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The Morphology of Pre-hatching Embryos of *Caecilia orientalis* (Amphibia: Gymnophiona: Caeciliidae)

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Running head: Pre-hatching caecilian embryo morphology

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ABSTRACT
A clutch of advanced embryos of the direct-developing caecilian *Caecilia orientalis* (Caeciliidae: Gymnophiona: Amphibia) was collected in the field in Ecuador. Specimens were cleared and stained in order to evaluate skeletal development (each element of the chondrocranium, dermatocranium, jaws and teeth, hyobranchial apparatus, vertebrae) at near-hatching. Because it is established that development is correlated with reproductive modes in a number of features, we included comparison with taxa that represent the major reproductive modes and all of the modern normal tables and ossification sequences. The embryos most closely resembled stage 47/48 *Gegeneophis ramaswamii*, an Indian caeciliid, and stage 45-48 *Hypogeophis rostratus*, a Seychellian caeciliid, both direct developers, in details of bone mineralization, chondrocranial degeneration, and vertebrogenesis, and stage 42 *H. rostratus* in external features (gills, pigmentation, etc.). They were less like pre-hatchlings of *Ichthyophis kohtaoensis*, an ichthyophiid with free-living larvae, and fetuses of the viviparous caeciliid *Dermophis mexicanus* and the viviparous typhlonectid *Typhlonectes compressicauda* at comparable total lengths. A correlation of developmental features with mode of life history is implied. A noteworthy feature is that the direct-developing *C. orientalis* has an armature of multiple rows of teeth on the lower jaw with tooth crowns that resemble the “fetal” teeth of viviparous taxa and that are covered with a layer of oral mucosal epithelium until full development and eruption, but the upper jaw bears a single row of widely spaced, elongate, slightly recurved teeth that resemble those of the adult.

Key words: caecilian, direct development, bone mineralization, tooth crowns
Caecilians (Amphibia: Gymnophiona) are elongate, limbless, tailless or nearly so animals that inhabit most of the tropical regions of the world. They are not often observed because they are fossorial or secondarily aquatic to semi-aquatic. However, information is accruing about their biology and the nature of their adaptive radiation. In particular, their reproductive biology is of interest because it includes several different modes. Apparently all caecilians have internal fertilization via the male intromittent organ inserted into the vent of the female to transport sperm. Eggs may be laid terrestrially shortly after fertilization, with the mother guarding the clutch until hatching, when larvae wriggle into streams for a relatively lengthy period up to one year before metamorphosing and returning to land. Alternatively, development through metamorphosis ensues before hatching and a juvenile emerges, obviating the aquatic, free-living larval stage. In addition, a number of species are viviparous, retaining the developing embryos in the maternal oviducts through metamorphosis, so that juveniles are born. Yolk is resorbed early during the gestation period, which may be 7-11 months depending on the species, and nutrient secretions from the mother’s oviductal mucosa nourish the fetuses, which actively “forage” in the oviducts (see Wake, 1977ab; Himstedt, 1996; Exbrayat, 2000, 2006 for summaries). Recently, it has been observed that two distantly related direct-developing caeciliid species, the east African *Boulengerula taitanus* and the South American *Siphonops annulatus*, have precocial hatching and the young eat the skin and its secretions of the mother (Kupfer, et al. 2006; Wilkinson et al., 2008).

*Caecilia orientalis* (Taylor, 1968) occurs in the eastern lowlands of Ecuador, and its reproductive mode was unknown until the discovery of an egg clutch at
the Yanayacu Biological Station in January 2001 (Funk et al., 2004). The rare availability of a clutch of living embryos of the direct-developing *C. orientalis* allowed examination of several aspects of their biology. For example, an observation derived from the single clutch of *C. orientalis* has yielded information about caecilian evolution. The lamina-associated polypeptide 2 (LAP2) is an integral protein of the inner nuclear membrane in mammals, fish and frogs (reviewed by Dechat et al., 2000; Prüfert et al., 2004). LAP2 expression in somatic cells of *C. orientalis* and *C. guntheri* is strikingly different from that of anuran and urodele amphibians (del Pino et al., 2002). Caecilian somatic cells include three LAP2 isoforms with electrophoretic motilities comparable to those of the LAP2 α, β, and γ of mammals (del Pino et al., 2002). Mammalian cells express mainly LAP2 α, β, and γ whereas in somatic cells of the frog *Xenopus laevis* only LAP2 β isoforms have been detected (Lang et al., 1999). Consequently, LAP2α is considered a mammalian character, and has not been detected in cells of frogs, fish and the chick (del Pino et al., 2002; Lang et al., 1999; Prüfert et al., 2004). Further analysis of the LAP2 isoforms found in somatic tissues of caeciliids and other caecilians is required to determine whether the caeciliid LAP2 pattern of somatic cells is molecularly similar to that of mammals. We mention this example to support the necessity of fieldwork for molecular studies and the multidisciplinary approach to research on rare taxa.

We present information on the developmental morphology of the embryos of *C. orientalis* based on examination of living and preserved members of that one clutch known to science. Caecilian development is known from only a few normal tables and descriptions of various stages of embryos and larvae for very few species. Because so
little is known, this report on a clutch at a particular stage of development presents new information to contribute to the comparative developmental morphology of caecilians.

**MATERIALS AND METHODS**

**Specimens**

Three adults (two males and a female), an uncharacterized individual, and an egg clutch were found under a large decomposing log at the Yanayacu Biological Station, Napo Province, Ecuador at 00°35’S 77°53’ W. The station is located in a region of cloud forest at an altitude of approximately 2100 m on the east side of the Cordillera Oriental of the Andes. The adults and two embryos were preserved and deposited in the Museum of Zoology of the Pontificia Universidad Católica del Ecuador (QCAZ). The remaining five embryos were maintained for further study. The simultaneous occurrence of adults and the egg clutch under a log allowed identification of the specimens as *Caecilia orientalis* (see Funk et al., 2004).

**Culture medium and fixation**

The embryos of *C. orientalis* were cultured by two methods: (1) the egg clutch was maintained in a humid chamber that consisted of a 10 cm Petri dish with a bottom covered by wet filter paper. The egg clutch was placed over a small piece of plastic foil to prevent the egg jelly from sticking to the filter paper. (2) An embryo, dissected from the jelly capsule, was maintained in 0.15x Steinberg's solution (58 mM NaCl, 0.65 mM KCl, 0.85 mM MgSO₄, 5 mM Tris (pH 8.0), 0.34 mM Ca(NO₃)₂₂⁻, [Rugh, 1962]).

Two methods of fixation also were used: for bone and cartilage staining, the embryos were fixed in 10% formalin in phosphate buffered saline (PBS: 137 mM NaCl, 3 mM KCl, 1.5 mM KH₂PO₄, 7 mM Na₂HPO₄, pH 7.4). One embryo was fixed for
scanning electron microscopy in MEMFA buffer at room temperature for 12 hours, and
stored in methanol at -20 °C (Harland, 1991).

**Clearing and staining**

Fixed embryos were eviscerated and stained with Alizarin Red and Alcian Blue. The specimens were cleared in 0.5 % KOH and stored in glycerol at – 20 °C according to (Jegalian and de Robertis, 1992).

**Maintaining the clutch**

One embryo was released with forceps from the jelly capsule and was immersed in 0.15x Steinberg’s solution, a dilute saline solution used for the culture of the aquatic embryos of frogs. The Petri dish was tilted to allow the hatchling to remain in the aquatic medium or to move away to the dry environment. That hatchling was processed for molecular analysis of proteins (del Pino et al., 2002), and for the morphological study of the skeletal system.

In the absence of information about the developmental biology of *C. orientalis*, the remaining embryos were cultured in a humid chamber at room temperature (approximately 20 °C) for four days. Fungal growth was discovered that covered the jelly capsules, and the embryos died. Two dead embryos were released from the jelly capsules and were fixed and stained for bone and cartilage. The remaining embryos were fixed in 10% formalin within the jelly capsules.

**SEM**

The left ramus of the lower jaw was excised. The fixed tissue was dehydrated through a series of 80%, 95% and 100% ethanol concentrations followed by critical point drying in an Autosamari-815 critical point dryer. The dried tissue was mounted onto
aluminum stubs with conductive carbon tape then sputter coated to 8.4 nm with iridium in a Med 020 sputter coater. Observations and photomicrographs were made with a Philips XL-30 scanning electron microscope. Photomicrographs were labeled using Adobe PhotoShop.

**Staging the embryo**

We followed Müller et al.’s (2005) assessment that the normal tables and developmental sequences reported for species that are egg-laying with free-living larvae (e. g., Dünker et al., 2000) and viviparous species (e. g., Wake and Hanken, 1982; Sammouri et al., 1990) are not appropriate for comparison with direct-developing taxa. Therefore, we refer to Brauer’s (1899) evaluation of development in the direct-developing *Hypogeophis rostratus* and, like Müller et al. (2005), used Brauer’s ‘stages’ for our assessment of the state of development of our embryo of *C. orientalis*.

**RESULTS**

*Caecilia orientalis* egg clutch

The egg clutch consisted of nine egg capsules. Seven capsules contained embryos; two capsules were empty and collapsed. The egg capsules were elongated with an average length of 10.4 mm and a mean diameter of 8.4 mm (Funk et al., 2004), and were connected to each other by thick cords of egg-jelly (Fig. 1A). The jelly cords formed a central knot to which all of the capsules were attached, similar to the clutches of *Ichthyophis glutinosus* (Sarasin and Sarasin, 1887-90; Breckenridge and de Silva, 1973; Breckenridge and Jayasinghe, 1979), *I. kohtaoensis* (Himstedt, 1996), *G. carnosus* (Seshachar, 1942), and *S. annulatus* (Gans, 1961), for example. The mother moves the eggs in the nest, leading to the formation of the central knot of egg cords, in *I.*
kohtaoensis (Himstedt, 1996). A similar feature of parental care may occur in C. orientalis.

When an embryo was released from its capsule in the lab, it rapidly moved away from the water in its tilted Petri dish. It reacted in a similar way whenever it was immersed in the solution. The hatchling was vigorous and moved with an undulating motion. The water avoidance behavior observed and the empty capsules suggest that the embryos of this egg clutch were near the time of hatching. In fact, the empty jelly capsules suggest that two embryos already hatched.

The hatchling measured 40.0 mm total length (TL). The body was light brown with obvious melanocytes, and the small eyes were darkly pigmented (Fig. 1A-D). It had three pairs of gills with numerous branches and an obvious vascular network. Two gills on each side were large (left: 3.4 mm long with 25 filaments and 2.6 mm with 19 filaments; right: 4.1 mm long with 28 filaments and 3.4 mm with 21 filaments) and the third gills were very small (left: 0.7 mm long with 7 filaments, right: 0.3 mm with 4 filaments) (Fig. 1BCD). The hatchling had a small dorsal tail fin (Fig. 1B), comparable to that of stage 36 embryos of Ichthyophis kohtaoensis (Dünker et al., 2000).

**Developmental osteology**

*The skull*

*Chondrocranium:* The chondrocranium is still well formed at stage 47 (Figs. 2AB, 3ABC). There is limited chondroclastic activity despite some ossification. The ventral trabeculae and parachordals are well established; the orbital cartilages are elongate and supported by stout pilae; posteriorly the orbital cartilages connect to the
taenia marginales, which connect posteriorly to the large otic capsules (Figs. 2AB, 3AC). Anteriorly, large nasal capsules attach to the trabeculae ventrally and the orbital cartilages dorsally (Figs. 2AB, 3A). The capsules appear to lack copulae anteriorae, and the prenasal process is short and blunt (Fig. 3A). The capsule cartilage is eroded ventrally, but not yet becoming invested with bone (Fig. 3C). The otic capsules are cartilaginous and complete, have started ossification around their peripheries, and are attached to the taeniae and the parachordals (Figs. 2AB, 3A). The taenial extension that curves ventrally and connects to the capsule is ossifying and the cartilage is resorbing, as is that of the parachordals between the postoptic and preotic pilae (Figs. 2AB, 3A). The quadrate is cartilaginous medially and dorsally, with some bone investing it, but its articular component is well ossified and the cartilage is resorbed, except for the articular cap (Figs. 2AB, 4A). A pterygoid process is not apparent. The columella/stapes is largely cartilaginous with some slight ossification beginning on the anterior head (Fig. 2AB). Meckel’s cartilages remain pronounced, with large medial bosses and a shaft that extends to the level of the articulation, but its retroarticular process is nearly fully resorbed (Figs. 2AB, 4A). Extensive dermal ossification around the Meckel’s cartilage elements is established (see below). The occipital condyles are ossified (Figs. 2AB, 3A), and the ossifications represent the exoccipital elements. We see no evidence of a chorda dorsalis extending into the skull cavity, but, despite the limited cartilage resorption of the posterior part of the skull, it may have been resorbed.

**Dermatocranium:** In dorsal view, lateral strips of bone representing the frontals (over the eyes) and the parietals (much the longer) have formed (Fig. 2B); posteriorly, the parietal has some ossification that is spreading medially. There is an ossification site anterior to
the frontal slip that likely represents the nasal. The squamosal appears to be forming from a medial low, lateral slip that overlies the quadrate and is barely mineralizing anteriorly. The maxilla is well formed, with a strong dentigerous process bearing a number of teeth in a single row and a flared flat plate anterior to the orbit (Figs. 2B, 3A). Paired premaxillae are formed; the dentigerous processes are well ossified, each bearing 4 teeth, two attached to pedicels and two crowns forming between the well-developed teeth (fig. 4B). Ossification of the dorsal processes has commenced. Ventrally, the vomers are small mineralizing patches that lack dentigerous processes (Fig. 3A), and the palatines are well ossified dentigerous arcs that bear a single row of teeth (Fig. 3AC). The palatines have faintly staining ossification that extends from the dentigerous process toward the maxilla (fig. 3AC), but the elements are not yet fully fused to the maxilla. The os basale and the sphenoid process are beginning to mineralize as thin struts of ossification (Fig. 3A).

*Lower jaw:* The lower jaws are well developed. The pseudangular with the articulation facet and the retroarticular process are well ossified, covering the short Meckel’s cartilage retroarticular process which is nearly fully eroded by chondroclasts (Figs. 3AB, 4A). A cartilage cap remains on the articular surface; it contacts the cartilaginous end of the quadrate (Fig. 4A). The dentigerous plate is stout and extends from the anterior end of the jaw nearly to the articular facet (Fig. 2AB). It bears several rows of numerous teeth of several different shapes (see Dentine).

*Hyobranchial apparatus:* The hyobranchium is nearly completely formed (fig. 3D); the cerathyals are fused to a small remnant of the basihyal by long descending processes; the medial halves of the blades are flared, tapering laterally. The paired
ceratobranchial (CB) I is fused to a lighter-staining basibranchial I remnant at its lateral edges. The blades are moderately broad and taper slightly laterally. Ceratobranchials II are free, fused medially to the lighter-staining remnant of basibranchial II, the blade width similar to that of CB I. Ceratobranchials III and IV are fused together broadly medially, obliterating any remnant of CB III-IV, and the blades of both elements are fused continuously from medial to lateral except at their ends, where CB III still have broad, free ends, and the recurved part of IV is expanded and ventral, rather than lateral. A small remnant of CB V appears to be fused medially to the margin of each CB IV. These elements are filling in with cartilage to form the rounded plates typical of the adult cartilaginous hyobranchium. Paired laryngeal cartilages are centered between CB III-IV. See figure 3D.

**Dentition:** As observed in cleared and stained specimens, all of the teeth are pedicellate. Those on the lower jaw are ‘fetal’, in the sense that none have the elongate recurved unicuspid teeth of an adult. The crown shapes are all elongate but variously have peg-like narrowing apices, to spatulate apices, to apices that broaden slightly and have small spicule-like projections (Fig. 3B), reminiscent of early fetal teeth in the viviparous *Gymnopus multiplicata* and *Dermophis mexicanus* (Wake, 1976, 1977ab, 1980). However, all shapes can occur in a given row of teeth, unlike the situation in the latter taxa. Teeth posterior on the dentigerous process are as well developed and mineralized as those medially (Figs. 2AB, 4AB). There appear to be at least four rows of teeth on the jaws (Fig. 4B), similar to the aggregations seen in fetuses of viviparous caecilians. However, there is a single row of teeth, both fixed to pedicels and ‘replacement’ crowns, in the maxillary-premaxillary arcade (Fig. 3B) and on the palatines.
(Fig. 3AC), similar to the adult condition and more well formed than in fetuses of viviparous taxa observed. Premaxillary teeth are widely spaced, elongate but not recurved structures (Fig. 3B). The apices of the crowns are rounded rather than pointed, the latter the adult condition. Newly mineralizing crowns from alternate tooth loci occur between pedicellate teeth (Fig. 3B), presumably the ‘replacement’ teeth that will become associated with pedicels when the currently attached crowns are shed. The palatine teeth have much shorter crowns that are flat with lateral points, some associated with pedicels, some newly developing.

Scanning electron microscopy of the dentition of the lower jaw reveals that many teeth that appear to be fully erupted are still covered with a thin layer of cellular epithelium that extends over the elongate tooth crowns (Fig. 4C). Five rows of teeth are present medially, three postero-laterally. Typical of caecilian ‘fetal’ dentitions, the teeth on the labial margin are more fully developed and erupted than those closer to the lingual margin of the jaw. Only the nearly fully developed crowns have penetrated their epithelial covering (Fig. 4D). Those of the three labial-most tooth rows are bare of the epithelium, which surrounds only the bases of those crowns (Fig. 4D). Newly erupted crowns are covered with cellular debris (Fig. 4D), which is not present on ‘older’ crowns. Crown shapes are nearly uniform, with a basal crown stalk that expands to a slightly bulbous, expanded apex that bears four to six low spicules, more pronounced on some crowns than on others (fig. 4D). The labial face of each crown is slightly cupped.

The vertebrae and ribs

The embryo has 125 vertebrae in various stages of development (Fig. 5AB), most advanced in anteriormost vertebrae in the typical highly cephalized pattern of caecilians
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(Wake and Wake, 2000). Taylor (1968) in his description of the species reported that 120-128 vertebrae are present in adults (based on x-rays of two specimens). Our count of 125 vertebrae in the stage 45 embryos indicates that all vertebrae present in the adult are formed by that stage of development. The first three vertebrae have extensive ossification evident (Fig. 2A). The cartilaginous neural arch of the atlas is fully invested with bone, the centrum and neural arch rudiments of the axis have a thin coat of bone, but retain considerable cartilage, and the next vertebra has a well ossified centrum with mineralization of neural arch pedicels and rudiments. The atlas retains a notochordal rudiment that projects anteriorly, but it does not reach the foramen magnum (Fig. 3A).

Behind those, centrum ossification is evident in at least 40 vertebrae (Fig. 5A), strongest in the anterior half, progressively lighter and more restricted in the posterior half, then more posteriorly ossification is present only investing the anterior half of the centrum (Fig. 5A) (posterior sclerotomite-half of the more anterior somite: see Wake and Wake, 2000). Ventral keels are apparent, although not well developed. Only the anterior-most neural arches have significant ossification. Clearly, centrum ossification precedes that of the neural arches. More posteriorly, vertebrae are cartilaginous with well-formed centra, neural arches, and rib-bearing processes (Fig. 5B). Cartilage density varies along the column, being most extensive anteriorly, and a boss of dense cartilage typically forms at the juncture of the apex of the neural arch. In the most posterior vertebrae, the centrum is a slender cartilaginous structure that forms a continuous ring with the neural arches, and the notochordal cartilage is prominent, both between vertebrae and through the centers of the centra (Fig. 5B). Dorsal and ventral rib-bearers have developed on the anterior vertebrae and are associated with bifid rib heads; the ribs of the anterior-most vertebrae
have grown longer and more curved latero-ventrally (Fig. 5A). On the vertebrae of the posterior half of the column, neither rib heads nor rib-bearers have differentiated, and the connection is continuous, with rib struts more poorly developed, especially in length (Fig. 5B), concomitant with the antero-posteriorly graded development of the entire column.

DISCUSSION

Comparison with development in other species of caecilians

The embryos in the clutch of *C. orientalis* present a mosaic of developmental features. Because we have only one stage of development, we cannot comment on the ossification sequence relative to that of other caecilians, except to compare the presence, developmental state, or absence of elements. Furthermore, because our embryos are in a relatively late stage of osteogenesis, we cannot definitively assess some issues of homology of certain relevant elements (e.g., some of those of the lower jaw and of the anterior membrane bones of the skull). The difficulty is compounded because there currently are only three relatively comprehensive accounts of skull development available in the literature, those of Wake and Hanken (1982) for the viviparous, New World caeciliid *Dermophis mexicanus*, Müller et al. (2005) for the direct-developing, Indian caeciliid *Gegeneophis ramaswamii*, and Müller (2006) for the direct-developing, Seychellian caeciliid *Hypogeophis rostratus*. Each of these presents summary discussions, and those by Müller include excellent, current summaries of the history of ideas about skull development and of differences in reported sequences. Wake (2003) also reviewed the data and the ideas. The incomplete developmental sequence of the direct-developing, Seychellian *Hypogeophis rostratus* (which included a stage of
Grandisonia [then Hypogeophis] alternans, a form with free-living larvae) that Marcus (1933), Eifertinger (1933) and Marcus et al. (1935) described and interpreted was highly influential in considerations of comparative skull development for some time, preceding the work of de Beer (1937) and many others, until Wake and Hanken (1982) and then especially Müller (2006) questioned both data and interpretations. Each species that is reported presents new information about skull development, so we believe it appropriate to compare the stage of development of the skeleton of C. orientalis with that of the paucity of other taxa for which there is information.

Because it is now well established that there is a correlation of some features of development in amphibians with their general reproductive mode (Wake and Hanken, 1982; Hanken, 1992; Wake, 1982, 1989, 1993, 2006; Wake and Dickie, 1998; Müller, 2006), we compare our data for C. orientalis with the direct-developing H. rostratus (Brauer, 1897, 1899; Marcus, 1933; Marcus et al., 1935; Müller, 2006) and G. ramaswamii (Müller et al., 2005), and then with the viviparous D. mexicanus (Wake and Hanken, 1982) (all terrestrial caeciliids, but from the Seychelles, India, and Central America, respectively), and then in part with the aquatic, viviparous typhlonectid T. compressicauda (Sammouri et al. [1990], external morphology only; Wake et al. [1985], chondrocranium of a few stages only) at the comparable stage of general development (given our caveats about staging). Our hypothesis is that the state of skeletal development of C. orientalis will most closely resemble that of the similarly direct-developing caeciliids, then that of the viviparous caeciliid, and least like that of the aquatic, viviparous typhlonectid, which is very different in several respects from the
viviparous caeciliid. We briefly consider the effects of phylogenetic relationship, reproductive modes, and biogeographic sphere.

Predictably, not all external characters ‘fit’ a particular stage in diverse taxa. We found that our specimens of *C. orientalis* generally agree with stage 42 of *Hypogeophis rostratus* in terms of external morphological features (three gills, one reduced, on each side; pigmentation; head morphology; etc.), although it resembles ‘stage’ 48 in yolk resorption. However, the embryo most closely resembles the direct-developing *Gegeneophis ramaswamii* of ‘stage’ 47-8 in many aspects of skeletal development, although there are interesting variations in ossification states.

Because we are comparing only one example of development (two embryos at nearly the same stage of development from a single clutch), we must consider those embryos in the context of the several stages reported for the comparator taxa. The more complete reports of skull development in comparator taxa allows cross-stage comparison in order to “locate” the point in the developmental trajectory of *C. orientalis*. The state of ossification of the skull of *C. orientalis* is much like that of a stage 47/8 *G. ramaswamii*, with most of the same elements present (see Table 4, Müller et al., 2005). That stage in turn resembles that of 45-48 of *H. rostratus*, so Müller’s (2006) comparison of *G. ramaswamii, H. rostratus*, and *D. mexicanus* is apt. We see several similarities of *C. orientalis* to *G. ramaswamii* and to *H. rostratus* in terms of state of choncrocranium development and degeneration as endochondral bone development ensues, and dermal elements invest the skull and lower jaw. However, we do not see separate prootics or lacrimals present in both *G. ramaswamii* (Müller et al., 2005) and *H. rostratus* (Müller, 2006), nor do we see indications that such elements are already fused to others; this might
reflect either 1] their absence, 2] development later, which would be unusual, or 3] fading staining. We find that in one embryo of *C. orientalis* the os basale is a scant but fully distributed sheet of mineralization, unlike that in the comparator taxa. This suggests that ossification of the entire os basale occurs virtually uniformly (probably except for the elements surrounding the brainstem-spinal cord), in contrast to the pattern of the dorsal membrane bone elements in which mineralization spreads medially from lateral, linear sites of ossification. Furthermore, the posterior region of the skull of *C. orientalis* has considerably less degeneration of the chondrocranium (e. g. of the otic capsules, etc.) than does that of *G. ramaswamii* at the comparable stage. The prominent and elongate chorda dorsalis of the stage 45 (and preceding) *G. ramaswamii* and *H. rostratus* is not present in our embryos, either because it is not so extensively developed, or because it has already resorbed.

However, more substantial differences exist in postcranial development, at least between *C. orientalis* and *G. ramaswamii* (Müller [2006] restricted his analysis of ossification in *H. rostratus* to that of the skull and hyobranchial apparatus). In *C. orientalis*, the atlas, the axis, and the next two vertebrae are well ossified, the axis-atlas complex being well ossified including the neural arches, the latter two vertebrae having ossified centra and neural arch pedicels. The first 40 or so vertebrae have ossifying centra with mineralization progressively slighter posteriorly, as noted above. The anterior 60 or so vertebrae have rib-bearers and ribs, progressively less well developed further away from the head. Cartilage of the neural arches and centra of the posteriormost 30 vertebrae is weakly stained, and the elements are more fragile in appearance, retaining a prominent notochord. In contrast, in *G. ramaswamii* (Müller et al., 2005), at stage 38 the atlas and
the anterior vertebrae are cartilaginous; at stage 40, neural arches of all vertebrae are almost fully developed, and the atlas and first 70 vertebrae have ossified centra; at stage 45 all neural arches are chondrified, and all centra except those of the last five vertebrae and most neural arches are ossified; at stage 47/8 all neural arches are ossified and the cartilaginous ribs are well developed, but not ossified; at stage 49, there is complete ossification of all vertebrae and ribs with no vestiges of cartilage. These differences suggest that development in *C. orientalis* may be somewhat more cephalized than in *G. ramaswamii*, with slower development of postcranial elements relative to that of the skull.

The teeth of the lower jaw of the near-hatching embryos of *C. orientalis* strongly resemble fetal teeth in viviparous species in having several rows with development of those rows from the lingual aspect so that the first-developed rows are more labial, and newer ones more lingual; addition is also antero-medial to postero-lateral, as is typical of both fetal and adult dentitions. Crown shape in lower jaw teeth of *C. orientalis* also resembles that of fetal teeth, particularly those of the aquatic, viviparous typhlonectids *Typhlonectes natans* and *T. compressicauda* (Wake, 1976, 1977a; Hraoui-Bloquet and Exbrayat, 1996), and to a limited extent, the paroral teeth of apparently newborn *Scolecomorphus vittatus* (Loader et al., 2003). The pattern of development with the extensive epithelial covering of the elongated crown before eruption has not been noted previously for caecilians (or amphibians in general, to our knowledge). This suggests that the epithelium is stretched over the crown as it develops, and only is broken through at the end of crown development, based on our SEMs of newly erupted teeth (Fig. 4CD). Conversely, the single row of widely spaced teeth on the upper jaw arcade and the
elongate peg-like shape of the crowns does not resemble fetal teeth so much, but is perhaps more similar to the peg-like teeth of *B. taitanus*, whose hatchlings forage on the skin of the mother (Kupfer et al., 2006). However, those of *C. orientalis* are not bicuspid like those of *B. taitanus*. Teeth in newly hatched young of other direct-developers that bear a resemblance to the fetal teeth of pre-birth viviparous species have been reported for *Caecilia* (see Wake, 2003). In any case, these teeth differ markedly from the adult dentition in crown shape (elongate, monocuspid, and recurved) and in numbers of rows (a single row on the premaxillary-maxillary and vomeropalatine arcades, and on the dentary, with a very few teeth in an “inner mandibular row”, called the splenial). Tooth crown morphology is a poorly explored area of systematic and functional biology of caecilians.

Comparison of the developmental stage of *C. orientalis* with development in the viviparous *Dermophis mexicanus* is limited by 1) having only the one stage of the former, and 2) the use of total length rather than character-defined states in Wake and Hanken’s (1982) description of development. However, the comparison that Müller et al. (2005) made of development in *G. ramaswamii* with that of the data for *D. mexicanus* gives a significant point of departure, because of the above-noted similarities (and differences) of *C. orientalis* to *G. ramaswamii*. We cannot ascertain the sequence of development in *C. orientalis*, as we have noted, but the absence of prootics and lacrimals is similar to the condition in *D. mexicanus* but not *G. ramaswamii* (given our statement above that our *C. orientalis* resembles a stage 47/48 *G. ramaswamii*). The lower jaw elements are well ossified but not yet fully fused, similar to the other two direct-developing species,
suggesting that development may be faster in *D. mexicanus* (possibly associated with its early intraoviductal feeding).

Comparison with the chondrocranium of *T. compressicauda* basically points up those features that distinguish its chondrocranial structure from that of other caecilians studied, including the enlarged and nearly enclosed nasal capsules, the flange of cartilage that extends from the orbital cartilage and the nasal capsule to roof the orbit, and the lateral walls of the braincase being extensively cartilaginous. *T. compressicauda* lacks a prenasal process, a lamina perpendicularis of the mesethmoid, and a septum nasi. The anterior part of the chondrocranium is extensively cartilaginous, more so than in other caecilians reported (Wake et al., 1985), and this is especially apparent at Stage III-5 (42 mm TL), a stage that would seem to be comparable to that of our *C. orientalis*. The degeneration of the palatoquadrate, Meckel’s cartilage, the parachordal plate and the occipital arches is less extreme than in *C. orientalis*. We noted previously that crown shape in lower jaw teeth of *C. orientalis* resembles that of fetal teeth of some viviparous taxa, particularly those of the aquatic, viviparous typhlonectids *Typhlonectes natans* and *T. compressicauda* (Wake, 1977a; Hraoui-Bloquet and Exbrayat, 1996). These typhlonectids have several rows of fetal teeth on a tooth plate formed of the fused tooth pedicels surmounting the large medial bosses of the cartilage. The plate of typhlonectids is unlike that of other caecilians that have fetal or ‘larval’ dentitions, and concomitantly is not present in *C. orientalis*. Aside from the tooth crown shapes and perhaps the relatively early development of the palatoquadrate and the articular bones (M.H. Wake, pers. obs.), there are few obvious correlates with reproductive mode or comparisons with other taxa for either *T. compressicauda* or *C. orientalis* that would give any reliable phylogenetic
signal regarding development, but that is largely a consequence of the paucity of material
for *C. orientalis* and the absence of published ossification sequences for *T. compressicauda* and for most other caecilians at this time.

Consequently, we see more similarities of our specimens of *C. orientalis* to *G. ramaswamii* and *H. rostratus* at comparable stages than to either *D. mexicanus* or *T. compressicauda*. This greater resemblance among the direct-developing taxa may well have more to do with the difficulties of having only one stage of *C. orientalis* and limited information about ossification in *T. compressicauda* than with any correlation with reproductive mode. Müller et al. (2005) also commented on the insufficiency of data on caecilian ossification sequences to develop hypotheses about the evolution of ossification patterns and any correlates with life history in caecilians. We await more ontogenetic material for caecilians, especially Latin American taxa, and look forward to the time that the several laboratories working on caecilian biology will have sufficient material for collaborative assessments of caecilian development, ecology, behavior, life history, and relationships.

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Figure Legends

Figure 1. Late embryos of *Caecilia orientalis*. A. Clutch with embryos in egg membranes bound together by coiled strings of ‘egg cases’. B. Embryo extracted from egg membrane. Note slight lateral compression of terminus of body. C. Close-up of head of embryo. Note vascular supply of gills and extent of pigmentation of skin, especially concentrations of melanocytes in annular margins. D. Dorsal view of anterior of embryo to illustrate head shape and biramous filamented gills. Scale bars: A, B, D = 2.0mm; C = 1.25mm.

Abbreviations: ag = anterior gill ramus; agl = anterior gill ramus; ap = annular groove pigmentation; cd = coiled egg case strands; e = eye; em = embryo; m = mouth; op = otic placode; pg = posterior gill ramus; pg1 = left posterior gill; pgr = right posterior gill.

Figure 2. A. Right lateral view of head of cleared and stained embryo. B. Left lateral view. Mineralization is Alizarin Red stained; cartilage alcian blue. Scale bars = 0.6mm. Abbreviations: ar = articular; at = atlas; d = dentary; f = frontal; ma = maxilla; moc = mineralized otic capsule; nc = nasal capsule; oc = otic capsule; or = orbital cartilage; p = parietal; pan = pila antotica; ppor = pila postorbitalis; q = quadrates; s = stapes; sn = solum nasi; tm = taenia marginalis.

Figure 3. Ventral elements of the skull. A. Ventral view of entire skull. B. Frontal view of nasal region, premaxillary and pseudodentary teeth. C. Palatine shelf (see text). D. Hyobranchial apparatus. Note that posteriormost ceratobranchials have not yet completed fusion with the third ceratobranchial, and cartilage has not filled in to
smooth to the rounded shape of the compound element of the adult. Scale bars: \textbf{A} = 1.0mm; \textbf{B} = 0.25mm; \textbf{C} = 0.65mm; \textbf{D} = 0.5mm. Abbreviations: \textit{ac} = arytenoid cartilage; \textit{bh} = basihyal; \textit{cbI} = ceratobranchial I; \textit{cbII} = ceratobranchial II; \textit{cbIII} = ceratobranchial III; \textit{cbIV} = ceratobranchial IV; \textit{cbV} = ceratobranchial V; \textit{ch} = ceratohyal; \textit{dt} = dentary tooth; \textit{ept} = ectopterygoid; \textit{mps} = maxillopalatine shelf; \textit{nc} = nasal capsule; \textit{pal} = palatine; \textit{pm} = premaxilla; \textit{pmt} = premaxillary tooth; \textit{pp} = prenasal process; \textit{ob} = os basale; \textit{oc} = otic capsule.

Figure 4: Articulation and dentition. \textbf{A}. Articular region; note extent of ossification of elements. \textbf{B}. Tooth crowns and pedicels of lower jaw. \textbf{C}. Scanning electron micrograph (SEM) of tooth of lower jaw. Note epithelial covering. \textbf{D}. SEM of lower jaw tooth erupted from its epithelium. Scale bars: \textbf{A} = 0.25mm; \textbf{B} = 0.15mm; \textbf{C, D} = 50\mu m. Abbreviations: \textit{ac} = articular cartilage; \textit{ar} = articular; \textit{dr} = dentigerous ramus; \textit{h} = hinge; \textit{ma} = maxilla; \textit{mc} = Meckel’s cartilage; \textit{ome} = oral mucosal epithelium; \textit{q} = quadrate; \textit{rp} = retroarticular process; \textit{tc} = tooth crown; \textit{tp} = tooth pedicel.

Figure 5. Vertebrae. \textbf{A}. Mid-column vertebrae. The centra have begun to ossify; the cartilaginous rib-bearers and ribs are well formed. \textbf{B}. Posteriormost vertebrae, illustrating extreme cephalization of caecilian development such that posteriormost elements are least developed. In more anterior vertebrae, the centrum is well formed, neural arches are connected and have medial crests; posteriorly, the centra and neural arch elements form circles, and are not completely linked in terminal vertebrae. The notochord is still present, but resorbing. Scale bars: \textbf{A} = 0.3mm; \textbf{B} = 0.7mm. Abbreviations: \textit{c} = centrum; \textit{na} =
neural arch; ncr = notochordal rudiment; ns = neural spine; r = rib; rb = rib-bearer;
z = zygapophysis.
83x109mm (600 x 600 DPI)
162x93mm (600 x 600 DPI)
170x106mm (600 x 600 DPI)