Developmental morphological diversity in caecilian amphibians: systematic and evolutionary implications

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CHAPTER 2

Ontogeny of the skull, lower jaw and hyobranchial skeleton of *Hypogeophis rostratus* (Amphibia: Gymnophiona: Caeciliidae) revisited

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ABSTRACT Few detailed descriptions of the development of the head skeleton in caecilian amphibians are available. One of those is the work of Marcus and students (e.g., Gehwolf, 1923; Marcus, 1933; Marcus et al., 1935) on the morphology and development of the skull, lower jaw and hyobranchial skeleton in the Seychellean caeciliids *Hypogeophis rostratus* and *Grandisonia alternans*. These workers described a high number of individual ossifications that fuse during ontogeny to form the adult skull. Although later studies have doubted the generality of those observations, the work of Marcus and his students has been hugely influential in subsequent studies of caecilian skull morphology and amphibian evolution. Based on new observations on an ontogenetic series of 32 sectioned and cleared and stained specimens, ranging from the beginning of chondrification to the adult, the development of the skull, lower jaw and hyobranchial skeleton of *H. rostratus* are described. The new results are largely incompatible with those of Marcus and students and no evidence for several of the reported ossifications, including supra-, infra- and basioccipital, epiotic, pleurosphenoid, preethmoid, posterior vomer, prepalatine, quadratojugal, postparietal, second coronoid, supraangular and complementare, is found. It is argued that most of Marcus et al.'s reports of non-existent ossifications are based on false phylogenetic preconception, misinterpretation of the observed morphology and technical error. Data on the ossification sequence of the skull and lower jaw in *H. rostratus* are provided and briefly compared to published information on *Dermophis mexicanus* and *Gegeneophis ramaswamii*.

INTRODUCTION

At a time when biosciences as a whole are a fast moving field, morphology and morphological systematics draw both from the most current studies using advanced analytical techniques, as well as from studies that sometimes date back as far as the 19th century. In some cases, such as rare and seldom studied animals for which new material is limited, old accounts often represent the only source of primary morphological data. These, however, as with almost all scientific studies, are the products of their time and are often as much a reflection of contemporary trends as they are documentations of the observed morphology. One possible case in point concerns the skull morphology of caecilian amphibians, where new studies (Wake and Hanken, 1982; Müller et al., 2005) have produced results largely incongruent with earlier studies of skull development (e.g., Marcus et al., 1935).

Caecilians (or Gymnophiona), frogs and salamanders constitute the three extant clades of the Amphibia. Caecilians comprise about 170 named species in six families (Wilkinson and Nussbaum, 2005). They are the least known, major living tetrapod clade, which is probably mainly due to their largely subterranean habits and confinement to parts of the wet and seasonal tropics of South and Middle America, Africa and Asia. The first extensive comparative studies of caecilian morphology were undertaken by Wiedersheim (1879) and Peters (1880). Taylor (1969b) provided brief descriptions of adult skull morphology for a broad range of taxa and, most recently, Wake (2003) reviewed and summarized known adult skull morphology of all caecilian genera. However, most studies on caecilian morphology have been restricted to investigations on adult material and were usually carried out on small samples (e.g., Brand, 1956). This led to uncertainties about bone homologies because the heavily ossified, burrowing-adapted caecilian skulls are highly modified compared to the skulls of other amphibians.

Little ontogenetic information about caecilians was available when Marcus et al. (1935) published a lengthy account on the development of the skull in what were then thought to be two species of *Hypogeophis*. Based on their observations of a very limited number of three ontogenetic stages (early and late embryo and juvenile), they described a high number of individual bones that occur during the development of the skull. These bones were reported to fuse during ontogeny to form a highly compact adult skull that is made up of a relatively small number of bones, like the os basale, which is comprised of the dermal parasphenoid and most of the posterior part of the neurocranium. The formation of the large compound bones from individual ossifications had been already postulated by early students of caecilian skull morphology (Dugès, 1834; Wiedersheim, 1879), and was later corroborated by Peter (1898). However, the unusually high number of separate ossification centers reported by Marcus et al. (1935), aroused much interest (de Beer, 1937) and featured prominently in the still unsettled debate concerning the systematic relationships of living amphibians (Schoch and Milner, 2004 for most recent review). More importantly, because of the general lack of developmental studies in caecilians, the results of Marcus et al. (1935) were seen as representing the standard in caecilian cranial development. Several authors of subsequent studies on caecilian skull morphology (e.g. Ramaswami, 1948; Brand, 1956; Visser, 1963) commented on presumably fused bones, whose presence was assumed rather than actually observed. Some of the results of Marcus et al. (1935) were at least occasionally doubted (Brand, 1956) but were nonetheless largely accepted until Wake and Hanken's (1982) study of skull development in *Dermophis mexicanus*, a viviparous Central American caeciliid, in which they were unable to confirm several ossifications (e.g. basi- and supraoccipital, pleurosphenoid, postorbital, quadratojugal) reported by Marcus et al. (1935).

Recently, Müller et al. (2005) investigated the development of the skull in the *Gegeneophis ramaswamii*, an Indian direct-developing caeciliid more closely related to the Seychellean caecilian radiation comprising *Hypogeophis rostratus* and *Grandisonia* (Hass et al., 1993; Wilkinson et al., 2002) than *Dermophis mexicanus*. They too, found no evidence for many of the ossifications reported by Marcus et al. (1935) that Wake and Hanken (1982) did not find in *D. mexicanus*, which raised further doubt about the validity of many of Marcus et al.'s observations. To clarify these conflicting observations I analyzed a nearly complete ontogenetic series of *H. rostratus* with regard to the ossifications, their homology, and their sequence of appearance.

MATERIALS AND METHODS

The material used in this study was collected by A. Brauer in 1896 in the Seychelles. The material is deposited in the Museum für Naturkunde, Berlin (ZMB) and also represents the source for the studies by H. Marcus and co-workers (e.g., Marcus, 1909:105, 110). I studied an ontogenetic series of embryos, juveniles and adults of *Hypogeophis rostratus* (Appendix). Embryos selected for clearing and staining were double stained for bone and cartilage using a slightly modified protocol based on Taylor and Van Dyke (1985). Specimens selected for serial sections were decalcified, embedded using a Shandon Hypercenter XP tissue processor, sectioned at 8 µm using a Leica SW 2000R microtome equipped with Feather N35H disposable blades and sections stained with azocarmine-red and anilin-blue (AZAN) following standard procedures (Romeis, 1989). For scanning electron microscopy (SEM), the skull of a cleared and stained juvenile was partly disarticulated and, using tweezers, the bones were gently freed from adhering fibrous tissue. Glycerin was washed out in ethanol and the bones air-dried, mounted, and sputter coated with gold-palladium. Cleared and stained specimens were observed under a Nikon SMZ-U stereomicroscope equipped with a camera lucida and a digital camera (Nikon Coolpix 995) or a Zeiss DR stereomicroscope. Photos of cleared and stained specimens were taken with a Zeiss Tessovar with a Nikon Coolpix 995 digital camera attached. Serial sections were observed under a Nikon Eclipse E600 microscope and SEM prepared specimens observed and photographed under a Hitachi S2500 SEM with a digital image capture system.

The overall preservation of the material was generally very good, given the long time in storage. Some of the cleared and stained specimens did not or not completely retain the alizarin red bone stain. This was particularly the case in earlier embryos. These were observed under indirect illumination (e.g. dark field), under which developing and unstained or decalcified bone appears as a light structure (Haas, 1996). In the serial sections, bones and other elements were assessed based on their histological appearance rather than staining. This pertains especially to the distinction between cartilage, precartilage and mesenchyme in the hyobranchial skeleton. As Cartilage I recognized tissue characterized by the expression of cartilage extracellular matrix (ECM). Precartilage is an aggregation of densely packed nuclei that resemble those of cartilage but without visible cartilage ECM, whereas mesenchyme is a more diffuse yet recognizable aggregation of cells. Although useful as descriptors, these distinctions are somewhat arbitrary as they pertain to certain sections of a developmental continuum. I have therefore tried to avoid any over-interpretation based on these structures

In the description of the development of the skull, I distinguish between endoskeletal bone, dermal bone and membrane bone (following Patterson, 1977). Endoskeletal bone is bone that forms by peri- or endochondral ossification of a cartilaginous precursor, such as the exoccipital, which forms as a perichondral ossification of the cartilaginous exoccipital arch. Dermal bone develops without a cartilaginous precursor and has no connection to an endoskeletal element. Typical dermal bones are nasal and maxilla. Membrane bone is a form of bone that ossifies without a cartilaginous precursor but is phylogenetically part of the endocranium. All membrane bones in *Hypogeophis rotratus* and other caecilians investigated (see Müller et al., 2005) develop as a laminar outgrowth from endoskeletal bone, such as the dorsomedial outgrowth of membrane bone from the ossified otic capsule, above the foramen magnum. In all instances covered here, membrane bone extends from, and is always connected to, endochondral bone. For a reference to the morphology of the endocranium discussed in here, see Figure 2E.

To facilitate comparison with published accounts of development in *Hypogeophis rostratus*, embryos were staged according to Brauer (1899). Brauer's description of development in *Hypogeophis* is more of an overview, rather than a staging table in a modern sense, where development is divided into discrete intervals defined by morphological, physiological, and behavioral markers (e.g., Nieuwkoop and Faber, 1967; Bartsch et al., 1997). Brauer's detailed figures and descriptions, however, were subsequently referred to as stages (e.g., Marcus, 1909:111; Eifertinger, 1933) and can be used as such. The published staging tables for the biphasic *Ichthyophis kohtaoensis* (Dünker et al., 2000) and viviparous *Typhlonectes compressicauda* (Sammouri et al., 1990) were inadequate for the description of development in *H. rostratus*, as several of the stage-defining characters (e.g., development of lateral line organs in *I. kohtaoensis*, formation of the sack-like gills in *T. compressicauda*) are not expressed in the direct developing *H. rostratus.* When comparing specimens to Brauer's (1899) account, I established approximate correspondences with his figured 'stages' based on features such as the development of the external gills, head flexure, and the amount of yolk. Marcus and co-workers staged their material in a similar way, and their specimens were found to match the newly staged material well in terms of skull development. I observed some intraspecific variation in skull development in my material, and although external development did not always reflect similar skull development in all specimens investigated, reference to Brauer's stages proved to be a better descriptor of development than a reference to size, which would have significantly increased the degree of intraspecific variation observed. In the following description, I describe skull development based on Brauer's 'stages' (abbreviated BS herein), as reference to BS is preferable to a comparison based on size, which is neither a reliable indicator of developmental progress nor a useful facilitator of interspecific comparisons.

Many caecilian species are fairly poorly defined (Nussbaum and Wilkinson, 1989; Gower and Wilkinson, 2005). One exception is the caecilians of the Seychelles archipelago (*Grandisonia alternans*, *G. brevis*, *G. larvata*, *G. seychellensis*, *Hypogeophis rostratus* and *Praslinia cooperi*), which are relatively well known taxonomically (Parker, 1958; Nussbaum, 1984; Wilkinson and Nussbaum, 2005). *Hypogeophis rostratus* is the most widespread species and occurs on every Seychellean island from which caecilians are known, and is often the only caecilian species present (Nussbaum, 1984). Although all these populations are treated as belonging to *H. rostratus*, morphological divergences between several island populations have been recognized and led to the description of several subspecies (Parker, 1958; Taylor, 1968, 1969a). There is sufficient evidence that all of the specimens collected by Brauer (and used here) originate from Mahé and/or Silhouette, and thus would belong to the nominate subspecies *H. r. rostratus*. Here, however, I follow Nussbaum and Wilkinson (1989) in only recognizing nominal species, given that the biological and taxonomical meaning of subspecies is poorly defined.

RESULTS

Development of the skull

At BS 38 (the earliest developmental stage available) the chondrocranium is relatively incomplete. Chondrification proceeds from posterior to anterior. The occipital arch, together with the palatoquadrate, is most prominently developed but does not contact the otic capsule and parachordal cartilage (*cf.* Fig. 1A). The otic capsule, especially its medial wall, is only weakly chondrified, has a large fenestra ovalis, and is connected to the parachordal cartilage at its anterior and posterior ends, albeit via very weakly developed cartilaginous bars. A small, weakly chondrified, Y-shaped cartilaginous stapes is present at the anteroventral end of the fenestra ovalis. The palatoquadrate is a fairly large element and oriented dorsoventrally. In lateral view, its ventral half is slightly broader than its dorsal half. It further has a clearly discernible articular facet and a small pterygoid process, which is continuous with the main body of the palatoquadrate ventromedially. The parachordal cartilage is only weakly chondrified anterior to the otic capsule. The taenia marginalis shows the same degree of chondrification as the parachordal cartilage and extends from the anterodorsal end of the otic capsule to the level of the palatoquadrate. It is continuous with the otic capsule although the connection is narrow and almost unstained.

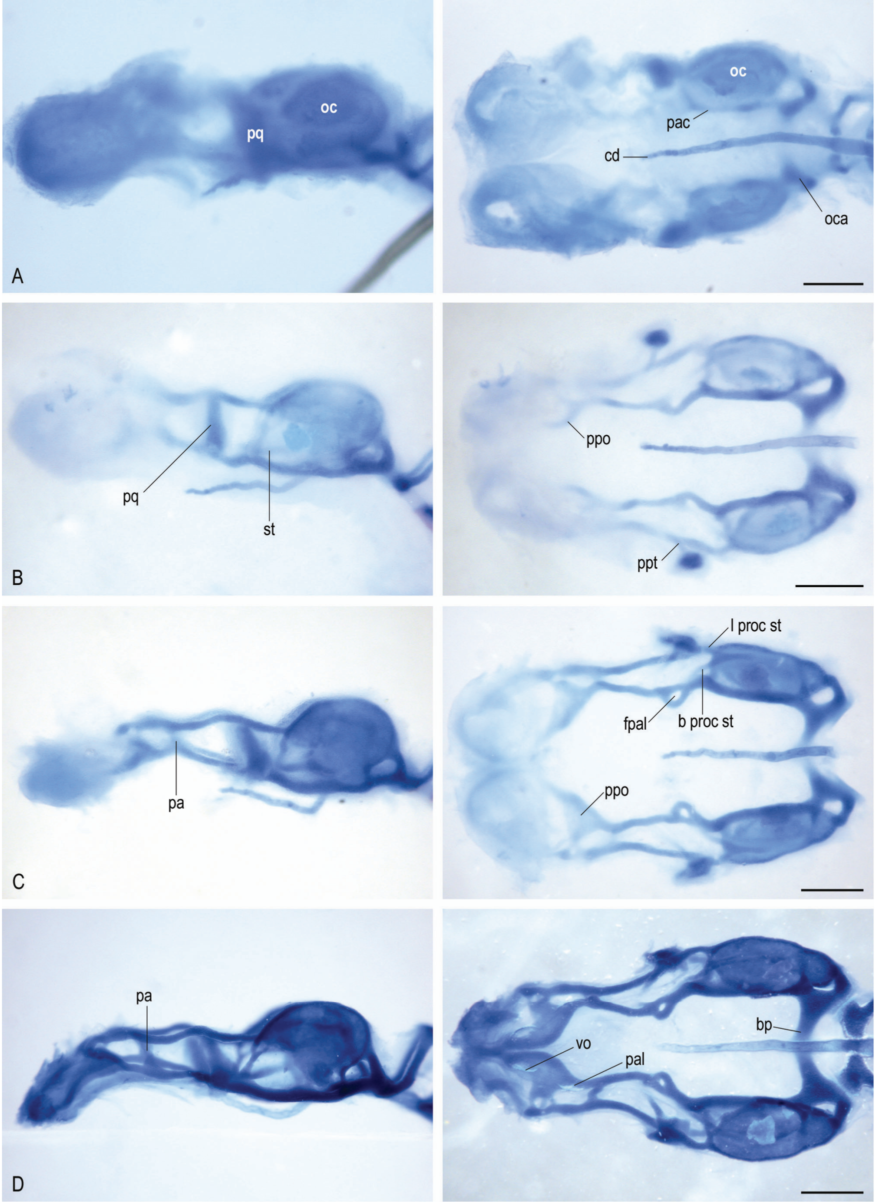

Fig. 1. Development of the skull in *Hypogeophis rostratus*; lateral views on the left and ventral views on the right. **A**: BS 38/39 (Hyro 26); note the unfused occipital arch (oca) and parachordal cartilage (pac). **B**: BS 40 (Hyro 25). **C**: BS 40 (Hyro 27). **D**: BS 41/45 (Hyro 14); vomer (vo) and palatine (pal) are present but unstained because of decalcification/insufficient ossification. bp, basal plate; b proc st, basal process of the stapes; cd, chorda dorsalis; fpal, palatine foramen; l proc st, lateral process of the stapes; oc, otic capsule; pa, pila antotica; ppo, pila preoptica; ppt, pterygoid process of the palatoquadrate/quadrate; pq, palatoquadrate; st, stapes. Scale bar equals 0.5 mm.

Chondrocranial development has progressed little in a BS 38/39 embryo (Fig. 1A), however, chondrification of the otic capsules is more extensive and a basal plate is forming ventrally between the occipital arches. The parachordal cartilage remains weakly developed anterior to the otic capsule. The taenia marginalis is more chondrified and extends further rostrally than in the previous embryo. The connection to the otic capsule, however, is constricted and stained less intensely than the rest of the taenia marginalis.

The embryos of BS 40 available for study (Hyro1, sectioned; Hyro 25 and Hyro 27, cleared and stained) show some variation in the degree of skeletal development, as can be seen in Fig. 1B and C. A very weakly chondrified pila antotica is discernible just posterior to the position of the eye in Hyro 25 (*cf.* Fig. IB), connecting the taenia marginalis and parachordal cartilage. Anterior of the pilae antoticae, the trabecular cartilages bend inwards and converge towards the midline. Anterior of the pila antotica, anlagen of the trabecular cartilage, pila preoptica, and nasal capsule are just visible in Hyro 25. Hyro 27 shows a more advanced development of the trabecular cartilage, pila preoptica and nasal capsule (*cf.* Fig 1C). The occipital arch is fused with the otic capsule and parachordal cartilage. The basal plate is more chondrified than in the previous embryo although the part closest to the chorda dorsalis remains only faintly stained. The parachordal cartilage anterior to the otic capsule has a foramen for the palatine branch of the facial nerve. Also, the basal process of the stapes is beginning to fuse to the parachordal cartilage and the palatoquadrate is more anteriorly inclined than in the previous specimens. The sectioned specimen (Hyro 1) exhibits the same degree of chondrification as Hyro 27. In addition, small ossifications of vomer and palatine are present. The vomer forms as a simple blade anterolaterally of the convergence of the preoptic plates. The palatine consists of a simple sliver of bone, only half the size of the vomer, and forms just posterolaterally of the choana.

In embryos of BS 40 to BS 40/41 (Fig. 1D), most of the chondrocranium except the anterior nasal capsule is well developed, although the otic capsule remains weakly chondrified ventrolaterally. Perichondral ossification is apparent at the posterior part of the chondrocranium, where the exoccipital forms as a thin layer of bone sheathing the occipital arch. Ossification of the prootic is apparent in the anterodorsal part of the otic capsule and the posterior taenia marginalis. Anterior to the otic capsule, the parachordals are somewhat compressed dorsoventrally and curve gently inwards. A zone of weak chondrification is apparent just anterior to the otic capsule, at the point where the basal process of the stapes joins the parachordals (processus columello-trabecularis *sensu* Visser, 1963). From histological sections, this area consists of cell-rich cartilage and the parachordal and stapes are fused rather than in articulation. Just anteriorly is the foramen for the palatine nerve that pierces the parachordal. A laterally-directed, short cartilaginous process is anterior to the palatine foramen and will form the basipterygoid articulation from BS 45 onwards. Further anteriorly, the parachordal becomes more rounded and curves laterally towards the pila antotica. The pila antotica forms a well chondrified but slender bar. Anteriorly, trabecular cartilages are well developed and expand into a pair of broad and somewhat weakly chondrified pilae preopticae, which elongate anteriorly and eventually fuse to form the nasal septum that extends beyond the anterior copulae into the processus prenasalis. The nasal septum also forms a posterodorsally-directed process. Except for the nasal septum and oblique cartilage, most of the anterior and lateral parts of the nasal capsule remain only weakly chondrified. The stapes is a well-chondified, triradiate cartilage. The footplate is rod-like and sits within the widely open fenestra ovalis. Anterior to the fenestra ovalis, the stapes bifurcates and forms a short, anteriorly-expanded lateral process that articulates with an inconspicuous process at the posterior margin of the palatoquadrate, and a basal process that fused with the parachordal. The ventral half of the palatoquadrate, including the well-developed processus pterygoideus, is covered by a thin layer of perichondral bone. A thin, blade-like premaxilla is present anteriorly, ventral of the anterior copula. It is followed by more developed and larger vomer and palatine ossifications posteriorly. The vomer has enlarged and is crescent-shaped in ventral view. It consists of a dental lamina and a narrow palatine shelf that is growing towards the midline. The plate-like palatine has also

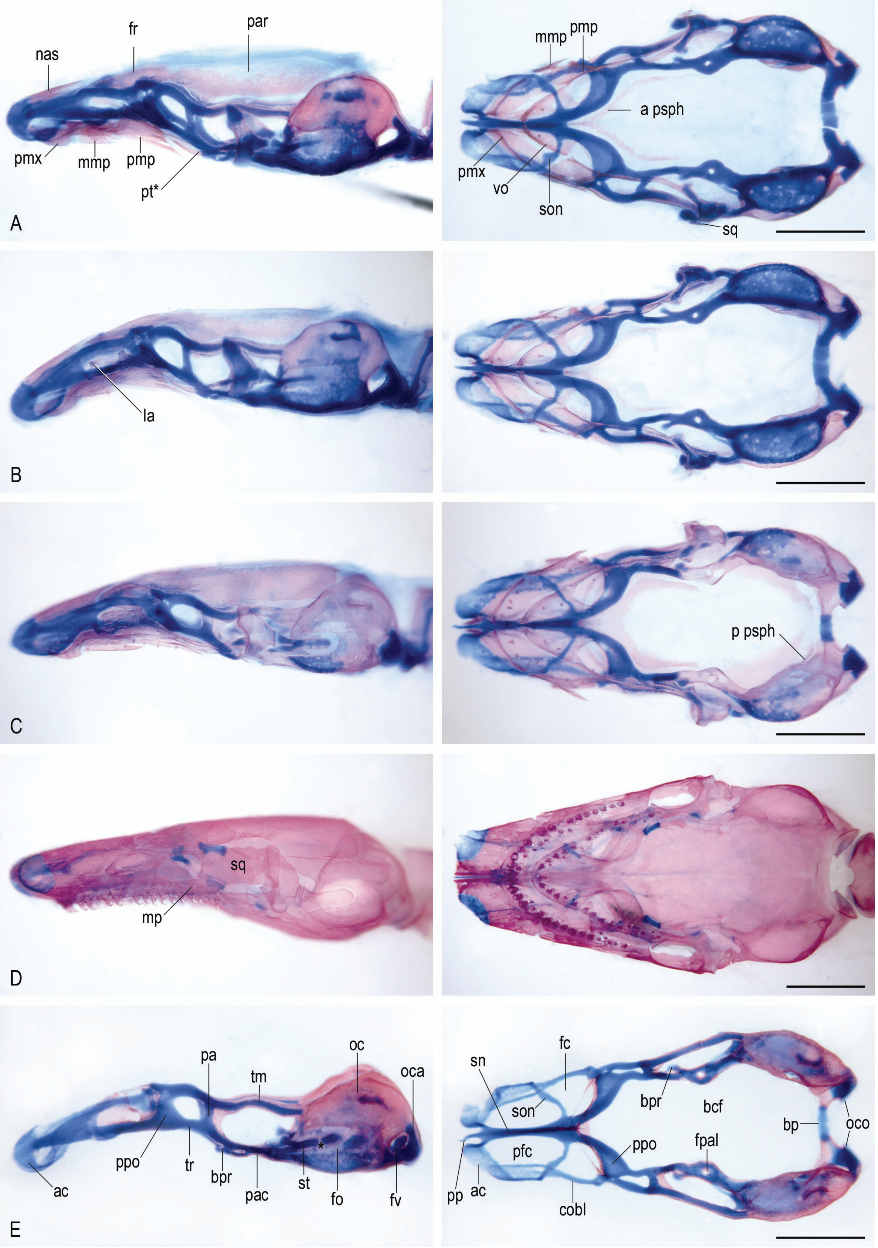
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Fig. 2. Development of the skull in *Hypogeophis rostratus*; lateral views on the left and ventral views on the right. **A**: BS 45 (Hyro 11). **B**: BS 46 (Hyro 19). **C**: BS 47 (Hyro 17). **D**: Juvenile (Hyro 24); the squamosal (sq) covers the cheek region and is in contact with the maxillopalatine (mp). **E**: BS 47 (Hyro 21); endocranium, the quadrate/palatoquadrate and all dermal elements are removed. a psph, anterior part of parasphenoid; ac, anterior copula; bfc basicranial fenestra; bp, basal plate; bpr, basal process; cobl, oblique cartilage; fc, choanal foramen; fpal, palatine foramen; fo, fenestra ovalis; fr, frontal; fv, vagus foramen; la, lacrimal; mmp, maxillary part of the maxillopalatine; nas, nasal; oc, otic capsule; oca, occipital arch; oco, occipital condyles; pa, pila antotica; par, parietal; pfc, prechoanal foramen; pmp, palatine part of the maxillopalatine; pmx, premaxillary; p psph, posterior part of parasphenoid; pac, parachordal cartilage; pp, prenasal process; ppo, pila preoptica; pt*, fused pterygoid; sn, septum nasi; son, solum nasi; st, stapes; tm, taenia marginalis; tr, trabecular cartilage; vo, vomer. * marks a damage due to handling. Scale bar equals 1 mm.

enlarged and its anterior half is twisted against its posterior half, giving it an hourglass-like shape if seen from ventral. Further posterior to the palatine is a long and thin, plate-like pterygoid (Fig. 3D) that stretches to the anterior tip of the pterygoid process of the palatoquadrate.

By late BS 41/45, ossification of the quadrate has started (Fig. 3E) with a thin layer of perichondral ossification surrounding the pterygoid process and the ventromedial and ventrolateral sides of the palatoquadrate cartilage. A small frontal is present as a small, narrow sheet of bone laterally, above the oblique cartilage. A parietal is present too as a relatively long sliver of bone above the taenia marginalis. A small, short plate-like maxilla is found laterally of the posterior half of the nasal capsule. The dermal pterygoid is fused to the perichondral ossification surrounding the pterygoid process of the quadrate.

The available BS 45 specimens do also show variation in the degree of skeletal development. In the least advanced embryo (Hyro 5), chondrocranial development has greatly progressed as compared to previous stages. The fenestra ovalis remains comparatively large and not yet filled by the stapes. The occipital arch is completely sheathed with perichondral bone, as are the posterior part and most of the medial wall of the otic capsule. A layer of perichondral bone also covers the anterodorsal surface of the otic capsule and posterior parachordals. The stapes, still unossified, tightly abuts the palatoquadrate. The connection between the stapes and the parachordal cartilage is robust and well chondrified, fusing the stapes to the parachordals (Fig. 3F). The palatoquadrate cartilage is partly replaced by the quadrate bone, and the dermal pterygoid is fused to the ossified endoskeletal pterygoid process of the quadrate. The nasal capsule is fully formed and is composed of anterior copulae and well chondrified lateral walls. A cartilaginous roof is absent and the sola nasi consist of thin strands of cartilage (Cornu laterale *sensu* Marcus et al., 1935). The premaxilla has grown considerably and become crescent-shaped, with a distinct dental lamina. A maxilla is present as a simple, relatively deep but short, plate-like ossification lateral of the posterior nasal capsule. Vomer and palatine have also enlarged and their dental laminae are very distinct. The palatine shelf of the vomer is much enlarged and has a notch for the palatine branch of the facial nerve on its medial edge. The palatine has a large, vertically oriented lamina medial to the eye and lateral to the nasal epithelium. Posterior of the choana, a medially directed process has formed through the accretion of bone, which gives the palatine its characteristic Y-shape when viewed from ventral. One to two premaxillary, vomerine, and maxillary tooth crowns are present but not fused to their respective bones as their pedicels are not yet developed. The anterior part of the parasphenoid is starting to form as a V-shaped bone at the anterior edge of the basicranial fenestra. Frontal and parietal bones remain long and narrow plates of bone dorsolaterally, above the oblique cartilage and taenia marginalis respectively. In the most advanced BS 45 specimen (Hyro 11; Fig 2A), skeletal development has progressed further. The dorsal and medial part of the otic capsule is almost completely ossified, with cartilage mainly confined to the ventrolateral region. Membrane bone extends dorsomedially from the posterior dorsomedial margin of the otic capsules, forming the dorsal rim of the foramen magnum. The anterior footplate of the stapes is perichondrally ossified. A lamina of membrane bone, extending from the taenia marginalis, lies dorsally behind the pila antotica. A sphenethmoid ossification is present and consists of a median bony lamina anterior of the pilae preopticae (trabecular plates) that is continuous with perichondral ossification of the cartilaginous nasal septum. Laterally, thin lamellae of membrane bone, which are part of the sphenethmoid, form the anterior wall of the brain cavity. Small nasals are present and lie dorsomedially of the nasal capsule. The anterior parasphenoid extends caudally to the level of the

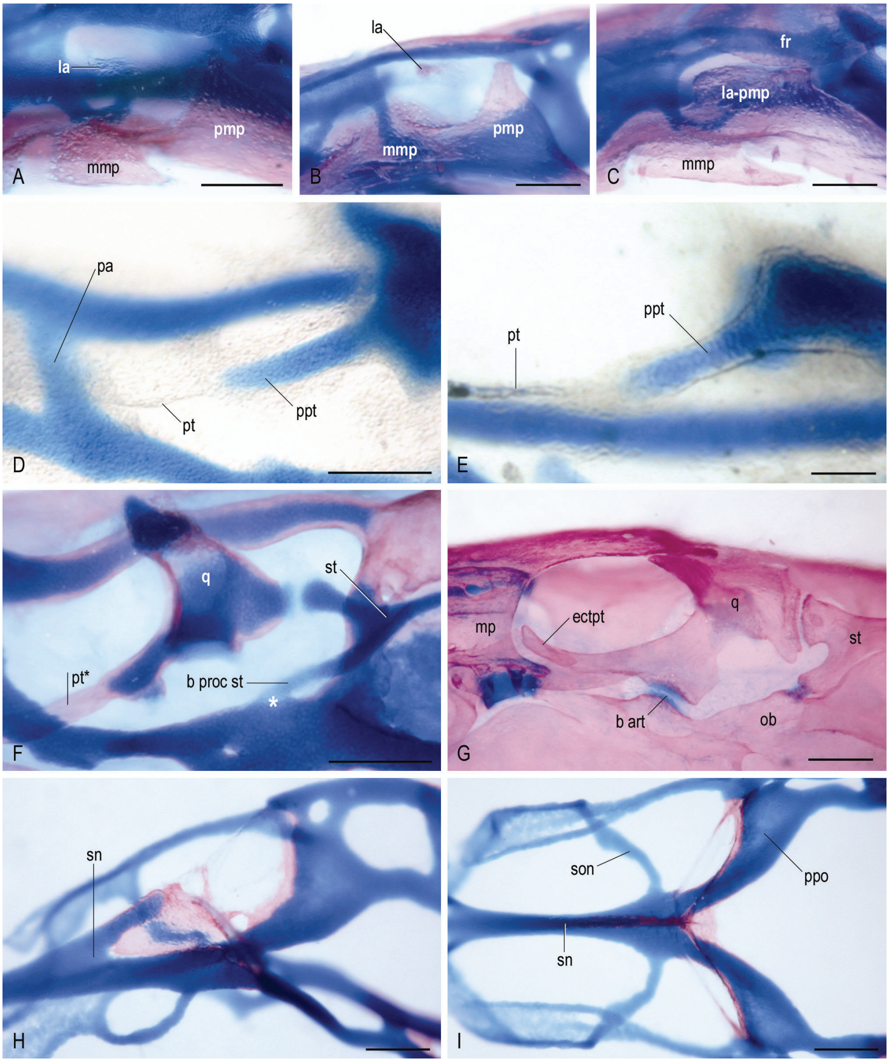

Fig. 3. Aspects of skull development in *Hypogeophis rostratus*. Close-up of the development of the lacrimal (la) from its first occurrence at BS 45 (**A**, lateral view; Hyro11), through BS 46 (**B**, slightly ventrolateral view; Hyro 19) and its eventual fusion to the palatine part of the maxillopalatine (pmp) at BS 47 (**C**, lateral view; Hyro 17). The free pterygoid (pt) in an embryo of BS 41 (**D**, ventrolateral view; Hyro 28) and BS 41/45 (**E**, lateral view; Hyro31); note the extensive perichondral ossification around the pterygoid process (ppt) and quadrate in **E**. Fusion of the basal process of the stapes (b proc st) is marked with an asterisk (*) and shown in **F** in a BS 45 embryo (Hyro 11). **G** Juvenile skull (Hyro 24) in ventral view, showing the ectopterygoid (ectpt) just posterior of the maxillopalatine (mp); note the cartilage covered articular facets of the basal articulation (b art) and the cartilaginous connection between the stapes (st) and os basale (ob). Dorsolateral (**H**) and lateral view (**I**) of the sphenethmoid ossification in a BS 47 embryo (Hyro 21). Additional abbreviations: mmp, maxillary part of the maxillopalatine; pa, pila antotica; ppo, pila preoptica; pt*, fused pterygoid; q, quadrate; sn, septum nasi; son, solum nasi. Scale bar equals 250 µm in all pictures.

basal process and has also developed an anterior directed process that extends rostrally between the vomers up to the palatine foramen of the vomer. Maxilla and palatine are fused to form the maxillopalatine. The maxillary part of the maxillopalatine, however, remains short. A few maxillary, premaxillary, vomerine, and palatine tooth crowns are present but not attached to their respective bones. The squamosal is present as a distinct, albeit small, elliptical bone lateral to the dorsal half of palatoquadrate. A small lacrimal bone is present above the maxilla, anterior to the orbital shelf of the palatine (Fig. 3A). The premaxilla has developed an alary process that extends dorsally between the anterior copula and the prenasal process. The vomer has completely encircled the palatine branch of the facial nerve. Frontal and parietal have approached each other more closely and have also grown further towards the dorsal midline. The leading, medial edges of the frontal and especially the parietal are less intensely stained than the lateral parts and show a reticulated staining pattern.

At BS 46 (Fig. 2B) endocranial ossification has progressed further. Most of the endocranium posterior to the pila antotica is covered with a layer of perichondral bone. The lamina of membrane bone behind the pila antotica has enlarged, extends onto the pila antotica, and is fused ventrally to the perichondral bone layer around the parachordal. In the ethmoidal region, the sphenethmoid has expanded further and forms a well-developed bony nasal septum. The bony nasal septum is continuous with the perichondral ossification around the cartilaginous nasal septum and the perichondral ossification of the pila preoptica (*cf.* Fig. 3H,I).

The palatoquadrate is completely covered by a layer of perichondral bone, i.e. the quadrate bone. The stapes is also completely sheathed with a thin layer of perichondral bone, except for the sites of articulation with the quadrate and parachordal. Most dermal bones have expanded, particularly the nasal, frontal and parietal. Whereas the frontal and parietal are growing in a lateral to medial direction, the nasal is growing from medial to lateral. The squamosal remains comparatively small and still has not grown out to cover the cheek region. A small dermal ectopterygoid ossification (*cf.* Fig. 3G) is present lateral to the anterior pterygoid process and posterolaterally to the maxillopalatine. Splints of bone are present in the anlagen of the posterior parasphenoid, anterolaterally of the basal plate.

At BS 47 (Fig. 2C), most of the posterior part of the endocranium up to the pila antotica is ossified, with small remnants of cartilage confined to the occipital arch, the medial part of the basal plate, and the anteroventral wall of the otic capsule. The footplate of the stapes has broadened and the stapes is completely covered by perichondral bone except for the articulations with the quadrate and the former parachordal, which has been integrated into the endocranial part of the os basale. The sphenethmoid ossification has expanded and replaced the cartilage in the centre of the pila preoptica. The posterior and anterior parts of the parasphenoid have enlarged and additional small splints of bone are present just posteromedial of the palatine foramen. The anterior part of the parasphenoid completely fills the anterior basicranial fenestra, between the preoptic plates. The lacrimal is fused posteriorly to the orbital shelf of the palatine part of the maxillopalatine (Fig. 3C). Nasals and premaxillae are fused between the anterior copulae. The squamosal remains comparatively small and covers only the anterodorsal part of the quadrate laterally. In the most advanced BS 47 specimen (Hyro 7), the separate parts of the parasphenoid have fused and expanded and this bone covers the entire basicranial fenestra, apart from a relatively small, medial area at the level of the quadrate, where ossification is poor.

In juveniles (Figs. 2D, 3G), only part of the orbital and trabecular cartilages, part of the orbitonasal orifice, the prenasal process, the basal articulation, and the connection of stapes and os basale remain cartilaginous. The posterior endocranium and parasphenoid are fused and form the os basale. The skull has a morphology comparable to adult specimens in that the maxillopalatine has grown caudally to contact the squamosal, which has grown rostrally and covers the cheek region laterally. Both bones do also form the orbit and completely encircle the eye. The pedicels of most teeth are fused to their respective bones, thus anchoring the tooth crowns, and the dental arcades have the same extent and shape as in the adult cranium. Most of the dermal bones, however, are not as tightly sutured as in the adult skull. The membrane bone that forms the upper margin of the foramen magnum is still not fully developed, leaving a midline gap.

Development of the lower jaw

At BS 38, Meckel’s cartilage is a well chondrified but simple rod. The anterior tips are separated by a fairly broad gap. Posteriorly, the retroarticular process of Meckel’s cartilage does extends only a short distance beyond the jaw articulation. Meckel’s cartilage becomes lyre-shaped (in ventral view) at BS 38/39 and a broad symphyseal area has formed at the anterior midline. A processus condyloideus is present and articulates with the palatoquadrate, and the processus retroarticularis is prominently developed and extends well beyond the jaw articulation. The anlage of a dentary, the first ossification to appear in the skull, is present laterally at the anterior tip of, and in very close proximity to, Meckel’s cartilage.

The dentary has enlarged in a BS 40 embryo, and is continuous with perichondral ossification that has developed at the anterior tip of Meckel’s cartilage. An angular is present along the ventral side of Meckel’s cartilage. It is a long, rod-like bone that is almost rectangular in transverse sections. A thin, plate-like bone covers the lingual side of Meckel’s cartilage anterior of the jaw articulation. In the youngest embryo where it is visible (Hyro 1, BS40), this bone has a very narrow connection to the angular below the articular surface of the lower jaw.

At BS 40/41, the dentary has expanded further and the angular is fused to the lingual bony lamina, except for a very large medial foramen through which the mandibular branch of the trigeminal nerve passes.

At BS 45, the anterior tip of Meckel's cartilage is extensively endochondrally and perichondrally ossified. Most of its labial and ventral side is covered by the dentary, which extends back to the level of the jaw articulation. A well-ossified coronoid is present and fused to the perichondral ossification at the tip of Meckel’s cartilage. Dentary tooth crowns appear first, followed shortly after by coronoid tooth crowns. Neither set of teeth is attached to the bones yet. The angular has greatly expanded and covers most of the lingual and ventral side of Meckels cartilage. It is fused to the perichondral articular close to the jaw articulation, and the retroarticular process is completely perichondrally ossified.

The dentary, angular, and coronoid are slightly larger at BS 46, and additional dentary tooth crowns are present. By BS 47, Meckel's cartilage has been replaced by bone in the anterior part of the lower jaw and also the area of the jaw articulation. The retroarticular process is heavily perichondrally ossified and no cartilage remains in its posterior part. The coronoid is more extensively fused to the dentary.

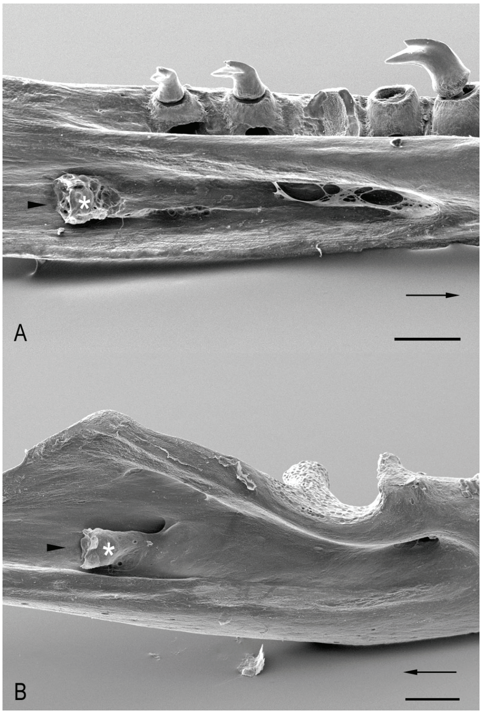

Fig. 4. The meckelian bone in the left ramus of the lower jaw of a juvenile *Hypogeophis rostratus* (Hyro 9); SEM photograph. **(A)** pseudodentary and (**B**) pseudoangular. Meckel's cartilage has been removed and arrowheads indicate its former position. Note the fusion of the ossified part of Meckel's cartilage (meckelian bone, marked with an * in **A** and **B**) to the dermal bones. Arrows point rostrally in both pictures. Scale bar equals 100 µm

In the juveniles, Meckel's cartilage is eroding and is gradually transformed into a meckelian bone that gets incorporated into the pseudodentary and pseudoangular (Fig. 4). The ossification of Meckel's cartilage seems to proceed slowly and gradually from both ends and its length is inversely correlated with that of the postembryonic specimens examined. The retroarticular process is completely ossified. Pedicels of the dentary and coronoid teeth are present and attached to their respective dental laminae.

Branchial and hyoid skeleton

The hyobranchial skeleton is first visible as very faintly stained rods of prechondral condensations and weakly chondrified cartilage at BS 38/39 (Fig. 5A). Ceratohyals and ceratobranchials I to IV are readily identifiable. The ceratohyals are medially connected by a basihyal. The basihyal connects posteriorly to a very faintly indicated basibranchial that connects ceratobranchials I and II medially. One specimen (Hyro 27) of BS 40 shows additional mesenchymatic condensation posterior to ceratobranchial IV (Fig. 5C). It is unclear whether this is an independent entity or part of the ceratobranchial IV anlage. From BS 40/41 on, most elements are well chondrified. A basibranchial is present only as a very faintly stained, thin, thread-like strand of tissue that can be seen in several specimens (Fig 4B,C,D). This thin strand of cell-rich, precartilaginous mesenchyme shows well in histological sections and connects ceratobranchials I, II and III to the basihyal along the midline. It was, however, found to be weakly chondrified in one specimen (Fig. 5C). The embryonic basihyal is somewhat triangular in shape. The ceratohyal and ceratobranchial I are simple, slightly dorsoventrally flattened, cartilaginous rods. Ceratobranchial II is much more strongly compressed. Ceratobranchial III, and particularly ceratobranchial IV are broadened medially but with very slender distal ends that are slightly bend in- and outwards. By BS 45 (Fig. 5E) all elements are well developed. In the sectioned specimens (Hyro 5 and subsequent specimens), the prechondral connection is lost between ceratobranchials I and II, but ceratobranchials II and III are still connected by a small but distinct strand of precartilage. The distal ends of ceratobranchials III are more strongly twisted dorsally and inwards. The distal ends of ceratobranchials IV are also bend dorsally. Ceratobranchial IV is well developed, articulates with ceratobranchial III medially at about one third the length of ceratobranchial III, and is comparatively broad. This appearance does not change much in later stages, although all traces of prechondral connections are gone by BS 46 (in sectioned specimens). BS 46 (Fig. 5F) is very similar to BS 45 (Fig.5 E) in that both have essentially the same shape except that the small, anteromedially directed process

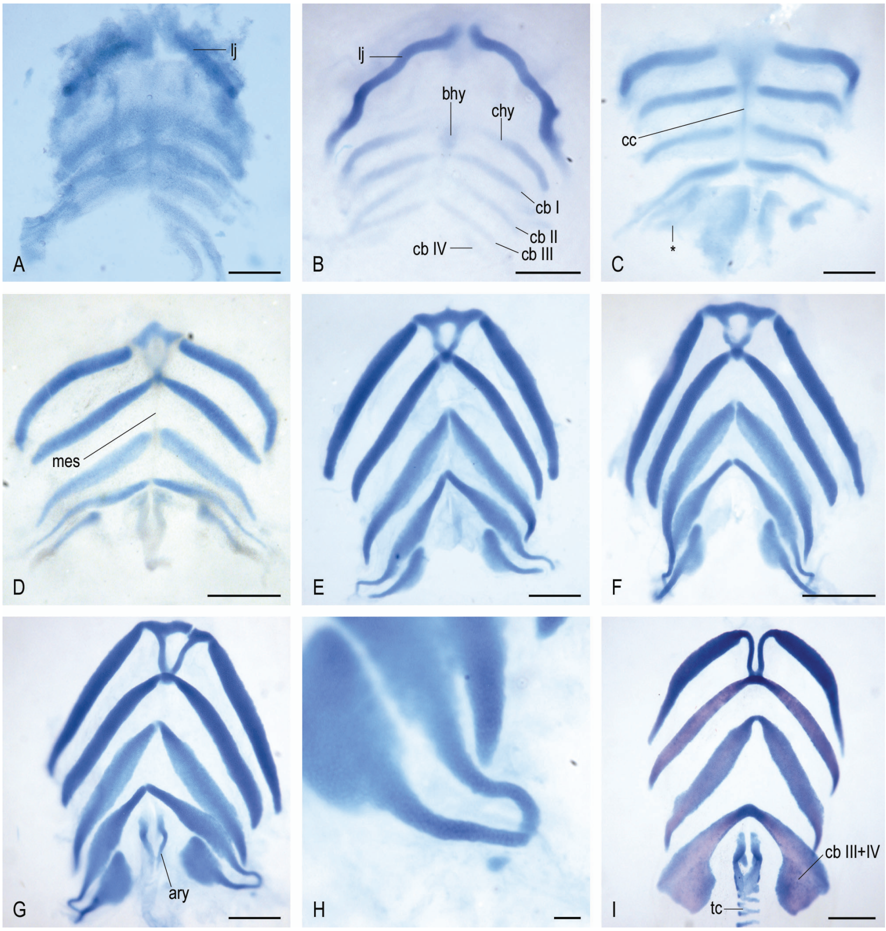

Fig. 5. Development of the hyobranchial skeleton in *Hypogeophis rostratus*, all in ventral view. **A**: BS 38/39 (Hyro 26); note that ceratobranchial III and IV are missing on the right side. **B**: BS 40 (Hyro 25). **C**: BS 40 (Hyro 27); the asterisk (*) marks the mesenchymatic condensation behind ceratobranchial IV. **D**: BS 41/45 (Hyro 31); note the median strand of mesenchyme (mes). **E**: BS 45 (Hyro 11). **F**: BS 46 (Hyro 19). **G**: BS 47 (Hyro 17). **H**: Close-up of **G** showing the cartilaginous nodule posterior of ceratobranchiale IV. **I**: juvenile (Hyro 24); note the fusion of ceratobranchiale III and IV (cb III+IV). ring cartilages posterior of the arytenoid cartilages are tracheal cartilages (tc). **A** and **B** both show the lower jaw (lj) as well. ary, arytenoid cartilage; bhy, basihyale; chy, ceratohyale; cb I, ceratobranchiale I; cb II, ceratobranchiale II; cb III, ceratobranchiale III; cb IV, ceratobranchiale IV; cc, copula communis. Scale bars equal 0.5 mm, except in **H** where the scale bar equals 100 µm.

of the basihyal is absent by BS 46. By BS 47 (Fig. 5G) ceratobranchials II, III, and especially the medial part of ceratobranchial IV, have broadened and are more plate-like. A small, cartilaginous nodule posterior to ceratobranchial IV is present in a single BS 47 specimen (Fig. 5H), but only on the left side. In juveniles (Fig. 5I), the hyobranchial skeleton has a morphology typical of most adult caeciliid caecilians (Nussbaum, 1977; Wake, 2003), in that the fused ceratohyal and basihyal are M-shaped and fused with the ceratobranchial I while the fused ceratobranchial III+IV is strongly dorsoventrally compressed and greatly expanded.

DISCUSSION

Chondrocranium

Most previous students of caecilian cranial development have remarked on the relatively similar architecture of the chondrochranium among different species (e.g. Peter (1898) and Winslow (1898) for *Ichthyophis glutinosus* and Wake and Hanken (1982) for *Dermophis mexicanus*). All conform to the general pattern of nasal and otic capsules that are chondrified to varying degrees and connected by relatively slender bars of cartilage, the taenia marginalis dorsally and trabecular cartilage ventrally that are interconnected by the pila antotica. *Hypogeophis rostratus* does not deviate greatly from this common pattern, and differs only in some minor aspects from other species. The foramen for the palatine nerve that pierces a conspicuous, broad extension of the parachordal cartilage anterior of the otic capsule, is shared with the Seychellean caeciliid *Grandisonia alternans* (Marcus et al., 1935). Reiss (1996) depicted a similar foramen in a comparable position in the rhinatrematid *Epicrionops petersi*, but considered it to be a carotid foramen. No such foramen is found in the ichthyophid *Ichthyophis glutinosus* (Peter, 1898), the caeciliids *Dermophis mexicanus* (Wake and Hanken, 1982) and *Gegeneophis ramaswamii* (Müller et al., 2005), or the typhlonectid *Typhlonectes compressicauda* (Wake et al., 1985). An unusual characteristic of the chondrocranium of *H. rostratus* is the fusion of the stapes to the parachordal cartilage. This cartilaginous connection persists in juveniles (Fig. 3G). The most variable part of the caecilian chondrocranium seems to be the nasal capsules. Compared to *Ichthyophis* (Ramaswami, 1948; Jurgens, 1971; pers. obs.), the nasal capsule of *H. rostratus* also lacks a dorsal roof but has the floor reduced to a thin, thread-like solum nasi (cornu laterale *sensu* Marcus et al., 1935), with only the lateral wall being fairly complete. The nasal septum and anterior copula are well developed and the long prenasal process extends beyond the anterior margin of the copula (*contra* Jurgens, 1971). Overall, the extent to which the nasal capsule is developed in *H. rostratus* resembles that of *G. ramaswamii* (Müller et al., 2005) and, to a lesser extent, *D. mexicanus* (Wake and Hanken, 1982). Among "higher caecilians", an informal group comprising the derived families Caeciliidae, Scolecomorphidae and Typhlonectidae (see Nussbaum, 1977, 1979; Duellman and Trueb, 1986), studied so far, *T. compressicauda* has a more extensive nasal capsule but lacks a nasal septum and prenasal process (Wake et al., 1985).

Marcus et al. (1935) described the cranium and Eifertinger (1933) and Marcus (1933) the lower jaw of a "stage 40" embryo of *Grandisonia alternans* based on a reconstruction from serial sections. The extent to which the precartilaginous nasal capsule is developed differs slightly compared to *Hypogeophis rostratus*, although this might be related to differences in differentiating precartilage from other tissue. In my specimens, I furthermore do not see the prechondral condensations above the taenia marginales that Marcus et al. (1935) interpreted as a rudimentary cartilaginous skull roof. More pronounced are the differences in the lower jaw, where Eifertinger (1933) and Marcus (1933) described a large, inward-directed process of Meckel's cartilage just posterior of the symphysis. This process is absent from all specimens of *H. rostratus* examined in this study.

The skull and lower jaw and their constituent bones

The lofty, almost fragile construction of the embryonic chondrocranium is in stark contrast with the heavily ossified skull of adult caecilians. The chondrocranium ossifies almost completely in adult caecilians, with typically only parts of the nasal capsule and anterior nasal septum, and sometimes parts of the orbital and trabecular cartilages, remaining cartilaginous (Wake, 2003). Dermal bones are extensively developed and form a complete skull roof in most adult caecilians (Taylor, 1969b).

In adult caecilians, most of the neurocranium is ossified and forms two large bones, the sphenethmoid and the os basale (Wiedersheim, 1879). These encapsulate the brain and most of the sensory organs and, together with the dermal bones, form the compact caecilian skull. A further characteristic of caecilian skulls is the high degree of fusion that occurs among the neighboring bones. In some cases, as in the os basale, dermal elements fuse with endocranial elements. In this section, I will summarize the composition of the skull and lower jaw of adult of *Hypogeophis rostratus* as proposed by Marcus et al. (1935) and compare their assumptions against my new results. For a comprehensive discussion of bone homologies see Müller et al. (2005).

Parietal. The parietals are large, paired, dermal bones that cover most of the dorsal side of the posterior half of the skull. Marcus et al. (1933, 1935) described the occurrence of a parietal foramen, sometimes closed by a separate interparietal that subsequently fuses with the parietals, and they also described additional postparietal elements. Although not observed in my material, additional smaller bones forming within the suture between larger bones are frequently found in other amphibians and their morphological interpretation is currently debated (e.g., Smirnov, 1997). If present at all, the postparietal is considered to represent a separate centre of ossification of the parietal (Straub, 1985; but see Schoch, 2002).

Frontal. According to Marcus et al. (1935), the frontal in *Hypogeophis* actually represents a temporal because it is derived from the fusion of frontal and prefrontal. They figured a very large prefrontal in a stage 47 embryo (Fig. 6A), which was not observed in similar embryos or any other *H. rostratus* embryo studied herein. According to the observations presented here, the adult frontal of *H. rostratus* arises as a single element and receives no contribution from other ossification centres.

Nasopremaxillary. According to Marcus et al. (1933), the nasopremaxillary of *Hypogeophis rostratus* is composed of the nasal, premaxilla and occasionally the septomaxilla. Subsequent studies have accepted these homologies for caeciliid caecilians in general (e.g., Ramaswami, 1948) although the septomaxillary was hardly ever observed. None of the *H. rostratus* specimens examined for this study exhibits a clearly distinct septomaxilla. When present in caeciliids, current evidence indicates that its occurrence is rather irregular and transient (Marcus et al., 1935; Wake and Hanken, 1982). Marcus et al. (1935) described most parts of the nasal and premaxilla to be of perichondral, rather than dermal, origin as commonly accepted. Examination of my histological sections shows that the perichondral lamina of the nasal capsule cartilages is unossified by the time nasal and premaxilla are well developed and that the latter two elements are separated from the underlying cartilage, although intimately associated with it. This clearly shows that both nasal and premaxilla do form as dermal bones as in all other tetrapods. Marcus et al. (1935) further suggested a separate origin of the "perichondral" part of the premaxilla and the tooth bearing alveolar process, but from the material examined here it is evident that the premaxilla forms from a single ossification centre. A so-called prepalatine, which Marcus et al. (1935) claimed to be integrated into the oral shelf of the premaxilla is absent.

Vomer. According to Marcus et al. (1935), the vomer is composed of a dermal ossification, the actual vomer, and a replacement ossification at its anterior end, which they homologized with a preethmoid. Such an element is not found in the *Hypogeophis rostratus* specimens examined here, in which the vomer arises as a single, dermal ossification.

Maxillopalatine. The maxillopalatine is a complex bone that incorporates the maxilla, palatine, and lacrimal. The largest contribution to the maxillopalatine in *Hypogeophis* is made by the palatine, with the maxillary forming the outer tooth row and parts of the orbital shelf. The maxilla and palatine each form from single centers of ossification. Marcus et al. (1935) described the palatine to be composed of the palatine and a posterior vomer, which forms the medial part of the choana, but no such separate ossification was observed herein, and the medial wing of the palatine seems to form by simple accretion. Marcus et al. (1935) further described the contribution of a lacrimal to the maxillopalatine. A small, separate ossification is present in the specimens of *Hypogeophis rostratus* observed herein and corresponds in position to the lacrimal described in *Gegeneophis ramaswamii* (Müller et al., 2005 for detailed discussion).

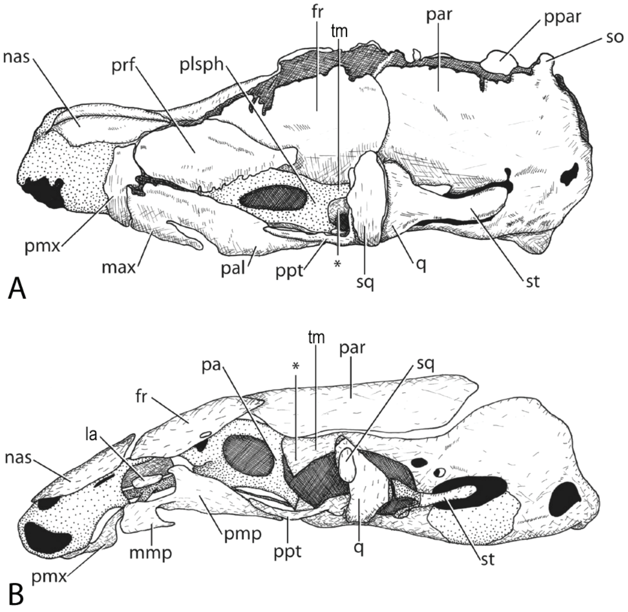

Fig. 6. Skull of embryonic *Hypogeophis rostratus* of stage 47 in lateral view. **A**: redrawn from Marcus et al. (1935). **B**: Results of this study (Hyro 18). Cartilage stippled, bone hatched. fr, frontal; la, lacrimal; max, maxillary; mmp, maxillary part of the maxillopalatine; nas, nasal; pa, pila antotica; pal, palatine; par, parietal; plsph, pleurosphenoid; pmp, palatine part of the maxillopalatine; pmx, premaxillary; ppar, postparietal; ppt, pterygoid process of the palatoquadrate/quadrate; prf, prefrontal; q, quadrate; so, supraoccipital; sq, squamosal; st, stapes; tm, taenia marginalis; asterisk (*) marks the endochondral bone lamina around the taenia marginalis that stretches onto the pila antotica. Labelling in **A** follows Marcus et al. (1935).

Squamosal. The squamosal (paraquadrate *sensu* Marcus et al., 1935) covers most of the cheek region between the eye and the quadrate. It is a dermal ossification that arises lateral to the quadrate and seems to expand rapidly at the time of hatching. Marcus et al. (1935) described the formation of periorbital elements that subsequently fuse to each other and the squamosal. Available specimens of *Hypogeophis rostratus* however did not exhibit any indication of periorbital elements, although I cannot completely rule out their occurrence in embryos very close to hatching, which are missing in my sampling.

Ectopterygoid. First described for caecilians by Wiedersheim (1879), who termed it the postpalatinum, this element was also figured and described by Marcus et al. (1933, 1935) as a free pterygoid bone that is sometimes incorporated into the maxillopalatine. The element they refer to in fact represents the ectopterygoid, as it is formed laterally of the pterygoid process of the pterygoquadrate. The true pterygoid fuses early to the pterygoid process of the quadrate (see below). The ectopterygoid was overlooked by Lawson (1963) and Müller (2003) but correctly identified by Straub (1985). The ectopterygoid is a small bone bordering the posterolateral margin of the maxillopalatine, lateral to the pterygoid process of the pterygoquadrate (Fig. 3G).

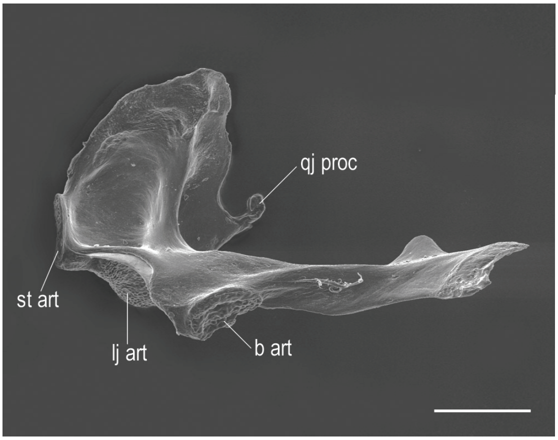

Fig. 7. SEM of medial side of left pterygoquadrate of a juvenile *Hypogeophis rostratus* (Hyro 9) showing the quadratojugular process and the articular facets of the pterygoquadrate. b art, basal articulation; lj art, lower jaw articulation; qj proc, quadratojugular process; st art, stapes articulation. Scale bar equals 250 µm.

Pterygoquadrate. The pterygoquadrate is a complex bone that forms through the fusion of the palatine to the pterygoid process of the quadrate. This fusion has also been reported for *Dermophis mexicanus* (Wake, 2003) and *Gegeneophis ramaswamii* (Müller et al., 2005). The main body of the pterygoquadrate is dorsally connected to the os basale via dense connective tissue. It has three well developed articulatory facets (Fig. 7) for articulation with the stapes (posteriorly), the pseudoangular (ventrally) and the os basale (ventroanteromedially = basipterygoid or basal articulation). The pterygoid portion extends anterior well beyond the pila antotica, overlapping the maxillopalatine dorsally. Anteroventrally a small process is found on the main body of the pterygoquadrate and assists in supporting the squamosal, which covers most of the pterygoquadrate laterally (Fig. 7). This process was described as a quadrato-maxillary (quadratojugal) by Marcus et al. (1935; see also Peter, 1898 and Reiss, 1996). However, it seemingly forms as membrane bone continuous with the quadrate and homology with a dermal quadratojugal is rejected.

Stapes. Compared to that of other amphibians, the caecilian stapes is a relatively large bone that completely fills the fenestra ovalis in adult specimens. It articulates with the os basale via an anteroventrally directed process and with the posterior side of the quadrate via an anteriorly directed process. It forms as a single element and receives no contributions from the otic capsule, contrary to Marcus et al. (1935) and Marcus (1935). Confirming previous reports (Lawson, 1963; Straub 1985), the stapes of *Hypogeophis rostratus* does not have a foramen for the stapedial artery (*contra* de Beer, 1937).

Os basale. The os basale is by far the largest and most complex bone of the adult skull and comprises the otic capsule, most of the posterolateral neurocranium, and the dermal parasphenoid. According to Marcus et al. (1935) it develops form the following individual elements: basi-, pleuro- (ex-), supra- and infraoccipital, as well as the epiotic, otic capsule, pleuro-, and parasphenoid. Based on my observations, I recognize only the following ossifications as contributing to the adult os basale of *Hypogeophis rostratus*: exoccipital, prootics (likely the otic capsule ossification of Marcus et al., 1935), and parasphenoid. A basioccipital does not occur as a separate ossification. A cartilaginous tectum synoticum is absent and the area above the foramen magnum is covered by membrane bone that extends from the exoccipital and cannot be homologized with a supraoccipital, which is a separate replacement ossification of the cartilaginous tectum synoticum (see also Brand, 1956). Marcus et al. (1935) further considered a short, dorsal process of the otic capsule to represent a rudimentary tectum synoticum, and homologized its ossified successor structure with an epiotic. A separate epiotic ossification is, however, absent. A separate infraoccipital ossification is also absent because the structure in question forms as membrane bone. A pleurosphenoid ossification is likewise absent (see Müller et al., 2005 for details).

Sphenethmoid. I fully agree with Marcus et al. (1935) that this bone is the most difficult to assess in the caecilian cranium. Marcus et al. (1935) considered the adult sphenethmoid to be composed of unpaired mesethmoid and basiethmoid and paired orbitosphenoid ossifications. The sphenethmoid is the last of the endocranial bones to form. It is first present in late BS 45 embryos, where it consists of a median bony lamina anterior to the pilae preopticae, and extends from the perichondral ossification of the nasal septum. Additionally, continuous lamellae of membrane bone extend laterally and form the anterior wall of the brain cavity (Fig. 3H,I). This membrane bone extends onto the pila preoptica and is in later stages continuous with the perichondral ossification of that structure. In the available specimens, it was not possible to demonstrate more than a single continuous ossification, and it seems possible that this bone, previously considered to be among the most complex products of fusion of multiple ossifications, is formed from only a single (or paired) ossification centre. A final decision requires a more comprehensive ontogenetic series.

Pseudodentary.—The pseudodentary is the tooth-bearing bone of the lower jaw and forms the anterior part and most of the labial side of the jaw from the anterior terminus to the jaw articulation. According to Eifertinger (1933) and Marcus (1933), it is formed by the mentomeckelian, dentary, splenial, coronoid and supraangular. Based on my observations, only the dermal dentary and coronoid (splenial of Eifertinger, 1933 and Marcus, 1933) and the endoskeletal mentomeckelian contribute to form the pseudodentary (Fig. 4).

Pseudoangular.—The remainder of the lower jaw, including the jaw articulation and the retroarticular process, is formed by the pseudoangular, which is, according to Eifertinger (1933) and Marcus (1933) formed by the angular, prearticular, complementary and articular. Of these, only the angular and articular were observed in the material examined here. In BS 40 embryos, a plate-like ossification is present on the lingual side of Meckel's cartilage, anterior of the jaw articulation. It is continuous with the angular via a narrow bony bridge. This situation resembles Eifertinger's (1933) reconstruction where it was interpreted as a prearticular already fused to the angular. Wake and Hanken (1982) did not find a prearticular in *Dermophis mexicanus* but Müller et al. (2005) reported one in *Gegeneophis ramaswamii*. Meckel's cartilage is transformed gradually into a meckelian bone that is incorporated into the pseudodentary and pseudoangular (Fig. 4).

Hyobranchial skeleton

Knowledge of the hyobranchial skeleton in caecilians stems largely from descriptions of adult morphology (e.g. Wiedersheim, 1879; Brand, 1956; Nussbaum, 1977; Wilkinson and Nussbaum, 1997). The development of the hyobranchial skeleton has been described in *Ichthyophis* spp. (Sarasin and Sarasin, 1887-1890; Peter, 1898; Visser 1963), *Gegeneophis ramaswamii* (Ramaswami, 1948; Müller at al., 2005), *Typhlonectes compressicauda* (Wake et al., 1985), and *Dermophis mexicanus* (Wake, 2003). Wake (1989) further described the metamorphosis of the hyobranchial skeleton in *Epicrionops* spp.

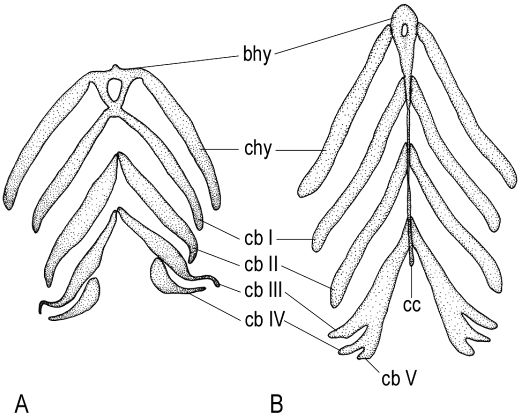

Fig. 8. Hyobranchial skeleton of *Hypogeophis rostratus* at BS 45. **A**: results of this study (Hyro 11). **B**: Redrawn from Gehwolf (1923). bhy, basihyale; chy, ceratohyale; cb I, ceratobranchiale I; cb II, ceratobranchiale II; cb III, ceratobranchiale III; cb IV, ceratobranchiale IV; cb, V ceratobranchiale V; cc, copula communis.

Marcus (1922), summarizing the work of his student S. Gehwolf, and Gehwolf (1923) described the development of the hyobranchial skeleton in *Hypogeophis*. Their descriptions differ from my observations in several points. Marcus (1922) and Gehwolf (1923) described the presence of a fifth ceratobranchial arch that fuses with the fourth and third during ontogeny (Fig. 8B). One of my specimens (Hyro 27, Fig. 5C) shows an undefined prechondral condensation posterior of the fourth arch, although it is unclear if this is a separate entity or an extension of the fourth ceratobranchial. Another specimen (Hyro 17, Fig. 5H) has a tiny cartilaginous nodule on the left side. However, well-developed fifth arch does not seem to be present in general, and Marcus' (1922) and Gehwolf's (1923) descriptions (Fig. 8) are likely based on an aberrant specimen, if correct at all (see critique of Marcus et al. below). Both workers also described a cartilaginous copula communis that connects all arches medially. Such a connection does indeed occur in the form of mesenchyme and precartilage, which may also chondrify to a certain extent (Fig. 5C). A well-developed, cartilaginous copula communis as described for older stages by Marcus (1922) and Gehwolf (1923) is, however, absent in specimens that I examined, although it must be noted that some interspecific variability might be expected here. In general, the observations and interpretations of Marcus (1922) and Gehwolf (1923) differ substantially from those presented here (Fig. 8).

Although the adult morphology of the hyobranchial skeleton of *Hypogeophis rostratus* is similar to that of other caeciliids and typhlonectids, the embryonic morphology as described here shows some obvious differences to *Dermophis mexicanus* (Wake, 2003), *Gegeneophis ramaswamii* (Müller et al., 2005) and *Typhlonectes compressicauda* (Wake et al., 1985). The embryonic basihyal, which usually is shaped like an open V, is triangular with a frontal transverse bar in *H. rostratus*. Ceratobranchial IV is much more prominently developed than in other caeciliids and typhlonectids, in which ceratobranchial IV fuses early in ontogeny to ceratobranchial III and remains vestigial throughout development. In this respect, embryonic *H. rostratus* more closely resemble larvae of *Ichthyophis glutinosus* (Sarasin and Sarasin, 1887–1890; pers. obs.).

Critique of Marcus et al.

The results presented here differ in many instances profoundly from the Marcus et al. (1935) study of skull development in *Hypogeophis* and other studies by Harry Marcus and his students (e.g., Marcus, 1933; Eifertinger, 1933; Marcus et al., 1933; Fig. 6). Marcus et al. (1935) studied only three stages, a BS 40 embryo of *Grandisonia alternans*, a BS 47 embryo of *Hypogeophis rostratus* and a 68 mm long juvenile of *G. alternans*, although it appears that they had more material available, as indicated by reference to a 42 mm "larva" of *H. rostratus* (a species with direct development). In these specimens, the skull of the BS 40 embryo is just beginning to ossify with only small ossifications of vomer and palatine present. The BS 47 embryo, however, shows a high degree of ossification of the skull and the beginning of the formation of the compound bones, such as the maxillopalatine and nasopremaxillary. Given the lack of stages crucial to the analysis if ossification, i.e. stages between BS 40 and 47, it seems unlikely that many of the ossifications reported by Marcus et al. (1935) could have actually been observed.

One of the difficulties in evaluating the results of Marcus and his co-workers is their liberal mingling of two different, though closely related, species. For their studies, Marcus and co-workers used specimens of *Hypogeophis rostratus* and *H. alternans*, of which the latter is now referred to the genus *Grandisonia* (Taylor, 1968). Although such a procedure might not be so problematic under certain circumstances, Marcus viewed both species as interchangeable, as is made clear by his statement: “…in this study both species will not be separated but the most instructive specimen will be chosen, unconcerned of the species.” (Marcus, 1908:696 [author’s translation]) and specimens were thus often simply referred to as *Hypogeophis*, without indicating which species was actually examined (e.g. Marcus, 1909). It appears that the two species do indeed have a very similar early embryogenesis (Brauer, 1897, 1899), but differences in later ontogeny are obvious, and advanced embryos of *H. rostratus* are easily distinguished from those of *G. alternans*. *Grandisonia*, which today comprises an additional 3 species (Nussbaum and Wilkinson, 1989;

Wilkinson and Nussbaum, 2005; but see Straub, 1985), furthermore seems to differ in life history from *H. rostratus*. At least some species of *Grandisonia* possess larval characters (e.g., neuromasts, gill slits) upon hatching (Parker, 1958; pers. obs.), which are lacking in the direct-developing *H. rostratus*. Some of the differences between the observations presented here and those of Marcus and co-workers might therefore be the result of specific differences, although it seems unlikely that *G. alternans* or indeed any *Grandisonia* spp. shows the characteristics described by Marcus et al. (1935), given that these are neither present in *H. rostratus* nor in the closely related *G. ramaswamii* (Müller et al., 2005). Straub (1985) called into doubt the identity of some the material identified as *G. alternans* by Marcus et al. (1933, 1935) and tentatively treated all specimens used by Marcus and co workers as *H. rostratus*.

In some instances, Marcus and co-workers explicitly mention studying *Hypogeophis rostratus*. Here, other explanations must be sought for the reported differences. A substantial proportion appear to stem from the different methods used to observe and document developmental morphology, paired with over-interpretation of the evidence. Marcus and co-workers based their observations on reconstructions from serial sections. Although this method can produce greatly magnified and very instructive models, it sometimes fails to reproduce the correct morphology (compare head curvature in Fig. 6 and hyobranchial skeleton shape in Fig. 8), because serial sections can be difficult to align and interpret. Separate elements, such as bones that are in close proximity, can be mistakenly interpreted as a single element, whereas a single but complicated structure might appear to be formed by several individual elements. For instance, Marcus et al. (1935) described the palatoquadrate to be continuous with the stapes. From my observations, it is clear that both elements are never continuous with each other, but at times they tightly abut each other, with the border between them being easily overlooked in sectioned specimens. In another instance, Eifertinger (1933) and Marcus (1933) interpreted the dorsal tip of the angular to represent a so-called complementare because it appears somewhat irregular and can sometimes be found to form separately. A more probable explanation in line with my observations is that Eifertinger (1933) wrongly interpreted a mere process of the angular as an independent element. This is supported by Eifertinger’s (1933) remark that it could only be seen in single sections. Eifertinger (1933) sectioned his material at 24 µm, which is rather thick and can therefore easily obscure continuity of an element. Where mentioned, most of the specimens used by Marcus and co-workers were also sectioned quite thickly (up to 30 µm), which is even more relevant in the very small, embryonic skulls considered here, where the anlagen of individual bones are sometimes just a few micrometers apart.

Most cases of disagreement between this and previous studies stem from what seems to be over-interpretation of the observed morphology by Marcus and co-workers that, together with the very limited number of embryonic stages studied, led to several errors. It is evident that weakly ossified areas, narrowing and broadening of an element, or indentations within an element were in many cases interpreted as evidence for a previous fusion between two independent elements, even though those postulated elements were never observed as independent structures prior to their presumed fusion. Reports of a quadratojugal (quadrato-maxillary *sensu* Marcus et al., 1935; Fig. 7) and a posterior vomer (Marcus et al., 1935) are such cases. In other instances, the presence of individual bones during ontogeny was assumed if bone was found in the skull in a position that corresponds to a separate ossification in the skull of Palaeozoic forms. The description by Marcus et al. of a pleurosphenoid and basi- and supraoccipital seem to be based on such an assumption. As can be seen in Figure 6A, Marcus et al. (1935) correctly figured the bone that envelops the pila antotica as a lamella of membrane bone that extends from the taenia marginalis onto the pila. In their description, however, they interpreted this bone to be a pleurosphenoid although no separate ossification occurs in the pila antotica. The description of these elements without their actual observation was greatly facilitated by Marcus’ assumption that caecilians are direct descendants of Palaeozoic forms (so-called stegocephalians), specifically aistopods (Marcus et al., 1933; Marcus, 1933) and unrelated to other living amphibians. He was followed in that by all of his students, which resulted in a sometimes bizarre distortion of the evidence.

Eifertinger (1933), for example, in the concluding remarks to his study of the caecilian lower jaw, stated that the observed high number of individual elements supports a grouping with aistopods, yet acknowledges that lower jaws of aistopods were unknown at that time.

stage	skull	lower jaw
38/39		dentary
40/41	palatine vomer	angular mentomeckelian
	exoccipital premaxilla prootics pterygoid	
41/45	quadrate	articular
	frontal maxilla parietal pterygoquadrate	
45	anterior part of parasphenoid squamosal	coronoid
	lacrimal maxillopalatine nasal sphenethmoid stapes	pseudoangular pseudodentary
46	posterior part of parasphenoid	
47	ectopterygoid lacrimal-maxillopalatine	
	nasopremaxillary	
>47	os basale	

Table 1. Ossification sequence of *Hypogeophis rostratus*. Elements are listed according to their first appearance. The sequence of the elements within boxes could not be resolved.

Ossification sequence (Table 1)

Some of the available earlier embryos are decalcified to various degrees, likely as a result of fixation or storage, so I have relied mostly on the sectioned material and later, better-preserved embryos for reconstructing the ossification sequence of *Hypogeophis rostratus*. The dentary is the first ossification to appear, followed by the angular, vomer, palatine, mentomeckelian, exoccipital, pterygoid and premaxilla. These are followed by the prootics. Shortly after ossification of the articular and quadrate, the parietal, frontal, and maxilla appear and the pterygoid fuses with the quadrate to form the pterygoquadrate. This is followed by ossification of the coronoid, parasphenoid, squamosal, and later by the nasal, lacrimal, sphenethmoid and stapes, and the formation of the maxillopalatine, pseudoangular and pseudodentary. The last dermal elements to appear are the ectopterygoid and the posterior part of the parasphenoid, at which point the lacrimal fuses to the maxillopalatine and, at a later stage, the nasopremaxillary and os basale form.

The ossification sequence reported here for *Hypogeophis rostratus* is relatively similar to that of *Gegeneophis ramaswamii* (Müller et al., 2005). Differences include the early formation of the squamosal as compared to the nasal. Information on ossification sequences of caecilians is very fragmentary and comprehensive ossification sequences are only available for *Dermophis mexicanus* (Wake and Hanken, 1982) and *G. ramaswamii* (Müller et al., 2005). The direct developing *H. rostratus* and *G. ramaswamii* have a more similar ossification sequence as compared to that of the viviparous *Dermophis mexicanus*. All three species are characterized by derived reproductive modes and a functional correlation between these and the observed ossification sequences might be expected. In *D. mexicanus*, for example, the comparatively early onset of the ossification of the jaw articulation is seemingly correlated with intrauterine feeding at an early stage (Wake and Hanken, 1982). Müller et al. (2005) reviewed the literature with regards to ossification sequences in caecilians, and concluded that the available data is currently insufficient to enable a robust inference of the evolution of ossification sequences in caecilians.

Conclusion

From the mid 1900s until his emigration from Germany in 1938, fleeing persecution by the Nazi regime (Tonutti, 1977), Harry Marcus and his students conducted a very extensive research program on the morphology of just two caecilian species, *Hypogeophis rostratus* and *Gegeneophis alternans*. They contributed a sizeable amount of data that forms a considerable proportion of our still relatively scant knowledge of caecilian morphology. Their work, due to the paucity of data on other species, was often seen as representative for all caecilians and thus has been very influential in other workers' interpretations of caecilian morphology (e.g., Ramaswami, 1948; Visser, 1963; Carroll and Currie, 1975), and still resonates in the debate about the phylogenetic position of caecilians. Subsequent workers, however, have pointed out inconsistencies (Brand, 1956) and, more recently, incongruence (Wake and Hanken, 1982; Müller et al., 2005) with results reported by Marcus and students. Here I have shown that many of the reported ossification centers supposedly forming the adult cranium are absent during development, and that their description was based largely on a misinterpretation of the observed morphology promoted by misplaced phylogenetic assumption. In my work, I have focussed on works of Marcus and his students that concern the morphology and development of the skull, lower jaw and hyobranchial skeleton (Marcus, 1922, 1933, 1935; Gehwolf, 1923; Marcus et al. 1933, 1935; Eifertinger, 1933). Marcus and students also worked on other organ systems in caecilians (e.g., Marcus, 1923; Marcus and Albrecht, 1936) and although no attempt has been made to assess the accuracy of those observations, I suggest that these publications should be approached carefully and caution against their uncritical use. Problems with previous works and the small amount of available comparative data should encourage new, sorely needed investigations into caecilian developmental biology.

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Appendix. List of material examined.

stage	study ID (collection number)	total length in mm	preparation
37	Hyro 30 ex. ZMB 35003-016	19	cleared and stained
38	Hyro 12 ex. ZMB 34665-760	24	cleared and stained
38/39	Hyro 13 ex. ZMB 34665-760	24	cleared and stained
38/39	Hyro 26 ex. ZMB 34665-760	25	cleared and stained
40	Hyro 1ex. ZMB 35044-070	26	serial sections
40	Hyro 27 ex. ZMB 34665-760	24	cleared and stained
40	Hyro 25 ex. ZMB 34665-760	25	cleared and stained
40/41	Hyro 15ex. ZMB 35003-016	27	cleared and stained
41	Hyro 16ex. ZMB35003-016	28	cleared and stained
41	Hyro 28 ex. ZMB 34665-760	27	cleared and stained
41	Hyro 14ex. ZMB 34665-760	28	cleared and stained
41/45	Hyro 29 ex. ZMB 34665-760	28	cleared and stained
41/45	Hyro 4ex. ZMB 35044-070	36	serial sections
41/45	Hyro 31ex. ZMB 35003-016	30	cleared and stained
45	Hyro 32ex. ZMB 35003-016	30	cleared and stained
45	Hyro 5ex. ZMB 35044-070	38	serial sections
45	Hyro 11ex. ZMB 34865-887	42	cleared and stained
46	Hyro 2ex. ZMB 35044-070	50	serial sections
46	Hyro 19ex. ZMB 34665-760	47	cleared and stained
46	Hyro 20ex. ZMB 34665-760	45	cleared and stained
46+	Hyro 6ex. ZMB 35044-070	50.7	serial sections
47	Hyro 21ex. ZMB 34665-760	45	cleared and stained
47	Hyro 18 ex. ZMB 34665-760	45	cleared and stained
47	Hyro 17ex. ZMB 34665-760	51	cleared and stained
47	Hyro 7ex. ZMB 35044-070	56	serial sections
juvenile	Hyro 22ex. ZMB 34761-864	73	cleared and stained
juvenile	Hyro 8ex. ZMB 34761-864	73	serial sections
juvenile	Hyro 23ex. ZMB 34761-864	82	cleared and stained

juvenile	Hyro 24ex. ZMB 34761-864	82	cleared and stained
juvenile	Hyro 9 ex. ZMB 34761-864	86	cleared and stained; SEM
juvenile	Hyro 10ex. ZMB 34761-864	127	cleared and stained
adult	Hyro 33 ex. ZMB 34565-578	240	manual dissection