

A New Species of *Chaetostoma* (Siluriformes: Loricariidae) Expands the Distribution of Rubbernose Plecos Eastward into the Lower Amazon Basin of Brazil

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A new species of the rubbernose pleco genus *Chaetostoma* is described from the Maicuru and Seiko Rivers, a northern tributary of the lower Amazon River and a tributary of the lower Xingu River, respectively, both in Pará State, Brazil. The new species is diagnosed from all congeners, except members of the *Chaetostoma anale* species group, by having an enlarged second unbranched anal-fin ray with posterior paired dermal flaps. Additionally, the new species is distinguished from its only other currently recognized congeners from rivers draining the Guiana Shield (*C. jegui* and *C. vasquezi*) by having a smaller opercle and a supraoccipital excrescence undeveloped, comprising a simple skin area present in juveniles and absent in adults. A revised multi-locus phylogeny for the species of *Chaetostoma* is presented, and the *Chaetostoma anale* species group is discussed and rearranged.

Uma nova espécie de *Chaetostoma* é descrita dos rios Maicuru, um afluente norte do baixo rio Amazonas, e Seiko, um afluente do baixo rio Xingu, ambos no estado do Pará, Brasil. A nova espécie é diagnosticada de todos os seus congêneres, exceto membros do grupo *Chaetostoma anale*, por apresentar o segundo raio não ramificado da nadadeira anal aumentado e com abas dérmicas posteriores. Além disso, a nova espécie se distingue dos únicos outros congêneres atualmente reconhecidos do Escudo das Guianas (*C. jegui* e *C. vasquezi*) por ter o opérculo menor e a protuberância supraoccipital não desenvolvida, composta por uma área de pele simples em juvenis e ausente em adultos. Uma filogenia multilocus revisada para as espécies de *Chaetostoma* é apresentada e o grupo *Chaetostoma anale* é discutido e reorganizado.

SPECIES of *Chaetostoma* are popularly known as rubbernose plecos because they lack either plates, odontodes, or fleshy tentacles on the anterior and anterolateral portions of the snout, which is instead covered by soft skin and slime coat. *Chaetostoma* is the third most species-rich genus in the Hypostominae subfamily of the sucker-mouth armored catfish family Loricariidae (Lujan et al., 2015; Salcedo and Ortega, 2015; Ballen et al., 2016; Urbano-Bonilla and Ballen, 2021), and currently includes 49 valid species. Most species of *Chaetostoma* are distributed between approximately 80 to 2,500 m asl either in rivers along the eastern (cis-Andean) flanks of the Andes Mountains from Venezuela to southern Peru ($n = 24$) or western (trans-Andean) flanks of the Andes from Panama to northern Peru ($n = 18$). However, five species are restricted to Caribbean coastal drainages in Colombia and Venezuela, two are endemic to the Lake Valencia drainage in Venezuela, and two occur in Guiana Shield tributaries of the Amazon and Orinoco basins in Brazil and Venezuela. The two eastern, Guiana Shield species are *C. jegui* from the upper Branco River in northern Brazil (Amazon River basin) and *C. vasquezi* from the Caura and Caroni Rivers

in southern Venezuela (Orinoco River basin). This Andean/Guiana Shield distribution and elevated species richness have been of interest for studying the impact of Andean orogeny on biological diversification (Salcedo et al., 2011) and for helping to resolve the historical biogeographical processes affecting freshwater biodiversity in the Andes and adjacent areas (Rodríguez-Olarte et al., 2011; Schaefer, 2011).

Ballen (2011) described *Chaetostoma formosae* from the Meta River drainage in Colombia and identified a putatively monophyletic group of species comprising *C. formosae*, *C. anale*, and *C. jegui*, which he called the *Chaetostoma anale* species group. Ballen (2011) diagnosed this group by mature males having a modified anal fin where the second unbranched ray is broader and longer than the first unbranched ray, and the posterior pelvic-fin margin has a distinct shape in which middle rays are longest. These dimorphic conditions had already been observed and described by Rapp Py-Daniel (1991) and are shared by the latter two species.

Lujan et al. (2015) proposed a phylogenetic hypothesis for 20 valid and seven putatively undescribed species of *Chaetostoma* based on multi-locus molecular data (using

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Table 1. Primers and annealing temperatures for amplifications. Ta, annealing temperature.

| Locus | Primer name | Primer sequence (5' → 3') | Ta (°C) | Reference |
|-------------|-------------|------------------------------|---------|------------------------|
| 16S | 16sa | CGCCTGTTTATCAAAAACAT | 50, 48 | Palumbi et al. (2002) |
| | 16sb | CCGGTCTGAACTCAGATCACGT | | |
| <i>cytb</i> | cytbFa | TCCCACCCGGACTCTAACCGA | 56, 54 | Lujan et al. (2015) |
| | cytbRa | CCGGATTACAAGACCGGCGCT | | |
| | FishcytB-F | ACCACCGTTGTTATTCAACTACAAGAAC | | |
| | TruccytB-R | CCGACTTCCGGATTACAAGACCG | | |
| <i>nd2</i> | nd2_Dist_f | AGCTTTTGGGCCCATACCCCA | 58 | Arroyave et al. (2013) |
| | nd2_Dist_r | AGGRACTAGGAGATTTTCACTCCTGCT | | |
| <i>col</i> | L6252-Asn | AAGGCGGGGAAAGCCCCGGCAG | 50, 48 | Melo et al. (2011) |
| | H7271-COXI | TCCTATGTAGCCGAATGGTCTTTT | | |
| | FishF1 | TCAACCAACCACAAAGACATTGGCAC | | |
| | FishR1 | TAGACTTCTGGGTGGCCAAAGAATCA | | |
| <i>myh6</i> | myh6_F459 | CATMTTYTCCATCTCAGATAATGC | 53 | Li et al. (2007) |
| | myh6_F507 | GGAGAATCARTCKGTGCTCATCA | 62 | |
| | myh6_R1325 | ATTCTCACCACCATCCAGTTGAA | | |
| | myh6_R1322 | CTCACCACCATCCAGTTGAACAT | | |
| <i>rag1</i> | RAG1Fa | CCTGGTTTTTCATGCATTTGAGTGGCA | 53 | Lujan et al. (2015) |
| | RAG1R1186 | ACGCTCTTCTGARGGAATA | | |
| <i>rag2</i> | RAG2Fc | ATGGAGGCCGAAACACCCAACA | 58 | |
| | RAG2R961 | CGCTGCTGWACTCCATTT | | |

genes 16S, *cytb*, *rag1*, *rag2*, and *myh6*). Lujan et al. (2015) found *Chaetostoma* to be monophyletic as long as three genera are considered junior synonyms (*Lipopterichthys*, *Hypocolpterus*, and *Loraxichthys*) and that *Chaetostoma* was sister to a clade containing five Central and Northern Andean genera with the following phylogenetic interrelationships: (*Andeancistrus* + *Transancistrus* (*Leptoancistrus* + (*Dolichancistrus* + *Cordylancistrus*))). In their hypothesis, Lujan et al. (2015) added two more species to the *Chaetostoma anale* species group, *C. dermorhynchum* and *Chaetostoma* sp. “Xingu,” which nested in a clade with *C. formosae*, as sister to their new species *C. trimaculineum*.

The *Chaetostoma* sp. “Xingu” of Lujan et al. (2015) was first collected during an expedition by the Museu de Zoologia of the University of São Paulo (MZUSP) in 2011, when five specimens of this putatively new *Chaetostoma* were caught in the Seiko River, a tributary of the Jarauçu River, which is itself a left-bank (western) tributary of the lower Xingu River. A second expedition by the Academy of Natural Sciences of Drexel University (ANSP) collected a few additional specimens in 2012, and a third independent collecting expedition by the Instituto Nacional de Pesquisas da Amazônia (INPA) collected an additional 23 specimens in 2018, all at the same locality. A few years before, in 2015, an expedition of the NSF-funded project Aquatic Faunal Survey of Lower Amazon basin in Pará State, Brazil revealed 16 additional specimens of an unknown species of *Chaetostoma* from the Maicuru River, an independent northern tributary of the lower Amazon River that drains the southern flank of the Guiana Shield, approximately 800 km southeast of the geographically closest congener (*C. jegui*). In this study, we demonstrate that populations of *Chaetostoma* from the Maicuru and Xingu Rivers are conspecific, describe it as a new species, investigate this species’ phylogenetic placement within the genus, and redefine the *Chaetostoma anale* species group.

MATERIALS AND METHODS

Taxonomic sampling.—The data matrix of the *Chaetostoma* Clade (Lujan et al., 2015) was used to add new specimens and

additional molecular markers. Ingroup taxa spanned 37 valid species of *Chaetostoma*, representing 75% of the known diversity of the genus, and eight putatively undescribed species. Outgroup taxa included representatives of all other genera in the *Chaetostoma* Clade (*sensu* Lujan et al., 2015; *Andeancistrus*, *Cordylancistrus*, *Dolichancistrus*, *Leptoancistrus*, and *Transancistrus*). Fish collections providing specimens for this study are identified using acronyms in Sabaj (2020). Tissues used in the molecular analysis were obtained from the collections of ANSP, INPA, LBP, MCP, and ROM. See Supplemental Table 1 (see Data Accessibility) for a list of species, catalog number, locality, and GenBank accession numbers of all samples. Taxonomic identities of the voucher specimens of tissues used in the molecular analyses were confirmed by direct examination. Conservation status of the new species was assessed following the categories and criteria of the International Union for Conservation of Nature (IUCN Standards and Petitions Subcommittee, 2019). The geographic distribution map was created with shape and raster files downloaded from the databases of ANA (Agência Nacional de Águas, Brazil: <https://www.snirh.gov.br/hidroweb>) and GADM (Geographic Database of Global Administrative Areas; <https://gadm.org>) with the software QGIS (v. 2.14.5), using the tutorial of Calegari and Fontenelle (2017).

DNA sequencing.—Molecular sequences of the new species were obtained from four mitochondrial markers (16S, *cytb*, *col*, and *nd2*) and three nuclear markers (*rag1*, *rag2*, *myh6*). Total DNA was extracted in a volume of 100 µl using DNeasy Blood & Tissue kit (Qiagen®, Hilden, Germany). The polymerase chain reaction (PCR) was performed in 25 µl of 10 mM of each primer (Table 1), 1X Platinum® PCR SuperMix (Invitrogen™), and 15–30 ng of 1:9 diluted DNA. The fragments were amplified under the following thermocycler conditions: initial denaturation of 2 min at 94°C; 35 cycles of 1 min at 95°C, 30 s at annealing temperature (Table 1), and 2 min at 72°C; followed by a final extension of 10 min at 72°C and storage step of 5 min at 4°C. The amplicons were purified and sequenced in both directions at Functional Biosciences,

Inc. (WI, USA). Estimates of interspecific genetic divergence within the *Chaetostoma anale* species group were calculated by comparing *col* sequence data in MEGA7 (Kumar et al., 2016) using the Kimura 2-parameter model (Kimura, 1980). Paratypes of the new species (MCP 50363; voucher 562, from the Maicuru River and ANSP 199686; voucher B1472 from the Xingu basin) were sequenced for various genes (GenBank accession numbers below) and therefore constitutes genseq-2 (Chakrabarty et al., 2013).

Phylogenetic analysis.—Sequences for each gene were aligned automatically using Geneious (v.10, Biomatters, Inc.) then manually edited, trimmed to reading frame when characterized as a coding gene, and checked to ensure the absence of internal stop codons. The expanded data matrix was analyzed by maximum likelihood with the software RAXML (Stamatakis, 2006) in the CIPRES platform. The analysis was conducted on a concatenated dataset partitioned by gene, by first running a 200-replicate search for the best tree followed by a 2000-replicate bootstrap analysis, in both cases using a generalized time-reversible model of nucleotide substitution with rate heterogeneity modeled by a gamma distribution (GTR + gamma).

Morphometric data collection.—Morphometric and meristic data were taken from 25 specimens following Pereira et al. (2007) and Rapp-Py-Daniel et al. (2019). Measurements were taken point-to-point to the nearest 0.1 mm with digital calipers and expressed as percent of standard length (SL) or head length (HL). Osteological data and counts of vertebrae and ribs were obtained from five individuals, including the holotype, via radiographs taken in dorsal and lateral view. In the list of examined material, museum abbreviation and catalog number come first, followed by the number and SL range of specimens in that lot, the number and SL range of specimens measured for the morphometric comparisons, in parentheses, if different, followed by locality data. Morphological comparisons were made with specimens housed at INPA, LBP, MCP, MUSM, and ROM.

***Chaetostoma orientale*, new species**

urn:lsid:zoobank.org:act:35BE1946-45EA-4805-B159-689EA296AA9B

Figure 1

Chaetostoma sp. “Rio Maicuru” L413: Seidel (2008). Aquarium description, photos.

Chaetostoma sp. “Rio Xingu” L416: Seidel (2008). Aquarium description, photos.

Chaetostoma sp. “Xingu”: Lujan et al. (2015). Species included in phylogeny.

Holotype.—MCP 54585, male, 132.4 mm SL. Brazil, Pará, Monte Alegre, Maicuru River at road PA-254, Amazon River basin, 1°36'47"S, 54°22'37"W, elevation 33 m asl, W. Crampton, R. Reis, B. Calegari, F. Lima, C. Oliveira, J. Bogotá, and E. Cerdeira, 3 October 2015.

Paratypes.—Maicuru River, Monte Alegre, Pará, Brazil: MCP 50363, 10, 20.0–114.8 mm SL (5 measured 53.9–114.8 mm SL), ROM 111498, 2, 47.8–90.0 mm SL (1 measured 90.0 mm SL), ZUEC 17331, 3, 38.9–87.5 mm SL (1 measured 87.5 mm SL), collected with holotype; INPA-ICT 58110, 1, 48.0 mm SL, Maicuru River at Três Marias Village, 1°36'35.53"S,

54°22'15.78"W, L. Rapp Py-Daniel, R. Oliveira, C. Silva-Oliveira, F. Ribeiro, A. Canto, and A. Gonçalves, 13 October 2018; INPA-ICT 59582, 7, 57.0–147.5 mm SL, Maicuru River at bridge, 1°36'33.6"S, 54°22'22.4"W, P. Viana, 15 November 2020. Xingu River basin, Medicilândia, Pará, Brazil: ANSP 199686, 2, 74.4–133.3 mm SL, INPA-ICT 37928, 1, 105.2 mm SL, Seiko River, tributary to Jarauçu River, itself a tributary to Xingu River, ca. 3.3 km west of Medicilândia on Trans-Amazon road, 3°27'22.53"S, 52°55'33.61"W, elevation 98 m asl, M. H. Sabaj, M. Arce and L. M. Sousa, 5 October 2012; INPA-ICT 58146, 19, 36.1–170.0 mm SL (19 measured, 69.2–170.0 mm SL), MPEG 38952, 2 (2 measured 69.2–142.0 mm SL), ROM 111499, 2 (2 measured 75.3–89.9 mm SL), same locality as above, L. Rapp Py-Daniel, R. Oliveira, C. Silva-Oliveira, F. Ribeiro, A. Canto, and A. Gonçalves, 16 October 2018; MZUSP 111724, 5, 53.3–143.0 mm SL, same locality as above, O. Oyakawa, J. Birindelli, C. Moreira, A. Akama, L. Sousa, and H. Varela, 19 November 2011.

Genseq-2 GenBank accession numbers.—The following DNA sequences were obtained from paratype tissue vouchers 562 (MCP 50363, Maicuru basin) and B1472 (ANSP 199686, Xingu basin), respectively. 16S: —, OL303552; *col*: OK514642, OK514639; *cytb*: OL303598, OL303680; *nd2*: OL303349, OL303359; *rag1*: —, OL303244; *rag2*: OL303809, OL303885; *myh6*: OL303718, OL303773.

Diagnosis.—*Chaetostoma orientale* is distinguished from most congeners, except for members of the *Chaetostoma anale* species group (*C. anale*, *C. dermorhynchum*, *C. formosae*, and *C. trimaculineum*) and *C. jegui* by having an enlarged second unbranched anal-fin ray with posterior paired dermal flaps (Fig. 2; vs. second unbranched anal-fin ray not hypertrophied, lacking posterior dermal flaps). The new species differs from species of the *Chaetostoma anale* species group by having cheek odontodes relatively short, thick, and hooked, type 3 of Lujan et al. (2015; Fig. 3; vs. thinner, more elongate, and slightly hooked, type 2; see Lujan et al., 2015 for more details). *Chaetostoma orientale* differs from *C. formosae* by the possession of light spots on the posterior portion of dorsal fin (vs. light marks on dorsal fin absent). The new species also differs from *C. jegui* by having a longer lower lip reaching the level of the ventral end of the branchial membrane, buccal papilla with accessory digitate lobes, pectoral-fin leading ray reaching to the proximal third of pelvic-fin leading ray when adpressed, and head color pattern consisting of dark spots (vs. lower lip falling short of the branchial membrane, buccal papilla simple and without accessory lobes, pectoral-fin leading ray reaching beyond the middle of pelvic-fin leading ray when adpressed, and head color pattern consisting of light dots). *Chaetostoma orientale* is distinguished from Guiana Shield congeners, *C. jegui* and *C. vasquezi*, by having the exposed portion of the opercle narrower than one orbital width, anterior margin of exposed opercle at vertical line through anterior orbital margin (vs. opercle and orbit of approximately same width, anterior margin of exposed opercle conspicuously behind vertical line through anterior orbital margin), and the supraoccipital excrescence unpigmented and undeveloped, a simple skin area in juveniles and absent in adults (vs. supraoccipital excrescence, or skin dermal keel, conspicuous, present on supraoccipital region of juveniles and adults). Additionally, among cis-Andean congeners, the new species is distinguished from the Amazon



Fig. 1. *Chaetostoma orientale*, holotype, MCP 54585, male, 132.4 mm SL. Brazil, Pará, Monte Alegre, Maicuru River.

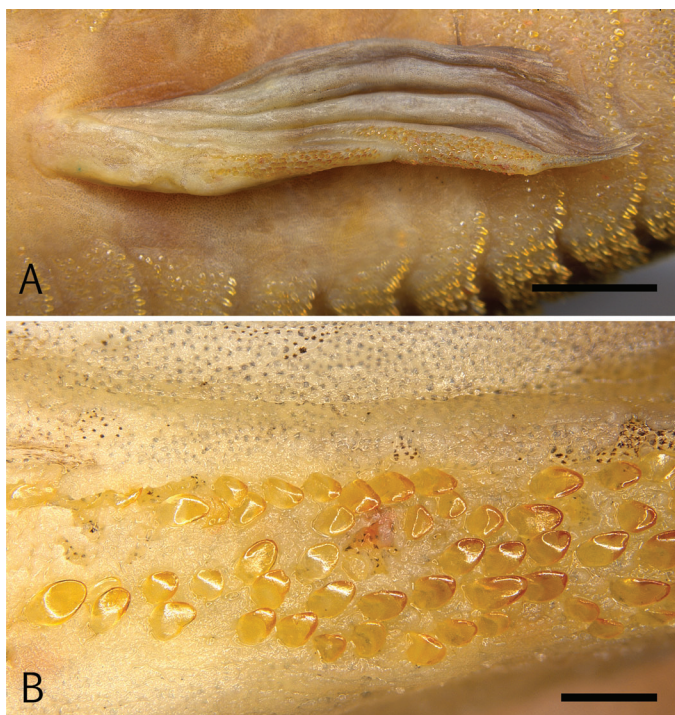


Fig. 2. Photomicrograph of the modified anal fin of mature male of *Chaetostoma orientale*, holotype, MCP 54585, male, 132.4 mm SL. (A) Anal fin in ventral view, scale bar = 5 mm; (B) odontodes of anal fin in detail, scale bar = 0.5 mm.

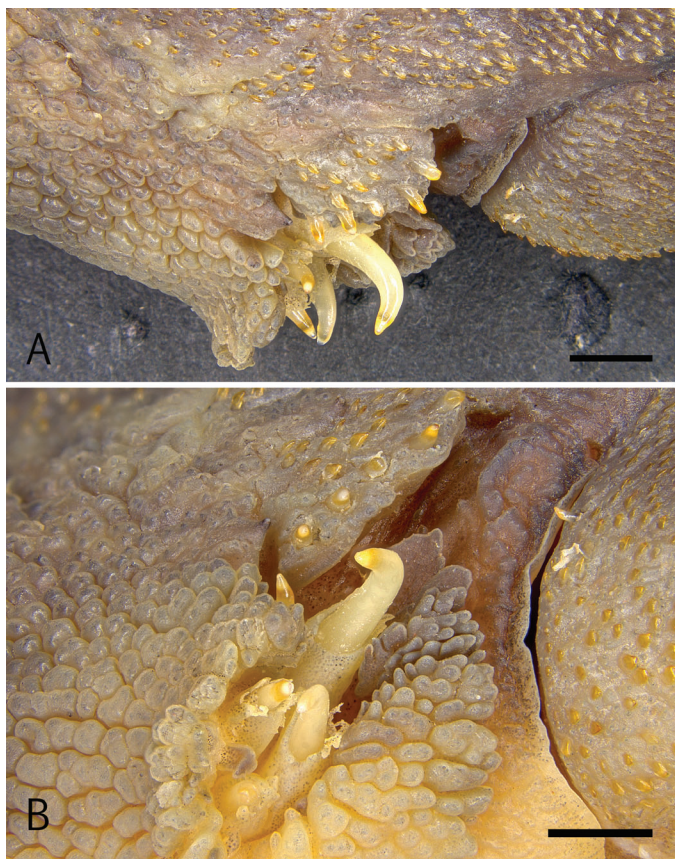


Fig. 3. Photomicrograph of type 3 cheek odontodes of mature male of *Chaetostoma orientale*, holotype, MCP 54585, 132.4 mm SL. (A) Dorsal view; and (B) lateral view. Scale bar = 2 mm.

basin species *C. branickii*, *C. breve*, *C. carrioni*, *C. changae*, *C. jegui*, *C. lexa*, *C. lobarhynchos*, *C. marmorescens*, *C. microps*, and *C. taczanowskii*, and those from Orinoco basin *C. dorsale*, *C. platyrhynchus*, and *C. vasquezzi* by having small faded black spots on the head (vs. absence of spots in *C. branickii*, *C. changae*, *C. jegui*, *C. lexa*, *C. marmorescens*, *C. taczanowskii*, and *C. vasquezzi*; or spots of different colors: light green to gray spots in *C. lobarhynchos*; white spots in *C. breve* [when present], *C. carrioni*, *C. dorsale*, and *C. platyrhynchus*; and white/blue/yellow spots in *C. microps*). *Chaetostoma orientale* further differs from *C. anale*, *C. chimu*, *C. daidalmatos*, *C. joropo*, *C. lineopunctatum*, *C. milesi*, *C. strompoulos*, and *C. trimaculineum* by lacking spots on the trunk, except for plates of the dorsal series surrounding the dorsal-fin base (vs. color pattern consisting of conspicuous spots throughout the body). See Table 2 for a summary of interspecifically variable characters of within the *C. anale* species group.

Description.—Morphometric and meristic data in Table 3. Dorsal profile of head convex from snout tip to dorsal-fin origin, then straight and gradually descending from that point to adipose fin. Dorsal peduncle profile slightly concave. Ventral profile straight from snout to caudal fin. Body depressed, greatest body depth at dorsal-fin origin; lowest body depth at caudal peduncle. Greatest body width at pectoral girdle. Caudal peduncle slightly compressed, triangular in cross section.

Head wide, rounded anteriorly in dorsal view; snout moderately depressed. Anterior margin of snout unplated and lacking odontodes. Cheek covered by plates. Orbit moderate in size (10.7–18.5% HL), dorsolaterally positioned. Eye distinctly anterior to vertical line through pectoral-fin origin. Dorsal margin of orbit not elevated. Mouth wide, occupying almost entire head width. Oral disk elliptical ending just anterior to origin of gill opening. Oval papillae covering upper lip; roundish papillae covering lower lip; papillae becoming smaller toward lip margins. Lower lip margin crenulate. Buccal cavity with very large digitate papillae (Fig. 4); one central conical papilla immediately posterior to premaxillary symphysis; one or two smaller papillae posterolateral to each premaxillary tooth row; and one large rugose patch of papillae dorsomedial to each dentary tooth row, followed by smaller papillae laterally; maxillary barbel short, approximately one eye diameter (Fig. 4). Premaxillary tooth row straight, joining contralateral tooth row at 180° angle. Dentary straight, joining contralateral tooth row at 160° angle. Teeth small and villiform, asymmetrically bicuspid; lateral cusp approximately half size of mesial cusp. Posterior border of opercular with single row of four to six straight hypertrophied odontodes, slightly larger than those elsewhere on opercle (Fig. 3). Three to five hypertrophied evertible cheek odontodes hooked anteriorly (Fig. 3, type 3 of Lujan et al., 2015), not reaching cleithrum. Supraoccipital excrescence consisting of vestigial longitudinal unplated area present in juveniles, absent in adults, which have contralateral predorsal plates meeting medially.

Trunk covered by five longitudinal series of plates, naked around dorsal-fin base. Body plates flat or gently curved, lacking any keel or ridge. Dorsal-fin base bordered laterally by seven to nine dorsal plates. Region between dorsal and adipose fin with three to six dorsal plates. Abdomen naked.

Dorsal fin II, 8–9 (mode 8); spinelet V-shaped, spine locking mechanism functional, dorsal-fin origin slightly

Table 2. Interspecifically variable characters of species of *Chaetostoma* of the *C. anale* species group.

| | <i>C. anale</i> | <i>C. dermorhynchum</i> | <i>C. formosae</i> | <i>C. orientale</i> | <i>C. trimaculineum</i> |
|---|--|--|--|---|---|
| Head | | | | | |
| Color pattern (color; border; shape; size) | black; distinct; round; 1/2 naris size | black; distinct; round; naris size | black; distinct; round; naris size | black; distinct; irregular, round to elongate; 1/2 naris size | black; distinct; round; 1/2 naris size |
| Male snout enlargement | present | present | absent | absent | absent |
| Supraoccipital excrescence | present | present | present | absent in adults | present |
| Cheek odontodes | | | | | |
| Shape | Type 2 | Type 2 | Type 2 | Type 3 | Type 2 |
| Number | 5 | 2–5 | 5–6 | 5 | 4–5 |
| Mouth | | | | | |
| Premaxillary teeth | 60 | 105±14 | 57±22 | 84±12 | 100±31 |
| Dentary teeth | 75 | 131±14 | 85±35 | 107±16 | 138±44 |
| Buccal papillae | single | single | single | complex | single |
| Lower lip length | elongate | short | elongate | elongate | short |
| Body | | | | | |
| Width (% SL) | 32.5±1.8 | 31.4±0.8 | 35.8±2.1 | 34.5±1.0 | 31.7±1.2 |
| Spots (color; border; shape; size; pattern) | black; less distinct than on head; round; 3/4 naris size; spots forming 5–6 irregular rows on dorsal, mid-dorsal, median, and mid-ventral plate series | white to gray; indistinct; round; diameter of orbit; arranged in 4–5 rows from pelvic to caudal fin; more distinct posteriorly | black; distinct; round; 1/2 naris size; few scattered on predorsal plates and around dorsal-fin base, restricted to dorsal and mid-dorsal plate series | black; indistinct; round; 1/2 naris size; few scattered on predorsal plates and around dorsal-fin base; flanks mottled but lacking distinct spots | black; distinct; round; 1/2 naris size; spots forming 3–4 linear rows restricted to dorsal, mid-dorsal, and median plate series |
| Fins | | | | | |
| Branched dorsal-fin rays | 8 | 8 | 8 | 8 or 9 | 8 |
| Dorsal-fin coloration | uniform | each ray with up to 10 white spots; < 1/2 diameter of naris; less distinct in mature adults | rays uniform, membranes hyaline or with few black spots | rays uniform, membranes hyaline or gray | rays uniform, membranes hyaline or with few black spots |
| Branched anal-fin rays | 5 | 4 | 3 (4) | 4 | 4 |
| Mature pelvic-fin margin | W-shaped | W-shaped | W-shaped | rounded | rounded |

anterior to pelvic-fin origin, last dorsal-fin ray reaching adipose fin when adpressed. Pectoral-fin rays I, 6; pectoral-fin spine reaching to first third of pelvic fin when adpressed in juveniles and to first half in adults. Pelvic-fin rays i, 5; unbranched rays surpassing anal-fin origin; skin fold extending along posterodorsal surface of first unbranched pelvic-fin ray. Pectoral- and pelvic-fin spine with thicker odontodes irregularly distributed along entire dorsal, anterior, and ventral surface of spine, and one aligned row of larger odontodes along posterodorsal margin of spine. Odontodes present on all but last branched pectoral- and pelvic-fin rays. Anal-fin rays ii, 4; first unbranched anal-fin ray almost same length as second, but still shorter in immature males and females (see Sexual Dimorphism section). First branched anal-fin ray longest, with remaining branched rays becoming shorter. Caudal fin obliquely forked, i, 7+7, i rays; lower lobe longer than upper. Procurrent caudal-fin rays four to six dorsal, two to five ventral. Ribs eight. Vertebrae 28.

Color in alcohol.—Head and body base color grayish brown; whitish pale ventrally. Head with dense small black dots smaller than pupil diameter, some connected forming vermiculations in interorbital area. Scattered dark dots on body restricted to dorsum. Supraoccipital excrescence black or dark gray when present in juveniles. Longitudinal faded gray stripe along trunk from first median plate to caudal peduncle. Skin between body plates darker gray, forming somewhat reticulated aspect. Naked area behind compound pterotic dark brown to almost black. Dorsal-fin membrane light brown with darker dots similar to those on head scattered on rays and nearby membrane. Remaining fins plain brownish gray with indistinct darker dots. Caudal fin mostly plain brown. Tip of unbranched dorsal- and caudal-fin rays whitish pale in some individuals.

Color in life.—Head with conspicuous dark gray to matte black vermiculate dots along entire dorsal surface, more dot-shaped in lateral and anterior border of snout and along

Table 3. Morphometric and meristic data of 25 specimens of *Chaetostoma orientale*. Range includes holotype. SD = standard deviation.

| | Maicuru River (n = 7) | | | | | Seiko River (n = 18) | | | |
|-----------------------------------|-----------------------|------|-------|-------|-------|----------------------|-------|-------|-------|
| | Holotype | Min | Max | Mean | SD | Min | Max | Mean | SD |
| Standard length (mm) | 132.4 | 53.9 | 132.4 | 88.0 | — | 69.2 | 170.0 | 122.3 | — |
| Percent of standard length | | | | | | | | | |
| Head length | 35.6 | 34.7 | 36.7 | 35.7 | 0.69 | 30.6 | 35.2 | 32.6 | 1.34 |
| Predorsal length | 44.5 | 31.5 | 45.7 | 43.2 | 5.15 | 38.4 | 45.3 | 41.1 | 1.65 |
| Postanal length | 25.0 | 25.0 | 27.1 | 26.3 | 0.89 | 24.1 | 28.7 | 26.8 | 1.22 |
| Postdorsal length | 28.5 | 28.5 | 31.9 | 29.7 | 1.11 | 23.4 | 32.8 | 26.9 | 3.01 |
| Cleithral width | 36.3 | 33.1 | 36.3 | 34.5 | 1.07 | 31.6 | 35.1 | 32.9 | 0.93 |
| Cleithral process width | 4.3 | 3.3 | 4.4 | 3.9 | 0.37 | 3.0 | 4.9 | 3.9 | 0.48 |
| Body depth at dorsal-fin origin | 19.3 | 18.2 | 21.5 | 19.6 | 1.17 | 16.7 | 21.8 | 18.9 | 1.24 |
| Body width at dorsal-fin origin | 29.0 | 25.1 | 29.9 | 27.7 | 1.95 | 27.0 | 31.5 | 28.6 | 0.99 |
| Body width at anal-fin origin | 17.8 | 16.7 | 20.1 | 18.2 | 1.06 | 17.8 | 22.7 | 19.2 | 1.26 |
| Interbranchial distance | 26.9 | 23.8 | 26.9 | 25.2 | 0.96 | 22.3 | 26.0 | 24.1 | 1.07 |
| Dorsal-fin base length | 26.9 | 24.7 | 27.3 | 26.2 | 1.02 | 23.7 | 32.1 | 27.1 | 2.01 |
| Interdorsal distance | 15.9 | 13.6 | 16.8 | 15.1 | 1.15 | 9.8 | 15.6 | 13.2 | 1.45 |
| Caudal peduncle depth | 13.3 | 12.9 | 13.8 | 13.3 | 0.28 | 11.7 | 13.9 | 12.6 | 0.56 |
| Dorsal-fin spine length | 24.1 | 24.1 | 27.4 | 25.9 | 1.19 | 26.4 | 30.9 | 28.4 | 1.28 |
| Pectoral-fin spine length | 36.3 | 28.2 | 36.3 | 31.3 | 2.66 | 27.6 | 33.8 | 30.4 | 1.75 |
| Pelvic-fin spine length | 26.2 | 24.9 | 27.0 | 26.1 | 0.67 | 23.1 | 27.0 | 24.7 | 1.03 |
| First anal-fin ray length | 10.3 | 7.4 | 16.0 | 10.9 | 2.64 | 9.5 | 16.9 | 11.8 | 1.67 |
| Second anal-fin ray length | 16.1 | 11.5 | 19.0 | 14.0 | 2.76 | 10.5 | 21.6 | 14.3 | 2.62 |
| Adipose-fin spine length | 7.6 | 7.6 | 9.4 | 8.5 | 0.67 | 6.4 | 9.8 | 8.2 | 0.88 |
| Pectoral to pelvic origin length | 21.8 | 20.4 | 22.8 | 21.7 | 0.80 | 19.6 | 24.4 | 22.1 | 1.19 |
| Pelvic to anal origin length | 25.9 | 22.0 | 25.9 | 23.6 | 1.34 | 21.7 | 24.9 | 23.2 | 0.93 |
| Anus to anal-fin distance | 10.2 | 7.7 | 10.2 | 9.1 | 0.92 | 6.5 | 9.4 | 8.2 | 0.77 |
| Percent of head length | | | | | | | | | |
| Head depth | 51.6 | 48.9 | 53.2 | 51.0 | 1.70 | 49.6 | 61.1 | 53.4 | 2.53 |
| Head width at opercle | 93.3 | 86.5 | 93.3 | 90.2 | 2.40 | 91.2 | 104.1 | 99.0 | 3.64 |
| Head width at soft border | 99.9 | 89.8 | 99.9 | 94.9 | 3.50 | 92.1 | 117.2 | 103.2 | 5.55 |
| Snout length | 67.7 | 66.1 | 70.7 | 67.7 | 1.63 | 66.4 | 75.7 | 70.1 | 2.26 |
| Orbit diameter | 12.9 | 12.7 | 17.1 | 15.5 | 1.88 | 10.7 | 18.5 | 14.5 | 2.01 |
| Interorbital width | 30.7 | 25.9 | 30.7 | 27.9 | 1.72 | 25.2 | 32.0 | 29.1 | 1.71 |
| Internares width | 9.5 | 9.1 | 10.5 | 9.5 | 0.49 | 7.7 | 10.5 | 9.1 | 0.86 |
| Orbit to nare length | 14.4 | 12.6 | 15.5 | 14.2 | 0.90 | 13.1 | 16.6 | 14.6 | 1.02 |
| Lower lip width | 78.2 | 70.7 | 78.8 | 76.1 | 3.17 | 58.4 | 83.8 | 76.9 | 5.69 |
| Lower lip length | 14.0 | 14.0 | 18.7 | 16.1 | 1.45 | 14.6 | 22.9 | 18.2 | 2.07 |
| Dentary length | 28.3 | 26.1 | 29.8 | 28.1 | 1.08 | 25.7 | 31.2 | 28.1 | 1.55 |
| Premaxillary length | 23.9 | 23.7 | 27.5 | 24.9 | 1.31 | 19.2 | 25.8 | 23.2 | 1.60 |
| Count | | | | | | | | | |
| Median-ventral plates | 24 | 24 | 25 | 24.1 | 0.38 | 22 | 24 | 23.4 | 0.60 |
| Predorsal plates | 4 | 3 | 4 | 3.6 | 0.53 | 3 | 3 | 3.0 | 0.00 |
| Plates limiting supraoccipital | 2 | 2 | 2 | 2.0 | 0.00 | 2 | 2 | 2.0 | 0.00 |
| Plates between dorsal and adipose | 5 | 5 | 6 | 5.4 | 0.53 | 3 | 5 | 4.4 | 0.69 |
| Plates between adipose and caudal | 1 | 1 | 2 | 1.6 | 0.53 | 1 | 2 | 1.7 | 0.45 |
| Upper procurent caudal-fin rays | 5 | 5 | 5 | 5.0 | 0.00 | 4 | 6 | 5.4 | 0.77 |
| Plates between anal and caudal | 11 | 10 | 11 | 10.3 | 0.49 | 7 | 10 | 8.4 | 0.69 |
| Lower procurent caudal-fin rays | 2 | 2 | 3 | 2.4 | 0.53 | 3 | 5 | 3.7 | 0.58 |
| Plates at base of dorsal fin | 8 | 7 | 8 | 7.6 | 0.53 | 7 | 9 | 8.4 | 0.60 |
| Plates at base of anal fin | 2 | 2 | 3 | 2.1 | 0.38 | 2 | 3 | 2.2 | 0.37 |
| Hemiseries premaxillary teeth | 96 | 68 | 102 | 84.7 | 12.05 | 91 | 129 | 105.1 | 10.73 |
| Hemiseries dentary teeth | 112 | 76 | 127 | 107.3 | 16.49 | 96 | 141 | 121.5 | 13.70 |
| Branched anal-fin rays | 4 | 4 | 4 | 4.0 | 0.00 | 4 | 4 | 4.0 | 0.00 |
| Branched pectoral-fin rays | 6 | 6 | 6 | 6.0 | 0.00 | 6 | 6 | 6.0 | 0.00 |
| Branched pelvic-fin rays | 5 | 5 | 5 | 5.0 | 0.00 | 5 | 5 | 5.0 | 0.00 |
| Branched dorsal-fin rays | 8 | 8 | 8 | 8.0 | 0.00 | 8 | 9 | 8.3 | 0.45 |
| Branched caudal-fin rays | 14 | 14 | 14 | 14.0 | 0.00 | 13 | 14 | 13.9 | 0.23 |

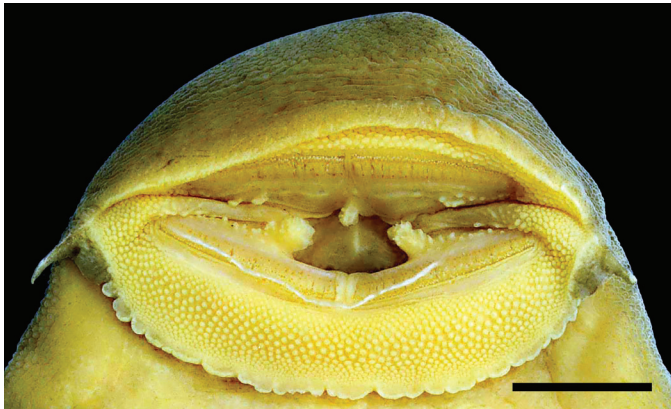


Fig. 4. Lips and buccal papillae of *Chaetostoma orientale*, holotype, MCP 54585, male, 132.4 mm SL. Ventral view. Scale bar 10 mm.



Fig. 5. Live coloration of *Chaetostoma orientale* at type locality shortly after capture. (A) Paratype, MCP 50363; (B) holotype, MCP 54585.

postorbital region (Figs. 5, 6). Posterior margin of compound pterotic and dorsum with dark dots larger than those on head, and distributed approximately one or two dots per plate, except for predorsal plates with three or four dots each. Base color of head and trunk varying from medium to light brown or grayish brown. Lateral-line pores between each plate of median series darker than plates, forming dashed line along entire trunk. Base color of fins yellowish brown, slightly lighter than remaining body. Large, indistinct black spot at origin of each dorsal-fin branched ray, rays slightly darker distally than proximally. Pectoral-fin branched rays having few black spots in distal half, those on anterior rays irregular in shape. Pelvic-fin rays uniformly yellowish brown. Adipose-fin spine and dorsal- and caudal-fin unbranched rays

slightly darker than branched rays. Caudal fin brown, more lightly colored than body, branched rays of ventral lobe darker toward distal margin.

Sexual dimorphism.—As in all species of *Chaetostoma*, the urogenital papilla of males is conical, while in females it is a small flat opening surrounded by an irregular dermal ridge (Ballen, 2011; see also fig. 1 in Lujan et al., 2015). In addition, as in all species in the *C. anale* species group, mature males of *C. orientale* have the second unbranched anal-fin ray broader and longer than first unbranched ray, second unbranched ray having small but conspicuous spatulate odontodes along anterior surface of free distal portion (Fig. 2). Finally, the posterior pelvic-fin margin of mature males has a distinct shape in which middle rays are longest (Fig. 1); pelvic-fin margin of females and immature males straight or almost straight. Males also develop a skin flap on the dorsal surface of the pelvic-fin leading ray that attaches to the body above the ray insertion, along with development of odontodes erupting from the skin on branched rays 1–3; these odontodes are less abundant and less developed in females, which also lack the skin flap on the leading ray. Males of *C. orientale* have a larger unplated area in the snout than females.

Ontogenetic variation.—*Chaetostoma orientale* ontogenetically varies in the condition of the supraoccipital excrescence. This structure is very low and composed of a patch of darkened skin that does not protrude between the predorsal plates in juveniles and is absent in adults, in which the predorsal plates develop and contact each other medially, closing the area where the excrescence usually grows. Additionally, adult males have a posterodorsally expanded skin flap on the pelvic-fin spine, which is present but smaller in juveniles.

Distribution.—*Chaetostoma orientale* is known from two localities in the Pará State of Brazil, on opposite sides of the Amazon River main stream. It occurs in the Maicuru River, a left-bank (northern) tributary to the lower Amazon, at the southern border of the Guiana Shield, and in the Seiko River basin, a left-bank (eastern) tributary of the lower Xingu River, itself a right-bank (southern) tributary to the lower Amazon, at the northern border of the Brazilian Shield (Fig. 7).

Etymology.—The new species is named *Chaetostoma orientale*, from the Latin *orient-*, *oriens*, *orientalis*, meaning eastern, from the east, of or belonging to the east, in reference to its easternmost distribution among species of the genus. An adjective in neuter form in agreement with genus gender.

Habitat and ecology.—The Maicuru River locality is 33 m above sea level, 40–60 m wide, and up to 1 m deep. The bottom was composed of pebbles, loose rocks, and boulders; water was clear and the current moderately strong. More than 40 fish species were collected with *Chaetostoma orientale* in the Maicuru River, such as the crenuchids *Characidium* cf. *crandellii* and *Melanocharacidium dispilomma*, the characids *Bryconops gracilis*, *Jupiaba atypindi*, and *Jupiaba* cf. *polylepis*, the heptapterids *Imparfinis* cf. *hasemani* and *Phenacorhamdia* sp., the pseudopimelodid *Pseudopimelodus bufonius*, and the loricariids *Ancistrus* sp., *Harttia* sp., *Hypancistrus* sp., *Lithoxus* sp., *Loricaria* cf. *cataphracta*, *Peckoltia* sp., and *Pseudancistrus* sp., among others. The Seiko River in the Xingu basin is a creek 8–30 meters wide (Fig. 8), with relatively well-preserved



Fig. 6. Live coloration of *Chaetostoma orientale* from the Seiko River, shortly after capture. ANSP 199686, photo by Mark Sabaj.

riparian gallery forest. The bottom has pebbles, rocks, and boulders with clear water and moderately strong current. More than 20 fish species were collected along with *C. orientale* in the Seiko River, such as the parodontid *Apareiodon argenteus*, the anostomid *Leporinus cf. megalepis*, the curimatid *Cyphocharax spilurus*, the crenuchid *Characidium* sp., the characids *Bryconops giacopinii*, *Ctenobrycon alleni*, *Hemibrycon surinamensis*, *Knodus* sp., and *Odontostilbe* sp., the heptapterids *Imparfinis hasemani* and *Phenacorhamdia* sp., the loricariids *Ancistrus* sp., *Hypostomus* sp., and *Lasiancistrus* sp., the

cichlid *Crenicichla* cf. *saxatilis*, and the synbranchid *Synbranchus marmoratus*.

Extinction risk assessment.—*Chaetostoma orientale* is currently known from the lower Maicuru River, draining the southern versant of the Guiana Shield and the Jarauçu River basin, draining the northern versant of the Brazilian Shield, both in Pará State, Brazil. The species is likely distributed downstream and upstream of these localities and is possibly even more widespread in other nearby rivers draining the Guiana and

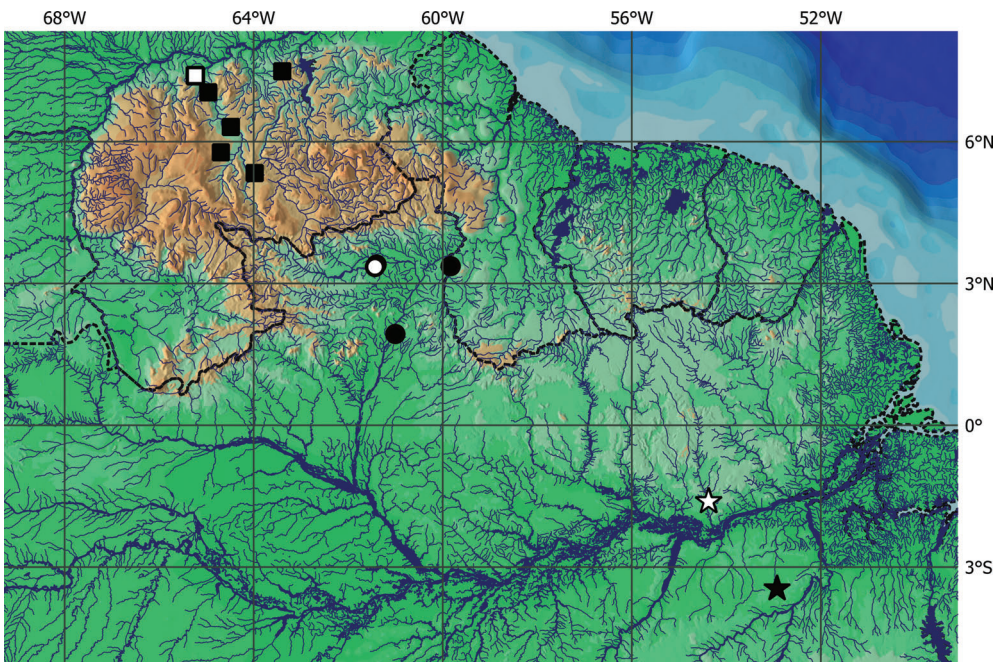


Fig. 7. Geographic distribution of species of *Chaetostoma* from the Guiana and Brazilian Shields. Star = *C. orientale*, from Brazil, Pará, Monte Alegre, Maicuru River and Medicilândia, Xingu River, Amazon basin; Dot = *C. jegui*, from Branco, Uraricoera and Takutu Rivers, Amazon basin; Square = *C. vasquezi*, from Caura and Caroni Rivers, Orinoco basin. White symbols represent type locality; symbols can represent more than one lot and locality.



Fig. 8. Seiko River, tributary to Jaraçu River, itself a tributary to Xingu River, ca. 3.3 km west of Medicilândia on Trans-Amazon road, 3°27'22.53"S, 52°55'33.61"W. Photo by Mark Sabaj.

Brazilian shields. The Extent of Occurrence calculated including the microbasins is approximately 37,500 km², and although some human disturbance is observable in the lower Maicuru River, this river's middle and upper courses are mostly inaccessible and ecologically intact. The upper tributary creeks of the Jaraçu River, where the Xingu specimens were collected, are cut by the busy Trans-Amazon road, but most of the lower portion of the Jaraçu basin is ecologically well preserved within the Reserva Extrativista Verde para Sempre conservation area. For these reasons, the species is tentatively assessed as Least Concern according to the categories and criteria of the International Union for Conservation of Nature (IUCN Standards and Petitions Subcommittee, 2019). Additional collecting efforts should be conducted in that region in order to better understand the species' geographic distribution.

Parataxonomic remark.—Small numbers of *Chaetostoma orientale* have been exported into the ornamental fish hobby, where the species has been assigned the L-numbers (= loricariid numbers) L413 from the Maicuru River and L416 from the Seiko River (Seidel, 2008).

DISCUSSION

Since the erection of *Chaetostoma* by Tschudi (1846) for the then new species *C. lobarhynchos* from the Peruvian Andes, this genus has been closely associated with rivers of the Andean piedmont, where it is often locally common and abundant. Until recently, the only two species of *Chaetostoma* known from rivers distant from the Andes or the coastal mountains of northern South America were *C. jegui* and *C. vasquezi*, which are allopatrically distributed in rivers draining opposite south/north flanks of the geologically ancient highlands of the Guiana Shield. *Chaetostoma jegui* is known from upper portions of the Branco River, a south-flowing tributary of the Amazon basin, and *C. vasquezi* occurs in the upper portions of the Caura and Caroni Rivers, north-flowing tributaries of the Orinoco basin.

Recently, several populations of *Chaetostoma* have been revealed in Brazilian rivers (Curuá-Una, Maicuru, Paru, and Xingu) of the eastern Amazon, even further removed from the Andes than the Branco, yet none of these species have been described until now. Our phylogenetic analysis (Fig. 9) demonstrates that populations of *Chaetostoma* of the Xingu and Maicuru Rivers are conspecific, despite the geographic distance and main channel of the Amazon River between them. Pairwise *col* genetic distances between the Xingu and the Maicuru populations are null (Table 4).

Chaetostoma orientale shares most body proportions with *C. vasquezi* and *C. jegui*, the species of *Chaetostoma* respectively distributed on northern and southern versants of the Guiana Shield, which themselves are morphologically very similar. *Chaetostoma orientale* differs from them, though, by having a narrower opercle, never reaching orbital width (vs. opercle wide, approximately as wide as orbit). Additionally, the new species had 12.6–12.8% pairwise *col* genetic distances from *C. jegui* and *C. vasquezi* (Table 4).

Despite the considerable contiguous river channel distance currently separating *Chaetostoma jegui* and *C. vasquezi*, comprising over 2,000 km of the lower Branco and upper Negro Rivers, the Casiquiare Canal, and the upper Orinoco River, these two species have *col* genetic distances of only 0.0–0.4%. No specimens of *Chaetostoma* have ever been collected throughout this intervening reach, yet genetic similarity suggests that until recently, these species must have been part of a single population. One possibility is that this historical population was once contiguously distributed throughout the intervening river channels, but has recently disappeared from these habitats without leaving any other relictual populations. An alternative hypothesis based on the relatively recent (Pliocene/Pleistocene) breakup of the proto-Berbice paleodrainage seems to make more sense. The proto-Berbice was an approximately Orinoco-sized watershed that united parts of the modern upper Caura and Caroni, the upper Branco (including the Uaricoera, Ireng, and Takutu), the Rupununi, and Berbice River. Existence of the proto-Berbice is hypothesized based on geomorphological evidence and a growing list of closely related fish species having disjunct modern distributions across Guiana Shield highlands that now separate once-contiguous segments of this paleodrainage (Lujan and Armbruster, 2011; Armbruster et al., 2021). Despite the genetic and phenotypic similarity of these species, and previous hypotheses that they are conspecific, we choose to continue recognizing them as distinct and valid species under the hypothesis that their

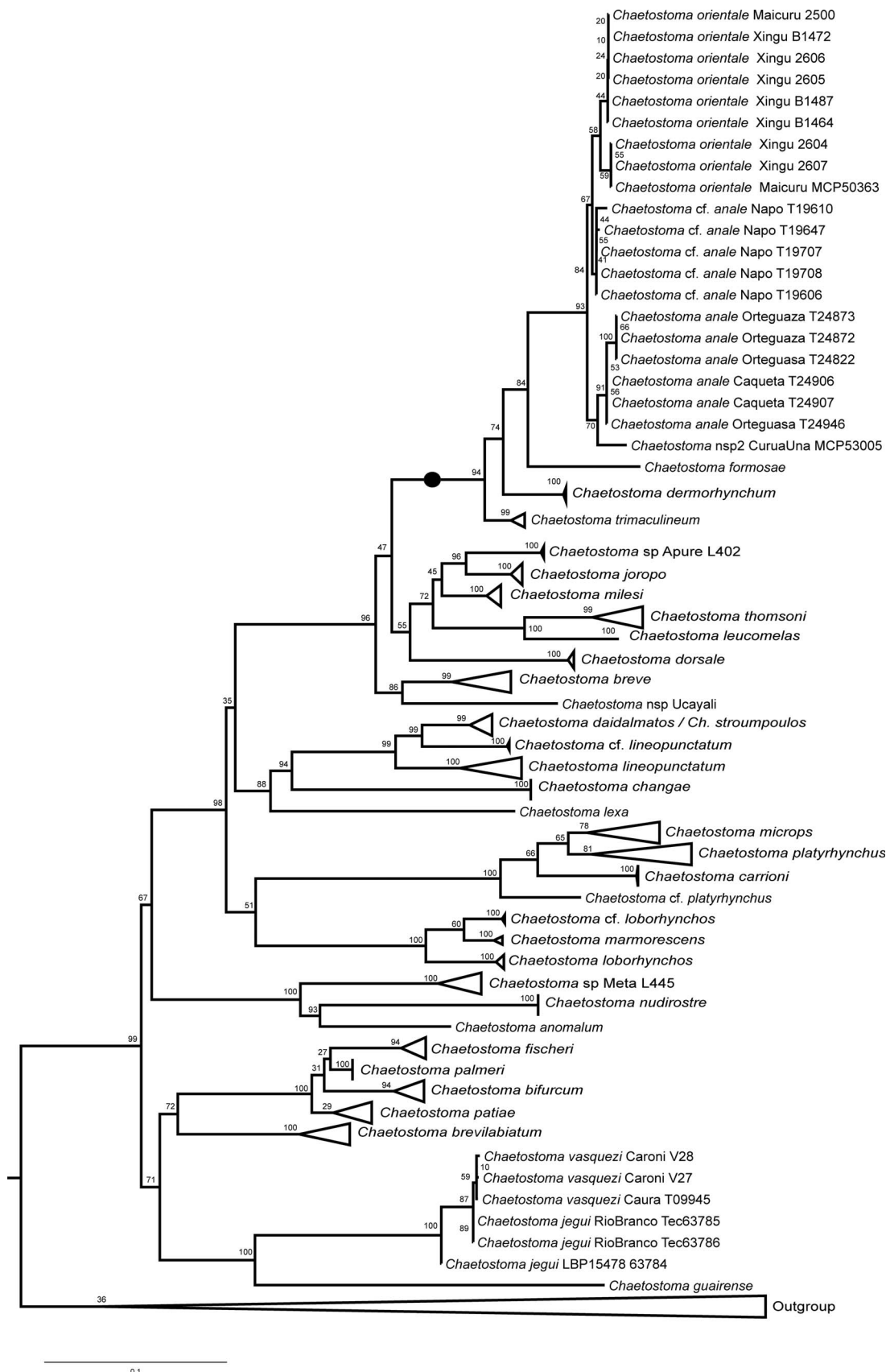


Fig. 9. Phylogenetic relationships within the genus *Chaetostoma* based on ML analysis of a 5,844 base alignment of four mitochondrial (*16S*, *col*, *cytb*, *nd2*) and three nuclear (*myh6*, *rag1*, *rag2*) gene regions. Bootstrap support values presented for each node. *Chaetostoma anale* species group indicated by a black circle. Respective nodes for all species except current and former members of the *C. anale* species group collapsed to single terminals. Complete results from ML analyses of the concatenated alignment (Fig. S1), alignment of mitochondrial genes only (Fig. S2), and alignment of nuclear genes only (Fig. S3) are available as supplemental files (see Data Accessibility). See Table 1 for additional data on each sample in the analysis. See Data Accessibility for tree file.

Table 4. Pairwise mtDNA genetic distances for the cytochrome oxidase c subunit 1 (*coI*) gene using the Kimura 2 parameter.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|--|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1 <i>Chaetostoma anale</i> | | | | | | | | | | | | | | | | | | | | |
| Caqueta_T24906 | | | | | | | | | | | | | | | | | | | | |
| 2 <i>Chaetostoma anale</i> | 0.000 | | | | | | | | | | | | | | | | | | | |
| Orteguasa_T24946 | | | | | | | | | | | | | | | | | | | | |
| 3 <i>Chaetostoma anale</i> | 0.004 | 0.004 | | | | | | | | | | | | | | | | | | |
| Orteguasa_T24822 | | | | | | | | | | | | | | | | | | | | |
| 4 <i>Chaetostoma anale</i> | 0.000 | 0.000 | 0.004 | | | | | | | | | | | | | | | | | |
| Caqueta_T24907 | | | | | | | | | | | | | | | | | | | | |
| 5 <i>Chaetostoma cf. anale</i> | 0.002 | 0.002 | 0.005 | 0.002 | | | | | | | | | | | | | | | | |
| Napo_T119606 | | | | | | | | | | | | | | | | | | | | |
| 6 <i>Chaetostoma cf. anale</i> | 0.002 | 0.002 | 0.005 | 0.002 | 0.000 | | | | | | | | | | | | | | | |
| Napo_T119647 | | | | | | | | | | | | | | | | | | | | |
| 7 <i>Chaetostoma cf. anale</i> | 0.002 | 0.002 | 0.005 | 0.002 | 0.000 | 0.000 | | | | | | | | | | | | | | |
| Napo_T119708 | | | | | | | | | | | | | | | | | | | | |
| 8 <i>Chaetostoma sp.</i> | 0.013 | 0.013 | 0.016 | 0.013 | 0.011 | 0.011 | 0.011 | | | | | | | | | | | | | |
| Curua-Una_MCP53005 | | | | | | | | | | | | | | | | | | | | |
| 9 <i>Chaetostoma orientale</i> | 0.004 | 0.004 | 0.007 | 0.004 | 0.002 | 0.002 | 0.002 | 0.002 | 0.009 | | | | | | | | | | | |
| Maicuru_MCP50363 | | | | | | | | | | | | | | | | | | | | |
| 10 <i>Chaetostoma orientale</i> | 0.004 | 0.004 | 0.007 | 0.004 | 0.002 | 0.002 | 0.002 | 0.002 | 0.009 | 0.000 | | | | | | | | | | |
| Maicuru_2500 | | | | | | | | | | | | | | | | | | | | |
| 11 <i>Chaetostoma orientale</i> | 0.004 | 0.004 | 0.007 | 0.004 | 0.002 | 0.002 | 0.002 | 0.002 | 0.009 | 0.000 | 0.000 | | | | | | | | | |
| Xingu_2607 | | | | | | | | | | | | | | | | | | | | |
| 12 <i>Chaetostoma orientale</i> | 0.004 | 0.004 | 0.007 | 0.004 | 0.002 | 0.002 | 0.002 | 0.002 | 0.009 | 0.000 | 0.000 | 0.000 | | | | | | | | |
| Xingu_2604 | | | | | | | | | | | | | | | | | | | | |
| 13 <i>Chaetostoma orientale</i> | 0.004 | 0.004 | 0.007 | 0.004 | 0.002 | 0.002 | 0.002 | 0.002 | 0.009 | 0.000 | 0.000 | 0.000 | 0.000 | | | | | | | |
| Xingu_2605 | | | | | | | | | | | | | | | | | | | | |
| 14 <i>Chaetostoma orientale</i> | 0.004 | 0.004 | 0.007 | 0.004 | 0.002 | 0.002 | 0.002 | 0.002 | 0.009 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | | | | | |
| Xingu_2606 | | | | | | | | | | | | | | | | | | | | |
| 15 <i>Chaetostoma orientale</i> | 0.004 | 0.004 | 0.007 | 0.004 | 0.002 | 0.002 | 0.002 | 0.002 | 0.009 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | | | | |
| Xingu_B1472 | | | | | | | | | | | | | | | | | | | | |
| 16 <i>Chaetostoma orientale</i> | 0.004 | 0.004 | 0.007 | 0.004 | 0.002 | 0.002 | 0.002 | 0.002 | 0.009 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | | | |
| Xingu_B1464 | | | | | | | | | | | | | | | | | | | | |
| 17 <i>Chaetostoma orientale</i> | 0.004 | 0.004 | 0.007 | 0.004 | 0.002 | 0.002 | 0.002 | 0.002 | 0.009 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | | |
| Xingu_B1487 | | | | | | | | | | | | | | | | | | | | |
| 18 <i>Chaetostoma jegui</i> | 0.128 | 0.128 | 0.133 | 0.128 | 0.128 | 0.128 | 0.128 | 0.128 | 0.124 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 |
| Branco_Tec63785 | | | | | | | | | | | | | | | | | | | | |
| 19 <i>Chaetostoma jegui</i> | 0.128 | 0.128 | 0.133 | 0.128 | 0.128 | 0.128 | 0.128 | 0.128 | 0.124 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 |
| Branco_Tec63786 | | | | | | | | | | | | | | | | | | | | |
| 20 <i>Chaetostoma vasquezi</i> | 0.130 | 0.130 | 0.135 | 0.130 | 0.131 | 0.131 | 0.131 | 0.131 | 0.126 | 0.128 | 0.128 | 0.128 | 0.128 | 0.128 | 0.128 | 0.128 | 0.128 | 0.128 | 0.128 | 0.004 |
| Caroni_V28 | | | | | | | | | | | | | | | | | | | | |
| 21 <i>Chaetostoma vasquezi</i> | 0.128 | 0.128 | 0.133 | 0.128 | 0.128 | 0.128 | 0.128 | 0.128 | 0.124 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 | 0.126 | 0.000 |
| Caroni_V27 | | | | | | | | | | | | | | | | | | | | |
| 1 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |

modern, highly disjunct distribution has placed them on divergent evolutionary paths.

Our phylogeny (Fig. 9) found *Chaetostoma orientale* to be deeply nested within the *C. anale* species group, as sister to another undescribed lineage from the Napo River in Ecuador. This species pair was recovered as sister to *C. anale* plus a second undescribed lineage from the Curuá-Una River in Pará, Brazil. Genetic divergence among these members of the *C. anale* species group is generally low (0.0–0.9%, Table 4). Complete results from maximum-likelihood (ML) analyses of the concatenated alignment (Fig. S1), alignment of mitochondrial genes only (Fig. S2), and alignment of nuclear genes only (Fig. S3) are available as supplemental files (see Data Accessibility).

Because of the strongly supported monophyly of our *Chaetostoma anale* clade, marked with a black dot in Figure 9, and the distance of this clade to *C. jegui* and *C. vasquezzi*, we reject the Ballen (2011) morphology-based hypothesis that these later species are members of the *Chaetostoma anale* species group and remove them from that group herein. Nonetheless, remaining members of the *C. anale* species group were, in fact, found to comprise a well-supported clade composed of *C. anale*, *C. dermorhynchum*, *C. formosae*, *C. orientale*, and *C. trimaculineum*, plus at least two additional undescribed species mentioned above from the Napo and the Curuá-Una Rivers.

Although the genus *Chaetostoma* has historically been closely associated with Andean rivers, and certainly remains a taxon with much to tell us about evolutionary diversification in response to Andean uplift, this study demonstrates that the history is more complex than formerly thought. Additional collecting efforts are much needed in the southern end of the Guiana Shield in northern Brazil in order to obtain a more clear picture of the diversity and distribution of non-Andean species of *Chaetostoma*.

MATERIAL EXAMINED

Material examined in addition to that listed in Ballen (2011), Ballen et al. (2016), Lujan et al. (2015), and Urbano-Bonilla and Ballen (2021).

Chaetostoma breve: MUSM 30221, 3, 52.0–84.5 mm SL, Peru, La Libertad, Ferreñafe, Marañon River, Quebrada Verma.

Chaetostoma jegui: Brazil, Roraima State, Branco River drainage: INPA 2822, holotype, 156.8 mm SL, Uraricoera River, Maracá Island, rocks above Furo Maracá; INPA 1958, paratype, 123.0 mm SL, collected with holotype; INPA 1968, paratypes, 69.8–168.8 mm SL, Uraricoera River, Maracá Island, Furo Santa Rosa; INPA 2013, paratype, 148.6 mm SL, Takutu River, Bonfim; INPA 33840, 4, 73.0–100.5 mm SL, Caracaraí, Ilha de Maracá, Furo do Maracá.

Chaetostoma cf. *jegui*: LBP 15478, 3, 26.2–68.4 mm SL. Brazil, Roraima, Branco River, Cachoeira do Bem Querer, 1°55'48.2"N, 61°00'10.2"W.

Chaetostoma lineopunctatum: MUSM 35905, 21, 92.4–106.7 mm SL, Peru, San Martín, Tocache, Huallaga River.

Chaetostoma vasquezzi: Venezuela, Bolívar State, Orinoco River drainage: FMNH 110073, 3 of 8, 62.6–68.2 mm SL, Río Caura, Salto Para-Quyuna Soodii, 6°18'38.88"N, 64°29'02.04"W; AUM 36555, 3, not measured, Río Claro, 50.5 km S of Ciudad

Bolívar, 7°55'24.89"N, 63°06'55.01"W; AUM 39882, 2, not measured, Río Nichare at La Raya Rapids, approx. 1 km upstream from mouth of Nichare, 6°32'60"N, 64°50'30.01"W; AUM 39895, 19, not measured, Río Claro east of 'los tanques,' 7°55'19.99"N, 63°06'05.00"W; AUM 53812, 1, 163.5 mm SL, Río Caura, raudales la Unión, Los Trincheros, 7°02'37.57"N, 64°57'40.82"W.

Chaetostoma sp. 1: ZUEC-PIS 16042, 1, 71.6 mm SL, Brazil, Amapá, Amapari River, downstream from town Pedra Branca do Amapari, 0°43'05"N, 53°53'22"W.

Chaetostoma sp. 2: MCP 53005, 1, 28.5 mm SL, Brazil, Pará, Curuá-Una River, at mouth of Curuá-Tinga River, 3°47'56.10"S, 54°21'19.30"W.

DATA ACCESSIBILITY

Supplemental material is available at <https://www.ichthyologyandherpetology.org/i2021068>. Unless an alternative copyright or statement noting that a figure is reprinted from a previous source is noted in a figure caption, the published images and illustrations in this article are licensed by the American Society of Ichthyologists and Herpetologists for use if the use includes a citation to the original source (American Society of Ichthyologists and Herpetologists, the DOI of the *Ichthyology & Herpetology* article, and any individual image credits listed in the figure caption) in accordance with the Creative Commons Attribution CC BY License. ZooBank publication urn:lsid:zoobank.org:pub:618E0A8D-AB6A-40E9-8546-071965770CA3.

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LITERATURE CITED

Armbruster, J. W., N. K. Lujan, and D. D. Bloom. 2021. Redescription of the Guiana Shield darter species *Characi-*

- dium crandellii* and *C. declivirostre* (Crenuchidae) with descriptions of two new species. *Ichthyology & Herpetology* 109:102–122.
- Arroyave, J., J. S. S. Denton, and M. L. J. Stiassny.** 2013. Are characiform fishes Gondwanan in origin? Insights from a time-scaled molecular phylogeny of the Citharinoidei (Ostariophysi: Characiformes). *PLoS ONE* 8:e77269.
- Ballen, G. A.** 2011. A new species of *Chaetostoma* Tschudi (Siluriformes: Loricariidae) from Colombia with a definition of the *C. anale* species group. *Papéis Avulsos de Zoologia* 51:383–398.
- Ballen, G. A., A. Urbano-Bonilla, and J. A. Maldonado-Ocampo.** 2016. Description of a new species of the genus *Chaetostoma* from the Orinoco River drainage with comments on *Chaetostoma milesi* Fowler, 1941 (Siluriformes: Loricariidae). *Zootaxa* 410:181–197.
- Calegari, B. B., and J. P. Fontenelle.** 2017. Tutorial de preparação de mapas de distribuição geográfica: Parte II—Mapa Hipsométrico. *Boletim Sociedade Brasileira de Ictiologia* 124:14–34.
- Chakrabarty, P., M. Warren, L. M. Page, and C. C. Baldwin.** 2013. GenSeq: an updated nomenclature and ranking for genetic sequences from type and non-type sources. *ZooKeys* 346:29.
- IUCN Standards and Petitions Subcommittee.** 2019. Guidelines for using the IUCN Red List Categories and Criteria. Version 14. Prepared by the Standards and Petitions Subcommittee. Available from: <https://www.iucnredlist.org/documents/RedListGuidelines.pdf> (accessed November 2019).
- Kimura, M.** 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16:111–120.
- Kumar, S., G. Stecher, and K. Tamura.** 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33:1870–1874.
- Li, C., G. Ortí, G. Zhang, and G. Lu.** 2007. A practical approach to phylogenomics: the phylogeny of ray-finned fish (Actinopterygii) as a case study. *BMC Evolutionary Biology* 7:44.
- Lujan, N. K., and J. W. Armbruster.** 2011. Two new genera and species of Ancistrini (Siluriformes: Loricariidae) from the Western Guiana Shield. *Copeia* 2011:216–225.
- Lujan, N. K., V. Meza-Vargas, V. Astudillo-Clavijo, R. Barriga-Salazar, and H. López-Fernández.** 2015. A multi-locus molecular phylogeny for *Chaetostoma* clade genera and species with a review of *Chaetostoma* (Siluriformes: Loricariidae) from the Central Andes. *Copeia* 103:664–701.
- Melo, B. F., R. C. Benine, T. C. Mariguela, and C. Oliveira.** 2011. A new species of *Tetragonopterus* Cuvier, 1816 (Characiformes: Characidae: Tetragonopterinae) from the rio Jari, Amapá, northern Brazil. *Neotropical Ichthyology* 9:49–56.
- Palumbi, S., A. Martin, S. Romano, W. O. McMillan, L. Stice, and G. Grabowski.** 2002. *The Simple Fool's Guide to PCR*. University of Hawaii, Honolulu, Hawaii.
- Pereira, E. H. L., F. Vieira, and R. E. Reis.** 2007. A new species of sexually dimorphic *Pareiorhaphis* Miranda Ribeiro, 1918 (Siluriformes: Loricariidae) from the rio Doce basin, Brazil. *Neotropical Ichthyology* 5:443–448.
- Rapp Py-Daniel, L. H.** 1991. *Chaetostoma jegui*, a new mailed catfish from Rio Uraricoera, Brazil (Osteichthyes: Loricariidae). *Ichthyological Exploration of Freshwaters* 2:239–246.
- Rapp-Py-Daniel, L. H., A. S. Oliveira, D. A. Bastos, P. M. M. Ito, J. Zuanon, and S. R. Briglia-Ferreira.** 2019. A new species of *Paralithoxus* (Siluriformes: Loricariidae: Ancistrini) from the highlands of Serra da Mocidade, Roraima State, Brazilian Amazon. *Neotropical Ichthyology* 17: e190041.
- Rodríguez-Olarte, D., J. I. Mojica, and D. C. Taphorn.** 2011. Northern South America: Magdalena and Maracaibo basins, p. 243–257. *In: Historical Biogeography of Neotropical Freshwater Fishes*. J. S. Albert and R. E. Reis (eds.). University of California Press, Berkeley, Los Angeles, London.
- Sabaj, M. H.** 2020. Codes for natural history collections in ichthyology and herpetology. *Copeia* 108:593–669.
- Salcedo, N. J., and H. Ortega.** 2015. A new species of *Chaetostoma*, an armored catfish (Siluriformes: Loricariidae), from the río Marañón drainage, Amazon basin, Peru. *Neotropical Ichthyology* 13:151–156.
- Salcedo, N. J., D. Rodríguez, R. E. Strauss, and R. J. Baker.** 2011. The Fitzcarrald Arch: a vicariant event for *Chaetostoma* (Siluriformes: Loricariidae) speciation? *Copeia* 2011: 503–512.
- Schaefer, S. A.** 2011. The Andes: riding the tectonic uplift, p. 259–278. *In: Historical Biogeography of Neotropical Freshwater Fishes*. J. S. Albert and R. E. Reis (eds.). University of California Press, Berkeley, Los Angeles, London.
- Seidel, I.** 2008. Die Gebirgsharnishwelse des Amazonas-Tieflandes. *Die Aquarien- und Terrarienzeitschrift* 2008: 24–29.
- Sevilla, R. G., A. Diez, M. Norén, O. Mouchel, M. Jérôme, V. Verrez-Bagnis, H. Van Pelt, L. Favre-Krey, G. Krey, and J. M. Bautista.** 2007. Primers and polymerase chain reaction conditions for DNA barcoding teleost fish based on the mitochondrial cytochrome b and nuclear rhodopsin genes. *Molecular Ecology Notes* 7:730–734.
- Stamatakis, A.** 2006. RAXML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22:2688–2690.
- Tschudi, J. J., von.** 1846. *Ichthyologie*, p. ii–xxx + 1–35, pls. 1–6. *In: Untersuchungen über die Fauna Peruana*. Scheitlin & Zollikofer, St. Gallen. 1844–46, in 12 parts.
- Urbano-Bonilla, A., and G. A. Ballen.** 2021. A new species of *Chaetostoma* (Siluriformes: Loricariidae) from the Orinoco basin with comments on Amazonian species of the genus in Colombia. *Journal of Fish Biology* 98:1091–1104.
- Ward, R. D., T. S. Zemlak, B. H. Innes, P. R. Last, and P. D. Hebert.** 2005. DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences* 360:1847–1857.