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# The tadpole of *Hylodes amnicola* Pombal, Feio & Haddad 2002 (Anura: Hylodidae): External morphology and buccopharyngeal anatomy

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Torrent frogs of the genus *Hylodes* Fitzinger are a very intriguing group. These frogs are adapted to live in fast-flowing waters and have a series of morphological and behavioral apomorphic character states associated with this lifestyle (Silva & Benmaman 2008). Adult *Hylodes* are diurnal and display a complex set of visual signals to communicate in noisy fast-stream environments (de Sá *et al.* 2016). Strong selection operating in torrent habitats is assumed to have posed evolutionary constraints, limiting the phenotypic diversity of adults. The 26 species currently assigned to *Hylodes* (see Frost 2023) show great similarity in their external morphology, body proportions, and coloration patterns (Montesinos 2017), which makes their taxonomic identification a complex task. Consequently, the taxonomic diversity within the genus remains highly underestimated and many cryptic lineages still await formal description (Montesinos 2017).

*Hylodes* tadpoles inhabit the same fast-flowing waters and creeks where the adult frogs of this genus breed (e.g., de Sá *et al.* 2015). Although some authors have argued that, as the adult frogs, tadpoles have a conservative body plan (e.g., Sazima & Bokermann 1982; Malagoli *et al.* 2020), recent studies have pointed out that the larval morphology is actually variable within Hylodidae and informative at different phylogenetic levels of inclusiveness (Montesinos *et al.* 2022). For instance, the presence of an inverted V upper jaw is a putative synapomorphy of Hylodidae, differentiating it from other members of Neoaustrarana, and *Hylodes* can be distinguished from other hylodids (i.e., *Crossodactylus* Duméril and Bibron, *Megaelosia* Miranda-Ribeiro and *Phantasmarana* Vittorazzi, Augusto-Alves, Neves-da-Silva, Carvalho-e-Silva, Recco-Pimentel, Toledo, Lourenço, and Bruschi) by lacking a medial emargination on the lower lip, besides exhibiting buccopharyngeal characters that allow for effective intrageneric diagnoses (Montesinos *et al.* 2022).

Larval morphology has not been fully appreciated in the hylodid literature, despite the existence of morphological descriptions for most tadpoles (Laia & Rocha 2012). Specifically, while tadpoles of 22 out of the 26 *Hylodes* species have been formally described, a significant portion of the morphological characters considered important for systematic analysis are either absent or poorly detailed in the original larval descriptions (Montesinos *et al.* 2022). In this research, we conduct an in-depth examination of the larval morphology of *Hylodes amnicola* Pombal, Feio & Haddad, a species whose larva was first described by Pombal *et al.* (2002). Our study offers a comprehensive reevaluation of its external morphology and presents, for the first time, a detailed description of its buccopharyngeal cavity. Furthermore, we introduce a previously undocumented oral character state for Hylodidae.

We analyzed the same two tadpoles of *Hylodes amnicola* used in the original description, collected at the Parque Estadual do Ibitipoca, Municipality of Lima Duarte, State of Minas Gerais, Brazil, on 26–29 October 1999 (MNRJ 24862: Stage 25; MNRJ 24999: Stage 31 after Gosner 1960), plus one tadpole from the same locality, collected on 09 February 2002 (CFBH 30972: Stage 34). The examined material is housed in the herpetological collection of the Brazilian Museu Nacional (MNRJ, Rio de Janeiro) and Coleção de Anfíbios Célio Fernando Baptista Haddad (CFBH, UNESP, Rio Claro). Tadpoles from lot CFBH 30972 were sequenced for the mitochondrial locus 16S and had their species identity confirmed based on molecular evidence (de Sá *et al.* 2022). Seventeen measurements were taken with a digital caliper to the nearest 0.1 mm: total length (TL), body length (BL), body width at the level of spiracle (BW), head width at the level of eyes (HW), body height (BH), internarial distance (IND), interorbital distance (IOD), eye diameter (ED), eye–naris distance

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(END), naris–snout distance (NSD), snout–spiracle distance (SSD), tail height (TAH), upper tail fin height (UTF), lower tail fin height (LTF), tail muscle height at base of tail (TMH), tail muscle width at base of tail (TMW), and oral disc width (ODW). One tadpole at Stage 34 (CFBH 30972) was dissected to study the buccopharyngeal anatomy (Wassersug (1976) and, after inspection under stereomicroscope, prepared to observation with scanning electron microscope (Dias & Anganoy-Criollo unpubl. data). Measurements and terminology followed Kok & Kalamandeen (2008), Altig & McDiarmid (1999), Schlosser (2002) for lateral lines, and Wassersug (1976) for buccopharyngeal structures.

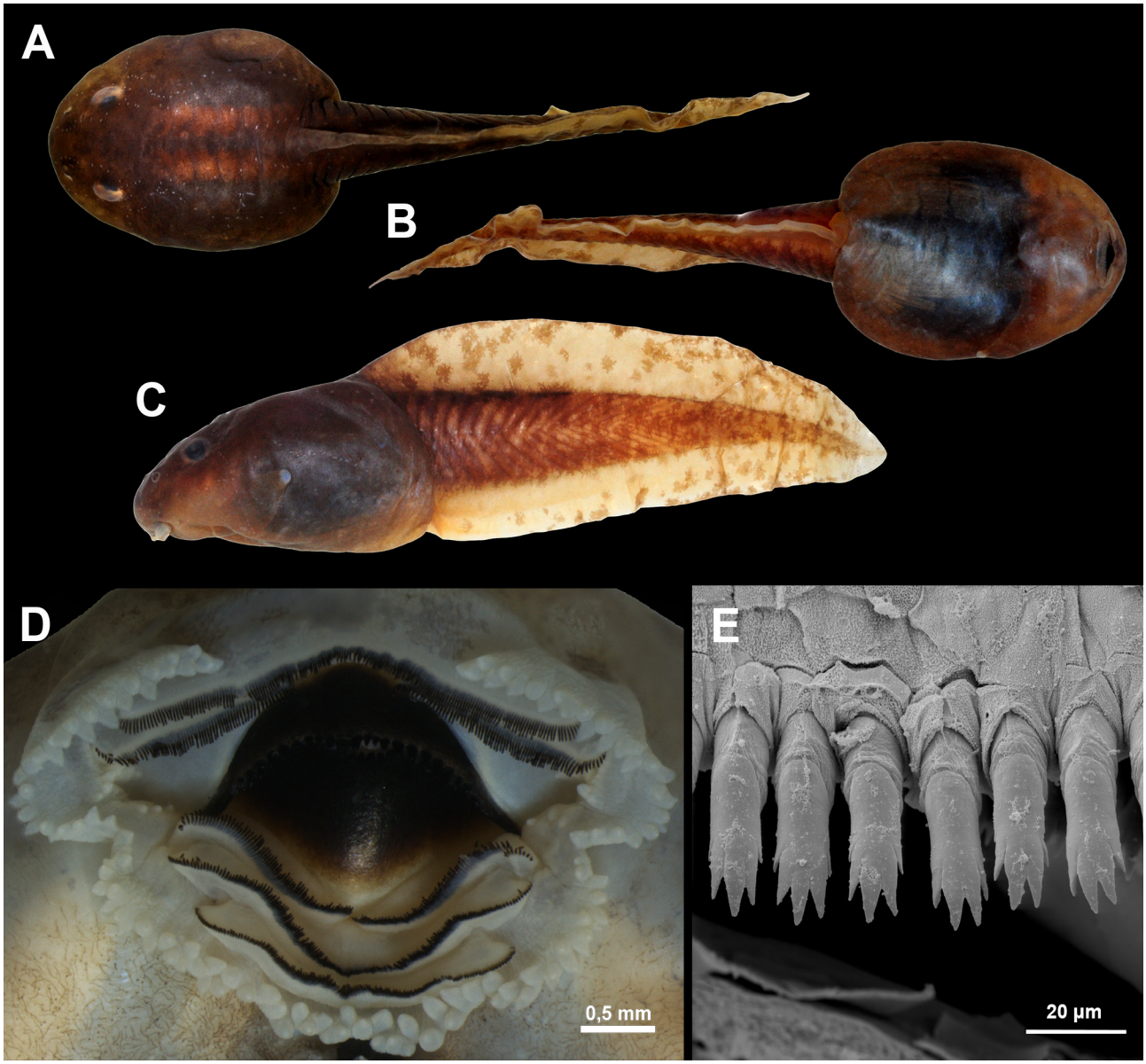


**FIGURE 1.** Tadpole of *Hylodes amnicola* (MNRJ 24862; Stage 25; TL = 52.42 mm). (A) dorsal view, (B) ventral view, (C) lateral view, (D) oral disc, (E) CFBH 30972 (Stage 34), detail of labial teeth.

**Tadpole redescription (Fig. 1).** Tadpoles body distinctively robust, oval in dorsal, ventral, and lateral views, wider posteriorly, wider than tall (BH/BW = 0.83 ± 0.06). Snout rounded. Eyes rounded, positioned dorsolaterally, diameter approximately 20% of head width (ED/HW = 0.20 ± 0.004), half the width of interorbital distance (ED/IOD = 0.50 ± 0.04), and 20% wider than eye–nostril distance (ED/END = 1.20 ± 0.12), iris black. Nostrils rounded, positioned dorsolaterally, about two times closer to eyes than to snout (END/NSD = 0.54 ± 0.01), dorsal region tumescent, rim white or slightly pigmented. Interorbital distance wider than internasal distance (IOD/IND = 1.12 ± 0.09). Ventral depression prior to the coiled intestine present. Spiracle sinistral, located medially at the level of the second third of the body (SSP/BL = 0.58 ± 0.01), oriented posterodorsally, opening without pigmentation. Anterior lateral line system composed of four pairs of lines (post-infra-orbital and post-supra-orbital lines present) and posterior lateral line system composed of three pairs of

lateral lines (oral divided from the beginning in an anterior sinuous line parallel to the infraorbital line, and a longitudinal line descending ventrally). Medial lateral line fused with dorsal lateral line between caudal myomeres 12 and 13. Stitches predominantly rounded, arranged in continuous lines, including post-supraorbital and post-infraorbital lines. Vent tube short, dextral, the inferior portion of the external wall extending beyond the opening, attached to the ventral margin of the fin. Tail represents approximately two thirds of the total length (TAL/TL = 0.63 ± 0.01). Caudal musculature robust, representing two thirds of the body heigh (TMH/BH = 0.64 ± 0.00), tapering gradually to become pointed, representing more than half of the tail height (TMH/MTH = 0.56 ± 0.10). Tail higher than body (TAH/BH = 1.16 ± 0.22), dorsal fin originates anteriorly to the end of the body, approximately 36% higher than the ventral fin (UTF/LTF = 1.36 ± 0.10). Tail muscle width approximately 37% of body width (TMW/BW = 0.37 ± 0.01). Oral disc directed ventrally, approximately 41% of head width at eye level in size (ODW/HW = 0.41 ± 0.01), emarginate, bordered by two rows of small papillae medially interrupted on the anterior lip, two rows of supernumerary papillae on the labial corner between tooth rows A2 and P1, submarginal papillae absent. Labial tooth row formula 2(2)/3(1). Labial teeth long, well developed, multicuspid; head and body with no distinct separation. Jaw sheaths strongly developed and serrated, fully keratinized, anterior upper jaw sheath arched in inverted "V" shape, with smooth lateral processes and a medial projection; lower jaw sheath V-shaped, completely keratinized. Serration of the anterior jaw sheath homogeneous in size. Pre-oral region pigmented with irregular blotches. In preservative, dorsum and flanks dark brown, with sparse irregular blotches randomly distributed. A pair of dark brown blotches between nostrils and pre-orbital lateral line. Ventral surface cream with slight silver sheen, immaculate. Tail muscle light brown, with dense pigmentation forming a reticulum; a dark brown stripe extends dorsally, not reaching half of the tail. Fins cream, slightly translucent, with irregular brown blotches, on the ventral fin blotches are mostly distributed on its final portion.

**Measurements (in mm).** TL = 51.99 ± 0.62; BL = 19.45 ± 0.59; BW = 12.45 ± 1.15; HW = 10.85 ± 0.37; BH = 10.26 ± 0.25; IND = 3.80 ± 0.03; IOD = 4.24 ± 0.38; ED = 2.13 ± 0.03; END = 1.78 ± 0.15; NSD = 3.27 ± 0.22; SSD = 11.23 ± 0.62; TAH = 11.92 ± 2.57; UTF = 4.30 ± 0.48; LTF = 3.19 ± 0.59; TMH = 6.53 ± 0.21; TMW = 4.65 ± 0.32; ODW = 4.41 ± 0.04.

**Buccopharyngeal anatomy (Fig. 2).** Buccal roof elliptical with prenarial arena oval, bearing a small crest (Fig. 2A). Prenarial papilla absent. Internal nares elliptical, transversely oriented regarding the longitudinal axis; posterior valve free, with low, conical projection. Vacuities present, circumscribed by the margins of the inner nares, covered with ciliated cells (Fig. 2C). Postnarial arena diamond-shaped, with two conical papillae. Lateral ridge papillae bifurcate. Median ridge tall, triangular, with irregular, pustulated apex. Buccal roof arena triangular, bordered with 6–7 papillae on each side; buccal arena covered with rounded and triangular pustulations. Glandular zone well-defined; secretory pits small, poorly marked. Dorsal velum arch-shaped, medially interrupted. Buccal floor triangular, larger at the caudal end (Fig. 2B). Two pairs of infralabial papillae; first pair (from medial to lateral) tall, conical, covered with pustulations; second pair hand-like, with four branches, covered with pustulations (Fig. 2D). Tongue anlage elliptical; single pair of lingual papillae long, finger-like. Buccal floor arena elliptical, lateral region with 14–17 tall, conical papillae; papilla at the same level as the buccal pocket branched; prepocket papillae present, 5–6, short, conical. Buccal floor arena covered with rounded and conical pustulations, denser on posterior half; few (3–4) conical papillae scattered in the posterior arena. Buccal pockets deep, oblique, slit-shaped. Ventral velum present; spicular support conspicuous; marginal projections present, parallel to filter plates; three projections on medial area long, triangular; medial notch not evident. Secretory pits poorly developed, scarce on velar surface. Glottis fully exposed. Branchial basket triangular, wider than long, about 1/4 of buccal length and 1/2 of buccal width. Three shallow filter cavities, partially covered by ventral velum.

A thorough comparison of larval morphology and morphometry of *Hylodes* is provided in Montesinos *et al.* (2022). The tadpole of *Hylodes amnicola* differs from all species, except *H. perplicatus,* by the dorsal fin originating before the ending portion of the body (after the ending portion in *H. fredi, H. pipilans,* and *H. meridionali;* and exactly at the posterior limit of the body in all other species). It also differs from all species, except *H. charadranaetes*, *H. fredi*, and *H. phyllodes*, by presenting an immaculate nostril (black ring complete or not in others). Morphometrical differences can also be observed: *H. amnicola* tadpoles are longer than *H. asper*, *H. babax*, *H. otavioi*, *H. perplicatus*, and *H. phyllodes*, and shorter than *H. meridionalis*. It differs from all species, except *H. babax*, *H. magalhaesi*, *H. ornatus*, and *H. perplicatus*, by the width of the tail muscle at the base of tail, which represents less than 40% of the body width.

Regarding the buccal cavity, tadpoles of *Hylodes amnicola* can be differentiated from its congeners mainly by the number of papillae on the buccal floor and roof, the shape of the medial ridge, and the morphology of the lateral ridge papillae (e.g., Montesinos *et al.* 2022 and literature therein). Bilate *et al.* (2012) suggested that two main arrangements of buccal floor arena papillae are present in *Hylodes* larvae: in U or V or as parallel lines. In *H. amnicola* papillae are

arranged in a U shape. Although this should be further investigated, it appears that this is the most well distributed condition in the genus, with few species (e.g., *H. charadranaetes*) presenting the parallel condition. The most remarkable feature in *H. amnicola* is the presence of narial vacuities. These have been found in several families: Ascaphidae (van Eeden 1951), Bufonidae (Dias & Anganoy-Criollo unpubl. data), Centrolenidae (Rada *et al.* 2019; Dias *et al.* 2020), Hylidae (e.g., Wassersug 1980; Kolenc *et al.* 2008; Dias & Pie 2021), Leptodactylidae (Nascimento *et al.* 2021), but never in Hylodidae. Our data provide further evidence that vacuities are more extensively distributed across the anuran tree of life than previously established. It is worth noting that while some authors (e.g., Wassersug 1980) have proposed a chemosensory role for the vacuities, the function of these structures remains undetermined (Dias & Pie 2021).

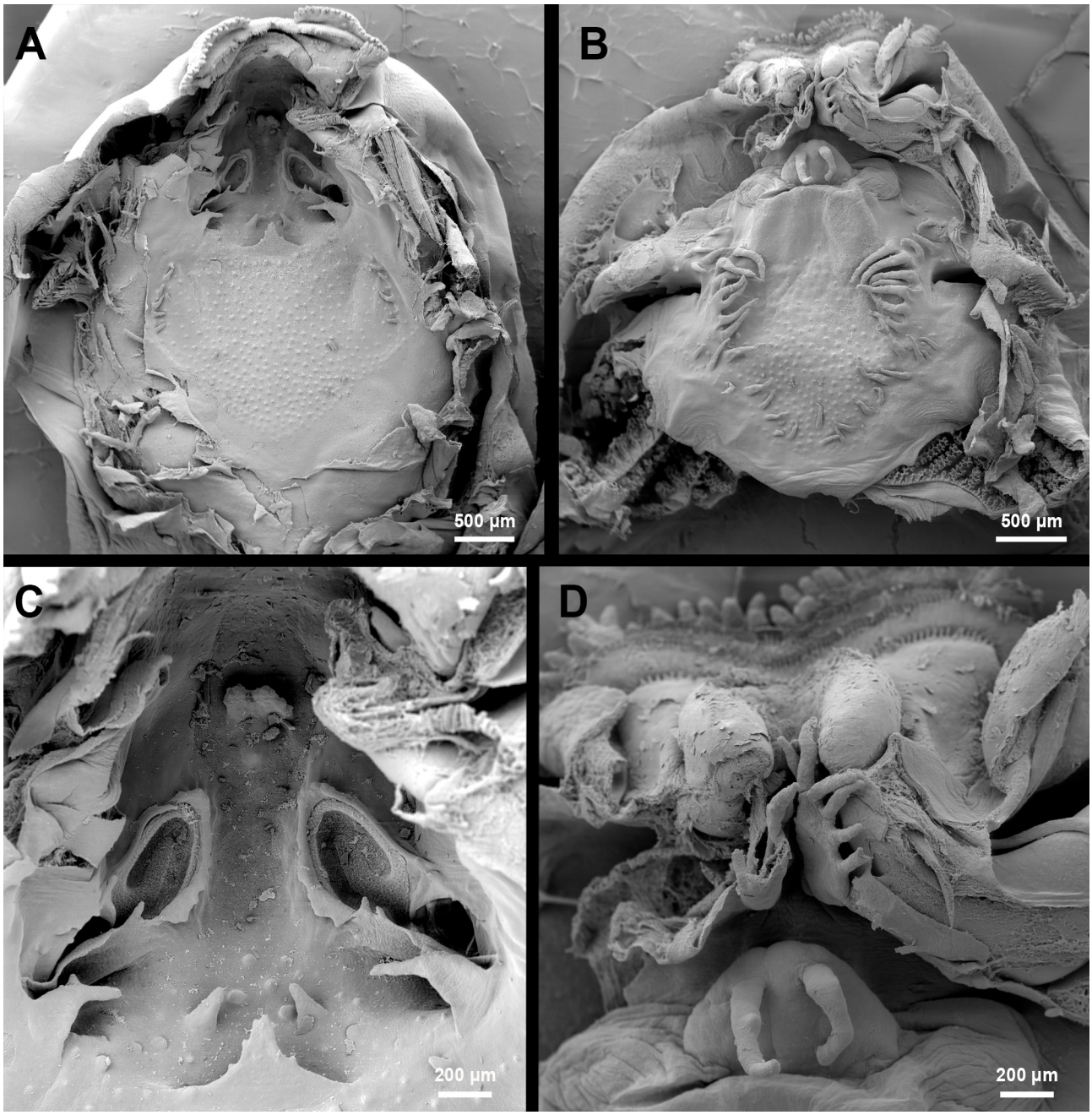


**FIGURE 2.** Buccopharyngeal anatomy of *Hylodes amnicola* (CFBH 30972; Stage 34). (A) buccal roof, (B) buccal floor, (C) detail of the narial vacuities on buccal roof, (D) detail of the infralabial and lingual papillae of buccal floor.

Finally, it is also interesting to note that the buccopharyngeal cavity of *Hylodes amnicola* seems to be less densely covered with pustulations and papillae in comparison with other hylodid larvae. Buccopharyngeal elements have been associated with trophic and/or sensorial function (e.g., Wassersug 1980). The reduction in the number and density of these elements could reflect a change in feeding habits in this species in comparison with its congeners. Unfortunately,

data on tadpoles' diet is quite rare and fragmented and we do not know what *Hylodes* tadpoles really eat (Altig *et al.* 2007; Montaña *et al.* 2019). Procedures complementary to analysis of trophic spectrum (e.g., stable isotopes, DNA metabarcoding), in association with robust morphological assessment can be useful to test whether phenotypic differences observed in tadpole of *H. amnicola* can be explained by shifts in its ecology.

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