

Historical biogeography of cynolebiasine annual killifishes inferred from dispersal–vicariance analysis

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ABSTRACT

Aim To analyse the biogeographical events responsible for the present distribution of cynolebiasine killifishes (Teleostei: Rivulidae: Cynolebiasini), a diversified and widespread Neotropical group of annual fishes threatened with extinction.

Location South America, focusing on the main river basins draining the Brazilian Shield and adjacent zones.

Methods Phylogenetic analysis of 214 morphological characters of 102 cynolebiasine species using TNT, in conjunction with dispersal–vicariance analysis (DIVA) based on the distribution of cynolebiasine species among 16 areas of endemism.

Results The basal cynolebiasine node is hypothesized to be derived from an old vicariance event occurring just after the separation of South America from Africa, when the terrains at the passive margin of the South American plate were isolated from the remaining interior areas. This would have been followed by geodispersal events caused by river-capturing episodes from the adjacent upland river basins to the coastal region. Optimal ancestral reconstructions suggest that the diversification of the tribe Cynolebiasini in north-eastern South America was first caused by vicariance events in the Paranã–Urucuia–São Francisco area, followed by dispersal from the São Francisco to the Northeastern Brazil area. The latter dispersal event occurred simultaneously in two different cynolebiasine clades, possibly as a result of a temporary connection of the São Francisco area before the uplift of the Borborema Plateau during the Miocene. The diversity of cynolebiasines inhabiting the Paraguay area is hypothesized to be derived from two processes: an older vicariance event (about 30 Ma) separating Paraguay from southern Amazonian areas (Guaporé–Xingu–Araguaia–Tocantins), and a series of more recent dispersal and vicariance events (about 15–11 Ma) caused by successive marine transgressions, which permitted alternating biotic exchange and isolation in the Paraguay, La Plata, Negro and Patos areas.

Main conclusions DIVA indicates there to have been a series of vicariance events congruent with tectonic episodes in South America, but the present distribution of cynolebiasines has also been shaped by a series of dispersal events. The effects of the combined action of dispersal and vicariance events were more conspicuous in the Eastern Brazil and Paraguay areas, thus generating reticulate biogeographical scenarios.

Keywords

Cynolebiasinae, Cyprinodontiformes, dispersal–vicariance analysis, killifishes, Neotropics, reticulate biogeographical history, Rivulidae, South American rivers.

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INTRODUCTION

The extensive river basins of South America contain the richest fauna of freshwater fishes of the world (e.g. Reis *et al.*, 2003). Freshwater fishes are biogeographically interesting owing to their limited ability to disperse beyond the limits of the river basins in which they originated (Myers, 1938; Vari, 1988). In South America, the great diversity of fish taxa and river basins combined with long past isolation from the remaining continents provide a particularly promising context for biogeographical studies. However, papers about the historical biogeography of Neotropical fishes are poorly represented in the literature, possibly as a result of insufficient knowledge of species-level taxonomy, phylogenetic relationships and geographic distributions of taxa (Vari & Weitzman, 1990).

The Rivulidae is the fourth most diversified family among the 71 fish families occurring in freshwater environments of the Neotropical region (Reis *et al.*, 2003). About half of all rivulid species are annual fishes, which are uniquely found in seasonal pools formed during the rainy season, with embryos surviving in resistant diapause eggs in dry periods (e.g. Wourms, 1972). The tribe Cynolebiasini is the most diversified and geographically widespread clade of annual fishes, comprising 107 species inhabiting seasonal pools of a great portion of South America, including Brazil, Bolivia, Paraguay, Uruguay and Argentina (Costa, 2001, 2002a, 2006a, 2007a). Cynolebiasine distribution is approximately coincident with the geographic limits of the Brazilian Shield, which is a vast ancient Precambrian crystalline basement occupying the greatest part of the continental South American Plate (Fig. 1), with

sedimentary basins not influenced by marine transgressions since the Triassic (e.g. Lundberg *et al.*, 1998; Almeida *et al.*, 2000). Cynolebiasines occur in the main South American river basins, including the Amazonas, Paraguay, Paraná and São Francisco river basins, in areas occupied by a large array of physiognomic and habitat diversity, from dense rain forests to semi-arid savannas (e.g. Costa, 2002b).

Cynolebiasines also exhibit the greatest morphological diversification among aplocheiloid killifishes, comprising both miniature species not exceeding 25 mm of standard length (SL) (Costa, 2007a) and the largest aplocheiloid species reaching 150 mm SL (Costa, 2006a). Recent ecological studies have revealed high diversification in feeding habits and trophic morphological specializations among species occurring in the same area (Costa, 2009a). More important for biogeographical studies, cynolebiasine species are typically restricted to small areas, which are often simultaneously inhabited by species of different cynolebiasine clades. In addition, owing to the geographically restricted areas occupied by species and their specialized life style, combined with the intensive natural habitat destruction, more than 50% of all cynolebiasine species are threatened with extinction (Costa, 2002b, 2009b).

Cynolebiasines have been classified into four genera, three of which are well corroborated in recent phylogenetic studies (e.g. Costa, 2006a,b): *Cynolebias*, from central and north-eastern Brazil; *Austrolebias*, from southern Brazil, southern Bolivia, Paraguay, Uruguay and north-eastern Argentina; and *Nematolebias*, from south-eastern Brazil (Costa, 2001, 2002a, 2006a). The fourth genus, *Simpsonichthys*, is weakly diagnosed, but



Figure 1 Approximate limits of the Brazilian Shield (dashed line) and major rivers of South America and other rivers cited in the text (modified from Lundberg *et al.*, 1998).

includes five well-supported subgenera (Costa, 2006b, 2007a): *Simpsonichthys*, from central Brazil; *Xenurolebias* and *Ophthalmolebias*, from eastern Brazil; *Hypsolebias*, from central and north-eastern Brazil; and *Spectrolebias*, from central Brazil, Bolivia and Paraguay (Costa, 2007a). Phylogenetic studies involving substantial cynolebiasine taxon samples are still lacking. Molecular data are available for only a few cynolebiasine species (e.g. Murphy *et al.*, 1999; García *et al.*, 2002), but morphological characters have been continuously applied to a series of phylogenetic studies of individual genera (e.g. Costa, 2002a, 2006a,b). The objective of this paper is to analyse historical biogeographical features based on a first broad phylogenetic analysis of the cynolebiasine killifishes under an event-based approach (Ronquist, 1997), in order to find evidence supporting vicariance and dispersal events responsible for the present distribution of cynolebiasines.

MATERIALS AND METHODS

Phylogenetic analysis

The phylogenetic analysis is based on 214 morphological characters extracted from independent phylogenetic analyses provided in the taxonomic revisions of *Cynolebias* (Costa, 2001), *Simpsonichthys* (Costa, 2006a) and *Austrolebias* (Costa, 2006b), where character states were discussed and illustrated. Characters were re-analysed to include species described in more recent years and reduce ambiguity, following Sereno (2007) for character statement formulation. Quantitative characters, previously expressed through discrete character states, are herein re-analysed as proposed by Goloboff *et al.* (2006). Terminal taxa include 102 of the 107 known valid species of the Cynolebiasini, and two rivulid species of other lineages (*Notholebias fractifasciatus* of the Cynopoeilini and *Rivulus janeiroensis* of the Rivulinae), as well as three outgroups (*Kryptolebias brasiliensis*, a basal taxon of the Rivulidae; *Scriptaphyosemion guignardi*, a Nothobranchiidae; and *Aplocheilus panchax*, an Aplocheilidae). Complete lists of species and specimens examined are provided in recent revisionary studies (Costa, 2001, 2006a,b).

The search for the most parsimonious trees (traditional search) and bootstrap analysis (1000 replicates) were performed with TNT 1.1 (Goloboff *et al.*, 2008a). The implied weighting option was used to find a single fully bifurcating tree, which is a pre-requisite for dispersal–vicariance analyses (Ronquist, 1996; see below) (see Goloboff *et al.*, 2008b, for further justification for the use of weighting techniques against homoplasy). Character states of all discrete characters were treated as unordered (listed in Appendix S1 in the Supporting Information). Morphometric and meristic data were median values taken from ranges available in revisionary studies (Costa, 2001, 2006a, 2007a), treated as ordered continuous characters, implemented in TNT analysis. The distribution of character states of discrete characters among taxa is given in Appendix S2, and that of continuous characters in Appendix S3.

Biogeography

The biogeographical history of the annual killifishes of the tribe Cynolebiasini is reconstructed using dispersal–vicariance analysis (Ronquist, 1996, 1997), a method recommended in studies aiming to infer the biogeographical history of an individual lineage in the absence of a general area cladogram (e.g. Sanmartín, 2007). It is an event-based parsimony method with an explicit treatment of multiple identified processes (vicariance, dispersal, extinction and sympatric speciation) incorporated in the analysis, in which relative costs are assigned (vicariance and sympatric speciation events with a cost of zero; dispersal and extinction events with a cost of one per unit area added or deleted from the distribution) (Ronquist, 1996).

The search for the optimal historical reconstruction, in which the total cost is minimized under parsimony criteria, was performed using the computer program DIVA 1.2 (Ronquist, 1996). In order to reduce the tendency of the root-node distribution to include most of the areas occupied by the terminals, making optimization of the root zone less reliable (Ronquist, 1996), distribution data for the sister group to the Cynolebiasini (the Cynopoeilini from the eastern Brazil area; Costa, 2008) were included in the analysis following Ronquist's (1996) recommendation. The exact search option of DIVA was used without restricting the number of areas in which the ancestor occurred, except in one instance (see text below), when the maximum number of unit areas was constrained to two, using the maxareas option of the optimize command in DIVA.

Several methods have been proposed to identify or delimit areas of endemism (e.g. Morrone, 1994; Szumik *et al.*, 2002; Szumik & Goloboff, 2004), and they are often based on grid cells projected on a distribution map. However, as in other biogeographical studies on freshwater fishes, areas of endemism are herein delimited through river basin watersheds, which are the natural boundaries for species distributions. Rivers have existed since the formation of the continents and are sensitive to contemporary tectonics (Potter, 1997), thus making them fundamental units in the effort to reconstruct geological and biotic history. Studies on the biogeography of freshwater fishes usually employ entire river drainages as areas of endemism (e.g. Vari, 1988; Hubert & Renno, 2006). However, different sections of a particular river drainage may have different ages (Potter, 1997), as evidenced in phylogenetic studies of annual killifishes (e.g. Costa, 2001). In the present study, an area of endemism is delimited by sections or groups of river basins showing congruent distributional ranges shared by two or more species of freshwater fishes.

The following 16 areas of endemism are analysed herein (Fig. 2). (A) Eastern Brazil: numerous small river basins of the eastern coastal plains of Brazil, as well as the lower courses of three larger rivers (Jequitinhonha, Pardo and Doce); (B) Central Brazilian Plateau: the plateau comprising the upper section of streams running to the Paraná, São Francisco,

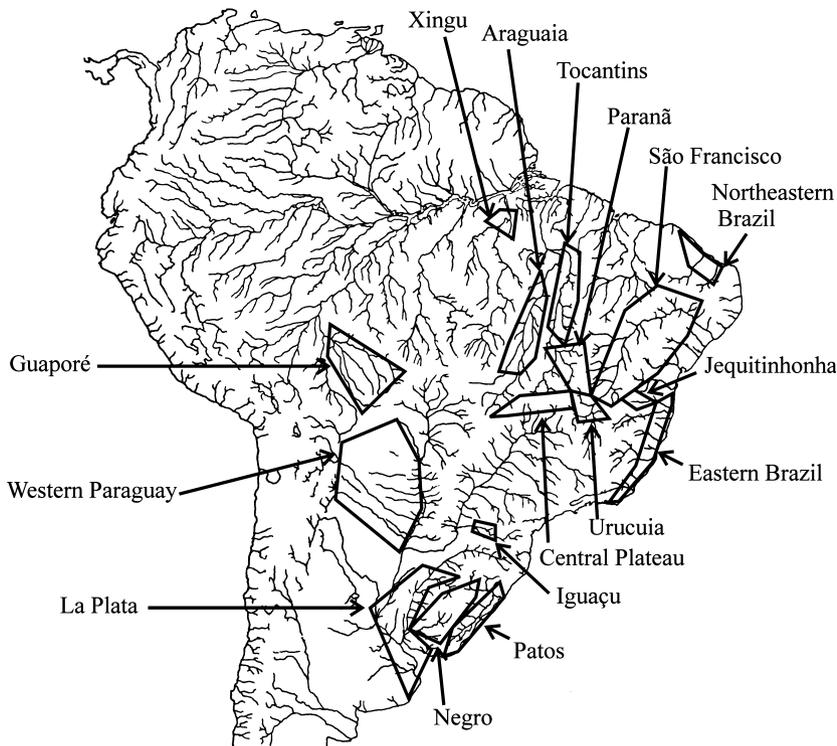


Figure 2 Areas of endemism of cynolebiasine killifishes.

Tocantins and Araguaia rivers; (C) Paraguay: the western part of the Paraguay River basin; (D) Guaporé: Guaporé River drainage of the Madeira River basin; (E) Xingu: the middle–lower section of the Xingu River basin; (F) Araguaia: the middle section of the Araguaia River basin; (G) Tocantins: the middle section of the Tocantins River basin; (H) Jequitinhonha: the middle section of the Jequitinhonha River basin above Salto da Divisa falls; (I) São Francisco: the middle section of the São Francisco basin north of the city of São Francisco, as well as the middle section of the Paraguaçu, Vaza-Barris and Itapicuru river basins; (J) Uruçua: Uruçua and Paracatu river drainages of the São Francisco River basin and adjacent areas; (K) Paranã: Paranã River drainage of the Tocantins River basin; (L) Northeastern Brazil: Jaguaribe River and smaller adjacent coastal basins; (M) La Plata: the lower La Plata River basin and middle–lower Uruguay River basin; (N) Negro: Negro River drainage of the Uruguay River basin, and upper and middle sections of the Jacuí, Santa Maria, Jaguarão and Quarai river drainages; (O) Patos: Patos lagoon system and adjacent coastal plains to the south; and (P) Iguaçú: the middle section of the Iguaçú River basin.

As the number of unit areas exceeded the limit established in DIVA, three combined analyses were performed, following Ronquist's (1996) suggestion. The first one encompassed all species of the genera *Spectrolebias* and *Hypsolebias*; the second included all species of the genera *Cynolebias* and *Austrolebias*; and the third analysis had as terminal taxa all species of the most basal genera (*Nematolebias*, *Xenurolebias*, *Ophthalmolebias*, *Simpsonichthys*) as well as the genera *Spectrolebias*, *Hypsolebias*, *Cynolebias* and *Austrolebias* as terminal taxa exhibiting the ancestral distribution determined in analyses 1 and 2.

RESULTS AND DISCUSSION

Phylogenetic analysis

The most parsimonious phylogenetic tree for 209 morphological characters and 107 aplocheiloid taxa is illustrated in Figs 3–6. This general tree of cynolebiasine relationships differs from isolated phylogenies for cynolebiasine genera (Costa, 2001, 2006a,b) by *Simpsonichthys* being paraphyletic. To accommodate a classification compatible with the present phylogeny, former subgenera of *Simpsonichthys*, all monophyletic, are herein considered as full genera.

General biogeographical reconstruction

The phylogeny of cynolebiasines supports eight lineages corresponding to formally recognized genera (Figs 3–6). The three basal lineages (*Nematolebias*, *Xenurolebias* and *Ophthalmolebias*) are endemic to the eastern Brazilian coastal plains (Fig. 3), whereas the other five lineages (*Simpsonichthys*, *Hypsolebias*, *Spectrolebias*, *Cynolebias* and *Austrolebias*) occur in the remaining South American areas analysed (Figs 3–6). DIVA provides two possibilities for the geographic distribution of the most recent ancestor of the cynolebiasine: either occurring in most analysed areas, or restricted to the Eastern Brazil area. The first possibility can be explained by an older sequence of vicariance events, while the second one indicates a long history of dispersals through South America, which is also the only explanation provided by DIVA when using the constrained exact search limiting maximum ancestral distributions to two areas. The latter possibility seems to be less

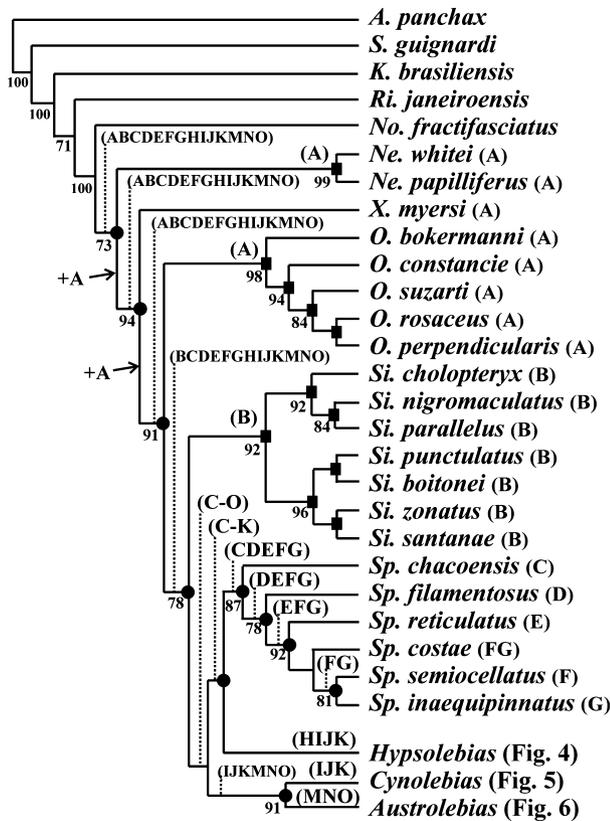


Figure 3 Most parsimonious cladogram of phylogenetic relationships among cynolebiasine fishes, representing outgroups and species of *Nematolebias* (*N. whitei* and *N. papilliferus*), *Ophthalmolebias*, *Simpsonichthys* (*Si.*), *Spectrolebias* (*Sp.*) and *Xenurolebias*, and summary of the optimal reconstructions of ancestral distributions using dispersal–vicariance analysis. Hypothesized vicariance (black circles) and duplication (black squares) events appear on nodes; rows indicate dispersal (+) events. Letters above branches are hypothesized ancestral distributions; numbers below branches are bootstrap values. Terminal taxa include two rivulid species of other lineages (*Notholebias fractifasciatus* and *Rivulus janeiroensis*), as well as the outgroups *Aplocheilus panchax*, *Scriptaphyosemion guignardi* and *Kryptolebias brasiliensis*. Areas of endemism: (A) Eastern Brazil, (B) Central Brazilian Plateau, (C) Paraguay, (D) Guaporé, (E) Xingu, (F) Araguaia, (G) Tocantins, (H) Jequitinhonha, (I) São Francisco, (J) Urucuia, (K) Paranã, (L) Northeastern Brazil, (M) La Plata, (N) Negro, (O) Patos.

plausible, because cynolebiasine species are not good dispersers, species being presently known from very small areas, therefore, possessing biological characteristics not compatible with long-distance dispersal.

The occurrence of the most basal cynolebiasine lineages in eastern Brazil, suggesting a former vicariance event between eastern Brazil and most other areas of the Brazilian Shield, is congruent with phylogenetic studies on some other Neotropical freshwater fish groups. Eastern Brazil is an important area of endemism for freshwater fishes (e.g. Weitzman *et al.*, 1988), containing several endemic taxa hypothesized to be the sister group of clades occurring in vast areas of South America, excluding eastern Brazil (Ribeiro, 2006). The

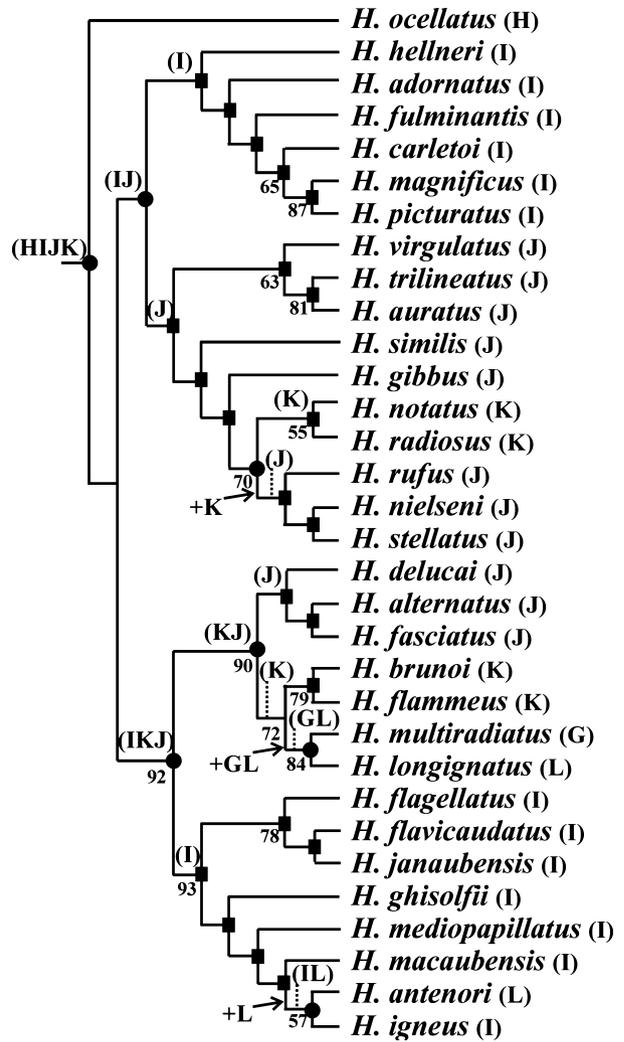


Figure 4 Most parsimonious cladogram of phylogenetic relationships among cynolebiasine fishes, representing species of *Hypsolebias*, and summary of the optimal reconstructions of ancestral distributions using dispersal–vicariance analysis. Hypothesized vicariance (black circles) and duplication (black squares) events appear on nodes; rows indicate dispersal (+) events. Letters above branches are hypothesized ancestral distributions; numbers below branches are bootstrap values. Areas of endemism: (G) Tocantins, (H) Jequitinhonha, (I) São Francisco, (J) Urucuia, (K) Paranã, (L) Northeastern Brazil.

biogeographical pattern shared by such different fish groups such as trichomycterids, callichthyids, loricariids, doradids and characids has been considered to be the result of the ancient isolation of the river basins of eastern Brazil (Ribeiro, 2006). The oldest event is related to the formation of the passive continental margin of South America, just after the break-up of South America and Africa, which isolated eastern Brazil for the first time (e.g. Potter, 1997; Ribeiro, 2006). Isolation of the South American and African plates was followed by the gradual formation of taphrogenic basins as a result of vertical movements between rifted blocks caused by the combination of an isostatic adjustment of the eastern

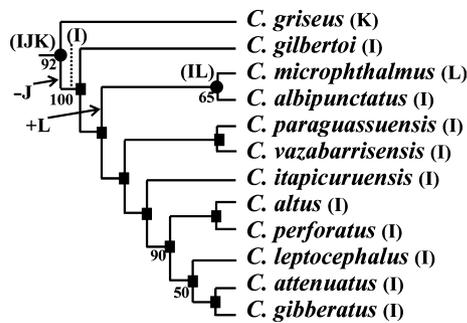


Figure 5 Most parsimonious cladogram of phylogenetic relationships among cynolebiasine fishes, representing species of *Cynolebias*, and summary of the optimal reconstructions of ancestral distributions using dispersal–vicariance analysis. Hypothesized vicariance (black circles) and duplication (black squares) events appear on nodes; rows indicate dispersal (+) and extinction (–) events. Letters above branches are hypothesized ancestral distributions; numbers below branches are bootstrap values. Areas of endemism: (I) São Francisco, (J) Uruçuia, (K) Paranã, (L) Northeastern Brazil.

margin of the platform and reactivations of ancient rifts, thus capturing adjacent upland river basins to eastern Brazil (Almeida & Carneiro, 1998; Ribeiro, 2006). This complex geological history provides a palaeogeographical reconstruction in which multiple biotic dispersals over time from inner regions to eastern Brazilian coastal plains were possible, thereby explaining the occurrence of subsequent basal cynolebiasine lineages in the Eastern Brazil area.

The putative vicariance events among basal cynolebiasines isolating eastern Brazilian taxa along the ancient passive South American margin coast implicate a minimum age for cynolebiasines as old as the Cretaceous. However, the fossil record for cyprinodontiform fishes is poor, and is thus not informative for the evaluation of the age of the group. Fossils are unknown for the whole suborder Aplocheiloidei, in which the cynolebiasines are included. The oldest known fossil of the Cyprinodontoidei, the hypothesized sister group of the Aplocheiloidei (Parenti, 1981; Costa, 1998), dates from the Oligocene of Europe (Parenti, 1981), apart from an unconfirmed record from the Eocene of Argentina (Arratia & Cione, 1996). These records just indicate Oligocene or Eocene as the minimum age for aplocheiloids and cyprinodontoids, and do not necessarily mean that aplocheiloids originated during that time (see Heads, 2005, for detailed criticisms of estimations of absolute ancestral taxon age through dating techniques using the fossil record).

Despite the absence of fossils, Parenti (1981) interpreted the Aplocheiloidei distribution pattern, consisting of taxa endemic to southern Asia, Madagascar, the Seychelles, sub-Saharan continental Africa, southern North America and Middle and South America, as derived from the fragmentation of Gondwana. This hypothesis was rejected by Lundberg (1993) based both on the absence of palaeontological evidence and on the incompatibility between the sequence of continental drift and

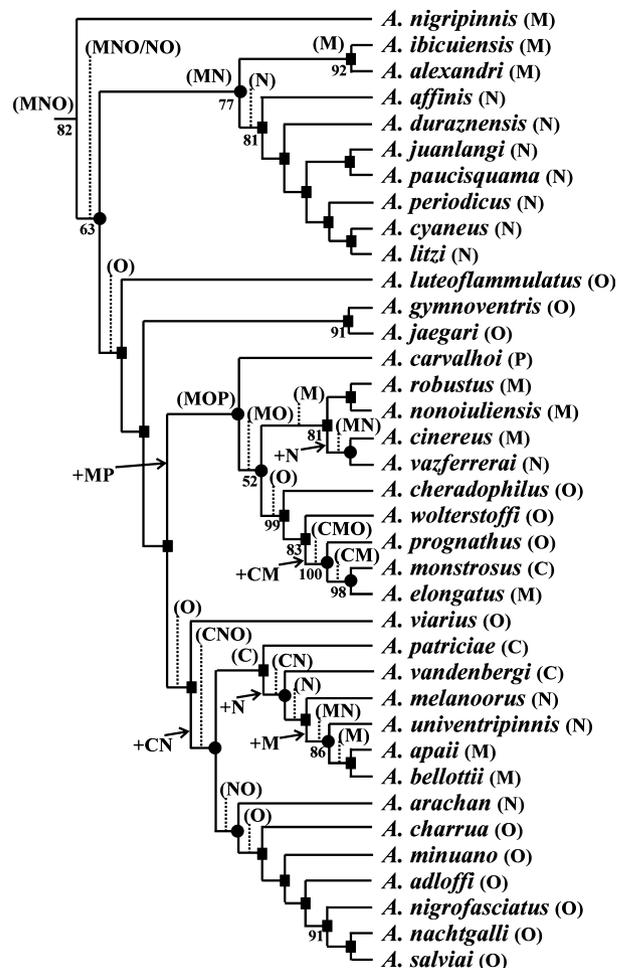


Figure 6 Most parsimonious cladogram of phylogenetic relationships among cynolebiasine fishes, representing species of *Austrolebias*, and summary of the optimal reconstructions of ancestral distributions using dispersal–vicariance analysis. Hypothesized vicariance (black circles) and duplication (black squares) events appear on nodes; rows indicate dispersal (+) events. Letters above branches are hypothesized ancestral distributions; numbers below branches are bootstrap values. Areas of endemism: (C) Western Paraguay, (M) La Plata, (N) Negro, (O) Patos, (P) Iguaçu.

Parenti’s aplocheiloid phylogeny (e.g. African taxa are more closely related to Asian taxa than to American taxa). He concluded that the ecology and physiology of cyprinodontiform fishes do not contradict a model of transoceanic dispersal. In fact, however, American and African aplocheiloid fishes are found in shallow freshwater swamps, with the exception of a rivulid clade containing three species inhabiting estuarine swamps (e.g. Costa, 2006c; Costa *et al.*, 2009), and none of these fishes exhibits any ability to survive in sea water. In addition, subsequent phylogenetic analysis of aplocheiloid fishes based on molecular data (Murphy & Collier, 1997) provided a well-corroborated phylogeny, also supported by morphology (Costa, 2004), in which aplocheiloid lineage relationships fit the Gondwanan continental break-up

sequence (i.e. taxa from southern Asia, Madagascar and Seychelles as the sister group to a clade containing Neotropical and African taxa). Owing to the consistency between the distribution of aplocheiloids and the vicariance hypothesis, as well as their inability to survive in salt-water environments, Sparks & Smith (2005) attributed the aplocheiloid distribution to a Gondwanan origin. This implies a Cretaceous ancestor for cynolebiasines.

Central areas

DIVA indicates a vicariance event separating the Central Brazilian Plateau area from the Northeastern Brazil–São Francisco–Urucuaia–Paraná–Tocantins–Araguaia–Xingu–Guaporé–Paraguay–Iguaçu–La Plata–Negro–Patos area (Fig. 3). This vicariance pattern is partly corroborated by the distribution of characid fishes of the tribe Diapomini, in which the genus *Planaltina*, endemic to the Central Brazilian Plateau, is the sister group to the clade comprising the genera *Acrobrycon* and *Diapoma*, which are endemic to an area encompassing the western Amazon, La Plata River floodplains, Uruguay River basin and Patos Lagoon system (Weitzman & Menezes, 1998).

The sister group taxa *Spectrolebias* and *Hypsolebias* have distributions that overlap in the Tocantins area (Figs 3 & 4). However, DIVA indicated an optimal distribution for a *Spectrolebias* ancestor involving the Tocantins–Araguaia–Guaporé–Paraguay area, while the *Hypsolebias* ancestor would be restricted to the São Francisco–Urucuaia–Paraná area, thus implicating a later dispersal to the Tocantins area within the *Hypsolebias* lineage. This is consistently corroborated by the distribution patterns of other rivulids occurring in the Tocantins area (*Trigonectes balzanii*, *Plesiolebias xavantei*, *Plesiolebias filamentosus*, *Plesiolebias canabravensis*, *Maratecoara formosa*, *Maratecoara splendida*, *Pituna compacta*), all having closest relatives in southern Amazonian tributaries (Araguaia, Xingu, Guaporé) and in the Paraguay River basin (Costa, 1990, 2007b).

DIVA indicated a series of vicariance events among species of *Spectrolebias*. The first event separated the Paraguay area from the southern tributaries of the Amazon River (Guaporé–Xingu–Araguaia–Tocantins), which is corroborated by sister group relationships between the genus *Papiliolebias*, from the Paraguayan Chaco, and the clade comprising *Maratecoara* and *Pituna*, from the southern Amazon basin (Murphy *et al.*, 1999). The shared biological pattern supports the Western Paraguay area, which is presently a part of the Paraguay–Paraná river system draining to the south, being more closely related to areas of the Amazon River basin than to other areas of the Paraná basin. The proposed former connection present before the hypothesized vicariance event is congruent with the palaeogeographic reconstruction of the cis-Andean region of Bolivia; it shows a rupture of the former connection between the Paraguay and Amazon basins after the bending of the Bolivian orocline (i.e. Chapare Buttress) between the late Oligocene and early Miocene (e.g. Sempere *et al.*, 1990; Lundberg *et al.*, 1998).

A sequence of suggested vicariance events among the Madeira, Xingu and Araguaia–Middle Tocantins areas is only partially supported by additional biogeographical data on Neotropical fishes. Close relationships among taxa inhabiting both the Araguaia and Tocantins areas are well represented by other rivulids, including the genera *Maratecoara* and *Plesiolebias* (Costa, 2007b) and species of the *Rivulus zygometes* species group (Costa, 2007c,d). In addition, the occurrence of a member of the *Hypsolebias flammeus–flavicaudatus* species group in the Tocantins area is interpreted as derived from dispersal, as indicated by DIVA, probably as a result of range expansion through diffusion along the Tocantins River basin from the Paraná to the Tocantins area.

North-eastern areas

Two clades, the *Hypsolebias flammeus–flavicaudatus* species group and the genus *Cynolebias*, have highly overlapping distribution patterns in north-eastern South America (Figs 4 & 5). The origin of the sympatric occurrence of two closely related cynolebiasine is unclear owing to the multiple possibilities indicated in the analysis. Both clades occur in the Paraná–São Francisco–Northeastern Brazil areas, having greater species diversification in the São Francisco area. More interestingly, DIVA reconstruction indicates that the ancestor of both clades was originally present in areas of the Tocantins and São Francisco river basins (i.e. the Paraná–Urucuaia–São Francisco area for the ancestor of the *H. flammeus–flavicaudatus* species group, and the Paraná–São Francisco area for the ancestor of *Cynolebias*), but absent in the Northeastern Brazil area, which was reached by later dispersal. The ancestral distribution of *Cynolebias* includes two non-contiguous areas (Paraná–São Francisco), possibly as a consequence of the fact that DIVA optimizations often failed to detect extinction events (Ronquist, 1996). A plausible explanation is that the ancestor of *Cynolebias* also occurred in the Urucuaia area, which is between the Paraná and São Francisco areas, thus assuming that *Cynolebias* depicts a pattern similar to the ancestral distribution of the *H. flammeus–flavicaudatus* species group.

DIVA ancestral area reconstructions support two dispersal events occurring from the São Francisco towards the North-eastern Brazil area, once in each clade. This shared dispersal event is congruent with palaeogeographical reconstructions of the palaeo-São Francisco basin, which temporarily drained to the north before the uplift of the Borborema Plateau during the Miocene (Brito-Neves *et al.*, 2004) redirected the São Francisco eastwards to follow its present course (e.g. Beurlen, 1970; Potter, 1997). Therefore, the geological history supports a geodispersal episode transferring biotic components of both clades from the São Francisco to the Northeastern Brazil area, followed by a vicariance event affecting both clades in the Miocene.

A third clade, the *Hypsolebias magnificus–notatus* species group, is geographically overlapped by the *H. flammeus–flavicaudatus* species group and *Cynolebias* (Figs 4 & 5). The three clades are present in the Paraná and São Francisco areas.

Therefore, all three clade distributions suggest former connections between a section of the Tocantins River basin and the São Francisco River basin, followed by fragmentation causing vicariance events. Palaeogeographical reconstructions indicate a connection between the present Tocantins and São Francisco river basins, which was broken during the Cenozoic (Beurlen, 1970; Campos & Dardenne, 1997).

Southern areas

A number of dispersal and vicariance events are hypothesized to explain the present-day distribution of the main *Austrolebias* lineages among the five endemism areas herein delimited (Fig. 6). DIVA optimal reconstructions show that the ancestor of *Austrolebias* originally occurred in the La Plata–Negro–Patos area, with subsequent dispersals to Paraguay and Iguazu (Fig. 6). The Paraguay area was then involved in three dispersal episodes: from the Patos to the Paraguay area through the La Plata area for the *Austrolebias elongatus* species group; from the Patos to the Paraguay area through the Negro area for the base of the clade comprising the *Austrolebias bellottii* and the *Austrolebias adloffii* species groups; and a reverse route, from the Paraguay area to the Negro and La Plata areas, again for the *Austrolebias bellottii* species group.

The complex dispersal–vicariance scenario suggested by DIVA (Fig. 6) is congruent with the complex geological history of the Paraguay–Paraná River basin, which was strongly influenced by the mid-Miocene uplift of the Andes (e.g. Lundberg *et al.*, 1998). After the tectonic episode beginning about 30 Ma, when part of the Paraguay River basin changed its northern-directed course to the south as a result of the bending of the Bolivian orocline, the Paraguay River basin became part of the present Paraná River basin (Sempere *et al.*, 1990; Lundberg *et al.*, 1998). This former palaeogeographic scenario modification is evidenced by the distribution of *Spectrolebias* (see above).

The southern region was later influenced by extensive marine transgressions caused by flexural subsidence along the Andes foreland basin (e.g. Lundberg *et al.*, 1998). During the middle and late Miocene (c. 15–11 Ma), three successive Atlantic marine transgressions occupied a broad region of the present lower La Plata basin, extending to Bolivia to form the ‘Paranan Sea’ (e.g. Donato, 2006). These marine transgressions caused alternate periods of isolation and connection among the Paraguay, Uruguay, Negro and Patos areas. Both biotic exchange and isolation were possible in this period, supporting the successive events of dispersal and vicariance indicated in the biogeographical reconstruction.

CONCLUSIONS

DIVA reconstructions in conjunction with data on palaeogeographic scenarios support a series of plausible explanations for the present-day distribution of cynolebiasine killifishes. DIVA indicates a series of vicariance events congruent with tectonic episodes in South America, but the present distribu-

tion of cynolebiasines has also been shaped by a series of dispersal events. The effects of the combined action of dispersal and vicariance events were more evident in the Eastern Brazil and Paraguay areas, producing reticulate biogeographical scenarios (Ronquist, 1997).

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SUPPORTING INFORMATION

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

Appendix S1 Character statements for discrete characters of cynolebiasine killifishes.

Appendix S2 Data matrix of discrete characters of cynolebiasine killifishes.

Appendix S3 Data matrix of continuous characters of cynolebiasine killifishes.

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