (Fig. 8). This clade can be organized into four monophyletic groups, D1, D2, D3, and D4. According to our phylogenetic tree, *H. formosae* together with *H. boulengeri*, *H. derbyi*, *H. paranensis*, *H. commersoni*, *H. plecostomus*, *H. watwata*, and seven unidentified *Hypostomus* specimens are grouped in the clade D2 (Fig. 8). Clade D2 is divided into two sub-clades. Inside the first subclade, *H. formosae* clusters with *H. plecostomus*, distributed in Guianese rivers (Weber et al., 2012). In turn, these two sister-species cluster with five *Hypostomus* spp. (named 177, 36, 49, 219 and 270) from the Amazonian Basin and Northeastern South America coastal rivers. Finally, this lineage has, as sister-group, *H. watwata* from French Guyana. The second sub-clade inside D2 (Fig. 8) includes species from Eastern South America coastal rivers (*H. punctatus*) and the La Plata Basin (*H. commersoni*, *H. derbyi*, *H. paranensis*, *H. boulengeri*, and two unnamed *Hypostomus* species: Tib13 and. 1211). As expected, the four sequences of *H. boulengeri* cluster together.

Discussion

Weber (1986) defined two groups of *H. boulengeri* inhabiting Paraguayan freshwaters: the 'chacoenien' and the 'oriental' groups. These groups were first defined by their geographical distribution but Weber noticed slight morphological differences. By examining the *Hypostomus* specimens analyzed by Weber in MHNG, we found that most of the specimens of the 'chacoenien' group correspond morphologically to *H. formosae*.

Hypostomus formosae is part of the *H. plecostomus* species group as defined by Muller & Weber (1992) in external morphology, with dark dots on the body, median sized mandible and short crowned teeth. Inside this group, *H. formosae* is morphologically very similar to *H. boulengeri*, *H. piratatu* and *H. plecostomus* (Fig. 6). *Hypostomus formosae* and *H. boulengeri* are sympatric species in part of their distribution range, at least. This is evidenced by the presence of representatives of the two species in a same lot collected in Asun-

Fig. 5. *Hypostomus boulengeri,* MHNG 2519.23, 151.4 mm SL; Paraguay: Paraguay River: Asuncion. Dorsal, lateral, and ventral views. (Photographs: Muséum d'histoire naturelle, Genève).

cion, Paraguay (MHNG 2519.23 contained four specimens of *H. boulengeri* and two of *H. formosae*) (Fig. 1). However, it is now easy to differentiate these two species morphologically: only using the character presence or absence of a naked zone in the snout (Fig. 3) allowed us to correctly determine 98 % of the specimens examined. Moreover, our results of the HSA highlighted the other morphological characters that differentiate *H. formosae* from *H. boulengeri*. This same analyses indicated which characters are highly correlated with allometric growth in both species (Fig. 7, axis 2).

In addition to the external morphological similarities between *H. formosae* and *H. plecostomus*, our molecular results showed that they are sister species (Fig. 8). The close relationship between these two species can be explained by past temporary connections or river captures between the southern tributaries of the Amazon and northern tributaries of the Paraguay River. As evidenced by several authors (e. g. Lovejoy & De Araújo, 2000; Sivasundar et al., 2001; Montoya-Burgos, 2003; Hubert et al., 2007; Cardoso et al., 2012), these past inter-basin connections are accumulating strong support. The evolutionary position of *H. formosae* in the phylogenetic tree of *Hypostomus* provides new evidence for faunal exchanges between the Amazon Basin and the Paraguay Basin that are

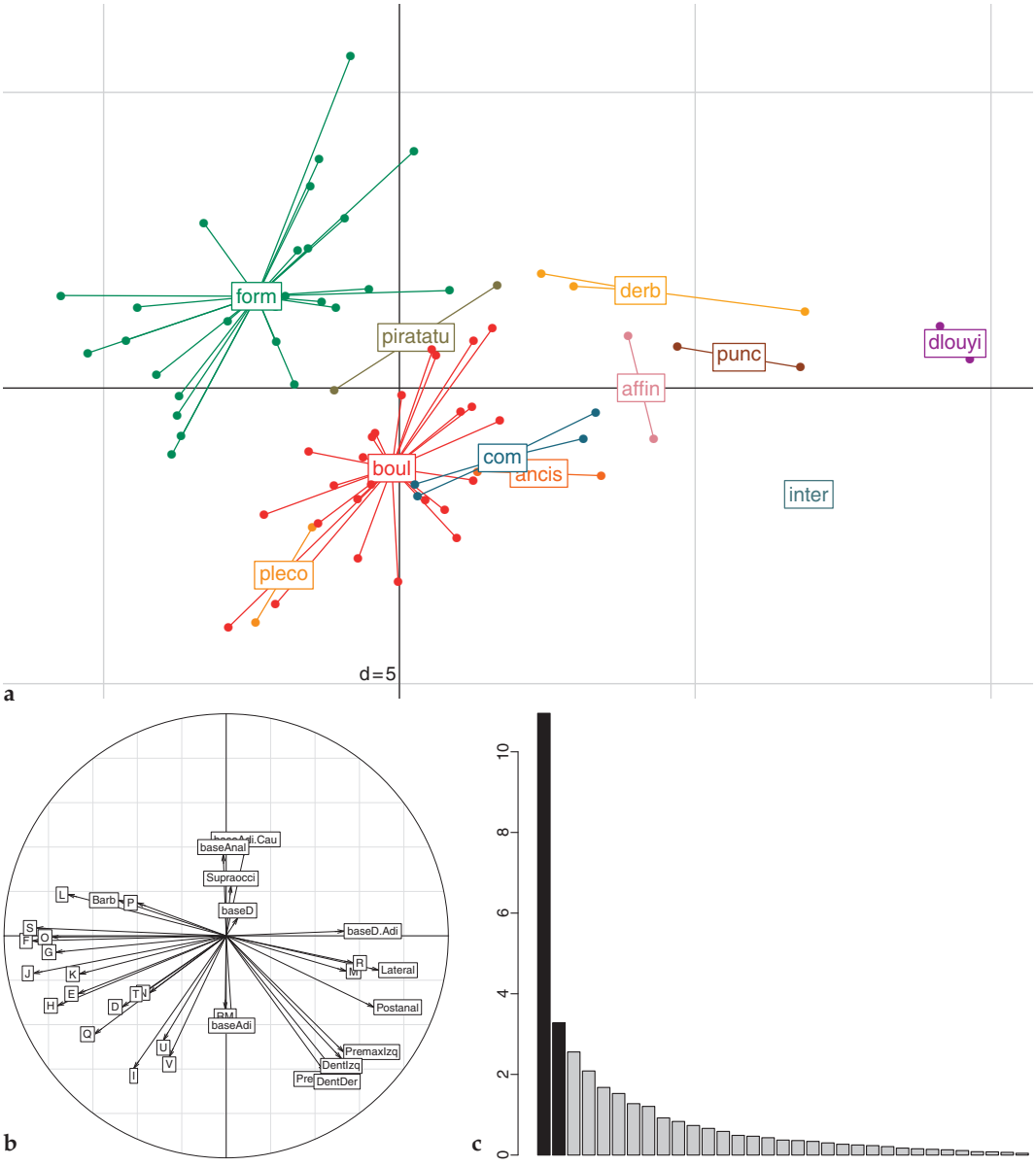

Fig. 6. Principal Component Analysis of morphological data table of '*Hypostomus plecostomus* group'. **a,** Projection of individuals' scores onto first factorial plane of PCA, axis 1 horizontal and axis 2 vertical; **b,** Correlation of the variables labelled as in Table 1; **c,** Eigenvalues. Species labels: **form**, *H. formosae*; **pleco**, *H. plecostomus*; **boul**, *H. boulengeri*; **comm**, *H. commersoni*; **ancis**, *H. ancistroides*; **pira**, *H. piratatu*; **derb**, *H. derbyi*; **inter**, *H. interruptus*; **affi**, *H. affinis*; **punc**, *H. punctatus*; and **dlouyi**, *H. dlouhyi*.

not connected today. The molecular analysis also revealed that the sequences of the specimens of *H. boulengeri* collected in Asuncion, Paraguay (type locality) and Formosa City, Argentina (this study), do not show high genetic divergence. These results and the revision of several specimens of this species allowed us to present here an extension of the distribution of *H. boulengeri*, which now includes the Paraguay River.

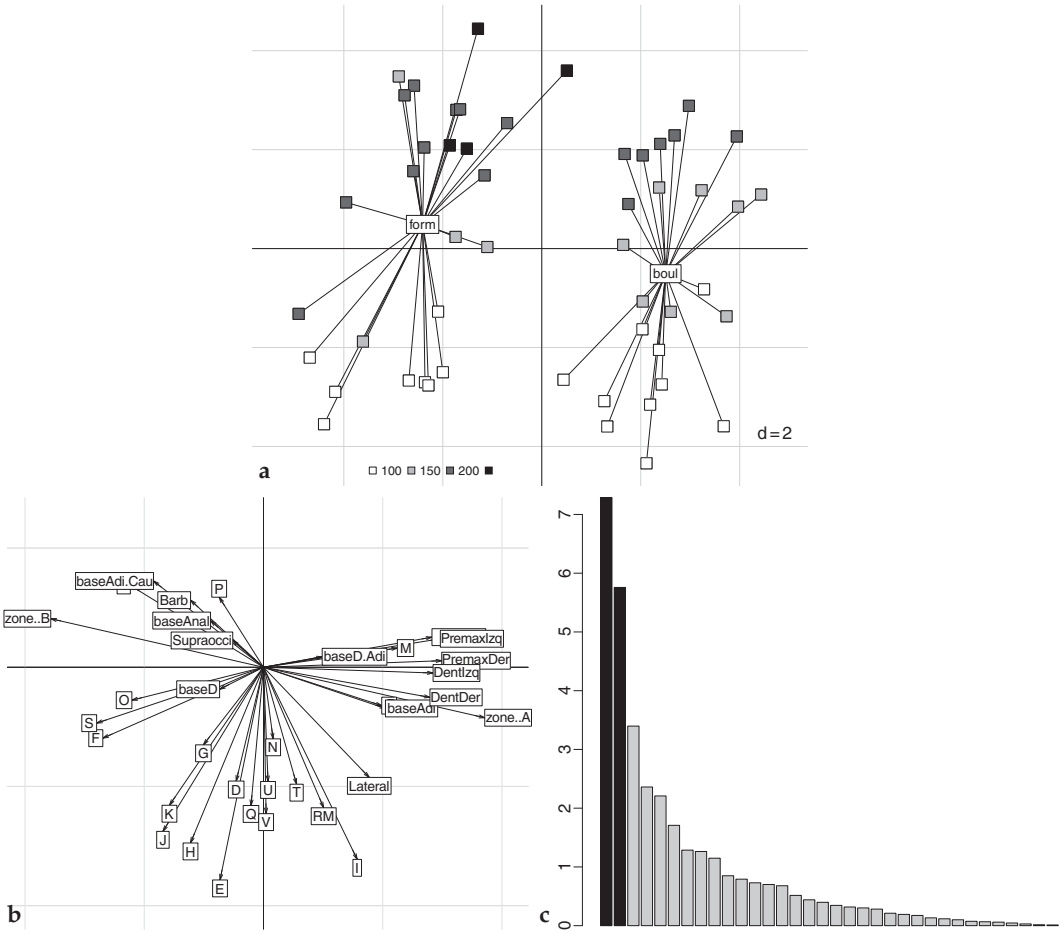

Fig. 7. Hill-Smith Analysis of morphological data of *Hypostomus boulengeri* and *H. formosae*. **a,** projection of individuals's scores onto first factorial plane of HSA, axis 1 horizontal and axis 2 vertical. Grey levels proportional to SL (in mm); **b,** scores of variables of HSA labelled as in Table 1; **c,** Eigenvalues. Species labels: **form**, *H. formosae*; and **boul**, *H. boulengeri*.

Comparative material. *Hypostomus affinis*: MHNG 2543.65, 3, 122–162.7 mm SL; Brazil: Rio Parita do Sul. – MHNG 2430.34, 1, 134.2 mm SL; Brazil: Rio de Janeiro: Paraiba do Sul River: Itaocara. – MHNG 2430.35, 1, 183.5 mm SL; Brazil: Rio de Janeiro: Paraiba do Sul River: Andra. – MHNG 2430.36, 1, 201.8 mm SL; Brazil: Rio de Janeiro: Paraiba do Sul River: Sao Fidelis.

H. albopunctatus: MHNG 2547.18, 3, 115.9–184.4 mm SL; Brazil: Sao Paulo: Cachoeira do Emas: Mogi-Guassu River.

H. ancistroides: MHNG 2602.91, 4, 24.2–44.8 mm SL; Brazil: Minas Gerais: affluent of Ribeirao da Ponte Alta. – MHNG 2645.28, 1, 94.4 mm SL; Brazil: Sao Paulo: Sao Paulo: Salto de Piracicaba: Piracicaba – MHNG 2587.10, 8, 20.4–93.8 mm SL; Brazil: Sao Paulo: Paraitinguinha River.

H. arecuta: MACN ict 9677, holotype, 1, 185.5 mm SL; Argentina: Corrientes Province: Ituzaingó: Parana River.

H. aspilogaster: CFA-IC 12154 (ex ILPLA 2154), 1, 395.0 mm SL; Argentina: Buenos Aires Province: Punta Lara: Río de la Plata Basin. – CFA-IC 12155 (ex ILPLA 2155), 1, 372.9 mm SL; Argentina: Buenos Aires Province: Punta Lara: Río de la Plata Basin. – CFA-IC 12156 (ex ILPLA 2156), 2, 281.2–285.2 mm SL; Argentina: Entre Ríos Province: Stream Mandisoví Grande: Federación department: Uruguay Basin.

H. commersoni: CFA-IC 11907 (ex ILPLA1907), 8, 50–87.5 mm SL; Argentina: Buenos Aires: La Plata City: El Pescado. – MACN Ict 9724, 1, 176 mm SL; Argentina: Formosa Province: El Colorado: Bermejo River. – MHNG 2517.62, 1, 127.5 mm SL; Brazil: Ibicui da Foxina – MHNG 2680.32, 5, 120.5–153.0 mm SL; Argentina:

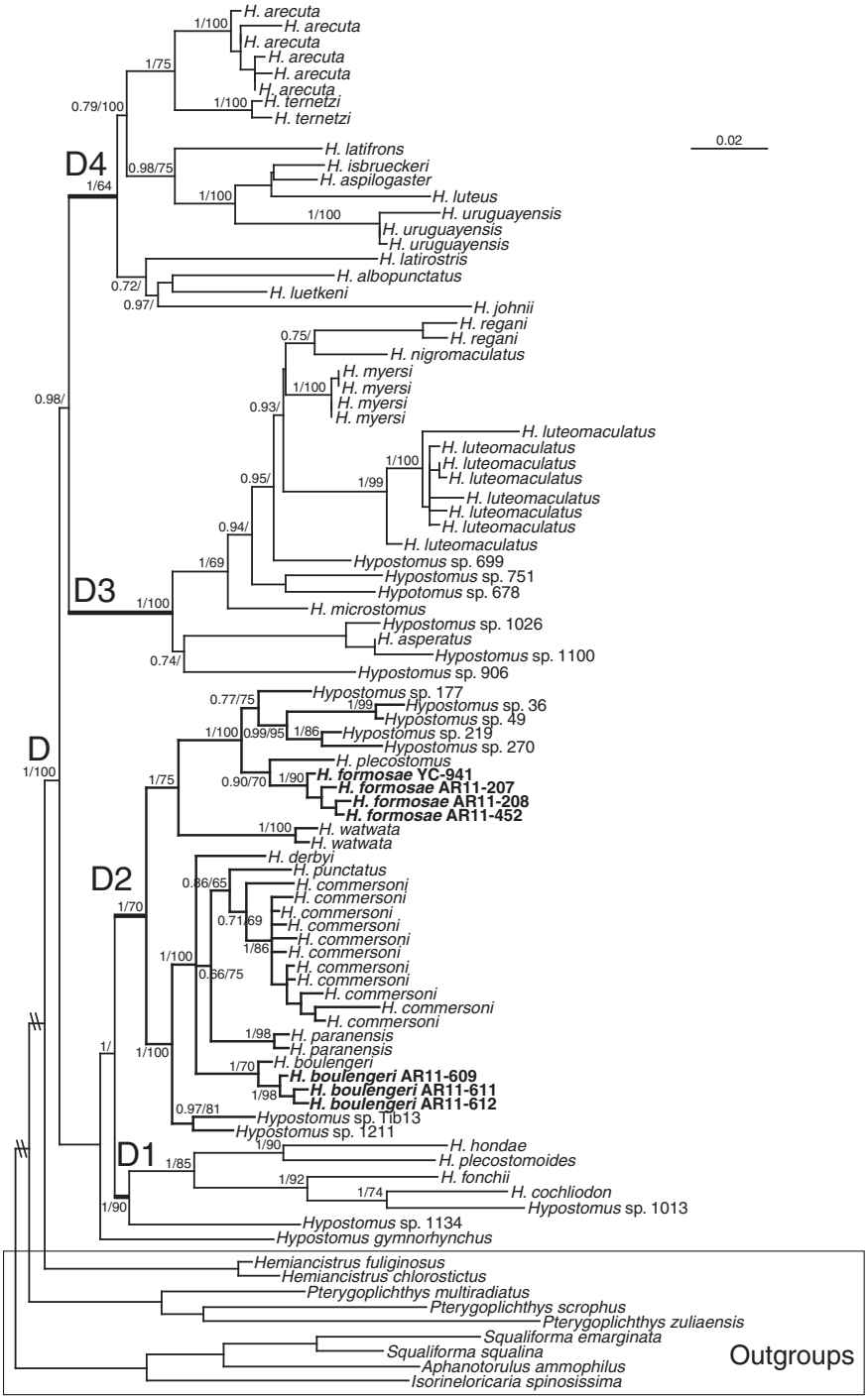

Fig. 8. *Hypostomus* maximun likelihood tree based on mitochondrial D-loop region. Numbers next to branches are Bayesian posterior probabilities followed by bootstrap values (1000 pseudoreplicates) when these are above 50 %. Bold letters are abbreviations used for naming clades (see text). Specimens of *H. formosae* and *H. boulengeri* sequenced in this work are in bold and their field number is given.

Buenos Aires: Reconquista River: Rio de la Plata. – MFA-ZV-I-1765, 1, 420 mm SL; Argentina: Santa Fe Capital: Manucho: Salado River. – MFA-ZV-I-102, 1, 200 mm SL; Argentina: Santa Fe Capital: Colastiné River. – MFA-ZV-I-2854, 1, 252 mm SL; Argentina: Santa Fe Capital: Parque del Sur. – MFA-ZV-I-797, 1, 252 mm SL; Argentina: Santa Fe Capital: Colastiné River.

H. cordovae: BMNH 1878.4.4.1, holotype (photographs only); Argentina: Cordoba Province.

H. derbyi: MHNG 2543.47, 3, 113.8–121.4 mm SL; Paraguay: Lake Itaipu; C. Dlouhy. – MACN Ict 7503, 3, 106–241 mm SL; Argentina: Misiones: Uruguai Stream: Isla Palacios.

H. dlouhyi: MHNG 2229.43, holotype, 137.6 mm SL; Paraguay: upper Parana: lake of Iguazu River. – MHNG 2229.45, 5 paratypes, 98.5–140.7 mm SL; Paraguay: Caaguazu: Yukyry Stream: Iguazu River.

H. interruptus: MHNG 2586.54, 2, 40.8–127 mm SL; Brazil: Parana: Piedade River. – MHNG 2586.70, 1, 82.78 mm SL; Brazil: Sao Paulo: Batari River.

H. isbrueckeri: MHNG 2448.39, 5 paratypes, 139.1–172.5 mm SL; Brazil: Santa Catarina: Canoas River: at Passo do Canoas.

H. laplatae: BMNH 1908.8.29.17, holotype (photographs only); Argentina: Buenos Aires Province: Rio de la Plata.

H. latifrons: MHNG 2256.67, holotype, 230.8 mm SL; Paraguay: Presidente Hayes: Aguaray-Guazu River: road Transchaco. – MHNG 2256.70, 1 paratype, 151.0 mm SL; Paraguay: Central: Paraguay River: Ita Enramada.

H. luteus: MCP 19991,1; Brazil: Santa Catarina: Uruguay River Basin: Uruguay River. – MCP 20751, 1; Brazil: Santa Catarina: Uruguay River Basin: Uruguay River.

H. microstomus: MHNG 2367.90, holotype, 196.1 mm SL; Paraguay: Itapua: Upper Parana River. – MHNG 2367.91, paratypes, 3, 105–106.7 mm SL; Paraguy: Itapua: Upper Parana River.

H. paranensis: CFA-IC 11914 (ex ILPLA1914), 2, 58–78.8 mm SL; Argentina: Córdoba Province: Córdoba Capital: Suquia River.

H. paulinus: MHNG 2198.45, 3, 127.4–150.7 mm SL; Paraguay: Itapua: Tembey.

H. piratatu: MHNG 2265.003, holotype, 212.9 mm SL; Paraguay: Paraguari: Sapucay: affluent of Tebicuary. – MHNG 2251.35, 3 paratypes, 182–183.1 mm SL; MHNG 2251.36, 1 paratype, 217.7 mm SL; Paraguay: Concepcion: Trementina Stream. – MHNG 2251.38, 3 paratypes, 214–215.5 mm SL; Paraguay: Concepcion: Tagatya-mi Stream. – MHNG 2251.34, 2 paratypes, 220–240.5 mm SL; Paraguay: Cordillera: Piribebuy River.

H. plecostomus: MHNG 2651.066, 2; Guyane: Berbice River, Dubulay Ranch. – MHNG 2651.077, 1; Guyana: Berbice River, Cambo Cambo creek. – MHNG 2708.046, 4, and MHNG 2708.047, 8, Suriname: Commewijne River Basin, Mapana creek. – MHNG 2621.023, 17; Suriname: Suriname River Basin, mouth of Paulus creek. – MHNG 2708.042, 1; Suriname: Suriname River Basin: Klass creek. – MHNG 2671.065, 16; Suriname: Corantijn River Basin, Lower Corantijn River at Matapi.

H. punctatus: MHNG 2543.17, 3, 97.7–98.0 mm SL; Brazil: Ubatiba River.

H. regani: MCP 19989, 1; Brazil: Santa Catalina: Uruguay River Basin: Uruguay River. – MCP 28628, 1; Brazil: Rio Grande do Sul: Uruguay River Basin: Uruguay River: in Remanso da Timbaúva. – MHNG 2547.017, 3, 141.86–162.09 mm SL; Brazil: Sao Pablo: Mogui Guazu.

H. roseopunctatus: MHNG 2414.10, 1 paratype, 121.9 mm SL; Brazil: Rio Grande do Sul: Comandai River: at Porto Lucena. – MHNG 2414.40, 2 paratypes, 184–185 mm SL; Brazil: Santa Catarina: Canoas River: road between Abdom Batista – Anita Garibaldi: Campos Novos.

H. strigaticeps: BMNH 1907.7.6.10, 1 syntype (photographs only); Brazil: Sao Paulo: Rio Piracicaba.

H. ternetzi: MACN Ict 9645, 1, 150 mm SL; Argentina: Corrientes Province: Yahape: Parana River.

H. uruguayensis: MACN Ict 9651, 1, 159.0 mm SL; Argentina: Corrientes Province: Yahape: Parana River. – MFA-ZV-I-1224, 1, 234 mm SL; Argentina: Santa Fe. – MFA-ZV-I-1231, 1, 254 mm SL; Argentina: Santa Fe. – MHNG 2448.41, 1 paratype, 213.1 mm SL; Brazil: Santa Catarina: Uruguay River: Ita. – MHNG 2430.73, 2 paratypes, 178.7–179.1 mm SL; Brazil: Rio Grande do Sul: Uruguay River: Sao Borja.

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Literature cited

Aljanabi, S. M. & I. Martinez. 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Research, 25: 4692–4693.

Cardoso, Y. P., A. Almirón, J. Casciotta, D. Aichino, M. Lizarralde & J. I. Montoya-Burgos. 2012. Origin of species diversity in the catfish genus *Hypostomus* (Siluriformes: Loricariidae) inhabiting the Parana River Basin, with the description of a new species. Zootaxa, 3453: 69–83.

Cardoso, Y. P., F. Brancolini, L. Protogino & M. Lizarralde. 2011. Actinopterygii, Siluriformes, Loricariidae, *Hypostomus aspilogaster* (Cope, 1894). Distribu-

tion extension and first record for Argentina. Check List, 7: 596–598.
Dray, S. & A. B. Dufour. 2007. The ade4 package: implementing the duality diagram for ecologists. Journal of Statistical Software, 22 (4): 1–20.
Hill, M. O. & A. J. E. Smith. 1976. Principal component analysis of taxonomic data with multi-state discrete characters. Taxon, 25: 249–255.
Hubert, N., F. Duponchelle, J. Nuñez, C. Garcia-Davila, D. Paugy & J. F. Renno. 2007. Phylogeography of the piranha *Serrasalmus* and *Pygocentrus*: implications for the diversifications of the Neotropical ichthyofauna. Molecular Ecology, 16: 2115–2136.
Knaack, J. 1999. A new species of suckermouth catfish (*Hypostomus* Lacépède 1803) from the Mato Grosso, Brazil (Pisces, Siluriformes, Loricariidae). Tropical Fish Hobbyist, 47: 102–108.
Liotta, J. 2005. Distribución geográfica de los peces de aguas continentales de la República Argentina. Serie Documentos N3, ProBiota FCNyM, UNLP, La Plata.
López, H. L., A. M. Miquelarena & J. Ponte Gómez. 2005. Biodiversidad y distribución de la ictiofauna mesopotámica. Insugeo Miscelánea, 14: 311–354.
Lovejoy, N. R. & L. G. De Araújo. 2000. Molecular systematics, biogeography and population structure of Neotropical freshwater needlefishes of the genus *Potamorrhaphis*. Molecular Ecology, 9: 259–268.
Montoya-Burgos, J. I. 2003. Historical biogeography of the catfish genus *Hypostomus* (Siluriformes: Loricariidae), with implications on the diversification of Neotropical ichthyofauna. Molecular Ecology, 12: 1855–1867.
Montoya-Burgos, J. I., C. Weber & P.-Y. Le Bail. 2002. Phylogenetic relationships within *Hypostomus* (Siluriformes: Loricariidae) and related genera based on mitochondrial D-loop sequences. Revue Suisse de Zoologie, 109: 369–382.
Muller, S. & C. Weber. 1992. Les dents des sous-familles Hypostominae et Ancistrinae (Pisces, Siluriformes, Loricariidae) et leur valeur taxonomique. Revue Suisse de Zoologie, 99: 747–754.
Oyakawa, O. T., A. Akama & A. M. Zanata. 2005. Review of the genus *Hypostomus* Lacépède, 1803 from rio Ribeira de Iguape Basin, with description of a new species (Pisces, Siluriformes, Loricariidae). Zootaxa, 921: 1–27.
R Development Core team. 2009. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org.
Reis, R. E., C. Weber & L. R. Malabarba. 1990. Review of the genus *Hypostomus* Lacepede, 1803 from Southern Brazil, with descriptions of the three new species (Pisces, Siluriformes, Loricariidae). Revue Suisse de Zoologie, 97: 729–766.
Sivasundar, A., E. Bermingham, & G. Ortí. 2001. Population structure and biogeography of migratory freshwater fishes (*Prochilodus*: Characiformes) in major South American Rivers. Molecular Ecology, 10: 407–417.
Thioulouse, J. & S. Dray. 2007. Interactive multivariate data analysis in R with the ade4 and ade4tkgui packages. Journal of Statistical Software, 22 (5): 1–14.
Weber, C. 1985. *Hypostomus dlouhyi*, nouvelle espèce, de poison chat cuirassé du Paraguay (Pisces, Siluriformes, Loricariidae). Revue Suisse de Zoologie, 92: 955–968.
— 1986. Révision de *Hypostomus boulengeri* (Eigenmann & Kennedy), et deux espèces nouvelles de poissons-chats du Paraguay (Pisces, Siluriformes, Loricariidae). Revue Suisse de Zoologie, 93: 979–1007.
— 1987. *Hypostomus microstomus* sp. nov. et autres poissons-chats cuirasés du Rio Parana (Pisces, Siluriformes, Loricariidae). Revue Suisse de Zoologie, 40: 273–284.
— 2003. Hypostominae. Pp. 351–372. In: R. E. Reis, S. O. Kullander, & C. J. Ferraris (eds.), Check list of the freshwater fishes of South and Central America. Edipucrs, Porto Alegre.
Weber, C., R. Covain & S. Fisch-Muller. 2012. Identity of *Hypostomus plecostomus* (Linnaeus, 1758), with an overview of *Hypostomus* species from the Guianas (Teleostei: Siluriformes: Loricariidae). Cybium, 36: 195–227.
Zawadzki, C. H., C. Weber & C. S. Pavanelli. 2010. A new dark-saddled species of *Hypostomus* (Siluriformes: Loricariidae) from the upper rio Paraguay Basin. Neotropical Ichthyology, 8: 719–725.

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