Description of a new species and phylogenetic analysis of the subtribe Cynopoecilina, including continuous characters without discretization (Cyprinodontiformes: Rivulidae)

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Received 31 October 2013; revised 22 April 2014; accepted for publication 16 July 2014

A new species of the subtribe Cynopoecilina is described from the rio Gravataí basin, laguna dos Patos system, southern Brazil. The relationships of the new species among taxa of the subtribe Cynopoecilina is discussed based on two analyses: one using 71 discrete characters and other with the addition of six continuous characters analyzed without discretization. The addition of the continuous characters resulted in the first fully resolved phylogenies for Cynopoecilus and Leptolebias species, not obtained in the analysis including only discrete characters. The new species is assigned to Cynopoecilus as sister group to the remaining species of the genus. A new diagnosis is proposed for Cynopoecilus to accommodate the new species. The resulting phylogeny indicates that the occupation of the grasslands of the Pampa biome by the species of Cynopoecilus occurred along the evolution of the genus and that this event was significant for the diversification of the genus.


INTRODUCTION

The Cynopoecilina is a subtribe of the subfamily Cynolebiatinae, Rivulidae, whose monophyly has been corroborated by Costa (1990a, b, 1998), Hrbek & Larson (1999) and Murphy, Thomerson & Collier (1999). It includes four genera (Table 1), all recently revised and phylogenetically analyzed in a series of papers by Costa. As such, the diversity and relationships of the species of this subtribe are relatively well known. Campellolebias Vaz-Ferreira & Sierra de Soriano, 1974 was revised and phylogenetically analyzed in a series of papers by Costa. Costa, 1995b, 2006a). Costa (2008) revised and phylogenetically studied Leptolebias Myers, 1952, which was redefined and restricted to six species, with three of the nine species previously assigned to that genus transferred to the Notholebias Costa, 2008, described therein. Cynopoecilus Regan, 1912 was revised by Costa (1995a, 2002a), with the recognition of five species. However, differently from other genera of the Cynopoecilina, no internal hypothesis of relationships has been provided for the species of Cynopoecilus.

Cynopoecilus species inhabit seasonal pools close to streams and rivers (Costa, 2002a), whereas species of Campellolebias, Leptolebias and Notholebias are usually associated with the Atlantic Forest (Costa, 1995b, 2008). Although all cynopoecilines are killifishes characterized by an annual life-cycle with a period of egg estivation during dry seasons (Costa, 1998), they differ in some reproductive aspects. The species of Campellolebias and Cynopoecilus present facultative

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Table 1. Genera and species of the subtribe Cynopoecilina (species marked with an asterisk are included in the phylogenetic analysis)

<table>
<thead>
<tr>
<th>Taxon</th>
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<td><em>Campellolebias chrysolineatus</em> Costa et al., 1989</td>
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<td><em>Cynopoecilus nigrovittatus</em> Costa, 2002</td>
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<td><em>Notholebias vermiculatus</em> Costa &amp; Amorim, 2013</td>
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internal fertilization (Parenti, 1981) and possess unique structures, such as a pseudogonopodium and modified anal-fin bones respectively, putatively associated to this reproductive mode, while Leptolebias and Notholebias are externally fertilizing species (Costa, 1998, 2008).

During an inventory of the fishes in a dense fragment of wetland forest near Porto Alegre, Rio Grande do Sul, Brazil, we found a new species of the Cynopoecilina. The new species possesses several of the synapomorphies assigned to Cynopoecilus by Costa (1995a, 2002a), but also some of the synapomorphies described by Costa (2008) and one of the synapomorphies described by Costa (2008) for Leptolebias, making a decision about its generic assignment difficult. Such situation demands a phylogenetic analysis including all the Cynopoecilina and especially all the species of Cynopoecilus, in order to test the relationships of the new species, as well as to review characters that phylogenetically diagnose Campellolebias, Cynopoecilus, Leptolebias and Notholebias.

Prior diagnoses and hypotheses of phylogenetic relationships of the genera of the Cynopoecilina were based on partially different sets of characters including a few continuous characters (e.g. the number of the supraorbital series of cephalic neuromasts) discretized in different and incongruent ranges in different papers. As the number of fin rays, scales, neuromasts, and vertebral counts also varies among the species of the subtribe, our phylogeny includes an analysis of six continuous characters without discretization as proposed by Goloboff, Mattoni & Quinteros (2006).

MATERIAL AND METHODS

Examined specimens belong to the Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre (MCP); and Departamento de Zoologia, Universidade Federal do Rio Grande do Sul, Porto Alegre (UFRGS).

Measurements and counts follow Costa (1988, 1995c) except for transverse series of scales that were counted between the anterior insertion of the dorsal fin and the anterior insertion of pelvic fin; predorsal length measured from the tip of the snout to anterior insertion of the dorsal fin; prepelvic length measured from the tip of the snout to the anterior insertion of the pelvic fin; lower jaw length measured between the anterior and posterior (lingual) borders of the lower jaw in a dorsal view; pelvic-fin length, pectoral-fin length and caudal-fin length, measured from fin base to the tip of the longest rays. Measurements are presented as percentages of standard length (SL), except for those related to head morphology whose are expressed as percentages of head length. Fin-ray counts include all elements. Number of vertebrae, gill-rakers, and caudal-fin rays were recorded from cleared and stained specimens. The number of counted specimens is presented in parentheses along the description. An asterisk indicates the count for the holotype.

The compound caudal centrum was counted as a single element. Osteological preparations (c&s) were made according to Taylor & Van Dyke (1985). Osteological nomenclature is according to Costa (1998). Terminology for frontal squamation follows Hoedeman (1958), and for cephalic neuromasts series Costa (2008: fig. 2).

The phylogenetic analysis was performed including all species of Campellolebias and Cynopoecilus; three of the four species of Notholebias; and four of the six species of Leptolebias for a total of 18 terminals in the ingroup (Tables 2 and 3). We have followed Costa (2006a) in using Nematolebias whitei (Myers, 1942), Neofundulus paraguayensis (Eigenmann & Kennedy, 1903), and Kryptolebias brasiliensis (Valenciennes, 1821) as outgroups, and rooting the tree in the last species. Data were extracted from the literature (Parenti, 1981; Costa, 1988, 1990a, b, 1995a, b, 1998, 2002a, b, 2006a, b, 2008) and direct examination of specimens (see Comparative Material), and the resulting matrix differs from all those presented previously.

Characters are described and standardized following Sereno (2007), where the character states are composed of four fundamental functional components identified as locator, variable, variable qualifier, and character states. These components exist in two patterns, neomorphic and transformational, being absence or presence assigned as states for neomorphic characters only. Comments are provided for characters 10, 12, 13, 15, 17, 18, 19, 22, 25, 28, 29, 30, 32, 33, 35, 36, 40, 44, 47, 48, 49, 50, 51, 52, 55, 56, 59, 64, 68, 69, 73 that possess different usages in the literature or that differ from our decision regarding character states (Appendix 1).

Continuous characters are new (characters 1 to 4) or reinterpreted in a new usage (characters 5 and 6), and were treated as continuous without discretization.

### Table 2. Continuous characters partition, showing observed range and range transformed from 0 (corresponding to smallest observed count) to 1 (corresponding to largest observed count) for each character

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results

The results of both analyses (excluding or including continuous characters) were congruent and supported the monophyly of the Cynopoecilina and of the four included genera, but with a low support for Leptolebias and Notholebias (Fig. 1). The analysis excluding continuous characters resulted in four equally parsimonious trees [tree length 105; consistency index (CI) = 0.74] and the consensus showed partial polytomies among the species of Campellolebias, Cynopoecilus, and Leptolebias, and an unsolved polytomy among all species of Notholebias. The addition of continuous characters resulted in a single tree (tree length 110.064; CI = 0.74) with a completely resolved phylogeny for all species of Cynopoecilus and Leptolebias, but the relationships among the species of Campellolebias and Notholebias remain unresolved (Fig. 1). The new rivulid described herein was recovered as sister group to all species of the genus Cynopoecilus and is assigned to that genus. The synapomorphies of the taxa obtained through phylogenetic analysis with 71 discrete characters and six continuous characters are listed in the Appendix 2.

Cynopoecilus Notabilis sp. nov.
(Figs 2–7)

Holotype

Paratypes
**Diagnosis**

*Cympopoeilus notabilis* is easily distinguishable from its congeners by several autapomorphies. The larger number of dorsal-fin rays (ch. 1: 20–23); the larger number of scales in a longitudinal series (ch. 3: 29–31); the larger number of transverse series of scales between the dorsal and pelvic-fin origins (ch. 4: 10–11); and the broad and approximately straight proximal portion of the epural and parhypural (Fig. 3 ch. 26: 0) diagnoses the species from all members of the Cynopoecilina. An elliptical to lanceolate caudal fin, much longer than deep (ch. 28: 1, Figs 2, 4) diagnoses the species from other members of the Cynopoecilina, except for the species of *Leptolebias*. The position of the second proximal radial of the dorsal fin between the 8th and 9th vertebrae (ch. 6: 8–9) diagnoses *C. notabilis* from other members of *Cynopoecilus*. Other characters useful in distinguishing *C. notabilis* from congeneric species are: the dorsal-fin origin at the vertical through the pelvic-fin origin in males (vs. dorsal-fin origin at the vertical through the genital bulb), the dorsal-fin origin at the vertical through the mid-length of the pelvic fin in females (vs. the dorsal-fin origin at the vertical through the anus), the anal-fin origin at the vertical between the bases of the 7th and
8th dorsal-fin rays in males (vs. the anal-fin origin at the vertical between the bases of the 2nd and 3rd dorsal-fin rays); the presence of a mid-lateral longitudinal black stripe on the head in males, with stripe passing close to the ventral border of eye and united to the lower black lateral body stripe at the pectoral-fin base and continuing anteriorly in a straight line reaching the articulation of the lower jaw (Fig. 2, vs. absence of such stripe); the ‘V’-shaped mark ventrally on the head, along the ventral margins of dentary in both sexes) (Fig. 5, vs. absence of this mark); the red brown stripe covering the base of all dorsal-fin rays and limited dorsally by a light yellow line in males (Fig. 2, vs. the dorsal fins spotted at its base); the nine or ten vertical bars from the region immediately posterior to the opercle to the caudal peduncle in females (Fig. 4, vs. the lateral surface of the body with a broad black stripe or black marks), and the black distal portion of the pelvic fin in males (Fig. 5, vs. pelvic fin brownish grey or dark grey).

Description

Morphometric data in Table 4. Males larger than females; largest male 40.5 mm SL; largest female 31.5 mm SL.
External morphology

See Figures 2, 4, 5 for general external morphology. Body elongate and slightly compressed. Greatest body depth near pelvic-fin base. Dorsal profile of head nearly straight. Ventral profile of head smoothly convex from tip of lower jaw to opercle in males, nearly horizontal and straight, and forming an angle of approximately 45° relative to the ventral profile of head in females. Lower jaw projecting far beyond upper jaw. Dorsal body profile convex from supraoccipital to dorsal-fin base; nearly straight along dorsal-fin base through caudal-fin base. Ventral body profile nearly straight to slightly convex from head through caudal-fin base.

Pectoral fin elliptical with posterior margin reaching vertical through urogenital region in males and vertical just beyond pelvic-fin base in females. Pectoral-fin rays 13 (6), 14 (12), 15* (9).

Pelvic fin pointed surpassing anus; reaching urogenital region in males and anus in females, not reaching anal-fin origin. Pelvic-fin rays 5 (3), 6* (24). Pelvic-fin bases in close proximity. Urogenital papillae bulb-shaped attached to anterior margin of anal fin in males (Figs 5, 6a) and with prominent pocket-like structure overlapping anterior margin of anal fin in females (Fig. 6b).

Dorsal-fin rays 20 (4), 21 (5), 22*(15), 23 (2). Dorsal fin in males larger than females. Dorsal fin long and pointed with most rays longer than greatest body depth; two anteriormost and three posteriormost rays shorter; two or three rays of posterior portion of dorsal fin longest, reaching posteriorly beyond half-length of caudal fin in males. Dorsal fin origin at vertical through pelvic-fin origin in males. Dorsal fin slightly pointed with two anteriormost and one posteriormost rays shorter in females. Dorsal fin origin through mid-length of pelvic fin in females.

Anal-fin rays 24 (1), 25 (2), 26 (8), 27* (9), 29 (1). Seven to nine anteriormost anal-fin rays unbranched followed by 18 to 20 longer branched rays in males (7) and 16 to 19 in females (13). Anal fin in males larger than females. Anal fin long and pointed with epidermis thickened anteriorly; length of most rays nearly equal to body depth in males; two or three rays in posterior portion of fin largest, reaching posteriorly beyond mid-length of caudal fin. Anal fin with membrane interrupted to isolate anterior and posterior portions of fin in males. Anal fin in females slightly pointed.

Caudal fin pointed, longer than deep. Caudal-fin rays 28* (1), 29 (2), 30 (8), 31 (7), 32 (6), 33 (3).

Squamation and contact organs

Scales large, cycloid. Body and head entirely scaled except on anteroventral surface of head. Scales of flank and pectoral fin with contact organs in males. Dorsal and anal-fin bases without scales. Two lines of scales covering anterior portion of caudal fin. Frontal squamation E-patterned; E-scales not overlapping. Supraorbital scales absent. Longitudinal series of scales 29 (4), 30 (8), 31* (15); transverse series of scales 10 (1), 11* (26); scale rows around caudal peduncle 16* (26).

Figure 6. Cynopoecilus notabilis sp. nov., ventral view, (A) male, UFRGS 16800, paratype, 40.5 mm SL; (B) female, UFRGS 16800, paratype, 23.9 mm SL. Abbreviations: UP, urogenital papillae, AF, anal fin. Scale bar = 1 mm.

Figure 7. Anal-fin skeleton of Cynopoecilus notabilis sp. nov., lateral view, anterior to left, (A and B) male, paratype, UFRGS 16302, 28.5 mm SL; (C) female, paratype, UFRGS 16301, 24.1 mm SL. Arrow indicates the first proximal radial. Scale bar = 1 mm.
Neuromasts
Transverse row of four or five neuromasts on caudal-fin base. One to four neuromasts on each scale of lateral series. Cephalic neuromasts: supraorbital 2–3 + 9–13, parietal 1–2, anterior rostral 1, posterior rostral 1, infraorbital 2 + 14–18, preorbital 2–3, otic 2, postotic 3, supratemporal 1, ventral opercular 2, preopercular 2 + 14–18, mandibular 5–6 + 2, and lateral mandibular 5–7.

Osteology

Premaxilla with conical, pointed and curved inward teeth; four teeth in outer row, longer than inner rows. Premaxilla with ascending process rectangular longer than wide and alveolar process slightly curved with expansion ventral-posteriorly. Maxilla elongate, curved with two processes posteriorly forming groove. Dentary rectangular with conical, pointed and curved inward teeth; eight teeth in outer row longer than inner rows. Angulo-articular with coronoid process expanded dorsally and ventral process absent. Retroarticular triangular and small.


Urohyal slender with dorsal process narrow and straight. Dorsal hypohyal absent. Ventral hypohyal triangular. Anterior ceratohyal and posterior ceratohyal separated by cartilage. Six branchiostegal rays (2); two connected in anterior portion of anterior ceratohyal, three connected in posterior portion of posterior ceratohyal and one connected in posterior ceratohyal. Interhyal small, rectangular and completely cartilaginous. Gill-rakers on first branchial arch 3 + 9 (2).

Basihyal broad, triangular with ossified portion larger than basihyal cartilage. Three basibranchials with cartilage at tips; basibranchial 1 short, basibranchials 2 and 3 elongate. Hypobranchial 1 united to basibranchial 2 by single cartilage and distal edge with articular face anteriorly expanded; hypobranchial 2 united to basibranchials 2 and 3 by single cartilage; hypobranchial 3 rectangular united to basibranchial 3. Four ceratobranchials elongate with cartilage at tips.

Table 4. Morphometric data from holotype (included in the males data) and 21 paratypes of Cynopoecilus notabilis sp. nov

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<tr>
<td>Body depth</td>
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<tr>
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Ceratobranchial 5 elongate expanded posteromedially with conical, elongate and pointed teeth; outer row with larger teeth. Four epibranchials elongate, slightly curved with cartilage at tips. Epibranchials 3 and 4 with short uncinate process. Intercarcal cartilage elongate. Pharyngobranchial 2 and 3 broad. Pharyngobranchial 2 with teeth absent. Pharyngobranchial 3 large with conical, elongate and pointed teeth connected to tooth plate with conical, elongate and pointed teeth.

Pre-caudal vertebrae 13, caudal vertebrae 17 (2). Pleural ribs 12 (2). Caudal vertebrae with neural prezygapophyses vestigial; postzygapophyses slightly longer than prezygapophyses.

Shoulder girdle with elongate posttemporal with ventral process absent; supracleithrum elongate and wider than posttemporal; cleithrum well-developed with wide dorsal portion and rounded margin postero-dorsally. Coracoid and scapula rectangular; narrow cartilage between coracoid and lower pectoral fin radial with ventral expansion. Radials disc-shaped, thin, weakly ossified completely surrounded by cartilage. Pelvic girdle with pelvic bone pointed and cartilage at tips; ischial process absent.

Dorsal-fin origin between neural spines of vertebrae 8 and 9 (2) and with 21 proximal radials (2). Anal-fin origin along vertical between base of 7th and 8th dorsal-fin rays in both sexes; and situated between pleural ribs 10 and 11 in males and 11 and 12 in females (2). Anal fin with 20–21 proximal radials; first proximal radial short, wide and approximately straight with eight unbranched anal-fin rays thin and weakly attached (2) (Fig. 7). Sixth and seventh anteriormost anal-fin rays anteriorly curved in males (1) (Fig. 7).

Epural and parhypural elongate with proximal edge broad and approximately straight; postero-basal portion of last neural spine with small process bearing liga ment attached to bent anterior tip of epural; minute accessory cartilages on caudal fin; hypural plates fused without median fissure (Fig. 3).

Eggs
Eggs with chorion surface reticulate; chorion projections mushroom-shaped.

Colour in life
Males possess two longitudinal black stripes along lateral surface of body; one extending from upper termination of opercle to proximal base of midventral caudal-fin rays and covering two to three longitudinal rows of scales; and second from lowermost termination of the pectoral-fin base across belly and base of all anal-fin rays (Fig. 2). Body red brown above lateral black stripe; whitish between two longitudinal black stripes and ventrally on belly and caudal peduncle. Scales along upper lateral black stripe and on belly between upper and lower black stripes iridescent blue. Head red brown dorsally through the dorsal border of eyes and snout. Iris golden. Sides of head bearing three black stripes. One from the posterior margin of eye and continuing through mid-lateral body black stripe. Two black stripes united to lower lateral body stripe at pectoral-fin base; one continuing anteriorly in straight line passing close to ventral border of eye and reaching articulation of lower jaw; other ventrally bordering margin of opercle and then extending anteriorly close to vertical through posterior border of eye. Ventral margin of dentary bordered with black, forming V-shaped mark from ventral view (Fig. 5). Spaces between stripes iridescent blue on sides of head changing to whitish on ventral surface. Dorsal, anal and caudal fins covered with reddish brown spots over light yellow to orangish background. Dorsal fin bearing reddish brown stripe covering base of all fin rays; stripe limited dorsally by light yellow line, and becoming progressively dark orange through tips of fin rays, resulting in dark brown dorsal-fin border. Dorsal fin with five large roundish spots forming series just above light yellow line followed dorsally by irregularly distributed smaller spots. Anal fin bearing black line covering base of all fin rays; stripe delimited by yellow line ventrally and progressively becoming dark orange to tip of fin rays, resulting in dark brown anal-fin border. Anal fin covered with red brown spots; spots larger near anal-fin base. Caudal fin covered with reddish brown spots; spots larger near caudal-fin base. Pectoral fins without marking. Pelvic fins yellow to orangish, becoming dark brown to black in their distal half.

Females possess nine or ten vertical bars from region immediately posterior to opercle to caudal peduncle (Fig. 4). Most bars restricted to midventral lateral surface, except for four bars between dorsal and anal fins that reach bases of both fins. Dorsal half of body surface uniform brown, except for vertical bars between anal and dorsal fins. Scales green-blue iridescent on ventral half of body, over bars and grayish background. Belly white ventrally.

Head brown dorsally through to dorsal border of eyes and snout. Iris golden. Sides of head with distinct and variable reticulated pattern covering check and opercle. Dorsal, anal and caudal fins covered with black spots over translucent or reddish background. Dorsal fin and anal fin bearing large black spots near bases and with scattered smaller spots distally. Caudal fin with scattered spots on proximal half and without marks distally. Pectoral and pelvic fins black distally.

Distribution and ecological notes
Known only from the type locality; a temporary pool in upper portion of rio Gravataí basin, laguna dos Patos system (Fig. 8). The type locality had clear water over a muddy bottom covered by large amount of litter within
a dense fragment of wetland forest (Fig. 9) connected and adjacent to a swamp where was collected the two only known specimens of *Listrura depinnai* Villa-Verde, Ferrer & Malabarba 2013. *Cynopoecilus nigrovittatus* was not found syntopic with *C. notabilis*, but is abundant in the grassland area that surrounds this fragment of wetland forest.

**Etymology**

From Latin *notabilis* meaning notable or remarkable in reference to the morphology of the fins and coloration in males; a unique pattern among the species of *Cynopoecilus*. An adjective.

**DISCUSSION**

**MONOPHYLY AND RELATIONSHIPS AMONG THE SPECIES OF *CYNOPOECELUS***

*Cynopoecilus notabilis* was recovered as the sister group to all other species of the genus. The analysis without

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**Figure 8.** Map of southeastern and southern Brazil showing laguna dos Patos (*), and type localities of the species of *Campellolebias* (stars), *Leptolebias* (squares), *Notholebias* (triangles), *Cynopoecilus notabilis* sp. nov. (circle 1), *C. nigrovittatus* (circle 2), *C. intimus* (circle 3), *C. melanotaenia* (circle 4), *C. fulgens* (circle 5), and *C. multipapillatus* (circle 6).

**Figure 9.** View of type locality of *Cynopoecilus notabilis* sp. nov., a temporary pool on wild life reserve ‘Refúgio de Vida Silvestre Banhado dos Pachecos’, rio Gravataí basin, laguna dos Patos system, Rio Grande do Sul, Brazil.
continuous characters resulted in the recognition of C. fulgens + C. multipapillatus as forming a clade in a polytomy with the remaining species, except for C. notabilis. The use of continuous characters resulted in a completely solved phylogeny for all the species of Cynopoecilus (Fig. 1).

Costa (2002a) listed 13 synapomorphies for Cynopoecilus. Among these, nine remain as diagnostic for the genus in the analyses (ch. 22: 1; ch. 27: 1, secondarily absent in C. fulgens and C. multipapillatus; ch. 32: 1; ch. 34: 1; ch. 35: 1; ch. 47: 1; ch. 51: 1; ch. 71: 1; ch. 74: 1). The anterior part of the anal fin of male thickened, probably with glandular tissue (34: 1) is homoplastically found in Leptolebias marmoratus (Ladiges, 1934), as already noted by Costa (2008). Two new synapomorphies are recognized for Cynopoecilus. The iris yellow to yellowish brown in males (ch. 67: 0) is interpreted as a reversal in the subtribe Cynopoecilina. The higher number of anal-fin rays (ch. 2: 23 or 24) is derived and an unambiguous synapomorphy of Cynopoecilus with further transformation among the species of the genus, reaching up to 29 anal-fin rays in C. notabilis.

Three characters listed by Costa (2002a) as synapomorphies for Cynopoecilus were not included in the analysis: the presence of a concavity on the median portion of the posterior border of autopallatine was found to be variable and was not analyzed; the zig-zag display during male courtship behaviour is unknown for C. notabilis; and the presence of several minute rays at the anterior portion of the anal fin is considered herein redundant with the number of anal-fin rays attached to the first proximal radial (ch. 32) and thus was not considered as a separate character. The posteriorly curved 10th to 12th anal-fin rays in males previously assigned as a synapomorphy for Cynopoecilus by Costa (2002a) is absent in C. notabilis and unknown in C. intimus Costa, 2002, thus being interpreted as an ambiguous synapomorphy for the species of Cynopoecilus excluding C. notabilis or for the species of Cynopoecilus excluding C. notabilis and in C. intimus (ch. 36: 1).

PHYLOGENETIC IMPLICATIONS ON THE DIAGNOSIS OF OTHER CLADES OF THE CYNOPOECILINA

The addition of a new species to the phylogeny of Cynopoecilina did not change the hypothesized relationships among its genera, but requires a reinterpretation of character transformation and of some characters diagnosing clades within the subtribe. Cynopoecilus notabilis shares three characters previously considered by Costa (2006a) as synapomorphies for Campellolebias: (ch. 24: 1) the posterobasal portion of last neural spine with a small process serving as the attachment point of ligament attached to the bent anterior tip of the epural; (ch. 69: 1) the dark lateroventral stripes on head; and (ch. 71: 1) the midventral stripe on the head and body) and one character previously considered by Costa (2008) a synapomorphy for Leptolebias: [ch. 28: 1] the elongate caudal fin, longer than deep. The placement of the new species in Cynopoecilus requires a reinterpretation of these characters within the phylogeny of the Cynopoecilina. The first three characters are no longer diagnostic for Campellolebias, but rather synapomorphies for Campellolebias plus Cynopoecilus, with subsequent loss in the ancestor of the clade containing C. fulgens, C. multipapillatus, C. nigrovittatus, C. melanotaenia and C. intimus. The fourth character is interpreted as a parallel gain in C. notabilis and members of Leptolebias. Although three of the synapomorphies of Campellolebias previously listed by Costa (2006a) were removed from the diagnosis, the genus is still corroborated with high support (Bremer support = 7) in the two analyses herein. The species of the genus share seven unique synapomorphies (ch. 30: 0; ch. 31: 1; ch. 33: 1; ch. 37: 1; ch. 50: 1; ch. 61: 0).

Only one of the synapomorphies listed for Notholebias by Costa (2008) was recovered in the present analysis: the narrow, approximately rectangular basihyal (ch. 14: 1). Costa (2008) listed two additional synapomorphies for the genus: the presence of iridescent bars or vertical lines on the caudal fin in males; however, information on that feature is not available for all species and it was not included in the present analyses; and the presence of pronounced contact organs on inner most the surface of the pectoral fin in males. Costa (2008: ch. 57), codified this last character on three states (contact organs absent, reduced or well-developed on most inner surface of pectoral fin in males), but we codified it only in two states (our ch. 59 – absence or presence of contact organs on the inner surface of pectoral fin in males). Our results support the presence of contact organs as primitive in the Cynopoecilina, and the absence of these structures as independently acquired in the clade consisting of Leptolebias aureoguttatus (Da Cruz, 1974), L. citrinipinnis (Costa, Lacerda & Tanizaki, 1988), L. marmoratus, and L. splendens (Myers, 1942) and the clade formed by Cynopoecilus fulgens, C. intimus, C. multipapillatus, C. nigrovittatus, and C. melanotaenia.

The monophyly of Leptolebias is supported by two unique synapomorphies previously listed by Costa (2008): a single anterior supraorbital neuromast (ch. 57: 1) and the distal third of the dorsal fin in males without dark pigmentation (ch. 76: 1). The elliptical to lanceolate caudal fin, much longer than deep (ch. 28: 1) was also recovered as a synapomorphy for...
Leptolebias, but is homoplasic present in Cynopoecilus notabilis.

**Is the phylogeny of Cynopoecilus indicative of a recent and successful invasion of the Pampa biome by the Cynopoecilina?**

*Cynopoecilus notabilis* was found exclusively inhabiting flooded and shaded areas of a fragment of wetland forest, while all the remaining species of *Cynopoecilus* are found abundant in flooded grasslands of the Pampa biome (IBGE Instituto Brasileiro de Geografia e Estatística, 2004) in southern Brazil. This pattern of habitat use was observed in the ‘Refúgio de Vida Silvestre Banhado dos Pachecos’ itself, with *C. notabilis* inhabiting the flooded and shaded areas of the forest while *C. nigrovittatus* is common and abundant in the surrounding insolated grasslands. The two species were never found to occur together, regardless the execution of monthly collections in the area for ecological studies by UFRGS team.

Given that the species of *Campellolebias*, *Leptolebias* and *Notholebias* are typically found in the Atlantic Forest biome associated with forested areas of southeastern and southern Brazil (Costa, 1995b, 2008) along with the Serra Geral Formation, a simple optimization of these environments in the obtained phylogeny (Fig. 1) supports the hypothesis that the invasion of the Pampa biome constitutes an important step in the evolution and diversification of *Cynopoecilus*. However, we have not found other fish groups whose distributions are associated to the Atlantic Forest and Serra Geral Formation along the Brazilian coast that share a similar pattern of distribution and relationships as demonstrated by the species of *Cynopoecilus*. *Cynopoecilus notabilis* has been found associated with two other fish taxa typical of the Atlantic Forest [these being one species of the siluriform genus *Listrura* (Villa-Verde, Ferrer & Malabarba, 2013) and one undescribed gymnotiform species of the *Gymnotus pantherinus* group (personal observation)], but these species are so far known only from these wetland forest fragments and do not possess other relatives occurring in the grasslands of the Pampa biome. Other fish taxa typical of the Atlantic Forest (e.g. *Deuterodon*, *Hollandichthys* and *Scleromystax*) have their southern limit of distribution occurring to the north of the distribution of *C. notabilis*, and no close relatives registered to the Pampa biome. So far the pattern described above is exclusive for the Cynopoecilina and *Cynopoecilus*.

**Conservation status**

Five species of the subtribe Cynopoecilina are threatened of extinction and included in the category ‘vulnerable’ – *Campellolebias brucei*, *Leptolebias opalescens* (Myers, 1942), *L. marmoratus*, and *L. splendens*, *Notholebias minimus* (Myers, 1942) – due to restricted area of distribution with severely fragmented habitats or because they are known to exist at only a single location. In addition they experience extreme fluctuations of their areas of occupancy and number of mature individuals (IUCN Red List of Threatened Species, 2013). The remaining species of Cynopoecilina were not formally evaluated by IUCN, but the restricted ranges of some of them and the possible anthropic disturbance in their environments make them potentially threatened. Although *Cynopoecilus notabilis* is known only from the type locality with a distribution of less than 20 km²; its occurrence within a conservation unit (Refúgio de Vida Silvestre Banhado dos Pachecos) reduces the immediate likelihood of adverse anthropogenic impacts.

**Acknowledgements**

We are grateful to people cited as collectors of the type material for help in field works and SEMA (Secretaria Estadual do Meio Ambiente) for collection permits and logistics support. Richard P. Vari and two referees offered valuable suggestions to this manuscript. Thanks for financial support from CNPq (Proc. 300705/2010-7 to JF and 477318/2012-6 to LRM) and CAPES (to JMW).

**References**


APPENDIX 1

Character list used in the phylogenetic analyses. Distribution of the characters states among terminal taxa is presented in the Tables 2 and 3.

Continuous (characters 1–6)

States for each species correspond to the observed range of a given character. Under the character description, the minimum observed value is equivalent to state 0 and the maximum observed value is equivalent to state 1. Intermediate values are decimal values between 0 to 1, proportionally calculated within the range of minimum and maximum values. Both values, the observed range and the assigned state for that range, are provided in the matrix for each species in the Table 2.

1. Dorsal fin, rays, number: (0) 11, (1) 23.
2. Anal fin, rays, number: (0) 14, (1) 29.
3. Scales, longitudinal series, number: (0) 25, (1) 31.
4. Scales, transverse series between dorsal and pelvic-fin origin, number: (0) 7, (1) 11.
5. Cephalic neuromasts, supraorbital series, number: (0) 6, (1) 20.
Costa (1998, 2006a, 2008) delimited three different classes of intervals to describe the states of this character in three papers dealing with the phylogeny of the Cynopoecilina. Costa (1998) splits the range in two states: $0 = 6–10$ and $1 = 12–25$ supraorbital neuromasts. Costa (2006a) recognized two states: $0 = 6–7$ and $1 = 12–19$ for supraorbital neuromasts. Costa (2008) considered three partially overlapping states: state $0 = 6–7$, state $1 = 9–13$ and state $2 = 12–16$ for supraorbital neuromasts. We treat this character as continuous and provide the range observed for each species as character states in the matrix.

6. Dorsal fin, second proximal radial, relative position to vertebrae (Costa, 1998: ch. 111; 2008: ch. 24): (0) between 7th and 8th vertebrae, (1) between 18th and 19th vertebrae. Even though this character is not a count properly, it presents a continuous range of variation in the position of the dorsal fin relative to the neural spines. Costa (2008) divided this variation in three arbitrary states: (0) between the 18th and 19th vertebrae, (1) between the 12th and 14th vertebrae, and (2) between the 9th and 11th vertebrae. We found a continuous variation from the 7th to 19th vertebrae, and treat this character as continuous.

7. Circumorbital series, dermosphenotic (Costa, 1990a, b; 1998: ch. 93; 2006a: ch. 1; 2008: ch. 1): (0) present, (1) absent.

8. Vomer, lateral wings, shape (Costa, 1998: ch. 79; 2006a: ch. 2; 2008: ch. 2): (0) broad, (1) narrow.

9. Vomer, teeth (Costa, 1998: ch. 76; 2006a: ch. 3; 2006b: ch. 4; 2008: ch. 3): (0) present, (1) absent.

10. Angulo-articulare, ventral process, shape (Costa, 1990a, b; 1998: ch. 16; 2006a: ch. 4; 2008: ch. 2): (0) long and wide, (1) long and narrow, (2) short and wide, (3) vestigial or absent. This character was treated by Costa (1998) as: not reduced, larger than coronoid process (state 0), slightly reduced, about equal size to coronoid process (state 1), reduced, smaller than coronoid process (state 2), and vestigial or absent (state 3).

11. Mesopterygoid, extension and relative position to quadrate (Costa, 2006a: ch. 5; 2008: ch. 4): (0) long, reaching metapterygoid and overlapping quadrate, (1) short, posterior tip at vertical through middle of quadrate, ventral portion overlapping quadrate, (2) very short, posterior tip at vertical through anterior portion of quadrate.

12. Symplectic, shape (Costa, 1998: ch. 43; 2006a: ch. 6; 2008: ch. 8): (0) short and deep, (1) long. This character was treated as short (state 0) vs. elongate (state 1) by Costa (1998).

13. Preopercle, dorsal portion, shape (Costa, 1990a, b, 2006a: ch. 7; 2008: ch. 9): (0) broad and rounded, (1) narrow and pointed. Costa (2008) used two additional characters related to the suspensorium: the posterior process of quadratoarticular narrow or wide (Costa, 2008: ch. 5) and the median portion of the autopalatine not constricted vs. constricted (Costa, 2008: ch. 7). We found these characters variable among analyzed specimens and were unable to recognize discrete states. These characters were not included in this analysis.

Hyoid and branchial arches


15. Interhyal, tissue (Parenti, 1981; Costa, 1990a, b; 1998: ch. 58; 2006a: ch. 9; 2008: ch. 11): (0) ossified, (1) cartilaginous. This character was treated as ossified (state 0) vs. cartilaginous or absent (state 1) by Costa (1998).


17. Epibranchials, length (Costa, 1998: ch. 61; 2006a: ch. 11); 2008: ch. 13): (0) short, (1) long. This character was treated as slender (state 0) vs. widened (state 1) epibranchials 1–3 in Costa (1998).

18. Third epibranchial, uncinate process, length (Costa, 1998: ch. 62; 2006a: ch. 12; 2008: ch. 14): (0) long, (1) short. This character was treated as not reduced (state 0) vs. reduced (state 1) in Costa (1998).

19. First hypobranchial, proximal edge (Costa, 1998: ch. 70; 2006a: ch. 13; 2008: 15): (0) bifid, terminating in cartilage united to the second basibranchial and another smaller cartilage united to the first basibranchial, (1) plain, terminating in single cartilage united to the second basibranchial. This character was treated as a cartilage extending along the entire medial border (state 0) vs. a cartilage with two distinct facets (state 1) by Costa (1998).

20. First hypobranchial, distal edge, articular face (Costa, 2006a: ch. 14; 2008: ch. 16): (0) articular face restricted to cartilaginous head of first ceratobranchial, (1) articular face anteriorly expanded.

This character was treated as elongate (state 0) vs. deep and short (state 1) in Costa (1998). Costa (1995a: ch. 8) listed the ‘urohyal slender, its largest depth about 20% of its longest length’ as an autapomorphy for *Cynopectus* and, subsequently Costa (2002a: 13) listed a slender urohyal (vs. deep [sic]) as a synapomorphy for *Cynopectus*. This character was not included in recent phylogenies for *Leptolebias* and *Notholebias* (Costa, 2008), and the state of this character is consequently unknown in both genera. As the character was previously assigned as an autapomorphy for *Cynopectus*, we have tentatively included this character in the analysis.

**Vertebræ and caudal fin**

23. Caudal vertebrae, neural prezygapophyses (Costa, 1990a, b; 2006a: ch. 16; 2008: ch. 18): (0) present and elongate, (1) vestigial or absent.


25. Caudal skeleton, hypurals, fusion (modified from Costa, 1998: ch. 103; 2006a: ch. 18; 2008: 20): (0) two symmetrical plates, (1) plates fused, showing or not vestiges of median fissure.

Costa (1998) divided the character ankylosis of the hypurals in four states: two symmetrical plates (state 0); plates in close proximity, sometimes partially ankylosed (state 1); plates united but with a persistent median fissure (state 2); and complete ankylosis forming a single plate (state 3). Costa (2006a) divided the character in three states: state 0 without modifications; state 1 equivalent to state 2 of Costa (1998); and state 2 equivalent to state 3 of Costa (1998). Costa (2008: ch. 20) considered three states: state 0 = two symmetrical plates; state 1 = plates fused with vestiges of a median fissure; and state 2 = 2 equivalent to state 3 of Costa (1998). We found these latter descriptive measurements of the elongation subjective. This character was split in to four states by Costa (2008: ch. 43): state 0 = about rectangular, extremity rounded and short; state 1 = about rectangular, extremity pointed; state 2 = about triangular, slightly elongated; and state 3 = about triangular, long. We found these latter descriptive measurements of the elongation subjective, and used the two character states as proposed in Costa (2006b).


27. Cartilages, minute accessory cartilages on caudal fin (Costa, 1998: ch. 107; 2002a): (0) absent, (1) present.

This character was treated as absence (state 0) vs. presence of a few, minute caudal cartilages (state 1) by Costa (1998).

28. Caudal fin, shape (Costa, 2008: 45): (0) round to subtruncate, approximately as deep as long, (1) elliptical to lanceolate, much longer than deep.

This character was listed as ‘caudal fin elongate, longer than deep (vs. short, deeper than long)’ [sic] in the diagnosis of *Leptolebias* by Costa (2008: 149), but was described as elliptical to lanceolate, much longer than deep, vs. round to subtruncate, approximately so deep as long in the Supplementary material of that paper.

**Anal fin**

29. Anal fin in males, shape (Costa, 2006a: ch. 36; 2006b: ch. 69): (0) rounded, (1) pointed.

This character was divided in to three states in Costa (2006a): state 0 = rounded and short; state 1 = pointed and slightly elongated; and state 2 = pointed and long, however this descriptive measurement of the elongation is subjective. This character was split in to four states by Costa (2008: ch. 43): state 0 = about rectangular, extremity rounded and short; state 1 = about rectangular, extremity pointed; state 2 = about triangular, slightly elongated; and state 3 = about triangular, long. We found these latter descriptive measurements of the elongation subjective, and used the two character states as proposed in Costa (2006b).


This character was treated in Costa (1998) as elongate, approximately same length and shape as second and third radials (state 0) vs. approximately triangular and much smaller than second and third radials (state 1).


32. Radials, first proximal radial, rays attached, number (Costa, 1995a, 1998: ch. 115, ch. 131; 2002a; 2008: ch. 25): (0) 2, (1) up to 8 or 9 min rays.

This character was divided in two characters in Costa (1998) who separately considered the number and the size of the rays attached to the first proximal radial [(4) first eight anal-fin rays attached...
33. Anal-fin rays in males, two anteriormost rays, ligamentous connections to radials (Costa, 1995b, 1998: ch. 118; 2006a: ch. 22; 2008: ch. 26): (0) rays thin and weakly connected to proximal radials, with small cartilaginous distal radials, (1) rays thickened and strongly attached to proximal radials, with large cartilaginous distal radials. This character was treated as the absence (state 0) vs. presence (state 1) of a strong ligamentous connections between first anal-fin ray and first and second proximal radials simultaneously by Costa (1998).

34. Anal-fin rays in males, anterior elements, epidermis (Costa, 1995a, 2002a, 2008: ch. 41): (0) not modified and similar to the posterior portion of the fin, (1) thickened (probably glandular in nature).

35. Anal-fin rays in males, sixth and seventh rays, shape (Costa, 1995a, 1998: ch. 124; 2002a): (0) Not distinctively curved, (1) anteriorly curved. This character was treated as approximately straight (state 0) vs. anteriorly curved or curved (state 1) in Costa (1998, 2008: ch. 27).

36. Rays of anal fin in males, 10th-12th rays, shape (Costa, 1995a, 1998: ch. 125; 2002a): (0) not distinctively curved, (1) distinctively curved posteriorly. This character was treated as approximately straight, not overlapping the posterior rays (state 0) vs. curved, directed anteriorly, and overlapping the posterior rays (state 1) in Costa (1998).

37. Muscles of anal-fin base, inclinators anales 1–3, shape (Costa, 1995b; Costa 1998: ch. 186; Costa, 2006a: ch. 30; 2008: ch. 35): (0) narrow, (1) expanded laterally to form fan-shaped structure.

38. Anal-fin membrane in males, shape (Costa, 2006a: ch. 34; 2008: ch. 40): (0) continuous, (1) interrupted to isolate anterior and posterior portions of fin.

Dorsal fin

39. Dorsal fin in males, shape (Costa, 2006a: ch. 35; 2008: ch. 42): (0) rounded, (1) pointed.

Pectoral fin

40. Shoulder girdle, posttemporal, ventral process (Costa, 1998: ch. 132; 2006a: ch. 23; 2008: ch. 28): (0) present, (1) absent. This character was treated as elongate (state 0) vs. vestigial or absent (state 1) in Costa (1998).

41. Shoulder girdle, supracleithrum, size (Costa, 1990a, b; 1998: ch. 134; 2006a: ch. 24; 2008: ch. 29): (0) short, about one half of longitudinal length of the posttemporal (1) long, approximately as long as the posttemporal.

42. Shoulder girdle, cleithrum, posterior flange (Costa, 1998: ch. 135; 2006a: ch. 25; 2008: ch. 30): (0) present, (1) vestigial or absent.

43. Shoulder girdle, lower pectoral fin radial and coracoid, shape (Costa, 2006b: ch. 59; 2008: ch. 32): (0) wide, (1) narrow, with narrow ventral expansion of cartilage.

44. Shoulder girdle, radials, shape (Costa, 1990a, b; 1998: ch. 145; 2006a: ch. 26; 2008: ch. 31): (0) cubic form, well-ossified (1) disc-shaped, thin, weakly ossified. This character was treated as robust, cubical (state 0) vs. thin, scale-like (state 1) by Costa (1998).

Pelvic fin

45. Pelvic fins, insertion of contralateral fins (Costa, 2006a: ch. 37; 2008: ch. 44): (0) in close proximity, (1) separated by short interspace.

46. Pelvic girdle, ischial process (Costa, 2006b: ch. 61; 2008: ch. 33): (0) present, (1) absent.

47. Pelvic fin in males, size (Costa, 1995a, 1998: ch. 152; 2002a): (0) not reduced, reaching anal-fin base, (1) reduced, not reaching anal-fin origin. Costa (1995a) described this character in the diagnosis of the genus *Cynopoecilus* as ‘reduced pelvic fin, its tip reaching the anus in males and not reaching it in females’. In Costa (1998) it was treated as ‘short, its tip not surpassing the anterior portion of the anal fin vs. elongate, its tip reaching the central or the posterior portion of the anal fin. Our treatment is comparable to that used by Costa (2002a) in the diagnosis of *Cynopoecilus*.

Urogenital papilla

48. Muscular fibres at base in males, arrangement (Costa, 1998: ch. 185; 2006a: ch. 29; 2008: ch. 34): (0) not circular, (1) circular, forming an ejaculatory pump. This character was treated in Costa (1998) as the presence of muscular fibres transversely arranged in front of the anal-fin support and above the urogenital papilla base; thereby probably constituting an ejaculatory pump (state 0) or the absence of this system (state 1).


Urogenital papilla in males, extension (Costa, 1995b, 1998: ch. 156; 2006a: ch. 32; 2008: ch. 37): (0) short, (1) long, to form pseudogonopodium. This character was treated as the presence of a ‘tubular, laterally compressed copulatory organ in the anterior portion of the anal fin of males, formed by the first two anal-fin rays, which are separate from the remaining portion of the fin by a rupture in the fin membrane’ (state 0) or its absence (state 1) by Costa (1998).

Urogenital papilla in males, bulb-shaped structure (Costa, 1998: ch. 171; 2006a: ch. 38): (0) absent, (1) present. This structure was referred as ‘fleshy baglike’ as a single character along with its position ‘proximate to anal-fin origin’ (Costa, 1995a) (see ch. 49 above), and as ‘pocket-like structure’ (Costa, 1998, 2008).

Urogenital papilla in females, shape (Costa, 1998: ch. 172; 2006a: ch. 33; 2008: ch. 39): (0) a transverse gap, (1) a prominent pocket-like structure overlapping anterior anal-fin origin. This character was treated as small pocket-like structure, not contacting anal-fin origin (state 0) and a prominent pocket-like structure, slightly overlapping the anterior portion of the anal fin (state 1) by Costa (1998).

Squamation

Head, frontal scales, arrangement (Parenti, 1981; Costa, 1990a, b; 1998: ch. 174; 2006a: ch. 38; 2008: ch. 46): (0) circular, (1) transversal.

Head, E-scales, relative position (Costa, 1998: ch. 175; 2006a: ch. 39; 2008: ch. 47): (0) overlapped, (1) not overlapped.

Head, supraorbital scales (Costa, 1998: ch. 176; 2006a: ch. 40; 2008: ch. 48): (0) present, (1) absent. This character is treated as scaled (state 0) and with one or three small scales or naked (state 1) in Costa (1998: ch. 176). Costa (2008: ch. 48) assigned state 0 to ‘one or more large scales’ and state 1 for ‘sometimes one small scale, often scales absent.’ We find these latter states difficult to recognise and kept the character as proposed by Costa (2006a). We found the character polymorphic in Cynopoecilus fulgens and C. multipapilatus.

Neuromasts and contact organs

Caudal-fin base, neuromasts, number (Costa, 1998: ch. 184; 2006a: ch. 42): (0) 1–2, (1) 4–7. Costa (2006a) reported 1 or 2 neuromasts in Cynopoecilus melanotaenia. We have found 5 to 7 in the species.

Supraorbital, neuromasts, anterior series, number (Costa, 2008): (0) 2 or 3, (1) one.

Scales of flank in males, contact organs (Costa, 2006a: ch. 43): (0) absent, (1) present.

Pectoral fin in males, contact organs (Costa, 2006a: ch. 44; Costa, 2008: ch. 57): (0) absent, (1) present. This character was divided into three states by Costa (2008: ch. 57): 0 = absent; 1 = minute, on uppermost fin rays; and 2 = pronounced, on most fin rays.

Reproduction and courtship behaviour

Egg, chorion surface, shape (Costa, 1990a, b, 1998: ch. 234; 2006a: ch. 53): (0) plain to verrucate, (1) reticulate.

Egg, chorion projections, shape (Costa, 1990a, b, 1998: ch. 235; 2006a: ch. 54): (0) spine to hair-like, (1) mushroom-like.

Insemination (Costa, 1995a, 1998: ch. 236; 2006a: ch. 55): (0) external, (1) internal.

Courtship behaviour in males, dorsal and anal fins, position (Costa, 1990a, b, 1998: ch. 238; 2006a: ch. 56): (0) antero-posteriorly expanded, (1) twisted.

Courtship behaviour in males, display (Costa, 1998: ch. 239, ch. 240; 2006a: ch. 57; 2008: ch. 74, ch. 75): (0) Not distinctive, (1) coiling retrorse motion during courtship, (2) Zig-zag display during courtship. Costa (1998, 2008) treated states 1 and 2 as described herein as two distinct characters in his analysis. We consider as alternative states of the same character.

Habitat

Habitat, seasonality (Costa, 1990a, b, 1998: ch. 242; 2006a: ch. 58): (0) non-long diapause stages, lifecycle in non-seasonal habitats, (1) long diapauses stages, lifecycle in seasonal pools.

Coloration

Eye, iris, median portion (Parenti, 1981; Costa, 1990a, b, 1998 ch. 204; 2006a: ch. 47): (0) distinctive dark marks absent, (1) obliquus, nearly vertical bar through centre of eye.

Eye in males, iris, iridescent colour (Costa, 1998: ch. 203; 2006a: ch. 48): (0) yellow to yellowish brown, (1) greenish blue to yellowish green.

Head in males, opercular region, colour pattern (Costa, 2008: 68): (0) red reticulation, (1) red bars. Costa (2008: 68) describes three states (0 – no pattern; 1 – bars; 2 – reticulate) but assessed only states 0 and 1 in the matrix.

Head in males, lateroventral portion, dark pigmentation pattern (Costa, 1995a; 1998: ch. 210; 2006a: ch. 51; Costa, 2008: ch. 67): (0) not forming stripes, (1) forming a stripe.
Costa (2008: ch. 67) described two states, (0) not distinctively pigmented, (1) concentrated to form midventral stripe, but marks state 2 (unknown) for all species of *Leptolebias*, *Campellolebias*, and *Cynopoecilus*. We do not know what state 2 means and changed the character to two states, the absence (0) or presence (1) of pigmentation following the information given in Costa (1995a, 1998, 2006a).

70. Head and trunk in males, midventral portion, dark pigmentation pattern (Costa, 1990a, b, 1998: ch. 210[part]; 2006a: ch. 50): (0) not distinctively pigmented, (1) pigmentation concentrated to form midventral stripe.

71. Head and body in males, flank from tip of lower jaw to caudal-fin base, (Costa, 1995a; 1998: ch. 190; 2002a): (0) not distinctively pigmented, (1) with broad longitudinal black stripe.

72. Body flank in males, iridescent colour pattern (Costa, 2006a: ch. 45): (0) iridescence over flank, (1) vertical rows of iridescent dots, (2) longitudinal rows of small iridescent spots.

73. Body flank in females, dark pigmentation (Costa, 1998: ch. 194; 2006a: ch. 46): (0) homogeneous, (1) vertically elongated blotches or bars, (2) narrow horizontal lines between scales, (3) broad stripe. Costa (1998: ch. 194) described state 0 – present; state 1 – reduced to minute dots of dark pigmentation; state 2 – absent.


75. Caudal peduncle, melanophores pattern (Costa, 2006a: ch. 52): (0) not distinctively aggregated, (1) aggregated to form black spots on posterior zone.

76. Dorsal fin in males, distal third, melanophores (Costa, 2008): (0) not concentrated, (1) strongly concentrated to form dark grey to black stripe.

77. Dorsal and anal fins in males, subdistal region, melanophores (Costa, 1995a, 2006a: ch. 49): (0) not concentrated, (1) strongly concentrated to form dark grey to black stripe.

APPENDIX 2

List of synapomorphies and autapomorphies.

*Subtribe Cynopoecilina*

Absence of vomerine teeth (ch. 9: 1); ventral process of the angulo-articular vestigial or absent (ch. 10: 3); symplectic elongate (ch. 12: 1); muscular fibres circularly arranged around the base of the urogenital papilla forming an ejaculatory pump (ch. 48: 1); presence of contact organs on the scales of the flank in males (ch. 58: 1); eggs with reticulate chorion surface (ch. 60: 1).

New synapomorphies discovered for Cynopoecilina: absence of supraorbital scales (ch. 55: 1, reversed to present in *Cynopoecilus nigrovittatus* plus *C. melanotaenia* and polymorphic in *C. fulgens* and *C. multipapillatus*); presence of four to seven neuromasts on the caudal-fin base (ch. 56: 1, reversed to state 0, one or two neuromasts, in *Leptolebias citrinipinnis* (Costa et al., 1988) plus *Leptolebias marmoratus* (Ladiges, 1934) plus *Leptolebias splendens* (Myers, 1942), mushroom-like projections on the chorion membrane of the eggs (ch. 61: 1, reversed to state 0, spine to hair-like projections, in the species of *Campellolebias*); dorsal and anal fins twisted during courtship in males (ch. 63: 1, reversed to state 0, antero-posteriorly expanded in *L. marmoratus*); iris greenish blue to yellowish green in males (ch. 67: 1, reversed to state 0, yellow to yellowish brown in *Cynopoecilus*).

Genus *Notholebias*

Basihyal narrow and nearly triangular (ch. 14: 1).

Clade *Leptolebias + Campellolebias + Cynopoecilus*

Absence of the dermosphenotic (ch. 7: 1).

Genus *Leptolebias*

Presence of a single anterior supraorbital neuromast (ch. 57: 1); lack of dark pigments along the distal third of the dorsal fin of males (ch. 76: 1); elliptical to lanceolate caudal fin, much longer than deep (Ch. 28: 1, acquired independently in *Cynopoecilus notabilis*).

Clade *Leptolebias aureoguttatus* (Cruz, 1974) + *L. citrinipinnis* + *L. marmoratus* + *L. splendens*

Absence of contact organs in the pectoral fin of males (ch. 59: 0; acquired independently in the clade formed by *Cynopoecilus fulgens*, *C. intimus* Costa, 2002, *C. melanotaenia*, *C. multipapillatus*, *C. nigrovittatus*.

Clade *Leptolebias citrinipinnis* + *L. marmoratus* + *L. splendens*

Presence of one or two neuromasts on the caudal-fin base (ch. 56: 0).

Clade *Leptolebias citrinipinnis* + *L. marmoratus*

Presence of only seven series of scales between the dorsal-fin and pelvic-fin origins (ch. 4: 7).

*Leptolebias aureoguttatus*

Longitudinal row of small iridescent spots on the lateral surface of the body of males (ch. 72: 2; independent acquired in *L. marmoratus* and in *Cynopoecilus + Campellolebias*).

*Leptolebias marmoratus*

Epidermis of the anterior anal-fin rays of males is thickened (ch. 34: 1; a character acquired independently in
Cynopoecilus; pelvic fins separated by a short interspace (ch. 45: 1; acquired independently in C. intimus and in the clade consisting of Campellolebias brucei, C. intermedius Costa & De Luca, 2006 and C. chrysoleineatus Costa, Lacerda & Brasil, 1989; urogenital papilla attached to the anterior margin of the anal fin in males (ch. 49: 1; acquired independently in Campellolebias and Cynopoecilus; dorsal and anal fins of males antero-posteriorly expanded during courtship (ch. 63: 0; a reversal among the members of the subtribe); presence of longitudinal rows of small iridescent spots along the lateral surface of the body of males (ch. 72: 2; acquired independently in Nematoelebias whitei.

Leptolebias splendens
Presence of only ten series of scales between the dorsal-fin and pelvic-fin origins (ch. 4: 10).

Clade Campellolebias + Cynopoecilus
Presence of 16 or 17 dorsal-fin rays (ch. 1: 16–17); presence of 13 or 14 neuromasts in the supraorbital series (ch. 5: 13–14); second proximal radial of the dorsal fin positioned at the 11th vertebra (ch. 6: 11); lateral wings of the vomer narrow (ch. 8: 1); anal-fin membrane of males divided into anterior and posterior portions (ch. 38: 1); urogenital papilla attached to the anterior margin of the anal fin in males (ch. 49: 1; acquired independently in Leptolebias marmoratus); internal fertilization (ch. 62: 1); presence of longitudinal rows of small iridescent spots on the lateral surface of the body of males (ch. 72: 2; acquired independently in L. aureoguttatus, L. marmoratus).

Genus Campellolebias
First proximal radial long, narrow (ch. 30: 0); first proximal radial curved posteriorly in males (ch. 31: 1); two anteriormost anal-fin rays in males thickened and strongly attached to the proximal radials with large cartilaginous distal radials (ch. 33: 1); inclinators anales 1–3 of the anal-fin base expanded laterally to form a fan-shaped structure (ch. 37: 1); urogenital papilla in males extended to form a pseudogonopodium (ch. 50: 1); eggs with spine to hair-like projections of the chorion (ch. 61: 0); melanophores of the caudal peduncle aggregated to form black spots on posterior zone (ch. 75: 1).

Clade Campellolebias brucei + C. intermedius + C. chrysoleineatus
Presence of a short interspace separating the pelvic-fin insertions (ch. 45: 1; acquired independently in Leptolebias marmoratus and Cynopoecilus intimus); presence of narrow, horizontal, black lines between the scales on lateral surface of the body in females (ch. 73: 2).

Campellolebias brucei
Presence of 16 to 19 cephalic neuromasts in the supraorbital series (ch. 5: 16–19).

Campellolebias dorsimaculatus
Presence of vertical rows of iridescent dots in the body flank of males (ch. 72: 1).

Genus Cynopoecilus
Presence of 23 or 24 anal-fin rays (ch. 2: 23–24); urohyal slender (ch. 22: 1); presence of minute accessory cartilages on the caudal fin (ch. 27: 1; reversed to absent in Cynopoecilus multipapillatus + C. fulgens); presence of 8 or 9 min rays attached to the first proximal radial of the anal fin (ch. 32: 1); epidermis of the anterior anal-fin rays in males thickened (ch. 34: 1; homoplastically observed in Leptolebias marmoratus); sixth and seventh anal-fin rays of males anteriorly curved (ch. 35: 1); pelvic fin reduced, not reaching the anal fin (ch. 47: 1); urogenital papilla of males bulbous (ch. 51: 1); iris yellow to yellowish brown of males (ch. 67: 0); presence of a broad longitudinal black stripe from the tip of the lower jaw to the caudal-fin base (ch. 71: 1); presence of an oblique longitudinal black stripe between the pectoral-fin base and the posterior part of the anal-fin base (ch. 74: 1).

Cynopoecilus notabilis sp. nov.
Presence of 20 to 23 dorsal-fin rays (ch. 1: 20–23); presence of 29 to 31 scales in longitudinal series (ch. 3: 29–31); presence of 10 to 11 transverse series of scales between the dorsal and pelvic-fin origins (ch. 4: 10–11); broad and approximately straight proximal portion of the epural and parhypural (ch. 26: 0); elliptical to lanceolate caudal fin, much longer than deep (ch. 28: 1, acquired independently in Leptolebias); position of the second proximal radial of the dorsal fin between the 8th and 9th vertebrae (ch. 6: 8–9).

Clade Cynopoecilus fulgens + C. multipapillatus + C. nigrovittatus + C. melanotaenia + C. intimus
Presence of teeth on the second pharyngobranchial (ch. 16: 0, a reversal); absence of contact organs on the pectoral fin of males (ch. 59: 0; a reversal, also observed in clade Leptolebias aureoguttatus + L. citrinipinnis + L. marmoratus + L. splendens); presence of a broad stripe of black pigments in the lateral surface the body of females (ch. 73: 3).

Clade Cynopoecilus fulgens + C. multipapillatus + C. nigrovittatus + C. melanotaenia
Presence of supraorbital scales (ch. 55: 0; polymorphic in C. fulgens and C. multipapillatus).
Clade Cynopoecilus fulgens + C. multipapillatus + C. nigrovittatus
Presence of 15 cephalic neuromasts in the supraorbital series (ch. 5: 15).

Clade Cynopoecilus fulgens + C. multipapillatus
Presence of a short, wide ventral process of the angulo-articular (ch. 10: 2); absence of minute accessory cartilages on the caudal fin (ch. 27: 0, a reversal in Cynopoecilus).

Cynopoecilus intimus
Pelvic fins separated by a short interspace is autapomorphic for this species (ch. 45: 1; homoplastically observed in Leptolebias marmoratus and in the clade Campellolebias brucei, C. intermedius, C. chrysolineatus).

Cynopoecilus multipapillatus
Presence of 17 to 20 cephalic neuromasts in the supraorbital series (ch. 5: 17–20).