

Patterns of larval development in Cretaceous pipid frogs

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A developmental series of nearly 250 tadpoles of *Shomronella jordanica* from the Early Cretaceous (Hauterivian) of the Shomron (Samaria) region of central Israel, a small collection (12) of *Thoraciliacus rostriceps* tadpoles from the Early Cretaceous (Aptian) of Makhtesh Ramon, Israel, and 13 tadpoles from the Late Cretaceous (Campanian/Maastrichtian boundary) of Stompoor, Marydale, South Africa were studied. These were compared with published data on the development of Paleogene (Middle Eocene–Early Oligocene) pipids from Patagonia, and with normal development of the contemporary pipid frog *Xenopus*. The comparisons of the developmental series of the Cretaceous and contemporary pipid frogs provided data on changes in the developmental pattern, namely of the degree of ossification and other developmental events. In general, it seems that ossification has become considerably delayed in the course of pipid evolution, whereas most anatomical features typical for free living pipid larvae were well established as early as in the Early Cretaceous. Comparisons with the developmental series of specimens from the Late Oligocene Palaeobatrachidae (closely related to the Pipidae) from Bechlejovice near Děčín, Czech Republic revealed that some morphological differences between the two families might be explained by their developmental mode (e.g., formation of the opisthocoelous vertebral centrum in *Shomronella*). The uncinat process on three anterior pairs of ribs in *Shomronella* is the character retained in primitive anurans, and indicates close phylogenetic relations of early pipoids to discoglossoids.

Key words: Anura, Pipidae, larval development, Cretaceous, Oligocene, Israel, South Africa.

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Introduction

Frogs related to extant Pipidae are known from as early as the Early Cretaceous of Israel (Neocomian, at least 122 Ma). They are the earliest anurans known in which tadpoles were preserved. A series of tadpoles (nearly 50), not associated with adults except for a fragmentary hind leg, HJZ 12029, comes from the Lower Cretaceous (Hauterivian; Lang and Mimran 1985) of Shomron (Samaria) region in central Israel. These were described as *Shomronella jordanica* Estes, Špinar, and Nevo, 1978, and their ossification sequence and growth rates were later investigated, together with a large additional series of nearly 200 specimens collected in 1990s, by Chipman and Chernov (2002). Unfortunately, these specimens represented mostly a single metamorphic stage (with few early larvae and one young postmetamorphic individual). Despite unequal representation of developmental stages in the sample, because of the preservation of soft parts of body, one can reconstruct the main anatomical transformations in the course of *Shomronella*'s development, and not only the ossification sequence.

Besides *Shomronella* Estes, Špinar, and Nevo, 1978, less numerous but a representative developmental series was recognized in the large collection of adult frogs from the Early Cretaceous of Makhtesh Ramon, Israel. This collection was first dated as Neocomian by Nevo (1968), Barremian by Estes

et al. (1978), and Aptian by Gvirtzman et al. (1996). Tadpoles from Makhtesh Ramon were noted, but not described, by Nevo (1956). Because these tadpoles represent the earliest stages of ossification as well as late metamorphic and early postmetamorphic stages, they can be easily associated with adults of *Thoraciliacus rostriceps* Nevo, 1968 (mostly on the basis of the distinctive shape of the parasphenoid and sacral and pelvic features). Moreover, specimen HJZ-Th04 bears an imprint of a tadpole on the upper surface of a slab (Fig. 1E) and a fragmentary posterior limb of an adult on the lower side, which helps associate the tadpoles with adults.

During the past several decades, large series of pipid frogs, including tadpoles, were recovered from the marginal facies deposits of a crater lake dated to the Campanian/Maastrichtian boundary around 74 Ma (Smith et al. 2002) on the Stompoor farm, Marydale, South Africa. A preliminary report was published by Van Dijk (1985). Excavations made by Roger Smith in 1999 yielded 243 specimens that were deposited in the South African Museum, in Cape Town. Earlier findings (Geoffrey Grantham in 1970s and Roger Smith in 1984) comprised several tens of specimens that are now deposited in the collections of the Department of Plant and Animal Biology, University of Stellenbosch and in the South African Museum (SAM material is from borehole Stompoor 109). In the early 1980s part of this older material was sent to Richard Estes but the

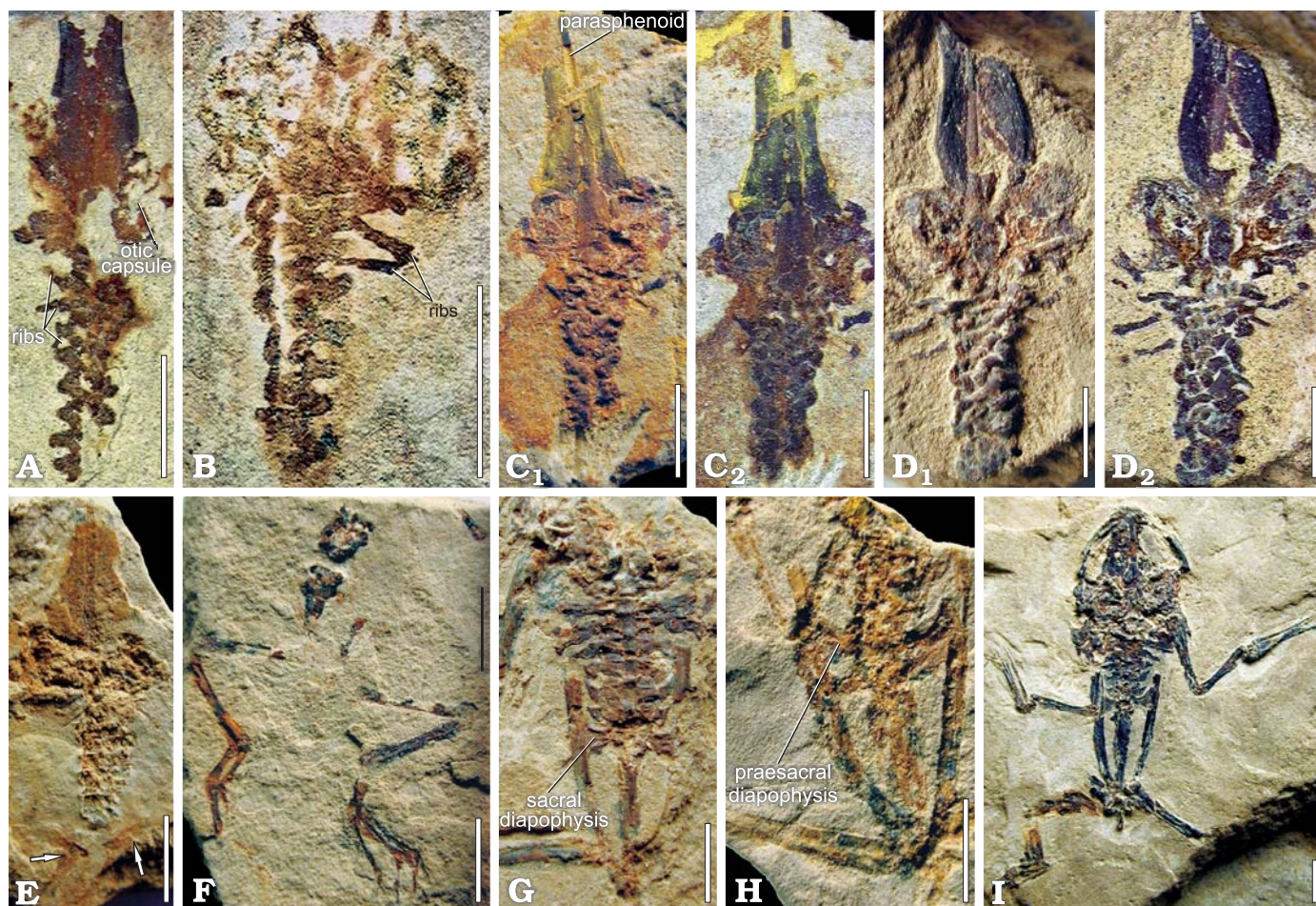


Fig. 1. Larval development in the Early Cretaceous pipid *Thoraciliacus rostriceps* from Makhtesh Ramon, Israel. **A.** The earliest recorded larva, with rudimentary ribs and not-yet fused neural arches; stage NF 59, dorsal aspect (HUIJZ-Th01). **B.** Same developmental stage (HUIJZ-FL3b). **C.** Stage NF 60, ventral view (HUIJZ-Th03); note the anterior tip of the parasphenoid exceeding beyond the both frontoparietals. The photo taken by Zeiss Stemi 2000C stereomicroscope (C₁). The photo taken by means of the image analysing software "Image Pro Plus" (C₂). **D.** Stage NF 60, dorsal view (HUIJZ-Th02). The photo taken by Zeiss Stemi 2000C stereomicroscope (D₁). The photo taken by means of the image analysing software "Image Pro Plus" (D₂). **E.** Stage NF 61, ventral view (HUIJZ-Th04). Rudiments of ilia and femora are marked by arrows. **F.** Estimated stage NF 63, according to ossified tips of toes, probably ventral aspect (HUIJZ-Th11). **G.** Postmetamorphic stage with the ilio-sacral articulation, ventral aspect (HUIJZ-F301). **H.** Postmetamorphic stage, ventral aspect (HUIJZ-F235); note fusion of sacral and praesacral diapophyses. **I.** Adult, ventral aspect (HUIJZ-F93; holotype, cf. also Trueb 1999). Scale bars 5 mm.

study was not completed because of his death, and this material is apparently lost. Frogs from Stompoor were recently described by Trueb et al. (2005). They are generally not well preserved, and the bones are usually shattered, apparently by crystallization of minerals. In spite of this, the series includes some adults in which important anatomical details may be observed, as well as tadpoles and metamorphosing froglets, which provide data on premetamorphic development.

The principal aim of the present study is to compare developmental patterns among these three Mesozoic pipid taxa and compare them with the development of later representatives of the pipid lineage in order to resolve evolutionary trends in pipid ontogeny. The ontogeny of Cenozoic pipids was documented from a series of 12 tadpoles of *Llankibatrachus truebae* Báez and Pügener, 2003 (Middle Eocene–Early Oligocene of Pampa de Jones and Confluencia sites, northwestern Patagonia, Argentina; Báez and Pügener 2003). This, together with extensive knowledge of the development

in contemporary *Xenopus* (Nieuwkoop and Faber 1967; Trueb and Hanken 1992) makes it possible to reconstruct principal changes in pipid development in the course of more than 120 million years of their history and to assess the evolutionary trends of their ontogenies.

Also, comparisons of Mesozoic pipids with a large developmental series of palaeobatrachids from the Late Oligocene of the Czech Republic (Roček 2003b) provides information that can help to solve the problem of pipid-palaeobatrachid relationships.

Material and methods

Developmental series of *Thoraciliacus* is represented by 12 larval and metamorphic specimens (HUIJZ Th01–Th11, FL3a, 3b), and several specimens (both catalogued and un-

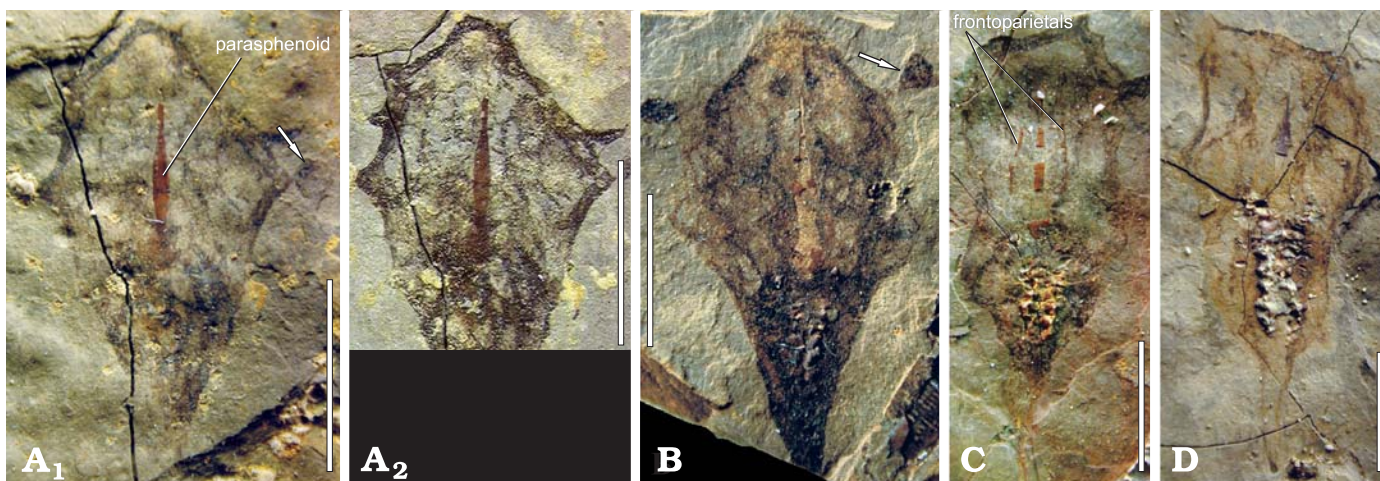


Fig. 2. Larval development of the Early Cretaceous pipid *Shomronella jordanaica* from the Shomron region, Israel. **A.** The earliest recorded larva, stage approximately NF 47–50, probably dorsal aspect (HUJZ-13150). Displaced eyeball marked by arrow. Note complete parasphenoid. The photo taken by Zeiss Stemi 2000C stereomicroscope (A₁). The photo taken by means of the image analysing software “Image Pro Plus” (A₂). **B.** Moderately older stage, approximately NF 51–54, dorsal view (HUJZ-13062). Displaced eyeball marked by arrow. **C.** Approximately same stage as the previous, dorsal view (HUJZ-13190). Rudimentary frontoparietals. **D.** Approximately same stage as the previous, dorsal view (HUJZ-13132). Scale bars 5 mm.

catalogued) illustrate postmetamorphic development. *Shomronella* is represented by a series of 44 tadpoles, most involving their counterparts (HUJZ 12001-12044); this series was collected in mid-1970s (Estes et al. 1978), and includes the holotype (HUJZ 12020). The second series was collected between 1989 and 2000 (Chipman and Tchernov 2002), and is represented by nearly 200 tadpoles (HUJZ 13001-13197). *Thoraciliacus* and *Shomronella* are deposited in the collections of the Department of Evolution, Systematics and Ecology, Hebrew University, Jerusalem.

The pipid from Stompoor was represented by 13 tadpoles (SAM-K6466, K9556, K9559, K9560, K9583, K9586, K9594, K9604, K9656, K9662, K9673, and K9679—2 individuals on a single slab), deposited in the collections of the South African Museum in Cape Town and 2 tadpoles (USP 484 and 485) deposited in the collections of the Department of Plant and Animal Biology, University of Stellenbosch.

Comparisons with *Xenopus laevis* (Daudin, 1802) were based on a series of several tens of individuals prepared as cleared-and-stained whole mounts covering the period between stages 46 and 66 of Nieuwkoop and Faber (1967). This material is deposited in the Department of Zoology, Faculty of Natural Sciences, Charles University, Prague.

Comparisons with *Palaeobatrachus* were based on a series of 171 individuals representing larval, metamorphic and postmetamorphic stages recovered from the Late Oligocene of Bechlejovice, Czech Republic (see details in Roček 2003b).

Where possible, identification of stages was made on the basis of external characters (such as development of limbs) in order to avoid individual variation of internal criteria. Some individuals from the Jerusalem collections were investigated and documented using “Image Pro Plus” software which made it possible to emphasize contrasts between the skeletal structures and surrounding matrix.

Institutional abbreviations.—DZ-FNSP, Department of Zoology, Faculty of Natural Sciences, Charles University, Prague, Czech Republic; HUJZ, The Department of Evolution, Systematics and Ecology, Hebrew University, Jerusalem, Israel; SAM, South African Museum, Cape Town, South Africa; USP, Department of Plant and Animal Biology, University of Stellenbosch, South Africa.

Other abbreviation.—NF, stages of normal development of contemporary pipid frog *Xenopus laevis*, as defined by Nieuwkoop and Faber (1967).

Results

Development in the Early Cretaceous pipid *Thoraciliacus* sp. (Fig. 1).—*Thoraciliacus* is the only Early Cretaceous pipid in which the adult may be associated with larval and metamorphic stages, principally on the basis of two facts: (1) the shape of the parasphenoid is the same in adult *Thoraciliacus* (Nevo 1968: fig. 10C) and in all tadpoles from the same locality in which this bone is preserved, and (2) specimen HUJZ-Th04, which includes both the skeleton of a tadpole as well as part of the adult skeleton (hind extremity fitting into the variation range of *Thoraciliacus*; Nevo 1968) on a single slab.

Although there are only 12 larvae preserved, they represent at least three developmental stages and thus provide important ontogenic information. The youngest preserved stage (HUJZ-Th01) is a complete skeleton in dorsal aspect (Fig. 1A), including the frontoparietal, which is still divided by a fontanelle anteriorly. Its parietal sections are fused with each other posteriorly, possibly leaving an indistinct median suture. The parasphenoid was either not yet developed or (more probably) it was not preserved. The medial walls of the otic capsules had only begun to ossify. There are nine vertebrae

developed and at least five posterior of them with separate neural arches. Three pairs of ribs (on the 2nd–4th vertebrae) are present. The limbs are not yet ossified. Considering only the degree of ossification, this larva is at stage NF 54.

However, comparison with the normal development of *Xenopus laevis* (Nieuwkoop and Faber 1967) suggests that limbs were probably developed but with only a cartilaginous skeleton (Fig. 4C); thus from the inferred external appearance this stage could correspond to stage NF 59. HUIJZ-FL3a and b (part and counterpart) represent about the same developmental stage as judged by nine vertebrae and three pairs of ribs; and its postsacral vertebrae were not yet ossified.

The next stage is represented by specimens HUIJZ-Th02, Th03, and Th05. HUIJZ-Th02 is in the dorsal aspect, the other two are in the ventral aspect. The parasphenoid is well developed, anteriorly exceeding the plane of the frontoparietals, which are fused to various degrees. The otic capsules were well ossified (HUIJZ-Th03) or at least calcified. Ten vertebrae may be discerned with their neural arches still separated from one another. The clavicles are present (HUIJZ-Th02, Th03). Although limbs are not present, it is possible that this may be due to preservation status. This seems to be evidenced by specimen HUIJZ-Th04 (Fig. 1E) in which the ilia are separated from one another and the femora are preserved, although other features (especially number of vertebrae) are the same as in other specimens of this stage. A typical feature is that the vertebral column is shorter than the skull measured to the rostral tip of the parasphenoid. These larvae may be roughly identified as stage NF 60–61.

Specimen HUIJZ-Th11 (Fig. 1F) represents a stage in metamorphosis (probably 63, judging by degree of ossification of the ilia in *Xenopus*), with well developed hind limbs, but the ilia are not yet articulated to the vertebral column. The sacral vertebra does not have diapophyses.

Postmetamorphic development is characterized by additional pair of ribs on the 5th vertebra (but not on the atlas) and occasional fusion of diapophyses of the the sacral and the most posterior presacral vertebrae (Fig. 1H).

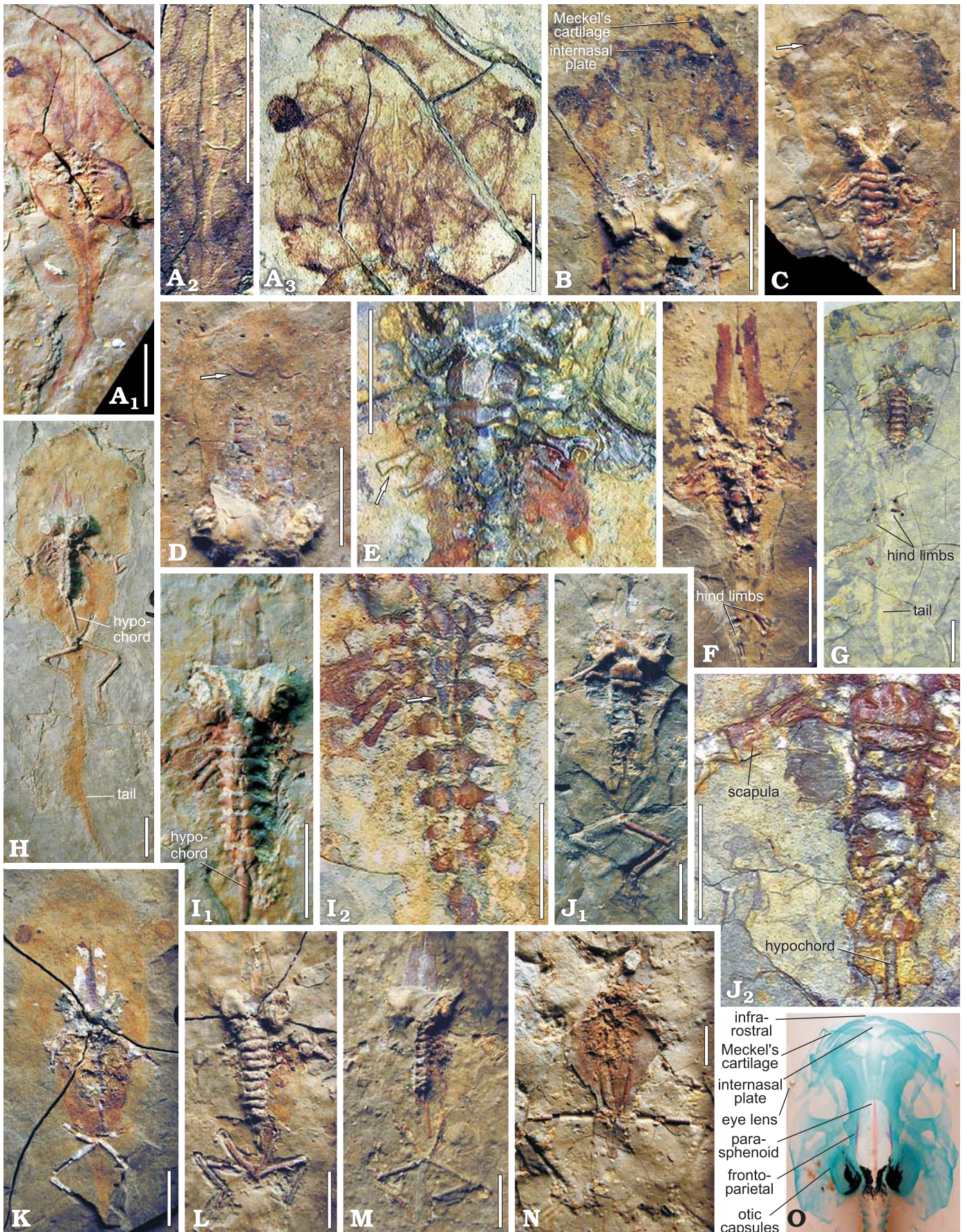
Development in the Early Cretaceous pipid *Shomronella jordanica* Estes, Špinar, and Nevo, 1978 (Figs. 2, 3, 5).—The earliest larva in the developmental series is HUIJZ-13150 (Fig. 2A) in which only the parasphenoid is ossified. It should

be noted that Chipman and Tchernov (2002) illustrated a still earlier tadpole, about 4 mm long with no ossification (see their fig. 2), but because they did not refer to the fossils by catalogue numbers, we were not able to relocate this specimen. Based on the external body shape, this individual may be in stages NF 47–50. The tail is not complete but the length of the trunk to the plane of the vent is about 10 mm. The anterior part of the parasphenoid is clearly delimited, which means that it was well ossified; its anterior tip reached beyond the plane of the eyes.

The frontoparietals begin to ossify as faint strips adjoining the constriction of the posterior half of the parasphenoid and soon extend anteriorly. Simultaneously, two ossification centers lateral to the posterior end of the parasphenoid (termed the exoccipitals by Chipman and Chernov 2003) and 6–8 pairs of neural arches can be seen. Externally, these larvae (HUIJZ-13062, 13190, 13132; Fig. 2B–D) do not differ much from the previous stage, except for size, but the progress in ossification suggests a more advanced stage (perhaps NF 51–54 based on distinct outlines of the body without posterior limb buds; Fig. 2B). Curiously enough, these larvae are characterized by pigmented eyes extruded from the outlines of the head (marked by arrows in Fig. 2A₁, B), which suggests that the head skeleton was not yet well chondrified. Also, the head in these early larvae is not oval (though more or less symmetrical) in shape, and their tail was apparently (as suggested by a slightly older larva HUIJZ-12036; Fig. 3A₁) shorter than the body.

The next stage (about NF 55) is represented by HUIJZ-12036 (Fig. 3A). Compared with the previous one, it had three pairs of ribs, although it seems that the number of vertebral rudiments did not increase. Faint imprints of cartilages suggest that the larval skull was already well chondrified; the lower jaw consisted of the infrarostral (possibly still paired) and Meckel's cartilages, both articulated with each other (compare Fig. 3A₃ and Fig. 3O). Interpretation of blood vessels and nerves by Jarošová (in Estes et al. 1978: fig. 2A) is largely confirmed, possibly except for what she identified as the vena capitis lateralis; our examination of the specimen suggests that it might be the outer outlines of the cartilaginous otic capsules. Further, the optic nerve extending to the inner surface of the eye (only slightly protruding from the

Fig. 3. Larval development of the Early Cretaceous pipid *Shomronella jordanica* from Shomron region, Israel (continued). A. Stage NF 55, dorsal view (HUIJZ-12036). The photo of complete specimen taken by Zeiss Stemi 2000C stereomicroscope (A₁). Imprint of parasphenoid taken by Zeiss Stemi 2000C stereomicroscope (A₂). The photo of the head with vessels and nerves taken by means of the image analysing software "Image Pro Plus" (A₃). B. Stage NF 59–60, head and anterior part of vertebral column, dorsal aspect (HUIJZ-13056). Note jaw cartilages comparable with those in corresponding stage of *Xenopus* (O); otic capsules and vertebrae are covered by thin layer of sediment. C. Stage NF 60 in dorsal view (HUIJZ-12012). Angulosplenic on the left side marked by arrow. Otic capsules still incompletely ossified. D. Stage NF 60, head in dorsal view; nasals marked by arrow (HUIJZ-12019). E. Stage NF 60, dorsal view (HUIJZ-12013). V1 and V2 fused. Rib with uncinat process marked by arrow. F. Stage NF 60, dorsal view (HUIJZ-13184). Hind limbs rudimentary and widely separated from vertebral column. G. Stage NF 61, dorsal view (HUIJZ-12009). H. Stage NF 63, ventral view (HUIJZ-12021). Note well developed but displaced hypochord. I. Stage NF 63, ventral aspect (HUIJZ-12017a). Note development of the vertebral centra (ossification within notochordal canal marked by arrow). The photo taken by Zeiss Stemi 2000C stereomicroscope (I₁). The vertebral column taken by means of the image analysing software "Image Pro Plus" (I₂). J. Stage NF 63, dorsal view (HUIJZ-12003). The neural arches of the posterior vertebrae are broken off so the inorganic filling of the vertebral canal is visible. V1 and V2 still separate. The photo of the complete specimen taken by Zeiss Stemi 2000C stereomicroscope (J₁). The vertebral column taken by means of the image analysing software "Image Pro Plus" (J₂). K. Stage NF 63–64 in ventral view (HUIJZ-13136). L. Stage NF 63–64 in dorsal view (HUIJZ-13073). M. Stage NF 63–64 in ventral view (HUIJZ-13080). N. Early postmetamorphic individual, probably in ventral view (HUIJZ-13053). Iliosacral articulation established. O. *Xenopus laevis*, stage NF 57, skull in dorsal view (DZ FNSP XL57-ex2). Scale bars 5 mm.



head outlines) indicate that the pigmented area represents the entire eye, not only the lens (compare with Fig. 3O where the eye lens is separated from the cartilaginous eye cup).

The subsequent stage is represented by specimen HUIJZ-13056 (Fig. 3B). It differs from the previous stage by having nine vertebrae and ossified posteromedial parts of the otic capsules. Meckel's cartilage and the internasal plate are comparable with those in stage NF 59 of *Xenopus* (Fig. 3O), and the lower jaw still lacks dermal ossification. The first dermal ossifications after the parasphenoid and frontoparietals are the angulosplenials adjoining Meckel's cartilages on their medial side, which appear only in stage NF 60 (HUIJZ-12012, Fig. 3C; HUIJZ-13040); the frontoparietals are still separated along their entire length. Nine to ten vertebrae may be recognized, the neural arches on both sides of anterior vertebrae are completely fused, while the more caudal trunk vertebrae are still separated in the midline. The first and second vertebrae are already fused into a single unit; fusion is documented by HUIJZ-12013 (Fig. 3E) where the first and second vertebrae still may be recognized on the right side, and by HUIJZ-1203 (Fig. 3J₁, J₂). There are three pairs of ribs articulated with the original vertebrae 2–4. It is noteworthy that the second pair of ribs bear a distinct uncinat process on its posterior side, as documented by HUIJZ-12013 (Fig. 3E) and numerous other specimens of this and an older age (HUIJZ-12007, 12010, 12017a, 12021, 12026, 12038, 13005, 13043, 13048, 13063, 13170, 13184). Still in stage NF 60, the tiny rudiments of the nasals may be recognized (HUIJZ-12019, Fig. 3D). Appearance of these dermal ossifications is accompanied with an increase in size of the frontoparietals so that the two bones approach one another and may ultimately fuse in their posterior parts (HUIJZ-12037).

The earliest ossified rudiments of the posterior limbs (with no pelvic elements) also appear in stage NF 60 (HUIJZ-13184, Fig. 3F). The diaphyses of the tibia and fibula (well separated) may be recognized and the elements of the pectoral girdle become ossified (among the first is the scapula). On the other hand, the hypochord is still absent. Stage NF 61 (HUIJZ-12009, Fig. 3G) is characterized by the appearance of the hypochord and rudimentary ilia. The hypochord usually appears simultaneously with the first caudal (i.e., 10th) vertebra.

The great majority of the specimens are in stage NF 63. These tadpoles still have a long tail (Fig. 3H), the hind limbs

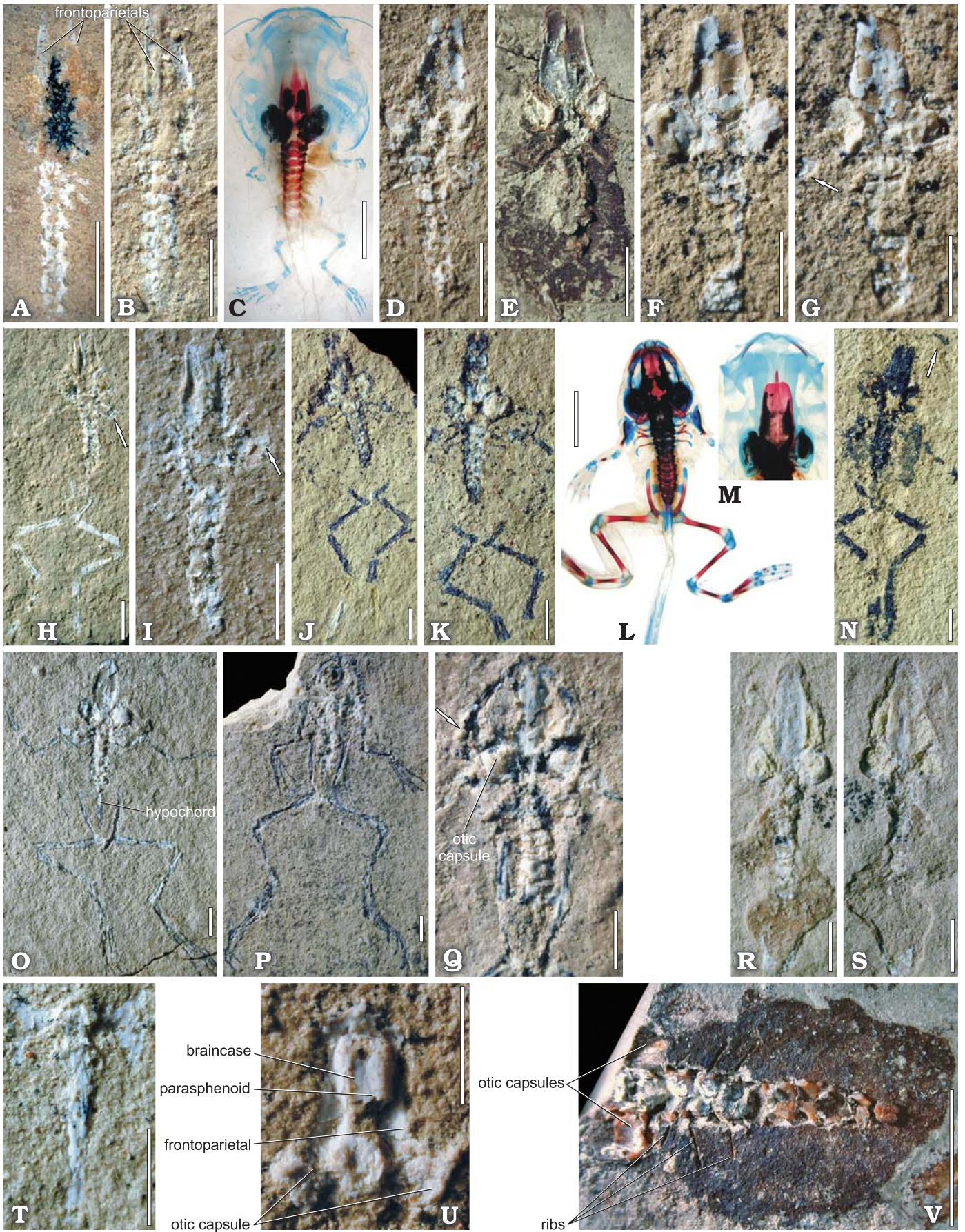
are ossified up to the distal tarsal elements, and the halves of the pelvis are in contact with each other. The forelimbs are ossified up to the distal ends of the separate ulna and radius. In specimen HUIJZ-12021 the columella is clearly visible. In all individuals of this developmental age, in which the vertebral column is exposed from the ventral side, one can see that the centra were ossified at the bases of the transverse processes and on the dorsal part of the notochordal canal (epichordal ossification). This condition results in a longitudinal groove on the ventral side of the vertebral column (Fig. 3H, I₁, M). The groove in the anterior vertebral centra was soon filled with ovoid calcifications or ossifications that replaced the notochord. Image analysing software reveals that those parts of the fillings corresponding to each centrum are separated from the lens-shaped fillings in the intervertebral spaces. The latter fillings fuse later with those within the adjacent posterior centrum, thus giving origin to the opisthocoealous centrum (Fig. 3I₂; see also Estes et al. 1978: fig. 6C). These fillings replacing the notochord should not be confounded with the inorganic fillings of the vertebral canal; these can be observed only from the dorsal side when the neural arches are broken off (Fig. 3J₁, J₂).

Further development may be characterized by gradual elongation of the ilia and their placement into an ultimate position nearly parallel with the axis (compare Fig. 3L with Fig. 3M, N). It is obvious that the hypochord was separated from the caudal vertebrae in the late stages of metamorphosis and that the tail was still as long as the hind limbs (Fig. 3M).

Development in a South African Late Cretaceous pipid

(Fig. 4).—The earliest larva (specimen USP 484; Fig. 4A), based on the presence of paired frontoparietal, is in stages NF 54 or 55; since Nieuwkoop and Faber (1967) characterized stage 56 by fused frontoparietals. However, they based their stages of this developmental period on external characters, namely on the relative size of the extremities. In our material of *Xenopus*, dermal ossification was considerably retarded and separate frontoparietals were still observed in stage 59 which is characterized by the extended forelimbs reaching to the base of the hind limbs. We believe that the body shape (including size of limbs) is more important for determining developmental stages than the degree of ossification, which may vary because of heterochrony (see Roček 2003b for discussion of variation of the internal characters in *Xenopus*), so we determined this larva as stage NF 59.

Fig. 4. Larval development in the Late Cretaceous pipid from Stompoor, South Africa, compared with normal development of Recent *Xenopus laevis*. A. The earliest recorded larva, stage NF 59, dorsal aspect (USP 484). Frontoparietals feebly ossified. B. Slightly older individual, stage NF 59–60, ventral aspect (SAM-PK-K9560). C. *Xenopus laevis*, stage NF 59 (DZ FN5P XL59-ex2). D. Beginning of metamorphosis, stage NF 60, ventral aspect (SAM-PK-K9656). E. Stage NF 60, ventral aspect (SAM-PK-K6466). Frontoparietal well ossified but still paired. F. Estimated stage NF 63, ventral aspect (SAM-PK-K9583a). G. Counterpart of the same specimen, dorsal aspect (SAM-PK-K9583b). Scapula marked by arrow. H. Stage NF 63, ventral aspect; note relative size of the parasphenoid (SAM-PK-K9662a). Scapula marked by arrow. I. Stage NF 63, ventral aspect (SAM-PK-K9662b). Scapula marked by arrow. J. Stage NF 63, dorsal aspect (SAM-PK-K9604a). K. Counterpart of the same specimen, ventral aspect (SAM-PK-K9604b). L. Recent *Xenopus laevis*, stage NF 63 (DZ FN5P XL63-ex3). M. Recent *Xenopus laevis*, stage NF 60, skull in ventral view (DZ FN5P XL60-ex2). N. Estimated stage NF 63–64, dorsal aspect (SAM-PK-K9620). Angulosplenic marked by arrow. O. End of the metamorphosis, stage NF 66, ventral aspect (SAM-PK-K9698). P. Adult, dorsal aspect (SAM-PK-K9610). Q. Adult, ventral aspect (SAM-PK-K9666). Arrow marks level of the jaw joint. R. Late metamorphic stage, dorsal view (SEM-PK-K9559a). S. Counterpart of the same specimen, ventral view (SEM-PK-K9559b). T. Disarticulated sacral vertebra with coalesced urostyle (SAM-PK-K9523). U. Adult, part of the skull in ventral view (SAM-PK-K9651). V. Tadpole, supposedly from the Cretaceous of Zaire (USP 493). Scale bars 5 mm.



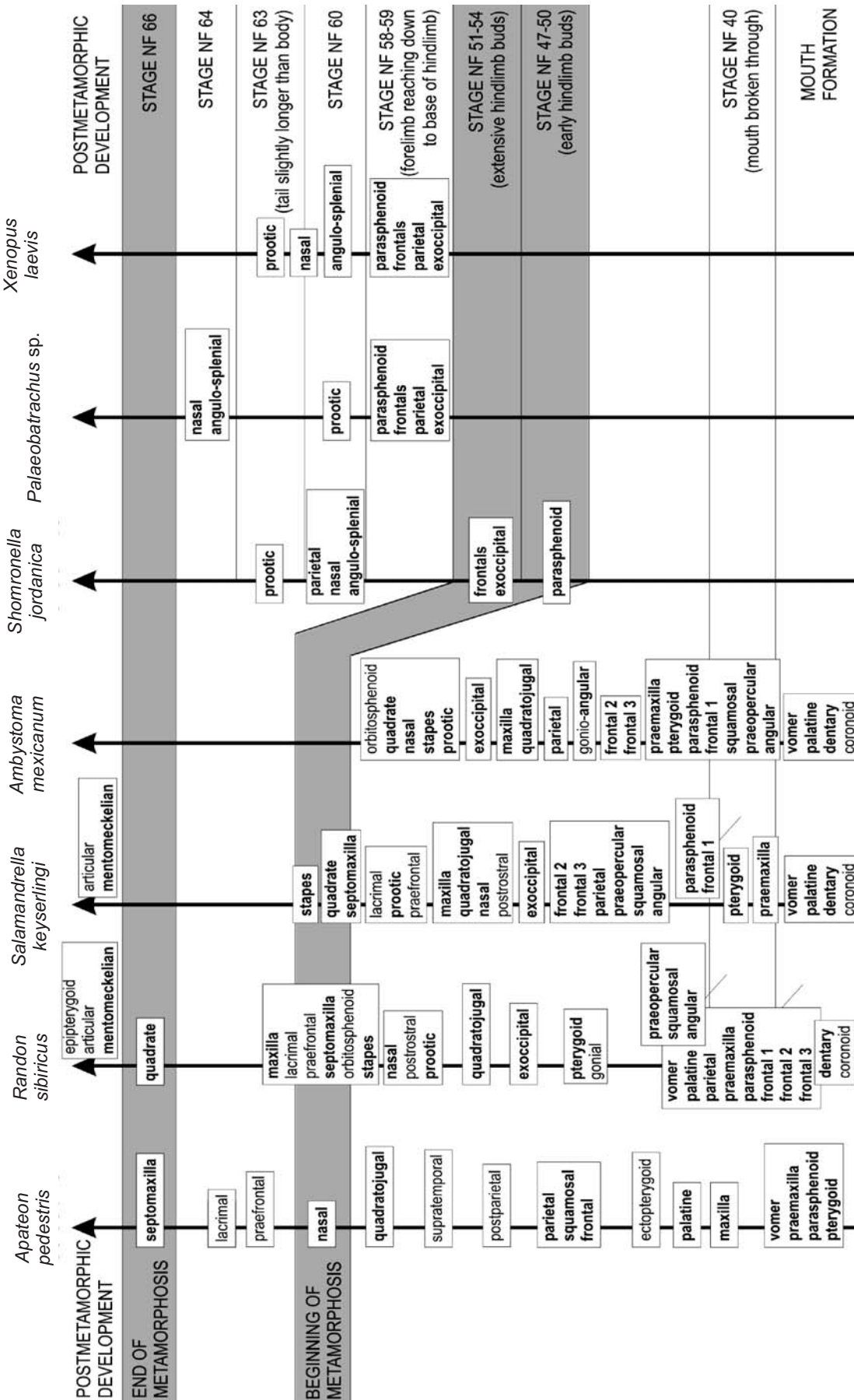


Fig. 5. Comparison of the relative sequence of some developmental events of the skull in the Cretaceous *Shomronella*, Oligocene *Palaeobatrachus*, and Recent *Xenopus*, with the Paleozoic *Apateon* (Temnospondyli: Branchiosauridae), primitive recent caudates *Randon* and *Salamandrella* (Cryptobranchidae: Hynobiidae), and neotenic caudate *Ambystoma* (Ambystomidae). Bones that are retained in the anurans are in bold. Because of differences in definition of metamorphosis among various authors, and because of different definition of developmental stages in the Paleozoic amphibians, caudates and anurans, comparison in terms of exactly corresponding anatomical stages is not possible. The most objective for staging in the amphibians is formation of the mouth, beginning of metamorphosis (associated with reduction of gills in caudates, development of the limbs in anurans), and the end of metamorphosis (loss of tail in the anurans). Data on *Apateon* from Schoch (1998), on the caudates from Lebedkina (2004), and on *Palaeobatrachus* from Roček (2003b).

This larva is characteristic by, besides paired frontoparietal, the posterior part of the otic capsules being ossified and by eight pairs of neural arches, all separated at the midline. It possesses two (plus developing 3rd) pairs of ribs on vertebrae 2–4.

A slightly more advanced larva (stage NF 59–60, suggested by its larger size, number of vertebrae and degree of ossification of the otic capsules), is preserved in ventral aspect (Fig. 4B); it has eight (plus developing 9th) vertebral centra. Frontoparietals are more massive but still separated by a fontanelle along their entire length, and the otic capsules are ossified in their medial parts. There are three pairs of ribs (the most posterior of them is still rudimentary). Specimens SAM-PK-K9586 and SAM-PK-K9594 are at the same stage.

The next stage (Fig. 4D) is characterized by early ossification of the rudiments of the ilia and femora (not included in Fig. 4D), more ossification of the frontoparietals, which are close to each other but still separated anteriorly, otic capsules ossified laterally and ventrally, and ribs clearly defined. Ten vertebral centra may be discerned, plus two (11th and 12th) rudimentary caudals. Based on the rudiments of the pelvis and hind limbs, it can be determined as stage NF 60 (see Roček 2003b: fig. 1M). This developmental stage is documented also by specimen SAM-PK-K9556.

The most frequent stage is about NF 63, based on the development of the ilia and posterior limbs (Fig. 4E–K). It may be characterized as an advanced metamorphic stage with a tail reduced considerably. The two frontoparietals are either separated by a midline suture or coalesced together. A remarkable feature of the frontoparietal is that it is depressed along the midline, a character maintained in adults. Its anterior margin is convex, with the tip of the parasphenoid extending from below it (Fig. 4H), as is the case with contemporary *Xenopus* in stage NF 60 (Fig. 4M). The diaphyses of the long bones of the posterior extremities are ossified, as are the distal digits. The anterior tips of the ossified portions of the ilia are posterior to the level of the most posterior vertebra (11th or 12th), and the two halves of the pelvis are still moderately separated. Development of the forelimb is considerably retarded, as inferred from the fact that only the scapula is ossified. A rudimentary humerus is present in SAM-PK-K9604 (Fig. 4J, K). In spite of the advanced development of the postcranial skeleton, the dermal ossification remains confined only to the frontoparietal and parasphenoid. Only in a slightly later stage (NF 63–64; Fig. 4N) can one observe the earliest rudiments of the dermal ossification of the lower jaw (angulosplenial). The two halves of the pelvis are in contact with one another by means of ossified ischia. This developmental stage is documented also by SAM-PK-K9673a, b and SAM-PK-K9679.

At the end of metamorphosis (stage NF 66; Fig. 4O), the fore- and hind limbs are almost completely ossified except for the carpal and tarsal elements; the urostyle is present. Dermal ossifications of the skull are still weak, based on the fact that dermal bones of the skull are not preserved in the specimen SAM-PK-K9698. The iliac shafts are not entirely ossified, and also the sacral diapophyses, if present, are not yet ossified.

Adults (Fig. 4P, Q) may reach about 40 mm snout-vent length and are characteristic by ossified carpal and tarsal elements and sacral diapophyses, the sacral vertebra co-ossified with the urostyle, and a completely ossified braincase (Fig. 4U). The braincase could be co-ossified with the frontoparietal (which is smooth on its dorsal surface, narrower in the middle of its length, and does not extend over the dorsal surface of the otic capsules; see SAM-PK-K9549a; SAM-PK-K9550a; SAM-PK-K9593; SAM-PK-K9651; SAM-PK-K9668a; SAM-PK-K9733) and with the parasphenoid. On the other hand, the astragalus and calcaneum are not coalesced even in fully ossified adults (e.g., SAM-PK-K9513; SAM-PK-K9568, SAM-PK-K9653a, SAM-PK-K9666, SAM-PK-K9717a). The parasphenoid reaches the level of the symphysis of the lower jaw (SAM-PK-K9549a; SAM-PK-K9658), but does not extend beyond the anterior margin of the frontoparietal (SAM-PK-K9669). The nasals are drop-shaped and moderately asymmetrical (SAM-PK-K9550a). The symphysis consists of the mentomandibulars (e.g., SAM-PK-9521; SAM-PK-9605; SAM-PK-K9653a). The jaw joint is located at the plane of the anterior portion of the otic capsules (SAM-PK-K9550a; SAM-PK-K9605; see also Fig. 4Q). The ribs are articulated with, but not co-ossified with, the transverse processes (SAM-PK-K9717b). The sacral diapophyses are moderately extended posteriorly (e.g., SAM-PK-K9625; SAM-PK-K9633; see also Fig. 4T). The vertebral centra are distinctly opisthocoeleous (e.g., SAM-PK-K9550a; SAM-PK-K9552; SAM-PK-K9637; SAM-PK-K9653a, SAM-PK-K9670a; SAM-PK-K9700; SAM-PK-K9707; see also Fig. 4Q). The vertebral column consists of eight presacrals, and vertebra 9 is the sacral (e.g., SAM-PK-K9576; SAM-PK-K9670; see also Fig. 4Q). The iliac shafts are straight, long, and extend nearly to the most posterior (3rd) rib (usually to vertebra 5) (e.g., SAM-PK-K9602; SAM-PK-K9610a). Specimen SAM-PK-K9687 provides valuable information on at least some carpal elements. The proximal row consists only of the ulnare and radiale; the radiale is distally adjoined by a comparatively small praeaxiale centrale, to which carpale distale 1 and 2 articulate. The praepollex is not preserved (terminology after Jarošová 1973).

There is one individual (SEM-PK-K9559a, b), preserved in dorsal and ventral aspects (Fig. 4R, S), apparently in an advanced metamorphic stage (judging by presence of unidentifiable ossification in the pelvic region), which differs from other specimens in having comparatively long, narrow and flat frontoparietal that lacks the typical median depression and by rather small otic capsules. Proportions of the skull suggest another pipid taxon at Stompoor. Similar proportions of the frontoparietal to the otic capsules were found in SAM-PK-K9669, which is a subadult (i.e., iliac shafts comparatively short, sacral diapophyses not ossified) but in which the frontoparietal is comparatively narrow and long, and the parasphenoid, although well preserved, does not extend beyond its anterior margin.

Another tadpole (USP 493, Fig. 4V) of uncertain age, is from crater lake deposits of Zaire, and is mentioned here to

complete the list of otherwise scarce Mesozoic anuran larvae. It was found together with several adult frogs by employees of Zaire mining companies. The mining companies were reluctant to allow localities to be published, as it might help their competitors. This is why we only guess that these frogs, including the tadpole, are also of the Cretaceous age and related to the break-up of Gondwana, as the other pipids are thought to be. The material of adults (see also Van Dijk 1995: fig. 2) was lent to R. Estes in 1980s but now it is considered lost.

Based on the number of vertebrae, presence of three pairs of ribs, and well ossified otic capsules, we estimate its developmental stage as NF 60 (compare ossification of the otic capsules in *Shomronella*, Fig. 3C). It is preserved as imprints of the skeleton in a matrix, with some fragments of bones preserved *in situ*. Judging from the preserved part of vertebra 7 that includes zygapophyses, the specimen is exposed ventrally. Ten ossified vertebrae can be seen, the most posterior (i.e., first caudal) being only an early rudiment. The otic capsules are well ossified and one can recognize the interior of the posterior semicircular canal and the prootic foramen. The most striking feature is the presence of three pairs of thin straight ribs, preserved as imprints in the matrix. Such "fish-like" ribs are quite unusual for pipids.

Discussion

There is a considerable variation in the appearance of anatomical features in the course of larval and metamorphic development of the Anura (see discussion in Roček 2003b). Variation is also caused by the preservation condition. For instance, non-skeletal structures are preserved in early larvae of *Shomronella*, which allow the reconstruction of their external appearance and thus principal criteria for definition of developmental stages like those in contemporary *Xenopus*. However, because the degree of development of skeletal features may vary in individuals with the same external morphology, and because external morphology defines developmental stages, staging of fossil tadpoles preserved exclusively as ossified skeletons, may not be accurate. Moreover, ossification sequence inferred from histological examinations may considerably precede that inferred from cleared and stained specimens. Taken all this into account, developmental comparisons of fossil taxa among themselves and comparisons with contemporary relatives may not be quite precise. Nevertheless, general information on morphology and sequence of ossification may be inferred.

Main developmental events are summarized in Table 2. They were selected mainly with regard to whether they may be observed in a majority of compared taxa. Obviously, in those taxa where only a few tadpoles (and thus few developmental stages) were available, the basis for comparisons is rather limited.

Xenopus was used to represent contemporary pipids not only because its development is well known and is the basis for staging development in other anuran taxa, but mainly be-

cause of its free-living larvae. In all Cretaceous pipids, as well as in Paleogene/Neogene *Palaeobatrachus*, larvae were also free-living. "Direct development" in *Pipa* is no doubt a secondary developmental strategy acquired comparatively recently. Free-living larvae were undoubtedly present in *Palaeobatrachus*, whose tadpoles could attain gigantic dimensions (as evidenced by several specimens from the Upper Vindobonian, Miocene, of Randecker Maar, Germany; Roček and Rage 2000). Tadpoles of all taxa included in this study, which possessed long tails even in late metamorphic stages, were undoubtedly free-living larvae as well.

Sequence of ossification.—The earliest centres in contemporary *Xenopus* are in the frontals, parasphenoid, and the posteromedial part of the otic capsules (exoccipitals). In our cleared-and-stained whole mounts the earliest traces of calcification can be observed in stages NF 54–57 (e.g., Fig. 3O), which approximately corresponds to data by Trueb and Hanken (1992). Nieuwkoop and Faber (1967) mentioned the earliest ossification at the stage NF 51. Later appearance of the frontoparietal was recorded by Sedra and Michael (1957) who histologically examined wild caught specimens. Hence, their results are comparable to those by Nieuwkoop and Faber (1967) and confirm the view expressed by Estes et al. (1978) that there is greater variability in the timing of ossification in wild, than laboratory bred, populations. Disregarding these circumstances, one may conclude that in most cases the earliest dermal ossifications (represented by the frontals and parasphenoid) precede the endochondral ossifications (exoccipitals) and these, in turn, precede the ossification of the anterior neural arches.

We cannot say much about *Thoraciliacus* and the South African pipid because their earliest larvae in the samples (Figs. 1A, 4A) obviously were more advanced than the earliest stage of *Shomronella* (Fig. 2A₁, A₂). The earliest stage of *Shomronella*, however, displays a well defined parasphenoid (except for its posterior part), which suggests its high degree of ossification, but there were no signs of frontals or exoccipitals. Judging by the shape of the body with a short tail and no legs, plus no traces of cartilages, one can conclude that this stage is younger than the earliest stage with recorded ossification in contemporary *Xenopus* (see above). We roughly estimate the earliest *Shomronella* tadpoles to be in stages NF 47–50 because during this period of development in *Xenopus* the body shape in dorsal aspect remains generally the same. We conclude from this and other early larvae of *Shomronella* that dermal ossification began much earlier in development in this Early Cretaceous pipid than in contemporary *Xenopus*. Comparison of this earliest preserved larva with two others, which are more developed (i.e., complete parasphenoid and ossified anterior neural arches), revealed that in *Shomronella* the frontoparietal began to ossify later than the parasphenoid and probably simultaneously with the anterior part of the vertebral column.

Among the last ossification events in the sequence, which may be recognized on the fossil material are those associated

Table 1. Sequence of the main developmental events, which can be recognized in *Shomronella jordanica*.

Developmental event	Stages 47–50	Stages 51–54	Stage 55	Stage 56	Stage 60	Stage 61	Stage 63	Stages 63–64	Stage 66
Parasphenoid well ossified	x								
Early frontoparietals		x							
Frontoparietals reach level of anterior tip of parasphenoid			x						
Frontoparietals fuse posteriorly					x				
Frontoparietals fuse completely							x		
Angulosplenials appear					x				
Nasals appear					x				
Beginnings of otic capsules		x							
Posteromedial part of otic capsules well ossified				x					
Otic capsules complete							x		
Sphenethmoid of 2 ossifications							x		
Up to 8 pairs of neural arches		x							
Up to 9 pairs of neural arches				x					
Up to 10 pairs of neural arches					x				
Anterior neural arches fuse					x				
2 anterior vertebrae fuse					x				
Formation of anterior centra							x		
Hypochord present						x			
3 pairs of ribs			x						
Femur, tibia and fibula appear					x				
Ilia appear						x			
Ilia in contact with one another							x		
Ilia in ultimate size and position									x
Femur in contact with ilium							x		
Suprascapula present					x				
Supposed coalescence of tibia and fibula								x	

with the cranial and postcranial parts of the endoskeleton (e.g., completion of the ossification of the otic capsules or of the ilia). Most of these ossifications are completed well before the end of metamorphosis (stage NF 63; see Table 2). Only the development of the urostyle is markedly delayed (a separate hypochord may be recognized in stage NF 66, whereas in *Xenopus* the cartilaginous neural arches within the urostyle may be recognized at the end of metamorphosis; Roček 2003b: fig. 3I). Fusion of the anterior two presacrals may vary. Whereas in *Shomronella* they obviously fused well before the end of metamorphosis (stage NF 60), they may still be separate in metamorphosed juvenile *Thoraciliacus* (Trueb 1999). It should be noted that fusion of the anterior presacrals is not correlated with fusion of the sacral either with the posterior presacral or with the most anterior caudal. This is evidenced by *Shomronella* and *Thoraciliacus* in which anterior two (1st and 2nd) presacrals fuse with one another; in contrast, their sacrum does not fuse with neighboring vertebrae.

The ossification sequence is remarkably stable in the anurans (with exception of dermal ossification of the jaws in larval pipids and *Eleutherodactylus* Duméril and Bibron, 1841 in which the jaw apparatus is deviated from a common scheme) for both fossil and recent taxa. Among the earliest bones are always the parasphenoid, pars frontalis of the frontoparietal, exoccipital and prooticum, whereas the pelvis and distal elements of the extremities (endochondral skeletal parts in general) are among the last—*Pelobates* cf. *decheni* Troschel, 1861 (see Maus and Wuttke 2004), *Ascaphus truei* Stejneger, 1899, *Alytes obstetricans* (Laurenti, 1768), *Hymenochirus boettgeri* (Tornier, 1896), *Pipa myersi* Trueb, 1984 (see Yeh 2002; Roček 2003a; and references therein).

Timing of developmental appearance of diagnostic characters in various pipids.—The Cretaceous, Paleogene/Neogene, and contemporary pipids share a certain number of characters common to all of them, characters by which they can be distinguished from each other, and characters that dis-

Table 2. Comparison of main developmental events in Mesozoic and Paleogene/Neogene pipids with free-swimming larvae, Palaeobatrachidae, and contemporary *Xenopus* (stages based on external characters according to Nieuwkoop and Faber 1967). ? – character cannot be observed on available material; 0 – character absent in the taxon. Data on *Palaeobatrachus* from Roček (2003b), data on *Xenopus* are based on material deposited in Department of Zoology, Charles University, Prague.

Developmental event	<i>Shomronella</i>	<i>Thoraciliacus</i>	South African pipid	<i>Llankibatrachus</i>	<i>Palaeobatrachus</i>	<i>Xenopus</i>
Parasphenoid well ossified	47–50	< 59	?	?	58	59
Early frontoparietals	51–54	?	?	51	58	57–59
Frontoparietals reach level of anterior tip of parasphenoid	55	?	?	?	58	59
Frontoparietals fuse posteriorly	60	59	59–60	?	59	59
Frontoparietals fuse completely	63	60–61 <	63	59	60	60
Angulosplenials appear	60	?	63–64	?	64	60
Nasals appear	60	?	?	?	64	62
Clavicles present	?	60–61	?	?	60	
Early ossification of otic capsules	51–54	59	59	51	59	59–60
Posteromedial part of otic capsules well ossified	56	?	59–60	?	59–60	62
Otic capsules complete	63	60–61		59	60	63
Sphenethmoid of 2 ossifications	63	?		?	?	63
Up to 8 pairs of neural arches	51–54	?	59	?	58	58
Up to 9 pairs of neural arches	56	< 59	59–60	51	59	59
Up to 10 pairs of neural arches	60	60–61	60	62	60	60
Anterior neural arches fuse	60	59		?	58	60
2 anterior vertebrae fuse with one another	60	0		< 59	0	0
Sacral and praesacral vertebrae fuse	0	66 <		?	65	0
Formation of anterior centra	63	?		?	?	< 60
Hypochord present	61	?		?	60	66
3 pairs of ribs	55	< 59	< 59–60	?	< 58	62
Femur appears	60	60–61	60	57–58	59	60
Ilia appear	61	60–61	60	< 62	59	60
Ilia in contact with one another	63	?	63 <	62	60	63
Ilia in ultimate size and position	66	?		?	63	66
Femur in contact with ilium	63	63	63	?	63	62–63
Suprascapula present	60	?		?	59	63
Supposed coalescence of tibia and fibula	63–64	63		?	64	60–61

tinguish them from the palaeobatrachids. These characters appear at various developmental levels and may thus indicate their phylogenetic history.

One of the features that are common to pipids and palaeobatrachids is the relation between the parasphenoid and frontoparietal. In *Thoraciliacus* (Fig. 1C; see also Nevo 1968; Trueb 1999), *Shomronella* (Estes et al. 1978: fig. 2), South African pipid, *Palaeobatrachus* (Roček 2003b: fig. 3A, B), and *Xenopus* (Fig. 4M) it extends anteriorly beyond the level of the anterior margin of the frontoparietals. Anterior extent of the parasphenoid, together with the shape of its posterior part, which is clearly distinctive not only in adults (see Nevo 1968: fig. 10) but also in tadpoles (Figs. 1D₁, 3A₂), are characters arising in early development. To a certain ex-

tent, this holds true also for the ultimate shape of the frontoparietal.

Presence of free ribs is another common feature of all pipids and palaeobatrachids: 3–4 pairs on vertebra 2–4/5 in pipids, 5 pairs on the anterior 5 vertebrae in *Palaeobatrachus* (Roček 2003b: figs. 1O, 2J). They develop, like all mesodermal derivatives and thus in accordance with the somitic axial skeleton, in a rostrocaudal direction. In comparison, three pairs of ribs are present in *Shomronella* at stage NF 55, and in other Cretaceous and Paleogene/Neogene pipids as well as in *Palaeobatrachus* appear somewhat later: i.e., stages NF 59–60, stage NF 58. In contemporary *Xenopus* this is shifted to stage NF 62. In *Palaeobatrachus* a complete set of five pairs of ribs is recognizable in stages NF 59–60.

Difference in the number of ribs is difficult to explain in a phylogenetic sense. Larger numbers of ribs may be reasonably considered a primitive state. In this respect, *Palaeobatrachus* should be more primitive than all pipids. However, there are undoubtedly five pairs of ribs in one *Shomronella* tadpole (HUJZ 12038; see Estes et al. 1978: fig. 7A) and regularly four pairs of ribs in Early Cretaceous adult *Thoraciliacus* and *Cordicephalus* (Nevo 1968; Trueb 1999). These suggest a decrease in number of ribs during the early evolution of pipoids and, therefore, larger numbers of ribs should be expected in ancestral pipoids earlier than *Shomronella*. Because palaeobatrachids retain five pairs of ribs, they supposedly had to split from the common pipoid stem before *Shomronella* (this would also correspond to molecular data indicating earlier, probably Laurasian origin of the common stock; Feller and Hedges 1998). This is, however, not confirmed by the fossil record in which the earliest palaeobatrachids are recorded only from the Late Cretaceous (Campanian and Maastrichtian) of France and Spain, respectively (Buffetaut et al. 1996; Astibia et al. 1990). The gap between primitive hypothetical Early Cretaceous pipoids and the earliest known palaeobatrachids is about 45 Ma.

One thing should be emphasized in considering ribs and their significance for phylogenetic reconstructions. The only other Mesozoic anurans that possess free ribs are discoglossoids. They also have three pairs of free ribs on vertebrae 2–4, but the second pair each bear a distinct uncinat process. This characteristic feature was maintained even in the Paleogene/Neogene discoglossid *Latonia* (Roček 1994) and in contemporary *Discoglossus*. It was also present in the earliest anuran *Prosalirus* from the Jurassic (Pliensbachian) of Arizona (Jenkins and Shubin 1998) and in *Vieraella* and *Notobatrachus* from the Early Jurassic (Liassic and the Callovian–Oxfordian, respectively) of South America (Báez and Basso 1996). Presence of the uncinat process on the ribs associated with vertebra 3 is therefore a primitive feature that is maintained only in the discoglossoid lineage (present also in contemporary *Ascaphus* and *Leiopelma*) but not in the pipoid lineage – with the exception of *Shomronella* (Fig. 3E). This may be taken as an evidence of phylogenetic relations of early pipoids to the discoglossoid lineage, and in general, a primitive feature retained in the most primitive anurans.

Comparison of developments in Cretaceous pipids and in *Palaeobatrachus*.—Comparisons of the beginnings of dermal ossification in *Shomronella* (and, curiously, also in Paleogene *Llankibatrachus*) revealed that they distinctly preceded those in *Palaeobatrachus*. This can be shown by the onset of ossification of most cranial elements (Table 2). In contrast, the periods during which single cranial elements (both dermal and chondral) developed were considerably longer in *Shomronella* than in *Palaeobatrachus*. For instance, the frontoparietals of *Shomronella* appeared in stages NF 51–54 and completed their fusion in stage NF 63, whereas in *Palaeobatrachus* they appeared as late as stage NF 58, but were completely fused as early as stage NF 60. Similarly, the otic capsules of *Shomro-*

nella begin to ossify in stages NF 51–54 and are completely ossified in stage NF 63, whereas in *Palaeobatrachus* they begin to ossify in stage NF 59 and are complete in stage NF 60. In *Thoraciliacus*, the situation is obscured by the lack of relevant specimens. It may be of some interest that the otic capsules seem to begin ossification rather late (i.e., stage NF 59; Fig. 1A) and are complete as early as stages NF 60–61. It is difficult to decide whether this was caused by incomplete preservation (stage NF 59 in the earliest available stage for *Thoraciliacus*) or if it reflects the real situation. The latter seems to be supported by Stompoor pipid development.

Regarding the postcranial skeleton, *Shomronella* has eight pairs of neural arches by stages NF 51–54 and 10 pairs in stage NF 60, whereas *Palaeobatrachus* has eight pairs of neural arches much later, at stage NF 58, and 10 pairs at stage NF 60. This means that also in the axial skeleton the development of *Palaeobatrachus* was abbreviated.

Remarkably, the hind limbs develop later in *Shomronella* than in *Palaeobatrachus*, which is the reverse of the situation to that in the cranium. However, like the skull and axial skeleton, also the hind limbs have longer development in *Shomronella* than in *Palaeobatrachus*. In *Shomronella*, the femur appears in stage NF 60 and the ilia are in their ultimate size and position only in stage NF 66, whereas in *Palaeobatrachus* the femur appears in stage NF 59, but iliac development ends as early as stage NF 63.

Shape of the vertebral centra is considered an important feature distinguishing pipids and palaeobatrachids from each other (opisthocoelous in the former, procoelous in palaeobatrachids). Procoelous shape of the vertebrae in *Palaeobatrachus* is established as early as stage NF 59 (Roček 2003b: fig. 4H). In *Shomronella*, the vertebral centra begin to ossify above the notochord (epichordal pattern according to Estes et al. 1978) so that the centra are incomplete ventrally in stage NF 63, indicating the presence of the notochordal canal. In the horizontal section, the centra at this stage are amphicoelous (see also Estes et al. 1978: fig. 6C). Soon, however, the notochordal canal is filled with calcified matrix, first in the anterior vertebrae and then added more caudally, and intervertebral spaces are filled with ovoid elements made of the same material. These ovoid elements soon fuse with the calcified matrix filling the notochordal canal of the adjacent posterior vertebrae. During later development (stage NF 63), these calcifications ossified and coalesced with earlier vertebral rudiments, giving rise to the opisthocoelous centrum. This process is shown in fig. 6C of Estes et al. (1978). It is also obvious that the opisthocoelous type of the Early Cretaceous *Shomronella* appears later in development than the procoelous type in the late Oligocene *Palaeobatrachus*, but in light of other heterochronic shifts this is not so important. It is more important that the procoelous condition of early palaeobatrachids could hypothetically arise in a similar way, when the ovoid calcification filling the intervertebral space could join and fuse with the vertebral segment adjacent anteriorly.

Comparison of *Palaeobatrachus* with *Xenopus* revealed that, whereas the beginnings of the ossification are about at

the same stage (NF 58–59) and both the parasphenoid and frontoparietals appear at about the same time, the dermal bones that develop later (e.g., angulosplenials, nasals) appear much later in *Palaeobatrachus* than in *Xenopus*.

General features of evolution of amphibian ontogenies.—

There is some information available about the development of the primitive amphibians, namely of the Branchiosauridae (Temnospondyli) from the Lower Permian of Germany (Boy 1974; Schoch 1998), that can be compared with development of Cretaceous as well as contemporary pipids. Data on development of primitive recent urodeles [*Ranodon sibiricus* Kessler, 1866, *Salamandrella keyserlingi* Dybowski, 1870, *Ambystoma mexicanum* (Shaw, 1789)] may be used for such comparisons as well (Lebedkina 2004). The main constraints in these comparisons is that one can use only relative sequence of developmental events, mainly onset of ossification, and not of corresponding developmental stages. Rarely can fossil amphibians be definitely staged. This restricts comparisons to ossification sequence only.

Despite this constraint, the parasphenoid is always the earliest, or among the earliest, cranial ossifications in Paleozoic branchiosaurids (see Schoch 1998: fig. 8 for *Apateon pedestris* Meyer, 1844; Boy 1974: fig. 1 and table 1 for *Branchiosaurus* sp.). However, onset of ossification of the parasphenoid is accompanied by vomers, praemaxillaries and pterygoids. The frontals, which appear always before the parietals in the anurans (Čihák et al. 2003; Lebedkina 2004), appear much later in the branchiosaurids; i.e., only after the pterygoid, maxilla, and palatine, which appear one after another. In the branchiosaurids, the parietals and squamosals appear simultaneously with the frontals, and are then followed by the quadratojugals shortly before metamorphosis, and the nasals at the beginning of metamorphosis. The septomaxillary appears only at the end of metamorphosis. It should be noted that in *Branchiosaurus* the neural arches appear rather early, shortly after the parasphenoid (but much earlier than the frontals), whereas the ilia appear only in late metamorphosis (Boy 1974). One can suppose that this developmental scheme might correspond to that in ancestors of the Anura and of the Caudata.

In the Caudata, the sequence of ossification is slightly different. In the earliest stage, when the mouth forms in *Ranodon*, the dentary and coronoid appear as in *Branchiosaurus*, but they are followed by the parasphenoid, three pairs of frontals, parietals, vomers and palatines only in the subsequent stage (stage III of Lebedkina; but cf. Schoch 1998: fig. 8). The preoperculars, squamosals and angulars appear only with the transition to active feeding, and the exoccipitals appear still later. In *Salamandrella*, the earliest bones to appear are again the dentary, coronoid, vomer and palatine, whereas the parasphenoid appears much later (with active feeding), followed still later by two posterior pairs of frontals and by the parietal. Only when these bones appear does the exoccipital begin to ossify. In *Ambystoma*, the onset of ossification sequence is the same as in *Salamandrella*, soon followed

by the parasphenoid and the anterior pair of frontals (besides other bones), with the two posterior pairs of frontals appearing later, and parietals still later. It is obvious that the beginnings of the ossification sequence (e.g., vomer, dentary, coronoid) is basically the same as in the Branchiosauridae, and only few elements (e.g., maxilla) are shifted to later stages.

In the anurans (including Early Cretaceous pipids) the skeleton is simplified and the whole ossification sequence is shifted to metamorphosis. The definition of metamorphosis in the temnospondyls and caudates, principally based on disappearance of gill-rakers that reflects a transition from gill breathing to lung breathing, differs from that in the anurans, because of external criteria available. In the anurans, the larval branchial skeleton is converted into a filter-feeding device, nevertheless, the beginning of metamorphosis in all groups can be defined with appearance of limbs in a larva (tadpole). The criterion of its end differs—in the temnospondyls and caudates it is a complete disappearance of gill rakers and in the anurans it is the complete disappearance of the tail. However, it is well known that in the anurans the transformation of the branchial skeleton into the hyoid, as well as its ossification (both dermal and endochondral) continues postmetamorphically (Roček 1981, 2003a). Despite these differently defining events associated with metamorphosis, it is obvious that the skeletal development in frogs is shifted to metamorphosis. This would be even more apparent if appearance of the limbs would be taken as the common criterion of the beginning of metamorphosis in all groups.

It may be inferred from comparing temnospondyls with anurans (see Fig. 5) that in the former group there was a markedly longer ossification period extending from initial mouth formation until the adult. In the anurans, in contrast, the ossification period was shorter and occurring in later developmental stages. Seemingly, this could have been caused by abbreviation of the ossification period and its shift to later developmental stages. In fact, this heterochrony can also be caused by a shift of metamorphosis to earlier developmental stages, which is evidenced, among other features, by the presence of juvenile teeth of temnospondyls in the adult anurans (Smirnov and Vasil'eva 1995), and which would better correspond to the idea of paedomorphosis (defined as acceleration of development accompanied with abbreviated somatogenesis). In pipids, whose developmental sequence is recorded from the Early Cretaceous (i.e., more than 120 Ma), the situation is similar—onset of ossification is seemingly postponed to later developmental stages (if *Xenopus* is compared with *Shomronella*) while its concluding phases occur in the corresponding stages. This means that the whole ossification process is abbreviated into a shorter period. This could, however, also mean that metamorphosis was shifted to earlier developmental stages in contemporary *Xenopus* (see also Wassersug and Hoff 1982).

Delayed dermal ossification of the jaws in the Anura (Yeh 2002), compared with the Paleozoic Temnospondyli

and recent Caudata (Fig. 5), is no doubt caused by adaptation to herbivory during their larval period. The herbivory of the anuran larvae is anatomically associated with the supra-rostral and infra-rostral cartilages (i.e., larval jaws) that are supported by enlarged horizontal palatoquadrates and by Meckel's cartilage (see Roček 2003a for review). Only during metamorphosis do these larval jaws become incorporated into the nasal region (supra-rostrals) and the adult lower jaw (infra-rostral, which ossifies into the mentomandibular), and the principal elements of the jaw apparatus in the adult anurans are the palatoquadrate and Meckel's cartilage, respectively, accompanied with their adjacent dermal ossifications. In pipids the larval jaw apparatus is not developed (due to absence of larval stages adapted to herbivory that occur in non-pipids) and this is the reason why their dermal ossification of the jaws seems to occur earlier than in non-pipids, and similar to the caudates.

It would be therefore interesting to know more about the larval development and metamorphosis in the Mesozoic non-pipid anurans (e.g., early discoglossoids such as *Eodiscoglossus*), namely whether their larval cranial structure deviated from the general scheme of larval temnospondyls as well as of pipids because of adaptation to herbivory (these deviations principally include anterior rotation of the palatoquadrate and presence of labial cartilages that precede jaws of adults; see Roček 2003a for a review). There is a representative developmental series of *Eopelobates* from the Upper Oligocene of the Czech Republic (Špíner 1972), and about 70 fossil tadpoles of *Pelobates* cf. *decheni* Traschel, 1861 from the Upper Oligocene of Germany (Maus and Wuttke 2004), which provide fairly good knowledge on the ossification sequence. These specimens come from the Paleogene, so that they are basically identical with contemporary pelobatids. However, they do not involve larval cartilaginous endocranial structures that are of key importance in this respect. Oligocene pelobatids are thus too advanced to provide insight into the development of the early non-pipoid anurans.

Summary.—The earliest ossified element in the Early Cretaceous *Shomronella* is the parasphenoid which may be well ossified as early as in stages NF 47–50. In contrast, in the contemporary *Xenopus* and in the Late Oligocene *Palaeobatrachus*, the first ossification can be recognized only in stages NF 59 and 58, respectively. This means that the onset of ossification was shifted into later developmental stages in the course of pipid evolution.

- The differences between the Early Cretaceous taxa and contemporary *Xenopus* are more pronounced in those ossification events that occur comparatively early (e.g., appearance of the parasphenoid), whereas in those which occur in later stages (e.g., angulosplenials and nasals) the difference is less pronounced.
- Among the last ossification events, the coalescence of the tibia and fibula, occurs in stages NF 63–64 in *Shomronella* and in 63 in *Thoraciliacus*, but occurs in stages NF 60–61 in *Xenopus*. Ossification thus extended over a longer period in

the development of early pipids than in the Paleogene palaeobatrachids and contemporary *Xenopus*.

- As a consequence of faster ossification of the skeleton, the ossification of the elements, which was sequential (e.g., parasphenoid before the frontoparietals, frontoparietals before the exoccipitals) became less pronounced and some elements ossify at the same stage.
- The developmental sequence for the ossification of the parasphenoid, frontoparietals, and exoccipitals, which occurs in that order, in the Early Cretaceous *Shomronella*, tend to be reversed in some *Xenopus* individuals (i.e., frontoparietals before parasphenoid).
- These heterochronic shifts in developmental timing concern mainly the skull and anterior vertebrae. The posterior vertebrae, hypochord, and appendages do not exhibit these heterochronic changes.
- This shift in ossification sequence is not obvious if Cretaceous and Paleogene/Neogene pipids are compared to each other.
- The ribs associated with the vertebra 3 in *Shomronella* bear a distinct uncinat process, similar to this in the Jurassic anurans *Prosalirus*, *Vieraella* and *Notobatrachus*, as well as contemporary discoglossoids *Ascaphus* and *Leiopelma*. Their presence in *Shomronella* might indicate phylogenetic relations of early pipoids.
- Development in *Shomronella* elucidates the mechanism for the origin of the opisthocoelous vertebral centra. The same mechanism, if directionally reversed, might explain the origin of the procoelous centra in *Palaeobatrachus*.
- Comparisons of the development in temnospondyl amphibians with that of pipoid anurans indicates that in the anurans the whole ossification sequence is shifted from pre-metamorphic stages to metamorphosis. This also could be interpreted in the context of the hypothesis of the paedomorphic origin of the anurans; i.e., seemingly the metamorphosis of frogs, including their entire ossification sequence, was shifted to the earlier developmental stages, due to overall abbreviation of their somatogenesis.

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