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Description of the tadpole of *Alsodes neuquensis* **Cei, 1976** and comparison with the sibling species *A. gargola* **Gallardo, 1970** **(Amphibia, Anura, Alsodidae)**

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This paper describes the external and buccal cavity morphology, chondrocranium, hyobranchial apparatus and cranial muscles of *Alsodes neuquensis* in comparison with the sibling species *A. gargola*. The tadpole of *A. neuquensis* presents a shorter and higher tail, and a relatively smaller oral disc than *A. gargola*. The external larval morphology of *Alsodes* may be characterised as follows: (i) tooth row formula 2(2)/3(1), (ii) single row of marginal papillae with a wide rostral gap, (iii) single row of submarginal papillae in the mental region, (iv) both supra- and infraangular submarginal papillae present. The buccal features do not show marked intrageneric differences. The anterior and/or posterior pair of infralabial papillae may be simple or forked. All known tadpoles present one prenarial papilla accompanied or not by few pustules, and the lateral ridges of floor can be three to penta-armed. The chondrocranial features shared by the species of the genus *Alsodes* are (i) tetrapartite cartilage suprarostralis, (ii) chondrified adrostral tissue mass, (iii) processus anterolateralis of the larval crista parotica well developed or larval processus oticus, and (iv) large processus pseudopterygoideus that may be either completely fused to the basi cranii (*A. vanzolinii*, *A. verrucosus* and some *A. neuquensis*) or fused only at the tip (*A. barrioi*, *A. gargola* and some *A. neuquensis*). Larval cranial muscles are for the first time described for the genus *Alsodes*. They do not show differences between both species studied herein.

Key words: buccal cavity morphology, cranial muscles, cranial skeleton, external morphology, larvae

INTRODUCTION

Alsodes Bell and *Eupsophus* Fitzinger are related frog genera endemic to the Patagonian region. A recent phylogenetic study suggested that both genera evolved from western regions of the Andes and radiated with multiple colonisation events towards the east, in areas currently within Argentina (Blotto et al., 2013). *Alsodes neuquensis* Cei, 1976 inhabits small shallows creeks and springs at the type locality Pampa de Lonco Luan and nearby areas such as Primeros Pinos, in Neuquén province, Argentina (Cei, 1976; 1987). *Alsodes neuquensis* was traditionally treated as a subspecies of *A. gargola* Gallardo, 1970 on the basis of a few and quite variable adult characters (Cei, 1976). Their status was recently addressed in the phylogenetic work of Blotto et al. (2013), in which the Bayesian analysis recovered them as sister taxa but not under parsimony.

Like most anuran genera, the knowledge of larval morphology in *Alsodes* remains fragmentary for most species. It is known for *A. barrioi* (Veloso et al., 1981; Díaz

& Valencia, 1985), *A. coppingeri* (Formas et al., 2008), *A. gargola* (Formas et al., 1997 as *A. australis,* see Blotto et al., 2013; Pillado et al., 2000), *A. hugoi* (Cuevas & Formas, 2001), *A. igneus* (Cuevas & Formas, 2005), *A. montanus* (Busse, 1980; Díaz & Valencia, 1985), *A. nodosus* (Cei, 1962; Formas, 1975; Díaz & Valencia, 1985), *A. pehuenche* (described as *Telmatobius montanus*; Cei & Roig, 1965; see Cei, 1976), *A. tumultuosus* (Díaz & Valencia, 1985), *A. valdiviensis* (Formas, 1975 and Díaz & Valencia, 1985 as *A. monticola,* see Formas & Brieva, 2004), *A. vanzolinii*, and *A. verrucosus* (Formas & Brieva, 2004). The external description of *A. verrucosus* was reported by Díaz & Nuñez (1988) from specimens collected at Wellington Island, but the species assignation was put in doubt by Formas & Brieva (2004) because the referred locality does not fit with the species distributional range. Lavilla & Scrocchi (1986) performed a morphometric analysis of tadpoles of *A. neuquensis* and Lavilla (1988) included larval specimens of *A. neuquensis* (in both cases as *A. gargola neuquensis*) in his diagnosis of *Alsodes*. On the other hand, internal features of the genus were rarely

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studied. The buccal cavity is known for *A. barrioi*, *A. gargola*, *A. neuquensis*, *A. vanzolinii* and *A. verrucosus*, whereas the chondrocranium was described in these same species except *A. gargola* and *A. neuquensis* (Wassersug & Heyer, 1988; Lavilla, 1992; Echeverría et al., 2001; Formas & Brieva, 2004). The larval cranial muscles were not described for the genus.

The purpose of the present work is to describe the external morphology, buccal cavity, chondrocranium, hyobranchial apparatus and its related muscles for the tadpole of *A. neuquensis*. In addition, we will compare the same set of characters for the larvae of *A. gargola* in order to provide characters for distinguishing both species. The results are compared and discussed with the available information on larval morphology of other species of *Alsodes* and with those of the sister genus *Eupsophus*.

MATERIALS AND METHODS

Tadpoles of *Alsodes neuquensis* and *A. gargola* were collected at the type locality of each species and nearby sites (see Appendix), euthanised with benzocaine and fixed in 10% formalin and housed at the Herpetological Collection of the Instituto de Diversidad y Evolución Austral (CNP, Chubut, Argentina), Museo Nacional de Ciencias Naturales "Bernardino Rivadavia" (MACN, Buenos Aires, Argentina) and Museo de La Plata (MLP, La Plata, Argentina). Ten tadpoles of *A. neuquensis* between Stages 28 and 33 of Gosner (1960), and six tadpoles of *A. gargola* between Stages 29–37 were used to obtain morphometric external measurements and for qualitative comparisons of external larval morphology. Four tadpoles of each species between Stages 29 and 35 were double

stained following the technique of Taylor & Van Dyke (1985) to study the chondrocranium and hyobranchial apparatus, interrupting the process before clearing in order to study the cranial muscles before continuing the process to reach complete clearing (see Appendix). Three specimens of *A. neuquensis* (Stages 28, 30 and 31) and one of *A. gargola* (Stage 26) were prepared for scanning electronic microscope (SEM) examination of the buccal cavity, oral disc, and external features following the protocol of Alcalde & Blotto (2006, Appendix). External and chondrocranial measurements were taken to the nearest 0.1 mm under a Zeiss Stemi SV11 stereomicroscope with measuring equipment. Terminology follows d'Heursel & de Sá (1999) and Haas (1995) for the chondrocranium and hyobranchial apparatus, Alcalde & Rosset (2003) for cranial measurements, Haas (2001) for mandibular muscles, Haas (2003) and Haas & Richards (1998) for hyobranchial muscles, Schlosser & Roth (1995) for innervation, Wassersug (1980) for buccal cavity features, Altig & McDiarmid (1999) and Van Dijk (1966) for external morphology and oral disc morphology, and Altig & Johnston (1989) for tadpole ecomorphological types.

We selected 22 external morphology measurements: total length (TL), body length (BL), tail length (TaL), maximum tail height (MTH), tail muscle height (TMH), tail muscle width (TMW), internarial distance (IND, measured between the internal edges of narial apertures), interorbital distance (IOD, measured between the external edges of pupils, all following Altig & McDiarmid, 1999); body maximum width (BMW), body width at nostrils (BWN), body width at eye level (BWE), body maximum height (BMH), rostro-spiracular distance (RSD, measured horizontally from the tip of the snout to the posterior edge of the spiracular tube), fronto-nasal distance (FN, from

Fig. 1. External morphology of the tadpole of *Alsodes neuquensis* (MACN 46544) at Stage 33 from Primeros Pinos (Neuquén, Argentina). (A) Lateral, (B) ventral, and (C) dorsal views of the larva, (D) oral disc, (E) scanning electronic microscope photographs show detail of labial teeth, and (F) the white spots of the body representing glandular zones. Scale bars: A-C=10mm; D=2mm; E=20 μm; F=50 μm). The arrows show the white spots representing glandular zones (see F for detail).

Table 1. Measurements (mm) for ten *Alsodes neuquensis* and six *A. gargola* tadpoles (see details in Appendix). Mean, standard deviation, and range given in parentheses.

| Character	*A. neuquensis*	*A. gargola*
Total length (TL)	48.60±5.97 (40.13–57.83)	55.53±4.45 (48.84–62.00)
Body length (BL)	19.17±2.09 (15.71–22.49)	20.70±1.44 (18.15–22.57)
Tail length (TaL)	29.43±3.92 (24.42–35.34)	34.83±3.17 (30.69–39.43)
Maximum tail height (MTH)	9.42±1.43 (7.41–11.44)	9.64±0.60 (8.99–10.29)
Internarial distance (IND)	4.42±0.68 (3.67–6.03)	4.31±0.35 (3.62–4.57)
Interorbital distance (IOD)	5.48±0.74 (4.45–6.63)	5.68±0.35 (5.11–6.20)
Tail muscle height (TMH)	4.15±0.65 (3.29–5.31)	4.70±0.53 (3.99–5.52)
Tail muscle width (TMW)	3.25±0.62 (2.54–4.12)	4.00±0.47 (3.51–4.81)
Body maximum width (BMW)	11.28±1.46 (8.01–13.17)	11.93±0.69 (11.04–13.05)
Body width at nostril level (BWN)	8.59±1.01 (6.93–10.12)	9.22±0.96 (7.33–10.07)
Body width at eye level (BWE)	10.68±1.34 (8.40–13.13)	10.85±1.05 (8.72–11.52)
Body maximum height (BMH)	9.35±1.16 (6.93–10.88)	10.56±0.50 (10.04–11.22)
Rostroespiracular distance (RSD)	11.61±1.41 (9.57–13.85)	12.97±0.77 (11.97–13.94)
Fronto-nasal distance (FN)	2.32±0.41 (1.60–3.03)	2.66±0.20 (2.42–2.95)
Eye-nostril distance (END)	2.19±0.22 (1.91–2.53)	2.36±0.26 (1.89–2.66)
Nostril major axis (N)	0.45±0.08 (0.35–0.56)	0.51±0.07 (0.43–0.64)
Eye diameter (E)	1.20±0.15 (1.04–1.55)	1.32±0.21 (0.97–1.56)
Interocular distance (IO)	4.37±0.40 (3.88–5.09)	3.87±0.62 (3.27–5.09)
Oral disc with (OD)	5.22±0.41 (4.63–5.97)	6.44±0.43 (5.78–6.91)
Dorsal gap length (DG)	2.89±0.21 (2.59–3.25)	3.70±0.42 (3.12–4.27)
Dorsal fin height (DFH)	2.74±0.69 (1.83–3.91)	2.71±0.53 (1.94–3.20)
Ventral fin height (VFH)	2.91±0.55 (2.12–3.78)	2.84±0.35 (2.34–3.32)

the tip of the snout to the anterior edge of nostrils), eye-nostril distance (END, from the posterior edge of nares to the anterior edge of eyes), nostril major axis (N), eye diameter (E), intraocular distance (IO, distance between interior edges of eyes), oral disc width (OD), and dorsal gap length (DG, all following from Lavilla & Scrocchi, 1986); dorsal fin height (DFH) and ventral fin height (VFH), both measured at a position coincident with MTH (according to Kolenc et al., 2009). We expressed most measures of *A. neuquensis* as proportions with their corresponding mean and standard deviation. In order to simplify comparisons, the main body of the tadpole description is based on *A. neuquensis*, highlighting features and measures of *A. gargola* in parentheses only when these species differ.

RESULTS

External morphology

Alsodes neuquensis has type IV, exotrophic, lentic and benthic larvae (Fig. 1), total length 48.6±5.97 mm, body ovoid in lateral view, slightly depressed (BMH/BMW=0.83±0.03), body length about 40% of total length (37% in *A. gargola*), body shape ovoid in dorsal view, the maximum width is placed at the posterior portion of the head or at the abdominal region (BMW=11.28±1.46). Snout rounded in dorsal and lateral profile. Eyes relatively small (E=1.20±0.15), diameter represents 11 % of body width at eye level, dorsally located and smaller than interorbital distance (IOD/E=4.58±0.41). Upper elygium (*sensu* Kruger et al., 2013) was identified in the 60%

of specimens whereas the remainder 20% have both upper and lower elygium; the remaining 20% lack both (upper elygium is present in 90% of the studied larvae of *A. gargola*). Interorbital distance represents 50% of body width at eye level (IOD/BWE=0.51±0.04). Nostrils circular, positioned dorsally, nostril aperture orients almost laterally; nostril diameter represent 5% of body width at nostril level (N/BWN=0.05±0.01). Fronto-nasal distance slightly larger than eye-nostril distance (FN/END=1.07±0.19). Internarial distance smaller than interorbital distance (IND/IOD=0.82±0.19). Spiracle sinistral, inner wall fused to body wall except for its distal end which folds to delimit spiracle opening. Spiracular tube caudally projected, slightly curved toward the dorsum, spiracle opening rounded with the same diameter that the tube and visible in dorsal view (Fig. 1A). Rostro-spiracular distance represents 58–65% of body length. Tail length about 60% of total length (TaL/TL= 0.60±0.01) (slightly longer in *A. gargola*, TaL/TL=0.63±0.01), tail axis straight and tail tip rounded. Caudal musculature width narrower than caudal musculature height at the beginning of the tail (TMW/TMH=0.78±0.06), not reaching tail tip. The maximum tail height occurs in its middle third and both fins are almost equal to maximum body height (MTH/BMH=1.01±0.09) (MTH/BMH=0.91±0.03; slightly lower in *A. gargola*). Both fins have straight margins, with the ventral fin being a bit taller than the dorsal (DFH/VFH=0.93±0.11). The trapezoidal-shape vent tube has a wide dextral opening. All measurements are shown in Table 1.

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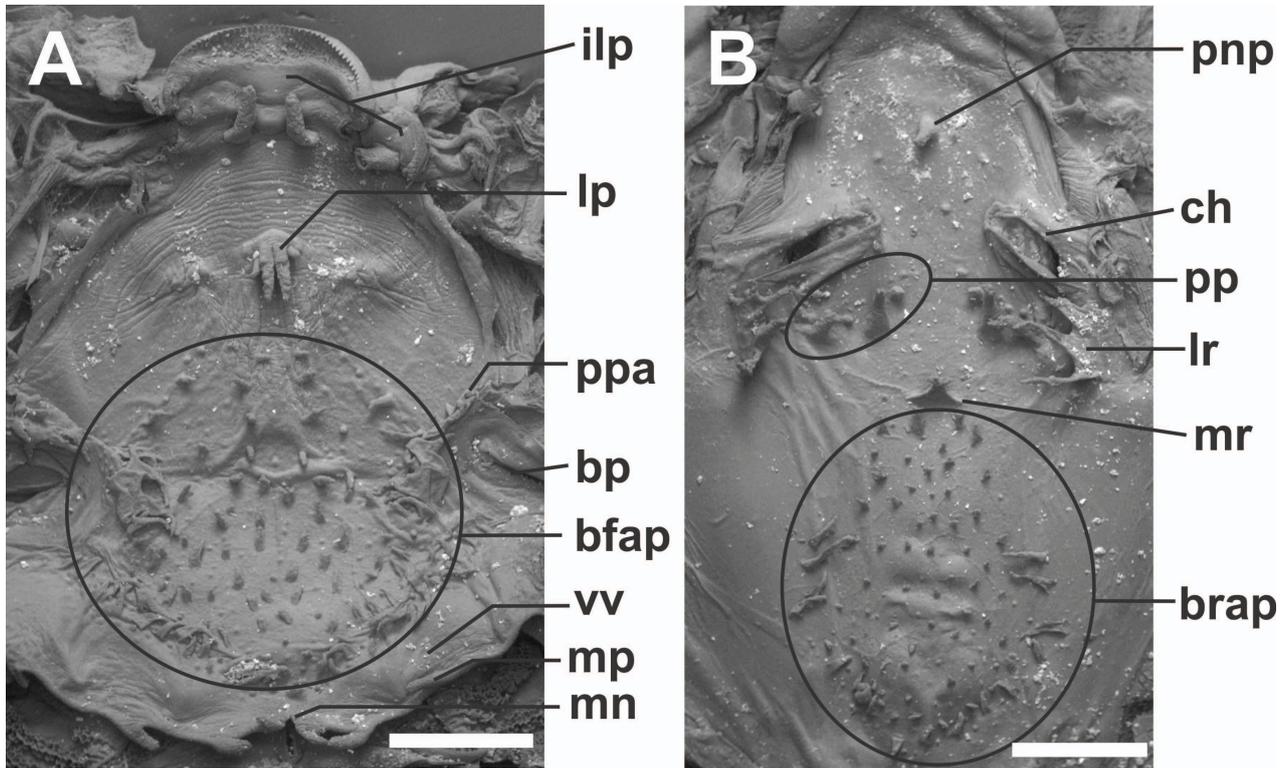
Fig. 2. Scanning electronic microscope photographs of the buccal cavity of *Alsodes neuquensis* at Stage 28 (MACN 38629) from Primeros Pinos, Neuquén, Argentina. (A) Buccal floor – bp: buccal pocket, bfap: buccal floor arena papillae, ilp: infralabial papillae, lp: lingual papillae, ppa: prepocket papillae, mn: median notch, mp: marginal projection, vv: ventral velum; (B) buccal roof – brap: buccal roof arena papillae, ch: choana, lr: lateral ridge, mr: median ridge, pnp: prenarial papillae, pp: postnarial papillae. Scale bars=1 mm.

Oral disc subterminal, ventrally positioned, not visible dorsally, and emarginated. Width of disc about 0.5 times of maximum body width (OD/BMW=0.47±0.09) (OD/BMW=0.54±0.03 in *A. gargola*). There is a single row of marginal papillae with medium to large dorsal gap (DG/OD=0.55±0.04), ventral gap absent. Most marginal papillae are simple, small, and longer than wide; but some specimens present few forked papillae scattered within the mental row of marginal papillae. Both supra and infra-angular regions present few submarginal long conical papillae (<5). A row of submarginal long and conical papillae, of same length as the P3 tooth row, is also present in the mental area. The curved, flattened and long-headed keratodonts bear 14–20 marginal cusps at their distal ends (Fig. 1E). Keratodonts arranged in tooth rows of about 20–25 teeth/mm each. The labial tooth row formula is LTRF 2(2)/3(1); A2 and P1 gaps are sub-equal. The relative lengths of the rows are A2≥A1 and P1≥P2>P3 (a single specimen with P2>P1>P3). Both jaw sheaths, the upper arch-shaped and the lower V-shaped, are robust and serrated (Fig. 1D).

In preservative, the dorsum of the body is brown with some brown blotches; in lateral view the brown colour lightens towards the ventral region of the abdominal area; the spiracle and gular region are translucent with some light brown spots. Lateral line system is poorly or not at all visible. The ventral skin lacks pigmentation making possible the visualisation of the hyobranchial apparatus, heart, intestine and the muscle rectus abdominis.

In the ventrolateral region there are two pairs of white circular spots, apparently corresponding to glandular tissue: the anteriormost pair is ventrolaterally placed between gular and abdominal regions, whereas the caudalmost pair is near the posterior end of body. In all *A. neuquensis* larvae studied by us the posterolateral pair was always present. The anterolateral spots were present in 60% of the specimens (90% in *A. gargola*), 20% of the specimens presented only one spot on the left side of the body near the beginning of the spiracular tube, and 20% did not present any anterolateral spots. Some specimens of *A. gargola* presented two or three spots on the right side of the anterolateral pair, and similar spots could be also found on dorsal and ventral caudal fins.

The vent tube is translucent with a few light brown spots. The tail muscle represents a continuum with the dorsal body pattern; myomeres are noticeable but slightly obscured by pigmentation, in particular the posteriormost, which are diffuse. The first half of the dorsal fin is translucent with some brown spots, the second half of both fins are densely pigmented with small brow flecks; the anterior half of the ventral fin is translucent with few brown spots. The edges of both fins lack pigmentation. The eyes are black.

Buccal cavity morphology
Buccal floor (Fig. 2A) with two pairs of long, simple but slightly serrated infralabial papillae (the right papilla of the lateral pair was cut accidentally on figure). The anterior pair is placed laterally than the posterior one,

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Table 2. Origin and insertion of cranial muscles of *Alsodes neuquensis* and *A. gargola* larvae. Comments: [1]The origin of muscles levator mandibulae longus superficialis and profundus overlaps; [2]The muscles interhyoideus and intermandibularis are clearly separated by a wide gap; [3]The m. subarcualis rectus II-IV is simple; it does not invade fourth interbranchial septum.

Muscle	Origin	Insertion
Nervus trigeminus (cranial nerve V), mandibular musculature		
Levator mandibulae internus	Capsula auditiva and processus ascendens	Cartilago meckeli, through a tendon
L. m. longus superficialis[1]	Arcus subocularis and processus ascendens	Cartilago meckeli
L. m. longus profundus[1]	Arcus subocularis	Pars alaris by common tendon with m. l. m. e. profundus
L. m. externus profundus	Processus muscularis	
L. m. externus superficialis	M. l. m. e. profundus	Adrostral tissue mass
L. mandibulae articularis	Processus muscularis	Cartilago meckeli
L. mandibulae lateralis	Processus articularis	Adrostral tissue mass
Submentalis	Undetectable at the studied stages	
Intermandibularis[2]	Cartilago meckeli	Median raphe
Mandibulolabialis inferior	Cartilago meckeli	Ventral area of the oral disc
Nervus facialis, (cranial nerve VII), hyoid musculature		
Suspensoriohyoideus	Processus muscularis and arcus subocularis	Ceratohyale
Suspensorioangularis	Processus muscularis	Processus retroarticularis
Orbitohyoideus	Processus muscularis	Ceratohyale
Quadratoangularis	Anteroventral on palatoquadrate	Processus retroarticularis
Hyoangularis lateralis	Ceratohyale	Processus retroarticularis
Hyoangulais medialis	Absent	
Interhyoideus[2]	Both distal ends of ceratohyale	Median raphe
Interhyoideus posterior	Undetectable under stereomicroscope dissections	
Diaphragmatopraecordialis	Undetectable under stereomicroscope dissections	
Nervus glossopharyngeus (cranial nerve IX), branchial musculature		
Levator arcuum branchialium I	Arcus subocularis forming a continuum with m. l. a. br. II	Commissura terminalis I
Subarcualis rectus I	Dorsal head on ceratobranchiale I. Ventral heads on processus branchiales II and III	Ceratohyal, the ventral and dorsal parts are overlap in ventral view
Constrictor branchialis I	Absent	
Nervus vagus (cranial nerve X), branchial musculature		
Constrictor branchialis II	Ceratobranchiale II	Commissura terminalis I
Constrictor branchialis III	Ceratobranchiale III	Commissura terminalis I
Constrictor branchialis IV	Ceratobranchiale III	Commissura terminalis II
Diaphragmatobranchialis	Peritoneal wall	Ceratobranchiale III
Levator a. branchialium II	Arcus subocularis and processus anterolateralis of crista parotica	Commissura terminalis II
Levator a. branchialium III	Lateral on capsula auditiva	Comissura terminalis III and ceratobranchiale IV
Levator a. branchialium IV	Posteroventral on capsula auditiva	Ceratobranchiale IV, from medial to proximal part
Subarcualis obliquus II	Processus branchiales II and III, one head	Processus urobranchialis
Subarcualis rectus II-IV[3]	Ceratobranchiale IV	Processus branchialis II
Tympanopharyngeus	M. l. a. branchialium IV	Pericardium
Dilatator laryngis	Posteroventral on capsula auditiva	Lateral sides of larynx
Constrictor laryngis	Forms an annulus surrounding the larynx	
Transversus ventralis IV	Absent	
Nervus hypoglossus (spinal nerve II), hypobranchial musculature		
Geniohyoideus	Hypobranchiale between ceratobranchiales II and III	Cartilago infrarostrale
Rectus cervicis	Peritoneal wall	Processus branchiales II – III

both of them subequal in size. Four long simple lingual papillae arranged in two pairs, the lateral pair greatly smaller than the central one. Buccal pockets large and transversely oriented. Anterior margins of the pockets display 3 or 4 simple, slightly serrated papillae per side. Buccal floor arena bounded by 40–50 papillae. The arena is completely scattered by a very dense field of a mixture of short and long conical papillae, some of them slightly forked. Medially to the buccal pockets there is a pair of tetra-armed, very well developed papillae. The ventral velum has digit-shape marginal projections, secretory pits and the large median-notch.
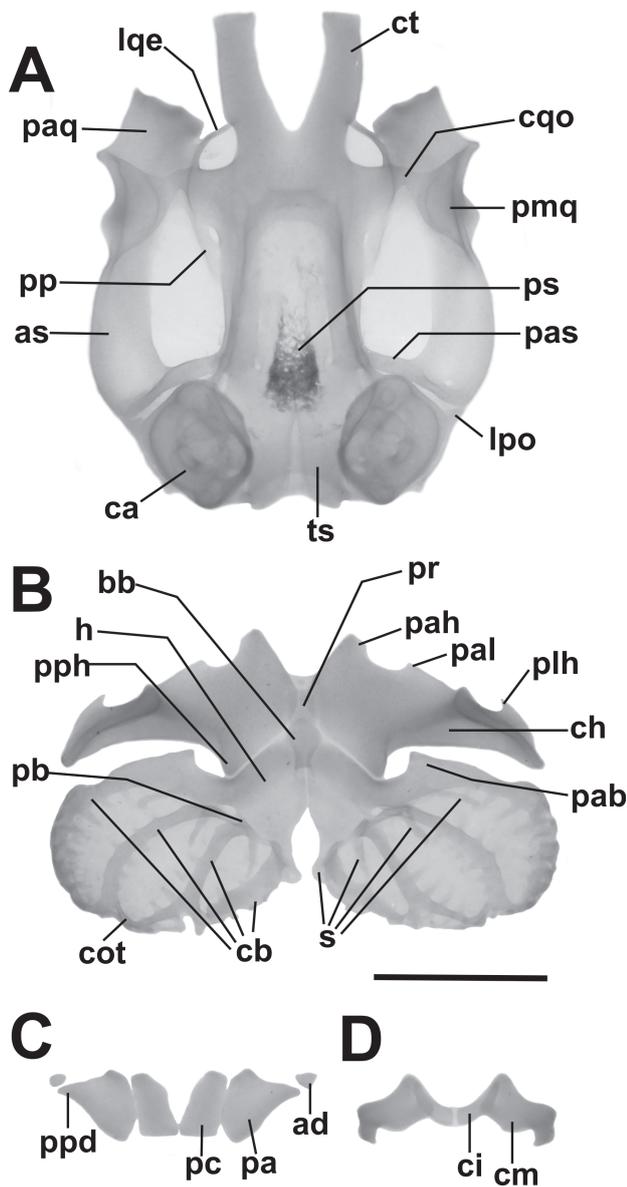
Fig. 3. Cranial skeleton of *Alsodes neuquensis* at Stage 31 (MACN 47279). (A) Dorsal view of the chondrocranium – ct: cornua trabeculae, lqe: ligamentum quadratoethmoidale, paq: pars articularis quadrati, cqo: commissura quadratoorbitalis, pmq: processus muscularis quadrati, pp: processus pseudopterygoideus, ps: parasphenoid, as: arcus subocularis, pas: processus ascendens, lpo: larval processus oticus, ca: capsula auditiva, ts: tectum synoticum; (B) ventral view of the hyobranchial apparatus – bb: basibranchiale and processus urobranchialis, cb: ceratobranchiales, ch: ceratohyale, cot: commissura terminalis, h: hypobranchiale, pab: processus anterior branchialis, pah: processus anterior hyalis, pal: processus anterolateralis hyalis, plh: processus lateralis hyalis, pph: processus posterior hyalis, pr: pars reuniens, pb: processus branchialis, s: spicula I-IV; (C) frontal view of the cartilago suprarostralis – ad: adrostral cartilage, pa: pars alaris, pc: pars corporis, ppd: processus posterior dorsalis; (D) frontal view of the cartilagines infrarostrales and cartilago Meckeli – ci: cartilago infrarostrale, cm: cartilago Meckeli. Scale bar: A-B=3mm; C–D are not to scale.

Buccal roof (Fig. 2B) with one simple large and stout unpaired papilla positioned at the midline of the prenarial arena. This papilla is flanked by two pustules positioned posterolateral to it. The choana is transversally arranged and limited by smooth elevated anterior and posterior margins. Each side of the postnarial arena has four to five simple papillae, two of them long and intercalated with the remaining two or three being shorter than the first-ones. The median ridge is triangular, tall, narrow at the base and with forked distal end. Lateral ridge papillae, paired, tetra-armed (posterior arm is the tallest, anterior arm is the shortest), of wide base, and serrated. Buccal roof arena presents a central triangular-shaped zone of high density of papilla (about 80 papillae, some of them slightly forked), but no pustules. Lateral and anteriormost papillae are large whereas central and posterior ones tend to be shorter. There is no gap between the median ridge and most anterior buccal roof arena papillae. The posterior half of BRA is limited by 8 to 10 long papillae per side arranged in a U-shape pattern. Lateral roof arena papillae absent. Dorsal velum glandular as suggesting the presence of secretory pits.

Chondrocranium, hyobranchial apparatus and cranial muscles

The following description is based on four *A. neuquensis* and four *A. gargola* larvae as detailed in the Appendix. The neurocranium is almost subquadrangular and depressed, with similar widths at both posterior (arcus subocularis) and anterior (processus articularis) levels. The tetrapartite cartilage suprarostralis possesses the medial partes corpora and the lateral partes alares without cartilaginous connections. Each pars alaris bears a short processus anterior dorsalis and a well-developed processus posterior dorsalis. A chondrified adrostral tissue mass is clearly present in both species.

The cornua trabeculae slightly diverge anteriorly from the planum ethmoidale and account for about 29.5% of the total neurocranium length in both species. Each cornu trabeculae is homogeneous in width and bears a very well-developed processus lateralis trabeculae. The slightly dilated anterior end of each cornu trabeculae articulates the cartilago suprarostralis. Nasal structures were not observed at the studied stages. In both species the foramina nervi olfatorius is limited by the planum precerebrale (*sensu* de Beer, 1937) medially, the pila ethmoidalis (*sensu* de Beer, 1937) laterally and the tectum anterius dorsally.

The fontanella frontoparietalis is closed by the tiny tectum anterius anteriorly, by the dorsal margins of the orbital cartilage anterolaterally, by the taenia tecti marginalis posterior and the capsula auditiva caudolaterally, and by the tectum synoticum caudally. Other tectal structures were not observed at the studied stages. The walls at orbital level are completely chondrified in the larger specimen of *A. neuquensis*. The studied specimen presented four pillars: ethmoid, prootic (*sensu* Cannatella, 1999), metoptic and antotic; and three foramina: optic, oculomotor and prootic. The foramen opticum and foramen oculomotorium are of similar size. The basi cranii is fully closed in the largest specimen of *A.*

neuquensis, which has both foramina carotica primaria and craneopalatina well defined. Only the foramina carotica primaria is formed at the studied stages of *A. gargola*. The parasphenoid is the only ossification centre present at the Stage 31 of *A. neuquensis* (Fig. 3).

The ovoid-shaped capsula auditiva represented 31.5% of the total neurocranium length in *A. neuquensis* (35% in *A. gargola*). The anterior copula of the capsula auditiva is slightly coupled to the processus ascendens. The larval crista parotica bears a very-well developed processus anterolateralis and a short processus posterolateralis, in some *A. neuquensis* the processus anterolateralis reach to arcus subocularis forming the larval processus oticus. The medial wall of the capsula auditiva is pierced by an undivided foramen acusticum, a small foramen endolymphaticum, and a medium size foramen perilymphaticum superior. The foramen perilymphaticum inferior and the foramen jugulare open caudally on the capsula auditiva. The fenestra ovalis lacks operculum at the studied stages.

The mandibular arch is represented by the cartilagines meckeli, cartilagines infrarostrales and the palatoquadrate. The sigmoid cartilago meckeli articulates medially with the cartilagines infrarostrales by the cavity of the processus ventromedialis, and caudally with the pars articularis quadrati by a stout condylus and the processus retroarticularis. The palatoquadrate possesses pars articularis, a wide and rounded processus muscularis quadrati attached to neurocranium via a low commissura quadratoorbitalis and a high ligamentum tectum (to the processus pseudopterygoideus), a wide commissura quadrato cranialis, a small triangular-shaped processus quadratoethmoidalis, a large processus pseudopterygoideus, a homogeneus arcus subocularis that caudally exceeds the level of the prootic foramen, and a processus ascendens of intermediate union that forms a straight angle with the neurocranium. The lateral and posterior margins of the arcus subocularis bent upwards. In both species the large processus pseudopterygoideus (*sensu* Haas, 2003) is medially fused to the basi cranii. In *A. gargola* and *A. neuquensis* the fusion is incomplete as indicated by the presence of a foramen. In one specimen of *A. neuquensis* (MLP.A 5748) from Moquehue stream the processus pseudopterygoideus is fused to the basi cranii along the entire length of the process.

On the hyobranchial apparatus, presence of a rounded basihyale varies between individuals. It is present in the largest specimen of *A. neuquensis* from Primeros Pinos and in the specimen of *A. gargola* from the Tonchek Lagoon, but it is absent in the specimen of *A. neuquensis* from Moquehue stream (MLP.A 5748) and in the smallest specimen of *A. neuquensis* near from Primeros Pinos (MACN 47279). All ceratohyale processes (anterohyal lateral, anterohyal, posterohyal and articular) are well developed. The ceratohyalia is medially joined by a diffuse pars reuniens. The hexagonal-shaped basibranchiale bears a short and wide processus urobranchialis. The ceratobranchiales I, II, III and IV join the planum hypobranchiale by a cartilaginous connection, but in *A. neuquensis* the third ceratobranchiale meets the planum by a very thin cartilage that under stereomicroscope

seems a ligamentous union. Comissurae proximales absent and a distal comissura is present between each pair. All ceratobranchials possesses cartilaginous lateral projections. The processus branchialis between ceratobranchiales II and III is open. The processus anterior branchialis is present on the ceratobranchiale I. Spiculae I–III are slender and long (*sensu* Haas, 2003) compared with the robust fourth spicula. The sinus posterior hypobranchiale is wide.

The cranial muscles in both species present the same arrangement; the ramus mandibularis of the nervus trigeminus runs laterally to all mm. levatorae mandibulae (see Table 2 for details about origin and insertion of each muscle).

DISCUSSION

The comparison of some of the studied proportions indicates that larvae of *A. neuquensis* present shorter and higher tails, and relatively smaller oral discs than *A. gargola.* External features of both species agree with larval characterisations of the genus proposed by early authors (Lavilla, 1988; Altig & McDiarmid, 1999; Formas & Brieva, 2004).

Marginal papillae of the oral disc are possible subjects of variation within species. Díaz & Valencia (1985) reported two submarginal papillae rows on posterior labium for *A. nodosus*, a feature that seems unique within the genus, but Formas (1975) described a single row of submarginal papillae for the same species. Similarly, the oral disc of *A. montanus* from La Parva (Santiago, Chile) was reported as not emarginated by Díaz & Valencia (1985), but the drawings of Busse (1980) on the same species and locality indicate a slightly emarginated oral disc. The same inconsistency was found for the oral disc of *A. valdiviensis* (as *A. monticola*), see controversy about the identity of this species and some material assigned to it in Formas & Brieva (2004) and Formas et al. (2008): Formas (1975) described an emarginated oral disc for *A. valdiviensis* (cited as *Eupsophus monticola*) from Cordillera Pelada, whereas Díaz & Valencia (1985) reported non-emarginated oral discs for *Alsodes* from the same locality.

With respect to the paired white spots present anteriorly and posteriorly at both sides of the ventral body parts in both species studied here, the posterior ones were previously mentioned for the larvae of *Alsodes hugoi* (Cuevas & Formas, 2001), and they are inferable from drawings (although not mentioned in the corresponding descriptions) in larvae of *A. verrucosus*, *A. vanzolinii* (Formas & Brieva, 2004), and *A. coppingeri* (Formas et al., 2008). Altig & McDiarmid (1999) have made a brief comment on similar spots present in some hylid larvae, which are apparently of glandular nature. Kolenc et al. (2008) provided scanning electron micrographs that prove a rather sensitive instead of glandular function for these spots in hylids; the posterior spots corresponding to aggregations of neuromasts in the larva of *Hypsiboas faber*. However, the posterior spots present in larvae of *Alsodes* studied herein may be of glandular nature according to our SEM images rather than the

sensitive tissue reported for several hylids (Kolenc et al., 2008; Sánchez, 2010) and dendrobatids (Anganoy-Criollo, 2013). Future studies are needed to confirm this assertion and to establish homologies between different taxonomic groups. As far as we know the anterior pair of spots observed in both species of *Alsodes* and the caudal spots observed in *Alsodes gargola*, are reported here for the first time in anuran larvae.

The buccal cavity has been described for *A. gargola* and *A. neuquensis* (Echeverría et al., 2001, current work), *A. vanzolinii* and *A. verrucosus* (Formas & Brieva, 2004). Wassersug & Heyer (1988) provided a description for a larva of *Alsodes* sp., as *A. monticola* from Lago Nahuel Huapi, Neuquén, Argentina; see comments on identity of *A. monticola* in Formas et al. (2008), and also described the buccal features of an *Alsodes* sp. from Parque Nacional Nahuelbuta, Cabrerías (Malleco, Chile), which presumably refers to *A. barrioi*. Thus, in light of the present knowledge, buccal features of the genus *Alsodes* show scarce variation among species. The most remarkable features are: (i) anterior pair of infralabial papillae may be either not forked (*A. barrioi, A. neuquensis, A. gargola* and *A. verrucosus*) or forked (*A. vanzolinii*); (ii) two pairs of infralabial papillae, posterior pair may be either not forked (*A. gargola, A. neuquensis* and *A. verrucosus*) or forked (*A. barrioi, A. vanzolinii*); (iii) lingual papillae arranged in two transversally arranged pairs, the medial larger, the lateral shorter (all species), (iv) the BFA bounded by 11–17 papillae per side (*A. vanzolinii*), and about 20–25 per side (*A. barrioi, A. gargola* and *A. neuquensis*, *A. verrucosus*); (v) ventral velum ends on large marginal projections (all species); (vi) prenarial papilla single accompanied by few pustules only in the pair *A. gargola* and *A. neuquensis*; (vii) triangular-shaped median ridge may be either bifid (*A. barrioi, A. gargola, A. neuquensis, A. vanzolinii*) or with rugosities (*A. verrucosus*); and (viii) lateral ridges from three to penta-armed (all species).

The chondrocranial morphology is known for five species of *Alsodes* including the two described in the present work: *A. barrioi, A. gargola, A. neuquensis, A. vanzolinii* and *A. verrucosus* (Lavilla, 1992; Formas & Brieva, 2004; this work). These species may be characterised by sharing the following characters: (i) neurocranium ovoid to subquadrangular, (ii) cornua trabeculae well developed (>20% of total length), (iii) cartilage suprarostralis tetrapartite, (iv) adrostral tissue mass present, (v) commissura quadratoorbitalis present, (vi) palatoquadrate extends posteriorly to the level of the pila antotica, and (vii) processus anterolateralis of the larval crista parotica well developed, and (viii) large processus pseudopterygoideus with tip fused to the basi cranii leaving a rounded foramen (*A. barrioi, A. gargola* and some *A. neuquensis*) or not (*A. vanzolinii, A. verrucosus* and some *A. neuquensis*). Formas & Brieva (2004) did not report the processus pseudopterygoideus for *A. vanzolinii* and *A. verrucosus* but a complete fusion can be inferred based on the original illustrations, (Alcalde & Blotto, 2006 made the same observation).

The hyobranchial apparatus of *A. gargola, A. barrioi, A. vanzolinii* and *A. verrucosus* present all ceratobranchiales fused to the hypobranchial plate. *Alsodes neuquensis* shows the same configuration for the connections of ceratobranchiales I, II and IV. The third ceratobranchiale of *A. neuquensis* has a sindesmotic connection; nevertheless, intraspecific variation must be additionally studied and considered in relation to this condition.

Finally, there are two additional features that represent informative variation within *Alsodes*: the type of attachment of the processus ascendens to the pila antotica, and the presence/absence of larval processus oticus. The processus ascendens may join the pila antotica either just at the level of the ventral margin of the foramen oculomotorium (*A. barrioi, A. gargola, A. neuquensis*) or immediately below this foramen (*A. vanzolinii, A. verrucosus*). Clearly, the point where the union occurs (just at the limit was used to distinguish between the character states "intermediate connection" and "low connection", see Haas, 2003) justifies the different character scoring made by other authors (Lavilla, 1992; Formas & Brieva, 2004). For example, the two species (*A. vanzolinii, A. verrucosus*) described with low attachment are those from Formas & Brieva (2004), whereas Figure 4c from that paper is concordant with an intermediate attachment as considered here. Similarly, the crista parotica bears a very wide and well developed processus anterolateralis that in *A. barrioi* and in some exemplars of *A. neuquensis* the process is fused to the palatoquadrate to form the larval processus oticus (Lavilla, 1992, this work).

Wassersug & Duellman (1984) stated that endotrophy implies either total or partial reduction of larval buccal structures. This is the case for the larvae of *E. emiliopugini*, which present reduced buccal papillation and both dorsal and ventral velums poorly marked (Vera Candioti et al., 2011). However, the buccal cavity description of a larvae as "*Eupsophus roseus*" (one specimen dissected, Stage 29; KU 162057, 11 km W Angol, Arroyo Los Lleulles, Malleco province, Chile) described by Wassersug & Heyer (1988) displays well developed buccal features, as is common for the larvae of many exotrophic larvae (Wassersug & Heyer, 1988; Echeverría et al., 2001; Formas & Brieva, 2004; this work). In addition to this unexpected buccal cavity morphology, *Batrachyla taeniata* (a species with exotrophic larvae) is also present in this area (Veloso et al., 1981), and the material described by Wassersug & Heyer (1988; KU 162057) as *E. roseus* is recorded as *Batrachyla* sp. in the database of the HerpNet2 Portal (data obtained from the University of Kansas Museum of Natural History; accessed through the HerpNet2 Portal, <http://www.HerpNet2.org>. On this basis, we consider that the identity of the material employed by Wassersug & Heyer (1988) must be re-evaluated.

In recent phylogenetic analyses, the sister group of the clade *Alsodes* + *Eupsophus* remains uncertain (Faivovich et al., 2005; Correa et al., 2006; Frost et al., 2006; Grant et al., 2006; Pyron & Wiens, 2011; Blotto et al., 2013; Fouquet et al., 2013; Pyron, 2014). Thus, we reviewed the available larval descriptions of the genus *Eupsophus* (Formas & Pugín, 1978; Formas, 1989a; Formas, 1989b; Formas, 1992; Vera Candioti et al., 2005; Cárdenas-Rojas et al., 2007; Nuñez & Úbeda, 2009; Vera Candioti et al.,

2011; Nuñez et al., 2012) in order to provide diagnostic characters for this genus and compare them with those of *Alsodes*. When the position of the *Alsodes* + *Eupsophus* clade is resolved, some of these characters must be revisited to see whether they represent synapomorphies for *Alsodes*, *Eupsophus* or some internal clades, although some possible synapomorphies for *Eupsophus* were advanced by Vera Candioti et al. (2011) and Blotto et al. (2013). In contrast with features above described for *Alsodes*, many of the larval traits of *Eupsophus* are shared with other endotrophic tadpoles as those of *Rhinoderma* and *Cyclorhamphus* (Lavilla, 1987; 1991), for example: (i) pigmentation absent, (ii) spiracle reduced or absent, and (iii) short cornua trabeculae (<20% of chondrocranium length).

Other traits of *Eupsophus* larvae that differ from those of *Alsodes* but that are not clearly linked to endotrophy are: (i) vent tube triangular with opening medial, (ii) LTRF 2(2)/2[1], (iii) keratodont body scarcely marked and head short with cusps reduced or absent, (iv) submarginal papillae absent, (v) dorsal gap subequal to tooth row A1, (vi) adrostral tissue mass absent, (vii) larval crista parotica absent, (viii) comissura quadrato-orbitalis absent, (ix) ceratobranchiale rays absent, (x) spicules absent or poorly developed, (xi) origin of m. levator mandibulae internus on processus ascendens only, (xii) m. levator externus superficialis either absent or very thin, (xiii) m. subcualis rectus I with two slips, and (xiv) m. interhyoideus and m. intermandibularis forming an X-shaped structure (*sensu* Haas, 2003). Despite the uncertain phylogenetic position of *Alsodes* + *Eupsophus*, an X-configuration of the m. interhyoideus and m. intermandibularis is a putative synapomorphy of *Eupsophus* (described for *E. emiliopugini* and inferred from figures of *E. roseus*; Vera Candioti et al., 2005; 2011), since a similar morphology was only reported for some Microhylidae (Haas, 2003; Vera Candioti, 2007).

Comparative studies between phylogenetically related exotrophic and endotrophic larvae are needed to discriminate among convergent-divergent features related to deviations to the exotrophy.

Note added in proof
While our article was in press, a new *Alsodes* species has been published by Charrier et al. (2015).

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APPENDIX

CNP.A, Herpetological Collection of Instituto de Diversidad y Evolución Austral (IDEAus-CONICET, Puerto Madryn, Chubut, Argentina); **MACN**, Herpetological Collection of Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (Buenos Aires, Argentina); **MLP**, Herpetological Collection of Museo de La Plata (Buenos Aires, Argentina).

Alsodes gargola. Six Stage 29 to 37 larvae for morphometrics measurement: one Stage 29 (**CNP.A 3771** from Tonchek Lagoon, Bariloche department, Río Negro province, Argentina; this specimen was first measured and then was cleared and stained), two Stage 33 (**MLP.A 4066** from Hielo Azul footpath refuge, Bariloche department, Río Negro province, Argentina), one Stage 35 (**MLP.A 4066**), and two Stage 37 (**MLP.A 4066**). One Stage 26 larva for SEM and stereomicroscope observations of external features, oral disc, and buccal cavity papillation (**CNP.A 3074**). Four larvae for cranial muscles, chondrocranium, and hyobranchial apparatus descriptions: One Stage 29 (**CNP.A 3771**), one Stage 30 (**CNP.A 3785** from Tonchek Lagoon, Bariloche department, Río Negro province, Argentina) one Stage 32 (**MLP.A 5749** from Arroyo Zanjon Hondo, Los Alerces National Park, Futaleufú department, Chubut province, Argentina), and one Stage 35 (**MLP.A 5750** from Hielo Azul footpath refuge, Bariloche department, Río Negro province, Argentina).

Alsodes neuquensis. Ten Stage 28 to Stage 33 larvae for morphometrics measurement: two Stage 28 (**MACN 46544**, n=1 Pampa de Lonco Luan, Aluminé department, Neuquén province, Argentina; and **MACN 46546**, n=1, 10 km W from Primeros Pinos, Aluminé department, Neuquén province, Argentina), one Stage 29 (**MACN 38629** 15 km W from Primeros Pinos, Aluminé department, Neuquén province, Argentina), three Stage 30 (**MACN 38629,** n=1, and **MACN 46544**, n=2), two Stage 31 (**MACN 38629** and **MACN 47279** 15 km W from Primeros Pinos, Aluminé department, Neuquén province, Argentina; the last specimen was first measured and then was cleared and stained), and two Stage 33 (**MACN 38629**, n=1, and **MACN 46544**, n=1). Three larvae used for SEM and stereomicroscope observations of external features, oral disc, and buccal cavity papillation: one Stage 28 (**MACN 38629**), one Stage 30 and one Stage 31 larvae (**MLP.A. 2530**, from Moquehue stream, Aluminé department, Neuquén province, Argentina). Four larvae for cranial muscles, chondrocranial, and hyobranchial apparatus descriptions: one Stage 32 (**MLP.A 5748** from Moquehue stream, Aluminé department, Neuquén province, Argentina); two Stage 32 (**MLP.A 4935** from Laguna La Atravesada, Aluminé department, Neuquén province, Argentina); and one Stage 31 (**MACN 47279**).