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Jenynsia luxata, a new species from Northwestern Argentina, with additional observations of *J. maculata* Regan and phylogeny of the genus (Cyprinodontiformes: Anablepidae)

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Jenynsia luxata, a new species from northwestern Argentina, is described. This species is diagnosable from all other *Jenynsia* by the medial processes of left and right pelvic bones relatively reduced and separated from each other. The new species resembles *J. multidentata*, but it is further distinguished from this species by the absence of a swelling between the urogenital opening and the anterior base of the anal fin in females and details of coloration. Phylogenetic analyses, both under implied and equal weighting, recover the subgenera *Plesiojenynsia* and *Jenynsia* as monophyletic units. New information on previously missing characters of *Jenynsia maculata* is added. These data and phylogenetic characters coded for the new species herein described contribute to a better resolution of the phylogenetic relationships within the subgenus *Jenynsia*, which is herein supported by additional synapomorphies relative to previous phylogenies.

Jenynsia luxata, una nueva especie del noroeste de Argentina, es descripta. Esta especie es diagnosticable por presentar los procesos mediales de los huesos pélvicos izquierdo y derecho relativamente reducidos y separados. La nueva especie se parece a *J. multidentata*, pero se distingue de ésta por la ausencia de un abultamiento entre la abertura urogenital y la base de la aleta anal en hembras y por detalles en el patrón de coloración. Los análisis filogenéticos, tanto bajo pesos implicados como iguales, recuperan los subgéneros *Plesiojenynsia* y *Jenynsia* como unidades monofiléticas. Se aporta nueva información sobre caracteres de *J. maculata* previamente codificados como entradas faltantes. Esos datos y los caracteres filogenéticos codificados para la nueva especie aquí descripta contribuyen a una mayor resolución de las relaciones filogenéticas dentro del subgénero *Jenynsia*, que está aquí soportado por sinapomorfías adicionales en relación a las filogenias previas.

Key words: Pelvic bones, Phylogeny, Systematics, Taxonomy, Tucumán.

Introduction

The number of species of the anablepid genus *Jenynsia* Günther has been increased in the last few years to thirteen, with the description of *J. diphyes* Lucinda, Ghedotti & Graça (Lucinda *et al.*, 2006). *Jenynsia* includes small viviparous fishes that possess tricuspidate teeth in the outer mandibular series in adults and an unscaled tubular gonopodium formed principally by anal-fin rays 3, 6, and 7 (Parenti, 1981).

The species of the genus *Jenynsia* are distributed latitudinally in South America from Rio de Janeiro, Brazil to Río Negro, Argentina, and longitudinally from coastal Atlantic drainages at sea level to rivers bordering the Andean region from southern Bolivia to central Argentina, reaching up to almost 2,300 m above sea level at Cachi, Salta, Argentina where the type locality of *Jenynsia maculata* Regan is located.

The sister-group relationship between *Jenynsia* and *Anableps* Scopoli was first proposed by Parenti (1981), and then corroborated by Ghedotti (1998) who also provided a hypothesis of phylogenetic relationships among the nine species of *Jenynsia* known at that time. Ghedotti (1998) also provided a hypothesis of the monophyly of *Jenynsia* and the genus was split in two clades, the subgenera *Plesiojenynsia* Ghedotti and *Jenynsia*. After Ghedotti (1998), all the new species of *Jenynsia* described, *J. diphyes*, *J. onca* Lucinda, Reis & Quevedo, *J. tucumana* Aguilera & Mirande, and *J. weitzmani* Ghedotti, Meisner & Lucinda were included into his morphological matrix and several new hypotheses of relationships were erected within the subgenus *Jenynsia*, but the two subgenera proposed by Ghedotti (1998) were always recovered as monophyletic. Lately Köerber & Azpelicueta (2009) recognized *J. pygogramma* Boulenger as a junior

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synonym of *J. obscura* (Weyenbergh). Currently there are five species in the subgenus *Plesiojenynsia* while the other eight species belong to the nominal subgenus *Jenynsia*.

Species of the genus *Jenynsia* have very restricted distributions, excepting *Jenynsia multidentata* (Jenyns) which is present from lowlands up to 1200 m above sea level in a large area in Argentina, Brazil and Uruguay (Aguilera & Mirande, 2005). Indeed, *J. multidentata* is considered as a eurytopic species according to Hued & Bistoni (2005) following the criteria of Karr *et al.* (1986), because it can tolerate a wide range of environmental conditions. This characteristic, which can justify the wider distributional pattern shown by this species can also mislead systematic studies in cases in which specimens distributed far from their type locality are considered to represent the same species. *Jenynsia multidentata* is probably a species-complex that should be better studied.

The objectives of this paper are to describe a new species belonging to the subgenus *Jenynsia* from Northwestern Argentina, with a coloration pattern that resembles *Jenynsia multidentata* and to propose a new phylogenetic hypothesis based on a reanalysis of the morphological matrix by Ghedotti (1998) including data for the new species herein described. In the matrix by Ghedotti (1998), *Jenynsia maculata* had several missing entries, which are herein coded from observations of cleared and stained specimens.

Material and Methods

Specimens were cleared and counterstained (C&S) following Taylor & Van Dyke (1985). Measurements are straight distances taken with caliper to nearest 0.1 mm, following Aguilera & Mirande (2005), and expressed as percents of standard length (SL) in Table 1. Nomenclature of the sensory canal system follows Gosline (1949). The last two rays in the anal fin of females and dorsal fin of all specimens were counted as separate elements following Ghedotti & Weitzman (1995). The number of vertebrae includes the hypural complex as one element, and the gill rakers were counted from the ventral limb of first gill arch. Numbers in brackets following the counts indicate the number of specimens for each count; and an asterisk indicates counts of the holotype. The cladistic analyses were performed under equal and implied weighting in a wide range of concavities (constant K) (Goloboff, 1993) with the same protocol followed by Aguilera & Mirande (2005). Clade support was estimated using both resampling methods and decay indices. For the former the matrix was pseudoreplicated with Symmetric Resampling and the supports are expressed as GC values (groups present/contradicted) (Goloboff *et al.*, 2003). The relative Bremer support was used as decay index, given that this considers both information supporting as contradicting each node and also because it gives values that are comparable between analyses under equal and implied weighting (Bremer, 1994; Goloboff & Farris, 2001). Multistate characters, whose states follow a logical sequence (*i.e.*, could be interpreted as nested homologies) were considered as additive (characters 19, 30, 40, 46, and 58 of Ghedotti's 1998 matrix). The analyses were rooted in *Profundulus labialis* (Günther), and the outgroup also includes *Fluviphylax obscurus* Costa, *Aplocheilichthys spilauchen* (Duméril) and *Alfaro cultratus* (Regan). The analyses were performed using TNT software (Goloboff *et al.*, 2008) both with and without constraints in the outgroup structure as used by Ghedotti (1998). We split the transformation series of character 46, as previously done by Aguilera & Mirande (2005), as:

Character 46: Length of anal-fin ray five in adult male: (0) long, approximately as long as ray three; (1) intermediate, between one-quarter and three-quarters length of ray three; (2) short, less than one-quarter length of ray three.

Character 71: Symmetry of anal-fin ray five in adult male: (0) symmetric; (1) asymmetric, with one side short and the other side intermediate.

Lucinda *et al.* (2006) considered that "this change violates the assumption of character-state independence". However, the character 46 is only applicable to the species whose males have a symmetric fifth anal-fin ray and these species can logically have any of the possible states of that character. Therefore, the assumption of independence is not violated because no coding of each of these characters determines logically some specific state in the other one. Thus, the critics by Lucinda *et al.* (2006) are unfounded and the same character definitions used in Aguilera & Mirande (2005) are applied in this paper. Following this criterion, species with an asymmetric anal-fin ray 5 in adult males were codified with missing entries for character 46.

We found that character 51 (protuberance on tip of tubular gonopodium formed by anal-fin ray eight) was incorrectly coded by Ghedotti (1998) and this mistake was transferred to subsequent papers dealing with the phylogeny of *Jenynsia*. In the data matrix by Ghedotti (1998) this character was coded as if all the species of *Anableps* and *Jenynsia unitaenia* Ghedotti & Weitzman, *J. eirmostigma* Ghedotti & Weitzman and *J. eigenmanni* (Haseman) lacked the protuberance on tip of gonopodium (*i.e.*, character state 1 = absent) and all the remaining species of *Jenynsia* have that protuberance (*i.e.*, character state 0 = present), inversely relative to the description of that character in the text. This mistake did not produce consequences on the original phylogeny by Ghedotti (1998), given that all zeroes became ones and *vice versa*. However, this error produced errors in the new species described after Ghedotti (1998), because their authors coded this character as described in the body text (*i.e.*, with the zeroes and ones inverted relatively with the data matrix). In the present analysis we re-code the character 51 for all the species in the data matrix, assigning the character state "0" (presence of a protuberance on tip of gonopodium formed by anal-fin ray eight) to all *Anableps* species and to *J. diphyes*, *J. eigenmanni*, *Jenynsia eirmostigma*, *J. unitaenia*, and *J. weitzmani*, and the character state "1" (absence of the protuberance on tip of gonopodium) to *J. alternimaculata* (Fowler), *J. lineata* (Jenyns), *J. maculata*, *J. multidentata*, *J. obscura*, *J. onca*, *J. sanctaecatarinae* Ghedotti & Weitzman and *J. tucumana*. As in the paper by Ghedotti (1998) the root of the analysis is coded as a missing entry ("?") for this character.

Abbreviations for ichthyological collections in which the examined specimens are deposited are the following: AI: Asociación Ictiológica, La Plata; ANSP: Academy of Natural Sciences of Drexel University, Philadelphia CI-FML: Fundación Miguel Lillo, Tucumán; and MACN: Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires.

Results

Jenynsia luxata new species
Fig. 1

Holotype. CI-FML 5464, 24.6 mm SL, male, Argentina, Tucumán, Burruyacu city, a small unnamed stream, río Tajamar basin, 26º30'13.47"S 64º44'8.75"W, Apr 2002. L. Lobo.

Paratypes. AI 234, 4, 28.0-30.7 mm SL; CI-FML 5465, 6 (2 C&S), 20.9-29.1 mm SL; and MACN-Ict 9769, 5, 25.1-35.9 mm SL, same data as for holotype. CI-FML 5466, 12 (3 C&S), 17.3-44.3 mm SL, Argentina, Santiago del Estero, Pellegrini, small flooded pools at Ruta Provincial 4, not connected to but near to río Urueña basin, Mar 2010, P. Calviño.

Diagnosis. *Jenynsia luxata* is diagnosable from all other members of the genus by the medial processes of left and right pelvic bones relatively reduced, not overlapping each other at ventral midline (Fig. 2). The separation between the medial processes is detectable in fresh and alcohol-preserved specimens by the independent movement of these bones when this region is gently pressed by a needle.

Among the species of the subgenus *Jenynsia*, the coloration pattern distinguishes *J. luxata* (four to six regular rows of small circular spots of dark brown chromatophores on body side, been more apparent along caudal peduncle) from *J. alternimaculata* (two or, occasionally, three rows of dorsoventrally elongate markings on lateral body surface), *J. lineata* (five to seven rows of elongate, horizontal dash-shaped markings on side), *J. maculata* (three or four irregular series of more or less oblong blackish spots), *J. multidentata* (five to seven rows and also lines formed by round or short, horizontal dash-shaped markings, more than four scales in length on ventral caudal peduncle), *J. onca* (distinct ovoid to circular dark spots confined to ventral half of flank posterior to pelvic fin; spots gradually more circular towards midventral line), and *J. tucumana* (a row of dark markings ranging from dots to small vertical stripes, on the lateral surface, from the tip of the adpressed pectoral fin to the margin of hypural). *Jenynsia luxata* is also distinguishable from *J. multidentata* and *J. lineata* by the absence of a swelling between the urogenital opening and the anterior base of the anal fin in females; from *J. obscura* by the lower number of predorsal scales (12-16 *vs*. 19-25); from *J. onca* by the absence of a large dorsal convex expansion at subdistal segments of right half of sixth anal-fin ray of adult males and the smaller eye diameter of females (26.1-33.1% HL *vs*. 33.5-40.0% HL); and from *J. lineata* and *J. sanctaecatarinae* by the absence of a distinct rounded spot on dorsal pectoral-fin base.

Fig. 1. *Jenynsia luxata*. Above: holotype CI-FML: 5464, male, 24.4 mm SL, Tucumán, Burruyacu city, a small unnamed stream, río Tajamar basin, Argentina; below: paratype CI-FML 5466, female, 34.8 mm SL, Santiago del Estero, Pellegrini, small flooded pools at Ruta Provincial 4, not connected to but near to río Urueña basin, Argentina.

Description. Body stout and laterally compressed posteriorly; greatest body depth slightly anterior to pelvic-fin origin. Head blunt; head squamation in Fig. 3. Mouth terminal to slightly oblique. Dorsal profile of body straight from snout tip to supraoccipital process, straight to concave to dorsal-fin origin, and slightly convex backwards to caudal-fin origin. Ventral profile of body slightly concave from snout tip to isthmus, more pronounced to pelvic-fin origin, oblique backward along anal-fin base, and almost straight to caudal-fin origin. Anal-fin insertion located slightly posterior to vertical line through dorsal-fin origin. Sexual dimorphism present, males smaller than females and with intromittent organ formed by first eight anal-fin rays. Dorsal-fin insertion in females at halfway between pelvic and anal fins, in males closer to anal-fin origin. Pelvic fin reaching anus in females and reaching base of gonopodium in males. Absence of swelling between urogenital opening and anal-fin base of females.

Pores of cephalic sensory system associated with lateral sensory system anterior branch of supraorbital sensory canal formed by pores 1 and 2a; middle part by 2b, 3, 4a, and posterior branch by 4b, 5, 6, 7; preopercular canal continuous, with 7 pores; infraorbital canal formed by 4 pores; mandibular canal with pores X, Z, W, and pores Ya and Yb separated or included, in small specimens, in open groove; tricuspid teeth in both premaxilla and dentary.

Morphometric data in Table 1. Dorsal-fin rays 8 (1) or 9 (24*). Anal-fin rays in females 10 (17). Principal caudal-fin rays 14 (5), 15 (9*), or 16 (11). Pectoral-fin rays 14 (3), 15 (12), or 16 (10*). Pelvic-fin rays 6 (25*). Lateral line 30 (4), 31 (11*), 32 (8), or 33 (2). Predorsal scales 12 (2), 13 (8*), 14 (11), 15 (3), or 16 (1). Circumpeduncular scales 16 (25*). Vertebrae 28 (1), 29 (2), or 30 (2). Epipleural ribs 9 (2), 10 (2), or 11(1). Pleural ribs 11 (1), 12 (1), or 13 (3). Gill rakers 13 (2), or 14 (1).

Coloration in alcohol. Body background yellowish, grading from brown or pale brown dorsally to cream ventrally. Head dorsum from snout tip to vertical line through anterior eye margin dark brown but paler than area between eyes to supraoccipital process, having very distinct concentration of dark brown chromatophores. Cheek and area posteriorly surrounding eye pale brown or cream. Concentration of dark brown chromatophores on upper margin of opercle. Scales on body, from dorsum to second row behind lateral line with concentration of dark brown chromatophores bordering scales on their posterior field, forming reticulated pattern. Body side with circular spots or short dash-shaped markings of dark brown chromatophores in center of each scale, which coalesce in some specimens forming continuous lines and arranged into 4 to 6 regular rows along caudal peduncle. Rows of chromatophores less evident on anterior portion of body. Midlateral row more evident than remaining ones. Belly unpigmented, only peritoneal coloration visible at midventral line through body wall. Absence of diffuse dark chromatophores on dorsal pectoral-fin base. All fins hyaline, but having scattered chromatophores following rays on dorsal, caudal, and pectoral fins. Scattered chromatophores on gonopodium from its base to tip.

Distribution. The new species is currently known to inhabit the endorheic río Tajamar or río Cajón basin near to Ruta Provincial 304, at Burruyacu, northeastern Tucumán, and also in small flooded pools not connected but near to río Urueña basin, in northwestern Santiago del Estero (Fig. 4). Despite several collecting expeditions to the area and extensive sampling by the authors, no additional specimens were found.

Etymology. The specific name "*luxata*" derives from the Latin, meaning dislocate, in allusion to the diagnostic character of the species herein described, the separate pelvic bones.

Phylogenetic relationships. The morphological data matrix is presented in Table 2. The assignments of character states exhibited by specimens of *Jenynsia maculata* showed some differences with that by Ghedotti (1998): the fifth anal-fin ray of adult males is approximately as long as the third (46-0), the gonopodium lacks a protuberance on its tip formed by anal-

Table 1. Descriptive morphometrics of *Jenynsia luxata*. SD = standard deviation.

Character	Holotype	Males Paratypes (n = 8)			Females Paratypes (n = 16)		
		Range	Mean	SD	Range	Mean	SD
Standard length (mm)	24.4	17.3 - 30.4	22.8		26.5 - 44.3	33.4	
Percents of standard length							
Head length	28.9	25.4 - 29.4	27.8	1.4	26.9 - 29.3	28.1	0.8
Predorsal length	60.0	60.0 - 64.5	62.2	1.8	63.7 - 68.3	65.9	1.4
Snout to pectoral fin	31.3	29.2 - 33.2	31.3	1.3	27.9 - 32.9	30.0	1.4
Snout to pelvic fin	51.4	48.3 - 56.4	52.8	2.3	52.8 - 58.3	55.6	1.6
Peduncle depth	16.6	15.0 - 17.4	16.4	0.9	13.8 - 16.5	14.8	0.7
Caudal peduncle	34.1	32.2 - 36.2	34.2	1.3	26.1 - 33.0	29.7	2.1
Gonopodium length	26.5	21.8 - 30.5	25.9	2.6	-	-	-
Percents of head length							
Snout length	25.2	19.3 - 29.9	25.9	3.9	25.3 - 33.3	28.8	2.2
Post orbital length	44.5	42.4 - 54.2	47.5	3.8	42.0 - 48.6	46.0	1.7
Eye diameter	33.1	31.0 - 39.2	34.6	2.8	26.1 - 33.1	29.2	1.7
Interorbital width	45.3	41.4 - 57.8	47.7	5.4	42.3 - 51.3	46.7	2.4

Fig. 2. Pelvic girdle of *Jenynsia luxata*, paratype, male, CI-FML 5465, 27.8 mm SL.

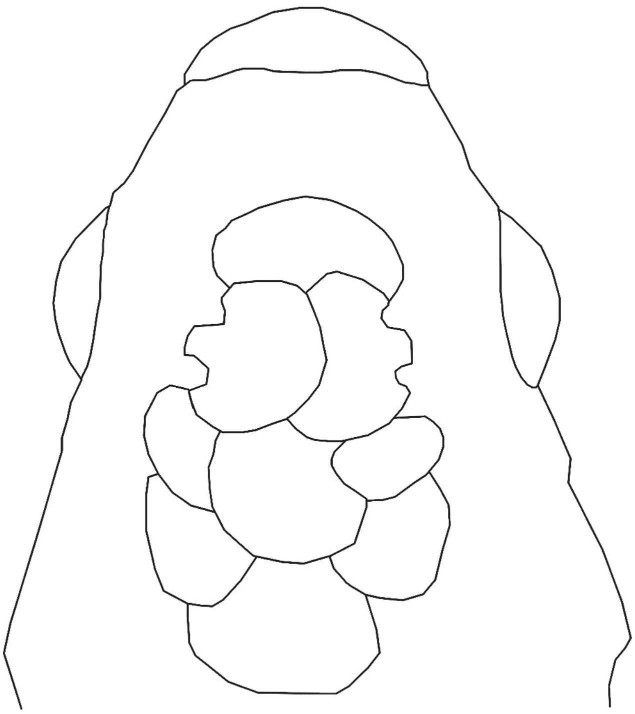

Fig. 3. Diagrammatic representation of head squamation of *Jenynsia luxata*.

fin ray eight (51-1), the hypurals are fused in adults, forming two symmetrical dorsal and ventral hypural elements (59-1), the pore W of the mandibular canal can be both present or absent (60-[01]), there is a series of three or more narrow lines composed of short, dash shaped marking (64-2), and the swelling between urogenital opening and anterior anal-fin base is absent (69-0).

Under equal weights, 12 equally most parsimonious trees of 154 steps were found (CI = 0.58; RI = 0.78). The topology of the consensus tree (Fig. 5A) is the same as the proposed by Lucinda *et al*. (2006) for the subgenus *Plesiojenynsia*, with *J. unitaenia* as the sister species of a polytomy including *J. diphyes*, *J. weitzmani*, and a clade composed of *J. eigenmanni* and *J. eirmostigma*. The subgenus *Jenynsia* is more resolved than in previous phylogenies, with a trichotomy at the base including *J. maculata*, *J. sanctaecatarinae*, and a clade composed of the remaining species of the subgenus. The latter clade also forms a trichotomy, composed of *J. luxata* and *J. onca* and two nodes including *J. lineata* and *J. multidentata*, and a trichotomy between *J. alternimaculata*, *J. obscura*, and *J. tucumana*, respectively.

Under implied weighting, two equally most parsimonious trees of 154 steps (CI = 0.58; RI = 0.78) were obtained with each concavity (K), in the range from 4 to 20. The strict consensus between these equally most parsimonious trees is completely resolved for the subgenera *Plesiojenynsia* and partially resolved for *Jenynsia* (Fig. 5B). Within *Plesiojenynsia*, *J. diphyes*, *J. unitaenia*, and *J. weitzmani* are successive sister species of a clade composed of *J. eigenmanni* and *J. eirmostigma*. In the subgenus *Jenynsia*, *J. maculata*, *J. sanctaecatarinae, J. onca*, and *J. luxata* are successive sister groups of a clade composed of *J. tucumana*, *J. alternimaculata*, and *J. obscura* as the sister group of a clade composed of *J. lineata* and *J. multidentata*.

Discussion

Jenynsia luxata exhibits the three synapomorphies considered by Ghedotti (1998) as diagnostic for the subgenus *Jenynsia*: (1) a modified sixth anal-fin ray segmented on its proximal quarter; (2) unsegmented on its distal quarter in adult males and (3) the vertically inclined proximal radials associated with the first six anal-fin rays in the gonopodium. These character-states are unique and unreversed in the subgenus *Jenynsia*. In the present analyses, both under equal and implied weighting, additional synapomorphies support the monophyly of the subgenus *Jenynsia*: a long and somewhat narrow, expanded medially and narrow laterally posterodorsal process of palatine in dorsal view (character state 17-1); left and right halves of anal-fin ray six in adults males not laterally paired, and with

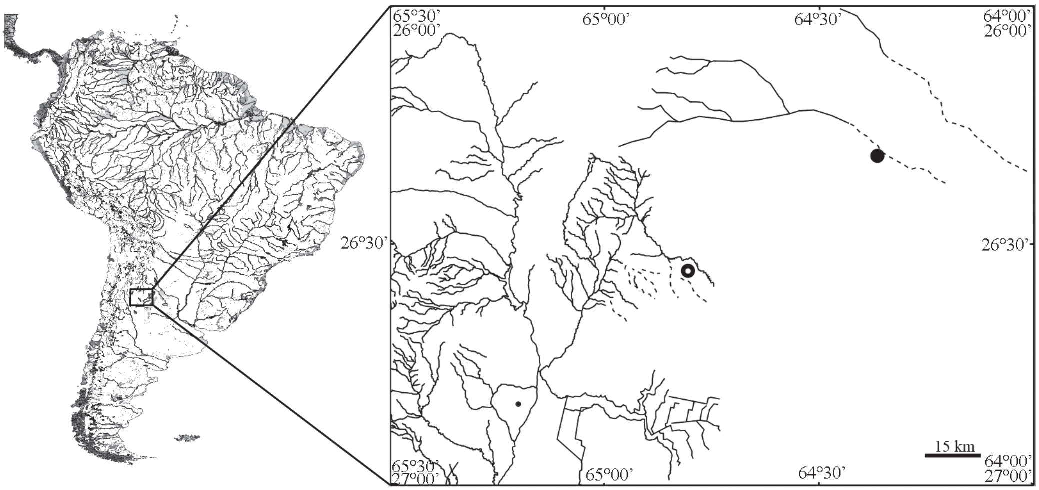

Fig. 4. Hydrographic map of South America showing the currently known distribution of *Jenynsia luxata*. The area delimited by the rectangle is enlarged at right, where the type locality is indicated by an open dot and an additional locality is indicated by a black dot. The small black dot indicates the capital of Tucumán.

both sides expanded and visible externally (character state 47-1); absence of a protuberance on the tip of gonopodium formed by anal-fin ray eight (character state 51-1); and presence of a series of three or more narrow lines not associated with distinct midlateral stripe on caudal peduncle, composed of short, dash shape markings (character state 64-2). The character 17 presents a reversion in *Jenynsia obscura* (character state 1 to 0); the character 47 has a transformation to state 2 in *J. onca* and a reversion to state 0 in the clade composed of *J. multidentata* and *J. lineata*; the character 51 is reversed to state 0 in *J. onca*; and the character 64 has a transformation to state 1 in *J. multidentata* and a reversion to state 0 in the clade composed of *J. tucumana*, *J altenimaculata*, and *J obscura*.

The medial process of left and right pelvic bones not overlapping each other is a unique character in *Jenynsia* that only can be found in the closely related genus *Anableps*, among the Anablepidae. This character is optimized in the phylogeny of the family as a parallelism between *Anableps* and *Jenynsia luxata*.

Table 2. Morphological data matrix. A= polymorphic character, states 0 and 1; B= polymorphic character, states 0 and 2; ?= missing character.

	1 – 10	11 – 20	21 – 30	31 – 40	41 – 50	51 – 60	61 – 70	71
Profundulus labialis	0000000000	0000000000	0000000000	0000000000	0000000000	??00000000	0000000000	0
Alfaro cultratus	0100000000	0000000010	0000000000	0000110102	2011000000	??0000021?	?000000001	0
Aplocheilichthys spilauchen	0100110001	00010000A1	0001100000	0000100000	0000000000	??00000010	?000000000	0
Fluviphylax obscurus	00002?0102	0000000020	0000100102	0000110004	0000000000	??0000020?	?000000000	0
Oxyzygonectes dovii	0100100011	0100000010	0101001000	0000001000	0000000000	??00000010	0000000100	0
Anableps dowi	1011111011	1111012112	1111001010	1011311111	1101000001	0010001210	1300111101	0
Anableps anableps	1011111011	1011112112	1111011010	1111111111	1101000001	0010001210	1001111101	0
Anableps microlepis	1011111011	1011112112	1111011010	1111111111	1101000001	0010001210	1001111101	0
Jenynsia unitaenia	0000110011	A100000001	0100100001	0000201002	1011110000	00100101A0	0100000101	0
Jenynsia weitzmani	0000010A11	0100000001	0100100001	0000201003	1011110000	0010110111	0100000101	0
Jenynsia onca	0000100001	0100001010	0101100100	0000001002	1011022110	0111010110	0002000101	0
Jenynsia eirmostigma	0000010111	0100000001	0100A00102	0000201003	1011110000	00101101A1	0210000101	0
Jenynsia eigenmanni	0000010111	1100000001	0100100101	0000201003	10111?0000	00101101A1	0210000101	1
Jenynsia sanctaecatarinae	0000110011	0100001010	0101100000	0000101002	10110?1110	1111010110	0002000101	1
Jenynsia alternimaculata	0000010001	0100001010	0101100000	0000B01002	10110?1110	11110101A1	0000000101	1
Jenynsia obscura	0000000012	0100000000	0101100000	0000201102	1011021110	1111010101	0110000111	0
Jenynsia multidentata	0000A10A11	A1000010A0	0101100000	0000001002	1010020110	1111010100	0001000111	0
Jenynsia lineata	0000010111	A100001010	0101100000	0000001002	1011020110	1111010100	0002000111	0
Jenynsia tucumana	0000010011	0100001010	0101100000	0000?01002	1011021110	1111010100	0010000101	0
Jenynsia diphyes	0000110011	0100000001	0100100000	0000201002	1011110000	1010110111	0210000101	0
Jenynsia maculata	0000000011	0100001010	0101100001	0000?01002	1011001110	101101011A	0002000101	0
Jenynsia luxata	0000100011	0100001010	0101100000	0000?01012	1011021110	1111010100	0001000101	0

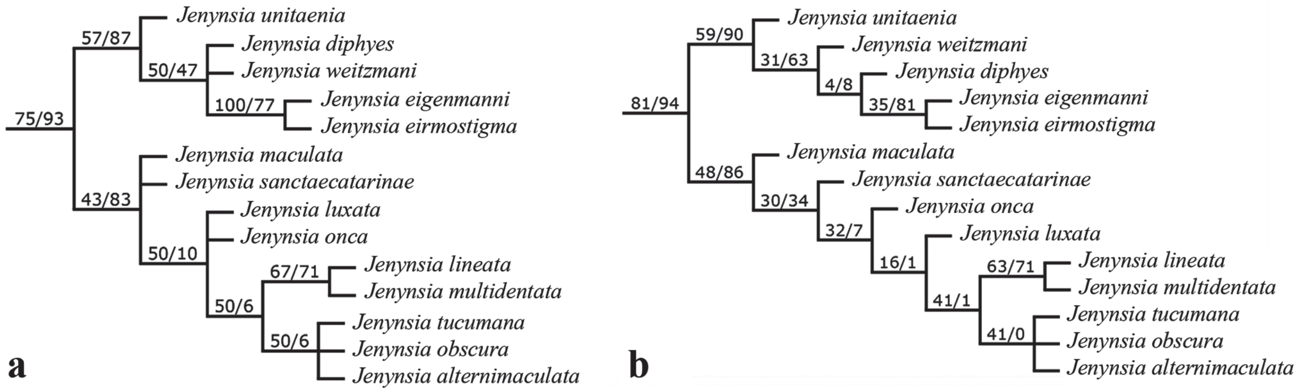

Fig. 5. Topologies from most equally parsimonious trees and relative Bremer support/GC values under (**a**) equal weights (strict consensus of 12 trees), and (**b**) implied weighting (strict consensus of two trees), with concavities K = 4 to 20 (with supports measured under K = 8). Unsupported nodes are shown as collapsed. The relationships of genera of the family are consistent with those proposed in previous studies, therefore species of *Anableps* and *Oxyzygonectes* are not shown.

Regarding the protuberance on the tip of gonopodium formed by anal-fin ray eight (character 51), only *J. tucumana* and *J. onca* were coded with the state 1 (= absence of that protuberance) in the latest published data matrix of the Anablepidae (Lucinda *et al.*, 2006), among the species belonging to the subgenus *Jenynsia*. However, close examination of specimens of *J. alternimaculata*, *J. lineata*, *J. maculata*, *J multidentata*, and *J. obscura* revealed that all these species also lack the protuberance on the tip of gonopodium. Based on this examination, all the above mentioned species were coded as "1" (= absent) for the character 51 of the original matrix of Ghedotti (1998). This change in the coding produces a higher resolution on the phylogenetic relationships among the members of the Anablepidae and especially in the subgenus *Jenynsia*. All the species of this subgenus lack the protuberance on the tip of gonopodium and it represents a new, unreversed synapomorphy for the subgenus *Jenynsia*.

The obtained topology for the subgenus *Jenynsia* differs from all previous analyses both under implied weighting and equal weights due to the addition of the new species herein described, the coding of several missing entries for *J. maculata*, and the correction of character 51 in the matrix from Ghedotti (1998). In the phylogenetic hypothesis herein proposed, based both on implied weighting and equal weights, *Jenynsia maculata* is the sister species of all the remaining species of the subgenus *Jenynsia*. Changes in the coding of several characters of *J. maculata*, compared with Ghedotti (1998), produced some differences in their optimization in the present analysis. The optimization of these characters is identical in the analyses under both equal and implied weighting. The change in coding of character 46, from state 2 (in Ghedotti's 1998 matrix) to 0 (in present codification) produced the migration of *Jenynsia maculata* to a basal position in the subgenus *Jenynsia* tree topology. In the present phylogenetic hypothesis the state 2 of character 46 is a synapomorphy of the clade including all the species of *Jenynsia*, excepting *J. maculata* and *J. sanctaecatarinae*.

The character-states 51-1 and 64-2 are herein proposed as new synapomorphies for the subgenus *Jenynsia*. The character 59 codified according to Ghedotti (1998) matrix (*i.e.*, character-state 0) was optimized as a synapomorphy for the subgenus *Jenynsia*, but it was not recovered given the present coding (*i.e.*, character-state 1). This character-state (59-1) is now a synapomorphy of a deeper node composed of *J. luxata* as the sister group of a clade including *J. alternimaculata*, *J. lineata*, *J. multidentata*, *J. obscura*, and *J. tucumana*. The character-state 69-1, as in the hypothesis of Ghedotti (1998), is a synapomorphy of a clade composed of *J. lineata* and *J. multidentata.*

Jenynsia luxata externally resembles *J. multidentata*, however, besides the medial process of pelvic bones not overlapping, the absence of swelling in *J. luxata* and several osteological differences between these species allow us to distinguish them. *Jenynsia multidentata* has a wide distributional range, from río Negro in Argentina to southern Brazil, and there is even a record from Chile (Quezada-Romegialli, 2009) where this species was introduced for mosquito control. Despite the fact that *J. multidentata* was unintentionally introduced in several rivers along its distributional range due to its use as bait, many native populations are in need of additional studies. Some of these populations likely constitute undescribed species.

Comparative material (numbers in parentheses indicate the number of measured specimens): *Jenynsia alternimaculata*. **Argentina.** CI-FML 3825, 16 (4 C&S), 20.8-43.7 mm SL, Salta, Orán, río Anta Muerta, tributary of río Blanco, río Bermejo basin. **Bolivia.** CI-FML 3831, 41 (10), 22.2-37.8 mm SL, Tarija, unnamed river in Acheralitos, which flows to río Cambarí, río Tarija basin. *Jenynsia lineata*.

Uruguay. CI-FML 5462, 26 (1 C&S), 20.9-38.3 mm SL, Maldonado, laguna del Diario. *Jenynsia maculata*. **Argentina.** CI-FML 3832, 10 (5), 21.0-30.2 mm SL, Catamarca, Fuerte Quemado, small tributary of río Santa María. CI-FML 4743, 20 (2 C&S), 15.9-30.4 mm SL, Salta, río Calchaquí, Cachi. *Jenynsia multidentata*. **Argentina.** CI-FML 1081, 3, 23.0-23.8 mm SL, Tucumán, Lules, arroyo Calimayo (2 km from Ruta Nacional 38). CI-FML 1569, 11 (5), 20.1-43.2 mm SL, Córdoba, arroyo Las Mojarras, 2 km from lago San Roque. CI-FML 3826, 15 (10, 2 C&S), 28.0-36.5 mm SL, Tucumán, Monteros, río Mandolo, río Salí basin. **Uruguay.** CI-FML 1440, 36 (10), 19.8-43.2, Canelones, Canteras de Carrasco. *Jenynsia obscura*. **Argentina.** CI-FML 2009, 288 (10), 21.8-54.7 mm SL, Catamarca, Hualfín, Los Nacimientos. CI-FML 5463, 10 (2 C&S), 27.6-57.8 mm SL, Catamarca, río Vís Vís basin. *Jenynsia tucumana*. **Argentina.** CI-FML 3828, holotype, 30.7 mm SL, Tucumán, Trancas, río Vípos, 5 km from Ruta Nacional 9. AI 163, paratypes, 6, 26.3-40.4 mm SL; ANSP 180781, paratypes, 6, 20.4-33.0 mm SL; CI-FML 3829, paratypes, 4, 26.2-32.8 mm SL; CI-FML 3840, paratypes, 2 C&S, 28.3 and 36.0 mm SL; CI-FML 3841, paratypes, 4 C&S, 20.4-38.5 mm SL, same data as holotype.

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