

Historical biogeography of aplocheiloid killifishes (Teleostei: Cyprinodontiformes)

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Abstract

Aplocheiloid killifishes, a diversified group of primary freshwater fishes occurring in tropical and subtropical regions of the Americas, Africa and south-eastern Asia, have been the focus of debates among biogeographers using dispersal and vicariance approaches. The aim of the present paper is to infer biogeographical events responsible for the present distribution of aplocheiloid killifishes using an event-based methodology (DIVA) in conjunction to a phylogeny combining mitochondrial DNA sequences and morphology. Optimal ancestral reconstructions support vicariance events chronologically congruent to northern Gondwana break-up, including separation of Madagascar, India, South America and Africa plates (about 121–84 Ma), as well as congruent to paleogeographical events within the Africa plate, such as the widening of the Benue Trough (about 90–80 Ma) and the start of activity of the East African Rift System (about 30 Ma), and within the South American plate, as the formation of Gaarland (about 35–33 Ma), uplift of the Andean Eastern Cordillera (11.8 Ma) and the interruption of the paleo-Amazonas river basin by the uplift of the Vaupés Swell (about 11–7 Ma). The reconstructions also support geodispersal events related to the colonization of the Greater Antilles (about 35–33 Ma) and Central America areas (3.7–3.4 Ma) by aplocheiloids through land connections, besides some dispersal events through the Zaire, East Africa, Amazon and Eastern Brazil areas.

Key words

Andean uplift, dispersal-vicariance analysis, East African Rift System, Gaarland, paleo-Amazonas, Vaupés Swell.

Introduction

The killifish suborder Aplocheiloidei comprises a diversified clade of small teleosts, usually about 25–50 mm of total length as maximum adult size, with over 620 species occurring in shallow freshwater or rarely brackish environments. Aplocheiloids are mostly known by many included species being popular aquarium fishes, but some of them are also often used as experimental animals in laboratories (*e.g.*, HARRINGTON & KALLMAN, 1968; WOURMS, 1972; PARK & KIM, 1984). However, the suggestive geographical distribution of the three families contained in the suborder, with Aplocheilidae endemic to Madagascar, Seychelles, and south-eastern Asia, Nothobranchiidae endemic to sub-Saharan Africa, and Rivulidae endemic to Middle and South America (COSTA,

2004), has brought the Aplocheiloidei to the focus of debates involving conflicting explanations about patterns of historical biogeography in the tropics (LUNDBERG, 1993; MURPHY & COLLIER, 1997; BRIGGS, 2003; SPARKS & SMITH, 2005).

Biogeographical relationships among aplocheiloid lineages were first discussed just after MYERS (1931) formally recognising the killifish group presently known as the suborder Aplocheiloidei. MYERS (1938), when noticing the possible closely relationships among some South American and African aplocheiloid taxa, claimed that trans-continental distribution of aplocheiloids could not be viewed as evidence of an ancient connection between South America and Africa, since those fish lineages

“... belong to the secondary division of fresh-water fishes, and that a fortuitous marine dispersal of one or more species at some time in the Tertiary is not impossible, particularly if a Brazil-Guinea ridge remained for a time as an island chain” (MYERS, 1938: 353). The term ‘secondary division of fresh-water fishes’ had been first proposed in that same paper, referring to fish groups, including cyprinodontiforms and cichlids, in which members were often tolerant to salt water. Later, MYERS (1958) considered the aplocheiloid Asiatic genus *Aplocheilus* as a basal lineage, suggesting its present geographic distribution as a result of recent dispersal from Africa.

A half century after MYERS recognising aplocheiloids as a group, PARENTI (1981) analysed aplocheiloid relationships, considering Asiatic taxa more closely related to African taxa than to American taxa, at same time defending a Gondwanan origin for the group followed by vicariance events derived from the ancestral Mesozoic continent break-up. This proposal was rejected by LUNDBERG (1993) based both on the absence of cyprinodontiforms and closely related teleost orders in Mesozoic fossil deposits and the incongruence between timing of phylogenetic events (*i.e.*, African taxa more closely related to Asiatic taxa than South American taxa) and the drift-vicariance model (India-Madagascar separating earlier from Africa-South America plate, South America separating later from Africa). In addition, LUNDBERG (1993) tentatively explained the present distribution pattern of aplocheiloids by marine dispersal favoured by the occurrence of some taxa in brackish water habitats, electing the Neotropical region as a centre of origin for aplocheiloid dispersal.

MURPHY & COLLIER (1997), based on a molecular phylogeny for 25 aplocheiloid species and two cyprinodontiform out-groups, in which sister group relationships between taxa from south-eastern Asia and Madagascar/Seychelles and between a clade of sub-Saharan Africa and another from South America were supported, considered again aplocheiloid distribution as a result of vicariance by fitting well the Gondwana drift model. This hypothesis was not accepted by BRIGGS (2003), who insistently argued again that the group is recent, a conclusion derived from cyprinodontiforms being represented in fossil record only after mid-Tertiary, besides belonging to the MYERS’ secondary freshwater fish category, citing as example *Rivulus marmoratus* POEY (= *Kryptolebias marmoratus*), which has been found in numerous coastal localities between Florida and Brazil. A subsequent contrary view was provided by SPARKS & SMITH (2005), who emphatically criticise the use of unmeaningful statements about dispersal ability and lack of fossils against phylogenetic evidence. Increasing controversy, HERTWIG’S (2008) analysis of morphological characters refuted aplocheiloid monophyly and supported the Asiatic genus *Aplocheilus* as the sister group to a clade comprising all other cyprinodontiforms.

The break-up of Gondwana, a sequence of geologic events between Jurassic and Cretaceous responsible for delimitating the present continents of the southern hemi-

sphere, has been an important paleogeographical scenario for our understanding about timing of biotic evolution in the tropics (*e.g.*, SANMARTÍN & RONQUIST, 2004). However, evidence extracted from aplocheiloid distribution patterns have some limitations, including reduced number of out-group and in-group taxa in phylogenetic analyses, not representing most lineages, and omission of analytical methods to infer chronological congruence among phylogenetic diversity and paleogeographical events after break-up of Gondwana. The objective of the present study is to analyse the historical biogeography of aplocheiloids using an event-based methodology in conjunction to a phylogeny based on a representative sample of aplocheiloid lineages and outgroups, combining DNA sequences and morphology, in order of checking the temporal congruence between diversification of aplocheiloids and our present knowledge about paleogeographical events in the region inhabited by them.

Material and methods

Phylogenetic analysis. Terminal taxa were select to encompass all the main aplocheiloid lineages, in order of to combine the greatest possible sample of different gene sequences available in GenBank with representative material available for morphological analysis (see COSTA, 2012 for a list of material used in morphological studies). Generic classification followed COSTA (1998a, 2010, 2011a) for rivulids and MURPHY & COLLIER (1999), MURPHY *et al.* (1999a) and COLLIER *et al.* (2009) for nothobranchiids. DNA sequences, including the mitochondrial genes cytochrome b, 12S rRNA and 16S rRNA, were obtained in GenBank for 61 aplocheiloid species representing all the main lineages of the suborder, and six outgroups, including three species of different cyprinodontoid families (Cyprinodontidae, Fundulidae, Goodeidae), two species belonging to the Adrianichthyidae, the most basal family of the order Beloniformes, the sister group of the Cyprinodontiformes, and one species representing the order Atheriniformes. Protocols for extraction, amplification and sequencing, and GenBank accession numbers are provided in MURPHY & COLLIER (1996, 1997), MURPHY *et al.* (1999a, b), MIYA *et al.* (2003) and SETIAMARGA *et al.* (2008). Identification of the species analysed by MURPHY *et al.* (1999) as *Rivulus caudomarginatus* and *Rivulus violaceus* were corrected to *Kryptolebias ocellatus* and *Melanorivulus crixas*, respectively, following recent taxonomical studies (COSTA, 2007, 2011b). Sequences were aligned using Clustal-W (CHENNA *et al.*, 2003) and subsequently optimized manually.

Morphological characters were extracted from phylogenetic studies involving aplocheiloid lineages and outgroups (PARENTI, 1981, 2008; ROSEN & PARENTI, 1981; COSTA, 1998b, 2004, 2006, 2008, 2009a, 2009b, 2011c, 2012), where character and character states are discussed and illustrated. Characters were reformatted to reduce

ambiguity following SERENO (2007) for character statement formulation. Morphological characters included osteology, laterosensory system and other cephalic structures, fin morphology and colour patterns. 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. Characters showing high levels of subjectivity in character state delimitation among the numerous terminal taxa (e.g. dorsal and anal fin morphology, flank colour patterns in males) were excluded from the analysis. All characters were treated as unordered. A total of 199 morphological characters were examined (Appendix 1), which were checked both in terminal taxa and in species closely related to terminal taxa, in a total of 280 species of aplocheiloids and 28 species of outgroups (see complete list in COSTA, 2012). Osteological preparations (c&s) were made according to TAYLOR & VAN DYKE (1985). Terminology for frontal squamation followed HOEDEMAN (1958) and for cephalic neuromast series COSTA (2001). Distribution of character states of morphological characters among terminal taxa appears in Appendix 2.

The search for most parsimonious trees (using ‘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 1,000 trees saved in each replicate) and bootstrap analysis (1,000 replicates) were performed with TNT 1.1 (GOLOBOFF *et al.*, 2008). Character states of all morphological characters were treated as unordered and genes were analysed giving equal weight to all sites.

Biogeography. Reconstruction of the biogeographical history of aplocheiloid lineages was inferred using the dispersal-vicariance analysis (DIVA) (RONQUIST, 1996, 1997), an event-based parsimony method with an explicit treatment of multiple identified processes (vicariance, dispersal, extinction, and sympatric speciation), with relative costs previously assumed (vicariance and sympatric speciation events with a cost of zero; dispersal and extinction events with cost of one per unit area added or deleted from the distribution) (RONQUIST, 1996). This method is recommended in studies aiming to reconstruct the biogeographical history of a single lineage in the absence of a general area cladogram (e.g., RONQUIST, 1997; SANMARTÍN, 2007). Although recent developments in geology make possible identification of several paleogeographical events, not much has been known from drainage evolution in the South American plate, as well as high level of controversy is still found in the literature about the drainages of the African plate (e.g., GOUDIE, 2005). Thus, DIVA methodology is herein chosen by having the advantage of not being constrained by particular modes of areas relationships (e.g., VAN BOCKLAER *et al.*, 2006). The search for the optimal historical reconstruction, in which the total cost is minimized under a

parsimony criterion, was performed using the computer program DIVA 1.2 (RONQUIST, 1996). The exact search of DIVA was used without restricting the number of areas in which the ancestor occurred.

Areas of endemism were delimited on the basis of congruence among distributional ranges exhibited by monophyletic groups of aplocheiloid fishes. The 11 areas of endemism herein analyzed, with the respective taxonomic units supporting them, were: 1) Greater Antilles (GA) – including Cuba, Pinos and Hispaniola islands, the geographic distribution of the genus *Rivulus*. 2) Central America (CA) – all extension of continental Central America, including southern Mexico in north, and the adjacent trans-Andean portion of South America containing the Magdalena River basin in the south, the geographic distribution of *Cynodonichthys*. 3) Orinoco (Or) – comprising the Orinoco river basin and adjacent coastal areas, including the Maracaibo region, the geographic distribution of the genera *Austrofundulus*, *Gnatholebias*, *Rachovia*, *Terranatos*, *Renova*, *Micromoema*, and one monotypic genus, *Llanolebias* that is closely related to *Gnatholebias* (HRBEK & TAPHORN, 2008) and was not included in the analysis. 4) Amazon (Am) – including the whole Amazonas river basin and adjacent river basins in Guianas and northeastern Brazil, the geographic distribution of *Anablepsoides*, *Aphyolebias*, *Laimosemion*, *Maratecoara*, *Moema*, *Neofundulus*, *Pituna*, *Plesiolebias*, *Pterolebias*, *Spectrolebias*, *Trigonectes*, and two rare plesiolebiasine genera, *Papiliolebias* and *Stenolebias*, closely related to *Maratecoara* and *Pituna* (COSTA, 2011c). 5) Brazilian Shield (BS) – including the Paraná, São Francisco, upper Tocantins, upper Araguaia, middle Jequitinhonha and Jaguaribe rivers basins, besides adjacent smaller basins in northeastern and southern Brazil, the geographic distribution of *Austrolebias*, *Cynolebias* and *Hypsolebias*, and another cynolebiasine genus *Simpsonichthys*, not included in the analysis. 6) Eastern Brazil (EB) – comprising the coastal rivers drainages between Paraguaçu river and Patos lagoon basins, the geographic distribution of the genera *Atlantirivulus*, *Campellolebias*, *Leptolebias*, *Nematolebias*, *Notholebias*, and *Kryptolebias* with exception of an estuarine species widespread along northeastern Brazil and Caribbean region (*K. marmoratus* Poey); also endemic to the area, but not included in the analysis, are *Cynopoecilus*, the sister group of *Campellolebias* (e.g. COSTA, 1998a), *Ophthalmolebias* and *Xenurolebias*, two basal cynolebiasine genera (e.g. COSTA, 2010), and *Prorivulus*, a basal rivulid genus of uncertain phylogenetic position. 7) West Africa (WA) – including river basins between Gambia and western Ghana, the geographic distribution of *Archiaphyosemion*, *Callopanchax* and *Scriptaphyosemion*. 8) Zaire (Za) – including the Zaire and Niger river basins, besides smaller adjacent coastal drainages between eastern Ghana and Cabinda (Angola), the geographic distribution of the genera *Aphyosemion* and *Fundulopanchax*, besides two small genera not included in the analysis, the rare *Episemion* and the monotypic *Foerschichthys*. 9) East Africa (EA) – including East African river basins between Somalia and

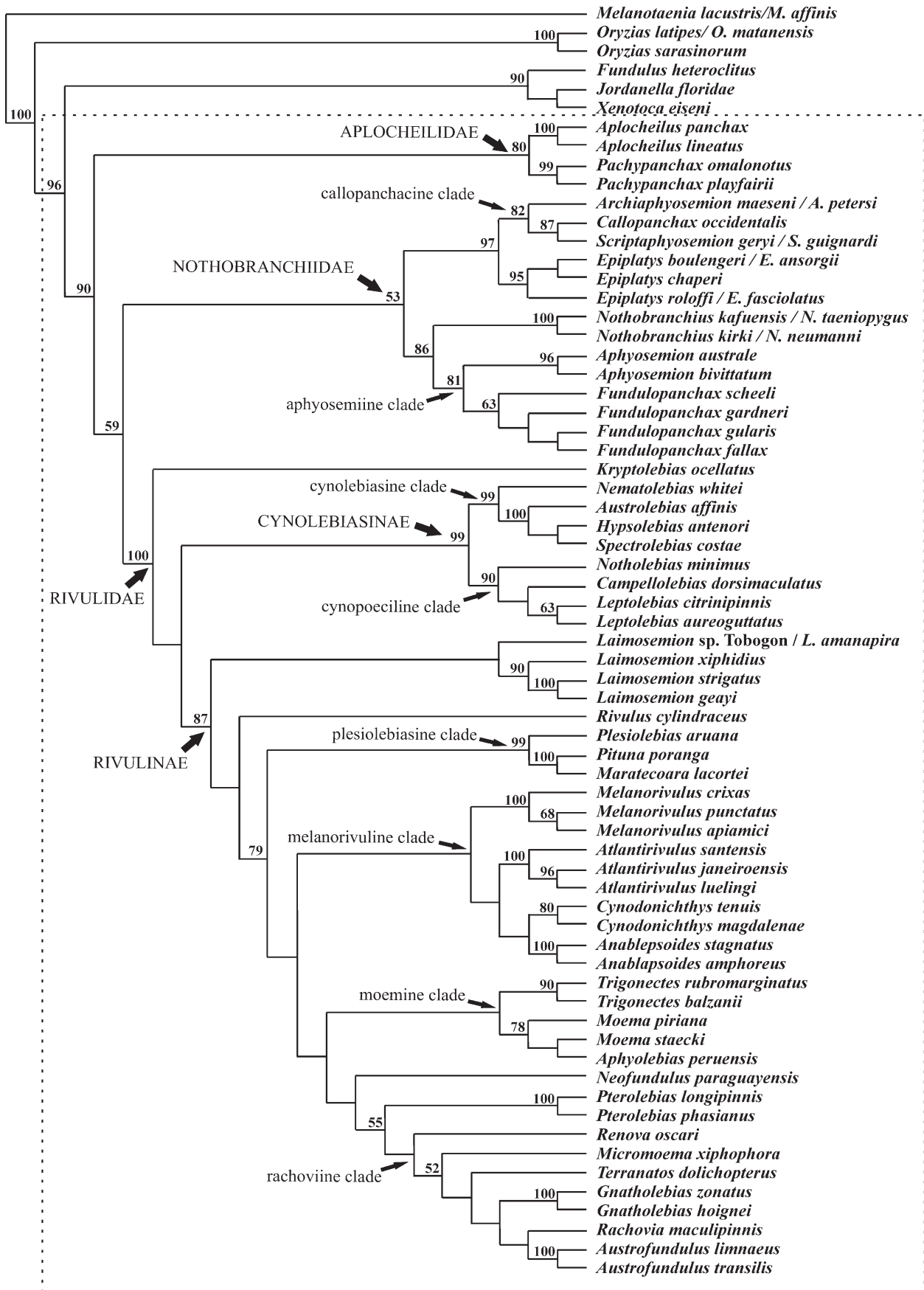


Fig. 1. Most parsimonious cladogram of phylogenetic relationships between 61 representatives of the Aplocheiloidei and six outgroups, based on a combined set of segments of three mitochondrial genes (cytochrome b, 12S rRNA and 16S rRNA; 1145 sites, of which 655 were parsimony-informative, 333 were constant and 158 were variable but not parsimony-informative) and 199 morphological characters (tree length: 7447). Numbers above branches are bootstrap values. Dotted rectangle delimits the Aplocheiloidei.

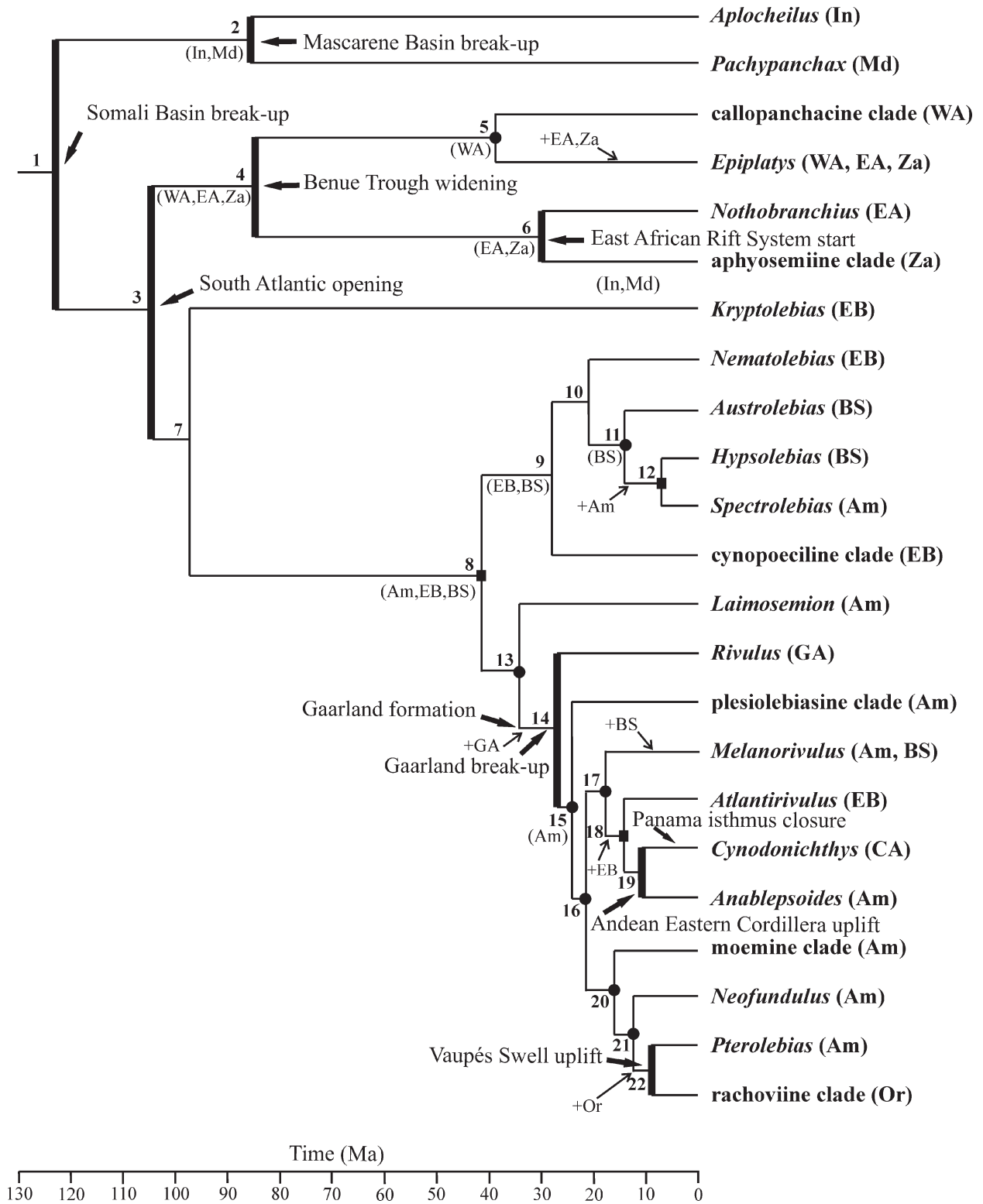


Fig. 2. Summary of the optimal reconstructions of ancestral distribution using dispersal–vicariance analysis. Broad vertical lines in the tree are hypothesized vicariance events associated to dated paleogeographical events indicated by broad arrows, whereas narrow vertical lines have arbitrary position relative to time scale; black squares on nodes are vicariance events not attributed to particular paleogeographical events and black circles are duplications; narrow arrows indicate dispersal (+) events. Nodes are sequentially numbered; ancestral distributions appear near nodes when unambiguously assessed. Areas of endemism: (Am) Amazon, (BS) Brazilian Shield, (CA) Central America, (EA) East Africa, (EB) Eastern Brazil, (GA) Greater Antilles, (In) India, (Md) Madagascar, (Or) Orinoco, (WA) West Africa, (Za) Zaire.

South Africa, Rift Lakes basins, White Nile River drainage, and Lake Tchad basin, the geographic distribution of *Nothobranchius*. 10) Madagascar (Md) – comprising Madagascar and Seychelles, the geographic distribution of *Pachypanchax*. 11) India (In) – comprising India and Sri Lanka, the geographic distribution of *Aplocheilus*, except for *A. panchax*, a salt tolerant species occurring since southern Pakistan to East Timor.

Terminal taxa for the biogeographical analysis were monophyletic units with consistent geographical distribution, usually genera. As a consequence, species were substituted by their respective genera. In order of to reduce the number of terminal taxa in the biogeographical analysis, some monophyletic groups of genera sharing the same geographical range were clustered into a single terminal taxa. Although two salt water tolerant species of the genera *Aplocheilus* and *Kryptolebias* being geographically widespread to extend their geographic range much beyond the areas of endemism herein delimited, since freshwater basal species of those genera are geographically restricted to India and Eastern Brazil areas, respectively, their distribution ranges were assigned to the latter areas. Since the South American Paraguay river basin has been considered as a recent composite biogeographical area (e.g., COSTA, 2010, 2011c), genera occurring both in the Amazon area and Paraguay basin were assigned as endemic to the former area, whereas the only genus simultaneously occurring in Paraguay basin and Brazilian Shield area, *Austrolebias*, was assigned to the latter one.

Results

The single most parsimonious phylogenetic tree for 61 aplocheiloid taxa and six outgroups is illustrated in Fig. 1. It corroborates monophyly of the Aplocheiloidei and included families. The biogeographical analysis indicates a series of vicariance events responsible for family distribution, whereas distribution of intra-familial groups is shaped both by vicariance and dispersal events (Fig. 2), which are below discussed and tentatively associated to paleogeographical scenarios.

Discussion

The phylogenetic tree herein generated by combining molecular and morphological data is similar to past phylogenies based on either morphology or molecular data alone, by supporting aplocheiloid monophyly (PARENTI, 1981, COSTA, 1998b, MURPHY & COLLIER, 1997) and subclades proposed in former partial analyses (e.g., COSTA, 1998a, 2008, 2010, 2011a; MURPHY & COLLIER, 1999; MURPHY *et al.*, 1999a, 1999b). In addition, DIVA supports

a series of vicariance events associated with aplocheiloid diversification that are chronologically congruent to the break-up of Somali Basin since 121 Ma, as summarized by SANMARTÍN & ROQUIST (2004).

The first three most basal vicariance events, represented by nodes 1, 2 and 3 in Fig. 2, are congruent to the separation of Madagascar and India areas from northern South America plus Africa, Madagascar area from India area (break-up of Mascarene basin, 84 Ma), and South America from Africa (opening of the South Atlantic Ocean, about 110 Ma), respectively. Within the African lineage, two vicariance events delimited by DIVA reconstruction may also be associated to paleogeographical events. The first one (node 4 in Fig. 2) separates West Africa from the remaining parts of Africa, thus isolating the ancestor of the clade comprising *Archiaphyosemion*, *Callopanchax*, *Scriptaphyosemion* and *Epiplatys*, an event also detected by MURPHY & COLLIER (1997). This event is here tentatively related to the widening of the Benue Trough, occurred still in the Cretaceous, about 90–80 Ma (e.g., FAIRHEAD, 1988; FAIRHEAD & BINKS, 1991; STANKIEWICZ & DE WIT, 2006), when Africa still moved as two plates separated by rifting in West Africa, erecting a potential geographical barrier for aplocheiloids. The reconstruction model supported by DIVA also demands a dispersal of *Epiplatys* lineages from West Africa to Zaire and East Africa areas, events already in part postulated in the recent phylogeographical analysis of the genus *Epiplatys* (COLLIER *et al.*, 2009). *Epiplatys* is a species-rich and widespread genus, with lineages endemic to both West Africa and Zaire areas (COLLIER *et al.*, 2009), besides some species inhabiting the White Nile and Tchad regions of the East area herein delimited.

The second vicariance event in Africa (node 6 in Fig. 2) indicates a fragmentation in central Africa, isolating the eastern area today inhabited by *Nothobranchius* (East Africa area), from the mid-western area inhabited by the apyosemiine clade comprising *Aphyosemion* and *Fundulopanchax* (Zaire area). The limit between both areas is congruent with multiple paleogeographical events associated with the start of activity of the East African Rift System in the Oligocene, about 30 Ma (BURKE, 1996). These events comprise a series of expressive transformations in the long Trans-African paleo-rivers, a large drainage network including at different times portions of the present Niger, Tchad, Zaire, Nile and Zambezi courses, formerly draining to east and northeast (e.g., GOUDIE, 2005; STANKIEWICZ & DE WIT, 2006). Sections of the system were successively disrupted by the uplift associated with the East African Rifting, imposing new barriers for aplocheiloids between Zaire and Eastern Africa areas.

In the Neotropical region, DIVA analysis indicates some possibilities for ancient isolation of basal lineages in Eastern Brazilian area, which may be considered as a consequence of the formation of the passive continental margin of South America just after the break-up of South America and Africa (POTTER, 1997; RIBEIRO, 2006). The basal divergence between the subfamilies Cynolebiasinae

and Rivulinae is parsimoniously hypothesized to be related to a vicariance event separating the former group in Eastern Brazil area from the latter in Amazon area (node 8). The evolution of each subfamily is predominantly characterized by a series of duplication events within those areas, with geodispersal events associated to vicariance events, some which are chronologically congruent with some major key paleogeographical events in South America. Distribution of the Cynolebiasinae implicates an ancestral distribution in East Brazil and Brazilian Shield areas, besides one dispersal event to the Amazon area. A recent biogeographical analysis of species of the tribe Cynolebiasini provides an alternative scenario (COSTA, 2010), in which the gradual formation of taphrogenic basins due to vertical movements between rifted blocks caused river capture from the adjacent upland (the Brazilian Shield area), and consequent multiple biotic dispersals from the Brazilian Shield area to the Eastern Brazil area (ALMEIDA & CARNEIRO, 1998; RIBEIRO, 2006). This paleogeographical model is congruent with the occurrence of successive basal lineages of the cynolebiasine clade in the Eastern Brazil area (COSTA, 2010).

DIVA indicates the most recent ancestor of the Rivulinae as endemic to the Amazon area, which is inhabited by representatives of most Rivulinae lineages, including *Laimosemion* that is supported as the sister group to the remaining rivulines. The second most basal split in the Rivulinae clade indicates a dispersal event from the Amazon area to the Greater Antilles area, followed by vicariance isolating a Greater Antilles area taxon, which may be attributed to the formation and later rupture of a long land connection between central Cuba and northern South America, known as Gaarland, during the transition Eocene–Oligocene, about 35–33 Ma (ITURRALDE-VINENT & MACPHEE, 1999; ITURRALDE-VINENT, 2005). During the Upper Oligocene (about 27–25 Ma), as a consequence of a higher sea level, this connection was interrupted, a paleogeographical scenario implicating in isolation of that rivuline lineage, the ancestor of *Rivulus*.

Duplication events within the Amazon area are supported by DIVA analysis, thus indicating that all the ancestors of each of the three most diversified clades of the Rivulinae were endemic to that area. Among lineages of the melanorivuline clade, a dispersal event within the genus *Melanorivulus* is proposed to be occurred from the Amazonian area to the Brazilian Shield area. This dispersal event is supported by a recent taxonomical study, in which *M. schuncki* (COSTA & DE LUCA), the only species of the genus endemic to the lower Amazon, is considered as the probable sister-group to the clade comprising the remaining about 35 species of the genus (COSTA & DE LUCA, 2011). Another dispersal event is hypothesized to have occurred from the Amazon area to the south-eastern Brazil area, followed by vicariance separating the latter area inhabited by the ancestor of *Atlantirivulus* (node 18).

More interestingly, the two latest events assigned to the melanorivuline clade (node 19) are more parsimoniously explained as dispersal–vicariance events involv-

ing the Central America, which has been connected to South America just recently. The uplift of the Andean Eastern Cordillera at 11.8 Ma (HOORN *et al.*, 1995), with the formation of the present Magdalena Valley and isolation of the plain areas of north-western corner of South America, is herein hypothesized as a first causal factor for the vicariance event originating the present genera *Anablepsoides* from Cis-Andean northern South America and *Cynodonichthys* endemic to the region encompassing Central America and Trans-Andean northern South American (Magdalena river basin). This paleogeographical scenario was chronologically followed by the formation of the connection between that northern corner of Trans-Andean South America and Central America through the closure of the Panama isthmus between 3.7 and 3.4 Ma (DUQUE-CARO, 1990; HOORN *et al.*, 1995), permitting geodispersal of the ancestor of *Cynodonichthys* through the newly formed Central America.

DIVA supports a later dispersal event to the Orinoco area, followed by vicariance separating both areas (node 22). A remarkable evolutionary radiation would then follow the initial colonization of the Orinoco area by a rivuline ancestor, thus originating the highly morphologically diverse genera (e.g. *Austrofundulus*, *Gnatholebias*, *Terranatos*) today found in the area, composing the rachoviine clade. These events are congruent with recent paleogeographical reconstructions, which have consistently shown that the paleo-Amazonas river flowed to north, thus continuous to the present Orinoco river basin (HOORN *et al.*, 1995, 2010; MORA *et al.*, 2010). This river basin configuration was broken with the uplift of the Vaupés Swell occurred between the Late Miocene and Pliocene, about 11–7 Ma, as a result of a sequence of processes related to the Andean Orogen (HOORN *et al.*, 2010; MORA *et al.*, 2010), separating the Orinoco and Amazonas river basins, and forming the present trans-continental Amazonas basin flowing to east. Therefore, biogeographical pattern exhibited by the rachoviine clade is congruent with the paleo-Amazonas–Orinoco basin, whereas the rupture of that basin is herein considered as a causal factor promoting the vicariance event assigned to the node 22.

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References

- ALMEIDA, F.F.M. & CARNEIRO, C.D.R. (1998): Origem e evolução da Serra do Mar. – *Revista Brasileira de Geociências*, **28**: 135–150.
- BRIGGS, J.C. (2003): Fishes and birds: Gondwana life rafts reconsidered. – *Systematic Biology*, **52**: 548–553.
- BURKE, K. (1996): The African plate. – *South African Journal of Geology*, **99**: 341–409.
- CHENNA, R.; SUGAWARA, H.; KOIKE, T.; LOPEZ, R.; GIBSON, T.J.; HIGGINS, D.G. & THOMPSON, J.D. (2003): Multiple sequence alignment with the Clustal series of programs. – *Nucleic Acids Research*, **31**: 3497–3500.
- COLLIER, G.E.; MURPHY, W.J. & ESPINOZA, M. (2009): Phylogeography of the genus *Epiplatys* (Aplocheiloidea: Cyprinodontiformes). – *Molecular Phylogenetics and Evolution*, **50**: 190–196.
- COSTA, W.J.E.M. (1998a): Phylogeny and classification of Rivulidae revisited: evolution of annualism and miniaturization in rivulid fishes (Cyprinodontiformes: Aplocheiloidei). – *Journal of Comparative Biology*, **3**: 33–92.
- COSTA, W.J.E.M. (1998b): Phylogeny and classification of the Cyprinodontiformes (Euteleostei: Atherinomorpha): a reappraisal. In: MALABARBA, L.R., REIS, R.E., VARI, R.P., LUCENA, Z.M.S. & LUCENA, C.A.S. (eds.): *Phylogeny and classification of Neotropical fishes*. Porto Alegre: Edipucrs, 537–560.
- COSTA, W.J.E.M. (2001): The neotropical annual fish genus *Cynolebias* (Cyprinodontiformes: Rivulidae): phylogenetic relationships, taxonomic revision and biogeography. – *Ichthyological Exploration of Freshwaters*, **12**: 333–383.
- COSTA, W.J.E.M. (2004): Relationships and redescription of *Fundulus brasiliensis* (Cyprinodontiformes: Rivulidae), with description of a new genus and notes on the classification of the Aplocheiloidei. – *Ichthyological Exploration of Freshwaters*, **15**: 105–120.
- COSTA, W.J.E.M. (2006): Descriptive morphology and phylogenetic relationships among species of the Neotropical annual killifish genera *Nematolebias* and *Simpsonichthys* (Cyprinodontiformes: Aplocheiloidei: Rivulidae). – *Neotropical Ichthyology*, **4**: 1–26.
- COSTA, W.J.E.M. (2007): Five new species of the aplocheiloid killifish genus *Rivulus*, subgenus *Melanorivulus*, from the middle Araguaia river basin, central Brazil (Teleostei: Cyprinodontiformes: Rivulidae). – *Aqua International Journal of Ichthyology*, **13**: 55–68.
- COSTA, W.J.E.M. (2008): Monophyly and taxonomy of the Neotropical seasonal killifish genus *Leptolebias* (Teleostei: Aplocheiloidei: Rivulidae), with the description of a new genus. – *Zoological Journal of the Linnean Society*, **153**: 147–160.
- COSTA, W.J.E.M. (2009a): Osteology of the African annual killifish genus *Callopanchax* (Teleostei: Cyprinodontiformes: Nothobranchiidae) and phylogenetic implications. – *Vertebrate Zoology*, **59**: 31–40.
- COSTA, W.J.E.M. (2009b): Species delimitation among populations of the eastern Tanzanian seasonal killifish *Nothobranchius korthausae* (Cyprinodontiformes: Nothobranchiidae). – *Ichthyological Exploration of Freshwaters*, **20**: 111–126.
- COSTA, W.J.E.M. (2010): Historical biogeography of cynolebiasine annual killifishes inferred from dispersal–vicariance analysis. – *Journal of Biogeography*, **37**: 1995–2004.
- COSTA, W.J.E.M. (2011a): Phylogenetic position and taxonomic status of *Anablepsoides*, *Atlantirivulus*, *Cynodonichthys*, *Laimosemion* and *Melanorivulus* (Cyprinodontiformes: Rivulidae). – *Ichthyological Exploration of Freshwaters*, **22**: 233–249.
- COSTA, W.J.E.M. (2011b): Identity of *Rivulus ocellatus* and a new name for a hermaphroditic species of *Kryptolebias* from southeastern Brazil (Cyprinodontiformes: Rivulidae). – *Ichthyological Exploration of Freshwaters*, **22**: 185–192.
- COSTA, W.J.E.M. (2011c): Comparative morphology, phylogenetic relationships and historical biogeography of plesiolebiasine seasonal killifishes (Teleostei: Cyprinodontiformes: Rivulidae). – *Zoological Journal of the Linnean Society*, **162**: 131–148.
- COSTA, W.J.E.M. (2012): The caudal skeleton of extant and fossil cyprinodontiform fishes (Teleostei: Atherinomorpha): comparative morphology and delimitation of phylogenetic characters. – *Vertebrate Zoology*, **62**: 161–180.
- COSTA, W.J.E.M. & DE LUCA, A.C. (2011): *Rivulus schuncki*, a new species of the killifish subgenus *Melanorivulus*, from eastern Brazilian Amazon (Cyprinodontiformes: Rivulidae). – *Ichthyological Exploration of Freshwaters*, **21**: 289–293.
- DUQUE-CARO, H. (1990): Neogene stratigraphy, paleoceanography and paleobiogeography in northwest South America and the evolution of the Panama Seaway. – *Palaeoclimatology, Palaeogeography, Palaeoecology*, **77**: 203–234.
- FAIRHEAD, J.D. (1988): Mesozoic plate tectonic reconstructions of the Central South Atlantic Ocean: the role of the West and Central African Rift system. – *Tectonophysics*, **155**: 181–191.
- FAIRHEAD, J.D. & BINKS, R.M. (1991): Differential opening of the Central and South Atlantic Oceans and the opening of the West African rift system. – *Tectonophysics*, **187**: 191–203.
- GOLOBOFF, P. A.; FARRIS, J. S. & NIXON, K. C. (2008): TNT, a free program for phylogenetic analysis. – *Cladistics*, **24**: 774–786.
- GOUDIE, A.S. (2005): The drainage of Africa since the Cretaceous. – *Geomorphology*, **67**: 437–456.
- HARRINGTON, R.W. & KALLMAN, K.D. (1968): The homozygosity of clones of the self-fertilizing hermaphroditic fish, *Rivulus marmoratus* Poey (Cyprinodontidae, Atheriniformes). – *American Naturalist*, **102**: 337–343.
- HERTWIG, S.T.H. (2008): Phylogeny of the Cyprinodontiformes (Teleostei, Atherinomorpha): the contribution of cranial soft tissue characters. – *Zoologica Scripta*, **37**: 141–174.
- HOEDEMAN, J.J. (1958): The frontal scalation pattern in some groups of toothcarps (Pisces, Cyprinodontiformes). – *Bulletin of Aquatic Biology*, **1**: 23–28.
- HOORN, C.; GUERRERO, J.; SARMIENTO, G.A. & LORENTE, M.A. (1995): Andean tectonics as a cause for changing drainage patterns in Miocene northern South America. – *Geology*, **23**: 237–240.
- HOORN, C.; WESSELINGH, F.P.; HOVIKOSKI, J. & GUERRERO, J. (2010): The development of the Amazonian mega-wetland (Miocene; Brazil, Colombia, Peru, Bolivia). In HOORN, C. & WESSELINGH, F.P. (eds.): *Amazonia: landscape and species evolution*. Oxford: Wiley-Blackwell, 123–142.
- HRBEK, T. & TAPHORN, D.C. (2008): Description of a new annual rivulid killifish genus from Venezuela. – *Zootaxa*, **1734**: 27–42.

- ITURRALDE-VINENT, M.A. (2005): La Paleogeografía del Caribe y sus implicaciones para la biogeografía histórica. – *Revista del Jardín Botánico Nacional*, **25–26**: 49–78.
- ITURRALDE-VINENT, M.A. & MACPHEE, R.D.E. (1999): Paleogeography of the Caribbean region: implications for Cenozoic biogeography. – *Bulletin of the American Museum of Natural History*, **238**: 1–95.
- LUNDBERG, J.G. (1993): African-South American freshwater fish clades and continental drift: problems with a paradigm. In: P. Goldblatt (ed.): *Biological relationships between Africa and South America*. New Haven: Yale University Press, 156–199.
- MORA, A., BABY, P., RODDAZ, M., PARRA, M.; BRUSSET, S.; HERMOZA, W. & ESPURT, N. (2010): Tectonic history of the Andes and sub-Andean zones: implications for the development of the Amazon drainage basin. In: HOORN, C. & WESSELINGH, F.P. (eds.): *Amazonia: landscape and species evolution*. Oxford: Wiley-Blackwell, 38–60.
- MURPHY, W.J. & COLLIER, G.E. (1996): Phylogenetic relationships within the aplocheiloid fish genus *Rivulus* (Cyprinodontiformes, Rivulidae): implications for Caribbean and Central American biogeography. – *Molecular Biology and Evolution*, **13**: 642–649.
- MURPHY, W.J. & COLLIER, G.E. (1997): A molecular phylogeny for aplocheiloid fishes (Atherinomorpha, Cyprinodontiformes): the role of vicariance and the origins of annualism. – *Molecular Biology and Evolution*, **14**: 790–799.
- MURPHY, W.J. & COLLIER, G.E. (1999): Phylogenetic relationships of African killifishes in the genera *Aphyosemion* and *Fundulopanchax* inferred from mitochondrial DNA sequences. – *Molecular Phylogenetics and Evolution*, **11**: 351–360.
- MURPHY, W.J.; NGUYEN, T.N.P.; TAYLOR, E.B. & COLLIER, G.E. (1999a): Mitochondrial DNA phylogeny of West African aplocheiloid killifishes (Cyprinodontiformes, Aplocheilidae). – *Molecular Phylogenetics and Evolution*, **11**: 343–350.
- MURPHY, W.J.; THOMERSON, J.E. & COLLIER, G.E. (1999b): Phylogeny of the neotropical killifish family Rivulidae (Cyprinodontiformes, Aplocheiloidei) inferred from mitochondrial DNA sequences. – *Molecular Phylogenetics and Evolution*, **13**: 289–301.
- MIYA, M.; TAKESHIMA, H.; ENDO, H.; ISHIGURO, N.B.; INOUE, J.G.; MUKAI, T.; SATOH, T.P.; YAMAGUCHI, M.; KAWAGUCHI, A.; MABUCHI, K.; SHIRAI, S.M. & NISHIDA, M. (2003): Major patterns of higher teleostean phylogenies: a new perspective based on 100 complete mitochondrial DNA sequences. – *Molecular Phylogenetics and Evolution*, **26**: 121–138.
- MYERS, G.S. (1931): The primary groups of oviparous cyprinodont fishes. – *Stanford University Publications Biological Sciences*, **6**: 1–14.
- MYERS, G.S. (1938): Fresh-water fishes and West Indian zoogeography. – *Smithsonian Reports*, **1937**: 339–364.
- MYERS, G.S. (1958): Trends in the evolution of teleostean fishes. – *Stanford Ichthyological Bulletin*, **7**: 27–30.
- PARENTI, L.R. (1981): A phylogenetic and biogeographic analysis of cyprinodontiform fishes (Teleostei, Atherinomorpha). – *Bulletin of the American Museum of Natural History*, **168**: 335–557.
- PARENTI, L.R. (2008): A phylogenetic analysis and taxonomic revision of ricefishes, *Oryzias* and relatives (Beloniformes, Adrianiichthyidae). – *Zoological Journal of the Linnean Society*, **154**: 494–610.
- PARK, E.H. & KIM, D.S. (1984): Hepatocarcinogenicity of diethylnitrosamine to the self-fertilizing hermaphroditic fish *Rivulus marmoratus* (Teleostomi: Cyprinodontidae). – *Journal of the National Cancer Institute*, **73**: 871–876.
- POTTER, P. E. (1997): The Mesozoic and Cenozoic paleodrainage of South America: a natural history. – *Journal of South American Earth Sciences*, **10**: 331–344.
- RIBEIRO, A.C. (2006): Tectonic history and the biogeography of the freshwater fishes from the coastal drainages of eastern Brazil: an example of faunal evolution associated with a divergent continental margin. – *Neotropical Ichthyology*, **4**: 225–246.
- RONQUIST, F. (1996): DIVA v. 1.1. Computer program for MacOS and Win32. Available from <http://morphbank.ebc.uu.se/DIVA>.
- RONQUIST, F. (1997): Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. – *Systematic Biology*, **46**: 195–203.
- ROSEN, D.E. & PARENTI, L.R. (1981): Relationships of *Oryzias*, and the groups of atherinomorph fishes. – *American Museum Novitates*, **2719**: 1–25.
- SANMARTÍN, I. (2007): Event-based biogeography: integrating patterns, processes, and time. In Ebach, M.C. & Tangney, R.S. (eds.): *Biogeography in a changing World*. Boca Raton, CRC Press, 135–159.
- SANMARTÍN, I. & RONQUIST, F. (2004): Southern Hemisphere biogeography inferred by event-based models: plant versus animal patterns. – *Systematic Biology*, **53**: 216–243.
- SERENO, P. C. (2007): Logical basis for morphological characters in phylogenetics. – *Cladistics*, **23**: 565–587.
- SETIAMARGA, D.H.E.; MIYA, M.; YAMANOU, Y.; MABUCHI, K.; SATOH, T.P.; INOUE, J.G. & NISHIDA, M. (2008): Interrelationships of Atherinomorpha (medakas, flyingfishes, killifishes, silversides, and their relatives): the first evidence based on whole mitochondrial genome sequences. – *Molecular Phylogenetics and Evolution*, **49**: 598–605.
- SPARKS, J.S. & SMITH, W.L. (2005): Freshwater fishes, dispersal ability, and nonevidence: “Gondwana life rafts” to the rescue. – *Systematic Biology*, **54**: 158–165.
- STANKIEWICZ, J. & DE WIT, M.J. (2006): A proposed drainage evolution model for Central Africa - Did the Congo flow east? – *Journal of African Earth Sciences*, **44**: 75–84.
- TAYLOR, W.R. & VAN DYKE, G.C. (1985): Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. – *Cybio*, **9**: 107–109.
- VAN BOCKLAER I., ROELANTS K.; BIJU S.D.; NAGARAJU J. & BOS-SUYT F. (2006): Late Cretaceous Vicariance in Gondwanan Amphibians. – *PLoS ONE*, **1**(1): e74. doi:10.1371/journal.pone.0000074.
- WOURMS, J.P. (1972): Developmental biology of annual fishes: III. Pre-embryonic and embryonic diapause of variable duration in the eggs of annual fishes. – *Journal of Experimental Zoology*, **182**: 389–414.

Appendix 1

List of morphological characters

Osteology

Jaws, jaw suspensorium and opercular series

- [1] Autopalatine, dorsal portion, prominent bony flange: (0) absent; (1) present.
- [2] Autopalatine, dorsal tip, shape and orientation relative to main longitudinal axis of bone: (0) slightly curved anteriorly, approximately continuous to main axis; (1) bent, displaced laterally relative to main axis.
- [3] Autopalatine, ventral extremity, extent relative to dorsal portion of quadrate: (0) contacting; (1) not contacting.
- [4] Autopalatine, anteroventral process, anterior expansion: (0) not expanded; (1) expanded, tear-shaped.
- [5] Autopalatine, posterior margin, bony flange: (0) absent; (1) present.
- [6] Autopalatine, head, shape and articulation with upper jaw: (0) relatively narrow, without strong connection to maxilla; (1) expanded and articulating with maxilla via dense ligament.
- [7] Entopterygoid, development: (0) well-developed; (1) rudimentary.
- [8] Entopterygoid, anterior portion, extent relative to autopalatine: (0) overlapping; (1) not overlapping.
- [9] Entopterygoid, posterior portion, extent relative to metapterygoid region: (0) long, reaching it; (1) short, not reaching it.
- [10] Entopterygoid, ventral portion, extent relative to quadrate: (0) overlapping; (1) not overlapping.
- [11] Entopterygoid, dorsal surface, extent relative to infraorbital region: (0) expanded to reach infraorbital region; (1) short, not reaching it.
- [12] Pterygoquadrate cartilage, dorsal projection: (0) absent; (1) present.
- [13] Metapterygoid: (0) present; (1) absent.
- [14] Metapterygoid, size relative to surrounding structures: (0) large, occupying space between entopterygoid and hyomandibula; (1) small to rudimentary, separated from entopterygoid and hyomandibula by wide spaces.
- [15] Metapterygoid, general shape: (0) about rectangular, dorsal and ventral portions wide, approximately equal in width; (1) about triangular, dorsal portion constricted; (2) whole bone narrowed.
- [16] Quadrate, posterior process, length relative to quadrate length without process: (0) longer; (1) equal or shorter.
- [17] Maxilla, main axis, torsion: (0) not twisted; (1) slightly twisted.
- [18] Maxilla, dorsal process, development: (0) well-developed; (1) rudimentary.
- [19] Maxilla, dorsal process, distal expansion: (0) absent; (1) present.
- [20] Maxilla, ventral process, anterior expansion: (0) absent; (1) present.
- [21] Maxilla, subdistal portion, anterior border, projection supporting ligament: (0) present; (1) absent.
- [22] Maxilla, distal part, abrupt widening: (0) absent; (1) present.
- [23] Premaxilla, subdistal portion, posterior margin, expansion: (0) present; (1) absent.
- [24] Premaxilla, ascending process, shape: (0); about rectangular (1) about triangular.
- [25] Premaxilla, alveolar arm, anterior expansion: (0) absent; (1) present.
- [26] Premaxilla, alveolar arm, shape: (0) slightly curved; (1) S-shaped.
- [27] Premaxilla (in males), teeth, outer row, shape and orientation: (0) slightly curved, directed inside mouth; (1) strongly curved, directed laterally, outside mouth.
- [28] Rostral cartilage: (0) present; (1) absent.
- [29] Rostral cartilage, shape: (0) sub-triangular or rounded, about as long as wide; (1) approximately circular.
- [30] Dentary, shape: (0) slender; (1) deep.
- [31] Dentary, coronoid process, extent relative to dorsal portion of angulo-articular: (0) extending; (1) not extending.
- [32] Dentary, coronoid process, shape: (0) robust; (1) slender.
- [33] Dentary, posteroventral process, shape: (0) pointed, narrow; (1) truncate, broad.
- [34] Dentary, robust ventral process anteriorly directed: (0) absent; (1) present.
- [35] Dentary, medial symphysis, kind of articulation: (0) ligamentous; (1) cartilaginous.
- [36] Dentary (in adult males), teeth, outer row, shape and orientation: (0) slightly curved, tip posteriorly directed; (1) strongly curved, tip postero-laterally directed; (2) slightly curved, tip anteriorly directed.
- [37] Dentary (in adult males), teeth, outer row, size related to adjacent teeth of inner rows: (0) two or three times bigger; (1) about five times bigger.
- [38] Angulo-articular, ventral process, shape: (0) wide, about rectangular; (1) narrow, about triangular.
- [39] Angulo-articular, ventral process, development: (0) well-developed; (1) rudimentary.
- [40] Angulo-articular, ventral process, axis orientation: (0) straight; (1) curved.
- [41] Angulo-articular, ventral process, direction and extent relative to dentary: (0) anteroventrally directed, not reaching dentary; (1) anteriorly directed, often terminating in long sharp point, surpassing or in close proximity to vertical through posterior limit of dentary.

- [42] Angulo-articular, coronoid process, development: (0) well-developed; (1) rudimentary.
- [43] Angulo-articular, concavity anterior to dorsal process: (0) absent; (1) present.
- [44] Meckel's cartilage, shape: (0) narrow and long; (1) broad and short.
- [45] Symplectic, general shape: (0) short and deep, about so deep as long; (1) slender and long, conspicuously longer than deep.
- [46] Symplectic, dorsal margin, expansion towards entopterygoid: (0) absent; (1) present; (?) rudimentary expansion, often absent.
- [47] Hyomandibula, lateral flange, development: (0) rudimentary; (1) expanded to form broad process.
- [48] Hyomandibula, posteroventral flap: (0) absent; (1) present.
- [49] Hyomandibula, posteroventral flap, vertical extent relative to articular condyle for symplectic: (0) short, reaching transverse line through middle of condyle; (1) long, reaching transverse line through distal portion of condyle.
- [50] Hyomandibula, posteroventral flap, horizontal extent expressed by width relative to main condyle of hyomandibula: (0) well developed, equally broad; (1) narrower; (2) rudimentary.
- [51] Hyomandibula, articulation with otic region of skull: (0) bifid head; (1) single head.
- [52] Hyomandibula, area adjacent to metapterygoid, foramen: (0) absent; (1) present.
- [53] Preopercle, shape: (0) robust, L-shaped, with a well developed anteromedian rim; (1) thin, C-shaped, with a reduced anteromedian rim.
- [54] Preopercle, dorsal arm, shape: (0) blunt; (1) pointed.
- [55] Preopercle, dorsal arm, length relative to anteroventral arm: (0) approximately equal or shorter; (1) distinctively longer.
- [56] Opercle, ventro-posterior part, expansion: (0) absent; (1) present. Remarks: all taxa exhibiting the apomorphic condition also have the dorsal portion of the opercle distinctively longer than the ventral portion (vs. about equal in length in the remaining taxa), thus considered as a dependent condition.
- [57] Opercle, dorsal margin: (0) slightly curved; (1) angular.
- Hyoid and branchial arches**
- [58] Interhyal, ossification: (0) ossified; (1) cartilaginous.
- [59] Interhyal, development: (0) well-developed; (1) rudimentary or absent.
- [60] Dorsal hypohyal: (0) present; (1) absent.
- [61] Basihyal, length relative to longitudinal length of three basibranchials: (0) shorter; (1) longer.
- [62] Basihyal, cartilaginous portion, length relative to total basihyal length: (0) about half or more; (1) about one third; (2) about one tenth.
- [63] Basihyal, shape: (0) sub-rectangular; (1) sub-triangular; (2) sub-pentagonal.
- [64] Urohyal, shape: (0) slender; (1) deep.
- [65] Urohyal, anterior process, expansion: (0) absent; (1) present.
- [66] Epibranchials 1-3, length relative to third pharyngobranchial width: (0) shorter; (1) longer.
- [67] First epibranchial, uncinat process: (0) present; (1) absent.
- [68] First epibranchial, flexion: (0) unbowed; (1) bowed.
- [69] First epibranchial, torsion: (0) untwisted; (1) twisted.
- [70] Second epibranchial, subdistal process: (0) present; (1) absent.
- [71] Second epibranchial, development: (0) well-developed; (1) minute.
- [72] Third epibranchial, uncinat process, angle formed with distal process: (0) about 90°; (1) about 45–60°.
- [73] Third epibranchial, uncinat process, development: (0) well-developed; (1) rudimentary.
- [74] Fourth epibranchial, posterior process: (0) absent; (1) present.
- [75] Fourth epibranchial, articular surface, extent: (0) narrow; (1) broad.
- [76] Fourth epibranchial, uncinat process: (0) present; (1) absent.
- [77] Interarcual cartilage: (0) present; (1) absent.
- [78] First pharyngobranchial: (0) present; (1) absent.
- [79] Second pharyngobranchial, articular facet for second epibranchial, position and shape: (0) on medial margin, not forming distinct articulation process; (1) continuous to proximal margin, forming distinct articulation process.
- [80] Second pharyngobranchial, distal border, notch close to condyle: (0) absent; (1) present.
- [81] Second pharyngobranchial, medial border, expansion: (0) not expanded; (1) expanded.
- [82] Second pharyngobranchial, medial flap: (0) absent; (1) present.
- [83] Second pharyngobranchial, teeth: (0) present; (1) absent; (?) present or absent.
- [84] Second pharyngobranchial, teeth, arrangement: (0) multiple rows on whole posterior region; (1) single row restricted to medial part of posterior border.
- [85] First basibranchial: (0) present; (1) absent.
- [86] Hypobranchials, extent: (0) narrow, separated by broad interspace; (1) broad, separated by narrow interspace.
- [87] First hypobranchial, medial edge: (0) unbranched or with slight division; (1) deeply branched.
- [88] First hypobranchial, lateral edge, articular facet extension relative to apical cartilage of first ceratobranchial: (0) restricted to articulation area; (1) extended.
- [89] Second and third hypobranchials, teeth: (0) absent; (1) present.

- [90] Second hypobranchial, medial process directed to second basibranchial: (0) absent; (1) present.
- [91] Second hypobranchial, medial edge: (0) unbranched or with slight division; (1) deeply branched.
- [92] Third basibranchial, posterior half, shape: (0) not constricted; (1) constricted.
- [93] Fourth ceratobranchial, ventral process: (0) present; (1) absent. Remarks: in all terminal taxa having the character state 1, teeth are absent on the fourth ceratobranchial, possibly constituting a single character.
- [94] Fourth ceratobranchial, ventral process, orientation: (0) ventral; (1) posterior.
- [95] Fourth ceratobranchial, antero-proximal process, extent: (0) narrow; (1) laterally displaced forming wide flap.
- [96] Fifth ceratobranchial, anterior portion, flaps adjacent to articular head: (0) absent; (1) present.
- [97] Fifth ceratobranchial, tooth plate, shape: (0) triangular; (1) rectangular or sub-oval.

Neurocranium

- [98] Neurocranium, general shape: (0) not flattened; (1) flattened.
- [99] Lateral ethmoid, medial extent expressed by cartilaginous space width between medial margin of bone and vomer and parasphenoid relative to anterior parasphenoid width: (0) wider; (1) narrower.
- [100] Lateral ethmoid, anterior retrorse process: (0) absent; (1) present.
- [101] Lateral ethmoid, anterior retrorse process, development: (0) rudimentary; (1) well-developed.
- [102] Lateral ethmoid, anterior retrorse process, extent relative to main lateral ethmoid axis: (0) not reaching; (1) contacting, often fused.
- [103] Parasphenoid, anterolateral process, extent relative to pterosphenoid: (0) short, not reaching; (1) long, touching or attached to.
- [104] Frontal, lateral border, shape: (0) approximately straight; (1) concave.
- [105] Vomer: (0) present; (1) absent.
- [106] Vomer, teeth: (0) present; (1) absent.
- [107] Vomer, teeth, distribution: (0) middle small patch; (1) along anterior border of bone.
- [108] Mesethmoid, ossification: (0) ossified; (1) unossified.

Infra-orbital series

- [109] Lachrymal, ventral portion, expansion: (0) absent; (1) present.
- [110] Second infra-orbital: (0) present; (1) absent.
- [111] Dermosphenotic: (0) present; (1) absent.

- [112] Dermosphenotic, development: (0) well developed; (1) minute.

Vertebrae and caudal skeleton

- [113] Anterior vertebrae, first pleural rib, position: (0) third vertebra; (1) second vertebra.
- [114] First vertebra, latero-dorsal laminar process: (0) present; (1) absent.
- [115] First vertebra, anteriorly directed paired process: (0) absent; (1) present.
- [116] Epipleural ribs, shape: (0) single; (1) bifid.
- [117] Compound centrum, central portion of side, keel-shaped process: (0) absent; (1) present.
- [118] Stegural, development: (0) well-developed; (1) minute.
- [119] Stegural, ventral portion, lateral process: (0) absent; (1) present.
- [120] Hypurals 4 and 5, degree of fusion: (0) unfused; (1) fused.
- [121] Upper hypurals and compound caudal centrum, degree of fusion: (0) attached, limited by cartilage edge; (1) complete ankylosis.
- [122] Upper and lower hypural plates, degree of fusion: (0) unfused; (2) fused.
- [123] Radial caudal cartilages: (0) present; (1) absent.
- [124] Ventral accessory bone: (0) absent; (1) present.
- [125] Caudal-fin rays, zone between upper and lower hypural plates, arrangement: (0) separated by broad interspace; (1) continuously arranged.
- [126] Epurals, number: (0) three or two; (1) one.
- [127] Epural, proximal region, width relative to distal region: (0) wider to slightly narrower; (1) conspicuously narrower.
- [128] Epural, shape: (0) rod-like; (1) blade-like.
- [129] Preural vertebra 2, neural spine, development: (0) absent; (1) well-developed, distal tip acting in support of caudal-fin rays.
- [130] Preural vertebra 2, neural spine, width relative to neural spines of preural vertebrae 4 and 5: (0) about equal; (1) wider.
- [131] Preural vertebra 2, hemal spine, width relative to hemal spines of preural vertebrae 4 and 5: (0) distinctively wider; (1) slightly wider.
- [132] Preural vertebra 3, neural and hemal spines, width relative to neural and hemal spines of preural vertebrae anterior to preural vertebra 4: (0) about equal; (1) wider.

Dorsal and anal fin support

- [133] Dorsal and anal fins, sexual dimorphism in number of rays: (0) not dimorphic; (1) more rays in males than in females.
- [134] Dorsal fin, first rays, number of rays attached to first proximal radial: (0) single long ray; (1) long ray preceded by one or two short fin rays.

- [135] Dorsal and anal fins, rays, branching: (0) most rays branched; (1) all or almost all rays unbranched.
 [136] Dorsal and anal fin, medial radials, ossification: (0) well-ossified; (1) poorly ossified or cartilaginous.

Shoulder and pelvic girdle

- [137] Pectoral-fin, insertion: (0) lateral; (1) ventrolateral.
 [138] Posttemporal and supracleithrum, co-ossification: (0): absent; (1) present.
 [139] Posttemporal, ventral process: (0) present; (1) absent.
 [140] Posttemporal, prominent lateral keel: (0) absent; (1) present.
 [141] First postcleithrum: (0) present; (1) absent.
 [142] First postcleithrum, shape: (0) rod-like; (1) scale-like.
 [143] Cleithrum, posterior flange: (0) present; (1) absent.
 [144] Coracoid, anteroventral condyle, shape: (0) entirely narrow; (1) widening towards extremity.
 [145] Proximal radials, cartilaginous interspace: (0) minute; (1) broad.
 [146] Fourth pectoral radial, ventral expansion: (0) absent; (1) present.
 [147] Pelvic bone, thickness and processes, development: (0) thick, processes well-developed; (1) thin, ischial and lateral processes rudimentary or absent.

External morphology

Head

- [148] Mouth, cleft position: (0) terminal; (1) superior.
 [149] Eye, orbital rim, attachment: (0) free; (1) partially attached (ventrally); (2) completely attached.
 [150] Anterior naris, shape: (0) flat; (1) tubular.
 [151] Membrane between preopercular laterosensory series and isthmus, middle gap: (0) present; (1) absent.
 [152] Membrane between preopercular laterosensory series and isthmus, middle gap posterior extent: (0) long, reaching opercle; (1) short, reaching transverse line through corner region of pre-opercular series or shorter; (?) gap absent.
 [153] Membrane between preopercular laterosensory series and isthmus, gap below corner of pre-opercular series: (0) present; (1) absent.
 [154] Ventral squamation, anterior extent: (0) reaching corner of pre-opercular series; (1) reaching anterior end of pre-opercular series; (2) reaching chin.
 [155] Frontal squamation, geometric arrangement: (0) transverse; (1) circular.

- [156] Frontal squamation, symmetry: (0) present; (1) absent.
 [157] Frontal squamation, modal arrangement-pattern: (0) G-H; (1) E; (2) D; (3) F.
 [158] Frontal squamation, E-scales, relative position: (0) overlapped; (1) not overlapped.
 [159] Frontal squamation, anteriormost scale, position relative to rostral region: (0) in close proximity; (1) separated by space without scales and with thickened epidermal tissue.

Trunk

- [160] Flank in males, skin contact organs on scale free margin: (0) absent; (1) present.
 [161] Venter in females, urogenital papilla, shape: (0) minute protuberance around urogenital opening; (1) prominent pocket-like structure around urogenital opening, often overlapping anal-fin origin.

Fins

- [162] Pectoral fin in males, rays, papillate contact organs: (0) absent; (1) present.
 [163] Pectoral fin in males, posterior extremity, shape: (0) rounded; (1) pointed.
 [164] Pelvic fin in males, tip, filamentous rays: (0) absent; (1) present.
 [165] Pelvic fin, number: (0) five or six; (1) 7; (2) 8; (3) 9.
 [166] First dorsal fin: (0) present; (1) absent.
 [167] Anal fin in males, anterior portion, expanded lobe: (0) absent; (1) present.
 [168] Anal fin in females, distal length relative to fin base: (0) shorter; (1) longer.
 [169] Anal fin in females, thickness relative to male anal fin: (0) equal; (1) distinctively thicker.
 [170] Anal fin in males, rays, hook-like contact organs: (0) absent; (1) present.
 [171] Caudal fin in males, dorsal and ventral portions, posterior expansion forming lyre-shape: (0) absent; (1) present.
 [172] Caudal fin in males, posterior margin, filaments forming fringed border: (0) absent; (1) present.
 [173] Caudal fin in males, length relative to standard length: (0) clearly less than half; (1) about half or longer.

Laterosensory system

- [174] Supra-orbital series, canal: (0) close; (1) open.
 [175] Infra-orbital series, lachrymal and dermosphenotic sections, canal: (0) close; (1) open; (?) canal open.
 [176] Infra-orbital series, lachrymal, number of pores: (0) three; (1) two.
 [177] Infra-orbital series, neuromasts, arrangement: (0) aligned; (1) on zigzag row.

- [178] Preopercular series, relative position of upper and lower sections: (0) separated by interspace; (1) continuous.
 [179] Mandibular series, posterior section, canal: (0) close; (1) open.

Colour patterns

- [180] Colour pattern, sexual dimorphism: (0) absent; (1) present.
 [181] Flank in males, scales, free margin, red pigment concentration: (0) absent; (1) present.
 [182] Flank in males, humeral region, small orange spots: (0) absent; (1) present.
 [183] Flank in females, dark brown stripes: (0) absent; (1) present.
 [184] Flank in females, antero-central portion, distinctive black spot: (0) absent; (1) present.
 [185] Caudal peduncle in females, posterodorsal end, round black spot with white margin: (0) absent; (1) present.
 [186] Head, iris, dark grey to black bar through centre of eye: (0) absent; (1) present.
 [187] Head in males, anterior suborbital region, distinctive dark grey to black bar: (0) absent; (1) present.
 [188] Head in males, post-orbital region, three oblique head stripes: (0) absent; (1) present.
 [189] Head, lower jaw, colour: (0) not distinctively coloured; (1) dark grey to black.
 [190] Head in males, middle opercular region, blue blotch: (0) absent; (1) present.
 [191] Unpaired fins in females, margins, black zone: (0) absent; (1) present.
 [192] Dorsal fin in females, basal region, black blotch: (0) absent; (1) present.
 [193] Anal fin in males, basal region, dark grey to black ground colour with row of white to yellow spots: (0) absent; (1) present.
 [194] Anal fin in females, colour: (0) hyaline; (1) bright yellow or orange.
 [195] Caudal fin in males, ventral portion, stripe pattern: (0) diffuse red stripe; (1) dark orange stripe with narrow light yellow and dark reddish brown upper margin; (2) light yellow stripe with broad dark red upper margin; (?) distinctive stripes absent.
 [196] Caudal fin in males, middle portion, red bars: (0) absent; (1) present.
 [197] Caudal fin in females, base, dorsal portion, rounded black spot with white dot on anterior margin: (0) absent; (1) present.
 [198] Pectoral fin in males, dark grey to black marks vertically crossing all fin extension: (0) absent; (1) present.
 [199] Pectoral fin, distal margin, white stripe: (0) absent; (1) present.

