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Morphology of the cranial skeleton and musculature in the obligate carnivorous tadpole of *Lepidobatrachus laevis* (Anura: Ceratophryidae)

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Abstract

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Lepidobatrachus laevis (Ceratophryidae: Ceratophryinae) is a bizarre frog endemic to the Chacoan desert of central South America. Its tadpole is an obligate carnivore that can catch and consume live prey nearly its own size. Morphological adaptations associated with this unique feeding mode, including the larval skull anatomy and associated cranial musculature, have only been partly described. We studied the head of Stages 26–27 larvae using gross dissection, immunohistochemistry, and standard histology. Derived features of this tadpole compared to the microphagous, herbivorous larvae of most other anurans include simplified chondrocranial cartilages and very robust jaw muscles. The *mm. suspensorio- et quadratoangularis* do not take their origin from the *processus muscularis* of the palatoquadrate, as in most other tadpoles, but instead originate from the corpus of the palatoquadrate caudal to this process. The jaw levators are unusually large. The tadpole of *Ceratophrys*, another member of the ceratophryine clade, also consumes large animal prey, but its morphology is very different. It probably has evolved independently from a generalized, mainly herbivorous tadpole similar to the larva of *Chacophrys*, the third ceratophryine genus. Most specialized features of the larval head of *Lepidobatrachus laevis* are adaptations for 'megalophagy'—ingestion of whole, very large animal prey.

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Introduction

With more than 5800 extant species, anurans are by far the most diverse and numerous group of lissamphibians (extant amphibians; Frost 2010). A wide range of reproductive modes is an important factor behind their evolutionary success (Cannatella 1999). Whereas most species exhibit a biphasic life cycle with a generalized herbivorous or omnivorous larva, several clades have evolved carnivorous larvae. The South American frog *Lepidobatrachus laevis* (Budgett 1899) from the Chacoan region of Paraguay and Argentina has an especially unusual, 'megalophagous' larva, which has adaptations that enable it to swallow live animal prey nearly as large as itself (Ruibal and Thomas 1988; Scott and Aquino 2004). The mature larva is very large, with an enlarged yet flattened head, and is an obligate carnivore that frequently cannibalizes larvae of its own species.

Lepidobatrachus (three species) and two other South American genera, *Ceratophrys* (eight species) and *Chacophrys* (one species), comprise the monophyletic subfamily Ceratophryinae (Haas 2003; Fabrezi 2006; Frost *et al.* 2006; Grant *et al.* 2006) within the family Ceratophryidae (formerly Leptodactylidae—Haas 2003; Ruibal and Thomas 1988). The latter clade also includes *Atelognathus*, *Batrachyla*, *Telmatobius*, and possibly *Insuetophrynus* (Frost *et al.* 2006). Phylogenetic relationships among the three ceratophryine genera are not resolved. There are two alternative hypotheses: (i) *Lepidobatrachus* is the basal taxon (Frost *et al.* 2006) or (ii) *Chacophrys* or *Ceratophrys* is basal (Wild 1999; Fabrezi 2006; Fabrezi and Quinzio 2008; Fabrezi and Lobo 2009). *Lepidobatrachus* is aquatic throughout life, whereas *Chacophrys* and *Ceratophrys* are terrestrial as adults. The first phylogenetic hypothesis implies that the fully aquatic lifestyle of *Lepidobatrachus* is a plesiomorphic trait for ceratophryines, whereas the second one implies that an aquatic adult stage is a derived trait in this clade. Megalophagy and cannibalism are shared characters of all adult Ceratophryinae (Ruibal and Thomas 1988; Hanken 1993). Larvae of *Lepidobatrachus* and *Ceratophrys* are macrophagous and specialized carnivores (Ruibal and Thomas 1988; Wassersug and Heyer 1988), whereas tadpoles of *Chacophrys pierottii* are generalized suspension feeders (Wassersug and Heyer 1988; Quinzio *et al.* 2006).

Adult ceratophryine frogs possess several features that are interpreted as examples of peramorphosis or overdevelopment (Fabrezi 2006; Fabrezi and Quinzio 2008; Fabrezi and Lobo 2009). Peramorphosis is a type of heterochrony that may result from an increase in rate (acceleration), a later offset time (hypermorphosis), or an earlier onset time (predisplacement) of development (Reilly *et al.* 1997). In *Lepidobatrachus,* this has produced a distinctive skull shape in the adult. Perhaps, its most remarkable feature is the caudal displacement of the jaw articulation, which lies posterior to the occipital joint (Fabrezi 2006; Fabrezi and Quinzio 2008). In contrast, the unusual head morphology of the tadpole of *Lepidobatrachus* results from precocious, embryonic development of characters, which typically form during metamorphosis in other ceratophryines (Hanken 1993; Fabrezi and Quinzio 2008; Fabrezi and Lobo 2009).

In an important paper, Ruibal and Thomas (1988) draw attention to the remarkable tadpole of *L. laevis* and describe certain aspects of its trophic morphology. However, their description of cranial cartilages and especially musculature is incomplete and partly inaccurate. For example, muscles that are not directly associated with the feeding mechanism are not considered. Furthermore, a novel nomenclature for jaw levators and depressors is introduced to circumvent difficulties in establishing homologies with the jaw muscles of more generalized anuran larvae. Ruibal and Thomas (1988) suggest the possible fusion of two angularis muscles (suspensorio- and quadratoangularis), but they are unable to resolve this and other issues. Thus, there is the need for additional study of the larval cranial musculature in this species, similar to the recent publication by Fabrezi and Lobo (2009) that describes the hyoid skeleton and associated muscles in an advanced larva.

Here, we present a comprehensive description of the larval cranial skeleton and musculature in Stage 26 and Stage 27 tadpoles of *L. laevis.* Our account, which incorporates both earlier reports and new data, establishes a baseline for comparisons of larval anatomy among related species. It also provides data that can be used for further studies of the development, larval adaptations, and evolution of these fascinating frogs. Investigations of anatomically extreme tadpoles are important for a deeper understanding of the evolution of the broad array of reproductive modes found in extant anurans.

Materials and Methods

Animals

Live adult *Lepidobatrachus laevis* were collected in Salta, Argentina, and maintained as a breeding colony in James Hanken's laboratory at Harvard University, Cambridge, Massachusetts, USA. Breeding was induced by injection of both male and female frogs with a luteinizing hormone-releasing hormone (LHRH) agonist (Sigma-Aldrich, St. Louis, MO, USA). The tadpoles were staged (Gosner 1960), sacrificed by brief immersion in 1% aqueous tricaine methanesulphonate (MS-222; Sigma-Aldrich), and preserved immediately in 4% paraformaldehyde. Animal care procedures were approved by the Harvard University/Faculty of Arts and Sciences Standing Committee on the use of Animals in Research and Teaching. An Animal Welfare Assurance statement is on file with the university's Office for Laboratory Welfare (OLAW). Anatomic terminology follows Haas (2001, 2003), unless noted otherwise. A total of five specimens, Stages 26–27, were used for the study. Feeding begins at those stages that display a functional larval chondrocranium and musculature. Metamorphic changes are apparent beginning at Stage 30.

Histology, immunohistochemistry, and dissection

External characters were observed in preserved larvae using a Zeiss Stemi SV 11 stereomicroscope (Zeiss, Germany). For serial sectioning, specimens were dehydrated in an ethanol series (50%, 70%, 90%, 95%, 100%, 100%; 1 h each), embedded in paraffin (2× Rotihistol, 1 h; Histoplast S, overnight at 54 °C; embedded in Histoplast S; Serva, Heidelberg, Germany), and sectioned at 7 μm on a Microm HM360 microtome (Microm, Waldorf, Germany). Sections were stained with Heidenhain's Azan technique (Böck 1989). Specimens for manual dissection were prepared using a clearing and staining protocol (Klymkowsky and Hanken 1991). Briefly, the skin and intestines were removed from the larvae, which then were dehydrated in an ethanol series. Following a 24-h staining with Alcian blue (20 mg Alcian blue 8GX [C.I. 74240], 70 mL absolute ethanol, and 30 mL glacial acetic acid), the specimens were washed with 0.5% KOH, digested for 2–4 days at room temperature with 1% trypsin, stained with alizarin red for 24 h, and bleached with 0.5% KOH and a few drops of 3% hydrogen peroxide. Before the larvae were transferred to glycerol, their muscles were stained using the monoclonal antibody 12/101 (Developmental Studies Hybridoma Bank, IA, USA), which was raised against newt skeletal muscle. Overnight incubation with the primary antibody (diluted 1 : 100 with DAKO antibody solution; DAKO, Hamburg, Germany) was followed by overnight incubation with a biotinylated secondary antibody (diluted 1 : 500 with DAKO antibody solution). The avidin–biotin system (DAKO) and incubation with DAB (3,3′-diaminobenzidin-tetrahydrochloride, DAKO) were used to detect muscle staining.

Dissections were performed with the aid of a Zeiss Stemi SV 11 stereomicroscope using watchmaker forceps. Photomicrographs were taken with a ColorViewIII digital camera (Soft Imaging System GmbH, Münster, Germany) installed on the Zeiss Stemi SV 11 stereomicroscope. The software analySIS® (Soft Imaging System GmbH) was used for calibration and data storage. Final versions of the illustrations were produced with Adobe® Illustrator® CS3 (13.0.0) and Inkscape.

Results

Morphology is described in Stage 26 and Stage 27 tadpoles. At these stages, the head is flattened, and eyes and nasal pits are located near the dorsal midline (Fig. 1A). The wide snout spans nearly the entire width of the head rostrally. The unusually large buccopharyngeal cavity created by the enlarged cartilages of the neurocranium is an adaptation to swallowing large prey. Synonyms for anatomic terms are provided in parentheses.

Chondrocranium

The larval *chondrocranium* is a cartilaginous case that protects the brain and supports the sense organs and jaw apparatus. The brain may be seen through the skin dorsally and is surrounded by elements of the *neurocranium*. The *neurocranium* consists of *cornua trabeculae*, *planum trabeculare anticum*, *trabeculae cranii*, *planum basale*, parachordal cartilages, and *capsula auditiva* (otic capsules). The *neurocranium* is flat, with its widest expanse at the level of the *processus muscularis palatoquadrati*. The *viscerocranium* is composed of *palatoquadratum*, *cartilago Meckeli*, *cartilago labialis inferior*, *cartilago labialis superior,* and elements of the hyobranchial skeleton. The chondrocranium has been described by Ruibal and Thomas (1988), and a detailed description of the hyoid apparatus can be found in Fabrezi and Lobo (2009). Our results are generally compatible with these accounts, but we present additional data regarding the *cartilago Meckeli*, the *palatoquadratum,* and the articulation of the *ceratohyale* with the *palatoquadratum*.

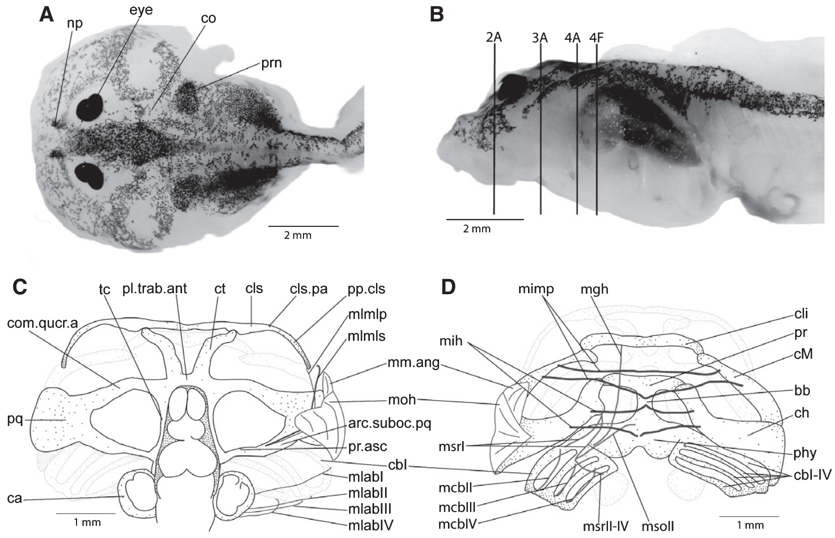

Fig. 1—Larva of *Lepidobatrachus laevis,* Stages 26–27. All cranial cartilages are well developed, but ossification of the skull has not yet begun. —**A**, **B**. The broad, flattened head, dorsal eyes, and nasal pits are the most distinctive external features of the tadpole **A**: Dorsal view. **B**: Left lateral view; vertical lines depict planes of section in Figs 2–4. —**C, D**. Drawings of the cleared-and-stained larval skull in dorsal (left) and ventral views, respectively. Note the striking discrepancy in size between the large jaws (cM, com.qucr.a, pr.asc) and hyoid elements (ch) and the small branchial baskets (cb, ceratobranchiale). Most of the muscles described in detail in the text are shown here overlaying the chondrocranium on the right side only. arc.suboc.pq, arcus subocularis palatoquadrati; bb, basibranchiale; ca, capsula auditiva; cbI (II, III, IV), ceratobranchiale I (II, III, IV); ch, ceratohyale; cli, cartilago labialis inferior; cls, cartilago labialis superior; cls.pa, pars alaris of the cartilago labialis superior; cM, cartilago Meckeli; co, cartilago orbitalis; com.qucr.a, commissura quadratocranialis anterior; ct, cornua trabecula; mcbII (III, IV), m. constrictor branchialis II (III, IV); mgh, m. geniohyoideus; mih, m. interhyoideus; mimp, m. intermandibularis posterior; mlabI (II, III, IV), m. levator arcuum branchialium I (II, III, IV); mlmlp, m. levator mandibulae longus profundus; mlmls, m. levator mandibulae longus superficialis; mm.ang, musculi angulari; moh, m. orbitohyoideus; msoII, m. subarcualis obliquus II; msrI (II–IV), m. subarcualis rectus I (II–IV); np, nasal pit; phy, planum hypobranchiale; pl.trab.ant, planum trabeculare anticum; pp.cls, processus posterior of the cartilago labialis superior; pq, palatoquadratum; pr, pars reuniens; pr.asc, processus ascendens palatoquadrati; prn, pronephros; tc, trabecula cranii.

Neurocranium

The braincase is open ventrally via the *fenestra basicranialis* (basicranial fenestra). The fenestra is flanked rostrally by the *planum trabeculare anticum*, laterally by the *trabeculae cranii,* and caudally by the *planum basale*. The dorsal projection of the *trabeculae cranii* is the *cartilago orbitalis*. The *planum trabeculare anticum* forms both the roof of the mouth and the floor of the *cavum cranii* in the anteriormost region (Figs 1C and 2A). The *cornua trabeculae* are two bars with a common origin at the *planum trabeculare anticum*. They join the *cartilago labialis superior* by a synchondrosis (Fig. 1C). The *trabeculae cranii* extend from the *planum trabeculare anticum* to the *planum basale* at the level of the fusion of the *processus ascendens palatoquadrati* (Fig. 1C). In the mature larva (Stage 30), the *planum basale* is a thick horizontal plate, but at Stages 26–27, fusion of the parachordal cartilages is not complete (Fig. 2E,F). The *capsula auditiva* is not yet enclosed by cartilages, although its cartilaginous wall is well developed laterally and ventrally (Figs 3C–4A). The *crista parotica* is a lateral ridge of the *capsula auditiva*, from which originate muscles of the branchial basket (e.g., *mm. levatores arcuum branchialium II, III et IV*; Fig. 4A–C).

Viscerocranium

Jaws of anuran tadpoles are unique among vertebrates in having labial cartilages (*cartilagines labiales superior et inferior*). Other elements of the jaw include the *cartilago Meckeli* and the *palatoquadratum*. In *L. laevis,* the *cartilago labialis superior* (suprarostral cartilage) comprises two lateral *partes alares* (Fig. 1C). A medial *pars corporis* is not present. Each *pars alaris* extends laterally and then turns caudally. The posterior part is elongated into a thin *processus posterior* (Figs 1C and 2A–F), which ends just before the articulation of the *palatoquadratum* with the *cartilago Meckeli*. The anteromedial part of the *pars alaris* articulates via a synchondrosis with the *trabecula cranii* and functions as the larva's moveable upper jaw (Fig. 1C).

The lower jaw consists of the *cartilago Meckeli* and *cartilago labialis inferior* (infrarostral cartilage, infralabial cartilage, mentomeckelian cartilage). In *L. laevis,* each *cartilago labialis inferior* is deployed posteriorly and slightly medial to the *processus posterior* of the *cartilago labialis superior* (Fig. 1D). The *cartilago labialis inferior* of both sides are joined at the midline by connective tissue. The *cartilago labialis inferior* is joined to the *processus dorsomedialis*, the anteromedial end of the *cartilago Meckeli,* via the *commissura intramandibularis* (Fig. 2A). The *cartilago Meckeli* is compact; its posterolaterally directed *processus retroarticularis* articulates with the *processus articularis palatoquadrati* (Fig. 3C–E). Four of the five *mm. levatores mandibulae* insert on the *processus retroarticularis.*

The larval *palatoquadratum* (pterygoquadrate) is attached to the neurocranium by two cartilaginous processes (Fig. 1C): anteriorly, by the *commissura quadratocranialis anterior* (quadratocranial commissure; Fig. 2A,B), and posteriorly, by the *processus ascendens palatoquadrati* (Fig. 2E,F). The *commissura*

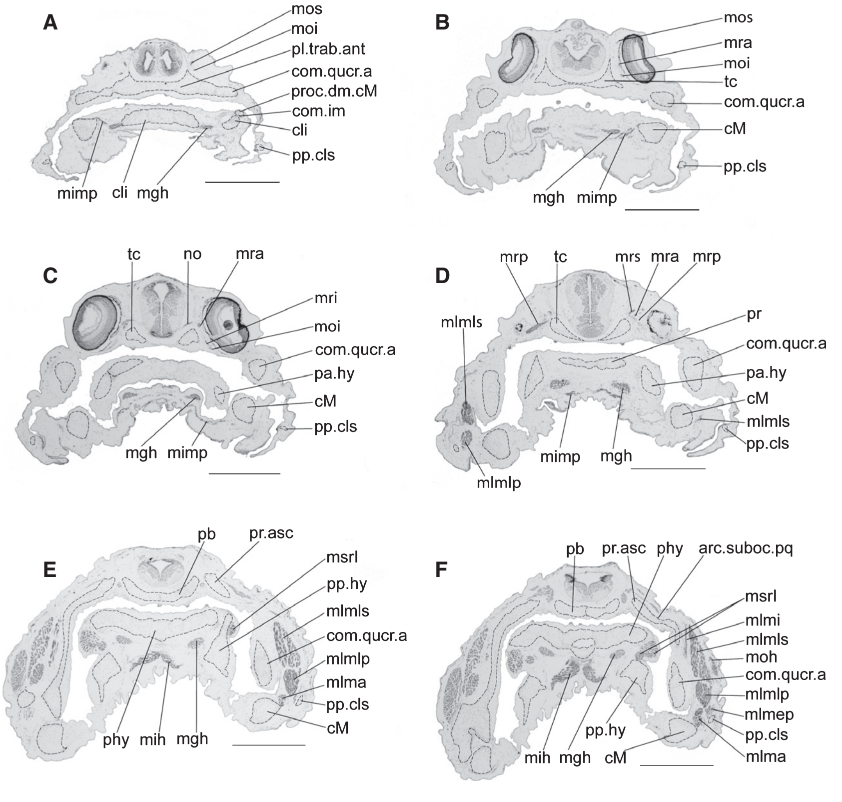

Fig. 2—Transverse sections through a tadpole of *Lepidobatrachus laevis*, Stage 26 (continued in Figs 3 and 4). —**A**–**F**. Sections at different levels, beginning from the anterior connection of the *palatoquadratum* with the neurocranium (**A**, com.qucr.a) and extending to the posterior connection (**F**: *pr.asc*). Plane of section **A** is shown in Fig. 1B. Additional abbreviations: com.im, commissura intramandibularis; mlma, m. levator mandibulae articularis; mlmep, m. levator mandibulae externus profundus; mlmi, m. levator mandibulae internus; moi, m. obliquus inferior; mos, m. obliquus superior; mra, m. rectus anterior; mri, m. rectus inferior; mrp, m. rectus posterior; mrs, m. rectus superior; no, nervus opticus; pa.hy, processus anterior hyalis; pb, planum basale; pp.hy, processus posterior hyalis; proc.dm.cM, processus dorsomedialis of the cartilago Meckeli. Scale bar, 1 mm.

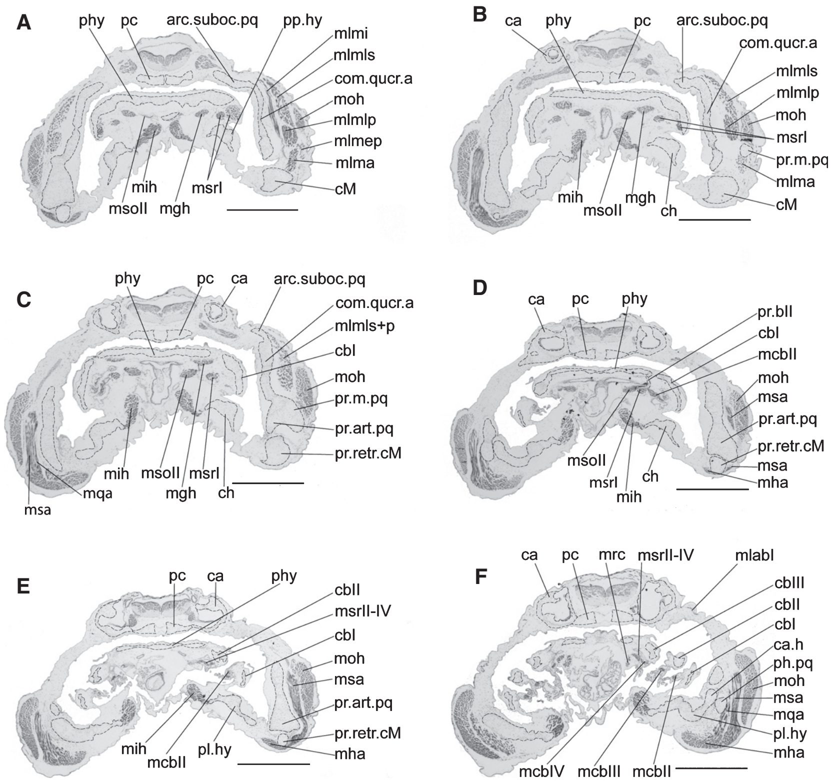

Fig. 3—Transverse sections through a tadpole of *Lepidobatrachus laevis*, Stage 26 (continued from Fig. 2). —**A**–**F**. Sections at different levels, beginning from the midpoint between eye and ear (**A**) and extending to the middle of the *capsula auditiva* (**F**). Plane of section **A** is shown in Fig. 1B. Additional abbreviations: ca.h, condylus articularis of the processus lateralis hyalis; mha, m. hyoangularis; mlmls + p, mm. levatores mandibulae longi superficialis et profundus; mqa, m. quadrato-angularis; mrc, m. rectus cervicis; msa, m. suspensorioangularis; pc, parachordal cartilage; ph.pq, processus hyoquadrati palatoquadrati; pl.hy, processus lateralis hyalis; pr.art.pq, processus articularis palatoquadrati; pr.bII, processus branchialis on ceratobranchialis II; pr.m.pq, processus muscularis palatoquadrati; pr.retr.cM, processus retroarticularis of the cartilago Meckeli. Scale bar, 1 mm.

quadratocranialis anterior projects caudally from the *planum trabeculare anticum*. The *arcus subocularis palatoquadrati* (otic process, *processus oticus*; Figs 2F–3C) lies between the *commissura quadratocranialis anterior* and the *processus ascendens palatoquadrati* (Fig. 2E,F). The most prominent part of the *palatoquadratum* is the *processus muscularis palatoquadrati* (muscular process; Fig. 3B,C), a short, lateral, and robust process that curves dorsally. The *processus articularis palatoquadrati* is the most anterior extension of the *palatoquadratum*; it articulates with the *processus retroarticularis* of the *cartilago Meckeli* (Fig. 3C–E). The *processus hyoquadrati* (hyoquadrate process) is a ventral condyle located at the posterior part of the *palatoquadratum,* which articulates with the *condylus articularis* of the *ceratohyale* (Fig. 3F).

Paired *ceratohyalia* and branchial baskets are the major components of the hyobranchial skeleton in ventral view (Fig. 1D). The *processus lateralis hyalis* lies lateroventral to the branchial basket and ends at the level of the second branchial arch (Fig. 4F). In the ventral region behind the eye, the *ceratohyale* articulates with the *facies hyoidis* of the *processus hyoquadrati* of the *palatoquadratum* via the *condylus articularis*, a dorsal projection of the *processus lateralis hyalis* (Fig. 3F). In most other anuran larvae, this articulation is located ventral to the eye. Ventromedially, a crista is formed where the *m. interhyoideus* originates from the *processus posterior hyalis* (Figs 3A–4D).

Rostral parts of the branchial baskets lie medially between the *processi posteriores hyales*. Each branchial basket is composed of a *planum hypobranchiale* and four *ceratobranchialia*, which together provide skeletal support for the gill and filter apparatus (Fig. 1D). The two *plana* are fused to each other, forming a single median plate. The proximal fusions of the *ceratobranchialia* are the *commissurae proximales;* distal fusions are the *commissurae terminales* (Fig. 4F). The *commissurae terminales* approach one another; thus, there seems to be only a single cartilage at the caudal end of the branchial basket (Figs 1C,D and 4F). Both *ceratobranchialia II et III* have a ventral *processus branchialis* rostrally (Fig. 3D).

The *cartilagines arytaenoideae* are elongated cartilages that flank the larynx anteriorly near the pharynx. These cartilages have started to develop by Stages 26–27, when they are composed mostly of chondroblasts (Fig. 4B).

Muscles

Table 1 lists the cranial muscles of larval *L. laevis* and their origins and insertions. As do most other vertebrates, larval *L. laevis* have six extrinsic eye muscles. The anterior two *mm. obliquii* originate together at an angle formed by the braincase and the *planum trabeculare anticum* (Fig. 2A). The dorsal muscle is the *m. obliquus superior*, which extends dorsally and caudally to insert on the anterodorsal part of the eye (Fig. 2B). The ventral *m. obliquus inferior* runs caudoventrally and inserts on the medioventral part of the *bulbus oculi* (Fig. 2C). The four *mm. recti* have a common origin at the *cartilago orbitalis* caudal to the passage of the *n. opticus* (N. II; Fig. 2D). The

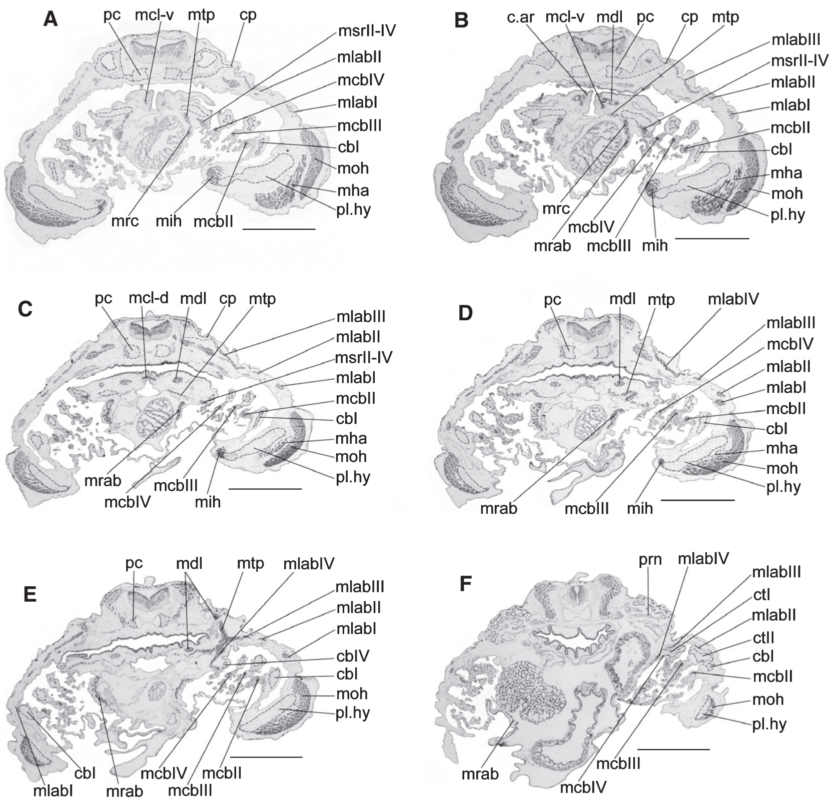

Fig. 4—Transverse sections through a tadpole of *Lepidobatrachus laevis*, Stage 26 (continued from Fig. 3). —**A–F**. Sections at different levels, beginning from the caudal part of the *capsula auditiva* (**A**) and extending to the end of the branchial basket at the level of the rostral portion of the pronephros (**F**). Planes of section **A** and **F** are shown in Fig. 1B. Additional abbreviations: c.ar, cartilago arytaenoidea; cp, crista parotica; ctI (II), commissura terminalis I (II); mcl-v (d), m. constrictor laryngis ventralis (dorsalis); mdl, m. dilatator laryngis; mrab, m. rectus abdominis; mtp, m. tympanopharyngeus. Scale bar, 1 mm.

m. rectus posterior is very short and inserts on the caudoventral border of the *bulbus oculi* (Fig. 2D). The *m. rectus superior* turns caudally and inserts dorsally on the *bulbus oculi* above the lens. The *m. rectus inferior* turns rostrally and inserts medioventrally on the *bulbus oculi* (Fig. 2C). The *m. rectus anterior* runs horizontally and rostrally and inserts anteromedially on the *bulbus oculi* (Fig. 2B). The *mm. recti superior*, *inferior et anterior*, and the *m. obliquus inferior* are innervated by the *n. oculomotorius* (N. III); the *m. rectus posterior* by the *n. abducens* (N. VI); and the *m. obliquus superior* by the *n. trochlearis* (N. IV). The *mm. obliquii* rotate the eye about the optical axis, whereas the *mm. recti* rotate the eye in the horizontal and vertical planes at right angles to its axis.

Muscles innervated by the *n. trigeminus* (N. V) are components of the mandibular arch. These are the jaw levator muscles (*mm. levatores mandibulae*) and the *m. intermandibularis*. The *m. levator mandibulae longus* is the largest of the jaw levators. It originates dorsocaudally from the *arcus subocularis palatoquadrati* (Figs 1C and 3C) and is divided into two parts: *superficialis* and *profundus*. Both portions run rostrally but then diverge at the level of the anterior border of the *capsula auditiva* (Fig. 3B). The *superficialis* part runs rostroventrally and inserts by a long tendon on the dorsolateral edge of the *cartilago Meckeli* (Figs 1C and 2D). The *profundus* part runs rostrally and inserts on the caudolateral *processus posterior* of the *pars alaris* of the *cartilago labialis superior* (Figs 1C and 2D). The *m. levator mandibulae internus* originates dorsally from the *arcus subocularis palatoquadrati*, rostromedially to the origin of the *m. levator mandibulae longus* (Fig. 3A). The *internus* muscle runs steeply ventrally and inserts by a long tendon on the lateral edge of the *processus retroarticularis* of the *cartilago Meckeli*. The *m. levator mandibulae articularis* originates on the anteriormost medial side of the *processus muscularis palatoquadrati*. It is a short, robust muscle that inserts on the dorsolateral surface of the *processus retroarticularis* of the *cartilago Meckeli* (Figs 2E–3B). The short *m. levator mandibulae externus profundus* originates just anterior to the *articularis* muscle. It runs ventrolaterally and inserts medially on the *processus posterior* of the *pars alaris* of the *cartilago labialis superior* (Figs 2F and 3A). The *m. levator mandibulae externus superficialis* develops later and inserts on the *cartilago Meckeli*. The jaw levators contribute to mouth closing by raising the anterior parts of the *cartilago Meckeli* and by pulling the suprarostral cartilage posteroventrally.

The *m. intermandibularis anterior* (submentalis) is not developed by Stage 26. In older larvae, it is a small, medial muscle attached to the posterior surface of the *cartilago labialis inferior* (Ruibal and Thomas 1988). The *m. intermandibularis posterior* has multiple origins from the *cartilago Meckeli* (Figs 1D and 2A–C). The anteriormost fibers arise at the dorsomedial edges of the *cartilago Meckeli*; additional fibers originate more caudally from the ventromedial border. Fibers from the rostral region run medially and meet contralateral fibers in a median raphe, but some fibers in the caudal area of the muscle extend

Table 1 Larval cranial musculature of *Lepidobatrachus laevis*, Stage 26 (Gosner 1960)

Muscle	Origin	Insertion
Eye muscles		
m. obliquus inferior	planum trabeculare anticum	medioventral bulbus oculi
m. obliquus superior	planum trabeculare anticum	anterodorsal bulbus oculi
m. rectus anterior	trabeculae cranii	anteromedial bulbus oculi
m. rectus posterior	trabeculae cranii	caudoventral bulbus oculi
m. rectus inferior	trabeculae cranii	medioventral bulbus oculi
m. rectus superior	trabeculae cranii	dorsomedial bulbus oculi
Mandibular arch muscles		
m. lev. mand. longus superficialis	arcus subocularis palatoquadrati	cartilago Meckeli
m. lev. mand. longus profundus	arcus subocularis palatoquadrati	cartilago labialis superior
m. lev. mand. internus	arcus subocularis palatoquadrati	proc. retroarticularis CM
m. lev. mand. externus profundus	processus muscularis palatoquadrati	cartilago labialis superior
m. lev. mand. ext. superficialis	processus muscularis palatoquadrati	cartilago Meckeli
m. lev. mand. articularis	processus muscularis palatoquadrati	proc. retroarticularis CM
m. intermandibularis anterior	cartilago labialis inferior	median raphe
m. intermandibularis posterior	cartilago Meckeli	median raphe
Hyoid arch muscles		
m. orbitohyoideus	processus muscularis palatoquadrati	processus lateralis hyalis
m. quadratoangularis	palatoquadrate	proc. retroarticularis CM
m. suspensorioangularis	palatoquadrate	proc. retroarticularis CM
m. hyoangularis	ceratohyale	proc. retroarticularis CM
m. interhyoideus anterior	ceratohyale	median raphe, anterior pericardial wall
Branchial arch muscles		
m. subarcualis rectus I	ceratobranchiale I + proc. br. II	processus posterior hyalis
m. subarcualis rectus II–IV	ceratobranchiale IV	proc. br. II
m. subarcualis obliquus II	proc. br. II	basibranchale + pericardial wall
m. lev. arcuum branchialium I	capsula auditiva – Crista parotica	ceratobranchiale I
m. lev. arcuum branchialium II	capsula auditiva	commissura terminalis II
m. lev. arcuum branchialium III	capsula auditiva	commissura terminalis III
m. lev. arcuum branchialium IV	capsula auditiva	ceratobranchiale IV
m. constrictor branchialium II	commissura terminalis I	ceratobranchiale I
m. constrictor branchialium III	commissura terminalis II	ceratobranchiale II
m. constrictor branchialium IV	commissura terminalis III	ceratobranchiale III
m. tympanopharyngeus	capsula auditiva	pericardial wall
Hypobranchial muscles		
m. geniohyoideus	hypobranchiale – ceratobranchiale II	cartilago labialis inferior
m. rectus cervicis	rostral continuation of the m. rectus abdominis	processi branchiales II et III
Laryngeal muscles		
m. dilatator laryngis	capsula auditiva	cartilago arytaenoidea
m. constrictor laryngis dorsalis	dorsal median raphe	cartilago arytaenoidea
m. constrictor laryngis ventralis	ventral median raphe	cartilago arytaenoidea

proc. retroarticularis CM, processus retroarticularis cartilago Meckeli; proc. br. II, processus branchialis of ceratobranchiale II.

diagonally to the median raphe of the *m. interhyoideus*. Contraction of the *m. intermandibularis posterior* elevates the floor of the mouth, causing water to flow caudally from the buccal cavity into the pharyngeal cavity.

Muscles of the hyoid arch are the *m. interhyoideus* and four jaw depressors: *m. orbitohyoideus*, *m. suspensorioangularis*, *m. quadratoangularis,* and *m. hyoangularis*. All are innervated by the facial nerve (N. VII, *n. facialis*). The *m. interhyoideus* (*m. interhyoideus anterior*, subhyoideus) is a transverse muscle, which originates from a medial ridge at the *ceratohyale* (Figs 1D and 2E–4D). Fibers run rostromedially, but only the anteriormost fibers join the contralateral muscle in a median raphe. The other fibers insert on the rostral pericardium wall (Fig. 3A). Contraction elevates the floor of the pharyngeal cavity and causes water to flow caudally into the branchial cavity. Thus, the *m. interhyoideus* provides force for the power stroke during gill irrigation. The *m. orbitohyoideus* is the most powerful cranial muscle of *L. laevis* (Fig. 1C,D). It originates from the dorsolateral tip (Fig. 3A,B) and from a large portion of the *processus muscularis palatoquadrati*. Its most rostral and dorsal fibers overlie partly both the origin and caudal parts of the *mm. levatores mandibulae longi superficialis et profundus*, which run in the *canalis muscularis* (Fig. 3C). The fibers are oriented rostrocaudally and curve slightly ventrally.

They insert caudoventral to the *m. hyoangularis* on the posteromedial part of the *ceratohyale* (*processus lateralis hyalis,* Fig. 4D–F). Contraction of the *m. orbitohyoideus* elevates the posterolateral parts of the *ceratohyale*. This lowers the more anteromedial parts, which depresses the branchial floor and expands the *cavum buccale,* causing water to flow caudally.

Three angularis muscles are present (*mm. angulari*; Fig. 1C,D). The *m. suspensorioangularis* originates from the *palatoquadratum* caudolateral to the origin of the *mm. levatores mandibulae longi* (Fig. 3D,E) and descends to insert ventrolaterally on the *processus retroarticularis* of the lower jaw (*cartilago Meckeli*; Fig. 3E). The *m. hyoangularis* originates ventrally on the *ceratohyale* (*processus lateralis hyalis*) rostromedial to the insertion of the *m. orbitohyoideus* (Fig. 4C,D). It inserts on the *processus retroarticularis* of the *cartilago Meckeli* just medial to the insertion of the *m. suspensorioangularis* and *m. quadratoangularis* (Fig. 3D,E). The *m. quadratoangularis* originates from the ventrolateral aspect of the posterior parts of the *palatoquadratum* (Fig. 3F). This muscle is delimited laterally by the body of the *m. suspensorioangularis* and inserts, together with the *m. suspensorioangularis*, ventrolaterally on the *processus retroarticularis* of the *cartilago Meckeli* (Fig. 3E). Although the three angularis muscles have different origins, those of the *m. suspensorioangularis* and the *m. quadratoangularis* are difficult to distinguish. The *m. hyoangularis* fuses rostrally with the *m. quadratoangularis,* but its fibers can always be discerned by the different fiber orientations of the two muscles (Fig. 3F). Contraction of each angularis muscle contributes to mouth opening. The *m. hyoangularis* retracts the *cartilago Meckeli*, causing the mouth to open slightly. The *mm. suspensorio- et quadratoangularis* elevate the posterior part of the *cartilago Meckeli,* thereby depressing the anterior part, which causes the mouth to open.

Muscles of the branchial arches (Fig. 1D) are the *mm. levatores arcuum branchiales I, II, III et IV, m. subarcualis rectus I, m. subarcualis rectus II–IV, m. subarcualis obliquus II, mm. constrictores branchiales II, III et IV,* and *m. tympanopharyngeus.* They are innervated by the *n. glossopharyngeus* (N. IX) and *n. vagus* (N. X). The *mm. levatores arcuum branchialium I, II, III et IV* form a flat band that covers the branchial basket dorsolaterally. The *m. levator arcuum branchialium I* originates ventrolaterally from rostral part of the *crista parotica* of the otic capsule (Figs 1D and 3F). The origins of the remaining branchial levators (*mm. levatores arcuum branchialium II, III et IV*) are caudal at the otic capsule and lie close together (Figs 1D and 4A–D). Therefore, a gap between the first branchial arch levator and the others is clearly visible. Extending caudoventrally, the *mm. levatores arcuum branchialium I, II et III* initially run parallel to each other, but they diverge approximately halfway to their separate insertions. The first branchial levator inserts on the caudoventral part of *ceratobranchiale I* before the *commissura terminalis I*. The second and third branchial levators insert dorsolaterally on the *commissurae terminales II et III* (Fig. 4F). The *m. levator arcuum branchialium IV* extends medioventrally from its origin (Fig. 4E,F) and inserts ventrally on the distal end of *ceratobranchiale IV* (Fig. 4E). Contraction of the four branchial arch levators extends the branchial chambers, which conducts water from the buccal cavity into the branchial cavity.

The *m. subarcualis rectus I* (Fig. 1D) originates ventrally from the proximoanterior part of *ceratobranchiale I* (Fig. 3B) and from the *processus branchialis II* (Fig. 3F). It runs rostrally and inserts on the dorsomedial side of the *processus posterior hyalis* (Fig. 2E). Contraction of the *m. subarcualis rectus I* brings *ceratobranchialia I et II* and the *ceratohyale* together. The *m. subarcualis rectus II–IV* is formed by the fusion of three muscles. It originates ventrally from *ceratobranchiale IV* (Figs 1D and 4C). The *m. subarcualis rectus II–IV* runs rostrally, ventral to the proximal parts of the more anterior *ceratobranchialia* and the *mm. constrictores branchiales* (Figs 3F–4B). It runs slightly posterior to the *processus branchialis III* and inserts ventrolaterally on the *processus branchialis II* of *ceratobranchiale II* (Fig. 3E). The subarcualis rectus muscles appear to be antagonists of the *mm. levatores arcuum branchialium* and stabilize the proximal ends of the *ceratobranchialia* when the branchial arch levators contract. The *m. subarcualis obliquus II* originates ventrally from *processus branchialis II* of the *ceratobranchiale II* (Figs 1D and 3D). It courses rostromedially and inserts ventrolaterally on the *basibranchiale* (*copula posterior*). Some fibers also insert on the anterior part of the pericardium dorsal to the fibers of the *m. interhyoideus* (Fig. 3A). This muscle supports the *m. subarcualis rectus I*. Thus, the *mm. subarcualis obliquus II et rectus I* bring the *ceratohyale* closer to the branchial basket and stabilize the proximal ends of the *ceratobranchialia,* forcing ingested water caudally.

The *m. constrictor branchialis I* is absent in *L. laevis*. The *mm. constrictores branchiales II, III et IV* originate ventrally from the three *commissurae terminales,* which connect the *ceratobranchialia* distally (e.g., *commissura terminalis I* connects *ceratobranchiale I* to *ceratobranchiale II;* Figs 1D and 4F). In *L. laevis*, all *commissurae terminales* are in close proximity; thus, the *mm. constrictores branchiales* all originate from a small area. All three muscles run rostromedially (Figs 1D and 3F–4E); each muscle runs close to its anterior *ceratobranchiale*, on which it inserts ventromedially (e.g., *m. constrictor branchialis IV* inserts on *ceratobranchiale III*: Fig. 3F). Consequently, the *mm. constrictores branchiales* connect two consecutive *ceratobranchialia*. Contraction expands the gill slits, causing water to flow caudally. Contraction of the *m. subarcualis obliquus II* adducts *ceratobranchiale II*. This, combined with contraction of the *mm. constrictores branchialis II, III et IV,* extends the gill slits. Thus, these muscles are antagonists of the *mm. levatores arcuum branchialium I, II, III et IV*.

The *m. tympanopharyngeus* originates from the *capsula auditiva* caudal to the *m. levator arcuum branchialium IV* and ventral to the *m. dilatator laryngis* (Fig. 4E). It is innervated by the *n. vagus* (N. X). The *m. tympanopharyngeus* and *m. levator arcuum branchialium IV* are difficult to separate at their origins and descend closely together. The *m. tympanopharyngeus* then turns rostromedially and inserts dorsally on the pericardium close to the medial part of the *ceratobranchiale IV* (Fig. 4A).

Hypobranchial muscles derive from somitic mesoderm of the trunk. In *L. laevis,* these are the *m. geniohyoideus* and the *m. rectus cervicis*. Both are innervated by branches of spinal nerves (*n. hypoglossus,* spinal nerve II). The *m. geniohyoideus* originates ventrolaterally from the *planum hypobranchiale* near its junction with *ceratobranchiale II* (Figs 1D and 3C) and extends rostrally to insert on the posterior lateral tip of the *cartilago labialis inferior* (Fig. 2A). It always lies dorsal to the *mm. intermandibularis et interhyoideus*. Contraction of the *m. geniohyoideus* retracts the *cartilago labialis inferior* and opens the mouth. The *m. rectus cervicis* (*sternohyoideus, diaphragmatobranchialis medialis*) is the anterior continuation of the *m. rectus abdominis*; its origin is defined by a change in fiber orientation of the *m. rectus abdominis* (Fig. 4B). The *m. rectus cervicis* courses initially close to the intestinal wall, then shifts medially near the *processus branchialis* of *ceratobranchiale III* where some of its fibers insert. Remaining fibers insert on the *processus branchialis* of *ceratobranchiale II*. Contraction of the *m. rectus cervicis* pulls the branchial basket to the rostral wall of the abdomen, thus stabilizing the branchial basket.

Muscles of the larynx are the *m. dilatator laryngis* and the *m. constrictor laryngis*. Both are innervated by the *n. vagus* (N. X). The *m. constrictor laryngis* is divided into two parts. The *m. constrictor laryngis dorsalis* originates from a median raphe dorsal to the laryngeal tract (Fig. 4C). It runs rostroventrally and inserts ventrolaterally on the *cartilago arytaenoidea*. The *m. constrictor laryngis ventralis* originates more anteriorly from a median raphe ventral to the larynx (Fig. 4A). It runs dorsocaudally and inserts with its dorsal part on the *cartilago arytaenoidea* (Fig. 4B). The *m. dilatator laryngis* originates from the *capsula auditiva* close and caudal to the *m. levator arcuum branchialium IV* (Fig. 4E). It descends ventrally, then turns rostromedially, and ultimately inserts on the *cartilago arytaenoidea* dorsal to the *m. constrictor laryngis dorsalis* (Fig. 4B). Contraction of this muscle extends the larynx.

Discussion

Data on larval morphology exist for most species of ceratophryine frogs, representing all three genera: *Lepidobatrachus laevis* (Ruibal and Thomas 1988; Haas 2003; Fabrezi and Lobo 2009), *L. llanensis* (Lavilla and Fabrezi 1992; Fabrezi and Lobo 2009), *Chacophrys pierottii* (Wild 1999; Quinzio *et al.* 2006; Fabrezi and Lobo 2009), *Ceratophrys cranwelli* (Lavilla and Fabrezi 1992; Vera Candioti 2005), *C. cornuta* (Duellman 1978; Duellman and Lizana 1994; Wild 1997), *C. aurita* (Wassersug and Heyer 1988), *C. calcarata* (La Marca 1986), and *C. ornata* (Haas 2003). Most accounts, however, are limited to a description of external tadpole morphology (e.g., Lynch 1982; La Marca 1986; Duellman and Lizana 1994; Quinzio *et al.* 2006). Furthermore, most studies describe tadpoles between Stages 36 and 40 (Gosner 1960), after metamorphosis has begun to effect changes in muscle and cartilage organization in the larval head (Wild 1997, 1999; Fabrezi and Quinzio 2008; Fabrezi and Lobo 2009). Fabrezi and Quinzio (2008), for example, report prometamorphic changes, such as reduction in oral structures. Our study is the first complete description of the chondrocranium and all associated musculature in the larval head of *L. laevis*.

All ceratophryine frogs have large tadpoles with dorsally placed eyes and nostrils, but external morphology differs in other characters. Typically, *Lepidobatrachus* spp. are the extreme forms, contrasting to the much more similar *Chacophrys* and *Ceratophrys*. For example, in *L. laevis,* the head is almost as long as the trunk and nearly twice as wide, whereas in *Ch. pierottii* and in *C. cranwelli,* the head, while relatively large, never reaches these excessive proportions (Wild 1999). All ceratophryids have Orton Type IV tadpoles (Ruibal and Thomas 1988; Lavilla and Fabrezi 1992). Even the tadpole of *L. laevis,* with its unusual asymmetric development of the branchial openings, resembles a sinistral, Orton Type IV larva (Ruibal and Thomas 1988). Morphological features of larval *Chacophrys* may be intermediate between those of *Ceratophrys* and *Lepidobatrachus* (Quinzio *et al.* 2006).

Whereas tadpoles of both *Lepidobatrachus* and *Ceratophrys* have specializations related to their carnivorous and macrophagous habits (Ruibal and Thomas 1988; Wassersug and Heyer 1988; Hanken 1993; Haas 2003), only *Lepidobatrachus* is an obligate carnivore; larval *Ceratophrys* are facultatively carnivorous. In *L. laevis,* the unusual head form and specialized morphology of the chondrocranium and cranial musculature, as well as the lack of keratinized jaw sheaths, are adaptations for consuming large animal prey, which are swallowed whole. *Ceratophrys* instead processes animal prey with its jaws before swallowing, while *Chacophrys* has a typically generalized, suspension feeding, microphagous, mostly herbivorous tadpole (Wild 1999; Quinzio *et al.* 2006). Reflecting this diversity of feeding habits, *Lepidobatrachus, Chacophrys,* and *Ceratophrys* share few features of oral anatomy. The only feature common to most known tadpoles of the Ceratophryinae, which also could be considered a morphological synapomorphy for the three genera, is a complete row of marginal papillae (Quinzio *et al.* 2006).

The larval chondrocranium of *Lepidobatrachus*, as in all other ceratophryine species studied to date, has a robust construction typified by short *cornua trabeculae* and strong jaw cartilages (Ruibal and Thomas 1988; Wild 1997, 1999; Vera Candioti 2005; Fabrezi and Quinzio 2008). In addition, in *Lepidobatrachus,* the primary jaw articulation is displaced posteriorly—caudal to the eye—relative to its typical position in anuran larvae, which dramatically increases the size of the larval lower jaw and gape. The *commissura quadratocranialis anterior* is longer than in other tadpoles, and the *arcus subarcualis palatoquadrati* is deployed posterior to the eye in a mediolateral orientation. Articulations of the enlarged *ceratohyale* with the *palatoquadratum* also have shifted posteriorly. Finally, the *processus lateralis hyalis* reaches ventrally into the region of the *capsula auditiva.* These morphological specializations, as well as the small branchial baskets and large head, are correlated with the unusual feeding mode of larval *Lepidobatrachus*; all are adaptations for 'megalophagy,' the consumption of very large prey, which are swallowed whole (Ruibal and Thomas 1988; Wassersug and Heyer 1988; Hanken 1992, 1993; Lavilla and Fabrezi 1992).

The link between external morphology and feeding type is recognized by a commonly used classification of larval ecomorphs (McDiarmid and Altig 1999). In this system, *Lepidobatrachus* belongs to the lentic carnivore guild, which also includes *Ceratophrys* (Vera Candioti 2005) and the pipid frog *Hymenochirus boettgeri* (Sokol 1962; Deban and Olson 2002). Within this guild, prey is manipulated in different ways. *Hymenochirus boettgeri* sucks in small prey using an unusual modification of the jaw apparatus, which is configured as a tube (Deban and Olson 2002). Macrophagous larvae such as *Lepidobatrachus* and *Ceratophrys* produce a very large suction force inside the buccal cavity. They have enlarged *ceratohyalia*, a modification also found in suctorial larvae (Haas and Richards 1998). Even more reduced branchial baskets and larger *ceratohyalia* are found in macrophagous tadpoles of *Hyla nana* (Vera Candioti *et al.* 2004).

Larson and Reilly (2003) studied the function of several muscles in aquatic feeding and gill irrigation in tadpoles of *Rana catesbeiana*. They report the *m. levator mandibulae longus superficialis* as active during feeding and hyperexpiration, thereby closing the mouth. Muscles of the levator mandibulae complex are very well developed in both *Lepidobatrachus* and *Ceratophrys* and could supply the force needed for mouth closure after ingesting large prey either whole or in smaller pieces, respectively. The *m. intermandibularis* of *C. cranwelli* is intermediate in size—larger than in *L. laevis* but smaller than in *Ch. pierottii*. In *C. cranwelli,* the muscle is divided into two slips, whereas in *L. laevis,* it has several origins from the *cartilago Meckeli* and is quite small. It might function to both modify jaw position and elevate the floor of the mouth, causing water and food to flow caudally from the buccal cavity to the pharyngeal cavity. *Lepidobatrachus laevis* ingests animal prey whole, whereas *C. cranwelli* bites off pieces of its prey before swallowing; the latter condition may necessitate a stronger *m. intermandibularis*. *Chacophrys pierottii* has a very well-developed *m. intermandibularis* and prominent keratinized sheaths, which are useful for herbivorous scraping.

Cranial musculature in larval *L. laevis* also differs significantly from that seen in more typical anuran larvae. The *m. suspensoriohyoideus* is absent. Ruibal and Thomas (1988) describe only two angularis muscles, angularis and hyoangularis, and suggest that the angularis muscle may represent fused *m. suspensorioangularis* and *m. quadratoangularis;* the latter muscle was reported absent by Fabrezi and Quinzio (2008) and by Haas (2003). The larvae described here are significantly younger than those in the above-mentioned studies. We were, however, able to resolve the *m. suspensorioangularis* and *m. quadratoangularis* as both present and distinct in *L. laevis*, although they indeed are difficult to differentiate and are fused rostrally. We suggest that these muscles fuse further as development proceeds and are no longer distinguishable in older larvae. Origin of the *m. orbitohyoideus* from the *commissura quadratocranialis anterior* (anterior process; Ruibal and Thomas 1988) by means of a flat tendon, as reported by Ruibal and Thomas (1988), is not visible in the specimens and stages considered in our study, but such a tendon might develop in older stages.

Anatomic differences among *Lepidobatrachus*, *Ceratophrys,* and *Chacophrys* exemplify the extreme diversity of larval adaptations and morphologies present within the Ceratophryinae. The derived cranial morphologies of *Lepidobatrachus* and *Ceratophrys* may represent independent instances of the evolution of larval carnivory from a generalized, herbivorous ancestor (Fabrezi 2006). In *Lepidobatrachus,* carnivory is manifest as megalophagy, whereas in *Ceratophrys,* animal prey is processed by the jaws before swallowing (Wassersug and Heyer 1988). Under this scenario, the contrasting carnivorous tadpole morphologies in these two genera evolved independently from a basal, herbivorous tadpole type exemplified today by *Chacophrys*.

Phylogenetic relationships among the three ceratophryine genera are not resolved (Fabrezi and Quinzio 2008), and different larval characters offer support for alternate schemes of relationship. For example, several features of the tadpole of *C. cranwelli* are in many respects intermediate between those of *L. laevis* and *Ch. pierottii.* These features include oral and gut anatomy and the size of the *m. intermandibularis* (Ruibal and Thomas 1988; Wassersug and Heyer 1988; Wild 1997). On the other hand, some features of the larval *chondrocranium* (*cartilago labialis superior*) and branchial skeleton (*spiculae* and cartilaginous projections along *ceratobranchialia*) of *Chacophrys* are not shared with either *Ceratophrys* or *Lepidobatrachus* (Lavilla and Fabrezi 1992; Wild 1999; Vera Candioti 2005; Fabrezi and Quinzio 2008).

Absence of the *m. quadratoangularis* was proposed as a synapomorphy of the clade *Ceratophrys* + *Lepidobatrachus* (Haas 2003; Fabrezi and Quinzio 2008). We show, however, that the *m. quadratoangularis* is initially present in *L. laevis*. It subsequently fuses with the *m. suspensorioangularis* and ultimately is indistinguishable from the latter muscle. Thus, the proposed taxonomic character is not valid. Nevertheless, there remain at least 19 additional larval characters that unite *Ceratophrys* + *Lepidobatrachus,* and both adult and larval characters support the clade *Chacophrys* + (*Ceratophrys* + *Lepidobatrachus*) (Fabrezi and Quinzio 2008). Fabrezi and Lobo (2009) describe differences of the adult hyoid skeleton and associated muscles between *Lepidobatrachus* (*L. laevis* and *L. llanensis)* and other ceratophryines. Those differences include reduction or loss of hyoid muscles in adult *Lepidobatrachus* and changes in the hyoid skeleton. *Lepidobatrachus* is the most derived ceratophryine genus with two possibilities of phylogeny: (i) *Lepidobatrachus* basal or (ii) *Ceratophrys* or

Chacophrys basal. Data in Fabrezi and Lobo (2009) support the latter scenario.

Despite its remarkable cranial morphology, the tadpole of *L. laevis* shows relatively minor modifications of the origins and insertions of cranial muscles in comparison with the pattern of muscle attachment seen in more generalized frog larvae. Instead, changes in the relative size of muscles are common, such as those that confer enormous jaw levators. A mechanistic understanding of the heterochronic changes in growth processes that cause this remodeling of both chondrocranium and cranial muscles is an important goal for future research.

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