PHYLOGENY AND CLASSIFICATION OF RIVULIDAE REVISITED: ORIGIN AND EVOLUTION OF ANNUALISM AND MINIATURIZATION IN RIVULID FISHES (CYPRINODONTIFORMES: APLOCHEILOIDEI)

Wilson J.E.M. Costa

Lab. Ictiologia Geral e Aplicada, Departamento de Zoologia,
Universidade Federal do Rio de Janeiro, Cidade Universitária, Cx. Postal 68049,
21944-970 Rio de Janeiro RJ, BRAZIL. E-mail: wcosta@acd.ufrj.br

Abstract. Costa, W.J.E. Phylogeny and classification of Rivulidae revisited: Origin and evolution of annualism and miniaturization in rivulid fishes (Cyprinodontiformes: Aplocheiloidei). J. Comp. Biol. 3(1):33-92. Phylogenetic relationships of rivulids are reexamined. The analysis comprises 243 characters in 46 terminal rivulid taxa. A total of 153 rivulid species were studied. Characters are primarily morphological, but some ecological and behavioral traits are also included. Monophyly of the Rivulidae is corroborated by 23 synapomorphies involving morphology and size of lateral ethmoid, frontal, lacrimal, dermosphenotic, autopalatine, preopercle, maxilla, first vertebra, absence of first postcleithrum, contiguity of branchiostegal and opercular membranes, and reduction of latero-sensory system of head. Twenty-seven genera are recognized in a formal classification obtained from the strict consensus tree for the 16 equally most parsimonious cladograms (consistency index 0.64, retention index 0.83, rescaled consistency index 0.51), presenting the following topology: *((Leptolebias (Cynopoecilus + Campellolebias)) (Spectrolebias ((Nematolebias gen. n. + Simpsonichthys) (Austrolebias gen. n. (Megalebias gen. n. + Cynolebias))))) (Rivulus (((Gnatholebias gen. n. + Pterolebias) (Aphyolebias gen. n. (Neofundulus + Trigonectes) (Micromoema gen. n. (Moema + Renova)))) (Millerichthys ((Rachovia (Austrofundulus + Terranatos)) (Pituna (Papiliolebias (Plesiolebias (Maratecoara + Stenolebias)))))))). Nematolebias* includes species previously placed in *Simpsonichthys, and Austrolebias and Megalebias* are erected to cover monophyletic species assemblages previously classified in *Cynolebias. Pterolebias* is restricted to only two species, the remaining being transferred to *Aphyolebias, Gnatholebias,* and *.Micromoema. Rivulus stellifer* is transferred to *Rachovia. Papiliolebias* is considered a genus independent *fiom Plesiolebias.* Monophyly of all proposed genera are supported. The hypothesis of annualism constituting a single evolutionary event within Rivulidae and lost in *Rivulus is* equally parsimonious to the hypothesis of annualism arising twice among rivulids. Apomorphic ecological and behavioral features are considered to be coadaptive acquisitions associated to annualism, as well as some morphological tendencies, such as deep body, anteriorly positioned dorsal fin and elaborate cephalic neuromast pattern. Annualism among rivulids is considered to have been achieved by the successive colonization of peripheral aquatic habitats. Four evolutionary events of miniaturization are hypothesized to have occurred along the rivulid evolution. The unique common occurrence of all known miniatures in a single area of endemism suggests that the phenomenon was favored in this area.

Key Words: Cladistics; Rivulidae; Neotropical Fishes; Historical Ecology; Miniaturization; Ichthyology; Annual fishes.

Introduction

The family Rivulidae comprises a speciose monophyletic assemblage of small primarily freshwater fishes (usually reaching about 30 mm, rarely surpassing 80 mm of standard length), with about 200 valid species (over 250 nominal species), currently placed in 20 genera (Costa. 1990a. 1995a; Thomerson & Taphorn, 1995; Costa & Nielsen, 1997; Costa, in press). Rivulids occur in Middle America, between central Mexico and Panama along Atlantic drainages and Costa Rica and Panama, in Pacific drainages. Some species are endemic to Caribbean Islands, and a member of a salt water tolerant species group lives in Florida. In South America, the family is widely distributed throughout most cis-Andean river basins, from northern Venezuela to south of Buenos Aires Province, Argentina, as well as trans-Andean Atlantic drainages of Colombia and Venezuela, with a few recorded occurrences in Pacific coastal basins of northwestern Colombia (Costa, 1990b. 1995b; Huber, 1992).

Males of several species exhibit gaudy color patterns and elongate fins, constituting popular aquarium fishes. At least two species are known to be the only hermaphroditic vertebrates with self-fertilization; one of them, *Rivulus marmoratus* Poey, is widely used as experimental fish (several references in Lazara & Smith, 1990). In one genus *(Campellolebias* Vaz-Ferreira & Sierra), a complex modification of urogenital papilla and anal-fin-rays, membrane, support and musculature, produced a unique and elaborate apparatus for internal fertilization (Costa, 1995c). However, despite these interesting features, diversity and wide geographical range, phylogenetic relationships of the group were poorly investigated until recent years (see Parenti, 1981; Costa, 1990a, 1995b for history on rivulid systematics).

The first description of a rivulid species was provided by Valenciennes (in Humboldt & Valenciennes, 1821), and only 22 nominal species were known at the beginning of the current century. Former revisionary taxonomic *(e.g.,* Myers, 1927) and phylogenetic morphological studies (Parenti, 1981) were based on very small collections. This low representation of museum collections was probably due to the specialized biotopes inhabited by the Rivulidae, making their capture difficult.

Except for the genus *Rivulus* Poey, all rivulids are annual fishes, a term applied to species living in temporary freshwater biotopes, which dry seasonally (Myers, 1942, 1952). During the middle to end of the rainy season, and the early phases of the subsequent dry season, adult individuals bury their eggs in substrate until the environment becomes almost completely dry. Whereas all adult fishes die, only the fertilized eggs survive and hatch at the start of the following rainy season. Embryonic development of annual fishes involves unusual stages, including dispersion and reaggregation of amoeboid blastomeres and three diapauses, being the first diapause pre-embryonic and facultative, the second embryonic, usually facultative but sometimes obligate, and the third, embryonic (pre-hatching stage) and obligate (Wourms, 1972a,b,c). These peculiar physiological, behavioral and ecological features, associated to small endemism areas make several species threatened with extinction (Costa, 1995b). Contrastingly, species *of Rivulus* are non annual, but usually found in rather shallow aquatic biotopes (frequently about five centimeters deep) close to the edges of streams, rivers and lakes *(e.g.,* Lüling, 1971; Bastos & Lourenço, 1983; Costa, 1987). Eventually, these peripheral biotopes may dry, but species of *Rivulus* are able to jump out of water, where they can survive for 25-60 hours in wet medium *(e.g.,* Seghers. 1978; Bastos, 1979). An exception to this habitat preference occurs in a species group of *Rivulus* inhabiting euryaline biotopes such as mangroves *(e.g.,* Huehner *et al.,* 1985; Taylor, 1988).

Recent efforts to collect fishes in these specialized biotopes revealed a greater diversity than recognized previously, indicating that the family is an important component of the Neotropical biota. Mainly based on discoveries of new taxa and new collections of previously known taxa, many short preliminary contributions proposed distinct phylogenetic schemes and discussed validity of relationships evidences described in these successive phyletic studies (Costa. 1989a, 1990a, 1991a, 1995a,c,d, 1996, 1998; Thomerson & Taphorn, 1995; Costa & Nielsen, 1997). In addition, data from these studies dealing with morphological characters *(e.g.,* Costa, 1995a.c,d, 1996.1998), have been tested by some molecular phylogenetic studies (Murphy & Collier, 1996, 1997; Hrbek, in press). The primary purpose of the present study is to reevaluate morphological characters used in previous phylogenetic studies among rivulids, also incorporating many undescribed characters, in a comprehensive phylogenetic analysis of the family. Another aim of this study is, based on a phylogenetic scheme, to erect hypotheses on the origin and evolution of specialized features of rivulids, comprising annualism and miniaturization.

Materials and Methods

The phylogenetic methodology used to propose phylogenetic hypotheses was Cladistics. The most parsimonious cladograms were obtained using the program Hennig86 (Farris, 1988). Outgroups included species of all cyprinodontiform families and some other atherinomorph fishes, as listed in Costa (in press). Character state polarization using multiple outgroups follows Maddison *et al.* (1984), using recent phylogenetic studies on interrelationships of cyprinodontiform families (Costa, in press) as a baseline. The immediate outgroup to Rivulidae was the Aplocheilidae (Parenti, 1981; Costa, in press), followed by a clade comprising all families of the Cyprinodontoidei, the sister group to the Aplocheiloidei (Rivulidae plus Aplocheilidae) (Parenti, 1981; Costa, in press). In the case of polymorphic characters within analyzed taxa (both ingroups and outgroups), the plesiomorphic state was inferred as that occurring in basal taxa, according to available phylogenetic hypotheses (Costa, in press). However, this was not employed to the Aplocheilidae, due to the very distinct available phylogenetic hypotheses involving relationships of its members (Parenti, 1981; Murphy & Collier, 1997). All character states of multistate characters were treated as unordered. Optimization of characters after analysis was made through ACCTRAN mode.

Terminal rivulid taxa were: (1) genera currently accepted as valid and with a single or few similar included species *(Austrofundulus* Myers, *Campellolebias* Vaz-Ferreira & Sierra, *Cynopoecilus* Regan, *Leptolebias* Myers, *Maratecoara* Costa, *Millerichthys* Costa, *Moema* Costa, *Neofundulus* Myers, *Pituna* Costa, *Rachovia* Myers, *Renova* Thomerson & Taphorn, *Spectrolebias* Costa & Nielsen, *Stenolebias* Costa, *Terranatos* Taphorn & Thomerson, and *Trigonectes* Myers); (2) monophyletic units of diversified but well-defined genera *(Plesiolebias* Costa divided into subgenera, *Plesiolebias* and *Papiliolebias* Costa; *Cynolebias* Steindachner divided into *C. griseus* Costa, Lacerda & Brasil, C. *porosus* species group, C. *bellottii* species group and C. *wolterstorffi* species group; and *Simpsonichthys* Carvalho divided into *S. whitei* species group and *S. constanciae* species group); (3) monophyletic units of a poorly defined genus *(Pterolebias* Garman divided into *P. longipinnis* Garman, *P. phasianus* Costa, *P. xiphophorus* Thomerson & Taphorn, *P. zonatus* species group, and *P. peruensis* species group); and (4) selected species or species groups representing the major lineages occurring in all geographical range of the most speciose and widespread genus of the family, which has been considered a paraphyletic group in some recent studies (Parenti, 1981; Hrbek, in press) *(Rivulus* Poey divided into 17 terminal taxa: *R. cylindraceus* Poey, *R. atratus* Garman, R. *brasiliensis* (Valenciennes), *R. caudomarginatus* Seegers, *R. ocellatus* Hensel, *R. urophthalmus* Günther, *R. cryptocallus* Seegers & Huber, *R. brunneus* Meek & Hildebrand, *R. amphoreus* Huber, *R. hartii* (Boulenger), R. *janeiroensis* Costa, *R. punctatus* Boulenger, *R. geayi* Vaillant, R. *bahianus* Huber, *R. tenuis* (Meek), R. *xiphidius* Huber, and R. *stellifer* Thomerson & Turner). Following the phylogenetic analysis, a new classification, including new generic names and combinations, is presented at the final part of this study. Therefore, the nomenclature adopted in the character analysis is according to the current literature, thus distinct from that proposed at the end of the present study.

To describe characters, they are grouped into 23 categories, comprising morphological, ecological and behavioral sets of characters. Thus, some ecological and behavioral traits are used as phylogenetic characters and included in the data matrix. This contrasts with statements provided by some authors who argue that circularity may result when characters related to evolutionary scenarios are placed along with other characters *(e.g.,* anatomical) in a data matrix *(e.g.,* Coddington, 1988; Brooks & McLennan, 1991). However, the present procedure follows the opinion that cladistic methods do not provide a way to select synapomorphies *a priori* (de Pinna & Salles, 1990), and that there is no logical justification for rejecting some characters from a phylogenetic analysis by claiming to preserve them to test subsequent secondary approaches (Deleporte, 1993). In fact, circularity is avoided by the independence of phylogeny from the evolutionary hypotheses to be tested (Deleporte, 1993), the null hypothesis consisting of the pattern of phylogenetic relationships itself (de Pinna & Salles, 1990).

Comparative material of Rivulidae is listed in Appendix 1. Outgroups are listed in Costa (in press). Fin-ray counts include all elements. The compound caudal centrum was counted as a single element in vertebrae numbers, which were recorded only on cleared and stained material. Osteological preparations were made following Dingerkus & Uhler (1977) and Taylor & Van Dyke (1985). Osteological nomenclature is that of Weitzman (1962), with modifications described by Vari (1989). Nomenclature for musculature is according to Winterbottom (1974). Nomenclature for cephalic neuromasts is according to Gosline (1949). Supraorbital neuromasts counts do not include the transverse neuromasts over the rostral region. Terminology for the pseudogonopodium and associated structures is according to Costa (1995c). Nomenclature for egg surface and ornamentation follows the nomenclature for pollen grains according to Punt *et al.* (1994). Concepts of cyprinodontiform suborders and families are according to Parenti (1981) and Costa (in press). Throughout the text, the abbreviation SL means standard length.

Character analysis

The 243 characters considered to be informative in the present phylogenetic analysis of Rivulidae are listed below. They are followed by a brief description of the included character states, and the consistency (CI) and retention indexes (RI), besides a discussion relative to the distribution of character states among terminal taxa. Character state distributions for rivulid taxa are presented in Appendix 2 and the consensus cladogram of most parsimonious phylogenetic hypotheses in Figs. 1-6.

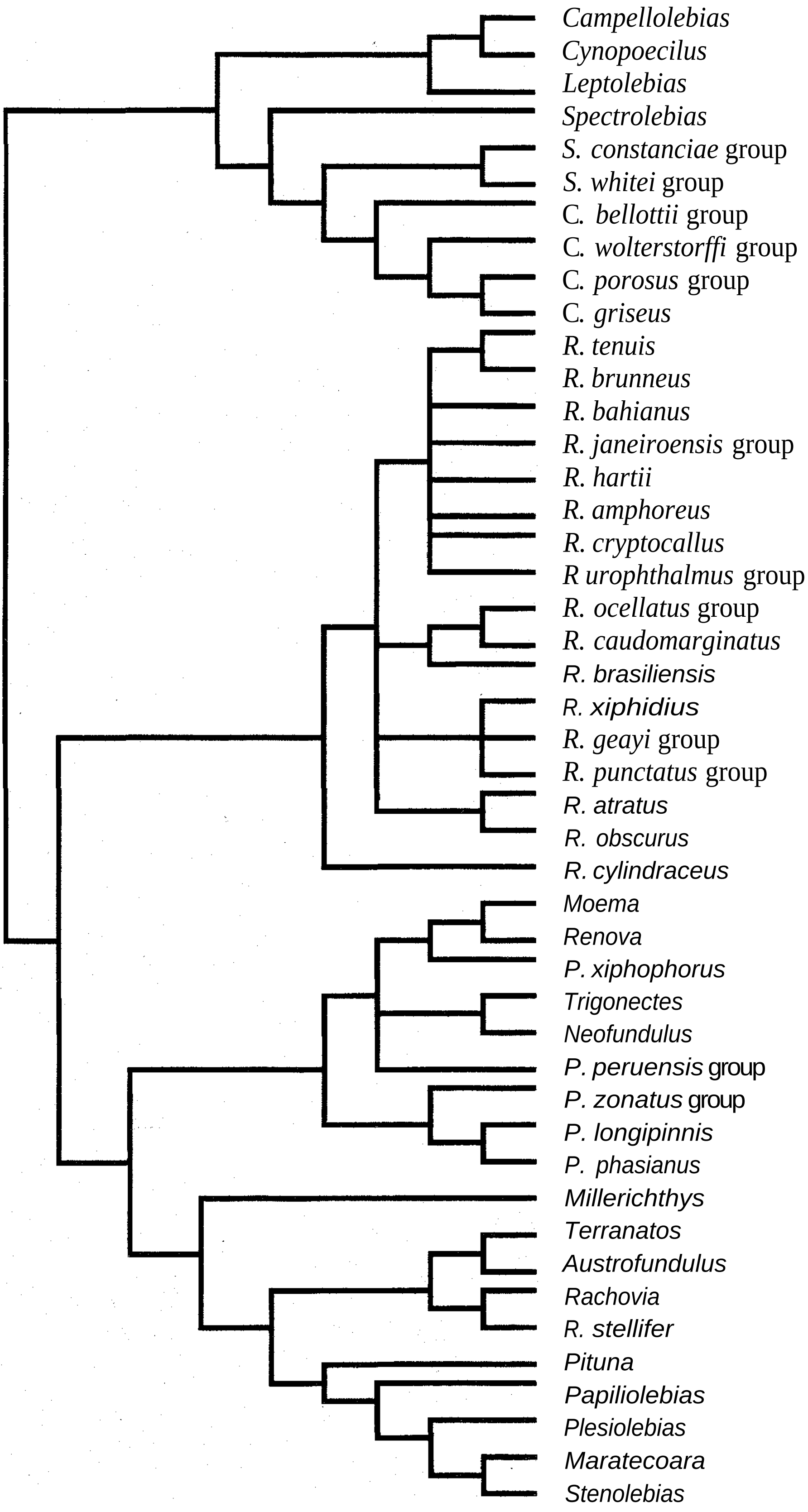

Figure 1. Phylogenetic relationships among 46 rivulid taxa, expressed in a consensus cladogram of the 16 equally *most* parsimonious trees (length 430, CI 64, RI 82). Apomorphies are listed in Figs. 2-6.

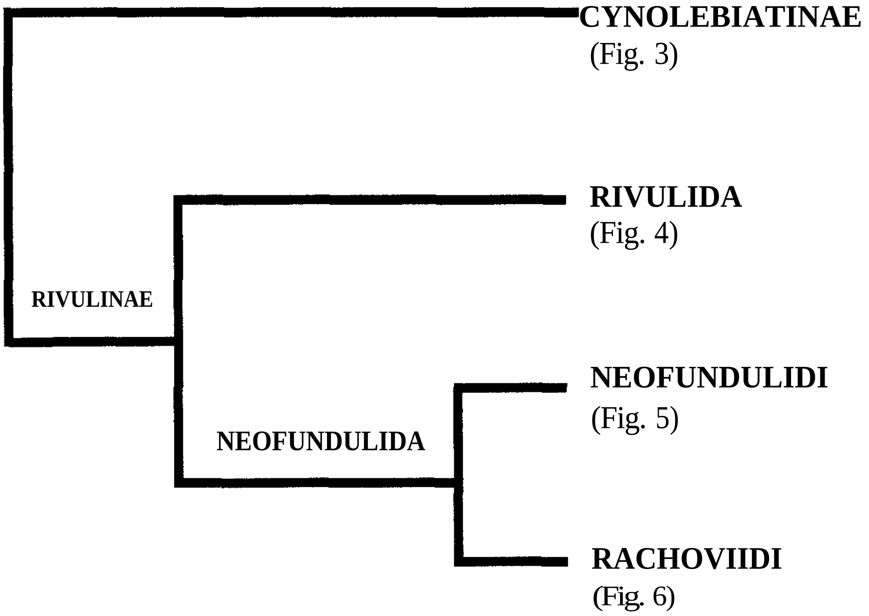

Figure 2. Interrelationships and classification of the major rivulid lineages. Cynolebiatinae: 28.1, 34.3,45.3, 60.1, 71.1, 72.1, 73.1, 76.1, 89.2, 93.1, 94.1*, 100.2, 105.1, 111.2, 117.1, 119.1, 132.1*, 134.1, 145.1, 169.2, 172.1, 176.1, 179.1, 180.1, 181.1*, 183.1, 199.1; Rivulinae: 39.1, 41.1, 70.1, 139.1, 148.1, 174.1; Rivulida: 50.1,204.1-, 242.1-; Neofundulida: 169.1; Neofundulidi: 83.1-*, 103.1-, 109.1*, 147.1*, 149.1*, 151.1*, 15S.1*, 237.1*; Rachoviidi: 45.2*, 100.1,194.1. Character numbers are according to the text (*=homoplastic features; -"reversals). Synapomorphies for included taxa are summarized in Figs. 3-6.

Jaws

1. Shape *of the premaxilla and dentarium.* State 0: elongate, producing a snout profile sharply pointed; state 1: short, producing a snout profile approximately rounded to slightly pointed (CI: 20; RI: 0). As discussed by Costa (1989a, 1990a), the genera *Trigonectes* and Moema differ from other rivulid taxa by the elongate jaw bones (Fig. 7B), producing an anteriorly pointed snout, which has been considered as synapomorphic. A reevalution of this character in outgroups revealed that a long and pointed snout is present in basal taxa of Beloniformes, and in part of the basal taxa of Aplocheilidae and Cyprinodontoidei, therefore considered plesiomorphic for Cyprinodontiformes. Other rivulids also having elongate jaw bones are the *Pterolebias zonatus* and *Cynolebias wolterstorffi* groups.

2. Shape *of the posterior border of the distal tip of premaxilla.* State 0: indented; state 1: straight (CI: 100; RI: 100). In rivulids, the posterior border of the distal portion of premaxilla is slightly curved and not interrupted by a concavity (Figs. 7B-F, 8). In aplocheiloids and all other cyprinodontiforms, the premaxilla has a posterodistal indentation (Fig. 7A), which also occurs in other atherinomorphs and is thus considered plesiomorphic.

3. *Shape of the alveolar arm of the premaxilla.* State 0: not expanded anteriorly; state 1: expanded anteriorly (CI: 100; RI: 100). The alveolar process of the premaxilla is distinctively expanded in *Spectrolebias* (Fig. 7C), a condition not found in other rivulids and outgroups.

4. Shape of the *basal portion of the ascending process of the premaxilla.* State 0: not or slightly constricted; state 1: constricted (CI: 100; RI: 100). Uniquely among aplocheiloids, the basal portion of the ascending process of the premaxilla is strongly constricted in R. *atratus* and R. *obscurus* (Fig. 7D). Costa (1989b) reported an apomorphic concavity on the base of the ascending process as diagnostic *for Pituna* (Fig. 7E), but this weakly constricted condition is not distinct from that found in other plesiolebiatins and many other rivulids, and thus is not phylogenetically informative.

5. *Shape and orientation of the main axis of the ventral process of maxilla.* State 0: approximately straight, directed anteromedially to slightly curved, dp directed posteriorly; state 1: bent and directed posteriorly, median portion expanded anteriorly, producing a triangular shape (CI: 100; RI: 100). As discussed by Costa (in press), the triangular ventral process of the

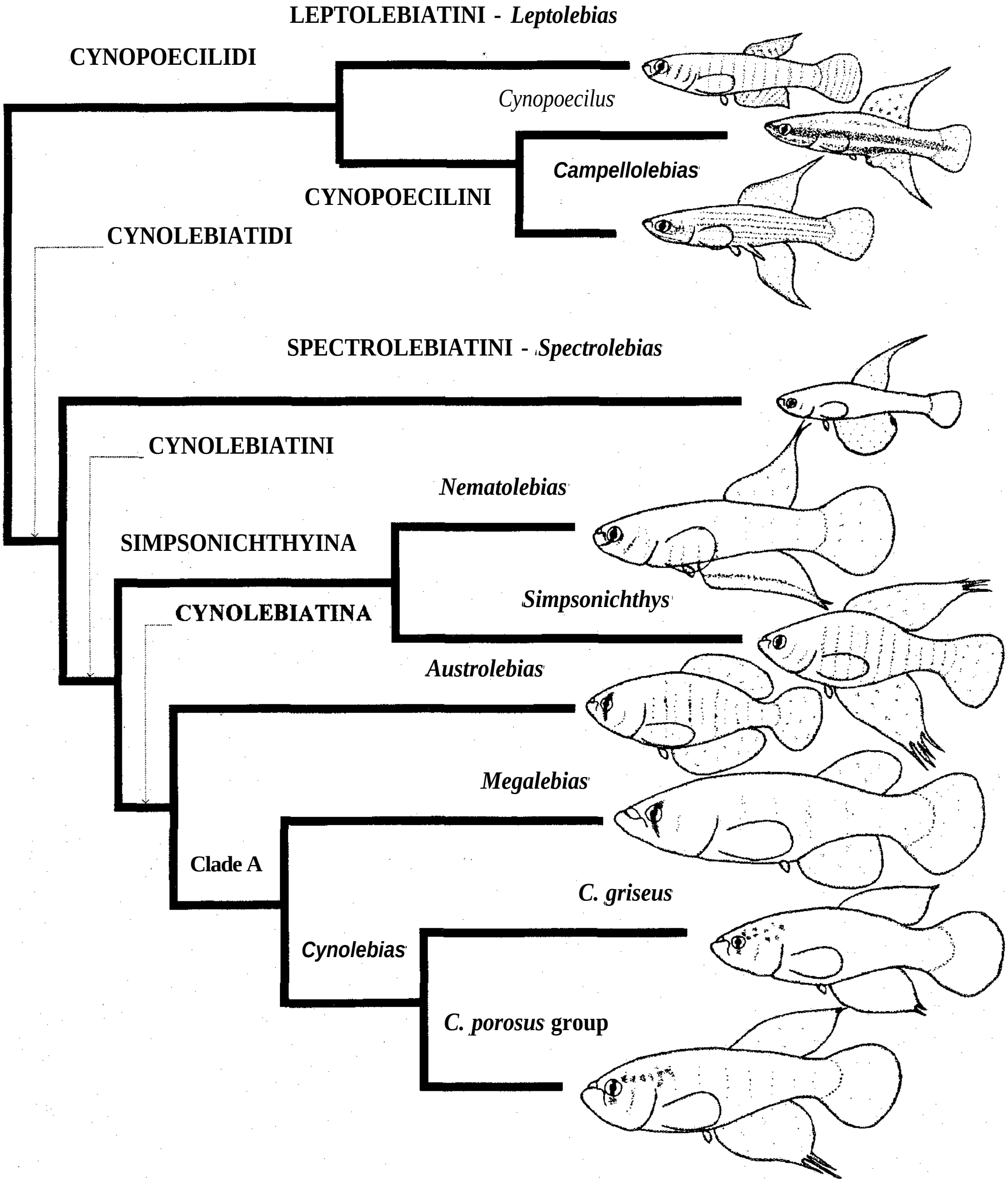

Figure 3. Interrelationships and classification of the Cynolebiatinae. Cynopoecilidi: 16.3, 175.1, 184.1, 203.1, 234.1, 235.1, 238.1; Leptolebiatini (Leptolebias): 7.1,42.1, 93.1-*, 141.1, 194.2, 206..2*; Cynopoecilini: 78.1, 79.1,114.1,155.1*, 185.1,199.1-, 236.1,237.2*; *Cynopoecilus:* 27.1,107.1,115.1,124.1,125.1,128.1*, 131.1, 152.1, 171.1, 190.1, 191.1, 203.1-, 239.1; *Campellolebias:* 80.1, 102.1, 103.2, 111.3*, 116.1, 117.1-, 123.1, 130.1,156.1,169.3,170.1,186.1,210.1,240.1; Cynolebiatidi: 14.1,47.1,113.1,126.1*, 127.1,128.1*, 136.1, 138.1, 140.1, 142.1, 182.1, 237.1; Spectrolebiatini *(Spectrolebias):* 3.1, 32.1, 44.1, 168.1, 173.1, 187.1, 205.1, 219.1; Cynolebiatini: 40.1, 55.1, 95.1*, 132.1-, 150.1, 193.1; Simpsonichthyina: 17.1, 21.1, 93.1-*, 155.1*, 225.1; *Nematolebias:* 8.1*, 17.2*, 67.1*, 95.1-, 206.2*; *Simpsonichthys:* 25.1, 122.2; Cynolebiatina: 16.2, 33.1, 38.1*, 89.3, 126.1-, 146.1, 206.3*, 207.3; *Austrolebias:* 49.1, 56.1-, 177.1*; Clade A: 37.1, 45.4, 67.1*, 85.1, 86.1,109.1*, 111.1*, 113-, 167.1*; *Megalebias:* 1.1-, 90.1, 143.1; *Cynolebias:* 15.1-, 16.2-, 18.1,126.1-, 206.3-, 207.3-, 214.1; *C. griseus:* 67.1-; *C. porosus* group: 66.1, 75.1,132.1-. Character numbers are according to the text (* = homoplastic features; - = reversals).

maxilla described as synapomorphic for rivulids by Parenti (1981) is interpreted as a character state derived from a condition shared by all aplocheiloids (slightly curved, tip directed posteriorly). It constitutes a curved process with the tip directed posteriorly, in contrast to

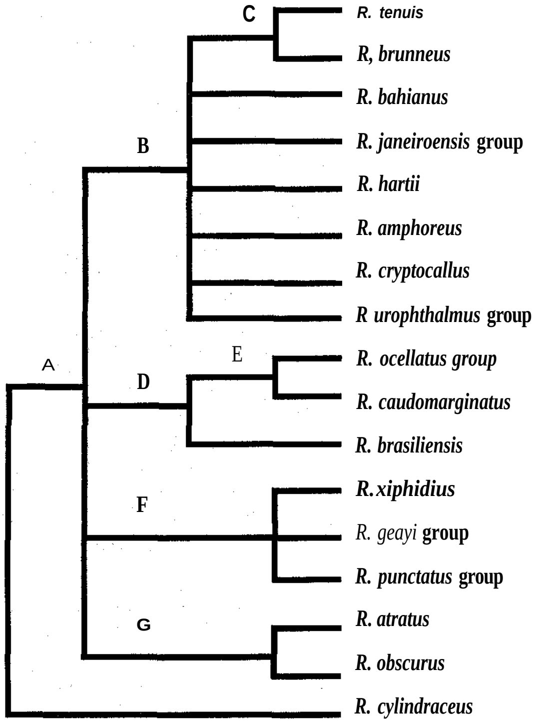

Figure 4. Phylogenetic relationships among 17 terminal taxa of the genus *Rivulus* (Rivulida). Node A: 101.1*, 103.1-; Node B: 19.1, 52.1*, 83.1-*, 109.1*, 221.1*, 227.1; Node C: 52.1-, 109.1-, 211.1, 227.2; Node D: 17.2*, 24.1, 50.2, 58.1-, 96.1, 135.1-, 192.2*; Node E: 148.1-, 222.2*, 224.1; Node F: 16.1*, 45.2*, 48.1, 132.1*, 213.1*; Node G: 4.1, 22.1*; 129.1, 132.1*, 197.1*; *R. tenuis:* no apomorphy; *R. brunneus: idem; R. bahianus:* 19.1-, 148.1- 188.2*, 197.1*; *R. janeiroensis* group: 19.1-, 20.1, 83.1-, 132.1*, 148.1-, 197.1*; *R. hartii:* 167.1*, *188.2*; R. amphoreus:* 110.1*; 227.1-; *R. cryptocallus:* 26.1, 188.2*, 197.1*; *R. urophthalmus group:* 19.1-, 188.2*, 197.1*; *R. ocellatus* group: 109.1*, 241.1; *R. caudomarginatus:* 228.1; -R. *brasiliensis:* 51.1, *70.1-*; R. xiphidius:* 101.1-, 147.1*; 149.1*, 202.1; *R. geayi* group: 148.1-, 196.1*, 200.1*; *R. punctatus group:* 63.1, 98.1-, 200.1*, 222.1*, 226.1*; *R. atratus:* 198.1, 230.1; *R. obscurus:* 211.1*; *R. cylindraceus:* 148-*, 196.1*, 213.1*, 222.2*. Character numbers are according to the text (* = homoplastic features; - = reversals).

approximately straight and directed anteromedially as in other cyprinodontiforms and outgroups. In rivulids, instead of slightly curved as in aplocheilids (Fig. 7A), the process is bent, acquiring a triangular shape (Figs. 7B-F, 8).

6. *Shape of the median portion of maxilla.* State **0:** approximately straight; state 1: curved (CI: 100; RI: 100). *Maratecoara* and *Stenolebias* have a distinctively twisted median portion of maxilla (Fig. 7F), in contrast to the approximately straight condition as in other rivulids, the plesiomorphic condition for cyprinodontiforms (Costa, in press).

7. *Shape of the maxilla.* State 0: narrow; state 1: broad (CI: 100; RI: 100). Cyprinodontiforms typically possess a narrow and elongate maxilla. *Leptolebias* is unique by possessing a robust maxilla (Fig. 8A). Costa (1995b) reported this derived condition only for two species of *Leptolebias,* but in fact it is present in adult specimens of all species of *Leptolebias.*

8. *Width of rostral cartilage.* State 0: moderate, longitudinal length longer than transversal length; state 1: broad, longitudinal length about as long as transversal length (CI: 50; RI: 0). *Moema* and species of the *Simpsonichthys whitei* group have a distinctively broad rostral cartilage (Fig. 7B), in contrast with the narrow cartilage of other rivulids and outgroups (Figs. 7A, C-F, 8).

9. *Shape of the anterior border of the rostral cartilage.* State 0: approximately straight; state 1: deeply concave (CI: 100; RI: 100). The straight or slightly convex anterior border of the rostral cartilage is plesiomorphic among atherinomorphs. In many species of *Aphyosemion* and *Epiplatys,* there is an anterior tip. In contrast, in the genera *Plesiolebias, Pituna, Stenolebias* and *Maratecoara,* the anterior border is concave (Fig. 7E-F).

10. *Pointed, lateral expansion in rostral cartilage.* State 0: absent; state 1: present (CI: 100; RI: 100). *Austrofundulus* is unique among rivulids and other cyprinodontiforms by the presence of prominent lateral tips on the rostral cartilage (Fig. 8B).

11. *Shape of the rostral cartilage.* State 0: short, approximately rounded; state 1: elongate, usually rectangular or hexagonal (CI: 100; RI: 100). Atherinomorphs typically have a rounded rostral cartilage (Fig. 7A). Parenti (1981) considered an elongate rostral cartilage as synapomorphic for a group of rivulids excluding *Rivulus.* However, an apomorphic longitudinally elongate rostral cartilage occurs in all rivulid taxa (Figs. 7 and 8). In *Moema* and in the *S. whitei group,* the apomorphic increase in width (character 8) causes an approximately rounded shape rather close to the rounded condition occurring in outgroups, differing from the typical elongate shape of rivulids. However, although approximately rounded, in this case it is clearly longer than in outgroups, thus considered within the condition above reported as apomorphic.

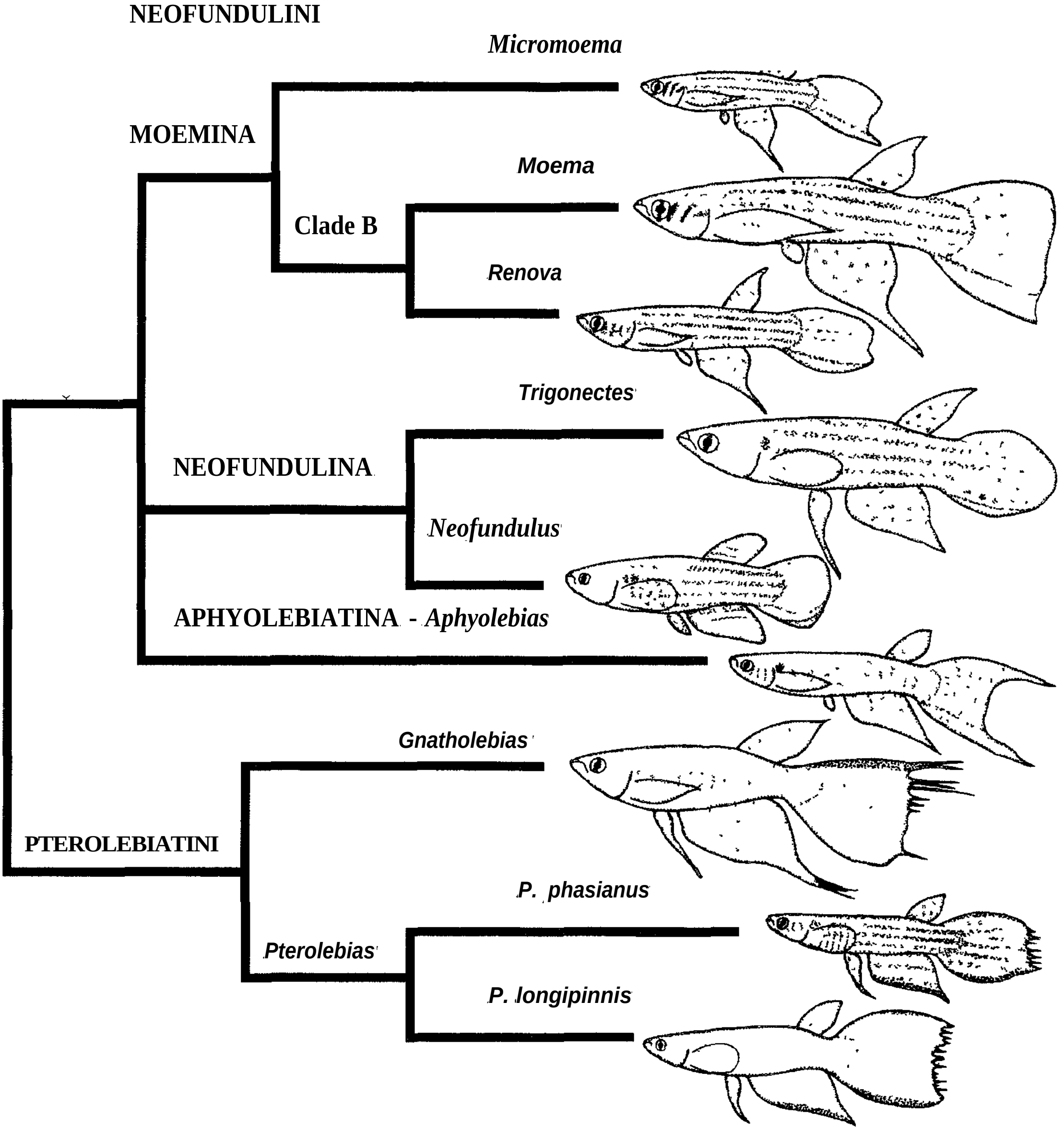

Figure S. Interrelationships and classification of the Neofundulidi. Neofundulini: 221.1*; Moemina: 158.1,188.1*, 206.4, 212.1; *Micromoema:* 30.1*; 109.1-*, 132.1*, 161.1*; Clade B: 77.1*, 110.*, 112.1*; *Moema:* I.I-*, 8.1*, 46.1*, 48.1,69.1*, 70.1-*, 74.1*, *167.1*; Renova:* 212.1-, 222.1*; Neofundulina: 46.1*, 112.1*, 149.1-*, 188.1*, 196.1*; *Trigonectes:* 1.1-*, 69.1*, 153.1*, 167.1*; *Neofundulus:* 147.1-, 216.1, 226.1*, 230.1*; Aphyolebiatina *(Aphyolebias):* 38.1*, 161.1*, 196.2*, 232.1*; Pterolebiatini: 22.1*, 65.1,153.1*, 159.1,162.1; *Gnatholebias:* 1.1-*, 103.3-, 121.1, 126.1*, 128.1*, 229.1, 232.1*, 233.1; *Pterolebias:* 30.1*, 38.1*, 41.2*, 53.1*, 54.1*, 109.1-*, 149.1-*, 196.3,231.1; *P. phasianus: 77.1, 104.1,188.1*; J' *longipinnis:* 200.1*. Character numbers are according to the text (* = homoplastic features; - = reversals).**

12. Mandibular canal. State 0: well-developed, externally represented by a close canal; state 1: absent, externally represented by minute neuromasts (CI: 100; RI: 100). As discussed by Parenti (1981) and Costa (1990a), all rivulids lack a mandibular canal, in contrast to the well-developed, closed canal with pores found in outgroups.

13. Width of the coronoid process of dentary. State 0: broad; state 1: narrow (CI: 100; RI: 100). Rivulids are unique among cyprinodontiforms by the presence of a narrow and short coronoid process of dentary (Figs. 9B-E, 10), about as narrow as the postero-ventral process of dentary. The plesiomorphic condition for cyprinodontiforms is a longer and wider coronoid process (Fig. 9A).

14. Width of the postero-ventral process of dentary.

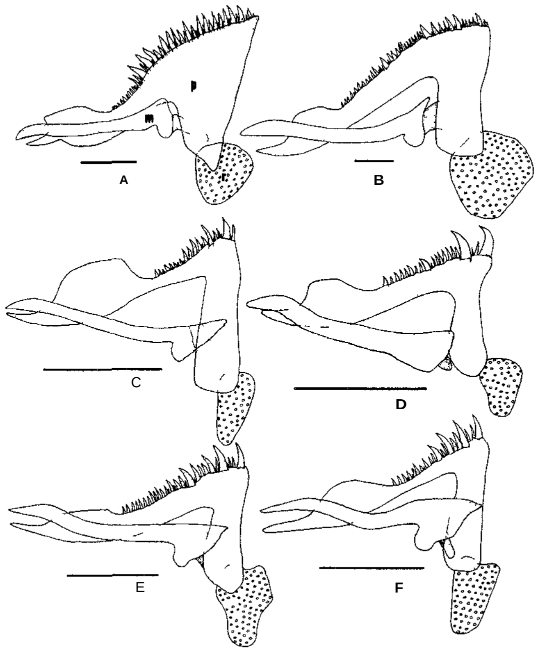

Figure 7. Left upper jaw, dorsal view. A. ***Aplocheilus lineatus;*** **B.** ***Moemapepatei; C. Spectrolebias semiocellatus;*** **D.** ***Rivulus obscurus;*** **E.** ***Pituna compacta;*** **E** ***Maratecoara lacortei.*** **Abbreviations: m, maxilla; p, premaxilla; r, rostral cartilage. Dots indicate bone; circles, cartilage. Scale bar = 1 mm.**

groups (Fig. 9E). An extremely reduced process (Fig. 10C) occurs in *Leptolebias, Campellolebias* and *Cynopoecilus.* In other rivulids, this process is not reduced, constituting the plesiomorphic condition for atherinomorphs. Costa (1990a) indicated a reduction of this process as a synapomorphy of a rivulid clade, but he did not separate the three derived states described above.

17. Width of the ventral process of angulo-articular. State 0: narrow; state 1: slightly widened; state 2: widened (CI: 66; RI: 66). Some rivulids have a distinctively widened ventral process of angulo-articular, in contrast to the plesiomorphic narrower shape of the ventral process of other cyprinodontiforms. This apomorphic condition occurs in *Rivulus brasiliensis* (Costa, 1990d), -R. *caudomarginatus* and R. *ocellatus,* and in species of the S. *whitei* group (9C). However, a wide ventral process of angulo-articular also occurs in aplocheilids (Costa, in press). Since Aplocheilidae constitutes the immediate sister group to rivulids, and molecular data indicate the Aplocheilidae to be a non monophyletic assemblage (Murphy & Collier, 1997), the character is coded as "?" in the data matrix for outgroup. In the & *constanciae* group the process is wide (Fig. 9B), but considered an intermediate state between the plesiomorphic condition and that apomorphic condition described above.

18. Shape of the coronoid process of angulo-articular. State 0: short; state 1: long (CI: 100; RI: 100). As discussed by Costa (in press), a short coronoid process of angulo-articular is synapomorphic for aplocheiloids. However, similarly to the plesiomorphic condition occurring in other cyprinodontiforms, there is an elongate coronoid process in species of the *C. griseus* and C. *porosus* group (Fig. 9D).

19. Extent of the anteroventral process of angulo-articular. State 0: short; state 1: elongate (CI: 25; RI: 25). *la Rivulus hartii, R. amphoreus, R. brunneus, R. tenuis* and *R. cryptocallus,* the anteroventral process of angulo-articular is distinctively longer than the condition found in other rivulids and outgroups (Fig. 10B).

20. Shape of the anteroventral process of angulo-articular. State 0: approximately straight; state

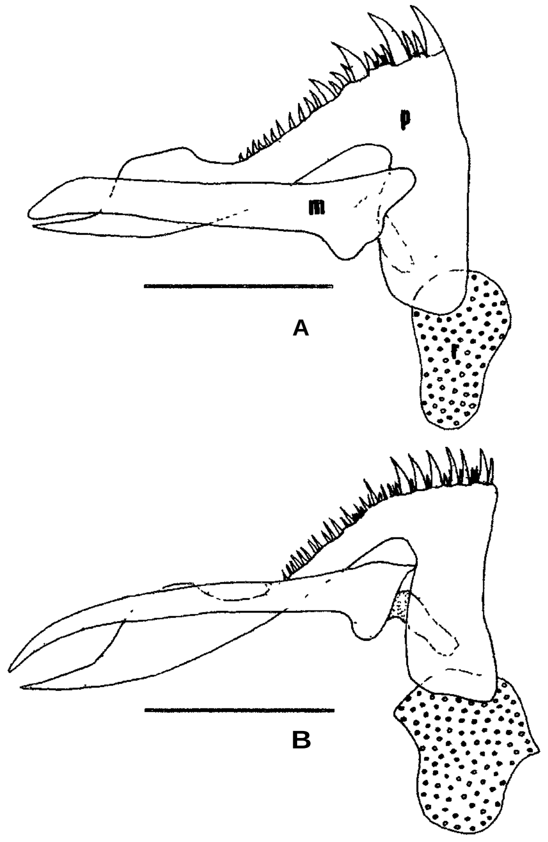

Figure 8. Left upper jaw, dorsal view. A. ***Leptolebias fluminensis; B. Austrofundulus limnaeus.*** **Abbreviations: m, maxilla; p, premaxilla; r, rostral cartilage. Dots indicate bones; circles, cartilage. Scale bar = 1** ***mm.***

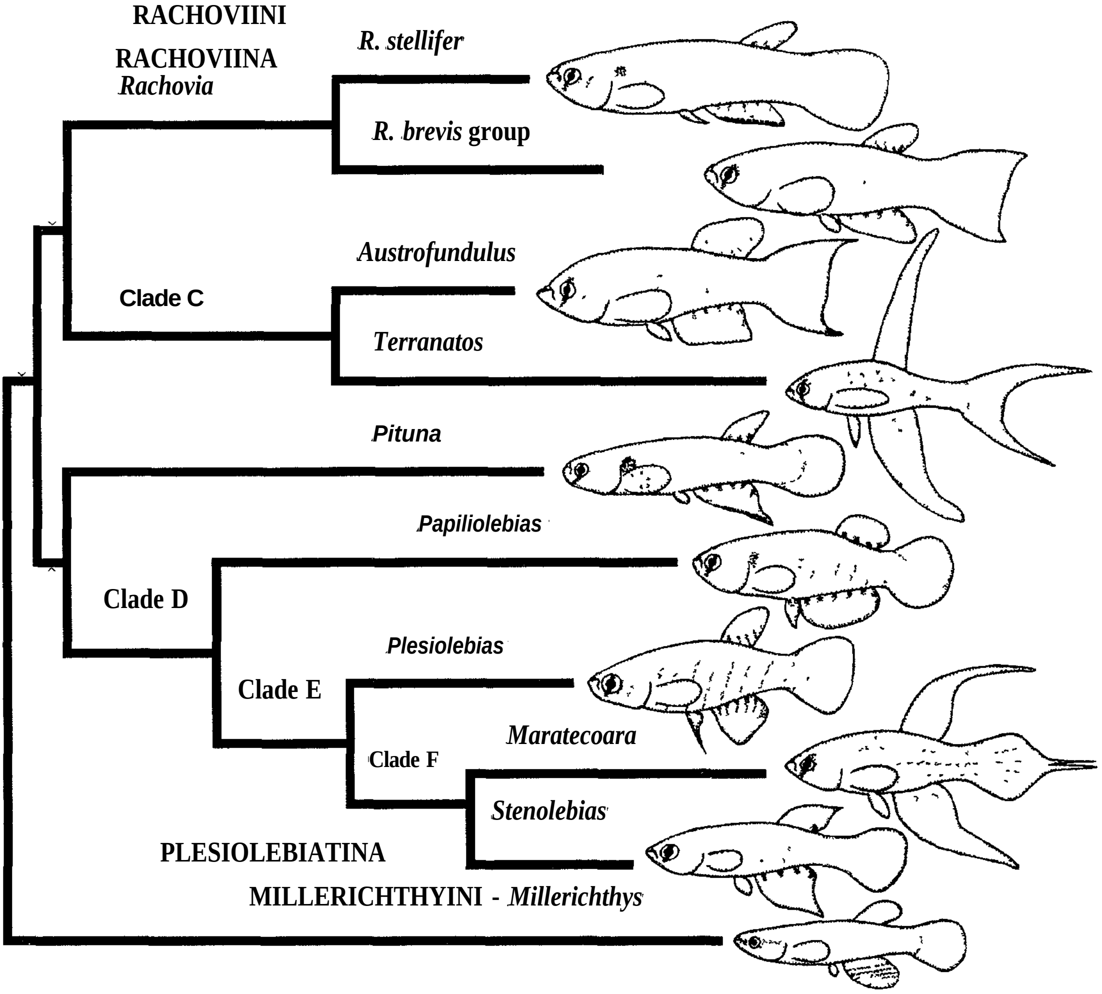

Figure 6. Interrelationships and classification of the Rachoviidi. Rachoviini: 91.1, 200.1*, 206.1, 218.1; Rachoviina: 45.2-, 52.1*, 103.1, 178.1, 207.1; *Rachovia:* 36.1, 83.11-; *R. stellifer:* 74.1*, 100.1-, 101.1*, 109.1*, 194.1-, 196.2*, 207.1-, 220.2, 222.1*; *R. brevis* group: 163.1*, 181.1*, 209.1, 221.1*; Clade C: 95.1*, 97.1, 161.1*; *Austrofundulus:* 10.1, 24.1*, 109.1*, 110.1*, 163.1*, 201.1; *Terranatos:* 39.1-, 52.1-, 88.1 111.3*, 120.1, 137.1, 139.1-*, 151.1*, 154.1*, 155.1*, 174.1-*, 178.1-, 206.3*, 218.1-*; Plesiolebiatina: 9.1, 16.1*, 35.1, 54.1*, 59.1, 94.1*, 103.2*; Pituna: 147.1-, 155.1*, 194.1-*, 196.1*, 215.1*, 220.1, 230.1*; Clade D: 53.1*, 103.3, 106.1, 139.1-*, 181.1*; *Papiliolebias:* 148.2; Clade E: 111.1*; 132.1*, 196.1-; *Plesiolebias:* 31.1, 41.2, 43.1, 61.1, 64.1, 108.1, 157.1, 166.1, 174.1-, 177.1*, 189.1, 195.1; Clade F: 6.1, 82.1, 155.1*, 215.1*, 237.1-; *Stenolebias:* 57.1; *Maratecoara:* 29.1, 68.1, 87.1, 95.1*, 103.3-*, 111.2*, 117.1*, 154.1*, 160.1, 165.1, 174.1-, 192.1, 207.2, 208.1, 218.1-; Millerichthyini *(Millerichthys):* 34.2, 133.1, 181.1*, 204.1-, 217.1, 223.1. Character numbers are according to the text (* = homoplastic features; - = reversals).

State 0: narrow; state 1: broad (CI: 100; RI: 100). *Simpsonichthys, Cynolebias and Spectrolebias* share a widened postero-ventral process of dentary, as pointed out by Costa (1990a) (Fig. 9B-E), instead of the narrow process, plesiomorphic for cyprinodontiforms.

IS. Shape of the postero-ventral process of dentary. State 0: short; state 1: expanded (CI: 50; RI: 0). Species of the C. *wolterstorffi* group and C. *bellottii* have a distinctively expanded posteriorly postero-ventral process of dentary (Fig. 9E). The plesiomorphic condition for cyprinodontiforms is a shorter and narrower process.

16. Reduction of the ventral process of angulo-articular. State 0: not reduced, larger than coronoid process; state 1: slightly reduced, about equal in size to coronoid process; state 2: reduced, smaller than coronoid process; state 3: vestigial or absent (CI: 60; RI: 80). A reduced ventral process of angulo-articular occurs in some rivulid taxa. A slightly reduced state (Fig. 10D) occurs in the genera *Pituna, Plesiolebias, Maratecoara, Stenolebias, R. xiphidius*, and species of the *R. punctatus* group and *R. geayi* group. A more reduced process is present in the C. *bellottii* and C. *wolterstorffi*

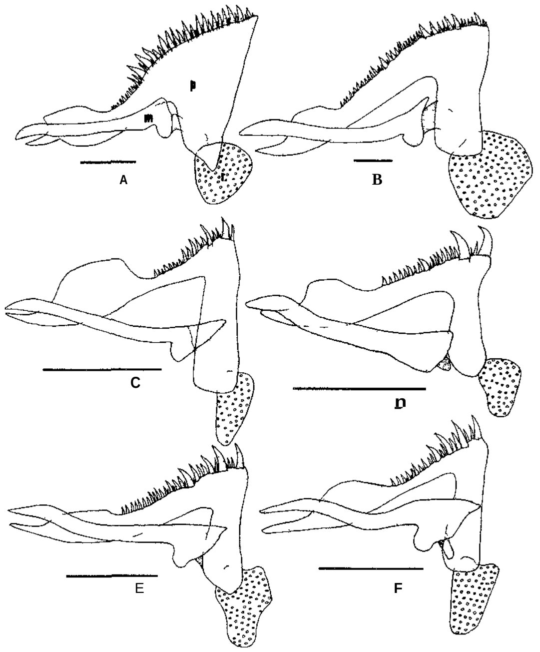

Figure 7. Left upper jaw, dorsal view. A. *Aplocheilus lineatus;* B. *Moema pepatei; C. Spectrolebias semiocellatus;* D. *Rivulus obscurus;* E. *Pituna compacta;* F. *Maratecoara lacortei.* Abbreviations: m, maxilla; p, premaxilla; r, rostral cartilage. Dots indicate bone; circles, cartilage. Scale bar = 1 *mm.*

groups (Fig. 9E). An extremely reduced process (Fig. 10C) occurs in *Leptolebias, Campellolebias* and *Cynopoecilus*. In other rivulids, this process is not reduced, constituting the plesiomorphic condition for atherinomorphs. Costa (1990a) indicated a reduction of this process as a synapomorphy of a rivulid clade, but he did not separate the three derived states described above.

17. Width of the ventral process of angulo-articular. State 0: narrow; state 1: slightly widened; state 2: widened (CI: 66; RI: 66). Some rivulids have a distinctively widened ventral process of angulo-articular, in contrast to the plesiomorphic narrower shape of the ventral process of other cyprinodontiforms. This apomorphic condition occurs in *Rivulus brasiliensis* (Costa, 1990d), *R. caudomarginatus* and *R. ocellatus,* and in species of the *S. whitei* group (9C). However, a wide ventral process of angulo-articular also occurs in aplocheilids (Costa, in press). Since Aplocheilidae constitutes the immediate sister group to rivulids, and molecular data indicate the Aplocheilidae to be a non monophyletic assemblage (Murphy & Collier, 1997), the character is coded as "?" in the data matrix for outgroup. In the *S. constanciae* group the process is wide (Fig. 9B), but considered an intermediate state between the plesiomorphic condition and that apomorphic condition described above.

18. Shape of the coronoid process of angulo-articular. State 0: short; state 1: long (CI: 100; RI: 100). As discussed by Costa (in press), a short coronoid process of angulo-articular is synapomorphic for aplocheiloids. However, similarly to the plesiomorphic condition occurring in other cyprinodontiforms, there is an elongate coronoid process in species of the *C. griseus* and *C. porosus* group (Fig. 9D).

19. Extent of the anteroventral process of angulo-articular. State 0: short; state 1: elongate (CI: 25; RI: 25). *In Rivulus hartii, R. amphoreus, R. brunneus, R. tenuis* and *R. cryptocallus,* the anteroventral process of angulo-articular is distinctively longer than the condition found in other rivulids and outgroups (Fig. 10B).

20. Shape of the anteroventral process of angulo-articular. State 0: approximately straight; state

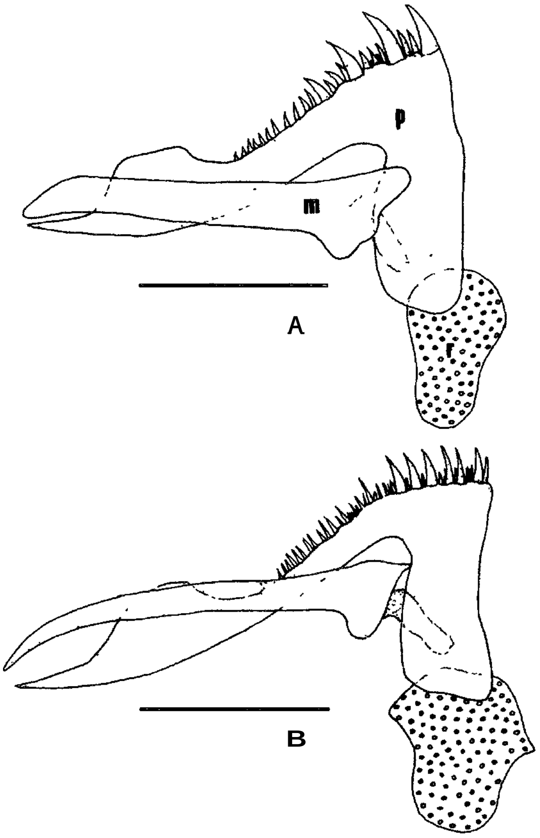

Figure 8. Left upper jaw, dorsal view. A. *Leptolebias fluminensis;* B. *Austrofundulus limnaeus.* Abbreviations: m, maxilla; p, premaxilla; r, rostral cartilage. Dots indicate bones; circles, cartilage. Scale bar = 1 mm.

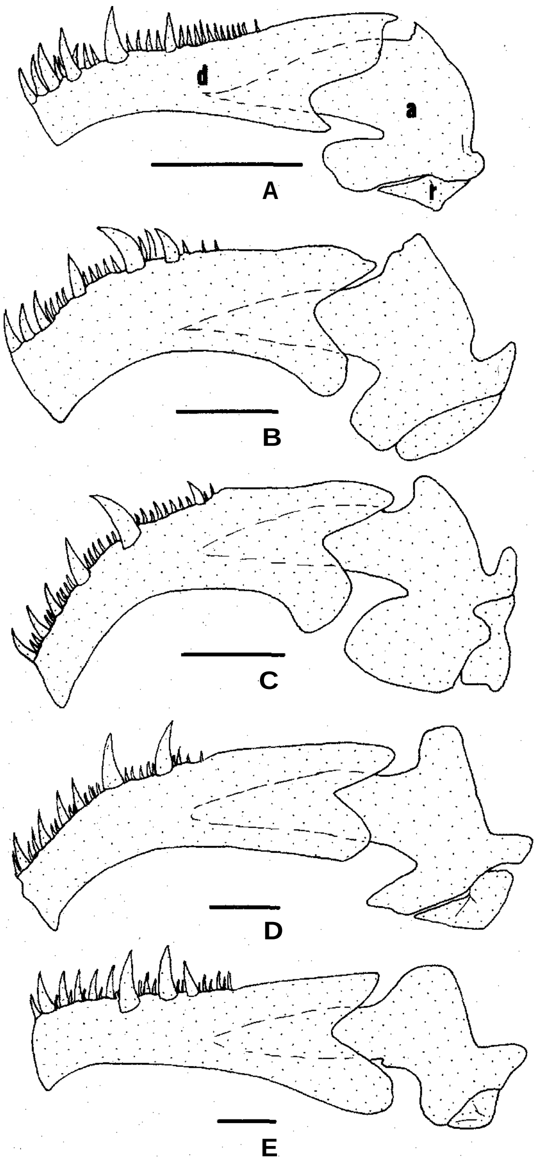

Figure 9. Left lower jaw, ventrolateral view. A. ***Aphyosemion guignardi;*** **B.** ***Simpsonichthys flavicaudatus;*** ***C. Simpsonichthys whitei;*** **D.** ***Cynolebias perforatus;*** **E.** ***Cynolebias wolterstorffi.*** **Abbreviations: a, angulo-articular; d, dentary; r, retroarticular. Meckel's cartilage not represented. Scale bar = 1 mm.**

1: curved (CI: 100; RI: 100). As noted by Costa & Brasil (1991a: fig. 5a) and Costa (1991c), the anteroventral process of the angulo-articular of *R. janeiroensis* and closely related species from eastern Brazil is distinct from the plesiomorphic condition for cyprinodontiforms. In this group it is curved, in contrast to approximately straight.

21. A large tooth on the corner of dentary with its tip anteriorly directed. State 0: absent; state 1: present (CI: 100; RI: 100). The presence of a large conical tooth with an anteriorly directed tip on the dentary (Fig. 9B, C) is a condition unique among aplocheiloids, recorded for *Simpsonichthys* by Costa (1996).

22. Large teeth on dentary and premaxilla strongly curved laterally. State 0: absent; state 1: present (CI: 50; RI: 75). Occurring only in *Pterolebias longipinnis, P. phasianus,* species of the P *zonatus* group, *R. atratus,* and *R. obscurus,* among aplocheiloids and other cyprinodontiforms, there are large teeth on the strongly curved laterally jaws, with their bases displaced outside of the line where other teeth are attached (Fig. 10A).

Jaw suspensorium and opercular series

23. Extent of the ventral portion of autopalatine. State 0: long, overlapping dorsal portion of quadrate; state 1: short, not contacting quadrate (CI: 100; RI: 100). Parenti (1981) considered a long ventral extension of the autopalatine, covering part of the quadrate, as a synapomorphy of cyprinodontoids. However, according to the present study (also Costa, in press), the ventral extension of the autopalatine is so long in cyprinodontoids as in aplocheilids and other atherinomorph groups (Fig. 11A). Therefore, only rivulids among cyprinodontiforms have a reduced ventral extension

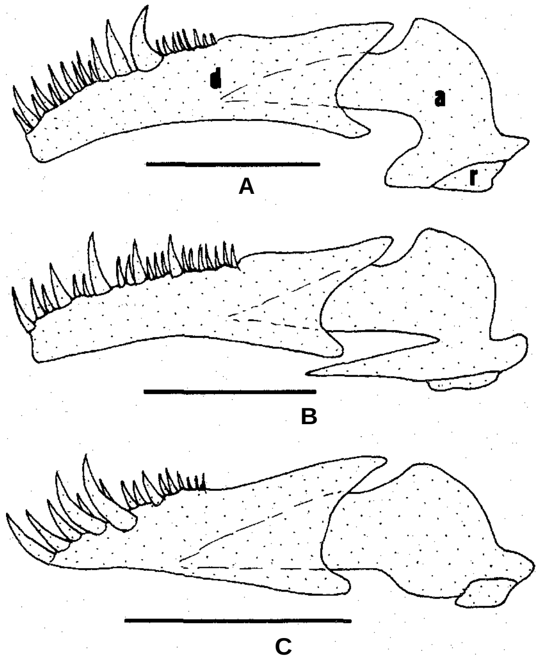

Figure 10. Left lower jaw, lateral view.. A. ***Pterolebias phasianus;*** **B.** ***Rivulus hartii;*** **C.** ***Campellolebias brucei;*** **D.** ***Pituna compacta.*** **Abbreviations: a, angulo-articular; d, dentary; r, retroarticular. Meckel's cartilage not represented. Scale bar = 1 mm.**

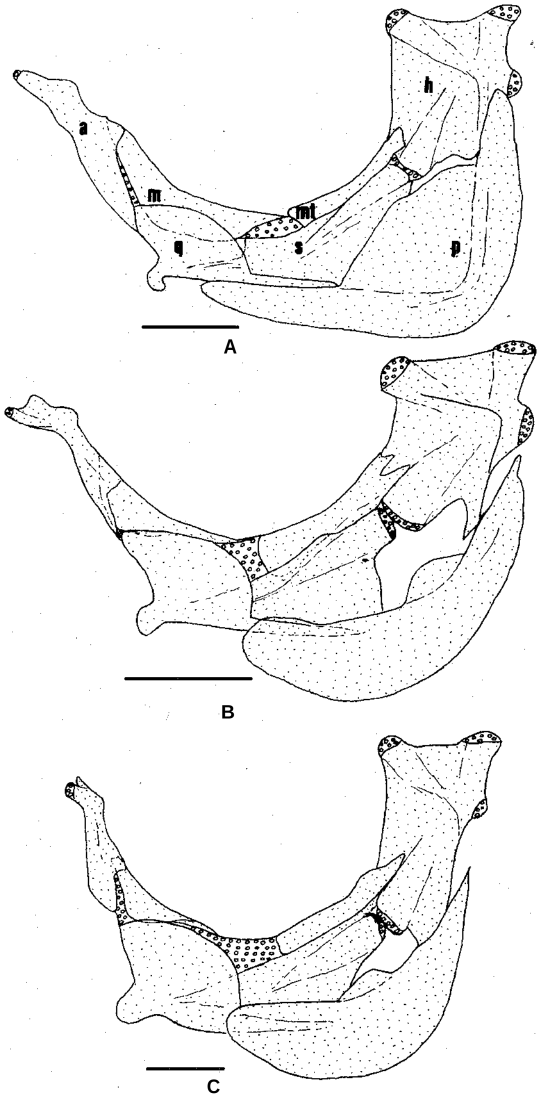

Figure 11. Left jaw suspensorium, lateral view. A. *Pachypanchax playfairi;* B. *Rivulus caudomarginatus* C. *Simpsonichthys chacoensis.* Abbreviations: a, autopalatine; h, hyomandibula; m, mesopterygoid; mt, metapterygoid; p, preopercle; q, quadrate; s, sympletic. Dots indicate bone; circles, cartilage. Scale bar = 1 mm.

of the autopalatine (Figs. 11-15).

24. A prominent flange on the dorsal portion of autopalatine. State 0: absent; state 1: present (CI: 100; RI: 100). *Rivulus brasiliensis, R. caudomarginatus* and *R. ocellatus* are unique among aplocheiloids in having an expanded flange on the dorsal portion of the autopalatine (Fig. 11B).

25. A distinctive process on the dorsal tip of autopalatine. State 0: absent; state 1: present (CI: 100; RI: 100). Species of the & *constanciae* group have prominent process on the dorsalmost tip of the autopalatine, something unique among aplocheiloids (Fig. 11C).

26. Shape of the autopalatine. State 0: slender and curved; state 1: broad and bent (CI: 100; RI: 100). *Rivulus cryptocallus* presents a distinctively robust and bent autopalatine (Fig. 12A). No other aplocheiloid has this autopalatine morphology.

27. A concavity on the median portion of the posterior border of autopalatine. State **0:** absent; state 1: present (CI: 100; RI: 100). *Cynopoecilus* has a deep constriction on the posterior border of the autopalatine, producing a sinuous lateral profile (Fig. 12B). This apomorphic shape does not occur in other aplocheiloids.

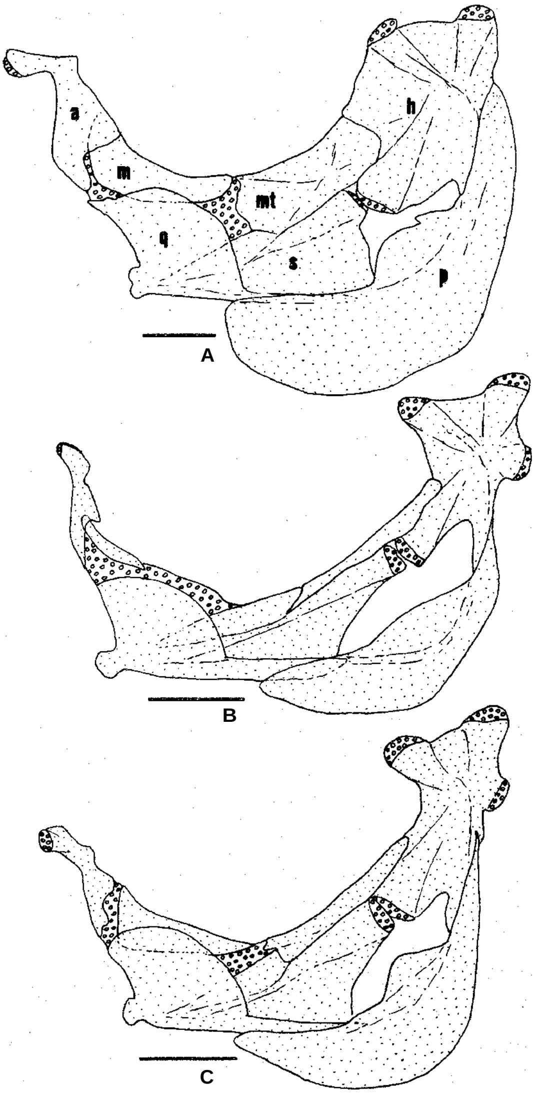

Figure 12. Left jaw suspensorium, lateral view.. A. *Rivulus cryptocallus;* B. *Cynopoecilus melanotaenia;* C. *Maratecoara lacortei.* Abbreviations: a, autopalatine; h, hyomandibula; m, mesopterygoid; mt, metapterygoid; p, preopercle; q, quadrate; s, sympletic. Dots indicate bone; circles, cartilage. Scale bar = 1 mm.

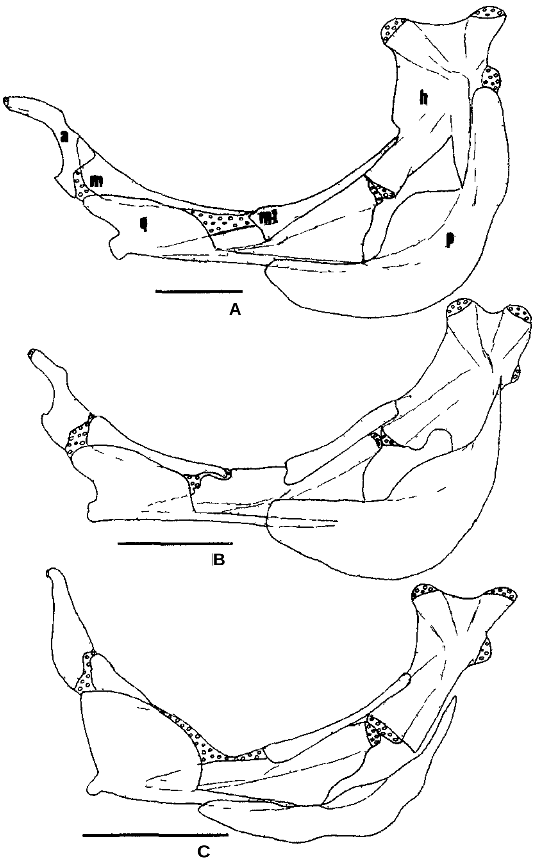

Figure 13. Left jaw suspensorium, lateral view. A. ***Pterolebias phasianus;*** **B.** ***Plesiolebias glaucopterus; C. Spectrolebias semiocellatus.*** **Abbreviations: a, autopalatine; h, hyomandibula; m, mesopterygoid; mt, metapterygoid; p, preopercle; q, quadrate; s, sympletic. Dots indicate bone; circles, cartilage. Scale bar = 1 mm.**

28. Position of the dorsomedial process of autopalatine. State 0: close to central portion of the bone; state 1: close to the dorsal end of the bone (CI: 100; RI: 100). The genera *Spectrolebias, Cynolebias, Simpsonichthys, Leptolebias, Cynopoecilus* and *Campellolebias* are the only aplocheiloids having an anterodorsal displacement of the dorsomedial process of the autopalatine (Figs. 11C, 12B, 13C, and 14A, C).

29. Shape of the anterior arm of the autopalatine. State 0: narrow; state 1: broad (CI: 100; RI: 100). .*Maratecoara* has an apomorphically widened anterior arm of the autopalatine (Fig. 12C).

30. *Shape of the ventral tip of autopalatine.* State 0: narrow; state 1: expanded anteriorly (CI: 50; RI: 50). *Pterolebias phasianus, P. xiphophorus* and *P. longipinnis* are unique among aplocheiloids by the anteriorly expanded ventral tip of the autopalatine (Fig. 13A).

31. Shape of autopalatine. State 0: elongate; state 1: shortened, ventral portion widened (CI: 100; RI: 100). A distinct shape of the autopalatine, consisting of a short bone with a widened ventral portion, as noted by Costa (1998), occurs in species of the subgenus *Plesiolebias,* constituting a condition unique among aplocheiloids (Fig. 13B).

32. Shape of the anteroventral border of autopalatine. State **0:** short; state 1: expanded

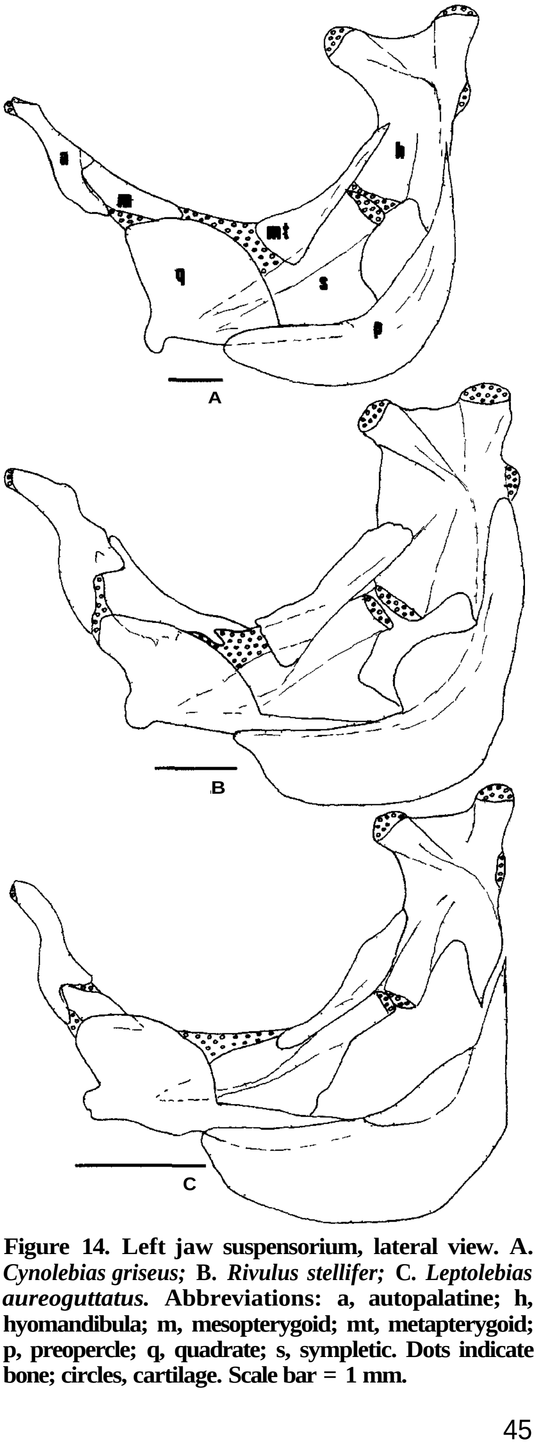

Figure 14. Left jaw suspensorium, lateral view. A. ***Cynolebias griseus;*** **B.** ***Rivulus stellifer;*** **C.** ***Leptolebias aureoguttatus.*** **Abbreviations: a, autopalatine; h, hyomandibula; m, mesopterygoid; mt, metapterygoid; p, preopercle; q, quadrate; s, sympletic. Dots indicate bone; circles, cartilage. Scale bar = 1 mm.**

anteriorly (CI: **100; RI:** 100) *Spectrolebias is* unique among aplocheiloids by the anteriorly expanded anteroventral border of the autopalatine (Fig 13C) The autopalatine of the subgenus *Plesiolebias* is ventrally widened (character 31), but this is a condition distinct from that of *Spectrolebias* In *Plesiolebias,* the ventral portion of the main axis of the bone is entirely widened, whereas only the anterior run of the bone is expanded in *Spectrolebias*

33 Shape of the dorsomedial process of autopalatine State 0 small, state 1 large (CI 100, RI 100) Most aplocheiloids and other cyprinodontiforms have a small dorsomedial projection of the autopalatine In *Cynolebias,* there is a distinctive expansion, forming a pronounced process (Fig 14A)

34 Reduction of mesopterygoid State 0 mesopterygoid large, longer than metapterygoid and autopalatine, its posterior tip very close or in contact with metapterygoid, and ventral portion greatly overlapping quadrate, state 1 mesopterygoid of moderate size, smaller than metapterygoid, longer than autopalatine, its posterior tip very close or in contact with metapterygoid, and ventral border slightly overlapping quadrate, state 2 mesopterygoid small, about as long as autopalatine, posterior tip not contacting metapterygoid, and ventral border slightly overlapping quadrate, state 3 mesopterygoid very small, sometimes half length of autopalatine, posterior tip very distant from metapterygoid, and ventral border slightly overlapping or not contacting quadrate (CI 100, RI 100) As noted by Costa (m press), rivulids have a slightly reduced mesopterygoid (Figs 11-15), when compared to the condition exhibited by other cyprinodontiforms (Fig 11A) However, m *Millerichthys, Spectrolebias, Cynolebias, Simpsonichthys, Leptolebias, Campellolebias* and *Cynopoecilus,* the mesopterygoid is much more reduced (Costa, 1990a), therefore considered distinct character states In *Millerichthys,* the mesopterygoid is about equal in longitudinal length to the autopalatine (Costa, 1995b fig 4B), whereas in *Spectrolebias, Cynolebias, Simpsonichthys, Leptolebias, Campellolebias* and *Cynopoecilus,* it is much smaller than the autopalatine (Figs 11C. 12B. 13C, and 14A, C)

35 Reduction of the anterior portion of mesopterygoid State 0 not reduced, state 1 reduced (CI 100, RI 100) The genera *Plesiolebias, Pituna, Maratecoara,* and *Stenolebias* are unique by having a reduced anterior portion of mesopterygoid (Figs 12C and 13C) In consequence of this reduction, the mesopterygoid and autopalatine are not overlapped, m contrast to other aplocheiloid taxa and outgroups, in which the mesopterygoid and autopalatine are slightly overlapped

36 Shape of the ventral border of mesopterygoid. State 0 smooth, state 1 Jagged (CI 100, RI: 100) *Rivulus stellifer* and *Rachovia* have a Jagged ventral border of the mesopterygoid (Fig 14B), a condition contrasting with the approximately smooth mesopterygoid border of other rivulids and outgroups

37 Shape of the ventral portion of metapterygoid. State 0 not expanded, state 1 expanded, ventral border close to quadrate (CI 100, RI 100). Only in *C griseus* and species of the C. *wolterstorffi* and C *porosus* groups, the ventral portion of the metapterygoid is very close **to** the posterodorsal border of quadrate (Fig 14A).

38 Constriction on the dorsal portion of metapterygoid State 0 metapterygoid about rectangular, dorsal and ventral portions approximately equal in width, state 1 about triangular, dorsal portion very constricted (CI: 3 3, RI 66) *Cynolebias* and *Pterolebias phasianus, P longipinnis,* and the group *P peruensis* have a very narrow dorsal portion of metapterygoid (Figs 13A and 14A) The plesiomorphic condition for aplocheiloids is a rectangular metapterygoid, present in other rivulids.

39 Angle formed between anterior border and ***main*** *axis of posterior process of quadrate in a lateral new.* State **0 90-130,** state **1 140-160** (CI **50, RI:** 90) Costa (1990a) pointed out an anteriorly expanded quadrate as synapomorphic for an assemblage of rivulids not including cynolebiatines A reexamination of this character, analyzed through the angle formed by the anterior and ventral borders, indicate this apomorphic condition is also present m some cynolebiatines sensu Costa (1995b) Therefore, among rivulids only the genera *Spectrolebias, Cynolebias, Simpsonichthys, Leptolebias, Campellolebias, Cynopoecilus* and *Terranatos* have a condition similar to that occurring as a plesiomorphy in cyprinodontoids and other atherinomorphs (Figs 11C, 12B, 13C, 14A, C) Some aplocheilids may possess a large angle of quadrate (Fig 11A), but not forming the typical concave anterior border present m rivulids with an expanded quadrate (Figs. 11B, 12A. C. 13A, B. 14B, 15).

40. Reduction of the posterior process of quadrate., expressed by the proportion between length of the process and the total longitudinal length of quadrate not including anterior process. State 0: about 50%; state 1: about 30% (CI: 100; RI: 100). In the genera *Simpsonichthys* and *Cynolebias,* the posterior process of the quadrate is notoriously reduced, producing a longitudinally shortened suspensorium (Figs. 11C and 14A), distinct from the longer process in outgroups.

41. Expansion of the posterior process of quadrate, expressed by the proportion between length of the process and the total longitudinal length of quadrate not including anterior process. State 0: about 50%; state 1: about 60%; state 2: about 70% (CI: 66; RI: 91). Costa (1995a) considered an elongate posterior process as synapomorphic for the genera *Stenolebias, Maratecoara* and *Plesiolebias.* In a more detailed comparison, this apomorphic condition (state 1) is interpreted as present in most rivulids (not including the genera *Spectrolebias, Cynolebias, Simpsonichthys, Leptolebias, Campellolebias,* and *Cynopoecilus),* being longer (state 2) in *Plesiolebias, Pterolebias longipinnis,* and *P. phasianus* (Figs. 13A, B).

42. Width of the posterior process of quadrate. State 0: narrow; state 1: broad (CI: 100; RI: 100). Cyprinodontiforms plesiomorphically have a narrow posterior process of quadrate. Only *Leptolebias,* among rivulids, has a widened process (Fig. 14C).

43. Size of sympletic. State 0: short; state 1: elongate (CI: 100; RI: 100). Species of the subgenus *Plesiolebias* have an exceptionally enlarged sympletic (Fig. 13B), yielding a very elongate suspensorium. Costa (1990a) defined a more inclusive rivulid clade by an elongate sympletic, but this was not confirmed in the present study.

44. Orientation of the posterior portion of suspensorium. State 0: directed dorsally; state 1: directed posteriorly (CI: 100; RI: 100). *Spectrolebias* is unique among aplocheiloids by having the posterior portion of the suspensorium posteriorly displaced (Fig. 13C).

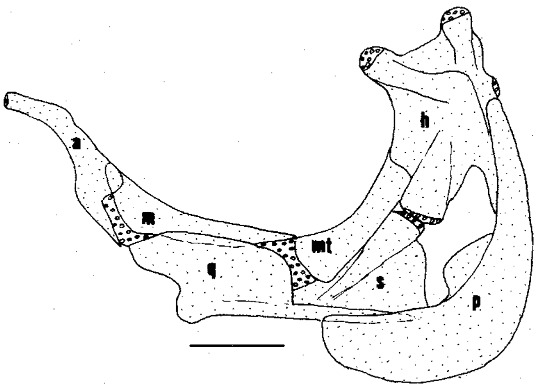

Figure 15. ***Trigonectes balzanii,*** **left jaw suspensorium, lateral view. Abbreviations: a, autopalatine; h, hyomandibula; m, mesopterygoid; mt, metapterygoid; p, preopercle, q, quadrate; s, sympletic. Dots indicate bone; circles, cartilage. Scale bar = 1 mm.**

45. Reduction of preopercle and sensory canal. State 0: robust, L-shaped, with an expanded median rim and well-developed sensory canal, neuromasts usually hidden by a closed canal; state 1: thin, C-shaped, with a reduced median rim, and reduced sensory canal, restricted to the upper portion of the bone, externally represented by two large exposed neuromasts; state 2: thin, C-shaped, with a reduced median rim and vestigial sensory canal, thus acquiring a pointed shape dorsally, and with minute exposed neuromasts; state 3: thin, C-shaped, with a very reduced median rim and vestigial sensory canal, with a shortened pointed tip dorsally, and minute exposed neuromasts; state 4: thin, C-shaped, with a vestigial or absent median rim, vestigial sensory canal, and with a short pointed tip dorsally, and minute exposed neuromasts (CI: 66; RI: 87). All aplocheilids have a well-developed preopercle (Fig. 11A), with a complete sensory canal, constituting the plesiomorphic state for atherinomorphs. Parenti (1981) noted the weakly developed preopercular sensory canal of rivulids as synapomorphic. Subsequently, Costa (1990a) added an apomorphic C-shaped preopercle, with reduced median flange, to the diagnosis of the family. Parenti (1981) also defined *Cynolebias* based on the loss of a preopercular canal, corroborated in subsequent studies *(e.g.,* Costa, 1990a). However, only under a detailed analysis it is possible to detect all the proposed transformation series, between the unmodified preopercle of outgroups and different degrees of preopercle reduction noted in rivulid taxa. State 1 corresponds to the basal condition for rivulids (Fig. 11B. 12A. 13A, 14B and 15); state 2 is present in *.Millerichthys, Plesiolebias, Maratecoara, Stenolebias, Pituna, R. xiphidius,* and species of the *R. punctatus* and the *R. geayi* groups (Figs. 12C and 13B); state 3 occurs in *Spectrolebias, Simpsonichthys, Leptolebias, Campellolebias, Cynopoecilus,* and the *Cynolebias bellottii* group (Figs. 11C, 12B, 13C, and 14C); and state 3 is unique for C. *griseus* and species of the C. *porosus* and C. *wolterstorffi* groups (Fig.

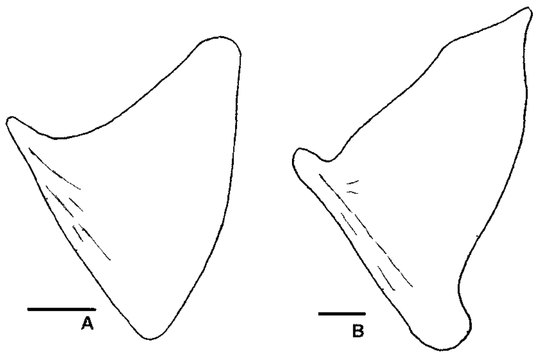

Figure 16. Left opercle, lateral view. A. *Cynolebias wolterstorffi;* B, *Moema pepatei.* Scale bar = 1 *mm.*

14A).

46. Relative size of dorsal and ventral portions of preopercle. State 0: about equal in size or ventral portion slightly longer than dorsal portion; state 1: dorsal portion longer than ventral portion (CI: 50; RI: 50). As discussed by Costa (1989a), the genera *Trigonectes, Moema* and *Neofundulus* have an apomorphically elongate dorsal portion of preopercle (Fig. 15).

47. Shape of the anterodorsal margin of opercle. State 0: convex to about straight; state 1: concave (CI: 100; RI: 100). In the plesiomorphic condition, the opercle has an approximately straight anterodorsal margin. The genera *Spectrolebias, Cynolebias* and *Simpsonichthys* have a distinctively concave anterodorsal margin of opercle (Fig. 16A).

48. A postero-ventral projection on the opercle. State 0: absent; state 1: present (CI: 100; RI: 100). *Moema* is the only member of the Aplocheiloidei exhibiting a posterior projection on the postero-ventral edge of the opercle (Fig. 16B).

Hyoid arch

49. Shape of the urohyal. State 0: elongate; state 1: deep and short (CI: 100; RI: 100). Species of the *Cynolebias bellottii* group have a deep and short urohyal (Fig. 17B), contrasting with the elongate shape of most other cyprinodontiforms and outgroups. However, in most species of the *C. wolterstorffi* group the urohyal has a uniquely derived morphology, consisting of an anteriorly directed and narrowed anterior portion. However, C. *cheradophilus* has an urohyal similar to the derived state above described. Thus, the C. *wolterstorffi* group is coded as P in the data matrix.

50. Anterodorsal process of urohyal. State **0:** long; state 1: short; state 2: vestigial to absent (CI: 100; RI: 100). The dorsal process of the urohyal is usually long in the Cyprinodontoidei, but

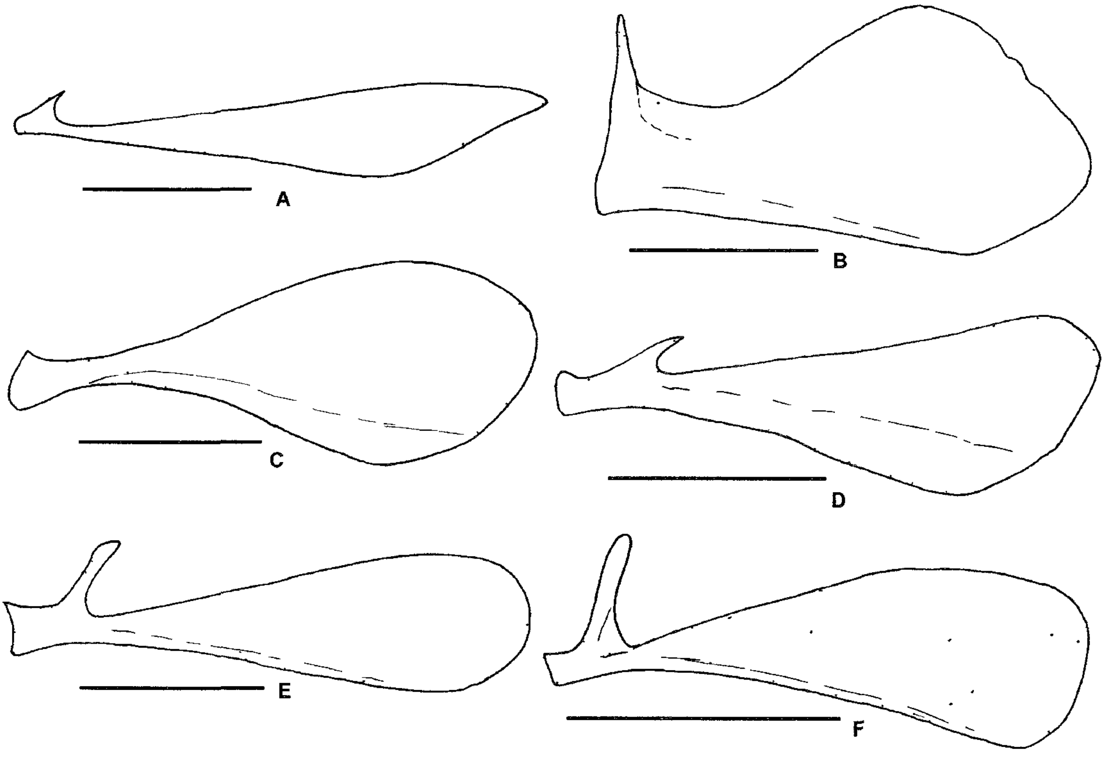

Figure 17. Urohyal, left lateral view.. *A. Aplocheilus lineatus;* B. *Cynolebias cyaneus; C. Rivulus brasiliensis; D. Rivulus santensis;* E. *Rivulus stellifer;* F. *Pituna compacta.* Scale bar = 1 **mm.**

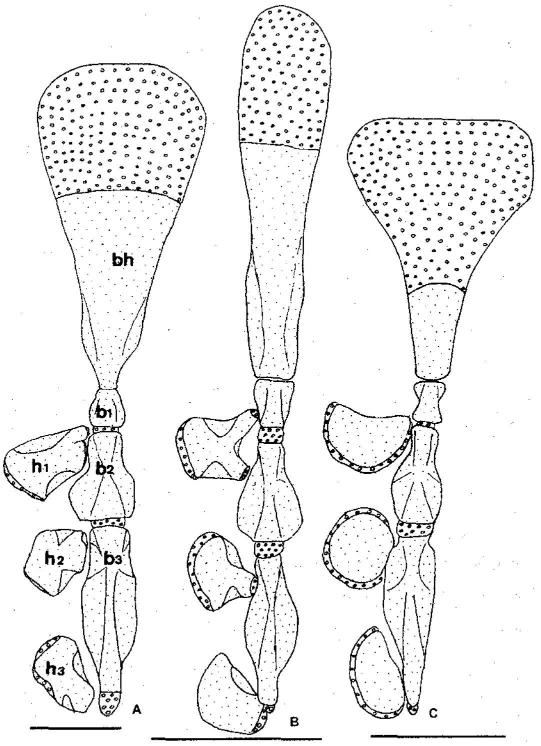

Figure 18. Basihyal (bh), basibranchials (bl-3) and left hypobranchials (hl-3). A. *Trigonectes balzanii;* B. *Plesiolebias bitteri; C. Cynolebias vandenbergi.* Dots indicate bone; circles, cartilage. Scale bar = 1 mm.

variable in length among aplocheilids (Costa, in press). All *non Rivulus* rivulid taxa (including *Millerichthys* and *Pituna,* Fig. 17F, both with species previously assigned to *Rivulus)* have elongate anterodorsal process of urohyal (Fig. 17B). In most *Rivulus* there is a minute process (state 1) (Fig. 17D), similar to that of some aplocheilid taxa *(e.g., Aplocheilus lineatus,* and several species *of Epiplatys and Aphyosemion,* Fig. 17A), but distinct from that of most other aplocheilid taxa *(e.g., Pachypanchax, Fundulopanchax,* and *Nothobranchius).* That process is extremely reduced or absent in *Rivulus caudomarginatus, R. ocellatus* and R. *brasiliensis* (state 2) (Fig. 17C).

51. *Vertical expansion of the posterior portion of urohyal.* State **0:** absent; state **1:** present (CI: 100; RI: 100). *Rivulus brasiliensis* is unique by having a rather expanded posterior portion of urohyal (Fig. 17C).

52. *Anterior expansion of the anterior arm of urohyal.* State **0:** absent; state **1:** present (CI: 25; RI: 62). In *Rivulus santensis, R. urophthalmus, R. amphoreus, R. cryptocallus, R. janeiroensis, R. bahianus, R. stellifer, Rachovia* and *Austrofundulus,* the anterior arm of the urohyal is clearly elongate (Fig. 17D, E), contrasting with the short anterior arm of most other aplocheiloid taxa.

53. *Shape of basihyal.* State 0: distal portion wide, about triangular; state 1: distal portion narrow, about rectangular (CI: 50; RI: 80). As discussed by Parenti (1981), the plesiomorphic condition for cyprinodontiforms is an entirely narrow basihyal. An anteriorly widened basihyal occurs in most aplocheiloids (Fig. 18A, C). However, as pointed out by Costa (1990a), some rivulids have a narrow basihyal. This latter condition is confirmed for *Plesiolebias, Maratecoara, Stenolebias, Pterolebias longipinnis* and *P. phasianus* (Fig.18B).

54. *Size of basihyal.* State 0: short; state 1: long (CI: 50; RI: 83). As discussed by Costa (1995a), some rivulid taxa have a derived, long basihyal, if the length of the basihyal is compared with the space between anterior border of first basibranchial and posterior border of third basibranchial (Fig. 18B). This was confirmed for *Plesiolebias, Maratecoara, Pituna, Stenolebias, Pterolebias longipinnis* and *P. phasianus.*

55. *Reduction of basihyal.* State **0:** not reduced; state 1: reduced (CI: 100; RI: 100). *Simpsonichthys* and *Cynolebias* have a reduced basihyal proportional to the space between anterior border of first basibranchial and posterior border of third basibranchial (Fig. 18C), a condition not observed in other aplocheiloids.

56. *Basihyal cartilage.* State 0: cartilaginous portion larger than osseous portion; state 1: cartilage reduced, restricted to anterior part of basihyal (CI: 50; RI: 0). Parenti (1981) described the markedly reduced bony portion of the basihyal as synapomorphic for aplocheilids. However, this is the condition occurring in beloniforms and most atheriniforms, therefore considered plesiomorphic. In contrast, most cyprinodontoids and rivulids have a reduced basihyal cartilage. Among rivulids, an expanded cartilaginous portion of basihyal occurs only in species of the *Cynolebias bellottii* complex (Fig. 18C).

57. *Reduction of the number of branchiostegal rays,* State 0: six; state 1: five (CI: 100; RI: 100). As discussed in Costa (1995a), *Stenolebias* is unique among aplocheiloids by possessing only five branchiostegal rays, instead of six.

58. *Interhyal.* State 0: ossified; state 1: cartilaginous or absent (CI: 50; RI: 66). As

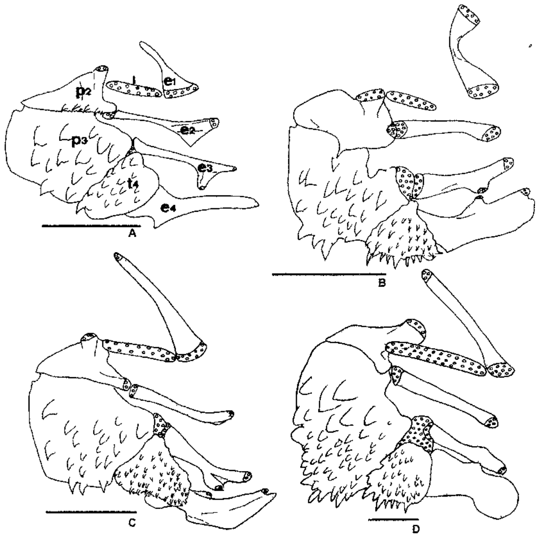

Figure 19. Left dorsal portion of branchial arches, ventral view. A. *Epiplatys chaperi;* B. *Maratecoara lacortei; C. Simpsonichthys whitei;* D. *Cynolebias perforatus.* Abbreviations: e1-4, epibranchials 1-4; I, interarcual cartilage; p2-3, pharyngobranchials 2,3; t4, toothplate pharyngobranchial 4. Dots indicate bone; circles, cartilage. Scale bar = 1 mm.

discussed by Parenti (1981: fig 28), an ossified interhyal is plesiomorphic for cyprinodontiforms. Parenti (1981) reported an ossified interhyal for R. *cylindraceus, R. marmoratus* and *R. beyet* (= *R. marmoratus),* but according to the present study, this condition is not present in the former species. Parenti (1981) associated presence of interhyal with an assemblage of *Rivulus* comprising smaller species, and absence of interhyal with larger species of *Rivulus.* However, several small species with cartilaginous interhyal have been observed, as well as a large species, R. *brasiliensis,* with an ossified Interhyal, refuting such association. Among rivulids, an ossified Interhyal is present only in R. *brasiliensis, R. ocellatus, R. marmoratus* and *R. caudomarginatus.*

Branchial arches

59. Shape of the anteromedial border of first epibranchial. State 0: straight to slightly concave; state 1: strongly concave (CI: 100; RI: 100). As discussed by Costa (1995a), the first epibranchial of the genera *Plesiolebias, Pituna, Maratecoara* and *Stenolebias* has a uniquely derived morphology (Figs. 19B).

60. Elongate epibranchials and interarcual cartilage. State 0: absent; state 1: present (CI: 100; RI: 100). The genera *Spectrolebias, Cynolebias, Simpsonichthys, Leptolebias, Campellolebias* and *Cynopoecilus* have epibranchials and interarcual cartilage distinctively elongate (Fig. 19C, D), a condition not occurring in any other aplocheiloid taxon nor in outgroups.

61. Shape of epibranchials 1-3. State **0:** slender; state 1: widened (CI: 100; RI: 100). As discussed by Costa (1990a, 1995a: fig. 1B). species of the subgenus *Plesiolebias* have widened epibranchials, an apomorphic condition not occurring in other aplocheiloid taxa.

62. Reduction of the uncinate process of the third epibranchial. State **0:** not reduced; state **1:** reduced (CI: 100; RI: 100). Costa (in press) indicated that rivulids have a very reduced uncinate process of the third epibranchial (Fig. 19A), constrasting with the plesiomorphic, elongate uncinate process of other atherinomorphs (Figs. 19B-D, 20).

63. Shape of the first epibranchial. State 0: straight to slightly curved; state 1: strongly curved (CI: 100; RI: 100). Species *of the R. punctatus group* are unique among cyprinodontiforms by having this peculiar shape of the first epibranchial (Fig. 20A).

64. Interarcual cartilage. State 0: present; state 1: absent (CI: 100; RI: 100). Parenti (1981) reported the absence of rostral cartilage for *Pterolebias* and Costa (1991a, 1995a: fig. 1B) for *Plesiolebias* and *Stenolebias.* However, only the subgenus *Plesiolebias* lacks an interarcual cartilage, although it is very reduced in some *Pterolebias.* Since there are not vestiges of an interarcual cartilage indicating an extremely reduced condition, the reduction of this cartilage in *Pterolebias* is considered under a distinct character (67).

65. Reduction of interarcual cartilage. State 0: not reduced; state 1: reduced (CI: 100; RI: 100). *Pterolebias* was diagnosed by Parenti (1981) by the absence of interarcual cartilage. However, all specimens examined possessed a cartilage, although very reduced in some species. Actually, the apomorphic, reduced condition occurs only in *P. longipinnis, P. phasianus, P. zonatus* and *P. hoignei* (Fig. 20B), but not in the *P. peruensis* group (Fig. 20C). Since this cartilage is absent in the subgenus *Plesiolebias,* the character was coded as "?" for this taxon.

66. Posterior displacement of the interarcual cartilage. State 0: absent; state 1: present (CI: 100; RI: 100). Species of the *Cynolebias porosus* group are unique among aplocheiloids by having

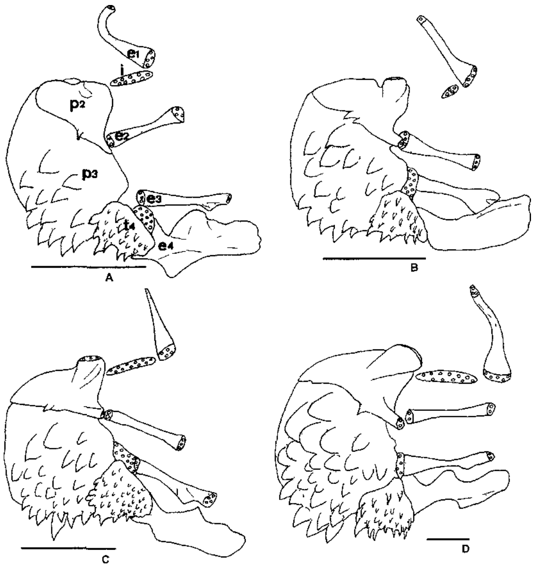

Figure 20. Left dorsal portion of branchial arches, ventral view. A. *Rivulus pictus;* B. *Pterolebias phasianus;* C. *Pterolebias obliquus; D. Moema pepotei.* Abbreviations: e1-4, epibranchials 1-4; I, interarcual cartilage; p2-3, pharyngobranchials 2,3; t4, toothplate pharyngobranchial 4. Dots indicate bone; circles, cartilage. Scale bar = 1 mm.

the medial tip of the interarcual cartilage attached to the posterior portion of the second pharyngobranchial (Fig. 19D), instead of attached to the tip of the anterior border of that bone, as in other cyprinodontiforms. Due to the absence of an interarcual in *Plesiolebias,* the character was coded as "?" for this subgenus.

67. Teeth of the second pharyngobranchial. State 0: present; state 1: absent (CI: 33; RI: 0). Although always reduced in number (Costa, in press), like other cyprinodontiforms, rivulids usually have teeth on the second pharyngobranchial. These teeth are always absent in the *Simpsonichthys whitei, Cynolebias porosus* and C. *wolterstorffi* groups (Fig. 19C, D).

68. Pharyngobranchial 2 anteriorly expanded. State 0: absent; state 1: present (CI: 100; RI: 100). *Maratecoara* has a unique morphology of the second pharyngobranchial, in which the anteromedial portion is anteriorly expanded (Fig. 19B).

69. Shape of teeth on the third pharyngobranchial and fifth ceratobranchial. State 0: base of teeth not widened, all teeth conical; state 1: base of teeth of the anterior portion of the dentigerous plate very widened, teeth of central portion molariform (CI: 50; RI: 0). Costa (1990a) described the presence of molariform teeth on the branchial arches of *Trigonectes* as an synapomorphy for the genus. However, this derived tooth morphology also occurs *in Moema* (Fig. 20D).

70. Arrangement of cartilage on the medial border of the first hypobranchial. State 0: cartilage on the entire medial border; state 1: cartilage on two distinct facets (CI: 50; RI: 90). A divided medial border of the first branchial was considered by Costa (1990a) as an apomorphy for a rivulid assemblage. In fact, most rivulids have a divided first branchial medial border to some extent (Fig. 18A, B). The plesiomorphic undivided medial border occurs in aplocheilids except for *Pachypanchax,* most cyprinodontoids, *Spectrolebias, Cynolebias, Simpsonichthys, Leptolebias, Campellolebias, Cynopoecilus,* and *Moema* (Fig. 18C).

71. Shape of the hypobranchial 2. State 0: about rectangular; state 1: rounded (CI: 100; RI: 100). *Spectrolebias, Cynolebias, Simpsonichthys, Leptolebias, Campellolebias* and *Cynopoecilus* are unique among aplocheiloids by having thin hypobranchials, the second hypobranchial being distinctively rounded (Fig. 18C). This is a modification of the plesiomorphic condition for cyprinodontiforms, in which this bone is robust and rectangular.

72. Teeth on fourth ceratobranchial. State 0: present; state 1: absent (CI: 100; RI: 100). Parenti (1981) reported a reduced dentition on the fourth ceratobranchial *in Austrofundulus* and *Terranatos,* considering it as an intermediate condition between the absence of teeth in *Cynolebias* and the normally developed dentition in the remaining rivulids. However, Costa (1990a) noted that both *Austrofundulus* and *Terranatos* have a completely developed dentition on the fourth ceratobranchial (Fig. 21A). Absence of teeth on the fourth ceratobranchial is confirmed for *Spectrolebias, Cynolebias, Simpsonichthys, Leptolebias, Campellolebias* and *Cynopoecilus* (Fig. 21B).

73. Postero-ventral process on the anterior portion of fourth ceratobranchial. State 0: present; state 1: absent (CI: 100; RI: 100). As discussed by Costa (1990a), the anterior portion of the fourth ceratobranchial of *Spectrolebias, Cynolebias, Simpsonichthys, Leptolebias, Campellolebias* and *Cynopoecilus* is narrowed, lacking a ventral process found in other cyprinodontiforms (Fig. 21B).

74. Number of gill-rakers in the ventral portion of first gill arch. State 0: usually seven to ten, rarely 11; state 1: 12-15 (CI: 33; RI: 0). *Moema, Austrofundulus* and *Rivulus stellifer* have more

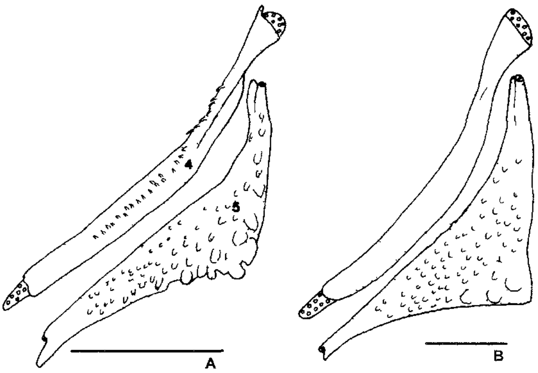

Figure 21. Left ceratobranchials 4 and 5, dorsal view. A. *Austrofundulus limnaeus;* B. *Simpsonichthys flavicaudatus.* Dots indicate bone; circles, cartilage. Scale bar = 1 mm.

gill-rakers than most other cyprinodontiforms. Other rivulids also have the plesiomorphic condition, with no more than ten gill-rakers. In the three taxa presenting the apomorphic condition, the number varies from 12 to 15.

75. *Small denticles on gill-rakers of the first gill arch.* State 0: absent; state 1: present (CI: 100; RI: 100). Species of the *Cynolebias porosus* group are unique among aplocheiloids by having small denticles on the gill-rakers.

Neurocranium

76. *Vomerine teeth.* State 0: absent; state 1: present (CI: 100; RI: 100). As discussed by Costa (in press), many aplocheiloids are unique among cyprinodontiforms in having vomerine teeth, considered an apomorphic condition for this group by not occurring in immediate outgroups to cyprinodontiforms. However, as noted by Costa (1995d) vomerine teeth are absent in an assemblage of rivulids. It includes the genera *Spectrolebias, Cynolebias, Simpsonichthys, Leptolebias, Campellolebias* and *Cynopoecilus,* except in *Simpsonichthys whitei* and some species of the *Cynolebias porosus* group *(e.g., C. perforatus),* which are polymorphic for this character and were coded as P.

77. *Number of vomerine teeth.* State 0: few teeth (usually one to three, sometimes four to six); state 1: numerous teeth (eight to 12) (CI: 50; RI: 50). Costa (1993) described high counts of vomerine teeth to corroborate the monophyly of an assemblage comprising *Moema* and *Trigonectes.* This was followed by Thomerson & Taphorn (1995), who also included *Renova* in that clade. However, numerous vomerine teeth are also present in *Pterolebias phasianus,* but they are absent in some species of *Trigonectes* (coded as P in the data matrix). The character is coded as "?" for those taxa without vomerine teeth.

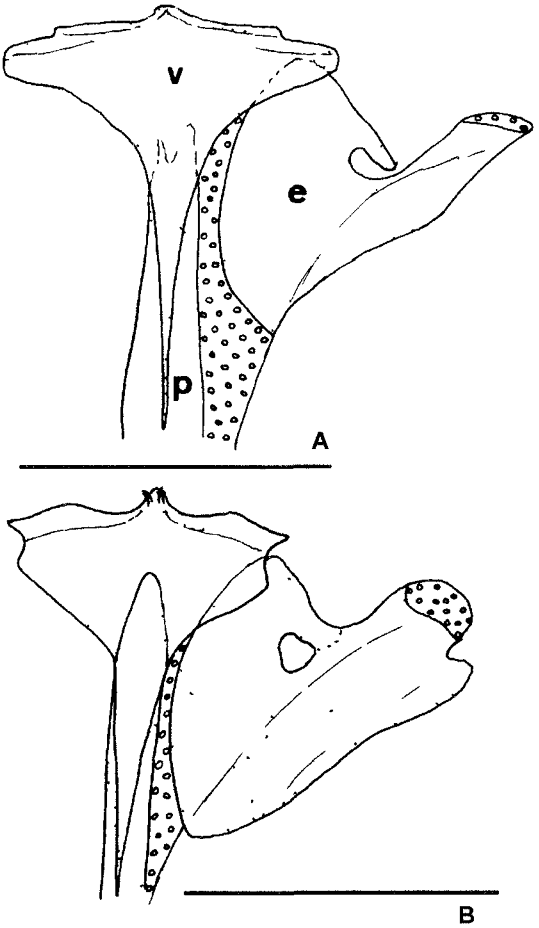

Figure 22. Vomer (v), anterior portion of parasphenoid (p) and left lateral ethmoid (e), ventral view. A. *Campellolebias brucei;* B. *Maratecoara lacortei.* Dots indicate bone; circles, cartilage. Scale bar = 1 mm.

78. *Extent of the posterior process of vomer.* State **0:** short, its tip close to a transverse line through posterior margin of lateral ethmoid; state 1: elongate, its tip in much surpassing that transverse line (CI: 100; RI: 100). Costa (1995c) pointed out that an elongate posterior process of vomer would be autapomorphic for *Campellolebias* (Fig. 22A). However, *Campellolebias* possesses a unique morphology of the vomer (character 82), but *Cynopoecilus,* with a different shape of the vomer, also presents an elongate process.

79. *Shape of the lateral wings of vomer.* State **0:** broad; state 1: narrow (CI: 100; RI: 100). *Campellolebias* and *Cynopoecilus* possess an apomorphic narrowing of the lateral wings of the vomer, yielding to a shape unique among

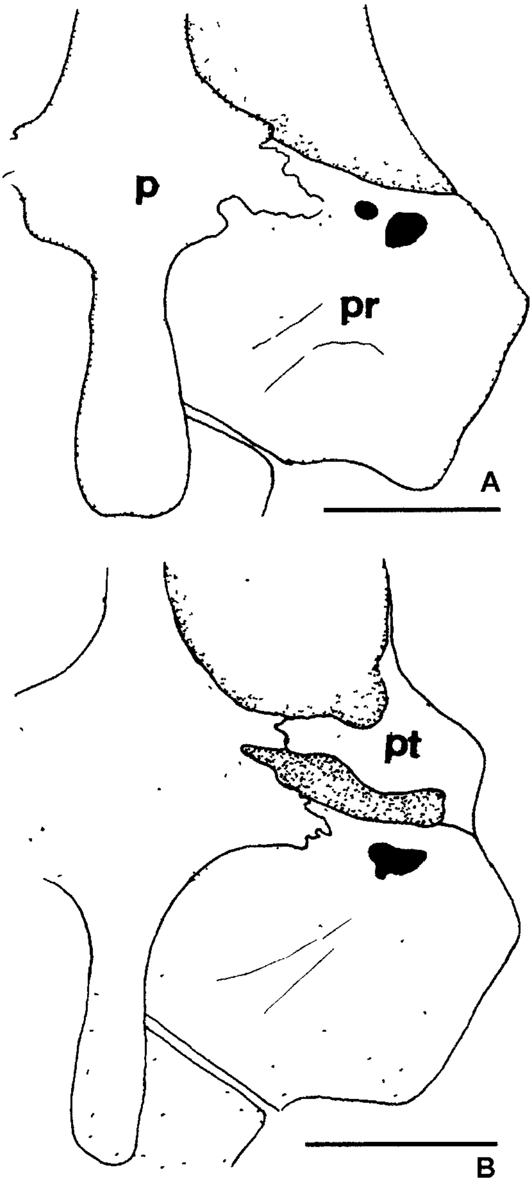

Figure 23. Posterior portion of parasphenoid (p), left prootic (pr) and pterosphenoid (pt), ventral view. A. *Rivulus brasiliensis;* B. *Rivulus urophthalmus.* Scale bar = 1 mm.

aplocheiloids (Fig. 22A).

80. Paired anteroventral projection on vomer. State 0: absent; state 1: present (CI: 100; RI: 100). *Campellolebias* is unique among aplocheiloids by the presence of a paired anteroventral projection on vomer (Fig. 22A).

81. Size of an anterior process on lateral ethmoid, directed posteriorly, carrying a ligament attached to autopalatine. State 0: small; state 1: median to elongate, sometimes contacting lateral arm of the bone (CI: 100; RI: 100). As discussed by Costa (in press), the presence of an anterior process on the lateral ethmoid is synapomorphic for aplocheiloids. This process is short in aplocheilids and elongate in rivulids, as noted by Costa (1990a).

82. Anterior process of lateral ethmoid elongate, firmly attached to lateral arm of the bone. State **0:** absent; state 1: present (CI: 100; RI: 100). In *Maratecoara* and *Stenolebias,* this process is firmly attached to the lateral arm of the bone (Fig. 22B), a condition not occurring in any other aplocheiloid. For outgroups lacking this process, the character was coded as "?"' in the data matrix.

83. Extent of lateral process of parasphenoid. State 0: vestigial or absent, not contacting any structure; state 1: well-developed, contacting pterosphenoid (CI: 25; RI: 82). The presence of well-developed lateral process of parasphenoid is plesiomorphic for cyprinodontiforms, although very reduced or absent in aplocheilids and most rivulids (Fig. 23A). A well developed lateral process occurs in *Rivulus urophthalmus, R. bahianus, R. cryptocallus, R. brunneus, R. amphoreus, R. hartii, R. stellifer, Pterolebias, Moema, Trigonectes, Neofundulus, Renova and Rachovia* (Fig. 23B).

84. Reduction of the lateral border of frontal. State 0: lateral border well-developed and well-defined; state 1: lateral border shortened and ill-defined (CI: 100; RI: 100). As discussed by Costa (in press), the frontal of rivulids is laterally reduced, a unique condition among cyprinodontiforms, forming a concave lateral border of the neurocranium. In the plesiomorphic condition, the border is approximately straight, producing a rectangular shape of neurocranium in a dorsal view.

85. Size and shape of sphenotic. State 0: narrow and small, much smaller than autopterotic; state 1: broad and large, about equal in size to autopterotic (CI: 100; RI: 100). Species of the *Cynolebias porosus* and C. *wolterstorffi* groups, and C. *griseus* have a uniquely enlarged sphenotic, which reaches a size similar to that of autopterotic (Fig. 24A). Besides being enlarged, in this apomorphic morphology, the lateral process of the sphenotic is widened, with its anterior tip anteriorly expanded.

86. Shape of posterolateral rim of autopterotic. State 0: rounded or slightly triangular, not expanded laterally; state 1: triangular, expanded laterally (CI: 100; RI: 100). *Cynolebias griseus* and the C. *porosus* and C. *wolterstorffi* groups are unique among aplocheiloids in having the laterally

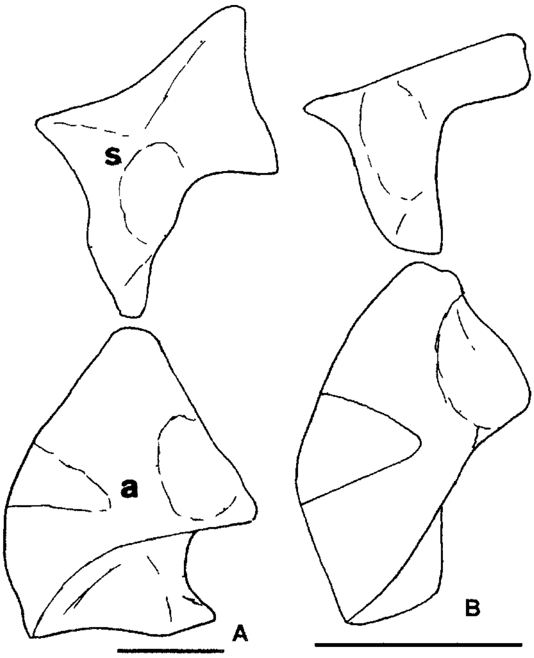

Figure 24. Left sphenotic (s) and left autopterotic (a), ventral view. A. *Cynolebias perforatus;* B. *Simpsonichthys multiradiatus.* Scale bar = 1 nun.

expanded posterolateral rim of autopterotic, which usually forms a lateral sharp tip (Fig. 24A). The plesiomorphic condition among cyprinodontiforms presents this flange short, usually rounded and without a process (Fig. 24B).

87. Shape and orientation of the supraoccipital process of males. State 0: slender, directed posteriorly; state 1: deep, directed dorsally (CI: 100; RI: 100). As discussed by Costa (1995a: fig. 9), *Maratecoara* is unique among aplocheiloids by having a deep supraoccipital process in males. The plesiomorphic condition for cyprinodontiforms is a narrow, posteriorly directed process.

88. A median laminar process between supraoccipital processes. State 0: absent; state 1: present (CI: 100; RI: 100). *Terranatos* is unique among the aplocheiloids by possessing a laminar, median process between and parallel to the supraoccipital processes.

Infraorbital series

89. Reduction and torsion of lacrimal. State 0: flat, posterior rim wide (cyprinodontoids) to slightly twisted, posterior rim reduced, bone formed mainly by canal (aplocheilids); state 1: very twisted and narrow, slender, canal vestigial; state 2: thin, lower portion very narrow, sensory canal vestigial; state 3: median and lower portions strongly narrowed (CI: 100; RI: 100). Atherinomorphs typically have a robust and triangular lacrimal, with a well-developed sensory canal. Parenti (1981) reported a narrow and twisted lacrimal as synapomorphic for aplocheiloids, and a lacrimal with obsolescent sensory canal as synapomorphic for rivulids. This was corroborated in a recent study (Costa, in press), and corresponds respectively to states 0 (in part) (Fig. 25A) and 1 (Fig. 25B) of this character. State 2 was already reported by Costa (1990a) for a derived assemblage of rivulids, herein confirmed for *Spectrolebias, Simpsonichthys Leptolebias, Campellolebias,* and *Cynopoecilus* (Fig. 25C). A further modification of the lacrimal still may be distinguished for *Cynolebias griseus,* and the C. *porosus, C. wolterstorffi* and C. *bellottii* groups, herein interpreted as a distinct character state (3), consisting of a strongly narrowed bone (Fig. 25D, E).

90. Curved lacrimal. State **0:** absent; state 1;

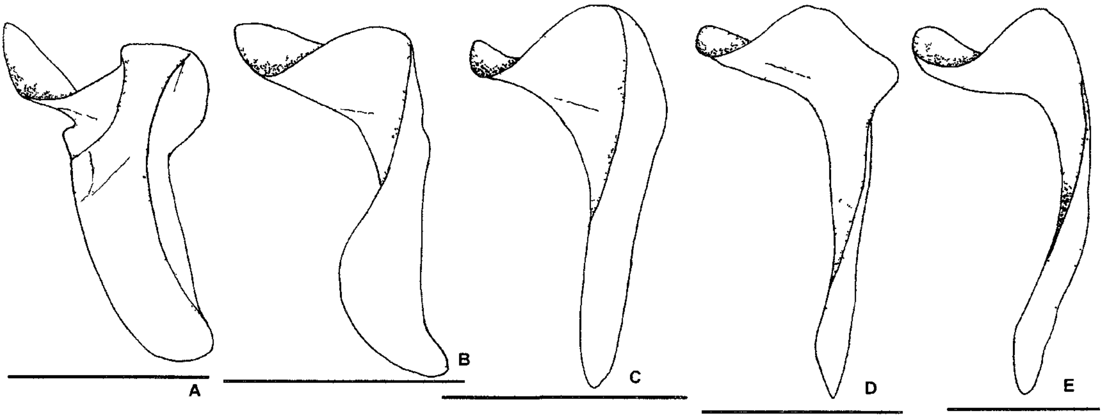

Figure 2S. Left lacrimal, lateral view. A. *Aplocheilus lineatus;* B. *Pterolebias zonatus;* C. *Simpsonichthys fulminantis;* D. *Cynolebias adloffi;* E. *Cynolebias wolterstorffi;* F. *Pituna compacta.* Scale bar = 1 nun.

present (CI: 100; RI: 100). A curved lacrimal is a unique condition for the species of the *Cynolebias wolterstorffi* group (Fig. 25E).

91. Shape of the ventral portion of lacrimal. State 0: not posteriorly expanded; state 1: posteriorly expanded (CI: 100; RI: 100). The ventral portion of the lacrimal of *Rivulus stellifer, Rachovia, Austrofundulus, Pituna, Papiliolebias, Plesiolebias, Maratecoara* and *Stenolebias* is posteriorly expanded (Fig. 25F). This apomorphic condition is not present in other rivulids.

92. Reduction of dermosphenotic. State 0: elongate to short, sensory canal well-developed; state 1: minute, sensory canal absent (CI: 100; RI: 100). Parenti (1981) reported the reduction of the dermosphenotic as apomorphic for rivulids. However, according to Costa (in press), the dermosphenotic of aplocheilids is also reduced, but intermediate between the condition of other cyprinodontiforms (elongate) and the condition exhibited by rivulids (minute, state 1). This character is coded as "?" for those rivulid taxa lacking a dermosphenotic (character 93).

93. Dermosphenotic. State 0: present; state 1: absent (CI: 33; RI: 66). A dermosphenotic is absent in all species of *Spectrolebias, Cynolebias, Campellolebias* and *Cynopoecilus.* The presence of the dermosphenotic (sixth infraorbital bone) is plesiomorphic among the atherinomorphs.

Vertebrae, ribs and caudal-fin skeleton

94. Paired, pointed, anteriorly directed process on first vertebra, corresponding to the neural prezygapophyses. State 0: present; state 1: absent (CI: 50; RI: 92). As discussed by Costa (1990a, 1995a), in the genera *Plesiolebias, Pituna, Maratecoara, Stenolebias, Spectrolebias, Cynolebias, Simpsonichthys, Leptolebias, Campellolebias* and *Cynopoecilus,* the first vertebra lacks neural prezygapophyses, thus the neural portion of this vertebra is formed uniquely by the neural spine (Fig. 26A). The remaining rivulids exhibit the plesiomorphic condition for cyprinodontiforms, in which the pointed laterodorsal processes are easily distinguished from the median neural spine (Fig. 26B)

95. Size of the neural spine of first vertebra. State 0: short; state 1: elongate (CI: 25; RI: 57). Parenti (1981) reported an elongate first neural spine as a synapomorphy of a group including *Austrofundulus, Terranatos* and *Cynolebias* (in Parenti's system including *Leptolebias, Campellolebias, Cynopoecilus, and Simpsonichthys).* However, a distinctively elongate first neural spine is confirmed for *Cynolebias,* species of the *Simpsonichthys constanciae group. Austrofundulus, Terranatos and Maratecoara* (Fig. 26).

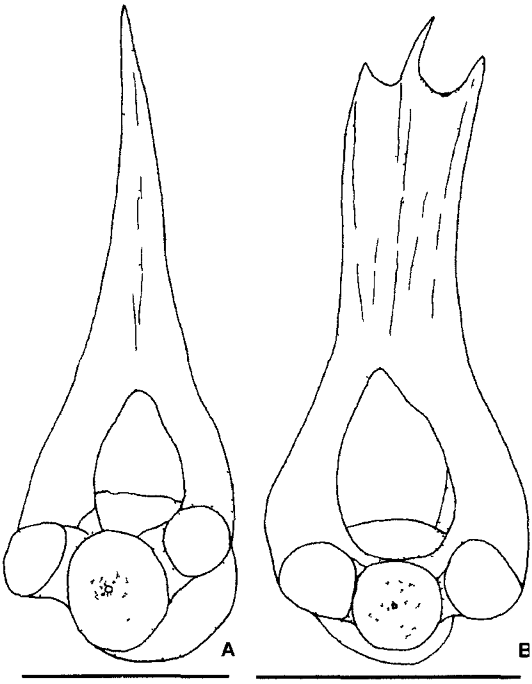

Figure 26. First vertebra, anterior view. A. *Maratecoara lacortei;* B. *Terranatos dolichopterus.* Scale bar = 1 *mm.*

96. Anterior expansion of the neural spine of the first vertebra. State 0: absent; state 1: present (CI: 100; RI: 100). In *Rivulus brasiliensis, R. caudomarginatus* and R. *ocellatus,* the neural spine of the first vertebra is anteriorly expanded (Fig. 27A), a condition not occurring in other rivulids (Fig. 27B).

97. A dorsally directed process on the base of anterior epipleural ribs. State 0: absent; state 1: present (CI: 100; RI: 100). *Terranatos* and *Austrofundulus* are unique in having a distinctive process, dorsally directed, on the base of the epipleural rib (Fig. 28A).

98. Shape of the distal portion of epipleural ribs. State 0: not bifid; state 1: bifid (CI: 50; RI: 0). Parenti (1981) defined an aplocheilid assemblage comprising *Aphyosemion, Fundulopanchax* and *Nothobranchius* by all sharing bifid epipleural ribs. However, according to the present study, it also occurs in *Epiplatys,* but not in *Nothobranchius.* Among rivulids, a similar derived condition of epipleural ribs morphology is present in *Rivulus xiphidius* and in *the Rivulus geayi* group, as noted by Costa (1995f) (Fig. 28B).

99. Neural spine on the first vertebra. State 0:

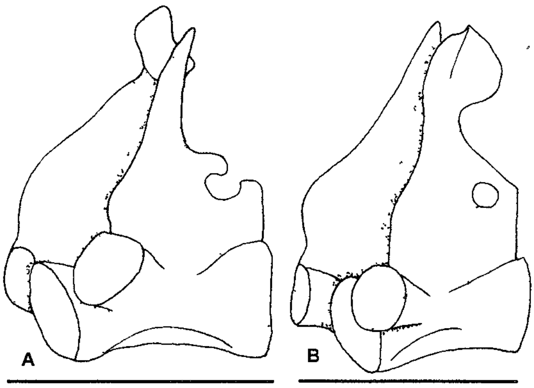

Figure 27. First vertebra, left lateral view. A. *Rivulus caudomarginatus;* B. *Rivulus santensis.* Scale bar = 1 nun.

absent; state 1: present (CI: 100; RI: 100). As discussed by Costa (1990a), in the Rivulidae there is a distinctive median neural spine in the first vertebra (Fig. 26). The plesiomorphic condition for the cyprinodontiforms is a first neural arch with lateral processes, sometimes united by a bridge forming a closed arch, but never with a prominent median spine.

100. Reduction in size of the neural prezygapophyses of caudal vertebrae. State 0: moderate to elongate; state 1: reduced; state 2: vestigial or absent (CI: 66; RI: 94). Cyprinodontiforms typically have well-developed neural prezygapophyses in caudal vertebrae (Fig. 29A). As discussed by Costa (1990a), the apomorphic, reduced neural prezygapophyses of caudal vertebrae occur in an assemblage of rivulids. Under a detailed comparison, it is possible to distinguish reduced prezygapophyses in the genera *Terranatos, Millerichthys, Rachovia, Austrofundulus, Plesiolebias, Maratecoara, Stenolebias and Pituna* (Fig. 29B), from minute or absent prezygapophyses in *Spectrolebias, Cynolebias, Simpsonichthys, Leptolebias, Campellolebias* and *Cynopoecilus* (Fig. 29C), herein interpreted as two distinct derived states.

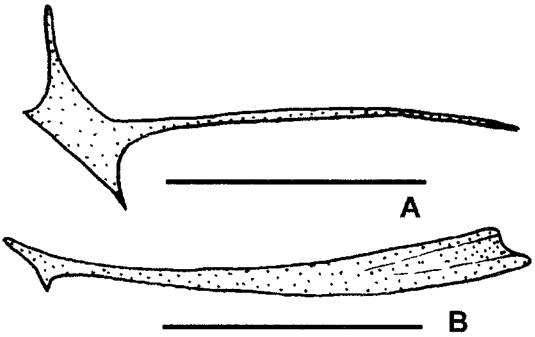

Figure 28. Left epipleural rib of fifth vertebra, lateral view. A. *Terranatos dolichopterus;* B. *Rivulus xiphidium.* Scale bar = 1 mm.

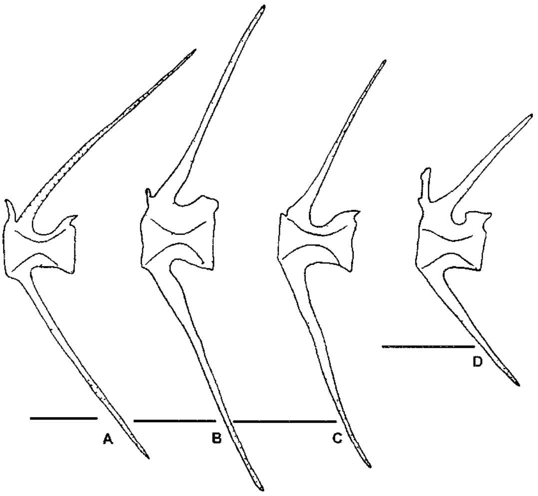

Figure 29. Fifth caudal vertebra, left lateral view. A. *Moema pepatei;* B. *Plesiolebias bitteri; C. Campellolebias brucei;* D. *Rivulus caudomarginatus.* Scale bar = 1 mm.

101. Shape of the neural prezygapophyses of caudal vertebrae. (CI: 33; RI: 87). State 0: short; state 1: long. As discussed by Costa (1990a), species of *Rivulus* (including *R. stellifer)* have a long neural prezygapophysis in caudal vertebrae (Fig. 29D). An exception occurs in *R. cylindraceus,* which has short neurapophyses. These apomorphic, long-shaped prezygapophyses are not present in other cyprinodontiforms.

102. A distinctive process on the posterobasal portion of the last neural spine. State **0:** absent; state **1:** present (CI: 100; RI: 100). This apomorphic condition is unique for *Campellolebias* (Fig. 30A).

103. Ankylosis of hypurals. State 0: two symmetrical plates; state 1: plates in close proximity, sometimes partially ankylosed; state 2: plates united but with a persistent median fissure; state 3: complete ankylosis forming a single plate (CI: 42; RI: 78). Most rivulids have hypurals ankylosed to form two plates separated by a median gap (state 0) (Fig. 3OB), the probable plesiomorphic condition for atherinomorph fishes (Rosen, 1964). In *Terranatos, Austrofundulus, Rachovia,* and *R. stellifer* the two plates are very close, sometimes partially ankylosed (state 1). In *Pituna, Maratecoara,* and *Campellolebias,* the plates are ankylosed, but a fissure is always visible (state 2) (Fig. 30 A), *la Millerichthys, Plesiolebias, Stenolebias, Spectrolebias, Cynolebias, Simpsonichthys, Leptolebias, Cynopoecilus, Rivulus cylindraceus, Pterolebias hoignei* and *P. zonatus,* the ankylosis is complete, forming a single plate

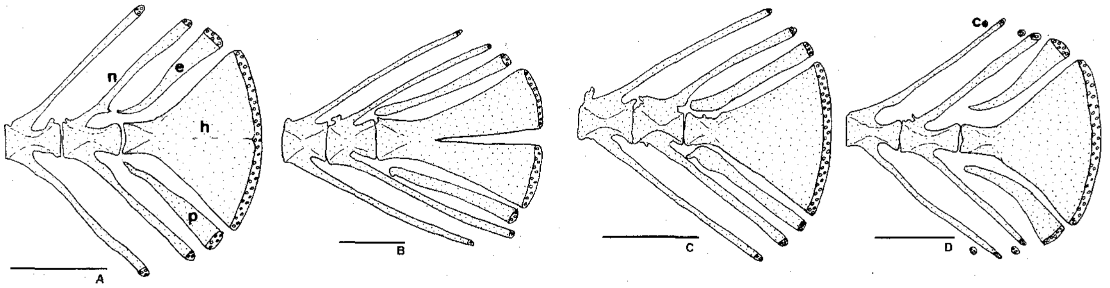

Figure 30. Caudal skeleton, left lateral view. A. *Campellolebias brucei;* B. *Pterolebias phasianus; C. Plesiolebias aruana;* **D. *Cynopoecilus melanotaenia.* Abbreviations: c, caudal-fin accessory cartilage; e, epural; h, hypural plate; n, last neural spine; p, parahypural. Dots indicate bone; circles, cartilage. Scale bar = 1 nun.**

without vestiges of a gap (state 3) (Fig. 30 **C,** D). However, this character is rather variable in immediate outgroups to Rivulidae. Among aplocheilids, there is a single plate in *Pachypanchax,* some species of *Aplocheilus,* and in *Nothobranchius.* There is a median gap in all other aplocheilids, besides an additional fissure dividing the dorsal plate in some species of *Aplocheilus* and most species of *Epiplatys.* As discussed by Costa (in press), a totally fused plate occurs in the majority of cyprinodontoid taxa, but a short gap is also present in some poeciliids, and two symmetrical plates in some anablepids. Therefore, this character is coded as P for the outgroup.

104. Shape of hypural fan. State **0:** deep; state **1:** slender (CI: 100; RI: 100). *Pterolebias phasianus* has a narrowed hypural fan (Fig. 30B), an apomorphic condition unique for aplocheiloids.

105. Shape of the proximal region of epural and parahypural. State 0: broad and approximately straight; state 1: narrow and curved anteriorly (CI: 100; RI: 100). The genera *Spectrolebias, Cynolebias, Simpsonichthys, Leptolebias, Campellolebias* and *Cynopoecilus* have an apomorphic shape of the epural and parahypural, consisting of narrow medial tips, usually curved anteriorly (Fig. 30A, D). The plesiomorphic condition for cyprinodontiforms is to possess robust medial portion of epural and parahypural, directed to the closest vertebral centrum.

106. A pointed process, anteriorly directed, on the proximal tip of the parahypural. State **0:** absent; state 1: present (CI: 100; RI: 100). This distinctive process on the parahypural occurs in *Plesiolebias, Maratecoara* and *Stenolebias,* and is unique among aplocheiloids (Fig. 30C).

107. Caudal accessory cartilages. State **0:** absent; state 1: few and minute caudal cartilages (CI: 100; RI: 100). As discussed by Costa (in press), aplocheiloids typically lack caudal accessory cartilages. An exception occurs in *Cynopoecilus,* which has minute cartilages (Fig. 30D).

108. Reduction in number of vertebrae. State 0: usually 27-31, rarely 26 or 32; state 1: usually 23-25. rarely 26 (CI: 100; RI: 100). As discussed by Costa (1990a, 1991b, 1998), the species of the subgenus *Plesiolebias* have the lowest vertebrae counts among rivulids.

109. Increase in number of vertebrae. State 0: usually 27-31, rarely 26 or 32; state 1: 33-40 (CI: 11; RI: 52). As discussed by Costa (1989, 1990a), an apomorphic, higher vertebrae counts occur in some assemblages of rivulids. The character state 1 was confirmed in the following taxa: *Neofundulus, Moema, Renova, Trigonectes,* group *Pterolebias zonatus,* group *P. peruensis, Austrofundulus,* group *Cynolebias porosus,* group C. *wolterstorffi, C. griseus, Rivulus stellifer, R. urophthalmus, R. amphoreus, R. janeiroensis* and R. *ocellatus.*

110. Number of caudal-fin-rays. State 0: 23-31; state 1: 32-36 (CI: 33; RI: 33). Costa (1993) suggested 32 or 33 caudal-fin-rays as synapomorphic for the clade *(Trigonectes + Moema).* However, an examination of larger samples of cleared and stained material demonstrated greater variations in *Trigonectes* (27-33), with an overlap with the range considered plesiomorphic in the present study. Therefore, it is coded as polymorphic (P) in the data matrix. The apomorphic condition is confirmed for *Moema, Renova, Austrofundulus* and *Rivulus amphoreus.*

Dorsal and anal-fin skeleton

111. Relative position of dorsal-fin origin and vertebrae of males, expressed by the relative position of the second dorsal proximal radial and the nearest neural spine anterior to it. State 0: 16-22; state 1: 13-15; state 2: 10-12; state 3: 9-10 (CI: 50;

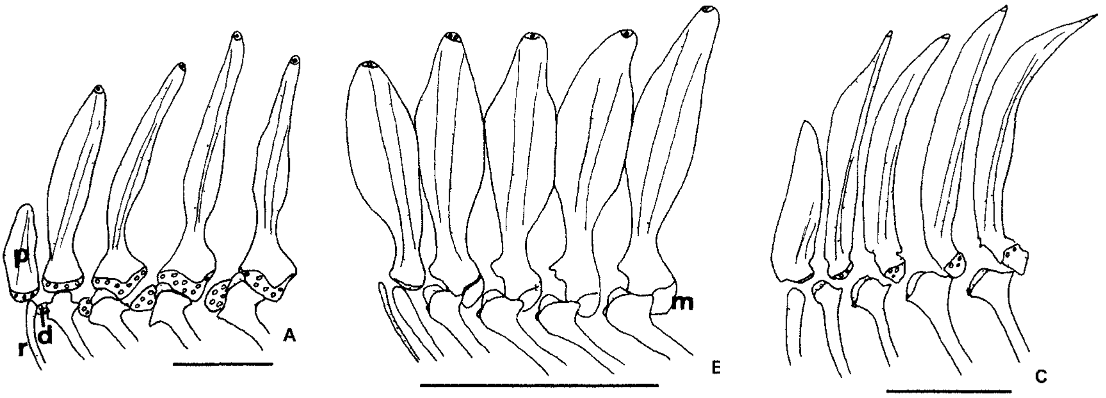

Figure 31. Anterior portion of anal-fin support, left lateral view. A. *Cynolebias alexandri;* B. *Terranatos dolichopterus;* C. *Pterolebias hoignei.* Abbreviations: d, first distal radial; m, fifth medial radial; p, first proximal radial; r, first anal-fin ray. Dots indicate bone; circles, cartilage. Scale bar = 1 nun.

RI: 72). This character was first described as dorsal-fin origin in front of anal-fin origin (Costa, 1990a), a simplification followed in subsequent studies *(e.g.,* Costa, 1995d). However, such description is herein considered imprecise, because anal-fin origin may be independently displaced posteriorly or anteriorly. Position of dorsal-fin origin relative to the vertebrae permits a more precise interpretation, including a transformation series with three character states, and consequently a distinct distribution of derived states among taxa. State 1 is present in *Stenolebias,* in the subgenus *Plesiolebias, C. griseus,* and in the C. *porosus* and C. *wolterstorffi* groups; state 2, in *Maratecoara, Leptolebias, Spectrolebias, Cynopoecilus, Simpsonichthys,* group *Cynolebias bellottii ;* state 3, in *Terranatos* and *Campellolebias.* Both in the S. *constanciae* group and C. *bellottii* group, many species exhibit the character state 3. However, phylogenetic studies on these groups *(e.g.,* Costa, 1996) indicate that the basal condition for each of these groups corresponds to the character state 2.

112. Posterior displacement of anal-fin origin of males, expressed in the relative position of second anal proximal radial and the nearest pleural rib or hemal spine anterior to it. State 0: 10-15; state 1: 16-18 (CI: 50; RI: 66). This character was first described as the snout/anal-fin length in percentage of standard length (Costa, 1990a). As discussed above for dorsal-fin position (character 111), the position of anal-fin origin relative to the vertebrae constitutes a more precise statement, presenting a distinct distribution among taxa. The apomorphic condition is confirmed for *Renova, Trigonectes, Moema* and *Neofundulus.*

113. Anterior displacement of the anal-fin origin of males, expressed in the relative position of second anal proximal radial and the nearest pleural rib or hemal spine anterior to it. State 0: 10-15; state 1: 6-9 (CI: 50; RI: 66). Costa (1990a) first described this character as the snout/anal-fin length in percentage of the standard length (Costa, 1990a). It is confirmed for the *Cynolebias bellottii* group, *Spectrolebias,* and *Simpsonichthys.*

114. Shape of proximal anal radials. State 0: moderate to wide, lateral margins convex; state 1: very narrow, rod-shaped, lateral margins usually straight (CI: 100; RI: 100). This apomorphic condition, only occurring in *Campellolebias* and *Cynopoecilus,* has been discussed by Costa (1995d: fig. 8).

115. Number of anal-fin-rays attached to the first proximal radial. State 0: two or three; state 1: eight (CI: 100; RI: 100). As discussed by Costa (1990, 1995d: fig. 8). this apomorphic condition is unique for *Cynopoecilus.* Parenti (1981) described this condition for males of *Cynopoecilus,* but it is also present in females. However, only in males these rays form a distinct fold. This structure is probably associated with the derived morphology of urogenital papilla of males, forming a morphologic apparatus for internal fertilization.

116. Shape of first proximal anal radial. State **0:** approximately straight; state 1: curved posteriorly (CI: 100; RI: 100). As discussed by Costa (1995c: fig. 1B), this apomorphic shape of the first proximal anal radial is unique for *Campellolebias,* and is probably related to the specialized anal musculature making pseudogonopodium movements possible.

117. Shape and size of the first proximal anal *radial.* State 0: elongate, approximately same length and shape as second and third radials; state 1: about triangular, much smaller than second and third radials (CI: 33; RI: 77). Costa (1990a) noted the apomorphic condition of the reduced first proximal anal radial (Fig. 30A) for a clade of cynolebiatines. This is confirmed for *Maratecoara, Spectrolebias, Cynolebias, Simpsonichthys, Leptolebias,* and *Cynopoecilus.*

118. Strong ligamentous connections between first anal-fin ray and first and second proximal radials simultaneously. State 0: absent; state 1: present (CI: 100; RI: 100). As discussed by Costa (1995c), these apomorphic robust ligaments are found only in *Campellolebias.*

119. Ossification of medial anal radials. State 0: ossified; state 1: cartilaginous to weakly ossified (CI: 100; RI: 100). In *Simpsonichthys, Cynolebias, Spectrolebias, Campellolebias, Cynopoecilus* and *Leptolebias,* the medial radials of the anal-fin are thin and cartilaginous or weakly ossified. In these rivulid genera, usually there are not clear limits between the medial and the proximal radials. This contrasts with the plesiomorphic condition for Cyprinodontiformes, in which medial radials are ossified and conspicuously separated from proximal radials.

120. Width of proximal anal radials. State 0: slender, usually first radials slightly widened; state 1: all radials strongly widened (CI: 100; RI: 100). *Terranatos* is unique among aplocheiloids by the extremely widened proximal anal radials (Fig. 31B). In some rivulids, the anteriormost radials may be slightly wider than the posterior ones (Fig. 31A), but distinct from the condition occurring in *Terranatos.*

121. Proximal anal radials curved, their tips directed posteriorly. State 0: absent; state 1: present (CI: 100; RI: 100). This apomorphic shape of proximal anal radials is unique for the group *Pterolebias zonatus,* in which all radials - except for the first- are curved (Fig. 31C). The plesiomorphic condition for cyprinodontiforms is of approximately straight radials.

122. Branching of dorsal and anal-fin-rays. State 0: most rays branched; state 1: most rays unbranched, usually only a few branched rays on the anteromedian portion of the fins (CI: 100; RI: 100). As discussed by Costa (1996), among aplocheiloids and outgroups, this apomorphic condition is unique for the group *Simpsonichthys constanciae.*

123. Size and ossification of the first two distal anal radials. State 0: small and cartilaginous; state 1: large and ossified (CI: 100; RI: 100). As indicated by Costa (1995c: fig. 1B), this apomorphic condition is unique for *Campellolebias.*

124. Shape of the sixth and seventh anal-fin-rays of males. State 0: approximately straight; state 1: curved anteriorly (CI: 100; RI: 100). As discussed by Costa (1995c: fig. 8), this apomorphic condition is unique for *Cynopoecilus.*

125. Shape of the 10th to 12th anal-fin-rays of males. State 0: approximately straight, not overlapping the posterior rays; state 1: curved, directed posteriorly, overlapping the posterior rays (CI: 100; RI: 100). Costa (1995c: fig. 8) has shown that this apomorphic condition is unique for *Cynopoecilus.*

126. Elongate filamentous rays on the dorsal and anal-fins of males. State 0: absent; state 1: present (CI: 25; RI: 40). Elongate filamentous rays on dorsal and anal-fins of males are found in *Simpsonichthys, Spectrolebias, Cynolebias griseus,* and in the *P. zonatus* and *C. porosus* groups (Fig. 31). The apomorphic condition is present in most species of *Simpsonichthys,* occurring in all basal clades of the genus (Costa, 1996). In *Cynolebias,* filamentous rays are absent in the group *C. bellottii* and group *C. wolterstorffi,* but present in *C. griseus* and the group *C. porosus,* although they are more conspicuous in the anal-fin of the latter group. In *Spectrolebias,* there is a long filamentous ray on the dorsal fin, but the anal-fin is apomorphically fan-shaped, without filaments. The apomorphic condition for this character is considered distinct from the elongate dorsal and anal-fins of *Terranatos* and *Maratecoara,* in which both fin-rays and fin membranes are elongate.

127. Sexual dimorphism in number of dorsal and anal-fin-rays. State 0: not dimorphic; state 1: more rays in males than in females (CI: 100; RI: 100). This dimorphism in the number of fin-rays was first described by Berg (1897), and indicated as synapomorphic for an assemblage of rivulids by Costa (1990a). This is confirmed for the genera *Spectrolebias, Cynolebias* and *Simpsonichthys.*

128. Number of anal-fin-rays in males. State **0:** 11 to 18; state 1: 19-32 (CI: 33; RI: 75). There is a clear apomorphic increase of anal-fin-rays of males in *Spectrolebias, Simpsonichthys, Cynolebias, Cynopoecilus* and the in the group

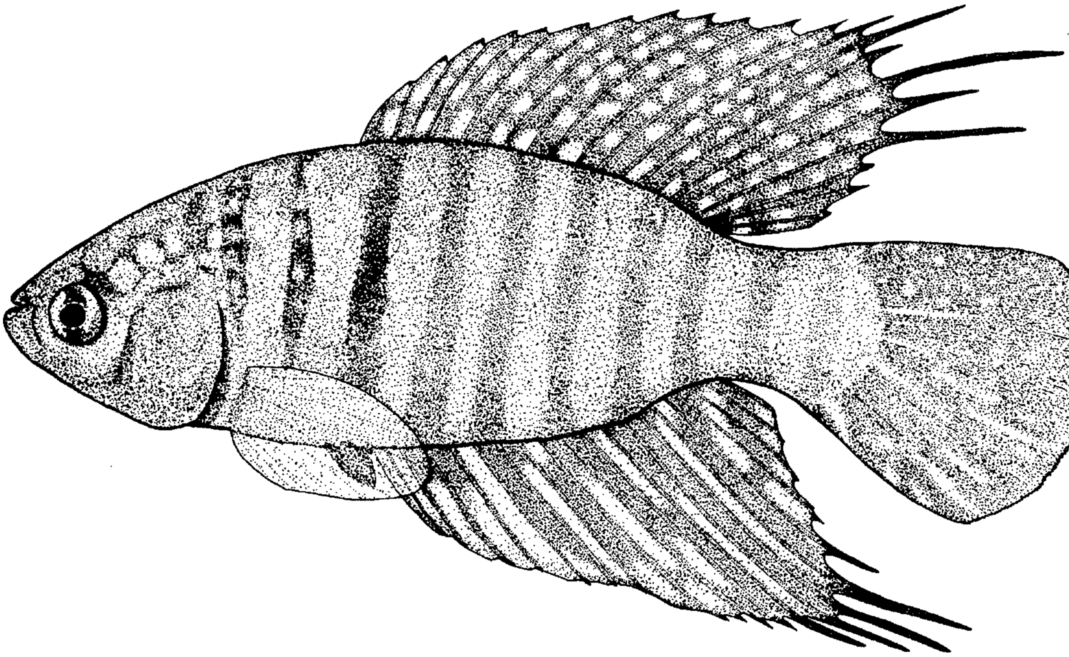

Figure 32. *Simpsonichthys hellneri*, male, 37.7 mm SL, UFRJ 2070.

Pterolebias zonatus.

129. Reduction in number of anal-fin-rays. State 0: 11 to 18; state 1: 8-10 (CI: 100; RI: 100). An apomorphic reduction of the anal-fin ray counts occurs *in Rivulus atratus and R. obscurus,* representing a unique condition among the aplocheiloids.

130. Thickness of first two anal-fin-rays of males. State 0: not thickened; state 1: thickened (CI: 100; RI: 100). Costa (1995c: fig. 8B) has shown that *Campellolebias* is unique for the males having a pseudogonopodium, a structure associated with internal fertilization, functionally similar to the gonopodia of the poeciliids. The two rays forming the pseudogonopodium are thickened.

131. Five minute rays at the anterior portion of anal-fin. State 0: absent; state 1: present (CI: 100; RI: 100). As observed by Costa (1995d: fig. 8), the presence of minute rays on the anterior portion of the anal-fin is apomorphic in *Cynopoecilus.*

Shoulder girdle

132. Reduction of the ventral process of posttemporal. State 0: elongate; state 1: vestigial or absent (CI: 11; RI: 46). As a plesiomorphic condition, the cyprinodontiforms possess a well-developed ventral process of posttemporal (Figs. 33 and 34). As discussed by Costa (1995b), this process was lost in some rivulids. This apomorphic condition is confirmed for the *Rivulus punctatus group, R. geayi* group, R. *obscurus, R. atratus, R. janeiroensis* group, *Pterolebias xiphophorus,* subgenus *Plesiolebias, Maratecoara, Stenolebias, Pituna, Cynopoecilus, Campellolebias, Leptolebias, Spectrolebias,* and *Cynolebias porosus* group (Figs. 35). The condition is variable in *C. wolterstorffi* (Fig. 36), but present in all other members of its species group, thus coded as 0.

133. Shape of the ventral process of posttemporal. State 0: elongate and rod-like; state 1: conical and very elongate (CI: 100; RI: 100). As described by Costa (1995b), *Millerichthys* is unique among aplocheiloids in having the apomorphic shape described here of the ventral process of posttemporal (Fig. 33B).

134. Size of supracleithrum. State 0: short, about half longitudinal length of posttemporal; state 1: elongate, approximately as long as posttemporal (CI: 100; RI: 100). Costa (1990a) has discussed that elongation of supracleithrum is synapomorphic for *Spectrolebias, Cynolebias, Simpsonichthys, Leptolebias, Campellolebias* and *Cynopoecilus* (Figs. 35 A and 36).

135. Posterior flange of cleithrum. State 0: present; state 1: absent (CI: 50; RI: 66). Atherinomorphs typically have a prominent rounded flange on the posterior margin of the upper portion of the cleithrum. In Rivulidae, this flange is present only in *Rivulus brasiliensis,*

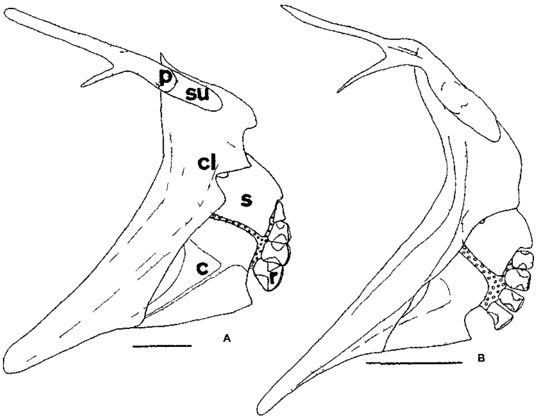

Figure 33. Left shoulder girdle, lateral view. A. *Rivulus brasiliensis;* B. *Millerichthys robustus.* Abbreviations: c, coracoid; d, cleithrum; p, posttemporal; r, fourth pectoral radial; s, scapula; su, supracleithrum. Postcleithrum is not represented. Dots indicate bone; circles, cartilage. Scale bar = 1 mm.

R. *caudomarginatus* and in the R. *ocellatus* group (Fig. 33A), but absent in all other rivulid taxa (Figs. 33B, 34-36).

136. Shape of the dorsal portion of cleithrum. State 0: short; state 1: elongate (CI: 100; RI: 100). The dorsal portion of the cleithrum of *Spectrolebias, Cynolebias,* and *Simpsonichthys* is distinctively elongate (Fig. 36). In other rivulids, the cleithrum is shorter, similar to the plesiomorphic condition for cyprinodontiforms.

137. Widened dorsal portion of cleithrum. State 0: absent; state 1: present (CI: 100; RI: 100). This wide shape of the cleithrum is an apomorphic condition uniquely exhibited by *Terranatos* (Fig. 34B).

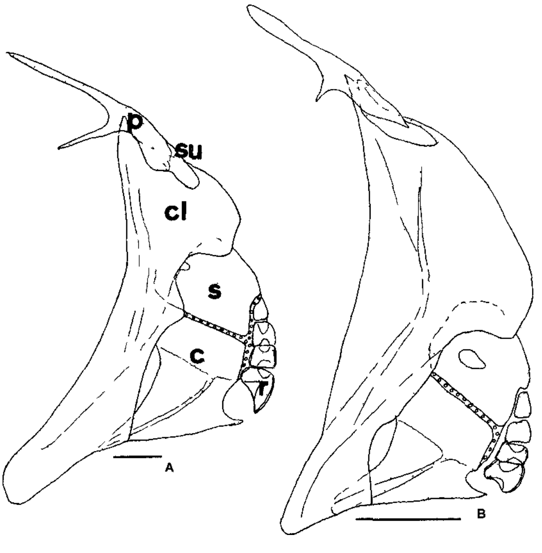

Figure 34. Left shoulder girdle, lateral view. A. *Trigonectes balzanii;* B. *Terranatos dolichopterus.* Abbreviations: c, coracoid; d, cleithrum; p, posttemporal; r, fourth pectoral radial; s, scapula; su, supracleithrum. Postcleithrum is not represented. Dots indicate bone; circles, cartilage. Scale bar = 1 mm.

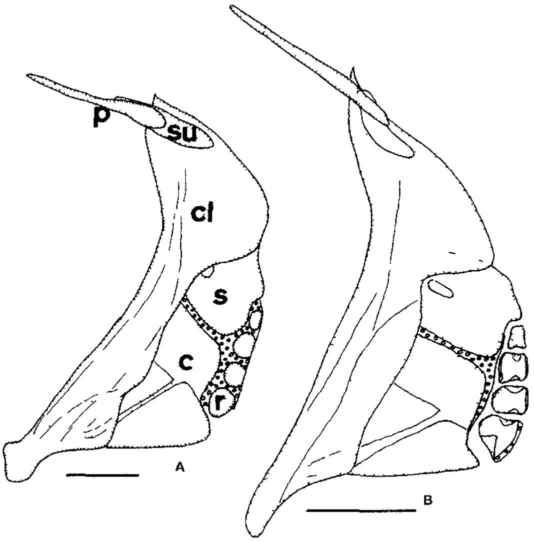

Figure 35. Left shoulder girdle, lateral view. A. *Leptolebias fluminensis;* B. *Maratecoara lacortei.* Abbreviations: c, coracoid; ci, cleithrum; p, posttemporal; r, fourth pectoral radial; s, scapula; su, supracleithrum. Postcleithrum is not represented. Dots indicate bone; circles, cartilage. Scale bar = 1 mm.

138. Postero-ventral flange of cleithrum. State **0:** present; state 1: absent (CI: 100; RI: 100). In contrast to other aplocheiloids, in *Spectrolebias, Cynolebias* and *Simpsonichthys,* there is not a postero-ventral flange on the cleithrum (Fig. 36).

***139.** Shape of the ventral tip of cleithrum.* State **0:** short; state 1: elongate anteriorly (CI: 33; RI: 86). Costa (1990a) pointed out that an anteriorly expanded ventral portion of the cleithrum is synapomorphic for *Rivulus* (Fig. 33A). However, an anteriorly elongate ventral portion of the cleithrum, with the tip anterior to the anteroventral tip of the coracoid, occurs not only in all species of *Rivulus* (including R. *stellifer),* but also in *Austrofundulus, Rachovia, Pterolebias, Trigonectes, Neofundulus, Renova, Moema, Pituna, and Millerichthys* (Figs. 33B and 34A).

140. Orientation of the ventral tip of cleithrum. State 0: directed anteriorly; state 1: directed ventrally (CI: 100; RI: 100). The apomorphic morphology of the cleithrum is unique for *Spectrolebias, Cynolebias* and *Simpsonichthys* (Fig. 36).

141. Narrowing of scapula and coracoid. State 0: absent; state 1: present (CI: 100; RI: 100). *Leptolebias* is unique among aplocheiloids and outgroups by having narrowed scapula and

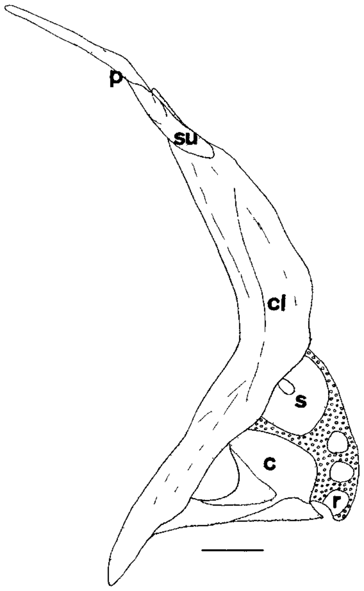

Figure 36. ***Cynolebias wolterstorffi*, left shoulder girdle, lateral view. Abbreviations: c, coracoid; ci, cleithrum; p, posttemporal; r, fourth pectoral radial; s, scapula; su, supracleithrum. Postcleithrum is not represented. Dots indicate bone; circles, cartilage. Scale bar = 1 mm.**

coracoid (Fig. 35A).

142. Shape of the anteroventral tip of coracoid. State 0: narrow; state 1: broad (CI: 100; RI: 100). *Spectrolebias, Simpsonichthys* and *Cynolebias* have an apomorphically widened anterior tip of the coracoid (Fig. 36). This contrasts with the narrow coracoid tip of other aplocheiloid taxa and outgroups.

143. Expanded anteroventral portion of coracoid. State **0:** absent; state **1:** present (CI: **100; RI:** 100). The group *Cynolebias wolterstorffi* is unique among aplocheiloids by the anteriorly expanded anteroventral portion of the coracoid (Fig. 36).

144. First postcleithrum. State 0: present; state 1: absent (CI: 100; RI: 100). As discussed by Parenti (1981) and Costa (1990a, in press), in all members of the Rivulidae, the first postcleithrum is absent. This condition contrasts with the presence of a scale-shaped first postcleithrum, which constitutes the plesiomorphic condition for cyprinodontiforms.

145. Shape of pectoral radials. State 0: robust, cubical; state 1: thin, scale-like (CI: 100; RI: 100). As discussed by Costa (1990a, fig. 30), the pectoral radials of the genera *Spectrolebias, Cynolebias, Simpsonichthys, Leptolebias, Campellolebias* and *Cynopoecilus* are reduced to form rounded, thin small bones. In fact, in those taxa, the scapula and coracoid are thinner than in other groups and are separated by a wider portion of cartilage (Figs. 35A and 36), all considered to constitute a single event of reduction, affecting the same taxa. In the plesiomorphic condition, atherinomorphs have robust radials, scapula and coracoid, separated by a tight cartilage.

146. First pectoral radial. State 0: present; state 1: usually absent, sometimes vestigial (CI: 100; RI: 100). Costa & Brasil (1990) have shown that *Cynolebias* is unique in having the uppermost pectoral radial lost or at most represented by a small point of ossification (Fig. 36).

147. Fourth pectoral radial expanded ventrally. State **0:** absent; state **1:** present (CI: 25; **RI:** 66). This apomorphic ventral expansion of the postero-ventral tip of the ventralmost pectoral radial occurs in *Pterolebias, Trigonectes, Moema, Renova* and *Pituna* (Fig. 34A). An expanded fourth radial is not present in other aplocheiloids and outgroups.

Pelvic girdle

148. Number of pelvic-fin-rays. State 0: six; state 1: usually seven, sometimes eight; state 2: nine (CI: 25; RI: 64). In the plesiomorphic condition, the cyprinodontiforms have six pelvic-fin-rays. Parenti (1981) reported seven or eight rays as a synapomorphy for a group comprising all rivulids minus some species of *Rivulus*. Costa (1990a) considered eight rays as apomorphic *for Pterolebias, Maratecoara,* and *Terranatos*. However, as pointed out by Thomerson & Taphorn (1992a) and Costa (1998), *Pterolebias* and plesiolebiatins may have seven rays. Thus, taxa with seven or eight rays were united here in a single character state in the data matrix. The plesiomorphic presence of six pelvic-fin-rays is confirmed for some species of *Rivulus* comprising R. *ocellatus, R. caudomarginatus, R. cylindraceus, R. geayi, R. bahianus,* and R. *janeiroensis*, but it also occurs in *Spectrolebias, Cynolebias, Simpsonichthys, Leptolebias, Campellolebias* and *Cynopoecilus.* All remaining genera of the family have seven or eight pelvic rays, except for the subgenus *Papiliolebias,* with nine rays (Costa, 1998).

Paired fins morphology

149. Shape of pectoral fin. State 0: rounded; state 1: pointed (CI: 33; RI: 50). Costa (1989a, 1990a) noted the apomorphic presence of a pointed pectoral fin in some rivulids. This condition is confirmed for the group *Pterolebias*

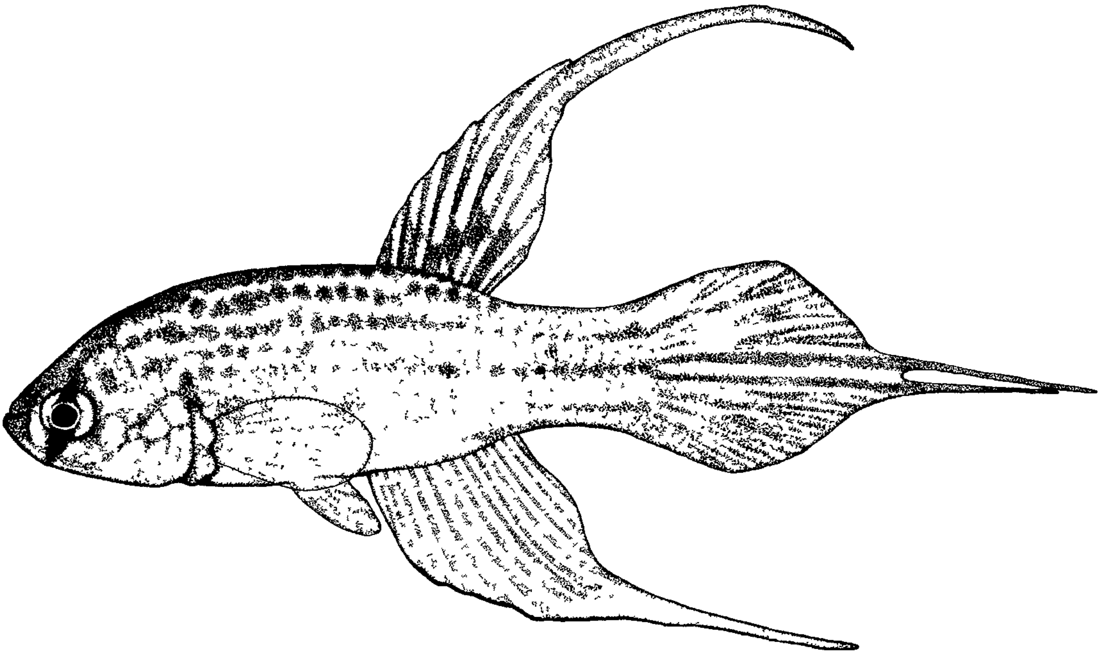

Figure 37. Maratecoara lacortei, male about 30 mm SL, not preserved.

zonatus, P. xiphophorus, the group P. peruensis, Moema and Renova. All other rivulids and outgroups have a rounded pectoral fin.

150. *Papillae on the internal surface of upper pectoral-fin-rays of larger males.* State 0: absent; state 1: present (CI: 100; RI: 100). The apomorphic presence of pectoral papillae, reported by some authors (e.g., Carvalho, 1957), occurs in *Simpsonichthys* and *Cynolebias.* There is no similar structures in other aplocheiloids and outgroups.

151. Size *of pectoral fin of males, expressed* by the *relative position of its tip and vertebrae.* State **0:** between vertebrae 8 and 11; state 1: between vertebrae 12 and 15 (CI: 50; RI: 88). Parenti (1981) reported an elongate pectoral fin, reaching the pelvic-fin base, as synapomorphic for a group comprising all rivulids minus *Rivulus.* In contrast, Costa (1989b. 1990a) considered the reduced pectoral fin *of Rivulus, Plesiolebias, Campellolebias* and *Cynopoecilus,* expressed as percentages of standard length, as an apomorphic condition. Both characters, as expressed in those studies, may introduce some error when comparing fishes as to distinct position of pelvic and anal-fins and different degrees of body elongation. The use of the relative position of tip of pectoral fin and vertebrae are herein adopted in view to minimize these errors, producing a different distribution of character states among rivulid taxa. The apomorphic condition is confirmed for *Trigonectes, Moema, Neofundulus, Renova, Pterolebias* and *Terranatos.*

152. *Reduction of pelvic fin.* State 0: short to elongate; state 1: minute (CI: 100; RI: 100). As discussed by Costa (1995d), the pelvic fin of *Cynopoecilus* is reduced (Costa, 1995b: fig. 122), constituting an apomorphic condition. One species of the group *Rivulus janeiroensis* lacks the pelvic fin (Costa & Brasil, 1991), and this fin is also extremely reduced or absent in a clade *of Simpsonichthys* of the S. *constanciae* group (Costa, 1996). However, the apomorphic condition of the character does not belong to the ground plan of these genera, thus coded as 0 in the data matrix.

153. *Extent of the pelvic fin of males.* State 0: short, its tip not surpassing the anterior portion of the anal-fin; state 1: elongate, its tip reaching the central or the posterior portion of the anal-fin (CI: 50; RI: 66). The plesiomorphic condition for pelvic fin morphology in cyprinodontiforms is a short fin. *Trigonectes* and some taxa within *Pterolebias* (P. *longipinnis, P. phasianus* and the group. P. *zonatus)* are known to have very elongate pelvic fins in males (Thomerson, 1974; Costa 1988b, 1990c), and somewhat elongate in females too. A pelvic fin with an elongate filamentous ray is present in *Plesiolebias xavantei,* but this condition is not present at a basal level within the genus (Costa,

Austrofundulus and Rachovia. However, it is not known if that head morphology in rivulid genera other than Austrofundulus and Rachovia is caused by diseases, since many old specimens of those genera do not possess a fatty protuberance, thus not considered homologous.

164. *Association between branchiostegal and opercular membranes.* State 0: not united; state 1: united (CI: 100; RI: 100). As discussed by Parenti (1981) (see also Costa, 1990a, in press), rivulids are unique among cyprinodontiforms by possessing an apomorphic, united branchiostegal and opercular membranes. In other atherinomorph taxa, the membranes are separate.

165. Size *of opercular membrane of males.* State 0: short, slightly projecting beyond posterior border of opercle; state 1: expanded, surpassing pectoral-fin base (CI: 100; RI: 100). As discussed by Costa (1995a), *Maratecoara* is unique among aplocheiloids and outgroups by having an expanded opercular membrane (Fig. 37), a condition easily visible during the courtship behavior.

166. *Shape of ventral profile of head.* State 0: approximately rounded; state 1: angular (CI: 100; RI: 100). As discussed by Costa (1995a, 1998), this apomorphic morphology of head is unique for the subgenus *Plesiolebias.*

Size

167. *Increase in standard length (SL).* State **0:** usually 25-50 mm SL, rarely reaching 65 mm SL; state 1: reaching 80-90 mm SL (CI: 25; RI: 40). As discussed by Costa (1989, 1990a). an apomorphic increase in size (SL) occurs in some rivulid groups. This condition is confirmed for *Moema, Trigonectes,* group C. *porosus,* group C. *wolterstorffi* and *Rivulus hartii.*

168. Reduction in standard length. State 0: usually 25-50 nun SL; state 1: slightly surpassing 20 mm SL (CI: 100; RI: 100). A reduced size of adult occurs in *Spectrolebias,* since the largest specimen known has 22.2 mm SL (Costa &Nielsen, 1997). However, males *of Spectrolebias semiocellatus* have elaborate fin morphology fully developed at about 16 mm SL, as well as females with 15 mm SL have mature ovaries. Pygmy species also occur in *Plesiolebias* and *Simpsonichthys,* but it is not a ground plan condition for any of these genera (Costa, 1996, 1998).

Urogenital papilla

169. Size and shape *of the urogenital papilla of adult males.* State 0: a small rounded protuberance enclosed by tegument folds; state 1: a prominent papilla; state 2: a short, cylindrical papilla; state 3: a long, cylindrical papilla (CI: 100; RI: 100). Like other aplocheiloids and most cyprinodontoids, in *Rivulus* (excluding R. *stellifer),* the urogenital papilla of males is not enlarged, not easily visible, in contrast to the very conspicuous papilla of other rivulids. It is somewhat elongate, on the other hand, reaching a cylindrical shape, in *Spectrolebias, Cynolebias, Simpsonichthys, Leptolebias* and *Cynopoecilus.* In *Campellolebias,* it is associated with the pseudogonopodium, being very elongate (Costa, 1990a. 1995c: fig. 1A). This character is not applicable to *Cynopoecilus* (character 171), which has an apomorphic pocket-like structure around the urogenital region, thus coded as "?" in the data matrix.

170. *Attachment of urogenital papilla with anal-fin in males.* State 0: free; state 1: attached to the anterior margin of anal-fin (CI: 100; RI: 100). As discussed by Costa (1995c: fig. 1A), in *Campellolebias* the urogenital papilla of males is completely attached to the anterior portion of the anal-fin (pseudogonopodium).

171. A pocket-like structure around urogenital region of males. State 0: absent; state 1: present (CI: 100; RI: 100). As discussed by Costa (1995d), this structure is unique for *Cynopoecilus.* It externally involves the whole urogenital region, remaining a small, posteriorly directed aperture, close to anal-fin origin.

172. Size and shape *of urogenital papilla of adult females.* State 0: a small pocket-like structure, not contacting anal-fin origin; state 1: a prominent pocket-like structure, slightly overlapping anterior portion of anal-fin (CI: 100; RI: 100). This apomorphic morphology of female urogenital papilla occurs in *Spectrolebias, Cynolebias, Simpsonichthys, Leptolebias, Campellolebias* and *Cynopoecilus.*

Swimbladder

173. *Position of posterior extent of swimbladder.* State 0: reaching a vertical through a region between pelvic-fin base and anterior portion of anal-fin; state 1: reaching a vertical approximately through middle of anal-fin base (CI: 100; RI: 100). As discussed by Costa & Nielsen (1997), the swimbladder *of Spectrolebias* is posteriorly elongate, an apomorphic condition not observed in other analyzed taxa.

Scales

174. Arrangement of frontal scales. State 0: scales arranged to form a transverse pattern, without a distinct central scale; state 1: scales arranged to form a circular pattern, with a distinct central scale (CI: 25; RI: 76). The derived, circular pattern of frontal scales arrangement occurring in most rivulids was noted by Hoedeman (1956) and Scheel (1969). Hoedeman (1961) proposed the separation of the Neotropical Rivulins *(*= Rivulidae) into two tribes based on the presence of that circular pattern in Rivulini and its absence in Cynolebiatini. However, Parenti (1981) argued that the circular pattern is shared by all members of the Rivulidae, considering it a synapomorphy for the family. Costa (1990a) revised this character, confirming the non occurrence of the circular pattern in some rivulid genera, as observed by Hoedeman (1961) and Scheel (1969). The circular pattern is confirmed for *Rivulus* (including *R. stellifer), Pterolebias, Trigonectes, Moema, Renova, Neofundulus, Rachovia, Pituna, Stenolebias,* and *Papiliolebias.* In *Austrofundulus* and *Millerichthys,* the arrangement of frontal scales is very irregular, thus coded as "?" in the data matrix.

175. *Arrangement of E-scales.* State **0:** overlapped; state 1: non overlapped, separated by a third, median scale (CI: 100; RI: 100). Cyprinodontiforms typically have two overlapped frontal scales situated in a traversal line through the posterior border of eye (E-scales). Costa (1990a) noted an apomorphic non overlapped pattern for E-scales in an assemblage including *Leptolebias, Campellolebias* and *Cynopoecilus,* confirmed in the present study. Taxa with a circular pattern of frontal scales (character 174), are coded as "?".

176. Squamation between eye and supraorbital series of neuromasts. State 0: scaled; state 1: one or three small scales or naked (CI: 100; RI: 100). Plesiomorphically, cyprinodontiforms have a scaled region between Supraorbital neuromasts and orbit. An apomorphically reduced supraorbital squamation occurs in *Spectrolebias, Cynolebias, Simpsonichthys, Leptolebias, Campellolebias* and *Cynopoecilus.*

177. An anterior frontal scale with all borders free. State **0:** absent; state **1: present** (CI: **50; RI:** 0). As discussed by Costa (1998), the members of the subgenus *Plesiolebias* have a derived frontal squamation pattern, in which an anterior scale has all borders free. This also occurs in the *Cynolebias bellottii* group. Typically among aplocheiloids, all free borders scales are situated closer to a transversal line through eye.

178. Increase of caudal-fin squamation in older males. State 0: usually restricted to the basal portion of fin, not reaching a vertical through the center of the fin; state 1: expanded, squamation over about anterior three quarters of the fin (CI: 50; RI: 0). Myers (1932) first described this apomorphic condition for *Austrofundulus,* also noted by Scheel (1969). Costa (1990a) reported this condition as an apomorphy for *Austrofundulus, Rachovia* and *Neofundulus.* However, it is confirmed only for *Austrofundulus* and R. *stellifer,* in which small scales are distributed over a great portion of the caudal fin. In *Rachovia brevis,* the caudal squamation is also expanded, and therefore the character is coded as P for *Rachovia.* Those taxa lacking scales on the caudal fin (character 179) are coded as "?" in the data matrix.

179. Kind of caudal-fin squamation. State 0: body squamation extending on caudal fin through minute, vertically elongate scales; state 1: body squamation abruptly interrupted on caudal-fin base, without minute, vertically elongate scales (CI: 100; RI: 100). This character substitutes the character state "absence of caudal-fin scales" reported by Parenti (1981) (already noted by Taphorn & Thomerson, 1978), and expressed as length of caudal-fin squamation as percentages of caudal-fin length in Scheel (1969) and Costa (1990a), herein considered imprecise and ambiguous. The apomorphic condition occurs in *Spectrolebias, Simpsonichthys, Cynolebias, Leptolebias, Campellolebias,* and *Cynopoecilus.*

Neuromasts

180. Kind of association between anterior and posterior portions of supraorbital neuromasts series. State 0: separated by a space into two distinct series; state 1: united, forming a single row (CI: 100; RI: 100). As discussed by Costa (1990a), this apomorphic pattern of arrangement of neuromasts uniquely occurs in some rivulids. It is confirmed for *Spectrolebias, Cynolebias, Simpsonichthys, Leptolebias, Campellolebias* and *Cynopoecilus.*

181. Arrangement of neuromasts of the supraorbital posterior series. State 0: arranged in a short L-shaped line; state 1: arranged in a long, straight to C-shaped line (CI: 25; RI: 80). The plesiomorphic condition for the supraorbital sensory canal is a short posterior canal (Gosline, 1949). Both, in taxa with a closed and an open canal, the three neuromasts of that series are arranged in an L-shaped line. As discussed by Costa (1995b), an apomorphic arrangement of supraorbital neuromasts is found in some rivulid taxa, in which neuromasts are arranged in a slightly curved line. It is confirmed for *Millerichthys, Plesiolebias, Maratecoara, Stenolebias, Spectrolebias, Cynolebias, Simpsonichthys, Leptolebias, Cynopoecilus* and *Campellolebias.*

182. Increase in number of supraorbital neuromasts. State **0:** usually **6 to 10** neuromasts; state **1:** usually 12 to about 25 (CI: 100; RI: 100). Parenti (1981) noted an elaborate supraorbital neuromast pattern for *Cynolebias.* As discussed by Costa (1990a), *Cynolebias, Simpsonichthys* and *Spectrolebias* present an apomorphic increase in number of Supraorbital neuromasts. The reduced number of supraorbital neuromasts of other rivulid taxa is similar to the number of pores found in the supraorbital canal of other cyprinodontiforms, thus considered plesiomorphic.

183. Size of neuromast on dermosphenotic. State 0: large; state 1: small (CI: 100; RI: 100). Although always having an open sensory canal on dermosphenotic -a bone always reduced, when present (character 92)- most rivulids have a large neuromast on that bone. Costa *et al.* (1988, fig. 8) first noted the large dermosphenotic neuromast in *Cynolebias xavantei* (now in *Plesiolebias),* distinct from the minute neuromast of other species of *Cynolebias.* Costa (1990) reported this reduction as synapomorphic for a rivulid assemblage, confirmed for a group comprising *Spectrolebias, Cynolebias, Simpsonichthys, Leptolebias, Campellolebias* and *Cynopoecilus.*

184. Number of neuromasts on caudal-fin base. State 0: one or two; state 1: five to seven (CI: 100; RI: 100). As discussed by Costa (1995c). the genera *Leptolebias, Campellolebias* and *Cynopoecilus* present an apomorphic increase in the number of caudal neuromasts.

Muscles of anal-fin base

185. Muscular fibers transversely arranged in front of anal-fin support and above urogenital papilla base, probably constituting an ejaculatory pump. State 0: absent; state 1: present (CI: 100; RI: 100). As discussed by Costa (1995c: fig. 1C), the genera *Cynopoecilus* and *Campellolebias* are unique among aplocheiloids by this apomorphic muscle morphology, which is probably associated with a mechanism for internal fertilization.

186. Shape of inclinatores anales *1-3.* State 0: narrow; state 1: expanded laterally, forming a fan-shaped structure (CI: 100; RI: 100). This apomorphic morphology of the *inclinatores anales* is unique for *Campellolebias* among aplocheiloids, as discussed by Costa (1995c: fig. 1C).

Color patterns

187. Translucent body, vertebrae and swimbladder visible through body wall in life. State **0:** absent; state 1: present (CI: 100; RI: 100). This autopomorphic condition of *Spectrolebias* was discussed by Costa & Nielsen (1997). This reduction of pigment is typical of miniature species, as discussed by Wetzman & Vari (1987).

188. Longitudinal rows of dark spots (dark reddish brown in males, dark brown in females). State 0: rows absent; state 1: three longitudinal rows from the anterior portion of body sides to caudal-fin base, alternated with shorter rows of similar spots restricted to the anterior portion of the body; state 2: five to seven longitudinal rows of dark spots from the anterior portion of body sides to caudal-fin base, without intermediate shorter rows of spots (CI: 28; RI: 37). The apomorphic color pattern of state 1 was briefly described by Parenti (1981), suggesting it has arisen three times independently, thus being homoplastic for *Trigonectes* and two species of *Neofundulus,* which was considered a paraphyletic genus. However, Costa (1988a) found evidences supporting the monophyly of *Neofundulus,* and that derived color pattern was considered synapomorphic *for Neofundulus* plus *Trigonectes.* Subsequently, other taxa sharing this pattern were described as close relatives to *Neofundulus* and *Trigonectes* (Costa, 1989a; Thomerson & Taphorn, 1995). This apomorphic pattern is now confirmed for *Neofundulus, Trigonectes, Moema, Renova, Pterolebias phasianus,* and *P. xiphophorus* (see figures in Costa, 1995e). Another similar apomorphic color pattern (state 2) occurs *in Rivulus urophthalmus, R. amphoreus, R. bahianus, R. cryptocallus,* and *R. hartii.* The two patterns are distinguished by state 1 being comprised of three main, complete series, alternated by shorter, truncate series, usually with distinct, lighter coloration, whereas in the state 2 all the rows (five to seven) are similar in length and coloration (see figs. in Huber, 1992).

189. A longitudinal red stripe on the anterodorsal portion of body of males. State 0: absent; state 1: present (CI: 100; RI: 100). As discussed and illustrated by Costa (1991b; 1995a; 1998). this apomorphic color pattern is unique for the subgenus *Plesiolebias.*

190. A broad longitudinal black stripe from the tip of lower jaw to the caudal-fin base. State 0: absent; state 1: present (CI: 100; RI: 100). As discussed by Costa (1995d), this apomorphic color pattern is unique for *Cynopoecilus* (Costa, 1995b: fig. 122). Regan (1912) first diagnosed *Cynopoecilus,* distinguishing it from *Cynolebias,* by males and females *of Cynopoecilus* possessing similar color patterns. This was followed by Costa (1990a), who listed this condition as autapomorphic. However, under examination of live specimens, it is possible to prove that males have a color pattern more elaborate than females, constituting the plesiomorphic condition for aplocheiloids. However, in preserved material, the notorious longitudinal zone of melanophores in both sexes causes the aspect of similar color patterns.

191. An oblique longitudinal black stripe from the pectoral-fin base to the posterior part of anal-fin base. State 0: absent; state 1: present (CI: 100; RI: 100). As discussed by Costa (1995d), this apomorphic color pattern is unique for *Cynopoecilus* (Costa, 1995b: fig. 122).

192. *Three longitudinal rows of orange spots on the anterodorsal portion of the body of males.* State 0: absent; state 1: present (CI: 100; RI: 100). Costa (1995d) has shown that this apomorphic color pattern is unique for *Maratecoara* (Costa, 1995: fig. 19).

193. One to three black blotches, approximately on the central portion of body sides of juveniles, usually kept in adult females and lost in adult males. State 0: absent; state 1: present (CI: 100; RI: 100). Described in many papers *(e.g.,* Costa, 1995b) and considered apomorphic by Costa (1990a), this color pattern is unique for *Cynolebias* and *Simpsonichthys.*

194. Dark pigmentation on the body sides and fins of females. State 0: present; state 1: reduced to minute dots of dark pigmentation; state 2: absent (CI: 50; RI: 71). Described as diagnostic by Myers (1952) and pointed out as apomorphic by Costa (1990a), *Leptolebias* is unique by the lack of dark marks on body and fins of females (state 2). When compared with most rivulids assemblages and outgroups, reduced dark pigmentation is also found in females of *Plesiolebias., Maratecoara, Rachovia. Austrofundulus, Terranatos* and *Millerichthys.* However, in a detailed examination of well-preserved specimens of these groups, it is possible to find minute patches of dark chromatophores forming dark dots (state 1), contrasting with the unpigmented derived condition *of Leptolebias* females.

195. *Oblique raws of dark dots on the body sides of females.* State 0: absent; state 1: present (CI: 100; RI: 100). As discussed by Costa (1998), the subgenus *Plesiolebias* is unique among aplocheiloids by having oblique rows of dark brown dots on the body sides of females.

196. Humeral spot. State 0: absent; state 1: a large, metallic blue or green blotch in males; state 2: a dark brown to black spot; state 3: metallic orange spots (CI: 33; RI: 40). The absence of a humeral spot in closer outgroups to rivulids suggests that the presence of this spot is apomorphic for some rivulid taxa. However, these spots may have very distinct coloration, probably constituting non-homologous conditions. These different color spots are treated as different states. State 1 is present in *Pituna, Papiliolebias, Rivulus cylindraceus,* group R. *geayi, Trigonectes,* and *Neofundulus;* state 2 in R. *ocellatus, R. caudomarginatus, R. brasiliensis, R. stellifer* and *group P. peruensis;* and state 3 *in Pterolebias longipinnis* and P. *phasianus.*

197. Alternated dark gray and light yellow spots on dorsum. State 0: absent; state 1: present (CI: 20; RI: 20). This apomorphic condition, illustrated in several papers *(e.g.,* Huber, 1992), is found in *R. urophthalmus, R. cryptocallus,* the group R. *janeiroensis, R. bahianus, R. obscurus,* and R. *atratus.*

***198.** Dark pigmentation concentrated on ventrum.* State **0:** absent; state **1:** present (CI: **100; RI:** 100). *Rivulus atratus* has an autapomorphic dark ventrum, unique among aplocheiloids and outgroups.

199. *Vertical bars on the body sides of juveniles, sometimes also present in adults.* State **0:** absent; state 1: present (CI: 50; RI: 85). Conspicuous vertical bars are present in juveniles of *Leptolebias, Spectrolebias, Cynolebias* and *Simpsonichthys,* a condition not occurring in other rivulids or outgroups.

200. Oblique pattern of stripes or rows of spots or dots on the body sides. State 0: absent; state 1: present. In some taxa of the Rivulidae, there is a clear oblique arrangement of color marks on body sides, a condition not recorded in outgroups (CI: 25; RI: 72). This pattern occurs in *Plesiolebias, Pituna, Maratecoara, Stenolebias, Terranatos, Rachovia, Austrofundulus, R. stellifer, P. longipinnis,* and in the *Rivulus punctatus, R. geayi, R. janeiroensis,* and *P. peruensis* groups.

201. Melanophores concentrated on perianal papilla of males. State 0: absent to scarcely concentrated; state 1: densely concentrated (CI: 100; RI: 100). This pigmentation pattern was described as diagnostic for *Austrofundulus* by Taphorn & Thomerson (1978), subsequently considered apomorphic by Parenti (1981), and confirmed in the present study.

202. A metallic purplish blue stripe along ventral portion of body sides of males. State 0: absent; state 1: present (CI: 100; RI: 100). This color pattern occurs only in R. *xiphidius* (Huber, 1992),

203. Color of iris of males. State 0: yellow to pale orange, rarely with green shine; state 1: intense metallic greenish blue (CI: 50; RI: 0). This apomorphic color of iris occurs in *Campellolebias* and *Leptolebias.*

204. A transverse dark bar crossing the eye. State 0: absent; state 1: present (CI: 33; RI: 88). Parenti (1981) reported a vertical bar through eye as a synapomorphy for a group including *Rachovia, Austrofundulus, Cynolebias* and *Neofundulus.* This condition, however, was observed to occur in all the Rivulidae minus *Rivulus,* but was present in R. *stellifer.*

205. A transverse dark bar through posterior portion of iris. State 0: absent; state 1: present (CI: 100; RI: 100). Costa & Nielsen (1997) have shown that this condition is unique for *Spectrolebias.*

206. A transverse suborbital bar. State **0:** absent; state 1: black, long, slightly directed anteriorly; state 2: dark red, long, anteriorly directed; state 3: black, long, slightly directed posteriorly; state 4: brown, short, anteriorly directed (CI: 57; RI: 75). In contrast to aplocheilids and most other cyprinodontiforms, a suborbital bar is present in some rivulid taxa. However, distinct position and coloration suggest non-homologous conditions. Thus, each pattern is analyzed as distinct, not ordered character states. In *R. stellifer, Rachovia, Austrofundulus, Pituna, Plesiolebias, Maratecoara, Stenolebias,* it is black and slightly directed anteriorly, usually preceded by a light zone (state 1). In *Leptolebias* and in the *Simpsonichthys whitei* group, it is dark red and strongly directed anteriorly (state 2). In *Terranatos,* and in the *Cynolebias bellottii* and C. *wolterstorffi* groups, the bar is also black, but ventrally to slightly directed posteriorly (state 3). In *Moema, Renova,* and in *Pterolebias xiphophorus,* it is brown, very short, and weakly directed anteriorly (state 4).

207. A transverse supraorbital bar. State **0:** absent; state 1: a dark brown to black bar; state 2: an orange bar (CI: 60; RI: 33). A supraorbital dark bar occurs in *Terranatos, Rachovia, Austrofundulus,* and in the *Cynolebias bellottii* and C. *wolterstorffi* groups, whereas in *Maratecoara* there is an orange bar. Plesiomorphically, cyprinodontiforms lack a supraorbital bar.

208. Color of the branchiostegal membrane of males. State 0: not distinctively colored; state 1: metallic blue (CI: 100; RI: 100). As discussed by Costa (1995a), *Maratecoara* is unique among aplocheiloids and outgroups by having a metallic blue branchiostegal membrane. This condition is very conspicuous during aggressive and courtship behaviors.

209. Orange pigmentation on the anteroventral region of the head of males. State 0: absent; state 1: present (CI: 100; RI: 100). As discussed by Thomerson and Taphorn (1992a: figs. 17 and 22; 1992b: fig. 2), *Rachovia* males have an apomorphic color pattern of the chin.

210. Three dark longitudinal stripes on the ventral part of head, the median stripe from lower jaw to anus, the lateral stripes from lower jaw to a vertical through upper section of preopercular neuromast series. State **0:** absent; state 1: present (CI: **100;** RI: 100). This color pattern was first described by Vaz-Ferreira & Sierra (1974: fig. 4A) and later reported as synapomorphic for *Campellolebias* by Costa (1990a; 1995c).

211. Ventral portion of opercle intense blue. State 0: absent; state 1: present (CI: 50; RI: 50). This apomorphic color of the opercle occurs in some species of *Rivulus: R. brunneus, R. tenuis,* and *R. obscurus* (see illustrations in Huber, 1992).

212. Two oblique reddish brown stripes on the preopercular region of males. State 0: absent; state 1: present (CI: 50; RI: 0). This derived color pattern occurs only in *Moema* and *Pterolebias xiphophorus* (see Thomerson & Taphorn (1992: **fig.** 1).

213. A black stripe from snout to opercular region, sometimes reaching anterior portion of body sides. State 0: absent; state 1: present (CI: 50; RI: 66). This apomorphic color pattern (Costa, 1995f), resembling a black mask, occurs in *R. xiphidius, R. cylindraceus,* and in *the R. punctatus* and *R. geayi* groups (see illustrations in Huber, 1992).

214. *Dark brown to black blotches on the dorsoposterior portion of the head.* State **0:** absent; state 1: present (CI: 100; RI: 100). This apomorphic color pattern (Costa *et al.*, 1990: fig. 9; Costa & Brasil, 1991: figs. 1 and 3), as discussed by Costa (1995b), occurs in *Cynolebias griseus* and in the C. *porosus* group.

215. *Bright red pigmentation on the anterior margin of the dorsal fin of larger males.* State 0: absent; state 1: present (CI: 50; RI: 50). This apomorphic color pattern was observed in *Maratecoara, Stenolebias,* and *Pituna.*

216. *A subbasal, longitudinal white to yellow stripe on the anal-fin of males.* State **0:** absent; state 1: present (CI: 100; RI: 100). As discussed by Costa (1988a), this apomorphic color pattern is unique for *Neofundulus.*

217. *Three transverse yellow stripes on the anal-fin of males.* State 0: absent; state 1: present (CI: 100; RI: 100). This apomorphic condition, described for *Millerichthys* by Miller & Hubbs (1974), is not found in other rivulids.

218. *Alternate black and white spots on the basal and posterior part of the anal-fin of males.* State **0:** absent; state 1: present (CI: 25; RI: 57). As discussed and illustrated by Costa (1998), this apomorphic color pattern occurs in *Pituna, Stenolebias* and *Plesiolebias. Rivulus brasiliensis, Rachovia, Austrofundulus,* and *R. stellifer* also possess a similar pattern.

219. *A black spot, anteriorly bordered by a blue line, on the posterior portion of the distal margin of the anal-fin of males.* State 0: absent; state 1: present (CI: 100; RI: 100). Costa & Nielsen (1997) have shown that this condition of *Spectrolebias* is unique among aplocheiloids.

220. *A bicolor longitudinal stripe on the distal margin of anal-fin of males.* State **0:** absent; state **1:** light blue **to** green above, dark red below; 2: white above, black below (CI: 100; RI: 100). The presence of a bicolor stripe on the anal-fin of males was discussed by Costa (1991b). Due to the differences in the pigmentation patterns found, they are coded as distinct character states. State 1 is apomorphic for *Pituna,* while state 2 is apomorphic for R. *stellifer.*

221. *An orange stripe (sometimes pink or red) along lower margin of the caudal fin of males.* State **0:** absent; state 1: present (CI: 50; RI: 83). This apomorphic condition is found in *Trigonectes, Moema, Renova, Neofundulus, Pterolebias xiphophorus* and in the *P. peruensis* group. Only part of the species of *Rachovia* present a similar condition and the character is coded as P in the data matrix.

222. *A black spot on the upper portion of the caudal-fin base of females.* State 0: absent; state 1: a black spot preceded by a white area; state 2: an ocellate spot (CI: 33; RI: 66). Parenti (1981) considered the presence of an ocellate spot as plesiomorphic for Rivulidae, based on the supposed occurrence of this color pattern in aplocheilids. However, as discussed by Costa (1990a), this condition is not present in any member of the Aplocheilidae, thus constituting an apomorphy for rivulids. Costa (1990a) considered *Rivulus* as a monophyletic lineage, pointing out this spot as diagnostic for the genus. However, a similar spot has been subsequently reported for non *Rivulus* taxa (Costa, 1995b; Thomerson and Taphorn, 1995). In the present study, however, two distinct spot patterns are established. A true ocellate caudal spot is confirmed only for *Rivulus caudomarginatus, R. ocellatus,* and R. *cylindraceus. Renova* and all other *Rivulus* analyzed (including R. *stellifer)* except for R. *brasiliensis,* the *R. geayi* group, *R. atratus, R. obscurus* and R. *xiphidius* present a black spot that is only in part edged by a light area. The female caudal spots of *Millerichthys* (a series of black spots along caudal-fin base) are considered a distinct character (223).

223. *Small black spots along caudal-fin base of females.* State 0: absent; state 1: present (CI: 100; RI: 100). Only females of *Millerichthys* possess the apomorphic condition of this character.

224. *An ocellate black spot on the upper portion of caudal-fin base of males.* State **0:** absent; state **1:** present (CI: 100; RI: 100). This ocellate spot, common in *Rivulus* females, is also found in males of some species of *Rivulus.* In the species herein analyzed, it is present in R. *caudomarginatus* and *R. ocellatus,* as noted by Huber (1992). However, Soto & Noakes (1994) reported the absence of this spot in males of R. *marmoratus,* a species commonly regarded as synonym of R. *ocellatus* by recent authors *(e.g.,* Seegers, 1984). However, in a photo published by Soto & Noakes (1994: fig. 1b), it is possible to see a faint caudal spot in a R. *marmoratus* male.

225. *A narrow blue margin on the posterior border of caudal fin of males.* State **0:** absent; state **1:** present (CI: 100; RI: 100). As discussed by Costa (1996), this apomorphic color pattern is unique for *Simpsonichthys.*

226. Transverse black bars on the caudal fin of females. State 0: absent; state 1: present (CI: 50; RI: 0). As discussed by Costa (1988a. 1990a, 1995f), this apomorphic color pattern of female caudal fin occurs in *Neofundulus* and in the group *Rivulus punctatus*.

227. Yellow stripe on the upper and lower edges of the caudal fin of males. State **0:** absent; state **1:** present; state 2: present, with a reddish brown medial border (CI: 66; RI: 80). A simple yellow stripe on the dorsal border, and another on the ventral border of caudal fin of males are present in R. *urophthalmus, R. hartii,* the group *R. janeiroensis, R. bahianus,* and R. *cryptocallus.* Similar stripes, but with dark reddish brown margins occur in *R. tenuis* and *R. brunneus* (see illustrations in Huber, 1992).

228. A black border, medially edged with a narrow white zone on the caudal fin of males. State **0:** absent; state 1: present (CI: 100; RI: 100). Among aplocheiloids, this pattern is unique for *R. caudomarginatus* (Seegers, 1984).

229. *A horizontal reddish brown stripe close to the dorsal border of caudal fin of males.* State 0: absent; state 1: present (CI: 100; RI: 100). This apomorphic color pattern is uniquely found in *the group Pterolebias zonatus* (Thomerson, 1974: figs. 1 and 5).

230. Black spots scattered over pectoral fin of males. State 0: absent; state 1: present (CI: 33; RI: 0). Costa (1988a, 1989a. 1990a) noted this derived color pattern in some rivulid taxa. This apomorphic condition is confirmed to be present in *R. atratus, Pituna* and *Neofundulus.*

231. Black vertical bars on the pectoral fin of males. State 0: absent; state 1: present (CI: 100; RI: 100). Among aplocheiloids and outgroups, black bars on pectoral fin occur only in *Pterolebias longipinnis* and *P. phasianus* (Costa, 1988b: fig 1).

232. A narrow black margin on the ventral border of the pectoral fin of males. State 0: absent; state 1: present (CI: 50; RI: 0). This apomorphic condition is present in the *Pterolebias zonatus* and *P. peruensis* groups (Costa *et al.*, 1996: fig. 1).

233. White to light green border on the lower edge of the pectoral fin of males. State 0: absent; state 1: present (CI: 100; RI: 100). This apomorphy is found only in the *Pterolebias zonatus* group (Costa, 1995e: fig. 1).

234. Kind of chorion surface. State **0:** plain **to** verrucate; state 1: reticulate (CI: 100; RI: 100). This apomorphic morphology of the chorion surface, described by Wourms (1976: figs. 2 and 3), was suggested as apomorphic by Costa (1990a). This is confirmed for *Cynopoecilus, Campellolebias* and *Leptolebias.*

235. Shape of chorion projections. State 0: spined to hair-like; state 1: mushroom-like (CI: 100; RI: 100). Discussed and illustrated by Wourms (1976: figs. 1, 2, 4 and 5) and Wourms & Sheldon (1976: fig. 7), it was pointed as a synapomorphy for a rivulid clade, by Costa (1990a). This is confirmed for *Cynopoecilus, Campellolebias* and *Leptolebias.*

Reproductive behavior

236. Kind of fertilization. State 0: external; state 1: internal (CI: 100; RI: 100). As discussed by Parenti (1981) and Costa (1995c), among aplocheiloids only *Campellolebias* and *Cynopoecilus* have internal fertilization.

237. *Site of spawning.* State 0: On plants or on the bottom; state 1: within substrate; state 2: at midwater, not contacting a substrate (CI: 40; RI: 82). The plesiomorphic condition for aplocheiloids is the spawning occurring on plants or on the bottom, in shallow places, as in most cyprinodontoid groups (Breder & Rosen, 1966). In *Neofundulus, Pterolebias, Trigonectes, Renova, Moema, Cynolebias* and *Simpsonichthys,* the spawning occurs within the substrate *(e.g.,* Carvalho, 1957). On the other hand, in *Plesiolebias, Cynopoecilus* and *Campellolebias (e.g.,* Breitfeld, 1988; Costa *et al.,* 1988; Sommer, 1988; Costa, 1990a), the spawning is not in contact with a substrate, but at the midwater This behavior is not known for *Millerichthys* and *Spectrolebias,* thus coded as "?" in the data matrix.

238. Position of fins during male courtship behavior. State 0: unpaired fins expanded, lateral movements of body predominating; state 1: unpaired fins contracted, dorsal and anal-fins twisted, vertical and lateral movements of body occurring simultaneously (CI: 100; RI: 100). This apomorphic courtship behavior pattern, described and illustrated by Costa *et al.* (1989: fig. 19), was considered by Costa (1990a) synapomorphic for a clade comprising *Leptolebias, Campellolebias* and *Cynopoecilus.* This is corroborated here, but a similar pattern also occurs in *S. myersi* (Carvalho), so the character coded as P for the group *S. whitei.* In S. *boitonei,* the male also presents the apomorphic display for courtship, but it is not the ground plan for the *S. constanciae* group.

239. A zigzag display during male courtship behavior. State 0: absent; state 1: present (CI: 100; RI: 100). As described by Vaz-Ferreira *et al.* (1985) and confirmed in the present study, *Cynopoecilus* has this unique courtship behavior.

240. A coiled retrorse motion during male courtship behavior. State 0: absent; state 1: present (CI: 100; RI: 100). This behavioral pattern is unique for *Campellolebias.*

241. Hermaphroditism with internal self-fertilization. State 0: absent; state 1: present (CI: 100; RI: 100). As reported by Harrington (1961) for *R. marmoratus,* this apomorphic reproductive pattern, unique among vertebrates, is found only in the group *R. ocellatus.*

Development and habitat

242. Kind of development and seasonally of habitats. State 0: non annual development, without diapause stages, life cycle in nonseasonal habitats; state 1: annual development, with clear diapause stages, life-cycle in temporary pools (CI: 50; RI: 94). Annual life style has been recorded since first half of the 20th century by German aquarists and taxonomists (Ahl, 1922), but annual development was first described in detail by Wourms (1972a,b,c). It occurs in many rivulid taxa and in the aplocheilid clade comprising *Fundolopanchax* and *Nothobranchius (e.g.,* Scheel, 1968), considered independently derived by Parenti (1981) and Costa (1990a). However, Parenti (1981) hypothesized an annual development for some *Rivulus (i.e.,* species with a cartilaginous interhyal). Non annual development or life cycles with nonseasonal habitats is confirmed for *Rivulus,* except for R. *stellifer.* The apomorphic condition is confirmed for *R. stellifer* and all other rivulid taxa except for *Rivulus.* In addition, field data suggest that *R. nicoi* Thomerson & Taphorn, a species not available for the present study, may be an annual (Thomerson & Taphorn, 1992a). However, the absence of osteological characters in the original description makes doubtful the inclusion of this species in *Rivulus.* No data about this life style is available *for Millerichthys,* thus coded as "?" in the data matrix.

243. Kind of habitat. State 0: freshwater; state 1: marine (CI: 100; RI: 100). Non cyprinodontiforms atherinomorphs are typically marine. Among cyprinodontoids, basal taxa of the major clades *(e.g.,* fundulids, cyprinodontids, poeciliids and anablepids) (Costa, in press) are often saltwater resistant, found in brackish coastal habitats. In contrast, aplocheilids are typically freshwater fishes. Among rivulids, only *R caudomarginatus* and the group *R. ocellatus* live in saltwater habitats *(e.g.,* Seegers. 1984).

Phylogeny and Classification

The analysis of the data matrix including the 243 characters listed and discussed above (Appendix 2), through the combination of the heuristic algorithms mh*; bb*; (Farris, 1988), yielded 16 equally most parsimonious phylogenetic trees (length 430, consistency index 0.64, retention index 0.82, rescaled consistency index 0.52). The consensus tree is presented in Figure 1. The cladograms of Figures 2-6 are the basis for the classification below. Character numbers in diagnoses are according to those presented in the character analysis.

Family Rivulidae Myers

(Fig. 2)

Diagnosis. A short snout (1.1), a straight posterior border of the distal tip of premaxilla (2.1), a triangular ventral process of maxilla (5.1), an elongate rostral cartilage (11.1), an absent mandibular canal (12.1), a reduced coronoid process of dentary (13.1), a short ventral portion of autopalatine (23.1), a reduced mesopterygoid (34.1), a thin, C-shaped preopercle (45.1), a reduced basihyal cartilage (56.1), a cartilaginous interhyal (58.1), a reduced uncinate process of the third epibranchial (62.1), a well-developed process on the anterior border of lateral ethmoid (81.1), a reduced lateral border of frontal (84.1), an extremely twisted and narrowed lacrimal (89.1), a minute dermosphenotic (92.1), a neural spine on the first vertebra (99.1), hypurals ankylosed to form a single hypural plate (103.3); absence of a posterior flange on cleithrum (135.1), absence of the first postcleithrum (144.1), united branchiostegal and opercular membranes (164.1), a transverse dark bar crossing the eye (204.1), and annual development (242.1).

Included taxa. Twenty-seven genera as described below.

Subfamily Cynolebiatinae Hoedeman, new usage

Diagnosis. A dorsally positioned dorso-medial process of autopalatine (28.1), a very reduced mesopterygoid (34.3), a thin, C-shaped opercle with reduced dorsal tip, median rim and sensory canal (45.3), elongate epibranchials and interarcual cartilage (60.1), a rounded second hypobranchial (71.1), absence of fourth ceratobranchial teeth (72.1), absence of a postero-ventral process on the anterior portion of fourth ceratobranchial (73.1), absence of vomerine teeth (76.1), a thin lacrimal, with narrow lower portion and vestigial sensory canal (89.2), absence of dermosphenotic (93.1), absence of anterior processes of first vertebra (94.1), a vestigial or absent neural prezygapophysis of caudal vertebrae (100.2), a narrow and anteriorly curved epural and parahypural (105.1), an anteriorly placed dorsal-fin origin (111.2), a reduced first proximal anal radial (117.1), a reduced ossification of medial anal radials (119.1), a vestigial or absent ventral process of posttemporal (132.1), an elongate supracleithrum (134.1), scale-like pectoral radials (145.1), a cylindrical urogenital papilla in males (169.2), a prominent pocket-like urogenital papilla in females (172.1), a reduced supraorbital squamation (176.1), a reduced caudal-fin squamation (179.1), a continuous supraorbital series of neuromasts (180.1), along posterior series of supraorbital neuromasts (181.1), a minute neuromast on dermosphenotic (183.1), and vertical bars on body sides of juveniles (199.1).

Included taxa. The genera *Leptolebias, Cynopoecilus, Campellolebias, Spectrolebias, Simpsonichthys, Nematolebias, Austrolebias, Megalebias,* and *Cynolebias.*

Supertribe Cynopoecilidi Costa, new usage

Diagnosis. A vestigial or absent ventral process of angulo-articular (16.3), non overlapped E-scales (175.1), numerous (5-7) neuromasts on caudal-fin base (184.1), an intense metallic greenish blue iris in males (203.1), a reticulate chorion surface (234.1), mushroom-like chorion projections (235.1), and unpaired fins contracted during male courtship behavior (238.1).

Included taxa. The genera *Leptolebias, Cynopoecilus, Campellolebias.*

Tribe Leptolebiatini, new name

Type genus. *Leptolebias* Myers.

Diagnosis. A broad maxilla (7.1), a widened posterior process of quadrate (42.1), a reduced dermosphenotic (93.0, a reversal), narrowed scapula and coracoid (141.1), absence of dark pigmentation on body sides and fins of females (194.1), and a dark red, long, anteriorly directed suborbital bar (206.3).

Included taxa. Only the type genus, *Leptolebias.*

Genus *Leptolebias* Myers

Leptolebias Myers, 1952:140 (type species *Cynopoecilus marmoratus* Ladiges, by original designation; proposed as a subgenus of *Cynolebias).*

Diagnosis. Same as for the tribe.

Included taxa. Nine species: *L. marmoratus* (Ladiges), *L. sandrii* (Faria & Muller), *L. fluminensis* (Faria & Muller), *L. citrinipinnis* (Costa, Lacerda & Tanizaki), *L. minimus* (Myers), *L. fractifasciatus* (Costa), *L. cruzi* (Costa), *L. aureoguttatus* (Cruz), and *L. leitaoi* (Cruz & Peixoto). A taxonomic revision of the genus by the author is in progress.

Distribution. Endemic to the coastal plains of eastern Brazil, between Mucuri river basin (Bahia) and Paranagua (Parana).

Tribe Cynopoecilini Costa, new usage

Diagnosis. An elongate posterior process of vomer (78.1), narrow lateral wings of vomer (79.1), rod-shaped proximal anal radials (114.1), an elongate first proximal anal radial (117.0, a reversal), an elongate and pointed anal-fin of males (155.1), a putative ejaculatory pump (185.1), absence of vertical bars in juveniles (199.0, a reversal), internal fertilization (236.1), and a midwater spawning (237.1).

Included taxa. The genera *Cynopoecilus* and *Campellolebias.*

Genus *Cynopoecilus* **Regan**

Cynopoecilus Regan, 1912:642 (type species *Cynolebias melanotaenia* Regan, by original designation).

Diagnosis. A concavity on the median portion of the posterior border of autopalatine (27.1), minute caudal accessory cartilages (107.1), eight anal-fin-rays attached to the first proximal radial (115.1), anteriorly curved sixth and seventh anal-fin-rays of males (124.1), posteriorly curved 10th to 12th anal-fin-rays of males (125.1), numerous total anal-fin-rays (23-26) (128.1), five minute rays at the anterior portion of anal-fin (131.1), a minute pelvic fin (152.1), a pocket-like structure around urogenital region of males (171.1), a broad longitudinal black stripe from tip of lower jaw to caudal-fin base (190.1), an oblique longitudinal black stripe from pectoral-fin base to posterior part of anal-fin base (191.1), a yellow iris in males (203.0, a reversal), and a zigzag display during male courtship behavior (239.1).

Included taxa. Two species, C. *melanotaenia* (Regan) and an undescribed species from the upper Jacuí river basin, southern Brazil.

Distribution. It occurs along coastal plains of southern Brazil and Uruguay, and in the rio Jacuí basin, southern Brazil.

Genus *Campellolebias* Vaz-Ferreira & Sierra

Campellolebias Vaz-Ferreira & Sierra, 1974:1 (type species *Campellolebias brucei* Vaz-Ferreira & Sierra, by original designation).

Diagnosis. Paired anteroventral projection on vomer (80.1), a distinctive process on the posterobasal portion of the last neural spine (102.1), a persistent median fissure on hypural plate (103.1), dorsal-fin origin anteriorly positioned, second dorsal proximal radial between neural spines of vertebrae 9 and 10 (111.3), a posteriorly curved first proximal anal radial (116.1), a long first proximal anal radial (117.0, a reversal), strong ligamentous connections between first anal-fin ray and first and second proximal radials simultaneously (118.1), large and ossified first two anal distal radials (123.1), thickened first two anal-fin-rays of males (130.1), a tubular, laterally compressed copulatory organ in the anterior portion of the anal-fin of males, formed by the first two anal-fin-rays, which are separated from the remaining portion of the fin by a rupture in the fin membrane (156.1), a cylindrical and elongate urogenital papilla of males (169.3), urogenital papilla attached to anterior margin of anal-fin in males (170.1), laterally expanded *inclinatores anales* 1-3 (186.1), three dark longitudinal stripes on the ventral part of head (210.1), and a coiled retrorse motion during male courtship behavior (240.1).

Included taxa. Three species have been recognized in the last taxonomical studies of the group (Costa *et al.*, 1989; Costa, 1995c): C. *brucei* Vaz-Ferreira & Sierra, C. *dorsimaculatus* Costa, Lacerda & Brasil and C. *chrysolineatus* Costa, Lacerda & Brasil.

Distribution. Endemic to the southeastern Brazilian coastal plains, between the Ribeira river (São Paulo) and southern Santa Catarina.

Supertribe Cynolebiatidi Hoedeman, new usage

Diagnosis. A broad postero-ventral process of dentary (14.1), a concave anterodorsal margin of opercle (47.1), an anterior displacement of the anal-fin origin of males (113.1), elongate filamentous rays on the dorsal and anal-fins of males (126.1), sexual dimorphism in number of dorsal and anal-fin-rays (127.1), numerous anal-fin-rays in males (19-32) (128.1), an elongate dorsal portion of cleithrum (136.1), absence of a postero-ventral flange of cleithrum (138.1), a ventrally directed ventral tip of cleithrum (140.1), a broad anteroventral tip of coracoid (142.1), numerous Supraorbital neuromasts (12-25) (182.1), and spawning within substrate (237.1).

Included taxa. The genera *Spectrolebias, Simpsonichthys, Nematolebias, Austrolebias, Megalebias,* and *Cynolebias.*

Tribe Spectrolebiatini, new name

Type genus. *Spectrolebias* Costa & Nielsen

Diagnosis. An anteriorly expanded alveolar process of premaxilla (3.1), an anteriorly expanded anteroventral border of autopalatine (32.1), a posteriorly directed posterior portion of suspensorium (44.1), miniaturization (168.1), a posteriorly expanded swimbladder (173.1), a translucent body (187.1), a transverse dark bar through posterior portion of iris (205.1), and a black spot, anteriorly bordered by a blue line, on the posterior portion of the distal margin of anal-fin of males (219.1).

Included taxa. Only the type genus, *Spectrolebias.*

Genus Spectrolebias Costa & Nielsen

Spectrolebias Costa & Nielsen, 1997:258 (type species *Spectrolebias semiocellatus* Costa & Nielsen, by original designation).

Diagnosis. Same as for the tribe.

Included taxa. This recently described genus includes only its type species, *S. semiocellatus* Costa & Nielsen.

Distribution. Known from a single collection made close to Formoso river, Araguaia river basin, central Brazil. The absence of *S. semiocellatus* in extensive collections south to this locality, suggests the genus does not occur in the upper region of the Araguaia river basin.

Tribe Cynolebiatini Hoedeman, new usage

Diagnosis. A reduced posterior process of quadrate (40.1), a reduced basihyal (55.1), an elongate neural spine of first vertebra (95.1), an elongate ventral process of posttemporal (132.0, a reversal), papillae on the internal surface of upper pectoral-fin-rays of larger males (150.1), and one to three black blotches, approximately on the central portion of body sides of juveniles, usually kept in adult females (193.1).

Included taxa. The genera *Simpsonichthys, Nematolebias, Austrolebias, Megalebias,* and *Cynolebias.*

Subtribe Simpsonichthyina, new name

Type genus. *Simpsonichthys* Carvalho.

Diagnosis. A widened ventral process of angulo-articular (17.1), an anteriorly directed large tooth on the corner of dentary (21.1), a reduced dermosphenotic (93.0, a reversal), an elongate and pointed anal-fin of male (155.1), and a narrow blue margin on the posterior border of caudal fin of males (225.1).

Included taxa. The genera *Simpsonichthys* and *Nematolebias.*

Genus *Simpsonichthys* Carvalho

Simpsonichthys Carvalho, **1959:** 2 (type species *Simpsonichthys boitonei* Carvalho, by original designation).

Diagnosis. A prominent process on the dorsal tip of autopalatine (25.1), and unbranched dorsal and anal-fin-rays (123.1).

Included taxa. Twenty-three species: *Simpsonichthys constanciae* (Myers), *S. bokermanni* (Carvalho & Cruz), *S. flavicaudatus* (Costa & Brasil), *S. zonatus* (Costa & Brasil), *S. trilineatus* (Costa & Brasil), *S. alternatus* (Costa & Brasil), *S. stellatus* (Costa & Brasil), *S. magnificus* (Costa & Brasil), *S. fulminantis* (Costa and Brasil), *S. hellneri* (Berkenkamp), *S. ghisolfii* Costa, Cyrino & Nielsen, *S. antenori* (Tulipano), 5. *flammeus* (Costa), *S. notatus* (Costa, Lacerda & Brasil), *S. multiradiatus* (Costa & Brasil), *S. marginatus* Costa & Brasil, *S. costai* (Lazara), *S. boitonei* Carvalho, *S. santanae* (Shibata & Garavello), *S. filamentosus* Costa, Barrera & Sarmiento, and *S. chacoensis* (Amato), and two undescribed species (Costa & Hellner, in press). A taxonomic revision of the genus by the author is in progress.

Distribution. Temporary pools in Chaco (Paraguay), and Beni (Bolivia), Araguaia-Tocantins, Parana and São Francisco rivers, Jaguaribe, Mossoró, and Cachoeira basins (Brazil), and smaller basins of northeastern Brazil.

Nematolebias, new genus

Type species. *Cynolebias whitei* Myers.

Diagnosis. A broad rostral cartilage (8.1), a strongly widened ventral process of angulo-articular (17.2), absence of teeth on second pharyngobranchial (67.1), a reduced neural spine on first vertebra (95.0, a reversal), and a dark red, anteriorly directed suborbital bar (206.2).

Included taxa. Three species: *N. whitei* (Myers) and *N. myersi* (Carvalho), and an undescribed species. A taxonomic revision of the genus by the author is in progress.

Distribution. Coastal plains of eastern Brazil, between southern Bahia State and Rio de Janeiro State.

Etymology. From the Greek *nema* (thread), and *lebias,* a nominal cyprinodontid genus commonly used to compose generic names of the family Rivulidae, referring to the cylindrical and slender body shape of males. Gender masculine.

Subtribe Cynolebiatina, new usage

Diagnosis. An expanded postero-ventral process of dentary (15.1), a reduced ventral process of angulo-articular (16.2), a large dorsomedial process of autopalatine (33.1), a constricted dorsal portion of metapterygoid (38.1), a strongly narrowed lacrimal (89.3), absence of elongate filamentous rays on fins (126.0, a reversal), absence of the upper pectoral radial (146.1), a ventrally directed, black suborbital bar (206.2), and a transverse black supraorbital bar (201.1).

Included taxa. The genera *Austrolebias, Megalebias,* and *Cynolebias.*

Austrolebias, new genus

Type species. *Cynolebias bellottii* Steindachner.

Diagnosis. A deep and short urohyal (49.1), an expanded cartilaginous portion of basihyal (56.0, a reversal), and an anterior frontal scale with all borders free (177.1).

Included taxa. Twenty-four species: *A. carvalhoi* Myers, *A. nonoiuliensis* Taberner, Fernández & Castelli, *A. bellottii* Steindachner, *A. nigripinnis* Regan, *A. adloffi* Ahl, *A. cyaneus* Amato, *A. luteoflammulatus* Vaz-Ferreira, Soriano & Paulete, *A. viarius* Vaz-Ferreira, Soriano & Paulete, *A. alexandri* Castello & Lopez, *A. affinis* Amato, *A. melanoorus* Amato, *A. gymnoventris* Amato, *A. cinereus* Amato, *A. nioni* Berkenkamp, Reichert & Prieto, *A. vazferrerai*

Berkenkamp, Reichert & Prieto, *A. patriciae* Huber, and *A. vandenbergi* Huber, and seven undescribed species. A taxonomic revision of the genus by the author is in progress.

Distribution. Chacoan (Paraguay and Argentina), Iguaçú (Brazil), Uruguay (Argentina, Brazil and Uruguay), and Plata (Argentina and Uruguay) river basins, and coastal smaller basins of southern Brazil and Uruguay.

Etymology. From the Latin *australis* (from the South), and *lebias*, a nominal cyprinodontid genus commonly used to compose generic names of the family Rivulidae, referring to the distribution pattern of the genus, constituting the southmost occurrence for the Rivulidae. Gender masculine.

Unnamed clade A

(Megalebias + Cynolebias)

Diagnosis. An expanded ventral portion of metapterygoid (37.1), a vestigial or absent median preopercular rim (45.4), absence of teeth of the second pharyngobranchial (67.1), a broad and large sphenotic (85.1), a triangular posterolateral rim of autopterotic (86.1), numerous vertebrae (34-40) (109.1), a posteriorly positioned dorsal-fin origin (111.1), a posteriorly positioned anal-fin origin (113.0, a reversal), and an increase in standard length (80-110 mm SL) (167.1).

Megalebias, new genus

Type species. *Cynolebias wolterstorffi* Ahl.

Diagnosis. Elongate premaxilla and dentarium (1.0, a reversal), a curved lacrimal (90.1), and an expanded anteroventral portion of coracoid (143.1).

Included taxa. Five species: *M. wolterstorffi* Ahl, *M. cheradophilus* Vaz-Ferreira, Soriano & Paulete, *M. elongatus* Steindachner, *M. prognathus* Amato, and *M. monstrosus* Huber. A taxonomic revision of the genus by the author is in progress.

Etymology. From the Greek "mega" (large), and *lebias*, a nominal cyprinodontid genus commonly used to compose generic names of the family Rivulidae, due to the large size of its members, some of them constituting the biggest species of the family. Gender masculine.

Genus *Cynolebias* Steindachner

Cynolebias Steindachner, 1876:172 (type species *Cynolebias porosus* Steindachner, by original designation).

Diagnosis. A non reduced ventral process of angulo-articular (15.0, a reversal), a non reduced ventral process of angulo-articular (16.0, a reversal), a long coronoid process of angulo-articular (18.1), elongate filamentous rays on the dorsal and anal-fins of males (126.1), absence of a transversal suborbital bar (206.0, a reversal), absence of a transversal supraorbital bar (207.0, a reversal), and dark brown to black blotches on the dorsoposterior portion of the head (214.1).

Included taxa. Seven species: C. *porosus* Steindachner, C. *microphthalmus* Costa & Brasil, C. *albipunctatus* Costa & Brasil, C. *perforatus* Costa & Brasil, C. *leptocephalus* Costa & Brasil, *C. gilbertoi* Costa, and *C. griseus* Costa, Lacerda & Brasil. A taxonomic revision of the genus by the author is in progress.

Distribution. Upper Tocantins, São Francisco, Jaguaribe, Mossoró, Itapicurú and Vaza-Barris river basins (Brazil).

Remarks. The present study indicates that a clade comprising all species of *Cynolebias* except for C. *griseus* -the C. *porosus* group- is defined by a posteriorly displaced interarcual cartilage (66.1), small denticles on gill-rakers of the first gill arch (75.1), and absence of ventral process of posttemporal (132.1). *Cynolebias griseus* is defined by the presence of second pharyngobranchial teeth (67.0, a reversal).

Subfamily Rivulinae Myers, new usage

Diagnosis. An anteriorly expanded quadrate (39.1); an expanded posterior process of quadrate (41.1), a bifid medial border of first hypobranchial (70.1), an anteriorly elongate ventral tip of cleithrum (139.1), an increase in number of pelvic-fin-rays (seven or eight) (148.1), and a circular pattern of frontal scales (174.1).

Included taxa. The genera *Rivulus, Pterolebias, Gnatholebias, Aphyolebias, Trigonectes, Neofundulus, Micromoema, Moema, Renova, Millerichthys, Rachovia, Austrofundulus, Terranatos, Pituna, Papiliolebias, Plesiolebias, Maratecoara*, and *Stenolebias.*

Infrafamily Rivulida Myers, new usage

Diagnosis. A short anterodorsal process of urohyal (50.1), absence of a transverse dark bar crossing the eye (204.0, a reversal), and non annual development (242.0, a reversal).

Included taxa. Only the type genus, *Rivulus*.

Genus *Rivulus* Poey

Rivulus Poey, 1860: 307 (type species *Rivulus cylindraceus* **Poey,** by original designation).

Cynodonichthys Meek, **1904: 101** (type species *Cynodonichthys tenuis* Meek, by original designation).

Vomerivulus Fowler, 1944: 244 (type species *Rivulus leucurus* Fowler, by original designation).

Anablepsoides Huber, 1992: 43 (type species *Rivulus atratus* Garman, by original designation).

Diagnosis. Same as for the infrafamily.

Included taxa. About 80 valid species, most listed in Huber (1992). Most in need of revisionary taxonomic studies.

Distribution. Middle and South America, from Mexico to Argentina, including the Caribbean Islands, and Florida.

Infrafamily Neofundulida Costa, new usage

Diagnosis. A prominent urogenital papilla in adult males (169.1).

Included taxa. The genera *Pterolebias, Gnatholebias, Aphyolebias, Trigonectes, Neofundulus, Micromoema, Moema,* and *Renova.* The genera *Pterolebias, Gnatholebias, Aphyolebias, Trigonectes, Neofundulus, Micromoema, Moema,* and *Renova.*

Supertribe Neofundulidi Costa, new usage

Diagnosis. An expanded lateral process of parasphenoid (83.1), two symmetrical, separated hypurals plates (103.0, a reversal), numerous vertebrae (33-40) (109.1), a ventrally expanded fourth pectoral radial (147.1), a pointed pectoral fin (149.1), a long pectoral fin (151.1), an elongate and pointed anal-fin of males (155.1), and spawning within the substrate (237.1).

Included taxa. The genera *Rivulus, Pterolebias, Gnatholebias, Aphyolebias, Trigonectes, Neofundulus, Micromoema, Moema,* and *Renova.*

Tribe Pterolebiatini Costa, new usage

Diagnosis. Large teeth on dentary, and premaxilla strongly curved laterally (22.1), a reduced interarcual cartilage (65.1), long pelvic fin in males (153.1), posterior filaments on the posterior border of the caudal-fin-rays of males (159.1), and an enlarged caudal fin in males (162.1).

Included taxa. The genera *Pterolebias* and *Gnatholebias.*

Genus *Pterolebias* Garman

Pterolebias Garman, 1895: 141 (type species *Pterolebias longipinnis,* by monotypy).

Diagnosis. An anteriorly expanded ventral tip of autopalatine (30.1), a constricted dorsal portion of metapterygoid (38.1), a very long posterior process of quadrate (41.2), a narrow basihyal (53.1), a long basihyal (54.1), a reduced number of vertebrae (109.0, a reversal), a rounded pectoral fin (149.0, a reversal), a metallic orange humeral spot (196.3), and black vertical bars on the pectoral fin of males (231.1).

Included taxa. Two species: *P. longipinnis* Garman and *P. phasianus* Costa. A taxonomic revision of the genus by the author is in progress.

Distribution. Amazonas (Brazil and Bolivia) and Paraguay (Brazil, Bolivia and Argentina) river basins.

Gnatholebias, new genus

Type species. *Pterolebias zonatus* Myers.

Diagnosis. Long premaxilla and dentarium (1.0, a reversal), hypurals ankylosed to form a single plate (103.3), proximal anal radials curved, their tips directed posteriorly (121.1), elongate filamentous rays on the dorsal and anal-fins of males (126.1), numerous anal-fin-rays (22-26) (128.1), a horizontal reddish brown stripe close to the dorsal border of caudal fin of males (229.1), a narrow black margin on the ventral border of the pectoral fin of males (232.1), and a white to light green border on the lower edge of the pectoral fin of males (233.1).

Included taxa. Two species: *G. zonatus* Myers and *G. hoignei* Thomerson.

Distribution. Orinoco, Unare and Guanipa (Venezuela) river basins.

Etymology. From the Greek gnaqox (jaw), and *lebias,* a nominal cyprinodontid genus commonly used to compose generic names of the family Rivulidae, in reference to the robust and long jaws of its members. Gender masculine.

Tribe Neofundulini Costa, new usage

Diagnosis. An orange stripe (sometimes pink or red) along lower margin of the caudal fin of males (221.1).

Included taxa. The genera *Aphyolebias, Trigonectes, Neofundulus, Micromoema, Moema,* and *Renova.*

Subtribe Aphyolebiatina, new name

Type genus. *Aphyolebias,* new genus.

Diagnosis. A constricted dorsal portion of metapterygoid (38.1), elongate posterior extensions on the upper and lower borders of caudal fin of males (161.1), a dark brown to black humeral spot (196.2), and a narrow black margin on the ventral border of the pectoral fin of males (232.1).

Included taxa. Only the type genus, *Aphyolebias*.

Aphyolebias, new genus

Type species. *Pterolebias peruensis* Myers.

Diagnosis. Same as for the Subtribe.

Included taxa. Four species: *A. peruensis* (Myers), *A. wischmanni* (Seegers), *A. rubrocaudatus* (Seegers), and A. *obliquus* (Costa, Sarmiento & Barrera).

Distribution. Western Amazonian basin (Peru and Bolivia).

Etymology. From *Aphyosemion,* a nominal aplocheilid genus, and *lebias,* a nominal cyprinodontid genus frequently used to compose generic names of the family Rivulidae, referring to the superficial resemblance between the new genus and *Aphyosemion* (lyre-shaped caudal fin, slender body and brilliant stripe on border of caudal fin of males). Gender masculine.

Subtribe Neofundulina Costa, new usage

Diagnosis. An elongate dorsal portion of preopercle (46.1), a posterior displacement of anal-fin origin of males (112.1), a rounded pectoral fin (149.0, a reversal), three longitudinal rows of dark spots from the anterior portion of body sides to caudal-fin base, alternated with shorter rows of similar spots restricted to the anterior portion of the body (188.1), and a metallic green humeral spot (196.1).

Included taxa. The genera *Trigonectes* and *Neofundulus.*

Genus *Neofundulus* Myers

Neofundulus Myers, 1924:9 (type species *Fundulus paraguayensis* Eigenmann & Kennedy, by original designation).

Diagnosis. Fourth pectoral radial not expanded ventrally (147.0, a reversal), a subbasal, longitudinal white to yellow stripe on the anal-fin of males (216.1), transverse black bars on caudal fin of females (226.1), and black spots on pectoral fin of males (230.1).

Included taxa. Five species: *N. paraguayensis* (Eigenmann & Kennedy), *N. ornatipinnis* Myers, *N. parvipinnis* Costa, *N. guaporensis* Costa, and *N. acutirostratus* Costa. *Neofundulus* was revised by Costa (1988), but two species remained known only from the holotypes. Huber (1995) reported a probable undescribed species from the Paraguayan Chaco.

Distribution. Chaco (Paraguay and Argentina), Paraguay (Paraguay, Brazil and Bolivia), Guaporé (Brazil) and São Francisco (Brazil) river basins.

Genus *Trigonectes* Myers

Trigonectes Myers, 1925:371 (type species *Trigonectes strigabundus* Myers, by monotypy).

Rivulichthys Myers, 1927:118 (type species *Rivulus rondoni* Ribeiro, by original designation).

Diagnosis. Elongate premaxilla and dentarium (1.0, a reversal), molariform teeth on the third pharyngobranchial and fifth ceratobranchial (69.1), elongate pelvic fins in males (153.1), and increase in standard length (80-90 mm SL) (167.1).

Included taxa. Six species: *T. balzanii* (Perugia), *T. rogoaguae* (Myers), *T. strigabundus* Myers, *T. macrophthalmus* Costa, *T. rubromarginatus* Costa and *T. aplocheiloides* Huber. The genus was revised by Costa (1990), and Huber (1995) described a further species.

Distribution. Madeira (Bolivia and Brazil), Chaco (Paraguay, Bolivia and Argentina), Paraguay (Paraguay, Bolivia and Brazil) and Araguaia-Tocantins (Brazil) river basins.

Subtribe Moemina, new name

Type genus. *Moema* Costa.

Diagnosis. A rod-shaped tip of anal-fin of males (158.1), three longitudinal rows of dark spots from the anterior portion of body sides to caudal-fin base, alternated with shorter rows of similar spots restricted to the anterior portion of the body (188.1), a brown, short, anteriorly directed suborbital bar (206.4), and two oblique reddish brown stripes on the preopercular region of males (212.1).

Included taxa. The genera *Micromoema, Moema,* and *Renova.*

Micromoema, new genus

Type species. *Pterolebias xiphophorus* Thomerson & Taphorn.

Diagnosis. An anteriorly expanded ventral tip of autopalatine (30.1), reduced number of vertebrae (31) (109.0, a reversal), absence of the ventral process of posttemporal (132.1), and an elongate posterior extension on the lower border of the caudal fin of males (161.1).

Included taxa. Only the type species, *M. xiphophora.*

Distribution. Upper Orinoco river basin, Venezuela.

Etymology. From the Greek microx (small), and *moema,* a closely related rivulid genus, an allusion to the small size of the sole member of the genus and its close relationships and resemblance with the rivulid genus *Moema.* Gender feminine.

Unnamed clade B *(Moema + Renova)*

Diagnosis. Numerous vomerine teeth (8-12) (77.1), numerous caudal-fin-rays (32-36) (110.1), and a posteriorly displaced anal-fin origin of males (112).

Genus *Moema* Costa

Moema Costa, 1989a: 223 (type species *Moema piriana* Costa, by original designation).

Diagnosis. Long premaxilla and dentary (1.0, a reversal), a broad rostral cartilage (8.1), along dorsal portion of preopercle (46.1), a posteroventral projection on the opercle (48.1), molariform teeth on third pharyngobranchial and fifth ceratobranchial (69.1), an undivided medial border of first hypobranchial (70.1), numerous gill-rakers on the first gill arch (15) (74.1), and an increase in standard length (89-90 mm SL).

Included taxa. Five species: *M. piriana* Costa, *M. portugali* Costa, *M. staecki* (Seegers), and *M. pepotei* Costa, and an undescribed species from the Napo river basin, Ecuador. This genus was briefly revised by Costa (1992b).

Distribution. Endemic to the Amazonian basin, in the Negro (Brazil), Napo (Ecuador) and Madeira (Peru, Bolivia, and Brazil) basins, and lower Amazonian region (State of Pará, Brazil).

Genus *Renova* Thomerson & Taphorn

Renova Thomerson & Taphorn, 1995:186 (type species *Renova oscari,* by original designation).

Diagnosis. Absence of two well-defined oblique reddish brown stripes on the preopercular region of males (212.0, a reversal), and an ocellate caudal black spot in females (222.1).

Included taxa. Only the type species, R. *oscari* Thomerson & Taphorn.

Distribution. Orinoco river basin (Venezuela).

Supertribe Rachoviidi Costa, new usage

Diagnosis. A dorsally pointed preopercle (45.2), reduced neural prezygapophyses of caudal vertebrae (100.1), and a reduced dark pigmentation in females (194.1).

Included taxa. The genera *Millerichthys, Rachovia, Austrofundulus, Terranatos, Pituna, Papiliolebias, Plesiolebias, Maratecoara,* and *Stenolebias.*

Tribe Millerichthyini, new name

Type genus. *Millerichthys* Costa.

Diagnosis. A reduced mesopterygoid (34.2), a conical and elongate ventral process of posttemporal (133.1), a long posterior series of supraorbital neuromasts (181.1), absence of a transverse dark bar crossing eye (204.0, a reversal), three transverse yellow stripes on the anal-fin of males (217.1), and a series of black spots along caudal-fin base of females (223.1).

Included taxa. Only the type genus, *Millerichthys.*

Genus *Millerichthys* Costa

Millerichthys Costa, 1995b: 18 (type species *Rivulus robustus* Miller & Hubbs, by original designation).

Diagnosis. Same as for the tribe.

Included taxa. It comprises a single species, *M. robustus* (Miller & Hubbs).

Distribution. Papaloapan and Coatzacoalcos river basins (Mexico).

Tribe Rachoviini Costa, new usage

Diagnosis. A posteriorly expanded ventral portion of lacrimal (91.1), an oblique pattern of stripes or rows of spots or dots on the body sides (200.1), along, slightly anteriorly directed black suborbital bar (206.1), and alternate black and white spots on the basal and posterior part of the anal-fin of males (218.1).

Included taxa. The genera *Rachovia, Austrofundulus, Terranatos, Pituna, Papiliolebias, Plesiolebias, Maratecoara,* and *Stenolebias.*

Subtribe Rachoviina Costa, new usage

Diagnosis. A well-developed dorsal tip of preopercle (45.1, a reversal), an anteriorly expanded interhyal (52.1), hypural plates in close proximity, sometimes partially ankylosed (103.1), expanded caudal-fin squamation in older males (178.1), and a transverse dark brown supraorbital bar (207.1).

Included taxa. The genera *Rachovia, Austrofundulus,* and *Terranatos.*

Genus *Rachovia* Myers

Rachovia Myers, 1927: 119 (type species *Rivulus brevis* Regan, by original designation).

Diagnosis. A jagged ventral border of mesopterygoid (36.1), and a well-developed lateral process of parasphenoid (83.1).

Included taxa. Five species: R. *brevis* (Regan), *R. maculipinnis* (Weibezahn), R. *hummelinki* De Beaufort, *R. pyropunctata* Taphorn & Thomerson, and *R. stellifer* (Thomerson & Turner). This genus was revised by Taphorn & Thomerson (1978).

Distribution. Sinú, Magdalena (Colombia), Maracaibo, **Orinoco,** and Unare (Venezuela) river basins.

Unnamed clade C

(Austrofundulus + Terranatos)

Diagnosis. A long neural spine of first vertebra (95.1), a dorsally directed process on the base of anterior epipleural ribs (97.1), and elongate posterior extensions on the upper and lower borders of caudal fin of males (161.1).

Genus *Austrofundulus* Myers

Austrofundulus Myers, 1932: 159 (type species *Austrofundulus transilis* Myers, by original designation).

Diagnosis. Lateral pointed expansion on rostral cardlage (10.1), numerous gill-rakers on the first gill arch (3+13) (74.1), numerous vertebrae (14+20) (109.1), numerous caudal-fin-rays (33-34) (110.1), a fatty protuberance on top of the head of older males (163.1), and black perianal papilla (201.1).

Included taxa. Two species: *A. transilis* Myers and A. *limnaeus* Schultz. This genus was revised by Taphorn & Thomerson (1978). However, the vast and disjunct geographic distribution area of A. *limnaeus,* a poorly defined species with high degree of polymorphic characters, indicates the need of more detailed taxonomic studies.

Distribution. Sinú, Magdalena (Colombia), Maracaibo and Orinoco (Venezuela), and Branco (Guyana) river basins.

Genus *Terranatos* Taphorn & Thomerson

Terranatos Taphorn & Thomerson, 1978:384 (type species *Austrofundulus dolichopterus* Weitzman & Wourms, by original designation).

Diagnosis. Non anteriorly expanded quadrate (39.0, a reversal), a short anterior portion of urohyal (52.0, a reversal), a median laminar process between supraoccipital processes (88.1), dorsal fin anteriorly positioned, second dorsal proximal radial between neural spines of vertebrae 9 and 10 (111.3), anal proximal radials strongly widened (120.1), a widened dorsal portion of the cleithrum (137.1), a short ventral tip of cleithrum (139.0, a reversal), a long pectoral fin in males (151.1), elongate dorsal and anal-fins of males (154.1), elongate and pointed anal-fin of male (155.1), transverse arrangement of frontal scales (174.0, a reversal), reduced caudal-fin squamation (178.0, a reversal), a slightly posteriorly directed, black suborbital bar (206.2), and absence of alternate black and white spots on the basal and posterior parts of the anal-fin of males (218.0, a reversal).

Included taxa. It includes only the type species, *T. dolichopterus.* The best systematic account of this species is that presented in its original description (Weitzman & Wourms, 1967).

Distribution. Endemic to the Orinoco river basin (Venezuela).

Subtribe Plesiolebiatina Costa, new usage

Diagnosis. A deeply concave anterior border of rostral cartilage (9.1), a slightly reduced ventral process of angulo-articular (16.1), a reduced anterior portion of mesopterygoid (35.1), a long basihyal (54.1), a strongly concave anteromedial border of first epibranchial (59.1), absence of paired, pointed, anteriorly directed process on first vertebra, corresponding to the neural prezygapophyses (94.1), a median fissure in the hypural plate (103.2), and a metallic green humeral spot (196.1).

Included taxa. The genera *Pituna, Papiliolebias, Plesiolebias, Maratecoara,* and *Stenolebias.*

Genus *Pituna* Costa

Pituna Costa, 1989a: 225 (type species *Pituna poranga* Costa, by original designation).

Diagnosis. Fourth pectoral radial expanded ventrally (147.1), elongate and pointed anal-fin of males (155.1), non reduced dark pigmentation on the body sides and fins of females (194.0, a reversal), bright red pigmentation on the anterior margin of the dorsal fin of larger males (215.1), a green and red stripe on distal margin of anal-fin of males (220.1), and black spots on pectoral fin of males

(230.1).

Included taxa. According to the most recent revision of the genus (Costa, 1998b), *Pituna* includes only a single species, *P. compacta* (Myers).

Distribution. Das Mortes-Araguaia-Tocantins, Xingú and Parnaíba river basins (Brazil).

Unnamed clade D

(Papiliolebias + Plesiolebias +
Maratecoara + Stenolebias)

Diagnosis. A narrow basihyal (53.1), a complete ankylosis of hypurals, forming a single plate (103.3), a pointed process, anteriorly directed, on the proximal tip of the parahypural (106.1), a short ventral tip of cleithrum (139.0, a reversal), neuromasts of the supraorbital posterior series arranged in a long line (181.1), and spawning without contact with substrate (237.2).

Genus *Papiliolebias* Costa

Papiliolebias Costa, 1998a: 319 (type species *Plesiolebias bitteri* Costa, by original designation, proposed as a subgenus of *Plesiolebias).*

Diagnosis. Nine pelvic-fin-rays (148.2). Other autapomorphic conditions for *Papiliolebias*, not included in the present analysis, are: dark blue unpaired fins of males and a white line along distal margin of anal-fin of male (Costa, 1998a).

Included taxa. Only the type species, *P. bitteri* Costa.

Distribution. Chacoan river basins (Bolivia, Paraguay and Argentina).

Unnamed clade E

(Plesiolebias + Maratecoara + Stenolebias)

Diagnosis. An anteriorly positioned dorsal-fin origin (111.1), absence of the ventral process of posttemporal (132.1), absence of a humeral spot (196.0, a reversal),

Genus *Plesiolebias* Costa

Plesiolebias Costa, 1989b: 193 (type species *Cynolebias xavantei* Costa, Lacerda & Tanizaki, by original designation).

Diagnosis. Shortened autopalatine, ventral portion widened (31.1), a very elongate posterior process of quadrate (41.2), an elongate sympletic (43.1), widened epibranchials (61.1), absent interarcual cartilage (64.1), reduced number of vertebrae (23-26) (108.1). a long anterior portion of anal-fin in males (157.1), an angular ventral profile of head (166.1), a transversal arrangement of frontal scales (174.0, a reversal), an anterior frontal scale with all borders free (177.1), a longitudinal red stripe on the anterodorsal portion of body of males (189.1), and oblique rows of dark dots on the body sides of females (195.1).

Included taxa. Four species: *P. xavantei* (Costa, Lacerda & Tanizaki), *P. glaucopterus* (Costa & Lacerda), *P. lacerdai* Costa, and *P. aruana* (Lazara).

Distribution. Paraguay (Brazil) and das Mortes/ Araguaia/Tocantins (Brazil) river basins.

Unnamed clade F

(Stenolebias + Maratecoara)

Diagnosis. A twisted median portion of maxilla (6.1), anterior process of lateral ethmoid elongate, firmly attached to lateral arm of the bone (82.1), an elongate and pointed anal-fin of males (155.1), a bright red pigmentation on the anterior margin of the dorsal fin of larger males (215.1), and spawning on the substrate (237.0. a reversal).

Genus *Stenolebias* Costa

Stenolebias Costa, 1995a: 70 (type species *Plesiolebias damascenoi* Costa, by original designation).

Diagnosis. Five branchiostegal rays (57.1).

Included taxa. Two species: *S. damascenoi* (Costa) and *S. bellus* Costa.

Distribution. Endemic to the Paraguay river basin (Brazil).

Genus *Maratecoara* Costa

Maratecoara Costa, 1995a:68 (type species *Cynolebias lacortei* Lazara, by original designation).

Diagnosis. A broad anterior arm of the autopalatine (29.1), an anteriorly expanded pharyngobranchial 2 (68.1), a deep and dorsally directed supraoccipital in males (87.1), a long neural spine of first vertebra (95.1), a median fissure on the hypural plate (103.2, a reversal), an anteriorly positioned dorsal-fin origin (111.2), a small and triangular first proximal anal radial (117), long dorsal and anal-fins in males (154.1), a lozenge-shaped caudal fin in males, with two elongate filaments on the posterior tip (160.1), an expanded opercular membrane in males (165.1), a transversal arrangement of frontal scales (174.0, a reversal), three longitudinal rows of orange spots on the anterodorsal portion of body in males (192.1), an orange supraorbital bar (207.2), a metallic blue branchiostegal membrane in males (208.1), and absence of alternate black and white spots on the basal and posterior part of the anal-fin of males (218.0, a reversal).

Included taxa. Two species: *M. lacortei* (Lazara) and M. *formosa* Costa.

Distribution. Das Mortes/Araguaia/Tocantins river basin (Brazil).

Discussion

Monophyly and classification of rivulid taxa

The monophyly of the Rivulidae, first proposed by Parenti (1981), has been corroborated in subsequent phylogenetic studies, both using morphological (Costa, 1990a, in press) and molecular (Murphy & Collier, 1997; Hrbek, in press) characters. The present study corroborates once more this hypothesis, now supported by 23 synapomorphies, as listed in the diagnosis of the family.

While the monophyly of the Rivulidae and the suborder Aplocheiloidei are unanimously accepted, Parenti's (1981) hypothesis of Aplocheilidae monophyly has been rejected by molecular phylogenetic studies (Murphy & Collier, 1997; Hrbek, in press). These studies pointed out that African aplocheilids *(Aphyosemion, Fundulopanchax, Nothobranchius and Epiplatys)* are more closely related to rivulids than to Madagascan *(Pachypanchax)* and Asiatic *(Aplocheilus)* aplocheilids. However, according to Parenti's (1981) phylogenetic study, *Aplocheilus, Pachypanchax,* and *Epiplatys* would form a monophyletic assemblage. The new hypothesis on aplocheiloid relationships, besides fitting well the present geologic model for the tectonic break of Gondwana, may be in part corroborated by some morphological characters observed in the present study. *Pachypanchax* and *Aplocheilus* have a free orbital membrane (Parenti, 1981), whereas *Aplocheilus* (but not *Pachypanchax)* has a lateral pectoral fin and a well-developed dorsal rip of parahypural, which overlaps the centrum of the last vertebra. All three conditions are plesiomorphic for atherinomorphs and do not occur in African aplocheilids and rivulids. In addition, the apomorphic condition of the bifid epipleural ribs in *Epiplatys, Aphyosemion,* and *Fundulopanchax* (see character 98) suggests closer relationships between those African taxa than to *Aplocheilus* and *Pachypanchax.* On the other hand, the absence of an uncinate process on the fourth epibranchial, reported as synapomorphic for *Epiplatys, Aplocheilus* and *Pachypanchax* by Parenti (1981), and posttemporal and supracleithrum ankylosed, and premaxillary ascending processes tapered posteriorly, reported as synapomorphic for Aplocheilidae (Parenti, 1981, 1982; Costa, in press), are confirmed in the present study.

Among rivulid taxa, most controversy has been concentrated on the monophyly and relationships of the genus *Rivulus.* While Parenti (1981) (morphological analysis) and Hrbek (molecular analysis) (in press) claimed against monophyly, Costa (1990a) (morphological analysis) and Murphy & Collier (1996) (molecular analysis) found evidence supporting it. In addition, according to Parenti (1981), a group comprising all rivulids but excluding all species of *Rivulus* would be monophyletic, but according to Hrbek (in press), *R. caudomarginatus* would be the sister group to the remaining rivulids, the other species of *Rivulus* forming a paraphyletic assemblage within the Rivulinae (in the sense of the present study). However, both in Costa (1990a) and Murphy & Collier (1997), *Rivulus* would be a clade within the Rivulinae. The present hypothesis is similar to Costa's (1990a) and Murphy & Collier's (1997) in separating all rivulids into two large monophyletic lineages, but transferring *Plesiolebias* and *Terranatos* (taxa not included in Murphy & Collier's (1997) analysis) to the Rivulinae. On the other hand, in Hrbek's hypothesis, except for *R. caudomarginatus,* the rivulids would be divided into two major clades, exactly corresponding to the Cynolebiatinae and Rivulinae as proposed in the present study The present study corroborates the hypothesis of *Rivulus* being a clade, despite some plesiomorphic character states suggesting that the *Rivulus* assemblage, including *R. brasiliensis, R. caudomarginatus, R. ocellatus,* and *R. marmoratus,* is the sister group to all other rivulids (see characters 58 and 135).

Rivulus stellifer is transferred to *Rachovia,* as previously proposed by Wildekamp (1982), and placed in the Subtribe Rachoviina along with *Austrofundulus* and *Terranatos.* The monophyly of Rachoviina, as proposed here, was not supported in previous morphological analyses *(e.g.,* Costa, 1990a), but has some resemblance with a clade derived from a molecular analysis (Hrbek, in press). However, the clade obtained in Hrbek's molecular analysis is rather impressive in a biogeographic view because, besides comprising *R. stellifer, Rachovia, Austrofundulus* and *Terranatos,* it also includes *Pterolebias zonatus* (= *Gnatholebias zonatus)* and *P. xiphophorus* (= *Micromoema xiphophorus).* In other words, a clade encompassing all minus one *(Renova)* known species of annual rivulid fishes inhabiting Venezuela and Colombia. However, the inclusion of G. *zonatus* and *M. xiphophorus* in such clade, as proposed by Hrbek (in press), is strongly divergent from the results obtained herein, including the several well-corroborated clades both in Neofundulidi and Rachoviidi, as discussed below.

The monophyly of *Pterolebias,* weakly corroborated in previous studies (Parenti, 1981; Costa, 1990a), is not supported herein. A group comprising four species from western Amazon, herein included in the new genus *Aphyolebias,* is hypothesized to be more closely related to *Renova, Neofundulus, Trigonectes* and *Moema,* than to a clade containing the type species of the genus and other species currently placed in *Pterolebias.* Another species from the Venezuelan Orinoco river basin, also placed in a new genus, *Micromoema,* is hypothesized to be more closely related to *Moema* and *Renova.* The new genus *Gnatholebias* is erected to comprise a well-corroborated clade of two species *(G. zonatus* and G. *hoignei)* endemic to the Orinoco basin, the sister group to *Pterolebias,* now restricted to only two species (P. *longipinnis* and *P. phasianus)* endemic to the southern Amazonian and Paraguayan river basins. The monophyly of *Pterolebias* in the sense of the present study is corroborated by molecular data (Hrbek, in press).

The monophyly of a group comprising the large-sized Neofundulidi, the genera *Moema* and *Trigonectes,* proposed in previous morphological phylogenetic studies *(e.g.,* Costa, 1989a, 1990a) is not corroborated in the present study. *Moema* is indicated to be more closely related to *Renova and Micromoema,* and *Trigonectes* to be the sister group to *Neofundulus.* The molecular hypothesis strongly differs in the relationships of the first clade *(Moema* as the sister group to *Aphyolebias, Renova* as the sister group to *Pterolebias,* and *Micromoema* as the sister group to *Terranatos),* but it is identical in the sister group relationships between *Trigonectes* and *Neofundulus.*

The present hypothesis on phylogenetic relationships within the Neofundulidi, clade indicates an interesting, almost entirely disjunct historical biogeographic pattern. Among the Neofundulini, an area comprising the upper Orinoco river basin (inhabited *by Micromoema* and *Renova)* would be the sister area to an area comprising the central and eastern Amazon *(Moema),* then closely related to central South America *(Trigonectes* and *Neofundulus)* and to western Amazon *(Aphyolebias).* Among the Pterolebiatini, a partially congruent pattern indicates an area comprising the Orinoco river basin and adjacent basins *(Gnatholebias)* as the sister area to southern and eastern Amazon, and the Paraguay river basin *(Pterolebias).*

The close relationships between the Rachoviina and the Plesiolebiatina, and between these groups *with Millerichthys* is first proposed, strongly differing from other morphological analyses, in which plesiolebiatins would be more closely related to cynolebiatines *(e.g.,* Costa, 1990a. 1995b). Another difference from previous studies is the closer relationships of *Plesiolebias* with *Maratecoara* plus *Stenolebias* than to *Papiliolebias* (Costa, 1998a), which, therefore, is not considered a subgenus of *Plesiolebias,* but constitutes a distinct genus. Rachoviidi intrarelationships also indicate a biogeographic disjunct pattern, with the Maracaibo, Orinoco and adjacent basins (Rachoviina) as the sister area to central South America (Plesiolebiatina), but in this case being both closely related to central Mexico (Millerichthyini). The biogeographic pattern for the Plesiolebiatina, with Chaco *(Papiliolebias)* as the sister area to central Brazil *(Plesiolebias + Maratecoara + Stenolebias),* is the same as proposed by Costa (1998a).

Phylogenetic relationships within the Cynolebiatinae are in accordance with previous studies *(e.g.,* Costa, 1990a; Costa & Nielsen, 1997). A very similar hypothesis was also obtained from the molecular analysis (Hrbek, in press), but in that case, *Simpsonichthys* was considered more closely related to *Austrolebias* than to *Nematolebias.* In contrast, the present analysis indicates monophyly of the Simpsonichthyina clade *(Simpsonichthys* plus *Nematolebias),* defined by some unambiguous synapomorphies. In the present classification, the new genera *Nematolebias, Austrolebias* and *Megalebias* are erected to include well-corroborated unnamed clades. Biogeographic patterns extracted from Cynolebiatinae relationships are discussed by Costa (1995b,c, 1996).

Origin and evolution of annualism

The two most recent approaches on the evolution of annualism in aplocheiloids (Murphy & Collier, 1997; Hrbek, in press) indicate different hypotheses of annualism origin. According to Murphy & Collier (1997), annualism among all aplocheiloids corresponds to a single event, secondarily lost in four clades, three in aplocheilids and one in *Rivulus*. This hypothesis was based on a cladogram topology, in which some of the basal taxa of the African aplocheilid clade, the putative sister group to rivulids, are annuals. However, Hrbek (in press) suggested that, among rivulids, annualism arose in Cynolebiatinae and in Rivulinae independently. According to this author, based on the putative sister group relationships between *R. caudomarginatus,* a species inhabiting mangroves, and a group containing all other rivulids, those annual fish clades could be derived from a salt-tolerant ancestor, that would have dispersed from Africa.

The most parsimonious cladograms obtained here admit two equally parsimonious interpretations. Annualism may correspond to a single event in the evolution of the rivulids, with a reversal in *Rivulus,* or two independent events, in Cynolebiatinae and in Neofundulida. The first of those interpretations is in accordance with the phylogenetic studies by Costa (1990a) and Murphy & Collier (1997), and the second one is more congruent with Hrbek's (in press) hypothesis. However, a marine origin for the Rivulidae is completely rejected, since this condition does not occur in basal rivulid taxa nor in immediate outgroups, but, in contrast, constitutes a synapomorphy for a clade of species of *Rivulus (R. caudomarginatus, R. ocellatus,* and R. *marmoratus)* (character 243).

Hypotheses on the evolution of ecological and behavioral traits may be extracted from a hypothesis of phylogenetic analysis (Brooks, 1985; Brooks & McLennan. 1991). Thus, the use of the current phylogenetic scheme of Rivulidae as a historical basis could help to evaluate some evolutionary aspects about annualism origin. More interestingly, if annualism arose independently in distinct aplocheiloid lineages, as discussed above, homoplastic traits shared only by distinct annual fish clades could provide evidences for hypotheses of adaptive convergence. Thus, if the same traits uniquely arose in annual rivulids and in an ecological outgroup (African annuals), then these convergent traits are probably adaptive acquisitions associated with annualism.

The appearance of apomorphic states of distinct characters in a same clade does not allow the distinction between coadaptive and coincidental phylogenetic association between characters (Brooks & McLennan, 1991), but their combined occurrence at the basis of distinct monophyletic assemblages strengthen a coadaptation hypothesis of trait complexes. Annual embryonic development and ecological and behavioral traits are intrinsically related characters. The ability to survive in temporary pools is not practicable without the concomitant existence of the annual embryonic development pattern. On the other hand, spawning behavior is also convergent in annual rivulids and annual aplocheilids. Annuals spawn into the bottom, where eggs undergo diapause during dry seasons, whereas non annual aplocheiloids usually spawn on plant roots near the water surface (character 237). Therefore, the bottom-spawning behavior occurring in both annual fish groups, but not in their sister group taxa, indicates that it is homoplastic for both annual lineages, and probably constitutes a condition closely related to the existence of annualism.

Simultaneous unique occurrence of apomorphic morphological traits in some members of the two annual aplocheiloid lineages may reflect some kind of favoritism *(i.e.,* selective advantage) associated to annual life style *(e.g.,* life in temporary pools). Body depth, position of dorsal fin and neuromast pattern are potential candidates to corroborate adaptive convergent hypotheses. A deep body and a consequent elongate first neural spine does not occur in non annual aplocheiloids, but occurs in distinct annual rivulid lineages *(Terranatos + Austrofundulus), Maratecoara, Simpsonichthys,* and *Cynolebias* (see character 95), as well as in the annual aplocheilid genus *Nothobranchius.* Similarly, different grades of anteriorly positioned dorsal fin is present in *Terranatos,* most plesiolebiatins and cynolebiatines (see character 111), and also in *Nothobranchius,* but are absent in all non annual aplocheiloids. Additionally, elaborate cephalic neuromast patterns have gradually developed at different phylogenetic levels and in distinct clades comprising the Rachoviidi and the Cynolebiatinae (see characters 180-182), whereas a somewhat elaborate neuromast pattern occurs in species of *Nothobranchius*, which has the anterior and posterior supraorbital rows continuous (Scheel, 1968), a condition similar to that in Cynolebiatinae.

Non annual rivulids *(Rivulus)* live in permanent waters, usually in shallow aquatic biotopes (more frequently about five centimeters deep) on the periphery of streams, rivers, and lakes *(e.g.,* Lüling, 1971; Bastos & Lourenço. 1983; Costa. 1987; Costa. 1995f). Eventually, these peripheral permanent biotopes may dry, but species *of Rivulus* are able to jump out of water, often finding other appropriate places to their survival (Seghers, 1978; Bastos, 1979). An exception to this habitat preference occurs in a species group of *Rivulus* inhabiting euryaline biotopes such as mangroves *(e.g.,* Huehner *et al.,* 1985; Taylor. 1988), herein considered a derived and independent condition, not occurring in other aplocheiloids (character 243). Species *of Aphyosemion* Myers *and Adamas* Huber, the sister group of the annual African aplocheilid clade (Parenti, 1981), seem to also live in permanent peripheral biotopes (Huber, 1976; 1979). In contrast, rivulid annual fishes live in temporary pools usually associated with river floodplains (Vaz-Ferreira & Sierra, 1971; Costa, 1998a), but not in contact with permanent waters, sometimes some kilometers away. These evidences suggest that evolution of annualism is related to the colonization of peripheral biotopes in two steps, the first one occurring in shallow and permanent creeks and pools, the second in adjacent and most peripheral habitats, the temporary pools. The first peripheral biotope is shared by other teleosts *(e.g.,* the lebiasinid genus *Pyrrhulina* Valenciennes and the callichthyid genus *Callichthys* Scopoli) in the Neotropical region, but seasonal biotopes are inhabited exclusively by annual fishes, except in a few instances, when temporary pools are mixed with permanent waters after rain inundations. Thus, colonization of annual habitats seems to represent the conquest of a new environment by neotropical teleosts.

Miniaturization

The greatly diverse neotropical freshwater ichthyofauna is known by the frequent, usually unique specializations of its components. Among the most interesting features is the miniaturization, occurring in the major teleost lineages, as broadly discussed by Weitzman & Vari (1988). This phenomenon is characterized by an extremely reduced body size in adults (Hanken & Wake, 1993).

Weitzman & Vari (1988) established a criterion to recognize miniatures, combining an arbitrary standard of 26 mm SL as maximum adult size, and the presence of reductive characters, such as reduction of laterosensory canal system, body scales, fin-rays, and sculpturing on the surface bones of the head. That study was in great part dedicated to list miniatures of the South American freshwater fish fauna, and to evaluate the number of distinct evolutionary events of miniaturization. No miniature rivulid species was known on that occasion, although the maximum adult size of some species *(e.g., Leptolebias sandrii* and *L. minimus)* only slightly surpassed that standard.

At present, eight species with adults not exceeding 26 mm SL are known: *Leptolebias cruzi, Plesiolebias glaucopterus, P. lacerdai, P. aruana, Stenolebias damascenoi, S. bellus, Simpsonichthys costai,* and *Spectrolebias semiocellatus.* In fact, this criterion does not seem to be useful to distinguish miniatures from non-miniatures in the Rivulidae, since some are slightly below this standard, and many other species slightly surpass that measurement. In addition, there is usually a gradual reduction with overlapped measurements when comparison involves successive sister groups, not permitting to delimit a clear boundary where miniaturization begins. Exceptions are *Simpsonichthys costai, Spectrolebias semiocellatus, Plesiolebias lacerdai,* and *P. aruana,* all reaching about 20 mm SL, constituting the smallest species of the family, and strongly contrasting with about 25-30 mm SL in closer sister groups and the majority of other rivulids. Among aplocheilids, only two miniatures with equivalent reduced size - *Adamas formosus* Huber and *Foerschichthys flavipinnis* (Meinken)- are known. *Plesiolebias lacerdai* and *P. aruana,* although placed in the same genus, are not sister species. Therefore, through a phylogenetic criterion to find miniatures, four taxa among rivulids are miniaturized, corresponding to four independent events. On the other hand, although many reductive characters occur in all the smaller species, they are not unique to those small taxa. Reductive characters are very common in the Rivulidae, arising at different phylogenetic levels. For example, from the 23 diagnostic apomorphic conditions for the family Rivulidae, 15 are reductive. Therefore, reductive conditions exhibited by small rivulid species *(e.g.,* loss of the latero-sensory canal of the head, reduction of bones of jaws, jaw suspensorium, branchial arches, infraorbital series and neurocranium) arose before events of body size reduction, and thus are not associated with a miniaturization event. An exception is the reduction of body pigmentation in *Spectrolebias* (see character 187).

New possibilities for niche acquisition may be available to reduced size fishes (Miller, 1996). However, explanations on factors favoring or associated to miniaturization in neotropical fishes are not common in the literature. Roberts (1972) suggested a close relationship between the high frequency of miniatures in the Amazon with the rich biological interactions due to the high diversity of fishes. According to Roberts (1972), small size fishes could have access to food resources not available to the larger species, and an extremely reduced size would be below the size which predaceous fishes are instigated to make attack. Unfortunately, nothing is known about ecological interactions of miniature rivulid fishes to provide more detailed arguments for a discussion on feeding habits or potential predators of rivulids. The occurrence of ichthyophagous fishes *(e.g., Hoplias spp.)* in temporary pools is not common, but larger annual fishes may be very vulnerable to terrestrial predators. In the Province of Buenos Aires, in temporary pools at the beginning of the dry season, smaller annual fish species *(Austrolebias bellottii, A. nonoiuliensis,* and *A. nigripinnis)* are very abundant. However, the large *Megalebias elongatus* is extremely rare, with an individual presenting a deep mark of bird beak that showed to be fatal. Similar marks were observed in preserved specimens in ichthyological museum collections.

Besides all those four miniature rivulids being annuals, all are sympatric. In contrast, in more than one hundred areas of endemism for annual fishes scattered over all the Neotropical region, no similar event of miniaturization was recorded. Thus, the coincident unique occurrence of four independent, evolutionary events of miniaturization within a same area of endemism strongly suggests that something in this area may favor phenomenons of miniaturization.

Despite a reported relationship between small size in fishes and spatially restricted habitats (Warburton, 1989), among the seasonal aquatic habitats of annual fishes, the area inhabited by the rivulid miniatures is among the more vast temporary swamps. This area comprises a broad floodplain zone of the Araguaia river, in central Brazil, forming a long temporary swamp with more than 10 km of interconnected pools, about one meter deep. However, phytological, climatic and topological conditions are similar to those in other flooded areas inhabited by other annual fishes.

Furthermore, this area is also unique by covering the richest annual fish fauna, with seven species living in sympatry. Two of the miniature species, *S. costai* and *S. semiocellatus,* live at midwater, in places with dense aquatic vegetation; *P. lacerdai* and *P. aruana* are also midwater fishes, but scattered over most portions of the pool; *Pituna compacta* is found only at the borders of the pool, in shallow places (about five centimeters deep); *Trigonectes rubromarginatus* lives close to the surface; and *Maratecoara lacortei* is found close to the bottom, in the deeper parts of the pool. In other areas of endemism, usually the maximum number of sympatric rivulid species is three, rarely four or five, and spatial preferences are not so clear. Therefore, the degree of annual fish diversity in a temporary pool seems to be closely related to miniaturization.

Other possible topologies

As mentioned in "Materials and Methods", all multistate characters of the present analysis were analyzed as unordered. This option was chosen to avoid hypotheses of order, which aprioristically constrains the number of most-parsimonious trees. However, 16 of the 22 multistate characters could be interpreted as morphoclines, and analyzed as ordered, both in sequences tending to structure simplification (16, 34,45, 50, 89,100, and 194) or sequences directed to higher degrees of structure elaboration (17. 41, 103, 111, 148. 169, 222, and 227), according to outgroup comparison. The six other multistate characters (188. 196, 206. 207, 220, and 237) cannot be ordered because they are relative to distinct patterns without a clear sequence.

A distinct topology of the strict consensus cladogram of the equally most parsimonious trees was obtained when those 16 characters were ordered linearly and sequentially.

According to this topology, *Rivulus* would be paraphyletic and all annual fish taxa would form a clade; *Millerichthys* would be the sister group to the Cynolebiatinae; *Millerichthys* plus Cynolebiatinae, the sister group to Rachoviini; and Rachoviina, not monophyletic. Relationships within the Neofundulidi, Plesiolebiatina, and Cynolebiatinae would be the same as proposed here. That strict consensus cladogram, obtained from the 704 equally most parsimonious trees, presented length 451, consistency index 0.61, and retention index 0.82, being therefore 21 steps longer, and having CI 3% lower than the hypothesis followed in the present study.

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Appendix 1. Specimens of Rivulidae examined for the present study. Number of specimens in each lot is preceded by a comma after catalogue numbers, indicating between parentheses holotypes (H), paratypes (P), and number of specimens cleared and stained for bone and cartilage (C&S). Generic combinations are according to the present paper. Order is alphabetical. Abbreviations for institutions are: CAS, California Academy of Sciences, San Francisco; ILPLA, Instituto de Limnología Raúl Ringuelet, La Plata; MCP, Museu de Ciências da Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre; MCZ, Museum of Comparative Zoology, Cambridge; MLP, Museo La Plata, La Plata; MNK, Museo de Historia Natural Noel Kempff, Santa Cruz; MNRJ, Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro; MUSM, Museo de Historia Natural de la Universidad Mayor de San Marcos, Lima; MZUSP, Museu de Zoologia, Universidade de São Paulo, São Paulo; NRM, Naturhistoriska Riksmuseet, Stockholm; UFRJ, Laboratório de Ictiologia Geral e Aplicada, Universidade Federal do Rio de Janeiro, Rio de Janeiro; UMMZ, University of Michigan, Museum of Zoology, Ann Arbor; USNM, National Museum of Natural History, Smithsonian Institution, Washington; ZVCM, Facultad Ciencias, Zoologia Vertebrados, Montevideo.

Aphyolebias obliquus: UFRJ 3034,4 (P); UFRJ 3035, 2 (P; C&S); A. *peruensis:* MZUSP 38394,1; MZUSP 38457,1 (C&S); A *rubrocaudatus:* MUSM 3119, 7 (1 C&S); *A. cf. wischmanni:* MUSM 3116, 6 (1 *C&S); Austrofundulus limnaeus:* USNM 218055,17; USNM **122005, 2; UFRJ 3912,** 2 (C&S); **MZUSP** 37200, 2; MZUSP 38415, 2 (C&S); *A. transilis:* MZUSP 38389. 4; MZUSP 38429, 1 (C&S); *Austrolebias adloffi:* MCP 10933, 22 (2 C&S); *A. affinis:* MZUSP 36448, 1 (P); MZUSP 36449, 1 (P); A. *alexandri:* MZUSP 38473. 2 (C&S); MNRJ 11720, 4; A. *bellottii:* ILPLA 997, 9 (2C&S); MZUSP 38416,1 (C&S); A. *carvalhoi:* MNRJ 5760. 4 (P); UFRJ 4444. 2; UFRJ 4445, 2 (C&S); *A. cinereus:* MZUSP 38504,1 (C&S); A. *cyaneus:* UFRJ 270, 11; UFRJ 271, 1 (C&S); *A. gymnoventris:* MZUSP 36450, 1 (P); MZUSP 36451. 1 (P); A *luteoflammulatus:* MCP 15048, 2; UFRJ 4460, 2 (C&S); A. *nigripinnis:* UFRJ 2082.1 (C&S); UFRJ 4472, 4 (C&S); A. *nonoiuliensis:* UFRJ uncat., 10 (4C&S); A. *vanderbergi:* UFRJ 3028, 2 (P); UFRJ 3029, 4 (P; C&S); A *viarius:* MCP 15050. 4 (1 C&S); *Austrolebias sp.* 1: UFRJ 4671, 12; UFRJ 4672, 6 (C&S); *Austrolebias sp. 2:* MCP 10201, 2; *Austrolebias sp.* 3: UFRJ 4016, 9; UFRJ 4014. 7 *(C&S); Austrolebias sp. 4:* UFRJ 4723. 38; UFRJ 4724. 8 (C&S); *Campellolebias brucei:* UFRJ 293, 6; UFRJ 1854, 2 (C&S); UFRJ 4494, 6 (C&S); C. *chrysolineatus:* MZUSP 38344, 11 (P); MZUSP 38428,2 (P; C&S); UFRJ 284,2; C. *dorsimaculatus:* **UFRJ 2192, 2** (C&S); **MZUSP 38815, 6** (P); *Cynolebias albipunctatus:* UFRJ 160, 35; UFRJ 647, 2 (C&S); UFRJ 4467, 6 (C&S); *C. griseus:* UFRJ 2098.14; UFRJ 319,1 (C&S); UFRJ 150.2 (C&S); C. *leptocephalus:* UFRJ 687, 2 (P); UFRJ 688,1 (P; C&S); C. *microphthalmus:* MZUSP 38343, 3 (P); *C. perforatus:* UFRJ 2089. 7; UFRJ 2077, 2 (C&S); UFRJ 4468, 2 (C&S); C. *porosus:* UFRJ 648, 10; UFRJ 649,18; UFRJ 650, 2 (C&S); UFRJ 4514,2 (C&S); UFRJ 4467, 2 (C&S); *Cynolebias sp.:* UFRJ 4470, 3; UFRJ 4471, 2 (P; C&S); *Cynopoecilus* *melanotaenia:* UFRJ 4008, 25; UFRJ 1857, 2 (C&S); UFRJ 4653, 6 (C&S); *Cynopoecilus sp.:* UFRJ 4489,13; UFRJ 4490,6 (C&S); *Gnatholebias hoignei:* MZUSP 38391, 4; MZUSP 38439, 2 (C&S); UFRJ 4578. 5 (C&S); G. *zonatus:* MZUSP 38392. 4; MZUSP 38392, 2 (C&S); UFRJ 3166, 2 (C&S); *Leptolebias aureoguttatus:* UFRJ 199, 7; UFRJ 172, 2 (C&S); L. *cruzi:* UFRJ 173,1 (C&S); MZUSP 36298. 8 (P); UFRJ 3678, 2 (all C&S); *L. fluminensis:* UFRJ 2202, 24; UFRJ 175, 1 (C&S); UFRJ 3679, 4 (C&S); *L. leitaoi:* UFRJ 171, 1 (C&S); *L. marmoratus:* SU(CAS) 36524, 10; *L. minimus:* UFRJ 2201,8; UFRJ 174.1 (C&S); UFRJ 3680, 4 (C&S); *L. sandrii:* MNRJ 11302. 23; MZUSP 38443, 2 (C&S); *Maratecoara formosa:* UFRJ 2112. 4 (P); UFRJ 2111, 4 (P; C&S); *M. lacortei:* UFRJ 3551, 2; UFRJ 3555, 2; MZUSP 41390, 3 (C&S); UFRJ 3556,1 (C&S); UFRJ 3542. 1 (C&S); *Megalebias cheradophilus:* UFRJ 4458, 2 (C&S); M. *elongatus:* MNRJ 11400, 2; MLP 8287, 2 (C&S); *M. monstrosus:* UMMZ 201175, 2; UMMZ 216569, 1; M. *wolterstorffi:* MZUSP 38421, 1 (C&S); MNRJ 11381, 8; UFRJ 4593, 6 (C&S); *Micromoema xiphophorus:* UFRJ 3165, 1 (C&S); *Millerichthys robustus:* UMMZ 194706, 14 (P; 4 C&S); UFRJ 4597, 4 (P); UFRJ 4598. 2 (C&S); *Moema pepotei:* MUSM 3069,6 (1 *C&S); M. piriana:* UFRJ 315,2; MZUSP 38515,1 (P; C&S); MZUSP 38516, 1 (P; C&S); *M. portugali:* UFRJ 262, 13; MZUSP 38512, 1 (P; C&S); UFRJ 283. 1 (C&S); *M. staecki:* UFRJ 317. 1 (C&S); *Moema sp.:* UFRJ 4594, 2 (C&S); *Nematolebias myersi:* UFRJ 249, 28; UFRJ 3161, 3 (C&S); UFRJ 282, 1 (C&S); *N. whitei:* UFRJ 2071.15; UFRJ 2127,1 (C&S); UFRJ 3159, 3 (C&S); *Nematolebias sp.:* UFRJ 4465, 10; UFRJ 4652, 2 (C&S); *Neofundulus ornatipinnis:* MLP 31-X-73-11, 4; UFRJ 2113, 1 (C&S); *N. paraguayensis:* UFRJ 3647, 17; UFRJ 3648, 4 (C&S); MZUSP 36617. 1(C&S); *N. parvipinnis:* **MZUSP 36619,1** (P; C&S); **MZUSP 36620.5** (P); UFRJ 267, 1 (C&S); *Papiliolebias bitteri:* UFRJ 3031, 2; UFRJ 3171, 3 (C&S); *Pituna compacta:* UFRJ 2115.2 (C&S); UFRJ 247,21; UFRJ 294.1 (C&S); UFRJ 3543, 16; UFRJ 3563, 33; UFRJ 3545, 3 (C&S); UFRJ 3564,4 (C&S); UFRJ 3935, 3 (C&S); *Plesiolebias aruana:* UFRJ 387, 15; UFRJ 2079, 2 (C&S); UFRJ 3557. 27; UFRJ 3558. 5 (C&S); UFRJ 3681,6 (C&S); P. *glaucopterus:* UFRJ 120, 2 (C&S); UFRJ 3672. 6 (C&S); UFRJ 3638. 8; *P. lacerdai:* UFRJ 1156,27; UFRJ 121, 2 (C&S); UFRJ 3547. 27; UFRJ 3548, 5 (C&S); *P. xavantei:* UFRJ 2096, 86; MZUSP 38417. 4 (C&S); UFRJ 122, 1 (C&S); *Pterolebias longipinnis:* UFRJ 3644, 9; UFRJ 3676, 2 (C&S); MZUSP 38420,1 (C&S); UFRJ 272,1 *(C&S}; P. phasianus:* UFRJ 3649, 38; **MZUSP 38110, 1** (P; C&S); **MZUSP 38113, 1** (C&S); **UFRJ** 273.1 (C&S); **UFRJ 3673,**4 (C&S); UFRJ 3675, 3 (C&S); *Rachovia brevis:* UFRJ 295.1 (C&S); R. *maculipinnis:* MZUSP 38390. 3; MZUSP 38505, 1 (C&S); MZUSP 38433, 1 (C&S); *R. pyropunctata:* USNM 219618, 6 (1 C&S); MZUSP 37202. 2 (1 C&S); MZUSP 38456, 2 (C&S); R. *stellifer:* UFRJ 245, 5 (C&S); *Renova oscari:* UFRJ 3164, 1 (C&S); UFRJ 4596. 3 (C&S); *Rivulus amphoreus:* UFRJ 4606, 3 (C&S); R. *atratus:* MZUSP 38310, 1; MZUSP 38449, 1 (C&S); *R. bahianus:* UFRJ 3167, 46; UFRJ 277, 1 (C&S); UFRJ 4602. 2 (C&S); *R. beniensis:* UFRJ 3637, 3; *R. brasiliensis:* UFRJ 3458. 32; UFRJ 3682, 2 (C&S); MZUSP 38427, 2 (C&S); UFRJ 4603, 2 (C&S); *R. brunneus:* MZUSP uncat. 10 (2 C&S); R. *campelloi:* ZVCM uncat.. I (P; C&S); *R. caudomarginatus:* UFRJ 1806, 32; UFRJ 3683, 2 (C&S); MZUSP 38474, 1 (C&S); UFRJ 4604, 2 (C&S); R. *chucunaque:* USNM 293487, 68 (1 C&S); R. *cladophorus:* UFRJ 643, 4 (P); *R. cryptocallus:* UFRJ 359, 3; UFRJ 2126,1 *(C&S); R. cylindraceus:* USNM uncat, 2 (C&S); R. *decoratus:* UFRJ 2134, 3; UFRJ 2135, 3 (C&S); UFRJ 2291. 2 (C&S); *R. depressus:* UFRJ 2118, 1 (C&S); *R. derhami:* UFRJ 392, 2 (C&S); R. *dibaphus:* INPA 2370. 6 (1 C&S); *R. elongatus:* MZUSP 26211. 3; *R. erberi:* UFRJ 358, 3; *R. fuscolineatus:* USNM 219778.10 *(P); R. geayi:* UFRJ 1820.1 (C&S); R. *haraldsiolii:* UFRJ 125,8; MZUSP 38450,1 (C&S); R. *hartii:* MZUSP 37204, 3; MZUSP 38472, 2 (C&S); *R. hildebrandi:* USNM 92958, 1 (P); *R. holmiae:* USNM 66302, 1 (P); *R. igneus:* UFRJ 4595. 2 (C&S); *R. immaculatus:* USNM 308411, 2 (P); *R. janeiroensis:* UFRJ 187,11; UFRJ 130. 2 (P; C&S); R. *luelingi:* UFRJ 161, 8; UFRJ 4607, 2 (C&S); *R. marmoratus:* USNM 174968, 1; *R. micropus:* MZUSP 38307, 21; MZUSP 38448, 2 (C&S); R. *modestus:* UFRJ 2102, 6; UFRJ 2103, 5 (C&S); *R. nudiventris:* MNRJ 11740. 2 (C&S); *R. obscurus:* MZUSP 37208. 3; MZUSP 38440, 2 (C&S); *R. ocellatus:* UFRJ 1806, 3; MZUSP 38441. 1 (C&S); MZUSP 38451,1 (C&S); UFRJ 3684,1 (C&S); R. *ornatus:* MZUSP 38494, 12; R. *pictus:* UFRJ 2209,17; UFRJ 2278,4 (C&S); UFRJ 2280, 3 (C&S); UFRJ 2279, 4 (C&S); UFRJ 2110, 4 (C&S); *R. punctatus:* UFRJ 141, 7; UFRJ 2110, 4 (C&S); *R. rubrolineatus:* MZUSP 26371, 3; *R. santensis:* UFRJ 123, 4; UFRJ 278, 1 (C&S); -R. *stagnatus:* USNM 66303, 1 (P); UFRJ 4605, 4 (C&S); R. *tecminae:* UFRJ 20858, 2; UFRJ 2095, 1 (C&S); *R. tenuis:* UFRJ 4600, 4; UFRJ 4601, 2 (C&S); *R. urophthalmus:* INPA 2244, 20; MZUSP 38471,2 (C&S); UFRJ 3055,6 (C&S); R. *violaceus:* UFRJ 143,20; UFRJ 1852, 7 (C&S); R. *xiphidium:* **UFRJ 4608, 3** (C&S); **R. *zygonectes:* UFRJ 1414, 25; UFRJ 2108,** 4 (C&S); **UFRJ 2107,** 4 (C&S); *Simpsonichthys alternatus:* UFRJ 2130,11 (P); UFRJ 2131, 4 (P; C&S); *S. antenori:* MZUSP 38342, 29; MZUSP 38422, 1 (C&S); *S. boitonei:* UFRJ 3158. 1 (C&S); MZUSP 38418,1 (C&S); UFRJ 3158.1 (C&S); *S. bokermanni:* UFRJ 1836.14; UFRJ 3162, 1 (C&S); MZUSP 38432, 2 (C&S); *S. chacoensis:* UFRJ 3030,1; UFRJ 3170,1 (C&S); *S. constanciae:* **UFRJ 2199,**47; **UFRJ 1978,3** (C&S); **UFRJ 1851.**

6 (C&S); *S. costai:* MZUSP 38437, 2 (C&S); UFRJ 162, 2 (C&S); UFRJ 3549, 45; UFRJ 3350, 4 (C&S); UFRJ 3561 (C&S); *S. filamentosus:* CBF 3003. 7 (P); UFRJ 3989,4 (P); UFRJ 3990, xxx; *S. flammeus:* UFRJ 2081, 116; UFRJ 280, 1 (C&S); *S. flavicaudatus:* UFRJ 2094, 24; UFRJ 3160, 4 (C&S); UFRJ 281,1 (C&S); UFRJ 2125,4 (C&S); *S. fulminantis:* UFRJ 686, 3 (P); UFRJ 685, 2 (P; C&S); *S. ghisolffi:* UFRJ 3526, 2 (P); UFRJ 3527, 2 (P; C&S); *S. hellneri:* UFRJ 2093, 61; UFRJ 2080, 4 (C&S); S. *magnificus:* UFRJ 154. 12; UFRJ 260. 1 (C&S); *S. marginatus:* UFRJ 7 3536, 7 (P); UFRJ 3537, 4 (P; C&S); *S. multiradiatus:* UFRJ 2076. 8 (P); UFRJ 2075,6 (P; C&S); *S. notatus:* UFRJ 2068, 27; UFRJ 268,1 (C&S); *S. stellatus:* UFRJ 2066, 5 (P); UFRJ 2067, 4 (P; C&S); *S. trilineatus:* UFRJ 2133, 2 (P); UFRJ 2132,1 (P; C&S); UFRJ 4670, 5 males and 5 females (C&S); *S. zonatus:* UFRJ 142, 6; UFRJ 279, 1 (C&S); *Simpsonichthys sp.* 1: UFRJ 4667, 10 (C&S); *Simpsonichthys sp.* 2: UFRJ 4147, 5 (C&S); *Spectrolebias semiocellatus:* UFRJ 3632, 6 (P); UFRJ 3633, 5 (P; C&S); *Stenolebias bellus:* MZUSP 42310, 1 (H); UFRJ 386, 1 (P; C&S); *S. damascenoi:* MZUSP 41391,1 (H); MZUSP 41392, 2 (P; C&S); *Terranatos dolichopterus:* MZUSP 38414, 2 (C&S); USNM 219618.21; UFRJ 3910,1; UFRJ 3911, 3 (C&S); UFRJ 4597, 1 (C&S); *Trigonectes aplocheiloides:* NRM 15931, 2; USNM 230033. 1; USNM 230034, 1; MNKP 138. 4 (2 C&S); *T. balzanii:* UFRJ 3677, 18; UFRJ 3670. 1 (C&S); UFRJ 3671, 6 (C&S); MZUSP 38452,1 (C&S); *T. macrophthalmus:* MNRJ 11308, 1 (H); MZUSP 38453.1 (P; C&S); MZUSP 38454,1 (P; C&S); *T. rogoaguae:* CAS 42532, 20; *T. rubromarginatus:* UFRJ 3553. 13; MZUSP 38455, 1 (C&S); UFRJ 274, 1 (C&S); UFRJ 3554. 3 (C&S); *T. strigabundus:* UFRJ 2100,31; UFRJ 2114,2 (C&S).

Errata corrigenda. Include this Appendix 2 for the paper of Costa.

Appendix 2. Data matrix of 243 characters used in the phylogenetic analysis of the Rivulidae. Character and character state numbers are according to those presented in the text. "P" indicates polymorphic taxa and "?" character states not pertinent. OG = outgroup; RB = *Rivulus brasiliensis;* RC = *Rivulus caudomarginatus;* RO = *Rivulus ocellatus* group; RY = *Rivulus cylindraceus;* RU = *Rivulus urophthalmus* group; RR = *Rivulus cryptocallus;* RB = *Rivulus brunneus;* RA = *Rivulus amphoreus;* RH = *Rivulus hartii;* RJ = *Rivulus janeiroensis* group; RT = *Rivulus atratus;* RS = *Rivulus obscurus;* RP = *Rivulus punctatus* group; RG = *Rivulus geayi* group; RI = *Rivulus bahianus;* RE = *Rivulus tenuis;* RX = *Rivulus xiphidium;* PL = *Pterolebias longipinnis;* PP = *Pterolebias phasianus;* PZ = *Pterolebias zonatus* group (= *Gnatholebias);* PX = *Pterolebias xiphophorus* (= *Micromoema xiphophora);* PE = *Pterolebias peruensis* group (=*Aphyolebias);* NE = *Neofundulus;* RN = *Renova;* TR = *Trigonectes;* MO = *Moema;* RV = *Rachovia (R. brevis* group); RL = *Rivulus stellifer* (=*Rachovia stellifer);* AU = *Austrofundulus;* TE = *Terranatos;* MI = *Millerichthys;* PI = *Pituna;* PA = *Papiliolebias;* PS = *Plesiolebias;* MA = *Maratecoara;* ST = *Stenolebias;* SP = *Spectrolebias;* SW = *Simpsonichthys whitei* group (= *Nematolebias};* SC = *Simpsonichthys constanciae* group (= *Simpsonichthys);* CP = *Cynolebias porosus* group; CG = *Cynolebias griseus;* CW = *Cynolebias wolterstorffi* group (= *Megalebias}; Cynolebias bellottii* group (= *Austrolebias};* LE = *Leptolebias;* CY = *Cynopoecilus;* CA = *Campellolebias.*

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    00100 00000 00000 P0110 00010 0001? 00P00 10000 00000 00010 00001 00010 11010 00000 00100 10000 00000 00000 00000 010
RL  11001 00000 11100 00000 00100 00000 00010 10010 10001 00000 01000 10100 01000 00001 00010 00000 10110 00010 11000 00010 10100 00010 00000 00000 00000 00000 00001 00010 00010
    00100 00000 00000 00010 00010 0001? 00100 00000 00000 00000 20001 00010 10000 00000 00102 01000 00000 00000 00000 010
AU  11001 00001 11100 00000 00100 00000 00010 00010 10001 00000 01000 10100 01000 00001 00010 00000 10010 00010 11001 01011 00100 00011 00000 00000 00000 00000 00001 00010 00010
    00100 00000 00000 10110 00010 000?? 00100 00000 00000 00010 00001 10010 11000 00000 00100 00000 00000 00000 00000 010
TE  11001 00000 11100 00000 00100 00000 00010 00000 10001 00000 00000 10100 01000 00001 00000 00000 10010 00110 11001 01011 00100 00000 30000 00001 00000 00000 00001 01000 00010
    00100 10011 00000 10010 00010 00000 00000 00000 00000 00010 00001 00010 31000 00000 00000 00000 00000 00000 00000 010
Ml  11001 00000 11100 00000 00100 00000 00020 00010 10002 00000 00000 10100 01000 00001 00000 00000 10010 00010 01000 00011 00300 00000 00000 00000 00000 00000 00101 00010 00010
    00100 00000 00000 00010 00010 000?0 00000 10000 00000 00010 00000 00000 00000 00000 00000 01000 00100 00000 00000 0?0000?0
PI  11001 00010 11100 10000 00100 00000 00011 00010 10002 00000 00010 10110 01000 00001 00000 00000 10010 00010 11010 00011 00200 00000 00000 00000 00000 00000 00001 00010 00010
    01100 00001 00000 00010 00010 0001? 00000 00000 00000 00000 10001 00010 10000 00001 00101 00000 00001 00000 00000 010
PA  11001 00010 11100 10000 00100 00000 00011 00010 10002 00000 00110 10110 01000 00001 00000 00000 10010 00010 11010 00011 00300 10000 00000 00000 00000 00000 00001 00000 00010
    00200 00000 00000 00010 00010 0001? 00000 10000 00000 00010 10001 00010 10000 00000 00100 00000 00000 00000 02000 010
PS  11001 00010 11100 10000 00100 00000 10011 00010 20102 00000 00110 10110 11010 00001 00000 00000 10010 00010 11010 00011 00300 10100 10000 00000 00000 00000 01001 00000 00010
    00100 00000 01000 00010 10010 00000 01000 10000 00010 00011 00001 00010 10000 00000 00100 00000 00000 00000 02000 010
MA  11001 10010 11100 10000 00100 00010 00011 00010 10002 00000 00110 10110 01000 00101 00000 00000 11010 01010 11011 00011 00200 10000 20000 01000 00000 00000 01001 00000 00010
    00100 00011 00001 00011 00010 00000 00000 10000 00000 01010 00001 00010 12100 00001 00000 00000 00000 00000 00000 010
ST  11001 10010 11100 10000 00100 00000 00011 00010 10002 00000 00110 11110 01000 00001 00000 00000 11010 00010 11010 00011 00300 10000 10000 00000 00000 00000 01001 00000 00010
    00100 00001 00000 00010 00010 0001? 00000 10000 00000 00010 00001 00010 10000 00001 00100 00000 00000 00000 00000 010
SP  11101 00000 11110 00000 00100 00100 01030 00000 00013 01000 00000 10101 01000 00000 11100 1?000 10010 00020 0?110 00012 00301 00000 20100 01010 00000 11100 01011 10101 01011
    00000 00000 00000 00010 00120 01100 10?11 11100 01000 00000 00010 00011 00000 00000 00010 00000 00000 00000 OPOOO010
SW  11001 00100 11110 02000 10100 00100 00030 00001 00003 01000 00001 10101 01000 01000 11100 P?000 10010 00020 01010 00012 00301 00000 20100 01010 00000 11100 00011 10101 01011
    00001 00001 00000 00010 00020 01000 10?11 11100 00000 00100 00010 00010 20000 00000 00000 00001 00000 00000 01P00 010
SC  11001 00000 11110 01000 10101 00100 00030 00001 00003 01000 00001 10101 01000 00000 11100 1?000 10010 00020 01011 00012 00301 00000 20100 01010 01000 11100 00011 10101 01011
    00001 00001 00000 00010 00020 01000 10P11 11100 00000 00100 00010 00010 00000 00000 00000 00001 00000 00000 01000 010
CP  11001 00000 11110 00100 00100 00100 00130 01101 00004 01000 00001 10101 01000 11000 11101 P?000 10011 10030 O?111 00012 00301 00010 10000 01010 00000 11100 01011 10101 01011
    10001 00000 00000 00010 01020 01000 10?11 11100 00000 00100 00010 00010 00000 00010 00000 00000 00000 00000 01000 010
CG  11001 00000 11110 00100 00100 00100 00130 01101 00004 01000 00001 10101 01000 00000 11100 1?000 10011 10030 0?111 00012 00301 00010 10000 01010 00000 11100 00011 10101 01011
    10001 00000 00000 00010 01020 01000 10?11 11100 00000 00100 00010 00010 00000 00010 00000 00000 00000 00000 01000 010
CW  01001 00000 11111 20000 00100 00100 00130 01101 00004 010P0 00001 10101 01000 01000 11100 1?000 10011 10031 0?111 00012 00301 00010 10000 01010 00000 01100 00011 10101 01111
    10001 00000 00000 00010 01020 01000 10?11 11100 00000 00100 00010 00010 33000 00000 00000 00000 00000 00000 01000 010
CB  11001 00000 11111 20000 00100 00100 00130 00101 00003 01010 00001 00101 01000 00000 11100 1?000 10010 00030 0?111 00012 00301 00000 20100 01010 00000 01100 01011 10101 01011
    10001 00000 00000 00010 00020 01000 11?11 11100 00000 00100 00010 00010 33000 00000 00000 00000 00000 00000 01000 010
LE  11001 01000 11100 3?000 00100 00100 00030 00000 01003 00000 00000 10101 01000 00000 11100 1?000 10010 00020 01010 00012 00301 00000 20000 01010 00000 00000 01011 00000 10011
    00000 00000 00000 00010 00020 01001 10?11 10110 00000 00020 00010 00110 20000 00000 00000 00000 00000 00011 00100 010
CY  11001 00000 11100 3?000 00100 01100 00030 00000 00003 00000 00000 10101 01000 00000 11100 1?110 10010 00020 0?110 00012 00301 01000 20011 01010 00011 00100 11011 00000 00011
    00000 01001 00000 00010 00020 11001 10?11 10111 00001 10000 00000 00010 00000 00000 00000 00000 00000 00011 12110 010
CA  11001 00000 11100 3?000 00100 00100 00030 00000 00003 00000 00000 10101 01000 00000 11100 1?111 10010 00020 0?110 00012 01201 00000 30010 10110 00100 00001 01011 00000 00011
    00000 00001 10000 00010 00031 01001 10?11 10111 10000 00000 00000 00110 00001 00000 00000 00000 00000 00011 12101 010
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