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Descriptive morphology and phylogenetic relationships among species of the Neotropical annual killifish genera *Nematolebias* and *Simpsonichthys* (Cyprinodontiformes: Aplocheiloidei: Rivulidae)

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Structures of the external morphology and skeleton of the annual fish genera *Nematolebias* and *Simpsonichthys* are described and illustrated. Phylogenetic relationships among all included species of both genera and other cynolebiatins are estimated, based on 116 morphological characters in a total of 50 terminal taxa. *Nematolebias* is hypothesized to be the sister group of a clade including *Simpsonichthys*, *Austrolebias*, *Megalebias*, and *Cynolebias*, which is diagnosed by ventral portion of mesopterygoid not overlapping quadrate, third neural spine approximately as wide as fourth neural spine, ventral condyle of coracoid narrow, scales slightly extending over anal-fin base, loss of vomerine teeth, loss of neuromast anterior to infraorbital series, ventral process of the angulo-articular short and narrow, and dorsal portion of cleithrum elongated. *Simpsonichthys* is divided into five subgenera, including *Spectrolebias*, previously considered a separate genus, *Simpsonichthys*, and three new subgenera, *Xenurolebias*, *Ophthalmolebias*, and *Hypsolebias*. Monophyly of *Simpsonichthys* is supported by a small pointed dorsal process on the palatine, unbranched fin rays on the tip of the dorsal and anal fins in males, and frontal E-scales overlapped.

Estruturas da morfologia externa e esqueleto dos gêneros de peixes anuais *Nematolebias* e *Simpsonichthys* são descritas e ilustradas. Relações de parentesco entre todas as espécies incluídas em ambos gêneros e outros cinolebiatíneos são estimadas, com base em 116 caracteres morfológicos num total de 50 táxons terminais. *Nematolebias* é considerado como o grupo irmão hipotético de um clado incluindo *Simpsonichthys*, *Austrolebias*, *Megalebias* e *Cynolebias*, o qual é diagnosticado pela porção ventral do mesopterigóide não se sobrepondo ao quadrado, terceiro espinho neural aproximadamente tão largo quanto quarto espinho neural, côndilo ventral de coracóide estreito, escamas suavemente se estendendo sobre base de nadadeira anal, perda de dentes do vomer, perda de neuromasto anterior à série infra-orbital, processo ventral de ângulo-articular curto e estreito e porção dorsal de cleitro alongada. *Simpsonichthys* é dividido em cinco subgêneros, incluindo *Spectrolebias*, anteriormente considerado um gênero separado, *Simpsonichthys*, e três novos subgêneros, *Xenurolebias*, *Ophthalmolebias* e *Hypsolebias*. Monofiletismo de *Simpsonichthys* é sustentado por um pequeno processo dorsal pontiagudo no palatino, raios não ramificados na ponta das nadadeiras dorsal e anal em machos e escamas frontais E sobrepostas.

Key words: Systematics, cladistics, Cynolebiatinae, *Spectrolebias, Xenurolebias*, *Ophthalmolebias, Hypsolebias*.

Introduction

Nematolebias comprises two species endemic to the coastal plains of southeastern Brazil (Costa, 2002a). *Simpsonichthys* contains 43 valid species, constituting the most speciose genus of aplocheiloid annual fishes; it occupies a vast geographic range in northeastern, central and eastern South America (*e.g.*, Costa, 2003a). The greatest diversity of *Simpsonichthys* is concentrated in the area drained by the middle and upper Tocantins-Araguaia, middle São Francisco, and upper Paraná river basins, to where a total of 31 species are endemic. Species of *Simpsonichthys* are also present in the Madeira basin, Bolivia; Paraguay basin, Paraguay; Jaguaribe, Pardo, Jequitinhonha, and Xingu basins, and smaller coastal basins of northeastern and eastern Brazil. Two phylogenetic studies, mainly based on color patterns and some osseous structures, involved a meaningful number of species of the genera *Nematolebias* and *Simpsonichthys* (Costa, 1996, 2003).

The name *Simpsonichthys* was first proposed by de Carvalho (1959), but it was considered as a synonym of *Cynolebias* Steindachner (Parenti, 1981). Subsequently, based

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upon phylogenetic studies on rivulids, *Cynolebias* was divided (*e.g.*, Costa, 1990, 1996, 1998a). New genera, including *Spectrolebias* Costa & Nielsen, *Nematolebias* Costa, *Austrolebias* Costa, and *Megalebias* Costa were then erected (Costa & Nielsen, 1997; Costa, 1998a), and *Simpsonichthys* was resurrected (Costa, 1996). However, gradual addition of new taxa and new characters in most recent studies generated some impact in previous phylogenetic hypotheses. *Nematolebias* was first considered to be the sister group to *Simpsonichthys*, and *Spectrolebias*, to be the sister group to an assemblage including *Nematolebias*, *Simpsonichthys*, *Austrolebias*, *Cynolebias*, and *Megalebias* (Costa, 1998a), but according to a more recent hypothesis (Costa, 2003b), *Spectrolebias* would be the sister group to a clade including only *Nematolebias* and *Simpsonichthys*. Two independent molecular phylogenies of rivulids (Murphy *et al.*, 1999; Hrbek & Larson, 1999), including respectively three and four species of *Simpsonichthys* and *Nematolebias*, hypothesized some species of *Simpsonichthys* is more closely related to *Austrolebias* than to *Nematolebias*.

Until recent years, morphological traits were the only or the main source of characters to erect phylogenies and classifications of actinopterygian fishes. Different kinds of morphological characters have been progressively employed in killifish systematics, but Parenti (1981) first used them in a cladistic context. Subsequently, a variety of phylogenetically informative morphological traits, such as osseous features, scale and neuromast arrangement patterns, color patterns, besides data on behavior and ecology, were used to establish rivulid clades and formally diagnose taxa (*e.g.*, Costa, 1990, 1998a, 2004a). However, despite morphological characters being regularly listed in systematics studies on killifishes, morphological studies are usually restricted to selected features that were found to be phylogenetically informative, not making available the data considered uninformative (*e.g.*, Parenti, 1981; Costa, 1990, 1998a, 2001). Consequently, many structures are still undescribed or poorly known. Loureiro & de Sá (1998) conducted a descriptive osteological study of a broad range of taxa including some species today placed in *Austrolebias*, *Megalebias* and *Simpsonichthys*, mostly obtained from aquarium material. Unfortunately their descriptions were brief and some structures misidentified.

Phylogenetic studies of killifish using DNA sequence data have become much more common. However, these studies often neglect or omit previously published, conflicting morphological characters (*e.g.*, Hrbek & Larson, 1999). Much of the recent enthusiasm for molecular studies as a unique powerful source of phylogenetic information is due to the rapid production of data and the putatively greater number of informative characters that result, in comparison to morphological analyses. However, recent debates indicate that morphological characters are crucial for understanding phylogenetic relationships (Wahlberg & Nylin, 2003; Smith & Turner, 2005), molecular and morphological characters may have similar numbers of relevant characters (Jenner, 2004; Lee, 2004), and morphological phylogenies are important to detect common errors in molecular analyses, such as long-branch attraction, deviations between gene and species tree, and contamination and misidentification of specimens (Jenner, 2004; Wiens, 2004). However, most authors are in agreement about the striking necessity in to improve morphological phylogenies (*e.g.*, Dettai *et al.*, 2004; Wiens, 2004).

The main objectives of the present study are: to provide accurate descriptions of morphological features, including external morphology of body, head and fins, squamation, neuromasts, contact organs and skeleton in *Nematolebias* and *Simpsonichthys*; to find additional informative characters in order to improve phylogenetic analysis; and, to revise characters included in former analyses (*e.g.*, Costa, 1996, 2003) to eliminate subjectivities and to make them more accurate.

Material and methods

Specimens are listed in the Appendix I. Measurements and counts follow Costa (1995). Measurements are presented as percentages of standard length (SL), except for those relative to head morphology, expressed as percentages of head length (HL). Fin-ray counts include all elements; number of vertebrae, gill-rakers, and pectoral, pelvic and caudal-fin rays were recorded only from cleared and stained specimens; the compound caudal centrum was counted as a single element. Osteological preparations were made according to Taylor and Van Dyke (1985). Terminology for frontal squamation follows Hoedeman (1958), and for cephalic neuromast series Costa (2001).

The phylogenetic analysis followed cladistic methodology (see Kitching *et al.*, 1998 for a synthesis of recent advances). Terminal taxa were all species of *Nematolebias* (*N. papilliferus* Costa and *N. whitei* (Myers)) and *Simpsonichthys* (*S. adornatus* Costa, *S. alternatus* (Costa & Brasil), *S. antenori* (Tulipano), *S. auratus* Costa & Nielsen, *S. boitonei* de Carvalho, *S. bokermanni* (de Carvalho & da Cruz), *S. brunoi* Costa, *S. carlettoi* Costa & Nielsen, *S. chacoensis* (Amato), *S. cholopteryx* Costa, Moreira & Lima, *S. constanciae* (Myers), *S. costai* (Lazara), *S. delucai* Costa, *S. filamentosus* Costa, Barrera & Sarmiento, *S. flagellatus* Costa, *S. flammeus* (Costa), *S. flavicaudatus* (Costa & Brasil), *S. fulminantis* (Costa & Brasil), *S. ghisolfii* Costa, Cyrino & Nielsen, *S. hellneri* (Berkenkamp), *S. igneus* Costa, *S. izecksohni* (da Cruz), *S. magnificus* (Costa & Brasil), *S. marginatus* Costa & Brasil, *S. multiradiatus* (Costa & Brasil), *S. myersi* (de Carvalho), *S. nielseni* Costa, *S. notatus* (Costa, Lacerda & Brasil), *Simpsonichthys ocellatus* Costa, Nielsen & De Luca, *S. parallelus* Costa, *S. perpendicularis* Costa, Nielsen & De Luca, *S. picturatus* Costa, *S. radiosus* Costa & Brasil, *S. reticulatus* Costa & Nielsen, *S. rosaceus* Costa, Nielsen & De Luca, *S. rufus* Costa, Nielsen & De Luca, *S. santanae* (Shibata & Garavello), *S. semiocellatus* (Costa & Nielsen), *S. similis* Costa & Hellner, *S. stellatus* (Costa & Brasil), *S. suzarti* Costa, *S. trilineatus* (Costa & Brasil), and *S. zonatus* (Costa & Brasil), including species formerly placed in *Spectrolebias*. To test monophyly of *Nematolebias* and *Simpsonichthys*, their relationships to other cynolebiatins, and monophyly of the Cynolebiatini, were also included as terminal taxa *Cynolebias griseus* Costa, Lacerda & Brasil and *Austrolebias carvalhoi* (Myers), two basal species of the well-corroborated cynolebiatin clade *Cynolebias* Steindachner + *Megalebias* Costa + *Austrolebias* Costa. Most recent phylogenetic hypotheses for the Rivulidae genera (Costa, 1998a, 2004a) is the baseline for selecting out-groups to polarize character states (Nixon & Carpenter, 1993). Out-groups include *Leptolebias minimus* (Myers), a basal species of the Cynopoecilini, the sister group to Cynolebiatini, *Neofundulus paraguayensis* (Eigenmann & Kennedy), a member of a basal annual rivulid clade, and *Kryptolebias brasiliensis* (Valenciennes), a basal species of the most basal lineage of the Rivulidae (Costa, 1995, 1998a, 2004a).

All characters and character states used in the phylogenetic analysis are listed and coded in the Appendix II and are organized in the data matrix of Appendix IV. Characters with the derived state occurring in a single terminal taxon were not included in the data matrix. Characters of behavior (Belote & Costa, 2002) were not included in the phylogenetic analyses due to the scarcity of available data among the terminal taxa. Character states of multistate characters were treated as ordered whenever possible. Boundaries between states of quantitative characters were determined according to variations found among terminal taxa, in order to accommodate the real range observed for each terminal species included in that category (*i. e.*, character state). These characters (20, 21, 25, 35, 43, 44, 62, 63, 81) may contain some subjectivity, and consequently were excluded from a second analysis, which produced similar trees (see phylogenetic analysis below). Most parsimonious cladograms, consistency indices (CI) and retention indices (RI) were obtained using the heuristic algorithm mhennig*;bb* of the program Hennig86 (Farris, 1988). TreeGardener 2.2.1 (Ramos, 1996) was used for optimization of character state changes in the strict consensus tree of most parsimonious cladograms, using ACCTRAN. Bootstrap analysis (Felsenstein, 1985) was used to establish nodal support through the simple heuristic algorithm of PAUP 4.0 (Swoford, 1998) with 1000 replicates.

Descriptive and comparative morphology

Descriptions and comparisons are primarily based on males, except in morphological traits typical for females. Descriptions are focused on *N. papilliferus*, referring to other species of *Nematolebias* and *Simpsonichthys* when differences were noted. In view of to simplify information about distribution of morphological features among species of *Simpsonichthys*, they are grouped following the classification presented at the end of this paper, in which *Simpsonichthys* is divided into five subgenera: *Xenurolebias*, *Ophthalmolebias*, *Spectrolebias*, *Simpsonichthys*, and *Hypsolebias*. Monophyletic subunits of the latter subgenus, the most speciose, are referred as the following species groups: *S. magnificus* species group, including *S. hellneri*, *S. adornatus*, *S. fulminantis*, *S. carlettoi*, *S. magnificus*, and *S. picturatus*; *S. notatus* species group, including *S. stellatus*, *S. rufus*, *S. notatus*, *S. radiosus*, *S. similis*, *S. trilineatus*, *S. auratus*, and *S. nielseni* sp.; *S. antenori* species group, including *S. antenori*, *S. igneus*, *S. ghisolfii*, *S. flavicaudatus*, and *S. flagellatus*; and, *S. flammeus* species group, including *S. delucai*, *S. alternatus*, *S. multiradiatus*, *S. flammeus*, and *S. brunoi*.

Size. Species of *Nematolebias* and most species of *Simpsonichthys* are medium sized killifishes, reaching about 35-60 mm SL as the maximum adult size. However, *S. parallelus*, *S. cholopteryx*, *S. costai*, *S. reticulatus*, and *S. semiocellatus* are miniature species, among the smallest species of the Rivulidae. Their maximum size does not surpass 25 mm SL (Costa, 1996, 1998a, 2003). On the other hand, *S. ghisolfii* is clearly bigger than any other congener, reaching at least 73 mm SL (Costa, 2003b).

As in most aplocheiloid fishes, all species of *Nematolebias* and *Simpsonichthys* exhibit sexual dimorphism in size. Males are always bigger than females. Females approximately reach 80-85% of the size of males, but in *S. ocellatus*, females reach only about 65%.

General morphology of body. Species of *Nematolebias* differ from most other cynolebiatins by their slender body. The maximum body depth, which is always at the level of the pelvic-fin base, is 28% SL in larger males. Among species of *Simpsonichthys*, except *S. semiocellatus* that is a slender species like species of *Nematolebias*, all are deep-bodied, most reaching 32-40% SL. Exceptionally, in some species of the *S. flavicaudatus* group, males may reach 41-44% SL (Costa, 2003b).

Increasing in body depth is proportional to increasing in head depth, and therefore, whereas in *S. semiocellatus* the maximum head depth is about 90% of HL, in most *Simpsonichthys* the head depth is approximately equal or longer than the head length. The minimum caudal peduncle depth is 14-19% SL in larger males of *Nematolebias* and *Simpsonichthys*, except in *S. semiocellatus*, with 12% SL.

The body is compressed, its widest portion situated at the humeral region, which is slightly narrower than the widest portion of the head. Head width is about 65-80% HL in larger males of most species of *Simpsonichthys* and *Nematolebias*, but in *S. chacoensis*, *S. costai*, *S. reticulatus* and *S. semiocellatus*, the head is slightly more compressed, about 60% SL.

The dorsal profile of the head is slightly concave in juveniles, becoming approximately straight in adult specimens. Sometimes the dorsal profile of the head is gently convex in older males. Both the dorsal profile between nape and the end of dorsal-fin base and the ventral profile between the lower jaw and the end of anal-fin base are always convex. The ventral profile of the head is angular in *S. semiocellatus* and *S. filamentosus*, in which there is a prominent flap of thickened tissue at the level of the angulo-articular bone. The dorsal and ventral profiles of the caudal peduncle usually are

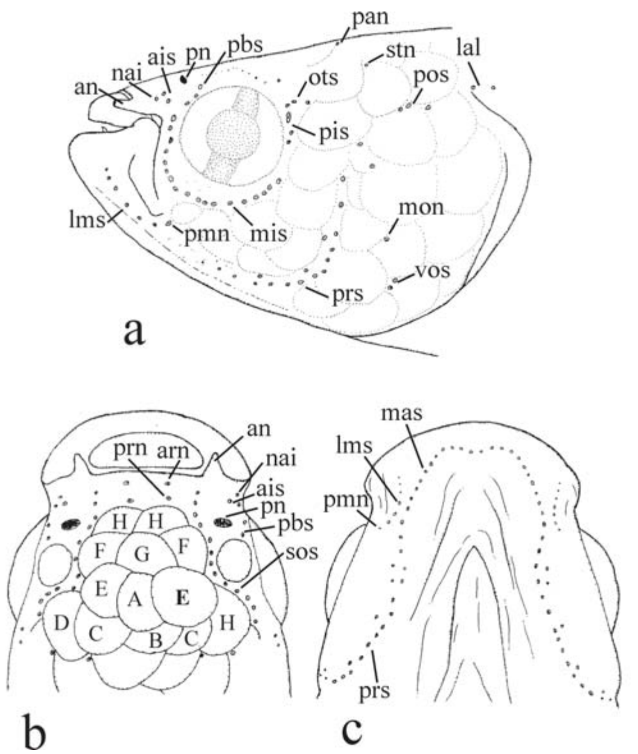

Fig. 1. Diagrammatic representation of cephalic structures of *Nematolebias papilliferus*, including latero-sensory system and cephalic squamation; UFRJ 5295, male, 36.1 mm SL. (a) lateral view; (b) dorsal view; (c) ventral view. ais = anterior infraorbital series; an = anterior naris; arn = anterior rostral neuromast; lal = lateral line neuromasts; lms = lateral mandibular series; mas = mandibular series; mis = median infraorbital series; mon = median opercular series; nai = neuromast anterior to infraorbital series; ots = otic series; pan = parietal neuromast; pbs = preorbital series; pis = posterior infraorbital series; pmn = paramandibular neuromast; pn = posterior naris; pos = post-otic series; prn = posterior rostral neuromast; prs = preopercular series; sos = supraorbital series; stn = supra-temporal neuromast; vos = ventral opercular series; A-H = frontal scales A-H; in bold, frontal scale with all borders free.

straight, sometimes weakly concave. The caudal peduncle is short, about half length of the head, except in *S. semiocellatus*, in which the caudal peduncle is distinctively elongated.

Cephalic structures (Fig. 1)**.** The eyes are large, dorsolaterally placed on the head, and the orbital rim is not free from the head rim. The eyes occupy 22-28% HL in larger males and 29-31% in juvenile males of species of *Nematolebias*. In *Simpsonichthys*, the eye diameter varies from 25 to 38% HL in adult males of median sized species, but in juveniles and adults of miniature species the orbital diameter is 34-42% HL. In the subgenus *Ophthalmolebias*, the eyes are slightly ventrally displaced, becoming more laterally positioned on the head (Figs. 2a-3a).

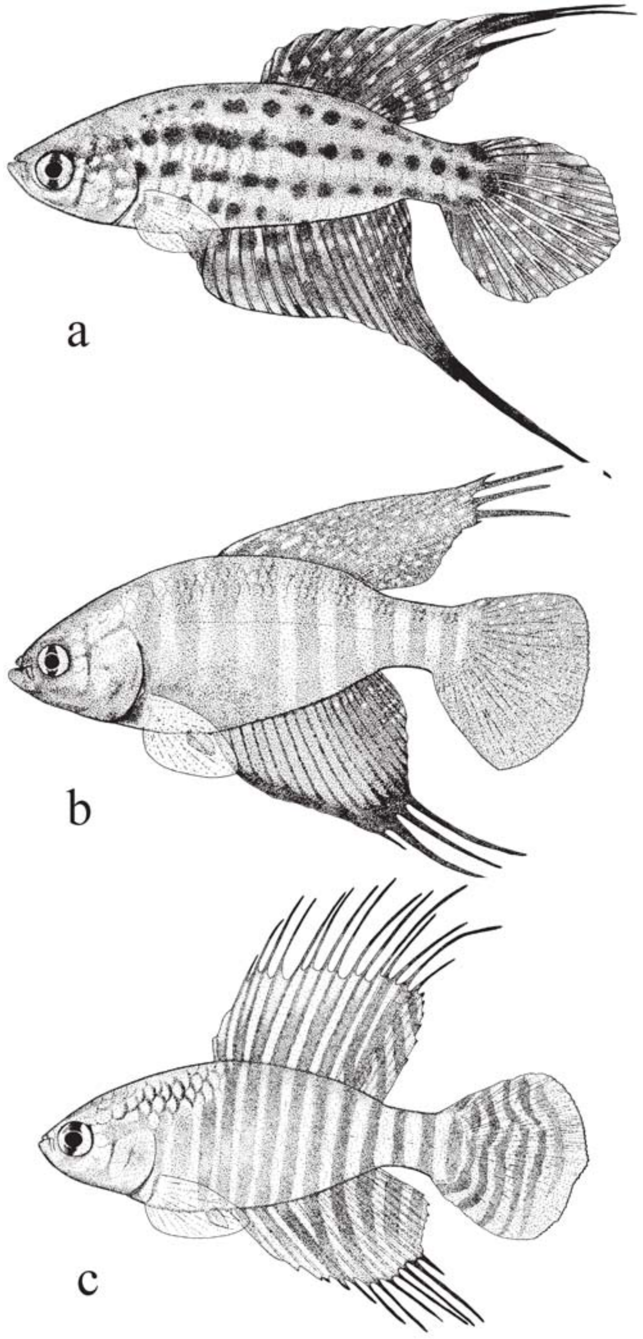

Fig. 2. Some members of the genus *Simpsonichthys*. (a) *S. constanciae*, UFRJ 2199, male, about 40 mm SL; Brazil: Rio de Janeiro: Barra de São João; (b) *S. flavicaudatus*, not preserved, male, topotype, about 40 mm SL; Brazil: Pernambuco: Lagoa Grande; (c) *S. flammeus*, not preserved, male, about 30 mm SL; Brazil: Goiás: Nova Roma.

The mouth is subdorsal, with the upper jaw moderately protractile. The lower jaw is robust, presenting a prominent fold at the mouth corner, and short, usually 15-28% HL. The mouth cleft extends ventroposteriorly, reaching a vertical through the anterior portion of the orbit. The snout is short, usually 10-17% of HL. The anterior naris is tubular and ante-

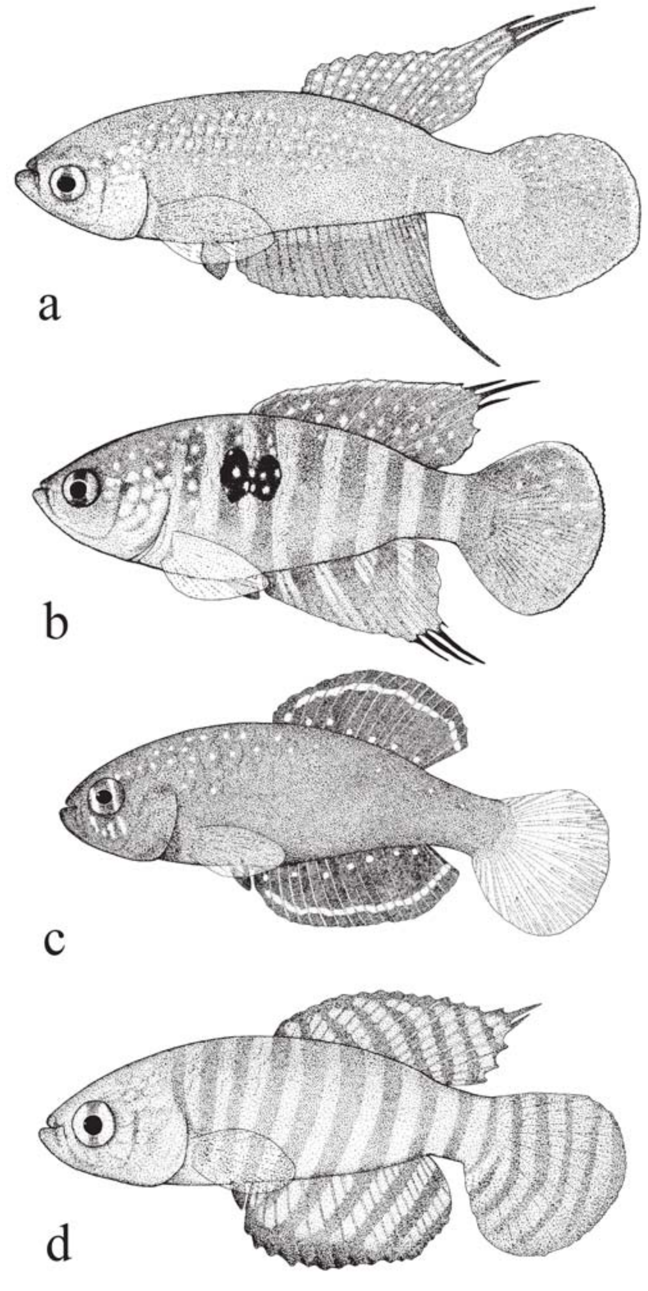

Fig. 3. Some members of the genus *Simpsonichthys*. (a) *S. bokermanni*, UFRJ 1836, male, about 35 mm SL; Brazil: Bahia: Ilhéus; (b) *S. notatus*, UFRJ 2068, male, about 30 mm SL; Brazil: Goiás: Nova Roma; (c) *S. costai*, not preserved, male, about 20 mm SL; Brazil: Mato Grosso: rio das Mortes floodplains; (d) *S. alternatus*, MNRJ 12523, male, holotype, 22.7 mm SL; Brazil: Minas Gerais: Brasilândia.

riorly directed; the posterior naris is an oval, transverse opening dorsally positioned in head at level of the anterior portion of the orbit.

The opercular opening is extensive, its dorsalmost point situated at the level of the dorsal portion of the orbit. The dorsal portion of the opercular membrane slightly extends behind posterior margin of opercle. The opercular and the branchiostegal membrane are united.

Urogenital papilla (Fig. 4)**.** In males, the urogenital papilla is tubular, slightly visible in a lateral view of the venter. However, in *Spectrolebias* the male urogenital papilla may be elongated (Fig. 4d). In females of all species, the urogenital opening is placed in a prominent pocket-like structure that usually slightly overlaps the anterior portion of the anal fin (Fig. 4C).

Fins. In males of both species of *Nematolebias* and most species of *Simpsonichthys*, the dorsal and the anal fins have a similar morphology (Figs. 2-3). Their distal extremity terminates in a long pointed tip, producing an approximately triangular shape. The tip of the dorsal and anal fins bears some short filamentous rays. In females, both fins are shorter than in males, the dorsal fin is rounded to slightly pointed, the anal fin is always rounded, and both fins lack filamentous rays on their distal border. The anal fin is hardened in females, often having a distinctive thickened tissue on its anterodistal portion.

Several species of *Simpsonichthys* exhibit the dorsal and anal fins morphologically similar to the fins in *Nematolebias* (Fig. 3a), although often not so elongated in males. In species of the *S. flammeus* group, the anal fin is elongate in females, acquiring a spatula-like shape. In some species of *Simpsonichthys*, both the dorsal and the anal fins are rounded in males (*S. costai*, *S. magnificus*, *S. picturatus*, *S. delucai*) (Fig. 3c), or only the dorsal fin is rounded (*S. reticulatus*), or only the anal fin is rounded (*S. semiocellatus*, *S. alternatus*, *S. suzarti*) (Fig. 3d). In some species, the filaments are absent in both fins (*S. costai*, *S. parallelus*, *S. cholopteryx*, *S. delucai*, *S. magnificus*, *S. picturatus*) (Fig. 3c), or are absent only in the anal fin (*S. reticulatus*, *S. alternatus*, *S. adornatus*, *S. semiocellatus*) (Fig. 3d). In other species, filaments on the tip of the dorsal and the anal fins may be rather long, reaching or surpassing the posterior margin of the caudal fin (*S. constanciae*, *S. izecksohni*, *S. filamentosus*, *S. semiocellatus*, *S. hellneri*, *S. flavicaudatus*, *S. flagellatus*, *S. igneus*, *S. flammeus*, *S. brunoi*) (Figs. 2a, b). In *S. chacoensis*, dorsal filaments are short, but the anal-fin filaments are long. In *S. flammeus* and *S. brunoi*, instead of filaments being restricted to the tip of the dorsal fin as in other congeners, they are present along the entire extension of the distal border of the fin (Fig. 2c). Uniquely in *S. myersi* and *S. izecksohni*, there are short filaments on the posterior margin of the anal fin in females (Fig. 4e).

Depending on the number of fin rays, the length of the dorsal and anal-fin bases may change strongly. The dorsal-fin base varies from about 30% SL in adult males of species with fewer rays, to about 60% in males of *S. adornatus*, the species with the greatest number of dorsal-fin rays (Costa, 2003b). Similarly, the anal-fin base ranges from 32% to 48% SL. The number of dorsal-fin rays also influences the predorsal length, which varies from 62% SL in adult males of species with fewer rays, to 28% in males of *S. adornatus*.

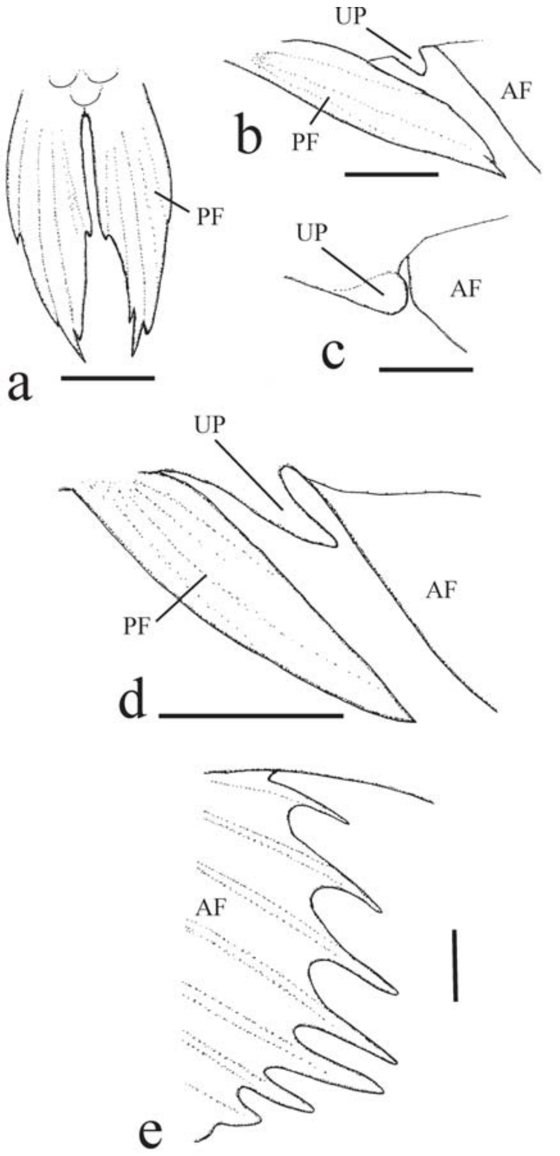

Fig. 4. Urogenital region, pelvic and anal fins in *Nematolebias* and *Simpsonichthys*. (a) pelvic-fin insertion, ventral view, of *N. papilliferus*, male, UFRJ 5361; (b) pelvic fin and urogenital papilla, lateral view, of *N. papilliferus*, male, UFRJ 5361; (c) pelvic fin and urogenital papilla, lateral view, of *N. papilliferus*, female, UFRJ 5361; (d) pelvic fin and urogenital papilla, lateral view, of *S. semiocellatus*, male, UFRJ 3933; (e) posterior margin of the anal-fin, lateral view, of *S. myersi*, female, UFRJ 4760. AF = anal fin; PF = pelvic fin; UP = urogenital papilla. Scale bar 1 mm.

Almost all species have the caudal fin rounded to subtruncate. An exception is the subgenus *Xenurolebias*, in which the caudal fin is asymmetrically lanceolate, the ventral portion being slightly expanded. The pectoral fin is elliptical and long. Its maximum length is 24-33% SL in adult males. The pelvic fin is usually short. Its length varies between 9 and 13% SL in adult males. In *S. zonatus* and *S. santanae*, the pelvic fin is very small, reaching only 3-5% SL (Costa, 1996, 2003). In *S. boitonei*, *S. parallelus*, and *S. cholopteryx* the pelvic fin is absent (Costa, 2003b). In larger males of *S. marginatus* (above 35 mm SL) the pelvic fin is long, its length reaching 16-25% SL. The pelvic-fin bases are medially in contact or their bases are slightly united.

Body squamation. The scales are large and cycloid, with numerous radii. The body and the head are entirely scaled, except on the anterior portion of the ventral surface of head. The body squamation slightly extends over caudal-fin base. In *Nematolebias* the scales do not extend over base of dorsal and anal fins, but in *Simpsonichthys*, a few scales extend on the median portion of the anal-fin base in males. The number of scales along the main longitudinal series is 28-31 in *Nematolebias* and 22-28 in *Simpsonichthys*. The number of scales in the longest transverse series is 8-11 in *Nematolebias* and *Simpsonichthys*, except in the *S. antenori* group, with 12-14. There are 12-16 scales around the caudal peduncle.

Frontal squamation. A circular frontal squamation pattern, unique among cyprinodontiforms, was considered a synapomorphy for rivulids (Parenti, 1981). This pattern consists of a central A-scale, with all borders overlapped by adjacent scales. However, although present in basal rivulid lineages (Costa, 2004a), a circular pattern does never occur in any cynolebiatin (Costa, 1990, 1998a). The A-scale always has a free posterior margin in all species of *Nematolebias* and *Simpsonichthys* (Figs. 1, 5).

In *Nematolebias* and in most species of *Simpsonichthys*, the frontal squamation is composed of five to 12 large scales between supraorbital series of neuromasts, of which three to eight scales are placed anterior to E-scales. Usually, there are two or one overlapped small scales, between supraorbital series of neuromasts and eye, but sometimes these supraorbital scales are absent. The paired E-scales are not overlapped in *Nematolebias* (Fig. 1b), except in a few specimens with E-scales slightly overlapped. In all species of *Simpsonichthys*, the E-scales are always overlapped (Fig. 5).

In *Nematolebias* and in the great majority of species of *Simpsonichthys*, the frontal squamation is E-patterned (*i.e.*, the E-scale has all margins free). Exceptions occur in the *S. notatus* group, in which all species are A-patterned, and in *S. semiocellatus*, that is F-patterned (Fig. 5a, b).

Laterosensory system (Fig. 1)**.** In all rivulids the laterosensory system is represented by rows of exposed neuromasts, and among cynolebiatins, cephalic neuromast are numerous (Parenti, 1981; Costa, 1990, 1998a, b). On the dorsal surface of the head, just posterior to the median portion of the snout, there are two neuromasts, the anterior and the posterior rostral neuromast (Costa, 2001). The anterior supraorbital series of neuromasts is longitudinally arranged and is united to the posterior supraorbital series, forming a single continuous series (Costa, 1990, 1998). Sometimes, the anteriormost supraorbital neuromasts may be bigger than the posterior ones. In *Ophthalmolebias*, however, the anterior and the posterior

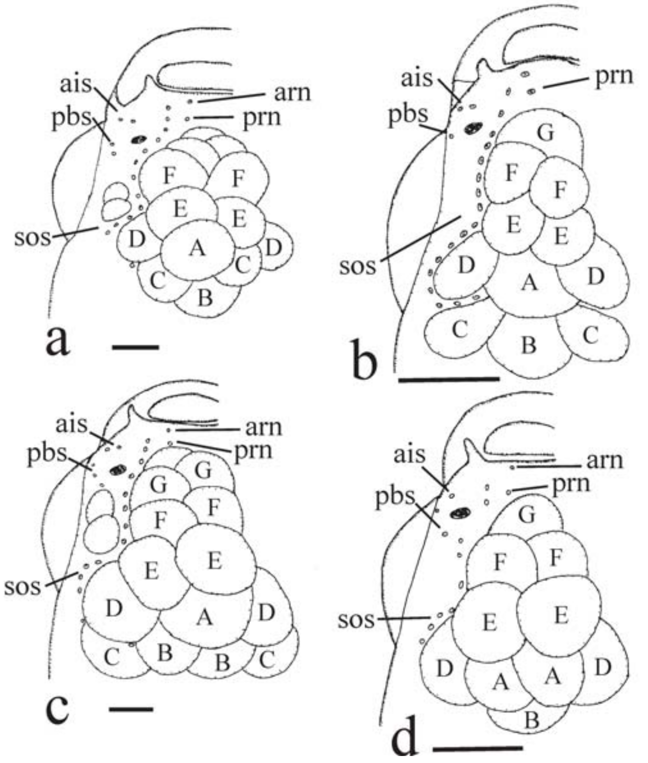

Fig. 5. Diagrammatic representation of the frontal squamation pattern and neuromasts in *Simpsonichthys*. (a) *S. radiosus*, male, UFRJ 6017; (b) *S. semiocellatus*, male, UFRJ 3933; (c) *S. perpendicularis*, male, UFRJ 5144; (d) *S. cholopteryx*, male, UFRJ 5428. ais = anterior infraorbital series; arn = anterior rostral neuromast; pbs = preorbital series; prn = posterior rostral neuromast; sos = supraorbital series; A-H = frontal scales A-H. Scale bar 1 mm.

supraorbital series are separated by an interspace (Fig. 5c). In *S. parallelus* and *S. cholopteryx*, the two anteriormost supraorbital neuromasts are separated from the remaining posterior neuromasts (Fig. 5d). The total number of supraorbital neuromasts usually varies from 10 to 16, rarely 9 or 17-18. Posterior to the supraorbital series, there is a single parietal neuromast, or double as in *S. costai*.

The infraorbital series comprises three segments. A short anterior segment, usually with two and sometimes with a single neuromast, lays anterolateral to the posterior nostril. In *Nematolebias* there is an additional neuromast just anterior to the anterior infraorbital series (Fig. 1), a condition not found in other cynolebiatins, but present in basal rivulids. The median segment runs near the anterior and ventral margin of the orbit, comprising 12-22 neuromasts. The short posterior segment includes two to four neuromasts, dorsally connected to a short longitudinal otic series comprising one or two neuromasts. Often the median and posterior segments of the infraorbital series are continuous. Adjacent to the anterodorsal margin of the orbit, there is a pre-orbital series with two or three neuromasts. In *S. parallelus* and in *S. cholopteryx*, the pre-orbital series is connected to the median portion of the supraorbital series (Fig. 5d).

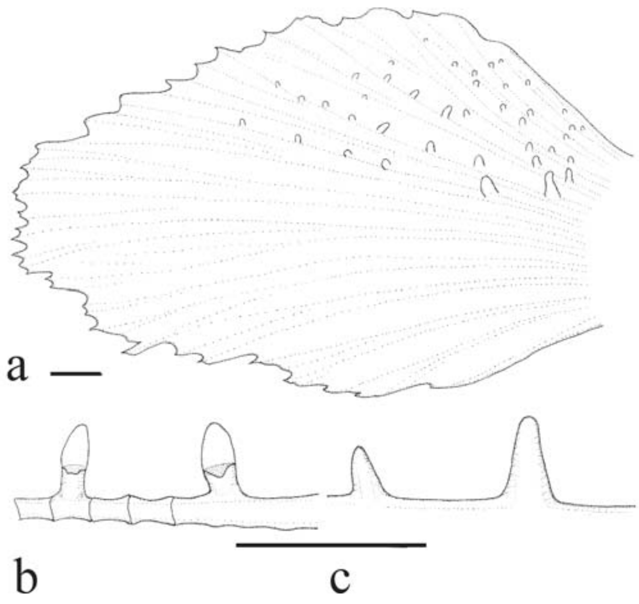

Fig. 6. Pectoral-fin contact organs of *Nematolebias whitei*. (a) general lateral view of the medial surface of the pectoral fin, UFRJ 5286; (b) osseous support, UFRJ 5283, and (c) external morphology of two contact organs of the subproximal portion of the 6th ray, ventrolateral view, UFRJ 5286. Scale bar 1 mm.

The opercle is dorsally bordered by a longitudinal post-otic series composed of two or three neuromasts. Dorso-anterior to the post-otic series there is a supra-temporal neuromast. One median opercular neuromast lies on the central portion of the opercle, and two ventral opercular neuromasts below it. The preopercular series comprises 9-15 aligned neuromasts, besides some slightly displaced from the main line. In *Nematolebias*, the preopercular series is continuous to the mandibular series (Fig. 1). The mandibular series includes 6-14 neuromasts. A lateral mandibular series comprises 3-5 aligned neuromasts. A single paramandibular neuromast is placed below the mouth cleft.

Contact organs. Dermal contact organs are common in atherinomorph fishes (Wiley & Collete, 1970). Possibly they are important to the maintenance of body contact between sexes during reproductive behavior. In rivulids, they are present on the scales and fin rays in males of some taxa, during all the adult stage.

Contact organs of flank scales of rivulids are minute dermal protuberances on the median portion of the posterior border of the scale. The flank scales bearing a contact organ have a superficial resemblance to a ctenoid scale, but this kind of scales is not present in atherinomorph fishes (Roberts, 1993). Scale contact organs are present in most species of *Simpsonichthys*, but are absent in *S. constanciae*, *S. semiocellatus*, *S. chacoensis*, *S. reticulatus*, *S. costai*, and in species of *Nematolebias*.

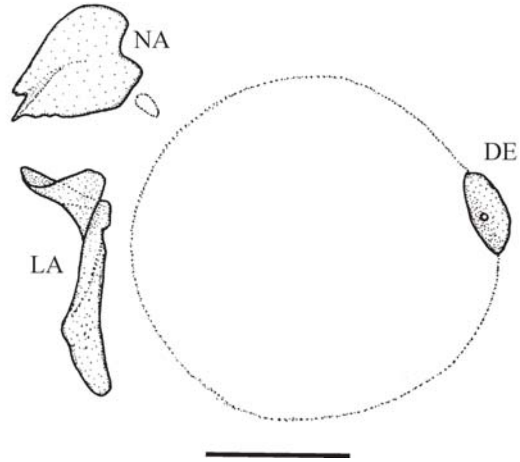

Fig. 7. Superficial layer of skin and dermal bones of the head of *Nematolebias papilliferus* (placed in a single plain), left side, external view, male, UFRJ 4652. DE = dermosphenotic; LA = lachrymal; NA = nasal. Scale bar 1 mm.

Contact organs of pectoral-fin rays are arranged in row along the inner surface of fin rays. They are small, papillate and restricted to the dorsalmost rays in *Simpsonichthys*, with the exception of *S. constanciae*, *S. cholopteryx*, *S. costai*, *S. semiocellatus*, and *S. filamentosus*, in which pectoral contact organs are absent. In *S. flagellatus*, the pectoral-fin contact organs are prominent and very conspicuous, in contrast to other congeners, in which the contact organs are hardly visible even under a dissecting microscope. In *Nematolebias*, the pectoral-fin contact organs are hypertrophied and distributed over most pectoral-fin rays (de Carvalho, 1957; Costa, 2002a). They are tubular, the base supported by a bone extension derived from the outgrowth of the lepidotrichium, and possess a mesenchyme-filled axial space continuous to the space in the center of the fin ray (Wiley & Collete, 1970; Costa, 2002a) (Fig. 6).

Superficial dermal bones. There are three ossified structures representing the superficial dermal bones in cyprinodontiform fishes. Among them, the nasal, present in all rivulids, is a scale-like, thin ossification on the anterodorsal portion of the head, with a short, longitudinal, ventral keel (Fig. 7).

The two other superficial dermal bones, the lachrymal and the dermosphenotic, correspond respectively to the first and sixty bones of the circumorbital series, whereas the second through fifth circumorbital bones are absent in all cyprinodontiforms. As in other rivulids, the lachrymal in *Nematolebias* and *Simpsonichthys* is a thin and twisted bone, adopting an almost cylindrical shape with the dorsal portion slightly widened and anteriorly directed (Parenti, 1981; Costa, 1998a, b) (Fig. 7). It is placed just anteriorly to the anterodorsal margin of the orbit. The dermosphenotic is very thin, ovoid, its outer surface gently concave to reach a shallow, open trough shape (Fig. 7). It is positioned just posteriorly to the posterodorsal margin of the orbit. It is always present in *Nematolebias*, but among species of *Simpsonichthys*, it is present only in the subgenera *Xenurolebias* and *Ophthalmolebias* (Costa, 1998a). A minute dermosphenotic is also found in *S. filamentosus*. The remaining species of *Simpsonichthys* do not present any sign of ossification on the dermosphenotic region.

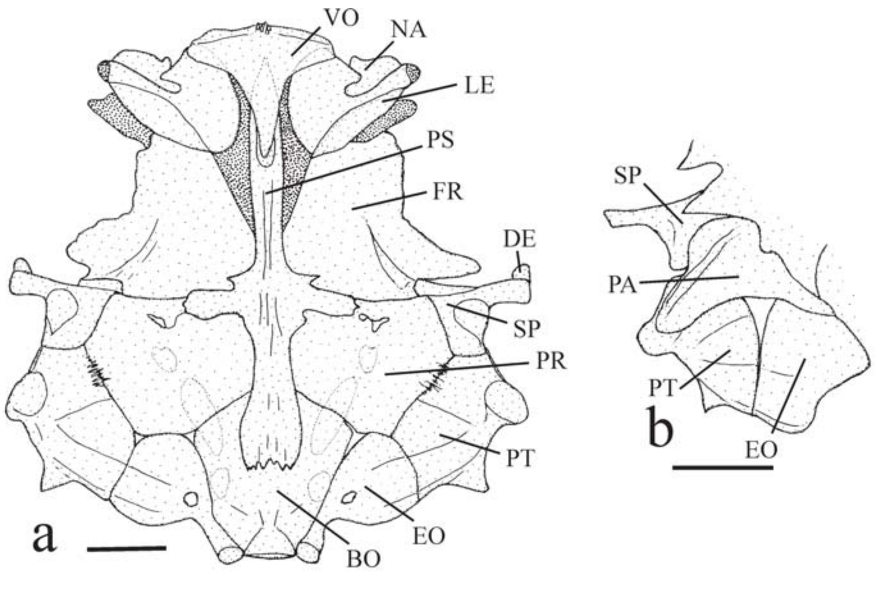

Fig. 8. Neurocranium of *Nematolebias papilliferus*, male, UFRJ 4652. (a) general ventral view; (b) left posterodorsal view. BO = basioccipital; DE = dermosphenotic; EO = exoccipital; FR = frontal; LE = lateral ethmoid; NA = nasal; PA = parietal; PR = prootic; PS = parasphenoid; PT = pterotic; SP = sphenotic; VO = vomer. Larger stippling indicates cartilage. Scale bar 1 mm.

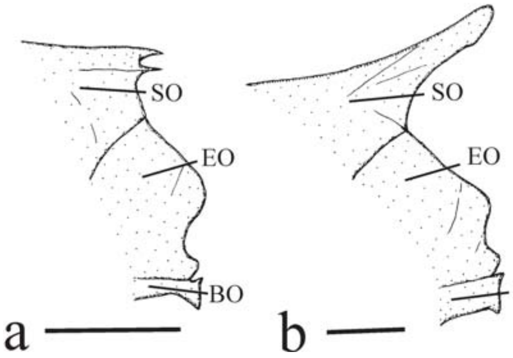

Fig. 9. Posterior portion of neurocranium, left lateral view. (a) *Nematolebias papilliferus*, male, UFRJ 4652; (b) *Simpsonichthys flammeus*, male, UFRJ 5117. BO = basioccipital; EO = exoccipital; SO = supraoccipital. Scale bar 1 mm.

Neurocranium. Bones of the neurocranium have little morphological variation in *Nematolebias* and *Simpsonichthys*. The neurocranium is depressed as in all aplocheiloid fishes (Costa, 1998b). Its dorsal surface is composed by poorly ossified structures with unclear limits, a condition present in all rivulids (Costa, 1998a). The frontal is approximately rectangular and occupies the anterior two thirds of the neurocranium roof. It is extremely thin, with reduced lateral borders. The parietal is subtriangular, possessing a distinctive transverse keel (Fig. 8b). The posterodorsal region of the neurocranium is formed medially by the supraoccipital, which have a paired posterior process, and a small epiotic, placed posterolaterally to the supraoccipital. The supraoccipital process is usually short (Fig. 9a), but it is distinctively long in species of the *S, flammeus* group, its narrow tip lying in close proximity to the neural spine of the first vertebra (Costa, 2003b) (Fig. 9b). The posterolateral edge of the neurocranium is formed by the sphenotic, with a prominent lateral process, the pterotic and the exoccipital.

The limits of bones along the ventral surface of the neurocranium are conspicuous (Fig. 8a). On the anterior portion, there is a triangular vomer, with an elongated posterior process. In *Nematolebias* there are two to five, small, conical teeth on the anteromedian portion of the vomer. Vomerine teeth are absent in all species of *Simpsonichthys*. The mesethmoid is unossified. The lateral ethmoid is compact, bearing a distinctive anterior retrorse process. The anteromedian portion of the lateral ethmoid slightly overlaps dorsally the lateral portion of vomer, and the medial margin sometimes abuts the lateral surface of the anterior process of the parasphenoid. The parasphenoid comprises a cross-shaped structure, in which the anterior process overlaps dorsally the posterior process of the vomer, the posterior process is firmly attached ventrally to the basioccipital, and each of their short lateral processes is ventrally attached to the prootic.

Jaws, jaw suspensorium and opercular apparatus. The jaws are connected to jaw suspensorium through the articulation between angulo-articular and quadrate, and through a ligamentous connection joining upper jaw and palatine (Fig. 10). The upper jaw is formed by the maxilla, the premaxilla and the rostral cartilage, which are joined by connective tissue. The premaxilla is an elongate bone with teeth on the medial half of its anterior face. It bears a posteriorly directed, rectangular flattened ascending process on its medial portion, and an anteriorly directed, trapezoidal process on its subdistal portion. The maxilla, which is placed dorsolaterally to the premaxilla, is elongate, bifid in its anterior medial portion, from

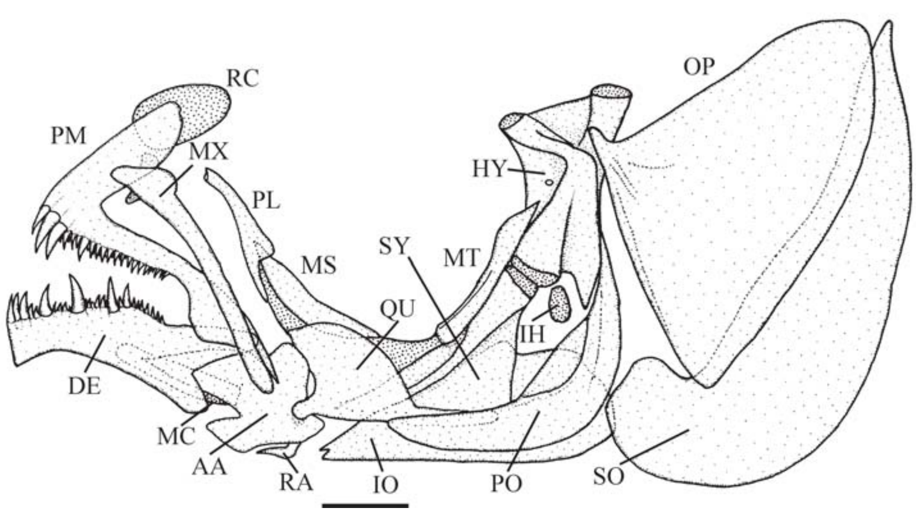

Fig. 10. Jaws, jaw suspensorium and opercular apparatus, left side, lateral view, of *Nematolebias papilliferus*, male, UFRJ 4652. AA = angulo-articular; DE = dentary; HY = hyomandibula; IH = interhyal; IO = interopercle; MC = Meckel's cartilage; MS = mesopterygoid; MT = metapterygoid; MX = maxilla; OP = opercle; PL = palatine; PM = premaxilla; PO = preopercle; QU = quadrate; RA = retro-articular; RC = rostral cartilage; SO = subopercle; SY = sympletic. Larger stippling indicates cartilage. Scale bar 1 mm.

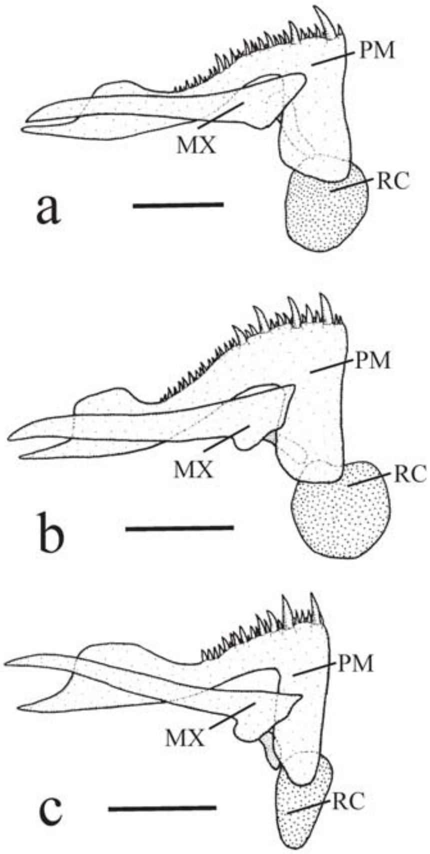

Fig. 11. Left upper jaw, dorsal view. (a) *Nematolebias whitei*, male, UFRJ 5283; (b) *Simpsonichthys myersi*, male, UFRJ 4759; (c) *S. bokermanni*, male, UFRJ 5863. Larger stippling indicates cartilage. MX = maxilla; PM = premaxilla; RC = rostral cartilage. Scale bar 1 mm.

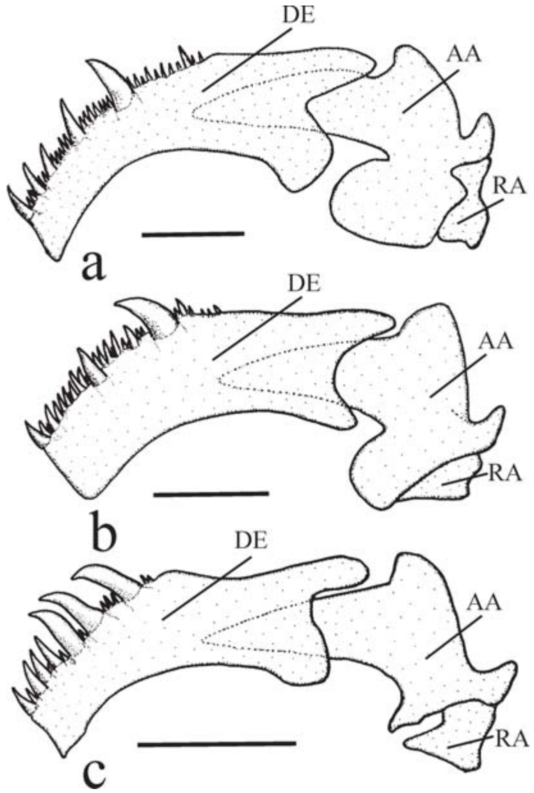

Fig. 12. Left lower jaw (Meckel's cartilage not represented), ventrolateral view. (a) *Nematolebias whitei*, male, UFRJ 5283; (b) *Simpsonichthys chacoensis*, male, UFRJ 3170; (c) *S. constanciae*, male, UFRJ 5285. AA = angulo-articular; DE = dentary; RA = retro-articular. Scale bar 1 mm.

where a subtriangular process projects dorsally to the premaxillary ascending process, whereas another process ventrally overlaps that premaxillary process. The rostral cartilage is elongate to about round in older males of *Nematolebias* and *Xenurolebias*, but it is approximately pentagonal and longitudinally elongated in the remaining species of *Simpsonichthys* (Fig. 11). Its anterior portion lies ventral to the posterior portion of the premaxillary ascending process.

The lower jaw is formed by the dentary, angulo-articular, retro-articular, and the coronomeckelian cartilage. The dentary is the longer bone, occupying the most portion of the lower jaw, with teeth on the distal two thirds of its anterodorsal surface. Both in *Nematolebias* and *Simpsonichthys* the posteroventral part of dentary terminates in a prominent process, a feature also present in *Cynolebias*, *Megalebias* and *Austrolebias* (Costa, 1990, 1998a). The angulo-articular is a triangular bone, with a ventral process, which is short in *Nematolebias* and *Simpsonichthys*, but wide in *Nematolebias* and moderately narrowed in *Simpsonichthys* (Fig. 12). In *S. constanciae*, the ventral angulo-articular process is narrower than in other congeners (Fig. 12c). Another smaller process is present on the posterior extremity of the angulo-articular, which supports the articulation with the quadrate. The retro-articular is a small bone placed posteroventrally to the angulo-articular. The coronomeckelian cartilage is elongate, attached medially along dentary and angulo-articular.

Both premaxillary and dentary teeth are arranged in irregular rows. A few distinctive larger teeth are placed on the most external row. All teeth are conical, with slightly curved tip, then directed to the inner of the mouth. The large tooth on the posterior portion of the dentary is slightly anteriorly directed in *Nematolebias* and strongly anteriorly directed in *Simpsonichthys* (Costa, 1996, 1998a), except in the *S. notatus* species group, in which all teeth are directed to the mouth. In *S. constanciae*, there are some larger teeth of the external row of premaxilla and dentary with tips anteriorly directed (Fig. 12c).

The palatine and the ectopterygoid are completely fused, as in other aplocheiloids (Parenti, 1981; Costa, 1998b) (Fig. 10). In *Simpsonichthys*, except *S. semiocellatus*, the anterodorsal tip of the palatine bears a small, pointed process, supporting a thick ligament connected to the maxilla (Costa, 1998a) (Fig. 13a-d). This process is longer in *S. ocellatus* (Fig. 13c) and may sometimes acquire a laminar shape in *S. flagellatus*, and it is absent in *Nematolebias*. In *Nematolebias* and in several species of *Simpsonichthys*, the medial surface of the palatine is approximately plain, but in

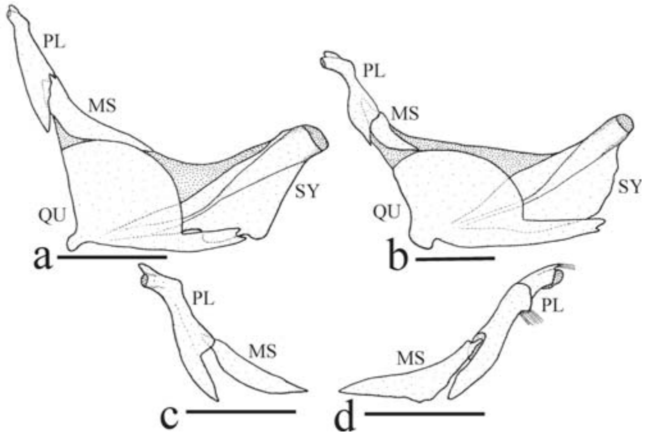

Fig. 13. Anterodorsal portion of left jaw suspensorium. (a) *Simpsonichthys filamentosus*, male, UFRJ 3990, lateral view; (b) *S. boitonei*, male, UFRJ 4988, lateral view; (c) *S. ocellatus*, male, UFRJ 5098, lateral view; (d) *S. perpendicularis*, male, UFRJ 5145, medial view. MS = mesopterygoid; PL = palatine; QU = quadrate; SY = sympletic. Larger stippling indicates cartilage. Scale bar 1 mm.

Ophthalmolebias and species of the *S. antenori* and *S. flammeus* groups, there is a medial semicircular crest, supporting a thick ligament connected to the lateral ethmoid. In *Ophthalmolebias*, the crest is more prominent, forming a ventral expansion (Fig. 13d).

The mesopterygoid is thin and posteriorly reduced, the posterior tip separated from metapterygoid by an interspace filled with cartilage. The ventral portion of the mesopterygoid slightly overlaps the dorsal portion of the quadrate in *Nematolebias* (Fig. 10). In *Simpsonichthys*, as well as in *Cynolebias*, *Austrolebias* and *Megalebias*, the ventral portion of the mesopterygoid only gently abuts the dorsal portion of the quadrate (Fig. 23a). In the subgenus *Simpsonichthys*, the mesopterygoid is shorter, its posterior end lying dorsal to the anterodorsal border of the quadrate (Fig. 13b).

The quadrate is approximately triangular, with the anterior and the ventral margins approximately straight, and the posterior margin rounded (Fig. 10). It bears a posterior short process. The angle formed between the anterior and the ventral margins is about 100º to 125º, but is variable within species, sometimes angle reaching about 80° or 90° in older males of deep bodied species (*e.g.*, *S. igneus*). The posterior process is shorter than in most other rivulids, usually reaching between 34 and 47 % of the total longitudinal length of the quadrate. The medial surface of the posterior portion of the quadrate is firmly attached to the lateral surface of the anterior portion of the sympletic, which usually is a deep, triangular bone. In the subgenera *Simpsonichthys* and *Spectrolebias*, the sympletic is elongate (Fig. 13a-b).

The posterior portion of the sympletic and the ventral portion of the hyomandibula are dorsally bordered by the metapterygoid. In *Nematolebias* and in the subgenera *Xenurolebias*, *Ophthalmolebias* (except *S. constanciae*), and *Simpsonichthys*, the metapterygoid is narrow in its ventral portion, becoming abruptly wider in its dorsal portion (Fig. 14a). In *Hypsolebias*, the metapterygoid is approximately rectangular, with the dorsal portion only slightly wider than the ventral portion (Fig. 14b). In *S. constanciae* and in the subgenus *Spectrolebias*, the entire bone is narrow, assuming a rod shape (Fig. 14c).

The hyomandibula is a somewhat compact bone (Fig. 14a-b), but elongate in the subgenus *Spectrolebias* (Fig. 14c). It comprises two robust dorsal condyles, the anterior articulating with the sphenotic and the posterior with the pterotic, a ventral condyle attached to the sympletic, and a posterodorsal process supporting the articulation of the opercle. Running laterally from the anterodorsal condyle to the posteroventral tip of the hyomandibula, there is a prominent curved crest. Its posteroventral tip is separated from the ventral condyle of the hyomandibula by a broad laminar extension (Fig. 14a). The laminar extension is reduced In *S. filamentosus*, *S. semiocellatus*, and species of *Hypsolebias* (Fig. 14c), except in species of the *S. antenori* group (not including *S. antenori*), in which the laminar extension is vestigial (Fig. 14b).

All the bones of the opercular apparatus are thin. The dorsal and posterior borders of the opercle and all the borders of the interopercle and subopercle are membranous and poorly ossified. The preopercle is firmly attached to the hyomandibula and to the posterior process of the quadrate. The preopercle of all the cynolebiatins is thin, gently curved, with a reduced anteromedian flap (Fig. 15a) (Costa, 1990, 1998a). Its dorsal end is narrow and pointed, and the ventral portion wide. The anteromedian flap is narrower in species of the *S. flammeus* group (Fig. 15b), and vestigial or absent in species of the *S. antenori* group (Fig. 15c). The anteroventral portion of the preopercle is attenuated in species of the *S. flammeus* and *S. antenori* groups (Fig. 15b-c).

Hyoid and branchial arches. The basihyal is triangular and flattened, with an anterior broad cartilaginous portion, and a narrow posterior portion. The cartilage occupies 18-33% of the total longitudinal length of the basihyal in *Nematolebias* and in most species of *Simpsonichthys* (Fig. 16a), but reaches 38-45% in *S. flammeus*, *S. brunoi* and *S. multiradiatus* (Costa, 1996). The width of the basihyal may be variable among species, the greatest width between 30-80% of the basihyal length. However, in larger males of *Nematolebias* and in *S. ghisolfii*, this width reaches 90-100%.

Both the dorsal and the ventral hypohyals are small, but always ossified (Figs. 16a, c). The anterior ceratohyal is wide and cylindrical in its anterior portion, narrowed in the median portion, with two narrow branchiostegal rays attached to the ventral border, and compressed in the posterior portion (Fig. 16c). The anterior and the posterior ceratohyals are separated by a broad space of cartilage, supporting four branchiostegal rays, each gradually become wider posteriorly (Fig. 16c). The posterior ceratohyal is compressed, subtriangular (Fig. 16c). An oval and cartilaginous interhyal

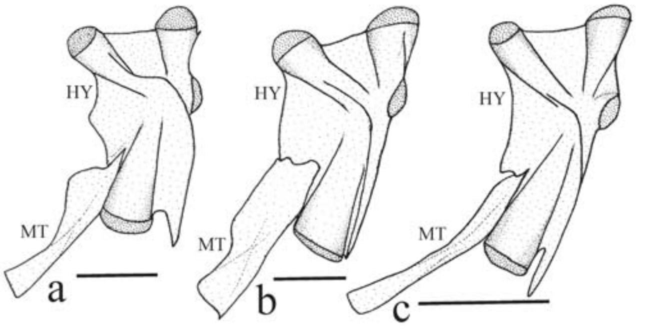

Fig. 14. Posterodorsal portion of left jaw suspensorium, lateral view. (a) *Nematolebias whitei*, male, UFRJ 5283; (b) *Simpsonichthys igneus*, male, UFRJ 4869; (c) *S. filamentosus*, male, UFRJ 3990. HY = hyomandibula; MT = metapterygoid. Larger stippling indicates cartilage. Scale bar 1 mm.

is attached to the posterior end of the posterior ceratohyal (Fig. 16c). The urohyal is thin, with short ventrolateral flap and an anterodorsal process (Fig. 16a).

The dorsal part of the branchial arches comprises a wide third pharyngobranchial with a well-developed dentigerous plate, anteriorly overlapped by the second pharyngobranchial and posteriorly by the small fourth pharyngobranchial tooth plate, three long epibranchials 1-3, a robust fourth epibranchial and a long interarcual cartilage (Fig. 16b). The second pharyngobranchial is approximately triangular, usually wider than long, but in *Hypsolebias*, it is clearly longer than wide (Fig. 17b). Often there are one to four teeth near the posterior edge of the second pharyngobranchial, but in the *S. antenori* species group there are five to ten teeth. In *Nematolebias*, *Xenurolebias* and in *S. parallelus* and *S. cholopteryx*, second pharyngobranchial teeth are always absent. In all species of *Nematolebias* and *Simpsonichthys*, except *S. costai*, the second pharyngobranchial possesses a small subdistal process (Fig. 16b). The third epibranchial bears a short uncinate process (Fig. 16b). The interarcual cartilage is laterally attached to the anterior tip of the second pharyngobranchial (Fig. 16b), but in *S. trilineatus*, *S. auratus*, *S. magnificus*, *S. picturatus*, *S. carlettoi*, and *S. adornatus*, the cartilage is attached to the medial margin of that bone (Fig. 17b).

The ventral part of the branchial arches comprises three ossified rectangular basibranchials, three rounded hypobranchials, four elongate ceratobranchials without teeth, and a fifth ceratobranchial with a well-developed dentigerous plate (Fig. 16a). The proximal edge of the first hypobranchial is rounded, without vestige of branching (Fig. 16a). The whole lateral border of the first hypobranchial is bordered by cartilage (Fig. 16a). The anterior portion of the second hypobranchial bears a small process anteroventrally directed. In *S. magnificus*, *S. picturatus*, *S. carlettoi*, and *S. fulminantis* there is an additional process directed to the second basibranchial (Fig. 17c). The proximal tip of the fourth ceratobranchial is clearly wider than the proximal tip of the first, second and third ceratobranchials (Fig. 16a), but in *Spectrolebias*, it is narrower (Fig. 17d). The fifth ceratobranchial is boomerang-shaped, its anterior portion on a longitudinal axis and the posterior portion slightly laterally displaced, on an axis forming angle of 125-135° with the longitudinal axis (Fig. 16a). The anterior portion is shorter than the posterior portion. In *S. filamentosus* and *S. semiocellatus*, the fifth ceratobranchial is slender and their anterior and posterior portions form an angle of 145° (Fig. 17d). In *S. filamentosus*, *S. semiocellatus*, and species of the *S. flammeus* and *S. antenori* groups, the anterior portion is longer that the posterior one (Fig. 17d).

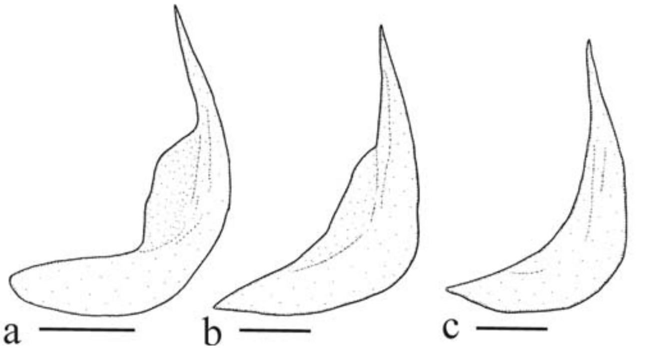

Fig. 15. Left preopercle, lateral view. (a) *Nematolebias whitei*, male, UFRJ 5283; (b) *Simpsonichthys flammeus*, male, UFRJ 5117; (c) *S. antenori*, male, UFRJ 4880. Scale bar 1 mm.

Vertebrae and caudal skeleton. The first vertebra bears a neural spine, which has anterior and posterior laminar extensions (Fig. 18a). It lacks neural prezygapophyses, but there is a prominent neural postzygapophysis directed to the ventral surface of the neural prezygapophysis of the second vertebra. As in other cyprinodontiforms, the pleural rib is present in all precaudal vertebrae, except the first. All precaudal vertebrae possess a rod-like epipleural rib. In *Nematolebias*, the neural spines of the second and the third vertebrae are wider than the first neural spine (Fig. 18a), but in all species of *Simpsonichthys*, as well as in *Cynolebias*, *Austrolebias* and *Megalebias*, the second neural spine is slightly narrower than the first, and the third is about so narrow as all posterior neural spines (Fig. 18a). Also only in *Nematolebias*, the neural prezygapophysis of the second vertebra is directly attached to the first neural spine (Fig. 18a). In *Simpsonichthys* and most other rivulids, there is a ligament between the first neural spine and the neural prezygapophysis of the second vertebra. In caudal vertebrae, the neural prezygapophyses are vestigial or absent and the neural postzygapophyses are short (Fig. 18b).

The caudal skeleton comprises an epural and a parhypural with similar shape, in which the proximal portion is narrowed and slightly curved, with the tip anteriorly directed (Fig. 18b). All the hypurals are ankylosed, without any vestige of gaps (Fig. 18b). Accessory cartilages are absent. There are 20-31 caudal-fin rays.

Dorsal and anal fins. Males have more dorsal and anal-fin rays than females. The number of dorsal-fin rays and the

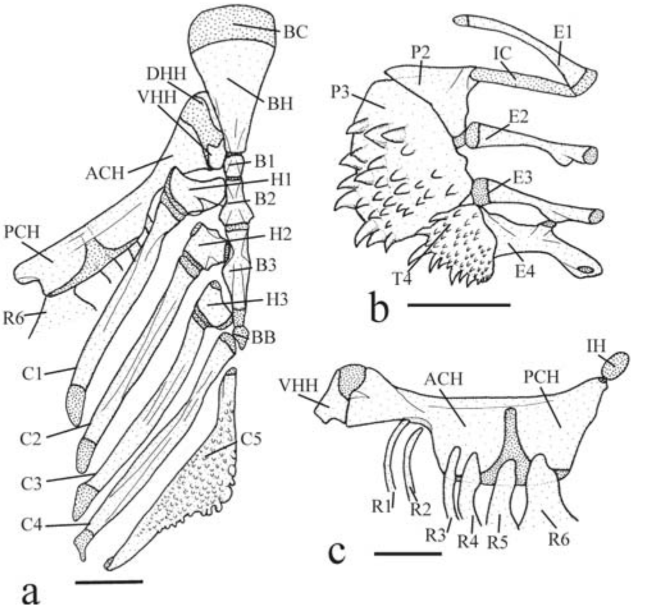

Fig. 16. Hyoid and branchial arches of *Nematolebias papilliferus*, male, UFRJ 4652. (a) left and median portion of hyoid arch and ventral part of branchial arches, dorsal view; (b) dorsal part of branchial arches, ventral view; (c) left hyoid bar, lateral view. ACH = anterior ceratohyal; B1-3 = basibranchials 1-3; BB = basibranchial cartilage; BC = basihyal cartilage; BH = basihyal; C1-5 = ceratobranchials 1-5; DHH = dorsal hypohyal; E1-4 = epibranchials 1-4; H1-3 = hypobranchials 1-3; IC = interarcual cartilage; IH = interhyal; P2-3 = pharyngobranchials 2-3; PCH = posterior ceratohyal; R1-6 = branchiostegal rays 1-6; T4 = tooth plate pharyngobranchial 4; VHH = ventral hypohyal. Larger stippling indicates cartilage. Scale bar 1 mm.

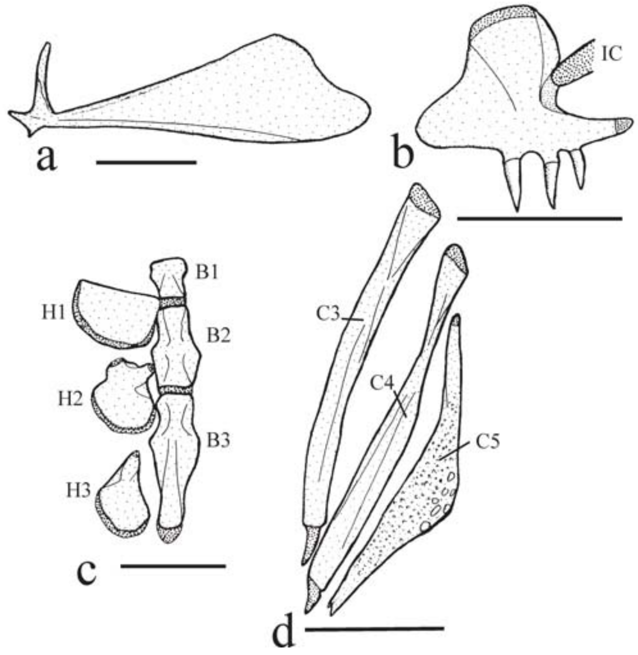

Fig. 17. Some osteological structures of the hyoid and branchial arches in *Nematolebias* and *Simpsonichthys*. (a) urohyal of *N. papilliferus*, left lateral view, male, UFRJ 4652; (b) left second pharyngobranchial, ventral view, of *S. magnificus*, male, UFRJ 4958; (c) left hypobranchials and basibranchials, dorsal view, of *S. magnificus*, male, UFRJ 4958; (d) left third, fourth and fifth ceratobranchials, dorsal view, of *S. filamentosus*, male, UFRJ 3990. B1-3 = basibranchials 1-3; C3-5 = ceratobranchials 3-5; H1-3 = hypobranchials 1-3; IC = interarcual cartilage. Larger stippling indicates cartilage. Scale bar 1 mm.

position of the dorsal-fin origin in males are extremely variable in species of *Simpsonichthys*. In *Nematolebias*, *Xenurolebias*, *Ophthalmolebias*, subgenus *Simpsonichthys* (except *S. boitonei* and *S. marginatus*), and *S. semiocellatus* and *S. filamentosus*, there are 15-21 rays and the dorsal-fin origin is between the neural spines of vertebrae 10 and 14; in *S. brunoi, S. delucai*, *S. alternatus*, *S. trilineatus*, and *S. auratus*, 18-22 rays, dorsal-fin origin between neural spines of vertebrae 7 and 9; in *S. chacoensis*, *S. costai*, *S. reticulatus*, *S. boitonei*, *S. marginatus*, *S. flammeus*, *S. antenori* species group, *S. magnificus* species group and *S. notatus* species group (except *S. auratus* and *S. trilineatus*), 20-25 rays, dorsal-fin origin between neural spines of vertebrae 6 and 9; in *S. multiradiatus*, 25-28 rays, dorsal-fin origin between neural spines of vertebrae 5 and 7; and, in *S. adornatus*, 28-32 rays, dorsal-fin origin between neural spines of vertebrae 3 and 4. In species of *Nematolebias* and *Simpsonichthys*, there are 17-25 anal-fin rays, and the anal-fin origin is placed between the pleural ribs of the vertebrae 5 and 9. In *Nematolebias*, except the two or three anteriormost rays that are unbranched, all other dorsal and anal-fin rays are branched. In *Xenurolebias*, most rays are also branched, but the longer rays forming the tip of the fin are unbranched, and in all other species of *Simpsonichthys* approximately all dorsal-fin rays are unbranched. In all species of *Simpsonichthys*, the rays on the tip of the anal fin are unbranched, except in *S. constanciae*, in which all anal-fin rays are unbranched.

Two rays are associated to the first proximal radial of the dorsal fin (Fig. 18c), and two or three associated to the first proximal radial of the anal fin (Fig. 18d). The first anal-fin proximal radials are widened (Fig. 18d). The dorsal-fin median and the distal radials are ossified (Fig. 18c). The anal-fin median radials are ossified, but the anal-fin distal radials are ossified only on the posterior portion of the fin; in the anterior portion of the fin, distal radials are absent or cartilaginous (Fig. 18d). In *S. magnificus*, *S. picturatus*, *S. carlettoi*, *S. fulminantis*, and *S. adornatus*, the median radials of the dorsal fin are longer than in other cynolebiatins (Fig. 19b). In all species of *Cynolebias*, *Megalebias*, and *Austrolebias*, the median radials of dorsal and anal fins are always cartilaginous.

Shoulder and pelvic girdles. The dorsal portion of the shoulder girdle is articulated with the neurocranium by an elon-

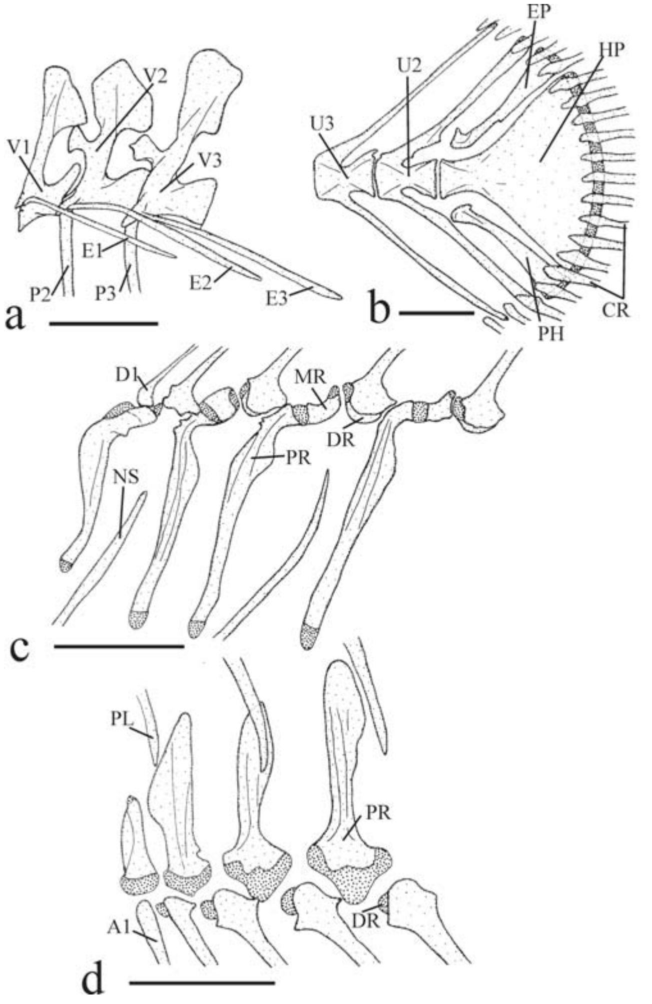

Fig. 18. Vertebrae and unpaired fin support of *Nematolebias papilliferus*, male, UFRJ 4652. (a) first three vertebrae, left lateral view; (b) last caudal vertebrae and caudal skeleton, left lateral view; (c) anterior portion of dorsal-fin support, left lateral view; (d) anterior portion of anal-fin support, left lateral view. A1 = first anal-fin ray; CR = caudal-fin rays; D1 = first dorsal-fin ray; DR = distal radial; E1-3 = epipleural ribs of vertebrae 1-3; EP = epural; HP = hypural plate; MR = middle radial; P2-3 = pleural ribs of vertebrae 2-3; NS = neural spine; PH = parhypural; PL = pleural rib; PR = proximal radial; U2-3 = preural centra 2-3; V1-3 = vertebrae 1-3. Larger stippling indicates cartilage. Scale bar 1 mm.

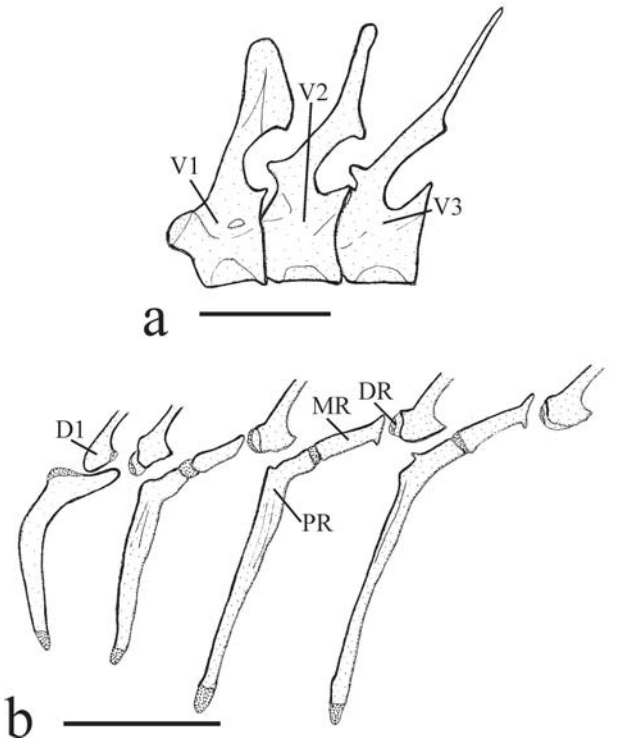

Fig. 19. Some osteological structures of the axial and unpaired fin skeleton in the genus *Simpsonichthys*. (a) first three vertebrae, left lateral view, of *S. myersi*, male, UFRJ 4759 (pleural and epipleural ribs not represented); (b) anterior portion of dorsal-fin support, left lateral view, of *S. magnificus*, male, UFRJ 4958. D1 = first dorsal-fin ray; DR = distal radial; MR = middle radial; PR = proximal radial; V1-3 = vertebrae 1-3. Larger stippling indicates cartilage. Scale bar 1 mm.

gated and forked posttemporal. The ventral process of the posttemporal is absent in *Nematolebias* and in *S. costai*, *S. reticulatus*, *S. parallelus*, and *S. cholopteryx* (Fig. 20a). An elongate supracleithrum is attached between the posttemporal and the cleithrum.

The dorsal portion of the cleithrum is about triangular, lacking a distinctive posterior flange present in basal rivulids (*i.e.*, *Kryptolebias* Costa) (Costa, 2004a). This dorsal portion of the cleithrum is short in *Nematolebias*, *Xenurolebias* and *S. semiocellatus*, its length shorter or about equal to the vertical length between the dorsal edge of scapula and the ventral edge of coracoid (Fig. 20a). In all other species of *Simpsonichthys*, the dorsal portion of the cleithrum is distinctively longer (Fig. 21). In *S. filamentosus*, *S. semiocellatus*, and *S. chacoensis*, there is an anteriorly expanded flap on the anterior portion of the cleithrum (Fig. 21). The ventral tip of the cleithrum and the ventral tip of the coracoid are placed in closed proximity. The coracoid is about triangular and is ventrally separated from the rounded scapula by a small interspace filled with cartilage. In *Nematolebias*, the ventral portion of the coracoid possesses a narrow condyle, which is broad in all species of *Simpsonichthys* (Fig. 21). The proximal radials of the pectoral fin are rounded and thin, the ventralmost radial lying in close proximity to the posteroventral margin of the coracoid (Fig. 20c). Medial to the main axis of the shoulder girdle, there is a rod-like and elongate third postcleithrum. The first and the second postcleithra are absent.

The pelvic girdle comprises a narrow pubic bone without a distinct ischial process (Fig. 20c). The pelvic girdle and the pelvic fin are vestigial in *S. zonatus* and *S. santanae*, and are absent in *S. boitonei*, *S. parallelus*, and *S. cholopteryx*. There are 11-16 pectoral-fin rays, and 5-7 pelvic-fin rays.

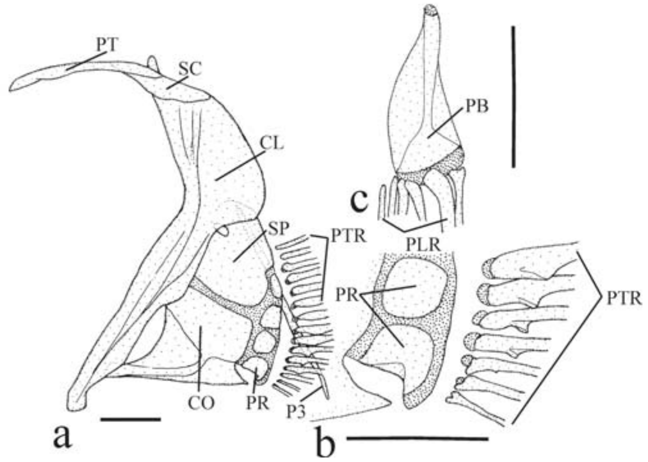

Fig. 20. Paired fin support of *Nematolebias papilliferus*, male, UFRJ 4652. (a) left shoulder girdle, left lateral view; (b) two ventralmost pectoral-fin proximal radials and adjacent area of coracoid, left lateral view; (c) left pelvic girdle, ventral view. CL = cleithrum; CO = coracoid; P3 = post-cleithrum 3; PB = pelvic bone; PLR = pelvic-fin rays; PR = proximal radials; PT = posttemporal; PTR = pectoral-fin rays; SC = supracleithrum; SP = scapula. Larger stippling indicates cartilage. Scale bar 1 mm.

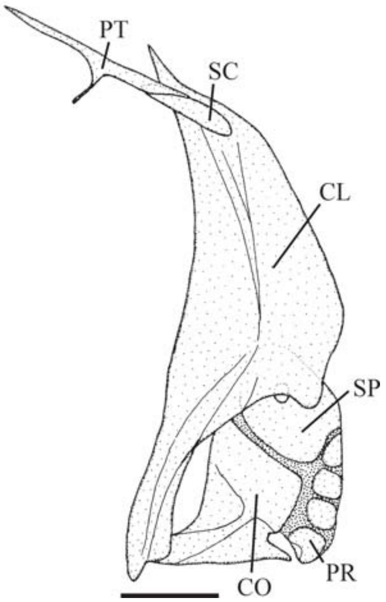

Fig. 21. Left shoulder girdle, left lateral view, of *Simpsonichthys filamentosus*, male, UFRJ 3990, (third post-cleithrum and pectoral-fin rays not represented). CL = cleithrum; CO = coracoid; PR = proximal radials; PT = posttemporal; SC = supracleithrum; SP = scapula. Larger stippling indicates cartilage. Scale bar 1 mm.

Phylogenetic analysis

This phylogenetic analysis is based on both a re-examination of all previously reported phylogenetically informative morphological characters and a series of morphological characters not identified in other studies. This study comprises a total of 116 characters (see Appendix II), that are included in the data matrix (Appendix IV).

Six most parsimonious trees (tree length = 292, CI = 54%, RI = 79%) were found. Conflicts are restricted to different topologies involving species of the *S. notatus* group (clade F'). Other polytomies correspond to unsolved nodes present in all most parsimonious tress. The strict consensus tree (tree length = 297, CI = 53%, RI = 79%) and node supports are presented in Fig. 22. Synapomorphies for each clade are presented in Appendix III. A second analysis was performed excluding quantitative characters, which are suspect to contain some degree of subjectivity. The same trees were found (consensus tree with length = 266, CI = 53%, RI = 79%), demonstrating that these characters had a lower impact on the analysis.

Taxonomic accounts

Nematolebias Costa

Nematolebias Costa, 1998a: 75 (type species *Cynolebias whitei* Myers, by original designation).

Diagnosis. Differs from all other members of the Cynolebiatini by the following unambiguous synapomorphies: widened ventral process of angulo-articular (*vs.* narrow), neural spine of the first vertebra directly attached to the neural prezygapophysis of the second vertebra (*vs.* connected by thin ligaments), hypertrophied tubular contact organs on the medial surface of the dorsal half of the pectoral fin in males, its basal portion ossified, forming a cup-shaped osseous structure (*vs.* papillate contact organs, when contact organs are present), and a subdistal orange stripe with transverse golden lines contrasting with a black zone dorsal to the orange stripe on the anal fin in males (*vs.* never a similar color pattern). Some synapomorphies of *Nematolebias*, homoplastically occurring in other cynolebiatins, are: widened basihyal in older males (also occurring in the *S. antenori* species group) (*vs.* narrow basihyal), absence of second pharyngobranchial teeth (also occurring in *Simpsonichthys*, subgenera *Xenurolebias* and *Simpsonichthys*) (*vs.* presence), preopercular and mandibular series of neuromasts continuous (also occurring in *Cynolebias*) (*vs.* preopercular and mandibular series of neuromasts separated), absence contact organs on the flank (also occurring in *Ophthalmolebias*, *Spectrolebias* and in the *S. antenori* species group) (*vs.* contact organs present), iridescent line on subdistal zone of caudal fin in males (also occurring in the subgenus *Simpsonichthys*) (*vs.* subdistal line absent), and pectoral-fin reddish brown with iridescent spots in males (reddish brown also in the *S. notatus* group, and iridescent spots also in the subgenus *Simpsonichthys*) (*vs.* never a similar color pattern).

Included species. *Nematolebias papilliferus* Costa and *N. whitei* (Myers).

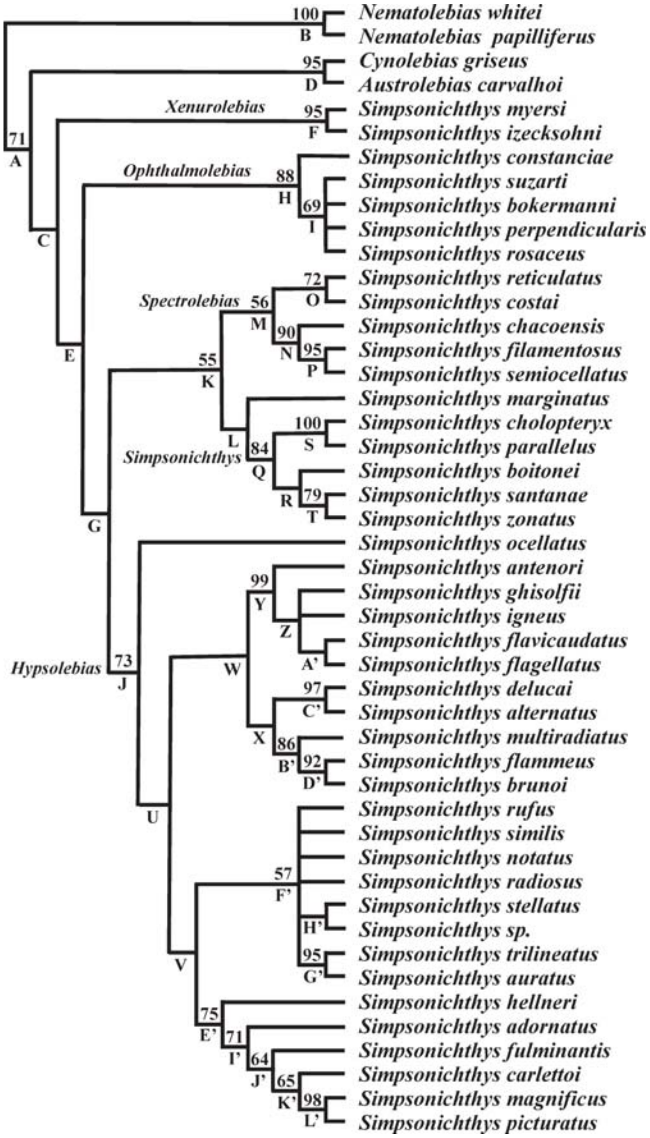

Fig. 22. Strict consensus of six equally parsimonious shortest phylogenies (L = 297; CI = 0.53 ; RI = 0.79). Letters left of branches represent different clades of the strict consensus tree, and numbers left of branches are bootstrap values. List of apomorphies by node is presented in Appendix III.

Distribution. Coastal plains of Estado do Rio de Janeiro, southeastern Brazil.

Simpsonichthys de Carvalho

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

Diagnosis. Differs from other cynolebiatins by having the following unambiguous synapomorphies: small pointed process on the dorsal portion of the palatine (*vs.* process absent), rays on the tip of the dorsal and anal fins unbranched in males (*vs.* branched), and E-scales overlapped (*vs.* not overlapped).

Included taxa. Five subgenera and 43 species, listed below.

Distribution. Southern tributaries of the rio Amazonas basin, including rios Madeira, Xingu, Araguaia and Tocantins drainages; western rio Paraguay basin; rio Paranaíba drainage, upper rio Paraná basin; middle rio São Francisco basin; rio Jaguaribe basin and adjacent smaller coastal drainages of northeastern Brazil; and, isolated rivers basins of eastern Brazil, including rios Pardo, Jequitinhonha, Mucuri, Itaúnas, São Mateus and smaller coastal basins.

Xenurolebias new subgenus

Type species. *Simpsonichthys myersi* (de Carvalho).

Diagnosis. Distinguished from all other rivulids by the following unambiguous synapomorphies: presence of filamentous rays on the posterior margin of the anal fin in females (*vs.* absence), caudal fin lanceolate and asymmetric due to an expansion of the ventral portion of the fin in males (*vs.* caudal fin rounded or subtruncate, dorsal and ventral portion symmetric), and a unique color pattern consisting of a bar on the posterior portion of the caudal peduncle, posteriorly extending over the dorsal and ventral margins of the caudal fin in males (*vs.* never a similar color pattern).

Included species. *Simpsonichthys izecksohni* (da Cruz) and *S. myersi* (de Carvalho).

Distribution. Rivers basins of eastern Brazilian coastal plains between rio Jucuruçu, southern Estado da Bahia, and rio Doce, Estado do Espírito Santo.

Etymology. From the Greek *xenos* (strange), *oura* (tail) and *lebias* (small fish, and a name commonly used to compose generic names of cyprinodontiform fishes), referring to the unique morphology of the caudal fin. Gender masculine.

Ophthalmolebias new subgenus

Type species. *Simpsonichthys constanciae* (Myers).

Diagnosis. Distinguished from the remaining cynolebiatins by the following unambiguous synapomorphies: a medial crest with a prominent ventral expansion on the palatine (*vs.* without ventral expansion when crest is present), eyes positioned laterally on head (*vs.* dorsolaterally), anterior and posterior sections of the supraorbital series of neuromasts separated by an interspace (*vs.* continuous), metallic blue bars on the flank in females (*vs.* metallic bars absent), and anal fin pink in females (*vs.* hyaline).

Included species. *Simpsonichthys constanciae* (Myers), *S. bokermanni* (de Carvalho & da Cruz), *S. perpendicularis* Costa, Nielsen & De Luca, *S. rosaceus* Costa, Nielsen & De Luca, and *S. suzarti* Costa.

Distribution. Brazilian eastern river basins, between rio Cachoeira, Estado da Bahia, and rio São João, Estado do Rio de Janeiro.

Etymology. From the Greek *ophthamus* (eye) and *lebias* (small fish, also a name used to compose generic names of rivulids), referring to the large eyes laterally placed on head. Gender masculine.

Subgenus *Simpsonichthys* de Carvalho, new usage

Diagnosis. Distinguished from all other cynolebiatins by having flank red with bright blue bars on the anterior portion and vertical rows of blue dots on the posterior portion in males (a condition modified in *S. cholopteryx*, in which there are blue bars on the entire flank).

Included species. *Simpsonichthys marginatus* Costa & Brasil, *S. zonatus* (Costa & Brasil), *S. santanae* (Shibata & Garavello), *S. boitonei* de Carvalho, *S. parallelus* Costa, and *S. cholopteryx* Costa, Moreira & Lima.

Distribution. Headwaters of rio Paraná, rio São Francisco and rio Araguaia basins, central Brazil.

Subgenus *Spectrolebias* Costa & Nielsen

Spectrolebias Costa & Nielsen, 1997: 258 (type species *Spectrolebias semiocellatus* Costa & Nielsen, by original designation; first proposed as a genus).

Diagnosis. Distinguished from all other cynolebiatins by two unambiguous synapomorphies: a long hyomandibula (*vs.* short) and a narrowed proximal tip of the fourth ceratobranchial (*vs.* wide). It may be also diagnosed by the absence of organs of contact on the flank in males (*vs.* presence), but secondarily present in *S. filamentosus*. Another synapomorphy of *Spectrolebias* is a narrowed metapterygoid (*vs.* wide), a condition that also occurs in *S. constanciae*.

Included species. *Simpsonichthys chacoensis* (Amato), *S. semiocellatus* (Costa & Nielsen), *S. filamentosus* Costa, Barrera & Sarmiento, *S. costai* (Lazara), and *S. reticulatus* Costa & Nielsen.

Distribution. Southern Amazonian river drainages, including rio Madeira, Xingu, Araguaia and Tocantins, and Paraguayan Chaco, río Paraguay basin.

Hypsolebias new subgenus

Type species. *Cynolebias flavicaudatus* Costa & Brasil.

Diagnosis. Distinguished from the other taxa of *Simpsonichthys* in having the second pharyngobranchial longer than wide (*vs.* wider than long). Another synapomorphy of *Hypsolebias* is the presence of a shortened lateroventral process of the hyomandibula, but that also occurs in some species of *Spectrolebias* (*vs.* wide process).

Included species. *Simpsonichthys ocellatus* Costa, Nielsen & De Luca, *S. hellneri* (Berkenkamp), *S. adornatus* Costa, *S. fulminantis* (Costa & Brasil), *S. carlettoi* Costa & Nielsen, *S. magnificus* (Costa & Brasil), *S. picturatus* Costa, *S. stellatus* (Costa & Brasil), *S. nielseni* Costa, *S. rufus* Costa, Nielsen & De Luca, *S. notatus* (Costa, Lacerda & Brasil), *S. radiosus* Costa & Brasil, *S. similis* Costa & Hellner, *S. trilineatus* (Costa & Brasil), *S. auratus* Costa & Nielsen, *S. antenori* (Tulipano), *S. igneus* Costa, *S. ghisolfii* Costa, Cyrino & Nielsen, *S. flavicaudatus* (Costa & Brasil), *S. flagellatus* Costa, *S. delucai* Costa, *S. alternatus* (Costa & Brasil), *S. multiradiatus* (Costa & Brasil), *S. flammeus* (Costa), and *S. brunoi* Costa.

Etymology. From the Greek *hypsi* (high), and *lebias* (small fish; also a nominal cyprinodontid genus commonly used to compose generic names of the family Rivulidae), an allusion to the deep body of the members to the new subgenus. Gender masculine.

Distribution. Central and northeastern Brazil, including the upper and middle rio Tocantins basin, middle rio São Francisco basin, lower rio Jaguaribe basin and adjacent coastal basins, and middle rio Jequitinhonha.

Discussion

Different topologies for hypotheses of relationships among the genera *Nematolebias* and *Simpsonichthys*, and other cynolebiatins have been proposed in recent studies (Costa, 1996, 1998a, 2002a, 2003; Murphy *et al.*, 1999; Hrbek & Larson, 1999). These hypotheses differ in the phylogenetic position of *Nematolebias* (*i.e.*, closely related to *Simpsonichthys* in Costa, 1996, 1998, 2003, or the sister group of the all other genera of the Cynolebiatini in Murphy *et al.*, 1999; Hrbek & Larson, 1999) and the position of *Xenurolebias* (*i.e.*, closely related to *Nematolebias* in Costa, 1998a, 2003; Hrbek & Larson, 1999, or closely related to *Simpsonichthys* in Costa, 2002a).

Monophyly of the Cynolebiatini. Monophyly of the Cynolebiatini, which comprises the genera *Nematolebias*, *Simpsonichthys*, *Cynolebias*, *Megalebias*, and *Austrolebias*, has been supported both in morphological and molecular phylogenetic studies (Costa, 1990, 1995a, 1998a, 2002b, 2003, 2004a; Murphy *et al.*, 1999; Hrbek & Larson, 1999). Monophyly of this assemblage is here corroborated by unambiguous synapomorphies: a broad posteroventral process of dentary; a shortened posterior process of quadrate; a long interarcual cartilage; hypurals completely ankylosed, forming a single plate without median fissure; and, males with more dorsal and anal-fin rays than females.

Phylogenetic position of *Nematolebias*. It is hypothesized that *Nematolebias* is the sister group of the clade including *Simpsonichthys*, *Cynolebias*, *Austrolebias*, and *Megalebias*. This hypothesis differs from previous studies based on morphological characters (Costa, 1996, 1998a, 2003), but is congruent with studies based on molecular characters (Murphy *et al.*, 1999; Hrbek & Larson, 1999), although the latter studies included only a few cynolebiatin species as terminal taxa.

The divergence between the present study and previous morphological studies is due to the introduction of five characters discovered during the course of this study, supporting the monophyly of the group comprising *Simpsonichthys*, *Cynolebias*, *Austrolebias*, and *Megalebias*: ventral portion of mesopterygoid not overlapping quadrate, third neural spine about as wide as fourth neural spine, a narrow ventral condyle of coracoid, loss of a neuromast anterior to the infraorbital series, and scales extending over anal-fin base. Other synapomorphies supporting the monophyly of the group including *Simpsonichthys*, *Cynolebias*, *Austrolebias*, and *Megalebias* were already in part reported in previous studies: loss of vomerine teeth (reversed in a group of *Cynolebias*, Costa, 2001), a short and narrow ventral process of the angulo-articular, and an elongated dorsal portion of cleithrum.

Derived conditions uniquely shared by *Nematolebias* and *Simpsonichthys* are: posterior outer dentary tooth anteriorly directed; anal-fin origin anteriorly placed, between pleural ribs of vertebrae 5 and 9; and, pelvic-fin bases medially in contact or united. These conditions are parsimoniously interpreted as having originated at the base of the Cynolebiatini, with a reversal in the clade comprising *Cynolebias*, *Megalebias*, and *Austrolebias*.

Phylogenetic position of *Xenurolebias*. *Xenurolebias* is herein erected to include two species, *S. myersi* and *S. izecksohni*, as a subgenus of *Simpsonichthys*. The two species share some synapomorphies, such as the presence of filamentous rays on the posterior border of the anal fin in females and an asymmetrical and lanceolate caudal fin in males.

Simpsonichthys myersi was considered to be closely related to *Nematolebias* both in morphological (Costa, 1996, 1998) and molecular (Hrbek & Larson, 1999) analyses. However, *Xenurolebias* is here hypothesized to be closer related to other species assemblages of *Simpsonichthys* than to *Nematolebias*. The hypothesis of *Xenurolebias* as a clade within *Simpsonichthys* is supported by all species of *Xenurolebias* possessing the four synapomorphies defining the genus *Simpsonichthys*: a distinctive, small pointed dorsal process on the palatine; unbranched fin rays on the tip of dorsal fin in males; unbranched fin rays on the tip of anal fin in males; and, frontal E-scales overlapped. The hypothesis of *Xenurolebias* as a member of the clade that includes *Simpsonichthys*, *Cynolebias*, *Megalebias*, and *Austrolebias*, but precludes *Nematolebias*, is corroborated by seven synapomorphies: ventral portion of mesopterygoid not overlapping quadrate; third neural spine about as wide as fourth neural spine; a narrow ventral condyle of coracoid; scales slightly extending over anal-fin base; loss of vomerine teeth (reversed in a group of *Cynolebias*, Costa, 2001); loss of neuromast anterior to infraorbital series; and, a short and narrow ventral process of the angulo-articular. The hypothesis of *Xenurolebias* as a basal clade within the genus *Simpsonichthys* is supported by all species of *Simpsonichthys*, except those placed in *Xenurolebias*, sharing two synapomorphies: most dorsal-fin rays unbranched in males, and loss of black spots over the posterior portion of the caudal peduncle in females (reversed in the *S. antenori* species group).

There are two derived conditions shared by *Nematolebias* and *Xenurolebias*: a widened rostral cartilage, and the loss of second pharyngobranchial teeth. However, all species of *Cynolebias* also have a widened rostral cartilage (Costa, 2001), and consequently the condition is parsimoniously interpreted as having been originated at the base of the Cynolebiatini, with reversals in *Simpsonichthys* and *Austrolebias*. The loss of second pharyngobranchial teeth is considered to be homoplastic.

Monophyly and phylogenetic position of *Ophthalmolebias*. The assemblage comprising *S. constanciae*, *S. bokermanni*, *S. perpendicularis*, and *S. rosaceus* was hypothesized to be a monophyletic group by Costa (2003). Subsequently, another species of this clade, *S. suzarti*, was described and the clade was named *S. constanciae* species group (Costa, 2004b). This clade is here corroborated and is considered as a subgenus of *Simpsonichthys*, named *Ophthalmolebias*. Monophyly of *Ophthalmolebias* is supported by five synapomorphies: a medial crest on the palatine, with a prominent ventral expansion; eye laterally placed on the head; anterior and posterior sections of the supraorbital series of neuromasts separated by interspace; metallic blue bars on the flank in females; and, anal fin pink in females.

Species of *Ophthalmolebias* were considered to be closely related to *Xenurolebias* and *Nematolebias* (Costa, 2003b), by all having pelvic-fin bases medially in contact or united, and by the putative common possession of pink anal fin in females. However, the condition of pelvic-fin bases medially in contact or united occurs in most species of *Simpsonichthys*, not constituting evidence of close relationships between *Ophthalmolebias*, *Xenurolebias*, and *Nematolebias*, and the character state anal fin pink in females is not present in *Xenurolebias* and in *Nematolebias*, except in a few specimens of *N. whitei*, in which the anal fin is pinkish hyaline.

Monophyly and phylogenetic position of *Spectrolebias*. Costa & Nielsen (1997) described *Spectrolebias* to include a new miniature species from central Brazil, *S. semiocellatus*, hypothesized to be the sister group of the clade comprising *Nematolebias*, *Simpsonichthys*, *Cynolebias*, *Megalebias*, and *Austrolebias* (Costa, 1998a). Costa (2003) found evidence supporting a close relationship between *S. semiocellatus* and

Simpsonichthys filamentosus, then transferring the latter species to *Spectrolebias*, which was considered to be the sister group to a clade including *Nematolebias* and *Simpsonichthys*.

Both species previously placed in *Spectrolebias*, *S. semiocellatus* and *S. filamentosus*, are considered closely related to *S. chacoensis*, *S. costai*, and *S. reticulatus*. *Spectrolebias* is redefined as a subgenus of *Simpsonichthys* to include *S. semiocellatus*, *S. filamentosus*, *S. chacoensis*, *S. costai*, and *S. reticulatus* by four synapomorphies: a narrowed metapterygoid (also occurring in *S. constanciae*); a long hyomandibula; a narrowed proximal tip of the fourth ceratobranchial; and, loss of organs of contact on the flank in males (reversed in *S. filamentosus*). *Spectrolebias* is parsimoniously considered to be the sister group of the subgenus *Simpsonichthys* by both sharing the following synapomorphies: an elongate sympletic, and four features of the color pattern of the dorsal and anal fins in males, including the presence of distal dark stripe and subdistal bright blue subdistal stripe in each fin, all reversed in some species of both *Simpsonichthys* and *Spectrolebias*. However, the black and blue distal spot on the posterior portion of the anal fin in *S. semiocellatus* (Costa & Nielsen, 1997) and also on the anterior portion of the dorsal fin of *S. reticulatus* (Costa & Nielsen, 2003) may be vestiges of those color patterns.

The hypothesized sister group of *Spectrolebias* is the monophyletic group including *S. boitonei*, the type species of the genus *Simpsonichthys*, and *S. marginatus*, *S. zonatus*, *S. santanae*, *S. parallelus*, and *S. cholopteryx*. It is herein established as the subgenus *Simpsonichthys*, weakly diagnosed by a flank red with bright blue bars on the anterior portion and vertical rows of blue dots on the posterior portion in males, a condition modified in *S. cholopteryx*, in which there blue bars on the entire flank. However, a clade within the subgenus *Simpsonichthys* including all species except *S. marginatus* is supported by three unambiguous synapomorphies: a shortened mesopterygoid, its posterior tip in a vertical through the anterior portion of the quadrate; pelvic fin and girdle vestigial or absent; and, side of head with alternating red and bright blue bars in males.

Monophyly and phylogenetic position of *Hypsolebias*. It is hypothesized that the sister group of the clade comprising the subgenera *Spectrolebias* and *Simpsonichthys* is a diversified monophyletic group, herein formally referred as *Hypsolebias*, a new subgenus. *Hypsolebias* shares two synapomorphies with *Simpsonichthys* and *Spectrolebias*: loss of dermosphenotic (also occurring in *Cynolebias*, *Austrolebias*, and *Megalebias*) and dorsal fin anteriorly positioned in males, between neural spines of vertebrae 3 and 9 (reversed in some species of *Spectrolebias* and *Simpsonichthys*). Monophyly of *Hypsolebias* is supported by two synapomorphies: a shortened lateroventral process of hyomandibula (also occurring in some species of *Spectrolebias*); and, second pharyngobranchial longer than wide. *Hypsolebias* is the most speciose subgenus of *Simpsonichthys*, including a total of 25 species.

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Appendix I

Below is presented the list of all material of the genera *Nematolebias* and *Simpsonichthys* and other terminal taxa examined in the present study. Species are listed in blocks according to genera, subgenera and species groups. Data on material is organized in the following sequence: catalog number, number of specimens, locality, collector and date of collection. Abbreviations are: c&s, specimens cleared and stained for bone and cartilage, H, holotype, N, neotype, and P, paratype(s). Institutional acronyms are: CAS (SU), California Academy of Sciences, San Francisco (previously deposited in Stanford University); CBF, Colección Boliviana de Fauna, Museo Nacional de Historia Natural, La Paz; CMK, private collection Maurice Kottelat, Cornol; MCP, Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre; MCZ, Museum of Comparative Zoology, Cambridge; MNHN, Muséum National d'Histoire Naturelle, Paris; MNRJ, Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro; MZUSP, Museu de Zoologia, Universidade de São Paulo, São Paulo; UMMZ, University of Michigan, Museum of Zoology, Ann Arbor; UFRJ, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro; USNM, National Museum of Natural History (former United States National Museum), Smithsonian Institution, Washington; and, ZVC-P, Facultad de Humanidades y Ciencias, Departamento Zoología de Vertebrados, Montevideo.

Nematolebias. *N. papilliferus* Costa: Brazil: Rio de Janeiro: MZUSP 52964, H; UFRJ 5361, 12 P; MZUSP 52965, P; UFRJ 2200, 2 P; MZUSP 52966, 4 P; UFRJ 4652, 2 P (c&s); UFRJ 4711, 3 P; UMMZ 234763, 15 P; UFRJ 4710, 17 P; MZUSP 38387, 1; MZUSP 38386, 19; UFRJ 5301, 32; UFRJ 4756, 1; UFRJ 4465, 10; UFRJ 5305, 19; UFRJ 5299, 43; UFRJ 5304, 6; Inoã. - UFRJ 5364, 71 P; UFRJ 5365, 10 P (c&s); Sampaio Correia. - UFRJ 5360, 12 P; UFRJ 5295, 16 P; UFRJ 5296, 4 P (c&s); Maricá. *N. whitei*: Brazil: Rio de Janeiro: CAS (SU) 36516, H; drying swamp 10 or 12 miles from near Cabo Frio. - MNRJ 11366, 28; Cabo Frio. - MNRJ 11398, 12; MNRJ 11399, 2; MNRJ 11361, 7; MZUSP 38338, 16; MZUSP 38436, 1 (c&s); UFRJ 5292, 59; UFRJ 5293, 8 (c&s); São Pedro da Aldeia. - MZUSP 38340, 41; MZUSP 38341, 63; UFRJ 2127, 1 (c&s); MZUSP 38413, 1 (c&s); UFRJ 2071, 15; UMMZ 230862, 4; MCZ 138930, 4; UFRJ 2198, 6; UFRJ 5286, 3; UFRJ 5294, 27; Barra de São João. - UFRJ 2197, 8; UFRJ 3159, 2 (c&s); Rio das Ostras. - UFRJ 4754, 20; UFRJ 5307, 4 (c&s); Bom Sucesso. - UFRJ 5363, 68; UFRJ 5362, 10 (c&s); Búzios. - MNRJ 25828, 1; km 11 of the road São Pedro da Aldeia-Barra de São João. - MNRJ 19468, 7; road Amaral Peixoto, km 150-152.

Genus *Simpsonichthys*. Subgenus *Xenurolebias*: *S. izecksohni*: Brazil: Espírito Santo: MNRJ 10613, H, male; MNRJ 10614 1 P; MNRJ 10615, 2 P; MZUSP 14721, 1 P; MZUSP 14722, 1 P; MZUSP 14723, 1 P; MZUSP 14724, 1 P; UFRJ 5236, 6; UFRJ 5812, 5 (c&s); Reserva Florestal da Companhia Vale do Rio Doce, Linhares. - MZUSP 38336, 3; UFRJ 5056, 25; UFRJ 5057, 2; UFRJ 5255, 4; UFRJ 5813, 2 (c&s); UFRJ 4761, 4; UFRJ 5290, 2; Conceição da Barra. *S. myersi*: Brazil: Espírito Santo: MNRJ 9849, H, male; MNRJ 9850, 1 P; MNRJ 9851, 53 P; road between Conceição da Barra and Itaúnas, 18 km from Conceição da Barra. - UFRJ 4760, 35; UFRJ 4759, 6 (c&s); UFRJ 5237, 10; 2 km N of Itaúnas. Bahia: UFRJ 249, 19; UFRJ 282, 1 (c&s); UFRJ 3161, 3 (c&s); UMMZ 231549, 4; MCZ 138936, 4; 1 km N of Mucuri. - UFRJ 377, 2; Mucuri. - UFRJ 4762, 15; UFRJ 5814, 4 (c&s); 5 km W of Mucuri. - UFRJ 250, 12; 10 km N of Caravelas. - UFRJ 1921, 1; UFRJ 5815, 4 (c&s); - UFRJ 5288, 4; UFRJ 6063, 25; 1 km N of Prado. Subgenus *Ophthalmolebias*: *S. bokermanni*: Brazil: Bahia: Município de Ilhéus: MNRJ 11167, H; MNRJ 11168, 16 P; MZUSP 28476, 1 P; MZUSP 28477, 1 P; MZUSP 28478, 1 P; MZUSP 28479, 1 P; MNRJ 19503, 22; MNRJ 11721, 9; MZUSP 38432, 2 (c&s); UFRJ 1836, 13; UFRJ 2124, 4 (c&s); UFRJ 3162, 1 (c&s); UFRJ 5863, 1 (c&s); CEPLAC. - MNRJ 19462, 6; Itabuna. *S. constanciae*: Brazil: Rio de Janeiro: CAS (SU) 36514, H; 10 miles N of Cabo Frio. - MZUSP 36295, 8; MZUSP 38345, 13; MZUSP, 38425, 1 (c&s); MZUSP 36300, 8; MZUSP 38337, 5; UFRJ 1851, 6 (c&s); UFRJ 1978, 3 (c&s); UFRJ 2072, 8; UFRJ 2199, 25; UFRJ 5809, 2; UMMZ 230858, 8; MCZ 138931, 8; CMK 11945, 4; UFRJ 4757, 6; Barra de São João. - UFRJ 2196, 3; near Rio das Ostras. - UFRJ 5284, 15; UFRJ 5285, 4 (c&s); between Barra de São João and Rio das Ostras. - MNRJ 15596, 2; road Amaral Peixoto, km 150-152. - MNRJ 19542, 5; road Amaral Peixoto, km 150. *S. perpendicularis*: Brazil: Bahia, rio Jequitinhonha basin: MZUSP 62570, H; MZUSP 62571, 5 P; UFRJ 5144, 10 P; UFRJ 5145, 5 P (c&s); ribeirão do Salto floodplains, road between Itarantim and Jordânia, about 6 km from Jordânia. *S. rosaceus*: Brazil: Bahia: MZUSP 62572, H; MZUSP 62573, 5 P; UFRJ 5146, 20 P; UFRJ 5147, 4 P (c&s); UFRJ 5289, 46; rio Pardo floodplains, road between Potiguará and Itapetinga. *S. suzarti*: Brazil: Bahia: MCP 34088, H; UFRJ 5810, 1 P; UFRJ 5811, 2 P (c&s); near Canavieiras, rio Pardo floodplains. Subgenus *Simpsonichthys*: *S. boitonei*: Brazil: Distrito Federal, rio São Bartolomeu drainage, rio Paraná basin: MNRJ 9012, H; MNRJ 9013/18, 6 P; MNRJ 11376, 3; area of the Zoological Garden, Brasília. - MZUSP 38418, 1 (c&s); Reserva do IBGE. - UFRJ 3158, 1 (c&s); aquarium material. - UFRJ 4071, 2; UFRJ 4988, 1 ex. (c&s); córrego Roncador, Reserva Ecológica IBGE. *S. cholopteryx*: Brazil: Mato Grosso: Município de Alto Araguaia, rio Araguaia drainage, rio Amazonas basin: MZUSP 78061, H; UFRJ 5428, 29 P; ribeirão do Sapo on road MT 100, 31 km S of Alto Araguaia. - MZUSP 73338, 16 P (6 c&s); Ribeirão do Sapo, km 464 of Ferronorte railway. - MZUSP 73323, 41 P; córrego do Sapinho, km 474 of Ferronorte railway. *S. marginatus*: Brazil: Goiás, rio Tocantins drainage, rio Amazonas basin: MNRJ 12440, H, male; MNRJ 12441, 1 P; MZUSP 50163, 2 P; UFRJ 3536, 7 P; UFRJ 3537, 4 P (c&s); UFRJ 3922, 1; MNRJ 18418, 12; Barro Alto, pool close to rio dos Patos, rio Maranhão drainage. *S. parallelus*: Brazil: Goiás, rio Paranaíba drainage, rio Paraná basin: MZUSP 57537, H; MZUSP 57538, 4 P; UFRJ 4838, 7 P; UFRJ 4839, 4 P (c&s); MCP 22681, 4 P; UFRJ 5196, 16 P; UFRJ 5197, 4 P; UFRJ 5386, 11; floodplains of rio Formoso, Parque Nacional da Emas. - MNRJ 18285, 5; rio Formoso, Chapadão do Céu. *S. santanae*: Brazil: Distrito Federal, rio Paraná basin: MZUSP 43777, H; MZUSP 43778, 41 P; ribeirão Santana. *S. zonatus*: Brazil: Minas Gerais, rio Urucuia drainage, rio São Francisco basin: MZUSP 40131, H; MZUSP 40132, 1 P; MNRJ 11558, 2 P; UFRJ 142, 6; UFRJ 279, 1 (c&s); UFRJ 2123, 3 (c&s); Garapuava. Subgenus *Spectrolebias*: *S. chacoensis*: Paraguay: UFRJ 3030, 2; UFRJ 3170, 1 (c&s); 92 km N of Mariscal Estigarriba, San Juan. - USNM 232295, 1; flooded Chaco, 15 km NE of Ochoa. *S. costai*: Brazil: Mato Grosso, rio Araguaia drainage: UFRJ 163, 4; UFRJ 162, 2 (c&s); UFRJ 1157, 4; close to rio das Mortes, road to Cocalinhos. - UFRJ 3549, 31; UFRJ 3350, 4 (c&s); MNHN 1997-0045, 8; USNM 343822, 6; 9 km E from rio das Mortes, road to Cocalinho. Tocantins: UFRJ 5050, 5; Ilha do Bananal. - UFRJ 3560, 8; UFRJ 3561, 3 (c&s); Barreira do Piqui. - UFRJ 3794, 4; Formoso do Araguaia. Rio Tocantins drainage: Tocantins: UFRJ 3798, 10; Brejinho de Nazaré. *S. filamentosus*: Bolivia: Santa Cruz, rio Mamoré drainage: CBF 3002, H; CBF 3003, 7 P; UFRJ 3989, 4 P; UFRJ 3990, 4 P (c&s); MZUSP 51328, 2 P; close to río San Pablo, road Santa Cruz-Trinidad, about 60 km N from Ascención de Guarayos. *S. reticulatus*: Brazil: Pará: Município de Altamira, rio Xingu drainage: MCP 34090, H; UFRJ 5871, 2 P; UFRJ 5872, 2 P (c&s); near the city of Altamira. *S. semiocellatus*: Brazil: Tocantins, rio Araguaia drainage: MZUSP 50654, H; MZUSP 50655, 3 P; UFRJ 3632, 6 P; UFRJ 3633, 5 P (c&s); UFRJ 3933, 2; close to rio Formoso, Formoso do Araguaia. - UFRJ 5266, 14; ilha do Bananal. Subgenus *Hypsolebias*: *S. ocellatus*: Brazil: Minas Gerais, rio Jequitinhonha basin: MZUSP 61232, H; MZUSP 61233, 20 P; UFRJ 5096, 9 P; UFRJ 5097, 60 P; UFRJ 5098, 8 P (c&s); UFRJ 5102, 208 P; UMMZ uncatalogued, 24 P; 1 km W from Itaobim, road BR-367. - UFRJ 5099, 4 P; 2.5 km W from Taquaral. - UFRJ 5100, 38 P; 9.5 km E from São Pedro do Jequitinhonha. - UFRJ 5101, 52 P; 20.5 km W from Almenara. - UFRJ 5270, 5; UFRJ 5148, 1; 2 km from rio Jequitinhonha, 18 km W from Salto da Divisa. - UFRJ 5056, 27; near rio Jequitinhonha, 1 km from Itaobim. *Simpsonichthys magnificus* species group: *S. adornatus*: Brazil: Bahia, rio São Francisco basin: MZUSP 54563, H; UFRJ 4805, 18 P; UFRJ 4806, 1 P; 8 km N of Sítio do Mato. - MZUSP 54564, 10 P; MCP 22262, 20 P; UFRJ 5188, 20 P; UFRJ 5189, 10 P; UFRJ 4807, 8 P (c&s); 9.5 km N from Sítio do Mato. *S. carlettoi*: Brazil: Bahia, rio São Francisco basin: MCP 34089, H; UFRJ 5945, 2 P (c&s); near Guanambi, road to Malhada, rio das Rãs drainage. - UFRJ 5946, 1 P; aquarium fish. - UFRJ 6065, 5; Guanambi, road to Malhada. - UFRJ 6070, 11; UFRJ 6071, 3; 14 km from Guanambi, road to Malhada. *S. fulminantis*: Brazil: Bahia, rio São Francisco basin: MZUSP 43674, H; MZUSP 43675, 2 P; UFRJ 685, 2 P; UFRJ 686, 3 P; Guanambi, rio das Rãs drainage. - UFRJ 2194, 1; temporary pool about 30 km S of Bom Jesus da Lapa. - UFRJ 3809, 6; UFRJ 5864, 4 (c&s) near Guanambi. - UFRJ 4802, 1; UFRJ 4847, 2; 4.5 km S from Guanambi. - UFRJ 6068, 6; UFRJ 6069, 2; Guanambi, road to Caitité. *S. hellneri*: Brazil: Minas Gerais, rio São Francisco basin: UFRJ 2070, 1 ; 25 km N from Itacarambi. - UFRJ 2120, 1; 7 km S from São Francisco. - UFRJ 2093, 33; UFRJ 2080, 4 (c&s); UMMZ 230856, 10; MNHN 1997-0050, 4 ex.; USNM 343819, 3; MCZ 138942, 10; CMK 11943, 4; 1 km N from São Francisco. - UFRJ 4800, 41; UFRJ 4989, 8 (c&s); 10 km N from Itacarambi. - UFRJ 4801, 16; 10.5 km N from Itacarambi. - UFRJ 4766, 9; 11 km N from Itacarambi. - UFRJ 3802, 7; 4 km N from São Francisco. - UFRJ 3807, 6; road Itacarambi-Manga. *S. magnificus*: Brazil: Minas Gerais, rio São Francisco basin: MZUSP 41374, H; MZUSP 41375, 9 P; UFRJ 154, 12; UFRJ 260, 1 (c&s); UFRJ 2119, 1 (c&s); Manga. - UFRJ 4959, 4; UFRJ 4958, 3 (c&s); 1 km E from Gado Bravo, rio Verde Grande floodplains. Bahia: UFRJ 5827, 1; Malhada. - MNRJ 16116, 10; MNRJ 16324, 8; Mocambinho, Manga. *S. picturatus*: Brazil: Bahia, rio São Francisco basin: MZUSP 59228, H; UFRJ 5053, 46 P; UFRJ 5194, 15 P; UFRJ 5195, 8 P; MZUSP 59229, 8 P; UFRJ 5054, 7 P (c&s); UFRJ 4875, 49; near Volta das Pedras. - UFRJ 5418, 3; UFRJ 5419, 8; near Igarité. *Simpsonichthys notatus* species group: *S. auratus*: Brazil: Minas Gerais, rio Paracatu drainage, rio São Francisco basin: MZUSP 52912, H; MZUSP 52913, 3 P; UFRJ 4666, 3 P; UFRJ 4667, 10 P (c&s); UMMZ 234240, 2 P; UFRJ 4635, 5; UFRJ 4668, 10 P; UFRJ 4669, 8; Lagoa Grande, close to rio Taboca. *S. nielseni*: Brazil: Minas Gerais, rio São Francisco basin: UFRJ 6060, H; UFRJ 6061, 7 P; UFRJ 6062, 5 P (c&s); MCP 37306, 2 P; Pirapora. *S. notatus*: Brazil: Goiás, rio Tocantins drainage: MZUSP 39985, H; MZUSP 39986, 3 P; MNRJ 11559, 2 P; UFRJ 155, 10; UFRJ 268, 1 (c&s); Alvorada do Norte, rio Paranã drainage. - MNRJ 11557, 3; rio Paranã floodplains, Nova Roma. - UFRJ 2068, 23; UMMZ 230860, 4; MCZ 138945, 6; rio Paranã floodplains, road Iaciara-Nova Roma. - UFRJ 5415, 17; UFRJ 5416, 5 (c&s); 2 km N of Ribeirão Canabrava. *S. radiosus*: Brazil: Goiás, rio Tocantins drainage: UFRJ 6017, H; UFRJ 6018, 9 P; UFRJ 6019, 6 P (c&s); UFRJ 6020, 6 P; UFRJ 6021, 5 P; Formosa, rio Crixás floodplains. *S. rufus*: Brazil: Minas Gerais, rio São Francisco basin: MZUSP 61234, H; MZUSP 61235, 1 P; UFRJ 5110, 3 P; UFRJ 5111, 2 P (c&s); UFRJ 5114, 10; UFRJ 5112, 3 P; UFRJ 511, 4 P (c&s); riacho do Barro floodplains, Ibiaí. *S. similis*: Brazil: Minas Gerais, rio Urucuia drainage, rio São Francisco basin: MZUSP 51834, H; MZUSP 51835, 1 P; UFRJ 4146, 1 P; UFRJ 4147, 5 P (c&s); Urucuia. - UFRJ 4148, 1 P; aquarium specimen. *S. stellatus*: Brazil: Minas Gerais, rio São Francisco basin: MNRJ 12446, H; UFRJ 2066, 5 P; UFRJ 2067, 4 P (c&s); MZUSP 45771, 4 P; MNRJ 12518, 3 P; 1 km N of the city of São Francisco. - UFRJ 2121, 6; UMMZ 230861, 4; MNHN 1997-0051, 4; MCZ 138940, 4; 7 km S from São Francisco. - UFRJ 2121, 101; UFRJ 5126, 8 (c&s); 1.4 km S from São Francisco. *S. trilineatus*: Brazil: Minas Gerais, rio Paracatu drainage, rio São Francisco basin: MNRJ 12525, H; UFRJ 2132, 1 P (c&s); MNRJ 12526, 1 P; UFRJ 2133, 2 P; UFRJ 4637, 140; UFRJ 4670, 10 (c&s); UFRJ 5272, 2; UMMZ 234762, 14; UFRJ 4749, 2; Brasilândia. *Simpsonichthys antenori* species group: *S. antenori*: Brazil: Estado do Ceará, rio Jaguaribe basin: MZUSP 56256, N; UFRJ 4864, 43; 2.5 km N from Limoeiro do Norte. - UFRJ 4878, 6; Limoeiro do Norte. - UFRJ 4867, 176; UFRJ 4880, 8 (c&s); 1.5 km N of Limoeiro do Norte. - UFRJ 4873, 25; road BR-304 near Aracati. - MNRJ 4542, 7; Russas. - MNRJ 21260, 4; road BR-116, km 138, Russas. - MNRJ 19465, 3; Pedras, perto de Russas. Coastal basins: MZUSP 38342, 29; Pacajus. - MNRJ 19478, 10; Aquirás, road BR-116, km 30. Rio Grande do Norte: ZVC-P uncat., 2; Mossoró. *S. flagellatus*: Brazil: Bahia, rio São Francisco basin: MCP 28578, H; UFRJ 4788, 54 P; MCP 28579, 5 P; UFRJ 4881, 10 P (c&s); UFRJ 4861, 67; 12 km W of Bom Jesus da Lapa. - UFRJ 4874, 93; UFRJ 5352, 10 (c&s); near Volta das Pedras. - UFRJ 4854, 43; about 20 km N of Ibotirama. - UFRJ 4786, 11; UFRJ 4790, 19; 25 km S of Ibotirama. - UFRJ 4785, 13; near Paratinga, 71.5 km N of Bom Jesus da Lapa. - UFRJ 4783, 9; 22 km S of Ibotirama. - UFRJ 4851, 7; near Paratinga. - UFRJ 4853, 3; about 19 km N of Ibotirama. - UFRJ 5425, 3; Correntina. - UFRJ 6045, 7; 48.5 km S of Bom Jesus da Lapa. Minas Gerais: UFRJ 4789, 45; UFRJ 4787, 96; UFRJ 5353, 10 (c&s); near Travessão, 24 km N of São Francisco. - UFRJ 3804, 6; UFRJ 4499, 2 (c&s); 3 km N of Travessão. - UFRJ 2125, 4 (c&s); UFRJ 4500, 2 (c&s); UFRJ 144, 22; UFRJ 2092, 6; Januária. - UFRJ 4782, 14; 11 km N of Itacarambi. - UFRJ 2094, 9; UFRJ 4564, 2 (c&s); MNHN 1997-0049, 3; MCZ 138937, 4; UMMZ 2094, 6; 25 km N of Itacarambi. - UFRJ 2091, 1; 20 km N of Manga. - UFRJ 4780, 4; 1 km E of Gado Bravo. - UFRJ 2090, 9; 1 km N of Itacarambi. - UFRJ 4784, 17; 10 km N of Itacarambi. - UFRJ 4781, 10; 2 km W from Gado Bravo. - MNRJ 16067, 64; Mocambinho, Manga. - UFRJ 5409, 3; UFRJ 5410, 6 (c&s); UFRJ 5411, 31; UFRJ 6076, 15; Janaúba. - UFRJ 6072, 3; 14 km from Guanambi, road to Malhada. *S. flavicaudatus*: Brazil: Pernambuco, rio São Francisco basin: MZUSP 40129, H; MZUSP 40130, 3 P; MNRJ 11556, 2 P; UFRJ 146, 2; UFRJ 281, 1 (c&s); UFRJ 4565, 2 (c&s); vicinities of Lagoa Grande, Santa Maria da Boa Vista. UFRJ 5186, 3; Irecê. *S. ghisolfii*: Brazil: Bahia, rio São Francisco basin: MZUSP 49403, H; MZUSP 49404, 1 P; UFRJ 3526, 2 P; UFRJ 3527, 2 P (c&s); UFRJ 3808, 1 (c&s); UFRJ 4768, 4; UFRJ 4849, 10; UFRJ 6066, 6; UFRJ 6067, 2; Guanambi. - UFRJ 5406, 2; UFRJ 5407, 3 (c&s); UFRJ 5408, 12; UFRJ 6064, 16; UFRJ 6074, 4; 8 km S of Pindaí. - UFRJ 6073, 12; UFRJ 6075, 1; 5 km N from Pindaí. - UFRJ 6105, 10/12 (c&s); Macaúbas. *S. igneus*: Brazil: Bahia: MZUSP 56254, H; MZUSP 56255, 8 P; MCP 23669, 8 P; UFRJ 4857, 100 P; UFRJ 5190, 15 P; UFRJ 5191, 6 P; UFRJ 5192, 10 P; UFRJ 5193, 10 P; 22 km N from Igarité. - UFRJ 4872, 33 P; 40 km N of Igarité. - UFRJ 4868, 28 P; UFRJ 4870, 62 P; UFRJ 4869, 10 P (c&s); 27.5 km N of Igarité. - UFRJ 4877, 14 P; UFRJ 5417, 7; 21.5 km N from Igarité. *Simpsonichthys flammeus* species group: *S. alternatus*: Brazil: Minas Gerais, rio Paracatu drainage, rio São Francisco basin: MNRJ 12523, H; MNRJ 12524, 2 P; MZUSP 46082, 3 P; UFRJ 2129, 1 P; UFRJ 3457, 9; MNHN 1997.0048, 6; UMMZ 231545, 6; UFRJ 2130, 11 P; UFRJ 2131, 4 P (c&s); UFRJ 4636, 25; UFRJ 4750, 4; Brasilândia, João Pinheiro. *S. brunoi*: Brazil: Estado de Goiás, rio Tocantins drainage: MCP 28576, H; MCP 28577, 7 P; UFRJ 5414, 17 P; UFRJ 5413, 15 P; UFRJ 5412, 8 P; near the city of Vila Boa, ribeirão Canabrava floodplains. *S. delucai*: Brazil: Minas Gerais, rio Urucuia drainage, rio São Francisco basin: MCP 28572, H; MCP 28573, 1 P; UFRJ 5426, 17 P; UFRJ 5427, 4 P (c&s); near the city of Urucuia. *S. flammeus*: Brazil: Tocantins, rio Tocantins drainage: MNRJ 1152, H; MNRJ 11553, 2 P; MCP 12795, 2 P; UFRJ 280, 1 ex. (c&s); Arraias, swamp in the confluence of rio Bezerra and rio Paranã. - UFRJ 5116, 4; UFRJ 5117, 2 (c&s); Peixe. Goiás: UFRJ 2081, 75; UFRJ 2116, 4 (c&s); MNHN 1997.0047, 8; UMMZ 230857, 12; USNM 343825, 5; MCZ 138944, 2; UFRJ 157, 33; swamp close to rio Paranã, road Iaciara-Nova Roma. *S. multiradiatus*: Brazil: Tocantins, rio Tocantins drainage: MNRJ 12519, H; MNRJ 12520, 9 P; MZUSP 46080, 8 P; UFRJ 2074, 7 P (c&s); UFRJ 2075, 6 P; UFRJ 2076, 8 P; UFRJ 2097, 63; UMMZ 230858, 12; MCZ 138938, 2; Brejinho de Nazaré. **Other Cynolebiatini.** *Cynolebias griseus*: Brasil: Goiás: UFRJ 150, 2 (c&s); UFRJ 319, 1 (c&s); UFRJ 2098, 12; UFRJ 5124, 1 (c&s); Nova Roma, rio Tocantins basin. *Austrolebias carvalhoi*: Brazil: Santa Catarina: UFRJ 4444, 2; UFRJ 4444, 2 (c&s); UFRJ 4967, 4 (c&s); União da Vitória, rio Iguaçu drainage, rio Paraná basin.

Appendix II

Characters (between brackets) and character states (between parentheses) used to erect the phylogenetic hypothesis among species of *Nematolebias* and *Simpsonichthys* are listed below, with the respective reference to papers where the character is first described or discussed. Distribution of character states among terminal taxa is presented in the data matrix in Appendix IV.

Superficial dermal bones

[1] Nasal ventromedial keel: (0) absent; (1) present.

[2] Dermosphenotic (Costa, 1990): (0) present; (1) absent.

Neurocranium

[3] Posterior process of supraoccipital (Costa, 2003b): (0) short, without a narrow posterior extension; (1) long, terminating in narrow posterior extension.

[4] Vomerine teeth (Costa, 1996): (0) present; (1) absent.

Jaws, jaw suspensorium and opercular apparatus

[5] Rostral cartilage width, expressed as proportion of rostral cartilage length in large males (modified from Costa, 1996): (0) longer than wide; (1) approximately so long as wide.

[6] Posteroventral process of dentary (Costa, 1990): (0) narrow; (1) broad.

[7] Posterior outer dentary tooth (Costa, 1996): (0) posteriorly directed; (1) anteriorly directed.

[8] Ventral process of angulo-articular (Costa, 2003b): (0) long and wide; (1) long and narrow; (2) short and wide; (3) short and narrow; (4) vestigial or absent [not ordered].

[9] Dorsal tip of palatine (Costa, 1998a): (0) without processes; (1) with distinctive, small pointed dorsal process.

[10] Medial crest of palatine: (0) absent; (1) present; (2) present, with prominent ventral expansion.

[11] Mesopterygoid extent and its relative position to quadrate (modified from Costa, 1990, 1998a): (0) long, reaching metapterygoid and overlapping quadrate; (1) short, posterior tip in vertical through middle of quadrate, ventral portion overlapping quadrate; (2) short, posterior tip in vertical through middle of quadrate, ventral portion only contacting quadrate; (3) very short, posterior tip in vertical through anterior portion of quadrate, not contacting it; (4) mesopterygoid consisting of minute ossification lying above quadrate.

[12] Length of posterior process of quadrate, expressed by proportion between process length and total longitudinal length of quadrate (modified from Costa, 1998a): (0) 50-56%; (1) 34-47%.

[13] Sympletic (Costa, 1990, 1998a): (0) short and deep (Fig. 10); (1) long (Fig. 13a).

[14] Metapterygoid (modified from Costa, 1998a): (0) approximately rectangular, dorsal and ventral portions broad; (1) dorsal portion narrow, ventral broad; (2) dorsal portion broad, ventral narrow; (3) rod-like, entire bone narrow [not ordered].

[15] Dorso-ventral axis of hyomandibula: (0) short (Fig. 10); (1) long (Fig. 14c).

[16] Lateroventral process of hyomandibula (Costa, 2003b): (0) posteriorly displaced, its ventral tip separated from ventral hyomandibula condyle by broad osseous flange; (1) not posteriorly displaced, its ventral tip in close proximity to ventral hyomandibula condyle; (2) completely fused to ventral hyomandibula condyle.

[17] Dorsal portion of preopercle (Costa, 1990, 1998): (0) rounded; (1) pointed.

[18] Anteromedian shelf of preopercle (Costa, 1998a): (0) broad, expanding behind preopercle arms; (1) narrow, with about some width as preopercle arms; (2) vestigial or absent.

[19] Anterior arm of preopercle: (0) long (Fig. 15a); (1) short (Fig. 15c).

Hyoid and branchial arches

[20] Length of cartilaginous portion of basihyal, expressed as percentage of longitudinal basihyal length (modified from Costa, 1996, 1998a): (0) 10-12%; (1) 18-33%; (2) 38-45%; (3) 55-60%.

[21] Basihyal width (expressed as percentage of basihyal length in adult male; modified from Costa, 1996): (0) 30-80%; (1) 90-100%.

[22] Interhyal (Parenti, 1981; Costa, 1990): (0) ossified; (1) cartilaginous.

[23] Second pharyngobranchial: (0) wider than long; (1) longer than wide.

[24] Second pharyngobranchial teeth (Costa, 1998a): (0) present; (1) absent.

[25] Number of second pharyngobranchial teeth (Costa, 1998a): (0) 1-4; (1) 5-10; (?) teeth absent.

[26] Epibranchials (modified from Costa, 1998a): (0) short; (1) long.

[27] Uncinate process of third epibranchial (Costa, 2004a). (0) long; (1) short.

[28] Interarcual cartilage (Costa, 1998a): (0) short; (1) long.

[29] Attachment between interarcual cartilage and second pharyngobranchial: (0) near distal pharyngobranchial cartilage; (1) on medial margin.

[30] Proximal edge of first hypobranchial (Costa, 1998a): (0) bifid, terminating in cartilage united to second basibranchial and another smaller cartilage united to first basibranchial, (1) plain, terminating in single cartilage united to second basibranchial.

[31] Distal edge of first hypobranchial (Costa, 2004a): (0) articular face restricted to cartilaginous head of first ceratobranchial, (1) articular face anteriorly expanded.

[32] Distinctive anteromedial process on second hypobranchial, directed to second basibranchial: (0) absent; (1) present.

[33] Teeth on fourth ceratobranchial (Parenti, 1981; Costa, 1990): (0) present; (1) absent.

[34] Fifth ceratobranchial shape: (0) anterior branch shorter than posterior branch; (1) anterior branch longer than posterior branch.

[35] Angle of the two main axes of fifth ceratobranchial: (0) 125-135°; (1) 145°.

[36] Proximal tip of fourth ceratobranchial: (0) wider than proximal tip of third ceratobranchial; (1) narrower than proximal tip of third ceratobranchial.

Vertebrae and caudal skeleton

[37] Connection between first neural spine and neural prezygapophysis of second vertebra: (0) thin ligaments; (1) directly attached.

[38] Third neural spine: (0) conspicuously wider than fourth neural spine; (1) about so wide as fourth neural spine.

[39] Neural prezygapophyses of first vertebra (Costa, 1990, 1998a): (0) present; (1) absent.

[40] Neural prezygapophyses of caudal vertebrae (Costa, 1990): (0) elongate; (1) vestigial or absent.

[41] Hypurals (modified from Costa, 1998a): (0) two symmetrical plates; (1) plates fused, but with conspicuous median fissure; (2) complete ankylosis forming a single plate, with-

out vestige of median fissure.

[42] Proximal region of epural and parhypural (Costa, 1998a): (0) broad and approximately straight; (1) narrow and curved anteriorly.

Dorsal and anal-fin squeleton

[43] Number of male dorsal-fin rays and relative position of dorsal-fin origin, expressed by position of second proximal radial and neural spines (modified from Costa, 1996, 1998a): (0) 7-9 rays, dorsal-fin origin between neural spines of vertebrae 18 and 19; (1) 12-16 rays, dorsal-fin origin between neural spines of vertebrae 11 and 14; (2) 15-21 rays, dorsal-fin origin between neural spines of vertebrae 10 and 14; (3) 18-22 rays, dorsal-fin origin between neural spines of vertebrae 7 and 9; (4) 20-25 rays, dorsal-fin origin between neural spines of vertebrae 6 and 9; (5) 25-28 rays, dorsal-fin origin between neural spines of vertebrae 5 and 7; (6) 28-32 rays, dorsal-fin origin between neural spines of vertebrae 3 and 4; (?) members of clades having great variation in number of rays and position of dorsal fin.

[44] Number of male anal-fin rays and relative position of anal-fin origin, expressed by position of second proximal radial and pleural ribs (modified from Costa, 1996, 1998a): (0) 11-14 rays, between pleural ribs of vertebrae 18 and 19; (1) 16-21 rays, between pleural ribs of vertebrae 10 and 11; (2) 20-23 rays, between pleural ribs of vertebrae 9 and 11; (3) 17-25 rays, between pleural ribs of vertebrae 5 and 9.

[45] Sexual dimorphism in number of dorsal and anal-fin rays (Costa, 1990): (0) not dimorphic; (1) more rays in male than in female.

[46] Dorsal-fin rays branching (modified from Costa, 1996): (0) most rays branched, including rays on fin tip; (1) most rays branched, but rays on fin tip unbranched; (2) all rays usually unbranched.

[47] Anal-fin rays branching: (0) most rays branched, including rays on fin tip; (1) most rays branched, but rays on fin tip unbranched.

[48] First proximal radials of anal fin: (0) long and narrow; (1) short and wide.

[49] Medial and distal radials of dorsal and anal fins: (0) ossified; (1) cartilaginous.

[50] Median radials of dorsal fin: (0) short; (1) long.

Shoulder and pelvic girdle

[51] Posttemporal ventral process (Costa, 1996): (0) present; (1) absent.

[52] Supracleithrum (Costa, 1990): (0) short; (1) long.

[53] Dorsal portion of cleithrum (modified from Costa, 1998a): (0) short; (1) long.

[54] Anterior flap of cleithrum: (0) narrow; (1) expanded.

[55] Posterior flange of cleithrum (Costa, 1998a): (0) present; (1) vestigial or absent.

[56] Relative position of ventral tip of cleithrum and ventral tip of coracoid (Costa, 1998a): (0) not in close proximity; (1) in close proximity.

[57] Ventral condyle of coracoid: (0) narrow; (1) wide.

[58] Pectoral-fin radials (Costa, 1990): (0) well-ossified, cub form; (1) thin, weakly ossified, disc shaped; (2) weakly ossified to form small disc shaped thin bones.

[59] Space between lower pectoral-fin radial and coracoid: (0) wide; (1) narrow, with narrow ventral expansion of cartilage.

[60] Pelvic bone (modified from Costa, 1996): (0) long; (1) short, fin long; (2) vestigial, fin short; (3) both fin and girdle absent.

[61] Ischial process: (0) present; (1) absent.

External morphology of body and head

[62] Maximum male standard length (modified from Costa, 1996): (0) 35-60 mm SL; (1) about 21-25 mm SL.

[63] Head width (expressed as percentage of HL in larger males): (0) 65-80%; (1) about 60%.

[64] Ventral profile of head: (0) curved; (1) with distinct obtuse angle at level of ventral tip of angulo-articular with prominent flap of thickened tissue.

[65] Eye position (Costa, 2003b): (0) dorsolateral; (1) lateral.

[66] Urogenital papilla of males (modified from Costa, 1998a): (0) globular; (1) tubular and short; (2) tubular and long.

[67] Urogenital papilla of females (Costa, 1998a): (0) a transverse gap; (1) a prominent pocket-like structure overlapping anterior anal-fin origin.

Fins

[68] Male dorsal-fin shape (Costa, 1996): (0) rounded; (1) pointed.

[69] Male anal-fin shape (Costa, 1996): (0) rounded; (1) pointed.

[70] Female anal-fin shape (Costa, 2003b): (0) short and rounded; (1) long, approximately spatula-shaped.

[71] Filamentous rays on male dorsal-fin tip (Costa, 1996): (0) absent; (1) short, reaching vertical through caudal-fin base; (2) long, reaching beyond posterior border of caudal fin.

[72] Filamentous rays along distal border of male dorsal fin (Costa, 1996): (0) absent; (1) present.

[73] Filamentous rays on male anal-fin tip (Costa, 1996): (0) absent; (1) short, reaching vertical through caudal-fin base; (2) long, reaching beyond posterior border of caudal fin.

[74] Filamentous rays on posterior border of female anal fin (Costa, 2003b): (0) absent; (1) present.

[75] Male caudal-fin shape (Costa, 1996): (0) rounded to subtruncate; (1) lanceolate.

[76] Pelvic-fin bases: (0) separated by small interspace; (1) barely medially touching each other or united medially.

Squamation

[77] Scales on anal-fin base: (0) absent; (1) present.

[78] Arrangement of frontal scales (modified from Parenti, 1981; Costa, 1990): (0) circular; (1) transversal.

[79] Frontal squamation pattern (modified from Costa, 2003b): (0) E; (1) A; (2) F [not ordered].

[80] Relative position of E-scales (Costa, 1998a): (0) overlapped; (1) not overlapped.

Neuromasts

[81] Total number of supraorbital neuromasts (Costa, 1998a): (0) 6-7; (1) 9-18; (2) 21-22.

[82] Relationship between anterior and posterior sections of supraorbital series of neuromasts (Costa, 1998a): (0) anterior section separated by interspace; (1) anterior and posterior section continuous.

[83] Distribution of neuromasts of anterior supraorbital series: (0) neuromasts regularly spaced, forming a continuous series; (1) two anteriormost neuromasts separated from posterior neuromasts by longer space, making series interrupted at level of posterior nostril.

[84] Neuromast anterior to anterior infraorbital series: (0) present; (1) absent.

[85] Relationship between supraorbital and pre-orbital series of neuromasts: (0) separated by interspace; (1) continuous.

[86] Relationship between preopercular and mandibular series of neuromasts: (0) separated by interspace; (1) continuous.

Contact organs

[87] Contact organs on scales of male flank (Costa, 2003b): (0) absent; (1) present.

[88] Male pectoral-fin contact organs (Costa, 2003b): (0) absent; (1) papillate, restricted to two or three uppermost fin rays; (2) tubular, dispersed along all rays of upper half of fin.

Male color patterns

[89] Dark pigmentation pattern of body side to form bars (modified from Costa, 1996): (0) similar bars on entire flank, often lost in adult specimens; (1) three dark blue bars alternating with red bars on anterior portion of flank, followed by pale, almost inconspicuous bars.

[90] Dark pigmentation pattern on anterior half of body side to form round spots (modified from Costa, 1996): (0) dark pigment never concentrated to for round spots; (1) melanophores concentrated to form single round black spot on anterocentral portion of flank; (2) melanophores concentrated to form round black spot on anterocentral portion of flank and other similar spots anteriorly.

[91] Concentration of red pigmentation on body side (modified from Costa, 1996): (0) not distinctively concentrated; (1) concentrated to form red bars; (2) most flank covered by red pigmentation.

[92] Iridescent color pattern on body side (modified from Costa, 1996): (0) brilliant spots or dots on scales over entire flank; (1) brilliant dots restricted to dorsal half of flank; (2) dorsovertically elongated brilliant dots on flank; (3) brilliant bars on anterior portion and vertical rows of brilliant dots on posterior portion of flank [not ordered].

[93] Dark pigmentation pattern on suborbital region (modified from Costa, 1996): (0) melanophores not forming distinctive narrow bar; (1) melanophores concentrated to form distinctive narrow bar.

[94] Dark pigmentation pattern on postorbital region: (0) melanophores not forming distinctive narrow bar; (1) melanophores concentrated to form distinctive narrow bar.

[95] Red pigmentation pattern on suborbital and opercular region (modified from Costa, 1996): (0) not forming distinctive red marks; (1) red suborbital, preopercular and opercular bars.

[96] Dark pigmentation pattern of median portion of iris (Parenti, 1981): (0) distinctive dark marks absent; (1) bar through center of eye.

[97] Iridescent color of iris (Costa, 2003b): (0) yellow; (1) anterior and posterior zones metallic blue.

[98] Concentration of melanophores on dorsal fin distal border (modified from Costa, 1996): (0) not concentrated; (1) strongly concentrated to form dark gray to black stripe.

[99] Concentration of melanophores on dorsal fin base (modified from Costa, 1996): (0) not concentrated; (1) concentrated to form black blotches.

[100] Iridescent color pattern on dorsal fin (modified from Costa, 1996): (0) dots; (1) bars; (2) transverse broken stripes; (?) not applicable patterns [not ordered].

[101] Iridescence on distal zone of dorsal fin (modified from Costa, 1996): (0) no iridescence; (1) forming distal stripe.

[102] Iridescence on subdistal zone of dorsal fin (modified from Costa, 1996): (0) no iridescence; (1) forming short subdistal stripe on anterior portion of fin; (2) forming subdistal stripe.

[103] Dark pigmentation pattern of anal fin (modified from Costa, 1996): (0) homogeneous; (1) oblique bars.

[104] Concentration of melanophores on distal border of anal fin (modified from Costa, 1996): (0) not concentrated; (1) concentrated to form gray to black stripe.

[105] Iridescent color pattern of anal fin (modified from Costa, 1996): (0) dots on entire fin; (1) dots restricted to posterior portion of fin; (2) oblique broken stripes; (3) oblique bars; (4) lines parallel to fin rays; (5) short lines parallel to fin rays on distal half of fin; (6) iridescence absent [not ordered].

[106] Iridescence on subdistal zone of anal fin (modified from Costa, 1996): (0) no iridescence; (1) forming subdistal stripe.

[107] Iridescent color pattern of caudal fin (modified from Costa, 1996): (0) dots; (1) bars; (?) not applicable patterns [not ordered].

[108] Iridescence on subdistal zone of caudal fin (modified from Costa, 1996): (0) no iridescence; (1) forming distinctive subdistal iridescent zone.

[109] Iridescent color pattern on pectoral fin (Costa, 1996): (0) iridescence absent; (1) small spots on basal portion of fin.

[110] Ground color on pectoral fin (modified from Costa, 1996): (0) hyaline; (1) reddish brown; (2) red [not ordered].

Female color patterns

[111] General dark pigmentation pattern on body side (modified from Costa, 1996): (0) dots; (1) bars; (2) broken bars or spots; (?) dark pigmentation absent [not ordered].

[112] Melanophore pattern on central portion of flank (modified from Costa, 1990): (0) not distinctively aggregated; (1) aggregated to form black spots on anterocentral zone.

[113] Iridescent color pattern on central portion of flank (modified from Costa, 1996): (0) iridescence absent; (1) metallic blue bars; (2) faint bars and bright golden circular mark [not ordered].

[114] Melanophore pattern of caudal peduncle (Costa, 1996): (0) not distinctively aggregated; (1) aggregated to form black spots on posterior zone.

[115] Iridescent color pattern of unpaired fins (modified from Costa, 1996): (0) iridescence absent; (1) bright blue spot on posterior region of anal fin; (2) small bright blue spots on posterior portion of dorsal and anal fins, and basal portion of caudal fin.

[116] Ground color on anal fin (modified from Costa, 1995): (0) hyaline, sometimes yellowish or pinkish hyaline; (1) pink.

Appendix III

List of apomorphies by node. Conditions not homoplastic and not suffering reversal in less-inclusive clades in bold, reversals in italic, conditions suffering reversal in less-inclusive clades underlined, and homoplastic conditions followed by asterisk. Character and state numbers are according to Appendix II. See Fig. 22 for included species in nodes.

A: 4.1*, **8.3**, **11.2**, **38.1**, 53.1, **57.1**, **77.1**, **84.1**; B: 21.1*, 24.1*, **37.1**, 86.1*, _87.0_, **88.2**, **105.5**, 108.1*, 109.1*, 110.1*; C: 9.1, **46.1**, **47.1**, _80.0_; D: 2.1*, _7.0_, **14.1**, **44.2**, **49.1**, **58.2**, **60.1**, _76.0_, **81.2**, 93.1*; E: _5.0_, **46.2**, _114.0_*; F: 24.1*, **74.1**, **75.1**, 105.3*; G: 2.1*, **43.4**; H: **10.2**, **65.1**, _82.0_, **113.1**, **116.1**; I: **115.2**; J: 16.1*, **23.1**; K: 13.1*, 98.1, 102.1, 104.1*, 106.1; L: 92.3; M: 14.3*; **15.1**, **36.1**, _87.0_; N: **54.1**, **66.2**; O: 51.1*, 62.1*, _68.0_*, _73.0_*, 93.1*, **94.1**; P: 16.1*, 34.1*, **35.1**, _43.2_*, **64.1**, _88.0_*, _98.0_*; Q: **11.3**, _43.2_, **60.2**, **95.1**; R: _98.0_*, _104.0_*, 109.2*; S: 51.1*, 60.3*, 62.1*, _71.0_*, _73.0_*, **83.1**, **85.1**, 97.1*, 108.1*; T: 24.1*, 99.1*; U: 115.1; V: 111.2*; W: **10.1**, **18.1**, **19.1**, 34.1*; X: **3.1**, **70.1**, 105.3*, 107.1*; Y: **18.2**, 104.1*, _114.1_; Z: _71.2_, _73.2_; A': **25.1**, **105.2**; B': **20.2**, **100.1**; C': _69.0_*, _73.0_*, _115.0_*; D': _71.2_*, **72.1**; E': **89.1**; F': **79.1**; G': 29.1*, **90.2**, 110.1; H': 99.1*; I': 29.1*, **50.1**, **91.1**; J': **32.1**; K': 109.2*; L': _68.0_*, _69.0_*, _71.0_*, _73.0_*.

Appendix IV

Matrix of 116 characters for 50 rivulid species. Characters and states are according to Appendix II. Autapomorphies were excluded from the analysis; 0 = plesiomorphic state; 1-9 = apomorphic states; ? = not pertinent or unknown state.

	1–10	11–20	21–30	31–40	41–50	51–60	61–70	71-80	81-90	91-100	101-110	111-116
K. brasiliensis	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	000000
N. paraguayensis	0000000100	0000000001	0100001000	1000000000	0000000000	0000100000	0000000000	0000000001	0000000000	0000010000	0000000000	000000
L. minimus	1001000400	401?001001	0101?11001	1010000011	1111000100	0100110110	1100011110	1000000101	110?001100	0000010000	0000301000	?00000
A. carvalhoi	1101010300	2101001003	0100011101	1010000111	21?2100110	0110111211	1000011000	0000001100	2101001100	0010010000	0000000000	210000
C. griseus	1101110300	2101001101	1100011101	1010000111	21?2100110	0110111211	1000011110	1010001100	2101011100	0010010000	0000000000	110100
N. papilliferus	1000111200	1102001001	1101?11101	1010001011	2123100100	1100110110	1000011110	1010010101	1100010200	0000010000	0000500111	110100
N. whitei	1000111200	1102001001	1101?11101	1010001011	2123100100	1100110110	1000011110	1010010101	1100010200	0000010000	0000500111	110100
S. adornatus	1101011310	2100011001	0110011111	1010000111	2163121101	0110111110	1000011110	1010011100	1101001100	1100010000	0000000000	210110
S. alternatus	1111011311	2100011111	0110011101	1011000111	2133121100	0110111110	1000011101	1000011100	1101001100	0000010000	0000301000	110000
S. antenori	1101011311	2100021211	0110111101	1011000111	2143121100	0110111110	1000011110	1010011100	1101001100	0000010000	0001000000	110110
S. auratus	1101010310	2100011001	0110011111	1010000111	2133121100	0110111110	1000011110	1010011110	1101001102	0000010000	0000000001	210000
S. boitonei	1101011310	3112001001	0100011101	1010000111	2143121100	0110111113	1000011110	10100?1100	1101001100	2200110000	1100010012	110000
S. bokermanni	1001011312	2102001001	0100011101	1010000111	2123121100	0110111110	1000111110	1010011100	1001001100	0000010000	0000000000	111021
S. brunoi	1111011311	2100011112	0110011101	1011000111	2133121100	0110111110	1000011111	2120011100	1101001100	0000010001	0000301000	110010
S. carlettoi	1101011310	2100011001	0110011111	1110000111	2143121101	0110111110	1000011110	1010011100	1101001110	1100010000	0000000002	210010
S. chacoensis	1101011310	2113101001	0100011101	1010010111	2143121100	0111111110	1010021110	1020011100	1101000100	0000010100	0101010000	110000
S. cholopteryx	1101011310	3112001001	0101?11101	1010000111	2123121100	1110111113	1100011110	00000?1100	1111101000	2000111100	0101010100	110000
S. constanciae	1001011312	2103001001	0100011101	1010000111	2123121100	0110111110	1000111110	2020011100	1001000000	0000010000	0000000000	111001
S. costai	1101011310	2113101001	0100011101	1010010111	2143121100	1110111110	1110011000	0000011100	1101000000	0011011100	0101010000	100000
S. delucai	1111011311	2100011111	0110011101	1011000111	2133121100	0110111110	1000011001	0000011100	1101001100	0000010000	0000301000	110000
S. filamentosus	1001011310	2113111001	0100011101	1011110111	2123121100	0111111110	1001021110	2020001100	1101001000	0000010000	0000000000	110000
S. flagellatus	1101011311	2100021211	0110111101	1011000111	2143121100	0110111110	1000011110	2020011100	1101001100	0000010010	0001200000	110110
S. flammeus	1111011311	2100011112	0110011101	1011000111	2143121100	0110111110	1000011111	2120011100	1101001100	0000010001	0000301000	110010
S. flavicaudatus	1101011311	2100021211	0110111101	1011000111	2143121100	0110111110	1000011110	2020011100	1101001100	0000010000	0001200000	110110
S. fulminantis	1101011310	2100011001	0110011111	1110000111	2143121101	0110111110	1000011110	1010011100	1101001110	1100010002	000040?000	210010
S. ghisolfii	1101011311	2100021211	1110111101	1011000111	2143121100	0110111110	1000011110	2020011100	1101001100	0000010000	0001000000	110110
S. hellneri	1101011310	2100011001	0110011101	1010000111	2143121100	0110111110	1000011110	1010011100	1101001110	1000010000	0000400000	210010
S. igneus	1101011311	2100021211	0110111101	1011000111	2143121100	0110111110	1000011110	2020011100	1101001100	0000010000	0001000000	110110
S. izecksohni	1001111310	2102001001	0101?11101	1010000111	2123111100	0100111110	1000011110	1011111100	1101001100	0000010000	0000300000	110100
S. magnificus	1101011310	2100011001	0110011111	1110000111	2143121101	0110111110	1000011000	0000011100	1101001110	1100010000	0000001002	210010
S. marginatus	1101011310	2112001001	0100011101	1010000111	2143121100	0110111110	1000011110	2020011100	1101001100	2200010100	0101010000	110000
S. multiradiatus	1111011311	2100011112	0110011101	1011000111	2153121100	0110111110	1000011111	1010011100	1101001100	0000010001	0000301000	110010
S. myersi	1001111310	2102001001	0101?11101	1010000111	2123111100	0100111110	1000011110	1011111100	1101001100	0000010000	0000301000	110100
S. nielseni	1101010310	2100011001	0110011101	1010000111	2143121100	0110111110	1000011110	1010011110	1101001100	0000010012	101030?000	210010
S. notatus	1101010310	2100011001	0110011101	1010000111	2143121100	0110111110	1000011110	1010011110	1101001101	0000010000	1010000000	210000
S. ocellatus	1101011310	2100011001	0110011101	1010000111	2143121100	0110111110	1000011110	1010011?0	1101001100	0000010000	0010301000	112000
S. parallelus	1101011310	3112001001	0101?11101	1010000111	2123121100	1110111113	1100011110	00000?1100	1111101100	2200111100	0101010100	110000
S. perpendicularis	1001011312	2102001001	0100011101	1010000111	2123121100	0110111110	1000111110	1010011100	1001001100	0000010000	0000000000	111021
S. picturatus	1101011310	2100011001	0110011111	1110000111	2143121101	0110111110	1000011000	0000011100	1101001110	1100010000	0000000002	210010
S. radiosus	1101010310	2100011001	0110011101	1010000111	2143121100	0110111110	1000011110	1010011110	1101001101	0000010000	101000?000	210000
S. reticulatus	1101011310	2113101001	0100011101	1010010111	2143121100	1110111110	1110011010	1000011100	1101000100	0011010100	0000000000	100000
S. rosaceus	1001011312	2102001001	0100011101	1010000111	2123121100	0110111110	1000111110	1010011100	1001001100	0000010000	0000000000	111021
S. rufus	1101010310	2100011001	0110011101	1010000111	2143121100	0110111110	1000011110	1010011110	1101001100	0000010000	1010000000	210010
S. santanae	1101011310	3112001001	0100011101	1010000111	2123121100	0110111112	1000011110	10100?1100	1101001100	2200110010	0100010002	110000
S. semiocellatus	1101011300	2113111001	0100011101	1011110111	2123121100	0101111110	1111021100	2000011120	1101000000	0000010000	0101010000	100000
S. similis	1101010310	2100011001	0110011101	1010000111	2143121100	0110111110	1000011110	1010011110	110100110?	0000010000	1000000000	210010
S. stellatus	1101010310	2100011001	0110011101	1010000111	2143121100	0110111110	1000011110	1010011110	1101001100	0000010010	1011000000	210010
S. suzarti	1001011312	2102001001	0100011101	1010000111	2123121100	0110111110	1000111100	1010011100	1001001100	0000010000	0000000000	111021
S. trilineatus	1101010310	2100011001	0110011111	1010000111	2133121100	0110111110	1000011110	1010011110	1101001102	0000010000	0000000001	210000
S. zonatus	1101011310	3112001001	0100011101	1010000111	2123121100	0110111112	1000011110	10100?1100	1101001100	2200110010	0100010002	110000

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