The evolution of intrauterine feeding in the Gymnophiona (Lissamphibia)

A comparative study on the morphology, function, and development of cranial muscles in oviparous and viviparous species

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Zusammenfassung

Die rezenten Amphibien (Lissamphibia) sind durch einen komplexen biphasischen Lebenszyklus gekennzeichnet. Sie durchlaufen eine Metamorphose, bei der sich eine aquatische Larve zum terrestrischen Adultus entwickelt. Im Zusammenhang mit dem biphasischen Lebenszyklus gilt Oviparie als ursprünglicher Fortpflanzungsmodus für die Lissamphibia. In allen drei Gruppen der Amphibien, d.h. innerhalb der Froschlurche (Anura), Schwanzlurche (Caudata) und Blindwühlen (Gymnophiona), sind abgeleitete Fortpflanzungsmodi (Oviparie mit direkter Entwicklung, Viviparie) evolviert. Innerhalb der Blindwühlen ist die Evolution von abgeleiteten Fortpflanzungsmodi mit neuen Beutefangmechanismen während der Ontogenese verbunden: Im ursprünglichen biphasischen Lebenszyklus nutzen die aquatischen Blindwühlenlarven bis zur Metamorphose Saugschnappen; der terrestrische Beutefang der Adulti ist Beißen. Frischgeschlüpfte Jungtiere von oviparen Blindwühlen mit direkter Entwicklung fressen zunächst an der Haut ihrer Mütter (Dermatophagie). Die Föten viviparer Blindwühlen fressen intrauterin indem sie das Uterusepithel abschaben. Es ist anzunehmen, dass die unterschiedlichen Verhaltensweisen einen Einfluss auf die Morphologie und Funktionsweise des Kopfes während der Entwicklung haben. In dieser Arbeit beschreibe ich den Einfluss der Evolution von Fortpflanzungsmodi auf die Transformationen in der Entwicklung des Craniums und der cranialen Muskulatur bei Blindwühlen. Während der ursprünglichen biphasischen Entwicklung von Blindwühlen gibt es in der Metamorphose fundamentale Umbauprozesse der cranialen Muskulatur. Ausschließlich auf die Larve beschränkte Merkmale der Kopfmuskulatur sind: das Vorhandensein eines M. ceratomandibularis, die Ausbildung eines separaten M. interhyoideus, der weite Teile des Hyobranchialapparates lateral umfasst, das Vorhandensein eines M. subarcualis rectus II-IV und der Ansatz des M. depressor

mandibulae posterior am distalen Ende des Ceratohyale. Der Vergleich zur Kopfmuskulatur der Larve von Schwanzlurchen zeigt, dass diese Merkmale plesiomorph für die Larve der Gymnophiona sind. Die Evolution von abgeleiteten Fortpflanzungsmodi ist mit der Reduktion larvaler Merkmale verbunden. Die Kopfmuskulatur von Embryonen, Föten und Jungtieren viviparer Arten und oviparer Arten mit direkter Entwicklung ist identisch zur Kopfmuskulatur adulter Tiere. Die frühzeitige Entwicklung von Muskelmerkmalen des Adultus bei Arten mit Direktentwicklung oder Viviparie kann als Umstrukturierung der ursprünglichen Ontogenie ('*ontogenetic repatterning*') verstanden werden. Verglichen zum übrigen Schädel sind zahntragende Knochen im Cranium von Föten viviparer Arten früh in der Ontogenese angelegt und auffallend groß; die relative Größe nimmt im Verlauf der Ontogenese ab. Die Ausbildung großflächiger zahntragender Knochen zu Beginn der Entwicklung des Schädels wird als Konsequenz aus der intrauterinen Ernährung bei viviparen Blindwühlen angesehen. Mit dieser Studie konnte gezeigt werden, dass die aquatische Lebensweise der Larve bei Blindwühlen andere Anforderungen an die Funktionsweise des Cranium und der cranialen Muskulatur stellt, als die terrestrische Lebensweise adulter Tiere. Besonders der M. levator mandibulae longus und der M. interhyoideus posterior unterscheiden sich in ihrer Funktion bei der Larve von adulten Tieren. Die Mechanik des Kieferapparates bei Jungtieren einer oviparen Art mit direkter Entwicklung und Neugeborenen einer viviparen Art ist nahezu identisch zu der des Adultus. Die Folgen der Evolution von direkter Entwicklung und Viviparie bei Blindwühlen sind eine vom Grundmuster abgeleitete Bildung der Kopfmuskulatur, Unterschiede im Ablauf von Entwicklungsprozessen des Kopfes und der Verlust larvaler Funktionalität des Kopfes und der Kopfmuskulatur.

Summary

The extant amphibians (Lissamphibia) are characterized by a complex biphasic life-cycle in that an aquatic larvae passes through a metamorphosis to become a terrestrial adult. Oviparity with biphasic life-cycle is the ancestral mode of reproduction in the Lissamphibia. The derived reproductive modes oviparity with direct development and viviparity evolved independently in the three amphibian groups, i.e. frogs (Anura), salamanders (Caudata), and caecilians (Gymnophiona). In caecilians, the evolution of derived modes of reproduction is correlated to new feeding habits over ontogeny. In the ancestral condition, aquatic larvae use suction feeding for prey capture before they pass through metamorphosis to become terrestrial predators that feed by biting. Hatchlings from direct developing caecilians have been observed to feed on the skin of their mothers before they switch to the diet of adults (dermatophagy). In viviparous caecilians, fetuses feed intrauterine by scraping the uterus epithelium. Different feeding modes over ontogeny are likely to have an impact on the morphology and the function of the developing head of caecilians. This study examines the transformations in caecilian cranial and cranial muscle development that can be related to the evolution of derived modes of reproduction and new feeding strategies. The ancestral biphasic life-cycle involves substantial modifications during metamorphosis in the cranial musculature of caecilians. Characters that are exclusively present in larvae are: presence of a m. ceratomandibularis, presence of a well developed m. interhyoideus that covers the hyobranchium laterally, presence of a m. subarcualis rectus II-IV, insertion of the m. depressor mandibulae posterior on the distal tip of the ceratohyal. The comparison to salamander larvae reveals that these muscle characters are plesiomorphic for larvae of the Gymnophiona. The evolution of derived modes of reproduction involves the evolutionary loss of larval muscle characteristics. The cranial musculature in embryos, fetuses, and juveniles of

direct developing and viviparous caecilians is identical to adults. The precocious development of adult muscle features in embryos of direct developing and viviparous caecilians is considered to be a case of ontogenetic repatterning. In viviparous species, fetal skull development is modified which results in the presence of large tooth bearing bones early in ontogeny. The relative size of tooth bearing bones to the remainder of the cranium decreases during intrauterine caecilian development. The early formation of large tooth bearing bones is likewise related to intrauterine feeding. I further demonstrate that larval feeding puts different functional demands on the jaw closing muscles, especially the m. levator mandibulae longus and the m. interhyoideus posterior, than adult feeding. Skin feeding juveniles of a direct developing caecilian and neonates of a viviparous species have an almost identical jaw closing system as adult caecilians. The evolution of direct development and viviparity in caecilians can be linked to derived patterns of cranial muscle formation, differences in the timing of cranial development, and to the release from functional constraints that are introduced by the aquatic larval stage of biphasic caecilians.

Introduction

The extant amphibians (i.e. Lissamphibia) comprise caecilians (Gymnophiona), salamanders (Caudata), and frogs (Anura). A complex biphasic life-cycle is characteristic for amphibians. It comprises an aquatic larval stage and metamorphosis to the terrestrial adult (Hanken, 1992; Duellman and Trueb, 1994). Although the presence of a biphasic lifecycle is the ancestral condition for amphibians, direct development and viviparity evolved independently within the three amphibian groups (Wake, 1993, 2003a). Studies on modifications of the ancestral biphasic ontogeny in frogs (Hanken, 1992; Hanken et al., 1992, 1997) and salamanders (Gould, 1977; Wake, 1982; Wake and Hanken, 1996) provided insights on the complex relationships between development and the evolution of morphologies in vertebrates (Hanken, 1989, 1999). Caecilians have been neglected in most of this studies and many aspects of caecilian development and evolution are cryptic.

Recent hypothesis on the phylogeny of the Lissamphibia regard the Gymnophiona as sister-taxon to the Batrachia, i.e. Caudata plus Anura (San Mauro et al., 2005; Zhang et al., 2005; Frost et al., 2006; Roelants et al., 2007). Caecilians have a highly derived morphology as consequence to their fossorial lifestyle. The skull is heavily ossified and characterized by several fusions of bones (e.g. the os basale; see Wake, 2003b for a review on caecilian skull morphology). Caecilians are unique among vertebrates in having a dual jaw closing mechanism in that a hyobranchial muscle is recruited as jaw closing muscle (Bemis et al., 1983; Nussbaum, 1983). The vertebral musculature in caecilians is independent of the muscles of the body wall (Nussbaum and Naylor, 1982), that allows for a unique hydrostatic locomotion (O'Reilly et al., 1997). Modes of reproduction in caecilians comprise the ancestral oviparity with larvae and metamorphosis, and the derived oviparity with direct development,

and viviparity. In direct developing oviparous caecilians the eggs are deposited on land and terrestrial juveniles emerge from the eggs (based on the definition by Hanken, 1992). The free living aquatic larval stage and metamorphosis are absent in direct developing species. In viviparous caecilians the eggs develop in utero. Intrauterine feeding stages (i.e. fetuses) hatch from the eggs. Fetuses develop in the uterus until the female gives birth to terrestrial juveniles (Wake, 1993).

Feeding modes in caecilians comprise biting, suction feeding, skin feeding, and intrauterine feeding. The differences in reproductive modes in caecilians are closely related to different feeding habits during ontogeny. Adult caecilians are known to be generalist predators (Hebrard et al., 1992; Verdade et al., 2000; Presswell et al., 2002; Kupfer and Maraun, 2002; Gaborieau and Measey, 2004; Measey et al., 2004; Kupfer et al., 2005) that use biting for prey capture (O'Reilly, 2000; O'Reilly et al., 2002; Measey and Herrel, 2006). The use of suction for prey capture was documented in larvae of the caecilian genus *Epicrionops* (O'Reilly, 2000). Skin feeding, was shown for juveniles of the direct developing oviparous species *Boulengerula taitana* and *Siphonops annulatus* (Kupfer et al., 2006; Wilkinson et al., 2008). In these species, the juveniles feed on the skin of the mother before they switch to the diet of adults. Based on the distinct positions of *B. taitana* and *S. annulatus* in caecilian phylogeny (Frost et al., 2006; Roelants et al., 2007; fig. 1), Wilkinson et al. (2008) concluded that skin feeding is a widespread feeding mode in oviparous species with direct development. Viviparous caecilians feed intrauterine during development, i.e. fetuses scrape on the uterus epithelium and stimulate the segregation of a secretion that often is referred to as 'uterine milk' (Wake, 1977; 1980a). For intrauterine feeding, the fetuses have a specialized dentition that is replaced at birth (Parker, 1956; Parker and Dunn, 1964; Wake, 1980b) and comparable to the teeth in juveniles of skin feeding species (Wilkinson and

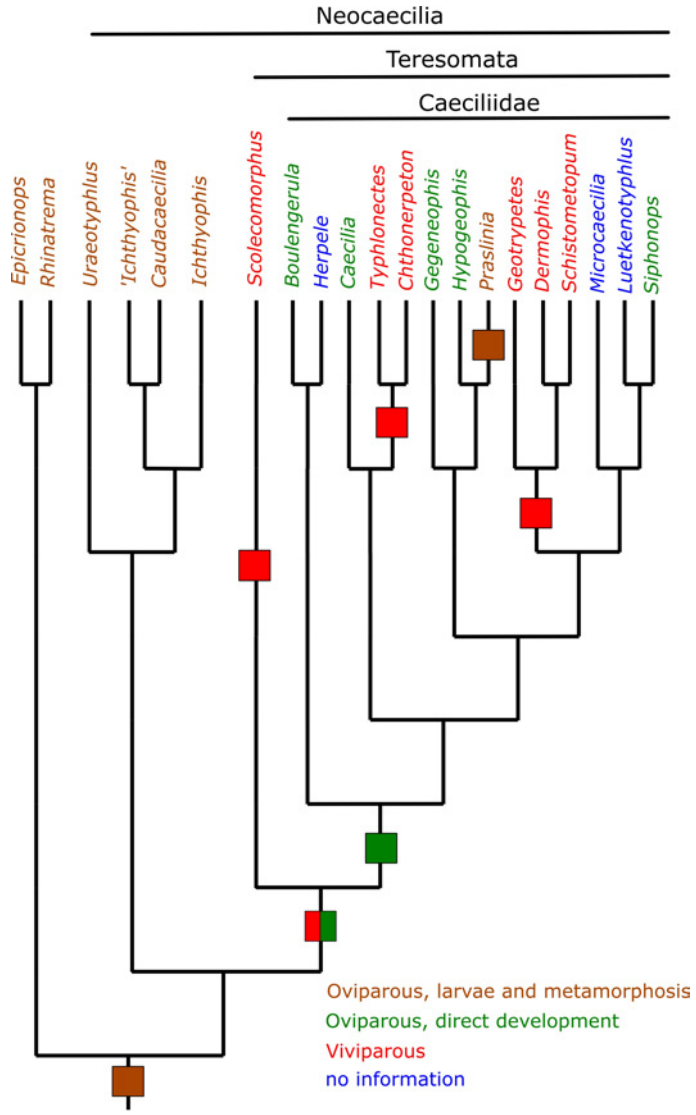

Figure 1. Evolution of reproductive modes in caecilians according to Müller (2007). Phylogeny from Roelants et al. (2007). Reproductive modes are color coded: brown - oviparity with larvae and metamorphosis; green - oviparity with direct development; red - viviparity. The ancestral biphasic ontogeny was lost at the root of the Teresomata. Viviparity most likely evolved three times independently and at least two times from ancestors that had direct development. Larvae are present in the derived genus Praslinia that is nested within a clade of direct developing species.

Nussbaum, 1998; Kupfer et al., 2006). Wilkinson et al. (2008) suggested that intrauterine feeding in viviparous caecilians evolved from skin feeding ancestors. Müller et al. (2009) found evidence that viviparous species of the Scolecomorphidae (genus *Scolecomorphus*) show juvenile skin feeding rather than fetal intrauterine feeding. This was based on the observation of postnatal transformations of the premaxillary-maxillary arcade in the skull of *Scolecomorphus kirkii*.

Based on recent hypothesis on caecilian relationships (Roelants et al., 2007), vivparity evolved at least three times within the Gymnophiona, i.e. in the genus *Scolecomorphus*, the Typhlonectidae (the genera *Typhlonectes* and *Chthonerpeton*), and a clade that comprises the caeciliid genera *Dermophis*, *Gymnopis*, and *Schistometopum* (Müller, 2007; fig. 1). Two of the three clades that evolved viviparity are nested within direct developing species. Based on the phylogeny presented by Roelants et al. (2007) the ancestral biphasic lifecycle was lost at the root of the Teresomata ('higher caecilians'; sensu Wilkinson and Nussbaum, 2006). This hypothesis also was discussed to involve the re-evolution of larvae in species of the Caeciliidae (Müller, 2007).

Because of the tight correlation between reproductive modes and feeding habits over caecilian ontogeny, the evolution of derived reproductive modes is likely to have an impact on the morphology and development of the cranium and the associated cranial musculature. Several studies described the development of the skull in oviparous species with biphaisc lifecycle (Peter, 1898; Visser, 1963; Müller, 2007), with direct development (Marcus et al., 1935; Wake, 1986; Müller et al., 2005; Müller, 2006, 2007), and in viviparous species (Wake and Hanken, 1982; Wake et al., 1985; Müller et al., 2009). Müller (2007) presented a first discussion on the modifications in cranial development that are related to the evolution of derived reproductive modes, with an emphasis on direct development. The comparison between oviparous species with larval stage and metamorphosis, and species with direct development revealed that direct development is related to a gradual formation of an 'adult-like' skull morphology instead of showing dramatic changes during metamorphosis (Müller, 2007).

One would expect to find alterations in cranial muscle development that can be treated as consequences to the evolution of derived reproductive modes and that are related to

the modifications in the ontogenetic trajectory (i.e. the relationship between the shape and age of an individual; Alberch et al., 1979) of cranial development. However, there is surprisingly little information published on the development of muscles in caecilians. Wake (1986) gave a short description on cranial muscles in the direct developing species *Idiocranium russeli* including some juvenile specimens. However, there is no information available on the development of cranial muscles in oviparous species with direct development and in viviparous species. Knowledge on the development of cranial muscles in relation to the modes of reproduction, however, is crucial to understand the functional integration of the skull and how skull morphology is effected by different functional demands over development.

To investigate the evolution of development for a character complex, e.g. the cranium and the cranial musculature, it is essential to first define ancestral character states. The ancestral mode of reproduction is oviparity in combination with a complex biphasic life-cycle (Duellman and Trueb, 1994). However, there is only limited information available on the cranial musculature in larvae of the most recent common ancestor of amphibians, i.e. in the ground pattern of the Lissamphibia. The study by Haas (2001) reconstructed the jaw closing musculature in the ground pattern of the Lissamphibia. Kleinteich and Haas (2007) published a discussion on the complete cranial musculature, including muscles of the hyobranchial apparatus, for larvae of the most recent common amphibian ancestor. Haas (2001) and Kleinteich and Haas (2007) examined the cranial muscle morphology in larvae of the caecilian *Ichthyophis kohtaoensis* and concluded from this species on the larvae of the ancestor of all caecilians. Müller (2007) described the cranial musculature in several caecilian larvae, including specimens of *Epicrionops lativittatus*. The genera *Epicrionops* plus *Rhinatrema* (i.e. the Rhinatrematidae, fig. 1) are considered to be in a sister-group

relationship to the remainder caecilians (Neocaecilia sensu Wilkinson and Nussbaum, 2006) and thus are very important for discussions on the most recent common ancestor of caecilians. However, Müller (2007) focused on the muscles that are related to jaw movements without considering the hyobranchial musculature.

Aims of this study

This study analyses the modifications in the development of cranial muscles in caecilians that have derived modes of reproduction, i.e. oviparity with direct development and viviparity. Based on a reconstruction of the cranial musculature in larvae of the most recent common ancestor of the Lissamphibia, the results presented herein are interpreted within a phylogenetic framework. Further, differences in the ontogenetic trajectories for skull development that are related to the evolution of viviparity and intrauterine feeding in caecilians are examined. The functional consequences of modifications in the development of the cranium and the cranial musculature are evaluated herein.

Chapter 1 provides a first comparison of the hyal and ventral branchial muscles in larvae of the caecilian *Epicrionops bicolor* with salamander larvae and juveniles of neotene salamanders. A terminology for hyal and ventral branchial muscles is suggested that is supposed to reflect homologies between muscles in caecilians and salamanders. The results presented in chapter 1 contribute to the discussion on the larval cranial musculature in the ground pattern of the Lissamphibia.

Chapter 2 contains the first description of cranial muscle development in caecilians with derived modes of reproduction, i.e. oviparity with direct development and viviparity. To reconstruct the modifications in cranial muscle development, the results presented in chapter 2 are compared to the cranial musculature in larvae of the most recent common ancestor of amphibians and caecilians.

Chapter 3 is a geometric morphometric study that examines the changes in cranial shape over development (i.e. the ontogenetic trajectory) in an oviparous caecilian with biphasic life-cycle and in a viviparous species. The comparison and quantification of ontogenetic trajectories gives further insights in the alteration of developmental pathways that can be related to the evolution of viviparity.

Chapter 4 compares the feeding biomechanics between caecilian larvae and embryos and juveniles of a direct developing species and juveniles of a viviparous caecilian. The results presented in chapter 4 display the functional demands on the cranium and the cranial musculature that are based on different reproductive modes and the related feeding habits during development.

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Chapter 1

The hyal and ventral branchial muscles in caecilian and salamander larvae: homologies and evolution

ABSTRACT: Amphibians (Lissamphibia) are characterized by a bi-phasic life-cycle that comprises a larval stage and metamorphosis to the adult. The ancestral feeding behavior of amphibian larvae is suction feeding. The negative pressure that is needed for ingestion of prey is created by depression of the hyobranchial apparatus and thus by the hyobranchial musculature. Understanding the homologies of hyobranchial muscles in amphibian larvae is a crucial step in understanding the evolution of this important character complex. Here I will describe the hyal and ventral branchial musculature in larvae of caecilians (Gymnophiona) and salamanders (Caudata) and in juveniles of two aquatic salamander species. I will propose a terminology for the hyal and ventral branchial muscles that reflects the homologies of muscles in different caecilian and salamander species. A discussion on the hyal and ventral branchial muscles in larvae of the most recent common ancestor of amphibians (i.e. the ground pattern of Lissamphibia) is presented. The hyal and ventral branchial musculature comprises the following muscles: m. depressor mandibulae, m. depressor mandibulae posterior, m. ceratomandibularis, m. ceratohyoideus externus, m. interhyoideus, m. interhyoideus posterior, m. ceratohyoideus internus, m. subarcualis obliquus II, m. subarcualis obliquus III, m. subarcualis rectus II-IV, and m. transversus ventralis IV. Except for the m. ceratohyoideus externus, all of these muscles are supposed to be present in the ground-pattern of the Lissamphibia. It remains unresolved, whether the m. ceratohyoideus externus is autapomorphic for the Batrachia (frogs plus salamanders) or the Caudata, or present in the ground pattern of the Lissamphibia remains unresolved.

INTRODUCTION

In the three groups of extant amphibians (Lissamphibia), i.e. caecilians (Gymnophiona), salamanders (Caudata), and frogs (Anura), a complex life cycle with larvae and metamorphosis is present. It is most parsimonious to assume that the most recent common ancestor of amphibians had a larval stage (Duellman and Trueb, 1994; Wake, 1993; Wilkinson et al., 2002). Amphibian larvae are aquatic and their ancestral feeding strategy is suction feeding. All salamander larvae studied so far use suction for prey capture (Deban and Wake, 2000; Deban et al., 2001; O'Reilly et al., 2002). In caecilians, suction feeding was documented for larvae of species within the genus *Epicrionops* (Rhinatrematidae) and is supposed to be the feeding mode in other caecilian taxa (O'Reilly, 2000; O'Reilly et al., 2002). Frog tadpoles use mucous entrapment suspension feeding, which, however, is a derived feeding mode within amphibian larvae (O'Reilly et al., 2002).

The negative pressure needed for suction feeding is caused by depression of the hyobranchial apparatus and thus the hyal and branchial musculature (Deban and Wake, 2000). Besides suction, the hyobranchial musculature is important for tongue movements and ventilation and thus has a double function in feeding and breathing (Wake, 1982; Roth and Wake, 1985).

A first important study on hyal and branchial muscles in amphibians was the study by Drüner (1901, 1904) that comprised larval and adult specimens of 10 salamander species. The study on caecilian cranial nerves by Norris and Hughes (1918), was the first study that applied the terminology from Drüner (1901, 1904) to caecilian cranial musculature. Later, Edgeworth (1935) published his monograph on cranial muscles in vertebrates. Edgeworth (1935) compared many vertebrate species, including amphibians. Although, part of

Edgeworths terms were based on Drüners (1901, 1904) study, Edgeworth (1935) further introduced several new terms. The terms that are used in recent studies on amphibian cranial muscles (Bauer, 1997; Haas, 1997, 2001, 2003; Kleinteich and Haas, 2007) are still derived from the studies by Drüner (1901, 1904) and Edgeworth (1920, 1935). However, comparative anatomists at the beginning of the last century used the terms for cranial muscles as description of topology or function, and not for homology. In Drüners (1901, 1904) and Edgeworths (1935) terminology it was possible, that homologous structures got different names, in case they showed ontogenetic or phylogenetic variation.

Today, characters from morphological studies are used in analyses on morphological character evolution (Rieppel and Kearney, 2002); names of structures are used as homology statements, i.e. primary homologies (De Pinna, 1991; Brower and Schawaroch, 1997). The lack of information on homology makes it difficult to apply the terminology from Drüner (1901, 1904) and Edgeworth (1935) to questions on mophological evolution in a phylogenetic context without a thorough revision on the validity of terms.

Recent hypotheses on the relationships within the Lissamphibia suggest that caecilians are the sister taxon to salamanders plus frogs (Batrachia hypothesis; Trueb and Clothier, 1991; Zardoya and Meyer, 2000, 2001; Frost et al., 2006; Roelants et al., 2007). The divergence of the three amphibian groups is supposed to be very old (351 - 266 mya; Marjanovic and Laurin, 2007) and each lineage has evolved highly specialized morphologies.

A previous study on the hyobranchial apparatus in tadpoles by Haas (1997) showed the potential that larval hyobranchial characters have in the study of amphibian evolution. Especially the ventral branchial muscles of the subarcualis muscle system showed a high degree of character state evolution. Several studies gave descriptive accounts on the hyal and branchial muscles in salamanders (Drüner, 1901, 1904; Litzelmann, 1923; Edgeworth, 1920,

1935; Piatt, 1938; Fox, 1958; Bauer, 1997). Caecilians, however, have been neglected in most of the previous studies, although their position in amphibian phylogeny is crucial to explore questions on the evolution of the hyobranchium in amphibians. A list of cranial muscles in larvae of *Epicrionops bicolor* was provided by Wake (1989) in a study on the development of the skeletal elements of the hyobranchium. A first description of the entire cranial musculature in larvae of a caecilian that includes the hyobranchial musculature was published only recently (Kleinteich and Haas, 2007).

This study describes the hyal and ventral branchial muscles in larvae of the caecilian *Epicrionops bicolor*, that is a species within the Rhinatrematidae, the sister group to the remainder caecilians (San Mauro et al., 2004, 2005; Frost et al., 2006; Roelants et al., 2007). Further, I examined the hyal and ventral branchial muscles in salamander larvae of the species *Salamandrella keyserlingii* (Hynobiidae) and *Desmognathus quadramaculatus* (Plethodontidae) and in juvenile specimens of the neotene species *Siren intermedia* (Sirenidae) and *Amphiuma means* (Amphiumidae). A terminology that can be universally applied to the musculature in caecilian and salamander larvae is suggested herein. This study contributes to the discussion on the cranial muscles in larvae of the most recent common ancestor of amphibians with an emphasis on the hyal and ventral branchial muscles.

MATERIALS AND METHODS

I examined larval specimens of the caecilian *Epicrionops bicolor* Boulenger, 1883 (Gymnophiona: Rhinatrematidae), larvae of the salamander species *Salamandrella keyserlingii* Dybowski, 1870 (Caudata: Hynobiidae) and *Desmognathus quadramaculatus* (Holbrook, 1840) (Caudata: Plethodontidae), and juveniles of the neotene salamander species *Siren intermedia* Barnes, 1826 (Caudata: Sirenidae) and *Amphiuma means* Garden *in* Smith,

1821 (Caudata: Amphiumidae). Table 1 contains a list of specimens that were examined herein. Specimens have been made available by the Zoological Museum Hamburg (ZMH), the Museum of Vertebrate Zoology Berkeley (MVZ), Alexander Haas (Sala, University of Hamburg), and by Marvalee H. Wake (MHW, University of California, Berkeley).

Specimens have been available as serial sections, enzyme cleared and stained animals, or were dissected by hand. Table 1 shows the preparation techniques that were applied for each specimen. Specimens MHW341 and MHW367 have been vertically bisected along the body axis before sectioning; the two halves of the body were serial sectioned in different planes of section. Serial sectioned animals were stained in Azan standard stain (Böck, 1989). The serial sections of the *Epicrionops bicolor* specimens were stained alternating in Picro Ponceau, Haemalaun Eosin, and Azan stain. Enzyme clearing and staining followed the procedure in Dingerkus and Uhler (1977); bones were stained red with Alizarin red, cartilages were stained blue by Alcian blue. Prior to dissection by hand, the cartilages of specimen ZMH A09702 were stained blue with Alcian blue by following the first steps of the Dingerkus and Uhler (1977) protocol until the enzyme maceration step. Pencil drawings of the dissected specimen were created at a dissecting microscope and redrawn on a computer with the open source vector graphics software Inkscape 0.45.

A computer aided 3D computer reconstruction was performed for the *Salamandrella keyserlingii* specimen ZMH A09801. Digital photographs of the serial section from specimen ZMH A09801 were taken for every third section with a Canon PowerShot S50 digital camera that was mounted on a microscope. The general procedure of digitizing the serial section on screen into contour lines and alignment of the contours followed the protocol in Haas and Fischer (1997). I used Alias® Maya™ 6.0 for drawing the contour lines, alignment, modeling and rendering. The stack of contour lines was used as template for the modeling process. The

Table 1. Specimens used in this study.

ID	Species	TL [mm]	Preparation	Stain	Thickness [µm]
MHW341	*Epicrionops bicolor*	89	serial section frontal	Picro Ponceau / H+E / Azan	10
MHW341	*Epicrionops bicolor*	89	serial section sagittal	Picro Ponceau / H+E / Azan	10
MHW367	*Epicrionops bicolor*	161	serial section transversal	Picro Ponceau / H+E / Azan	10
MHW367	*Epicrionops bicolor*	161	serial section frontal	Picro Ponceau / H+E / Azan	10
ZMH A09801	*Salamandrella keyserlingii*	26	serial section transversal	Azan	8
Sala2	*Salamandrella keyserlingii*	29	cleared and stained	Alcian blue / Alizarin red	-
Sala3	*Salamandrella keyserlingii*	31	cleared and stained	Alcian blue / Alizarin red	-
MVZ226908	*Desmognathus quadramaculatus*	59	serial section transversal	Azan	10
MVZ213004	*Desmognathus quadramaculatus*	57	serial section transversal	Azan	10
ZMH A08377	*Amphiuma means*	105	serial section transversal	Azan	10
ZMH A09702	*Siren intermedia*	216	dissection by hand	Alcian blue	-
ZMH A09701	*Siren intermedia*	142	serial section transversal	Azan	10

surface modeling procedure was based on the method described in Kleinteich and Haas (2007). The enzyme cleared and stained specimens of *S. keyserlingii* were used as reference for the alignment of contours and for comparison during the modeling process. Bones, cartilages, and muscles have been reconstructed for the *S. keyserlingii* specimen. Tooth, although present in the animal, have not been considered for 3D modeling.

The innervation of hyal and ventral branchial muscles in the specimens examined was not in the focus of this study. However, innervation often is crucial for the identification and homologization of muscles; the innervation patterns were confirmed by identifying cranial nerves in the serial sections.

There is no terminology for hyal and ventral branchial muscles available that can be applied for caecilians and salamanders simultaneously. The terms I use herein are derived from the studies by Drüner (1901, 1904) and Edgeworth (1935). Table 2 shows a list of synonyms for the hyal and ventral branchial musculature; synonymous terms are discussed in the discussion section of this chapter.

Table 2. Synonymous terms for hyal and ventral branchial muscles in caecilians and salamanders.

This study	Drüner (1902, 1904)	Edgeworth (1935)	Kleinteich and Haas (2007)	Other examples
m. depressor mandibulae	m. cephalodorsomandibularis (superficial layer) m. cephalohyomandibularis	m. depressor mandibulae	m. depressor mandibulae	m. digastricus (Fox, 1959)
m. depressor mandibulae posterior	m. cephalodorsomandibularis (deep layer) m. levator hyoidei	m. depressor mandibulae m. levator hyoidei	m. levator hyoidei	
m. ceratomandibularis	m. ceratomandibularis part of m. cephalohyomandibularis	m. hyomandibularis m. branchiomandibularis	m. hyomandibularis	m. ceratohyoideus externus (Norris and Hughes, 1913); posterior depressor mandibulae (Erdmann, 1984)
m. ceratohyoideus externus	m. ceratohyoideus externus	m. branchiohyoideus externus	-	
m. interhyoideus	m. interhyoideus	m. interhyoideus	m. interhyoideus	m. gularis (Eaton, 1936)
m. interhyoideus posterior	m. interbranchialis I	m. interhyoideus posterior	m. interhyoideus posterior	
m. ceratohyoideus internus	m. ceratohyoideus internus	m. subarcualis rectus I	m. subarcualis rectus I	
mm. subarcuale obliqui II and III	mm. subarcuale obliqui II and III	mm. subarcuale obliqui II and III mm. subarcuale recti II and III	mm. subarcuale obliqui II and III	
m. subarcualis rectus II-IV	m. subarcualis rectus I, II and III	m. subarcualis rectus IV	m. subarcualis rectus II-IV	
m. transversus ventralis IV	m. interbranchialis IV	m. transversus ventralis IV	m. transversus ventralis IV	

RESULTS

M. depressor mandibulae group

The m. depressor mandibulae group comprises four muscles; i.e. the m. depressor mandibulae, the m. depressor mandibulae posterior, the m. ceratomandibularis and the m. ceratohyoideus externus. Muscles of this group are innervated by cranial nerve VII (n. facialis).

Caecilians: In *Epicrionops bicolor*, the m. depressor mandibulae originates from the lateral face of the squamosal, the dorsal surface of the parietal, and parts of this muscle originate from the trunk fascia. The m. depressor mandibulae in *E. bicolor* larvae inserts along the dorsal edge of the processus retroarticularis of the lower jaw (fig. 1).

The m. depressor mandibulae posterior in *E. bicolor* has its origin at the lateral side of the capsula auditiva and at the dorsal trunk fascia. It inserts distally on the ceratohyal and wraps around the dorsal tip of the ceratohyal like a hood (fig. 2). Some fibers of the m. depressor mandibulae posterior are attached to the dorsal edge of the processus retroarticularis of the lower jaw, medial to the m. depressor mandibulae.

The m. ceratomandibularis is a voluminous muscle that originates from the lateral face of the ceratohyal in *E. bicolor* (fig. 2). The m. ceratomandibularis inserts ventrolaterally on the pseudoangular along an area that reaches from the mandibular joint to the caudal tip of the processus retroarticularis (fig. 1).There is no m. ceratoyhoideus externus in larval *E. bicolor*.

Salamanders: In all salamanders examined herein, the m. depressor mandibulae and the m. depressor mandibulae posterior are incompletely separated from each other. The m. depressor mandibulae posterior can be identified as layer of muscle fibers that lies close to

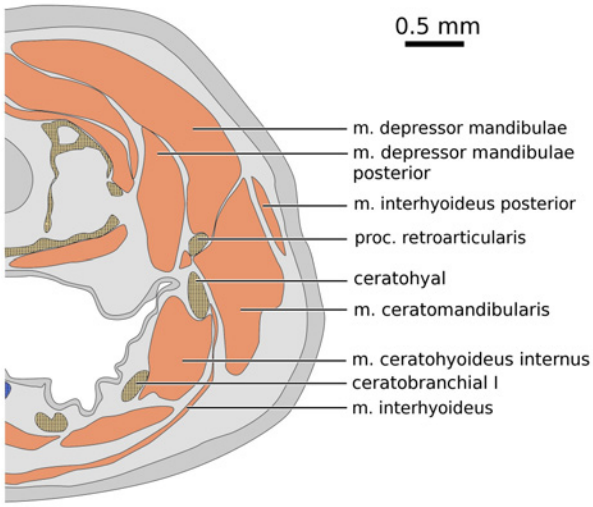


Figure 1. *Epicrionops bicolor* (MHW367), schematic drawing of a transversal section in plane with the ear capsule. The m. depressor mandibulae inserts on the processus retroarticularis of the lower jaw. The m. ceratomandibularis is a voluminous muscle that connects the lower jaw and the ceratohyal. The m. interhyoideus posterior has no insertion on the lower jaw.

fibers of the m. depressor mandibulae posterior are attached to the dorsal edge of the processus retroarticularis of the lower jaw, medial to the m. depressor mandibulae.

The m. ceratomandibularis is a voluminous muscle that originates from the lateral face of the ceratohyal in *E. bicolor* (fig. 2). The m. ceratomandibularis inserts ventrolaterally on the pseudoangular along an area that reaches from the mandibular joint to the caudal tip of the processus retroarticularis (fig. 1).There is no m. ceratoyhoideus externus in larval *E. bicolor*.

Salamanders: In all salamanders examined herein, the m. depressor mandibulae and the m. depressor mandibulae posterior are incompletely separated from each other. The m. depressor mandibulae posterior can be identified as layer of muscle fibers that lies close to the mediocaudal side of the m. depressor mandibulae (fig. 3). Both muscles originate from the lateral face of the squamosal and the ear capsule. In *Amphiuma means* and *Siren intermedia*, the m. depressor mandibulae inserts on the dorsal edge of the processus retroarticularis immediately caudal to the mandibular joint (fig. 4); in *Salamandrella* edge

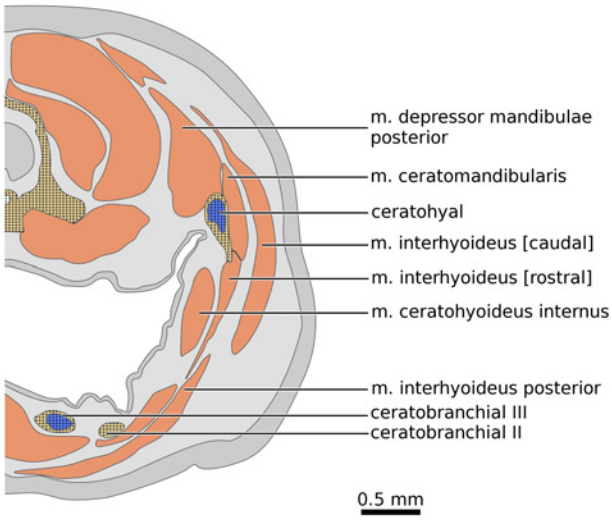


Figure 2. *Epicrionops bicolor* (MHW367), schematic drawing of a transversal section through the first vertebra. The m. depressor mandibulae posterior acts on the ceratohyal. The m. interhyoideus is clearly separated into a rostral and a caudal layer.

the mediocaudal side of the m. depressor mandibulae (fig. 3). Both muscles originate from the lateral face of the squamosal and the ear capsule. In *Amphiuma means* and *Siren intermedia*, the m. depressor mandibulae inserts on the dorsal edge of the processus retroarticularis immediately caudal to the mandibular joint (fig. 4); in *Salamandrella keyserlingii* and *Desmognathus quadramaculatus* the m. deprossor mandibulae inserts on the ventral edge of the articluar via a tendon that reaches rostral to the mandibular joint (fig 5A). The m. depressor mandibulae posterior shares its insertion with the m. depressor mandibulae in *A. means*, *S. keyserlingii*, and *D. quadramacualtus*. In *D. quadramaculatus*, however, some fibers of the m. depressor mandibulae posterior are attached to the distal tip of the ceratohyal (fig. 3). In *S. intermedia*, the m. depressor mandibulae posterior inserts on the distal part of the ceratohyal and has no insertion on the lower jaw.

The m. ceratomandibularis is present in *Siren intermedia*, *Amphiuma means*, and *Desmognathus quadramaculatus*; it is absent in *Salamandrella keyserlingii*. In *Siren intermedia*, the m. ceratomandibularis originates from the ceratohyal by covering the dorsal edge and lateral face of the cartilage entirely (figs. 3, 4, 6). In *A. means* and *D.*

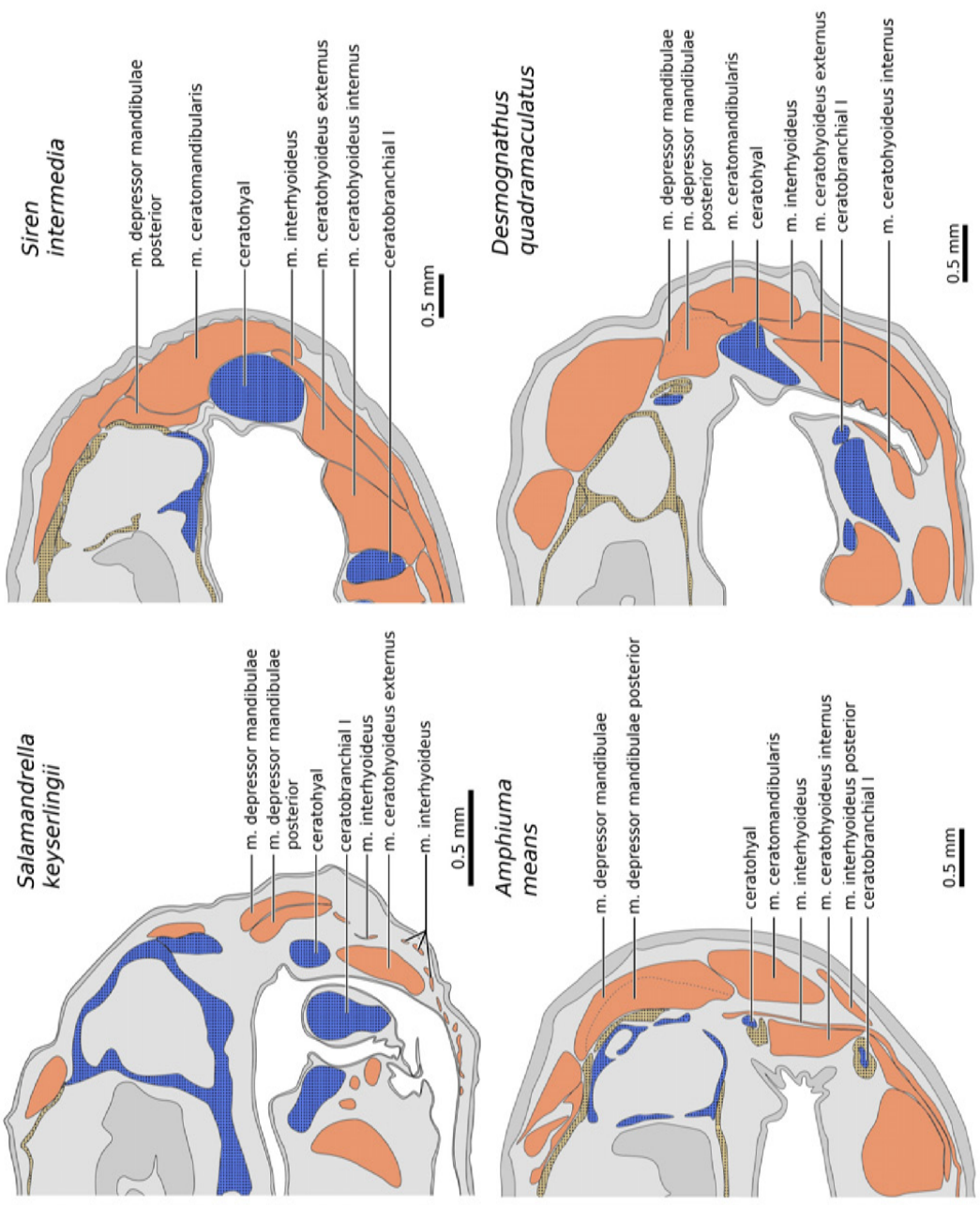


Figure 3. *Salamandrella keyserlingii* (ZMH A09801), *Siren intermedia* (ZMH A09701), *Amphiuma means* (ZMH A08377), and *Desmognathus quadramaculatus* (MVZ226908), schematic drawings of transversal sections in plane with the ear capsule. The m. depressor mandibulae and the m. depressor mandibulae posterior can be barely separated, except in *S. intermedia*. However, the dashed line shows the boarder between the two muscles that can be traced through the muscle sections. In *S. intermedia* and *D. quadramaculatus*, the fibers of the m. depressor mandibulae posterior are attached to the ceratohyal.

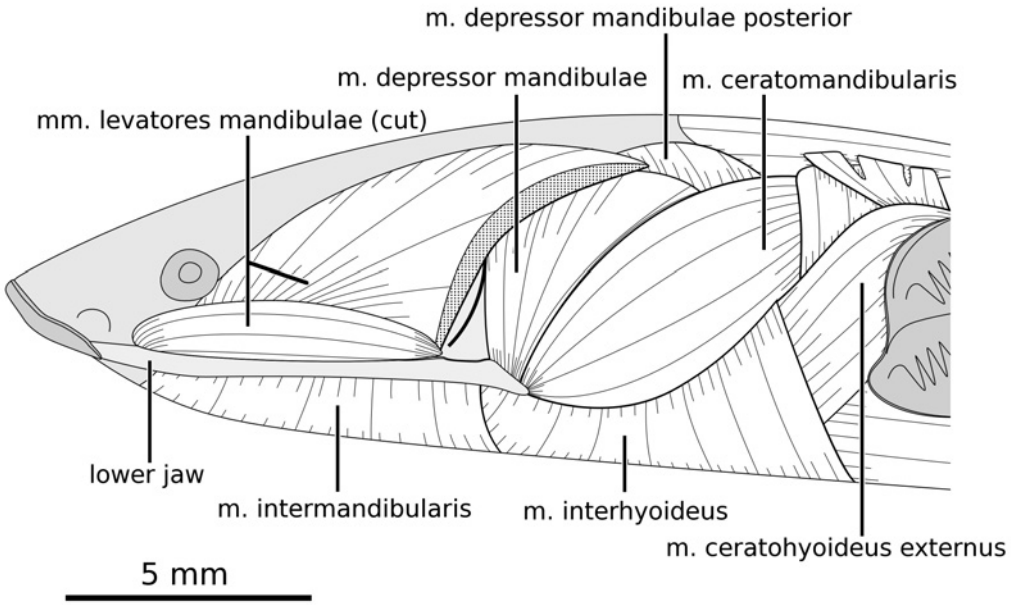


Figure 4. *Siren intermedia* (ZMH A09702), drawing of skinned specimen in lateral view. Parts of the jaw closing musculature (mm. levatores mandibulae) removed. The m. ceratomandibularis has a similar position to the m. depressor mandibulae. Both muscles insert on the dorsal edge of a small processus retroarticularis of the lower jaw.

quadramaculatus, the m. ceratomandibularis originates from the lateral face of the distal part of the ceratobranchial I (fig. 6). The fibers of this muscle run rostrad and ventrad. The m. ceratomandibularis inserts ventrally on the distalmost tip of the processus retroarticularis in *S. intermedia* and *A. means* (fig. 4, 6); in *D. quadramaculatus* it inserts via a tendon on the ventral edge of the articular, ventral and rostral to the mandibular joint.

The m. ceratohyoideus externus is present in *Salamandrella keyserlingii*, *Siren intermedia*, and *Desmognathus quadramaculatus*, it is absent in *Amphiuma means*. This muscle originates from the lateral face of the ceratobranchial I distally, where it covers most of the cartilage laterally (figs. 4, 5, 6). The m. ceratohyoideus externus inserts along the ventral side of the ceratohyal from proximal to distal, following the bended shape of the ceratohyal (figs. 4, 5).

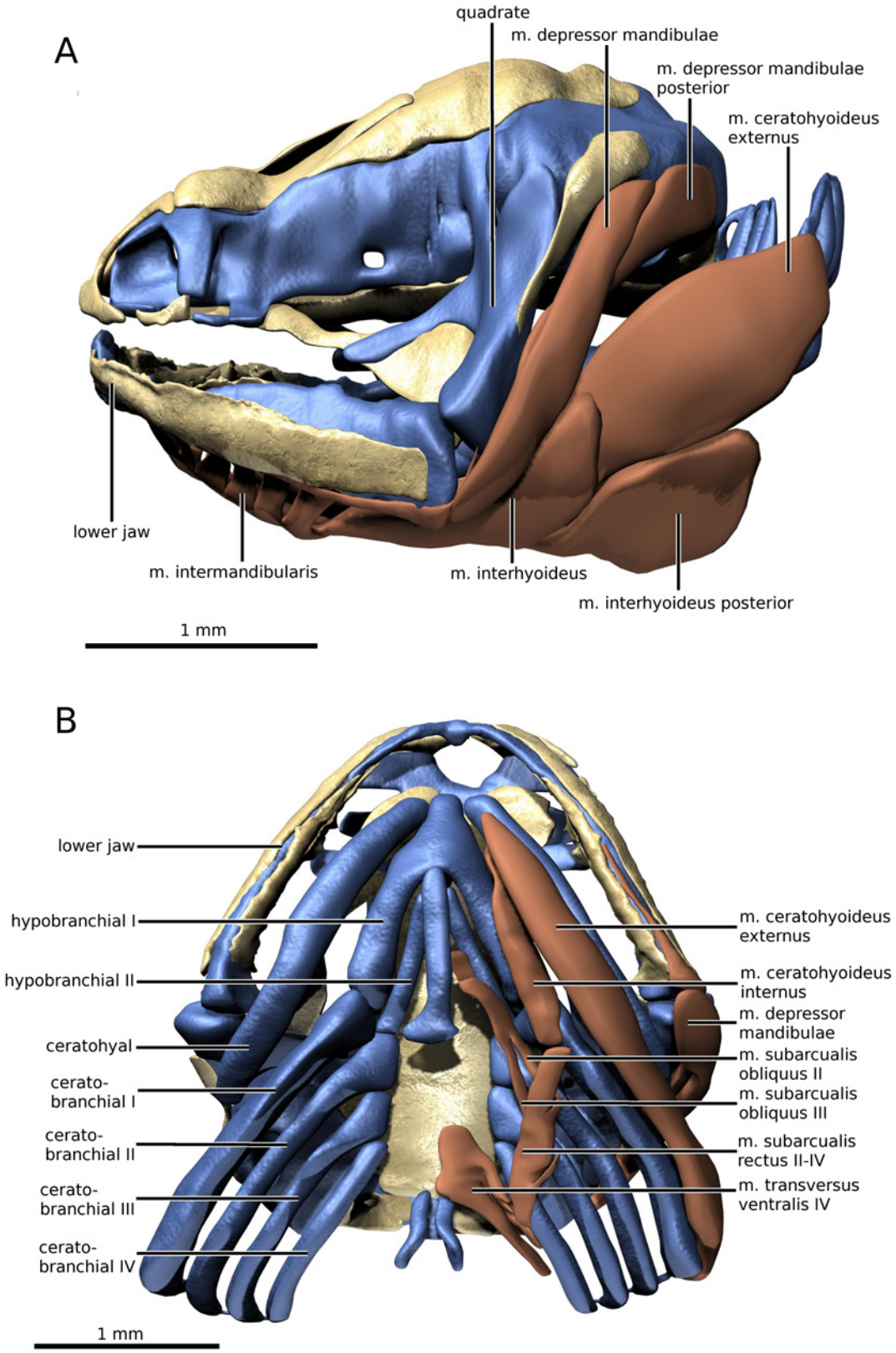


Figure 5. *Salamandrella keyserlingii* (ZMH A09801), 3D reconstruction, jaw closing and dorsal branchial muscles removed. A: lateral view. The m. depressor mandibulae inserts with a tendon on the ventral edge of the lower jaw. B: ventral view, m. interhyoideus and m. interhyoideus posterior removed. The m. ceratohyoideus externus and the m. ceratohyoideus intenus have a very similar orientation. The m. subarcualis rectus II-IV inserts on the ceratobranchials III, II, and I. The mm. subarcuale obliqui II and III share their insertion.

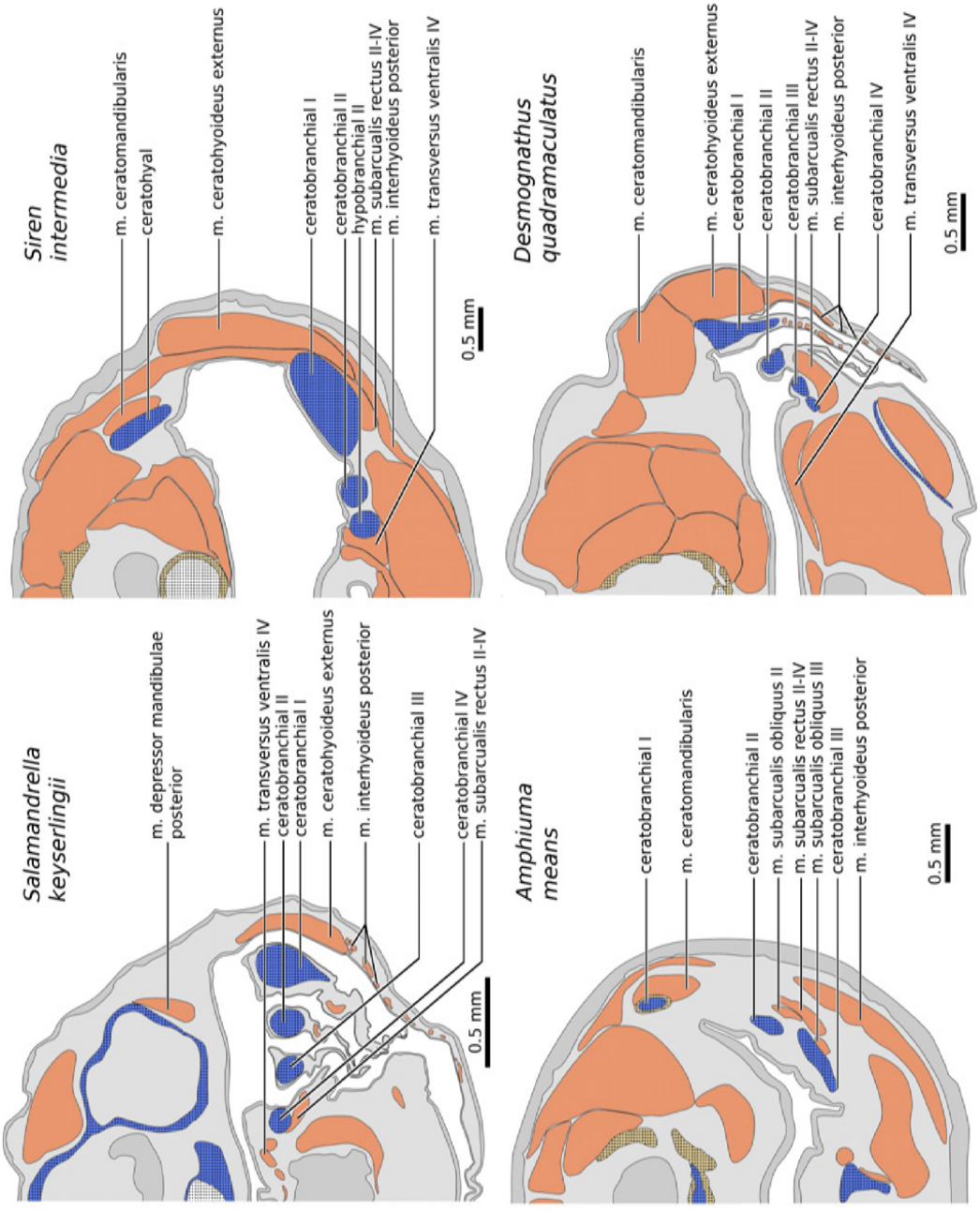


Figure 6. *Salamandrella keyserlingii* (ZMH A09801), *Siren intermedia* (ZMH A09701), *Amphiuma means* (ZMH A08377), and *Desmognathus quadramaculatus* (MVZ226908), schematic drawings of transversal sections through the branchial basket. The two larval specimens of *S. keyserlingii* and *D. quadramaculatus* are very similar. The most notable difference between the two species is the absence of the m. ceratomandibualris in *S. keyserlingii*.

The ventral hyal muscles

This group contains the m. interhyoideus and the m. interhyoideus posterior. Both are innervated by the n. facialis.

Caecilians: In *E. bicolor*, the m. interhyoideus has two origins. A rostral bundle of muscle fibers is attached by a fascia to the ventral edge of the ceratohyal; a caudal fiber bundle originates from the dorsal fascia (fig. 2). The fiber bundles of both parts of the muscle run ventrally and merge into each other. The m. interhyoideus meets its contralateral counterpart at the ventromedian midline of the animal.

The m. interhyoideus posterior in *E. bicolor* covers a wide area of the hyobranchium laterally. The m. interhyoideus posterior originates along a tendon that covers the posterior part of the m. depressor mandibulae, the m. depressor mandibulae posterior and the dorsal trunk musculature laterally. The m. interhyoideus posterior runs in ventral and caudal direction. The muscle inserts on the ventromedian raphe where it meets the m. interhyoideus posterior of the contralateral side of the animal (fig. 2).

Salamanders: In all Salamanders examined, a m. interhyoideus and a m. interhyoideus posterior are present. Both muscles share muscle fibers and the separation of the two muscles is weak. The m. interhyoideus originates from the ventrolateral edge of the ceratohyal; in *Amphiuma means*, some muscle fibers originate dorsal to the distal tip of the ceratohyal from the ventral edge of the squamosal. The m. interhyoideus posterior originates caudal and medial to the m. interhyoideus (figs. 3, 6). The fibers of the m. interhyoideus posterior are attached to the lateral side of the fascia of the m. ceratohyoideus externus (fig. 6) or of the m. ceratomandibularis in *A. means* where a m. ceratohyoideus externus is absent. The fibers of the m. interhyoideus and the m. interhyoideus posterior run ventrally and meet their counterparts from the contralateral side at the ventral midlines of the animals.

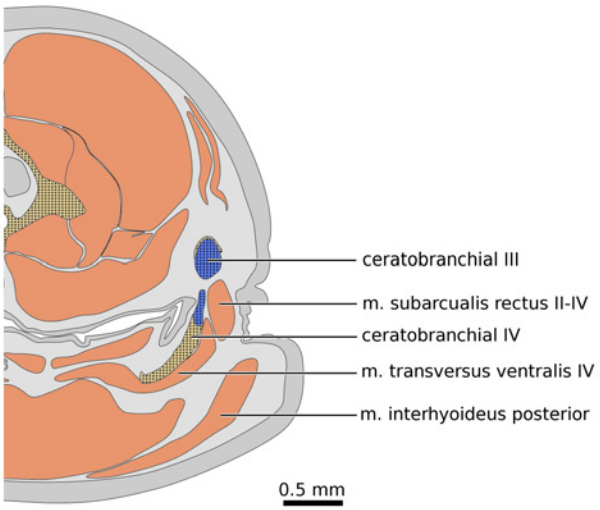


Figure 7. *Epicrionops bicolor* (MHW367), schematic drawing of a transversal section immediately rostral to the tracheal cartilages. M. transversus ventralis IV originates from the lateroventral face of ceratobranchial IV, immediately ventral to the origin of m. subarcualis rectus II-IV

The ventral branchial muscles

The ventral branchial muscles comprise the m. ceratohyoideus internus, the mm. subarcuales obliqui II and III, the m. subarcualis rectus II-IV, and the m. transversus ventralis IV. Except for the m. ceratohyoideus internus, all muscles of this group are innervated by the Xth cranial nerve (n. vagus), m. ceratohyoideus internus is innervated by cranial nerve IX (n. glossopharyngeus).

Caecilians: The m. ceratohyoideus internus originates along the entire lateral face of ceratobranchial I (fig. 1). The fibers of the m. ceratoyhoideus internus are obliquely oriented and run in rostral and ventral direction. The m. ceratohyoideus internus inserts on the ceratohyal over an area that extends from a ventral edge in the distal region to the entire ventral surface in the proximal region of the ceratohyal (fig. 1).

In *E. bicolor* there are no mm. subarcuales obliqui II and III.

The m. subarcualis rectus II-VI is a thin muscle that in *E. bicolor* originates from the lateroventral edge of the ceratobranchial IV (fig. 7). Its fibers run rostrad and cover two segments of the hyobranchium. Some muscle fibers insert on the lateral face of ceratobranchial III, the remainder fibers are attached to ceratobranchial II.

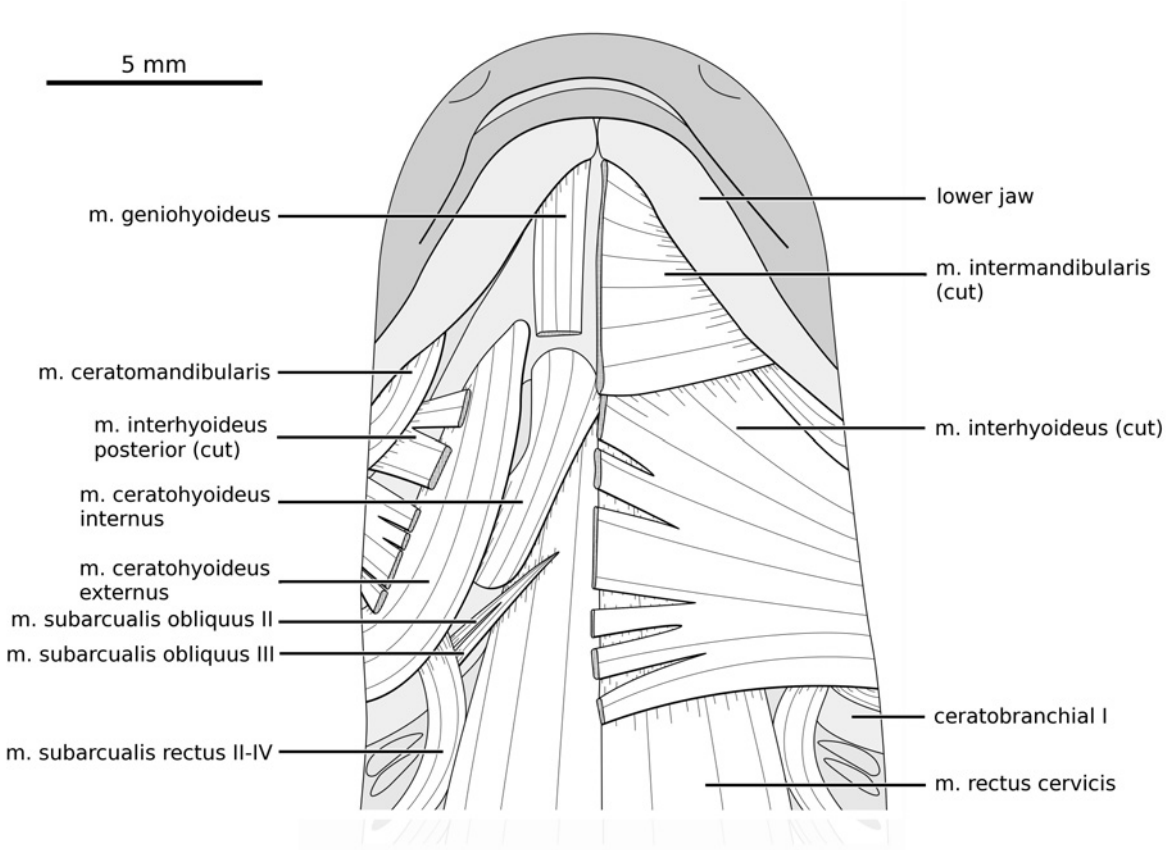


Figure 8. *Siren intermedi*a (ZMH A09702), drawing of skinned specimen in ventral view, m. intermandibularis, m. interhyoideus, m. interhyoideus posterior, and m. geniohyoideus cut on the right side of the animal. The mm. subarcuale obliqui II and III insert on the fascia of the m. rectus cervicis in *S. intermedia*.

In *E. bicolor* the m. transversus ventralis IV originates from the distal and caudal tip of the ceratobranchial IV (fig. 7). Its fibers run in caudal and ventral direction and insert on the lateral wall of the trachea, caudal to the tracheal cartilages.

Salamanders: The m. ceratohyoideus internus in Salamanders is highly variable. In *Salamandrella keyserlingii* and *Desmognathus quadramaculatus*, the m. ceratohyoideus internus is a thin bundle of muscle fibers on the ventral side of the specimens (fig. 3, 5B); in *Siren intermedia* and *Amphiuma means*, the same muscle is more voluminous and dorsolaterally extended (fig. 3, 6). The m. ceratohyoideus internus in *S. keyserlingii* originates from the ventromedial edge of the ceratobranchial I (fig. 5); in *D. quadramaculatus* this muscle originates ventrolaterally from the ceratobranchial I. In *S. intermedia*, the m. ceratohyoideus internus originates along the lateral face of the

ceratobranchial I and hypobranchial I (fig. 8); in *A. means* the origin is at the dorsolateral edge of ceratobranchial I and II. The m. ceratohyoideus internus inserts with a tendon on the medial side of the proximal tip of the ceratohyal in *S. keyserlingii* and *D. quadramaculatus* (fig. 5). In *S. intermedia* it inserts on the ventrolateral edge of the basibranchial I and the ventromedial face of the proximal part of the ceratohyal (fig. 8). The m. ceratohyoideus internus in *A. means* inserts on the ventral face of the ceratohyal (fig. 3) and stretches from proximal parts of the cartilage distally.

The m. subarcualis obliquus II is a thin muscle that originates from the ventral side of the ceratobranchial II in its proximal region, immediately caudal to the articulation between ceratobranchial II and ceratobranchial III (fig. 5) in all salamander specimens examined herein. In *Salamandrella keyserlingii* and *Desmognathus quadramaculatus*, this muscle inserts via a tendon on the lateral side of the caudal tip of the basibranchial I (fig. 5). In *Siren intermedia*, the m. subarcualis obliquus II has its insertion on the ventrolateral side of the fascia that covers the m. rectus cervicis (fig. 8). The insertion site of the m. subarcualis obliquus II in *S. intermedia* is immediately medial to the hypobranchial I. In *A. means* this muscle inserts on the ventral side of the proximal tip of the ceratobranchial I.

In all salamander specimens examined herein, the m. subarcualis obliquus III originates from the ventrolateral side of the proximal ceratobranchial III (figs. 5, 6). Its fibers run in rostral and medial direction and merge with the m. subarcualis obliquus II.

The m. subarcualis rectus II-IV originates from the ventromedial face of the proximal ceratobranchial IV in *Salamandrella keyserlingii* and *Desmognathus quadramaculatus* (figs. 5, 6). In *Siren intermedia* and *Amphiuma means*, the origin of the m. subarcualis rectus II-IV has its position on the ventrolateral side of the distal ceratobranchial IV (fig. 6). In all salamander specimens examined herein, the fibers of the m. subarcualis rectus II-IV run in

rostral direction. Bundles of muscle fibers attach to the ventrolateral faces of the ceratobranchials I, II, and III (fig. 5), except for *S. intermedia*. In *S. intermedia*, the m. subarcualis rectus II-IV only inserts on ceratobranchial I (fig. 8).

The m. transversus ventralis IV is similar in all specimens examined, except for *Amphiuma means*. The muscle originates from the ventromedial face of the proximal ceratobranchial IV, runs ventromediad and rostrad and inserts together with its contralateral counterpart on the dorsomedial edge of the fascia of the m. rectus cervicis immediately rostral to the arytaenoid cartilages and the laryngeal muscles (fig. 5, 6). In *A. means*, this muscle originates from the distal tip of ceratobranchial IV. Its fibers run in ventromedial and caudal direction and meet with their contralateral counterparts on the linea alba caudal to the arytaenoid cartilages.

DISCUSSION

A universally valid terminology of cranial muscles in caecilians and salamanders

M. depressor mandibulae and *m. depressor mandibulae posterior*. These muscles have been extensively discussed for salamander specimens in Bauer (1997) and a comprehensive list of synonyms from literature was provided there. Drüner (1901) used the term m. cephalodorsomandibularis for the jaw opening muscle and separated deep and superficial layers of this muscle. The superficial layer of Drüners m. cephalodorsomandibularis represents the m. depressor mandibulae, the deep layer the m. depressor mandibulae posterior in my study. Confusingly, in the second part of his study, Drüner (1904) examined *Siren lacertina* and defined a m. cephalo*hyo*mandibularis and a m. levator hyoidei for this species only. The m. cephalohyomandibularis originates from the dorsocaudal region of the skull and the ceratohyal and inserts on the lower jaw. Drüner (1904) mentioned that the m.

cephalohyomandibularis represents the m. cephalodorsomandibularis (= m. depressor mandibulae; table 2) in other salamanders. Obviously, Drüner (1901, 1904) created a terminology that reflects topology or function, rather than homologies. The same applies for the m. levator hyoidei in *S. lacertina*, that is the homologue to the deep layer of the m. cephalodorsomandibularis in Drüner (1901, 1904) and the m. depressor mandibulae posterior herein. I suggest to use the term m. depressor mandibulae posterior because this muscle is incompletely separated from the m. depressor mandibulae and merges with the m. depressor mandibulae during metamorphosis (Norris and Hughes, 1918; Edgeworth, 1935; Eaton, 1936; Piatt, 1938; Fox, 1959; Bauer, 1997).

M. ceratomandibularis and *m. ceratohyoideus externus*. There is much confusion about these two muscles in literature. Drüner (1901, 1904) introduced the term m. ceratomandibularis for a muscle that originates from the ceratobranchial I in salamander larvae and inserts on the lower jaw. However, in the ancestral condition, this muscle did not originate from the ceratobranchial I but from the ceratohyal (Edgeworth, 1935; Bauer, 1997). Species of the genus *Siren* show the ancestral condition. However, in *Siren lacertina*, Drüner (1904) did not find a m. ceratomandibularis. Instead Drüner (1904) described the m. cephalohyomandibularis to originate in part from the ceratohyal. I suggest to interpret the m. cephalohyomandibularis as compound muscle that, besides the m. depressor mandibulae also is partially homologous to the m. ceratomandibularis. In *S. intermedia* both muscles are very close to each other (fig. 5) and difficult to separate. Edgeworth (1935) introduced different terms for the hyal muscles: a muscle that connects the ceratohyal and the lower jaw in caecilians and salamanders of the genus *Siren* is called m. hyomandibularis; in salamanders other than *Siren* the same muscle is called m. branchiomandibularis. Those names in Edgeworths (1935) study clearly are synonyms to Drüners (1901, 1904) m.

ceratomandibularis. Erdmann and Cundall (1984) in a study on *Amphiuma* cranial morphology used the name *posterior depressor mandibulae* for a muscle that originates from the ceratobranchial I and inserts on the lower jaw. Based on my results, the posterior depressor mandibulae in *Amphiuma* is considered a m. ceratomandibualris in other salamanders and caecilians. The presence of a second fascialis innervated muscle in similar position to the m. ceratomandibularis caused some confusion, especially in studies on caecilian cranial muscles. Drüner used the term m. ceratohyoideus externus for this second muscle. The m. ceratohyoideus externus originates from ceratobranchial I and inserts on the ceratohyal in salamanders. In caecilians only one muscle is present and homologies have been cryptic, either the m. ceratomandibularis or the m. ceratohyoideus externus is absent compared to salamanders. For larvae of the caecilian *Ichthyophis beddomii*, Norris and Hughes (1918) mentioned a m. ceratohyoideus externus. This muscle was shown to be equivalent to the m. hyomandibularis in larvae of *I. glutinosus* (Edgeworth, 1935) and *I. kohtaoensis* (Kleinteich and Haas, 2007). However, m. hyomandibularis is a synonym to m. ceratomandibularis and consequently can not be a synonym to m. ceratohyoideus externus at the same time, because those are two different muscles. By direct comparison of caecilian and salamander specimens it becomes clear, that the m. ceratohyoideus externus as defined by Drüner (1901, 1904) is absent in caecilians, because there is no muscle that is innervated by cranial nerve VII and connects the ceratobranchial I to the ceratobyal (Edgeworth, 1935; Kleinteich and Haas, 2007; this study). It is likely that Norris and Hughes (1918) falsely used the term m. ceratohyoideus externus for a muscle that actually should have been called m. ceratomandibualris.

M. interhyoideus and *m. interhyoideus posterior*. The term m. interhyoideus is used consistently throughout the literature (Drüner, 1901, 1904; Edgeworth, 1935; Piatt, 1938;

Fox, 1958 Nussbaum, 1977; Erdmann and Cundall, 1984; Kleinteich and Haas, 2007). However, Drüner used the synonym m. interbranchialis I for m. interhyoideus posterior. This is confusing because this muscle is weakly separated to the m. interhyoideus and also innervated by cranial nerve VII (Drüner 1901). Another synonym of m. interhyoideus posterior is m. gularis (Eaton, 1936). I suggest to stay with the term m. interhyoideus posterior to account for the often incomplete separation of this muscle to the m. interhyoideus.

M. ceratohyoideus internus, *m. subarculis obliquus II*, *m, subarcualis obliquus III*, and *m. subarcualis rectus II-IV*. The term m. ceratohyoideus internus was introduced by Drüner (1901, 1904) to account for the topology of this muscle medial to the m. ceratohyoideus externus. Although both muscles, the m. ceratohyoideus externus and the m. ceratohyoideus internus, connect ceratobranchial I and ceratohyal, they can be separated based on their innervation from cranial nerve VII (m. ceratohyoideus externus) or cranial nerve IX (m. ceratohyoideus internus), respectively (fig. 9). Drüner (1904) further introduced the terms m. subarcualis rectus I, II, III and mm. subarcuales obliqui. The mm. subarcuales recti in Drüner (1904) originate from the ceratobranchials I, II, and III and share their insertion on ceratobranchial IV. Those muscles are homologous to the m. subarcualis rectus II-IV in this study. Edgeworth (1935) adopted Drüners terms. However, Edgeworth (1935) used the names m. subarcualis obliquus II and III, as synonym to m. subarcualis rectus II and III. Further, it is important to note that Edgeworth defined the more caudal branchial bar as origin of the muscles and not as insertion. Consequently, the consecutive numbering that reflects the origins of those muscles differs to Drüner (1904). In Edgeworth (1935) the m. subarcualis rectus I originates from ceratbranchial I and inserts on the ceratohyal; in Drüner (1904) the m. subarcualis rectus I originates from ceratobranchial I and inserts on ceratobranchial IV. Drüners m. subarcualis rectus I is actually a m. subarcualis IV in

Edgeworths definition. Although Edgeworth (1935) adopted the term m. subarcualis rectus I from Drüner (1904), this muscle actually is homologous to the m. ceratohyoideus internus. This was previously pointed out by Lawson (1965). To avoid potential conflicts between Drüners (1904) and Edgeworths (1935) terminology, Kleinteich and Haas (2007) suggested to use the terms m. subarcualis *rectus* for muscles that are located on the lateral side of the hyobranchium and m. subarcualis *obliquus* for muscles that connect the ceratobranchials on the ventrally in *Ichthyophis kohtaoensis*. Based on Kleinteich and Haas (2007) the ventral branchial musculature contains a m. subarcualis rectus I that connects ceratobranchial I and ceratohyal, a m. subarcualis obliquus II, that connects ceratobranchial II and I, a m. subarcualis obliquus III, in between ceratobranchial III and II, and finally a m. subarcualis rectus II-IV that runs from ceratobranchial IV to ceratobranchial I (ceratobranchial II in *I. kohtaoensis*). I follow the definition in Kleinteich and Haas (2007), except for the m. subarcualis rectus I. The first of the proposed sequential ventral muscles is called m. ceratohyoideus internus herein. This term reflects the topology of this muscle that is much comparable to the m. ceratohyoideus externus. The m. ceratohyoideus internus differs notably in its orientation of muscle fibers, its size, and innervation from the m. subarcualis rectus II-IV.

M. transversus ventralis IV. This term was adopted from Edgeworth (1935) and is synonym to m. interbranchialis IV in Drüner (1901, 1904).

Hyal and branchial musculature in larvae of the most recent common ancestor of amphibians

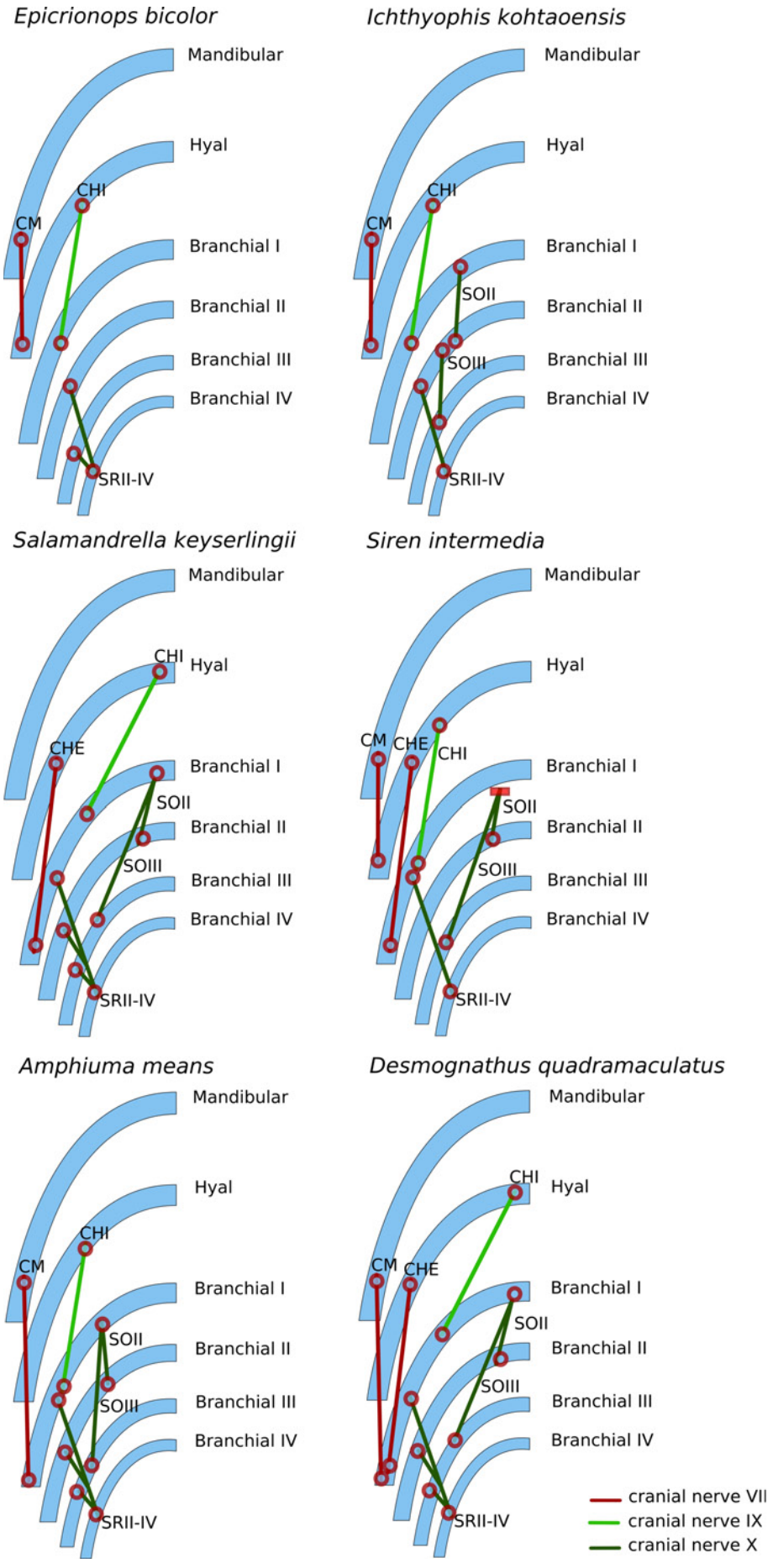
A first preliminary discussion on larval muscles in the ground pattern of the Lissamphibia, i.e. in the most recent common ancestor of todays amphibians, was presented

in Haas (2001) for the jaw closing muscles and in Kleinteich and Haas (2007) for the entire cranial musculature. Here I will complement my results to the discussion in Kleinteich and Haas (2007). The phylogeny that is used for discussion is from Roelants et al. (2007) and was based on mitochondrial (16S rRNA) and nuclear (CXCR4, NCX1, RAG1, SLO8A3) markers. Figure 9 is a schematic representation of the homology hypotheses for the ventral branchial muscles of the species studied herein and *Ichthyophis kohtaoensis* (data from Kleinteich and Haas, 2007).

Most of the hyal and branchial muscles were present in all specimens examined herein and it is very likely that those muscles were present in larvae of the most recent common ancestor of amphibians. However, some muscles need to be discussed more thoroughly because they were not present in all species: the mm. subarcuales obliqui II and III (absent in *Epicrionops bicolor*), the m. ceratomandibularis (absent in *Salamandrella keyserlingii*), and the m. ceratohyoideus externus (absent in *E. bicolor* and *Amphiuma means*) (fig. 9).

Mm. subarcuales obliqui II and III. In caecilians, those muscles are present in larvae of *Ichthyophis kohtaoensis* (Kleinteich and Haas, 2007) and have been described for adult individuals of the Ichthyophidae, Uraeotyphlidae, and Caeciliidae (Nussbaum, 1977, 1979; Wilkinson and Nussbaum, 1997). Based on the phylogeny in Roelants et al. (2007), it is most parsimonious to assume that the mm. subarcuales obliqui II and III have been present in the

Figure 9. Schematic representation of the mandibular, hyal, and branchial arches and the associated ventral musculature. Identical names indicate primary homologies. The scheme for *Ichthyophis kohtaoensis* is derived from the descriptions in Kleinteich and Haas (2007). CM - m. ceratomandibularis, CHE - m. ceratohyoideus externus, CHI - m. ceratohyoideus internus, SOII - m. subarcualis obliquus II, SOIII - m. subarcualis obliquus III, SRII-IV - m. subarcualis rectus II-IV. Caecilians do not have a m. ceratohyoideus externus; the muscle that connects the hyal and the first branchial arch is innervated by cranial nerve IX and thus homologous to the m. ceratohyoideus internus. The mm. subarcuales obliqui II and III are absent in *Epicrionops bicolor.* In *Salamandrella keyserlingii* there is no ventral muscle that inserts on the mandibular arch; a m. ceratomandibularis is absent. In *Amphiuma means, only* one muscle is present that connects the hyal and first branchial arches. Based on the innervation pattern, the m. ceratohyoideus externus is absent.


Epicrionops bicolor
Mandibular
Hyal
CHI
CM
Branchial I
Branchial II
Branchial III
Branchial IV
SRII-IV
Ichthyophis kohtaoensis
Mandibular
Hyal
CHI
CM
Branchial I
SOII
Branchial II
SOIII
Branchial III
Branchial IV
SRII-IV
Salamandrella keyserlingii
Mandibular
CHI
Hyal
CHE
Branchial I
SOII
Branchial II
SOIII
Branchial III
Branchial IV
SRII-IV
Siren intermedia
Mandibular
Hyal
CM
CHE
CHI
Branchial I
SOII
Branchial II
SOIII
Branchial III
Branchial IV
SRII-IV
Amphiuma means
Mandibular
Hyal
CHI
CM
Branchial I
SOII
Branchial II
SOIII
Branchial III
Branchial IV
SRII-IV
Desmognathus quadramaculatus
Mandibular
CHI
Hyal
CM
CHE
Branchial I
SOII
Branchial II
SOIII
Branchial III
Branchial IV
SRII-IV
cranial nerve VII
cranial nerve IX
cranial nerve X

most recent common ancestor of caecilians and thus are part of the ground-pattern of the Lissamphibia. The loss of those muscles in *Epicrionops bicolor* is supposed to be an autapomorphic character of the Rhinatrematidae. This was previously suggested by Nussbaum (1977) and Wilkinson and Nussbaum (2006).

M. ceratomandibularis. Kleinteich and Haas (2007) proposed that this muscle is present in the ground pattern of salamanders, caecilians, and frogs and thus part of the ground-pattern of the Lissamphibia. This was based on the assumption that this muscle is present in the ground pattern of salamanders. However, based on the phylogeny in Roelants et al. (2007), that proposed a sister-group relationship of the Hynobiidae (where the m. ceratomandibularis is absent) plus Cryptobranchidae to the remainder salamanders, there is an alternative interpretation of this muscle: the m. ceratomandibularis in salamanders was developed independently. Crucial taxa to solve this question are species within the Cryptobranchidae, as well as basal anurans. Drüner (1904) examined *Andrias japonicus* (Drüner used the species name *Cryptobranchus japonics*) and did not mention a m. ceratomandibularis. However, Drüner (1904) described three portions of the m. depressor mandibulae. It is well documented, that the m. ceratomandibularis becomes part of the m. depressor mandibulae in adult salamanders (Edgeworth, 1935; Bauer, 1997) and this might be the case in *A. japonicus*. The paedomorphic life cycle of *A. japonicus* complicates the comparison to 'true' salamander larvae as in *Salamandrella keyserlingii*. In frog tadpoles, there are five muscles that constitute to the m. depressor mandibulae complex and although homologies for those muscles have not been established yet, it is likely, that those group contains a homologous muscle to the m. ceratomandibularis in caecilians and salamanders other than *S. keyserlingii*. One potentially homologous muscle in frog tadpoles is the m. hyoangularis that originates from the ceratohyal and inserts on the retroarticular process of

the lower jaw (Edgeworth, 1935; de Jongh, 1968; Haas, 2003). If a homologous structure to the m. ceratomandibularis is present in tadpoles and/or the Cryptobranchidae, then the m. ceratomandibularis is part of the ground pattern of the Lissamphibia. In this case, the absence of this muscle is autapomorphic for the Hynobiidae.

M. ceratohyoideus externus. This muscle was originally described as being unique for salamander larvae (Drüner, 1901; Edgeworth, 1935). This is also supported herein; the m. ceratohyoideus externus is present in salamanders and absent in caecilians. However, it is unclear if there is a homologue to this muscle in frog tadpoles, leaving the question open, if the m. ceratohyoideus externus is apomorphic to the Batrachia (frogs + salamanders) or salamanders. The absence of the m. ceratohyoideus externus in *Amphiuma means* clearly is the derived condition within salamanders. Drüner (1904) and Erdmann and Cundall (1984) found this muscle absent in a second species of *Amphiuma*, i.e. *A. tridactylum.* Edgeworth (1935) claimed that the absence of this larval muscle is related to direct development and thus the absence of a distinct larval stage in *Amphiuma*. This relates to interesting questions in the evolution of neoteny in salamanders. Both, *A. means* and *Siren intermedia* are obligate neotene salamanders (Duellman and Trueb, 1994). However, *S. intermedia* retains this larval muscle; *A. means* does not. This expresses different patterns of partial metamorphosis with *S. intermedia* generally being more 'larval-like', e.g. by retaining external gills (see Duellman and Trueb, 1994 for a more complete list of metamorphosis patterns).

Larval morphology in caecilians and salamanders

Although the cranial musculature in caecilian and salamander larvae consists of muscles that can be homologized between the two groups, there are notable differences in the way the hyal and branchial muscles are organized in the two groups.

Epicrionops bicolor larvae are very similar to larvae of *Ichthyophis kohtaoensis* (Kleinteich and Haas, 2007) in their hyal and branchial musculature. This similarity was previously mentioned by Wake (1989). Wake (1989), however, did not describe a m. transversus ventralis IV in *E. bicolor* larvae, contrary to my results. A m. transversus ventralis IV clearly is present in the specimens studied herein (fig. 7). Given that the m. transversus ventralis IV in *E. bicolor* and *I. kohtaoensis* larvae are homologous, the only notable differences between the two species are the absence of the mm. subarcuales obliqui in *E. bicolor* (discussed above) and the insertion of the m. interhyoideus posterior. The attachment of the m. interhyoideus posterior on the ventral side of the processus retroarticularis is autapomorphic for adult caecilians and contributes to their unique jaw closing mechanism (Bemis et al., 1983; Nussbaum, 1983; Summers and Wake, 2005; Kleinteich et al., 2008). Larvae of *E. bicolor* show the ancestral character state, i.e. the m. interhyoideus posterior is an exclusively branchial muscle (Müller, 2007; this study) and will shift its insertion at metamorphosis. In *I. kohtaoensis* the unique caecilian jaw closing mechanism is present in larvae; i.e. the ancestral adult character state is expressed earlier in development.

S*alamandrella keyserlingii* and *Desmognathus quadramaculatus* both have a larva. Although, both species are well separated from each other in salamander phylogeny (Frost et al., 2006; Roelants et al., 2007), their larvae are very similar. Characters in which salamander larvae differ from caecilian larvae are: (1) insertion of the m. depressor mandibuale ventral to the mandibular joint in salamanders; (2) insertion of the m. depressor mandibulae posterior mainly on the lower jaw with only a few fibers on the ceratohyal (in caecilians the entire muscle inserts on the ceratohyal); (3) m. ceratohyoideus internus inserts with a tendon (in caecilian larvae fleshy insertion); (4) m. subarcualis obliquus III shares its insertion with the

m. subarcualis obliquus II in salamanders; (5) m. subarcualis rectus II-IV spans three segments (in caecilians two); (6) fibes of the m. transversus ventralis IV are oriented rostrad and ventrad (in caecilians caudad and ventrad).

My results for the musculature of salamander larvae are consistent with previous studies on salamander cranial morphology (Drüner, 1901, 1904; Litzelmann, 1923; Edgeworth, 1935; Piatt, 1938; Fox, 1959; Bauer, 1997). The two neotene species *Siren intermedia* and *Amphiuma means* are different from salamander larvae in that the insertion of the m. depressor mandibuale is at the dorsal edge of the retroarticular process, which is comparable to larval and adult caecilians (Lawson, 1965; Wilkinson and Nussbaum, 1997; Kleinteich and Haas, 2007; Müller et al., 2009). Further, the two neotene species have a fleshy insertion of the m. ceratohyoideus internus that was proposed to be typical for adult salamanders (Drüner, 1901) and is similar to the condition in caecilians.

Special attention has to be paid to the m. depressor mandibulae posterior. In salamander larvae, the m. depressor mandibulae posterior shows an ontogenetic relocation of muscle fibers from the ceratohyal to the lower jaw (Litzelmann, 1923; Edgeworth, 1935; Piatt, 1938; Fox, 1958; Bauer, 1997). In larval salamanders the majority of muscle fibers inserts on the lower jaw and only a few fibers are still attached to the ceratohyal. This pattern was confirmed by my results on *Desmognathus quadramaculatus* larvae and although this was not the case in the *Salamandrella keyserlingii* specimen examined herein, the insertion of m. depressor mandibulae posterior fibers on the ceratohyal was previously reported in species of the Hynobiidae by Fox (1958). The same transition of muscle fibers from the ceratohyal to the lower jaw occurs in caecilians (Edgeworth, 1935; own observation in *Ichthyophis* cf. *kohtaoensis*). However, in caecilians the transition from the ceratohyal to the lower jaw does not occur before metamorphosis; i.e., in caecilian larvae the entire m.

depressor mandibuale posterior inserts on the ceratohyal. The neotene salamander *Siren intermedia* shows the same condition as caecilian larvae (Drüner, 1904; this study), suggesting that the insertion of the m. depressor mandibuale posterior at the ceratohyal is ancestral for amphibian larvae. This was previously proposed by Edgeworth (1935) and Bauer (1997). Salamander larvae show the derived condition in that the transition of muscle fibers towards the lower jaw happens at an earlier developmental stage. *Amphiuma means* has no attachment of m. depressor mandibuale posterior muscle fibers on the ceratohyal, which is similar to fully metamorphosed caecilians and salamanders (Drüner, 1901, 1904; Edgeworth, 1935; Bauer, 1997).

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Chapter 2

Cranial muscle development in direct developing oviparous and in viviparous caecilians

ABSTRACT: The evolution of direct development and viviparity in caecilians correlates to derived feeding habits during ontogeny (skin feeding, intratuerine feeding) that put new functional demands on the cranial musculature during development. This study compares cranial muscle development in oviparous species with direct development and in viviparous species. In caecilians with direct development or viviparity, I found several muscles absent over ontogeny that were previously described for caecilian larvae; i.e. the m. levator mandibulae externus, the m. ceratomandibularis, the m. subarcualis obliquus II, and the m. subarcualis rectus II-IV. Contrary, a m. genioglossus, that is not reported in caecilian larvae is present in direct developing and viviparous species. All species studied herein have the jaw closing muscles situated in an adductor chamber that is covered laterally by the squamosal. The cranial muscle morphology of embryos, fetuses, and juveniles of oviparous direct developing and viviparous species is almost identical to adult specimens. The precocious formation of adult muscle features in embryology represents ontogenetic repatterning and is proposed to be correlated to the mode of reproduction. This also suggests that functional demands of skin feeding and intrauterine feeding are similar to the demands of adult feeding behavior (biting).

INTRODUCTION

In chapter 1, I compared the hyal and branchial musculature of caecilian larvae with larvae and juveniles of salamanders and concluded on the cranial musculature in the ground-pattern of the Lissamphibia. The results in chapter 1 contribute to the discussion by Kleinteich and Haas (2007) on cranial muscles in larvae of the most recent common ancestor of todays amphibians. The cranial musculature in caecilian larvae is very similar to what was reconstructed for the most recent common ancestor of all amphibians.

Although oviparity with larvae and metamorphosis is ancestral for caecilians, larvae are not generally present in caecilians. Oviparity in combination with direct development and viviparity evolved within the derived caecilian taxa Scolecomorphidae and Caeciliidae (including the aquatic Typhlonectidae) (Wake, 1977a; Wilkinson and Nussbaum, 1998, Exbrayat, 2006). The evolution of direct development and viviparity was correlated to new feeding habits during development (i.e. skin feeding, intrauterine feeding) (Wake, 1977b; Kupfer et al., 2006; Wilkinson et al., 2008).

The cranium of direct developing oviparous species was shown to develop gradually (Müller et al., 2005; Müller, 2006, 2007) whereas the larval skull morphology shows dramatic changes during metamorphosis (e.g. remodeling of the hyobranchium, development of a closed temporal region; Visser, 1963; Müller, 2007). The differences in the development of the skull between species with larval stage and direct developing species were supposed to be due to ontogenetic repatterning, i.e. the modification of an ancestral biphasic trajectory to a steady ontogenetic trajectory as seen in other direct developing amphibians (Roth and Wake, 1985; Hanken et al., 1992, 1997).

Cranium and cranial musculature are integrated in function and changes in the ontogenetic repatterning of the cranium (metamorphosis vs. gradual development) are likely to have an impact on the development of the cranial musculature. The entire cranial musculature of direct developing oviparous species or viviparous species was described by Edgeworth (1935), Lawson (1965), and Wilkinson and Nussbaum (1997). Those studies, however, focused on adult specimens. Descriptions of muscle development in direct developing and viviparous species have not been published before.

This study will provide a description and comparison of the cranial musculature in embryos and juveniles of the direct developing oviparous species *Boulengerula taitana* and *Gegeneophis ramaswamii*. and in fetuses and juveniles of the viviparous species *Dermophis mexicanus* and *Gymnopis multiplicata*. The cranial musculature of an adult individual of *G. ramaswamii* is examined and compared to the subadult specimens. The aim of this study is to evaluate the differences in cranial muscle development between species with oviparity and metamorphosis, oviparity and direct development, and viviparity.

MATERIALS AND METHODS

I examined 19 specimens that comprised embryos and juveniles of the direct developing oviparous species *Boulengerula taitana* Loveridge, 1935 (4 specimens) and *Gegeneophis ramaswamii* Taylor, 1964 (5 specimens) and embryos and fetuses of the viviparous species *Dermophis mexicanus* (Duméril and Bibron, 1841) (6 specimens) and *Gymnopis multiplicata* Peters, 1874 (3 specimens). Further, one adult individual of *G. ramaswamii* was examined (table 1). The specimens were donated by Hendrik Müller (HM; University of Jena), Mark Wilkinson (MW and RWW; Natural History Museum London), and Marvalee Wake (MHW; University of California Berkeley).

Table 1. Specimens used in this study.

ID	Species	TL [mm]	Preparation	Stain	Thickness [µm]
HM0063	*Boulengerula taitana*	18	serial section transversal	Bunke	3
MW03877	*Boulengerula taitana*	20	SRµCT at 9keV	-	-
HM0036	*Boulengerula taitana*	36	serial section transversal	Heidenhain's Azan	8
MW03912	*Boulengerula taitana*	49	SRµCT at 9keV	-	-
MW1601	*Gegeneophis ramaswamii*	25	serial section transversal	Heidenhain's Azan	8
MW1340	*Gegeneophis ramaswamii*	26	serial section transversal	Heidenhain's Azan	8
MW1332	*Gegeneophis ramaswamii*	30	serial section transversal	Heidenhain's Azan	8
MW1600	*Gegeneophis ramaswamii*	55	serial section transversal	Heidenhain's Azan	8
MW1600B	*Gegeneophis ramaswamii*	58	serial section transversal	Heidenhain's Azan	8
RWW	*Gegeneophis ramaswamii*	-	serial section transversal	Green Masson's Trichrome / Haemalaun Eosin / Mallory's PTAH	8
MHW_111	*Dermophis mexicanus*	23	serial section transversal	Picro Ponceau / Haemalaun Eosin	10
MHW_050	*Dermophis mexicanus*	30	serial section transversal	Heidenhain's Azan / Haemalaun Eosin	7
MHW_110	*Dermophis mexicanus*	34	serial section transversal	Picro Ponceau / Haemalaun Eosin	10
MHW_104	*Dermophis mexicanus*	44	serial section transversal	Picro Ponceau / Haemalaun Eosin	10
MHW_432	*Dermophis mexicanus*	49	serial section transversal	Mallory's Azan / Haemalaun Eosin	10
MHW_435	*Dermophis mexicanus*	61	serial section transversal	Mallory's Azan / Haemalaun Eosin	10
MHW_038	*Gymnopis multiplicata*	54	serial section transversal	Heidenhain's Azan	7
MHW_524	*Gymnopis multiplicata*	30	serial section transversal	Heidenhain's Azan	7
MHW_040	*Gymnopis multiplicata*	84	serial section transversal	Heidenhain's Azan	7

Boulengerula taitana hatches at about 30 mm total length and feeds actively at the skin of the mother until the juveniles are about 120 mm in total length (Kupfer et al., 2006; Müller, 2007). In *Gegeneophis ramaswamii* hatching occurs at approximately 55 mm (Müller, 2007). The viviparous species examined here hatch *in utero* at about 25 - 30 mm (Wake, 1977a). Total length at birth is 100 - 125 mm in *Gymnopis multiplicata* and *Dermophis mexicanus* (Wake, 1977a, 1980). Details on localities and staging of the *G. ramaswamii* specimens examined herein have been provided by Müller et al. (2005).

Specimens were available as serial sections, except the 20 mm and 49 mm *Boulengerula taitana*. All serial sections were sliced in transverse plane. Slice thickness and applied staining techniques are listed in table 1.

The serial section of the 44 mm *Dermophis mexicanus* specimen (MHW_104) was used for computer aided interactive 3D reconstruction. The 3D reconstruction was based on the protocol described by Haas and Fischer (1997). I used the 3D design software Autodesk®

Maya™ 8.0. Surface modeling followed exactly the steps described in Kleinteich and Haas (2007). The interactive alignment of subsequent slices introduced minor errors to the shape of the resulting surfaces; the reconstructed surfaces appear more rippled than they are supposed to be in the living animal. The reconstructed model displays cartilages, bones, and musculature of the skull in the 44 mm *D. mexicanus*. Teeth, although present in the specimen examined, have not been reconstructed. The m. genioglossus is a loose bundle of muscle fibers. However, due to incomplete alignment for 3D reconstruction, it was not possible to reconstruct single muscle fibers. Instead I modeled the outline of the muscle.

High resolution synchrotron radiation based x-ray micro computed tomography (SRμCT) was performed for the 20 mm and 49 mm *Boulengerula taitana* specimens (MW03877 and MW03912). Prior to SRμCT imaging, I decapitated and and freeze dried (after Meryman, 1960, 1961) the specimens. Freezing of the samples was done at -80 °C. After freezing, the samples were dried under vacuum with a Lyovac® GT2 freeze drying system. SRμCT imaging was performed at beamline BW2 of the DORIS III storage ring at the German Electron Synchrotron (DESY) Hamburg. The GKSS research center Geesthacht operates the SRμCT setup at beamline BW2. Energies for SRμCT imaging were set to 9 keV. The resulting 3D volume datasets had isometric voxels with edge lengths of 2.3 μm (20 mm *B. taitana*) and 2.0 μm (49 mm *B. taitana*).

X-ray radiation μCT imaging has limitations in the visualization of soft tissues (Hörnschemeyer et al., 2002; Betz et al., 2007). By using monochromatic synchrotron based x-ray radiation the detail within soft-tissues is significantly increased. However, especially small muscles, or muscles that are incompletely separated from other structures (e.g. other muscles, nerves), remain cryptic in SRμCT data. The detailed analysis of histological serial sections is proposed to be more reliable for identification of muscles than SRμCT imaging.

Table 2. Cranial muscles in specimens examined and in larval *Ichthyophis kohtaoensis* (data from Kleinteich and Haas, 2007). + presence; - absence. Muscles in boldface differ in their absence/presence to *I. kohtaoensis*. Muscles that are marked by an asterisk have different origins or insertions compared to *I. kohtaoensis*.

	B. taitana 18 mm	*B. taitana* 20 mm	*B. taitana* 36 mm	*B. taitana* 49 mm	*G. ramaswamii* 25 mm	*G. ramaswamii* 26 mm	*G. ramaswamii* 30 mm	*G. ramaswamii* 55 mm	*G. ramaswamii* 58 mm	*G. ramaswamii* adult	*D. mexicanus* 23 mm	*D. mexicanus* 30 mm	*D. mexicanus* 34 mm	*D. mexicanus* 44 mm	*D. mexicanus* 49 mm	*D. mexicanus* 61 mm	*G. multiplicata* 30 mm	*G. multiplicata* 54 mm	*G. multiplicata* 84 mm	*I. kohtaoensis* larva
m. levator mandibulae longus	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
m. levator mandibulae internus	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+
m. levator mandibulae articularis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
m. levator mandibulae externus	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**+**
m. levator quadrati	-	-	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+
m. pterygoideus	-	-	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
m. intermandibularis	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
m. depressor mandibulae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
m. depressor mandibulae posterior *	-	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
m. ceratomandibularis	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**+**
m. interyhoideus *	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+
m. interyhoideus posterior	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
m. ceratohyoideus internus	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
m. subarcualis obliquus II	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**+**
m. subarcualis obliquus III	-	-	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
m. subarcualis rectus II-IV	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**+**
m. transversus ventralis IV	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+
m. cephalodorsosubpharyngeus *	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
m. constrictor laryngis	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
m. dilatator laryngis	-	-	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
m. genioglosssus	**+**	**+**	**+**	**+**	**-**	**-**	**+**	**+**	**+**	**+**	**-**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**-**
m. geniohyoideus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

The terminology that I apply for skeletal elements follows Müller et al. (2005) and Müller (2006). Nomenclature of cranial muscles is according to the suggestions in chapter 1 of this thesis and to Kleinteich and Haas (2007).

RESULTS

The presence of cranial muscles in the specimens examined herein is summarized in table 2 and compared to the musculature in larvae of the caecilian *Ichthyophis kohtaoensis*.

Mm. levatores mandibulae

The mm. levatores mandibulae comprise the m. levator mandibulae longus, the m. levator mandibulae internus and the m. levator mandibulae articularis. Muscles of this group adduct the lower jaw. The mm. levatores mandibulae are situated in an adductor chamber that is covered laterally by the squamosal (figs. 1, 2, 3A). The m. levator mandibulae longus originates along an area that covers the distal tip of the frontal, the lateral edge of the parietal and the taenia marginalis of the neurocranium. The m. levator mandibulae internus is attached to the lateral and ventral sides of the taenia marginalis, medial to the m. levator mandibulae longus (fig. 1). The m. levator mandibulae articularis is the caudalmost muscle of the mm. levatores mandibulae group (fig. 3). It originates from the ventral and rostral face of the otic process of the quadrate and inserts on the pseudoangular immediately rostral to the mandibular joint. The m. levator mandibulae longus plus the m. levator mandibulae internus insert along the dorsal edge of the pseudoangular, rostral to the m. levator mandibulae articularis.

Boulengerula taitana: In the 18 and 20 mm specimens, a squamosal is not present and the adductor chamber is open laterally (fig. 4). It was not possible to distinguish the m. levator mandibulae longus from the m. levator mandibulae internus. The m. levator mandibulae longus plus internus originates from the ventrolateral part of the taenia marginalis, caudal to the pila praeoptica and the lateral edge of the parietal. In the 36 mm and

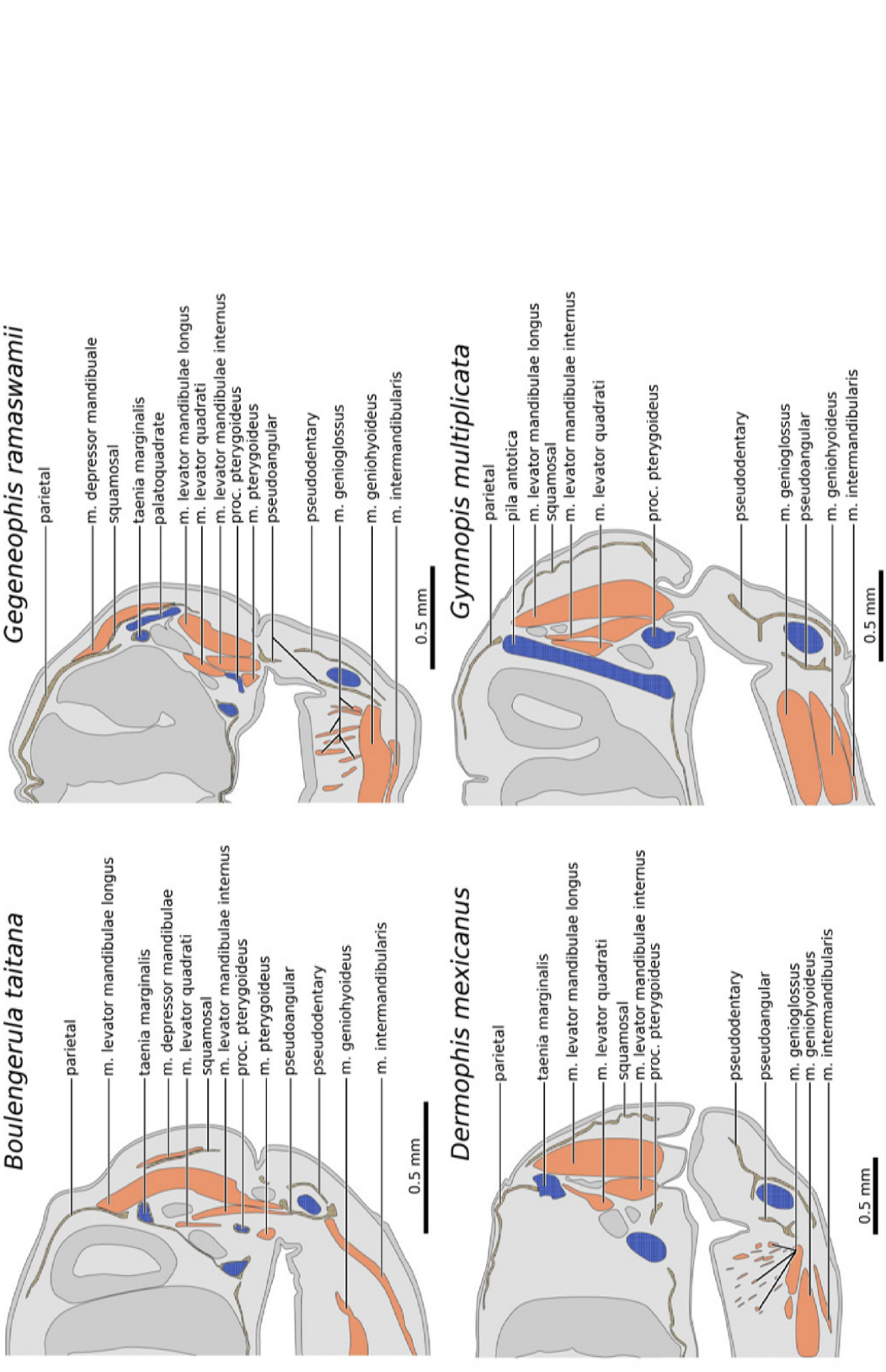

Figure 1. *Boulengerula taitana* 36 mm TL (HM0036), *Gegeneophis ramaswamii* 55 mm TL (MW1600), *Dermophis mexicanus* 49 mm TL (MHW_432), and *Gymnopis multiplicata* 54 mm TL (MHW_038), schematic drawings of transversal sections through the adductor chamber. The mm. levatores mandibulae are covered laterally in the specimens examined. The m. levator mandibulae longus is the lateralmost muscle of the mm. levatores mandibulae. Medial to the m. levator mandibulae longus are the m. levator mandibulae internus and the m. levator quadrati. The m. genioglossus is a loose bundle of muscle fibers that pass through the tongue, dorsal to the m. geniohyoideus and the m. intermandibularis.

49 mm specimens, the caudalmost fibers of the m. levator mandibulae longus originate from the rostral face of the distal tip of the otic process of the quadrate. Additional to the insertion of the m. levator mandibulae longus on the pseudoangular, some fibers are connected to the pseudodentary.

Gegeneophis ramaswamii: A squamosal is absent in the 25 mm, 26 mm, and 30 mm specimens; the adductor chamber is open laterally. In the 26 mm specimen of *G. ramaswamii* there is only the m. levator mandibulae articularis present. The muscle ends blind dorsal to the pseudoangular; there are no fibers of this muscle that insert to the bone. In the older specimens (55 mm and larger), the m. levator mandibulae longus additionally has some fibers attached to the pseudodentary.

Dermophis mexicanus: In all specimens examined, the mm. levatores mandibulae are situated in a chamber medial to the squamosal, or as in the smallest specimen, medial to a layer of connective tissue that presumably ossifies and becomes the squamosal in later stages. In the 23 mm specimen of *D. mexicanus*, there are only two mm. levatores mandibulae present: the mm. levatores mandibulae longus and internus can not be separated in this early stage. In the older specimens examined, all three mm. levatores mandibulae are differentiated.

Gymnopis multiplicata: All specimens of *G. multiplicata* possessed the three mm. levatores mandibulae. The muscles are covered laterally by the squamosal; in the youngest specimen examined herein (30 mm), the ossification of the squamosal is incomplete and covers only the caudal region of the mm. levatores mandibulae.

M. levator quadrati and m. pterygoideus

The m. levator quadrati and m. pterygoideus act on the quadrate. The m. levator quadrati is situated medial to the m. levator mandibulae internus. The m. levator quadrati originates

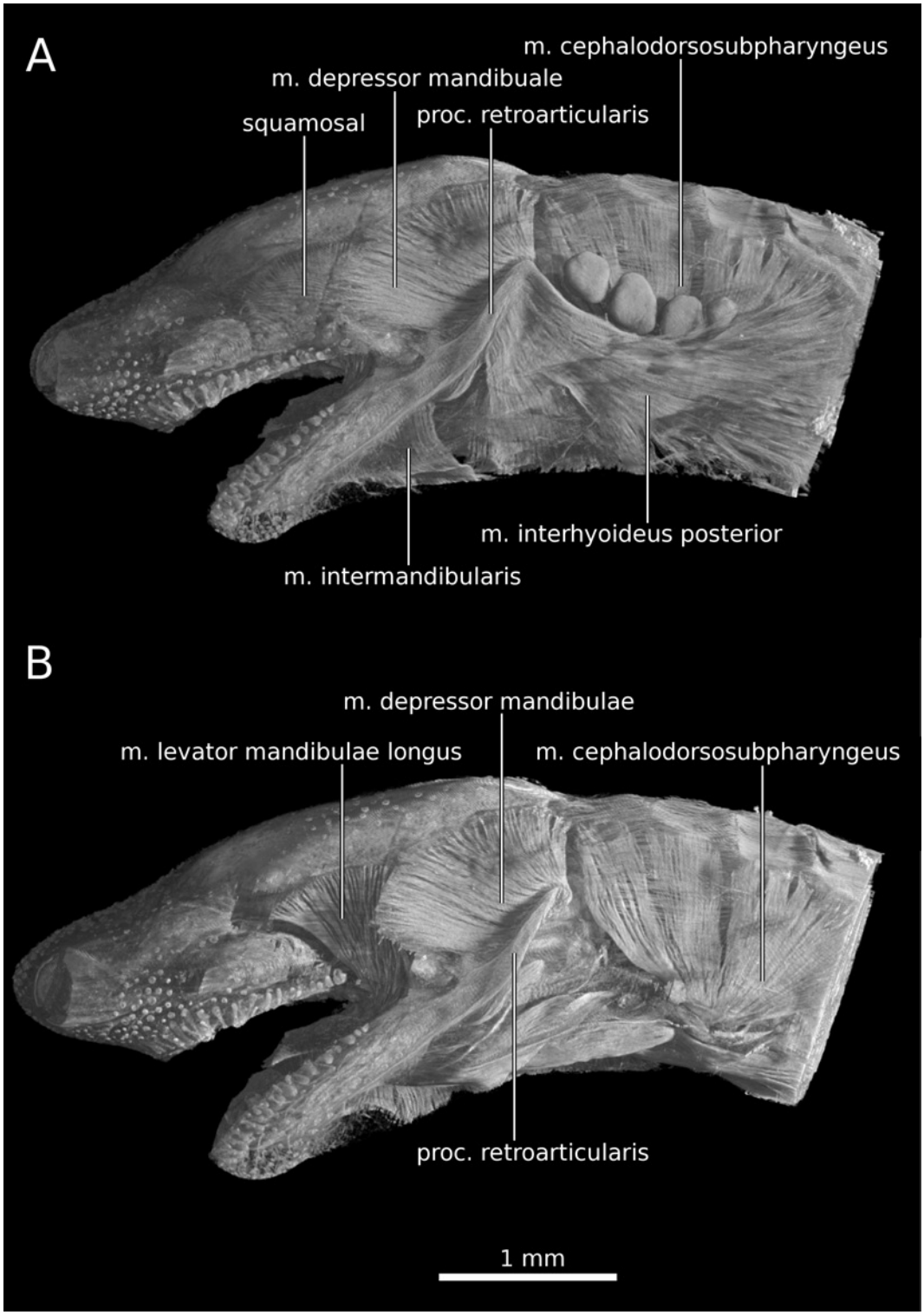

Figure 2. *Boulengerula taitana* 49 mm TL (MW03912). Volume renderings of SRμCT data in lateral view. **A:** Skin removed. The mm. levatores mandibulae are hidden by the squamosal. The m. interhyoideus posterior is a voluminous muscle that inserts on the ventral edge of the processus retroarticularis of the lower jaw and covers the entire hyobranchial apparatus laterally. **B:** Squamosal, m. interhyoideus posterior, parts of the m. intermandibularis, and thymus buds removed. The m. cephalodorsosubpharyngeus runs from the fascia of the dorsal trunk musculature ventrad and inserts on the lateral wall of the pharynx.

from the caudal and dorsal face of the pila antotica and with some fibers from the ventral side of the taenia marginalis of the neurocranium (figs. 1, 3B). The m. levator quadrati inserts on the dorsal edge of the processus pterygoideus of the quadrate. The m. pterygoideus originates along the medial face of the processus retroarticularis of the lower jaw. Its fibers run parallel to lower jaw in rostral direction (fig. 5, 6). The m. pterygoideus inserts on the ventral edge of the processus pterygoideus of the quadrate, opposite to the insertion of the m. levator quadrati (fig. 1).

Boulengerula taitana: The m. levator quadrati and m. pterygoideus are not present in the 18 mm and 20 mm specimens. In the remainder of the specimens examined both muscles can be identified.

Gegeneophis ramaswamii: In *G. ramaswamii*, the m. levator quadrati and m. pterygoideus are present in the 55 mm, 58 mm, and adult specimen; the 30 mm individual has a m. levator quadrati but no m. pterygoideus. Both muscles are absent in the 25 mm and 26 mm specimens. The m. levator quadrati originates immediately medial to the m. levator mandibulae internus. In the adult *G. ramaswamii*, the origin of the m. levator quadrati is more ventral relative to the m. levator mandibulae internus than in the younger stages.

Dermophis mexicanus: The m. levator quadrati and the m. pterygoideus of all specimens of *D. mexicanus* examined are very similar in shape and position, except for the 23 mm specimen, where a separate m. levator quadrati is not discernible. In older individuals, the origin of the m. pterygoideus is more caudal than in younger specimens.

Gymnopis multiplicata: All specimens examined herein have a m. levator quadrati and m. pterygoideus.

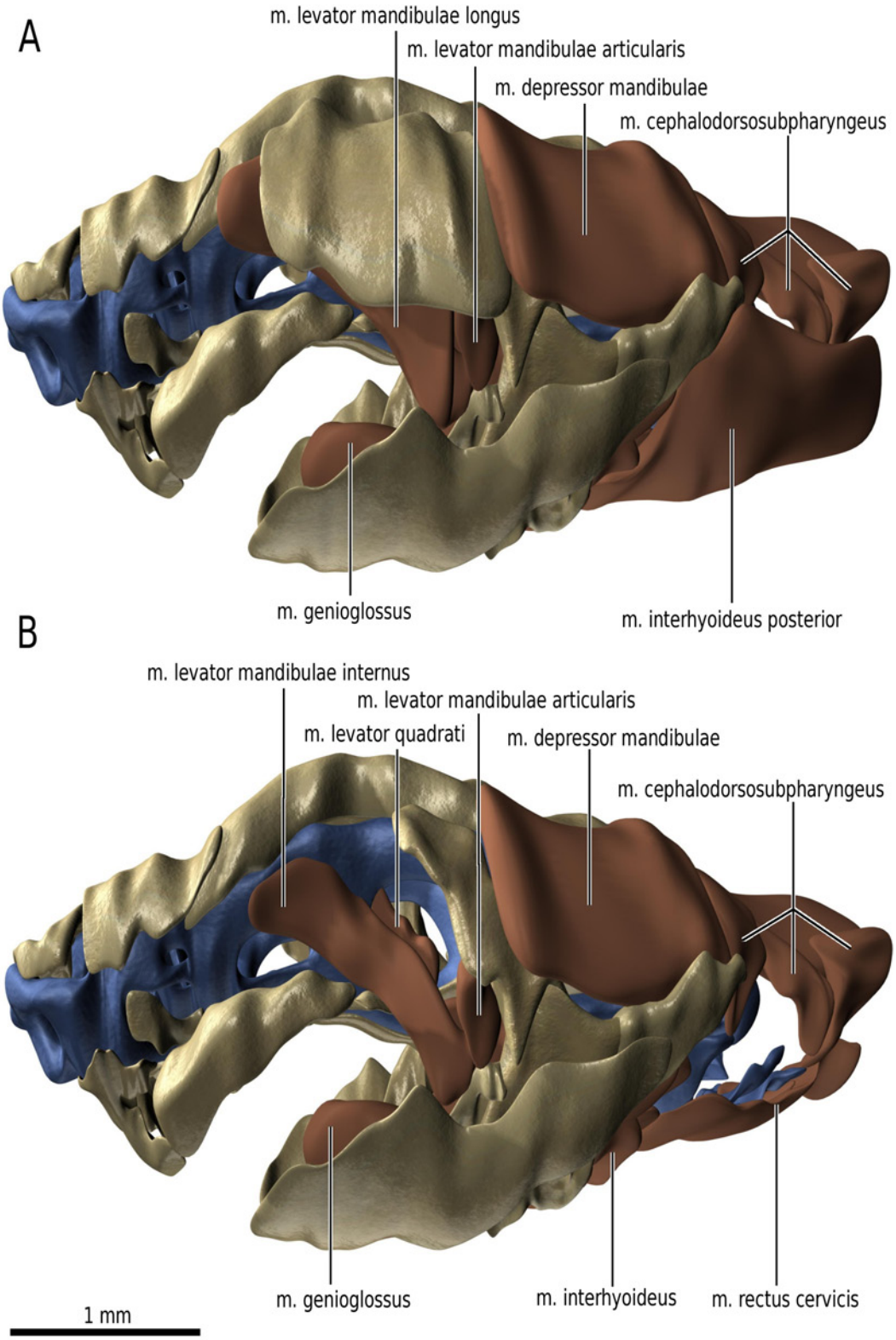

Figure 3. *Dermophis mexicanus* 44 mm TL (MHW_104). Surface renderings of 3D reconstructed specimen in lateral view. **A:** Superficial musculature. The mm. levatores mandibulae are covered laterally by the squamosal. The m. interhyoideus posterior is wrapped around the ventrolateral side of the head, caudal to the mandibular joint. **B:** Squamosal, m. levator mandibulae longus, and m. interhyoideus posterior removed. The m. levator quadrati originates from the caudal face of the pila antotica. In this specimen, the m. cephalodorsosubpharyngeus has three separated heads; the rostral head of the muscle has its fibers attached to ceratobranchial I.

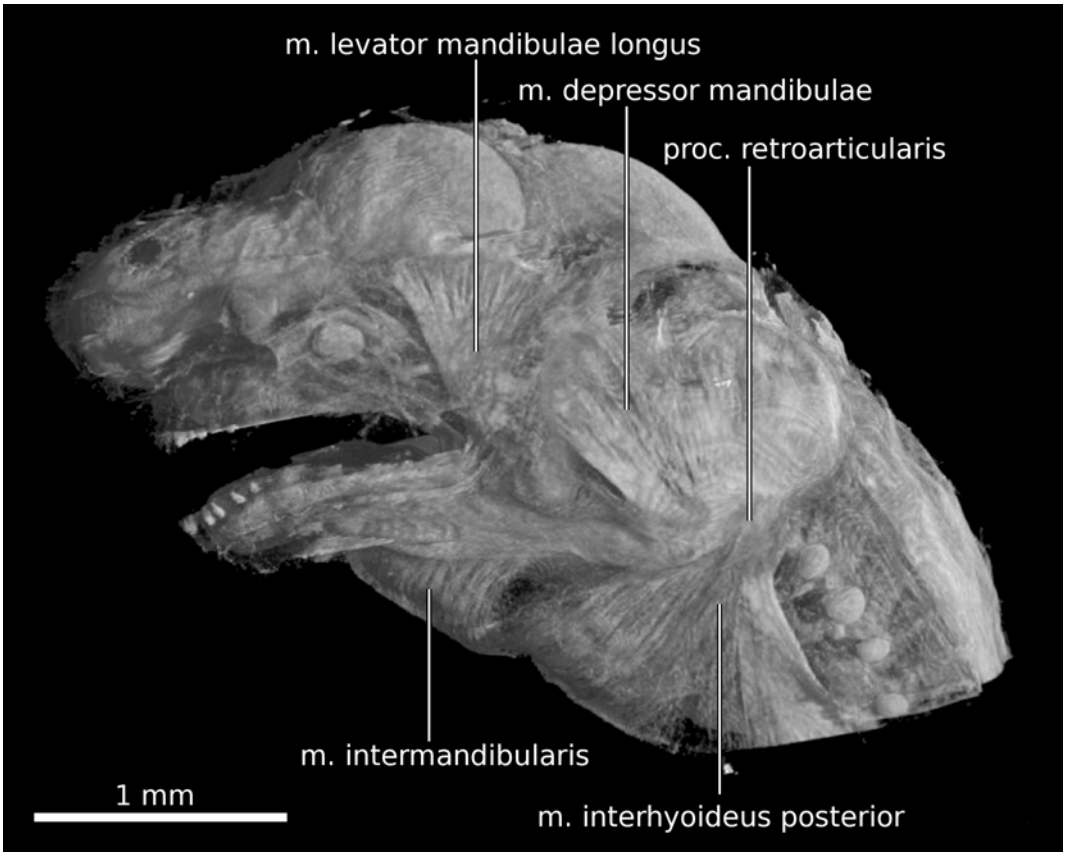

Figure 4. *Boulengerula taitana* 20 mm TL (MW03877). Volume rendering of SRμCT data in lateral view. Skin removed. The adductor chamber is opened laterally. The mm. levatores mandibulae are covered in part by the m. depressor mandibulae.

M. intermandibularis

The m. intermandibularis originates along the medioventral side of the pseudoangular over an area that reaches from the rostral tip of the pseudoangular to the mandibular joint (figs. 1, 2, 5). Its fibers run mediad and meet with the fibers of the m. intermandibularis of the contralateral body side in the ventral midline of the animal.

Boulengerula taitana: All specimens of *B. taitana* examined have a m. intermandibularis.

Gegeneophis ramaswamii: In *G. ramaswamii* this muscle is present in the 25 mm, 30 mm, 55 mm, 58 mm, and the adult specimen. In the 25 mm animal where an ossified pseudoangular is absent, the m. intermandibularis originates from Meckel's cartilage.

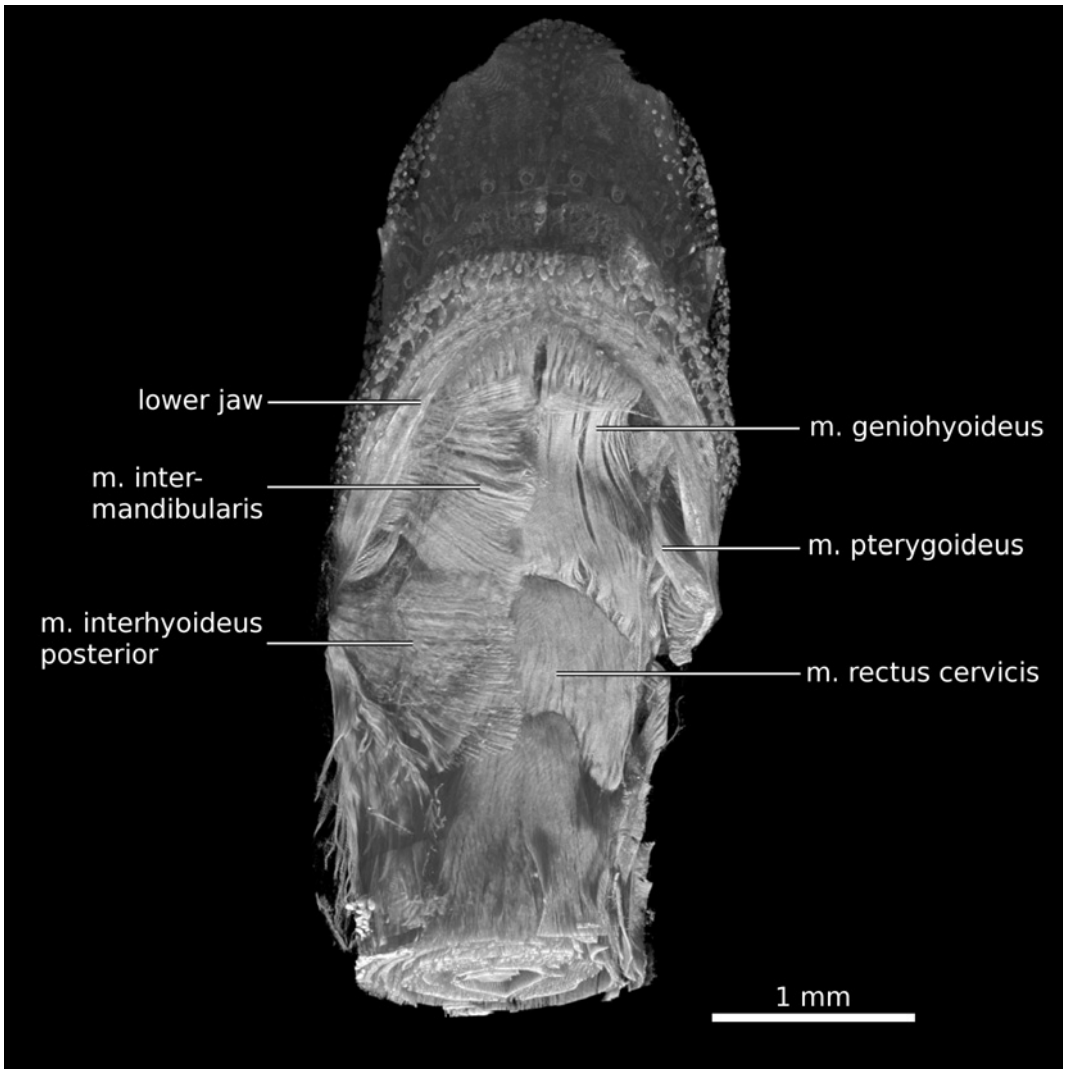

Figure 5. *Boulengerula taitana* 49 mm TL (MW03912). Volume rendering of SRμCT data in ventral view. Skin removed. M. intermandibularis and m. interhyoideus posterior were reomved from the left side of the specimen. The m. pterygoideus runs parallel to the lower jaw at the ventral side of the animal. The m. geniohyoideus originates from the fascia of the m. rectus cervicis.

Dermophis mexicanus: The m. intermandibularis is present in all *D. mexicanus* specimens examined and shows little variation among developmental stages.

Gymnopis multiplicata: All specimens of *G. multiplicata* have a m. intermandibularis and there are no ontogenetic differences.

M. depressor mandibulae group

The m. depressor mandibulae group comprises the m. depressor mandibulae and the m. depressor mandibulae posterior. The m. depressor mandibulae originates along an area that

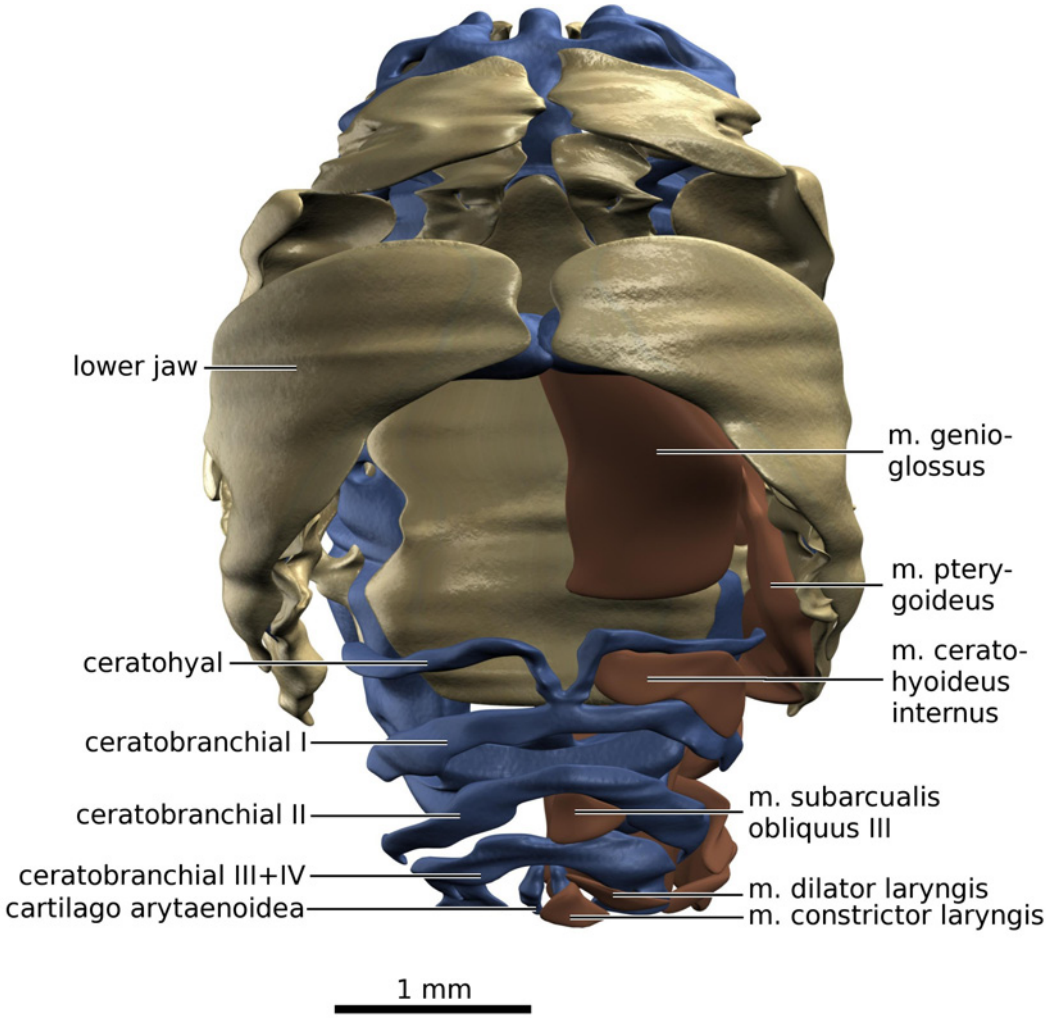

Figure 6. *Dermophis mexicanus* 44 mm TL (MHW_104). Surface renderings of 3D reconstructed specimen in ventral view. M. intermandibularis, m. interhyoideus, m. interhyoideus posterior, and m. transversus ventralis IV not shown. The m. ceratohyoideus internus originates from the rostral edge of ceratobranchial I and inserts on the caudal face of the ceratohyal. The m. subarcualis obliquus III is a small muscle that connects ceratbranchial III+IV to ceratobranchial II on the ventral side of the hyobranchial apparatus.

reaches from the laterocaudal face over the squamosal, the laterodorsal face of the parietal and the dorsolateral region of the ear capsule to the rostral part of the fascia that covers the dorsal trunk musculature (figs. 2, 3, 4). The m. depressor mandibulae posterior is an incompletely separated mediocaudal part of the m. depressor mandibulae that can be recognized by a slightly different orientation of muscle fibers. The m. depressor mandibulae and the m. depressor mandibulae posterior insert along the dorsal edge of the processus

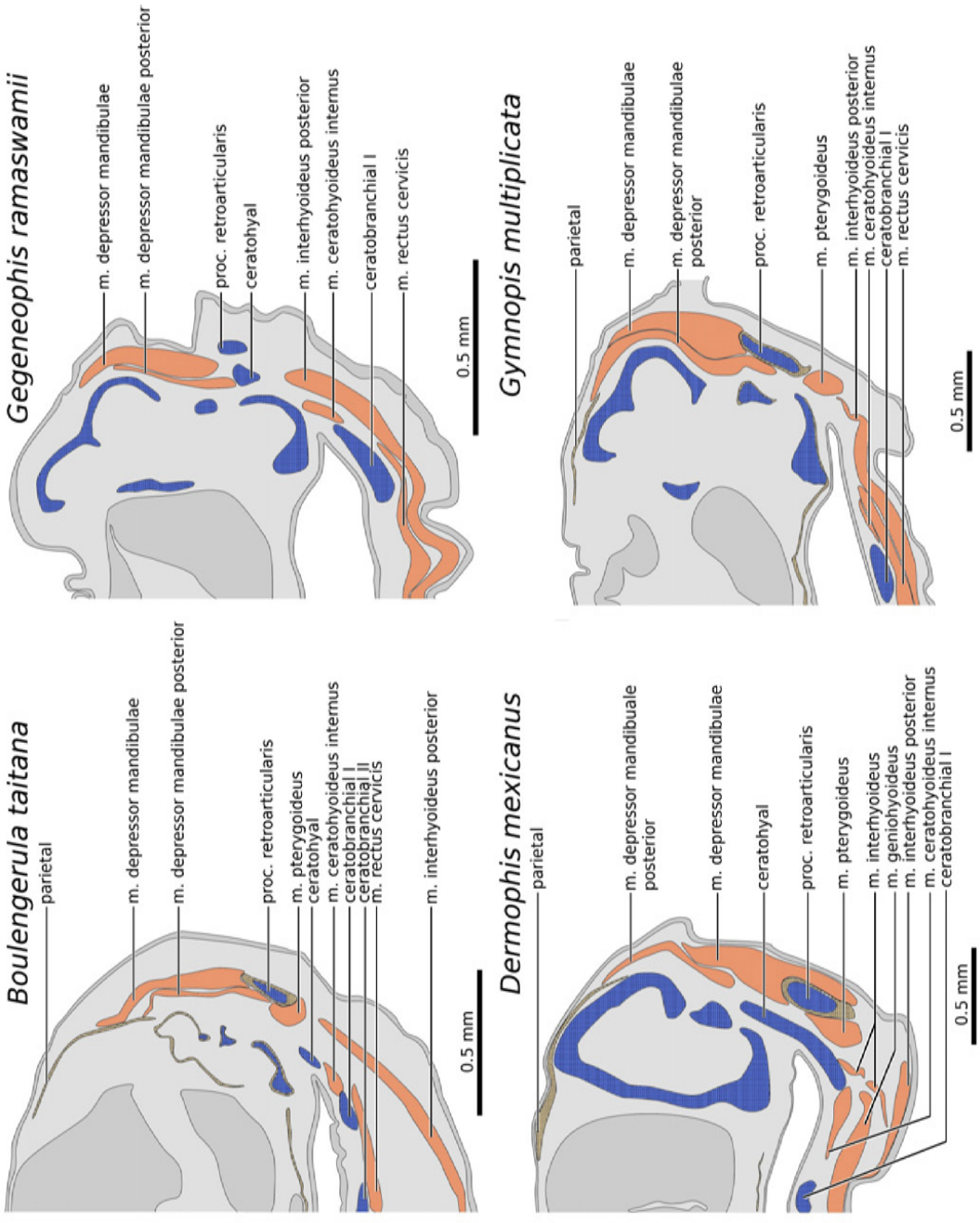

Figure 7. *Boulengerula taitana* 36 mm TL (HM0036), *Gegeneophis ramaswamii* 30 mm TL (MW1332), *Dermophis mexicanus* 34 mm TL (MHW_110), and *Gymnopis multiplicata* 54 mm TL (MHW_038), schematic drawings of transversal sections through the ear capsule. In all specimens, a m. depressor mandibulae and a m. depressor mandibulae posterior can be identified. Both muscles are closely associated and insert on the processus retroarticularis of the lower jaw. In the 30 mm *G. ramaswamii* specimen, the m. depressor rmandibuae posterior inserts on the distal tip of the ceratohyal.

retroarticularis of the lower jaw, the m. depressor mandibulae posterior additionally inserts with some fibers along the medial face of the processus retroarticularis (fig. 7).

Boulengerula taitana: The 18 mm and 20 mm specimens examined only have one muscle of the m. depressor mandibulae group. In the 36 mm and 49 mm specimens, it is possible to distinguish between the m. depressor mandibulae and the m. depressor mandibulae posterior. However, there is no clear boarder between the two muscles and the m. depressor mandibulae posterior can only be recognized as few muscle fibers that insert on the medial face of the processus retroarticularis. Both muscles originate exclusively from the parietal in *B. taitana* (fig. 2).

Gegeneophis ramaswamii: The m. depressor mandibulae and the m. depressor mandibulae posterior are present in all specimens of *G. ramaswamii* examined, except for the 26 mm specimen. In the youngest specimens (25 mm and 26 mm), the fibers of both muscles are not connected to skeletal elements at their origins. In the 30 mm animal, where most of the neuro- and dermatocranial ossifications are still incomplete, both muscles originate directly from the lateral face of the cartilaginous ear capsule. Additionally to the insertion at the lower jaw, the m. depressor mandibulae posterior inserts with some fibers on the distal tip of the ceratohyal in the 25 mm, the 30 mm, and the 55 mm specimens (fig. 7).

Dermophis mexicanus: In the 23 mm specimen of *D. mexicanus*, it is not possible to assign muscle fibers to the m. depressor mandibulae posterior. In the 30 mm and larger specimens both muscles are present.

Gymnopis multiplicata: All specimens of *G. multiplicata* examined herein have both muscles. The m. depressor mandibulae posterior can easily be recognized in *G. multiplicata* because of its insertion along the medial face of the processus retroarticularis ventral and medial to the insertion of the m. depressor mandibulae. In the 54 mm specimen, the two muscles appear as well separated heads.

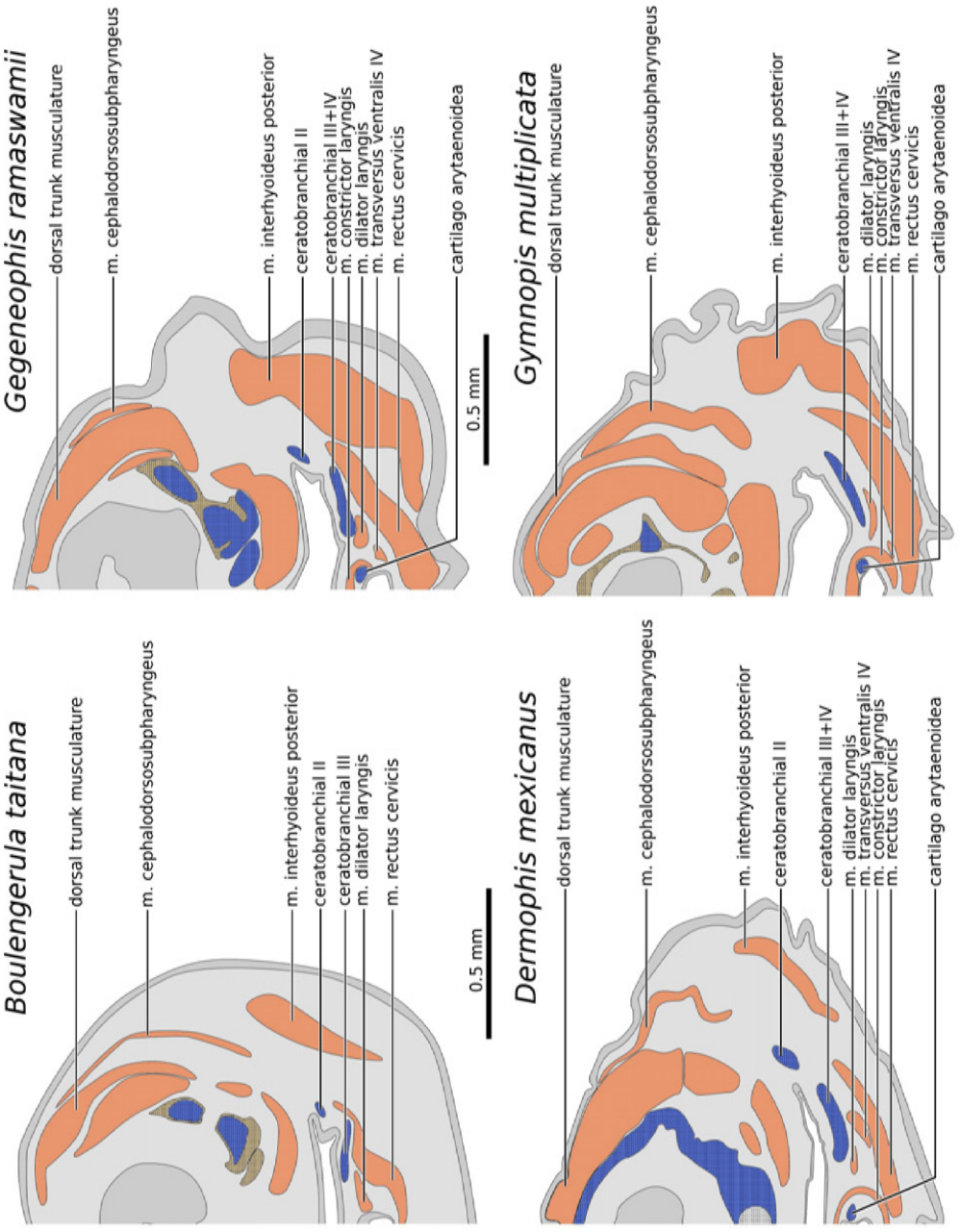

Figure 8. *Boulengerula taitana* 36 mm TL (HM0036), *Gegeneophis ramaswamii* 58 mm TL (MW1600B), *Dermophis mexicanus* 44 mm TL (MHW_104), and *Gymnopis multiplicata* 84 mm TL (MHW_040), schematic drawings of transversal sections through the larynx. The m. cephalodorsosubpharyngeus originates from the fascia of the dorsal trunk muscles. A m. transversus ventralis IV is present in all species examined, except *B. taitana*. The m. constrictor laryngis forms a ring that wraps around the cartilago arytaenoidea.

Ventral hyal muscles

The ventral hyal muscles contain the m. interhyoideus and the m. interhyoideus posterior. The m. interhyoideus originates along the ventral side of the proximal ceratohyal and inserts with its contralateral counterpart along the ventral midline of the animal (fig. 3B, 7). The m. interhyoideus posterior shares its origin with its contralateral counterpart on the ventral midline and inserts along the ventral side of the elongated processus retroarticularis (figs. 2A, 3A, 4, 5, 7, 8).

Boulengerula taitana: The specimens of *B. taitana* examined herein lack the m. interhyoideus. The m. interhyoideus posterior is present in all developmental stages studied.

Gegeneophis ramaswamii: All specimens have a well developed m. interhyoideus posterior that covers the entire hyobranchial apparatus ventrally and laterally. In *G. ramaswamii*, a separate m. interhyoideus is not present. However, the ceratohyal is closely associated to the processus retroarticularis and close to the insertion of the m. interhyoideus posterior in all *G. ramaswamii* specimens examined, except for the adult; a few medial fibers of the m. interhyoideus posterior insert on the ventral margin of the distal ceratohyal.

Dermophis mexicanus: Both muscles of the ventral hyal musculature are present in all *D. mexicanus* specimens examined. The m. interhyoideus is a delicate muscle that is almost entirely covered ventrally and laterally by the m. interhyoideus posterior (figs 3, 7).

Gymnopis multiplicata: In *G. multiplicata*, both muscles are present in the smallest specimen examined (30 mm). In the remainder specimens, only the m. interhyoideus posterior could be recognized.

Ventral branchial muscles

The ventral branchial musculature comprises the m. ceratohyoideus internus, the m. subarcualis obliquus III, and the m. transversus ventralis IV. The m. ceratohyoideus internus originates along the rostral face of the ceratobranchial I and inserts along the caudal edge of the ceratohyal; it connects both cartilages ventrally and laterally (figs. 6, 7). The m. subarcualis obliquus III has its origin in the proximal part of the ceratobranchial III along its rostral edge. Its fibers run rostrad and insert on the caudal face of ceratobranchial II (fig. 6). The m. transversus ventralis IV originates from the medial side of the distal tip of the ceratobranchial IV, or the merged ceratobranchial III+IV in specimens in that a separate ceratobranchial IV is absent (all *Dermophis mexicanus* and *Gymnopis multiplicata* specimens examined, adult *Gegeneophis ramaswamii*). The fibers of the m. transversus ventralis IV run ventrad and insert on the lateroventral wall of the trachea (fig 8).

Boulengerula taitana: Among the specimens examined, the m. subarcualis obliquus III only could be recognized in the 36 mm of *B. taitana*. The m. transversus ventralis was absent in all specimens.

Gegeneophis ramaswamii: None of the ventral branchial muscles was present in the 25 mm and 26 mm specimens of *G. ramaswamii*. In the remainder of the specimens examined, except for the 30 mm specimen, the m. ceratohyoideus internus, the m. subarcualis obliquus III, and the m. transversus ventralis IV could be identified. The 30 mm specimen, lacked the m. subarcualis obliquus III.

Dermophis mexicanus: The m. ceratohyoideus internus, the m. subarcualis obliquus III, and the m. transversus ventralis IV were present in all specimens of *D. mexicanus* examined.

Gymnopis multiplicata: In the 54 mm and 84 mm specimens of *G. multiplicata*, the m. ceratohyoideus internus, the m. subarcualis obliquus III, and the m. transversus ventralis

are present. The smallest specimen of *G. multiplicata* (30 mm) lacks a m. transversus ventralis IV.

Dorsal branchial muscles

The m. cephalodorsosubpharyngeus is the only dorsal branchial muscle in the caecilian species examined. It originates from the fascia of the dorsal trunk musculature, immediately caudal to the m. depressor mandibulae (figs. 2, 3, 8). The m. cephalodorsosubpharyngeus inserts on the lateral and ventral wall of the pharynx. Separate mm. levatores arcuum branchialium that are defined by their insertion on the distal tips of the ceratobranchial bars have not been found in the specimens examined.

Boulengerula taitana: In the 20 mm specimen, it was not possible to recognize this muscle. The insertion of the m. cephalodorsosubpharyngeus on the pharynx is dorsal to the distal tips of the ceratobranchials III and IV in all specimens.

Gegeneophis ramaswamii: The 30 mm, 55 mm, 58 mm, and adult specimens of *G. ramaswamii* have a m. cephalodorsosubpharyngeus. In the 30 mm specimen, this muscle originates from the fascia of the dorsal trunk muscles dorsal to the caudal region of the hyobranchial apparatus. In the older specimens, the origin of this muscle has shifted rostrad; it originates immediately caudal to the m. depressor mandibulae posterior. The insertion of the m. cephalodorsosubpharyngeus on the pharynx in *G. ramaswamii* is dorsal and caudal to the hyobranchial apparatus.

Dermophis mexicanus: The m. cephalodorsosubpharyngeus is present in all specimens of *D. mexicanus* examined. In the smaller specimens (23 mm, 30 mm, 34 mm, 44 mm) the m. cephalodorsosubpharyngeus is incompletely separated in two or three (in the 44 mm specimen) muscle heads of which the rostral head inserts on the distal tip of

ceratobranchial I (fig. 3). The fibers of the caudal heads of this muscle in the smallest specimens insert on the lateral side of the pharynx, dorsal and caudal to the hyobranchial apparatus.

Gymnopis multiplicata: All specimens of *G. multiplicata* examined have a m. cephalodorsosubpharyngeus. In *G. multiplicata* the fibers of this muscle cover the lateral and ventral side of the pharynx and mingle with the fibers of the m. cephalodorsosubpharyngeus from the contralateral side of the body.

Laryngal musculature

The larynx muscles comprise the m. constrictor laryngis and the m. dilator laryngis. The m. constrictor laryngis encompasses the caudal part of the cartilago arytaenoidea and meets with its contralateral counterpart dorsal and ventral to the trachea. The fibers of the m. constrictor laryngis form a ring (figs 6, 8); contraction of the muscle fibers will constrict the larynx. The m. dilator laryngis originates from the ventral face of the ceratobranchial IV or the merged ceratobranchials III + IV. The origin of the m. dilator laryngis is medial to the origin of the m. transversus ventralis IV (fig. 8). The fibers of the m. dilator laryngis run ventrad and rostrad and insert on the rostral tip of the cartilago arytaenoidea immediately rostral to the m. constrictor laryngis (fig. 6). The m. dilator laryngis depresses the larynx.

Boulengerula taitana: Among the *B. taitana* specimens examined, only the 36 mm specimen had a m. dilator laryngis. A m. constrictor laryngis could not be discerned in any of the specimens. I failed to recognize the larynx musculature in the SRμCT datasets of the 20 mm and 49 mm individuals. The m. dilator laryngis in the 36 mm specimen originates from the ventral side of the pharynx-wall medial to the ceratobranchial IV.

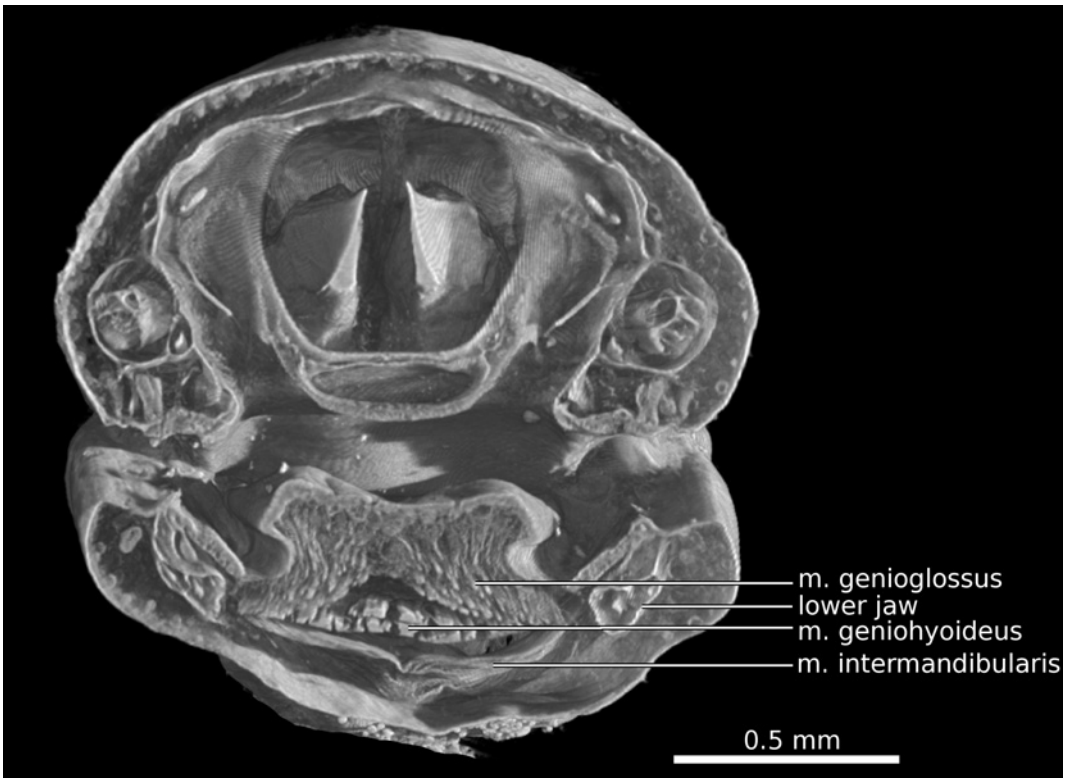

Figure 9. *Boulengerula taitana* 49 mm TL (MW03912). Volume renderings of SRμCT data in frontal view, sectioned in plane with the tongue and the eyes. The muscular part of the tongue consists of the m. genioglossus and the m. geniohyoideus. The m. genioglossus is a loose arrangement of muscle fibers that pass through the tongue.

Gegeneophis ramaswamii: In the 30 mm, 55 mm, 58 mm, and adult specimens, both muscles of the larynx are present; in the smaller specimens (25 mm and 26 mm) the two muscles are absent.

Dermophis mexicanus: All *D. mexicanus* specimens examined have a m. constrictor laryngis and a m. dilator laryngis.

Gymnopis multiplicata: Both larynx muscles are present in all specimens examined.

Muscles of the tongue

The tongue muscles in the caecilians examined comprise the m. genioglossus and the m. geniohyoideus. The m. genioglossus builds the muscular tongue (fig. 9). This muscle originates from the lingual face of the rostral region of the pseudodentary (fig. 6). The m.

genioglossus is a loose bundle of muscle fibers that fan out in dorsal direction and pass through the tongue (figs. 1, 9). Fibers of the m. genioglossus are either directly attached to the basal side of the dorsal epithelium of the tongue or via fasciae that pass through the tongue. The m. geniohyoideus originates from the fascia of the dorsorostral side of the m. rectus cervicis and with some fibers from the ventral face of the proximal ceratobranchial I (fig. 5). The fibers of the m. geniohyoideus run rostrad and insert in the rostral region of the pseudodentary on the lingual side of the bone, ventral to the origin of the m. genioglossus.

Boulengerula taitana: All specimens of *B. taitana* examined, have a m. genioglossus and a m. geniohyoideus. In the 18 mm specimen, however, the lingual face of the pseudodentary is not ossifed and the fibers of the two muscles are attached to Meckels cartilage or are blind ending. All fibers of the m. geniohyoideus in *B. taitana* originate from the ceratobranchial I immediately rostral to the insertion of the m. rectus cervicis; in the 49 mm specimen, fibers of the m. geniohyoideus are directly attached to the fascia of the m. rectus cervicis.

Gegeneophis ramaswamii: The m. genioglossus is present in the 30 mm and larger specimens. In the 30 mm animal this muscle is weakly developed and contains only a few fibers that span through the tongue. The m. geniohyoideus is present in all *G. ramaswamii* specimens examined. In the 25 mm, 26 mm, and 30 mm specimens, the m. geniohyoideus originates from the ceratobranchial I; in larger specimens, fibers of the m. geniohyoideus are additionally attached to the rostral margin of the m. rectus cervicis. The 25 mm and 26 mm specimens lack the ossification of the lingual pseudodentary; in the 25 mm *G. ramaswamii*, the m. geniohyoideus inserts on Meckels cartilage, in the 26 mm specimen, the muscle does not reach to the rostral part of the lower jaw - its fibers end blind.

Dermophis mexicanus: All specimens, except the 23 mm individual, have a m. genioglossus. The m. geniohyoideus is present in all developmental stages of *D. mexicanus* examined herein.

Gymnopis multiplicata: Both tongue muscles are present in all *G. multiplicata* specimens examined. The origin of the m. geniohyoideus shows variation over development of the muscle. In the 30 mm and 54 mm specimens, the origin is restricted to the ceratobranchial I; in the 84 mm specimen, the m. geniohyoideus originates from the rostral area of the m. rectus cervicis and ceratobranchial I.

DISCUSSION

Kleinteich and Haas (2007) presented a description of the entire cranial musculature in a larval caecilian. The descriptions on cranial muscles during development in direct developing oviparous and viviparous species presented herein show fundamental differences in the topology and presence or absence of muscles compared to the study by Kleinteich and Haas (2007) (tab. 2).

Several muscles that were described for caecilian larvae are absent in the specimens examined; i.e. the m. levator mandibulae externus, the m. ceratomandibularis, the m. subarcualis obliquus II, and the m. subarcualis rectus II-IV. The presence of the m. genioglossus was not confirmed for caecilian larvae by Kleinteich and Haas (2007). Other muscles, though present in the specimens examined and in caecilian larvae, differ in their appearance. Those are the m. depressor mandibulae posterior (insertion on the lower jaw and not the ceratohyal), the m. interhyoideus (restricted to the ventral side of the animal), and the m. cephalodorsosubpharyngeus (no separate mm. levatores arcuum branchialium present). Further, in caecilian larvae, the mm. levatores mandibulae originate in part from the dorsal

surface of the skull (Haas, 2001; Kleinteich and Haas, 2007; Müller, 2007); in the specimens I examined herein, the mm. levatores mandibulae are restricted to an adductor chamber that is covered laterally by the squamosal. Contrary, in caecilian larvae parts of the squamosal are covered laterally by the m. levator mandibulae longus (Kleinteich and Haas, 2007; Müller, 2007). My specimen sample comprised developmental stages from embryos to juveniles or adults of four different caecilian species and it seems very unlikely that the differences to caecilian larvae (Kleinteich and Haas, 2007; chapter 1) are due to incomplete sampling of developmental stages herein.

Based on the Batrachia-Hypothesis (Trueb and Clothier, 1991; Zardoya and Meyer, 2000, 2001; Frost et al., 2006; Roelants et al., 2007), it is most parsimonious to assume presence of the m. levator mandibulae externus, the m. subarcualis obliquus II, and the m. subarcualis rectus II-IV in larvae of the most recent common ancestor of amphibians (i.e. ground pattern of Lissamphibia); presence of this muscles is plesiomorphic for caecilians (Haas, 2001; Kleinteich and Haas, 2007; chapter 1). Although the existence of the m. ceratomandibularis in larvae of the most recent common lissamphibian ancestor is still ambiguous, there is clear evidence that the m. ceratomandibularis can be assigned to the ground pattern of the Gymnophiona (chapter 1). The absence of the m. levator mandibulae externus, the m. ceratomandibularis, the m. subarcualis obliquus II, and the m. subarcualis II-IV is the derived condition within caecilians. The m. genioglossus is absent in caecilian and salamander larvae (Kleinteich and Haas, 2007) and the presence of this muscle in young developmental stages of direct developing oviparous and viviparous species is derived.

The m. depressor mandibulae posterior inserts entirely on the distal tip of the ceratohyal in caecilian larvae and salamanders of the genus *Siren* (Drüner, 1904; Kleinteich and Haas, 2007; chapter 1). The insertion on the ceratohyal is considered to be ancestral for

amphibian larvae (Edgeworth, 1935; Bauer, 1997; chapter 1). From the specimens studied herein, none shows the ancestral character state for the m. depressor mandibulae posterior. In the 25 mm, 30 mm, and 55 mm specimens of *Gegeneophis ramaswamii*, a few fibers of the m. depressor mandibulae posterior insert on the ceratohyal; in the remainder of the specimens the entire muscle is attached to the lower jaw.

The m. interhyoideus is a well developed muscle in caecilian larvae. In larval *Epicrionops bicolor* and *Ichthyophis kohtaoensis*, this muscle covers wide areas of the hyobranchial apparatus laterally (Kleinteich and Haas, 2007; chapter 1). The condition in caecilian larvae was shown to be similar to salamander larvae and is supposed to be the ancestral character state for caecilians. In the specimens I examined herein, the m. interhyoideus is either a delicate muscle that is restricted to the ventral side of the animal (*Gegeneophis ramaswamii*, *Dermophis mexicanus*, *Gymnopis multiplicata*) or it is absent (*Boulengerula taitana*, adult *G. ramaswamii*, fetal *G. multiplicata*).

Kleinteich and Haas (2007) described four mm. levatores arcuum branchialium in larval *Ichthyophis kohtaoensis*. They considered these muscles as ancestral for amphibian larvae. In the specimens examined herein, the mm. levatores arcuum branchialium are absent. However, another muscle, the m. cephalodorsosubpharyngeus, is present. Kleinteich and Haas (2007) suggested that the m. cephalodorsosubpharyngeus is synonym to the m. levator arcus branchialis I in caecilian larvae. This is not confirmed herein. The m. cephalodorsosubpharyngeus extends over a wide area of the hyobranchial apparatus and is more likely to be a compound muscle, that besides the m. levator arcus branchialis I also consists of the mm. levatores arcuum branchialium II, III, and IV. In the 23 mm, 30 mm, 34 mm, and 44 mm specimens of *Dermophis mexicanus*, it is possible to separate a rostral head (presumably the homologous structure to the m. levator arcus branchialis I) and one or two

caudal heads of the muscle. I propose that the presence of the compound m. cephalodorsosubpharyngeus in early developmental stages of direct developing oviparous and viviparous species is derived within caecilians.

The differences between caecilian larvae and the specimens examined herein are similar to the differences between the larval and adult morphology of oviparous biphasic species; in direct developing and viviparous caecilians adult characters form precociously. Cranial muscles in adult caecilians have been described in Sarasin and Sarasin (1887-1890), Wiedersheim (1879), Luther (1914), Edgeworth (1920, 1935), Marcus et al. (1933), Ramaswami (1941, 1942), Lawson (1965), Nussbaum (1977, 1983), Bemis et al. (1983), Wake (1986, 1989), Iordanski (1996), Wilkinson and Nussbaum (1997), and Müller et al. (2009).

In all adult caecilians studied so far, independent of the mode of reproduction, the mm. levatores mandibulae are restricted to an adductor chamber, even though the lateral coverage by the squamosal might be incomplete and temporal fossae are present (zygokrotaphy; see Nussbaum (1983) or Wake (2003) for examples).

A m. levator mandibulae externus has never been described in adult caecilians; neither in oviparous species with biphasic development (Luther, 1914; Edgeworth, 1935; Ramaswami, 1941; Iordanski, 1996) nor in direct developing oviparous or in viviparous species (Luther, 1914; Marcus et al., 1933; Edgeworth, 1935; Lawson, 1965; Bemis et al., 1983; Iordanski, 1996; Wilkinson and Nussbaum1997; Müller et al., 2009). The term m. adductor mandibulae externus that was used by Luther (1914), Ramaswami (1941), Bemis et al. (1983), Iordanski (1996), and Wilkinson and Nussbaum (1997) is synonym to m. levator mandibulae longus (Haas, 2001; Kleinteich and Haas, 2007) and does not indicate homology to the m. levator mandibulae externus as defined here.

The m. ceratomandibularis that was described by Kleinteich and Haas (2007; Kleinteich and Haas used the term m. hyomandibularis) and in chapter 1 for caecilian larvae was proposed to merge with the m. depressor mandibulae during caecilian metamorphosis; absence of a m. ceratomandibularis is a feature of adult caecilians (Edgeworth, 1935).

The m. depressor mandibulae posterior is known to shift its insertion during metamorphosis from the ceratohyal to the lower jaw in amphibians. This was observed in salamanders (Litzelmann, 1923; Edgeworth, 1935; Piatt, 1938; Fox, 1958; Bauer, 1997) and caecilians (Edgeworth, 1935), and is most likely the case in frogs, given that the m. suspensorihyoideus in tadpoles (de Jongh, 1968; Haas, 1997) is the homologue to the m. depressor mandibulae posterior.

A well developed m. interhyoideus that covers the ceratohyal laterally has never been described for adult caecilians. In adult caecilians the m. interhyoideus is incompletely separated from the m. interhyoideus posterior or absent (Lawson, 1965; Wilkinson and Nussbaum, 1997).

Presence of a m. genioglossus and a m. cephalodorsosubpharyngeus are additional characteristics of the cranial musculature in adult caecilians independent on the reproductive mode. The m. genioglossus was previously described for adult caecilians by Lawson (1965), Bemis et al. (1983), and Wilkinson and Nussbaum (1997). The presence of the m. cephalodorsosubpharyngeus in adult caecilians has been confirmed by Lawson (1948), Bemis et al. (1983; m. levator arcus branchiales), and Wilkinson and Nussbaum (1997).

The presence of a m. subarcualis obliquus II and a m. subarcualis rectus II-IV in caecilian larvae (Kleinteich and Haas, 2007) and absence of this muscles in the specimens herein do not provide clues on the evolution of reproductive modes. Nussbaum (1977) described a m. subarcualis rectus II to be present in adult specimens within the genus

Ichthyophis. Presence of this muscle in adult individuals of the genus *Ichthyophis* and possibly in species of the Caeciliidae (Wilkinson and Nussbaum, 1997) indicates that the m. subarcualis obliquus II is not exclusive for caecilian larvae. For the m. subarcualis rectus II-IV, a similar muscle was described by Lawson (1965) in adult *Hypogeophis rostratus*. The potential m. subarcualis rectus II-IV in *H. rostratus* connects the first and the third ceratobranchials (Lawson, 1965). Presence of the m. subarcualis obliquus II and the m. subarcualis rectus II-IV in adult caecilians of species different from the ones examined herein suggests that the presence of the two muscles is not restricted to a larval stage.

All specimens considered herein are from species within the derived Caeciliidae; the larval caecilians studied so far belong to the more basal Rhinatrematidae (*Epicrionops bicolor*, chapter 1) and Ichthyophiidae (*Ichthyophis kohtaoensis*, Kleinteich and Haas, 2007). One could argue that the differences in muscle development evolved within the Caeciliidae and might not be directly related to the mode of reproduction. If this is true, one would expect to find larvae of species within the Caeciliidae that are more similar to the specimens examined herein than to larvae of *E. bicolor* or *I. kohtaoensis*. Müller (2007) gave an account on cranial skeleton and muscle morphology in caecilians, including larvae of species within the Caeciliidae (i.e. *Sylvacaecilia grandisonae*, *Praslinia cooperi*, *Grandisonia* cf. *larvata*, and *Grandisonia sechellensis*). All of the caeciliid larvae examined by Müller (2007) differed from the specimens examined herein by: (1) presence of an open adductor chamber, (2) presence of the m. ceratomandibularis, and (3) a well developed m. interhyoideus. For the m. depressor mandibulae posterior and the m. levator mandibulae externus it remains unresolved whether the observed differences are directly related to reproductive modes. The m. depressor mandibulae posterior (m. levator hyoideus in Müller, 2007) in larvae of *P. cooperi* and *S. grandisonae* is similar to non-caeciliid larvae and different to the specimens examined

herein by its insertion on the ceratohyal. However, in larval *Grandisonia* cf. *larvata* and *Grandisonia sechellensis* the m. depressor mandibulae posterior is absent (Müller, 2007). The m. levator mandibulae externus could only be identified in larvae of *P. cooperi* and is absent in larvae of the remainder species of the Caeciliidae examined by Müller (2007).

However, from the characteristics of muscle development described herein, at least the position of the mm. levatores mandibulae in an adductor chamber, the absence of a m. ceratomandibularis, and the reduced or absent m. interhyoideus are characteristics of adult caecilians. The shift in the timing of the appearance of these muscle characters from metamorphosis to the early onset of muscle development in the specimens examined herein can be related to direct development and viviparity with some confidence. The absence of larval muscle characters indicates a gradual development of the cranial musculature in direct developing and viviparous species.

The direct developing frog *Eleutherodactylus coqui* was shown to have precocious development of adult characters in early development (Hanken et al., 1992, 1997) and thus provides an interesting system for comparison. Like the caecilian specimens examined herein, larval specific muscle characters never form during muscle development in *E. coqui* (Hanken et al., 1997). Ontogenetic repatterning was further described for the evolution of direct development in salamanders (Roth and Wake, 1985). My results and published data on salamanders and frogs suggest that ontogenetic repatterning is a widespread phenomenon in the evolution of derived reproductive modes in amphibians. This was previously predicted by Wake and Hanken (1996). However, in another direct developing species of frog, *Philautus silus*, Kerney et al. (2007) did not find drastic changes for cranial development from the ancestral biphasic ontogenetic trajectory. Direct development of the skull in *P. silus* is similar to larval development and metamorphosis in other frog species. It remains unclear, whether

ontogenetic repatterning is a general mechanism for alterations in ontogenetic trajectories that relate to derived life histories.

The modifications in cranial muscle development in species with derived reproductive modes are likely to be related to differences in feeding behavior. Aquatic feeding in larvae will have different functional demands than skin, intrauterine, or terrestrial feeding. The similarities in the cranial musculature between juveniles of the oviparous *Boulengerula taitana*, fetuses of *Dermophis mexicanus* and *Gymnopis multiplicata*, and adult caecilians indicate identical feeding biomechanics for skin, intrauterine, and terrestrial feeding. A functional interpretation of the differences between larval morphology and the morphology of embryos and fetuses is given in chapter 4 of this thesis.

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Chapter 3

Allometric growth and heterochrony in the cranial development of oviparous and viviparous caecilians – a geometric morphometric study

ABSTRACT: Different reproductive modes in caecilians have been proposed to influence the cranial development by altering ontogenetic pathways. Geometric morphometrics provides powerful tools to quantify changes in the ontogenetic trajectories of animals by using landmark coordinates that describe the shape of structures in a geometric sense. Linear morphometrics shows patterns of relative growth. Herein, I use geometric and linear morphometrics to compare the changes in skull shape over ontogeny between the oviparous caecilian *Epicrionops bicolor* and the viviparous species *Dermophis mexicanus*. Both species show allometric growth of the skull. However, there are differences in the general shape of the skulls, the degrees of allometry, and the regions of growth between the two species. *D. mexicanus* shows more ontogenetic shape changes than *E. bicolor*. Most of the allometry observed in *D. mexicanus* is due to a notable growth of the nose relative to the remainder of the cranium and a relative decrease in the size of the premaxillary and maxillary bones after hatching. I suggest that some of the observed differences can be related to the different reproductive strategies (oviparity vs. viviparity) in the two species. The differences between *E. bicolor* and *D. mexicanus* development can be explained as heterochronic shifts in the sequence of development.

INTRODUCTION

Geometric morphometrics provides a method to quantify changes in shape and to test differences in shape for statistically significance (Zelditch et al., 2004; Slice, 2007). Shape in a geometric sense is defined as the geometric information that remains when effects of location, orientation, and scale are filtered out from an object (Kendall, 1977). The objects are described as a set of landmark coordinates. By comparing the shape of a species at different stages of development, geometric morphometrics will reflect changes in shape when the animal gets older, i.e. allometry (see the studies by Zelditch et al., 1993, 2000, 2003, and references therein). The ontogenetic change of shape from an initial point is referred to as ontogenetic trajectory (Alberch et al., 1979; Alberch, 1980; Zelditch and Fink, 1996). Comparisons of ontogenetic trajectories between different species have been used to conclude on the evolution of development (Alberch and Alberch, 1981; Nehm, 2001; Zelditch et al., 2003), especially within the focus of heterochrony (Klingenberg, 1998 and references therein; but see articles in Zelditch, 2001).

In the previous chapters of this thesis, I described the differences in the cranial muscle morphology during ontogeny of oviparous caecilians with larval stages, direct developing oviparous, and viviparous species. There are notable differences in cranial muscle development related to the reproductive mode: muscles that are present in caecilian larvae are never developed during direct development or viviparity, a result of ontogenetic repatterning (Roth and Wake, 1985; Hanken et al., 1992, 1997). Geometric morphometrics can be used to prove the idea of alterations in the ontogenetic trajectory and to quantify the changes.

A previous morphometric study on skull development in *Dermophis mexicanus* by Lessa and Wake (1992), showed a high degree of allometry, and a high level of integration of

concordant measurements (e.g. paired cranial elements). In another morphometric study on the development of the vertebral column in caecilians, Wake (1980a) linked the developmental patterns of caecilian vertebrae to their function in terrestrial and aquatic caecilians. The shape of a structure, e.g. the vertebrae, has been shown to reflect the function.

Due to the different modes of reproduction and differences in the cranial architecture during early development, the shape of the skulls over ontogeny (i.e. the ontogenetic trajectory) is supposed to differ between species. The comparison of ontogenetic trajectories between species with different modes of reproduction will potentially link morphological characters to functional constraints and makes conclusions on the evolution of development possible.

Here I compare the larval development of the oviparous species *Epicrionops bicolor* with the fetal development of the viviparous species *Dermophis mexicanus* by applying geometric morphometrics. The aims of this study are: 1) to test, whether the shape of the skulls of *Epicrionops bicolor* and *Dermophis mexicanus* over development is different by using a principal components analysis, 2) to validate or falsify the hypothesis that both species show allometric growth, i.e. that shape alters with size, by multivariate regression, 3) to trace the alterations of shape over ontogeny for the two species investigated herein by using the thin plate spline approach, and 4) to quantify allometric growth patterns by using linear morphometrics with an allometric growth model that is based on Huxley's (1932) equation for relative growth.

Table 1. Specimens used in this study.

ID	Species	TL [mm]	Comments
LSUMZ27293	*Epicrionops bicolor*	66.0	
LSUMZ27291	*Epicrionops bicolor*	89.0	
LSUMZ27289	*Epicrionops bicolor*	125.0	
LSUMZ27272	*Epicrionops bicolor*	146.0	
LSUMZ27267	*Epicrionops bicolor*	165.0	
LSUMZ27268	*Epicrionops bicolor*	165.0	no lateral view
LSUMZ27283	*Epicrionops bicolor*	190.0	
LSUMZ27245	*Epicrionops bicolor*	206.0	
MHW_772a	*Dermophis mexicanus*	54.5	no dorsal view
MHW_772b	*Dermophis mexicanus*	56.4	no dorsal view
MHW_792a	*Dermophis mexicanus*	60.1	no dorsal view
MHW_772c	*Dermophis mexicanus*	62.0	no dorsal view
MHW_730b	*Dermophis mexicanus*	68.6	
MHW_789b	*Dermophis mexicanus*	70.8	
MHW_730a	*Dermophis mexicanus*	73.1	
MHW_789a	*Dermophis mexicanus*	76.4	
MHW_930a	*Dermophis mexicanus*	77.3	
MHW_930b	*Dermophis mexicanus*	80.0	
MHW_959c	*Dermophis mexicanus*	101.0	
MHW_958g	*Dermophis mexicanus*	112.0	
MHW_D1007g	*Dermophis mexicanus*	120.0	
MHW_D1078	*Dermophis mexicanus*	123.0	
MHW_939	*Dermophis mexicanus*	134.0	
MHW_D1062	*Dermophis mexicanus*	148.0	
MHW_D1037	*Dermophis mexicanus*	150.0	
MHW_D1038	*Dermophis mexicanus*	150.0	
MHW_971e	*Dermophis mexicanus*	151.0	
MHW_1315	*Dermophis mexicanus*	154.0	
MHW_M856	*Dermophis mexicanus*	171.0	

MATERIALS AND METHODS

Specimens used for this study

The specimens that I examined for the geometric morphometric study are listed in table 1. All specimens were previously enzyme cleared and stained to specifically visualize bones and cartilages (Wassersug, 1976; Dingerkus and Uhler, 1977).

I examined 8 larval specimens of *Epicrionops bicolor* Boulenger, 1883 from the collection of the Louisiana State University Museum of Natural Sciences (LSUMZ), made available by Marvalee H. Wake (University of California at Berkeley). The same specimens were used in a previous study on the sequence of palatal development in *E. bicolor* by Reiss

Table 2. Description of Landmarks in dorsal and lateral views. Landmarks that are not strictly homologous between *Epicrionops biocolor* and *Dermophis mexicanus* are marked with an asterisk.

Landmark	*Epicrionops bicolor*	*Dermophis mexicanus*
	dorsal view	
1	rostromedial tip of the snout	
2	internasal suture at caudal edge of nasal	
3	interfrontal suture at caudal edge of frontal	
4	interparietal suture at caudal edge of parietal	
5	caudalmost tip of exoccipital (os basale)	
6	lateral bulge of ear capsule, caudal edge	
7	lateral bulge of ear capsule, rostral edge	
8*	transition of taenia marginalis to ear capsule	dorsal connection of squamosal to neurocranium
9	frontoparietal suture at lateral edge of parietal	
10*	dorsocaudal tip of maxillary	dorsocaudal tip of prefrontal
11	nasofrontal suture at rostrolateral tip of frontal	
12	rostral bulge of nasal capsule	
	lateral view	
1	rostral tip of nasal	
2	nasofrontal suture at dorsal edge of nasal	
3	frontoparietal suture at dorsal edge of frontal	
4	dorsocaudal tip of parietal	
5	caudalmost tip of exoccipital (os basale)	
6	ventrolateral tip of quadrate	
7	ventrocaudal tip of palatine	ventrocaudal tip of maxillopalatine
8	premaxillar-maxillar suture at ventrocaudal tip of premaxillary (nasopremaxillary)	
9	ventrorostral edge of premaxillary (nasopremaxillary)	
10	rostralmost tip of premaxillary (nasopremaxillary)	

(1996). Specimen LSUMZ27268 was damaged laterally, so I recorded landmark coordinates only from the dorsal side of the skull in this specimen. Further, two juvenile/adult specimens of *E. bicolor* were available (LSUMZ27265 and LSUMZ27266). However, the skull of juvenile/adult *E. bicolor* is very different from the larval skull and it was not possible to assign all landmarks that I used to describe the larval skull shape to the juvenile/adult skulls. I deleted the two juvenile/adult specimens from the geometric morphometric analysis and considered them for qualitative comparisons only.

I included 21 specimens of *Dermophis mexicanus* (Duméril and Bibron, 1841) in this study. *D. mexicanus* is known to start intrauterine feeding at about 25 mm and juveniles are born at 110 mm to 150 mm (Wake, 1977a, 1980b, c), thus the specimen sample comprises

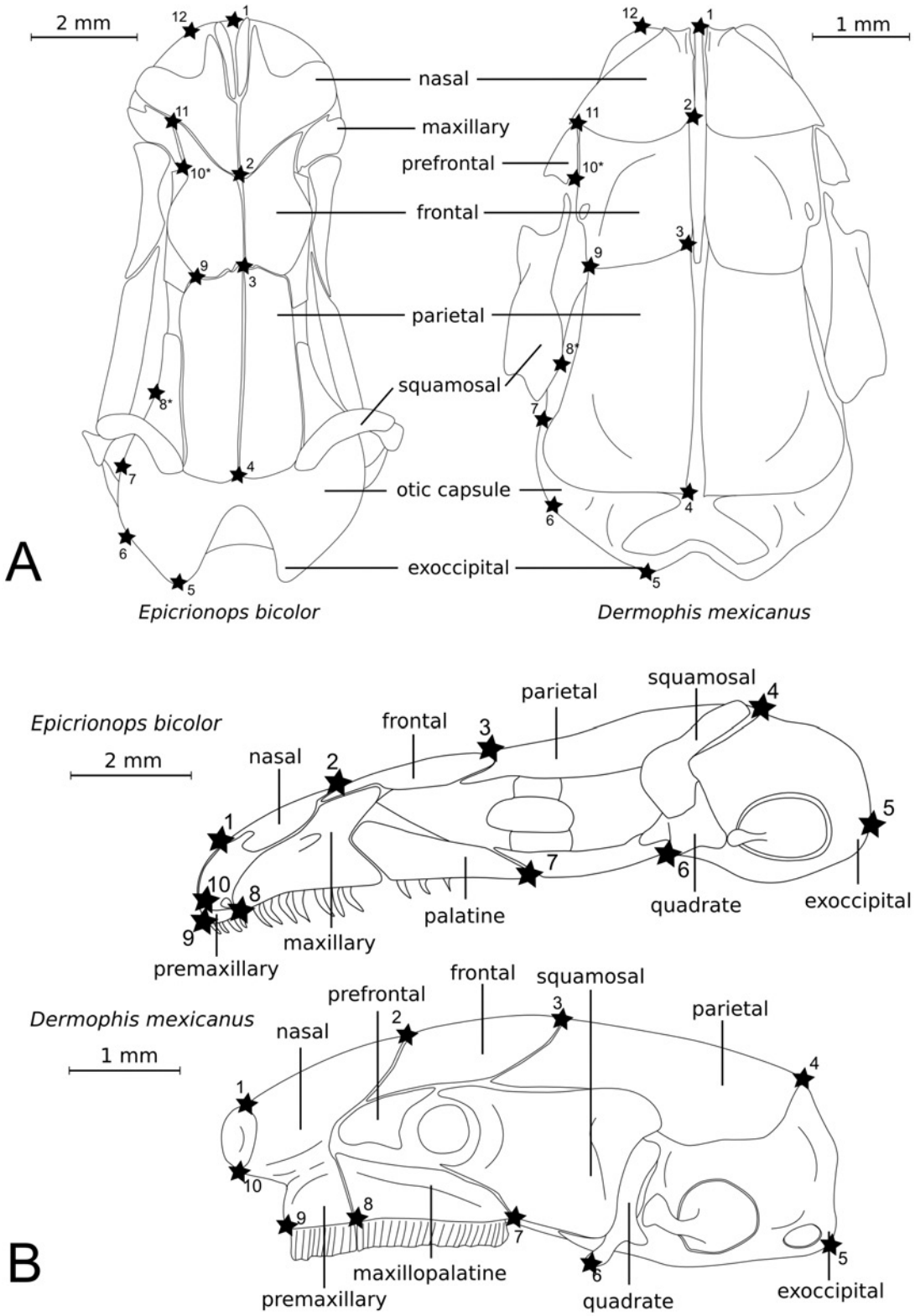


Figure 1. Landmarks used in this study. **A:** dorsal view and **B:** lateral view shown for *Epicrionops bicolor* (LSUMZ27245, 206 mm) and *Dermophis mexicanus* (MHW789a, 76 mm). Definitions of landmarks are provided in table 2. Landmarks were assigned to homologous structures in the two species. However, for an appropriate description of the shape of the skull in these species, it was necessary to include non-homologous landmarks (marked with an asterisk) as well for analysis.

fetal and juvenile animals. The specimens are from the collection of Marvalee H. Wake (MHW) and were examined at the University of California Berkeley. The specimens used herein were described previously in a study on cranial development of *D. mexicanus* by Wake and Hanken (1982). The dermal bones in the skull roof in the specimens MHW_772a, MHW_772b, MHW_772c, and MHW_792a are weakly ossified and it was not possible to assign all landmarks in dorsal view to this animals.

Image capturing, assignment of landmarks, and superimposition

Digital photographs of the skulls of the specimens were captured in dorsal and lateral views with a Nikon Coolpix E4500 digital camera that was mounted on a dissecting microscope. I positioned all skulls on a millimeter grid to capture the size of the objects in the photographs.

To describe the form of the skulls, I defined 12 landmarks in dorsal view and 10 landmarks in lateral view. Landmarks are shown in figure 1; landmark definitions are presented in table 2. The skull architecture of larval *Epicrionops bicolor* and fetal *Dermophis mexicanus* is strikingly different and not all landmarks mark homologous spots in both species. In *D. mexicanus* the squamosal attaches with a dorsomedial curvature to the neurocranium (dorsal landmark 8); the squamosal in *E. bicolor* has a much different shape and landmark 8 can not be assigned. However, I used the transition between ear capsule and the taenia marginalis in *E. bicolor* as landmark 8 due to its similar position in the animal as landmark 8 in *D. mexicanus*. Further, *E. bicolor* lacks prefrontals (Nussbaum, 1977) and thus landmark 10 (dorsocaudal tip of prefrontal) not can be assigned. However, the dorsocaudal tip of the maxillary in *E. bicolor* is in a similar position to the tip of the prefrontal in *D. mexicanus.* Landmarks that are on different cranial elements are marked with an asterisk in table 2. Exclusion of those landmarks would have had resulted in a loss of information on

cranial shape in the investigated species. For the assignment of landmarks, I used TpsDig 2.12 (Rohlf, 2008).

Skulls are three dimensional; two dimensional images of them are only projections of the real object to a plane. However, caecilian skulls are typically dorso-ventrally flattened and anterio-posteriorly elongated. The effect of landmarks shifting perpendicular to the image plane is supposed to have rather minor effects on the geometric morphometric analysis. Further, the integration of dorsal and lateral views helps to estimate the impact of landmark position shifts in the third, non-considered, dimension.

I used generalized least squares Procrustes superimposition (GLS) to remove differences in location, orientation, and scale from the objects (Rohlf, 1990). GLS was done with CoordGen6F (Sheets, 2001a) which is part of the freely available IMP software series. I used tpsSmall 1.2 (Rohlf, 2003) to correlate the Euclidean distances between all pairs of specimens of both species with the Procrustes distances between all pairs of specimens. The correlation was very high ($r > 0.9999$), which indicates that the area that the specimens occupy in Kendall's shape space is small enough to not cause distortions when projected to the tangent plane (see Zelditch et al., 2004 for a summary on shape space theory).

Principal components analysis

To test for differences in the shape of the specimens, I performed a principal components analysis (PCA) with the software PCAGen6N (Sheets, 2001b) from the IMP software series. The PCA with PCAGen6N produces a new set of variables (principal components, PCs). Each PC represents progressively less variance within the dataset, i.e. the Eigenvalues for each PC become smaller. I plotted the Eigenvalues of the PCs over the number of PCs (scree plot) to determine the inflection point (Zelditch et al., 2004). Only PCs left of the inflection

point have been considered for the analysis of shape since those contain most of the useful information on variation within the data. A Mann-Whitney *U* test was used to test for statistically significant differences in the scores along the PCs between the two species. I applied this test because the scores of the PCs are not normally distributed. The specimen sample comprises different ages and different sizes of animals within each species. To estimate effects on the PCA that are due to ontogenetic shape differences, the PC scores were regressed over the natural logarithm of centroid size. The mean shape of all specimens in this study was used as reference shape in the PCA. To depict changes in shape along the PCs, I used the thin plate spline approach.

Multivariate regression

I performed a multivariate regression with the IMP software Regress6L (Sheets, 2001c) to calculate the ontogenetic trajectories for *Epicrionops bicolor* and *Dermophis mexicanus*. This approach is based on Zelditch et al. (2003, 2004). Size was treated as independent variable, shape was considered to be the dependent variable. The natural logarithm of centroid size was used as a measure of size that is independent of shape. Partial warp scores were used as shape variables. I used the mean landmark configuration of the two smallest animals of each species as reference shape. Regress6L performs a MANOVA to test for statistical significance of allometry by calculating Wilk's λ (i.e. the variation in shape that can not be explained by the multivariate regression). Further, Regress6L calculates the ratio of the sum of Procrustes distances between the observed shape and the shape that is predicted by multivariate regression for all specimens. This ratio gives an estimate for the variation in shape that is explained by size based on multivariate regression (Zelditch et al., 2004). Multivariate regression with Regress6L assumes a linear relationship of shape over size. To

test for a linear correlation of shape over size, I computed linear regressions of procrustes distances from the reference shape over size for each species in dorsal and lateral view.

Zelditch et al. (2003, 2004) proposed to calculate the angle between ontogenetic trajectories and to test this angle for statistical significance in order to quantify the comparison between ontogenetic trajectories of different species. This, however, assumes, that there is a stage in ontogeny of both compared species where they share the same shape. Based on my studies of caecilian cranial development, I doubt that there is an early ontogenetic stage in which the skull of the larva of an oviparous caecilian has the same shape as the skull of a fetal viviparous caecilian. Herein, I compare the ontogenetic trajectories only qualitatively by comparing the changes in shape when the animals grow and not quantitatively by calculating an angle for the growth-vector.

Linear morphometrics

I used linear or traditional morphometrics to study the relationship of measurements between landmarks to the growth along the long-axis of the skull. In dorsal and lateral view, the distance between landmarks 1 and 4 was used as a measure for the length of the skull along its long-axis, i.e. the reference length for further calculations. Landmark data were converted to linear morphometric, i.e. distance, measurements with the software TMorphGen6B (Sheets, 2000). Distances between landmarks over ontogeny were log transformed and regressed over the natural logarithm of the reference distance. The equations for the linear regressions of each distance over ontogeny have the form:

(1) $\ln(Y) = k*\ln(X) + \ln(b)$

With *X* being the independent variable (reference length) and *Y* being the dependent variable (distance between landmarks). Equation (1) is the logarithmic representation of Huxleys relative growth equation (Huxley, 1932):

(2) $Y = b*X^k$

The variable *k* (slope of the regression) is the growth rate of *Y* relative to *X*. Values for $k > 1$ indicate positive allometry relative to the length of the skull, $k < 1$ indicates negative allometry relative to the length of the skull, and $k = 1$ shows isometry, i.e. measurement lengths scale with skull length over ontogeny. Treating growth rates of exactly $k = 1$ as isometry only, seems unnecessary strict in a biological content. I used the standard error of the growth rate *k* for the linear regression (equation 1) to get an estimate for more realistic thresholds that allow to determine between allometry and isometry. I calculated the correlation coefficients r^2 for each linear regression to test the reliability of the calculated growth rates *k*. I considered only growth rates *k* that were based on linear regressions with values for $r^2 > 0.8$ for further discussion.

RESULTS

Patterns of shape space occupation during development – PCA

Principal components analysis of all specimens in dorsal view results in 20 principal components (table 3). The first two PCs explain 76.61% of the variation in shape within the data. PC1 explains 51.41% of the variation and separates both species in shape space (figure 2A). The visual separation of the two species in shape space is also supported by a high statistical significance based on the Mann-Whitney *U* test ($p < 0.01$). Low values of PC1 (i.e. the position of *Epicrionops bicolor* individuals in shape space) represent a narrow nasal

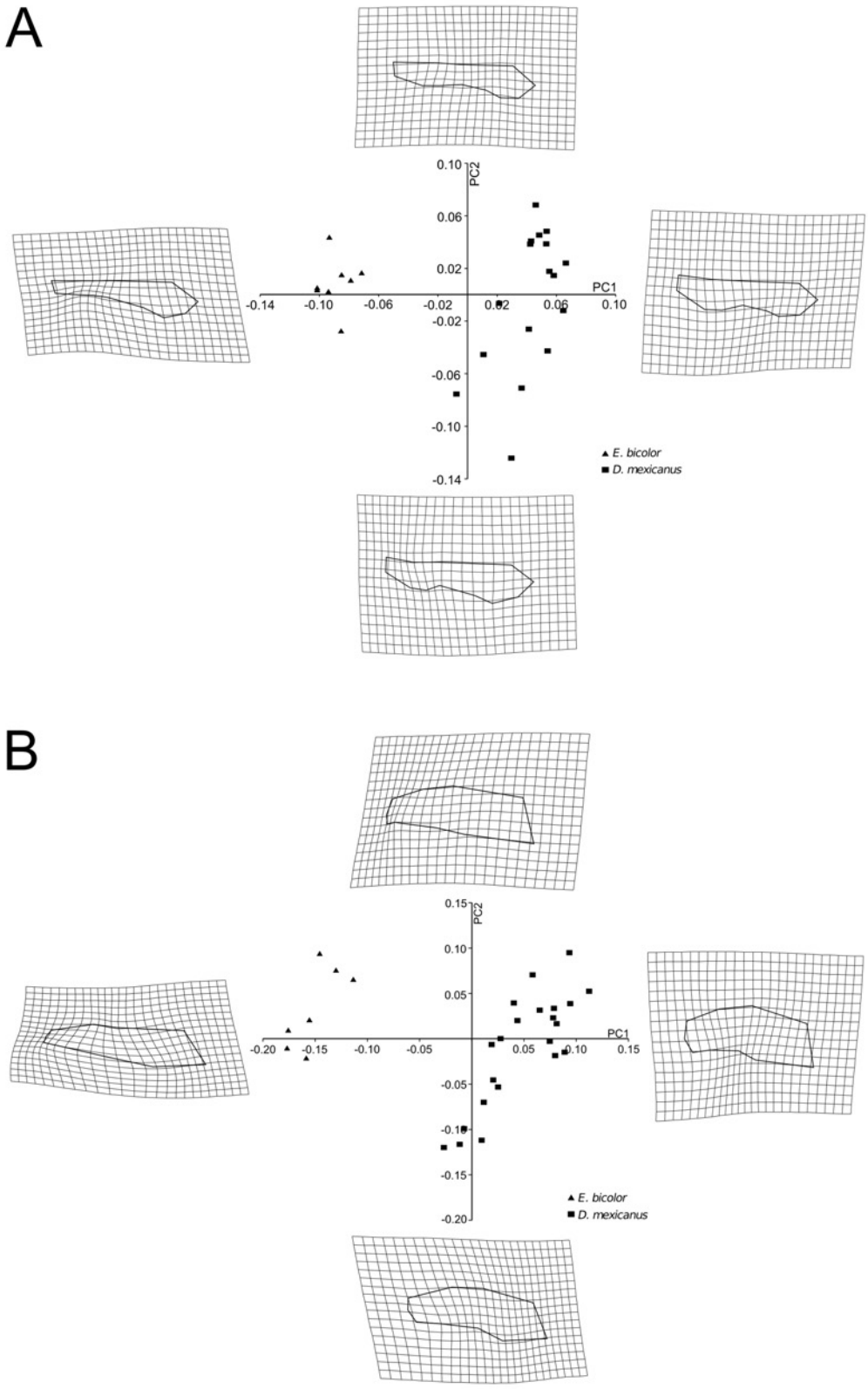


Figure 2. Plot of principal components showing all specimens in this study. **A:** dorsal view and **B:** lateral view. PC2 is plotted over PC1. The shapes at the end of the axes represent the extreme shapes along the principal components. The deformation grids show the deformation of the mean shape of all specimens (origin of coordinate system) to the extreme shapes. *Epicrionops bicolor* and *Dermophis mexicanus* are clearly separated by PC1 in dorsal and lateral view.

Table 3. Eigenvalues and percentage of explained variation for principal component analysis.

PC	Eigenvalue	% variation explained
	dorsal view	
1	4.17E-003	51.41
2	2.05E-003	25.20
3	3.88E-004	4.78
4	3.35E-004	4.13
5	2.95E-004	3.63
6	2.39E-004	2.94
7	1.76E-004	2.17
8	1.38E-004	1.70
9	8.13E-005	1.00
10	6.51E-005	0.80
11	5.33E-005	0.66
12	3.62E-005	0.45
13	2.95E-005	0.36
14	1.92E-005	0.24
15	1.54E-005	0.19
16	1.17E-005	0.14
17	8.27E-006	0.10
18	5.36E-006	0.07
19	2.28E-006	0.03
20	1.16E-006	0.01
	lateral view	
1	8.78E-003	53.53
2	3.71E-003	22.59
3	1.68E-003	10.22
4	6.06E-004	3.69
5	5.14E-004	3.13
6	3.44E-004	2.10
7	2.23E-004	1.36
8	1.74E-004	1.06
9	1.63E-004	1.00
10	6.63E-005	0.40
11	5.86E-005	0.36
12	3.09E-005	0.19
13	2.36E-005	0.14
14	1.82E-005	0.11
15	1.03E-005	0.06
16	8.43E-006	0.05

region that gives the skull in dorsal view a bottle-like shape. As PC1 increases the rostral part of the skull becomes wider towards a broad nasal and frontal region (figure 2A).

PC2 in dorsal view explains 25.2% of the shape variation that is contained in the dataset. The distribution of the two species along PC2 is not significantly different ($p < 0.95$). PC2 includes information on the width and the length of the dorsal skull shapes (figure 2A).

Linear regression of PC1 and PC2 for dorsal skull shape over the natural logarithm of centroid size results in correlation coefficients r^2 of 0.05 for PC1 and 0.34 for PC2. This indicates that some variation of the specimens along PC2 can be explained by differences in the size of the animals, although the correlation of PC2 over size is not very strong.

In lateral view, the PCA results in 16 PCs (table 3). The first three PCs explain 86.34% of the variation in the lateral shapes of the specimens. The first PC accounts for 53.53% of the total variation and separates both species significantly in shape space ($p < 0.01$; figure 2B). An increase in the scores for PC1 in lateral view shows a transformation from a flat and elongated skull shape (*Epicrionops bicolor*) to a high, short, and ventrally bended shape (*Dermophis mexicanus*; figure 2B).

The distributions of the shapes in lateral view along PC2 (22.59%) and PC3 (10.22%) do not differ significantly between *Epicrionops bicolor* and *Dermophis mexicanus* (PC2: $p < 0.17$; PC3: $p < 0.39$). Decrease in PC2 from the reference shape (i.e. PC2 < 0) shows a relatively shortened rostral part of the skull shape; increase in PC2 (PC2 > 0) depicts an elongation in the rostral skull regions and a shortening in the caudalmost parts of the skull (figure 2B).

Linear regressions of the lateral view PCs over the natural logarithm of centroid size results in correlations with r^2 values of 0.39 for PC1, 0.27 for PC2, and 0.13 for PC3. This indicates that besides the differences in shape between the two species, PC1 also accounts for some amount of variation in size of the specimens. The distribution of the specimens along PC2 also shows a weak correlation with size.

Reconstruction of ontogenetic trajectories – multivariate regression

Epicrionops bicolor and *Dermophis mexicanus* show a linear relationship of shape over size in dorsal and lateral view (figure 3). All linear regressions result in high correlation

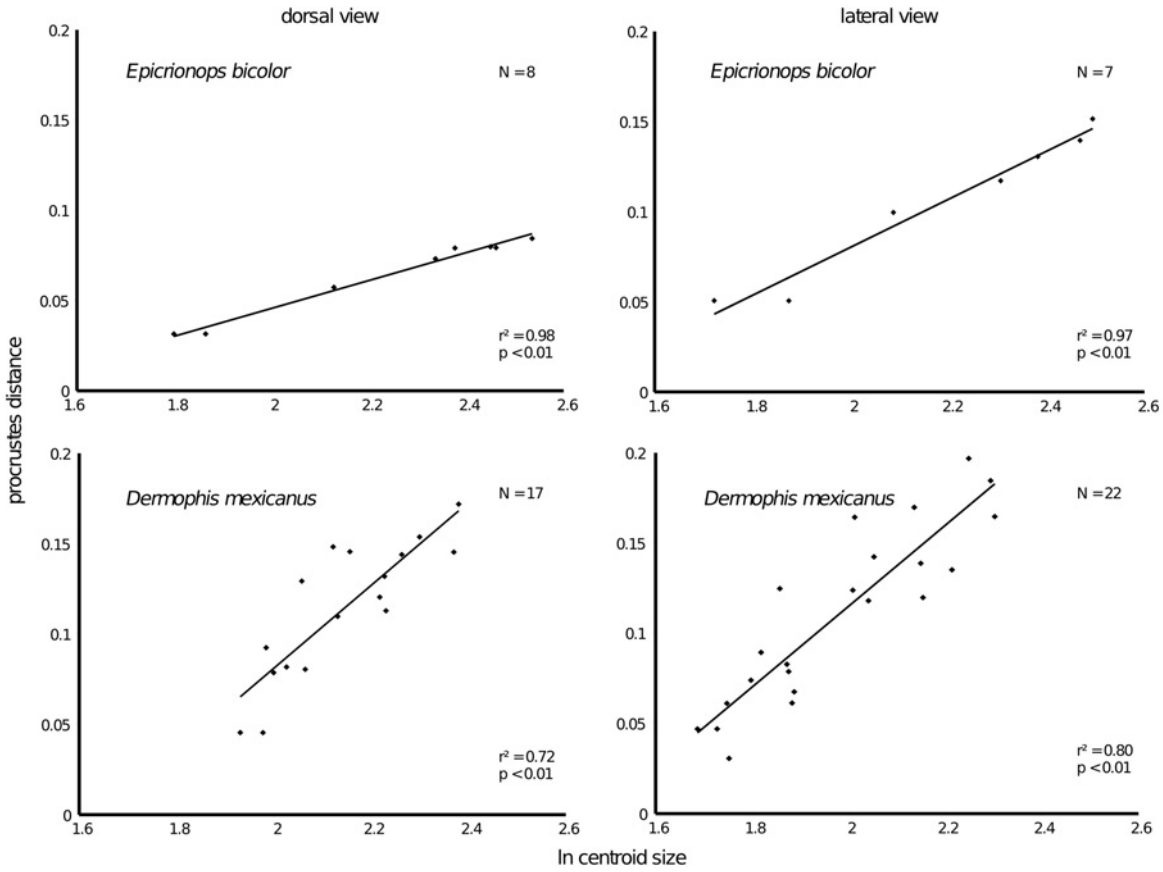
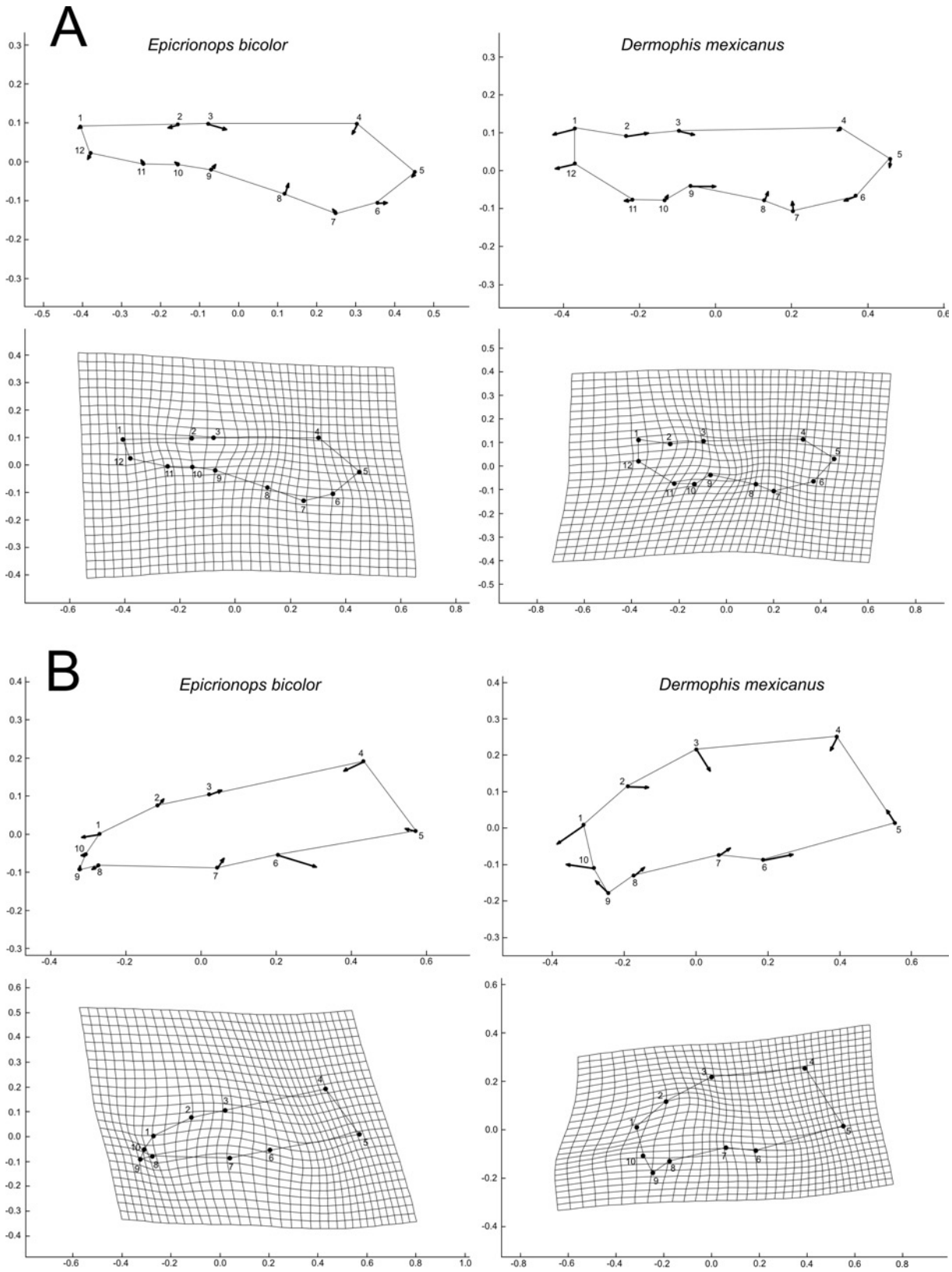


Figure 3. Test for linear relationship of shape over size for *Epicrionops bicolor* (upper row) and *Dermophis mexicanus* (lower row) in dorsal (left hand side) and ventral (right hand side) views. Procrustes distance to a reference shape (mean shape of 2 smallest individuals) is drawn over the natural logarithm of centroid size. Both species show a significant allometric growth pattern, i.e. shape varies with size. The high correlation coefficients r^2 indicate a linear relationship of shape with the logarithm of centroid size.

coefficients r^2 (dorsal view: 0.98 for *E. bicolor*, 0.72 for *D. mexicanus*; lateral view: 0.97 for *E. bicolor*, 0.80 for *D. mexincanus*) and significant relationships of shape and size ($p < 0.01$ for both species and both views).

Multivariate regression of *Epicrionops bicolor* dorsal and lateral skull shape shows significant allometry. The multivariate regression in dorsal and lateral view results in Wilk's λ scores of 0.0, assuming a high correlation between size and shape in *E. bicolor*. The variation of shape in *E. bicolor* that can be explained by size is 33.4% in dorsal and 38.3% in lateral view.


A
Epicrionops bicolor
Dermophis mexicanus
B
Epicrionops bicolor
Dermophis mexicanus

Most notable changes in dorsal shape over ontogeny are an elongation of the frontal bone (between landmarks 2 and 3) and a narrowing of overall skull shape (most vectors on landmarks point inward; figure 4A). In lateral view of *Epicrionops bicolor* skull shape, multivariate regression depicts a ventrocaudal shift of the jaw articulation (landmark 6) and a decrease in the height of the lateral skull face (figure 4B).

In *Dermophis mexicanus*, multivariate regression shows a significant relationship between shape and size of the specimens. The MANOVA result in scores for Wilk's λ of 0.0 (dorsal view) and 0.005 (lateral view) which shows a high correlation of size and shape in both views. Size predicts 38.6% of the variation in shape in dorsal view and 43.6% in lateral view.

In dorsal view the most obvious shape changes in the development of *Dermophis mexicanus* are the rostral elongation of the nasal (landmarks 1, 2 and 12) and a relative shortening of the parietal (landmarks 3 and 9). The dorsal skull shape (figure 4A) becomes narrower over ontogeny (most vectors on landmarks point to the inside). In lateral view (figure 4B), *D. mexicanus* skulls change their shapes over ontogeny by a substantial growth of the nasal (landmarks 1 and 10), a decrease in the height of the lateral skull face (most

Figure 4. Graphical representation of growth patterns in *Epicrionops bicolor* (left side) and *Dermophis mexicanus* (right side). **A:** dorsal and **B:** lateral view. Relative growth over ontogeny was calculated by multivariate regression of partial warp scores over the natural logarithm of centroid size. Upper rows: vectorplot with vectors pointing to the direction of landmark shifts during ontogeny; lower row: interpolation of vectors over all landmarks by using the thin plate spline. The mean shape of the two smallest specimens is used as reference (position of landmarks). The size of growth vectors and the deformation of the thin plate spline is higher in *D. mexincanus* than in *E. bicolor*. In *E. bicolor*, most of the ontogenetic shape change is due to the outgrowth of the frontal bone (dorsal landmarks 2 and 3) and a narrowing of the caudal region of the skull (most vectors point to the inside of the skull shape, especially evident at dorsal landmarks 4 and 8). In lateral view *E. bicolor* shows high ontogenetic shape change due to the elongation of the nasal (between lateral landmarks 1 and 2), a relative shortening of the parietal (between lateral landmarks 3 and 4), and a ventrocaudal shift of the jaw articulation (lateral landmark 6). In *D. mexicanus* the major ontogenetic shape changes happen in the rostral region of the skull (dorsal landmarks 1and 12 shifting rostrally, dorsal landmarks 2, 3, and 9 shifting caudally). The lateral skull shape of *D. mexicanus* becomes flattened (most vectors point to the inside) and elongated due to growth of the nasal region (lateral landmarks 1 and 10).

Table 4. Parameters of allometric growth in the caecilian species *Epicrionops bicolor* and *Dermophis mexicanus*. Growth coefficient k (slope of regression) and Pearsons correlation coefficient r^2 for regression of the growth equation. Standard errors of the shape are used for confidence; k > 1 positive allometry, k < 1 negative allometry, k = 1 isometry

Landmarks	*Epicrionops bicolor*			*Dermophis mexicanus*		
	k	allometry	r^2	k	allometry	r^2
dorsal view						
1-4	used as reference					
1-2	0.91 ± 0.06	negative	0.95	2.06 ± 0.13	positive	0.88
2-3	1.97 ± 0.18	positive	0.92	0.61 ± 0.11	negative	0.45
3-4	0.79 ± 0.04	negative	0.97	0.62 ± 0.08	negative	0.63
4-5	0.94 ± 0.12	-	0.85	0.95 ± 0.17	-	0.47
5-6	0.60 ± 0.09	negative	0.79	1.06 ± 0.09	-	0.78
6-7	1.31 ± 0.15	positive	0.88	0.32 ± 0.12	negative	0.17
7-8	0.92 ± 0.19	-	0.67	0.58 ± 0.26	negative	0.12
8-9	0.97 ± 0.13	-	0.83	0.11 ± 0.14	negative	0.01
9-10	1.30 ± 0.19	positive	0.8	1.77 ± 0.19	positive	0.7
10-11	0.96 ± 0.12	-	0.85	1.44 ± 0.10	positive	0.84
11-12	1.01 ± 0.11	-	0.89	1.08 ± 0.08	-	0.83
1-12	1.17 ± 0.10	positive	0.92	0.79 ± 0.13	negative	0.5
2-11	0.68 ± 0.11	negative	0.79	1.14 ± 0.11	positive	0.76
3-9	0.72 ± 0.12	negative	0.75	0.83 ± 0.10	negative	0.64
4-7	0.77 ± 0.07	negative	0.92	0.59 ± 0.04	negative	0.86
lateral view						
1-4	used as reference					
1-2	1.49 ± 0.12	positive	0.94	1.81 ± 0.09	positive	0.95
2-3	1.19 ± 0.24	-	0.71	0.53 ± 0.11	negative	0.53
3-4	0.70 ± 0.07	negative	0.9	0.68 ± 0.06	negative	0.87
4-5	0.97 ± 0.13	-	0.85	0.52 ± 0.07	negative	0.74
5-6	0.51 ± 0.08	negative	0.81	0.49 ± 0.09	negative	0.61
6-7	1.47 ± 0.26	positive	0.78	1.37 ± 0.11	positive	0.88
7-8	1.18 ± 0.16	positive	0.86	0.83 ± 0.13	negative	0.67
8-9	0.50 ± 0.24	negative	0.31	1.42 ± 0.36	positive	0.43
9-10	0.49 ± 0.25	negative	0.29	1.20 ± 0.13	positive	0.8
1-10	0.53 ± 0.31	negative	0.23	-0.12 ± 0.14	negative	0.03
2-8	1.27 ± 0.11	positive	0.93	0.74 ± 0.06	negative	0.87
3-7	0.95 ± 0.11	-	0.88	0.46 ± 0.07	negative	0.7

landmarks pointing inward), a decrease in the expansion of the premaxillary (landmark 9), and a caudal shift of the mandibular joint (landmark 6).

Determination of regions with allometric growth – linear morphometrics

Table 4 contains the allometric growth coefficients *k* for measured lengths between landmarks and the correlation coefficients r^2 for the linear regressions on which the values for *k* are based. Figure 5 shows the measured distances and the according growth coefficients *k*.

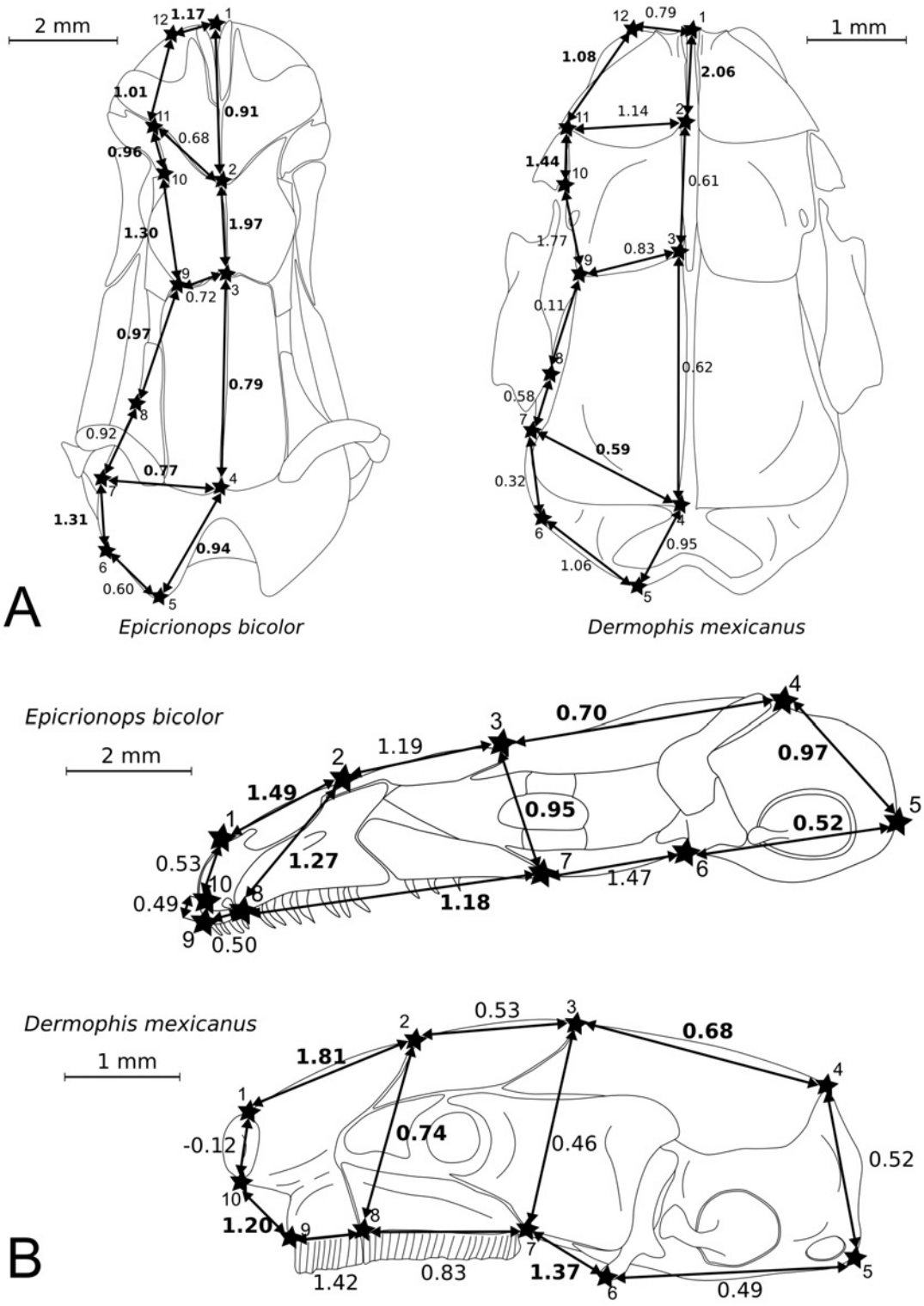
The highest positive allometric growth coefficients *k* on the dorsal shape of the skull are observed for the length of the nasal region along the midline in *Dermophis mexicanus* (landmarks 1-2, $k = 2.06$) and for the growth of the frontal in *Epicrionops bicolor* (landmarks 2-3, $k = 1.97$), i.e. those structures become elongated relative to the entire skull over ontogeny.

Notable negative allometry in dorsal view is seen for both species in the width of the ear capsule (landmarks 4-7, $k = 0.77$ in *Epicrionops bicolor;* $k = 0.59$ in *Dermophis mexicanus*) and the length of the parietal along the midline (landmarks 3-4, $k = 0.79$ in *E. bicolor* and $k = 0.62$ in *D. mexicanus*). In *D. mexicanus*, however, the statistical support for *k* is weak ($r^2 = 0.63$).

Isometry is the growth pattern for the distance between the rostrolateral tip of the frontal and the rostral tip of the nasal capsule in dorsal skull shape of both species (landmarks 11-12, $k = 1.01$ in *Epicrionops bicolor*; $k = 1.08$ in *Dermophis mexicanus*). In *E. bicolor*, the distances between the mediocaudal tip of the parietal and the caudal tip of the exoccipital (landmarks 4-5, $k = 0.94$), between the rostral tip of the ear capsule and the rostrolateral tip of the parietal (landmarks 8-9, $k = 0.97$), between the dorsocaudal tip of the maxillary and the rostrolateral tip of the frontal (landmarks 10-11, $k = 0.96$) grow isometrically.

In lateral view the highest values for *k* are seen in the length of the nasal bone in both species (landmarks 1-2, $k = 1.49$ in *E. bicolor*; $k = 1.81$ in *D. mexicanus*).

Negative allometry in lateral views seen for growth of the length of the parietal in both species (landmarks 3-4, $k = 0.70$ in *E. bicolor*; $k = 0.68$ in *D. mexicanus*). The distance between the caudal tip of the nasal and the ventrocaudal tip of the premaxillary scales with negative allometry in *D. mexicanus* (landmarks 2-8, $k = 0.74$).


A
2 mm
1.17
1.01
0.91
0.68
0.96
1.97
1.30
0.72
0.97
0.79
0.92
0.77
1.31
0.94
0.60
Epicrionops bicolor
1 mm
0.79
1.08
2.06
1.14
1.44
0.61
1.77
0.83
0.11
0.62
0.58
0.59
0.32
0.95
1.06
Dermophis mexicanus
Epicrionops bicolor
2 mm
0.70
1.19
0.97
1.49
0.95
0.52
0.53
1.27
1.47
0.49
1.18
0.50
B
Dermophis mexicanus
1 mm
0.53
0.68
1.81
0.74
0.46
0.52
-0.12
1.20
1.42
0.83
1.37
0.49

Isometric growth in lateral skull shape is seen in *Epicrionops bicolor* for the height of the skull, at the center (landmarks 3-7, k = 0.95) and caudal (landmarks 4-5, k = 0.97) measurements.

In *D. mexicanus*, the distance between the caudal tip of the maxillopalatine and the jaw articulation at the quadrate scales with positive allometry (landmarks 7-6, k = 1.37), the distance of the jaw articulation to the caudal tip of the os basale scales with negative allometry in *E. bicolor* (landmarks 6-5, k = 0.52). This indicates a caudal shift of the jaw articulation (landmark 6) in both species.

DISCUSSION

Epicrionops bicolor and *Dermophis mexicanus* show allometric growth during ontogeny, i.e. the shape of the specimens alters with size. Among amphibians, allometry was identified by using landmark data (i.e. geometric morphometrics) for salamander larvae (Djorovic and Kalezic, 1996) and frog tadpoles (Larson, 2002, 2005) and seems to be a general pattern of amphibian development. In accordance with myresults, Lessa and Wake (1992) previously reported a high degree of allometry in *Dermophis mexicanus*.

However, both species examined herein show notable differences in the degree of allometry and in the way bones are developed when the animal and its skull growths. In the viviparous *Dermophis mexicanus*, changes in shape during ontogeny are more evident than

Figure 5. Allometric growth between landmarks calculated for linear morphometric measurements of *Epicrionops bicolor* and *Dermophis mexicanus*. **A:** dorsal and **B:** lateral view. Numbers are growth coefficients k; k-values that are based on a regression with $r^2 > 0.8$ are in bold face. In dorsal view *E. bicolor* and *D. mexicanus* differ notably in growth patterns of the nasal capsule (dorsal landmarks 1 - 2; positive allometry in *E. bicolor*, negative allometry in *D. mexicanus*). In lateral view the most notable difference in growth of the two species is widening (positive allometry) of the rostral part of the skull (lateral landmarks 2 - 8) in *E. bicolor* and a narrowing (negative allometry) of the same region in *D. mexicanus*.

during larval development in *Epicrionops bicolor*. The grids in the multivariate regression analysis are deformed to a higher degree in *D. mexicanus* than in *E. bicolor* (figure 4) and linear morphometric analysis revealed that *D. mexicanus* has more structures that grow allometrically compared to *E. bicolor* (figure 5, table 4).

I suggest that the differences in the degree of allometry between both species are linked to the modes of reproduction (i.e. oviparity with larvae and metamorphosis versus viviparity). The development of the viviparous *Dermophis mexicanus* is not strictly divided into a larval and an adult stage and can be considered to be continuous. The continuous development of viviparous caecilians demands a smooth transition from a cranium that is used for intrauterine feeding to the cranium of a fossorial terrestrial predator, i.e. the adult condition. The smooth transition of the cranial morphology for different demands (intrauterine rasping vs. digging and biting) in viviparous species might explain the more substantial ontogenetic shape changes in *D. mexicanus* fetal development compared to *Epicrionops bicolor*. Larval stages of *E. bicolor* show only little allometry and the adult skull shape is formed abruptly at metamorphosis. During larval development of *E. bicolor*, the skull shows only little allometry prior to the onset of metamorphosis, which I propose is linked to the functional demands on the skull that remain constant (aquatic feeding).

For frog development it was proposed that the ontogenetic trajectory is divided by metamorphosis to a larval and an adult section (Harris, 1999); the overall ontogenetic trajectory therefore is non-linear. The slopes of the trajectory depend on the life-history stage. When considering metamorphic and adult specimens of *E. bicolor*, I expect to see similarities to the ontogenetic trajectories of frog tadpoles. The segmentation of an ontogenetic trajectory in distinct life-history stages (i.e. larva and adult) can weaken the functional constraints that larval morphology puts on adult structures and this is suggested to be closely related to the

evolution of specializations in the adult. The impact of functional and developmental constraints on adult morphology in amphibians was previously discussed by Wake (1982).

The most obvious differences in the ontogenetic trajectories of *Epicrionops bicolor* and *Dermophis mexicanus* are (1) the growth of the nasal capsule and (2) growth of tooth bearing bones. Both differences can be explained by heterochrony, i.e. changes in the timing of developmental events (Gould, 1977).

The substantial outgrowth of the nasal capsule during development is one major cause of allometry in *Dermophis mexicanus.* The skull becomes proportionally elongated by positive allometric growth of the nasopremaxillary. The same pattern was observed by Lessa and Wake (1992). In *Epicrionops bicolor* the shape of the nasal capsule shows only little ontogenetic alteration – the nasal capsule in young larvae is similar to the nasal capsule in older specimens and the elongation of the skull over ontogeny is mainly due to positive allometric growth of the frontal (figures 4 and 5).

The differences in the development of the nasal capsule relative to the remainder of the cranium can be related to the ecology of these animals. Amphibians are known to use their olfactory sense in aquatic and terrestrial habitats; olfaction plays a major role in the detection of prey items in aquatic amphibians (Jørgensen, 2000). Although there are no data available on olfaction in larval caecilians, I expect free living aquatic larvae of *E. bicolor* to rely on olfaction for the search for prey items. I suggest that the necessity of the olfactory sense in caecilian larvae demands nasal structures that are fully functional at hatching. For this purpose, the nasal capsule in *E. bicolor* has to be developed in the embryo before hatching, i.e. the onset of feeding. Thus there are no evident changes in the structure of the nasal capsule during (post-hatching) larval development. Fetuses of *D. mexicanus* develop in utero; they live in a constant and protected environment and they have no need to search

actively for prey. There is no pressure to develop a functional nasal region at the onset of feeding in viviparous species; the nasal capsule grows after the onset of feeding during fetal development. This can be considered to be a heterochronic shift of nasal capsule development from the embryo in basal caecilian taxa to the fetus in more derived clades of the Gymnophiona.

The second major difference between the ontogenetic trajectories of *Epicrionops bicolor* and *Dermophis mexicanus* is the development of the premaxillary and maxillary (figures 4 and 5). Both bones bear teeth in caecilians. Tooth bearing bones are developed substantially in early developmental stages of *D. mexicanus*. The decrease in the height of the lateral face of the skull in *D. mexicanus* is due to negative allometry of the tooth bearing bones. *E. bicolor* shows only little ontogenetic shape change in tooth bearing elements. Viviparous caecilians are known to have the smallest amounts of yolk in their eggs compared to oviparous species (Wake, 1977b, 1993; Exbrayat, 2006) and thus are likely to start (intrauterine) nutrition earlier in development. The early onset of feeding explains the accelerated development of feeding structures (i.e. teeth and tooth bearing bones) in the viviparous *D. mexicanus*. It has been previously shown that mobility has an impact on the way skeletal structures form (Morris and Gaudin, 1982; Amprino, 1985; Hall, 1986) – the early onset of feeding in viviparous caecilians is presumed to accelerate the ossification of bones of the feeding apparatus.

A striking similarity exists between caecilians and marsupial mammals. In marsupials, structures of the feeding apparatus, like the tongue and bones of the feeding apparatus, are accelerated in their development, compared to the same structures in placental mammals due to the earlier onset of feeding in marsupials (Clark and Smith, 1993; Smith, 1997, 2001, 2006). In marsupials, most changes in developmental timing affect the

development of the somatic head structures relative to the nervous system (Smith, 1997). Although, nervous system development was not in the focus of this paper, I suggest that the relatively large size of tooth bearing bones and thus the relatively small size of the brain case in fetuses of viviparous caecilians can be related to a delay of nervous system development compared to cranial bone development.

Multivariate regression revealed that in both species the jaw articulation is shifted to the posterior along the long axis of the skull as the animal grows (figure 4B). This was mentioned previously by Reiss (1996) for *Epicrionops bicolor* palate development. A posterior shift of the jaw articulation also occurs in frog development (de Jongh, 1967; Hanken and Summers, 1988) and Reiss (2002) considered the anterior position of the jaw joint a '*new larval*' character for the Lissamphibia in general. The presence of the posterior shift in the viviparous *Dermophis mexicanus* suggests that this general pattern in amphibian development is independent of the feeding mode during early ontogeny. This is surprising because a shift in the jaw articulation along the long axis of the skull will have an impact on the lever-arm ratios of the jaw and thus on the function of the feeding apparatus.

Epicrionops bicolor and *Dermophis mexicanus* differ significantly in the shape space they occupy during ontogeny (figure 2). In dorsal and lateral views the highest amount of variation within the data (PC1) is due to interspecific shape differences. The skulls of *E. bicolor* specimens are narrower and more flattened at all stages of larval development considered herein than the skulls of *D. mexicanus*. Based on the assumption that evolutionary novelty can be detected as a non-overlap of regions in the shape spaces between ancestral and derived ontogenies (Nehm, 2001), the development of *D. mexicanus* seems to be an example of novelty. However, I rather interpret the differences in the occupation of shape space as an example for adultation, i.e. the acceleration of development of adult features relative to larval

characters (Jägersten, 1972; Jeffery and Swalla, 1992) and not as novelty. As I have shown in previous chapters of this thesis, viviparous species develop adult-like cranial muscle characters in early embryology; species that have a larval stage develop adult characters not before metamorphosis. Thus, the '*novelty*' of *D. mexicanus* development is based on the formation of adult-like characters in embryology that do not appear before metamorphosis in *E. bicolor*. With a more complete sampling that also includes metamorphic and adult specimens of *E. bicolor*, larvae of other species and more specimens of direct developing and viviparous caecilians I expect to see an overlap of the shape spaces that different caecilian species occupy during development. This will test the idea that the evolution of caecilian development progresses by means of heterochrony.

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Chapter 4

Feeding biomechanics during caecilian development: functional consequences for suction feeding, scraping, and biting

ABSTRACT: Caecilians have an unique dual jaw closing system in that jaw closure is driven by the ancestral jaw closing muscles (mm. levatores mandibulae) plus a secondarily recruited hyobranchial muscle (m. interhyoideus posterior). The quadrate-squamosal complex in caecilians was shown to be kinetic (streptostyly); the attachment of the squamosal to the remainder skull must be understood as an additional joint in the caecilian jaw closing system. The dual jaw closing system and streptostyly have been shown to increase the biting performance in fossorial adult caecilians. Although all adult caecilians use biting for prey capture, there are different feeding habits (suction feeding; skin feeding; intrauterine scraping) in different phases of caecilian ontogeny that correlate to reproductive modes. This study examines the cranial biomechanics of caecilians in the suction feeding larva of *Ichthyophis* cf. *kohtaoensis*, in the embryo and juvenile of the skin feeding *Boulengerula taitana*, and in a newborn of the intrauterine feeder *Typhlonectes natans*. I applied a lever arm model to calculate effective mechanical advantages of jaw closing muscles over gape angles and to predict total bite force in developing caecilians. The comparison of Embryo and juvenile of *B. taitana* and larva and adult of *I.* cf. *kohtaoensis* revealed differences in the jaw closing mechanics during caecilian ontogeny. Gape angles above which the caecilian jaw closing system becomes destabilized increase with age, which results in a limitation of maximum gape angle in subadult specimens. Force transmission (effective mechanical advantages) and interaction of the two jaw closing systems over gape angle in larval *I.* cf.

kohtaoensis are notably different to any other caecilian studied so far, which supposedly is due to suction feeding. The skin feeding juvenile of *B. taitana* and the neonate *T. natans* are very similar to adult caecilians in the feeding parameters considered herein.

INTRODUCTION

Caecilians (Gymnophiona) are the only vertebrates that have two sets of jaw closing muscles that are integrated in function (Nussbaum, 1983). This so called dual jaw closing mechanism consists of the ancestral mm. levatores mandibulae and the additionally recruited m. interhyoideus posterior (Bemis et al., 1983; Nussbaum, 1983). The mm. levatores mandibulae act as third class levers by inserting on the dorsal edge of the lower jaw rostral to the mandibular joint; the m. interhyoideus posterior is a first class lever that inserts on the ventral side of a prolonged caudal process of the lower jaw, the processus retroarticularis (Lawson, 1965; Nussbaum, 1977, 1983; Bemis et al., 1983; Wilkinson and Nussbaum, 1997; Müller et al., 2009). The evolution of the unique dual jaw closing mechanism was proposed to be related to the fossorial lifestyle of caecilians and the compact skull architecture. Nussbaum (1983) argued that the compactness of the skull restricted the space for the mm. levatores mandibulae and thus necessitated an accessory jaw closing system.

Despite its compactness, the skull in caecilians is known to be kinetic; the quadrate can be slightly rotated (streptostyly) (Luther, 1914; Edgeworth, 1925; Marcus et al., 1933; De Villiers, 1936; Wake and Hanken, 1982; Iordanski, 1990, 2000). By using a lever arm modeling approach, Summers and Wake (2005) showed that streptostyly in caecilians is related to the jaw closing function of the m. interhyoideus posterior. Streptostyly increases the contribution of the m. interhyoideus posterior to bite force at small gape angles. The lever arm model by Summers and Wake (2005) was modified by Kleinteich et al. (2008) to account for different fiber orientations of the m. interhyoideus posterior and to also include the mm. levatores mandibulae. It turned out, that streptostyly amplifies the force that is generated by the mm. levatores mandibulae. Kleinteich et al. (2008) demonstrated that the

mm. levatores mandibulae will tend to dislocate the jaw, if the gape angle exceeds some critical value. This dislocation is compensated by the m. interhyoideus posterior. Streptostyly and the function of the m. interhyoideus posterior also have an impact on the shape of the mandibular joint; the axis of rotation has an oblique orientation and the condyle of the joint is entirely flanked by the fossa (Kleinteich et al., 2008).

Although most adult caecilians are fossorial terrestrial predators, there is a high diversity of feeding habits during ontogeny, depending on the mode of reproduction. Reproductive modes in caecilians are: oviparity with larvae and metamorphosis, oviparity with direct development, and viviparity (Wake, 1977a, 1993). Larvae of oviparous caecilians feed in aquatic habitats (Himstedt, 1991) and are supposed to use suction for prey capture (O'Reilly, 2000; O'Reilly et al., 2002). In two direct developing oviparous species, an unique mode of juvenile feeding was reported. Juveniles in the species *Boulengerula taitana* and *Siphonops annulatus* have been found to feed on the skin of their mothers until they switch to the diet of adults (Kupfer et al., 2006; Wilkinson et al., 2008). Wilkinson et al. (2008) argued that skin feeding might be a general feeding mode in direct developing caecilians. Viviparous caecilians have prenatal intrauterine feeding by rasping the uterus epithelium (Parker, 1956; Wake, 1976, 1977b). Both, skin feeding direct developing and viviparous caecilians are known to have a specialized teeth that differ from the adult dentition (Parker, 1956; Parker and Dunn, 1964; Wake, 1980; Wilkinson and Nussbaum, 1998; Kupfer et al., 2006). Differences in feeding modes and morphology that depend on ontogeny are likely to have functional consequences on the skull in larval, fetal or juvenile caecilians.

In this study, I apply the lever arm model that was developed by Kleinteich et al. (2008) for adult caecilians to caecilian embryos, larvae, and juveniles. Morphometrical values, including physiological cross sectional area of muscles as estimates for bite forces

were derived from high resolution synchrotron based x-ray CT data. The results of bite force modeling are directly compared with the data published by Kleinteich et al. (2008) for adult caecilians. The aims of this study are: (1) to test the hypothesis that effective mechanical advantages of muscles over different gape angles alter with age and size in caecilians and (2) to investigate, whether there are differences in the function of the caecilian jaw closing mechanism in suction feeding larvae of *Ichthyophis* cf. *kohtaoensis*, in embryos and juveniles of the skin feeding direct developing species *Boulengerula taitana*, and in juveniles of the viviparous species *Typhlonectes natans* that are generalist predators.

MATERIALS AND METHODS

Specimens used for this study

Table 1 contains a list of specimens that were examined herein. Specimens are stored in the collection of the Zoological Museum Hamburg (ZMH) or were provided by Mark Wilkinson (MW; Natural History Museum London).

The taxonomy of the species *Ichthyophis kohtaoensis* is highly debated. This species was originally described to be endemic to Koh Tao Island in Thailand (Taylor, 1960). However, the specimen studied herein was collected in July 1995 by Werner Himstedt at Ban Na Sabaeng in the Khemmerat district (Ubon Ratchathani province, North-eastern Thailand). It is unclear, if the mainland populations are a different species than *I. kohtaoensis* (pers. comm. Alexander Kupfer, Jena). To account for the vague taxonomic status of the specimen studied herein, I will use the name *Ichthyophis* cf. *kohtaoensis* throughout this chapter.

For species names I follow the taxonomy in Frost (2009). Although taxonomy discussions are out of focus of this paper, it shall be noted that the name *Boulengerula taitana* is valid based on Frost (2009); the often used name *Boulengerula taitanus* (e.g.

Table 1. Specimens used in this study including energies and voxel sizes for SRμCT imaging.

ID	Species	TL [mm]	LH Stage	Energy [keV]	Voxelsize [μm]
MW03877	*Boulengerula taitana*	20	Embryo	9	2.3
MW03912	*Boulengerula taitana*	49	Juvenile	9	2.0
ZMH A08978	*Ichthyophis* cf. *kohtaoensis*	69	Larva	9	2.9
ZMH A04346	*Typhlonectes natans*	64	Juvenile	19	3.9

Nussbaum and Hinkel, 1994; Kupfer et al., 2006; Gaborieau and Measey, 2004; Measey and Herrel, 2006) is a synonym. A discussion on the valid species name, was presented by Marjanovic and Laurin (2008).

Specimen ZMH A04346 is a 64 mm juvenile *Typhlonectes natans* that, according to its label, was fixed immediately after birth. This animal is surprisingly small for a newborn *Typhlonectes*. Juveniles in the closely related species *Typhlonectes compressicauda* have been reported to be about 100 mm (Moodie, 1978; Exbrayat and Delsol, 1985; Wake et al., 1985) to up to 200 mm (Wake, 1977a). However, there is high intraspecific variation in the size at birth (95 - 200 mm) and the small size might be due to interspecific size differences between *T. compressicauda* and *T. natans*. Despite its small size, the external gills that typically drop off at birth in the genus *Typhlonectes* (Exbrayat, 2006) have been absent in the specimen so that there is clear evidence that this specimen indeed is a juvenile.

High resolution synchrotron radiation based x-ray μCT imaging

The specimens examined were decapitated and freeze dried prior to CT imaging. Freeze drying followed the procedure described by Meryman (1960, 1961). For freezing, the samples were exposed to -80 °C for 3 hours. Vacuum drying was performed with a Lyovac® GT2 freeze drying system. The samples were kept for 24 hours in the vacuum chamber. Freeze drying is known to increase the contrast within tissues for x-ray based imaging

methods (Follett, 1968) and shrinkage artifacts are rather minor, compared to other drying techniques (Boyde, 1978).

High resolution synchrotron radiation based x-ray micro computed tomography (SRμCT) was performed at beamline BW2 of the DORIS III storage ring at the German Electron Synchrotron (DESY) Hamburg. The SRμCT setup was operated by the GKSS research center Geesthacht. The setup of the SRμCT facility was published previously by Beckmann et al. (2006). The samples were penetrated by the x-ray beam. X-ray radiation was converted to visible light by a fluorescent screen that is positioned behind the sample in the SRμCT setup. Images on the fluorescent screen (CdWO4 single crystal, 500μm thick) were magnified by a lens (Nikkor, focal length 35 or 50 mm) onto a CCD camera (KX2, Apogee Instruments, Inc.; 14 bit digitalization at 1.25 MHz). In total, 720 images were captured over 180° in 0.5° increments. For every 8th step, the sample was moved out of the x-ray beam and a reference image was captured. Absorption images were calculated by subtraction of the reference images from the images with sample.

Parameters for SRμCT imaging are listed in table 1. Energies ranged in between 9 and 19 keV. Voxels of the resulting volume datasets were isometric with edge lengths in between 2.0 μm and 3.9 μm. The size of the raw datasets was reduced by merging neighboring voxels (binning) to increase the accessibility of the data; measurements herein were taken from 3 fold binned datasets.

Lever arm modeling

The lever arm model herein is identical to the model by Kleinteich et al. (2008) (fig. 1). All calculations were performed with the open source software package for numerical computations Scilab 4.1.2. Gape angles were simulated to range in between 0° to 90°. The

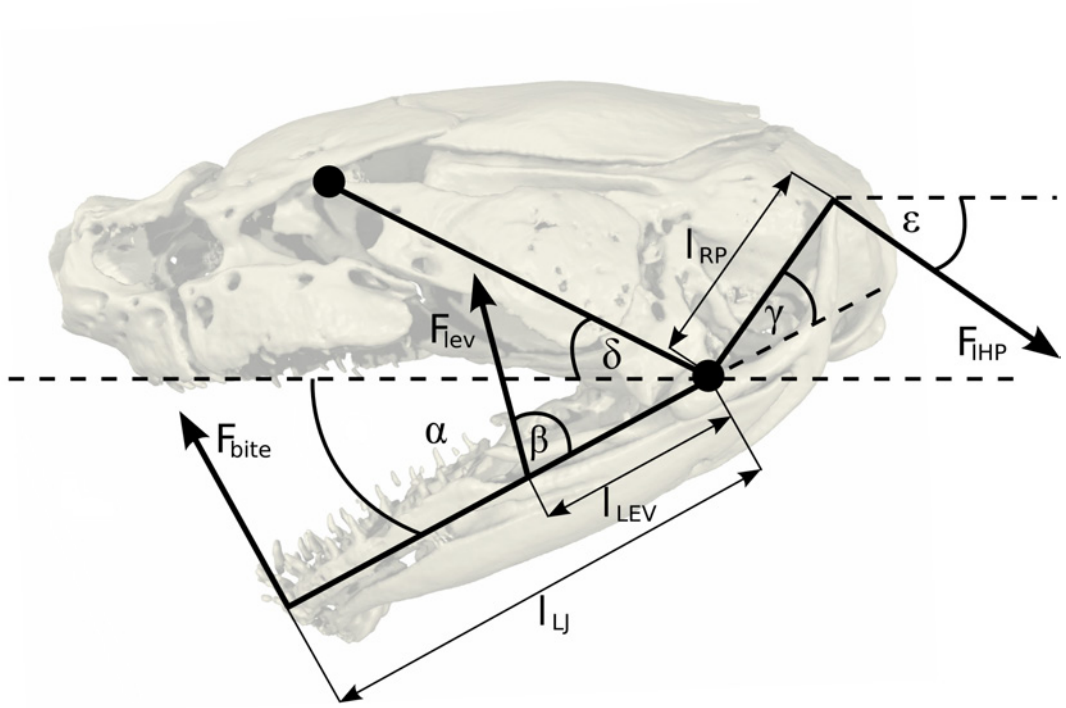

Figure 1. Lever arm model to calculate effective mechanical advantages and bite forces (F_{bite}) over gape angles (α) in caecilians (Kleinteich et al., 2008) drawn over the skull of *Typhlonectes natans* specimen ZMH A04346 in lateral view. Gape angles range from 0° to 90°. In caecilians, two jaw closing mechanisms are integrated: (1) the mm. levatores mandibulae with the fiber angle β, the lever arm l_{LEV}, and force F_{LEV} and (2) the m. interhyoideus posterior with fiber orientation ε, lever arm lRP, and force F_{IHP}. The lever arm model also accounts for two anatomical measurements of the caecilian skull: the angle of the quadrate-squamosal complex (δ) and the angle of the retroarticular process of the lower jaw with a line drawn from the lower jaw joint to the tip of the lower jaw (γ).

ratio of inlever length, to outlever length is called mechanical advantage (MA). For the m. interhyoideus posterior, the mechanical advantage is calculated by:

(1) $MA_{IHP} = l_{RP} / l_{LJ}$

With MA_{IHP} = mechanical advantage of the m. interhyoideus posterior, l_{RP} = length of the retroarticular process of the lower jaw, and l_{LJ} = distance from the rostral tip of the lower jaw to the jaw articulation.

The ratio of output force to force generated by a muscle (input force) is called effective mechanical advantage (EMA) herein (based on Biewener, 1989; Westneat, 2003). EMA for the m. interhyoideus posterior is calculated by:

(2) $EMA_{IHP} = F_{bite} / F_{IHP} = \sin(\alpha + \gamma + \varepsilon) * MA_{IHP} + \sin(\delta - \varepsilon) * \cos(\alpha + \delta)$

With F_{bite} = output bite force, F_{IHP} = force generated by the m. interhyoideus posterior, MA_{IHP} = mechanical advantage of the m. interhyoideus posterior, α = gape angle, γ = retroarticular angle with respect to the anteroposterior axis, δ = quadrate-squamosal angle with respect to the anteroposterior axis, and ε = muscle fiber orientation of the *m. interhyoideus posterior* with respect to the anteroposterior axis.

The MA and EMA for the mm. levatores mandibulae are calculated by:

(3) $MA_{LEV} = l_{LEV} / l_{LJ}$

(4) $EMA_{LEV} = F_{bite} / F_{LEV} = \sin(\beta - \alpha) * MA_{LEV} + \sin(\beta + \delta) * \cos(\alpha + \delta)$

With MA_{LEV} = mechanical advantage of the mm. levatores mandibulae, l_{LEV} = distance from the insertion of the muscles to the jaw articulation, l_{LJ} = distance from the rostral tip of the lower jaw to the jaw articulation, F_{bite} = output bite force, F_{LEV} = force generated by muscles of the *mm. levatores mandibulae* group, α = gape angle, β = muscle fiber orientation of the muscles with respect to the anteroposterior axis, and δ = quadrate-squamosal angle with respect to the anteroposterior axis.

Theoretical forces that can be generated by a single muscle were calculated by:

(5) $F_{muscle} = (V / l) * p_{MIS}$

Where F_{muscle}= force generated by a muscle, V = the volume of the muscle, l = length of the muscle in the direction of the fiber orientation, and p_{MIS} = the maximal isometric stress (p_{MIS} = 250 kPa; Herzog, 1995). The ratio of muscle volume to muscle length is a measure for physiological cross-sectional area (PCSA).

Total bite force for the entire jaw closing system (i.e. both jaw closing systems and both sides of the skull) is calculated as the doubled sum of bite forces from single muscles, assuming bilateral symmetry.

The jaw closing musculature of caecilians comprises three mm. levatores mandibulae: m. levator mandibulae longus, m. levator mandibulae internus, and m. levator mandibulae articularis (Luther, 1914; Edgeworth, 1935; Lawson, 1965). In larvae of *Ichthyophis* cf. *kohtaoensis* there is a fourth muscle, the m. levator mandibulae externus. This muscle, however, is incompletely separated from the m. levator mandibulae longus (Haas, 2001; Kleinteich and Haas, 2007). In this study, I did not separate between the two muscles; measurements of muscle volume and fiber orientation are done for the entire muscle unit of the m. levator mandibulae longus plus externus. Two other muscles in the jaw closing system of caecilians are the m. levator quadrati and the m. pterygoideus (Luther, 1914; Edgeworth, 1935; Iordanski, 1996; Kleinteich and Haas, 2007). Both muscles insert on the quadrate and are not directly involved in jaw movements and their role in the jaw closing system of caecilians can not be examined with the lever arm model applied in this study. The m. levator quadrati and the m. pterygoideus are not considered herein.

In the 20 mm specimen of *Boulengerula taitana*, the m. levator mandibulae internus could not be identified (chapter 2). Thus, there are no measurements for this muscle in this specimen available.

Angle, volume, and length measurements

Measurement of muscle fiber angles followed the procedure described in Kleinteich et al. (2008). All measurements were taken for both sides of the body and the mean values of both sides were used for calculations. Stacks of images of virtual sections through the muscles parallel to the muscle fiber orientation were captured with the *Oblique Slices Tool* in Amira® 4.1 (Mercury Computer Systems). The resulting image stacks were imported into the image editing tool ImageJ 1.41 (NIH, available at http://rsbweb.nih.gov/ij/). Areas of the images

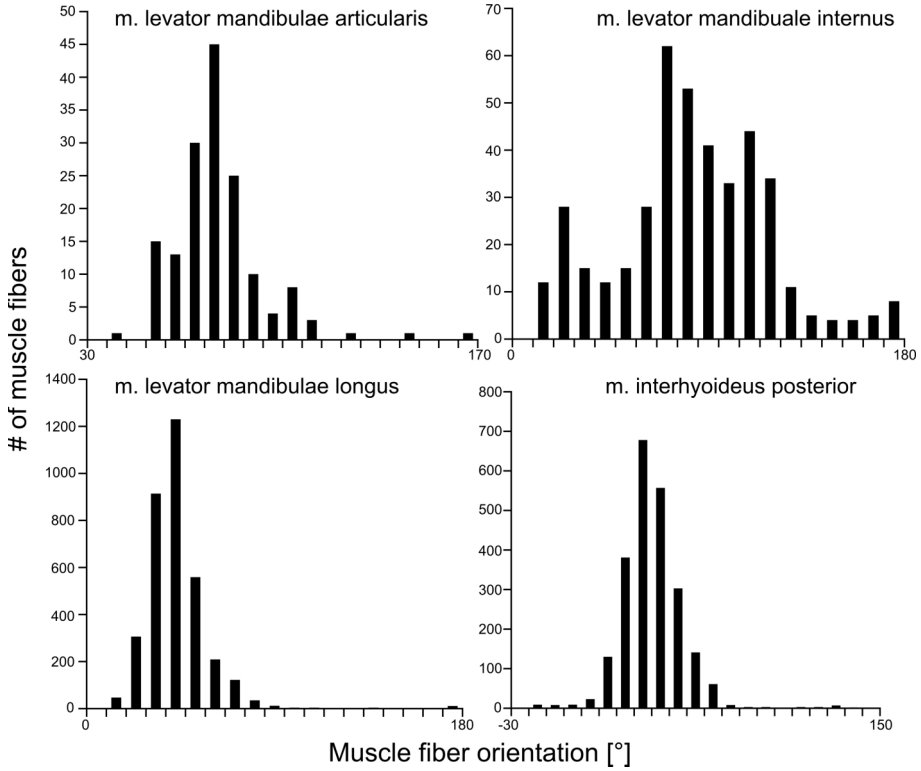

Figure 2. Frequency distributions of muscle fiber orientations using larval *Ichthyophis* cf. *kohtaoensis* (ZMH A08978) as example. Fiber orientations were measured for the m. levator mandibulae articularis, the m. levator mandibulae internus, the m. levator mandibulae longus and the m. interhyoideus posterior. Frequencies of muscle fiber angles within a muscle are distributed around a mean value. The width of the frequency distribution pattern is supposed to reflect muscle architecture. Muscles with parallel oriented fibers have a narrower distribution of fiber orientations than fan shaped muscles. Mean values and standard derivation from the mean for muscle fiber angles are presented in table 2.

that contained information on musculature were segmented by the *Polygon Selection* and *Clear Outside* functions of ImageJ. After segmentation, images of muscles were inverted and then converted into binary images with the *Adjust Threshold* tool. The threshold for conversion into binary images was adjusted in a way that muscle fibers were within the threshold (black); background structures were outside the threshold (white). The resulting binary images of muscle fibers were analyzed with the *Analyze Particles* function of ImageJ that, among other output, showed the orientation of muscle fibers as a result. For analysis, only particles that were larger than 5x5 pixels and that had a circularity (i.e. the ratio of width to height) of less than 0.3 were considered. Distribution of muscle fiber angles (fig. 2) is a

Table 2. Measurements used in calculations of effective mechanical advantages and bite forces. Standard derivation of the mean was calculated for muscle fiber angles.

		Boulengerula taitana 20 mm	*Boulengerula taitana* 49 mm	*Ichthyophis* cf. *kohtaoensis* 69 mm	*Typhlonectes natans* 64 mm
m. levator mandibulae articularis	MA	0.17	0.16	0.12	0.18
	Volume [µm³]	1.10E+06	2.68E+06	1.50E+07	2.46E+07
	length [µm]	303	321	508	847
	PCSA [µm²]	3.63E+03	8.33E+03	2.97E+04	2.90E+04
	Force [N]	0.001	0.002	0.007	0.007
	Angle β [°]	68 ± 33	81 ± 19	71 ± 17	65 ± 34
m. levator mandibulae internus	MA		0.32	0.47	0.37
	Volume [µm³]		8.25E+06	9.23E+07	6.11E+07
	length [µm]		645	938	1035
	PCSA [µm²]		1.28E+04	9.87E+04	5.90E+04
	Force [N]		0.003	0.025	0.015
	Angle β [°]		80 ± 20	78 ± 37	121 ± 28
m. levator mandibulae longus	MA	0.27	0.32	0.47	0.37
	Volume [µm³]	7.55E+06	1.71E+07	3.36E+08	3.30E+08
	length [µm]	655	770	1412	1521
	PCSA [µm²]	1.15E+04	2.23E+04	2.38E+05	2.17E+05
	Force [N]	0.003	0.006	0.060	0.054
	Angle β [°]	86 ± 38	92 ± 17	36 ± 17	84 ± 32
m.interhyoideus posterior	MA	0.34	0.52	0.50	0.49
	Volume [µm³]	1.05E+07	1.30E+08	4.98E+08	1.35E+09
	length [µm]	614	2036	2369	2514
	PCSA [µm²]	1.71E+04	6.36E+04	2.10E+05	5.36E+05
	Force [N]	0.004	0.016	0.053	0.134
	Angle ε [°]	83 ± 37	37 ± 33	34 ± 16	65 ± 43
Angle quadrate-squamosal δ [°]		75	36	54	30
Angle proc. retroarticularis γ [°]		24	15	20	32

good indicator for the quality of measurements; randomly measured particles are supposed to show no notable distribution of measured angles. Standard derivations from the mean of muscle fiber angle are an indicator of muscle architecture; parallel fibered muscles are supposed to have a narrow standard derivation, fan shaped muscles will have a wide standard derivation (fig. 2; tab. 2).

Anatomical measurements, i.e. the angles of the quadrate and the processus retroarticularis, and the lengths of the lower jaw, the processus retroarticularis, and the inlevers of the mm. levatores mandibulae were taken from volume rendered images with the open source image editing software GIMP 2.4.5.

For volume and length measurements of single muscles, I segmented each muscle interactively in Amira® 4.1 from the SRμCT data with the *brush* of the *Labels Field* function. Surfaces of the segmented muscles were calculated by Amira® 4.1 with the *SurfaceGen* module. Volumes of the resulting surfaces were analyzed with the *SurfaceArea* tool, lengths of the muscle surfaces were measured with the *LineProbe* tool of Amira® 4.1.

RESULTS

Measurements and parameters for lever arm modeling

Table 2 contains all important parameters that were measured for the specimens examined herein and used as input data for the lever arm model.

The MAs of the m. interhyoideus posterior are higher compared to the mm. levatores mandibulae in all specimens examined. Values for the MA of the m. interhyoideus posterior range from 0.34 in the 20 mm *Boulengerula taitana* specimen to 0.52 in the 49 mm *B. taitana*. MAs for the mm. levatores mandibulae are in between 0.12 (m. levator mandibulae articularis in the 69 mm *Ichthyophis* cf. *kohtaoensis* specimen) and 0.47 (mm. levatores mandibulae internus and longus in larval *I.* cf. *kohtaoensis*).

The angle of the quadrate is very similar in the 49 mm *Boulengerula taitana* (36°), and in the 64 mm (30°) specimens. The highest quadrate angle is measured in the 20 mm *B. taitana* (75°). The quadrate angle of the 69 mm larva of *Ichthyophis* cf. *kohtaoensis* is in between (54°).

Inclination of the processus retroarticularis of the lower jaw ranges from 15° (49 mm *Boulengerula taitana*) to 32° (64 mm *Typhlonectes natans*).

Muscle fiber orientations to the horizontal and standard derivations from the mean are listed in table 2. The fibers of the m. interhyoideus posterior run rather oblique in the 49 mm *Boulengerula taitana* (37°) and the 69 mm *Ichthyophis* cf. *kohtaoensis* (34°) and almost vertical in the 20 mm *B. taitana* (83°). The m. levator mandibulae articularis shows fiber orientations of 65° (64 mm *Typhlonectes natans*) up to 81° (49 mm *B. taitana*). The m. levator mandibulae internus has its smallest fiber angle in the 69 mm *I.* cf. *kohtaoensis* individual (78°); the highest value is measured in 64 mm *T. natans* (121°). The m. levator mandibulae longus ranges in its muscle fiber orientation from 36° (69 mm *I.* cf. *kohtaoensis*) to 92° (49 mm *B. taitana*).

Measurements of physiological cross sectional area and the corresponding calculated forces that can be theoretically generated by single muscles are rendered in table 2. In all specimens examined herein, the m. levator mandibulae articularis is the smallest muscle. Forces that can be generated by this muscle have been calculated to range in between 0.001 N (20 mm *Boulengerula taitana*) and 0.007 N (69 mm *Ichthyophis* cf. *kohtaoensis* and 64 mm *Typhlonectes natans*). The muscle with the highest physiological cross sectional area and highest calculated force is the m. interhyoideus posterior for all specimens, except the 69 mm *I.* cf. *kohtaoensis*. In *I.* cf. *kohtaoensis* the m. levator mandibulae longus is the most powerful muscle. The m. interhyoideus posterior in larval *I.* cf. *kohtaoensis* has a calculated force of 0.053 N; the m. levator mandibulae longus is expected to generate 0.06 N.

Effective mechanical advantages over gape angles

The relationships of effective mechanical advantages (EMA) and gape angles are shown for *Boulengerula taitana* in fig. 3, for *Ichthyophis* cf. *kohtaoensis* in fig. 4, and for *Typhlonectes*

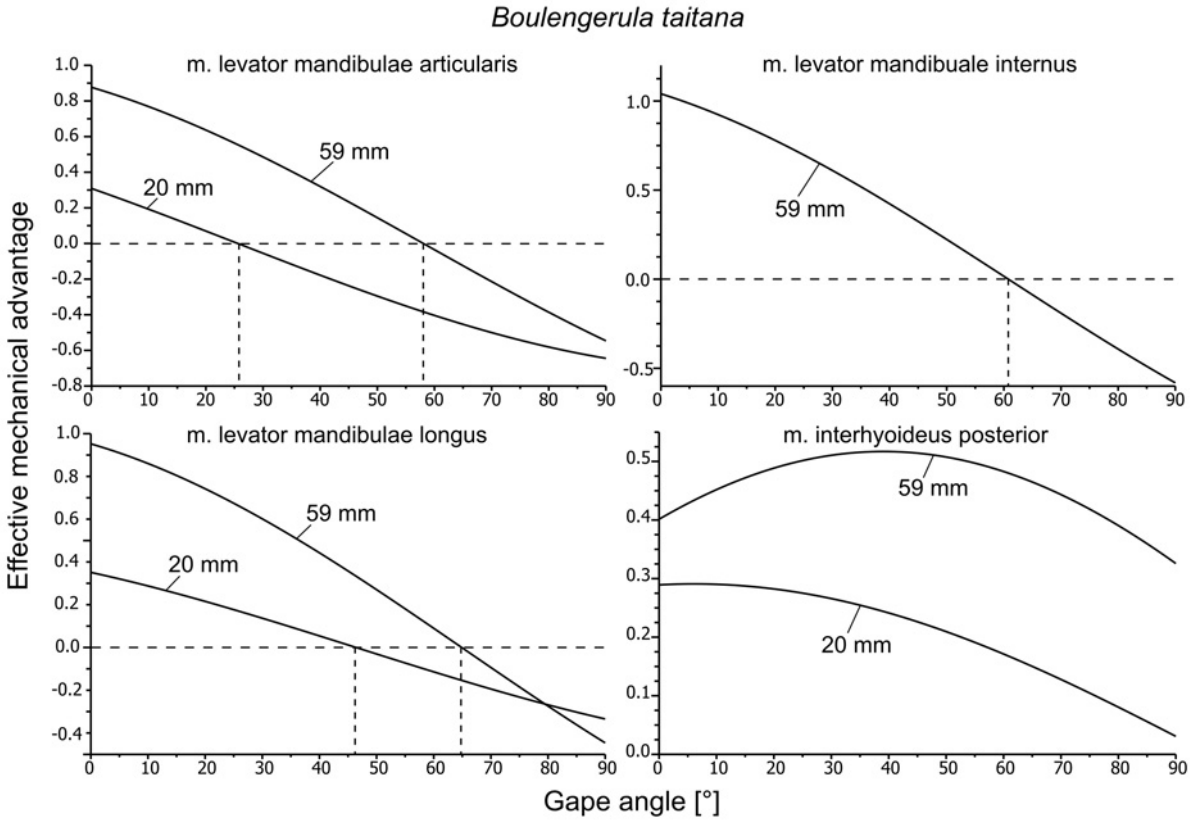

Figure 3. Effective mechanical advantages over gape angles in *Boulengerula taitana*. Graphs are drawn for the 20 mm (MW03877) and 49 mm (MW03912) specimens. Separate calculations were performed for the m. levator mandibulae articularis, the m. levator mandibulae internus, the m. levator mandibulae longus, and the m. interhyoideus posterior. Gape angles were simulated to range from 0° to 90°. The mm. levatores mandibulae show critical gape angles (indicated by dashed lines) above those, the effective mechanical advantages will have negative values. In *B. taitana*, the critical gape angles increase with age. The m. interhyoideus posterior has no critical gape angle. The gape angle at which the effective mechanical advantage is highest, increases with age.

natans in fig. 5. Maximal EMA and corresponding gape angles, as well as critical gape angles are presented in table 3.

In all specimens examined, all muscles of the mm. levatores mandibulae group have their highest EMA at a closed gape (gape angle 0°). The 20 mm *B. taitana* specimen has the lowest EMA for the mm. levatores mandibulae (0.31 for the m. levator mandibulae articularis and 0.35 for the m. levator mandibulae longus) among the specimens studied. The highest EMA is calculated for the m. levator mandibulae longus in *T. natans* (1.15). With higher gape angles, the EMA of the mm. levatores mandibulae decreases. The mm. levatores mandibulae of all specimens show a critical gape angle above which the EMA becomes

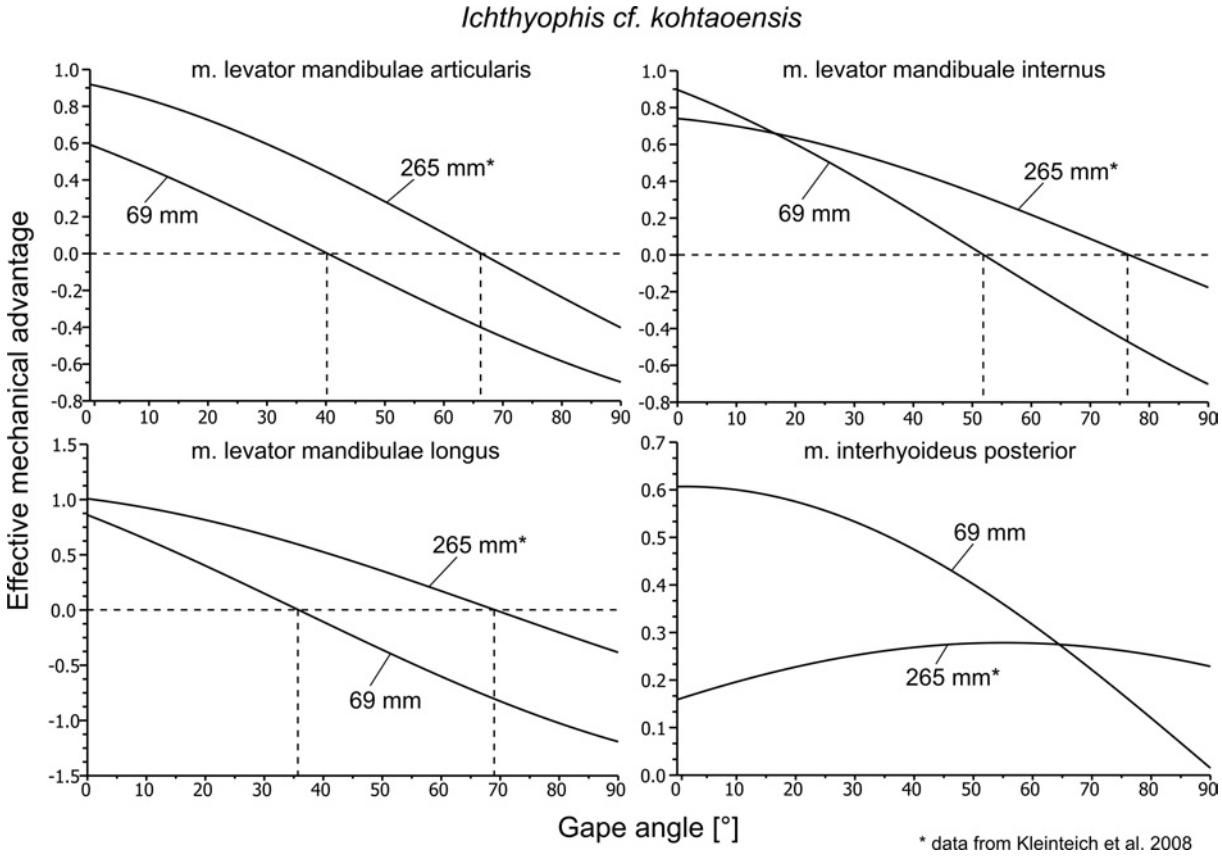

Figure 4. Effective mechanical advantages over gape angles in *Ichthyophis* cf. *kohtaoensis*. Graphs are drawn for the 69 mm specimen studied herein (ZMH A08978) and a 265 mm adult specimen (data from Kleinteich et al., 2008). Separate calculations were performed for the m. levator mandibulae articularis, the m. levator mandibulae internus, the m. levator mandibulae longus (plus externus), and the m. interhyoideus posterior. Gape angles were simulated to range from 0° to 90°. The mm. levatores mandibulae show critical gape angles (indicated by dashed lines) above those, the effective mechanical advantages will have negative values. The critical gape angles of the mm. levatores mandibulae in the adult is higher, than in the larva of *I.* cf. *kohtaoensis*. The m. interhyoideus posterior has no critical gape angle. In larval *I.* cf. *kohtaoensis*, the effective mechanical advantage of the m. interhyoideus posterior is higher, than in the adult.

negative. This critical gape angle has its lowest value for the m. levator mandibulae articularis in the 20 mm *B. taitana* (27°) and its highest value for the m. levator mandibulae internus in the 64 mm *T. natans* (87°).

The m. interhyoideus posterior has its optimal gape angle at an almost closed gape in the 20 mm *Boulengerula taitana* (6°) and the 69 mm *Ichthyophis* cf. *kohtaoensis* (1°) specimens. The EMA of the m. interhyoideus posterior decreases with increasing gape in these two specimens. In the 49 mm *B. taitana* specimen, the EMA of the m. interhyoideus

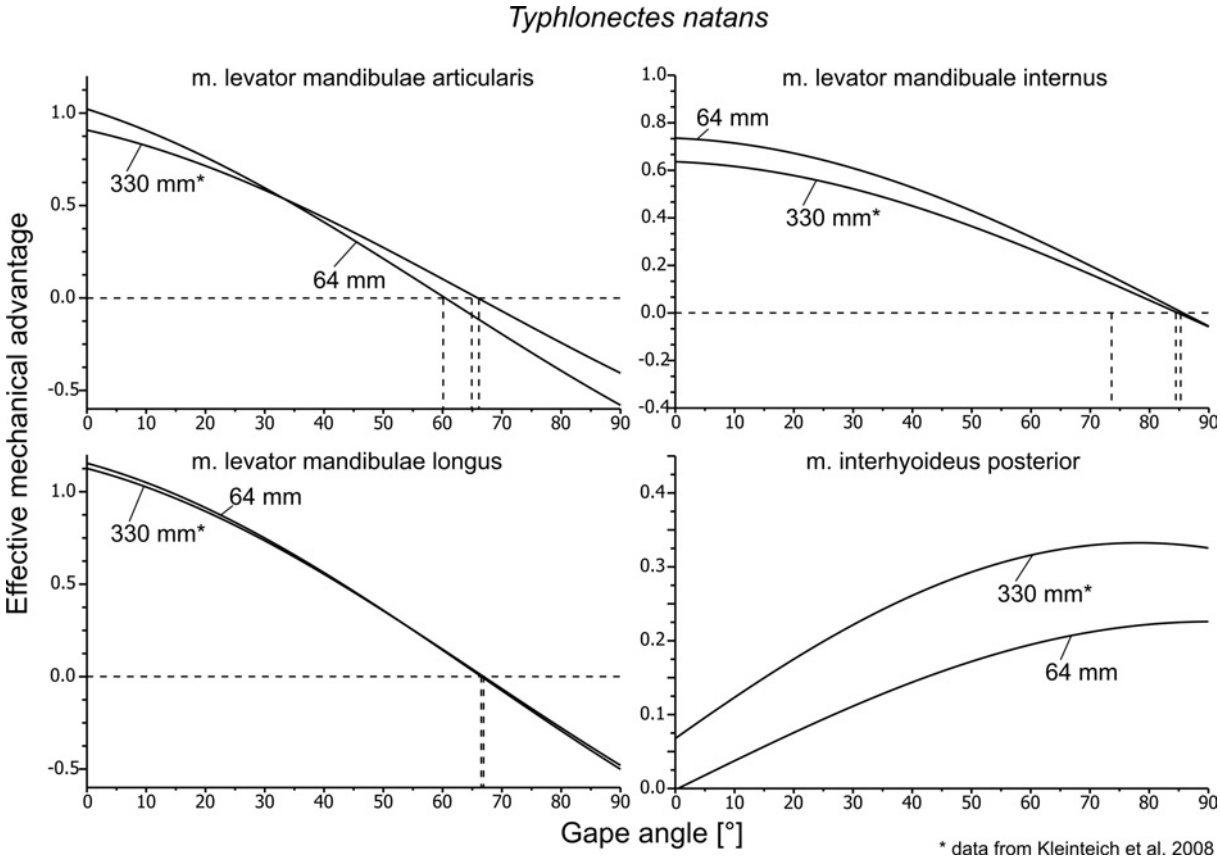

Figure 5. Effective mechanical advantages over gape angles in *Typhlonectes natans*. Graphs are drawn for the 64 mm specimen studied herein (ZMH A04346) and a 330 mm adult specimen (data from Kleinteich et al., 2008). Separate calculations were performed for the m. levator mandibulae articularis, the m. levator mandibulae internus, the m. levator mandibulae longus (plus externus), and the m. interhyoideus posterior. Gape angles were simulated to range from 0° to 90°. The mm. levatores mandibulae show critical gape angles (indicated by dashed lines) above those, the effective mechanical advantages will have negative values. Both specimens show very similar correlations of effective mechanical advantages and gape angles. For the m. levator mandibulae articularis, the critical gape angle slightly increases with age. The m. interhyoideus posterior in 64 mm *T. natans* reacts similar to different gape angles as in the adult specimen; only the values for effective mechanical advantages are different.

posterior increases with increasing gape, until it reaches an optimal gape angle of 40° above that the EMA decreases. In the 64 mm *Typhlonectes natans* specimen examined herein, the EMA of the m. interhyoideus posterior is 0 for a closed gape and increases with increasing gape angles The maximum EMA of the m. interhyoideus posterior in 64 mm *T. natans* is 0.23 at a gape angle of 90°.

Table 3. Effective mechanical advantages, bite forces and important gape angles.

		Boulengerula taitana 20 mm	*Boulengerula taitana* 49 mm	*Ichthyophis* cf. *kohtaoensis* 69 mm	*Typhlonectes natans* 64 mm
m. levator mandibulae articularis	EMA max	0.31	0.88	0.59	1.02
	Gape angle α EMA max [°]	0	0	0	0
	Force EMA max [N]	3.00E-04	0.002	0.004	0.007
	Critical gape angle α [°]	27	69	41	60
m. levator mandibulae internus	EMA max		1.04	0.9	0.74
	Gape angle α EMA max [°]		0	0	0
	Force EMA max [N]		0.003	0.022	0.011
	Critical gape angle α [°]		62	53	87
m. levator mandibulae longus	EMA max	0.35	0.95	0.86	1.15
	Gape angle α EMA max [°]	0	0	0	0
	Force EMA max [N]	0.001	0.006	0.052	0.062
	Critical gape angle α [°]	47	66	37	68
Sum mm. levatores mandibulae	**Force max [N]**	**0.001**	**0.011**	**0.078**	**0.081**
	Gape angle α Force max [°]	**0**	**0**	**0**	**0**
	Critical gape angle α [°]	**40**	**62**	**40**	**68**
m.interhyoideus posterior	EMA max	0.29	0.52	0.61	0.23
	Gape angle α EMA max [°]	6	40	1	90
	Force EMA max [N]	0.001	0.008	0.032	0.030
Total bite force	**Force max [N]**	**0.005**	**0.034**	**0.221**	**0.161**
	Gape angle α Force max [°]	**0**	**0**	**0**	**0**
	Gape angle α Force 50% [°]	**33**	**59**	**28**	**59**
	Critical gape angle α [°]	**59**	**89**	**50**	**89**

Bite force over gape angle

Figures 6, 7, and 8 show bite forces over gape angles for the species examined herein. Bite force values for each muscle, the entire mm. levatores mandibulae complex, and total bite force are given in table 3.

Total bite force, i.e. the sum of all muscles for both halves of the body, is maximal at a closed gape in all specimens considered herein. Total bite force decreases with increasing gape. In all specimens, total bite force will have negative values above a critical gape angle

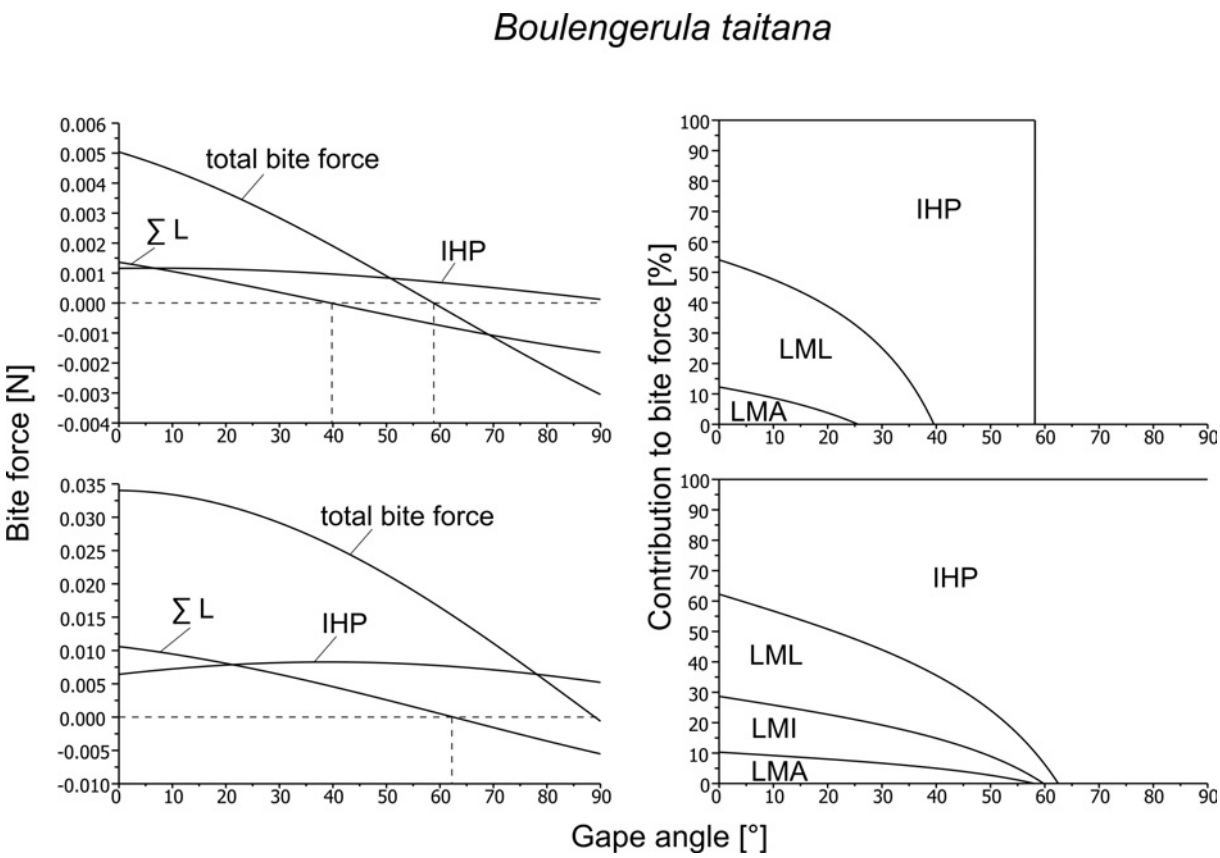

Figure 6. Bite forces (left side) and relative contribution of single jaw closing muscles to total bite force (right side) over gape angles in *Boulengerula taitana*. Upper row: *B. taitana* 20 mm (MW03877); lower row *B. taitana* 49 mm (MW03912). Bite forces over gape angles were calculated for the sum of the mm. levatores mandibulae (Σ L), the m. interhyoideus posterior (IHP), and the sum of all jaw closing muscles for both sides of the body (total bite force). Percentage of contribution to total bite force over gape angle was calculated for the m. levator mandibulae articularis (LMA), the m. levator mandibulae internus (LMI), the m. levator mandibulae longus (LML), and the m. interhyoideus posterior (IHP). With increasing gape angle, bite force decreases and the percentage contribution of the m. interhyoideus posterior increases. Dashed lines indicate critical gape angles. In the 20 mm specimen, total bite force has a critical gape angle of app. 60°; in the 49 mm specimen, three is no critical gape angle in the range of gape angles considered herein (0° - 90°).

for the entire jaw closing system. This critical gape angle for the entire system is lowest in the 20 mm *Boulengerula taitana* (59°) and the 69 mm *Ichthyophis* cf. *kohtaoensis* (50°) specimens.

In all specimens, the relative contribution of the entire mm. levatores mandibulae complex decreases with increasing gape angle; the contribution of the m. interhyoideus posterior increases. In larval *Ichthyophis* cf. *kohtaoensis*, the contribution of the m. levator

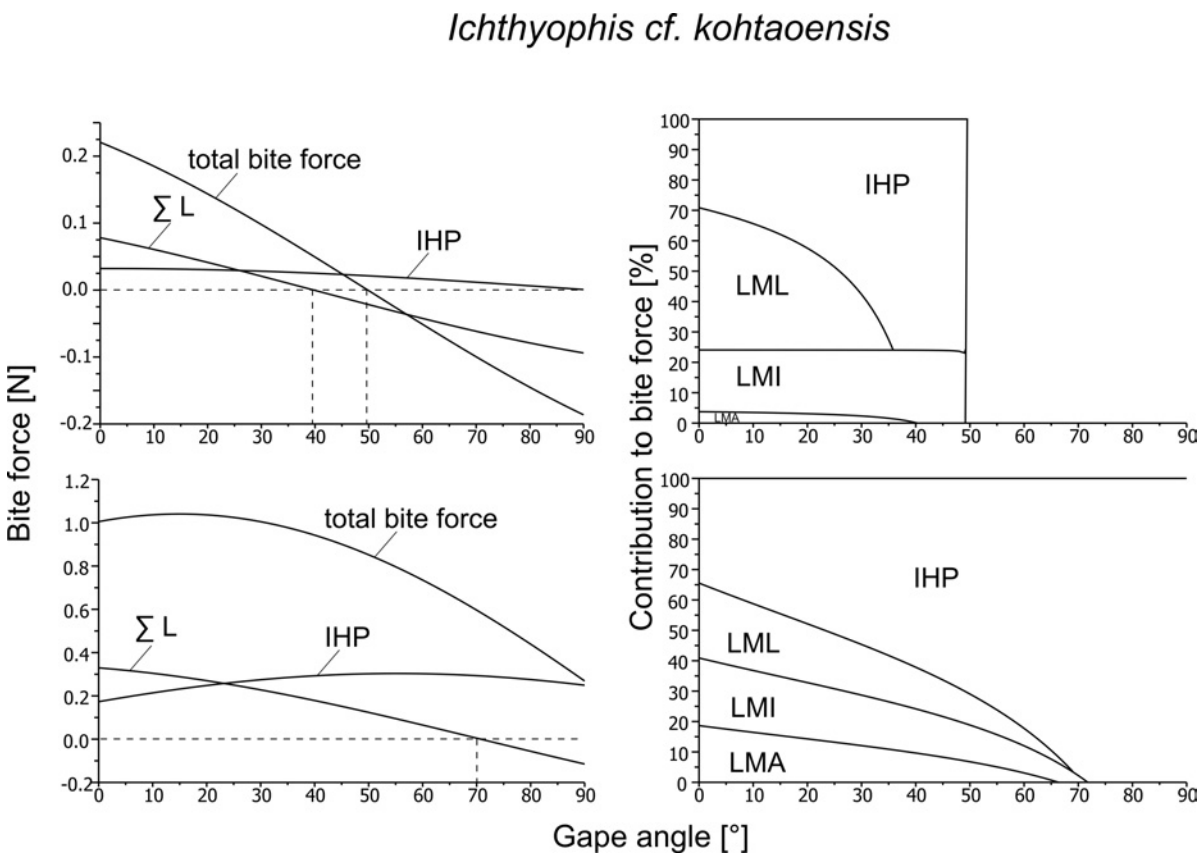

Figure 7. Bite forces (left side) and relative contribution of single jaw closing muscles to total bite force (right side) over gape angles in *Ichthyophis* cf. *kohtaoensis*. Upper row: *I*. cf. *kohtaoensis* 69 mm (ZMH A08978); lower row *I*. cf. *kohtaoensis* 265 mm (data from Kleinteich et al., 2008). Bite forces over gape angles were calculated for the sum of the mm. levatores mandibulae (Σ L), the m. interhyoideus posterior (IHP), and the sum of all jaw closing muscles for both sides of the body (total bite force). Percentage of contribution to total bite force over gape angle was calculated for the m. levator mandibulae articularis (LMA), the m. levator mandibulae internus (LMI), the m. levator mandibulae longus (LML), and the m. interhyoideus posterior (IHP). With increasing gape angle, bite force decreases and the percentage contribution of the m. interhyoideus posterior increases. In the 69 mm specimen, the relative contribution of the m. levator mandibulae internus plus the m. levator mandibulae articularis to total bite force remains almost constant (app. 25%). Dashed lines indicate critical gape angles. The 69 mm larval specimen has a critical gape angle for total bite force of app. 50°.

mandibulae longus, decreases to a higher degree than in the other muscles and the other specimens studied herein. The contribution of the mm. levatores mandibulae internus plus articularis is almost constant in larval *I*. cf. *kohtaoensis*.

The highest total bite force calculated herein is 0.221 N in the 69 mm *Ichthyophis* cf. *kohtaoensis* specimen; the lowest value was calculated with 0.005 N for the 20 mm *Boulengerula taitana*.

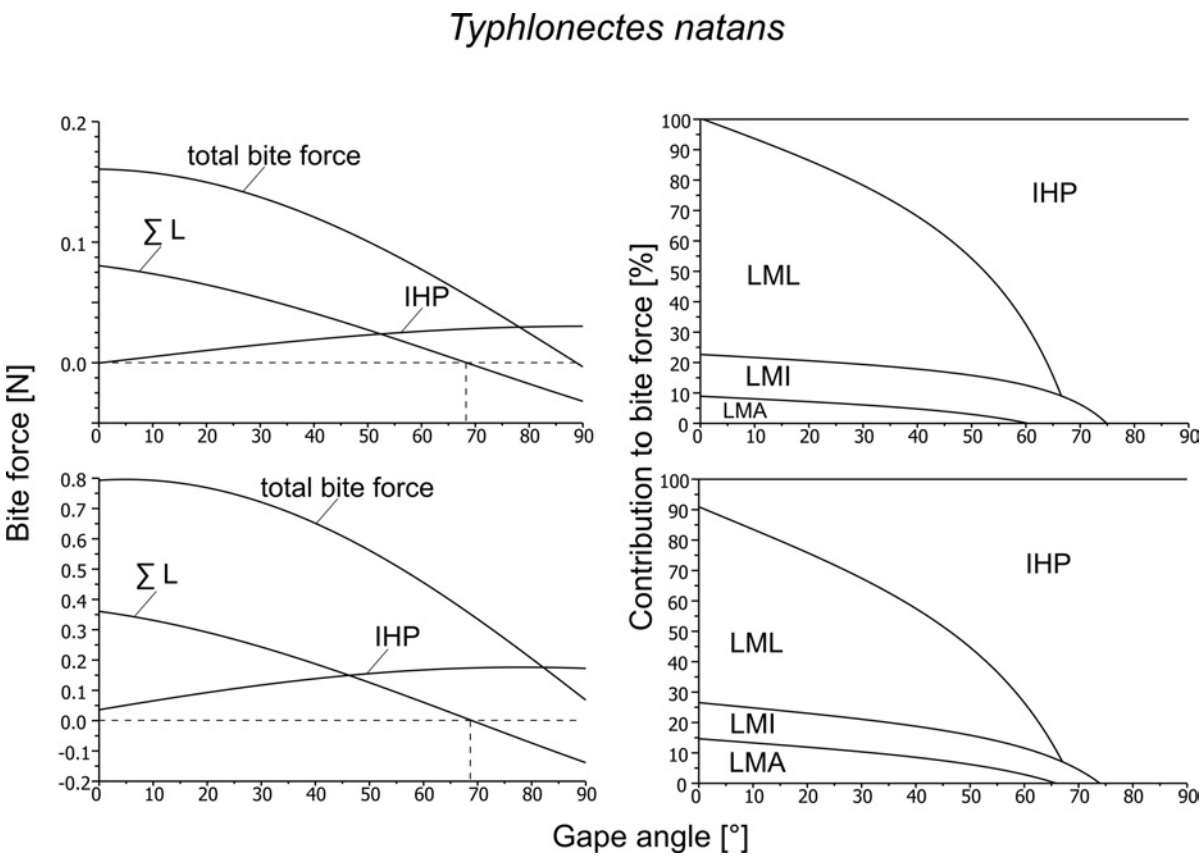

Figure 8. Bite forces (left side) and relative contribution of single jaw closing muscles to total bite force (right side) over gape angles in *Typhlonectes natans*. Upper row: *T. natans* 64 mm (ZMH A04346); lower row *T. natans* 330 mm (data from Kleinteich et al., 2008). Bite forces over gape angles were calculated for the sum of the mm. levatores mandibulae (Σ L), the m. interhyoideus posterior (IHP), and the sum of all jaw closing muscles for both sides of the body (total bite force). Percentage of contribution to total bite force over gape angle was calculated for the m. levator mandibulae articularis (LMA), the m. levator mandibulae internus (LMI), the m. levator mandibulae longus (LML), and the m. interhyoideus posterior (IHP). With increasing gape angle, bite force decreases and the percentage contribution of the m. interhyoideus posterior increases. Dashed lines indicate critical gape angles for the mm. levatores mandibulae. The 64 mm and 330 mm specimens are very similar in their bite force characteristics over different gape angles.

The specimens differ notably in the gape angles at that values for total bite force decreases to 50% of its maximum (α_{50}). In the 20 mm *Boulengerula taitana* and in the 69 mm *Ichthyophis* cf. *kohtaoensis*, the value for α_{50} is rather low, at 33° or 28°, respectively. In the 49 mm *B. taitana* and the 64 mm *Typhlonectes natans* specimens, the value for α_{50} is identical - 59°.

DISCUSSION

Jaw closing mechanics in *Boulengerula taitana* and *Ichthyophis* cf. *kohtaoensis* transform through ontogeny; the juvenile specimen of *Typhlonectes natans*, however, shows the same feeding mechanics as the adult. In both species that show transformation, the gape angle above which the mm. levatores mandibulae will tend to open the jaw, i.e. the critical gape angle (Kleinteich et al., 2008), increases with age. Different to juvenile or adult specimens (Kleinteich et al., 2008), the m. interhyoideus posterior cannot entirely compensate for jaw depression by the mm. levatores mandibulae in the range of gape angles considered herein. Embryos of *B. taitana* and larvae of *I.* cf. *kohtaoensis* are predicted to open the lower jaw less wide than juvenile or adult individuals; at gape angles of more than only about 30°, the bite force is decreased to less than 50% of its maximal value.

In the 20 mm *Boulengerula taitana* specimen, the calculated limitation in gape angle has no biological significance because this specimen was an embryo and did not feed actively. Hatching occurs at about 28 mm total length in *B. taitana* (Kupfer et al., 2006). Unfortunately, I did not have a freshly hatched specimen available for comparison. However, the larger specimen studied herein (TL 49 mm), showed a very similar pattern for effective mechanical advantages and bite forces over gape angles as have been reported for adult caecilians (Kleinteich et al., 2008). In *B. taitana*, the juveniles hatch at a premature stage and feed on the skin of their mothers until they reach about 80-90 mm in total length (Kupfer et al., 2006; Müller, 2007). My results suggest, that from the perspective of lever arm mechanics, skin feeding is similar to adult feeding. The 49 mm *B. taitana* specimen deals with the same advantages (high effective mechanical advantages of the mm. levatores mandibulae, wide range of almost constant bite forces) and limitations (critical gape angle) of the unique caecilian dual jaw closing system, just as adults.

In larval *Ichthyophis* cf. *kohtaoensis*, the low critical gape angle is supposed to be more crucial than in the 20 mm *Boulengerula taitana* specimen because this specimen actually feeds (Himstedt, 1991). In vivo gape angle measurements in caecilian larvae are rare. However, it has been reported for larvae of an unidentified species of the genus *Epicrionops* that the maximum gape angle is less than 25° (O'Reilly, 2000). This fits well into the range of gape angles that is predicted by the study herein; based on the lever arm model, the maximum gape angle is supposed to be less than 50° (critical gape angle for the sum of all muscles), and most likely is less than 37° (critical gape angle of the m. levator mandibulae longus; table 3). It is assumed that larvae of *I.* cf. *kohtaoensis* are similar in their feeding habits to larvae of the genus *Epicrionops*, that use suction feeding (O'Reilly, 2000; O'Reilly et al., 2002). Success in suction feeding highly depends on the steepness of the pressure gradient; the pressure gradient can be increased by decreasing the size of the mouth aperture (Wainwright, 2007; Van Wassenbergh and Aerts, 2009). A smaller gape angle will result in a smaller cross sectional area of the mouth opening and thus in a higher suction feeding performance. Kupfer et al. (2005) reported that caecilian larvae are generalist predators. Thus they are supposed to easily respond to limits in their jaw closing performance by choosing small prey items.

The effective mechanical advantage of the m. interhyoideus posterior is age dependent in *Boulengerula taitana* and *Ichthyophis* cf. *kohtaoensis*. In the smaller individuals of both species considered herein, the m. interhyoideus posterior has its maximal effective mechanical advantage at an almost closed gape (table 3). In larger specimens, the optimal gape angle for the m. interhyoideus posterior is 40° (*B. taitana*, table 3) and 55° (*I.* cf. *kohtaoensis*, Kleinteich et al., 2008), respectively. The optimal gape angle for function of the m. interhyoideus posterior increases with age. It remains unresolved if the observed increase

in optimal gape angle for the m. interhyoideus posterior is typical for caecilian development in general. However, there is one important difference between the two species: in *B. taitana*, the total effective mechanical advantage of the m. interhyoideus posterior *increases* during ontogeny; in *I.* cf. *kohtaoensis*, the effective mechanical advantage *decreases* for gape angles less than 65°.

The increase in the effective mechanical advantage over ontogeny in *Boulengerula taitana* is presumably to be due to an unfinished development of the jaw closing system prior to hatching. Especially the fiber orientation of the m. interhyoideus posterior, the angle of the processus retroarticularis of the lower jaw and the articulation of the quadrate squamosal and, thus, the angle of the quadrate show notable differences between the 20 mm and the 49 mm specimen. These differences are assumed to have no impact on the animal because they are observed in a non-feeding stage.

Contrary, the larval specimen of *Ichthyophis* cf. *kohtaoensis* feeds actively. The m. interhyoideus posterior has a crucial role in larval suction feeding: contraction of the muscle elevates the depressed buccal cavity and adducts the ceratobranchials (Lauder and Shaffer, 1985), thus it compresses the buccal cavity volume. Compression of the buccal cavity by the m. interhyoideus posterior in suction feeding animals occurs only when the mouth is closed (Lauder and Reilly, 1988; O'Reilly, 2000; Deban and Wake, 2000). This however, would imply that the m. interhyoideus posterior in caecilian larvae is activated with some delay relative to the mm. levatores mandibulae during the gape cycle, i.e. that the m. interhyoideus posterior becomes active after the mouth is closed. Contrary, EMG data on adult caecilians suggest, that the m. interhyoideus posterior is simultaneously active to the mm. levatores mandibulae (Bemis et al., 1983). In salamanders of the species *Ambystoma tigrinum* it was previously demonstrated by EMG measurements that general motor patterns of muscle

activity are conserved throughout ontogeny (Lauder and Shaffer, 1988). However, the study by Lauder and Shaffer (1988) did not consider the m. interhyoideus posterior and adult salamanders do not include the m. interhyoideus posterior in their jaw closing system. Whether the timing of m. interhyoideus posterior activation differs through ontogeny in caecilians remains to be resolved.

Suction feeding in larval *Ichthyophis* cf. *kohtaoensis* is also suggested to be the cause for the relatively large size of the m. levator mandibulae longus (including the m. levator mandibulae externus; see Kleinteich and Haas, 2007) and the orientation of its muscle fibers. Nussbaum (1983) argued that the size of the m. interhyoideus posterior and its contribution to bite force, increases if the mm. levatores mandibulae are restricted in size by the squamosal. In caecilian larvae, the squamosal does not cover the mm. levatores mandibulae (Haas, 2001; Kleinteich and Haas, 2007; Müller, 2007; chapter 1). Thus, reciprocal to Nussbaums (1983) argumentation, one would expect only a minor role of the m. interhyoideus posterior in jaw closure in caecilian larvae. Accordingly, the larval specimen of *I.* cf. *kohtaoensis* is the only caecilian studied so far (including Kleinteich et al., 2008), in which the physiological cross-sectional area and thus the generated force of the m. interhyoideus posterior is smaller than in the m. levator mandibulae longus (table 2). In larvae of the caecilians *Epicrionops bicolor* (chapter 1) and *E. lativittatus* (Müller, 2007) the m. interhyoideus posterior has no insertion at the processus retroarticularis and clearly does not contribute to bite force; jaw closure is driven by the mm. levatores mandibulae only.

Among caecilians, only in larvae the fibers of the m. levator mandibulae longus cover the m. depressor mandibulae laterally (Kleinteich and Haas, 2007; Müller, 2007). The orientation of m. levator mandibulae longus muscle fibers in larval *I.* cf. *kohtaoensis* is unusually oblique (36° in larva vs. 92° in the adult, see Kleinteich et al., 2008). Based on a

literature survey (Wiedersheim, 1879; Luther, 1904; Edgeworth, 1935; Bemis et al., 1983; Nussbaum, 1983; Wilkinson and Nussbaum, 1997; Haas, 2001; Kleinteich and Haas, 2007; Müller, 2007; Müller et al., 2009) I suggest that the oblique orientation of the m. levator mandibulae longus is unique for caecilian larvae and related to larval feeding. The oblique orientation of muscle fibers in larvae decreases the effective mechanical advantage of the m. levator mandibulae longus. However, it increases the velocity transmission of the muscle, that is reciprocal to the effective mechanical advantage (Westneat, 1994; 2003). The gape cycle in suction feeding caecilian larvae is much shorter than in adults (O'Reilly, 2000) and a high velocity transmission is supposed to be more crucial for suction feeding than high bite forces.

Surprisingly, in larval *Ichthyophis* cf. *kohtaoensis*, the relative contribution of the m. levator mandibulae internus plus the m. levator mandibulae articularis to total bite force is almost constant over different gape angles (app. 25%, fig. 7), i.e. the force of the m. levator mandibulae internus plus articularis decreases to the same degree over gape angle as total bite force. In adult *I.* cf. *kohtaoensis*, the relative contribution of the m. levator mandibulae internus plus articularis is higher at a closed gape (app. 40%) and decreases with increasing gape angle (Kleinteich et al., 2008). Without further knowledge on feeding mechanics in other caecilian larvae, it is impossible to decide whether this pattern was observed coincidentally or is related to suction feeding. If there is a delay in m. interhyoideus posterior action relative to the mm. levatores mandibulae, then this result could be artificial because total bite force is calculated based on the simultaneous action of the entire jaw closing system.

The 64 mm specimen of the viviparous species *Typhlonectes natans* has an almost identical distribution of effective mechanical advantages and bite forces over gape angle as the adult specimen studied by Kleinteich et al. (2008). The high value of the gape angle,

where 50% of the total bite force is transmitted, indicates, that as in adult caecilians (Kleinteich et al., 2008) and in the 49 mm *Boulengerula taitana*, the two jaw closing systems are well integrated in function in the 64 mm *T. natans*. Although it is impossible to conclude from the results of a newborn to the cranial function during fetal feeding, I would expect to find the same feeding biomechanics in even smaller, prenatal, specimens of *T. natans*, than the one examined herein. Kupfer et al. (2006) and Wilkinson et al. (2008) concluded that intrauterine feeding in caecilians evolved from skin feeding in direct developing ancestors and for *B. taitana* I showed that, skin feeding also is very similar to adult feeding.

Typhlonectes natans has been shown to have a higher contribution of the mm. levatores mandibulae to total bite force at small gape angles than other caecilians (Kleinteich et al., 2008). This was proposed to be correlated with the zygokrotaphic skull. The contribution pattern of single muscles to total bite force over different gape angles in *T. natans* is confirmed herein. However, it still remains unclear, if there is a tight link between this pattern and the aquatic lifestyle of *T. natans*. Aquatic larvae of *Ichthyophis* cf. *kohtaoensis* are substantially different in their cranial muscle morphology and feeding habits (suction vs. biting; O'Reilly, 2000) to *T. natans*, which is reflected in much different patterns of muscle contributions to total bite force.

Bite forces that I calculated herein are very small. Unfortunately, in vivo bite force measurements are exclusively available for *Shistometopum thomense* and *Boulengerula taitana* (Measey and Herrel, 2006). In adult *Boulengerula taitana* (total length: 240.3 ± 28.01 mm) bite forces are 0.62 ± 0.31 N (Measey and Herrel, 2006). This is an 18 fold difference to the calculated maximum bite force in the 49 mm juvenile specimen studied herein. However, given that physiological cross sectional area of jaw muscles scales to the square of the increase in total length, one would expect a 24 fold increase in bite force between the 49 mm

and a 240 mm specimen. Isometric scaling of the 49 mm specimen by a factor of 24 would result in a calculated maximum bite force of 0.82 N. This lies well in the range of the bite forces reported by Measey and Herrel (2006). With increasing availability of data on in vivo bite forces in caecilians and morphometrical parameters for bite force modeling it will be possible to further test the robustness of the lever arm model applied herein.

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Synopsis

The evolution of intrauterine feeding in caecilians

This study was designed to examine transformations in the head architecture of caecilians over development that depend on the modes of reproduction. The results were used to conclude on functional and developmental constraints which correlate to the evolution of viviparity and intrauterine feeding. Development of the cranium and the cranial musculature was altered by the evolution of direct development and viviparity. Modifications are: (1) the loss of muscles that are characteristic for caecilian larvae, (2) changes in the ancestral patterns of cranial muscle development, (3) alterations in the relationship between shape and size (i.e. the ontogenetic trajectory; Alberch et al., 1979) of the cranium, and (4) shifts in the timing of development (i.e. heterochrony; Gould, 1977) of cranial bones. The modifications in cranial development that can be linked to the evolution of derived reproductive modes have an impact on the jaw closing mechanics during caecilian development.

Figure 1 shows the recent hypotheses on caecilian relationships based on the study by Roelants et al. (2007). Suggested modifications from the ancestral mode of muscle development which can be concluded from my results are written on the nodes in the phylogeny. However, the topology of caecilian phylogeny, especially for the Teresomata and Caeciliidae is still under debate. Recent studies (Wilkinson et al., 2003; Frost et al., 2006; Roelants et al., 2007) differ in the position of the Scolecomorphidae and the seychellan caecilians (genera *Hypogeophis*, *Grandisonia*, and *Praslinia*). The phylogenetic position of these taxa is crucial to examine questions on the evolution of reproductive modes within Caecilians: the Scolecomorphidae contain the viviparous genus *Scolecomorphus* (Nussbaum, 1985); the seychellen genera *Grandisonia* and *Praslinia* show the ancestral biphasic life-

cycle (Parker, 1958). Consequently, the discussion on the evolutionary transformations in cranial muscle development can only be preliminary.

In chapter 1 I reconstructed the hyal and ventral branchial musculature in larvae of the most recent common ancestor of the three amphibian groups (i.e. caecilians, salamanders, and frogs) by comparing caecilian larvae to larval and neotene salamanders. Caecilian and salamander larvae are very similar in their cranial musculature. Except for the m. ceratomandibularis, each muscle in the hyal and branchial musculature of caecilian larvae has a homologous muscle in salamander larvae. The m. ceratomandibularis, is present in caecilians and salamanders too, but whether this muscle evolved twice remains unresolved. Based on the terminology for cranial muscles that I suggested in chapter 1, it is possible to directly compare caecilian and salamander muscle development. Based on published data on larval and adult salamanders (Drüner, 1901, 1904; Edgeworth, 1935; Fox, 1953; Bauer, 1997) and adult caecilians (Edgeworth, 1935; Nussbaum, 1977) it turned out that, besides the presence of homologous hyal and ventral branchial muscles, also the developmental processes are very similar in caecilians and salamanders. Congruent developmental processes can be considered to be ancestral for amphibian ontogeny. Ancestral developmental processes within the hyal and ventral branchial muscles are: (1) the loss of a separate m. ceratomandibularis during metamorphosis, (2) the m. depressor mandibulae posterior shifts its insertion from the ceratohyal in larvae to the lower jaw in adults, (3) the m. subarcualis rectus II-IV atrophies. Future comparative studies between caecilian, salamander, and frog development will potentially reveal additional patterns for amphibian cranial muscle development.

The cranial musculature in caecilians is unique among vertebrates because of the dual jaw closing mechanism, i.e. the contribution of a hyobranchial muscle (m. interhyoideus

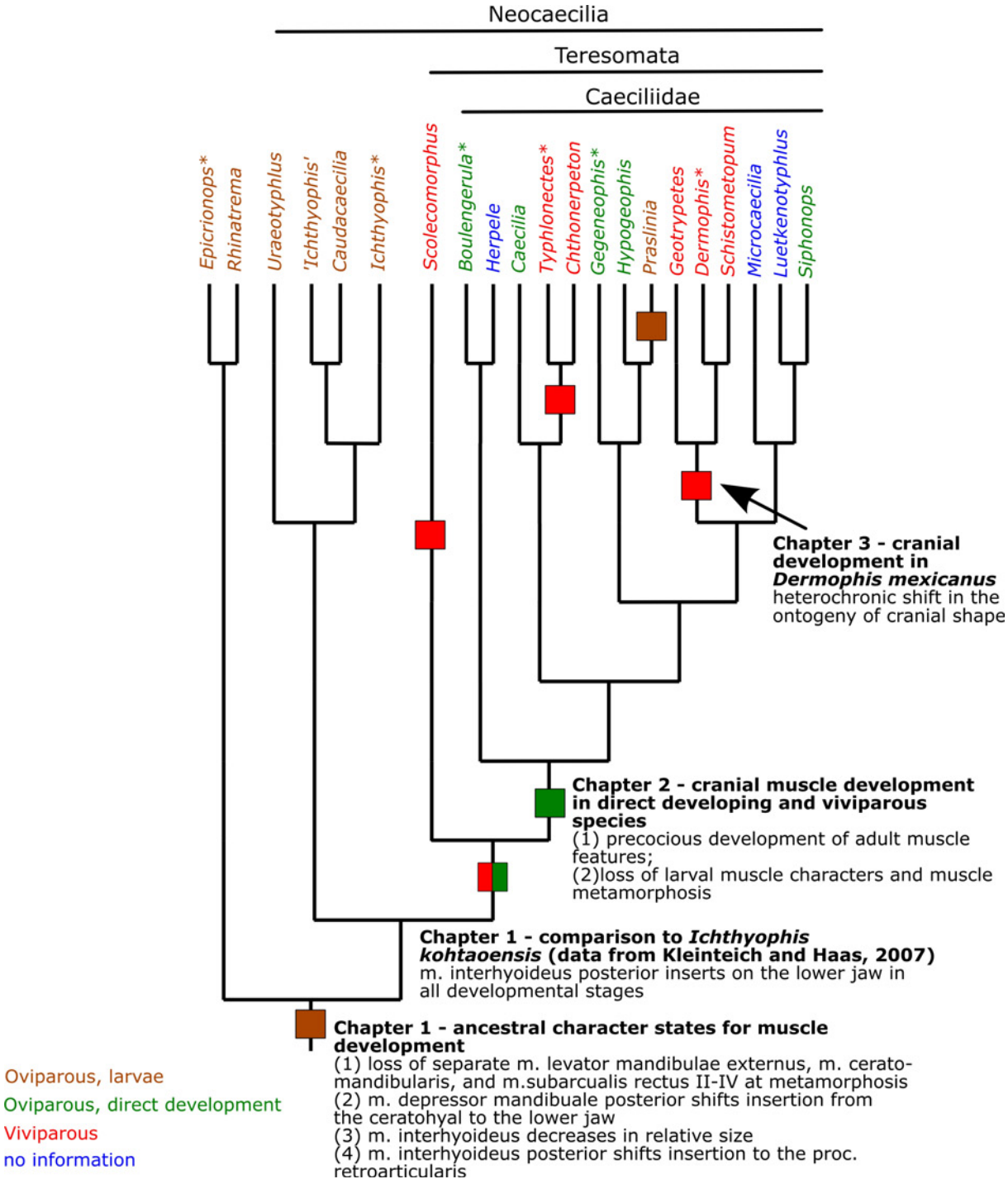

Figure 1. Evolution of reproductive modes in caecilians based on the phylogeny by Roelants et al. and the character optimization by Müller (2007). Reproductive modes are color coded: brown - oviparity with larvae and metamorphosis; green - oviparity with direct development; red - viviparity. Genera that comprise species examined in this thesis are marked by an asterisk. The transformations in cranial development that are based on my results are written to the nodes were they presumably evolved. The different chapters of this thesis contributed to the discussion on the evolution of reproductive modes at different nodes of caecilian phylogeny. Because species within the genus *Scolecomorphus* were not examined herein, it is not possible to conclude on the development of cranial muscles at the root of the Teresomata where the free living caecilian larvae is supposed to be lost.

posterior) to jaw closure. The insertion of the m. interhyoideus posterior at the lower jaw was found in all adult caecilians studied so far and is an apomorphy for the Gymnophiona (Wilkinson and Nussbaum, 2006). Different to the adult condition, however, larvae of *Epicrionops bicolor* do not have the m. interhyoideus posterior inserting at the lower jaw (chapter 1). Absence of the dual jaw closing mechanism in larvae of *E. bicolor* and of salamanders suggests that in larvae of the most recent common ancestor of caecilians the dual jaw closing mechanism was not present. In larvae of *Ichthyophis kohtaoensis* the m. interhyoideus posterior inserts on the ventral edge of the processus retroarticularis of the lower jaw (Kleinteich and Haas, 2007; chapter 4), which is presumably the derived condition for caecilian larvae (fig. 1).

The evolution of direct development at the root of the Caeciliidae (fig. 1) is suggested to be related to derived modes of cranial muscle development (chapter 2). In oviparous species with direct development, cranial muscles show adult characters from the onset of muscle development; muscle characters that are exclusive to the larval stage of caecilians are never expressed. Viviparous species show the same derived patterns for cranial muscle development as oviparous species with direct development (chapter 2).

The results presented in chapter 2 suggest that (1) oviparity with direct development and viviparity have similar effects on cranial muscle development and (2) ontogenetic repatterning, i.e. the modification of an ancestral ontogenetic trajectory, occurred during the evolution of derived modes of reproduction.

Kupfer et al. (2006) and Wilkinson et al. (2008) proposed that viviparity in caecilians evolved from ancestors which had direct development and that showed skin feeding as juveniles. This argumentation was based on the distinct positions of the skin feeding species *Boulengerula taitana* and *Siphonops annulatus* in caecilian phylogeny (Frost et al., 2006;

Roelants et al., 2007) and the presence of specialized teeth in juveniles of direct developing species and in fetuses of viviparous caecilians (Wilkinson and Nussbaum, 1998). If intrauterine feeding in viviparous caecilians evolved from skin feeding ancestors, than the similarities in cranial muscle development between direct developing and viviparous species potentially evolved once at the root of the Teresomata ('higher caecilians'; fig. 1). However, I did not consider species of the Scolecomorphidae (genera *Crotaphatrema* and *Scolecomorphus*) in this study and only limited information is available on cranial muscle development in this group (Müller et al., 2009). Based on my taxon sampling it is not possible to conclude on muscle development in the ground pattern of the Teresomata; precocious development of adult musculature and the reduction of larval muscle characteristics can only be assigned with some confidence to the ground pattern of the Caeciliidae (fig. 1).

Ontogenetic repatterning was previously suggested to be a consequence of the evolution of direct development in amphibians (Wake and Hanken, 1996). The derived precocious appearance of adult characters that form without a preceding larval morphology was also reported in direct developing salamanders (Roth and Wake, 1985) and frogs (Hanken et al., 1992, 1997). In salamanders, ontogenetic repatterning correlates to a highly derived adult morphology (Wake, 1982; Roth and Wake, 1985). However, for caecilians, previously published data suggests that the skulls of adults are rather similar independent on the mode of reproduction (see Wake, 2003a for a review on caecilian skull morphology). Kleinteich et al. (2008) did not find differences in the jaw closing function in adults of the biphasic oviparous species *Ichthyophis* cf. *kohtaoensis*, the direct developing oviparous *Siphonops annulatus*, and the viviparous *Typhlonectes natans*. This indicates that within caecilians the evolution of derived reproductive modes is not necessarily related to derived

adult morphologies. Similar to caecilians, in direct developing frogs of the genus *Eleutherodactylus*, ontogenetic repatterning was observed without a direct relation to a derived adult morphology (Hanken, 1992; Hanken et al., 1992, 1997).

Fetal development in the viviparous species *Dermophis mexicanus* was shown to involve an ontogenetic trajectory for cranial shape that is very different to the trajectory in larvae of the oviparous *Epicrionops bicolor* (chapter 3). Most of the differences between the two species can be assigned to heterochronies in the development of cranial bones that affect especially nasal capsule development and the formation of tooth bearing elements. Wake (2003b) proposed that the evolution of viviparity is related to the development of functions and structures that relate to a fetal lifestyle. It was previously reported that precocious ossification of tooth bearing bones in the viviparous *D. mexicanus* is a consequence of intrauterine feeding (Wake and Hanken, 1982; Hanken, 1989). I propose that, besides the early presence of these bones, also their large size relative to the remainder of the cranium can be linked to viviparity and fetal nutrition in *D. mexicanus*.

The geometric morphometric study in chapter 3 only comprises two species that are very distinct in caecilian phylogeny (fig. 1). It remains unresolved, whether the heterochronic shifts in the development of cranial bones in *Dermophis mexicanus* that are derived from the presumably ancestral ontogeny of *Epicrionops bicolor* are generally present in viviparous caecilians. A quantitative analysis on cranial shape over ontogeny in other caecilians species than the ones considered herein is not available yet. However, images of enzyme cleared and stained specimens from different stages of development of *Gegeneophis ramaswamii* and *Hypogeophis rostratus* (Müller et al., 2005; Müller, 2006) suggest a rather isometric cranial development in direct developing caecilians; the large size of the tooth bearing bones early in development is not found. This indicates that the observed heterochronies can not be

interpreted as present in the most recent common ancestor of the Teresomata or Caeciliidae. It is more parsimonious to assign the shift in the timing of tooth bearing bone development to the root of the clade that comprises the genera *Geotrypetes*, *Dermophis*, and *Schistometopum* (fig. 1). This, however, involves the presence of similar ontogenetic trajectories for cranial development in the genera *Geotrypetes* and *Schistometopum*, which is not confirmed yet.

The modifications in cranial and cranial muscle development that link to the evolution of derived modes of reproduction also have functional consequences (chapter 4). Besides the similarities in muscle topology between embryos, fetuses, juveniles, and adults of direct developing and viviparous species (chapter 2), there is a high degree of accordance in functionally important muscle characters (i.e. fiber orientation, lever arm ratios, and physiological cross-sectional area); the similarities in functional muscle morphology result in identical patterns of jaw closing biomechanics.

Caecilian larvae are supposed to have different feeding biomechanics than adult caecilians (chapter 4) which can be related to suction feeding. Suction feeding likewise involves the m. interhyoideus posterior, which might be in conflict with the jaw closing function of this muscle in adult caecilians. The evolutionary loss of a free living aquatic larvae at the root of the Teresomata (fig. 1) and the related modifications in cranial and cranial muscle development are suggested to remove the larval functional constraint of suction feeding from the m. interhyoideus posterior and thus the unique jaw closing mechanism in caecilians.

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