

Cranial Ontogeny in *Philautus silus* (Anura: Ranidae: Rhacophorinae) Reveals Few Similarities With Other Direct-Developing Anurans

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ABSTRACT Direct development has evolved in rhacophorine frogs independently from other anuran lineages, thereby offering an opportunity to assess features associated with this derived life history. Using a developmental series of the direct-developing *Philautus silus* (Ranidae: Rhacophorinae) from Sri Lanka, we examine features of cranial morphology that are part of a suite of adaptations that facilitate feeding in free-living tadpoles, but have been changed or lost in other direct-developing lineages. Larval-specific upper jaw cartilages, which are absent from many non-rhacophorine direct-developing species (such as *Eleutherodactylus coqui*), develop in embryos of *P. silus*. Similarly, lower jaw cartilages initially assume a larval morphology, which is subsequently remodeled into the adult jaw configuration before hatching. However, the cartilaginous jaw suspension and hyobranchial skeleton never assume a typical larval morphology. The palatoquadrate, which suspends the lower jaw, lacks the posterior connections to the braincase found in many metamorphosing species. Unlike in metamorphosing species, bone formation in *P. silus* begins before hatching. However, the sequence of bone formation resembles that of metamorphosing anurans more than that of other direct developers. In particular, *P. silus* does not exhibit precocious ossification of the lower jaw, which is characteristic of some frogs and caecilians that lack a free-living tadpole. These data reveal some similarities between *Philautus* and other direct-developing anurans. However, the departure of *Philautus* embryos from the generalized tadpole skeletal morphology is less pronounced than that observed in other direct-developing taxa. *J. Morphol.* 268:715–725, 2007. © 2007 Wiley-Liss, Inc.

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Life history evolution in amphibians may entail dramatic changes in development. In anurans, the most pronounced developmental changes are associated with the evolution of direct development, a reproductive mode in which the free-living larval stage is absent and the embryo hatches instead as a fully formed froglet. In some anurans, the evolutionary transition to direct development involves repatterning of the initial (embryonic) anlagen of

the cartilaginous skeleton, which is associated with both the loss of larval-specific cartilages and a change in the timing and sequence of bone formation (Hanken et al., 1992). However, the extent to which such changes are inevitable correlates of the evolutionary shift from indirect to direct development has not been evaluated. Despite repeated independent evolution of direct development among anurans (Duellman and Trueb, 1994; Hanken, 1999), few direct-developing species have been studied in detail. Instead, the vast majority of research has concentrated on one species of New World frogs, *Eleutherodactylus coqui* (Leptodactylidae). Focus on *E. coqui* may be attributed both to its suitability as a laboratory model (Elinson et al., 1990) and to its conspicuous loss of larval features, which provides a particularly compelling contrast to the typical tadpole morphology (for review see Orton, 1951; Hughes, 1959; Elinson, 1987; Wake, 1993; Hanken, 1999; Callery et al., 2001). Here we offer evidence that evolution of direct development in anurans is not invariably linked to dramatic changes in skeletal ontogeny, such as those found in *E. coqui*. Instead, *Philautus silus*, a rhacophorine frog native to Sri Lanka, shares only a few derived features of skeletal development with other direct-developing species while retaining several conspicuous features of a generalized tadpole morphology.

The Old World ranid subfamily Rhacophorinae includes at least three genera with abbreviated larval stages: *Nyctixalus*, *Theleoderma*, and *Philautus* (Duellman and Trueb, 1994). Species in the

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Indian Subcontinent and neighboring Sri Lanka evolved direct development at least once, and possibly twice, from an ancestral foam-nesting (metamorphic) ancestor (Meegaskumbura et al., 2002). We chose to examine *Philautus* because it has terrestrial development that is at least superficially similar to that of *Eleutherodactylus coqui* (Alcala and Brown, 1982; Patil and Kanamadi, 1997; Bahir et al., 2005). Little is known about the skeletal development of rhacophorines except for the wooden histological reconstructions of the tadpoles of *Buergeria buergeri* (Okutomi, 1937), an account of the hyobranchial skeleton of *Rhacophorus leucomystax* (Ridewood, 1898), and limited descriptions of the direct-developing *Philautus variabilis* (Ramaswami, 1938, 1956). The cartilaginous jaw suspension (palatoquadrate) in *P. variabilis* appears similar to that in *E. coqui* in at least one important respect: both species lack the posterior processes of the palatoquadrate (Ramaswami, 1938; Hanken et al., 1992), which typically connect the palatoquadrate to the braincase in larval anurans (Haas, 2003). These processes are present in metamorphosing *Buergeria buergeri* (Okutomi, 1937), which belongs to a genus that is basal to the direct-developing clade of rhacophorines (Meegaskumbura et al., 2002). Loss of these same features in two direct-developing species from different lineages leads to the immediate question of whether convergent evolution of terrestrial direct development in *Philautus* has led to other changes in skeletal development that parallel those found in *Eleutherodactylus* and other direct-developing frogs.

We characterized early skeletal development in *Philautus silus*, which were collected and bred in the central highlands of Sri Lanka. We concentrated on cranial features that are repatterned in *Eleutherodactylus coqui*. Many of these features are associated with aquatic feeding by tadpoles and serve no apparent trophic function in a direct-developing embryo. In particular, the presence or absence of tadpole-specific jaws, morphology of the jaw suspension and hyobranchial skeleton, and the sequence and timing of cranial ossification were investigated through examination of cleared-and-stained embryos and early hatchlings. Only some of these cranial features deviated from the generalized pattern of skeletal development in metamorphosing anurans, indicating that the evolution of direct development does not, in and of itself, mandate the loss of these tadpole-specific features.

MATERIALS AND METHODS

Because of legal prohibitions that prevent export of many biological materials from Sri Lanka, this entire study was carried out at the Wildlife Heritage Trust's Agra Arboretum in Agarapatana, Sri Lanka. All specimens used have been accessioned in the Wildlife Heritage Trust collections in the Arboretum (Table 1).

Twenty-five adult *Philautus silus* were collected from Agarapatana in the Central Montane region of Sri Lanka (elevation

1650 m, 6°50'N, 80°40'E) and maintained in captivity. Frogs were housed in glass terraria within an open-air vivarium and allowed to mate spontaneously over a 30-month period. They were kept under dim natural light to simulate the natural forest understory. Local potted plants and branches were added to enhance the habitat, and humus-rich topsoil was provided as a substratum. Relative humidity was maintained above 90% by frequent sprayings with a hand-operated horticultural sprayer (Bahir et al., 2005). Fifteen embryos and 10 hatchlings from a single clutch were reared to different stages of development over 55 days, euthanized by freezing, and fixed in a mixture of 5% chalk-buffered formaldehyde and 35% ethanol (formalcohol). Specimens were staged according to Bahir et al.'s (2005) staging table for *Philautus* and Townsend and Stewart's (1985) table for *Eleutherodactylus coqui* (Table 1). Stage 1 of Bahir et al. (2005) includes from oviposition through neurulation. Stages 2–7 correspond to tail elongation, eye and limb formation, and digit differentiation. Stage 8 corresponds to the onset of frontoparietal, limb, ilium, and neural arch ossification, and Stage 13 is characterized by the beginning of tail resorption and mouth elongation. Hatching typically occurs during Stage 14, when the tail is fully resorbed. Snout-vent and total lengths were recorded for each specimen after fixation.

Each sample was removed from the surrounding jelly coat with watchmaker forceps, cleared, and differentially stained for bone (red) and cartilage (blue) following the protocol of Dingerkus and Uhler (1977). Specimens were dehydrated and submerged in Alcian blue overnight, followed by rehydration and transfer to a trypsin solution in sodium borate for clearing. They then were placed in a solution of alizarin red and incubated overnight, bleached with hydrogen peroxide, and stored in glycerol. Yolk was manually removed from most samples younger than Stage 13.

Each specimen was scored for the presence of bone within 1 day of the staining process. Initial ossifications were detectable as faint purplish to red stains, which were clearly visible with a dissecting microscope. While the anlage of some superficial bones may be visible before they stain with alizarin, we did not use this criterion for "ossification," which would have biased our results in favor of relatively earlier onsets for dermal bones. Cranial ossification sequences are summarized in Tables 1 and 3. Cleared and double-stained specimens were photographed with a Nikon Coolpix 900 digital camera mounted on an Olympus-MZ40 dissecting microscope. Images were visualized with both NIH Image and Adobe Photoshop.

The skull of a single female *Philautus silus* (WHT6942) was drawn using a camera lucida (Fig. 3). The flesh was loosened by brief boiling and removed with forceps. This damaged the septomaxilla and anterior portions of the nasal capsule, which are not shown in the illustration.

RESULTS

Cartilaginous Skeleton

Suprarostrals. These small, paired cartilages extend anteroventrally from the trabecular horns and comprise the larval upper jaw. They are small yet distinct in Stages 6 and 7 (not shown). The cartilages persist in only one of two Stage-8 embryos and are absent in all subsequent stages.

Infrarostrals. These paired cartilages comprise the medial portion of the lower jaw of larval anurans. They are initially distinct from laterally adjacent Meckel's cartilages, but fuse to them during metamorphosis as the bony jaw of the adult replaces the cartilaginous larval jaw. This ancestral sequence is retained in *Philautus silus* embryos. Infrarostrals are distinct and unfused to Meckel's cartilage between Stages 6 and 9 (Fig. 1). In two of

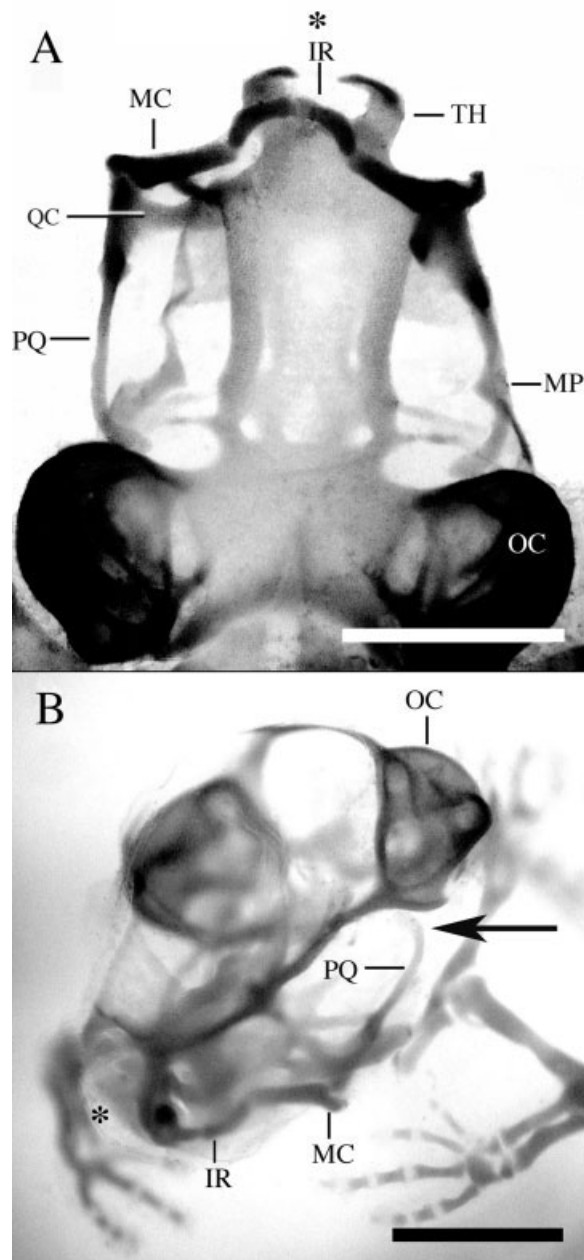


Fig. 1. Alcian blue-stained skull of a Bahir Stage-9 embryo of *Philautus silus*. **A:** Ventral view of the skull revealing the lower jaws and the palatoquadrate. **B:** Oblique anterolateral view revealing the unattached posterior palatoquadrate (arrow), and lower jaw on the left side of the embryo. Asterisk denotes anterior end of head. IR, infrarostral cartilage; MC, Meckel's cartilage; MP, muscular process of the palatoquadrate; OC, otic capsule; PQ, palatoquadrate; QC, quadratocranial commissure of the palatoquadrate; TH, trabecular horns. Scale bar, 1 mm.

three Stage-12 embryos, both infrarostrals are fused laterally to the adjacent Meckel's cartilages. By Stage 14, the infrarostrals are indistinguishable from adjacent Meckel's cartilages. The mentomeckelian bones replace the medial infrarostrals through endochondral ossification (Fig. 4).

Meckel's cartilages. Paired Meckel's cartilages are located on either side of the lower jaw between the larval infrarostral and palatoquadrate (Fig. 1). During embryonic development, each Meckel's cartilage elongates caudally and fuses to the adjacent infrarostral anteriorly and medially. There is a distinct joint between Meckel's cartilage and the ventral end of the palatoquadrate cartilage, which represents the single jaw articulation of the adult. Meckel's cartilage elongates caudally between Stages 12 and 14, as it is invested by the angulosplenial bone (Fig. 4).

Palatoquadrates. These cartilages suspend the lower jaw in both tadpoles and early direct-developing frog embryos. In the metamorphosing rhacophorine *Buergeria buergeri*, they are connected to the neurocranium by an anterior quadratocranial commissure and posterior otic and ascending processes (Okutomi, 1937). In *Philautus silus*, the palatoquadrate forms initially as a thin, horizontal cartilage that extends posteriorly beneath the eye toward the otic capsule (Fig. 1). Later in embryonic development (Stages 12–14), the palatoquadrate shortens and assumes a vertical orientation. Unlike most metamorphosing species, including *B. buergeri*, the posterior end of the palatoquadrate in *P. silus* does not connect to the neurocranium via ascending or otic processes (Fig. 1B). Instead, the posterior palatoquadrate remains separate from the neurocranium through all stages examined.

The anterior portion of the palatoquadrate retains some larval features (Fig. 1A). It has a distinct muscular process, which is the origin of the orbitohyoideus muscle used in tadpole feeding and respiration (Wassersug and Hoff, 1979). This process persists in one Stage-12 embryo, but it is fully resorbed in all later stages. There also is a distinct quadratocranial commissure, which is continuous with the orbitonasal lamina and connects the palatoquadrate to the ethmoidal region of the chondrocranium.

Hyobranchial skeleton. The hyobranchial skeleton is composed of a wide pair of ceratohyal cartilages anteriorly and four pairs of thin ceratobranchial cartilages posteriorly. The ceratohyal is used as the main lever for buccal pumping in larval anurans (Wassersug and Hoff, 1979). By Stage 6, the lateral edge of each ceratohyal curves slightly posteriorly, ventral to the muscular process of the palatoquadrate (Fig. 2). Its medial portion is fused to the hyobranchial plate posteriorly. As embryonic development proceeds, the lateral end of the ceratohyal grows posteriorly past the anteriormost ceratobranchials. By the time of hatching (Stage 14), each ceratohyal has transformed into the paired hyale of the adult hyoid apparatus. The posterior process of the ceratohyal is prominent between Stages 6 and 12, but resorbed by Stage 13. The anterior process of the ceratohyal is distinct by Stage 9 and remains pronounced in the hatchling

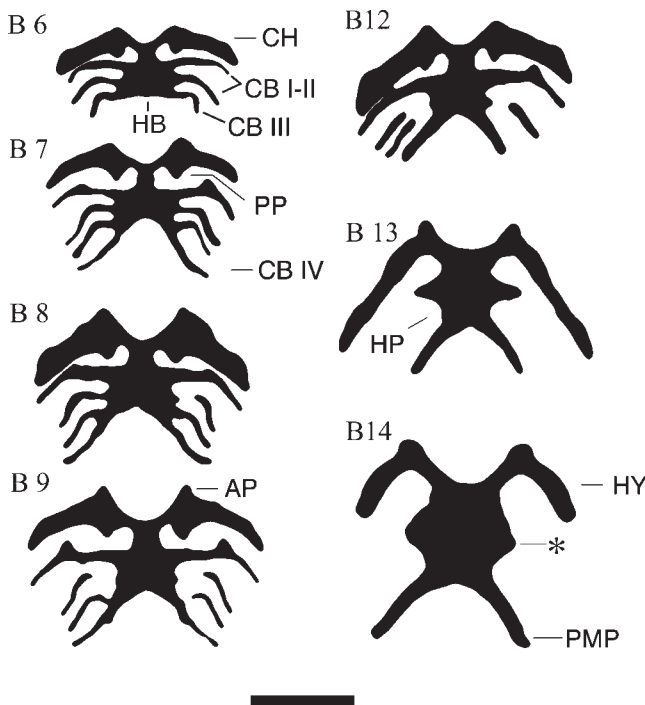


Fig. 2. Development of the hyobranchial skeleton of *Philautus silus* over seven developmental stages as seen in ventral view (Bahir Stage 6, B6; etc.). Images are contrast enhanced from photographs of dissected Alcian blue-stained hyobranchial skeletons. Anterior is at the top. Asterisk indicates the remnant of CB I on the hyoid plate. AP, anterior process of the ceratohyal; CB, ceratobranchial; CH, ceratohyal; HB, hypobranchial plate; HP, hyoid plate; HY, hyale; PMP, posteromedial process; PP, posterior process of the ceratohyal. Scale bar, 1 mm.

froglet (Fig. 2). The ceratobranchial cartilages and hypobranchial plate comprise the branchial basket in larval anurans. In *Philautus silus*, Ceratobranchials I–III are present by Stage 6, but they are nearly completely resorbed by Stage 13 (Fig. 2). The first ceratobranchial (CB I) has a distinct anterior process that appears in Stage 7 and persists through Stage 12. CB I is the last ceratobranchial to be resorbed, although its base persists as a lateral projection of the hyoid plate in hatchlings. CB IV has a different fate from the first three. It forms between Stages 6 and 7 and elongates into the posteromedial process of the hyoid (Fig. 2). The ceratobranchials of larval anurans often bear irregular cartilaginous rays that support the branchial arteries and veins. These rays are absent in both *P. silus* and *Eleutherodactylus coqui* embryos (Hanken et al., 1992).

Cranial Ossification

Skull bones in *Philautus silus* are described below in order of their developmental appearance. These are followed by a description of endolymphatic calcium deposits. Vomer, sphenethmoid, palatine, and quadratojugal bones were not seen in

any of the early posthatching froglets, although all are present in the adult. The first bones to ossify were the frontoparietals, exoccipitals, and parasphenoid, and the last to form was the columella (Tables 1 and 3).

Frontoparietals. The frontoparietal bones in rhacophorine frogs form initially as paired dorsolateral ossifications (Ramaswami, 1956). These dermal ossifications grow toward the midline to create the primary bones of the skull roof. In the adult, these bones fuse with the more lateral and posterior prootics (Fig. 3). Paired frontoparietal ossifications first appear in two Stage-8 embryos, along the anterior border of the cartilaginous taenia tecti marginalis and anterior to the tectum transversum. Each ossification has anterior, posterolateral, and medial projections. By Stage 12, the anterior and medial projections have extended toward the midline (Fig. 4). A second medial process appears during Stage 13, anterior to the original medial process and dorsal to the anterior ascending process of the endolymphatic calcium deposits, which are continuous with the endolymphatic sacs. The second medial process initially stains less intensely with alizarin than the original three processes (Fig. 4). It grows towards the midline by early Stage 14. In late stage 14 hatchlings, the frontoparietals are paired sheets of lightly stained and highly reticulated bone that extend over most of the braincase dorsally. Each sheet is distinguishable from the original three frontoparietal processes and continuous with the second medial process.

Exoccipitals. Paired exoccipital bones constitute the posterior end of the skull. In the adult they fuse with the prootics, forming the bony otic capsule (Fig. 3). They form initially by Stage 12 as faint slivers of endochondral bone on the posteromedial side of each cartilaginous otic capsule. These early ossifications closely resemble neural arches of the vertebral column (Kemp and Hoyt, 1969). They are absent at Stage 9 but are present in all Stage-12 specimens.

Parasphenoid. The parasphenoid is a large, median bone that invests the braincase ventrally. In the adult this bone has an anteroposterior cultriform process and paired lateral alae (Fig. 3). The base of the cultriform process is thin and translucent at the level of the alae. Initially (early Stage 12), the cultriform process appears thin and faint, but its staining intensifies as it grows anteriorly and posteriorly during subsequent stages (Fig. 4). The posterolateral alae subsequently expand toward the otic capsules in more advanced Stage-12 embryos. The parasphenoid is present in all Stage-12 specimens but in none of the younger embryos (Stage 9 and earlier).

Dentaries. These paired dermal bones form initially at Stage 13 as thin slivers on the anterior edge of each infrarostral cartilage. They appear at the same time as the angulosplenic and squamosal

TABLE 1. Ossification data, body size in millimeters (snout-vent length, SVL; total length, TL), and developmental stage (Bahir et al., 2005; Townsend and Stewart, 1985) for embryos used in this study

WHT number	Bahir stage	T & S stage	SVL	TL	EX	PS	FP	SM	PM	MX	AN	DE	PR	MM	SQ	PT	NA	CO	ECD
7038	6	8	5	9															
7039	7	9	6	11															
7040	7	9	4	9															
7041	7	9	3.5	8															
7042	7	9	4	9.5															
7043	7	9	4.5	9															
7044	8	10	5	10					X										
7045	8	10	5.5	11					X										
7046	9	11	5	10															
7047	12	13	5	12	X	X	X												X
7048	12	13	6	11	X	X	X		X										X
7049	12	13	6	12	X	X	X		X										X
7050	12	13	5	12	X	X	X		X										X
7051	13	14	5	11	X	X	X		X	X	X	X			X				X
7052	13	14	6	12	X	X	X		X	X	X	X			X				X
7053	14	15	5.3	8.3	X	X	X	X	X	X	X	X	X		X				
7054	14	15	5.5	7.8	X	X	X		X	X	X	X	X		X				
7055	14	15	6.5	11	X	X	X	X	X	X	X	X			X				X
7056	14	15	6.3	9.3	X	X	X	X	X	X	X	X		X	X				X
7057	14	15	6	11	X	X	X	X	X	X	X	X		X	X				X
7058	14	15	6	6	X	X	X	X	X	X	X	X		X	X				X
7059	14	15	6	6	X	X	X	X	X	X	X	X	X	X	X	X	X		X
7060	14	15	6	6	X	X	X	X	X	X	X	X	X	X	X	X	X		X
7061	14	15	7	7	X	X	X	X	X	X	X	X	X	X	X	X	X		X
7062	14	15	6	6	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

"X" denotes bone present on one or both sides. Abbreviations: AN, angulosplenic; CO, columella; DE, dentary; ECD, endolymphatic calcium deposits; EX, exoccipital; FP, frontoparietal; MM, mentomeckelian; MX, maxilla; NA, nasal; PM, premaxilla; PR, prootic; PS, parasphenoid; PT, pterygoid; SM, septomaxilla; SQ, squamosal. Vomer, palatine, and quadratojugal bones were not seen in any of the specimens examined.

bones (Fig. 4). Each dentary elongates posteriorly during Stages 13 and 14, as the articulation between Meckel's and palatoquadrate cartilages is displaced posteriorly from a position rostral to the eye to a point beneath the midline of the eye (Bahir et al., 2005).

Angulosplenials. Paired angulosplenic bones form on the lingual side of the lower jaw at Stage 13 (Fig. 4). They invest Meckel's cartilage and grow anteriorly toward the dentaries through Stages 13 and 14. The anterior end of the angulosplenic and posterior end of the dentary grow past one another on opposite sides of each Meckel's cartilage during Stage 14. The angulosplenic covers most of the lingual side of the jaw, whereas the dentary invests the labial side.

Squamosals. Paired squamosal bones, which comprise part of the adult suspensorium, also appear at Stage 13. They form on the anterolateral side of each palatoquadrate cartilage. The earliest ossification observed covers the middle third of the palatoquadrate and has a distinct posterior curve near the eye. As embryogenesis proceeds and the angle of articulation between the jaw and jaw suspension approaches perpendicular, the squamosal extends dorsally and ventrally toward the otic capsule and jaw joint, respectively. The dorsal extension of the squamosal does not reach the otic capsule, and otic and zygomatic rami are not present in any of the stages examined.

Mentomeckelians. Paired mentomeckelian bones first appear in the more advanced tailless hatchlings of Stage 14 (Fig. 4). The bones form as periosteal collars that replace the medial portion of each infrarostral cartilage through endochondral ossification.

Septomaxillae. Paired septomaxillary bones form on the edge of each faintly stained cartilaginous nasal capsule. They form initially as slivers of bone, which are found in all but one of the Stage-14 hatchlings. In later-stage hatchlings these slivers become U-shaped and open dorsally. In one advanced Stage-14 hatchling, each septomaxilla forms a nearly complete ring of dermal bone with a slight posterior opening between its two branches.

Premaxillae. Paired premaxillary bones appear on the rostral edge of the skull as distinct dorsoventral slivers of bone in all but one of the Stage-12 specimens. By Stage 13, the ventral border of each sliver expands laterally, forming the anterior portion of the pars dentalis (Fig. 4). After hatching (Stage 14), the dorsal alary process begins to expand laterally, forming an hourglass shape, and the palatine process begins to extend caudally from the ventral edge. No teeth were observed on the pars dentalis portion of the premaxilla in any of our Stage 14 hatchlings.

Maxillae. Paired maxillary bones first form immediately lateral to the premaxillae. They appear as slight dermal ossifications with dorsal and medial projections (partes facialis and palatina,

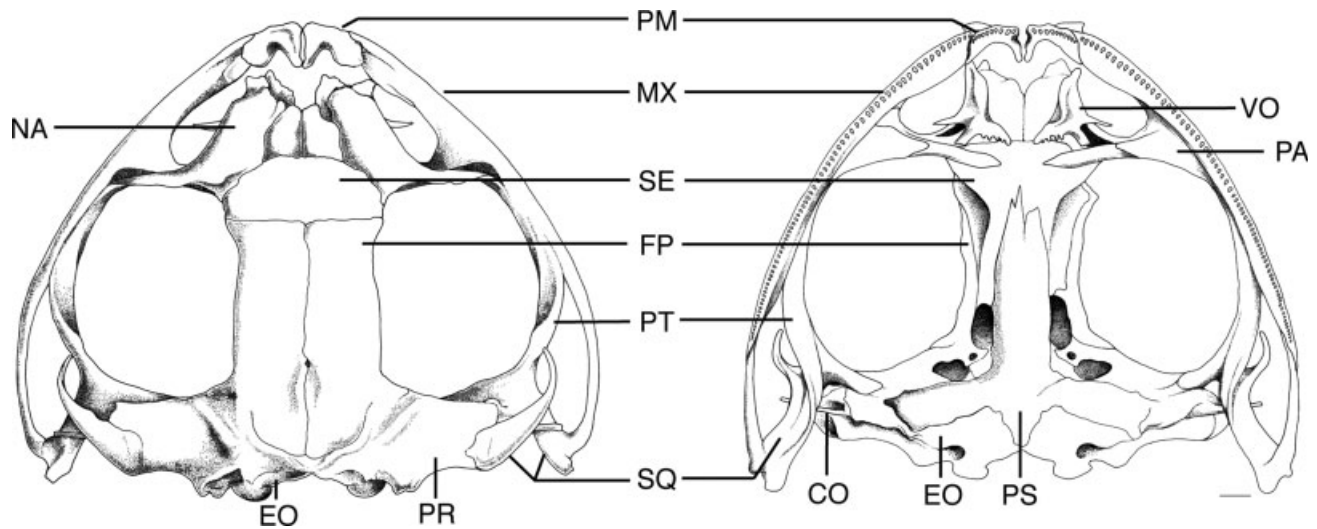


Fig. 3. Dorsal (left) and ventral (right) illustrations of an adult *Philautus silus* skull. CO, columella; EO, exoccipital; FP, frontoparietal; MX, maxilla; NA, nasal; PA, palatine; PM, premaxilla; PR, prootic; PS, parasphenoid; PT, pterygoid; SE, sphenethmoid; SQ, squamosal; VO, vomer. Scale bar equals 1 mm.

respectively). The maxillae are found in all Stage-13 and all but one Stage-14 specimens (Table 1). By late Stage 13, the maxillae extend laterally and posteriorly beneath the eyes. On each maxilla in later staged hatchlings, the pars facialis and a separate pars palatina extend toward the nasal and premaxilla bones, respectively.

Prootics. The prootic and exoccipital bones together compose the adult otic capsule, with the prootic primarily enclosing the anterior and lateral portions (Fig. 3). Paired prootic bones first form as thin sheets anterior to the cartilaginous otic capsules in 6 of 10 hatchling froglets (Stage 14). Additional ossifications may appear later in development (Kemp and Hoyt, 1969), although only the anterior ossification is apparent in our samples.

Pterygoids. Paired pterygoid bones form initially as a long thin dermal ossification on the medioventral edge of the pterygoid process of each palatoquadrate cartilage. They are present in four of five tailless hatchlings (late Stage 14). The medial process, which later attaches the pterygoid to the otic capsule, was not found in any specimen.

Nasals. Paired nasal bones form as faint dermal ossifications dorsal to the olfactory capsule. They are found only in four of five tailless hatchlings (late Stage 14). They remain faint and separate from the surrounding frontoparietals and premaxilla in all of the specimens examined, although later expand to roof the olfactory capsule (Fig. 3).

Columellae. Paired columellae form as endochondral ossifications of the pars media of the chondrocranium and contribute to the auditory apparatus of the adult frog (Duellman and Trueb, 1994). Initial ossification of these bones is present in only one tailless hatchling froglet (late Stage 14).

Endolymphatic calcium deposits. Endolymphatic calcium deposits are found in both tadpoles and direct-developing embryos. These deposits originate in the sacculus of the inner ear and distribute within the posterior neurocranium and down the vertebral column in interconnected endolymphatic sacs (Dempster, 1930). Their presence and distribution vary among specimens of a given developmental stage. Beginning in Stage 12, the deposits are located posterior to the braincase and extend down the vertebral column to varied extents. In one Stage-13 specimen, the anterior portion of the deposit extends farther rostrally and medially to create a bridge over the midbrain. This anterior medial process (Haas, 2003) is found in all but two of the subsequent Stage-14 specimens.

DISCUSSION

Reviews of anuran developmental diversity stress investigations of *Eleutherodactylus coqui* as a model for analyzing ontogenetic changes associated with direct development (Callery et al., 2001; Elinson, 2001; Schlosser, 2001; Elinson and Beckham, 2002; Hanken, 2003; Desnitskiy, 2004; Callery, 2006). However, our results from *Philautus* indicate that the many departures from a metamorphic developmental mode found in *E. coqui* are not inevitable consequences of the evolution of direct development. Jaw and suspensorium patterning, hyobranchial skeleton development, and ossification sequence and timing indicate that embryonic *P. silus* does not achieve the radical departure from a generalized tadpole morphology (e.g., *Bombina orientalis*) found in the highly derived direct-developing embryo of *E. coqui*. While both *P. silus* and *E.*

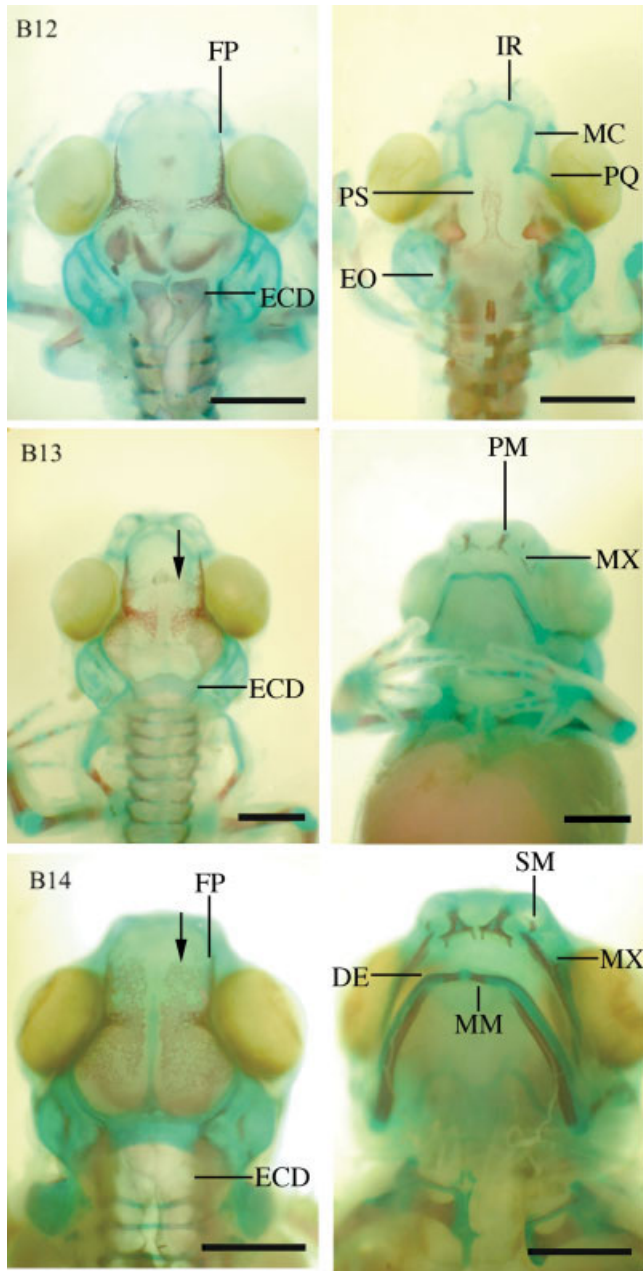


Fig. 4. Cranial bone development of *Philautus silus* over three developmental stages (Bahir Stage 12, B12; etc.) seen in dorsal (left) and ventral views. The ventral neurocranium is obscured by the hyobranchial skeleton and connective tissue in Stages 13 and 14. Arrows in B13 and B14 indicate location of the anterior second medial process of the frontoparietal. Abbreviations as in Figures 1 and 3. Additional abbreviations: DE, dentary; ECD, endolymphatic calcium deposits; MM, menotomeckelian; SM, septomaxilla. Scale bar equals 1 mm.

coqui have modified tadpole-specific feeding structures, with the exception of the posterior attachments of the jaw suspensorium to the skull, most of these structures are merely reduced in *P. silus*, while they are lost entirely in *E. coqui*. Additionally, the order of cranial bone formation is more closely aligned with the pattern found in metamorphic

anurans, instead of the unique sequences observed in other direct-developing or nonfeeding larval forms.

The jaw cartilages and suspensorium are highly specialized in the tadpoles of metamorphosing anurans, and represent unique adaptations for aquatic larval feeding (Wassersug and Hoff, 1979). However, direct-developing individuals do not feed until after they have hatched as terrestrial froglets. Their nonfeeding embryos do not utilize the specialized jaw morphology of a tadpole. Despite being a direct developer, the embryonic jaws of *Philautus silus* initially resemble those of tadpoles. Both upper and lower tadpole jaw cartilages, the paired suprarostrals and infrarostrals, are present in *P. silus* embryos. Suprarostrals are subsequently resorbed and infrarostrals fuse to Meckel's cartilages in a manner similar to the development of metamorphosing species. *Philautus silus* shares this pattern of jaw development with the metamorphosing rhacophorine *Buergeria buergeri* (Okutomi, 1937), although the suprarostrals of *P. silus* are greatly reduced, and the infrarostrals fuse to Meckel's cartilage early in development. The jaw suspension of *P. silus* exhibits the greatest departure from the typical tadpole morphology in its modifications of the palatoquadrate cartilage. The larval palatoquadrate cartilage of metamorphosing frogs has three attachments to the neurocranium via the quadratocranial commissure and the otic and ascending processes, and a fourth muscular process for the origin of the orbitohyoideus muscle. *Philautus silus* retains the muscular process and the anterior quadratocranial commissure, but it lacks posterior ascending and otic processes that typically connect the posterior palatoquadrate to the neurocranium.

Supra- and infra-rostral cartilages are variable features in anurans that lack a feeding tadpole stage ("endotrophic" anurans, Altig and Johnston, 1989). Although several endotrophic anurans have independently lost larval jaw cartilages, this loss is not invariably associated with any specific life history (Table 2). Within endotrophic anurans, species of varying developmental modes either lack (*Eleutherodactylus* spp., *Leiopelma* spp., and *Breviceps adspersus*) or retain (*Philautus silus*, *Flectonotus goeldii*, and possibly *Pipa pipa*, *Nectophrynoides vivipara*, and *Ceratobatrachus guentheri*) the jaw cartilages used by feeding tadpoles.

All endotrophic anurans examined have lost at least one of the four main larval processes of the palatoquadrate (Table 2). However, loss of these processes is not invariably linked to loss of the feeding larval stage. The ascending process of the palatoquadrate is also absent in the feeding tadpoles of both *Heleophrynidae* and *Otophryne* microhylids, (Ramaswami, 1944), and several metamorphosing genera have lost larval otic processes (reviewed in Roček, 2003). Given the small number of studies that have examined the tadpole-specific suspenso-

TABLE 2. Comparison of larval feeding structures in endotrophic embryos and larvae of various anurans

Species	Larval jaws	Palatoquadrate larval processes	Ceratobranchial rays	Developmental mode	Method	Citation
<i>Philautus silus</i>	IR, SR	QC, MP, (AP), (OP)	Absent	Direct-developing	Clear and stain	This study
<i>Eleutherodactylus coqui</i>	(IR), (SR)	(QC), (MP), (AP), (OP)	Absent	Direct-developing	Histology, immunohistochemistry	Hanken et al., 1992
<i>Eleutherodactylus nubicola</i>	(IR), (SR)	(QC), (MP), (AP), (OP)	Absent	Direct-developing	Histology	Lynn, 1942
<i>Eleutherodactylus guentheri</i>	(IR), (SR)	(QC), MP?, AP?, OP?	Unknown	Direct-developing	Histology	Lynn and Lutz, 1946
<i>Ceratobatrachus guentheri</i>	IR, (SR)	Unknown	Unknown	Direct-developing	Clear and stain	Ten Eyck, 1992
<i>Leiopelma archeyi</i>	(IR), (SR)	QC, (MP), AP, OP	Absent	Terrestrial larvae ^a	Histology	Stephenson, 1951
<i>Leiopelma hochstetteri</i>	(IR), (SR)	QC, (MP), AP, OP	Unknown	Nidicolous	Histology	Stephenson, 1955
<i>Breviceps adspersus</i>	(IR), (SR)	QC, MP, (AP), OP	Absent	Nidicolous	Histology	Swanepoel, 1970
<i>Flectonotus goeldii</i>	IR, SR	Unknown	Absent	Nidicolous	Histology, clear and stain	Haas, 1996
<i>Pipa pipa</i>	IR?, SR ^b	? ^c	Present ^d	Direct-developing ^e	Histology, clear and stain	Ridewood, 1898; Trueb et al., 2000
<i>Nectophrynoides vivipara</i>	IR, SR?	QC, MP, AP, (OP)	Absent	Ovoviviparous	Clear and stain	Blackburn, 2004

Parenttheses indicate loss of a larval cartilage or process. Abbreviations as in Figure 1, except: AP, ascending process; OP, otic process; SR, suprarostrials.

^aTerminology follows Bell (1985) for *Leiopelma archeyi*. Terrestrial hatchlings have both tails and legs.

^bSuprarostrial homologues in Pipidae are contested; see Roček and Vesely (1989) and Roček (2003).

^cThe quadratoacranial commissure is present in *Pipa pipa* (Roček and Vesely, 1989), but the muscular process has not been described. Pipids have fused otic and ascending processes (Sokol, 1977).

^dRidewood (1898) did not report reduction of ceratobranchial rays in his detailed comparison of hyobranchial skeletons of *P. pipa* and *Xenopus laevis*.

^eUnlike *Ph. silus* and *E. coqui*, *Pipa pipa* does not have terrestrial direct development. Instead, young are brooded in chambers on the mother's back and emerge as froglets.

rium in endotrophic anurans, it is difficult to infer definitive correlations between the absence of individual elements and a given life history mode.

The hyobranchial skeleton of metamorphosing anurans serves initially as a support for the gill arch vasculature and musculature used early in larval respiration (Viertel and Richter, 1999) and later as food traps in buccal pumping (Wassersug and Hoff, 1979). Larval anurans have a wide ceratohyal anterior to four thinner ceratobranchials that have secondary and tertiary rays to support the gill arch vasculature. These rays are reduced or absent in most of the endotrophic anurans that have been examined in detail (Table 2). The hyobranchial skeleton is subsequently remodeled during metamorphosis into the hyoid apparatus, which supports the adult tongue.

The embryonic hyobranchial skeletons of both *Philautus silus* and *Eleutherodactylus coqui* are remarkably similar. Both species develop all four larval ceratobranchials. These are connected to each other medially through a hypobranchial plate, which is continuous with the anterior ceratobranchials early in development. The separate posterior copula and planum hypobranchiale, which fuse to form the hypobranchial plate in many larval anurans (Roček, 2003), never form in these two taxa (Hanken et al., 1992). The hyobranchial skeleton of *P. silus* consists initially of three ceratobranchial cartilages posterior to a wider ceratohyal cartilage (Fig. 2). A fourth ceratobranchial cartilage appears between Stages 6 and 7, and then rapidly elongates to form the posteriomedial process. This sequence closely follows the ontogenetic pattern found in *E. coqui*. Both taxa fully form the first three ceratobranchials before the appearance of the fourth. However, the rapid extension of the fourth ceratobranchial in *P. silus* is noticeably faster than the slower elongation of this element in *E. coqui*.

The initial sequence of cranial ossification in metamorphosing anurans follows a highly conserved pattern in which the braincase begins to ossify before the rest of the skull. The exoccipital, parasphenoid, and frontoparietal are the first three bones to form in nearly all metamorphosing anurans examined to date (reviewed by Trueb, 1985; Hanken and Hall, 1988; Moore and Townsend, 2003). Initiation of bone formation coincides with the onset of metamorphosis, and there is no example of bone formation beginning before hatching in a metamorphosing anuran. The first cranial bone to ossify in *P. silus* is the frontoparietal, which is soon followed by the exoccipital and parasphenoid in a sequence similar to metamorphosing species (Trueb, 1985; Roček, 2003). Unlike metamorphosing species, however, *P. silus* begins bone formation as an embryo prior to hatching (Table 3). *Philautus silus* also has advanced the formation of several upper and lower jaw bones and the jaw suspension in relation to the remaining bones of the skull. The

TABLE 3. Comparison of ossification sequence and relative timing of bone formation between *Bombina orientalis*, a metamorphosing species (Maglia and Pügener, 1998) and direct-developing *Philautus silus* (this study), *Eleutherodactylus coqui* (Hanken et al., 1992), and *Pipa pipa* (Trueb et al., 2000)

<i>Bombina orientalis</i>	<i>Philautus silus</i>	<i>Eleutherodactylus coqui</i>	<i>Pipa pipa</i>
Hatching	Frontoparietal	Angulosplenia	Frontoparietal
Frontoparietal	Parasphenoid	Squamosal	Parasphenoid
Parasphenoid	Exoccipital	Parasphenoid	Maxilla
Exoccipital	Premaxilla	Premaxilla	Angulosplenia
Septomaxilla	Maxilla	Frontoparietal	Exoccipital
Premaxilla	Angulosplenia	Exoccipital	Prootic
Nasal	Dentary	Maxilla	Premaxilla
Vomer	Squamosal	Pterygoid	Dentary
Maxilla	Hatching	Dentary	Nasal
Angulosplenia	Septomaxilla	Septomaxilla	Septomaxilla
Squamosal	Prootic	Quadratojugal	Pterygoid
Dentary	Mentomeckelian	Hatching	Sphenethmoid
Quadratojugal	Pterygoid	Prootic	Squamosal
Pterygoid	Nasal	Nasal	Stapes
Sphenethmoid	Columella	Vomer	Tympanic annulus
Prootic	Vomer		"Hatching"
Mentomeckelian	Quadratojugal		

"Hatching" in *P. pipa* denotes emergence from brood chambers. Brackets enclose bones that ossify during the same developmental period.

premaxilla, maxilla, angulosplenia, dentary, and squamosal all ossify before hatching and are shifted earlier in development in comparison with the metamorphosing *Bombina orientalis*. As in other endotrophic species in which jaw ossification begins before hatching (see below), in *P. silus* this modification presumably facilitates use of the jaws in active feeding by the newly emerged froglet (Yeh, 2002).

Changes in cranial ossification in *Philautus silus* are less dramatic overall than those seen in the two other endotrophic species examined to date (Table 3; Hanken et al., 1992; Trueb et al., 2000). In *Eleutherodactylus coqui*, the first bones to ossify are the angulosplenia of the lower jaw and the squamosal of the jaw suspensorium. Indeed, most bones form before hatching in this species (Hanken et al., 1992). In *Pipa pipa* both the maxilla and angulosplenia ossify before the exoccipital, and all cranial bones begin to ossify before the young froglet emerges from the mother's dorsum (Trueb et al., 2000; Yeh, 2002). Thus, despite its independent loss of a free-living larval stage, in *P. silus* the initial ossification sequence is more similar to that of a metamorphosing frog and bone formation is only partially advanced into the embryo (Table 3).

"Ontogenetic repatterning" is a concept that was offered originally to account for the numerous morphological novelties associated with direct development in some plethodontid salamanders (Roth and Wake, 1985). It posits the de novo formation in direct-developing species of adult features that are

not preceded by an ancestral larval-specific morphology, and has since been applied to other amphibians (Alberch, 1987; Hanken et al., 1992, 1997; Marks, 2000). Because of the limited number of taxa analyzed in sufficient detail, it is unknown whether the developmental loss of larval-specific features is a frequent, if not predictable, outcome of the evolution of direct development. Orton (1951) reviewed the development of several endotrophic anurans and identified terrestrial direct-developing *Eleutherodactylus* as the most extreme departure from a metamorphic ontogeny. Cranial development in *Philautus silus* indicates that many of the specific features of ontogenetic repatterning found in *E. coqui* are not inevitable consequences of the evolution of terrestrial direct development. Instead, *Philautus* offers a more modest perturbation of a metamorphic ontogeny. The developmental differences between these two taxa may denote varying retention of ancestral features between their respective lineages. Unfortunately, this hypothesis cannot be tested with existing data. Further studies of other direct-developing species will greatly enhance our understanding of the mechanisms that underlie life history evolution and mediate the repatterning of ontogeny.

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