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Skeletal Development in *Xenopus laevis* (Anura: Pipidae)

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ABSTRACT Postembryonic skeletal development of the pipid frog *Xenopus laevis* is described from cleared-and-stained whole-mount specimens and sectioned material representing Nieuwkoop and Faber developmental Stages 46–65, plus postmetamorphic individuals up to 6 months old. An assessment of variation of skeletogenesis within a single population of larvae and comparison with earlier studies revealed that the timing, but not the sequence, of skeletal development in *X. laevis* is more variable than previously reported and poorly correlated with the development of external morphology. Examination of chondrocranial development indicates that the rostral cartilages of *X. laevis* are homologous with the suprarostral cartilages of non-pipoid anurans, and suggests that the peculiar chondrocranium of this taxon is derived from a more generalized pattern typical of non-pipoid frogs. Derived features of skeletal development not previously reported for *X. laevis* include 1) bipartite formation of the palatoquadrate; 2) precocious formation of the adult mandible; 3) origin of the angulosplenial from two centers of ossification; 4) complete erosion of the orbital cartilage during the later stages of metamorphosis; 5) development of the sphenethmoid as a membrane, rather than an endochondral bone; and 6) a pattern of timing of ossification that more closely coincides with that of the pelobatid frog *Spea* than that recorded for neobatrachian species.

The literature describing skeletal development in the pipid frog *Xenopus laevis* is extensive and, in recent years, the species has become a "model system" for experimental studies of skull development (e.g., on laryngeal development: Sassoon and Kelley, '86; Sassoon et al., '86; Kelley et al., '89; on Meckel's cartilage: Thomson, '86, '87, '89; on tooth development: Shaw, '79, '85, '86, '88). The first accounts of the skull were provided more than a century ago by Parker (1876, 1881; = *Dactylethra capensis, auctorum*). The development of the hyobranchial skeleton in *X. laevis* was described by Ridewood (1897) and that of the larval chondrocranium by Kotthaus ('33). Paterson ('39) reviewed the development of the head of larval and adult *X. laevis* and corrected several errors of Kotthaus ('33), which unfortunately had been perpetuated by de Beer ('37) in his book on the development of the vertebrate skull.

The first comprehensive description of cranial and postcranial ossification in *Xenopus laevis* was by Bernasconi ('51), who based his observations on laboratory-reared frogs. Ossification sequence was tabularized for 32 cleared-and-single–stained specimens ranging in age from 20–365 days and arranged in four stages: premetamorphic Stages I (20–29 d) and II (31–39 d), metamorphic Stage III (40–49 d), and postmetamorphic Stage IV (56–365 d). Bernasconi ('51) did not define his stages with morphological criteria; thus they are not easily compared to those of Nieuwkoop and Faber ('56). Moreover, no data are provided on the development of the cartilaginous precursors of the bony skeleton, and Bernasconi's ('51) illustrations reveal several errors and misinterpretations of the anatomy of the developing and adult skull.

A detailed account of the ontogenesis of the vertebral column of *Xenopus laevis* was provided by Smit ('53), who examined sectioned specimens representing Stages 24–66 of Nieuwkoop and Faber ('56) of individuals collected from natural populations in South Africa and staged by J. Faber. As part of their study of the visceral arches, larynx, and visceral muscles of *Xenopus laevis,* Sedra and Michael ('57) described changes in the skull during metamorphosis. Their specimens also were collected from wild populations in South Africa and comprise a total of 12 Nieuwkoop and Faber ('56) stages: viz., Stage 55 (±32 d; "typical larval stage"), Stages 56–57 (±38–41 d; "premetamorphic" stages before the exposure of the forelimbs), Stages 58–65 (±44–54 d; "metamorphosing" stages), and Stage 66 (±58 d; "newly metamorphosed"). The authors sectioned the heads of an unspecified number of specimens and based their descriptions and illustrations on hand-generated graphical reconstructions from these sections. Given the tedious nature of this method and the absence of any statements about variation, it seems likely that Sedra and Michael's ('57) descriptions may be based on only a single specimen of each stage.

Cranial osteological data from Sedra and Michael's study were included among the criteria used to define the developmental stages in the widely used normal table of development of *Xenopus laevis* (Nieuwkoop and Faber, '56). This work also contains general, but abbreviated, narrative accounts of the development of various parts of the skeleton. These were written by a variety of contributors and are based on examination of sectioned material obtained from natural populations. The editors (Nieuwkoop and Faber, '56:7) carefully pointed out that intrastage variation was not studied, but that "It has . . . become evident . . . that a general variation of approximately half a stage to either side has to be taken into account."

Variation in osteological development in *Xenopus laevis* was addressed briefly by Brown ('80), who examined small samples of wild-caught and laboratory-reared animals between Stages 49 and 60. Brown noted discrepancies in the timing and sequence of ossification of certain elements when he compared his results with those of Bernasconi ('51). Brown ('80) reported that ossification is delayed in laboratory-reared *X. laevis* as compared with wild-caught specimens and noted specific differences between his observed timing and sequence of osteological development in wild-caught *X. laevis* and those recorded by Nieuwkoop and Faber ('56). Brown ('80:28–29) correctly observed that there is "considerably more variation in time of ossification than the one half stage stated in the normal table . . . [and that this] . . . lessens the utility of the normal tables for staging wild populations."

Given these extensive and diverse research efforts, one would expect the osteology and development of *Xenopus laevis* to be well known. However, comparison of figures and descriptions in Kotthaus ('33), Paterson ('39), Weisz ('45a,b), Bernasconi ('51), Nieuwkoop and Faber ('56), Sedra and Michael ('57), Deuchar ('75), and Reumer ('85) reveals significant discrepancies in the terminology of both the larval chondrocranium and the adult skeleton. Moreover, none of these authors described the striking differences in the developing and adult cranium of *X. laevis* as compared to non-pipid[1] anurans.

Sokol ('75) pointed out that the ethmoidal region of the skull of larval pipids is distinct from that of all other anurans; he suggested that on the basis of this and many other characters (e.g., opercular structure, hyobranchial and filter apparatus, prootic foramina and their nerves, palatoquadrate suspensorium), pipoid (i.e., Rhinophrynidae + Pipidae) larvae were derived with respect to all other anurans exclusive of the Microhylidae. The peculiarities of the ethmoidal region with respect to the formation of the nasal capsule in pipids were emphasized by Roček ('89) and development of the ethmoidal area in *Pipa pipa* was described by Roček and Veselý ('89). Roček ('90) likened the ethmoidal endocranium of pipids to that of "labyrinthodont-grade" tetrapods, claiming that the two larval ethmoidal configurations in anurans (i.e., the pipoid and non-pipoid types) could not have been derived from one another (contra Sokol, '75).

An understanding of the structure of the mature skeleton (especially the cranium) requires knowledge of its development. Further, one can expect interspecific osteological variation among adults to be correlated with variation in developmental patterns. In the case of pipid frogs, the studies of *Xenopus laevis* clearly have historical precedence in the literature as a baseline against which

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[1] Throughout this paper, reference will be made to pipid and pipoid frogs versus non-pipid and non-pipoid frogs. There are five Recent genera in the Family Pipidae—viz., *Pipa* in the New World, *Hymenochirus, Pseudhymenochirus, Xenopus,* and *Silurana* in the Old World (Cannatella and Trueb, '88a,b). The superfamily Pipoidea is composed of the Pipidae plus its sister taxon Rhinophrynidae (containing only one Recent taxon, the New World *Rhinophrynus dorsalis*). Currently, pipoid frogs are considered to be a highly derived group of primitive frogs that are thought to be most closely related to pelobatoid anurans (e.g., Pelobatidae and Pelodytidae) (Cannatella, '85).

other pipid data will be compared. However, several problems must be resolved before such comparisons can be made. Disparities among published anatomical descriptions must be resolved and intraspecific variation in the timing and sequence of osteogenic events should be evaluated.

Herein, skeletal development of *Xenopus laevis* from Stage 46 (premetamorphic, feeding larva) through Stage 66 (metamorphosed froglet) and for 6 additional months is described and compared with results of previous studies on *X. laevis* and other anuran taxa. The account is organized into four major sections—chondrocranial development, metamorphic development of the cranium, postmetamorphic development of the cranium, and postcranial development. Within chondrocranial development, a description of the chondrocranium at Stage 53 immediately prior to the appearance of ossification precedes an account of its development. Metamorphic development of the cranium is organized regionally (e.g., dermal investing bones, nasal capsule), whereas remarks on postmetamorphic development are brief chronological summaries of the major changes that occur. Postcranial development is described regionally—the axial column, anterior appendicular skeleton, and posterior appendicular skeleton. Major developmental patterns observed are summarized following the detailed regional and temporal accounts. Chronologies of developmental events and their variation are presented in Tables 2–6 and Appendices A–D, respectively.

The Discussion includes comments on the events of skeletogenesis in *Xenopus laevis* that are unusual relative to what is known about the process in other anurans, and consideration of the homologies of some cranial elements of *X. laevis.* There are reasonably extensive comparisons between the results of this study and those of previous studies on *X. laevis,* along with comparisons of the skeletal development between *X. laevis* and other anurans. The paper concludes with a brief commentary on the reliability of ossification events as staging criteria.

TABLE 1. Comparison of three common normal tables of development for anurans[1]

Taylor and Kollros ('46)	Nieuwkoop and Faber ('56)	Gosner ('60)
I	46	26
II	47	27
—	48	—
III	49	28
—	50	—
IV	51	29
V	52	30
VI	53	31
VII	—	32
VIII	—	33
IX	54	34
X	55	35
XI	—	36
XII	—	37
XIII	56	38
XIV	57	39
XV	—	40
XVI	58	—
XVII	59	—
XVIII	60	41
XIX	—	—
XX	61	—
XXI	62	42
XXII	63	43
XXIII	64	44
XXIV	65	45
XXV	66	46

[1] An "—" indicates absence of equivalent stage. Adapted from Just et al. ('81)

MATERIALS AND METHODS

Adult *Xenopus laevis* were obtained from Carolina Biological Supply and maintained in the laboratory of James Hanken at the University of Colorado at Boulder. Breeding was induced by hormone injection and the eggs obtained were reared at 18°C. Specimens were preserved in 10% neutral-buffered formalin. Larvae were staged according to the normal table of Nieuwkoop and Faber ('56); to facilitate comparison of these data with those of other taxa, developmental stages of the three most commonly used normal tables of development (Taylor and Kollros, '46; Nieuwkoop and Faber, '56; Gosner, '60) are compared in Table 1. Larval snout-vent and tail lengths were measured; total length of larvae, as cited herein, is the sum of the snout-vent and tail lengths, but total length of postmetamorphic individuals is snout-vent length. Specimens were stained differentially for bone and cartilage as whole mounts, following the techniques of Dingerkus and Uhler ('77) and Wassersug ('76), as modified by Hanken and Wassersug ('81).

One hundred and fourteen individuals representing Stages 48–66 + 6 months of *Xenopus laevis* were measured; 102 specimens representing Stages 46–66 + 6 months were examined for osteological development and are deposited in the herpetological collection of the Museum of Natural History at The University of Kansas (KU 217886–987). For Stages 46–54 and 66 + 4 months to 66 + 6 months, only two specimens per stage were available for osteological examination; each of the other stages is represented by at least five individuals.

Serial cross sections of the heads of three individuals were prepared (KU 219922, Stage 63; KU 219923, Stage 64; KU 219921, Stage 65). Specimens were fixed and preserved in 10% formalin. The heads were removed and decalcified in a 5% formic acid–formalin solution for 3 d, after which they were dehydrated in an ascending ethyl alcohol series, cleared with Histoclear, and embedded in Paraplast Plus. Specimens were transversely sectioned at 5 and 10 μ and stained following the modified Heidenhain's Azan technique of Baldauf ('58).

Drawings were prepared with the aid of a dissecting microscope equipped with a camera lucida.

RESULTS

Chondrocranial development

The most complete descriptions of the chondrocranium of *Xenopus laevis* are those of Kotthaus ('33), Paterson ('39), and Sedra and Michael ('57). Kotthaus ('33) described chondrocranial development from newly hatched larvae (ca. Stage 40?) up to approximately Stage 53. Paterson's ('39) description of chondrocranial structure was secondary to the main point of her paper—viz., an account of cranial nerves. Sedra and Michael's ('57) account is the most complete and accurate, but the earliest stage that they describe is Stage 55 when cranial metamorphic changes are well underway. The descriptions in all of these studies are based on examination of sectioned material and illustrations are hand-drawn graphical reconstructions from sections, rather than illustrations of whole-mount specimens. The material examined herein has revealed some subtle anatomical differences in chondrocranial structure between our specimens and those described in earlier accounts.

Larval chondrocranium at Stage 53 (Fig. 1)

The last larval stage prior to appearance of cranial ossification in whole-mount specimens is Stage 53; hence, this stage is selected to represent the "mature" larval chondrocranium prior to the onset of metamorphic changes in the skull. The chondrocranium is longer than wide, depressed, and wedge-shaped in lateral aspect with the auditory region being the highest part of the skull.

One of the most distinctive and controversial (see Discussion) parts of the chondrocranium of pipids is the so-called "ethmoid" region that lies anterior to the braincase between the palatoquadrate cartilages and extends forward above the lower jaw as the suprarostral plate. The anterior margin of this plate is slightly convex and supports the upper lip of the larvae. In dorsal aspect, the rostral portion is broad, thin, and flat; its lateral portions form distinct wings that are distally attenuate and united with an anterior process of the palatoquadrate to form the long, distinctive tentacular cartilage of pipid larvae (Fig. 1). Posterior to the rostrum and between the palatoquadrate cartilages, the cartilage is thicker and bears a pair of longitudinal channels that house the olfactory nerves. The channels are confluent at their origin from the anterior end of the brain, but they diverge anterolaterally from one another and are separated medially by a thickened area of cartilage termed the vertical septum. At this stage, the olfactory channels are unroofed anteriorly; a narrow strip of cartilage, the tectum anterius, forms the anterior margin of the frontoparietal fontanelle and roofs the most posterior part of the common olfactory channel. The cartilage is broadly united to the palatoquadrate cartilage on each side by a robust commissura quadratocranialis anterior (Fig. 1).

The broad, arcuate mandible projects anterior to the dorsal portion of the skull and is composed of three elements that are synchondrotically united: paired Meckel's cartilages separated by a single infrarostral (fused infrarostrals *fide* Sokol, '75; inferior labial cartilage of Kotthaus, '33, Paterson, '39, Sedra and Michael, '57). The posterior end of Meckel's cartilage is united with the anterior end of the palatoquadrate via the larval pars articularis.

The palatoquadrate is short and consists of two distinct parts; the anterior portion lies lateral to the "ethmoid" portion of the skull, whereas the posterior portion is associated with the trabecula anterior to the otic capsules. The anterior palatoquadrate articulates with the posterior end of Meckel's cartilage via the larval pars articularis. It bears an anteromedial process, the processus cornu quadratus medialis, and an anterolateral process, the processus cornu quadratus lateralis (quadratoethmoidal cartilage of Paterson, '39); the latter unites with the lateral end of the rostral cartilage to form the tentacular cartilage (Fig. 1). The commissura quadrato-

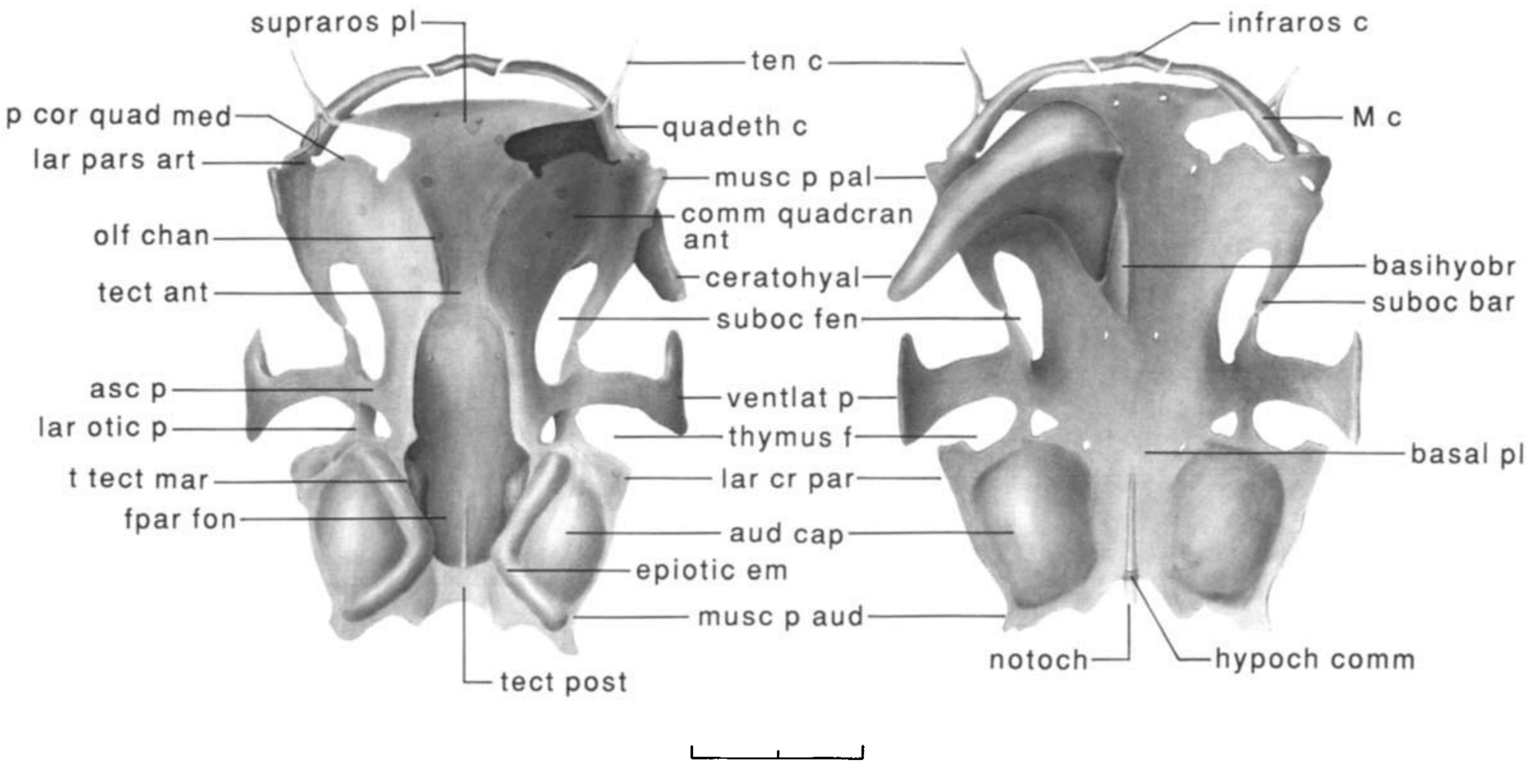

Fig. 1. *Xenopus laevis*. Dorsal (**left**) and ventral (**right**) views of the chondrocranium of a Stage-53 larva. Branchial baskets have been removed in both views and only the right half of the ceratohyal is shown in the ventral view. asc p, ascending process of the palatoquadrate; aud cap, auditory capsule; basal pl, basal plate; basihyobr, basihyobranchiale; comm quadcran ant, commissura quadratocranialis anterior; epiotic em, epiotic eminence of auditory capsule; fpar fon, frontoparietal fontanelle; hypoch comm, hypochordal commissure; infraros c, infrarostral cartilage; lar cr par, larval crista parotica; lar otic p, larval otic process; lar pars art, larval pars articularis of the palatoquadrate; M c, Meckel's cartilage; musc p aud, muscular process of auditory capsule; musc p pal, muscular process of palatoquadrate; notoch, notochord; olf chan, olfactory channel; p cor quad med, processus cornu quadratus medialis; quadeth c, quadratoethmoidal c; suboc bar, subocular bars of the palatoquadrate; suboc fen, subocular fenestra; supraros pl, suprarostral plate; t tect mar, taenia tecti marginalis; tect ant, tectum anterius; tect post, tectum posterius; ten c, tentacular cartilage; thymus f, thymus foramen; venlat p, ventrolateral process of the palatoquadrate. Scale = 2 mm.

cranialis anterior forms a broad bridge between the anterior palatoquadrate and the medial part of the chondrocranium; posterior to the commissura, the palatoquadrate forms a flat subocular bar that encloses the anterior half of the subocular fenestra. The posterior part of the palatoquadrate consists of a robust ventrolateral process that is expanded distally (basal process of Kotthaus, '33); proximally, the ventrolateral process is united to the braincase via the ascending process and to the otic capsule via the larval otic process. Previous workers (Paterson, '39; Sedra and Michael, '57) restricted the term ventrolateral process to the expanded ventral portion of the process, and referred to the straplike, proximal cartilage as the ascending process. It is clear from the specimens examined that the ascending process terminates at the level of the otic process and subocular bar; thus, the ventrolateral process is the band of cartilage with an expanded base that lies distolateral to the ascending process. Anterior to the ascending process, there is a flat anterior projection that represents the posterior half of the subocular bar. The two parts of the subocular bar have a tenuous synchondrotic connection; thus, there is not a single, robust subocular bar as depicted in the illustrations of Kotthaus ('33), Paterson ('39), and Sedra and Michael ('57).

The braincase is broad and shallow, especially anteriorly. The floor is formed by the basal plate, which at the level of the subocular fenestra is pierced by the pair of craniopalatine foramina anteriorly, and a pair of carotid foramina posteriorly. Posteromedially, between the otic capsules, the notochord persists in the floor of the braincase; the posterior border of the braincase is formed by a narrow cartilage bridge between the halves of the chondrocranium, the hypochordal commissure (Fig. 1). The lateral wall of the braincase is pierced by four foramina. The optic foramen separates the pila preoptica from the pila metoptica posteriorly, which, in turn, is delimited posteriorly by the oculomotor foramen. Slightly posterior and dorsal to the oculomotor foramen is the small trochlear foramen. Both foramina exit the wall of the braincase at the posterior level of the subocular fenestra just anterior to the ascending process. The pila antotica forms the lateral wall of the braincase between the oculomotor foramen and the large prootic foramen posterior to the ascending process. The wall separating the braincase and auditory capsule bears three foramina—two acoustic foramina ventrally and an endolymphatic foramen dorsal to and between the acoustic foramina. The jugular foramina exit the skull posteriorly at the level of the occipital arch. The pilae preoptica, metoptica, and antotica are united dorsally by the orbital cartilage, which forms the lateral margin of the frontoparietal fontanelle anterior to the otic capsule. The posterolateral margin of the fontanelle is formed by a narrow strip of cartilage medial to the epiotic eminence—the taenia tecti marginalis; the posterior margin of the fontanelle is composed of a delicate, transverse strip of cartilage between the otic capsules—the tectum posterius (tectum synoticum of Kotthaus, '33).

The auditory capsules are well developed with prominent epiotic eminences (Fig. 1). Anteromedially, a straplike bridge of cartilage—the larval otic process—joins the otic capsule to the ventrolateral process of the palatoquadrate ventral and posterior to the ascending process of the palatoquadrate. The larval crista parotica (larval otic process of Sedra and Michael, '57) is a platelike expansion of cartilage at the anterolateral corner of the otic capsule. A small flange of cartilage borders the lateral and posterolateral margin of the capsule and terminates in a slightly expanded process posterolaterally; together, these constitute the muscular process of the otic capsule. The immense fenestra ovalis lies in the ventrolateral wall of the otic capsule.

Development of the chondrocranium, Stages 46–52

Stage 46. In this, the earliest stage available for examination, the parachordals flank the notochord and unite anterior to it to form a narrow basal plate. The parachordals diverge anterolaterally from this point to form the posterolateral margins of the broad basicranial fenestra (Fig. 2). (Parachordal terminology follows Paterson ['39].) The lateral margins are formed by the cranial trabeculae, which extend longitudinally forward from the parachordals to the large, flat ethmoidal or trabecular plate. Lateral to the union of the anterior parachordal and the trabecula, there is a diffuse condensation of cartilage that represents the proximal part of the ventrolateral process of the posterior palatoquadrate. The anterior, posterior, and posteromedial parts of the auditory capsule are present lateral to the posterior parachordals (labelled parachordal in Fig. 2).

The anterior palatoquadrate is well developed and united with the ethmoid plate via a broad commissura quadratocranialis anterior. Posteriorly, the anterior palatoquadrate bears a subocular bar; anteriorly, the muscular process, processus cornu quadratus medialis, and larval articular process are evident. The processus cornu quadratus lateralis is represented only by a diffuse condensation of cartilage anterior to the palatoquadrate.

The suprarostral plate is well developed, but lacks a cartilaginous connection with the rudimentary tentacular cartilage. The lower jaw is represented by paired Meckel's cartilages separated by the single infrarostral.

Stage 47. The floor of the braincase is expanded (and the size of the basicranial fenestra decreased) by proliferation of cartilage between the anterior parachordals. The trabeculae are thickened to form a trough in which the anterior brain is lodged. The ventrolateral process of the posterior palatoquadrate is united with the medial edge of the trabecula. A proliferation of cartilage along the dorsum of the trabecula represents the incipient lateral walls of the braincase. Anteriorly, the processus cornu quadratus lateralis extends forward from the palatoquadrate to the tentacular cartilage; a connection between the tentacular cartilage and the ala of the suprarostral cartilage is absent. There is chondrification around the entire auditory capsule.

Stage 48. The basicranial fenestra is small. The ventrolateral process of the posterior palatoquadrate is lengthened, but not expanded distally; proximally, the posterior palatoquadrate bears a suborbital bar that nearly meets the suborbital bar of the anterior palatoquadrate. The ascending process of the palatoquadrate appears as a lateral cartilaginous projection of the wall of the braincase dorsal to the ventrolateral process. The walls of the braincase are thicker and longer, curving anteromedially around the front end of the brain. Anteriorly, the tentacular cartilage is united with both the cornu quadratus medialis and the lateral flange of the suprarostral cartilage. The epiotic eminences are scarcely visible in the otic capsule. The hypochordal commissure constitutes a band of cartilage ventral to the notochord

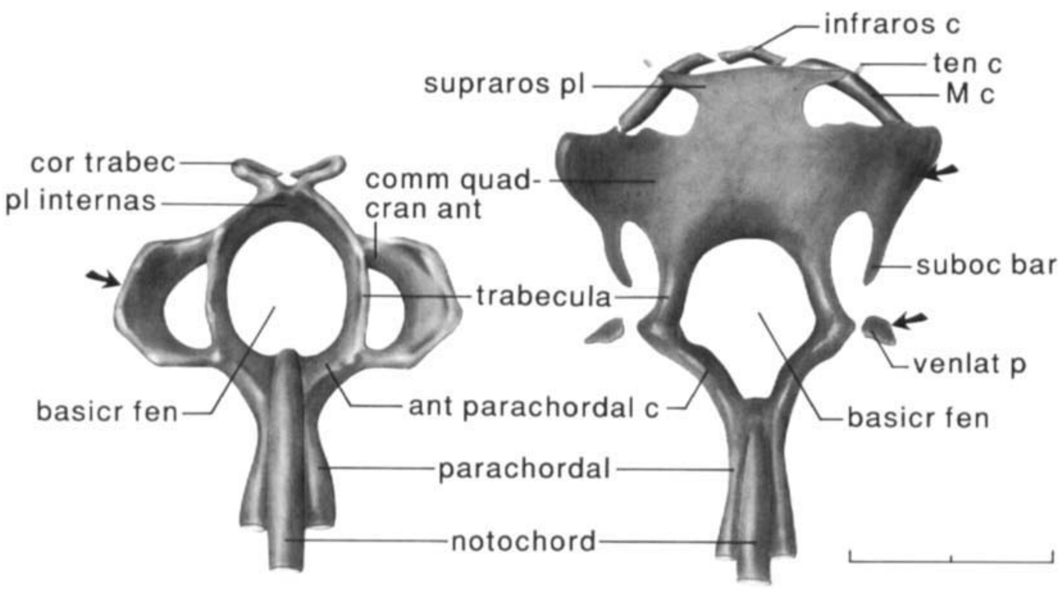

Fig. 2. *Rana temporaria* (**left;** 7.5-mm larva) and *Xenopus laevis* (**right**; Stage 46), dorsal views of chondrocrania of young larvae to illustrate differences between non-pipoid and pipoid taxa. Arrows indicate palatoquadrate cartilages. Note in particular, differences in palatoquadrate and rostral cartilages. Illustration of *Rana* adapted from Gaupp ('06); lower jaw not shown. ant parachordal c, anterior parachordal cartilage; basicr fen, basicranial fenestra; c, cartilage; comm quadcran ant, commissura quadratocranialis anterior; cor trabec, cornu trabecula; infraros c, fused infrarostral cartilages; M c, Meckel's cartilage; pl internas, planum internasale; suboc bar, subocular bar of palatoquadrate; supraros pl, suprarostral plate; ten c, tentacular cartilage; venlat p, ventrolateral process of palatoquadrate. Scale = 1 mm (*Xenopus*).

TABLE 2. Comparison of schedules of cranial ossification and mineralization in Xenopus laevis[1]

Stage	This study	Bernasconi ('51)	Sedra and Michael ('57)	Brown ('80)
54	1. Frontoparietal	1. Frontoparietal	—	—
55	2. Parasphenoid **Exoccipital** (55–56) **Prootic** (55–57)	2. Parasphenoid 3. **Exoccipital** Angulosplenial (medial) 4. Maxilla Teeth Premaxilla	1. Frontoparietal Parasphenoid	1. Frontoparietal Parasphenoid **Exoccipital** **Prootic** Angulosplenial (medial)
56	3. Angulosplenial (medial)	5. **Prootic**	—	—
57	4. Maxilla	—	—	2. Maxilla Premaxilla Teeth
58	5. Premaxilla (58–59) Nasal (58–60)	6. Nasal	2. **Prootic** **Exoccipital** Maxilla Nasal	—
59	—	7. Dentary	3. **Pars interna plectri** **Operculum**	3. Nasal
60	6. Septomaxilla Teeth Dentary (60–62) Angulosplenial (lateral) (60–61)	8. Angulosplenial (lateral) Septomaxilla Squamosal Pterygoid	4. Septomaxilla Premaxilla Teeth Angulosplenial Dentary	—
61	7. Pterygoid (61–62) **Tympanic annulus** (61–62) **Pars externa plectri** (61–62)	9. **Columella**	5. **Pars media plectri** **Pars externa plectri** **Tympanic annulus**	—
62	8. **Pars media plectri** (61–63) Squamosal (62–63)	10. Vomer	—	—
63	9. Vomer (63–64)	—	6. Pterygoid	—
64	10. Sphenethmoid (64–66)	—	7. Squamosal	—
65	11. **Pars articularis**	—	—	—
66	—	—	8. Spenethmoid	—
66 + 1 month	12. **Operculum**	11. Sphenethmoid **Pars articularis**	—	—
66 + 4 months	—	12. **Alary cartilage**	—	—
66 + 10 months	—	13. **Planum antorbitale**	—	—

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[1] Endochondral elements are in boldface. Developmental stages are those of Nieuwkoop and Faber ('56) and as applied to Bernasconi's ('51) data are only estimations. Groups of elements associated with a single number appeared simultaneously in the specimens examined. Ranges of stages in which elements were observed to appear in this study are indicated in parentheses.

and unites the otic capsules. The occiput of the chondrocranium has begun to form and the jugular foramina are evident.

Stage 49. The basicranial fenestra is closed, and the paired craniopalatal and carotid foramina are visible in the basal plate. Differential thickening of the intertrabecular plate begins to form the channels for the olfactory nerves in the ethmoid area. The anterior and posterior palatoquadrates are united by fused subocular bars, which enclose the subocular fenestra. The ascending process of the palatoquadrate has fused with the ventrolateral process, and the larval otic process appears as a posterior projection of cartilage from the ventrolateral process near its union with the ascending process. Elaboration of the auditory capsule includes the appearance of the larval crista parotica anterolaterally, along with the lateral muscular process.

Stage 50. The primary change involves elaboration of the anterodistal end of the ventrolateral process of the palatoquadrate to produce the expanded base.

Stages 51–52. The tectum anterius, a narrow transverse band of cartilage roofing the olfactory channel just anterior to the brain, forms the anterior margin of the frontoparietal fontanelle. The fontanelle is open posteriorly, although a small spur of cartilage associated with the posteromedial margin of each otic capsule signals the beginning of the tectum posterius. The larval otic process, which unites the otic capsule with the ascending process of the palatoquadrate, is complete. The base of the ventrolateral process of the palatoquadrate is expanded posteriorly. Until Stage 51, the anterior margin of the lower jaw lay approximately at the level of the anterior margin of the suprarostral cartilage; however, the larger mandible now extends beyond the rostral cartilages.

Metamorphic development of the cranium, Stages 54–66

The sequence of cranial ossification is presented in Table 2.

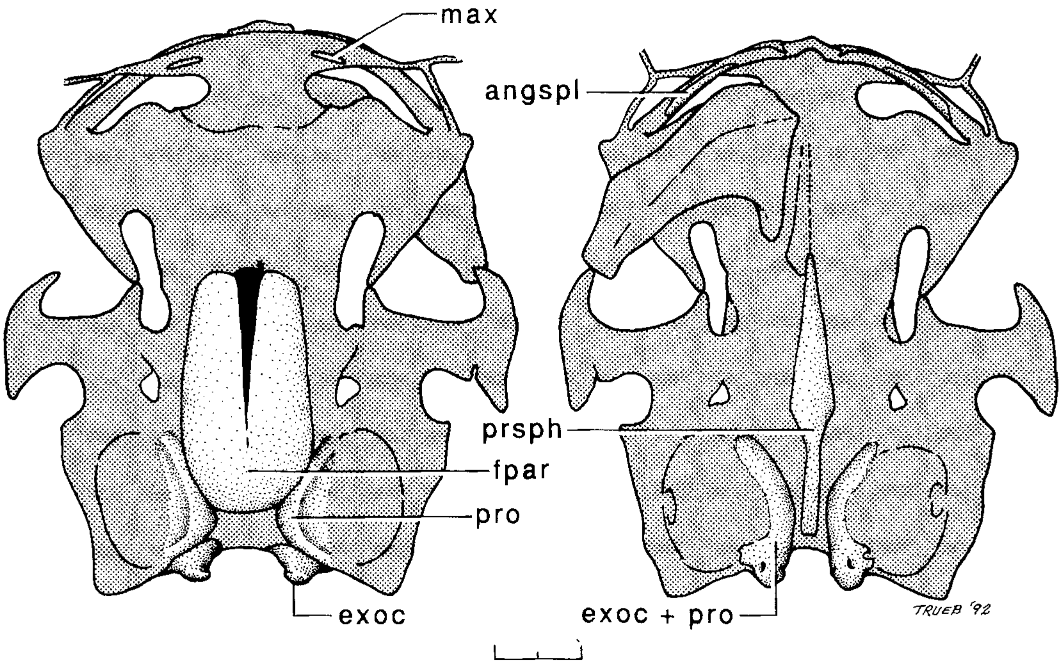

Fig. 3. *Xenopus laevis.* Dorsal (**left**) and ventral (**right**) views of skull of metamorphosing larva (Stage 57; KU 217919). Cartilaginous chondrocranial elements shown in stipple pattern are identified in Fig. 1. Only right ceratohyale and right half of basihyobranchial keel are depicted. angspl, angulosplenial; exoc, exoccipital; exoc + pro, fused exoccipital and prootic; fpar, frontoparietal; max, maxilla; pro, prootic; prsph, parasphenoid. Scale = 2 mm.

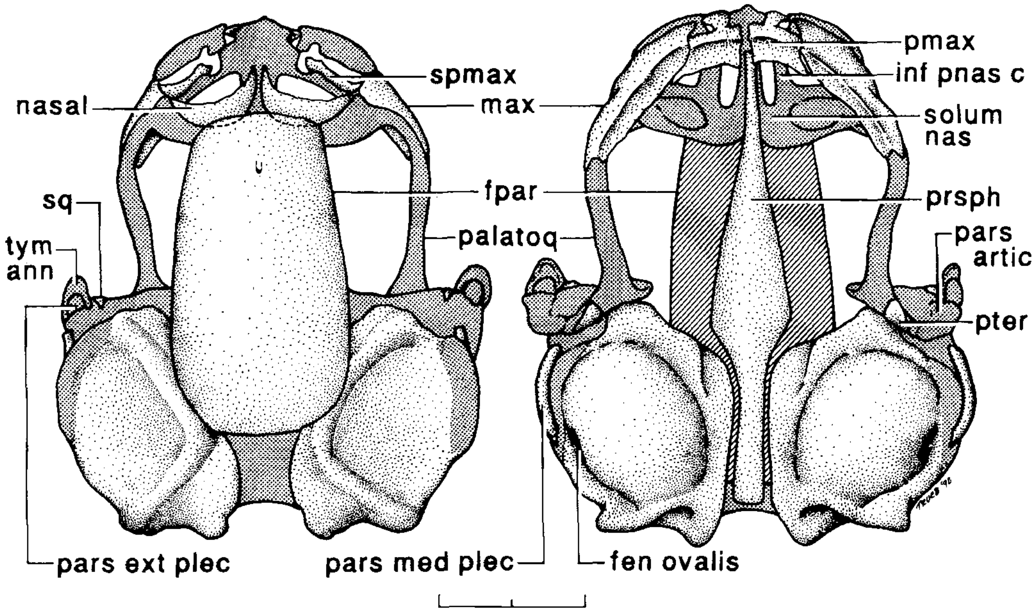

Fig. 4. *Xenopus laevis.* Dorsal (**left**) and ventral (**right**) views of skull of metamorphosing larva (Stage 63; KU 217948). Position of dental ridge is shown by broken line along venter of maxillae and premaxillae; individual teeth are not depicted. Cross-hatched pattern indicates venter of frontoparietal visible owing to absence of both cartilage and bone in the orbital region of the braincase at this stage of development. Cartilage is shown in stipple pattern. fen ovalis, fenestra ovalis; fpar, frontoparietal; inf pnas c, inferior prenasal cartilage; max, maxilla; pars artic, pars articularis of the palatoquadrate; pars ext plec, pars externa plectri; pars med plec, pars media plectri; palatoq, palatoquadrate; pmax, premaxilla; prsph, parasphenoid; pter, pterygoid; solum nas, solum nasi; spmax, septomaxilla; sq, squamosal; tym ann, tympanic annulus. For identities of other bony elements refer to Fig. 9. Scale = 2 mm.

Dermal investing bones

Frontoparietal. This azygous bone is the first cranial element to appear. It develops in Stages 54 and 55 from paired centers of ossification, each of which is long and slender and located above the orbital cartilage along the lateral margin of the frontoparietal fontanelle. The bones grow in length and breadth, and by Stage 57 they are narrowly separated medially and extend forward to the level of the tectum anterius (Fig. 3). They begin to fuse medially in Stage 58, and by Stage 60 the union is complete save for the small parietal foramen. Anterior growth over the tectum anterius begins in Stage 60; by Stage 62 the frontoparietal lies posteriorly adjacent to the nasals in most specimens, and in Stage 63 it overlaps the posteromedial margins of these bones (Fig. 4). Posterior growth is pronounced from Stage 60, and by Stage 63 the bone roofs the entire fontanelle and has achieved its postmetamorphic configuration.

Parasphenoid. This ventral bone appears contemporaneously with the exoccipitals and prootics in Stage 55. The parasphenoid forms from a single elongate, narrow center of ossification; it extends along the midventral floor of the braincase from between the otic capsules (but does not reach the occiput) to the anterior edge of the developing frontoparietals. In the next four stages, the bone lengthens and develops its characteristic spear shape (Fig. 3), in which the posterior end (between the otic capsules) is narrow and parallel-sided and the midsection (just anterior to the otic capsules) is relatively broad with laterally convex margins. The sides of the parasphenoid converge to an acuminate anterior end that lies beneath the trabecular plate anterior to the braincase. Anterior growth is pronounced in Stages 60–62, and by Stage 63 the tip of the parasphenoid dorsally overlaps the partes palatinae of the premaxillae (Fig. 4).

Nasals. Narrowly separated, paired centers of ossification that give rise to the nasals appear in Stages 58–60. Initially, each nasal forms from an arcuate ossification posterior

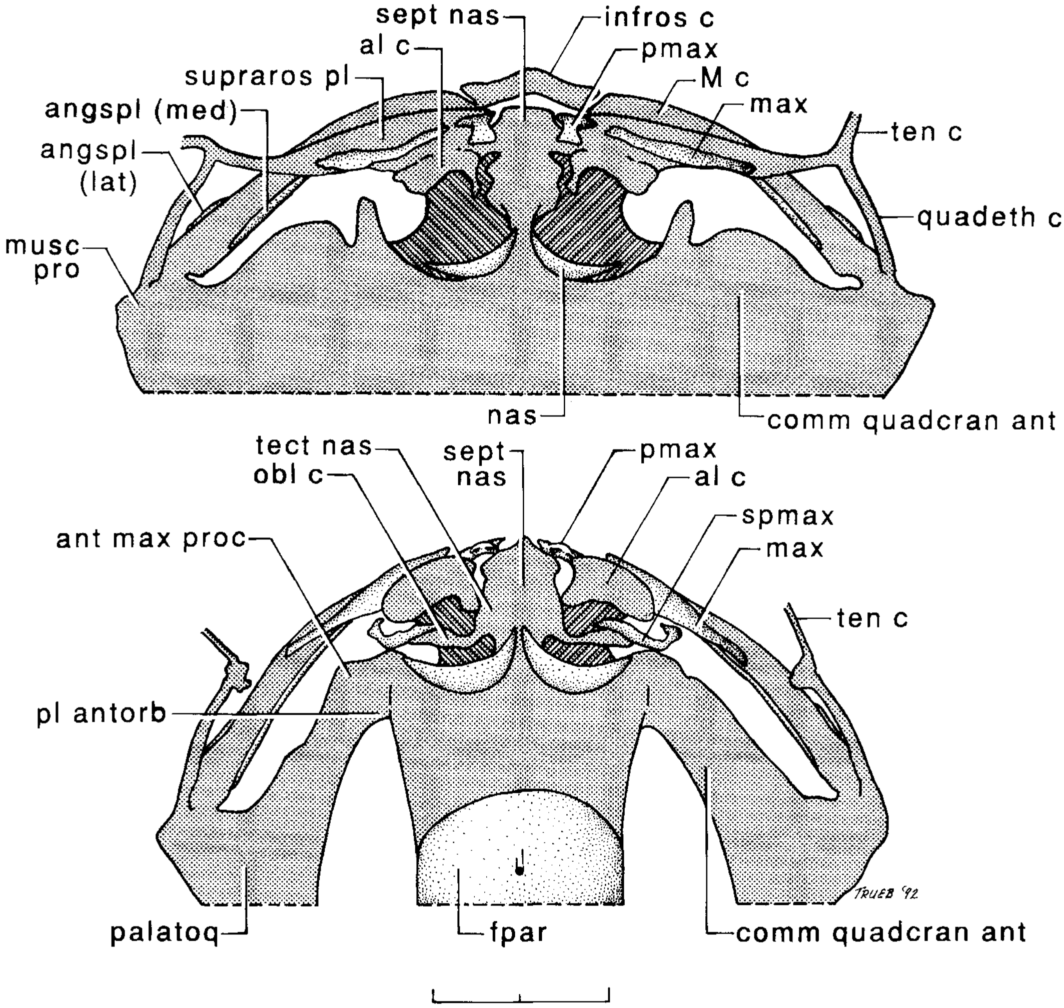

Fig. 5. *Xenopus laevis.* Development of rostral cartilages and anterior part of the bony skull during Stage 59 (**top**; KU 217927) and Stage 60 (**bottom**; KU 217936). In Stage 59, note the origin of the alary cartilages from the posteromedial margin of the suprarostral plate; the premaxillae and maxillae lie on the dorsal surface of the plate. Cross-hatched area between the alary cartilage and the nasal represents the solum nasi. By Stage 60, the suprarostral plate is absent and the septomaxilla, tectum nasi, and oblique cartilage have appeared. Posterior migration of the palatoquadrate is well advanced at this stage. Cartilage is shown in stipple pattern. al c, alary cartilage; angspl (lat), lateral angulosplenial; angspl (med), medial angulosplenial; ant max proc, anterior maxillary process; comm quadcran ant, commissura quadratocranialis anterior; fpar, frontoparietal; infros c, infrarostral cartilage; M c, Meckel's cartilage; max, maxilla; musc pro, muscular process of palatoquadrate; nas, nasal; obl c, oblique cartilage; palatoq, palatoquadrate; pl antorb, planum antorbitale; pmax, premaxilla; quadeth c, quadratoethmoidalis cartilage; sept nas, septum nasi; spmax, septomaxilla; supraros pl, suprarostral plate; tect nas, tectum nasi; ten c, tentacular cartilage. Scale = 2 mm.

to the external naris (Figs. 4,5). Growth seems to occur at an approximately equal rate at the anterolateral and anteromedial ends of this crescentic bone so that the symmetry of the developing nasal is maintained until the completion of metamorphosis (Stage 65), when the anteromedial tip of the nasal grows rapidly forward between the nasal capsules to produce the rostral process characteristic of the adult. Fusion of the two nasals begins with the anterior parts of the rostral processes and commences as early as Stage 66.

Vomer. The single adult vomer originates late in development (Stages 63–64) from a pair of small ossification centers that flank the parasphenoid at the level of the planum antorbitale. Growth occurs medially and by Stage 66, the two centers are fused to one another beneath the parasphenoid (Fig. 6).

Nasal capsule and septomaxilla

The development of the nasal capsule involves complex changes in the trabecular and suprarostral plates. By Stage 56, approximately the posterior two-thirds to three-fourths of the olfactory channels are roofed in cartilage; in effect, the tectum anterius extends anteriorly over the intertrabecular region to form a roof above the olfactory tracts and the vertical septum of cartilage that separates them (Fig. 3). Anterior growth of these elements continues into Stage 57, accompanied by remodeling of the trabecular plate at the level of the external nares (Fig. 5). This part of the plate, which lies at the lateral base of the suprarostral plate anterior to the olfactory foramina and anterior to the level of the commissura quadratocranialis anterior, is depressed ventrally to form the medial portion of the solum nasi. Concurrently, the medial cartilage, which represents the developing septum nasi, extends anteriorly between the external nares.

In Stage 58, the nasals appear along the anterior margin of the tectum anterius; an anterolateral proliferation of cartilage from the septum nasi represents the beginning of the tectum nasi posterolateral to the external naris. The alary cartilage arises from the posterior margin of the suprarostral cartilage anterior to the external naris. The oblique cartilage is an independent chondrification in the dorsolateral area of the developing nasal capsule; it fuses with the tectum nasi medially in Stage 59. Anteroventral growth of the portion of the trabecular plate medially adjacent to the commissura quadratocranialis anterior produces the posterolateral portion of the solum nasi.

Major changes occur in the rostral portion of the chondrocranium in Stage 60. Although the tentacular cartilage, along with the processus cornu quadratus lateralis and suprarostral process, is still present, most of the suprarostral plate has disappeared. At the same time the septum nasi extends anteriorly over the eroding suprarostral plate. Lateral to the developing nasal capsule, the commissura quadratocranialis anterior is eroding, and the septomaxilla has appeared within the nasal capsule. Disappearance of the commissura quadratocranialis anterior in Stage 61 identifies the posterolateral edge of the trabecular plate (or the anterior margin of the subocular fenestra) as the planum antorbitale (lamina orbitonasalis of some authors)—that is, the posterior wall of the nasal capsule. All traces of the tentacular cartilage disappear by Stage 61, but a remnant of the cornu quadratus lateralis persists into Stage 62.

By Stage 63, the posteroventral part of the septum nasi is fused to the medial planum antorbitale. The floor of the nasal capsule is smaller and consists of a band of cartilage that extends from the medial part of the planum forward along the medial margin of the choana and that is broadly separated from the septum nasi medially. The lateral solum nasi consists of a strip of cartilage that lies along the anterior margin of the choana. It is fused with the lateral end of the oblique cartilage to form the planum terminale. The anterior solum nasi is represented by a cartilaginous process that grows forward toward the premaxilla during Stage 64. By Stage 66, the process extends dorsad behind the alary process of the premaxilla to unite with the alary cartilage. This looped connection between the alary cartilage and solum nasi has been termed the superior prenasal cartilage by Paterson ('39) and Sedra and Michael ('57). It may represent both the superior and inferior prenasal cartilages of non-pipid anurans.

In Stages 64 and 65, there is a proliferation of the alary cartilage posterolaterally and the septum nasi anteriorly. By Stage 66, the anterior end of the septum nasi is a robust, expanded rostral cartilage that lies between the alary cartilages and the alary processes of the premaxillae, and the alary cartilage forms the anterior and lateral walls of the nasal capsule. The dorsolateral roof of the nasal capsule is composed of the expanded septomaxilla, and the dorsomedial roof by the nasal, oblique cartilage, and small tectum nasi. In contrast to the expansion of its dorsal roofing components, the cartilaginous floor of the nasal capsule becomes progressively more restricted.

Posterior braincase and ear

The exoccipital and prootic both form early in Stages 55–57 (Table 2). The appearance of the prootic may follow that of the exoccipital; the latter is present in all specimens by Stage

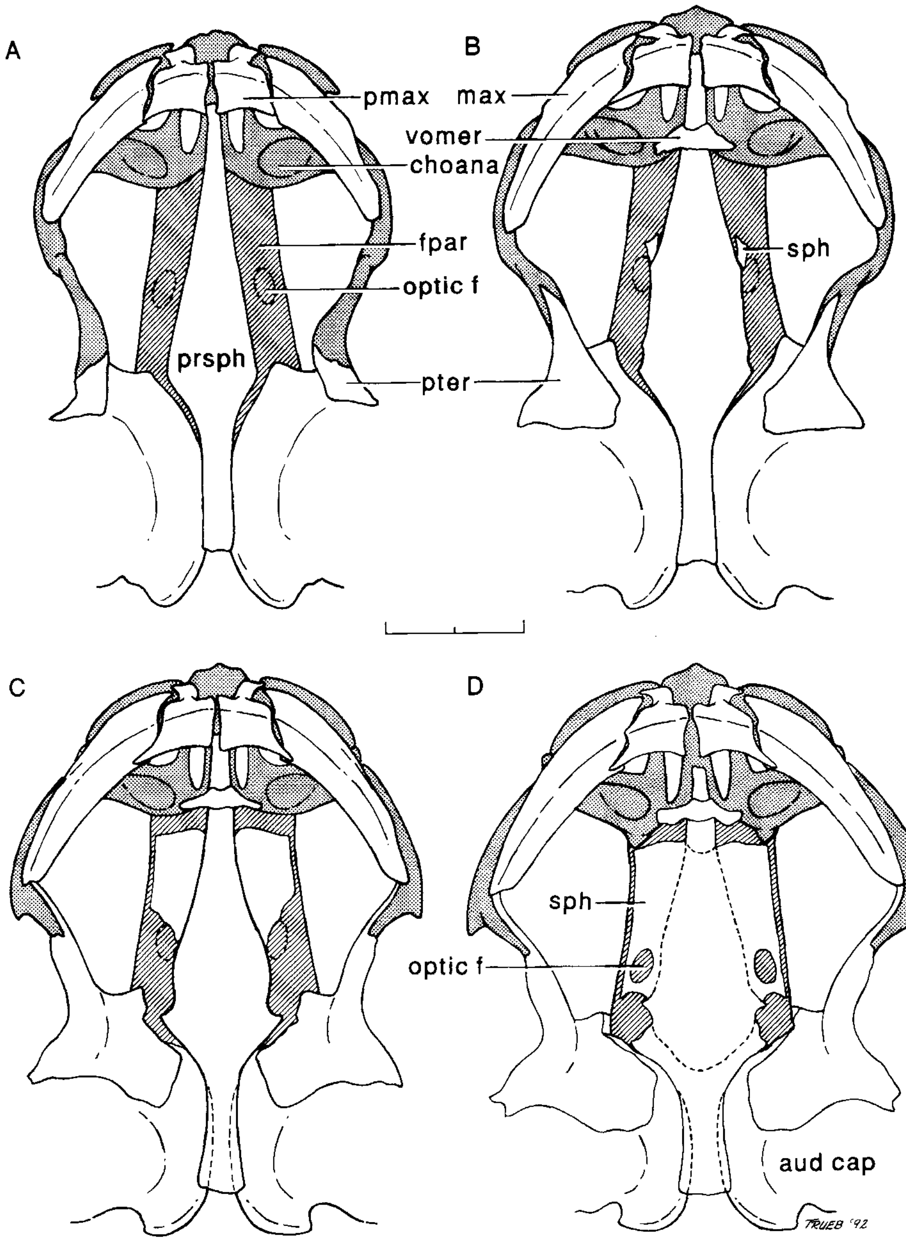

Fig. 6. *Xenopus laevis*. Development of the orbital region of the braincase and palate between Stages 64 and 66 + 1 month (ventral views). **A:** Stage 64 (KU 217955), prior to appearance of vomer and sphenethmoid. **B:** Stage 65 (KU 217957). Vomer and sphenethmoid are present, and parasphenoid has broadened between optic foramen and auditory capsules. **C:** Stage 66 (KU 217964). Note expansion of sphenethmoid and base of parasphenoid, and elaboration of lateral processes on parasphenoid in region of prootic foramen. Broken lines inside parasphenoid indicate margins of auditory capsules visible through the bone. **D:** Stage 66 + 1 month (KU 217971). The optic foramen is enclosed completely by the sphenethmoid, which also forms the anterior border of the prootic foramen. Anterolateral margins of parasphenoid and posterior margin of united sphenethmoids beneath parasphenoid are indicated by broken lines because these bones are fused at this stage. Position of dentition on maxilla and premaxilla is shown by light contour line; individual teeth are not drawn. Cartilage shown in stipple and ventral surface of frontoparietal by cross-hatched pattern. aud cap, auditory capsule; fpar, frontoparietal; max, maxilla; optic f, optic foramen; pmax, premaxilla; prsph, parasphenoid; pter, pterygoid; sph, sphenethmoid. Scale = 2 mm.

56, whereas the prootic is not present in all specimens until Stage 57. The exoccipital begins to ossify in the occipital arch in close association with the prootic, which commences ossification in the medial wall of the auditory capsule (Fig. 3). Ossification from the medial hemisphere of the otic capsule progresses to the lateral hemisphere, and by Stage 61 the capsule is well ossified with the exoccipital nearly completely fused with the prootic. The subspherical auditory capsule bears a marginal cartilage anteriorly and laterally (Fig. 4). This cartilage gradually diminishes in size and becomes restricted to a narrow edge along the anterolateral margin of the auditory capsule by Stage 66, at which point it represents the narrow crista parotica of the adult.

External and middle ear elements appear abruptly in Stage 62 (Table 2). The tympanic annulus forms as a slender crescent of cartilage that is vertically oriented and lies anteriorly adjacent to the small, round pars externa plectri. The latter is associated with the distal end of the pars media plectri and, at this stage, is ossified only distally. In succeeding stages, the pars externa plectri expands while the tympanic annulus grows around it and the pars media plectri ossifies in a distal-to-proximal direction. By Stage 66, the tympanic annulus forms a nearly complete ring around the pars interna plectri; it remains incomplete posteriorly, at the union of the pars externa plectri and pars media plectri. The pars media plectri at this stage is completely ossified and slightly expanded basally in the area of the fenestra ovalis. The pars media plectri abuts a delicate disc of cartilage, the pars interna plectri, that nearly fills the fenestra ovalis. Sedra and Michael ('57:46) identified the operculum as a "feebly developed outgrowth from the posterior border of the fenestra ovalis" in Stage 65. We find no indication of an operculum in either Stage 65 or 66 specimens in which the large fenestra ovalis seems to be bordered completely in bone; however, the structure described by Sedra and Michael ('57), de Villiers ('32), and Paterson ('39) is obviously present in 1–2-month-old postmetamorphic juveniles and in adult specimens.

Orbital region of the braincase

Coincident with the restructuring of the rostral chondrocranium between Stages 59 and 60, the cartilage forming the lateral walls of the braincase in the orbital region disappears. Thus, in the sections and the wholemounts examined, the preorbital portion of the neurocranium is associated with the postorbital part only by the frontoparietal dorsally and the parasphenoid ventrally, with the side walls of the braincase lacking any cartilage (Fig. 6A). In Stages 64–66, ossification appears in the ventrolateral area of the braincase adjacent to the parasphenoid in front of the optic foramen in the ventrolateral area of the braincase (Fig. 6B). From this center, the thin, sheetlike sphenethmoid grows anteriorly and dorsally to form the lateral wall of the neurocranium between the optic foramen and the planum antorbitale. In Stage 66, the ventral margins of the sphenethmoids articulate with the lateral margins of the parasphenoid and the dorsal margins lie adjacent to the frontoparietal; the bones do not enclose the orbitonasal foramina (Fig. 6C). In one specimen (KU 217966), the anteroventral corners of each sphenethmoid have proliferated medially to form a thin ventral bridge uniting the two sides of the braincase above the parasphenoid just posterior to the planum antorbitale. In the same specimen, there is posterodorsal proliferation of the bone to form the dorsal margin of the optic foramen; there is no evidence of cartilage or bone between the optic and prootic foramina. The posterior margin of the prootic foramen is formed by the prootic at the anteromedial corner of the otic capsule.

Upper jaw

Maxilla. The first ossification of the adult upper jaw appears in Stage 57 as a slender spindle of bone located along the posterior margin of the suprarostral cartilage anterior to the naris in the region where the alary cartilage will develop (Fig. 3). The bone lengthens laterally in Stages 58 and 59 (Fig. 5). By Stage 60, when the suprarostral plate has been replaced by the nasal cartilages, the maxilla is displaced ventrally and bears distinct partes facialis and palatina, and teeth. Subsequently the bone grows posteriorly; at Stage 60, the maxilla terminates at the anterior end of the orbit, and by Stage 66 it subtends the eye.

Premaxilla. The premaxilla forms in Stages 58 and 59. The paired bones are located at the tip of the snout and, initially, they are more closely associated with the maxillae laterally than with one another medially (Fig. 5). The first part of the bone to appear is the alary process, which is underlain by a tiny, transverse bar of bone representing the pars dentalis. By Stage 60, teeth are associated with the premaxillae and the pars palatina is present. During Stages 61–66, the premaxillae grow until they are narrowly separated from one another medially and from the maxillae laterally, and the pars palatina is as deep as the maxilla. The alary processes, which initially are uniform in width, slender, and only slightly laterally divergent from one another in frontal aspect, become expanded dorsally; growth seems to be more rapid at the dorsolateral and dorsomedial corners of each alary process so that by Stage 66, the processes are dorsally bifurcate.

Mandible

Angulosplenial. This dermal bone (angulare or goniale of some authors) invests Meckel's cartilage anteromedially and posteriorly; unlike its counterpart in other anurans, it arises from two centers of ossification in *Xenopus laevis.* The primary center appears in Stages 56 and 57 as a bony splint along the medial surface of Meckel's cartilage (Fig. 3). By Stage 59, this ossification is approximately centered on the length of Meckel's cartilage and covers about three-fourths its length; the bone is acuminate anteriorly, and blunt and wide posteriorly. In Stages 59 and 60, a secondary center of ossification (prearticular of Bernasconi, '51) appears lateral to the posterior third of Meckel's cartilage (Fig. 5). Proliferation of this center along with the posterior part of the medial center results in their fusion dorsally and ventrally (Fig. 7) so that by Stage 66, the posterior third of Meckel's cartilage is encased in the cylindrical angulosplenial with only a knob of cartilage protruding posteriorly. The coronoid process first appears in Stage 63 as a dorsomedial elaboration of bone along the primary center of ossification adjacent to the developing pterygoid. By Stage 66, the angulosplenial invests nearly the entire medial surface of Meckel's cartilage. Its anterior tip remains acuminate and is underlain by an expansion of the anterior end of Meckel's cartilage that appears in Stage 62.

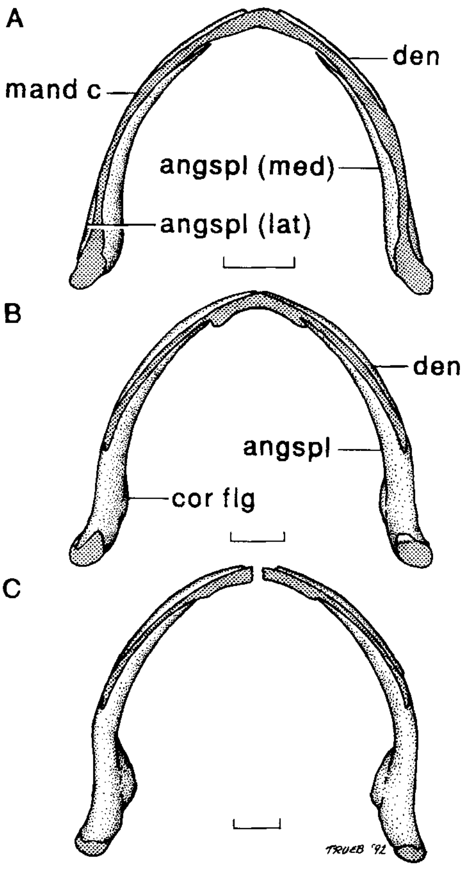

Fig. 7. *Xenopus laevis.* Development of the mandible (ventral aspect) during metamorphosis and early postmetamorphosis. **A:** Stage 63 (KU 217948). The angulosplenial forms from lateral and medial centers of ossification that are not united at this stage of development. The infrarostral is synchondrotically united to Meckel's cartilage (mandibular cartilage). **B:** Stage 64 (KU 217953). The two centers of ossification of the angulosplenial are fused at this stage and the coronoid flange has begun to form. The dentary has increased in length anteriorly and posteriorly, and the mandibular cartilage has expanded near the anterior tip of the angulosplenial. **C:** Stage 66 + 1 month (KU 217969). Note the erosion of cartilage at the mandibular symphysis, the increased size of the coronoid flange, and recurvature of the posterior part of the mandible. The angulosplenial extends anteriorly, dorsal to a narrow shelf formed by the mandibular cartilage. Cartilage is shown in stipple pattern. angspl (lat), lateral angulosplenial; angspl (med), medial angulosplenial; cor flg, coronoid flange of the angulosplenial; den, dentary; mand c, mandibular cartilage. Scale = 1 mm.

Dentary. The appearance of the dentary in Stages 60–62 is associated with changes in the infrarostral cartilage. Prior to Stage 60, this cartilage is distinct from Meckel's cartilages and has a characteristic inverted chevron shape (Figs. 1,3). At Stage 60, the infrarostral becomes straight and fuses dorsally with Meckel's cartilages; a partial separation between these cartilages is visible in ventral aspect through Stage 66. The dentary appears as a slender splint of bone on the outer surface of the mandible at the union of Meckel's cartilage and the infrarostral. In subsequent stages (Fig. 7), the dentaries retain a distinct medial separation and grow posteriorly along the lateral aspect of the mandible. By Stage 66, the bone covers approximately the anterior two-thirds of the external surface of Meckel's cartilage, leaving only a small area of the cartilage exposed laterally between the posterior end of the dentary and the massive angulosplenial posteriorly.

Suspensorium

The massive changes in the suspensorium associated with metamorphosis commence in Stage 60 with the initial erosion of the posterior parts of the palatoquadrate (ventrolateral process and its subocular bar), along with its connections to the neurocranium—the ascending and larval otic processes. As the posterior palatoquadrate diminishes and completely disappears by Stage 63, the anterior part undergoes extensive modification and migrates posteriorly beneath the eye and then posterodorsally to become associated with the anterolateral corner of the otic capsule. This transformation involves the disappearance of the muscular process and the erosion of the commissura quadratocranialis anterior and elaboration of its remnants into a robust suborbital bar (Fig. 4). The anterior portion of this bar is united to the planum antorbitale and laterally adjacent to the maxilla, and is termed the posterior maxillary process. The continuation of this cartilage diverges posteromedially from the maxilla, extends to the palatoquadrate cartilage, and comes to be underlain by the pterygoid bone; this posterior portion of the bar is identifiable as the pterygoid process of the palatoquadrate cartilage in Stage 63 (Fig. 4). In Stage 66, a spur of cartilage develops from the dorsolateral aspect of the pterygoid process. The process is directed posterodorsally and later in development serves as a point of articulation for the zygomatic ramus of the squamosal; hence, it is termed herein the zygomatic spur of the pterygoid process. Owing to its small size, we did not observe the otic process in the premetamorphic whole-mount specimens; according to Sedra and Michael ('57), the adult otic process is produced from the dorsal end of the palatoquadrate cartilage in Stage 64 and fuses with the crista parotica of the auditory capsule in Stage 65. Concomitantly, the medial region of the palatoquadrate grows toward a ventral cartilage that extends ventrolaterally from beneath the prootic foramen at the anteromedial corner of the otic capsule; this is the postpalatine commissure of Sedra and Michael ('57) and pseudobasal process of Paterson ('39).

Pterygoid. This massive ventral component of the suspensorium first appears in Stages 61 and 62 as a diffuse center of ossification on the ventromedial surface of the palatoquadrate (Figs. 4, 6). It rapidly proliferates anteriorly and medially during Stages 64 and 65. Anterior ossification lies along the ventromedial surface of the pterygoid process and represents the anterior ramus of the pterygoid. Medial ossification proceeds in a dorsomedial direction toward the ventral surface of the otic capsule. By Stage 66, the anterior ramus of the pterygoid extends along the pterygoid process adjacent to the maxilla, and the medial ramus is an extensive plate underlying the lateral otic capsule and Eustachian canal.

Squamosal. This element appears as early as Stage 62, but is not present in all specimens examined until Stage 63, when the ventral ramus appears as a sliver of bone along the anterolateral edge of the palatoquadrate medial to the developing pars externa plectri and tympanic annulus (Fig. 4). During Stage 64, the squamosal grows dorsally and assumes an arcuate (curved anteriorly) form in the lateral plane. Small zygomatic and otic rami are present in Stage 65 and the base of the ventral arm is expanded. In Stage 66, the squamosal is still small. The slender zygomatic process is directed anterolaterally from the ventral shaft of the squamosal and lies medially adjacent to the dorsal edge of the tympanic annulus. The otic ramus is posteromedially oriented toward the zygomatic spur of the pterygoid process and lies between the posterodorsal edge of the tympanum and the crista parotica. The expanded base of the ventral arm of the squamosal is configured into a broad, lateral flange that embraces the posteroventral rim of the tympanic annulus.

Pars articularis of the palatoquadrate. Ossification first invades the ventral, articular end of the palatoquadrate cartilage in Stage 66.

Postmetamorphic development of the cranium, Stages 66 + 1 month to 6 months

Stage 66 + 1 month (Fig. 8)

Within the first month of postmetamorphic development there are marked changes in several cranial elements. The rostral pro-

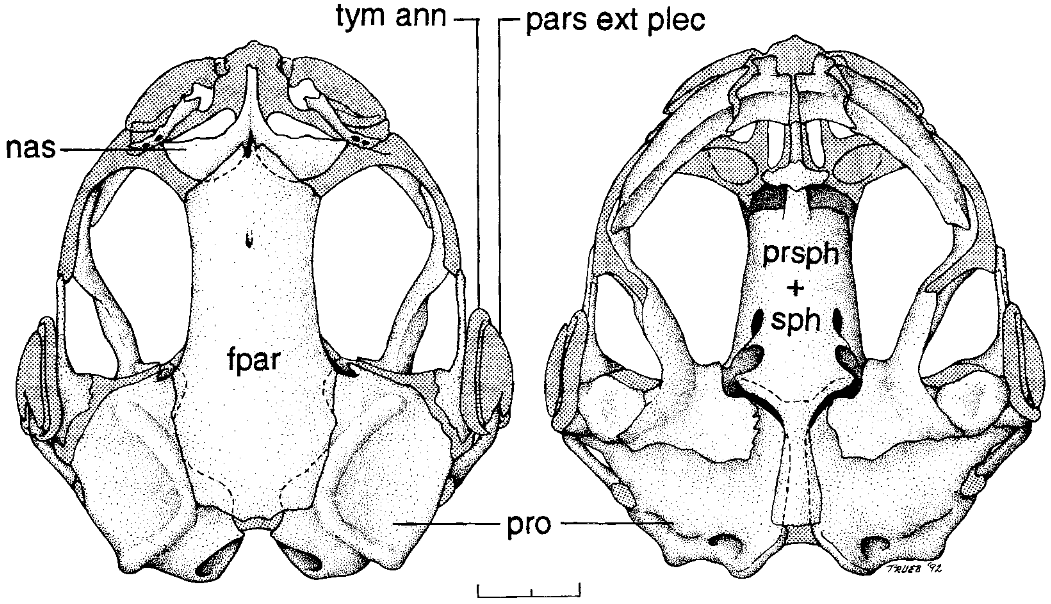

Fig. 8. *Xenopus laevis.* Dorsal (**left**) and ventral (**right**) views of the skull of an early postmetamorphic individual, Stage 66 + 1 month (KU 217969). Note the incomplete fusion of the nasals and the size of tympanic annulus and pars externa plectri relative to those of adult (Fig. 9). The parasphenoid is fused with the sphenethmoid, the posterior extent of which is indicated by the dashed transverse line in the ventral view. The margins of the prootic bones beneath the frontoparietal (dorsal view) and parasphenoid (ventral view) are indicated by dashed lines. Elements are identified in Fig. 9. Line along maxillae and premaxillae indicates position of teeth. Cartilage is indicated by stipple pattern. fpar, frontoparietal; nas, nasal; pars ext plec, pars externa plectri; pro, prootic; prsph + sph, fused parasphenoid and sphenethmoid; tym ann, tympanic annulus. Scale = 2 mm.

cesses of the nasals fuse with one another medially. The frontoparietal expands such that it widely overlaps the posteromedial surfaces of the nasals anteriorly and covers the anterior half of the tectum posterius. Anterolaterally, the frontoparietal develops a small, flangelike process on each side in the region of the planum antorbitale. In cross section, the roof of the braincase is distinctly vaulted, rather than shallowly domed. In the lateral braincase, the sphenethmoid grows posteriorly and posteroventrally to enclose completely the optic foramen and form a bony anterior margin to the prootic foramen. The sphenethmoids unite with one another ventromedially and fuse to the parasphenoid below; thus, the orbital region of the braincase now is completely enclosed by the sphenethmoid laterally and ventrally, and the frontoparietal dorsally.

The skull in the area of the prootic foramen is undergoing modification. At the anteromedial corner of the otic capsule, the prootic expands anteromedially (Figs. 6,8). Dorsally, the bone forms a distinct articulation with the frontoparietal and ventrally it is growing toward the parasphenoid. The parasphenoid develops two pairs of acuminate, lateral flanges—one pair on each side of the bone. The anterior pair lies between the levels of the optic and prootic foramina, whereas the posterior pair lies beneath the prootic foramina; each of these posterior flanges is directed posterolaterally toward the developing prootic.

Elsewhere in the skull, the prootic ossifies along the dorsolateral margin of the auditory capsule in the cartilage of the crista parotica. The otic ramus of the squamosal develops a narrow otic plate that is associated with the cartilaginous lateral margin of the crista parotica. The ventral ramus of the bone bears a broad lateral flange that is associated with the funnel-shaped tympanic annulus laterally, and the zygomatic ramus now approaches the maxilla where it articulates with the zygomatic spur of the pterygoid process of the palatoquadrate. The tympanic annulus and pars externa plectri are greatly enlarged relative to the size of the skull. A cartilaginous pars interna plectri forms an expanded base to the pars media plectri and nearly fills the fenestra ovalis. There is a narrow margin of cartilage along the poste-

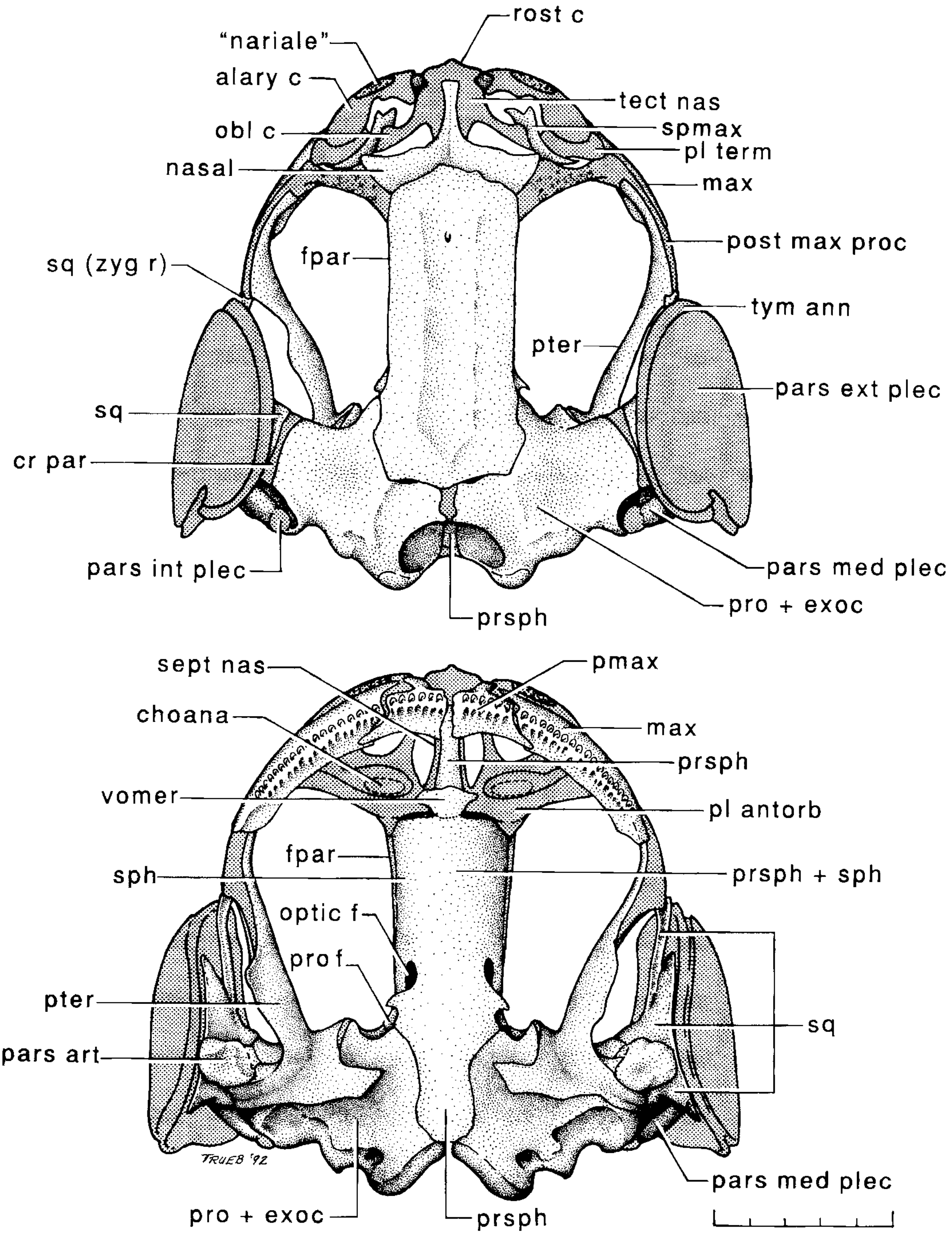
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Figure 9

rior margin of the fenestra ovalis that may represent an operculum.

In the lower jaw, the infrarostral cartilage unites completely with Meckel's cartilages laterally. Anteromedially, there is a partial erosion of the infrarostral cartilage to form an incomplete mandibular symphysis. The pars palatina of the premaxilla increases in depth and the lateral palatine process of this element now underlies the anteromedial process of the pars palatine of the maxilla. The pars articularis of the palatoquadrate is robustly ossified.

Stage 66 + 2 months

Changes in the cranium during the second month postmetamorphosis primarily involve modification of the prootic foramen. The sphenethmoid grows posterodorsally over the foramen to form a bony dorsal margin. At this stage, the sphenethmoid remains independent of the prootic and frontoparietal bones. The posterior flange of the parasphenoid nearly meets the anteromedial flange of the prootic to form an incomplete bridge between the braincase and auditory capsule beneath the prootic foramen. Continued anteromedial ossification of the margin of the prootic between its articulation with the parasphenoid ventrally and the frontoparietal dorsally forms the posterior border of the prootic foramen. The width of the crista parotica is now about one-third ossified. Despite the elaboration of the prootics anteriorly and laterally, there is no evidence of medial elaboration of the prootics or exoccipitals to complete the foramen magnum dorsally and ventrally or to unite the auditory capsules. Dorsally, the auditory capsules are bridged by the frontoparietal, and ventrally by the posterior end of the parasphenoid, which expands laterally across the medial edges of the prootic and forms the floor of the posterior braincase.

Stage 66 + 3 months to adult (Fig. 9)

Other than increase in overall size, changes in the skull after 3 months postmetamorphosis are relatively minor. The most conspicuous change is growth of the external plectral unit, the pars externa plectri and the tympanic annulus. At 3 months, the pars externa plectri is clearly separate from the tympanic annulus. In dorsal aspect, the unit as a whole extends about half the length of the zygomatic ramus of the squamosal toward the eye from the posterior tip of the otic ramus (ca. midlength of pars media plectri). In lateral view, the dorsal margin lies at the level of the head of the squamosal and the ventral margin is approximately even with the plane of the upper jaw. The structures increase slightly in relative size during the next 3 months, but at 6 months are still much smaller than they are in the adult. In mature specimens (Fig. 9), the pars externa plectri completely fills the area circumscribed by the tympanic annulus. The plectral unit occupies the entire posterolateral aspect of the skull behind the eye and extends nearly to the level of the occipital condyles. Its dorsal margin lies well above the squamosal and crista parotica, and the ventral margin below the level of the jaw articulation.

Other, less striking modifications include lateral expansion of the ossified portion of the crista parotica, completion of the bony bridge between the parasphenoid and prootic ventral to the prootic foramen, and expansion of the posterior end of the parasphenoid to form the ventral margin of the foramen magnum and the posteromedial floor of the braincase. Even in fully mature individuals, a gap exists between the anteromedial floor of the otic capsule and the parasphenoid and sphenethmoid medially, and the sphenethmoid does not fuse to the overlying frontoparietal. In larger individuals, mineralization occurs in the cartilaginous planum antorbitale. The nasals are completely fused medially and largely covered by the frontoparietal, which develops an irregular group of medial, longitudinal striations.

Fig. 9. *Xenopus laevis.* Dorsal (**upper**) and ventral (**lower**) views of the adult skull. KU 20957, male, 52.0 mm SVL, cleared and stained. Cartilage is shown in regular stipple pattern; irregular stippling (e.g., "nariale") indicates mineralized cartilage. alary c, alary cartilage; cr par, crista parotica; fpar, frontoparietal; max, maxilla; obl c, oblique cartilage; optic f, optic foramen; pars art, pars articularis of palatoquadrate; pars ext plec, pars externa plectri; pars int plec, pars interna plectri; pars med plec, pars media plectri; pl antorb, planum antorbitale; pl term, planum terminale; pmax, premaxilla; post max proc, posterior maxillary process; pro + exoc, fused prootic and exoccipital; pro f, prootic foramen; prsph + sph, fused parasphenoid and sphenethmoid; prsph, parasphenoid; pter, pterygoid; rost c, rostral cartilage; sept nas, septum nasi; spmax, septomaxilla; sph, sphenethmoid; sq (zyg r), zygomatic ramus of squamosal; sq, squamosal; tect nas, tectum nasi; tym ann, tympanic annulus. Scale = 5 mm.

Postcranial development

Axial column

For a detailed account of the ontogenesis of the vertebral column in *Xenopus laevis,* refer to Smit ('53), who also provided a review of previous work on *Xenopus* (Ridewood, 1897) and other anurans. The schedule of chondrification and ossification of the axial column observed in this study is recorded in Table 3.

Vertebrae. The first parts of the vertebrae to form are the neural arches, which in the youngest specimens available for examination (Stage 48) are present for Presacral Vertebrae I–VII. The neural arches of the last presacral, the sacrum, and three postsacral vertebrae appear in an anterior-posterior sequence, with that of the last vertebra (XII) occurring at Stage 62 (Table 3). Ossification commences in all the presacrals in Stages 55–57, and follows an anterior-to-posterior sequence throughout the remainder of the column. The neural arches of the sacrum (IX) ossify in Stage 57, and those of the first postsacral (X) in Stage 58. Ossification begins as early as Stage 59 for XI, but is not present in all specimens until Stage 64; XII does not begin to ossify until Stages 62–64. Fusion of Vertebrae XI and XII first occurs in Stage 63, followed by fusion of XI, X, and IX in Stage 64. At Stage 66, all the neural arches are imbricate, although those of Presacrals I and II are narrowly separated. Within the first month after metamorphosis, neural spines that overlap the posteriorly adjacent vertebra appear on Presacrals IV–IX. They form on Presacrals III and II during the second and third months postmetamorphosis, respectively. The neural process of Presacral I does not develop until the fifth month posthatching and initially appears as a pair of small spines on either side of the midline; subsequent ossification occurs between these spines to produce the bluntly truncated, flat neural spine of the adult.

The first vertebral centrum (I) is identifiable in cartilage in Stage 53; those of Presacrals II–VI appear in Stage 54, and VII–IX in Stage 57. Ossification of Centra I–IV occurs between Stages 55 and 57, followed by Centra V–IX in Stage 57.

Cartilaginous transverse processes are present on Presacrals II–IV in Stage 61 and ossification commences in Stage 64. Although the cartilaginous transverse processes of Presacrals V–VIII do not appear until Stage 63, they begin to ossify at the same time as the anterior presacrals. Ossification of the processes of Presacral VIII proceeds more rapidly than that of Presacrals V–VII, which are not ossified in all specimens until Stage 66. In most specimens, the transverse processes begin to lose their short, knobby appearance by Stage 64, and are elaborated into anterolaterally oriented spinous processes by Stage 66. The sacral diapophyses develop in cartilage in Stage 62, prior to the formation of the transverse processes of the posterior presacrals, but after those of the anterior presacrals. The sacral diapophyses are the first transverse elements of the vertebral column to begin ossification in Stage 63, but do not assume their expanded adult configuration until the first month postmetamorphosis.

Ribs. Ribs develop on Presacrals II–IV before the transverse processes and at about the same time that the centra and neural arches begin to ossify in Stage 57. By Stage 58, the rib on Presacral III is ossified in all specimens. Development of the ribs of Presacrals II and IV is delayed by comparison; ossification does not appear in all specimens until Stage 60 (Table 3). The ribs are synostotically united with the transverse processes in Stages 64 and 65. At Stages 65 and 66, the ribs of Presacrals III and IV are only about 30% longer than those of Presacral II and scarcely more robust. In the first month postmetamorphosis, the posterior two pairs of ribs become expanded distally and increase greatly in length until they are approximately twice the length of the anterior pair of ribs. The distal end of Presacral Rib IV develops a posterolateral curvature in Stage 64. In specimens 1 month postmetamorphosis, this rib is strong deflected posterolaterally at approximately its midlength, suggesting that growth in this rib is occurring between the distal curvature and the expanded distal end rather than at the distal tip.

Urostyle. This terminal element of the vertebral column is formed by fusion of Postsacral Vertebrae X–XII with the hypochord. The hypochord forms in cartilage and bone between Stages 60 and 62. By Stage 63, it is ossified in all specimens, and in Stage 66 the hypochord fuses with the block of postsacral vertebrae that lie dorsal and anterior to it.

Anterior appendicular skeleton

The anterior limb bud is present as early as Stage 48, the youngest specimens available for examination. The long bones of the fore-

TABLE 3. Comparison of schedules of axial chondrification and ossification in Xenopus laevis[1]

Stage	Bernasconi ('51)	This study		Smit ('53)	
		Cartilage	Bone	Cartilage	Bone
48	—	Neural Arches I–VII	—	Neural Arches V–VI	—
49	—	Neural Arch VIII	—	Neural Arches VII–IX	—
50	—	—	—	—	—
51	—	Neural Arch IX	—	Neural Arches I–III, X	—
52	—	—	—	Neural Arch XI	—
53	—	Neural Arches X–XI Centrum I	—	—	—
54	1. Neural Arches I–III Centrum I	Centra II–VI	—	—	Neural Arches I–IX
	2. Neural Arches IV–VII	—	—	—	—
	3. Neural Arches VIII–IX	—	—	—	—
	4. Neural Arch X Centra II–IV	—	—	—	—
55	—	—	Neural Arches I–VIII Centra I–IV	Neural Arch XII Transverse Processes II–IV	—
56	5. Centra V–X 6. Rib, Presacral II	—	—	Ribs, Presacral III Centra I–IX	— Centra I–IX
57	7. Ribs, Presacral III Neural Arch XI Centrum XI	Ribs, Presacrals II–IV	Neural Arches IX–X Centra V–IX	Ribs, Presacrals II, IV Hypochord	—
58	8. Ribs, Presacral IV Neural Arch XII Centrum XII	—	Ribs, Presacrals II–IV	—	Ribs, Presacrals II–III
59	—	—	Neural Arches V–XI	—	—
60	—	Hypochord	Hypochord	Neural Arch XII	Hypochord
61	—	Transverse Processes II–IV	—	—	Neural Arch XI
62	—	Neural Arch XII Sacral diapophysis	—	Sacral diapophysis	—
63	9. Sacral diapophysis	Transverse Processes V–VIII	Sacral diapophysis	—	—
64	—	—	Transverse Processes II–VIII	—	Sacral diapophysis Transverse Processes V–VIII

[1] Developmental stages are those of Nieuwkoop and Faber ('56) and as applied to Bernasconi's ('51) data are only estimations. Groups of elements associated with a single number or stage appeared simultaneously in the specimens examined.

limb and the ulnare and pre- and postaxial centralia are the first elements of the anterior appendicular skeleton to differentiate, and are followed by the distal carpals and metacarpals primarily during Stages 54–57. Formation of the pectoral girdle is approximately contemporaneous with that of the phalanges and takes place primarily after Stage 56 (Table 4). Sesamoid cartilages are present beginning in Stage 62.

Humerus, radioulna, ulnare, pre- and postaxial centralia and metacarpals. Many limb buds in specimens of Stages 52–54 were damaged during preparation and lack the forelimb; hence, we cannot determine exactly at which of these stages the elements appear. The humerus, radioulna, ulnare, and Metacarpals I–IV are the earliest elements to differentiate and in our sample are present in Stages 55 and 56. These are followed slightly later by the pre- and postaxial centralia and Distal Carpals I–III in Stages 55–57. The first elements of the forelimb to ossify are the humerus and radioulna in Stages 57–58, followed by the metacarpals. Metacarpals II–IV ossify in Stages 58–59, whereas the ossification of Metacarpal I extends through Stages 58–62.

Phalanges. The earliest phalangeal elements to differentiate are the proximal phalanges of Digits III and IV in Stage 56; by Stage 57, all phalanges are present in cartilage. Ossification proceeds more gradually and begins in Stage 60 when bone appears in the proximal phalanges of Digits I–IV; however, ossification of these elements is not present in all specimens until Stage 63. The second proximal phalangeal elements of Digits III and IV ossify between Stages 61 and 65; bone first appears in the second proximal phalangeal element of Digit II in Stages 63–65—approximately the same time frame during which the terminal phalangeal elements ossify in Digits III and IV. The final phalangeal element to ossify is the terminal member of Digit I in Stages 64–66.

Pectoral girdle. The first evidence of the pectoral girdle is the scapular cartilage in Stage 56, and by Stage 57, the coracoid, epicoracoid, procoracoid, and suprascapular cartilages are present. Ossification proceeds rapidly in Stages 58–62. The coracoid begins to ossify in Stages 58–60 at the same time the clavicle and cleithrum appear. The last part of the girdle to ossify is the scapula in Stages 62–65; during Stages 64 and 65, the scapula and clavicle become synostotically united (Fig. 10). The final component to differentiate is the sternum which arises from a pair of chondrifications associated with the posterior epicoracoid cartilages between Stages 62 and 65.

At Stage 66, the clavicle is a slender, curved element; the medial end of the bone is acuminate and curves anteriorly along the margin of the procoracoid cartilage. The epicoracoid cartilages are separate throughout most of their lengths but overlap posteriorly between the coracoid bones. The sternum is a single, shallow, arcuate cartilage. The glenoid end of the slender coracoid is about equal in width to the sternal end, but ossified more completely. In the first month postmetamorphosis, the medial configuration of the clavicle changes markedly; the medial end of the bone grows posteriorly over the procoracoid-epicoracoid cartilage, thereby becoming broad and blunt. At the same time, the sternal end of the coracoid has ossified and now is slightly larger than the glenoid terminus. The sternum has increased in depth by growth along the posterior margin, and the epicoracoid cartilages are synchondrotically united with one another between the coracoids. By the fifth month postmetamorphosis, the procoracoid cartilages are united with one another anteromedially.

Posterior appendicular skeleton

Like the forelimb, the limb bud of the hind limb is present in Stage 48, but the elements are preformed in cartilage about one stage earlier than their counterparts in the forelimb. The long bones of the hind limb differentiate first along with the metatarsals and naviculare, followed by the other tarsals and finally the phalangeal elements and the pelvic girdle (Table 5).

Femur, tibiafibula, fibulare, tibiale, tarsal elements, and metatarsals. The long bones (femur, tibiafibula, tibiale, and fibulare), naviculare, and Metatarsals II–V are differentiated in all specimens by Stage 55 and in one specimen by Stage 54; the posterior limbs of Stage-53 specimens were damaged, thus preventing observations. Metatarsal I differentiates along with the tarsal elements in Stages 55–56, and the prehallux is apparent in all specimens by Stage 57. By Stage 58, all of the long bones and metatarsals are ossified; all tarsal elements remain cartilaginous through Stage 66.

Phalanges. All phalangeal elements differentiate between Stages 55 and 57, contem-

TABLE 4. Comparison of schedules of forelimb chondrification and ossification in Xenopus laevis[1]

Stage	Bernasconi ('51)	This study		Nieuwkoop and Faber ('56)		Brown ('80)
		Cartilage	Bone	Cartilage	Bone	
55	—	Humerus Radioulna Ulnare Postaxial centrale Distal Carpals II–III Metacarpals I–IV	—	—	—	—
56	—	Distal Carpal I Phalanx III-1 Phalanx IV-1 Scapula	—	Humerus Radioulna Carpalia Metacarpals I–IV Scapula Procoracoid Coracoid	Humerus Radioulna Corocoid	—
57	1. Humerus Radioulna	Phalanges I-1–2 Phalanges II-1–2 Phalanges III-2–3 Phalanges IV-2–3 Coracoid Epicoracoid and procoracoid Suprascapula	Humerus (57–58) Radioulna (57–58)	Suprascapula	—	Humerus Radioulna Metacarpals
58	2. Metacarpals Phalanges Clavicle 3. Coracoid 4. Cleithrum	—	Metacarpal I (58–60) Metacarpals II–IV (58–59) Clavicle (58–59) Cleithrum (58–60) Coracoid (58–60)	Clavicle	Metacarpals I–IV — Cleithrum Scapula Phalanges	Clavicle
59	—	—	—	—	—	Cleithrum Coracoid
60	5. Scapula	—	Scapula (60–62) Phalanx I-1 (60–63) Phalanx II-1 (60–62) Phalanx III-1 (60–63) Phalanx IV-1 (60–63)	—	—	—
61	—	—	Phalanx III-2 (61–64) Phalanx IV-2 (61–64)	Sternum	—	—
62	—	Sternum Sesamoids				
63	—	—	Phalanx II-2 (63–65) Phalanx III-3 (63–66 + 1 month) Phalanx IV-3 (63–66 + 1 month)	—	—	—
64	—	—	Phalanx I-2 (64–66 + 1 month)	—	—	—

[1] Developmental stages are those of Nieuwkoop and Faber ('56) and as applied to Bernasconi's data are only estimations. Groups of elements associated with a single number or stage appeared simultaneously in the specimens examined. Ranges of stages in which elements were observed to appear in this study are indicated in parentheses. Bernasconi ('51) and Brown ('80) recorded only ossification events.

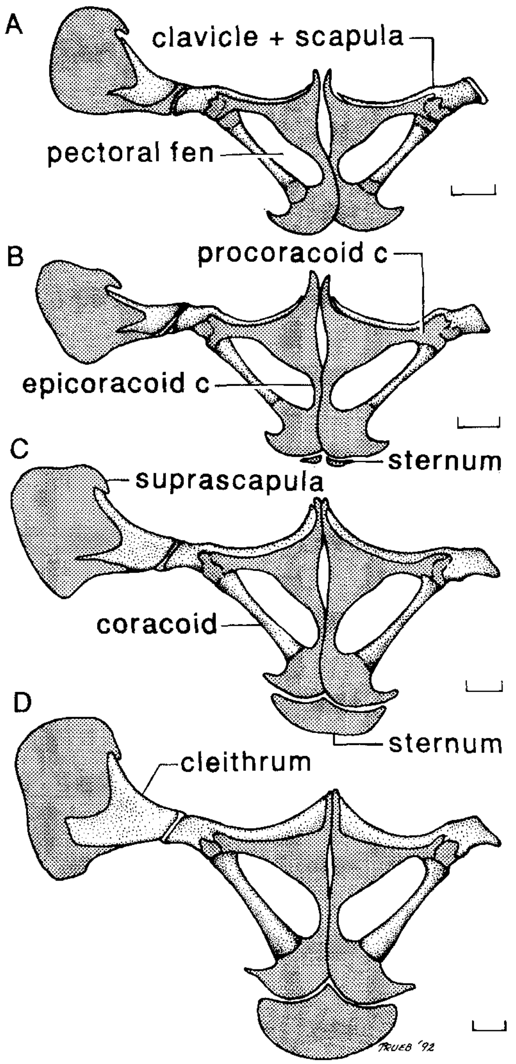

Fig. 10. *Xenopus laevis*. Development of the pectoral girdle during late metamorphosis and early postmetamorphosis. Girdles are drawn in ventral view with left suprascapulae removed, and scapulae and right suprascapulae deflected into the ventral plane. **A:** Stage 64 (KU 217955), prior to appearance of sternal elements. **B:** Stage 65 (KU 217958). Sternum appears as two posteromedial chondrifications adjacent to expanded ends of epicoracoid cartilages. **C:** Stage 66 (KU 217963). There is a marked expansion in the suprascapular cartilage and the thickness of the clavicular portion of the fused clavicle and scapula; the sternal chondrifications have united to form a single, shallow sternal plate. **D:** Stage 66 + 1 month (KU 217969). Note development of posterior arm of cleithrum and medial end of clavicle. Cartilage is shown in regular stipple pattern. c, cartilage; fen, fenestra. Scale = 1 mm.

poraneous with those of the forelimb. The first proximal phalanges of Digits II–V are the first to form (Stage 55) and are followed by the first phalanx of Digit I, second phalanges of Digits II and V, and second and third phalanges of Digits III and IV. The last phalangeal elements to appear in cartilage are the terminal phalanges of Digits I, IV, and V in Stage 57. Phalangeal ossification begins in Stage 58 when it is present in all the phalanges of Digits II and III and the proximal two phalangeal elements of Digit IV, and the first proximal phalanx of Digit V. Ossification appears in the third phalangeal element of Digit IV and second phalangeal element of Digit V in Stage 59. The terminal phalanges of Digits IV and V are the last to commence ossification in Stage 60. By Stage 65, all phalangeal elements of the hind limb have begun ossification in all specimens examined, whereas those of the forelimb have not.

Pelvic girdle. The ilium forms relatively early (Stage 55) in cartilage and commences ossification in the posterior part of the ilial shaft by Stage 57. The ischium and pubis are not apparent before Stage 60. Although ischial ossification can appear posterodorsal to the acetabulum as early as Stage 60, it is not developed in all specimens until Stage 63. Bone does not appear in the pubis until postmetamorphosis (Stage 66), at which time a disclike center of ossification develops in the pubic cartilage. The disc is oriented vertically with its flat surfaces facing anteriorly and posteriorly. The epipubis appears in Stages 60–63 as a small, triangular plate of cartilage. The truncate apex is attached to the midventral surface of the cartilaginous pubes. The epipubis lengthens, and by Stage 66 it has a moderately narrow proximal shaft and expanded terminus that is pointed anteriorly; the anterior margin becomes more blunt in subsequent development.

Within the first month postmetamophosis, the paired ischia are united synostotically, whereas the ilia and pubes remain separate from one another and the ischia. The ilial shaft is expanded anteriorly and develops a small crest along its dorsolateral margin. By Stage 66 + 2 months, the posteromedial area of the pubes behind the epipubis and anterior pubic ossifications has begun to mineralize. By 6 months postmetamorphosis, the ilial crest is well developed, but the pubic ossifications remain discrete from one another as they do in the adult.

SUMMARY OF OSTEOLOGICAL DEVELOPMENT

Chondrification and ossification sequences are presented in Tables 2–5; variation in these features is assessed in Appendices A–D. Figure 11 depicts a generalized scheme of ossification timing on a regional basis. The earliest and most protracted sites of bone formation are the cranium (Stage 54) and the axial column (Stage 55). The appendicular skeletons commence ossification two or three stages later (Stage 57), with the posterior appendicular skeleton ossifying more rapidly

TABLE 5. Comparison of schedules of hind-limb chondrification and ossification in Xenopus laevis[1]

		This study		Nieuwkoop and Faber ('56)		
Stage	Bernasconi ('51)	Cartilage	Bone	Cartilage	Bone	Brown ('80)
54	—	Femur Tibiafibula Tibiale Fibulare Naviculare Metatarsals II–V	—	Femur Tibiafibula Tibiale Fibulare Ilium Ischium	—	—
55	—	Metatarsal I Tarsals I–II Phalanx II-1 Phalanx III-1 Phalanx IV-1 Phalanx V-1 Ilium	—	—	—	—
56	—	Prehallux Phalanx I-1 Phalanx II-2 Phalanx III-1 Phalanges IV-2–3 Phalanx V-2	—	—	Femur Tibiafibula Tibiale Fibulare Metatarsals	—
57	1. Femur Tibiafibula Tibiale Fibulare 2. Ilium	Phalanx I-2 Phalanx IV-4 Phalanx V-3	Femur (57–58) Tibiafibula Tibiale (57–58) Fibulare (57–58) Metatarsals I–V (57–58) Ilium (57–58)	—	Phalanges	Femur Tibiafibula Ilium
58	3. Metatarsals Phalanges	—	Phalanges II-1–2 (58–60) Phalanx III-1 (58–59) Phalanx III-2 (58–60) Phalanx IV-I (58–59) Phalanx IV-2 (58–60) Phalanx V-1 (58–60)	—	—	—
59	—	—	Phalanges I-1–2 (59–60) Phalanx IV-3 (59–62) Phalanx V-2 (59–62)	—	—	—
60	4. Ischium 5. Pubis	Ischium Pubis Epipubis	Phalanx IV-4 (60–65) Phalanx V-3 (60–65) Ischium (60–63)	Epipubis	Ilium Ischium Pubis	Tibiale Fibulare Metatarsals
61–65	—	—	—	—	—	—
66	—	Pubis	—	—	—	—
66 + 1 month	—	—	—	—	—	—
66 + 2 months	6. Prehallux	—	—	—	—	—
66 + 2–5 months	—	—	—	—	—	—
66 + 6 months	7. Tarsals	—	—	—	—	—

[1] Developmental stages are those of Nieuwkoop and Faber ('56) and as applied to Bernasconi's ('51) data are only estimations. Groups of elements associated with a single number or stage appeared simultaneously in the specimens examined. Ranges of stages in which elements were observed to appear in this study are indicated in parentheses. Bernasconi ('51) and Brown ('80) recorded only ossification events.

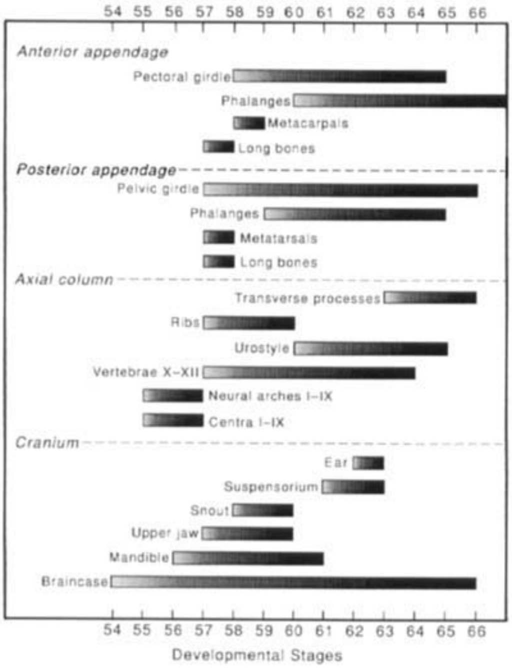

Fig. 11. *Xenopus laevis.* Timing of ossification events. Data plotted represent only the initiation of ossification within each skeletal unit; thus, all elements of the braincase, for example, have begun to ossify by Stage 66, but ossification of this structure is not complete by Stage 66. Feeding begins at Stage 45.

than the anterior, some components of which do not begin to ossify until the first month after metamorphosis.

Cranial patterns

Of all parts of the skeleton, the bony housing for the brain undergoes the earliest and most protracted period of development (Stages 54–66; Fig. 11). The first parts to form are the roof and the floor represented by the frontoparietal and parasphenoid, respectively. Thereafter, the auditory capsules and posterior skull begin to ossify as the prootics and exoccipitals. Ossification of the lateral walls of the braincase does not commence until Stages 64–66 when the sphenethmoid appears. The braincase is not fully formed until 2 months postmetamorphosis and undergoes significant alteration in the otic-prootic region during the 6 months following metamorphosis.

The mandible and upper jaw are the second and third parts of the osseous skull to form, followed by the bones of the nasal capsule, the nasals and septomaxillae. The suspensorium is the next unit to develop; whereas the formation of the pterygoid is complete at Stage 65, that of the squamosal and the plectral apparatus occupies several months postmetamorphosis.

Axial patterns

As expected, the core of the axial column—the centra and neural arches—includes the first elements to ossify; however, the development of the postsacral components (Vertebrae X–XII) is delayed and occupies considerably more developmental time than the anterior part of the vertebral column. The separate designation of the urostyle in Figure 11 is somewhat misleading, given that this element is formed by a fusion of the hypochord with the fused postsacral vertebrae. The onset of urostyle ossification is defined by the appearance of ossification in the hypochord, and the terminus as the fusion of the postsacral vertebrae with the hypochord.

If one considers the number of stages required for at least partial ossification of an element in all specimens examined as an index to the speed of formation, then ossification of the ribs and transverse processes does not follow the anterior-to-posterior sequence that one might expect. Thus, the second pair of ribs ossifies more rapidly than either the first or third pairs. All ribs develop prior to the appearance of transverse processes. The transverse processes of all presacral vertebrae commence ossification simultaneously, but the process is most rapid in Presacrals II–IV and VIII (1–2 stages), whereas ossification of the processes of Presacrals V–VII requires three stages.

Appendicular patterns

The last parts of the skeleton to ossify are the girdles and appendages beginning in Stage 57. The posterior appendicular skeleton ossifies more rapidly than the anterior skeleton (Fig. 11). In the posterior appendicular skeleton, the long bones, metatarsals, and girdle commence ossification simultaneously, whereas in the anterior skeleton, the beginning of metacarpal and pectoral-girdle ossification is delayed a stage relative to the posterior skeleton. Also, phalangeal ossification of the manus is delayed by a stage relative to that of the pes and requires one stage more for completion. In the manus, the proximal phalangeal elements form first and contemporaneously with one another, followed by the second phalangeal elements of Digits III and IV. The second (terminal) phalangeal element of Digit II then ossifies at the same time as the third (terminal) elements of Digits III and IV. The terminal phalanx of Digit I is the last to ossify. Thus, the general pattern is proximal to distal with ossification proceeding more rapidly in longer digits and in a postaxial-to-preaxial sequence. The same pattern is evident in the pes.

DISCUSSION

Novel features of cranial development in Xenopus laevis

There are several noteworthy and some unique features of chondrocranial development in *Xenopus laevis* relative to non-pipid anurans. First, the area anterior to the trabecular plate is developed as a single plate of cartilage, the suprarostral plate, instead of one or more suprarostral cartilages derived from paired cornu trabeculae (Fig. 2). Second, the trabecular plate lying between the commissurae quadratocranialis anterior is much longer (and hence, also the commissurae) than its counterpart in non-pipids. Whereas the anterior margin of the basicranial fenestra lies between the commissurae in most anurans, in *X. laevis* it lies well posterior to these structures (Fig. 2). Third, and perhaps most unusual, the palatoquadrate forms in two parts rather than as a single unit as in non-pipids. Each part forms in the same position relative to the parachordals and trabeculae (and later, the braincase), but development of the posterior portion is delayed relative to the anterior portion and to its formation in other anurans.

Even before feeding, which commences at Stage 45 in *Xenopus laevis,* the mandible (Paterson, '39:Fig. 22b) resembles in many respects that of the mature premetamorphic tadpole of Stage 54 and the adult. Meckel's cartilages are long, curved structures that are separated anteromedially by the single infrarostral that later fuses with them to form a continuous mandibular arcade to which dermal investing bones are applied medially and laterally to form the adult lower jaw (Fig. 7). In effect, these cartilages assume an initial configuration similar to that of non-pipid anurans at the end of metamorphosis. This was recognized by Sokol ('75:19), who observed that "a cenogenetic structural complex is no longer being maintained by selection pressure and is being eliminated in favor of a more direct developmental pattern." Early formation of the adult jaw configuration, which primitively occurs in late metamorphosis, in the embryonic period of *X. laevis* represents an example of precocious metamorphosis similar to that which occurs in the leptodactylid frog *Lepidobatrachus.* This derived developmental pattern was defined by Hanken ('92) as one in which metamorphorphic events in typical anurans are advanced into the embryonic period, and the development of larval components is correspondingly eliminated.

The timing of palatoquadrate development in *Xenopus laevis* also is peculiar. In most anurans, the palatoquadrate develops early as a massive bar of cartilage that is firmly attached posteriorly to the postorbital neurocranium by the ascending process and anteriorly to the trabecular or ethmoidal part of the neurocranium by the commissura quadratocranialis anterior. In *X. laevis,* the anterior palatoquadrate develops early (ca. by Stage 39). However, the posterior part of the palatoquadrate is transient with respect to the anterior part; it develops several stages later (Stages 45–46), begins to erode at Stage 61, and has disappeared at Stage 63, three stages before the completion of metamorphosis. In non-pipid anurans such as the pelobatid *Spea,* the configuration of the posterior part of the palatoquadrate and the nature of its attachment to the neurocranium change during the late stages of metamorphosis, but the structure does not disappear (Wiens, '89). Thus, there seems to have been a structural and temporal repatterning of palatoquadrate development in *X. laevis* relative to most other anurans.

Probably the most bizarre feature of cranial development in *Xenopus laevis* is the orbital region of the braincase. As in other anurans, the orbital region initially forms in cartilage. Whereas in non-pipid anurans, the cartilage usually is replaced partially or wholly by endochondral bone (sphenethmoid and anterior part of the prootics), in *X. laevis* the cartilage is resorbed between Stages 59 and 60 and the orbital walls of the braincase then are formed by membrane bones that develop at metamorphosis.

Questions of homology

The rostral chondrocranium

In *Xenopus,* the rostral portion of the chondrocranium anterior to the braincase has been variously termed the ethmoid cartilage or plate (Paterson, '39; Sedra and Michael, '57; Roček and Veselý, '89), planum internasale (Roček, '89, '90), and *Gaumendach* (palatal roof; Kotthaus, '33). Sokol ('75) referred to the anterior plate as a suprarostral, reasoning that it is homologous with the suprarostral cartilages of non-pipid anurans (contra Starrett ['73], Roček ['89], and Roček and Veselý ['89]). He maintained that in pipids, as well as microhylids, this cartilage arises as an anterior extension of the trabeculae and that it fails to differentiate into the complex and variable multipartite suprarostral system characteristic of beaked larvae. For reasons discussed below, we adopt the terminology of Sokol ('75).

Roček and Veselý ('89) argued that topographical and developmental differences between the cornua trabeculae of non-pipid anurans and the planum internasale or ethmoid plate of pipids precluded their derivation from one another, despite the fact that both seem to be derived from the same region of cranial neural crest (Sadaghiani and Thiébaud, '87). Roček ('89) pointed out that in non-pipid anuran larvae, all adult ethmoidal structures except the solum nasi are derived from new cartilaginous tissue that appears between the cornua trabeculae and ultimately fuses with them; the solum nasi arises from the cornua trabeculae. In pipids, the adult ethmoidal structures also arise from new cartilaginous tissue (Roček, '89); the cartilage appears dorsal to the ethmoid plate in the same relative position as it does in non-pipids. The major developmental difference between pipids and non-pipids is that the larval ethmoid cartilage, unlike that of non-pipids, eventually disappears and does not contribute to the nasal capsule of the adult (Kotthaus, '33; Sedra and Michael, '57; this paper). Thus, it seems highly likely that one of two situations prevails; the larval rostral cartilage either represents a fusion and simplification of the cornua trabeculae of beaked tadpoles, or in pipids, the cornua trabeculae fail to develop and a flat, platelike, anterior extension of the planum internasale develops in their place.

It is clear that the configuration of the rostral chondrocranium in *Xenopus laevis* differs from that typical of non-pipoid anurans; however, we argue that the developmental differences between *X. laevis* and nonpipoid anurans are less trenchant than Roček and Veselý ('89) would have us believe. Moreover, deviations from the ancestral developmental pattern do not intrinsically constitute an argument against homology of the resulting structures (Patterson, '77). Parsimony and the phylogenetic logic of other evidence (Cannatella and Trueb, '88a,b; Cannatella, '85) argue for the derivation of the pipid condition from the plesiomorphic larval condition of their archaeobatrachian relatives (e.g., pelobatoids, discoglossids, bombinatorids, and ascaphids). To argue the alternate hypothesis—that is, that the chondrocrania of pipid and nonpipids could not have been derived from one another—is tantamount to suggesting a diphyletic origin of the Anura; we know of no evidence in support of this hypothesis, but there is a substantial body of evidence supporting the monophyly of the Anura (Trueb and Cloutier, '91a,b). Thus, it seems most reasonable to assume that the rostral cartilage of *Xenopus* is homologous with the suprarostral of non-pipoids, pending the discovery of evidence to the contrary such as a tadpole possessing both a flat platelike anterior extension of the planum internasale and suprarostral cartilage. In the unlikely event that such a larva were found, the hypothesized homology would fail the "conjunction test" of Patterson ('82) because both structures would have been found to occur in the same organism.

Orbital region of the braincase

The peculiar development of the orbital neurocranium in *Xenopus laevis* has been noted in passing by other workers. Paterson ('39:184) described the sphenethmoid region of young *Xenopus* as being occupied by "a very thin bone which forms the lateral wall of the cranium anterior to the optic foramen" and which "joins the fronto-parietalia with the cartilaginous floor of the cranium in front, and rather more posteriorly it is confluent with the parasphenoid." She commented that the bone "is obviously homologous with the os en ceinture, but it does not possess the large marrow cavities that characterize it in other Anura." Sedra and Michael ('57:49) similarly observed that "Each side wall [of the braincase] is mainly ossified in front of the optic foramen" by a bone they called the orbitosphenoid. They reported that the remainder of the side wall is membranous, but depicted the unossified portions of the orbital neurocranium as cartilage (Sedra and Michael, '57:Figs. 32,35–37,39). Our sections of heads in Stages 63–65 similarly reveal that cartilage is absent and that in Stages 64 and 65 there is thin bone lacking marrow cavities and surrounded by connective tissue in the orbital region of the braincase.

One can argue that the orbitosphenoid of salamanders and the sphenethmoid of anurans are homologous because both are endochondral elements that form from a pair of ossifications—one on either side of the braincase medial to the orbit. In the case of salamanders and most anurans, ossification appears anterodorsally and spreads posteriorly and ventrally toward the optic foramen and parasphenoid, respectively. Because the cartilage in which the sphenethmoid or orbitosphenoid forms is continuous with the lamina orbitonasalis or planum antorbitale and the septum nasi of the olfactory capsule, anterior ossification variably encloses the orbitonasal foramen in bone and may invade the septum nasi. In most taxa, the pair of ossifications unite ventrally above the parasphenoid and dorsally anterior to the level of the frontoparietal fontanelle to form the girdle bone that houses the anterior end of the brain. *Xenopus laevis,* however, differs from salamanders and non-pipid anurans by a combination of significant features: 1) ossification of the sphenethmoid seems to be membranous rather than endochondral; 2) the sphenethmoid forms only the posterolateral border of the orbitonasal foramen, and is distinct from rostral cartilages and any ossification that might occur in them; 3) it commences ossification posteroventrally rather than anterodorsally; 4) after metamorphosis, the sphenethmoid spreads posteriorly toward the prootic such that it surrounds the optic foramen and forms the anterior border of the prootic foramen (discussed above); and 5) the dorsal margin of each sphenethmoid fuses with the frontoparietal, and midventrally, the bones unite with one another and fuse with the parasphenoid below. If one were to use these developmental data to speculate that the sphenethmoid of *X. laevis* (and possibly other pipids as well; Trueb, personal observation) is not homologous to the sphenethmoid of other anurans, this would imply that the bone was missing in the ancestor of pipids. Were this the case, the anterior braincase could be considered a neomorphic structure that is topologically homologous and functionally analogous, but not phylogenetically homologous, to the sphenethmoid of nonpipid anurans. In the absence of evidence of a pipid ancestor that lacks a sphenethmoid, however, one must assume instead that development of the sphenethmoid has been altered and represents a derived feature, but that the bone is homologous with the sphenethmoid of other anurans. Thus, following Patterson ('77), the sphenethmoid of *X. laevis* is an endoskeletal bone that is not preformed in cartilage owing to a regression of cartilage or change in growth pattern; as such, it is similar to the membrane basisphenoid of teleosts, which is considered a phylogenetic homologue of the endochondral basisphenoid of *Amia,* paleoniscoids, and primitive pholidophorids (Patterson, '77).

Comparison to previous studies of skeletal development in Xenopus laevis

Comparisons among the various studies of chondrification and ossification of *Xenopus laevis* are problematic. First, the sources of the specimens examined varies; thus, Sedra and Michael ('57), Nieuwkoop and Faber ('56), Smit ('53), and part of Brown's ('80) material were wild-caught, whereas Bernasconi's ('51) material and the specimens reported on herein were reared in the laboratory. Brown's ('80) results suggest that ossification in wild-caught larvae begins earlier and proceeds more rapidly than it does in laboratory-reared populations; however, these developmental differences (as well as differences among laboratory-reared populations) simply may have resulted from one or more environmental variables such as temperature, density of larvae, and photoperiod. Second, specimens have been prepared in a variety of ways that may not be directly comparable. Bernasconi ('51) used alizarin-stained whole mounts, whereas the specimens examined by Brown ('80) and most of those examined herein are whole mounts double-stained for cartilage and bone; Sedra and Michael ('57), Paterson ('39), Nieuwkoop and Faber ('56), and Smith ('53) examined sectioned material. Third, Bernasconi's ('51) and Paterson's ('39) results are calibrated by larval size and age and, thus, cannot be compared readily with one another or with studies based on the normal table of *Xenopus laevis* development (Nieuwkoop and Faber, '56). As shown in Figure 12, the total lengths of *X. laevis* larvae are highly variable, especially during the stages of cranial ossification (Stages 54–64). Fourth, the range of ossification events in Brown ('80) is limited owing to the author having only Stages 55–60 available for examination. Fifth, none of the previous studies addressed individual variation in developmental timing. We found that the sequence of events does not vary, but that the onset of chondrification and ossification

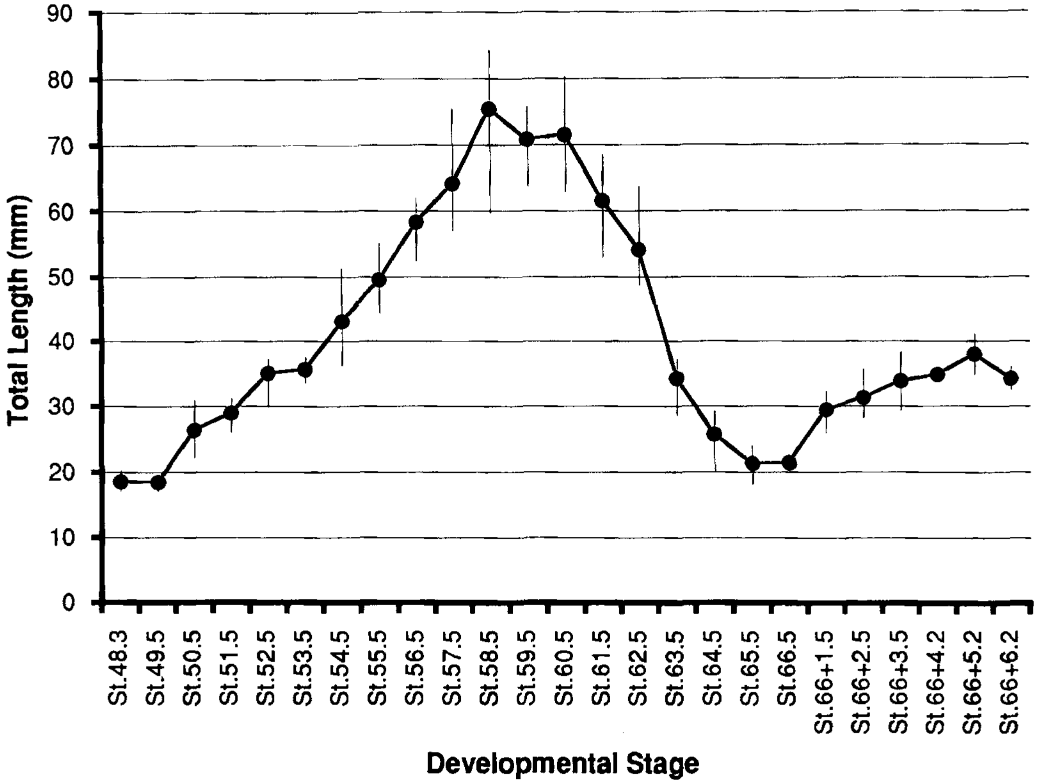

Fig. 12. *Xenopus laevis*. Variation in total length of larvae plotted against developmental stage. Means are indicated by closed circles; ranges are represented by vertical lines. The sample size measured in each stage is indicated by the terminal digit of the categories on the X-axis; thus, "St.48.3" indicates that three specimens of Stage 48 were measured.

varies depending on the skeletal element (Appendices A–D). Finally, the terminology of cranial elements is inconsistent and/or incorrect (Table 6). Bernasconi ('51) apparently noted two centers of ossification for the angulosplenial—viz., his goniale and articular. Sedra and Michael ('57) did not distinguish between the exoccipital and prootic, referring to both as "occipito-prootic ossification." Paterson ('39) distinguished parts of the frontoparietal and sphenethmoid regionally, but not developmentally. Bernasconi ('51) apparently misidentified several elements. Thus, his supraethmoid is the nasal, his nasale is the septomaxilla, and his palatinum and nariale are mineralized cartilage of the planum antorbitale and alary cartilage, respectively.

Notwithstanding these limitations, it is possible to make some general comparisons. The cranial ossification data of this study, Sedra and Michael ('57), Bernasconi ('51), and Brown ('80) are compared in Table 2 and Figure 13. The general chronology of ossification of Sedra and Michael seems to be delayed by two or three stages relative to the data from this study and that of Brown ('80). This result is somewhat surprising given that in a comparative assay of osteogenic events in *Bombina*, Hanken and Hall ('88) found that bones were fully differentiated in sectioned specimens some six stages before they were detectable in cleared-and-stained wholemounts. Exceptions to this generalization for *Xenopus laevis* are the parasphenoid, part of the angulosplenial, septomaxilla, and dentary, which appear at the same stages, and the plectral apparatus and premaxilla, which appear earlier and later, respectively, than in this study. Except for the plectral apparatus, the sequence of appearance of cranial elements is the same in this study and Sedra and Michael's. Curiously, Sedra and Michael do not mention the vomer. Relative to this study and those of Sedra and Michael ('57)

TABLE 6. Comparison of nomenclature used in this and other descriptions of osteological development in Xenopus laevis[1]

This study	Bernasconi ('51)	Brown ('80)	Paterson ('39)	Sedra and Michael ('57)
Angulosplenial, medial	Goniale	Prearticular	Angulare	Goniale
Angulosplenial, lateral	Articular	—	Angulare	Goniale
Exoccipital	Pleuroccipitale	—	—	Occipito-prootic ossification
Frontoparietal	—	—	Frontoparietal (part) Supraethmoid (part)	—
Nasal	Supraethmoid	—	—	—
Pars articularis of the palatoquadrate	Quadrate	—	—	—
Prootic	—	—	—	Occipito-prootic ossification
Sphenethmoid	Sphenethmoid	Sphenethmoid	Os en ceinture (part) Pleurosphenoid (part)	Orbitosphenoid
Septomaxilla	Nasale	—	—	—
Mineralization of alary cartilage	Nariale	—	—	—
Mineralization of planum antorbitale	Palatinum	—	—	—

[1] Bones not listed are named in the same way in all five studies.

and Brown ('80), Bernasconi's chronology is peculiar in that the prootic ossifies late and the squamosal and pterygoid bones ossify early.

In general, we think that Smit ('53) achieved a finer resolution of skeletal development of the axial column based on his study of sectioned material than we did in our examination of whole-mount specimens. The overall pattern of events is similar in this study and Smit's ('53) except for the timing of the chondrification of the transverse processes of Presacrals II–IV; in his schedule these structures appear in Stage 55, whereas we did not find them until Stage 61 (Table 3). Furthermore, we were unable to identify the cartilage precursors of Centra VII–IX, which were first evident in bone at Stage 57.

The development of the anterior appendicular skeleton reported in Nieuwkoop and Faber ('56) deviates somewhat from the results of this study, Bernasconi ('51), and Brown ('80) (Table 4). For example, Nieuwkoop and Faber ('56) reported the proximal limb bones to chondrify and ossify in Stage 56, whereas we noted chondrification in Stage 55 and ossification in Stage 57. Similarly, Nieuwkoop and Faber ('56) reported the coracoid present in bone and cartilage in Stage 56, whereas we did not find it chondrified until Stage 57 and ossified until Stage 58. Relative to this study, Nieuwkoop and Faber ('56) recorded the scapula and phalangeal elements to ossify earlier and the sternum to chondrify earlier. The primary difference between our results and Bernasconi's ('51) is the earlier ossification of the phalangeal elements in his schedule.

With respect to the posterior appendicular skeleton (Table 5), there are some striking differences between the results reported herein and those of Nieuwkoop and Faber ('56). They reported the ilium and the ischium to chondrify one and five stages earlier, respectively, than we found them. According to Nieuwkoop and Faber ('56), the long bones, metatarsals, and phalangeal elements ossify one stage earlier than reported here, and ossification of the ilium occurs three stages later in their developmental scheme, but the ischium ossifies at the same stage. They also reported the pubis to be ossified at Stage 60, whereas we found it to chondrify at this stage but not ossify until Stage 66. The sequences of ossification observed by Bernasconi ('51) and Brown ('80) correlate exactly with that of this study; however, Brown did not observe ossification of the tibiale, fibulare, and metatarsals until three stages later than in this study. This developmental hiatus in Brown's ('80) chronology is peculiar, given that each of the other studies reported the long bones and metatarsals to ossify early and nearly simultaneously.

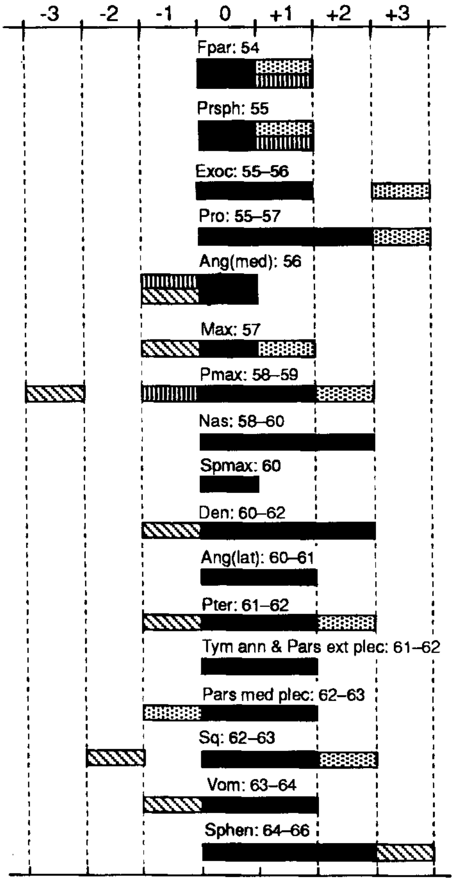

Fig. 13. *Xenopus laevis.* Timing of ossification of major cranial elements in this study (black bars) compared with data from Sedra and Michael ('57; stippled bars), Brown ('80; vertically hatched bars), and Bernasconi ('51; diagonally hatched bars). Zero refers to the stage at which we observed first ossification; the timing of earlier observations by other authors is indicated by negative values (e.g., −1 = 1 stage earlier), whereas the timing of later observations is indicated by positive values (e.g., +1 = 1 stage later). For detailed comparisons, see Table 2. Ang(lat), lateral angulosplenial; Ang(med), medial angulosplenial; Den, dentary; Exoc, exoccipital; Fpar, frontoparietal; Max, maxilla; Nas, nasal; Pars ext plec, pars externa plectri; Pars med plec, pars media plectri; Pmax, premaxilla; Pro, prootic; Prsph, parasphenoid; Pter, pterygoid; Sphen, sphenethmoid; Spmax, septomaxilla; Sq, squamosal; Tym ann, tympanic annulus; Vom, vomer.

Development of Xenopus laevis *compared to other anurans*

Pipoid taxa

There are limited data published for three other pipoid taxa. Brown ('80) provided information on ossification sequences of *Xenopus borealis* and *Silurana* (*Xenopus, auctorum*) *tropicalis* between Stages 55 and 60, and Trueb ('85) provided a partial summary of cranial development of *Rhinophrynus dorsalis* (Rhinophrynidae). Early ossification (i.e., the first 5 cranial bones) is the same in the three pipid (*Xenopus* and *Silurana*) taxa; however, the maxilla, premaxilla, and septomaxilla develop one or two stages later in *X. borealis* and *S. tropicalis* than in *X. laevis* (Brown, '80). Timing of ossification of the long bones of the fore- and hind limbs, metacarpals, clavicle, cleithrum, and the ilium varies slightly, but all these bones develop within one or two stages of one another. The most striking difference noted by Brown ('80) was in the timing of ossification of the tibiale, fibulare, and metatarsals, which ossify between Stages 56 and 57 in *X. borealis* and *S. tropicalis,* but not until Stage 60 in *X. laevis.* (See Trueb, '85 for a summary.) However, the results of this study which show these elements to ossify in Stage 57 in *X. laevis* do not substantiate this claim.

Published data on the development of the living sister taxon of the pipids, *Rhinophrynus dorsalis,* are incomplete. Nonetheless, the information provided by Trueb ('85) indicates some interesting similarities and differences. The prootic of *Rhinophrynus* develops later than the exoccipital rather than at the same time, as it does in *X. laevis.* Ossification of the sphenethmoid is evident in *Rhinophrynus* at the onset of metamorphosis (ca. Gosner Stage 41 = Nieuwkoop and Faber Stage 60), whereas the sphenethmoid of *X. laevis* does not develop until metamorphic climax (Nieuwkoop and Faber Stages 64–66). The septomaxilla and pterygoid of *Rhinophrynus* form later than in *X. laevis.*

Non-pipoid taxa

Pelobatid frogs are a member of the superfamily Pelobatoidea, which is the sister group of the Pipoidea (i.e., Rhinophrynidae + Pipidae fide Cannatella ['85]). Using the ontogenetic study of the pelobatid frog, *Spea bombifrons,* by Wiens ('89), it is possible to compare skeletogenesis in these archaeobatrachian taxa.

The same bones form first in the cranium of both *Spea* and *Xenopus laevis* (viz., the frontoparietal, parasphenoid, exoccipital, and the prootic); however, the prootic forms earlier in *X. laevis* than it does in *Spea.* The upper jaw, nasal, and septomaxilla form in the mid-sequence of cranial development in both taxa. In *X. laevis*, the angulosplenial of the mandible ossifies from two centers (unreported in any other anuran) and the larger, medial part ossifies before the upper jaw, nasal, or septomaxilla. In *Spea*, the angulosplenial ossifies after them, at approximately the same time the lateral part of the angulosplenial appears in *X. laevis*. The vomer develops late in *X. laevis* in comparison with *Spea*—that is, after the pterygoid rather than before. The plectral apparatus of *X. laevis* appears well before metamorphosis (Nieuwkoop and Faber Stages 59–61), whereas that of *Spea* develops postmetamorphically (i.e., after Gosner Stage 46), along with the sphenethmoid. With regard to timing (but not composition) of ossification of the braincase, *Spea* and *X. laevis* are more similar to one another than the latter is to *Rhinophrynus*.

All bony elements of the postcranial skeleton of *Spea*, except the prehallux, ischium, carpals, and tarsals, begin to ossify contemporaneous with the first three skull bones and in the stage preceding ossification of the prootic (ca. Gosner Stage 36 ≅ Nieuwkoop and Faber Stage 55). In the hylid, *Hyla lanciformis*, and the ranid, *Rana pipiens*, ossification of the skull and vertebral column is present in Gosner Stages 29 and 30 (≅ Nieuwkoop and Faber Stages 51 and 52), respectively, and the appendicular skeleton develops in Gosner Stages 35–38 (≅ Nieuwkoop and Faber Stages 55–56) (de Sá, '88; Kemp and Hoyt, '69). On the basis of these data, Wiens ('89) hypothesized that the ossification of the skull and vertebral column is delayed in *Spea* relative to *Hyla* and *Rana*. Insofar as we can equate the developmental stages of the various staging tables (Table 1), it is apparent in Figure 11 that in *Xenopus laevis*, the skull and vertebral column form slightly earlier (ca. Gosner Stages 34 and 35) than in *Spea*, but significantly later than in *Rana* or *Hyla*. However, *X. laevis* differs from each of these taxa in the late development of

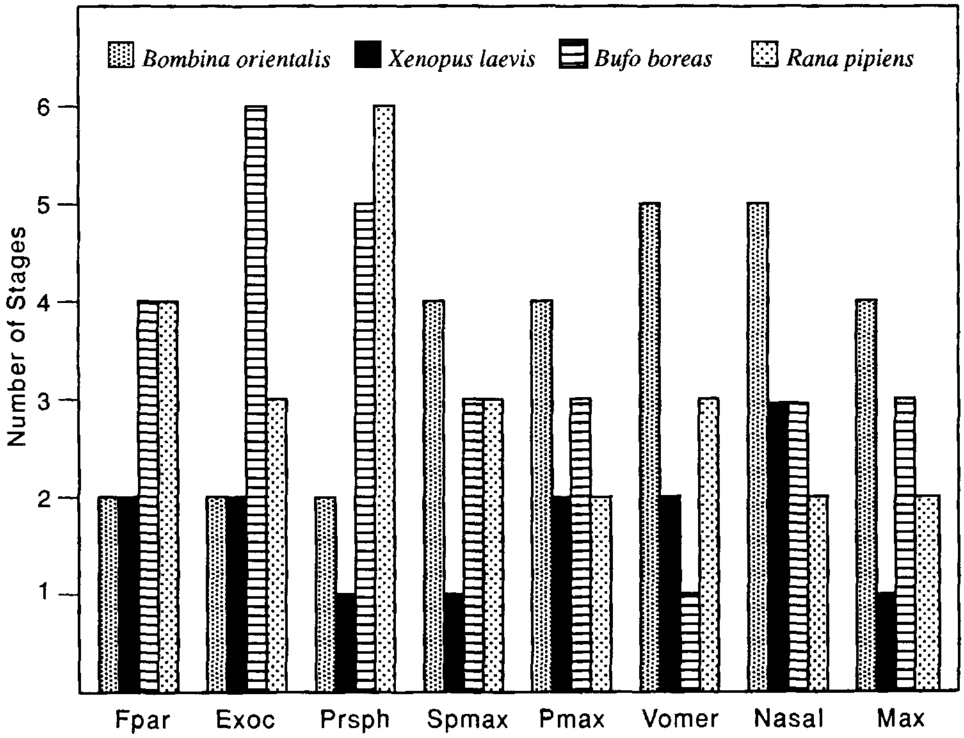

Fig. 14. Temporal variation in the ossification of cranial bones among four taxa of anurans. Numbers on the ordinate refer to the number of developmental stages in which an element has been observed to ossify. Data for *Bombina orientalis* are from Hanken and Hall ('84), for *Bufo boreas* from Gaudin ('78), and for *Rana pipiens* from Kemp and Hoyt ('69). Exoc, exoccipital; Fpar, frontoparietal; Max, maxilla; Pmax, premaxilla; Prsph, parasphenoid; Spmax, septomaxilla.

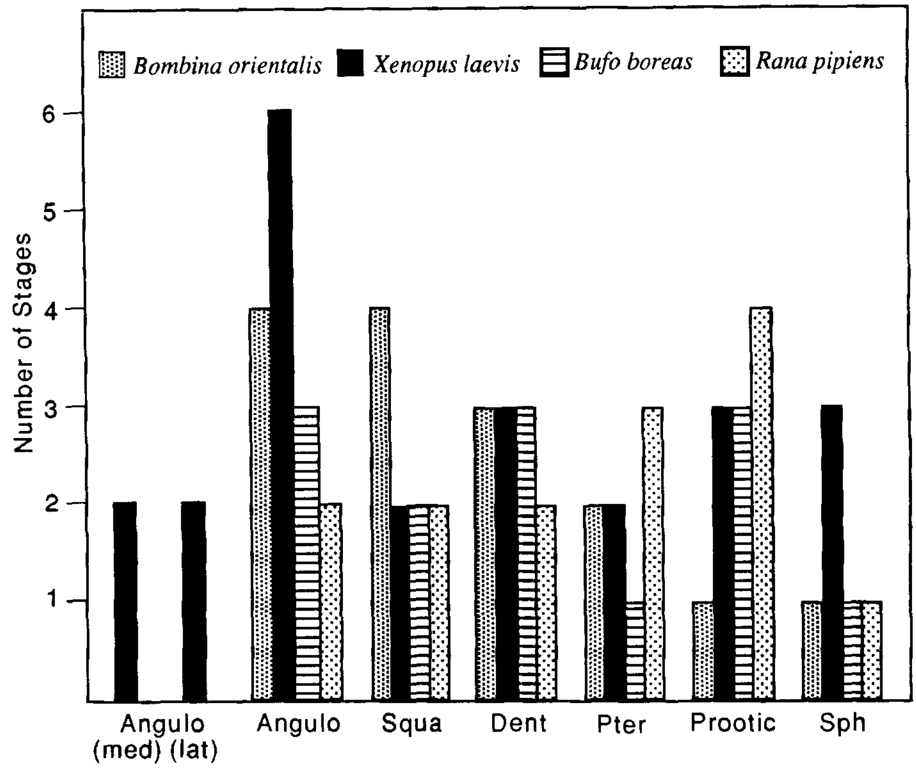

Fig. 15. Temporal variation in the ossification of cranial bones among four taxa of anurans. Numbers on the ordinate refer to the number of developmental stages in which an element has been observed to ossify. Data for *Bombina orientalis* are from Hanken and Hall ('84), for *Bufo boreas* from Gaudin ('78), and for *Rana pipiens* from Kemp and Hoyt ('69). In *Xenopus laevis*, the angulosplenial forms from two centers of ossification (medial and lateral), which are represented separately; the data for the two centers are combined for comparison with the formation of the bone in the other taxa. Angulo, angulosplenial; Dent, dentary; (lat), lateral portion; (med), medial portion; Pter, pterygoid; Sph, sphenethmoid; Squa, squamosal.

the appendicular skeleton which does not begin to ossify until Gosner Stages 39–40.

Timing of ossification relative to developmental stages

The developmental table of Nieuwkoop and Faber ('56) uses external morphology for staging living and whole preserved specimens of *Xenopus laevis*, as do Gosner's ('60) and other schemes for non-pipid anurans. However, Nieuwkoop and Faber's table also includes internal criteria, of which ossification and chondrification events are an important part in their diagnoses of various stages. Hanken and Hall ('84) demonstrated that cranial ossification in *Bombina orientalis* is poorly correlated with the development of external morphology. Given the importance of skeletal developmental features in Nieuwkoop and Faber's ('56) table of normal development, the question arises as to the reliability of these features as staging criteria. The data for cranial bones in *X. laevis* are arrayed with those for laboratory-reared developmental series of *Bombina, Bufo,* and *Rana* in Figures 14 and 15. Examination of these figures shows that cranial development in *X. laevis* is, in many instances, less variable than that observed in the other taxa, although based on the sample examined here, only three cranial bones show fidelity to single stages: the parasphenoid, septomaxilla, and maxilla. Even greater variation is apparent in the development of the postcranial skeleton (Appendices B–D). These results suggest that ossification is an imprecise staging criterion and that features of soft anatomy may be more reliable diagnostic tools of stages of larval development than ossification events in

Nieuwkoop and Faber's ('56) developmental table for *X. laevis.*

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LITERATURE CITED

Baldauf, R.J. (1958) A procedure for the staining and sectioning of the heads of adult anurans. Texas J. Sci. *10:*448–451.

Bernasconi, A.F. (1951) Über den Ossifikationsmodus bei Xenopus laevis Daud. Mem. Soc. Helvet. Sci. Nat. *79:*191–252 + 2 pls.

Brown, S.M. (1980) Comparative Ossification in Tadpoles of the Genus *Xenopus* (Anura, Pipidae). Master's thesis. San Diego State University, San Diego, CA.

Cannatella, D.C. (1985) A Phylogeny of Primitive Frogs (Archaeobatrachians). Ph.D. dissertation, University of Kansas, Lawrence.

Cannatella, D.C., and L. Trueb (1988a) Evolution of pipoid frogs: Intergeneric relationships of the aquatic frog family Pipidae (Anura). Zool. J. Linnean Soc. *94:*1–38.

Cannatella, D.C., and L. Trueb (1988b) Evolution of pipoid frogs: Morphology and phylogenetic relationships of *Pseudhymenochirus.* J. Herpetol. *22:*439–456.

de Beer, G.R. (1937) The Development of the Vertebrate Skull. Oxford: Oxford University Press.

de Sá, R.O. (1988) Chondrocranium and ossification sequence of *Hyla lanciformis.* J. Morphol. *195:*345–355.

de Villiers, C.G.S. (1932) Über das Gehörskelett der Aglossen Anuren. Anat. Anz. *74:*33–55.

Deuchar, E.M. (1975) *Xenopus:* The South African Clawed Frog. New York: John Wiley & Sons.

Dingerkus, G., and L.D. Uhler (1977) Enzyme clearing and Alcian blue stained whole small vertebrates for demonstration of cartilage. Stain Technol. *52:*229–232.

Gaudin, A.J. (1978) The sequence of cranial ossification in the California toad, *Bufo boreas* (Amphibia, Anura, Bufonidae). J. Herpetol. *12:*309–318.

Gaupp, E. (1906) Die Entwicklung des Kopfskelettes. In O. Hertwig (ed): Handbuch der Vergleichenden und Experimentellen Entwicklungslehre der Wirbeltiere, Vol. 3, Part 2. Jena: Verlag von Gustav Fischer, pp. 573–890.

Gosner, K.L. (1960) A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica *16:*183–190.

Hanken, J. (1992) Life history and morphological evolution. J. Evol. Biol. (in press).

Hanken, J., and B.K. Hall (1984) Variation and timing of the cranial ossification sequence of the Oriental fire-bellied toad, *Bombina orientalis* (Amphibia, Discoglossidae). J. Morphol. *182:*245–255.

Hanken, J., and B.K. Hall (1988) Skull development during anuran metamorphosis: I. Early development of the first three bones to form—the exoccipital, the parasphenoid, and the frontoparietal. J. Morphol. *195:* 247–256.

Hanken, J., and R.J. Wassersug (1981) The visible skeleton. Funct. Photog. *16:*22–26, 44.

Just, J.J., J. Kraus-Just, and D.A. Check (1981) Survey of chordate metamorphosis. In L.I. Gilbert and E. Frieden (eds): Metamorphosis: A Problem in Developmental Biology. New York and London: Plenum Press, pp. 265–326.

Kelley, D., D. Sassoon, N. Segil, and M. Scudder (1989) Development and hormone regulation of androgen receptor levels in the sexually dimorphic larynx of *Xenopus laevis.* Dev. Biol. *131:*111–118.

Kemp, N.E., and J.A. Hoyt (1969) Sequence of ossification in the skeleton of growing and metamorphosing *Rana pipiens.* J. Morphol. *129:*415–444.

Kotthaus, A. (1933) Die Entwicklung des Primordial-Craniums von Xenopus laevis bis zur Metamorphose. Z. Wissensch. Zool. *144:*510–572.

Nieuwkoop, P.D., and J. Faber (1956) Normal Table of Xenopus laevis (Daudin). A Systematical and Chronological Survey of the Development from the Fertilized Egg till the End of Metamorphosis. Amsterdam: North-Holland Publ. Co.

Parker, W.K. (1876) On the structure and development of the skull in the Batrachia. Part II. Proc. Zool. Soc. London *24:*601–669.

Parker, W.K. (1881) On the structure and development of the skull in the Batrachia. Part III. Philos. Trans. Roy. Soc. Lond. [Biol.] *1:*1–266 + 44 pls.

Paterson, N. (1939) The head of Xenopus laevis. Q. J. Microsc. Sci. *81:*161–234.

Patterson, C. (1977) Cartilage bones, dermal bones and membrane bones, or the exoskeleton versus the endoskeleton. In S.M. Andrews, R.S. Miles, and A.D. Walker (eds): Problems in Vertebrate Evolution. London and New York: Academic Press, pp. 71–121.

Patterson, C. (1982) Morphological characters and homology. In K.A. Joysey and A.E. Friday (eds): Problems of Phylogenetic Reconstruction. London and New York: Academic Press, pp. 21–74.

Reumer, J.W.F. (1985) Some aspects of the cranial osteology and phylogeny of *Xenopus* (Anura, Pipidae). Rev. Suisse Zool. *92:*969–980.

Ridewood, W.G. (1897) On the structure and development of the hyobranchial skeleton and larynx in *Xenopus* and *Pipa;* with remarks on the affinities of the Aglossa. Zool J. Linnean Soc. London *26:*53–128.

Roček, Z. (1989) Developmental patterns of the ethmoidal region of the anuran skull. In H. Splechtna and H. Hilgers (eds): Trends in Vertebrate Morphology. Stuttgart and New York: Gustav Fischer Verlag, pp. 412–415.

Roček, Z. (1990) Ethmoidal endocranial structures in primitive tetrapods: Their bearing on the search for anuran ancestry. Zool. J. Linnean Soc. *99:*389–407.

Roček, Z., and M. Veselý (1989) Development of the ethmoidal structures of the endocranium in the anuran *Pipa pipa.* J. Morphol. *200:*301–319.

Sadaghiani, B., and C.H. Thiébaud (1987) Neural crest development in the *Xenopus laevis* embryo, studied by intraspecific transplantation and scanning electron microscopy. Dev. Biol. *124:*91–110.

Sassoon, D., and D.B. Kelley (1986) The sexually dimorphic larynx of *Xenopus laevis:* Development and androgen regulation. Am. J. Anat. *177:*457–472.

Sassoon, D., N. Segil, and D. Kelley (1986) Androgen-induced myogenesis and chondrogenesis in the larynx of *Xenopus laevis.* Dev. Biol. *113:*135–140.

Sedra, S.N., and M.I. Michael (1957) The development of the skull, visceral arches, larynx and visceral muscles of the South African clawed toad, *Xenopus laevis* (Daudin) during the process of metamorphosis (from Stage

55 to Stage 66). Verhand. Koninklijke Nederlandse Akad. Wetenschappen Natuurkinde *51:*1–80.

Shaw, J.P. (1979) The time scale of tooth development and replacement in *Xenopus laevis* (Daudin). J. Anat. *129:*323–342.

Shaw, J.P. (1985) Tooth replacement in adult *Xenopus laevis* (Amphibia: Anura). J. Zool. Lond. (A) *207:*171–179.

Shaw, J.P. (1986) A longitudinal study of tooth resorption in newly metamorphosed *Xenopus laevis,* with comments on tooth resorption in amphibians. J. Zool. Lond. (A) *208:*215–228.

Shaw, J.P. (1988) A quantitative comparison of osteoclasts in the teeth of the anuran amphibian *Xenopus laevis.* Arch. Oral Biol. *33:*451–453.

Smit, A.L. (1953) The ontogenesis of the vertebral column of Xenopus laevis (Daudin) with special reference to the segmentation of the metotic region of the skull. Ann. Univ. Stellenbosch *29:*79–136.

Sokol, O.M. (1975) The phylogeny of anuran larvae: A new look. Copeia *1975:*1–23.

Starrett, P.H. (1973) Evolutionary patterns in larval morphology. In J.L. Vial (ed): Evolutionary Biology of the Anurans. Columbia, MO: University of Missouri Press, pp. 251–271.

Taylor, A.C., and J.J. Kollros (1946) Stages in the normal development of Rana pipiens larvae. Anat. Rec. *94:*7–23.

Thomson, D.A.R. (1986) Meckel's cartilage in *Xenopus laevis* during metamorphosis: A light and electron microscope study. J. Anat. *149:*77–87.

Thomson, D.A.R. (1987) A quantitative analysis of cellular and matrix changes in Meckel's cartilage in *Xenopus laevis.* J. Anat. *151:*249–254.

Thomson, D.A.R. (1989) A preliminary investigation into the effect of thyroid hormones on the metamorphic changes in Meckel's cartilage in *Xenopus laevis.* J. Anat. *162:*149–155.

Trueb, L. (1985) A summary of osteocranial development in anurans with notes on the sequence of cranial ossification in *Rhinophrynus dorsalis* (Anura: Pipoidea: Rhinophrynidae). South African J. Sci. *81:*181–185.

Trueb, L., and R. Cloutier (1991a) Toward an understanding of the amphibians: Two centuries of systematic history. In H.-P. Schultze and L. Trueb (eds): Origins of the Higher Groups of Tetrapods: Controversy and Consensus. Ithaca, New York: Cornell University Press, pp. 175–193.

Trueb, L., and R. Cloutier. (1991b) A phylogenetic investigation of the inter- and intrarelationships of the Lissamphibia (Amphibia: Temnospondyli). In H.-P. Schultze and L. Trueb (eds): Origins of the Higher Groups of Tetrapods: Controversy and Consensus. Ithaca, New York: Cornell University Press, pp. 223–313.

Wassersug, R.J. (1976) A procedure for differential staining of cartilage and bone in whole formalin-fixed vertebrates. Stain Technol. *51:*131–134.

Weisz, P.B. (1945a) The development and morphology of the larva of the South African clawed toad, Xenopus laevis. I. The third-form tadpole. J. Morphol., *77:*163–192.

Weisz, P.B. (1945b) The development and morphology of the larva of the South African clawed toad, Xenopus laevis. II. The hatching and the first- and second-form tadpoles. J. Morphol. *77:*193–217.

Wiens, J.J. (1989) Ontogeny of the skeleton of *Spea bombifrons* (Anura: Pelobatidae). J. Morphol. *202:*29–51.

APPENDIX A. Schedule of ossification of cranial elements in Xenopus laevis[1]

Bone	Developmental stages (Nieuwkoop and Faber, '56)												
	54	55	56	57	58	59	60	61	62	63	64	65	66
Frontoparietal	1/2	7/7	+	+	+	+	+	+	+	+	+	+	+
Parasphenoid		7/7	+	+	+	+	+	+	+	+	+	+	+
Exoccipital		4/7	6/6	+	+	+	+	+	+	+	+	+	+
Prootic		4/7	5/6	5/5	+	+	+	+	+	+	+	+	+
Angulosplenial (medial)			5/6	5/5	+	+	+	+	+	+	+	+	+
Maxilla				5/5	+	+	+	+	+	+	+	+	+
Premaxilla					4/5	5/5	+	+	+	+	+	+	+
Nasal					3/5	4/5	5/5	+	+	+	+	+	+
Septomaxilla							5/5	+	+	+	+	+	+
Teeth							5/5	+	+	+	+	+	+
Dentary							2/5	3/5	5/5	+	+	+	+
Angulosplenial (lateral)							2/5	5/5	+	+	+	+	+
Pterygoid								2/5	5/5	+	+	+	+
Tympanic annulus								3/5	5/5	+	+	+	+
Pars externa plectri								3/5	5/5	+	+	+	+
Pars media plectri									4/5	5/5	+	+	+
Squamosal									1/5	5/5	+	+	+
Vomer										4/5	5/5	+	+
Sphenethmoid											4/5	2/5	5/5
Pars articularis													5/5

[1] The number to the left of solidus indicates the number of individuals in which the element is ossified; the number to the right is the total number of specimens examined in that stage. A "+" indicates that the bone was present in all individuals examined.

APPENDIX B. Schedule of axial skeletogenesis in Xenopus laevis[1]

Element	Developmental stages (Nieuwkoop and Faber, '56)																		
	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Neural Arch I																			
Cartilage	22	+	+	+	+	+	+	+	+										
Bone								4/7	5/6	5/5	+	+	+	+	+	+	+	+	+
Neural Arch II																			
Cartilage	2/2	+	+	+	+	+	+	+	+										
Bone								4/7	5/6	5/5	+	+	+	+	+	+	+	+	+
Neural Arch III																			
Cartilage	2/2	+	+	+	+	+	+	+	+										
Bone								4/7	5/6	5/5	+	+	+	+	+	+	+	+	+
Neural Arch IV																			
Cartilage	1/2	+	+	+	+	+	+	+	+										
Bone								3/7	5/6	5/5	+	+	+	+	+	+	+	+	+
Neural Arch V																			
Cartilage	1/2	1/2	2/2	+	+	+	+	+	+										
Bone								3/7	5/6	5/5	+	+	+	+	+	+	+	+	+
Neural Arch VI																			
Cartilage	1/2	1/2	2/2	+	+	+	+	+	+										
Bone								2/7	4/6	5/5	+	+	+	+	+	+	+	+	+
Neural Arch VII																			
Cartilage	1/2	1/2	1/2	2/2	+	+	+	+	+										
Bone								2/7	3/6	5/5	+	+	+	+	+	+	+	+	+
Neural Arch VIII																			
Cartilage		1/2	–	1/2	1/2	2/2	+	+	+										
Bone								1/7	1/6	5/5	+	+	+	+	+	+	+	+	+
Neural Arch IX																			
Cartilage				1/2	1/2	2/2	+	6/7	+										
Bone										5/5	+	+	+	+	+	+	+	+	+
Neural Arch X																			
Cartilage						2/2	+	+	+	+									
Bone										1/5	5/5	+	+	+	+	+	+	+	+
Neural Arch XI																			
Cartilage						1/2	1/2	2/6	1/6	–	–	–	–	–	–	1/5			
Bone												1/5	4/5	4/5	4/5	4/5	5/5	+	+
Neural Arch XII																			
Cartilage															3/5	1/5			
Bone															1/5	3/5	5/5	+	+

(continued)

APPENDIX B. Schedule of axial skeletogenesis in Xenopus laevis[1] *(continued)*

Element	Developmental stages (Nieuwkoop and Faber, '56)																		
	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Centrum I																			
Cartilage						2/2	+	+	+										
Bone								4/6	5/6	5/5	+	+	+	+	+	+	+	+	+
Centrum II																			
Cartilage							2/2	1/6	1/6										
Bone								2/6	4/6	5/5	+	+	+	+	+	+	+	+	+
Centrum III																			
Cartilage							1/2	1/6	1/6										
Bone								1/6	2/6	5/5	+	+	+	+	+	+	+	+	+
Centrum IV																			
Cartilage							1/2	1/6	–										
Bone								1/6	–	5/5	+	+	+	+	+	+	+	+	+
Centrum V																			
Cartilage							1/2	–	–										
Bone										5/5	+	+	+	+	+	+	+	+	+
Centrum VI																			
Cartilage							1/2	–	–										
Bone										5/5	+	+	+	+	+	+	+	+	+
Centrum VII																			
Cartilage																			
Bone										5/5	+	+	+	+	+	+	+	+	+
Centrum VIII																			
Cartilage																			
Bone										5/5	+	+	+	+	+	+	+	+	+
Centrum IX																			
Cartilage																			
Bone										4/5	5/5	+	+	+	+	+	+	+	+
Hypochord																			
Cartilage														1/5	2/5				
Bone													1/5	3/5	2/5	5/5	+	+	Fused
Urostyle																			
Cartilage																			
Bone																		5/5	Fused
Rib PSV II																			
Cartilage										2/5	+	+							
Bone											3/5	4/5	5/5	+	+	+	+	+	+
Rib PSV III																			
Cartilage										3/5									
Bone											5/5	+	+	+	+	+	+	+	+
Rib PSV IV																			
Cartilage										2/5	+	+							
Bone											3/5	4/5	5/5	+	+	+	+	+	+
TP PSV II																			
Cartilage														5/5	+	+			
Bone																	5/5	+	+
TP PSV III																			
Cartilage														5/5	+	+			
Bone																	5/5	+	+
TP PSV IV																			
Cartilage														5/5	+	+			
Bone																	5/5	+	+
TP PSV V																			
Cartilage																1/5	+	+	
Bone																	2/5	4/5	5/5
TP PSV VI																			
Cartilage																1/5	+	+	
Bone																	2/5	4/5	5/5
TP PSV VII																			
Cartilage																1/5	+	+	
Bone																	3/5	4/5	5/5
TP PSV VIII																			
Cartilage																1/5	+		
Bone																	4/5	5/5	+
Sacral diapophysis																			
Cartilage															2/5				
Bone																5/5	+	+	+

[1] The number to the left of the solidus indicates the number of individuals in which the element is either present in cartilage or bone; the number to the right is the total number of specimens examined in that stage. A "+" indicates that either bone or cartilage was present in all individuals examined, whereas a "–" indicates absence. PSV, presacral vertebra; TP, transverse process.

APPENDIX C. Pectoral girdle and forelimb skeletogenesis in Xenopus laevis[1]

Element	Developmental stages (Nieuwkoop and Faber, '56)																		
	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Coracoid																			
Cartilage										5/5	+	+							
Bone											3/5	4/5	5/5	+	+	+	+	+	+
Scapula																			
Cartilage									4/6	5/5	+	+	+	+					
Bone													1/5	2/5	5/5	+	+	+	+
Clavicle											4/5	5/5	+	+	+	+	+	+	+
Epicoracoid and Procoracoid										4/5	5/5	+	4/5	5/5	+	+	+	+	+
Sternum															3/5	5/5	?4/5	5/5	+
Suprascapula										5/5	+	+	+	+	+	+	+	+	+
Cleithrum											3/5	4/5	5/5	+	+	+	+	+	+
Humerus																			
Cartilage	2/2[2]	+	+	+	?	?1/2	?	?6/7	+	2/5									
Bone										3/5	5/5	+	+	+	+	+	+	+	+
Radioulna																			
Cartilage					?	?	?	?6/7	6/6	2/5									
Bone										3/5	5/5	+	+	+	+	+	+	+	+
Ulnare																			
Cartilage					?	?	?	?5/7	6/6	+	+	+	+	+	+	+	+	+	+
Bone																			
Postaxial centrale																			
Cartilage					?	?	?	?2/7	5/6	5/5	+	+	+	+	+	+	+	+	+
Bone																			
Preaxial centrale																			
Cartilage					?	?	?	?1/7	5/6	5/5	+	+	+	+	+	+	+	+	+
Bone																			
Distal Carpal I																			
Cartilage								?	1/6	5/5	+	+	+	+	+	+	+	+	+
Bone																			
Distal Carpal II																			
Cartilage							?	?1/7	2/6	5/5	+	+	+	+	+	+	+	+	+
Bone																			
Distal Carpal III																			
Cartilage							?	?2/7	5/6	5/5	+	+	+	+	+	+	+	+	+
Bone																			
Metacarpal I																			
Cartilage							?	?1/7	4/6	5/5	+	+		+	+				
Bone											1/5	2/5	5/5	4/5	5/5	+	+	+	+
Metacarpal II																			
Cartilage							?	?3/7	6/6	+	+								
Bone											3/5	5/5	+	+	+	+	+	+	+
Metacarpal III																			
Cartilage							?	?3/7	6/6	+	+								
Bone											3/5	5/5	+	+	+	+	+	+	+
Metacarpal IV																			
Cartilage							?	?3/7	6/6	+	+								
Bone											3/5	5/5	+	+	+	+	+	+	+
Phalanx I-1																			
Cartilage										5/5	+	+	+	+	+				
Bone													2/5	1/5	2/5	5/5	+	+	+
Phalanx I-2																			
Cartilage										5/5	+	+	+	+	+	+	+	+	+
Bone																	3/5	4/5	4/5
Phalanx II-1																			
Cartilage										5/5	+	+	+	+					
Bone													3/5	3/5	5/5	+	+	+	+
Phalanx II-2																			
Cartilage										5/5	+	+	+	+	+	+	+		
Bone																3/5	3/5	5/5	+
Phalanx III-1																			
Cartilage								?	1/6	5/5	+	+	+	+	+				
Bone													3/5	3/5	4/5	5/5	+	+	+

(continued)

APPENDIX C. Pectoral girdle and forelimb skeletogenesis in Xenopus laevis[1] *(continued)*

Element	Developmental stages (Nieuwkoop and Faber, '56)																		
	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Phalanx III-2																			
Cartilage										5/5	+	+	+	+	+	+			
Bone														3/5	1/5	3/5	5/5	+	+
Phalanx III-3																			
Cartilage										5/5	+	+	+	+	+	+	+	+	+
Bone																1/5	2/5	2/5	4/5
Phalanx IV-1																			
Cartilage								?	1/6	5/5	+	+	+	+	+				
Bone													4/5	3/5	3/5	5/5	+	+	+
Phalanx IV-2																			
Cartilage										5/5	+	+	+	+	+	+			
Bone														1/5	1/5	3/5	5/5	+	+
Phalanx IV-3																			
Cartilage										5/5	+	+	+	+	+	+	+	+	+
Bone																1/5	2/5	2/5	4/5
Sesamoids															4/5	5/5	+	+	+

[1] The number to the left of the solidus indicates the number of individuals in which the element is either present in cartilage or bone; the number to the right is the total number of specimens examined in that stage. A "+" indicates that either bone or cartilage was present in all individuals examined. A "?" indicates that some or all of the specimens were damaged in preparation such that elements that might be expected to be present could not be documented.

[2] Limb bud.

APPENDIX D. Pelvic girdle and hind-limb skeletogenesis in Xenopus laevis[1]

Element	Developmental stages (Nieuwkoop and Faber, '56)																		
	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Ilium																			
Cartilage								2/7	6/6	+									
Bone										1/5	5/5	+	+	+	+	+	+	+	+
Ischium																			
Cartilage													2/5	+	+				
Bone													1/5	2/5	2/5	5/5	+	+	+
Pubis																			
Cartilage													2/5	4/5	5/5	+	+	+	
Bone																			5/5
Epipubis													1/5	2/5	4/5	5/5	+	+	+
Femur																			
Cartilage	2/2[2]	+	+	+	?1/2	?	?1/2	7/7	+	+									
Bone										4/5	5/5	+	+	+	+	+	+	+	+
Tibia and fibula																			
Cartilage					?	?	?1/2	7/7	+										
Bone										5/5	+	+	+	+	+	+	+	+	+
Tibiale and fibulare																			
Cartilage					?	?	?1/2	7/7	+	+									
Bone										1/5	5/5	+	+	+	+	+	+	+	+
Naviculare																			
Cartilage						?	?1/2	7/7	+	+	+	+	+	+	+	+	+	+	+
Bone																			
Tarsal I																			
Cartilage								1/7	6/6	+	+	+	+	+	+	+	+	+	+
Bone																			
Tarsal II																			
Cartilage							?	3/7	6/6	+	+	+	+	+	+	+	+	+	+
Bone																			
Prehallux																			
Cartilage									4/6	5/5	+	+	+	+	+	+	+	+	+
Bone																			
Metatarsal I																			
Cartilage						?	?	4/7	6/6	+									
Bone										1/5	5/5	+	+	+	+	+	+	+	+
Metatarsal II																			
Cartilage						?	?1/2	7/7	+	+									
Bone										1/5	5/5	+	+	+	+	+	+	+	+

(continued)

APPENDIX D. Pelvic girdle and hind-limb skeletogenesis in Xenopus laevis[1] *(continued)*

Element	Developmental stages (Nieuwkoop and Faber, '56)																		
	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Metatarsal III																			
Cartilage						?	?1/2	7/7	+	+									
Bone										1/5	5/5	+	+	+	+	+	+	+	+
Metatarsal IV																			
Cartilage						?	?1/2	7/7	+	+									
Bone										1/5	5/5	+	+	+	+	+	+	+	+
Metatarsal V																			
Cartilage						?	?1/2	7/7	5/6	+									
Bone										1/5	5/5	+	+	+	+	+	+	+	+
Phalanx I-1																			
Cartilage									2/6	5/5	+	+							
Bone												2/5	5/5	+	+	+	+	+	+
Phalanx I-2																			
Cartilage										5/5	+	+							
Bone												2/5	5/5	+	+	+	+	+	+
Phalanx II-1																			
Cartilage								1/7	5/6	5/5	+	+							
Bone											4/5	4/5	5/5	+	+	+	+	+	+
Phalanx II-2																			
Cartilage									2/6	5/5	+	+							
Bone											3/5	4/5	5/5	+	+	+	+	+	+
Phalanx III-1																			
Cartilage								3/7	6/6	+	+								
Bone											4/5	5/5	+	+	+	+	+	+	+
Phalanx III-2																			
Cartilage									4/6	5/5	+	+							
Bone											2/5	4/5	5/5	+	+	+	+	+	+
Phalanx III-3																			
Cartilage									2/6	5/5	+	+		+					
Bone											1/5	2/5	5/5	4/5	5/5	+	+	+	+
Phalanx IV-1																			
Cartilage								3/7	6/6	+	+								
Bone											3/5	5/5	+	+	+	+	+	+	+
Phalanx IV-2																			
Cartilage									4/6	5/5	+	+							
Bone											2/5	4/5	5/5	+	+	+	+	+	+
Phalanx IV-3																			
Cartilage									1/6	5/5	+	+		+					
Bone												1/5	5/5	4/5	5/5	+	+	+	+
Phalanx IV-4																			
Cartilage										5/5	+	+	+	+	+	+	+		
Bone													1/5	1/5	1/5	4/5	3/5	5/5	+
Phalanx V-1																			
Cartilage								1/7	5/6	5/5	+	+							
Bone											2/5	4/5	5/5	+	+	+	+	+	+
Phalanx V-2																			
Cartilage									2/6	5/5	+	+		+					
Bone												1/5	5/5	4/5	5/5	+	+	+	+
Phalanx V-3																			
Cartilage										5/5	+	+	+	+	+	+	+		
Bone													1/5	1/5	0/5	3/5	4/5	5/5	+

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[1] The number to the left of the solidus indicates the number of individuals in which the element is either present in cartilage or bone; the number to the right is the total number of specimens examined in that stage. A "+" indicates that either bone or cartilage was present in all individuals examined. A "?" indicates that some or all of the specimens were damaged in preparation such that elements that might be expected to be present could not be documented. Separate entries for chondrification of parts of pelvic girdle are noted to indicate when cartilage elements were distinct from one another.

[2] Limb bud.