The tadpole of *Sphaenorhynchus caramaschii*, with comments on larval morphology of *Sphaenorhynchus* (Anura: Hylidae)

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Abstract

We describe the tadpole of *Sphaenorhynchus caramaschii*. It differs from tadpoles of other species of *Sphaenorhynchus* in having a short spiracle, submarginal papillae, and alternating short and large marginal papillae in the oral disc. Some larval characteristics, like morphology and position of the nostrils, length of the spiracle, and size of the marginal papillae on the oral disc are discussed for tadpoles of other species of *Sphaenorhynchus*.

Key words: Hylinae, Dendropsophini, *Sphaenorhynchus*, taxonomy, systematics

Introduction

The Neotropical hylid frog genus *Sphaenorhynchus* Tschudi includes small greenish treefrogs that inhabit temporary, permanent, or semi-permanent ponds in open areas where males vocalize while perched on the floating vegetation or partially submerged in the water (e.g. Lutz & Lutz 1938; Bokermann 1973; Cruz & Peixoto 1980). *Sphaenorhynchus* is currently composed of 14 species, three of which are widespread throughout the Amazon basin [*S. carneus* (Cope), *S. dorisae* (Goin), and *S. lacteus* (Daudin)]. The latter is also present in northeastern Brazil (states of Maranhão and Piauí; Caramaschi et al. 2009; Benício et al. 2011) and Trinidad (recorded as *Hyla orophila* by Kenny 1969). The other 10 species [*S. botocudo* Caramaschi, Almeida & Gasparini, *S. bromelicola* Bokermann, *S. caramaschii* Toledo, Garcia, Lingnau & Haddad, *S. mirim* Caramaschi, Almeida & Gasparini, *S. orophilus* (Lutz & Lutz), *S. palustris* Bokermann, *S. pauloalvini* Bokermann, *S. planicola* (Lutz & Lutz), *S. prasinus* Bokermann, and *S. surdus* (Cochran)] are distributed in the Atlantic Forest, from the northern portion of the State of Rio Grande do Sul to the State of Pernambuco. The remaining species, *S. platycephalus* (Werner), is of uncertain taxonomic status and is known solely from the poorly preserved holotype assigned to South America (Harding 1991; Frost 2014).

The monophyly of *Sphaenorhynchus* has been tested on the basis of only limited taxon sampling (Faivovich et al. 2005; Wiens et al. 2006; Pyron & Wiens 2011) and the relationships among its species have not been studied in a quantitative phylogenetic framework. Nevertheless, many morphological synapomorphies have been suggested for *Sphaenorhynchus* (Duellman & Wiens 1992; Faivovich et al. 2005), including some related to larval morphology such as structure and position of the nostrils, and the presence of large marginal papillae in some species. Also, Cruz & Peixoto (1980) tentatively grouped *S. bromelicola* with *S. orophilus* and *S. planicola* with *S. prasinus* based on some larval similarities as total length, color pattern, spiracle length, and marginal papillae size.
The tadpoles of all known species of *Sphaenorhynchus* have been described except those of *S. botocudo*, *S. caramaschii*, and *S. mirim* (see Toledo et al. 2007; Caramaschi et al. 2009). In the present study, we describe the external larval morphology of *S. caramaschii* and provide comments on larval morphology of *Sphaenorhynchus* in general on the basis of new observations contrasted with data from the literature.

**Material and methods**

**Collection site.** The type locality of *Sphaenorhynchus caramaschii* is Fazenda São Luís, Municipality of Ribeirão Branco, State of São Paulo, Brazil. In the State of São Paulo, the species also occurs in the municipalities of Pilar do Sul, Iporanga, Apiaí, Ribeirão Grande, Botucatu, Lençóis Paulista and Avaré (Bertoluci & Rodrigues 2002; Pombal & Haddad 2005; Toledo et al. 2007; Almeida et al. 2008). For this study, tadpoles of *S. caramaschii* were collected from a semi-permanent pond at Fazenda Intermontes (24°11'58.4" S, 48°26'08.6" W, 905 m elevation; ca. 30 km from the type locality), Municipality of Ribeirão Grande, State of São Paulo, Brazil in April, May, and October 2010, by the second author. The identity of the tadpole was corroborated comparing the fragment AR-Wilk2/BR (~580 bp) of the mitochondrial ribosomal gene 16S rRNA of one adult paratopotype (CFBH 11285; GenBank accession number KP096219) and one tadpole (CFBHT 12419 collected with Lot CFBH 26552, see below; GenBank accession number KP096220). Our comparison showed that these specimens are 100% similar (overlap = 546 bp, mismatch = 0). Moreover, tadpoles of *S. caramaschii* are promptly distinguished from those of *S. surdus* by possessing a short spiracle and alternating short and large marginal papillae in the oral disc (medium-sized spiracle and small and homogenous papillae in *S. surdus*). Tadpoles were fixed in 5% formalin and deposited in the Célio F. B. Haddad Collection (CFBH), Universidade Estadual Paulista, Rio Claro, São Paulo, Brazil (CFBH 26552, 28652, 35923–35924).

**External morphology.** Terminology for larval morphology follows Altig & McDiarmid (1999), with the exception of the position of the intestinal mass, which follows Faivovich (2002). Methylene blue was employed to enhance visualization of oral disc structures. Tadpoles were staged following Gosner’s (1960) table. Eighteen measurements were taken from 42 tadpoles ranging from stages 26−36 (Table 1). Twelve measurements follow Lavilla & Scrocchi (1986): TL (total length), BL (body length), TAL (tail length), MTH (maximum tail height), TMH (tail muscle height), BH (body height), BW (body width), ED (eye diameter), ODW (oral disc width), END (eye-nostril distance), NSD (nostril to tip of snout distance), and ND (nostril diameter: distance of inner margins of the largest nostril axis). We also used six other measurements: TMW (tail muscle width), IND (intermaxilar distance), and IOD (interorbital distance) following Altig & McDiarmid (1999); DFH (dorsal fin height), and VFH (ventral fin height) following Grosjean (2005), and we also measured spiracle length (SL; distance between the origin of the external wall of the spiracle and its posterior margin). Measurements (in millimeters) were taken using an ocular grid to the nearest 0.1 mm in a Zeiss stet microscope (Stemi SV 11), except TL, BL, and TAL, which were measured to the nearest 0.01 mm using digital calipers. Comments on the larval morphology in *Sphaenorhynchus* are based on preserved specimens (see Appendix 1) complemented with descriptions by Bokermann (1966, 1973), Cruz (1973), Cruz & Peixoto (1980), Heyer et al. (1990), Suárez-Mayorga & Lynch (2001), Nunes et al. (2007), and Caramaschi (2010).

**Results**

**Description.** Measurements taken from tadpoles of *Sphaenorhynchus caramaschii* are presented in Table 1. The description is based on a tadpole in stage 31 (CFBH 35924, Fig. 1A–C), which has the following measurements (all in mm): TL 36.7; BL 10.7; TAL 25.9; MTH 6.7; TMH 4.5; BH 6.0; BW 7.0; ED 1.5; ODW 2.0; END 1.4; NSD 1.0; ND 0.6; TMW 0.4; IND 3.2; IOD 4.4; DFH 2.3; VFH 2.3; SL 1.0. Tadpole body wider than deep, oval in dorsal view, with a slight constriction behind the eyes and slightly cuneiform in lateral view (Fig. 1A–B); BL 29.1% of TL; snout sub-elliptical in lateral view and elliptical in dorsal view. Eyes dorsolateral, visible in ventral view. Nostrils elliptical (minimum diameter about 55% of maximum diameter), small (maximum diameter about 40% of ED), located nearer the tip of snout than the eyes, forwardly directed, with their longer axis forming an acute angle with respect to the main body axis; nasal rim white, with an irregular fleshy flange along its inner margins.
### TABLE 1. Measurements (in millimeters) of *Sphaenorhynchus caramaschii*. (Mean ± standard deviation; range into parenthesis).

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Stages</th>
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<tr>
<td></td>
<td>26–28 (n = 21)</td>
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<tr>
<td>TL</td>
<td>25.22 ± 4.98 (12.7–31.7)</td>
</tr>
<tr>
<td>BL</td>
<td>8.65 ± 1.08 (6.1–10.0)</td>
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<tr>
<td>BH</td>
<td>5.19 ± 0.61 (4.0–6.0)</td>
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<tr>
<td>BW</td>
<td>5.97 ± 0.69 (4.8–7.0)</td>
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<tr>
<td>TAL</td>
<td>16.45 ± 4.22 (6.5–21.6)</td>
</tr>
<tr>
<td>MTH</td>
<td>5.81 ± 1.02 (3.9–7.3)</td>
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<tr>
<td>DFH</td>
<td>1.80 ± 0.42 (0.8–2.4)</td>
</tr>
<tr>
<td>VFH</td>
<td>1.91 ± 0.41 (1.1–2.4)</td>
</tr>
<tr>
<td>TMH</td>
<td>2.97 ± 0.50 (2.0–3.9)</td>
</tr>
<tr>
<td>TMW</td>
<td>2.49 ± 0.49 (1.1–3.2)</td>
</tr>
<tr>
<td>ED</td>
<td>1.15 ± 0.17 (0.8–1.4)</td>
</tr>
<tr>
<td>END</td>
<td>1.12 ± 0.08 (1.0–1.2)</td>
</tr>
<tr>
<td>NSD</td>
<td>0.79 ± 0.14 (0.6–1.1)</td>
</tr>
<tr>
<td>ND</td>
<td>0.45 ± 0.10 (0.3–0.6)</td>
</tr>
<tr>
<td>IOD</td>
<td>3.22 ± 0.54 (2.0–4.1)</td>
</tr>
<tr>
<td>IND</td>
<td>2.41 ± 0.31 (1.7–2.8)</td>
</tr>
<tr>
<td>ODW</td>
<td>1.69 ± 0.12 (1.5–1.9)</td>
</tr>
<tr>
<td>SL</td>
<td>0.73 ± 0.12 (0.6–1.0)</td>
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Oral disc anteroventral, not emarginate, width 28.6% of BW. Labial tooth row formula 2(2)/3(1). A-2 interrupted medially by a wide gap, P-1 interrupted medially by a narrow gap. A-1 and P-1 almost of equal length, P-2 approximately 80% of P-1, P-3 one third of P-2. Marginal papillae arranged as a single row, with a few large marginal papillae in the antero- and posterolateral margins (large papillae about twice larger than the small papillae and arranged alternating with the small ones); anterior labium with a wide, median gap; few submarginal papillae in the posterior portion of the oral disc, near P2 and P3. Jaw-sheaths finely serrated; upper jaw-sheath with a small median projection; lower jaw-sheath U-shaped (Fig. 1C). Spiracle lateral, sinistral, short (about 9.3% of BL), with a small opening oriented slightly dorsally, its inner wall attached to body wall, except for its posterior extreme, and longer than the external wall. Vent tube medial, quite broad, funnel-shaped (two times wider at the base than at the tip), with both walls attached directly at the margin of the ventral fin. The intestinal mass is positioned at a right
angle to the longitudinal body axis, visible through the skin. Dorsal fin continuous, arising just anterior to body-tail junction; ventral fin origin at the most postero-ventral tip of the body, concealed by the vent tube; tail fins of similar depth.

**FIGURE 1.** Tadpole of *Sphaenorhynchus caramaschii* (CFBH 35924, stage 31. TL = 36.7 mm). A: Dorsal view. B: Lateral view. C: Oral disc. Scale bar = 1mm. Photos A and B: V. Loredam

The lateral line is visible as a series of elliptical, vertical whitish stitches. The V-shaped supraorbital line includes 10–13 stitches that bend medially on the head between the nares and continues anteroventrally onto the snout, where it approaches the prenasal series of 5–7 stitches, which are irregularly arranged. The postorbital series consist of two dorsal rows of stitches; a short row of 4–5 stitches is located dorsally and parallel to the large dorsolateral row with 13–16 stitches. This dorsolateral series projects obliquely until reaching the tail-body junction. The caudal series consists of 24–26 stitches. It begins just anterior to the dorsal fin and continues posteriorly along its base until the anterior third of the tail.

In life, body dorsally brown with irregular pale blotches, dark brown spots, and scattered iridophores (more evident around the lateral line stitches). Coloration slightly marbled on snout, whitish laterally with some scattered brown spots on the dorsal third of the body. Brown blotches around the eyes and near the nostril and oral disc. Iris black with a wide golden ring around the pupil. Venter cream with iridescent white elongate blotches on the posterolateral half. Vent tube white laterally and dark brown ventrally; its opening with black pigmentation on the margin. Tail musculature with three longitudinal dark brown stripes extending from the tail-body junction until
almost half of the tail: one on the longitudinal axis between the epaxial and hypaxial musculature and the other two on its dorsal and ventral margins respectively; brown blotches on the posterior half. Dorsal fin with cream and brown blotches; ventral fin transparent along first third and with cream and brown blotches from the middle until its posterior end. Iridescence white spots are also present on the tail musculature and fins (Fig. 2C). Coloration changes to green (adult coloration), when the larvae reach stage 40.

In preservative the color pattern is similar to that of living tadpoles, but fades and loses its golden and iridescent tones. The venter becomes light cream and the iris becomes black.

**Variation.** In addition to the few submarginal papillae on the posterior portion of the oral disc, near P2 and P3, some individuals present many submarginal papillae, almost forming a second row of papillae. Also, the submarginal papillae can be present anteriorly, near A1 and A2. A few large marginal papillae (about twice as large as the small ones) of the oral disc can be arranged together in some specimens instead of alternating with the small ones. The total length (excluding the gap) of A2 is 15–35% of A1; P3 is 30–50% of P2. The fleshy flange of the nasal rim is entirely unpigmented or partially pigmented and broad and/or slender along the inner margin of nostrils (Fig. 3A–D; see Discussion below). In some specimens the peribranchial region has scattered brown spots around the oral disc. The three dark brown stripes of the tail musculature are either interrupted or incomplete; its extension varies between the anterior third and half of the tail musculature.

Some individuals show loss of labial teeth, presence of a gap in P2 (one specimen), distinct growth of P3 (P3 grew and took place on one side of P1; one specimen), and damage in the papillae. Discolored keratinized structures (teeth, and less common upper and lower jaw-sheaths) are present in 15% of the specimens. Tadpoles in captivity, even for a short period of time, might show discoloration of the oral parts, as noted by Altig & McDiarmid (1999).

**Natural history notes.** Tadpoles of *Sphaenorhynchus caramaschii* were obtained in a semi-permanent pond located in an open field. The area of the pond was ca. 7500 m², with a minimum depth of 0.3 m during dry season (April-September) and maximum depth of 1.2 m in the rainy period (October-March). Large portions of the pond contained dense aquatic vegetation, mainly Thypaceae, Cyperaceae and *Brachiaria* sp. (Fig. 2B). The tadpoles were observed swimming close to the surface, amidst floating vegetation. When disturbed, they swam to the bottom of the pond. We detected some injuries in the tails of some tadpoles of *S. caramaschii* (25%, especially in earlier developmental stages) at several stages of regeneration.

Adult males were observed at night when calling, perched on emergent vegetation, about 20–30 cm from the water surface. They start calling near sunset and finish around sunrise. Amplexus is axillary (Fig. 2A). In addition, we observed the presence of green bones in juveniles and adult specimens.

**Comparison with other species.** The tadpole of *Sphaenorhynchus caramaschii* differs from those of some species of the genus by its short spiracle (extremely long in *S. palustris*; at least 28% of BL; medium-sized in *S. bromelicola, S. orophilus*, and *S. surdus*; 16–24% of BL; Figs. 1B and 5A–D). The dorsal fin originating at the body-tail junction distinguishes larvae of *S. caramaschii* from *S. orophilus, S. planicola, S. surdus* (on the body), *S. bromelicola* and *S. pauloalvini* (on the tail musculature). The labial tooth row formula 2(2)/3(1) distinguishes *S. caramaschii* from *S. carneas* [1/2-3(1)] and *S. lacteus* [2/3(1)]. The width of dorsal gap on marginal papillae is almost as long as A-1 in *S. caramaschii*, whereas it is wider than A-1 and A-2 in *S. bromelicola* and almost as short as A-2 gap in *S. pauloalvini*. The absence of a ventral gap in the marginal papillae differentiates *S. caramaschii* from *S. dorisae, S. palustris* and *S. planicola*. The presence of submarginal papillae distinguishes *S. caramaschii* from *S. bromelicola, S. dorisae, S. palustris, S. planicola*, and *S. prasinus*. The marginal papillae composed of alternating large and small papillae (large papillae about twice as large as the small ones) distinguishes larvae of *S. caramaschii* from *S. orophilus, S. pauloalvini, S. bromelicola, S. dorisae, S. surdus* (small and homogeneously sized marginal papillae; Figs. 1C and 4A–B).

The spiracle is positioned midway between the eye and the posterior margin of the body in *Sphaenorhynchus caramaschii*, whereas it is closer to the posterior margin of the body in *S. carneas, S. orophilus, S. palustris* and *S. surdus* and closer to the eyes in *S. pauloalvini* (see Bokermann 1973; Cruz & Peixoto 1980; Suárez-Mayorga & Lynch 2001; Nunes et al. 2007; Caramaschi 2010). The presence of dorsal brown blotches on separates tadpoles of *S. caramaschii* from those of *S. bromelicola, S. pauloalvini* (olive brown), *S. carneas* (green with red canthal line), *S. dorisae* (marbled brown with a sepià shovel-shaped blotch), and *S. lacteus* (dark purple). *Sphaenorhynchus caramaschii* tadpoles can be distinguished by the absence of well-defined stripes on the body from *S. orophilus* (presence of a well-defined subocular stripe), *S. palustris* (white stripe from the snout to body-tail junction), *S.
surdus (two black stripes from the corner of the mouth to the medium third of the body) and *S. pauloalvini* (oblique black stripe, posterior to the nostrils, that extends longitudinally until the vent tube, a canthal stripe that extends posteriorly to the eyes, and a ventrolateral stripe from the snout until near the spiracle, see Bokermann 1973). The presence of three stripes on the anterior third of the tail musculature separates larvae of *S. caramaschii* from those of *S. carneus* (single red stripe), *S. pauloalvini* (single dark stripe on the inferior border of the tail musculature), *S. dorisae*, *S. lacteus* and *S. planicola* (no distinctive pattern of pigmentation). Further, the absence of pigmentation on the first third of ventral fin can differentiate the larvae of *S. caramaschii* from *S. dorisae* (less pigmented) *S. lacteus*, *S. orophilus*, *S. palustris*, *S. planicola* and *S. prasinus* (fully pigmented).

**FIGURE 2.** A: Amplexus of *Sphaenorhynchus caramaschii*. B: Semi-permanent pond at Fazenda Intermontes, Municipality of Ribeirão Grande, State of São Paulo, Brazil, where tadpoles of *S. caramaschii* were collected. C: Tadpole of *S. caramaschii* in life. Photo C: D. Loebmann.

**Discussion**

Tadpoles of *Sphaenorhynchus caramaschii* swim amidst floating vegetation near the water surface. Similar behavior is known for tadpoles of other species of *Sphaenorhynchus* that are found associated with roots of floating vegetation and under films of algae (*S. carneus*, *S. dorisae*, *S. lacteus* and *S. planicola*; Cruz 1973; Suárez-Mayorga & Lynch 2001). However, larvae of *S. prasinus* prefer darker and deeper places (Bokermann 1973), similar to those of *S. surdus* that inhabit amidst aquatic vegetation at the bottom of the water bodies (Caramaschi 2010). Altig & McDiarmid (1999) suggested that larvae of *Sphaenorhynchus* belong to the ecomorphological guild of lentic and benthic larvae based mainly in the descriptions of the larva of *S. orophilus*. However, the original descriptions (Cruz & Peixoto 1980; Heyer *et al.* 1990) do not mention that larvae of this species are bottom dwellers, but only that they are found in large ponds with abundant vegetation (Heyer *et al.* 1990).

A large number of tadpoles of *Sphaenorhynchus caramaschii* presented injured tails, especially in early developmental stages. Suárez-Mayorga & Lynch (2001) observed similar injuries in larvae of *S. carneus* (15%), *S. lacteus* (15%) and *S. dorisae* (6%), also mainly in earlier stages of development (stages 25–32), and suggested that
probably they are due to the coexistence of some predators (fish and nymphs of Odonata). As observed by Suárez-Mayorga & Lynch (2001) in *S. carneus*, there is no pigmentation on the regenerated portions of the tail in tadpoles of *S. caramaschii*.

Whereas most species of *Sphaenorhynchus* have been described as green dorsally (e.g. Lutz & Lutz 1938; Bokermann 1966), it has been widely overlooked that green bones are also common in juveniles and adults of most, if not all, species of *Sphaenorhynchus* (Lutz & Lutz 1938; KA V and JF pers. obs.). The green coloration in bones is likely due to their impregnation with different concentrations of biliverdin, as described by Barrio (1965) for some species of *Hypsiboas*, *Lysapsus*, and *Pseudis*, and more recently for several species of Cophomantini (e.g. Ruiz-Carranza & Lynch 1982; Lötters et al. 2005; Garcia et al. 2003, 2007, 2008; Garcia & Haddad 2008; Antunes et al. 2008; Faivovich et al. 2013), most Lophiohylini (JF, pers. obs.), and several centrolenids (e.g. Cisneros-Heredia & McDiarmid 2007). The physiological basis and possible biological role of this phenomenon are unknown.

Available phylogenetic studies based on molecular evidence for three of the 14 known species recovered *Sphaenorhynchus* as a monophyletic taxon closely related to *Dendropsophus*, *Scarthyla*, *Scinax*, *Lysapsus*, *Pseudis* and *Xenohyla*, but with different topologies (Faivovich et al. 2005; Wiens et al. 2006; Wiens et al. 2010; Pyron & Wiens 2011). Cruz & Peixoto (1980) pointed out morphological similarities (total length, color pattern, spiracle length, and marginal papillae size) between larvae of *S. bromelicola* and *S. orophilus*, and between those of *S. planicola* and *S. prasinus*. Faivovich et al. (2005), based on the results of their comprehensive phylogenetic analysis of Hylidae and also on previous records (Kenny 1969; Bokermann 1973; Cruz 1973; Cruz & Peixoto 1980; Suarez-Mayorga & Lynch 2001), suggested some larval character states as putative morphological synapomorphies of at least some species of *Sphaenorhynchus*: the morphology and position of the nostrils and the presence of some notably large marginal papillae. These and another character, the elongation of the spiracle, are discussed below.

**FIGURE 3.** Lateral views of the nostril of larvae of *Sphaenorhynchus caramaschii* showing the fleshy flange of the internal margin of the nasal rim. A and B: Left and right nostril of CFBH 26652, stage 29. C: Left nostril of CFBH 28652, stage 31. D: Left nostril of CFBH 28652, stage 39. Scale bars = 0.05mm.
Nostril morphology (Fig. 3A–D). The fleshy flanges of the nostril of *Sphaenorhynchus caramaschii* larvae give the nasal rim the appearance of a “valve” (sensu Cruz 1973). Larvae of *S. orophilus*, *S. palustris*, *S. planicola*, and *S. surdus* also present these flanges on the nostrils (Cruz 1973; Cruz & Peixoto 1980; Nunes et al. 2007; Caramaschi 2010). The morphology of the fleshy flanges varies in extension, width and pigmentation in larvae of *S. caramaschii* (Fig. 3A–D). The flanges can be long, extending along the entire margin (Fig. 3A, C), or short (Fig. 3B, D), and they can also be broad (Fig. 3A, B) or slender (Fig. 3D). Pigmentation can be absent, partially present, or cover the entire flange. These characteristics are independent of the larval stages available to us. We have observed the flanges to be “opened” or “closed” (Fig. 3A and C, respectively). Although this could be due to individual variation, we suggest that this might indicate that the flanges have some degree of mobility allowing them to open or close, depending on their level of lateral expansion. Detailed observations of the nostrils in live individuals are necessary to test this hypothesis.

Nunes et al. (2007) mentioned the absence of a fleshy flange in the nostril of larvae of *Sphaenorhynchus bromelicola*, *S. carneus*, *S. dorisae*, *S. lacteus*, *S. pauloalvini* and *S. prasinus* based on information taken solely from literature (Bokermann 1966; Bokermann 1973; Suárez-Mayorga & Lynch 2001). Our own observations of larvae of *S. bromelicola*, *S. pauloalvini*, and *S. prasinus* indicate that they do have a fleshy flange in the inner margin of the nostrils similar to that observed in *S. caramaschii*. Kenny (1969) describes the same structure in the larva of *S. lacteus* (as *Hyla orophila*; contra Duellman 2005, who states “narial papillae are absent”). Suárez-Mayorga & Lynch (2001) did not report details on the structure of the nostrils of the tadpoles of *S. carneus* and *S. dorisae*, so the nostril morphology of these species remains unknown.

Nostril position (Figs. 3 and 5). Nostril position was determined relative to body axis. Tadpoles of *Sphaenorhynchus caramaschii* present anteriorly directed nostrils with their longer axis forming an acute angle with respect to the main body axis, as also observed in other known larvae of *Sphaenorhynchus* (*S. bromelicola*, *S. carneus*, *S. dorisae*, *S. lacteus*, *S. orophilus*, *S. palustris*, *S. pauloalvini*, *S. planicola*, *S. prasinus*, and *S. surdus*; see Kenny 1969; Suarez-Mayorga & Lynch 2001; Caramaschi 2010). This position of the nostril is a putative synapomorphy of *Sphaenorhynchus*, as larvae of its putative sister taxa according to different phylogenetic analyses (Faivovich et al. 2005; Wiens et al. 2010) have nostrils with their longer axis parallel to the main body axis (Lavilla 1990; de Sá & Lavilla 1997; Duellman & de Sá 1988; Rossa-Feres & Nomura 2006).

Marginal papillae (Fig. 4A–D). Larvae of *Sphaenorhynchus caramaschii*, *S. palustris*, *S. planicola* and *S. prasinus* have a few larger marginal papillae, at least about twice the size of the smaller ones, located mainly in the posterior portion of the oral disc and arranged together or alternating with the small ones. Larvae of *S. carneus* and *S. lacteus* also seem to have this character state as indicated by available descriptions (see Kenny 1969; Suarez-Mayorga & Lynch 2001). The large marginal papillae in *S. planicola* seem to be somewhat larger than those of other larvae of *Sphaenorhynchus* that share this character state (Fig. 4C–D). *Sphaenorhynchus bromelicola*, *S. dorisae*, *S. orophilus*, *S. pauloalvini*, and *S. surdus* present small and homogeneous marginal papillae (Fig. 4A–B; see also Suarez-Mayorga & Lynch 2001; Caramaschi 2010). Therefore, there are at least two character states related to the size of marginal papillae as described above. The presence of some notably large marginal papillae might be a synapomorphy of at least an internal clade within *Sphaenorhynchus*, as first observed by Cruz & Peixoto (1980) and suggested by Faivovich et al. (2005).

Relative spiracle length (Fig. 5A–D). A short spiracle, 3–11% of BL, fused to the body wall, except at its posterior extreme, occurs in larvae of *Sphaenorhynchus caramaschii*, *S. pauloalvini*, *S. planicola*, and *S. prasinus* (Fig. 5A; Bokermann 1973, Cruz 1973). We tentatively consider that *S. carneus*, *S. dorisae*, and *S. lacteus* also have a short spiracle, on the basis of the observations and figures provided by Kenny (1969), and Suarez-Mayorga & Lynch (2001). *Sphaenorhynchus bromelicola* and *S. orophilus* share a medium-size spiracle, 16–24% of BL (Fig. 5C–D; Bokermann 1966; Cruz & Peixoto 1980). On the basis of the description and figures provided by Caramaschi (2010), we tentatively consider that *S. surdus* also has a medium-size spiracle. The spiracle in *S. orophilus* and *S. surdus* is somewhat shorter than in *S. bromelicola*, whereas the spiracle in *S. palustris* is notably larger, reaching at least 28% of BL. It is directed backwards and entirely separated from the body, except at its base (Fig. 5B).

Tadpoles of *Otophryne robusta* bury themselves in the sand of streambeds, and if active, they bury when disturbed (Wassersug & Pyburn 1987; MacCulloch *et al.* 2008). Larvae of *Cardioglossa* occur on both sandy bottoms and leaf litter in shallow streams (Amiet 1972; Blackburn 2008). Blackburn (2008) noticed other similarities among larvae of *Cardioglossa* and *Otophryne*, including a dorsoventrally flattened body, small eyes, a ventrally positioned mouth, and a long tail (Blackburn 2008 reports 2/3 of total length in larvae of *C. manengouba*). With the exception of tail length and the elongate spiracle in larvae of some species, those similarities are not shared with larvae of *Sphaenorhynchus*. Larvae of *Sphaenorhynchus* are known to be nektonic generally and to be closely associated with roots of floating vegetation or, sometimes, to occur at the bottom of ponds (Bokermann 1973; Cruz 1973; Suárez-Mayorga & Lynch 2001), but detailed information is lacking on their habits and possible biological function, if any, of the elongate spiracle.

Despite Caramaschi’s (2010) assertions to the contrary, our observations corroborate Faivovich *et al.*’s (2005) suggestion that the nostril with fleshy flanges and anteriorly directed are putative synapomorphies of *Sphaenorhynchus*, as these are known so far only in this genus among hylines. The two other characters related to the presence of enlarged marginal papillae and the elongation of the spiracle could be synapomorphies of less inclusive clades within *Sphaenorhynchus*.

![FIGURE 4. Oral discs of larvae of *Sphaenorhynchus* showing the configuration of the marginal papillae. A: *S. orophilus* (MZUSP 57915, stage 34). B: *S. bromelicola* (MZUSP 79559, stage 35). C: *S. planicola* (MZUSP 79552, stage 37). D: *S. prasinus* (CFBH 38057, stage 26). Scale bars = 1.0 mm. Photos A, B and C: B. Blotto.](image-url)

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References


APPENDIX 1. Larvae examined.

*Sphaenorhynchus bromelicola*—BRAZIL: Bahia: Maracás, Fazenda Canabrava, MZUSP 79559.
*Sphaenorhynchus palustris*—BRAZIL: Bahia: Porto Seguro, Estação Vera Cruz, RPPN, MNRJ 42616.
*Sphaenorhynchus pauloalvini*—BRAZIL: Bahia: Ilhéus, MZUSP 79580, 79568.
*Sphaenorhynchus planicola*—BRAZIL: Rio de Janeiro: Itaguaí, MZUSP 79552.
*Sphaenorhynchus prasinus*—BRAZIL: Bahia: Ilhéus, MZUSP 79571, 79553; CFBH 38057.