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The tadpole of *Hylorina sylvatica* (Anura: Cyclorhamphidae) in southern Chile

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Abstract

The tadpole of *Hylorina sylvatica* is redescribed. The current description includes larval external morphology, internal oral features examined by SEM, and description of the chondrocranium and hyobranchial apparatus. The external and internal characteristics of the tadpoles of *H. sylvatica* are compared with others members of the subfamily Alsodinae (family Cycloramphidae) and used to improve the generic diagnosis which until now has been based on adult morphology.

Key words: Chondrocranium, internal oral anatomy, larval morphology, systematics

Introduction

Hylorina sylvatica Bell is a monotypic cycloramphid frog endemic to the *Nothofagus* forests of temperate South America (southern Chile and Argentina). Formas and Pugín (1978) described the external morphology of *H. sylvatica* tadpoles based on two specimens (IZUA 1383-A; stages 36 and 38) from Pargua, Llanquihue Province, Chile, and Wassersug and Heyer (1988) described the larval internal oral features of this species based on a single specimen. The external and internal morphology of tadpoles has been successfully used to improve generic diagnosis and to infer phylogenetic relationships among anurans (Larson & de Sá 1998; Haas 1995, 2001, 2003; Maglia *et al.* 2001; Púgener *et al.* 2003; Grosjean *et al.* 2004).

Herein, the tadpoles of *H. sylvatica* are redescribed based on 87 specimens (stages 25–43) from three different localities in southern Chile (Volcán Osorno, Llanquihue, and Puyuhuapi). We include a redescription of the external morphology and internal oral features, and provide new characters derived from the analysis of the chondrocranium and hyobranchial apparatus. We use the larval characters to complement the generic diagnosis of *Hylorina* within the family Cycloramphidae.

Material and methods

Eighty-seven tadpoles of *Hylorina sylvatica* (Instituto de Zoología, Universidad Austral de Chile; IZUA) were collected at: La Picada, Volcán Osorno (Provincia de Osorno) 41°04'S, 72°26'W, on December 1977 (IZUA 1808); Puyuhuapi (Provincia de Aysén) 44°15'S, 72°31'W, on 27th February 1990 (IZUA 2898 – 2899); Lenca (Provincia de Llanquihue) 41°14'S, 73°1'W (IZUA 2963), on 27th June 1995.

Tadpoles were fixed in 10% buffered commercial formaldehyde solution. Most of the larvae were preserved shortly after collection, but some were reared in the laboratory to verify species identification. Tadpoles were staged following the Gosner's table of normal development (Gosner 1960). Description is based on Gosner's Stage 35 and illustrated tadpole is at Gosner's Stage 36 (IZUA 2898).

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Eight measurements were taken with a dial caliper (to the nearest 0.1 mm) following Altig and McDiarmid (1999): total length, body length, tail length, maximum tail height, tail muscle height, tail muscle width, internarial distance, and interocular distance. Morphometric data are given as mean ± standard deviation.

Seven tadpoles at stages 27, 31, 32, 38, and 41 (from lots IZUA 1808 and 2898) were dissected for observation of the internal oral anatomy under scanning electron microscopy (SEM), following the methodology described by Larson *et al.* (2003) with minor modifications. Specimens were dissected and subsequently fixed in a 2-3% glutaraldehyde solution for 3-4 h at room temperature, followed by three 15 min washes in 0.1 M phosphate buffer. Next, specimens were dehydrated in a graded ethanol series as follows: 35%, 50%, 70%, 80%, 95%, three changes at 100%, for 15 min each, and a final 5 min wash in acetone 100%. Specimens were critical point dried in CO_2, mounted on aluminium stubs and sputter coated with gold. Features of dorsal and ventral internal oral anatomy were examined and photographed using a scanning electron microscope (Leo-420), attached to a computer. Additionally, two tadpoles (stage 36) were dissected, stained with Coomassie Blue, and examined using a stereosmicroscope (Wild 308700). Terminology used to describe features of the oral cavity follows Wassersug (1976, 1980) and Wassersug and Heyer (1988).

Chondrocrania were cleared and double stained with Alcian Blue and Alizarin Red (Song & Parenti 1995). The internal oral anatomy, chondrocranium, and hyobranchial anatomy were observed under stereomicroscope and drawings were made with the aid of a camera lucida. Chondrocranial anatomy was based on the examination of larvae at Gosner stages 25 (n= 2), 35 (n= 3), 38 (n=1), 41 (n= 1), 43 (n=1), and the overall description was based on larvae at stage 35 (IZUA 2898), illustrations on specimen at stage 38 (IZUA 2899). Chondrocranial terminology follows Larson and de Sá (1998) and D'Heursel and de Sá (1999).

Results

The tadpole of Hylorina sylvatica

The description is based on seven specimens (IZUA 2898, mean total length 63.3 ± 1.90 mm; stage 35). Body ovoid in lateral and dorsal view (Fig. 1A, B). Tail length twice of body length. Tip of snout gently rounded. Nostrils oval in shape, surrounded by a thin cutaneous marginal black fringe; dorsal and anterolaterally located. Internarial distance approximately 42% of interocular distance. Nostrils slightly closer to the tip of the snout than to the anterior border of eye. Pupils circular, eyes dorsolaterally situated. Oral disc emarginate (Fig. 1C), anteroventrally situated (about 40°); maximum width 135.1% of the internarial distance. A single row of marginal papillae surrounding the oral disc, rostral gap present, ventral gap absent. A cluster of submarginal papillae (8-9) is present laterally on the posterior labium and a single submarginal papilla is seen on the lateral lower corner of the upper labium. Upper and lower jaw sheath serrated. Posterolateral extension of the upper sheath thin, non-serrated, reaching beyond the lower sheath. Labial tooth row formula is 2(2) / 3[1]; balance value -1. Labial rows are almost equal in length. Labial teeth linearly arranged in one row, labial tooth spatuled with 15–17 cusps on their tips (Fig. 1D). Spiracle sinistral and ventrolateral, about 1.4X the internarial distance, with an ovoid opening posterolaterally directed at an approximately 55 degrees angle. Spiracular tube not free, but with the inner wall of the spiracle attached to the body, its tip not raised. Vent tube with a dextro-ventral aperture, laterally directed (Fig. 1E, F), representing approximately half of the internarial distance; posterior border continuous with the ventral margin of the tail fin (Fig. 1F). Dorsal fin originating as a fleshy crest reaching the level of the spiracular opening at stage 26, and midway between the posterior border of the spiracle and the vent tube at stages 37–39. Ventral fin originating at the posterior margin of vent. Tail tip gently rounded. At stage 26, the maximum tail height at the middle of the tail and exceeding dorsal surface of the body, maximum tail height about 0.53X the tail length; whereas at stage 34 and 39 about 0.35X and 0.29X, respectively. Tadpole measurements are shown in table 1.

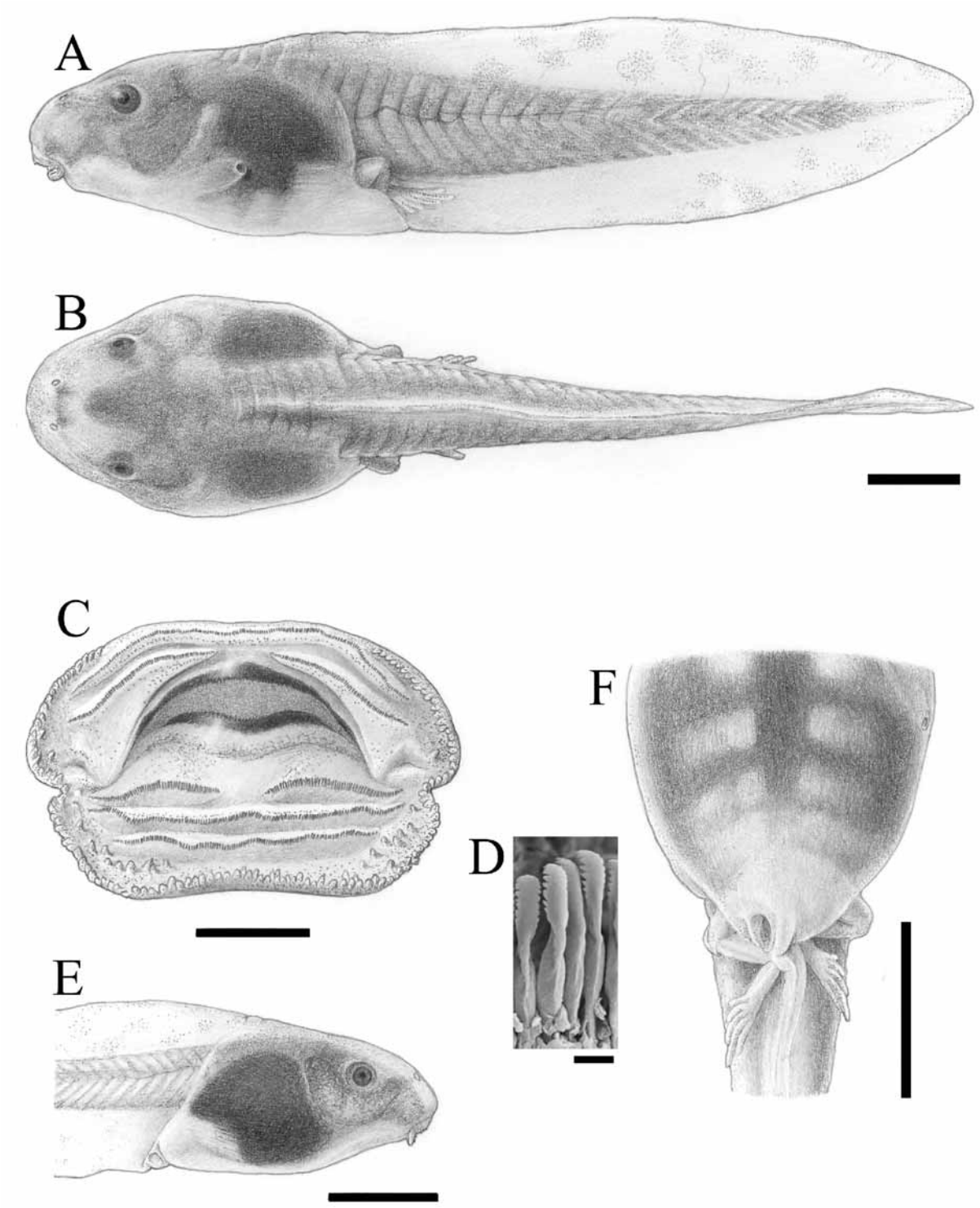

FIGURE 1. Tadpole of *Hylorina sylvatica* (Stage 36, IZUA) (A) Lateral view, (B) dorsal view, (C) oral disc, (D) labial teeth, (E) body lateral view (stage 27), (F) ventral view. Bar A,B,E,F= 5mm; C= 1mm; D= 20µm.

Internal oral anatomy

Internal oral anatomy is described at Gosner (1960) stage 41 and compared with the characteristics at stage 27.

Buccal roof: The buccal roof (Fig. 2A) is diamond shaped; its maximun width is approximately equal to its length. The prenarial arena is shallow with a low transverse ridge; a pair of single and blunt papillae is located anterior to the ridge. The nares are oriented at about 45 degrees angle with the main axis of the oral

cavity; narial valve projections are absent (Fig. 2A, C). At stage 27, the postnarial arena presents one pair of pustulations and three pairs of papillae; the most anterior papillae are small, the second pair is medium-sized, and the most posterior pair is large; their distal tips are blunt. A pair of smaller papillae and a pair of postulations are found in the posterior area of the postnarial arena close to the distal tip of the medium. At stage 27, the median ridge is a large and triangular structure extending ventrally from the buccal roof and its margin presents two notches (Fig. 2C). Whereas, at stage 41 the medium ridge has reduced in size and is vestigial (Fig. 2D). A pair of lateral ridge is at level of the median ridge; it is trifurcate, medially curved. Overall, the buccal roof arena is U-shaped, defined by 11–16 simple, conical, slightly medially, and anteriorly curved papillae on each side. The rows of papillae are almost parallel to each other and to the main axis of the body; they extend posteriorly to a point about three-quarters of the way back on the buccal roof. Buccal roof arena is covered by numerous pustulations (50–60). A distinct glandular area is found on the posterior half of the dorsal velum; this area is uniform in width and the secretory pits are dense. Posteromedially, the dorsal velum bears 16–17 marginal projections.

TABLE 1. Morphometric data (mean ± SD ranges) of the tadpoles of *Hylorina sylvatica* (all data in mm).

Stage	n	Total length	Body length	Tail length	Maximum tail heigth	Tail muscle height	Tail muscle width	Internarial distance	Interocular distance
25	29	26.3 ± 2.8	9.6±0.9	16.7 ± 2.6	8.9 ± 1.0	2.1 ± 0.3	2.0 ± 0.2	2.1 ± 0.2	4.0 ± 0.3
26	9	30.4± 1.8	10.7 ± 0.4	20.1 ± 1.8	10.1 ± 0.7	2.6 ± 0.2	2.5 ± 0.3	2.3 ± 0.1	4.7 ± 0.3
27	2	31.5–34.1	10.6–11.0	20.5–23.5	9.7–11.5	2.7–2.7	2.4–2.6	2.4–2.4	4.7–4.7
28	3	36.4 ± 1.9	12.0 ± 0.4	24.8 ± 1.9	10.7 ± 0.52	2.8 ± 0.1	2.9 ± 0.9	2.4 ± 0.1	4.8 ± 0.2
29	6	38.6 ± 3.1	13.4 ± 1.1	26.3 ± 3.5	10.6 ± 1.1	3.2 ± 0.3	3.2 ± 0.3	2.6 ± 0.3	5.2 ± 0.4
31	3	51.5± 11.9	16.6 ± 2.9	33.5 ± 9.4	12.2 ± 0.4	4.4 ± 1.4	3.9 ± 0.9	3.0 ± 0.4	5.9 ± 0.2
34	5	61.0 ± 3.0	20.9 ± 1.2	40.1 ± 1.7	14.0 ± 0.4	6.3 ± 0.4	5.6 ± 0.4	3.5 ± 0.2	7.9 ± 0.7
35	7	63.3 ± 1.9	21.5 ± 0.7	42.5 ± 2.1	15.3 ± 0.7	6.7 ± 0.4	5.8 ± 0.3	3.5 ± 0.1	8.4 ± 0.6
36	2	65.9– 66.3	21.9– 22.2	45.3 – 45.7	15.8 – 15.9	7.3 – 7.5	6.3 – 6.6	3.3 – 3.4	8.8 – 9.0
38	6	57.5±14.2	19.5 ± 4.6	39.2 ± 10.0	13.3 ± 2.9	6.0 ± 2.3	5.2 ± 1.8	2.8 ± 0.8	7.7 ± 2.5
39	3	70.2 ± 2.7	22.6 ± 0.4	48.6 ± 2.2	14.5 ± 0.7	6.9 ± 0.4	6.3 ± 0.2	3.0 ± 0.2	9.0 ± 0.3
40	5	69.3 ± 1.9	24.2 ± 6.4	54.3 ± 14.1	13.1 ± 1.1	6.9 ± 0.4	6.6 ± 0.6	2.5 ± 0.1	9.1 ± 0.3
43	2	66.2– 68.0	35.2– 37.3	73.3 – 77.9	10.8 – 11.0	6.0 – 6.1	6.1– 6.5	2.5– 2.6	8.0 – 8.8

Buccal floor: (Fig. 2B) The buccal floor is ovoid in shape; it is slightly longer than wide. Two pairs of infralabial papillae are present, the anteromedial pair is simple with rugose surfaces, and their tips are blunt, while the posterolateral pair is trifurcate with the branches connected basally. At stage 27, two pairs of lingual papillae project dorsally from the anteromedial region of the buccal floor, but no lingual button is present. The anterior pair is longer than the posterior. Their margins are smooth and the distal tips are slightly pointed. At stage 41, they are shorter and the lingual button is developed (Fig. 2E, F). The buccal floor arena is large and U-shaped, narrowing slightly posteriorly. The buccal floor arena is defined by a well-developed row of large and blunt buccal floor papillae that run approximately parallel to one another and extend posteriorly from a point anterior to the buccal pockets, almost to the edge of the velum. The anterior two-thirds of the buccal floor arena are smooth and the posterior third is pustulated (16–20 pustulations) with medium-sized papillae. Several small papillae (anterior) and large papillae (posterior) are located lateral to the buccal floor papillae. The buccal pockets are transversally oriented and 10–12 median-sized prepocket papillae are visible on the buccal roof (Fig. 2A). The posterior margin of the ventral velum seems smooth laterally and it has four to five marginal projections on each side. Neither distinct median notch nor secretory pits were observed on the posterior margin of the ventral velum.

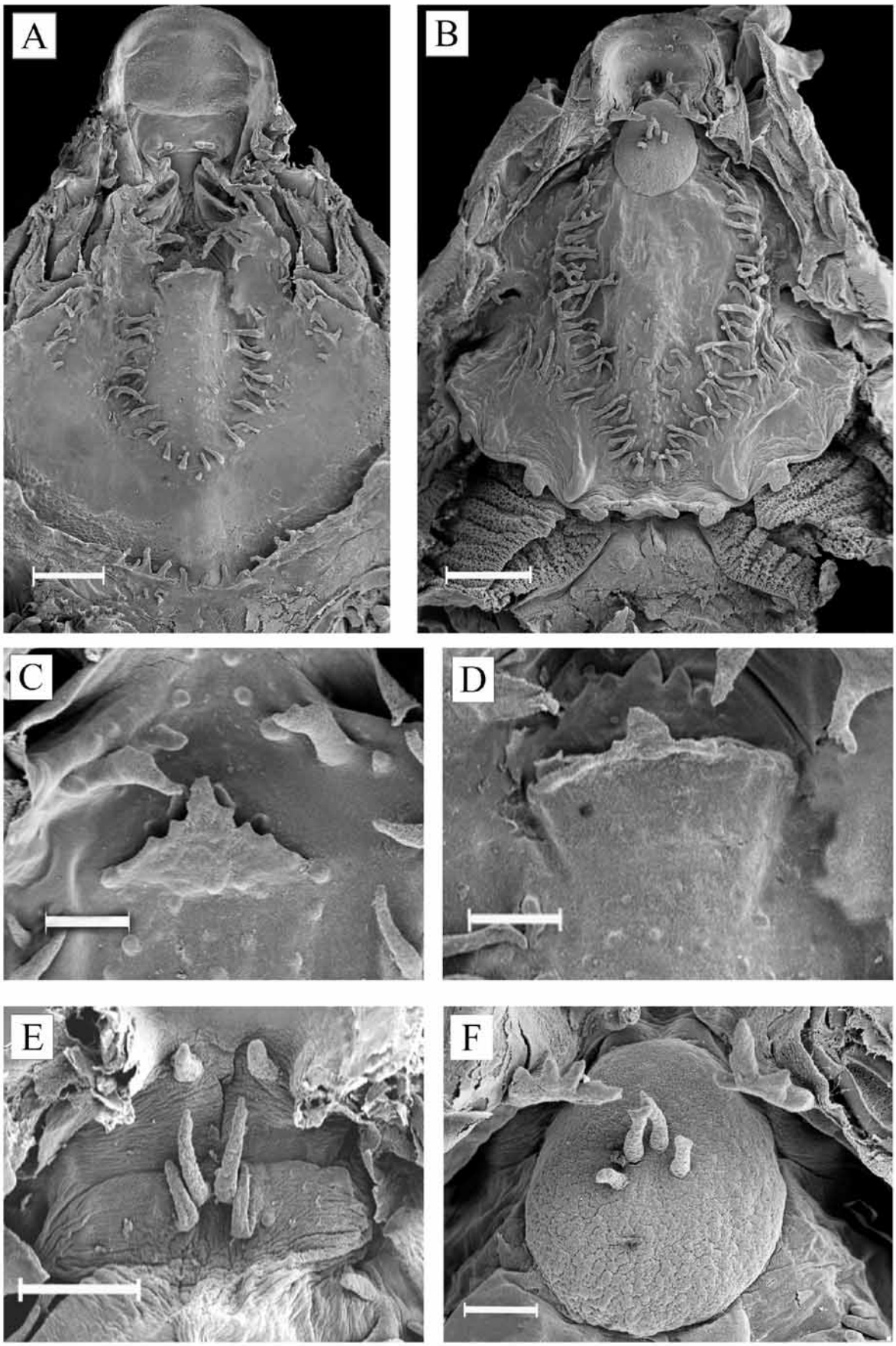

FIGURE 2. Roof (A) and floor (B) of the buccal cavity of *Hylorina sylvatica* (Stage 41). Medial ridge: stage 27 (C), stage 41 (D). Lingual bud: stage 27 (E), stage 41 (F). Bar 300μm.

Chondrocranium

At stage 35 the chondrocranium is completely cartilaginous, whereas by stage 38 the ossification of the parasphenoid is visible (Fig. 3B). The maximum width (at the level of the middle region of the palatoquadrates) is approximately 87.3% of its total length (Fig. 3A). The chondrocranium is low in lateral view; its maximum height (at the level of the cornua trabeculae) is approximately 29.8% of the total length (Fig. 3C).

Neurocranium

Ethmoidal region. The paired cartilagines labiales superior support the upper horny beak; each consists of two distinct elements, a medial corpus and a lateral alae (Fig. 3E). These elements are dorsal and ventromedially fused by a narrow, poorly chondrified, stripe of cartilage, but they are not medially fused. The partes corpores are subrectangular in shape, ventrally rounded, and almost in contact ventromedially; in frontal view the two corpora form an open "V". The partes alares are flat, ventrally rounded, and they flex posteriorly from their point of contact with the corpus; they bear a well-developed and triangular processus posterior dorsalis. The cornua trabeculae represent approximately 22.7% of the total chondrocranial length; they are approximately 2.3X longer than wide and project anteriorly, from the planum trabeculare anticum, diverging from each other and forming a wide "V". The cornua trabeculae are almost uniform in width along their length. A small processus lateralis trabeculae is visible on the lateral margin of each cornu. The distal tips of the cornua are curved and contact with the posterior edge of the alae (Fig. 1C). Posteriorly, the cornua trabeculae are confluent with the planum trabeculare anticum and the planum ethmoidale. The tectum nasi grows laterally from the planum ethmoidale and expands further ventrolaterally.

Orbitotemporal region. (Fig. 3B–C). At stage 35 a thin cartilage, the planum intertrabeculare, closes the basicranial fenestra. The planum intertrabeculare is formed by extensions of the trabeculae cranii laterally and the planum trabeculare anticum anteriorly. At stage 38, the parasphenoid is ossified on the cranial floor. Two sets of foramina are found on the cranial floor; the anterior smaller pair are the foramina craniopalatina, while the larger posterior pair corresponds to the foramina carotica primaria. The lateral walls of the braincase are formed by the orbital cartilages, which connect posterodorsally to the otic capsules. This connection forms the dorsal margin of the foramen prooticum located between the anterior margin of the otic capsule and the posterior margin of the orbital cartilage. The cartilago orbitalis has three foramina. The larger, anterior, and ovoid foramen is the foramen opticum, whereas the smaller and posterior opening is the foramen oculomotorium; a small trochlear foramen is visible dorsal to the foramen opticum.

The frontoparietal fontanelle is large and ovoid, approximately 55% of the total chondrocranial length (Fig. 3A). It is bordered anteriorly by the planum ethmoidale, laterally by the taeniae tecti marginales, and posteriorly by the tectum synoticum. The taeniae tecti marginales converge anteriorly forming a well-developed keel. At stage 35, the tectum sinoticum is completely formed and the taenia tecti transversalis is visible, whereas at stage 38, the parietal region is roofed by a layer of cartilage, the tectum parietale.

Otoccipital region. The otic capsules are rhomboid in shape and represent approximately 29% of the total chondrocranial length. In dorsal view the otic capsule bears a laterally projecting crista parotica, with a well-developed processus anterolateralis that contacts or not the posterior border of palatoquadrate; the processus posterolateralis is poorly developed. The ovoid fenestra ovalis is visible ventrolaterally on the otic capsule, below the crista parotica (Fig. 3C). The arcus occipitalis extends ventrally from the posteromedial margin of the otic capsule, fusing with the planum basale and giving rise to the occipital condyles. The planum basale and the arcus occipitalis form the ventral and medial margins of the ovoid foramen jugulare. The foramen perilymphaticum inferior is found lateral to the foramen jugulare on the ventromedial margin of the otic capsulae (Fig. 3B).

Visceral components

Palatoquadrate. The palatoquadrate has two distinctive anterior processes: the pars articularis and the processus muscularis quadrati (Fig. 3A–C). The former is robust and articulates broadly with the posterior margin

of Meckel's cartilage, whereas the latter is wide and its dorsal edge inclines medially and attaches to the commissura quadratocranialis anterior via the commissura quadratoorbitalis. In lateral view, the processus muscularis quadrati is triangular and its tip is located at the end of the septum nasi, not reaching the dorsal surface of the chondrocranium (Fig. 3C). The commissura quadratocranialis anterior extends between the palatoquadrate and the floor of the neurocranium. The processus antorbitalis projects dorsolaterally at the level where the commissura quadratocranialis anterior attaches to the orbital cartilage (Fig. 3A). A low hyoquadrate process is observed below the processus muscularis quadrati.

Posteriorly, the palatoquadrate attaches to the orbital cartilage via the processus ascendens, which is narrow and connects to the posterior margin of the palatoquadrate to the braincase. The processus ascendens attaches posterior and slightly below the foramen oculomotorium. The posterior margin of the processus ascendens forms an angle about 75-80° with the axis of the chondrocranium.

Cartilago Meckeli and cartilago labialis inferior. The cartilago Meckeli and cartilago labialis inferior form the lower jaw (Fig. 3B, C, E). The cartilago Meckeli is sigmoid in shape, short, placed ventral to the cornu trabecularum, and posterior to the lateral alae of the cartilago labialis superior; it is oriented perpendicular to the chondrocranial longitudinal axis. Anteriorly, the cartilago Meckeli articulates with the posterior margin of the cartilago labialis superior and posteriorly with the pars articularis quadrati.

The cartilago labialis inferior provides support to the lower horny beak. These cartilgines are rectangular, slightly longer than wide; with curved anterior and posterior margins (Fig. 3B) and they laterally contact with cartilago Meckeli.

Hyobranchial apparatus. The ceratohyalia (Fig. 3D), subtriangular in shape, are wide and flat cartilages; laterally they are dorsally twisted to articulate with the palatoquadrate at the facies articularis hyalis. Each ceratohyal bears two anterior processes; the processus anterior hyalis and the processus anterolateralis hyalis. Both processes are almost triangular in shape, the former larger than the latter. Additionally, each ceratohyale bears a well-developed triangular processus posterior hyalis. The ceratohyalia are joined medially by the pars reuniens, which is poorly chondrified; the basihyal is absent and the basibranchial articulates with the planum hypobranchiale and bears a well-developed urobranchial processus. The planum hypobranchiale are wide, flat sheets of cartilage, subtriangular in shape, which serve as the point of attachment for the ceratobranchials. The planum hypobranchiale are separated medially along their lengths. Posteriorly they diverge leaving an inverted narrowly U-shaped separation between their posterior edges.

The branchial baskets have four ceratobranchials that are distally continuous via the commissurae terminales. Ceratobranchiale IV is the widest; ceratobranchials I–III bear dorsally directed spicula.

Chondrocranium developmental modifications

Stage 25.– Throughout development, chondrocranial architecture undergoes modifications associated with osteocranial development and the shift from larval to adult feeding and breathing mechanisms. At this stage, the cranium is poorly chondrified; the pars alaris and the processus posterior dorsalis are poorly developed and the semicircular canals are visible through the cartilaginous walls of the otic capsules.

Stage 38.– The parasphenoid bone is ossified and its lateral alae are cover the ventral surfaces of the otic capsules. Also at this stage, the tectum parietale is fully developed.

Stage 41.– The processus posterior dorsalis of the pars alaris and the cornua trabeculae are smaller than in Stage 35. The frontoparietal fenestra is totally covered by cartilage and projecting from the larval crista parotica only the processus anterolateralis is observed.

Stage 43.– The cartilage labialis superior are almost eroded. The cornua trabeculae, the larval crista parotica, and its processus anterolateralis are absent. The posterolateral margins of the taeniae tecti marginales are covered by the ossification of the frontoparietal, the cultriform process and the alae of the parasphenoid are ossified.

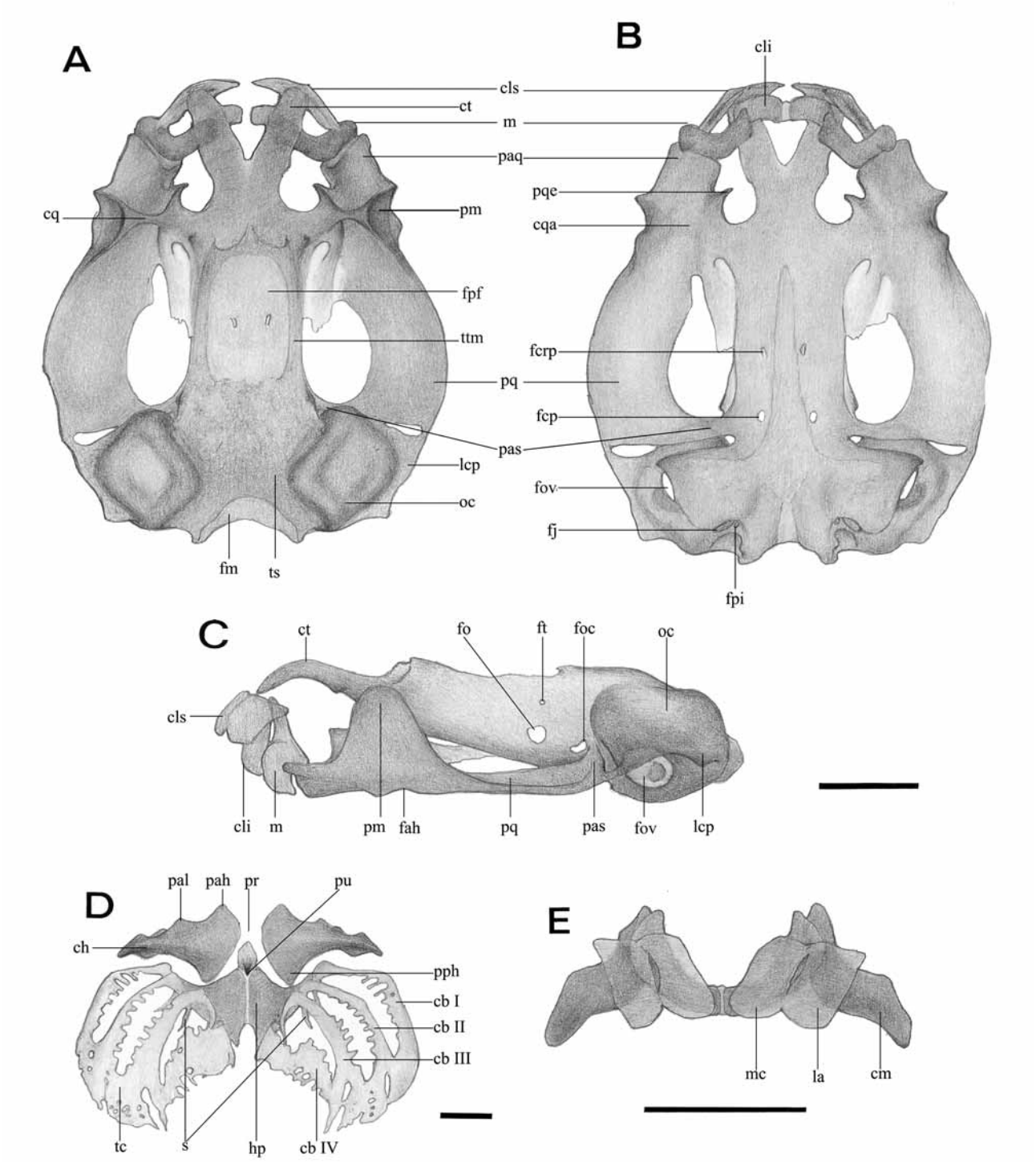

FIGURE 3. Chondrocranium and hyobranchial apparatus of *Hylorina sylvatica* (Stage 38, IZUA-2899). (A) dorsal, (B) ventral, and (C) lateral views; (D) hyobranchial apparatus, (E) suprarostral cartilages. Abbreviations: cb I-IV = ceratobranchials I-IV, ch = ceratohyal, cli = cartilago labialis inferior, cls = cartilago labialis superior, cq = commissura quadratoorbitalis, cqa = commissura quadratocranialis anterior, ct = cornua trabeculae, fah = facies articularis hyalis, fcp = foramen caroticum primarium, fcrp = foramen craniopalatinum, fj = foramen jugulare, fm = foramen magnum, fo = foramen opticum, foc = foramen oculomotorium, fov = fenestra ovalis, fpf = frontoparietal fenestra, fpi = foramen perilymphaticum, ft= foramen trochlear, hp = planum hypobranchiale, la = lateral alae, lcp = larval crista parotica, m = cartilago Meckeli, mc = medial corpus, oc = otic capsule, pah = processus anterior hyalis, pal = processus anterolateralis hyalis, paq = pars articularis quadrati, pas = processus ascendes, pm = processus muscularis quadrati, pph = processus posterior hyalis, pq = palatoquadrate, pqe = processus quadratoethmoidalis, pr = pars reuniens, pu = processus urobranchialis, s = spiculum, tc = commissura terminalis, ts = tectum synoticum, ttm = taenia tecti marginalis. Bar = 2mm.

Discussion

The tadpole of *Hylorina sylvatica* is a generalized pond-type larva (Duellman & Trueb 1986) with a roughly ovoid body, a tail about twice as long as the body, and with dorsal and ventral fins each about as high as the caudal musculature. The mouth is anteroventrally directed and the oral disc is lateral and ventrally bordered by a row of small papillae. Our analysis of the external morphology of *H. sylvatica* tadpoles overall agrees with previous descriptions (Formas & Pugín 1978); however we observed a few characteristics not reported before. Most remarkable are the fusion of the inner wall of the spiracle to the body wall; vent tube with a dextro-ventral aperture, laterally directed, and with the posterior border continuous with the ventral margin of the tail; at stage 26, the maximum tail height about 0.53X of the tail length, whereas at stage 34 and 39 it is only about 0.35X and 0.29X of the tail length, respectively. The external morphology of tadpoles of the family Cycloramphidae shows several diagnostic characters that are useful for identification and comparison among genera. The tadpoles of *H. sylvatica*, like most members of the subfamily Alsodinae [(*Alsodes, Limnomedusa, Macrogenioglottus, Odontophrynus, Proceratophrys*); family Cycloramphidae, sensu Frost *et al.* 2006; Frost 2007] are exotrophic and can be differentiated from those of *Eupsophus* and *Rhinoderma* (Cycloramphinae) that have endotrophic larvae (Formas 1989; Lavilla 1987; Vera-Candioti *et al.* 2005; Cárdenas-Rojas *et al.* 2007). *Hylorina sylvatica* shows a dextral vent tube, character shared with other members of the subfamily Alsodinae; however, it differs from *Limnomedusa* which has a medial vent tube (Cei 1980; Lavilla 1988); *Odontophrynus* and *Macrogenioglottus* have dextral vent tubes, but the inner wall is free and laterally displaced, the vent tube is also longer in *Odontophrynus* than in *Macrogenioglottus* tadpoles (Cárdenas-Rojas per. obs.) The tail tip of *Odontophrynus, Macrogenioglottus,* and *Proceratophrys cristiceps* (Müller) tadpoles is pointed and the tail musculature extends to the posterior tip of tail (Abravaya & Jackson 1978; Izeckzohn *et al.* 1979; Cei 1980; de Sá & Langone 2002; Vieira *et al.* 2007; Cárdenas-Rojas per. obs.). In *H. sylvatica* the tail tip is rounded and the tail musculature is shorter, not reaching the tip of the tail. *Alsodes verrucosus* (Philippi), *A.vanzolini* (Donoso-Barros), *Crossodactylus schmidtii* Gallardo, and *Proceratophrys boiei* (Wied-Neuwied) tadpoles have a "V-shaped" lower jaw sheath (Izecksohn *et al.* 1979; Faivovich 1998; Formas & Brieva 2004); however, it is "U-shaped" in *H. sylvatica.*

Our results on the internal oral anatomy of the tadpole of *H. sylvatica* are in agreement with previous descriptions (Wassersug & Heyer 1988), but we are adding new characters from the roof of oral cavity. Wassersug and Heyer (1988) stated that the median ridge, the lateral ridge papillae and the postulations within the buccal roof arena may have been destroyed during dissection or SEM preparation.

A comparison among the internal oral features of *H. sylvatica, Alsodes gargola* Gallardo, *A. verrucosus, A. vanzolinii, A.* sp (probably *A. barrioi*), *Limnomedusa macroglossa* (Duméril & Bibron), *Macrogenioglottus alipioi* Carvalho, *Proceratophrys appendiculata* (Günther), *P. avelinoi* Marcadal de Barrio and Barrio, *P. cristiceps,* and *Odontophrynus occidentalis* (Berg) shows that tadpoles of *H. sylvatica* have unique characteristics (Peixoto & Da Cruz 1980; Wassersug & Heyer 1988; Turner 1999; Echeverría *et al.* 2001; de Sá & Langone 2002; Formas & Brieva 2004; Alcalde & Blotto 2006; Vieira *et al.* 2007). In *Alsodes, Limnomedusa, Proceratophrys appendiculata, P. avelinoi,* and *Odontophrynus* the buccal floor arena is broad and "U-shaped" and in *Macrogenioglottus* and *P. cristiceps* it is ovoid-triangular shaped, whereas in *H. sylvatica* it is narrow and elongate. The buccal floor and roof arena present a wide range in the number of papillae among the genera considere. However, *H. sylvatica* shows clear differences in papillae length and pustulations; they have between 25-40 simple, conical, and median size papillae on the buccal floor arena and 11–16 on the buccal roof arena, each arena is relatively smooth with pustulation scattered over their surfaces. *Limnomedusa macroglossa* has 50–60 buccal floor arena papillae and 15–17 buccal roof arena papillae; these are longer than those of *H. sylvatica*. Within the buccal floor arena, *L. macroglossa* tadpoles have many papillae scattered among the pustulations, whereas the buccal roof arena is mostly covered with pustulations (Turner 1999; Alcalde & Blotto 2006); *P. appendiculata*, *P. avelinoi* and *P. cristiceps* are different by the dense pustulations

found in the buccal floor and buccal roof arenas, among they are different in number and size of papillae (Wassersug & Heyer 1988; de Sá & Langone 2002; Vieira *et al.* 2007). *Macrogenioglottus alipioi* have only 15–25 buccal floor papillae per side and 10 buccal roof papillae per side along with a few pustulationt (Wassersug & Heyer 1988). Also, the internal oral features of *H. sylvatica* show remarkable differences when compared to species of *Eupsophus* (Vera-Candioti *et al.* 2005; Cárdenas-Rojas *et al.* 2007). In *E. calcaratus* (Günther) and *E. queulensis* Veloso, Celis-Diez, Guerrero, Méndez, Iturra, and Simonetti, most buccal floor and buccal roof papillae and pustulations are reduced, having only 2–5 short papillae and 2-4 pustulations on the buccal floor and roof arenas; only infralabial and lingual papillae are present. This drastic reduction might be associated with the endotrophic development mode of *Eupsophus* larvae.

Comparing the chondrocranial morphology, especially the cartilago labialis superior, of *H. sylvatica*, *Eupsophus calcaratus*, *Alsodes barrioi* Veloso, Diaz, Iturra, and Penna, *A. vanzolinii*, *A. verrucosus*, and *Limnomedusa macroglossa* (all members of the Alsodinae), these genera can be easily differentiated. The cartilago labialis superior of *Alsodes* consists of pars corporis and pars alaris clearly separated from each other (Formas & Brieva 2004); in *E. calcaratus* the medial corpus is single and the alae is fused to the corpus by a cartilaginous dorsal connection (Vera-Candioti *et al.* 2005); in *L. macroglossa* the two corpora are fused ventromedially, but they are not laterally continuous with the alae, which bear a well-developed processus posterior dorsalis, a rounded processus anterior dorsalis, and adrostrals cartilages (Turner 1999; Alcalde & Blotto 2006). In *H. sylvatica* the corpora are medially separated; and alae and corpora are fused both in their dorsal and ventral tips. The hyobranchial apparatus has a basihyal in *Alsodes* (Formas & Brieva 2004) and *Limnomedusa* (Turner 1999; Alcalde & Blotto 2006), but this element is absent in *Hylorina sylvatica*.

Lynch (1971) diagnosed the genus *Hylorina* based on 33 characters, 20 of them derived from adult skull morphology and only two from tadpoles (labial tooth row formula and rostral gap). Lavilla (1988) presented a diagnosis of the genus *Hylorina* based on 13 external morphological larval features. Altig and McDiarmid (1999) summarized larval characteristics for *H. sylvatica* based on the descriptions given by Formas and Pugín (1978) and Lavilla (1988). Based on the characters described herein (i.e., external morphology, chondrocranium, hyobranchial apparatus, and internal oral features) the genus *Hylorina* can be diagnosed by a generalized pond-type exotrophic, lentic and benthic larva, with a roughly ovoid body; tail about twice as long as the body; dorsal and ventral fins about as tall as the caudal musculature; maximum total length 63.3$\pm$1.9 mm (stage 35); oral disc emarginate; maximum width about 134% of internarial distance; rostral gap present; ventral gap absent; single row of marginal papillae; intramarginal papillae absent; 8–9 submarginal papillae on posterior labium and a single one on anterior labium; lower jaw sheath "U"-shaped; labial tooth row formula 2(2)/ 3[1]; spiracle sinistral and about 1.4X the length of the internarial distance, not raised; inner wall of the spiracle fused to the skin of the body; vent tube with dextral aperture, laterally directed and representing about half of the internarial distance; buccal floor arena narrow, elongated, "U"-shaped; 25–40 buccal floor papillae per side; 10–12 prepocket papillae per side; corpora of suprarostral cartilages medially separated, alae and corpora fused by their dorsal and ventral tips.

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Specimens examined

Abbreviations

AMNH	American Museum Natural History
DRCR	Diana R. Cárdenas-Rojas (personal collection)
IZUA	Instituto de Zoología Universidad Austral de Chile
RdS	Rafael de Sá (personal collection) Richmond, Virginia USA
USNM	Smithsonian National Museum of Natural History

Alsodes vanzolinii: IZUA 3206, 3334: Ramadillas, Cordillera de Nahuelbuta, Provincia de Arauco, Chile.
Alsodes verrucosus: IZUA 2219: Puyehue. Provincia de Osorno, Chile.
Eupsophus calcaratus: IZUA 2896-2957: Puntra Chiloé, Chile; IZUA 2897: La Picada Volcán Osorno, Chile.
Eupsophus queulensis: IZUA 3708: Reserva Nacional Los Queules, Chile
Eupsophus roseus: IZUA 1809: Huachocopihue Valdivia, Chile; IZUA 2969: Cuesta de Soto Valdivia, Chile. DRCR 0009-0015-0022: Reserva Costera Valdiviana, Chile.
Hylorina sylvatica: IZUA 1808: La Picada, Volcán Osorno, Chile. IZUA 2963: Lenca, Provincia de Llanquihue, Chile. IZUA 2898-2899: Lago Risopatrón Puyuhuapi Provincia de Aysén, Chile.
Limnomedusa macroglossa: RdS UR Ind. 1, 2, 5, 7.
Macrogenioglottus alipio: AMNH A92809: Espirito Santo: Reserva Nova Lombardia Sta. Tereza. Brazil.
Odontophrynus americanus: RdS UR Ind. 1, 3, 4, 1W.
Rhinoderma darwinii: IZUA 515-518, 520, 524-526, 530, 533-534: Valdivia, Chile.