

Embryonic and Larval Development in the Caecilian *Ichthyophis kohtaoensis* (Amphibia, Gymnophiona): A Staging Table

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ABSTRACT Little is known about the developmental biology of caecilians—tropical, elongate, limbless, mostly fossorial amphibians that are members of the Order Gymnophiona. *Ichthyophis kohtaoensis* (Family Ichthyophiidae; southeast Asia) is an oviparous species in which maternal care of the clutch is provided. The clutch is laid in a burrow on land, and the embryos develop in their egg membranes, curved around a large yolk mass. Larvae are aquatic and exhibit characteristic features that are not present in the terrestrial adults. Because accurate descriptions of ontogenies and the establishment of standardized stages of embryonic and larval development are useful for both experimental and comparative embryology, a staging table for *I. kohtaoensis* was developed based on external morphological features. Development from the end of neurulation to metamorphosis was divided into 20 stages. Principal diagnostic features include development

of the lateral line organs, formation of three pairs of external gills, development of the eyes, changes in yolk structure, changes in the structure of the cloacal aperture and growth of the tail, including the formation and regression of the tail fin. This study provides a comparison with descriptions of embryonic stages of *I. glutinosus* and *Hypogeophis rostratus* and with a recent staging table for the aquatic, viviparous caecilian *Typhlonectes compressicauda*, the only other caecilians for which reasonably complete ontogenetic information exists in the literature. Comparisons with established staging tables for selected frogs and salamanders are also presented. *J. Morphol.* 243:3–34, 2000. © 2000 Wiley-Liss, Inc.

KEY WORDS: *Ichthyophis kohtaoensis*; amphibians; caecilians; ontogeny; development; staging

Caecilians, members of the Order Gymnophiona, are tropical, limbless, elongate, tailless or nearly so, amphibians that occupy subterranean, leaf-litter, or semi-aquatic to aquatic habitats. They are among the least studied of vertebrates. Rather little is known of the development of caecilians, especially compared to the relative wealth of knowledge about selected frogs and salamanders.

The development of *Ichthyophis glutinosus* (Family Ichthyophiidae) was described and beautifully illustrated by Sarasin and Sarasin (1887–1890). Cleavage in amphibian eggs is typically holoblastic and unequal. In *I. glutinosus*, the only caecilian for which cleavage has been described (Sarasin and Sarasin, 1887–1890), cleavage is nearly meroblastic, dividing the egg into numerous separate blastomeres and a residual multinucleate mass of cytoplasm. At the tail-bud stage, caecilians are similar to salamanders and have a relatively long head and pharyngeal region, including the gill plates, that projects above the yolk mass, but they have only a short tail bud. In contrast, in anurans the gill plate region lies dorsal to the yolk mass with only the anterior most part of the head projecting beyond the yolk, but a relatively long tail bud extends beyond

the yolk. A number of specific features of development are evaluated below.

There are very few descriptions of caecilian development from early stages to metamorphosis or birth (Sarasin and Sarasin, 1887–1890; Brauer, 1897, 1899; Sammouri et al., 1990); some authors have described only one or a few specimens (not staged) available for study. Sarasin and Sarasin (1887–1890) and Brauer (1899) presented detailed descriptions of the changes in the course of development, referring variously to the formation of gills, eyes, the tentacle, the lateral line organs, changes in yolk structure, and general external morphology. Brauer (1899) provided a rather complete series of drawings of early embryos of *Hypogeophis rostratus*, with

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some later stages of that species and a few for *Grandisonia alternans*; Marcus described the development of specific elements of *Hypogeophis rostratus*, but mostly internal features so only his work relevant to external morphology is cited herein; Sarasin and Sarasin (1887–1890) described various developmental stages of *Ichthyophis glutinosus*, including those of very early development. However, these studies did not present their results in the form of a staging table.

The continuously changing appearance of embryos and larvae during ontogenesis necessitates a method of quantifying the progress of development. Tables of normal stages of development have been established for a number of amphibian species, nearly all anuran and urodelan (see Duellman and Trueb, 1986). Each normal stage is designated by a number and represents a specific (but highly subjective) interval in ontogeny, defined by the presence or absence of ontogenetic character states, such as body size or morphology (Bartsch et al., 1997). By subdividing development into discrete stages, it is possible to group and compare individuals at approximately the same point in ontogeny. Staging tables are useful tools and have long been used in the study of amphibian development. Only one such table exists for caecilians; it is for a highly derived taxon. Sammouri et al. (1990) presented a detailed description of the embryonic development of *Typhlonectes compressicauda*, a viviparous aquatic caecilian of the family Typhlonectidae that has many developmental features unique to the family. They included a formal staging table and compared development in *T. compressicauda* with that of the oviparous frog *Alytes obstetricans*.

We describe the development of *Ichthyophis kohtaoensis*, an oviparous species with free-living larvae from Thailand, and compared it to that reported for *Ichthyophis glutinosus*, *Hypogeophis rostratus*, and *Typhlonectes compressicauda*. This comparison affords an examination of development in caecilians representing both basal and derived taxa, and provides a basis for evaluation of evolutionary trends in development in caecilians. The two species of *Ichthyophis* are members of the Family Ichthyophiidae. The families Rhinatrematidae (the most basal caecilian family) and Ichthyophiidae constitute the sister-group to all other caecilians. *H. rostratus* is oviparous and direct-developing (i.e., development through metamorphosis occurs before hatching in terrestrially laid clutches), thus obviating the free-living aquatic larval stage, and is a member of the derived family Caeciliidae. The monotypic *Hypogeophis* occurs in the Seychelles Islands. *T. compressicauda*, with its obligate viviparity, and with other features of its biology that are construed as correlates of its aquatic habitus, represents a highly derived clade of caecilians; it occurs in South America. The present study provides developmental information, including a staging table, for a

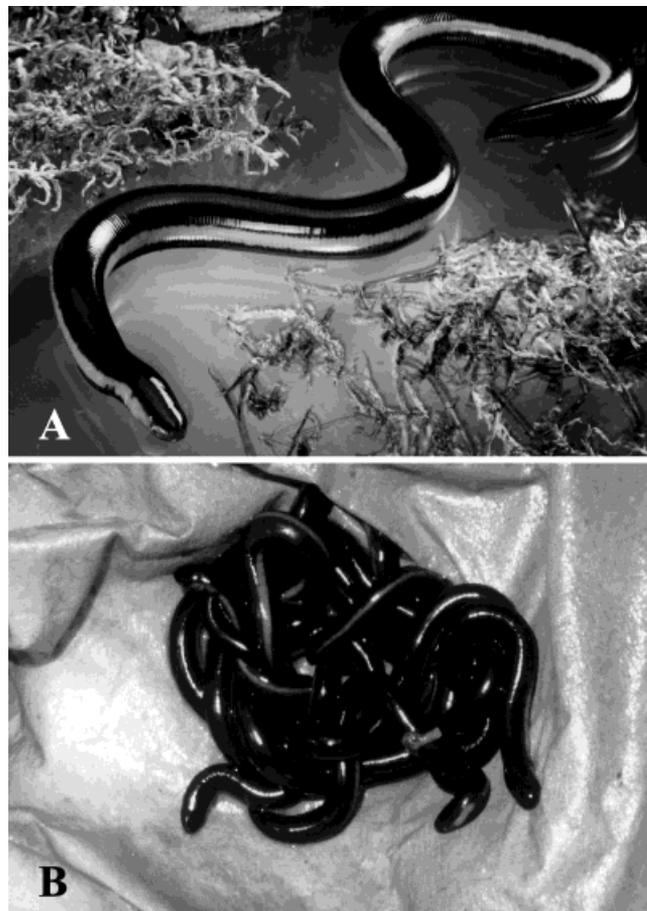


Fig. 1. *Ichthyophis kohtaoensis*. An adult specimen with a total body length of approximately 300 mm is shown in **A**. Larvae, approximately 100–150 mm in length, are shown in **B**. Photos by W. Himstedt.

second oviparous ichthyophiid caecilian, *I. kohtaoensis* (Fig. 1A), thus facilitating comparison within a genus and family of caecilians.

Ichthyophis kohtaoensis typifies the biology of oviparous caecilians, so far as is known (see Himstedt, 1996). Copulation in *I. kohtaoensis* has never been observed, but internal fertilization is presumed, as for all caecilians, via the male's inserting his phallodaeum into the vent of the female to effect sperm transport (see Wake, 1972, for summary). In the course of the rainy season from May to October, during which 90% of the annual rainfall in northeast Thailand occurs and floods the rice fields, the females lay their fertilized, developing eggs in moist cavities in the ground. *I. kohtaoensis* practices maternal care of the laid clutch, as has been reported for several egg-laying species (e.g., Sarasin and Sarasin, 1887–1890; Sanderson, 1936). The eggs of a clutch are bound together with strands that extend from the jelly coats, forming a cluster; the parental female curls around the clutch and turns the eggs at regular intervals. Similar to embryos of

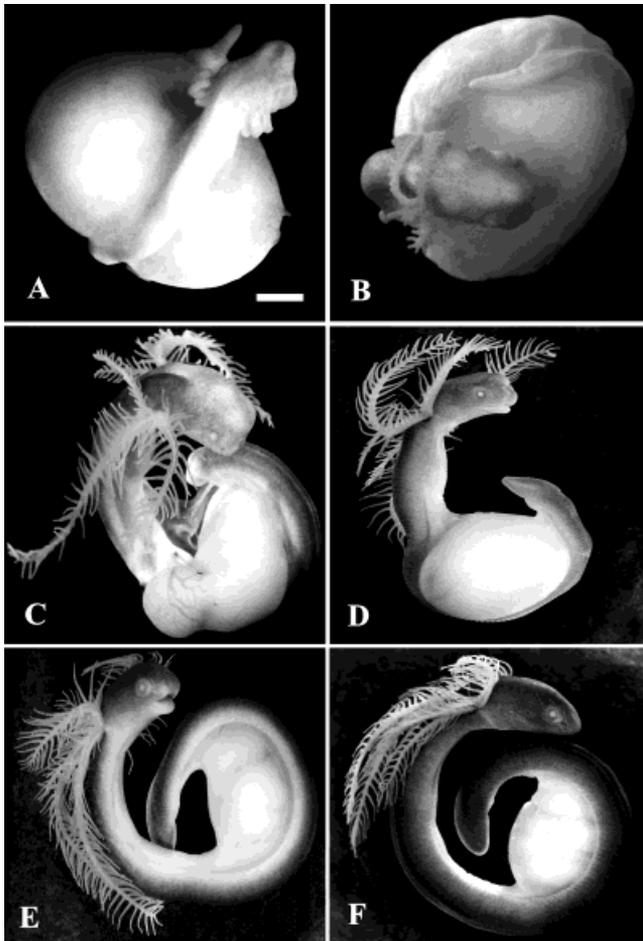


Fig. 2. Photomicrographs of embryonic stages of *Ichthyophis kohtaoensis*: **A**, stage 21; **B**, stage 23; **C**, stage 26; **D**, stage 28; **E**, stage 30; **F**, stage 31. Note the progressive development of the gills and gill filaments, and the reduction of the yolk mass. Scale bar = 1 mm.

F2-F6

salamanders, embryos of *I. kohtaoensis* and apparently of most caecilians, except for typhlonectids, develop three pairs of external gills (Figs. 2–6). Later in the wet season, the *Ichthyophis* embryos hatch and, as larvae, move out of the burrows to ponds and small streams. Also similar to the salamander condition, but unlike anurans, larvae of *I. kohtaoensis* (Fig. 1B) closely resemble their respective adults morphologically, physiologically, and trophically. In many caecilians that have aquatic larvae, such as *I. kohtaoensis*, the gills are lost soon after hatching, leaving only a gill chamber that includes gill rudiments on either side. Hatchling *Ichthyophis* larvae also possess a well-developed caudal tail fin, and electroreceptive (ampullary organs) and mechanoreceptive (neuromasts) lateral-line sensory organs on the head and trunk. The lateral yellow stripes (see Fig. 1A) and the unique sensory tentacle characteristic of adults do not develop until metamorphosis.

To our knowledge, there is not yet available a table of normal stages of development for oviparous caecilians. Consequently we evaluate our data on the development of *Ichthyophis kohtaoensis* and present a staging table based on external morphological characters (Table 1). Because the present study is intended to provide a staging table for a very poorly known species, a detailed description of the embryonic and larval development is given and as many characters are described as possible. Those features facilitate a comparison to other caecilians, and to frogs and salamanders, allowing a much broader comparison of development among amphibians. TI

MATERIALS AND METHODS

Collection and Maintenance of the Specimens

Because oviparous caecilians that provide parental care have proven difficult to breed in captivity to

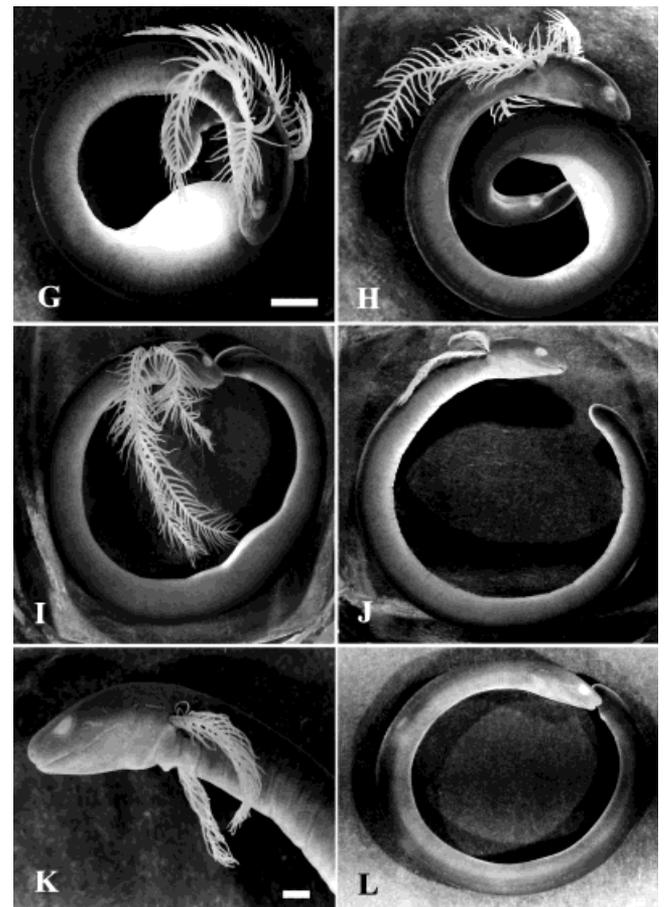


Fig. 3. Photomicrographs of embryonic stages of *Ichthyophis kohtaoensis*. Lettering sequence of embryos continues from Figure 2: **G**, stage 32; **H**, stage 33; **I**, stage 34; **J** and **K**, stage 36; **L**, stage 37. Note further reduction of the yolk, with complete enclosure in the abdominal folds occurring at stage 37 (**L**). Only two external gills are present in stage 36 (**J**, **K**); gills are stripped off after hatching (stage 37; **L**). Scale bar = 1 mm.

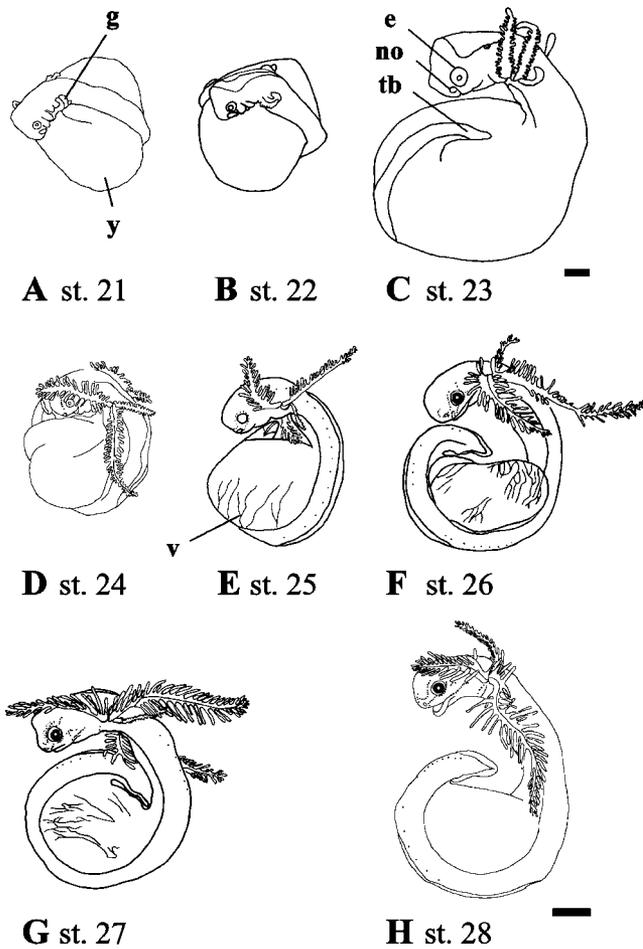


Fig. 4. *Ichthyophis kohtaoensis*. Lateral view drawings of details of embryonic stages 21–28 (A–H). The development of the gills and gill filaments characterizes stage 21–24 (A–D). The inception of vascularization of the yolk is apparent in E (stage 25). In F (stage 26), the eye is clearly pigmented. Refer to text for further details. e, eye; g, gill; no, nasal opening; tb, tail bud; v, vascular system; y, yolk. Scale bar in C = 1 mm; scale bar in H = 2 mm (also applies to A, B and D–G).

date, Prof. Werner Himstedt (Department of Zoology, Technical University of Darmstadt, Germany) collected the specimens of *Ichthyophis kohtaoensis* in Thailand, where the species is widespread and occupies diverse geographical and climatic regions of the country. *I. kohtaoensis* occurs in the warm plains near sea level in the vicinity of Bangkok and in south Thailand, in regions with moderate temperatures between 25–30°C, and at elevations of 2,000 m in the mountains of north Thailand, where the temperatures in January drop to the freezing point. In the summers of 1994 and 1995, during July or August, females with egg clutches were collected in the province of Ubon (district Khemarat) in northeast Thailand. Embryos and larvae were raised from the fertilized eggs in the laboratory. Clutches average approximately 30–40 eggs. Twelve out of 30 egg clutches collected were selected for our study. Stages

of development at the time of collection ranged from unpigmented embryos to stages close to hatching. In the field, the females and their egg clutches were usually found nestled in small cavities in the ground or under moist moss; in the laboratory, females were not separated from their eggs, but were maintained with their clutches in tanks with moist moss. Clutches were raised at room temperature (approximately 20°C) and ambient light cycle.

Preparation of Developmental Series, Observations, and Data Collection

We took eggs from clutches at various stages of development regularly during each week before hatching. Descriptions are based on preserved material. Embryos were dissected free from the surrounding egg membranes using forceps, fixed in Bouin's fixative (picric acid, formaldehyde, and glacial acetic acid; see Presnell and Schreiber, 1997), then measured and described. Larvae were kept in aquaria at about 27°C and fed pieces of meat, then

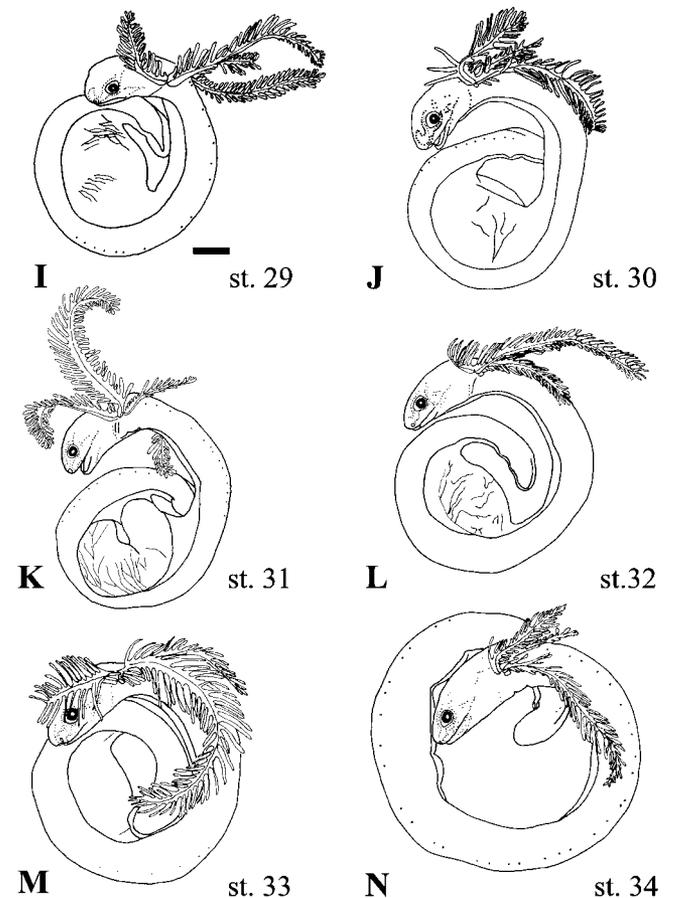


Fig. 5. *Ichthyophis kohtaoensis*. Lateral view drawings showing details of embryonic stages 29–34 (I–N). Lettering sequence of embryos continues from Figure 4. Note the changes in yolk volume and yolk sac vascularization, tailbud morphology, etc.; refer to text for details. Scale bar = 2 mm.

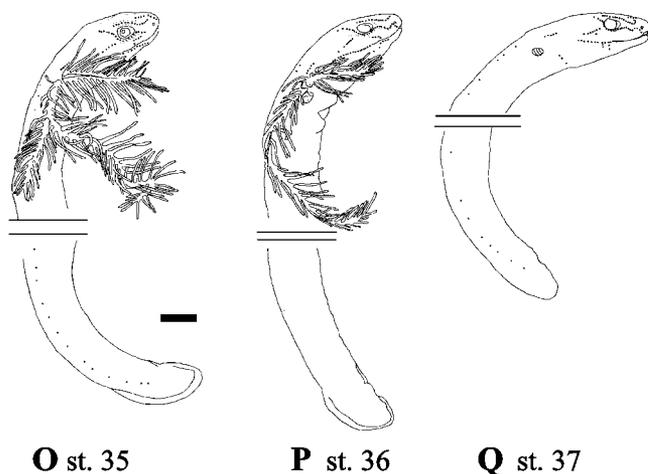


Fig. 6. *Ichthyophis kohtaoensis*. Lateral views of details of heads and tails of embryonic stages 35–37 (O–Q). Lettering sequence continues from Figure 4. Modification of gills, changes in the eye region, and developmental features at the time of hatching are emphasized. Refer to text for details. Scale bar = 2 mm.

selected specimens were fixed from the day of hatching until metamorphosis on a monthly basis and were similarly measured and described. Therefore, a nearly complete chronological survey of development from early embryonic stages through larval metamorphosis was available. Additionally, juveniles were preserved 1 month after metamorphosis. They were not described in the staging table, but their morphology is considered in the Results section. Early developmental stages corresponding to blastulation, gastrulation, and the beginning of neurulation are not represented in our series. In some cases, only one specimen per stage was available due to the rarity of the material.

Photographs were taken using a Wild stereomicroscope with a Photoautomat. Drawings of embryonic and larval stages were made using a Wild stereomicroscope and a camera lucida. Measurements of total body length of embryos were taken from camera lucida drawings. Larvae were measured directly. Total length of embryos is the longest dimension of the specimens in dorsal view, and was measured to the most posterior edge of the curled tail stem. The lengths in millimeters given in Table 2 are averages in round numbers, as there is sufficient variation to invalidate the use of measurements to tenths of millimeters. Additionally, the maximum length and width of the yolk mass and the maximum gill length were measured, and the number of gill filaments was counted.

For scanning electron microscopy (SEM), the specimens were dehydrated in a graded series of ethanol and dried in a Samdri-PVT-3B (Tousimis Research Corp., Rockville, MD) critical point dryer. Specimens were mounted on stubs using double-sided tape and silver paste, and then sputter coated with gold palladium in a Polaron E5400 (Energy Beam Science,

Agawam, MA) unit. Specimens were viewed with a ISI-DS130 scanning electron microscope and photographed with a Polaroid camera. The SEM was equipped with the SEMICAPS imaging system so images could be stored digitally and sent to the lab computer for further processing.

Because no egg clutches were observed being oviposited, the exact developmental time and age of specimens is not known. Total developmental time was estimated by combining data from several clutches with overlapping developmental stages. Counting backwards from the day of hatching, the estimated time of development ranges between 85 and 90 days following oviposition. Metamorphosis occurs approximately 9 to 12 months after hatching.

Many authors use the staging table of Gosner (1960) to identify developmental stages of frogs, and that of Harrison (1969) for salamanders. Because we could not specify stages of *Ichthyophis glutinosus* based on Sarasin and Sarasin's (1887–1890) description, or that by Brauer (1899) of *Hypogeophis rostratus*, we compared our material of *I. kohtaoensis* to that presented in the staging table for the viviparous aquatic caecilian *Typhlonectes compressicauda* (Sammouri et al., 1990). Principal diagnostic features were those from Sammouri et al. and Nieuwkoop and Faber (1967), and include development of the lateral line organs, formation of external gills, including the size of the gills and the number of gill filaments, development of the eyes, changes in yolk structure, and growth of the tail, including the formation and regression of the tail fin (Table 1). For the description of the development of the lateral line organs, we refer to the terminology of Hetherington and Wake (1979). Because few data are available for caecilians, Table 2 states total length, weight of the specimens, length and width of the yolk, and size of the gill and the number of gill filaments, though in some cases only one sample per stage was available.

RESULTS

We recognize 20 discrete developmental stages in our *Ichthyophis kohtaoensis* specimens, from embryos at the end of neurulation through mid-metamorphosis, based on readily discernible changes in major aspects of external morphology (e.g., lateral line organs, mouth opening, eyes, nasal openings, gills, tail, tail fin, cloacal region, yolk). The major features of each stage are summarized in Table 1 and described in detail herein. The table begins with stage 21 for two reasons: absence of material representing early stages of development through neurulation, and comparability of our earliest embryo with stage 21 of *Typhlonectes compressicauda* (Sammouri et al., 1990) (see Discussion).

Body Pigmentation

Earliest embryos of *Ichthyophis kohtaoensis* are almost white; at stage 21 they have only a few dif-

TABLE 1. Normal table of development of *Ichthyophis kohtaoensis*

Stage 21 (n = 1)

- embryo curls around a large yolk mass; head and tail tip nearly touch
- pigmentation covers anterior third of the body; melanophores scattered diffusely
- neural folds in contact in tail and forebrain region, approaching but not touching in hindbrain region
- mandibular arch divided into paired club-shaped upper maxillary buds and paired mandibular elements; hyoid arch and three branchial arches developed; paired mandibular elements just touch, forming a deep heart-shaped angle at the site of contact
- optic vesicles stand out distinctly; no eye pigmentation; lens discernible as central, dense, round disc
- otic vesicles, small white dots with a dense central disc, are discernible on either side of the neural folds in the region of the rhombencephalic groove
- olfactory pits evidenced by folds
- three short external gills; no gill filaments
- tail bud short, without tail fin, elevated from yolk
- cloacal opening is triangular-shaped, bordered by two lateral cloacal swellings
- yolk mass homogeneous, without constrictions; vascular system not discernible

Stage 22 (n = 2)

- paired mandibular elements continuous ventrally; club-shaped maxillary buds expanded ventro-laterally to border the stomodeum
- nasal pits large, lateral, round to oval
- gill filaments present on base (or base and top) of first gill and base of second gill

Stage 23 (n = 2)

- pigmentation covers anterior two-thirds of body
- club-shaped protrusions of the maxillary buds flank the lateral sides of the stomodeum; close contact between maxillary buds and lateral nasal walls forms a nasobranchial rim
- protrusion of optic vesicles and lenses; grooves encircle eyes and separate them from surrounding tissue
- first and second gill more elongate and bearing more filaments
- cloacal opening slit-shaped, bordered by two lateral cloacal swellings

Stage 24 (n = 2)

- pigmentation covers anterior 75% of the body
- first appearance of lateral line organs (neuromasts)
- mandibular elements fully continuous; maxillary buds connected; formation of laterally closed mouth opening, clearly distinguishable in lateral view
- third gill bears gill filaments
- tail bud pointed; fin formation begins
- yolk mass with smooth constrictions

Stage 25 (n = 1)

- pigmentation more dense, extends over all the body
- nasal, supra- and infraorbital neuromast rows developed; anlage of oral and postorbital neuromast rows; first appearance of neuromasts on trunk, more developed anteriorly; 24 elevated neuromasts distributed regularly on anterior half of the body, then 2–4 flat neuromasts with broader distance between them, then a gap lacking neuromasts, then 3 elevated neuromasts on tail tip; trunk neuromasts situated more laterally than dorsally
- otic vesicles no longer distinguishable
- gill chamber begins to form, as epithelial outpocketing at the base of the second and third gills
- vascular system now discernible on yolk surface

Stage 26 (n = 2)

- anterior part of the body clearly elevated from yolk
- initiation of two additional lateral line rows above the eyes; development of oral and postorbital neuromast rows; neuromasts on trunk still more elevated anteriorly; 40–42 elevated neuromasts regularly arranged along 75% of trunk; gap between neuromasts on anterior part of the trunk and tail tip is reduced
- eye pigmentation clearly discernible
- triangular-shaped rostro-ventral nasal openings surrounded by nasal swellings
- tail fin higher, further developed
- vascular system of the yolk further developed

Stage 27 (n = 1)

- lateral line organs in head region distinguishable as rows of white dots; anlage of V-shaped mandibular neuromast row on chin; first appearance of ampullary organs; three rows of lateral line organs above the eyes; neuromasts on trunk situated slightly more dorsally than laterally
- lower jaw elongates and extends posteriorly; mouth opening appears narrower
- nasal openings triangular to tear-shaped, moved to a more frontal position
- tail bud thickened, round; tail fin broadened, more delineated

Stage 28 (n = 2)

- appearance of gular neuromast rows on throat and supraspiracular neuromast rows above the gills; trunk neuromasts evenly distributed along anteroposterior axis; trunk neuromasts situated more dorsally than laterally on anterior part of trunk, more laterally in posterior region and especially on tail tip
- roundish lower jaw not fully developed, forms lip-like structure
- anlage of tentacle apparent near eye
- yolk is slightly apple-shaped

Stage 29 (n = 1)

- head elongated
- inception of ampullary organ row below the eyes; neuromasts become elevated in head region

TABLE 1. (Continued)

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- mouth development further advanced; narrow lower jaw extends posteriorly; mouth deeply notched laterally
 - yolk mass elongated with heart-shaped curvatures (shape similar to Sarasins' fig. 1b)
- Stage 30* (n = 6)
- straightening of head curvature
 - neural folds connected in head region; suture still present
 - two rows of lateral line organs (one row of neuromasts and one row of ampullary organs) below the eyes and three rows (one neuromast and two ampullary) projecting behind the eyes
 - tentacle anlage apparent as an opacity or small indentation anterior to the eye
 - cloacal region further developed; cloacal slit elongated, encircled by rim of swollen tissue, bordered laterally by two oval elongated buds
 - first bending of yolk mass, resulting in a heart- to U-shaped curvature (similar to Sarasins' fig. 2); late stage 30, yolk mass with S-shaped curvature to complete S-shaped twist (similar to Sarasins' fig. 3)
- Stage 31* (n = 1)
- head clearly flattened and elongated
 - initiation of skin glands on chin
 - inception of second row of ampullary organs below the eyes
 - occlusion of the jaws; mouth resembles the deeply notched, flat subterminal structure of larvae
 - clearly reduced double S-shaped yolk tube with curvatures transverse to the longitudinal axis of the embryo (similar to Sarasins' fig. 4)
- Stage 32* (n = 3)
- skin glands on chin are distinct
 - three complete rows of lateral line organs (one neuromast and two ampullary rows) below the eyes; dark gray pigmentation allows distinction between neuromasts (large solid white dots) and ampullary organs (small fine white dots)
 - tail tip becomes arrow-shaped and slightly curved ventrally; tail fin broadened; ventral incision between cloacal region and end of tail fin
 - cloacal aperture further differentiated; slit-shaped cloacal opening encircled by elongated elevated rim and bordered by two pronounced button-shaped swellings, which are now also visible in lateral view
 - beginning of yolk enclosure in abdominal folds and formation of spindle-shaped yolk mass; yolk mass spiralized; spirals run parallel to longitudinal axis of embryo; two symmetrical yolk halves separated by gut
- Stage 33* (n = 4)
- skin covering the eyes thickened; tear-shaped skin configuration overlies eyes and tentacle anlagen
 - elongated and retracted cloacal opening bordered by crescent- to bean-shaped cloacal swellings
 - large parts of reduced yolk enclosed in abdominal folds; externally, yolk discernible as large elevation; yolk mass spindle- to corkscrew-shaped
- Stage 34* (n = 3)
- neural folds in head region connected without suture
 - trunk neuromasts are larger, more accentuated, beginning to sink into the skin
 - tear-shaped skin pad thicker and cloudier; eyes barely discernible
 - reduced yolk mass nearly completely enclosed in abdominal fold; external yolk visible as one or several elevation(s); yolk mass resembles zigzag-shaped tube (similar to Sarasins' fig. 5a)
- Stage 35* (n = 3)
- neuromasts have central circular indentations; ampullary organs begin to sink into the skin
 - tear-shaped frontal nasal openings enlarged, becoming deeper
 - yolk mass nearly to completely enclosed in abdominal folds; no external yolk visible or only a slim yellow stripe; beginning of compartmentation of yolk tube; yolk mass appears to be segmented into several portions, which are aligned as a stack of coins
- Stage 36* (n = 3)
- neuromasts and ampullary organs barely discernible, sunken into skin; some neuromasts represented by small holes, especially in nasal row and anterior part of infraorbital row
 - only two external gills remain; third gill degenerated and internalized in the gill chamber; gill chamber nearly fully covered by epithelial fold
 - elongated, deeply retracted cloacal slit bordered by flattened, elongated wall of thickened tissue lacking pigment; wall is flattened at posterior end and more elevated anteriorly
- Stage 37* (n = 3)
- hatching begins
 - V-shaped mandibular neuromast row represented by small holes
 - two remaining gills are stripped off immediately after hatching; no external gills; opening to gill chamber elongates
 - tail tip more rounded than arrow-shaped; tail fin less broad, no longer delineated
- Stage 38* (n = 4)
- inception of lateral yellow stripes
 - neuromasts, resembling thick white dots with small central grooves, are considerably larger and are circumscribed by a dark circle or groove; no ampullary organs on chin
 - further enlargement of the tear-shaped frontal nasal openings
 - narrow tail fin curved dorsally
- Stage 39* (n = 3)
- broadened, intensively yellow lateral stripes, clearly discernible also on head
 - ventral side becomes pigmented, including chin; skin glands barely discernible
 - supra- and infraorbital rows have fewer neuromasts, and the supraorbital row is no longer curved
 - tail fin no longer discernible, only a narrow dorsal fimbria in region of former fin; tail tip no longer compressed laterally but begins to resemble rounded tail of adults
-

TABLE 1. (Continued)

- cloacal region pigmented; slit-shaped cloacal opening bordered by notched folds arranged transversely to cloaca, resembling a scar

Stage 40 (n = 1)

- metamorphic stage
- clearly reduced lateral line organs; no ampullary organs discernible on dorsal head, so only one row of lateral line organs visible above the eyes; neuromasts no longer accentuated, indicating beginning of degeneration of lateral line system; neuromasts resorbed posteriorly in the dorsal supra- and infraorbital rows; some neuromasts missing in the oral, postorbital, and supraspiracular rows; entire nasal neuromast row is resorbed
- funnel-shaped tentacle sheath clearly discernible; appearance of tentacle orifice with distinct tentacle fold
- gill chamber opening begins to close
- round tail resembles adult tail

Characteristic features of embryonic and larval stages from the end of neurulation (stage 21) through the onset of metamorphosis (stage 40) are summarized.

fusely scattered melanophores on the dorsum (Fig. 2A). Pigmentation covers the anterior third of the body at this stage. Pigmentation extends posteriorly, covering the anterior two-thirds of the body at stage 23. At stage 24 pigmented cells cover the anterior 75% of the body and at stage 25, the pigmentation is denser and extends over the entire body. The dark gray pigmentation of older embryonic stages (see stage 32; Fig. 3G) allows the distinction between elements of the lateral line system because denser neuromast rows are indicated by larger white dots, and fainter peripheral ampullary rows are represented by smaller white dots. At stage 39 the ventral side of the body becomes pigmented, including the cloacal region. The characteristic paired lateral yellow stripes of adult *I. kohtaoensis* (Fig. 1A) begin to develop on either side of the trunk at stage 38. The stripes broaden, become intensively yellow, and are clearly discernible on the head as well as the body at stage 39 (Figs. 10HI, 14IJ).

Neural Folds

At stage 21, the neural folds are in contact in the tail and forebrain regions, and approach but do not yet touch in the hindbrain region (Figs. 2A, 7A). The neural folds are in contact in the head region at stage 30, but a suture and a small triangular-shaped region at the anterior end of the suture, both lacking pigment, are still present in the hindbrain region. At stage 34 the neural folds in the head region are finally fully fused, without an apparent suture zone.

Lateral Line Organs

Lateral line organs are not discernible at stages 21 to 23 (Figs. 4A–C, 7A–C, 9AB). At stage 24 the first lateral line organs appear, represented by lines of pigmentless dots near the eyes (Figs. 4D, 7D). In the tail bud region, three pronounced elevated dots are observed, but the structures are absent on either side of the trunk. Based on their position and size, the organs are neuromasts, and are larger and more linearly arranged than ampullary organs, which first appear at stage 27 (see below). The neuromast

rows of stage 25 (Fig. 9C), running from the nasal openings to and encircling the eyes, are well developed and represent the nasal, supra- and infraorbital neuromast rows described by Hetherington and Wake (1979; their terminology will be followed in this description). Two additional rows of neuromasts occur, one projecting from the mouth opening (oral row) and one projecting from the eye (postorbital row), forming a “V.” The first neuromasts also appear on the trunk (Fig. 4E). They are more fully developed anteriorly and are situated more laterally than dorsally. At this stage, 24 elevated neuromasts are regularly distributed on the anterior half of the body, followed by two to four flat neuromasts that are more widely separated. Then a gap, lacking neuromasts, is observed, followed by the three elevated neuromasts on the tail tip.

At stage 26, two additional lateral line rows above the eyes appear, and the oral and postorbital neuromast rows are further developed (Fig. 7E). The neuromasts on the trunk are still more elevated anteriorly. Forty to 42 elevated neuromasts are regularly arranged along 75% of the trunk, and the gap between the neuromasts on the anterior part of the trunk and tail tip is reduced, with increased numbers of neuromasts proceeding posteriorly. The neuromast organs of the head region are distinguishable as rows of larger light dots; the first ampullary organs, resembling small fine dots, appear at stage 27 (Fig. 9D). Subsequently, a V-shaped row of neuromasts is established on the chin (mandibular neuromast row; Fig. 7F–H) and three rows of lateral line organs (one row of neuromasts and two rows of ampullary organs) are present above the eyes. However, the organs occur only above the right eye of our specimen; asymmetric development is not unusual (see Hetherington and Wake, 1979). Rows of neuromasts on the throat (gular neuromasts) and groups of neuromasts above the gills (supraspiracular rows) appear at stage 28 (Figs. 7F, 9E). The neuromasts on the trunk are evenly distributed and situated more dorsally than laterally on the anterior part of the trunk. However, they are found more laterally on the trunk in posterior regions and especially on the tail tip. The gaps between the anterior neuromasts

TABLE 2. Meristic data for *Ichthyophis kohtaoensis*, stages 21 to 40, including sample size

Stage	Sample size n	Total length (mm)	Weight (g)	Length of yolk (mm)	Width of yolk (mm)	Size of first gill (mm)/number of filaments	Size of second gill (mm)/number of filaments	Size of third gill (mm)/number of filaments
21	1	16.6	0.1	5.4	6.9	approx. 1/none	approx. 1/none	smaller than 1/none
22	2	18.5, 23.6	0.13	5.1	7.1	approx. 1/2–4 buds	approx. 1/few buds	smaller than 1/none
23	2	25.9, 29.6	0.16, 0.17	8.0, 9.0	6.1	3.2/37, 39 (5 only buds)	3.2, 3.5/34, 38 (4 only buds)	1.0, 2.0/none
24	2	18.3, 22.2	0.13, 0.14	5.4, 5.5	6.7, 7.6	3.1, 4.0/37, 44	3.1, 6.1/38, 61	1.6, 2.3/23, 27
25	1	23.3	0.18	5.7	7.9	4.9/38	5.8/50	2.5/31
26	2	24.9, 30.2	0.1, 0.16	7.0, 7.9	5.2, 5.6	4.4, 5.5/37, 40	6.8, 7.1/63, 64	3.2, 3.3/33, 34
27	1	32.5	0.19	5.7	5.2	5.5/36	8.3/56	3.5/26
28	2	27.4, 33.2	0.18	6.0, 6.5	5.5	5.1, 6.9/36, 41	8.5, 9.5/53, 59	4.0, 4.3/26, 28
29	1	36.3	0.21	6.9	4.9	6.9/48	12.0/74	5.9/40
30	6	31.7–41.5	0.18–0.23	5.8–7.4	5.0–5.8	5.7–7.5/36–50	10.2–13.0/54–74	4.0–6.0/28–34
31	1	40.8	0.23	5.9	5.4	5.3/34	10.7/63	7.0/36
32	3	43.8–45.9	0.24–0.27	6.2–6.8	4.1–4.9	6.2–8.2/4–40	12.2–17.2/65–74	5.5–7.5/30–36
33	4	49.8–53.8	0.29–0.34	6.9–8.7	3.0–4.4	7.0–8.3/28–41	12.2–16.0/52–67	5.0–8.2/28–35
34	3	54.9–78.3	0.31–0.37	11	3.2	6.0–8.5/38–41	10.8–21.0/56–70	4.5–9.0/26–37
35	3	74.8–80.0	0.39	—	—	7.0–8.0/37–54	12.5–16.0/56–81	4.5–7.0/25–38
36	3	70.1–83.0	0.42	—	—	6.0/41	12.0/66	—
37	3	65.1–76.2	0.42–0.47	—	—	6.5/47	13.0/55	—
38	4	78.2–120.0	0.60–1.63	—	—	—	—	—
39	3	133.1–163.0	2.66–4.81	—	—	—	—	—
40	1	156.2	4.15	—	—	—	—	—

and the posterior group, and between that group and those on the tail tip, are closed by a continuous line of neuromasts at regular intervals. The neuromast rows in the head region become elevated and resemble rows of dome-shaped protrusions rather than lines of unpigmented dots at stage 29 (Fig. 7G). Additionally, a row of ampullary organs forms below the eyes. At stage 30 several new rows develop: one row of neuromasts and one row of ampullary organs now lie below the eyes, and a row of neuromasts and two rows of ampullary organs extend behind the eyes (Figs. 7H, 9F). A second row of ampullary organs occurs below the eyes at stage 31 (Fig. 8I). At stage 32 the rows of lateral line organs include additional structures below the eyes.

As the body pigmentation progressively becomes darker, the lateral line organs are more prominent and stand out strikingly as large solid white dots (neuromasts) or small fine white dots (ampullary organs). By stage 32, the dark gray pigmentation permits distinction between neuromast and ampullary rows. At stage 34 the neuromasts of the trunk are sunken into the skin and are not as elevated as in previous stages, though they are slightly larger and more accentuated at this stage of development (Fig. 5N). The ampullary organs begin to sink into the skin at stage 35. At this stage the neuromasts have central, circle-like indentations. At stage 36 both kinds of lateral line organs are well sunken into the skin and are barely discernible. Some neuromasts are represented by small holes, especially in the nasal region and the anterior part of the infraorbital row. The V-shaped mandibular neuromast row resembles a row of small holes at stage 37. At stage 38 the neuromasts are considerably larger, but well sunken, and resemble thick white dots with small

central grooves; the dots are circumscribed by dark circles. Ampullary organs on the chin are no longer discernible at this stage (Fig. 8L). The supra- and infraorbital lateral line rows exhibit fewer neuromasts at stage 39; the supraorbital rows are no longer curved and thus are no longer in contact with the nasal row (Fig. 8M). At metamorphic stage 40 the lateral line organs are clearly reduced. No ampullary organs are discernible on the dorsum of the head and only one row of lateral line organs is observable above the eyes (Fig. 8N). Additionally, the neuromasts are no longer accentuated, indicating degeneration of the lateral line system. Neuromasts are resorbed posteriorly in the supra- and infraorbital rows; in the oral, postorbital, and supraspiracular rows some neuromasts are missing, and the entire nasal row is absent. Finally, 1 month after metamorphosis apparently commences, the postorbital, supraspiracular, gular, and the body neuromast rows are fully resorbed, the mandibular, supraorbital, and infraorbital rows are reduced, and the oral row consists only of scattered neuromasts, no longer forming a row projecting from the mouth opening.

We examined the development of the lateral line system at the ultrastructural level at stages 27, 30, and 35. In scanning electron micrographs, ampullary organs resemble small, deep pits (see Fig. 15A). At stages 27 and 30, the early neuromasts are represented by dome-shaped protrusions (Fig. 15B–D) that are covered by epidermis because the organs have not yet broken through the skin. At stage 35, however, the organs resemble shallow grooves, as they have sunken into the skin (Fig. 15E,F). The kinocilia and stereocilia in the neuromasts and the central position of several bundles of microvilli in

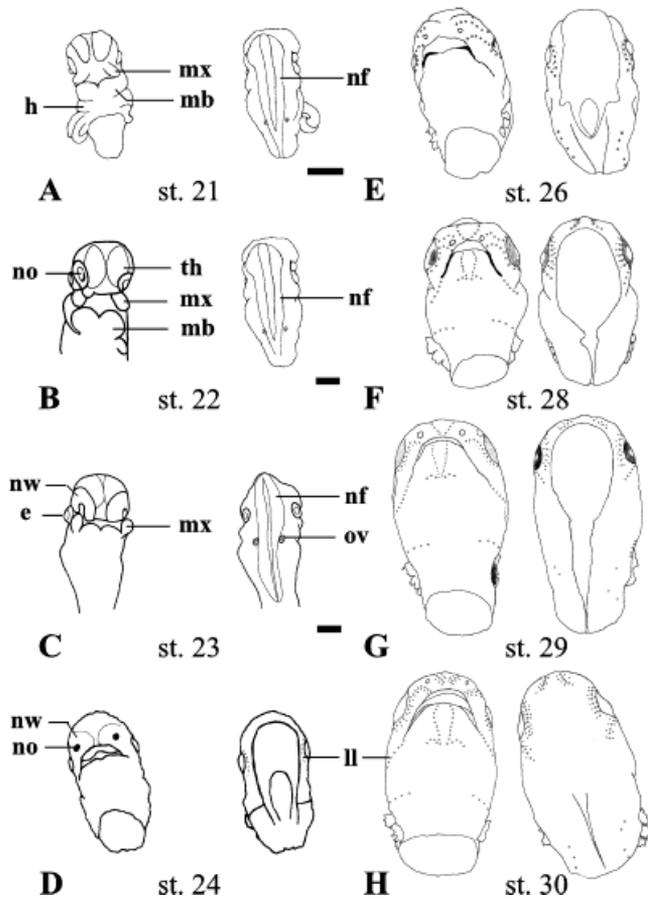


Fig. 7. *Ichthyophis kohtaoensis*. Line drawings of details of the development of the head region in embryonic stages 21–30 (A–H). For each stage, dorsal (right column) and ventral (left column) views are provided. Gills are severed to reveal head morphology on all specimens except the embryo in A. Note the development of the mouth components and the changing appearance of the nasal openings. The inception of the lateral line system is indicated in D (stage 24); in E–H progressively developing neuromast rows are represented by solid, large dots and ampullary organs are finer, small dots. The medial outlines in dorsal views indicate the neural folds, which are not yet closed in the hindbrain region. See text for further details. Abbreviations for Figures 7–10: e, eye; g, gill; gf, gill folds; gs, gill spiraculum; h, hyoid arch; ll, lateral line organs; mb, mandibular arch; mx, maxillary arch; nf, neural fold; no, nasal opening; nr, nasobranchial rim; nw, nasal wall; ov, otic vesicle; t, tentacle; th, telencephalic hemisphere; ys, yellow stripe. Scale bar in A = 2 mm (also applies to D–H); scale bar in B and C = 1 mm.

the ampullary receptors can be seen (Fig. 15A,F) because the amorphous substance secreted by specialized cells of the lateral line organs has dried and retracted during dehydration. At all stages examined, the epidermis includes the ciliated cells (Fig. 15A) typical of amphibian skin development.

Stomodeum

At stage 21, the mandibular arch is already divided into paired upper maxillary buds and paired

mandibular elements. Additionally, the hyoid arch and three branchial arches are well developed (Fig. 7A). However, the components of the lower jaw are not connected; the mandibular elements only touch, forming a deep, heart-shaped angle at the site of contact (Fig. 7A). The paired mandibular elements are continuous ventrally at stage 22, and the club-shaped maxillary buds have expanded ventrolaterally to border the stomodeum (Figs. 7B, 9A). At stage 23 the maxillary buds flank the lateral sides of the stomodeum (Fig. 7C). The maxillary buds and the lateral nasal walls are in close contact, forming a nasobranchial rim (Fig. 9B). The maxillary buds are connected at stage 24, and the mandibular elements are fully continuous, so that the lateral juncture of the mouth opening is clearly distinguishable in the lateral view (Fig. 7D). As the lower jaw elon-

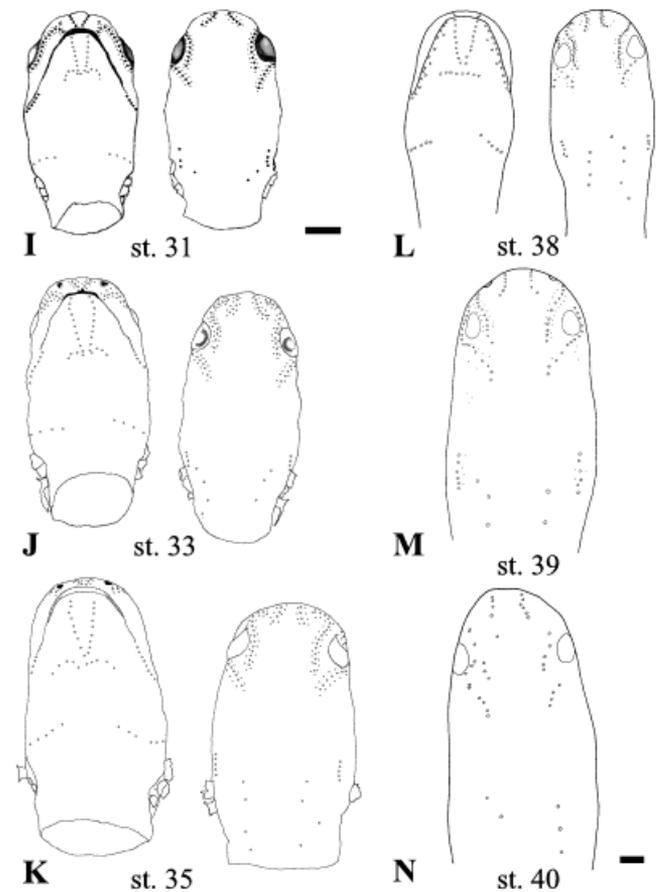


Fig. 8. *Ichthyophis kohtaoensis*. Line drawings of details of the development of the head region in stages 31–40 (I–N). Lettering sequence continues from Figure 7. In I–L, dorsal (right column) and ventral (left column) views are provided; M and N show dorsal views only. Gills are severed in I–K; hatched larvae (L–N) lack external gills. In the stages presented, subterminality of the mouth increases, lateral line organs begin to degenerate (L–N), and, as the tear-shaped skin configuration overlying the eyes and tentacle anlagen becomes thicker and more cloudy, eyes are barely discernible in K–N. See text for further details. Scale bar in I = 2 mm (also applies to G, K); scale bar in N = 1 mm (also applies to L, M).

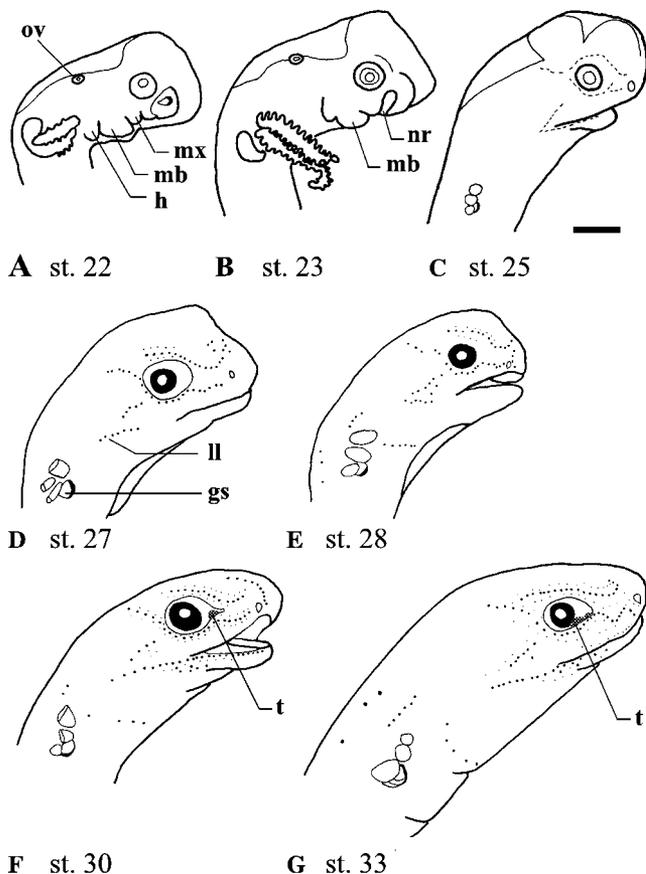


Fig. 9. *Ichthyophis kohtaoensis*. Line drawings of details of the head region of embryonic stages 22–33 (A–G) in lateral view. Gills are severed in C–G; the gill chamber opening is apparent in C (stage 25). Note the changing shape of the head and the jaw region and the development of the tentacle anlage in F and G. The inception of lateral line organs is indicated by dashed lines in C (stage 25); the developing neuromast rows are the more solid, larger dots and the ampullary organs are fine, small dots in D–G. See text for further details. Abbreviations are listed in Figure 7. Scale bar = 1 mm.

gates and extends caudally, the mouth opening appears narrower at stage 27. At stage 28 the lower jaw, which is roundish and not yet fully developed, forms a lip-like structure (Figs. 9E, 7F). Mouth development is further advanced at stage 29; the narrow lower jaw extends further caudally, and the mouth opening becomes deeply notched laterally (Fig. 7G). The upper and lower jaw occlude, closing the mouth, at stage 31 (Fig. 8I). At this stage the mouth presents the deeply notched flat subterminal structure characteristic of larvae.

Eyes

The eyes of *Ichthyophis kohtaoensis* embryos are apparent in stage 21 (Fig. 2A). At that stage the optic vesicles lack pigment but stand out distinctly, and the lens is discernible as a dense round central

disc. Protrusion of eyes and lenses is observable at stage 23, at which grooves encircle the optic vesicles and separate them from the surrounding tissue (Figs. 2B, 9B). Eye pigmentation appears at stage 26 (Figs. 2C, 4F). The eyes are covered by unpigmented, cloudy, translucent skin. At stage 33 the skin covering the eyes is thickened, forming a tear-shaped white configuration that overlies the eyes and the tentacle anlagen (Fig. 3H). The tear-shaped skin pad becomes cloudier and the eyes are barely discernible at stage 34 (Fig. 3I).

Tentacle

The tentacle anlage is first discernible externally at stage 28. At stage 30, the tentacle anlage is an opacity in a small indentation or groove near the eye at the frontal border of the cloudy translucent skin that covers the eyes (Fig. 9F). At stage 33 the tentacle aperture is represented by a white dense tissue fold, which is continuous with the thickened tear-shaped configuration that covers the eyes (Fig. 9G). At metamorphic stage 40 a funnel-shaped tentacle sheath is clearly discernible, and the tentacle fold appears in the tentacle orifice (Fig. 10I).

F10

Otic Vesicles

Otic vesicles are discernible from stage 21 to stage 24 on either side of the neural folds in the region of the rhombencephalic groove as small white dots with dense central discs (Figs. 7A–C, 9AB). At stage 25 otic vesicles are no longer distinguishable externally. They are sunken somewhat into the developing cranium and covered with a denser epidermal layer.

Nasal Openings

The olfactory pits of early embryonic stages are evidenced by folds (see stage 21; Fig. 4A). At stage 22 large, lateral, oval nasal pits are present. At stage 23 the nasal walls open laterally to form a nasobranchial rim, a connection between the nasal pits and the maxillary buds (Figs. 4C, 7C, 9B). The triangular rostro-ventral nasal openings are surrounded by enlarged nasal swellings at stage 26 (Fig. 7E). At stage 27 the triangular to tear-shaped nasal openings have moved to more frontal positions on the head (Fig. 9D). At stage 35, prior to hatching, the enlarged tear-shaped nasal openings become deeper, and further enlargement is observed at stage 38.

Gills and Gill Chamber

The embryos of *Ichthyophis kohtaoensis* bear three pairs of external gills, which first appear as slightly curved knobs or short elongated swellings situated laterally in the cephalic region on either

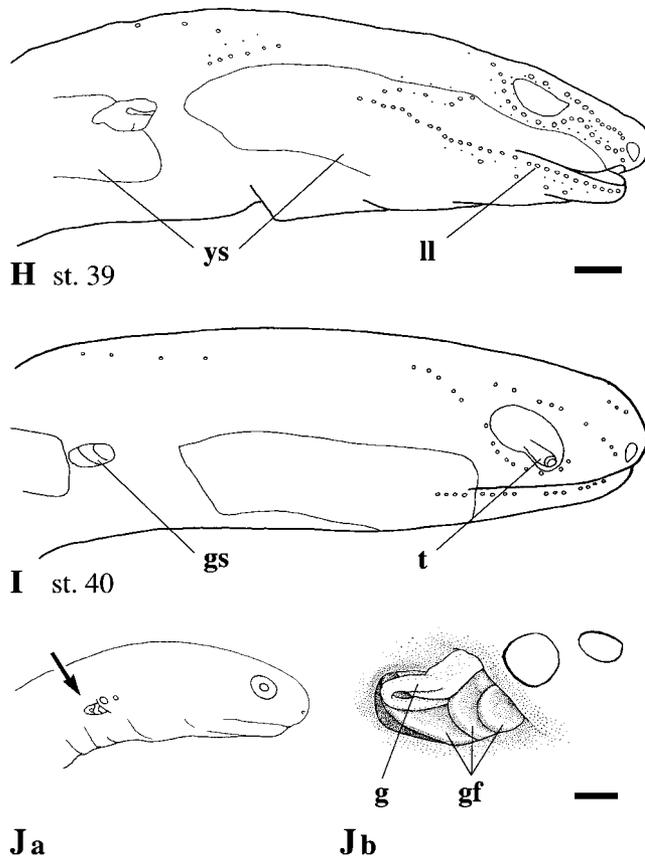


Fig. 10. *Ichthyophis kohtaoensis*. Line drawings showing details of the head region. Lettering sequence continues from Figure 9. In stage 39 (**H**) and metamorphic stage 40 (**I**), a broadened lateral yellow stripe and a regression of the lateral line organs occurs. Neuromast rows are represented by circles; see text for further details. **Jb** is a close-up of the gill chamber region (indicated by arrows in **Ja**) of a stage 36 embryo, showing the three gill folds and the internalization of the third gill. The first and second external gills have been severed. Abbreviations as for Figure 7. Scale bar in **H** = 1 mm (also applies to **I**); scale bar in **Jb** = 0.2 mm.

side of the head by stage 21 (Figs. 2A, 4A). At this stage the short gills lack filaments and gill slits between gill bars are not perforated. The gill filaments form in sequence, proximal to distal, as outpocketings of the gill tissue. They extend at right angles to the length of the ramus and are spaced nearly equidistantly, reaching approximately 3 mm in length. The first bud-shaped gill filaments appear either on the base or the base and top of the first gill and the base of the second gill at stage 22 (Fig. 4B). At stage 23 the first and second gills are more elongated and bear more filaments (Figs. 2B, 4C, 9B). Filaments of the third gill appear at stage 24, when the gills are already elongated (Fig. 4D). From stage 29 until hatching (stage 37), the second (middle) gill is considerably longer, sometimes double in size, compared to the first (anterior) and third (posterior) gills (see Table 2). Similarly, the filaments of the second gill are more numerous and appear longer

than those of the first and third gills (Table 2), a trend which first becomes evident at stage 24. The third gill is internalized into the gill chamber, which is nearly fully covered by its epithelial fold at stage 36 (Figs. 3K, 6P, 10Ja,b) and is resorbed prior to hatching (stage 37) (see Discussion). When crawling over the ground on their way to a pond or a stream, larvae strip off the remaining gills after hatching, leaving only the gill chamber openings visible on either side of the head (Figs. 3L, 6Q).

Although it is mostly covered by the external gills, the gill chamber is well developed in embryos. The gill chamber begins to form at stage 25 with the development of an epithelial outpocketing at the base of the second and third gills (Fig. 9C–F). Subsequently, three overlapping gill folds form (Fig. 10Jb); they then fuse, forming a skin fold that is the lateral wall of the shallow gill chamber, which is open dorsally and from which the bases of the gills extend. At stage 37 (hatching) the gill chamber opening elongates, and at metamorphosis (stage 40), the flat superficial chamber opening begins to close (Fig. 10I). One month after metamorphosis ensues, the chamber opening is still not completely closed, but is smaller in size and only discernible as a superficial indentation.

Somites

Somites can be counted through the translucent skin of our earlier embryos. Our stage 21 specimen has 125 pairs, the stage 22 specimen also 125, and the stage 23 specimens 127 and 128. A cleared and stained (skeletonized whole mount) hatchling larva in our collection has 123 vertebrae. We therefore infer that the full complement of somites is developed by stage 21.

Tail and Tail Fin

From stage 21 to stage 23 a short tail bud is partly elevated from the large yolk mass (Figs. 2AB, 4C). **F12** The tail tip is round and no tail fin is observable (Fig. 16A). At stage 24, the tail bud is no longer round but pointed, and the beginning of fin formation and lateral compression (more distinct in the dorsal region) becomes evident (Fig. 13B). **F13** A more developed, higher tail fin is observed at stage 26 (Fig. 4F). The tail bud thickens and becomes rounder again, exhibiting a broadened, more delineated tail fin at stage 27 (Fig. 4G). At stage 32 the tail tip is arrow-shaped and slightly curved (Fig. 3G; see also Fig. 16C). **F16** A ventral incision between the cloacal region and the end of the broadened tail fin is observable. The tail tip is more rounded than arrow-shaped, and the tail fin is no longer delineated and is narrower at stage 37. At stage 38 the narrow tail fin curves ventrally (Fig. 14H). Finally, a tail fin is no longer discernible at stage 39 (Fig. 14I). The tail, which is no longer compressed laterally but resem-

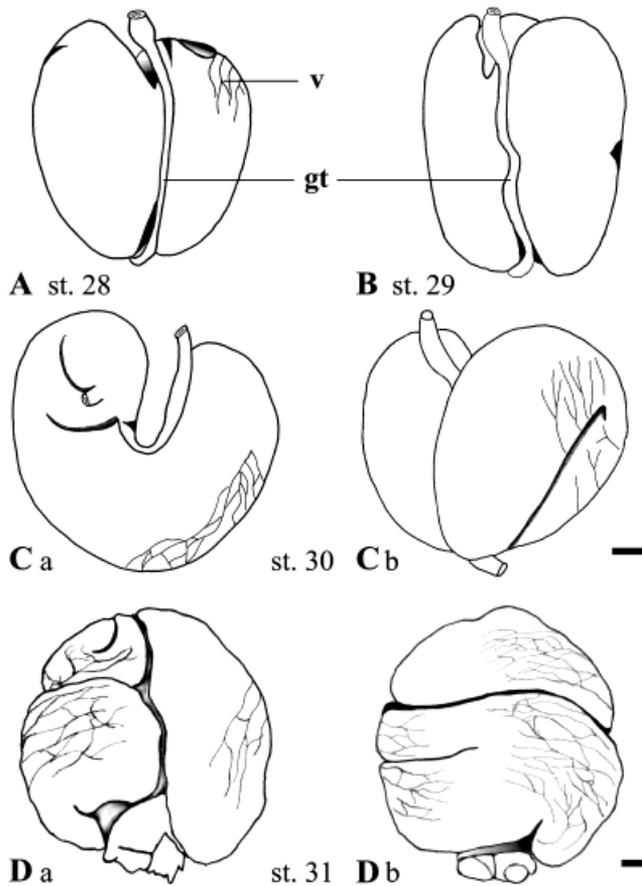


Fig. 11. *Ichthyophis kohtaoensis*. Line drawings of excised yolk of embryonic stages 28–31 (A–D): **Ca**, early stage 30; **Cb**, late stage 30; **Dab**, stage 31; different views of the same yolk mass. Spiralization of the yolk is described in the text. gt, gut tube; v, vascularization. Scale bar in **Cb** = 3 mm (also applies to **A, B**); scale bar in **D** = 3 mm.

bles the round tail of adults, exhibits a narrow dorsal fimbris in the region of the former fin. At metamorphic stage 40 the tail has achieved the circumference of the adult tail. Finally, 1 month after metamorphosis began, the tail tip is no longer pointed but obtuse, similar to the tail tip of adults.

Cloacal Region

Differentiation of the cloacal region is evident at stage 21. At stages 21 and 22, the triangular-shaped cloacal opening is bordered by two lateral round to elongated cloacal swellings (Figs. 13A, 16A). The cloacal opening, still bordered by lateral cloacal swellings, becomes slit-shaped at stage 23 (see also stage 24 in Fig. 13C). At stage 30 the elongated cloacal slit is encircled by a rim of swollen tissue and bordered by two lateral elongated buds (Figs. 13D, 16B). The cloacal aperture further differentiates at stage 32; the slit-shaped cloacal opening is surrounded by an elongated elevated rim and is bor-

dered by two pronounced button-shaped swellings. The latter are also clearly visible in the lateral view of the tail tip (Fig. 13E). At stage 33 the elongated and clearly retracted cloacal opening is bordered by crescent- to bean-shaped cloacal swellings (Figs. 3H, 13F; see also stage 35 in 16C). The elongated wall of thickened tissue which borders the retracted cloacal slit at stage 36 lacks pigment and is elevated anteriorly but flattened posteriorly (Fig. 14G). At stage 39 the cloacal region is pigmented and the slit-shaped cloaca is bordered by notched folds, arranged transversely to the slit (Fig. 14I); the cloacal aperture has a scar-shaped configuration at this developmental stage and through metamorphosis (Fig. 14J). Finally, 1 month after metamorphosis, the retracted cloacal slit is bordered by faint wrinkles or folds.

Yolk

From stage 21 to stage 23 the large yolk mass is homogeneous, slightly oval to bilobed (heart-

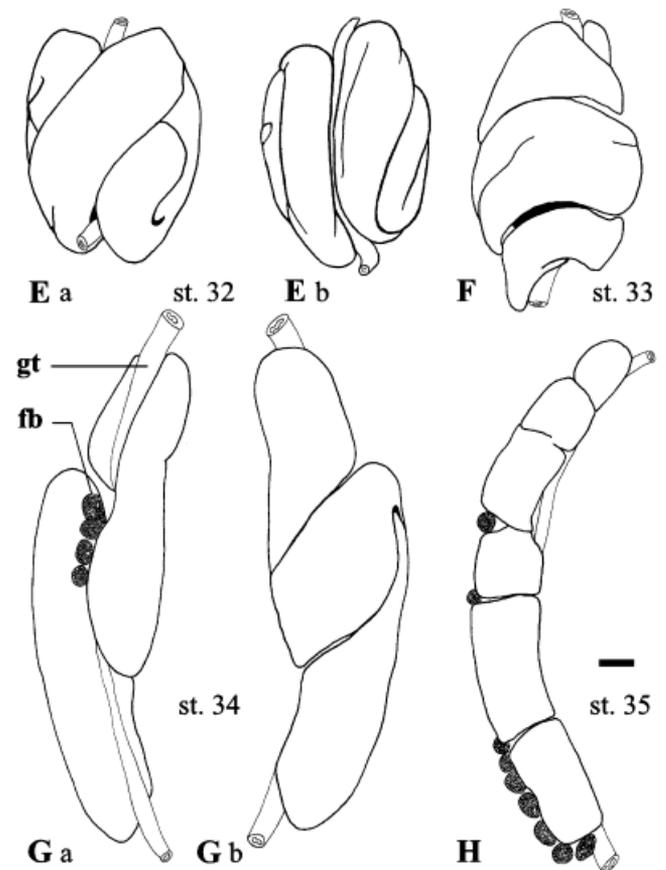


Fig. 12. *Ichthyophis kohtaoensis*. Line drawings of excised yolk of embryonic stages 32–35 (E–H). Lettering sequence continues from Figure 11. **Eab** and **Gab** provide different views of the yolk mass of stages 32 and 34, respectively. Increased spiralization occurs in stages 32 and 33 (E,F); elongation and compartmentalization of the yolk occurs in stages 34 and 36 (G,H). fb, fat body; gt, gut tube. Scale bar = 3 mm.

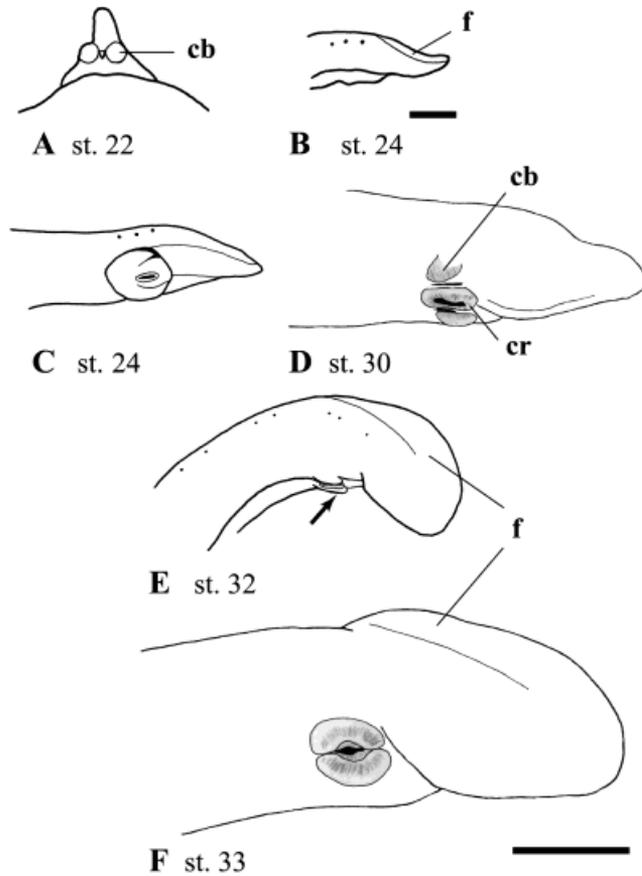


Fig. 13. *Ichthyophis kohtaoensis*. Line drawings of details of the cloacal region of embryonic stages 22–33 (A–F). The arrow in **E** points to cloacal buds, which are clearly visible in lateral view. Shading in **D** and **F** indicates the protrusion of the cloacal rim and the cloacal buds. See text for further details. cb, cloacal bud; cr, cloacal rim; f, fin. Scale bar in **B** = 3 mm; scale bar in **F** = 1 mm (also applies for **C–E**).

shaped); neither constrictions nor blood vessels are observed (Figs. 2AB, 4A–C). The yolk has smooth constrictions at stage 24. A vascular system is first discernible on the yolk surface at stage 25 and is further developed by stage 26 (Figs. 2C, 4EF). The yolk mass is less distinctly bilobed, but has slight central curvatures on top and bottom, where the gut intersects at stage 28 (Fig. 11A). At stage 29 the elongated yolk mass has distinct curvatures (Fig. 11B). The first bending of the yolk mass at stage 30 results in a U-shaped curvature (Fig. 11Ca). At late stage 30, the yolk tube exhibits an S-shaped curvature or a complete S-shaped twist (Fig. 11Cb). The reduced double S-shaped yolk tube of stage 31 bears curvatures transverse to the longitudinal axis of the embryo (Fig. 11Dab). At stage 32 the beginning of yolk enclosure in the abdominal folds is evident (Fig. 3G). The spindle-shaped yolk mass spiralizes, whereby the spirals run parallel to the longitudinal axis of embryo (Fig. 12Eab). Two symmetrical yolk halves separated by the gut are discernible and both

helical halves curl several times. At stage 33 large parts of the reduced yolk are enclosed in the abdominal folds (Fig. 3H). The spindle to corkscrew-shaped yolk (Fig. 12F) is discernible externally as a large elevation in the abdominal region. The further reduced yolk mass is nearly completely enclosed in the abdominal folds at stage 34, and the external yolk is visible only as one or several elevations (Fig. 3I). The yolk resembles a zigzag-shaped tube at this stage (Fig. 12G). At stage 35 yolk enclosure is complete, or nearly complete, and either no external yolk or only a slim yellow stripe is visible externally. At this stage, compartmentalization of the yolk begins and the yolk tube appears segmented into several portions, which are aligned like a stack of coins (Fig. 12H). Table 2 illustrates that, in general, the width of the initially large, round to oval yolk mass progressively decreases during the course of development, whereas the length of the yolk increases si-

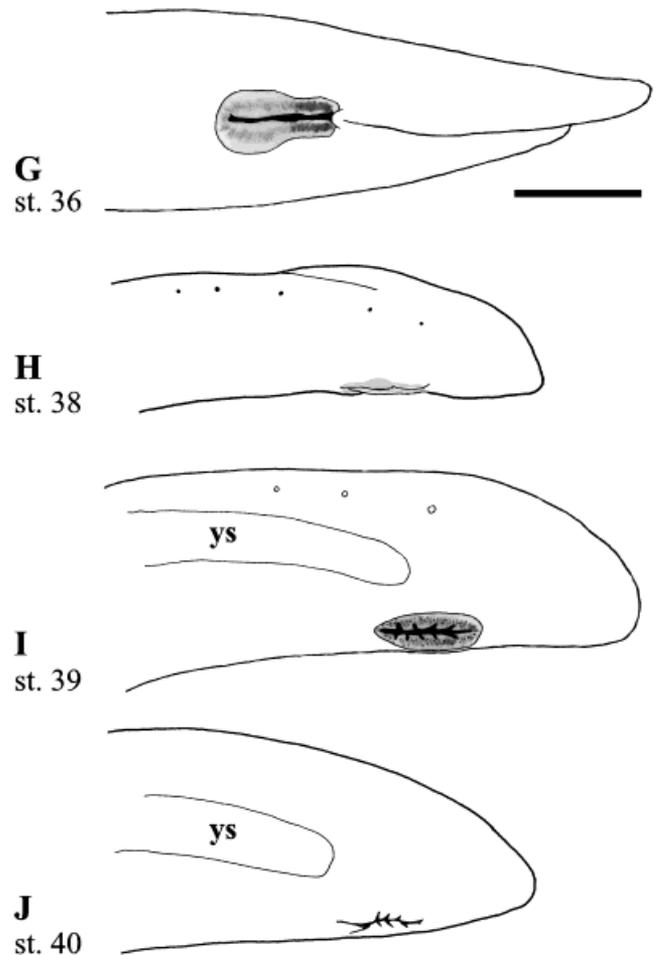


Fig. 14. *Ichthyophis kohtaoensis*. Details of the cloacal region of stages 36–40 (G–J). Lettering sequence continues from Figure 13. Shading in **G–I** indicates the protrusion of the cloacal region; dots in **I** represent first signs of pigmentation in the cloacal region. Black dots (**H**) and circles (**I**) along the dorsal trunk indicate neuromasts. ys, yellow stripe. Scale bar = 1 mm.

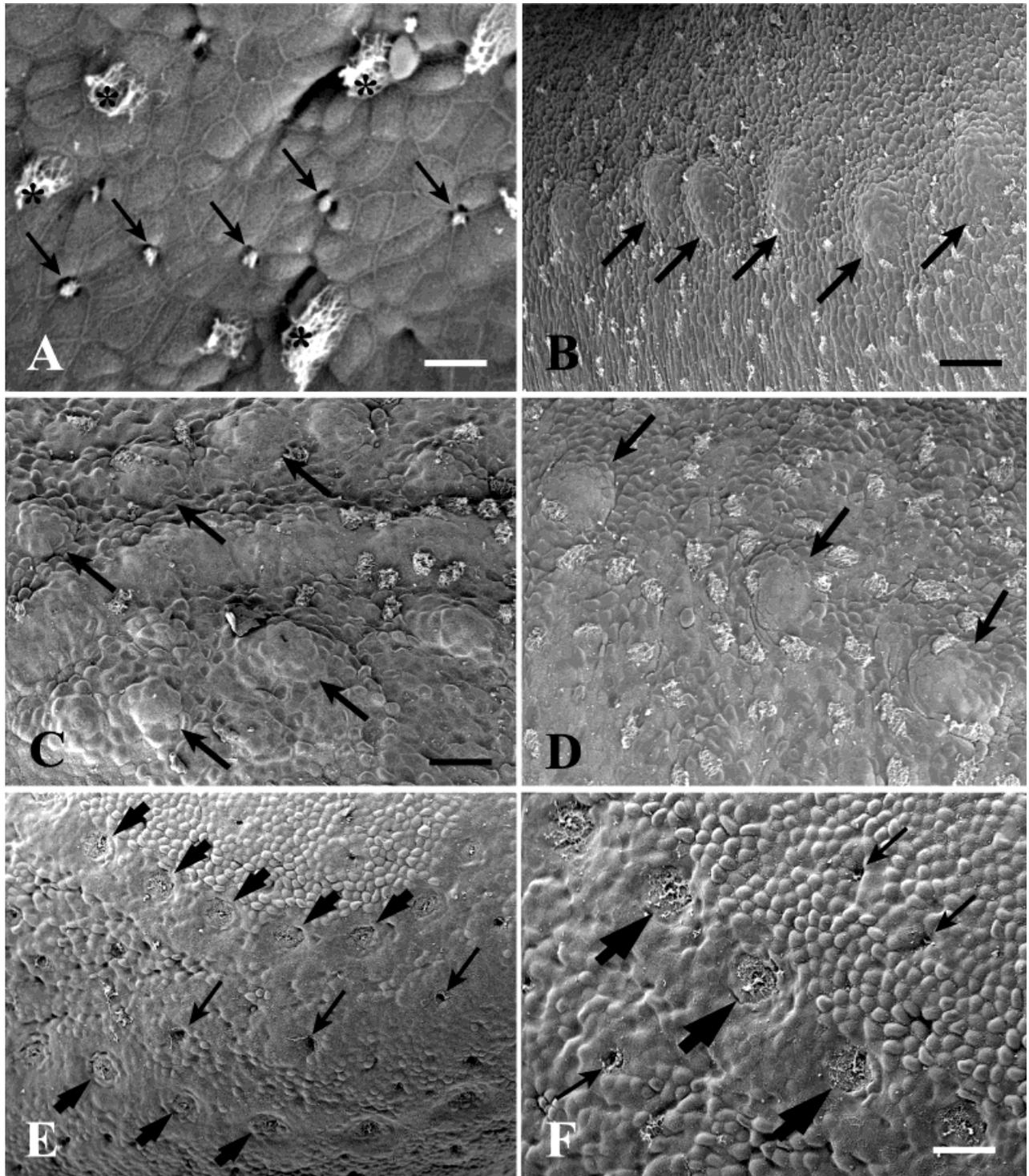


Fig. 15. *Ichthyophis kohtaoensis*. Scanning electron micrographs (SEMs) of the development of the lateral line organs: **A** and **B**, stage 27; **C** and **D**, stage 30; **E** and **F**, stage 35. Small arrows in **A**, **E**, and **F** indicate ampullary organs. Arrows in **B**, **C**, and **D** point to early, dome-shaped neuromasts in the head (**C**) and tail region (**B**, **D**). Large arrowheads in **E** and **F** (close-up of region shown in **E**) point to sunken neuromasts in the head region. Kinocilia and stereocilia of neuromasts (**F**) and ampullary organ microvilli (**A**) are visible because the amorphous substance secreted by specialized cells of the lateral line organs has dried and retracted during dehydration. Ciliated cells, indicated by asterisks in **A**, are visible in **A**–**D**. Scales: **A** = 30 μm ; **B** = 150 μm ; **C** = 70 μm also applies to **D** and **E**; **F** = 25 μm .

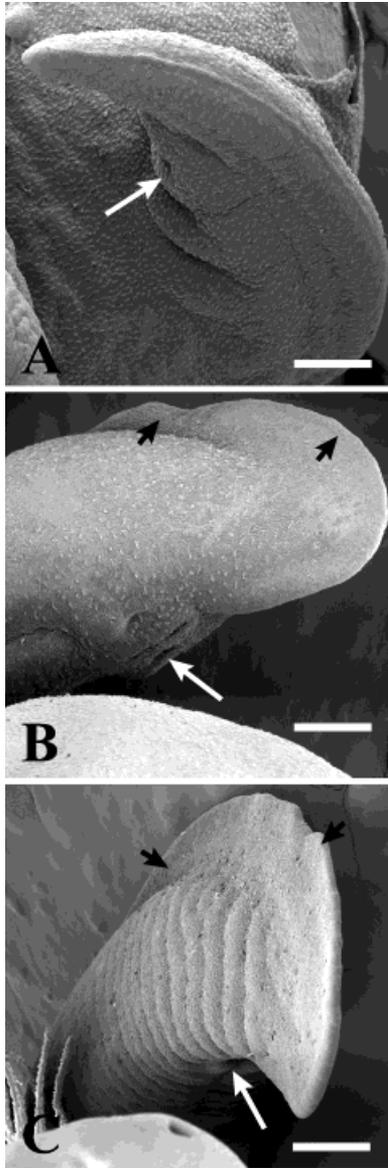


Fig. 16. *Ichthyophis kohtaoensis*. SEM's of the tail bud and the cloacal region: **A**, stage 22; **B**, stage 30; **C**, stage 35. White arrows point to the cloacal region, black arrows denote the tail fin. The cloacal rim and the cloacal buds are visible in **B**; see Figure 13 D and text for further details. Scale in **A** = 350 μm ; scale in **B** and **C** = 250 μm .

multaneously, resulting in the formation of an elongated yolk tube which is finally incorporated by the abdominal folds.

Total Body Length and Weight

Table 2 indicates that developmental size and weight vary considerably among individuals, and size ranges for different stages often overlap. As one might expect, both total body length and weight increase progressively during embryogenesis and larval development.

DISCUSSION

A number of tables of developmental stages for vertebrate species have been developed. Some have comparability of staging criteria, but many do not, either because of the particular features of the species (species-specificity) or the level of detail of the examination and description of those features. Further, the selection of staging criteria varies, depending on the intended use of the table. If it is to be used mainly to describe the development of a particular species (e.g., the detailed staging table of Nieuwkoop and Faber [1967] for *Xenopus laevis*), a set of criteria different from those used to group and compare individuals of different species (e.g., the less detailed but more general staging table of Gosner [1960]) will be used.

Stages are established according to a set of criteria, such as external morphology. Body sizes of specimens or physiological characters are considered by many researchers to be too variable to be used as staging criteria (Nieuwkoop and Faber, 1967; Townsend and Stewart, 1985; Grillitsch, 1989). External morphology provides more reliable and consistent staging criteria, although it too is subject to variation. Because of the inherent variation in developmental systems, individuals at a given stage often do not share all diagnostic features characteristic for that stage, and may present conflicting sets of characters. For example, the gill region of an embryo may appear well advanced, indicating a later stage in development, but the yolk is not correspondingly developed. Further, the development of certain organ systems, such as the lateral line system or the gills, has left–right asymmetry. By subdividing development into stages, it is possible to group and compare individuals and species.

The normal table that we have developed permits comparison of *Ichthyophis kohtaoensis* to other amphibian species, although the morphology of that caecilian species presents considerable variation relative to either viviparous caecilians or anuran or urodelan amphibians for which staging tables are available. We describe 20 stages to designate those between neurulation and mid-metamorphosis. They are chosen so as to be readily recognizable and are based mainly on external form. In addition to external morphological features, we include total length as a useful initial guideline for staging because so few data are available for caecilians. We recognize that factors such as crowding, temperature, light cycle, food, and water conditions can effect great variation in size and developmental rate. Developmental size varies, and size ranges for different stages often overlap. Thus, we avoid identifying stages based largely on size. Tables 1 and 2 list total body length for each stage, but more weight should be given to morphological features.

We discuss the ontogeny of selected morphological features (e.g., lateral line organs, gills, etc.) of *Ich-*

thyophis kohtaoensis and compare it to that of the congeneric *I. glutinosus*, then to the development of other caecilians, especially *Typhlonectes compressicauda*, a representative of a more derived family with a viviparous, rather than egg-laying, reproductive mode. We analyze our data in two ways: first, a direct comparison of each of the selected morphological features across species; second, a comparison of overall ontogeny of *I. kohtaoensis* with that of other species of caecilians in order to summarize salient similarities and differences in ontogenies. Finally, in those cases in which developmental morphology is particularly illustrative, we compare the ontogeny of *I. kohtaoensis* to that of selected oviparous and direct-developing frogs and salamanders, with specific reference to normal tables, in order to illustrate similarities and differences among taxa of amphibians. These several comparisons provide a baseline for further study of the evolution of patterns of development.

Ontogeny of External Morphological Features

Lateral line system. The lateral line system is a collection of epidermal sense organs (mechanoreceptive neuromasts and electroreceptive ampullary organs) and their nerves, distributed over the head and along the body in many aquatic amphibians. Early work on the lateral line system involved only the identification of mechanoreceptors, the neuromasts. The ampullary organs or "Nebenohren" of gymnophiones were described in 1887 by Sarasin and Sarasin, and Coggi (1905) compared them to the ampullae of Lorenzini of sharks. However, ampullary organs were not examined again for nearly a century. In fact, it was long assumed that amphibians lacked ampullary organs and had only neuromasts (Bennett, 1971). Hetherington and Wake (1979) reviewed the biology of lateral-line receptors in caecilians and corroborated the work of the Sarasins and Coggi, finding both neuromasts and ampullary organs in the lateral line system of gymnophiones. It is now understood that, in general, salamanders and caecilians have both neuromasts and ampullary organs as the receptors in their lateral line systems; anurans have only neuromasts (Fritzsich, 1981; Fritzsich and Wahnschaffe, 1983; Fritzsich and Münz, 1986).

Free-living larvae of several caecilian genera (e.g., *Ichthyophis*, *Caudacaecilia*, *Epicrionops*, *Sylvacaecilia*; Taylor, 1960, 1970; Hetherington and Wake, 1979; Fritzsich and Wake, 1986; Himstedt and Fritzsich, 1990; Wake, pers. obs.) possess a lateral-line system composed of both neuromasts and ampullary organs. Ampullary organs appear to be restricted to the head (Sarasin and Sarasin, 1887–1890; Fritzsich and Wake, 1986), where they are less numerous than neuromasts. Neuromasts occur in series along the body as well as on the head; how-

ever, they are more concentrated on the head. Our observations of ampullary organs associated with rows of neuromasts on the heads of *I. kohtaoensis* embryos at both light microscopic and ultrastructural levels (see Results) parallel those of Fritzsich et al. (1985) and Fritzsich and Wake (1986) for larvae of that species. In addition, lateral line organs (unspecified, but likely neuromasts) are reported for embryonic *Hypogeophis* (Brauer, 1899) and for *Sylvacaecilia* (then *Geotrypetes*) *grandisonae* (Largen et al., 1972; however, both neuromasts and ampullary organs are present in the latter [Wake, pers. obs.]). Further, Fritzsich and Wake (1986) observed structures that conform to descriptions of ampullary organs found in larval urodeles and caecilians in embryos of *Typhlonectes compressicauda* and *T. natans*, as well as on the heads of adult *T. natans* and *H. rostratus*. These organs lie within the epidermis and have a characteristic prominent central pit that resembles the flask-like ampullary organs of various freshwater fishes. Fritzsich and Wake (1986) remarked upon the association of the presence of ampullary organs with aquatic life and proposed that adults of *Typhlonectes* retain ampullary organs for electroreception in the murky, slow-flowing waters in which the animals live.

Sarasin and Sarasin's (1887–1890) examination of the formation of lateral line organs revealed that in early embryos of *Ichthyophis glutinosus*, represented by their figure 38 on Plate IV (comparable to our stage 26), neuromasts ("Hügelorgane") resemble dome-shaped structures, slightly protruding beyond the epidermis (also see Sarasins' fig. 11, Plate VI), whereas later the tip of the organ sinks in to form a depression or shallow groove (see Sarasins' fig. 12, Plate VI), very similar to the pattern of development in *I. kohtaoensis*. In larvae of *I. kohtaoensis* we found a distribution of lateral line organs rather similar to the pattern observed for *Ichthyophis* sp. by Hetherington and Wake (1979). However, they state that ampullary organs occasionally occur only along one side of a neuromast row, as in the instance of organs occurring only lateral to the supraorbital series. In contrast, three rows of lateral line organs (one row of neuromasts and two rows of ampullary organs), not two, are consistently discernible above and below the eyes of *I. kohtaoensis* larvae. Additionally, the distribution of ampullary organs differs from that in Hetherington and Wake's material; in *I. kohtaoensis* larvae ampullary organs are no longer concentrated on the chin, as they are in embryos of that species, but disappear or have sunk into the skin. The small, round pigmentless structures discernible in the chin region are skin glands. During the course of larval development the lateral line organs (neuromasts and ampullary organs) degenerate and by metamorphosis are no longer discernible (Sarasin and Sarasin, 1887–1890, and our observations).

Arrangements of neuromasts and ampullary organs vary among salamanders, though they bear

some similarities to the caecilian condition. For example, in *Triturus alpestris* and *Ambystoma mexicanum*, a linear arrangement of up to eight neuromasts occurs (Fritzsich and Bolz, 1986; Fritzsich and Wake, 1986). In these species the ampullary organs form groups of two to five organs, in contrast to the situation observed in *Ichthyophis*, in which the organs are in a more linear arrangement. In the axolotl *A. mexicanum*, ampullary organs occur at the bases of the gills. However, similar to their distribution in *I. kohtaoensis*, the ampullary organ clusters accompany the rows of neuromasts, are concentrated around the external nares and around the eyes, and are absent on the body and the tail. At the ultrastructural level, salamander ampullary organs are small, irregularly shaped pits distinct from large neuromasts (see figs. 5 and 6 in Fritzsich and Wahnschaffe, 1983), comparable to our SEM results (Fig. 15). The linear arrangement of kinocilia and stereocilia in the neuromasts and the central position of several bundles of microvilli in the ampullary receptor are visible when the amorphous substance (consisting of neutral mucopolysaccharides) filling the canal has dried and retracted during dehydration (see Fig. 15, and Fritzsich and Wahnschaffe's figures). Neuromast morphology in caecilians is generally similar to that of fishes and salamanders; however, the ampullary organs of salamanders and teleost fishes, lack kinocilia, but have many microvilli (Fritzsich and Wahnschaffe, 1983).

In general, those amphibians that adopt a predominantly terrestrial life history at metamorphosis lose their lateral line systems, whereas entirely aquatic amphibians retain their lateral line organs after metamorphosis (Shelton, 1970; Russel, 1976). There are some exceptions to this generalization; newts that are terrestrial but return to water to breed have large neuromasts that break through the skin and occupy a superficial position in a shallow depression during the aquatic phase, whereas they sink below the epidermal surface, are covered by one or two layers of epidermal cells, and appear to "de-differentiate" during terrestrial life (Fritzsich and Wahnschaffe, 1983). However, the generalization holds for caecilians; terrestrial animals lose their lateral line sensory structures at metamorphosis, as we observed in *Ichthyophis kohtaoensis* and Sarasin and Sarasin (1887–1890) and Breckenridge et al. (1987) saw in *I. glutinosus*, but the aquatic *Typhlonectes* has ampullary organs (but not neuromasts) in both pre-birth and adult states (summarized in Fritzsich and Münz, 1986).

Tentacle. Caecilians possess a sensory organ unique among vertebrates, the tentacle, which is apparently involved in tactile and chemoreceptive functions (see Billo and Wake, 1987). This small protrusile organ lying between the nasal opening and the eye is associated with the vomeronasal organ and its glands, and develops from several structures usually associated with the eye, such as the

lower eyelid, the interpalpebral space, the conjunctiva, the Harderian gland, the retractor and levator muscles, and their nerves (summarized in Billo and Wake, 1987). The tentacle apparatus is similar in its bauplan among all adult gymnophiones; however, sizes and shapes of the structures and their proportions differ among species and the tentacle aperture varies in its position relative to the eye and the nostril. The tentacle of *Ichthyophis glutinosus* was described by Sarasin and Sarasin (1887–1890). In that species, the specialized fold is recurved, elongate, and highly protrusile. Sarasin and Sarasin (1887–1890) also examined tentacle development in *I. glutinosus* and reported that in embryonic stages near hatching, the epithelium along the frontal edge of the eyes is thickened, resembling a tissue knot (see Sarasins' fig. 61, Plate XIX). In a 9.5 cm larva the tissue proliferation enlarges and a central cavity appears, which has no external contact (Sarasins' figs. 62 and 63, Plate XIX). In the course of development the growth cone continues sinking in and a small indentation develops from the outer surface (Sarasins' fig. 73, Plate XIX). At that stage, shown in Sarasins' figure 58 (Plate XIX), only a small pit in front of the eyes is visible externally in larvae, similar to the situation in our stage 30 and 33. According to the Sarasins, the formation of the tentacle fold begins in embryos prior to hatching, illustrated in figure 59 (Plate XIX). In those stages a tentacle orifice and a small "papilla," the tentacle fold, are discernible in front of the eye, similar to the situation in metamorphic stage 40 of *I. kohtaoensis*. Finally, in late larval stages, which exhibit the external features of young terrestrial adults (see the Sarasins' fig. 60, Plate XIX), the tentacle aperture moves in the direction of the upper lip, and the tentacle sheath and the involuted tentacle fold elongate. The tentacle of *I. kohtaoensis* is constructed very similarly to that of *I. glutinosus*, based on our observations of external developmental and adult morphology.

Billo and Wake (1987) examined tentacle development in *Dermophis mexicanus* using transverse, sagittal, and frontal head sections. They reported that a tentacle anlage appeared to be present ventro-rostral to the eye in the form of a minute ectodermal elevation in 14–15 mm TL *D. mexicanus* embryos, which appears comparable to stage 28 of *Ichthyophis kohtaoensis*, at which the tentacle anlage first appears. In 22–23 mm *Dermophis* embryos the tentacle anlage is surrounded by an ectodermal rim and resembles a depressed plug. This stage of tentacle development appears comparable to the situation found in *Ichthyophis* embryos of stage 33, in which the tentacle aperture represents a white, dense tissue fold. Billo and Wake (1987) reported that in 34–35 mm fetuses the tentacle anlage has differentiated by evagination into a tiny fold within a small sheath. In 44 mm fetuses the tentacle aperture still has the same ventrorostral position rela-

tive to the eye, which is covered by skin. By comparison, at our metamorphic stage 40 a funnel-shaped tentacle sheath is clearly discernible and the tentacle appears in the tentacle orifice.

A direct comparison of tentacle development in the species examined in these studies is difficult because they have different modes of reproduction and development (oviparous vs. viviparous); embryos, fetuses or larvae, and adults are different sizes at approximately the same developmental stages; and neither Billo and Wake (1987) nor Sarasin and Sarasin (1887–1890) provided staging criteria other than total body length. Furthermore, we did not have sectioned material of *Ichthyophis kohtaoensis* available, which would have permitted a more specific comparison. We do note that, like the condition reported by Sarasin and Sarasin (1887–1890) but contradicted by Breckenridge et al. (1987) in *I. glutinosus* (they state that the tentacle appears externally at metamorphosis), in *I. kohtaoensis* a tentacle anlage is visible externally in embryonic stages, although the development of the tentacle apparatus continues after hatching and metamorphosis. We do not have more advanced larval stages, at which the tentacle moves rostrally in the direction of the lip, available for examination.

Gills. Three pairs of external gills develop in salamanders and caecilians, whereas external gills are poorly developed or transitory, if present at all, in anuran embryos. Among caecilian taxa that have terrestrial adults, the gills of embryos typically are triradiate, elongate and plumose, no matter what the reproductive mode (oviparity, direct development, or viviparity), but they are a single pair of highly dilated sacs in the viviparous aquatic typhlonectids (Peters, 1874, 1875; Parker, 1956; Taylor, 1968; Wake, 1969; Nussbaum and Wilkinson, 1989; Wilkinson, 1989; Sammouri et al., 1990; Exbrayat and Hraoui-Bloquet, 1991, 1992; Hraoui-Bloquet and Exbrayat, 1994; Wilkinson and Nussbaum, 1997). Embryos of *Ichthyophis glutinosus* (Sarasin and Sarasin, 1887–1890), *Hypogeophis rostratus* (Brauer, 1897, 1899) and *Geotrypetes seraphinii* (Parker, 1956) have three pairs of gills throughout their embryonic development until the gills are lost at hatching or metamorphosis; in larvae of some species, three gill rudiments persist (e.g., *Epicrionops petersi* and *E. bicolor*; Wake, pers. obs.). However, Seshachar (1942) and Ramaswami (1954) stated that *Gegenophis carnosus* embryos have only two pairs of gills (also M. Wake, pers. obs.). In *Gymnopsis multiplicata* the external gills have three fringed rami arising from a short stump attached to the head (Wake, 1969). In contrast, gill rami in embryos of most taxa reported in the literature are directly attached to the head.

Brauer (1899) described the development of tiramous, filamentous gills from three knobs on each side of the head of embryos of *Hypogeophis rostratus*. He reported that the three rami develop

from these outpocketings of the gill tissue, as we and the Sarasins observed in *Ichthyophis*. It was not possible for us to examine early gill development because we lack early stages of development in our series, so we are not certain whether the three gills in *Ichthyophis* embryos develop in parallel or with a time delay. However, in contrast to gill development in *Hypogeophis*, where the third gill does not appear until the time that the gill filaments have appeared on the first gill, in *Ichthyophis* embryos all three gills are well developed at that stage. Brauer (1899) stated that at “full” development in *Hypogeophis* the middle ramus is slightly longer than the anterior and posterior. Brauer reported a maximum length of only 7 mm for the second gill and a maximum length of 5 mm for the first gill in a 40 mm total length *Hypogeophis* embryo; he stated that the filaments of the third gill remain small in size and number. According to Brauer and to Breckenridge and Jayasinghe (1979), the differences in lengths of gill rami are even more pronounced in *I. glutinosus* (which we also observed in *I. kohtaoensis*; see Table 2). Marcus (1908) observed gill development in *Hypogeophis* and *Grandisonia*, but mostly added information to Brauer’s report and commented on comparative gill homologies.

Wake (1967, 1969) described gill ontogeny and structure in embryos of the viviparous *Gymnopsis multiplicata*; total length was her sole “staging” criterion, so it is used here as a basis for comparison. A 10.5 mm embryo has two 2.5–3.5 mm long gill rami with 20–25 filaments on each side of the head. A 16.5 mm *Ichthyophis* embryo, at our earliest developmental stage (21), has only 1 mm long gill buds without any filaments, but has three rather than two external gills. A 37 mm *Gymnopsis* embryo has a 10 mm long posterior gill, a 6 mm long anterior gill, and a newly developed 2.5 mm long middle ramus with 10–12 short filaments that extend at right angles the length of the ramus, similar to the situation in *Ichthyophis*. The 36.3 mm stage 29 of *Ichthyophis* has first (6.3 mm) and third (5.9 mm) gills of comparable size, but the second or middle gill is much longer at 12 mm and its numbers of gill filaments is higher, between 40 and 74. Similar to Wake’s (1967, 1969) 52 mm and 54 mm embryos, our comparable stages 33 (53.8 mm) and 34 (54.9 mm) have a middle gill (12 mm in stage 33; 10.8 mm in stage 34) that is nearly twice as long as the anterior gill (6.8 mm in stage 33, 6 mm in stage 34) and the posterior gill (6 mm in stage 33, 4.5 mm in stage 34). All three external gills are significantly longer and have considerably more filaments in *Ichthyophis* embryos than do *Gymnopsis* embryos at approximately the overall size.

The unique large, sac-like gills of typhlonectids have frequently been described (see above), with conjecture about their function and their restriction to that aquatic lineage. Sammouri et al. (1990) provide a detailed description of the development of the

gills of *Typhlonectes compressicauda*, which we summarize for comparison with gill development in other taxa. Stages 15–20 illustrate development of four branchial arches (though the fourth is apparently transitory, not seen in the dorsal view of stage 19, and a slight expansion attached to the body wall in stage 20). Fusion of the three primary gill components begins at stage 21, forming the initially leaf-like gills that expand into large sacs as development proceeds. From stage 21 to stage 25, three full gill arches and one posterior hemibranch with gill slits are illustrated, though the paired gills are much expanded. Stage 27 appears to have three primary blood vessels serving the gill sac; these may be the extensions of the three aortic arches that serve the gills as reported by Wake (1967). Hraoui-Bloquet and Exbrayat (1994) described the development of the cellular structure of the gills of *T. compressicauda*; Exbrayat and Hraoui-Bloquet (1991, 1992) examined the modification of the gill surface during development in that species, concluding that branchial cells in embryos of later stages are involved in maternal-fetal exchange, perhaps including uptake of nutrient material. There is species-specific variation in the attachments of the gills to the heads among typhlonectids (Wilkinson and Nussbaum, 1997); numerous reports detail the presence of the large gills at birth in typhlonectids and the subsequent, rapid regression of circulation in them followed by their sloughing off, leaving a scar that is obliterated usually within days. Consequently, the fundamental structure of the gills of typhlonectids is comparable to that of other caecilians (and vertebrates in general), but the fusion of the gills and the expansion of the gill epithelium into the large sac-like gills is virtually unique (a comparison should be made to the structure of the bell gills of the hylid frog *Gastrotheca*, in which the female retains developing embryos in a “marsupium” of the skin of her back).

Gill degeneration and loss. Marcus (1908) analyzed the pattern of gill degeneration and loss in certain caecilians. It has long been assumed that gills are resorbed by the embryo or larva (Sarasin and Sarasin, 1887–1890; Brauer, 1899; Marcus, 1908; Wake, 1969; Breckenridge and Jayasinghe, 1979). Brauer (1899) reported that near the end of embryonic development the gills decrease in length; the third gill is the first to degenerate. He apparently assumed that the remaining first and second gills are progressively resorbed rather than stripped off. Marcus (1908) reported confirmation of Sarasin and Sarasin’s and Brauer’s suppositions that the gills are not stripped, but resorbed. Duellman and Trueb (1986) stated that in caecilians gill degeneration occurs during late embryonic, larval, or fetal life and gills are lost, either by resorption or breakage, soon after hatching. We note that an exception to this generalization is the situation in viviparous

taxa, in which fetuses retain gills for lengthy periods, even after “metamorphosis” and birth.

However, in *Ichthyophis kohtaoensis* only the third, posterior gill is resorbed and internalized in the gill chamber. The two remaining gills, the first or anterior gill and the second or middle gill, are often lost immediately after hatching. They are fragile and are stripped off when the larvae crawl over the ground on their way to a pond or a stream and the gills come into contact with substrate (Himstedt, pers. comm.). Those gills did not show signs of degeneration, either in total length or in the number of gill filaments. However, the diameters and lengths of the gill filaments slightly decreased prior to hatching and the gills sometimes appear reduced in length compared to the increasing body size. Gill growth ceases at about stage 32–33, but the growth in body length continues and accelerates, so that from stage 26 to stage 32 the ratio of length of the first gill to body length is 1:6, but is 1:8 at stage 34, and 1:11 at premetamorphic stage 37. We suggest that in *Hypogeophis* as well (see Brauer’s 1899 discussion), no significant reduction in gill size may have occurred, because our analysis, including examination of his figures, shows that not only the number of filaments but also gill size varies considerably among embryos of similar body size and mode of development. Because the embryos in figures 48 and 49 of Brauer’s (1899) description differ only slightly in total body lengths and in lengths of the first and second gills, these differences may not be significant, but within normal variation. Further, Breckenridge and Jayasinghe (1979) and Breckenridge et al. (1987) comment that the gills of *I. glutinosus* are lost in about 2 days after hatching, though the former state that they are “probably reabsorbed” but without evidence that this is the case.

Gill chamber. Müller (1835) first discovered gill apertures in larval stages of *Ichthyophis glutinosus* (*Coeilia hypocyanea*). Sarasin and Sarasin (1887–1890) reported that a large gill slit or “spiraculum” is discernible in late embryonic stages of that species (see Sarasin’s fig. 48, Plate V, and p. 23). The authors stated that the gill slit is also present in earlier embryos but at those developmental stages the groove is narrower and covered by the bases of the three external gills. According to Sarasin and Sarasin, in *I. glutinosus* the gill slit resembles a groove, bordered by a low tissue fold (“Hautwulst”), as shown in their figure 120 (Plate XXIV). They also described the presence of three tile-shaped overlapping tissue lobes, which they call “leaflets” (“Blättchen”). We found such structures in *I. kohtaoensis* embryos as soon as the gill chamber formed (stage 25), as three overlapping gill folds. Sarasin and Sarasin compared the gill folds of *I. glutinosus* to the gill “plates” of salamander larvae, previously described by Dugés (1835) as “ailerons,” or “little wings.” They assumed that these structures, which, according to Dugés (1835), bear blood vessels, par-

ticipate in respiration at least to some degree and may be interpreted as vestigial inner gills of fishes; they puzzled about lung respiration and the concomitant passage of water through the gill slits. Conversely, Wake (1967) found that neither side of the head of 52 mm and 54 mm TL fetuses of *Gymnopsis* had an open gill slit.

We interpret these conflicting pieces of information as a misconception of the nature of gill slits. We restrict the definition of “gill slit” to the branchial openings that allow the passage of water from the environment into the pharynx—the classic gill slits of vertebrate embryology. Our analysis of the older literature, including the illustrations and our own material, suggests that open gill slits of the ancestral sort have not been demonstrated to be present in the specimens that Sarasin and Sarasin, Brauer, and Marcus had available. We consider the gill chamber (the “spiraculum” of Sarasin and Sarasin, 1887–1890) to be a separate and distinct structure that is formed of an epithelial fold at the base of the gills, and into which the third gill is internalized (see *Ichthyophis kohtaoensis* description above). It is an external epithelial pocket, not open to the pharynx.

The cloaca. External sexual differences are not easily discernible in most caecilians. Taylor (1968) suggested that the morphology of the vent region, especially glands, might provide means of sexing animals. In typhlonectids, the anal region of the male is modified to form a circular depression and strong sexual dimorphism exists in the anal region of members of *Potomotyphlus* (Taylor, 1968; Wilkinson and Nussbaum, 1997). The cloaca in caecilians is internally dimorphic; further, a portion of the cloaca in males is eversible as a phallosome (literature summarized in Wake, 1972). Partial eversion of the cloaca also has been observed in near-term viviparous females (Wilkinson and Nussbaum, 1997; Wake, pers. obs.).

Cloacal development commences with the breakdown of the proctodeal plate and the appearance of swellings that differentiate into specific cloacal structures. We describe the different shapes and degrees of elevation of the swellings and their associated structures during development in embryos and larvae of *Ichthyophis* (see Table 1). At stage 30, for example, the elongated cloacal slit is encircled by a rim of swollen tissue and bordered by lateral oval, elongated cloacal buds (Fig. 13D); at stage 32, the two pronounced button-shaped cloacal swellings, which border the cloacal rim, are also clearly visible in lateral view (Fig. 13E). Brauer (1899) described two elongated swellings or elevations in a lateral position of the vent (the “Kloakenwülste”), indicating the cloacal anlage, in *Hypogeophis* embryos. Sarasin and Sarasin (1887–1890) described parts of the structure as “wulstige Erhebungen der Kloakenränder.” Sarasin and Sarasin (1887–1890) and Brauer (1899) indicated that such structures

marked the transitory appearance of hind limbs. We reject that assumption because the entire structures shown in the Sarasins’ figures 44 and 45 (Plate V) and in Brauer’s figures 38c and 39c strongly resemble the two pronounced button-shaped swellings we observed at stages 30–32, and we directly trace their development into the cloaca.

The yolk mass. Sarasin and Sarasin (1887–1890) described a complex bending and spiralization of the yolk mass during development, then reduction of the mass, in *Ichthyophis glutinosus*. Brauer (1899) described yolk development in *Hypogeophis* embryos and found that at the time the ventral body walls begin to enclose the yolk, the yolk mass reduces faster than in previous stages, flattens, and becomes spindle-shaped. Brauer’s study revealed that the abdominal folds close ventrally prior to full resorption of the yolk mass. In his figure 48, which is developmentally comparable to our stage 34 or 35, the resorbed yolk mass is completely enclosed in the abdominal fold and no yolk is discernible externally except as a slight thickening of the mid-body. At stage 29 in *I. kohtaoensis*, when the large yolk mass becomes slightly elongated, with deep curvatures on the dorsal and ventral sides (Fig. 11B), the shape of the yolk closely resembles the Sarasins’ (1887–1890) figure 1b in Plate XII. The first bending of the yolk mass at stage 30 results in a U-shaped curvature (Fig. 11Ca), resembling the Sarasins’ figure 2 (Plate XII). At late stage 30, the yolk tube exhibits an S-shaped curvature or a complete S-shaped twist (Fig. 11Cb), similar to the yolk shown in the Sarasins’ figure 3 (Plate XII). The reduced double S-shaped yolk tube of stage 31 bears curvatures transverse to the longitudinal axis of the embryo (Fig. 11Dab), resembling the yolk in the Sarasin’s figure 4 (Plate XII). It appears that the U-shaped yolk tube curves to form an S, which then further rolls to a double S-shaped form. The further reduced yolk mass is nearly completely enclosed in the abdominal folds at stage 34 and the external yolk is visible only as one or several elevations. At this stage the yolk resembles a zigzag-shaped tube (Fig. 12Gab), similar to the Sarasins’ figure 5 (Plate XII). Sarasin and Sarasin (1887–1890) assumed that the rotations and windings of the yolk result from tensions (“Spannungen”) in the yolk mass rather than from a spiralization of the gut. They stated that the changes in the shape of the yolk occur quickly and embryos of similar body sizes and developmental states often show significant differences in yolk shape. Those observations characterize yolk development in embryos of *I. kohtaoensis* as well. We do not have comparable information on yolk mass modification in other species.

Metamorphosis. Metamorphosis in at least some caecilians appears to be somewhat more protracted than in other amphibians (Breckenridge et al., 1987; Fritzsche, 1990; Wake, 1993; Exbrayat and Hraoui-Blouet, 1994). From those articles, the earlier work

of Sarasin and Sarasin (1887–1890), Brauer (1899), and Taylor (1968) and our data we briefly summarize existing knowledge of metamorphosis in terrestrial, oviparous taxa. The gills usually are much reduced or lost (either by resorption or breakage) soon after hatching, leaving only the gill chamber, often containing gill rudiments, on either side. Upon metamorphosis, the gill chambers and gills stubs are resorbed. The lateral line structures are resorbed. During larval or fetal life the sensory tentacles develop in front of the eyes. A dorsal caudal fin, absent in embryos of the direct-developing *Hypogeophis rostratus* (Brauer, 1899) and embryos and fetuses of several viviparous taxa (Wake, pers. obs.), is present in larval ichthyophiids and rhinatrematids and some caeciliids; it degenerates during metamorphosis. Breckenridge et al. (1987) assumed that the well-developed caudal fin enables hatchling *Ichthyophis* larvae to swim effectively. Alternatively, the caudal fin may not be functional at all, because the tail is very short and body undulation of the larvae appears to effect locomotion. Thus, we speculate that the tail fin is a plesiomorphy, shared with anuran and many urodele larvae, present only in basal taxa of caecilians because they retain small but true tails as adults, as well as during the larval stage (see Nussbaum and Wilkinson, 1989).

Comparison of *Ichthyophis kohtaoensis* Developmental Stages to Those of Other Caecilians

Development in caecilians, as discussed above, presents a number of differences within members of the order, as well as when compared to other amphibians. We summarize some of these differences by making direct comparisons of developmental stages in *Ichthyophis kohtaoensis*, first with other caecilians, then with oviparous-with-larvae and direct-developing frogs and salamanders (staging tables have not been developed for the few viviparous frogs and salamanders). We provide the latter in order to allow a general comparison and elucidation of major differences among developmental patterns in members of all of the three orders of amphibians.

***Ichthyophis kohtaoensis* and *Typhlonectes compressicauda*.** In order to evaluate the various developmental stages of *Ichthyophis kohtaoensis* as presented in our staging table, we compared them to those in the staging table for the viviparous aquatic caecilian *Typhlonectes compressicauda* (Sammouri et al., 1990). Sammouri et al. (1990) lacked specimens representing early developmental stages in their series, so they based their staging table for *T. compressicauda* on a table for *Alytes obstetricans*, a pelobatid frog, by Cambar and Martin (1959), and began with late neurulation stage 14. They further justified their comparison with that table, rather than the more frequently cited normal tables of Gosner (1960) or Harri-

son (1969) (or presumably Nieuwkoop and Faber, 1967), on the fact that Delsol et al. (1981) and Exbrayat et al. (1981) had already done a preliminary description of development in *T. compressicauda* based on comparison to Cambar and Martin's table. We designated our earliest stage as number 21 to account both for the absence of early developmental stages in our series of *I. kohtaoensis* and to provide a direct comparison with development in *T. compressicauda*, because our earliest stage most closely resembles their stage 21. We had some difficulties in comparing development in the two species because of significant differences in pattern between the oviparous and the viviparous modes of development, and because of different modes of description (criteria and characters) by French and English-speaking workers. We therefore evaluate some of the similarities and differences between the two species through a combined consideration of Sammouri et al.'s textual description and their figures of *T. compressicauda* with our series of *I. kohtaoensis*. For purposes of these comparisons, we refer to our material by the generic name *Ichthyophis*; this indicates only the species *kohtaoensis* unless otherwise indicated, just as *Typhlonectes* refers to the species *compressicauda* as described by Sammouri et al. (1990).

1. The general body shape of embryos of both caecilian species is quite similar, especially at stage 21, at which the embryo curls around a large yolk mass and the head and tail tip nearly touch each other.
2. Somitogenesis is faster in *Ichthyophis*; the full complement of more than 120 pairs of somites is present at stage 21, whereas *Typhlonectes* has only 65 pairs at stage 21, and 85 pairs at stage 24. The full complement of somites for *Typhlonectes* likely is more than 90 pairs, based on adult vertebral and annular counts.
3. Pigmentation of the body appears earlier in *Ichthyophis*; it covers the anterior third of the body at stage 21, but no pigmentation is discernible in *Typhlonectes* embryos at that stage. The inception of dorsal pigmentation in *Typhlonectes* embryos is reported at stage 23, and pigment does not cover the entire body until stage 28, whereas it extends all over the body at stage 25 in *Ichthyophis*. Further, in *Ichthyophis* pigment chromatophores are not arranged in lines, as occurs in *Typhlonectes*.
4. Formation of the lens appears earlier in *Ichthyophis*; it is present at stage 21. Although distinct optic vesicles are present in *Typhlonectes* embryos at stage 21, differentiation of the lens does not occur until stage 24.
5. Tentacle development is established earlier and progresses faster in *Typhlonectes*. In stage 27 embryos of *Typhlonectes*, the tentacle anlage is apparent. A tentacle anlage is first visible near the eye in *Ichthyophis* at stage 28. Unlike the

- situation in *Typhlonectes*, the tentacle in *Ichthyophis* does not approach the nasal opening during embryonic development and a tentacle orifice is not discernible until metamorphic stage 40.
6. In contrast to prehatching stages in *Ichthyophis*, *Typhlonectes* embryos do not have either lateral line organs (though the time of development of their adult ampullary organs has not been established; see Fritzsche and Wake, 1986) or a tail fin.
 7. Tooth buds or teeth, respectively, were not found in embryonic stages in *Ichthyophis*, though they are present in larvae; *Typhlonectes* has a well-developed fetal dentition with tooth buds established during embryogenesis.
 8. The stage of stomodeal development is similar at stage 21 in both *Typhlonectes* and *Ichthyophis*, but progress in mouth development differs between the species as the mandibular elements fuse earlier in *Typhlonectes* (at stage 23) to establish the lower jaw. However, the maxillary buds of *Ichthyophis* embryos are connected at stage 24, with the laterally closed mouth opening clearly distinguishable in lateral view, whereas the upper jaw of *Typhlonectes* forms at stage 27. In *Ichthyophis* the maxillary buds and the nasal walls are in close contact, forming a nasobranchial rim at stage 23, but in *Typhlonectes*, the fusion of the upper jaw elements with the olfactory placodes is not discernible until stage 26.
 9. Otic vesicles appear at the same stage (21) in both species, but in *Ichthyophis* embryos they are not pigmented, as is reported for otic placodes in *Typhlonectes* at stage 23.
 10. The development of the nasal openings not only starts earlier in *Ichthyophis* embryos but also proceeds faster. At stage 21 fold-shaped olfactory pits are present in *Ichthyophis*, whereas in stage 21 *Typhlonectes* the olfactory placode is evidenced only by a depression that indicates the invagination of the olfactory pit, the latter not differentiated until stage 25.
 11. Although external gills are present in embryos of both species, they are very different in structure. *Ichthyophis* embryos possess three pairs of gill rami-bearing filaments, whereas embryonic and fetal stages of *Typhlonectes* have sac-like gills. In the live-bearing *Typhlonectes*, the paired external gills are not lost until after metamorphosis and birth, and are therefore functional throughout the pre-juvenile ontogeny. In contrast, in late embryonic stages of the oviparous *Ichthyophis* (stage 36) the third gill is internalized into the gill slit and the remaining two gills are stripped off after hatching and the inception of the free-living larval period (stage 37), so that gaseous exchange is via lung-breathing and transport across the skin during the aquatic larval period.
 12. The cloacal region is similar in early stages (stages 21–30) of both species. However, differences occur at later stages, as in *Typhlonectes* the cloacal opening is not only retracted earlier (stage 31 compared to stage 33 in *Ichthyophis*) but at metamorphic stage 34 it is surrounded by ten radiating folds. In *Ichthyophis*, embryonic stage 39 has transversely arranged folds that border the slit-shaped cloaca.
 13. Predictably for a viviparous species like *Typhlonectes*, its small quantity of yolk is resorbed early (stage 28), compared to the situation in *Ichthyophis* embryos, which still possess a large amount of yolk at stage 28 and do not resorb the yolk until after stage 34. (Fully yolked ovarian ova are 8×10 mm in *Ichthyophis*, and 2 mm dia in *Typhlonectes* [Wake, pers. obs.]).
- Ontogenesis in general appears to be shortened in *Typhlonectes*, though some aspects of development occur earlier or faster in *Ichthyophis* (e.g., somitogenesis, appearance and extent of pigmentation, appearance of the lens). These disjunctions may be correlated with life history features, in that embryos and fetuses of the viviparous *Typhlonectes* are maintained for 7–9 months from fertilization to birth (fully metamorphosed), whereas the oviparous *Ichthyophis* have a post-oviposition, pre-hatching period of approximately 3 months, followed by a larval period of approximately a year (Himstedt, 1996).
- Ichthyophis kohtaoensis* and *I. glutinosus*.**
The Sarasins' (1887–1890) magnificent study of the development of *Ichthyophis glutinosus*, together with recent work by Breckenridge and colleagues, form the basis for our comparisons. The Sarasins did not state total lengths of their embryos (lengths of only two larvae are mentioned) or use scale bars on their illustrations, and they illustrated only a few developmental phases of embryos and larvae, but they described those, and some not illustrated, in considerable detail. Breckenridge and Jayasinghe (1979) also described development of embryos and larvae of *I. glutinosus*; Breckenridge et al. (1987) added information about histological structure in larvae. They considered only a few features of development, and emphasized the heart, the yolk, and the gills. They illustrated only two embryos at different stages of development, but present useful information about several developmental stages, including measurements of total lengths and gills. Consequently, our comparisons of development of *I. kohtaoensis* with *I. glutinosus* are more general than those with *Typhlonectes compressicauda*. We first consider Breckenridge and Jayasinghe's (1979) information, then pay particular attention to the embryos illustrated by the Sarasins, and discuss them in their sequence of presentation as compared to *I.*

kohtaoensis. We emphasize developmental features and size comparisons where possible.

Both of the embryos illustrated by Breckenridge and Jayasinghe (1979) represent earlier stages in development than those reported by the Sarasins and those that we have available. Their earliest embryo figured is 8.0 mm long, has only 21 pairs of somites; three gill rudiments, the optic cup and lens, the otic vesicles, and the heart are visible. The embryo was taken from the clutch 9 days after collection. The developing eggs were collected 9 days previous to that of the report; the embryos at the time of collection are described as "visible as a thin grey streak on the surface of the yolk. . . an irregular outline and rudiments of eyes, gills and heart are not visible." Breckenridge and Jayasinghe described general features of development of embryos of another clutch at 4, 9, 12, 15, and 16 days after collection, and then at intervals for 2 months, and conclude with a description of changes in larval development. Their second embryo illustrated is 9.0 mm long, has 73 pairs of somites (a second embryo examined has 58), and has a well-formed brain with an open neural tube. The eyes, otic vesicles, and gill rudiments are formed. The embryo was taken from its clutch 4 days post-collection. The embryo that they describe at 16 days after collection resembles stage 21–22 *Ichthyophis kohtaoensis* in its stage of gill development (three gills developed, posterior-most still lacks filaments) and total length (17 mm). The number of somites in that embryo is not reported, so we infer that it may be the full complement of more than 120. The pigmentation pattern of their 26.0 mm embryo, covering the entire body, is comparable to that of stage 25–26 *I. kohtaoensis*, which has approximately the same total length. Reduction of the yolk mass appears to occur more rapidly in *I. glutinosus* than in *I. kohtaoensis*. Breckenridge and Jayasinghe first report lateral line organs in their 40 mm total length embryos of *I. glutinosus*, which is the size equivalent of stage 30–31 *I. kohtaoensis*. However, lateral line organs first appear at stage 24 in *I. kohtaoensis*, and the numbers and distribution of neuromasts described by Breckenridge and Jayasinghe are comparable to that of stage 25 *I. kohtaoensis* embryos, which are approximately 23 mm total length. It seems likely that lateral line structures developed earlier, but were noted for the later stage by Breckenridge and Jayasinghe. Their description of the newly hatched larva, the hatching process, inception of the yellow stripes, and gill loss is comparable to our observations of *I. kohtaoensis* at stages 34 and 35. Thus, the size/stage comparisons provide tantalizing clues regarding interspecific variation in developmental timing. In general, the descriptions by Breckenridge and Jayasinghe, the Sarasins, and ourselves are not comparable in most ways, because Breckenridge and Jayasinghe focused on changes in yolk structure and that of the egg cases, heart morphology and beating,

and other aspects of visceral development, but they thereby provided the only, therefore highly valuable, data extant on these elements.

The descriptions of *Ichthyophis glutinosus* embryos by the Sarasins (1887–1890) allow more extensive comparison with our information on *I. kohtaoensis* because they illustrated more phases of development and described more of the components of development than did Breckenridge and Jayasinghe. The embryo shown in the Sarasins' figures 30 and 36 (Plate IV) curves around a large round yolk mass and the head and tail tip nearly touch, resembling stage 21 specimens of *I. kohtaoensis*. The mandibular arch is divided into paired upper maxillary buds and paired mandibular elements in the embryo in the Sarasins' figure 30. The hyoid and three branchial arches are also developed at this stage, but the arches become further elongated in the embryo shown in the Sarasins' figure 36, which is comparable developmentally to stage 21 of *I. kohtaoensis*. At that stage of development, the gills of *I. glutinosus* are short protrusions, which curve slightly dorsally. The third gill, shorter than the first and second, is covered by the two anterior gills. No gill filaments are developed in either stage 21 of *I. kohtaoensis* or in the Sarasins' embryo in figure 36. The Sarasins did not mention the state of development of the neural folds at this stage but, referring to their figure 37, which shows the head of the embryo of figure 36 in dorsal view, we assume that the neural folds are closed only in the tail and fore-brain region, but are not connected in the hindbrain region, similar to stage 21 *I. kohtaoensis*. Also comparable to stage 21, the optic vesicles of the Sarasins' figure 36 stand out distinctly and the lens is discernible. In both species otic vesicles are discernible on either side of the neural folds in the region of the rhombencephalic groove. The Sarasins described huge lateral nasal pits for the young embryo in figure 30. In contrast, in stage 21 *I. kohtaoensis* the olfactory pits are indicated only by folds; large ventro-lateral round-to-oval nasal pits are not discernible until stage 22.

There is a large gap in the developmental series between the embryo shown in the Sarasins' figure 36 and that in figure 38 (also Plate IV), as evidenced by the many changes that have apparently taken place during the interval, so our comparison of *Ichthyophis glutinosus* with *I. kohtaoensis* includes only mid-developmental and late-developmental stages. In the Sarasins' figure 38 the anterior part of the embryo is clearly elevated from the yolk, as we described for stage 26 embryos of *I. kohtaoensis*. The Sarasins described the head of the embryo in figure 38 as blunt, higher than long, and well developed, although still far from the final flattened shape of heads of larvae. The eyes are large and clearly pigmented in the embryo in figure 38, as described for stage 26 *I. kohtaoensis*. Three pairs of external gills,

described by the Sarasins as eye-catching, are elongate and filamentous.

The Sarasins stated that lateral line organs first appear at the stage of development illustrated in figure 38. However, exact timing of their inception is uncertain, because of the apparent several developmental stages not represented in the Sarasins' series between those illustrated in figures 36 and 38; i.e., lateral line inception may have occurred earlier, but cannot be documented. The Sarasins described several rows and groups of lateral line organs present in the specimen in figure 38. The pattern of lateral line distribution in a ventral view of the head and on the chin, respectively, shown in the Sarasins' figure 39 (Plate IV), strongly resembles the pattern described for stage 28 *Ichthyophis kohtaoensis*, as the V-shaped mandibular row as well as the gular row on the throat and ampullary organs accompanying the neuromast rows are developed. However, in lateral view of the head in the Sarasins' figure, only one row of lateral line organs is discernible above the eyes, whereas at stage 27 of *I. kohtaoensis*, at which the anlage of the mandibular row appears and the gular row is not yet developed, three rows of lateral line organs are developed above the eyes. There apparently is interspecific variation in the timing of development and overall pattern of components of the lateral line system.

The inception of the tail fin, described for the embryo in the Sarasins' figure 38, occurs at stage 24 in *Ichthyophis kohtaoensis*. However, similar to the appearance of lateral line organs, fin formation might actually occur in earlier stages which were not available to the Sarasins. We suggest that the Sarasins' figure 38 of *I. glutinosus* is a stage that is developmentally "between," or a combination of, stages 24 and 26 of *I. kohtaoensis*, based on the similarities and differences in their development.

In Sarasins' figure 42 (Plate V), the head is flattened, elongated, and closer to the larval form, resembling the flattened and elongated head of stages 30 and 31 of *Ichthyophis kohtaoensis*. The Sarasins reported that the yolk mass in their example is still comparatively large, but has a well-developed vascular system and some torsion. As we discussed, in *I. kohtaoensis* the first bending of the yolk occurs at stage 30; at stage 31 the clearly reduced yolk has a double S-shaped curvature. Additionally, the Sarasins distinguished between the appearance of the hind limbs at the tail tip and the presence of swellings in the cloacal region in their example. The slit-shaped cloacal opening bordered by two lateral swellings, which are clearly visible in lateral view in the Sarasins' figures 43 and 44 (Plate V) and the cloacal structures shown in our Figure 13DE are effectively the same. Both sets of figures show the pronounced button-shaped cloacal swellings observed at stage 32 *I. kohtaoensis*; our study shows that these swellings are cloacal components, not hind limb buds. The shape of the tail tip of the

embryo shown in the Sarasins' figure 42 resembles the arrow-shaped tail tip of stage 32 *I. kohtaoensis*. However, at stage 32 we found three complete rows of lateral line organs above and below the eyes; the Sarasins' figure 42 shows one or two rows, again suggesting different rates and/or patterns of development. The state of development of the embryo shown in figure 42 appears to be a stage approximately that of stage 31 *I. kohtaoensis* because the beginning of yolk enclosure in the abdominal folds is not discernible but the yolk is clearly reduced (and based on head shape, as noted above).

The larva shown in the Sarasins' figure 46 (Plate V), measuring 7 cm total body length, is comparable to stage 35 *Ichthyophis kohtaoensis* because in both species the yolk is enclosed in the abdominal folds. Further, the gills appear to be reduced compared to the increased body size, the third gill is not yet internalized in the gill cavity, and the total body length is similar (see Table 2).

The total length of the developmental stage shown in the Sarasins' figure 49 (Plate V), the smallest larva available in their study, is less than 7 cm, similar to the size of just-hatched *Ichthyophis kohtaoensis* larvae (stage 37). A further similarity is that only a gill cavity, but not external gills, is discernible on either side of the head. Breckenridge and Jayasinghe (1979) report that the newly hatched larvae of the *I. glutinosus* that they raised are 7.5 to 8 cm total length and have three pairs of gills, the middle one longest; the gills are lost 2 days after hatching. The tentacle is not indicated in the Sarasins' figure 49. In *I. kohtaoensis* the tentacle is first visible at stage 30 and its development continues at stage 33, but a clearly visible funnel-shaped tentacle sheath with an orifice and a distinct tentacle is not discernible until stage 40. The distribution of neuromasts shown in Sarasins' figures 51–54 (Plate V) is quite similar to the distribution found in hatched larvae of *I. kohtaoensis* at stage 37, except that the latter have three rows of lateral line organs above and below the eyes, but differs from that illustrated by Breckenridge and Jayasinghe (1979); the latter may be unclear because of the angle at which it was illustrated. Taylor's (1960), Hetherington and Wake's (1979), and Breckenridge et al.'s (1987) descriptions of lateral line series in species of *Ichthyophis* also indicate interspecific differences in pattern, though none of those studies report developmental comparisons, being restricted to well-developed larvae.

The 17 cm TL larval stage shown in the Sarasins' figure 50 (Plate V) is the largest stage represented in their study and is comparable to stage 38 *Ichthyophis kohtaoensis*. A narrow tail fin in *I. glutinosus* is still discernible, whereas a tail fin is absent at stage 39 in *I. kohtaoensis*. Because descriptions in addition to body length are not given, and because the Sarasins likely would have mentioned signs of metamorphosis, such as the beginning of gill chamber

closure (stage 40 *I. kohtaoensis*), we assume that their figure 50 is of a late embryonic stage similar to *I. kohtaoensis* at stage 36.

***Ichthyophis kohtaoensis*, and *Hypogeophis*, and *Grandisonia*.** Brauer (1897, 1899) examined the development of two species of caeciliids from the Seychelles Islands, the moderate-sized, direct-developing *Hypogeophis rostratus*, and the smaller, oviparous-with-larvae *H.* (now *Grandisonia*) *alternans*. He had abundant specimens representing early development in *H. rostratus*, but noted that he lacked material representing much of later development; further, he had only a few near-metamorphic specimens of *G. alternans*. Brauer concentrated his study on a detailed examination of early brain and ganglion development, as well as that of otic and nasal structures (curiously, he did not describe eye development), the elements of the mouth and hyoid, the gills, and the tail tip. He did not comment on many other features of development in his text. Brauer (1899) presented careful illustrations of developmental features of early embryos, and the heads and tail tips, and occasionally entire specimens, of later stages. Because he did not present complete descriptions in his text, and because he characterized his illustrations primarily by total body length, we have made several inferences about development through our detailed examination of his illustrations. His specimens, however, clearly were chosen to reflect the progression of development in the species, and each has slight changes (e.g., more somites, gill development changes) relative to preceding and succeeding specimens illustrated. We therefore compare the features that Brauer discussed in his text and those that we observed in his illustrations with the comparable features of *Ichthyophis* development. Because of the availability of few data other than total body length, we use those data to establish our baseline for comparisons of external morphological features of development among the taxa.

Brauer (1899) reported the appearance of otic vesicles in an early developmental stage, shown in his figure 11 (6.5 mm TL). He described them as thickenings of tissue above the hyoid arches. According to Brauer, the otic vesicle deepens and extends, becoming a pit (see Brauer's fig. 12; 7 mm TL). As development proceeds, the otic vesicles begin to close, the opening narrows, becoming a small hole, and finally the hole also disappears (Brauer's figs. 13–16; 8.0–9.0 mm TL). The otic vesicles remain visible externally as round swellings at the rostral border of the hyoid arches at embryonic stages shown in Brauer's figures 18, 20, and 22 (9.5–15 mm TL), but finally disappear as they sink into deeper tissue parts. Thus, otic vesicles are no longer discernible at stages that are comparable to stage 21 (16.6 mm TL) *I. kohtaoensis*. At that stage, *Ichthyophis* embryos have otic vesicles on either side of the neural folds in the region of the rhombencephalic groove, repre-

sented by white small dots with a dense central disc (Fig. 7A); the otic vesicles are no longer distinguishable at stage 25. It appears that otic vesicle development occurs at a faster rate relative to other features, such as nasal and stomodeal development, in *Hypogeophis* than in *Ichthyophis*.

Brauer did not discuss development of the eye in *Hypogeophis*, but we note that the lens is first apparent in the optic vesicle in his figure 26 (approx. 20 mm TL); the lens is visible in the optic vesicle in our stage 21 *Ichthyophis*, and because we do not have earlier material, we cannot say specifically when it develops. However, we infer that the lens develops later in *Hypogeophis* than in *Ichthyophis* because the former's lens appears at a stage in which other features are better developed than they are in *Ichthyophis* when the lens develops. We cannot assess the appearance of pigment in the eye from Brauer's figures.

Brauer reported that the nasal pits are represented by large, deep, delineated grooves, separated from the stomodeum by a thick rim of tissue, the nasal wall, as shown in his figures 18–25 (9.5–15 mm TL), and similar to the situation in stages 21 and 22 (16.6–23.6 mm TL) of *Ichthyophis kohtaoensis*. In older embryos, represented by Brauer's figures 26, 28c, and 32 (19–20 mm TL), the nasal wall starts to flatten and a nasobranchial rim begins to form. Brauer described the formation process as follows: the rim of tissue, which encircles the nasal pits, separates ventrally, forming a horseshoe-shaped wall bordering the nasal openings. The lateral parts of the "horseshoes" later fuse with the maxillary buds in developmental stages represented by Brauer's figures 27, 34a, 35, 35a, and 36 (18–21 mm TL); the embryo shown in his figures 34 and 34a (presumably approx. 20 mm TL, but not stated; figs. 26–35 are all the same body length) is definitely comparable to *Ichthyophis* stage 23 (26–29.6 mm TL) in naso-stomodeal development. In the course of further development the external nasobranchial rim narrows, becomes a deep slit, and finally disappears in Brauer's figure 37 (22 mm TL). The maxillary buds fuse and form a laterally closed mouth opening, which is clearly separated from the nasal openings in Brauer's figure 38a (25 mm TL). This is even more obvious in the embryo shown in figure 39a (26 mm TL), in which the nasal openings are clearly circumscribed and separated from the mouth opening. That phase in the development of Brauer's embryos is comparable to stage 24 (18.3–22.2 mm TL) of *I. kohtaoensis* with regard to stomodeal development, the stage of development of the nasal openings and of the tail bud (see below). The nasal openings in *Hypogeophis* reach their definitive shape in the embryo shown in figure 41a (33 mm TL), at which they are represented by two small frontal openings. In *Ichthyophis* embryos, the nasal openings are situated rostro-ventrally and are surrounded by nasal walls at stage 26 (24.9–30.2 mm TL). They move to

a more frontal position at stage 27 (32.5 mm TL), which may be comparable to the embryo shown in Brauer's figure 41 (33 mm TL), which may be comparable to the embryo shown in Brauer's figure 41 (33 mm TL).

Brauer reported that although mandibular, hyoid, and branchial arches are clearly developed in embryos shown in his figures 18b, 20a, and 21 (9.5–12 mm TL), the stomodeum (die "Mundbucht") lacks lateral walls between the nasal walls and the mandibular elements. This situation changes in the embryo shown in Brauer's figure 22a (15 mm TL), which is comparable to stage 21 (16.6 mm TL) of *Ichthyophis kohtaoensis*, at least with respect to the stage of stomodeal development. At that stage, the mandibular elements touch ventrally, and, according to Brauer, the hemispherical maxillary buds begin to fill the gap between the eyes, the nasal walls, and the components of the lower jaw. In Brauer's figure 23a (18 mm TL), the maxillary buds have moved ventro-laterally to border the stomodeum, similar to the situation in *Ichthyophis* at stage 22 (18.5–23.6 mm TL). Brauer stated that the mandibular elements are fused at this stage. However, referring to figure 23a, the paired mandibular elements, although continuous ventrally, still form a deep heart-shaped angle at the dorsal site of contact. We found the same state in *Ichthyophis* at stage 22, at which the maxillary buds are not yet connected, substantiating the comparability of the *Hypogeophis* embryo shown in Brauer's figure 23a to stage 22 of *I. kohtaoensis*.

Brauer (1899) paid particular attention to gill development, as have others interested in caecilian embryology. In *Hypogeophis*, the first and second gills appear shortly after each other. They grow from small button-shaped swellings in the embryo shown in Brauer's figure 20 (approx. 12 mm TL). The third gill develops considerably later, in Brauer's figure 34 (20 mm TL). In that embryo, the first two gills already bear filaments, which have initiated growth from little buds present in embryos represented by Brauer's figures 26–30 (also 20 mm TL). In *Ichthyophis kohtaoensis*, all three gills are developed as small knobs at stage 21 (16.6 mm TL), before the first gill filaments are discernible at stages 22 and 23 (18.5–29.6 mm TL). The pattern of gill development in *H. rostratus* differs slightly from that in *I. kohtaoensis*, because gill filaments develop a bit later and appearance of the third gill is delayed and disjunct from that of the first two in *H. rostratus*. Brauer reported that near the end of embryonic development, when the shape of the head resembles the shape of larval heads and the yolk is reduced, the gills become shorter and finally are resorbed (figs. 47–49; embryos 58–65 mm TL). This may be comparable to the relative shortening seen in premetamorphic *Ichthyophis*, in which the gills stop increasing in size at about stage 32 (44–46 mm TL), but body growth continues, so that the ratio of gill

length to body length reduces (see above). The third gill is finally resorbed in embryos shown in Brauer's figures 48 and 49 (60–65 mm TL), which resemble *Ichthyophis* stage 36 (70.1–83.0 mm TL) in many features of development that characterize the premetamorphic state.

Brauer's only reference to the lateral line system is his notation of the presence of lateral line organs in the embryo illustrated by his figure 40 (approx. 30 mm TL). Lateral line organs first are apparent in *Ichthyophis kohtaoensis* at stage 24 (18.3–22.2 mm TL), and we have described their development herein, with comparison to other *Ichthyophis*. Because of the brevity of Brauer's comment, and the absence of lateral line structures in any of his figures, we cannot assess their developmental pattern in *Hypogeophis*. However, it is noteworthy that this direct-developing species does retain lateral line structures at all.

Brauer paid considerable attention to the development of the tail bud and the tail in *Hypogeophis rostratus*, and also commented briefly on the condition of the yolk mass. In *Ichthyophis* embryos a short tail bud is elevated from the yolk at stage 21, and the formation of the tail fin begins at stage 24 (18.3–22.2 mm TL). Tail tip development supports comparability of Brauer's 33 mm TL embryos (fig. 41) to stage 27 *I. kohtaoensis*, because the tail has thickened and became round in both sets of animals. Although neither *Hypogeophis* and *Grandisonia* exhibit the tissue fimbris which is clearly discernible in *Ichthyophis*, Brauer reported a faint ridge or carina in the embryos shown in his figures 38c and 39c (25–26 mm TL), which may be interpreted as a vestigial tail fin. The yolk mass is large in both caeciliid taxa (approx. 9×6 mm; Wake, 1977), though their reproductive modes differ. In Brauer's specimens, the enclosure of the yolk in the abdominal folds begins and continues in embryos shown in his figures 45–47 (45–58 mm TL), proceeding with a change in yolk shape as the yolk mass flattens and becomes spindle-shaped. This condition is similar to *Ichthyophis* embryos of stage 33 (50–54 mm TL). By stage 36 in *Ichthyophis*, and in figures 48 and 49 of *H. rostratus*, the reduced yolk mass is completely enclosed in the abdominal folds and no longer discernible externally.

Unfortunately, somitogenesis in *Hypogeophis* cannot be compared with that of other caecilians, because Brauer (1899) indicated the numbers of pairs of somites only in illustrations of his early embryos (e.g., nine pairs in a 5.5 mm total length specimen [fig. 6], 14 pairs at 6.0 mm [fig. 8], 19 at 6.5 mm [fig. 11], and 32 pairs at 8.5 mm [fig. 15]). We presume that the increasing size of the embryos dictated the head-and-tail illustrations presented by Brauer, and we do not find the somite counts explicitly listed in his text. All of the specimens for which data on somite number in *Hypogeophis* are available are much earlier developmentally than our earliest

specimens of *Ichthyophis*. Sammouri et al. (1990) report 35 pairs of somites in their stage 16, 3.6 mm TL *Typhlonectes*; Brauer's 8.5 mm specimen is figured as having 32 pairs of somites, but is otherwise much more advanced than the *Typhlonectes* stage that is comparable in terms of somite number. This comparison is tenuous, because it is not clear that Brauer specifically included all somites in his illustrations.

Because Brauer illustrated individuals that reflected changes in developmental features, but did not fully describe each individual or present any information about sample sizes or overall variation at particular sizes, we do not construe his illustrations as stages in the modern context. Therefore, comparison with *Ichthyophis kohtaoensis*, and other caecilians, is limited to the details that we can directly evaluate. We summarize our comparison as follows:

1. Extensive development occurs in embryos of *Hypogeophis rostratus* by the time they reach approximately 20 mm TL.
2. Patterns of development of otic vesicles and gills in particular, but also other features, are dissimilar in *H. rostratus* and *Ichthyophis* (and *Typhlonectes*; see comparison of *I. kohtaoensis* and *Typhlonectes* above).
3. Embryos of both *Hypogeophis* and *Ichthyophis* are approximately the same lengths from stages 21 through 27, but *Ichthyophis* grows larger, perhaps faster, than does *Hypogeophis* from the equivalent stage until metamorphosis.
4. Despite these differences, certain general similarities exist that allow direct comparison of *H. rostratus* and *I. kohtaoensis*. Brauer's figure 22a is equivalent to stage 21 of *I. kohtaoensis*, based on mandible-maxilla relationships and nasal pit conformation; figure 23a resembles *I. kohtaoensis* stage 22, and figures 34 and 34a resemble stage 23, based on the advancement of stomodeum and nasal development; figures 38a–39a resemble stage 24, based on stomodeal and nasal features and stage of tail formation. Figure 41a of *H. rostratus* resembles stage 27 of *I. kohtaoensis*, based on the frontality of the nasal openings and the conformation of the tail tip; figures 45–47 are similar to stage 33, especially in terms of several features of head development and also yolk mass shape changes. Finally, the premetamorphic embryos of *H. rostratus* illustrated in figures 48–49 resemble stage 36 of *I. kohtaoensis* in having the third gill resorbed and the yolk enclosed in the abdominal folds, among other features.

Comparison of Caecilian Normal Tables With Those of Other Amphibians

One of the advantages of normal tables is that they facilitate comparisons both within and among

taxa. By comparing normal tables, it is possible to describe and compare developmental trends across all three groups of amphibians. Detailed comparisons are difficult, however. Caecilians, anurans, and urodeles show considerable variation in both developmental and adult morphology. Within each group, there is also variation in reproductive mode and life history. Amphibians with a biphasic life history pattern possess a free-living larval stage; their ontogeny is often characterized by the development of a suite of larval specializations that are then lost (or transformed) at metamorphosis. Direct development, or the loss of a free-living larval form, has also evolved in many groups, and is often coupled with varying degrees of ontogenetic repatterning. All of this developmental variation is reflected in normal tables.

Normal tables thus facilitate comparative ontogenetic studies, but cannot form the sole basis for those studies. Normal tables vary considerably, both in level of detail and in the criteria used to define individual stages. Different authors focus on different organ systems, and as illustrated by our within-caecilian comparison, tables for different species typically do not contain comparable sets of information. In spite of this variation, general ontogenetic patterns do emerge. We present a general comparison of developmental patterns in anurans and urodeles, based on selected normal tables of species selected to represent different life histories comparable to those in caecilians (except for viviparity). We describe, within each group, changes in ontogenetic pattern associated with biphasic and direct-developing modes. We compare the normal table of *Ichthyophis kohtaoensis* with those of the selected anurans and urodeles, then compare and contrast the biphasic and direct-developing modes, across all Amphibia. These broad comparisons are presented to establish a baseline for study of developmental patterns among members of the three extant orders of amphibians, in general and as correlated with life histories and patterns of evolutionary diversification.

General features of urodele development. Comparisons are based on either normal tables or ontogenetic descriptions for the following seven species: *Ambystoma maculatum* (Harrison, 1969), *A. mexicanum* (Schreckenberg and Jacobson, 1975; Bordzilovskaya and Dettlaff, 1991), *Batrachoseps wrighti* (Stebbins, 1949), *Desmognathus aeneus* (Marks and Collazo, 1998), *Plethodon cinereus* (Dent, 1942), *Pleurodeles waltlii* (Vassetsky, 1991), and *Triturus vulgaris* (Liozner and Dettlaff, 1991). This selection encompasses three families of salamanders (Ambystomatidae, Plethodontidae, and Salamandridae) and includes three direct developers (*B. wrighti*, *D. aeneus*, *P. cinereus*), three biphasics (*A. maculatum*, *P. waltlii*, *T. vulgaris*) and one neotenic (*A. mexicanum*).

For all species examined, the major organ systems appear in the same general sequence during ontogeny, with the exception of the limb buds. The optic vesicles are visible externally as distinct bulges, shortly after neural tube closure. The visceral arches then develop, giving rise to the external gill buds and stomodeal elements. The tailbud appears, followed by the otic, then olfactory vesicles. The tail fin develops and broadens. Pigment cells appear and spread over the head and flank. In biphasic urodeles, the limb buds appear late in ontogeny, when the external gills are branched and fairly well developed; in direct-developers, limb buds appear earlier in ontogeny, shortly after the formation of the tailbud and the subdivision of the branchial plate. Within each organ system, organs follow similar patterns of development, although morphological details may vary by species. For example, in all urodeles examined, the external gills form as buds, which elongate, branch, then develop filaments along each branch. The pattern is similar, but the timing and extent of branching, as well as the rate of gill regression, varies by species. The overall pattern of limb development is also highly conserved, although onset time varies. There is slight interspecific variation in the timing of specific ontogenetic events. For example, the mouth and external gills develop earlier in *Ambystoma mexicanum* than they do in *A. maculatum*, whereas the limb buds develop later (Bordzilovskaya and Dettlaff, 1991); these differences are possibly correlated with life history (neotenic vs. biphasic forms, respectively). The three direct-developing plethodontid species vary primarily in the timing of branchial plate subdivision and the development of pigmentation patterns (Dent, 1942; Stebbins, 1949; Marks and Collazo, 1998).

Overall body shape during development is also similar across species. During early development, urodele embryos are curled around a large volume of yolk. As the body elongates and the yolk mass is depleted, the curvature of the head and tail region decreases and the body axis eventually straightens. In biphasic (and neotenic) urodeles, most of the elongation and straightening of the body axis occurs prior to the appearance of the limb buds. In direct developers, the limb buds appear early, when the embryo is still curled around a substantial amount of yolk.

There are marked developmental differences between the biphasic (including neotenic) and direct-developing forms. In direct-developing urodeles, limb buds develop early in ontogeny, relative to other ontogenetic features, and differentiate more rapidly. The tail bud and external gills also appear earlier, and the mouth opening may perforate earlier, relative to the time of hatching. Many larval characters either do not develop or develop to a lesser extent. For example, in *Desmognathus aeneus*, the larval balancers, larval pigmentation, and tail fin do not develop (Marks and Collazo, 1998).

During ontogeny, the tail remains short and round; the gills are shorter and less branched than they are in biphasic forms, and may regress sooner and/or more rapidly. Direct developers resemble the adult form at hatching, and begin to feed shortly thereafter.

General features of anuran development. Comparisons of anuran development are based on the following three staging tables: the highly detailed table of Nieuwkoop and Faber (1967; *Xenopus laevis*, a biphasic, secondarily fully aquatic anuran), the generalized table of Gosner (1960; widely used for staging of biphasic anurans), and the table of Townsend and Stewart (1985; *Eleutherodactylus coqui*, a direct-developer).

In anurans with a biphasic life history pattern, larval ontogeny is partitioned into distinct phases, each with a different set of growth priorities. From egg to hatching, emphasis is on development of the locomotor and sensory systems. The eye, tailbud, tail fin, hatching and cement glands are well developed at hatching, but the gill buds and mouth parts are still at early stages of development. From hatching till onset of feeding, priority is given to development of the feeding and respiratory systems, as well as further refinement of the sensory and locomotor systems. The gills branch, elongate, then are covered by the operculum just prior to feeding; the gut and feeding apparatus develop, and the lateral line system and pigmentation patterns appear. The transition to the adult state typically accompanies a change in ecology, from an aquatic tadpole to a semi-terrestrial adult, and necessitates an abrupt shift in feeding, locomotor, and respiratory organs. Thus, metamorphosis includes the loss of many larval features, such as the tail, tail fin, gills, and lateral line system; limbs develop, and the feeding apparatus is transformed to the adult condition.

There is considerable variation across biphasic anurans in both timing and morphology of ontogenetic structures that assist the free-living larval stage, such as the external gills, feeding apparatus, tail and tail fin, as well as overall body shape. But they share the tendency to hatch at a relatively undeveloped stage, with later stages of organogenesis concentrated in post-hatching larvae. The basic tadpole form consists of a short, rounded body with a long, narrow tail. Yolk, if present, is fully contained in the gut region, and the gills are typically reduced and covered by an operculum. The majority of torso elongation begins after neurulation, but before the appearance of the limb buds.

Eleutherodactylus coqui, a direct-developing anuran, has eliminated the free-living larval stage and hatches as a fully formed, juvenile frog (Townsend and Stewart, 1985). Similar to many urodeles and caecilians, the embryo develops curled around a large mass of yolk. Onset of development of the eyes, gills, and limb buds occurs shortly after the neural folds have fused. The embryo develops a well-

vascularized tail fin that regresses prior to hatching and shows direct development of the adult pigment pattern. At hatching, a tiny froglet emerges, although it retains remnants of the yolk and the larval tail.

Limited data in the *Eleutherodactylus coqui* staging table makes detailed comparisons to biphasic anurans somewhat difficult; the majority of the staging criteria are based on aspects of limb development. However, there are some notable differences between *E. coqui* and biphasic anurans. First, and most obviously, the limb buds appear very early in *E. coqui*. The majority of nongrowth related elongation of the torso thus occurs prior to neural tube closure, rather than after, and is minimized once the limb buds appear. The gill buds appear earlier, but follow the same general pattern of branching, elongation, and regression that is evident in biphasic anurans. The gills also develop more rapidly in *E. coqui*, disappearing by the time the tail is fully developed; in some *Eleutherodactylus* species, the gills do not appear to develop at all (Townsend and Stewart, 1985). Development of the gut and regression of the yolk occur in later stages in *E. coqui*, relative to biphasic anurans. However in both groups these features are directly correlated with the onset of feeding.

Ontogenetic comparisons across Amphibia.

Ichthyophis kohtaoensis has a biphasic life history pattern in which free-living, aquatic larvae metamorphose into terrestrial adults. Compared to congeneric *I. glutinosus*, there is slight variation in pattern, and possibly timing, of the development of the lateral line. The nasal pits are larger and develop earlier in *I. glutinosus*. Otherwise, based on the information available, the development of these two, basal, oviparous caecilians is similar.

Greater differences are revealed when *Ichthyophis kohtaoensis* is compared to the more derived, direct-developing genera, such as the oviparous *Hypogeophis* and the viviparous *Typhlonectes compressicauda*. In both of the direct-developing caecilians, the lens forms later in ontogeny, whereas the yolk appears to be resorbed both earlier and faster; embryonic tail fins are lacking. In *Hypogeophis*, the otic vesicle develops more rapidly and the third gill forms later, relative to *I. kohtaoensis*, but otherwise development of the two taxa is fairly similar. The development of *I. kohtaoensis* and *T. compressicauda* appears to be the most divergent of the four caecilian taxa examined in detail. In *T. compressicauda*, the tentacle develops earlier and faster, whereas pigmentation and the olfactory pits develop later, relative to *I. kohtaoensis*. Fetal *T. compressicauda* do not possess lateral line series, and the ontogenetic period is shorter, overall. There are also differences in the structure and development of the stomodeum, cloacal region, and major differences in the gills. However, in both species the gills are lost

at hatching or birth, the transition to the free-living stage.

There are two major differences in ontogenetic pattern between *Ichthyophis kohtaoensis* and urodele amphibians. In *I. kohtaoensis*, the neural folds do not fuse until shortly before hatching, when the head and jaws are well developed. Gill development, on the other hand, appears to be accelerated. Stage 21 of *I. kohtaoensis* is comparable to Harrison (1969) stage 36, based primarily on gill characters, or Marks and Collazo (1998) stage 21, based on a combination of pigment, optic, and gill characters. Compared to biphasic urodeles, the tail fin is reduced and forms later in *I. kohtaoensis*, whereas the lateral line system, as well as the mouth and jaws, develop at an earlier stage. There are more similarities between *I. kohtaoensis* and direct-developing urodeles than with biphasic salamanders, especially in the development of the olfactory and mouth openings, as well as in the absorption of the yolk.

The extreme morphological differences between the free-living larval and adult stages of biphasic anurans are reflected in different ontogenetic priorities, making a stage-by-stage comparison with *Ichthyophis kohtaoensis* difficult. Depending on which characters are used, stage 21 *I. kohtaoensis* is comparable either to Nieuwkoop and Faber (1967) stage 25 (optic, otic, and tailbud characters) or stage 35/36 (pigment, stomodeal, olfactory, and gill characters). Compared to the biphasic anuran pattern, *I. kohtaoensis* shows early development of the gills, mouth, and lateral line, but late fusion of the neural folds. *Ichthyophis kohtaoensis* also hatches at a later, more developed stage. The development of *I. kohtaoensis* is more similar to that of the direct-developing anuran, *Eleutherodactylus coqui*, in that organogenesis begins early and is more or less complete at hatching. Stage 21 of *I. kohtaoensis* is directly comparable to Townsend and Stewart (1985) stage 5, although there are few corresponding staging criteria between the two tables. Eye and gill development is similar, but complete fusion of the neural folds still occurs much later in *I. kohtaoensis*.

As a general trend, the development of *Ichthyophis kohtaoensis* is more similar to that of urodeles than to that of anurans. The neural folds fuse later in *I. kohtaoensis* (shortly before hatching), and the gills develop earlier and faster than in the other two groups. There are also many generic-level differences, in that each group has distinguishing larval and adult morphological characteristics. For example, limbs, balancers, egg teeth, and cement glands do not develop in *I. kohtaoensis*; the covering of the eyes and development of the tentacular apparatus are unique to caecilians. External gills, which are common to all three groups of amphibians, vary greatly in their morphology. Gills are lost at hatching in *I. kohtaoensis*, but are retained in most salamander larvae, and are reduced and internalized in anuran tadpoles. In both frogs and

salamanders, gills are lost at metamorphosis (but are retained in neotenic forms).

Interestingly, the ontogenetic pattern of *Ichthyophis kohtaoensis*, a caecilian with a biphasic life history, more strongly resembles the patterns of direct-developing anurans and urodeles, compared to the ancestral biphasic forms. However, this may be an artifact of the normal tables themselves. The tables of direct-developing anurans and urodeles are typically highly generalized and encompass large intervals of ontogeny. Such tables tend to emphasize limb development as the main staging criterion, so there are relatively fewer criteria to match with data for limbless caecilians. As a result, it is often easier to compare the tables to those of other species and to assign comparable stages. The strong resemblance of the biphasic caecilian pattern to the direct-developing anuran and urodele patterns may also reflect a fundamental ontogenetic difference between the three groups of amphibians. Biphasic caecilians, in general, show less divergence of larval and adult forms during ontogeny, relative to biphasic anurans and urodeles. The metamorphic transition is thus less abrupt and exerts less influence on the timing and compartmentalization of ontogenetic events.

We stress that the evolution of development is an integrated and integrative field of study, rich in empirical and heuristic value. We present our comparisons of patterns of development among diverse amphibians to help to establish a baseline for further study of species with different evolutionary and developmental histories that result in markedly different phenotypes. We anticipate that such studies will facilitate a more intensive and better informed analysis of pattern and process of the evolution of development.

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