

Chondrocranium and cranial muscle morphology in *Lysapsus* and *Pseudis* tadpoles (Anura: Hylidae: Hylinae)

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Abstract

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The chondrocranium and cranial muscles of *Lysapsus limellum*, *Pseudis cardosoi*, *P. minuta* and four subspecies of *P. paradoxa* (*caribensis*, *occidentalis*, *paradoxa* and *platensis*) are described. *Lysapsus* and *Pseudis* are related to the Hylidae by: (1) vertical processus anterolateralis of the larval crista parotica, and (2) presence of m. mandibulolabialis inferior and superior (the latter reversed in *P. cardosoi*). We confirm some larval synapomorphies previously proposed for the pseudids, except the m. subarcualis obliquus II having one head and the absence of copula anterior. We add three new features for the pseudids: (1) the distal end of ceratobranchiales III and IV projects towards either the capsula auditiva (*Lysapsus*) or the processus basicapsularis – new term – (*Pseudis*); (2) the arcus subocularis is twice as wide at the level of the processus ascendens as it is just posterior to the processus muscularis quadrati, and (3) the lateral corner of the processus articularis quadrati largely projects laterally. We diagnose *Lysapsus* larvae (the states of *Pseudis* in parentheses) as follows: (1) short processus pseudopterygoideus (long), (2) absence of lateral development of larval crista parotica (presence), (3) absence of commissura proximalis II (presence), (4) presence of processus lateralis hyalis – new term – (absence), (5) absence of processus basicapsularis (presence), (6) processus ascendens slightly curved along its entire extent (curved at the union with the basi cranii only), and (7) absence of m. tympanopharyngeus (presence).

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Introduction

Pseudis and *Lysapsus* are a natural group of aquatic frogs, distributed throughout South America on the eastern Andean slopes from Venezuela and Colombia to northern Argentina (Gallardo 1961, 1964). Early on, the pseudids were classified either as Leptodactylidae, Hylidae or as a distinct family, the ‘Pseudidae’, e.g. Savage and de Carvalho (1953). The subsequent phylogenetic analyses of Duellman and Trueb (1986) and Ford and Cannatella (1993) indicated that Pseudidae should be placed at a common node with Hylidae and Centrolenidae. Hay *et al.* (1995) performed a molecular phylogenetic analysis and found pseudids to be a sister taxon to a clade including Dendrobatidae, Rhinodermatidae, Bufonidae, Hylidae and Centrolenidae. A molecular phylogeny presented by Ruvinsky and Maxon (1997) placed pseudids

and Rhinodermatidae in a weakly supported trichotomy with Pelodryadinae + Phyllomedusinae. Manzano (1997) compared the limb musculature of several Hylidae (including pseudids) and described interesting characters, but she did not perform a phylogenetic analysis based on her data. da Silva (1998) demonstrated that pseudids are nested within the Hylinae hylids based on adult morphology. Duellman (2001) considered pseudids as a separate subfamily ‘Pseudinae’ within the Hylidae, taking into account a set of shared characters from the adult appendicular skeleton. Barg (2003) suggested that pseudids form a clade not nested within Hylinae. Haas (2003) supported Duellman’s proposal with additional evidence: presence of a larval musculus (m.) mandibulolabialis superior and an apical portion of the adult m. intermandibularis. Burton (2004) analysed the muscles of the pes of a broad sample of hylid frogs and of frogs from

related taxa. He used these data to explore the relationships of Hyliidae and their allies (e.g. *Allophryne*, Centrolenidae) and found a well-supported clade containing the Hyliinae and Pseudinae. Darst and Cannatella (2004) presented a molecular phylogeny in which *Pseudis paradoxa* forms a well-supported clade with *Scarthyla goinorum*, and proposed the inclusion of pseudids within Hyliinae. Garda *et al.* (2004) studied the ultrastructure of the spermatozoa of pseudids and proposed a close relationship between them and the Leptodactylidae.

The works listed below state five distinct hypotheses about the relationships of pseudids: (1) pseudids are related to Leptodactylidae (Garda *et al.* 2004), (2) pseudids are related to Rhinodermatidae (Ruvinsky and Maxon 1997), (3) pseudids are related to other families of Neobatrachia (Hay *et al.* 1995), (4) pseudids are related to the Hyliinae hylids but not nested within them (Duellman 2001; Barg 2003; Haas 2003; Burton 2004), and (5) pseudids are nested within Hyliinae hylids (Darst and Cannatella 2004; da Silva 1998; Faivovich *et al.* 2005). However, a critical analysis of the cladograms used to support the fourth hypothesis ('pseudids are related to the Hyliinae hylids but not nested within them') does not show major topological differences from those cladograms that have found pseudids as a clade nested within the Hyliinae hylids. In the context of the present paper we prefer to consider pseudids within Hyliinae because most morphological characters and phylogenies provide synapomorphies to support this relationship.

Previous studies have investigated both larval chondrocranium and cranial musculature in *Pseudis minuta*, *P. paradoxa* and *Lysapsus limellum* (Parker 1882; Ridewood 1898; Lavilla and de Sá 1999; Haas 2003; Vera Candioti 2004). According to these works, *P. minuta* and *P. paradoxa* share the following character states: flat expansion of the larval crista parotica greatly developed (absent in *Lysapsus*), ultra-low suspensorium, long processus pseudopterygoideus (short in *Lysapsus*), fusion of pars alaris and pars corporis of the cartilago suprarostralis, absence of copula anterior, presence of commissura proximalis II (absent in *Lysapsus*), processus branchialis closed, m. subarcualis obliquus II comprising one head, m. subarcualis rectus II–IV divided in two portions by a gap, and m. levator mandibulae lateralis inserted onto the nasal sac.

This paper describes and compares the larval chondrocranium and cranial muscles of *L. limellum*, *P. cardosoi*, *P. minuta* and four subspecies of *P. paradoxa* (*caribensis*, *occidentalis*, *paradoxa* and *platensis*), to: (1) verify whether the larval cranial synapomorphic character states that have previously been proposed for pseudids are shared by all the species studied here, and (2) add some new chondrocranial characters for this group.

Materials and Methods

Tadpoles were staged using the table of Gosner (1960) and cleared and stained for cartilage and bone following the

technique of Taylor and Van Dyke (1985). For muscular observations, the procedure was interrupted before clearing and tadpoles were dissected under a Wild stereomicroscope. Terminology follows D'heursel and de Sá (1999) and Haas (1995) for chondrocranium, Haas (2001) for mandibular musculature, Haas and Richards (1998) and Haas (2003) for branchial and hyoid musculature, and Schlosser and Roth (1995) for muscular innervation. See Appendix 1 for reference material. Chondrocranial measurements were taken according to Alcalde and Rosset (2003) using a Reichert–Wien stereomicroscope equipped with micrometric ocular (to the nearest 0.1 mm).

Results

The chondrocranium of all species is described with reference to a representative common pattern at stage 35 (except *P. paradoxa paradoxa* – stage 38). Specific morphological divergences are considered with reference to the common chondrocranial pattern. Ontogenetic differences of some structures (e.g. operculum) were considered only when the temporal gap among species was larger than two developmental stages (e.g. stage 35 versus stage 38).

Neurocranium morphology

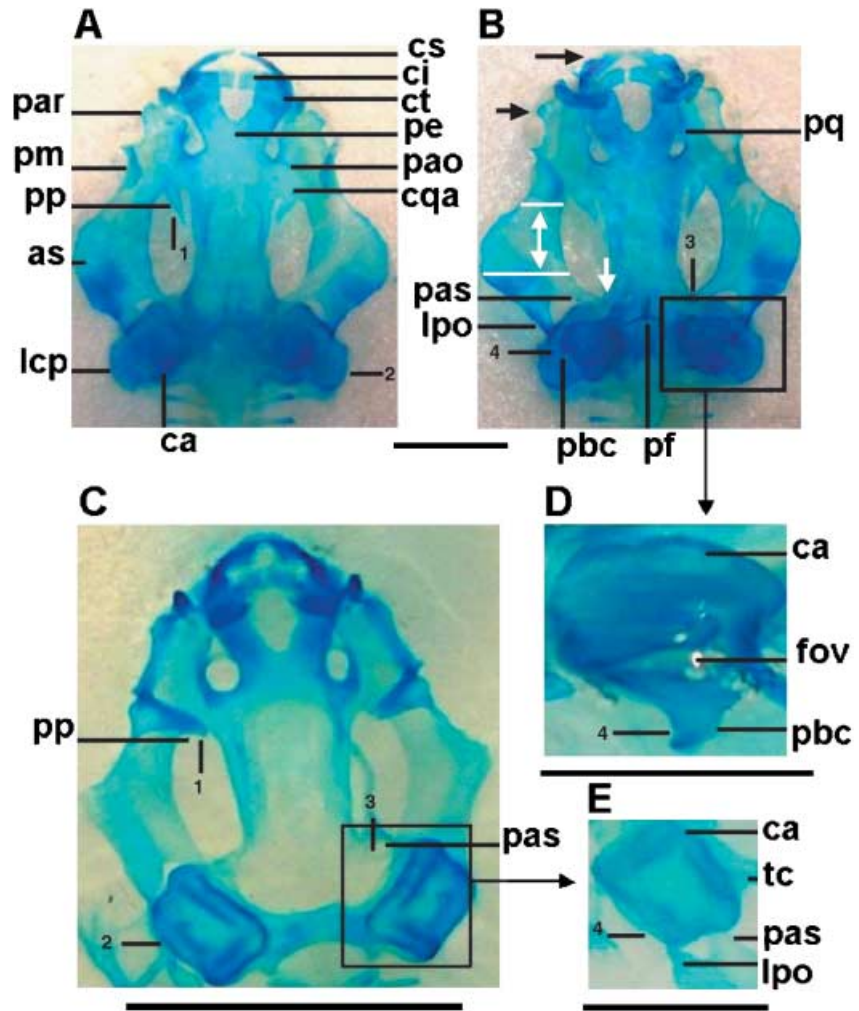
The neurocranium is square-shaped (width/length = 1) in *Pseudis* spp., and it is almost rectangular (width/length = 0.85) in *L. limellum*. In both genera, the neurocranium is depressed (height/width range 0.22–0.29) and its greatest width occurs at the level of the processus ascendens (Fig. 1A–C).

The upper horny beak is supported by an unpaired cartilago suprarostralis (cs) (Fig. 1A,B). Both partes corporis of this cartilage are fused and the partes alaris are continuous with them, sometimes bearing a small distal notch. The distal notch is absent in *L. limellum*, *P. paradoxa caribensis*, *P. p. paradoxa* and some specimens of *P. minuta*, *P. p. occidentalis*, and *P. p. platensis*. The pars alaris bears a well-developed processus posterior dorsalis.

The cornua trabeculae (ct) account for about 18–22% of chondrocranial length. They diverge anterolaterally from the planum ethmoidale (pe) (Fig. 1A). Their distal ends curve ventrally and expand slightly anteriorly, to articulate syndesmotically with the cartilago suprarostralis. Each cornu bears a distinct processus lateralis that receives the ligamentum quadrato-ethmoidale.

The anteromedial fusion of the trabeculae cranii (tc) forms the planum ethmoidale. At the time when the trabeculae cranii fuse to each other to form the planum intertrabeculare, the fenestra basicranialis becomes fully closed, except in two pairs of foramina: the foramen craniopalatinum (anterior) and the foramen caroticum primarium (posterior) (Fig. 1B). The basi cranii of *P. p. caribensis* and *P. p. platensis* are narrower caudally than at the posterior end of the planum ethmoidale,

Fig. 1—Chondrocranium of —**A**, **B** and **D**. *Pseudis paradoxa occidentalis* (MLP 3839, stage 35), and —**C** and **E**. *Lysapsus limellum* (MLP 3835) at stage 35. —**A** and —**C**. dorsal view of the neurocranium and mandibular arch. —**B**. ventral view of the neurocranium and mandibular arch. —**D** and **E**. lateral view of the otic region of the neurocranium. Arrows indicate synapomorphies of pseudids (see text). Notice the character states that vary between *Pseudis* and *Lysapsus*: long/short processus pseudopterygoideus (1), presence/absence of lateral development of larval crista parotica (2), type of curvature of the processus ascendens (3), and presence/absence of processus basicapsularis (4). Scale bars 5 mm; as, arcus subocularis; ca, capsula auditiva; ci, cartilago infrarostrale; cqa, commissura quadrato-cranialis anterior; cs, cartilago suprarostralis; ct, cornu trabeculae; fov, fenestra ovalis; lcp, larval crista parotica; lpo, larval processus oticus; pao, processus antorbitalis; par, processus articularis; pas, processus ascendens; pbc, processus basicapsularis; pe, planum ethmoidale; pf, parasphenoid; pm, processus muscularis quadrati; pp, processus pseudopterygoideus; pq, processus quadrato-ethmoidalis; tc, trabecula cranii.



whereas the width of the basi cranii is uniform in the other *Pseudis* spp. and in *L. limellum*.

The lateral walls of the cavum cranii are formed by the cartilagine orbitales, whose degree of chondrification varies among species. The posterior margin of the cartilago orbitalis, the pila antotica, is always present and delimits the fissura prootica anteriorly. The pila metooptica separates the foramen opticum from the foramen oculomotorium and is present in all species except *P. p. occidentalis* which presents poorly chondrified lateral walls. The pila ethmoidalis (*sensu* Cannatella 1999) closes the lateral wall of the cavum cranii anteriorly and delimits the foramen olfactorium posteriorly. At this level, a lateral projection forms the processus antorbitalis (pao). The pila preoptica (*sensu* Cannatella 1999) is absent and the foramen opticum is not well delimited anteriorly in *L. limellum* and *P. p. occidentalis*. The remaining species possess a pila preoptica; *P. p. platensis* and *P. p. caribensis* also have a small foramen trochleare located anterodorsal to the foramen opticum. The septum nasi arises dorsal to the planum ethmoidale and delimits the foramen olfactorium

anteriorly (Fig. 1A). This foramen is roofed by the tectum anterius.

In all species the fenestra frontoparietalis is enclosed by the tectum anterius anteriorly, the dorsal margins of the cartilagine orbitales laterally (except *P. p. occidentalis*), and the tectum synoticum posteriorly (Fig. 1A). In addition to this pattern, between stages 35 and 37, some species possess other cartilagine tecti such as taenia tecti medialis (except *P. p. caribensis*) and taenia tecti transversalis (only *P. p. platensis*, *P. minuta* and *P. cardosoi*). The taenia tecti transversalis is a transverse strut that extends across the fenestra frontoparietalis at the level of the pila antotica, subdividing it into two anterior and posterior portions. The taenia tecti medialis is an axial strut placed between the tectum synoticum and the taenia tecti transversalis, subdividing the posterior part of the fenestra frontoparietalis into left and right parietal fenestrae.

The capsulae auditivae (ca) are subspherical and comprise about 23–30% of the chondrocranial length (Fig. 1A). The operculum is present in *P. cardosoi* (stage 35) and *P. minuta* (stage 37). A very well-developed flat projection of the outer

wall of the capsula auditiva represents the expanded larval crista parotica (lcp) in *Pseudis* spp. (Fig. 1A,B). In this genus, the processus posterolateralis of the larval crista parotica is obscured by the large development of the crista. The larval crista parotica of *L. limellum* does not exhibit lateral development and only bears processus anterolateralis (Fig. 1C,E). The processus anterolateralis of both genera is vertically placed, reaching the posterolateral region of the palatoquadrate and forming the larval processus oticus (lpo) (Fig. 1A–E). In *Pseudis* spp., the ventral border of the fenestra ovalis (fov) forms a ventral projection, which we propose to call the processus basicapsularis (pbc, – new term –) (Fig. 1B,D). This process is well developed in all subspecies of *P. paradoxa* and in *P. minuta* but it is short and blunt in *P. cardosoi*. The distal end of the processus basicapsularis coincides topographically with the distal projection of ceratobranchiales III and IV and receives neither muscles nor ligaments. The complex of m. levator arcuum branchialium IV, m. tympanopharyngeus and m. dilatator laryngis originate just behind the processus basicapsularis. Tadpoles of *L. limellum* lack this process.

The medial wall of the capsula auditiva of *P. p. occidentalis* is poorly chondrified. In all other species, it is pierced by four or five foramina. The foramen acusticum possesses a complete dorsoventral septum dividing it into two portions: the acusticum anterius and acusticum posterius foramina. In *P. p. caribensis* and *P. minuta* there is a small foramen acusticum medius piercing the septum. The foramen endolymphaticum lies dorsal to both acoustic foramina. The foramen perilymphaticum superius opens posteriorly to the foramen acusticum posterius. The foramen perilymphaticum inferius opens into the foramen jugulare.

Mandibular arch morphology

The palatoquadrate is attached to the neurocranium by three structures: the commissura quadrato-cranialis anterior (cqa), the processus ascendens (pas), and the larval processus oticus. The commissura quadrato-cranialis anterior connects the medial margin of the palatoquadrate to the planum ethmoidale. The processus pseudopterygoideus (pp) is a posterior projection of the commissura quadrato-cranialis anterior. In *Pseudis* spp., the processus pseudopterygoideus is triangular, slightly curved medially and three times longer than its base (Fig. 1A). In *L. limellum* it is short (width and length subequal) and blunt (Fig. 1C). Both genera lack commissura quadrato-orbitalis.

The arcus subocularis (as) is twice as wide at the level of the processus ascendens as it is just posterior to the processus muscularis quadrati (pm) (Fig. 1B). The arcus subocularis lacks a fossa at the origin of both levatorae mandibulae longus muscles (lmlp, lmls).

In both genera, the processus ascendens and the trabecula cranii form an acute angle between them. In *L. limellum*, the processus ascendens curves along its entire length, appearing

as a ‘C’-shape in dorsal view (Fig. 1C) but in *Pseudis* spp. it runs straight, curving only when joining the trabecula cranii (Fig. 1B). The larval processus oticus is very well developed and joins the posterolateral corner of the palatoquadrate to the processus anterolateralis of the larval crista parotica (Fig. 1A–E).

The palatoquadrate articulates anteriorly with the cartilago meckeli by means of the processus articularis quadrati (par) (Fig. 1A,B). The lateral corner of the processus articularis quadrati largely projects laterally. A small and triangular processus quadrato-ethmoidalis (pq) projects from the medial margin of the processus articularis quadrati and receives the ligamentum quadrato-ethmoidale (Fig. 1B).

A well-developed processus muscularis quadrati lies lateral to the commissura quadrato-cranialis anterior (Fig. 1A). It is a smooth triangle projected dorsally and posteriomedially towards the neurocranium.

The lower jaw comprises the cartilagine meckeli and the cartilagine infrastrales (ci). The cartilago meckeli is a sigmoid structure with three processes, the retroarticularis, dorsomedialis and ventromedialis processes. The processus retroarticularis articulates with the processus articularis quadrati. The processus dorsomedialis and the processus ventromedialis articulate with the cartilago infrastralis by way of the commissura intramandibularis. The cartilagine infrastrales are ventromedially connected to each other by an elastic symphysis without copula.

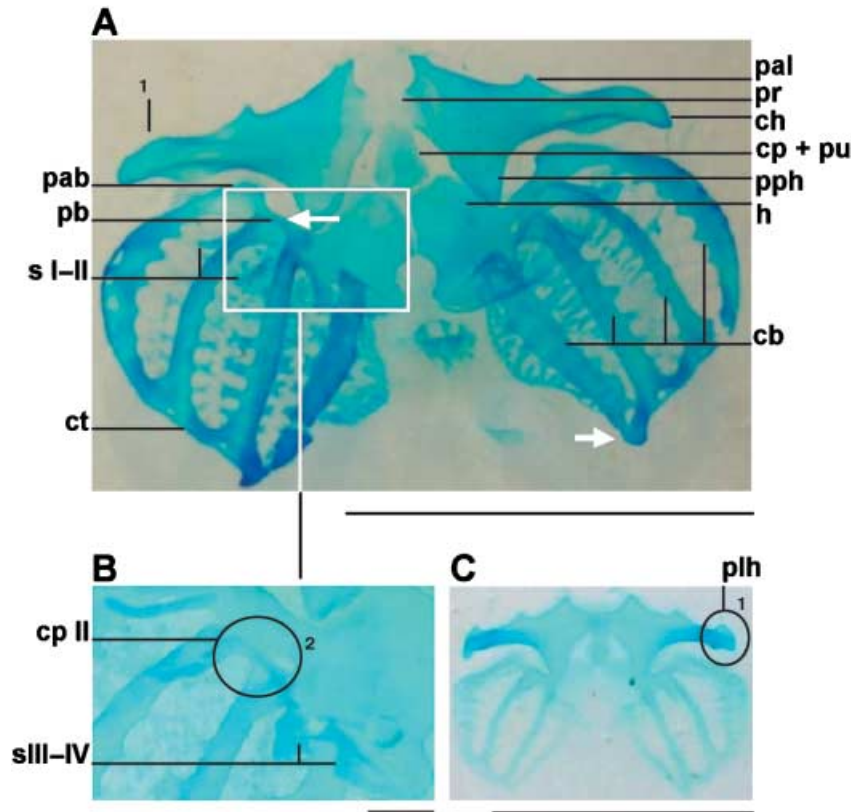
Hyobranchial morphology

The hyobranchial apparatus comprises the ceratohyalia and four branchial arches. The ceratohyalia are medially connected by a rectangular pars reuniens (pr) composed of diffuse cartilage (*sensu* Haas and Richards 1998) usually Alcian Blue negative (Fig. 2A). Each ceratohyale (ch) possesses processus anterior hyalis, processus anterolateralis hyalis (pal) and processus posterior hyalis (pph) (Fig. 2A). The processus anterolateralis hyalis is shorter than the processus anterior hyalis. The ceratohyale articulates by a processus articularis with the ventral notch of the processus muscularis quadrati. The ceratohyale of *L. limellum* has a small and pointed processus lateralis hyalis (plh, – new term –) located dorsolateral to the condylus (Fig. 2C). This process increases the surface for m. orbitohyoideus (oh).

The copula anterior is a transverse and slender cartilaginous bar placed in the ligamentum interhyale, just anterior to the pars reuniens. The copula anterior was found in *P. cardosoi* and in some specimens of *P. p. occidentalis*. The copula posterior (cp) is a pentagonal cartilage continuous with the pars reuniens. A short and rounded processus urobranchialis (pu) projects from its ventral surface (Fig. 2A).

The plana hypobranchiales (h) are medially separated and laterally continuous with the first ceratobranchiale. The sinus posterior hypobranchialis is U-shaped and limited by the rounded posterior ends of each hypobranchiale (Fig. 2A).

Fig. 2—Hyobranchial apparatus of—**A.** and **B.** *Pseudis paradoxa caribensis* (MHNLS 7903), and—**C.** *Lysapsus limellum* (MLP 3835) at stage 35. —**A.** and **C.** ventral view. —**B.** dorsal view. Arrows indicate synapomorphies of pseudids (see text). Notice the character states that vary between *Pseudis* and *Lysapsus*: presence/absence of processus lateralis hyalis (1), and presence/absence of commissura proximalis II (2). Scale bars 5 mm; cb, ceratobranchiales; ch, ceratohyale; cp, copula posterior; cp II, commissura proximalis II; ct, commissura terminalis; h, hypobranchiale; pab, processus anterior branchialis; pal, processus anterolateralis hyalis; pb, processus branchiales; plh, processus lateralis hyalis; pph, processus posterior hyalis; pr, pars reuniens; pu, processus urobranchialis; sI–II, spicula I and II; sIII–IV, spicula III and IV fused.



All ceratobranchiales (cb) are distally connected by a commissura terminalis (ct) which is laterally projected towards either the ventral side of the capsula auditiva (*Lysapsus*) or the processus basicapsularis (*Pseudis* spp.), at the level of ceratobranchiales III and IV (Fig. 2A,C). Ceratobranchiales II and III of *Pseudis* spp. are joined by a commissura proximalis (cp II) that is syndesmotically attached to the hypobranchiale (Fig. 2B). Ceratobranchiales II, III and IV of *L. limellum* and ceratobranchiales III and IV of *Pseudis* spp. are not proximally joined and meet the hypobranchiale by syndesmosis. A processus anterior branchialis (pab) is present at the anterior margin of the first ceratobranchiale. The processus branchiales (pb) of ceratobranchiales II and III are closed (Fig. 2A). Ceratobranchiales I and II have slender spicula (sI–II) that project dorsally from their proximal end. The spicula III and IV (sIII–IV) are joined by a reticulating cartilaginous connection (Fig. 2A,B).

Cranial musculature morphology

Table 1 presents the pattern of cranial muscles of *P. cardosoi* (Fig. 3) and provides origin and insertion of each muscle. Deviations from this pattern found in *L. limellum* and the remaining species of *Pseudis* are indicated in the following paragraphs.

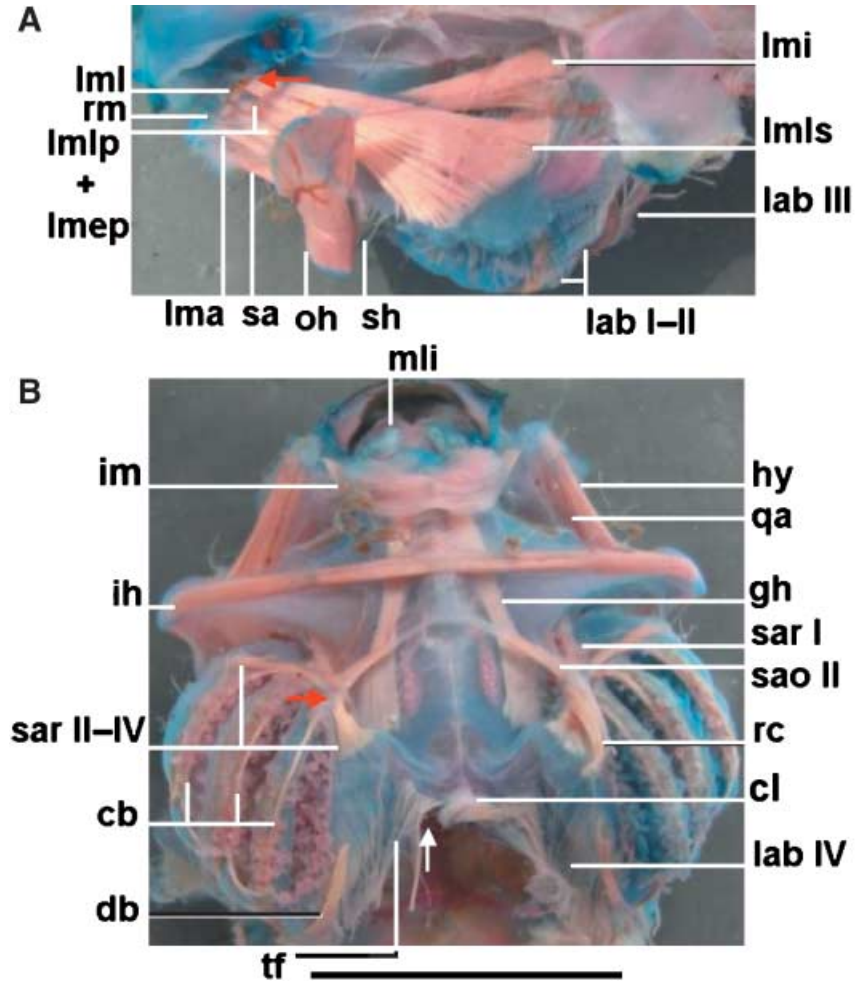
Mandibular musculature. The mandibular muscles appear similar in all species but they vary in composition. The lack of m. mandibulolabialis superior in *P. cardosoi* and the presence of m. submentalis in *P. p. paradoxa* at stage 38 result in the occurrence of nine muscles in *P. cardosoi*, 11 in *P. p. paradoxa* and 10 in *L. limellum*, *P. minuta*, *P. p. caribensis*, *P. p. occidentalis* and *P. p. platensis*. The m. mandibulolabialis superior originates in the cartilago meckeli together with the m. mandibulolabialis inferior (mli). The fibres of both muscles insert on two sites in the rostral region of the oral disc: at the level of the second row of keratodonts and near the dorsolateral row of marginal papillae. All muscles levatorae mandibulae are internal to the ramus mandibularis (rm) of the trigeminal nerve.

Hyobranchial musculature. The hyoid muscles of all species are structurally similar. The branchial musculature comprises 15 muscles in *Pseudis* spp. and 14 in *L. limellum* because the latter species lacks m. tympanopharyngeus (tf). The only structural difference among species is the number of heads of m. subarcualis obliquus II (sao II). The m. subarcualis obliquus II of *L. limellum*, *P. paradoxa caribensis*, *P. p. occidentalis*, *P. p. paradoxa* and *P. p. platensis* has one head (sometimes with a small notch at the origin), a condition shared with *P. cardosoi*. The m. subarcualis obliquus II of

Table 1 Mandibular and hyobranchial musculature of *Pseudis cardosoi* (SMNS 9301, stage 35)

Muscle	Origin	Insertion
Nervus trigeminus (cranial nerve V), mandibular musculature		
Levator mandibulae externus superficialis	M. levator mandibulae externus profundus	Ligamentum mandibulo-suprarostrale
Levator mandibulae externus profundus	Processus muscularis	Pars alaris by a common tendon with the m. levator mandibulae longus profundus
Levator mandibulae articularis	Processus muscularis	Cartilago meckeli
Levator mandibulae lateralis	Processus articularis	Nasal sac
Levator mandibulae longus profundus	Arcus subocularis obscured by the m. levator mandibulae longus superficialis	Pars alaris by a common tendon with the m. levator mandibulae externus profundus
Levator mandibulae longus superficialis	Arcus subocularis	Processus dorsomedialis
Levator mandibulae internus	Processus ascendens and trabecula cranii	Cartilago meckeli
Intermandibularis	Cartilago meckeli	Median raphe
Submentalis	Absent at the studied stages	
Mandibulolabialis inferior	Processus ventromedialis of cartilago meckeli	Oral disc at the level of the second ventral row of keratodonts
Mandibulolabialis superior	Absent	
Nervus facialis (cranial nerve VII), hyoid musculature		
Suspensioangularis	Processus muscularis	Processus retroarticularis
Quadratoangularis	Ventral face of processus articularis	Processus retroarticularis
Hyoangularis lateralis	Ceratohyale	Processus retroarticularis
Hyoangularis medialis	Absent	
Orbitohyoideus	Processus muscularis	Ceratohyale
Suspensiohyoideus	Processus muscularis and arcus subocularis	Ceratohyale
Interhyoideus	Ceratohyale distal ends	Median raphe
Interhyoideus posterior	It forms a diffuse sheath of fibres placed posteriorly in the wall of the branchial chamber behind the m. interhyoideus	
Diaphragmatopraecordialis	M. interhyoideus posterior	Pericardium
Nervus glossopharyngeus (cranial nerve IX), branchial musculature		
Levator arcuum branchialium I	Arcus subocularis continuous to the origin of the m. l. a. branchialium II	Commissura terminalis I at level of ceratobranchiale I
Subarcualis rectus I	The dorsal head originates at ceratobranchiale I. Both ventral heads originate at ceratobranchiales II and III	All heads insert at the posterior side of ceratohyale
Constrictor branchialis I	Absent	
Nervus vagus (cranial nerve X), branchial musculature		
Constrictor branchialis II	Ceratobranchiale II	Commissura terminalis between ceratobranchiales II and III
Constrictor branchialis III	Ceratobranchiale III	Commissura terminalis between ceratobranchiales II and III
Constrictor branchialis IV	Ceratobranchiale III	Ceratobranchiale III
Constrictor laryngis	It forms an annulus surrounding the larynx	Larynx
Dilatator laryngis	Posterolateral wall of capsula auditiva	Distal end of ceratobranchiale III
Diaphragmatobranchialis	Peritoneal wall	Commissura terminalis between ceratobranchiales II and III
Levator arcuum branchialium II	Arcus subocularis and larval processus oticus	Commissura terminalis between ceratobranchiales III and IV
Levator arcuum branchialium III	Larval crista parotica and larval processus oticus	Ceratobranchiale IV
Levator arcuum branchialium IV	Posterolateral wall of capsula auditiva	Ceratobranchiale I. This muscle presents a gap at level of ceratobranchiale III
Subarcualis rectus II–IV	Ceratobranchiale IV	Pericardium
Tympanopharyngeus	M. levator arcuum branchialium IV	
Transvs. ventralis IV	Absent	
Nervus hypoglossus (spinal nerve II), branchial musculature		
Geniohyoideus	Hypobranchiale	Cartilago infrarostrale by a short ligament
Rectus cervicis	Peritoneal wall	Ceratobranchiale III

Fig. 3—Cranial muscles of *Pseudis cardosoi* at stage 35 (SMNS 9301). —**A.** dorsolateral view of muscles related to neurocranium and mandibular arch. —**B.** ventral view of muscles related to hyobranchial apparatus and mandibular arch. Red arrows indicate synapomorphies of pseudids (see text). The white arrow shows the m. tympanopharyngeus which is absent in *Lysapsus limellum*. In —**A.**, m. levator mandibulae externus superficialis, was removed. In —**B.**, mm. interhyoideus posterior, diaphragmatopraecordialis and dilatator laryngis (both sides), and rectus cervicis (left side), were removed. Scale bars 5 mm; cb constrictor branchiales II, III and IV; cl, constrictor laryngis; db, diaphragmatobranchialis; gh, geniohyoideus; hy, hyoangularis lateralis; ih, interhyoideus; im, intermandibularis; lab I–II, levatorae arcuum branchialium I and II fused; lab III, levator arcuum branchialium III; lab IV, levator arcuum branchialium IV; lma, levator mandibulae articularis; lmepl, levator mandibulae externus profundus; lmi, levator mandibulae internus; lml, levator mandibulae lateralis; lmlp, levator mandibulae longus profundus; lmls, levator mandibulae longus superficialis; mli, mandibulolabialis inferior; oh, orbitohyoideus; qa, quadratoangularis; rc, rectus cervicis; rm, ramus mandibularis of trigeminus nerve; sa, suspensorioangularis; sao II, subarcualis obliquus II; sar I, subarcualis rectus I; sar II–IV, subarcualis rectus II–IV; sh, suspensoriohyoideus; tf, tympanopharyngeus.



P. minuta has two heads almost completely separated along its entire length.

Ossifications

Pseudis minuta, *P. p. occidentalis*, *P. p. platensis* and *P. p. paradoxa* possess the following ossification centres between stages 38 and 40: exoccipitals, frontoparietals, parasphenoid (pf) (Fig. 1B), and prootics. At stage 35, *P. cardosoi* and *P. p. caribensis* have parasphenoid only. *Lysapsus limellum* does not have ossification centres at the stages studied.

Discussion

According to previous work (Haas 2003; Vera Candioti 2004), *P. minuta*, *P. p. platensis* and *L. limellum* possess the following characters (when differing, *Lysapsus* states are in parentheses): (1) greatly developed flat expansion of larval crista parotica (absence), (2) ultra-low suspensorium, (3) long processus pseudopterygoideus (short), (4) fusion of

pars alaris and pars corporis of cartilago suprarostralis, (5) absence of copula anterior, (6) presence of commissura proximalis II (absence), (7) closed processus branchialis, (8) m. subarcualis obliquus II composed of one head, (9) m. subarcualis rectus II–IV divided into two portions by a gap, and (10) m. levator mandibulae lateralis inserted on the nasal sac.

We found that characters 5 and 8 are too variable to characterize groups within pseudids. With regard to the absence of copula anterior (character 5), an Alcian Blue-positive copula anterior occurs in *P. cardosoi* (stage 35) and in some specimens of *P. p. occidentalis* (stages 35 and 38). The intraspecific variation of this character was pointed out by several authors (Haas 1995; Larson *et al.* 2003), and consequently we dismissed this character. We verified character 8 in *L. limellum*, *P. cardosoi* and *P. paradoxa* but not in *P. minuta*. Thus, when our data are taken into account, only characters 1–4, 6, 7, 9 and 10 are sustained.

In light of the additional data presented, we re-diagnosed pseudids, *Lysapsus* and *Pseudis* larvae as follows:

Pseudids: (1) ultra-low suspensorium, (2) complete or almost complete fusion of pars alaris and pars corporis of cartilago suprarostralis, (3) closed processus branchialis, (4) m. subarcualis rectus II–IV divided into two portions by a gap, (5) insertion of m. levator mandibulae lateralis on nasal sac, (6) distal end of ceratobranchiales III and IV greatly projecting toward either the ventral surface of the capsula auditiva (*Lysapsus*) or the processus basicapsularis (*Pseudis* spp.), (7) the arcus subocularis is twice as wide at the level of processus ascendens as it is just posterior to processus muscularis quadrati, and (8) the lateral corner of the processus articularis quadrati largely projects laterally.

Lysapsus: (*Pseudis*' states in parentheses) (1) short processus pseudopterygoideus (long), (2) lack of lateral development of the larval crista parotica (presence), (3) lack of commissura proximalis II (presence), (4) presence of processus lateralis hyalis (absence), (5) absence of processus basicapsularis (presence), (6) processus ascendens slightly curved along its entire length (curved at the union with the basi cranii only), and (7) absence of m. tympanopharyngeus (presence). The m. tympanopharyngeus was reported for *L. limellum* by Vera Candiotti (2004); however, the evidence presented in that paper (see Table 1, last paragraph and Fig. 4A in Vera Candiotti 2004) indicates that the structure identified as m. tympanopharyngeus of *L. limellum* is actually the m. levator arcuum branchialium IV.

The processus basicapsularis of the capsula auditiva is an interesting feature that is only present in *Pseudis*. Despite its conspicuous size, it was not reported previously in studies of the species *P. minuta* and *P. paradoxa* (Parker 1882; Lavilla and de Sá 1999; Haas 2003). The distal end of the processus basicapsularis coincides topographically with the greatly projected distal end of ceratobranchiales III and IV. Even though this process does not receive directly either muscles or ligaments, a strong sheath of fibrous tissue can be observed behind this process where the complex of mm. levator arcuum branchialium IV, dilatator laryngis and tympanopharyngeus originate. The function of this process seems to be muscular insertion; however, further studies will provide additional functional information about this rare structure, unique within anurans.

The occurrence of a reticulate cartilaginous connection between the spicules III and IV in *Hyla annectans*, *H. cinerea*, *L. limellum*, *Pseudis* spp. and *Smilisca baudinii* (Haas 1996, 2003; Lavilla and de Sá 1999; Vera Candiotti 2004) strengthens the relationship between some Hyline genera and the pseudids, but the presence of m. mandibulolabialis superior is a unique larval feature shared by all subfamilies of Hylidae (except for Hemiphractinae) (Haas 2003). In addition to this character (here found to be reversed in *P. cardosoi*), we provide a new chondrocranial character shared by all Hylidae (except Hemiphractinae): the position of the processus anterolateralis of the larval crista parotica. This feature was recorded in all hylids (except Hemiphractinae) in which the chondrocranium is known (Lavilla and Fabrezi 1987; de Sá

1988; Fabrezi and Lavilla 1992; Fabrezi and Vera 1997; D'heursel and de Sá 1999; Lavilla and de Sá 1999; Alcalde and Rosset 2003; Vera Candiotti *et al.* 2004). The processus anterolateralis also runs vertical in a few non-hylid species, such as *Hyperolius puncticulatus* (Haas 2003) and *Rana temporaria* (De Jongh 1968). However, these should be considered convergences because according to various phylogenetic schemes (Ford and Cannatella 1993; Haas 2003) these species are not related to Hylidae. Other authors (Ford and Cannatella 1993; Fabrezi 1996) have reported additional convergent characters between hylids and hyperoliids, such as the presence of claw-shaped terminal phalanges and intercalary elements in digits. The fact that the vertical processus anterolateralis occurs in hylids with different tadpole ecological types (macrophagous, microphagous, suspension-rasper, suctorial, benthic, nektonic, neustonic) supports the idea of a close phylogenetic relationship as opposed to adaptive convergence.

The relationships of pseudids are the subject of intense research, particularly from a cladistic viewpoint (see Introduction). Two recent molecular cladistic analyses have presented well-supported clades containing *Scarthyla goinorum* and *P. paradoxa* (Darst and Cannatella 2004), and *S. goinorum* and pseudids (Faivovich *et al.* 2005). The relationship between *Scarthyla* and pseudids was first found by da Silva (1998) and Barg (2003); however, these analyses differ in certain aspects. da Silva (1998) placed *Scarthyla* and pseudids in a polytomy with other hylids. Barg (2003) presented the pseudids as a well-supported monophyletic group nested in a more inclusive clade containing *Dendropsophus minutus* (30-chromosome group) and *S. goinorum*.

Barg's results are of great interest, since Vera Candiotti *et al.* (2004) reported that the tadpoles of *Dendropsophus namus* (other species of the 30-chromosome group) have muscle subarcualis rectus II–IV divided into two portions by a gap at the level of ceratobranchiale III, and the m. levator mandibulae lateralis inserted on the nasal sac, conditions similar to the typical pseudids' pattern. Faivovich *et al.* (2005) have found an inclusive clade containing pseudids, *Scarthyla*, the species of the group of the 30-chromosome, and the species of *Scinax*. The position of *Scinax* as a clade related to the monophyletic group of pseudids plus *Scarthyla* is very interesting because Alcalde and Rosset (2003) have found that some species of *Scinax* possess processus lateralis hyalis, the same process described herein for *Lysapsus limellum*.

As a final point, we want to remark that the knowledge of the chondrocranium and the cranial muscles of the genus *Scarthyla* and of more species of the 30-chromosome group is needed. To know if these species possess the characters here described for pseudids, *Lysapsus* and *Pseudis* is a necessary step to improve the knowledge of the pseudids relationships.

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Appendix I. Specimens examined and collection data.

Museum abbreviations: **CM** = Carnegie Museum (USA); **MACN** = Museo Argentino de Ciencias Naturales Bernardino Rivadavia (Argentina); **MHNLS** = Museo de Historia Natural de la fundación La Salle (Venezuela); **MLP** = Museo de La Plata (Argentina); **SMNS** = Staatliches Museum für Naturkunde (Germany).

Lysapsus limellus. From Laguna Benítez, Corrientes, Argentina. **MLP 3835**: stained and dissected ($n = 2$), stages 35 and 36; stained and cleared ($n = 3$), stages 32, 35 and 37.

Pseudis cardosoi. From Sao Francisco de Paulo, Pró-Mata, Rio Grande do Sul, Brazil. **SMNS 9301**: stained and dissected ($n = 1$), stage 35. **SMNS 9302**: stained and cleared ($n = 1$), stage 35.

Pseudis minuta. From El Palomar, Buenos Aires, Argentina. **MACN 15602–604**: stained and cleared ($n = 3$), stages 35, 36 and 37.

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Pseudis minuta. From Nueva Palmira, Uruguay. **MLP 3837**: stained and dissected ($n = 2$), stages 35 and 38.

Pseudis minuta. From Punta Indio, Buenos Aires, Argentina. **MLP 3838**: stained and dissected ($n = 1$), stage 40.

Pseudis paradoxa caribensis. From San Martín de Turumban (Anacoco), Rio Cuyuni, Bolívar, Venezuela. **MHNLS 7903**: stained, dissected and cleared ($n = 1$), stage 35.

Pseudis paradoxa occidentalis. From north-eastern Chaco Province, Argentina (26°53'27.5"S 59°01'06.1"W). **MLP 3839**: stained and dissected ($n = 1$), stage 38; stained and cleared ($n = 5$), stages 32, 35, 36, 38 and 39.

Pseudis paradoxa platensis. From Santa Ana, Corrientes, Argentina. **MLP 3841**: stained, dissected and cleared ($n = 6$), stages 26 ($n = 2$), 31, 35, 38 and 39.

Pseudis paradoxa paradoxa. Locality unknown. **CM 56000**: stained, dissected and cleared ($n = 1$), stage 38.