A new annual killifish species of the *Hypsolebias flavicaudatus* complex from the São Francisco River basin, Brazilian Caatinga (Cyprinodontiformes: Rivulidae)

Wilson J. E. M. Costa & Pedro F. Amorim

Laboratório de Sistemática e Evolução de Peixes Teleósteos, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Caixa Postal 68049, CEP 21944-970, Rio de Janeiro, RJ, Brasil;
wcosta(at)acd.ufrj.br

Accepted on March 26, 2011.

Published online at www.vertebrate-zoology.de on June 22, 2011.

> Abstract

The *Hypsolebias flavicaudatus* species complex is a clade of annual killifishes inhabiting seasonal pools associated to São Francisco River basin, in the semi-arid Caatinga region of northeastern Brazil. The analysis of both morphological and molecular data supports recognition of a new species of that complex, herein named as *Hypsolebias guanambi*. It was collected in the upper section of the Carnaíba de Dentro River drainage and is distinguished from all other species of the complex by having the dorsal fin more posteriorly placed in females, presence of short filamentous rays on the dorsal and anal fins in males, and fewer teeth on the second pharyngobranchial bone. The recognition of a species of the *H. flavicaudatus* complex endemic to the upper Carnaíba de Dentro River drainage corroborates this region as an important area of endemism for annual killifishes.

> Resumo

O complexo de espécies *Hypsolebias flavicaudatus* é um clado de peixes anuais que habitam poças sazonais associadas à bacia do Rio São Francisco, na região semi-árida da Caatinga do nordeste do Brasil. A análise de caracteres tanto morfológicos quanto moleculares sustenta o reconhecimento de uma nova espécie deste complexo, aqui nomeada como *Hypsolebias guanambi*. Ela foi coletada na seção superior da drenagem do Rio Carnaíba de Dentro e se distingue de todas as outras espécies do complexo por possuir a nadadeira dorsal posicionada mais posteriormente em fêmeas, presença de raios filamentosos curtos nas nadadeiras dorsal e anal em machos e menos dentes no osso segundo faringobranquial. O reconhecimento de uma espécie do complexo *H. flavicaudatus* endêmica da drenagem do alto Rio Carnaíba de Dentro corrobora esta região como uma importante área de endemismo para peixes anuais.

> Key words

Caatinga, Killifishes, Neotropica, Species delimitation, São Francisco River, Systematics, Taxonomy.

Introduction

Hypsolebias comprises a rich-species clade of annual killifishes endemic to the savannas of central and north-eastern Brazil (Costa, 2006a, 2007, 2010a). It includes over 35 species occurring in an area encompassing the São Francisco, Tocantins, and Itapicuru river basins and the coastal plain areas adjacent to the Jaguaribe and Piranhas river basins (Costa, 2008, 2010a, 2010b, in press), but about 75% of all species are endemic to the São Francisco River basin. Three species of that basin, *H. flavicaudatus* (Costa & Brasil), *H. flagellatus* (Costa) and *H. janaubensis* (Costa), constitute a well-corroborated clade, hereafter termed the *H. flavicaudatus* species complex, with members easily recognized by having iridescent dots on the anal fin restricted to the posterior portion of the fin in males (vs. on the whole fin) and anterior portion of the anal fin pink and posterior portion yellow in males (vs. never a similar colour pattern) (Costa & Brasil, 1990; Costa, 2003, 2006b).

Species of the *H. flavicaudatus* occupy a broad geographic region, between Santa Maria da Boa Vista, Pernambuco state (about 9° 00′ S) and the city of São Francisco, Minas Gerais state (about 15° 30′ S). Species of the *S. flavicaudatus* complex have been identified by a few morphological characters, usually comprising colour patterns and some morphometric data (COSTA, 2003, 2006b, 2007). Specimens of an undetermined population from Guanambi, Bahia, northeastern Brazil, exhibit a peculiar combination of morphological features, which suggests they belonging to a distinct undescribed species. The objective of the present study is to analyse morphological and molecular data under different methods of species delimitation in order of determining the status of the Guanambi population.

Table 1. Material used in the molecular analysis.

Species	Catalog number	GenBank accession number
H. flagellatus	UFRJ 6704.1	HQ833479
H. flagellatus	UFRJ 6704.3	HQ833480
H. flagellatus	UFRJ 6704.4	HQ833481
H. flavicaudatus	UFRJ 6822.1	HQ833491
H. flavicaudatus	UFRJ 6822.2	HQ833492
H. guanambi	UFRJ 6782.1	HQ833483
H. guanambi	UFRJ 6782.2	HQ833484
H. guanambi	UFRJ 6782.3	HQ833485
H. guanambi	UFRJ 6782.4	HQ833486
H. igneus	UFRJ 6709	HQ833482
H. janaubensis	UFRJ 6787.1	HQ833487
H. janaubensis	UFRJ 6787.2	HQ833488
H. janaubensis	UFRJ 6787.3	HQ833489
H. janaubensis	UFRJ 6787.4	HQ833490
H. mediopapillatus	UFRJ 6701	HQ833478

Material and methods

Material. All material is deposited in the ichthyological collection of the Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro (UFRJ), and is listed below. Comparative material is listed in COSTA (2007).

Morphology. Morphological characters were obtained from specimens of the type series and comparative material, which were fixed in formalin just after collection, for a period of 10 days, and then transferred to 70 % ethanol. Data on colour patterns were taken both from direct examination of live specimens in aquaria just after collection, and photographs of both sides of live individuals (two males and two females of each collection) taken in aquaria some hours after collection, just before fixation in formalin. Measurements and counts follow COSTA (1995); measurements are presented as percent of standard length (SL), except for those related to head morphology, which are expressed as percent of head length. Fin-ray counts include all elements. Number of vertebrae and gill-rakers were recorded from cleared and stained specimens; the compound caudal centrum was counted as a single element. Osteological preparations (c&s) were made according to TAYLOR & VAN DYKE (1985), but about half of all prepared specimens were not stained to avoid ossification damage produced by the acetic acid present in the Alcian Blue solution. Terminology for bones follows COSTA (2006), for frontal squamation HOEDEMAN (1958) and for cephalic neuromast series COSTA (2001).

Genetics. Molecular data were obtained from specimens fixed in 99.8 % ethanol just after collection; they were preserved in the same ethanol solution. List of specimens and respective GenBank accession numbers appears in the Appendix 1. 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 fragment of the mitochondrial DNA were used the primers COX1F (5'–CATAAA GAYATYGGCACCCTY–3') and COX1R (5–GGYTCTTCRAARGTGTGATAS–3'), specific for the mitochondrial gene cytochrome C oxidase I (COX-I). Polymerase chain reaction (PCR) was performed in 15μl reaction mixtures containing 5 × Green GoTaq Reaction Buffer (Promega), 3.2 mM MgCl2, 1 µM of each primer, 75 ng total DNA, 0.2 mM of each dNTP, 30 µg of BSA (bovine serum albumin) and 1U of Taq polymerase. The thermocycling profile was: (1) 1 cycle of 4 minutes at 94 °C; (2) 30 cycles of 1 minute at 92 °C, 1 minute at 54 °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 Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare). To the sequencing reactions was used 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 (10–40 ng), 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. Control region sequences (N = 15) were deposited in GenBank (Tab. 1). Sequences were edited

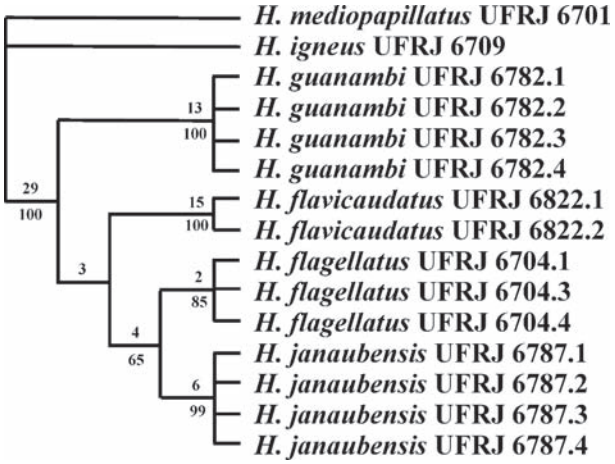

Fig. 1. Most parsimonious tree resulting from the analysis of the mt-DNA gene cytochrome C oxidase I (three length: 202; consistency index excluding uninformative characters: 0.8163; retention index: 0.8989). Numbers above the branches are branch lengths and below, bootstrap values (equal or above 50%) based on 1000 replicates.

using MEGA 4 (Tamura *et al*., 2007) and aligned using ClustalW (Chenna *et al*., 2003). Alignments were subsequently optimized manually.

Species concept and species delimitation. The species concept herein adopted is that known as the 'unified species concept' (e.g., de Queiroz, 2007), in which operational criteria for species delimitation are excluded from species conceptualization. Delimitation of species follows two independent methods: the population aggregation analysis (Davis & Nixon, 1992), a character-based method in which species are delimited by a unique combination of stable morphological character states occurring in one or more populations; and, the phylogenetic method described by Wiens & Penkrot (2002), consisting of a tree-based species delimitation using mitochondrial DNA, in which species limits are inferred from highly supported genealogical relationships with concordant geographic distribution. For this latter approach, the search for most parsimonious trees (implicit enumeration search) and bootstrap analysis (1000 replicates) were performed with TNT 1.1 (Goloboff, Farris & Nixon, 2008). Terminal taxa included topotypes of all nominal species of the *H. flavicaudatus* complex, besides topotypes of *H. mediopapillatus* and *H. igneus* as outgroups.

Results

The population aggregation analysis of morphological characters supports the Guanambi population as a distinct species, through a unique combination of character states of the following characters: position of dorsal fin relative to anal-fin rays in females; extent of dorsal and anal fin filamentous rays in males; number of caudal-fin rays; number of teeth on the second pharyngobranchial bone; number of grey bars on the flank in males; and, presence of short blue stripes and black spots on the anterior portion of the dorsal fin in males (see diagnosis below to distribution of character states among species of the *H. flavicaudatus* complex).

The cladistic analysis of a segment of the mt-DNA gene COX-I containing 1286 nucleotides, among which 1106 were constant and 79 parsimoniously informative, highly supports the Guanambi population as an exclusive lineage (Fig. 1). This analysis also strongly supports monophyly of the *H. flavicaudatus* species complex as well as exclusiveness of all proposed nominal species of the complex, as proposed in previous papers (e.g., Costa, 2003, 2006b, 2007).

Taxonomic accounts

Hypsolebias guanambi n. sp.

Figs. 1–2

Holotype. UFRJ 6861, male, 39.2 mm SL; Brazil: Estado da Bahia: Município de Guanambi: seasonal pool close to the road BR-030, 14 km W of the city of Guanambi, upper Carnaíba de Dentro River drainage, São Francisco River basin, 14° 13′42″ S, 42° 55′12″ W, altitude 502 m; W.J.E.M. Costa, C.P. Bove & B.B. Costa, 13 January 2005.

Paratypes. UFRJ 6072, 1 male, 29.3 mm SL, 1 female, 34.6 mm SL; collected with holotype. UFRJ 6783, 1 male, 32.5 mm SL, 1 female, 27.6 mm SL; UFRJ 6862, 2 males, 27.3–30.6 mm SL, 2 females, 26.6–29.1 mm SL (c&s); UFRJ 6782, 2 males, 27.8–30.4 mm SL, 6 females, 22.8–28.2 mm SL; same locality as holotype; W.J.E.M. Costa *et al*., 30 January 2010.

Diagnosis. *Hypsolebias guanambi* is distinguished from all other species of the *H. flavicaudatus* complex in having the dorsal fin more posteriorly placed in females (dorsal-fin origin in the vertical through the base of the 6th or 7th anal-fin ray, vs. between the base of the 1st and 5th rays), dorsal and anal fins often with short filamentous rays, reaching between the basal and middle portion of the caudal fin (vs. filaments always present, long, reaching the posterior portion of the caudal fin or surpassing its posterior limit) and fewer teeth on the second pharyngobranchial bone (4–6 vs. 7–11). Also distinguished from *H. flavicaudatus* by having more grey bars on the flank in males

Table 2. Morphometric data of *Hypsolebias guanambi*.

	holotype	paratypes	
	male	males (n = 4)	females (n = 3)
Standard length (mm)	39.2	29.3 – 32.5	26.6 – 34.6
Percent of standard length			
Body depth	39.5	35.8 – 37.9	36.8 – 40.4
Caudal peduncle depth	14.7	13.1 – 13.5	13.9 – 14.8
Predorsal length	52.0	48.4 – 51.5	60.5 – 62.3
Prepelvic length	46.6	42.0 – 47.6	49.1 – 51.4
Length of dorsal-fin base	37.5	34.4 – 37.8	20.3 – 24.4
Length of anal-fin base	39.4	35.1 – 40.5	26.1 – 29.6
Caudal-fin length	35.4	36.1 – 39.2	38.2
Pectoral-fin length	28.5	27.8 – 30.1	28.5 – 30.2
Pelvic-fin length	10.5	9.5 – 10.8	11.8 – 13.0
Head length	30.7	30.2 – 33.0	31.7 – 34.4
Percent of head length			
Head depth	97.9	91.8 – 102.2	92.0 – 95.7
Head width	65.3	58.8 – 60.5	60.8 – 61.9
Snout length	14.1	13.2 – 14.8	13.9 – 14.5
Lower jaw length	20.7	16.9 – 19.0	16.7 – 18.8
Eye diameter	29.3	27.1 – 32.6	29.1 – 34.8

(11 – 14 vs. 8 – 10) and fewer caudal fin rays (22 – 23 vs. 24 – 26); and, from *H. flagellatus* and *H. janaubensis*, by the absence of short blue stripes and black spots on the anterior portion of the dorsal fin in males (vs. presence).

Description. Morphometric data appear in Table 2. Largest male examined 39.2 mm SL; largest female examined 34.6 mm SL. Dorsal profile gently convex on head, convex from nape to end of dorsal-fin base, nearly straight on caudal peduncle. Ventral profile convex from lower jaw to end of anal-fin base, approximately straight on caudal peduncle. Body moderately deep, compressed, greatest body depth at level of pelvic-fin base. Eye positioned on dorsal portion of head side. Snout short, subtriangular in lateral view. Urogenital papilla cylindrical in males, short, slightly longer than wide; urogenital opening of females placed in pocket-like structure, slightly overlapping anal-fin origin.

Dorsal fin subtriangular in males, posterior extremity sharply pointed, sometimes with short filamentous rays on tip reaching vertical between base and middle of caudal fin; dorsal fin subtriangular in females, posterior extremity rounded to slightly pointed. Anal fin subtriangular in males, posterior extremity sharply pointed, often with short filamentous rays reaching vertical between base and middle of caudal-fin base; anal fin semicircular in females; distal portion of anterior rays strongly thickened. Caudal fin rounded. Pectoral fin long, elliptical to slightly pointed, tip reaching vertical on base of 7th or 8th anal-fin ray. Tip of pelvic fin reaching base of 3rd anal-fin ray. Pelvic-fin bases medially fused. Dorsal-fin origin in vertical between base of 2nd and 4th anal-fin ray in males, between base of 6th or 7th anal-fin ray in females. Dorsal-fin origin between neural spines of vertebrae 8 and 9 in males, and neural spines of vertebrae 11 and 13 in females. Anal-fin origin between pleural ribs of vertebrae 7 and 8 in males, and pleural ribs of vertebrae 8 and 10 in females. Dorsal-fin rays 21 – 23 in males, 13 – 17 in females; anal-fin rays 22 – 24 in males, 19 – 21 in females; caudal-fin rays 22 – 23; pectoral-fin rays 13 – 14; pelvic-fin rays 6.

Frontal squamation E-patterned; E-scales overlapping medially; no row of scales anterior to H-scale; supra-orbital scales 2 – 3. Longitudinal series of scales 29 – 30; transverse series of scales 13; scale rows around caudal peduncle 16. No contact organs on flank; papillate contact organs on three dorsal most rays of pectoral fin in males.

Cephalic neuromasts: supraorbital 15 – 18, parietal 2 – 3, anterior rostral 1, posterior rostral 1, infraorbital 2 + 19 – 22, preorbital 2 – 3, otic 1, post-otic 3, supratemporal 1, median opercular 1, ventral opercular 1, preopercular 17 – 18, mandibular 13 – 14, lateral mandibular 7, paramandibular 1. One neuromast on each scale of lateral line. Two neuromasts on caudal-fin base.

Basihyal subtriangular, width about 60 – 65 % of length; basihyal cartilage about 30 % of total length of basihyal. Six branchiostegal rays. Second pharyngobranchial teeth 4 – 6, arranged into two series. Gill-rakers on first branchial arch 3 + 11. Vomerine teeth absent. Dermosphenotic absent. Ventral process of posttemporal long. Total vertebrae 29 – 30.

Coloration. **Males**. Side of body light grey, with 11 – 14 approximately straight gray bars. Venter purplish white. Opercular region pale greenish golden. Iris light yellow, with dark brown bar. Dorsal fin pink, pale orange on posterior portion; light blue small spots on basal portion of fin. Anal fin pink on anterior portion, dark yellow on posterior portion; light blue to white small spots on posterobasal portion of fin; distal tip black. Caudal fin dark greenish yellow with light blue small spots on dorsal portion of fin base. Pectoral fin hyaline. Pelvic fin pink. **Females**. Side of body pale brownish grey, with 12 – 13 faint grey bars; one or two black spots on anterocentral portion of flank, in vertical between pelvic-fin base and anterior portion of anal-fin base; two or three black spots on caudal peduncle. Venter yellowish white. Opercular region pale golden. Iris light yellow, with grey bar. Fins hyaline.

Distribution and ecological notes. *Hypsolebias guanambi* is known only from the type locality area, where some seasonal pools are adjacent to a small

Fig. 2. *Hypsolebias guanambi*, UFRJ 6783, paratype, male, 33.5 mm SL; Brazil: Bahia: Guanambi.

Fig. 3. *Hypsolebias guanambi*, UFRJ 6783, paratype, female, 27.6 mm SL; Brazil: Bahia: Guanambi.

stream tributary to the Carnaíba de Dentro River, which is a right tributary of the São Francisco River, 13 km W of Guanambi, Bahia, north-eastern Brazil. The type locality is placed in the semi-arid Caatinga, a region with an irregular rainy season between November and May, annual precipitation reaching about 650–830 mm. During dryer periods, both swamps and streams of the region dry out.

At the time of collections, the pools were shallow, about 0.8 m in the deepest places. The water was clear to slightly turbid, dark yellow. Dense aquatic vegetation was present in all parts of the pools. Other species found in the same pool were *Hypsolebias carlettoi* (Costa & Nielsen) and *Cynolebias leptocephalus* Costa & Brasil. *Hypsolebias guanambi* was never found in the several well-sampled seasonal swamps around the city of Guanambi inhabited by *H. fulminantis* (Costa & Brasil) and *H. ghisolfii* (Costa, Cyrino & Nielsen), which are placed in the same river drainage, just about 10 km from the type locality of *H. guanambi*.

Etymology. The name *guanambi* refers to the city of Guanambi, around which vast plain areas flooded by the Caunaúba de Dentro River drainage are inhabited by five endemic annual fish species, including *H. guanambi*, thus constituting an important area of endemism for the Rivulidae. The name *guanambi* is derived from the Tupi-Guarani, meaning hummingbird.

Discussion

Recognition of *Hypsolebias guanambi* as a distinct species, member of the geographically widespread *H. flavicaudatus* complex, is consistent with the distribution pattern of other congeners. *Hypsolebias carlettoi* and *H. ghisolfii* are only known from the upper Carnaíba de Dentro drainage, whereas *H. fulminantis*, besides occurring in this area, is also found in localities near the main channel of the São Francisco River (Costa, 2007). This distribution pattern suggests a long past isolation of that area.

Acknowledgements

We are grateful to Claudia P. Bove, Bruno B. Costa, Maria Anais Barbosa, Jéssica C. Gomes, and Axel M. Katz, for help in collecting trips, to Carlos E. G. Schrago, for support in molecular analysis, and, to José Leonardo O. Mattos, Letícia Loss, Joana Zanol, Aretusa A. Macedo and Anderson Vasconcellos for technical assistance in the laboratory. This study was supported by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico - Ministério de Ciência e Tecnologia).

References

Chenna, R., Sugawara, H., Koike, T., Lopez, R., Gibson, T.J., Higgins, D.G. & Thompson, J.D. (2003): Multiple sequence alignment with the Clustal series of programs. – Nucleic Acids Research, **31**: 3497–3500.

Costa, W.J.E.M. (1995): Pearl killifishes – the Cynolebiatinae: systematics and biogeography of the neotropical annual fish subfamily. – tfh-publications, Neptune City, 128 pp.

Costa, W.J.E.M. (2001): The neotropical annual fish genus *Cynolebias* (Cyprinodontiformes: Rivulidae): phylogenetic relationships, taxonomic revision and biogeography. – Ichthyological Exploration of Freshwaters, **12**: 333–383.

Costa, W.J.E.M. (2003): The *Simpsonichthys flavicaudatus* species group (Cyprinodontiformes: Rivulidae: Cynolebiatinae): phylogenetic relationships, taxonomic revision and

biogeography. – Ichthyological Exploration of Freshwaters, **14**: 31–60.

COSTA, W.J.E.M. (2006a): Descriptive morphology and phylogenetic relationships among species of the Neotropical annual killifish genera *Nematolebias* and *Simpsonichthys* (Cyprinodontiformes: Aplocheiloidei: Rivulidae). – Neotropical Ichthyology, **4**: 1–26.

COSTA, W.J.E.M. (2006b): Three new species of seasonal killifishes of the *Simpsonichthys antenori* species group (Teleostei: Cyprinodontiformes: Rivulidae) from the rio São Francisco basin, Brazil. – Zootaxa, **1306**: 25–39.

COSTA, W.J.E.M. (2007): Taxonomic revision of the seasonal South American killifish genus *Simpsonichthys* (Teleostei: Cyprinodontiformes: Aplocheiloidei). – Zootaxa, **1669**: 1–134.

COSTA, W.J.E.M. (2008): *Simpsonichthys longignatus*, anew seasonal killifish of the *S. flammeus* group from the Pacoti River basin, northeastern Brazil (Cyprinodontiformes: Rivulidae). – Ichthyological Exploration of Freshwaters, **19**: 155–159.

COSTA, W.J.E.M. (2010a): Historical biogeography of cynolebiasine annual killifishes inferred from dispersal-vicariance analysis. – Journal of Biogeography, **37**:1995–2004.

COSTA, W.J.E.M. (2010b): *Simpsonichthys harmonicus*, a new seasonal killifish from the São Francisco River basin, northeastern Brazil (Cyprinodontiformes: Rivulidae). – Ichthyological Exploration of Freshwaters, **21**: 73–78.

COSTA, W.J.E.M. (in press). *Hypsolebias xxxxxxxxx*, a new seasonal killifish of the genus *Hypsolebias* from the Caatinga of northeastern Brazil, Itapicuru River basin (Cyprinodontiformes: Rivulidae). – Ichthyological Exploration of Freshwaters.

COSTA, W.J.E.M. & BRASIL, G.C. (1990): Description of two new annual fishes of the genus *Cynolebias* (Cyprinodontiformes: Rivulidae) from the Sao Francisco basin, Brazil. – Ichthyological Exploration of Freshwaters, **1**: 15–22.

DE QUEIROZ, K. (2007): Species concepts and species delimitation. – Systematic Biology, **56**: 879–886.

HOEDEMAN, J.J. (1958): The frontal scalation pattern in some groups of toothcarps (Pisces, Cyprinodontiformes). – Bulletin of Aquatic Biology, **1**: 23–28.

TAYLOR, W.R. & VAN DYKE, G.C. (1985): Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. – Cybium, **9**: 107–109.

TAMURA, K., DUDLEY, J., NEI, M. & KUMAR, S. (2007): MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. – Molecular Biology and Evolution, **24**: 1596–1599.

WIENS, J.J. & PENKROT, T.A. (2002): Delimiting species using DNA and morphological variation and discordant species limits in spiny lizards (*Sceloporus*). – Systematic Biology, **51**: 69–91.