The caudal skeleton of extant and fossil cyprinodontiform fishes (Teleostei: Atherinomorpha): comparative morphology and delimitation of phylogenetic characters

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Abstract
The caudal skeleton of teleost fishes of the order Cyprinodontiformes is described and compared on the basis of 394 extant and eight fossil species, supporting delimitation of 21 phylogenetic characters, of which 13 are firstly reported. The Cyprinodontiformes are unambiguously diagnosed by the presence of a single, blade-like epural, and by principal caudal-fin rays continuous on upper and lower hypural plates. Monophyly of the suborder Cyprinodontodei is supported by the widened neural and hemal spines of the preural centrum 3 and presence of a spine-like process on the stegural, and monophyly of the Aplocheiloidei by the absence of radial caudal cartilages. A keel-shaped lateral process on the compound centrum supports monophyly of the Nothobranchiidae. Some characters of the caudal skeleton in combination to other osteological features indicate the cyprinodontiform fossil genus †Prolebias to be a paraphyletic assemblage; †P. aymardi, †P. delphinensis and †P. stenoura, the type species of the genus, all from the Lower Oligocene of Europe, possibly are closely related to recent valenciids; †"P". meridionalis from the Upper Oligocene of France is an incertae sedis cyprinodontid; and, †"P". cephalotes, †"P". egemus and †"P". malzi from the Upper Oligocene-Lower Miocene of Europe are closely related to poeciliids, probably closely related to the recent African genus Pantanodon due to they sharing unique derived features of pelvic fin, branchial arches and jaws.

Key words
Cyprinodontiformes, killifishes, Miocene, morphology, Oligocene, osteology.

Introduction
Characters of the caudal skeleton play a relevant role in studies on systematics of teleost fishes, often providing useful phylogenetic information at different taxonomic levels (e.g., MONOD, 1968; ROSEN, 1973, 1985; PATTERSON & ROSEN, 1977; JOHNSON & PATTERSON, 1996; DE PINNA, 1996; ARRATIA, 1999). The broad use of the complex morphology of the caudal skeleton in phylogenetic studies may be explained by it being first easily studied in dry skeletons and via dissection, and later through radiographs and standard techniques for clearing and staining small vertebrates. In addition, the caudal skeleton is frequently well-preserved in fossil material, making possible to evaluate the evolution of comparable osteological characters in a vast array of extinct fish lineages (e.g., PATTERSON & ROSEN, 1977; ARRATIA, 1997; HILTON & BRETZ, 2010).

The Cyprinodontiformes are a diversified order of teleost fishes comprising about 1,120 species, today classified in 125 genera and ten families occurring in freshwater and brackish environments of Asia, Europe, Africa and Americas (e.g., NELSON, 2006; COSTA, 2008). Until 1981, all living oviparous cyprinodontiforms from the whole geographic distribution of the order were classified in a single family, the Cyprinodontidae, whereas American specialised viviparous taxa were placed in four families (Anablepidae, Goo-deidae, Jenynsiidae, Poeciliidae) (e.g., ROSEN, 1964). The Cyprinodontidae then comprised the great
majority of extant cyprinodontiform taxa, as well as all fossil cyprinodontiform taxa. Cyprinodontiform classification suffered drastic changes after the first phylogenetic analysis of the order hypothesizing the broad Cyprinodontidae as a paraphyletic assemblage (Parenti, 1981), which has been corroborated by all subsequent studies (e.g., Meyer & Lydeard, 1993; Parker, 1997; Costa, 1998a; Ghedotti, 2000). Extant taxa previously placed in the Cyprinodontidae are today distributed among all the ten cyprinodontiform families (Parenti, 1981; Costa, 2004). Whereas New World fossil taxa have been classified in families according to the most recent cyprinodontiform classification (e.g., Parenti, 1981), Old World taxa have been kept in the Cyprinodontidae without criticisms.

The cyprinodontiforms may be unambiguously diagnosed by the unique morphology of the caudal skeleton (Parenti, 1981; Costa, 1998a). However, characters of the caudal skeleton have been only sporadically employed in phylogenetic studies of cyprinodontiform groups (Costa, 1998a, 1998b), and with rare exceptions (e.g., Ghedotti, 1998), the derived character states of the cyprinodontiform caudal skeleton have not been checked in most cyprinodontiform fossils. The objective of this study is to describe and to compare morphological traits of the caudal skeleton of all extant lineages of the Cyprinodontiformes, evaluating potentially informative phylogenetic characters, and checking the distribution of derived character states in species of uncertainly positioned fossil genera.

Material and methods

Delimitation of the order Cyprinodontiformes is according to Parenti (1981) and Rosen & Parenti (1981), and classification of included suborders and families follows Nelson (2006), which is based on Parenti (1981) with modifications proposed by Costa (2004). Intrafamilial classification follows Parenti (1981) for the Goodeidae and Anablepidae; Parenti (1981) for the Cyprinodontidae, except for the inclusion of a separate tribe Aphanini, thus reflecting phylogenetic evidence provided later by Costa (1997); Ghedotti (2000) for the Poeciliidae; and, Costa (2004) for the Nothobranchiidae and Rivulidae. The classification adopted here is given in the Appendix S1, where appears the complete list of 394 extant and eight fossil species of the order Cyprinodontiformes examined, and 10 extant species belonging to other orders (Atheriniformes, Beloniformes and Mugiliformes). Fossil taxa are identified by the symbol † before the taxon name. Osteological preparations of specimens of recent taxa were made according to Taylor & Van Dyke (1985). Terminology for osteological structures follows Schultz & Arratia (1989) and Arratia & Schultz (1992). Descriptions focus on characters with some variation among formally recognised taxa (e.g., genera, families, suborders). In descriptions, the words ‘often’ and ‘usually’ refer to the occurrence of variability of a certain character state among included species of a given taxon. Characters refer to the morphology of adult specimens, except where noted. Character statements, listed in the Discussion, were formulated according to Sereno (2007). First author to propose characters under a phylogenetic context are cited after character statements, following recommendations described in Sereno (2009).

Results

Preural vertebra 1 and associated structures

The preural vertebra and posterior elements of the caudal skeleton form a compact compound centrum, in which the limits of the ural centrum are never conspicuous (Figs. 1, 2, 3A, B), even in embryos with about 10 mm of total length. Attached to it, there is a rudimentary stegural with poorly visible limits on the basal portion of the dorsal margin of the uppermost hypural (Fig. 4). In cyprinodontoids, except in some cyprinodontids (Cabanichthys, Orestias, Jordanella, Megapsilon), there is a lateral, short spine-shaped process on the stegural (Fig. 4A). In all nothobranchiids, there is a keel-shaped process on the central portion of the side of the compound centrum (Fig. 4B).

Hypurals

The caudal skeleton of cyprinodontiforms usually shows high degree of fusion among the hypural elements. The proximal part of all the hypurals is always ankylosed to the compound caudal centrum, where limits between the hypurals and the compound centrum are imperceptible (Figs. 1, 2, 3A, B). The lower hypurals (i.e., hypurals 1 + 2) are always ankylosed to form a single plate. The upper hypurals are equally ankylosed in most cyprinodontiforms, except in some species of the aplocheilid genus Aplocheilus (A. lineatus (Valenciennes) and A. panchax (Hamilton)) and the nothobranchiid genera Epilapia (E. chaperi (Sauvage), E. fasciollatus (Günther), E. neumanni Berken-
kaMP, and E. steindachneri (Svensson)) and Pseu-
depiplatys (P. annulatus (BoulenGer)), in which there are
two separated elements (Figs. 2C, D). In those species
of Aplocheilus (Fig. 2D), the ventral element of the
upper hypurals, possibly corresponding to hypurals
3 + 4, is wider than the dorsal element, which is here

Fig. 1. Caudal skeleton, left lateral view. A: Anableps dowi; B: Brachyrhaphis cascajalenis; C: Valencia letourneuxi; D: Fun-
dulus sciadics. Abbreviations: e, epural; h2–4, hemal
spine of preural centra 2–4; hp, hypural plate; lhp, lower hy-
prural plate; n2–4, neural spine of preural centra 2–4; p, parhy-
pural; r, radial cartilage; uhp, upper hypural plate. Arrow indi-
cates hypurapophysis. Scale bar = 1 mm.

Fig. 2. Caudal skeleton, left lateral view. A: Aphanias dispar; B: Cualac tesselatus; C: Epiplatys steindachneri; D: Aplochei-
lus lineatus. Abbreviations: e, epural; h2–4, hemal spine of preural centra 2–4; hp, hypural plate; hy3–5, hypurals 3–5;
lhp, lower hypural plate; n2–4, neural spine of preural centra
2–4; p, parhypural; r, radial cartilage; uhp, upper hypural plate. Arrow indicates hypurapophysis. Scale bar = 1 mm.

Fig. 3. Caudal skeleton, left lateral view. A: Rivulus bahianus; B: Hypsolebias trilineatus; C: Oryzias matanensis; D: Cratero-
cephalus honoriae. Abbreviations: e, epural; eo, extra caudal
ossicle; h2–4, hemal spine of preural centra 2–4; hp, hypural
plate; hy3–5, hypurals 3–5; lhp, lower hypural plate; n2–4,
neural spine of preural centra 2–4; p, parhypural; r, radial car-
tilage; s, stegural; uhp, upper hypural plate. Arrow indicates
hypurapophysis. Scale bar = 1 mm.

Fig. 4. Compound caudal centrum, left lateral view. A: Aplo-
cheilichthys spilacuen; B: Epiplatys sangmelinensis. Abbre-
viation: kp – keel-shaped process; lhp – lower hypural plate;
p – parhypural; sp – spine-shaped process; uhp upper hypural
plate. Scale bar = 0.5 mm.
in the European cyprinodontiform fossil †Prolebias cephalotes (Agassiz) (Fig. 5C).

Among aplocheiloids, the upper and lower plates are usually separated (Figs. 2C, D, 3A), but they are fused to compose a single hypural plate in the aplocheilid Pachypanchax, and in Aplocheilus blockii (Arnold), A. dayi (Steindachner) and A. werneri Meinke; in the nothobranchiid Nothobranchius; and, in several rivulids, including all Cynolebiasinae and Plecioslebiasini genera (Fig. 3B). A partial posterior fusion as that above described for poeciliids and profundulids is never found among aplocheiloids.

Epural

Cyprinodontiforms have a single, elongate epural bone (Figs. 1, 2, 3A, B). Its distal extremity bears a cartilaginous terminal and supports some caudal-fin rays, whereas its proximal extremity is placed close to the preural centrum 1. The epural is a blade-like bone with a flat core abruptly narrowing ventrally and a thin flap on the anteroventral portion, which may be close or in contact with the neural spine of preural centrum 2–4. In anablepids embryos the plates are separated.

A similar partially fused hypural, with a conspicuous anterior gap between hypurals, is found in most poeciliids, but several species have a complete fusion, whereas others a complete separation. Complete fusion is common in miniature species of the Procatopodinae reaching about 20 mm as maximum adult size. Embryos of viviparous species have partially fused hypural, even in species having separate hypurals when adults. A similar anterior gap is present in adult specimens of some species of Profundulus, embryos of viviparous species of the Goodeidae, and in the European cyprinodontiform fossil †Prolebias cephalotes (Agassiz) (Fig. 5C).

In cyprinodontoids, the upper and lower hypural plates are often completely fused (Figs. 1C, D, 2A, B). Exceptions are concentrated in the Anablepidae, Poeciliidae and Profundulidae. Among anablepids, Anableps (Anablepidae) have the plates always separated by an interspace (Fig. 1A) and Jenynsia (Anablepidae) may have plates separated or partially fused. The latter condition consists of a middle gap between the upper and lower plates restricted to the anterior portion, whereas the posterior portion the plates are in contact (Fig. 1B) or are fused. In anablepid embryos the plates are separated.

A similar partially fused hypural, with a conspicuous anterior gap between hypurals, is found in most poeciliids, but several species have a complete fusion, whereas others a complete separation. Complete fusion is common in miniature species of the Procatopodinae reaching about 20 mm as maximum adult size. Embryos of viviparous species have partially fused hypural, even in species having separate hypurals when adults. A similar anterior gap is present in adult specimens of some species of Profundulus, embryos of viviparous species of the Goodeidae, and
nodontoids, in anablepids, poeciliids, profundulids, valenciids, most species of the fundulid *Fundulus*, and the goodeid *Crenichthys* the proximal end of the parhypural overlaps the preural centrum 1, and it bears a pointed dorsoposteriorly directed hypurapophysis (Fig. 3A–D). A similar condition is present in the fossil taxa *†Prolebias aymardi* (SAUVAGE), *†P. cephalotes*, *†P. delphinensis* GAUDANT, and *†P. stenoura* SAUVAGE (Fig. 5C). In the remaining extant goodeids, the fundulids *Leptolucania* and *Lucania*, and all extant cyprinodontids, the proximal part does not reach the preural centrum 1, whereas the hypurapophysis is rudimentary or absent (Figs. 2A, B).

**Parhypurals**

The parhypural of the cyprinodontiforms is a subrectangular bone, in which the distal end is always truncate, terminating in a cartilaginous edge supporting some caudal-fin rays (Figs. 1, 2, 3A, B). Among cyprinodontoids, in anablepids, poeciliids, profundulids, valenciids, most species of the fundulid *Fundulus*, and the goodeid *Crenichthys* the proximal end of the parhypural overlaps the preural centrum 1, and it bears a pointed dorsoposteriorly directed hypurapophysis (Fig. 1A–D). A similar condition is present in the fossil taxa *†Prolebias aymardi* (SAUVAGE), *†P. cephalotes*, *†P. delphinensis* GAUDANT, and *†P. stenoura* SAUVAGE (Fig. 5C). In the remaining extant goodeids, the fundulids *Leptolucania* and *Lucania*, and all extant cyprinodontids, the proximal part does not reach the preural centrum 1, whereas the hypurapophysis is rudimentary or absent (Figs. 2A, B).
Among aplocheiloids, in species of the Aplocheilidae the parhypural is similar to those in poeciliids (Fig. 2D); in nothobranchiids and rivulids, the proximal end of the parhypural does not touch the preural centrum 1, it is usually narrowed and directed to the basal portion of the hemal spine of the preural centrum 2, and the hypurapophysis is absent (Fig. 3A, B), except in some species of Epiplatys (E. fasciolatus and E. steindachneri) and Pseudepiplatys (P. annulatus), that have their parhypural slightly abutting the preural centrum 1 and the hypurapophysis is rudimentary (Fig. 2C).

**Preural vertebrae 2–5 and associated cartilages**

In most cyprinodontiforms there are four or five preural vertebrae participating in the caudal skeleton; these vertebrae are easily distinguished from the remaining vertebrae not associated to the caudal skeleton by the former ones having the tips of the neural and hemal spines slightly longer and connected to caudal-fin rays (Figs. 1B, C, 2A, B). Exceptions are found in all species of the genera Anableps, Fundulus, and Orestias, in which there are six preural vertebrae (Figs. 1A, D). The neural spine of the preural centrum 2 is always well-developed, long, its tip supporting some caudal-fin rays (Figs. 1, 2A, B). In cyprinodontoids, the neural and hemal spines of the preural centra 2 and 3 are wider than the spines of the vertebrae anterior to them (Figs. 1A, 2A, B), whereas in aplocheiloids, only the neural and hemal spines of the preural centrum 2 are distinctively wider (Figs. 2C, D, 3A, B). In cyprinodontids (except Cualac tesselatus Miller, and species of Cubanichthys and Orestias), poeciliids, anablepids, profundulids (except Profundulus guatemalensis), the fundulid Fundulus luciae (Baird), and in the goodeids Chapalichthys encaustus (Jordan & Snyder) and Characodon lateralis Günther there is a constriction on the proximal portion of the neural spine of the preural centrum 2 (Figs. 1A, B, 2A, B). A similar constriction on the proximal portion of the hemal spine of the preural centrum 2 occurs in cyprinodontids (except Cualac tesselatus, and species of Cubanichthys and Orestias) (Fig. 2A, B) and in †Brachylebias persicus and †Prolebias meridionalis.

In cyprinodontoids, there are large radial cartilages between both neural spines and hemal spines of preural centra (Figs. 1, 2A, B). Usually there is one or two dorsal and one or two ventral cartilages, which are positioned between the anteriormost preural centrum spines (Figs. 1, 2A), but minute accessory cartilages adjacent to radial cartilages are also often present (Fig. 1D). Exceptions are the species of the cyprinodontid Cualac, Cyprinodon and Megupsilon, which have three dorsal and three ventral radial cartilages (Fig. 2B). In aplocheiloids, radial cartilages are always absent (Figs. 2C, D, 3A, B).

**Discussion**

The Cyprinodontiformes

Gosline (1963) characterized the caudal skeleton of the Cyprinodontiformes by the presence of a “plate-like hypural fan”, formed by the fusion of terminal vertebrae and hypurals (Gosline, 1961a). In addition to the fusion of hypurals, subsequently, Rosen (1964) described a unique symmetry among some bones of the dorsal and ventral parts of the caudal skeleton of the cyprinodontiforms, in which a single bladelike epural forms the symmetrical dorsal counterpart of the parhypural, a condition previously reported by Hollister (1940). Monophyly of the order Cyprinodontiformes was later discussed by Parenti (1981), who diagnosed that order on the basis of an apomorphic symmetrical caudal-fin support, in which a single epural mirrors the parhypural in shape and position, and an upper hypural plate formed by the fused hypurals 3-5 opposed to a lower hypural plate formed by the fused hypurals 1 and 2. She noted that complete fusion of all hypurals occurs in several monophyletic groups within the Cyprinodontiformes as well as unfused hypurals 4 and 5 are present in some species of Epiplatys and Aphryosemion, as already recorded for Aplocheilus panchax by Rosen (1964).

In fact, the character proposed by Parenti (i.e., symmetry of caudal-fin support) comprises four independent characters relative to the number of epurals, shape of the epural, fusion of hypurals 1 and 2, and fusion of hypurals 3, 4 and 5. Each of these characters contains a derived character state that would be diagnostic for the Cyprinodontiformes: one epural; epural shaped as parhypural (i.e., blade-like as described by Rosen, 1964); lower hypurals (1 and 2) fused; and, upper hypurals (3, 4 and 5) fused. The two latter character states cannot be unambiguously considered as synapomorphic for cyprinodontiforms, since lower hypurals fused also occurs in all other atherinomorphs, fusion of upper hypurals occurs in several beloniforms (e.g., Parenti, 2008), which is hypothesized to be the sister group of the cyprinodontiforms (Rosen & Parenti, 1981), but not in some species of Aplocheilus and
Epistromus (Parenti, 1981; Costa, 1998a). Characters useful to diagnose the Cyprinodontiformes are listed and discussed below.

1. Epurals, number: (0) three or two; (1) one (Rosen, 1964; Parenti, 1981). The presence of three or fewer epurals has been considered as a synapomorphy for a group comprising living teleosts and some fossil lineages (e.g., de Pinna, 1996). Mugilids and non-cyprinodontiform atherinomorphs have two epurals (e.g., Gosline, 1961b; Parenti, 1981, 2008; Saeed, Ivantsoff & Allen, 1989; Stiasny, 1990; Ivantsoff et al., 1997) (Figs. 3C, D) or sometimes three in beloniforms (Rosen, 1964), whereas all cyprinodontiforms have a single epural (Figs. 1, 2, 3A, B), thus confirming that condition as diagnostic for the order.

2. Epural, shape: (0) rod-like; (1) blade-like (Rosen, 1964). Non-cyprinodontiform teleosts have narrow rod-like epurals (Figs. 3C, D), which contrasts with the typical cyprinodontidomorph blade-like shape (Figs. 1, 2, 3A, B), thus confirming the derived condition as diagnostic for the Cyprinodontiformes.

3. Caudal-fin rays, zone between upper and lower hypural plates, arrangement: (0) separated by broad interspace; (1) continuously arranged. A distinctive condition of cyprinodontiform caudal skeleton involving the middle hypural zone is the continuous arrangement of adjacent caudal-fin rays (Figs. 1, 2, 3A, B). This morphology contrasts with the typical condition of most advanced teleosts, including atherinomorphs and most beloniforms, in which a wider interspace between hypural 2 and 3 is reflected by a hiatus between the corresponding caudal-fin rays (e.g., de Pinna, 1996; Arratia, 1999) (Figs. 3C, D).

4. Preural vertebra 2, neural spine: (0) absent; (1) well-developed, distal tip acting in support of caudal-fin rays. The presence of a fully developed neural spine on the preural vertebra 2 is a derived condition occurring in all cyprinodontiforms (Figs. 1, 2, 3A, B), but is also present in adrianichthyids (Fig. 3C). The neural spine of the preural vertebra 2 is absent in atherinomorphs and most beloniforms (e.g., Chernoff, 1986; Saeed et al., 1989; Stiasny, 1990) (Fig. 3D), whereas it is poorly developed in percomorphs (e.g., Gosline, 1961b). Since true epurals have been considered as those bones ontogenetically derived from the detachment of the neural spine of the adjacent vertebrae (e.g., Schultze & Arratia, 1989), the most anterior epural of atheriniforms and non-adrianichthyids beloniforms may be derived from the detachment of the neural spine of the preural vertebra 2. On the other hand, the long neural spine of the preural centrum 2 occurring in cyprinodontiforms and adrianichthyids may be either an early ontogenetic condition retained in adult individuals, or a secondary lengthening of the spine, a question only explained after long range ontogenetic studies.

5. Stegural, development: (0) well-developed; (1) minute. Another derived character state of the caudal skeleton occurring in all cyprinodontiforms, but also in adrianichthyids, is the minute uroneural (stegural). The presence of uroneurals (i.e. modified ural neural arches into paired bones) has been considered as a synapomorphy of teleosts, with a tendency to number reduction from seven to fewer in some recent teleost lineages (de Pinna, 1996). A long stegural bordering most dorsal margin of the hypural 5, bearing an anterodorsal membranous growth (Fig. 3D), which may be diagnostic for euteleosts (Wiley & Johnson, 2010), is found in Atheriniformes and most Beloniformes. In all the Cyprinodontiformes and in adrianichthyid beloniforms, the stegural is rudimentary, restricted to the basal portion of the adjacent hypural plate (Figs. 1, 2, 3A, B, D).

6. Preural vertebra 2, neural spine, width relative to neural spines of preural vertebrae 4 and 5: (0) about equal; (1) wider. A condition uniquely occurring in all cyprinodontiforms is the presence of a wide neural spine of the preural centrum 2, which is wider than the anterior neural spines (Figs. 1, 2, 3A, B). In adrianichthyids, that spine is not widened (Fig. 3D), but the condition is not comparable in atheriniforms and other beloniforms, in which the spine is absent (Fig. 3C). Therefore, this condition may be useful to diagnose cyprinodontiforms, but its polarization is doubtful.

7. Upper hypurals and compound caudal centrum, degree of fusion: (0) attached, limited by cartilage edge; (1) complete ankylosis. Only in cyprinodontiforms, the proximal part of all the hypurals is ankylosed to the compound caudal centrum, being imperceptible the limits between the hypurals and the compound centrum (Figs. 1, 2, 3A, B). In other atherinomorphs, only the lower hypurals are fused to the compound caudal centrum, whereas the upper hypurals are often separated by a cartilaginous contact area (Fig. 3D).
The Cyprinodontoidei

Monophyly of the Cyprinodontoidei has been consistently supported by apomorphic character states of the branchial and hyoid arches, jaws, and jaw suspensorium (Parenti, 1981). Costa (1998a) included among the cyprinodontiform synapomorphies the fusion of dorsal and ventral hypurals plates. This character and others corroborating the Cyprinodontoidei clade are listed and discussed below.

8. Upper and lower hypural plates, degree of fusion: (0) unfused; (1) partially fused (anterior portion unfused, posterior portion fused); (2) completely fused (modified from Costa, 1998a: character 88). Costa (1998a) assumed the fusion of all hypural elements as a synapomorphy of the Cyprinodontoidei (Figs. 1C, D, 2A, B), with reversals in poeciliids and anablepids that frequently have upper and lower hypurals plates unfused or partially fused (Figs. 1A, B). However, fusion of dorsal and ventral hypurals plates is also present in lineages of all aplocheiloid families (Fig. 3B). Therefore, fusion of upper and lower hypurals cannot be assumed as synapomorphic for cyprinodontoids without ambiguity.

9. Preural vertebra 3, neural and hemal spines, width relative to neural and hemal spines of preural vertebrae anterior to preural vertebra 4: (0) about equal; (1) wider. The neural and hemal spines of the preural centrum 3 usually are wider than the spines of the vertebra anterior to the preural vertebra 4 in all cyprinodontoids (Figs. 1, 2A, B), a condition not occurring in aplocheiloids, which have narrow spines of preural vertebra 3 (Figs. 2C, D, 3A, B).

10. Stegural, ventral portion, lateral process: (0) absent; (1) present. The presence of a lateral spine-like process on the stegural (Fig. 4A), previously reported for the poeciliid genus Gambusia by Rauchenberger (1989), is a derived condition uniquely found in cyprinodontoids, although absent or rudimentary in some cyprinodontids (see results above).

Among Cyprinodontoidei families, members of the Cyprinodontidae concentrate some informative morphological variability as discussed below.

11. Radial caudal cartilages, number: (0) one or two; (1) three. An increasing in the number of radial caudal cartilages, from one or two on the dorsal portion and one or two on the ventral portion of the caudal skeleton to three well-developed cartilages on each portion of the caudal skeleton, occurs in the American cyprinodontid genera Cuacul, Cyprinodon and Megupsilon (Fig. 2B).

12. Parhypural, proximal part, relative position to preural centrum 1: (0) overlapped; (2) not overlapped (modified from Costa, 1998a: character 91). An apomorphic reduced proximal part of the parhypural, in which it does not overlap the preural centrum 1 and the hypurapophysis is rudimentary or absent, besides occurring in all cyprinodontids (Figs. 2A, B), is found in some fundulids (Leptolucania and Lucania), most gouramids, and all nothobranchiids (Fig. 2C) and rivulids (Figs. 3A, B).

13. Caudal skeleton preural vertebrae, number: (0) 4–5; (1) 6. An apomorphic increasing in the number of vertebrae participating of the caudal skeleton from four or five to six vertebrae occurs both in the anablepoid genus Anableps, cyprinodontid genus Orestias and in the fundulid genus Fundulus (Figs. 1A, D), supporting independent acquisitions in those three distantly related genera (e.g., Parenti, 1981; Costa, 1998a).

14. Preural vertebra 2, hemal spine, sub-basal region, deep constriction: (0) absent; (1) present (modified from Costa, 1998a: character 92). An apomorphic deep constriction in the sub-basal region of the hemal spine of the preural vertebra 2 supports sister group relationships between American (Cyprinodontini) (Fig. 2B) and Eurasian cyprinodontids (Aphanini) (Fig. 2A) as proposed by Costa (1997).

15. Preural vertebra 2, neural spine, sub-basal region, deep constriction: (0) absent; (1) present. A similar constriction as discussed in the character 14 above, also occurs in the neural spine of the same preural vertebra of cyprinodontines and aphaninines, but also is present in other taxa of the suborder Cyprinodontoidei (e.g., poeciliids, anablepids, profundulids) (Figs. 1B, 2A, B), thus not informative to unambiguously support monophyly of formally designated taxonomic units.

16. Epural, core part, extent and position: (0) long, at same axis of whole bone; (1) short, restricted to dorsal portion of bone, posteriorly placed. A unique morphology of the epural is found in Aphanius dispar, A. isfahanensis, A. richardsoni, A. splendens, and A. sureyanus (Fig. 2A). However, according to the molecular phylogeny
proposed by Hrbek & Meyer (2003), these species do not form a clade.

The Aplocheiloidei

Monophyly of the Aplocheiloidei has been supported both by morphological and molecular characters (Parenti, 1981; Murphy & Collier, 1997; Costa, 1998a), although recently contrary view based on morphology was published (Hertwig, 2008), in which the Aplocheiloidei may be a paraphyletic assemblage. Monophyly hypothesis was first established by Parenti (1981) based on characters of the external anatomy, neurocranium, pelvic girdle, infraorbital series, cephalic laterosensory system, hyoid arch, and colour pattern. Costa (1998a) found additional derived character states supporting monophyly of the Aplocheiloidei, among which was a unique derived character state of the caudal skeleton (i.e., absence of radial caudal cartilages). Characters with informative distribution among aplocheiloids are discussed below.

17. Radial caudal cartilages: (0) present; (1) absent (Costa, 1998a: character 89). Radial caudal cartilages are commonly found in atherinomorphs (e.g., Stiassny, 1990), a condition also found among several other acanthomorph lineages. In all the aplocheiloids examined here, radial cartilages are absent (Figs. 2C, D, 3A, B), confirming this diagnostic feature of aplocheiloids.

18. Hypurals 4 and 5, degree of fusion: (0) unfused; (1) fused (modified from Parenti, 1981). Parenti (1981: 395) considered upper hypural plate divided as evidence of close relationships between the aplocheiloid genera Aplocheilus, Epiplatys and Pachypanchax, since this condition does never occur in cyprinodontoids, the immediate sister group to aplocheiloids. However, upper hypural plate divided is usually present in outgroups to cyprinodontiforms, thus being considered as a plesiomorphic condition, retained in some aplocheilids and nothobranchiids (see Results above to character state distribution among examined taxa).

19. Preural vertebra 2, hemal spine, width relative to hemal spines of preural vertebrae 4 and 5: (0) distinctively wider; (1) slightly wider (modified from Costa, 2004: character 43). The clade comprising the genera Aplocheilus and Pachypanchax was first hypothesized to be the sister group of the clade including nothobranchiids and rivulids in a phylogeny based on mitochondrial DNA (Murphy & Collier, 1997). Costa (2004) found morphological evidence supporting the clade comprising nothobranchiids and rivulids, describing eight derived character states, among which the hemal spine of preural centrum 2 being narrow, only slightly wider than the hemal spines of anteriorly adjacent vertebrae (Figs. 2C, 3A, B), which is herein corroborated. Another derived condition of the caudal skeleton shared by rivulids and nothobranchiids described by Costa (2004) and herein confirmed is the shortened proximal end of the parhypural, not overlapping the preural centrum, with a rudimentary or absent hypurapophysis (Figs. 2C, 3A, B), a condition also occurring in some cyprinodontoid lineages (see character 12 above). The plesiomorphic state for both characters are exhibited by Aplocheilus and Pachypanchax (Fig. 2D).

20. Compound centrum, central portion of side, keel-shaped process: (0) absent; (1) present. Monophyly of all the aplocheiloids endemic to continental Africa was first proposed based upon mitochondrial DNA phylogeny (Murphy & Collier, 1997); Costa (2004) first formally recognized that group as the Nothobranchiidae, which was diagnosed on the basis of bifid pleural ribs, already reported to occur in some nothobranchiid lineages by Parenti (1981), but later confirmed to occur in all nothobranchiids (Costa, 2004). In addition, all nothobranchiids have a prominent keel-shaped lateral process on the middle part of the compound centrum (Fig. 4B). This process is never present in any other cyprinodontiform and outgroups.

21. Epural, proximal region, width relative to distal region: (0) wider to slightly narrower; (1) conspicuously narrower (Costa, 1998b: character 105). The rivulid subfamily Cynolebiasinae has been diagnosed by a series of apomorphic morphological characters, including the unique shape of the proximal region of the epural (Fig. 3B). Possibly associated to this character is the absence of neural prezygapophyses and postzygapophyses on preural vertebrae.

The caudal skeleton of cyprinodontiform fossil taxa

Cyprinodontiform fossil taxa have been recorded from Americas, Europe and west Asia (e.g., Parenti,
1981). New World fossil record includes a few North American Pliocene taxa belonging to recent genera (e.g., Miller, 1945; Parenti, 1981) and \textit{Carrionellus diurnotus} White from the Lower Miocene of Ecuador, recently considered as closely related to \textit{Orestias} (Costa, 2011), being only known from impression fossils with no resolution for details of the caudal skeleton. Therefore, no informative data on the caudal skeleton could be extracted from New World taxa.

Old World cyprinodontiform fossils have been placed in five genera: \textit{Aphanius} Nardo, \textit{Brachylebias} Priem, \textit{Cryptolebias} Gaudant, \textit{Prolebias} Sauvage and \textit{Aphanolebias} Reichenbacher \& Gaudant, all currently considered as members of the Cyprinodontidae (e.g., Parenti, 1981; Reichenbacher \& Gaudant, 2003). \textit{Aphanius} comprises about 20 living species from an area comprising southern Europe, western Asia and northern Africa and at least four valid fossil species (not including taxa only known from otoliths) from the Oligocene-Miocene of southern, central and western Europe, and western Asia (Hrbeck \& Meyer, 2003; Gaudant, 2009; Reichenbacher \& Kowalke, 2009). The only fossil species herein examined, \textit{Aphanius illunensis} Gaudant, osteological features concordant to those above described for living species of \textit{Aphanius}. Similar morphology was found in \textit{Brachylebias persicus}, the only species of the genus, known from the Miocene of northwestern Iran, corroborating its current position among cyprinodontids.

\textit{Cryptolebias} is known from a single species, \textit{C. senogalliensis} (Coschi) from the Miocene of Italy, which was not available for the present study. This species has a unique morphology among cyprinodontiforms, combining a very slender body with dorsal and anal fins positioned anteriorly to the middle of the trunk (Gaudant, 1978). Caudal skeleton morphology cannot be fully appreciated from the original description of the genus (Gaudant, 1978), but the presence of a long parhypural articulating with the preural centrum, as illustrated in that paper, suggests that it is not a cyprinodontid.

\textit{Prolebias} from the lower Oligocene–Middle Miocene of Europe was first described by Sauvage (1874) to include some species formerly described by Agassiz (1839) and Sauvage (1869), but some others have been incorporated to the genus since then (e.g., Gaudant, 2009). \textit{Prolebias} has not been diagnosed by unique derived features, but by plesiomorphic character states (i.e., jaw teeth conical and absence of an anteroventral process on the dentary) (e.g., Gaudant, 2003) opposed to those apomorphic states occurring in the cyprinodontid genus \textit{Aphanius} (i.e., teeth tricuspidate and a conspicuous process on the dentary; Parenti, 1981; Costa, 1997). Although previous authors had suggested close relationships between \textit{Prolebias} and fundulines (then comprising species today placed in Fundulidae and Valenciidae) (Woodward, 1901; Regan, 1911), \textit{Prolebias} was kept in the Cyprinodontidae by Parenti (1981), which was followed by subsequent authors (e.g., Gaudant, 1989, 1991, 2003; Reichenbacher \& Gaudant, 2003; Reichenbacher \& Prieto, 2006).

A great diversification in the caudal skeleton morphology was observed among species of \textit{Prolebias} herein examined. \textit{Prolebias aymardi}, \textit{P. delphinensis} and \textit{P. stenoura}, all from the Lower Oligocene of Western Europe, do not exhibit the derived features of the caudal skeleton of cyprinodontids. There is no constriction on the basal portion of the hemal spine of the preural centrum 2 and the parhypural overlaps the preural centrum 1 (Fig. 5A) (vs. a pronounced constriction in that hemal spine and parhypural not reaching preural centrum 1 in Eurasian and North American cyprinodontids; Figs. 2A, B). In fact, on the basis of caudal skeleton characters, those three species of \textit{Prolebias} cannot be unambiguously placed in any cyprinodontiform group by not exhibiting any of the derived character states described above. The jaw dentition consisting of multiple series of conical teeth precludes the placement in the Cyprinodontidae (e.g., Costa, 1997). The ascending process of the premaxilla is long as that occurring in valenciids, profundulids and fundulids (Costa, 1998a), contrasting with the shorter ascending process of the remaining cyprinodontoids. In fact, the jaws, fins and the caudal skeleton of \textit{P. aymardi}, \textit{P. delphinensis} and \textit{P. stenoura} (Fig. 6A) are similar to those exhibited by recent valenciids (Fig. 1C). However, the apomorphic feature used to diagnose the family Valenciidae, long and narrow dorsal process of maxilla (Parenti, 1981), could not be observed in the examined material, thus preventing the unambiguous transference of those three species to the Valenciidae. Consequently, since \textit{P. stenoura} is the type species of \textit{Prolebias}, the latter name should be considered as an incertae sedis cyprinodontoid genus, probably closely related to or part of the Valenciidae. An identical situation is found in \textit{Aphanolebias meyeri} (Agassiz) from the Lower Miocene of central Europe, not available for the present study. Characters described and illustrated by Reichenbacher \& Gaudant (2003) are concordant with those described above to \textit{P. aymardi}, \textit{P. delphinensis} and \textit{P. stenoura}, supporting \textit{Aphanolebias} as an incertae sedis cyprinodontoid genus, probably close to recent valenciids.

The fourth species of \textit{Prolebias} examined, \textit{P. meridionalis}, from the Upper Oligocene of France, has the caudal skeleton similar to that described for Eurasian and North American cyprinodontids, with a constriction on the basal portion of the hemal spine of the preural centrum 2 and a short proximal part of the parhypural, not reaching the preural centrum 1 (Fig.
5B). The morphology of the unpaired fins, including the dorsal-fin origin anterior to the anal-fin origin (Fig. 6B), is typical among cyprinodontids. However, †P. meridionalis differs from aphanines by having conical teeth (vs. tricuspidate). Therefore, †“Prolebias” meridionalis is considered as an incertae sedis cyprinodontid, not a congener of the other three species discussed in the above paragraph.

The fifth nominal species of †Prolebias examined, †P. cephalotes also from the Upper Oligocene of France, has a different caudal skeleton. There is an anterior gap between the dorsal and hypural plates (Fig. 5C), a condition also recorded for †P. egeranus Laube and †P. malzi Reichenbacher & Gaudent from the Upper Oligocene–Lower Miocene of central Europe, not available to this study, but finely described by Obrìelová (1985) and Reichenbacher & Gaudent (2003), respectively. As described above, among extant cyprinodontiforms this morphology of hypurals is found in some American anablepsids, American profulvids, and American and African poeciliids (see distribution of characters states among taxa in Results above), but never in cyprinodontids, fundulids and valenciids (Fig. 1B). In addition, uniquely among species of †Prolebias, †P. cephalotes, †P. egeranus and †P. malzi have the pectoral-fin base laterally placed (vs. latero-ventrally placed) (Fig. 6C) and pelvic-fin base nearer pectoral-fin base than to anal-fin origin (vs. nearer anal-fin origin or midway between pectoral-fin base than to anal-fin origin), two derived conditions uniquely found in poeciliids among cyprinodontoids (Parenti, 1981; Costa, 1998a), which support the transference of those taxa for the family Poeciliidae. Thus, †“Prolebias” cephalotes, †“P.” egeranus and †“P.” malzi are considered as incertae sedis poeciliids.

The Poeciliidae is today geographically restricted to Africa and Americas (Parenti, 1981; Costa, 1998a; Ghedotti, 2000). In Africa, it is represented by the subfamily Aplocheilichthyinae and the greatest part of the subfamily Procatopodinae, whereas in North, Middle and South America it is represented by the Poeciliinae (e.g., Rosen & Bailey, 1963; Parenti, 1981), and in South America by the procatopodine genus Fluviphylax Whitley (e.g., Costa, 1996; Ghedotti, 2000). A phylogenetic analysis involving representatives of the several poeciliid lineages, which is beyond the scope of the present study, would be necessary to establish rigorous hypotheses about the placement of †“P.” cephalotes, †“P.” egeranus and †“P.” malzi among poeciliids. However, some morphological evidence of possible phylogenetic relationships deserves attention. The subfamily Poeciliinae has been diagnosed by the presence of a complex organ in males for internal insemination, the gonopodium, mainly formed by the anal-fin rays 3–5 (e.g., Rosen & Bailey, 1963; Ghedotti, 2000). The absence of any vestige of that complex structure in those three fossil species precludes relationships with the Poeciliinae. On the other hand, †“P.” cephalotes, †“P.” egeranus and †“P.” malzi have thickened pelvic-fin rays (Gaudent, 2009), a unique condition, similar to that occurring in the recent African procatopodine poeciliid genus Pantanodon Myers (Whitley, 1962; Rosen, 1965). Among those three species, osteological structures of the branchial arches were described only for †P. egeranus (Obrìelová, 1985), including the presence of a wide dentigerous plate on the fifth ceratobranchial and third pharyngobranchial, with small teeth regularly arranged in transverse rows, each of which is separated from the adjacent row by regular interspaces, a condition occurring only in Pantanodon (Whitehead, 1962; Parenti, 1981). In addition, Obrìelová (1985: figs. 5D, F) described and illustrated a dentary bone with a long coronoid process, a condition uniquely found in Pantanodon (Rosen, 1965) among living cyprinodontoids. The derived morphology of the pelvic fin, branchial arches and dentary strongly suggest close relationships between the European fossil taxa †“P.” cephalotes, †“P.” egeranus and †“P.” malzi, and the recent African poeciliid genus Pantanodon.

The occurrence of a poeciliid taxon in the Miocene of central Europe closely related to extant African poeciliids is not surprising. Records of terrestrial and freshwater vertebrate faunal exchanges between Africa and Europe during the Paleogene are well documented and hypotheses of dispersal routes are supported by partial land connections resulted from the displacement of the African Plate to north combined to sea-level falls (Gheerbrant & Rage, 2006). Among freshwater fishes, for example, the alestids are today restricted to Africa and South America (e.g., Zanata & Vari, 2005; Malabarba & Malabarba, 2010), but alestid-like teeth have been often consistently identified in different outcrops of the Paleogene of Europe (e.g., De la Peña Zarzuelo, 1996; Monod & Gaudent, 1998; Otero, 2010).

Conclusion

The comparative morphology of the caudal skeleton of the Cyprinodontiformes provides useful phylogenetic information. Among the 22 characters delimited in the present study, characters 1–10, 12, 14, 17, 20 and 21 corroborate formally recognized cyprinodontiform groups when their states are optimized on a phylogenetic tree condensing hypotheses generated in previous studies (Fig. 7). Other characters (11, 13, 16, 22) are potentially informative but its use is either only applicable to small assemblages within the principal
lineages or they are very variable among different lineages (15 and 18) (see Discussion above).

The morphology of the caudal skeleton combined to other osteological features indicates that the cyprinodontiform fossil genus †Prolebias is a paraphyletic assemblage, probably comprising taxa closely related to three distinct families, the Cyprinodontidae, the Valenciidae, and the Poeciliidae.

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References


The Pantanodontinae, edentulous (Cyprinodontinae: Ily Cyprinodontinae: Jenynsia multidentata [2006: UFRJ 8079, 1; Iran: Ezhych, Zayadeh Rud. Miocene]).


**Appendix**

List of material examined. Most material is deposited in the ichthyological collection of Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro (UFRJ). Abbreviations for other institutions are: BMNH.P, Natural History Museum, Paleontology, London; CAS, California Academy of Sciences, San Francisco; MNHN.P, Muséum national d’Histoire naturelle, Paleontology, Paris; MRAC, Musée Royal de l’Afrique Centrale, Tervuren; USNM, National Museum of Natural History (former United States National Museum), Smithsonian Institution, Washington. Number of specimens is indicated after catalog number.


ciennes, 1846: UFRJ 3895, 2; Bolivia: Copacabana, Lago Titicaca.

**Family Fundulidae:** Fundulus chrysotus (Günther, 1866): UFRJ 8128, 2; USA: Massachusetts, Seminole basin. Fundulus diaphanous (Lesueur, 1817): UFRJ 8127, 2; USA: Massachusetts, Bristol. Fundulus heteroclitus (Linnaeus, 1766): UFRJ 3359, 2; USA: Massachusetts, Oyster Pond. Fundulus majalis (Walbaum, 1792): UFRJ 3312, 2; USA: Massachusetts, Matta poissett. Fundulus notti (Agassiz, 1854): UFRJ 8129, 2; USA: Georgia, Lake Landon. Fundulus sciadiceus Copi, 1865: UFRJ 3321, 2; USA: Nebraska, near Luray. Fundulus zebrinus (Linnaeus, 1758): UFRJ 4006, 2; Brazil: Soto La Marina, Río Tamesi system.


heusensis

semiocellatus

jariensis
tocantinensis

Acre.

lebias boticarioi

6674, 3; Brazil: Pará, Mosqueiro.

UFRJ 6452, 2; Brazil: Rio de Janeiro, Igarapé do Baré.

CaRValho 5811, 2; Brazil: Bahia, Canavieiras.

molebias perpendicularis

Brazil: Rio de Janeiro, Nova Iguaçu.

UFRJ 5145, 5; Brazil: Bahia, Ribeirão Guará. Symposionchthys nigromaculatus Costa, 2007: UFRJ 6749, 3; Brazil: Goiás, Chapadão do Céu.

