

# Morphology of the teleost pharyngeal jaw apparatus in the Neotropical annual killifish genus *Cynolebias* (Cyprinodontiformes: Aplocheiloidei: Rivulidae)

by

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**ABSTRACT.** - The osteological and myological structure of the pharyngeal jaw apparatus (PJA) of a species assemblage of the Brazilian annual killifish genus *Cynolebias*, known as the *Cynolebias*-clade  $\alpha$ , is first described. The *Cynolebias*-clade  $\alpha$  comprises all species of the genus except *C. griseus*, and includes species in which males produce sounds during courtship behaviour. The PJA of the *Cynolebias*-clade  $\alpha$  is characterized by a series of morphological traits unique among aplocheiloid cyprinodontiforms: 1) anterior portion of the fifth ceratobranchial narrow and long, 2) robust teeth on the second pharyngobranchial and fifth ceratobranchial, 3) a pronounced process on the ventral surface of the fifth ceratobranchial, 4) fifth ceratobranchials medially in contact, 5) infrabranchial ossification, 6) fourth epibranchial L-shaped and with a process for insertion of the *levator posterior* muscle, and 7) third and fourth *levator externi* and *protractor pectoralis* widened to make levator posterior not visible laterally. All these features are present in all species of the *Cynolebias*-clade  $\alpha$  and absent in all other cynolebiatines, thus should be considered as morphological innovations simultaneously arising at the base of that clade. Some of these derived traits are morphologically convergent to similar traits found in other teleost fishes able to produce sounds through the PJA.

**RÉSUMÉ.** - Morphologie des mâchoires pharyngiennes des killis du genre néotropical annuel *Cynolebias* (Cyprinodontiformes: Aplocheiloidei: Rivulidae).

La structure ostéologique et myologique des mâchoires pharyngiennes (PJA) d'un assemblage d'espèces de killis brésiliens du genre *Cynolebias*, connu comme le clade *Cynolebias*  $\alpha$ , est décrite pour la première fois. Le clade *Cynolebias*  $\alpha$  comprend toutes les espèces du genre à l'exception de *C. griseus* et inclut les espèces chez lesquelles les mâles produisent des sons durant la parade nuptiale. Le PJA du clade *Cynolebias*  $\alpha$  est caractérisé par une série de traits morphologiques uniques parmi les cyprinodontiformes aplocheiloïdes : portion antérieure du cinquième cératobranchial long et étroit, dents robustes sur le second pharyngobranchial et le cinquième cératobranchial, processus prononcé sur la surface ventrale des cinquièmes cératobranchiaux qui sont en contact dans leur région moyenne, ossification infrabranchiale, quatrième épibranchial en forme de L avec un processus pour l'insertion du muscle *levator posterior*, et troisième et quatrième *levator externi* et *protractor pectoralis* élargis rendant invisible latéralement le *levator posterior*. Ces traits uniques sont considérés comme des innovations morphologiques apparues simultanément à la base du clade *Cynolebias*  $\alpha$ . Certains de ces traits dérivés sont morphologiquement convergents avec des traits similaires retrouvés chez d'autres téléostéens capables de produire des sons grâce au PJA.

Key words. - Rivulidae - *Cynolebias* - Killifishes - Pharyngeal jaws - Teeth - Osteology - Myology.

Aplocheiloid killifishes constitute a diverse assemblage of cyprinodontiform teleosts, with about 620 species occurring in most tropical and subtropical areas of the world (Costa, 2008). They primarily inhabit shallow parts of streams and swamps, but species of some African and South American lineages, known as annual fishes, live in temporary pools. During the dry season, all individuals of the population die, but resistant eggs survive in the substrate. This unique life style, known as annualism, comprises a combination of specialized biological features, such as diapausing eggs, quick development and specialized behavior (e.g., Costa, 1998).

A dramatic radiation in morphological traits has been recently reported for speciose clades of annual fishes. The best example is the tribe Cynolebiasini (Rivulidae), which is

endemic to central and eastern South America and includes over 95 species in four genera (*Austrolebias* Costa, 1998, *Cynolebias* Steindachner, 1876, *Nematolebias* Costa, 1998, and *Simpsonichthys* Carvalho, 1959) (Costa, 2001, 2002, 2006a, 2007). The cynolebiasines exhibit a great diversity of sexually dimorphic colour patterns and elaborated reproductive behavior. Cynolebiasine males are territorial and invite females to spawn in the bottom sediment of their territories through a large array of courtship behavioural patterns (Belote and Costa, 2002, 2003, 2004). However, uniquely among fishes of the order Cyprinodontiformes, *Cynolebias albipunctatus* Costa & Brasil, 1991 was recently reported as producing sounds during male courtship behaviour. Synchronized with a vertical head shaking movement, a crack-

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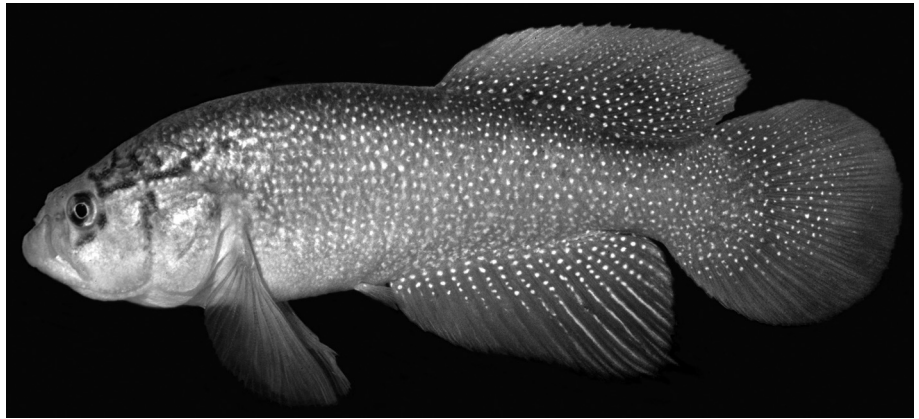


Figure 1. - *Cynolebias albipunctatus*, male, UFRJ 5127, 81.4 mm SL; Brazil: Bahia: Juazeiro.

like sound is produced by the male, which immediately attracts the female, thus starting a behavioural sequence which culminates in spawn and egg fertilization (Belote and Costa, 2003). No sound production was observed in *Cynolebias griseus* Costa, Lacerda & Brasil, 1990 the most basal taxon of the genus (Costa, 2001).

Although the sound production mechanism in *Cynolebias albipunctatus* was not explicitly demonstrated, it was

attributed to the action of a specialized pharyngeal region, which bears distinctively widened teeth both in the fifth ceratobranchial and third pharyngobranchial (Belote and Costa, 2003). *C. albipunctatus* is a basal member of a clade, named as clade  $\alpha$  by Costa (2001), endemic to the semi-arid Brazilian Caatinga region. Species of the clade  $\alpha$  are among the biggest aplocheiloids, usually reaching about 100-130 mm SL, with a characteristic robust head and dorsally prominent anterior portion of trunk (Fig. 1) (Costa, 2001). It is diagnosed by six synapomorphies involving striking modifications of the pharyngeal bones (Costa, 2001), including the fifth ceratobranchials, which are medially in contact and have a concave dorsal surface, a long anterior process and a pronounced, triangular ventral process (Costa, 2001). This structure is similar to the fifth ceratobranchials that comprise the pharyngeal jaw apparatus (PJA) of labroid fishes (*sens.* Nelson, 2006), which are known to produce sounds in behavioural contexts (e.g., Rice and Lobel, 2003). Even though some features of the PJA of *Cynolebias* were used in a phylogenetic study (Costa, 2001), the whole PJA structure was never described. The objective of the present study is to describe the osteological and myological components of the complex pharyngeal jaw apparatus of the *Cynolebias* clade  $\alpha$  (hereafter termed  $C\alpha$ -PJA), evaluating their origin and evolution through a phylogenetic context.

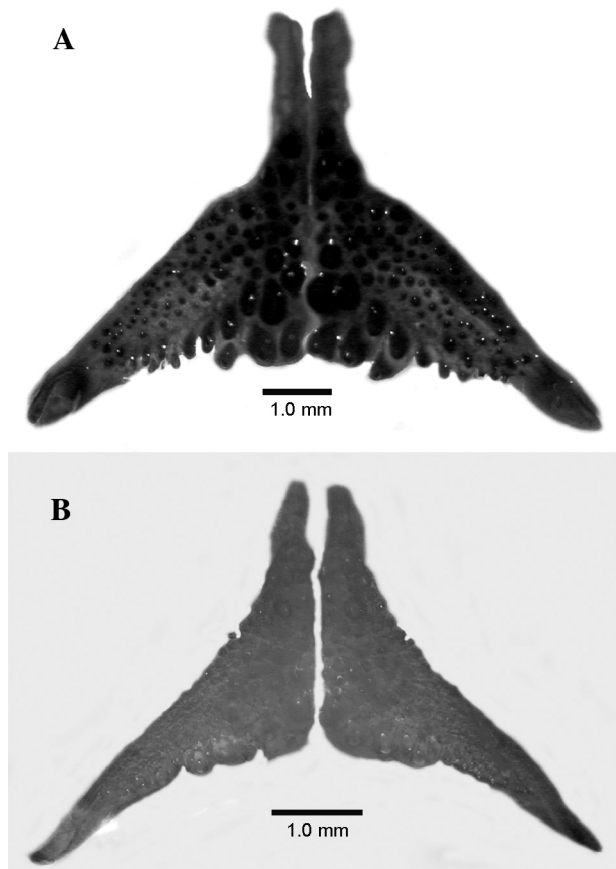


Figure 2. - Fifth ceratobranchials, dorsal view. **A:** *Cynolebias perforatus*; **B:** *C. griseus*.

## MATERIALS AND METHODS

The following material was prepared for examination of morphological details of the *Cynolebias*-PJA: *C. albipunctatus*: UFRJ 4467 (6 ex.), UFRJ 4515 (1 ex.) UFRJ 5128 (1 ex.); *C. altus*: UFRJ 5131 (3 ex.), UFRJ 5132 (3 ex.); *C. attenuatus*: UFRJ 4779 (2 ex.), UFRJ 5143 (8 ex.); *C. gibbus*: UFRJ 5133 (2 ex.), UFRJ 5134 (1 ex.); *C. gilbertoi*: UFRJ 4471 (2 ex.), UFRJ 4773 (5 ex.); *C. griseus*: UFRJ 150 (2 ex.), UFRJ 319 (1 ex.), UFRJ 5124 (1 ex.); *C. itapicuruensis*: UFRJ 5113 (3 ex.), UFRJ 4514 (1 ex.), UFRJ

5120 (3 ex.); *C. leptocephalus*: UFRJ 3810 (2 ex.); *C. microphthalmus*: UFRJ 5125 (3 ex.); *C. perforatus*: UFRJ 4771 (3 ex.), UFRJ4776 (8 ex.), UFRJ 2077 (2 ex.), UFRJ 4468 (2 ex.); *C. vazabarrisensis*: UFRJ 5121 (1 ex.), UFRJ 4467 (2 ex.). Examined material also includes all species of the genera *Nematolebias*, *Simpsonichthys* and *Austrolebias*, including about equal number of males and females, that are listed in Costa (2002, 2006a, 2007).

Osteological preparations were made according to Taylor and Van Dyke (1985). Terminology for bones and laterosensory neuromasts follows Costa (2001) and for muscles, Winterbottom (1974). The abbreviation UFRJ connotes material deposited in Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro.

## RESULTS

Some morphological features of the  $C\alpha$ -PJA make it notably distinct from the PJA of other aplocheiloid killifishes, including *Cynolebias griseus* that is not a member of the  $\alpha$ -clade of *Cynolebias*, as compared below.

### Fifth ceratobranchial and third pharyngobranchial

The fifth ceratobranchial is always triangular in a dorsal view, bearing a well-developed tooth-plate (Fig. 2). It is positioned ventral to the third pharyngobranchial, which also bears a similar tooth-plate. However, in species of the  $C\alpha$ -clade, the anterior portion of the fifth ceratobranchial is distinctively narrower and longer than in other cynolebiasines, and without teeth (Fig. 2A). In species of the  $C\alpha$ -clade, the dorsal surface of the fifth ceratobranchial is concave, with most teeth approximately conical, but those on the postero-medial portion are hypertrophied and have a molariform aspect (Fig. 3A); similar teeth are found in the dorsally adjacent portion of the third pharyngobranchial. In *C. griseus* and in the remaining cynolebiasines, the dorsal surface of the fifth ceratobranchial is approximately plain and all teeth are conical (Fig. 3B).

Uniquely among cynolebiasines, the ventral surface of the fifth ceratobranchial has a pronounced triangular process in species of the  $C\alpha$ -clade (Fig. 4), and the fifth ceratobranchials are in contact along the ventromedial edge (Fig. 2A) (Costa, 2001). In other cynolebiasines, there is a short and rounded ventral process and ceratobranchials are medially separated by a small interspace (Fig. 2B).

### Infrabranchial ossification

Costa (2001) first described a small unpaired cylindrical ossification present on the ventral portion of the branchial skeleton of some species of *Cynolebias*, hereafter termed as infrabranchial ossification. This ossification has a peculiar morphology, with dorsal cartilage head and ventrolateral

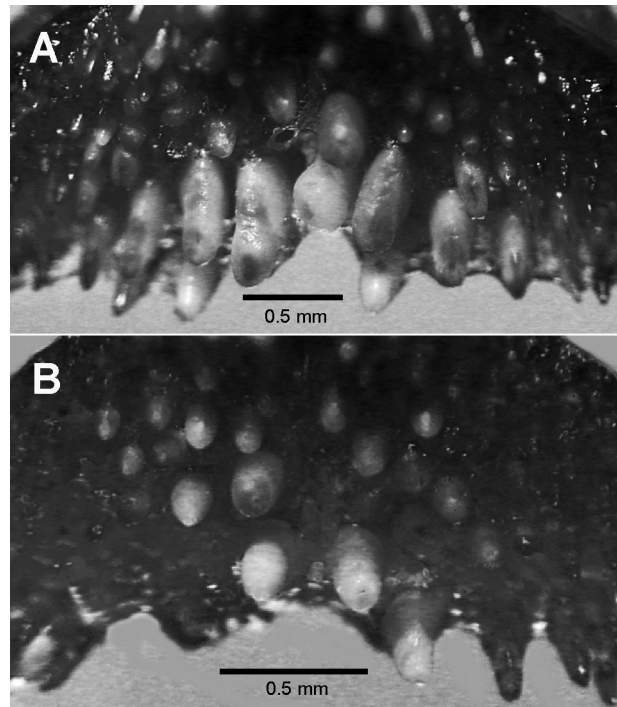


Figure 3. - Detailed postero-dorsal view of the posterior portion of the fifth ceratobranchial dentition. A: *Cynolebias perforatus*; B: *C. griseus*.

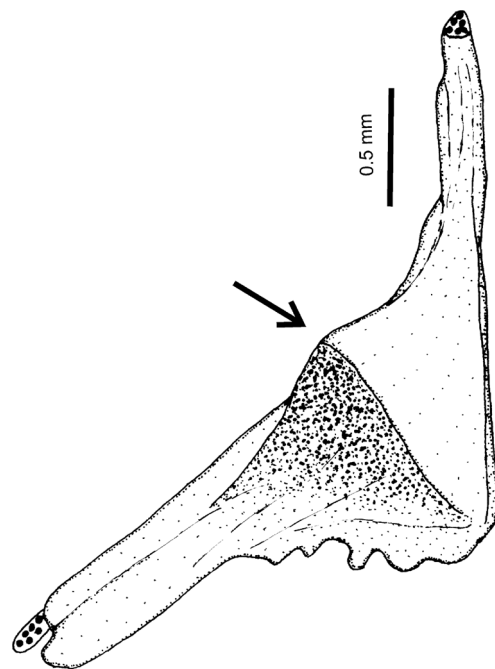
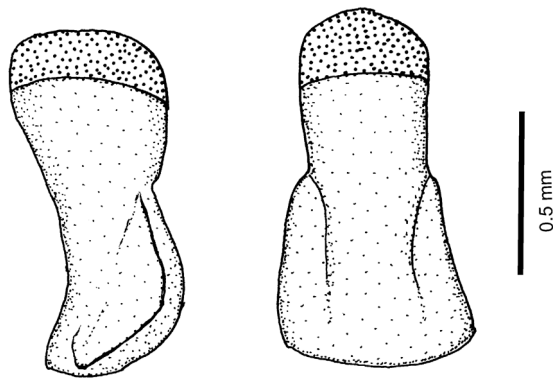


Figure 4. - Ventral surface of the right fifth ceratobranchial of *Cynolebias gilbertoi*. Arrow indicates triangular process.

wings (Fig. 5). Its longer axis is vertically positioned anteriorly to the fifth ceratobranchial and below the fourth basi-branchial cartilage, and it is attached by dense ligaments to



**A** **B**

Figure 5. - Infrabranchial ossification of *Cynolebias perforatus*. **A**: Lateral view; **B**: Posterior view.

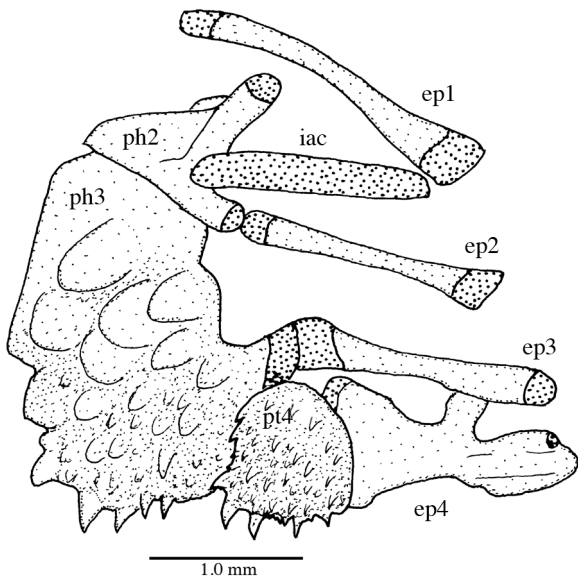


Figure 6. - Left dorsal portion of branchial skeleton of *Cynolebias leptocephalus*: ep1-4, epibranchials 1-4; iac, interarcual cartilage; ph2-3, pharyngobranchials 2-3; pt4, pharyngobranchial tooth plate 4.

the surrounding structures. The dorsal portion is attached to the anterior tip of each fifth ceratobranchial and to the fourth basibranchial cartilage. The ventral portion is attached by dense connective tissue to a wide, ventrally positioned, longitudinal muscle, which presently has not its homology identified among common branchial muscles of other cynolebiasines. Each lateral wing is attached to the nearest third hypobranchial. The infrabranchial ossification is present in all species of the  $C\alpha$ -clade and it is absent in all other cynolebiasines.

#### Fourth epibranchial

In contrast to the first, second and third epibranchials that

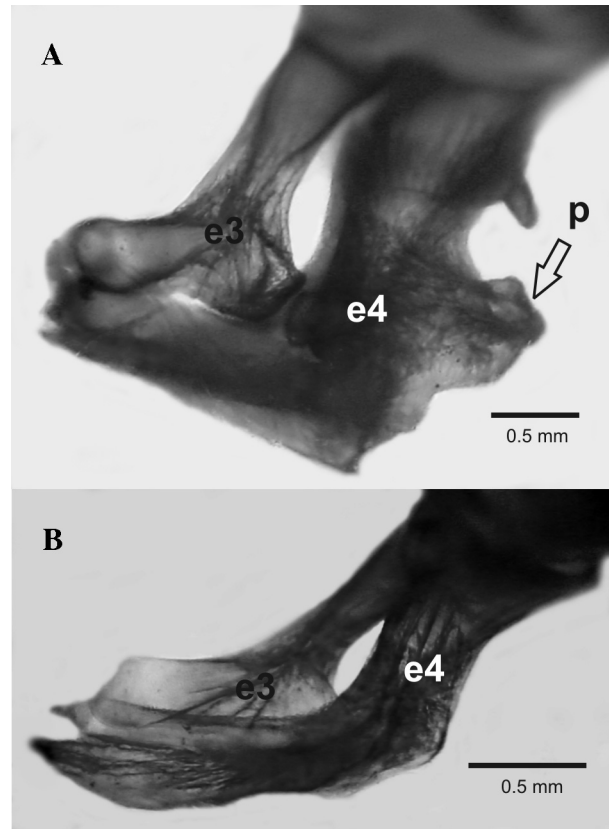


Figure 7. - Left third (e3) and fourth (e4) epibranchials, dorsal view. **A**: *Cynolebias perforatus*; **B**: *C. griseus*. p: process for insertion of the *levator posterior* muscle.

are narrow, without processes and approximately straight, the fourth epibranchial in species of the  $C\alpha$ -clade is a robust bone (Fig. 6), bent in its longest axis (Fig. 7A). In addition, there is a distinct process on its dorsal margin for insertion of the *levator posterior* muscle (Fig. 7A). Although a great variation in the fourth epibranchial width occurs among aplocheiloids, the fourth epibranchial is only slightly curved, never presenting a process on the dorsal margin in other cynolebiasines (Fig. 7B).

#### Muscles associated to the dorsal branchial skeleton and dorsal portion of the pectoral girdle

In species of the  $C\alpha$ -clade, the first and second *levatores externi* are significantly narrower than the third and fourth *levatores externi*, but the second *levator externus* is about twice wider than first one (Fig. 8A). The third and fourth *levatores externi* form a robust muscular mass, about six times wider than the second *levator externus*. The *levator posterior* is slightly narrower than fourth *levator externus*. The *protractor pectoralis* is robust, slightly narrower than the third plus fourth *levator externus* muscles; the *protractor pectoralis* and the *levator externus* 4 are slightly overlapping, thereby hiding the *levator posterior* under a lateral



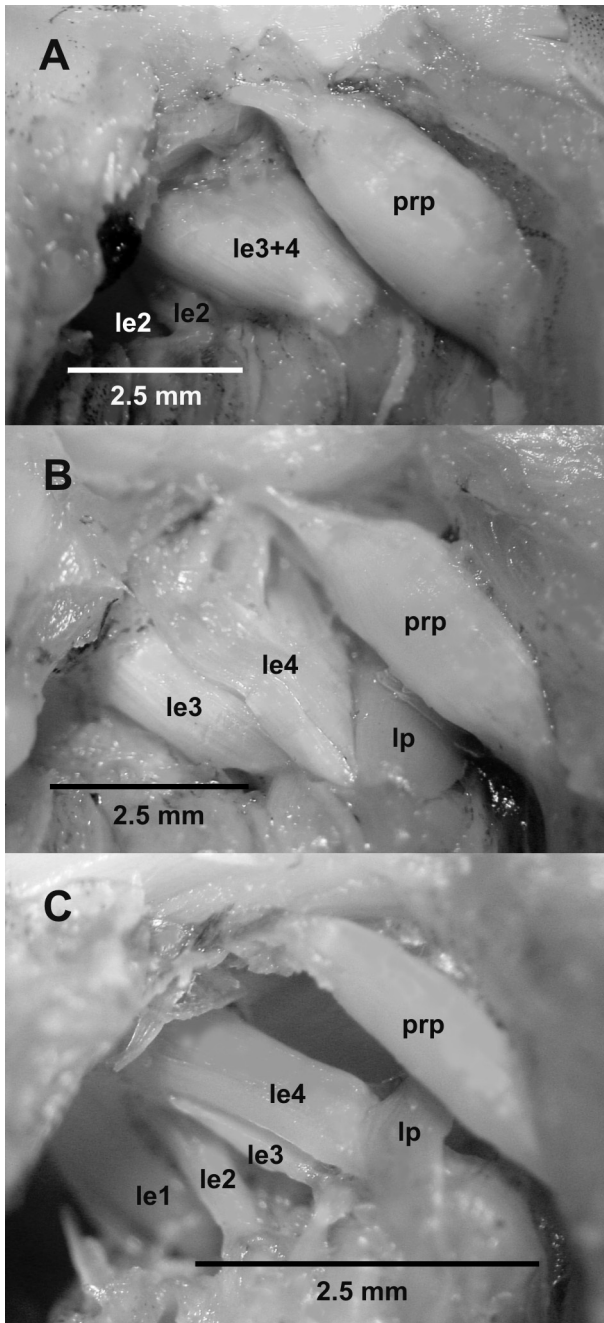


Figure 8. - Muscles associated to the dorsal skeleton and dorsal portion of the pectoral girdle, left lateral view. **A:** *Cynolebias perforatus*; **B:** *C. griseus*; **C:** *Nematolebias whitei*: le1-4, levator externi 1-4; lp, levator posterior; prp, protractor pectoralis.

view. Both in species of the  $C\alpha$ -clade and *Cynolebias griseus* (Fig. 8A-B), the muscles associated to the posterior portion of the dorsal branchial skeleton and the dorsal portion of the pectoral girdle are wider than in other cynolebiasines (Fig. 8C). However, these muscles are not so widened in *C. griseus*, making the levator posterior visible laterally (Figs. 8B).

DISCUSSION

Elaborated PJA morphology is often found among percomorph fishes, such as carangids, sciaenids, haemulids, and labroids (e.g., Liem and Sanderson, 1986; Rosen and Patterson, 1990; Grubich, 2003). However, the PJA of athrinomorphs is composed of unspecialized structures, except in species of the  $C\alpha$ -clade, which exhibits a specialized morphological design. All species of the  $C\alpha$ -clade have: 1) the anterior portion of the fifth ceratobranchial narrow and long, 2) robust teeth on the second pharyngobranchial and fifth ceratobranchial, 3) a pronounced process on the ventral surface of the fifth ceratobranchial, 4) fifth ceratobranchials medially in contact, 5) infrabranchial ossification, 6) fourth epibranchial L-shaped and with process for insertion of the levator posterior muscle, and 7) the third and fourth levator externi and the protractor pectoralis widened to make levator posterior not visible laterally, features strongly deviating from the typical PJA of cyprinodontiform fishes. Character states 1-4 and 7 may be convergently acquired in percomorphs lineages comprising sound producing species, but character states 5 and 6 have no similar representatives, making the  $C\alpha$ -PJA morphologically unique among teleosts.

The combination of the unique occurrence of a series of specialized osteological traits of the PJA in all species of the *Cynolebias* clade  $\alpha$  and the absence of intermediate stages in the remaining cynolebiasines suggests that morphological innovation has suddenly and simultaneously arisen at the base of *Cynolebias* clade  $\alpha$  (Fig. 9). However, the origin of robust muscles dorsally positioned to branchial arches, occurring to a lesser extent in *C. griseus*, seems to be arisen at the base of the genus (Fig. 9), with a subsequent elabora-

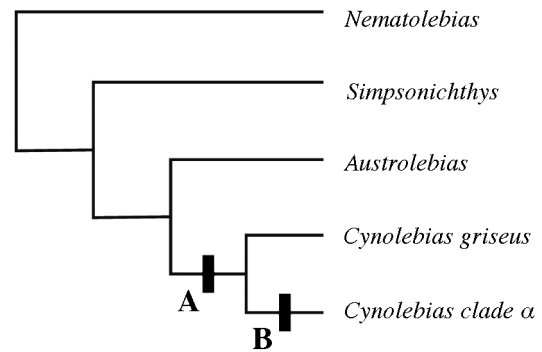


Figure 9. - Cladogram among Cynolebiasini lineages based on Costa (2001, 2006a, 2006b), showing PJA derived character states. **A:** Robust muscles dorsally positioned to branchial arches; **B:** Anterior portion of fifth ceratobranchial narrow and long; robust teeth on second pharyngobranchial and fifth ceratobranchial; pronounced process on ventral surface of fifth ceratobranchial; fifth ceratobranchials medially in contact; infrabranchial ossification; fourth epibranchial L-shaped, with process for insertion of levator posterior muscle; third and fourth levator externi and protractor pectoralis widened to make protractor pectoralis overlapping levator externus 4.

tion (*protractor pectoralis* overlapping *levator externus* 4) in the *Cynolebias* clade  $\alpha$ . All these character states are present both in males and females, but sound production in females is presently unknown.

Stridulation of pharyngeal teeth as a mode of sound production has been reported for some teleost lineages (e.g., Burkenroad, 1930; Luh and Mok, 1986). Among labroids, records of sound production using morphologically elaborated PJA are broadly reported in the literature, although the exact mechanism is still unclear (Rice and Lobel, 2003). Although data on *Cynolebias* sound production is restricted to a few observations during *C. albipunctatus* courtship behavior (Belote and Costa, 2003), the widespread occurrence of PJA elaborated morphological traits among species of the *Cynolebias* clade  $\alpha$  suggests that sound production is shared by all members of this clade.

Elaborated pharyngobranchial bone structure and its evolutionary flexibility has been considered as an important factor to the explosive trophic diversification occurring in labroid percomorphs (e.g., Liem and Sanderson, 1986; Galis and Metz, 1998), and the fusion degree of the fifth ceratobranchials is interpreted as directly proportional to the ability to exploit durable prey (Hulsey, 2006). In addition, a combination of stronger pharyngeal toothplates and larger *protractor pectoralis* muscle has been convergently acquired by molluscivore species of different percomorph lineages (e.g., Grubich, 2003). Therefore, it is possible that the same osseous and muscular specializations in *Cynolebias* are also associated to molluscivory (primarily or secondarily), but data on the feeding biology of species of *Cynolebias* are still unavailable.

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