



Comparative morphology, phylogenetic relationships, and historical biogeography of plesiolebiasine seasonal killifishes (Teleostei: Cyprinodontiformes: Rivulidae)

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Phylogenetic and biogeographical analyses were performed for the Plesiolebiasini, a group of 20 small and rare species of South American annual killifishes, some threatened with extinction, occurring in river basins of Brazil, Bolivia, Paraguay, and Argentina. The results of a maximum parsimony analysis of 142 morphological characters highly corroborate monophyly of the Plesiolebiasini. Monophyly of each plesiolebiasine genus is supported and *Plesiolebias* is hypothesized to be the sister group to a clade comprising the remaining plesiolebiasine genera (*Papiliolebias*, *Pituna*, *Maratecoara*, and *Stenolebias*), corroborating studies based on mitochondrial genes. The phylogenetic analysis supports sister group relationships between *Papiliolebias* and the clade containing *Pituna*, *Maratecoara*, and *Stenolebias*, and between *Maratecoara* and *Stenolebias*. The biogeographical analysis indicates a complex historical biogeographical scenario for plesiolebiasines. A vicariance event between the western Paraguay area and northern river basins may be related to the isolation of the Chaco region from the Amazon between the Late Oligocene and Early Miocene. A vicariance event involving the Paraguay River basin and northern rivers, and the multiple occurrence of area hybridism along the Araguaia depression are tentatively identified as a consequence of tectonic subsidence events occurring during the Pleistocene.

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INTRODUCTION

Annual fishes are specialized members of the killifish suborder Aplocheiloidei, with their entire biological cycle occurring in seasonal freshwater swamps of tropical and subtropical areas of South America and Africa. Typical annual fish habitats are temporary pools or swamps that dry once or twice a year, when all specimens die (Costa, 1995a). The eggs, protected by a thickened chorion, undergo diapause until the beginning of the next rainy season, when egg eclosion is followed by quick development. Evolutionary innovations related to annualism involve a combination of

morphological, physiological, and behavioural features, which allow the colonization of temporary swamps, a kind of habitat mostly inaccessible for other teleost fishes (Costa, 1998a). Despite the complexity of this lifestyle, annualism has been hypothesized to be independently acquired in different lineages of aplocheiloid killifishes (Parenti, 1981; Costa, 1990, 1998a; Hrbek & Larson, 1999).

Plesiolebiasini is a clade of small annual fishes (maximum adult size 40 mm standard length [SL]), inhabiting temporary swamps of central and north-eastern South America (Brazil, Bolivia, Paraguay, and Argentina) (Costa, 1998a, 2007a). It comprises five genera and 20 species. *Papiliolebias* Costa, with a single species, *Papiliolebias bitteri* (Costa, 1989), is

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endemic to the Chaco region of the Paraguay River basin (Costa, 1998a, b). *Stenolebias* Costa, with two species, *Stenolebias damascenoi* (Costa, 1991) and *Stenolebias bellus* (Costa, 1995), is known from a few specimens collected in the Brazilian Pantanal region of the Paraguay River basin (Costa, 1991, 1995b). *Maratecoara* Costa comprises *Maratecoara formosa* Costa & Brasil, 1995 and *Maratecoara splendida* Costa, 2007 from the Tocantins River basin, and *Maratecoara lacortei* (Lazara, 1991) from the Araguaia and Mortes river basins (Costa, 2007a). *Pituna* Costa includes *Pituna xinguensis* Costa & Nielsen, 2007 from the Xingu River basin, *Pituna compacta* (Myers, 1927) from the Tocantins River basin, *Pituna poranga* Costa, 1989 and *Pituna obliquoseriata* Costa, 2007 from the Araguaia River basin, *Pituna schindleri* Costa, 2007 from the Parnaíba River basin, and *Pituna brevirostrata* Costa, 2007 from the upper Paranaíba River drainage of the Paraná River basin (Costa, 2007a). *Plesiolebias* Costa includes *Plesiolebias xavantei* (Costa, Lacerda & Tanizaki, 1988), *Plesiolebias filamentosus* Costa & Brasil, 2007 and *Plesiolebias canabravensis* Costa & Nielsen, 2007 from the Tocantins River basin, and *Plesiolebias aruana* (Lazara, 1991), *Plesiolebias lacerdai* Costa, 1989, and *Plesiolebias fragilis* Costa, 2007 from the Araguaia River basin, *Plesiolebias altamira* Costa & Nielsen, 2007 from the Xingu and Tapajós river basins, and *Plesiolebias glaucopterus* (Costa & Lacerda, 1988) from the Paraguay River basin (Costa, 2007a).

Amongst the 20 species of the Plesiolebiasini, only one was described before 1988 (Myers, 1927), whereas eight were described in 2007 (Costa, 2007a). Plesiolebiasines are often rare in their habitats and some species endemic to central Brazil are highly threatened with extinction. The greatest diversification of the Plesiolebiasini is concentrated in the Araguaia and Tocantins river basins, where species of *Pituna*, *Maratecoara*, and *Plesiolebias* are often sympatric. Species of this area also exhibit the most expressive divergence in morphology and habitat preference, where the slender species of *Pituna* inhabit the shallowest parts of the pools, the long-finned and deep-bodied species of *Maratecoara* are only found at the deepest parts of the pool, and the miniature species of *Plesiolebias* live at the mid-water column, close to dense clumps of aquatic plants (Costa, 1998a, 2007a).

In spite of its morphological diversity, monophyly of the Plesiolebiasini is supported both by morphology (Costa, 1998a) and by DNA sequences (Hrbek & Larson, 1999; Murphy, Thomerson & Collier, 1999). However, phylogenetic studies involving plesiolebiasine taxa have been based on very few terminal taxa (between four and six). Molecular data are available for only five plesiolebiasine species (Hrbek & Larson, 1999; Murphy *et al.*, 1999) and morphological studies

included a few characters considered as informative for reconstruction of phylogenetic relationships amongst the plesiolebiasines known at that time (Costa, 1995b, 1998a). The objective of this study was to provide a phylogenetic analysis based on a detailed survey of morphological characters of all the species of the Plesiolebiasini, which was then used as a historical background for the first biogeographical analysis of the group.

MATERIAL AND METHODS

PHYLOGENETIC ANALYSIS

Terminal taxa included all species of the Plesiolebiasini, which were delimited in previous taxonomic studies by a unique combination of morphological character states, following the methodology formally known as population aggregation analysis (Davis & Nixon, 1992). In order to test the hypothesis of Plesiolebiasini monophyly, additional taxa included were *Pterolebias longipinnis* Garman, 1895, *Rachovia maculipinnis* (Radda, 1964), *Rivulus janeiroensis* Costa, 1991, *Rivulus pictus* Costa, 1989, and *Trigonectes rubromarginatus* Costa, 1990, each representing distinct rivuline lineages (Costa, 1998a; Hrbek & Larson, 1999; Murphy *et al.*, 1999). Additional outgroups were four nonrivuline aplocheiloid taxa: *Kryptolebias brasiliensis* (Valenciennes, 1821), *Nematolebias whitei* (Myers, 1942), *Scriptaphyosemion guignardi* (Romand, 1981), and *Aplocheilus panchax* (Hamilton-Buchanan, 1822). A list of material examined appears in Appendix S1. Material herein illustrated is deposited in the ichthyological collection of the Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro (UFRJ). Osteological preparations were made according to Taylor & Van Dyke (1985). Nomenclature for cephalic laterosensory series follows Costa (2001). Terminology for frontal squamation follows Hoedeman (1958); the E-scale was determined as that scale adjacent to the anterior-most neuromast of the posterior section of the supraorbital series.

Character statements were formulated according to Sereno (2007). Colour patterns when consistently identified (i.e. all specimens of two or more terminal taxa sharing a similar colour pattern, composed of one or more colours, at the same position of a morphological structure) were treated as independent characters. Character states of all discrete characters were treated as unordered and are listed in Appendix S2. Morphometric and meristic data were median values taken from ranges available in taxonomic studies (Costa, 1991, 1995b, 2007a), treated as ordered continuous characters as proposed by Goloboff, Mattoni & Quinteros (2006). The distribu-

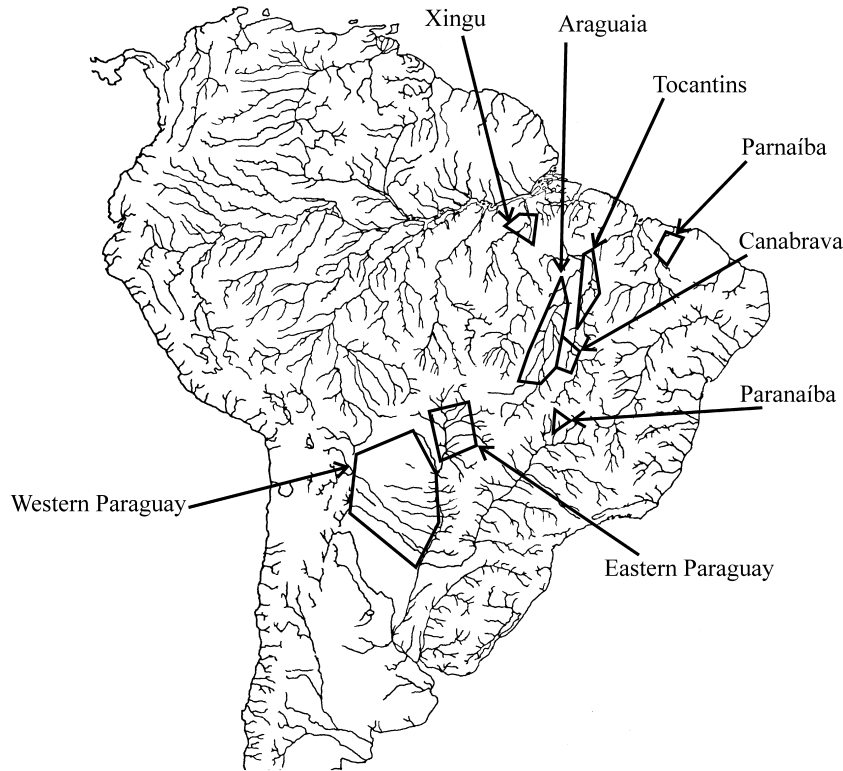


Figure 1. Areas of endemism of plesiolebiasine killifishes.

tion of characters states of discrete characters amongst taxa appears in Table S1, and continuous characters in Table S2. The search for most parsimonious trees (traditional search), bootstrap analysis (1000 replicates), and distribution of synapomorphies amongst nodes were performed with TNT 1.1 (Goloboff, Farris & Nixon, 2008).

BIOGEOGRAPHY

High congruence amongst the distribution patterns of the two most basal plesiolebiasine groups (the clade comprising all species of *Plesiolebias* and the clade comprising *Papiliolebias*, *Stenolebias*, *Maratecoara*, and *Pituna*) suggests a shared biogeographical history derived from a series of vicariance events. To search for a general vicariance biogeographical pattern for plesiolebiasines, each basal plesiolebiasine clade was analysed as an individual taxon. The analysis follows the component analysis methodology (e.g. Nelson & Platnick, 1981; Morrone, 2009), adopting a combination of assumptions (Page, 1990; Morrone & Crisci, 1995), in which Assumption 0 is used for redundant taxa, Assumption 1 for missing taxa, and Assumption 2 for widespread taxa. This combination is herein preferred by best preserving the biogeographical information available in the taxon area cladogram (see Fig. 7 in Results below).

Areas of endemism were sections or groups of river basins delimited by congruent distributional range shared by two or more rivulid species, usually corroborated by nonplesiolebiasine taxa; an exception is the Canabrava area, which was uniquely delimited by the congruent biogeographical pattern shared by two plesiolebiasine species. The following eight areas of endemism are herein analysed (Fig. 1): Parnaíba: middle section of the Parnaíba River basin; Tocantins: middle section of the Tocantins River basin; Canabrava: Canabrava River drainage, a western tributary of the Tocantins River; Araguaia: middle section of the Araguaia River basin; Xingu: middle-lower section of the Xingu River basin; Paranaíba: upper section of the Paranaíba River drainage, an upper tributary to the Paraná River; Eastern Paraguay: eastern part of the Paraguay River basin, corresponding to the Pantanal; and Western Paraguay: western part of the Paraguay River basin, corresponding to the Chaco.

RESULTS

CHARACTER ANALYSIS

The morphological description below focuses on structures presenting informative variability for the phylogenetic analysis. Characters and character states,

separated by a dot, appear in brackets and are numbered according to Appendix S2. Characters formerly discussed and illustrated elsewhere are briefly presented. The matrix of character state distribution amongst terminal taxa appears in Tables S1 and S2.

Autopalatine

As in other cyprinodontiforms (Parenti, 1981; Costa, 1998c), the autopalatine and the ectopterygoid are completely fused. The anteroventral pointed process, corresponding in position to the ectopterygoid of other teleost fishes, is short as in all other rivulids (Costa, 1998c), its tip not reaching the quadrate [1.1] (Fig. 2A). The anterior margin of the autopalatine is slightly concave, but in *Plesiolebias* and *Pterolebias* the concavity is pronounced because of an anterior projection of the anteroventral process [2.1] (Fig. 2A) (Costa, 1998a). The posterior margin of the autopalatine is straight or slightly concave in most aplocheiloids, whereas there is a deep concavity in *Plesiolebias* [3.1] (Fig. 2A). The posterior margin is formed by the main condyle of the autopalatine (Fig. 2C) in all terminal taxa except in plesiolebiasines and in *Ra. maculipinnis*, in which there is a distinct bony flange on the posterior marginal region [4.1] (Fig. 2D). In rivulids and nothobranchiids, there

is a process on the medial surface of the autopalatine, which supports a ligament attaching the autopalatine to the lateral ethmoid [5.1] (Fig. 2C–E). This process, absent in *Aplocheilus*, was named as the dorsomedial process of the autopalatine by Costa (1998a). It is primitively placed adjacent to the posterior margin of the bone, but in *Maratecoara*, the process is anteriorly expanded, projecting beyond the anterior margin of the autopalatine [6.1] (Fig. 2E).

Entopterygoid

The entopterygoid is a thin ossification on the dorsal border of the jaw suspensorium. Its anterior portion overlaps the ventral part of the autopalatine in aplocheiloids except plesiolebiasines (Costa, 1998a), in which the anterior portion of the entopterygoid is shorter, not reaching or gently touching the autopalatine [7.1] (Fig. 2A, B). The posterior tip of the entopterygoid is firmly attached to the anterior portion of the metapterygoid in most aplocheiloids; amongst the exceptions are all the species of *Plesiolebias*, *Stenolebias*, and *Pi. brevirostrata*, in which the posterior tip of the entopterygoid is not in contact with the metapterygoid [8.1] (Fig. 2A) (Costa, 1998a).

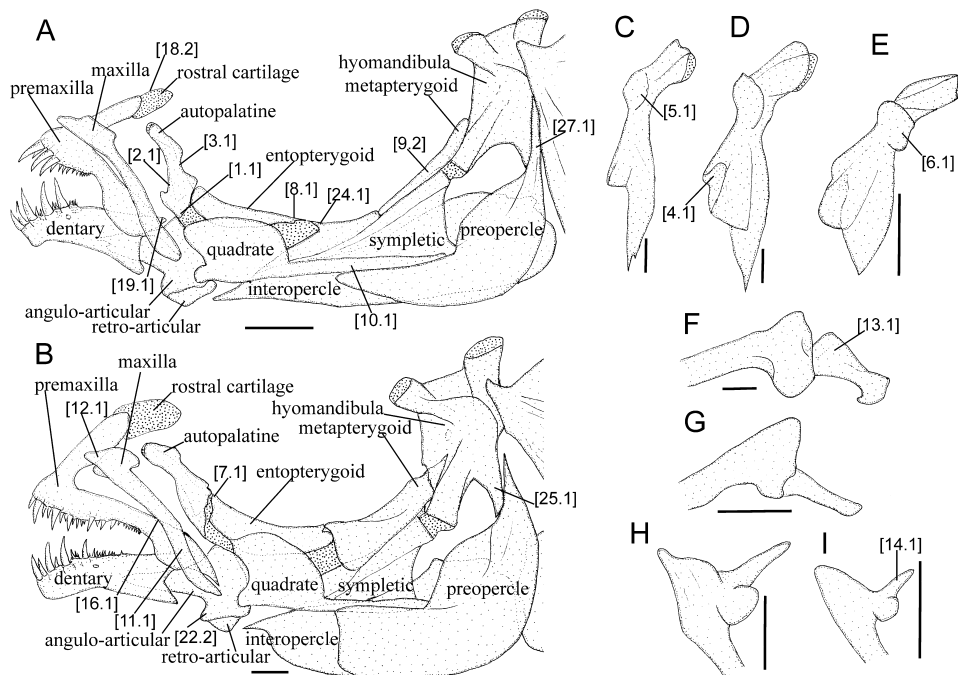


Figure 2. Jaws, jaw suspensorium, and opercular series: left jaw suspensorium and anterior part of opercular series, lateral view, of *Plesiolebias filamentosus* (A), and *Pituna compacta* (B); left autopalatine, medial view, of *Kryptolebias brasiliensis* (C), *Pi. compacta* (D), and *Maratecoara splendida* (E); left maxilla, proximal portion, dorsal view, of *Rachovia maculipinnis* (F), and *Pi. compacta* (G); left maxilla, proximal portion, posterior view, of *M. splendida* (H), and *Pl. filamentosus* (I). Numbers in brackets are characters and character states numbered according to Appendix S2. Larger stippling indicates cartilage. Scale bars = 0.5 mm.

Metapterygoid

The metapterygoid is a thin, approximately rectangular bone in basal aplocheiloids. In *Pt. longipinnis* and *Ra. maculipinnis*, the ventral portion is distinctively wider than the dorsal portion [9.1] (e.g. Costa, 2005). In plesiolebiasines, the metapterygoid is rectangular, except in *Plesiolebias*, in which the whole bone is narrowed [9.2] (Fig. 2A).

Quadrate

The quadrate is triangular as in other rivulids, bearing a posterior process, which is longer than the main body of the quadrate, a derived condition shared by other rivulids [10.1] (Fig. 2A, B).

Jaws

As in other rivulids, the maxilla is elongate and twisted (Parenti, 1981) [11.1], dorsally terminating as a bifid structure, in which a flat and usually triangular process projects dorsally to the premaxillary ascending process [12.1], and another process projects ventrally. The ventral process is flattened and approximately triangular in rivulids (Parenti, 1981) [13.1] (Fig. 2F), with the exception of the plesiolebiasines, in which the process is about cylindrical (Fig. 2G). In addition, the process is distinctively shorter in *Plesiolebias* than in any other aplocheiloid (i.e. longer than dorsal process, vs. shorter than dorsal process in *Plesiolebias*) [14.1] (Fig. 2H, I). The jaws are shorter than the jaw suspensorium [15.1], a condition occurring in most rivulids and nothobranchiids (Fig. 2A, B) (Costa, 1998c). The premaxilla is slender, with posterior border continuous without expansions [16.1], and bears a posteriorly directed, flattened ascending process, which is always rectangular [17.1], and overlaps a rectangular, longitudinally elongated rostral cartilage [18.2], as in other rivulids (Costa, 1998a, b) (Fig. 2A, B). Also as in other rivulids, the coronoid process of the dentary is short, not extending beyond the dorsal portion of the angulo-articular [19.1], and slender, approximately as deep as the angulo-articular [20.1] (Fig. 2A, B). Premaxillary and dentary teeth are arranged in irregular rows; teeth are conical, slightly curved, and all directed to the inner of the mouth (Fig. 2A, B), instead of laterally directed as occurs in the external teeth of *Pt. longipinnis* and *Ra. maculipinnis* (Costa, 2005) [21.1].

Angulo-articular

The angulo-articular is triangular with a ventral process, which is rudimentary in plesiolebiasines and in *Ri. pictus* (Costa, 1998a) [22.2] (Fig. 2A, B).

Symplectic

The symplectic is triangular, about as deep as long, or slightly longer than deep, except in all species of

Plesiolebias, in which the symplectic is distinctively more slender, about twice longer than deep [23.1] (Fig. 2A). In all the plesiolebiasine genera except *Pituna*, the symplectic has a process towards the posterior tip of the entopterygoid (Fig. 2A) that is also present in *Pt. longipinnis* and *Ra. maculipinnis* [24.1]. In *Pi. brevirostrata*, the process is absent, and in the remaining species of *Pituna*, the process is variably reduced or absent (Fig. 2B).

Hyomandibula

The posteroventral process of the hyomandibula is long as in all other rivulids [25.1] (Fig. 2A, B).

Preopercle

The preopercle of all the plesiolebiasines is thin (Costa, 1990, 1998a) [26.1], with a short and pointed dorsal portion [27.1] (Fig. 2A, B), a condition also occurring in *N. whitei* and *Ri. pictus*.

Interhyal

The interhyal is cartilaginous [28.1] and rudimentary [29.1] as in all other rivulines (Fig. 3A) (Costa, 2005). An ossified and well-developed interhyal is found in aplocheilids, nothobranchiids, and in *Kryptolebias*, whereas in cynolebiatines the interhyal is well developed but cartilaginous.

Branchiostegal rays

The presence of six branchiostegal rays in most plesiolebiasines is a condition shared by almost all aplocheiloids. This generalized aplocheiloid condition comprises two narrow and short rays situated on the anterior portion of the anterior ceratohyal, followed by four wider and longer rays along the ceratohyal bar. Fewer rays are present in *Stenolebias* and most species of *Plesiolebias*, in which there is one ray instead of two on the anterior part of the anterior ceratohyal [30.1] (Fig. 3A); just *Pl. glaucopterus* has six rays amongst all congeners.

Basihyal

Basihyal morphology is variable amongst plesiolebiasines. The basihyal is longer than the longitudinal length comprising the three basibranchials in *Papiliolebias*, *Plesiolebias*, and *Stenolebias* [31.1] (Fig. 3B). Only species of *Pituna* exhibit a plesiomorphic, triangular and wide basihyal, in which the anterior portion is wider than half the basihyal length (Fig. 3C). In the remaining plesiolebiasines, the basihyal is narrower anteriorly, assuming a subtriangular or rectangular shape [32.1] (Fig. 3B). The cartilaginous portion is always shorter than the ossified portion as occurring

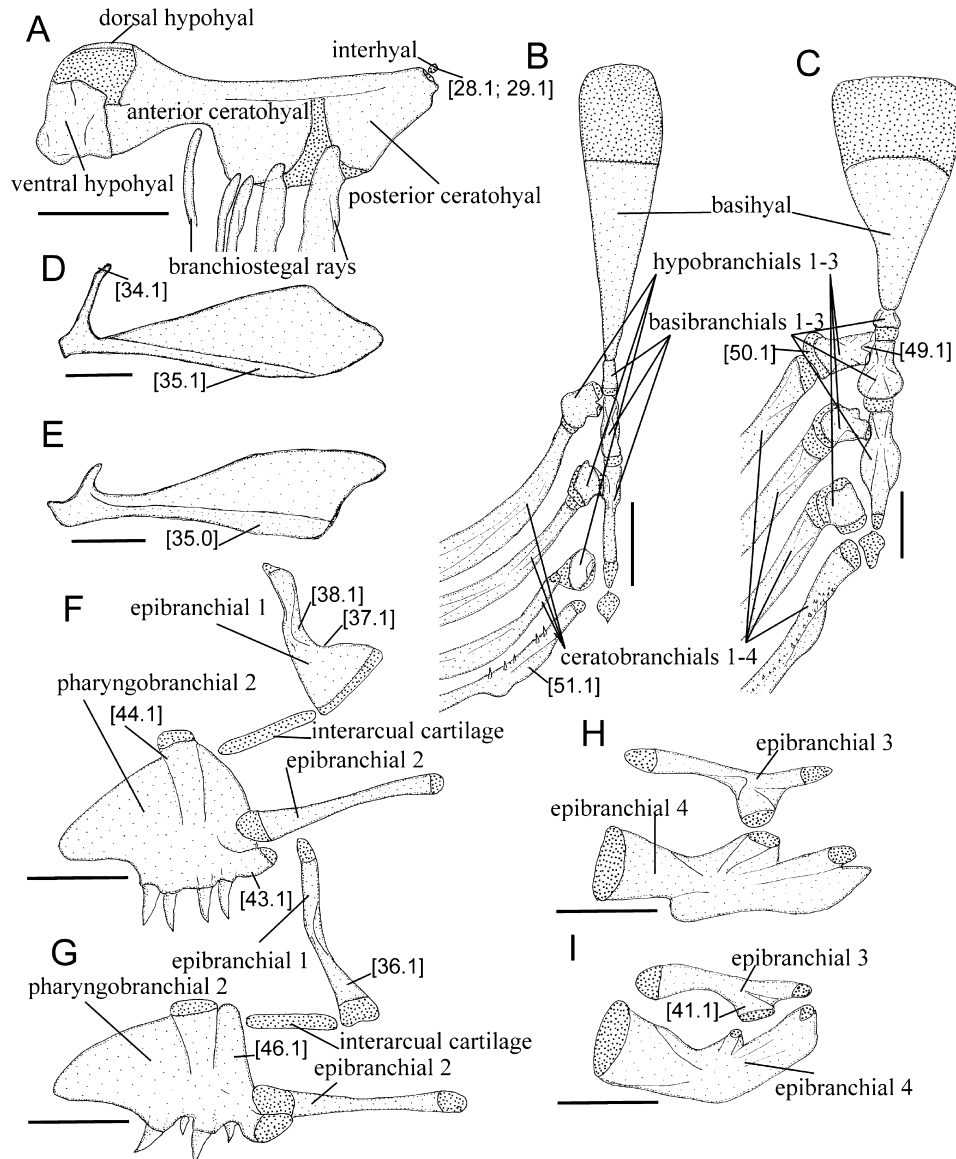


Figure 3. Hyoid and branchial arches: left hyoid bar, lateral view, of *Plesiolebias filamentosus* (A); basihyal and ventral part of branchial arches (fifth ceratobranchial excluded), central and left portion, dorsal view, of *Pl. filamentosus* (B), and *Pituna poranga* (C); urohyal, left lateral view, of *Papiliolebias bitteri* (D), and *Rivulus pictus* (E); dorsal branchial arches, anterior portion, ventral view, of *Maratecoara splendida* (F), and *Pi. poranga* (G); dorsal branchial arches, posterior portion, ventral view, of *M. splendida* (H), and *Pa. bitteri* (I). Numbers in brackets are characters and character states numbered according to Appendix S2. Larger stippling indicates cartilage. Scale bars = 0.5 mm.

in most rivulids [33.1] (vs. longer in other aplocheiloids) (Costa, 1998c) (Fig. 3B, C).

Urohyal

The dorsal process of the urohyal is bifid [34.1] (Fig. 3D) in *Papiliolebias* and in all the species of *Pituna* except *Pi. brevirostrata*, a derived condition also occurring in *Ra. maculipinnis*. The ventral flap is laterally directed as in most other rivulids and nothobranchiids [35.1] (Fig. 3D); amongst rivulids, the ple-

siomorphic condition, ventral flap ventrally directed, is found only in *Kryptolebias* and *Rivulus* (Fig. 3F).

First epibranchial

The proximal tip of the first epibranchial is slightly wider than the distal tip in most rivulids [36.1] (Fig. 3G); exceptions are *K. brasiliensis* and *M. splendida*, in which the proximal tip is distinctively wider, about five or six times wider than the distal tip (Fig. 3F), a condition similar to that present in

nonrivuline aplocheiloids. The first epibranchial is gently bowed [37.1] and slightly twisted [38.1] in all plesiolebiasines (Fig. 3F, G). A bowed first epibranchial is also present in *Ri. pictus* (Costa, 1998a) and a slightly twisted first epibranchial is shared by all rivulines.

Second epibranchial

As in all other rivulines, the second epibranchial of plesiolebiasines lacks a short process near the distal end of the bone [39.1] (Fig. 3F, G), which is present in the remaining aplocheiloids.

Third epibranchial

The uncinat process of the third epibranchial is shorter than the posterior process as in all rivulids and nothobranchiids [40.1] (Fig. 3H, I). The angle formed between the uncinat process and the distal process is about 90° in most plesiolebiasines, contrasting to 45°–60° in *Pa. bitteri* and most rivulines [41.1] (Fig. 3I).

Interarcual cartilage

Uniquely amongst aplocheiloids, the interarcual cartilage is absent in all species of the genus *Plesiolebias* [42.1] (Costa, 1998a).

Second pharyngobranchial

The second pharyngobranchial of the plesiolebiasines has two short projections bearing a cartilaginous head. The first one is an articular facet for the second epibranchial, which is continuous to the proximal margin of the bone as occurs in all other rivulids [43.1] (Fig. 3F, G) (vs. placed on the medial border in *A. panchax*), and the second is a condyle on the distal border of the bone, which is laterodistally directed as in other rivulines [44.1] (Fig. 3F, G) (vs. distally orientated in nonrivuline aplocheiloids). The medial border is not expanded as that present in *Pt. longipinnis* and *Ra. maculipinnis* [45.1] (Costa, 2005), but there is a distinctive medial flap, as occurs in most rivulines [46.1] (Fig. 3F, G). Second pharyngobranchial teeth are always present (vs. absent in *Sc. guignardi*, *N. whitei*, and *Ri. pictus*) [47.1].

Basibranchials and hypobranchials

All the basibranchials and hypobranchials are robust bones, except in species of *Plesiolebias*, in which they are narrow and thin [48.1] (Fig. 3B), a condition not occurring in other aplocheiloids. The medial edge of the first hypobranchial is bifid in all the plesiolebiasines [49.1] (Fig. 3B, C), a feature shared by all other rivuline terminal taxa. A rudimentary bifid morphology was noted in some specimens of *K. brasiliensis*. The cartilage of the first hypobranchial articular facet for the first ceratobranchial is extended beyond the

articular region, as occurs in all rivulids except *Kryptolebias* [50.1] (Fig. 3B, C) (Costa, 2004).

Fourth ceratobranchial

In all plesiolebiasines except *Maratecoara*, the ventral process of the fourth ceratobranchial is posteriorly directed (vs. ventrally directed, not or poorly visible in dorsal view of the fourth ceratobranchial), sometimes extending ventrally to the fifth ceratobranchial, a condition found in all other rivuline terminal taxa [51.1] (Fig. 3B, C). In species of *Maratecoara*, the process is short and ventrally directed, and in *N. whitei*, the process is absent (Costa, 1990).

Lateral ethmoid

The lateral ethmoid extends medially towards the parasphenoid, making the cartilaginous interspace narrower than the anterior parasphenoid width as in all other rivulids [52.1] (Fig. 4A) (Parenti, 1981) (vs. cartilaginous interspace wider than anterior parasphenoid width in other aplocheiloids). There is a well-developed anterior retrorse process in the lateral ethmoid, a condition shared by all rivulids [53.1] (Fig. 4A) (Costa, 1990). However, uniquely amongst rivulids, the retrorse process of the plesiolebiasines touches the main axis of the lateral ethmoid [54.1] (Fig. 4A).

Parasphenoid

The parasphenoid of all species of *Plesiolebias* is unique because it has a dorsal, triangular process on its anterior portion [55.1] (Fig. 4B) (vs. process absent in all other taxa) (Fig. 4C). On the middle portion of the parasphenoid, there is a short anterolaterally directed process (Fig. 4D), differing from that in some other rivulines, including *Pt. longipinnis* and *Ra. maculipinnis*, in which the process is long and attached to the pterosphenoïd [56.1] (Costa, 1998a, 2005).

Frontal

The frontal is thin with a concave lateral margin as in all other rivulids [57.1] (vs. straight lateral edge) (Costa, 1998a, 2005).

Supraoccipital

The supraoccipital has a paired, pointed posterior process, a condition present in all aplocheiloids. The process is longer, reaching the neural process of the first vertebra, sometimes fused to it, in all species of *Plesiolebias* [58.1] (Fig. 4E), as well as in *A. panchax* (vs. process shorter, not reaching the first vertebrae). In all species of *Maratecoara*, the processes are medi-

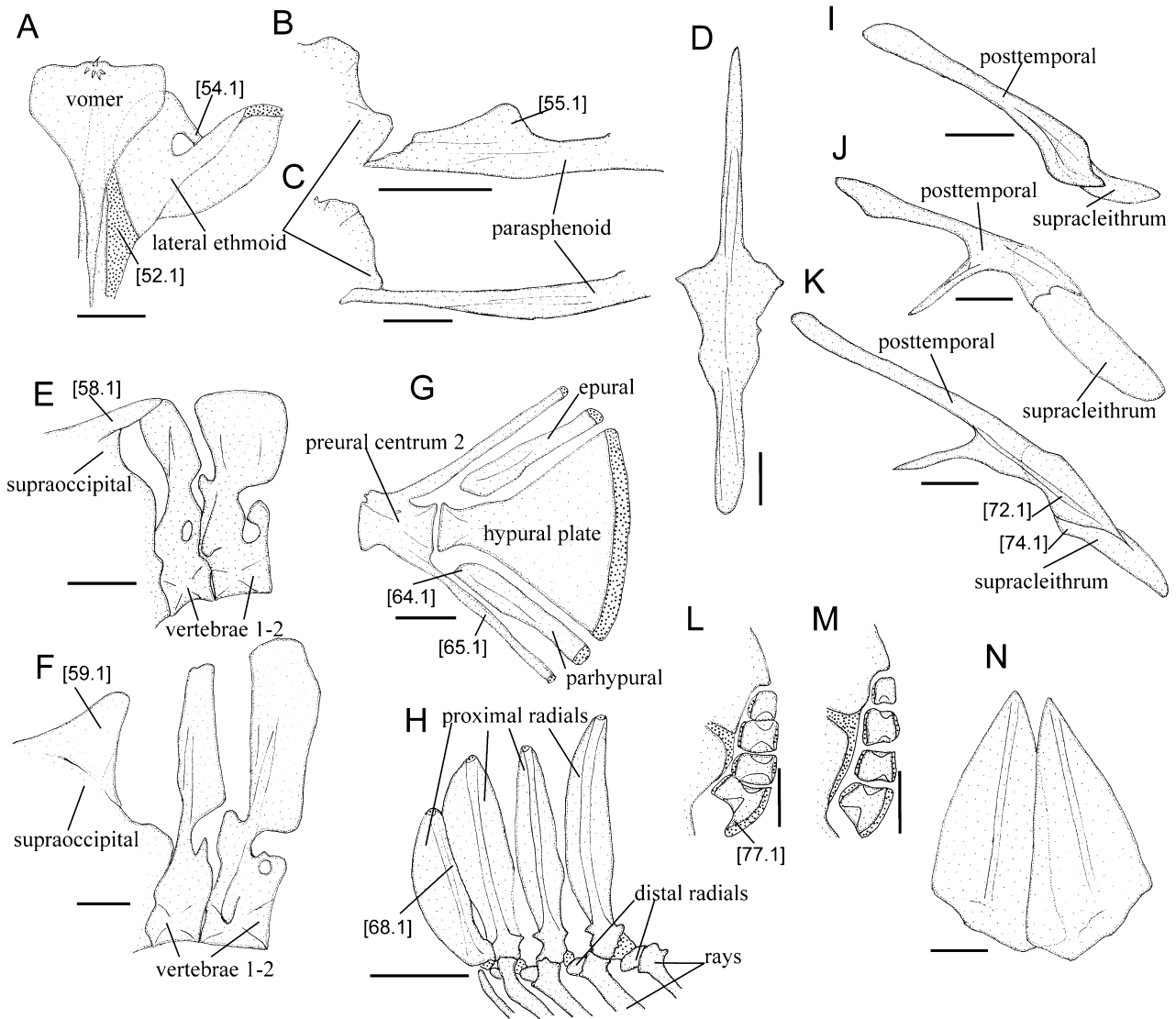


Figure 4. Neurocranium, vertebrae, and fins: anterior portion of neurocranium, central and left portion, ventral view, of *Papiliolebias bitteri* (A); median portion of ventral part of neurocranium, left lateral view, of *Plesiolebias filamentosus* (B), and *Maratecoara splendida* (C); parasphenoid, ventral view, of *Pa. bitteri* (D); neurocranium, posterior part, and first two vertebrae, lateral view (ribs excluded), of *Pl. filamentosus* (E), and *Ma. splendida* (F); caudal skeleton (fin rays excluded), left lateral view, of *Stenolebias damascenoi* (G); anal-fin base, anterior portion, left lateral view, of *Plesiolebias glaucopterus* (H); left post-temporal and supracleithrum, lateral view, of *Maratecoara formosa* (I), *Pa. bitteri* (J), and *Pterolebias longipinnis* (K); left proximal radials of pectoral fin, lateral view, of *Pituna poranga* (L), and *Maratecoara lacortei* (M); pelvic bones, dorsal view, of *Pituna obliquoseriata* (N). Numbers in brackets are characters and character states numbered according to Appendix S2. Larger stippling indicates cartilage. Scale bars = 0.5 mm.

ally fused, forming a single medial triangular process [59.1] (Fig. 4F) (Costa, 1995a).

First vertebra

All plesiolebiasines share two morphological features of the first vertebra: there is no laterodorsal laminar process like that present in aplocheilids and nothobranchiids [60.1], and there is no anteriorly directed

paired process like that present in most rivulines [61.0] (Costa, 1998a) (Fig. 4E, F).

Hypurals

The hypurals are usually fused to form a single hypural plate (Fig. 4G), neither presenting gaps to delimit two isolated dorsal hypural plates [62.1] as in adult specimens of *A. panchax* (Parenti, 1981), nor a

single median gap [63.1] as in most rivulids and nothobranchiids (Costa, 1990, 1998a). A narrow gap on the middle of the hypural plate may be present or not in all species of *Pituna* and *Maratecoara*, as well as in *Ra. maculipinnis*.

Parhypural and preural centrum 2

The proximal tip of the parhypural is unpaired and does not overlap the preural centrum [64.1], and the haemal spine of the preural centrum 2 is as wide as the haemal spines anterior to it [65.1] (Fig. 4G), two conditions contrasting with those occurring in *Aplocheilus* (Costa, 1998c), in which the proximal tip of the parhypural is paired and slightly overlaps the preural centrum and the haemal spine of the preural centrum 2 is distinctively wider (Costa, 2009: Fig. 4G).

Dorsal-fin rays

As in other rivulids and nothobranchiids, there is a long fin ray attached to the first proximal radial, which is preceded by one or two short fin rays [66.1], a condition contrasting with that in *Aplocheilus*, in which there is a single ray attached to the first proximal radial (Costa, 2005).

Proximal radials of the anal fin

The anterior proximal radials of the anal fin are wide as a consequence of the expansion of their anterior and posterior convex margins, making radials separated by short interspaces [67.1] (Fig. 4H), a feature also found in *Pt. longipinnis* and *Ra. maculipinnis* (vs. proximal radials narrow, margins almost straight). In addition, in all plesiolebiasines the first and the second proximal radials, and sometimes the third, are fused [68.1] (Fig. 4H).

Pectoral fin

The pectoral fin is ventrolaterally inserted as in all rivulids and nothobranchiids [69.1] (vs. laterally inserted in *Aplocheilus*) (Costa, 1998c). As a consequence, the fin insertion is nearer the ventral end of the pectoral girdle than the dorsal one.

Post-temporal and supracleithrum

The post-temporal and the supracleithrum are not co-ossified as occurs in other rivulids, in contrast to aplocheilids and nothobranchiids, which have these bones co-ossified [70.1] (Parenti, 1981). Although firmly attached, the contour of each bone may be viewed in all plesiolebiasines. The supracleithrum is always shorter than the post-temporal, but in *Maratecoara*, the supracleithrum is conspicuously shorter than half the post-temporal length (Fig. 4I), whereas in the remaining plesiolebiasines, and also in *K. brasiliensis* and *N. whitei*, it is longer [71.1] (Fig. 4J, K).

There is no prominent lateral keel along the post-temporal in contrast to *Ra. maculipinnis*, *Pt. longipinnis*, and *T. rubromarginatus* [72.1] (Costa, 2005). The ventral process of the post-temporal is absent or rudimentary in all plesiolebiasines except *Pa. bitteri* [73.1] (Fig. 4J). The supracleithrum never has an anterior expansion like those observed in *Ri. janeiroensis*, *Ri. pictus*, *Ra. maculipinnis*, *Pt. longipinnis*, and *T. rubromarginatus* [74.1] (Fig. 4K).

Cleithrum and first postcleithrum

As for most other rivulids, the cleithrum in plesiolebiasines lacks a posterior flange [75.1] (Costa, 1998a) (CI: 1.00; RI: 1.00) and the first postcleithrum is absent [76.1] (Parenti, 1981).

Fourth pectoral radial

The fourth pectoral radial is trapezoidal. In *Pituna* (except *Pi. brevirostrata*), it has a ventral expansion (Fig. 4L), a condition similar to that in *Ra. maculipinnis*, *Pt. longipinnis*, and *T. rubromarginatus* [77.1] (Costa, 2005), whereas in other plesiolebiasines this expansion is absent (Fig. 4M).

Pelvic bone

The pelvic bone is thin, and the ischial and lateral processes are minute or absent, conditions shared by all rivulids [78.1] (Costa, 1998a). Uniquely in plesiolebiasines, the pelvic bones are medially overlapped [79.1] (Fig. 4N).

Pelvic-fin rays

The number of pelvic-fin rays is variable amongst species of the Plesiolebiasini, between seven and nine, but never six as in aplocheilids and nothobranchiids. Seven rays [80.1] are present in all species of *Pituna* and *Stenolebias*, and in *Pl. glaucopterus*, *Pl. aruana*, and *Pl. canabravensis*; eight rays [80.2] are present in all species of *Maratecoara* and in *Pl. altamira*, *Pl. fragilis*, *Pl. lacerdai*, *Pl. filamentosus*, and *Pl. xavantei*; and nine rays [80.3] in *Pa. bitteri*.

Mouth

The mouth cleft is always dorsally directed [81.1] (Fig. 5F), a condition present in other rivulids and nothobranchiids, but contrasting with the terminal mouth cleft of *Aplocheilus* (Costa, 2004).

Eye

As in all other rivulids, the orbital rim is attached to the eye, with no gap in the membrane around the eye [82.1]; amongst aplocheiloids, a partially free orbital rim is uniquely found in species of *Aplocheilus* (Parenti, 1981; Costa, 2004). The pupil is approxi-

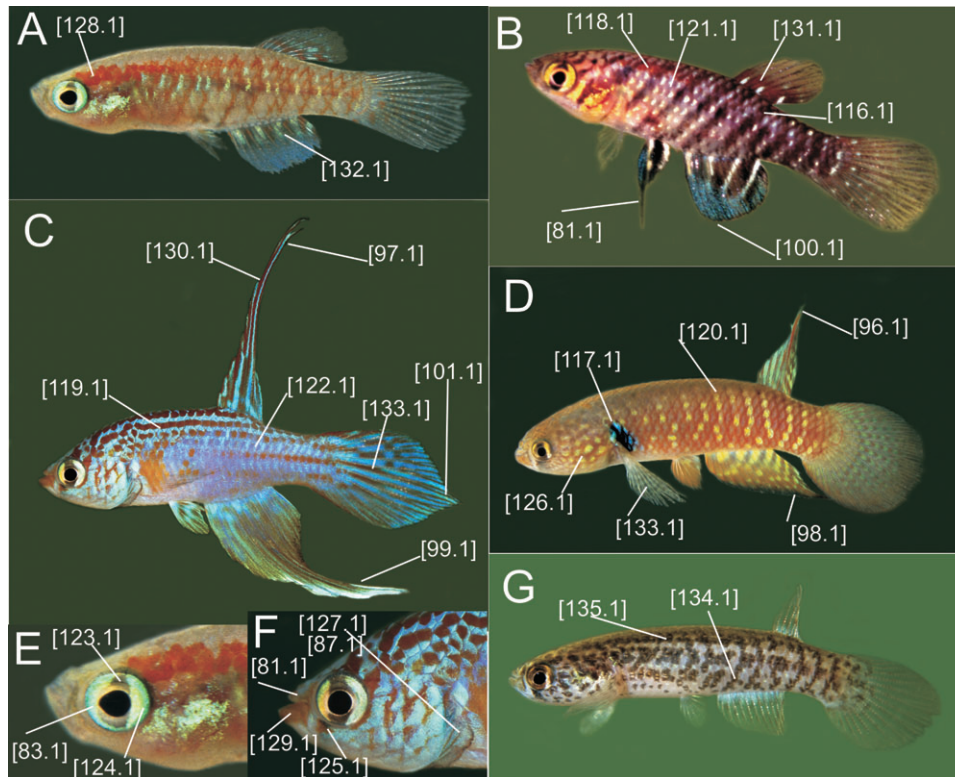


Figure 5. External morphology: A, *Plesiolebias canabravensis*, male holotype, 17.6 mm SL (standard length) (UFRJ 6424); B, *Plesiolebias filamentosus*, male, about 20 mm SL (not preserved); C, *Maratecoara lacortei*, male, 28.8 mm SL (UFRJ 6406); D, *Pituna compacta*, male, 39.7 mm SL (UFRJ 6391); E, head of same individual as in (A); F, head of same individual as in (C); G, *Pituna poranga*, female, 28.0 mm SL (UFRJ 6395). Numbers in brackets are characters and character states, numbered according to Appendix S2.

mately round, except in species of *Plesiolebias*, in which it has a minute anterior expansion [83.1] (Fig. 5E).

Ventral surface of head and branchiostegal membrane

The epidermal membrane covering the ventral surface of the head is continuous in all plesiolebiasines, neither having a middle gap between the preopercular laterosensory series and isthmus [84.1] as in nonrivuline aplocheiloids, nor a gap just below the corner of the preopercular laterosensory series [86.1] as in *Aplocheilus*. Uniquely in *Maratecoara*, the branchiostegal membrane is strongly expanded, extending out of the opercular aperture to overlap the pectoral-fin base [87.1] (Fig. 5E) (Costa, 1995b), a condition not found elsewhere amongst aplocheiloids. The anterior portion of the ventral surface of the head is naked, scales being placed posteriorly to a point adjacent to the anterior extremity of the preopercular series [88.1]. In *N. whitei*, nothobranchiids, and aplocheilids, the naked area is longer, extending to the region near the corner of the preopercular series.

Frontal scales

The frontal scales are circularly arranged around an A-scale without free borders in species of the genera *Pituna*, *Papiliolebias*, and *Stenolebias* [89.1] (Fig. 6A), whereas in species of *Maratecoara* and *Plesiolebias*, they are transversely arranged, the A-scale always having a free border (Fig. 6B, C). Most plesiolebiasines have a F squamation pattern [90.3] (Fig. 6A, B); exceptions are *Pi. brevirostrata* with the frontal squamation D-patterned and all species of *Plesiolebias*, which are G-patterned [90.0] (Fig. 6C). As in other rivulines (Hoedeman, 1958), the E-scales are not medially overlapped [91.1].

Contact organs on scales

Males of all plesiolebiasine species have minute contact organs on the border of trunk scales [92.3], a condition not occurring in basal aplocheiloids (e.g. Costa, 2005).

Contact organs on pectoral fin

Nematolebias whitei and *Ra. maculipinnis* share the presence of contact organs on the inner surface of the

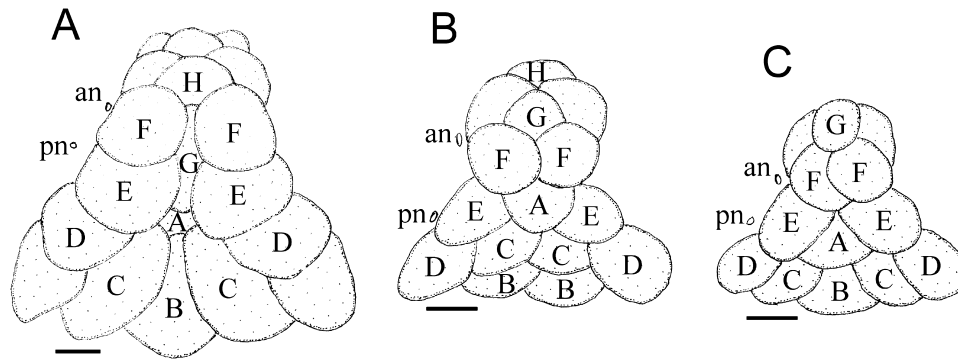


Figure 6. Frontal scales (A–H) and cephalic squamation patterns amongst plesiolebiasines: A, *Pituna obliquoseriata*; B, *Maratecoara lacortei*; C, *Plesiolebias xavantei*. Abbreviations: an, anterior supraorbital series, posterior-most neuromast; pn, posterior supraorbital series, anterior-most neuromast. Scale bars = 1.0 mm.

pectoral fin in males [93.1], although previous studies indicate that this condition is not homologous in these species. Pectoral contact organs are never present in plesiolebiasines.

Pelvic fin

Plesiolebias xavantei, *Pl. filamentosus*, *Pl. lacerdai*, *Pl. fragilis*, and *Pl. altamira* differ from all other plesiolebiasines by two features of the pelvic-fin morphology in males: there are filamentous rays on the fin tip [94.1] (Fig. 5B) and the fins are medially united [95.1]. In the remaining plesiolebiasines, the pelvic fins lack filaments and are separated medially.

Dorsal and anal fins

The morphology of the dorsal and the anal fins in males is variable amongst genera of the Plesiolebiasine. In *Plesiolebias* and *Papiliolebias*, the distal borders of both fins are rounded (Fig. 5B), whereas in the other genera the fins are pointed [96.1; 98.1] (Fig. 5D). In *Maratecoara*, both fins are long, the tip surpassing the posterior edge of the caudal fin [97.1; 99.1] (Fig. 5C). In *Plesiolebias*, the anal fin exhibits a peculiar shape, in which the anterior portion is longer than the posterior one [100.1] (Fig. 5B).

Caudal fin

All species of *Maratecoara* possess a unique morphology of caudal fin in males, in which the fin is lanceolate [101.1] (Fig. 5C), its tip often terminating in a long filamentous ray, a condition not occurring in other aplocheiloids. In other plesiolebiasines, the caudal fin is never lanceolate and fin-rays are not projected to form filaments. Instead of a single long filamentous ray, in *Pt. longipinnis* and *Ra. maculipinnis* there are short filaments along the posterior margin of the caudal fin, forming a fringed border [102.1] (Costa, 1998a, 2005). In *Pituna*, the body squamation extends posteriorly, reaching the middle

part of the caudal fin in older males [103.1], a condition contrasting with other plesiolebiasines, in which the caudal-fin squamation is restricted to the basal portion of the fin.

Laterosensory system of head

Plesiolebiasines possess a reduced pattern of cephalic sensory canals, as in most rivulids, but exhibit an elaborated arrangement of neuromasts, a condition often occurring in annual fish groups of the Rivulidae and Nothobranchiidae (Costa, 1998a). There are no prominent epidermal trenches around the supra-orbital series of neuromasts [104.1] and the anterior and posterior infra-orbital canals [107.1; 108.1] and the upper section of the preopercular canal [110.1] are opened; as occurs in all other rivulids and in nothobranchiids, the posterior section of the mandibular canal is opened [111.1]. In plesiolebiasines, the anterior section of the supra-orbital series of neuromasts extends to the middle portion of the frontal region, and contains five to seven neuromasts [105.2], contrasting with the three neuromasts restricted to the anterior frontal region in basal aplocheiloids; there are four to six supra-orbital neuromasts in the posterior series [106.1], except in *Pl. aruana* and *Pl. canabravensis*, both with seven or eight neuromasts [106.2], in contrast to three neuromasts in basal aplocheiloids. The number of preorbital neuromasts in species of *Plesiolebias* is similar to that in basal aplocheiloids (1–3), but smaller than in *Maratecoara*, *Pituna*, and *Stenolebias* (4–6) [109.1] and in *Papiliolebias* (8) [109.2]. In species of *Maratecoara*, *Pituna*, and *Stenolebias*, the upper and lower sections of the preopercular series of neuromasts are not separated by an interspace [111.1] (vs. interspace present in *Papiliolebias*, *Plesiolebias*, and most other aplocheiloids). The number of preopercular neuromasts is higher in plesiolebiasines than in most other aplocheiloids, the latter having between four and six

neuromasts, in contrast to seven to ten in all plesiolebiasines [112.1], except in species of *Maratecoara*, with 13 to 15 neuromasts. The anterior and the posterior sections of the mandibular series are continuous [114.1], except in species of *Plesiolebias*, in which the anterior and posterior sections are separated by an interspace, configuring the condition present in most basal aplocheiloids. In addition, all plesiolebiasines, except *Pl. glaucopterus*, *Pl. xavantei*, *Pl. filamentosus*, *Pl. lacerdai*, *Pl. fragilis*, and *Pl. altamira*, have numerous mandibular neuromasts (seven to 11), thus differing from the condition observed in most basal aplocheiloids (five to six).

Male colour patterns

Flank: The following colour patterns are unique for assemblages of plesiolebiasine species: black oblique bars along the whole flank in *Pl. xavantei*, *Pl. filamentosus*, *Pl. lacerdai*, *Pl. fragilis*, and *Pl. altamira* [116.1] (Fig. 5B); a vertically elongated iridescent blue blotch on the humeral region in all species of the genera *Pituna* and *Papiliolebias* [117.1] (Fig. 5D); a red stripe on the anterodorsal region of the flank in all the species of *Plesiolebias* with the exception of *Pl. lacerdai* and *Pl. fragilis* [118.1] (Fig. 5A, B); three horizontal rows of brilliant orange spots on the anterodorsal region of the flank in all the species of *Maratecoara* [119.1] (Fig. 5C); small yellow spots arranged in irregular oblique rows on the whole flank in all the species of *Pituna* [120.1] (Fig. 5D); white dots regularly arranged in straight oblique rows on flank in *Pl. xavantei*, *Pl. filamentosus*, and *Pl. lacerdai* [121.1] (Fig. 5B); and intense light blue iridescence covering most of flank surface in all species of *Maratecoara* and *Stenolebias* [122.1] (Fig. 5C).

Head: All the plesiolebiasines have a dark grey to black bar through the centre of the eye [123.1] (Fig. 5E), a condition shared by all annual rivulids (Parenti, 1981). In species of *Maratecoara* and in *Pl. glaucopterus*, *Pl. aruana*, and *Pl. canabravensis* [124] (Fig. 5A, C), and also in *Pt. longipinnis*, the iris is green, in contrast to yellow as in other rivulids or orangish yellow as in *Pa. bitteri*. There is a dark grey to black bar on the antero-suborbital region in all plesiolebiasines [125], except *Pl. glaucopterus*, *Pl. aruana*, *Pl. canabravensis*, and species of *Stenolebias*, although not always well developed in species of *Maratecoara* (Fig. 5); this colour pattern is also found in *Pt. longipinnis* and *Ra. maculipinnis*. In *Pituna* and *Papiliolebias*, the scales of the opercular region have a light iridescent spot on the centre and a dark orangish brown border, forming regular reticulate pattern [126.1] (Fig. 5D). In *Maratecoara*, the branchiostegal membrane is bright blue [127.1] (Fig. 5C) (vs. not brightly coloured in all other aplocheiloids).

In *Pl. glaucopterus*, *Pl. aruana*, and *Pl. canabravensis*, there is a red stripe on the post-orbital region [128.1] (Fig. 5A), continuous with the anterodorsal red stripe of the flank. In all plesiolebiasines except *Plesiolebias*, the jaws are distinctively coloured with dark orange pigment [129.1] (Fig. 5F).

Fins: Five unique colour patterns exist for plesiolebiasine assemblages: the dorsal fin of species of the genera *Pituna*, *Maratecoara*, and *Stenolebias* has bright red lines on the distal part of some fin rays [130.1] (Fig. 5C); red pigment covers the whole basal region of the dorsal fin in *Pl. xavantei*, *Pl. filamentosus*, *Pl. lacerdai*, *Pl. fragilis*, and *Pl. altamira* [131.1] (Fig. 5B); the basal portion of the anal fin is dark grey to black with a row of white to yellow spots in species of all plesiolebiasines except *Maratecoara* and *St. damascenoi* [132.1] (Fig. 5A); there are longitudinal orange stripes on the basal portion of the caudal fin in *M. splendida* and *M. lacortei* [133.1] (Fig. 5C); and there are vertical rows of dark grey to black small spots, sometimes coalesced to form bars, on the pectoral fin of all species of *Pituna* except *Pi. brevirostrata* [134.1] (Fig. 5D).

Female colour patterns

Flank: In all species of the genera *Pituna* and *Plesiolebias* there are oblique rows of dots on the flank, sometimes united, forming oblique bars [135.1] (Fig. 5E). Only in *Pi. compacta* and *Pi. poranga*, the dark pigment is longitudinally arranged to form horizontal rows of small spots or short stripes [136.1] (Fig. 5E) (Costa, 2007a).

PHYLOGENETIC RECONSTRUCTION

The single most parsimonious cladogram obtained from the phylogenetic analysis appears in Figure 7. The Plesiolebiasini is well supported; it is diagnosed by four unambiguous synapomorphies: anterior portion of the entopterygoid not overlapping the autopalatine [7.1], anterior retrorse process of the lateral ethmoid contacting the main axis of the bone [54.1], first two proximal radials of the anal fin fused [68.1], and pelvic bones medially overlapped [79.1]. Anal fin with basal region dark grey to black with a row of white to yellow spots [132.1] is synapomorphic for plesiolebiasines, but reversed in species of *Maratecoara* and in *St. damascenoi*, in which there is no vestige of this colour pattern. In addition, absence of an anterior expansion on the ventral process of the maxilla [13.0] is considered a reversal involving all the plesiolebiasines. An increase in the number of neuromasts, between five and seven in the anterior [105.2] and four or more in the posterior [106.1] sections of the supraorbital series was also indicated

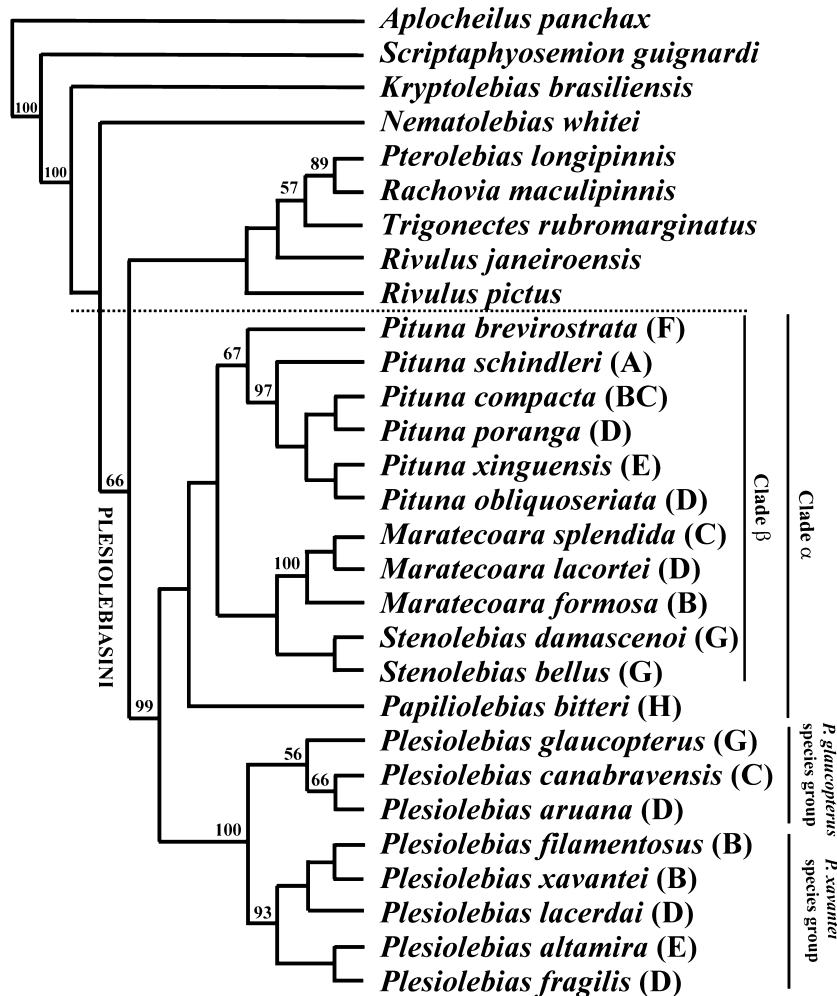


Figure 7. Most parsimonious tree from the analysis of 142 morphological characters of plesiolebiasines and outgroup taxa. Numbers above the base of a node are bootstrap percentages higher than 50%. Letters in parentheses after plesiolebiasine species are areas of endemism: A, Parnaíba; B, Tocantins; C, Canabrava; D, Araguaia; E, Xingu; F, Parnaíba; G, eastern Paraguay; H, western Paraguay.

as an unambiguous synapomorphy in the present analysis. Although there are also numerous supra-orbital neuromasts in several cynolebiasine taxa (e.g. Costa, 1998a), the condition is not comparable because the anterior and posterior series are continuous. An increase of seven or more neuromasts in the lower section of the preopercular series [112.1] may also support monophyly of the Plesiolebiasini, but a parallel increase occurs in *N. whitei*, *Pt. longipinnis*, and *T. rubromarginatus*. Other derived character states supporting the Plesiolebiasini clade but also found in *Ra. maculipinnis*, are as follows: a bony flange on the posterior margin of the autopalatine [4.1]; dorsal margin of the symplectic with an expansion towards entopterygoid [24.1], reversed in *Pituna*; anterior portion of basihyal narrow, less than 50% basihyal length [32.1], reversed in *Pituna*; anterior

proximal radials of the anal fin widened [67.1]; character state 67.1 was also present in *Pt. longipinnis*. The presence of contact organs on the flank in males [92.1] was consistently indicated as synapomorphic for plesiolebiasines, although occurring homoplastically in *Ri. janeiroensis*. However, previous studies involving other terminal taxa of the rivulids showed that this derived condition is present in different lineages of the Cynolebiasinae (Costa, 2006a, b) and Rivulinae (Costa, 2005, 2006c).

The phylogenetic reconstruction supports *Plesiolebias* as the sister group to a clade including all other plesiolebiasines. The clade comprising the genera *Papiliolebias*, *Maratecoara*, *Stenolebias*, and *Pituna* (hereafter termed clade- α) is supported by the dark orange jaws [129.1], and two derived features of the laterosensory system of the head, also occurring

in *N. whitei*: upper and lower sections of the preopercular series continuous [111.1] and anterior and posterior sections of the mandibular series continuous [114.1]. *Plesiolebias* is diagnosed by the following unambiguous synapomorphies: posterior margin of the autopalatine with a deep concavity [3.1]; metapterygoid narrowed [9.2]; ventral process of the maxilla shorter than dorsal process [14.1]; symplectic slender, about twice as long as deep [23.1]; interarcual cartilage absent [42.1]; basibranchials and hypobranchials thin [48.1]; parasphenoid with a dorsal process [55.1]; pupil with an anterior pointed extension [83.1]; and, anterior portion of the anal fin longer than the posterior portion [100.1]. Monophyly of *Plesiolebias* is also supported by: anterior margin of the autopalatine with a distinctive notch [2.1] (also present in *Pt. longipinnis*); posterior portion of the entopterygoid not reaching the metapterygoid [8.1] (also occurring in *N. whitei*, both species of *Stenolebias*, and *Pi. brevirostrata*); posterior process of the supraoccipital touching the neural spine of the first vertebrae [58.1] (also occurring in *A. panchax*); flank with oblique rows of dark spots in females [135.1] (also occurring in most species of *Pituna*). The clade containing *Pl. glaucopterus*, *Pl. aruana*, and *Pl. canabravensis* (hereafter termed *Pl. glaucopterus* species group) is supported by a green iris in males [124.1] (also occurring in *Maratecoara*) and the presence of a red stripe on the post-orbital region [128.1]. The clade comprising *Pl. xavantei*, *Pl. filamentosus*, *Pl. lacerdai*, *Pl. fragilis*, and *Pl. altamira* (hereafter termed *Pl. xavantei* species group) is corroborated by: pelvic fin with eight rays [80.2] (also occurring in *Pt. longipinnis*, *Ra. maculipinnis* and all species of *Maratecoara*); pelvic fin with a filamentous ray in males [94.1] (also in *Pt. longipinnis* and *Ra. maculipinnis*); pelvic fins medially fused [95.1] (also in *Pt. longipinnis*); flank with black oblique bars in males [116.1]; and basal region of the dorsal fin red in males [131.1].

The clade comprising *Stenolebias*, *Maratecoara*, and *Pituna* (hereafter termed clade- β) is supported by the pointed dorsal [96.1] and anal [98.1] fins of all included species, a condition occurring in several other annual rivulids, but herein parsimoniously supported as independently derived; and, by the presence of red lines on the distal region of the dorsal-fin rays in males [130.1] (reversed in *St. bellus*), a condition unique amongst rivulids (Fig. 5C, D; see also Costa, 1991: fig. 14 for the pattern exhibited by *St. damascenoi*). Another feature supporting this hypothesis is the rudimentary or absent ventral process of the post-temporal in *Pituna*, *Maratecoara*, and *Stenolebias*, contrasting with the primitive long process in *Pa. bitteri* [73.0]. However, this process is also rudimentary or absent in all species of *Plesiolebias*, thus making polarization ambiguous. A clade

including *Stenolebias* and *Maratecoara* is supported by an expanded dorsomedial process of the autopalatine, which projects anteriorly beyond autopalatine margin [6.1], by a ventrally directed ventral process of the fourth ceratobranchial [51.0] (a reversal), and the presence of intense light blue iridescence on the flank in males [122.1]. The clade containing all species of *Maratecoara* is corroborated by posterior supraoccipital processes medially fused [59.1]; supracleithrum distinctively shorter than post-temporal half length [71.0] (a reversal); pelvic fin with eight rays [80.2] (also occurring in *Plesiolebias*, *Pt. longipinnis*, and *Ra. maculipinnis*); branchiostegal membrane expanded to overlap pectoral-fin base [87.1]; frontal scales arranged transversely [89.0] (a reversal); dorsal [97.1] and anal [99] fins long in males, tip surpassing the posterior margin of the caudal fin; caudal fin lanceolate in males, tip terminating in filamentous ray shape; lower section of the preopercular series with numerous neuromasts (13–15) [112.2]; anterodorsal region of the flank with three horizontal rows of orange spots in males [119.1]; iris green in males [124.1] (also occurring in *Plesiolebias*); and branchiostegal membrane bright blue in males [127.1]. *Stenolebias* is supported by the posterior tip of the entopterygoid not reaching the metapterygoid [8.1] (also occurring in *Plesiolebias* and *Pi. brevirostrata*) and a single anterior branchiostegal ray [30.1] (also occurring in *Plesiolebias*).

Monophyly of *Pituna* is supported by a wide, triangular basihyal, its anterior width more than 50% of its length [32.0] (a reversal); caudal-fin squamation extending over about 50% of fin length in males [103.1] (also occurring in *Ra. maculipinnis* and *T. rubromarginatus*); flank with small yellow spots arranged in tortuous oblique rows in males [120.1]; and, flank with oblique rows of dark spots in females [135.1] (also in *Plesiolebias*). In addition, all species of *Pituna* share a large blue humeral blotch [117.1], but a similar mark is also present in *Pa. bitteri*. A clade containing all species of *Pituna* except *Pi. brevirostrata* is diagnosed by: dorsal process of urohyal branched [34.1] (also in *Pa. bitteri*); fourth pectoral radial ventrally expanded [77.1] (also in *Pt. longipinnis*, *T. rubromarginatus*, and *Ra. maculipinnis*); opercular region with reticulate colour pattern in males, formed by iridescent colour on middle of scales contrasting with dark orangish brown pigment along entire scale margins [126.1] (also in *Pa. bitteri*); and pectoral fin with dark grey to black marks vertically crossing whole fin extension [134.1] (also in *Pt. longipinnis*).

BIOGEOGRAPHICAL RECONSTRUCTION

Direct inspection of the geographical distribution of plesiolebiasine taxa (Fig. 7) reveals a high congruence

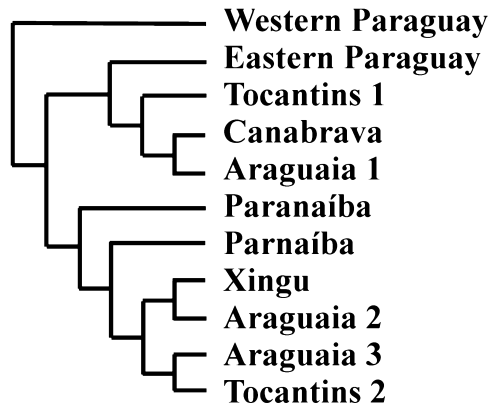


Figure 8. General area cladogram for plesiolebiasine areas of endemism.

between subclades of the *Plesiolebias* and the α -clade, suggesting a series of vicariance events. Both clades are present in the Araguaia, Canabrava, Tocantins, Xingu, and eastern Paraguay areas, but *Plesiolebias* is missing in the western Paraguay, Paranaíba, and Parnaíba areas.

A similar biogeographical pattern is shared by the *Pl. glaucopterus* species group and the clade containing *Maratecoara* and *Stenolebias*. In both clades, the taxon occurring in the Araguaia area (*Pl. aruana*, *M. lacortei*) is the sister group to the taxon occurring in the Canabrava area (*Pl. canabravensis*, *M. splendida*). In the *Maratecoara*–*Stenolebias* clade, the species occurring in the Tocantins area (*M. formosa*) is sister to the clade occurring in the Araguaia–Canabrava areas, but a species of the *Pl. glaucopterus* group is missing in the Tocantins area. Again in both groups, the phylogenetic reconstruction supports a sister group relationship between the taxa found in the eastern Paraguay area (*Pl. glaucopterus*, and *St. damascenoi* and *St. bellus*) and the clades occurring in the Araguaia and Tocantins river basins.

Another biogeographical pattern occurs in the *Pl. xavantei* species group and in *Pituna* (Fig. 7). Both clades indicate an identical redundant distribution involving the Araguaia area, comprising sister group relationships between Xingu (*Pl. altamira*, *Pi. xinguensis*) and Araguaia (*Pl. fragilis*, *Pi. obliquoseriata*) areas, as well as between Araguaia (*Pl. lacerdai*, *Pi. poranga*) and Tocantins (*Pl. filamentosus*, *Pl. xavantei*, *Pi. compacta*) areas. In addition, species of the *Pl. xavantei* group are missing in the Parnaíba and Paranaíba areas, and the *Pituna* clade contains a widespread species, *Pi. compacta*, which is found both in the Tocantins and the Canabrava areas.

The distribution patterns of *Plesiolebias* and the α -clade corroborate a single general area cladogram (GAC) (Fig. 8). The GAC comprises three Araguaia

and two Tocantins areas, thus supporting a reticulate biogeographical scenario, where the present day Araguaia and Tocantins areas are the fusion of distinct historical entities, thus constituting composite areas. The GAC also implicates some extinction events, alternatively interpreted as absence of collections, of a member of the *Pl. glaucopterus* species group in the Tocantins area, members of the *Pl. xavantei* species group in the Paranaíba and Parnaíba areas, and a basal species of *Plesiolebias* in the western Paraguay area, besides a dispersal event of *Pi. compacta* from the Tocantins to the Canabrava area.

DISCUSSION

PHYLOGENETIC RELATIONSHIPS

Monophyly of the Plesiolebiasini is well supported, as already indicated in all previous phylogenetic studies (Costa, 1998a; Hrbek & Larson, 1999; Murphy *et al.*, 1999). The present morphological data also corroborate the hypothesis obtained from independent analyses of mitochondrial gene sequences, in which *Plesiolebias* is the sister group to a clade comprising all other plesiolebiasines (Hrbek & Larson, 1999; Murphy *et al.*, 1999; Vermeulen & Hrbek, 2005). However, those analyses included just five plesiolebiasine species as terminal taxa, and species of *Stenolebias* were not included. In the previous analysis of morphological data (Costa, 1998a), the phylogenetic position of *Pituna* and *Plesiolebias* changed in the single most parsimonious cladogram (*Pituna* as the sister group to the remaining plesiolebiasines and *Plesiolebias* as the sister group to *Maratecoara* and *Stenolebias*). Differences in the topology of the cladograms of the two morphological analyses are a result of the number of analysed characters responsible for the plesiolebiasine topology (43 in the previous analysis, vs. 69 in the present).

Excluding *Stenolebias*, which was not included in any DNA phylogenetic analyses, the present phylogenetic reconstruction is similar to that proposed by Murphy *et al.* (1999) because *Papiliolebias* is the sister group of *Pituna* and *Maratecoara*. This contrasts with other analyses using different sets of mt-DNA genes, where *Papiliolebias* is the sister group of *Maratecoara* alone (Hrbek & Larson, 1999) or *Pituna* alone (Hrbek, Deus & Farias, 2004; Vermeulen & Hrbek, 2005).

HISTORICAL BIOGEOGRAPHY

The GAC indicates a complex biogeographical history involving two composite areas, Araguaia and Tocantins (Fig. 8). The proposed biogeographical scenario cannot be adequately tested by palaeoge-

graphical reconstructions because data on the origin and development of most South American river basins are still poorly documented (e.g. Potter, 1997), despite papers focusing on some South America regions (e.g. Lundberg *et al.*, 1998 for western river basins associated with the Andes uplift; Ribeiro, 2006 and Menezes *et al.*, 2008 for eastern river basins). However, some recorded geological events and biogeographical patterns exhibited by nonplesiolebiasine taxa may tentatively be related to the biogeographical reconstruction herein proposed.

The first divergence in the GAC indicates a vicariance event between the western Paraguay area, which corresponds to the Chaco region inhabited by *Pa. bitteri*, and the remaining areas analysed, including areas placed at the southern Amazonian tributaries (Xingu, Araguaia, Tocantins), the Parnaíba and Paranaíba areas, as well as the eastern Paraguay area, which corresponds to the Pantanal region. The divergence of this basal node may be associated with the separation of the Chaco area from the northern river drainages, after the Paraguay River changed its flow direction from north to south as a consequence of the Andes uplift process between the Late Oligocene and Early Miocene (e.g. Sempere *et al.*, 1990; Lundberg *et al.*, 1998). This putative vicariance event is also indicated by biogeographical reconstructions for cynolebiasine killifishes (Costa, 2010), which is supported by the sister group relationships between *Spectrolebias chacoensis* (Amato, 1986) endemic to the Chaco and a clade comprising species endemic to the southern tributaries of the Amazon basin.

The basal node of the GAC also indicates that the area today occupied by the Pantanal region, formerly comprising rivers draining to north and east, is a more recent addition to the present Paraguay River basin. Therefore, the GAC supports a more recent connection between the Pantanal region and tributaries of the Amazon River basin, which possibly was broken after subsidence of the Pantanal depression during the Pleistocene (e.g. Saadi, 1993), thus separating the eastern Paraguay area from the Araguaia–Canabrava–Tocantins area. The Xingu–Araguaia–Canabrava–Tocantins–Parnaíba–Paranaíba area is partly supported both by the killifish *Rivulus zygonectes* Myers, 1927 species group and by a clade of the killifish genus *Spectrolebias*, including *Spectrolebias reticulatus* (Costa & Nielsen, 2003), *Spectrolebias costai* (Lazara, 1991), *Spectrolebias semiocellatus* (Costa & Nielsen, 1997), and *Spectrolebias inaequipinnatus* (Costa & Brasil, 2008). The *R. zygonectes* species group is endemic to a similar area, but also including the middle Tapajós River basin, and excluding the Paranaíba area (Costa, 2007b, c); whereas, that clade of *Spectrolebias* inhab-

its the seasonal pools along that same area, but excluding the Parnaíba and Paranaíba areas (Costa, 2010).

The GAC supports the Araguaia area as derived from the fusion of three distinct ancient areas, each of them more closely related to different adjacent areas (Fig. 8). The relative central position of the present Araguaia area (Fig. 1) suggests that it has captured and coalesced adjacent drainages. This is congruent with data on the Araguaia subsidence that occurred in the Pleistocene (Hales, 1981; Saadi, 1993), which may have concatenated different areas adjacent to the Araguaia depression into the newly formed lowland.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Data matrix of distribution of character states of discrete characters amongst taxa

Table S2. Distribution of character states of continuous characters amongst taxa

Appendix S1. List of material examined.

Appendix S2. List of character statements used to reconstruct the phylogenetic hypothesis amongst the Plesiolebiasini.

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