

## Taxonomy and phylogenetic relationships among species of the seasonal, internally inseminating, South American killifish genus *Campellolebias* (Teleostei: Cyprinodontiformes: Rivulidae), with the description of a new species

WILSON J. E. M. COSTA

Laboratório de Ictiologia Geral e Aplicada, Departamento de Zoologia, Universidade Federal do Rio de Janeiro, Caixa Postal 68049, CEP 21944-970, Rio de Janeiro, Brasil. E-mail: wcosta@acd.ufrj.br

### Abstract

*Campellolebias*, a genus of South American annual killifishes, is diagnosed by a unique specialized structure, the pseudogonopodium, on the anterior part of the anal fin in males, which is used for internal insemination; a derived caudal skeleton morphology; an apomorphic color pattern on the ventral portion of the head in males; and a unique courtship behavior pattern in males. Four species are included, all endemic to Brazil's southern coastal plains: *C. dorsimaculatus* and *C. intermedius* n. sp., from the rio Ribeira de Iguape basin, São Paulo; *C. chrysolineatus*, from northern Santa Catarina; and *C. brucei*, from southern Santa Catarina. A clade comprising *C. intermedius*, *C. chrysolineatus* and *C. brucei* is defined by the pelvic-fin bases medially separated by a short interspace, seven longitudinal rows of iridescent marks on the flank in males, and dark horizontal lines between flank scales on females. A clade comprising *C. chrysolineatus* and *C. brucei* is diagnosed by long dorsal, anal and pelvic fins in males.

**Keywords:** Killifishes, Cyprinodontiformes, Rivulidae, *Campellolebias*, Neotropica, Atlantic forest, internal insemination, morphology, phylogenetic relationships

### Resumo

*Campellolebias*, um gênero de peixes anuais sul-americanos, é diagnosticado por uma estrutura especializada exclusiva, o pseudogonopódio, na parte anterior da nadadeira anal em machos, o qual é utilizado para inseminação interna; uma morfologia derivada de esqueleto caudal; um padrão apomórfico de colorido na porção ventral da cabeça em machos; e um padrão exclusivo de comportamento de corte em machos. Quatro espécies são incluídas, todas endêmicas das planícies costeiras do sul do Brasil: *C. dorsimaculatus* e *C. intermedius* n. sp., da bacia do rio Ribeira de Iguape, São Paulo; *C. chrysolineatus*, do norte de Santa Catarina; e *C. brucei*, do sul de Santa

Catarina. Um clado compreendendo *C. intermedius*, *C. chrysolineatus* e *C. brucei* é definido pelas bases das nadadeiras pélvicas medialmente separadas por um curto interespaço, sete fileiras longitudinais de marcas iridescentes no flanco em machos e linhas horizontais escuras entre as escamas do flanco em fêmeas. Um clado compreendendo *C. chrysolineatus* e *C. brucei* é diagnosticado pelas nadadeiras dorsal, anal e pélvicas longas em machos.

## Introduction

*Campellolebias* is a genus of cynolebiatin killifishes inhabiting seasonal pools with dark acid waters (pH 4.5–5.0) in dense forests of the coastal river basins of southern Brazil (Costa *et al.*, 1989; Costa, 1995a, 2003). It constitutes a unique, reproductively specialized aplocheiloid lineage, characterized by elaborate morphological and behavioral traits related to internal insemination (Costa, 1995a, 1998). Three species are presently included in the genus, all described during the last quarter of the 20th century (Vaz-Ferreira & Sierra, 1974; Costa *et al.*, 1989). A fourth new species is herein described.

*Campellolebias* was first erected to include a single species, *C. brucei* Vaz-Ferreira & Sierra, characterized by a unique structure of the anal fin in males, in which the first two rays are separated from the posterior portion of the fin and attached to a long urogenital papilla; and dark stripes on the ventral portion of the head in males (Vaz-Ferreira & Sierra, 1974). *Campellolebias* was placed in the synonymy of *Cynolebias* Steindachner by Parenti (1981), but considered a valid genus in subsequent studies (*e. g.*, Costa *et al.*, 1989; Costa, 1990, 1995a, 2003). Costa *et al.* (1989) described *Campellolebias dorsimaculatus* and *Campellolebias chrysolineatus*, and Costa (1995a) revised the genus, based on the few available specimens of the genus then deposited in scientific collections. His revision provided an osteological description and new data on the morphology of structures involved in internal insemination (*i. e.*, bones, ligaments and muscles); and he introduced the term “pseudogonopodium” for the specialized anal-fin structure utilized in internal insemination (Costa, 1995a). Costa (1998) later listed some synapomorphies for *Campellolebias*, including bones and muscles of the pseudogonopodium and a unique reproductive courtship behavior pattern.

On the basis of morphological data, *Campellolebias* was considered to be the sister group to *Cynopoecilus* Regan, and a member of a clade including *Cynopoecilus* and *Leptolebias* Myers, which would be the sister group to a clade comprising the remaining cynolebiatines (*i. e.*, *Cynolebias*, *Austrolebias* Costa, *Simpsonichthys* de Carvalho, and *Nematolebias* Costa) (Costa, 1990, 1998). This hypothesis was also later supported by molecular data (Hrbek *et al.*, 2004). However, relationships among species of *Campellolebias* are still poorly defined (Costa, 1995a, b).

The objectives of the present study are: to revise and to update taxonomic and morphological knowledge on the genus, based on the study of recent collections; describe a new species from the rio Ribeira de Iguape basin; and reanalyze phylogenetic

relationships among included species, based on morphological and behavioral characters described in past studies (*e. g.* Costa *et al.*, 1989; Costa, 1990, 1995b, 1998, 2006).

## Material and methods

Material is deposited in MCP, Museu de Ciências e Tecnologia da Pontifícia Universidade Católica, Porto Alegre; MNRJ, Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro; MZUSP, Museu de Zoologia, Universidade de São Paulo, São Paulo; UFRJ, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro; and, ZVC-P, Facultad de Humanidades y Ciencias, Departamento Zoología de Vertebrados, Montevideo. Comparative material used in the phylogenetic analysis is listed in Appendix 1.

Measurements and counts follow Costa (1995a); anal-fin base length in males is measured between the anteriormost point of the pseudogonopodial base and base of the last anal-fin ray. Measurements are presented as percentages of standard length (SL), except for those related to head morphology, which are expressed as percentages of head length. Fin-ray counts include all elements. Number of vertebrae, gill-rakers, 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 follows Costa (2001).

The phylogenetic analysis follows cladistic methodology. Terminal taxa were all species of *Campellolebias*; *Cynopoecilus melanotaenia* (Regan), the type species of *Cynopoecilus* and which is hypothesized to be the sister group to *Campellolebias* (Costa, 1990, 1998); and *Leptolebias marmoratus* (Ladiges), the type species of *Leptolebias*, which is hypothesized to be the sister group to *Campellolebias* plus *Cynopoecilus* (Costa, 1990, 1998). Outgroups were: *Nematolebias whitei* (Myers), a basal member of a clade that is hypothesized to be the sister group to *Campellolebias* + *Cynopoecilus* + *Leptolebias* (Costa, 1998, 2006); *Neofundulus paraguayensis* (Eigenmann & Kennedy), a basal member of an annual fish clade (Costa, 2005); and *Kryptolebias brasiliensis* (Valenciennes), a basal species of the most basal lineage of the Rivulidae (Costa, 2004).

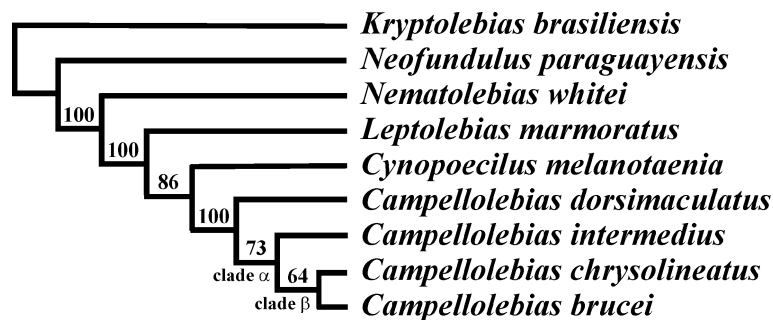
Characters were taken from recent morphological phylogenetic analyses, including cynolebiatins (Costa, 1998, 2006). Characters and character states are listed and coded in Appendix 2, and are plotted in the data matrix of Appendix 3. Character and character state numbers, separated by a point, in the text (*e. g.*, diagnoses) are according to Appendix 2. Character states of multi-state characters were treated as ordered when possible. Most parsimonious cladograms, consistency indices (CI) and retention indices (RI) were obtained using the exact algorithm *ie\** 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 (Swofford, 1998) with 1000 replicates.

## Results

### Phylogenetic analysis

Most of the 58 morphological and behavioral characters used to analyze the phylogenetic relationships among species of *Campellolebias* and closely related genera are congruent among themselves. Consequently, this analysis produced a single highly corroborated cladogram (tree length = 76, CI = 0.88, RI = 0.91) (Fig. 1). Synapomorphies for *Campellolebias* and included subclades are listed in diagnoses below, following character and character state numbers (between parentheses), as listed in Appendix 2.



**FIGURE 1.** Most parsimonious phylogeny among 6 species of the Rivulidae (L = 76, CI = 0.88, RI = 0.91). Numbers left of branches are bootstrap values.

### Taxonomic accounts

#### *Campellolebias* Vaz-Ferreira & Sierra

*Campellolebias* Vaz-Ferreira & Sierra, 1974: 14 (type species: *Campellolebias brucei* Vaz-Ferreira & Sierra, 1974; type by original designation and monotypy).

#### Diagnosis

Distinguished from all other genera of the suborder Aplocheiloidei in having a pseudogonopodium, a structure present in the anterior portion of the anal fin in males, which is mobile and acts in internal insemination (Costa, 1995a: fig. 1). It is a long, subcylindrical structure, slightly compressed laterally, free from the remaining portion of the unmodified anal-fin. It consists of skin tissue involving the first two anal-fin rays, which are thickened (22.1), and a long urogenital papilla (32.1). Internally the first

proximal radial is narrowed and slightly curved posteriorly (21.1), and is firmly connected to pseudogonopodial rays by enlarged cartilaginous medial radials and thick ligaments (Costa, 1995a: fig. 1). The adjacent *inclinatores anales* are widened to form a fan-shaped muscular structure (30.1) (Costa, 1995a: fig. 1).

Other derived conditions present in *Campellolebias* that distinguish it from all other rivulids are: posterobasal portion of the last neural spine with a small process bearing a thin ligament attached to the anterior tip of epural, which is distinctively bent (17.1) (Costa, 1998: fig. 30a); a dark ventral stripe between lower jaw and pseudogonopodium (50.1) (Vaz-Ferreira & Sierra, 1974: pl. 2); a dark lateroventral stripe on head (51.1) (Vaz-Ferreira & Sierra, 1974: pl. 2); and a derived behavior pattern, termed “coiled retrorse motion” by Costa (1998) (57.1), which consists of a typical courtship involving repetitive series of male coiling movements before copulation, in which males move simultaneously backwards and upwards, with the body at an angle of about 45° from the substrate. *Campellolebias* also differs from the remaining cynolebiatin genera (*Cynopocilus*, *Leptolebias*, *Nematolebias*, *Simpsonichthys*, *Cynolebias*, and *Austrolebias*) in possessing long and narrow first proximal radials of the anal fin (20.0) (Costa, 1995a: fig. 1). Derived conditions hypothesized to occur independently in *Campellolebias* and in other rivulids (*i. e.*, homoplastic features), or with uncertain polarity, are: narrow basihyal, longest width 35–40 % (8.2) (Costa, 1995a: fig. 2); second pharyngobranchial teeth absent (10.1); numerous neuromasts on the caudal-fin base (42.1); contact organs on the pectoral-fin rays in males (44.1); melanophores concentrated on the subdistal portion of dorsal and anal fins in males (49.1); and, black spots on the posterior portion of the caudal fin in females (52.1).

### Key to species of the genus *Campellolebias*

- 1a. Pelvic-fin bases medially separated by interspace about half pelvic-fin base width; iridescent marks arranged in horizontal rows on flank in males; dark chromatophores arranged in horizontal lines on flank in females; no distinctive dark bars on flank in males; no pale purple stripe on flank in males; no distinctive black spot on posterior portion of dorsal fin..... 2
- 1b. Pelvic-fin bases medially in contact; iridescent dots arranged in vertical rows on flank in males; dark chromatophores arranged in vertically elongated spots on flank in females; 9–10 dark brown bars on flank in males; two pale purple stripes on flank in males; black spot on posterior portion of dorsal fin. .... *C. dorsimaculatus*
- 2a. Dorsal and anal fins long in males, tip reaching posterior half of caudal fin; pelvic-fin tip reaching base of 4th anal-fin ray in males. .... 3
- 2b. Dorsal and anal fins moderately long in males, tip reaching basal portion of caudal fin; pelvic-fin tip reaching anterior base of pseudogonopodium in males. ... *C. intermedius*
- 3a. Supraorbital neuromasts 2 + 10–12; sides of body purplish pink, with 7 horizontal

- golden lines in males; pale orange stripes on flank in females; black spots just below lateral midline, on antero-central portion of flank in females; 29 vertebrae. .... *C. chrysolineatus*
- 3b. Supraorbital neuromasts 3 + 13–16; sides of body dark purplish red, with 7 horizontal rows of metallic green spots in males; no pale orange stripe on flank in females; no black spot on flank in females; 27–28 vertebrae. .... *C. brucei*

***Campellolebias dorsimaculatus* Costa, Lacerda & Brasil**

(Figs. 2–3)

*Campellolebias dorsimaculatus* Costa, Lacerda & Brasil, 1989: 66 (type locality: temporary pool in border of forest [near Icapara, lower rio Ribeira de Iguape basin], Município de Iguape, Estado de São Paulo, Brazil, 24°37'S 47°30'W [correctly 24°40'0.4"S 47°26'4.4"W, altitude 10 m]; holotype: MZUSP 38813).

*Material examined*

Brazil: Estado de São Paulo: MZUSP 38813, holotype; MZUSP 38814, 1 paratype; MZUSP 38815, 12 paratypes; temporary pool in border of forest, near Icapara, Município de Iguape, lower rio Ribeira de Iguape basin; G. C. Brasil & D. Nielsen, 31 Mar. 1988. MZUSP 38816, 1 paratype; MZUSP 11493, 2 paratypes; MNRJ 11493, 2 paratypes; same locality and collectors, 19 Jul. 1988. UFRJ 6309, 2; UFRJ 6310, 3 (c&s); same locality, A. C. De Luca, 2001. UFRJ 6311, 1; same locality, W. J. E. M. Costa, B. B. Costa & C. P. Bove, 12 Dec. 2005.

*Diagnosis*

Distinguished from all other congeners by having: pelvic-fin bases medially in contact (*vs.* separated by interspace), iridescent dots arranged in vertical rows on flank in males (*vs.* iridescent spots arranged in horizontal rows), dark chromatophores arranged in vertically elongated spots on flank in females (*vs.* arranged in horizontal lines), 9–10 dark brown bars on flank in males (*vs.* no dark brown bars on flanks), two pale purple stripes on flank in males (*vs.* no pale purple stripe on flanks), and black spot on posterior portion of dorsal fin (*vs.* no distinctive black spot on posterior portion of dorsal fin).

*Description*

Morphometric data given in Table 1. Males larger than females, largest male 28.4 mm SL, largest female 26.5 mm SL. Dorsal profile convex from snout to end of dorsal-fin base, approximately straight on caudal peduncle. Ventral profile slightly convex from lower jaw to end of anal-fin base, nearly straight on caudal peduncle. Body moderately slender, slightly compressed. Greatest body depth at level of pelvic-fin base.



**FIGURE 2.** *Campellolebias dorsimaculatus*, UFRJ 6311, male, 26.1 mm SL (one day after collection); Brazil: São Paulo: Iguape.



**FIGURE 3.** *Campellolebias dorsimaculatus*, UFRJ 6309, female, 22.0 mm SL; Brazil: São Paulo: Iguape.

Dorsal and anal fins pointed, moderately elongate in males, tip reaching vertical through caudal-fin base; dorsal and anal fins rounded, short and without filamentous rays in females. Caudal fin rounded. Pectoral fins elliptical. Posterior margin of pectoral fins reaching vertical between pelvic-fin base and anus in males, through vertical just in front pelvic-fin base in females. Tip of pelvic fins reaching anterior portion of pseudogonopodium base in males, reaching urogenital papilla in females. Pelvic-fin bases medially in contact. Dorsal-fin origin anterior to anal-fin origin, anal-fin origin on vertical between base of 2nd and 3rd dorsal-fin rays in males, between base of 3rd and 5th dorsal-fin rays in females. Dorsal-fin origin between neural spines of vertebrae 9 and 11. Anal-fin origin between pleural ribs of vertebrae 10 and 11 in males, between pleural ribs of vertebrae 11 and 12 in females. Dorsal-fin rays 16–18; anal-fin rays 2 + 13–15 in male, 15–16 in females; caudal-fin rays 26–30; pectoral-fin rays 13–14; pelvic-fin rays 5–6.

TABLE 1. Morphometric data of species of the genus *Campellolebias*.

	<i>C. dorsimaculatus</i>		<i>C. intermedius</i>	
	males (8)	females (6)	males (2)	females (2)
Standard length (mm)	17.0–28.4	17.0–26.5	20.8–23.9	17.0–20.7
Percents of standard length				
Body depth	24.3–29.4	22.6–24.8	27.6–28.3	24.5–26.6
Caudal peduncle depth	12.8–13.7	11.3–13.2	14.9–15.1	13.2–13.4
Pre-dorsal length	49.3–55.2	52.9–59.2	56.4	58.9–59.9
Pre-pelvic length	51.7–54.0	53.5–59.6	55.2–56.0	55.8–58.8
Length of dorsal-fin base	27.8–32.2	27.2–31.3	28.7–28.9	24.1–24.3
Length of anal-fin base	23.3–28.3	17.9–20.8	22.3–25.7	18.7
Caudal-fin length	34.3–38.3	33.9–39.1	37.3–37.4	33.1–39.2
Pectoral-fin length	19.6–24.6	20.0–22.1	24.5–25.1	20.1–21.7
Pelvic-fin length	10.0–11.2	8.7–9.4	10.8–11.0	9.7–10.0
Head length	28.3–30.7	29.5–31.1	30.6–31.5	30.6–31.5
Percents of head length				
Head depth	67.9–82.0	64.9–73.5	74.5–78.4	68.9–71.8
Head width	68.9–71.2	69.4–79.6	65.9–75.8	68.3–72.4
Snout length	10.9–14.6	10.7–11.8	11.7–11.8	11.2–11.7
Lower jaw length	14.6–17.8	15.1–15.2	15.6–16.8	14.4–15.7
Eye diameter	32.4–39.8	36.2–42.3	37.1–37.5	37.9–39.2
continued.				
	<i>C. chrysolineatus</i>		<i>C. brucei</i>	
	males (10)	females (10)	males (10)	females (10)
Standard length (mm)	19.9–32.3	19.0–32.3	21.4–30.9	17.5–20.5
Percents of standard length				
Body depth	23.3–28.4	24.0–26.9	26.1–31.1	25.0–28.8
Caudal peduncle depth	12.6–14.8	12.0–14.3	14.0–16.4	13.7–15.4
Pre-dorsal length	54.2–56.2	54.7–59.2	53.2–56.7	56.3–58.7
Pre-pelvic length	51.1–55.5	53.8–57.8	52.8–56.1	54.5–57.3
Length of dorsal-fin base	24.2–29.0	23.2–26.6	27.8–30.4	24.0–28.6
Length of anal-fin base	20.9–26.1	18.2–21.2	22.9–27.1	18.1–22.5
Caudal-fin length	36.3–40.4	34.3–38.7	37.5–42.0	35.0–40.9
Pectoral-fin length	23.4–25.0	21.8–24.7	22.8–24.0	20.7–22.8
Pelvic-fin length	12.6–14.7	9.0–10.6	12.9–16.2	10.2–11.2
Head length	24.9–33.2	28.3–32.5	29.0–32.6	29.3–32.2
Percents of head length				
Head depth	72.8–78.2	72.2–78.9	70.7–80.6	68.3–73.2
Head width	68.4–75.4	72.8–76.8	67.3–72.8	67.8–75.0
Snout length	10.9–13.6	11.1–13.2	11.4–12.8	11.0–12.3
Lower jaw length	15.5–19.8	15.9–17.0	16.1–19.3	15.6–17.2
Eye diameter	33.9–38.1	31.9–39.6	33.4–41.3	37.9–40.7



Scales large, cycloid. Body and head entirely scaled, except on anteroventral surface of head. Dorsal and anal-fin bases without scales. Scales covering anterior 10 % of caudal fin. Frontal squamation E-patterned, E-scales not overlapping. Longitudinal series of scales 26–27; transverse series of scales 7–8; scale rows around caudal peduncle 12. Three to five minute contact organs on exposed margin of each scale of ventral portion of flank in males. Minute papillate contact organs on internal surface of distal portion of pectoral-fin rays 1–4 in males.

Cephalic neuromasts: supraorbital 2 + 10–13, parietal 1, anterior rostral 1–2, posterior rostral 1–2, infraorbital 1–2 + 21–26, preorbital 3, otic 1–2, post-otic 2–3, supratemporal 1, median opercular 1, ventral opercular 3, preopercular 18–22, mandibular 8–12, lateral mandibular 2–5, paramandibular 1. Two to six neuromasts on each scale of lateral series. Transverse row of five neuromasts on caudal-fin base.

Basihyal subtriangular, longest width about 35 % of length; basihyal cartilage about 30 % of total length of basihyal. Six branchiostegal rays. Second pharyngobranchial absent. Gill-rakers on first branchial arch 2 + 8. Vomerine teeth absent. Dermosphenotic absent. Ventral process of posttemporal absent. Total vertebrae 27–28.

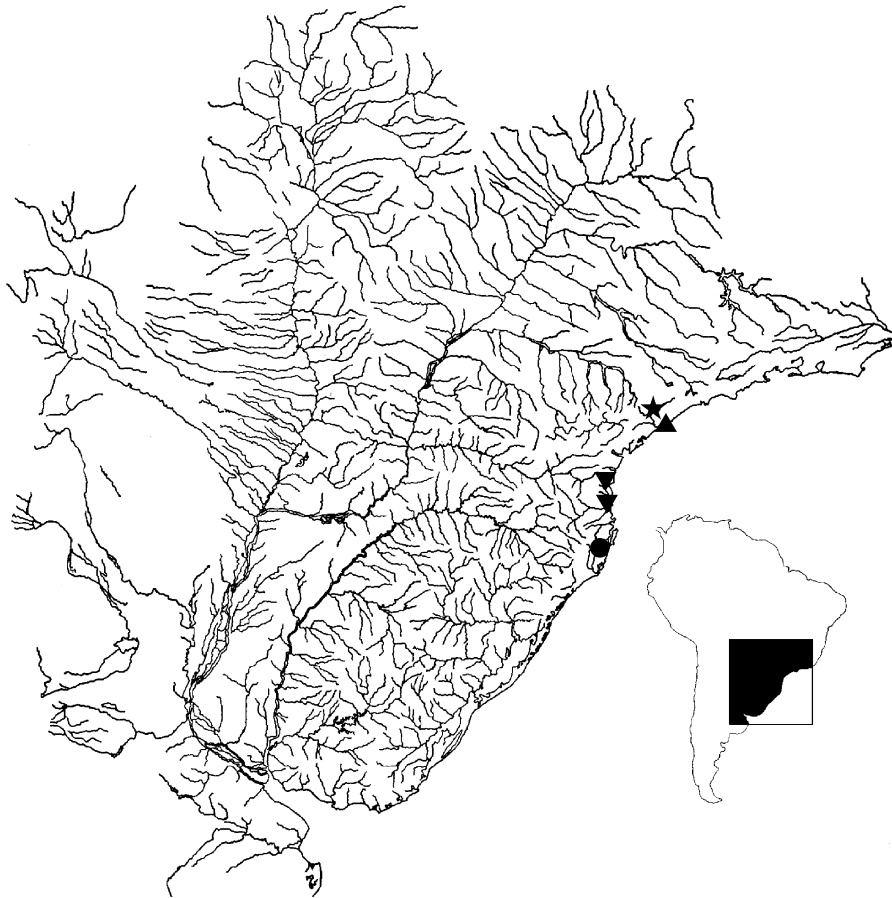
#### *Coloration*

Males: Sides of body purplish brown, with 9–10 brown bars alternating with vertical rows of golden dots, all overlapped by narrow pale purple stripe on lateral midline and other similar stripe just below lateral midline. Sides of head dark purplish red, scales of opercular region golden. Iris bright yellowish green, with dark reddish brown bar through center of eye. Dorsum light brown. Venter white to bluish white; dark reddish gray stripe along ventrolateral region of head; dark brown midventral stripe from lower jaw to pseudogonopodium. Dorsal and anal fins dark orangish pink green, with small reddish brown spots on basal and posterior portions, and one or two black spots on posterior portion of dorsal fin; light blue iridescence on distal and anterior portions, narrow submarginal zone and narrow golden line along anterior edge of fins. Caudal fin light green, with small reddish brown spots, and narrow golden marginal line. Pelvic fins dark reddish brown, with narrow golden line on anterior margin. Pectoral fins hyaline.

Females: Sides of body light yellowish gray, with dark gray vertically elongated blotches; small black spots on posterior portion of caudal peduncle. Venter white to pale golden. Opercular region greenish golden. Iris light yellow, with brown bar through center of eye. Unpaired fins hyaline, with small black spots on basal region, larger on caudal-fin posterior portion of dorsal fin. Pectoral fins hyaline. Pelvic fins gray.

#### *Distribution*

Temporary pools in the lower rio Ribeira de Iguape basin, coastal plains of São Paulo, Brazil (Fig. 4).



**FIGURE 4.** Geographic distribution of the genus *Campellolebias*. Triangles = *C. dorsimaculatus*; stars = *C. intermedius*; inverted triangles = *C. chrysolineatus*; dot = *C. brucei*. Some symbols may represent more than one locality.

### Clade $\alpha$

#### *Diagnosis*

Pelvic-fin bases medially separated by short interspace (37.1; also occurring in *Leptolebias marmoratus* but not in other species of *Leptolebias*); seven longitudinal rows of iridescent marks on the flank in males (45.2); and narrow dark horizontal lines between scales on the flank in females (46.2).

#### *Included species*

*C. intermedius* sp. n., *C. chrysolineatus*, and *C. brucei*.

***Campellolebias intermedius* Costa & De Luca, new species**

(Figs. 5–6)

Holotype. UFRJ 6312, male, 23.9 mm SL; Brazil: Estado de São Paulo: temporary pool in road about 6 km W of Juquiá, rio Juquiá drainage, rio Ribeira de Iguape basin, about 24°20'S 47°35'W; A. C. De Luca *et al.*, 27 Oct. 2002.

Paratypes. Brazil: Estado de São Paulo: rio Ribeira de Iguape basin, rio Juquiá drainage: UFRJ 6313, female, 20.7 mm SL; collected with holotype. UFRJ 6314, 1 male, 20.8 mm SL; 1 female, 17.0 mm SL; UFRJ 6315, 2 males, 19.1–22.9 mm SL; 2 females, 15.4–16.8 mm SL; temporary pool along road, about 3 km W of Juquiá; A. C. De Luca & C. G. C. Wenceslau, 25 Jan. 2003.

Additional material (non types). UFRJ 6316, 12 males, 16.6–20.6 mm SL, 21 females, 14.0–17.6 mm SL (in poor state of conservation, equivocally first fixed in 40 % ethyl alcohol just after collection); road about 3 km W of Juquiá; A. C. De Luca *et al.*, 27 Oct. 2002.

*Diagnosis*

Distinguished from *C. dorsimaculatus* in having: pelvic-fin bases medially separated by interspace (*vs.* in contact), iridescent marks arranged in horizontal rows on flanks in males (*vs.* iridescent dots arranged in vertical rows), dark chromatophores arranged in horizontal lines on flank in females (*vs.* arranged in vertically elongated spots), no dark brown bars on flanks in males (*vs.* 9–10 dark brown bars on flanks), no pale purple stripe on flanks of males (*vs.* two pale purple stripes), and no distinctive black spot on posterior portion of dorsal fin on posterior portion of dorsal fin (*vs.* black spot present). Distinguished from *C. chrysolineatus* and *C. brucei* by having dorsal and anal fins moderately long in males, tip reaching basal portion of caudal fin (*vs.* dorsal and anal fins long, reaching tip posterior half of caudal fin) and pelvic-fin tip reaching anterior base of pseudogonopodium in males (*vs.* reaching base of 4th anal-fin ray). Also distinguished from *C. chrysolineatus* by the absence of black spots on the flanks in females (*vs.* presence), and flank light reddish brown in males (*vs.* purplish pink). Also distinguished from *C. brucei* by having fewer supraorbital neuromasts (2 + 12 *vs.* 3 + 13–16), fewer anal-fin rays (14–16 *vs.* 16–18), and vertical golden lines on flanks in males (*vs.* vertical rows of metallic green spots).

*Description*

Morphometric data given in Table 1. Males larger than females, largest male 23.9 mm SL, largest female 20.7 mm SL. Dorsal profile convex from snout to end of dorsal-fin base, approximately straight on caudal peduncle. Ventral profile gently convex from lower jaw to end of anal-fin base, nearly straight on caudal peduncle. Body moderately slender, slightly compressed. Greatest body depth at level of pelvic-fin base.



**FIGURE 5.** *Campellolebias intermedius*, UFRJ 6312, holotype, male, 23.9 mm SL; Brazil: São Paulo: Juquiá.



**FIGURE 6.** *Campellolebias intermedius*, UFRJ 6313, paratype, female, 20.7 mm SL; Brazil: São Paulo: Juquiá.

Dorsal and anal fins pointed, slightly elongated, tip reaching vertical through basal portion of caudal fin in males, rounded and short in females. Caudal fin rounded. Pectoral fins elliptical. Posterior margin of pectoral fins reaching vertical between pelvic-fin base and anus in males, through vertical just in front pelvic-fin base in females. Tip of pelvic fins reaching anterior base of pseudogonopodial rays in males, reaching urogenital papilla in females. Pelvic-fin bases medially separated by interspace, with length equal to half pelvic-fin base width. Dorsal-fin origin anterior to anal-fin origin, anal-fin origin on vertical between base of 3rd dorsal-fin ray. Dorsal-fin origin between neural spines of vertebrae 11 and 12 in males, 9 and 10 in females. Anal-fin origin between pleural ribs of vertebrae 11 and 12 in males, between pleural ribs of vertebrae 11 and 12 in females. Dorsal-fin rays 15–17; anal-fin rays 2 + 13–14 in males, 14–16 in females; caudal-fin rays 25–27; pectoral-fin rays 14; pelvic-fin rays 6.

Scales large, cycloid. Body and head entirely scaled, except on anteroventral surface of head. Dorsal and anal-fin bases without scales. Scales covering anterior 10 % of caudal

fin. Frontal squamation E-patterned, E-scales not overlapping. Longitudinal series of scales 26–27; transverse series of scales 7; scale rows around caudal peduncle 12. Three to five minute contact organ on exposed margin of each scale of ventral portion of flank in males. Minute papillate contact organs on internal surface of distal portion of four uppermost pectoral-fin rays in males.

Cephalic neuromasts: supraorbital 2 + 12, parietal 1, anterior rostral 1, posterior rostral 1, infraorbital 1 + 22–23, preorbital 2, otic 2, post-otic 2, supratemporal 1, median opercular 1, ventral opercular 1, preopercular 17–18, mandibular 8–9, lateral mandibular 3–4, paramandibular 1. Two to four neuromasts on each scale of lateral series. Transverse row of five neuromasts on caudal-fin base.

Basihyal triangular, longest width about 40 % of length; basihyal cartilage about 30 % of total length of basihyal. Six branchiostegal rays. Second pharyngobranchial absent. Gill-rakers on first branchial arch 2 + 8. Vomerine teeth absent. Dermosphenotic absent. Ventral process of posttemporal absent. Total vertebrae 26–28.

#### *Coloration*

Males: Sides of body light reddish brown, with 7 horizontal golden lines. Sides of head dark reddish brown, central portion of opercular region metallic green. Iris bright yellowish green, with dark reddish brown bar through center of eye. Dorsum brown. Venter white; dark brown stripe along ventrolateral region of head; dark brown midventral stripe from lower jaw to pseudogonopodium. Dorsal and anal fins light green, with small dark brown spots on basal and posterior portions, light blue with dark brown bars on distal and anterior portions; narrow submarginal black line and narrow bluish white marginal line along anterior edge of fins. Caudal fin light green, with small reddish brown spots. Pelvic fins dark bluish gray. Pectoral fins hyaline.

Females: Sides of body light yellowish brown, with 5–7 dark gray horizontal lines; small black spots on posterior portion of caudal peduncle. Venter white to pale golden. Opercular region greenish golden. Iris light yellow, with brown bar through center of eye. Unpaired fins hyaline, with small black spots on basal region, larger on caudal-fin base. Pectoral fins hyaline. Pelvic fins dark gray.

#### *Distribution*

Temporary pools in rio Juquiá drainage, rio Ribeira de Iguape basin, São Paulo, Brazil (Fig. 4).

#### *Etymology*

From the Latin *intermedius* (intermediate), referring to its phylogenetic position among congeners.

**Clade  $\beta$** *Diagnosis*

Dorsal and anal fins long in males, a condition sometimes more conspicuous in the anal fin, which has most rays long, tips reaching posterior half of caudal fin (36.2).

*Included species*

*C. chrysolineatus* and *C. brucei*.

***Campellolebias chrysolineatus* Costa, Lacerda & Brasil**

(Figs. 7–8)

*Campellolebias chrysolineatus* Costa, Lacerda & Brasil, 1989: 69 (type locality: temporary pool at border of forest, Município de Araquari, Estado de Santa Catarina, Brazil, 26°25'S 48°38'W; holotype: MZUSP 38817).

*Material examined*

Brazil: Estado de Santa Catarina: MZUSP 38817, holotype; MZUSP 38818, 2 paratypes; MZUSP 38819, 1 paratype; MNRJ 11494, 2 paratypes; temporary pool in border of forest, Município de Araquari; G.C. Brasil *et al.*, 21 Nov. 1987. MZUSP 38344, 11 paratypes; same locality; C. Tatsuta *et al.*, April 1986. UFRJ 5210, 6; UFRJ 5211, 2 (c&s); temporary pool between Araquari and Balneário Barra do Sul, 26°24'45.9"S 48°38'23.3"W; W. J. E. M. Costa, C. P. Bove & B. B. Costa, 4 Nov. 2000. UFRJ 6317, 2; UFRJ 6318, 23; UFRJ 6319, 6 (c&s); temporary pool between Araquari and Balneário Barra do Sul, 26°24'32.9"S 48°38'30.9"W; W. J. E. M. Costa, C. P. Bove & B. B. Costa, 17 Dec. 2005. UFRJ 284, 2; Itapema; G. C. Brasil, 19 Aug. 1988.

*Diagnosis*

Similar to *C. brucei* and distinguished from *C. dorsimaculatus* and *C. intermedius* by having dorsal and anal fins long in males, tip reaching posterior half of caudal fin (*vs.* reaching basal portion of caudal fin) and pelvic-fin tip reaching base of 4th anal-fin ray in males (*vs.* reaching anterior base of pseudogonopodium). Distinguished from *C. brucei* in possessing 2 + 10–12 supraorbital neuromasts (*vs.* 3 + 13–16), sides of body purplish pink, with 7 horizontal golden lines in males (*vs.* dark purplish red with 7 horizontal rows of metallic green spots), pale orange stripes on flank in females (*vs.* no pale orange stripe), and black spots just below lateral midline on anterocentral portion of flank in females (*vs.* no black spot, sometimes small black dots).

*Description*

Morphometric data given in Table 1. Males larger than females, largest male 38.1 mm

SL, largest female 32.3 mm SL. Dorsal profile convex from snout to end of dorsal-fin base, approximately straight on caudal peduncle. Ventral profile gently convex from lower jaw to end of anal-fin base, nearly straight on caudal peduncle. Body moderately slender, slightly compressed. Greatest body depth at level of pelvic-fin base.



**FIGURE 7.** *Campellolebias chrysolineatus*, UFRJ 5210, male, 30.1 mm SL; Brazil: Santa Catarina: Araquari.



**FIGURE 8.** *Campellolebias chrysolineatus*, UFRJ 5210, female, 23.3 mm SL; Brazil: Santa Catarina: Araquari.

Dorsal and anal fins pointed and elongate in males, terminating in short filament reaching vertical through posterior half of caudal fin; dorsal and anal fins rounded, short and without filamentous rays in females. Caudal fin rounded. Pectoral fins elliptical. Posterior margin of pectoral fins reaching vertical between pelvic-fin base and anus. Tip of pelvic fins reaching base of 3rd or 4th anal-fin rays in male, reaching between base of 1st and 3rd anal-fins ray in females. Pelvic-fin bases medially separated by interspace about half pelvic-fin base width. Dorsal-fin origin anterior to anal-fin origin, anal-fin origin on

vertical between base of 2nd and 3rd dorsal-fin rays in males, between base of 3rd and 4th dorsal-fin rays in females. Dorsal-fin origin between neural spines of vertebrae 10 and 11. Anal-fin origin between pleural ribs of vertebrae 11 and 12. Dorsal-fin rays 15–18; anal-fin rays 2 + 14–15 in males, 15–17 in females; caudal-fin rays 27–29; pectoral-fin rays 14–15; pelvic-fin rays 6.

Scales large, cycloid. Body and head entirely scaled, except on anteroventral surface of head. Dorsal and anal-fin bases without scales. Scales covering anterior 10 % of caudal fin. Frontal squamation E-patterned, E-scales not overlapping. Longitudinal series of scales 27; transverse series of scales 7; scale rows around caudal peduncle 12. Three to seven minute contact organs on exposed margin of each scale of ventral portion of flank in male. Minute papillate contact organs on internal surface of distal portion of pectoral-fin rays 1–4 in males.

Cephalic neuromasts: supraorbital 2 + 10–12, parietal 1, anterior rostral 1–2, posterior rostral 1–2, infraorbital 3 + 25–26, preorbital 3, otic 2, post-otic 2, supratemporal 1, median opercular 1–2, ventral opercular 1–3, preopercular 19–21, mandibular 9–10, lateral mandibular 3–4, paramandibular 1. Three to seven neuromasts on each scale of lateral series. Transverse row of neuromasts on caudal-fin base 5–7.

Basihyal triangular, longest width about 40 % of length; basihyal cartilage about 35 % of total length of basihyal. Six branchiostegal rays. Second pharyngobranchial absent. Gill-rakers on first branchial arch 2–3 + 9–10. Vomerine teeth absent. Dermosphenotic absent. Ventral process of posttemporal absent. Total vertebrae 29.

#### *Coloration*

Males: Sides of body purplish pink, with 7 horizontal golden lines. Sides of head dark purplish pink, scales of opercular region golden. Iris bright yellowish green, with dark reddish brown bar through center of eye. Dorsum light brown. Venter white to bluish white; dark reddish gray stripe along ventrolateral region of head; dark brown midventral stripe from lower jaw to pseudogonopodium. Dorsal and anal fins light pink, with small reddish brown spots on basal and posterior portions, dark gray with blue to golden iridescence on distal and anterior portions; narrow golden marginal line along anterior edge of fins. Caudal fin light pink, with small reddish brown spots, and narrow golden marginal line. Pelvic fins dark bluish gray, sometimes with narrow golden line on anterior margin. Pectoral fins hyaline.

Females: Sides of body light brownish gray, with 6 gray horizontal lines alternating with pale orange stripes; black spots on anteroventral portion of flank just below lateral midline, sometimes coalesced to form short black stripes; small black spots on posterior portion of caudal peduncle. Venter white to pale golden. Opercular region greenish golden. Iris light yellow, with brown bar through center of eye. Unpaired fins hyaline, with small black spots on basal region, larger on caudal-fin base. Paired fins hyaline.



*Distribution*

Coastal plains of northern Estado de Santa Catarina, between baía de Guaratuba and Itapema (Fig. 4).

***Campellolebias brucei* Vaz-Ferreira & Sierra**

(Figs. 9–10)

*Campellolebias brucei* Vaz-Ferreira & Sierra, 1974: 1 (type locality: temporary swamp between Criciúma and Tubarão [road BR-101, near road to Rincão], Estado de Santa Catarina, Brazil [approximately 28°45'S 49°15'W]; holotype: ZVC-P 2116).

*Material examined*

Brazil: Estado de Santa Catarina: ZVC-P 2126, 1 paratype; ZVC-P 2127, 1 paratype; temporary swamp between Criciúma and Tubarão, road BR-101, near road to Rincão; G. C. Brasil, 28 Nov. 1972. UFRJ 293, 7; UFRJ 1854, 4 (c&s); same locality; G. C. Brasil & M. T. C. Lacerda, Aug. 1988. UFRJ 4493, 111; UFRJ 4494, 6 (c&s); same locality; W. J. E. M. Costa, R. D'Arrigo & D. Belote, 15 Sep. 1997.

*Diagnosis*

Similar to *C. chrysolineatus* and distinguished from *C. dorsimaculatus* and *C. intermedius* by having dorsal and anal fins long in males, tip reaching posterior half of caudal fin (*vs.* reaching basal portion of caudal fin) and pelvic-fin tips reaching base of 4th anal-fin ray in males (*vs.* reaching anterior base of pseudogonopodium). Distinguished from *C. chrysolineatus* in possessing 3 + 13–16 supraorbital neuromasts (*vs.* 2 + 10–12), sides of body dark purplish red, with 7 horizontal rows of metallic green spots (*vs.* purplish pink, with 7 horizontal golden lines in males), no pale orange stripes on flank in females (*vs.* pale orange stripes present), and no black spots on anterocentral portion of flank in females (*vs.* black spots, sometimes coalesced forming short black stripes).

*Description*

Morphometric data given in Table 1. Males larger than females, largest male 37.8 mm SL, largest female 20.5 mm SL. Dorsal profile convex from snout to end of dorsal-fin base, approximately straight on caudal peduncle. Ventral profile slightly convex from lower jaw to end of anal-fin base, nearly straight on caudal peduncle. Body moderately slender, slightly compressed. Greatest body depth at level of pelvic-fin base.

Dorsal and anal fins pointed and elongate in males, terminating in short filament reaching vertical through posterior half of caudal fin; dorsal and anal fins rounded, short and without filamentous rays in females. Caudal fin rounded. Pectoral fins elliptical. Posterior margin of pectoral fins reaching vertical between pelvic-fin base and anus in males, through vertical just in front pelvic-fin base in females. Tip of pelvic fins reaching

base of 3<sup>rd</sup> or 4<sup>th</sup> anal-fin rays in males, reaching base of 1st or 2nd anal-fin ray in females. Pelvic-fin bases medially separated by interspace slightly shorter than pelvic-fin base. Dorsal-fin origin anterior to anal-fin origin, anal-fin origin on vertical between base of 2nd and 3rd dorsal-fin rays in males, between base of 3rd and 4th dorsal-fin rays in females. Dorsal-fin origin between neural spines of vertebrae 9 and 11. Anal-fin origin between pleural ribs of vertebrae 10 and 11 in males, between pleural ribs of vertebrae 11 and 12 in females. Dorsal-fin rays 16–19; anal-fin rays 2 + 14–16 in males, 16–18 in females; caudal-fin rays 26–30; pectoral-fin rays 13–14; pelvic-fin rays 5–6.



**FIGURE 9.** *Campellolebias brucei*, UFRJ 4493, male, 26.9 mm SL; Brazil: Santa Catarina: Criciúma.



**FIGURE 10.** *Campellolebias brucei*, UFRJ 4493, female, 18.0 mm SL; Brazil: Santa Catarina: Criciúma.

Scales large, cycloid. Body and head entirely scaled, except on anteroventral surface of head. Dorsal and anal-fin bases without scales. Scales covering anterior 10 % of caudal fin. Frontal squamation E-patterned, E-scales not overlapped. Longitudinal series of scales 25–26; transverse series of scales 7–8; scale rows around caudal peduncle 12. Three to six minute contact organs on exposed margin of each scale of ventral portion of flank in

males. Minute papillate contact organs on internal surface of distal portion of pectoral-fin rays 2–4 in males.

Cephalic neuromasts: supraorbital 3 + 13–16, parietal 1, anterior rostral 1–2, posterior rostral 1–2, infraorbital 3 + 26–28, preorbital 3–5, otic 5, post-otic 3, supratemporal 1–2, median opercular 1, ventral opercular 3, preopercular 20–24, mandibular 7–10, lateral mandibular 3–5, paramandibular 1. Three to six neuromasts on each scale of lateral series. Transverse row of five neuromasts on caudal-fin base.

Basihyal subtriangular, longest width about 35 % of length; basihyal cartilage about 35 % of total length of basihyal. Six branchiostegal rays. Second pharyngobranchial absent. Gill-rakers on first branchial arch 2 + 8. Vomerine teeth absent. Dermosphenotic absent. Ventral process of posttemporal absent. Total vertebrae 27–28.

#### *Coloration*

Males: Sides of body dark purplish red, with 7 horizontal rows of metallic green spots; sometimes scarcely visible narrow, dark purplish red bars on posterior portion of sides of body. Sides of head dark purplish red, scales of opercular region metallic green. Iris bright yellowish green, with dark reddish brown bar through center of eye. Dorsum light brown. Venter white to bluish white; dark reddish gray stripe along ventrolateral region of head; dark brown midventral stripe from lower jaw to pseudogonopodium. Dorsal and anal fins light green, with small reddish brown spots on basal and posterior portions, light blue on distal and anterior portions; narrow submarginal black line and narrow bluish white marginal line along anterior edge of fins. Caudal fin light green, with small reddish brown spots and narrow light blue marginal line. Pelvic fins dark bluish gray with narrow light blue line on anterior margin. Pectoral fins hyaline.

Females: Sides of body light brownish gray, with 5–7 dark brown to black vertical zigzag lines, sometimes interrupted by narrow pale brown bars; sometimes melanophores concentrated to form black dots over vertical lines on anteroventral portion of flank; small black spots on posterior portion of caudal peduncle. Venter white to pale golden. Opercular region greenish golden. Iris light yellow, with brown bar through center of eye. Unpaired fins hyaline, with small black spots on basal region, larger on caudal-fin base. Paired fins hyaline.

#### *Distribution*

Known only from the type locality region, temporary channels near Criciúma, coastal plains of southern Santa Catarina, Brazil (Fig. 4).

#### **Discussion**

Reevaluation of phylogenetic morphological characters used in previous studies (*e. g.*, Costa, 1990, 1998) indicates an hypothesis similar to those formerly presented, in which

*Campellolebias* is a strongly supported clade, and the sister group to *Cynopoecilus* (Fig. 1). *Campellolebias intermedius*, not available in previous phylogenetic studies of the genus, is hypothesized to be the sister group to a clade including *C. chrysolineatus* and *C. brucei*. Therefore, although *C. intermedius* and *C. dorsimaculatus* are known from neighboring regions of the rio Ribeira de Iguape basin, they are not closely related taxa. However, records for *Campellolebias* are still scarce, thus preventing biogeographic analyses.

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## Appendix 1

The list below includes material examined of the species used as terminal taxa in the phylogenetic analysis, except those of the genus *Campellolebias*, which are listed in the “Taxonomic accounts” above. Data are organized in the following sequence: catalog number, number of specimens, locality. Abbreviations are: c&s, specimens cleared and stained for bone and cartilage; H, holotype; N, neotype; P, paratype(s). Abbreviations of institutions are: MCP, Museu de Ciências e Tecnologia da Pontifícia Universidade Católica, Porto Alegre; UFRJ, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro.

*Cynopoecilus melanotaenia*: UFRJ 4837, 14; UFRJ 5225, 4 (c&s); Brazil: Rio Grande do Sul: Estação Quinta. *Kryptolebias brasiliensis*: UFRJ 3458, 32; UFRJ 3682, 1 (c&s); UFRJ 4603, 2 ex.; Brazil: Rio de Janeiro: Magé. *Leptolebias marmoratus*: MCP 28604, N; UFRJ 5404, 1; UFRJ 5355, 24; UFRJ 5356, 4 (c&s); Brazil: Rio de Janeiro: Vila de Cava. *Nematolebias whitei*: UFRJ 5292, 59; UFRJ 5293, 8 (c&s); Brazil: Rio de Janeiro: São Pedro da Aldeia. *Neofundulus paraguayensis*: UFRJ 3647, 10; UFRJ 3648, 4 (c&s); Brazil: Mato Grosso do Sul, about 70 km NW from de Aquidauana.

## Appendix 2

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

### Superficial dermal bones and neurocranium

- [1] Dermosphenotic (Costa, 1990) (CI: 1.00; RI: 1.00): (0) present; (1) absent.
- [2] Lateral wings of vomer (Costa, 1998a) (CI: 1.00; RI: 1.00): (0) broad; (1) narrow.
- [3] Vomerine teeth (Costa, 1996) (CI: 1.00; RI: 1.00): (0) present; (1) absent.

### Jaws, jaw suspensorium and opercular apparatus

- [4] Ventral process of angulo-articular (Costa, 1998) (CI: 1.00; RI: 1.00): (0) long and wide; (1) long and narrow; (2) short and wide; (3) vestigial or absent [not ordered].
- [5] Mesopterygoid extent and its relative position to quadrate (modified from Costa, 1990, 1998) (CI: 1.00; RI: 1.00): (0) long, reaching metapterygoid and overlapping quadrate; (1) short, posterior tip in vertical through middle of quadrate, ventral portion overlapping quadrate; (2) very short, posterior tip in vertical through anterior portion of quadrate.
- [6] Symplectic (Costa, 1990, 1998) (CI: 1.00; RI: 1.00): (0) short and deep (Fig. 10); (1) long.
- [7] Dorsal portion of preopercle (Costa, 1990, 1998) (CI: 1.00; RI: 1.00): (0) broad and rounded; (1) narrow and pointed.

### Hyoid and branchial arches

- [8] Basihyal width (expressed as percentage of basihyal length in adult male; modified from Costa, 1998) (CI: 1.00; RI: 1.00): (0) 45–80 %; (1) 90–100 %; (2) 35–40 % [not ordered].
- [9] Interhyal (Parenti, 1981; Costa, 1990) (CI: 1.00; RI: 1.00): (0) ossified; (1) cartilaginous.
- [10] Second pharyngobranchial teeth (Costa, 1998) (CI: 0.50; RI: 0.50): (0) present; (1) absent.
- [11] Epibranchials (Costa, 1998) (CI: 1.00; RI: 1.00): (0) short; (1) long.
- [12] Uncinate process of third epibranchial (Costa, 2004) (CI: 1.00; RI: 1.00): (0) long; (1) short.
- [13] Proximal edge of first hypobranchial (Costa, 1998) (CI: 1.00; RI: 1.00): (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.
- [14] Distal edge of first hypobranchial (Costa, 2004) (CI: 1.00; RI: 1.00): (0) articular face restricted to cartilaginous head of first ceratobranchial, (1) articular face anteriorly expanded.
- [15] Teeth on fourth ceratobranchial (Parenti, 1981; Costa, 1990) (CI: 1.00; RI: 1.00): (0) present; (1) absent.

### Vertebrae and caudal skeleton

- [16] Neural prezygapophyses of caudal vertebrae (Costa, 1990) (CI: 1.00; RI: 1.00): (0) elongate; (1) vestigial or absent.
- [17] Posterobasal portion of last neural spine (Costa, 1998) (CI: 1.00; RI: 1.00): (0) without process; (1) with small process bearing ligament attached to bent anterior tip of epural.
- [18] Hypurals (modified from Costa, 1998) (CI: 1.00; RI: 1.00): (0) two symmetrical plates; (1) plates fused, but with vestiges of median fissure; (2) complete ankylosis forming a single plate, without vestige of median fissure.
- [19] Proximal region of epural and parhypural (Costa, 1998) (CI: 1.00; RI: 1.00): (0) broad and

approximately straight; (1) narrow and curved anteriorly.

#### Dorsal and anal-fin skeleton

- [20] First proximal radials of anal fin (Costa, 2006) (CI: 0.50; RI: 0.50): (0) long and narrow; (1) short and wide.
- [21] First proximal radial of anal fin in male (Costa, 1995a, 1998) (CI: 1.00; RI: 1.00): (0) approximately straight; (1) curved posteriorly.
- [22] First two anal-fin rays in male, ligamentous connections and radials (Costa, 1995a, 1998) (CI: 1.00; RI: 1.00): (0) thin and weakly connected to proximal radials, with small cartilaginous distal radials; (1) thickened and strongly attached to proximal radials, with large cartilaginous distal radials.

#### Shoulder and pelvic girdle

- [23] Posttemporal ventral process (Costa, 1998) (CI: 1.00; RI: 1.00): (0) present; (1) absent.
- [24] Supracleithrum (Costa, 1990, 1998) (CI: 1.00; RI: 1.00): (0) short; (1) long.
- [25] Posterior flange of cleithrum (Costa, 1998) (CI: 1.00; RI: 1.00): (0) present; (1) vestigial or absent.
- [26] Pectoral-fin radials (Costa, 1990, 1998) (CI: 1.00; RI: 1.00): (0) well-ossified, cub form; (1) thin, weakly ossified, disc shaped.
- [27] Space between lower pectoral-fin radial and coracoid (Costa, 2006) (CI: 1.00; RI: 1.00): (0) wide; (1) narrow, with narrow ventral expansion of cartilage.
- [28] Ischial process (Costa, 2006) (CI: 1.00; RI: 1.00): (0) present; (1) absent.

#### Muscles of anal-fin base

- [29] Muscular fibers circularly arranged around urogenital papilla base to form ejaculatory pump (Costa, 1995a, 1998) (CI: 1.00; RI: 1.00): (0) absent; (1) present.
- [30] *Inclinatores anales* 1-3 (Costa, 1995a, 1998) (CI: 1.00; RI: 1.00): (0) narrow; (1) expanded laterally to form fan-shaped structure.

#### External morphology of body and fins

- [31] Urogenital papilla of male (Costa, 1998) (CI: 1.00; RI: 1.00): (0) free of anal fin; (1) attached to anal fin.
- [32] Extent of urogenital papilla in male (Costa, 1995a, 1998) (CI: 1.00; RI: 1.00): (0) short; (1) long, to form pseudogonopodium.
- [33] Urogenital papilla of female (Costa, 1998) (CI: 1.00; RI: 1.00): (0) a transverse gap; (1) a prominent pocket-like structure overlapping anterior anal-fin origin.
- [34] Anal-fin membrane in male (modified from Costa, 1998) (CI: 1.00; RI: 1.00): (0) continuous; (1) interrupted to isolate anterior portion of fin.
- [35] Male dorsal-fin shape (Costa, 2006) (CI: 1.00; RI: 1.00): (0) rounded; (1) pointed.
- [36] Male anal-fin shape (Costa, 2006) (CI: 1.00; RI: 1.00): (0) rounded and short; (1) pointed and slightly elongated; (2) pointed and long.
- [37] Pelvic-fin bases (Costa, 2006) (CI: 0.50; RI: 0.50): (0) in close proximity; (1) separated by short interspace.

#### Squamation

- [38] Arrangement of frontal scales (modified from Parenti, 1981; Costa, 1990, 1998) (CI: 1.00; RI: 1.00): (0) circular; (1) transversal.
- [39] Relative position of E-scales (Costa, 1998) (CI: 1.00; RI: 1.00): (0) overlapped; (1) not overlapped.
- [40] Supraorbital scales (modified from Costa, 1998) (CI: 1.00; RI: 1.00): (0) present; (1) absent.

## Neuromasts

- [41] Total number of supraorbital neuromasts (Costa, 1998) (CI: 1.00; RI: 1.00): (0) 6–7; (1) 12–19.  
[42] Neuromasts on caudal-fin base (Costa, 1995a, 1998) (CI: 1.00; RI: 1.00): (0) 1–2; (1) 5–7.

## Contact organs

- [43] Contact organs on scales of male flank (Costa, 2006) (CI: 1.00; RI: 1.00): (0) absent; (1) present.  
[44] Male pectoral-fin contact organs (Costa, 2006) (CI: 0.50; RI: 0.66): (0) absent; (1) present.

## Color patterns

- [45] Iridescent color pattern on body side in male (modified from Costa, 2006) (CI: 0.66; RI: 0.50): (0) iridescence over flank; (1) vertical rows of iridescent dots; (2) longitudinal rows of small iridescent spots [not ordered].  
[46] Dark pigmentation on flank in female (modified from Costa, 1998) (CI: 0.75; RI: 0): (0) homogeneous; (1) vertically elongated blotches or bars; (2) narrow horizontal lines between scales; (3) broad stripe; (?) dark pigmentation absent.  
[47] Dark pigmentation pattern of median portion of iris (Parenti, 1981; Costa, 1990, 1998) (CI: 1.00; RI: 1.00): (0) distinctive dark marks absent; (1) bar through center of eye.  
[48] Iridescent color of iris in male (Costa, 2006) (CI: 0.50; RI: 0.66): (0) yellow to yellowish brown; (1) greenish blue to yellowish green.  
[49] Concentration of melanophores on dorsal and anal fins subdistal region in male (Costa, 1995a) (CI: 1.00; RI: 1.00): (0) not concentrated; (1) strongly concentrated to form dark gray to black stripe.  
[50] Dark pigmentation pattern on midventral portion of head and trunk (Costa, 1990, 1995a, 1998) (CI: 1.00; RI: 1.00): (0) not distinctively pigmented; (1) concentrated to form midventral stripe.  
[51] Dark pigmentation pattern on lateroventral portion of head (Costa, 1995a, 1998) (CI: 1.00; RI: 1.00): (0) not distinctively pigmented; (1) concentrated to form stripe.  
[52] Melanophore pattern of caudal peduncle (Costa, 2006) (CI: 0.50; RI: 0.66): (0) not distinctively aggregated; (1) aggregated to form black spots on posterior zone.

## Egg

- [53] Chorion surface (Costa, 1990, 1998) (CI: 1.00; RI: 1.00): (0) plain to verrucate; (1) reticulate.  
[54] Chorion projections (Costa, 1990, 1998) (CI: 0.50; RI: 0): (0) spine to hair-like; (1) mushroom-like.

## Reproductive behavior

- [55] Insemination (Costa, 1995a, 1998) (CI: 1.00; RI: 1.00): (0) external; (1) internal.  
[56] Position of dorsal and anal fins during male courtship behavior (Costa, 1990, 1998) (CI: 1.00; RI: 1.00): (0) antero-posteriorly expanded; (1) twisted.  
[57] Coiling retrorse motion during male courtship behavior (Costa, 1998) (CI: 1.00; RI: 1.00): (0) absent; (1) present.

## Development and habitat

- [58] Development and seasonality of habitats (Costa, 1990, 1998) (CI: 1.00; RI: 1.00): (0) non long diapause stages, life-cycle in non-seasonal habitats; (1) long diapause stages, life-cycle in seasonal pools.



### Appendix 3

Matrix of 58 morphological characters for 9 rivulid species. Characters and states are according to Appendix II. 0 = plesiomorphic state; 1–3 = apomorphic states; ? = not pertinent or unknown state.

	1–10	11–20	21–30
<i>Kryptolebias brasiliensis</i>	0000000000	0000000000	0000000000
<i>Neofundulus paraguayensis</i>	0001000010	0101000000	0000100000
<i>Nematolebias whitei</i>	0002101111	1111110211	0011111100
<i>Leptolebias marmoratus</i>	1013211011	1111110111	0011111110
<i>Cynopoecilus melanotaenia</i>	1113211010	1111110111	0011111110
<i>Campellolebias brucei</i>	1113211211	1111111110	1111111111
<i>Campellolebias chrysolineatus</i>	1113211211	1111111110	1111111111
<i>Campellolebias dorsimaculatus</i>	1113211211	1111111110	1111111111
<i>Campellolebias intermedius</i>	1113211211	1111111110	1111111111

continued.

	31–40	41–50	51–58
<i>Kryptolebias brasiliensis</i>	0000000000	0000000000	00000000
<i>Neofundulus paraguayensis</i>	0000000010	0000021000	00000001
<i>Nematolebias whitei</i>	00101101?0	1001111000	01000001
<i>Leptolebias marmoratus</i>	1010111111	10102?1100	00110001
<i>Cynopoecilus melanotaenia</i>	1011110111	1010231000	00111101
<i>Campellolebias brucei</i>	1111121111	1111221111	11101111
<i>Campellolebias chrysolineatus</i>	1111121111	1111221111	11101111
<i>Campellolebias dorsimaculatus</i>	1111110111	1111111111	11101111
<i>Campellolebias intermedius</i>	1111111111	1111221111	11101111