

A new small barb (Cyprininae: Smiliogastrini) from the N'sele and Mayi Ndombe rivers in the lower reaches of the middle Congo basin (Democratic Republic of Congo, Central Africa)

MELANIE L.J. STIASSNY,¹ TOBIT L.D. LIYANDJA,²
AND RAOUL J.C. MONSEMBULA IYABA²

ABSTRACT

A new species of smiliogastrin cyprinid is described from tributaries of the middle Congo River in the Democratic Republic of Congo. Restriction of the genus name, *Barbus*, to certain large-bodied, (polyploid) barbines, and current uncertainty regarding phylogenetic relationships among the numerous small-bodied African (diploid) barbs, renders generic assignment for the new species problematical. Pending the results of ongoing systematic analyses, and to reduce short-term nomenclatural instability, the new species is described here as a species of "*Barbus*."³ "*Barbus*" *validus*, new species, is readily distinguished from all other small-bodied African barbs by the combined possession of scales in midlateral series that are not enlarged relative to those along the impinging rows above and below; well-developed barbels, with the maxillary pair extending beyond the level of mideye, and the mandibular pair reaching the level of midopercle; the presence of numerous conical tubercles over the snout, cheek, and dorsum of head; a small circular occipital fontanel located medially at the parietal suture; well-developed gill rakers, with 8 or 9 on the hypo- and ceratobranchial elements of the first arch; a last

¹ Department of Ichthyology, American Museum of Natural History, New York.

² Faculté des Sciences, Département de Biologie, Université de Kinshasa, Democratic Republic of Congo.

³ Because the ICZN has issued no ruling on the use of single vs. double quotation marks with binomials, the adoption of double quotation marks around the genus name here is in accord with American usage and this journal's style; in other publications the name may appear as '*Barbus*' until the status and composition of that genus has been established.

unbranched dorsal-fin ray that is weakly ossified and lacking serrations along the posterior border; and a dorsal fin that is creamy white proximally and with the distal half to two thirds darkly pigmented.

KEYWORDS: Congo River basin, cyprinine biodiversity, COI barcodes, taxonomy

INTRODUCTION

In a recent review of the fishes of the N'sele River, a large affluent tributary entering the Congo River at Pool Malebo, Monsembula Iyaba et al. (2013) reported on the presence there of five smiliogastrin cyprinids identified as *Barbus humeralis*, *Barbus matthesi* (= *Clypeobarbus matthesi* after Stiassny and Sakharova, in press), *Barbus miolepis*, *Barbus vanderysti*, and *Clypeobarbus pleuropholis*. The identification of *Barbus humeralis* was based initially on examination of two small individuals collected in a right bank tributary of the N'sele River, and subsequent collections in the neighboring Mayi Ndombe (Black) River, have rendered numerous additional samples of this taxon available for study. Comparison of these specimens with type and comparative materials of *Barbus humeralis*, indicate that our initial identification was made in error. Specimens from the N'Sele and Mayi Ndombe rivers originally identified as *B. humeralis* (e.g., Monsembula Iyaba et al., 2013: fig. 7E), although superficially similar to that taxon, do not conform to Boulenger's (1902) original description and instead represent an undescribed species for which a formal taxonomic description is provided herein. Comparative DNA sequence data in the form of COI barcodes (partial fragments of the mitochondrial cytochrome *c* oxidase I gene) (see table 1, fig. 1) confirm the distinctiveness of the new species, which differs in sequence from *B. humeralis* (from the Lulua River, Kasai basin) by more than 8%, a value considerably higher than the standard, albeit somewhat arbitrary, metric of a maximum of 3% COI sequence divergence for conspecifics (Song et al., 2008).

Since the restriction of the cyprinine genus *Barbus* sensu stricto to certain of the large bodied, (polyploid) barbin species of Europe, the African Magreb, and southwestern Asia (Berrebi et al., 1996), an appropriate generic designation for the approximately 300 small bodied (diploid) African barbs has remained highly problematical. Recently, however, Yang et al. (2015) have proposed the resurrection of the oldest available genus name, *Enteromius*, for these African "small barbs," and some authors have adopted that proposal (e.g., Decru et al., 2015). While Yang et al.'s (2015) study represents progress toward resolution of tribal-level relationships among the more the 1300 species of the subfamily Cyprininae, their sampling of African members of the newly erected tribe Smiliogastrini (viz., all African small barbs and their allies, and most members of the Asian genus *Puntius* and allies) was limited. And, as noted by Schmidt and Bart (2015), the current lack of resolution of relationships within the tribe renders the adoption of the name *Enteromius* for the numerous small-bodied African barbs problematical. If classification and nomenclature are to reflect phylogenetic relationships and monophyly is to prevail then, based on the trees presented by Yang et al. (2015), certain putatively monophyletic genera such as *Barboides* and *Clypeobarbus* will either need to be sunk into *Enteromius*, or various *Enteromius* species will need to be reassigned to *Barboides* and *Clypeobarbus*. In the absence of a well-supported tree that includes

TABLE 1. Taxa, voucher catalog numbers, and GenBank accession numbers for COI sequences.

Taxon	AMNH catalog	Tissue code	COI
<i>"Barbus" vanderysti</i>	AMNH 255251	223650	KT369040
<i>"Barbus" vanderysti</i>	AMNH 255257	223662	KT369039
<i>"Barbus" vanderysti</i>	AMNH 255259	223668	KT369038
<i>Clypeobarbus pleuropholis</i>	AMNH 256387	221131	KT369043
<i>Clypeobarbus matthesi</i>	AMNH 250728	072-7130	KT369042
<i>Clypeobarbus matthesi</i>	AMNH 250565	072-7124	KT369041
<i>"Barbus" validus</i> , n. sp.	AMNH 259315	213001	KT369036
<i>"Barbus" validus</i> , n. sp.	AMNH 259315	213004	KT369033
<i>"Barbus" validus</i> , n. sp.	AMNH 259240	212944	KT369034
<i>"Barbus" validus</i> , n. sp.	AMNH 259315	213000	KT369035
<i>"Barbus" miolepis</i>	AMNH 259266	212983	KT369032
<i>"Barbus" miolepis</i>	AMNH 250897	073-7231	HM880223
<i>"Barbus" humeralis</i>	AMNH 251138	074-7381	KT369032
<i>"Barbus" humeralis</i>	AMNH 251138	074-7382	KT369030
<i>"Barbus" humeralis</i>	AMNH 253143	080-7965	KT369029

considerably more African taxa, such action appears premature and likely to cause considerable nomenclatural instability. Therefore, in the short term we consider the admittedly far from satisfactory, but pragmatically transparent option to continue to refer to these species as *"Barbus"* as the more conservative nomenclatural choice. For these reasons we have opted to designate the species described herein as a new *"Barbus"* species, in the anticipation that ongoing phylogenetic studies incorporating considerably more of the African small-bodied *"Barbus"* species (e.g., Hayes and Armbruster, in litt., July 2015) will provide a solid phylogenetic framework upon which to base intratribal classification, generic assignments, and a stable nomenclature for the numerous African smiliogastrin species.

MATERIALS AND METHODS

Thirteen standard morphometric measurements and 15 meristic counts were taken following Lévêque et al. (1987). Specimens were pinned flat, and photographed on the left side with a Nikon Digital SLR camera and 60 mm f/2.8 AF Micro-Nikkor lens. Linear measurements were taken using the open access software ImageJ v1.48 (Schneider et al., 2012). Vertebral and fin-ray counts were taken from radiographed and/or cleared and stained specimens using a modified protocol of Taylor and Van Dyke (1985). Last branched + $\frac{1}{2}$ ray of the dorsal fin articulating with the same pterygiophore were counted as a single element. Vertebral counts include the four Weberian centra and the compound terminal centrum. Lateral-line counts exclude the pored scales on the caudal peduncle and fin distal to the point of caudal flexion.

Geometric morphometrics were used to compare variation in overall body shape between adult male and female specimens. Body shape variation was assessed with a set of 13 fixed

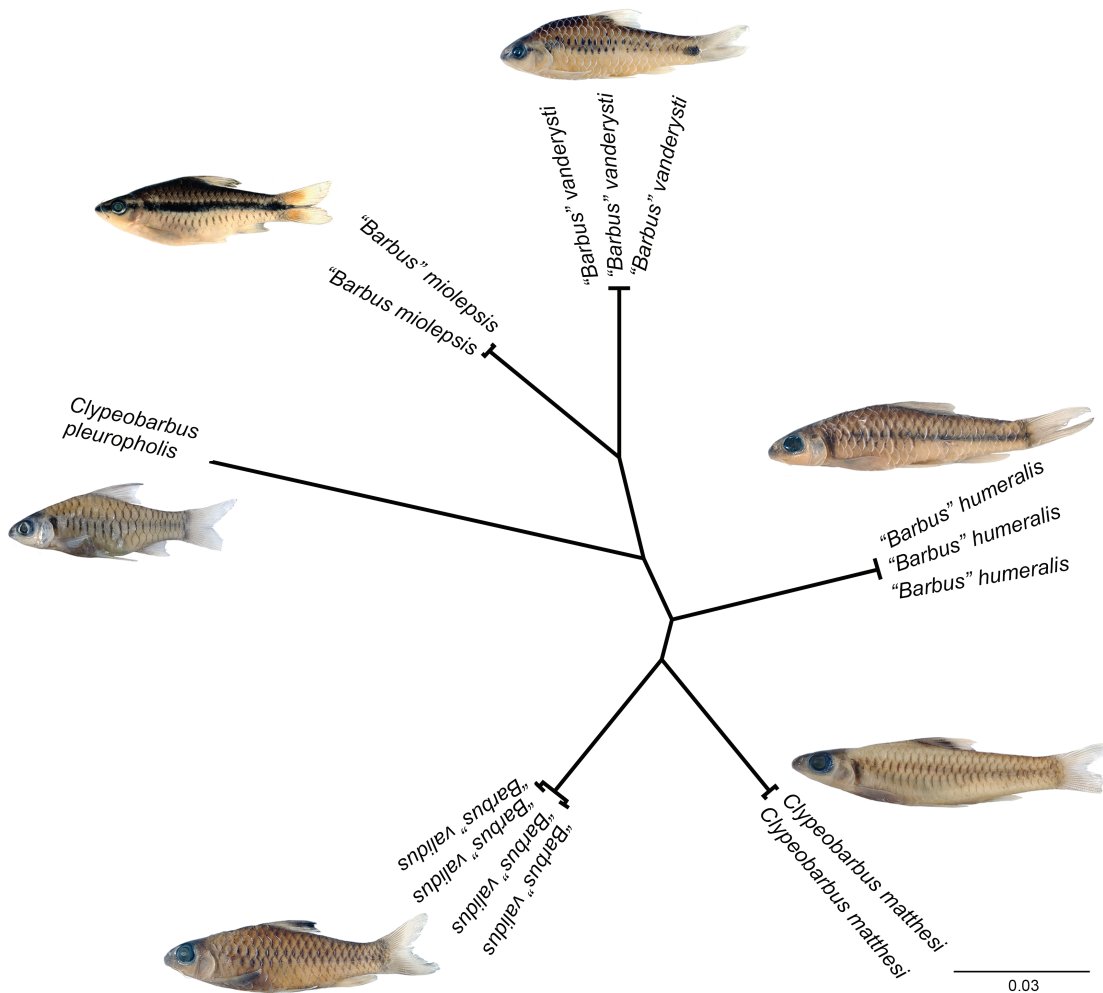


FIGURE 1. Unrooted UPGMA network of partial cytochrome c oxidase subunit I (COI) sequences for representatives of all N'sele River smiliogastrins and representative samples of *"Barbus" humeralis* from the Lulua River (Kasai basin).

landmarks digitized using version 2.17 of the program *tpsDIG2* (Rohlf, 2013). Shapes were aligned and visualized as deformation grids, computed with thin-plate spline transformations in the geomorph package (Adams and Otárola-Castillo, 2013) in R (R Core Team, 2013).

Abbreviations used are: **C&S**, cleared and stained preparations; **SL**, standard length; **ph**, photograph. Institutional abbreviations follow Sabaj Perez (2014).

Total genomic DNA was extracted from fresh tissue samples with DNeasy Tissue Extraction Kit (Qiagen) following the manufacturer's protocol. Amplification and sequencing of partial cytochrome c oxidase subunit I (COI) was carried out using Folmer et al.'s (1994) universal primers LCO1490 (50-GGTCAACAAATCATAA AGATATTGG-30) and HCO2198 (50-TAAACTTCAGG GTGACCAAAAAATCA-30). DNA amplification was performed in a 25 mL volume containing one Ready-To-Go PCR bead (GE Healthcare), 21 mL of PCR-grade

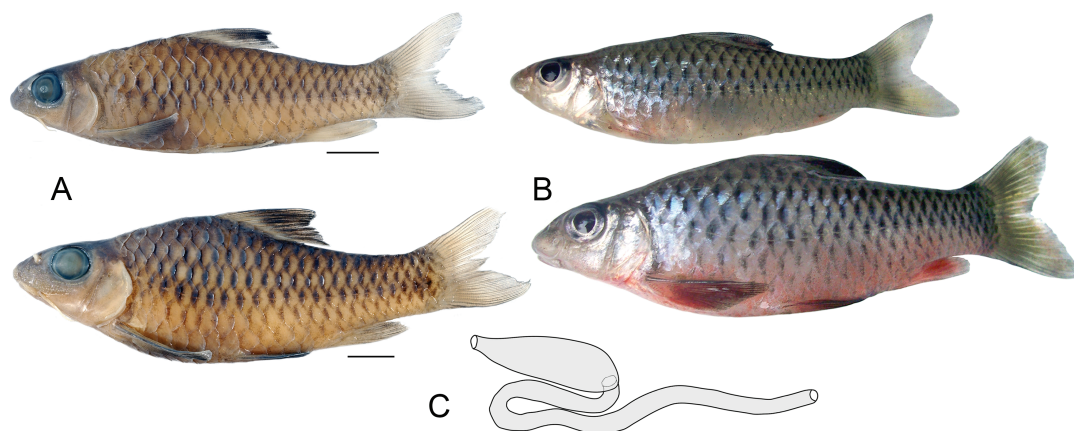


FIGURE 2. “*Barbus*” *validus*, new species: A, AMNH 259318, holotype (female) and AMNH 254600, paratype (male) in preservation; B, female and male immediately postmortem; C, digestive tract (slightly unraveled for clearer depiction of morphology), after removal of liver and adherent tissues (not to scale). Scale bars = 1 cm.

water, 1 mL of each primer (10 mmol/L), and 2 mL of genomic DNA, with the following thermal profile: 5 min initial denaturation at 95° C, followed by 35 cycles of denaturation at 95° C for 60 s, annealing at 42° C for 60 s, and extension at 72° C for 90 s, followed by a 7 min final extension at 72° C. Double-stranded PCR products were purified using AMPure (Agencourt). Sequencing of each strand of amplified product was performed in a 5 mL volume containing 1 mL of primer (3.2 mmol/L), 0.75 mL of BigDye Ready Reaction Mix, 1 mL of BigDye buffer, and 2.25 mL of PCR-grade water. Sequencing reactions consisted of a 2 min initial denaturation at 95° C, followed by 35 cycles of denaturation at 95° C for 30 s, annealing at 45° C for 60 s, and extension at 72° C for 4 min, followed by a 3 min final extension at 72° C. Sequencing reactions were purified using CleanSEQ (Agencourt) and electrophoresed on an Applied Biosystems 3700 automated DNA sequencer in the AMNH Molecular Systematics Laboratories. Bioinformatics Contig assemblage and sequence editing were performed using Geneious Pro v7.1.5 (Biomatters, available from <http://www.geneious.com/>). A UPGMA distance network was calculated in Geneious Tree Builder (Geneious Pro v7.1.5), using the Tamura-Nei genetic distance model and with no outgroup assigned. These data are represented in figure 1, which highlights the distinctiveness of the new taxon. Specimen voucher data and GenBank accession numbers for sequences generated in the current study are given in table 1.

“*Barbus*” *validus*, new species

Figures 2–6; table 2

HOLOTYPE: AMNH 259318, female, 73.9 mm SL, Democratic Republic of Congo, Kinshasa Province, Mayi Ndombe River at bridge (04° 16′ 39.9″ S, 015° 58′ 06.1″ E), T. Liyandja, 9 September 2011.

PARATYPES: AMNH 254600, 61.8 mm SL, Democratic Republic of Congo, Kinshasa Province, N’Sele River, small right bank stream flowing into main channel (04° 17′ 59.6″ S, 015° 41′

09.2" E), R.J.C. Monsembula Iyaba et al., 07 August 2011. — AMNH 254601, 56.3 mm SL, same data as AMNH 254600. — AMNH 259299, 93.2 mm SL, same locality as holotype, 7 September 2011. — AMNH 259274, 80.4 mm SL, same locality as holotype, 01 July 2011. — MCZ 171830, 78.3 mm SL, same locality as holotype, 01 July 2011. — AMNH 263755, 2 specimens, 73.0–91.1 mm SL, same data as holotype. — AMNH 259310, 3 specimens, 77.5–90.1 mm SL, same locality as holotype, 8 March 2011. — BMNH 2015.8.17.1, 76.0 mm SL, same locality as holotype, 8 March 2011. — AMNH 259315, 2 specimens, 79.4–93.8 mm SL, same locality as holotype, 22 August 2011. — ZSM 43946, 82.1 mm SL, same locality as holotype, 22 August 2011. — AMNH 258019, 4 specimens, 49.5–63.2 mm SL, Democratic Republic of Congo, Mai-Ndombe Province, Mayi Ndombe River, main channel just downstream of second rapid (03° 55' 51.4" S, 015° 58' 06.1" E), T. Liyandja, 01 May 2011. — AMNH 258076, 5 specimens, 49.6–94.8 mm SL, same locality as holotype, 30 June 2011. — MRAC B5-025-P-0001-0002, 2 specimens, 58.2–82.2 mm SL, same locality as holotype, 30 June 2011. — AMNH 259240, 6 specimens, 56.1–85.2 mm SL, 2 C&S, Democratic Republic of Congo, Mai-Ndombe Province, Mayi Ndombe River, main channel (04° 18' 22.2" S, 015° 59' 20.8" E), T. Liyandja et al., 01 July 2011. — AMNH 258938, 2 C&S, 49.1–52.8 mm SL, Democratic Republic of Congo, Kinshasa Province, Lumene River, ICCN base (04° 25' 09.7" S, 016° 02' 50.3" E), T. Liyandja et al., 05 March 2011. — AMNH 259332, 71.5 mm SL, Democratic Republic of Congo, Kinshasa Province, Mayi Ndombe River, Butu Bunkiene (04° 17' 45.2" S, 015° 58' 18.8" E), T. Liyandja et al., 09 September 2011.

OTHER MATERIAL EXAMINED: AMNH 263756, 10 specimens, 4 nonformalin fixed, same locality as holotype, 30 June 2011. — AMNH 263757, 8 specimens, 2 nonformalin fixed, Democratic Republic of Congo, Mai-Ndombe Province, Mayi Ndombe River, main channel, 04° 18' 22.2" S, 015° 59' 20.8" E, Coll. T. Liyandja et al., 01 July 2011 (4 as gift to University of Kinshasa teaching collection). — AMNH 257942, 4 specimens, Democratic Republic of Congo, Kinshasa Province, River Lumene (ICCN base) (04° 25' 09.7" S, 016° 02' 50.3" E), T. Liyandja et al., 05 March 2011. — AMNH 258062, 11 specimens, 4 nonformalin fixed, Democratic Republic of Congo, Kinshasa Province, Mayi Ndombe River, main channel at bridge (04° 16' 39.9" S, 015° 58' 06.1" E), T. Liyandja et al., 29 June 2011. — AMNH 263758, 3 specimens, same locality as holotype, 22 August 2011. — AMNH 263759, 3 specimens, nonformalin fixed, Democratic Republic of Congo, Mai-Ndombe Province, Mayi Ndombe River, main channel just downstream of second rapid (03° 55' 51.4" S, 015° 58' 06.1" E), T. Liyandja, 01 May 2011. — AMNH 258041, same locality as AMNH 258019, 22 May 2011. — AMNH 259318, same data as holotype. — AMNH 259236, 4 specimens, nonformalin fixed, Democratic Republic of Congo, Mai-Ndombe Province, Mayi Ndombe River, T. Liyandja, 30 June 2011.

DIFFERENTIAL DIAGNOSIS: "*Barbus*" *validus* is distinguished from all other African smilio-gastrin species by the following features that, in combination, uniquely diagnose the species: scales in midlateral series not enlarged relative to impinging rows above and below; barbels well developed, maxillary pair extending beyond level of mideye, mandibular pair reaching level of midopercle; numerous conical tubercles over snout and cheek, and dorsum of head; small circular occipital fontanel often present and located medially at parietal suture; gill rakers well developed, 8 or 9 on hypo- and ceratobranchial elements of the first arch; last unbranched

TABLE 2. Morphometric measurements and meristic data for the holotype and 34 paratypes of “*Barbus*” *validus*, new species.

Morphometric measurements	Holotype	Paratypes				
		min	max	n	mean	SD
Standard length (mm)	73.9	49.1	94.8	34		
% SL						
Head length	28.5	24.8	30.1	34	27.4	1.5
Predorsal length	49.4	47.2	52.3	34	50.1	1.2
Preanal length	76.5	74.3	77.9	34	76	1.2
Prepelvic length	52.1	50.9	54.9	34	53	1.2
Dorsal-fin base	15.7	14.1	17.4	34	15.9	0.9
Anal-fin base	7.8	6.5	10.2	34	8.3	0.9
Caudal-peduncle depth	14.2	13.6	15.6	34	14.6	0.6
Caudal-peduncle length	17.9	16.6	20.6	34	17.7	1.0
Body depth	31.5	26.3	35.5	34	31.3	2.3
%HL						
Eye diameter	39.6	37.2	46.9	34	40.4	2.8
Snout length	34.4	21.3	35.9	34	31.2	4.1
Head depth (through pupil)	58.2	50.6	64	34	57.5	3.2
Meristic counts	Holotype	Paratypes				
Gill rakers (total) on first arch	9	8(4), 9(12), 10(4)				
Lateral-line scales	23	22(5), 23(10), 24(4), 25(1)				
Body scale-rows	3.5/1/2.5	3.5/1/2.5(25)				
Predorsal scale-rows	7	7(14), 8(6)				
Circumpeduncular scale-rows	12	12(15), 11(5)				
Dorsal-fin rays	iii.8	iii.8(25)				
Anal-fin rays	iii.5	iii.5(25)				
Principal caudal-fin rays	9+10	9+10(20)				
Procurent caudal-fin rays	8+9	7+8(1), 7+9(6), 8+9(12), 8+10(1)				
Pectoral-fin rays	i.14	i.14(18), i.15(2)				
Pelvic-fin rays	i.7	i.7(20)				
Total vertebrae	35	35(12), 34(3)				
Abdominal vertebra	19	18(3), 19(11), 20(1)				
Caudal vertebra	16	15(3), 16(11), 17(1)				
Pleural ribs	14	13(9), 14(6)				

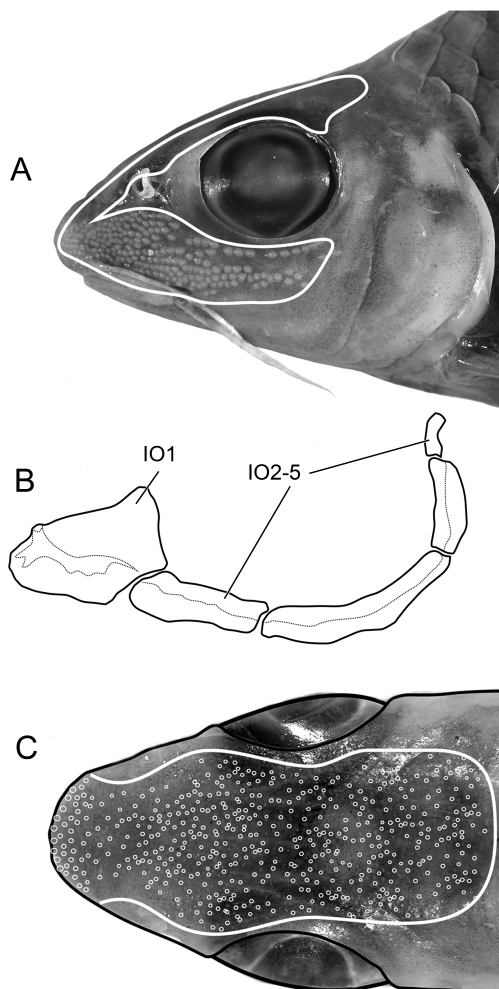


FIGURE 3. “*Barbus*” *validus*, new species: **A**, head in lateral view; **B**, isolated infraorbital series; **C**, head in dorsal view. White outlines indicate the location of tubercles.

dorsal-fin ray weakly ossified, flexible, and lacking serrations along posterior edge; dorsal fin creamy white proximally, distal half to two thirds darkly pigmented.

DESCRIPTION: A robust, deep-bodied “*Barbus*” attaining a maximum-recorded size of 95.8 mm SL (mature male, AMNH 258076), with general appearance as in figure 2. Proportional measurements and meristic counts for holotype and 34 paratypes given in table 2. Head relatively small, eyes large, mouth subinferior. Barbels robust and prominent, maxillary pair extending beyond level of mideye, mandibular pair reaching level of midopercle. Mature males with rows of large conical tubercles over snout, and numerous smaller tubercles scattered over dorsum of head (fig. 3A, C). Tubercles present, generally smaller and less prominent in females and juveniles. Small, circular, occipital fontanel usually present, located in midline at parietal suture, contained entirely within parietals (compare fig. 4A and 4B, C, see discussion). Five infraorbitals (IO): IO1 deepest of series, IO2–4 with infra-

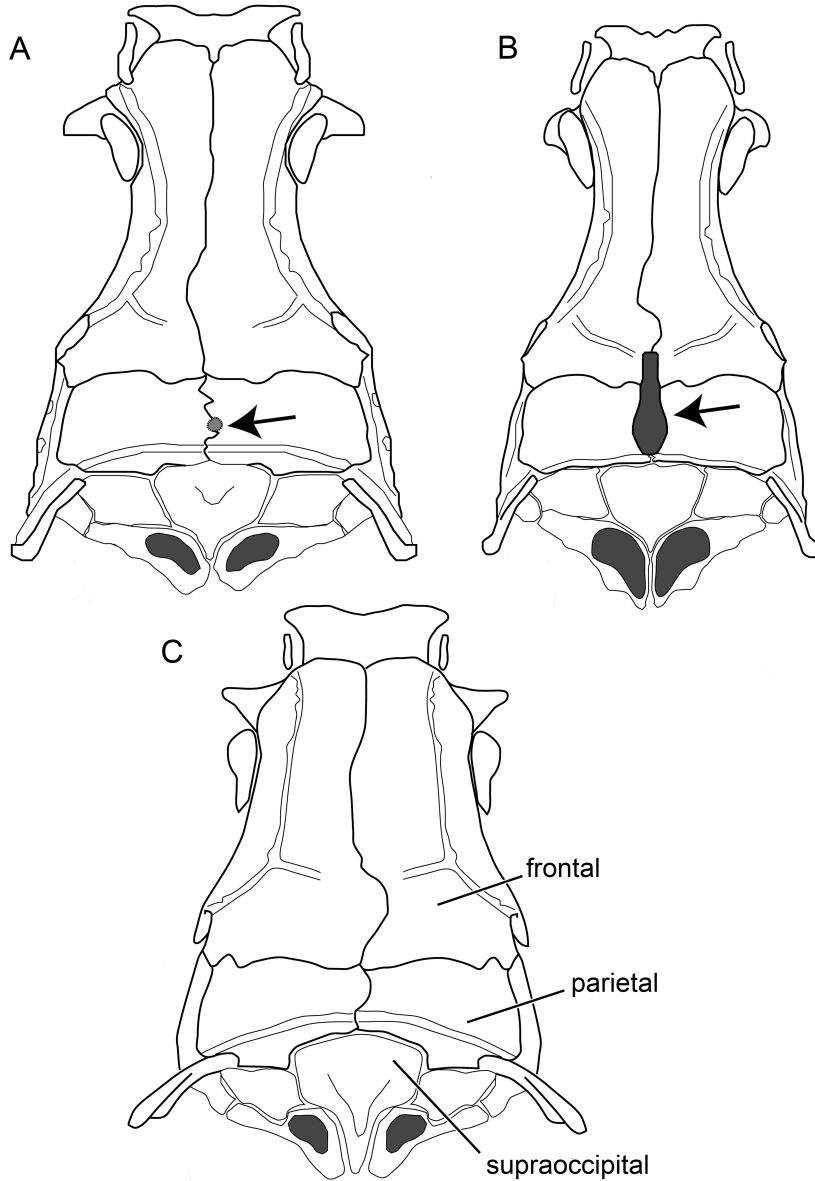


FIGURE 4. Neurocrania in dorsal view: **A**, “*Barbus*” *validus*, AMNH 258938; **B**, “*Barbus*” *humeralis*, AMNH 247409; **C**, “*Barbus*” *miolepis*, AMNH 250765. Arrows indicate location of occipital foramina.

orbital canal portion of bone occupying dorsal half of each element, IO3 longest of series, twice length of IO1, IO5 reduced to canal (fig. 3B). Scales radially striated, 22–25 in lateral line (+2–3 scales over caudal fin base), 3.5/1/2.5 body rows, 11–12 circumpeduncular rows, and 7–8 predorsal rows. Lateral line complete, scales not enlarged relative to those along impinging rows above and below.

Dorsal fin iii.8, positioned midway between snout and caudal-fin base, origin anterior to pelvic-fin insertion. Last unbranched ray long and flexible, lacking serrations on posterior

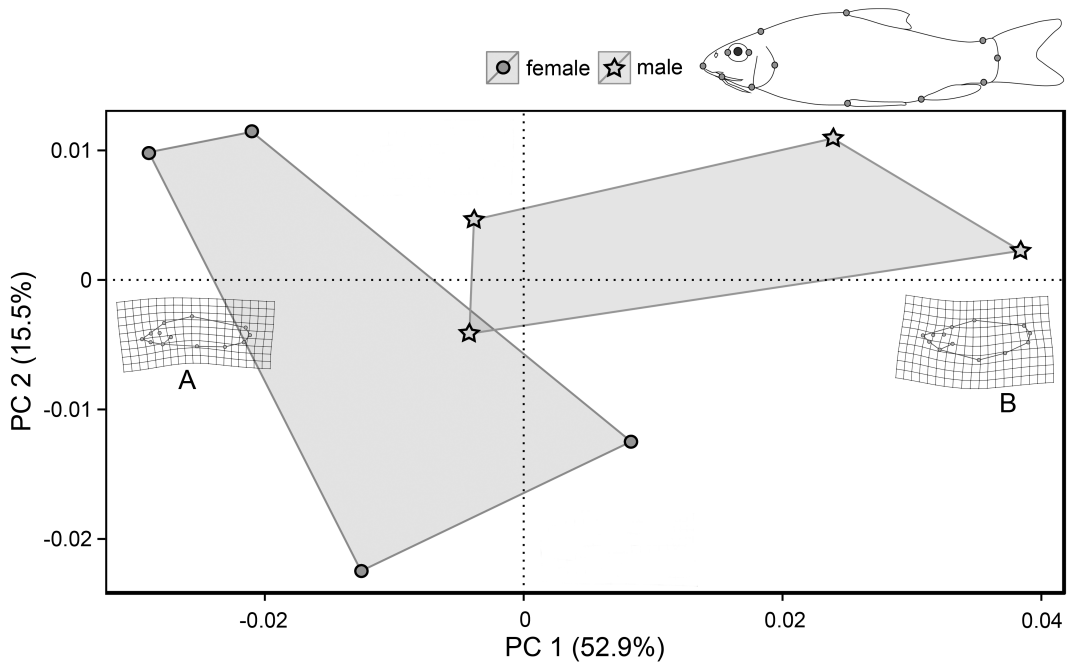


FIGURE 5. Principle components (PC) 1 and 2 displaying variation in body shape for mature male (stars) and female (circles) specimens of "*Barbus*" *validus*. Visualization of shape variation is provided as warped grids of (A) minimum and (B) maximum PC 1 values relative to mean shape.

border. Anal fin iii.5, forked caudal fin, 9 + 10 principal rays, 7–8 + 8–10 procurrent rays. Pelvic fin, 8 rays (i.7), pectoral fin, 15–16 rays (i.14–15).

Gill rakers well developed, 8–9 arrayed along hypo- and ceratobranchial of first arch, one smaller epibranchial raker usually present.

Total vertebrae 34–35, 18–20 abdominal, 15–17 caudal (holotype: 19 + 16). Thirteen or 14 pleural ribs, 4–5 supraneurals located between neural spines of vertebral centra 4–8. First dorsal-fin pterygiophore between neural spines of vertebral centra 9–10, and first anal-fin pterygiophore directed at hemal spine of vertebral centra 19 or 20. Caudal-fin rays supported by neural and hemal spines of preural centra 2–3. Pleurostyle, single epural, parhypural + seven hypural elements present.

Coloration in preservation (fig. 2A); base body coloration yellowish brown, darker dorsally, becoming pale ventrally and over belly, cheek, and jaws. Snout and dorsum of head, dark grayish brown. Faint trace of a cleithral stripe present in large individuals but often obscured by opercular margin. Body scales with a dark chevron-shaped marking along proximal edge, strongest dorsally and particularly along lateral line, less clearly marked ventrally. Dorsal fin creamy white proximally, distal half to two thirds darkly pigmented. Pectoral and pelvic fins smoky gray, strongly so in larger males. Scales over region of caudal flexion somewhat darker than preceding scales and forming a trace of a caudal bar. Base of caudal fin pale gray proximally, hyaline distally. Coloration immediately post mortem (fig. 2B) similar to that in preservation but overlain with a silvery reflectance and pale rose flush on ventrum. Rosy flush particularly evident in large males where it extends onto pectoral, pelvic, and anal fins.

GUT MORPHOLOGY AND DIET: Digestive tract short; esophagus leads to large, elongate, thick-walled stomach. Intestine exits directly from distal end of stomach, making a single rostrocaudal loop before descending to anus (fig. 2C). Total length of tract (unraveled but not stretched) ca. 80%–90% SL. Review of gut contents (preserved specimens) indicates that “*B. validus*” is omnivorous and opportunistic. Most gut contents contained disarticulated insect remains, including many tricopteran larvae and fragmented sand cases, numerous chironomid larval head cases and bodies, and various unidentifiable insect remains. In addition, woody debris, seeds, and macerated grasses, and few terrestrial arthropod remains of spiders and ants are also present, suggesting the species is opportunistically harvesting both aquatic and exogenous resources.

SEXUAL DIMORPHISM AND REPRODUCTION: Adult male specimens are generally larger than females (largest male, 95.8 mm SL, AMNH 258076; largest female, 88.9 mm SL, AMNH 259310) and, in addition to possessing larger conical tubercles over the snout, they tend also to be deeper bodied than females of similar size (fig. 5). Specimens collected in June, July, and August showed no sign of maturation of ovaries or testes. Most large individuals collected in March had enlarged testes or ovaries filled with numerous small, round eggs suggesting that peak breeding occurs at that time.

DISTRIBUTION: Currently known only from the N’sele and Mayi Ndombe Rivers (fig. 6). Further collecting in additional tributaries of the Middle Congo and Kwango River (Kasai drainage) will likely extend this distributional range. We note, however, that no specimens of the species are present in collections from the main channel of the Congo River either above or below Pool Malebo.

ETYMOLOGY: Specific name, *validus*, from the Latin meaning strong or powerful, in reference to the robust appearance of the species.

COMPARATIVE MATERIALS EXAMINED: “*Barbus*” *humeralis*: AMNH 6137 (holotype of *Barbus dolichosoma*), 52.2 mm SL, Democratic Republic of Congo, Tshopo Province, Avakubi. — BMNH 1901.12.26, syntype, 64.8 mm SL, Democratic Republic of Congo, Nord-Ubangi Province, Yembe River at Banzyville. — AMNH 253265, 51.5 mm SL, Democratic Republic of Congo, Kasai Central Province, Miao River, tributary of Lulua River (05° 56′ 23.0″ S, 022° 13′ 17.7″ E). — AMNH 247409, 38.5–49.1 mm SL, 2C&S, Democratic Republic of Congo, Kasai Central Province, Katende, Lulua River (06° 00′ 16.2″ S, 022° 23′ 25.8″ E). — AMNH 253143, 51.2 mm SL, Democratic Republic of Congo, Kasai Central Province, Tshiyoyi, Lulua River. — AMNH 247497, 51.4 mm SL, Democratic Republic of Congo, Lulua Province, Ntumba Shambuyi (05° 43′ 59.4″ S, 023° 19′ 06.0″ E). — AMNH 252779, 48.5 mm SL, Democratic Republic of Congo, Kasai Occidental Province, Kampaya, Lulua River (06° 25′ 32.9″ S, 022° 24′ 58.98″ E). — AMNH 247409, 2 cleared and stained specimens, 45.9–48.2 mm SL, Democratic Republic of Congo, Lulua Province, Katende, Lulua River (06° 00′ 16.2″ S, 022° 23′ 25.8″ E).

“*Barbus*” *miolepis*: AMNH 254599, 56.0 mm SL, Democratic Republic of Congo, Kinshasa Province, small rightbank stream flowing into main channel of N’sele River (04° 17′ 59.6″ S, 015° 41′ 09.2″ E). — AMNH 255139, 2 specimens, 48.5–56.8 mm SL, Democratic Republic of Congo, Mai-Ndombe Province, Mayi Ndombe River, Nganda Banga Nzambe above second rapid (03° 56′ 54.5″ S, 015° 59′ 20.3″ E). — AMNH 250765, 2 C&S, 56.1–57.8 mm SL, Demo-

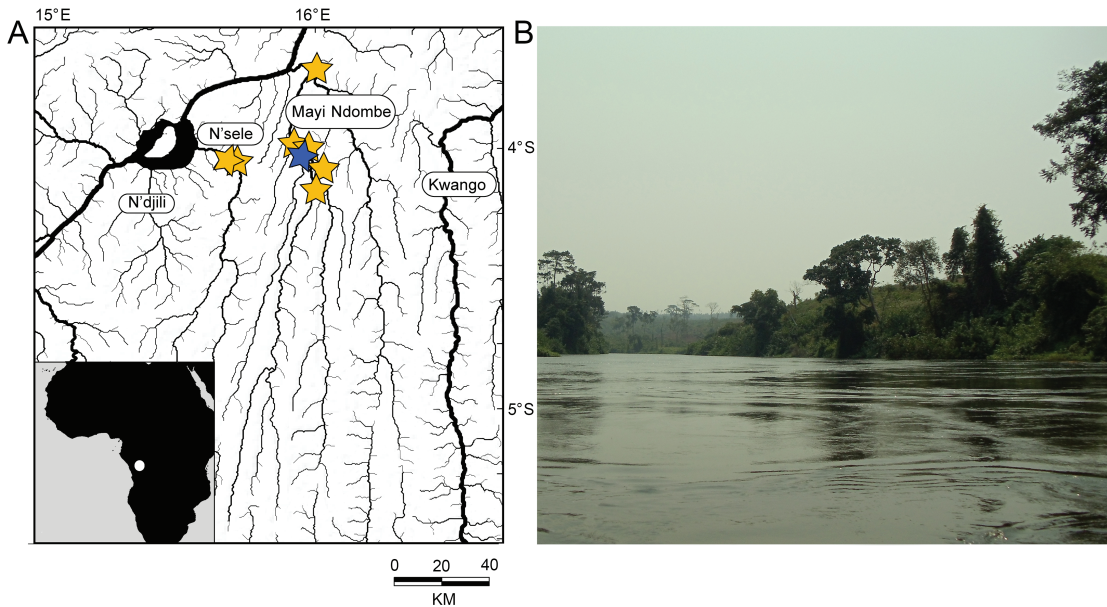


FIGURE 6. **A**, Distributional range of *Barbus* *validus*: yellow stars indicate collection sites and blue star indicates collection locality of holotype; **B**, typical habitat of *Barbus* *validus* in the Mayi Ndombe River.

cratic Republic of Congo, Kinshasa Province, small stream flowing into N'sele River (04° 26' 41.0" S, 015° 41' 00.4" E).

Barbus *vanderysti*: AMNH 257140, 4 specimens, 2 C&S, 48.5–56.4 mm SL, Democratic Republic of Congo, Kinshasa Province, River Binkukiti, N'Sele River drainage (05° 12' 43.1" S, 015° 34' 21.8" E). — AMNH 255251, 12 specimens, 46.4–62.0 mm SL, Democratic Republic of Congo, Kinshasa Province, River Nkengi, N'Sele River drainage (05° 14' 21.1" S, 015° 32' 49.1" E). — AMNH 255259, 5 specimens, 43.4–59.5 mm SL, Democratic Republic of Congo, Kinshasa Province, River Zodi, N'Sele River drainage (05° 12' 47.9" S, 015° 34' 24.8" E).

Clypeobarbus matthesi: AMNH 256172, 5 specimens, 43.1–65.0 mm SL, Democratic Republic of Congo, Kwilu Province, Kwilu River (05° 20' 54.2" S, 018° 56' 16.0" E). — AMNH 250565, 4 specimens, 48.2–58.6 mm SL, Democratic Republic of Congo, Kinshasa Province, N'sele River, below rapids at Kisangani Village, 04° 21' 31.9" S, 015° 42' 54.1" E. — AMNH 258000, 7 specimens, 2 C&S, 49.5–53.0, Democratic Republic of Congo, Kinshasa Province, Mayi Ndombe River at Mayi Ndombe Village. — AMNH 240447, 81.7 mm SL, Democratic Republic of Congo, Tshuapa Province, Salonga National Park, Nkombe-Dunda, Luilaka River (02° 40.289' S, 021° 43.259' E).

Clypeobarbus pleuropholis: AMNH 250741, 8 specimens, 34.4–47.8 mm SL, Democratic Republic of Congo, Kinshasa Province, Right bank N'Sele River, just below rapids (04° 21' 31.9" S, 015° 42' 54.1" E). — AMNH 237086, 5 C&S, 34.3–39.9 mm SL, Republic of Congo, Main channel of Congo River at confluence of Djoue River (05° 17' 32.4" S, 018° 56' 29.6" E). — AMNH 254854, 5 specimens, 36.8–48.5 mm SL, Democratic Republic of Congo, Kinshasa Province, main channel Congo River en route to Mayi Ndombe River (04° 01' 31.4" S, 015° 36' 37.1" E).

DISCUSSION

Resolution of phylogenetic relationships among the more than 300 species of African smiliogastrins remains an outstanding problem in cyprinid systematics (Berrebi et al., 1996; Schmidt and Bart, 2015). While no attempt is made here to place the newly discovered “*Barbus*” *validus* phylogenetically, we do note some apparent similarities between this species and certain members of the smiliogastrin genus, *Clypeobarbus*, as recently rediagnosed by Stiassny and Sakharova (in press). Similarly, “*Barbus*” *humeralis*, the species with which “*B.*” *validus* was initially confused, also shares features in common with *Clypeobarbus* species. For example, “*B.*” *humilis* shares with *Clypeobarbus* the derived presence of an elongate, occipital fontanel bordered anteriorly and laterally by the frontals and parietals, and posteriorly by the supraoccipital (fig. 4B). An occipital fontanel is also usually present in “*B.*” *validus*. However, that fontanel is small and circular, contained entirely within the parietals and located at the parietal suture in the midline (compare fig. 4A and B, C). “*Barbus*” *humilis* additionally shares with *Clypeobarbus* the derived presence of a distinctive pigmentation streak located posterodorsal to the cleithrum (cleithral streak, fig. 1). While a faint cleithral streak is often present in large specimens of “*B.*” *validus*, it is less strongly marked, narrower, and often obscured by the posterior margin of the opercle. “*Barbus*” *validus* has a faint caudal bar in the region of caudal flexion, a feature considered diagnostic of *Clypeobarbus* by Stiassny and Sakharova (in press). Finally, although not strictly diagnostic for *Clypeobarbus*, neither species share the pattern of free neuromasts arranged in sensory pit lines on the head as seen in *Clypeobarbus* and certain (*Enteromius*) “*Barbus*” species, but both exhibit numerous conical tubercles over the snout and cheek, and additionally share with the species of *Clypeobarbus* the presence of a last unbranched dorsal-fin ray that is weakly ossified, flexible, and without serrations along its posterior edge. However, both “*B.*” *humilis* and “*B.*” *validus* lack the characteristic enlargement of midline scales diagnostic for *Clypeobarbus*, and in both species the scales in the midlateral series are not enlarged relative to those along impinging rows above and below. As indicated previously, phylogenetic relationships among the numerous small-bodied “*Barbus*” are far from resolved, and pending the results of ongoing analyses we refrain here from proposing any generic reassignment for these two species.

ACKNOWLEDGMENTS

We are grateful to Julien Punga Kumanenga, Jean Malekani Mukulire, Koffi Mulumba, and all staff of the Biology Department at the University of Kinshasa for assistance with permits and logistical support during collecting trips to the N’sele and Mayi Ndombe rivers. Our thanks also to Emmanuel Vreven (MRAC) for providing photographs of the holotype “*Barbus*” *humeralis*, and to Oliver Crimmen and James Maclaine (BMNH) for access and hospitality during stays at the Natural History Museum, London. At the AMNH Jairo Arroyave and Christopher Martinez provided much helpful assistance with genetic data collection and geometric morphometric analyses. Barbara Brown, Radford Arrindell, Tom Vigliotta, and Lowell Flanders are acknowledged for their curation and care of specimens used in this study. We are particularly

grateful to Victor Mamonekene (University of Marien Ngouabi, Brazzaville) and José Justin Mbimbi Mayi Munene (University of Kinshasa) for their companionship in the field and for helpful input. Finally, we gratefully acknowledge the Else-Kröner-Fresenius-Stiftung for providing a BEBUC fellowship to T.L.D.L. The Axelrod Research Curatorship (M.L.J.S.) provided additional financial support for this study.

REFERENCES

- Adams, D.C., and E. Otárola-Castillo. 2013. Geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Methods in Ecology and Evolution* 4: 393–399.
- Berrebi, P., M. Kottelat, P. Skelton, and P. Rab. 1996. Systematics of *Barbus*: state of the art and heuristic comments. *Folia Zoologica* 45: 5–12.
- Boulenger, G.A. 1902. Additions à la faune ichthyologique de bassin du Congo. Matériaux pour la faune du Congo. *Annales du Musée du Congo (Zoology)* 2 (2): 19–57.
- Decru, E., et al. 2015. Taxonomic challenges in freshwater fishes: a mismatch between morphology and DNA barcoding in fish of the north-eastern part of the Congo basin. *Molecular Ecology Resources*. [early view edition doi: 10.1111/1755-0998.12445]
- Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299.
- Lévêque, C., G.G. Teugels, and D.F.E. Thys Van Den Audenaerde. 1987. Revision de quelques *Barbus* à trois taches (Pisces, Cyprinidae) d'Afrique de l'Ouest avec la description de trois espèces nouvelles: *B. tiekoro* n.sp., *B. traorei* n.sp. et *B. trispiloides* n.sp. *Revue d'Hydrobiologie Tropicale* 20: 165–184.
- Monsembula Iyaba, R.J.C., T. Liyandja, and M.L.J. Stiassny. 2013. Fishes of the N'sele River (Pool Malebo, Congo basin, Central Africa): a list of species collected in the main channel and affluent tributaries, Kinshasa Province, Democratic Republic of Congo. *Check List* 9: 941–956.
- R Core Team. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Online resource (<http://www.R-project.org/>), accessed 3 November 2015.
- Rohlf, F.J. 2013. tpsDIG2, v2.17. Freeware. Online resource (<http://life.bio.sunysb.edu/morph>), accessed 3 November 2015.
- Sabaj Perez, M.H. (2014). Standard symbolic codes for institutional resource collections in herpetology and ichthyology: an online reference. Version 5.0. Washington, D.C.: American Society of Ichthyologists and Herpetologists. Online resource (<http://www.asih.org/>).
- Schneider, C.A., W.S. Rasband and K.W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9: 671–675.
- Schmidt, R.C., and H.L. Bart. 2015. Nomenclatural changes should not be based on equivocally supported phylogenies: Reply to Yang et al. 2015. *Molecular Phylogenetics and Evolution* 90: 193–194 (<http://dx.doi.org/10.1016/j.ympev.2015.05.025>).
- Song, H., J. E. Buhay, M. F. Whiting, and K. A. Crandall. 2008. Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified. *Proceedings of the National Academy of Sciences of the United States of America* 105: 13486–13491.
- Stiassny, M.L.J., and H. Sakharova. In press. Review of the smiliogastrin cyprinids of the Kwilu River (Kasai basin, central Africa), revised diagnosis for *Clypeobarbus* (Cyprinidae: Cyprininae: Smiliogastrini), and description of a new species. *Journal of Fish Biology*.

- Taylor, W.R., and G.C. Van Dyke. 1985. Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybium* 9: 107–119.
- Yang, L., et al. 2015. Phylogeny and polyploidy: Resolving the classification of cyprinine fishes (Teleostei: Cypriniformes). *Molecular Phylogenetics and Evolution* 85: 97–116.

All issues of *Novitates* and *Bulletin* are available on the web (<http://digitallibrary.amnh.org/dspace>). Order printed copies on the web from:

<http://shop.amnh.org/a701/shop-by-category/books/scientific-publications.html>

or via standard mail from:

American Museum of Natural History—Scientific Publications
Central Park West at 79th Street
New York, NY 10024

Ⓒ This paper meets the requirements of ANSI/NISO Z39.48-1992 (permanence of paper).