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Marcelo Loureiro

Rafael O. de Sá
University of Richmond, rdesa@richmond.edu

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Osteological Analysis of the Killifish Genus *Cynolebias* (Cyprinodontiformes: Rivulidae)

MARCELO LOUREIRO* AND RAFAEL O. DE SÁ
Department of Biology, University of Richmond, Richmond, Virginia

ABSTRACT Relationships among the species of the annual fish *Cynolebias* are unclear. An analysis of the variation and utility of osteological characters for phylogenetic analysis was done using cleared and double-stained specimens representing 21 species of *Cynolebias*. This analysis showed that some of the characters previously used to diagnose this genus and some of the species are polymorphic. Osteologically, *Cynolebias* can be diagnosed by the following synapomorphies: (1) triangular-shaped parietal, (2) vomer positioned ventral to the parasphenoid, (3) long ventral process of the dentary, (4) teeth on fourth ceratobranchial, and (5) teeth on first epibranchial. In addition, characters that help define some of the currently recognized species complexes were identified. Species in the "*antenori* complex" share at least five synapomorphies, such as ossified medial radials of dorsal and anal fins, four pectoral radials, ventral process of the maxillae enlarged, mesopterygoid long relative to the autopalatine, and proportion of cartilage in the basihyal. The "*bellottii* complex" is characterized by having a reduce basihyal and a deep urohyal, whereas species in the "*elongatus* complex" possess a caudal fin supported by four vertebrae and have unique modifications of jaw bones. The following osteological features are useful as diagnostic characters at the specific level: (1) Two vertebrae supporting the caudal fin (*C. nigripinnis*); (2) cartilaginous pelvic bones (*C. notatus*); (3) short third postcleithrum and broad lateral process of the sphenotic (*C. wolterstorffi*); (4) thick third postcleithrum (*C. gymnoventris*); (5) crest on the parietal and reduced in the upper portion of the lacrimal (*C. whitei*); (6) anteriorly curved lacrimal (*C. cheradophilus*); (7) second dermosphenotic (*C. bellottii*); and (8) expansion of the ventral tip of the maxillae and long basyhial (*C. constanciae*). J. Morphol. *238:*245–262, 1998. © 1998 Wiley-Liss, Inc.

KEY WORDS: *Cynolebias*; synapomorphies; species complexes

The genus *Cynolebias* belongs to the family Rivulidae (Order Cyprinodontiformes) and possesses a characteristic annual life cycle. This life cycle is shared by most of the species in the family Rivulidae and with some species of the African family Aplocheilidae. This unique life cycle for vertebrates includes drought-resistant eggs laid by the adults in the substrate of the temporary ponds they inhabit. Embryos develop and survive dry periods between wet seasons by undergoing diapause (Wourms, '72). Subsequently, larvae will complete development and hatch after the ponds are filled by heavy rains. Juveniles can reach maturity as early as 2 months after hatching.

The first phylogenetic and biogeographic analysis of the order Cyprinodontiformes was done by Parenti ('81), who provided uniquely derived characters to define the family Rivulidae. In addition, she provided two synapomorphies for *Cynolebias*: (1) caudal fin not scaled, and (2) preopercular canal closed. Furthermore, she included *Cynopoecilus*, *Leptolebias*, *Simpsonichthys*, *Campellolebias*, and *Terranatos* in the synonymy of *Cynolebias*. A more comprehensive study of the family Rivulidae was done by Costa ('90), who recognized *Cynopoecilus*, *Leptolebias*,

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M. Loureiro is currently at the Facultad de Ciencias, Sección Vertebrados, Iguá 4225, Montevideo 11400, Uruguay.

*Correspondence to: Marcelo Loureiro, Facultad de Ciencias, Sección Vertebrados, Iguá 4225, Montevideo 11400, Uruguay.

Campellolebias, *Terranatos*, ”*Cynolebias*” (= *Plesiolebias*, Costa ’91), and *Cynolebias* as monophyletic lineages and grouped them into a monophyletic subfamily, Cynolebiatinae. In the analysis of *Cynolebias*, Costa (’90) proposed five generic synapomorphies: (1) males with more rays in the dorsal fin than females; (2) juveniles with a dark spot on each side in the middle of the body; (3) at least 16 neuromast in the supraorbital series; (4) preanal length representing 55% of the standard length; and (5) postero-lower process of dentary elongated. A phylogenetic analysis of the subfamily was done by Costa (’95), in which he proposed three autapomorphies for *Cynolebias*: (1) male with more dorsal fin rays than female; (2) female with black blotch on center of body sides; and (3) anal fin base of males enlarged. Species of *Cynolebias* have been clustered in “species groups” by Amato (’86) and in “species complexes” by Costa and Brasil (’90, ’93) (Table 1), whereas interspecific relationships were analyzed by Costa (’95).

TABLE 1. Species complexes of Cynolebias *(Costa and Brasil, ’90, ’93)*

Plesiomorphic species
- *C. constanciae*[1]
- *C. whitei*[1]
- *C. myersi*[1]
- *C. izecksoni*

Bellottii complex
- *C. affinis*[1,2]
- *C. alexandri*[1,2]
- *C. cyaenus*[1,2]
- *C. gymnoventris*[1,2]
- *C. luteoflamulatus*[1,2]
- *C. nigripinnis*[1,2]
- *C. adloffi*[1,3]
- *C. bellottii*[1,3]
- *C. viarius*[1,3]
- *C. cinereus*[3]
- *C. carvalhoi*[3]

Species related
- *C. melanoorus*
- *C. vazferreirai*
- *C. nonoiuliensis*
- *C. costai*
- *C. duraznensis*[1]

Antenori complex
- *C. fulminantis*
- *C. antenori*
- *C. flavicaudatus*[1]
- *C. flammeus*[1]
- *C. magnificus*

Species related
- C. stellatus
- C. alternatus
- C. notatus[1]
- *C. bokermanni*[1]
- *C. boitonei*[1]
- *C. zonatus*
- *C. chacoensis*[1]
- *C. hellneri*
- *C. santanae*

Elongatus complex
- *C. elongatus*
- *C. prognathus*
- *C. cheradophilus*[1]
- *C. wolterstorffi*[1]
- *C. holmbergi*

Porosus complex
- *C. porosus*
- *C. albipunctatus*
- *C. griseus*
- *C. leptocephalus*
- *C. perforatus*

Unknown relations
- *C. trilineatus*

[1]Species used in this analysis.
[2]Species of luteoflamulatus group.
[3]Species of adloffi group (Amato, ’86). Species related according to Wildekamp (’95).

Reports on the characteristics of isolated bones for various species are available in the literature; however, the complete osteology has not been previously described for any species of *Cynolebias*. The present study has three goals: first, to provide a complete osteological description of *Cynolebias luteoflamulatus* that could serve as baseline for comparative purposes; second, to describe some of the inter- and intraspecific osteological variation present in *Cynolebias*; and third, to analyze the utility of osteological characters for phylogenetic analyses of the genus *Cynolebias* as was defined by Costa (1995).

MATERIALS AND METHODS

Specimens of *Cynolebias* used in this study were in part collected in the field in Uruguay and in part borrowed from Facultad de Ciencias, Montevideo, Uruguay, with the rest obtained in the pet trade (see the Appendix).

For osteological analysis specimens were cleared and double-stained for cartilage and bone following Dingerkus and Uhler (’77). Specimens were observed under a dissecting microscope (Leica Wild M3C), and drawings were made with a camera lucida attached to the microscope. Bone and cartilage terminology follows that of Parenti (’81) and Costa (’90).

RESULTS

Osteological description of Cynolebias luteoflamulatus

Vertebrae

The number of vertebrae varied between 27 and 32 (x = 30, sd = 1, m = 30). The first vertebra lacks neural prezygapophyses and pleural ribs but has a laterally compressed neural spine (Fig. 1A). The second vertebra also has a laterally compressed neural spine. The first pleural rib originates from the second vertebra. Neural prezygapophyses are present on the abdominal vertebrae. These structures are larger on the most anterior vertebrae, decreasing posteriorly. The neural prezygapophysis is one fourth the height of the neural spine. The caudal vertebrae have reduced prezygapophyses (Fig. 1C).

Caudal fin

The caudal fin is homocercal and rounded. The skeleton of the caudal fin is almost perfectly symmetrical due to the fusion of the hypural plates into a single fan-shaped structure flanked by a dorsal free epural and

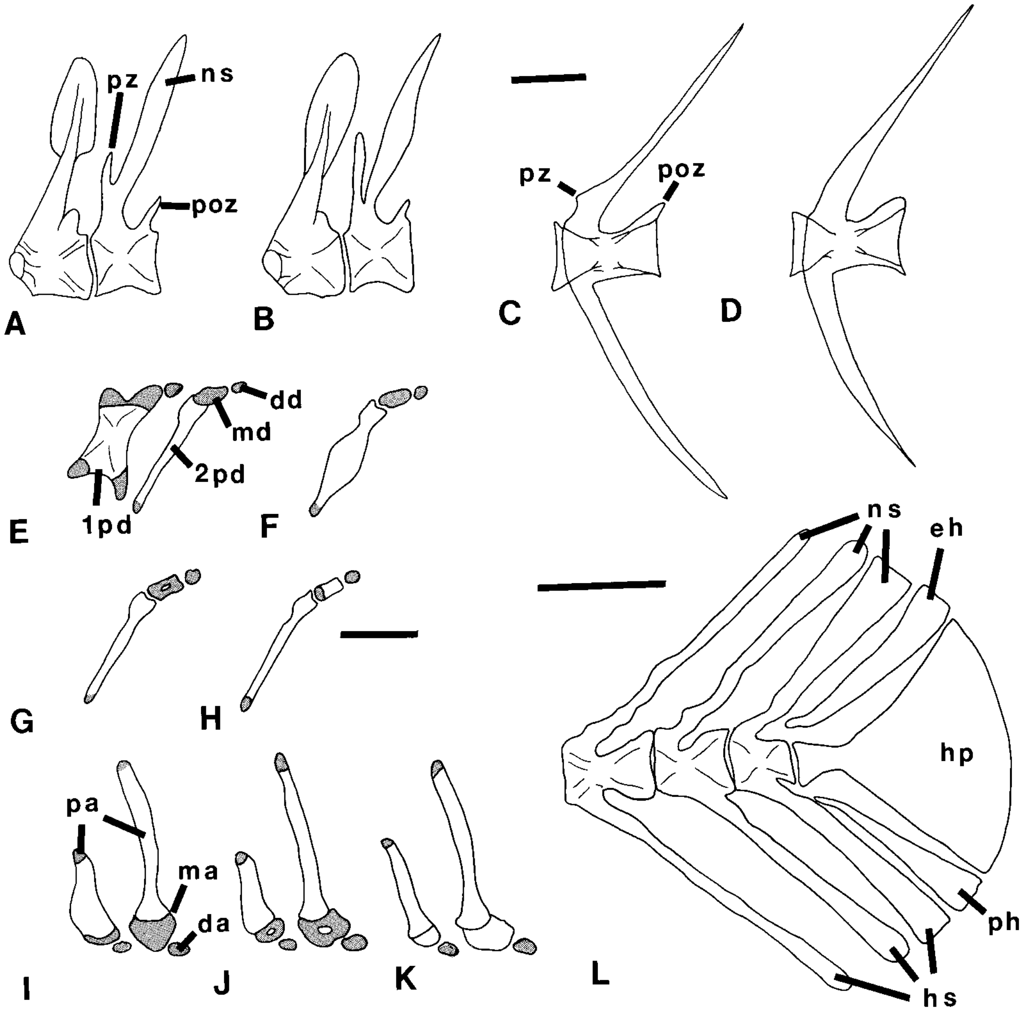

Fig. 1. Lateral view of first and second vertebrae (ribs have been removed) of (**A**) *Cynolebias luteoflamulatus*; (**B**) *Cynolebias viarius*. Lateral view of caudal vertebrae of (**C**) *Cynolebias luteoflamulatus*; (**D**) *Cynolebias bokermanni*. Lateral view of first and second radials of dorsal fin of *Cynolebias luteoflamulatus* (**E**). Lateral view of second radials of dorsal fin of (**F**) *Cynolebias constanciae*; (**G**) *Cynolebias flavicaudatus*; (**H**) *Cynolebias bokermanni*. Lateral view of first and second radials of anal fin of (**I**) *Cynolebias luteoflamulatus*; (**J**) *Cynolebias notatus*; (**K**) *Cynolebias chacoensis*. Lateral view of caudal skeleton of *Cynolebias luteoflamulatus* (**L**). da, distal radial of anal fin; dd, distal radial of dorsal fin; eh, epural; hp, hypural plate; hs, hemal spines of vertebrae; ma, medial radial of anal fin; md, medial radial of dorsal fin; ns, neural spine; pa, proximal radial of anal fin; ph, parhypural; poz, neural postzygapophisis; pz, neural prezygapophisis; 1pd, first proximal radial of dorsal fin; 2pd, second proximal radial of dorsal fin. Shaded area represents cartilage. Scale bars = 1mm.

a ventral free parhypural. The last three vertebrae, and their corresponding neural and hemal spines articulate with the fin rays participating in the support of the caudal fin (Fig. 1L).

Dorsal and anal fin

The proximal radials of dorsal and anal fins are ossified and elongated, except for their ventral tips, which are cartilaginous (Fig. 1E,I), whereas the medial radials are short and cartilaginous. Each radial supports one ray, except the first and the last ray of both fins that support two rays each. The first and second proximal radials of anal and dorsal fins are partially or completely fused into a single element. The distal radials of both fins are small cartilaginous elements found at the base of the fin rays. The proximal radials and the rays of the anal fin are thicker in females than in males and therefore appear closer to each other in females. Females have reduced medial and distal radials.

Pectoral fin and pectoral girdle (Fig. 2A)

The pectoral girdle articulates with the skull through the supracleithrum and the post-temporal bones. The post-temporal articulates with the epiotic in the skull. The post-temporal bone is thin, elongated, and partially fused to a flattened supracleithrum; these two bones are about equal in length. The post-temporal has a ventral process that points anteriorly. The first and second postcleithral bones are absent. Between the girdle and the first pleural rib, there is a thin, elongated, and free third postcleithrum bone. The third postcleithrae is slightly curved on its ventral end and is as thick as the first pleural rib. The main body of the girdle is formed by the cleithrum, scapula, coracoid, and pectoral radials. The cleithrum is curved and tall, extending from the ventral region of the body to the origin of the neural spine of the second vertebra. The

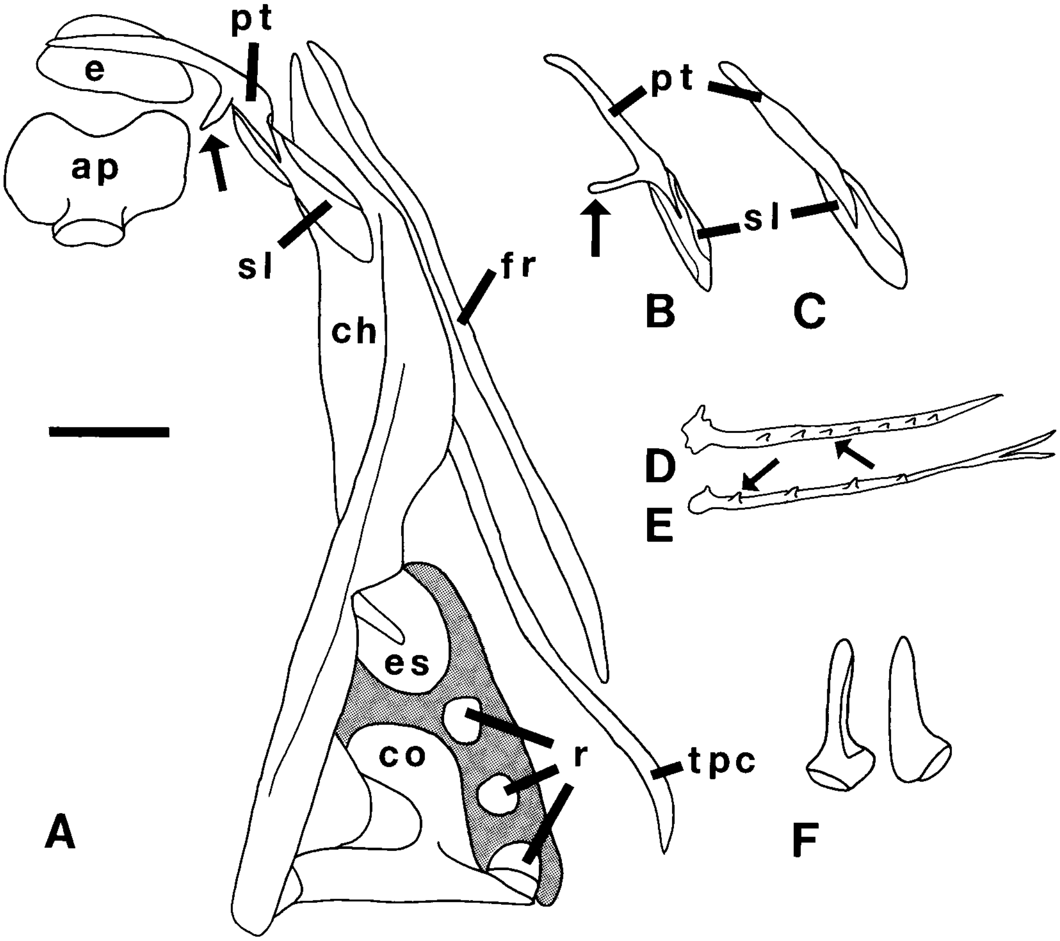

Fig. 2. Lateral view of pectoral girdle of *Cynolebias luteoflamulatus* (**A**). Lateral view of posttemporal and supracleithrum of (**B**) *Cynolebias bellottii*, (**C**) *Cynolebias wolterstorffi*. Anal fin ray of *Cynolebias cheradophilus* (**D**). Pectoral fin ray of *Cynolebias luteoflamulatus* (**E**). Ventral view of pelvic bones of *Cynolebias luteoflamulatus* (**F**). ap, autopterotic; ch, cleithrum; co, coracoid; e, epiotic; es, scapula; fr, first pleural rib; pt, posttemporal; r, pectoral radials; sl, supracleithrum; tpc, third postcleithrum. Symbols: big arrow, posttemporal spine; small arrow, ray protuberances. Shaded area represents cartilage. Scale bar = 1mm.

ventral tip of the cleithrum extends below the level of the ventral edge of the coracoid. The cleithrum has anterior and posterior flat expansions. The scapula is rounded and has a notch on its anterior margin. The coracoid is also rounded with a posterior spine-like projection. There are three rounded pectoral radials. However, in one individual examined, there are four pectoral radials. The pectoral rays of males have small and ossified projections arising from the internal side of the pectoral fin (Fig. 2E). Those projections are on rays 1–5. The scapula and radials articulate with the cleithrum and coracoid through a cartilaginous plate. Right and left cleithra articulate with each other ventrally. The main axis of the post-temporal–supracleithrum complex forms a 45° angle with the main axis of the dorsal edge of the cleithrum.

Pelvic fin and pelvic girdle

The two halves of the pelvic girdle do not overlap due to reduction of the medial process (Fig. 2F). Each bone is posteriorly expanded and lacks the ischial process. Rays supporting the pelvic fins articulate directly with each one of these bones.

Neurocranium

Dorsal view (Fig. 3A). The nasals are paired broad bones, forming an approxi-

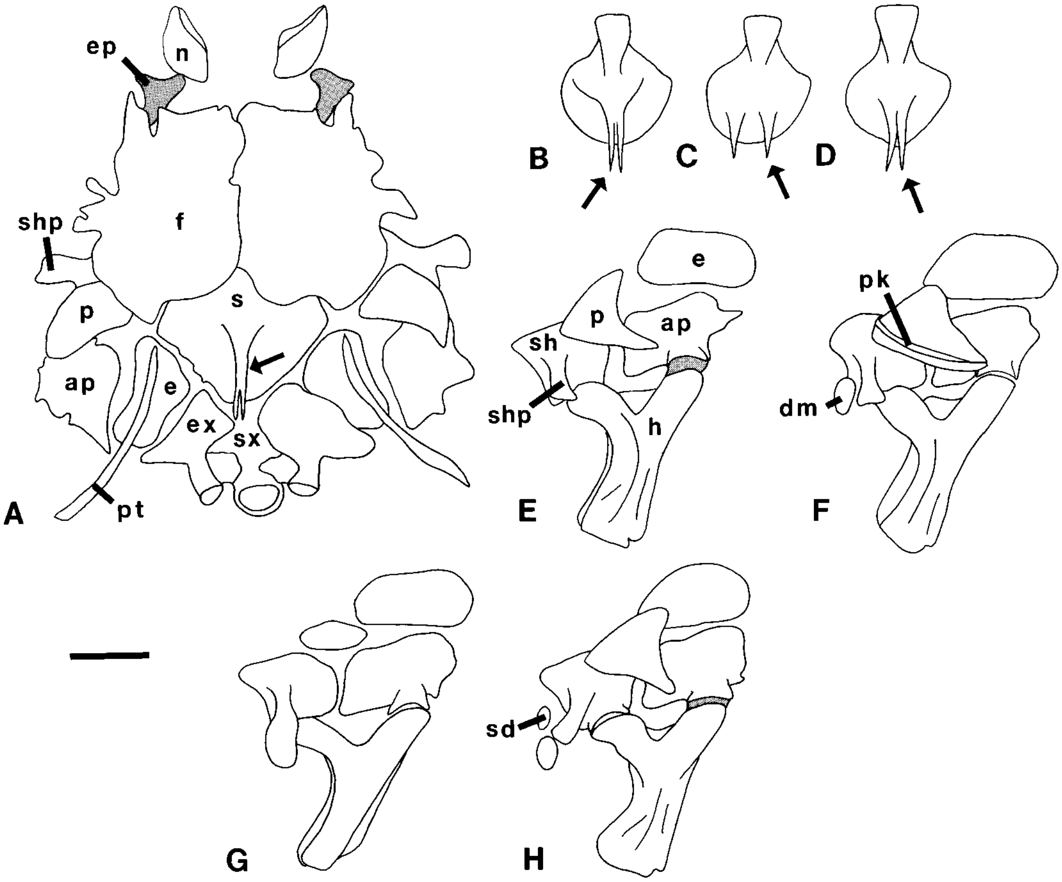

Fig. 3. Dorsal view of the neurocranium of *Cynolebias luteoflamulatus* (jaws have been removed) (**A**). Dorsal view of supraoccipital of (**B**) *Cynolebias luteoflamulatus*, (**C**) *Cynolebias whitei*, (**D**) *Cynolebias bellottii*. Lateral view of the hyomandibular-skull articulation of (**E**) *Cynolebias luteoflamulatus*, (**F**) *Cynolebias whitei*, (**G**) *Cynolebias wolterstorffi*, (**H**) *Cynolebias bellottii*. ap, autopterotic; dm, dermosphenotic; e, epiotic; ep, ethmoid process; ex, exoccipitals; f, frontal; h, hymandibular; n, nasal; p, parietal; pk, parietal crest; pt, posttemporal; s, supraoccipital; sd, "second dermosphenotic"; sh, sphenotic; shp, sphenotic process; sx, suboccipital. Symbol: arrow, supraoccipital spine. Shaded area represents cartilage. Scale bars = 1mm.

mately 45° angle with the horizontal plane. Nasals and frontals do not contact each other and are separated by cartilaginous ethmoid processes. The upper tip of this process is triangular in shape and is placed anterior and lateral to the frontals. The frontals are broad rectangular bones. They are truncated anteriorly and extend from the nasal to the otic region. Laterally, the frontals fold inward into the orbital region and form irregular-shaped lateral projections. The supraoccipital is a single triangular shaped bone found posterior of the frontals. The supraoccipital has an anterior triangular-shaped projection that underlies the frontals, and a dorsal posterior process. The posterior process, the supraoccipital spine, is formed by two parallel "wings" (Figs. 3A,B, 4A). They are connected to each other by a transverse bony bridge.

The sphenotic (Figs. 3A,E, 4A) articulates with the anterior arm of the hyomandibular.

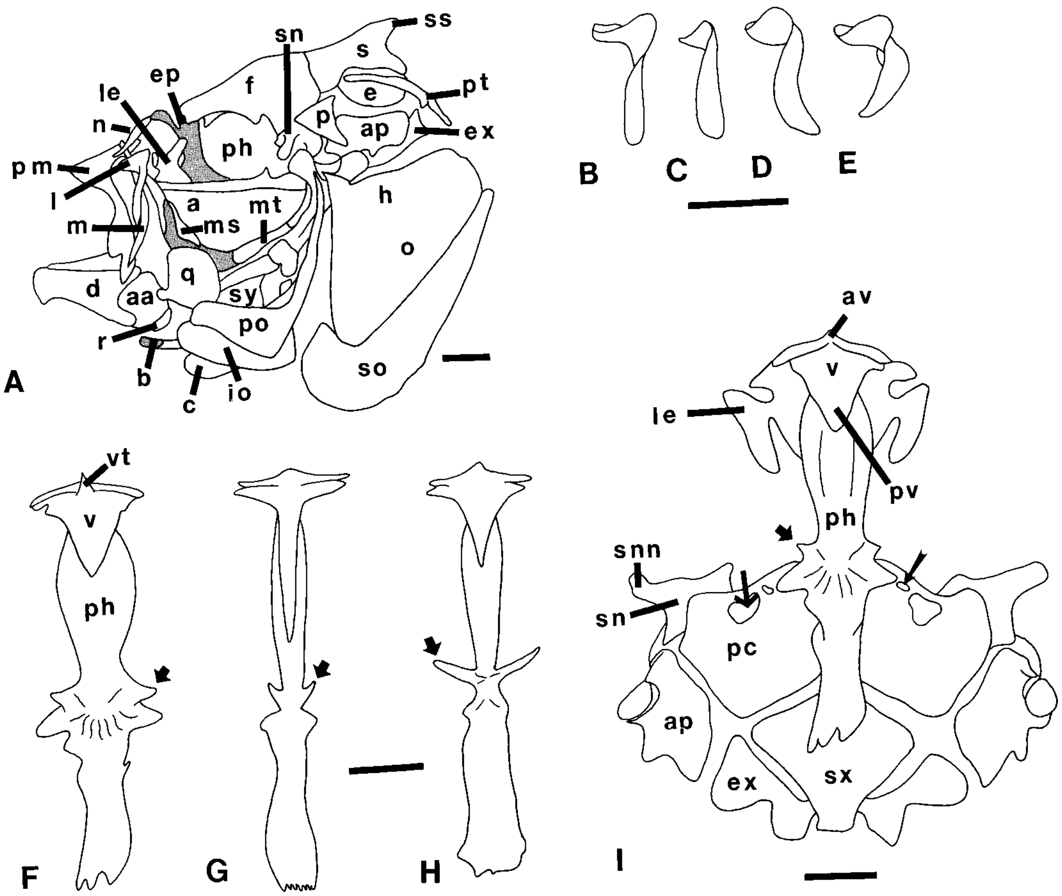

Fig. 4. Lateral view of the skull *Cynolebias luteoflamulatus* (**A**). Lateral view of the lacrimal of (**B**) *Cynolebias luteoflamulatus*, (**C**) *Cynolebias whitei*, (**D**) *Cynolebias constanciae*, (**E**) *Cynolebias cheradophilus*. Ventral view of vomer and parasphenoid of (**F**) *Cynolebias luteoflamulatus*, (**G**) *Cynolebias cyaenus*, (**H**) *Cynolebias wolterstorffii*. Ventral view of the neurocranium of *Cynolebias luteoflamulatus* (**I**). a, autopalatine; aa, anguloarticular; ap, autopterotic; av, anterior process of the vomer; b, basihyal; c, ceratohyal; d, dentary; e, epiotic; ep, ethmoid process; ex, exoccipital; f, frontal; h, hyomandibular; io, interopercular; l, lacrimal; le, lateral ethmoid; m, maxilla; ms, mesopterygoyd; mt, metapterygoyd, n, nasal; o, opercular; p, parietal; pc, prootic; ph, parasphenoid; pm, premaxilla; po, preopercular; pt, posttemporal; pv, posterior process of the vomer; q, quadrate; r, retroarticular; s, supraoccipital; sn, sphenotic; snn, sphenotic process; so, subopercular; ss, supraoccipital spine; sx, suboccipital; sy, symplectic; v, vomer; vt, vomer tooth. Symbols: big arrow, orbital artery foramen; small arrow, trigeminofascialis recess; thick arrow, processus ascendens of the parasphenoid. Shaded area represents cartilage. Scale bars = 1mm.

The lateral process of the sphenotic protrudes from the skull, directed ventrally and posteriorly. The parietal is variably present; if present, it is triangular shaped (Figs. 3A,E, 4A) and partially covers the frontal, the sphenotic, the autopterotic, and the epiotic. *Cynolebias luteoflamulatus* lacked parietals in two of the examined individuals, while the parietals in one specimen were reduced. The autopterotic articulates with the posterior arm of the hyomandibular and with the epiotic. The back of the skull comprises the exoccipitals, which articulate with the first vertebra through two occipital condyles, and the basioccipital, which articulates with the centrum of the first vertebra.

Ventral view (Fig. 4I). In the ventral view, the skeletal elements on the midline axis (from anterior to posterior) are: vomer, rostral cartilage, paired lateral ethmoids, parasphenoid, and basioccipital. The vomer is overall rhomboid shaped with posterior and anterior processes. The anterior edge of the vomer is thick (Fig. 4F,I). A single tooth is located on the anterior process of the vomer in four of the individuals examined (Fig. 4F). The posterior process of the vomer lies ventral of the anterior arm of the parasphenoid. The lateral ethmoid expands anteriorly, articulating with the vomer and with the anterior arm of the parasphenoids. It also has an expanded dorsal process that articulates with the autopalatine anteriorly. The parasphenoid is long, extending from the nasal region to the back of the skull, and has two lateral projections on its medial region (processus ascendens of the parasphenoid), which are triangular shaped. Medially the parasphenoid is expanded laterally (Fig. 4F, I).

Otic region (Fig. 4I). Lateral to the medial series are the following bones: prootic, sphenotic, autopterotic, and exoccipital (all paired bones). The prootic articulates laterally with the sphenotic (anteriorly) and the autopterotic (posteriorly); posteriorly with the basioccipital, and medially with the parasphenoid. Two pairs of fenestrae are found in the prootic, the larger one carries the orbital artery and the smaller one the trigeminofascialis recess.

Orbital rim

The dorsal bones of the orbital rim are attached to the skull. The infraorbital bones are represented by the lacrimal, a free bone located anteriorly. The lacrimal overlaps the medial portion of the maxilla laterally. The lacrimal is a narrow and elongate bone, its upper portion twisted in one direction and ventrally on the opposite direction, the ventral tip of this bone is rounded (Fig. 4A,B). The dermosphenotic is absent.

Jaw structure

Three bones are considered dorsal elements: premaxillae (paired), maxillae (paired), and rostral cartilage (single). The premaxillae have an ascending process that extends dorsally and posteriorly. This process is flat and broad and covers the rostral cartilage in its anterior portion (Fig. 5A). The alveolar arm of the premaxilla is anteriorly expanded. The maxillae are thin and perpendicular to the main body axis (Fig. 4A). Anteriorly, the maxillae have dorsal and ventral processes (Fig. 5E). The ventral process is concave posteriorly. It is located below the ascending process of the premaxillae and above the anterior portion of the rostral cartilage. The rostral cartilage has a discoidal shape (Fig. 5A). In large adults, it may be elongate and constrained medially. The rostral cartilage covers the anterior region of the vomer and it is free, allowing for the protrusion of the upper jaw.

There are four ventral elements: dentary, Meckel's cartilage, anguloarticular, and retroarticular (all paired). The dentary possesses an elongated ventral process and articulates posteriorly with the anguloarticular (Fig. 5I). The anguloarticular bone has two anterior processes: a medial (large) and a ventral (small). The retroarticular is small, located below and posteriorly to the anguloarticular. Anguloarticular and retroarticular articulate with the quadrate. Meckel's cartilage runs between the dentary and the anguloarticular along the length of the mandible.

Jaw suspensorium (Fig. 5L)

The jaw suspensorium consists of the autopalatine, mesopterygoid, quadrate, symplectic, metapterygoid, and hyomandibular. The autopalatine is elongate and covers part of the mesopterygoid; the latter is slender and curved. The autopalatine and mesopterygoid articulate with the quadrate ventrally. The mesopterygoid is about the same size of the autopalatine (Figs. 4A, 5L). The quadrate is triangular shaped and has a small anterior process that articulates with the anguloarticular and the retroarticular. The

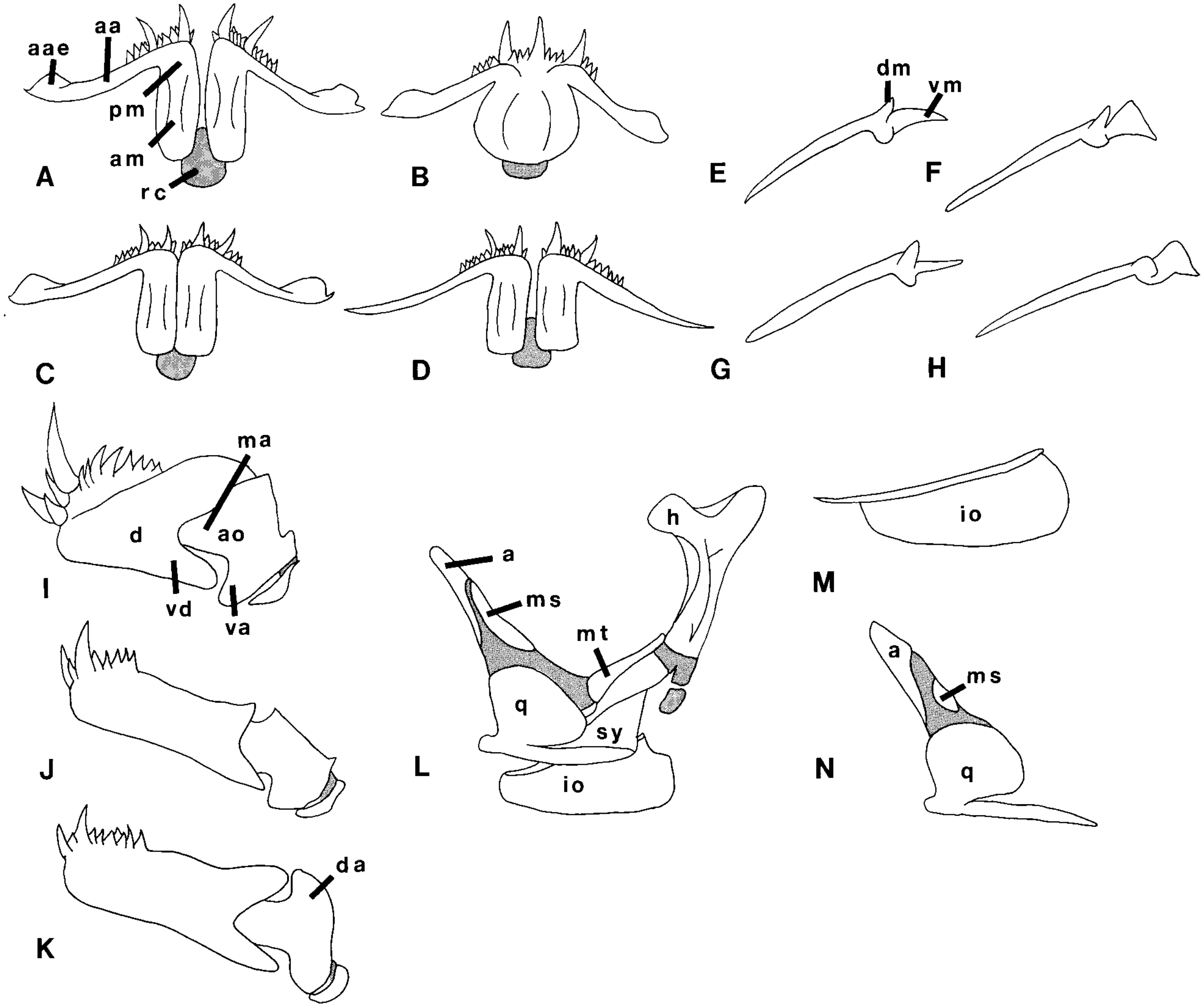
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Figure 5

quadrate possesses a thick, elongate, posterior process that articulates with the symplectic, which is partially overlapped by the quadrate. The length of the posterior process of the quadrate is equal to the bone itself without the process. Metapterygoid and mesopterygoid are separated by cartilage. The metapterygoid bone is elongated and slightly curved and contacts the symplectic ventrally. The symplectic is long, partially covers the metapterygoid, and has a posterior cartilaginous articulation to the hyomandibular. The hyomandibular is a Y-shaped bone; the upper arms articulate with the sphenotic anteriorly and with the autopterotic posteriorly. The ventral arm of the hyomandibular articulates with the symplectic anteriorly and with the interhyal posteriorly.

Opercular series (Fig. 4A)

The opercular is triangular shaped and articulates with the hyomandibular anteriorly. In the place of articulation the opercular has a small spine. The upper portion articulates with the hyomandibular and the opercular bones. The horizontal segment is laterally compressed and partially covers the interopercular. The interopercular is rounded and has a thick dorsal margin (Fig. 5L).

Gill arches

Ventral components (Fig. 6A). The interhyal is cartilaginous. The basihyal is trapezoidal or triangular shaped. Laterally, the basihyal articulates with the ventral and dorsal hypohyals. Ventrally, it articulates with the urohyal. Posteriorly, it articulates with the first basibranchial. The ratio basihyal length/ventral hypohyal length is three to one (Fig. 6B). The thickness of the basihyal is about 50% of its length. The basihyal consists of two parts, a posterior ossified component and a cartilaginous anterior one. The proportion of cartilage and bone is approximately one to two.

There are three ossified basibranchials. The hypobranchials are square shaped. There are five ceratobranchials, all bearing teeth. The first, second, and third have two rows of teeth, the fourth has one row, and the fifth is fully covered with teeth. The first ceratobranchial articulates with the first epibranchial and the interarcual cartilage dorsally. The second, third, and fourth ceratobranchials articulate with the second, third, and fourth epibranchials, respectively. The fourth ceratobranchial articulates with a triangular-shaped cartilage ventrally. The latter is located posteriorly to the third basibranchial. The fifth ceratobranchial is triangular shaped, has a ventral process, and does not articulate with any epibranchial. The urohyal bone is laterally compressed, is found ventral to the basihyal, and has an anterior and dorsally directed process. The height of the urohyal is 25% of its length (Fig. 6H). Ventral and dorsal hypohyals articulate with a long ceratohyal. The ceratohyal is divided in two (Fig. 6N). The anterior half is slender and has a posterior, expanded, and deep process, while the posterior half is deep in all its length. The anterior half supports two branchiostegal rays in 50% of the specimens examined, whereas in the other 50% it supports three branchiostegal rays. The deep process of the anterior half supports two rays. The posterior half of the ceratohyal supports one branchiostegal ray, and articulates posterodorsally with a cartilaginous interhyal. The processes of the anterior half and the posterior half of the ceratohyal are embedded in a cartilaginous plate. This plate supports another branchiostegal ray between the two portions.

Dorsal components (Fig. 6O). The first epibranchial is the only element of this series that possesses teeth. There are two teeth on this element. The interarcual cartilage and the second epibranchial articulate medially with pharyngobranchial 2. The third epibranchial articulates with pharyngobranchial 3 and the fourth epibranchial articu-

Fig. 5. Dorsal view of the premaxillae and rostral cartilage of (**A**) *Cynolebias luteoflamulatus*, (**B**) *Cynolebias myersi*, (**C**) *Cynolebias chacoensis*, (**D**) *Cynolebias wolterstorffi*. Dorsal view of maxillae of (**E**) *Cynolebias luteoflamulatus*; (**F**) *Cynolebias myersi*; (**G**) *Cynolebias cheradophilus*; (**H**) *Cynolebias chacoensis*. Lateral view of lower jaw of (**I**) *Cynolebias luteoflamulatus*, (**J**) *Cynolebias constanciae*, (**K**) *Cynolebias cheradophilus*. Lateral view of jaw suspensorium of *Cynolebias luteoflamulatus* (**L**). Lateral view of interopercular of *Cynolebias bokermanni* (**M**). Lateral view of anterior jaw suspensorium of *Cynolebias wolterstorffi* (**N**). a, autopalatine; aa, alveolar arm of the premaxilla; aae, expansion of the alveolar arm; am, ascending process of the premaxillae; ao, anguloarticular; d, dentary; da, dorsal process of the anguloarticular; dm, dorsal process of the maxilla; h, hyomandibular; io, interopercular; ma, medial process of the anguloarticular; ms, mesopterygoid; mt, metapterygoyd; q, quadrate; rc, rostral cartilage; sy, symplectic; va, ventral process of the anguloarticular; vd, ventral process of the dentary; vm, ventral process of the maxilla. Shaded area represents cartilage. Scale bar = 1 mm.

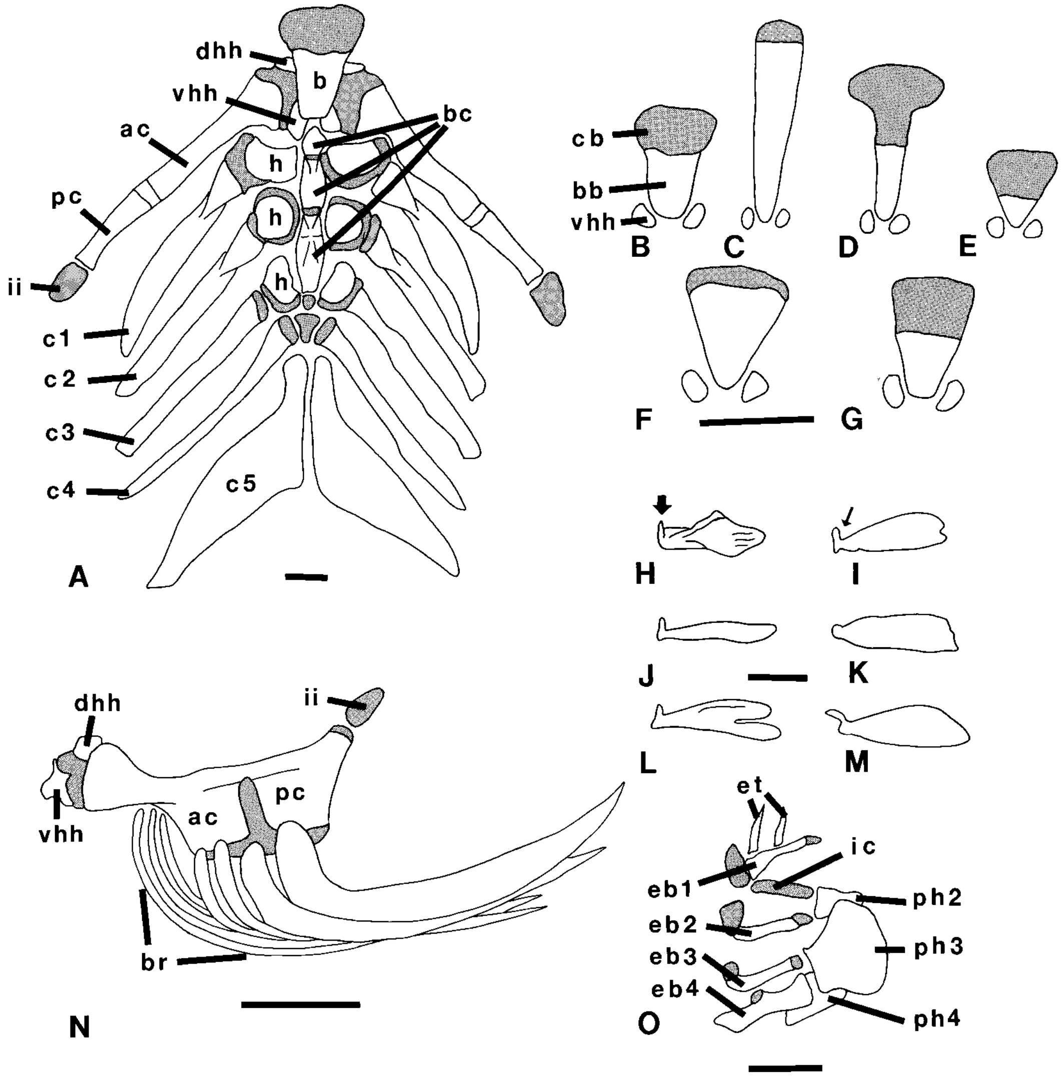

Fig. 6. Dorsal view of ventral gill arch of *Cynolebias luteoflamulatus* (**A**). Ventral view of basihyal of (**B**) *Cynolebias luteoflamulatus*, (**C**) *Cynolebias constanciae*, (**D**) *Cynolebias wolterstorffi*, (**E**) *Cynolebias cheradophilus*, (**F**) *Cynolebias whitei*, (**G**) *Cynolebias affinis*. Lateral view of urohyal of (**H**) *Cynolebias luteoflamulatus*, (**I**) *Cynolebias constanciae*, (**J**) *Cynolebias whitei*, (**K**) *Cynolebias nigripinnis*, (**L**) *Cynolebias cheradophilus*, (**M**) *Cynolebias bokermanni*. Lateral view of ceratohyal of *Cynolebias luteoflamulatus* (**N**). Dorsal view of dorsal gill arch of *Cynolebias luteoflamulatus* (**O**). ac, anterior ceratohyal; b, basihyal; bb, ossified basihyal; bc, basibranchials; br, branchiostegal rays; c, ceratobranchials 1, 2, 3, 4, 5; cb, cartilaginous basihyal; dhh, dorsal hypohyal; eb, epibranchials 1, 2, 3, 4; et, epibranchial teeth; h, hypobranchials; ic, interarcual cartilage; ii, interhyal; pc, posterior ceratohyal; ph, pharyngobranchials 2, 3, 4; vhh, ventral hypohyals. Symbols: big arrow, interhyal; small arrow, indentation of urohyal; thick arrow, anterior process of urohyal. Shaded area represents cartilage. Scale bars = 1mm.

lates with pharyngobranchial 4. Third and fourth epibranchials also articulate with each other medially. The fourth epibranchial is the thickest. The pharyngobranchials articulate with each other and are covered with teeth ventrally. The first pharyngobranchial is absent. The third pharyngobranchial is the largest and lies dorsal to the second and the fourth pharyngobranchials, covering them partially.

Teeth

Teeth are present on the premaxillae and the dentaries. The premaxillae has four rows of conical teeth. These teeth are all equal in size, except those on the first row, which has four larger, medial teeth. The dentaries also have four rows of conical teeth, all equal in size, except for four teeth that are located at the front and are three times the size of others. The gill arches teeth are also conical. The teeth of the ceratobranchials are larger on the first ceratobranchial, gradually decreasing posteriorly to the fourth ceratobranchial. Then, on the fifth ceratobranchial, the teeth increase in size again. The vomer tooth, when present, it is oriented anteriorly (Fig. 4B).

Osteological comparisons among species of Cynolebias

Vertebrae

Neural prezygapophyses are present in the abdominal vertebrae in most species examined; however, they are absent in *Cynolebias cheradophilus*, *C. myersi*, and *C. wolterstorffi*. Abdominal vertebrae of *C. bellottii*, *C. boitonei*, and *C. viarius* have well-developed neural prezygapophyses that are one-half the height of the neural spines (Fig. 1B). In other species, the neural prezygapophysis are only one-fourth the height of the neural spines (e.g., *C. luteoflamulatus*, Fig. 1A). Neural prezygapophyses of the caudal vertebrae are absent in *C. bokermanni*, *C. cheradophilus*, *C. constanciae*, *C. myersi*, and *C. wolterstorffi* (Fig. 1D). Other species present reduced neural prezygapophyses in the caudal vertebrae.

Caudal fin

Most species have a caudal fin similar to that described for *Cynolebias luteoflamulatus*. In *C. myersi* and *C. nigripinnis*, the caudal fin is supported only by the two last vertebrae, whereas in *C. cheradophilus* and *C. wolterstorffi*, the support is given by the last four vertebrae.

Dorsal fin

The proximal radials have lateral flat flanges in *Cynolebias constanciae* (Fig. 1F). The medial radials are short and variably ossified. In *C. chacoensis* and *C. constanciae*, they are completely ossified, whereas in *C. flavicaudatus*, *C. notatus*, and *C. whitei*, only the central region of the medial radials is ossified (Fig. 1G). In *C. bokermanni* and *C. myersi*, the medial radials remain cartilaginous only in their ventral tip (Fig. 5H). In other species examined the medial radials are similar to those of *C. luteoflamulatus*.

Anal fin

The medial radials are completely cartilaginous in most species, except in *Cynolebias bokermanni*, *C. constanciae*, and *C. notatus*, in which a center of ossification is found at the center of the bone (Fig. 1J), and in *C. chacoensis* where the medial radials are completely ossified (Fig. 1K). Fin rays of males of *C. cheradophilus* have small, spine-like, ossified projections (Fig. 2D).

The posterior rays of anal and dorsal fins of males of *Cynolebias boitonei*, *C. bokermanni*, *C. constanciae*, *C. chacoensis*, *C. flavicaudatus*, and *C. whitei* are elongated, with their distal fourth cartilaginous. *Cynolebias chacoensis* has a single, elongated, anal ray that extends beyond the posterior margin of the caudal fin.

Pectoral fin and pectoral girdle

Post-temporal and supracleithrum bones are of the same length in all species except in *Cynolebias boitonei*, in which the post-temporal is shorter. In *C. bellottii*, *C. bokermanni*, *C. duraznensis*, *C. flavicaudatus*, *C. viarius*, and *Cynolebias* sp., the post-temporal ventral process is long and reaches the back of the skull (Fig. 2B), whereas in *C. boitonei*, *C. myersi*, *C. whitei*, and *C. wolterstorffi*, this process is absent (Fig. 2C). In other species, the process is similar to *C. luteoflamulatus*. *Cynolebias wolterstorffi* has the shortest third postcleithrum, less than one-half the height of the cleithrum. In other species, the third postcleithrum is about 75% of the cleithrum height. In *C. gymnoventris*, he postcleithrum is thicker than the rib, whereas in the rest of the species postcleithrum and rib have the same thickness. The dorsal tip of the postcleithrum of *C. bellottii* can be fused to the first pleural rib (fused in 15 of the examined specimens). The number of pectoral radials is variable. *Cynolebias boitonei*, *C. bokermanni*, *C. chacoensis*, *C. constanciae*, *C. flavicaudatus*, *C. myersi*, *C. notatus*, and *C. whitei* have four rounded, pectoral radials whereas *C. cheradophilus*, *C. gymnoventris*, *C. wolterstorffi*, and *Cynolebias* sp. have three, rounded, pectoral radials. The latter species have lost the upper radial. In the other species, the number of radials varied between three and four among individuals. The percentage of individuals examined having four radials was: *C. alexandri* = 10%, *C. affinis* = 20%, *C. bellottii* and *C. nigripinnis* = 45%, *C. cyaenus* and *C. duraznensis* = 25%. In these species with a variable number of radials, the radials are rounded as in the species with three pectorals. Like *C. luteoflamulatus*, the pectoral rays of males of *C. boitonei*, *C. cheradophilus*, and *C. whitei* have small, ossified projections on the internal side of the pectoral fin. Similar structures were found on the anal fin of *C. cheradophilus*. The arrangement of these projections is species specific. In *C. boitonei*, they are present on rays 1, 2, and 3 (counting dorsal to ventral), in *C. whitei* on rays 1–6, and in *C. cheradophilus* on rays 1 through 11. Species with four pectoral radials have a more curved cleithrum, with a well-developed posterior projection that covers the dorsal half of the scapula. In these species, the scapula is least rounded.

Pelvic fin and pelvic girdle

Most species of *Cynolebias* have ossified pelvic bones, but the posterior three-fourths of the bone are cartilaginous in *C. wolterstorffi*, and in *C. notatus* the bones are completely cartilaginous. *Cynolebias boitonei* is unique among *Cynolebias* species because it lacks pelvic girdle and pelvic fins.

Neurocranium

Dorsal view. The “wings” of the supraoccipital spine can be completely separated from each other or they can be dorsally free but joined at their origin. They are free in *Cynolebias myersi* and *C. whitei* (Fig. 3C). They are joined at the origin in *C. boitonei*, *C. bellottii*, *C. cheradophilus*, *C. viarius*, and *C. wolterstorffi* (Fig. 3D). Other species exhibit a condition similar to that of *C. luteoflamulatus*.

The lateral process of the sphenotic is similar to the condition in *Cynolebias luteoflamulatus* in most species examined; however, in *C. bellottii*, *C. cheradophilus*, and *C. viarius*, the process points ventrally and anteriorly, and in *C. wolterstorffi* the process has a broad edge (Fig. 3G). The parietal of *C. affinis* is fused to the autopterotic, whereas in *C. bokermanni* it fuses with the epiotic. *Cynolebias whitei* has a parietal crest that runs from the anterior to the ventral apices of this bone (Fig. 3F). The parietal presents two states in *C. viarius*; it is reduced in five of the individuals and not reduced in the rest. *Cynolebias wolterstorffi* has a reduced and circular-shaped parietal in the only specimen examined (Fig. 3G). In other species the parietal is similar to that of *C. luteoflamulatus*. Parietals are absent in *C. cheradophilus* and *C. constanciae*. The bones of the posterior and dorsal region of the skull are mostly separated from each other in *Cynolebias*; however, in *C. boitonei*, *C. bokermanni*, *C. chacoensis*, *C. flavicaudatus*, and *C. notatus*, these bones contact with each other.

Ventral view. The posterior process of the vomer is thin and long in *Cynolebias chacoensis*, *C. cyaenus*, and *C. gymnoventris*. In these species, the length of the posterior process is three times the width of the bone (Fig. 4G). If present, the anterior process is rounded, but it is absent in *C. boitonei* and *C. flavicaudatus*. A single tooth may be present on the anterior process of the vomer. The tooth was observed in *C. nigripinnis* (4.4%) and *C.* sp.(6.7%). One specimen of *C. viarius* (2.6%) has two teeth on the vomer. In *C. wolterstorffi*, the processus ascendens of the parasphenoid is long and thin (Fig. 4H), while in other species examined it is similar to that of *C. luteoflamulatus*. In *C. luteoflamulatus*, *C. cheradophilus*, *C. bellottii*, and *C. viarius*, the parasphenoid expands laterally in its medial region; in other species, this bone is slender (Fig. 4G, H).

Orbital rim

In most species, the lacrimal has the same characteristics described for *Cynolebias luteoflamulatus*. However, in *C. whitei*, the upper portion of the lacrimal is reduced (Fig. 4C), in *C. bokermanni* and *C. constanciae*, the ventral portion curves posteriorly (Fig. 4D), while in *C. cheradophilus*, it is curved anteriorly (Fig. 4E). The dermosphenotic, when present, is a small, rounded, free bone next to the sphenotic (Fig. 3F). Species with a dermosphenotic are *C. whitei*, *C. constanciae*, *C. bokermanni*, *C. myersi*, *C. chacoensis*, and *C. bellottii*. *Cynolebias bellottii* has a second ossification similar and dorsal to the dermosphenotic (Fig. 3H).

Jaw structure

The rostral cartilage is discoidal in all species examined in this study. The ascending processes of left and right premaxillae are fused into a single structure in *Cynole-*

bias myersi (Fig. 5B). In *C. chacoensis*, the premaxillae overlap medially but do not fuse (Fig. 5C). Most species have the alveolar arm of the premaxilla anteriorly expanded (Fig. 5A,B,C). This anterior expansion is reduced in *C. cheradophilus* and *C. wolterstorffi* (Fig. 5D). *Cynolebias boitonei*, *C. bokermanni*, *C. chacoensis*, *C. constanciae*, *C. flavicaudatus*, *C. myersi C. notatus*, and *C. whitei* have the ventral process of the maxilla enlarged anteriorly (Fig. 5F), whereas in *C. cheradophilus* and *C. wolterstorffi* it is thin and straight (Fig. 5G). The dorsal process of the maxilla is oriented dorsally, but it is reduced to a rounde protuberance in *C. chacoensis* (Fig. 5H). The ventral tip of the maxilla is expanded in *C. constanciae*.

The ventral process of the dentary is reduced in *Cynolebias constanciae* (Fig. 5J). *Cynolebias cheradophilus* and *C. wolterstorffi* also have a dorsal process on the anguloarticular bone (Fig. 5K) that it is not found in any other species.

Jaw suspensorium

The mesopterygoid is longer than the autopalatine in *Cynolebias boitonei*, *C. bokermanni*, *C. chacoensis*, *C. constanciae*, *C. flavicaudatus*, *C. notatus*, and *C. whitei*. In *C. cheradophilus* and *C. wolterstorffi*, the mesopterygoid is reduced (Fig. 5N), whereas in other species, the two bones are about the same size as described for *C. luteoflamulatus* (Fig. 5L).

Opercular series

The opercular bones of all species are as those described for *Cynolebias luteoflamulatus*. *Cynolebias bokermanni* is unique in having the thicker segment of the interopercular extending beyond the anterior end of the bone like an anterior spine (Fig. 5M).

Gill arches

Ventral components. The basihyal is of variable length. In *Cynolebias constanciae* it is long, representing about five times the length of the ventral hypohyal (Fig. 6C). In *C. wolterstorffi* the relation is four to one (Fig. 6D), and in most other species, the relation is three to one (Fig. 6B). *Cynolebias cheradophilus* has a reduced basihyal. Its length is about the same as that of the hypohyal (Fig. 6E). The basihyal is of variable thickness. It is thickest in *C. cheradophilus*, being twice its length. It is about 25% of the length in *C. constanciae*, 75% of the length in *C. boitonei*, *C. bokermanni*, *C. chacoensis*, *C. myersi*, *C. notatus*, and in *C. whitei* (Fig. 6F), and the thickness is about the same as the length in other species. The proportion of cartilage and bone also varies among the species. In *C. boitonei*, *C. bokermanni*, *C. chacoensis*, *C. constanciae*, *C. flavicaudatus*, *C. myersi*, *C. notatus*, and *C. whitei*, the cartilaginous component represents 25% of the total length (Fig. 6C,F). In *C. nigripinnis* and *Cynolebias* sp., 33% is cartilaginous, as it occurs in *C. luteoflamulatus* (Fig. 6B). In *C. affinis*, *C. alexandri*, *C. bellottii*, *C. cyaenus*, *C. duraznensis*, and *C. gymnoventris*, 50% is cartilaginous (Fig. 6G), and in *C. viarius*, and *C. wolterstorffi* the cartilaginous component reaches 65% of the total length. *Cynolebias wolterstorffi* also has the cartilaginous segment widened anteriorly (Fig. 6D).

The height of the urohyal represents 20% of its length in *Cynolebias chacoensis*, *C. constanciae*, *C. flavicaudatus*, *C. myersi*, *C. whitei*, and *C. wolterstorffi* (Fig. 6I). It is 25% in other species (Fig. 6H). In *C. whitei*, the posterior part of the bone splits in two (Fig. 6L). The urohyal of *C. affinis*, *C. alexandri*, *C. boitonei*, *C. bokermanni*, *C. duraznensis*, *C. nigripinnis*, and *C. notatus* has an indentation next to the anterior process (Fig. 6I). *Cynolebias cheradophilus* lacks the anterior process (Fig. 6K), while in *C. bokermanni* this process points anteriorly (Fig. 6M). The anterior half of the ceratohyal supports two branchiostegal rays in all species, except in *C. notatus* and *C. wolterstorffi*, where it supports three branchiostegal rays. In *C. bokermanni*, it supports only one ray.

Dorsal components. The first epibranchial is the only element of this series that possesses teeth. The number of teeth is variable among species, *Cynolebias chacoensis* and *C. flavicaudatus* have four teeth. *Cynolebias boitonei*, *C. bokermanni*, *C. viarius*, and *C. whitei* have three teeth. *Cynolebias gymnoventris*, *C. luteoflamulatus*, *C. myersi*, and *C. wolterstorffi* have two teeth. In the other species, the number of epibranchial teeth is intraspecifically variable from two to three (*C. affinis*, *C. alexandri*, *C. constanciae*, *C. cyaenus*, *C. duraznensis*, *C. nigripinnis*, and *C. notatus*), three to four (*C. bellottii and C. cheradophilus*), and two to four (*Cynolebias* sp.).

Teeth

The characteristics of the teeth of all species examined are similar to those described for *Cynolebias luteoflamulatus*. In *C. myersi*, where the ascending processes of the premaxillae are fused, there are only three larger teeth, one of them located medially (Fig. 5B).

DISCUSSION

Overall, the osteological characteristics reported here for *Cynolebias* agree with the few and isolated observations previously reported for the genus (Vaz-Ferreira and Sierra, '73; Parenti, '81; Costa '90, '94). However, there are noteworthy deviations from the general pattern and characteristics found here that have not been previously reported.

The absence of neural prezygapophysis on the first vertebra has been suggested as a derived condition for the family Rivulidae (Costa, '90), whereas the presence of pleural rib on the second vertebrae was considered a unique characteristic uniting all Cyprinodontiformes (Parenti, '81). In the species studied here, these characters agree with previous reports. The lack of neural prezygapophyses on the caudal vertebrae has been considered as a synapomorphy for Cynolebiatini (Costa, '90, '94). However, these structures although reduced, were present in twelve of the twenty species studied here. Thus, the presence of neural prezygapophyses on the caudal vertebrae in *Cynolebias* may be a plesiomorphic state for the genus. Otherwise the reduction, instead of the absence of neural prezygapophyses, could be considered a derived state for *Cynolebias*.

The caudal fin of *Cynolebias cheradophilus* and *C. wolterstorffi* is supported by four vertebrae. This character could be diagnostic for the "*elongatus* complex," as it was not found in any other species examined. *Cynolebias nigripinnis* has two vertebrae supporting the caudal fin, differentiating it from the similar *C. affinis*, which has three vertebrae supporting the caudal fin. These two species were differentiated by Amato ('86) using external morphology.

The ossified medial radials of dorsal and anal fins have not been previously reported for Rivulidae, and represent a derived condition among *Cynolebias*. This condition was found in species of the "*antenori* complex" and in *C. constanciae*, *C. myersi*, and *C. whitei*, species previously suggested to be related to the "*antenori* complex" (Costa and Brasil, '93). Furthermore, it distinguishes the "*antenori* complex" species from other groups. Cartilaginous radials in *C. boitonei*, a species related to the "*antenori* complex," could be interpreted as an autapomorphy (secondary loss/reversal). The ossified medial radials of *C. bokermanni* and *C. notatus* could be interpreted either as relating them to the "*antenori* complex" or as a convergence among the species. The small bony projections on the rays of the anal fin of males of *C. cheradophilus* are a characteristic that needs to be examined further in other species of the "*elongatus* complex." Similar structures are also in the pectoral fin of this species and could have a sensory function in courtship (see below).

The loss of pelvic fins in *Cynolebias boitonei* was used by Costa and Brasil ('90) to relate this species to *C. zonatus*, which has reduced pelvic fins. Cartilaginous pelvic bones were found only in *C. notatus*, a character diagnostic of the species.

The short third postcleithrum of *Cynolebias wolterstorffi* and the thick third postcleithrum of *C. gymnoventris* are diagnostic characteristics for those species respectively. The absence/reduction of the upper pectoral radial has been suggested as a synapomorphy for the "*bellottii* complex" (Costa and Brasil, '93). However, five species of this complex—*C. affinis*, *C. alexandri*, *C. bellottii*, *C. luteoflamulatus*, and *C. nigripinnis*—show unreduced upper radials. Small structures on the pectoral fin-rays of males have been described as tactile organs in *Cynolebias whitei* (Carvalho, '57) and in other genera of killifishes (Foster, '67). These structures include nerve endings and could function during reproductive behavior (Foster, '67). The presence of these structures in *C. whitei* has been defined by Costa ('95) as autopomorphic; however, these structures were also found in *C. boitonei*, *C. cheradophilus*, and *C. luteoflamulatus*. The four species were placed in different clades by Costa, ('95), so its acquisition is considered independent.

The supraoccipital spines are highly variable, having the same configuration in different species groups. However, the state where both wings of the spine are free is shared only by *Cynolebias whitei* and *C. myersi*. This character could suggest a close relationship between these two species. Among studied species, *C. wolterstorffi* is diagnosed by a broad lateral process of the sphenotic and a unique shape of the processus ascendens of the parasphenoid. The parietal bone is not always present among Cyprinodontiformes. When present, its long axis extends obliquely and forward from the supraoccipital (Rosen, ’64). Costa (’94) illustrated the parietal reaching and partially covering the supraoccipital, and extending between the supraoccipital and the epiotic in the tribe Plesiolebiatini (sister group of Cynolebiatini). In *Cynolebias*, the parietal is overall triangular shaped and does not resemble the parietal of other Cyprinodontiformes. A triangular shaped parietal is a synapomorphy of *Cynolebias*. The parietal fusion with the autopterotics in *C. affinis* and with the epiotics in *C. bokermanni* is independently derived in those species. A crest on the parietal in *C. whitei* is an autapomorphy for the species. Reduction of the parietal is seen in less than 6% among the specimens of only two species within the “*bellottii* complex,” *C. luteoflamulatus* and *C. viarius* . If Amato’s (’86) placement of these species in different subgroups of the “*bellottii* complex” is correct, the parietal reduction is independent in both species. *Cynolebias wolterstorffi* also possesses reduced parietals. All species with a compact skull, i.e., no spaces between bones, belong to the “*antenori* complex” or are related to it.

The absence of teeth on the vomer is a synapomorphy for the tribe Cynolebiatini (Costa, ’94). The presence of a tooth found on this bone in *Cynolebias luteoflamulatus*, *C. nigripinnis*, and *C. viarius* is derived within the genus. In the suborder Aplocheiloidei, the vomer is dorsal to the anterior arm of the parasphenoid (Parenti, ’81); however, in *Cynolebias* the vomer is ventral to the parasphenoid. Thus, this condition is a synapomorphy for *Cynolebias*.

The shape and torsion of the lacrimal are a synapomorphy for Aplocheiloidei (Parenti, ’81). A lacrimal with reduced torsion and with a wide upper portion is a synapomorphy of the tribe Cynolebiatini (Costa, 90,’94). However, the lacrimal of the species examined herein showed no reduction in torsion, resembling the typical lacrimal of Rivulinae (Costa, ’90). The lacrimal shows characteristics that can help to diagnose some species. For example, the reduction in the upper portion of the lacrimal is diagnostic of *Cynolebias whitei* and an anteriorly curved lacrimal diagnoses *C. cheradophilus*. The lacrimals of *C. bokermanni* and *C. constanciae* are curved posteriorly and this condition could reflect a close relation between these two species. Costa (’90) reported the lack of a dermosphenotic in all *Cynolebias* except some large species. A dermosphenotic was not found in the large species examined herein, *C. cheradophilus* and *C. wolterstorffi*, but it was present in several of the “small” species, *C. bokermanni*, *C. chacoensis*, *C. constanciae*, *C. myersi*, and *C. whitei*. All species with a dermosphenotic are in the “*antenori* complex” or are related to this complex. The “small” *C. bellottii*, not only has a dermosphenotic but also another ossification in the same region. This second ossification has not been reported previously for Rivulidae. The homology of this bone is unclear. It could represent (1) a new ossification of a “second dermosphenotic,” (2) another bone of the orbital rim, or (3) the division of the original dermosphenotic into two separate centers of ossification. The analysis of a developmental series in this species may help to understand the homology of this ossification.

Three jaw characteristics not previously reported were found in *Cynolebias cheradophilus* and *C. wolterstorffi*: (1) the reduction of the expanded process of the alveolar arm of the premaxillae, (2) a thin and straight ventral process of the maxillae, and (3) a dorsal process of the anguloarticular. These three characteristics could be derived conditions within the “*elongatus* complex.” The modifications of jaw structure in *C. cheradophilus* and *C. wolterstorffi* could reflect differences in feeding behavior with other *Cynolebias* species or could be the consequence of allometric growth since these species are among the largest in the genus.

An enlarged ventral process of the maxillae is shared by all species of the “*antenori* complex” and the plesiomorphic species. A long ventral dentary process is a synapomorphy for *Cynolebias* (Costa, ’90). The reduction of this process and the expansion of the ventral tip of the maxillae found in *C. constanciae* are autopomorphies diagnostic of the species. As suggested for the “*elongatus* complex” species, these characters could reflect a change in the feeding habits of *C. constanciae* in relation to other *Cynolebias*.

A small mesopterygoid, relative to the autopalatine, has been considered as a synapomorphy for Cynolebiatini (Costa, '90). However, the mesopterygoid is longer than the autopalatine in the "*antenori* complex" and related species. The only species examined with a reduced mesopterygoid are *Cynolebias cheradophilus and C. wolterstorffi*. Species of the "*bellottii* complex" have a mesopterygoid about equal in size to the autopalatine, this state being the most common among cyprinodontiforms (Costa, '90).

Cynolebias myersi has the ascending processes of the parasphenoids fused into a single structure. The existence of possible variation in this character could not be tested because only one specimen was available.

A long basihyal is diagnostic of *Cynolebias constanciae*. This character is also considered a synapomorphy of the tribe Plesiolebiatini (Costa, '94). Reduction of the bony part of the basihyal and a deep uruhyal were considered synapomorphies for the "*bellottii* complex" (Costa and Brasil, '93). This analysis agrees with that interpretation. The lack of teeth on the fourth ceratobranchial was considered a synapomorphy for Cynolebiatini (Costa, '90). A row of teeth in the species studied here suggests that the presence of teeth could be a derived character within the tribe and a synapomorphy for *Cynolebias*. However, the presence of teeth on this bone is common in other genera of the family, so their presence could also be interpreted as primitive and the absence in other Cynolebiatini as the derived condition. The presence of teeth on the first epibranchial has not been previously reported for Cyprinodontiformes. This character can be considered a synapomorphy for *Cynolebias*.

Costa's phylogeny of the genus *Cynolebias* shows a common ancestry of the "*bellottii* complex," the "*antenori* complex," *C. boitonei*, *C. zonatus*, and *C. constanciae* based on one character, i.e., the elongation of the upper portion of the cleithrum. This study found at least five characters, e.g., ossification of dorsal fin medial radials, four pectoral radials, ventral process of the maxillae enlarged, mesopterygoyd bigger than autopalatine, and proportion of cartilage in the basihyal, that relate the "*antenori* complex," *C. boitone*, and *C. constanciae* to the clade comprised of *C. bokermanni*, *C. myersi* and *C. whitei* and separates all of them from the "*bellottii* complex."

The present study found that several osteological characters, including some of the characters previously use as diagnostic, either for the genus or the species, are polymorphic, e.g., shape of the parietal bone, number of pectoral radials, epibranchial teeth, and vomerine teeth. Polymorphisms have not been taken into account in most phylogenetic studies and their proper use could change some of the relations among taxa (Wiens, '95; Rannala, '95). Previous studies in Rivulidae have been based on very few specimens per species and consequently polymorphisms have been overlooked. Furthermore, Parenti and Tigano ('93), reported polymorphism in the rostral cartilage in an Old World cyprinodontiform. Polymorphic characters seem not to be infrequent in this order and should be taken into consideration in any phylogenetic reconstruction. Herein we have shown that the osteological characters can be used to diagnose species as well as address phylogenetic relationships among species of *Cynolebias*.

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APPENDIX

List of species used in this study. Part of the material examined in this study will be deposited in the U.S. National Museum (Smithsonian Institution). The remainder belong to Facultad de Ciencias, Depto. Zoologia de Vertebrados, Montevideo (ZVC.P). The material is listed below; number of specimens and their localities are specified.

Cynolebias affinis Amato—Pet trade, 25 specimens examined, 13 cleared and stained.

Cynolebias alexandri Castello and Lopez—Uruguay: Salto: Parque Indígena, 30 specimens examined, all cleared and stained.

Cynolebias bellottii Steindachner—Uruguay: Salto: Bañado Verocay, 37 specimens examined, all cleared and stained. ZVC.P 876; Uruguay: Colonia: Carmelo, 61 specimens examined, 19 cleared and stained.

Cynolebias boitonei Carvalho—Pet trade, 1 specimen examined, cleared and stained.

Cynolebias bokermanni Carvalho and Cruz—Pet trade, 2 specimens examined, all cleared and stained.

Cynolebias chacoensis Amato—Pet trade, 2 specimens examined, all cleared and stained.

Cynolebias cheradophilus Vaz-Ferreira, Sierra and Scaglia—Uruguay: Rocha: Ruta 10, Arroyo Valizas, 17 specimens examined, all cleared and stained.

Cynolebias constanciae Myers—Pet trade, 45 specimens examined, 18 cleared and stained.

Cynolebias cyaenus Amato—Pet trade, 4 specimens examined, all cleared and stained

Cynolebias duraznensis Reichert—Uruguay: Durazno: Durazno, 41 specimens examined, 26 cleared and stained. Uruguay: Durazno: Sarandi del Yi, 17 specimens examined, 4 cleared and stained.

Cynolebias flavicaudatus Costa and Brasil—Pet trade, 3 specimens examined, all cleared and stained.

Cynolebias gymnoventris Amato—Uruguay: Rocha: Velazquez, 7 specimens examined, 5 cleared and stained.

Cynolebias luteoflamulatus Vaz-Ferreira—Uruguay: Rocha: Camino de los botes, 17 specimens examined, all cleared and stained. Uruguay: Rocha: Ruta 16, 26 specimens examined, all cleared and stained. Uruguay: Rocha: Ruta 9 km 200, 4 specimens examined, all cleared and stained.

Cynolebias myersi Carvalho—Pet trade, 1 specimen examined, cleared and stained.

Cynolebias nigripinnis Regan—Uruguay: Colonia: Higueritas, 36 specimens examined all cleared and stained. ZVC.P 848; Uruguay: Colonia: Higueritas, 44 specimens examined, 4 cleared and stained.

Cynolebias notatus Costa and Brasil—Pet trade, 2 specimens examined, all cleared and stained.

Cynolebias viarius Vaz-Ferreira, Sierra and Scaglia—Uruguay: Rocha: Ruta 10 y

Ruta a Aguas Dulces, 25 specimens examined, all cleared and stained. ZVC.P 525; Uruguay: Rocha: Palmeras gemelas, 50 specimens examined, 8 cleared and stained. ZVC.P 596; Uruguay: Rocha: Totora, 14 specimens examined, 2 cleared and stained. Uruguay: Rocha: Ruta 10, 7 specimens examined, 2 cleared and stained.

Cynolebias whitei Myers—Pet trade, 55 specimens examined, 18 cleared and stained.

Cynolebias wolterstorffi Ahl—Pet trade, 1 specimen examined, cleared and stained.

Cynolebias sp.—Uruguay: Rocha: Ruta 16 km 26, 26 specimens examined, 6 specimens cleared and stained. Uruguay: Rocha: Chuy, 19 specimens examined, 5 cleared and stained. Uruguay: Treinta y Tres: Charqueada, 8 specimens examined, 2 cleared and stained. Uruguay: Rocha: Arroyo San Miguel, 24 specimens examined, 6 cleared and stained.