

Osteology of *Priocharax* and Remarkable Developmental Truncation in a Miniature Amazonian Fish (Teleostei: Characiformes: Characidae)

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ABSTRACT Establishing phylogenetic relationships of miniature fishes is challenging in taxa with developmental truncation. Within the Characiformes, developmental truncation appears to be relatively rare, with the Neotropical genus *Priocharax* being an example. *Priocharax* includes three miniature species among the smallest of the order and has been hypothesized to belong to the Heterocharacinae. The pronounced reduction in its skeleton, however, prevented a clearer evaluation of its relationships. The present detailed osteological study was designed to address this question and revealed that 21 bones are absent and nine other skeletal structures are simplified in *Priocharax* when compared to other characids. Comparison of the skeleton of adult *Priocharax* with early developmental stages of other characids demonstrated that most of the absences and simplifications can be interpreted as developmental truncations. The most striking developmental truncations are in the pectoral girdle, in which the endoskeleton remains entirely cartilaginous. Other interesting truncations are in the ethmoid region of the skull, infraorbital series, and Weberian apparatus, in which the claustrum is absent. Our study also revealed some unusual sexual dimorphisms in the pelvic girdle. Two cladistic analyses were performed to assess the relationships of *Priocharax* within the Heterocharacinae. The first consisted of a traditional analysis in which all absences and reductions of *Priocharax* were coded in the same way as in the remaining taxa. This resulted in three equally most parsimonious topologies, all of which have *Priocharax* as the most basal taxon of the Heterocharacinae. The second analysis incorporated ontogenetic information, and most absences and reductions of *Priocharax* were reinterpreted as apomorphic conditions and thus, coded differently from similar conditions in outgroups. This resulted in a single phylogenetic hypothesis with *Priocharax* and *Gnathocharax* as sister groups based on seven synapomorphies. Our approach demonstrates the importance of developmental studies to better understand morphological evolution of miniaturized, truncated taxa, and to generate hypotheses of their relationships. *J. Morphol.* 000:000–000, 2015. © 2015 Wiley Periodicals, Inc.

KEY WORDS: anatomy; heterochrony; miniaturization; Neotropical; phylogenetic systematics; terminal deletion

INTRODUCTION

The last 10 years have witnessed the discovery of a large number of miniaturized vertebrates, with remarkable examples especially from the world of bony fishes (Kottelat et al., 2006; Britz et al., 2009; Mattox et al., 2013). Most miniaturized fishes have been convincingly assigned systematically to monophyletic groups without major problems, but it has proved much more difficult to establish the phylogenetic relationships of miniature fishes that also exhibit a certain level of developmental truncation or progenesis (see Johnson and Brothers, 1993 for *Schindleria*; Siebert, 1997 for *Sundasalanx*; Britz and Conway, 2009; Britz et al., 2014 for *Paedocypris*). Although the teleost order Characiformes includes a large number of miniature taxa (Weitzman and Vari, 1988; Conway and Moritz, 2006; Toledo-Piza et al., 2014), developmental truncation appears to be extremely rare among them. One miniature characiform taxon with some level of developmental truncation and unresolved phylogenetic affinities is *Priocharax*, a genus described by Weitzman and Vari (1987) from the Amazon. The two originally

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included species do not exceed a standard length (SL) of 17 mm: *Priocharax ariel* from the upper reaches of Río Orinoco and Río Negro in Venezuela, and *P. pygmaeus* from the Upper Rio Amazonas near the border between Brazil, Colombia, and Peru. A third miniature species, *Priocharax nanus*, was recently described from the surroundings of Santa Isabel do Rio Negro (Toledo-Piza et al., 2014), showing that the species-level diversity of the genus is far from being completely known.

Weitzman and Vari (1987) listed five diagnostic characters for *Priocharax*, the most conspicuous of which is i) the presence of a larval pectoral fin in the adults. *Priocharax* also has ii) a high number of conical teeth on the upper (45–90 including both premaxilla and maxilla) and lower jaws (30–50), iii) the adults are miniature in size being among the smallest characiforms known and iv) the anal fin has 16–22 branched rays. An additional diagnostic character listed by Weitzman and Vari (1987) to define *Priocharax* is v) the presence of only five branched pelvic-fin rays, an unusual number within characiforms with usually seven branched pelvic-fin rays. Many of the features used by Weitzman and Vari (1987) and Toledo-Piza et al. (2014) to diagnose *Priocharax* and its species are related to reductive characters (*sensu* Myers, 1958), such as loss of scales, lower number of pelvic-fin rays, absence of lateral-line canals and some bones (i.e., part of the infraorbital series and pectoral-fin skeleton), and retention of a larval pectoral fin in the adults, among others. Reductive characters in *Priocharax* pose an obstacle to the comparison with adult representatives of other Characidae and hence, to our understanding about its phylogenetic position (Weitzman and Vari, 1987; Mattox and Toledo-Piza, 2012).

When Weitzman and Vari (1987) described *Priocharax*, they referred to the presence of numerous conical teeth and the elongate maxilla as characters potentially relating the new genus to representatives of the Characinae (*sensu* Géry, 1977) such as *Charax*, *Galeocharax*, *Acestrocephalus*, *Heterocharax*, *Gnathocharax*, and *Lonchogenys*. However, those authors pointed out that this was a provisional hypothesis since a proper phylogenetic study of the pertinent taxa was not available. More than 10 years later, Lucena (1998) was the first to include *Priocharax* in a phylogenetic analysis when investigating the relationships of the scale-eating characine *Roeboides*. He recovered *Priocharax* as the sister group to a clade with another eleven genera, and Lucena and Menezes (2003) later classified all of them in the subfamily Characinae. This basal position of *Priocharax* in Lucena's hypothesis (1998) resulted from the absence in the genus of the derived states of many characters included in his analysis, which were coded as zero and attracted *Priocharax* to the all-

zero outgroup taxon in that study. The high amount of absences of derived states in *Priocharax*, however, may be related to the specialized truncated condition of this miniaturized fish rather than to the presence of a large number of plesiomorphies.

More recently, Mirande (2009, 2010) presented comprehensive studies on the phylogenetic relationships of the family Characidae, and although not all pertinent taxa for each subfamily were included, evidence was presented suggesting that the Characinae as proposed by Lucena and Menezes (2003) should be split into two distantly related taxa: the Characinae in a more restricted sense and the Heterocharacinae. *Priocharax* was not included in Mirande's (2009, 2010) analyses, therefore the question of its relationships to either the Characinae or the Heterocharacinae remained unresolved. Independent of Mirande (2009, 2010), Mattox and Toledo-Piza (2012) performed a phylogenetic analysis focused on the Characinae (*sensu* Lucena and Menezes, 2003) including representatives of all 12 genera assigned to this subfamily as well as a number of outgroup taxa. They arrived at similar results, except that *Gilbertolus* and *Roestes* were included in the Heterocharacinae by Mattox and Toledo-Piza (2012) and *Exodon*, *Bryconexodon*, and *Roebonexodon* were included in the Characinae by Mirande (2009, 2010). In addition, *Priocharax* was hypothesized by Mattox and Toledo-Piza (2012) as being more closely related to the Heterocharacinae based on five synapomorphies, and more specifically to the tribe Heterocharacini, a group of small characids including the genera *Heterocharax*, *Hoplocharax*, *Gnathocharax*, and *Lonchogenys*. However, the relationship of *Priocharax* among those four genera could not be resolved (Mattox and Toledo-Piza, 2012: 894). That was due to the impossibility of coding a large number of character states for *Priocharax* due in part to the absence, reduction, or modification of many skeletal structures, possibly related to miniaturization, which resulted in a high number of missing entries in the data matrix. As already recognized by Weitzman and Vari (1987: 648), "*Priocharax* is an example of the problems inherent in studies of the phylogenetic relationships of miniature, paedomorphic species."

Two ways of investigating phylogenetic relationships of miniature species and overcoming any issues related to their reductive condition have been suggested (e.g., Fink, 1982; Britz and Conway, 2009; Britz et al., 2014). The first is searching for characters in structures that have not suffered ontogenetic truncation in the miniature taxon and that may be informative regarding its relationships to nontruncated taxa, an approach followed by Mattox and Toledo-Piza (2012). The second is comparing adult specimens of the miniature taxon to early ontogenetic stages of nontruncated closely

related taxa. With this approach, putative plesiomorphic absences or reductions can be identified as derived ontogenetic truncations and result in better justified and more precise hypotheses of homology (Fink, 1982; Reilly et al., 1997; Britz et al., 2014).

Britz and Conway (2009) applied the second approach to *Paedocypris*, a highly ontogenetically truncated Asian cyprinid, by analyzing its anatomical structure in relation to the osteological development of a closely related nonminiature representative of the Cyprinidae (*Danio rerio*; Cubbage and Mabee, 1996; Bird and Mabee, 2003). This comparison suggested that the majority of the absent bones in *Paedocypris* are due to simple developmental truncation of terminal stages in the ossification sequence. Britz and Conway (2009: 410) suggested that the same approach should be applied to other lineages of miniature fishes, highlighting the vast diversity of South American characiforms and siluriforms as promising candidates for such an investigation. We applied Britz and Conway's (2009) approach in our study of *Priocharax* to evaluate its anatomical structure in comparison to the ontogenetic sequence of the closely related characid *Salminus* (Mattox et al., 2014). Such an analysis was expected to aid in the understanding of the phylogenetic relationships of this miniature characiform.

MATERIALS AND METHODS

This study is based mostly on new, recently collected material of *Priocharax* sp. (Fig. 1) from the vicinity of Santa Isabel do Rio Negro, Amazonas State. Specimens were preserved in buffered formalin for 48 h, rinsed in water and transferred to 70% alcohol. Although the specimens studied herein are similar to *P. ariel*, a few anatomical differences exist between the former and paratypes of the latter species. We, therefore, refer to the material used in this study as *Priocharax* sp.

A total of 53 specimens were included in the study, 18 of which were cleared and double stained (c&s) following the method of Taylor and Van Dyke (1985). Specimens were then, gradually transferred in a series of glycerin and stored in 80% glycerin. The SL of all specimens was measured. Selected specimens were fully dissected and details of their skeleton were photographed with a Zeiss Axiocam HRc attached to a Zeiss Discovery V20 Stereomicroscope and images of the same specimen at different levels of focus were combined into a single extended depth of focus image using the Z-Stack option.

Nomenclature of cartilages followed de Beer (1927, 1985) and Bertmar (1959) and that for bones was based on Weitzman (1962) with the following modifications introduced by subsequent authors (i.e., Vari, 1979; 1995; Fink and Fink, 1981; 1996; Zanata and Vari, 2005; Conway and Britz, 2007): mesethmoid instead of ethmoid, vomer instead of prevomer, intercalar instead of opisthotic, endopterygoid instead of mesopterygoid or entopterygoid, anterior ceratohyal instead of ceratohyal, posterior ceratohyal instead of epiphyal, inner arm of os suspensorium instead of os suspensorium, outer arm of os suspensorium instead of rib of fourth vertebra. Terminology for parts of the Weberian ossicles followed Chranilov (1927).

Material used in this study is deposited in the ichthyological collection of Museu de Zoologia da Universidade de São Paulo (MZUSP): *Priocharax* sp.: MZUSP 109199, 10 specimens, 12.5–14.7 mm SL (5 c&s, 13.6–14.3 mm SL), Right bank of Rio Daraá in front of inspection post, affluent of Rio Negro, Santa

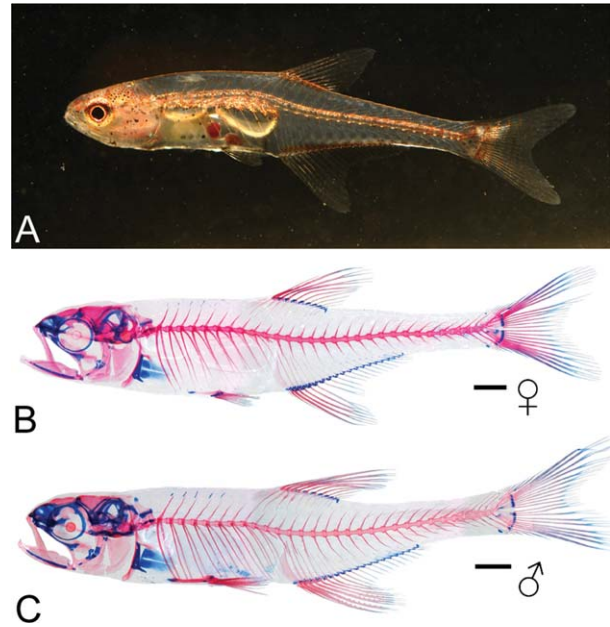


Fig. 1. **A:** *Priocharax* sp., live specimen photographed right after capture. **B:** Female, MZUSP 109199, 13.3 mm SL, c&s, lateral view of whole skeleton. **C:** Male, MZUSP 109199, 13.5 mm SL, c&s, lateral view of whole skeleton. Gill arches, hyoid arches and right side opercular series and pectoral girdle were removed from c&s specimens. Scale bars: 1 mm.

Isabel do Rio Negro (0°26'01"S 64°45'40"W), collected by M. Toledo-Piza, O.T. Oyakawa, G.M.T. Mattox, M.M.F. Marinho, and J. Santana, February 10, 2011.—MZUSP 111125, 38 specimens, 11.9–14.1 mm SL (8 c&s, 12.7–15.2 mm SL), lake at right bank of Rio Urubaxi, near Igarapé Tapage (0°33'44.2"S 64°49'40.8"W), collected by M. Toledo-Piza, G.M.T. Mattox, M.M.F. Marinho, and R. Britz, October 29, 2011. *P. ariel*: MZUSP 55142, 5 c&s specimens, 11.8–13.4 mm SL, paratypes, Venezuela, Territorio Federal Amazonas, Departamento Río Negro, Caño Manu, tributary of Río Casiquiare approximately 250 m upstream from Solano, 02°N 66°57'W, collected by R. P. Vari, C. J. Ferraris Jr., O. Castillo, J. M. Fernandez, December 7, 1984.

RESULTS

Neurocranium

The ethmoid region is weakly ossified and consists of a large cartilaginous median ethmoid plate with an anterior rounded projection that reaches the anteriormost ventral portion of the mesethmoid and supports the premaxilla (Fig. 2). The plate's anterolateral projection articulates with the anterior end of the autopalatine and with the tip of the ascending process of the maxilla through a small separate cartilage. Posterolaterally, the ethmoid plate connects to the *lamina orbitonasalis*. The plate projects posteriorly into a very short *trabecula communis*, which is confluent with a ventral cartilaginous process from the orbitosphenoid. The mesethmoid is short and relatively broad. Its anterior median projection is very short, as is its lateral wing that articulates with the ascending process of the premaxilla. The mesethmoid lacks

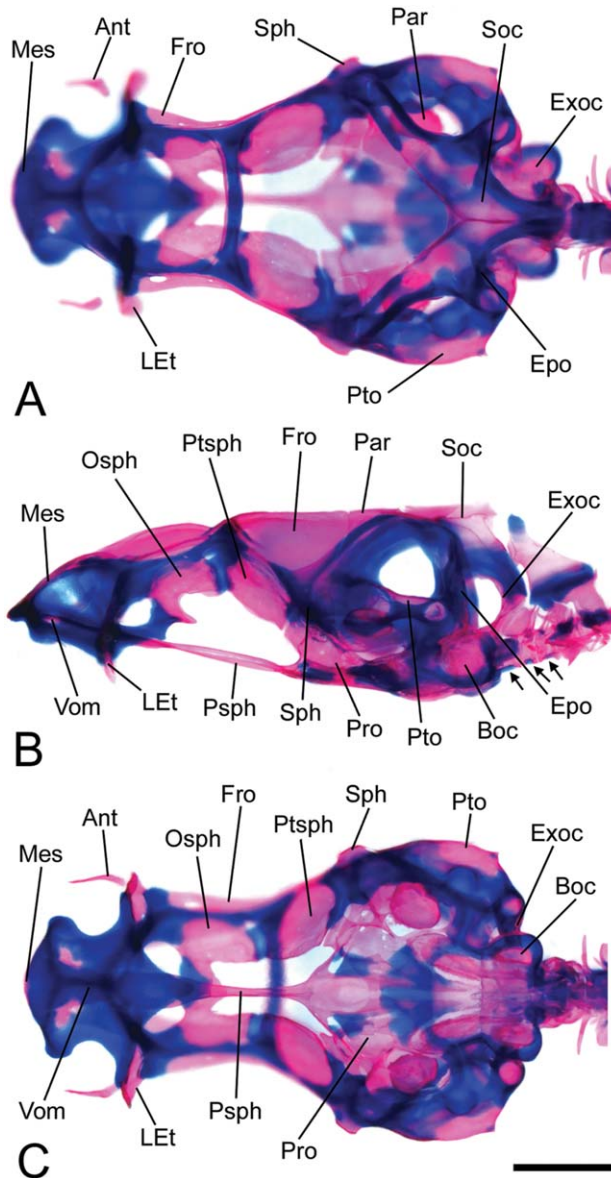


Fig. 2. *Priocharax* sp., male, MZUSP 109199, 15.0 mm SL, c&s. neurocranium. **A**: Dorsal view. **B**: Lateral view. Arrows indicate autogenous cartilages ventral to basioccipital and vertebral centra; **C**: Ventral view. Ant, antorbital; Boc, basioccipital; Epo, epiotic; Exoc, exoccipital; Fro, frontal; LEt, lateral ethmoid; Mes, mesethmoid; Osph, orbitosphenoid; Par, parietal; Pro, prootic; Psph, parasphenoid; Ptsph, pterosphenoid; Pto, pterotic; Sph, sphenotic; Soc, supraoccipital; Vom, vomer. Intercalar not visible in figures. Scale bar: 0.5 mm.

ossified ventral lamellae, but a median cartilaginous flange, the nasal septum, connects its ventral surface to the dorsal surface of the ethmoid plate. A small and rounded lateral ethmoid that lacks the anterior process of other characiforms is developed at the lateralmost corner of the *lamina orbitonasalis*. Posterodorsally, the *lamina orbitonasalis* is continuous with the *taenia marginalis*. A cartilaginous bridge, the epiphyseal bar, connects the contralateral *taeniae marginales* across the roof of the neu-

rocranium (Fig. 2A). The long laminar frontal extends from the posterior margin of the mesethmoid to the anterior otic capsule and thus, covers most of the *taenia marginalis*. It is connected to its contralateral part via a bony bridge along the epiphyseal bar. The small parietal is a roughly triangular bone posterior to the frontal. The wide preepiphyseal cranial fontanel is bordered anteriorly by the mesethmoid and laterally and posteriorly by the medial margin and epiphyseal bar of the frontal. An equally wide postepiphyseal fontanel is developed posterior to the bar and bordered by the frontal, parietal, and the anterior margin of the supraoccipital.

The orbitosphenoid is ossified in the *taenia marginalis* and bears a ventral process of membrane bone that contacts its contralateral part with its posterior margin notched (Fig. 2B). The orbitosphenoid is almost completely surrounded by cartilage even in the largest specimens. The rhinosphenoid is absent. The thin laminar pterosphenoid is developed in the posterior part of the *taenia marginalis*, posterior to the orbitosphenoid, from which it is separated by a narrow band of cartilage. In some specimens, the pterosphenoid extends to the dorsal margin of the *taenia marginalis*, but in others, a narrow cartilage band remains. The sphenotic lies posterior to the pterosphenoid and ventral to the parietal and is surrounded by cartilage. It has a small sphenotic spine that projects ventrally from its lateral surface. The pterotic is located posterior to the sphenotic and forms the lateral part of the otic capsule. The pointed process projecting from its posterior margin is very small. The pterotic lacks its laterosensory canal, indicating that the dermopterotic is most likely absent. The prootic forms a large part of the floor of the cranium (Fig. 2C). It is pierced by many foramina of which the largest, the auditory foramen, is located posteriorly on the ventral surface of the otic capsule, and behind which the lapillus is partially visible. The median elongate parasphenoid extends from the anterior margin of the basioccipital to the base of the *trabecula communis*. Its posterior end extends as two thin processes with a deep notch inbetween. The pointed ascending process projects laterodorsally along the anterior margin of the prootic reaching up to its midlength. The parasphenoid contacts the vomer at its anterior end. The weakly ossified vomer is wider anteriorly where it carries a pair of heads that fail to reach the level of the premaxilla as in most characiforms.

The supraoccipital contacts the parietal anteriorly and forms the posterior margin of the posterior fontanel (Fig. 2A). Posteriorly it bears a short supraoccipital crest (Fig. 2B). The epiotic forms a bony tube around the upper part of the posterior semicircular canal of the inner ear (Fig. 3). It lacks the anterolateral arm of most other

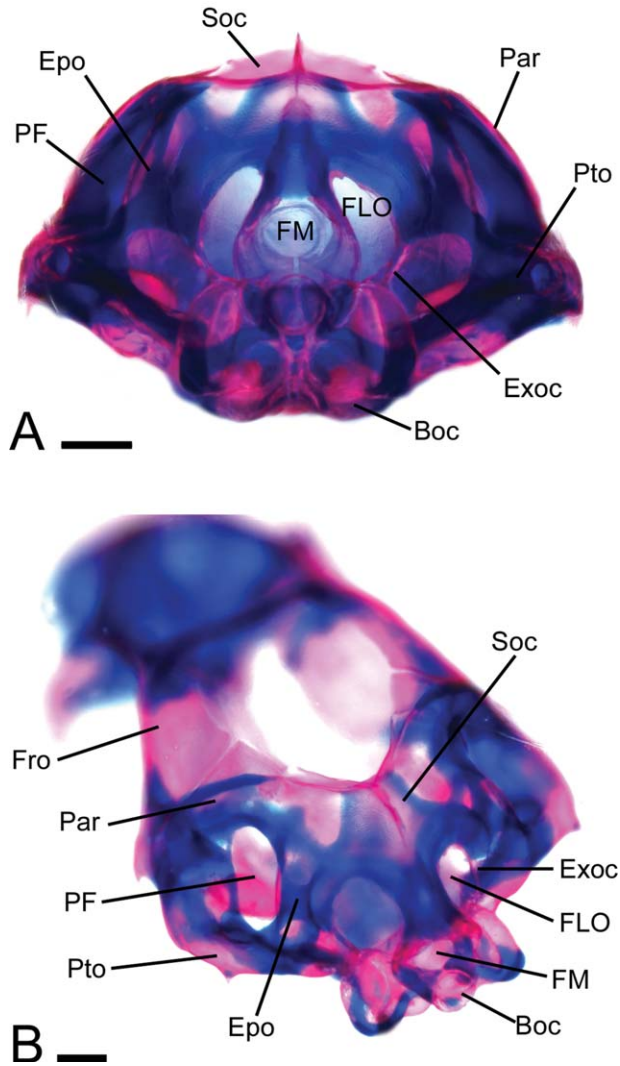


Fig. 3. *Priocharax* sp., male, MZUSP 109199, 15.0 mm SL, c&s posterior portion of neurocranium. **A**: Posterior view. **B**: Dorsolateral view. Boc, basioccipital; Epo, epiotic; Exoc, exoccipital; FLO, lateral occipital foramen; FM, foramen magnum; Fro, frontal; Par, parietal; PF, posttemporal fossa; Pto, pterotic; Soc, supraoccipital. Scale bars: 0.2 mm.

characiforms, which typically reaches the parietal. As a consequence *Priocharax* sp. only has a single, large posttemporal fossa (Fig. 3B), while in the majority of characiforms this fossa is divided into two by the anterolateral arm. The exoccipital forms most of the lateral bony wall of the lagenar part of the otic capsule. Together with the posteroventral margin of the supraoccipital, it surrounds the large lateral occipital foramen. Ventrally the exoccipital contacts the median basioccipital, which forms the posterior portion of the floor of the cranium. The posterior portion of the basioccipital has a median autogenous cartilage on its ventral surface. A small intercalar, approximately cylindrical in shape, is developed near the posterior articulation between pterotic and exoccipital

and is the site of attachment for the ligament from the lower limb of the posttemporal. There are no laterosensory canals in any of the bones in the neurocranium (Fig. 2).

Hyopalatine Arch and Opercular Series

The hyomandibular has a narrow vertical flange of membrane bone along its anterior margin and a second, smaller one, along its posterior margin. It has two articular heads. Its dorsal head is elongate, cartilaginous, and articulates with the sphenotic and pterotic. Its opercular head is much smaller (Fig. 4A). The large foramen for the passage of the hyomandibular branch of the facial nerve pierces the dorsal portion of the hyomandibular medially. Hyomandibular and symplectic are separated by a remnant of the hyosymplectic cartilage, a wide zone of cartilage that forms the articulation for the interhyal. The elongate, rod-shaped symplectic is slightly wider posteriorly. It supports a cartilage at its anterior tip and a small flange of membrane bone projects ventrally from its anterior half. The L-shaped metapterygoid in the *pars metapterygoidea* of the palatoquadrate forms the dorsal margin of the quadrate-metapterygoid fenestra. The metapterygoid carries a small cartilaginous posterior head directed toward the cartilage separating hyomandibular and symplectic and a cartilaginous bar connects it with the posterior tip of the quadrate. Anteriorly, metapterygoid and quadrate are separated by remnants of the palatoquadrate cartilage. The latter extends between endopterygoid and ectopterygoid anteriorly to the palatine region. The metapterygoid lacks the dorsal membrane bone portion typical of other characiforms. The quadrate has a similar L-shape as the metapterygoid. Its dorsal margin is concave and forms the ventral border of the large quadrate-metapterygoid fenestra. The posteroventral process of the quadrate covers the anterior half of the symplectic laterally. The broad and thin, oval, laminar endopterygoid extends from the *pars autopalatina* to the anterior part of the metapterygoid and is located dorsal to the remnants of the palatoquadrate cartilage. The endopterygoid is well ossified along its ventral margin but gradually thins out toward its medial edge. The narrow and elongate ectopterygoid runs along the ventral margin of the palatoquadrate cartilage from the *pars autopalatina* to the anterodorsal tip of the quadrate. The autopalatine is ossified around the *pars autopalatina*, which is in contact with a separate small cartilage medially. This cartilage is situated between the anterior tip of *pars autopalatina*, the anterior tip of the ascending process of the maxilla and the ethmoid plate.

The opercle is well ossified around its articular condyle, from where a medial longitudinal crest extends. It has an approximately triangular shape

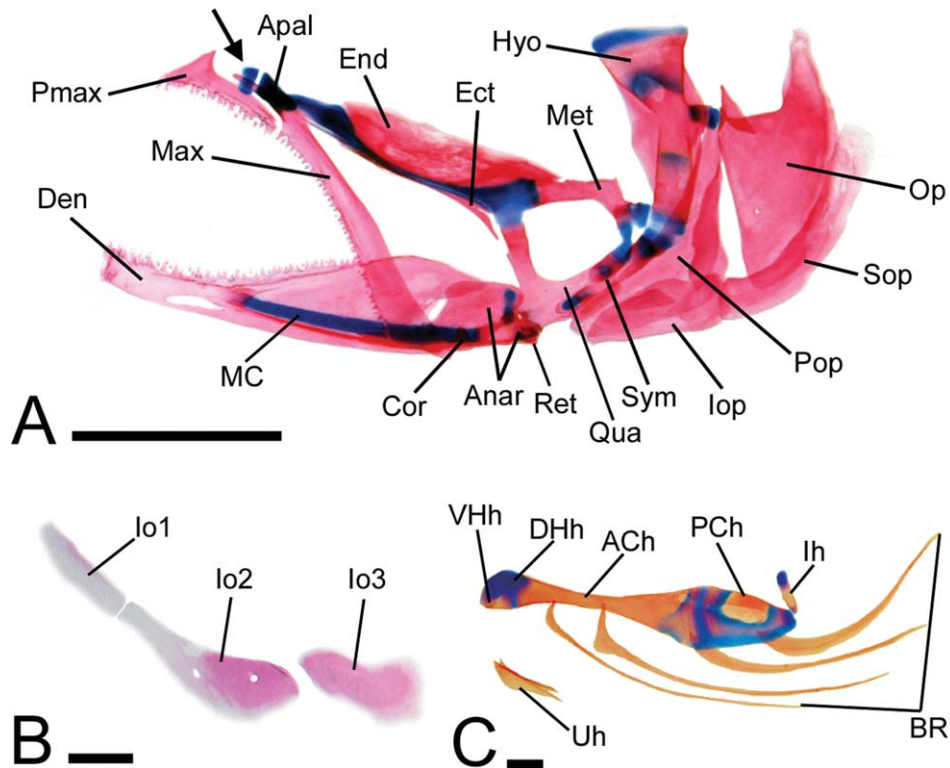


Fig. 4. *Priocharax* sp., all MZUSP 109199 dissected elements of the skull. **A:** Hyopalatine arch and opercular series, right side, lateral view (image reversed), female, 13.3 mm SL, c&s. Arrow indicates autogenous cartilage. **B:** Infraorbital series, left side, lateral view, antorbital not shown, male, 15.0 mm SL, c&s. **C:** Hyoid arch, left side, lateral view, male, 15.0 mm SL, c&s. ACh, anterior ceratohyal; Anar, anguloarticular; Apal, autopalatine; BR, branchiostegal rays; Cor, coronomeckelian; Den, dentary; DHh, dorsal hypohyal; Ect, ectopterygoid; End, endopterygoid; Hyo, hyomandibular; Ih, interhyal; Io1–3, infraorbital 1–3; Iop, interopercle; MC, Meckel's cartilage; Met, metapterygoid; Max, maxilla; Op, opercle; PCh, posterior ceratohyal; Pmax, premaxilla; Pop, preopercle; Qua, quadrate; Ret, retroarticular; Sop, subopercle; Sym, symplectic. Uh, urohyal; VHh, ventral hypohyal. Scale bars: A: 1 mm; B and C: 0.2 mm.

with a rounded posterior margin and a wide notch on its dorsal margin (Fig. 4A). This notch is limited anteriorly by a dorsolaterally directed, triangular process. Posterior to the notch, the opercle extends dorsally as a narrow bony lamella. The subopercle is weakly ossified on its posterior end and partially overlapped laterally by the opercle along its dorsal margin. Its anterior portion extends further anteriorly than the opercle. Its ventral outline runs parallel to the curvature of the ventral margin of the opercle. The subopercle is slightly wider anteriorly, from where a tiny nubbin projects dorsally in some specimens. The preopercle is a relatively large boomerang-shaped bone. Its vertical arm tapers dorsally gradually and terminates as a pointed tip at the level of the opercular condyle. It has a small posterior lamellar extension. The preopercle covers part of the ventral margin of the symplectic and the posterior margin of the hyomandibular laterally. The interopercle is thin and relatively short, wider posteriorly and gradually tapering anteriorly where the interoperculo-mandibular ligament originates. There is no suprapreopercle.

The large dentary is conspicuously wider posteriorly, tapers toward its anterior third from where it remains the same depth up to its anterior tip. It car-

ries a single series of 35–45 small, similar-sized, conical teeth. It lacks the replacement tooth trenches typical of other characiforms. The posterior portion of the dentary has a large triangular coronoid process that is partially covered laterally in females by the anguloarticular. In males, the coronoid process is smaller and is not covered to the same extent by the anguloarticular. There is a narrow gap between the dentary and the anguloarticular below the coronoid process of the dentary. A conspicuous elongate foramen is developed at the anterior portion of the dentary. The mandibular fossa on the medial surface of the dentary and anguloarticular is relatively shallow and houses the long cylindrical Meckel's cartilage along its ventral portion. A small trapezoidal coronomeckelian is developed near the posterior tip of Meckel's cartilage. The anguloarticular is approximately rectangular and articulates posteroventrally with the quadrate. It is separated from the retroarticular by a small cartilaginous portion ventral to this articulation. The small triangular retroarticular has a rounded posterior surface on which the interoperculo-mandibular ligament inserts.

The premaxilla is relatively long and bears a single series of 18–25 small, conical teeth of similar size. Its ascending process is short and

triangular (Fig. 4A) and articulates with the anterolateral margin of the mesethmoid and its weakly developed lateral wing. The elongate maxilla is slightly wider posteriorly and gradually tapers anteriorly. It has a single series of 37–45 small conical teeth of similar sizes along its anterior margin. The anterior toothless process of the maxilla extends along the dorsoposterior margin of the lateral arm of the premaxilla.

Infraorbital Series and Sclerotic Bones

There are only four bones in the infraorbital series, the antorbital and infraorbitals 1, 2, and 3 (Figs. 1 and 4B). The flat lamellar antorbital is located anterior to the lateral ethmoid. It is broader ventrally and becomes gradually narrower dorsally. Its ventral tip is curved medially. Infraorbital 1 is relatively short, thin, and poorly ossified. Infraorbital 2 is longer than infraorbital 1 and extends along the ventral margin of the orbit from the posterior tip of infraorbital 1 to approximately the vertical through the posterior tip of the maxilla. Infraorbital 2 is thin and weakly ossified anteriorly, but widens toward its posterior triangular tip which is better ossified. It is pierced by one or two openings in some specimens. Infraorbital 3 is short, slightly elongate, and well ossified. Although it occupies the region normally covered by infraorbital 3 in other characiforms, it does not reach ventrally to the preopercle. Laterosensory canals are not developed on any of the infraorbitals. Infraorbitals 4, 5, and 6 and the supraorbital are missing. The sclerotic bones are not developed and the sclerotic cartilage band extends around the entire orbit.

Hyoid, Urohyal, and Branchial Arches

The cylindrical interhyal articulates with the cartilage between hyomandibular and symplectic in the hypopalatine arch and with the posterior ceratohyal. The posterior ceratohyal is mostly perichondrally ossified and separated from the anterior ceratohyal by a continuous band of cartilage that also extends along the ventral margin of the bone (Fig. 4C). There is an elongate foramen on the medial surface of the posterior ceratohyal which represents the posterior opening of a canal extending from the anterior ceratohyal. The elongate anterior ceratohyal is wider posteriorly than anteriorly but gradually tapers toward the center. The anterior ceratohyal carries the anterior continuation of the canal described above which has its anterior opening on the posterior portion of its dorsal margin (Fig. 4C). The anterior end of the anterior ceratohyal contacts a cartilage that wraps around the dorsal and ventral hypohyals. The dorsal hypohyal is only a small flange of bone on the medial margin of its cartilaginous precursor that projects toward the basihyal. The ventral hypohyal is small and its ventromedial surface serves as an

attachment point for the ligament from the urohyal. The thin lamellar urohyal is triangular in lateral view, with the posterior margin irregularly concave. A pair of ventrolateral flanges project from the ventral margin of the urohyal. Its anterior end is slightly bifurcated to accommodate the paired ligament to the ventral hypohyals. There are four slender branchiostegal rays, the anterior two associated with the anterior ceratohyal, the third near the synchondral articulation between anterior and posterior ceratohyals and the fourth articulated with the posterior ceratohyal. They become sequentially shorter but wider caudally. Although slender, all four bear a small projection on their anterior margin, especially evident in the two anteriormost rays. The basihyal cartilage is elongate with a wider anterior portion that tapers posteriorly. Its anterior portion has a small median notch giving it a heart-shaped appearance in dorsal view and the basihyal ossification is restricted to its cylindrical posterior half (Fig. 5A).

The rod-like basibranchials are developed around much of the anterior copular cartilage in the ventral midline of the branchial arches (Fig. 5A). Basibranchial 1 is short and located just posterior to the basihyal between hypobranchials 1. It is separated from basibranchial 2 by an elongate cartilage, which exhibits a shallow lateral notch to accommodate the posteromedial tip of the cartilage at the tip of hypobranchial 1. Basibranchial 2 is much longer and extends from the region just posterior to hypobranchial 1 to the anterior end of hypobranchial 2. Basibranchial 2 is separated from basibranchial 3 by an elongate zone of cartilage. Basibranchial 3 is also elongate with its anterior tip slightly wider but tapering posteriorly and reaching the anterior margin of the cartilaginous bridge between hypobranchials 3. A short cartilaginous tip covered dorsally by the cartilage bridge connecting hypobranchials 3 marks the posterior limit of the anterior copula. Basibranchial 4 cartilage (or posterior copula) is well developed, elongate and triangular, and extends from the posterior end of the cartilage bridge between hypobranchials 3 to the anterior end of ceratobranchial 5. There are four pairs of hypobranchial elements. Hypobranchial 1 is developed around the center of the first hypobranchial cartilage and has a small elongate gill-raker. Hypobranchial 2 cartilage is approximately triangular and shows a weak perichondral ossification along the middle of its lateral margin. Its medial margin, however, remains cartilaginous. Hypobranchial 2 has two small and elongate gill-rakers. The well-ossified triangular hypobranchial 3 has a ventromedially directed horn-shaped process. As mentioned above, the posterior portion of hypobranchial 3 cartilage is confluent with its contralateral part through a transverse bridge anterior to basibranchial 4 cartilage. Hypobranchial 3 carries a single small gill-raker. Hypobranchial 4 cartilage is small and rod-shaped, lacks any ossification

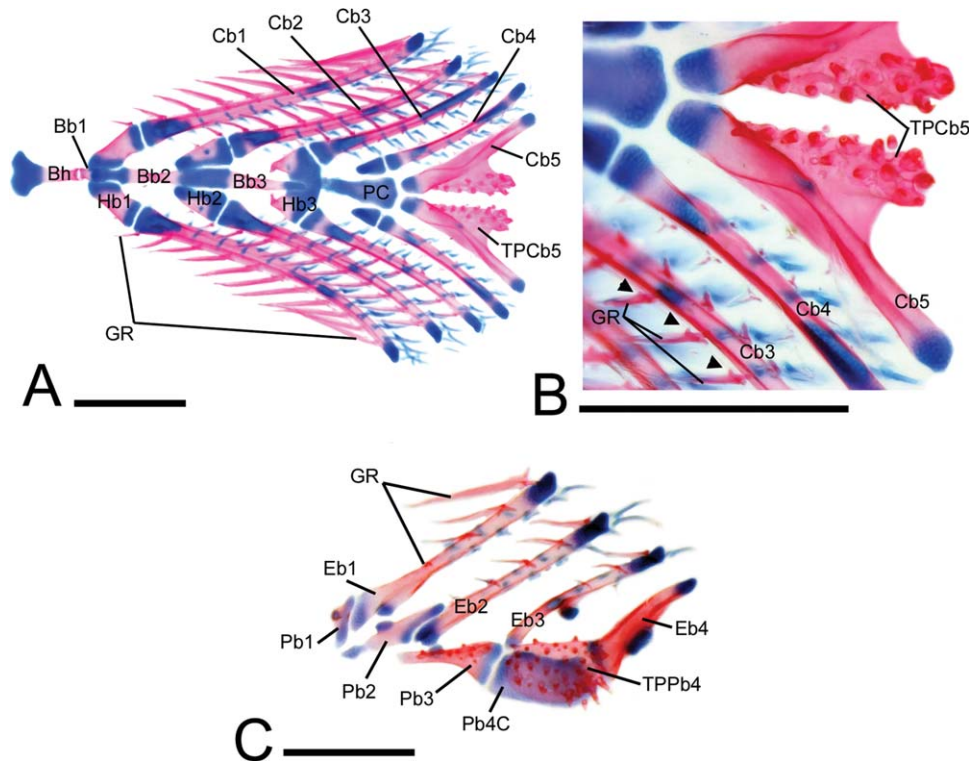


Fig. 5. *Priocharax* sp., female, MZUSP 109199, 13.3 mm SL, c&s branchial skeleton. **A:** Ventral gill-arch skeleton, dorsal view. **B:** Close-up of lower pharyngeal toothplate, left side, dorsal view. Arrowheads indicate spike-like process at base of gill raker. **C:** Dorsal gill-arch skeleton, left side, ventral view, fifth epibranchial cartilage not shown. Bb1–3, basibranchial 1–3; Bh, basihyal; Cb1–5, ceratobranchial 1–5; Ep1–4, epibranchial 1–4; GR, gill rakers; Hb1–3, hypobranchial 1–3; Pb1–3, pharyngobranchial 1–3; PbC, pharyngobranchial cartilage; PC, posterior copula; TPCb5, tooth plate of fifth ceratobranchial; TPPb4, tooth plate of fourth pharyngobranchial. Scale bars: 0.5 mm.

and does not support any gill-rakers. Ceratobranchials 1–4 are rod-shaped with ceratobranchial 1 much more massive than and twice as long as ceratobranchial 4. All ceratobranchials support a series of elongate gill-rakers along their leading edges that become sequentially longer caudally. Although these rakers lack denticles on their surfaces, each has a small spike-like process near its base (Fig. 5B, arrowheads). Ceratobranchial 1 has ten to twelve, ceratobranchial 2 nine to ten, and ceratobranchial 3 eight to nine gill-rakers (with variation between contralateral elements). Ceratobranchial 4 bears seven poorly ossified, small gill-rakers. Ceratobranchials 3 and 4 also support a series of five to six small rakers on their trailing edge; these series are absent in ceratobranchials 1 and 2. The rod-shaped ceratobranchial 5 has a small flange of membrane bone along its lateral edge and its anterior and posterior ends are tipped in cartilage. The toothed area of ceratobranchial 5 is triangular and supports two or three rows of rather large, conical teeth toward the medial margin of the toothplate (Fig. 5B). Ceratobranchial 5 has a single series of 5–6 small, weakly-ossified gill-rakers along its leading edge.

Epibranchials 1–4 are elongate with epibranchial 4 slightly more robust and better ossified

(Fig. 5C). All epibranchials have cartilaginous proximal and distal tips and an uncinatous process best developed on epibranchials 3 and 4. Epibranchials 1, 2, and 3 each have a single series of 5, 4, and 3 elongate gill-rakers, respectively, along their leading edges. Epibranchial 4 has no gill rakers. Pharyngobranchials 1–3 are only weakly ossified perichondrally. Pharyngobranchial 4 cartilage is a wide and flat element. Pharyngobranchial 1 is much broader at the base and tapers dorsally. Pharyngobranchials 2 and 3 have a small dorsal process ending in a cartilaginous tip. Pharyngobranchial 3 is better ossified than the other two. Upper pharyngeal tooth plates are present on the ventral surfaces of pharyngobranchial 3 and pharyngobranchial 4 cartilage, but remain separate even in larger specimens. They have a number of rather large, conical teeth. A few smaller teeth are also present on pharyngobranchial 2 on one side only in four of the specimens.

Weberian Apparatus Skeleton

The skeletal part of the Weberian apparatus includes the four anterior abdominal vertebral centra and associated elements (Fig. 6A,B). The

first centrum is the shortest of the four Weberian centra. It has a small median cartilage ventrally. The second centrum is asymmetrical in lateral view, with its dorsal portion slightly shorter than the ventral portion. The second centrum supports a large lateral process and bears a small median cartilage ventrally. The second centrum also supports a pair of small ventral processes, triangular in shape and directed posteriorly. The third centrum is as long as the second centrum. The neural arch of the third vertebra is relatively broad in all specimens, but there is a large gap between it and the subsequent neural arch. This gap is partially occupied by a pointed dorsal process from the third centrum (Fig. 6A, arrowhead). The third neural arch has a slender and anterolaterally-directed transverse process. Dorsally, this neural arch is connected to the large supraneural 3 via a band of cartilage, a remnant of the neural complex cartilage. The fourth centrum is longer than preceding centra. Its neural arch is much wider and larger than that of the third. Dorsally, neural arch four supports a large mass of cartilage connecting it to supraneural 3. Unusually for characids, the fourth neural spine is short barely reaching the dorsal margin of the posterior end of the cartilage of the neural complex. Supraneural 4 is absent.

The claustrum is absent (Fig. 6A,B). The scaphium is large with a short pointed ascending process and a wide *concha scaphii* that fits into a notch along the posterior margin of the exoccipital. Its short *processus articularis* is connected to the first centrum via a small articular knob of cartilage. The thin L-shaped intercalarium has also a cartilage capped articular process. Its ascending process projects dorsally from the centrum and its manubrium is directed anterolaterally toward the interossicular ligament (Fig. 6B). The tripus is relatively large and triangular with a cartilage capped articular process. Its anterior process is inconspicuous and not differentiated from the main body of the tripus. Its posterior process is much longer and ends in a greatly tapering transformer process which is connected to the anterior chamber of the swimbladder. The articular process of the tripus is slightly turned posterodorsally toward the gap between neural arches 3 and 4. The inner arm of the os suspensorium is long and enlarged, with its anterior half positioned vertically rather than horizontally as in most characiforms. It has a concavity on its anterior margin. The outer arm of the os suspensorium is slightly larger than the twisted portion of the inner arm, triangular and inclined posteroventrally. Its outer arm connects the os suspensorium to the fourth centrum through a cartilaginous base at the site of articulation. Supraneural 3 is relatively large, triangular and weakly ossified and sits on the cartilaginous remnant of the neural complex where it contacts the third and fourth neural arches. Distally it does not reach up to the tip of the neural complex cartilage. A prominent anterior bony process of mem-

brane bone projects off the body of supraneural 3 approaching the posterior portion of the supraoccipital.

Vertebral Column and Intermuscular Bones

There are 14–15 abdominal and 19–21 caudal vertebrae, with a total of 33–35 vertebrae. All centra, except those associated with the Weberian ossicles and the compound centrum, are hourglass-shaped and similar in size (Fig. 6C,D). However, preural centrum 3 is shorter than preceding centra. Ossified neural arches are developed at the anterior end of all post-Weberian centra. Inconspicuous neural prezygapophyses are present on centra 6–12, and are confluent with the proximal portion of the neural arches. Small dorsally directed neural postzygapophyses are developed on the posterior edge of all centra. Neural postzygapophyses on preural centra 2–4 connect with the posterior edge of respective neural arches via large flanges of membrane bone. Neural spines become gradually more posteriorly inclined caudally and have approximately the same size (Fig. 6D,E), except for preural neural spines 2 and 3 which are longer than preceding neural spines. Preural neural spine 2 has a flange of membrane bone along its anterior edge (Fig. 6E). There are small haemal prezygapophyses on centra 5–11 near the base of the parapophyses, and on centra 26–30 near the base of the haemal arches. Nine to ten ribs are present starting on vertebra 5. The rib on vertebra 5 is slightly more robust than subsequent ribs. All ribs are of similar length and are directed ventrally, except for the rib on the last abdominal centrum (14 or 15) which is much smaller and directed posteriorly. Ribs on centra 5–9 show a cartilaginous ventral tip. All ribs except the posteriormost possess a small anterior flange near their articulation with the respective parapophyses. Parapophyses are short, and become sequentially shorter caudally. Haemal spines begin on the fifteenth or sixteenth centra, are of similar size and gradually more posteriorly inclined caudally, except for haemal spines on preural centra 2 and 3 which are longer than preceding ones. There are five to six supraneural cartilages, beginning with supraneural 5, none of them with signs of ossification. Epineural and epipleural intermuscular bones are developed beginning on centra 17–22 and extending to centra 30–33 (Fig. 6D). They are more conspicuous in the posterior half of the intermuscular series.

Pectoral Girdle

The pectoral girdle consists of posttemporal, supra-cleithrum, cleithrum, one postcleithrum, and the scapulocoracoid cartilage (Fig. 7A). Extrascapular, scapula, coracoid, mesocoracoid, pectoral radials, or pectoral-fin rays are absent. The thin and elongate

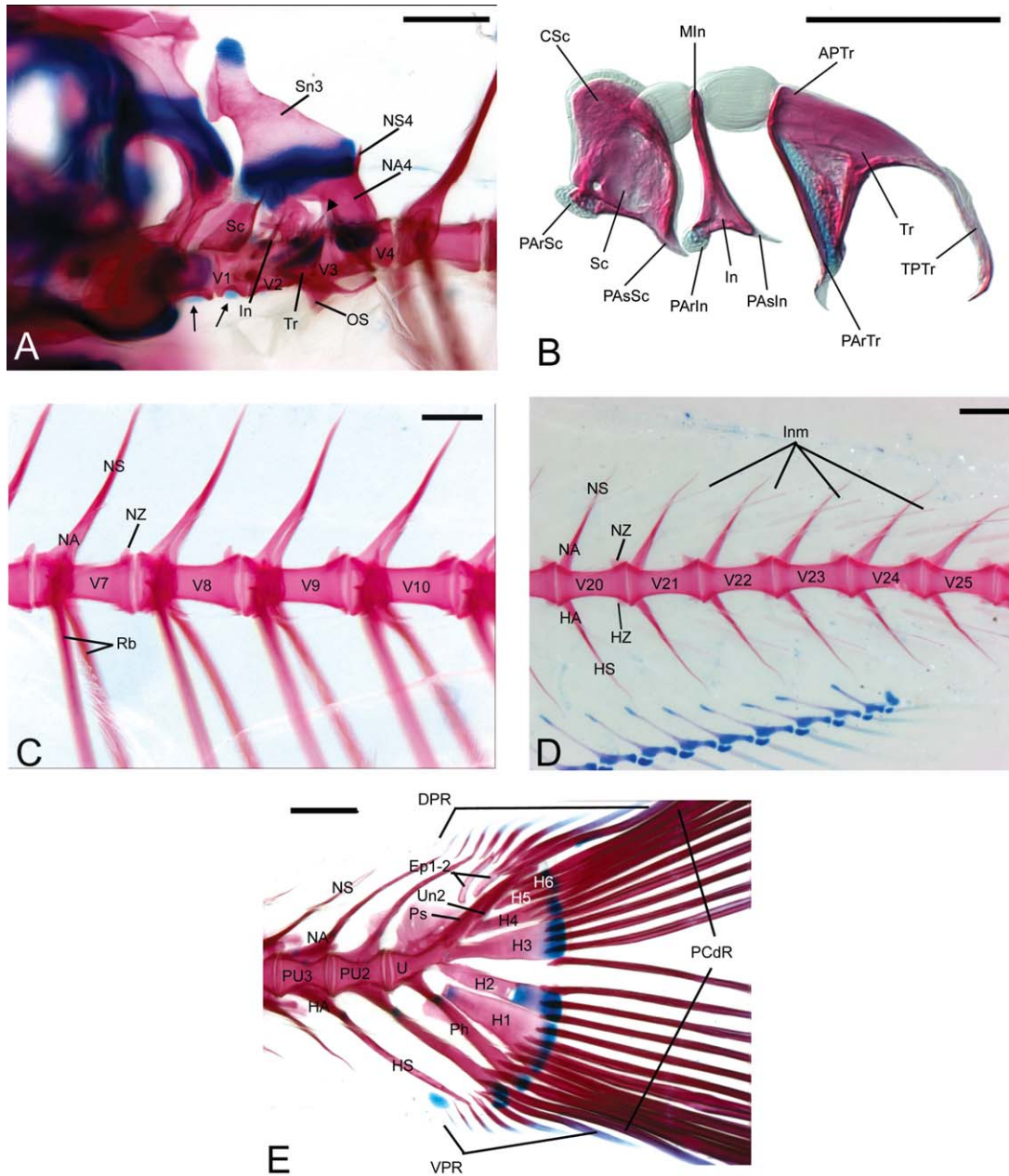


Fig. 6. *Priocharax* sp, female, axial and caudal skeleton. **A** and **E**: MZUSP 111125, YY mm SL, **B**: MZUSP 111125, 13.0 mm SL, **C** and **D**: MZUSP 109199, 13.3 mm SL, c&s. **A**: Weberian apparatus, left side, lateral view. Arrowhead indicates gap between neural arches 3 and 4, partially filled by a pointed dorsal process. Arrows indicate autogenous cartilages ventral to basioccipital and first centrum. **B**: Close-up of Weberian ossicles, left side. **C**: Close-up of vertebral column from seventh to tenth vertebrae, left side, lateral view. **D**: Close-up of vertebral column from twentieth to twenty-fifth vertebrae, left side, lateral view. **E**: Caudal fin skeleton and supporting elements, left side, lateral view. APTr, anterior process of tripus; CSc, *concha scaphii*; DPR, dorsal procurvent rays; Ep1–2, epural 1–2; H1–6, hypural 1–6; HA, haemal arch; HS, haemal spine; HZ, haemal zygapophysis; In, intercalarium; Inm, intermuscular bones; MIn, *manubrium intercalarii*; NA, neural arch; NS, neural spine; NZ, neural zygapophysis; OS, os suspensorium; PArIn, *processus articularis* of intercalarium; PArSc, *processus articularis* of scaphium; PArTr, *processus articularis* of tripus; PAsIn, *processus ascendens* of intercalarium; PAsSc, *processus ascendens* of scaphium; PCdR, principal caudal-fin rays; Ph, parhypural; Ps, pleurostyle; Pu2–3, preural centrum 2–3; Rb, Rib; Sc, scaphium; Sn, supraneural; TPTr, transformator process of tripus; Tr, tripus; U, compound ural centrum; Un2, uroneural 2; V1–4, vertebral centra 1–4; V7–10, vertebral centra 7–10; V20–25, vertebral centra 20–25; VPR, ventral procurvent rays. Scale bars: 0.2 mm.

posttemporal has a pointed dorsal tip that articulates with the dorsal surface of the skull near the meeting point of parietal and supraoccipital. Its ventral tip is

slightly broader and more rounded and articulates with the dorsal tip of the supracleithrum. It also has a small medial process from which a short ligament

originates to insert on the intercalar. The narrow, elongate supracleithrum articulates dorsally with the posttemporal and is slightly wider ventrally. Its ventral tip is slightly curved posteriorly and overlaps the dorsal tip of the cleithrum laterally. Baudelot's ligament originates medially on this region of the supracleithrum and inserts on the posterior part of the lateral surface of the basioccipital anterior to the lagenar capsule. There are no laterosensory canals on the posttemporal and supracleithrum. The large cleithrum articulates dorsally with the supracleithrum, and with its counterpart in the ventral midline. It supports the scapulocoracoid cartilage (Fig. 7A). The cleithrum is narrower dorsally tapering to a pointed dorsal tip and wider ventrally. It has a prominent, hook-shaped and ventrally curved, posterior process (Fig. 7A, arrowhead) just above the dorsal base of the scapulocoracoid cartilage with a rounded distal tip. The single oval postcleithrum has an anterodorsal pointed margin and is laterally covered by this prominent posterior process of the cleithrum. The scapulocoracoid cartilage is dorsoventrally elongate and articulates with most of the posterior margin of the cleithrum along its medial surface. It has a long posterior coracoid process at its posteroventral corner where it is pierced by a comparatively large foramen. The posterior margin of the scapulocoracoid cartilage articulates with the cartilage of the pectoral radial plate. This cartilage plate shows a deep median incisure that almost completely separates an upper from a lower half (Fig. 7A).

Pelvic Girdle

The pelvic girdle consists of a pair of basiptyergia, each comprising a triangular base and an anterior elongate rod-like process (Fig. 7B,C). There is also a small flange of membrane bone along the medial aspect of the anterior process, which in females fails to reach its anterior end. The medial tip of the base has a short and well-ossified process directed ventrally. In males (Fig. 7C), the basiptyergium is much better ossified and more robust than in females (Fig. 7B), and the ventral process at its medial tip is more conspicuous. The medial flange of membrane bone is also wider in males and reaches the anterior end of the basiptyergium. There is no ischiatic process. The basiptyergium articulates with two or three pelvic radial cartilages of which only the lateral most is ossified at least partially. A pelvic splint and six pelvic-fin rays are present, of which the lateralmost is unbranched. The medialmost pelvic-fin radial is developed as a small cartilage in one of the females, but is absent from other specimens. Males have only two fully ossified pelvic radials. They also have more robust pelvic-fin rays with series of conspicuous bony hooks along the medial margins of fin rays 2–5 (Fig. 7C). The anterior tip of the basiptyergium is located approximately at

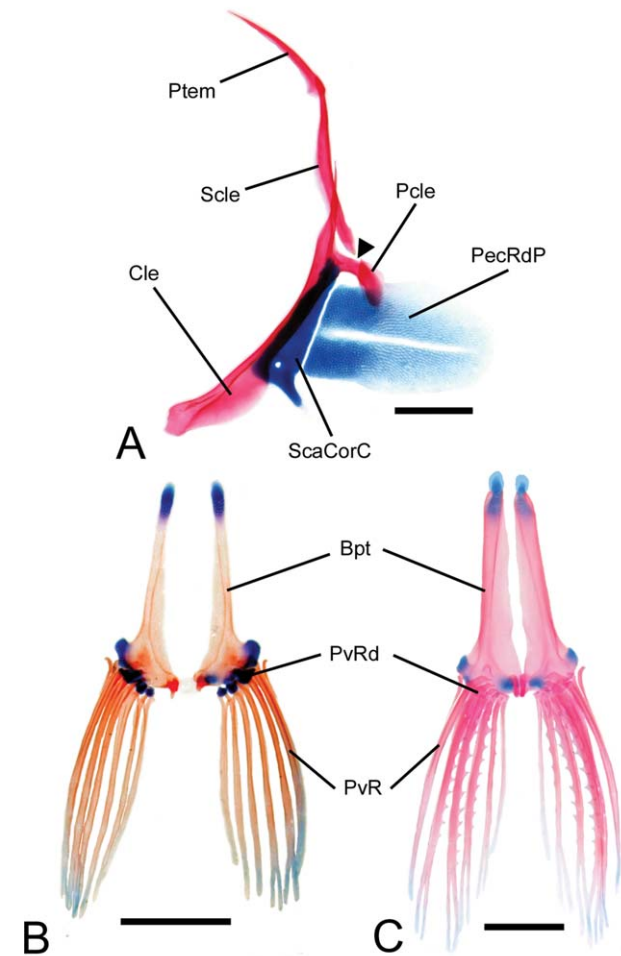


Fig. 7. *Priocharax* sp., all MZUSP 109199, paired fins. A: Pectoral girdle, right side, lateral view (image reversed), female, 13.3 mm SL, c&s. Arrowhead indicates posterior process of cleithrum. B: Pelvic girdle, ventral view, female, 13.3 mm SL, c&s. C: Pelvic girdle, ventral view, male, 13.5 mm SL, c&s. Bpt, basiptyergium; Cle, cleithrum; Pcle, postcleithrum; PecRdP, pectoral radial plate; Ptem, posttemporal; PvR, pelvic-fin rays; PvRd, pelvic-fin radials; ScaCorC, scapulocoracoid cartilage; Scle, supracleithrum. Scale bars: 0.5 mm.

the level of the tip of the sixth rib (Fig. 1B) in females, but reaches further anteriorly beyond the fifth rib in males (Fig. 1C). Males have both basiptyergia articulating with each other (Fig. 7C), while those of females are separated and connected only via a short ligament (Fig. 7B).

Dorsal Fin

There are 10 proximal-middle radials with 10 serially associated distal radials (Fig. 8A). The first pterygiophore supports two unbranched dorsal-fin rays, of which the anterior ray is in a supernumerary position. It inserts between the neural spines of vertebrae 12–13 (between 11 and 12 in one female). The remaining nine pterygiophores each support one branched ray. The supernumerary, first dorsal-fin

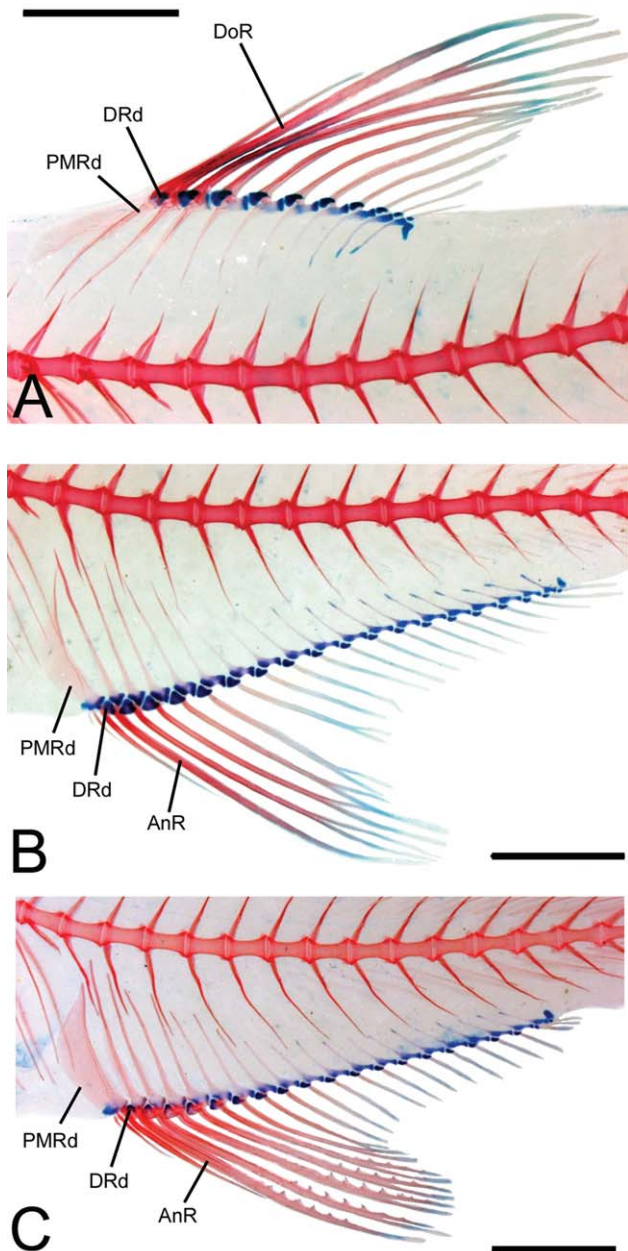


Fig. 8. *Priocharax* sp., all MZUSP 109199, unpaired median fins. **A**: Dorsal fin of female, left side, lateral view, 13.3 mm SL, c&s. **B**: Anal fin of female, left side, lateral view, 13.3 mm SL, c&s. **C**: Anal fin of male, left side, lateral view, 13.5 mm SL, c&s. AnR, anal-fin ray; DoR, dorsal-fin ray; DRd, distal radial; PMRd, proximal-middle radial. Scale bars: 1 mm.

ray is approximately half the length of the second, serially associated, dorsal-fin ray. The seven anteriormost proximal-middle radials are well ossified, but the three posterior proximal-middle radials have only thin perichondral lamellae around the middle of their cartilages precursors. The first proximal-middle radial supports an anteriorly directed median flange of membrane bone along its anterior margin. The anterior tip of this flange extends up to the level

of centra 12 and 13. The subsequent 2–3 proximal-middle radials show flanges of membrane bone along their anterior, posterior, and lateral edges. The proximal-middle radials of the dorsal fin appear to be relatively better ossified in males, with the four anteriormost elements completely ossified, and the fifth to eighth proximal-middle radials with only small cartilaginous portions at their dorsal tips. The dorsal-fin stay is short, completely cartilaginous, and confluent with the distal tip of the posteriormost proximal-middle radial cartilage.

Anal Fin

There are 20 proximal-middle radials with 20 serially associated distal radials (19 proximal-middle and distal radials in four females). The first anal-fin pterygiophore inserts in front of the hemal spine of vertebra 15 or 16 and supports four unbranched anal-fin rays, of which the first three are in a supernumerary position. All remaining pterygiophores each support one branched or segmented anal-fin ray in a serial association (Fig. 8B,C). The three anterior supernumerary anal-fin rays are very short, with the third reaching approximately half the length of the anteriormost serially associated anal-fin ray. The first unbranched, serially associated anal-fin ray and the subsequent four to six branched anal-fin rays are much longer than more posterior fin rays, creating the appearance of a notch at the anterior third of the ventral profile of the anal fin. This notch is more conspicuous in males (Fig. 8C). Males also possess four to eight pairs of bony hooks along the posterior margins of their first serially associated unbranched and four subsequent branched anal-fin rays (Fig. 8C). The anterior eight to nine proximal-middle radials are completely ossified except for their ventral tips which remain cartilaginous. The posteriormost ten to twelve proximal-middle radials are only developed as thin perichondral ossifications around the midlength of their cartilage precursors, with the degree of ossification decreasing sequentially posteriorly. The first proximal-middle radial shows a posteriorly concave curvature and bears a curved flange of membrane bone along its anterior margin. This anterior flange is more pronounced in males and large females and extends up to the dorsal tip of the first proximal-middle radial. The anterior seven to ten distal radials are predominantly cartilaginous, with small perichondral ossification on their middle portions. The posteriormost six to ten distal radials are cartilaginous. The anal-fin stay is cartilaginous and almost entirely separated from the distal tip of the posteriormost proximal-middle radial cartilage.

Caudal Fin and Caudal Fin Skeleton

Priocharax sp. has 19 (10 + 9) principal caudal-fin rays and seven to ten dorsal and six to eight ventral procurrent rays. Caudal-fin rays are supported by the neural and haemal spines of preural centra 2 and 3, the two epurals, the pleurostyle, uroneural 2, the six hypurals, and the parhypural (Fig. 6E). There is usually a small single dorsal caudal radial cartilage and a single, slightly larger, ventral caudal radial cartilage. The haemal spine of preural centrum 3 supports the ventral-most procurrent caudal-fin rays. The pleurostyle bears a flange of membrane bone along its dorsal margin. The base of the parhypural is fused to the compound centrum and its distal tip is cartilaginous. It bears a small flange of membrane bone on its anterior edge. The first hypural is separated from the compound centrum by a gap. The second hypural is fused to the compound centrum and has its distal third largely cartilaginous, with perichondral ossification in some specimens. There are four hypurals above the second hypural, each successively smaller dorsally. The third hypural of one specimen retains cartilage remnants near its anterior tip, and all hypurals show a cartilaginous distal tip. An opisthural cartilage is present in all specimens at the posterior tip of the notochord.

DISCUSSION

Priocharax: Proportioned Dwarf or Developmentally Truncated Characiform

Evolutionary decrease of body size results in miniature organisms that may be grouped into two categories (Rüber et al., 2007; Britz and Conway, 2009; Britz et al., 2014): the organism is either a miniature copy of the adult of a close relative, a proportioned dwarf (*sensu* Gould, 1971) or it resembles early ontogenetic stages of larger close relatives. Britz and Conway (2009) introduced a method to determine the level of skeletal truncation of a miniature fish by mapping its lost and reduced bones onto an ontogenetic sequence of ossification of a nonminiaturized close relative. Applying this method to Asian cyprinids, they concluded that the miniature *Paedocypris* and *Danionella* species are highly developmentally truncated and that species of *Boraras* of the same size-class are proportioned dwarves. We were interested in evaluating the level of skeletal truncation of *Priocharax* sp. because it is characterized by numerous bone losses when compared to other nonminiature characids. We utilized Mattox et al.'s (2014) study on the sequence of ossification of the entire skeleton of *Salminus brasiliensis*, a large putative basal characid (*sensu* Mirande, 2009, 2010) for comparison. Of the 147 bones that are present in the adult *Salminus*, 18 are missing in *Priocharax* sp.: claustrum, coracoid, dorsal-fin bony stay, extrascapular, infraorbitals 4, 5, and 6, meso-

coracoid, nasal, pectoral-fin radials, pectoral-fin rays, pelvic-fin medial radial, two postcleithra, post-Weberian supraneurals, scapula, sclerotic bones, and supraorbital. In addition, *Priocharax* sp. also lacks the rhinosphenoid and suprapreopercle, two bones present in many characiforms, but absent in *Salminus* (Roberts, 1969). When bone losses in *Priocharax* sp. are mapped onto the sequence of ossification of *Salminus* (Fig. 9), it is evident that most bones that are missing in *Priocharax* sp. are those that ossify late in the ontogeny of *Salminus*. The bone losses in *Priocharax* sp. can thus be interpreted as a typical case of developmental truncation through terminal deletion at an organism-wide level similar to, but not nearly as extensive as, the developmental truncation hypothesized for *Paedocypris* (Britz and Conway, 2009; Britz et al., 2014) and *Danionella* (Britz et al., 2009). This provides additional support to the idea that the last elements to form in development are often the first to be lost in evolution (e.g., Mabee et al., 2000; Engeman et al., 2009; Mattox et al., 2014).

Developmental truncation is not only reflected in the complete loss of ossifications as those mentioned above but also characterized by several simplifications of bones, features that can also be related to miniaturization (e.g., Myers, 1958; Weitzman and Fink, 1983; Weitzman and Vari, 1988; Hanken and Wake, 1993; Britz and Conway, 2009; Britz et al., 2009). Although the neurocranium of *Priocharax* sp. has most of the ossifications expected in a characid skull, many bones are less ossified (e.g., basioccipital, mesethmoid, orbitosphenoid, parasphenoid, vomer; Fig. 2). The ethmoid region is largely cartilaginous with parts of the ethmoid plate and *lamina orbitonasales* persisting. In the otic region, most bones are bordered by extensive areas of cartilage and the epiotic lacks the typical lateral arm present in most characiforms (i.e., epioccipital bridge *sensu* Weitzman and Fink, 1983: 354), resulting in a single wide posttemporal fossa (Fig. 3B) instead of the two separate fossae present in most members of the order. A similar condition has already been interpreted as related to miniaturization in a few characids such as *Iotabrycon praecox* (Roberts, 1973) *Paracheirodon simulans*, *Tytocharax madeirae* (Weitzman and Fink, 1983), *Hasemanian nana* (Mirande, 2010), *Cyanogaster noctivaga* (Mattox et al., 2013), in addition to a few miniature crenuchids such as *Klausewitzia aphanes* (= *Odontocharacidium aphanes*) (Weitzman and Fink, 1983) and the lebiasinid *Pyrrhulina australis* (Mirande, 2010). During the development of *Salminus* (Mattox et al., 2014) the lateral arm of the epiotic is the last part of that bone to develop, so its absence in these many miniatures may be interpreted as an ontogenetic truncation at the character level, independently acquired in these different genera. Another simplification of the skeleton of *Priocharax* sp. is the lack of

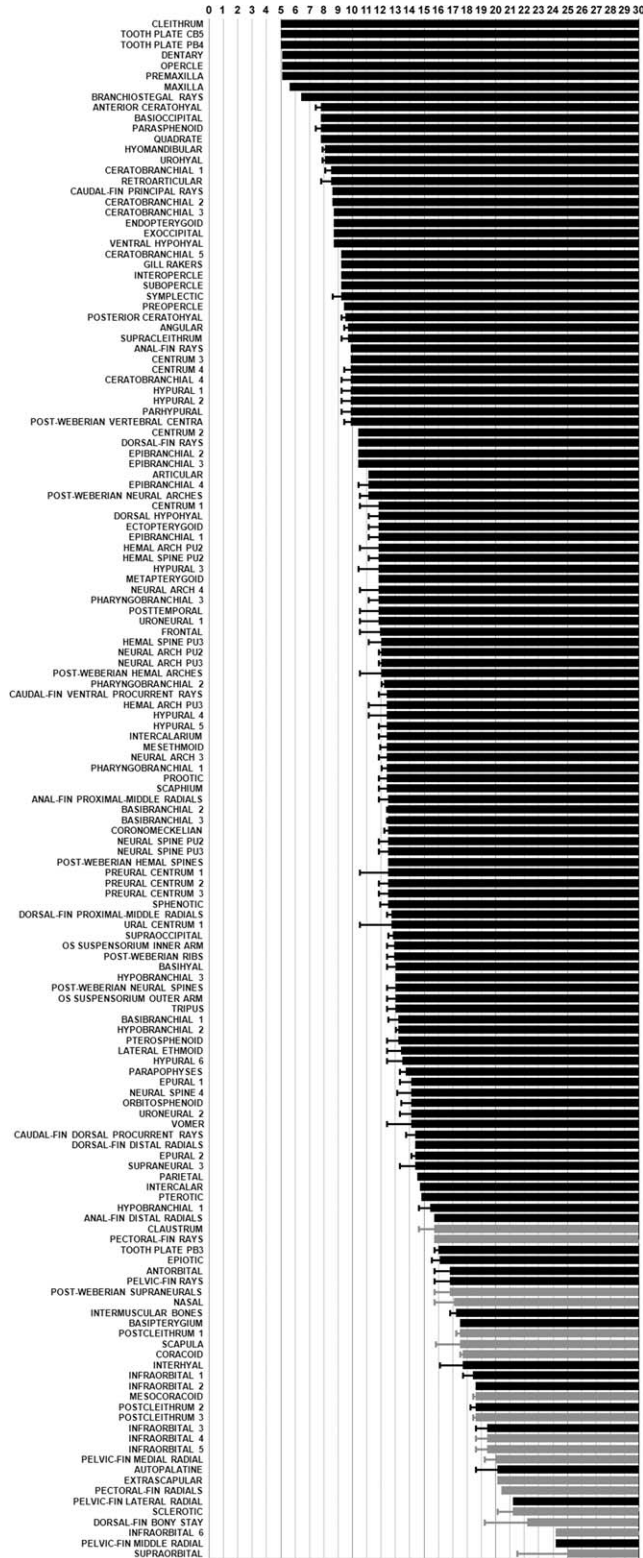


Fig. 9. Diagram to illustrate relation between whole sequence of ossification of *Salminus brasiliensis* (from Mattox et al., 2014) and losses in the skeleton of *Priocharax* sp. First appearance of ossifications shown as thin vertical line, fixed presence of ossifications shown as thick horizontal line during ontogeny of *Salminus*. Black bars represent ossifications present and gray bars ossifications absent in *Priocharax*.

most laterosensory canals that are typically present in adult characiforms (e.g., *Brycon meeki*, Weitzman, 1962: 65) on the body, the pectoral girdle, and the head (except for a rudimentary canal on the preopercle), another feature related to miniaturization (e.g., Myers, 1958; Weitzman and Fink, 1983; Weitzman and Vari, 1988).

The shape of the opercle in *Priocharax* sp. is also noteworthy because it greatly resembles that of early ontogenetic stages of *Salminus brasiliensis* (Mattox et al., 2014: 118, Fig. 8). In the latter species, the opercle starts to develop as a splint of bone from the hyomandibular condyle which will later become the medial longitudinal crest of the opercle. In early stages, the ossification of the opercle expands ventrally over the branchial chamber, leaving a gap in the dorsal region of the branchial chamber that is only covered by skin. This dorsal region of the branchial chamber starts to get covered by bone only when the opercle starts to grow dorsally from the crest later in ontogeny. The larval shape of the opercle in *Priocharax* sp. (Fig. 4A) is thus another example of developmental truncation at the character level. Additional examples may be found in the hyoid arch and the branchial arches, where the dorsal hypohyal (Fig. 4C) and the hypo- and pharyngobranchials remain predominantly cartilaginous (Fig. 5A and 5C). Parts of the axial and fin skeletons also show a developmentally truncated condition, such as the cartilaginous supraneurals, the persistence of largely cartilaginous portions in the dorsal- and anal-fin pterygiophores (Fig. 8), and in the pelvic-fin radials. The most conspicuous developmental truncation in *Priocharax* sp. is the persistence of the scapulocoracoid cartilage and the cartilaginous pectoral radial plate in the pectoral girdle, which will be discussed in detail below (Fig. 7A).

The 18 missing bones in the skeleton of *Priocharax* sp. represent 12% of the full complement of bones in *Salminus*, and there are 15 additional simplifications in the skeleton. The Asian miniature cyprinid *Paedocypris*, studied in detail by Britz & Conway (2009), was found to lack 37 out of the 145 bones present in the nonminiature and nontruncated cyprinid *Danio rerio*. This is more than 25% of the full complement of bones, with 18 additional reductions of bone complexity. Although *Priocharax* sp. is not as developmentally truncated as *Paedocypris*, it is a miniaturized fish with some level of organism-wide developmental truncation and not a proportioned dwarf (*sensu* Gould, 1971). Of the large number of miniature characiforms (compare list in Toledo-Piza et al., 2014), the osteology of only few has been studied in sufficient detail. The miniature *Cyanogaster noctivaga* was considered a proportioned dwarf by Mattox et al. (2013) due to its mostly complete skeleton and the same seems to apply to other miniature characiforms

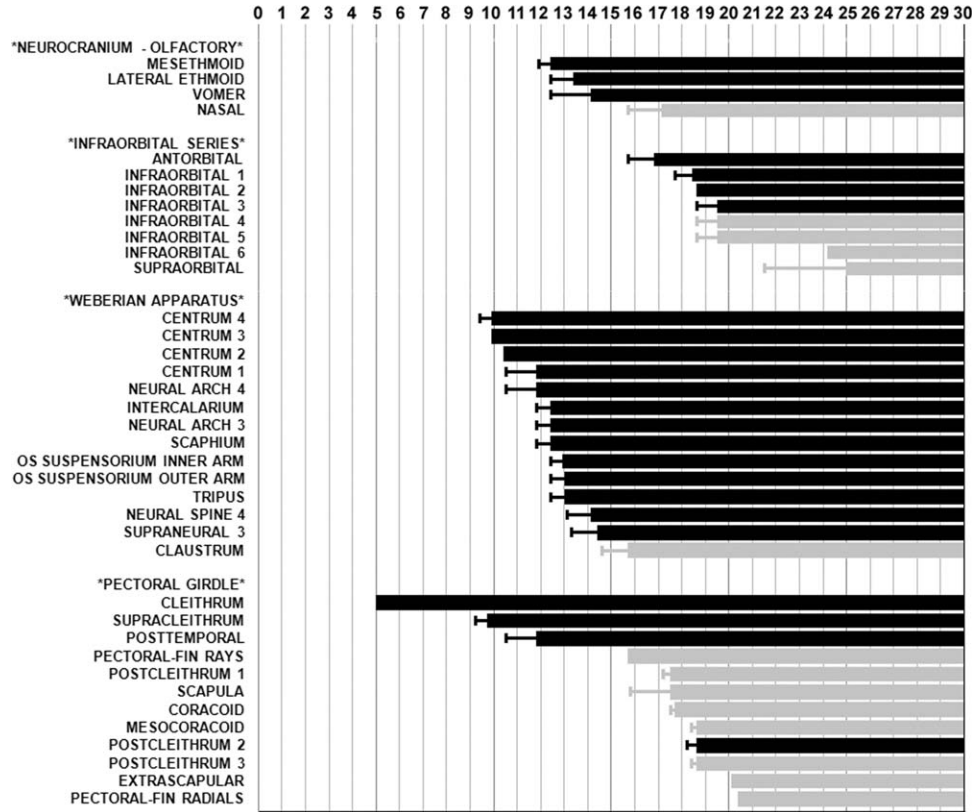


Fig. 10. Diagram of selected anatomical complexes showing relation between sequence of ossification of *Salminus brasiliensis* (from Mattox et al., 2014) and losses in the skeleton of *Priocharax* sp. First appearance of ossifications shown as thin vertical line, fixed presence of ossifications shown as thick horizontal line during ontogeny of *Salminus*. Black bars represent ossifications present and gray bars ossifications absent in *Priocharax*.

such as *Lepidarchus adonis* (Roberts, 1966), *Iotabrycon praecox* (Roberts, 1973), *Paracheirodon innesi* and *P. simulans* (Weitzman and Fink, 1983), and *Amazonspinther dalmata* (Bührnheim et al., 2008). Therefore, *Priocharax* sp. may still hold the title of the most developmentally truncated characiform.

Truncations at the Level of Anatomical Complexes

The theme of terminal deletion in development when the entire skeleton is considered is even more prominent when the different anatomical complexes are evaluated separately (Fig. 10). Truncation of the pectoral fin in *Priocharax* sp. is the most conspicuous example of this pattern (Fig. 7A). The presence of a larval pectoral fin has been used as one of the outstanding diagnostic features of *Priocharax* (Weitzman and Vari, 1987; Toledo-Piza et al., 2014). In fact, adults of *Priocharax* are unique among characiforms in lacking the entire bony endoskeleton of the pectoral fin (i.e., coracoid, mesocoracoid, radials, scapula, propterygium) in addition to a number of dermal elements such as the extrascapular, one or two postcleithra and, uniquely, pectoral-fin radial

plate combined with absence of pectoral-fin rays and any ossification in the pectoral endoskeleton is a highly unusual feature even among teleosts and shared, as far as we know, only with the highly developmentally truncated teleosts *Schindleria* (Johnson and Brothers, 1993) and *Sundasalanx* (Roberts, 1981). There is a remarkable similarity between the structure of the pectoral girdle of the adult *Priocharax* sp. and that of a larval *Salminus brasiliensis* at approximately 11 mm SL (see Mattox et al., 2014: 135, fig. 17). In other words, out of the 12 bones in the pectoral girdle of the adult *Salminus*, *Priocharax* sp. has only four. Three of these actually develop before 11 mm SL in *Salminus*. Also noteworthy is the fact that the first bone of the pectoral-fin endoskeleton of *Salminus* to develop is the scapula at 17.5 mm SL, a size that exceeds the maximum known SL of the three species of *Priocharax* (17.1 mm SL in *P. ariel*, 16.4 mm SL in *P. pygmaeus* and 15.4 mm SL in *P. nanus*, Weitzman and Vari, 1987: 642; Toledo-Piza et al., 2014: 231).

Characiforms, in general, have a delayed completion of the ossification of the endoskeletal pectoral girdle in relation to the rest of the skeleton. The first endoskeleton bone to develop in the pectoral girdle of the cyprinid *Danio*, for instance, is

the coracoid (Cubbage and Mabee, 1996; Grandel and Schulte-Merker, 1998), which is the 102nd element to ossify in the whole skeleton (Britz and Conway, 2009), while in *Salminus* the first endoskeleton bone to appear in the pectoral girdle is the scapula as the 127th element to develop in the whole skeleton (Mattox et al., 2014). The endoskeletal elements in *Salminus* only ossify much later in ontogeny when most of the remaining skeleton is already ossified (Mattox et al., 2014). In fact, this striking delay to complete the development of the endoskeletal pectoral girdle in characiforms is evidenced by the retention of a larval pectoral fin fold and radial cartilage plate in juveniles between 22 and 41 mm SL of species of some genera such as *Charax*, *Roeboides*, *Phenacogaster*, *Cynopotamus*, *Galeocharax*, and *Oligosarcus* (Lucena, 1998: 44). It seems that this heterochronic shift of pectoral-fin development in characiforms has been taken to an extreme in *Priocharax* sp.

In the original description of *Priocharax*, Weitzman and Vari (1987: 644–645) mentioned the lack of all ossified elements other than the cleithrum, posttemporal, and supracleithrum in the pectoral girdle. In all specimens examined herein, however, there is one postcleithrum associated with the posterior process of the cleithrum (Fig. 7A). Examination of paratypes of *P. ariel* revealed that these specimens, although in poor condition, lack postcleithra as mentioned by Weitzman and Vari (1987). In contrast, the recently described *P. nanus* has two postcleithra (Toledo-Piza et al., 2014): a thin elongate rod more ventrally located, identified as postcleithrum 3, and a large, round dorsal element associated with the posterior process of the cleithrum. Based on position only, Toledo-Piza et al. (2014) could not decide if that element represented postcleithrum 1 or 2, but they noted that in characiforms with only two postcleithra, these are either postcleithra 1 and 2, or postcleithra 2 and 3. This led Toledo-Piza et al. (2014) to suggest that *P. nanus* had postcleithra 2 and 3, but that additional evidence was needed.

All specimens of *Priocharax* sp. lack the elongate, rod-like postcleithrum 3, and have only the round element, which is associated with the posterior process of the cleithrum. Based on its position and shape, the single postcleithrum of *Priocharax* sp. is hypothesized here as homologous to the dorsal postcleithrum of *P. nanus*, which we interpret as postcleithrum 2.

Priocharax sp. has a conspicuous truncation in the development of infraorbitals (Figs. 4B and 10). As discussed by Mattox et al. (2014), infraorbitals start to develop relatively late in the ontogeny of *Salminus*, with infraorbital 6 and the supraorbital being among the last three bones to develop in the entire skeleton (Fig. 9). Consequently, infraorbital 6 and supraorbital are more likely to be lost due to developmental truncation, and these bones are

indeed absent in many miniature characiforms (e.g., Vari, 1979; Weitzman and Fink, 1983; Zanata and Vari, 2005; Mattox and Toledo-Piza, 2012). Loss of bones of the infraorbital series is even more extreme in *Priocharax* sp., which lacks infraorbitals 4, 5, and 6, in addition to the supraorbital. Of the remaining infraorbitals, infraorbital 3 of *Priocharax* sp. is also greatly reduced in size and complexity when compared to the common condition in characiforms (e.g., *Brycon meeki*, Weitzman, 1962: 64–65; *Salminus brasiliensis*, Mattox et al., 2014: 113–115). Few other characids have such a reduction in which all posterior infraorbitals are missing and only infraorbitals 1, 2, and 3 are present. These include the stevardiini *Iotabrycon praecox* (Roberts, 1973), the cheirodontin *Amazonspither dalmata* (Bührnheim et al., 2008) in addition to *Axelrodia riesei*, *Hemigrammus erythrozonus*, the species of *Paracheirodon* and *Tytocharax madeirae* (Weitzman and Fink, 1983), all of which are tiny or miniature species.

Interestingly, Weitzman and Vari (1987) mentioned that the antorbital was the only bone in the infraorbital series of both *P. ariel* and *P. pygmaeus*. All specimens examined herein, however, have three poorly ossified infraorbitals in addition to the antorbital. These infraorbitals are very thin and inconspicuous and can easily be overlooked. Still, examination of paratypes of *P. ariel*, although in poor condition, corroborate the observations of Weitzman and Vari (1987). The recently described *P. nanus* also lacks all infraorbital bones except for the antorbital (Toledo-Piza et al., 2014: 234). Whether the variation in the presence/absence of infraorbital bones in *Priocharax* has taxonomic relevance depends on a study focused on the taxonomy of the genus, a matter that lies beyond our present scope.

Although less conspicuous, other anatomical complexes of *Priocharax* sp. also show evidence of terminal deletion, the Weberian apparatus being one of them (Figs. 6A,B and 10). To our knowledge, *Priocharax* is the only characiform genus for which species have been recorded to lack the claustrum, a feature already mentioned by Weitzman and Vari (1987) for *P. ariel* and *P. pygmaeus*. *P. nanus* is unique among its congeners in having the claustrum (Toledo-Piza et al., 2014), a diagnostic feature for this species. The claustrum is the last element of the Weberian apparatus to ossify in the development of *Salminus* and its absence in *P. ariel*, *P. pygmaeus* (Weitzman and Vari, 1987) and all specimens examined herein is interpreted as another terminal deletion at the level of an anatomical complex. In addition to the loss of the claustrum, the neural complex is only weakly ossified and extensive cartilaginous portions remain dorsal to the neural arches of centra 3 and 4 (Fig. 6A). The neural spine of centrum 4 is also very short. Another conspicuous feature putatively associated with

developmental truncation is the wide gap between neural arches 3 and 4. This condition was illustrated, but not described, in *P. ariel* by Weitzman and Vari (1987: 645) and was also noted in *P. nanus* (Toledo-Piza et al., 2014: 235). In addition to *Priocharax*, the only other characid known to us to show this unusual gap in the Weberian apparatus is the miniature *Cyanogaster noctivaga* (Mattox et al., 2013), a species probably more closely related to the Stevardiinae than to the Heterocharacinae.

Priocharax sp. lacks the nasal (Figs. 2A and 10), which is the last bone to appear in the olfactory region of the neurocranium of *Salminus* (Mattox et al., 2014). This bone is present in most other characiforms except in some miniatures such as *Lepidarchus adonis* (Roberts, 1966: 210), *Hyphessobrycon elachys* and the tiny *Coptobrycon bilineatus* (Mirande, 2010: 400). Given the closer relationship of *Lepidarchus* to other Alestidae (Zanata and Vari, 2005) and of *Hyphessobrycon* and *Coptobrycon* to other clades within the Characidae (Mirande, 2010) rather than to the Heterocharacinae, the loss of the nasal likely occurred independently in *Priocharax* sp. and reflects an additional case of terminal deletion at the level of an anatomical complex.

Miniaturization, Morphological Novelty, and Sexual Dimorphism

Miniaturization is an important and widespread process among animals and the miniaturized phenotype is usually characterized by a combination of structural reductions or simplifications and morphological novelty, sometimes resulting in “novel *bauplans* associated with the origin of higher taxa” (Hanken and Wake, 1993: 513). Extreme examples of such morphological novelties include complex modifications of the os suspensorium and pelvic girdle in the miniature *Paedocypris* (Britz and Conway, 2009) and the presence of pointed bony expansions of the dentary and upper jaw bones resembling teeth in *Danionella dracula* (Britz et al., 2009), both southeast Asian cyprinids with a high degree of developmental truncation. Although *Priocharax* sp. has lost 18–20 bones compared to other characiforms, it does not have the degree of organismal truncation seen in *Paedocypris* and *Danionella*, in which 42 and 44 bones, respectively, fail to develop in addition to many other simplifications of the skeleton (Britz and Conway, 2009; Britz et al., 2009, 2014). *Priocharax* sp. also does not show any striking morphological novelties. The only exception here is the presence of a small autogenous articular cartilage between the autopalatine and the ascending process of the maxilla (Fig. 4A, arrow). This cartilage is present on both sides of all specimens and, as far as we know, it is not developed in any other characid. Similarly, autogenous small unpaired cartilages are present ventral to the posterior portion of the basioccipital and the two anteriormost vertebral

centra (Figs. 2A and 6B), which are unique among characiforms and may constitute subtle novelties.

Another common feature of miniaturized species is the presence of conspicuous sexual dimorphism (Hanken and Wake, 1993). Several characters related to sexual dimorphism were observed in *Priocharax* sp., many of which show only subtle differences between males and females, while others appear as striking differences reported here for the first time. Among the subtle sexual dimorphisms are the relatively larger size of the coronoid process in females and the more developed ossification of dorsal-fin pterygiophores and of the flange on the first anal-fin pterygiophore in males. Males of *Priocharax* sp. show several hooks on the anal-fin rays (Fig. 8C), a feature already mentioned for *P. ariel* by Weitzman and Vari (1987: 645) and widespread among the Characidae (e.g., Malabarba and Weitzman, 2003; Mirande, 2010; Mattox and Toledo-Piza, 2012; Camelier and Zanata, 2014). Contrary to what Weitzman and Vari (1987) observed for both *P. ariel* and *P. pygmaeus* [no mature males of *P. nanus* were examined by Toledo-Piza et al. (2014)], all of our male specimens of *Priocharax* sp. show also conspicuous hooks on the pelvic fin (Fig. 7C), a feature less common than anal-fin hooks (Camelier and Zanata, 2014). The basipterygium of males is also much better ossified and reaches further anteriorly extending beyond the vertical through the rib of the fifth centrum (Fig. 1C) while that of females is less ossified and ends further posteriorly falling short of the vertical through the rib of the sixth centrum (Fig. 1B). In addition, the contralateral basipterygia of males are in direct contact at their median processes (Fig. 7C), while those of females approach each other but fail to make contact with only a ligament between them (Fig. 7B).

Phylogenetic Relationships of *Priocharax*

As with other miniature species (e.g., *Danionella*, *Paedocypris*, Rüber et al., 2007; Britz and Conway, 2009; Britz et al., 2014), the phylogenetic position of *Priocharax* is difficult to establish due to the difficulties associated with the coding of characters in highly truncated taxa. Britz et al. (2014) demonstrated how different coding strategies for reductive and absent characters influence the outcome of a phylogenetic analysis.

Lucena (1998) was the first to include *Priocharax* in a phylogenetic analysis along with members of 11 additional genera that were later classified as the Characinae (Lucena and Menezes, 2003). His study was based on 59 characters and 25 terminal taxa including an all-zero outgroup. Seven of the 59 characters were coded as inapplicable for *Priocharax*, and only four of the remaining 52 characters were coded differently from the all-zero outgroup taxon. It is hardly surprising

TABLE 1. Coding of 150 characters of *Mattox & Toledo-Piza(2012)* in *Priocharax* sp.

	1-10	11-20	21-30	31-40	41-50	51-60	61-70
A	10?2?10?01	1????????	?????????	?????????	?????????	?????????	?????????
71-80	81-90	91-100	101-110	111-120	121-130	131-140	141-150
?????????	?????????	?????????	?????????	????1????	?????????	?????????	??????1?1?
B	1-10	11-20	21-30	31-40	41-50	51-60	61-70
	1002000001	1????????	?????????	?????0000-	00101-00-0	0000001003	1---1---0-
71-80	81-90	91-100	101-110	111-120	121-130	131-140	141-150
0001001000	0000001010	1001-10000	1000111121	0101110100	0101--0001	-----00001	0-01111000
C	1-10	11-20	21-30	31-40	41-50	51-60	61-70
	1002000001	1????????	?????????	?????0000-	<u>20101-00-0</u>	000000 <u>2334</u>	1---1---0-
71-80	81-90	91-100	101-110	111-120	<u>121-130</u>	<u>131-140</u>	141-150
0001001000	000000 <u>2010</u>	<u>1202-20000</u>	1000111121	0101110100	010 <u>2</u> --0001	-----00001	0-011110 <u>30</u>

A: original coding by Mattox and Toledo-Piza (2012); **B:** new coding presented herein based on recently collected specimens of *Priocharax* sp., character states as in Mattox and Toledo Piza (2012) except for character 60, which is coded as 3 (i.e. absence of IO4); **C:** new coding presented herein incorporating a priori interpretations based on ontogenetic data of *Salminus brasiliensis*. The 11 characters highlighted in bold and underlined refer to those reinterpreted under the ontogenetic scheme explained in the text. Question marks refer to characters that could not be scored due to poor condition of specimens (most ? in A) or because they are myological characters (? for characters 12-35 in A, B, C) not studied in *Priocharax* sp. Hyphens in B and C refer to unapplicable characters due to reduction or absence of given structures.

that such a coding practice resulted in *Priocharax* being resolved as the sister group to all remaining characines in Lucena’s (1998) cladogram. Britz et al. (2014) pointed out that the interpretation of lack of ossifications as primary absences had also lead Mayden and Chen (2010) to erroneously consider numerous absences of *Paedocypris* as plesiomorphies, while Britz and Conway (2009) had already demonstrated that they were secondary absences. Two of the four characters coded in *Priocharax* differently from the outgroup taxon (Lucena, 1998) are more widespread and reflect two general conditions in the Characinae and the Heterocharacinae (*sensu* Mattox and Toledo-Piza, 2012): the presence of more than 20 teeth on the maxilla and the absence of multiple cusps on the teeth (i.e., presence of unicuspid, conical teeth). The third character is the large size of the pseudotympanum extending ventrally, a condition shared with *Gnathocharax*, *Heterocharax*, *Hoplocharax*, and *Lonchogenys*. Such a condition of the pseudotympanum was hypothesized as synapomorphic for the Heterocharacinae including *Priocharax* by Mattox and Toledo-Piza (2012). The fourth character is the presence of two rows of gill rakers on ceratobranchial 2. All specimens examined herein, however, have only a single row of gill rakers on ceratobranchial 2 (Fig. 5A), a difference that may be due to geographic or species-level variation.

More recently, Mattox and Toledo-Piza (2012) restricted the Characinae based on the analysis of 150 characters and 64 taxa and included *Priocharax* in the distantly related Heterocharacinae (with *Heterocharax*, *Hoplocharax*, *Gilbertolus*, *Gnathocharax*, *Lonchogenys*, and *Roestes*). However, due to the poor condition of the available material at that time and the reductive nature of most of the characters in *Priocharax*, 139 of the 150 characters were coded as inapplicable for this taxon. Nevertheless, two hypotheses of relation-

ships of *Priocharax* were discussed by Mattox and Toledo-Piza (2012: 894), both including *Priocharax* in a clade with *Heterocharax*, *Hoplocharax* and *Lonchogenys*. In one of the hypotheses, *Priocharax* appears as the sister group to the clade (*Lonchogenys* (*Heterocharax* *Hoplocharax*)). The other hypothesis identified *Priocharax* as derived among the three species of *Heterocharax*. The two hypotheses varied only in terms of optimization of ambiguous characters.

In this study, new and better preserved material of *Priocharax* was available and we were able to reassess the results of Mattox and Toledo-Piza (2012). Based on the examination of new specimens, *Priocharax* was recoded in the matrix of Mattox and Toledo-Piza (2012). This decreased the number of missing entries from 139 to 43 (Table 1). In addition, two characters were reinterpreted in relation to the states proposed by Mattox and Toledo-Piza (2012): characters 6 and 149. A scale sheath along the anal-fin base (character 6) was coded as present in *Priocharax* by Mattox and Toledo-Piza (2012: 815) based on sparse scales seen in some poorly preserved specimens. None of the specimens studied herein, however, have such a scale sheath and this character was coded as absent. Similarly, Mattox and Toledo-Piza (2012) coded the contact of hypurals 2 and 3 (character 149) as present along the entire length of both bones, but observations of more and better preserved specimens revealed that these bones are actually separate along most of their length.

A phylogenetic analysis of the new matrix produced after most of the previously inapplicable characters of Mattox and Toledo-Piza (2012) were recoded (Table 1B), using the same parameters, resulted in three equally most parsimonious hypotheses of relationships among the Heterocharacinae (Fig. 11A–C). In all three hypotheses, *Priocharax* was recovered as the most basal taxon

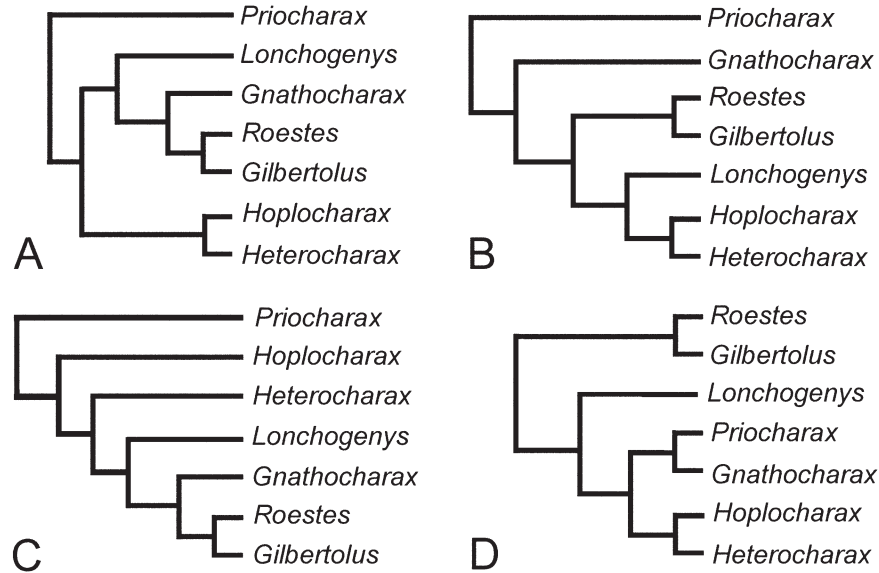


Fig. 11. Hypotheses of *Priocharax* relationships with other Heterocharacinae (*sensu* Mattox and Toledo-Piza, 2012). **A–C**: Three equally most parsimonious hypotheses based on matrix modified from Mattox and Toledo-Piza (2012), Table 1B, with 98 characters recoded based on recently collected specimens of *Priocharax* examined herein. **D**: Single most parsimonious hypothesis based on matrix modified from Mattox and Toledo-Piza (2012), Table 1C, with 98 characters recoded based on recently collected specimens of *Priocharax* examined herein and eleven characters reinterpreted following ontogenetic information based on Mattox et al. (2014). See text for further explanation.

of the Heterocharacinae with the tribe Roestini (*Roestes* and *Gilbertolus*) nested among genera previously included in the tribe Heterocharacini (*Heterocharax*, *Hoplocharax*, *Gnathocharax*, and *Lonchogenys*) by Mattox and Toledo-Piza (2012).

As an alternative approach, we also used evidence from the skeletogenesis and sequence of ossification of the characid *Salminus brasiliensis* recently described by Mattox et al. (2014), and reinterpreted a priori many of the reductive characters of *Priocharax* as conditions exclusive to that genus that resulted from developmental truncations due to the process of miniaturization (Table 1C).

In other words, whenever we had ontogenetic evidence from *Salminus* to interpret a given absence or reduction in *Priocharax* as a developmental truncation secondarily acquired due to miniaturization, the character was coded as an exclusive autapomorphic state for *Priocharax*. Eleven characters were thus coded differently from the previous matrix (Table 1B,C, bold underlined characters). The rhinosphenoid (character 41) and the autogenous infraorbital 4 (character 60) were both coded as secondarily absent in *Priocharax*. The absence of postcleithrum 3 (character 124) in *Priocharax* sp. (discussed above) was also coded as a secondary loss in this species based on the fact that at least one species of the genus, *P. nanus*, possesses that ossification. The remaining eight characters represent simplifications of bony elements that clearly resemble early ontogenetic stages of *Salminus* (see Mattox et al., 2014): character 57, loss of overlap between laminar expansion of infraorbital 1 and dorsolateral portion of

maxilla; character 58, loss of anteroventral projection of infraorbital 2; character 59, loss of contribution of infraorbital 2 to outer border of orbital ring; character 87, loss of anterodorsal projection of metapterygoid; character 92, loss of spiniform projection on posterior margin of preopercle; character 94, loss of bony lamella on anterior margin of basihyal; character 96, canal on anterior ceratohyal opening on posterodorsal region of this bone; character 149, loss of contact between hypurals 2 and 3.

The analysis of the matrix using information from development yielded a single most parsimonious hypothesis of relationships within the Heterocharacinae (Fig. 11D). Both tribes (Heterocharacini and Roestini) were recovered as monophyletic as proposed by Mattox and Toledo-Piza (2012), and *Priocharax* is included within the Heterocharacini as sister group of *Gnathocharax*. The sister group relationship between *Priocharax* and *Gnathocharax* proposed herein is based on seven synapomorphies. Three of them are bone losses shared by both taxa: absence of infraorbital 6, supraneural 4, and postcleithrum 1 (*Gnathocharax* lacks all three postcleithra). Two of them are considered reversals to plesiomorphic conditions from derived states present in other heterocharacines: neural prezygapophyses and postzygapophyses diminutive, approximately triangular and without contact with adjacent zygopophyses, and profile of posterior margin of opercle slightly concave, further reversed to straight to convex in *Priocharax*. Two other synapomorphies represent derived features: lower pharyngeal tooth plate with denticles concentrated along medial and

posterior margins (Fig. 5B), and articulation between contralateral basiptyrgia, the latter being sexually dimorphic and present only in males of *Priocharax* (Fig. 7C).

Comparison between our two analyses reinforces that many of the seemingly plesiomorphic features in *Priocharax* cannot be distinguished from derived paedomorphic character states without developmental information (e.g., Weitzman and Vari, 1987: 649). Coding absences and reductions in *Priocharax* in the same way as absences in outgroups leads to a basal position of this genus in relation to the remaining heterocharacines in our analysis B (Fig. 11A–C). Similarly, the large amount of putative plesiomorphic states attracted *Priocharax* to the base of the cladogram in Lucena's (1998) analysis. Alternatively, when ontogenetic information is incorporated and characters are revised accordingly, the misleading interpretation produced by these putatively plesiomorphic conditions is avoided resulting in a more plausible hypothesis of relationships of this miniature characiform.

CONCLUSION

Our results show that *Priocharax* sp. is a developmentally truncated species with many conspicuous reductive features, the most striking of them being the larval pectoral-fin skeleton. Although not as truncated as some miniatures from southeast Asia, *Priocharax* sp. is the most truncated characiform known to date. We used developmental information from a putative basal characid to reinterpret a number of morphological characters of *Priocharax*, yielding a novel hypothesis of a close relationship with the diminutive heterocharacine *Gnathocharax*. The Neotropical ichthyofaunal region harbours more than 200 miniature fishes, the richest in the world in terms of miniature fish species (Toledo-Piza et al., 2014). These include members of all the main lineages of Neotropical fishes as potential candidates for similar studies (Britz and Conway, 2009), and we hope that our results will stimulate others to follow this line of research.

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