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A new genus of miniature cynolebiasine from the Atlantic Forest and alternative biogeographical explanations for seasonal killifish distribution patterns in South America (Cyprinodontiformes: Rivulidae)

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Abstract

The analysis of 78 morphological characters for 16 species representing all the lineages of the tribe Cynopoecilini and three out-groups, indicates that the *incertae sedis* miniature species '*Leptolebias*' *leitaoi* Cruz & Peixoto is the sister group of a clade comprising the genera *Leptolebias*, *Campellolebias*, and *Cynopoecilus*, consequently recognised as the only member of a new genus. *Mucurilebias* gen. nov. is diagnosed by seven autapomorphies: eye occupying great part of head side, low number of caudal-fin rays (21), distal portion of epural much broader than distal portion of parhypural, an oblique red bar through opercle in both sexes, isthmus bright red in males, a white stripe on the distal margin of the dorsal fin in males, and a red stripe on the distal margin of the anal fin in males. *Mucurilebias leitaoi* is an endangered seasonal species endemic to the Mucuri river basin. The biogeographical analysis of genera of the subfamily Cynolebiasinae using a dispersal-vicariance, event-based parsimony approach indicates that distribution of South American killifishes may be broadly shaped by dispersal events. The analysis supports south-eastern Brazilian coastal plains as the centre of dispersal for cynolebiasine killifishes. Alternative biogeographical explanations are compared and possible dispersal routes and means of dispersal are discussed.

Key words

Atlantic Forest, Centre of origin, Dispersal, Mucuri river, Vicariance.

Introduction

The tribes Cynolebiasini and Cynopoecilini are well-corroborated clades of killifishes, belonging to the seasonal killifish subfamily Cynolebiasinae, with the Cynolebiasini comprising seven genera (*Austrolebias* Costa, 1998, *Cynolebias* Steindachner, 1877, *Hypsolebias* Costa, 2006, *Nematolebias* Costa, 1998, *Simpsonichthys* Carvalho, 1959, *Spectrolebias* Costa & Nielsen, 1997, *Xenurolebias* Costa, 2006) and the Cynopoecilini comprising four genera (*Campellolebias* Vaz-Ferreira & Sierra, *Cynopoecilus* Regan, 1912, *Leptolebias* Myers, 1952, and *Notholebias* Costa, 2008, 2010). In addition, Costa (2008) recognised an *incertae sedis* cynopoeciline species, '*Leptolebias*' *leitaoi* (Cruz & Peixoto, 1991) that could not be properly placed in any genera. '*Leptolebias*' *leitaoi* is known only from the floodplains of the Mucuri river, an area about 500 km north from the geographically nearest cynopoecilines (Costa, 2008). All the known specimens of '*L*'. *leitaoi* were collected in 1988, within a cacao farm close to the Mucuri river bank (Cruz & Peixoto, 1991). The whole region was subsequently highly modified by a cellulose industry and great part of the original forest was substituted by cattle pastures few years after the discovery of '*L*'. *leitaoi*. As a consequence, the species was never found again (Costa, 1995, 2009). Since the type series was lost, determination of the phylogenetic position of '*L*'. *leitaoi* depends on the single specimen of the species presently known to be preserved in a fish collection (Costa, 2008). This specimen was collected with the type series, kept in aquaria and fixed, cleared and stained just after dying. Consequently, some diagnostic characters could not be examined and the phylogenetic position of the species was not evaluated, preventing a tentative generic placement (Costa, 2008). However, after six years, '*L*'. *leitaoi* is still an *incertae sedis* taxon, which is a undesirable situation since '*L*'. *leitaoi* is a focal species within an important Brazilian governmental program to conserve killifishes threatened with extinction (ICMBio, 2013). To solve this problem, characters of that specimen are re-analysed in order to find the best phylogenetic hypothesis for the position of '*L*'. *leitaoi* among cynopoecilines, which then is used to provide a conventional generic placement.

The great distance between the only record of '*L*'. *leitaoi* and other members of the tribe Cynopoecilini, about 500 km, is not an uncommon pattern in the Atlantic Forest, similar to distributional gaps recorded for other cynolebiasine killifishes of the Atlantic Forest: 1) all species of the genus *Ophthalmolebias*, except *O. constanciae* (Myers, 1942), occurs in an area of north-eastern Brazil between the Almada and Jequitinhonha river basins, whereas *O. constanciae* is endemic to an area 800 km distant, in south-eastern Brazil (Costa, 2007); 2) the sister species *L. aureoguttatus* (Cruz, 1974) and *L. itanhaensis* Costa, 2008 from southern Brazil are separated from other congeners in south-eastern Brazil by a 400 km gap (Costa, 2008); 3) the genus *Xenurolebias* from eastern Brazil is separated from its sister group genus *Nematolebias* from south-eastern Brazil by a 400 km gap. These broad gaps may be explained both by large-scale extinction or long-distance dispersal, since incomplete sampling becomes improbable after over 200 collecting trips in about 30 years (June 1993 – February 2014), directed to killifish habitats of all parts of the Atlantic Forest. Costa (2010) analysed the biogeographical patterns of the Cynolebiasini, concluding that they are predominantly derived from old vicariance events. On the basis of a more inclusive analysis to comprise all genera of the Cynolebiasinae, alternative biogeographical historical explanations are here compared.

Material and methods

Phylogeny. The phylogenetic position of '*L*'. *leitaoi* was inferred through examination of a single topotype specimen, UFRJ 171, male, 22 mm SL, cleared and stained for bone and cartilage according to Taylor & Van Dyke (1985), deposited in the Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro; characters examined included data on squamation and contact organs taken just after fixation in formalin, and data on osteological characters taken after clearing and staining procedures. Data on colour patterns were taken from colour photos of live topotypes, and on reproductive behaviour characters from direct observation of one male and one female kept in aquarium, not preserved. The phylogenetic analysis was conducted using the same data matrix provided in Costa (2008), just inserting '*L*'. *leitaoi* as a terminal taxon (Appendix 1); characters were modified to follow Sereno's (2007) proposal for formulating character statements (Appendix 2); characters of anal-fin musculature and neuromast patterns, not recorded for '*L*'. *leitaoi*, were coded as a question mark for this species in the data matrix. A Maximum Parsimony analysis was performed with TNT 1.1 (Goloboff *et al*., 2008), using the 'traditional' search and setting random taxon-addition replicates to 10, tree bisection-reconnection branch swapping, multitrees in effect, collapsing branches of zero-length, characters equally weighted, and a maximum of 100,000 trees saved in each replicate. Branch support was assessed by bootstrap analysis, using a heuristic search with 1,000 replicates and the same settings used in the MP search, but saving a maximum of 1,000 trees in each random taxon-addition replicate.

Biogeography. Biogeographical distribution patterns of the subfamily Cynolebiasinae is analysed under a dispersal-vicariance, event-based parsimony approach (DIVA) (Ronquist, 1996, 1997). The search for the optimal historical reconstruction was performed using the computer program DIVA 1.2 (Ronquist, 1996). The phylogenetic tree used in the analysis was a summary of our present knowledge on cynolebiasine relationships, using genera as terminal taxa. This tree follows the phylogeny of the Cynopoecilini as herein proposed (Fig. 1) and the phylogeny of the Cynolebiasini as proposed by Costa (2010), except for the sister group relationship between *Nematolebias* and *Xenurolebias*, as strongly supported in an unpublished phylogenetic study using nuclear and mitochondrial genes by Amorim and Costa. The analysis also included the ancestral distribution of the most basal rivulid genus *Kryptolebias* Costa to follow Ronquist's (1996) recommendation to include distribution data for sister groups to reduce the tendency of the root node distribution to include most of the areas occupied by the terminals. The analysis included 14 terminal taxa (Fig. 2), representing all the genera of the subfamily Cynolebiasinae and *Kryptolebias*. The exact search of DIVA was used both without reducing the number of ancestral areas and restricting them to two areas through the maxareas option of the optimize command in DIVA. Areas of endemism were delimited according to the distribution of monophyletic groups of the Cynolebiasinae and other killifishes. In order to detect potential dispersal routes, areas of endemism (Fig. 3) were represented

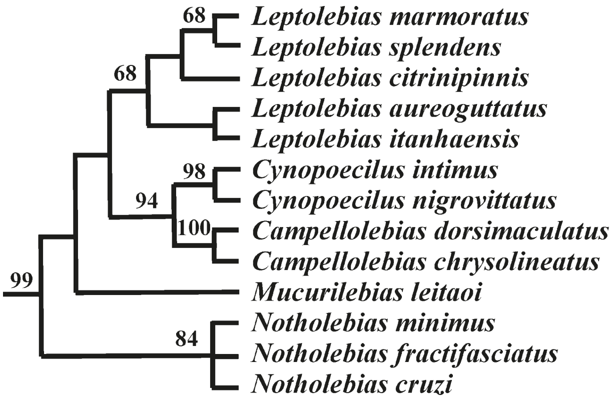

Fig. 1. Most parsimonious phylogeny among 13 species of the Cynopoecilini. Out-groups not depicted. Numbers above branches are bootstrap values.

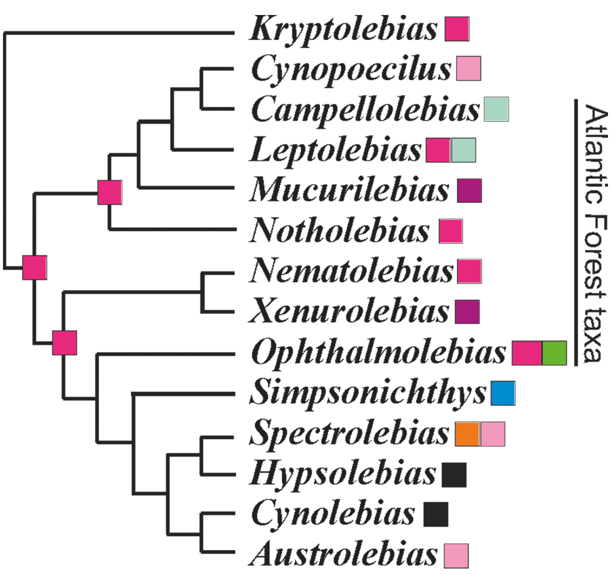

Fig. 2. Phylogenetic relationships among genera of the Cynolebiasinae used in the biogeographical analysis. Coloured squares refer to the geographical distribution of terminal taxa and nodes, in areas of endemism represented in Fig. 3: green, Almada–Jequitinhonha; purple, Jucuruçu–Doce; red crimson, São João–Guanabara; light greenish blue, Itanhaém–Jundiá; black, Caatinga-eastern Cerrado; orange, southern Amazon; blue, Brazilian Central Plateau; pink, Chaco-Pampas.

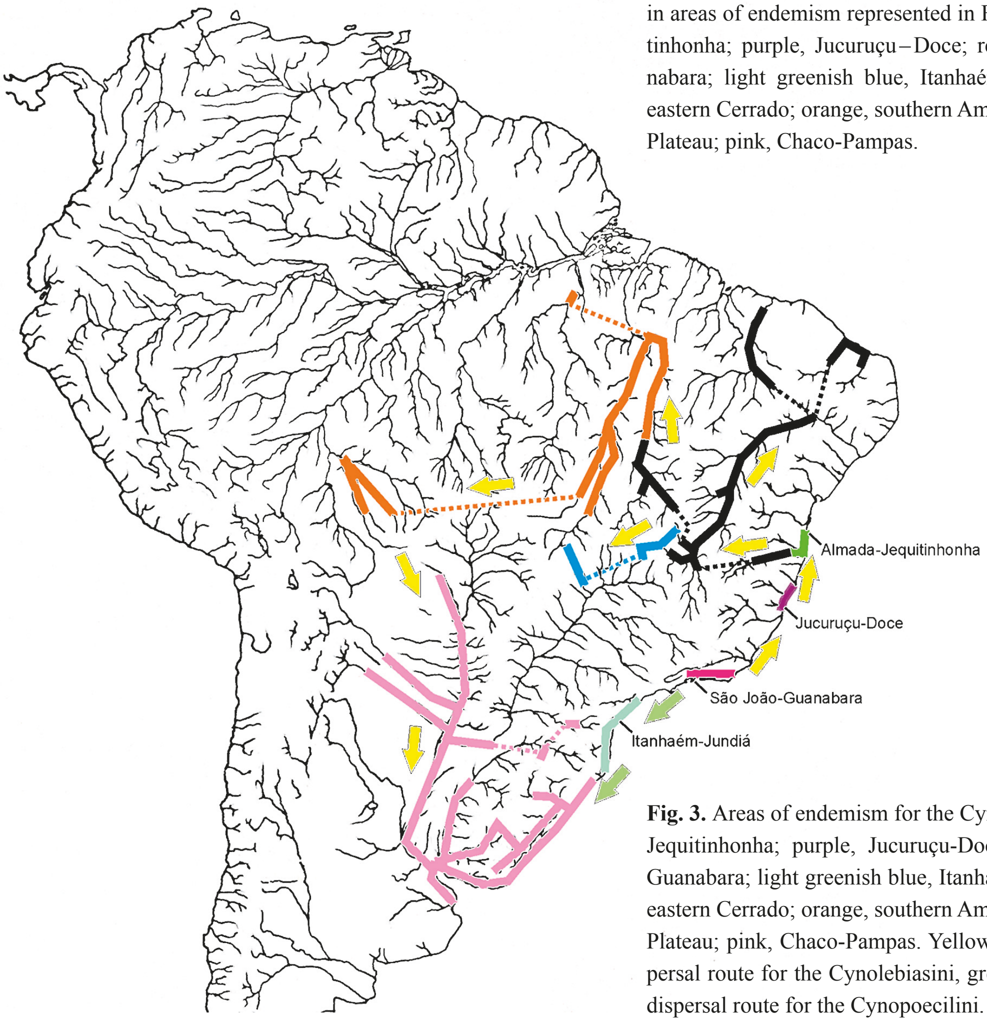

Fig. 3. Areas of endemism for the Cynolebiasinae: green, Almada-Jequitinhonha; purple, Jucuruçu-Doce; red crimson, São João-Guanabara; light greenish blue, Itanhaém-Jundiá; black, Caatinga-eastern Cerrado; orange, southern Amazon; blue, Brazilian Central Plateau; pink, Chaco-Pampas. Yellow rows indicate the main dispersal route for the Cynolebiasini, green arrows indicate the main dispersal route for the Cynopoecilini.

as tracks uniting collecting sited through river drainages or through plain areas. Four areas were recognised in the Atlantic Forest, named according to the river drainages limiting it to north and south, respectively: Almada-Jequitinhonha, Jucuruçu-Doce, São João-Guanabara and Itanhaém-Jundiá; four areas out of the Atlantic Forest were recognised: Caatinga-eastern Cerrado, southern Amazon, Brazilian Central Plateau, and Chaco-Pampas.

Results

Phylogenetic position of '*L*'. *leitaoi*. The phylogenetic analysis produced a single most parsimonious tree (Fig. 1), in which '*L*'. *leitaoi* appears as the sister group of a clade including the genera *Leptolebias*, *Campellolebias* and *Cynopoecilus*. This phylogenetic position supports recognition of '*L*'. *leitaoi* as a member of a new genus, described below.

Biogeographical reconstruction. The biogeographical analysis indicated several possibilities for ancestral areas of more inclusive clades, when the number of ancestral areas was not previously restricted, making difficult to chose one mostly plausible explanation among equally parsimonious possibilities. When using the constrained exact search limiting maximum ancestral distributions to two areas, DIVA unambiguously supported the São João–Guanabara area as the centre of origin for both the most recent ancestor of the Cynolebiasini and the Cynopoecilini (Fig. 2). Whereas Cynolebiasini dispersal is primarily directed to north, later following a clockwise route to reach the inner biogeographical provinces of the Caatinga, Cerrado, the transitional zone of southern Amazon, Chaco and the Pampas, the Cynopoecilini dispersal occur in a single northern area, the Jucuruçu-Doce area, followed by a southern route finishing in the Pampas (Fig. 3).

Taxonomic accounts

Mucurilebias gen. nov.

Type species: *Cynolebias leitaoi* Cruz & Peixoto, 1991.

Diagnosis. Distinguished from all other cynopoecilines by seven unique conditions: eye large, occupying great part of head side, dorsal and ventral margins of orbit in close proximity to dorsal and ventral surface of head, respectively, in lateral view (*vs*. eye never so large, margins separated by dorsal and ventral surfaces by broad space); low number of caudal-fin rays (21 *vs*. 23–30); distal portion of epural much broader than distal portion of parhypural (*vs*. epural and parhypural equal in width); presence of an oblique red bar through opercle in both sexes (*vs*. absence); isthmus bright red in males (*vs*. never red pigmented); presence of a white stripe on the distal margin of the dorsal fin in males (*vs*. absence); and presence of a red stripe on the distal margin of the anal fin in males (*vs*. absence).

Discussion

Phylogenetic position of *Mucurilebias*. *Mucurilebias leitaoi* possesses some morphological conditions unique among cynopoecilines, considered autapomorphic: eye large, occupying great part of head side, dorsal and ventral margins of orbit in close proximity to dorsal and ventral surface of head, respectively, in lateral view (Cruz & Peixoto, 1991: figs. 2–4; Costa, 1995: fig. 112; *vs*. eye never so large, margins separated by dorsal and ventral head surfaces by broad space); low number of caudal-fin rays (21 *vs*. 23–30); distal portion of epural much broader than distal portion of parhypural (*vs*. epural and parhypural equal in width); presence of an oblique red bar through opercle in both sexes (Fig. 2; *vs*. absence); isthmus bright red in males (Fig. 2; *vs*. never red pigmented); presence of a white stripe on the distal margin of the dorsal fin in males (Fig. 2; *vs*. absence); and presence of a red stripe on the distal margin of the anal fin in males (Fig. 2; *vs*. absence). Inclusion of *Mucurilebias* in the Cynopoecilini is supported by the following synapomorphic conditions: vomerine teeth absent, ventral process of angulo-articular rudimentary, posterior process of quadrate broad, mesopterygoid short, posterior tip in vertical through anterior portion of quadrate, and sympletic long and narrow (Costa, 2008), and iris green (Cruz & Peixoto, 1991). In addition, *M. leitaoi* does not exhibit the several synapomorphic conditions used to diagnose the genera *Campellolebias*, *Cynopoecilus*, *Leptolebias* and *Notholebias* (Costa, 2008).

Comparing conflicting biogeographical explanations. Costa (2010) found two possible interpretations for the distribution patterns of seasonal fishes of the tribe Cynolebiasini: the most recent ancestor of the clade was a geographically widespread taxon, occurring in a vast region of South America, or it was geographically restricted to eastern Brazil. The first explanation would implicate in an old sequence of vicariance events (hereafter OSVE), starting still in the Cretaceous, at the time of the separation of the South American and African plates, whereas the second one supported a history of more recent long dispersal events (hereafter RLDE) through great part of South America. The present analysis also indicated two similar possibilities.

Costa (2010) considered OSVE explanation more plausible, since cynolebiasine species are poor dispersers. All species of the Cynolebiasinae are seasonal killifishes, uniquely found in temporary pools formed during the rainy seasons, one or two depending on the region, usually one in the Amazon, Cerrado, Chaco and Pampas, two in the Atlantic Forest and Caatinga (Costa, 1995, 2001, 2002). In over 30 years of field studies in the whole geographical range of the Cynolebiasinae, their species were always found in temporary pools, never in streams, rivers, lakes, lagoons or in any salt-water environment (person. observ.). On the other hand, field studies also

Fig. 4. *Mucurilebias leitaoi*, male, not preserved, about 20 mm SL: Brazil: Bahia: Mucuri.

have consistently indicated that cynolebiasine species are confined to small areas of distribution, often few kilometres long, which has been positively tested by molecular studies (Costa *et al*., 2012, 2014; Costa & Amorim, 2013, in press). Considering this biological characteristic occurring in all the over 100 cynolebiasine extant species, it is not plausible to infer a single cynolebiasine ancestor species distributed nearly along the whole extension of the present day distribution of the Cynolebiasinae, as supported by OSVE explanation.

The OSVE explanation requires tests of congruency with historical distribution patterns of other biotic groups with similar biological attributes and similar general geographical distribution (*e.g.*, Nelson & Platnick, 1981). Among South American freshwater fish species groups, sister group relationships between taxa endemic to eastern Brazil, in the Atlantic Forest, and taxa occurring in the vast inner areas of South America, considered by Ribeiro (2006) as resulting from ancient isolation, seem to corroborate OSVE explanation for cynolebiasine geographical distribution (Costa, 2010). However, when analysing all the Cynolebiasinae together, two factors become evident: each major clade (*i.e.*, Cynolebiasini and Cynopoecilini) has multiple basal lineages occurring in the Atlantic Forest, more specifically in the São João – Guanabara area; each of the two major clades exhibit different area relationships (Fig. 2). Searching for a common shared pattern under an OSVE approach, it would implicate assuming both multiple duplication events and numerous and broad extinction events, becoming RLDE more plausible (Bremer, 1992). In addition, no other organism is known to have a similar biogeographical pattern for inner areas of South America as that exhibited by cynolebiasines.

Another limiting factor for the OSVE explanation is the lack of fossil record supporting an old existence for the group (Lundberg, 1993). Fossils are unknown for rivulids and all other taxa belonging to the cyprinodontiform suborder Aplocheiloidei (Costa, 2010). However, rich fossil material belonging to different families of the Cyprinodontoidei, the sister group to the Aplocheiloidei (Parenti, 1981; Costa, 1998), has been recorded from the Oligocene and Miocene of Europe (Costa, 2012), representing the oldest confirmed fossil records for the order Cyprinodontiformes. Therefore, these records may only be seen as the minimum age for aplocheiloids and cyprinodontoids, not necessarily meaning that aplocheiloids had their origin in the Oligocene or Eocene (Costa, 2010).

Means of dispersal. Recent studies have inferred long-distance dispersals for poor disperser organisms, without providing plausible explanations about the means of dispersal that would make possible those putative dispersal events (Heads, 2005). Among freshwater fishes of the order Cyprinodontiformes and the family Cichlidae, dispersals have been justified by the aprioristic assumption that members of this group are able to survive in brackish waters (*e.g.*, Myers, 1931; Lundberg, 1993; Briggs, 2003), making possible dispersals through estuarine zones. This simplistic explanation is refuted by almost all members of the Rivulidae and Cichlidae being uniquely found in freshwater, not exhibiting any evidence indicating some tolerance to life in saltwater (Sparks & Smith, 2005; Costa, 2013). Cynolebiasines are always found in seasonal pools with acid freshwater, pH usually 4.5-6.0 (*e.g.*, Costa, 1995), never in coastal alkaline aquatic habitats, therefore dispersal through estuarine zones should be discarded.

Since the 19th century, birds have been considered as dispersal agents of plant seeds and some animals. Darwin (1959) reported bird accidental transport of seeds attached to earth adhered to their beaks and feet, suggesting that the same mode of dispersal could occur with eggs of small freshwater animals. Concordantly, Wallace (1876) suggested freshwater fish dispersal by eggs adhered to the body of aquatic birds. Considering that cynolebiasine eggs are large (usually about 1.0–2.0 mm of diameter), able to survive in dry places for long periods and protected by a thick chorion, often exhibiting prominent ornamentations (*e.g.*, Fava & Toledo-Piza, 2007; Costa & Leal, 2009), dispersal of cynolebiasine eggs by birds is a possible option. Cynolebiasine eggs are adapted to survive in dry soil, in embryonic diapause stage (Wourms, 1972), during dry seasons, often three to seven months long. Therefore long-distance dispersal of eggs attached do dry earth of the temporary pool bottom through aquatic birds is a plausible possibility. However, if cynolebiasine egg transportation by birds was a common mean of dispersal, the distribution of cynolebiasine species would probably be wider and would not be so restricted to some regions. Thus egg dispersal by birds is an acceptable explanation since only considering it as accidental and rare events.

Dispersal routes in the Atlantic Forest and cynolebiasine habitat preference. Killifishes of the Cynolebiasinae are not broadly distributed in the regions where they are found, but are uniquely found in temporary pools formed along the floodplains of rivers and streams (Fig. 3). Under a RLDE scenario, cynolebiasines had the São João–Guanabara area as its primary centre of origin. Members of the most basal lineages, *Nematolebias* of the Cynolebiasini and *Notholebias* of the Cynopoecilini, are commonly found sharing the same habitat in the São João–Guanabara area, consisting of temporary pools situated in open vegetation or close to forest border, in the coastal Restinga formation, always in altitude close to the sea level (*e.g.,* Costa, 2002; Costa & Amorim, 2013; Costa *et al.*, 2014). Species of *Xenurolebias*, the sister group of *Nematolebias*, is endemic to the Jucuruçu-Doce area, and are found in similar coastal habitats (Costa & Amorim, in press). The Jucuruçu-Doce area is separated by 400 km from the São João–Guanabara area, a disjunction supported by DIVA as result of a dispersal event. The intermediate area contains long extensions of natural and modified coastal habitats not significantly different from those inhabited by species of *Nematolebias* and *Xenurolebias*, but cynolebiasines were never recorded for there although numerous attempts.

All species of *Ophthalmolebias*, except *O. constanciae*, are endemic to the Almada-Jequitinhonha area. The disjunctive distribution of *O. constanciae*, over 800 km distant to south, is remarkable, suggesting again a long-distance dispersal. In addition, whereas most species of *Ophthalmolebias* are found near the coast, in habitats like that described for *Nematolebias* and *Xenurolebias*, *O. rosaceus* and *O. perpendicularis* are found along the Pardo and Jequitinhonha river basins, about 100 km from the coast. More interestingly, the area extension occupied by that species pair is continuous to the area of the Jequitinhonha river drainage inhabited by *Hypsolebias ocellatus*, a member of the clade sister to *Ophthalmolebias*, suggesting a possible dispersal route from the Atlantic Forest to the neighbouring inner Caatinga. Important to note that other Atlantic Forest areas of endemism of cynolebiasines are limited to the east by large mountain ranges (*i.e.*, Mar and Mantiqueira mountain ranges), with peaks reaching over 1,000 and 2,500 m above sea level, which could impede cynolebiasine dispersal.

Among the Cynopoecilini, the analysis support both dispersal of *Mucurilebias* to north and components or the remaining clade (*Leptolebias* + *Campellolebias* + *Cynopoecilus*) to south, always through plain areas at the sea level, in habitats as described for the Cynolebiasini. The only exceptions are some species of *Leptolebias* (*L. aureoguttatus*, *L. itanhaensis*, *L. marmoratus*, and *L. splendens*) that live in temporary channels within dense rain forest, thus herein considered as the only true forest dweller among members of the Cynolebiasinae.

The RLDE approach supports the coastal plains of south-eastern Brazil as a centre of origin for cynolebiasines, besides allowing a much more recent age for the group than estimated by the OSVE approach (Costa, 2010). Considering that: all the basal lineages of the Rivulidae are primarily restricted to the Atlantic coastal plains of South America; all the basal lineages of the Nothobranchiidae, the sister group of the Rivulidae, are equally found in the Atlantic coastal plains of Africa; and the Aplocheilidae, the sister group of Rivulidae plus Nothobranchiidae, is endemic to Madagascar and south-eastern Asia, under a RLDE approach it would be consistent to interpret: the Atlantic coastal plains of Africa as a putative centre of origin for the common ancestor of the Rivulidae plus Nothobranchiidae; and a subsequent dispersal of the ancestor of Rivulidae to South America. However, this interpretation is merely speculative without support of convincing time estimates, still not available for aplocheiloids. On the other hand, this dispersal hypothesis may be part of a general pattern of synchronised dispersal, temporarily favoured by geoclimatic changes. Recent studies have consistently invoked trans-oceanic dispersal among freshwater and terrestrial vertebrates from Africa to South America approximately during the Eocene-Oligocene transition (*e.g.,* Gamble *et al.*, 2010; Loss-Oliveira *et al.*, 2012; Friedman *et al.*, 2013), a time scale congruent with our knowledge about cyprinodontiform fossils (*e.g.*, Costa, 2012).

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Appendix 1

Matrix of 78 morphological characters for 16 rivulid species

Kryptolebias brasiliensis
0000000000000000000000000000000000000000000000000000000000?0000000000000000000

Neofundulus paraguayensis
0001000000100101000000000100000000000000000001000000001000?0001000−00000000001

Nematolebias whitei
000201001011111111021100000111111000001001100010001101100111001000000110000001

Leptolebias marmoratus
1013120110111111110111000001111111010010111111111012111010?2011201−00201100001

Leptolebias citrinipinnis
1013120110111111110111000001111111000010011010111012111010?0011101300301101001

Leptolebias splendens
1013120110111111110111000001111111000010011011111012111010?0011201200201101001

Leptolebias aureoguttatus
1013120110111111110111000001111111000010011010111012111110?2011101400301101001

Leptolebias itanhaensis
1013120110111111110111000001111111000010011010111012111110?2011101400301101001

Notholebias fractifasciatus
001312011110111111011100000111111100001001100011101201111110011100100101101001

Notholebias minimus
001312011110111111011100000111111100001001100011101201111110011100100101101001

Notholebias cruzi
001312011110111111011100000111111100001001100011101201111110011100100101101001

Cynopoecilus intimus
1113121110101111110111011011111111010111112100111112012110?0101000000301111101

Cynopoecilus nigrovittatus
1113121110101111110111011011111111010111112000110122022110?0101000000301111101

Campellolebias dorsimaculatus
111312011011111111111011010111111111101101200011101202211101001110011311011011

Campellolebias chrysolineatus
111312011011111111111011010111111111101101310011101202211102001110011311011011

Mucurilebias leitaoi
1013120110111111110111000001111111??000100110011110??????111001110000010??01001

Appendix 2

List of characters and character states

Superficial dermal bones and neurocranium

[1] Dermosphenotic: (0) present; (1) absent.
[2] Vomer, lateral wings, shape: (0) broad; (1) narrow.
[3] Vomer, teeth: (0) present; (1) absent.

Jaws, jaw suspensorium and opercular apparatus

[4] Angulo-articular, ventral process, shape: (0) long and wide; (1) long and narrow; (2) short and wide; (3) rudimentary [not ordered].
[5] Quadrate, posterior process, shape: (0) narrow; (1) wide.
[6] Mesopterygoid, extent relative to quadrate: (0) long, reaching metapterygoid and overlapping quadrate; (1) short, posterior tip in vertical through middle of quadrate, ventral portion overlapping quadrate; (2) very short, posterior tip in vertical through anterior portion of quadrate.
[7] Autopalatine, median portion, constriction: (0) absent; (1) present.
[8] Sympletic, shape: (0) short and deep; (1) long and narrow.
[9] Pre-opercle, dorsal portion, shape: (0) broad and rounded; (1) narrow and pointed.

Hyoid and branchial arches

[10] Basihyal, shape: (0) broad, triangular; (1) narrow, about rectangular.
[11] Interhyal, ossification: (0) ossified; (1) cartilaginous.
[12] Second pharyngobranchial, teeth: (0) present; (1) absent (Costa, 1998).
[13] Epibranchials, shape: (0) short; (1) long.
[14] Third epibranchial, uncinate process, shape: (0) long; (1) short.
[15] First hypobranchial, proximal edge, shape: (0) bifid, terminating in cartilage connected to second basibranchial and another smaller cartilage connected to first basibranchial; (1) plain, terminating in single cartilage connected to second basibranchial.
[16] First hypobranchial, distal edge, articular face extent: (0) restricted to cartilaginous head of first ceratobranchial; (1) anteriorly expanded.
[17] Fourth ceratobranchial, teeth: (0) present; (1) absent.

Vertebrae and caudal skeleton

[18] Caudal vertebrae, neural pre-zygapophyses, development: (0) well-developed; (1) rudimentary.
[19] Caudal vertebrae, last neural spine, postero-basal portion, small process bearing ligament attached to anterior tip of epural: (0) absent; (1) present.
[20] Hypurals, fusion degree: (0) two separate symmetrical plates; (1) two plates fused, with vestige of median fissure; (2) complete ankylosis forming single plate, without vestige of median fissure.
[21] Epural and parhypural, proximal region: (0) broad and approximately straight; (1) narrow and curved anteriorly.

Dorsal and anal-fin skeleton

[22] Anal fin, first proximal radials: (0) long and narrow; (1) short and wide.
[23] Anal fin in males, first proximal radial: (0) approximately straight; (1) curved posteriorly.

[24] Dorsal fin, relative position on body mid-length: (0) posterior; (1) anterior.
[25] Anal fin in males, anterior portion, multiple minute rays (8–9): (0) absent; (1) present.
[26] Anal fin in males, first two rays in male, ligamentous connections to radials: (0) thin, weakly connected to proximal radials; (1) thickened and strongly attached to proximal radials.
[27] Anal fin in males, anterior rays: (0) straight; (1) curved.

Shoulder and pelvic girdle

[28] Pectoral fin, posttemporal, ventral process: (0) present; (1) absent.
[29] Pectoral fin, supra-cleithrum, shape: (0) short; (1) long.
[30] Pectoral fin, cleithrum, posterior flange: (0) present; (1) absent.
[31] Pectoral fin, radials, shape: (0) well-ossified, cub form; (1) thin, weakly ossified, disc shaped.
[32] Pectoral fin, radials, relative extent between ventral pectoral-fin radial and coracoid: (0) wide; (1) narrow.
[33] Pelvic fin, ischial process: (0) present; (1) absent.

Muscles of anal-fin base

[34] Muscular fibres circularly arranged around urogenital papilla base to form ejaculatory pump: (0) absent; (1) present.
[35] *Inclinatores anales* 1–3, shape: (0) narrow; (1) expanded laterally to form fan-shaped structure.

External morphology of body and fins

[36] Urogenital papilla in males, attachment to anal fin: (0) free; (1) attached.
[37] Urogenital papilla in males, extent: (0) short; (1) long.
[38] Urogenital papilla in males, shape: (0) globular to tubular; (1) prominent pocket-like structure overlapping anterior anal-fin origin.
[39] Urogenital papilla in females: (0) minute gap; (1) prominent pocket-like structure overlapping anterior anal-fin origin.
[40] Anal fin in males, membrane shape: (0) continuous; (1) interrupted, making anterior portion of fin separated.
[41] Anal fin in males, anterior portion, thickened tissue possibly glandular: (0) absent; (1) present.
[42] Dorsal fin in males, posterior extremity, shape: (0) rounded; (1) pointed.
[43] Anal fin in males, shape: (0) about rectangular, extremity rounded and short; (1) about rectangular, extremity pointed; (2) about triangular, slightly elongated; (3) about triangular, long.
[44] Pelvic fin, medial relative position of bases: (0) in close proximity; (1) separated by short interspace.
[45] Caudal fin, shape: (0) approximately so deep as long; (1) much longer than deep.
[46] Caudal fin, posterior margin: (0) rounded; (1) pointed

Squamation

[47] Frontal scales, arrangement: (0) circular; (1) transversal.
[48] E-scales, relative medial position: (0) slightly overlapped; (1) not overlapped.
[49] Supraorbital squamation: (0) one or more large scales occupying whole supraorbital area; (1) rudimentary or absent.
[50] Head, area adjacent to isthmus, row of scales: (0) present; (1) absent.

Neuromasts

[51] Supraorbital neuromasts, number: (0) 6–7; (1) 9–13; (2) 12–16.
[52] Supraorbital series, arrangement: (0) two separate sections; (1) continuous series; (2) continuous, with short anterior interruption [not ordered].
[53] Supraorbital neuromasts, anterior-most section, number: (0) 2–3; (1) 1.
[54] Infraorbital neuromasts, section around eye, number: (0) 12–16; (1) 17–20; (2) 21–28.
[55] Pre-opercular neuromasts, number: (0) 6–8; (1) 10–16; (2) 17–24.
[56] Caudal fin, base, neuromasts, number: (0) 1–2; (1) 4–7.

Contact organs in males

[57] Flank, scale margin: (0) absent; (1) present.
[58] Pectoral fin, inner surface: (0) absent; (1) present.
[59] Pectoral fin, inner surface, shape, development and ossification: (0) minute, not ossified; (2) pronounced, basal portion ossified.

Colour patterns

[60] Flank in males, iridescent colour patterns: (0) diffuse over flank; (1) vertical rows of dots; (2) longitudinal rows of small spots [not ordered].
[61] Flank in males, melanophore pattern: (0) not distinctively patterned; (1) two black stripes.
[62] Flank in females, dark pigmentation: (0) present; (1) absent.

[63] Iris, bar through centre of eye: (0) absent; (1) present.
[64] Iris in males, colour: (0) yellow to yellowish brown; (1) green; (2) blue.
[65] Dorsal and anal fins in males, sub-distal region, distinctive concentration of melanophores: (0) absent; (1) present.
[66] Dorsal fin in males, extent of dark red pigmentation: (0) reaching antero-distal margin; (1) not surpassing sub-distal portion.
[67] Caudal fin in males, iridescent colour pattern: (0) dots; (1) bars or vertical lines; (2) vermiculate marks; (3) iridescence homogeneously arranged over fin; (4) horizontal lines; (?) no iridescence [not ordered].
[68] Head and trunk, mid-ventral portion, stripe: (0) absent; (1) present.
[69] Head in males, latero-ventral portion, stripe: (0) absent; (1) present.
[70] Head in males, opercular region, red pigmentation pattern: (0) not patterned; (1) bars; (2) stripes; (3) reticulate [not ordered].
[71] Caudal peduncle in females, dark grey to black spots: (0) absent; (1) present.

Egg

[72] Chorion, surface: (0) plain to verrucate; (1) reticulate.
[73] Chorion projections, shape: (0) spine to hair-like; (1) mushroom-like.

Reproductive behaviour

[74] Insemination, kind: (0) external; (1) internal.
[75] Male courtship behaviour, performance of dorsal and anal fins: (0) antero-posteriorly expanded; (1) twisted.
[76] Male courtship behaviour, zigzag display: (0) absent; (1) present.
[77] Male courtship behaviour, coiling retrorse motion: (0) absent; (1) present.

Life style

[78] Embryologic diapause and habitat: (0) without long diapause stages, life-cycle in perennial habitats; (1) long diapause stages, life-cycle in seasonal pools.