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External morphology, chondrocranium, hyobranchial skeleton, and external and internal oral features of *Rhinoderma rufum* (Anura, Rhinodermatidae)

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The species of *Rhinoderma* Duméril & Bibron are endemic to the temperate forests of South America in southern Chile and Argentina (Formas *et al.* 1975). Both have specialized reproductive modes, *Rhinoderma darwinii* Duméril & Bibron undergoes complete embryonic and larval development in the mouth of the male: newly metamorphosed frogs are expelled into the terrestrial environment (Jorquera *et al.* 1972). In contrast, embryos of *R. rufum* (Philippi) do not remain in the male's mouth, but instead are expelled into the water as larvae (Jorquera *et al.* 1974). Jorquera *et al.* (1972, 1974) described the normal development of both species. The chondrocranial morphology and visceral skeleton of *R. darwinii* was described by Lavilla (1987**)** and its internal oral features by Wassersug & Heyer (1988). The table of normal development of *R. rufum* (Jorquera *et al.* 1974) emphasized the duration of each stage of development: however, some features currently used for comparative purposes in tadpole morphological studies were not included or described only briefly. In this work, I include a detailed description of the morphology, particularly of the mouthparts, of the tadpoles of *R. rufum*, and describe the chondrocranium, hyobranchial skeleton, and the internal features of the oral cavity in this species. In addition, I compare the morphology of the mouth, chondrocranium, and hyobranchial skeleton of the congeneric tadpoles, considering different modes of feeding (endotrophic in *R. darwinii* versus exotrophic in *R. rufum*). Last, I present some thoughts about the evolution of larvae of *Rhinoderma*.

Tadpoles of *Rhinoderma rufum* (n =10) were collected by Emilio Pugin in Chiguayante (36º54’ S, 73° 01' W, Concepción Province, southern Chile; February 1972) and deposited in the Institute of Embryology at Universidad Austral de Chile (IEUA-011). Two tadpoles were raised in captivity until complete metamorphosis. Tadpoles were staged following the development table of Gosner (1960). The external morphology of four tadpoles of *R. darwinii* (IZUA 3528) were examined (Stage 32) for comparative purposes. The measurements (two tadpoles Stage 36) and terminology follow those of Altig & MacDiarmid (1999). The chondrocranium of *R. rufum* was studied in two cleared–and–stained tadpoles (Stage 32) following the protocol of Song & Parenti (1995) in which cartilage is stained with Alcian blue. The chondrocranial terminology follows Larson & de Sá (1998). The jaw sheaths of one tadpole (Stage 32) for each species of *Rhinoderma* were also studied under scanning electronic microscope (SEM; Leo-420). The same specimens were used to observe the labial teeth. Features of internal oral cavity were examined and photographed with a stereoscopic microscope Olympus SZ61 after having applied lugol solution (1%). Terminology of oral structures follows that of Wassersug (1976) and Wassesug & Heyer (1988).

The external description of *Rhinoderma rufum* is based on a Stage 32. In lateral view, the total length of the oval body is 31.7 mm (Fig. 1A), and the tail is 1.7 times the length of the body. The head is gently rounded with non-protruding, rounded dorsal nostrils. The interorbital distance is 134% of the internarial distance. The distance between the front edge of the nostrils and the end of the snout is 1.7 times the distance between the anterior edge of the eye and the posterior edge of the nostril. The diameter of the anterolateral eyes is 0.57 times the internarial distance. The oral disc (Fig. 2A) is anteroventral and not emarginated: the rostral gap is wide and the mental gap is absent and transparent. The marginal conical papillae are distributed in a single row over both the upper and lower lips. The supramarginal and inframarginal papillae are scarce (1 or 2), and the intramarginal papillae are absent. The dark brown upper and lower jaw sheaths are wider than long: they are well keratinized and have a serrated edge (Fig. 2B).The tips of the serrations are rounded; the length is 1.5 times its width. There are 60–70 serrations/mm. The labial tooth row formula is 2 (2) / 3. The labial teeth are three times longer than wide and their edges are jagged (Fig. 2D). There are 60–80 labial teeth/mm. The sinistral spiracular tube has an oval aperture, the length of which is 1.3 times the internarial distance; its diameter corresponds to 42% of the diameter of the eye, and its inner wall is attached to the body. The vent tube is as long as wide, and the ovoid medial opening is subsequently continuous with the margin of the ventral fin (Fig. 1C). The dorsal fin does not extend over the body, and the ventral fin starts at the distal end of the vent tube. The end of the tail is rounded, and the

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maximum width of the dorsal fin does not exceed the height of the body. The dorsal surface is light brown (in 10% formalin) with groups of melanophores and guanophores; the ventral area is transparent and the digestive tract and the interhyoideus muscle are visible. The measurements (mm) of two tadpoles at Stage 36 are: total length 37.5-38.1, body length 8.8–9.2, tail length 16.8–17.2, maximum body height 5.1–6.0, maximum tail height 4.8–5.1, tail muscle height 2.8–3.0, interorbital distance 2.5–2.7, internarial distance 1.2–1.4, oral disc width 1.8–2.1, and vent tube length 1.0–1.2. Webbing was observed in Stage 36 (Fig. 1B).

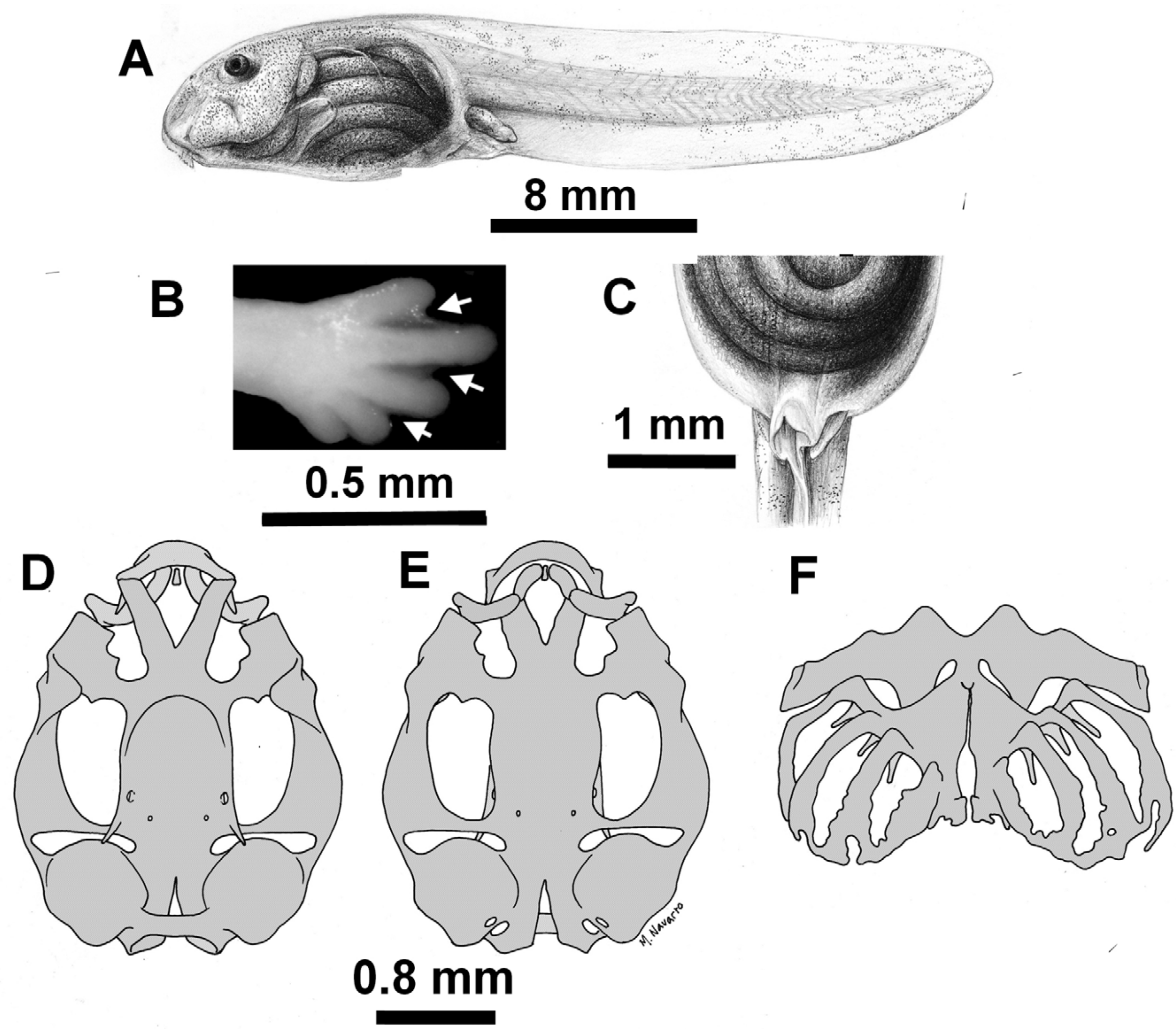

FIGURE 1. Tadpole of *Rhinoderma rufum* (Stage 32): Lateral view (A), vent tube (C), dorsal view chondrocranium (D), ventral view of chondrocranium (E), ventral view of hyobranchial apparatus (F). Foot of *Rhinoderma rufum* (Stage 36), arrows indicate the webbing (B).

The internal oral anatomy description of *Rhinoderma rufum* is based on a single Stage 32. The oral roof is oval-shaped, 1.4 times longer than wide. The prenarial arena has a single, short blunt papilla (Fig. 2E). The nares are, approximately 3.2 times longer than wide and oriented at about a 15 degree angle with respect the longitudinal axis of the oral cavity (Fig. 2G). The internarial distance is 0.6 times the length of the nares. Posterior to the nares there is a row of 12 short papillae. The median ridge is triangular, four times wider than high, and bears a scalloped edge (Fig. 2F). Lateral ridge papillae are absent. The oral roof arena is U-shaped and surrounded by elongated papillae, 15–19 per side (Fig. 2H), beginning almost as far forward as median ridge; the posterior edge of oral roof has 16 short papillae (Fig. 2I). The oral roof arena presents 21 postulations (Fig. 2H). Secretory pits were not observed. The dorsal velum is short and widely separated at the midline.

The floor of the mouth is oval; 1.3 times wider than long. Infralabial papillae are absent. At the anterior region, the rounded tongue has six short papillae (Fig. 2K). The U-shaped oral floor arena has 12–17 subequal simple papillae on each side (Fig. 2J); the posterior part of the floor bears postulations and short papillae (Fig. 2L). The ventral velum is undulated (Fig. 2M). Oral pockets were not defined.

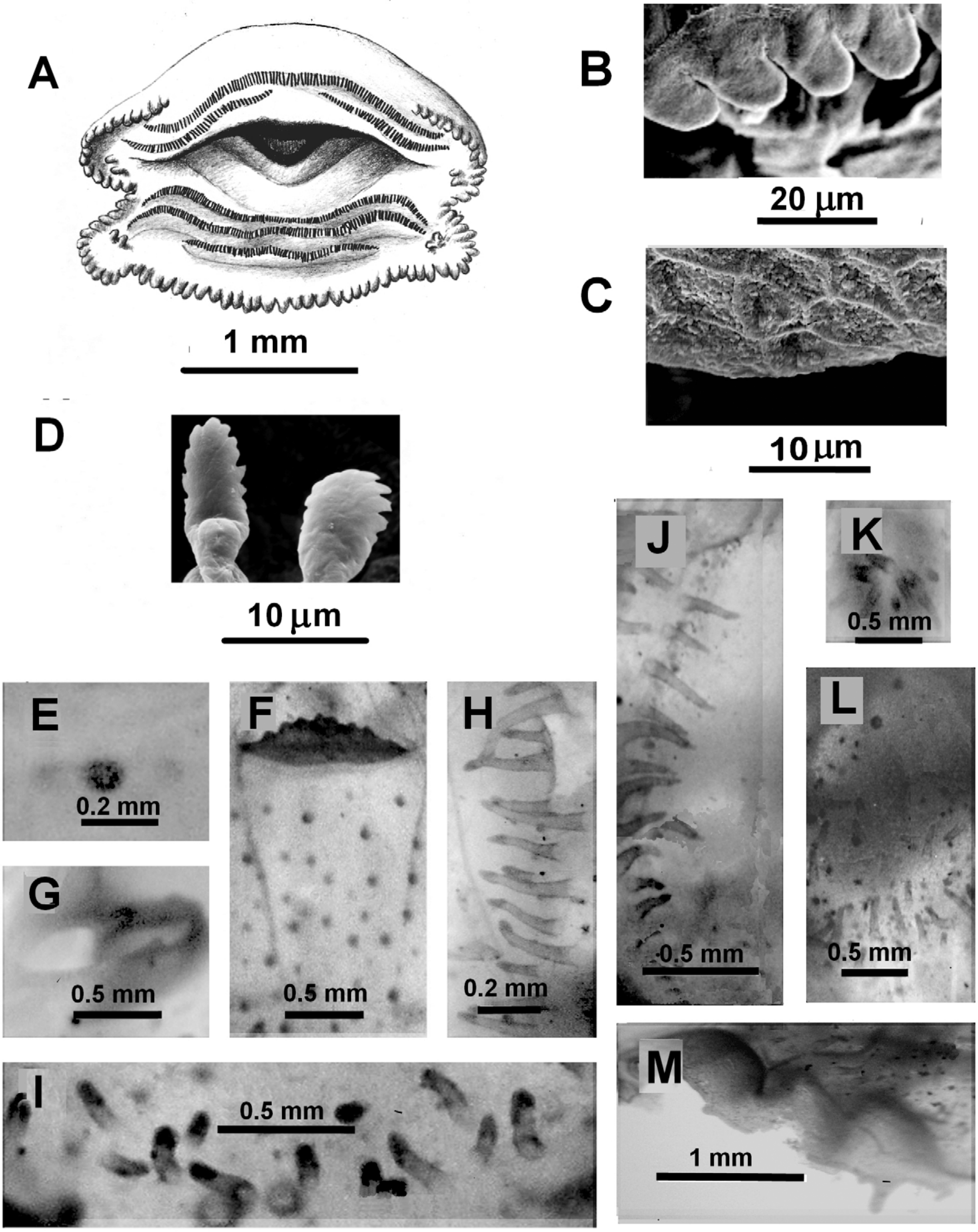

FIGURE 2. Morphology of the external and internal oral structures of *Rhinoderma rufum*: Oral disc (A), upper jaw sheath (B), labial teeth (D); Oral roof: prenarial papillae (E), median ridge and postulations of the oral roof arena (F), right narina (G), row of the lateral papillae of oral roof arena (H), papillae of the posterior border of the oral roof arena (I); Oral floor: right papillae of the oral floor arena (J), lingual bud and lingual papillae (K), oral floor arena and posterior papillae (L), ventral velum (M). Upper jaw sheath of *Rhinodermadarwinii* (C).The specimens of both species are in Stage 32.

The chondocranium of *Rhinoderma rufum* (Stage 32) (Fig. 1D,E) is 1.3 times longer than wide. The suprarostral cartilage is poorly chondrified and consists of a single plate with dorsal and ventral notches scarcely visible. It is anteriorly convex and articulates with the trabecular horns; each ala possesses a well-developed processus posterior dorsalis. The proximal and distal ends of the cornua trabeculae are thin, and uniform in width; their length is about 19% of the length of the chondrocranium. The anterior parts of the cornua trabeculae are curved; the cornua diverge from one another anterolaterally to form a V shape. Posterioly, the cornua converge in the trabecular planum and ethmoidal planum. The thin orbital cartilages formed the walls of the cranium and their caudal edges reach the anterior margins of the otic capsules. The orbital cartilages have two foramina (not figured) the foramen opticum and the foramen oculomotorius; the former is 2.5 times longer than the latter. The prootic foramen is large, positioned dorsally and laterally visible. These connections formed the dorsal margin of the foramen prooticum. The oval frontoparietal fenestra is about 53% of the total length of the chondrocranium. It is limited anteriorly by the ethmoidal planum, laterally by the taenia tecta marginalis and posteriorly by the tectum synoticum. The occipital arch is thin and fused to the otic capsule, the fenestra ovalis (0.26 times the total length of the chondrocranium) is well defined. The occipital condyles are prominent. The otic capsules, 1.1 times longer than wide and 0.25 times the total length of the chondrocranoium, are posteromedially connected to the occipital condyles via the tectum synoticum, forming the dorsal margin of the foramen magnum. Dorsally, each occipital arch is continuous with the tectum synoticum and the planum basale and centrally formed the foramen magnum and the foramen jugulare.

The palatoquadrate is joined to the skull anteriorly by the corner of the quadratocranialis and posteriorly by the processus ascendens to the pila antotica. The pars articularis is robust and articulated anteriorly with Meckel's cartilage. The processus articularis quadrati is wide, and its dorsal edge is bent medially. From the side, the processus muscularis quadrati is triangular with a rounded tip and does not reach the dorsal surface of the chondrocranium. Just below the process muscularis quadrati and centrally on the palatoquadrate, there is a triangular notch, the facies articularis quadrati, which serves as a point of articulation of the ceratohyal to palatoquadrate. Meckel's cartilage is shallowly in a sigmoid; it articulates with the posterior margin of the infrarostral cartilage, and its posterior margin is articulated broadly with the anterior portion of the palatoquadrate. The infrarostral cartilages, which provided support for the lower jaw, is curved and joined together by an intramandibular cupula.

The ceratohyalia are wide and bent slightly backward (Fig. 1F). The margin of the ceratohyal has two processes, the processus anterior hyalis and the processus anterolateralis hyalis. Both are triangular and bear a processus anterior hyalis that is slightly higher than the processus anterolateralis hyalis. The posterior margin of the ceratohyal has a well-developed processus posterior hyalis. The margin of the posterior hyalis slightly overlaps with the planum hypobranchial. The ceratohyalia are united medially with the pars reuniens. The pars reuniens and Copula II are chondrified, forming an elongated structure. The processus urobranchialis is small and rounded. Copula I is clear. The hypobranchial plates are wide and separated over their entire length. Posteriorly they do not diverge, but nonetheless are separate. The branchial baskets consisted of four well-chondrified ceratobranchials, which are bound to the corners centrally via the commissura terminalis. Ceratobranchials II–IV are attached to the hypobranchial plate. Ceratobranchial IV is wider than the others, and II–V have well-developed spicules.

A morphological comparison between the tadpoles of *Rhinoderma rufum* and *R. darwinii* is summarized in Table 1. The chondrocranial and hyobranchial skeletons of both species are similar; however, the suprarostral cartilages differ. In *R. darwinii*, there is a single plate with a triangular processus posterior dorsalis, obvious dorsal and ventral notches, and a deep ventral notch between the body and suprarostral alae. By contrast in *Rhinoderma rufum*, the single plate has scarcely visible dorsal and ventral notches, and a well-developed hardly visible and well developed processus posterior dorsalis. These differences are not thought to be associated with the different modes of feeding; instead, these seem to represent interespecific variation. Additional examples of the variation in the shape of the suprarostral cartilage have also been reported by Larson & de Sá (1998) and Vera-Candioti (2007). The morphological similarity observed provides generically diagnostic characters and suggests that the chondrocranium may be useful in the elucidation of taxonomic problems of congeneric species differing in their modes of feeding (e.g., *Cycloramphus*, see Verdade 2005).

Comparison between the internal oral structures of *Rhinoderma* species indicates that although *R. darwinii* has an endotrophic tadpole, the internal oral structures are the same as those of the exotrophic tadpole of *R. rufum* suggesting that these features are not associated with the endotrophy. However, the absence of labial teeth, supramarginal and inframarginal papillae, and the jaw sheaths without serrations (Fig. 2C), may be correlated with the endotrophy of *R. darwinii*.

The phylogenetic relationships of the frogs of the genus *Rhinoderma* are controversial (see Frost *et al*. 2006; Grant *et al*. 2006; Pyron and Wiens 2011). Thus, it is difficult to establish an appropriate phylogenetic framework within which to propose hypotheses of polarization of the larval characters. Despite these restrictions, a remarkable behavioural synapomorphy distinguishing the *Rhinoderma* linage may be considered the transportation of tadpoles in the vocal sacs of the males (Duellman 2007). Comparison of the tadpoles of the two *Rhinoderma* species reveals notable differences between them. For example, *R. rufum* possesses a generalized pond-type tadpole, whereas *R. darwinii* has significant reductions in some external morphological features (e.g., oral structures, tail, spiracle and pigmentation). These observations raise a question concerning the morphology of the tadpole of the common ancestor of both species. It seems more parsimonious to postulate the remodelling of previously existing structures than the generation of new ones. Given this principle I posit that the ancestral tadpole of two species of *Rhinoderma* had a generalized tadpole with characteristics to those of *R. rufum.*

TABLE 1. Morphological differences between the tadpoles of *Rhinoderma rufum* and *Rhinoderma darwinii*.[(1), Wassersug & Heyer 1988; (2), Lavilla 1987)].

Characters	*R. rufum*	*R. darwinii*
Total length (Stage 35)	28–31 mm	16–18 mm
Snout-vent lenth (Stage 46)	12–13 mm	8.5 mm
Upper and lower sheaths	well-developed, with serrations, pigmented	vestigial, nearly smooth edge (Fig. 2C), slightly pigmented
Tooth row formula	2(2)/3	0/0
Borders of the labial teeth	serrated	-
Submarginal papillae	present	absent
Spiracle	dextral, tubular, inner surface attached to the skin	absent
Proctodeal tube	tubular, as long as wide, posterior edge continuous with caudal fin	lobed, twice as long as wide, dorsal skin fused to the caudal fin
Tail length	two times the body length	1.2 times the body length
Maximum tail height	reaches the dorsal level of the body	does not reach the dorsal level of the body
Oral roof	12–14 postulations	absent (1)
Suprarostral cartilage	a single plate backward curved	a single plate with dorsal and ventral notches (2)
Otic capsule	1.1 times longer than wide	1.5 times longer than wide (2)

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