

## Early Development of Chondrocranium in the Tailed Frog *Ascaphus truei* (Amphibia: Anura): Implications for Anuran Palatoquadrate Homologies

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**ABSTRACT** Chondrocranial development in *Ascaphus truei* was studied by serial sectioning and graphical reconstruction. Nine stages (21–29; 9–18 mm TL) were examined. Mesodermal cells were distinguished from ectomesenchymal (neural crest derived) cells by retained yolk granules. Ectomesenchymal parts of the chondrocranium include the suprarostrals, pila preoptica, anterior trabecula, and palatoquadrate. Mesodermal parts of the chondrocranium include the orbital cartilage, posterior trabecula, parachordal, basiotic lamina, and otic capsule. Development of the palatoquadrate is as follows. The pterygoid process first connects with the trabecula far rostrally; their fusion progresses caudally. The ascending process connects with a mesodermal bar that extends from the orbital cartilage to the otic capsule, and forms the ventral border of the dorsal trigeminal outlet. This bar is the “ascending process” of *Ascaphus* adults; it is a neurocranial, not palatoquadrate structure. The basal process chondrifies in an ectomesenchymal strand running from the quadrate keel to the postpalatine commissure. Later, the postpalatine commissure and basal process extend anteromedially to contact the floor of the anterior cupula of the otic capsule, creating separate foramina for the palatine and hyomandibular branches of the facial nerve. Based on these data, and on comparison with other frogs and salamanders, the anuran anterior quadratocranial commissure is homologized with the pterygoid process of salamanders, the anuran basal process (=“pseudobasal” or “hyobasal” process) with the basal process of salamanders, and the anuran otic ledge with the basitrabecular process of salamanders. The extensive similarities in palatoquadrate structure and development between frogs and salamanders, and lacking in caecilians, are not phylogenetically informative. Available information on fossil outgroups suggests that some of these similarities are primitive for Lissamphibia, whereas for others the polarity is uncertain. *J. Morphol.* 231:63–100, 1997. © 1997 Wiley-Liss, Inc.

The palatoquadrate cartilage forms the endoskeletal framework of the upper jaw in all osteichthyans, including tetrapods (Arratia and Schultze, '91). In this study, I attempt to resolve the historical controversy over the homologies between particular palatoquadrate processes in anurans (frogs and toads) and those of other tetrapods. Both larval and adult palatoquadrate homologies have been controversial (reviewed by de Beer, '37; Pyles, '88; Reiss, '93). In larvae, two fundamentally different schemes of homology have been proposed for the connection between the palatoquadrate and the trabecula in the ethmoidal region (Edgeworth,

'23, '25; de Beer, '37; Pusey, '38, '43; Jarvik, '42). In adults, at least six different schemes of homology have been proposed for the connection between the palatoquadrate and the base of the neurocranium (Gaupp, 1893, '06; de Beer, '26, '37; Kruijtz, '31; Pusey, '38, '43; N.G. Stephenson, '51; van der Westhuisen, '61; Swanepoel, '70).

Resolving this controversy is important because it allows one to establish valid synapomorphies involving the palatoquadrate,

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and thereby reconstruct its evolution. For example, within the Anura, two of the five morphological characters supporting the *Leiopelmatanura* involve the palatoquadrate (Ford and Cannatella, '93). Within the Lissamphibia (frogs, salamanders, and caecilians) as a whole, palatoquadrate homologies are particularly important for reconstructing the evolution of anuran metamorphosis; palatoquadrate remodelling is one of the most notable metamorphic events in anurans (Wassersug and Hoff, '82; Rose and Reiss, '93).

When alternative hypotheses of homology exist, there are at least two possible approaches to resolving the conflict. First, knowledge of phylogenetic relationships can help. Such knowledge cannot actually falsify particular schemes of homology (in the sense of topographical correspondence), because these schemes are logically prior to phylogenetic analysis (Rieppel, '88, fig. 4). It can, however, lead us to seek another scheme more congruent with the phylogeny (e.g., Shubin, '94). Second, additional information can help. Learning more about the feature and its topographical relation to other features within individual taxa, or about variability among taxa, may make one scheme clearly preferable to another. Developmental information can be particularly helpful, because ontogeny often reveals individual elements, and relations between these elements, that are obscured in mature forms (e.g., Rieppel, '77; Shubin and Alberch, '86; but see Hall, '94).

A recent molecular analysis embeds *Ascaphus truei*, the tailed frog of the Pacific Northwest, within a monophyletic archaeobatrachian clade (Hay et al., '95). Morphological characters, however, strongly support the traditional viewpoint that *Ascaphus* and the New Zealand genus *Leiopelma* are the most basal living frogs (Ford and Cannatella, '93). Because of its presumed basal phylogenetic position, *Ascaphus* historically has played an important role in the controversy over anuran palatoquadrate homologies (de Villiers, '34; de Beer, '37; Pusey, '38, '43; van Eeden, '51; van der Westhuizen, '61). Structural peculiarities of the palatoquadrate of *Ascaphus* larvae have been considered primitive by some workers (Pusey, '43); others (van Eeden, '51; van der Westhuizen, '61) have interpreted the same features as adaptations correlated with the oral sucker mechanism that larvae use to cling to rocks in fast-flowing mountain streams (Gradwell, '73).

In the present study, I describe the early development of the *Ascaphus* chondrocranium, and determine the mesodermal versus ectomesenchymal (neural crest) origin of its parts. I also describe notable features of mouth, nasal sac, and otic sac ontogeny. This description provides a basis for future comparison with development in other, more derived, anurans. My primary focus, however, is on the palatoquadrate and adjacent structures. I identify the individual developmental components that contribute to the highly fused larval palatoquadrate, and examine the relations of these components to other elements of the head, such as nerves, blood vessels, and gill pouches. These data, when analyzed in a comparative context, suggest a resolution of the controversy over anuran palatoquadrate homologies.

#### MATERIALS AND METHODS

*Ascaphus* mates in the fall; females store sperm through the winter and lay fertilized eggs the following spring (Metter, '64a,b). Gravid females were collected from tributaries of Cougar Reservoir, Lane County, Oregon, in early July. Ovulation was induced by intraperitoneal injection of 250 IU human chorionic gonadotropin (Sigma), following Brown ('75). Embryos were separated from the female and reared either at 11° or 15–17°C in dechlorinated tap water. Larvae were collected from the same streams as the adults, as well as from Source Creek and Denny Creek, in the Snoqualmie Pass area, King County, Washington. Additional specimens provided by Dr. H. Brown of Western Washington University came from the Anderson-Watson Lakes Trail, Whatcom County, Washington. Both reared and field-collected larvae were kept in dechlorinated tap water at 15–17°C and fed on algae-covered rocks from a colleague's fish aquaria.

*Ascaphus* does not conform to the standard developmental stages of Gosner ('60), and alternative criteria have been defined only for prehatching development through stage 21 (Brown, '89; see also Wernz and Storm, '69). Therefore new morphological criteria were defined for embryonic and larval development through stage 29 (Table 1). Sectioned specimens were prepared for all stages from 21–29. Specimens of stages 23 and 24 showed a similar degree of chondrocranial development, and thus are treated together. Cleared-and-stained specimens were prepared for stages 25–29. In addition, both sectioned and cleared-and-stained se-

TABLE 1. Staging criteria used

Stage	Approximate age and length (15°C)	Initial criterion used here for <i>Ascaphus</i>	Gosner ('60) initial criterion	Corresponding Brown ('89) age and length (11°C)
20	16–18 days 9–10 mm	Vitelline circulation present (Brown, '89)	Circulation in external gills	23 days 9.7 mm
21	18–20 days 9–10 mm	Operculum present	Cornea transparent	
22	20–24 days (hatching) 10–11 mm	Operculum covers branchial arches, fused with yolk sac	Tail fin circulation	28 days (hatching) 11 mm
23	24–26 days 11–12 mm	External nasal tube present	Opercular fold present	32 days 12 mm
24	26–28 days 12–13 mm	Oral disc overlaps anterodorsal margin of adhesive organ	Operculum fused on right side	
25	30–34 days 13–15 mm	Oral disc protrudes ventrally beyond adhesive organ (functional)	Operculum fused bilaterally; spiracle formed	46 days 16.7 mm
26	34–40 days 15–16 mm	Anal flap present ventral to hind limbs	Hindlimb bud appears; length < ½ times diameter	57 days 18 mm
27	40–45 days 16–17 mm	Opercular openings fused; median spiracle formed	Hindlimb length > ½ times diameter	66 days 19.7 mm
28	45–54 days 17–19 mm	Dental formula (2–3/6); upper and lower beak keratinized	Hindlimb length > diameter	120 days
29	54+ days 18+ mm	Hindlimb length > 1½ times diameter	Hindlimb length > 1½ times diameter	

ries of larval and metamorphic stages were prepared for reference purposes.

Specimens to be sectioned were fixed in aqueous Bouin's solution, embedded in Paraplast (Monoject Scientific), and sectioned transversely at 8 µm (stages 21–26), 10 µm (stages 26–29), or 15 µm (older larvae and juveniles). Sections were stained with Gill's hematoxylin and eosin or Mallory's triple connective tissue stain (Humason, '79). Sections were drawn with the aid of a camera lucida or microprojector. Graphical reconstructions of selected stages were prepared following the method of Pusey ('39). Sections were aligned by the midline of the brain for ventral reconstructions, and by comparison with lateral views of the specimen for lateral and medial reconstructions. Specimens to be cleared-and-stained were fixed in 10% neutral buffered formalin (Humason, '79), and processed following Dingerkus and Uhler ('77).

Recognition of tissue origin (neural crest derived versus mesodermal) was based on the presence of abundant large yolk granules in mesodermal tissues, compared with few or no small yolk granules in ectomesenchymal tissues. This histological distinction has been noted by others working with mesolecithal vertebrates (e.g., Platt, 1897; Stone, '22, '26, '29; Bertmar, '59); it is quite clear in the younger stages of *Ascaphus*, but becomes less so with advancing development. The method has one major limitation.

Whereas individual mesodermal cells in the midst of neural crest cells are readily identifiable, individual neural crest cells in the midst of mesodermal cells are obscured by the yolk granules. Moreover, by the time chondrification occurs only some of the mesodermal cells still contain yolk; thus any neural crest cells in a predominantly mesodermal region would escape detection. Due to this limitation, the "mesodermal" structures identified here may contain some neural crest cells. A recent study of *Bombina orientalis* using vital labeling (Olsson and Hanken, '96) found neural crest contributions to regions (the posterior trabecula, pila antotica, and dorsal end of the ascending process) that appear mesodermal based on histological criteria in *Rana palustris* (Stone, '29) and *Ascaphus*; these regions are most likely of mixed origin.

## RESULTS

The following description is organized by region; the regions are identical to those used by Pusey ('43) and van Eeden ('51) in describing later developmental stages. Each section begins with a general orientation, followed by a description of the conditions in the earliest stage examined and their subsequent modifications. A more complete description of the individual stages can be found in Reiss ('93).

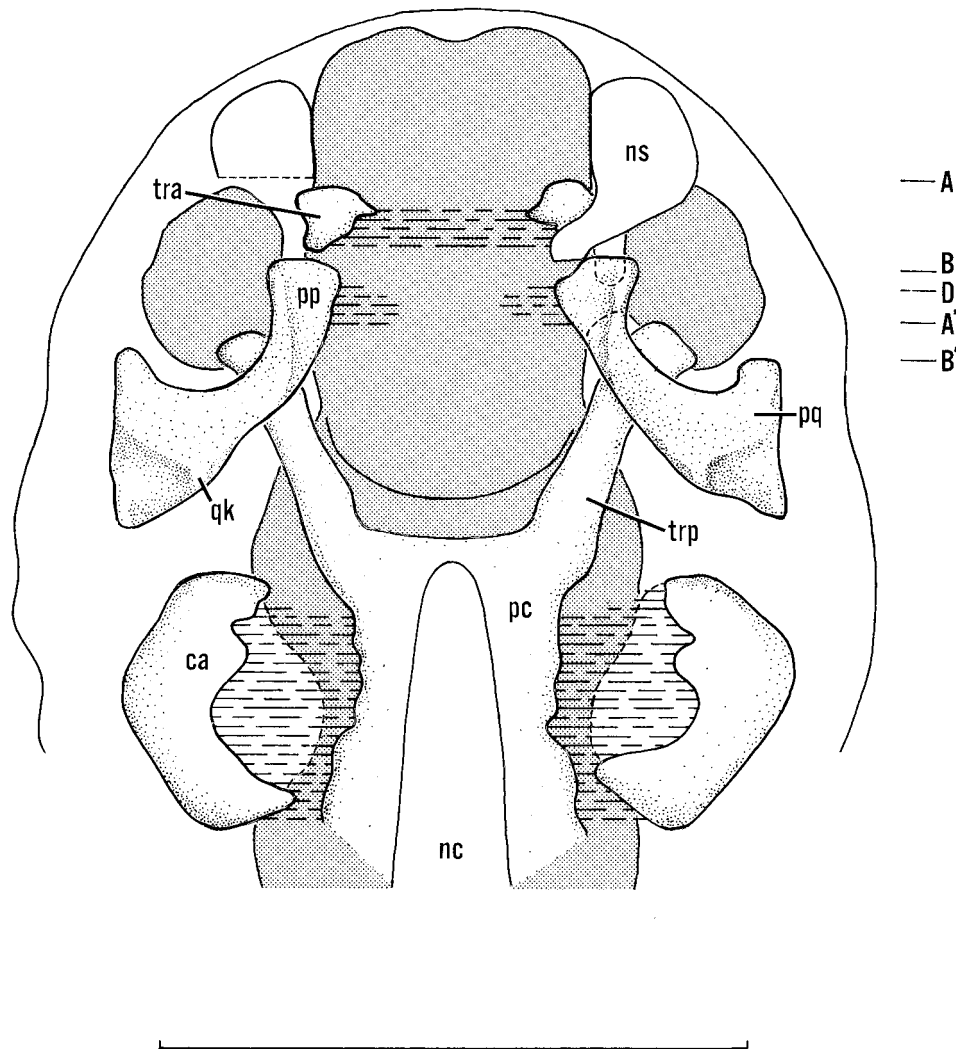


Fig. 1. *Ascaphus truei*. Stage 21. Graphical reconstruction of mesenchymal chondrocranium, ventral view. Mesenchymal condensations represented by stippling, areas of loose mesenchyme by broken horizontal lines. Suprarostal mesenchyme omitted. Scale = 1 mm. Let-

ters A-D, A'-B' indicate levels of sections in Figures 3 and 4. ca, auditory capsule; nc, notochord; ns, nasal sac; pc, parachordal; pp, pterygoid process; pq, palatoquadrate; qk, quadrate keel; tra, anterior trabecula; trp, posterior trabecula.

#### *Anterior end of the neurocranium and the suprarostal system*

The anterior neurocranium consists of the following basic parts: 1) the anterior trabeculae, forming the floor of the neurocranium, 2) the pilae preoptica, forming the sides of the neurocranium between olfactory and optic nerves, and 3) the medial and lateral suprarostals, supporting the upper lip. This region forms exclusively from neural crest mesenchyme.

At the earliest stage examined (stage 21), the anterior trabeculae are just beginning to condense (Figs. 1, 2). The pilae preoptica are still mesenchymal (Fig. 3A, B). The medial and lateral suprarostals are represented by a layer of dense mesenchyme (Fig. 3A, mes) overlying the ectodermal roof of the stomodaeum, just anterior to the tip of the saclike preoral gut.

As development proceeds (stage 22), the anterior trabeculae condense further as

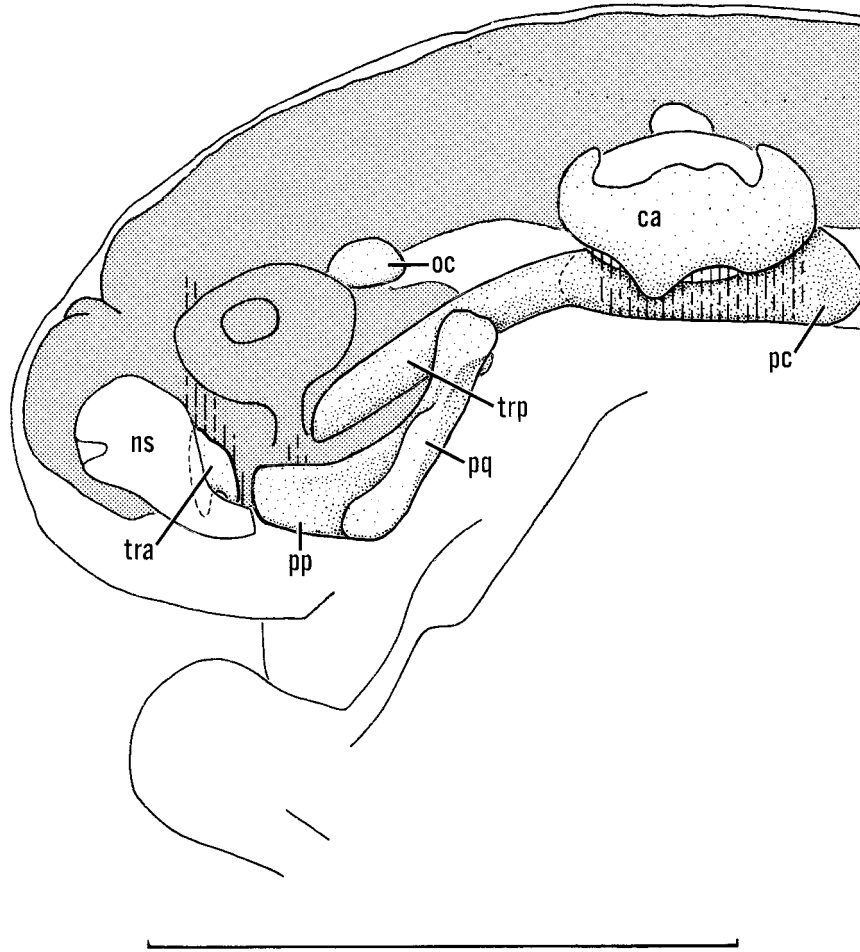


Fig. 2. *Ascaphus truei*. Stage 21. Graphical reconstruction of mesenchymal chondrocranium, lateral view. Conventions as in Figure 1. Scale = 1 mm. ca, auditory capsule; ns, nasal sac; oc, orbital cartilage; pc, parachordal; pp, pterygoid process; pq, palatoquadrate; tra, anterior trabecula; trp, posterior trabecula.

paired rods beneath the telencephalon (Fig. 5, tra). The pilae preoptica (Fig. 5A, ppo) condense as mesenchymal sheets on either side of the brain, behind the olfactory nerve. As the brain separates from the stomodeal roof, the trabecula develops a more and more prominent ventral flange, continuous with the mesenchyme representing the medial suprarostal. This flange is the rudiment of the trabecular horn (Fig. 5B, trh). The "inrolling" of the upper lip (described below) carries the mesenchymal rudiments of the medial suprarostals ventrally and caudally, so that they come to lie ventrolateral to the tip of the preoral gut (Fig. 5C, msr, g).

With further development (stages 23–25), the trabecula and pila preoptica begin to chondrify from behind forwards (Figs. 7, 10, 11). By stage 26, chondrification has spread forward past the olfactory nerve, thereby enclosing it in a foramen. Early on, a mesenchymal intertrabecular ligament connects the two trabeculae (stage 23, Fig. 7), and this soon chondrifies as the trabecular plate (stage 25, Fig. 10). In the oldest specimen examined (stage 29) the cranial floor remains incomplete rostrally (Figs. 17, 19A), and lacks the "subcranial pit" described in older larvae by Pusey ('43).

Meanwhile, the trabecular horns extend further ventrally, and begin to chondrify

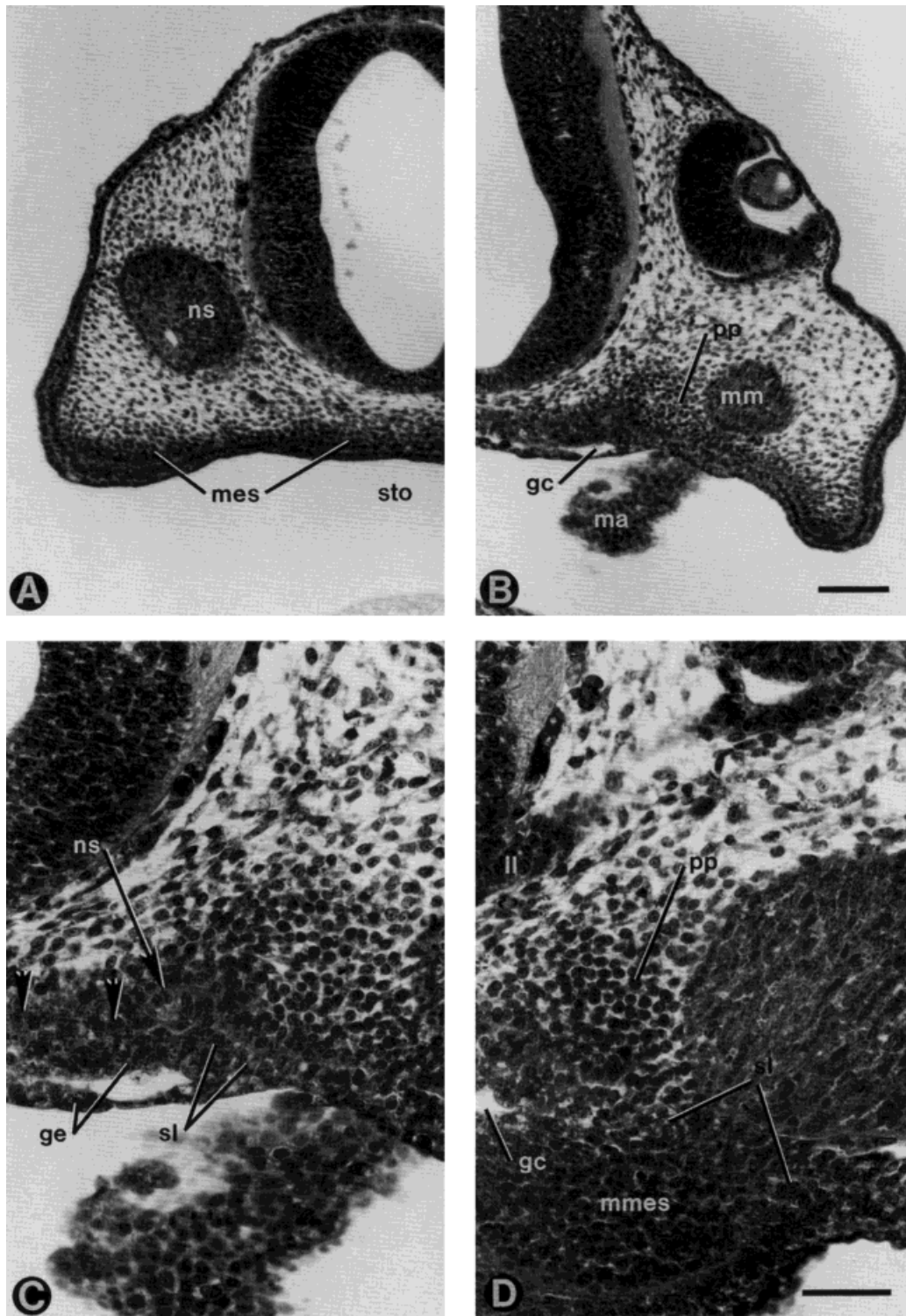


Figure 3

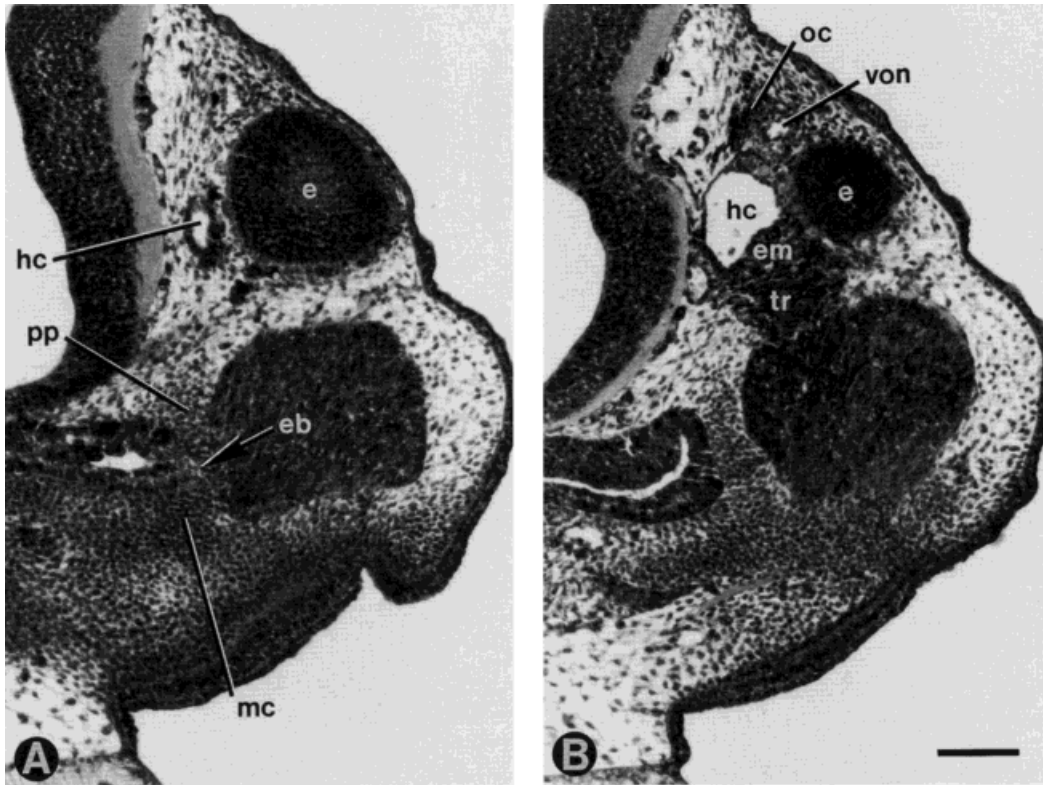


Fig. 4. *Ascaphus truei*. Stage 21. Transverse sections through rear of eye (e). Levels of sections are indicated in Figure 1. **A:** Section through rostral part of premandibular head cavity (hc). Shows common blastema of pterygoid process of palatoquadrate (pp) and Meckel's cartilage (mc). Note lightly staining ectodermal band

(eb) along ventrolateral corner of the gut. **B:** Section through middle of head cavity. Note condensations of orbital cartilage (oc) and posterior trabecula (tr), which are continuous with the eye muscle mesenchyme (em). The orbital cartilage lies dorsomedial to the orbitonasal vein (von). Scale = 100  $\mu$ m.

Fig. 3. *Ascaphus truei*. Stage 21. Serial transverse sections through rostral end of head. Levels of sections are indicated in Figure 1. **A:** Section behind external naris. There is not yet a trabecular condensation between brain and nasal sac (ns) at this level. Dense ectomesenchyme (mes) overlies the roof of the stomodeum (sto). **B:** Section through rostral end of pterygoid process rudiment (pp), which lies medial to the mandibular muscle mass (mm). The front of the mandibular process (ma) is cut tangentially. Note the gut cavity (gc), surrounded by the endodermal wall of the preoral gut. **C:** Higher magnification of B to show caudal end of nasal sac (ns) lying on the dorsal wall (arrowheads) of the gut (ge). The tip of the arrow indicates the basal lamina of the nasal epithelium. Note also the mediad extension of the sensory layer of the surface ectoderm (sl) above the gut, toward the nasal epithelium. **D:** Section through optic nerve (II), 32  $\mu$ m caudal to C. Shows mandibular arch mesenchyme (mmes) separating edge from remainder of sensory layer of ectoderm. Scale for A–B = 100  $\mu$ m, for C–D = 50  $\mu$ m.

(stage 23). Chondrification spreads from the trabecular horns down into the medial suprarostals, which now form an L-shaped flange wrapping around the ventrolateral corner of the preoral gut (Figs. 7, 8A, 10, 12B, msr). At no time is any boundary between trabecular horns and medial suprarostals apparent. By stage 26, the two medial suprarostals are beginning to fuse in the midline, beneath the preoral gut. This fusion is largely complete by stage 29 (Figs. 17, 19B).

At the same time, two longitudinal ridges form along the lateral side of the trabecular horn and medial suprarostal. The dorsal ridge lies just below the nasal sac and above the dorsal border of a large lymph space. This is the "subnasal ridge" (Pusey, '43). The ventral ridge lies along the ventral surface of the lymph space. It is much larger than the dorsal ridge, and forms the articulation with the lateral suprarostal (Fig. 19B).

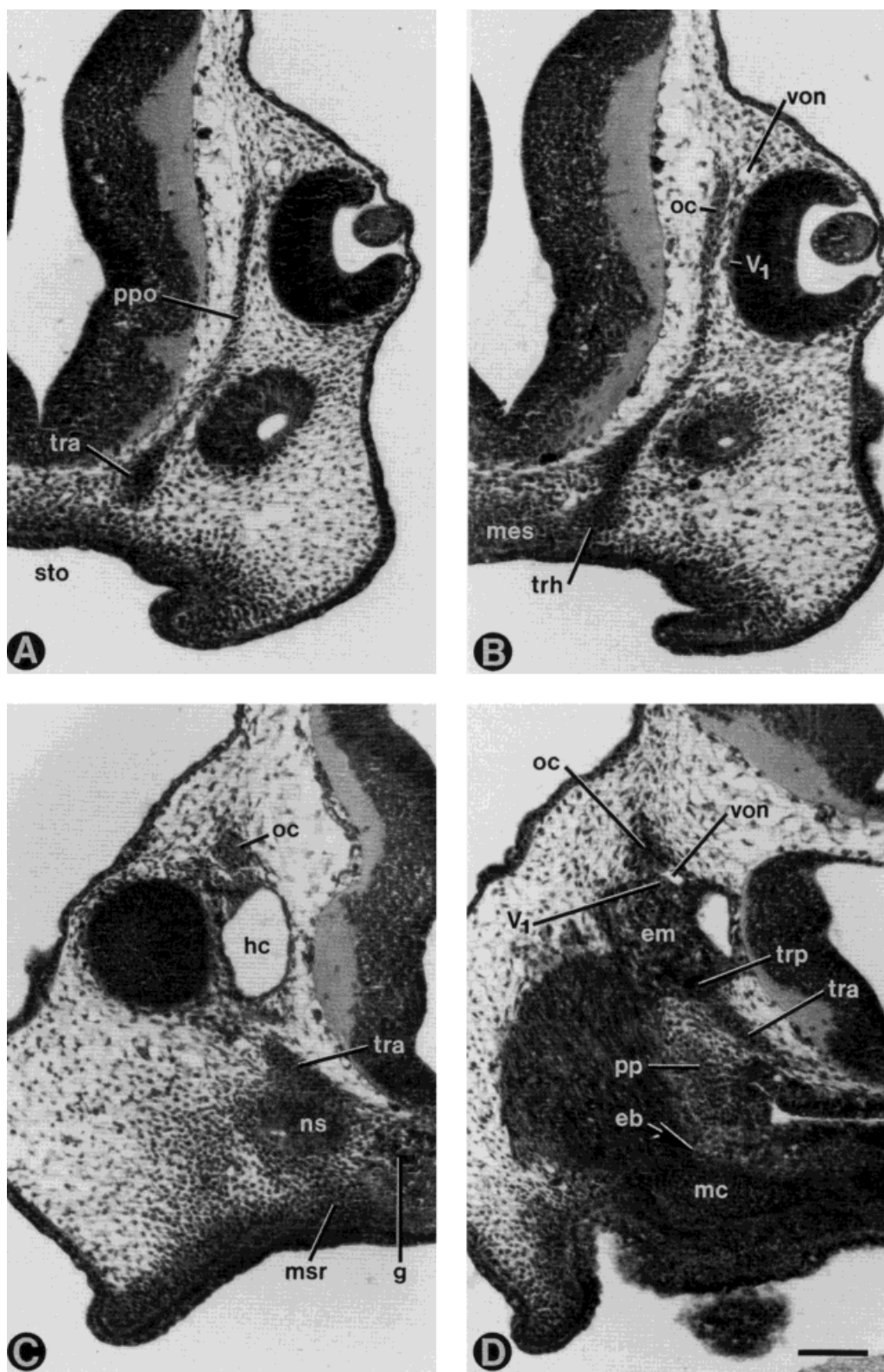


Figure 5



The lateral suprarostrals begin to chondrify after the medial suprarostrals (stage 25, Figs. 10, 11, 12B, lsr). With further development they become increasingly robust; in the last stage examined they articulate directly with the medial suprarostrals (Figs. 17, 19B).

#### *Nasal sac and preoral gut*

The nasal sac and preoral gut of *Ascaphus* larvae are unusual (Pusey, '43; Baard, '82). In larvae, the gut tube extends well in front of the mouth, which opens into its ventral surface. This creates a preoral gut ("pre-oral buccal cavity" of Pusey, '43) enclosed dorsally by the trabeculae and ventrolaterally by the medial suprarostrals. The nasal sac has a long, membranous (nonsensory) caudal extension (the "posterior narial tube" of Pusey, '43) leading to the choana. The choana opens into the lateral side of the preoral gut through a notch between the medial suprarostal and the trabecula (cf. Fig. 11). Most unusually, a band of ciliated epithelium innervated by the olfactory nerve (the "epithelial band" of Pusey, '43) runs forward from the choana in the wall of the preoral gut.

At the earliest stage examined (stage 21), the stomodeum is a shallow depression on the ventral surface of the head. The preoral gut is a flattened pouch of endoderm lying exposed in the roof of the stomodeum (Fig. 3B, C, gc, ge). Its edge abuts the superficial layer of the surrounding ectoderm, but lies

ventral to the free edge of the sensory layer. The dorsal wall of the stomodeum is much thicker than the ventral wall. The mouth opening has not yet broken through.

The nasal sac runs back from the external naris; it has a lumen only rostrally (Figs. 1, 2, 3A, ns). Caudad, the sac is continued by a wedge of epithelium—the choanal process—that contacts the dorsolateral surface of the preoral gut (Fig. 3C). The tip of the choanal process is also in contact with the free edge of the sensory layer of the surrounding ectoderm (Figs. 3C, D, sl). The sensory layer extends caudad from this level along the ventrolateral corner of the gut as a narrow ectodermal band (Fig. 4A, eb). This band appears comparable to the "ectodermal collar" around the foregut in salamanders (Adams, '24; Medvedeva, '61; Bertmar, '66).

As development proceeds, the stomodeum deepens (Figs. 5A, 8A). The preoral gut assumes the shape of a box, rather than a flattened pouch. Moreover, tissue now appears beneath its anterior tip, which previously lay free. This tissue contains the developing medial suprarostrals. All of these effects appear to result from an inrolling of the expanding upper and lower lips. The mouth breaks through to the gut cavity late in stage 22.

Due to the change in gut shape, the choanal process now contacts the lateral side of the gut, rather than its roof. As development proceeds, the lumen of the nasal sac extends into the choanal process, breaking through to the gut lumen to form the choana at stage 23 (Fig. 8C, cho). At the same time, the walls of the rostral end of the choanal process thin, taking on the characteristics of the larval posterior narial tube (Fig. 8A). The caudal part of the choanal process, by contrast, expands greatly. It extends forward from the choana in the wall of the preoral gut, gradually taking on the characteristics of the larval epithelial band (Figs. 12B, 19B, C). The lateral appendix of the nasal sac (cf. Baard, '82) first appears in stage 25, and Jacobson's organ in stage 26.

The band of sensory ectoderm running along the gut wall can be followed through stage 24, after which the distinction of ectodermal from endodermal tissues is difficult.

#### *Floor of the neurocranium*

The floor of the neurocranium consists of the following basic parts: 1) paired anterior (ectomesenchymal) and posterior (mesodermal) trabeculae, underlying the prosen-

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Fig. 5. *Ascaphus truei*. Stage 22. Serial transverse sections through head. **A:** Section through condensations of anterior trabecula (tra) and pila preoptica (ppo). Note deepening of stomodeum (sto) compared with previous stage. **B:** Section 48  $\mu$ m behind A. The condensation of the trabecular horn (trh) extends down from the trabecula and blends with the ectomesenchyme of the upper lip (mes). The dorsal end of the cranial side wall is the mesodermal orbital cartilage (oc). **C:** Section 56  $\mu$ m behind B. The large premandibular head cavity (hc) fills the gap between condensations of orbital cartilage and anterior trabecula. The nasal sac (ns) lies ventral to the trabecula, and is approaching the preoral gut (g). The dense ectomesenchyme beneath the nasal sac is the rudiment of the medial suprarostal (msr). **D:** Section behind eye, to show contact of anterior (ectomesenchymal) and posterior (mesodermal) trabeculae (tra, trp). Both orbital cartilage and posterior trabecula are still continuous with the eye muscle mesenchyme (em), which is continuous with the mandibular muscle mass. The orbitonasal vein (von) and deep ophthalmic nerve ( $V_1$ ) here run ventromedial to the orbital cartilage, but further rostrally (B) run laterally to it. The condensation of the pterygoid process (pp) is distinct from that of the anterior trabecula. It is separated from Meckel's cartilage (mc) by the intervention of the ectodermal band (eb) laterally and gut endoderm medially. Scale = 100  $\mu$ m.

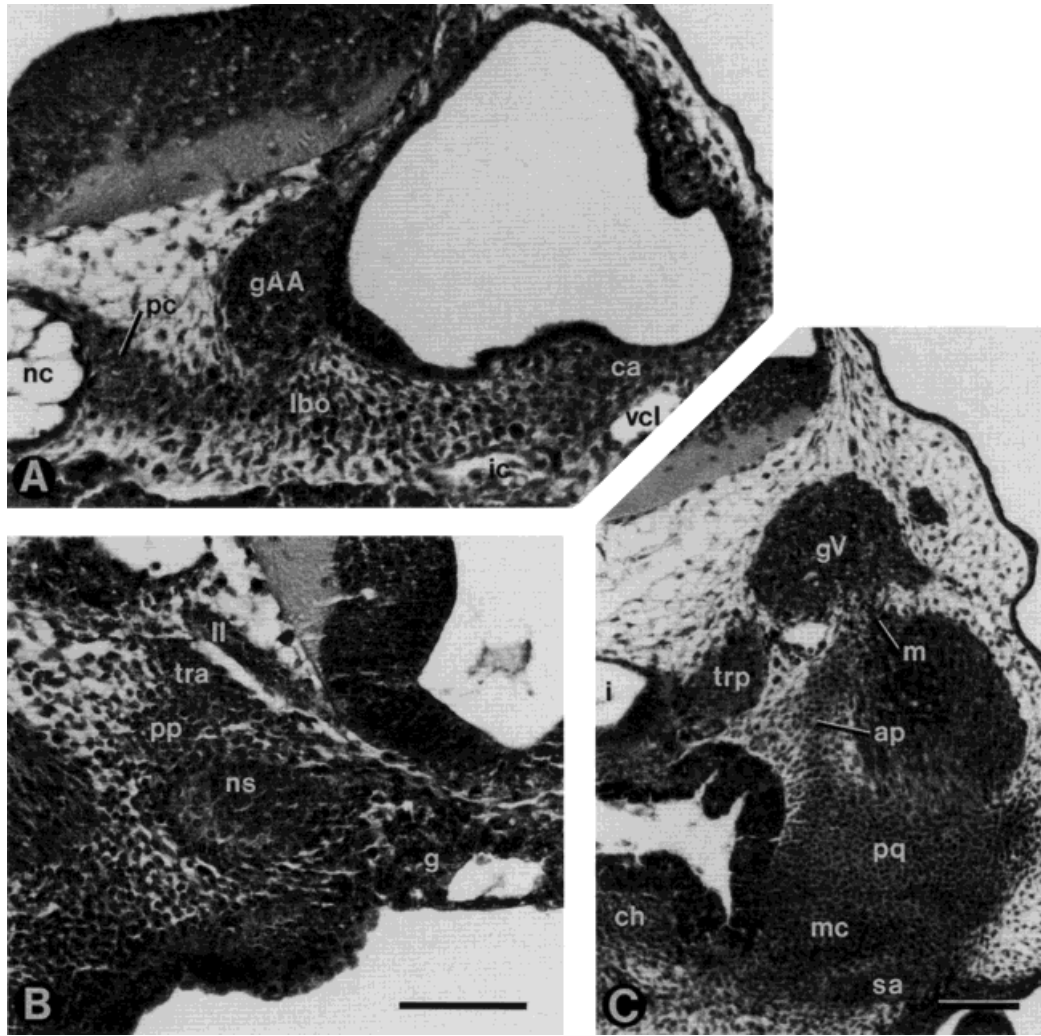


Fig. 6. *Ascapheus truei*. Stage 22. **A:** Section through otic vesicle and anterior acoustic ganglion (gAA). The parachordal (pc) is just beginning to chondrify at the lateral side of the notochord (nc). The otic capsule (ca) is still procartilaginous. The mesenchymal precursor of the basiotic lamina (lbo) can be seen stretching between them. Note the position of the internal carotid artery (ic) and the vena capitis lateralis (vcl). **B:** Section through preoral gut (g) in region of future mouth opening (between levels of Figs. 5C and 5D). The rostral tip of the pterygoid process condensation (pp) can be seen extending down from the anterior trabecula (tra), above the

nasal sac (ns). Note optic nerve (II). **C:** Section through trigeminal ganglion (gV). The mesodermal posterior trabecula (trp) lies below the ganglion, and lateral to the infundibulum (i). More laterally, the condensation of the ascending process (ap) extends up from the palatoquadrate body (pq). Its dorsal tip meets a mesodermal condensation (m) extending back from the orbital cartilage. The condensation of Meckel's cartilage (mc) is separating from the palatoquadrate. The m. suspensorioangularis (sa) can be seen beneath Meckel's cartilage. More medially, the ceratohyal condensation is visible (ch). Scales for A–B and C = 100  $\mu$ m.

cephalon, 2) paired parachordals, lying on either side of the notochord, and 3) the acrochordal cartilage, connecting the parachordals in front of the notochord. I also consider the basiotic laminae to be part of the skull base, but for ease of description they will be

treated with the otic capsule. All these structures are mesodermal, with the exception of the anterior trabeculae.

In the earliest stage examined (stage 21), the floor of the neurocranium is represented by a continuous prochondral condensation of

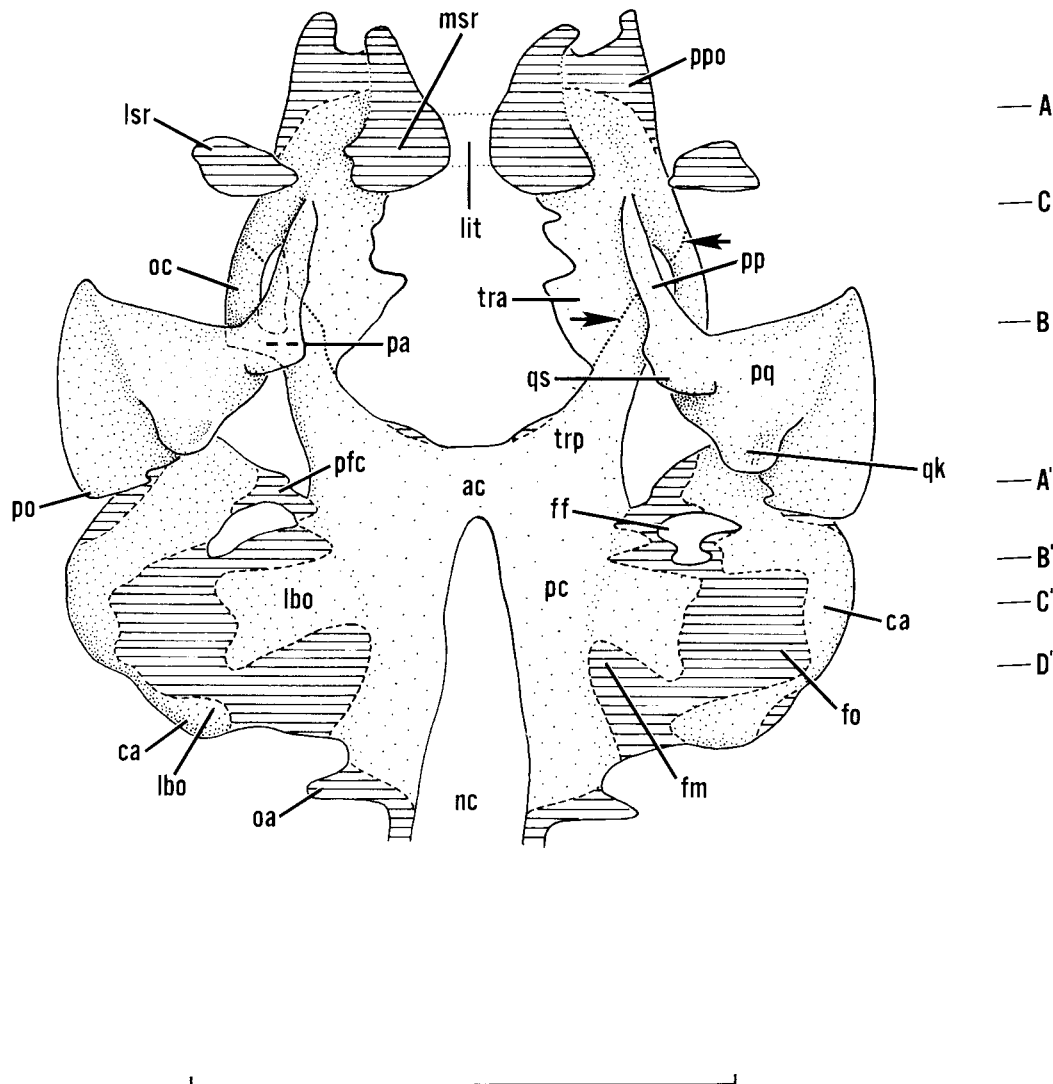


Fig. 7. *Ascaphus truei*. Stage 23. Graphical reconstruction of chondrocranium, ventral view. Regions of cartilage stippled, mesenchymal regions indicated by horizontal lines. Dotted lines (arrows) indicate approximate boundary between mesodermal and ectomesenchymal tissues. Scale = 1 mm. Letters A-C, A'-D' indicate levels of sections in Figures 8, 9. ac, acrochordal cartilage; ca, auditory capsule; ff, facial foramen; fm, mesotic

fissure; fo, fenestra ovalis; lbo, basiotic lamina; lit, intertrabecular ligament; lsr, lateral suprarostral; msr, medial suprarostral; nc, notochord; oa, occipital arch; oc, orbital cartilage; pc, parachordal; pfc, prefacial commissure; po, otic process; pp, pterygoid process; ppo, pila preoptica; pq, palatoquadrate; qk, quadrate keel; qs, quadrate spur; tra, anterior trabecula; trp, posterior trabecula.

the posterior trabeculae, acrochordal cartilage, and parachordals (Figs. 1, 2). As noted above, the anterior (ectomesenchymal) trabeculae have just begun to condense. The short posterior (mesodermal) trabeculae extend from the caudal border of the eye back along the lateral side of the infundibulum,

well above the gut roof. The rostral end of the posterior trabecula is continuous with the eye muscle mesenchyme, which derives from the lateral wall of an extensive premandibular head cavity (Fig. 4B, tr, em, hc). Caudally, the posterior trabeculae are continuous with the parachordals, which ex-

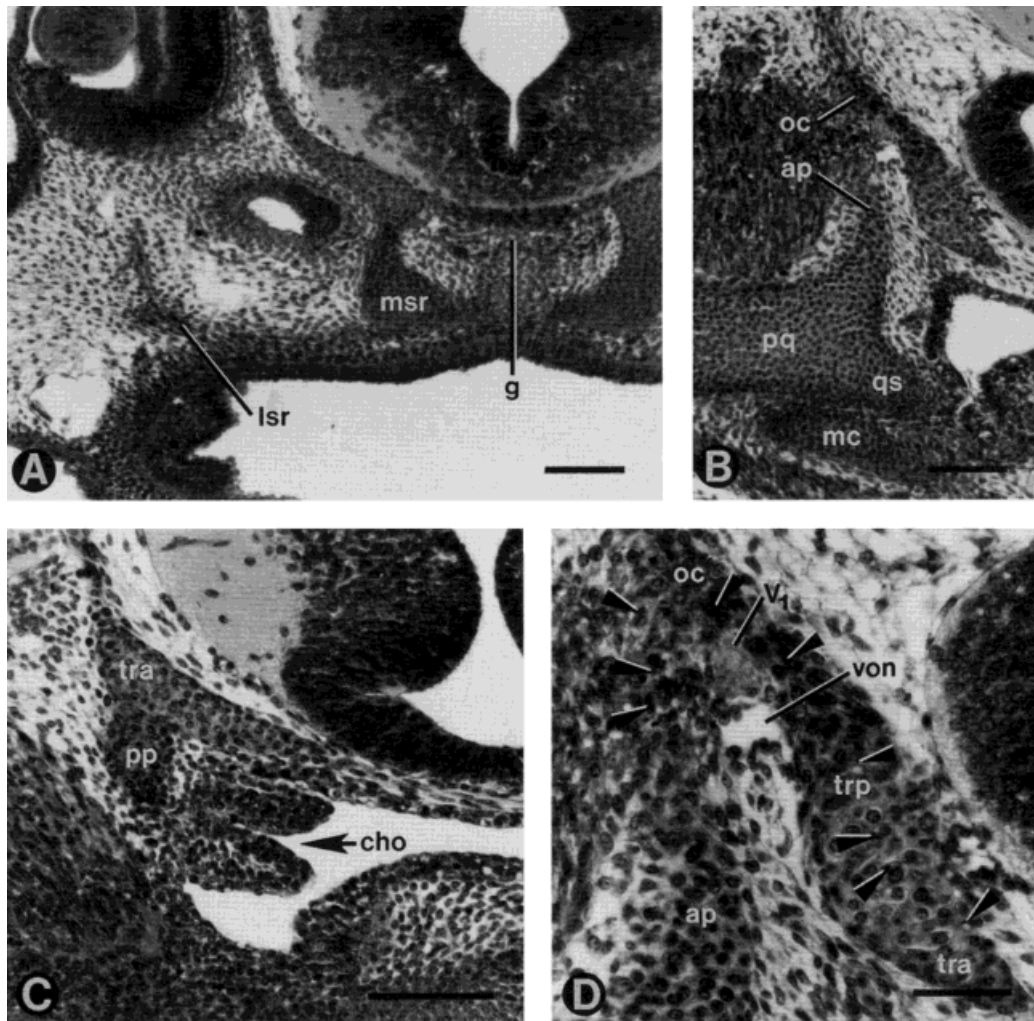


Fig. 8. *Ascaaphus truei*. Stages 23 and 24. Transverse sections; levels indicated in Figure 7. **A:** Stage 23. Section through trabecular horns and medial suprarostals (msr), which extend beneath the preoral gut (g). Lateral suprarostal (lsr) just condensing. **B:** Stage 23. Section through articulation of palatoquadrate (pq) and Meckel's cartilage (mc). Note mesenchymal connection of ascending process (ap) and orbital cartilage (oc), also quadrate spur (qs). **C:** Stage 24. Section through choana (cho). The pterygoid process (pp) and anterior trabecula (tra) are now chondrified. **D:** Higher magnification of B,

to show yolk granules (arrowheads) in mesodermally derived tissues. They are present at the dorsal end of the ascending process, and in condensations of the orbital cartilage (oc), and posterior trabecula (trp). They are absent in the ascending process proper and the anterior trabecula. The mesenchymal connection of orbital cartilage and trabecula, medial to the deep ophthalmic branch of the trigeminal ( $V_1$ ) and the orbitonasal vein (von), is the caudal border of the pila antotica. Scale for A–C = 100  $\mu$ m, for D = 50  $\mu$ m.

tend back to slightly behind the level of the vagal roots. The rudiment of the acrochordal cartilage lies just anterior to the tip of the notochord.

As development proceeds (stage 22), the anterior trabeculae appear as paired strips of procartilage. Their caudal ends are in

contact with the ventromedial edge of the eye muscle mesenchyme and posterior trabeculae (Fig. 5D, tra). At the same time, chondrification begins in the parachordals, acrochordal cartilage, and caudal end of the trabeculae, and spreads forward into the anterior trabeculae (stage 23, Fig. 7). The

acrochordal cartilage becomes quite wide rostrocaudally, apparently as a result of the retreat of the notochordal tip. The parachordals fuse in the midline dorsal to the notochord in stage 23, thus completing the basal plate. They fuse ventral to the notochord sometime after stage 29.

The completion of the cranial floor occurs gradually. As noted above, the trabecular plate chondrifies in stage 25, thereby enclosing the hypophysial fenestra rostrally (Fig. 10). By stage 26, the trabecular plate has grown back much further, but is not in contact with the posterior (mesodermal) trabeculae. By stage 27, the hypophysial fenestra is almost gone; a narrow shelf has grown forward from the acrochordal cartilage to meet the trabecular plate at the level of the craniopalatine foramina (for the palatine arteries). At this late stage, I could not determine the neural crest versus mesodermal origin of the newly formed parts of the cranial floor. It seems likely, however, that the craniopalatine foramina (Fig. 17, fcp) mark the approximate boundary between these cell populations.

Caudally, the parachordals chondrify in continuity with the base of the atlantal neural arches. The occipital arches appear in mesenchyme in stage 23 (Fig. 7, oa) and chondrify in stage 25 (Fig. 10). In stage 26, the arches fuse with the caudal end of the capsule, enclosing the jugular foramen for the glossopharyngeal and vagus nerves (cf. Fig. 18). The first spinal nerve exits between occipital and atlantal arches. It has both dorsal and ventral roots and a dorsal root ganglion, as well as a more distal ganglion, presumably sympathetic.

Secondarily, a separation arises between the occiput and the atlas, formed by a zone of cells resembling procartilage that appears in the parachordal (Fig. 16C). Sometime after stage 29 this develops into a synovial articulation.

#### *Side wall of the neurocranium and its foramina*

The side wall of the neurocranium consists of the following basic parts: 1) dorsally, the orbital cartilage, which lies above the optic and oculomotor nerves, 2) ventrally, the anterior and posterior trabeculae, 3) rostrally, the pila preoptica, and 4) caudally, the pila antotica, lying between oculomotor and trigeminal nerves. Later a pila metoptica forms, separating the optic and oculomotor nerves, and the taenia marginalis connects

the orbital cartilage with the otic capsule, completing the prootic foramen. The pila preoptica and trabeculae are described above; the present description focuses on the orbital cartilage and pila antotica. The prefacial commissure is treated with the otic capsule.

The side wall of the neurocranium is ectomesenchymal rostrally and mesodermal caudally. The boundary between these populations passes caudoventrally through the rear of the optic foramen (Figs. 7, 10, 11). The pila metoptica is predominantly mesodermal in origin, but contains some crest cells anteroventrally, at its junction with the trabecula.

In the earliest stage examined (stage 21), the mesodermal mesenchyme that gives rise to the caudal end of the orbital cartilage forms a condensation lying along the dorso-medial border of the orbitonasal vein. This condensation extends from the level of the optic nerve to just in front of the trigeminal ganglion (Figs. 2, 4B, oc). For most of its extent, the condensation is continuous ventrally with the eye muscle mesenchyme. At its caudal end, the orbital cartilage is connected by loose mesodermal mesenchyme with the posterior trabecula. This connection is the rudiment of the pila antotica.

As development proceeds (stage 22), the mesodermal orbital cartilage becomes better defined (Fig. 5). Rostrally, it meets the ectomesenchymal tissue of the newly formed pila preoptica. Caudally, the orbital cartilage is continued as a cord of mesodermal cells along the lateral side of the trigeminal ganglion, below the maxillomandibular nerve. This cord connects with the mesenchymal ascending process of the palatoquadrate (Fig. 6C, m, ap; see below).

By stage 23, the orbital cartilage and pila antotica are chondrifying (Figs. 7, 8B), thereby enclosing a large foramen for the optic and oculomotor nerves. As in earlier stages, this foramen is filled almost entirely by the premandibular (eye muscle) mesenchyme, though the premandibular head cavity itself is considerably reduced.

With further development, chondrification of these structures advances. A mesenchymal extension grows out from the caudal end of the orbital cartilage toward the otic capsule in stage 23. This is the taenia marginalis. It begins to chondrify in stage 25 (Fig. 11, tm), and reaches the otic capsule in stage 26 (Fig. 14B, C). This contact encloses the prootic foramen (for the trigeminal, abdu-

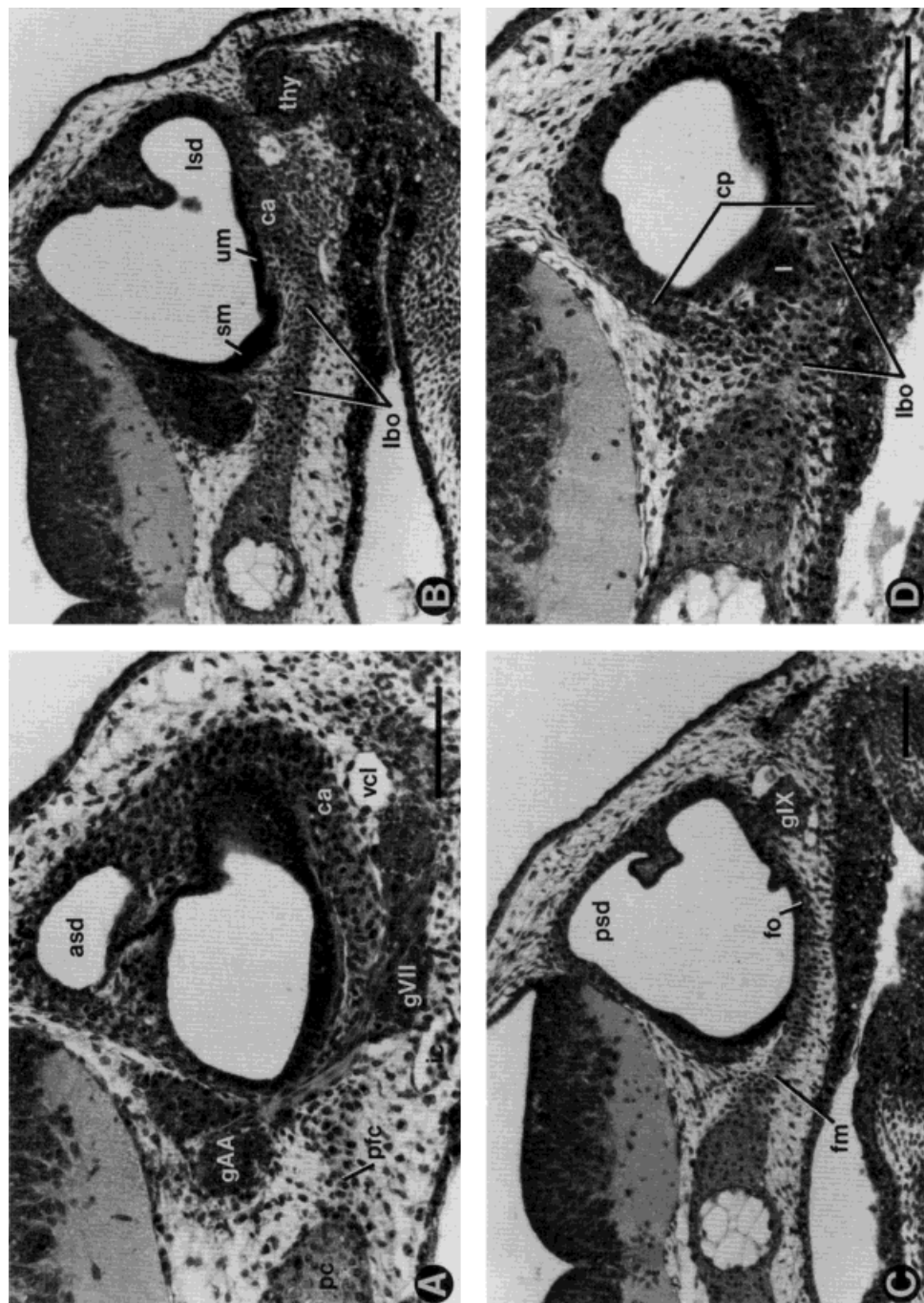


Figure 9

cens, and anterodorsal lateral line nerves) dorsally (cf. Fig. 18).

Meanwhile, the pila metoptica appears (stage 26). It begins to chondrify from its dorsal end, and chondrification is complete by stage 27. It separates a relatively small optic foramen, which transmits only the optic nerve, from a relatively large oculomotor foramen, which transmits the greater ophthalmic artery as well as the oculomotor nerve. Aside from increasing chondrification, no additional changes occur in the side wall of the cranium.

#### *Otic capsule*

As treated here, the otic capsule includes two basic components: 1) the otic capsule proper, surrounding the utricle and semicircular ducts, and 2) the basiotic lamina, underlying the sacculus and lagena. In addition, a number of other structures are most easily treated here. These include the prefacial commissure, which connects the skull base with the otic capsule in front of the facial root, and the palatobasal connection, joining the palatoquadrate to the basiotic lamina.

All of these structures seem to be mesodermal in origin, with the exception of the distal (lateral) part of the palatobasal connection. However, the cells of the otic capsule lose their distinguishing yolk granules relatively

early, before chondrification begins; it is possible that a significant number of neural crest cells contribute to the capsule but are undetectable.

At the earliest stage examined (stage 21), the otic capsule is represented by a dense cloud of mesodermal mesenchyme curving around the ventrolateral side of the otic vesicle (Figs. 1, 2). The otic vesicle consists of the endolymphatic sac and a large cavity representing the combined utricle and sacculus.

By stage 22, the mesenchyme along the ventrolateral surface of the vesicle condenses into procartilage (Fig. 6A, ca). A sheet of mesenchyme extends laterally from the parachordal beneath the future sacculus; this is the precursor of the basiotic lamina (Fig. 6A, lbo).

As development proceeds (stage 23), the otic capsule chondrifies rostrally (the anterior cupula, surrounding the anterior semicircular duct), laterally (along the future lateral semicircular duct), and caudally (the posterior cupula, surrounding the posterior semicircular duct) (Figs. 7, 9). Separate centers of chondrification for each of these regions were not observed.

Meanwhile, the basiotic lamina begins to chondrify in continuity with the parachordal (Fig. 7). It remains separated from the capsule proper by a large unchondrified gap that represents the fenestra ovalis (Figs. 7, 9C, fo). The connection with the parachordal is the anterior mesotic commissure (cf. Fig. 21). Rostrally, the basiotic lamina is in mesenchymal continuity with the mesenchymal prefacial commissure, medial to the facial foramen (Fig. 7), and with the anterior cupula, lateral to the facial foramen. The connection with the anterior cupula is the rudiment of the anterior basicapsular commissure. Caudally, the basiotic lamina separates from the parachordal and extends a prong back toward the rear of the capsule. The notch between the lamina and the parachordal I term the mesotic fissure (Fig. 9C, fm). At its caudal end, the basiotic lamina is still largely mesenchymal (Fig. 9D). In cross section, a poorly condensed dorsal extension along the medial wall of the sacculus (lagenar rudiment) gives the basiotic lamina the form of a V lying on its side. The limbs of the V join the posterior cupula of the capsule, whereas the apex joins the parachordal (by way of dense mesenchyme). These connections are the basivestibular and posterior mesotic commis-

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Fig. 9. *Ascaphus truei*. Stages 23 and 24. Serial transverse sections through the otic region. Levels of sections indicated in Figure 7. **A:** Stage 24. Section through anterior cupola of otic capsule (ca) and anterior semicircular duct (asd). Note facial root passing from anterior acoustic ganglion (gAA) to facial ganglion (gVII). The root of the mesenchymal prefacial commissure (pfc) is continuous with the parachordal cartilage (pc) medially. Note position of internal carotid artery (ic) and vena capitis lateralis (vcl). The caudal end of the otic process appears just below the vena capitis lateralis. **B:** Stage 23. Section through anterior acoustic ganglion, behind anterior acoustic and facial nerves. The basiotic lamina (lbo) is chondrified in continuity with the parachordal, but is separated by an unchondrified gap from the capsule. Note lateral semicircular duct (lsd), sacculus (sm), and utricular macula (um). Also note thymus (thy) budding off dorsal end of first branchial pouch. **C:** Stage 24. Section behind acoustic ganglia, through posterior semicircular duct (psd). The basiotic lamina is separated from the parachordal by an unchondrified gap, the mesotic fissure (fm). A similar gap between basiotic lamina and capsule is the fenestra ovalis (fo). The glossopharyngeal ganglion (gIX) is seen between internal carotid and vena capitis lateralis. **D:** Stage 24. Section through posterior cupula (cp). The unchondrified basiotic lamina stretches between parachordal and capsule, below the lagenar rudiment (l). Scales = 100  $\mu$ m.

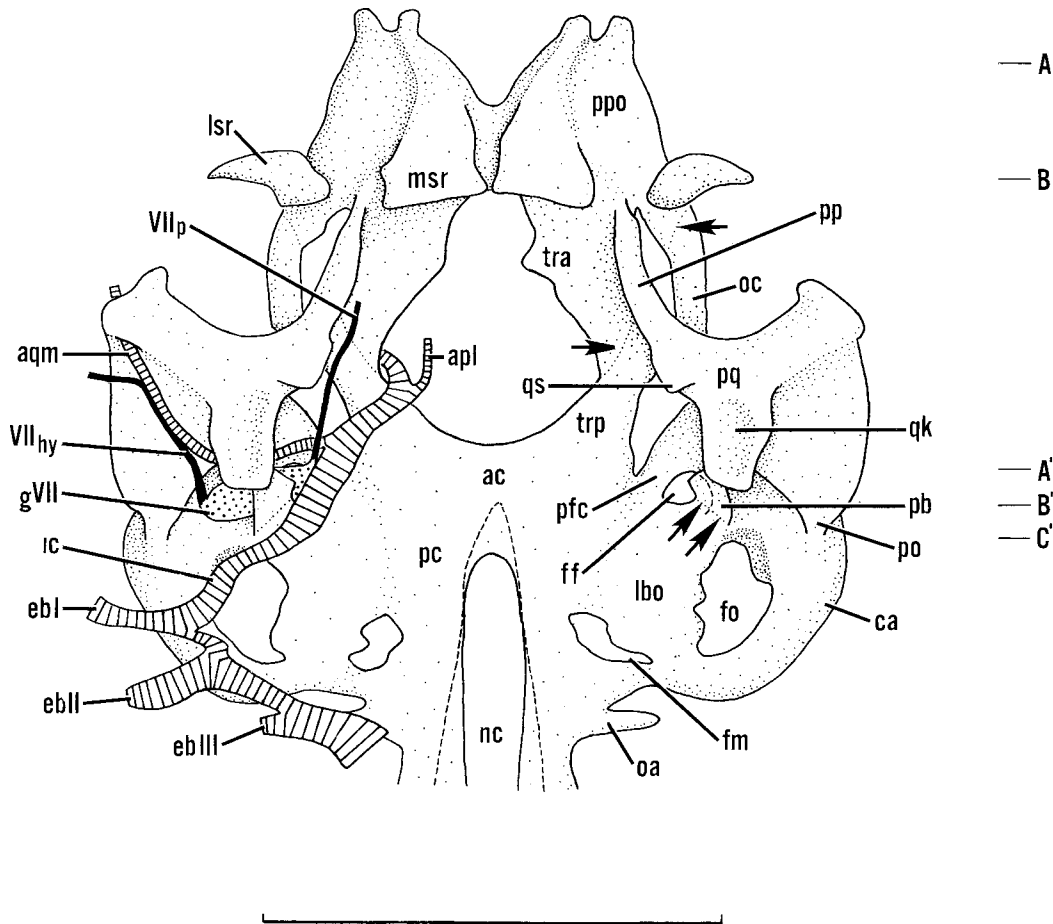


Fig. 10. *Ascaphus truei*. Stage 25. Graphical reconstruction of chondrocranium, ventral view. Mesenchymal palatobasal connection also reconstructed. Dotted lines (arrows) indicate approximate boundary between mesodermal and ectomesenchymal regions. Scale = 1 mm. Letters A-B, A'-C' indicate levels of sections in Figures 12, 13. ac, acrochordal cartilage; apl, palatine artery; aqm, quadratomandibular artery; ca, auditory capsule; ebl, first efferent branchial artery; ff, facial foramen; fm, mesotic fenestra; fo, fenestra ovalis; gVII,

facial (geniculate) ganglion; ic, internal carotid artery; lbo, basiotic lamina; lsr, lateral suprarostal; msr, medial suprarostal; nc, notochord; oa, occipital arch; oc, orbital cartilage; pb, basal process; pc, parachordal; pfc, prefacial commissure; po, otic process; pp, pterygoid process; ppo, pila preoptica; pq, palatoquarata; qk, quadrate keel; qs, quadrate spur; tra, anterior trabecula; trp, posterior trabecula; VII<sub>hy</sub>, hyomandibular branch of facial nerve; VII<sub>p</sub>, palatine branch of facial nerve.

tures, respectively. Behind the lagenar rudiment, the limbs of the V meet to form a complete caudal wall to the future lagenar chamber. This wall is beginning to chondrify, apparently in continuity with the posterior cupula (Fig. 7).

By this stage (23), the anterior and lateral semicircular ducts are pinched off from the otic vesicle, but the posterior is not (Fig. 9). A constriction in the wall of the vesicle marks the boundary between the dorsal utricle and the ventral saccus. Two compartments of the saccus are forming; a groove

along the medial wall below the utriculosaccular constriction is the rudiment of basilar and amphibian recesses, whereas the shallow pouch at its caudal end is the rudiment of the lagena.

By stage 25, important changes have occurred in this region. The otic capsule proper is well chondrified (Figs. 10, 11), consisting of anterior and posterior cupulae and a lateral wall; the medial wall is still largely mesenchymal. Complete cartilaginous septa are present for the anterior and lateral semicircular canals; they form by ingrowth of carti-



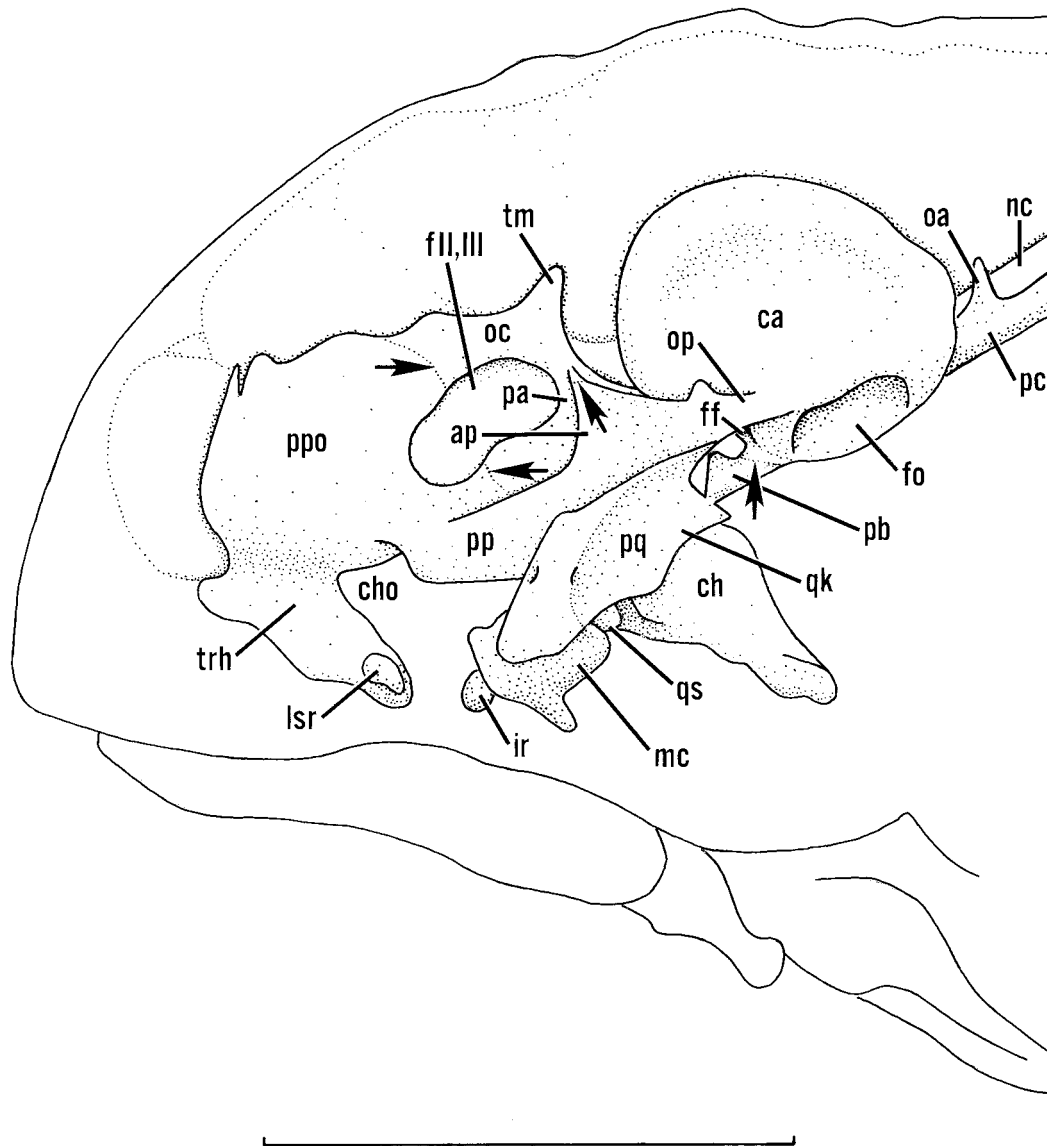


Fig. 11. *Ascaphus truei*. Stage 25. Graphical reconstruction of chondrocranium, lateral view. Mesenchymal palatobasal connection also reconstructed. Dotted lines (arrows) indicate approximate boundary between mesodermal and ectomesenchymal (neural crest derived) parts. Scale = 1 mm. ap, ascending process; ca, auditory capsule; ch, ceratohyal; cho, choana; ff, facial foramen; fII, III, optic + oculomotor foramen; fo, fenestra ovalis;

ir, infrarostral; lsr, lateral suprarostal; mc, Meckel's cartilage; nc, notochord; oa, occipital arch; oc, orbital cartilage; op, otic process; pa, pila antotica; pb, basal process; pc, parachordal; pp, pterygoid process; ppo, pila preoptica; pq, palatoquadrate; qk, quadrate keel; qs, quadrate spur; tm, taenia marginalis; trh, trabecular horn.

lage from either side. A septum for the posterior canal forms later (stage 26).

The basiotic lamina is chondrified along its entire length. Medially, the posterior mesotic commissure also is chondrified, enclosing the mesotic fenestra caudally (Fig. 10,

fm). Laterally, the lamina is connected to the capsule by chondrified anterior basicapsular (Fig. 13C) and basivestibular commissures, which complete the fenestra ovalis rostrally and caudally, respectively. The prefacial commissure is chondrified as a triangular bar

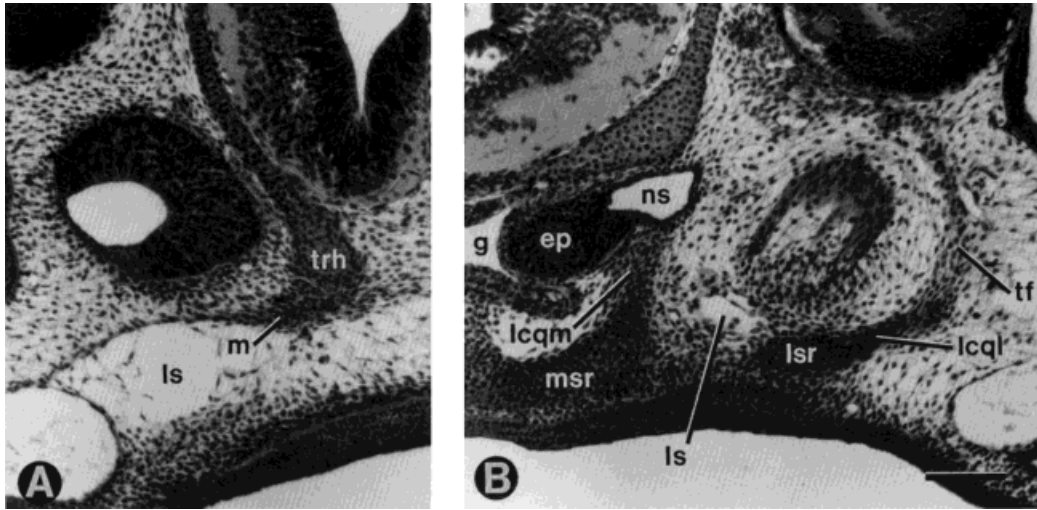


Fig. 12. *Ascaphus truei*. Stage 25. Transverse sections through rostral end of head. Levels of sections indicated in Figure 10. **A:** Section through trabecular horn (trh), showing mesenchymal extension (m) associated with roof of adjoining lymph space (ls). **B:** Section through medial and lateral suprarostrals (msr, lsr). Note mesenchymal temporal fascia (tf) surrounding levator

musculature. The ventral border of this fascia is the precursor of the ligamentum cornu-quadratum laterale (lcql). Also note precursor of ligamentum cornu-quadratum mediale (lcqm) below the nasal sac (ns). The rudiment of the epithelial band (ep) is visible between the lumen of the posterior narial tube and the preoral gut (g). Scale = 100  $\mu$ m.

connecting the parachordal with the anterior cupula of the otic capsule (Figs. 10, 13, pfc).

Further development of the otic capsule primarily involves its medial wall (see Fig. 18). In stage 25, a low process appears near the medial margin of the basiotic lamina. By stage 26, this process joins a process growing down from the roof of the capsule to form a rudimentary medial wall. This wall separates an anterodorsal foramen for the facial and auditory nerves and the endolymphatic duct from a posteroventral perilymphatic foramen. By stage 28, the anterodorsal foramen is divided into three foramina by the formation of two horizontal bars of cartilage. The dorsal foramen transmits nothing; later, it fills in with cartilage. The middle foramen transmits the endolymphatic duct. The ventral foramen transmits the facial and auditory nerves; it is further subdivided by vertical bars of cartilage into three foramina. From rostral to caudal these are the anterior acoustic meatus (the "anterior acoustic foramen" of van Eeden, '51), which transmits the facial and anterior acoustic nerves, the middle acoustic foramen, and the posterior acoustic foramen. By stage 29 (Fig. 18), cartilage divides the perilymphatic foramen into separate anterior and posterior perilym-

phatic foramina. Inside the capsule, a narrow shelf grows forward from the floor of the posterior cupula, forming a roof over the back of the lagenar chamber (Fig. 20C).

Fig. 13. *Ascaphus truei*. Stage 25. Serial transverse sections through rostral otic region and palatobasal connection. Levels of sections indicated in Figure 10. **A:** Section through anterior cupula of otic capsule (ca). The capsule is connected to the basal plate (bp) by a mesenchymal prefacial commissure (pfc). The palatoquadrate is represented by the otic process (op), suspended by the otic ligament (ol), and the quadrate keel (qk), a mesenchymal cap on the ceratohyal (ch). The palatine branch of the facial nerve (VII<sub>p</sub>) is seen emerging from the facial ganglion. The quadratomandibular artery (qma) is seen running laterally, over the quadrate keel. **B:** Section through anterior acoustic and facial ganglia (gAA, gVII). Note mesenchymal condensation of basal process (pb), extending up from the quadrate keel to contact the root of the prefacial commissure and the basiotic lamina. Arrowhead indicates lateral limit of cells containing yolk granules. Also note spiracular pouch (sp), running forward lateral to palatobasal connection, and the entrance of the temporal vein (vt) into the vena capitis lateralis, above the otic process. **C:** Section through anterior basicapsular commissure, where the basiotic lamina (lbo) and capsule meet behind the facial foramen. Note caudal end of otic process fused to capsule, and giving origin to caudal fibers of the m. suspensorioangularis (sa). Also note origin of spiracular pouch from dorsal wall of gut, and greater separation of sacculus (s) and utricle (u) than in previous stage. Scale = 100  $\mu$ m.

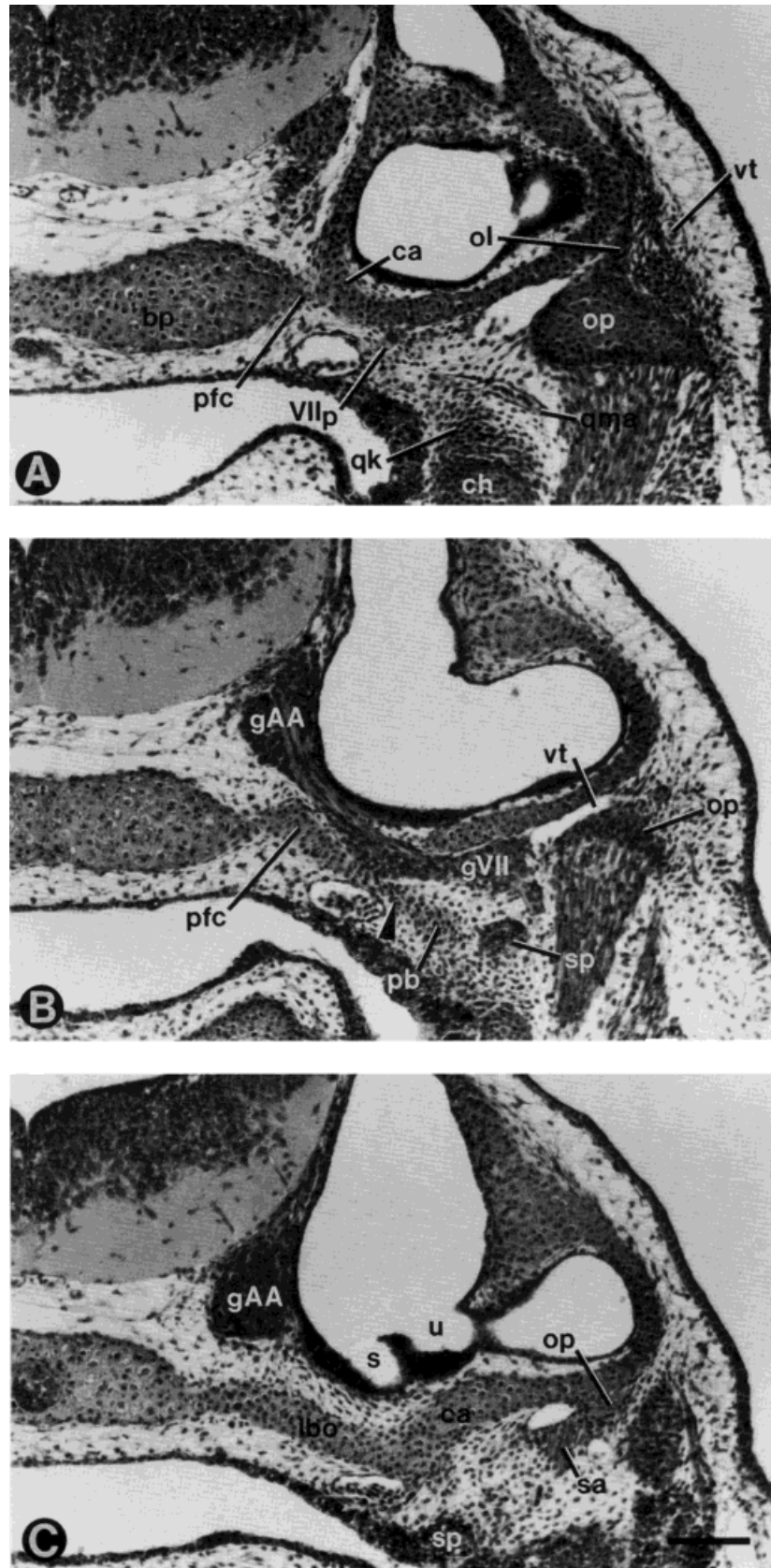


Figure 13

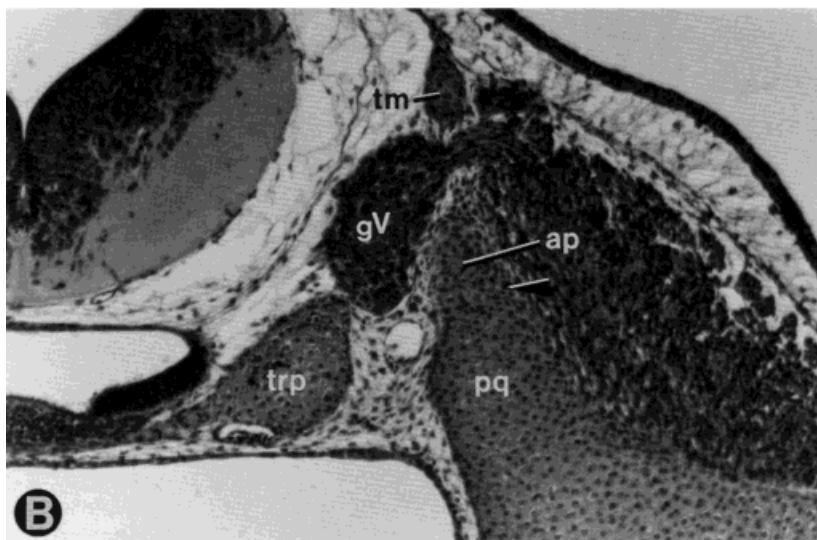
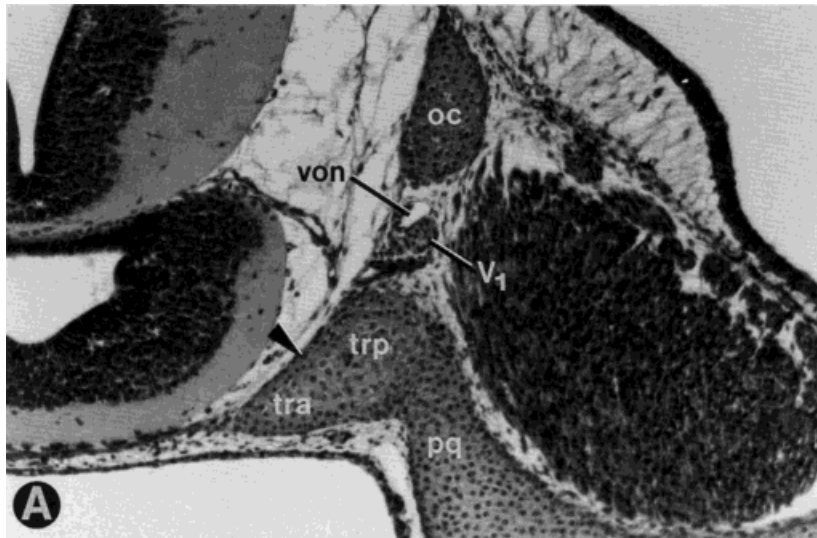


Figure 14

While these changes occur in the capsule, development of the otic vesicle continues. The posterior semicircular duct pinches off in stage 25. The utriculus and sacculus become progressively more distinct, as do their sensory maculae. The dorsal wall of the groove along the medial wall of the sacculus and lagena develops into the amphibian papilla, the ventral wall into the basilar papilla. The perilymphatic duct begins to form in stage 26, and is complete by stage 29.

The formation of the palatobasal connection is closely connected with the development of the rostral end of the otic capsule and the prefacial commissure. The relations of the facial nerve root, ganglion, and branches to the chondrocranial structures are especially important for understanding the changing morphology of this region.

When the otic capsule begins to chondrify in stage 23, the facial (geniculate) ganglion lies entirely extracranially, below the capsular floor (Fig. 9A, gVII). The facial root runs over the rostral edge of the basiotic lamina in company with the anterior acoustic nerve, passing through a space at the ventromedial border of the capsule that is the future facial canal. From here, the anterior acoustic nerve runs laterad into the capsule, whereas the facial root runs ventrad through the facial foramen (Figs. 7, 9A).

By stage 25, the proximal part of the facial ganglion is lodged in the facial foramen (Fig. 13B). The palatobasal connection forms as a mesenchymal strand connecting the rostral end of the basiotic lamina to the palatoquadrate keel, below the facial ganglion (Figs. 10, 11, 13). At this stage, the palatine branch of the facial nerve passes down from the ganglion *in front of* the palatobasal connection, whereas in older larvae the nerve passes

down through a foramen in the base of the connection (Pusey, '43; van Eeden, '51). The spiracular (hyoidean) gill pouch is represented by a solid endodermal cord that arises from the dorsal wall of the gut (Fig. 13C, sp), passes *behind* the palatobasal connection (Fig. 13B), and extends forward along the lateral side of the palatoquadrate.

The mesenchymal precursor of the palatobasal connection arises at a stage when much of the mesodermal mesenchyme has lost its distinguishing yolk granules. Nonetheless, many yolk granules are found in cells throughout the basal plate and basiotic lamina, including the chondrified proximal part of the palatobasal connection. By contrast, the distal part of the palatobasal connection lacks yolk granules. The boundary between these two regions is indicated by arrowheads in Figures 10, 11, and 13B. In earlier stages, this boundary occurs as a region in which the internal carotid artery and facial ganglion approach each other closely, and separate mesodermal cells (medially) from ectomesenchymal cells (laterally). I consider the mesodermal component of the palatobasal connection to be part of the basiotic lamina, and the ectomesenchymal component to be the basal process of the palatoquadrate (see discussion).

By stage 26, the proximal part of the facial ganglion is lodged farther up in the facial foramen (Fig. 15B). The palatobasal connection is expanded rostrally and medially, so that it is in contact with the floor of the otic capsule at a point just lateral to the origin of the palatine nerve (Fig. 15A). I term this contact the prepalatine connection; it divides the facial foramen into a palatine foramen (rostrally), and a hyomandibular foramen (caudolaterally). The hyomandibular foramen is the "secondary facial foramen" of van Eeden ('51). No other important changes occur in the palatobasal connection, although it is some time before the prepalatine connection is well chondrified (cf. Figs. 16A, B, 20B). By stage 29 (Figs. 17, 18, 20B) conditions closely resemble those in older larvae (Pusey, '43; van Eeden, '51).

#### *Cartilaginous cranial roof*

The cranial roof includes all parts of the chondrocranium above the brain. For a long time, a cranial roof is lacking. By stage 26, two distinct sections are present. Rostrally, the taenia marginalis extends from the orbit to contact the medial wall of the otic capsule. Caudally, a low ridge (the crista occipitalis

Fig. 14. *Ascaphus truei*. Stage 26. Serial transverse sections through rear of orbit. **A:** Section in front of ascending process, to show fusion of pterygoid process of palatoquadrate (pq) with trabecula. Approximate boundary between anterior and posterior trabeculae (tra, trp) indicated by arrowhead. Note orbitonasal vein (von) and deep ophthalmic branch of trigeminal ( $V_1$ ) below orbital cartilage (oc). **B:** Section through back end of mesodermal part of ascending process (ap), to show separation (arrowhead) from the palatoquadrate. Note maxillomandibular branch of trigeminal passing from the trigeminal ganglion (gV) out through the dorsal trigeminal outlet, below the taenia marginalis (tm). Behind this level, the mesodermal part of the ascending process fuses with the rostral tip of the otic capsule. **C:** Section 50  $\mu$ m behind B, to show contact of otic process (op) of palatoquadrate with otic capsule (ca). Scale = 100  $\mu$ m.

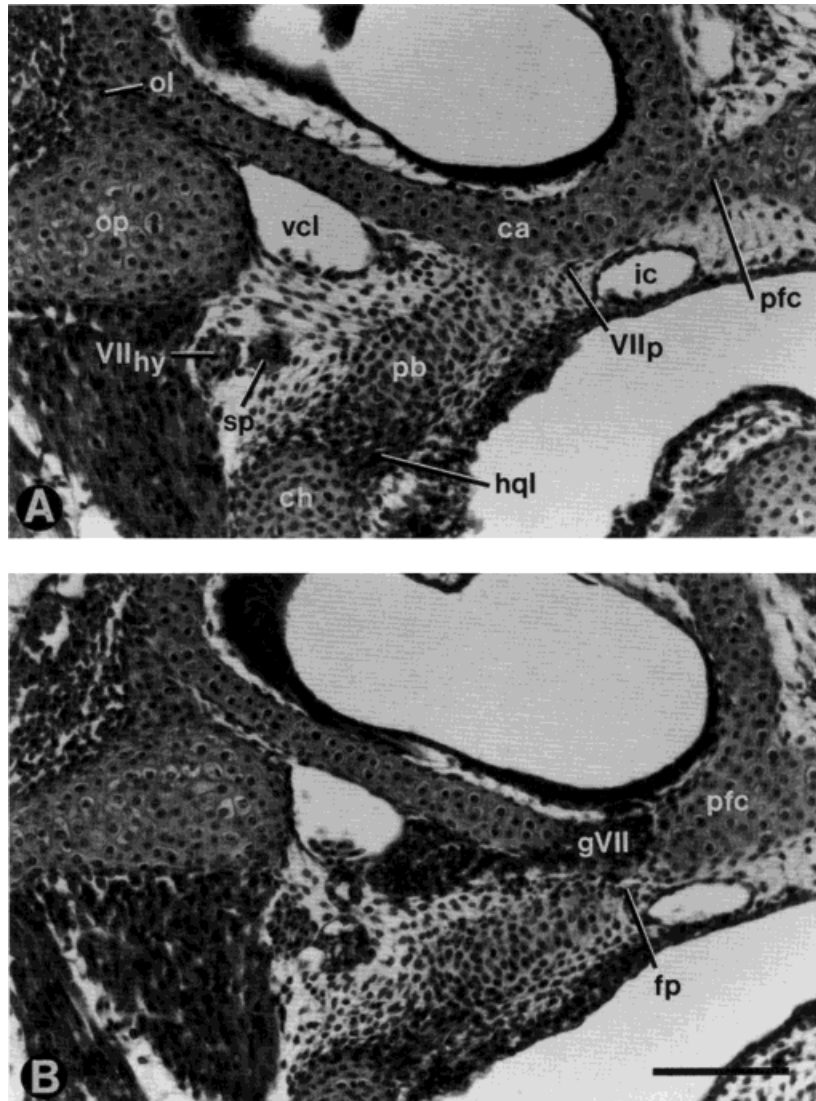


Fig. 15. *Ascaphus truei*. Stage 26. Transverse sections through palatobasal connection. **A**: Section in front of facial foramen, to show mesenchymal prepalatine connection of basal process of palatoquadrate (pb) with otic capsule (ca), above palatine branch of facial nerve (VII<sub>p</sub>). Note position of internal carotid artery (ic) and vena capitis lateralis (vcl). Also note presence of spiracular pouch (sp) and hyomandibular branch of facial nerve (VII<sub>hy</sub>) lateral to basal process, and mesenchymal hyo-

quadrate ligament (hql) connecting to ceratohyal (ch). The otic ligament (ol) is now chondrifying, connecting the otic process of the palatoquadrate (op) with the capsule. Chondrification of the prefacial commissure (pfc) has extended further forward. **B**: Section 30  $\mu$ m behind A, to show palatine foramen (fp). Note how rostral tip of facial ganglion (gVII) is now lodged in facial foramen. Scale = 100  $\mu$ m.

lateralis) develops along the medial wall of the capsule, and fuses with the occipital arch. The rostral and caudal parts are still widely separated from each other.

With further development, a number of distinct projections into the membranous cranial roof appear. A particularly large projec-

tion above the caudal end of the ventral trigeminal outlet represents the rudiment of the synotic tectum (Fig. 18).

#### *Palatoquadrate Complex*

Among the unusual features of the palatoquadrate of *Ascaphus* larvae is its extensive

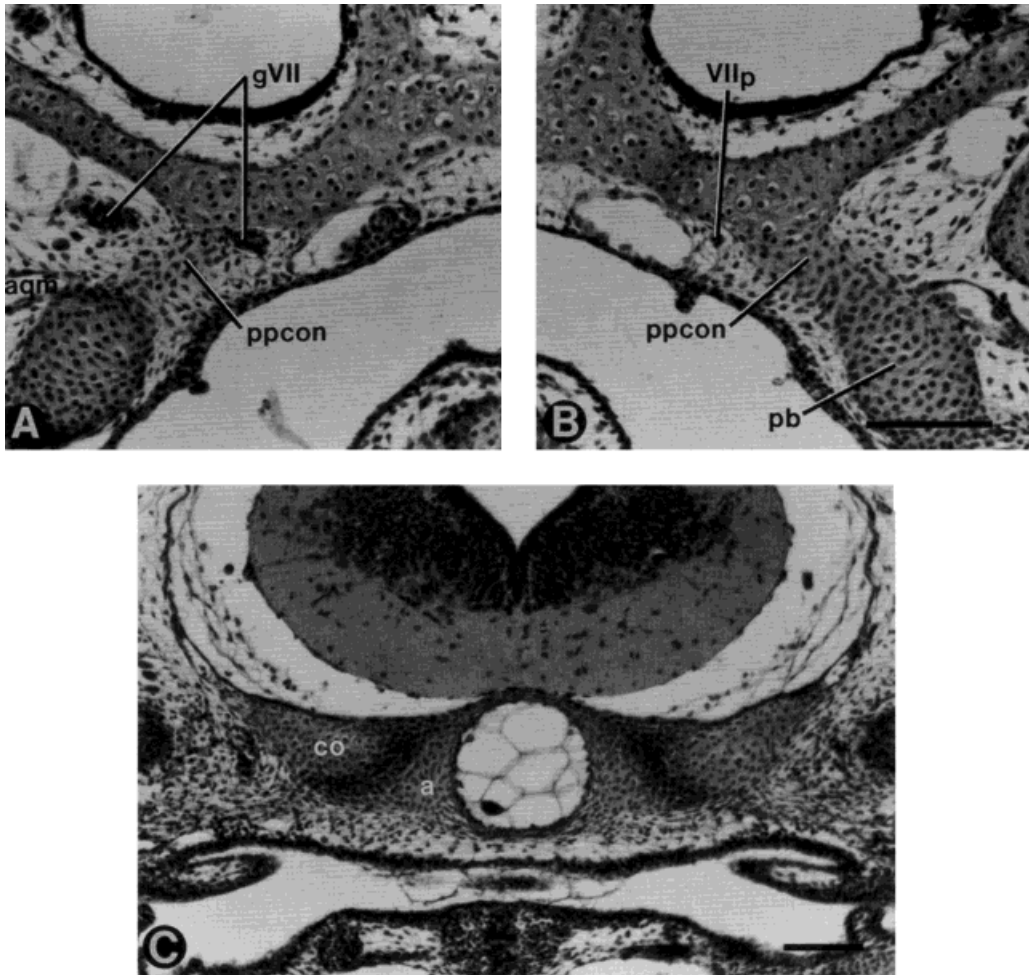


Fig. 16. *Ascapus truei*. Stage 27. **A, B:** Transverse sections through prepalatine part of palatobasal connection (ppcon) of right (A) and left (B) sides. Note that connection is formed of much denser mesenchyme in B than in A (it is fully chondrified just behind this level). In both sections one can see the quadratomandibular artery (aqm) passing over the basal process (pb). In A the

rostral tip of the facial ganglion (gVII) has been cut. In B only the palatine branch of the facial (VII<sub>p</sub>) is visible. **C:** Section through developing atlanto-occipital joint. The cartilage of the atlas (a) lies against the notochord. It is separated by a zone of procartilage from the occipital condyle (co). Scales for A-B and C = 100  $\mu$ m.

fusion with the neurocranium, which makes determination of its boundaries and relations difficult (Pusey, '43; van Eeden, '51). One can distinguish the following major regions: 1) the quadrate body, which forms the main mass of the palatoquadrate and articulates with the lower jaw; 2) the pterygoid process (anterior quadratocranial commissure), which is fused to the trabecula from just behind the choana back to the trigeminal region; 3) the ascending process, which extends up to meet the pila antotica, above the "profundus tunnel" (Pusey, '43) for the

deep ophthalmic branch of the trigeminal; and 4) the otic process, which extends back to contact the anterior and lateral walls of the otic capsule. Ascending and otic processes are broadly fused. The quadrate spur is a small flange that projects mediad from the pterygoid process ventral to the buccal cavity. The quadrate keel is a larger flange that projects caudad from the quadrate body, and articulates with the ceratohyal. The basal process, which connects the quadrate keel with the basiotic lamina, has been treated above. All parts of the palatoquad-

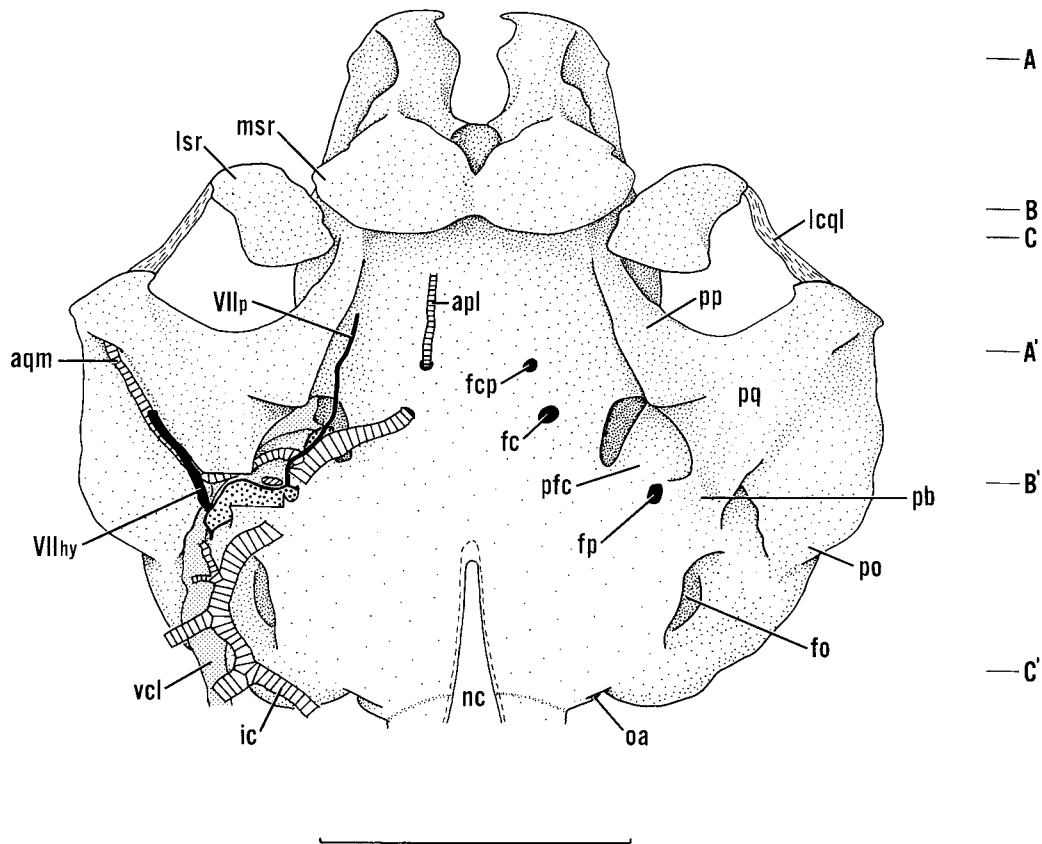


Fig. 17. *Ascaphus truei*. Stage 29. Graphical reconstruction of chondrocranium, ventral view. Palatobasal connection and internal carotid artery cut away on right side to show facial ganglion. Diagonal lines show cut surface of prepalatine part of palatobasal connection in front of facial ganglion. Scale = 1 mm. Letters A-C, A'-C' indicate levels of sections in Figures 19 and 20. apl, palatine artery; aqm, quadratomandibular artery;

fc, carotid foramen; fcp, craniopalatine foramen; fo, fenestra ovalis; fp, palatine foramen; ic, internal carotid; lcql, ligamentum cornu-quadratum laterale; lsr, lateral supra-rostral; msr, medial supra-rostral; nc, notochord; oa, occipital arch; pb, basal process; pfc, prefacial commissure; po, otic process; pp, pterygoid process; pq, palatoquadrate; vcl, vena capitis lateralis; VII<sub>hy</sub>, hyomandibular branch of facial; VII<sub>p</sub>, palatine branch of facial.

rate complex are ectomesenchymal in origin, except for the region at the dorsal end of the ascending process.

At the earliest stage examined (stage 21; Figs. 1, 2), the palatoquadrate is mesenchymal throughout. Boundaries between the palatoquadrate and the adjoining suprarostal cartilages, lower jaw, and ceratohyal are not always clear. The pterygoid process is wedged between the gut wall and the mandibular muscle mass (Figs. 3B, C, D, 4A, pp). Caudally, it is partly separated from the condensation of Meckel's cartilage by the intervention of the ectodermal band running along the gut wall (Fig. 4A, eb). The palatoquadrate body and Meckel's cartilage are largely continuous rostrally (Fig. 4B) but

are separate caudally. The palatoquadrate condensation ends freely at the level of the caudal tip of the infundibulum.

As development proceeds, the anterior trabecula condenses further (stage 22). The condensation of the pterygoid process runs forward along the lateral gut wall as a laterally compressed plate (Fig. 5D), with its tip contacting the trabecula above the hind end of the nasal sac (Fig. 6B). The condensation of the ascending process appears medial to the mandibular muscle mass; its dorsal tip contacts the mesodermal cord extending back from the orbital cartilage (Fig. 6C, ap, m).

By stage 23, the palatoquadrate complex is chondrifying throughout (Fig. 7), although its morphology has changed little since the



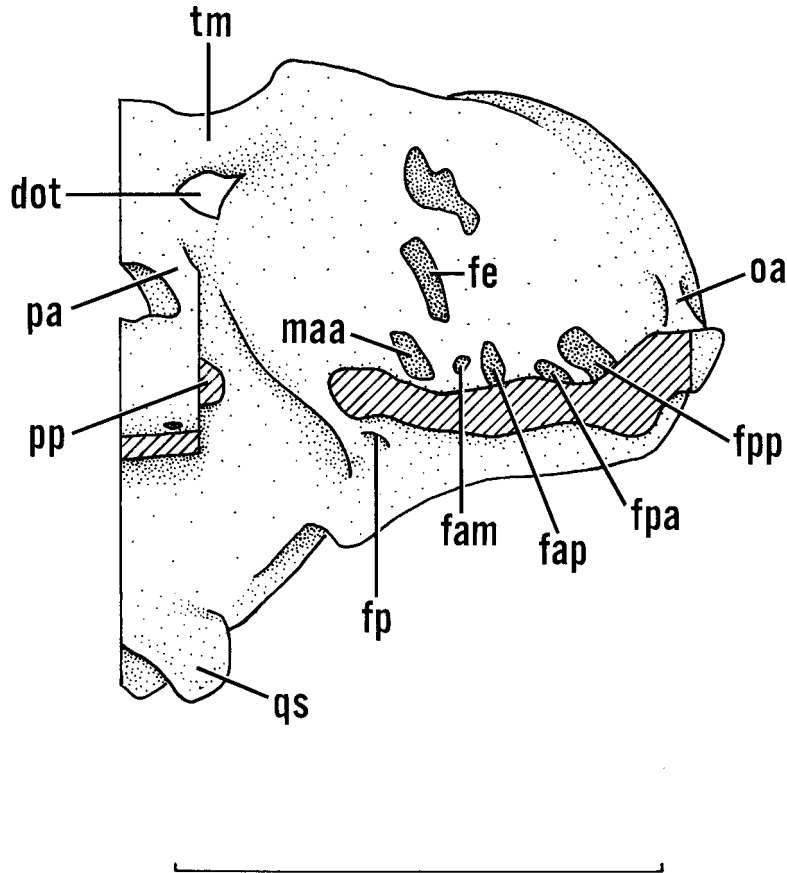


Fig. 18. *Ascapbus truei*. Stage 29. Graphical reconstruction of right otic capsule and caudal orbital region, medial view. Diagonal lines indicate cut surfaces; from anterior to posterior these are: 1) the midline of the trabecular plate, 2) the zone of fusion between pterygoid process and trabecula, and 3) the basal plate. Scale bar is 1 mm. dot, dorsal trigeminal outlet; fam, middle

acoustic foramen; fap, posterior acoustic foramen; fe, endolymphatic foramen; fp, palatine foramen; fpa, anterior perilymphatic foramen; fpp, posterior perilymphatic foramen; maa, anterior acoustic meatus; oa, occipital arch; pa, pila antotica; pp, pterygoid process; qs, quadrate spur; tm, taenia marginalis.

previous stage. The quadrate spur has appeared; it extends ventromedially from the quadrate body to contact the lateral gut wall (Figs. 7, 8B). The ascending process is connected by the mesenchymal mesodermal bridge to the orbital cartilage and pila antotica (Fig. 8B, D). The ceratohyal and lower jaw are still connected to the palato-quadrate by procartilage. The quadrate keel is distinguishable as a cap of procartilage running back along the dorsal border of the ceratohyal. The caudal end of the palato-quadrate, representing the otic process, ends freely alongside the otic capsule (Fig. 9A).

By stage 25 (Figs. 10, 11), the dorsal surface of the pterygoid process has begun to fuse with the anterior trabecula, apparently

by chondrification of the dense mesenchyme lying between them. This fusion progresses caudally, and by stage 26 has almost reached the floor of the profundus tunnel (Fig. 14A). By stage 29 (Figs. 17, 18), the zone of fusion is difficult to detect histologically (Fig. 20A).

The quadrate keel continues to develop, extending caudally. It has completely separated from the ceratohyal by stage 26. At the same time, the basal process arises as a mesenchymal strand connecting the quadrate keel with the basiotic lamina, and soon chondrifies. Its development is described above, with the otic capsule.

Fusion of the ascending process with the orbital cartilage, above the deep ophthalmic nerve, begins in stage 25 (Fig. 11), and gradu-

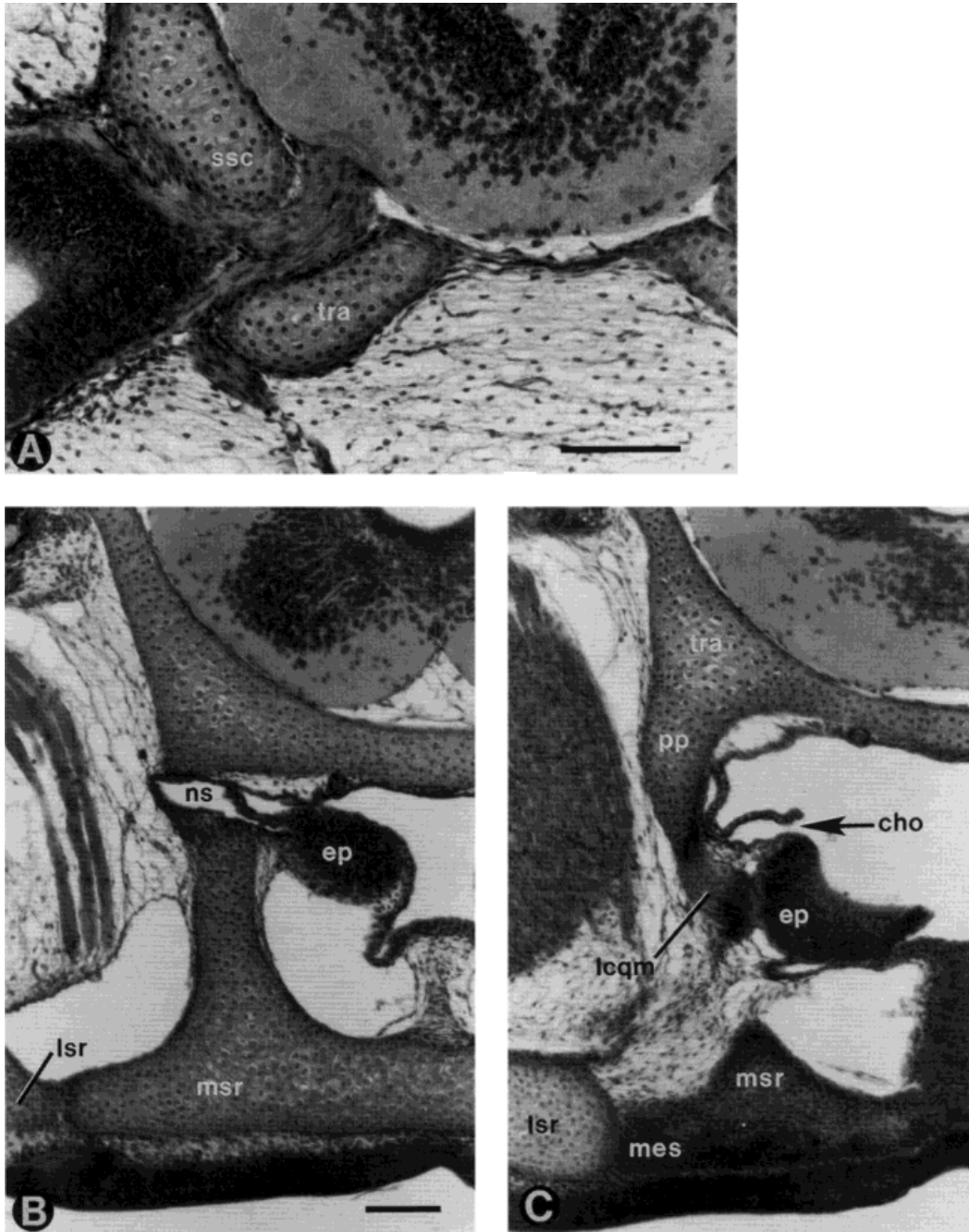


Fig. 19. *Ascaphus truei*. Stage 29. Transverse sections through rostral end of cranium. Levels of sections indicated in Figure 17. **A:** Section through olfactory foramen. Note robustness of cranial side wall (sphenoseptal commissure) (ssc) and trabecula (tra). The cranial floor is still membranous. **B:** Section through fused medial suprarostrals (msr), showing articulation with

lateral suprarostrals (lsr). **C:** Section through caudal end of medial suprarostrals, just in front of mouth. Note continuity of pterygoid process (pp) with trabecula. Also note dense mesenchyme of upper lip (mes), ligamentum cornu-quadratum mediale (lcqm), choana (cho), and well differentiated ciliated epithelium of the epithelial band (ep). Scales for A and B-C = 100  $\mu$ m.

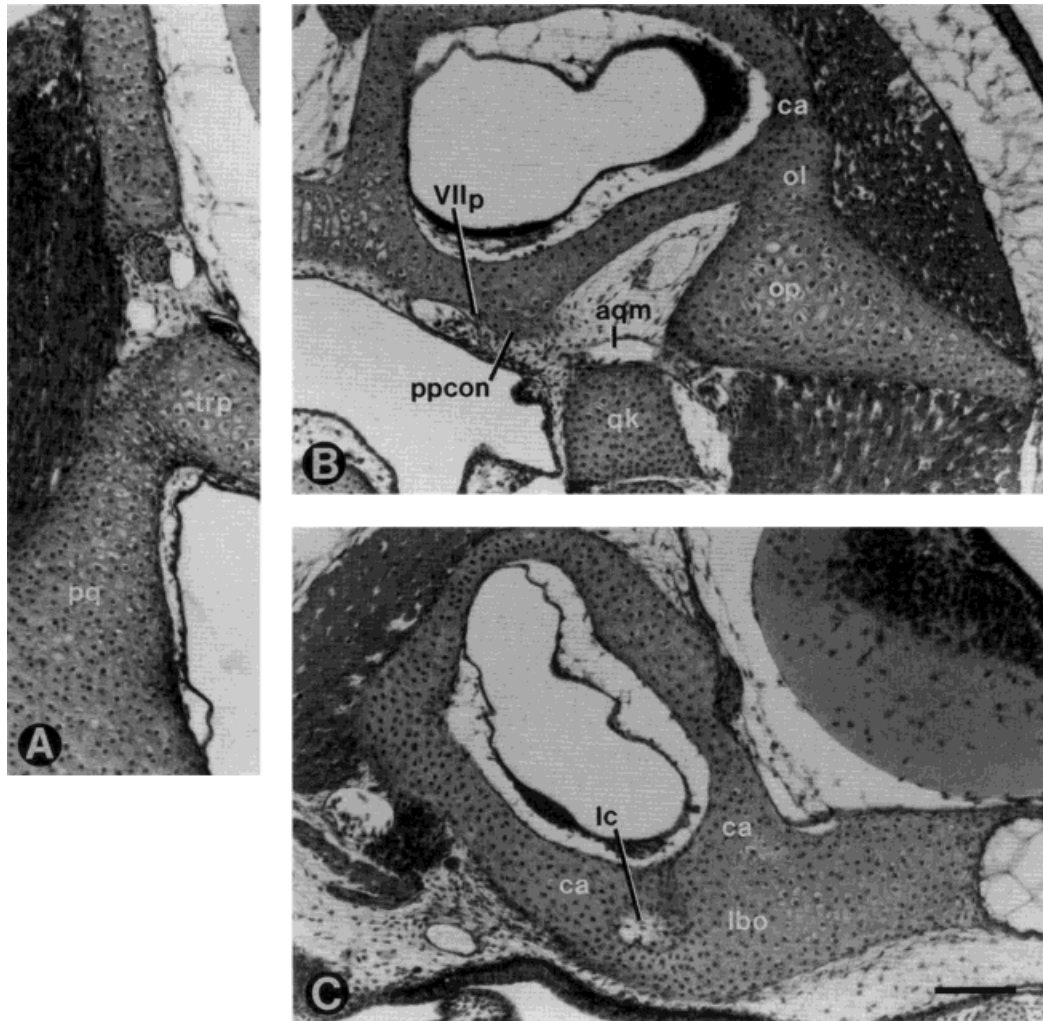


Fig. 20. *Ascapus truei*. Stage 29. Transverse sections; levels indicated in Figure 17. **A:** Section through fusion of pterygoid process of palatoquadrate (pq) and posterior trabecula (trp). **B:** Section through anterior cupula of otic capsule (ca). Note well chondrified quadrate keel (qk), palatine branch of facial nerve (VII<sub>p</sub>) running forward ventral to prepalatine connection (pp-

con), and quadratomandibular artery (aqm) running laterally. The otic ligament (ol) is now well chondrified, and the boundaries between otic process (op), ligament, and capsule are difficult to discern. **C:** Section through posterior cupula of otic capsule. Note floor of capsule (ca) forming roof over lagenar cavity (lc). The floor of the lagenar cavity is the basiotic lamina (lbo). Scale = 100  $\mu$ m.

ally becomes more complete. As noted above, the most dorsal part of the morphological "ascending process" is mesodermal, and should be considered a ventral extension of the orbital cartilage. By stage 26, this mesodermal part of the ascending process is chondrified all the way back to the rostral tip of the otic capsule (Fig. 14B). It thereby forms the ventral border of the dorsal trigeminal outlet, which transmits the maxillomandibular branch of the trigeminal and the anterodorsal lateral line nerve. Aside from an

increase in chondrification, little additional change occurs here.

Fusion of the otic process with the otic capsule also begins in stage 25 (Fig. 11). This connection is rather complicated, involving three distinct articulations. The first connection to form involves the caudal end of the otic process, which fuses to the otic capsule as a small ridge of chondrifying connective tissue running back along the ventrolateral surface of the capsule, above the fenestra ovalis (Figs. 10, 11, 13C, op). More rostrally,

the otic process is suspended by a ligament (the otic ligament; see Sokol, '81) from the crest of the ampulla of the anterior semicircular canal (Fig. 13A, ol). By stage 25, chondrification spreads upward into this ligament from the otic process, forming a nipplelike process (Fig. 11). The temporal vein (the "critical vein" of Pusey, '43) pierces the ligament behind the nipplelike process and in front of the region of fusion with the capsule (Fig. 13B). By stage 26, the otic ligament is fully chondrified. It is fused with the otic capsule, thereby forming the second otic connection (Figs. 15A, B). This chondrified otic ligament is the "larval otic process" (Sokol, '81). In front of the otic ligament, the dorsal surface of the otic process itself closely approaches the underside of the otic capsule. The incipient connection thus formed is the third otic connection, and is apparently the true one (Sokol, '75, '81). By stage 26, the otic process is in contact with the capsule in this region, although a layer of perichondrium separates them (Fig. 14C).

Thus by stage 26 three distinct otic connections have formed, in addition to the connection of the mesodermal part of the ascending process with the tip of the capsule. The boundaries between these connections soon are obscured, owing to the disappearance of perichondrium and the filling in of mesenchymal spaces with cartilage. By stage 27, a single broad sheet of cartilage wraps around the front and side of the otic capsule; it is pierced only by the foramen for the temporal vein (see Pusey, '43; van Eeden, '51).

#### DISCUSSION

##### *Embryology of the head in Ascapus*

Three features of cephalic ontogeny in *Ascapus* are particularly unusual for anurans. The first is the presence of an extensive premandibular head cavity. Although only a few other frog species have been examined at appropriate stages, such cavities have not been described in any. The head cavity is retained well into the time of chondrocranial formation, and the orbital cartilage and posterior trabecula seem to derive directly from the wall of the cavity. It is possible that the presence of head cavities in *Ascapus* is a primitive character; they also occur in lungfish, at least one species of salamander (*Triturus vulgaris*; Scott and Osborn, 1879; cited in Edgeworth, '35), and many amniotes (Edgeworth, '35).

The manner in which the mouth forms, in particular the relations of the foregut to surrounding tissues, is also peculiar in *Asca-*

*pus*. The shallow stomodeum and freely exposed, flattened foregut seen in stage 21 have not been described in any other amphibian, nor has the subsequent growth of the expanding upper lip caudally, beneath the foregut. However, early head development has not been studied in other frogs with ventrally placed mouths. The presence of ectodermal bands running back along the gut wall from the corners of the mouth is also unusual. As noted above, these somewhat resemble the ectodermal collar around the foregut of salamanders (Adams, '24; Medvedeva, '61; Bertmar, '66). However, in salamanders this collar surrounds the entire foregut.

The third unusual feature of *Ascapus* embryology is the formation of ciliated epithelial bands that run forward in the wall of the preoral gut, and are derived from the olfactory placode. Their origin could have been predicted, given their innervation by the olfactory nerve (Pusey, '43). These bands are almost certainly chemosensory, but the biological role of their exposure in the buccal cavity is not clear.

##### *Homologies of anuran palatoquadrate processes*

De Beer ('37: 416) noted the "remarkable tangle of confusion and mistaken identity" that existed in early studies on the amphibian palatoquadrate. The situation has not improved in the last 60 years—the homologies of anuran palatoquadrate processes are still controversial (reviewed by Pyles, '88; Reiss, '93). In tetrapods, the palatoquadrate is considered to have four processes by which it can attach to the neurocranium (Goodrich, '30; de Beer, '37). These are as follows: (1) the *pterygoid process* connects with the ethmoidal region of the neurocranium. (2) The *ascending process* connects with the pila antotica or orbital cartilage of the neurocranium; it lies lateral to the deep ophthalmic branch of the trigeminal, and medial to maxillary and mandibular branches. (3) The *otic process* connects with the otic capsule; it lies lateral to all branches of the trigeminal and facial nerves, the vena capitis lateralis, and the orbital (stapedial) artery. (4) The *basal process* connects with the basitrabecular process of the neurocranium; it lies anterior and dorsal to the palatine branch of the facial nerve, ventral to the deep ophthalmic branch of the trigeminal nerve and the vena capitis lateralis.

In the following discussion, I briefly review the history of controversy regarding each process, then consider the relevance of the present results on *Ascaphus*. As noted in the introduction, previous hypotheses of phylogeny can help decide between conflicting schemes of topographical correspondence. Recent analyses suggest that the sister group of Anura is either Caudata (salamanders) or [Gymnophiona (caecilians) + Caudata], and that the three living amphibian groups together comprise a monophyletic group Lissamphibia (Milner, '88, '93; Bolt, '91; Trueb and Cloutier, '91). I therefore compare anurans with salamanders, and when possible, caecilians, throughout.

#### The pterygoid process

In early work on skull development in frogs there was thought to be a single rostral process of the palatoquadrate in both larvae and adults—the “palatine process” (Parker, 1871). However, later workers realized that during metamorphosis the larval rostral process, which connects directly to the trabecula, is partly replaced by, rather than converted into, the more lateral rostral process of adults (Gaupp, 1891, 1893, '06). The larval process was called the “commissura quadrato-cranialis anterior” by Gaupp (1891), because he considered its homology uncertain. For brevity, I will refer to this as the “commissura” in the following discussion.

Most investigators since Gaupp's time have considered there to be two rostral processes of the palatoquadrate in anurans. Edgeworth's ('25, '35) views on the pterygoid process of adult anurans are difficult to interpret, but he considered the commissura homologous to the pterygoid process of salamanders. Others have considered both the commissura and the pterygoid process to represent the pterygoid (palatal) process, with the commissura corresponding to a distinct medial articulation of the palatoquadrate with the neurocranium in adult crossopterygian fishes, and the pterygoid process corresponding to a more lateral articulation with the postnasal wall (e.g., Jarvik, '42; Roček, '93). Finally, some investigators have considered the commissura to correspond to the basal process, shifted far forward in association with the rostral location of the jaw joint in anuran larvae (de Beer, '37; Pusey, '38, '43).

*Ascaphus* has played an important role in this debate. In particular, the discovery that *Ascaphus* larvae have both a commissura

and a more posterior basal process connecting with the otic region forced Pusey ('43) to revise his previous proposal ('38). He now ('43) distinguished the commissura as the “anterior basal process,” and proposed that in phylogeny a single ancestral basal process had split into two—the condition preserved in *Ascaphus* larvae. In subsequent anuran evolution, the larval anterior basal process (commissura) moved further forward, and the posterior basal process was lost. To support this view, Pusey ('43) cited the extreme caudal location of the posterior border of the commissura in *Ascaphus*. However, van Eeden ('51) noted that in one of his *Ascaphus* tadpoles the posterior part of the commissura was separated from the trabecula by a layer of perichondrium. Van Eeden ('51) considered this evidence that the extensive fusion of the commissura to the trabecula is secondary, and proceeds rostrocaudally during development. He considered the commissura homologous to the pterygoid process.

My observations on *Ascaphus* confirm van Eeden's expectation. In early stages, the commissura extends forward from the jaw joint, with only its tip contacting the trabecula; fusion with the trabecula proceeds rostrocaudally. Except for its platelike shape, the commissura of *Ascaphus* embryos closely resembles the pterygoid process present in a number of cryptobranchoid salamander larvae (Edgeworth, '23, '25; Aoyama, '30; Fox, '54; Regel, '68), and I have named it such in the results section. There is no reason to think that the commissura represents the basal process. When one wishes to distinguish them, it seems most reasonable to refer to the commissura as the “larval pterygoid process,” by contrast with the more lateral “adult pterygoid process.”

As in anurans, in the hynobiid salamander *Ranodon sibiricus* a medial larval pterygoid process is partly replaced by a more lateral adult pterygoid process during metamorphosis (Lebedkina, '63, '64; Regel, '68). In other metamorphosing salamanders (e.g., *Hynobius*, *Ambystoma*, *Triturus*, *Desmognathus*; Edgeworth, '25; de Beer, '37; Fox, '59; Regel, '64) the ontogeny is similar, although part or all of the pterygoid process fails to chondrify. Rhinatrematid caecilians of the genus *Epicrionops* have a poorly developed pterygoid process, whereas ichthyophiid caecilians have a variably developed process in larval stages that is mostly resorbed during metamorphosis; both, however, show a laterad shift of the

pterygoid bone during metamorphosis (Visser, '63; Reiss, '96).

#### The ascending and otic processes

Homologies of the ascending and otic processes of frogs were resolved by Stöhr (1881), who showed that the "pedicle" of Parker (1871) corresponds to the ascending, rather than the basal process of salamanders, based on its relation to the branches of the trigeminal nerve. However, the morphological variation in these processes among larvae has raised some question as to appropriate terminology (Sokol, '75, '81).

In *Ascaphus* larvae, the ascending and otic processes are represented by a continuous sheet of cartilage, broken only by the dorsal trigeminal outlet and the foramen for the temporal vein (Pusey, '43; van Eeden, '51). My observations show that this sheet arises from five distinct rudiments. One of these is mesodermal; it forms the bridge between the orbital cartilage and the rostral tip of the otic capsule, below the dorsal trigeminal outlet.

A mesodermal region at the top of the ascending process is shown in most previous figures of chondrocranial elements derived from neural crest in frogs (Stone, '29) and salamanders (e.g., Stone, '26; Raven, '31; Hörstadius and Sellman, '46), although Olsson and Hanken ('96) found this region to have a neural crest contribution in *Bombina*. This region chondrifies separately from the remainder of the ascending process in some salamanders; it is the processus antoticus of Regel ('62, '64, '68), who homologized it with a similar process of the neurocranium in crossopterygian fishes. In *Ascaphus*, the mesodermal region of the ascending process/otic process complex is retained in the adult as a bony bridge ossified by the prootic (de Villiers, '34; van Eeden, '51). The fact that it is (at least partly) mesodermal in origin implies that this bridge is part of the neurocranium, rather than the palatoquadrate.

Thus *Ascaphus*, like most other living frogs (Sokol, '81), has an otic process in both larvae and adults, and an ascending process in larvae, but lacks an ascending process as an adult (contra Pusey, '38; van Eeden, '51). By contrast, salamanders have otic and ascending processes both as larvae (e.g., de Beer, '37; Regel, '64, '68) and adults (Trueb, '93). Caecilians have an otic process, but lack an ascending process, both as larvae (Visser, '63; Reiss, '96) and adults (e.g., Els, '63); the "ascending process" of the earlier literature is in fact the otic process (Els, '63).

#### The basal process

The homology of the basal process has been more controversial than that of any other anuran palatoquadrate process (reviewed by Pyles, '88; Reiss, '93). The basal articulation is primitive for osteichthyans (Arratia and Schultze, '91). It is formed by the contact of the basal process—extending medially from the palatoquadrate—and the basitrabecular (basipterygoid) process—extending laterally from the caudal end of the posterior trabecula (Goodrich, '30; de Beer, '37). In contrast, the palatobasal connection (to use a non-committal term) of adult anurans lies relatively further back; it involves an articulation of the palatobasal process with an "otic ledge" at the anterior end of the otic capsule. This articulation may be a diarthrosis, a synchondrosis, or be functionally replaced by the pterygoid bone (Pyles, '88; Trueb, '93). A larval palatobasal connection has been described in only a few, unrelated tadpoles: *Ascaphus* (Pusey, '43; van Eeden, '51), *Heleophryne* (Ramaswami, '44; van der Westhuizen, '61), *Rana curtipes* (Ramaswami, '40), and *Amolops afghanus* (Ramaswami, '43). Of these *Ascaphus*, *Heleophryne*, and *A. afghanus* are torrent-dwelling forms with a well-developed oral sucker; *R. curtipes* is a mountain stream form, but does not have a sucker (Sekar, '90). Only in *Ascaphus* is it possible that the presence of a larval palatobasal connection is primitive.

Before proceeding, I must introduce some terminology for the neurocranial structures in the region of the palatobasal connection (Fig. 21). These structures include: (1) the basiotic lamina<sup>1</sup>, forming the floor of the otic capsule medial to the foramen ovale; (2) the anterior cupola of the otic capsule proper; (3)

<sup>1</sup>My distinction of a "basiotic lamina" is unconventional; in most current literature on tetrapods the otic region of the skull is considered to have only two components: the parachordals (basal plate) and the otic capsules (e.g., Bellairs and Kamal, '81). In this view both the fenestra ovalis and the perilymphatic foramen are completely surrounded by capsular cartilage (Noordenbos, '05; Goodrich, '30; de Beer, '37). However, an alternative view exists, in which the floor of the otic capsule is considered a lateral extension of the middle region of the basil plate, and the fenestra ovalis passes between true capsular ("periotic") and parachordal cartilage (Gaupp, 1893, 1900, '06). These views can be reconciled by distinguishing the floor of the otic capsule as the basiotic lamina; this region forms from a separate anlage in many chondrichthyans (de Beer, '37), as well as actinopterygians and dipnans (Bertmar, '59). This scheme better reflects the facts of development in *Ascaphus*, and has the additional advantage of making the tetrapod fenestra ovalis homologous to the basicapsular fenestra of fishes, rather than a neomorphic structure. The basiotic lamina corresponds approximately to the "mesotic cartilage" of Stöhr (1880, 1881), and includes the "anterior and posterior basicapsular commissures" of de Beer ('37) and Regel ('64, '68).

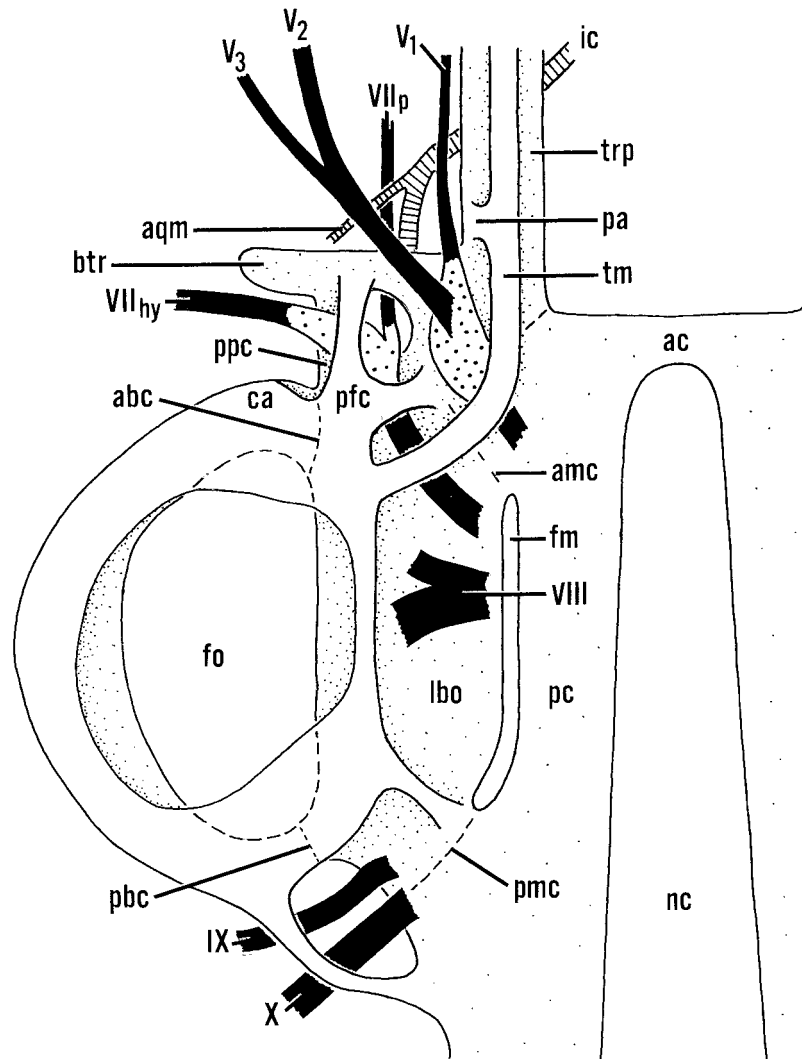


Fig. 21. *Ascaphus truei*. Diagram of left otic and temporal regions of generalized skull in dorsal view, to show relations of important structures. See text for discussion. abc, anterior basicapsular commissure; ac, acrochordal cartilage; amc, anterior mesotic commissure; aqm, quadratomandibular artery; btr, basitrabecular process; ca, auditory capsule (cupola anterior); fm, mesotic fissure (fenestra); fo, fenestra ovalis; ic, internal carotid; lbo, basiotic lamina; nc, notochord; pa, pila

antotica; pbc, posterior basicapsular commissure; pc, parachordal; pfc, prefacial commissure; pmc, posterior mesotic commissure; ppc, postpalatine commissure; tm, taenia marginalis; trp, posterior trabecula; V<sub>1</sub>, ophthalmic branch of trigeminal; V<sub>2</sub>, maxillary branch of trigeminal; V<sub>3</sub>, mandibular branch of trigeminal; VII<sub>hy</sub>, hyomandibular branch of facial; VII<sub>p</sub>, palatine branch of facial; VIII, auditory nerve; IX, glossopharyngeal nerve; X, vagus nerve.

the prefacial commissure, connecting the basiotic lamina and/or anterior parachordal to the anterior cupola of the otic capsule, thereby separating the facial and trigeminal nerves; (4) the postpalatine commissure, extending anterolaterally from the basiotic lamina behind the palatine branch of the facial nerve, but in front of the hyomandibular branch.

Gaupp (1893, '06) considered the anuran palatobasal connection a true basal connection. However, de Beer ('26) pointed out that whereas in salamanders and amniotes the palatine branch of the facial nerve passes ventrally *behind* the palatobasal connection, and then runs forward beneath it, in the anurans that had been studied at the time, the palatine nerve passes *in front* of the

palatobasal connection. Because of this, de Beer ('26, '37) considered the anuran connection a "pseudobasal connection," between the basal process of the palatoquadrate and the postpalatine commissure of the neurocranium. This concept was extended further by Pusey ('38, '43), who considered the palatoquadrate process itself to be a "pseudobasal" process, formed from the detached end of the basitrabecular process. The presence of a distinct palatine foramen in adults (de Villiers, '34) and larvae (Pusey, '43) of *Ascaphus* was considered proof that *Ascaphus*, alone among living anurans, possesses a true basal connection (de Beer, '37; Pusey, '38, '43).

Meanwhile, study of larval and metamorphosing stages of *Megophrys* led Kruijtzter ('31) to propose that the palatobasal process of anurans is a hyoid arch structure, based on similarities to the infrapharyngohyal cartilage of the lungfish *Neoceratodus*. These similarities included its independent origin, and its relation to the hyomandibular nerve and the stapedia (orbital) artery. Since then, several other authors have supported the idea of a hyoid origin for part or all of the palatobasal connection (N. G. Stephenson, '51; van der Westhuizen, '61; Swanepoel, '70). Whereas Kruijtzter ('31) apparently considered the otic ledge a neurocranial structure, N.G. Stephenson ('51) and Swanepoel ('70) considered both the palatobasal process and the otic ledge hyoid derivatives. Van der Westhuizen ('61) considered the otic ledge a hyoid derivative, but the palatobasal process a mandibular derivative.

This brief historical review reveals three basic issues with respect to the homology of the anuran palatobasal connection. The first issue is which visceral arches are involved—mandibular, hyoid, or both? The second issue is where the boundary between the neurocranium and the visceral arches (mandibular or hyoid) should be drawn—is the otic ledge a visceral or neurocranial structure? The final issue is which neurocranial structures (if any) are involved—the basitrabecular process, or the postpalatine commissure? I deal with each of these in turn.

The first issue concerns the possibility of hyoid arch involvement in the anuran palatobasal connection (Kruijtzter, '31; N.G. Stephenson, '51; van der Westhuizen, '61; Swanepoel, '70). My observations of *Ascaphus*—combined with a review of the literature—argue strongly against this possibility. The fundamental criterion of whether an

element belongs to a particular arch is not its relation to various nerves and vessels, but its relation to the gill slits. In *Neoceratodus*, the spiracular (hyoid) pouch is present only in the early embryo, but at this time it lies in front of the lateral end of the infrapharyngohyal, as one would expect (Greil, '13; Bertmar, '59). By contrast, in *Ascaphus* the spiracular pouch runs laterally *behind* the rudiment of the palatobasal connection (Fig. 13). Similar relations of the spiracular pouch and palatobasal connection are found in the direct-developing anurans *Leiopelma* (N.G. Stephenson, '51, pl. III, fig. 11) and *Brevicipes* (Swanepoel, '70, fig. 31C), and in the salamander *Ranodon sibiricus* (Regel, '68, fig. 16). The Eustachian tube of adult anurans, which develops during metamorphosis from the remnants of the spiracular pouch (Villy, 1890; Gaupp, 1893; Barry, '56), always passes up behind the adult palatobasal connection. Finally, the artery described in *Megophrys* (Kruijtzter, '31), *Leiopelma* (N.G. Stephenson, '51, '55), *Ascaphus* (van Eeden, '51) and *Bufo* (Barry, '56) as the stapedia (orbital) artery, a hyoid arch vessel, is actually the quadratomandibular (efferent mandibular) artery (cf. Shishkin, '67, '68). The quadratomandibular artery is well-developed in salamanders, at least in cryptobranchoids (Fox, '54, '59; Regel, '64, '68), and its relations to the palatobasal connection resemble those of anurans. Taken together, these observations provide no support for the derivation of the palatobasal connection from the hyoid arch.

The second issue concerns the boundary between the neurocranium and the palatoquadrate in the region of the palatobasal connection. The palatoquadrate of osteichthyans is primitively a free element that articulates with the neurocranium (Arratia and Schultze, '91). In contrast, all of the hypotheses for homology of the palatobasal process in anurans—except the hypothesis that it is homologous to the basal process of salamanders *and* other tetrapods—involve fusion of the palatoquadrate and neurocranial structures. Where such fusion exists, three criteria for the boundary seem possible: (1) a criterion of tissue origin (neural crest versus mesodermal), (2) a developmental criterion, in which neurocranial elements are considered to be those that develop in continuity with the rest of the neurocranium, and visceral arch elements those that form independently and second-



arily fuse with the neurocranium, and (3) a comparative criterion, in which related taxa with no fusion are used to determine the boundary.

The criterion of tissue origin seems especially useful, because the base of the skull in the region of the palatobasal connection is of mesodermal origin, whereas the palatoquadrate is of neural crest origin (reviewed by Hall, '87; see also Olsson and Hanken, '96). In *Ascaphus*, the larval palatobasal connection develops by the chondrification of a mesenchymal strand that connects the rear of the quadrate keel with the anterolateral part of the basiotic lamina (i.e., the postpalatine commissure). No independent centers of chondrification within this strand are apparent. The most proximal part of the strand is mesodermal; the distal part is ectomesenchymal (Figs. 10, 11). I consider the ectomesenchymal part to represent the basal process of the palatoquadrate; much of this has previously been considered part of the "larval otic ledge" (Pusey, '43; van Eeden, '51).

The boundary between neural crest and mesoderm within the palatobasal connection of other amphibians is unknown. Thus, a combined use of developmental and comparative criteria is necessary to relate the neurocranium/palatoquadrate boundary defined in *Ascaphus* to that of salamanders and other frogs. Among salamanders, the ontogeny of the larval palatobasal connection varies (Stadtmüller, '36). In cryptobranchids, the basal process of the palatoquadrate grows caudally and medially to contact the lateral edge of the basal plate in front of the prefacial commissure (Edgeworth, '23; Aoyama, '30; Fox, '54). In other salamanders, the palatobasal connection forms either by initial chondrification of the basal process in continuity with the palatoquadrate, and subsequent fusion to the chondrocranium in the region of the prefacial and/or postpalatine commissure (e.g., *Salamandra salamandra*: Stadtmüller, '24), or by chondrification of a band of mesenchyme stretching between the quadrate body and the prefacial and/or postpalatine commissure (e.g., *Hynobius* spp.: Edgeworth, '23, '25; Fox, '59; Regel, '64; *Ranodon sibiricus*: Edgeworth, '25; Regel, '68). This latter mode of development closely resembles that seen in *Ascaphus*.

The adult otic ledge and basal process of *Ascaphus* arise during metamorphosis from distinct centers of chondrification in a common blastema (van Eeden, '51). The adult

otic ledge chondrifies in continuity with the floor of the otic capsule (i.e., the basiotic lamina). It occupies a similar position as the "larval otic ledge," in part attaching to its remnants. The basal process of the adult chondrifies from a more distal center that subsequently fuses with the palatoquadrate body. Thus, at least the distal part of the adult otic ledge is derived from the larval basal process, and should be considered a palatoquadrate derivative. The identical conclusion was reached by van Eeden ('51). The adult otic ledge and basal process form similarly in *Heleophryne purcelli*, the only other anuran with a larval palatobasal connection in which metamorphosis has been described (van der Westhuizen, '61). Development is also similar in species that lack a larval connection (reviewed by van der Westhuizen, '61; Swanepoel, '70; Pyles, '88). Thus, in all anurans, both the basal process and the distal otic ledge seem to derive from the palatoquadrate.

Available evidence suggests that in all salamanders, a syndesmotric joint develops in the palatobasal connection in the mid-larval period (Edgeworth, '25; Stadtmüller, '36); this joint may be diarthrotic or synchondrotic in the adult (Stadtmüller, '36; Trueb, '93). In *Salamandra salamandra* (in which the basal process chondrifies independently of the neurocranium) the joint develops in the middle of the basal process, lateral to the original point of its fusion to the neurocranium; the freely projecting part of the "basitrabecular process" is actually a piece of the original basal process (Stadtmüller, '24). Morphological comparison suggests that the joint in other salamanders forms in the same relative position (Stadtmüller, '36). Thus, both the "basitrabecular process" of adult salamanders and the "otic ledge" of adult anurans seem to be partly derived (in ontogeny) from the basal process of the palatoquadrate.

The third issue concerns the precise relation of the palatobasal connection to the neurocranium. In particular, the relation of the connection to the palatine branch of the facial nerve, which de Beer ('26) proposed as the criterion for a "true" basal connection, must be considered more explicitly. As noted above, the presence of a palatine foramen has been considered proof that *Ascaphus*—and salamanders—have a true basitrabecular process (de Beer, '37; Pusey, '38, '43). This view overlooks an important, purely topologi-

cal, point. There seems to be no way to distinguish between a prefacial commissure that extends far laterally, contacting the postpalatine commissure, a true basitrabecular process, and a basitrabecular process fused with the prefacial commissure. This can be seen by reference to Figure 21, in which it is clear that no nerve or vessel passes through the (hypothetical) foramen I have shown between the prefacial commissure and the root of the basitrabecular process. Given this ambiguity, the term "basitrabecular process" should be restricted to parts of the skull base that project laterally beyond the side wall of the skull. I have just given reasons for believing that the freely projecting part of the "basitrabecular process" in adult salamanders, and the majority of the "otic ledge" of anurans, are both ontogenetically derivatives of the basal process. With this preliminary caution, let us examine the relations of the facial nerve in *Ascapus*, other frogs, and salamanders.

In *Ascapus* larvae, as well as adults, the facial root and anterior auditory nerve exit the cranial cavity through a common foramen (de Beer, '37; Pusey, '43; van Eeden, '51). This foramen, the "anterior auditory foramen" of Pusey ('43), and van Eeden ('51), is more properly called the anterior auditory meatus (Fig. 18). It opens into a space (the facial canal) that houses the proximal end of the facial ganglion, and is continuous dorsally with the cavity of the otic capsule. From this space the anterior auditory nerve runs dorsolaterally through the anterior auditory foramen (proper) into the otic capsule, whereas the facial ganglion emerges ventrally through the facial foramen. What I term the prepalatine connection—part of the palatobasal connection—divides the facial foramen into separate palatine and hyomandibular foramina.

Based on the above discussion, my interpretation of the facts of development in *Ascapus* is as follows: When it first forms (Fig. 10), the palatobasal connection lies entirely behind the palatine nerve; it involves contact of the basal process and the postpalatine commissure. Later, the rostral margin of the palatobasal connection grows forward, lateral to the palatine nerve, and fuses with the ventral surface of the otic capsule, just in front of the facial foramen. This prepalatine connection creates the larval palatine foramen. At least a few mesodermal cells are present immediately lateral and

anterolateral to the palatine foramen, and participate in forming the prepalatine connection. More distal parts of the palatobasal connection are composed of ectomesenchymal cells, and represent the basal process of the palatoquadrate.

Among other frogs, a palatine foramen is known to occur only in adults of the genera *Leiopelma* (E.M. Stephenson, '51, '55) and *Hymenochirus* (Paterson, '45). In *Leiopelma* the facial ganglion lies extracranially, and the palatine foramen transmits both the facial root, exiting the skull, and the hyomandibular nerve, reentering the skull to pass laterally through the hyomandibular canal. As noted by van Eeden ('51, 124), when compared with *Ascapus* it appears as though the facial ganglion of *Leiopelma* has been drawn downwards through the palatine foramen. The "anterior wall of the hyomandibular canal" (equivalent to the prepalatine connection of *Ascapus*) is lacking in some specimens of *Leiopelma hochstetteri* (E.M. Stephenson, '51), thereby eliminating the separation of palatine and hyomandibular foramina. This produces a situation closely resembling that found in discoglossid frogs, in which the facial ganglion is also extracranial (reviewed by van Eeden, '51). A facial canal is absent in *Leiopelma*, but present in all the discoglossids.

In salamanders, as in *Ascapus*, the facial and auditory ganglia are closely associated. The facial nerve also exits the skull through a facial canal, running between the rostral end of the otic capsule caudodorsally, the prefacial commissure anteriorly, and the basiotic lamina ventrally (Goodrich, '30, figs. 260, 261, 453D). The anterior acoustic foramen lies in the ventromedial wall of the otic capsule, above the proximal end of the facial canal. In the early development of some species (e.g., *Salamandra salamandra*: Stadtmüller, '24) the floor of the anterior cupula is not chondrified above the facial canal, and the facial nerve appears to pass through the otic capsule (Gaupp, 1893; 385–386; Goodrich, '30, fig. 453D; de Beer, '37). As noted previously (e.g., de Villiers, '34; de Beer, '37; Pusey, '43), the latter condition greatly resembles that of *Ascapus*. A similar condition also occurs in young larvae of the discoglossid frog *Alytes obstetricans* (de Beer, '37).

In most salamanders, the distal end of the facial canal is formed by the postpalatine commissure, which is connected anteriorly to the prefacial commissure ("basitrabecular

process"). This connection separates the palatine and hyomandibular foramina, and is directly comparable to the prepalatine connection of *Ascaphus*. The basal process connects either with the prefacial or postpalatine commissure (or both), and the palatine nerve thus pierces the root of the palatobasal connection. Species of the genus *Siren* are an exception to this pattern; here the palatine nerve passes forward above the palatobasal connection, which is entirely postpalatine (Norris, '13: 297–298; Bourque, '39).

In summary, I agree with van Eeden ('51) that there is no evidence that *Ascaphus* has a basitrabecular process at any stage of development (contra de Beer, '37; Pusey, '38, '43). However, the "otic ledge" of *Ascaphus* and other anurans does correspond to the "basitrabecular process" of salamanders. Both are most likely a part of the basal process of the palatoquadrate, fused to the neurocranium. The presence or absence of a "prepalatine" part of the palatobasal connection is determined by the presence or absence of a connection between the postpalatine and prefacial commissures. Such a prepalatine connection is present in *Ascaphus* and *Leiopelma*, as well as most salamanders.

The palatobasal connection of caecilians is quite different from that in frogs and salamanders. In the most basal living caecilians (rhinatrematids, ichthyophiids) the palatobasal connection is represented only by a sheet of connective tissue between the pterygoid bone and the base of the skull, both in larvae and adults (Visser, '63; Reiss, '96). In adults of other caecilians a well-developed basal articulation is present, formed by contact of the pterygoid bone and ossified pterygoid process with the basitrabecular process of the basal bone, in front of the otic capsule (Trueb, '93). The palatine nerve runs forward dorsal to the articulation (e.g., Norris and Hughes, '18), or accompanies the internal carotid artery through a canal in its base (e.g., Ramaswami, '41; Brand, '56; Els, '63; Visser, '63).

The development of the caecilian basal articulation is not well known. In caecilian embryos, the facial ganglion is lodged in a large fenestra in the side of the chondrocranium, below the tip of the anterior cupula, and the palatine nerve passes down through its own foramen in the floor of this fenestra (Peter, 1898; de Beer, '37). However, the rela-

tion of this foramen to the later-developed basal articulation is unclear. From the work of Marcus et al. ('35), it seems that the basal articulation of *Grandisonia* may form rostral to this region. In any case, it is clear that there is little resemblance between conditions in caecilians and those in salamanders and frogs, save a general adherence to the tetrapod Bauplan. In particular, there is no relation between the palatobasal connection and the otic capsule in caecilians, unlike frogs and salamanders, but like most other tetrapods.

#### CONCLUSIONS

The homologies I propose for anuran palatoquadrate processes are relevant both to relationships among frogs, and to relationships among the three groups of living amphibians (the Lissamphibia). For relationships among frogs, salamanders are the appropriate outgroup. Monophyly of the Bombinanura (all living frogs except *Ascaphus* and *Leiopelma*) of Ford and Cannatella ('93) is supported by the loss of the prepalatine connection, although this loss occurs convergently within *Leiopelma*, and is reversed in *Hymenochirus*. The extracranial position of the facial ganglion (van Eeden, '51) supports the monophyly of the Leiopelmatanura (all frogs except *Ascaphus*). Two other characters for Leiopelmatanura listed by Ford and Cannatella ('93)—loss of the ascending process, and the replacement of a basal by a pseudobasal process—are based on misinterpretations of homology in the literature on *Ascaphus*. The "ascending process" of *Ascaphus* adults is a neurocranial structure; it is unclear whether its presence is primitive for anurans or a derived feature of *Ascaphus*. The basal process of *Ascaphus* and the "pseudobasal process" of other anurans are homologues.

Turning to lissamphibian relationships, it is clear that *Ascaphus* and salamanders show a number of striking similarities in the structure and development of the basal connection and adjacent otic capsule. These include: (1) the location of the basal connection at the anterior end of the otic capsule (prefacial or postpalatine commissures), (2) the formation of a facial canal at the anterior end of the otic capsule, and (3) the division of the basal process at a point lateral to its initial attachment to the chondrocranium. The first two of these are unique among living tetrapods. However, the best outgroups for the Lissamphibia are fossil disso-

rophoid amphibians, including *Amphibamus*, *Dolesempetron*, and branchiosaurids (Milner, '88, '93; Bolt, '91; Treub and Cloutier, '91). These dissorophoids also have a basal articulation with the anterior end of the otic capsule, though the detailed structure of the region is unknown. If we accept the monophyly of Lissamphibia, caecilians have secondarily derived a more anterior basal articulation. The other two characters uniting frogs and salamanders are not known from the fossil taxa, so their polarity is uncertain. Thus, the notable similarities between salamanders and frogs in the structure and development of this region do not provide any support for a sister group relationship of frogs (Salientia) and salamanders (Caudata).

In conclusion, this study of early chondrocranial development in a basal anuran provides fundamental information for phylogenetic studies of lissamphibian cranial morphology. I have focused in particular on palatoquadrate development. Analysis of these data in a comparative context suggests a resolution of the long-standing controversy over the homologies of anuran palatoquadrate processes, and thereby allows reinterpretation of palatoquadrate characters used in phylogenetic analyses of the Anura and the Lissamphibia. This study thus provides a specific example of the utility of developmental analysis in establishing homology. I have shown elsewhere that palatal metamorphosis in general is a derived feature of lissamphibians (Reiss, '96). Metamorphic remodeling of the palatoquadrate in anurans is dramatic compared with that in salamanders and caecilians. In the future, I hope to use the palatoquadrate homologies established here to reconstruct the evolution of palatoquadrate metamorphosis within the Lissamphibia.

#### ACKNOWLEDGMENTS

This study would not have been possible without the help of a great number of people. P. Alberch provided inspiration, laboratory facilities for much of the work, and financial support. Additional support came from an NSF dissertation improvement grant, and emergency funds granted by the Department of Organismic and Evolutionary Biology, Harvard University. D.K. McClearn, A.R. McCune, and F.H. Pough generously provided laboratory facilities during my stay at Cornell University. D.M. Noden loaned a microscope with camera lucida attachment

for the drawing of sections. A. Bass loaned a microprojector for the same purpose. H. Brown provided a number of *Ascaphus* tadpoles, as well as a pleasant walk in the mountains. Finally, K.Z. Reiss was an able field assistant, and provided both moral and logistical support throughout. For comments on part or all of the manuscript I thank G. Graffin, F.A. Jenkins, Jr., A.R. McCune, K.Z. Reiss, C.S. Rose, and two anonymous reviewers.

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