Hyoid Skeleton, Its Related Muscles, and Morphological Novelties in the Frog *Lepidobatrachus* (Anura, Ceratophryidae)

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ABSTRACT

Many traits of the skull of ceratophryines are related to the capture of large prey independently of aquatic or terrestrial feeding. Herein, detailed descriptions of the development of hyoid skeleton and the anatomy of muscles responsible for hyoid and tongue movements in *Lepidobatrachus laevis* and *L. llanensis* are provided and compared with those of other neobatrachians. The aquatic *Lepidobatrachus* has special features in its hyoid skeleton that integrates a set of derived features convergent with the conditions observed in non-neobatrachian anurans and morphological novelties (e.g., dorsal dermal hyoid ossification) that deviate from the generalized pattern found in most frogs. Further, reduction of fibers of muscles of buccal floor, reduction or loss of hyoid muscles (m. geniohyoideus rama lateralis, anterior pair of m. petrohyoideus posteriores), small tongue, and simplified tongue muscles are also morphological deviations from the pattern of terrestrial ceratophryines, and other aquatic ceratophryids (e.g., *Telmatobius*) that seem to be related to feeding underwater. The historical derived features shared with *Chacophrys* and *Ceratophrys* involved in megalophagy are conserved in *Lepidobatrachus* and morphological changes in the hyoglossal apparatus define a unique functional complex among anurans. Anat Rec, 292:1700–1712, 2009. © 2009 Wiley-Liss, Inc.

Key words: feeding; tongue; hyoid; aquatic life style; amphibian

Frog genera *Lepidobatrachus* (three species), *Chacophrys* (one species), and *Ceratophrys* (eight species) are included in Ceratophryinae. Most phylogenetic hypotheses agree in considering this lineage as monophyletic (Reig and Limeses, 1963; Laurent, 1986; Maxson and Ruibal, 1988; Haas, 2003; Fabrezi, 2006; Frost et al., 2006; among others). Adults are aquatic in *Lepidobatrachus* (Fig. 1A) and terrestrial in *Chacophrys* and *Ceratophrys*. Within Ceratophryinae, two alternative hypotheses have been proposed: (1) *Lepidobatrachus* is the basal taxon (Reig and Limeses, 1963; Laurent, 1986; Frost et al., 2006), implying that aquatic life style is the plesiomorphic condition and (2) *Chacophrys* or *Ceratophrys* are basal (Maxson and Ruibal, 1988; Fabrezi, 2006; Fabrezi and Quinzio, 2008), and the aquatic life style would have derived from terrestrial adults.

Lepidobatrachus spp., *Chacophrys pierottii*, *Ceratophrys cranwelli*, and *C. ornata* are endemic to lowlands of the semiarid Chaco region in South America. All adults of ceratophryines are megalophagous and cannibals (Ruibal and Thomas, 1988; Hanken, 1993),
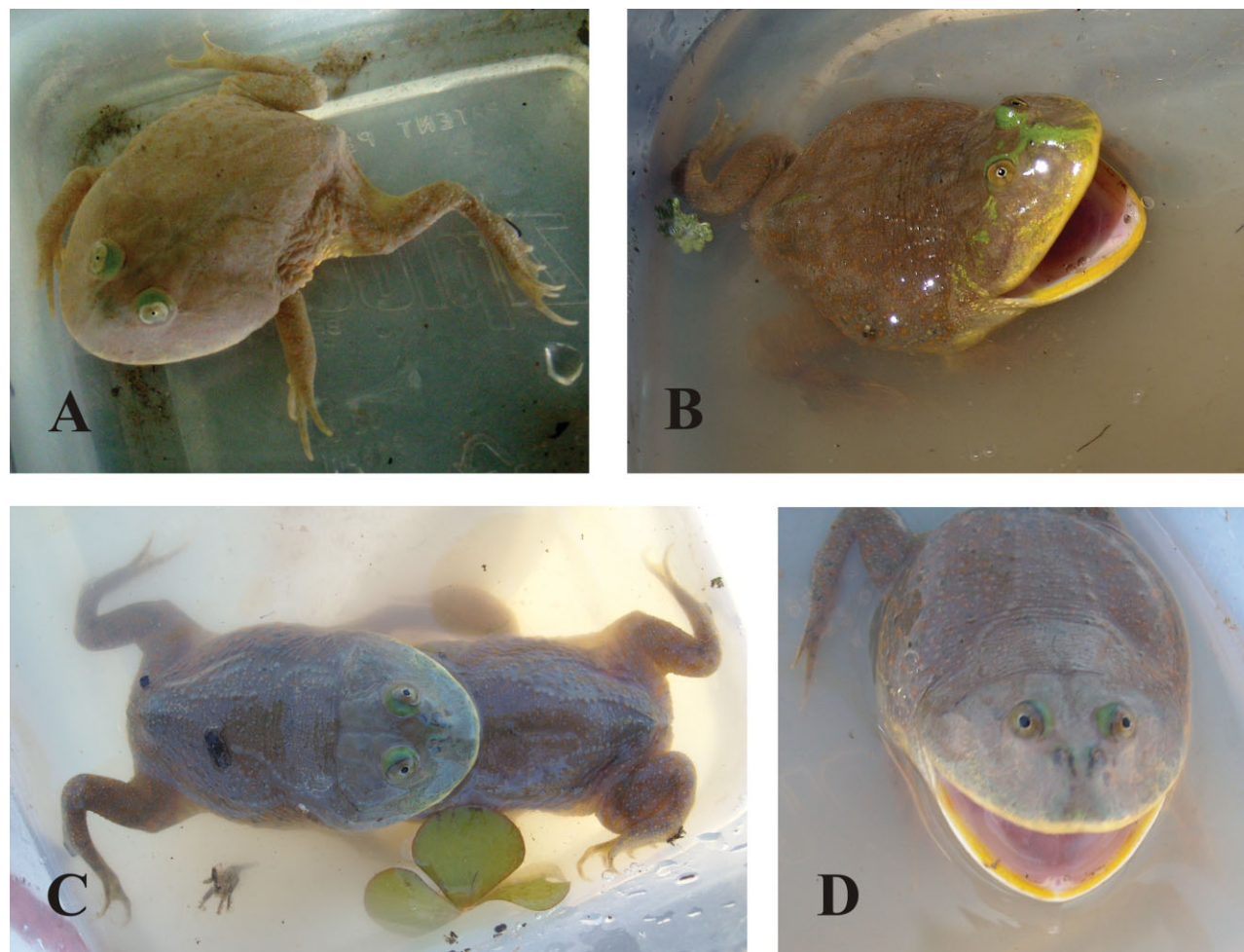
Fig. 1. The aquatic frog *Lepidobatrachus laevis*. **A:** During the wet season, postmetamorphic specimens are found in temporary ponds, where they eat tadpoles, frogs, snails, and large insects. **B:** The aggressive behavior is characteristic of ceratophryines. **C:** Cannibalism. One specimen makes effort to eat another one of similar size. **D:** The opened mouth displays fangs in lower jaw.

whereas tadpoles are very different from each other; macrophagous and specialized carnivorous in *Ceratophrys* and *Lepidobatrachus* (Ruibal and Thomas, 1988; Wassersug and Heyer, 1988), and typical generalized suspension feeders in *Chacophrys pierottii* (Faivovich and Carrizo, 1992; Quinzio et al., 2006). In the three genera, tadpole development is fast, and some metamorphic changes are predisplaced during larval growth producing an adult-like morphology in *Lepidobatrachus* (Fabrezi and Quinzio, 2008).

Adults of ceratophryines share a set of morphological traits related to feeding on large prey (Cei, 1981; Fabrezi, 2001; Fabrezi and Emerson, 2003; Fabrezi, 2006). They have extremely large and wide mouths (Fig. 1B,C) with the lower jaw articulating posteriorly to the craniovertebral joint. The mentomeckelian and the dentary are indistinguishably fused, and firmly sutured with the angulosplenial. The mandibular symphysis joins the irregularly ossified anterior end of each hemi mandible by dense fibrous connective tissue, forming an almost immovable suture. The pair of spur-like odontoids flanks the mandibular symphysis (Fig. 1D). Each odontoid projects anteriorly from the mentomeckelian-dentary ossification and is presumably used to bite the prey. The upper jaw is saw-like and bears numerous monocuspid and nonpedicellate teeth.

Other anatomic elements that could play a direct or indirect role in feeding, such as the hyoid skeleton, present shared traits among the three genera, and particular features in *Lepidobatrachus* (Fabrezi, 2006). Shared traits are the absence of the anterolateral processes and the strong ossification of the posteromedial processes, which in older specimens invades anteriorly the hyoid plate and reaches the hyoglossal sinus. Special features in *Lepidobatrachus* are the discontinuous hyalia and presence of a transverse dermal ossification in the hyoid plate, which was considered the parahyoid bone (Fabrezi, 2006).

Emerson (1977) showed that the hyoid apparatus may represent a morphological compromise for three functions (vocalization, breathing, and feeding) rather than an optimal morphology for any particular one. Nishikawa (2000) proposed that the hyoid apparatus

forms a base on which the tongue rests, and even though movements of the hyoid plate appear to play an important role in the buccal floor expansion and contraction during breathing and calling, the role of the hyoid in anuran feeding remains unclear. She (Nishikawa, 2000) described three mechanisms of tongue protrusion (mechanical pulling, inertial elongation, and hydrostatic elongation) which would be responsible for prey capture in most anurans. Nishikawa (2000) mapped the feeding mechanism in the anuran phylogeny in which *Ceratophrys* is the basal ceratophryine and found elongate tongues would have evolved independently in *Chacophrys* and *Ceratophrys*, with *Lepidobatrachus* being a mechanical puller. However, tongue protrusion was never observed during aquatic feeding in *Lepidobatrachus* but it would be able to protrude its tongue during terrestrial feeding (O'Reilly et al., 2002).

O'Reilly et al., (2002) discussed scenarios for the evolution of aquatic feeding in adult frogs, from which, based on distribution of aquatic behavior alone, it is plausible that aquatic foraging is a shared primitive character among many basal frogs. Forearm scooping to capture prey in water, as it was observed in *Bombina, Lepidobatrachus, Calyptocephalella, Rana, Pipa*, and *Xenopus* (Gray et al., 1997), appears to be an ancestral behavior used for prey transport, while tongue protrusion, jaw bending, and head flexion have evolved for terrestrial feeding (Nishikawa, 2000).

As mentioned, many traits of the skull of ceratophryines are related to capturing large prey independently of aquatic or terrestrial feeding; however, morphological variation of the hyoid apparatus and tongue within ceratophryines is not sufficiently explored and perhaps some of this variation could be related to distinctive feeding mechanism and/or life style. Given that the aquatic *Lepidobatrachus* has special features in its hyoid skeleton that deviate from the generalized pattern found in neobatrachians, and considering these skeletal changes could be complemented with changes in the hyoid and tongue muscles, a comprehensive understanding of hyoid development and hyoid and tongue musculature is critical. Herein, a detailed description of development of hyoid skeleton in *Lepidobatrachus laevis* and *L. llanensis* is presented and compared with that of other ceratophryines. In addition, anatomical interespecific variation of muscles responsible of hyoid and tongue movements within ceratophryines is provided and compared with taxa belonging to proposed outgroups. It is hoped that discussion of novel morphologies, homology, and interspecific variation may be useful for understanding morphological complexes that could be involved in the evolution of different functions concomitant with life styles.

MATERIAL AND METHODS

Data in this study were obtained from two different sources: (1) description of the ontogeny of hyoid skeleton in ceratophryines and (2) comparisons of adult hyoid musculature between ceratophryines and other selected neobatrachians. Species, specimen numbers, and collection data are listed in Appendix I.

The study of the ontogeny of the hyoid skeleton was performed in skeletal preparations of both larvae and adults of cleared and double-stained whole-mount speci-mens for cartilage and bone of *Lepidobatrachus laevis, Lepidobatrachus llanensis*, and *Chacophrys pierotti*. Specimens were collected, euthanatized in an aqueous solution of chloretone, and fixed in neutral-buffered formalin (4%) in the field. Larval series were staged following the standard table of Gosner (1960) from stages in which all five toes are separated (Stage 36) up to stages when tail is completely lost (Stage 46). Skeletal preparations were obtained following the protocol by Wassersug (1976). Data on the ontogeny of hyoid skeleton in *Ceratophrys cranwelli* and *C. cornuta* were taken from literature (Fabrezi and García, 1993; Wild, 1997).

The study of hyoid musculature was conducted in adult specimens of four ceratophryines (*Ceratophrys cranwelli, Chacophrys pierottii, Lepidobatrachus laevis*, and *Lepidobatrachus llanensis*). For comparisons, adult specimens of *Telmatobius oxycephalus, Odontophrynus americanus*, and *Pseudis platensis* were also included in this analysis. *T. oxycephalus* is an aquatic taxon belonging to the Telmatobiinae clade that is considered the sister group of Ceratophryinae (Grant et al., 2006). Terrestrial *Odontophrynus americanus* has been considered to be related to ceratophryines (Laurent, 1986). *Pseudis platensis* is an aquatic taxon unrelated with ceratophryines, which offer the possibility of describing morphological convergence. Anatomical descriptions of muscles were based on manual dissections of whole alcohol preserved specimens that were double stained with Alcian Blue and Alizarin Red S but not cleared. These preparations were preserved in 70% ethanol and at the time of observation, temporarily stained with iodine solution to obtain a better contrast of muscles (Bock and Shear, 1972).

Observations and illustrations were made with a Nikon SMZ 800 stereomicroscope with attached camera lucida. Terminology and criteria to identify skeletal structures follow those of Haas (2003) and Maglia and Pugener (1998), and for muscle descriptions Trewavas (1933), Horton (1982), and Burton (1998).

To reconstruct the phylogenetic history of the observed variation, we used two alternative hypotheses of relationships among ceratophryines (Maxson and Ruibal, 1988; Frost et al., 2006) and considered data from a recent molecular phylogenetic study that proposed Ceratophryinae and Telmatobiinae are forming a monophyletic clade (Grant et al., 2006). Character change was traced along those alternative hypotheses using a parsimony software (TNT of Goloboff et al., 2003), applying deltran optimization in those cases of ambiguous assignation for internal nodes. Optimization of characters (hyoglossal morphology) allowed us to describe a hypothesis of evolutionary change taking simultaneously into consideration life style (terrestrial versus aquatic) and feeding features in the Ceratophryinae.

RESULTS
The Hyoid Skeleton

Ontogeny of the hyoid skeleton in Chacophrys pierottii. The larval hyobranchial skeleton is formed by the paired ceratohyalia that meet medially in a single pars reuniens; the pars reuniens is continued in the basibrachial that bears a posteroventral pointed projection named processus urobranchialis. The basibranchial

attaches by connective tissue to the hypobranchial plates. The hypobranchial plates are articulated and each hypobranchial plate supports four ceratobranchialia. The ceratohyale presents four processes: anterior, posterior, lateralis, and hyoquadrate. The latter is a large condylus that articulates with the ventral side of palatoquadrate. The ceratobranchialia bear numerous cartilaginous rays, and are joined with each other by commisurae terminalis. The ceratobranchialia are fused to the hypobranchial plate, and the Ceratobranchialia II and III are connected proximally by the cartilaginous processus branchialis. Spiculae I–IV are horizontal elongate cartilages originating dorsally from the proximal ends of the ceratobranchialia. The described hyobranchial skeletal features do not change until metamorphosis has begun (Fig. 2A).

At the time of emergence of forelimbs, spiculae, processus branchialis and commissurae terminalis start to erode. After resorption of the ceratobranchialia, each hypobranchial plate has two processes (lateral and posterior) resulting from reduction of the Ceratobranchialia I and IV, respectively (Fig. 2B). Next, the anterior and posterior processes of the ceratohyale are less evident, and the ceratohyale tapers gradually although the hyoquadrate processus retains its condylar shape. The proximal portions of the ceratohyalia (between the pars reuniens and the processus anterior) become slender, weak, and finally disrupt. The pars reuniens and the basibranchial form the anterior piece of an incipient hyoid plate delimiting the hyoglossal sinus (Fig. 2C). The pars reuniens + basibranchial expand in an open U-shaped cartilage in which the arms represent the larval pars reuniens and the medial portion, transversally enlarged, corresponds to the basibranchial extended anteriorly to the hypobranchial plates (Fig. 2C,D). As metamorphosis advances, the ceratohyale acquires uniform width for complete disappearance of the anterior and posterior processes, and bears vestiges of the hyoquadrate condylus in its rounded distal tip (Fig. 2D). The pars reuniens + basibranchial cartilage remains separated by sutures from ceratohyalia and hypobranchial plates (Fig. 2D,E). The residual larval processes (lateral and posterior) representing attachments of the Ceratobranchialia I and IV to the hypobranchial plate present changes. The posterior process is well differentiated in the adult posteromedial process, with a distal tip insinuating its epiphysis. Adjacent to the lateral process, a cartilaginous condensation appears indicating the adult posterolateral process develops from new cartilage (Fig. 2D,E). At the end of metamorphosis (Fig. 2F), the ceratohyalia are already slender, longer, and curved; and vestiges of the hyoquadrate process appears separated from the ceratohyale. The medial fusion of the hypobranchial plates is incomplete, and a suture dividing the anterior components of the hyoid (ceratohyale-pars reuniens-basibranchial) is conserved. After completion of metamorphosis, ossification in the posteromedial processes advances rapidly from the middle of the diaphysis in proximal and distal directions (Fig. 2G). Adults of *Chacophrys pierottii*, as well as *Ceratophrys cranwelli*, present posteromedial ossifications invading the hyoid plate and part of the posterolateral processes (Fig. 4A,B).
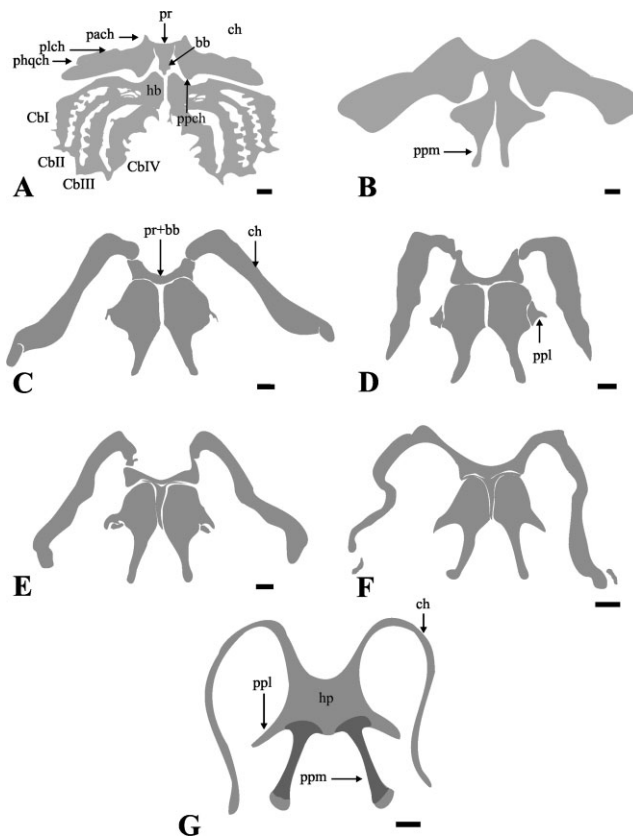

Fig. 2. Hyobranchial apparatus and its metamorphosis in *Chacophrys pierottii*. **A:** Fully developed larval skeleton at Stage 39. **B:** Advanced disappearance of larval ceratobranchialia at the end of larval Stage 42. **C:** Larval Stage 43. Elongation of ceratohyalia is evident. **D:** Larval Stage 44. Additional new cartilage participates in the differentiation of posterolateral processes. **E:** Larval Stage 45. Formation of the posterolateral process is incomplete. **F:** Stage 46. At the end of metamorphosis the hypobranchialia are not fully fused. The larval ceratohyale, pars reunions, and basibranchial are forming the anterior portion of the adult hyoid. **G:** Adult male. The hyoid is a single piece with well ossified posteromedial processes. Abbreviations: bb, basibranchial; CbI–CbIV, ceratobranchialia I to IV; ch, ceratohyale; hb, hypobranchial; hp, hyoid plate; pach, processus anterior of ceratohyale; phqch, processus hyoquadrate of ceratohyale; plch, processus lateralis of ceratohyale; ppch, processus posterior of ceratohyale; ppl, posterolateral process; ppm, posteromedial process; pr, pars reuniens. Dark gray areas indicate ossification. Scale bar is 1 mm.

Ontogeny of the hyoid skeleton in Lepidobatrachus llanensis and L. laevis. The larval skeleton of *Lepidobatrachus* is a single piece with its components entirely fused (Fig. 3A). The ceratohyalia converge medially in the pars reuniens, which has long arms that delimit a V-shaped sinus. The basibranchial is short and bears the stout processus urobranchialis. The hypobranchial plates are fused. The ceratohyale has no processus lateralis. The spiculae are also absent. The ceratobranchialia lack cartilaginous rays. These features do not change until metamorphosis has begun.

Disto and proximal erosion of the ceratobranchialia denotes the beginning of the hyobranchial skeleton metamorphosis (Fig. 3B,C). The incipient hyoid plate, formed by the fused hypobranchial plates, presents blunt
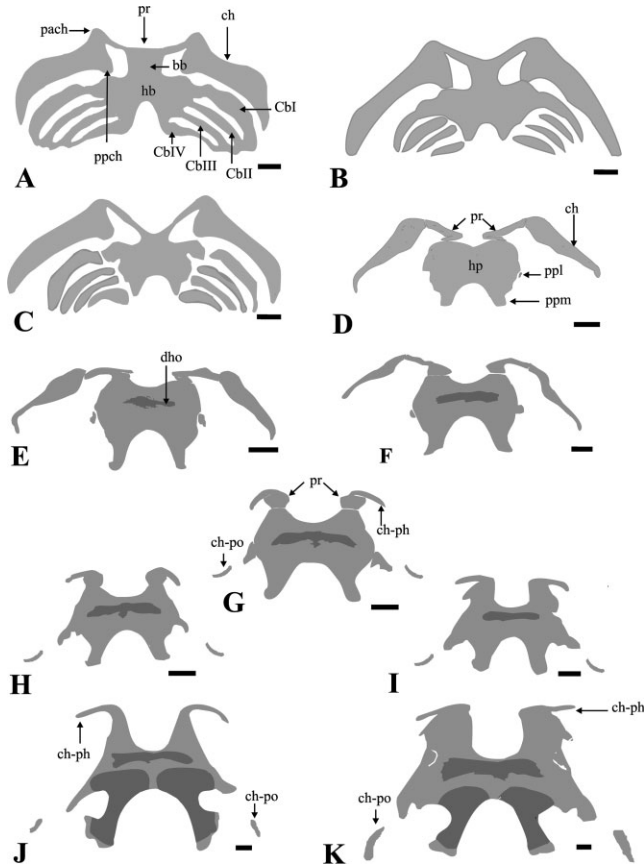
Fig. 3. **A-G:** Hyobranchial apparatus and its metamorphosis in *Lepidobatrachus llanensis*. **H, J:** Juvenile hyoid of *L. llanensis*. **I, K:** Juvenile hyoid of *L. laevis*, with the same pattern of development. A: Fully developed larval skeleton at Stage 39. B and C: Disappearance of larval ceratobranchialia during larval Stage 42. The basibranchial is reduced and elongation of the pars reuniens is remarkable. D: Larval Stage 43. Elongation of ceratohyalia is evident as well as the lateral displacement of the pars reuniens. E: Larval Stage 44. Differentiation of posterolateral processes from de novo cartilages begins. Dermal dorsal ossification has appeared. F: Larval Stage 45. The ceratohyale is shorter, and the pars reuniens is wider in the limits of the hyoid plate. G: Stage 46. The larval ceratohyale is reduced to two short pieces, the otic and the hyoid. The pars reuniens is sutured to the hyoid plate. H: Juvenile hyoid in *L. llanensis* before ossification of posteromedial processes. I: Juvenile hyoid in *L. laevis* before ossification of posteromedial processes. J: Juvenile in *L. llanensis*. The hyoid is a single piece with well ossified posteromedial processes. K: Juvenile in *L. laevis*. Abbreviations: ch-ph, pars hyoid of the hyale; ch-po, pars otic of hyale; dho, dorsal hyoid ossification; other abbreviations as in Fig. 1. Dark gray areas indicate ossification. Scale bar is 2 mm.
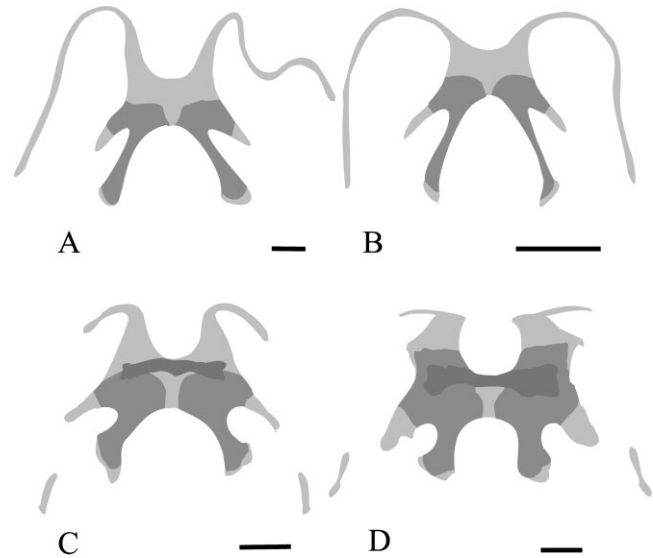

Fig. 4. Adult hyoid skeleton in ceratophryines. **A:** *Ceratophrys cranwelli* (female (SVL 85 mm). **B:** *Chacophrys pierottii* (male, SVL 53 mm). **C:** *Lepidobatrachus llanensis* (male, SVL 76 mm). **D:** *Lepidobtrachus laevis* (female, SVL 99 mm). Dark gray areas indicate ossification. Scale bar is 5 mm.

with the ceratohyale and the hyoid plate (Fig. 3E). An incipient dermal ossification starts to develop on the dorsal face of the hyoid plate which has developed a bulky posterior process insinuating the adult posteromedial process (Fig. 3E). Simultaneously, two *de novo* cartilaginous condensations appear laterally to the hyoid plate that will form the posterolateral processes of the adult hyoid (Fig. 3E). Before finishing the metamorphosis, the ceratohyale is slender and shorter and conserves the weak cartilaginous connection with the pars reuniens (Fig. 3F). The dorsal dermal ossification attached to the hyoid plate grows transversally (Fig. 3F). At the end of metamorphosis, each ceratohyale is reduced to a short and slender segment fused to the broad pars reuniens (Fig. 3G). A cartilaginous segment, representing vestiges of the distal disruption of the ceratohyale, becomes attached to the otic capsule. The cartilaginous posterolateral process and the ceratohyale + pars reuniens remain weak cartilaginous unions with the hyoid plate (Fig. 3G). Postmetamorphic growth of the hyoid skeleton involves ossification of the posteromedial processes, which invades proximally the hyoid plate reaching the hyoglossal sinus in older specimens and growth of the dorsal ossification (Figs. 3H–K and 4C,D).

Buccal Floor, Hyoid, and Tongue Muscles

The three genera of ceratophryinae share a common pattern of superficial throat musculature in which the most anterior fibers of m. intermandibularis are directed posteromedially, forming an apical supplementary slip and the more posterior fibers of m. intermandibularis radiate from the median aponeurosis (Figs. 5A and 6A). This pattern has already been observed in these taxa and in *Batrachophrynus, Calyptocephalella, Insuetophrynus, Odontophrynus*, and *Telmatobufo* by Burton (1998).

and short processes (lateral and posterior) resulting from reduction of the ceratobranchialia I and IV, respectively. The former disappears immediately. During subsequent metamorphic stages (Fig. 3D), the reduced basibranchial is incorporated into the hyoid plate and simultaneously each half of the pars reuniens enlarge. The anterior and posterior processes of ceratohyale are eroded and the ceratohyale becomes slender distally by reduction of the hyoquadrate processus. Each part of the pars reuniens first placed obliquely turns to a transverse position, and presents weak cartilaginous connection
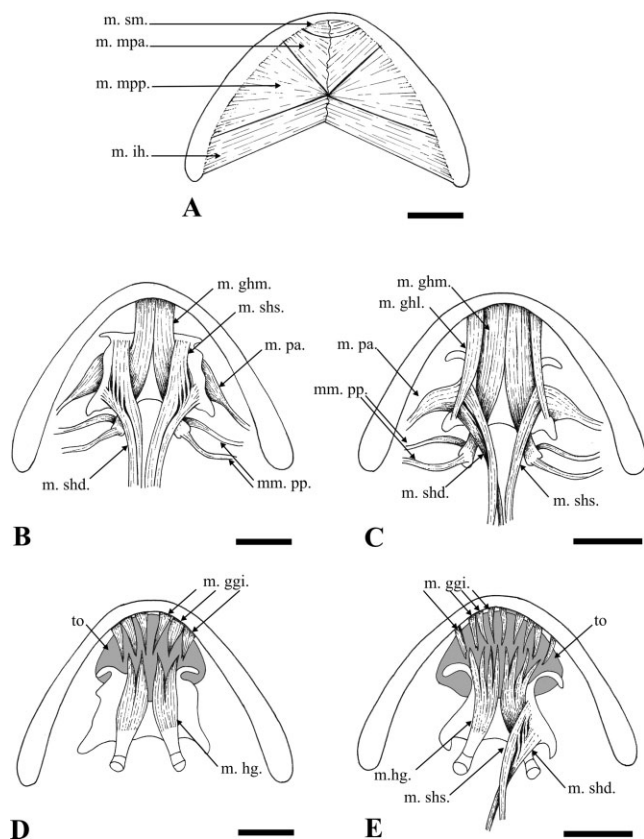
Fig. 5. Buccal floor, hyoid, and tongue muscles in *Lepidobatrachus laevis* (**A–C**), *L. llanensis* (**D–E**). A: Buccal floor muscles. The M. intermandibularis posterior is divided in two portions that differ in the orientation of the fibers. The muscles intermandibularis and interhyoideus are formed by loose fibers. B and D: Hyoid muscles. B: The m. geniohyoideus rama lateralis is absent. D: The m. geniohyoideus rama lateralis is reduced. B and D: There are two pairs of m. petrohyoidei posteriores. The m. sternohyoideus have two ramii (ventral and dorsal) that vary in their relationships and attachments on the hyoid. C and E: Tongue muscles. The m. hyoglossus is completely divided. The m. genioglossus is simple, only formed by interdigitating fibers. Abbreviations: m. ggi., m. genioglossus pars interdigitated; m. ghl., m. geniohyoideus lateralis; m. ghm., m. geniohyoideus medialis; m. hg., m. hyoglossus; m. ih., m. interhyoideus; m. mpa, m. intermandibularis pars anterior; m. mpp, m. intermandibularis pars posterior; m. pa., m. petrohyoideus anterior; mm. pp., mm. petrohioidei posteriores; m. shd., m. sternohyoideus dorsalis: m. shv., m. sternohyoideus ventralis; m. sm., m. submentalis; to, tongue. Scale bar is 10 mm.

Fig. 6. Buccal floor, hyoid, and tongue muscles in *Chacophrys pierottii*. **A**: Buccal floor muscles. The M. intermandibularis posterior is divided in two portions that differ in the orientation of the fibers. **B**: Hyoid muscles. The m. geniohyoideus rama lateralis is well developed. Three pairs of m. petrohyoidei posteriores are present. The m. sternohyoideus have two ramii (ventral and dorsal) without shared fibers. **C**: Tongue muscles. The m. hyoglossus with internal fibers forming a medial group. The m. genioglossus has two components: the deep interdigitating fibers and the superficial ventral component. Abbreviations: m. ggv., m. genioglossus pars ventralis; and as in Fig. 5. Scale bar is 10 mm.

In *Chacophrys* and *Ceratophrys*, the numerous fibers of superficial throat muscles (m. submentalis, m. intermandibularis, and m. interhyoideus) form a thick and compact arrangement, but in *Lepidobatrachus*, the m. intermandibularis and m. interhyoideus have fewer fibers forming a transparent cover. In other species, for example, *Pseudis platensis*, fibers of the m. intemandibularis and m. interhyoideus are transversally oriented and no subdivision of m. intermandibularis is present (Fig. 7A).

The m. geniohyoideus is divided into m. g. lateralis (ventral and superficial) and m. g. medialis in the ceratophryines *Chacophrys, Ceratophrys cranwelli*, an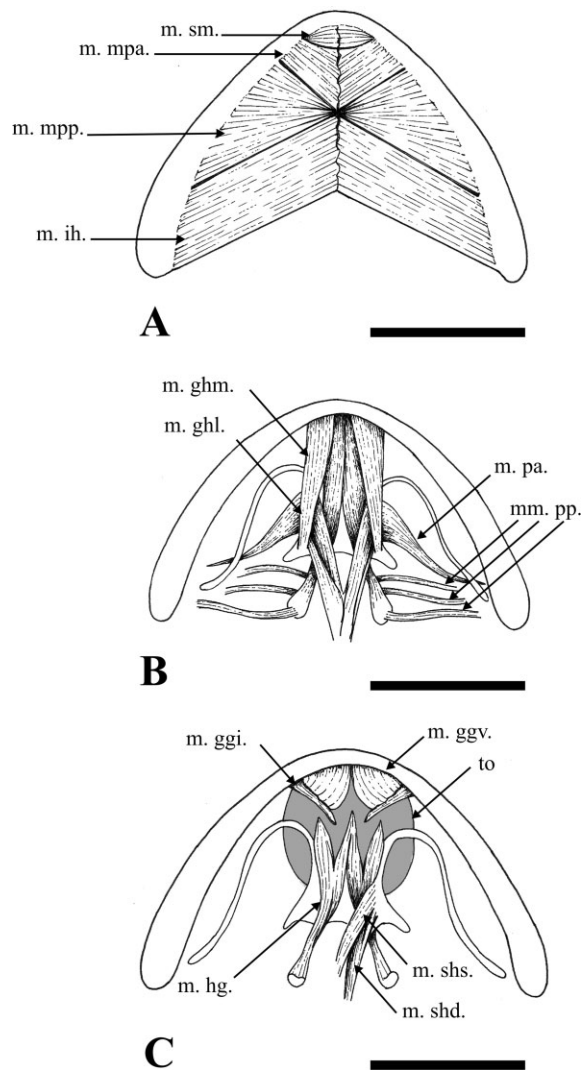d *Lepidobatrachus llanensis* (Figs. 4D and 5B), and also in *Odontophrynus americanus, Telmatobius oxycephalus*, and *Pseudis platensis* (Fig. 7B,D). The division of the m. geniohyoideus is complete, and the m. sternohyoideus crosses ventrally to the m. g. medialis. Both divisions are attached in front of the lower jaw, the m.g. medialis near the symphysis, and the m. g. lateralis more laterally. Fibers of the m. g. lateralis run to the posterolateral process of the hyoid and fibers of the m. g. medialis insert proximal to the epiphysis of the posteromedial process. In *L. llanensis*, the m. g. lateralis presents fewer fibers than in *C. cranwelli* and *Chacophrys*. In *L. laevis*, the m. g. lateralis is absent in adults (Fig. 5B),
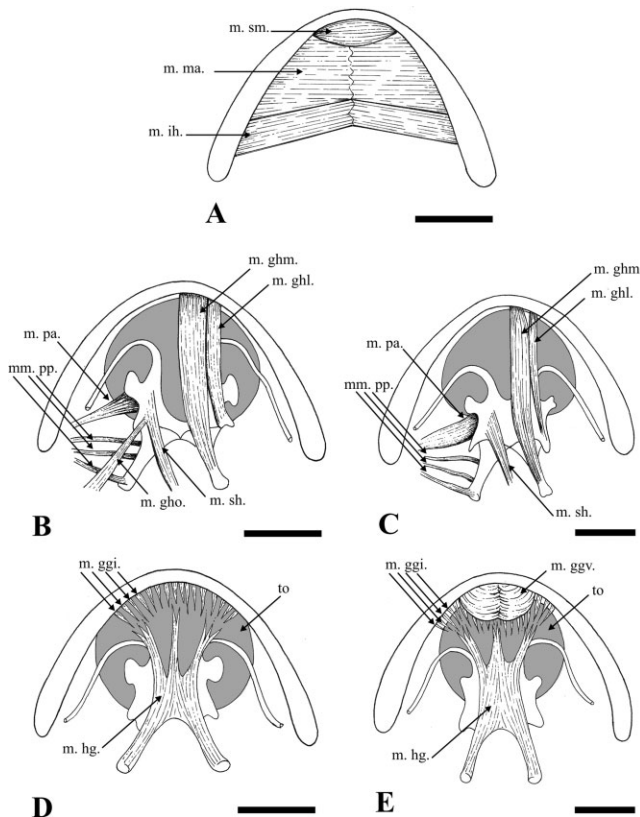
Fig. 7. Buccal floor, hyoid, and tongue muscles of other neobatrachians. **A:** Buccal floor muscles. The muscle intermandibularis is single in *Pseudis platensis*. **B:** Hyoid muscles in *P. platensis*. The m. geniohyoideus rama lateralis is well developed. Three pairs of m. petrohyoidei posteriores are present. The m. sternohyoideus is single and the m. omohyoideus is present. **C** and B: Hyoid muscles in *Odontophrynus americanus*. The m. geniohyoideus rama lateralis and three pairs of m. petrohyoidei posteriores are present. The m. sternohyoideus is single. **D:** Tongue muscles in *P. platensis*. The m. hyoglossus with internal fibers forming a medial group. The m. genioglossus has only differentiated many divisions of deep interdigitating fibers. **E:** Tongue muscles in *O. americanus*. The m. hyoglossus with internal fibers forming a medial group. The m. genioglossus has two components: the deep interdigitating fibers and the superficial ventral component. Abbreviations: m. ma, m. intermandibularis; m. gho., m. omohyoideus, m. sh., m. sternohyoideus, other abbreviations as in Figs. 5 and 6. Scale bar is 5 mm.

but few fibers of this division are present in recently metamorphosed specimens.

In *Odontophrynus americanus, Telmatobius oxycephalus*, and *Pseudis platensis*, the m. sternohyoideus is single and inserts along the lateral side of the hyoid plate (Fig. 7B,D). In ceratophryines, the m. sternohyoideus is formed by two divisions, dorsal (deep) and ventral (superficial) divisions (Figs. 5B,D and 6B). Fibers of the dorsal division are attached on the ventral surface of the hyoid plate, along the base of the posterolateral process, oriented medially and posteriorly, and joined on the dorsal face of the cartilaginous epicoracoid, with fibers that continue with the m. rectus abdominus. The ventral division has fibers originating along margins of the hyoid plate, anterior to the origin of fibers of the dorsal divi-

sion. The ventral division of the m. sternohyoideus crosses obliquely the dorsal component and inserts on the epicoracoid. In *Lepidobatrachus laevis*, the dorsal and ventral divisions of the m. sternohyoideus are well separated at the origin (Fig. 5B). Fibers of the ventral component are attached anteriorly and transversally, almost on the limit between the hyoid plate and the hyale. Few lateroexternal and deep fibers of this ventral division diverge to be incorporated into the dorsal component of the m. sternohyoideus in both species of *Lepidobatrachus*.

The m. omohyoideus is absent in ceratophryines and other studied species. This muscle is present in *Pseudis platensis*, with fibers attached to the ventral surface of the hyoid plate, near the base of the posterolateral and posteromedial processes (Fig. 7B). Insertion of this muscle is located on the ventral face of the scapula.

The m. petrohyoideus anterior originates on the otic region of skull and its fibers diverge ventrally to insert along the lateral edge of the hyoid plate in ceratophryines (Figs. 5B,D and 6B). This muscle is less compact with few fibers in *L. laevis*. In the other studied species (*Odontophrynus americanus, Telmatobius oxycephalus*, and *Pseudis platensis*), the insertion of this muscle is located on the anterior edge between the anterolateral and posterolateral processes (Fig. 7B,D).

There are three pairs of mm. petrohyoidei posteriores in *Ceratophrys cranwelli, Chacophrys pierottii, Odontophrynus americanus, Telmatobius oxycephalus*, and *Pseudis platensis* that are attached to the skull, near and posterior to the attachment of the m. petrohyoideus anterior (Figs. 6B and 7B,D). The most anterior of mm. petrohyoidei posteriores inserts proximally, on the dorsal face of the posteromedial process; the second one inserts medially, on the edge of the posteromedial process; and the third on the epiphysis of this process (Fig. 6B). In *Lepidobatrachus* spp., the most anterior pair is absent (Fig. 5B,D).

The m. hyoglossus originates from the ventral surface of the posterior end of the posteromedial process, with fibers running anteriorly to divide into a variable number of discrete portions, which radiate out to different parts of the tongue. Medial fibers of this muscle converge to join with the opposite in a single segment that penetrates into the tongue in *Ceratophrys cranwelli, Chacophrys pierottii, Odontophrynus americanus, Telmatobius oxycephalus*, and *Pseudis platensis* (Figs. 6C and 7C,E). In *Lepidobatrachus* spp., the medial fibers remain separated and each m. hyoglossus conserves its individuality (Fig. 5C,E). The lateral fibers diverge from the medial division before running into the tongue in a single group in *Chacophrys* and *L. laevis*, span in two or three groups in *Ceratophrys* and *L. llanensis*, and in three or more groups in the other species. The m. hyoglossus attaches proximally to the origin of the m. geniohyoideus. In *Lepidobatrachus* spp., the m. hyoglossus presents a connective sheath covering the entire length of the posteromedial process and its fibers differentiate at level of the hyoid plate (Fig. 5C,E).

The m. genioglossus originates on the lingual edge of the mandible and extends caudally for a short distance before entering in the tongue. The m. genioglossus has a complex structure and it is formed by different components. Horton (1982) has described four patterns of organization of the m. genioglossus among anurans. In

Ceratophrys, Chacophrys, *Telmatobius*, and *Odontophrynus*, the most prominent component corresponds to that named m. genioglossus ventralis that forms a solid structure with fibers oriented antero–posteriorly changing gradually to transversal orientation (Figs. 6C and 7E). Dorsal to this muscle, there is an interdigitated component, which has fibers radiating caudally from their origin on the mandible. Fibers of this component interdigitate with fibers of the m. hyoglossus and insert into the tongue. The m. genioglossus in *Lepidobatrachus* spp. and *Pseudis platensis* is only formed by the interdigitated component (Figs. 5C,E and 7C). This component presents many divisions in *P. platensis*, whereas it has few divisions and loose fibers in *Lepidobatrachus* spp.

Interspecific comparisons of the relative size of the tongue with respect to the lower jaw length reveal that *Telmatobius oxycephalus, Odontophrynus americanus, Pseudis platensis*, and *Ceratophrys cranwelli* have larger tongues (more than 50% of the lower jaw length). In *C. pierottii* and *Lepidobatrachus* spp., tongues are shorter.

DISCUSSION

Hyoid Skeleton

In most anurans, the hyoid apparatus consists of a central cartilaginous corpus (hyoid plate) that bears three pairs of cartilaginous processes (hyale, anterolateral, and posterolateral), and a pair of ossified processes (posteromedial). Only the pipid *Hymenochirus* has ossified hyalia and the pipid *Pipa* lacks hyalia (Ridewood, 1897b; Cannatella and Trueb, 1988). Hyalia are discontinuous in some anurans (scaphiopodids, *Pelodytes*, *Rhinophrynus*, and *Lepidobatrachus* spp.) having two segments, the otic (caudal) and the hyoid (anterior), whereas in megophryids, the otic segment is absent (Cannatella, 1985). In *Pelodytes punctatus* (Ridewood, 1897a) and *Spea bombifrons* (Wiens, 1989), the intermediate segment of the ceratohyale is eroded to separate the otic segment, which is a conspicuous cartilage with the anterior end expanded and connected by ligaments with the hyoid segment. Differently, in *Lepidobatrachus*, the segments of the hyale are short and disconnected to each other. Proposed functional explanation of the disjunct hyalia was that they allow the hyoid to move further anteriorly during feeding in *Rhinophrynus dorsalis* and scaphiopodids (Trueb and Cannatella, 1982; Trueb and Gans, 1983; O'Reilly and Nishikawa, 1995; Nishikawa, 2000).

There is variation in the participation of the larval components that are integrated in the hyoid plate. The pars reuniens is reduced in basal anurans (Wiens, 1989; Haas, 1997; Maglia and Pugener, 1998; Pugener and Maglia, 1998) and in most anurans, the pars reuniens and basibranchial are incorporated into the anterior half of the hyoid plate (Sedra and Michael, 1958; Chacko, 1965; de Jongh, 1968; Haas, 1999; Fabrezi and Goldberg, 2009; among others). In *Chacophrys pierottii* and *Ceratophrys* spp. (Fabrezi and García, 1993; Wild, 1997), these elements only form the anterior margin of the hyoid plate. In *Lepidobatrachus* spp., the cartilaginous larval pars reuniens constitutes the base of each reduced adult hyale, and the basibranchial could be reduced to minimal and medial portion of the margin of the hyoid plate. A minor participation of the anterior larval cartilages in the formation of the hyoid plate in the cerato-

phrynes could have consequence in the shortness of the adult structure, which is extreme in *Lepidobatrachus*.

Two interesting features concerning the larval pars reuniens in *Lepidobatrachus* were recorded: (1) metamorphic changes suggest it is paired. This fact provides support to confirm its identity as hypohyalia (de Beer, 1937); and (2) unlike in most anurans, the pars reuniens is incorporated into the reduced hyale. Thus, the discontinuous hyale of *Lepidobatrachus* does not present ontogenetic similitude with those of pelodytids and scaphiopodids, in which the larval pars reuniens is almost absent and the hyale forms an expanded anterior cartilage by fusion with the anterolateral process (Ridewood, 1897a; Wiens, 1989).

The anterolateral process develops at the end of metamorphosis by differentiation of additional new cartilage that is incorporated to the hyoid plate. The anterolateral processes are absent in pipids (Ridewood, 1897b; Cannatella and Trueb, 1988), *Rhinophrynus* (Trueb and Cannatella, 1982), *Ascaphus* (Canatella, 1985), *Leiopelma* (Trewavas, 1933), megophryids (Trewavas, 1933; Cannatella, 1985), the three ceratophryine genera, and *Phyllomedusa* spp. (Trewavas, 1933; Fabrezi, 2006). The absence of anterolateral process seems to be more frequent among basal anurans.

Differentiation of the posterolateral process occurs in the place where the larval Ceratobranchial I was attached to the hypobranchiale plate (Sedra and Michael, 1958; Chacko, 1965; de Jongh, 1968). This process may grow projecting from the hypobranchiale cartilage or develop from additional new cartilage that fuses to the hyoid plate at the end of metamorphosis in *Chacophrys* and *Lepidobatrachus*. Absence of the posterolateral processes is found within Arthroleptidae and Hyperoliidae (Laurent, 1978).

Differentiation of the posteromedial process occurs from the hypobranchial, in the place where the Ceratobranchial IV joined with the hypobranchial plate (Ridewood, 1897a,b; Sedra and Michael, 1958; Chacko, 1965; de Jongh, 1968). Proximal ossification of the posteromedial process reaches the hyoid plate in most anurans, invades it anteriorly or conserves a cartilaginous segment. In ceratophryines, during postmetamorphic life, ossification of the posteromedial processes advances gradually until it reaches the anterior half of the hyoid plate or the anterior margins of the hyoid plate in *Chacophrys* and *Lepidobatrachus* (Fig. 3 in Fabrezi, 2006). This extensive ossification of the posteromedial processes may provide strength to the hyoid plate.

The bony transverse bar that attaches on the dorsal surface of the hyoid plate during the metamorphosis of *Lepidobatrachus* spp. and older specimens of *Chacophrys pierottii* (Fabrezi, 2006) reinforces the robustness of the hyoid plate in these taxa. Fabrezi (2006) erroneously identified this dermal ossification as the anuran parahyoid. The anuran parahyoid is a dermal bone attached medially on the ventral surface of the hyoid plate of some basal anurans, and fibers of m. sternohyoideus may originate on it (Trewavas, 1933). Cannatella (1985) depicted the parahyoid in *Leiopelma, Barbourula, Alytes*, and *Pelodytes* as a V or Y shaped bone, a small and irregular ossification in *Bombina*, and a transversally elongated bone in *Ascaphus*. The parahyoid of *Rhinophrynus* is a transverse ossification extending between lateral margins of the hyoid plate (Trueb and

TABLE 1. Noteworthy features in the hyoid skeleton of Ceratophryines compared with respect to data of hyoid skeleton in other anurans

Hyoid skeleton	*Ceratophrys*/*Chacophrys*	*Lepidobatrachus* spp.	Literature data
Anterolateral process	Absent		Absent in pipoids, *Ascaphus, Leiopelma,* megophryids, and *Phyllomedusa*
Posterolateral process	Outgrowth of the incipient hyoid plate in *Ceratophrys*; additional new cartilage that fuses to the hyoid plate in *Chacophrys* and *Lepidobatrachus*		Outgrowth of the incipient hyoid plate or additional new cartilage that fuses to the hyoid plate. Absent in Arthroleptidae and Hyperoliidae
Posteromedial process	Proximal ossification of the posteromedial process invading the anterior half of the hyoid plate up to anterior margins of the hyoid		Proximal ossification of the posteromedial process extended up to the hyoid plate in most anurans. In hyperoliids, the ossification does not reach the hyoid plate and the posteromedial process presents a proximal cartilaginous segment
Larval pars reuniens and basibranchial	Forming the most anterior portion of the hyoid plate	Basibranchial reduced to the anterior margin of the hyoid plate. Pars reuniens incorporated at the base of the hyale	For most anurans, forming the anterior half of the adult hyoid plate
Hyalia	Cartilaginous and continuous	Cartilaginous and discontinuous (short hyoid and otic segments)	Cartilaginous and continuous in most anurans. Discontinuous with expanded hyoid and otic segments in scaphiopodids, *Pelodytes,* and *Rhinophrynus*; without otic segment in megophryids
Dermal ossification attached to the hyoid plate	Absent. However, older individuals of *Chacophrys* may bear a dorsal transversal ossification	Dorsal transversal ossification	Parahyoid: Ventral small ossification in *Bombina.* Ventral Y or V shaped ossification in *Barbourula, Alytes,* and *Pelodytes.* Ventral transversal ossification in *Ascaphus* and *Rhinophrynus*

Cannatella, 1982) and shares certain resemblance with the ossification of some ceratophryines. However, the parahyoid is a ventral dermal ossification whereas the ossification in *Lepidobatrachus* is dorsal. This dorsal hyoid ossification has not been described in other anurans and should be considered a morphological novelty (Müller and Wagner, 1991). Fabrezi (2006) and Fabrezi and Quinzio (2008) described acceleration and early onset of adult-like features in tadpoles of *Lepidobatrachus* spp., among these features the dorsal hyoid bone appears during metamorphosis, before ossification of the posteromedial processes. Differently, this bone is present in older specimens of *Chacophrys pierottii* (Fabrezi, 2006). The early presence of the dorsal hyoid bone in *Lepidobatrachus* and its occasional occurrence later in the ontogeny of other ceratophryines suggest this morphological novelty is part of the developmental program of ceratophryines, with two alternative hypotheses for the later onset of this bone, implying that it either is disappearing or it is appearing (Fabrezi, 2006).

Table 1 summarizes noteworthy features in the hyoid skeleton of Ceratophryines: (1) absence of anterolateral processes; (2) development of posterolateral processes from new cartilaginous condensation at the end of metamorphosis; (3) invasive ossification of posteromedial processes toward the hyoid plate; and (4) poor representation of the pars reuniens (hypohyalia) and basibrachial in the adult hyoid plate. Furthermore, *Lepidobatrachus* spp. have discontinuous hyalia (with a distinctive developmental pathway) and a dermal ossification on the dorsal face of hyoid plate, both representing morphological novelties not reported for other anurans. Thus, the hyoid of *Lepidobatrachus* spp. results in a modified skeletal apparatus which integrates traits shared with other ceratophryines; other traits resembling conditions of basal anurans; and traits representing morphological novelties.

Buccal Floor, Hyoid, and Tongue Muscles

Muscles of the buccal floor (m. submentalis, m. intermandibularis, and m. interhyoideus) act during feeding and breathing. The m. submentalis connects the anterior ends of the mandibles. During feeding, it bends the mandibles downwards by depressing their tips, and during breathing, it closes the nares by lifting the mentomeckelian upward (Nishikawa, 2000). Contraction of the m. intermandibularis and m. interhyoideus raises the

buccal floor (Nishikawa, 2000). Most anurans have a movable joint between the mentomeckelian and dentary, which would be related to mandibular bending (Nishikawa, 2000). Pipoids lack the mentomeckelian bones (Cannatella and Trueb, 1988), and ceratophryines have indistinct mentomeckelian. Furthermore, the mandibular symphysis in ceratophryines is strong and unmovable (Duellman and Trueb, 1986; Fabrezi and Emerson, 2003). The absence of a movable joint between the mentomeckelian and dentary would preclude the mandible bending.

Organization of the buccal floor muscles in ceratophryines seems to indicate a similar function in the movements of the buccal floor that were described in other anurans. However, the features of the lower jaw symphysis suggest that the role of the m. submentalis could be different.

In most anurans, the m. geniohyoideus is composed of medial and lateral components (Figs. 5D, 6B, and 7B,C). This muscle is single in *Leiopelma*, *Discoglossus* (Trewavas, 1933), some pipoids (Ridewood, 1897b; Trueb and Gans, 1983), and *Lepidobatrachus laevis* (Fig. 5B). The lateral component would only be absent or reduced in *Lepidobatrachus* and *Rhinophrynus* (Trueb and Gans, 1983).

The m. sternohyoideus presents great variation among anurans (Trewavas, 1933). Unlike in other taxa (*Telmatobius, Odontophrynus, Pseudis*), in ceratophryines, this muscle has two well-separated components, both with fibers running obliquely and attached along and lateral to the hyoid plate. However, some fibers of the superficial component are incorporated into the deep component in *Lepidobatrachus* spp. In *L. laevis*, the fibers of the superficial component are displaced towards the base of the hyale, like in *Rhinophrynus* (Trueb and Gans, 1983).

The absence of m. omohyoideus is a shared feature of ceratophryines, *Odontophrynus*, *Telmatobius*, as well as many taxa mentioned in the literature, such as leiuperids, myobatrachids, bufonids, brachycephalids, dendrobatids, hemisotids, microhylids, and *Rhinophrynus* (Trewavas, 1933; Trueb and Gans, 1983).

Chacophrys, Ceratophrys, Pseudis, Odontophrynus, and *Telmatobius* have a complete set of mm. petrohyoidei. In *Lepidobatrachus*, the m. petrohyoidei have few fibers and the most anterior pair of the mm. petrohyoidei posteriores is absent, as occurs in *Rhinophrynus* (Trueb and Gans, 1983). Trewavas (1933) described the absence of a pair of mm. petrohyoidei posteriores, probably the second pair, in some leiuperids, bufonids, *Brachycephalus, Cacosternum*, hemisotids, brevicipitids, and microhylids. Reduction in the number and fibers of the mm. petrohyoidei in *Lepidobatrachus* diminishes the attachments between the hyoid and skull.

Horton (1982) described patterns for the tongue musculature in which complexity in the division of fibers of the muscles genioglossus and hyoglossus at the insertion in the tongue mass displays intraordinal variation. The simplest state for the m. genioglossus is that observed in *Lepidobatrachus* spp. and *Pseudis platensis*. This state is characterized by only one interdigitated element separating into a number of flattened divisions (with few fibers in *Lepidobatrachus*), each of which lies between adjacent groups of the division of the m. hyoglossus. A more complex state is displayed by *Ceratophrys, Chacophrys, Telmatobius*, and *Odontophrynus*. In these taxa, the ventral component of m. genioglossus is added to a more complicated interdigitated element.

The m. hyoglossus also exhibits variation related to the number of divisions at the insertion (Horton, 1982). The most conspicuous feature of the m. hyoglossus is exhibited by *Lepidobatrachus* spp., in which its medial fibers (right and left) run and enter into the tongue separately (in most anurans medial fibers are contiguous for most of their length, and diverge only at level of the posteromedial processes).

Table 2 summarizes morphological variation of hyoglossal musculature in ceratophryines compared with data for other anurans. Of great concern regarding the Ceratophryinae is the fact that features of these anurans provide evidence to compare morphological traits that may be ordered as transformational series among different species (Fabrezi, 2006; Fabrezi and Quinzio, 2008). The observed variation in the hyoglossal musculature within ceratophryines suggests a transformational series indicating simplification of hyoid and tongue muscles within *Lepidobatrachus*.

Phylogenetic and Evolutionary Implications

Among the morphological synapomorphies that define Ceratophryinae (Fabrezi, 2006) several seem to be related to jaw specializations (i.e., monocuspid teeth, shared with some scaphiopodids, pipids, ranids, bufonids, and hylids; absence of the pars palatina of premaxilla, as in some pipids; strong fusion of the dentary and mentomeckelian, also in pyxicephalids and dicroglossids; and spur-like fangs formed by the dentary and mentomeckelian bones, only observed in this clade). Furthermore, ceratophryines have elongated jaws with a decrease in the skull length, which result in the unique mouth morphology of *Lepidobatrachus* where lower jaw articulation is displaced posterior to the occipital joint (Fabrezi, 2006). These derived and/or unique traits related to feeding converge in the capability of eating large prey, or megalophagy that would had evolved in the group (Hanken, 1993). Curiously, this evolutionary trend seems to be also implicated in larval feeding.

O'Reilly et al., (2002) described in anuran tadpoles, the suction feeders, derived from suspension feeders, that use single, explosive buccal expansion to capture relatively large prey (that are targeted individually). Suspension feeders use a relatively slow buccal-pumping mechanism to filter numerous small food particles simultaneously from a generally targeted part of the water column or substrate. These authors proposed macrophagy as a prerequisite for suction feeding. Even though numerous anuran tadpoles are macrophages, suction feeding in larvae has been reported only in *Hymenochirus* and *Lepidobatrachus* (Sokol, 1977; Ruibal and Thomas, 1988). Following the hypothesis proposed by O'Reilly et al., (2002), within ceratophryines larval feeding presents the three evolutionary steps: (1) suspension feeding (*Chacophrys*), (2) macrophagy (*Ceratophrys*), and (3) suction feeding and megalophagy (*Lepidobatrachus*). These morphological data provide arguments to propose megalophagy as a derived specialization of feeding that has also evolved in larvae of ceratophryines.

Emerson (1977) proposed a generalized model in which the hyoid muscles sternohyoideus and geniohyoideus act during tongue protrusion: static hyoid position is maintained by the m. sternohyoideus, and initial contraction of the m. geniohyoideus opens the lower jaw.

TABLE 2. Noteworthy features in the hyoglossal muscles of Ceratophryines compared with respect to data of hyoglossal muscles in other anurans

Muscle	*Ceratophrys/Chacophrys*	*Lepidobtrachus llanensis*	*Lepidobtrachus laevis*	Literature data
Intermandibularis	With a supplementary slip formed by fibers oriented posteromedially			Single in most anurans. With posterior supplementary slip in *Batrachophrynus, Caliptocephallela, Insuetophrynus, Odontophrynus, Telmatobius,* and *Telmatobufo*
Geniohyoideus medialis and geniohyoideus lateralis	Both divisions are present	M. genioihyoideus lateralis reduced	M. genioihyoideus lateralis absent	Both are present in most anurans. M. genioihyoideus medialis absent in *Leiopelma* and *Discoglossus*. M. geniohyoideus lateralis absent in *Rhinophrynus*
Sternohyoideus	Two components aligned at the origin	Two components aligned at the origin	Two components well separated at the origin, superficial component originating at the base of the hyale.	Great variation among anurans. Single in *Rhinophrynus*, with fibers originating at the base of the hyale.
	Two components without shared fibers	Fibers of superficial component are incorporated into the deep component.		
Omohyoideus	Absent			Present in most anurans. Absent in *Odontophrynus* and *Telmatobius*
Petrohyoidei posteriores	Three pairs	Two pairs, the anterior one is absent		Three pairs in most anurans. The anterior one is absent in *Rhinophrynus* and probably, the second pair is absent in some leiuperids, bufonids, *Brachycephalus, Cacosternum*, hemisotids, brevicipids, and microhylids
Hyoglossus	With medial fibers fused in a single group	Left and right muscles completely separated		With medial fibers fused in a single group
Geniohyoideus	Two components; ventral and interdigitated element.	Single, only deep fibers of the interdigitated element.		There are four patterns of organization of m. genioglossus among anurans. It may be formed by different components

For most anurans, experimental studies have demonstrated that tongue protrusion is only controlled by tongue muscles (Nishikawa, 2000). Exceptionally, in *Rhyinophrynus dorsalis* (Trueb and Gans, 1983) and *Spea multiplicata* (O'Reilly and Nishikawa, 1995), the m. geniohyoideus and m. genioglossus are necessary for normal tongue protrusion, and disjunct hyalia of these taxa allows the hyoid to move further anteriorly during feeding, unlike what happens in other frogs. Hyoid skeleton and muscles involved in tongue protrusion described in *Rhinophrynus* and *Spea* resemble those observed in *Lepidobatrachus*, but curiously, unlike *Lepidobatrachus*, these taxa are terrestrial and eat small prey.

As mentioned before, ceratophryines constitute a monophyletic clade, for which recent molecular (Frost et al., 2006) and morphological data (Fabrezi, 2006; Fabrezi and Quinzio, 2008) have suggested different relationships among the three genera. Relationships within ceratophryines are still controversial, and optimization of new characters from this study produces ambiguous results (Fig. 8) with some questions remaining unsolved; for example, if the aquatic life style is the derived condition in the group. Among basal anurans, adult pipids are fully aquatic because they are capable to feed underwater and retain the lateral line system. *Lepidobatrachus* feed underwater and at least the adult of *L. laevis* posses the lateral line system (Cei, 1981). In *Lepidobatrachus* spp., reduction of fibers of the muscles of the buccal floor, configuration of the hyoid skeleton, reduction or loss of some hyoid muscles (m. geniohyoideus rama lateralis, anterior pair of petrohyoideus posteriores), small tongue, and simplified tongue musculature are morphological deviations from the pattern of terrestrial ceratophryines and other aquatic ceratophryids (e.g., *Telmatobius*), which seem to be related to feeding
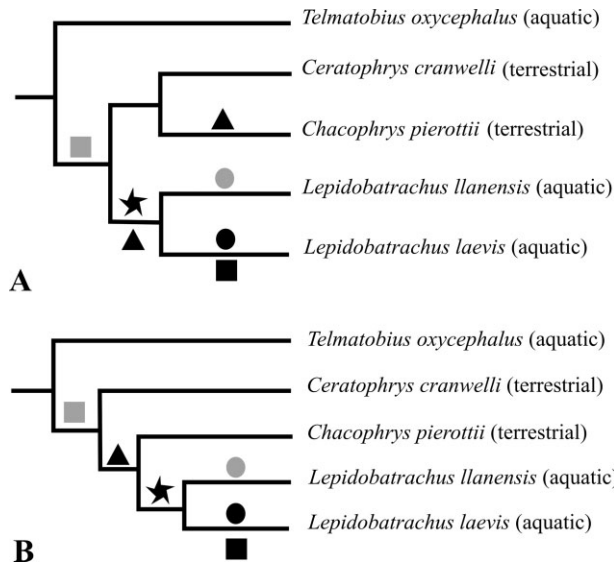
Fig. 8. Two alternative hypotheses of the relationships within Ceratophryinae considering Telmatobiinae is the sister group (Grant et al., 2006). **A:** *Lepidobatrachus* is the sister taxon of the clade formed by *Ceratophrys* and *Chacophrys* (Frost et al., 2006). **B:** *Ceratophrys* is the basal taxon within ceratophryines (Maxson and Ruibal, 1988). Optimization of five characters reported in this study (discontinuos hyale, dorsal, and ventral ramii of m. sternohyoideus sharing fibers; absence of a pair of m. petrohyoideus posterior; m. hyoglossus not fused medially; and m. genioglossus only formed by interdigitating fibers) are changes produced once in *Lepidobatrachus* in both hypotheses (black star). Optimization of reduction (gray circle) or absence (black circle) of the m. geniohyoideus rama lateralis requires two steps in the *Lepidobatrachus* clade (Deltran optimization). The orientation of the fibers of m. sternohyoideus pars ventralis crossing those of pars dorsalis (gray square) and parallel (black square) results in two steps, one implied a novelty for the ceratophryines and later an extra change happened in L. laevis. The tongue became shorter in *Chacophrys* and *Lepidobatrachus* (black triangle) this reduction implies two steps in the hypothesis shown in A (independent changes, Deltran optimization) and only one step in B an unambiguous optimization.

underwater and swallowing large prey. Further, the three mechanisms of tongue protrusion described by Nishikawa (2000) would differ in morphological aspects. Transition from mechanical pulling to inertial elongation could have involved, among others, a decrease in relative tongue mass (Nishikawa, 2000). *Lepidobatrachus* is mentioned as a mechanical puller that protrudes its tongue during terrestrial feeding (Nishikawa, 2000; O'Reilly et al., 2002), but during aquatic feeding uses only forearm scooping to capture prey (Gray et al., 1997; O'Reilly et al., 2002). Reduction of tongue mass in *Chacophrys* and *Lepidobatrachus* could be related to the evolution toward terrestrial feeding that diverges later within the clade formed by *Chacophrys* + *Lepidobatrachus* with changes in the hyoglossal apparatus of *Lepidobatrachus* in which terrestrial feeding is lost (but not the capability). Tongue size reduction was the only character that exhibited different hypotheses of evolution between both trees of Fig. 8. In the optimization of Fig. 7B, reduction of tongue size would occur once.

Unlike pipids, in which the absence of tongue and the aquatic life style might constrain the evolution of feeding, in *Lepidobatrachus* the historical derived features shared with *Chacophrys* and *Ceratophrys* involved in megalophagy seem to precede the morphological changes in feeding in *Lepidobatrachus*. Thus, the hyoglossal apparatus and related muscles of *Lepidobatrachus* integrate a set of derived features convergent to the conditions observed in non-neobatrachian anurans, and morphological novelties (dorsal dermal hyoid ossification) that define a functional complex unique among anurans to feed underwater.

ACKNOWLEDGMENTS

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APPENDIX

Abbreviations of Museums

FML, Instituto de Herpetología, Fundación Miguel Lillo, Tucumán, Argentina; MCN, Museo de Ciencias Naturales, Universidad Nacional de Salta, Argentina.

Specimens Examined

Skeleton whole mounts

Chacophrys pierottii: MCN 1029 (56 larval specimens, Stages 35–46), FML 2651 (male); MCN 987 (3 males); FML 9011 (2 males).

Ceratophrys cranwelli: MCN 188 (male), MCN 206 (male), MCN 206 (female), MCN 819 (female), MCN 933 (juvenile).

Lepidobatrachus laevis: MCN 109 (2 males), MCN 695 (female), MCN 815 (male), MCN 831(female and male), MCN 934 (male), MCN 935 (male), MCN 936 (male), MCN 647 (25 larval specimens, Stages 33–40), MCN 666 (7 larval specimens, Stages 38–43 and 2 froglets); MCN 668 (froglet), MCN 695 (2 juvenile), MCN 696 (juvenile), MCN 1109 (11 larval specimens, Stages 39–46).

Lepidobatrachus llanenesis: MCN 081 (female); MCN 667 (male), MCN 932 (larval specimens, Stages 45 and 46), MCN 567 (larval specimens, Stages 33–39); MCN 970 (larval specimens, Stages 33–46); MCN 081 (female), MCN 1118 (45 larval specimens, Stages 36–46).

Telmatobius oxycephalus: MCN 438 (male).

Odontophrynus americanus: MCN 105 (female).

Pseudis platensis: MCN 812 (female).

Muscle dissections

Chacophrys pierottii: MCN 987 (3 males).

Ceratophrys cranwelli: MCN 819 (3 males); MCN 933 (male), MCN 943 *Lepidobatrachus laevis:* MCN 815 (male and 3 juveniles), MCN 817 (3 juveniles), MCN 989 (female).

Lepidobatrachus llanensis: MCN 081 (female), MCN 932 (male), MCN 986 (female and 2 males), MCN 1118 (3 froglets).

Telmatobius oxycephalus: MCN 313 (3 males and juvenile).

Odontophrynus americanus: MCN 267 (male), MCN 315 (4 males).

Pseudis platensis: MCN 1110 (2 males and female).