Developmental morphological diversity in caecilian amphibians: systematic and evolutionary implications

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Citation

Müller, H. (2007, November 8). *Developmental morphological diversity in caecilian amphibians: systematic and evolutionary implications*. Leiden University Press. Retrieved from https://hdl.handle.net/1887/12462

CHAPTER 4

Heterochrony, ontogenetic repatterning, and the evolution of direct development in caecilian amphibians

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To be submitted to *Evolution and Development*

ABSTRACT Direct development is one of the most striking developmental adaptations in amphibians, involving heterochrony and ontogenetic repatterning. Despite it being of universal importance in amphibians – direct development is present in all three living major groups (frogs, salamanders and caecilians) – very little detailed information is available on direct development in caecilians. We here describe the postembryonic development of the skull in *Boulengerula taitanus*, a direct developing caecilian with an extended period of post-hatching parental care. Postembryonic skull development is compared with that of *Gegeneophis ramaswamii*, a direct developing species without post-hatching parental care, and *Ichthyophis* cf. *kohtaoensis*, a species with a free-living aquatic larva. Compared with *G. ramaswamii*, hatchling *B. taitanus* have a far less developed skull and are unlikely to be able to burrow. Skull development, especially the closure of the cheek region continues during the early postembryonic phase. The general trajectory of skull development in *B. taitanus* is nonetheless similar to that of *G. ramaswamii*, indicating a heterochronic shift in hatching time in the former. Skull development in both species is further characterized by the absence of larval-specific traits seen in larvae of *I.* cf. *kohtaoensis*, which shows that direct development in caecilians is also characterised by ontogenetic repatterning.

INTRODUCTION

One of the classic characteristics of amphibians is the possession of a complex life-cycle that is characterized by a free-living larval stage, which undergoes a complex transformation process (metamorphosis) into the adult (Duellman and Trueb 1986). However, this belies the great diversity of amphibian life-histories and associated ontogenies that have evolved within the group. The biphasic life-cycle of amphibians, with a primarily aquatic larva followed by a primarily terrestrial adult, offers many opportunities for natural selection to act upon. Depending on environmental conditions, an evolutionary advantage should be gained by extending or shortening the aquatic larval phase as opposed to the terrestrial adult, or by maintaining the equilibrium between the two. At the two extremes of this trajectory lie paedomorphosis – sexual maturity is attained by the larva, which fails to undergo a metamorphosis and remains permanently aquatic (e.g. Duellman and Trueb 1986; Denoël et al. 2005) – and direct development – a free-living larva is absent from the ontogeny and an adult-like juvenile hatches out of a terrestrially deposited egg (e.g. Wake and Hanken 1996). Between these two extremes, many different reproductive strategies have evolved in all three orders of living amphibians (Anura, Caudata and Gymnophiona) that differ widely in, for example, egg deposition sites, larval habitat, duration of the larval phase and parental care (see e.g., Nussbaum 1985; Duellman and Trueb 1986, Prado and Haddad 2005).

Direct development, heterochrony and ontogenetic repatterning

Among the plethora of developmental adaptations and modifications, direct development is one of the most fascinating evolutionary innovations. In virtually all major groups of animals, several lineages have developed ways to circumvent the free-living larval stage and transform more or less directly into an adult-like organism (e.g. Raff and Wray 1989; Wake and Hanken 1996; Collin 2004). Direct development in amphibians, as the name suggests, is characterized by the absence of a free-living, usually aquatic larva. Most adult features that do not form until metamorphosis (or later) in transforming species (Hanken et al. 1992; Wake and Hanken 1996) develop already during the embryonic period, resulting in, for example, the hatching of a small, but more or less fully formed frog rather than a tadpole.

Direct development appears to be an evolutionary successful strategy. It is a widespread phenomenon and known to occur in all three orders of modern amphibians. Within frogs, direct development has evolved independently in several groups (e.g. Thibaudeau and Altig, 1999, Bossuyt and Milinkovitch 2000; Müller et al. in press) and several authors have suggested that direct development was the key evolutionary innovation that enabled the radiation of the mega-diverse leptodactylid genus *Eleutherodactylus*, the rhacophorid *Philautus* and other groups (e.g., Hanken et al. 1992; Meegaskumbura et al. 2002). In salamanders, direct development occurs only within the lungless plethodontid salamanders, but might have evolved several times in this group (Wake and Hanken 1996, Chippendale et al. 2004; Mueller et al. 2004). Direct developing plethodontids, however, make up the majority of all living salamanders and the evolution of direct development has been implicated in both their evolutionary success in terms of species numbers, and the dispersal of bolitoglossine plethodontids into tropical Central and South America (Wake & Hanken, 1996).

The evolution of direct development is not well understood. It is generally assumed that it evolves through an increasing terrestrialisation of development as a way of bypassing the aquatic environment. Although the ancestral biphasic life-history has a tremendous potential for adaptive diversification (e.g. Wassersug 1975; Hanken et al. 1997; Hoff et al. 1999), living in aquatic habitats also carries a significant risk due to possible desiccation and predation. Direct development can be viewed as adaptive in terms of being able to ensure reproductive success by controlling the timing of reproduction to avoid desiccation, as well as through prolonging embryonic development (leading to developmentally more advanced offspring) and/or decreasing the time spent in the aquatic environment (e.g. Duellman and Trueb, 1986, Haddad and Prado, 2005).

Developmentally, two mechanisms are primarily involved in the evolution of direct development: heterochrony and repatterning. Heterochrony leads to a shift in the on- or offset of events during development as compared to the ancestral ontogeny (e.g., Alberch et al. 1979; Raff and Wray 1989; Reilly et al. 1997). In *Eleutherodactylus coqui*, the majority of bones forming the skull are already present at hatching, whereas they do not form until metamorphosis in biphasic anurans (Hanken et al. 1992). However, in *E. coqui*, there is not only a heterochronic shift of the larval into the embryonic period, such that the tadpole would develop inside the egg and undergo a metamorphosis before hatching. Instead, the embryonic development is characterized by large scale ontogenetic repatterning: the development is highly modified (Townsend and Stewart 1985) and results in a loss of most larval-specific features and a precocious formation of the adult morphology (Ellinson, 1990; Hanken, 2003). Many regions of the skull assume a postmetamorphic, adult-like morphology from their inception, while other structures, such as the hyobranchial skeleton, initially assume a mid-metamorphic morphology (Hanken et al. 1992). This is also reflected in the development of the jaw adductor musculature, which initially assumes a mid-metamorphic configuration and larval-type myofibres are entirely absent (Hanken et al. 1997). Ontogenetic repatterning also seems to characterize the development of direct-developing bolitoglossine salamanders, where the hyobranchial skeleton forms in an adult-like configuration during embryogenesis (Alberch 1987).

Direct development in caecilian amphibians

All major reproductive modes – biphasic, direct development and viviparity – are found in caecilian amphibians. Free-living, largely aquatic larvae occur in rhinatrematid, ichthyophiid, uraeotyphlid and some caeciliid caecilians (Sarasin and Sarasin 1887-1890; Parker 1958; Largen et al. 1972; Wilkinson and Nussbaum 1996), indicating that this is the ancestral condition for the group (Fig. 1). Viviparity occurs in scolecomorphids, typhlonectids and some caeciliids (e.g. Peters 1875; Barbour and Loveridge 1928; Parker 1936; Taylor 1968). Direct development seems to be restricted to caeciliids, but very little information is available beyond the fact that a larval stage is seemingly absent in several species. Direct development is known, or is suspected to occur, in *Boulengerula taitanus* (Nussbaum and Hinkel 1994; Malonza and Measey 2005), *Caecilia orientalis* (Funk et al. 2004), *Gegeneophis ramaswamii* (Müller et al. 2005), some species of *Grandisonia* (Wake 1977), *Hypogeophis rostratus* (Brauer 1897), *Idiocranium russeli* (Sanderson 1937) and *Siphonops annulatus* (Goeldi 1899; Jared et al. 1999).

Little information is available regarding the mechanisms or consequences of direct development in caecilians. An aquatic life-history stage is apparently absent and juveniles of the abovementioned species are fully terrestrial upon hatching. This implies that most, if not all, of the characters thought to be associated with a burrowing life-style in caecilians, such as a well-ossified skull and the chemo- and mechanosensory tentacle (Himstedt 1996 and references therein) are fully functional upon hatching. This indeed seems to be the case, based on the limited evidence available. Brauer (1899) described the precocious development of the tentacle in embryonic *Hypogeophis rostratus*, a character known to develop only during metamorphosis in larvae of ichthyophiid caecilians (Sarasin and Sarasin 1887-1890; Dünker et al. 2001). Müller et al. (2005) and Müller (2006; Chapter 2) described the development of the skull in the direct developing *Gegeneophis ramaswamii* and *Hypogeophis rostratus*, respectively, and several characters in both species show signs of precocious development as compared to free-living larvae of biphasic species. For example the maxilla and palatine fuse well before hatching to form the maxillopalatine, a compound bone characteristic of adult caecilians that forms during metamorphosis in biphasic species. Both species have a very similar ossification sequence of the skull, which is different from that of the viviparous *Dermophis mexicanus* (Wake and Hanken 1982; Müller et al. 2005; Müller 2006). Hatchling *G. ramaswamii* furthermore have a well-developed skull that resembles the adult condition in that species. The skull morphology of hatchling *H. rostratus* is unknown.

That direct development in caecilians is more diverse than previously thought is demonstrated by recent discoveries in *Boulengerula taitanus*. Recently, Malonza and Measey (2005) reported *B. taitanus* to have altricial young that are seemingly unable to burrow in soil. Kupfer et al. (2006; see Chapter 5) established that hatchling *B. taitanus* are very small (around 28 mm) compared to those of other direct developing caecilians (Brauer 1899; Müller et al. 2005) and receive extended parental care from the guarding female, in the form of feeding of their offspring with their own modified skin. Kupfer et al. (2006; Chapter 5) further noted that juveniles will stay with their mother until a length of about 86 mm, by which time they resemble miniature, albeit less pigmented adults.

To gain a better understanding of direct development in caecilians, we investigated its impact on the postembryonic morphology in *G. ramaswamii* and *B. taitanus*, focussing on the development of the skull as a particularly rich source of characters known to be influenced by heterochronic shifts associated with direct development in other amphibians (Hanken et al. 1992). Postembryonic skull development in the two direct developing species is compared with *Ichthyophis* cf. *kohtaoensis*, which has a free-living aquatic larval stage.

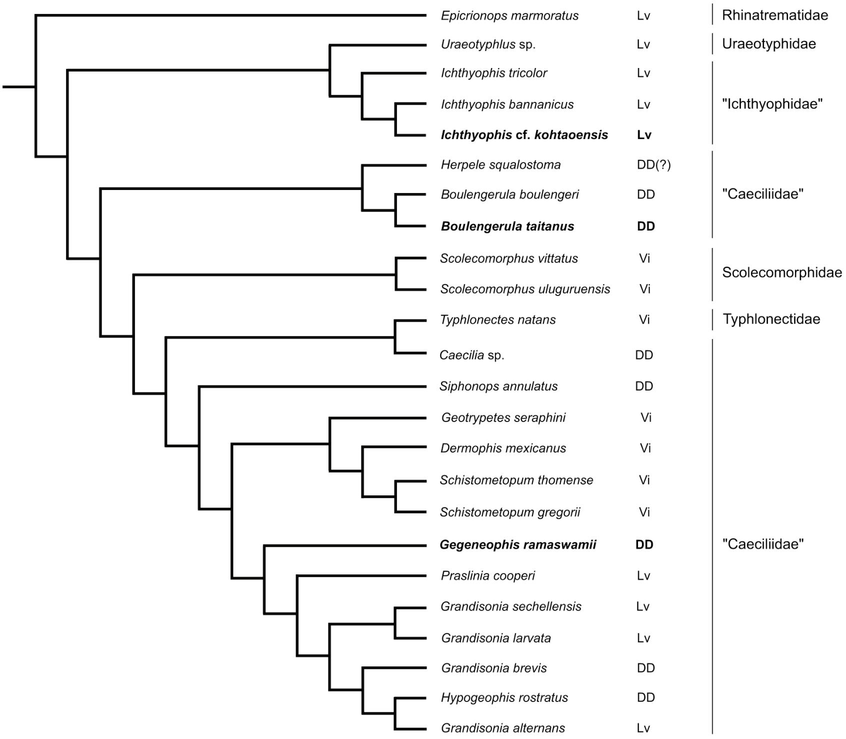

Fig. 1. Phylogenetic relationships of caecilians, from Wilkinson et al. (2003), modified according to Gower et al. (2002) regarding *Ichthyophis* spp. relationships. Taxa in investigated here are in bold face; note that taxon “16 *Ichthyophis* sp.” of Gower et al. (2002) corresponds to *I.* cf. *kohtaoensis* in this study. Lv – biphasic with a free-living larva, DD – direct development, Vi – viviparity.

MATERIAL AND METHODS

Available for this study were ontogenetic series ranging from embryos to large adults of the indirect developing *Ichthyophis* cf. *kohtaoensis*, and the direct developing *Boulengerula taitanus* and *Gegeneophis ramaswamii*. Specimens of *G. ramaswamii* were collected from Kerala, India (for further details see Müller et al. 2005), *B. taitanus* from the vicinities of Wundanyi and Maghimbinyi, Taita Hills, Kenya, and *I.* cf. *kohtaoensis* from the vicinity of Na Sabaeng village, Khemmarat District, Ubon Rathchathani Province, Thailand (for further details on locality and egg collection see Kupfer et al. 2004). Specimens were either fixed in the field or reared in the lab from field collected clutches and fixed in Bouin's fluid, 4% neutral buffered formalin or 70% ethanol and all subsequently stored in 70% ethanol. Embryos of *I.* cf. *kohtaoensis* and *B. taitanus* were removed form their egg capsules prior to fixation. Specimens are housed in the herpetological collections of the Natural History Museum, London (BMNH). For specimens details see Appendix.

Staging

Little information is available on the development of Gymnophiona. The only available staging tables are for *Typhlonectes compressicauda* (Sammouri et al. 1990), a highly derived viviparous species, and *Ichthyophis kohtaoensis* (Dünker et al. 2001), the latter of which was used for staging of *Ichthyophis* cf. *kohtaoensis* embryos and larvae used here. Both staging tables proved inadequate for the description of development in *B. taitanus* and *G. ramaswamii*, because several of the stage-defining characters (e.g., development of lateral line organs in the *I. kohtaoensis* staging table) are not expressed in embryos of these species. Embryos of *B. taitanus* and *G. ramaswamii* were therefore staged according to Brauer (1899). Brauer's description is more of an overview of the external development in *Hypogeophis rostratus*, a direct-developing Seychellean caeciliid, rather than a staging table in a modern sense, but his detailed figures and descriptions have subsequently been referred to as stages and can be used as such (see Müller 2006; Chapter 2).

Specimen preparation and investigation

Embryos of *B. taitanus* and *G. ramaswamii* have very little pigment and were usually surface stained with Borax carmine to enhance contrast for the examination of external characters, if necessary. Specimens used for the analysis of skeletal development were skinned and eviscerated (with the exception of embryos and very small specimens) and cleared and stained using standard procedures (Taylor & van Dyke 1985). All specimens were investigated under a

Nikon SMZ-U stereomicroscope equipped with a camera lucida and a Nikon Coolpix 995 for digital image capture.

Reconstruction of ancestral reproductive modes

The most recent comprehensive studies on caecilian intrarelationships (Wilkinson et al. 2003; Frost et al. 2006; Roelants et al. 2007) were used to establish most parsimonious optimizations of reproductive modes on the given phylogenies. Optimizations were calculated using the modular ancestral state reconstruction packages within the Mesquite system for phylogenetic computing (Maddison and Maddison, 2004). Reproductive mode was treated as a single character with three discrete character states (biphasic, direct developing, viviparous) that were treated as unordered or ordered in separate analyses. Information on reproductive modes was taken from Wake (1977) and Wilkinson and Nussbaum (1998), and references cited in the introduction to this paper. *Praslinia cooperi* was scored based on the assumed presence of larvae (Nussbaum and Gerlach 2004). Generally, knowledge about caecilian reproductive biology is still in its infancy and some of the assigned reproductive modes might prove incorrect.

RESULTS

External morphology of embryos

Embryos of *Boulengerula taitanus* and *Gegeneophis ramaswamii* share several features indicative of a direct mode of development (Fig. 2). In both species, the tentacle appears early during development and is clearly visible in older embryos. This is in contrast to *Ichthyophis* cf. *kohtaoensis*, where only a weakly developed tentacle anlage is present at hatching, which develops fully only during metamorphosis. Both direct developing species further show no lateral line organs expressed in their early development and also lack a well-developed gill slit and tail fin, all of which are characteristic for embryos and larvae of *I.* cf. *kohtaoensis*. At a comparable stage of development based on external features (e.g. gill differentiation), embryos of *B. taitanus* and *G. ramaswamii* are considerably smaller than those of *I.* cf. *kohtaoensis*, associated with larger egg, hatchling and adult sizes in the latter (Taylor 1968; Kupfer and Müller 2002; Kupfer et al. 2004).

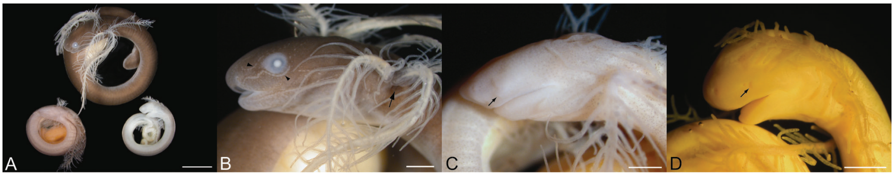

Fig. 2. A. Embryos of *Ichthyophis* cf. *kohtaoensis* (top), *Gegeneophis ramaswamii* (bottom left) and *Boulengerula taitanus* (bottom right) at a comparable stage of development with regard to the remaining yolk. The embryo of *G. ramaswamii* has been fixed inside the egg capsule and is more coiled than that of *B. taitanus*, which makes it appear smaller than it is. Scale bar equals 5 mm. B close-up of the head of the embryo of *I.* cf. *kohtaoensis* figured in A. Arrow heads point to the lateral lines and the arrow to the gill slit, note also the lip folds. C close-up of the head of the embryo of *G. ramaswamii* figured in A. Arrow points to the tentacle. D close-up of the head of an embryo of *B. taitanus*, slightly younger than the one figured in A. Arrow points to the developing tentacle. Scale bars in B, C and D equal 1 mm.

Postembryonic skull development in *Ichthyophis* cf. *kohtaoensis* and *Gegeneophis ramaswamii*

Postembryonic development in *Ichthyophis* cf. *kohtaoensis* includes a free-living, primarily aquatic larva that subsequently metamorphoses into a terrestrial, burrowing juvenile. Larval and adult skulls of different *Ichthyophis* species are very similar and have been described by several workers (e.g., Sarasin and Sarasin 1887-1890; Visser 1963; Wake 2003). Larval *Ichthyophis* cf. *kohtaoensis* are characterized by a short maxilla that is anterior to, and separate from, the palatine. The cheek region is not covered by bone and the squamosal is sickle-shaped, extending from the lateral side of the quadrate posteriorly onto the parietal, just in front of the otic capsule (Fig. 3). During metamorphosis, the maxilla starts to extend posteriorly, almost reaching the level of the posterior edge of the frontal in the adult. The maxilla further fuses with the palatine to form the maxillopalatine bone characteristic of all adult caecilians (Wake 2003), and its dental ridge extends posteriorly in parallel along the entire length of the dental ridge of the palatine part of the maxillopalatine. At about the same time, the dorsoposterior process of the squamosal that attaches to the parietal disappears, and the part of the squamosal lateral to the quadrate expands anteriorly to cover the cheek region. In the adults of most caecilians (Taylor 1969), the entire cheek region is covered by the squamosal, which contacts the parietal dorsally and the maxillopalatine ventrally. The maxillary part of the maxillopalatine will also form a canal for the tentacle, which starts migrating at the beginning of metamorphosis and presumably becomes functional around this time. The lateral wall of this tentacular canal can either be closed or open, depending on the position of the tentacle and species (Taylor 1969). In adult *I.* cf. *kohtaoensis*, the tentacular canal is closed laterally.

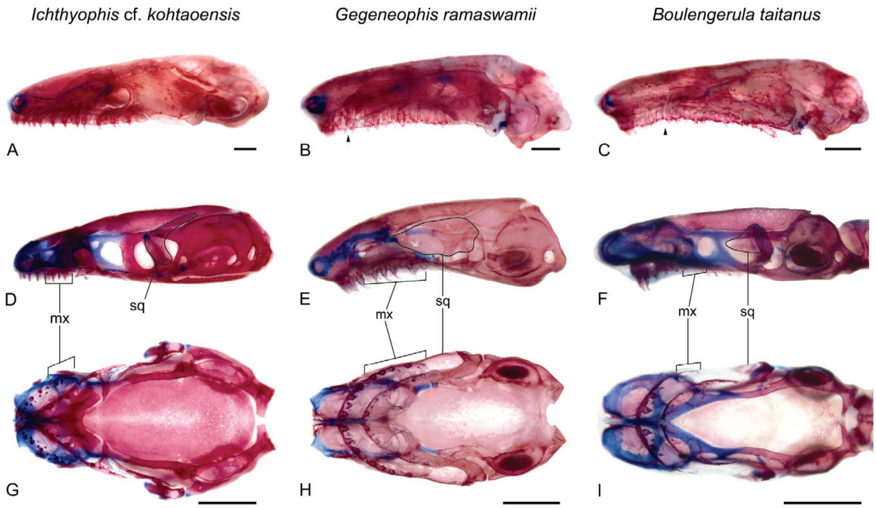

Fig. 3. Comparison of adult and hatchling skulls in *Ichthyophis* cf. *kohtaoensis*, *Gegeneophis ramaswamii* and *Boulengerula taitanus*. A B and C adult skulls. Arrow heads point to the posterior end of the premaxillary part of the nasopremaxillary. Lateral (D, E, F) and ventral view (G, H, I) of the skull of hatchling *I.* cf. *kohtaoensis*, *G. ramaswamii* and *B. taitanus*. The length of the dental lamina of the maxilla (mx) is indicated and outlines indicate the shape of the squamosal (sq) in lateral view. Scale bars equal 1 mm.

As in *Ichthyophis* cf. *kohtaoensis*, female *Gegeneophis ramaswamii* do not seem to provide care to their offspring for any extended period after hatching (DJG, MW pers. obs.; O. V. Oommen pers. comm.). Unlike for *Boulengerula taitanus* and *I.* cf. *kohtaoensis*, no *G. ramaswamii* specimen was available for which hatching was directly observed. One specimen, still encapsulated in the egg but with fully resorbed external gills, appears to be very close to hatching and has been described in detail by Müller et al. (2005). This specimen has an essentially adult-like morphology. The endocranium is well-ossified except for parts of the nasal capsule, and all dermal elements are almost adult-like: nasal and premaxilla are fused and form the nasopremaxillary; maxilla and palatine are also fused, with the maxillary arcade extending backwards to the level of the posterior edge of the frontal bone. The cheek region is completely covered by the squamosal, which is only separated by relatively narrow gaps from the maxillopalatine, frontal and parietal (Fig. 3). The only difference to the adult skull is an open tentacular groove in the maxillopalatine, which closes during further development to form the tentacular canal, and a weakly ossified central area of the parasphenoid part of the os basal. Two additional *G. ramaswamii* specimens of similar and even slightly smaller size, which were found unaccompanied by adults or other juveniles (see Table 1), show more advanced development of the skull than the previous specimen. In both specimens the squamosal and maxillopalatine are in closer contact and the tentacular groove is almost closed, with just a narrow gap remaining between the dorsal and ventral parts of the maxillopalatine. The parasphenoid part of the os basal is fully ossified. Endocranial ossification is also more advanced, with cartilage restricted to the anterior copula of the nasal cartilage, the orbitonasal, cartilage and the orbital and trabecular cartilage, similar to the adult condition. Although hatching has not been observed in *G. ramaswamii* and the exact developmental stage of the skull at hatching is therefore unknown, it seems certain that the skull at hatching is more or less adult-like in its morphology apart from the tentacular groove. Some variation in hatching size is also likely to occur, as is indicated by the three smallest available specimens, and some variation in the degree of development of the skull might also exist. The tentacular groove is fully closed in a specimen of 85 mm total length but a specimen 100 mm total length still retains a narrow suture in the maxillopalatine. Another specimen of 101 mm, however, has the tentacular groove completely closed as in all other, larger specimens examined.

Embryonic and postembryonic skull development in *Boulengerula taitanus*

A *Boulengerula taitanus* embryo of Brauer stage 45 is externally similar to embryos of *Gegeneophis ramaswamii* of the same stage (Müller et al. 2005), but is far less advanced in its skeletal development. The endocranium is well chondrified and appears slightly more robust than in *G. ramaswamii*, but unossified. Of the dermal ossifications, only premaxilla, vomer, palatine, parietal, dentary and angular are present, of which the premaxilla and dentary are the most prominent. The hyobranchial skeleton is only weakly chondrified but resembles that of Brauer stage 45 *G. ramaswamii* (Müller et al. 2005).

Two hatchling *B. taitanus* of 28 mm and 30 mm total length, respectively, were available for study. Both are at a similar, comparatively early stage of development. The entire endocranium anterior to, and including, the pila antotica is cartilaginous except for a very small, perichondral sphenethmoid ossification (Fig. 3). The footplate of the stapes has a thin perichondral ossification. All of the dermal bones are present, but poorly developed. The nasal and premaxilla are narrowly fused at the snout tip, via the alary process of the premaxilla, but widely separated laterally. The frontal and parietal are very thin and weakly ossified, and widely separated from their antimeres, leaving the brain exposed between them. Ventrally, the parasphenoid is very poorly developed, the anterior and posterior parts of the parasphenoid are still widely separated and only the posterior part of the parasphenoid is narrowly fused to the basal plate posteriorly, thus initiating the formation of the os basal (the compound bone formed from the posterior endocranium and parasphenoid). The maxilla is only a narrow sliver of bone and separate from the palatine. Maxillary teeth are absent. The squamosal is a small, triangular plate of bone lateral to the quadrate, and does not reach the pila antotica anteriorly. The premaxilla is comparatively large and is the only tooth bearing bone that has a well developed dental lamina with two fully developed teeth with ankylosed pedicels on each side. The palatine also bears two ankylosed teeth, but much smaller than the premaxillary teeth, while the vomer lacks ankylosed teeth. Some variation exists between the two hatchlings in the extent as to which the premaxilla covers part of the underside of the rostrum and in the development of the parts of the parasphenoid.

In a specimen of 35 mm total length, development of the dermal and endoskeletal ossification has generally progressed. The anterior and posterior parts of the parasphenoid have fused but still leave a large central area unossified. Maxilla and palatine are still separate and the nasal and premaxilla are also still separated laterally. By 37 mm total length, the maxilla is partly fused to the palatine at its anterior end and a well-developed maxillary tooth is present, but not yet ankylosed. Nasal and premaxilla are fused laterally and the parasphenoid is more extensively fused to the endocranium ventral to the otic capsules, and shows a reticulate ossification pattern in its centre, with numerous interconnected bone trabeculae. The squamosal has expanded to the anterior limit of the pila antotica, while the maxilla has grown posteriorly, approaching the anterior tip of the squamosal in lateral view. One small, ankylosed vomerine tooth is present on each side. A 42 mm and a 48 mm specimen show a similar degree of skull development to each other. Maxilla and palatine are fused along their entire length, except for a few larger foramina between the maxillary and palatine dental laminae. The maxillary part of the maxillopalatine has expanded posteriorly, reaching the level of the anterior margin of the pila antotica, while the squamosal has extended anteriorly beyond the anterior margin of the pila antotica. However, the orbital area lateral to the large optical foramen remains uncovered by dermal bone. In a specimen of 56 mm, and all larger ones, the squamosal has covered the orbital area and overlaps with the dorsal part of the maxillopalatine. The ossification of the anterior part of the squamosal is initially characterized by several larger foramina, which gradually decrease in size and number. Larger gaps are present between the squamosal and frontal and parietal dorsally, and maxillopalatine ventrally, but these continue to narrow, until all elements are tightly sutured by 90-100 mm total length, essentially resembling the adult condition.

From hatching on, the lower jaw is well developed and each dentary bears at least three large, ankylosed teeth. The hyobranchial skeleton appears weakly chondrified at hatching but resembles that of the adult apart from a shallow indentation between the tips of former ceratobranchials III and IV.

DISCUSSION

Adult *Boulengerula taitanus* and *Gegeneophis ramaswamii* are very similar in their overall morphology and ecology. Both species have a completely closed, stegokrotaphic skull. The eye is greatly reduced and the orbital region is completely covered by the squamosal and the maxilloplatine (Fig. 3). Both species are well adapted burrowers that spend almost their entire life under ground (Measey et al. 2004; Gower et al. 2004) and have similar adult sizes (Taylor 1968). Both species also develop directly, in that a free-living larval stage is absent. Direct development in *G. ramaswamii* and *B. taitanus* is characterized by a precocious development of the tentacle and a lack of typical larval labial folds, lateral line organs and a tail fin. The gill slit found in larvae of biphasic species is comparatively small and closes early during development, with no traces remaining at hatching. Despite these many similarities, the skulls of *B. taitanus* and *G. ramaswamii* are very different in their degree of differentiation upon hatching. Hatchling *G. ramaswamii* closely resemble adults with regard to skull shape and differentiation, but those of *B. taitanus* are much less advanced in their development and resemble embryos of *G. ramaswamii* (Müller et al. 2005) and other direct-developing species such as *Hypogeophis rostratus* (Müller 2006). These differences correlate with extended brood care provided by the mother in *B. taitanus*. After hatching, young *B. taitanus* stay with their mother and feed on her specially modified skin until they become independent, by which time their size has increased threefold (Kupfer et al. 2006; Chapter 5).

Superficially, hatchlings and young juveniles of *B. taitanus* show several similarities to larvae of the biphasic *Ichthyophis* cf. *kohtaoensis*. Both have the cheek region largely uncovered by bone, separate maxillae and palatines upon hatching, and the anterior endocranium and especially the nasal capsule is largely cartilaginous. However, larval *I.* cf. *kohtaoensis* undergo a metamorphosis somewhat like those of other amphibians – the tentacle develops and the gill slit closes, lateral line organs, labial folds and the tail fin are reduced, the larval squamosal and hyobranchial skeleton are remodelled into the adult-like shape, and the maxilla fuses to the palatine and expands rapidly posteriorly – juvenile *B.*

taitanus show none of these dramatic changes but instead gradually develop the adult-like morphology. Moreover, in hatchling *B. taitanus*, the squamosal and hyobranchial skeleton do not have a larval configuration but instead seem to assume a mid-metamorphic morphology from the beginning of ossification or chondrification, respectively. In this respect, the ontogeny of *B. taitanus* is similar to that of *G. ramaswamii*, and can be interpreted as a case of ontogenetic repatterning as commonly seen in direct developing species (Hanken 2003).

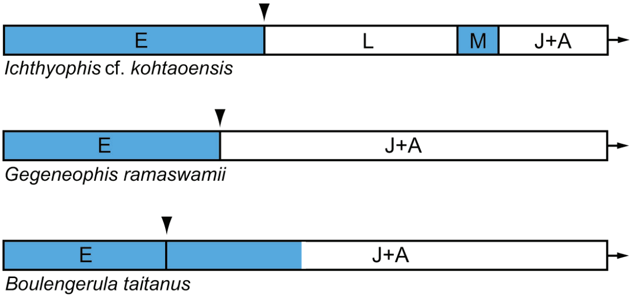

Fig. 4. Schematic representation of the life histories of *Ichthyophis* cf. *kohtaoensis*, *Gegeneophis ramaswamii* and *Boulengerula taitanus*. The arrow head marks the point where hatching occurs and blue indicates periods of morphological change. E – embryonic period; L – larval period; M – metamorphosis; J+A – juvenile and adult life.

Even though *Boulengerula taitanus* is a true direct developer like *Gegeneophis ramaswamii*, rather than possessing a curious land-larva that undergoes a postembryonic metamorphosis, the two species are nonetheless very different in their life-history and postembryonic ontogeny (Fig. 4). In *G. ramaswamii* the whole morphological development is condensed into the embryonic period (here defined as the time spent inside the egg), with very little morphological development occurring after hatching, apart from growth. In contrast, morphological development continues during most of the postembryonic care period in *B. taitanus*. However, the difference between *B. taitanus* and *G. ramaswamii* does not solely seem to be attributable to a shift in hatching time. Not only do *B. taitanus* hatch at a much smaller size than *G. ramaswamii* (~30 mm vs. ~55 mm) but the postembryonic developmental phase also seems to be prolonged. Juvenile *B. taitanus* are larger than those of *G. ramaswamii* (~80-90 mm vs. ~55 mm) by the time they have attained the same degree of skull development, with tight sutures between the squamosal and adjacent bones. This is directly correlated with the time spend under maternal care and the specialized feeding (Kupfer et al. 2006). At hatching, juvenile *B. taitanus* have a well-developed premaxilla that carries large teeth, whereas the other tooth bearing bones are smaller and carry either no teeth or much smaller ones than the premaxilla. Too little information is available on the embryonic development of these bones for a thorough analysis, but it seems nonetheless as if the development of the premaxilla in *B. taitanus* is somewhat accelerated compared to *G. ramaswamii* (Müller et al. 2005) and also *Hypogeophis rostratus* (Müller 2006). It is tempting to attribute this, together with the large teeth that show a specialized morphology (Kupfer et al. 2006), to the skin feeding seen in the juveniles. While the premaxilla (or premaxillary part of the nasopremaxilla) decreases in relative size compared to the other tooth bearing bones during further development, it remains relatively larger in *B. taitanus* as compared to *G. ramswamii* throughout the entire ontogeny (see Fig. 3B,C), which accounts for one of the most obvious differences between the adult skulls of both species.

In sum, the direct developing *Boulengerula taitanus* and *Gegeneophis ramaswamii* show ontogenetic repatterning and heterochronic shifts in their ontogeny, as compared to that of the ancestral biphasic ontogeny. It is currently unclear how the different developmental patterns observed in *B. taitanus* and *G. ramaswamii* relate to each other. The two species are only distantly related (Fig. 1 and Fig. 5) and little detailed information is available on the ontogeny of other direct developing caecilians other than *Hypogeophis rostratus* (Müller 2006) or indeed any other caecilians (see Wake 2003). At the moment, it is unclear if the degree of postembryonic skull development seen in *B. taitanus* is a special adaptation of this taxon or merely plesiomorphic, though the former hypothesis seems more plausible.

Evolution of direct development in caecilian amphibians

Several studies have recently investigated caecilian intrarelationships (Wilkinson et al. 2003; Frost et al. 2006; Roelants et al. 2007) with sufficiently dense taxon sampling to enable a preliminary discussion of the evolution of direct development in caecilians. All studies recovered a number of identical clades but differ in the relationships of these clades to each other, which complicates a consistent reconstruction of the evolution of reproductive modes. What is clear, however, is that the vast majority of 'higher caecilians' ("Caeciliidae", Scolecomorphidae and Typhlonectidae) are either known or thought to be direct developing or viviparous (Fig. 5; Wilkinson and Nussbaum 1998).

For the phylogeny of Wilkinson et al. (2003), parsimony optimization of the three main reproductive modes mapped onto the phylogeny unequivocally reconstructs direct development for the common ancestor of higher caecilians. *Herpele squalostoma*, the sister taxon to *Boulengerula*, is also reconstructed as direct developing. Viviparity evolved three times independently from a direct developing ancestor in *Scolecomorphus*, *Typhlonectes natans*, and the clade comprising *Geotrypetes seraphinii*, *Dermophis mexicanus* and *Schistometopum*. It further implies that a larva has re-evolved at least once among higher caecilians, within the Seychellean radiation, and possibly as many as three times (Fig. 5). Alternatively, assuming a free-living larva as the ancestral condition for higher caecilians requires two extra steps explaining the distribution of life-history modes on the given phylogeny. Based on the phylogeny of Frost et al. (2006), however, the reconstruction of the developmental mode of the last common ancestor of higher caecilians is ambiguous. According to this phylogenetic hypothesis, direct development, like viviparity, might have evolved only once or at least as many as three times. The reconstruction is further complicated by the unclear developmental mode of *Crotaphatrema tchabalmbaboensis*. When treating biphasic, direct developing and viviparous as ordered character states, however, the last common ancestor of higher caecilians is reconstructed as direct developing.

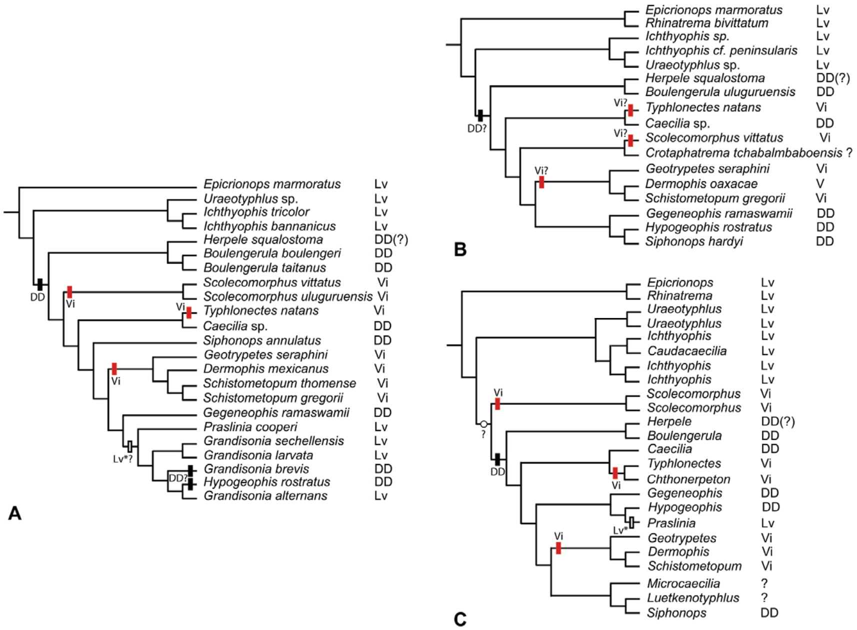

Fig. 5. Recent hypotheses of caecilian intrarelation ships. (A) from Wilkinson et al. (2003), (B) from Frost et al. (2006) and (C) from Roelants et al 2007. For each tree, the most-parsimonious character optimization is indicated, but in some cases other, equally parsimonious optimizations are also possible. DD – direct development, Lv – biphasic with a free-living larvae, Lv* – re-evolved free-living larva, Vi – viviparity, ? – indicates the possibility of alternative optimizations.

For the phylogeny of Roelants et al. (2007), the ancestral state of the last common ancestor of higher caecilians is also ambiguous for unordered character states, primarily because the viviparous *Scolecomorphus* clade is sister to all other higher caecilians. Direct development, however, is unequivocally reconstructed for the last common ancestor of the remaining higher caecilians and may have evolved only once within the group, with the possibility of a re-evolution within the Seychellean clade. Most parsimoniously, viviparity evolved three times independently, at least twice from a direct developing ancestor, and a free-living larva re-evolved from a direct developing ancestor on one occasion. *Herpele*, *Microcaecilia* and *Luetkenotyphlus* are reconstructed as being direct developing, but the developmental mode of these taxa is unknown at present (Wilkinson and Nussbaum, 1998), and the optimization changes if one or more of them are coded as viviparous. For ordered character states, direct development is reconstructed as the ancestral condition for the last common ancestor of all higher caecilians.

The results of the ancestral state reconstructions partly demonstrate the sensitivity of the analyses to the given phylogeny and the coding of the terminal taxa and more data on both are clearly needed for a comprehensive discussion of the evolution of development in caecilians. However, even though the recent hypotheses of caecilian intrarelationships are partly incongruent, and with them the interpretations of the evolution of direct development, it seems that direct development might have evolved relatively early among higher caecilians. This interpretation is complicated by the presence of free-living larvae in some caeciliid caecilians – *Grandisonia alternans*, *G. larvata*, *G. sechellensis*, *Praslinia cooperi* (Parker 1958; Nussbaum and Gerlach 2004), and also in *Sylvacaecilia grandisonae* (Largen et al. 1972), whose phylogenetic position is unknown. Character optimization suggests the re-evolution of a free-living larva in these taxa, rather than a plesiomorphic retention. A similar reversal to a free-living larva from a direct developing ancestor has recently been proposed for some plethodontid salamanders (Chippindale et al. 2004; Mueller et al. 2004). However, caecilians are different from other amphibians in that large eggs and brood care are a characteristic of all oviparous species in the group, whether biphasic or direct developing, and were likely present in the last common ancestor of living caecilians. Although not an absolute prerequisites, direct development in frogs and salamanders is strongly positively correlated with the presence of brood care and large, yolk rich eggs (Callery et al. 2001; Nussbaum 1985). In caecilians, the evolution of direct development was probably greatly facilitated by the presence of large eggs and brood care. Modifying the ontogeny towards direct development from a biphasic, *Ichthyophis*-like ancestor seems far less challenging than altering frog ontogeny to increase egg size and delete the tadpole stage from the ontogeny. Thus, the evolution of direct development in caecilians might have been more plastic than the most parsimonious ancestral state reconstructions suggest, with possibly a repeated independent evolution in various groups within higher caecilians. Caecilians might be much better suited to the study of the evolution of direct development than frogs or salamanders. In frogs for instance, it is difficult to dissociate changes associated specifically with direct development from those effected by an increased egg size (Callery et al. 2001). In caecilians however, biphasic and direct-developing forms share several traits such as large egg size with a presumably similar early embryogenesis and the presence of brood care, which should greatly facilitate the investigation of factors directly related to direct development. Caecilians thus have the potential to form a model comparative system for the evolution of reproductive modes in amphibian, and vertebrates in general.

Acknowledgments

We are grateful to D. Rotich and P. Malonza, and the National Museums of Kenya, the Kenyan Wildlife Service for issuing collecting permits, and the local authorities at Taita-Taveta district for their permission to carry out fieldwork. Fieldwork in Thailand was greatly facilitated by J. Nabhitabhata, National Science Museum Bangkok, and we are grateful to The Royal Forestry Department for issuing the relevant collection and export permits. We further wish to thank B. Wongsduang and the villagers of Na Sabaeng, Thailand; D. Mwaghania and J. Mwandoe and the people of Wundanyi, Kenya for access to their land and help in carrying out our fieldwork. We thank O. V. Oommen and the University of Kerala for granting access to material of *Gegeneophis ramaswamii*. This study was funded partly by a Department of Zoology Studentship, The Natural History Museum London, to HM, and a European Commission Marie Curie Fellowship to AK (MEIF-CT-2003-501675).

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Appendix

List of specimens

Taxon	Number	Size (in mm)	Life history stage	Remarks
Boulengerula taitanus	MW03884	-	embryo	clutch of 4 embryos
	AK1003	28	hatchling	
	AK1006	30	hatchling	
	HM0036A	35	juvenile	
	HM0036B	35	juvenile	
	HM0050B	37	juvenile	
	HM0050A	42	juvenile	
	MW03912A	48	juvenile	
	MW03899	56	juvenile	
	MW03895	59	juvenile	
	MW03926	69	juvenile	
	MW03920	69	juvenile	
	MW03904	89	juvenile	
	MW03890	99	juvenile	solitary
	HM0007	117	juvenile	solitary
	MW03889	129	juvenile	solitary
	HM0042	172	subadult	
	MW03905	218	adult	
	MW3914	313	adult	
Gegeneophis ramaswamii	MW01341	42	embryo	
	MW01349	56	almost ready to hatch embryo	erroneously reported as 61mm TL in Müller et al. (2005)
	MW01280	52	hatchling	
	MW01394	56	hatchling	

	MW01063	85	juvenile
	MW00420	100	juvenile
	MW01382	101	juvenile
	MW01054	111	juvenile
	MW01587	128	juvenile
	MW01072	144	juvenile
	MW01079	165	juvenile
	MW01581	189	subadult
	MW01451	198	subadult
	MW01291	235	adult
	MW01431	240	adult
	MW01095	277	adult
	MW01560	291	adult
Ichthyophis cf. *kohtaoensis*			embryo
			hatchling
			larva
			metamorph
	MW04086		juvenile
			adult