# *Enteromius pinnimaculatus* (Cypriniformes: Cyprinidae), a new species from southern Gabon

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## Abstract

With more than 407 species of freshwater and brackish water fishes, Gabon is a country rich in ichthyological biodiversity, but its aquatic environments remain poorly explored. We present and describe a new species of *Enteromius*, adding to the 16 species of *Enteromius* currently recorded from that country. This new species is distinguished from all other Gabonese *Enteromius* by the presence of several distinct spots on the dorsal fin in combination with three or four round spots on the flanks. In Africa, it is superficially similar to *Enteromius walkeri*, and shares with that species an unusual allometry in which the proportional length of the barbels decreases as the fish grows. Nevertheless, one can distinguish these species by vertebral number, maximum standard length, the length of the anterior barbels, the length of the caudal peduncle, and in most specimens, the number of lateral-line and circumpeduncular scales. These two species also inhabit widely separated drainages, with *E. walkeri* occurring in coastal drainages of Ghana including the Pra and Ankobra Rivers, and the new species occurring in tributaries of the Louetsi and Bibaka rivers of Gabon, which are part of the Ogowe and Nyanga drainages, respectively. Despite extensive collections in those drainages the new species is known from only two localities, suggesting the importance of conservation of its known habitat.

## Significance Statement

This contribution recognizes and describes a new species of *Enteromius* from just two locations in southern Gabon, one of which is in proximity to a planned hydroelectric dam site. The discovery highlights our incomplete knowledge of the central African fish fauna and underscores the importance of conserving the known habitat of this newly discovered, range restricted and vulnerable animal.

**Key Words**

Allometry, biodiversity, conservation, Central Africa, endemic, morphometrics, systematics

# Introduction

The African country of Gabon extends overly nearly 270,000 $km^2$ and possesses exceptional natural resources (Fermon, 2013). Gabon protects nature in the form of national parks and reserves, but also exploits natural resources through forestry, agriculture, and oil and mineral extraction (Gabon MAEPDR, 2011). Though rich with 407 known fresh and brackish water species (Stiassny *et al.*, 2007; Fermon, 2013), Gabon's fish fauna nevertheless remains poorly inventoried as evidenced by the discovery of many new species over the last twenty years (e.g. *Chromidotilapia mrac* Lamboj 2002, *Aphyosemion etsamense* Sonnenberg & Blum 2005, *Episemion krystallinoron* Sonnenberg *et al.* 2006, *Synodontus woleunensis* Friel & Vigliotta 2006, *Atopodontus adriaensi* Friel & Sullivan, 2008, *S. acanthoperca* Friel & Vigliotta, 2008, *S. punu* Vreven & Milondo 2009 and *Cryptomyrus ogoouensis* Sullivan *et al*., 2018).

Cypriniformes represents about 7% of Gabon's freshwater fish fauna. Its largest family Cyprinidae (30 species in Gabon), represents the third richest family overall, after Nothobranchidae (53 species) and Cichlidae (31 species). After the recent shift of *Raiamas* and *Opsaridium* into Danionidae (Tan & Armbruster, 2018), the remaining cyprinids of Gabon are distributed among three genera: *Labeobarbus*, *Labeo* and *Enteromius*.

*Enteromius* Cope 1867 was until recently subsumed under *Barbus* Cuvier and Cloquet 1816 (Yang *et al.*, 2015). Revisionary work by Yang et al. (2015), Stiassny & Sakharova (2016), and Hayes & Armbruster (2017) used new genetic tools to clarify the systematics of its containing tribe Smiliogastrini Bleeker, 1863, and assigned the majority of African species to *Enteromius,* with some species placed in *Barboides*, *Barbopsis*, *Caecobarbus*, *Clypeobarbus* and *Pseudobarbus*. Following this revision, *Enteromius* became the most diverse cyprinid genus in

Africa with 350 nominal species (Eschmeyer *et al.*, 2018) and 216 valid species (Hayes & Armbruster, 2017). These all possess small or moderate adult body size, a diploid genome, few striations on the scales, 7 or 8 branched rays in the dorsal fin, weakly developed gill rakers, zero, one or two pairs of barbels, and weakly developed lips (De Werdt & Teugels, 2007).

Due to its great diversity, this group of fishes has posed a systematic challenge for decades (Lévêque *et al.*, 1987; Berrebi *et al.*, 1996; Berrebi & Tsigenopoulos, 2003). Nevertheless, those early revisions and more recent work (Lederoun & Vreven, 2016; Stiassny & Sakharova, 2016; Van Ginneken *et al.*, 2017; Schmidt *et al.*, 2018) have permitted the recognition of numerous synonyms among the 350 nominal species. They have also recognized and described many new species, such as *E. validus* Stiassny *et al.* 2016, *Enteromius vandewallei* Lederoun & Vreven 2016 and *E. walshae* Mamonekene et al. 2018. Such revisions typically depend upon morphological characteristics and coloration for the identification and classification of newly collected specimens (Lévéque *et al.*, 1990; Stiassny *et al.*, 2007). This work continues along a similar perspective, and adds to the 16 *Enteromius* species known currently from Gabon (Mbega, 2004; Stiassny *et al.*, 2007).

Among the specimens collected during an inventory of the Louetsi River (Ngounie subdrainage of the Ogowe) of southern Gabon in April and May of 2017, before the potential construction of a planned hydroelectric dam at or in the vicinity of the Mioki Rapids (les Chutes de Mioki), two specimens of *Enteromius* from near Ndoubi village stood out. These specimens possessed three or four small round spots on the flanks, two pairs of moderately developed barbels, and multiple dark markings on the dorsal fin: a combination of characters otherwise unknown among the *Enteromius* of Gabon. A second sampling expedition at the same site during September 2017 increased the sample size of this important and interesting fish. In November of the same year, the consulting company BIOTOPE completed an inventory of the ichthyofauna and herpetofauna of the Birougou RAMSAR site, approximately 65 kilometers to the east. That mission to the Ngounié and Nyanga watersheds (Mbigou - Malinga sector), collected several individuals in the catchment of the Bissina River, which flows into the Bibaka River (Nyanga drainage), that appeared identical to those collected near the Mioki Rapids (Fig. 1). After a suite of morphometric, meristic, geographic and color-based comparisons reported herein, the team concluded that this enigmatic *Enteromius* was undescribed. This contribution demonstrates the evidence and formally describes the species.

# Methods

## Specimen Collection

Field collections (Fig. 2) in the Louetsi area were carried out under research permits AR0019/17 and AR0035/17 from MESRSFC/CENAREST/CG/CST/CSAR, while those in the Nyanga (Bissina) drainage were conducted under permit AR0044/17 from MESRSFC/CENAREST/CG/CST/CSAR and AE/17027 from Parcs Gabon. Specimens were collected using dip nets and a Halltech HT-2000 backpack electrofisher. All activities followed Animal Care and Use Protocol (ACUP) 4909, authorized by Oregon State University, with the exception that the BIOTOPE team used eugenol rather than MS-222 as the euthanizing agent. Specimens were euthanized, provisionally identified to species and counted. Some were photographed in an immersion tank following the protocol of Sabaj Perez (2009), and muscle and fin samples were preserved in cryotubes containing 95% ethanol. Samples from the Louetsi were transported to Oregon State University under export permits

12/05/2017/MESRFC/CENAREST/IRAF/LHI and 001/01/2018/MESRS/CENAREST/IRAF/LHI/JDM for laboratory identification.

**Data Collection**

Fourteen morphometrics and nine meristic counts followed the method of Lévêque *et al.* (1987). Total lateral-line scale counts following Lévêque et al. (1987) included all elements in the series, typically including one or two scales posterior to the structural base of the caudal fin. We also report scale lateral line scale counts to the point of caudal flexion, as many recent *Enteromius* descriptions (e.g.  Mamonekene et al. 2018) use that version of the count. Transverse scale counts include the middorsal and midventral scales as a half element, which follows most revisionary or synthetic treatments of *Enteromius*  (Lévêque *et al.*, 1987; Lévéque *et al.*, 1990; De Werdt & Teugels, 2007), though it is worth noting that Armbruster's (2012) general recommendations for cyprinids omit the half element at the midline.  The Weberian Apparatus was counted as four vertebrae, and the terminal compound centrum as a single element. As customary in species descriptions for the genus *Enteromius*,  the lengths of the anterior and posterior barbels were codified following Lévêque et al. (1987). These codes are as follows: 1 - the barbel not reaching the anterior border of the eye, 2 - the barbel reaching between the anterior border of the eye and the middle of the eye, 3 - the barbel reaching the posterior half of the eye, 4 - the barbel surpassing the posterior border of the eye. Certain individuals were photographed in an immersion tank following the protocol of Sabaj Perez (2009)

A literature search for *Enteromius* known from West and Central Africa was carried out, beginning with the most comprehensive systematic references for those regions (Lévéque *et al.*, 1990; Stiassny *et al.*, 2007). The search then expanded to other more recent publications dealing with *Enteromius* in these parts of Africa (Dankwa *et al.*, 1999; Mamonekene & Stiassny, 2012; Munene & Stiassny, 2016; Mamonekene *et al.*, 2018). As much as possible, references dealing with other regions in Africa (Poll, 1967; Eccles, 1992; Skelton, 2001) were consulted as were the online databases Fishbase and Eschmeyer's Catalog of Fishes. The team sent photographs of the putatively new species to other specialists on this genus to inquire whether they had previously encountered the fish. This work determined that the combination of characters present in the specimens from southern Gabon does not match any other known species in Gabon or Central Africa.

The most morphologically similar species appears to be *Enteromius walkeri* (Boulenger, 1904), a species that occurs only in coastal rivers in Ghana. (Fig. 3). These species share possession of multiple black spots on the flanks, many spots on the dorsal fin, pigmentation associated with the anterior lateral-line pores, and two pairs of barbels (Boulenger, 1904; Lévêque *et al.*, 1987; Lévéque *et al.*, 1990; Dankwa *et al.*, 1999). These appear to be the only two *Enteromius* species that possess this combination of characters, and indeed the only two with more than one dark spot on the dorsal fin. The California Academy of Sciences (CAS) and the University of Michigan (UMMZ) loaned specimens of *Enteromius walkeri* for examination. Two co-occurring and phenetically similar *Enteromius*, *E. camptacanthus* (Bleeker, 1863) (Fig. 4) and *E. chiumbeensis* (Pellegrin, 1936) (Fig. 5) were also included in morphometric and meristic comparisons. The examined specimens of *E. camptacanthus* and *E. chiumbeensis* were captured during the same expedition to the Louetsi that yielded the specimens of the putatively new species, and are accessioned and cataloged at Oregon State University (OS). Catalog numbers and full locality details of the examined material can be found at the end of the manuscript. Acronyms follow Sabaj (2016).

**Data Analysis**

The morphometric characteristics of the potentially new species and three others (*E. camptacanthus, E. chuimbeesis* and *E. walker*i) were compiled. Allometric coefficients for each nominal species were calculated via standardized major axis regression of the natural log transformed morphometrics versus the natural log of standard length in the SMATR package (Warton *et al.*, 2012) within the R computing environment (R Core Team, 2018). For tabular comparisons, measurements such as total length, body depth, and head length were expressed as percentages of standard length. Head width and the lengths of other elements of the head were expressed as percentages of head length.

Multivariate statistical analyses were conducted using Past 3 (Hammer *et al.*, 2001). The morphometrics were log10 transformed, and a principal components analysis (PCA) was completed using the variance-covariance matrix. This analysis requires a complete data matrix without missing values. Thus, total length was excluded from PCA due to the presence of several specimens with missing data due to damaged caudal fins. Two other specimens were removed from the multivariate analysis due to damaged dorsal fins. Because the four species differed greatly in the allometry of barbel length (see results), the morphospace could not be size-standardized with those measurements included. Size-standardization methods such as Burnaby's projection against the first principal component (Burnaby, 1966), shearing (Humphries *et al.*, 1981), or analysis of the residuals from regression of each measurement against standard length assume that all nominal species share a common allometric coefficient (Klingenberg, 1996; McCoy *et al.*, 2006). Though discarding barbel length from the analysis would permit size-standardization of the remaining characters, the two barbel length measurements were among the most discriminatory variables. As such, we retained barbel length in the multivariate analysis and did not size-standardize the dataset.

A PCA also treated a subset of the meristic data. For this analysis, invariant characteristics (such the number of anal-fin rays) were removed, as were individuals with missing data for any count. These were typically specimens that has lost their circumpeduncular scales, or those for which no radiograph was available. The PCA revealed the lateral-line scale counts to be the most discriminatory variables. Thus, box-plots of those counts in all available specimens visualized those data.

A cleared and stained individual was prepared according the protocol of Taylor and Van Dyke (1985). Photographs of the cleared and stained specimen were taken under a Zeiss V20 microscope with an Axiocam 105 color. An illustration of the left infraorbital series was prepared from a tracing of such a photograph in Adobe Illustrator. Finally, the GPS data were used to produce a map showing the sites inhabited by the putatively new species, as well as the sites where the teams sampled, but did not collect that species (Fig. 2).

# Results

**Meristic and Morphometric Analysis**

Examination of meristic counts indicated that with the exception of one outlier, the putatively new species has fewer scales in the lateral line series (Fig. 6) and fewer circumpeduncular scales (Table 1) than *Enteromius walkeri* or either of the most similar species in Gabon. That outlier (OS22150) had 23 total lateral line scales (21 to the point of caudal flexion) and 12 circumpeduncular scales. All other individuals of the putatively new species had 19 or 20 total lateral line scales (18 or 19 to the point of caudal flexion) and 10 circumpeduncular scales.

In the meristic PCA, the first axis indexed 71.4% variance and the second indexed 17.9% of the variance. The first axis described primarily the number of scales in the lateral-line, and also correlated positively with the number of circumpeduncular scales (Table 2). Vertebral counts influenced the second axis most strongly, followed by the number of branched pectoral-fin rays. A scatterplot of these two axes (Fig. 7) revealed that all but the aberrant individual of the putatively new species segregated from the other three species on PC1. *Enteromius chiumbeensis* separated completely from the other three species on PC2, indicating a vertebral count of 32 in that species, versus 33 to 35 in the others.

The standardized major axis regressions in SMATR indicated that the four species differ substantially ($p<0.001$) in the allometric trajectories of anterior and posterior barbel length (Fig. 8). Taking anterior barbel length as the example, *Enteromius chiumbeensis* and *E. camptacanthus* exhibit strong positive allometry (coefficients of 1.56 and 1.39, respectively) while the putatively new species and *E. walkeri* exhibit weak negative allometry (coefficients of 0.84 and 0.96, respectively). Thus, it was not possible to size-standardize the morphospace without distorting the differences among specimens (see discussion above under methods), and multivariate analyses did not incorporate an allometric correction. As a result, the first axis resulting from the principal components analysis indexes the size of the specimens and summarizes 96.11% of the total variance in the dataset (Table 3). All variables load positively on this axis, and the largest individuals appear to the right of Figure 9.

The second component, indexing 1.29% of the total variance (but a third of the variance remaining after excluding PC1), primarily describes the length of the barbels. Both the anterior and posterior barbel length load negatively on this axis (Table 3), and as such the specimens with the most positive PC2 scores have the proportionally smallest barbels. A plot of PC2 versus PC1 (Fig. 9, lower panel) highlights the difference between the positive barbel allometry of *Enteromius camptacanthus* and *E. chiumbeensis* on one hand, and the negative barbel allometry of *Enteromius walkeri* and the potentially new species on the other. In other words, in the two species with hyaline dorsal fins, the proportional length of the barbels increases as the fish grows, while the reverse is true in the species with spotted dorsal fins.

Despite indexing relatively small percentages of the overall variance in the dataset, two additional axes contain interpretable information. The most important measurement on PC3 (0.66% of variance) is interorbital width, but this axis does not discriminate any groups and is not shown graphically. PC4 (0.50% of variance) primarily indexes the length of the caudal peduncle, which loads positively on this axis. A plot of PC4 versus PC1 (Fig. 9, upper panel) readily distinguishes the putatively new species from Gabon from the other three, as do univariate regressions of caudal peduncle length against standard length (Fig. 8, lower panel). These results indicate that the potentially new species can be separated morphometrically by a 3% proportionately longer caudal peduncle relative to similarly sized specimens of the other examined species. Based on these and several other discriminatory species, a formal taxonomic description of the new species appears below.

# *Enteromius pinnimaculatus* sp. nov.

**Zoobank ID:** urn:lsid:zoobank.org:pub:5D06B0F7-FB54-4F3E-BBC3-BBA095A5B0C4

**Figures 1, 10, 11, 12 and 13 and Table 1**

**Holotype**

OS 22149 (1 specimen, tissue voucher GAB17-486, 41.05 mm SL), Gabon, Province de la Ngounié, small swampy right bank affluent of the Louetsi River upstream from the Chutes de Mioki, 2.09669° S, 11.60085° E, collected April 30, 2017 by Hans Kevin Mipounga and Jean Hervé Mve Beh.

**Paratypes**

Thirteen specimens, same locality as holotype: OS21870, 1 specimen, 17.4 mm SL; OS21889, 1 specimen, 18.97 mm SL; OS 22150, 1 specimen, (tissue voucher GAB17-250), 39.94 mm SL; OS 22151, 3 specimens, 19.42-20.79 mm SL, collected September 3, 2017; OS 22152, 26.98 mm SL (cleared & stained female, CT scan Gabon 4T), collected with OS 22151. OS 22153, 38.27 mm SL, collected with OS 22151; OS 22154, 1 specimen, (tissue voucher GAB17-1378), 15.0 mm SL, fixed directly in 95% ethanol, not included in morphometric table, collected with OS 2215; OS 22155, 1 specimen, (tissue voucher GAB17-1379), 17.0 mm SL, fixed directly in 95% ethanol, not included in morphometric table, collected with OS 22151. CAS 245836, 1 specimen, 22.89 mm SL, out of OS 22151; MRAC 2018-030-P-0001, 1 specimen, 28.20 mm SL, out of OS 22151; UMMZ 251024, 1 specimen, 22.98 mm SL, out of OS 22151.

**Non-type material**

These specimens were collected by the separate expedition to the Bissina River subdrainage of the Nyanga drainage. Because they were placed directly in alcohol after euthanasia, they have experienced shrinkage and cannot be included in morphometric comparisons. Though they appear to be conspecific with the specimens from the Louetsi, they occur in an adjacent drainage that flows into the Nyanga River, not into the Ngounie and then the Ogowe. They are therefore excluded from the paratype series.

OS uncataloged, (3 specimens, preserved directly in 90% ethanol), Gabon, Province de la Ngounié, swampy lowland stream in the Bissina River watershed, Nyanga River drainage. 2.20861°S, 12.17837°E.  Collected November 11, 2017 by Benjamin Adam.

**Differential Diagnosis**

A series of three or four dark spots along the flanks and a dorsal fin with multiple dark spots separates *Enteromius pinnimaculatus* from all other known species of *Enteromius* except *E. walkeri*. Nevertheless, *E. pinnimaculatus* sometimes has one or more spots on the anal fin and lacks the dark spot immediately ventral to the dorsal-fin origin, while *Enteromius walkeri* lacks pigmentation on the anal fin and has an additional dark spot ventral to the dorsal fin origin. Larger *E. pinnimaculatus* have noticeable pigmentation along the dorsal and ventral margins of most scale rows (Fig 10A and 10B), while adult *Enteromius walkeri* have two narrow bands of dark pigmentation dorsal and ventral to the lateral-line scale series on the anterior part of the body (Fig. 3A), but much less pronounced pigmentation at the intersection of other scale rows. The two species separate on the number of branched pectoral fin rays (11-12 in *E. pinnimaculatus* versus 13-14 in *E. walkeri*) and the number of unbranched dorsal fin rays (iii in *E. pinnimaculatus* versus iv in *E. walkeri*), though the extra element at the anterior of the dorsal fin in *E. walkeri* is minute and only observed on radiographs. With the exception of developmentally aberrant individuals, specimens of *Enteromius pinnimaculatus* have 33 vertebrae, while specimens of *E. walkeri* have 34. *Enteromius pinnimaculatus* differs modally from *E. walkeri* in the number of total lateral line scales (mode 20 versus mode 24), the number of lateral line scales to the point of caudal flexion (mode 18 versus mode 22), the number of circumpeduncular scales (mode 10 versus mode 12), and the number of branched dorsal-fin rays (mode 7 versus mode 8). *Enteromius pinnimaculatus* reaches only half the maximum body size (41.4 mm SL) of *E. walkeri* (78.5 mm SL). *Enteromius pinnimaculatus* has smaller pectoral fins (20.4 ± 1.2% SL) than *Enteromius walkeri* (24.3 ± 1.3% SL) as well as shorter anterior barbels (32.5 ± 3.0% HL vs. 43.7 ± 4.5% HL), with the difference in barbel length very pronounced in individuals of similar size (Fig. 8). *Enteromius pinnimaculatus* also has, on average, a shallower body depth (28.0 ± 0.9% SL) than E. *walkeri* (30.0 ± 1.1% SL) and a longer caudal peduncle (24.3 ± 1.2% SL vs. 21.4 ± 1.3% SL). Additional morphometric and meristic comparisons between the two species are reported in Table 1.

**Description**

Relatively small species, maximum known standard length of 41.4 mm. Greatest body depth immediately anterior to dorsal-fin origin. Dorsal body profile convex anterior to dorsal fin and concave and slightly depressed immediately posterior to dorsal fin, then straight from that point to dorsal procurrent rays of caudal fin. Dorsal-fin origin positioned slightly in advance of midpoint between the snout and the base of the caudal fin, just barely anterior to the pelvic-fin origin. Anus situated one scale width anterior to anal-fin origin, and just posterior to tip of adpressed pelvic fin. Pelvic fins abdominal. Pectoral fin origin low on body, at horizontal through ventral procurrent rays of caudal fin and one scale's height ventral to lateral-line scale row. Three branchiostegal rays, with most of their margin free of the isthmus, but joined to isthmus at ventral midline. Mouth moderately-sized and terminal, with posterior margin of maxilla at vertical through anterior margin of eye. Two pairs of moderately developed barbels. Posterior barbels extend beyond posterior margin of eye (27.1 – 35.6% SL, code 4 of Lévêque et al. 1987) and anterior barbels reach or exceed midpoint of eye (35.7 – 50.4% HL, code 2). Smallest specimens possessing proportionately longest barbels. Head and eye proportionately larger in smaller individuals. Eye diameter 27.2 – 38.9% of head length. These and other morphometrics (ranges, averages and standard deviations) in Table 1.

In cleared and stained specimen (OS22152) cranial fontanelle entirely closed, with sinuous medial suture between contralateral frontals and parietals. Infraorbital series broad and platelike, with clear flanges flanking sensory canal (Fig. 11). Two sensory pores on first infraorbital, one pore on second infraorbital, three pores on third infraorbital, one pore on fourth infraorbital, and none on fifth infraorbital (Fig. 11). Five triangular gill rakers on lateral ceratobranchial. Pharyngeal teeth in three rows, with five teeth in medial row, three teeth in central row, and one or two teeth in lateral row (contralateral sides of the cleared and stained specimen differ in the tooth count on this third row).

Meristics in Table 1. Typically, iii,7 dorsal-fin rays, including an unbranched rudiment and two longer unbranched soft rays (Fig. 12). Eighth branched ray present in holotype. Longest unbranched dorsal-fin ray flexible and non-serrate. Four supraneurals in cleared and stained specimen. Typically, iii,5 rays in the anal fin, with unbranched count including one rudiment and two longer unbranched rays. Cleared and stained specimen (OS22152) exhibits tiny additional rudiment buried beneath skin and anterior to counted elements of anal fin, not included in meristic count. Thirteen (rarely twelve) pectoral-fin rays, of which dorsalmost unbranched and remainder branched. One unbranched and seven branched pelvic-fin rays. Nine upper and nine lower principal caudal-fin rays. Eight upper procurrent and eight lower procurrent caudal-fin rays in cleared-and-stained specimen. Lateral line complete and runs along midlateral scale row without ventral deflection, 19 or 20 total scales in most specimens. Count includes one full sized scale posterior to posterior margin of hypural plate, and sometimes one smaller terminal scale. One specimen with 23 total lateral line scales, including two posterior of point of caudal flexion. 3.5 scales between lateral line and dorsal midline; 4.5 scales between lateral line and ventral midline; 2.5 between lateral line and pelvic-fin origin; 10 circumpeduncular scales in most specimens (12 in specimen with unusually high lateral-line scale count). Scale formula and fin-ray counts of three specimens from the Nyanga drainage verified by B.A. to match ranges reported herein for Louetsi (Ogowe) specimens. Typically thirty-three vertebrae, and exceptionally 35 in individual with visible spinal malformation on radiograph (OS22153). Twelve pairs of full pleural ribs in cleared and stained specimen, not including elements of Weberian Apparatus.

**Internal Soft Anatomy**

Gasbladder two chambered, with anterior chamber slightly smaller and posterior chamber tapering posteriorly. Stomach without clear differentiation from intestine. Intestine S-shaped. From pharynx, gastrointestinal track runs posteroventrally, then bends towards left lateral flank and runs anteriorly almost to anterior margin of stomach, then turns dorsally and reverses direction, continuing straight from that point to vent (Fig. 13). Spleen darkly pigmented and triangular, positioned dorsomedial to anterior bend in gastrointestinal tract. Ovaries elongate, positioned ventral to gasbladder and dorsal to intestine. Eggs relatively large (roughly 0.1

millimeters in diameter) and easily visible within ovary at 100x magnification. All observations of internal anatomy based on viscera removed from OS22152, an adult female specimen 27.0 mm standard length, prior to clearing and staining.

**Coloration in Preservative**

In preservation (Fig. 10), dorsum dark black to dark brown, particularly dark at dorsal-fin base. Flanks brown to yellowish, ventrum mustard yellow. Three or four round black spots on flanks: first anterior to dorsal-fin origin and centered on third and fourth scale in scale row dorsal to lateral-line scale row. Second spot posterior to dorsal-fin insertion, overlapping lateral line and centered on ninth or tenth scale of scale row dorsal to lateral-line scale row. Third (when present) faintest, dorsal to anal-fin insertion when present and centered on 13th or 14th scale. Third spot absent in some small individuals. Fourth intensely dark and located at posterior of caudal peduncle, centered on lateral-line scale row between procurrent caudal-fin rays. Lateral-line scales dark proximally around pores, forming a thin dotted line beginning just posterior to opercle and running to 14th or 16th lateral-line scales, typically reaching position of third major spot when four spots present on flanks. Numerous small black spots on all dorsal-fin rays and extending onto membranes, sometimes forming two lines (Fig 10B). One or several small black spots at midpoint of anal fin in most specimens, though holotype with only a single faint spot (Fig. 10A). Anal-fin otherwise hyaline with a dusky margin. Caudal-fin rays slightly dark at bases. Pectoral and pelvic fins hyaline.

**Coloration in Life**

The only photograph of *Enteromius pinnimaculatus* in life (Fig. 1) is of an individual from the Bibaka population (Bissina subdrainage of the Nyanga drainage). Opercle red, body color ranging from white on ventrum to light pink at midflank, dorsum light brown. Multiple small black spots over dorsal fin. Fins otherwise yellowish, with color most intense near bases and middle sections of paired and anal fins. Lateral-line scales with black spots on bases and surrounding pores, forming dashed black line along lateral-line scale row. Three to four dark spots on flanks, less intensely obvious in life than in preservative.

**Generic Placement**

The pigmentation along the scales of the lateral line series in *Enteromius pinnimaculatus* is reminiscent of some species in *Clypeobarbus*, a genus recently reaffirmed as distinct from *Barbus* and *Enteromius* (Conway & Stiassny, 2008; Stiassny & Sakharova, 2016; Hayes & Armbruster, 2017). However, the new species does not fit the current diagnosis of that genus (Stiassny & Sakharova, 2016) because it lacks an occipital fontanelle and has well developed intraorbital bones with flanges that extend far beyond the sensory canal (Fig. 10). It also lacks the distinctive cleithral pigmentation of *Clypeobarbus* and its lateral line scales are of a similar size to those adjacent, in contrast to the enlarged midlateral scales of *Clypeobarbus*. As such, the new species best fits the current concept of *Enteromius*.

**Diet**

The stomach of the cleared and stained specimen (OS22152) was full of unidentifiable flocculent material, and its intestine contained more of the same flocculence plus a few chitinous fragments and three mostly-digested dark objects that might have been seeds. Though these data are very limited, they suggest that the species is omnivorous, with plant and insect material in the diet.

**Etymology**

The specific epithet *pinnimaculatus* refers to the multiple small dark spots on the dorsal fin, which is a rare characteristic within *Enteromius*. An adjective in the nominative singular.

**Distribution and Habitat**

*Enteromius pinnimaculatus* is currently known only from two sites (Fig. 2). The first collection site is a small stream that drains into the Loueti River near the Mioki Rapids (11.60085°E; 2.09669°S), near Ndoubi village. The second is a small stream near Leyonga village in the Bissina River watershed (12.178365°E; 2.208614°S). Both sites are at moderate elevation, between 400 and 700 meters above sea level. Both streams drain primary forest (Fig. 14), and each is approximately 1 meter wide and about 30 cm deep with the substrate a slurry-like mud mixed with dead leaves. In the Bibaka, the banks are vertical with substantial underbank, dead wood and roots. The sites are in two different major watersheds (Ogowe and Nyanga) but both in Ngounie province, which borders Congo-Brazzaville.

The Chaillu Massif, a mountain range that straddles the border of Gabon and Congo, dominates this region. The relief of the Chaillu Massif consists of a metamorphic formation incised by steep hills and high mountain regions. Most of the massif is covered in dense forest with interspersed savannah formations, although these are mainly confined to the eastern parts (Vicat & Gioan, 1989; Mamonekene & Stiassny, 2012). The Chaillu Massif may have served as a refugium for species from climatic changes during ancient glaciation events and the rivers of this region contain a rich diversity of fishes. Despite forming part of the Lower Guinea ichthyofaunal province, the rivers of this region contain a ichthyofaunal community that appears to share some affinity with the Congo, as evidenced by the presence of fishes like *Enteromius chiumbeensis*, which is common further south (Poll, 1967; Mamonekene & Stiassny, 2012) in the Congo drainage but unknown in more northerly areas.

**Co-occurring species**

Other fish species collected syntopically at the Louetsi site (Ogowe drainage) include *Aphyosemion ocellatum*, *A. primigenium*, *Microctenopoma nanum*, and *Enteromius chiumbeensis*. All these are widespread throughout the Louetsi. Other fish species collected syntopically at the Bibaka site (Nyanga drainage) include a young *Clarias* (probably *C. camerunensis*) and two rare *Aphyosemion*: *A. hofmanni* and *A. wuendschi*. *Aphyosemion hofmanni* is only known from about ten localities in the region, and *A. wuendschi* is otherwise known only from its type locality in the Louetsi watershed, where it was captured in 1985 (Radda & Pürzl, 1985).

**Conservation Status**

Even though the two known localities for *Enteromius pinnimaculatus* correspond to two different major watersheds (Ogowe and Nyanga), the collection sites are actually separated by only 65 km. A polygon enclosing the two localities and encompassing sites at similar altitude estimates an extent of occurrence of approximately 1,500km$^2$. Even if polygon were expanded substantially, it would be hard to construe a reasonable extent of occurrence exceeding 5,000km$^2$.

While no information exists on the population size of the new species, its habitat appears restricted to small first or second order streams and wetlands, particularly shallow swampy areas at the confluence of streams with rivers (Fig. 14). Certainly, the sampling locations at which the species was not found (Fig. 2, purple circles) outnumber substantially those where the species was collected (Fig. 2, red star and target). That apparent habitat restriction implies that its extent of occupancy is considerably less than its extent of occurrence. With only two known localities, it is impossible to estimate occupancy precisely, but it is probable that the true area of occupancy for *Enteromius pinnimaculatus* falls short of 500 $km^2$.

In comparing these data to the IUCN Red List criteria (International Union for Conservation of Nature, 2001), we find that the species nearly qualifies for Endangered status via criterion B (geographic distribution), because the extent of occurrence is less than 5,000 $km^2$ and meets subcriterion A in being known from fewer than five localities. However, there is currently no evidence for a decline or fluctuation in occupancy, occurrence or population size, meaning that the species triggers only one of two needed subcriteria for endangered status.

*Enteromius pinnimaculatus* does meet criterion D (very small or restricted population) of the IUCN standards for Vulnerable status, as it is known from fewer than five localities. This puts the species at substantial risk of becoming endangered due to modifications to its habitat, and recommends a formal IUCN classification at the level Vulnerable (VU).

The known collection site in the Louetsi drainage falls within the proposed Dibwangui hydroelectric dam development. If that hydropower project proceeds, it is likely that the area will be fully deforested for the purposes of construction and operation of dam infrastructure, and the critical habitat for the species might be inundated or otherwise altered. If that habitat alteration causes the decline or local extinction of the Louetsi population, only one known healthy population would remain in the Nyanga watershed, and criterion B, subcriterion B of the IUCN standards (decline in occupancy, occurrence or population size) would be triggered. It is therefore reasonable to assume that the construction of the Dibwangui dam has the potential to change the status of this species from Vulnerable (VU) to Endangered (EN).

## Discussion

*Enteromius pinnimaculatus*, a new species of cyprinid fish from tributaries of the Louetsi (Ogowe) and Bissina (Nyanga) rivers of southern Gabon is readily distinguished from all known *Enteromius* species except *E. walkeri* by its color pattern in life and in preservative, with multiple small black spots on the dorsal fin and three to four dark spots on the flanks. As described above, numerous other differences easily separate these two species, including differences in maximum body size, meristics, morphometrics and nuances of coloration, as well as complete allopatry, with *E. walkeri* known only from coastal rivers of West Africa, most notably the Pra and Ankobra rivers of Ghana.

It is worth noting that records of *E. walkeri* from Ivory Coast are unconfirmed, and appear to refer to a single lot (MNHN a-4430) at the Muséum National d'Histoire Naturelle in Paris, collected in 1882 from an unknown location in "CI", and thus far before modern political boundaries were established. Teugels *et al.* (1988) indicate that the species inhabits the Tano River system, the mouth of which lies on the border between Ivory Coast and Ghana, but also indicate that the species is "known only in the west of Ghana, never observed in Ivory Coast." One putative record of *Enteromius walkeri* from Sierra Leone (FMNH73943) is based on a set of scales, with a note in the jar indicating uncertain identification (pers. comm. C. McMahan, March 5, 2018). Otherwise, all records of this species appear to be from Ghana.

**Barbel Allometry**

*Enteromius walkeri* and *E. pinnimaculatus* share an intriguing allometry of the barbels, which are quite elongate in juveniles, but grow more slowly than other parts of the head. *Enteromius camptacanthus* and *E. chiumbeensis* show the opposite pattern, with the barbels lengthening faster than other parts of the head over ontogeny. These different allometric coefficients explain the very different slopes for each pair of species in the morphometric scatterplot of PC1 (size) versus PC2 (barbel length) (Fig. 8), and illustrate that barbel length can be used to separate these species if size is considered (Fig. 7).

The biological reason for the difference in allometry is not clear, though developmental changes in the relative importance of chemosensation may play a role. In goatfishes, (which use mental barbels to locate food) barbel length increases up to 50% after larval settlement, coinciding with the onset of benthic foraging (McCormick, 1993). It is therefore possible that different dietary or habitat shifts among these species of *Enteromius* may explain why two species have proportionately larger barbels as juveniles, while two others have longer barbels as adults. Perhaps the adults of *E. camptacanthus* and *E. chiumbeensis* spend more time foraging benthically than do adults of the other two species?

Intriguingly, the co-occurring *Enteromius pinnimaculatus* and *Enteromius chiumbeensis* differ substantially in allometric coefficients, Does the allometric difference between the syntopic species hint at underlying trophic diversification, which might in turn help them occupy different niches in their tiny stream habitats? No detailed data on microhabitat preferences or the developmental biology of these species exist, so this and any other hypothesis for the difference in barbel allometry is speculative at best. Future studies should characterize the diet of adult and juvenile specimens to test the hypotheses of ontogenetic shifts in diet, and of niche partitioning.

The similarity in fin pigmentation and allometry between the geographically distant *Enteromius walkeri* and *E. pinnimaculatus* may hint at a close evolutionary relationship, but may also arise from convergence. Because no tissue samples of *E. walkeri* appear to exist in the world's ichthyology collections, these alternative possibilities cannot currently be tested. As more of Africa's fish diversity becomes accessible to genetic investigation (e.g. Van Ginneken *et al.*, 2017), future studies should assess whether phylogenetic signal in barbel allometry exists within *Enteromius*. If so, a reconstruction of the evolutionary history of this fascinating character may help to reveal the factors that have promoted the impressive diversification of the genus.

## Conclusion: perspectives on the diversity and conservation of fishes of Gabon

The discovery of this and other new species in Gabon is not surprising, because many areas of this country have not yet been inventoried. Most collections have been carried out along major highways or on major rivers, so most sampling stations occur along roads, or in the navigable sections of larger rivers such as the middle Ogowe (Fermon, 2013). Sampling in remote rivers and smaller water bodies will undoubtedly lead to the discovery of more new species, and in particular new range-restricted species and vulnerable species like *Enteromius pinnimaculatus*, or the co-occurring *Aphyosemion wuendschi*, both of which are known from only two sampling localities from small streams in primary forests within Gabon's Ngounie province.

At a time when the country is embarking on an ambitious all-out development program in line with the vision of the Gabon Emergent Strategic Plan (République Gabonaise, 2012), the discovery of this new species demonstrates that the aquatic ecosystems of Gabon have yet to deliver all their secrets. This discovery challenges scientists to continue exploring undersampled or unsampled regions, with particular attention to the small and ephemeral habitats that harbor miniature, easily missed species. Increased knowledge about this region's rich biodiversity will improve the ability to recommend effective management plans that balance conservation with the need to develop sustainable natural resources for the benefit of Gabon's people.

## Comparative Material Examined

*Enteromius camptacanthus.* All from Gabon, Province de la Ngounie, Soungou stream near Mabanga village, small stream on the left bank of the Ngounie River, with a large waterfall between this sampling site and the confluence, 2.27860°S, 11.61192°E. OS20935, 46 specimens, (tissue vouchers GAB17-998 and GAB17-999), 2 specimens photographed but not included in morphometric or meristic table, 31.47 - 95.81 mm SL, collected September 1, 2017; OS 21855, 1 specimen, (tissue voucher GAB17-375), 57.21 mm SL, collected May 4, 2017; OS 21877, 1 specimen, (tissue voucher GAB17-283), 74.98 mm SL), collected with OS21855; OS 21881, 12 specimens, (tissue voucher GAB17-274), 24.44 - 79.33 mm SL, collected with OS21855.

*Enteromius chiumbeensis:* All from Gabon, Province de la Ngounie. OS 21285, 1 specimen, 35.02 mm SL, small swampy stream on the right bank of the Louetsi River just upstream from the Chutes de Mioki, 2.0966°S, 11.60085°E, collected September 3, 2017; OS 21879, 8 specimens (tissue voucher GAB 17-282), 21.48 - 55.12 mm SL, Soungou stream near Mabanga village, small stream on the left bank of the Ngounie river, with a large waterfall between this sampling site and the confluence, 2.27860°S, 11.61192°E, collected May 4, 2017.

*Enteromius walkeri:* All from Ghana. CAS-SU 62769; 15 of 43 specimens examined and measured, 32.99 - 72.90 mm SL, cascades zone of stream near Asiakwa, Akim-Abuakwa, collected January 19, 1963; UMMZ 195011, 10 of 26 specimens examined and measured, 31.65 - 84.15 mm SL, Adansu River near Kibi, collected March 20, 1971.

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# Contributions

H.K.M. wrote the initial draft of most sections of the manuscript, collected and assembled data, performed statistical analyses, took and edited specimen photographs, prepared figures, and helped edit the manuscript. J.C. helped collect data, searched for relevant literature, wrote sections of the results and discussion, commented extensively on manuscript drafts, and helped translate the manuscript from French to English. J.H.M.B. drafted the section on the conservation status of the new species. B.L.S. supervised all aspects of the project, collected and analyzed data, prepared figures, edited photographs, led the morphometric and allometric analysis, wrote text in all manuscript sections, translated, and critically edited the manuscript. H.K.M., J.H.M.B., J.C. and B.L.S. collected specimens from the Louetsi drainage and helped sort, identify and catalog specimens at the Oregon State Ichthyology Collection. B.A. collected the specimens from the Nyanga drainage, provided site photographs and the live photograph of the new species, and contributed text to several manuscript sections. All authors helped edit the manuscript and all approved the submission.

**References**

Armbruster, J. W. (2012). Standardized measurements, landmarks, and meristic counts for cypriniform fishes. *Zootaxa* **3586**, 8-16.

Berrebi, P., Kottelat, M., Skelton, P. & Ráb, P. (1996). Systematics of *Barbus*: state of the art and heuristic comments. *Folia Zoologica* **45**, 5-12.

Berrebi, P. & Tsigenopoulos, C. S. (2003). Phylogenetic organization of the genus *Barbus* sensu stricto: A review based on data obtained using molecular markers. In *The freshwater fishes of Europe* (Banarescu, P. M. & Bogutskaya, N. G., eds.), pp. 11-22. Wiebelsheim: AULA-Verlag.

Boulenger, G. A. (1904). Descriptions of new West-African freshwater fishes. *Annals and Magazine of Natural History (Series 7)* **14**, 16-20.

Burnaby, T. P. (1966). Growth-invariant discriminant functions and generalized distances. *Biometrics* **22**, 96-110.

Conway, K. W. & Stiassny, M. L. (2008). Phylogenetic diagnosis of the African cyprinid genus *Clypeobarbus* (Ostariophysi: Cyprinidae), with the rehabilitation of Clypeobarbus bomokandi. *Ichthyological Research* **55**, 260-266.

Dankwa, H. R., Abban, E. K. & Teugels, G. G. (1999). *Freshwater fishes of Ghana: identification, distribution, ecological and economic importance*.

De Werdt, D. & Teugels, G. G. (2007). Genus *Barbus* Cuvier & Cloquet, 1816. In *The Fresh and Brackish Water Fishes of Lower Guinea, West-Central Africa* (Stiassny, M., Teugels, G. G. & Hopkins, C. D., eds.), pp. 471-511. Paris: IRD, MRAC.

Eccles, D. H. (1992). Field guide to the freshwater fishes of Tanzania. *FAO Species Identification Sheets for Fishery Purposes*.

Eschmeyer, W. N., Fricke, R. & Van der Laan, R. (2018). Catalog of Fishes electronic version (14 November 2018), https://www.calacademy.org/scientists/projects/catalog-of-fishes. California Academy of Sciences.

Fermon, Y. (2013). Caractérisation ichthyologique des bassin versants gabonais. p. 303p. Paris: WWF and Association Aimara.

Gabon MAEPDR (2011). Evaluation des stocks, Gestion des ressources halieutiques et mise en place d'un observatoire des pêches-ESOP. Rapport phase ii : Résultats des campagnes d'évaluation des stocks en milieu continental. . (Ministère de l'Agriculture, d. l. É., de la Pêche et du Développement Rural, ed.), p. 118p.: COFREPECHE – France Aquaculture.

Hammer, Ø., Harper, D. A. T. & Ryan, P. D. (2001). PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* **4**, 1-9.

Hayes, M. M. & Armbruster, J. W. (2017). The taxonomy and relationships of the African small barbs (Cypriniformes: Cyprinidae). *Copeia* **105**, 348-362.

Humphries, J. M., Bookstein, F. L., Chernoff, B., Smith, G. R., Elder, R. L. & Poss, S. G. (1981). Multivariate discrimination by shape in relation to size. *Systematic Zoology* **30**, 291-308.

International Union for Conservation of Nature (2001). *IUCN Red List categories and criteria*: IUCN.

Klingenberg, C. P. (1996). Multivariate allometry. . In *Advances in morphometrics* (Marcus, L. F., Corti, M., Loy, A., Naylor, G. J. P. & Slice, D. E., eds.), pp. 23-49. New York: Plenum.

Lederoun, D. & Vreven, E. (2016). *Enteromius vandewallei*, a new species of minnow from the Volta River basin, West Africa (Cypriniformes: Cyprinidae). *Ichthyological Exploration of Freshwaters* **27**, 97-106.

Lévéque, C., Paugy, D. & Teugels, G. G. (1990). *Faune des poissons d'eaux douces et saumâtres d''Afrique de l'Ouest*. Tervuren: Musée Royal de l'Afrique Centrale.

Lévêque, C., Teugels, G. & Thys Van Den Audenaerde, D. (1987). Révision de quelques *Barbus* à trois taches (Pisces, Cyprinidae) d'Afrique de l'Ouest, avec la description de trois nouvelles espèces: *B. tiekoroi* sp. n., *B. traorei* sp. n. et *B. trispiloides* sp. n. *Revue d'Hydrobiologie Tropicale* **20**, 165-184.

Mamonekene, V. & Stiassny, M. L. (2012). Fishes of the Du Chaillu Massif, Niari Depression, and Mayombe Massif (Republic of Congo, west-central Africa): A list of species collected in the tributaries of the upper Ogowe and middle and upper Kouilou-Niari River basins. *Check List* **8**, 1172-1183.

Mamonekene, V., Zamba, A. I. & Stiassny, M. L. J. (2018). *A New Small Barb (Cyprininae: Smiliogastrini) from the Louesse, Lekoumou (Upper Niari Basin), and Djoulou (Upper Ogowe Basin) Rivers in the Republic of Congo, West-Central Africa*: SPIE.

Mbega, J.-D. (2004). Biodiversité des poissons du bassin inférieur de l'Ogooué (Gabon). Volume 1 et Volume 2. Thèse de Doctorat en Sciences In *Faculté des Sciences.* , p. 614p. Namur: Université de Namur.

McCormick, M. I. (1993). Development and changes at settlement in the barbel structure of the reef fish, *Upeneus tragula* (Mullidae). *Environmental Biology of Fishes* **37**, 269-282.

McCoy, M. W., Bolker, B. M., Osenberg, C. W., Miner, B. G. & Vonesh, J. R. (2006). Size correction: comparing morphological traits among populations and environments. *Oecologia* **148**, 547-554.

Munene, J. J. M. M. & Stiassny, M. L. J. (2016). Fishes of the Kwilu River (Kasai basin, central Africa): A list of species collected in the vicinity of Kikwit, Bandundu Province, Democratic Republic of Congo. *2016* **7**, 9.

Poll, M. (1967). Contribution à la faune ichthyologique de l'Angola. *Publicoes Culturais da Comapnhia de Diamantes de Angola* **75**, 1-381.

R Core Team (2018). R: A language and environment for statistical computing.

Radda, A. & Pürzl, E. (1985). Zwei neue Formen der Gattung *Aphyosemion* aus Süd-Gabun. *Aquaria: Vivaristische Fachzeitschrift für die Schweiz und Österreich* **32**, 157-160.

République Gabonaise (2012). Plan Strategique Gabon Emergent. Available at www.sgg.gouv.ga/plan-strategique-gabon-mergent. pp. 1-149. Libreville: République Gabonaise,.

Sabaj, M. (2016). Standard symbolic codes for institutional resource collections in herpetology and ichthyology: an Online Reference. *Version* **6**, 802-832.

Sabaj Pérez, M. H. (2009). Photographic atlas of fishes of the Guiana Shield. *Bulletin of the Biological Society of Washington* **17**, 52-59.

Schmidt, R. C., Bart, H. L. & Nyingi, W. D. (2018). Integrative taxonomy of the red-finned barb, *Enteromius apleurogramma* (Cyprininae: Smiliogastrini) from Kenya, supports recognition of *E. amboseli* as a valid species. *Zootaxa* **4482**, 566-578.

Skelton, P. H. (2001). *A complete guide to the freshwater fishes of southern Africa*: Struik.

Stiassny, M. & Sakharova, H. (2016). 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* **88**, 1394-1412.

Stiassny, M. L. J., Teugels, G. G. & Hopkins, C. D., eds. (2007). *The Fresh and Brackish Water Fishes of Lower Guinea, West-Central Africa*. Paris: IRD, MRAC.

Tan, M. & Armbruster, J. W. (2018). Phylogenetic classification of extant genera of fishes of the order Cypriniformes (Teleostei: Ostariophysi). *Zootaxa* **4476**, 6-39.

Taylor, W. R. & Van Dyke, G. C. (1985). Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybium* **9**, 107-119.

Teugels, G. G., Levêque, C., Paugy, D. & Traore, K. (1988). Etat des connaissances sur la faune ichtyologique des bassins côtiers de Côte d'Ivoire et de l'ouest du Ghana. *Revue d'Hydrobiologie Tropicale* **21**, 221-237.

Van Ginneken, M., Decru, E., Verheyen, E. & Snoeks, J. (2017). Morphometry and DNA barcoding reveal cryptic diversity in the genus *Enteromius* (Cypriniformes: Cyprinidae) from the Congo basin, Africa. *2017*.

Vicat, J. P. & Gioan, P. (1989). La chaîne du Mayombe en République Populaire du Congo: Géologie, métallogénie, perspectives de développement Revue des connaissances sur le Mayombe: Synthèse préparée pour le Projet PNUD/Unesco PRC/85/002 et PRC/88/003. (UNESCO, ed.), p. 343p. Paris: Organisation des Nations Unies pour l'Éducation, la Science et la Culture.

Warton, D. I., Duursma, R. A., Falster, D. S. & Taskinen, S. (2012). smatr 3– an R package for estimation and inference about allometric lines. *Methods in Ecology and Evolution* **3**, 257-259.

Yang, L., Sado, T., Vincent Hirt, M., Pasco-Viel, E., Arunachalam, M., Li, J., Wang, X., Freyhof, J., Saitoh, K., Simons, A. M., Miya, M., He, S. & Mayden, R. L. (2015). Phylogeny and polyploidy: Resolving the classification of cyprinine fishes (Teleostei: Cypriniformes). *Molecular Phylogenetics and Evolution* **85**, 97-116.

**Table 1.** Counts and measurements, including ranges, means and standard deviations. Values for *Enteromius pinnimaculatus* represent the holotype and nine paratypes from the Louetsi drainage available for measurements at Oregon State University, and exclude the three specimens from the Nyanga drainage.

| | | *E. pinnimaculatus* (n=10) | | | *E. walkeri* (n = 24) | | |
|---|---|---|---|---|---|---|---|
| I. Morphometric measurements | Holotype | Range | Mean | SD | Range | Mean | SD |
| Standard length (mm) | 41.1 | 19.4-41.4 | 27.9 | 8.5 | 31.7-78.5 | 47.3 | 14.0 |
| *Percentages of standard length* | | | | | | | |
| Total length | 123.0 | 122.2-128.7 | 125.4 | 0.02 | 122.9-133.7 | 129.0 | 3.7 |
| Body depth | 28.3 | 26.5-29.6 | 28.0 | 0.9 | 27.0-33.5 | 30.0 | 1.1 |
| Head length | 24.7 | 24.5-27.9 | 26.5 | 1.3 | 25.5-30.0 | 28.3 | 1.1 |
| Pectoral-fin length | 19.1 | 18.1-22.0 | 20.4 | 1.2 | 22.7-28.0 | 24.3 | 1.3 |
| Length of dorsal-fin | 22.3 | 21.5-27.2 | 24.8 | 1.9 | 22.6-29.6 | 25.6 | 1.8 |
| Length of caudal peduncle | 24.3 | 22.3-26.3 | 24.3 | 1.2 | 19.0-23.8 | 21.4 | 1.3 |
| Depth of caudal peduncle | 12.9 | 11.0-15.0 | 13.1 | 1.3 | 13.8-16.1 | 15.1 | 0.7 |
| Head length (mm) | 10.2 | 5.4-10.2 | 7.3 | 1.88 | 9.22-21.53 | 13.3 | 3.6 |
| *Percentages of head length* | | | | | | | |
| Head width | 59.7 | 56.8-70.5 | 61.3 | 4 | 57.5-69.5 | 62.7 | 3.2 |
| Eye diameter | 27.2 | 27.2-38.9 | 34.0 | 3.6 | 27.4-39.9 | 32.4 | 3.5 |
| Snout length | 26.4 | 21.5-29.5 | 26.4 | 2.8 | 23.4-32.9 | 28.8 | 2.4 |
| Interorbital width | 39.0 | 30.1-39.1 | 33.5 | 3.4 | 29.9-43.5 | 38.8 | 2.9 |
| Length of posterior barbel | 39.0 | 35.7-50.4 | 42.9 | 4.6 | 38.7-59-6 | 49.5 | 4.4 |
| Length of anterior barbel | 29.8 | 27.1-35.6 | 32.5 | 3.0 | 36.4-51.9 | 43.7 | 4.5 |
| II. Meristic counts | Holotype | Range | Mode | SD | Range | Mode | SD |
| Total lateral-line scales | 19 | 19-23 | 20 | 1.2 | 22-26 | 24 | 1.1 |
| Lateral-line scales to caudal flexion | 18 | 18-21 | 18 | 1.0 | 20-24 | 22 | 1.0 |
| Upper transverse scales | 3.5 | 3.5 | 3.5 | 0.0 | 3.5 | 3.5 | 0 |
| Lower transverse scales to midventrum | 4.5 | 4.5 | 4.5 | 0.0 | 4.5 | 4.5 | 0 |
| Lower transverse scales at pelvic-fin origin | 2.5 | 2.5 | 2.5 | 0.0 | 2.0-2.5 | 2.5 | 0.2 |
| Circumpeduncular scales | 10 | 10-12 | 10 | 0.8 | 12 | 12 | 0 |
| Posterior barbel posterior (code) | 4 | 4 | 4 | 0 | 4 | 4 | 0 |
| Anterior barbel length (code) | 2 | 2 | 2 | 0 | 4 | 4 | 0 |
| Number of vertebrae | 33 | 33-35† | 33 | 0.7 | 34 | 4 | 0 |
| Number of unbranched dorsal-fin rays | iii | iii | iii | 0 | iv | iv | 0 |
| Number of branched dorsal-fin rays | 8 | 7-8 | 7 | 0.3 | 8 | 8 | 0 |
| Number of unbranched anal-fin rays | | iii | iii | 0 | iii | iii | 0 |
| Number of branched anal-fin rays | | 5 | 5 | 0 | 5 | 5 | 0 |
| Number of unbranched pectoral-fin rays | | i | i | 0 | i | i | 0 |
| Number of branched pectoral-fin rays | 12 | 11-12 | 12 | 0.3 | 13-14 | 13 | 0.4 |

†Specimen with 35 vertebrae has a clear developmental abnormality

| | *E. camptacanthus* (n=14) | | | *E. chiumbeensis* (n=9) | | |
|---|---|---|---|---|---|---|
| I. Morphometric measurements | Range | Mean | SD | Range | Mean | SD |
| Standard length (mm) | 24.4-79.3 | 56.8 | 16.2 | 21.5- 55.1 | 41.4 | 12.9 |
| *Percentages of standard length* | | | | | | |
| Total length | 121.3-137.3 | 131.2 | 3.9 | 124.6-131.6 | 128.6 | 2.7 |
| Body depth | 27.5-32.3 | 29.8 | 1.4 | 29.2-35.5 | 32.0 | 2.1 |
| Head length | 25.7-31.5 | 27.8 | 1.7 | 26.5-29.9 | 27.9 | 1.3 |
| Pectoral-fin length | 19.0-26.4 | 22.1 | 1.8 | 17.7-23.7 | 20.5 | 2.2 |
| Length of dorsal-fin base | 22.9-30.0 | 26.3 | 1.8 | 25.3-30.5 | 28.2 | 1.6 |
| Length of caudal peduncle | 17.5-21.0 | 20.0 | 0.9 | 19.1-23.5 | 21.3 | 1.6 |
| Depth of caudal peduncle | 11.5-14.6 | 13.4 | 0.9 | 12.8-14.9 | 13.6 | 0.6 |
| Head length (mm) | 7.61-21.5 | 15.6 | 4.0 | 6.3-15.11 | 11.4 | 3.2 |
| *Percentages of head length* | | | | | | |
| Head width | 49.3-70.1 | 63.8 | 6.2 | 42.0-63.6 | 55.1 | 7.5 |
| Eye diameter | 22.7-35.5 | 27.5 | 3.3 | 28.0-33.6 | 30.7 | 2.0 |
| Snout length | 22.3-31.9 | 28.4 | 2.8 | 24.3-30.6 | 28.0 | 2.2 |
| Interorbital width | 26.7-37.0 | 33.7 | 3.1 | 25.7-34.6 | 30.5 | 2.8 |
| Length of posterior barbel | 27.5-49.7 | 41.4 | 6.4 | 26.4-59.8 | 43.7 | 10.2 |
| Length of anterior barbel | 19.8-49.7 | 39.3 | 7.9 | 24.2-51.8 | 39.5 | 8.9 |
| II. Meristic counts | Range | Mode | SD | Range | Mode | SD |
| Total lateral-line scales | 22-25 | 25 | 1.1 | 22-24 | 22 | 0.9 |
| Lateral-line scales to caudal flexion | 20-23 | 22 | 1.1 | 20-22 | 20 | 0.7 |
| Upper transverse scales | 3.5 | 3.5 | 0 | 3.5-4.5 | 3.5 | 0.5 |
| Lower transverse scales to midventrum | 4.5 | 4.5 | 0 | 3.5-4.5 | 3.5 | 0.3 |
| Lower transverse scales at pelvic-fin origin | 2.5-3.0 | 2.5 | 0.1 | 2.0-2.5 | 2.5 | 0.2 |
| Circumpeduncular scales | 12 | 12 | 0 | 11-12 | 12 | 0.4 |
| Posterior barbel posterior (code) | 3-4 | 4 | 0.4 | 2-4 | 4 | 0.7 |
| Anterior barbel length (code) | 1-3 | 3 | 0.6 | 1-3 | 2 | 0.7 |
| Number of vertebrae | 33-35 | 34 | 0.6 | 32 | 32 | 0 |
| Number of unbranched dorsal-fin rays | iii-iv | iii | 0.3 | iv | iv | 0 |
| Number of branched dorsal-fin rays | 8 | 8 | 0 | 8 | 8 | 0 |
| Number of unbranched anal-fin rays | iii | iii | 0 | iii | iii | 0 |
| Number of branched anal-fin rays | 5 | 5 | 0 | 5 | 5 | 0 |
| Number of unbranched pectoral-fin rays | i | i | 0 | i | i | 0 |
| Number of branched pectoral-fin rays | 11-14 | 13 | 0.7 | 13-14 | 13 | 0.4 |

**Table 2.** Loadings and percent variance explained for the first two principal component axes resulting from analysis of the meristic data. The three measurements with highest loadings on each axis appear in bold.

| Count | PC 1 | PC 2 |
|---|---|---|
| Percent variance explained | 71.42% | 19.83% |
| Total lateral-line scales | **0.708** | 0.051 |
| Lateral-line scales to caudal flexion | **0.635** | -0.043 |
| Upper transverse scales | -0.006 | 0.093 |
| Lower transverse scales to midventrum | 0.029 | -0.291 |
| Lower transverse scales at pelvic fin origin | -0.009 | -0.003 |
| Circumpeduncular scales | **0.247** | 0.185 |
| Vertebral number | 0.134 | **-0.761** |
| Unbranched dorsal-fin rays | 0.043 | **0.315** |
| Branched dorsal-fin rays | 0.093 | 0.120 |
| Branched pectoral-fin rays | 0.076 | **0.419** |

**Table 3.** Loadings and percent variance explained for the first four principal component axes resulting from analysis of the morphometric data. All loadings on PC1 are positive and roughly equivalent, and this axis can be interpreted as indexing primarily specimen size. The two measurements with highest loadings appear in bold for PC2 through PC4.

| Measurement | PC 1 | PC 2 | PC 3 | PC 4 |
|---|---|---|---|---|
| Percent variance explained | 96.11% | 1.29% | 0.66% | 0.50% |
| Standard length | 0.265 | 0.278 | -0.100 | 0.157 |
| Body depth | 0.277 | 0.179 | -0.264 | -0.065 |
| Head length | 0.255 | 0.229 | -0.123 | -0.168 |
| Head width | 0.299 | 0.151 | 0.092 | 0.190 |
| Eye diameter | 0.197 | -0.178 | 0.231 | -0.185 |
| Snout length | 0.283 | 0.058 | -0.146 | -0.253 |
| Interorbital width | 0.297 | -0.001 | **0.637** | -0.235 |
| Pectoral-fin length | 0.291 | 0.020 | 0.231 | -0.080 |
| Length of dorsal-fin | 0.249 | 0.275 | **-0.425** | -0.164 |
| Length of caudal peduncle | 0.236 | 0.255 | 0.069 | **0.687** |
| Depth of caudal peduncle | 0.285 | 0.074 | 0.202 | -0.169 |
| Length of posterior barbel | 0.302 | **-0.523** | 0.005 | **0.450** |
| Length of anterior barbel | 0.342 | **-0.600** | -0.370 | -0.132 |

## Figure Captions

**Figure 1**. Live coloration of *Enteromius pinnimaculatus* sp. nov. Uncatalogued specimen from swampy lowland tributary of the Bissina River, Nyanga River drainage, Gabon, 2.208614° S, 12.178365° E

**Figure 2.** Distribution map for *Enteromius pinnimaculatus* sp. nov., illustrating the two known collection localities and nearby localities at which comprehensive sampling did not capture this species.

**Figure 3.** UMMZ 195011, *Enteromius walkeri.* Adult, 58.96 mm SL and juvenile, 31.7 mm SL. Photographs are to scale.

**Figure 4.** OS 20935, *Enteromius camptacanthus*. Adult, tissue voucher GAB17-999, 89.0 mm SL and juvenile, 31.5 mm SL. Photographs are to scale.

**Figure 5.** OS 21879, *Enteromius chiumbeensis*. Adult, tissue voucher GAB17-282, 55.1 mm SL and juvenile, 23.5 mm SL. Photographs are to scale.

**Figure 6.** Boxplot showing median, middle quartiles and range of lateral line scale counts for four species of *Enteromius*. Quartiles calculated with the interpolation option in PAST v3.

**Figure 7.** Scatterplots showing results of principal components analysis of meristic data, color coded by species and with minimum spanning polygons shown. The star marks the holotype of *Enteromius pinnimaculatus* sp. nov., which is the largest measured individual of that species. PC1 (71.4% variance) indexes the number of lateral line and circumpeduncular scales, and PC2 (17.9% variance) indexes primarily the number of vertebrae. Examined specimens of *Enteromius walkeri* all have 34 vertebrae, and vary little in other counts. As such, single points

represent more than one individual of that species, and the species varies very little on the second axis.

**Figure 8.** Standardized major axis regressions of the natural log of the lengths of the anterior and posterior barbels and the caudal peduncle against the natural log of standard length, color coded by species.

**Figure 9**. Scatterplots showing results of principal components analysis of morphometric data, color coded by species and with minimum spanning polygons shown. The star marks the holotype of *Enteromius pinnimaculatus* sp. nov., which is the largest measured individual of that species. Top: PC2 (1.26%) versus PC1(96.11%). Bottom: PC2 (0.50%) versus PC1 (96.11%). PC1 indexes the size of the specimen, PC2 primarily corresponds with the length of the barbels, and PC4 describes primarily the length of the caudal peduncle.

**Figure 10.** Adults and juvenile of *Enteromius pinnimaculatus* sp. nov. A. OS22149, holotype, tissue voucher GAB17-486, 41.4 mm SL. B. OS22153, paratype, 37.6 mm SL. C. OS22152, paratype, 27.0 mm SL, prior to clearing and staining. Photographs are to scale.

**Figure 11.** Left infraorbital series of OS22152, *Enteromius pinnimaculatus* sp. nov.

**Figure 12.** Dorsal fin, supraneurals and pterygiophores of OS22152, *Enteromius pinnimaculatus* sp. nov.

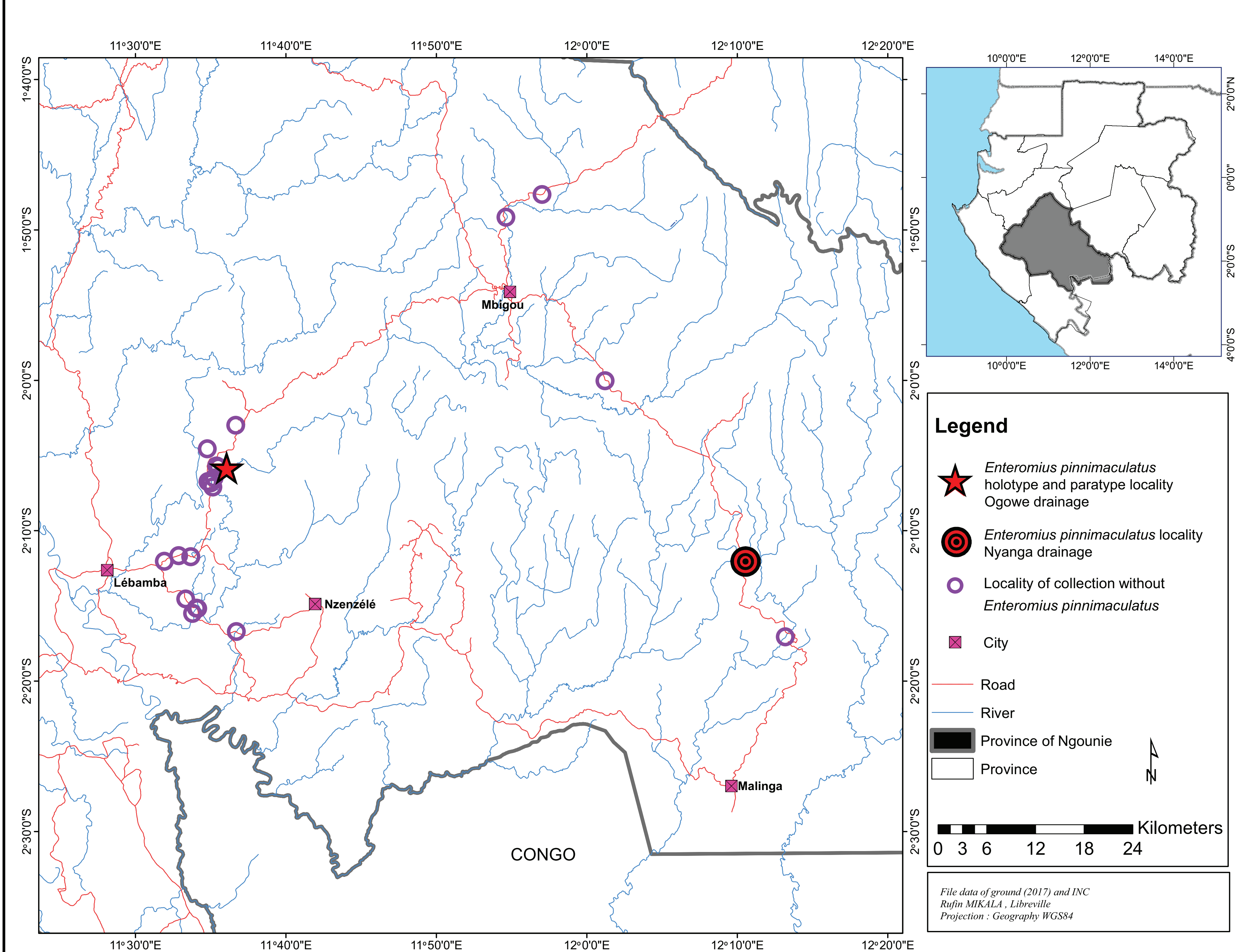
**Figure 13.** Schematic drawing of gasterointestinal tract removed from OS22152, *Enteromius pinnimaculatus* sp. nov. Arrows show direction of food passage.

**Figure 14.** Collection localities for *Enteromius pinnimaculatus sp. nov.* in Gabon, Ngounie Province. **Left:** Type locality, small swampy right bank affluent of the Louetsi River, Ngounie

subdrainage of the Ogowe drainage, upstream from the Chutes de Mioki. 2.09669° S, 11.60085° E. **Right:** swampy lowland tributary of the Bibaka River, Bissina subdrainage of the Nyanga drainage. 2.208614° S, 12.178365° E

Figure 1 - live photo

Figure 2 - map



11°30'0"E
11°40'0"E
11°50'0"E
12°0'0"E
12°10'0"E
12°20'0"E
1°40'0"S
1°50'0"S
2°0'0"S
2°10'0"S
2°20'0"S
2°30'0"S
Mbigou
Lébamba
Nzenzélé
Malinga
CONGO
10°0'0"E
12°0'0"E
14°0'0"E
2°0'0"N
0°0'0"
2°0'0"S
4°0'0"S
Legend
Enteromius pinnimaculatus holotype and paratype locality Ogowe drainage
Enteromius pinnimaculatus locality Nyanga drainage
Locality of collection without Enteromius pinnimaculatus
City
Road
River
Province of Ngounie
Province
N
0 3 6 12 18 24
Kilometers
File data of ground (2017) and INC
Rufin MIKALA , Libreville
Projection : Geography WGS84

Figure 3 - Enteromius walkeri

Figure 4- Enteromius camptacanthus

Figure 5 - Enteromius chiumbeensis

Figure 6 - boxplot

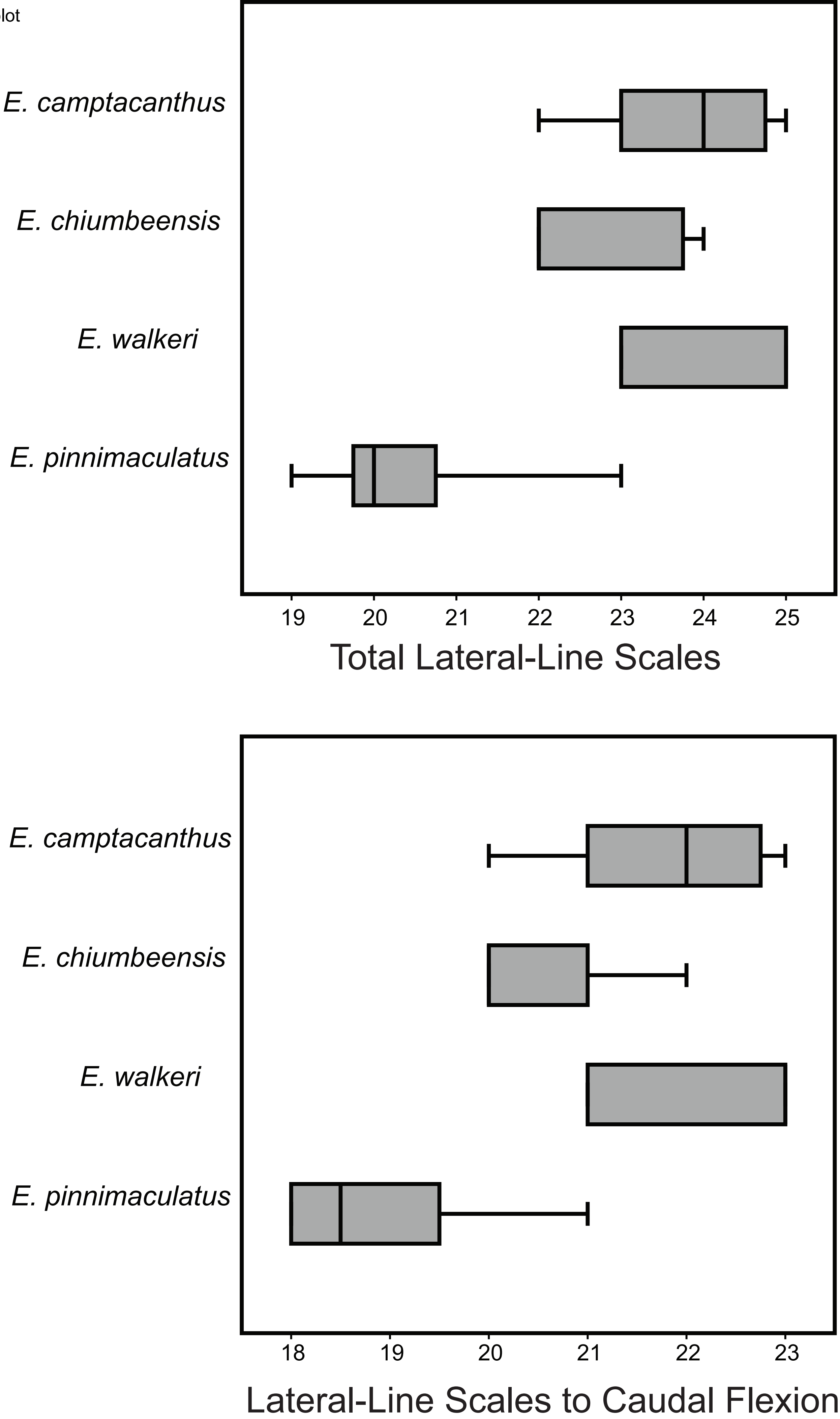


Figure 7 - Meristic PCA

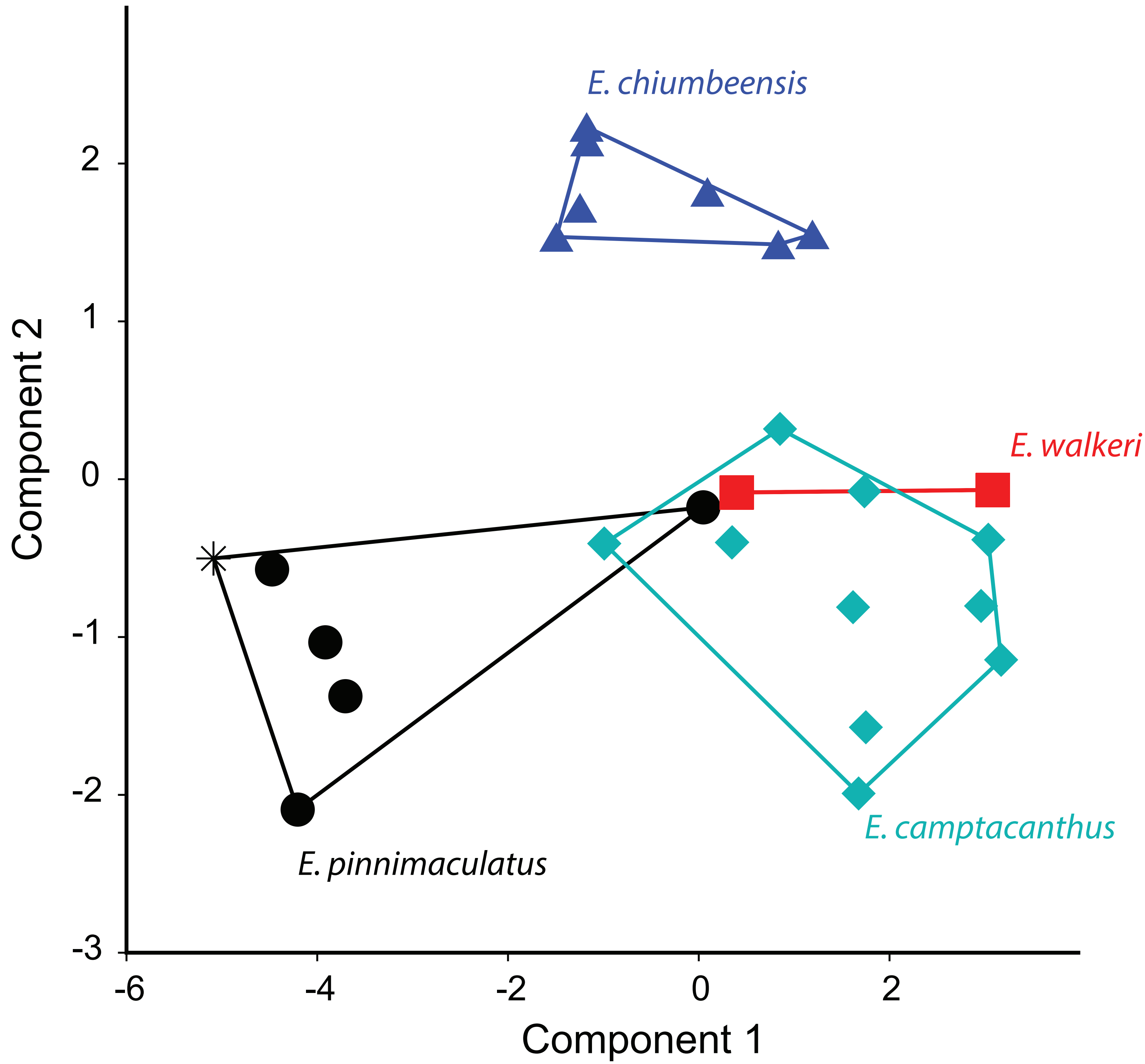


Figure 8 - Allometry

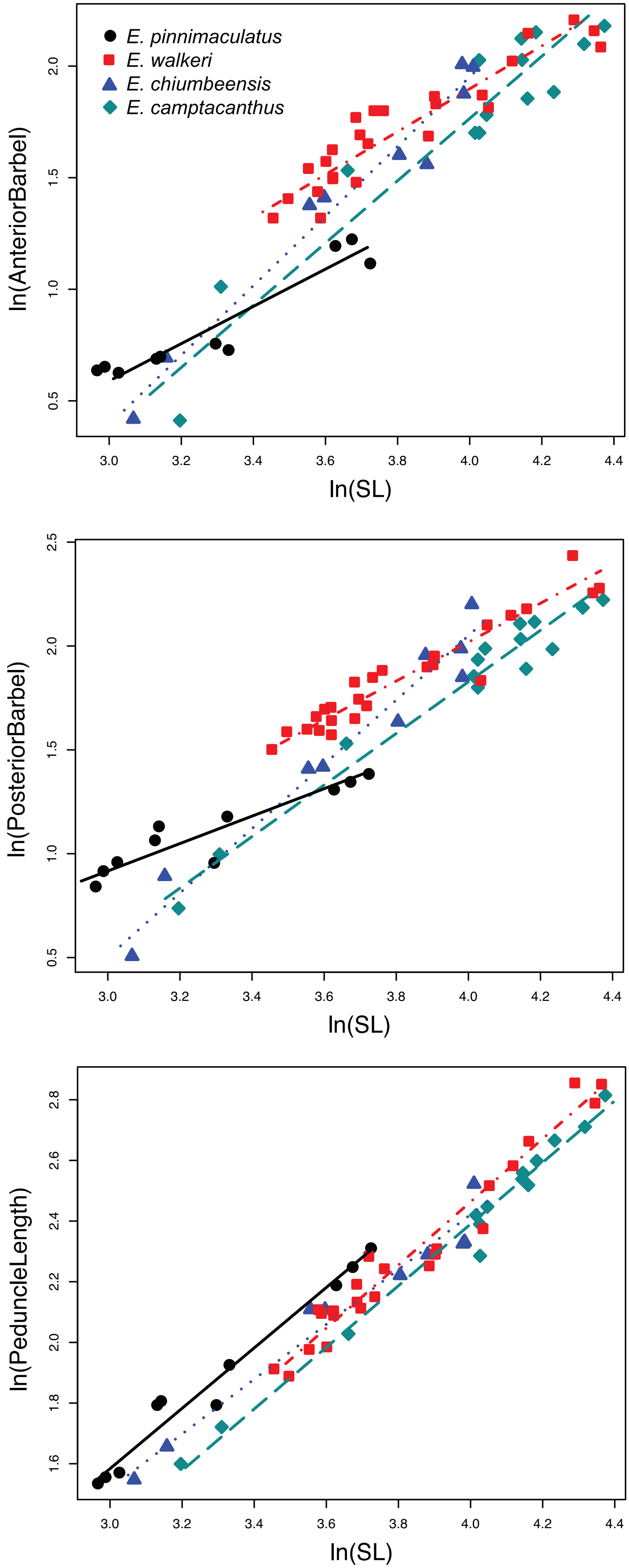


Figure 9 - Morphometrics PCA

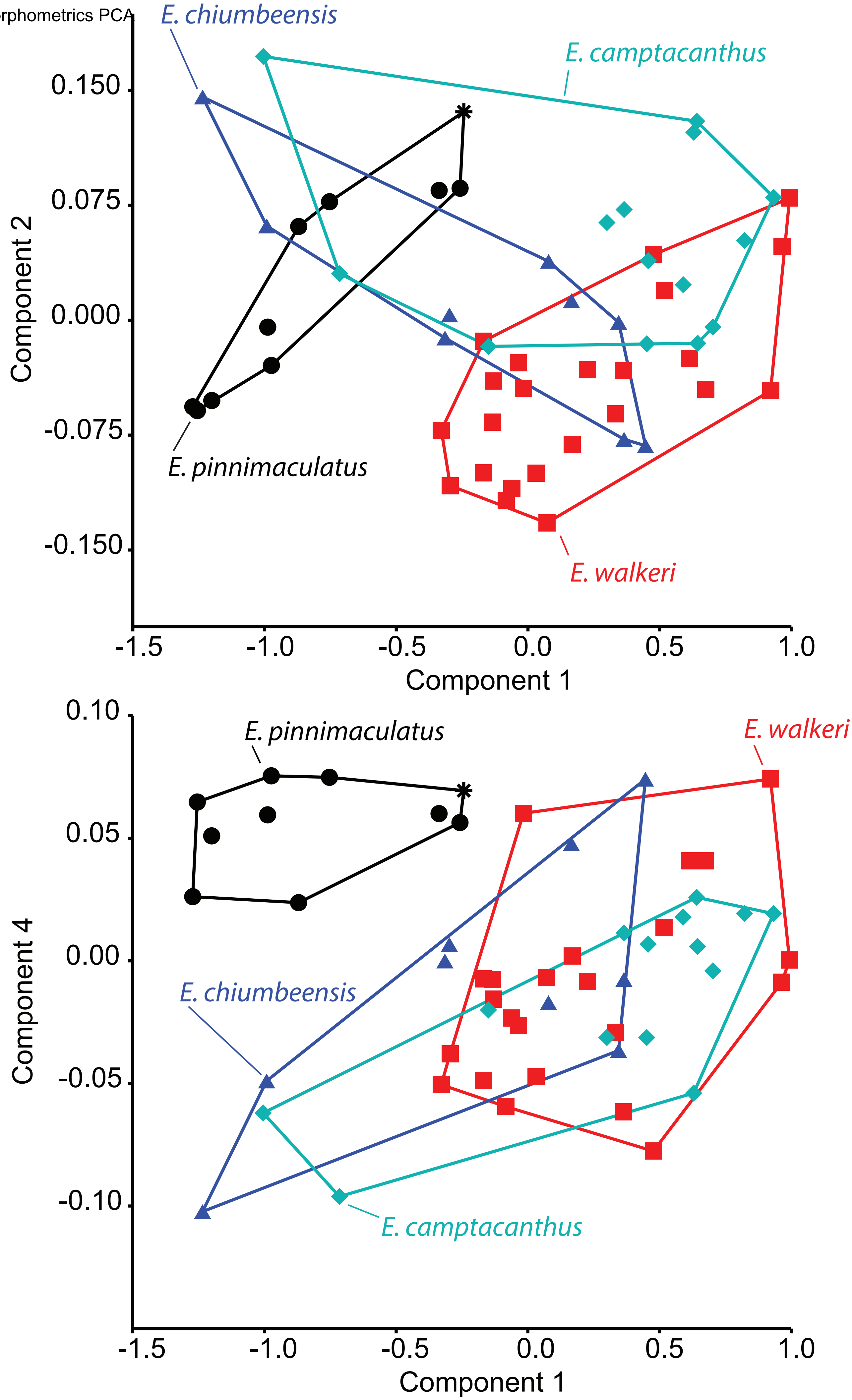


Figure 10 - Enteromius pinnimaculatus

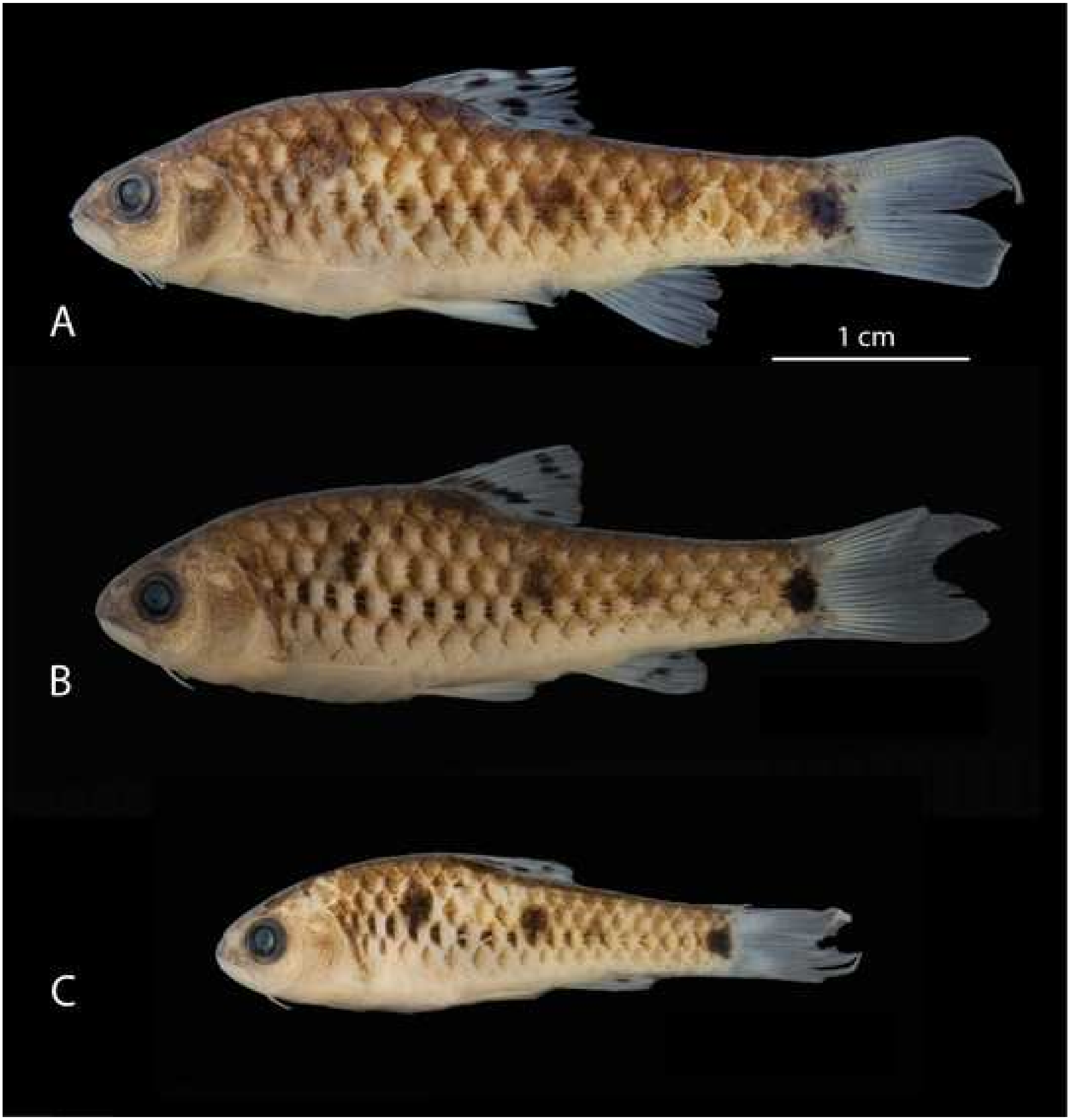


Figure 11 - infraorbitals

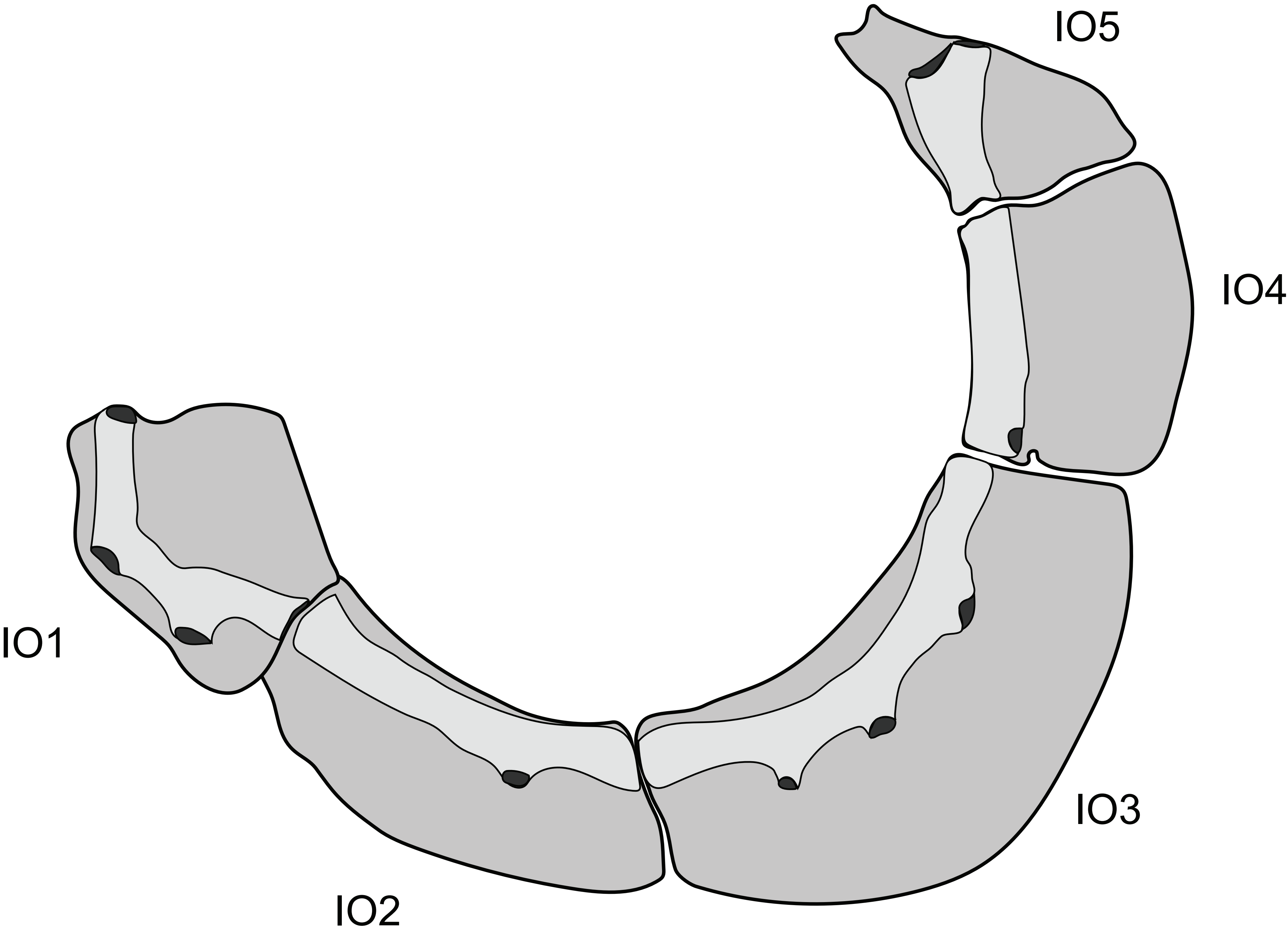


Figure 12 - dorsal fin

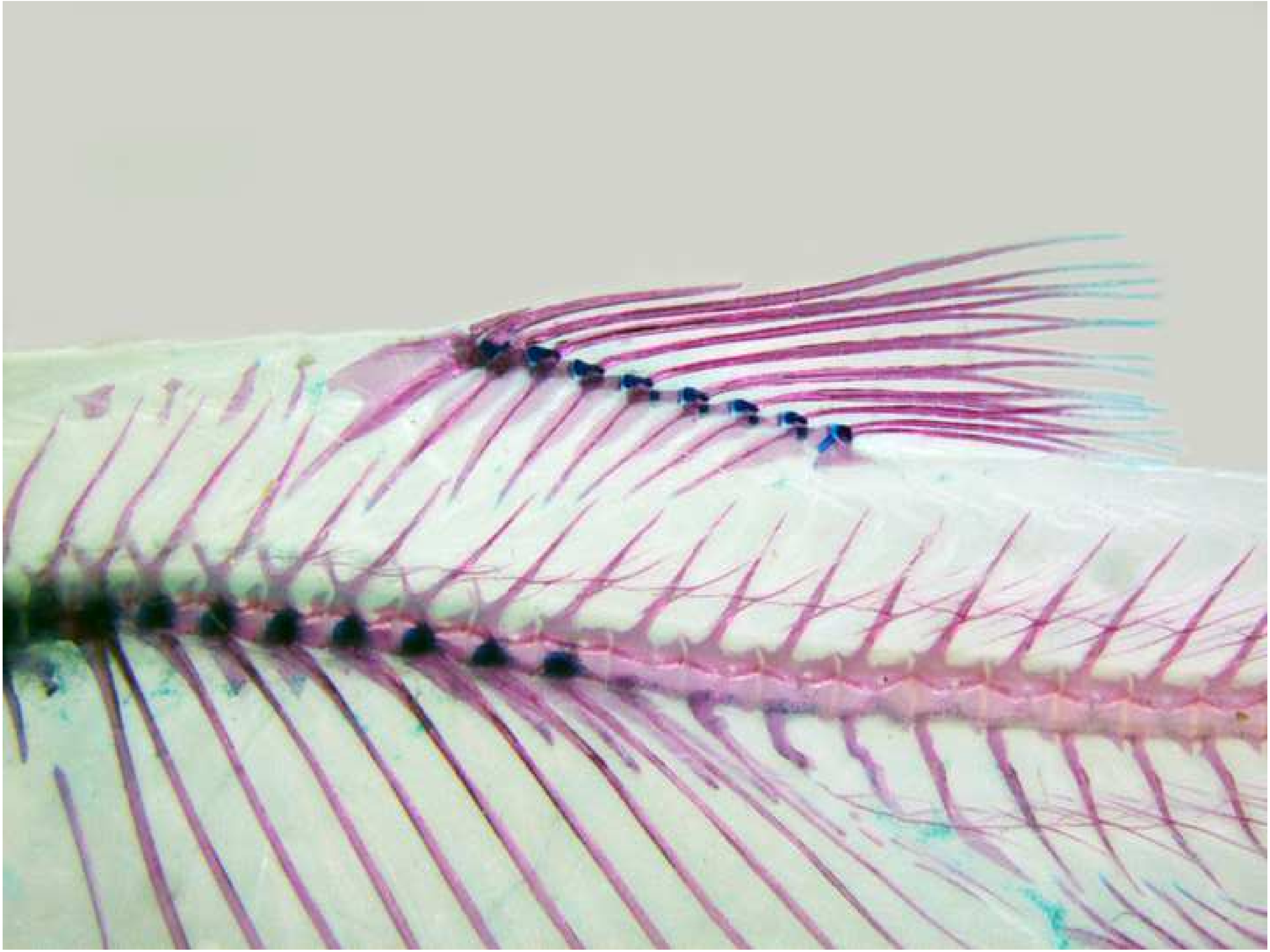


Figure 13 - intestine

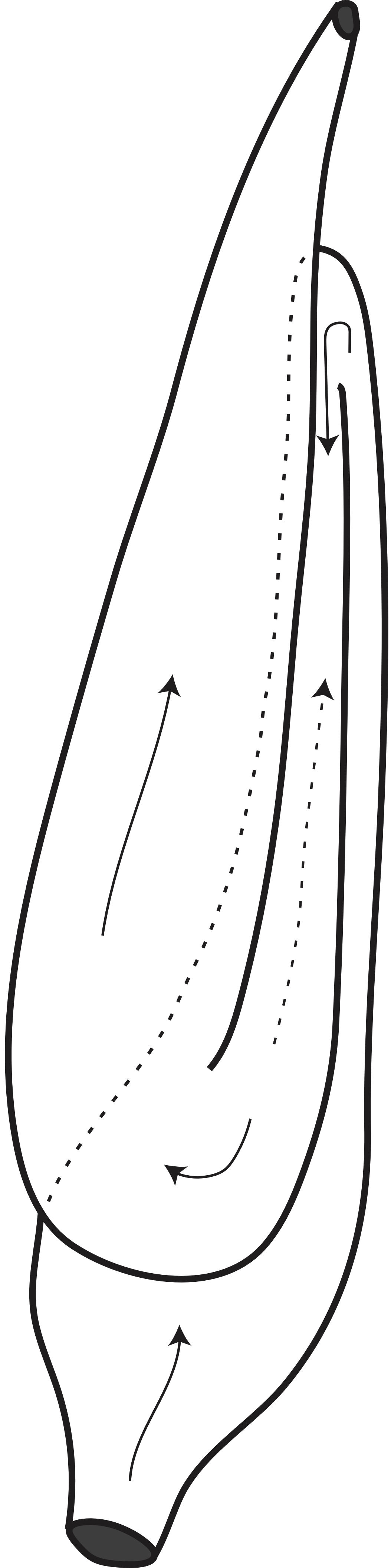


Figure 14 - Site photos