

Correspondence

The tadpole of *Alsodes* cf. *norae* (Anura: Alsodidae) with comments on the diagnosis of the genus *Alsodes*

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Manuscript received: 19 December 2014

Accepted: 12 May 2015 by ARNE SCHULZE

The Cordillera de Mahuidanche (about 55 km long and 20 km wide, maximum altitude 715 m a.s.l.) is a small segment of the Chilean Coastal Range situated between the mouths of the rivers Queule (39°01' S, 73°06' W, Cautín province) and Valdivia (39°52' S, 73°23' W, Valdivia province), southern Chile (Fig. 1). In the recent past, the largest portion of this mountain range used to be covered by temperate *Nothofagus* forests (*Drymis winteri*, *Laurelia phyllipiana*, *Weinmannia trichosperma*, *Dendrologotrichum dendroides*, and *Cladonia* spp.). However, today, their western slopes exhibit a high degree of human intervention (*Pinus* and *Eucalyptus* plantations, cattle pastures, and clearing of the native forest). On 25 May 2013, we captured a series of tadpoles in a stream (Llnehue, 39°35' S, 73°16' W, 400 m a.s.l.) that were identified as members of the genus *Alsodes* BELL based on the following traits: labial keratodont row formula (LKRF) 2(2)/3(1), oral disc intra-angular, rostral gap present, intramarginal, lateral and mental papillae present, and vent opening dextrally (LAVILLA 1988). In this area, we also captured adults of the following endemic species: *Eupsophus vertebralis* GRANDISON, 1961, *E. altor* NÚÑEZ, RABANAL & FORMAS, 2012 [both taxa have endotrophic tadpoles and the LKRF formula is 2(2)/2, FORMAS 1992, NÚÑEZ et al. 2012], and *Insuetophrynus acarpicus* BARRIO, 1970 [LKRF 2(2)/2(1–2), FORMAS et al. 1980]. However, we could not collect adult specimens of *A. norae* CUEVAS, 2008. We collected adult specimens of *A. norae* at the border of a humid and cold forest of *Nothofagus* in Cerro Oncol, (39°41' S, 73°18' W, type locality of *A. norae*), however. At the time of the capture (from June to September of 2012–2013; austral winter), the frogs were found sheltering under decomposing logs far from water. Since the distance between Cerro Oncol and Llnehue is only 5.7 km (beeline), the *Alsodes* tadpoles from Llnehue are temporarily named *Alsodes* cf. *norae*. Their identification should be confirmed by means of chromosomal stud-

ies (*A. norae* is the only known species of the genus to have 30 chromosomes, CUEVAS 2008) and sequences of DNA fragments. Specific identification via DNA barcoding was impossible, because the tadpoles were fixed in formalin (10%) right after capture and PCR was unsuccessful after six months in fixing solution. The presence of *A. cf. norae* tadpoles in this restricted area, the inability to capture larvae again (we explored the streams in the closer vicinity of Llnehue bimonthly during 2013 and 2014), and the extent of human intervention exerted on the stream and its surroundings are arguments adequate to justify the description of this tadpole.

Our aim in the present contribution is to provide information about the tadpole of *A. cf. norae*, including its external morphology, internal oral features, chondrocranium, and hyobranchial apparatus along with a review of the information available for these characteristics in tadpoles of the genus *Alsodes*.

A total of ten *Alsodes* cf. *norae* tadpoles (stages 31–37, GOSNER 1960) were collected from a stream (Llnehue, see above) that runs from east to west along a forest with a high degree of human-inflicted degradation. Tadpoles were preserved in 10% formaldehyde and deposited in the collection of the Laboratory of Zoology, Universidad Austral de Chile (IZUA–3528). Our morphological terminology and keratodont formula follow ALTIG & MCDIARMID (1999a), the terminology of oral disc follows ALTIG (1970), and our terminology of internal oral features follows WASSERSUG (1976). The chondrocranial structures are referred to according to LARSON & DE SÁ (1998). The measured parameters and their terminology follow those of ALTIG & MCDIARMID (1999a). Eight measurements were taken from two tadpoles (stage 31) with a dial calliper (to the nearest 0.1 mm) following ALTIG & MCDIARMID (1999a): total length, body length, tail length, maximum tail height, tail muscle height, tail muscle width, internarial distance, and

interorbital distance. The chondrocranium of *A. cf. norae* was studied in three cleared and stained tadpoles (stage 32) following the protocol of SONG & PARENTI (1995) in which cartilage is stained with Alcian blue. The rostrodonts, keratodonts, and external oral features of three tadpoles (stage 32) of *A. cf. norae* were studied. The buccopharyngeal features of the same tadpoles were also studied under a scanning electron microscope (SEM; Leo-420).

Alsodes cf. norae tadpoles ($n = 2$, stage 37) have an elliptical, slightly depressed and elongated body. The tail is 2.2 times the body length (Figs 2A–C). The head is gently rounded. The nostrils are ovoid, dorsally positioned, and located in a depression of the body wall with a continual dark brown marginal rim; they project upward and are closer to the snout than to the eye. The interorbital distance is 148.5% of the internarial distance. The eyes are dorso-laterally located, and their diameters correspond to 0.54 times the internarial distance. The sinistral spiracle is short, approximately 1.2 times the eye diameter, directed posterodorsally, located right below the median line, and its inner wall is attached to the body; the aperture is oval and its diameter corresponds to 36% of the eye diameter. The vent tube is short, dextral, and its aperture corresponds to 0.5 times the internarial distance. At its distal end, the right wall of the tube is folded towards the right side of the body and continues posteriorly along the margin of the ventral fin. The dorsal fin does not extend beyond the body. The

end of the tail is rounded, and the maximum width of the dorsal fin does not exceed the height of the body. In life, the tadpoles have a light brown dorsal face with clusters of melanophores; the ventral face is transparent, the digestive tract is visible, and tiny melanophores can be discerned. The coloration in formalin (10%) is the same as in live tadpoles. The oral disc is followed by a transparent area with minute melanophores. The dorsal fin and myotomes show irregular patches of melanophores; the ventral fin is transparent. The measurements of the two tadpoles (stage 31) described here are as follows (in mm): total length 34.0–35.1, body length 13.1–13.2, tail length 16.8–17.2, maximum body height 7.0–7.2, maximum tail height 6.0–6.2, tail muscle height 2.7–2.8, interorbital distance 3.2–3.9, internarial distance 1.9–2.0, and oral disc width 3.8–3.9.

The oral disc of the tadpoles ($n = 3$, stage 32) is antero-ventrally located, and laterally emarginated (Fig. 3A). The marginal papillae are conical, arranged in a single row and laterally located. There is a wide anterior gap, as long as the A-1. There are 15 to 17 papillae on each side of upper labium and the lower labium is furnished with a continuous row of conical marginal papillae (42–47). There are groups of submarginal papillae at the corners of the upper (3–7) and lower (6–8) lips. There is a row of mental papillae (7–9) close to the marginal papillae. The jaw sheaths are keratinised, dark-brown, and their edges are clearly serrated. The upper jaw sheath is five times longer than

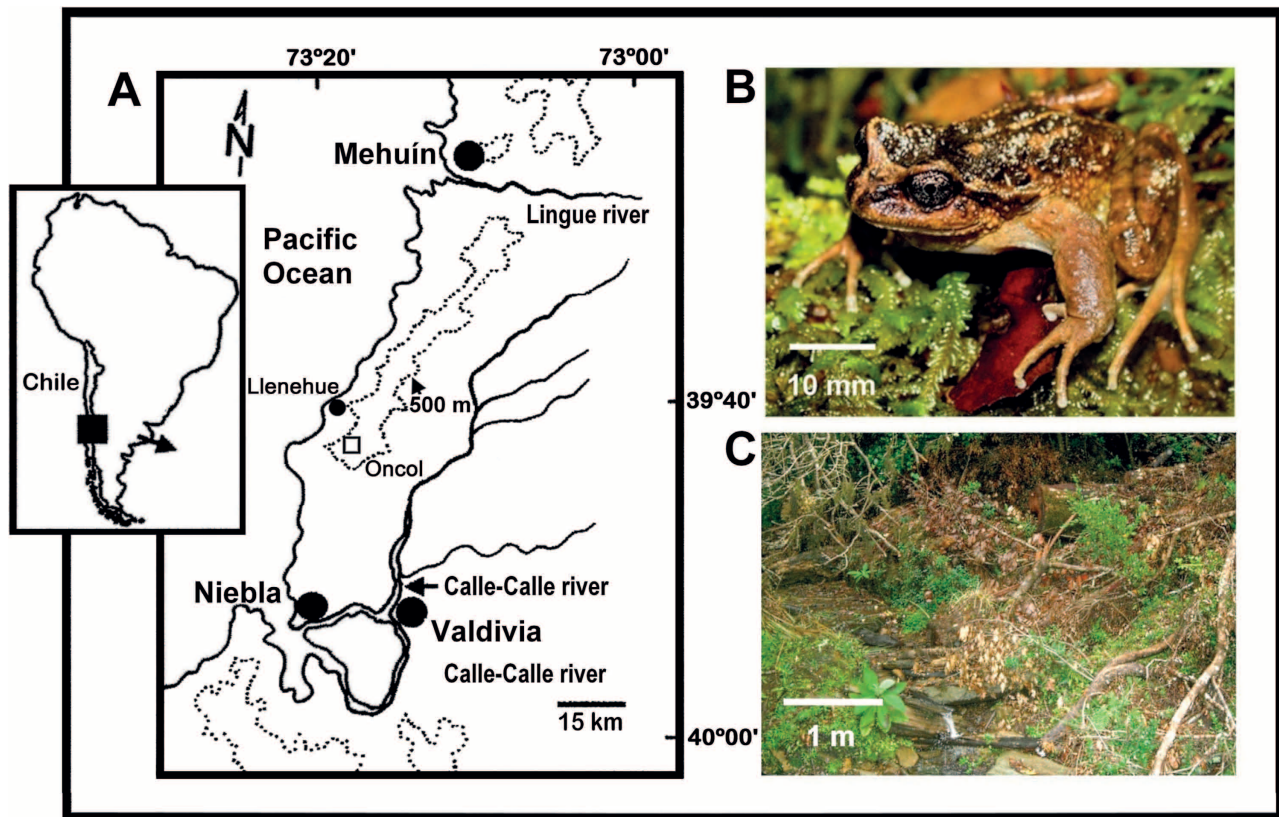


Figure 1. Distribution map of *Alsodes norae* (A), adult male of *A. norae* (B), habitat of *A. cf. norae* tadpoles (C).

wide. The rostradonts are triangular; their distal tips are pointed and slightly curved toward the mouth (Figs 2B, C). They are distributed in a palisade at a density of 5 teeth per 100 μm . The total tooth length is approximately 40–42 μm and their width is 19–21 μm . The keratodonts form continuous, homogeneous rows with a LKRF of $2(2)/3(1)$. The number of keratodonts in a row is approximately 10–11 per 100 μm . Each keratodont comprises a base, a neck, and a paddle with 12–14 short, subequal serrations (Fig. 3F). The heads of the keratodonts are incurved; their total length is 28–30 μm , and the maximum paddle width is 14–15 μm . The buccopharyngeal cavity floor of the tadpoles ($n = 2$, stage 32) is ovoid and about 9% wider than long (Fig. 3D). The tongue anlage is rounded but scarcely prominent. There are four long lingual papillae, and their height repre-

sents about 39% of the maximum width of the tongue. The buccal floor arena (BFA) is U-shaped and delimited by a twin row of about 20–26 pointed papillae, with the longest being located on the lateral edges; the posterior surface has about 28–30 pustulations. The buccal pockets are orientated at about 45 degrees towards the medial line and two times longer than wide. The velar border is slightly undulated and there are 5–7 lateral protections near the glottis. The buccal roof arena (BRA) is U-shaped, 1.5 times longer than wide and surrounded by about 22–24 elongated papillae (Fig. 3E). The choanae are approximately 3.8 times longer than wide and orientated at about 45 degrees relative to the medial line. The internarial distance is 0.5 times the length of the choanae. The postnarial arena sports a group of 7–9 median-sized papillae. The median ridge is

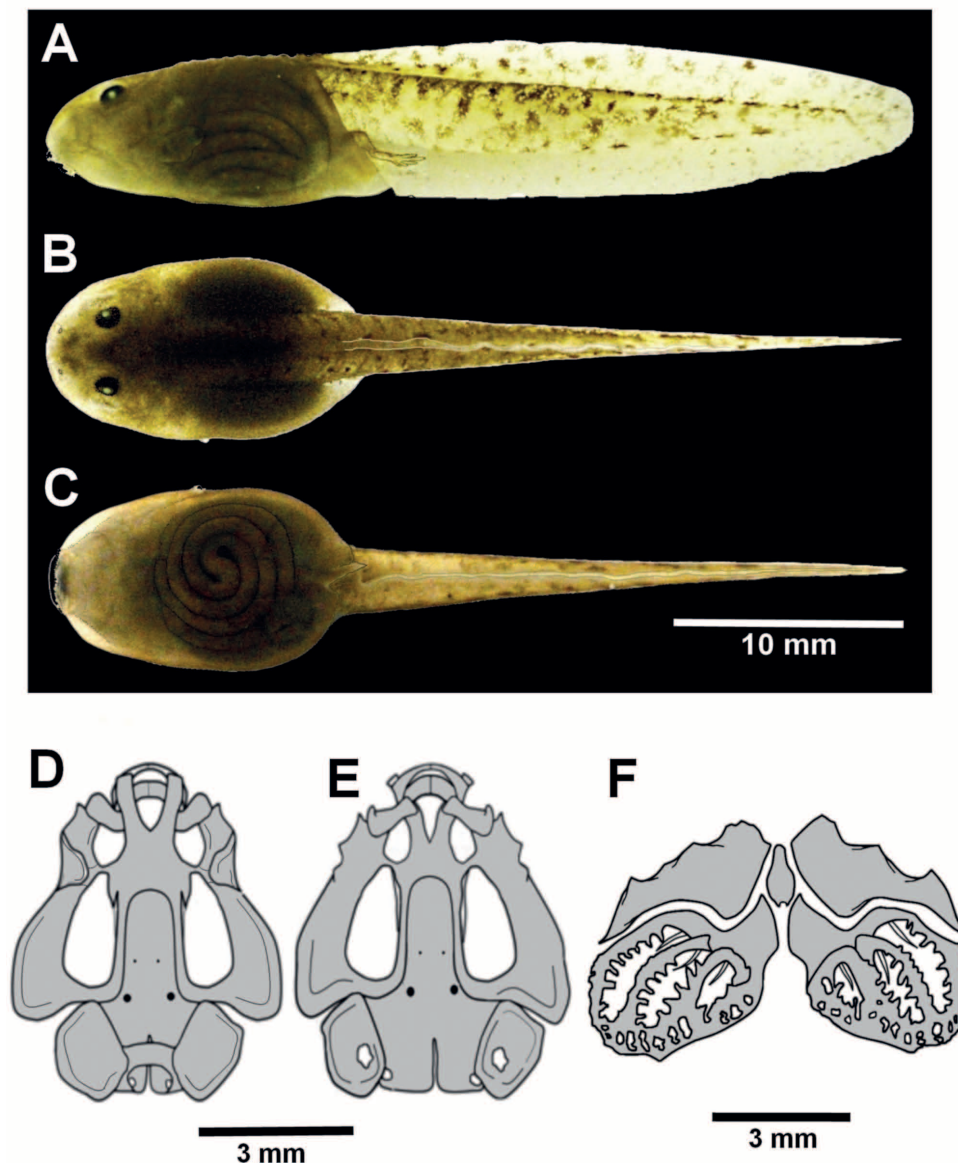


Figure 2. Tadpole of *Alsodes cf. norae* (stage 37): lateral (A), dorsal (B), ventral (C) views of a preserved specimen; chondrocranium in dorsal (D) and ventral (E) views, and ventral view of the hyobranchial apparatus (F).

triangular and four times wider than high; the anterior margin has four papillae per side. The buccopharyngeal roof arena presents about 25 pustulations. Secretory pits were not observed.

The cranium of *Alsodes cf. norae* tadpoles ($n = 3$, stage 32) (Figs 2D, E) is 1.2 times longer than wide (measured from the anterior tip of the suprarostrals to the posterior border of the otic capsule) and completely cartilaginous. The suprarostal cartilages support the upper horny beak and consist of two clearly separated elements, a central corpus, and a lateral ala. The partes corporeae are subrectangular in shape and the lateral ala bear a pointed processus posterior dorsalis. The cornua trabeculae comprise approximately 17% of the total chondrocranial length; they are approximately 2.5 times longer than wide, diverging from each other, and forming a wide V-shaped structure in dorsal view. The cornua are uniform in width and curved ventrally to articulate the suprarostrals. The posterior confluence of the cornua trabeculae is continuous with the planum trabeculae anticum, which, in turn continues with the planum ethmoidale that forms the anterior wall of the braincase. The frontoparietal fontanel is rectangular and represents approximately 42% of the total chondrocranium length. It is bordered laterally by the tecti marginales, posteriorly by the tectum synoticum, and anteriorly by the planum ethmoidale. The lateral walls of the braincase are formed by the orbital cartilages, which connect posterodorsally to the otic capsules. The posterior ends of the taenia tecti marginales are bent laterally. Two oval foramina are visible

in the posteroventral portion of the orbital cartilage. The foramen opticum is smaller than the foramen oculomotorium. The rhomboid otic capsules represent approximately 28% of the total chondrocranial length. They are fused to both the posterior floor and the braincase, and connected posteromedially via the tectum synoticum, forming the dorsal margin of the foramen magnum. The fenestra ovalis is located ventrolaterally on each otic capsule.

The Meckel's cartilage and cartilage labialis inferior form the lower jaw. The Meckel's cartilage is sigmoid, short, placed ventrally to the cornua trabecularum, and posteriorly to the lateral ala of the cartilage labialis superior; it is orientated perpendicularly to the chondrocranial medial axis. The processus retro articularis is well developed. The anterior part of the Meckel's cartilage articulates with the posterior margin of the cartilage labialis superior and posteriorly with the pars articularis quadrati. The cartilages labialis inferior are rectangular in shape and clearly delimited at the midline. The palatoquadrate is orientated approximately parallel to the medial line. Its anterior end is attached to the braincase via the commissura quadrato cranialis and its posterior end via the processus ascendens. The palatoquadrate bears two processes, the processus muscularis quadrati and the pars articularis quadratis. The processus muscularis quadrati is triangular and rounded and does not exceed the level of the suprarostal cartilage; its posterior end is inclined towards the medial plane and its dorsal border is inclined medially and attached to the braincase via the commissura quadrato orbitalis. Ventrally,

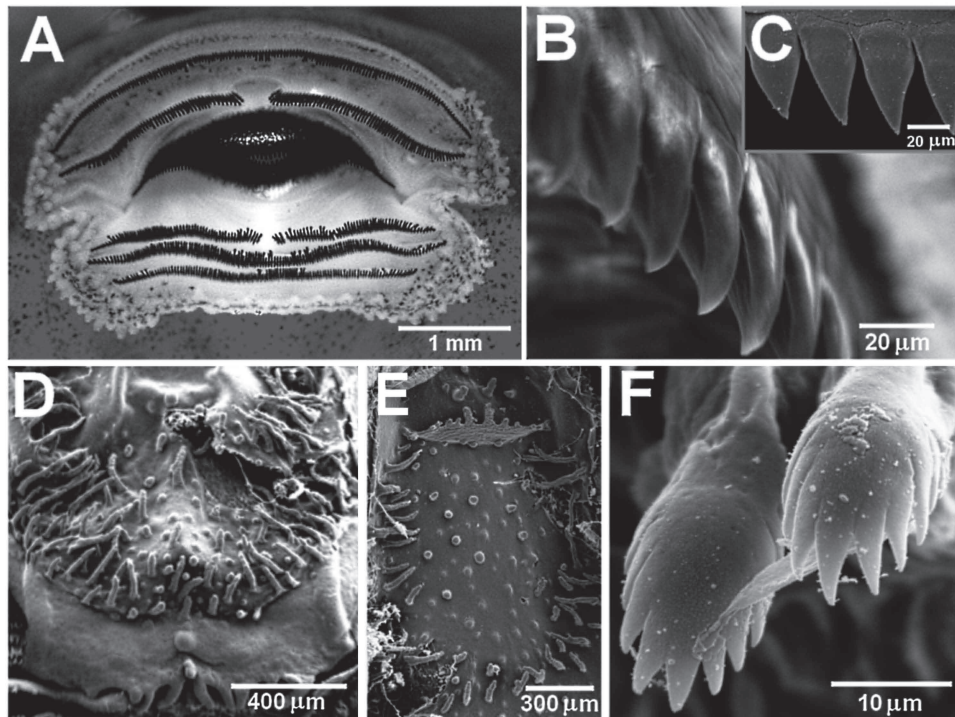


Figure 3. Morphology of the external and internal oral structures of the tadpole of *Alsodes cf. norae* (stage 32): oral disc (A), rostrants of the upper jaw (B, C), oral floor and velum (D); roof of the oral cavity: median ridge, pustulations, lateral papillae (E), and keratodonts of the A1-row (F).

the palatoquadrate bears a well-developed triangular hyoquadrate processus. The ceratohyalia (Fig. 2F) are wide and flat cartilages. Each ceratohyal bears two anterior processes of similar size: the processus anterior hyalis and the processus anterolateralis hyalis. Both are almost triangular in shape. The processus posterior hyalis is well developed. Medially the ceratohyalia are connected with the pars reuniens. The hypobranchial plates are wide, flat sheets of cartilage, which serve as the point of attachment for the ceratobranchials. The hypobranchial plates are separated medially along their length; they diverge posteriorly. The planum hypo branchiale shows a U-shaped posterior margin. The triangular-shaped processus anterior branchialis is present at ceratobranchial I. The ceratobranchialia are joined distally by a terminal commissure. Spicules I–III are present and associated with the cerato branchialia I–III.

The genus *Alsodes* comprises 18 species (FROST 2014) of which the tadpoles of 12 (67%) have been described (PILLADO et al. 2000), and added here is the larva of *A. cf. norae*. The tadpoles of *A. kaweshkari* FORMAS, CUEVAS & NUÑEZ, *A. neuquensis* CEI, *A. valdiviensis* FORMAS, CUEVAS & BRIEVA, and *A. vittatus* (PHILIPPI) are as yet unknown, and those previously assigned to *A. monticola* BELL are not included here because the taxonomic status of this species has not been adequately elucidated (see VELOSO et al. 1981 and FORMAS et al. 2008).

Larval characters have been successfully used to improve the generic diagnosis of various anurans (LARSON & DE SÁ 1998). LAVILLA (1988) and ALTIG & MCDIARMID (1999b) diagnosed the genus *Alsodes* based on external larval characters: exotrophic, lotic, and benthic; oral disc small; lateral margins intra-angular; rostral gap present; intramarginal lateral papillae present in intra- and supra-angular regions; intramarginal mental papillae present; rostradonts wider than deep; keratodont formula 2(2)/3(1); level of nostril aperture not raised and equidistant between eye and snout; dorsal fin originates distal to dorsal tail-body junction; tail tip broadly rounded to pointed; colour pattern uniformly dark with a few spots on tail. With the purpose of expanding the generic diagnosis we have included previously undescribed internal morphological characters (chondrocranium and oral internal larval features) of the following species: *A. cf. norae* plus *A. barrioi* VELOSO, DÍAZ, ITURRA-CONSTANT & PENNA, *A. gargola* GALLARDO, *A. nodosus* (DUMÉRIL & BIBRON), *A. vanzolinii* (DONOSO-BARROS), and *A. verrucosus* (PHILIPPI). In accordance with the internal and external morphology of tadpoles and adult features (osteology and secondary sex-indicative characters of males, LYNCH 1978), the genus *Alsodes* can now be diagnosed as follows: humeral crests well-developed; adult males with thorny structures on the fingers and rounded spiny patches on the chest; tadpoles type IV (ORTON 1953); exotrophic, lentic, and benthic; medium-sized (30.2–41.3 mm); body ovoid in lateral view; fins low and not exceeding body height; tail tip rounded; dorsal fin originating at the body-tail junction; vent tube short, dextral, the right wall displaced laterally; oral disc ventral, laterally emarginated, LKRF 2(2)/3(1); rostradonts triangu-

lar, distal tips pointed, slightly curved (*A. gargola*, *A. vanzolinii* [CUEVAS, personal observation] and *A. cf. norae*); keratodonts incurved with head, neck, and a paddle with 12–14 short serrations (*A. cf. norae*, *A. gargola*, and *A. nodosus*); rostral gap present; intramarginal lateral papillae in infra- and supra-angular areas; intramarginal mental papillae present; numerous BFA/BRA papillae and pustulations (approximately 35–45/28–37); hand-like lateral ridge papillae (4–5 branches), and a ventral velum with four projections; suparostral cartilages separated and rectangular-shaped; cornua trabeculae uniformly wide and diverging anteriorly (*A. barrioi*, *A. gargola*, *A. vanzolinii*, *A. verrucosus*, and *A. cf. norae*); ceratohyalia wide and their posterior margins with two well-developed posterior processes; branchial baskets with four ceratobranchials that are distally continuous via commissurae terminali.

DÍAZ & VALENCIA (1985) reported that *Alsodes tumultuosus*, *A. nodosus*, and *A. barrioi* larvae showed a remarkable homogeneity in their morphological external characteristics. This homogeneity was subsequently also described for the tadpoles of *A. australis*, *A. barrioi*, *A. gargola*, *A. montanus*, *A. nodosus*, *A. pehuenche*, *A. tumultuosus*, *A. vanzolinii*, *A. verrucosus* (see PILLADO et al. 2000), *A. hugoi* (CUEVAS & FORMAS 2001), *A. igneus* (CUEVAS & FORMAS 2005), *A. coppingeri* (FORMAS et al. 2008), and *A. cf. norae* (this paper). In spite of these morphological external similarities, the tadpoles of some of these species have distinctive characteristics. For example, *A. australis* has two dark spots behind the posterior borders of the nostrils, *A. hugoi* shows two whitish ventral spots near the proctodeal tube, and *A. barrioi* has two yellowish irregular spots on both sides of the vertebral line. *Alsodes nodosus* have a slightly pointed tail tip, in *A. coppingeri* and *A. gargola* the distal extreme of the spiracle does not adhere to the body wall, and in *A. verrucosus* and *A. vanzolinii* the lateral line system is prominent. Just like these species, the tadpole of *A. cf. norae* has distinctive characteristics that facilitate diagnosing it by the following combination of characters: (1) medium size (34.1–41.3 mm); (2) ventral fin transparent and devoid of melanophores; (3) dorsal fin and musculature with isolated groups of melanophores; (4) body transparent and internal organs visible; (5) lateral line system indistinct; (6) tail with yellowish coloration; (7) dorsum light brown. A comprehensive revision of *Alsodes* tadpoles is still lacking, but the new data presented here are useful for future systematic and phylogenetic studies of the genus *Alsodes*.

Acknowledgements

The authors would like to say special thanks to RICARDO SILVA who provided technical assistance with electronic scanning microscopy, and SOLEDAD PUENTE for assistance in the field.

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