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The Tadpole of *Scinax cardosoi* (Carvalho-e-Silva and Peixoto, 1991), with Description of Internal Oral Morphology and Taxonomic Considerations (Anura: Hylidae)

Juliana Kirchmeyer[1,*], Joice Ruggeri[2], Marcia dos Reis Gomes[1], Sergio Potsch de Carvalho-e-Silva[1]

[1] *Laboratório de Anfíbios e Répteis, Departamento de Zoologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Avenida Carlos Chagas Filho, 373 – Prédio do CCS, bloco A, sala A1-111, CEP 21941-902, Ilha do Fundão, Rio de Janeiro, RJ, Brazil.*
[2] *Laboratório de História Natural de Anfíbios Brasileiros, Departamento de Biologia Animal, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, SP, Brazil, CEP 13083-862.*
[*] *Corresponding author. Email: jullykp@gmail.com*

Abstract. Larval traits provide useful taxonomic characters. The only morphological synapomorphy that separates *Scinax* species from *Ololygon* species comes from larvae. However, tadpoles of *Ololygon* and *Scinax* remain undescribed; furthermore, there is a lack of information regarding their internal oral cavities. Herein, we describe the tadpole of *Scinax cardosoi* and its internal oral morphology. Overall, the body is triangular and depressed in lateral view and oval in dorsal view, the dorsal fin is slightly higher than the lower fin, and the tooth row formula is 2(1,2)/3. The tadpole of *S. cardosoi* can be distinguished from tadpoles of *Ololygon* and *Scinax* by its oral disc morphology and oral papillae arrangement. The position of the larval vent tube, as well as some adult features, suggest a close relationship between *S. cardosoi* and species of *Ololygon,* leading us to transfer the species from *Scinax* to *Ololygon.*

Keywords. Atlantic Forest; Larvae; New combination; *Ololygon.*

Resumo. Larvas fornecem caracteres importantes para identificação taxonômica. A única sinapomorfia morfológica que separa as espécies de *Scinax* das espécies de *Ololygon* é proveniente das larvas. Porém, existe pouca informação sobre as larvas desses táxons, principalmente sobre a morfologia oral interna, e muitos girinos de *Ololygon* e *Scinax* ainda não foram descritos. Aqui, nós descrevemos o girino e a morfologia oral interna de *Scinax cardosoi.* O girino de *S. cardosoi* possui corpo triangular e deprimido em vista lateral e oval em vista dorsal, nadadeira dorsal um pouco mais alta que a nadadeira ventral e fórmula dentária 2(1,2)/3. O girino de *S. cardosoi* pode ser distinguido dos girinos de *Ololygon* e dos girinos de *Scinax* por aspectos na morfologia oral e disposição das papilas orais. A posição do tubo anal do girino, assim como características relacionadas ao adulto, sugerem um relacionamento próximo entre *S. cardosoi* e as espécies de *Ololygon;* portanto, transferimos a espécie de *Scinax* para *Ololygon.*

INTRODUCTION

Within Hylidae Rafinesque, 1815, Scinaxinae Duellman et al., 2016 currently comprises 139 species divided into four genera: *Scinax* Wagler, 1830 (73 species), *Sphaenorhynchus* Tschudi, 1838 (15 species), *Ololygon* Fitzinger, 1843 (49 species), and *Julianus* Duellman et al., 2016 (2 species). The subfamily is widely distributed in Central and South America (Frost, 2018), with many species occurring in the Brazilian Atlantic Forest (Duellman and Wiens, 1992; Faivovich, 2002). Until recently, *Scinax* comprised two informal taxonomic groups referred to as the *S. catharinae* and *S. ruber* clades (Faivovich, 2002; Faivovich et al., 2005). However, Duellman et al. (2016) recognized the *S. catharinae* clade as the genus *Ololygon* and transferred the species of the *S. uruguayus* group from the *S. ruber* clade to the genus *Julianus;* the other species of the *S. ruber* clade remained in the genus *Scinax*.

Scinax cardosoi (Carvalho-e-Silva and Peixoto, 1991) is endemic to the southeastern Brazilian Atlantic Forest and has been reported from localities in Rio de Janeiro, São Paulo, Espírito Santo, and Minas Gerais State (Carvalho-e-Silva and Peixoto, 1991; Pinto et al., 2009; Linares et al., 2011; Moroti et al., 2017; Frost, 2018). Little is known about the biology and natural history of this species (Pinto et al., 2009; Linares et al., 2011; De Carvalho et al., 2015).

Larval features are widely used in the taxonomy of *Scinax* and *Ololygon* (Faivovich, 2002; Faivovich et al., 2005). The *S. ruber* clade (= *Scinax* and *Julianus* sensu Duellman et al., 2016) is supported by a single larval morphological synapomorphy: vent tube positioned above the margin of the ventral fin (Faivovich et al., 2005). However, tadpoles remain undescribed for 39 of the 73 species of

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Scinax, and internal oral morphology has been described for only four tadpoles of this group (Provete et al., 2012).

Herein, we describe the external morphology and internal oral features of *Scinax cardosoi* tadpoles from the type locality. We also compare them to tadpoles of *Scinax* (= *S. ruber* clade, except the *S. uruguayus* group) and *Ololygon* (= *S. catharinae* clade) described in the literature and discuss the position of this species in the genus *Scinax*.

MATERIALS AND METHODS

Tadpoles of *Scinax cardosoi* were collected at the type locality, currently within the Parque Estadual dos Três Picos (PETP), in March 1991 at Vale da Revolta (22°26′S, 42°56′W, Datum "Córrego Alegre"), municipality of Teresópolis, state of Rio de Janeiro, Brazil. One tadpole was reared through metamorphosis in the laboratory to confirm species identification (Fig. S1). A newly metamorphosed specimen was collected in May 2013 and four additional newly metamorphosed specimens were collected in April 2016 at Parque Nacional de Itatiaia (22°45′S, 44°50′W, Datum "Córrego Alegre"), municipality of Itatiaia, state of Rio de Janeiro, Brazil.

Tadpoles were preserved in 5% formaldehyde and the newly metamorphosed specimens, in 70% ethanol. Vouchers are deposited in the Amphibian Collection, Zoology Department, Biology Institute, Universidade Federal do Rio de Janeiro (ZUFRJ 4392, 4393, 14099, and 15678). Tadpole stages were determined following Gosner (1960). Descriptions are based on 13 individuals in stage 37. Drawings of external morphology and internal oral morphology are based on two different individuals. The tooth row formula follows Altig (1970) and larval terminology follows Altig and McDiarmid (1999), except body shape, which follows Rossa-Feres and Nomura (2006). Measurements are abbreviated as follows: total length (TL), body length (BL), body width (BW), body height (BH), tail length (TAL), maximum tail height (MTH), tail muscle height (TMH), tail muscle width (TMW), dorsal fin height (DFH), ventral fin height (VFH), internarial distance (IND), nostril diameter (ND), interorbital distance (IOD), eye diameter (ED), snout–eye distance (SED), eye–nostril distance (END), snout–nostril distance (SND), and oral disc width (ODW). Measurements for TL, BL, TAL, MTH, TMH, TMW, IND, and IOD follow Altig and McDiarmid (1999), ODW follows Scott and Jennings (1985), and those for BW, BH, ND, ED, END, and SND follow Lavilla and Scrocchi (1986). Measurements for SED, DFH, and VFH were added. Measurements were made with the aid of a graded ocular coupled to a stereomicroscope Leica MX-6 (except TL, BL, BW, BH, and MTH, which were taken with digital calipers accurate to 0.01 mm). Morphological structures of the oral cavity from two specimens at stage 37 were stained with 3% methylene blue solution for analysis, with terminology following Wassersug (1976). Dissections and drawings were made with the aid of a Leica MX-6 stereomicroscope and a camera lucida. Tadpoles were compared with available descriptions (Table S1) and with tadpoles of *Scinax* and *Ololygon* housed at ZUFRJ collection (Appendix S1).

RESULTS

External morphology

Measurements are presented in Table 1. Body triangular/depressed in lateral view (Fig. 1A) and oval in dorsal view (Fig. 1B); longer than wider (Table 1). BL approximately 34% of total length. Eyes directed dorsolaterally. IOD 62.3% of body width. Nares rounded, opening dorsally but visible laterally. IND 71% of IOD. Snout rounded in dorsal and lateral views. Oral disc ventral (Fig. 1D), not emarginate, ca. 50% of BW. Anterior labium with single row of rounded marginal papillae and medial gap. Lateral and dorsal submarginal papillae present. Posterior labium with two rows of uninterrupted marginal papillae. Tooth row formula 2(1,2)/3; A-1 and A-2 with same width; P-3

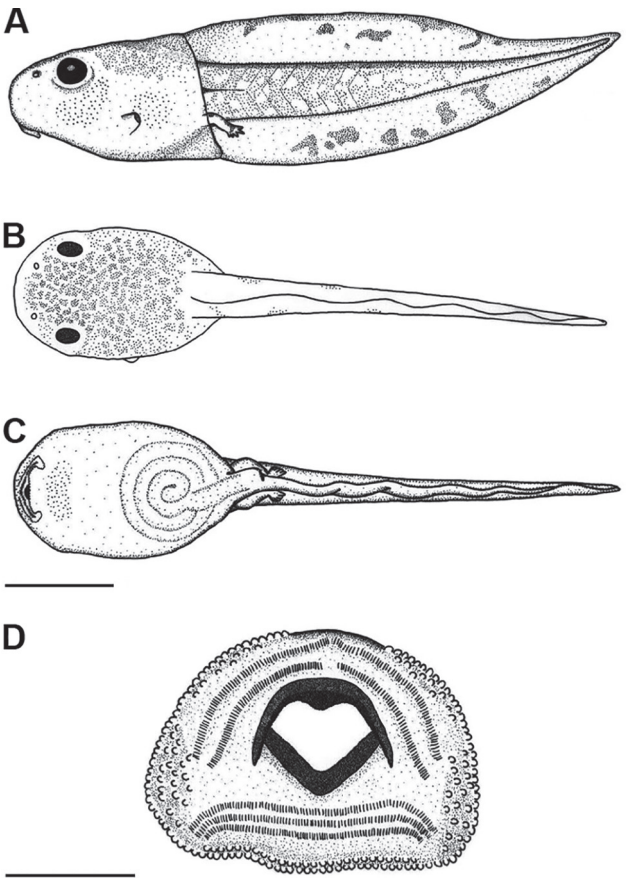

Figure 1. *Scinax cardosoi* tadpole in stage 37 (ZUFRJ 4393). **(A)** Lateral view; **(B)** Dorsal view; **(C)** Ventral view; **(D)** Oral disc. Scale bars: 5 mm (A, B, C) and 1 mm (D).

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Table 1. Measurements (in mm) of *Scinax cardosoi* tadpoles in stage 37 (n = 13).

Measurements	$\bar{x}$	SD	Minimum–maximum
Total length	28.7	1.2	24.3–30.9
Body length	9.8	0.3	9.4–10.3
Body width	5.6	0.5	4.8–6.6
Body height	5.2	0.4	4.5–6.2
Tail length	18.9	1.1	14.2–20.7
Tail height	7.5	0.3	7.0–8.2
Dorsal fin height	3.0	0.2	2.6–3.4
Ventral fin height	2.6	0.1	2.4–2.9
Muscle width	2.5	0.2	2.3–2.8
Muscle height	2.5	0.2	2.2–3.0
Nostril diameter	0.3	0.1	0.2–0.3
Internarial distance	2.5	0.1	2.4–2.7
Eye diameter	1.2	0.1	1.0–1.3
Interorbital distance	3.5	0.3	3.0–4.5
Eye–nostril distance	1.2	0.2	1.0–1.8
Snout–nostril distance	1.2	0.2	1.0–1.8
Snout–eye distance	2.7	0.3	2.3–3.5
Oral disc width	2.7	0.2	2.5–3.2

shorter than P-1 and P-2. Upper jaw sheath arch-shaped and lower jaw sheath V-shaped; upper jaw sheath less keratinized than lower jaw one; both serrate. Spiracle sinistral, fused to body wall, located on the posterior half of the body, ventral to lateral mid-line, its opening directed posterodorsally. Intestines arranged subparallel to longitudinal body axis. Vent tube sinistral, its opening reaching ventral fin margin (Fig. 2). Dorsal fin arched, slightly higher than lower fin, ascending gradually from posterior third of body. Tail with rounded tip, without flagellum. TMH 33.3% of MTH.

Color in preservation

Body brownish, lightening toward snout. Area below eyes and near the spiracle with dark brown spots (Fig. 1A).

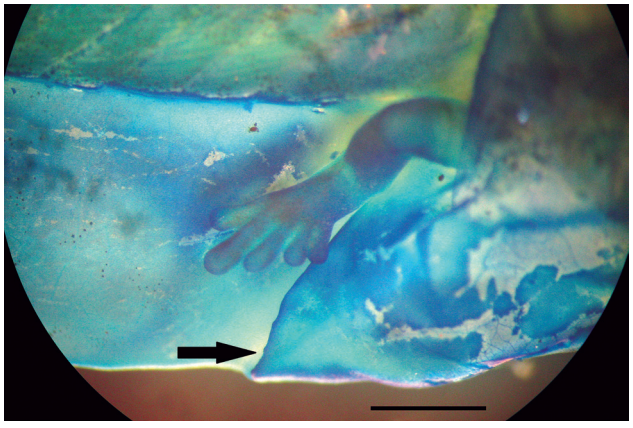

Figure 2. Vent tube of *Scinax cardosoi* tadpole (ZUFRJ 4393). Arrow indicates the opening of the vent tube. Scale bar: 1 mm.

Body translucent ventrally (intestine visible) with some dark brown spots close to oral disc (Fig. 1C). Fins translucent with irregular brownish blotches usually from middle third to the posterior tip. Dorsal fin with brownish pigmentation near body. Muscles with brownish pigmentation, lightening toward end of tail.

Internal oral features

Buccal roof (Fig. 3A)

Buccal roof triangular, longer than wide. Two pustulations in center of pre-narial arena. Internal nares elongated, oriented 45° from transverse plane. Presence of a smooth structure anterior to internal opening of the nostrils. Few small pustulations on the post-narial arena. Median ridge trapezoidal, wider than long, with irregular free margin. Two papillae on each side of the median ridge, one larger with some short branches in its distal margins, and one finger-like. Pustulations scattered uniformly over the surface of the buccal roof arena (BRA); BRA and lateral roof papillae absent. Glandular zone distinct. Dorsal velum well developed laterally, folded anteriorly, interrupted medially.

Buccal floor (Fig. 3B)

Buccal floor triangular, as long as wide. Presence of a non-keratinized spur behind the jaw on each side. One finger-like infralabial papillae on each side, projecting from the lateral wall. Tongue quite prominent anteriorly, ending in a wide and shallow central depression. Lingual papillae absent. One papillary row on each side of the Buccal Floor Arena (BFA), each row with ca. 10 papillae; papillae finger-like, except for pair located immediately medial to buccal pockets, which are large with several secondary projections; all papillae directed to center of arena. Small buccal pocket located on each side of the BFA, oriented horizontally. Five small pre-pocket papillae. Ventral velum round-shaped with a medium notch on the posterior margin (not visible in Fig. 3B).

Natural history

Tadpoles of *Scinax cardosoi* are found in temporary ponds or slow-flowing streams inside forests or in open areas near forest edges; they can also be found in disturbed areas, like the type locality, and in temporary ponds in agricultural fields. Despite ongoing research in the region, the species has not been found there since 1991. The snout–vent length of newly metamorphosed individuals is 11.2 ± 3.3 mm (10–12.2 mm, n = 5). At this stage, individuals already present some adult features, such as two

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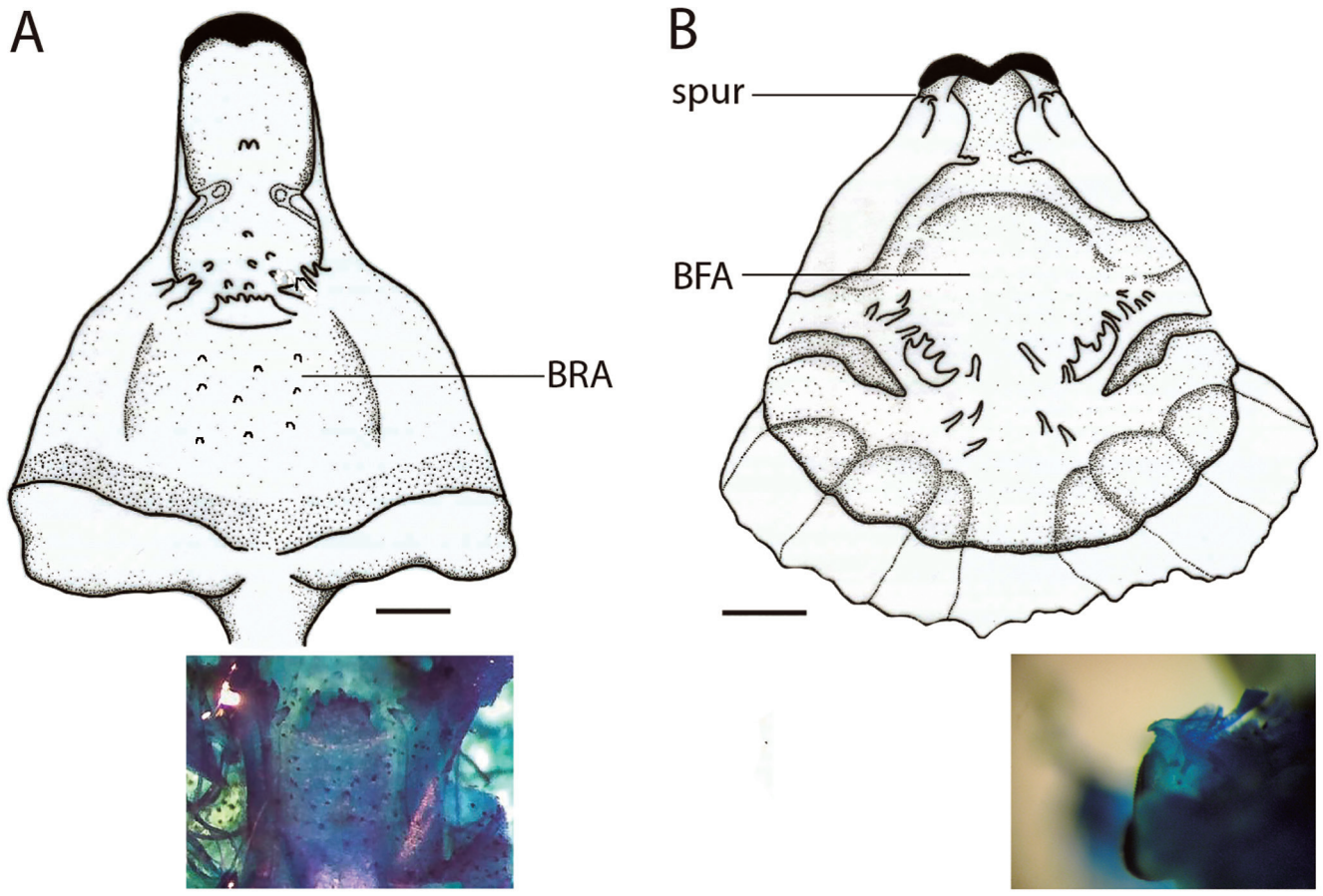

Figure 3. Buccal cavity of *Scinax cardosoi* tadpole in stage 37 (ZUFRJ 4393). **(A)** Buccal roof; inset: detail of the buccal roof arena (BRA), stained with methylene blue solution. **(B)** Buccal floor; inset: detail of the spur, stained with methylene blue solution. Scale bars: 1 mm. BFA = buccal floor arena; BRA = buccal roof arena.

lateral strips (one at each side) extending from the eyes to inguinal region and three dorsal strips extending from the eyes to the coccyx (Fig. S1). The forearms, arms, and thighs show transverse strips and the tibia has oblique strips.

DISCUSSION

Comparison between *Scinax cardosoi* and other tadpoles of *Scinax*

One synapomorphy—the vent tube of tadpoles positioned above the margin of the ventral fin (Faivovich et al., 2005)—supports the monophyly of the *Scinax ruber* clade (= *Scinax* and *Julianus* sensu Duellman et al., 2016). However, in tadpoles of *S. cardosoi* the vent tube reaches the margin of the ventral fin. The 2(1,2)/3 tooth row formula of *S. cardosoi* is similar only to that of *S. duartei* (Bokermann, 1967a). The presence of two rows of posterior papillae distinguishes *S. cardosoi* from other described *Scinax* tadpoles (Table 2). A row of posterior papillae without gap differentiates *S. cardosoi* from *S. auratus*, *S. crospedospilus*, *S. cuspidatus*, *S. juncae*, *S. maracaya*, *S. nebulosus*, and *S. rostratus* (row of posterior papillae with medial gap; Table 2). A triangular/depressed body in lateral view distinguishes *S. cardosoi* from *S. auratus*, *S. curicica*, *S. duartei*, *S. eurydice*, *S. fuscomarginatus*, *S. fuscovarius*, *S. hayii*, *S. maracaya*, *S. montivagus*, *S. pachycrus*, *S. perereca*, *S. rostratus*, *S. ruber*, *S. similis*, *S. squalirostris*, and *S. x-signatus* (body triangular, not depressed; Table 2). Also, the presence of a keratinized spur behind the jaw is a synapomorphy of the former *S. ruber* clade (Faivovich, 2002). However, this character has only been reported previously for *S. acuminatus*, *S. fuscovarius*, *S. hayii*, *S. nasicus*, and *S. similis* (Alves and Carvalho-e-Silva, 1999; Alcade et al., 2011). The tadpole of *S. cardosoi* differs from all other described species by having a non-keratinized spur.

Comparison between *Scinax cardosoi* and *Ololygon* tadpoles

The larval external morphology of *Scinax cardosoi* is similar to described tadpoles of *Ololygon*, all of which have a depressed body in lateral view, low fins and, vent

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Table 2. Tooth row formulae (TRF) and arrangement of oral papillae of 23 described tadpoles of *Scinax*.

Species	TRF	Anterior papillae row	Posterior papillae row	Reference
S. alter	2(2)/3(1)	Absent	Single, medial gap absent	Alves and Carvalho-e-Silva, 2002
S. auratus	2(2)/3(1)	Single, medial gap present	Single, medial gap present	Alves et al., 2004
S. crospedospilus	2(1,2)/3(1)	Single, medial gap present	Single, medial gap present	Heyer et al., 1990
S. curicica	2(2)/3(1)	Single, medial gap present	Single, medial gap absent	Pugliese et al., 2004
S. cuspidatus	2(2)/3(1)	Single, medial gap present	Single, medial gap present	Alves and Carvalho-e-Silva, 2002
S. duartei	2(1,2)/3	Absent	Single, medial gap absent	Bokermann, 1967a
S. eurydice	2(2)/3(1)	Single, medial gap present	Double, medial gap absent	Wogel et al., 2000
S. fuscomarginatus	2(2)/3(1)	Single, medial gap present	Single, medial gap absent	Rossa-Feres and Nomura, 2006
S. fuscovarius	2(2)/3(1)	Single, medial gap present	Single, medial gap absent	Rossa-Feres and Nomura, 2006
S. hayii	2(1,2)/3(1)	Single, medial gap present	Single, medial gap absent	Bokermann, 1967a
S. juncae	2(2)/3(1)	Single, medial gap present	Single, medial gap present	Mercês and Juncá, 2012
S. maracaya	2(2)/3(1)	Single, medial gap present	Single, medial gap present	Cardoso and Sazima, 1980
S. melanodactylus	2(2)/3	Single, medial gap present	Single, medial gap absent	Abreu et al., 2015
S. montivagus	2(2)/3(1)	Single, medial gap present	Single, medial gap absent	Juncá et al., 2015
S. nebulosus	2(2)/3(1)	Single, medial gap present	Single, medial gap present	Gomes et al., 2014
S. pachycrus	2(2)/3	Single, medial gap present	Single, medial gap absent	Carneiro et al., 2004
S. perereca	2(2)/3(1)	Absent	Single, medial gap absent	Pugliese and Bastos, 2001
S. rostratus	2(2)/3(1)	Single, medial gap present	Single, medial gap present	Hero and Mijares-Urrutia, 1995
S. ruber	2(2)/3(1)	Single, medial gap present	Single, medial gap absent	Lynch, 2006
S. rupestris	2(2)/3 or 2(2)/3(1)	Single, medial gap present	Single, medial gap absent	Araujo-Vieira et al., 2015
S. similis	2(2)/3(1)	Single, medial gap present	Single, medial gap absent	Alves and Carvalho-e-Silva, 1999
S. squalirostris	2(1,2)/3(1)	Absent	Single, medial gap absent	Bokermann, 1967b
S. x-signatus	2(2)/3(1)	Single, medial gap present	Single, medial gap absent	Lynch, 2006

Table 3. Tooth row formulae (TRF) and arrangement of oral papillae of *Scinax cardosoi* (boldface) and 20 described tadpoles of *Ololygon*.

Species	TRF	Anterior papillae row	Posterior papillae row	Reference
O. albicans	2(2)/3	Single, medial gap absent	Double, medial gap absent	Carvalho-e-Silva and Carvalho-e-Silva, 1994
O. angrensis	2(2)/3	Single, medial gap absent	Single, medial gap absent	Carvalho-e-Silva et al., 1995
O. argyreornata	2(2)/3	Single, medial gap present	Single, medial gap absent	Carvalho-e-Silva and Carvalho-e-Silva, 1998
O. aromothyella	2(2)/3	Single, medial gap present	Double, medial gap absent	Kolenc et al., 2007
O. belloni	2(2)/3	Single, medial gap present	Double, medial gap absent	Silva-Soares et al., 2010
O. berthae	2(2)/3	Single, medial gap present	Single, medial gap absent	de Sá et al., 1997
S. cardosoi	**2(1,2)/3**	**Single, medial gap present**	**Double, medial gap absent**	**This study**
O. catharinae	2(2)/3	Single, medial gap present	Double, medial gap absent	Conte et al., 2007
O. flavoguttata	2(2)/3	Single, medial gap absent	Double, medial gap absent	Carvalho-e-Silva and Carnaval, 1997
O. hiemalis	2(2)/3	Single, medial gap present	Double, medial gap absent	Haddad and Pombal, 1987
O. humilis	2(2)/3	Single, medial gap present	Single, medial gap absent	Carvalho-e-Silva and Carvalho-e-Silva, 1998
O. kautskyi	2(2)/3	Single, medial gap present	Double, medial gap absent	Carvalho-e-Silva et al., 1995
O. littoralis	2(2)/3	Single, medial gap present	Single, medial gap absent	Pombal and Gordo, 1991
O. luizotavioi	2(2)/3	Single, medial gap present	Single, medial gap absent	Bertoluci et al., 2007
O. machadoi	2(2)/3(1)	Single, medial gap absent	Single, medial gap absent	Bokermann and Sazima, 1973
O. obtriangulata	2(2)/3	Single, medial gap present	Double, medial gap absent	Heyer et al., 1990
O. ranki	2(2)/3	Single, medial gap present	Single, medial gap absent	Andrade and Cardoso, 1987
O. rizibilis	2(2)/3(3)	Single, medial gap present	Single, medial gap absent	Bokermann, 1964
O. strigilata	2(2)/3	Single, medial gap absent	Double, medial gap absent	Camurugi et al., 2013
O. trapicheiroi	2(2)/3	Single, medial gap present	Single, medial gap absent	Carvalho-e-Silva and Carvalho-e-Silva, 1994
O. tripui	2(2)/3	Single, medial gap present	Single, medial gap absent	Lourenço et al., 2010

tube reaching the margin of the ventral fin. However, the tadpole of *S. cardosoi* can be easily distinguished by its unique tooth row formula of 2(1,2)/3 (Table 3). The interrupted anterior row of papillae of *S. cardosoi* distinguishes it from *O. albicans, O. angrensis, O. flavoguttata, O. machadoi,* and *O. strigilata* (anterior papillae row uninterrupted; Table 3). Also, the presence of two posterior rows of marginal papillae on the posterior labium distinguishes *S. cardosoi* from *O. angrensis, O. argyreornatus, O. berthae, O. humilis, O. littoralis, O. luizotavioi, O. machadoi, O. ranki,*

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O. rizibilis, O. trapicheiroi, and *O. tripui* (single posterior papillae row; Table 3). The internal oral morphology has been described for only four species of *Ololygon: O. aromothyella, O. belloni, O. berthae,* and *O. catharinae* (Conte et al., 2007; Silva-Soares et al., 2010; Alcade et al., 2011). The presence of a smooth structure anterior to the internal opening of the nostrils distinguishes *S. cardosoi* from *O. aromothyella, O. belloni,* and *O. berthae* (smooth structure absent). Also, the presence of a non-keratinized spur behind the jaw differentiates *S. cardosoi* from *S. catharinae* (spur absent).

Taxonomic considerations and conclusions

Larval traits have shown to be important for comparative studies of amphibians (e.g., Wassersug, 1976; McDiarmid and Altig, 1999), including for the genera *Scinax* and *Ololygon* (Faivovich, 2002; Faivovich et al., 2005). However, there is a lack of information on tadpole morphology, particularly regarding internal oral anatomy. Currently, tadpoles have been described for about 70% species of *Ololygon* and 43% species of *Scinax*. Data on internal oral morphology are scarcer, existing for only 9% of *Ololygon* species and 6% of *Scinax* species.

Scinax cardosoi was considered more closely related to the *S. ruber* group (Carvalho-e-Silva and Peixoto, 1991) and subsequently included in the *S. ruber* clade (Faivovich et al., 2005). However, the vent tube of *S. cardosoi* is not positioned above the margin of the ventral fin, a synapomorphy for the *S. ruber* clade (Faivovich et al., 2005). In contrast, the vent tube of *S. cardosoi* reaches the margin of the ventral fin, a trait shared with tadpoles currently allocated to *Ololygon* (Duellman et al., 2016; Faivovich, 2002).

The *Scinax catharinae* clade (= *Ololygon;* sensu Duellman et al., 2016) is supported by four adult synapomorphies: (1) internal vocal sac, (2) absence of the anterior process of the suprascapula, (3) distal division of the middle branch of the *m. extensor digitorum comunis longus,* and (4) insertion of the medial side of this branch on the tendon of the *m. extensor brevis medius digiti IV* (Faivovich et al., 2005). Adult males of *S. cardosoi* have internal vocal sacs (Lourenço et al., 2014), and we report that the suprascapula does not have an anterior process (ZUFRJ 4125, 4126). In addition, *S. cardosoi* has a complex vocal repertoire (i.e., with multiple call types emitted in variable combinations), as has also been reported for species of *Ololygon;* furthermore, *Scinax* species tend to have simple call structures with a single type of note (De Carvalho et al., 2015). Based on the larval and adult characteristics, we placed *S. cardosoi* in the genus *Ololygon,* forming the original combination *Ololygon cardosoi* Carvalho-e-Silva and Peixoto, 1991 **comb. nov.**

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Appendix S1. Specimens examined.

Figure S1. A recently metamorphosed specimen of *Scinax cardosoi* (ZUFRJ 4392). Note that the specimen presents some external features of the adult, such as two lateral strips extending from the eyes to the inguinal region and three dorsal strips extending from the eyes to the coccyx. Scale bar: 1 mm. Photo by Cyro de Luna Dias Neto.

Table S1. Authors of tadpole descriptions of species from the genera *Ololygon* and *Scinax* used in the comparison with the tadpole of *Scinax cardosoi*.

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