JOURNAL OF MORPHOLOGY 270:1492–1502 (2009)

The Morphology of Prehatching Embryos of *Caecilia orientalis* (Amphibia: Gymnophiona: Caeciliidae)

Oscar D. Pérez,[1] Ngan Betty Lai,[2] David Buckley,[2] Eugenia M. del Pino[1] and Marvalee H. Wake[2]*

[1]*School of Biological Sciences, Pontificia Universidad Católica del Ecuador, Quito, Ecuador*
[2]*Department of Integrative Biology and Museum of Vertebrate Zoology, University of California, Berkeley, California 94720-3140*

ABSTRACT The state of development of advanced embryos of the direct-developing Ecuadorian caecilian *Caecilia orientalis* (Caeciliidae: Gymnophiona: Amphibia) was examined. 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 of *C. orientalis* most closely resemble those of stage 47/48 *Gegeneophis ramaswamii,* an Indian caeciliid, and stage 47/48 *Hypogeophis rostratus,* a Seychellian caeciliid, both direct developers, in details of bone mineralization, chondrocranial degeneration, and vertebrogenesis. They are most like stage 45 *H. rostratus* in external features (gills, pigmentation, etc.). They are less similar to prehatchings of *Ichthyophis kohtaoensis,* an ichthyophiid with free-living larvae, and to fetuses of the viviparous caeciliid *Dermophis mexicanus* and the viviparous typhlonectid *Typhlonectes compressicauda* at comparable total lengths in both skeletal development and external features. The similarity of developmental features among the direct-developers suggests a correlation with mode of life history. A noteworthy feature is that *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. J. Morphol. 270:1492–1502, 2009.

KEY WORDS: caecilian; direct development; bone mineralization; tooth crowns

INTRODUCTION

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 semiaquatic to aquatic. However, information is accruing about their biology. 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. Many species lay eggs terrestrially shortly after fertilization, with the mother guarding the clutch until hatching. In several taxa, larvae wriggle into streams for a relatively lengthy period up to 1 year before metamorphosing and returning to land. Some species, however, are direct-developers, with embryos in terrestrial clutches completing metamorphosis before hatching so that 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, 1977a,b, 1982, 1989, 1993, 2006; Himstedt, 1996; Wake and Dickie, 1998; Wilkinson and Nussbaum, 1998; Exbrayat, 2000, 2006 for summaries of caecilian reproductive modes). 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 a mode of parental care with precocial hatching, specialized dentition, and the young eating the mother's skin and its secretions for a time (Kupfer et al., 2006; Wilkinson et al., 2008).

Caecilia orientalis (Taylor, 1968) occurs in the eastern lowlands of Ecuador; its reproductive mode was unknown until the discovery of adults and an egg clutch (Funk et al., 2004). The rare

Contract grant sponsor: National Science Foundation; Contract grant numbers: IBN 02-12027, EF 03-34939; Contract grant sponsor: The Pontificia Universidad Católica del Ecuador.

*Correspondence to: Marvalee H. Wake, Department of Integrative Biology, 3060 VLSB, University of California, Berkeley, CA 94720-3140. E-mail: mhwake@berkeley.edu

Received 21 January 2009; Revised 13 May 2009; Accepted 18 May 2009

Published online 1 July 2009 in Wiley InterScience (www.interscience.wiley.com)
DOI: 10.1002/jmor.10772

availability of a clutch of living embryos revealed that *C. orientalis* is direct-developing and allowed examination of several aspects of its biology. Notably, the work of del Pino et al. (2002) on lamina-associated polypeptide 2 (LAP2) derived from specimens from the clutch illustrates that caecilian LAP2 expression may be similar to that in mammals rather than that of anurans and urodeles. Fieldwork benefits molecular studies and the multidisciplinary approach to research on rare taxa, as well as studies of development, ecology, and natural history.

We present information on the developmental morphology of the embryos of *C. orientalis* based on examination of living and preserved members of the one clutch discovered to date. Caecilian development is known from only a few normal tables and descriptions of various stages of embryos and larvae for very few species (e.g., Sarasin and Sarasin, 1887–1890; Brauer, 1897, 1899; Sammouri et al., 1990; Dünker et al., 2000; Müller et al., 2005; Müller, 2006); the major modes of reproduction (oviparity with free-living larvae, direct development, and viviparity) are represented among them. Our hypothesis is that the state of skeletal development of *C. orientalis* will most closely resemble that of the similarly direct-developing caeciliids and be less like that of species with other modes. Further, because so little is known, this report on a clutch at a particular stage of development presents new information to contribute to understanding of the comparative developmental morphology of caecilians.

MATERIALS AND METHODS

Specimens

Three adults (two males and a female), an uncharacterized individual, and the egg clutch of *C. orientalis* were found under a large decomposing log in cloud forest at the Yanayacu Biological Station, Napo Province, Ecuador, in January 2001 (see data in Funk et al., 2004). 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 briefly for further study.

Culture Medium and Fixation

Four embryos were cultured by placing the egg clutch in a humid chamber that consisted of a 10-cm Petri dish with a bottom covered by wet filter paper with the clutch placed over a small piece of plastic foil to prevent sticking to the filter paper. One embryo, dissected from its capsule, was maintained briefly in 0.15× Steinberg's solution (Rugh, 1962). It was then fixed for scanning electron microscopy (SEM) in MEMFA fixative at room temperature for 12 h and stored in methanol at −20°C (Harland, 1991). The remaining four embryos died from a fungal growth after 4 days and were fixed in 10% formalin in their capsules. Two were subsequently removed from their capsules for bone and cartilage staining. The material currently is maintained in the del Pino laboratory of developmental biology and will be deposited in QCAZ.

Clearing and Staining

Two formalin-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 (Jegalian and de Robertis, 1992). The specimens, hereafter referred to as "embryo 1" and "embryo 2," were photographed using a Carl Zeiss Stemi SV6 stereo microscope with a Sony Cyber-Shot DSC F707 camera. Because the embryos were at the same stage of development, photos were selected based on the nature and quality of the details presented.

SEM

The left ramus of the lower jaw of the MEMFA-fixed embryo was excised. The tissue was dehydrated through an ethanol series followed by critical point drying in an Autosamari-815 critical point dryer. The dried tissue was mounted onto aluminum stubs with conductive carbon tape and 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 SEM. All photomicrographs (clutch and embryo, cleared and stained, and SEM) were labeled using Adobe PhotoShop version 7.0.

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 the embryos of *C. orientalis*.

RESULTS

C. orientalis Egg Clutch

The clutch consisted of nine egg capsules. Seven capsules contained embryos (data in Funk et al., 2004); two capsules were empty and collapsed. We suspect that the embryos had hatched from those capsules, so we presume that the entire clutch was near hatching. The capsules were connected to each other by thick cords of egg membrane and dense "jelly" ("stalks," terminology of Breckenridge and Jayasinghe, 1979) (Fig. 1A). The stalks formed a central knot to which all of the capsules were attached, similar to the clutches of *Ichthyophis glutinosus* (Sarasin and Sarasin, 1887–1890; Breckenridge and Jayasinghe, 1979), *I. kohtaoensis* (Himstedt, 1996), *G. carnosus* (Seshachar, 1942), and *S. annulatus* (Gans, 1961), for example. The head and body of the 40.0 mm total length (TL) embryo extracted from its capsule and briefly held in Steinberg's were light brown, the skin having numerous large melanocytes concentrated at the annular borders, and the small eyes darkly pigmented (Fig. 1A–C). It had three pairs of gills with numerous filaments containing an extensive vascular network. The three gills on each side ramify from a central core; the shortest gill is central in the set, but internal aortic arch

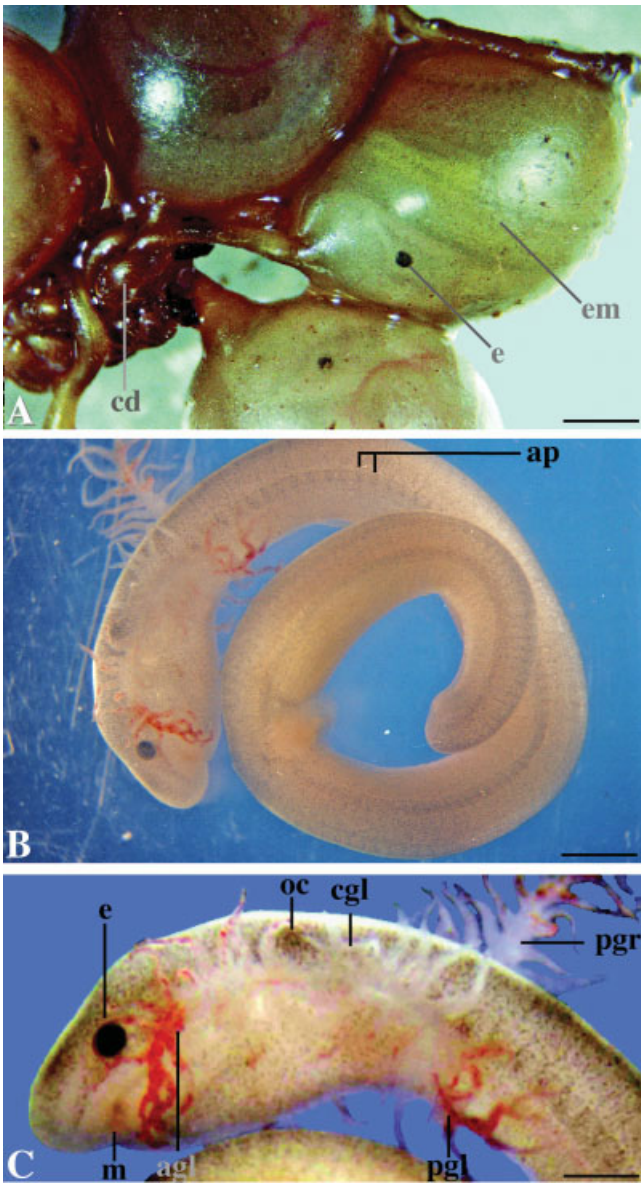

Fig. 1. Late embryos of *Caecilia orientalis*. **A**: Clutch with embryos in egg membranes bound together by coiled "stalks" of egg capsules. **B**: Embryo extracted from egg membrane, photographed immediately after death. Note slight dorsal keel on terminus of body. **C**: Close-up of head of embryo. Blood has pooled in some gill filaments. See text regarding gill orientation. Note extent of pigmentation of skin, especially concentrations of melanocytes in annular margins. Scale bars: A, B = 2.0 mm; C = 1.25 mm. Abbreviations: agl, anterior left gill ramus; ap, annular groove pigmentation; cd, coiled stalks connecting egg capsules; cgl, left central gill ramus; e, eye; em, embryo; m, mouth; oc, otic capsule; pgl, left posterior gill ramus; pgr, right posterior gill ramus.

contributions must be discerned to determine the order and absolute positions of the gills. The anterior and posterior gills on each side were large (left: 3.4 mm long, 25 filaments and 2.6 mm, 19 filaments; right: 4.1 mm long, 28 filaments and 3.4 mm, 21 filaments) and the third, central, pair was very small (left: 0.7 mm long, 7 filaments, right: 0.3 mm, 4 filaments) (Fig. 1B,C). The embryo had a small dorsal tail keel (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 47/48 (Figs. 2A,B and 3A–C). There is limited cartilage resorption despite some ossification. The ventral trabeculae and parachordals are well established; the orbital cartilages are elongate and supported by stout pilae (Fig. 2A,B); posteriorly, the orbital cartilages connect to the taenia marginales (Fig. 2B), which connect further posteriorly to the large otic capsules (Figs. 2A,B and 3A,C). Anteriorly, large nasal capsules attach to the trabeculae ventrally and the orbital cartilages dorsally (Figs. 2A,B and 3A). The copulae anteriorae appear to be fused to the capsules, and

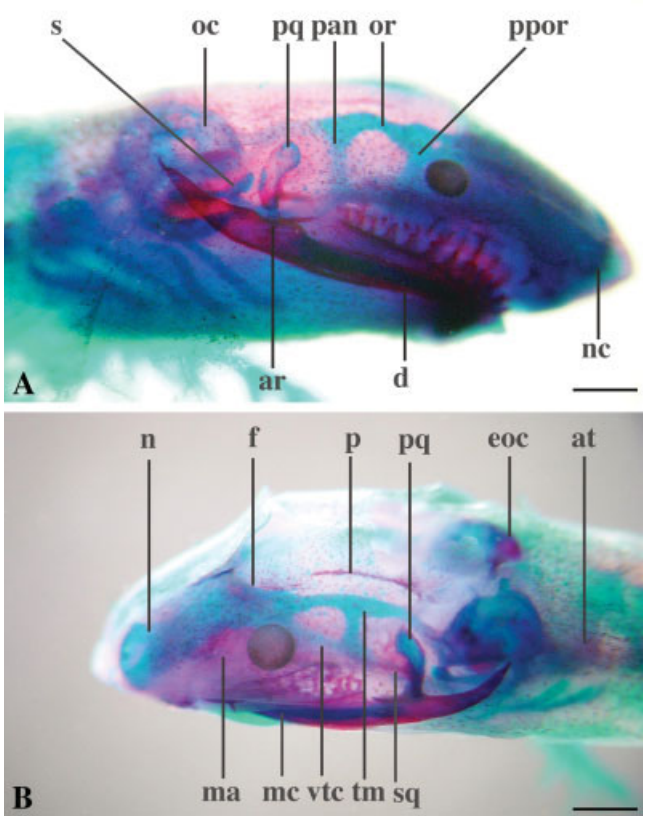

Fig. 2. **A**: Right lateral view of head of cleared and stained stage 47/48 embryo 1. **B**: Left lateral view, embryo 2. Mineralization is alizarin red-stained; cartilage Alcian Blue. Scale bars = 0.6 mm. Abbreviations: ar, articular process of pseudoangular; at, atlas; d, pseudodentary; eoc, exoccipital; f, frontal; ma, maxilla; mc, Meckel's cartilage; n, nasal; nc, nasal capsule; oc, otic capsule; or, orbital cartilage; p, parietal; pan, pila antotica; ppor, pila postorbitalis; pq, palatoquadrate; s, stapes; sq, squamosal; tm, taenia marginalis; vtc, trabecular cartilage.

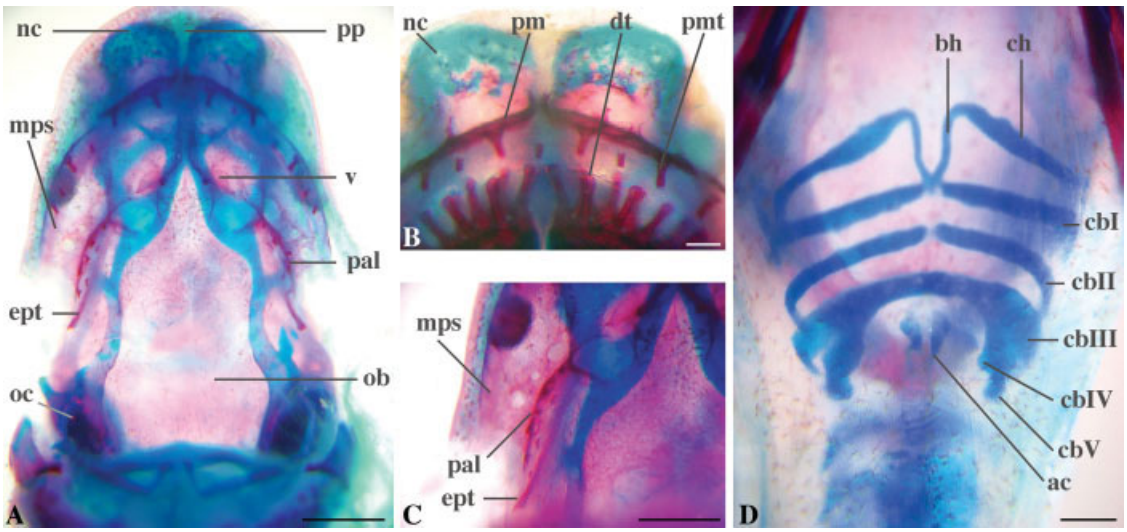

Fig. 3. Ventral elements of the stage 47/48 skull. **A**: Ventral view of cranium of embryo 1. **B**: Ventral view of nasal region, premaxillary and dentary teeth of embryo 2. **C**: Palatine area of skull of embryo 2. **D**: Hyobranchial apparatus of embryo 1. Basihyal is central at based of elongate processes. The small triangular element posterior to the basihyal but connected to it is basibranchial I. Note that ceratobranchials IV–V have not yet completed fusion with ceratobranchials III. Scale bars: A = 1.0 mm, B = 0.25 mm, C = 0.65 mm, and D = 0.5 mm. Abbreviations: ac, arytenoid cartilage; bh, basihyal; cbI, ceratobranchial I; cbII, ceratobranchial II; cbIII, ceratobranchial III; cbIV, ceratobranchial IV; cbV, ceratobranchial V; ch, ceratohyal; dt, dentary tooth; ept, ectopterygoid; mps, maxillopalatine shelf; nc, nasal capsule; ob, os basale (mostly parasphenoid part); oc, otic capsule; pal, palatine; pm, premaxilla; pmt, premaxillary tooth; pp, prenasal process of solum nasi; v, vomer.

the prenasal process of the solum nasi is short and slightly pointed, apparently eroding (Fig. 3A). The cartilage of the nasal capsules also is eroded ventrally, but not yet becoming invested with bone (Fig. 3C). The otic capsules are cartilaginous and complete and have started ossification around their peripheries (Figs. 2A,B and 3A). The taenial extensions that curve ventrally and fuse with the capsules are ossifying and the cartilage is resorbing, as is that of the parachordals between the postoptic and preotic pilae (Figs. 2A,B and 3A). The palatoquadrates are cartilaginous medially and dorsally, with some bone investing them, but their articular components are well ossified and the cartilage is resorbed, except for the articular caps (Figs. 2A,B and 4A). Pterygoid processes are well developed (Figs. 2A,B and 3A). The columellae/stapes are largely cartilaginous with some slight ossification beginning on the anterior heads (Fig. 2A,B). Meckel's cartilages remain pronounced, with large medial bosses and shafts that extend to the level of the articulations, but their retroarticular processes are nearly fully resorbed (Figs. 2A,B and 4A). Extensive dermal ossification around the Meckel's cartilage elements is established (see later). The occipital condyles are ossified (not shown), and the ossifications represent the exoccipital elements. We see no evidence of a chorda dorsalis extending into the skull cavity, but the chorda may have been resorbed.

Dermatocranium. In dorsal view, lateral strips of bone representing the frontals (above the eyes) and the parietals (nearly twice as long as the frontals) have formed (Fig. 2B). Posteriorly, the parietals have some ossification that appears to be spreading medially, based on denser lateral ossification and progressively weaker-staining mineralization toward the dorsal mid-line of the cranium. The nasals are weakly staining thin sheets of mineralization that overlie the nasal capsules (Fig. 2B). The squamosals appear to be forming from low, lateral slips that overlie the palatoquadrates and are barely mineralizing anteriorly (Fig. 2B). The maxillae are well formed, with stout dentigerous processes bearing a number of teeth in a single row and flared, flat plates that ascend anterior to the orbits (Figs. 2B and 3A). Paired premaxillae are formed; the dentigerous processes are well ossified, each bearing five teeth, two attached to pedicels and three crowns forming between the well-developed teeth (Fig. 4B). Ossification of the vertical processes of the premaxillae 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. 3A,C). The palatines have faintly staining ossification that extends from the dentigerous processes toward the maxillae, forming the palatine shelves (Fig. 3A,C), but the elements are not yet fully fused to the maxillae. The parasphenoids are thin struts of ossification that presage the parasphenoid part of the os basale, which is developing a thin, flat sheet of mineralization (Fig. 3A).

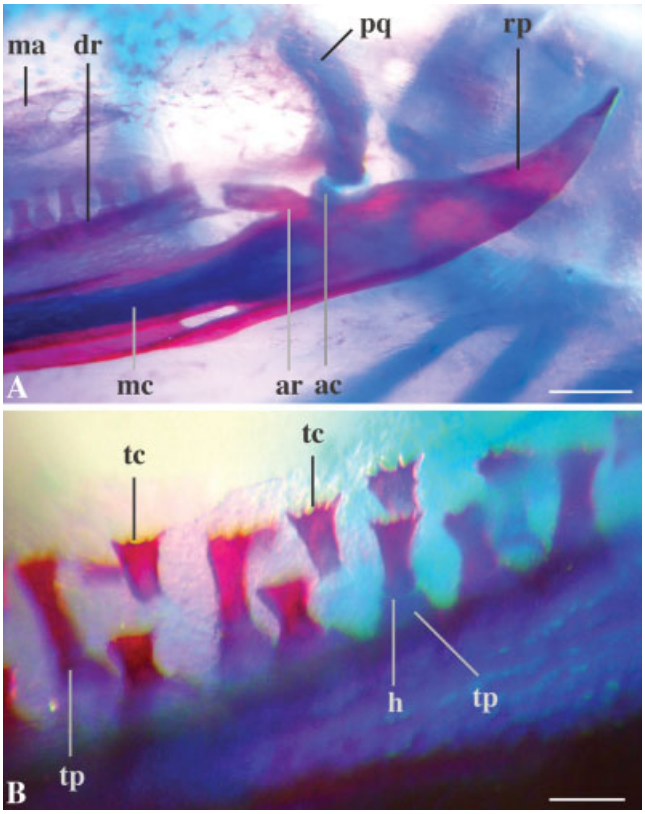

Fig. 4. Jaw articulation and dentition of stage 47/48 embryos. **A**: Articular region of embryo 2; note extent of ossification of elements. **B**: Tooth crowns and pedicels of lower jaw of embryo 1. The hinge ligaments between crowns and pedicels are faintly stained. Scale bars: A = 0.25 mm and B = 0.15 mm. Abbreviations: ac, articular cartilage; ar, articular process of pseudoangular; dr, dentigerous ramus of pseudodentary; h, hinge region; ma, maxilla; mc, Meckel's cartilage; pq, palatoquadrate; rp, retroarticular process; tc, tooth crown; tp, tooth pedicel.

Lower jaw. The lower jaws are well developed. The pseudoangulars with their retroarticular processes are well ossified, covering the short Meckel's cartilage retroarticular processes, which are nearly completely eroded (Figs. 3A,B and 4A). Cartilage caps remain on the articular surfaces; they contact the cartilaginous ends of the palatoquadrates (Fig. 4A). At least three ossified strips that are fusing to form the pseudodentary are present; their positions are not yet adequately defined, nor their ossification centers sufficiently obvious, to clearly identify elements other than the dentary. The dentigerous rami are stout and extend from the symphyseal ends of the jaws nearly to the articular facets (Fig. 2A,B), and as with fetal dentitions, are presumed to be dentary. They bear several rows of numerous teeth of slightly different shapes (see Dentition).

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 has the elongate recurved unicuspid teeth of adult *C. orientalis*. The crown shapes have a "body" with spatulate apices or apices that broaden slightly and have small spicule-like projections (Fig. 3B). The apex of the crown is effectively the primary cusp. The crowns with spiculate apices are reminiscent of early fetal teeth in the viviparous *Gymnopis multiplicata* and *Dermophis mexicanus* (Wake, 1976, 1977a,b, 1980). Teeth posterior on the dentigerous process are as well developed and mineralized as those medially near the jaw symphysis (Figs. 2A,B and 4A,B). There are five rows of teeth anteromedially on the lower jaws, reducing to 2–3 more posteriorly (Figs. 4B and 5A), similar to the aggregations seen in fetuses of vivipa-

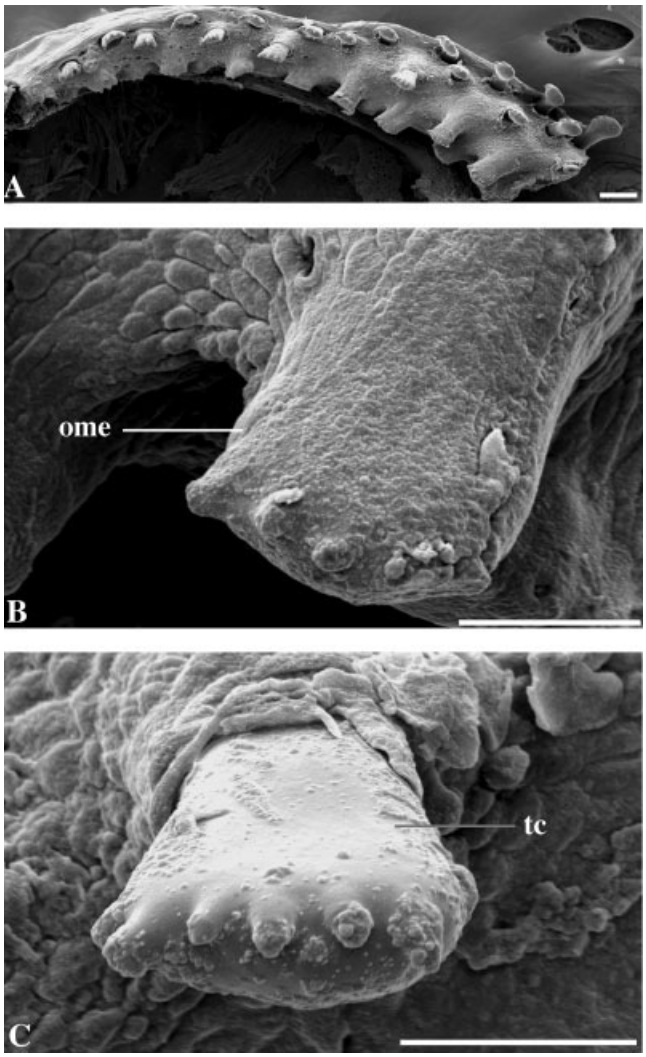

Fig. 5. Scanning electron micrographs (SEM) of teeth on lower jaw of stage 47/48 embryo 3. **A**: Left jaw ramus; note shapes of tooth crowns and number of rows of teeth. Labial side of the jaw is above, lingual below. Jaw symphysis is at right. **B**: Tooth crown covered by oral mucosa before eruption. **C**: SEM of lower jaw tooth erupted from its epithelium. Scale bars: A, B, C = 50 μm. Abbreviations: ome, oral mucosal epithelium; tc, tooth crown.

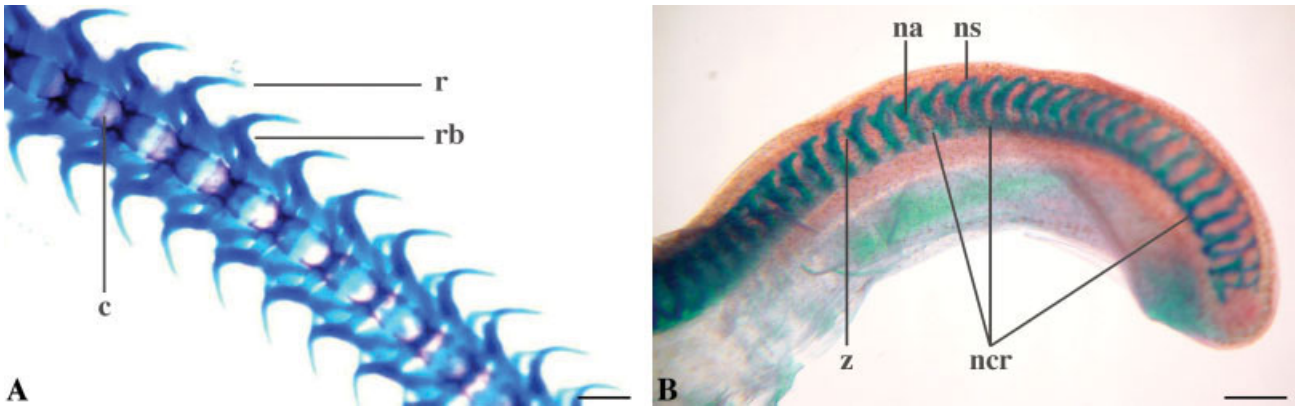

Fig. 6. Vertebrae of stage 47/48 embryos. **A**: Mid-column vertebrae of embryo 2. The centra have begun to ossify; the cartilaginous neural arches, rib-bearers, and ribs are well formed. **B**: Posterior vertebrae of embryo 1. Anterior-most cartilaginous centra in the series depicted are well formed, neural arches are connected and have mid-arch dorsal apices. More posteriorly, the centra and neural arch elements form complete circles; in posterior-most vertebrae they are not completely linked together. The notochord is still present between centra, but resorbing, and is weakly stained with Alcian blue. Scale bars: A = 0.3 mm; B = 0.7 mm. Abbreviations: c, centrum; na, neural arch; ncr, notochordal rudiment; ns, neural spine; r, rib; rb, rib-bearer; z, zygapophysis.

rous 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. 3A,C), more similar to the adult condition and better formed than in fetuses of viviparous taxa observed. Premaxillary and maxillary teeth are widely spaced, elongate, and angled but not recurved structures (Fig. 3B). The monocuspid 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) and are 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 somewhat lateral points, some associated with pedicels, some newly developing (Fig. 3A).

SEM 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 tooth crowns (Fig. 5A,B). Five rows of teeth are present medially near the symphysis, three posterolaterally (Fig. 5A). Typical of caecilian "fetal" dentitions, the teeth in more labial rows 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. 5C). Those of the three labial-most tooth rows are bare of the epithelium, which surrounds only the bases of those crowns (Fig. 5C). Newly erupted crowns are covered with cellular debris (Fig. 5C), which is not present on "older" crowns. Crown shapes vary a bit, but have 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. 5A,C). These crowns are similar to those of early fetuses of *D. mexicanus* and *G. multiplicata,* as mentioned earlier, and especially to those of *Typhlonectes compressicauda* and *T. natans* (Wake, 1976, 1977a; Hraoui-Bloquet and Exbrayat, 1996). The labial face of each crown is slightly cupped.

Hyobranchial apparatus. The hyobranchium is nearly completely formed (Fig. 3D); it remains cartilaginous in adults. The ceratohyals are fused to a small remnant of the basihyal by processes of the basihyal; the medial halves of the ceratohyals are flared, tapering laterally. The paired arms of ceratobranchial (CB) I are fused to the lateral edges of a lighter staining basibranchial I remnant posterior to and joined with the basihyal. The CBs I are moderately broad and taper slightly laterally. CBs II are free, fused medially to the lighter staining remnant of basibranchial II, their width similar to that of CB I. CBs III and IV are fused together broadly medially; both CB III and IV are fused continuously from medial to lateral except at their ends, where CB III still have broad, free ends, and the recurved parts of IV are expanded and ventral, rather than lateral. A small remnant of what may be CB V appears to be fused medially to the margin of each CB IV (see Wake, 2003). Paired arytenoid cartilages are centered between CB III and IV (see Fig. 3D).

The vertebrae and ribs. Both embryos have 125 vertebrae in various stages of development (Fig. 6A,B), most advanced in anteriormost vertebrae in the typical highly cephalized pattern of caecilians (Wake and Wake, 1986, 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 47/48 embryos indicates that all vertebrae present in the adult are formed by that stage of development. The first three verte-

brae have extensive ossification evident (Fig. 2A). The cartilaginous neural arch of the atlas is fully invested with bone, the centrum and neural arches 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 elements. 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. 6A), strongest in the anterior half, progressively slighter and more restricted in the posterior half (fig. 6A), then more posteriorly ossification is present only investing the anterior half of the spool-shaped centrum (posterior sclerotomite-half of the more anterior somite: see Wake and Wake, 2000). This pattern suggests that mineralization commences in the anterior half of each centrum, followed by that of the posterior half, with more anterior vertebrae further along in mineralization, reflecting the highly cephalized pattern of development of caecilians (Wake and Wake, 2000). Ventral keels are apparent, although not well developed. Only the anteriormost neural arches have significant ossification. Clearly, centrum ossification precedes that of the neural arches. More posteriorly, from about the 75th vertebra, vertebrae are cartilaginous with well-formed centra, neural arches, and rib-bearing processes (Fig. 6B). 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. 6B). Dorsal and ventral rib-bearers have developed on the anterior vertebrae and are associated with differentiating rib heads, but the joint for most is not fully formed. The ribs of the anterior-most vertebrae have grown longer and more curved lateroventrally (Fig. 6A). On the vertebrae of the posterior half of the column, neither rib heads nor rib-bearers have differentiated, and the ribs are fused to the vertebrae, with rib struts more poorly developed, especially in length (Fig. 6B), concomitant with the anteroposteriorly 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 effectively one stage of development, we cannot comment on the ossification sequence relative to that of other caecilians, except to compare the presence, developmental state (cartilaginous, cartilage degrading, mineralization), 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 currently there 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, Seychellean caeciliid *H. rostratus*. Each of these presents summary discussions, and those by Müller include excellent, current summaries of the history of ideas about caecilian 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 *H. 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; Wake, 1982, 1989, 1993, 2006; Hanken, 1992; Wake and Dickie, 1998; Müller, 2006), we compare our data for *C. orientalis* with caecilians with each of the major modes: oviparity with free-living larvae, direct development, and viviparity. We focus on taxa with derived reproductive modes, comparing the direct-developing *H. rostratus* (Brauer, 1897, 1899; Marcus, 1933; Marcus et al., 1935; Müller, 2006) and *G. ramaswamii* (Müller et al., 2005), the viviparous *D. mexicanus* (Wake and Hanken, 1982) (all terrestrial caeciliids, but from the Seychelles, India, and Central America, respectively), and 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) because of the availability of data tables for them. Our hypothesis is that the state of skeletal development

of *C. orientalis* will most closely resemble that of the similarly direct-developing caeciliids, but be less like that of viviparous species. We briefly consider the effects of phylogenetic relationships and reproductive modes.

Predictably, not all external characters "fit" a particular stage in diverse taxa. We found that our specimens of *C. orientalis* generally agree with stage 45 of *H. rostratus* in terms of external morphological features (three gills, one reduced, on each side; pigmentation; head morphology, etc.), although they resemble "stage" 48 in yolk resorption. However, the embryos most closely resemble those of the direct-developing *Gegeneophis ramaswamii* of "stage" 47/48, which in turn closely resemble "stage" 46–48 of *H. rostratus* (Müller, 2006) in many aspects of skeletal development, although there are interesting variations in ossification states. Because we are studying skeletal morphology, we characterize the *C. orientalis* embryos as "stage" 47/48 for convenience of reference and comparison.

Because we are comparing only one stage of skeletal and external development (two cleared and stained embryos for the former and the rest of the clutch, all at nearly the same stage of development and from the one 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 allow cross-stage comparison to "locate" the point in the developmental trajectory of *C. orientalis*. As noted earlier, the state of ossification of the skull of *C. orientalis* has most of the same elements present as a stage 47/48 *G. ramaswamii* (see Table 4, Müller et al., 2005). That stage in turn resembles that of 46–48 of *H. rostratus*. We see several similarities of *C. orientalis* to *G. ramaswamii* and to *H. rostratus* in terms of state of chondrocranium development and degeneration as endochondral bone development ensues, and dermal elements invest the skull and lower jaw. However, we do not see the 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 parasphenoid contributes to a scant sheet of mineralization over the fenestra basicranialis, presaging the parasphenoid part of the os basale, unlike that in the comparator taxa. The mineralization is visible only peripherally in the other specimen, but its staining is somewhat faded. The thin sheet suggests that ossification of the parasphenoid and the posterior plate of the os basale occurs virtually uniformly throughout the mesenchyme (probably except for the elements surrounding the brainstem-spinal cord and the attachment to the otic capsules), 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 ossified, the axis–atlas complex being well ossified including the complete neural arches, the two vertebrae that follow having ossified centra and neural arch pedicels. The first 40 or so vertebrae have ossifying centra with mineralization progressively slighter posteriorly, as noted earlier. 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 47/48 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.

Tooth crown morphology is a poorly explored area of systematic and functional biology of caecilians (see, e.g., Wake and Wurst, 1979; Greven, 1986; Wilkinson, 1991), especially that of fetuses and hatchlings (Wake, 1976, 1980; Kupfer et al., 2006; Wilkinson et al., 2008). The arrangement of teeth of the lower jaw of the near-hatching embryos of *C. orientalis* strongly resembles fetal teeth in viviparous species in being in several rows with earlier-developing rows more labial and newer ones more lingual. Tooth addition appears to proceed posteriorly in rows, as is typical of both fetal and adult dentitions. As noted earlier, tooth

crown shape resembles that of some viviparous species. The pattern of development with the extensive epithelial covering of the elongated crown before eruption is unusual in amphibians (but also recently reported in fetuses of the east African caecilian *Scolecomorphus kirkii:* Müller et al., 2009). The pattern 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. 5A–C).

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). Crown shape in lower jaw teeth of *C. orientalis* particularly resembles that of fetal teeth, those in the SEMs especially resembling those of the aquatic, viviparous typhlonectids *Typhlonectes natans* and *T. compressicauda* (Wake, 1976, 1977a; Hraoui-Bloquet and Exbrayat, 1996). Furthermore, the *C. orientalis* dentary teeth bear a strong resemblance to those of the direct-developing, hatched, skin-feeding *Siphonops annulatus* (Wilkinson et al., 2008). The *S. annulatus* teeth also resemble those of *Typhlonectes* in shape and arrangement. They are used to scrape the proliferated body skin of the mother to eat the skin and its secretions. Conversely, the condition of single rows of widely spaced teeth on the upper jaw and palatine arcades and the elongate peg-like shape of the crowns of *C. orientalis* bear much less resemblance to fetal teeth. They are perhaps more similar to the peg-like teeth of *B. taitanus,* whose hatchlings also forage on the skin of the mother (Kupfer et al., 2006). However, the upper jaw teeth of very young *C. orientalis* are not bicuspid like those of *B. taitanus,* which also has its lower jaw dentition arranged in a long dentary and a very short inner mandibular row, similar to the adult arrangement. The monocuspid paroral teeth of apparently newborn *Scolecomorphus vittatus* reported by Loader et al. (2003) differ from fetal teeth in both shape and placement, and in their placement outside of the mouth. Furthermore, the teeth on the jaws are heterogeneous, with single rows of adult-like monocuspid tooth crowns of different sizes, and some supernumerary teeth that include bicuspid as well as monocuspid teeth. Loader et al. (2003) postulated that the teeth may be associated with altriciality and some form of post-birth parental care in these viviparous animals. Given the variation in hatchling/birthling dentitions, and the presence of adult females (as with many species) and a male (unusual) with the clutch, there is temptation to speculate that *C. orientalis* might also have some form of posthatching parental care, including nutrition. However, because there did not appear to be any changes in skin color or texture of the adult females, and the absence of any observed care, we resist that temptation. Obviously, information on hatchling behavior in that and other species would be invaluable in further understanding of caecilian life-history variation.

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 TL 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 aforementioned 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 earlier 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 of the chondrocranium of *T. compressicauda* with that of *C. orientalis* basically reveals those features that distinguish *T. compressicauda*'s 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). 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 palatoquadrates, Meckel's cartilages, 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). However, the 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. Such a plate in 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 de-

velopment of the palatoquadrate and the articular bones (Wake, personal observation), there are few obvious shared derived similarities between *T. compressicauda* and *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.

Recent molecular (and morphological) phylogenetic reconstructions (e.g., Wilkinson et al., 2003; Wake et al., 2005; Frost et al., 2006; Wilkinson and Nussbaum, 2006; Roelants et al., 2007) support a sister-group relationship between *Caecilia* and the Typhlonectidae, usually represented by *Typhlonectes*, so the differences in developmental biology between at least those two genera may indeed be correlated with reproductive mode, and similarly some of the differences with *Dermophis* may be correlated with an independent evolution of viviparity in the *Dermophis-Gymnopis* (and *Schistometopum*) clade. In any case, we see many more similarities of our specimens of the direct-developing *C. orientalis* to the more distantly related direct-developing *G. ramaswamii* and *H. rostratus* at comparable stages than to the closer viviparous *D. mexicanus* or *T. compressicauda*, further supporting the hypothesis that development is correlated 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. More robust phylogenetic hypotheses based on more extensive taxon sampling are necessary to better understand patterns of evolution in caecilians. We also 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.

ACKNOWLEDGMENTS

The authors thank W. Chris Funk, George Fletcher-Lazo, Fernando Nogales-Sornosa, Diego Almeida-Reinoso, and Luis E. Coloma for the donation of the *C. orientalis* material. Alejandro Sánchez provided information on the method for bone and cartilage staining. Lai appreciates the training provided by the Electron Microscopy Laboratory, University of California, Berkeley.

LITERATURE CITED

Brauer A. 1897. Beiträge zur kenntniss der entwicklungsgeschichte und anatomie der gymnophionen. Zool Jahrb Anat 10:389–472.

Brauer A. 1899. Beiträge zur kenntniss der entwicklung und anatomie der gymnophionen. Zool Jahrb Anat 12:477–508.

Breckenridge WR, Jayasinghe S. 1979. Observations on the eggs and larvae of *Ichthyophis glutinosus*. Ceylon J Sci 13:187–202.

de Beer GR. 1937. The Development of the Vertebrate Skull. Oxford, UK: Oxford University Press.

del Pino EM, Sáenz FE, Pérez OD, Brown FD, Ávila M-E, Barragán VA, Haddad N, Paulin-Levasseur M, Krohne G. 2002. LAP2 (Lamina-associated polypeptide 2) expression in fish and amphibians. Int J Dev Biol 46:227–234.

Dünker N, Wake MH, Olson WM. 2000. Embryonic and larval development in the caecilian *Ichthyophis kohtaoensis* (Amphibia: Gymnophiona): A staging table. J Morphol 243:3–34.

Eifertinger L. 1933. Die Entwicklung des knöchernen unterkiefers von *Hypogeophis*. Beitrag zur kenntnis der gymnophionen XX. Zeit Anat Entwicklungsgesch 101:534–552.

Exbrayat J-M. 2000. Les Gymnophiones ces Curieux Amphibiens. Paris: Boubée.

Exbrayat J-M. 2006. Viviparity in *Typhlonectes compressicauda*. In: Jamieson BMG, Exbrayat J-M, editors. Reproductive Biology and Phylogeny of Gymnophiona (Caecilians). Enfield, NH: Science Publishers. pp 325–335.

Frost DR, Gran T, Faivovich J, Bain RH, Haas A, Haddad CFB, De Sá RO, Channing A, Wilkinson M, Donnellan SC, Rax worthy CJ, Campbell JA, Blotto BL, Moler P, Drewes RC, Nussbaum RA, Lynch JD, Green DM, Wheeler WC. 2006. The amphibian tree of life. Bull Am Mus Nat Hist 297:1–370.

Funk WC, Fletcher-Lazo G, Nogales-Sornosa F, Almeida-Reinoso D. 2004. First description of a clutch and nest site for the genus *Caecilia* (Gymnophiona: Caeciliidae). Herpetol Rev 35:128–130.

Gans C. 1961. The first record of egg laying in the caecilian *Siphonops paulensis* Boettger. Copeia 1961:490–491.

Greven H. 1984. The dentition of *Gegeneophis ramaswamii* (Amphibia: Gymnophiona) with comments on monocuspid teeth in the Amphibia. Zeit Zool Syst Evol 22:342–348.

Hanken J. 1992. Life history and morphological evolution. J Evol Biol 5:549–557.

Harland RM. 1991. In situ hybridization: An improved whole-mount method for *X. laevis* embryos. Methods Cell Biol 36:685–695.

Himstedt W. 1996. Die Gymnophionen. Marburg: Westarp Wissenschaften.

Hraoui-Bloquet S, Exbrayat J-M. 1996. Les dents de *Typhlonectes compressicaudus* (Amphibia: Gymnophiona) au cours du développement. Ann Sci Nat Zool Paris 17:11–23.

Jegalian BC, De Robertis EM. 1992. Homeotic transformations in the mouse induced by overexpression of a human Hox 3.3 transgene. Cell 71:901–910.

Kupfer A, Müller H, Antoniazzi MM, Jared C, Greven H, Nussbaum RA, Wilkinson M. 2006. Parental investment by skin feeding in a caecilian amphibian. Nature 440:926–929.

Loader SP, Wilkinson M, Gower DJ, Msuya CA. 2003. A remarkable young *Scolecomorphus vittatus* (Amphibia: Gymnophiona: Scolecomorphidae) from the North Pare Mountains, Tanzania. J Zool Lond 259:93–101.

Marcus H. 1933. Zur entstehung des unterkiefers von *Hypogeophis*. Beitrag zur kenntnis der gymnophionen XX. Anat Anz 77:178–184.

Marcus H, Stimmelmayr E, Porsch G. 1935. Die ossifikation des hypogeophisschädels. Beitrag zur kenntnis der gymnophionen XXV. Gegenbaurs Morphol Jb 76:375–420.

Müller H. 2006. Ontogeny of the skull, lower jaw, and hyobranchial sleleton of *Hypogeophis rostratus* (Amphibian: Gymnophiona: Caeciliidae) revisited. J Morphol 267:968–986.

Müller H, Oommen OV, Bartsch P. 2005. Skeletal development of the direct-developing caecilian *Gegeneophis ramaswamii* (Amphibia: Gymnophiona: Caeciliidae). Zoomorphol 124:171–188.

Müller H, Wilkinson M, Loader SP, Wirkner CS, Gower DJ. 2009. Morphology and function of the head in foetal and juve-

nile *Scolecomorphus kirkii* (Amphibia: Gymnophiona: Scolecomorphidae). Biol J Linn Soc 96:491–504.
Roelants K, Gower DJ, Wilkinson M, Loader SP, Biju SD, Guillaume K, Moriau L, Bossuyt F. 2007. Global pattern of diversification in the history of modern amphibians. Proc Natl Acad Sci USA 104:887–892.
Rugh R. 1962. Experimental Embryology: Techniques and Procedures, 3rd ed. Minneapolis, MN: Burgess Publishing.
Sammouri R, Renous S, Exbrayat JM, Lescure J. 1990. Développement embryonnaire de *Typhlonectes compressicaudus* (Amphibia: Gymnophiona). Ann Sci Nat Zool Paris 11:135–163.
Sarasin P, Sarasin F. 1887–1890. Ergebnisse Naturwissenschaftlicher Forschungen auf Ceylon in den Jahren 1884–1886. Band 2: Zur Entwicklungsgeschichte und Anatomie der Ceylonesischen Blindwühle *Ichthyophis glutinosus*. Wiesbaden: C W Kreidel's Verlag.
Seshachar BR. 1942. The eggs and embryos of *Gegenophis carnosus*. Bedd Curr Sci 11:439–441.
Taylor EH. 1968. Caecilians of the World A Taxonomic Review. Lawrence, KS: University of Kansas Press.
Wake MH. 1976. The development and replacement of teeth in viviparous caecilians. J Morphol 148:33–64.
Wake MH. 1977a. The reproductive biology of caecilians: An evolutionary perspective. In: Guttman S, Taylor D, editors. Reproductive Biology of the Amphibia. New York: Plenum Press. pp 73–102.
Wake MH. 1977b. Fetal maintenance and its evolutionary significance in the Amphibia: Gymnophiona. J Herpetol 11:379–386.
Wake MH. 1980. Fetal tooth development and adult replacement in *Dermophis mexicanus* (Amphibia: Gymnophiona): Fields versus clones. J Morphol 166:203–216.
Wake MH. 1982. Diversity within a framework of constraints. Amphibian reproductive modes. In: Mossakowski D, Roth G, editors. Environmental Adaptation and Evolution. Stuttgart: Gustav Fischer. pp 87–106.
Wake MH. 1989. Phylogenesis of direct development and viviparity. In: Wake DB, Roth G, editors. Complex Organismal Functions: Integration and Evolution in Vertebrates. Chichester: Wiley. pp 235–250.
Wake MH. 1993. Evolution of oviductal gestation in amphibians. J Exp Zool 266:394–413.
Wake MH. 2003. The osteology of caecilians. In: Heatwole H, Davies M, editors. Amphibian Biology, Vol. 5: Osteology. Chipping Norton, Australia: Surrey Beatty and Sons. Chapter 6, pp 1811–1878.
Wake MH. 2006. A brief history of research on gymnophionan reproductive biology and development. In: Jamieson BMG, Exbrayat J-M, editors. Reproductive Biology and Phylogeny of Gymnophiona (Caecilians). Enfield, NH: Science Publishers. pp 1–37.
Wake MH, Wurst GZ. 1979. Tooth crown morphology in caecilians (Amphibia: Gymnophiona). J Morphol 159:331–342.
Wake MH, Hanken J. 1982. The development of the skull of *Dermophis mexicanus* (Amphibia: Gymnophiona), with comments on skull kinesis and amphibian relationships. J Morphol 171:203–223.
Wake DB, Wake MH. 1986. On the development of vertebrae in gymnophione amphibians. Soc Zool France 43:67–70.
Wake MH, Dickie R. 1998. Oviduct structure and function and reproductive modes in amphibians. J Exp Zool 282:477–506.
Wake MH, Wake DB. 2000. Early developmental morphology of vertebrae in caecilians (Amphibia: Gymnophiona): Resegmentation and phylogenesis. Zool Anal Compl Syst 103:68–88.
Wake MH, Exbrayat J-M, Delsol M. 1985. The development of the chondrocranium of *Typhlonectes compressicaudus* (Gymnophiona), with comparison to other species. J Herpetol 19:68–77.
Wake MH, Parra Olea G, Sheen JP. 2005. Biogeography and phylogeny of certain New World caecilians. In: Donnelly MA, Crother BI, Guyer C, Wake MH, White M. editors. Ecology and Evolution in the Tropics: A Herpetological Perspective. Chicago: University of Chicago Press. pp 48–64.
Wilkinson M. 1991. Adult tooth crown morphology in the *Typhlonectidae* (Amphibia: Gymnophiona). J Zool Syst Evol Res 29:304–311.
Wilkinson M, Nussbaum RA. 1998. Caecilian viviparity and amniote origins. J Nat Hist 32:1403–1409.
Wilkinson M, Nussbaum RA. 2006. Caecilian phylogeny and classification. In: Jamieson BMG, Exbrayat J-M, editors. Reproductive Biology and Phylogeny of Gymnophiona (Caecilians). Enfield: Science Publishers. pp 39–78.
Wilkinson M, Loader SP, Gower DJ, Sheps JA, Cohen BL. 2003. Phylogenetic relationships of African caecilians (Amphibia: Gymnophiona): Insights from mitochondrial rRNA gene sequences. Afr J Herpetol 52:83–92.
Wilkinson M, Kupfer A, Marques-Porto R, Jeffkins H, Antoniazzi MM, Jared C. 2008. One hundred million years of skin feeding? Extended parental care in a Neotropical caecilian (Amphibia: Gymnophiona). Biol Lett 4:358–361.