Vertebrate Anatomy Morphology Palaeontology 11:101–112
ISSN 2292-1389

Hemigrammopetersius barnardi (Teleostei: Characiformes: Alestidae): a study of skeletal ontogeny and identification of homology for phylogenetic analysis

Meghan E. Dueck* and Alison M. Murray
Department of Biological Sciences, University of Alberta, Edmonton, Alberta, T6G 2E9, Canada; medueck@ualberta.ca; ammurray@ualberta.ca

Abstract: Members of Alestidae, a family of characiforms found in sub–Saharan Africa, have a wide range of adult body sizes. Because of this range of adult size, phylogenetic characters for the group may be difficult to distinguish from ontogenetic features, resulting in groups being united based on body size rather than evolutionary relatedness. Although previous studies have presented the morphology and osteology of some small and miniature taxa, these were pre-cladistic and did not attempt to distinguish between phylogenetic and ontogenetic features. Here we provide a study on the external morphology and osteology of a small alestid, *Hemigrammopetersius barnardi* which has reductions and losses of osteological features. We compared this species to juveniles of *Alestes dentex*, an alestid that attains much larger adult size, to identify characters in *H. barnardi* that potentially result from a decrease in body size rather than shared ancestry. We found that the loss of particular bones of the circumorbital series and postcranium, as well as a reduction of the sensory canal system, are likely the result of small body size, and therefore are not useful in establishing phylogenetic relationships among alestids.

INTRODUCTION

Of the four characiform families found in African fresh waters, Alestidae is the largest with over 100 valid species (Oliveira et al. 2011; Fricke et al. 2023). These are distributed throughout the lowland rivers in sub–Saharan Africa (Roberts 1975; Zanata and Vari 2005; Arroyave and Stiassny 2011). The family is considered to be monophyletic (e.g., Orti and Meyer 1997; Murray and Stewart 2002; Arroyave and Stiassny 2011); however, relationships among the constituent members remain unclear. Previous classifications of tribes and subfamilies based on dentition and body size were created prior to the prevalence of cladistics techniques, and resulted in polyphyletic groupings (Murray and Stewart 2002; Calcagnotto et al. 2005; Arroyave and Stiassny 2011). Intrafamilial relationships among alestids remain unresolved (e.g., Hubert et al. 2005; Zanata and Vari 2005). One aspect of alestid diversity that may contribute to the difficulty in establishing a natural classification is the great disparity in body size within the family. Alestids range in size from giants such as *Hydrocynus goliath* Boulenger, 1898, which reaches a standard length (SL) of 1300 mm (Hubert et al. 2005), to miniature species such as *Lepidarchus adonis* Roberts, 1966 (maximum 21 mm SL). The term 'miniature' when applied to fishes was arbitrarily defined by Weitzman and Vari (1988) as those species that do not exceed an adult size of 26 mm SL. These authors also noted that these species will exhibit the reduction and loss of morphological features. Following this definition, Alestidae contains three miniatures: *Lepidarchus adonis*, *Micralestes pabrensis* (Roman, 1966), and *Bathyaethiops baka* Moritz and Schliewan, 2016. In addition, other small alestid species exhibit similar losses and reductions of features despite having an adult size exceeding 26 mm SL; these include *Rhabdalestes rhodesiensis* (Ricardo-Bertram, 1943), with a maximum SL of 57 mm, and *Micralestes acutidens* (Peters, 1852), with a maximum SL of 90 mm, both of which have lost the circumorbital bones.

Previously, a number of alestid species of small size were grouped together in a single taxon, 'Petersiini' (e.g., Poll 1967; Géry 1995). However, more recently these size differences have been hypothesized to result from multiple independent miniaturization events within family (e.g., Hubert et al. 2005; Zanata and Vari 2005). If this latter hypothesis is correct, then the features previously used to

---

Published January 22, 2024
*corresponding author, © 2024 by the authors; submitted July 5, 2023; revisions received December 1, 2023; accepted December 21, 2023. Handling editor: Robert Holmes. DOI 10.18435/vamp29393

unite small alestids are likely related to small size (i.e., the byproduct of truncated ontogeny) and do not represent homology indicative of phylogenetic relationship. Since these ontogenetic characters are likely to be homoplastic, and therefore do not reflect phylogenetic relationships, they should be excluded from phylogenetic analyses. In this study, our goal is to distinguish the features of small alestids that are the result of ontogeny rather than shared evolutionary history. We do this by comparing small individuals of two representative species (Fig. 1), one species with a small maximum adult length, *Hemigrammopeterisus barnardi* (Herre, 1936), and a second with a much larger maximum length, *Alestes dentex* (Linnaeus, 1758).

The genus *Hemigrammopetersius* was erected by Pellegrin (1928) to include all those species previously included in *Petersius* which have an incomplete lateral line and an absence of inner dentary symphyseal teeth. In other words, these species were united in *Hemigrammopetersius* based on features that are potentially only evident because of the small maximum size of this species. *Hemigrammopeterisus barnardi* (Herre, 1936) has a reported maximum SL of 70 mm (Eccles 1992). Although not a miniature following the definition of Weitzman and Vari (1988), this species exhibits morphological reductions and bone losses commonly reported in miniature fishes, such as the loss of the supraorbital and third postcleithrum, and a reduced complexity of the sensory canal system (Zanata and Vari 2005). *Alestes dentex* (Linnaeus, 1758), on the other hand, is representative of the median size of species in the family, intermediate between the miniatures and giants. The largest adult SL reported for *Alestes dentex* is 410 mm (Paugy in Paugy et al. 2003). Similar studies, in which the osteology of a miniature fish species was compared to that of juveniles of a related species that attain a larger body size, have allowed researchers to identify features as potential synapomorphies because these are the features that are present during earlier developmental stages of larger relatives (e.g., Johnson and Brothers 1993; Britz and Conway 2009; Franz-Odendaal and Adriaens 2014).

Here we provide an osteological description of *Hemigrammopetersius barnardi* as a baseline for the comparison of alestid species. We then compare the osteology of adult specimens of *H. barnardi* with juveniles of *Alestes dentex*. This will allow us to identify characters that are the result of small size, and therefore not indicative of evolutionary relationships.

MATERIALS AND METHODS

Comparative Materials Examined

Alestes dentex: United States National Museum Smithsonian Institution, Washington DC, USA (USNM) 229863, three specimens cleared and stained (c&s), Sokoto-Rima Floodplain, Sokoto, Nigeria, 43.2–62.7 mm SL.

Hemigrammopetersius barnardi: Canadian Museum of Nature Fish Collection, Ottawa, ON, Canada (CMN F) 81–0188, 157 alcohol preserved, four c&s, Rufiji River, west end of Lake Ruwe, Tanzania, 19.2–34.9 mm SL.

Methods

Counts and measurements follow the methodology of Lunkayilakio and Vreven (2008). Transverse scale counts start

Figure 1. Cleared and stained whole-body specimens of Alestidae. Top, *Hemigrammopetersius barnardi* CMN F 81-0188; bottom, *Alestes dentex* USNM 229863.

at the dorsal fin origin. Measurements were made using a Nikon SMZ1000 stereomicroscope using a WD 123 Nikon Plan Apo 0.5x lens and a C –W 10xB/22 eyepiece reticle. Radiographs for vertebral counts were made using a Bruker micro CT Skyscan 1172 (no filter, 50 kV, and 201 µA).

Specimens were cleared and counterstained for bone and cartilage according to the procedure of Taylor and Van Dyke (1985). Photographs were taken using a Nikon DXM 1200C digital camera mounted on a Zeiss Stereo DiscoveryV8 stereomicroscope with a Carl Zeiss 44403 6

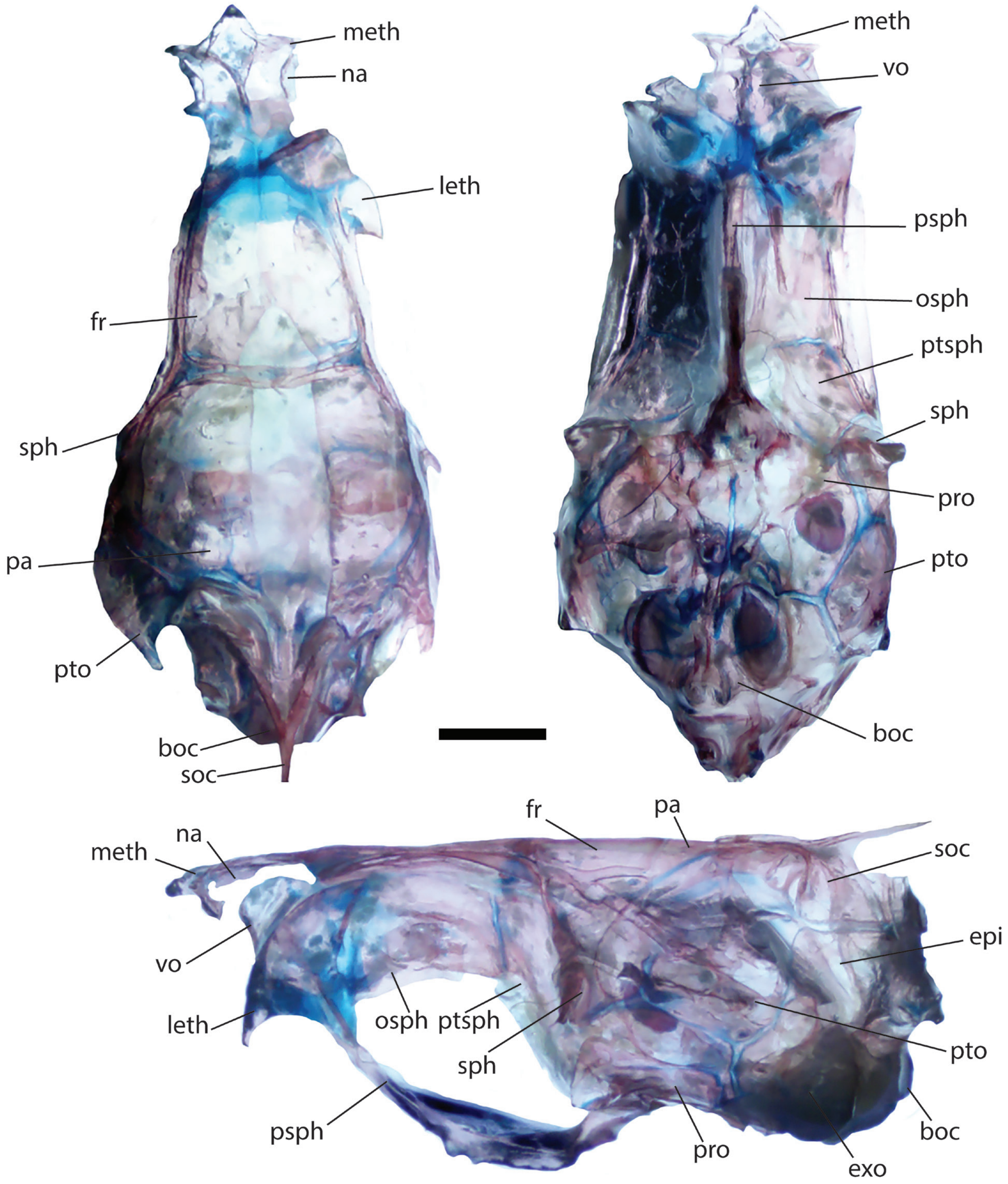


Figure 2. Photographs of the adult skull of *Hemigrammopetersius barnardi* CMN F 81-0188, specimen number 106, SL = 31.2 mm, in A, dorsal; B, ventral; and C, lateral views. Scale bar = 1 mm. Abbreviations: boc, basioccipital; epi, epioccipital; exo, exoccipital; fr, frontal; leth, lateral ethmoid; meth, mesethmoid; na, nasal; osph, orbitosphenoid; pa, parietal; pro, prootic; psph, parasphenoid; pto, pterotic; ptsph, pterosphenoid; soc, supraoccipital; sph, sphenotic.

–9000 eyepiece (8x) and a variety of lenses (Zeiss Achromat S 0.3x FWD 236 mm, Zeiss Plan Apo S 0.63x FWD 81 mm, and Zeiss Plan Apo S 1.0x FWD 60 mm), with NIS –Elements F package 2.20, version 5.03. Figures were prepared using Adobe Photoshop CS6. Osteological terminology follows Murray (2004), with the exception of endopterygoid instead of mesopterygoid and pubic processes from Prokofiev (2001).

Sexual maturity of individuals was assigned based on completion of ossification and size relative to maximum reported length. The smallest examined specimen of *A. dentex* (SL = 43.2 mm) is not fully ossified and the maximal length for the species (410 mm; Paugy in Paugy et al. 2003), is greater than six times larger than the examined material. Therefore, these are considered to be juveniles. In contrast, examined specimens of *H. barnardi* are considered to be adults because no cartilage persists along the vertebrae and ribs, and the maximum reported size of this species (70 mm, Eccles 1992) is only 2.0–3.6 times larger than the examined material.

OSTEOLOGY OF *HEMIGRAMMOPETERSIUS BARNARDI*

Skull

The mesethmoid has a rounded anteromedial tip in dorsal view projecting anteriorly past the pointed lateral projections (Fig. 2). Posteriorly, this bone narrows to a projection separating the left and right frontals, flanked by pointed posterolateral projections. The nasals are small, concave laterally. The lateral ethmoid has a rounded posterior edge which projects laterally.

The frontals narrow to a rounded tip anteriorly on either side of the mesethmoid. Above the orbit, they meet one another in a sinuous suture anterior to the large cranial fontanelle. The fontanelle separates the posterior parts of the frontals as well as the left and right parietals, and forms a notch in the anterior edge of the supraoccipital. A narrow epiphyseal bar divides the fontanelle between the frontals anterior to the level of the sphenotics. The triangular to rectangular parietals are less than one quarter the length of the frontals, and have curved posterior edges. The supraoccipital crest is narrow and extends posteriorly past the first vertebra to reach the level of the anterior edge of the second vertebra. The large, ovoid epioccipitals are perforated by a large foramen. The exoccipitals together with the basioccipital form the large lagenar capsule.

The short, wide vomer has a short posterior projection that ends in a pointed tip articulating with the parasphenoid. The parasphenoid bows deeply ventrally in lateral view. It bears a pair of small lateral projections which contact the prootics, and is bifurcated posteriorly where it meets the basioccipital. In ventral view, the orbitosphenoid is visible on either side of the parasphenoid; it is roughly rectangular and longer than the rhomboidal pterosphenoids. The ventrolaterally projections of the sphenotics behind the orbit are rounded. The pterotics each bear a prominent lateral projection. The prootics are relatively large, roughly rectangular to oval in shape and have an anterior ridge on the ventral surface.

Cranial Sensory Canals

The supraorbital sensory canal extends the entire length of each frontal; there are five pore openings, the anterior two opening anteriorly, and anteromedially, the third opening medially towards its counterpart on the other frontal at the level of the epiphyseal bar, and two posterior pores, one opening towards the parietal and the other towards the pterotic. The parietal branch of the supraorbital canal continues across the anterior portion of the parietal and the supratemporal sensory canal is positioned on the posterior edge of the parietal. Anteriorly, the sensory canals continue onto the nasals.

Jaws and Teeth

The premaxilla (Fig. 3) has a short triangular ascending process and bears an elongate ledge on the posteroventral

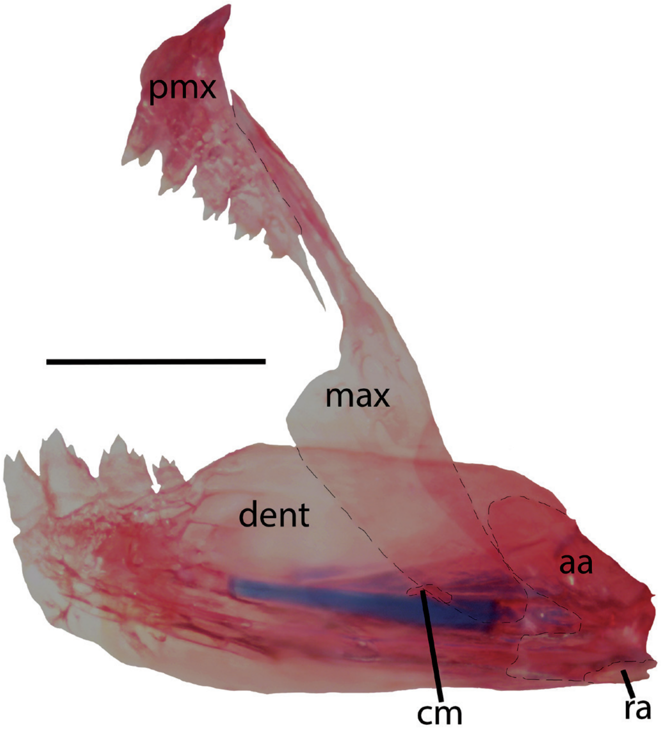


Figure 3. Photograph of the left jaw in lateral view of *Hemigrammopetersius barnardi* CMN F 81-0188, specimen number 106, SL = 31.2 mm. Dashed lines indicate the border of respective bone. Scale bar = 1.3 mm. Abbreviations: aa, anguloarticular, cm = coronomeckelian; dent, dentary; max, maxilla; pmx, premaxilla; ra, retroarticular.

portion (the premaxillary pedicle). There is no evidence of an interdigitating suture between left and right premaxillae. The premaxillary teeth are multicuspid (three or more cusps); there are four inner and three outer teeth on each bone. The hatchet-shaped, edentulous maxilla is narrow anteriorly with a large oval posterior portion.

There is an interdigitating suture between the left and right dentaries. Each dentary bears a single row of four multicuspid teeth; the teeth decrease substantially in size posteriorly. Each tooth is underlain by a tooth crypt containing a replacement tooth. There are no inner dentary symphyseal teeth. The long, cylindrical Meckel's cartilage is positioned on the medial surface of the dentary just dorsal to the level of the mandibular sensory canal. The small, oblong coronomeckelian is positioned on the medial side of the dentary dorsal to Meckel's cartilage. The anguloarticular has a rounded dorsal expansion but is much smaller than the dentary. The small, oval retroarticular is confined to the posteroventral region of the mandible. The mandibular sensory canal is positioned ventrally on the dentary and anguloarticular.

Opercular Bones

The two limbs of the preopercle form a right angle; both are of equal length and bear an enclosed sensory canal (Fig. 4). There is a sensory canal pore at the posteroventral corner of the preopercle. The triangular interopercle narrows anteriorly; it extends past the posterior edge of the preopercle to underlap the anterior edge of the subopercle. The slender subopercle extends under the posteroventral edge of the opercle. The roughly semi –circular opercle has a straight anterior edge and convex posterior edge.

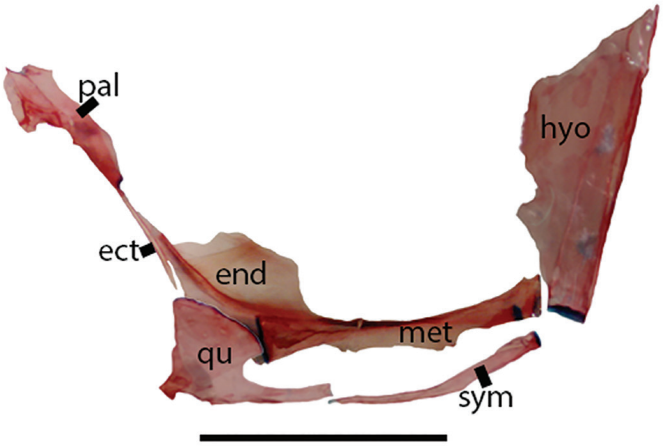


Figure 5. Photograph of the left suspensorium of *Hemigrammopetersius barnardi* CMN F 81-0188, specimen number 106, 31.2 mm SL in lateral view. Scale bar = 1 mm. Abbreviations: ect, ectopterygoid; end, endopterygoid; hyo, hyomandibula; met, metapterygoid; pal, palatine; qu, quadrate; sym, symplectic.

Suspensorium

The palatine is formed of an anterior plate of bone that narrows posteriorly (Fig. 5). The slender ectopterygoid curves slightly ventrally to lie along the narrow anterior projection of the endopterygoid. This latter has a strong curved ridge with a rectangular plate dorsally. The elongate metapterygoid stretches between the quadrate and hyoman-

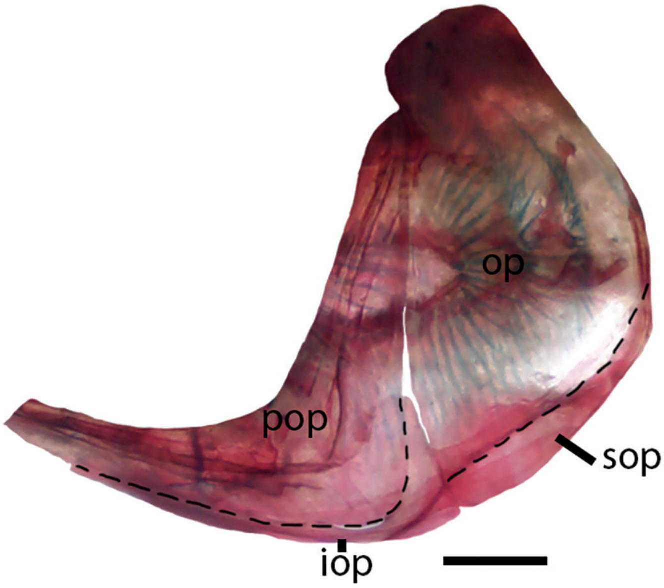


Figure 4. Photograph of the left opercular series of *Hemigrammopetersius barnardi* CMN F 81-0188, specimen number 2, 38.6 mm SL. Scale bar = 1 mm. Dashed lines indicate the border of respective bone. Abbreviations: iop, interopercular; op, opercle; pop, preopercle; sop, subopercle.

Figure 6. Photograph of the left infraorbital series of *Hemigrammopetersius barnardi* CMN F 81-0188, specimen number 2, 38.6 mm SL. Scale bar = 1 mm. Dashed lined indicate sensory canals. Abbreviations: ao, antorbital; dsph, dermosphenotic; io1-5, infraorbitals 1-5.

dibula. The anterior and ventral edges of the triangular body of the quadrate form a 90° angle, and the ventral edge projects to reach the symplectic. The symplectic is an elongate, slightly curved bone, tapering towards the quadrate. The hyomandibula bears a poorly –formed anterior flange.

Infraorbital Series

The infraorbital series (Fig. 6) consists of seven bones: the antorbital, lacrimal (infraorbital 1), infraorbitals 2–5 and the dermosphenotic (infraorbital 6). There is no supraorbital. The circumorbital sensory canal is present on all infraorbitals except the antorbital; the canal opens in a pore

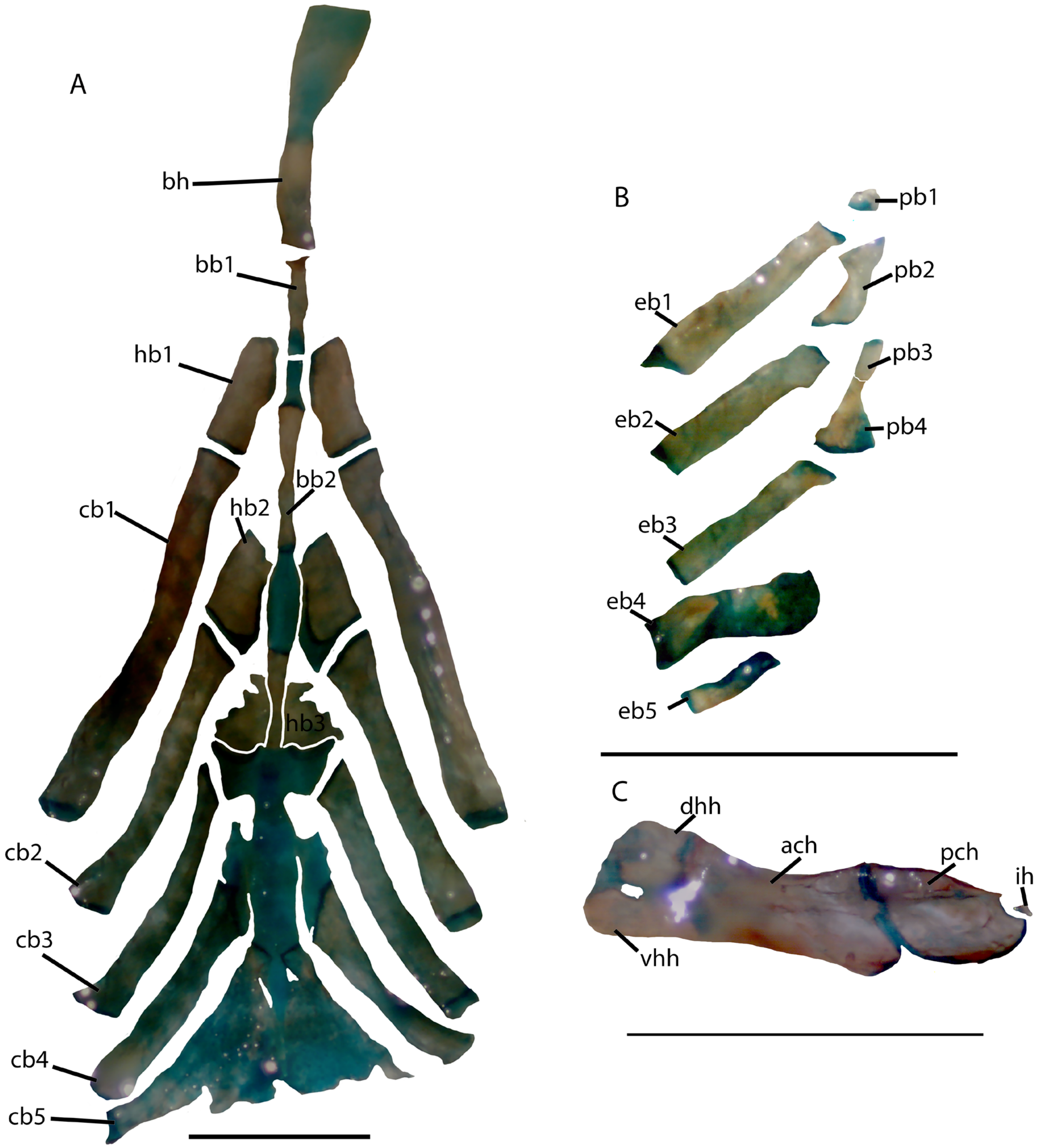


Figure 7. Photographs of the branchial arches and pharyngeal region of *Hemigrammopetersius barnardi* CMN F 81-0188, specimen number 106, 31.2 mm SL. A, lower pharyngeal bones in dorsal view; B, upper pharyngeal bones in ventral view; C, left ceratohyal in lateral view. Scale bar = 1 mm. Abbreviations: ach, anterior ceratohyal; bb1-3, basibranchials 1-3; bh, basihyal; brst, branchiostegal rays; cb1-5, ceratobranchials; dhh, dorsal hypohyals; eb1-5, epibranchials 1-5; hb1-2, hypobranchials 1-2; in, interhyal; pb 1-4, pharyngobranchials 1-4; pch, posterior ceratohyal; vhh, ventral hypohyals.

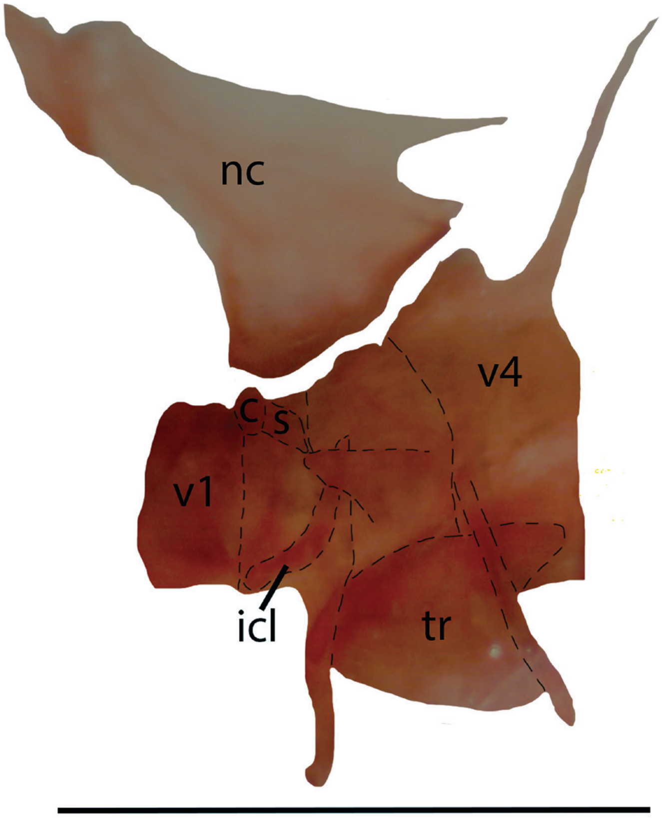


Figure 8. Photograph of the Weberian apparatus of *Hemigrammopetersius barnardi* CMN F 81-0188, specimen number 3, 26.2 mm SL, in left lateral view. Scale bar = 1 mm. Abbreviations: c, claustrum; icl, intercalarium; nc, neural complex; s, scaphium; tr, tripus; v1, vertebra 1; v4, vertebra 4.

at either end on each infraorbital bone, with no additional pores. Unlike most characiforms, the sensory canal on the dermosphenotic is not tripartite.

Branchial Arches and Pharyngeal Region

The basihyal is a large element, twice as long and much thicker than the first basibranchial (Fig. 7). Ceratobranchial 1 is a long, slender rod; ceratobranchials 2–4 become progressively shorter. The fifth ceratobranchial expands to form an anteromedial plate, which bears small conical teeth on its dorsal surface. There are five epibranchials and four pharyngobranchials. The first three epibranchials are long, rod –like bones and the fourth and fifth epibranchials are small, rectangular elements. All are capped with cartilage. The pharyngobranchials are all small and irregularly shaped with cartilaginous tips connected medially to the epibranchials.

The dorsal and ventral hypohyals curve so that they have a small opening between them, visible in lateral view. The anterior ceratohyal is concave dorsally and widens slightly posteriorly. A broad ridge on the posterior half of the lateral surface continues onto the posterior ceratohyal. The very small interhyal is triangular. There are four long, curved branchiostegal rays on each side; the first is the shortest. The anterior three branchiostegal rays contact the anterior ceratohyal and the fourth ray contacts the posterior ceratohyal. The urohyal is narrow anteriorly and expands posteriorly.

Weberian Apparatus and Vertebral Column

The neural complex of the Weberian apparatus (Fig. 8) bears a pointed posterior process dorsal to a concavity close to the base of the bone. The small scaphium is slightly larger than the oval claustrum. The intercalarium is long and gently curved. The transverse process on vertebra 3 extends anteriorly to overlap both the intercalarium and posteroventral tip of the scaphium. The tripus is rounded with a posterior concavity.

There are 34–36 centra in total, or which 15–17 are abdominal, including the four centra of the Weberian apparatus, and 18–20 are caudal (Tab. 1). In specimens that have been cleared and stained (n = 4), the presence of two transitional centra (following the terminology of Brewster 1986) can be confirmed; however, these are difficult to identify in radiographs and therefore transitional centra are here included in the abdominal counts. Each rib bears a dorsal flange where it articulates with the centrum.

Intermuscular bones are present in two series; the epineural series begins at centrum 9 or 10 and continue to preural centrum 5. The epiplural series is confined to the haemal spines on the anterior caudal centra and does not continue posterior to the fifth preural centrum; there 13–15 bones in the series.

Table 1. Counts for *Hemigrammopetersius barnardi*, CMN F 81-0188, from the Rufiji River basin (n = 158). The number of specimens assigned to each count is shown in brackets after the meristic.

	Sample Size					
Dorsal-fin rays	157	9 (25)	10 (132)			
Anal-fin rays	158	18 (3)	19 (64)	20 (21)	21 (70)	
Abdominal vertebrae	157	15 (28)	16 (117)	17 (12)		
Caudal vertebrae	157	18 (27)	19 (108)	20 (22)		
Total vertebrae	157	34 (36)	35 (106)	36 (15)		
Lateral line scales	120	8 (10)	9 (32)	10 (36)	11 (25)	12 (17)
Transverse scales	63	7 (19)	8 (37)	9 (7)		
Gill rakers	157	9 (25)	10 (132)			

Paired Fins and Girdles

The posttemporal has a narrow dorsal projection and lies dorsomedial to the oval extrascapular (Fig. 9). The supratemporal sensory canal extends the length of the extrascapular, crosses the posteroventral corner of the posttemporal and continues along the length of the elongate supracleithrum. The cleithrum narrows dorsally compared to the much wider anteroventral portion. The large coracoid bears an anterior concavity that forms a large opening where it meets the cleithrum. The T –shaped scapula is about half as long as the narrow mesocoracoid. This latter bone widens at its ventral end. There are four hourglass shaped radials, two on the scapula, and two on the coracoid, supporting one unbranched and eight or nine branched pectoral fin rays. There are two small, oval postcleithra; the first postcleithrum is positioned at the posteroventral base of the supracleithrum and the second postcleithrum is positioned medial to the posterior –most part of the cleithrum.

The long, narrow left and right pelvic bones narrow slightly anteriorly. The ischiac processes on the posterior edges contact each other medially at their anterior extents (Fig. 9). The pelvic fins each contain one unbranched and seven or eight branched fin rays, as well as a lateral pelvic splint.

Dorsal and Anal Fins and Supports

The rounded dorsal fin has two unbranched and eight or nine branched rays. The first unbranched dorsal –fin ray is approximately half the length of the second, which is the longest. The remaining rays progressively decrease in size.

The anal fin is larger than the dorsal fin and moderately falcate. It is positioned posteriorly on the body and has three unbranched rays and 15–18 branched rays. The first unbranched ray is small and just visible in unstained specimens under the scale covering; the remaining two unbranched rays are longer. The first branched anal –fin ray is the longest with the remaining 14–17 branched fin rays decreasing in size posteriorly.

Caudal Fin and Skeleton

The forked caudal fin is approximately one quarter of the total length of the fish, and has a fin ray formula of I,9,9,I. The compound centrum (u1+pu1) bears a urostyle which extends dorsally and posteriorly to form a concavity to accommodate the three epurals (Fig. 10). The posterior projection reaches the fin rays and lies dorsal to the long, thin uroneural. There are six hypurals, of which the triangular hypural 1 is the largest and hypurals 2 through 6 are more rectangular and decrease in size. There is a diastema between the first ventral (1 and 2) and dorsal (3–6) hypurals. None of the proximal tips of the hypurals reach the compound centrum. The parhypural and haemal spine of the second preural centrum have a small proximal flange on their anterior edges.

DISCUSSION

Morphological Variation in *Hemigrammopetersius barnardi*

Some species of miniature fishes have a higher

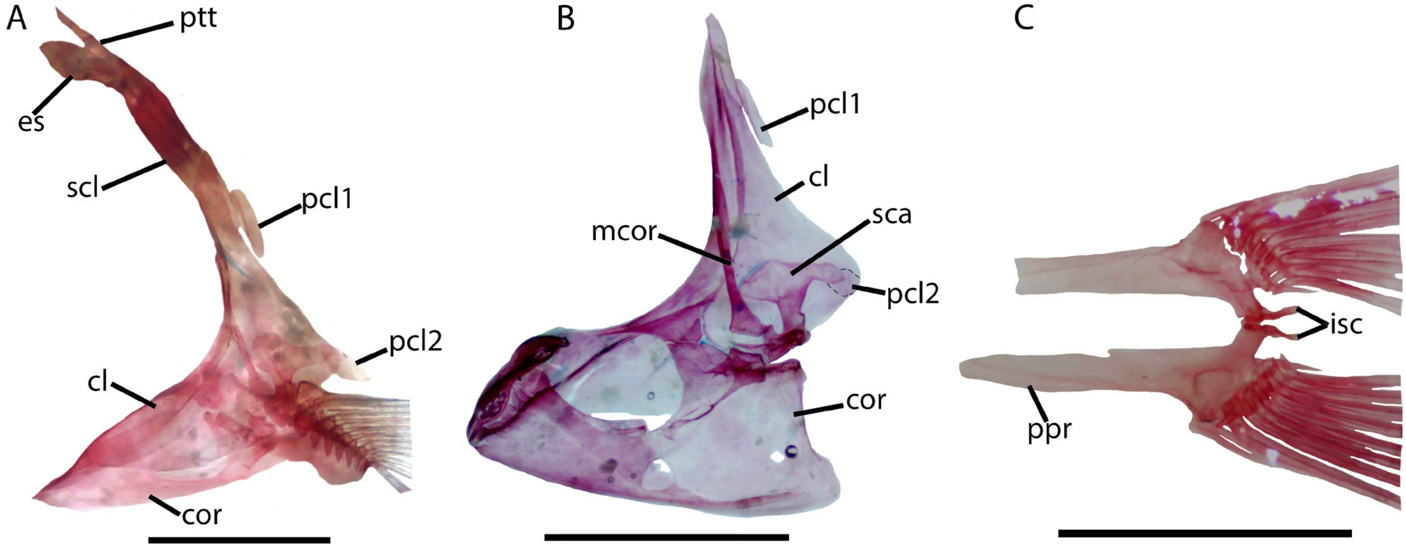


Figure 9. Photographs of the pectoral and pelvic girdle of *Hemigrammopetersius barnardi* CMN F 81-0188. A, left pectoral girdle in lateral view, specimen number 2, 38.6 mm SL; B, right pectoral girdle (supracleithrum, posttemporal and extrascapular removed) in oblique medial view, specimen number 106, 31.2 mm SL; C, pelvic girdle in ventral view, specimen number 106, 31.2 mm SL. Scale bar = 0.5 mm. Abbreviations: cl, cleithrum; cor, coracoid; es, extrascapular; isc, ischiac process of the pelvic girdle; mcor, mesocoracoid; pcl1-2 postcleithra 1-2; ppr, pubic process of the pelvic girdle; ptt, posttemporal; sca, scapula; scl, supracleithrum.

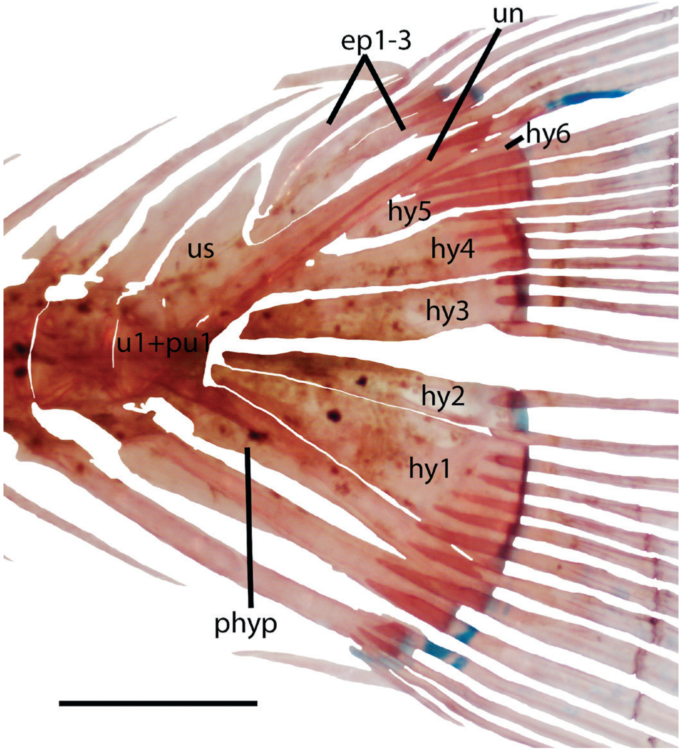


Figure 10. Photograph of the caudal fin of *Hemigrammopetersius barnardi* CMN F 81-0188, specimen number 4, 32.7 mm SL. Scale bar = 1 mm. Abbreviations: ep 1-3, epurals 1-3; hy 1-6; hypurals 1-6; phyp, parhypural; pu1, preural centrum 1; u1, ural centrum; un, uroneural; us, urostyle.

amount of variation in the population that expected in non-miniatures (e.g., Hanken and Wake 1993; Frobisch and Schoch 2009). However, we found limited variation among the examined specimens (n = 161) of *Hemigrammopetersius barnardi*. Two specimens were found to lack an adipose fin (specimens # 23 and 159), similar to that found in a population of another alestid, *Alestes stuhlmanni* Pfeffer, 1896 (Murray 2004).

Comparison with *Alestes dentex*

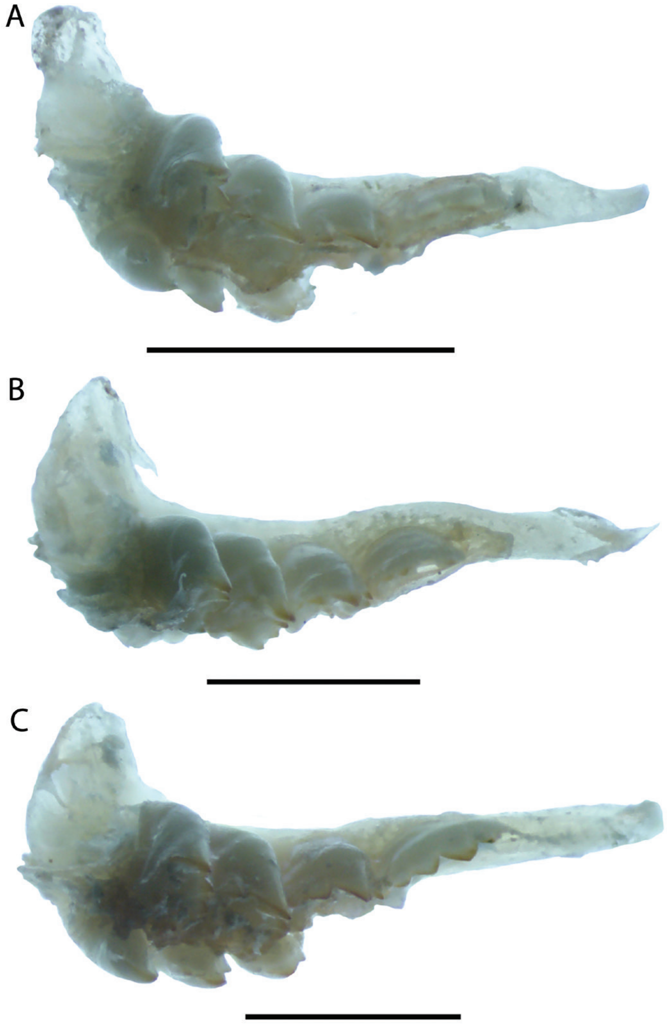
At least two miniaturization events are hypothesized to have occurred within Alestidae (Hubert et al. 2005; Zanata and Vari 2005), which makes this family a good candidate for assessing whether similar features among lineages reflect shared ancestry (and therefore are homologous), or are the result of truncated development associated with reduced adult body size (and therefore are potentially homoplastic). We focus here on osteological characters previously used in phylogenetic analyses of alestids (Murray and Stewart 2003; Zanata and Vari 2005) and compared these characters in adult specimens of *Hemigrammopetersius barnardi* and juvenile specimens of *Alestes dentex*, an alestid of much large adult body size, to identify potentially convergent characters in small alestids.

Hemigrammopetersius barnardi exhibits several characters that we here identify as a result of their small size, rather than their evolutionary history. The first is the loss of the supraorbital in *H. barnardi*. Loss of various circumorbital elements is noted as a homoplastic feature within miniature and small bodied characins (e.g., Malabarba and Weitzman 2003; Azevedo 2010; Marinho 2017). Zanata and Vari (2005) also found the loss of the supraorbital among alestid species to be convergent based on their phylogeny. The smallest juvenile specimens of *Alestes dentex* examined has no supraorbital; the largest juvenile specimen has a supraorbital bone present, but it is relatively smaller in size than that of larger specimens, indicating that this ossification is not fully formed in the juveniles, and likely appears later in ontogeny than other elements. Therefore, the loss of bones from the circumorbital series is most likely to be the result of miniaturization and not indicative of evolutionary relationships.

In contrast, the presence of a premaxillary pedicle is found in all examined specimens of both species here and is a synapomorphy of the family according to Murray and Stewart (2002). We find that this feature develops early in ontogeny and is present regardless of maximum adult size. The premaxillary pedicel is therefore identified as a robust phylogenetic character.

Dental characters have historically been problematic in classifications of alestids despite having been used to create classifications; the polyphyletic 'Petersiini' was diagnosed by lacking shoulders on the teeth or having reduced multicuspid teeth (Paugy 1990; Gery 1995). Studies of some alestids have shown that the teeth change throughout ontogeny (e.g., the labiolingual thickening observed in *A. stuhlmanni* (Murray 2004) or the fusion of unicuspid teeth into tricuspid teeth in which the middle cusp takes over the other two to form unicuspid dentition, as in *Hydrocynus* (Brewster 1986). *Alestes dentex* has been coded as having a posteromedial expansion of the third premaxillary tooth (character 62 in Zanata and Vari 2005). The smallest juvenile specimen of *A. dentex* examined here (SL = 43.6 mm) does not exhibit a posteromedial expansion; instead, in this specimen the character would be coded as 'expansion absent', just as it is *H. barnardi* (Fig. 3). The third tooth of the inner row of the premaxilla thickens with growth, as can be seen in the largest of our examined juvenile specimens of *A. dentex* (SL = 62.7 mm; Fig. 11C). This indicates that dental characters are greatly dependant upon stage of growth of the individual, and so are likely to give false phylogenetic signals in small –bodied species. Therefore, dental characters are likely to be homoplastic among alestids and do not reflect phylogenetic relationships.

Changes in the postcranial skeleton have also been reported to occur with changes in maximal body size of a species (Weitzman and Vari 1988); complete loss or simplifications of form are common. In *Hemigrammopetersius*

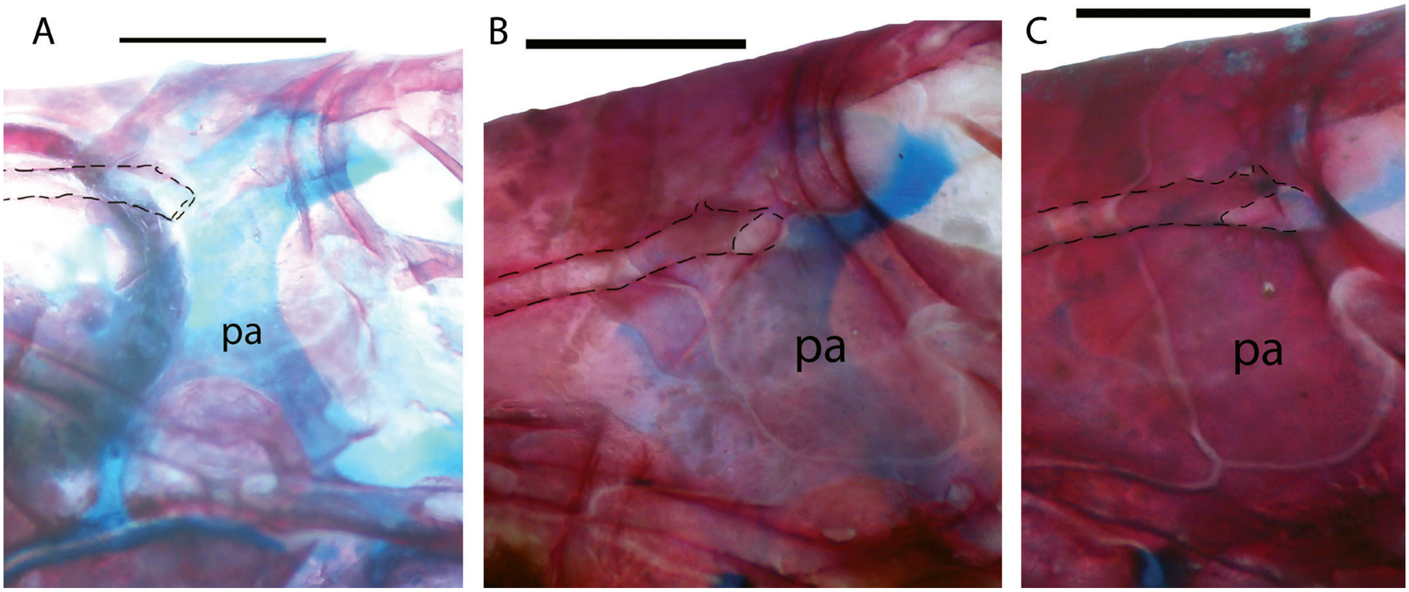


↑ **Figure 11.** Photographs of posteromedial expansion of third premaxillary tooth in a growth series of *Alestes dentex* USNM 229863. A, specimen number 3, SL = 43.2 mm; B, specimen number 1, SL = 55.8 mm; and C, specimen number 2, SL = 62.7 mm. Scale bar = 1 mm.

barnardi, there are only two postcleithra, in contrast to other larger sized alestids (e.g., *A. stuhlmanni*, Murray 2004). The absence of one or more postcleithra has also been reported for other alestids with a small maximal body size (e.g., *Rhabdalestes rhodesiensis* and *Lepidarchus adonis*; Zanata and Vari 2005). Therefore, we consider the loss of one or more postcleithra in these taxa to be correlated with small adult size and as such it is a homoplastic trait.

Finally, reductions to the sensory canal system have been reported for miniature characiforms (Weitzman and Vari 1988; Hanken and Wake 1993). This is also the case for *Hemigrammopetersius barnardi* which has a reduced sensory canal system in comparison to larger bodied alestids. In *H. barnardi*, this system forms a single, unbranched canal on the dermosphenotic with a pore at either end, and the supraorbital canal system is absent from the posterior portion of the parietal. This differs from the condition in larger bodied alestids which have a tripartite canal on the dermosphenotic with three canal pore openings, and the canal on the parietal extends from anterior to posterior edges of the bone (e.g., Murray 2004). Zanata and Vari (2005: character 157) coded the supraorbital sensory canal as present across the entire length of the parietal for *A. dentex*; however, we found that in the smallest juvenile specimen of *A. dentex* we examined, the supraorbital sensory canal is only present on the anterior portion of the parietal. In the larger juvenile *A. dentex* examined, the supraorbital sensory canal extends

↓ **Figure 12.** Photographs of the supraorbital sensory canal on the parietal bone in a growth series of *Alestes dentex* USNM 229863. A, specimen number 3, SL = 43.2 mm; B, specimen number 1, SL = 55.8 mm; and C, specimen number 2, SL = 62.7 mm. Scale bar = 1 mm. Dashed lines indicate the supraorbital sensory canal. Abbreviation: pa, parietal.



from the anterior edge of the parietal to its posterior border (Fig. 12). This feature, therefore, is appearing quite late in the growth series and the lack of the tripartite canal on the dermosphenotic and truncated canal on the parietal in *H. barnardi* are features clearly associated with the diminutive size of this species. This indicates that sensory canal morphology is prone to convergent evolution and should not be used in phylogenetic analyses.

CONCLUSIONS

The osteology of 161 specimens of *Hemigrammopetersius barnardi* from a single population from the Rufiji River, Tanzania is consistent among all specimens examined. There is little gross morphological variation noted, with only two specimens lacking an adipose fin. This alestid, which is characterized by small adult size, provides an opportunity to assess the effects of a truncated ontogenetic trajectory on the phylogenetic utility of anatomical characters. The comparison of the osteology of this species with small specimens of *Alestes dentex*, which attains much larger adult size, allows us to identify characters that change throughout ontogeny. Adults of miniature species with a truncated development tend to exhibit the juvenile state of such characters that are expressed in small individuals of larger species. As such, when comparing two miniature species, it is possible to misinterpret the common possession of an early developmental state as homology. Consequently, the common expression of such character states in miniature species is likely associated with small size rather than evolutionary relationships. In alestids, these features prone to homoplasy include the reduction of the sensory canals, the loss of bones from the circumorbital series, and the reduction or complete loss of postcranial bones such as the postcleithra. Additionally, similar dental characters including reduction of cusps on the teeth, may be associated with small maximal body size rather than shared ancestry. Other characters that do not change throughout ontogeny (e.g., the presence of a premaxillary pedicle) are a better indicator of evolutionary relationships.

ACKNOWLEDGEMENTS

We thank Sylvie Laframboise and Stéphanie Tessier (Canadian Museum of Nature) and Susan Jewett (USNM) for lending materials in their care, Murray Gingras (University of Alberta) for access to his CT –scanner, and Oksana Vernygora (Canadian Food Inspection Agency) for technical help with the CT –scanner. The manuscript was improved with helpful suggestions from two anonymous reviewers. Our thanks to Robert Holmes for many editorial suggestions. This project was funded by the Natural Sciences and Engineering Research Council of Canada Discovery Grant 327448 to AMM.

CONTRIBUTIONS

This project was part of the MSc programme of the first author under the supervision of the second author. MED collected and analysed data and took the photographs. AMM conceived the project. Both authors contributed to writing the manuscript and creating the figures in their current form.

LITERATURE CITED

Arroyave, J., and M.L.J. Stiassny. 2011. Phylogenetic relationships and the temporal context for the diversification of African characins of the family Alestidae (Ostariophysi: Characiformes): Evidence from DNA sequence data. Molecular Phylogenetics and Evolution 60:385–397.

Azevedo, M.A. 2010. Reproductive characteristics of characid fish species (Teleostei, Characiformes) and their relationship with body size and phylogeny. Iheringia Serie Zoologia 100:469–482.

Boulenger, G.A. 1898. Matériaux pour la faune du Congo. Poissons nouveaux du Congo. Deuxiéme Partie. Elopes, Characins, Cyprins. Annales du Musee du Congo (Series Zoology) 1:21–38.

Brewster, B. 1986. A review of the genus *Hydrocynus* Cuvier 1819 (Teleostei: Characiformes). Bulletin of the British Museum of Natural History (Zoology) 50:163–206.

Britz, R., and K.W. Conway. 2009. Osteology of *Paedocypris*, a miniature and highly developmentally truncated fish (Teleostei: Ostariophysi: Cyprinidae). Journal of Morphology 270:389–412.

Calcagnotto, D., S.A. Schaefer and R. DeSalle. 2005. Relationships among characiform fishes inferred from analysis of nuclear and mitochondrial gene sequences. Molecular Phylogenetics and Evolution 36:135 –153.

Eccles, D.H. 1992. FAO species identification sheets for fishery purposes. Field guide to the freshwater fishes of Tanzania. Prepared and published with the support of the United Nations Development Programme (project URT/87/016). Rome: FAO.

Franz-Odendaal, T.A., and D. Adriaens. 2014. Comparative developmental osteology of the seahorse skeleton reveals heterochrony amongst *Hippocampus* sp. and progressive caudal fin loss. EvoDevo 5:1–11.

Fricke, R., W.N. Eschmeyer, and J.D. Fong. 2023. Species by Family/Subfamily. Retrieved from: http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp

Frobisch, N.B., and R.R. Schoch. 2009. Testing the impact of miniaturization on phylogeny: Paleozoic dissorophoid amphibians. Systematic Biology 58:312–327.

Gery, J. 1995. Description of new or poorly known Alestinae (Teleostei: Characiformes: Alestidae) from Africa, with a note on the generic concept in the Alestinae. Journal of Ichthyology and Aquatic Biology 1:37–64.

Hanken, J., and D.B. Wake. 1993. Miniaturization of body – size – organismal consequences and evolutionary significance. Annual Review of Ecology and Systematics 24:501–519.

Herre, A.W. 1936. A new cyprinid genus and species and a new characin from Portuguese East Africa. Proceedings of the Biological Society of Washington 49:99–102.

Hubert, N., C. Bonillo, and D. Paugy. 2005. Early divergence among the Alestidae (Teleostei, Ostariophyses, Characiformes): mitochondrial evidences and congruence with morphological data. Animal biology and pathology 328:477–491.

Johnson, G.D., and E.B. Brothers. 1993. *Schindleria*: a paedomorphic goby (Teleostei: Gobioidei). Bulletin of Marine Science 52:441–471.

Linnaeus, C. 1758. Systema Naturae. 10th ed. (reprinted 1956). London: British Museum of Natural History.

Lunkayilakio, S.W., and E. Vreven. 2008. *Nannopetersius mutambuei* (Characiformes: Alestidae), a new species from the Inkisi River basin, Lower–Congo. Ichthyological Explorations of Freshwaters 19:367–376.

Malabarba, L.R., and S.H. Weitzman. 2003. Description of a new genus with six new species from Southern Brazil, Uruguay and Argentina, with a discussion of a putative characid clade (Teleostei: Characiformes: Characidae). Comunicações do Museu de Ciências e Tecnologia da PUCRS. Série Zoologia 16:67–151.

Marinho, M.M.F. 2017. Comparative development in *Moenkhausia pittieri* and *Paracheirodon innesi* (Ostariophysi: Characiformes) with comments on heterochrony and miniaturization in the Characidae. Journal of Fish Biology 91:851–865.

Moritz, T., and U.K. Schliewan. 2016. Description of two new *Bathyaethiops* species (Teleostei: Alestidae) from the Congo basin. ZooTaxa 4117:476–490.

Murray, A.M., and K.M. Stewart. 2002. Phylogenetic relationships of the African genera *Alestes* and *Brycinus* (Teleostei, Characiformes, Alestidae). Canadian Journal of Zoology 80:1887–1899.

Murray, A.M. 2004. Osteology and morphology of the characiform fish *Alestes stuhlmannii* Pfeffer, 1896 (Alestidae) from the Rufiji River basin, East Africa. Journal of Fish Biology 65:1412–1430.

Oliveira, C., G.S. Avelino, K.T. Abe, T.C. Mariguela, R.C. Benine, G. Orti, R.P. Vari, and R.M. Correa e Castro. 2011. Phylogenetic relationships within the speciose family Characidae (Teleostei: Ostariophysi: Characiformes) based on multilocus analysis and extensive ingroup sampling. BMC Evolutionary Biology 11:1–25.

Orti, G., and A. Meyer. 1997. The radiation of characiform fishes and the limits of resolution of mitochondrial ribosomal DNA sequences. Systematic Biology 46:75 –100.

Paugy, D. 1990. Note a propos des Petersiini (Teleostei: Characidae) d'Afrique occidentale. Ichthyological Explorations of Freshwaters 1:75–84.

Paugy, D., C. Lévêque, G.G. Teugels. 2003. Poissons d'eaux douces et saumâtres de l'Afrique de l'Ouest. Vol. I. IRD Éditions, Publications scientifiques du Muséum, MRAC, Paris, 457 pp.

Pellegrin, J. 1928. Mutanda ichthyologica: *Clupeocharax* Pellegrin. Revue Zoologique Africaine 16:1–82.

Peters, W.C.H. 1852. Diagnosen von neuen Flussfischen aus Mossambique. Monatsberichte der Akademie der Wissenschaft zu Berlin 1852, 275–276, 681–685.

Pfeffer, G.J. 1896. Die Thierwelt Ost–Afrikas und der Nachbargebiete. Lief. V. Die Fishche Ost–Africkas.

Poll, M. 1967. Revision des Characidae nains Africains. Annales du Musee Royal de l'Afrique Centrale Tervurum serie in 8 Sciences Zoologiques 162:1–158.

Prokofiev, A.M. 2001. A new genus of Neoscopelidae for *Eomyctophum gracile* Daniltshenko from the middle Eocene of Georgia (Pisces: Osteichthyes: Myctophiformes). Zoosyst Rossica 10:215–218.

Ricardo-Bertram, C.K. 1943. The fishes of the Bangweulu Region. Zoological Journal of the Linnean Society 41:183–217.

Roberts, T.R. 1966. Description and osteology of *Lepidarchus adonis*, a remarkable new characid fish from West Africa. Standford Ichthyological Bulletin 8:209–227.

Roberts, T.R. 1975. Geographical distribution of African freshwater fishes. Zoological Journal of the Linnean Society 57:249–319.

Roman, R. 1966. Les poisons des hauts bassins de la Volta. Annales du Museum royale d`Afrique Centrale (Sciences Zoologique) 150:1–191.

Taylor, W.R., and G.C. Van Dyke. 1985. Revised procedure for staining and clearing small fishes and other vertebrates for bone and cartilage study. Cybium 9:107–119.

Weitzman, S.H., and R.P. Vari. 1988. Miniaturization in South American freshwater fishes; an overview and discussion. Proceedings of the Biological Society of Washington 101:444–465.

Zanata, A.M., and R.P. Vari. 2005. The family Alestidae (Ostariophysi, Characiformes): a phylogenetic analysis of a trans-Atlantic clade. Zoological Journal of the Linnean Society 145:1–144.