



A new miniature cryptic species of the seasonal killifish genus *Spectrolebias* from the Tocantins River basin, central Brazil (Cyprinodontiformes, Aplocheilidae)

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Abstract

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The miniature seasonal killifish *Spectrolebias costae*, first described for the middle Araguaia River basin, has been also recorded from two areas in the middle Tocantins River basin, from where male specimens exhibit some differences in their colour pattern. Analyses directed to species delineation (GMYC and bPTP), using a fragment of the mitochondrial gene COI, strongly support two species, *S. costae* from the Araguaia River basin and a new species from the Tocantins River basin. *Spectrolebias gracilis* **sp. n.** is described on the basis of specimens collected from two localities separated by about 530 km, Canabrava River floodplains near Alvorada do Tocantins and Tocantins River floodplains near Palmeirante. Field inventories were unsuccessful in finding additional populations in the region, which is attributed to the high environmental degradation, including several large dams that have permanently inundated typical killifish habitats. *Spectrolebias gracilis* is member of a clade also including *S. costae*, *S. inaequipinnatus*, and *S. semicellatus*, diagnosed by having the dorsal and anal fins in males with iridescent dots restricted to their basal portion, caudal fin in males hyaline, and caudal-fin base with two pairs of neuromasts. Within this clade, a single miniaturisation event is supported for the most recent common ancestor of the subclade comprising *S. costae* and *S. gracilis*, which differ from other congeners by reaching only about 20 mm standard length as maximum adult size.

Introduction

The great species diversity, striking colour patterns and the broad array of unique biological specializations make aplocheiloid killifishes important members of the tropical biota of Americas, Africa, and southern Asia (Costa 2008). The subequatorial South American area comprising the largest southern tributaries of the Amazonas River (Tocantins, Araguaia, Xingu and Tapajós River drainages), is remarkable by concentrating numerous endemic aplocheiloid fishes (Costa 1990, 2007a, b, 2011, 2016), many of them consisting of miniatures not surpassing 30 mm of standard length (SL) when adults (Costa 1998). Some endemic groups, such as the genera *Maratecoara* Costa, 1995, *Pituna* Costa, 1989, *Plesiolebias* Costa,

1989, and part of the genus *Spectrolebias* Costa & Nielsen, 1997 are members of the aplocheiloid clades known as seasonal or annual killifishes (Myers 1942; Costa 2002a), that comprise species completing their whole life cycle in seasonal pools formed during the rainy seasons.

Spectrolebias is the most basal lineage and the only genus of the seasonal killifish tribe Cynolebiini that is geographically widespread along southern Amazon tributaries (Costa 2007a; Costa et al. 2017). *Spectrolebias costae* (Baker 1990) is the smallest member of the Cynolebiini, with its maximum adult size not surpassing about 20 mm SL (Costa 2007a). This species was first discovered in the early 1980's, near the central Brazilian town of Aruanã, in the middle Araguaia River floodplains, by local fishermen (L. Costa, pers. comm. to WJEMC, January 1986;

Costa 2002b). It was soon exported as an aquarium fish species and quickly became a worldwide popular species, but its formal description was only published about ten years later, in an American aquarium association journal (Lazara 1991). It was then named *Cynolebias costai* Lazara, 1991, in honour of the Brazilian fisherman L. C. Costa from Aruanã. However, one year before Lazara's description, Baker (1990) reported his experience with this species in aquarium referring to it as *Cynolebias costae* and providing a brief description of the colour pattern of males and females. Therefore, following the International Code of Zoological Nomenclature (ICZN 1999), Baker (1990) should be considered the author of *C. costae*, of which *Cynolebias costai* Lazara, 1991 is the junior synonym (Costa 2008). More recently, this species was transferred to *Spectrolebias* (Costa 2010).

Between 1986 and 1994, in addition to the type locality region around Aruanã, *S. costae* was also found in other localities of the Araguaia River basin in the Bananal Island and adjacent areas in the Formoso River basin, as well as in the das Mortes River floodplains, which is a main tributary of the Araguaia River (Costa 1995b). However, some years later new populations morphologically similar to *S. costae* were found in the middle section of the Tocantins River basin (Costa 2007a). More detailed comparison revealed that populations from the Tocantins basin differ from populations from the Araguaia basin in some characters of the male colour pattern, suggesting that it might be a distinct cryptic species (*sensu* Bickford et al. 2007). That hypothesis is herein corroborated by mitochondrial-DNA species delimitation analyses, and so we provide a formal description of this new species.

Materials and methods

Specimens

Field studies failed to find specimens of *S. costae* in the type locality region or in the das Mortes River basin, areas that were drastically modified in recent years (see Discussion). Consequently, for molecular studies, field collections were made only in the floodplains of the Formoso River, middle Araguaia River basin, where populations of *S. costae* are still abundant, and in two localities of the middle Tocantins River, in the Canabrava River floodplains and in the Tocantins River floodplains near the town of Palmeirante. For morphology, both recent and older collections deposited in the ichthyological collection of the Institute of Biology, Federal University of Rio de Janeiro (UFRJ), were analysed. Specimens were captured with small dip nets (40 × 30 cm) and euthanized soon after collection. Euthanasia was conducted in a buffered solution of tricaine methanesulfonate (MS-222) at a concentration of 250 mg/l, for a period of about 10 minutes, i.e., until opercular movements ceased. Collections were made with permits provided by ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade; permit number 20618-1 to WJEMC) and methods for euthanasia were approved

by CEUA-CCS-UFRJ (Ethics Committee for Animal Use of Federal University of Rio de Janeiro; permit number: 01200.001568/2013-87). See Costa (2007a) for a list of comparative material of *Spectrolebias*.

DNA extraction, PCR, and sequencing

Specimens were fixed in absolute ethanol and preserved in the same fixative. We used a fragment of the mitochondrial gene cytochrome oxidase c subunit I (COI), which is the single-locus marker most used for species delimitation. Total genomic DNA was extracted from muscle tissue of the right side of the caudal peduncle using the DNeasy Blood & Tissue Kit (Qiagen), according to the manufacturer's instructions. To amplify the fragments of the DNA were used the primers LCO1490, HCO2198 (Folmer et al. 1994) and Cox1R (Costa and Amorim 2011). Polymerase chain reactions (PCR) were performed in 30 µl reaction mixtures containing 5x Green GoTaq Reaction Buffer (Promega), 3.2 mM MgCl₂, 1 µM of each primer, 75 ng of total genomic DNA, 0.2 mM of each dNTP and 1U of Taq polymerase. The thermocycling profile was: (1) 1 cycle of 4 minutes at 94 °C; (2) 35 cycles of 1 minute at 92 °C, 1 minute at 47 °C and 1 minute at 72 °C; and (3) 1 cycle of 4 minutes at 72 °C. In all PCR reactions, negative controls without DNA were used to check contaminations. Amplified PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega). Sequencing reactions were made using the BigDye Terminator Cycle Sequencing Mix (Applied Biosystems). Cycle sequencing reactions were performed in 10 µl reaction volumes containing 1 µl BigDye 2.5, 1.55 µl 5x sequencing buffer (Applied Biosystems), 2 µl of the amplified products (10–40ng), and 2 µl primer. The thermocycling profile was: (1) 35 cycles of 10 seconds at 96 °C, 5 seconds at 54 °C and 4 minutes at 60 °C. The sequencing reactions were purified and denatured and the samples were run on an ABI 3130 Genetic Analyzer. Sequences were edited using MEGA 6 (Tamura et al. 2013) and aligned using ClustalW (Chenna et al. 2003). The DNA sequences were translated into amino acids residues to test for the absence of premature stop codons or indels using the program MEGA 6.0. A list of specimens with respective catalogue numbers, locality coordinates and GenBank accession numbers are given in Table 1.

Morphological studies

Specimens were fixed in formalin for a period of 10 days, and then transferred to 70% ethanol. Material is deposited in the ichthyological collections of Institute of Biology, Federal University of Rio de Janeiro, Rio de Janeiro (UFRJ) and Centre of Agrarian and Environmental Sciences, Federal University of Maranhão, Chapadinha (CIC-CAA). Measurements and counts follow Costa (1988). 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. Specimens were cleared and stained for osteological preparations following Taylor

Table 1. List of specimens used in molecular analysis, with respective catalogue numbers, locality coordinates and GenBank accession numbers.

Species	Catalogue number	Coordinates	GenBank
<i>Nematolebias whitei</i>	UFRJ 6841.1	22°34'34"S, 41°59'10"W	KF311352
<i>Spectrolebias semiocellatus</i>	UFRJ 9297.1	11°47'30"S, 49°45'56"W	MF441496
<i>Spectrolebias costae</i>	UFRJ 9298.1	11°47'30"S, 49°45'56"W	MF441497
	UFRJ 9298.2	11°47'30"S, 49°45'56"W	MF441498
	UFRJ 9298.3	11°47'30"S, 49°45'56"W	MF441499
<i>Spectrolebias gracilis</i> sp. n.	UFRJ 9299.1	07°53'02"S, 47°55'45"W	MF441500
	UFRJ 9299.2	07°53'02"S, 47°55'45"W	MF441501
	UFRJ 9299.3	07°53'02"S, 47°55'45"W	MF441502
	UFRJ 9299.4	07°53'02"S, 47°55'45"W	MF441503
	UFRJ 9300.1	12°29'45"S, 49°00'28"W	MF441504
	UFRJ 9300.2	12°29'45"S, 49°00'28"W	MF441505
	UFRJ 9300.3	12°29'45"S, 49°00'28"W	MF441506
	UFRJ 9300.4	12°29'45"S, 49°00'28"W	MF441507

and Van Dyke (1985); the abbreviation C&S in lists of material indicates those specimens prepared for osteological examination. Terminology for osteological structures followed Costa (2006), for frontal squamation Hoedeman (1958), and for cephalic neuromast series Costa (2001). Osteological characters used in species description are those showing informative variability among the *Cynolebiini* (Costa 2006). Characters involving colouration of living specimens were based on photographs taken in small aquaria between 12 and 48 hours after collections. Characters informative for distinguishing the cryptic species were formatted according to Sereno (2007).

Phylogenetic analysis and species delimitation

Phylogenetic analyses were performed in the programs Garli 2.0 (Zwickl 2006), for maximum likelihood (ML) and MrBayes 3.2.6 (Ronquist et al. 2012), for Bayesian inference (BI). Ingroup terminal taxa were three specimens of *S. costae* and four specimens of each population of *S. cf. costae*; outgroups were *Spectrolebias semiocellatus* Costa & Nielsen, 1997, a member of the sister group of *S. costae* (Costa, 2010) and *Nematolebias whitei* (Myers, 1942), a member of the basal-most lineage of the *Cynolebiini* (Costa et al. 2017). The best fitting evolutionary model of each codon position was determined using Akaike information criteria (AIC), with jModeltest version 2.1.7 (Darriba

et al. 2012); the best model found for each position was GTR+G. Support values of the ML analysis were calculated by 1000 bootstrap replications (Felsenstein 1985). For BI analysis, two independent Markov-Chain Monte Carlo (MCMC) runs were performed with 3 million generations each, sampling one of every 1,000 trees. The quality of the MCMC chains was evaluated in Tracer 1.6 (Rambaut et al. 2013). Posterior probabilities were obtained after applying a burn-in of the first 25% of the trees.

For unilocus species delimitations, the dataset was reduced to include unique haplotypes, comprising two haplotypes of *S. costae* and two of each population of *S. cf. costae*. These analyses were conducted using the generalized mixed Yule coalescent (GMYC) using both single and multiple threshold analyses (Pons et al. 2006; Fujisawa and Barraclough 2013) and the Bayesian implementation of the Poisson tree processes (bPTP; Zhang et al. 2013), which were performed in the Exelixis Lab's web server (<http://species.h-its.org/gmyc/> and <http://species.h-its.org/ptp/>, respectively). The ultrametric tree was generated with BEAST v1.8.4 and its user interface BEAUti 1.8.4 (Drummond et al. 2012). The clock model was set to the uncorrelated lognormal, the tree prior was set to a Coalescent Constant Population prior, the number of generations for MCMC was 10 million, with sampling of the trees every 1000 generations. Convergence was evaluated with Tracer v1.6. The value of parameters of the analyses, convergence of the MCMC chains, sample size and the stationary phase of the chains were evaluated using Tracer v. 1.5; a burn-in discarding the first 20% of the trees was applied using Tree-Annotator v1.8.4 (as for BEAST).

Results

Molecular data

The phylogenetic analyses supported the two populations of the Tocantins River basin as a single exclusive lineage with high support in both ML and BI analyses (Fig. 1). However, the two populations of the Tocantins River basin, although separated by about 530 km were not individually supported as exclusive lineages; the Canabrava population was supported by low bootstrap value for the ML analysis, and the Palmeirante population as a basal non exclusive lineage, which may be an indicative of persisting genetic flow (Wiens and Penkrot 2002). Concordantly, both the GMYC and bPTP models delimited the Tocantins River basin populations as two distinct specific entities, whereas haplotypes of the Canabrava and Palmeirante populations were clustered in a single species (Fig. 2).

Morphological characters

Morphometric and meristic data obtained from specimens representing the populations of the Tocantins River basin (see description below) were similar to data recorded for specimens collected along the Araguaia River basin (Costa 2007a). Females were nearly identical in all characters examined, including colour pattern. Only two characters

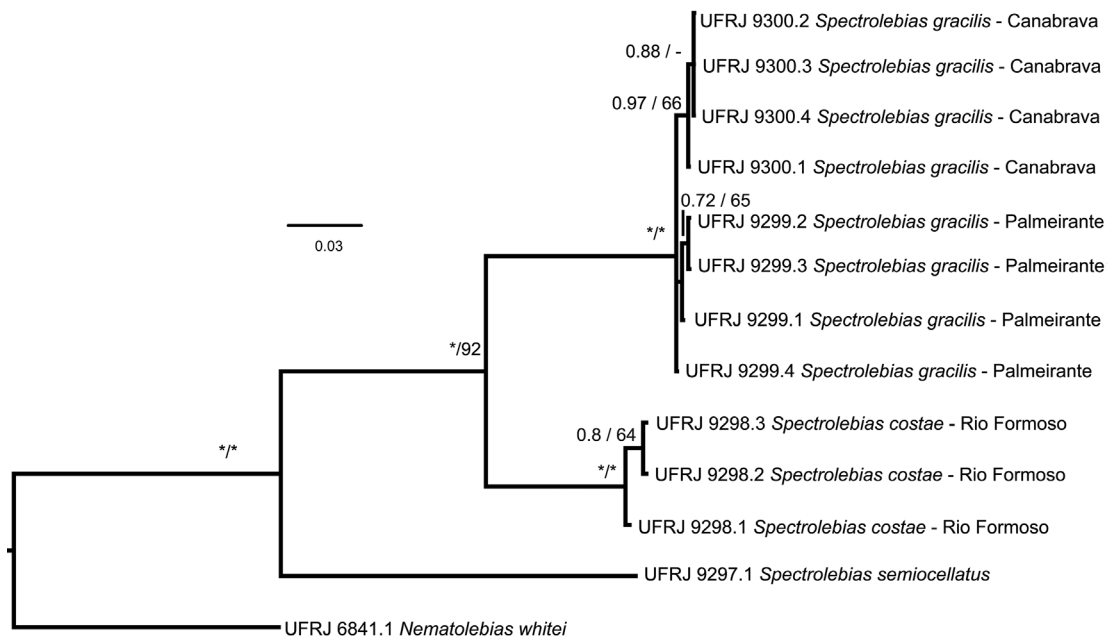


Figure 1. Bayesian phylogenetic tree for *Spectrolebias costae* sp. n., *S. gracilis*, *S. semicellatus* and *Nematolebias whitei*. Numbers above nodes are support values, including posterior probabilities for the Bayesian analysis, followed by bootstrap percentages for the maximum likelihood analysis; *, means maximum support value and, bootstrap value below 50.

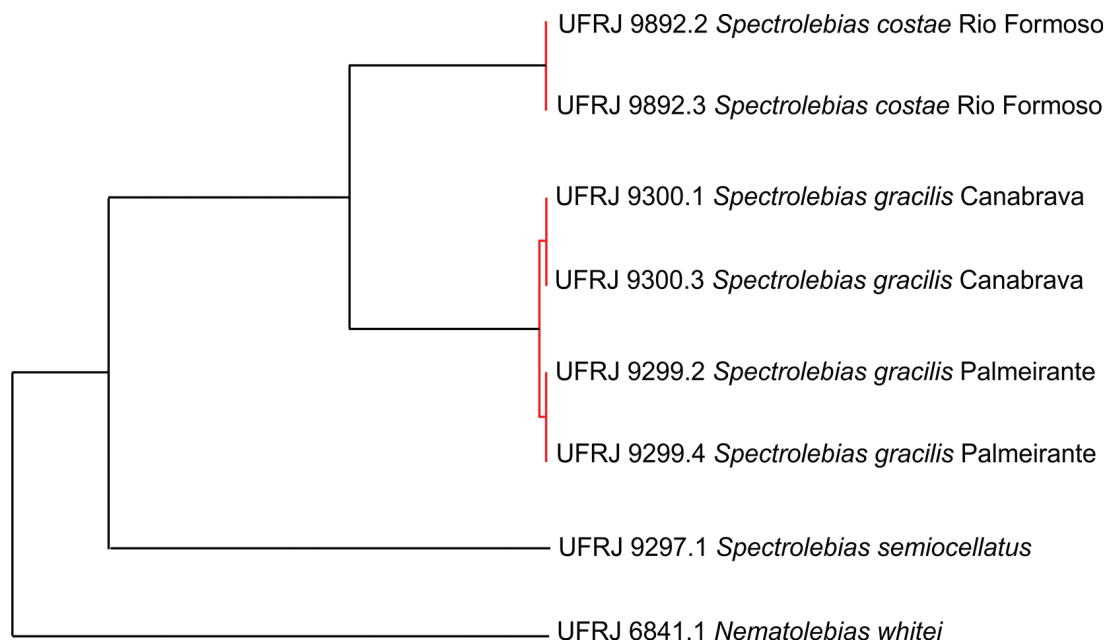


Figure 2. Species delimitation tree generated by the General Mixed Yule Coalescent (GMYC) and the Bayesian Poisson Tree Processes (bPTP) models, using a fragment of the gene COI. Black lines indicate branching processes among species, red lines indicate branching processes within species.

of the colour pattern of males were consistently effective to distinguish specimens of different populations:

1. Opercle in males, iridescent pattern: (0) 6–8 small blue dots usually arranged in three vertical rows over dark ground colour (in all populations of the Araguaia River basin; Fig. 3); (1) 10–12 blue spots in close proximity, irregularly arranged and surrounded by diffuse blue

iridescence (in all populations of the Tocantins River basin; Fig. 4).

2. Dorsal in males, basal portion, iridescent pattern: (0) blue dots regularly arranged in single longitudinal row (in all populations of the Araguaia River basin; Fig. 3); (1) blue dots irregularly arranged, often forming two rows (in all populations of the Tocantins River basin; Fig. 4).



Figure 3. *Spectrolebias costae*, UFRJ 3549, male, 18.8 mm SL; das Mortes River floodplains.

Taxonomic accounts

Spectrolebias gracilis sp. n.

<http://zoobank.org/D920DB7C-E3EA-477E-A744-3904651C2F8A>
Figs. 4–5; Table 2

Holotype. UFRJ 6440, male, 19.2 SL; Brazil: Tocantins state: Alvorada do Tocantins municipality: temporary lagoons close to the Canabrava River, a tributary of the Santa Teresa River, middle Tocantins River basin, road TO-373, 12°29'46"S, 49°00'51"W, altitude about 290 m asl; W. J. E. M. Costa et al., 16 Apr. 2006.

Paratypes. UFRJ 6441, 5 males, 18.9–20.8 mm SL, 3 females, 16.1–17.8 mm SL; UFRJ 6442, 3 males, 18.7–19.1 mm, 1 female, 16.8 mm SL (C&S); collected with holotype. UFRJ 9300, 2 males, 19.6–19.7 mm SL, 2 females, 18.4–18.5 mm SL; type locality area, 12°29'45"S, 49°00'28"W, altitude about 290 m; W. J. E. M. Costa et al., 26 Feb. 2013. – UFRJ 9593, 2 males, 19.9–20.0 mm SL, 2 females, 16.2–16.8 mm SL; UFRJ 9299, 4 males, 18.5–20.5 mm SL, 1 female, 19.2 mm SL; Goi- atins municipality, temporary pool in the floodplains of the right bank of the Tocantins River, near Palmeirante, 07°53'02"S, 47°55'45"W, altitude about 170 m asl; W. J. E. M. Costa et al., 28 Feb. 2013. – UFRJ 10802, 9 males, 15.8–17.6 mm SL, 22 females, 13.0–17.4 mm SL; UFRJ 10803, 3 males, 16.3–20.7 mm SL, 3 females, 13.9–15.3 mm SL (C&S); CICC AA 00692, 5 males, 16.2–17.2 mm SL, 5 females, 14.6–16.0 mm SL; same locality; A.C. de Luca, 2012.

Diagnosis. *Spectrolebias gracilis* is member of a clade endemic to the Araguaia-Tocantins River System, also including *S. costae*, *S. semiocellatus* Costa & Nielsen, 1997 and *S. inaequipinnatus* Costa & Brasil, 2008, and morphologically diagnosed by: dorsal and anal fins in males with iridescent dots restricted to the basal portion

of fins (vs. scattered over the whole fin), caudal fin in males hyaline (vs. variably coloured, usually dark red or grey), caudal-fin base with two pairs of neuromasts (vs. one). *Spectrolebias gracilis* is similar to *S. costae* and distinguished from *S. semiocellatus* and *S. inaequipinnatus* by having dorsal fin rounded in males (vs. pointed), dark brown to black pigmentation on the flank in males (vs. light brownish grey), and a subdistal bright blue stripe on the dorsal and anal fins in males (vs. subdistal bright blue absent). *Spectrolebias gracilis* differs from *S. costae* by the iridescent light blue colour pattern in males, comprising the presence of 10–12 small blue spots irregularly arranged on opercle, surrounded by diffuse blue iridescence (Fig. 4; vs. 6–8 small blue spots, usually arranged in three vertical series, contrasting with dark brown colour ground, Fig. 3) and one or two series of dots irregularly arranged on the basal portion of the dorsal fin (Fig. 4; vs. blue dots arranged in single longitudinal row close to fin base, Fig. 3).

Description. Morphometric data is given in Table 2. Largest male examined 20.8 mm SL; largest female examined 18.5 mm SL. Body relatively deep, compressed. Greatest body depth in vertical through pelvic-fin insertion. Dorsal profile convex between snout and posterior end of dorsal fin, nearly straight and horizontal on caudal peduncle; ventral profile convex between lower jaw and pectoral-fin base, approximately straight and moderately steep between pelvic-fin base and posterior end of anal fin, nearly straight and horizontal on caudal peduncle. Urogenital papilla short and cylindrical in males, globular in females. Head moderately wide, sub-triangular in lateral view. Jaws short, teeth numerous, conical, irregularly arranged; outer teeth hypertrophied, inner teeth small and numerous. Vomerine teeth 13. Gill-rakers on first branchial arch 2 + 7, gill-rakers short, straight, without denticles. Head narrow, sub-triangular in lateral view.

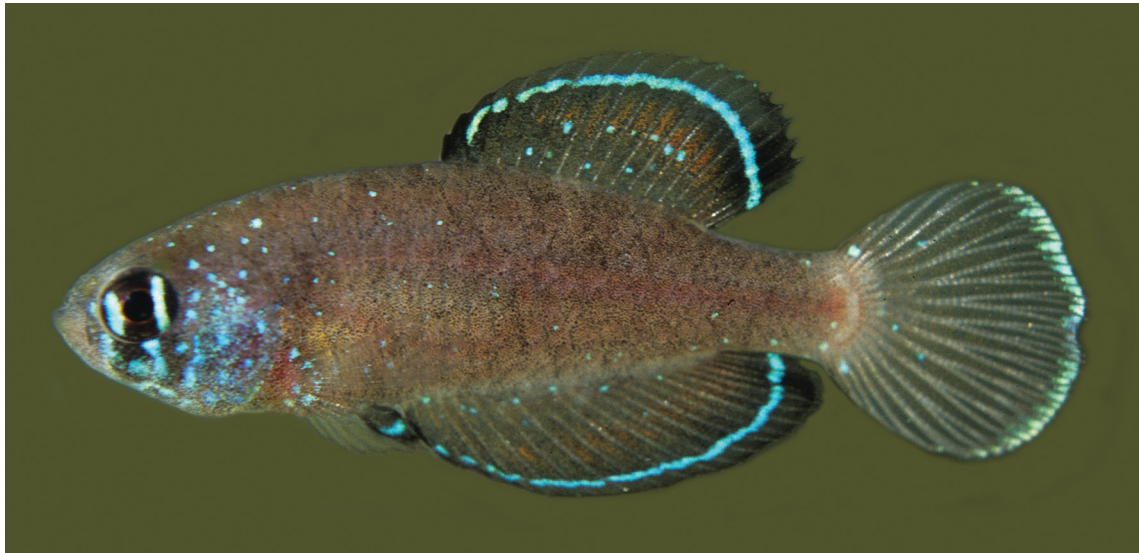


Figure 4. *Spectrolebias gracilis* sp. n., UFRJ 6440, holotype, male, 19.2 mm SL; Canabrava floodplains.



Figure 5. *Spectrolebias gracilis* sp. n., UFRJ 6441, paratype, female, 17.8 mm SL; Canabrava floodplains.

Snout short, blunt. Jaws short, premaxilla and dentary teeth conical, small, numerous, irregularly arranged, except for external series with longer fang-like teeth. Vomerine teeth absent. Dermosphenotic absent. Gill-rakers on first branchial arch 2 + 8–9. Six branchiostegal rays. Total vertebrae 26–27.

Dorsal and anal fins rounded, broader and fan-shaped in males, without filamentous rays. Caudal fin subtruncate, dorsal and ventral margins nearly straight, posterior margin gently convex. Pectoral fin elliptical, posterior margin reaching vertical between base of fifth and sixth anal-fin rays in males, reaching urogenital papilla in females; in males, minute contact organs on two uppermost pectoral-fin rays. Pelvic-fin small, tip reaching between second and third anus anal-fin ray in males, between first and second anal-fin ray in females; pelvic-fin bases me-

dially in close proximity. Dorsal-fin origin in vertical between base of 3rd and 5th anal-fin rays in males, between base of 4th and 6th anal-fin rays in females. Dorsal-fin origin between neural spines of vertebrae 7 and 8 in males, between neural spines of vertebrae 9 and 10 in females; anal-fin origin between pleural ribs of vertebrae 6 and 7 in males, between pleural ribs of vertebrae 7 and 8 in females. Hypurals ankylosed, forming single hypural plate. Ventral process of posttemporal absent. Dorsal-fin rays 21–23 in males, 15–18 in females; anal-fin rays 23–25 in males, 19–21 in females; caudal-fin rays 21–23; pectoral-fin rays 12–13; pelvic-fin rays 5–6.

Scales small, cycloid. Body and head entirely scaled, except anterior ventral surface of head. Body squamation extending over anterior 20% of caudal-fin base; no scales on dorsal, anal and pectoral-fin bases. Longitudinal series

Table 2. Morphometric data of *Spectrolebias gracilis* (sp. n.).

	Holotype	Paratypes	
	male	males (10)	females (6)
Standard length (mm)	19.2	17.3–20.8	16.1–17.8
Percent of standard length			
Body depth	33.6	31.3–36.7	32.1–33.6
Caudal peduncle depth	14.2	13.2–15.7	12.9–13.7
Pre-dorsal length	50.6	46.8–51.2	56.4–61.8
Pre-pelvic length	43.7	39.6–45.6	45.3–47.9
Length of dorsal-fin base	36.8	36.3–40.9	21.1–26.4
Length of anal-fin base	43.9	39.6–44.2	26.7–30.3
Caudal-fin length	35.7	33.8–38.1	33.0–37.1
Pectoral-fin length	24.8	21.6–24.8	19.0–21.3
Pelvic-fin length	11.1	9.8–11.7	9.9–11.8
Head length	30.5	29.8–33.7	31.7–33.5
Percent of head length			
Head depth	95.6	94.4–103.3	84.7–93.3
Head width	58.6	55.7–62.8	56.5–60.7
Snout length	12.1	11.9–14.9	9.4–13.4
Lower jaw length	17.4	15.2–19.2	14.8–17.2
Eye diameter	38.5	35.1–39.6	35.9–39.7

of scales 24–25; transverse series of scales 9–10; scale rows around caudal peduncle 12. No contact organs on scales. Total vertebrae 26–27. Frontal squamation E-patterned; E-scales overlapping medially; anterior-most frontal G-scale.

Latero-sensory canals absent. Cephalic neuromasts: supraorbital 11–13, parietal 3–4, anterior rostral 1, posterior rostral 1, infraorbital 1 + 16–20, preorbital 3, otic 2, post-otic 2, supratemporal 1, pre-opercular 11–14, median opercular 1, ventral opercular 1, mandibular 6–7, lateral mandibular 3–5, paramandibular 1. One or two neuromasts per scale of trunk lateral line. Two pairs of neuromasts on caudal-fin base.

Colouration in life. Males (Fig. 4). Body dark purplish brown to black; posterior-most extremity of caudal peduncle light pinkish brown; minute bright blue dots irregularly scattered over flank, more concentrated on its anterior portion. Head brown to black, with 10–12 small bright blue spots irregularly arranged on opercle, surrounded by diffuse blue iridescence; two bright blue bars on suborbital region. Iris dark brown, with two bright blue bars. Dorsal and anal fins dark reddish grey to black, with sub-distal bright blue line; light blue dots irregularly scattered over basal portion of both fins. Caudal fin hyaline, with light blue dots irregularly scattered over its basal portion; posterior margin bluish white. Pectoral fin hyaline with bright blue dots on basal portion. Pelvic fin dark grey to black, with subdistal bright blue stripe.

Females (Fig. 5). Body pale brown, with irregularly arranged, vertically elongated dark brown blotches, more concentrated on its anterior portion. Opercular region and venter with greenish golden iridescence. Two black bars on suborbital region. Iris dark brown, with two brownish yellow bars. Dorsal and anal fins hyaline, with small dark brown spots. Caudal and paired fins hyaline.

Etymology. From the Latin *gracilis*, meaning thin, referring to the thin body of the small-sized new species.

Distribution and habitat. *Spectrolebias gracilis* is known from temporary pools of two localities of the middle Tocantins River basin, central Brazil (Fig. 6). In both localities pools were shallow, about 80 cm in deeper places, and densely occupied by aquatic vegetation.

Discussion

Spectrolebias gracilis has been collected in two localities of the middle section of the Tocantins River basin, separated by about 530 km (Fig. 6). Our field inventories in temporary pools of middle, lower and upper parts of the basin indicated that this species is not present. Collecting trips in the last three decades in the middle Tocantins River region have shown that the typical habitat of seasonal killifishes, consisting of temporary pools with dense aquatic vegetation, is relatively rare, when compared to similar areas in the middle Araguaia River region, probably as result of the Araguaia River basin occupying vast plain areas not present on the Tocantins River basin. However, the scarcity of suitable habitats for the occurrence of seasonal killifishes in the entire region is due to major impacting environmental factors resulted from intensive anthropic activities.

In the Araguaia River basin, the region around Aruanã, an important regional touristic site, has been highly deforested and the temporary swamps have been extirpated. The same occurred in the das Mortes floodplains, where original vegetation was substituted by plantations and swampy areas were drained. In the Tocantins River basin, the dense forest previously present at the river floodplains was drastically removed in recent years, and large dams have inundated floodplain areas (Akama 2017). For example, in recent field studies we found that the Lajeado Dam, which shut off river flow in 2002, forming a reservoir that now occupies an area of 630 km² along 172 km of the middle section of the Tocantins River (Agostinho et al. 2007). As a result, the river has permanently inundated all seasonal killifish habitats around the dam, including the type locality of rare killifishes endemic to this region, such as *Hypsolebias multiradiatus* (Costa & Brasil, 1994), *Maratecoara formosa* Costa & Brasil in Costa, 1995, *Plesiolebias xavantei* (Costa, Lacerda & Tanizaki, 1988), and *Trigonectes strigabundus* Myers, 1925. The Tocantins River basin has been considered as the most impacted Amazon tributary, with a dense concentration of dams (Winemiller et al. 2016; Akama 2017). The five presently operating dams in the middle section of the Tocantins River probably promote wide distribution gaps for fish living in temporary pools situated close to rivers, putting in risk their existence.

Spectrolebias costae and *S. gracilis* are members of a species group endemic to central Brazil, also including *S. semiocellatus* and *S. inaequipinnatus*, diagnosed by some

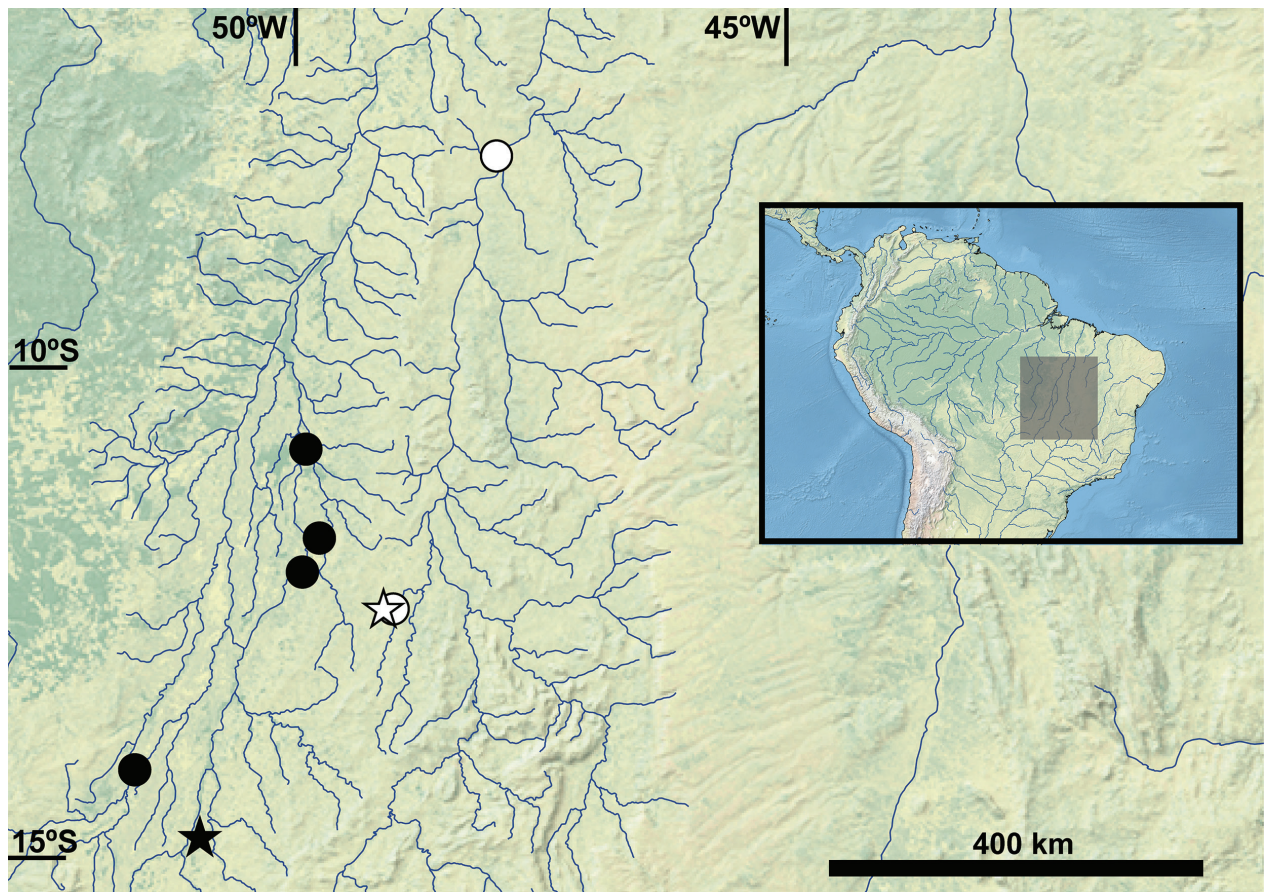


Figure 6. Geographical distribution of *Spectrolebias costae* (black symbols) and *S. gracilis* sp. n. (white symbols). Stars indicate type localities.

derived character states: dorsal and anal fins in males with iridescent dots restricted to the basal portion of fins (vs. scattered over the whole fin), caudal fin in males hyaline (vs. variably coloured, usually dark red or grey), caudal-fin base with two pairs of neuromasts (vs. one) (Costa 2010). *Spectrolebias costae* and *S. gracilis* are unique among species of this clade by possessing rounded dorsal fin in males, dark brown to black pigmentation on flank in males, and subdistal bright blue stripes on dorsal and anal fins in males. *Spectrolebias semiocellatus*, endemic to the Araguaia River basin, and *S. inaequipinnatus*, endemic to the Tocantins River basin, are closely related species, sharing the presence of a subtriangular dorsal fin in males with a long filamentous ray on its distal tip and frontal squamation F-patterned (vs. E-patterned), two derived conditions not occurring in other members of the tribe Cynolebiini (Costa & Brasil, 2008).

Weitzman and Vari (1988) reported a high incidence of events of miniaturization in Neotropical freshwater fishes, establishing an arbitrary standard of 26 mm SL as maximum adult size to recognise miniatures species. Costa (1998) argued that this standard value could be mostly useful in a phylogenetic context, particularly when detecting decreasing size gaps in sister lineages. *Spectrolebias costae* and *S. gracilis* reach about 20 mm SL as maximum adult size, thus contrasting with other closely related (*S. semiocellatus* and *S. inaequipinnatus*) and basal (*S.*

chacoensis (Amato, 1986) congeners that reach about 30 mm SL or more (Costa et al. 1997; Costa 2007a; Costa and Brasil 2008). This abrupt size gap suggests that a unique event of miniaturization occurred in the most recent common ancestor of the clade comprising *S. costae* and *S. gracilis*, which are also the smallest species among the about 100 species included in the tribe Cynolebiini

As discussed by Weitzman and Vari (1988), in addition to reduction in body size, miniaturization processes may also result in other morphological changes, more notoriously reduction of serial structures or structural simplification, which often occur in parallel in not closely related miniaturized taxa. The clade comprising *S. costae* and *S. gracilis* exhibits low counts of scales of the longitudinal (22–25) series and vertebrae (25–27), thus contrasting with higher values (usually 27 or more scales in the longitudinal series and 29 or more vertebrae) in congeners and species of closely related genera that reach 30 mm SL or more (Costa 2007). However, similar low counts are also found in *Spectrolebias reticulatus* (Costa & Nielsen, 2003) a species endemic to the Xingu River basin, Brazilian Amazon, as well as in the smallest species of *Simpsonichthys* Carvalho, 1959, all of them barely reaching 25 mm SL (Costa 2007). These data suggest that low scale and vertebra counts have independently arisen in at least three unrelated lineages of miniature cynolebiines.

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