

Osteology of the African annual killifish genus *Callopanchax* (Teleostei: Cyprinodontiformes: Nothobranchiidae) and phylogenetic implications

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> Abstract

Osteological structures of *Callopanchax* are first described and illustrated. Twenty-six characters derived from comparisons of osseous structures among some aplocheiloid fishes provided evidence supporting hypotheses of relationships among three western African genera (*Callopanchax*, *Scriptaphyosemion* and *Archiaphyosemion*), as proposed in recent molecular analysis. The clade comprising *Callopanchax*, *Scriptaphyosemion* and *Archiaphyosemion* is supported by a laterally displaced antero-proximal process of the fourth ceratobranchial. The sister group relationship between *Callopanchax* and *Scriptaphyosemion* is supported by a constriction on the posterior portion of the parasphenoid, an anterior expansion of the hyomandibula, a rectangular basihyal cartilage, an anterior pointed process on the first vertebra, and a long ventrally directed hemal prezygapophysis on the preural centrum 2. Monophyly of *Callopanchax* is supported by a convexity on the dorsal margin of the opercle, a long interarcual cartilage, and long neural prezygapophyses on the anterior caudal vertebrae.

> Key words

Killifishes, *Callopanchax*, Africa, Osteology, Annual fishes.

Introduction

The Aplocheiloidei is a diversified clade of cyprinodontiform fishes, with over 500 valid species occurring in freshwater biotopes of most tropical and subtropical areas of the world. Presently they are grouped into three families: the Aplocheilidae, endemic to southern Asia and Madagascar; the Nothobranchiidae, endemic to continental Africa; and, the Rivulidae, endemic to Americas (COSTA, 2004). All aplocheilids and several nothobranchiids and rivulids live in shallow streams and swampy areas, whereas other nothobranchiids and rivulids inhabit seasonal pools formed during rainy periods. The latter aplocheiloids are known as annual fishes, since during the dry season all individuals die, but eggs undergo in diapause, buried in the bottom sediment for months, waiting for the next rainy season. Thus, every year an entire new generation arises when eggs emerge after the first rains.

Osteology has been an important source of morphological characters to erect hypotheses of relationships among aplocheiloid families (PARENTI, 1981;

COSTA, 1998a, 2004) and among genera and species of the Rivulidae (*e. g.*, COSTA, 1998b, 2005, 2006a, b). However, only recently osteological traits have been fully described and illustrated for some rivulid lineages (*e. g.*, COSTA, 2005, 2006a, 2006b). On the other hand, systematics of the Nothobranchiidae is still incipient, with genera and subgenera poorly defined, and little is known about osteology (AMIET, 1987; AARN & SHEPHERD, 2001). However, a number of recent molecular studies provide hypotheses of nothobranchiid taxa relationships, corroborating monophyly of some previously proposed genera and subgenera (*e. g.*, MURPHY & COLLIER, 1997, 1999; MURPHY *et al.*, 1999; SONNENBERG *et al.*, 2006). Molecular data support nothobranchiid clades presently not diagnosed by morphological synapomorphies, making clear the need of improving the morphological data base for this group.

Callopanchax MYERS is an annual fish genus, endemic to the region of western Africa encompassing Guinea, Sierra Leone and Liberia. It has a central position in the most controversial debates on taxonomy and phylogeny of nothobranchiids. The name *Cal-*

hobranchius included all African annual killifishes. On the other hand, *Archiaphyosemion* and *Scriptaphyosemion* were considered as subgenera of *Aphyosemion*, which would be the sister group to the clade containing *Fundulopanchax* and *Nothobranchius*. However, most recent phylogenetic studies, based on molecular data, corroborate monophyly of clade comprising *Callopanchax*, *Archiaphyosemion* and *Scriptaphyosemion*, which were not closely related to the clade comprising *Aphyosemion* and *Fundulopanchax* (MURPHY & COLLIER, 1997, 1999; MURPHY *et al.*, 1999).

The aim of the present study is to describe and illustrate the osteological traits of *Callopanchax*, comparing to other aplocheiloid species in order of searching for characters useful to test monophyly of *Callopanchax* and its phylogenetic position among nothobranchiids.

Materials and methods

Material is deposited in Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro (UFRJ). All material listed here was prepared according to TAYLOR & VAN DYKE (1985). Generic placement of species follows MURPHY & COLLIER (1999) and MURPHY *et al.* (1999). Osteological descriptions were based on all species of *Callopanchax* (*Callopanchax monroviae*: UFRJ 6277, 2; *Callopanchax occidentalis*: UFRJ 6275, 2; UFRJ 6281, 2; *Callopanchax toddi*: UFRJ 6555, 2), but only osteological traits of *C. monroviae* were illustrated. Thirteen aplocheiloid species were used in the phylogenetic analysis. Since all informative characters were identical for the three species of *Callopanchax*, only two species were included in the data matrix. The analysis also included nine representatives of distinct nothobranchiid lineages (*Aphyosemion aureum*: UFRJ 4812, 1; *Aphyosemion herzogi*: UFRJ 4611, 1; *Archiaphyosemion petersi*: UFRJ 3907, 2; *Fundulopanchax gularis*: UFRJ 626, 1; *Fundulopanchax moensis*: UFRJ 6267, 1; *Epiplatys bifasciatus*: UFRJ 4111, 3 (c&s); *Epiplatys chaperi*: UFRJ 619, 2; *Nothobranchius korthausae*: UFRJ 6413, 2; *Scriptaphyosemion guignardi*: UFRJ 4110, 4) to test conflicting hypothesis about the phylogenetic position of *Callopanchax* (PARENTI, 1981; MURPHY & COLLIER, 1997); and, one basal rivulid species (*Kryptolebias brasiliensis*: UFRJ 3682, 1; UFRJ 4603, 2) and one basal aplocheilid species (*Aplocheilus lineatus*: UFRJ 3147, 2) as outgroups. All characters were binary, except characters 14 and 20, which were treated as non-ordered. The data matrix was analyzed with PAUP (D. L. SWOFFORD, PAUP*; Phylogenetic Analysis Using

Parsimony (*and other methods), Beta version 4.0b2, Sinauer Associates, Sunderland, Massachusetts), using the branch and bound algorithm, with accelerated transformation (ACCTRAN) to optimize characters. Branch support was evaluated using bootstrap analysis of 1,000 replicates.

Results

Superficial dermal bones and neurocranium

Figs. 1A–D

Nasal thin, scale-like. Lachrymal subtriangular, carrying distinctive lateral canal, anterior tip slightly twisted, posterior and ventral portions laminar. Dermosphenotic thin, ovoid, its outer surface gently concave. Frontal trapezoidal and thin. Parietal short, ovoid, without keels. Supraoccipital with short, paired posterior process. Epitotic small, without dorsal process. Sphenotic with prominent narrow lateral process. Vomer trapezoidal, with elongated posterior process; 4–5 teeth concentrated on anterocentral portion of vomer. Mesethmoid unossified. Lateral ethmoid compact, its anterior portion overlapping lateral portion of vomer; medial margin separated by broad space from anterior process of parasphenoid. Parasphenoid cross-shaped; distal portion of anterior process narrow and dorsally overlapping posterior process of vomer, proximal portion widened; posterior process firmly and ventrally attached to basioccipital, constricted on proximal portion, abruptly widening towards distal region; short lateral processes ventrally attached to prootic.

Jaws, jaw suspensorium and opercular apparatus

Figs. 2A–B

Premaxilla narrow, with teeth on medial half of anterior face; ascending process subtriangular, slightly curved distally to form slight concavity on posterior medial margin; subdistal portion of premaxilla with anteriorly directed, triangular process. Maxilla subcylindrical, proximal portion bifid. Rostral cartilage large, round. Dentary robust, with teeth on distal two thirds of its anterodorsal surface. Angulo-articular triangular, with wide ventral process. Retro-articular small. Coronomeckelian cartilage elongate. Premaxillary and dentary teeth conical, with slightly curved tip; teeth arranged in irregular rows, largest teeth on most external row.

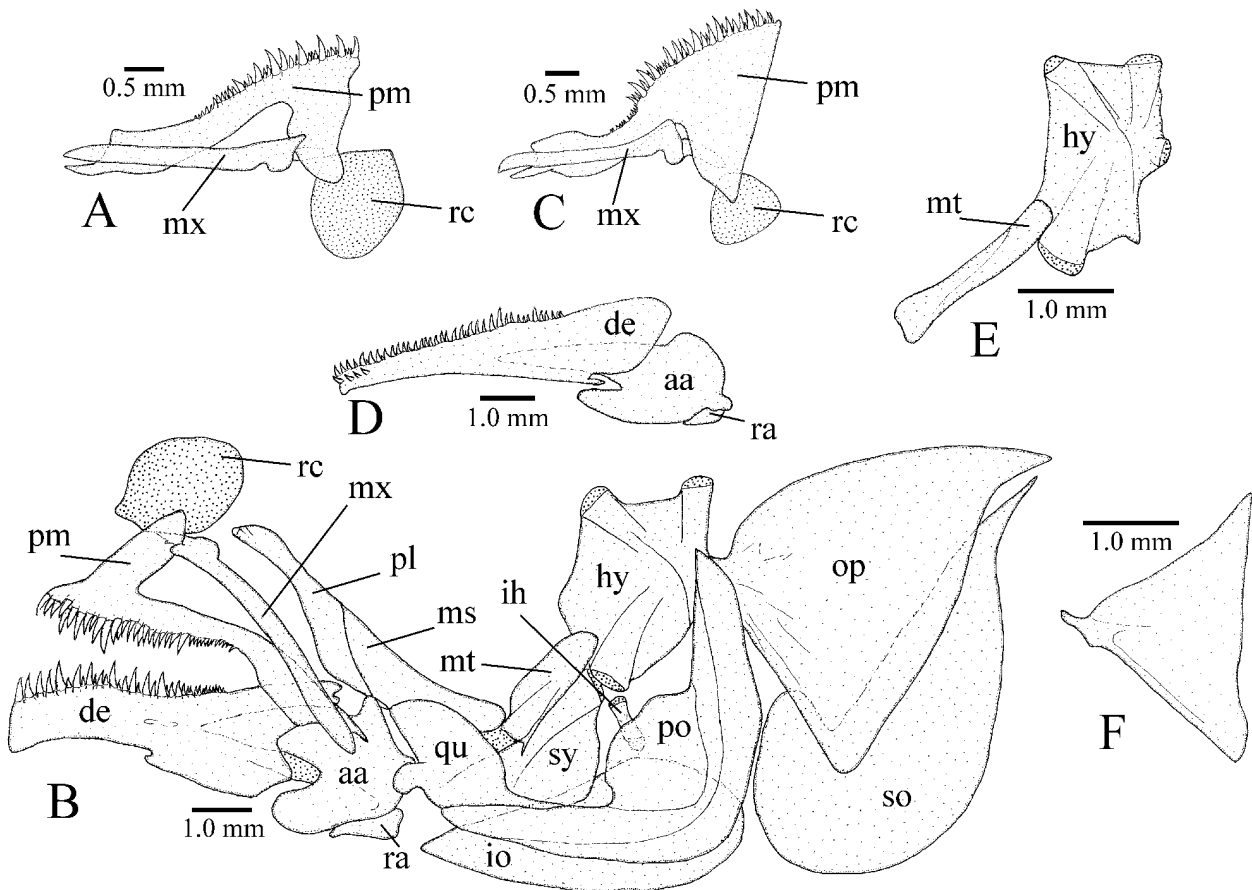


Fig. 2. Jaws, jaw suspensorium and opercular apparatus. **A–B:** *Callopanchax monroviae*; **C–D, F:** *Aplocheilus lineatus*; **E:** *Epiplatys bifasciatus* (A, C, left upper jaw, dorsal view; B, general lateral view of left jaws, jaw suspensorium and opercular apparatus; D, left lower jaw, lateral view; E, left hyomandibula and metapterygoid, lateral view; F, left opercle, lateral view); aa, angulo-articular; de, dentary; hy, hyomandibula; ms, mesopterygoid; mt, metapterygoid; mx, maxilla; op, opercle; pl, autopalatine; pm, premaxilla; po, preopercle; qu, quadrate; ra, retro-articular; rc, rostral cartilage; so, subopercle; sy, symplectic. Larger stippling indicates cartilage.

Autopalatine and ectopterygoid completely fused. Subdistal portion of autopalatine with small medial projection, supporting ligament connected to maxilla. Mesopterygoid thin, long, posteriorly abutting metapterygoid. Ventral portion of mesopterygoid overlapping dorsal portion of the quadrate. Quadrate approximately triangular, anterior and ventral margins approximately straight, dorso-posterior margin convex; posterior process of quadrate subcylindrical, short. Symplectic triangular, deep. Metapterygoid subrectangular, shorter than mesopterygoid. Hyomandibula compact, with prominent curved lateral crest between anterodorsal condyle and posteroventral tip of hyomandibula, separated from ventral condyle of hyomandibula by broad laminar extension; anterior margin of hyomandibula slightly expanded anteriorly. Opercle subtriangular, dorsal margin convex; posterior border of opercle and all borders of interopercle and subopercle membranous and poorly ossified. Preopercle C-shaped, robust, with distinct vertical canal and expanded anteromedian flap; dorsal and anterior ends slightly pointed.

Hyoid and branchial arches

Figs. 3A–D

Basihyal about pentagonal in dorsal view, flattened; cartilaginous portion subrectangular, about twice longer than triangular osseous portion; basihyal width about 60 % of total basihyal length. Dorsal and ventral hypohyals ossified. Anterior process of anterior ceratohyal cylindrical, abruptly narrowing posteriorly, with two narrow branchiostegal rays attached to ventral border; anterior ceratohyal compressed and deep in its posterior portion, rectangular in lateral view. Anterior and posterior ceratohyals separated by space of cartilage, supporting four branchiostegal rays gradually becoming wider posteriorly. Posterior ceratohyal compressed, subtriangular. Interhyal ossified, cylindrical, attached to posterior end of posterior ceratohyal. Urohyal thin, deep, with short ventrolateral flap; anterodorsal process minute or absent.

Second pharyngobranchial approximately triangular, small, wider than long, lacking teeth. Third

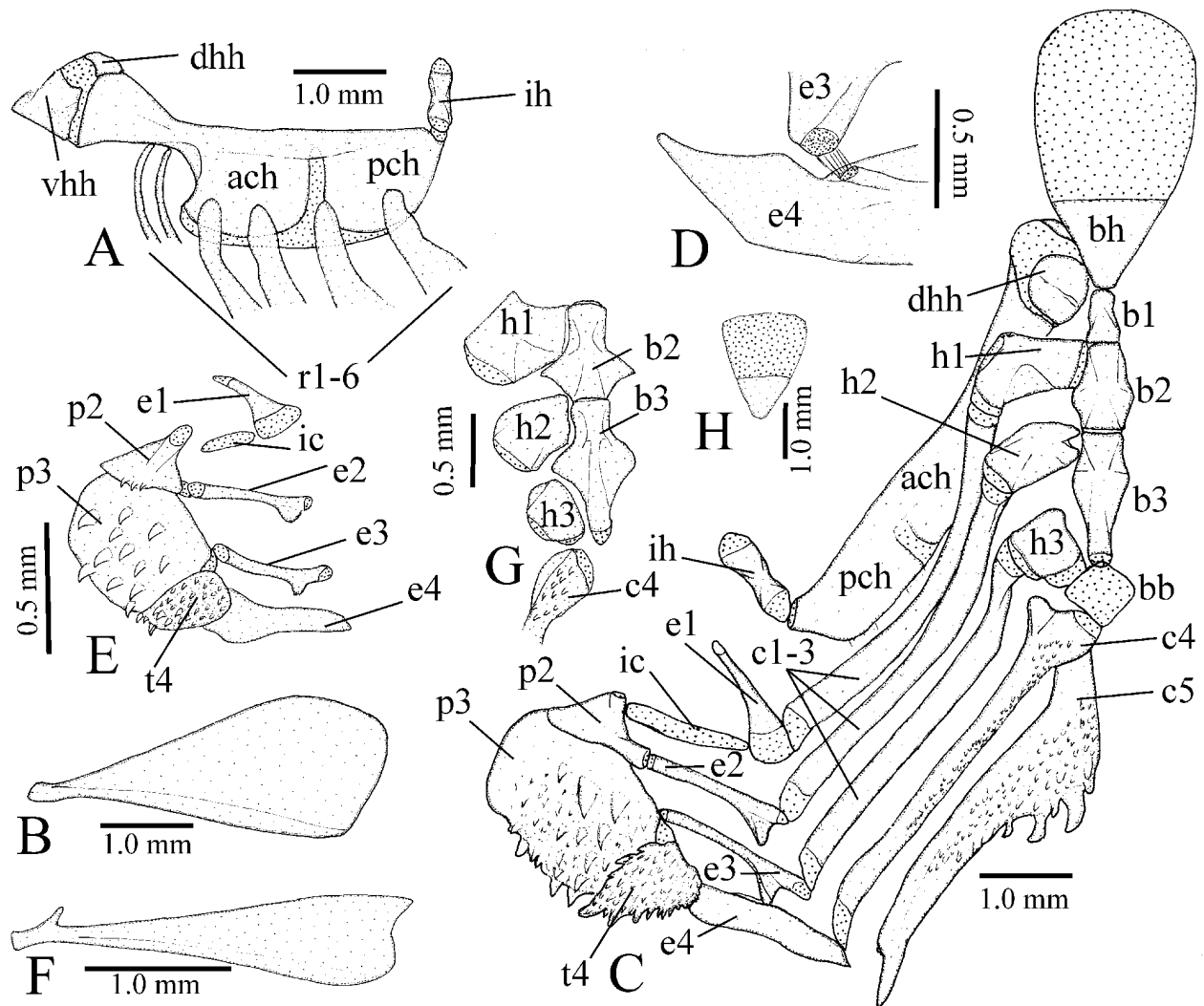


Fig. 3. Hyoid and branchial arches. **A–D:** *Callopanchax monroviae*; **E–F:** *Epiplatys bifasciatus*; **G:** *A. herzogi*; **H:** *Aplocheilus lineatus* (A, left hyoid bar, lateral view; B, F, urohyal, left lateral view; C, left and middle hyoid and branchial arches, ventral view of dorsal portion and ventral view of ventral portion; D, detailed dorsal view left epibranchials 3 and 4 connection; E, left dorsal portion of branchial arches, ventral view; G, mid-left portion of ventral branchial arches, dorsal view; H, basihyal, dorsal view); ach, anterior ceratohyal; b1–3, basibranchials 1–3; bb, basibranchial cartilage; bh, basihyal; c1–5, ceratobranchials 1–5; dhh, dorsal hypohyal; e1–4, epibranchials 1–4; h1–3, hypobranchials 1–3; p2–3, pharyngobranchials 2–3; pch, posterior ceratohyal; r1–6; branchiostegal rays 1–6; t4, tooth plate pharyngobranchial 4; vhh, ventral hypohyal. Larger stippling indicates cartilage.

pharyngobranchial wide, with well-developed denticerous plate containing large conical, slightly curved teeth. Fourth pharyngobranchial tooth plate with numerous small conical teeth. Epibranchials 2–3 and interarcual cartilage narrow and long, epibranchial 1 slightly shorter with proximal portion widened, fourth epibranchial longer and wider. Second epibranchial with pronounced triangular subdistal process. Third epibranchial with long uncinat process directly connected to fourth epibranchial by ligaments. Interarcual cartilage laterally attached to anterior tip of second pharyngobranchial and to proximal portion of 1st epibranchial.

Basibranchials 1–3 ossified; basibranchial 1 smaller, trapezoidal in dorsal view; basibranchial 2

wider, hexagonal; basibranchial 3 longer, hexagonal; basibranchial cartilage, wide, lozenge-shaped. Hypobranchial 1 pentagonal in dorsal view, anterior edge longer, straight, medial edge straight, without vestige of branching; hypobranchial 2 transversely elongated, medial edge branched; hypobranchial 3 lozenge-like. Ceratobranchials 1–3 elongate, without teeth; ceratobranchial 4 elongate, with numerous minute teeth; ceratobranchial 5 with well-developed denticerous plate containing conical, slightly curved teeth. Proximal tip of fourth ceratobranchial wider than proximal tip of ceratobranchials 1–3, with an anterior expanded flap. Fifth ceratobranchial boomerang-shaped, anterior portion shorter, on longitudinal axis, posterior portion longer, slightly laterally displaced.

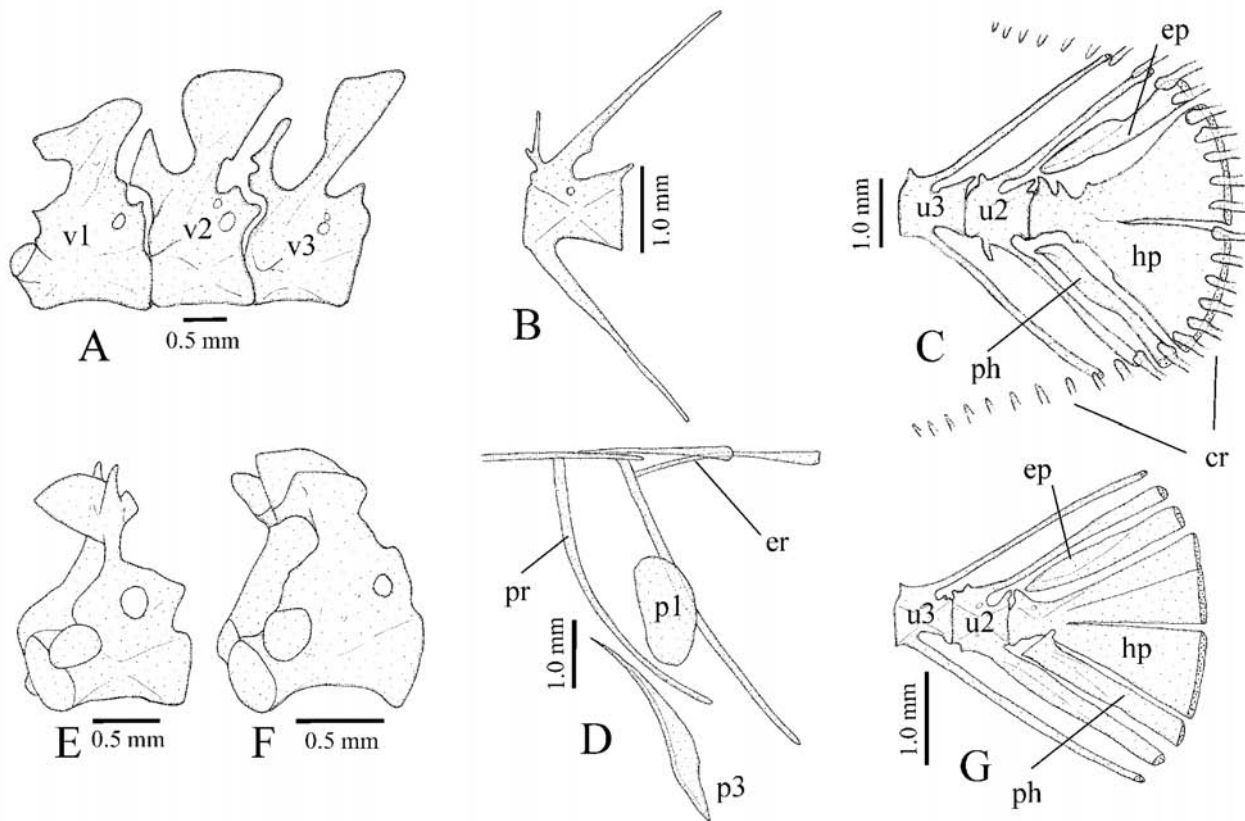


Fig. 4. Vertebrae and caudal fin skeleton. **A–D:** *Callopanchax monroviae*; **E:** *Scriptaphyseosmion guignardi*; **F:** *Epiplatys bifasciatus*; **G:** *Aplocheilus lineatus* (A, first three vertebrae, left lateral view; B, fourth caudal vertebrae, left lateral view; C, G, caudal fin skeleton, left lateral view; D, left ribs of first three vertebrae and associated post-cleithra, lateral view; E, D, first vertebra, anterolateral view); cr, caudal-fin rays; er, epipleural ribs; ep, epural; hp, hypural plate; pr, pleural rib; ph, parhypural; u2–3, preural centra 2–3; v1–3, vertebrae 1–3. Larger stippling indicates cartilage.

Vertebrae and caudal-fin skeleton

Figs. 4A–D

Neural spine of 1st vertebra with long anterior laminar extension, and short neural pre- and post-zygapophyses. Second neural spine wider than first and second, longer than first and so long as third; vertebrae posterior to third vertebra with rod-like neural spine. Neural prezygapophyses of vertebrae 2 and 3 long, neural postzygapophyses short. Pleural ribs on all precaudal vertebrae except the first; epipleural ribs narrow, gradually widening and flattening distally. Neural prezygapophyses of anterior caudal vertebrae long; neural postzygapophyses of caudal vertebrae short. Hemal spine of preural centrum 2 approximately so wide as hemal spines of anterior vertebrae. Hemal prezygapophysis of preural centrum 2 lengthened, directed ventrally. One or two pointed short processes on dorsal surface of compound caudal centrum. Epural and parhypural similar in shape, laminar, with proximal portion pointed. Hypurals ankylosed except for median horizontal gap to form two triangular plates without vestige of former hypural limits. Accessory caudal cartilages absent.

Shoulder and pelvic girdles

Figs. 5A–B

Posttemporal forked, fused to supracleithrum. Dorsal portion of cleithrum about triangular, somewhat elongated, with reduced posterior flange. Ventral tip of cleithrum distant from ventral tip of coracoid. Scapula about rectangular in lateral view, coracoid about triangular. Proximal radials quadrangular, ventralmost radial separated by interspace from coracoid. First postcleithrum scale-like, second postcleithrum absent, third postcleithrum rod-like, sometimes flattened. Pubic bone narrow, with minute ischial process.

Dorsal and anal-fin support

Figs. 5C–D

Dorsal-fin origin between neural spines of vertebrae 13 and 15; anal-fin origin between pleural ribs of vertebrae 14 and 15. Two rays associated to first proximal radial of dorsal fin, and two or three associated to first proximal radial of anal fin. Proximal radials narrow. Median and distal radials ossified.

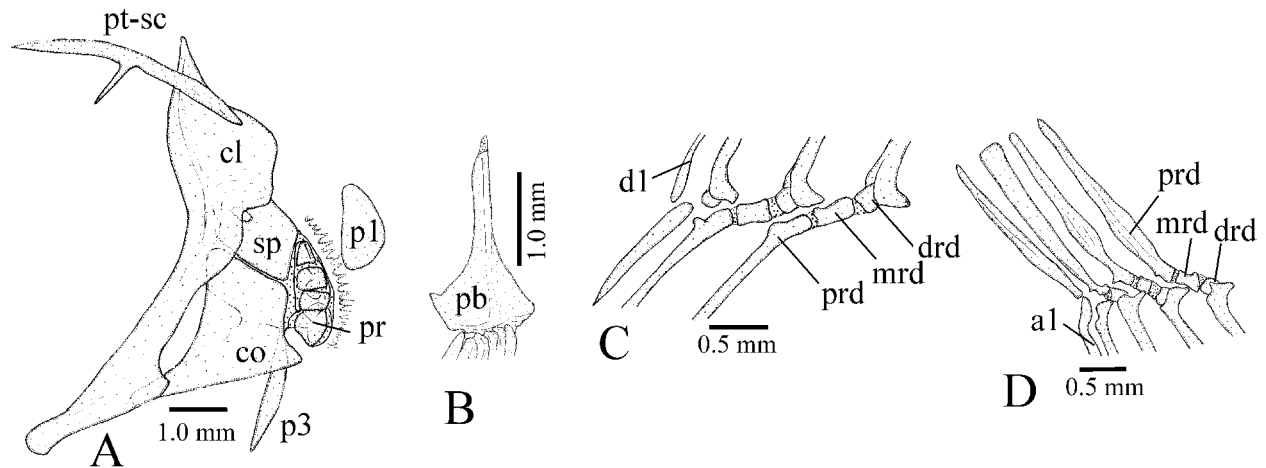


Fig. 5. Fin support of *Callopanchax monroviae*. **A:** left shoulder girdle, left lateral view; **B:** left pelvic girdle, dorsal view; **C:** anterior portion of dorsal-fin support, left lateral view; **D:** anterior portion of anal-fin support, left lateral view; a1, first anal-fin ray; cl, cleithrum; co, coracoid; d1, first dorsal-fin ray; drd, distal radial; mrd, median radial; p1–3, post-cleithra 1–3; pb, pelvic bone; prd, proximal radials; pt–sc, posttemporal-supracleithrum; sp, scapula. Larger stippling indicates cartilage.

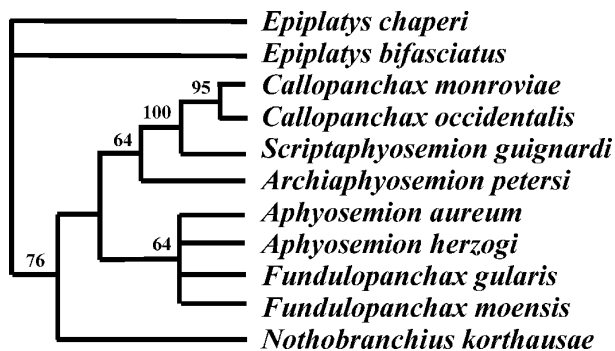


Fig. 6. Most parsimonious phylogeny among 11 species of the Nothobranchiidae (three length = 34; consistency index = 0.82; retention index = 0.86; rescaled consistency index = 0.71). Numbers above branches are bootstrap values. Outgroups not included.

Phylogenetic analysis

Characters found to have informative variability to erect the phylogenetic hypothesis among nothobranchiids lineages are listed below. Distribution of character states among terminal taxa is presented in the data matrix (Table 1). The most parsimonious cladogram of phylogenetic relationships among 11 terminal nothobranchiid taxa is illustrated in Fig. 6.

1. *Anterior arm of parasphenoid*: 0. subdistal portion not distinctively widened; 1. subdistal portion distinctively widened (Fig. 1B).
2. *Posterior arm of parasphenoid*: 0. subdistal portion gradually narrowing (Fig. 1G); 1. subdistal portion abruptly narrowing to form distinctively constricted zone (Fig. 1B).
3. *Parietal*: 0. long, rectangular (Fig. 1E, F); 1. short, elliptical (Figs. 1A).

4. *Anterior portion of parietal*: 0. widening (Fig. 1F); 1. not widening (Fig. 1A, E).
5. *Premaxilla and dentarium*: 0. long (Fig. 2A, B); 1. short (Fig. 2C, D).
6. *Coronoid process of dentary*: 0. posteriorly projecting above vertical through dorsal tip of angulo-articular (Fig. 2D); 1. posterior portion at the same level of dorsal tip of angulo-articular (Fig. 2B).
7. *Posterior portion of premaxillary ascending process*: 0. gradually narrowing posteriorly (Fig. 2C); 1. posterior portion distinctively narrowed, presenting conspicuous medial concavity (Fig. 2A).
8. *Metapterygoid*: 0. long (Fig. 2E); 1. short (Fig. 2B); 2. minute.
9. *Anterior margin of hyomandibula*: 0. straight (Fig. 2E); 1. convex (Fig. 2B).
10. *Dorsal margin of opercle*: 0. approximately straight to slightly convex (Fig. 2F); 1. strongly convex (Fig. 2B).
11. *Urohyal*: 0. slender (Fig. 3F); 1. deep (Fig. 3B).
12. *Basihyal*: 0. triangular (Fig. 3H); 1. pentagonal (Fig. 3C).
13. *Interarcual cartilage*: 0. short (Fig. 3E); 1. long (Fig. 3C).
14. *Second pharyngobranchial teeth*: 0. numerous (10–16), in two rows; 1. few (1–6), single row; 2. teeth absent.
15. *Hypobranchials*: 0. narrow, separated by broad interspace (Fig. 3C); 1. wide, separated by narrow interspace (Fig. 3G).
16. *Antero-proximal process of 4th ceratobranchial*: 0. close to main axis of bone (Fig. 3G); 1. laterally displaced, often forming wide flap (Fig. 3C).

17. Pointed, anteriorly projected median process on first vertebra: 0. absent (Fig. 4F); 1. present (Fig. 4A, E).
18. Pleural ribs: 0. rod-like; 1. distally widening, often bifid (Fig. 4D).
19. Neural prezygapophyses of anterior caudal vertebrae: 0. short; 1. long (Fig. 4B).
20. Hypurals: 0. two dorsal plates, one ventral plate (Fig. 4G); 1. one dorsal and one ventral plates, both entirely ankylosed (Fig. 4C); 2. single entirely ankylosed plate.
21. Proximal end of parhypural: 0. with paired dorsal processes (Fig. 4G); 1. shortened and laminar (Fig. 4C).
22. Hemal spine of preural centrum two: 0. wide (Fig. 4G); 1. narrow (Fig. 4C).
23. Hemal prezygapophysis of preural centrum 2: 0. minute, anteriorly directed (Fig. 4G); 1. long, ventrally directed (Fig. 4C).
24. Dorsal processes on compound caudal centrum: 0. absent (Fig. 4G); 1. one or two processes projecting towards epural (Fig. 4C).
25. Dorsal-fin rays attached to first proximal radial: 0. one; 1. two (Fig. 5C).
26. Pectoral-fin insertion: 0. lateral; 1. ventrolateral (Fig. 5A).

Discussion

The phylogenetic analysis of osteological characters support clades previously defined by molecular data (MURPHY & COLLIER, 1997, 1999; MURPHY *et al.*, 1999), such as the clades comprising *Callopanchax*, *Scriptaphyosemion* and *Archiaphyosemion*, and *Aphyosemion* plus *Fundulopanchax* (Fig. 7). The clade comprising *Callopanchax*, *Scriptaphyosemion* and *Archiaphyosemion*, three sympatric genera endemic to western Africa, is here supported by the derived shape of the antero-proximal process of the fourth ceratobranchial, which is laterally displaced to form a wide lateral flap (Fig. 3C; character: 16.1). The clade comprising *Aphyosemion* and *Fundulopanchax*, two genera from central-western Africa, is corroborated by the expanded hypobranchials, which occupy most interspace areas (Fig. 3G; ch: 15.1).

The proposed sister group relationship between *Callopanchax* and *Scriptaphyosemion* (MURPHY & COLLIER, 1997; MURPHY *et al.*, 1999) is highly supported by five synapomorphies: subdistal portion of the posterior arm of the parasphenoid distinctively constricted (Fig. 1B; ch: 2.1), anterior margin of the hyomandibula with a distinct convexity (Fig. 2B; ch: 10.1), cartilaginous portion of the basihyal rectangu-

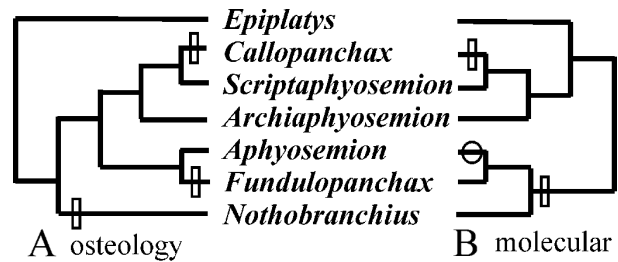


Fig. 7. Simplified cladograms depicting relationships among seven nothobranchiid genera: **A:** cladogram based on osteological data (present study; rectangles indicate origin of annualism); **B:** cladogram based on molecular data (MURPHY & COLLIER, 1999; MURPHY *et al.*, 1999; rectangles indicate origin of annualism, circle indicates loss of annualism, using ACCTRAN).

lar, yielding an overall pentagonal shape to basihyal (Fig. 3C; ch: 12.1), pointed, anteriorly projected median process on the first vertebra (Fig. 4A, E; ch: 17.1), and hemal prezygapophysis of the preural centrum 2 long and ventrally directed (Fig. 4C; ch: 23.2). An illustration of the parasphenoid of *Archiaphyosemion guineense* (AARN & SHEPHERD, 2001: fig. 11a), a species not available to the present study, shows a distinctively constricted posterior arm of the parasphenoid, as that herein described for *Callopanchax* and *Scriptaphyosemion* (Fig. 1B; ch: 2.1), but not present in *Archiaphyosemion petersi*. This observation supports the hypothesis indicating a paraphyletic condition of the genus *Archiaphyosemion*, in which *A. guineense* would be more closely related to *Callopanchax* and *Scriptaphyosemion* than to other species of *Archiaphyosemion* (MURPHY *et al.*, 1999).

The group comprising *Callopanchax*, *Scriptaphyosemion*, *Archiaphyosemion*, *Aphyosemion*, and *Fundulopanchax*, not corroborated in previous phylogenetic studies, is supported by the loss of second pharyngobranchial teeth (Fig. 3C; Ch: 14.1). No osteological character was found to support an assemblage containing *Nothobranchius*, *Aphyosemion* and *Fundulopanchax*, nor an assemblage including *Epiplatys*, *Callopanchax*, *Scriptaphyosemion* and *Archiaphyosemion*, as proposed by molecular studies (MURPHY & COLLIER, 1997, 1999; MURPHY *et al.*, 1999).

Monophyly of *Callopanchax* is supported by three synapomorphies: dorsal margin of the opercle with a conspicuous convexity (Fig. 2B; ch: 10.1), interarcual cartilage long (Fig. 3C; ch: 13.1), and neural prezygapophyses of the anterior caudal vertebrae long (Fig. 4B; ch: 19.1).

Annualism is a unique style of life cycle among teleost fishes, known to occur in African and South American aplocheiloids. The ability in to complete the entire life cycle in temporary pools implicates in the acquisition of a set of advanced characters, including behavior (bottom spawning), egg structure (thickened chorion), embryonic development (diapauses),

Tab. 1. Matrix of 26 osteological characters for 13 aplocheiloid taxa. Character and character states are numbered according text.

	1–10	11–20	21–26
<i>Aplocheilus lineatus</i>	0000000000	0000000000	000000
<i>Kryptolebias brasiliensis</i>	0000110000	1001001011	100011
<i>Aphyosemion aureum</i>	1011111100	1002100101	110111
<i>Aphyosemion herzogi</i>	1011111100	1002100101	110111
<i>Archiaphyosemion petersi</i>	1011111100	1002010101	110111
<i>Callopanchax monroviae</i>	1111111111	1112011111	111111
<i>Callopanchax occidentalis</i>	1111111111	1112011111	111111
<i>Epiplatys chaperi</i>	1001011000	0001000100	110111
<i>Epiplatys bifasciatus</i>	1001011000	0001000100	110111
<i>Fundulopanchax gularis</i>	1011111100	1002100101	110111
<i>Fundulopanchax moensis</i>	1011111100	1002100101	110111
<i>Nothobranchius korthausae</i>	0011111100	1001000102	110111
<i>Scriptaphyosemion guignardi</i>	1111111110	1102011101	111111

and physiology (rapid growth rate), making annualism a complex life style (*e. g.*, MURPHY & COLLIER, 1997; COSTA, 1998b). In a first phylogenetic approach (PARENTI, 1981), annualism was considered as having a double origin from non-annual aplocheiloid ancestors, arising independently once in South American and once in African aplocheiloids. Evidence supporting two independent origins of annualism among South American aplocheiloids was found by COSTA (1998b) and HRBEK & LARSON (1999), but a third independent annual Neotropical lineage has been recently reported (COSTA, 2006c).

Parenti's African annual clade comprised both *Nothobranchius*, a strict annual fish genus, and *Fundulopanchax* (including *Callopanchax* as subgenus), which contained both typical annual and semi-annual fishes (*i. e.* facultative annuals). However, under a new phylogenetic tree topology (MURPHY & COLLIER, 1997, 1999; MURPHY *et al.*, 1999), two alternative explanations for the evolution of annualism in African aplocheiloids may be provided. It would be equally parsimonious to assume three independent origins in the three annual fish genera (*i. e.*, *Callopanchax*, *Nothobranchius* and *Fundulopanchax*; DELTRAN option), or two independent origins, once in *Callopanchax* and once in the base of the clade *Nothobranchius* + *Fundulopanchax* + *Aphyosemion*, with a subsequent loss in *Aphyosemion* (ACCTRAN option, Fig. 7B). Among these two alternative hypotheses, the cladogram topology derived from the analysis of osteological features parsimoniously supports the hypothesis of a triple annualism origin, once in each annual fish genus (Fig. 7A).

Acknowledgments

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