

Development of the Ethmoidal Structures of the Endocranium in *Discoglossus pictus* (Anura: Discoglossidae)

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ABSTRACT We use histological techniques and computer-aided three-dimensional reconstructions made from serial histological sections to describe the ontogeny of the ethmoidal endocranium of discoglossid frog *Discoglossus pictus*. We identify a pattern of development for the suprarostreal cartilage that differs from previous findings and probably represents the ancestral anuran pattern. The nasal cartilages, including the inferior prenasal cartilage, are de novo adult structures. The only larva-derived structures of the adult nasal capsules are the posterior aspects of the solum nasi and septum nasi. We also identify patterns of development for the ethmoid plate and postnasal wall that occur during early in ontogenesis. These patterns are associated with development events during metamorphic climax. The pattern of timing of chondrification of the anterior nasal cartilages more closely coincides with that of the neobatrachian species than that recorded for the pelobatid frog *Spea*. In addition, this study supports a sister taxon relationship between *Discoglossus* and *Alytes*. *J. Morphol.* 271:1078–1093, 2010. © 2010 Wiley-Liss, Inc.

KEY WORDS: Anura; Discoglossidae; *Discoglossus*; development; cranium

INTRODUCTION

The ethmoid region is one of the structures that undergo dramatic remodeling during anuran development and metamorphosis. Although the anuran skeleton as well as the structural changes that tadpoles undergo during development and metamorphosis received much study in recent years (e.g., Trueb and Hanken, 1992; Hall and Larsen, 1998; Maglia et al., 2001; Haas, 2003), fewer studies have examined the skeletal anatomy of the adult nasal capsules or their development (e.g., Roček, 1981; Haas, 1996; Pugener and Maglia, 2007). Thus, there is a considerable lack of basic data on developmental morphology of the anurans, despite the fact that the ethmoid region of the anuran skull possesses morphological features that may provide important clues to the phylogeny and morphological evolution of the Anura (Jurgens, 1971). In view of the crucial phylogenetic position of the Discoglossidae (e.g., Duellman and Trueb,

1986; Ford and Cannatella, 1993; Maglia et al., 2001; Pugener et al., 2003), there is an evident need for such data for this taxon.

The painted frogs of the genus *Discoglossus* are small-sized frogs with long hind limbs and shorter, but stocky forelegs. The genus comprises at least five species distributed over western Europe, northwestern Africa, the Middle East, and some Mediterranean islands (e.g., Busack, 1986; García-París and Jockusch, 1999). *Discoglossus* is an extant member of an ancient frog lineage, represented for example by *Eodiscoglossus* from the Middle Jurassic to Early Cretaceous periods in Europe (Evans et al., 1990), more than 160 million years ago.

Several authors have described the adult osteology and larval development of *Discoglossus*. The oldest study that included a brief description of the adult skeleton of *Discoglossus pictus* was published by Boulenger (1896). Van Zyl (1950) described the adult cranium of *D. pictus* from a reconstruction based on serial sections. Also, Cannatella (1985) and Clarke (1988) used adult osteological characters in their phylogenetic analyses of archaeobatrachian frogs. Kraemer (1974) published a description of the chondrocranial development of *D. pictus*, and Pugener and Maglia (1997) described the skeletogenesis and the development of *D. sardus*. A number of studies (e.g., Bolkay, 1919; Pusey, 1938; Slabbert and Maree, 1945; Jarošová, 1974; Sokol, 1981; Fabrezi, 1992; Havelková and Roček, 2006) included

Contract grant sponsor: Ministry of Education of the Czech Republic; Contract grant number: MSM0021627502.

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Received 16 September 2009; Revised 17 March 2010;
Accepted 21 March 2010

Published online 7 May 2010 in
Wiley Online Library (wileyonlinelibrary.com)
DOI: 10.1002/jmor.10855

references to the development and larval and adult characters of *Discoglossus*. Among these, only Kraemer (1974) and Pugener and Maglia (1997) briefly described the development of the ethmoid region of the endocranium.

Here, we provide a detailed account of the development of all components of the ethmoidal endocranium of *D. pictus* and discuss these data in the context of classical descriptions of the ethmoidal endocranium development in other anuran species.

MATERIALS AND METHODS

We examined 14 individuals of the painted frog *D. pictus* (Otth, 1837) (a loan from the Hubrecht Laboratory of the Netherlands Institute for Developmental Biology, now at the Institut für Systematische Zoologie, Museum für Naturkunde, Berlin). Tadpoles were lab-raised and staged according to the developmental table of Nieuwkoop and Faber (1956); stages are abbreviated as NF in the text. To facilitate comparison with other frog species, approximate correspondences between stages of the general staging table of Gosner (1960) are given in Table 1. All stages were sectioned and stained. Histological sections were stained using Heidenhain's Azan trichrome (Gray, 1954). Catalog number and stage of the specimens examined are presented in Table 1.

Eight individuals of *D. pictus* (Stages 39, 40, 44/45, 46, 49, 55/56, 59, and 66) were used to develop 3D-models generated from serial histological sections using 3D Studio MAX 6.0; smoothing was done in Bone-Viewer, a program specially written for this purpose by Ysoft Co., Czech Republic.

Anatomical terminology, in general, follows that of the Amphibian Anatomical Ontology (Leopold et al., 2007; Maglia et al., 2007). Alternative terms to those used in the descriptions are indicated throughout the text and each is followed by one or more reference citations. Terms for higher taxonomic units within the Anura follow Ford and Cannatella (1993). Evolutionary considerations are based on the hypothetical phylogenetic relationships among the major clades of frogs given by Ford and Cannatella (1993).

Photographs were taken using a Nikon Eclipse 80i microscope fitted with a Nikon DS-5M digital camera. Illustrations were made from photographs using Corel Draw 11.0. The material is deposited in the collections of the Department of Zoology, Charles University in Prague and in the Department of Biological and Biochemical Sciences, University of Pardubice in Pardubice.

RESULTS

Timing of development of the ethmoidal endocranium is summarized in Table 2.

At NF Stage 39 (Figs. 1–3), early signs of mesenchymal condensation are detected. This early condensation corresponds with the rudimentary trabeculae (=trabeculae cranii *sensu* Roček, 2003) and mandibular arch. The mandibular arch is represented by a continuous, vertical strip of mesenchyme, located perpendicular to the trabeculae. It is difficult to differentiate, at this stage, among Meckel's cartilage, the palatoquadrate and the infraorbital (=lower labial cartilage *sensu* Roček, 1981, 2003; Haas, 1996, 2003). A rostrally directed process extends off of the ventral part of the mandibular arch (Figs. 1–3). This process seems to represent the prospective suprarorbital ala (=lateral suprarorbital *sensu* Reiss 1997).

TABLE 1. Comparison of two common normal tables of development for anurans and list of specimens examined

Features	Nieuwkoop and Faber (1956)	Gosner (1960)	Specimens examined	
			ZMB no.	NF stage
Neural tube	20	16		
Early tailbud	24	17		
Muscular response, olfactory pits	26	18		
	28	—		
Tailbud:length = breath	31	—		
Heart beat	33	19		
Circulation in 1st gill	36	—	DP 8	39
Circulation in both gills	40	20	DP 9	40
Mouth opens	—	21		
Circulation in tail fin	41	22		
Operculum anlage	42	—	DP 10	42/43
Mouth moves	—	—		
Beak, gut slings	43	23		
Operculum extends	—	23		
Denticles	—	23		
Operculum closes	44	24	DP 11	44/45
Spiracle (early larva)	45	25		
Hindlimb bud	46	26	DP 12	46
	47	27	DP 13	47
	48	—		
	49	28	DP 15	49
	50	—		
	51	29	DP 17	51
	52	30	DP 18	52
	53	31		
Midlarval stages	—	32		
	—	33		
	54	34		
	55	35	DP 20	55/56
	—	36		
	—	37		
	56	38		
	57	39		
	—	40		
	58	—		
Onset metamorphic climax	59	—	DP 22	59
	60	41		
	—	—		
	61	—	DP 23	61
	62	42		
	63	43		
	64	44		
	65	45		
	66	46	DP 25	66

The approximate correspondence between the developmental stages of *Xenopus laevis* (Nieuwkoop and Faber, 1956) and the general staging table for frogs (Gosner, 1960) is adopted from Schlosser and Roth (1997). The second part of the table starting with NF 46 is adopted from Trueb and Hanken (1992). ZMB no., Institut für Systematische Zoologie, Museum für Naturkunde, Berlin catalog number; NF stage, stages according to Nieuwkoop and Faber (1956).

At NF Stage 40 (Figs. 1–3), the skull still consists exclusively of mesenchyme. Its principal components are rudimentary trabeculae expanding anteroventrally. Judging from a position of the anterior quadrato-cranial commissure (=commissura quadrato-cranialis anterior *sensu* Sokol, 1975; Haas, 1996, 2003) and from the condition in Stage 46 when the trabecular

TABLE 2. The earliest records of some larval structures in the development of *Discoglossus pictus*

	NF stage	NF stage	NF stage	NF stage	NF stage	NF stage	NF stage	NF stage	NF stage	NF stage	NF stage			
	39	40	42/43	44/45	46	47	49	50	51	52	55/56	59	61	66
Earliest records	x													
Trabeculae (mesenchyme)	x													
Trabecular horns (mesenchyme)		x												
Supraorbital corpora (cartilage))-()-(
Supraorbital alae (mesenchyme))-((-)									
Trabecular plate (mesenchyme)								→						
Septum nasi (cartilage)														
Septum nasi (cartilage)														
Columnae ethmoidales (mesenchyme)						x						x		
Lamina orbitonasalis (cartilage)											x			
Undifferentiated mandibular pharyngeal arch (mesenchyme)	x													
Infrarostral (mesenchyme)														
Meckel's cartilage (mesenchyme))-(
Palatoquadratum (mesenchyme))-(
Anterior quadratoorbital commissure (mesenchyme))-(
Admandibular														
Inferior prenasal cartilage (cartilage)	x										x			
Superior prenasal cartilage (cartilage)												x		
Tectum nasi (cartilage)												x		
Solum nasi medial portion (cartilage)												x	→	
Solum nasi lateral portion (cartilage)												x		
Alary cartilage (cartilage)												x		
Oblique cartilage (cartilage)												x		
Anterior nasal wall (mesenchyme)												x		
Basimandibular														x

x is the earliest record of structure with no precursor;)-(structure originating by separation from precursor; (-) structure originating by fusion of preexisting structures; → structure originating by transformation of precursor.

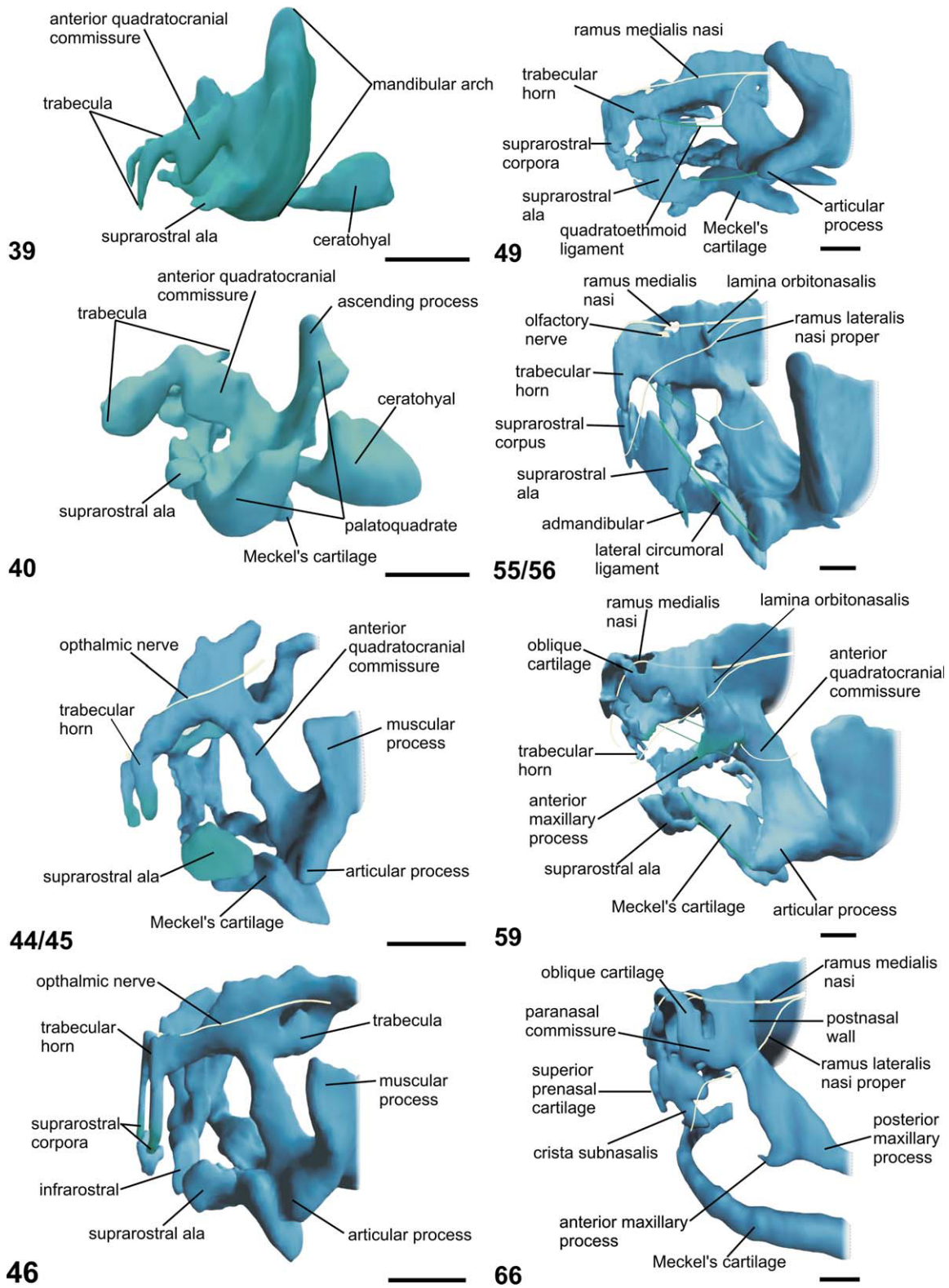


Fig. 1. Lateral view of a 3D-reconstruction of *Discoglossus pictus*. Numbers indicate NF stages. Stages 39 and 40 represent the complete endocranium, all others are ethmoid region only. Anterior is to the left. Cartilage is in blue, nerves are in yellow, and mesenchyme and ligaments are in green. Scales represent 200 μm.

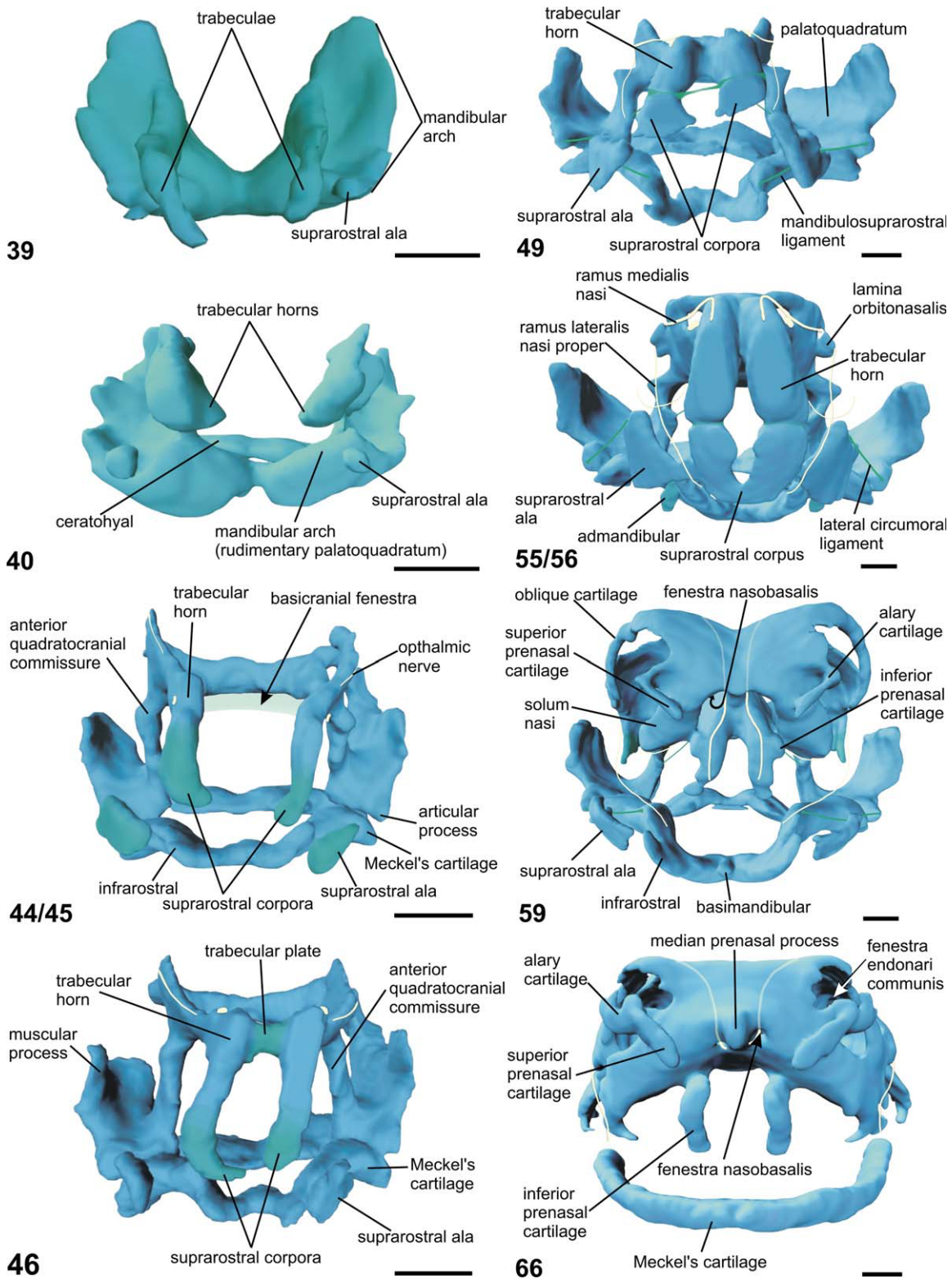


Fig. 2. Anterior view of a 3D-reconstruction of *Discoglossus pictus*. Numbers indicate NF stages. Stages 39 and 40 represent the complete endocranium, all others are ethmoid region only. Cartilage is in blue, nerves are in yellow, and mesenchyme and ligaments are in green. Scales represent 200 μ m.

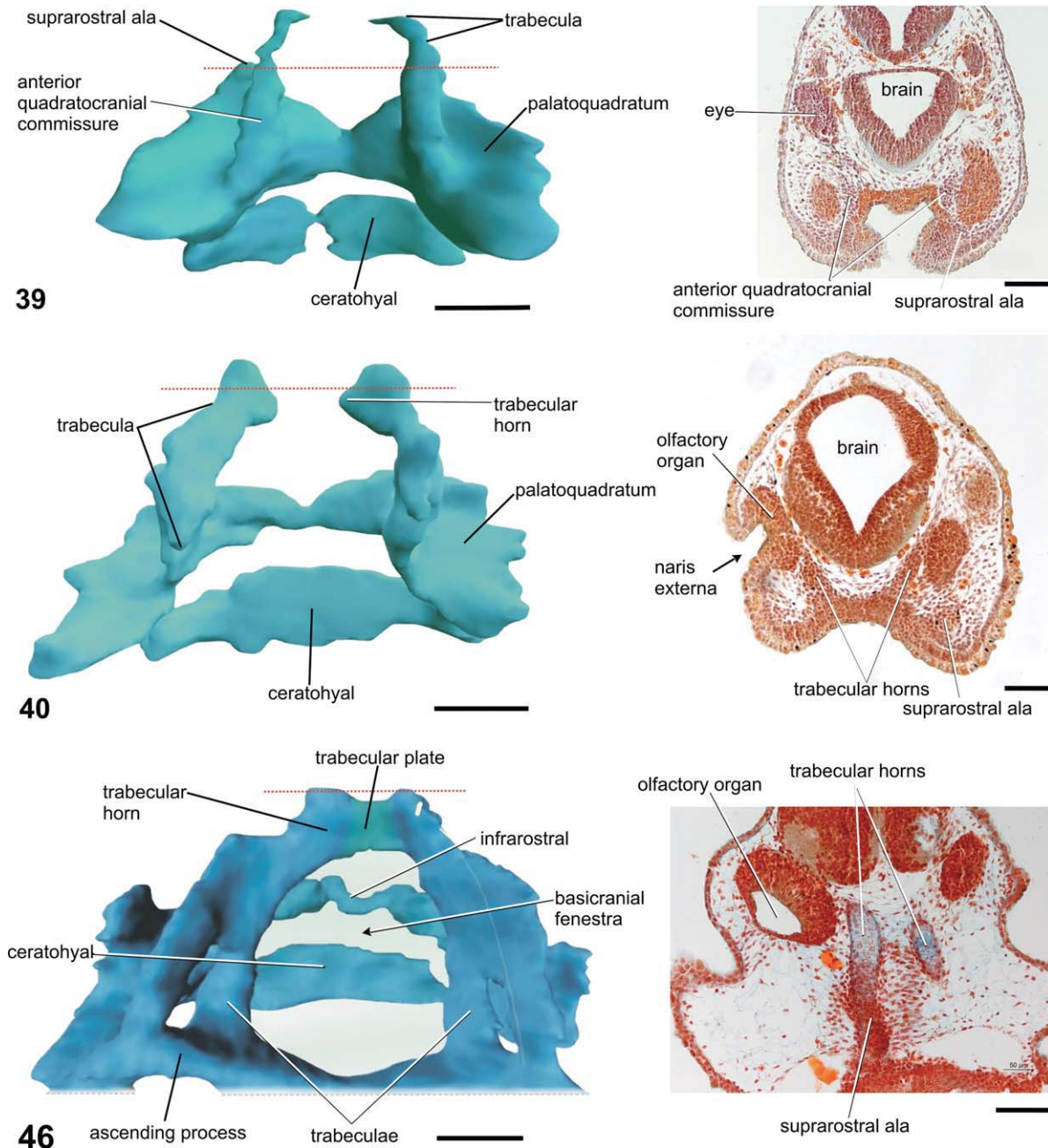


Fig. 3. Dorsal view of a 3D-reconstruction of *Discoglossus pictus*. Numbers indicate NF stages. Stages 39 and 40 represent the complete endocranium. Stage 46 represents the ethmoid region only. Frontal sections through the most anterior part of the ethmoidal endocrania (at levels indicated by red dotted lines) are illustrated in the right column. Cartilage is in blue, mesenchyme is in green, and nerves are in yellow. Scales on the right column represent 100 μm . Scales on the left column represent 200 μm .

plate appears (=planum basale *sensu* Higgins, 1920; = ethmoid plate *sensu* Sokol, 1981; Wild, 1997; = planum trabeculare anticum *sensu* Haas, 1996; = planum ethmoidale *sensu* Sheil, 1999; Sheil and Alamillo, 2005), the trabecular horns (=cornua trabecularum *sensu* Roček, 1981, 1989, 2003; Haas, 1996, 2003) are represented by only small outgrowths on the ventromedial tips of the trabeculae (Figs. 2 and 3).

Usually, the trabecular horns are defined as the parts of the trabeculae extending anteriorly beyond the trabecular plate (e.g., Stöhr, 1882). Other elements on either side are the mandibular arch (with the palatoquadrate, Meckel's cartilage, and infrarostral still not separated from each other) and a large and transversely located ceratohyal. The palatoquadrate starts to rotate anteriorly from its vertical to a horizontal

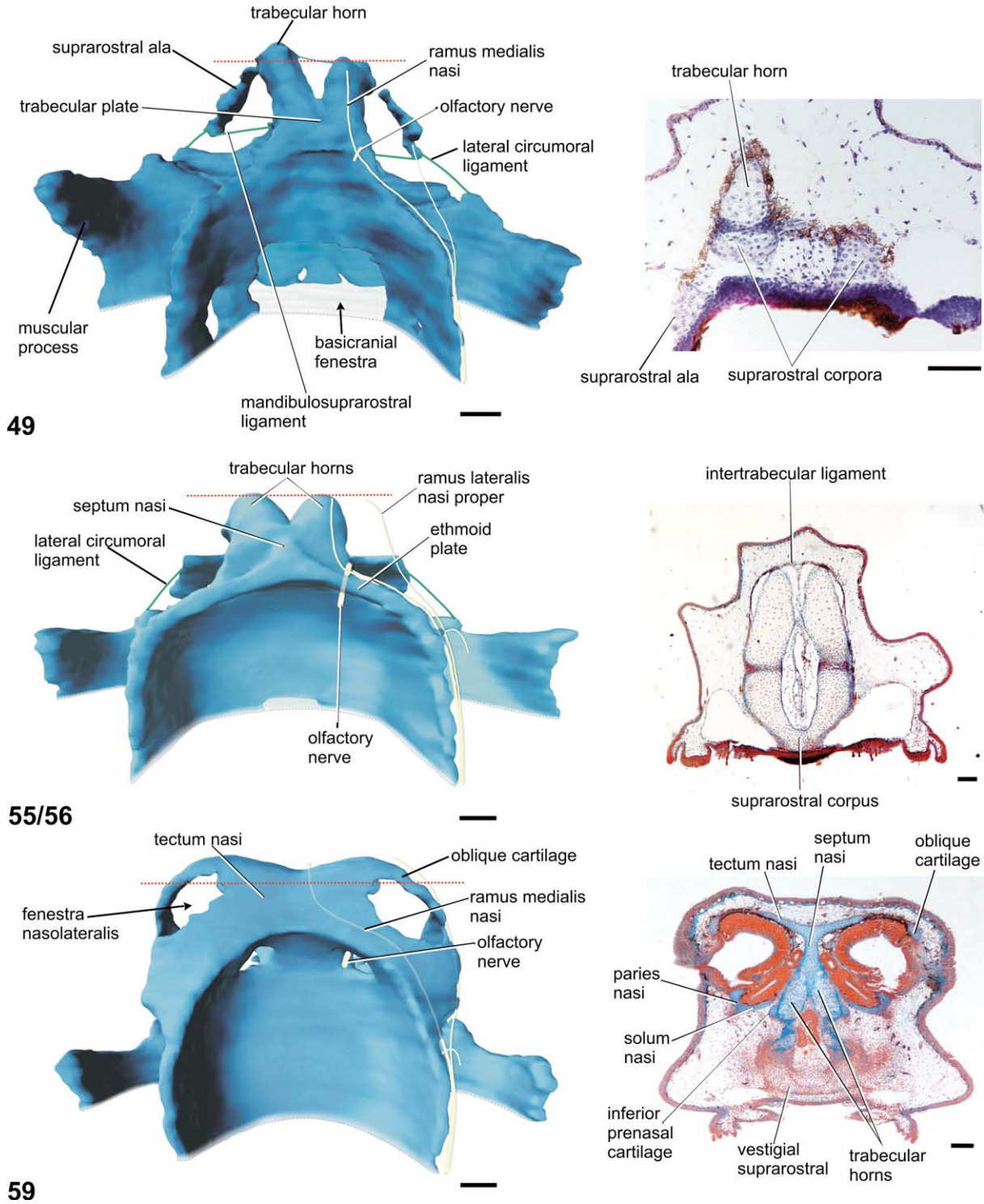


Fig. 4. Dorsal view of 3D-reconstruction of the ethmoidal endocranium of *Discoglossus pictus*. Numbers indicate NF stages. Frontal sections through the most anterior part of the ethmoidal endocrania (at levels indicated by red dotted lines) are illustrated in the right column. Cartilage is in blue, mesenchyme is in green, nerves in yellow. Scales on the right column represent 100 μm . Scales on the left column represent 200 μm .

position. The dorsal end of the palatoquadrate (its ascending process, which in further development takes part in the connection of the posterior section of

the palatoquadrate to the trabecle) is free (Fig. 1). Close to the anterior edge of the palatoquadrate, there is a small condensation that represents the rudiment

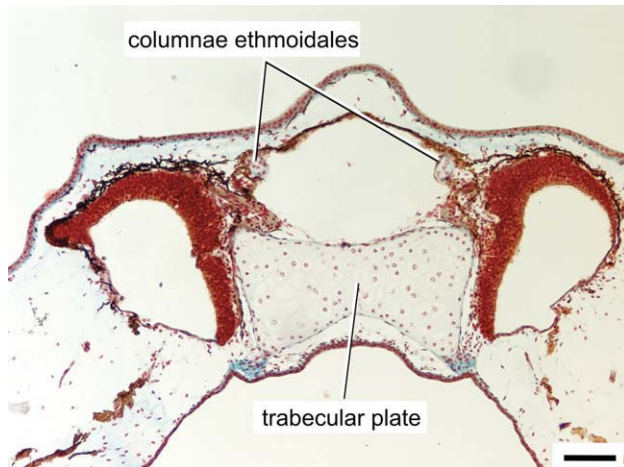


Fig. 5. Columnae ethmoidales of *Discoglossus pictus*, Stage 52, roofing the olfactory nerves. Scale bar represents 100 μm .

of the suprarostrals alae. Thus, it seems that the suprarostrals alae originate from the mandibular arch.

During NF Stage 42/43, the mandibular arch begins to differentiate and the palatoquadrate continues to rotate anteriorly. In further development (at NF Stage 44/45; Figs. 1 and 2), the trabecula, palatoquadrate, Meckel's cartilage, and infrarostrals chondrify, but the connection between Meckel's cartilage and the infrarostrals is mesenchymal. Likewise, the tip of the trabecular horn (the prospective suprarostrals corpus [=medial suprarostrals *sensu* Reiss, 1997]) and the suprarostrals alae are mesenchymal (Figs. 1 and 2). A short section of the lateral edge of the palatoquadrate expands anterodorsally to form the muscular process, which is triangular in shape. Both trabecles continue to expand anteriorly and posteriorly.

At NF Stage 46 (Figs. 1–3), a thin connection consisting of fibroblasts and chondroblasts is established between the trabeculae (Figs. 2 and 3). It represents the earliest rudiment of the trabecular plate. The suprarostrals alae chondrifies and the last parts of the ethmoidal endocranium, which are still formed by mesenchyme, are the tips of the trabecular horns. The palatoquadrate attains nearly a horizontal position, and the anterior quadratocranial commissure forms a little process on its anterior edge, which, in later stages, is a point of attachment of the quadratoethmoid ligament (=ligamentum quadrato-ethmoidale *sensu* Roček, 2003).

At NF Stage 49 (Figs. 1, 2, and 4), the ethmoidal endocranium consists completely of newly formed cartilage. The trabecular horns continue to expand anteriorly and their terminal vertical parts become separated (Fig. 4, right). Thus, it seems that the suprarostrals corpora are derivatives of the trabecular horns. The trabecular plate begins to chondrify in a posteroanterior

direction. The suprarostrals alae extend toward the medial suprarostrals (Fig. 2). The infrarostrals fuse with one another in an unpaired lower larval jaw. Its connections with Meckel's cartilages on both sides are transformed from mesenchymal to movable joints. The dorsolateral edges of the infrarostrals bear articular condyles, which fit only loosely into the cotyles of Meckel's cartilages. The anterior quadratocranial commissure becomes more robust. At this stage, ligaments can be recognized connecting structures of the ethmoidal endocranium. Generally, they consist of parallel collagen fibers and contain many spindle-shaped fibroblasts with prominent elongated nuclei. The quadratoethmoid ligament connects the quadratoethmoid process to the ventrolateral edge of the trabecular horn. A short and thick mandibulosuprarostrals ligament (=ligamentum mandibulosuprarostrale *sensu* Haas, 2003; Figs. 2 and 4) connects the posterior surface of the suprarostrals alae to the dorsomedial surface of Meckel's cartilage. The lateral circumoral ligament (=ligamentum cornu-quadratum laterale *sensu* Roček, 2003; = ligamentum circumoralis *sensu* Sokol, 1981; = ligamentum cornuquadratum *sensu* Haas, 2003) is stretched between the posterior dorsal edge of the suprarostrals alae and the articular portion of the palatoquadrate.

It should be noted that the medial suprarostrals was separated from the trabecular horns as early as in Stage 47 (not illustrated in this article). Obviously, there is some variation in timing of these events.

At NF Stage 55/56 (Figs. 1, 2, and 4), the ethmoid plate (=ethmoidal wall *sensu* Higgins, 1920), the anterior wall of braincase, is cartilaginous and complete with no signs of its origin. Its first paired rudiments may be recognized as early as NF Stage 51 as small condensations are detected above the olfactory nerves. It is still paired at Stage 52 when its rudiments become cartilage (Fig. 5). These structures may be termed columnae ethmoidales (*sensu* Higgins, 1920; Gaupp, 1893; = pilae ethmoidalis *sensu* Duellman and Trueb, 1986). Similarly, the rudimentary septum nasi (=planum verticale *sensu* Higgins, 1920; = nasal septum *sensu* Hall and Larsen, 1998) develops as a vertical partition between the posterior parts of the trabecular horns. Its earliest rudiment may be recognized as a dorsal protrusion from the trabecular plate at NF Stage 50 (Fig. 6). Later, it merges to the median margins of the columnae ethmoidales, forming a complete wall that separates the internasal space from the braincase. The ethmoid plate is pierced by a pair of large olfactory foramina, which are located anterolaterally to the braincase. The developing septum nasi gradually expands anteriorly below the intertrabecular ligament (=ligamentum intertrabeculare superius *sensu* Gaupp, 1893). This ligament connects the dorsal

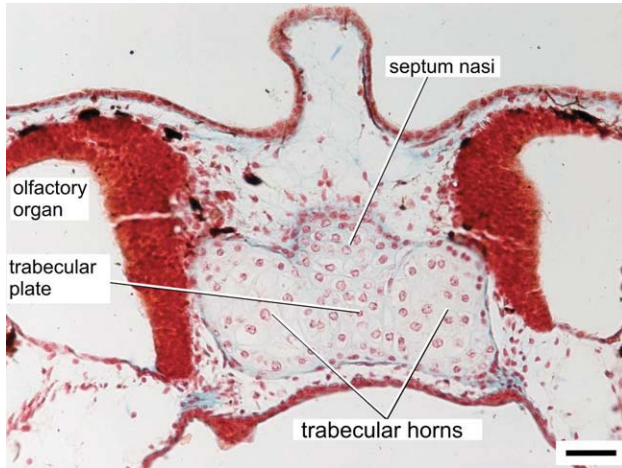


Fig. 6. *Discoglossus pictus*. Section through the posterior part of trabecular horns at NF Stage 50 illustrating arising septum nasi produced by the trabecular plate. Scale bar represents 50 μ m.

edges of the trabecular horns (Fig. 4, right) and continues laterally onto the dorsal side of the olfactory sacs. Also, the rudiment of the lamina orbitonasalis, the earliest part of the postnasal wall (=pars plana nasi *sensu* Gaupp, 1896) separating the nasal capsule from the orbit, may be recognized. The lamina orbitonasalis is distinguished clearly from the braincase wall by its young cartilage (young cartilage can be clearly distinguished from old cartilage by the small size of the chondrocytes and by the staining properties of the extracellular matrix, which is much more basophilic, i.e., stained more intensely). It joins to the braincase wall, thus enclosing the medial branch (=ramus medialis nasi) of the ophthalmic nerve in a slot (Fig. 7). The suprarostal corpora fuse with each other and become an anteroposteriorly compressed, U-shaped vertical plate (Figs. 1 and 2) termed the suprarostal corpus (=pars corporis *sensu* Haas, 2003). Its articulation with the trabecular horns is syndesmotic. The coalesced suprarostal corpora are fused laterally to the suprarostal alae. At this stage, the symphysis between the infrarostals is synchondrotic but the symphyseal cartilage differs from that of the infrarostals. A small element consisting of fibrous tissue, which we consider to be the admandibular (=paramandibular *sensu* de Beer, 1937), is joined to the anteroventral surface of Meckel's cartilage.

At NF Stage 59 (Figs. 1, 2, and 4), the ethmoidal endocranium undergoes profound changes. The original U-shaped suprarostal corpus is nearly completely disintegrated (Fig. 4, right). It is U-shaped but the extracellular matrix is resorbed and the tissue is not stained. It should be noted that the seemingly vestigial suprarostal corpora adjoined ventrally to the trabecular horns in Figure 2 are in fact posteriorly curved trabecular horns. The connection of the suprarostal corpus

with the suprarostal alae is lost. The suprarostal alae move ventrolaterally and are considerably resorbed along their edges (Figs. 1 and 2). The trabecular horns markedly change their shape due to resorption along their dorsolateral and ventral edges and become considerably shorter due to resorption of their anterior parts. In their posterior part, the erosion is less pronounced. The nasal capsules develop laterally to the trabecular horns. The ventral part of the septum nasi seems to be derived from the posterior part of the trabecular horns. A newly formed cartilage is deposited between the vestigial trabecular horns to form the anterior part of the septum nasi (Fig. 4, right). The septum nasi expands anteriorly and dorsolaterally to form the posterior part of the tectum nasi (=planum tectale *sensu* Higgins, 1920; = nasal roof *sensu* Hall and Larsen, 1998). However, in the anterior part of the nasal capsule, the tectum nasi arises independently of the septum nasi. During the following development, it extends medially to fuse with the tectum of the opposite side as well as with the septum nasi. Just anterior to the anterior end of the septum nasi, there is a completely isolated condensation of mesenchyme, which represents the anterior nasal wall (=solum anterius *sensu* Higgins, 1920; = divisio praenasalis communis *sensu* Roček, 1981; = prenasal wall *sensu* Roček, 1989) and the median prenasal process (=processus praenasalis medius *sensu* Roček, 1981). The lateral part of the solum nasi (=nasal floor *sensu* Hall and Larsen, 1998) consists partly of mesenchyme and partly of young cartilage of the nasal capsule, whereas its medial part is derived from the trabecular horn. The inferior prenasal cartilages (=processus praenasalis inferior lateralis *sensu* Stadtmüller, 1936; Jarvik, 1942; = cartilago praenasalis inferior *sensu* Roček, 1981, 2003; = in-

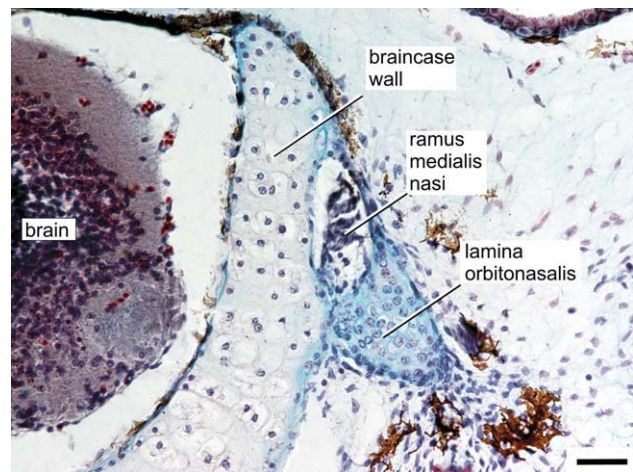


Fig. 7. *Discoglossus pictus*. Section through arising lamina orbitonasalis at NF Stage 55/56. Young cartilage of rudimentary lamina orbitonasalis is clearly distinguishable from old cartilage of the braincase. Scale bar represents 50 μ m.

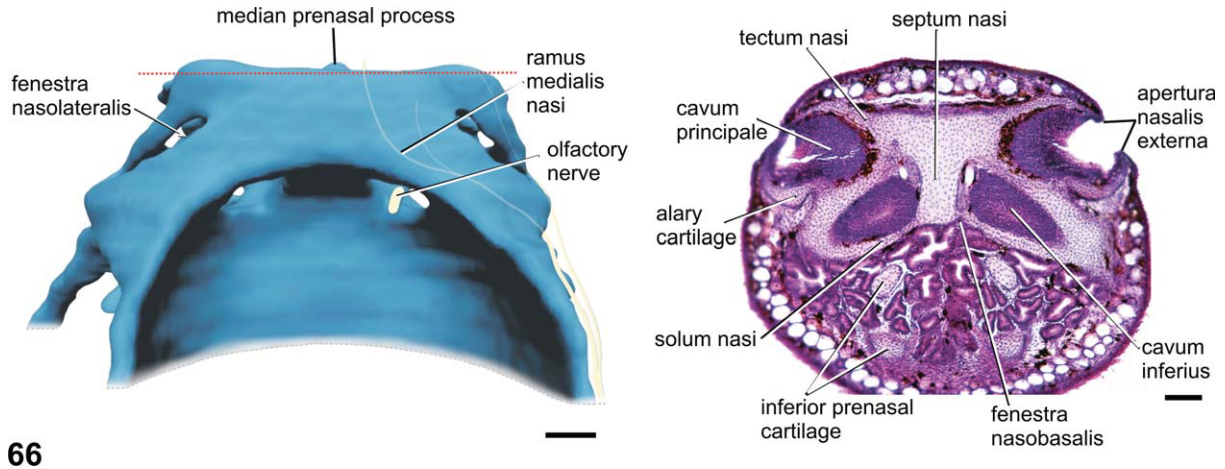


Fig. 8. Dorsal view of 3D-reconstruction of the ethmoidal endocranium of *Discoglossus pictus*, Stage 66. Frontal sections through the most anterior part of the ethmoidal endocranium (at levels indicated by red dotted lines) are illustrated in the right column. Cartilage is in blue, mesenchyme is in green, and nerves are in yellow. Scales on the right column represent 100 μm . Scales on the left column represent 200 μm .

ferior prenasal process *sensu* Reiss, 1998) develop close to the anterior ends of the disintegrating trabecular horns. They are formed by young cartilage clearly distinguishable from cartilage of the trabecular horns (Figs. 2 and 4, right). The anterior part of the developing nasal capsule consists of the solum and tectum nasi; in addition, there is also the alary cartilages (=cartilago alaris *sensu* Gaupp, 1896; Stadtmüller, 1936; Roček, 1981, 2003; = alinasal cartilage *sensu* Higgins, 1920; = processus alaris *sensu* Jarvik, 1942) and the superior prenasal cartilages (=processus prenasalis superior lateralis *sensu* Stadtmüller, 1936; Jarvik, 1942; = cartilago prenasalis superior *sensu* Roček, 1981, 2003). The lateral part of the nasal capsule is far from complete. It consists of the oblique cartilage (=cartilago obliqua *sensu* Gaupp, 1896; Stadtmüller, 1936; Jurgens, 1971; Roček, 1981, 2003; = dorsal process *sensu* Higgins, 1920; = lamina obliqua *sensu* Jarvik, 1942; = cartilago obliquo *sensu* Trueb, 1970) and a narrow strip of cartilage (=the paranasal commissure *sensu* Hall and Larsen, 1998; = connection between lamina nariochoanalis and postnasal wall *sensu* Jurgens, 1971), which connects the oblique cartilage to the anterior part of the postnasal wall. The postnasal wall expands distally, which passes to the planum triangulare (=pars plana *sensu* Parker, 1876; = lamina externa *sensu* Higgins, 1920; = processus antorbitalis *sensu* Roček, 1981; = triangular plane *sensu* Hall and Larsen, 1998). The planum triangulare bears two processes, the anterior maxillary process (=processus maxillaris anterior *sensu* Roček, 1993, 2003) is directed anteriorly, whereas the other, the posterior maxillary process (=the processus maxillaris posterior *sensu* Roček, 1993, 2003) is directed

posteriorly. The posterior maxillary process projects posteriorly along the anterior quadratocranial commissure. The commissure is eroded on its ventromedial side. The planum triangulare as well as the anterior and posterior maxillary processes are still formed by mesenchyme (Fig. 1).

During the following development, the posterior maxillary process elongates to the extent of half the length of the adult subocular bar. At NF Stage 61, the medial part of the subocular bar appears to be histologically older than the lateral part. This reflects fusion between the posterior maxillary process and the anterior quadratocranial commissure. The area is also marked by the posterior attachment of the quadratoethmoid ligament, although this ligament loses its anterior attachment. The posterior part of the subocular bar is formed exclusively by the anterior quadratocranial commissure, which becomes elongated due to rotation and reduction of the palatoquadrate. Both Meckel's cartilages are fused indistinguishably with the infrarostals; however, both halves of the lower jaw are interconnected by an unpaired, well-defined, and rather swollen tissue element, the basimandibulare (Fig. 2). The histological picture of the lower jaw suggests that its elongation (proportional to the elongation of the anterior quadratocranial commissure due to posterior rotation of the palatoquadrate) is mainly the result of growth of the infrarostal and of the anterior half of Meckel's cartilage, combined with erosion of the cartilage on its surface.

NF Stage 66 (Figs. 1, 2, and 8) represents a fully metamorphosed froglet without a tail. The main components of the ethmoidal endocranium are two nasal capsules separated from one another by the

septum nasi. Internally, each nasal capsule is formed by a complex of the cavities. The largest nasal cavity (=cavitas capsulae nasalis *sensu* Roček, 2003), which is also the most dorsal, is the cavum principale. Moreover, two accessory cavities, the cavum inferius and the cavum medium, are located ventromedially and laterally, respectively. Laterally, the septum nasi is continuous with the anterior nasal walls of the nasal capsules and extends out anteriorly in the midline as the median prenasal process. The lateral portion of the anterior wall of the nasal capsule is occupied by the alary cartilage, which is attached anteriorly to the superior prenasal cartilage and ventrally to the lamina superior (=lamina superior cristae intermediae *sensu* Stadtmüller, 1936; Roček, 1981, 2003). The lamina superior, as well as the lamina inferior (=lamina inferior cristae intermediae *sensu* Stadtmüller, 1936; Roček, 1981, 2003), is attached to the crista intermedia. The crista intermedia is a horizontal plate located ventrally to the alary cartilage. The crista intermedia serves as the skeletal boundary between the ventromedial part of the cavum principale and the cavum inferius. The medial margin of the crista intermedia is fused to the tectum nasi adjoining the septum nasi. The inferior prenasal cartilages run out anteroventrally from the solum nasi. The solum nasi of each capsule has a large fenestra endochoanalis (= endochoanal fenestra *sensu* Hall and Larsen, 1998) posteriorly and by a smaller fenestra nasobasalis anteriorly (=foramen apicale *sensu* Gaupp, 1896; Stephenson, 1951; = basal fenestra *sensu* Higgins, 1920; = nasobasal fenestra *sensu* Hall and Larsen, 1998). The lateral wall of the nasal capsule is incomplete and consists of several structures. The oblique cartilage runs posterolaterally across the dorsal and lateral part of the nasal capsule, separating the fenestra nasolateralis (=fenestra dorsalis *sensu* Michael, 1961; = lateral nasal fenestra *sensu* Hall and Larsen, 1998) from the area through which the external nostril opens (see also below). Anteriorly, the oblique cartilage is connected to the tectum nasi, whereas posteriorly it is connected to the anterior surface of the postnasal wall by the paranasal commissure. Additionally, the posterolateral end of the oblique cartilage is attached to the lamina inferior. The oblique cartilage, alary cartilage, tectum nasi, and the lamina inferior all forms the margin of a large fenestra endonarina communis (=apertura nasalis externa *sensu* Michael, 1961; =fenestra narina *sensu* Haas, 1996) that contains the external nostril (=apertura nasalis externa *sensu* Pugener and Maglia, 2007; = external naris *sensu* Trueb, 1970; Wiens, 1989). The postnasal wall is pierced by two openings: the large medial orbitonasal foramen (=foramen orbitonasale *sensu* Roček, 2003) housing medial ramus (=ramus medialis nasi) of the ophthalmic nerve, and the smaller lateral orbito-

nasal foramen housing the lateral ramus (=ramus lateralis nasi proper) of the ophthalmic nerve. The lower jaw is considerably elongated and its former divisions (the median tissue swelling in the midline, infrarostrals, and Meckel's cartilages) cannot be recognized any more.

DISCUSSION

Comparison of Larval Ethmoidal Structures Among the Anura

The upper jaw of frog tadpoles is supported by suprarostal cartilages. Suprarostal cartilages are absent in other vertebrates. They have a characteristic structure (ala and corpus part) and an articulation with the trabecular horns (e.g., Sokol, 1981; Haas, 2003). The developmental origin of these unique cartilages has been unclear for a long time. In the ranid frog, *Rana temporaria*, the suprarostal cartilage forms initially as paired procartilaginous primordia in the anterior ethmoid region, which later fuse with the trabecular horns posteriorly (Stöhr, 1882; Spemann, 1898). During development, these primordia extend medially and laterally to form the medial corpus and the lateral alae (Stadtmüller, 1936). Kerney et al. (2007) show that also the larval suprarostal plate of pipoid frogs develops from the paired anterior primordia. The development of the suprarostal cartilage is different in *D. pictus*. First, in *D. pictus*, suprarostal cartilage arises from tetrapartite primordia. Second, the suprarostal ala seems to be derived from the mandibular pharyngeal arch instead of the trabecular horn. Third, the suprarostal corpora develop in the same manner as in *R. temporaria*, but condensation of the mesenchyme proceeds in posteroanterior direction.

In discoglossids, bombinatorids, and *Ascaphus*, the suprarostal cartilage articulates with the trabecular horns by the medial corpus (van Seters, 1922; van Eeden, 1951; Sokol, 1981). In these phylogenetically basal families, the suprarostal ala does not articulate with the trabecular horn. Moreover, the lateral circumoral ligament is not attached to the trabecular horn but to the suprarostal ala in discoglossids, bombinatorids, and *Ascaphus* (van Eeden, 1951; Sokol, 1981). These similarities in suprarostal morphology suggest that *D. pictus* shares a developmental pattern for the suprarostal cartilage with *Ascaphus*, bombinatorids, and other discoglossids, thus it might represent a plesiomorphic condition for the Anura.

There are also other elements of the larval ethmoidal endocranium in some anurans, namely the adrostral and the admandibular. The adrostral is an ovoid cartilaginous or collagenous element that is situated lateral to the posterior dorsal process of the suprarostal ala (Sokol, 1981; Haas, 1995, 2003). *D. pictus* lacks adrostral. No adrostral is described for other discoglossids, bombinatorids,

pipids, microhylids, and *Ascaphus* (e.g., Sokol, 1981; Trueb and Hanken, 1992; Reiss, 1997; Haas, 2003). Haas (1995) suggested that the adrostral is an apomorphic character of Pelobatoidea + Bufonoidea + Ranoidea (taxa with type IV tadpoles, Orton, 1953), correlated with highly moveable upper jaw. Its absence in *Ascaphus*, bombinatorids, and discoglossids (taxa with type III tadpoles; Orton, 1953) may be interpreted as plesiomorphic character state for the Anura.

The admandibular is an independent element that appears only temporarily in *D. pictus* and remains precartilaginous. The admandibular tissue mass, regardless of whether a cartilaginous admandibular is present or not, is found in the Heleophrynidae (van der Westhuizen, 1961), Discoglossidae (van Seters, 1922; Sokol, 1981; Pugener and Maglia, 1997), and the genus *Bombina* (Bombinatoridae; Maglia and Pugener, 1998; Maglia et al., 2001). Sokol (1981) suggested that the admandibular has evolved as part of the oral adhesive mechanism. This may be true in heleophrynids which have mountain stream tadpoles. However, discoglossids and *Bombina* have typical pond-type tadpoles (Duellman and Trueb, 1986). The functional and phylogenetic significance of this character is obscure.

Ontogeny of the Nasal Capsules

During early development, the olfactory organ lies anterolaterally to the telencephalon. Because of differential growth between brain and the nasal sacs, the relationship changes in most Anura. Consequently, the nasal capsules usually lie forward and ventrolateral to the anterior braincase (Jurgens, 1971). In *D. pictus*, the anterior division of the braincase is still situated between the posterior divisions of the nasal capsules. This might be a plesiomorphic condition for the Anura. Such a condition was also found in Urodela (e.g., Schuch, 1934; Regel, 1968), and two in the primitive genera *Ascaphus* and *Leiopelma* (Stephenson, 1951; van Eeden, 1951). A similar arrangement was also observed in several species of the Ranidae (de Villiers, 1931), Myobatrachidae (du Toit, 1934), Heleophrynidae (van der Westhuizen, 1961), Leptodactylidae (Baldauf and Tanzer, 1965), and in species *Barbourula busagensis* (Bombinatoridae; Jurgens, 1971).

The nasal capsules are de novo, functionally adult structures that begin development during premetamorphosis and prometamorphosis (*sensu* Hall et al., 1997; Shi, 2000). Our observations are comparable with those presented by Kraemer (1974) for *D. pictus*, by de Jongh (1968) for *R. temporaria* (Ranidae), and by Haas (1999) for *Pyxicephalus adspersus* (Ranidae). In contrast, anterior nasal cartilages do not develop simultaneously but one after another in the pelobatid frogs, *Spea intermontana* (Hall and Larsen, 1998) and *Spea multiplicata* (Pugener and Maglia, 2007).

Around Stage 59, all the major elements of the nasal capsules are formed. This discrepancy seems to support the precocious onset of chondrogenesis of the nasal skeleton of *Spea* (Pugener and Maglia, 2007) when compared with *D. pictus* and neobatrachian species. Precocious onset of chondrogenesis of the nasal skeleton in *Spea* may correlate with the occurrence of rapid development in this genus (Dunlap and Sanchiz, 1996).

The development of the anuran ethmoid plate (wall separating the internasal space from the braincase) is not well known. The formation of the ethmoid plate in *D. pictus* starts with development of the septum nasi, which arises as a medial dorsal growth from the trabecular plate, subsequently uniting to the medial margins of the columnae ethmoidales and closing off the braincase from the internasal space. Thus, at no time does a tectum internasale (=pons ethmoidalis *sensu* Higgins, 1920) and a fenestra precerebralis exist in *D. pictus*, like that in *Rana fusca* (Ranidae; Gaupp, 1893) and *Pelobates fuscus* (Pelobatidae; Born, 1876; Plasota, 1974). On the other hand, *D. pictus* seems to share the developmental pattern for the ethmoid plate with the discoglossid frog, *Alytes obstetricans* (van Seters, 1922).

The development of the postnasal wall in *D. pictus* follows the same general developmental pattern recognized for all other anurans (e.g., Swanepoel, 1970; Roček, 1993, 2003). Recently, an alternative view was proposed by Pugener and Maglia (2007) who considered the postnasal wall to be composite structure composed of the lamina orbitonasalis ventrally and the tectum nasi dorsally.

Roček (1981, 1993) suggested that the dorsolateral part of the anuran postnasal wall incorporates a distinct element, the epipraemandibular. Initially, the epipraemandibular is present as a cartilaginous rodlike element next to the posterior margin of the suprarostal ala (Roček, 1981). During the later development, the epipraemandibular changes its location and fuses with the dorsolateral margin of the postnasal wall dorsally to the lateral branch of the ophthalmic nerve (Roček, 1981, 1993). Roček (2003) homologizes the epipraemandibular with adrostral. *D. pictus* does not have the adrostral (see above). Furthermore, given that in *D. pictus* entire postnasal wall (the anticipated location of the adult adrostral) arises from de novo condensation, we cannot support Roček's (1981, 1993, 2003) interpretation that anuran postnasal wall incorporates larval epipraemandibular (or adrostral).

Additionally, van Eeden (1951) suggested that the posterior maxillary process in anurans develops from the anterior quadratocranial commissure and that the entire subocular bar is formed from the commissure. In *D. pictus*, the posterior maxillary process is not derived from the anterior quadratocranial commissure because it develops prior to the resorption of the proximal part of the commissure.

Jarvik (1942, 1980) and Jurgens (1971) suggested that the side wall of the nasal capsule was fairly complete in anuran ancestors. During the ontogeny of many anurans, the nasal lateral wall is extensively formed and cartilage erosion takes place during late ontogeny (Pugener and Maglia, 2007). Such cartilage resorption most probably occurred during their phylogeny as well. Depending on the degree of cartilage resorption, the adult nasal capsule may possess a single lateral window (the fenestra nasolateralis) or two windows (the fenestra dorsalis and fenestra lateralis) separated from each other by a medial cartilaginous bridge reaching between the oblique cartilage and the tectum nasi (Jurgens, 1971). The fenestra nasolateralis may also be bounded ventrally by a cartilaginous bridge, the paranasal commissure, connecting the planum terminale and the postnasal wall. A comparable condition exists in *D. pictus* indicating that *D. pictus* is ancestral in this respect. In *D. pictus*, the lateral nasal wall of the nasal capsule is fairly complete at NF Stage 59. The tectum nasi, however, is resorbed during subsequent development, resulting in a large fenestra nasolateralis and more differentiated oblique cartilage. The paranasal commissure is present in several species of the families Bufonidae (Stadmüller, 1936; Sedra, 1950; Michael, 1961), Pelobatidae (Jurgens, 1971; Hall and Larsen, 1998; Pugener and Maglia, 2007), and in species *Brachycephalus ephippium* (Brachycephalidae; McLachlan, 1943) and *Gastrotheca riobambae* (Hylidae; Haas, 1996). *Brachycephalus ephippium* and *Gastrotheca riobambae* are the only species in which both bridges occur.

The nasal floor of anurans usually bears two pairs of fenestrae, the fenestrae endochoanales and fenestrae nasobasales. The position and the relative size of the fenestra nasobasalis are features that *D. pictus* shares with most ranids, hylids, bufonids, pelobatids (e.g., Parker, 1881; Gaupp, 1893; Higgins, 1920; Jurgens, 1971; Hall and Larsen, 1998; Pugener and Maglia, 2007), and with *Alytes obstetricans* (Discoglossidae; van Seters, 1922; Maree, 1945). In bombinatorids, the fenestra nasobasalis is positioned so far caudally that it lies medially to the attachment of the inferior prenasal cartilage (Born, 1876; Slabbert, 1945; Jurgens, 1971). A similar condition was described for *Ascaphus* and *Leiopelma* (Stephenson, 1951; van Eeden, 1951). It may be assumed that *Ascaphus* and *Leiopelma* represent the plesiomorphic condition with respect to this particular feature. Moreover, the condition obtaining in *Ascaphus* and *Leiopelma* and developmental pattern described here supports Jurgens' (1971) suggestion that the fenestra nasobasalis has shifted from caudal to the terminal position during phylogeny.

The crista intermedia is a characteristic feature of the Anura. The crista intermedia serves as skeletal boundary between the cavum principale and

the cavum inferius, especially that part of the cavum inferius which houses the diverticulum mediale. The developmental pattern described here for *D. pictus* resembles that of several neobatrachian species belonging to the Ranidae, Myobatrachidae, Brachycephalidae, Bufonidae, and Arthroleptidae (Gaupp, 1893; du Toit, 1934; McLachlan, 1943; Badenhorst, 1945; Maas, 1945; Jurgens, 1971). The position of the crista intermedia in the bombinatorids and in *Ascaphus* and *Leiopelma* differs significantly from that in *D. pictus*. In these genera, the diverticulum mediale lies far ventrolaterally, whereas the medial margin of the crista intermedia is fused to the medioventral nasal wall (Born, 1876; Stadmüller, 1936; Slabbert, 1945; Stephenson, 1951; van Eeden, 1951). In *A. obstetricans* (Discoglossidae; Maree, 1945), pelobatids (Jurgens, 1971; Roček, 1981; Pugener and Maglia, 2007), *Hypopachus cuneus* (Microhylidae; Jurgens, 1971), and *Gastrotheca riobambae* (Hylidae; Haas, 1996), however, the crista intermedia is fused to the mid-section of the septum nasi. The various positions occupied by the crista intermedia may support Jurgens' (1971) opinion on the phylogenetic migration of the diverticulum mediale. It is assumed that in correlation with the medial shifting of the diverticulum mediale, the medial margin of the crista intermedia shifted medially and subsequently dorsally. Comparing *D. pictus* to other discoglossids and bombinatorids, it can be assumed that *D. pictus* have achieved a far more derived condition.

The reports about the developmental origin of the inferior prenasal cartilages are contrasting. Parker (1876) believed that the inferior prenasal cartilages are in fact the preserved anterior portions of the trabecular horns. This was a view followed by Stephenson (1951) and Hall and Larsen (1998). Roček (1981) suggested that in the pelobatid frog, *Pelobates fuscus*, the inferior prenasal cartilages originate from the vestigial trabecular horns and from remnants of the suprarostrals cartilages. In *D. pictus*, the inferior prenasal cartilages develop lateral from the anterior ends of the trabecular horns, as de novo condensation, simultaneously with other parts of the nasal capsule. Accordingly, Haas (1996) and Pugener and Maglia (2007) disputed any possibility that the inferior prenasal cartilages would be derived from the trabecular horns and they suggested that they are de novo chondrifications.

CONCLUSIONS

Our study provides detailed information concerning the developmental dynamics of the ethmoid region of a basal frog *D. pictus*. Our findings may have consequences for interpretation of evolutionary patterns of morphological features that have been used to interpret relationship among

more inclusive group of anurans. For example, the pattern of development for the suprarostral cartilage described here supports the idea that larval Type III (*Ascaphus*, discoglossids, bombinatorids) closely resembles the tadpole of the hypothetical ancestral anuran (Sokol, 1975; Ford and Cannatella, 1993). Sokol (1975) rejected Orton's (1957) and Starrett's (1973) interpretation that the fused suprarostral cartilages of pipoid larvae (Type I; Orton, 1953) are plesiomorphic for anurans and that larval Types II, III, and IV are evolved from larval Type I. Sokol (1975) also rejected Starrett's (1973) opinion of structural simplicity as being plesiomorphic in anuran tadpoles. Consequently, Pugener et al. (2003) suggested that the straight, fused suprarostral (e.g., pelobatoids, pipids) would derive from taxa having larvae with beakbearing cartilages. Likewise, the pattern of development for the postnasal wall described here differs from previous findings.

We also identify patterns of development for the ethmoid plate, showing that important developmental events associated with metamorphic climax occur during premetamorphosis. However, available information concerning this character complex is incomplete and the phylogenetic significance of certain anatomical features (absence of the fenestra precerebralis and the tectum internasale) remain unclear. We have shown that nasal capsules of *Discoglossus* retain features that are plesiomorphic for the Anura; however, certain apomorphic features (arrangement of the fenestra nasobasalis and the crista intermedia) support the conclusion of some earlier investigators (Duellman, 1975; Ford and Cannatella, 1993; Pugener et al., 2003) that *Discoglossus* and *Alytes* are more similar to each other than they are to either *Bombina* or *Barbourula*.

ACKNOWLEDGMENTS

The authors thank Jenny Narraway, Hubrecht Laboratory, Utrecht, for the loan of sectioned material of *Discoglossus* under her care, and they thank Dr. Peter Giere, Institut für Systematische Zoologie, Museum für Naturkunde, Berlin, who kindly prolonged the loan. They also thank the anonymous reviewers for valuable comments which helped to improve the earlier version of the manuscript.

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