

THE DEVELOPMENT OF THE ETHMOIDAL REGION OF ASCAPHUS
TRUEI (STEJNEGER)

by

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Thesis presented for the degree of Master of Science

at the

University of Stellenbosch

Submitted: November, 1982

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ABSTRACT

This study deals with the development of the ethmoidal region of Ascaphus truei Stejneger with special interest to the development of the nasal sacs, the diverticulum medium and the Jacobson's organ. This is to gather more information regarding the phylogeny of the structures. The opinions concerning the phylogenetical migration of the Jacobson's organ, are corroborated by the development of the organ in Ascaphus. The possible origin of the diverticulum medium from the nasal end of the nasolacrimal duct also is commented on.

INTRODUCTION

Through the years many attempts have been made to explain the origin of the amphibians as a group. We have the situation that in the transition from an aquatic to a terrestrial life, various adaptations were necessary to enable the first land vertebrates to survive upon the land. In order to explain some of the adaptations and characteristics in the absence of sufficient paleontological evidence, it would be reasonable to study the development of primitive tetrapods.

Such an attempt has been made by studying one of the more primitive anurans, Ascaphus truei. The Ascaphidae, which consists of two genera, Ascaphus and Leiopelma, is regarded as the most primitive group of the Anura.

The type specimen of Ascaphus was first described by Stejneger in 1899 and after that much has been written on the phylogenetic position of Ascaphus. Interesting characteristics of Ascaphus are: the absence of vocal chords, an auditory apparatus with no tympanum and the peculiar sucking apparatus of the tadpole as an adaptation to the life in the mountain streams. Ascaphus, termed the "frog with a tail", has primitive characteristics such as the possession of "tail" muscles, the m. caudalipuboischiotibialis and m. caudalipubofemoralis, a processus

ascendens and the Jacobson's organ, which in position corresponds to that of the more advanced urodeles.

Ascaphus is found only in north-western North America where it lives in cold mountain streams.

Studies on the craniology of Ascaphus were carried out by De Villiers (1934), De Beer (1937), Pusey (1943), Van Eeden (1951) and Jurgens (1971). Van Dijk (1954) did a detailed study on the "tail" of Ascaphus. Work on the development and functions of the nasal sacs in Amphibia have been done by Born (1876), Seydel (1895), Bruner (1913), Broman (1919), Higgins (1920) and Hamlin (1929). Virtually no work has been done on the development of the nasal sacs in Ascaphus, therefore the need arose for the present study, dealing with the development of the ethmoidal region, with special interest to the development of the nasal sacs.

MATERIAL AND METHODS

Five different stages of development were studied:

Stage 1 : Snout-vent length 10,0 mm.

Stage 2 : Snout-vent length 13,2 mm.

Stage 3 : Snout-vent length 16,8 mm.

Stage 4 : Snout-vent length 14,8 mm.

Stage 5 : Snout-vent length 18,0 mm.

All the material were collected from Mason Country, Washington, U.S.A. Stages 3 and 5 were microtomed by Van Eeden (1951). Both specimens were microtomed at 15 microns and stained with boracscarmin and azan. Stages 2 and 4 were decalcified with 7,5% HNO₃ for 4 days and microtomed at a thickness of 10 and 15 microns respectively. Stage 1 was treated with Müller's fluid for 3 days and microtomed at a thickness of 10 microns. Stages 1, 2 and 4 were stained with the triple stain, haemalum, Bismarck brown and erythrosine. Graphic reconstructions were made from transverse and sagittal sections according to the method devised by Pusey (1939).

ACKNOWLEDGEMENTS

This investigation was suggested by and carried out under the guidance of Dr J.D. Jurgens, whom I wish to thank sincerely for his invaluable advice and many hours of fruitfull discussions.

I am most grateful to the Council for Scientific and Industrial Research for the award of a Research Scholarship, which made this study possible.

My cordial thanks to Prof. M.E. Malan for reading the manuscript.

My sincere thanks to Miss Stephany Baard and Mrs Kotzé for typing the manuscript.

Lastly, thanks to my mother who supported me through the years and encouraged me to achieve this.

DESCRIPTION OF DEVELOPMENTAL STAGESStage 1.

As is known the nasal sac of anurans is very complex. However, at this early stage of development the elongated nasal sac of Ascaphus has a relatively simple appearance (Fig. 1, 2). Posteromedially it has an evagination which opens into the preoral buccal cavity through the choana. This preoral buccal cavity is situated anterior to the main buccal cavity and is surrounded in front by the suprarrostral system (Fig. 4). In this young stage the anterior part of this cavity is paired (Fig. 4). E.M. Stephenson (1951) reports a paired preoral buccal cavity in an isolated specimen of the closely-related Leiopelma hochstetteri. The larger part of the nasal sac lies anterolaterally to the olfactory foramen through which the olfactory nerve passes.

The ramus ophthalmicus profundus, while traversing the orbital region, divides into a ramus medialis nasi and a ramus lateralis nasi proper (Figs. 4, 8, 13, 18, 20) (Jurgens, 1971). The ramus medialis nasi enters the nasal capsule through the foramen orbitonasale mediale, while the ramus lateralis nasi proper enters through the foramen orbitonasale laterale (Fig. 20 A, B, C). While running through the cavity of the nasal capsule, the

ramus medialis nasi gives off a lateral branch, the ramus externus nasi. The latter leaves the nasal capsule through the foramen frontale, while the ramus medialis nasi exits through the fenestra nasobasalis. Jurgens (1971) reports that in one of the specimens of Ascaphus studied, the ramus lateralis nasi proper gives off a branch within the the cavity of the nasal capsule (Jurgens, 1971, Figs. 1A and B, 2A). The ramus lateralis nasi proper will be dealt with later (See Discussion).

The primary function of the nasal sac is its perceptive ability through the olfactory epithelium. Generally in anurans this olfactory epithelium is differentiated into three layers of cells each with a specific function. The basal cells form a basal membrane, while the supporting cells support the third type, namely the olfactory cells (Leeson & Leeson, 1970). In this early stage of development in Ascaphus the olfactory epithelium is still undifferentiated. There is, however, already an indication of such a differentiation into three layers. The innermost layer of loosely arranged cells will eventually develop into the olfactory cells.

The nasal sac also has a ventral evagination, the organ of Jacobson. According to the theory put forth by Schmalhausen (1968), the Jacobson's organ originated in

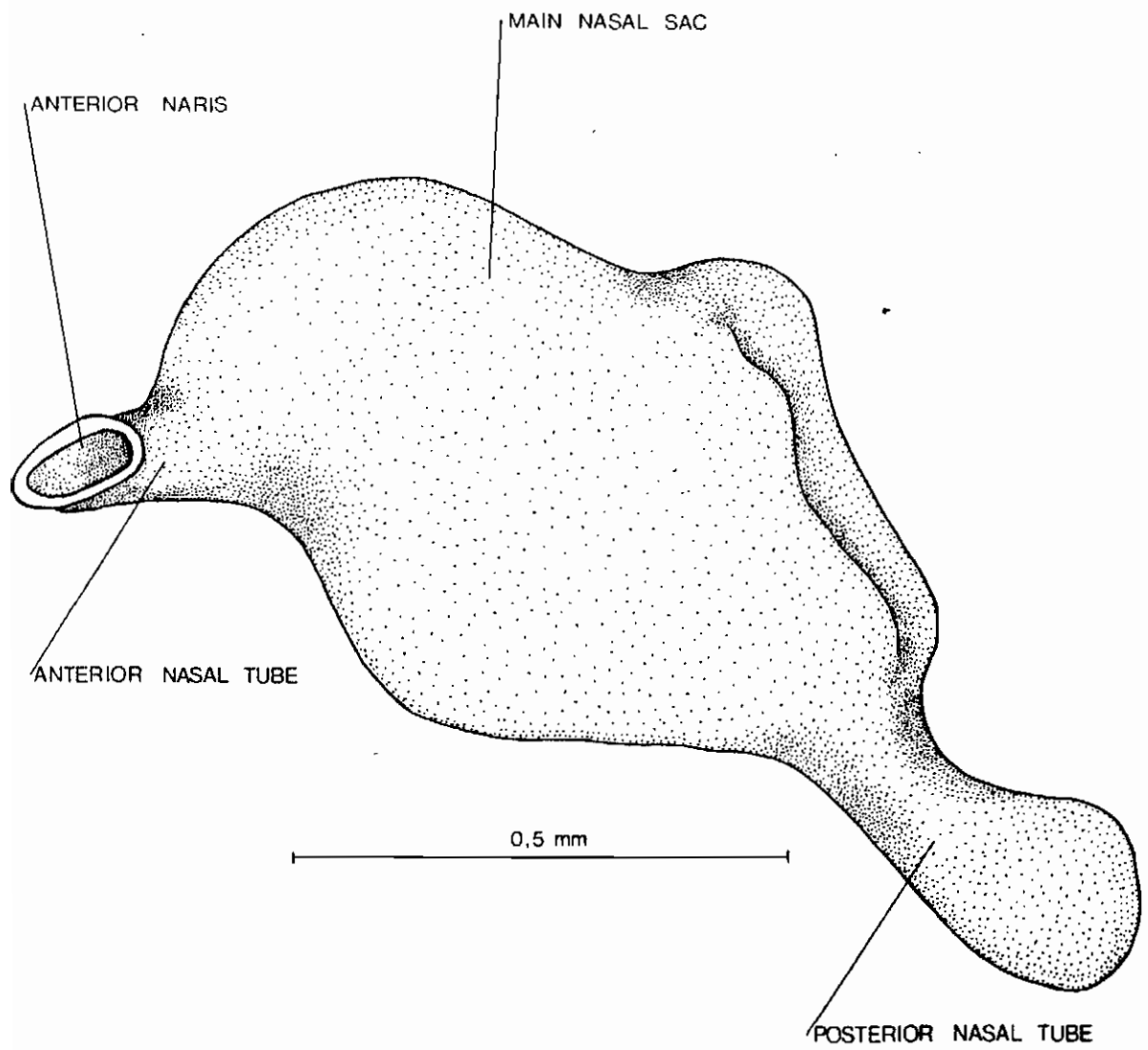


FIG. 1 STAGE 1 - DORSAL ASPECT OF THE LEFT NASAL SAC.

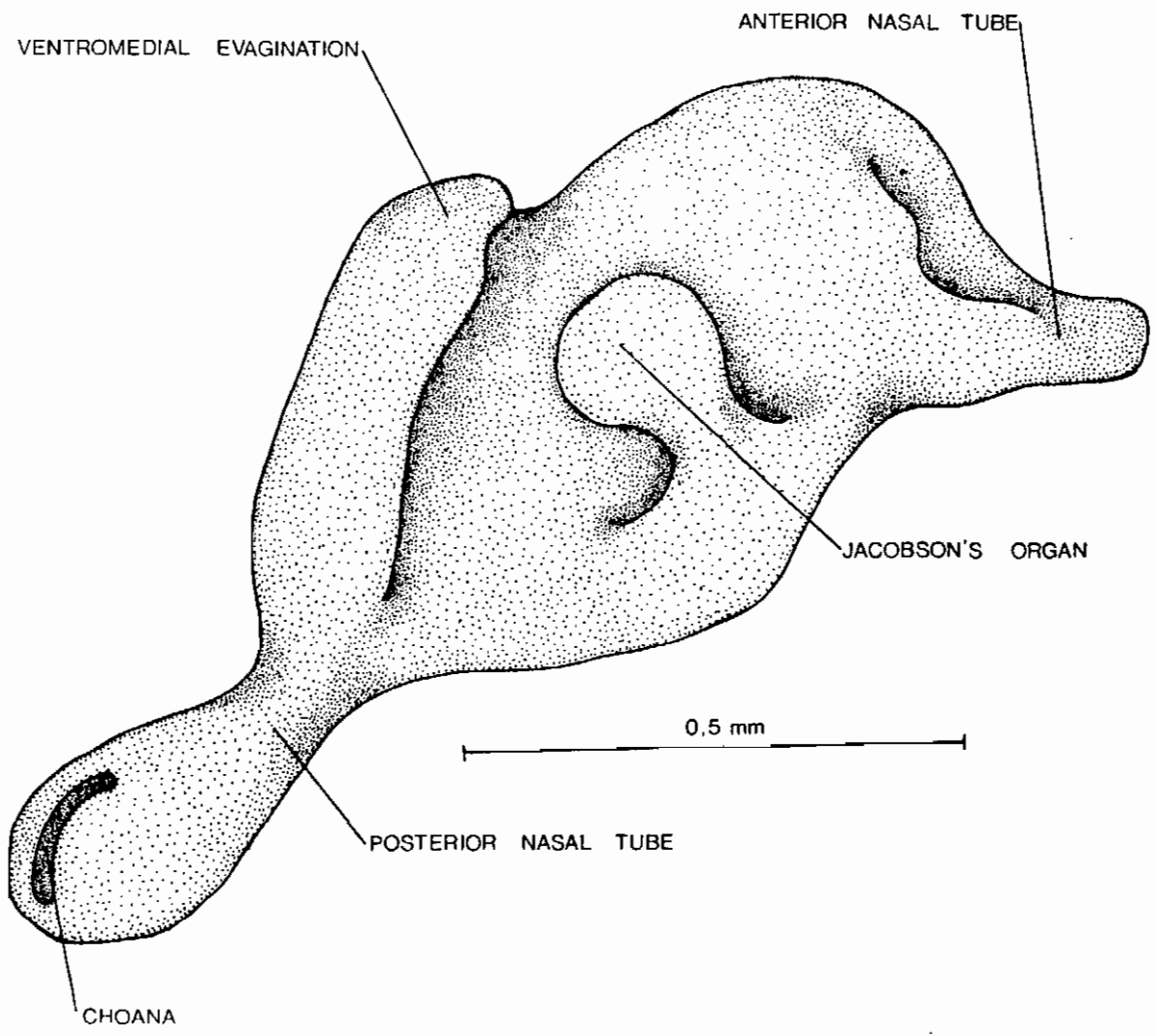


FIG. 2 STAGE 1 - VENTRAL ASPECT OF THE LEFT NASAL SAC.

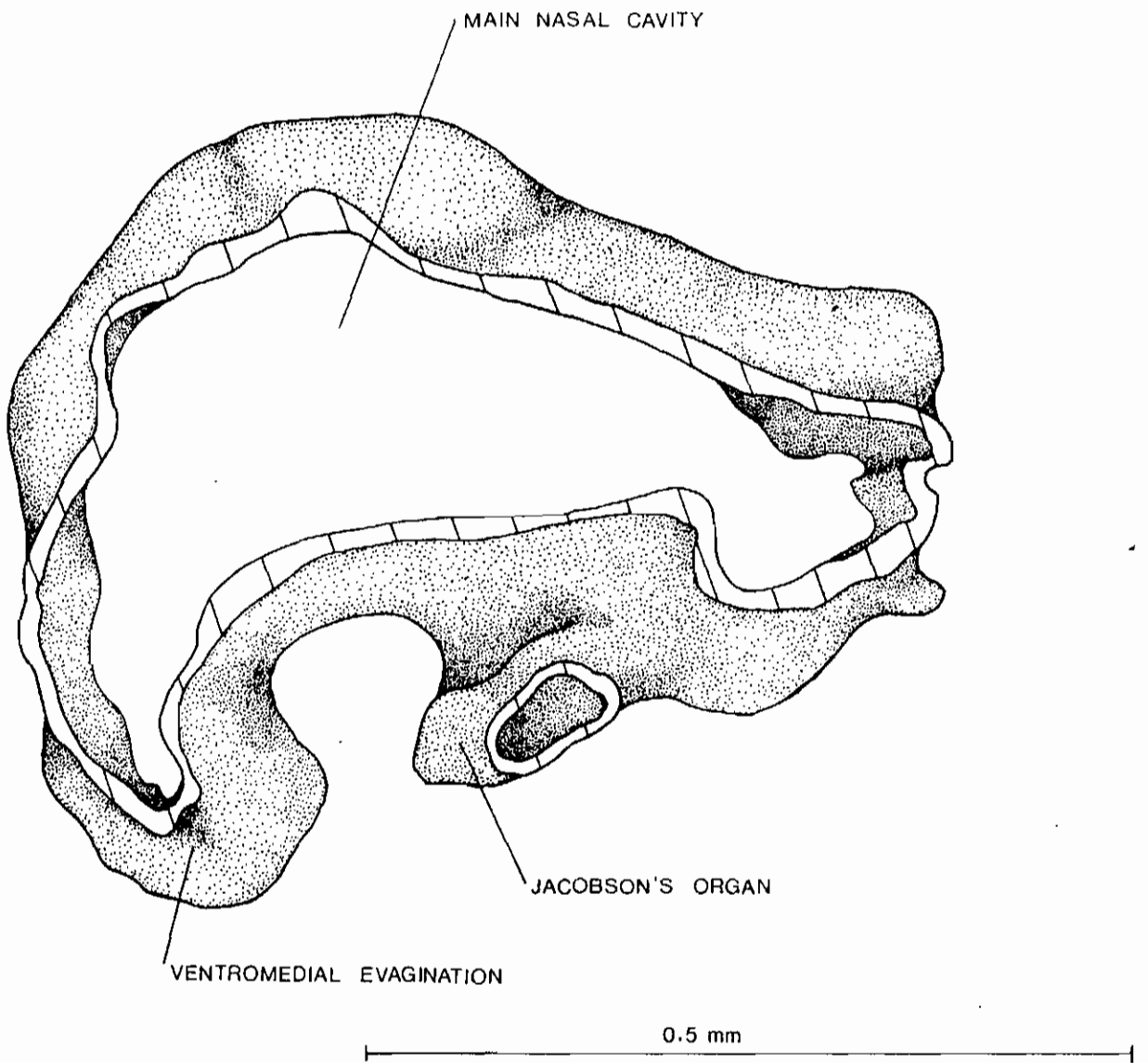


FIG.3 STAGE 1 - STEREOGRAM OF PART OF LEFT NASAL SAC , ANTERIOR VIEW .

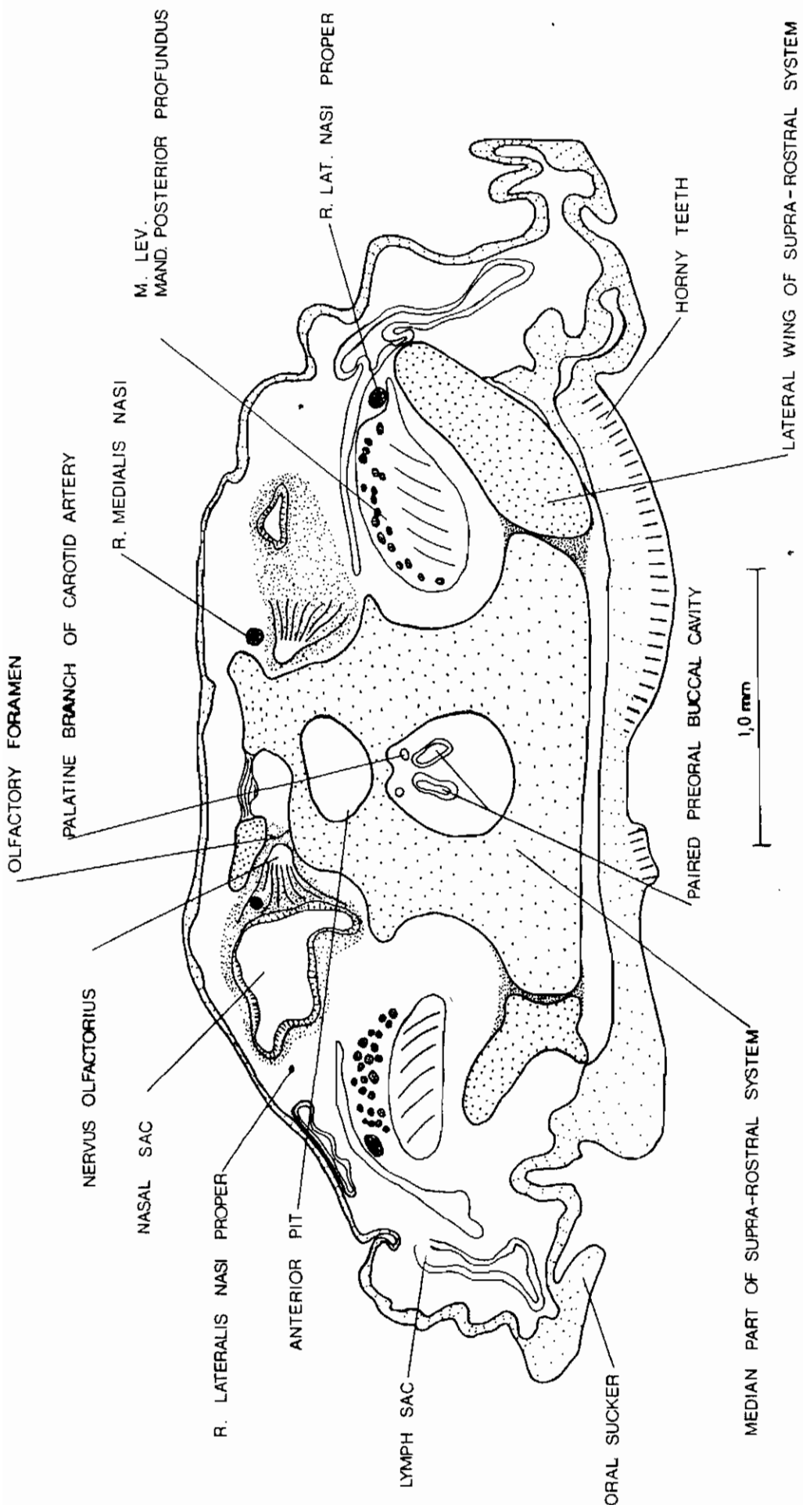


FIG. 4 STAGE 1 - CROSS-SECTION THROUGH THE REGION OF THE OLFACTORY FORAMEN.

the phylogeny as a ventral part of the main nasal sac. This part became specialized to facilitate odours from the buccal cavity.

A characteristic feature of terrestrial vertebrates which probable originated during the transition from an aquatic to a terrestrial life, the nasolacrimal duct, is still absent at this early stage of development.

Stage 2.

In this stage, as in stage 1, there is no pronounced differentiation of the nasal sacs. They have an elongated appearance with long anterior nasal tubes and long slit-like choanae (Van Eeden, 1951). The external naris lies on a projecting funnel, which leads to the anterior nasal tube (Pusey, 1943). This tube extends medially and opens into the main nasal sac.

In transverse section the main nasal sac has a more or less circular form with the lateral wall devoid of any olfactory epithelium. The rest of the sac is lined with olfactory epithelium, although not much differentiated. The three layers of basal, supporting and olfactory cells (Gaupp, 1896) are, however, clearly recognizable at this stage. The olfactory hair form a dense layer on the inner surface of the olfactory epithelium.

The dorsolateral wall of the main nasal sac shows a small evagination, which probably could be homologized with the lateral appendix, as described for Rana nigromaculata by Tsui (1946 b) (Fig. 8). This evagination has a lumen which at one stage of development is separated from the main nasal cavity. In Ascaphus, as in Rana nigromaculata (Tsui, 1946 b), the lumen is very small in relation to the rest of the appendix. Tsui states in his work that this appendix could be a forerunner to the Jacobson's organ, a view which will be dealt with in the Discussion.

The vomeronasal organ or Jacobson's organ is situated ventrally to the main nasal sac and is present as a small pouch (Fig. 6, 7). Although its epithelium has a granular appearance, it is definitely part of the olfactory epithelium lining the rest of the dorsal, medial and ventral walls of the nasal sac. Like the olfactory epithelium, the epithelium lining the Jacobson's organ also possesses cilia projecting into the lumen of the organ.

It is evident that the Jacobson's organ is an evagination of the ventral wall of the nasal sac. Its anterior part has a sac-like appearance, but posteriorly it flattens out into a ventrolateral orientated groove. The ventromedial evagination is still present, although relatively smaller than in the preceding stage (Fig. 6).

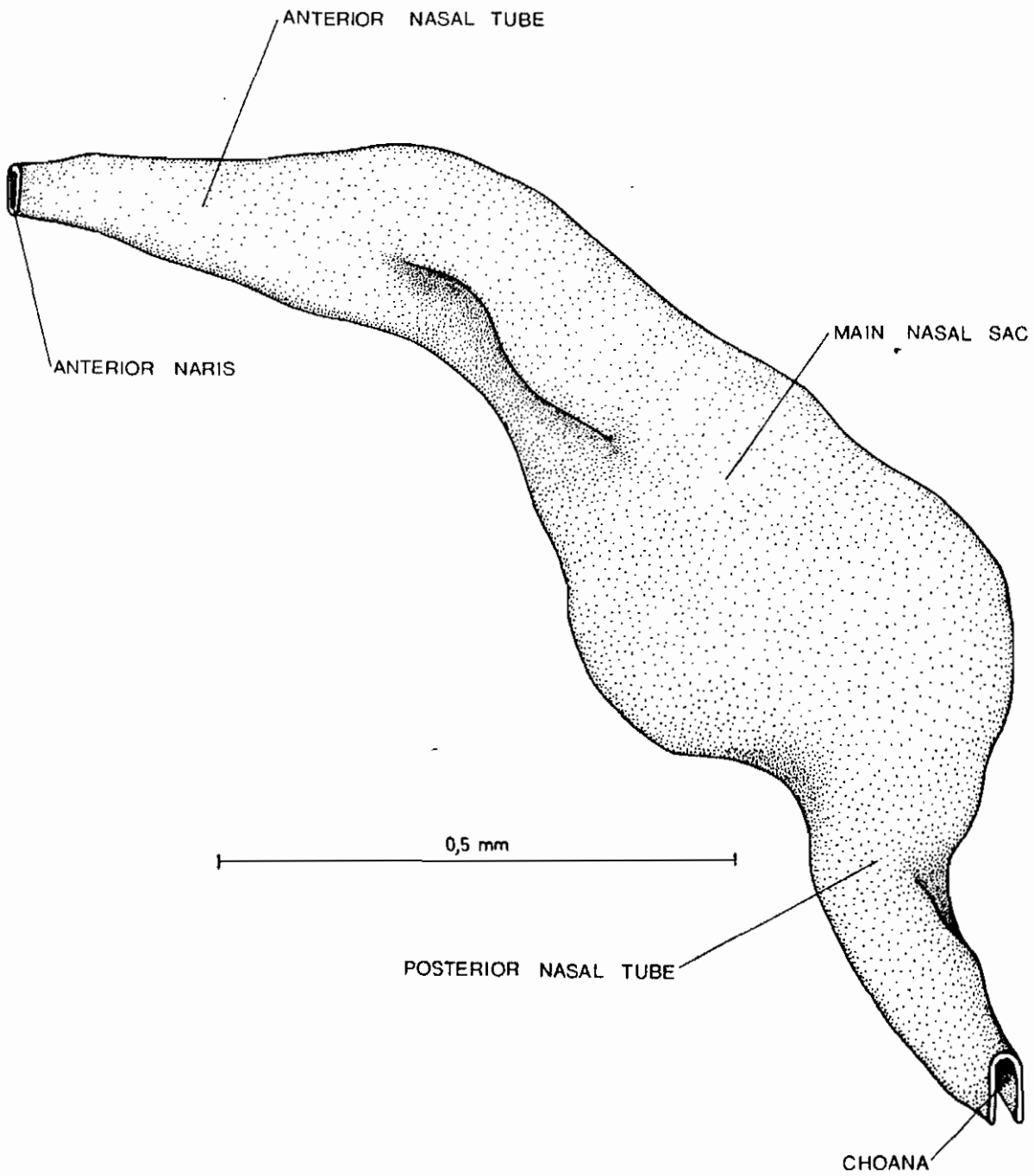


FIG. 5 STAGE 2 - DORSAL ASPECT OF THE LEFT NASAL SAC.

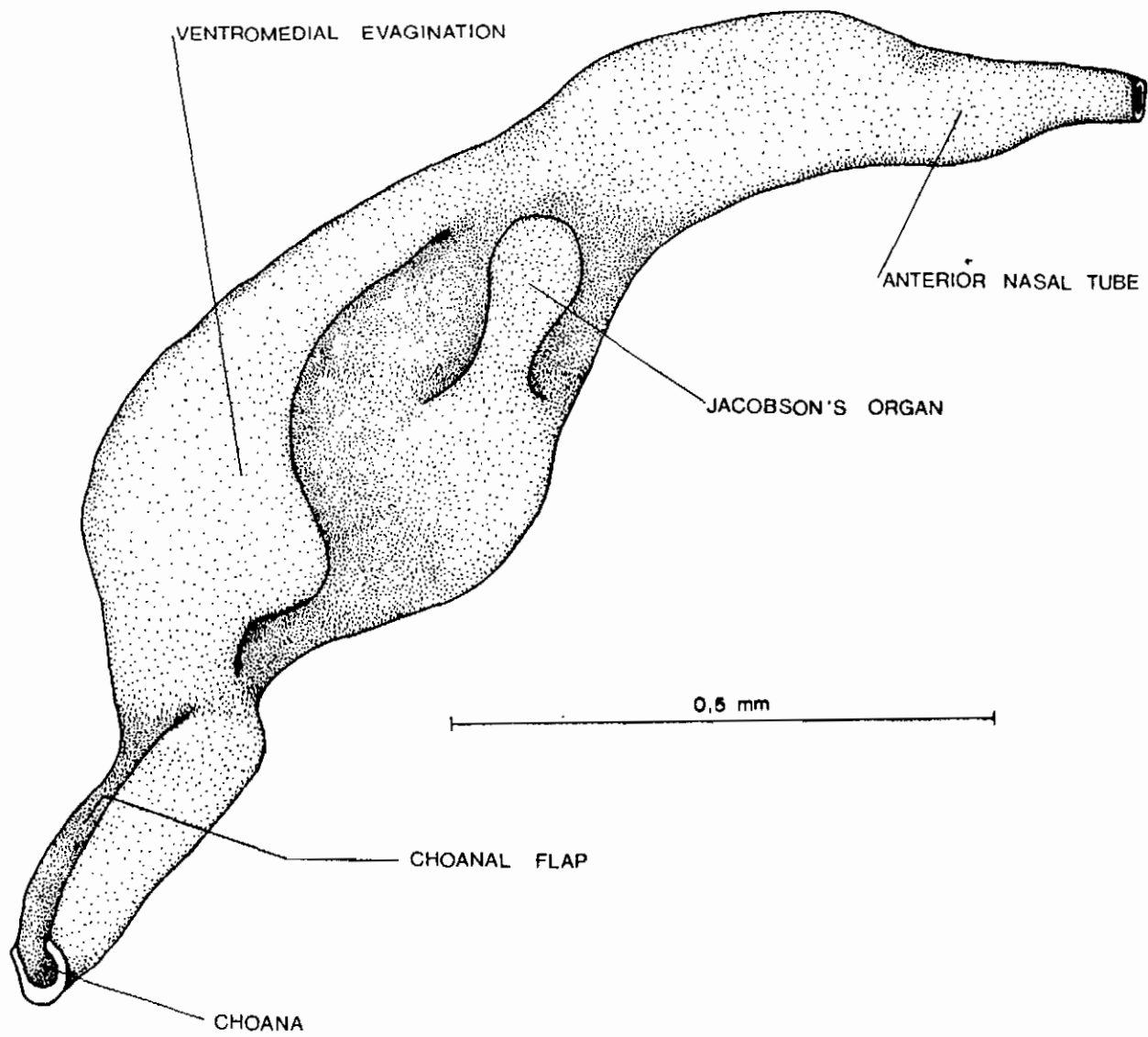


FIG. 6 STAGE 2 - VENTRAL ASPECT OF THE LEFT NASAL SAC.

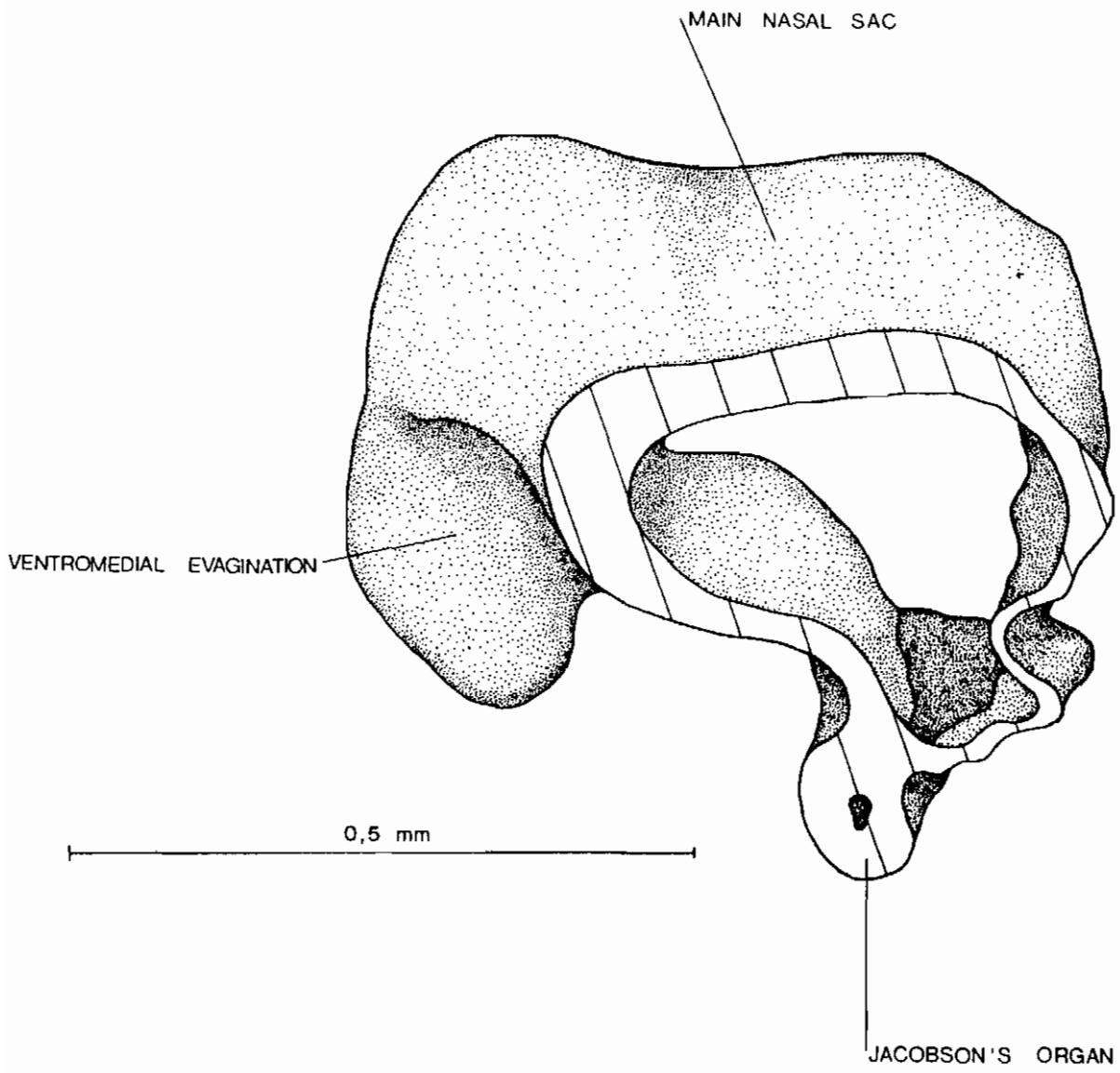


FIG. 7 STAGE 2 - STEREOGRAM OF PART OF LEFT NASAL SAC . ANTERIOR VIEW .

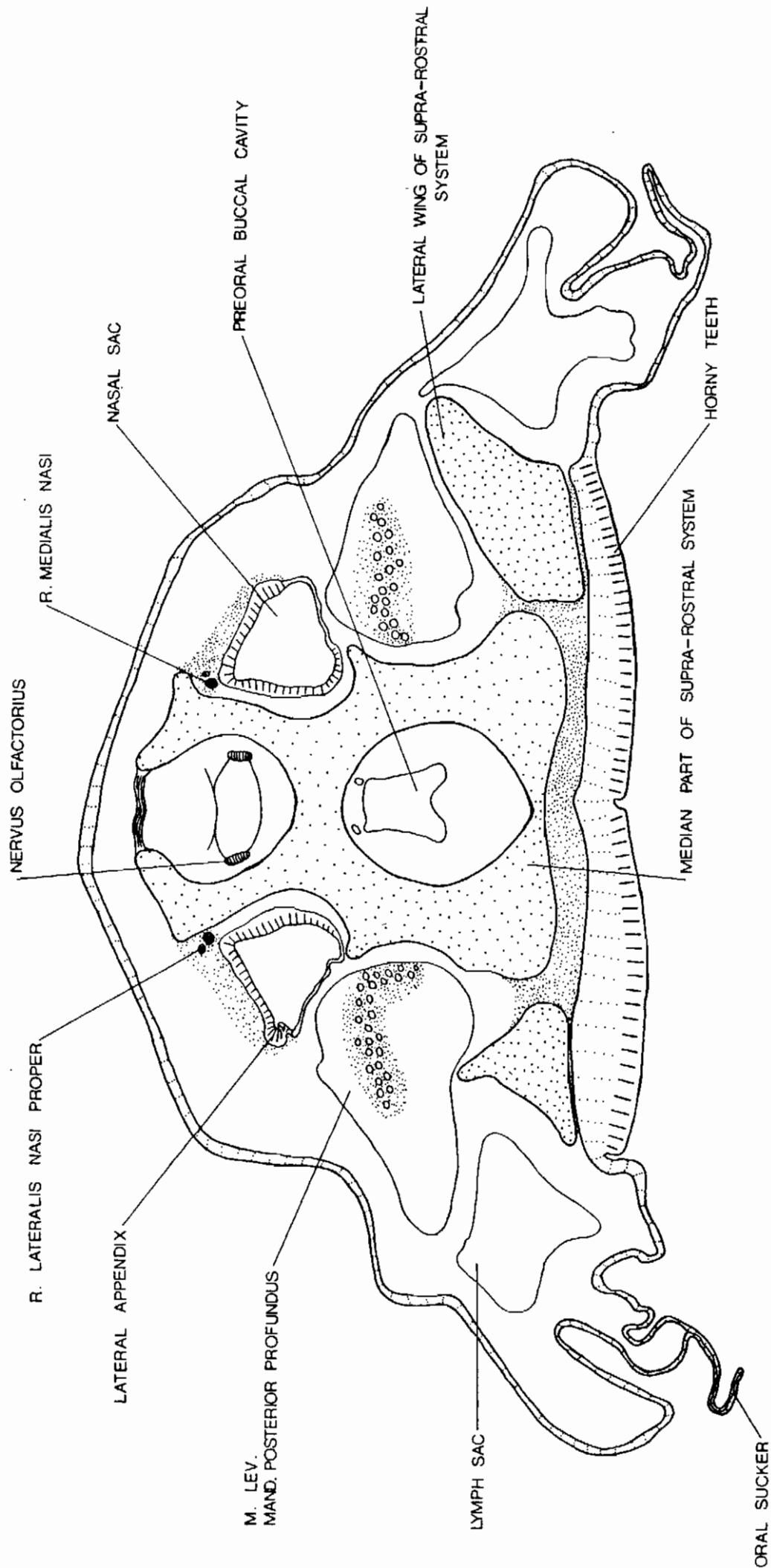


FIG. 8 STAGE 2 - CROSS-SECTION THROUGH THE REGION OF THE LATERAL APPENDIX

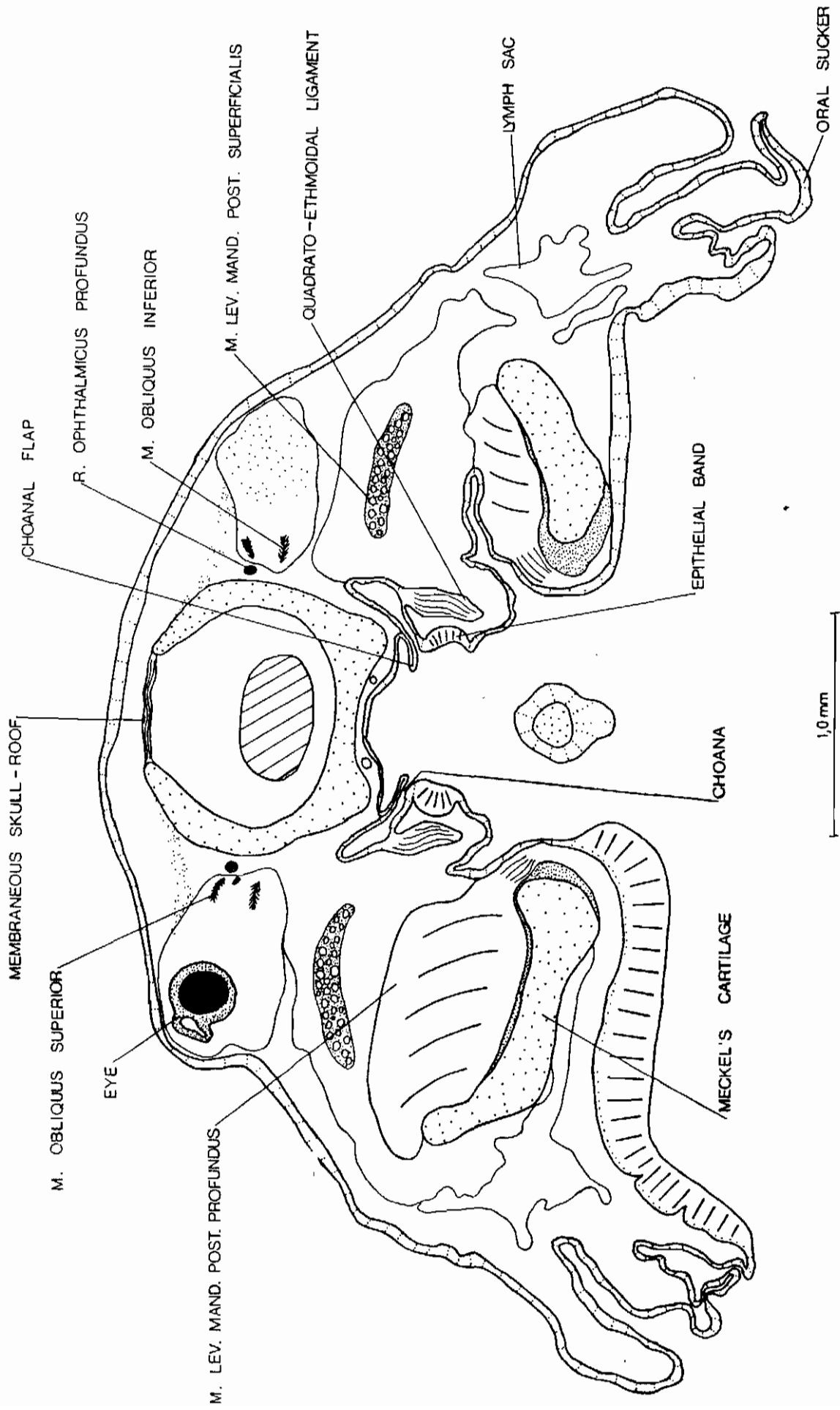


FIG. 9 STAGE 2 - CROSS-SECTION THROUGH THE CHOANAL REGION.

The intermaxillary and medial nasal glands are absent and no glandular epithelium can be seen anywhere in the nasal region. There still is no sign of the nasolacrimal duct. Each choana has a valvular flap with which it can be closed from medially (Fig. 9). Bruner (1913) describes choanal valves without a muscular mechanism for all larvae of lower and higher lungbearing urodelans. He also remarks that the choanal valves are temporary structures disappearing during metamorphosis. These observations made by Bruner (1913), are also applicable to Ascaphus. Own investigations show that, from the earliest stage, choanal valves are present and that they disappear during metamorphosis. The valves of Ascaphus also have no muscular mechanism and the closure of the valves would be merely mechanical, preventing a flow from the oral cavity to the cavity of the nasal sac.

Stage 3.

In this stage the external naris still opens on a projecting funnel. The latter leads to the undifferentiated nasal sac, which lies very far anteriorly. It is partly roofed over by a laterally projecting ledge. Support from ventrally is by another similar ledge projecting from the region where the suprarrostral flanges become confluent with the trabecular horns (Fig. 13)

(Van Eeden, 1951). Although no separate divisions are present, it is evident that differentiation of the nasal sac has started.

Anteriorly the brain capsule has no wall and therefore the cavity of the brain capsule opens freely into a wide internasal space. The olfactory foramen pierces the thick side-wall of the brain capsule far anteriorly (Fig. 13). The median part of the supra-rostral system, together with the trabecular horns and the cranial floor, enclose a cartilaginous tunnel in which the preoral buccal cavity lies (Fig. 8). It is into this cavity that the nasal sac, after tapering posteriorly to form a posterior nasal tube, opens by means of a slit-like choana (Fig. 12). This opening is valvular and can be closed (Bruner, 1914).

No intermaxillary gland is present and the space anterior to the neurocranium is filled up with connective tissue. The glandula nasalis medialis is absent. Just posterior to the supra-rostral system, there is a ventral notch (Pusey, 1943) through which the posterior nasal tube passes.

In the lateral walls of the preoral buccal cavity, an epithelial band is present (Fig. 9). It reaches from the choana up to the mouth opening and in function could probably be correlated with the ciliated epithelium in the

nasal sacs and posterior nasal tubes. Pusey (1943) refers to Noble (1927), who correlates the ciliated epithelium in the nasal sacs and the posterior nasal tubes, with the presence of food in these cavities. They are of the opinion that the cilia set up a feeding current through the nasal sac and nostrils.

Following it backwards, the lumen of the diverticulum principale enlarges and the wall is lined with a thick, very typical, olfactory epithelium as described by Gaupp (1896). This is true for the medial, ventral, dorsal and dorso-lateral walls. The ventrolateral wall is lined with an indifferent epithelium (Gaupp, 1896). If this part is followed backwards, it forms a ventrolateral evagination (Fig. 13). In a stage just older than the one under discussion, the dorsolateral part of the evagination comes into contact with the nasal end of the nasolacrimal duct, the anlage of which originates in this position. As described by Born (1876) and also dealt with by Gaupp (1896), the nasolacrimal duct "der Amphibien bildet sich durch Einwachsung und Abschnüring eines Epithelstreifens von der Nase bis zum Auge hin, der dann ein Lumen bekommt und sich mit der Nasenhöhlen in Verbindung setzt." (Born, 1876; p. 643).

In this older stage the cavum medium is also seen for the first time, becoming confluent with the cavum principale.

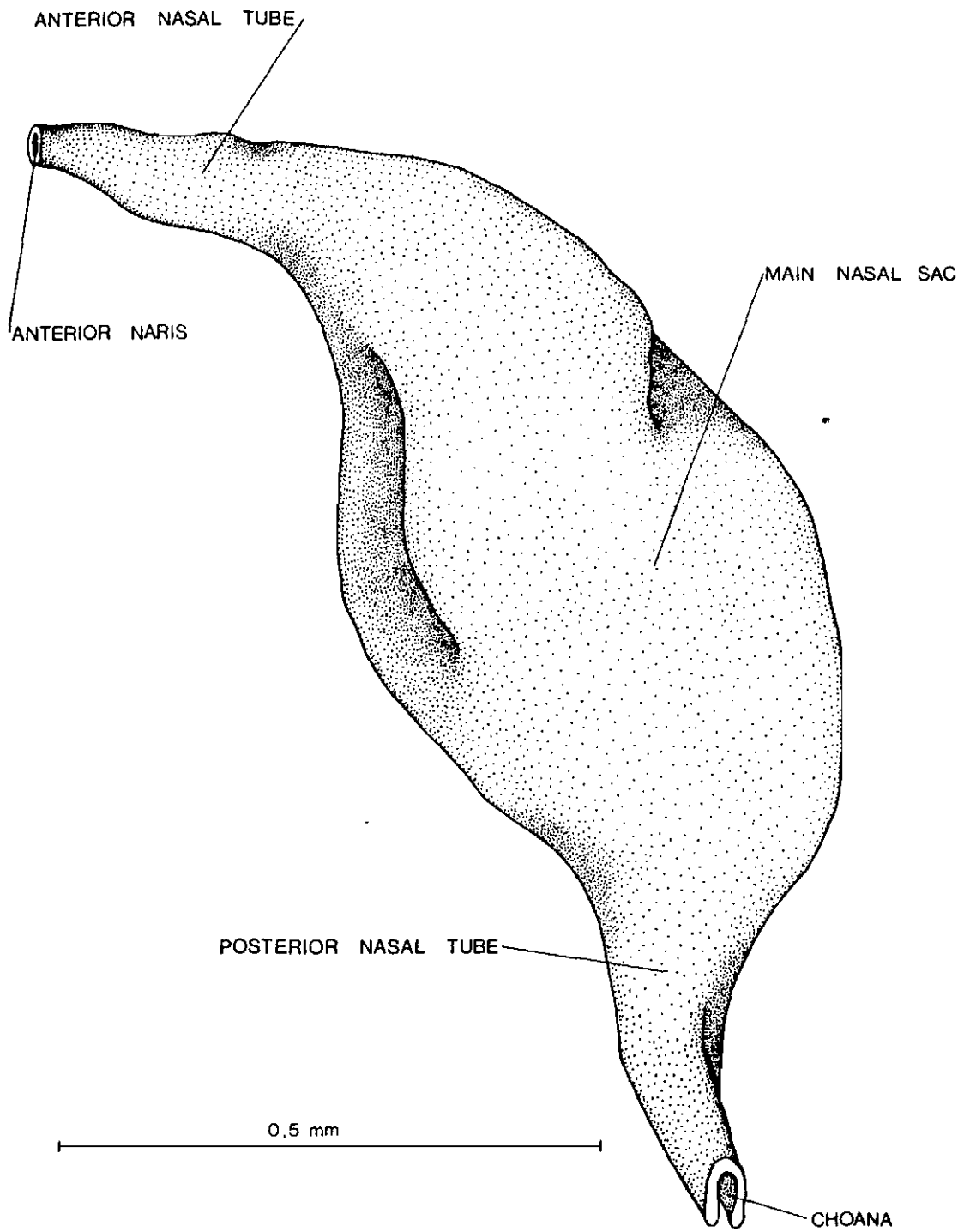


FIG. 10 STAGE 3 - DORSAL ASPECT OF THE LEFT NASAL SAC.

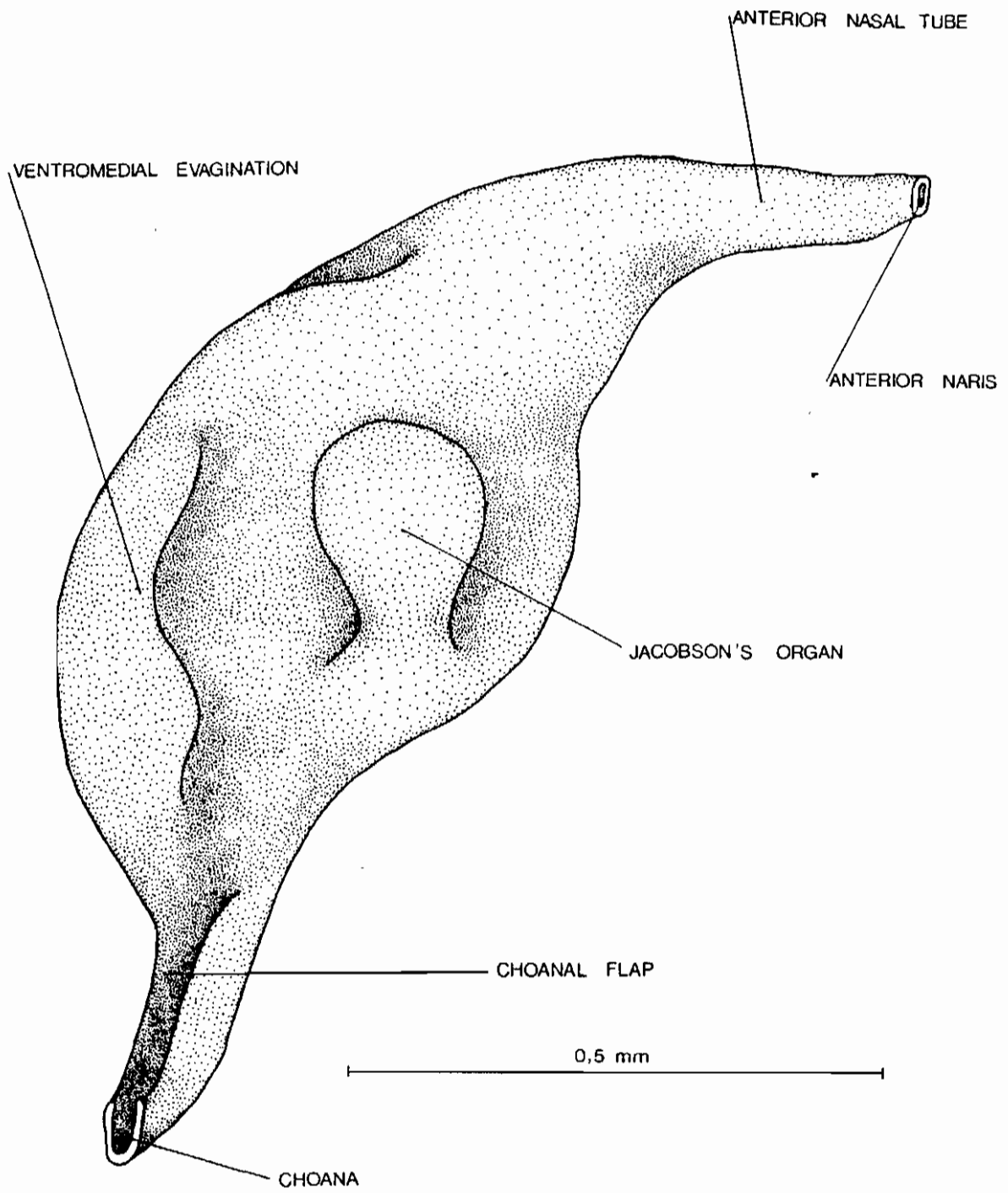


FIG. 11 STAGE 3 - VENTRAL ASPECT OF THE LEFT NASAL SAC.

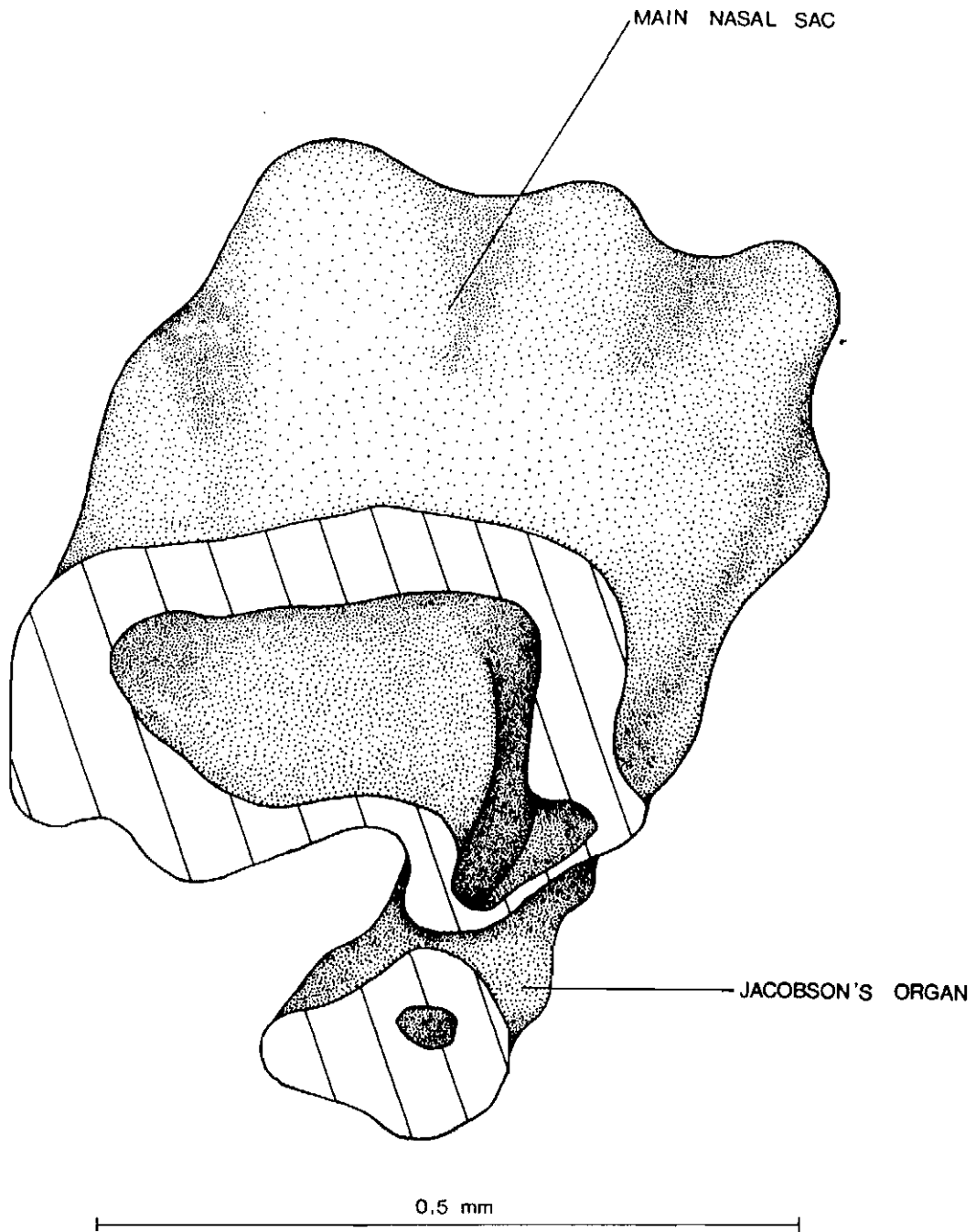


FIG. 12 STAGE 3 - STEREOGRAM OF PART OF THE LEFT NASAL SAC, ANTERIOR VIEW.

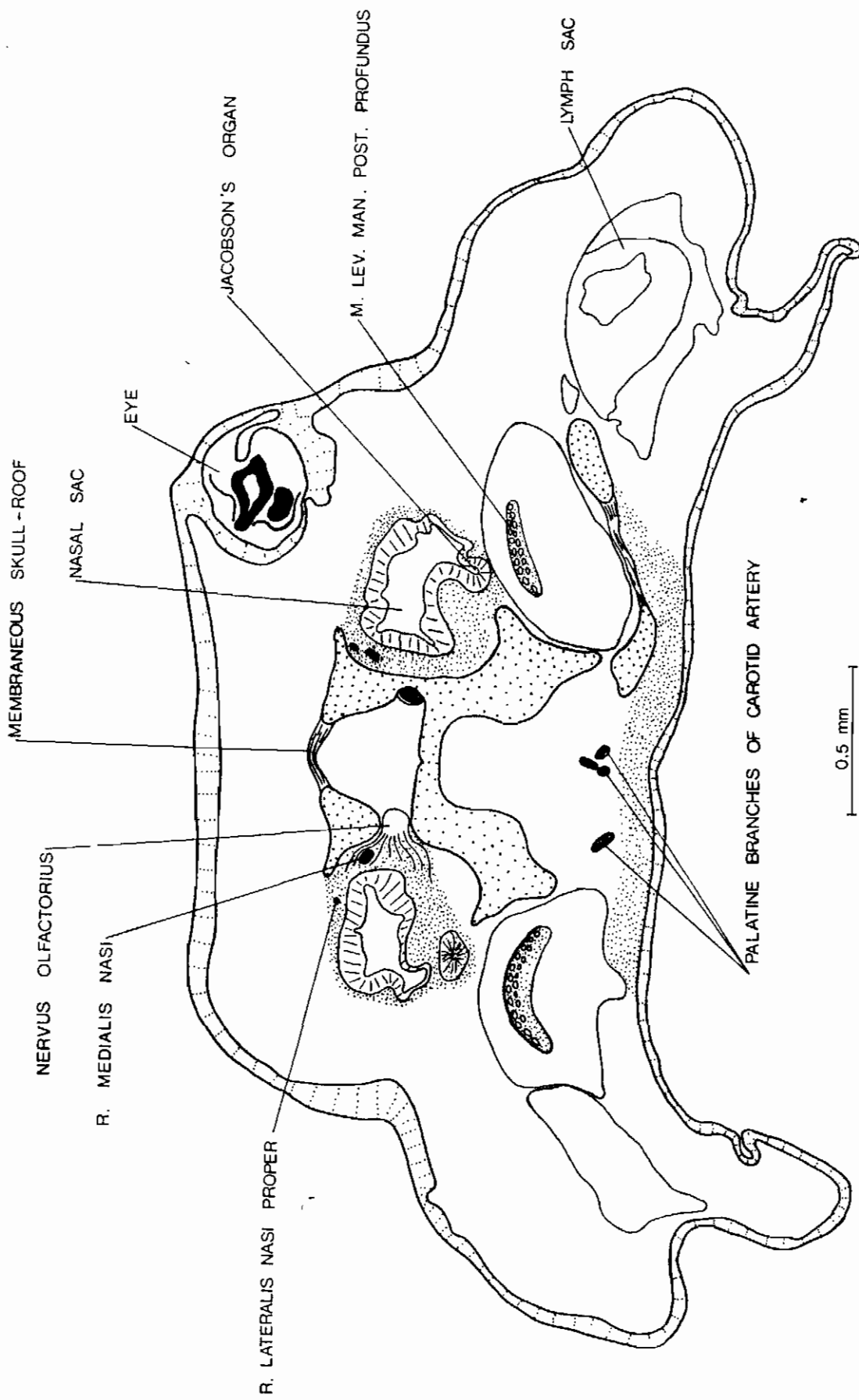


FIG. 13 STAGE 3 - CROSS-SECTION THROUGH THE REGION OF THE JACOBSON'S ORGAN .

The dorsolateral appendix (Tsui, 1946 b) of the cavum principale is smaller and less prominent than in the previous stage. Practically no lumen is present in the appendix.

As far as the diverticulum mediale or the Jacobson's organ is concerned, it is situated in a position ventrally to the diverticulum principale (Fig. 12). Its cavity is confluent with that of the diverticulum principale through an evagination which probably represents the infundibulum of later stages. The wall of the organ is lined with ciliated olfactory epithelium (Fig. 13). The presence of choanal valves leads us to consider Seydel's (1895) opinion that the Jacobson's organ only functions during adult life, although it develops during early ontogeny (Bruner, 1914). Obviously the valves prevent the olfactory medium, water, to pass from the oral cavity into the nasal sac and Jacobson's organ. It follows that in the tadpole, Jacobson's organ cannot analyse contents of the oral cavity.

Stage 4.

The external naris opens laterally. The cartilago alaris forms the skeletal support from ventrally, while the tectum nasi supports it from dorsally (Fig. 18). Both the

crista subnasalis and the processus lingularis are visible ventrally to the nasolacrimal duct and form part of the skeletal support surrounding the anterior nasal canal. The septomaxillary is also seen almost surrounding the anterior end of the nasolacrimal duct. From the external naris, the infundibulum continues as a dorsomedial evagination, which opens into the diverticulum principale (Fig. 16, 17).

In section the intermaxillary gland appears as a mass of tubules medioventrally to the nasal sacs and anteroventrally to the processus prenasalis medius. The broad septum nasi is visible posterodorsally to the intermaxillary gland. The tubules of the gland open from dorsally into the prechoanal sac. Stephenson (1951) remarks that in Leiopelma the tubule-cells forming the walls of the tubuli, are histologically characteristic in that their nuclei are situated close to the basal membrane. This also is true for Ascapthus.

Very prominent is the cartilago prenasalis inferior, anteroventrally to the nasal capsule. From the solum nasi, the crista intermedia extends laterally and posterolaterally, supporting the glandula nasalis medialis and wedging in between the diverticulum principale and Jacobson's organ. The lamina inferior is continued posteriorly as the processus lingularis.

The glandula nasalis medialis is situated just laterally to the septum nasi. Its ducts open from medially into the Jacobson's organ and not from ventrally or dorsally like in urodeles and some other anurans (Helling, 1938).

The diverticulum principale is practically surrounded by the nasal capsule. It is by far the largest of the three nasal sacs. The diverticulum medium is minute, while the Jacobson's organ is still not fully developed. The cavum principale is in connection with the cavum inferius of the diverticulum laterale and with the Jacobson's organ. No recessus sacciformes (Gaupp, 1896) as in Rana, is present. The diverticulum principale is lined with a thick olfactory epithelium. Its three layers of cells are clearly seen. The basal layer with a flat, squamous-like appearance, the supporting layer with the large, round cells and the layer of olfactory cells with their elongated bodies and the olfactory hair or "Flimmern" (Gaupp, 1896), which reach into the cavum principale. Bowman's glands seem to be present only in the dorsal wall of the diverticulum principale. The thickness of the epithelium also ranges from relatively thick in the anterodorsal part to thin in the posteroventral and posterodorsal walls. The diverticulum medium is from dorsally partly covered by the lamina superior and from ventrally by the lamina inferior.

Anteriorly the nasolacrimal duct in transverse section takes on an inverted, shallow U-shape. The anterolateral leg of the U continues to the external naris (Fig. 18), while the other leg is continued into the diverticulum medium (Fig. 20, A-D). There is a striking resemblance between the epithelia of the nasolacrimal duct and the diverticulum medium. Both have a basal layer of cells and a second layer with thick, columnar supporting cells. Posteriorly the nasolacrimal duct opens, like described by De Villiers (1934), as two ductlets on top of the lower eyelid. On its course medially to the nasal sac, the anterior part of the nasolacrimal duct is almost surrounded by the septomaxillary (Fig. 19). The nasolacrimal duct passes anterior to the cartilago obliqua into the nasal capsule. The large fenestra narina is incompletely divided into two fenestrae by the cartilago alaris, which does not join the tectum nasi (See fig. 2A, Jurgens, 1971). Through the anterodorsal one the anterior nasal canal passes, while through the lower one the nasolacrimal duct passes.

The diverticulum mediale or Jacobson's organ is situated almost ventrally to the diverticulum principale (Fig. 15). It lies, however, more medially than in the juvenile stage. The epithelium of the Jacobson's organ is a typical olfactory epithelium without Bowman's glands. Three layers of cells can be recognised, with the "Riechzellen" (Gaupp,

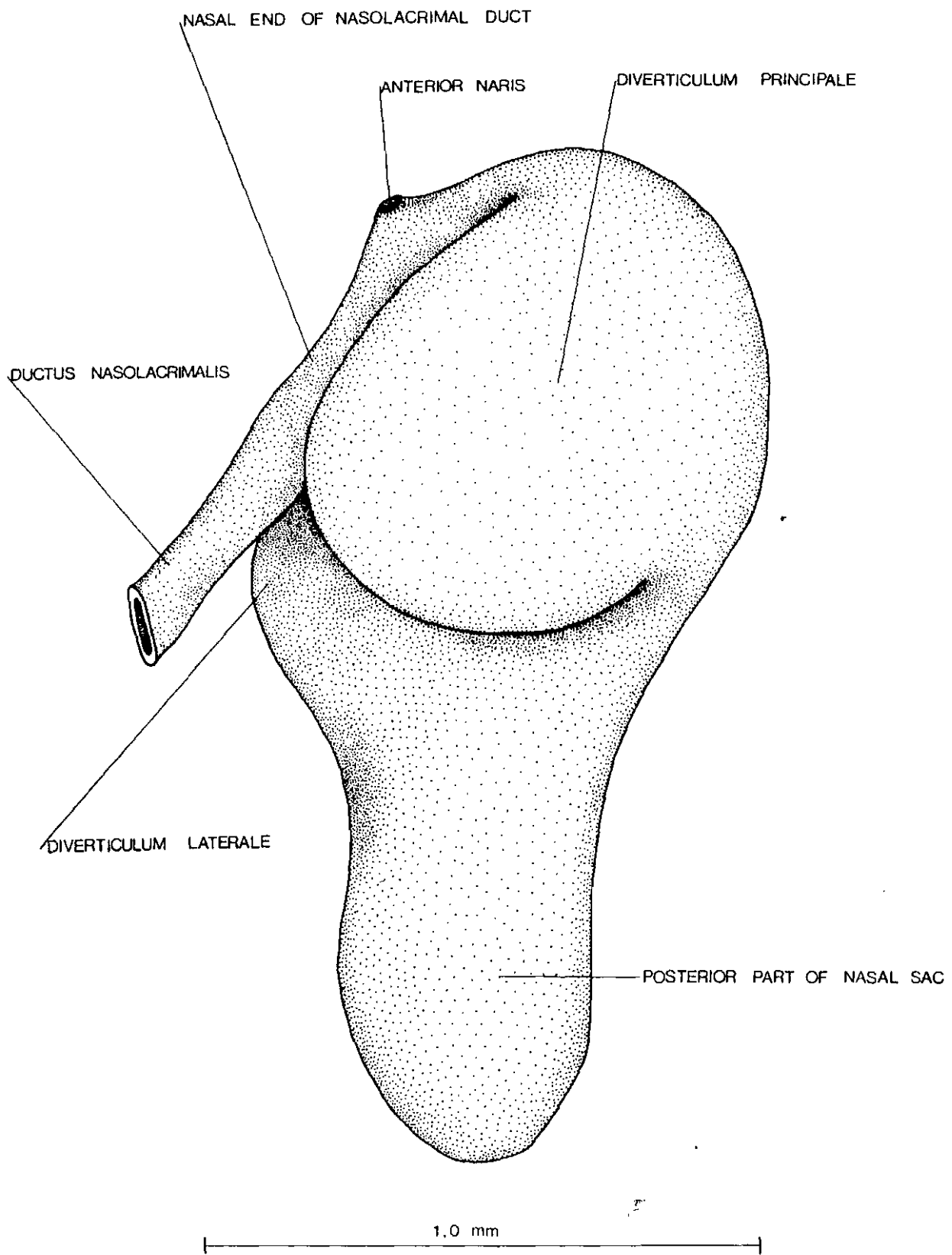


FIG. 14 STAGE 4 - DORSAL ASPECT OF LEFT NASAL SAC.

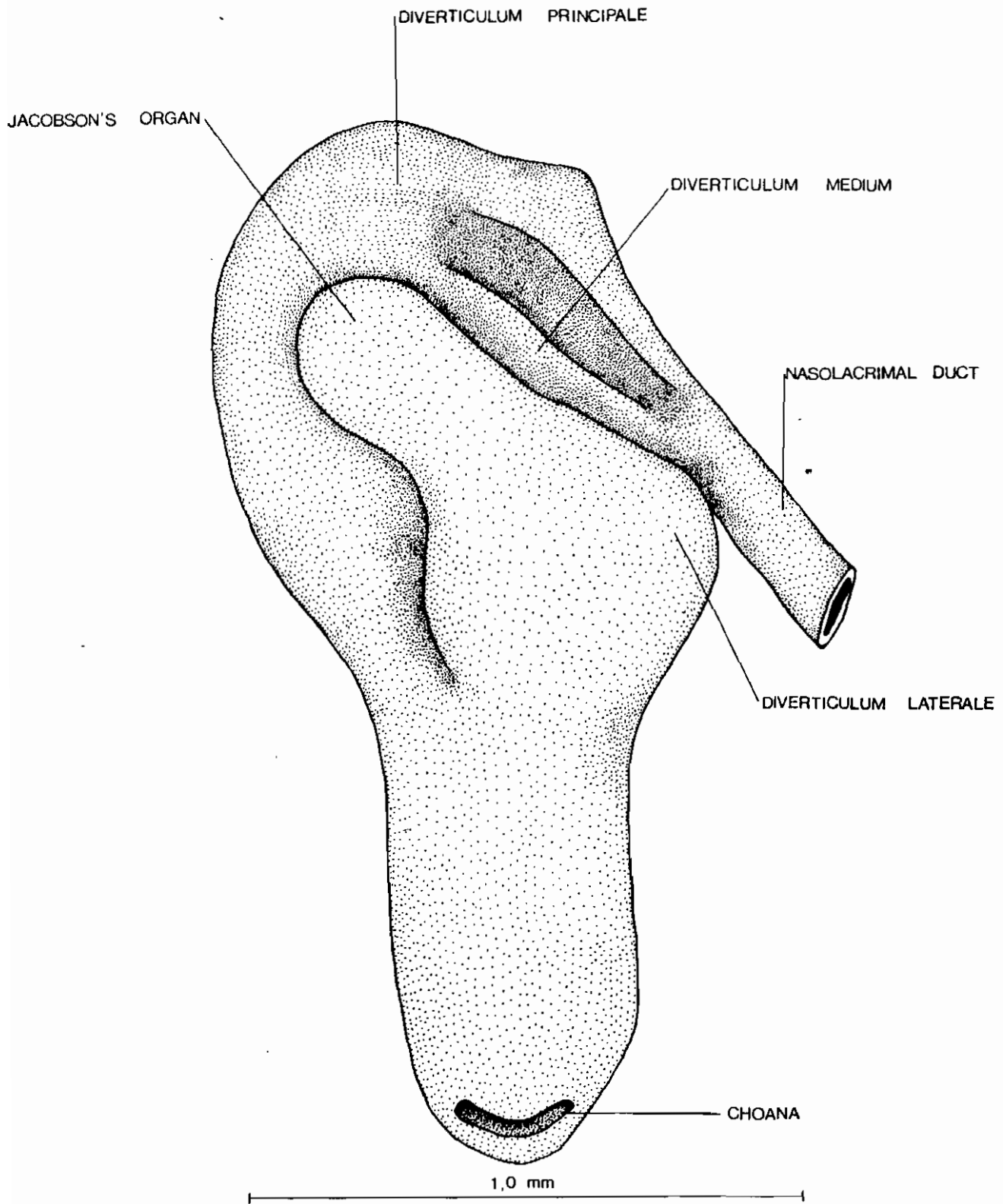


FIG. 15 STAGE 4 - VENTRAL ASPECT OF LEFT NASAL SAC .

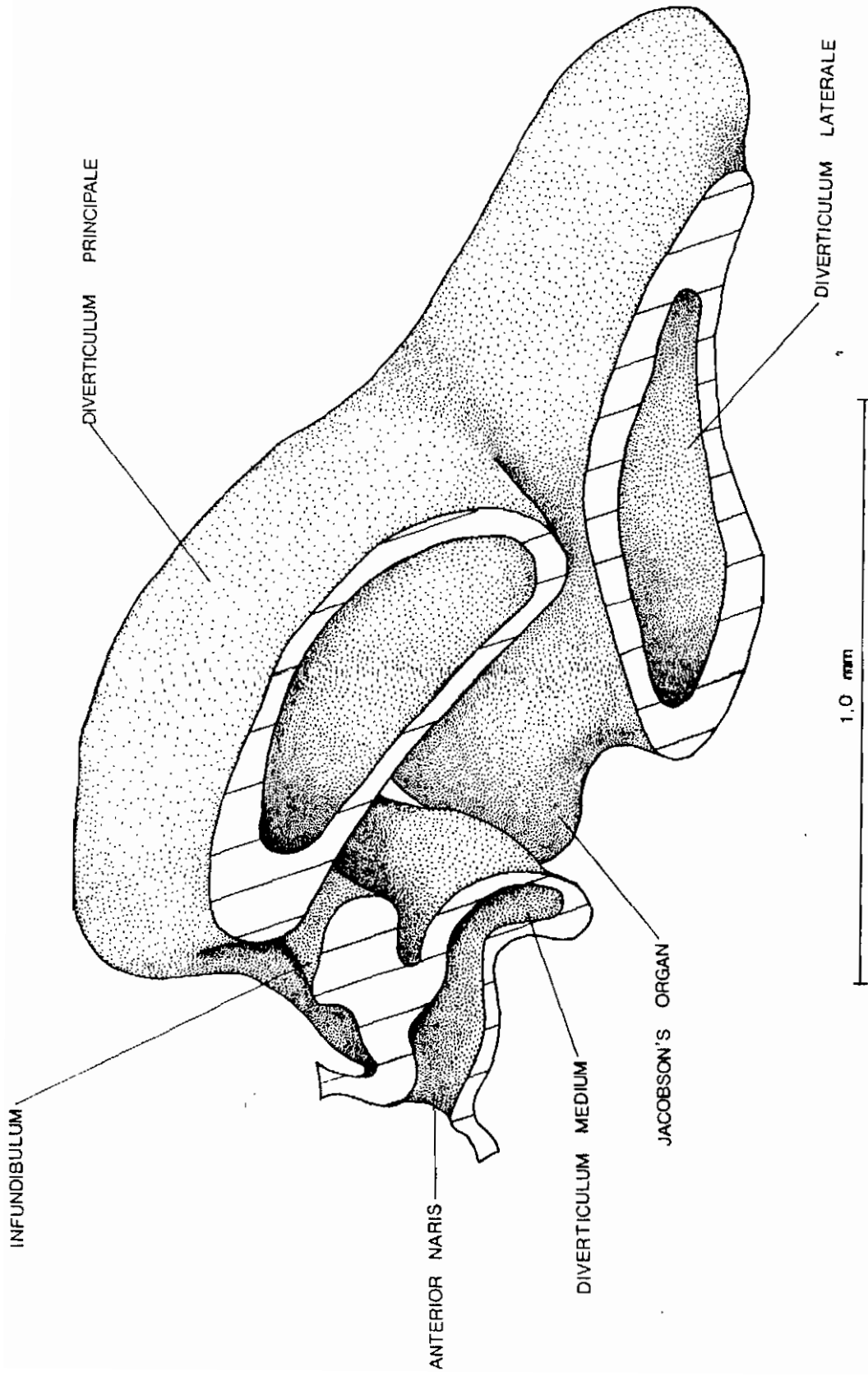


FIG. 16 STAGE 4 - STEREOGRAM OF PART OF LEFT NASAL SAC, LATERAL VIEW.

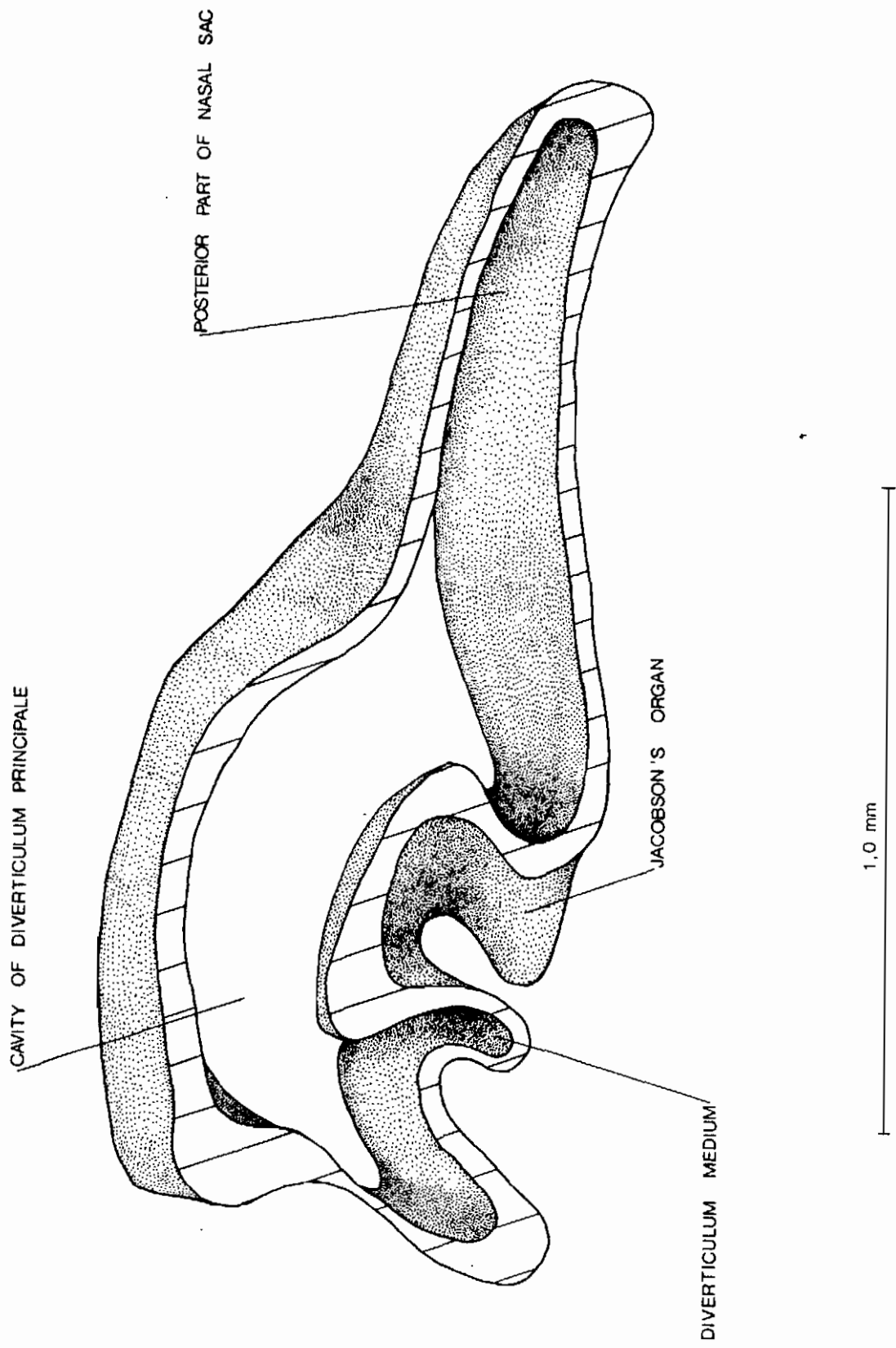


FIG. 17 STAGE 4 - STEREOGRAM OF PART OF NASAL SAC, LATERAL VIEW.

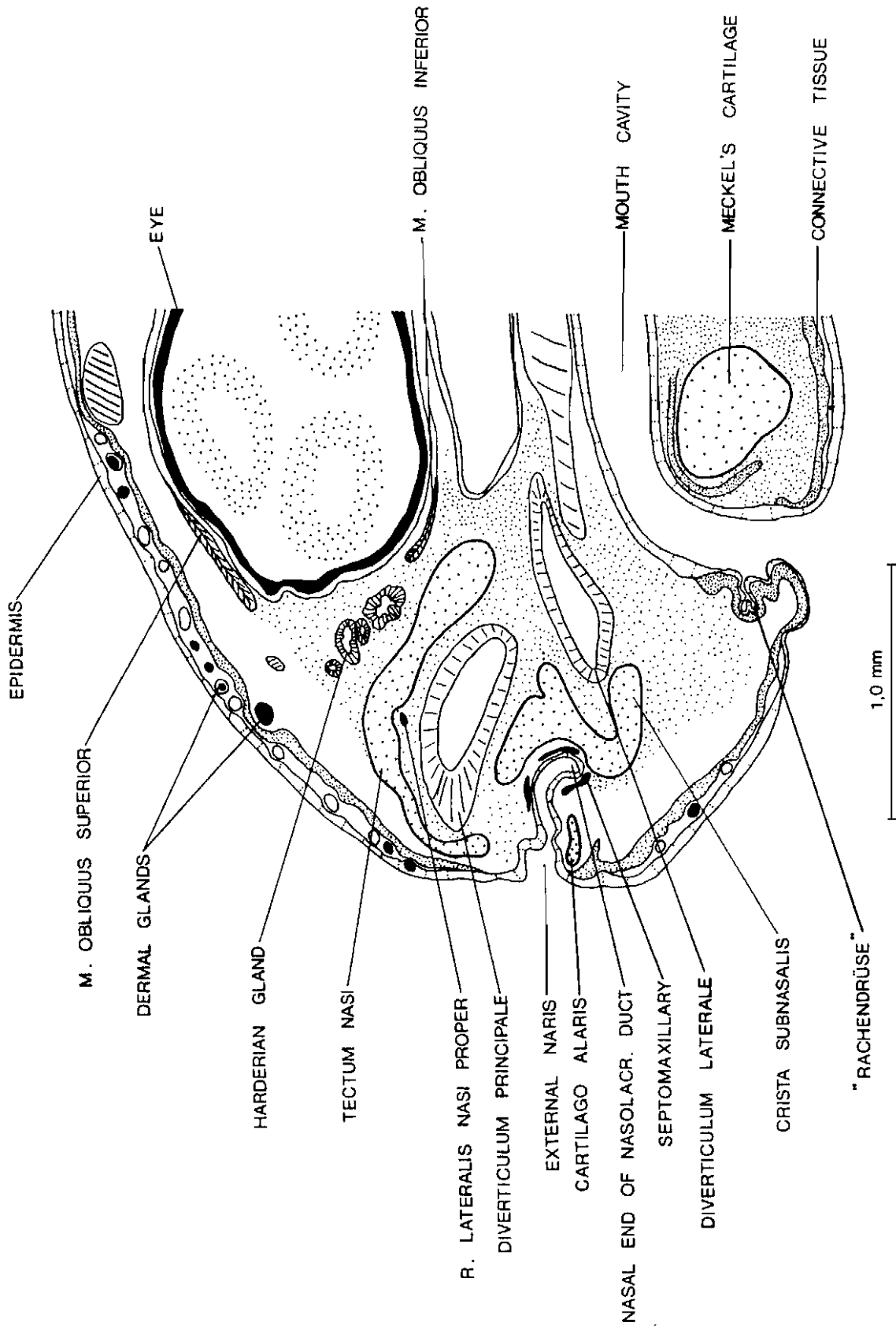


FIG. 18 STAGE 4 - SAGITTAL SECTION THROUGH THE REGION OF THE EXTERNAL NARIS .

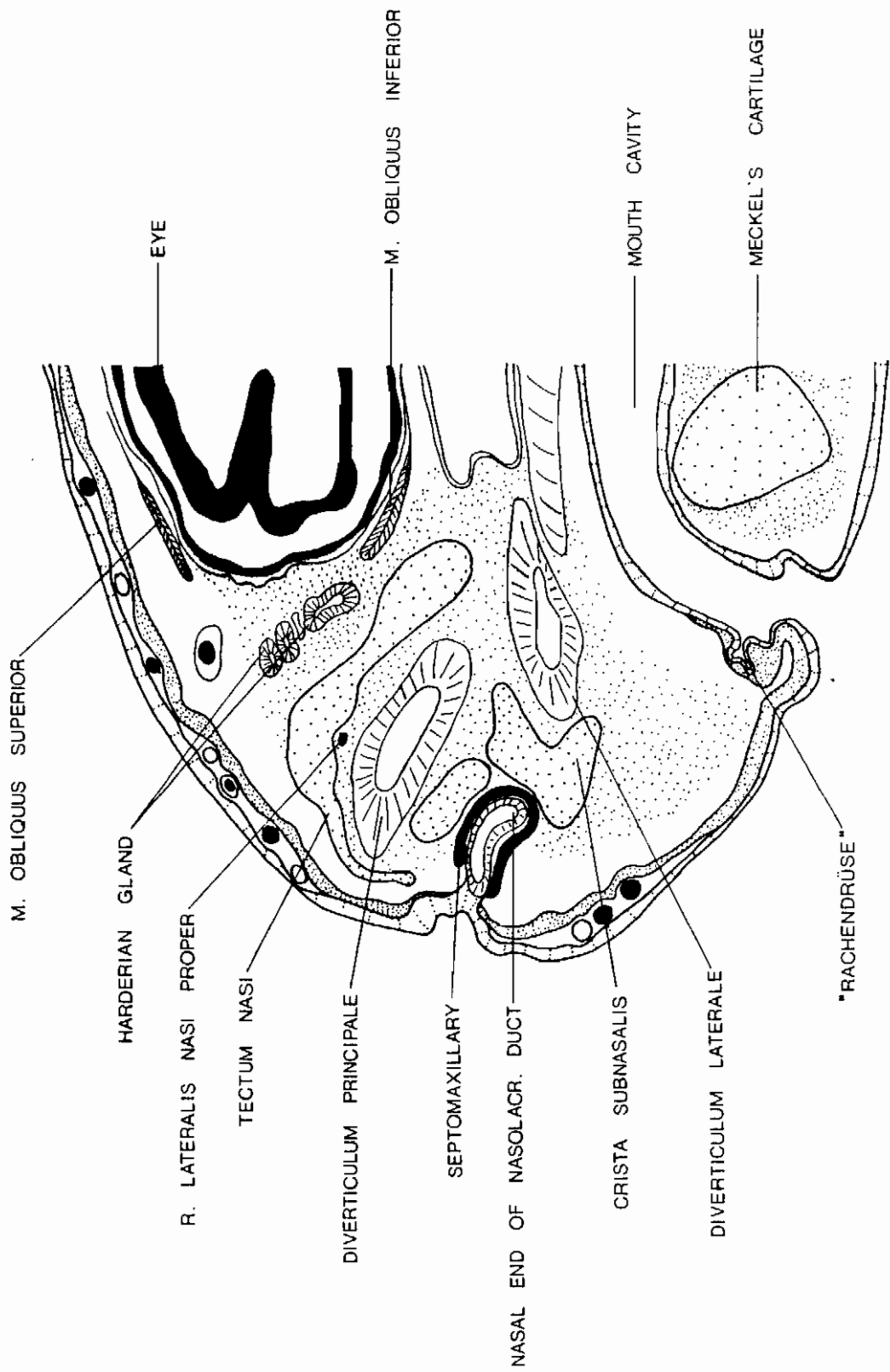


FIG. 19 STAGE 4 - SAGITTAL SECTION THROUGH THE REGION OF THE SEPTOMAXILLARY

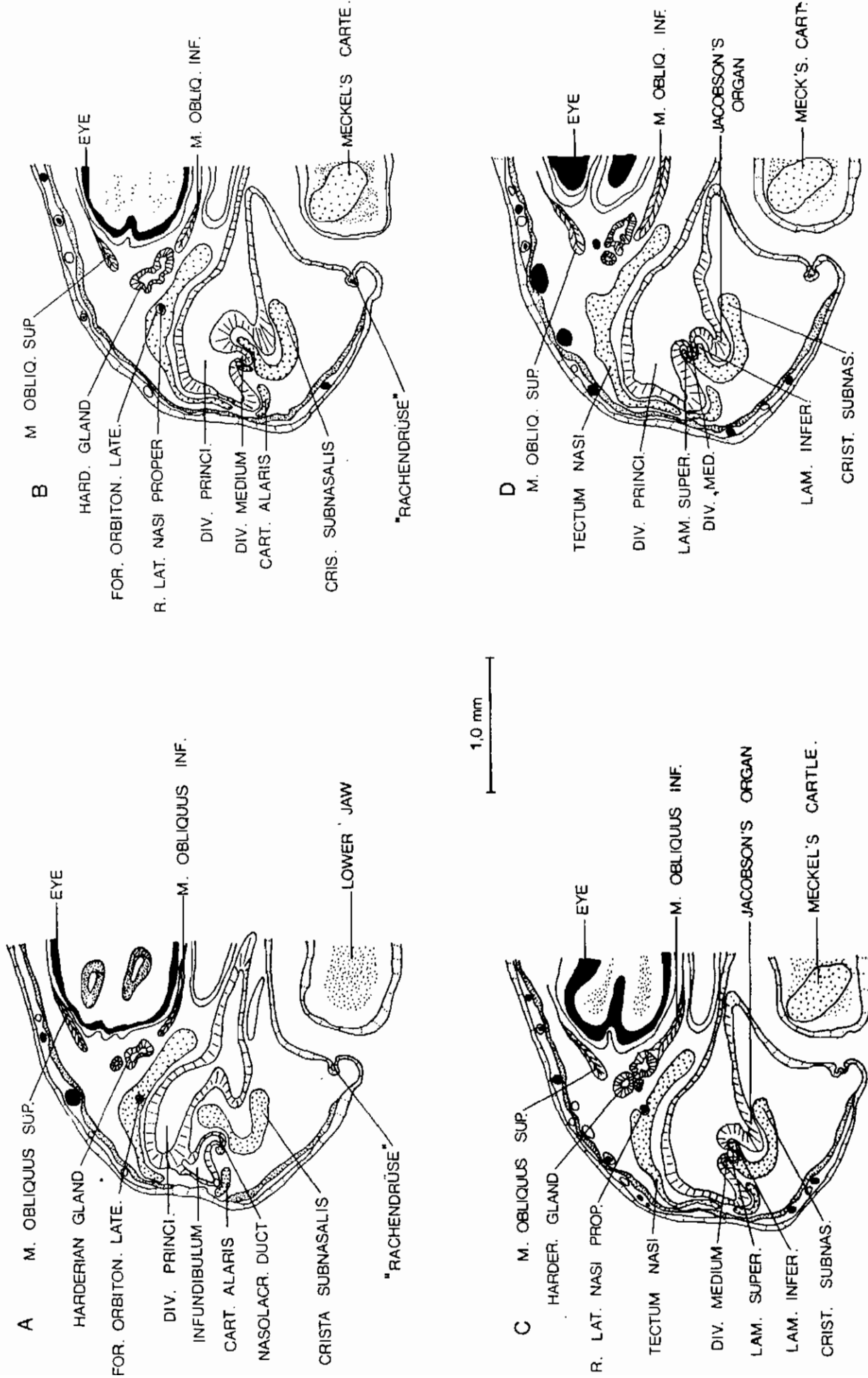


FIG. 20 A - D STAGE 4 - CONSECUTIVE SAGITTAL SECTIONS THROUGH THE REGION OF THE DIVERTICULUM MEDIUM AND JACOBSON'S ORGAN .

1896) very obvious. Olfactory hair are present also in the cavum mediale. The difference between the epithelia of the diverticulum principale, Jacobson's organ and the diverticulum medium is very obvious. Interesting to note is that the opening of the diverticulum medium into the cavum principale is slit-like, reaching posteriorly as far as the Jacobson's organ (Fig. 20, A-D).

The diverticulum laterale does not extend far laterally. Its ventral wall is lined with a respiratory epithelium. "Flimmern" (Gaupp, 1896) are very obvious in the cavum inferius. The choana is narrow and situated so far posteriorly that there practically is no sulcus maxillopalatinus (Fig. 15). In the juvenile, the choana lies further forward.

Stage 5.

The intermaxillary gland appears as a massive accumulation of tubules occupying practically the whole space in the anterior part of the snout. Very prominent also is the cartilago prenasalis superior and the cartilago prenasalis inferior, both supporting the premaxillary. Noble (1931) ascribes to the intermaxillary gland, the function of secreting a sticky substance to keep the tongue adhesive. Posteriorly the intermaxillary gland surrounds the preoral buccal cavity, being referred to by De Villiers

(1934) as the prechoanal sac. Some of the tubules of the intermaxillary gland open into this preoral cavity.

The external naris opens to the outside by piercing the cartilaginous capsule dorsolaterally. According to Gaupp (1896), the medial nasal gland opens through the ventral wall of the diverticulum mediale in Rana esculenta. In Ascaphus truei, however, the glandula nasalis medialis opens through the medial wall of the diverticulum mediale (Jacobson's organ), thus becoming closely associated with it (Fig. 26). Gaupp (1896, p. 665) divides this gland into three parts. He describes a part which reaches caudally "bis in das Gebiet des hinteren Blindsackes". In Ascaphus this part reaches so far posteriorly that it ends as a single tubule behind the choanal region in the same transverse plane as the foramen orbitonasale. Interesting to note is that Helling (1938) states that the glandula nasalis medialis in some urodeles and anurans opens from ventrally suggesting a primitive character, while in more advanced forms it opens from dorsally.

Posterior to the external naris, the crista intermedia forms the roof of the recessus inferior in which the Jacobson's organ lies. The recessus inferior is situated far laterally, lying ventrally to the diverticulum medium.

In the same transverse plane the septomaxillary, through which the nasolacrimal duct runs, is situated. The lamina superior extends laterally from the crista intermedia to form the roof of the recessus medius. The floor of this recessus is formed by the lamina inferior, which at the same time forms the roof of the underlying recessus inferior (Fig. 24). The lamina inferior is continued caudally as the processus lingularis (Jurgens, 1971). The cartilago prenasalis inferior unifies with the solum nasi just laterally to the fenestra nasobasalis through which the ramus medialis nasi leaves the nasal capsule.

The diverticulum principale or the main nasal sac, reaches caudally up to where the choana breaks through into the buccal cavity. From here on posteriorly it flattens out into a shallow groove confluent with the sulcus maxillopalatinus. Due to ineffective fixation, no true reflection of the extend of the olfactory epithelium in the main nasal sac could be ascertained.

Jacobson's organ lies far anteriorly. Following it posteriorly, it widens considerably. At the point where it joins the cavum medium, the Jacobson's organ no longer lies anteroventrally to the diverticulum principale, but in a more ventral position to it. The fact that the Jacobson's organ lies far anterolaterally, corresponds

with the observations of Helling (1938) and Jurgens (1971), who both describe this condition as a primitive anuran state. The wall of the Jacobson's organ consists of typical olfactory epithelium. It consists of three layers of cells. A basal layer, a supporting layer and a layer of olfactory cells or "Riechzellen" (Gaupp, 1896). In some regions ciliated cells can be seen. The olfactory cells are longer than the other and they form a layer of tightly-packed cells lining the cavum inferius. The supporting cells are also numeral, but not so tightly packed together. One can see the nerve fibers of the olfactory cells running through this layer. The basal cells form a typical squamous-like epithelium (Leeson & Leeson, 1970). Bowman's glands, which are typical of olfactory epithelium, are absent in the epithelium of the Jacobson's organ. This corresponds to what Helling says is typical of the Jacobson's organ, namely that "..... durch sein hohes Riechepithel und das ausnahmlose Fehlen von Bowmanschen Drüsen ausgezeichnet." (Helling, 1938 p. 623). Following the Jacobson's organ caudally, its lateral part is not lined with olfactory epithelium, but with true respiratory epithelium or typical "Flimmerepithel" (Gaupp, 1896). This consists of a layer of basal cells with elongated nuclei and a second layer of ciliated cells with a more cuboid appearance. The cilia of the cells reach into the cavum and are of reasonable length. Medially, the olfactory epithelium

with which the Jacobson's organ is lined, continues caudally almost as far as the choana.

The diverticulum medium is accommodated in the recessus formed between the laminae inferior and superior. Laterally to this the septomaxillary extends partly around the diverticulum medium (Fig. 24). The septomaxillary is large and the ventral part is continued posteriorly as a process lying between the infundibulum and lamina superior. In Leiopelma this bone is smaller (Jurgens, 1971). The cavum medium joins the cavum principale by means of the infundibulum. A lateral evagination or diverticulum, when followed posteriorly, narrows and becomes confluent with the nasolacrimal duct. This diverticulum is in fact nothing but the "nasal end of the ductus nasolacrimalis" (De Villiers, 1934).

The nasal end of the nasolacrimal duct is triangular in cross-section (Fig. 25), but as it continues caudally, it becomes rounded, terminating as two ductlets, a dorsal and a ventral one. While the ventral one opens in a groove in the lower eyelid, the dorsal ductlet opens on the eyelid, and not in the deepest part of the groove as described by Gaupp (1896) for Rana. It is obvious that the epithelium of the nasal end of the nasolacrimal duct corresponds significantly with that of the diverticulum medium. The epithelium, like that of the diverti-

culum medium, consists of two layers of cells. A basal layer with a squamous-like appearance and a layer of "Flimmerepithel" (Gaupp, 1896). The "Flimmerepithel" is found only in the nasal end of the duct.

The diverticulum laterale continues laterally so far, that it becomes wedged in between the pars facialis and pars palatinus of the maxillary bone (Fig. 27).

The posterior process of the crista subnasalis, for quite a distance, lies between the lateral part of the diverticulum medium and the maxillary (Fig. 27). The extensive expansion laterally of the nasal sac can be correlated with the flattening of the head and the skull during metamorphosis.

Behind the choanal region, the diverticulum laterale is continued posteriorly as the sulcus maxillopalatinus (Fig. 28). The sulcus maxillopalatinus and the plica palatina both are lined with respiratory epithelium, while the palatal fold and the rest of the buccal wall are covered with a more columnar epithelium.

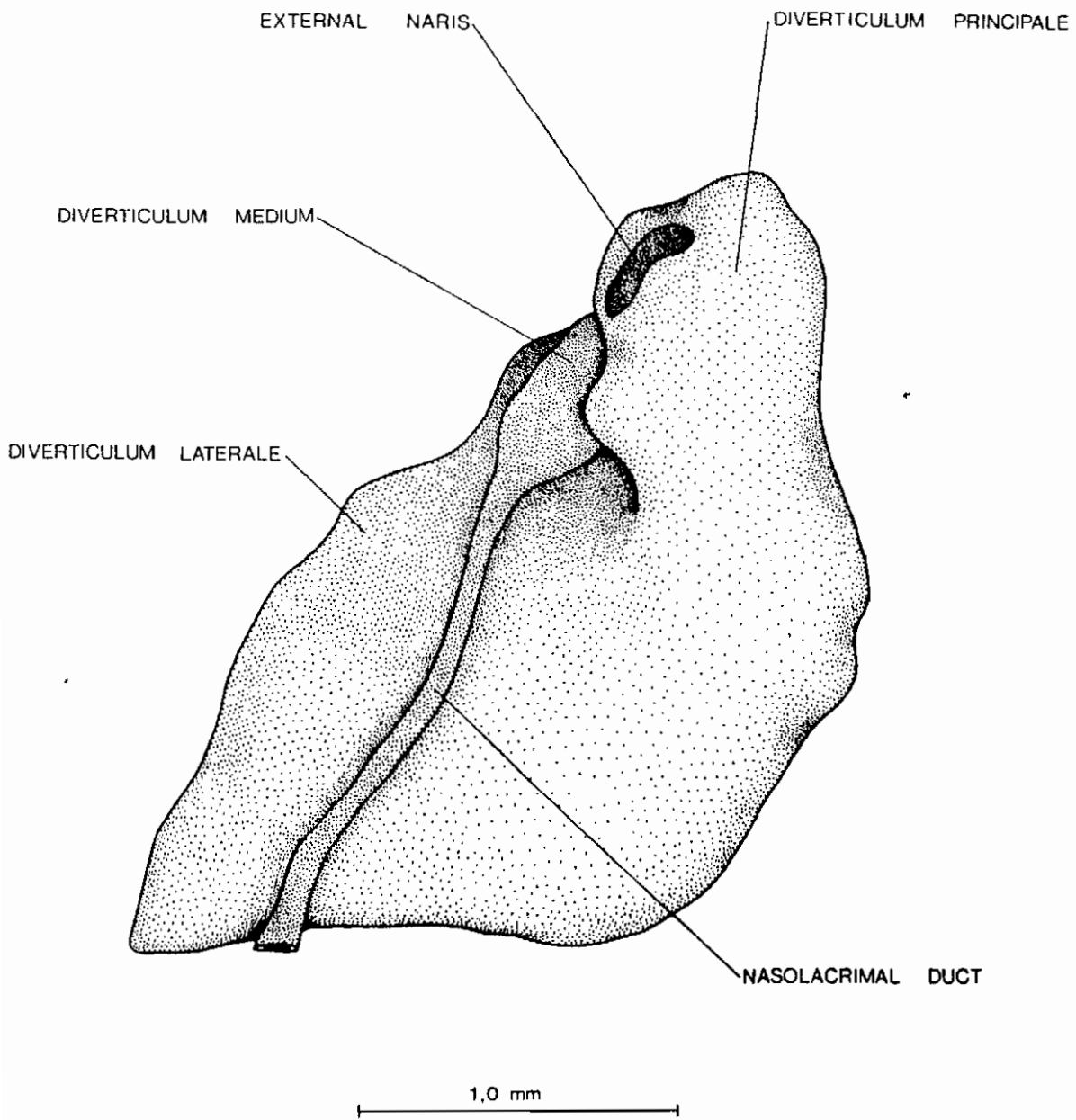


FIG. 21 STAGE 5 - DORSAL ASPECT OF THE LEFT NASAL SAC .

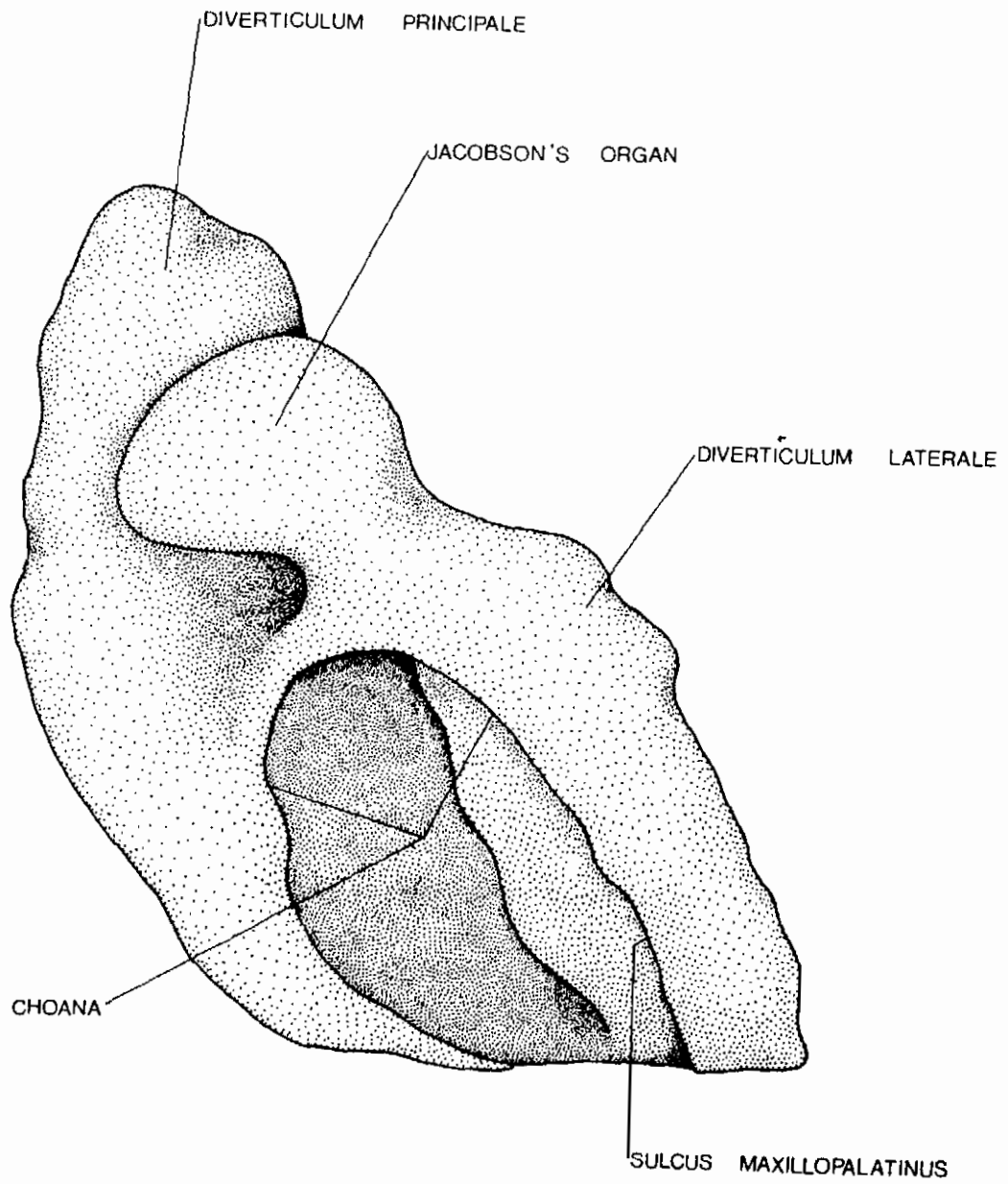


FIG 22 STAGE 5 - VENTRAL ASPECT OF THE LEFT NASAL SAC .

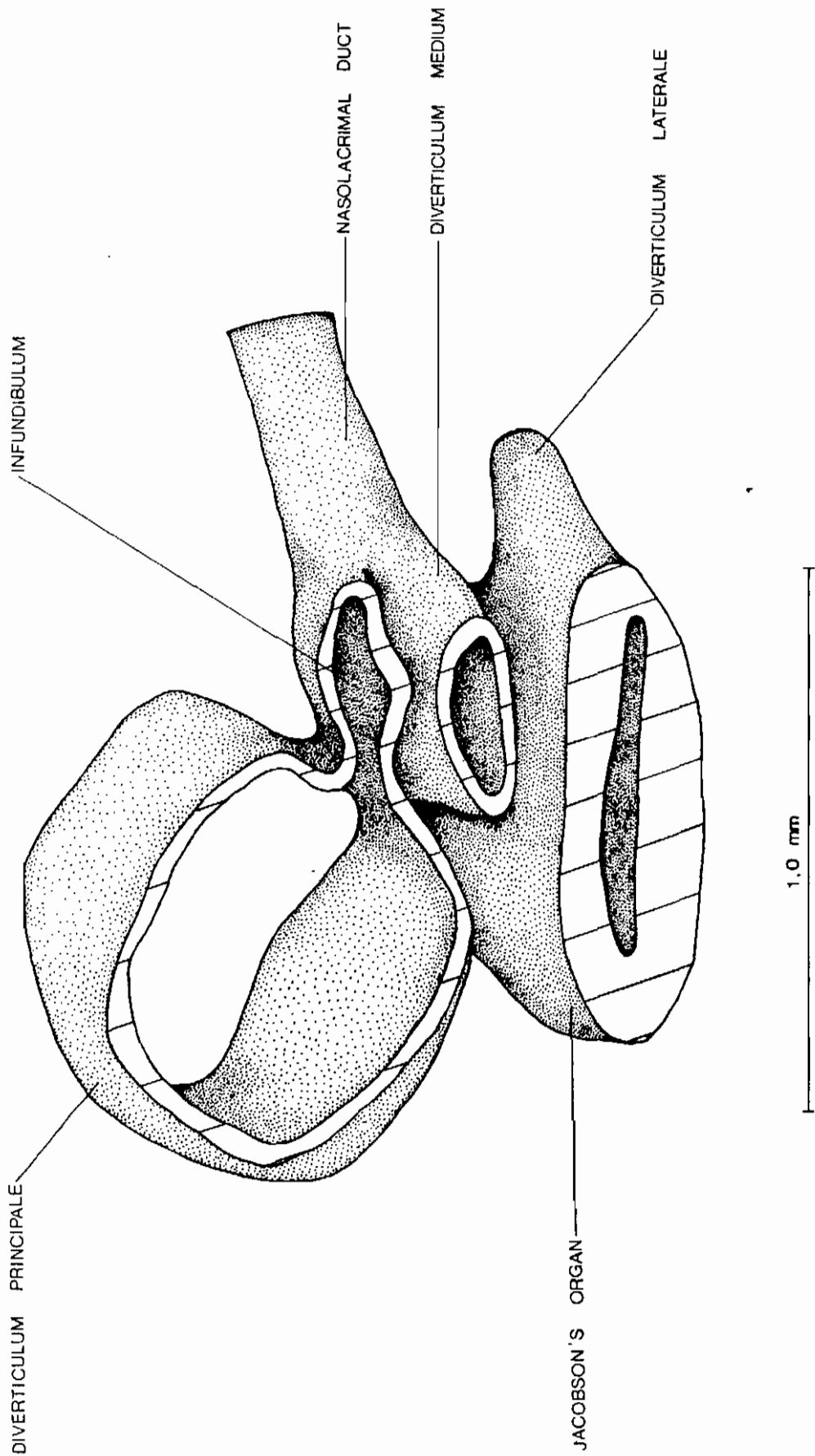


FIG. 23 STAGE 5 - STEREOGRAM OF PART OF LEFT NASAL SAC, ANTERIOR VIEW.

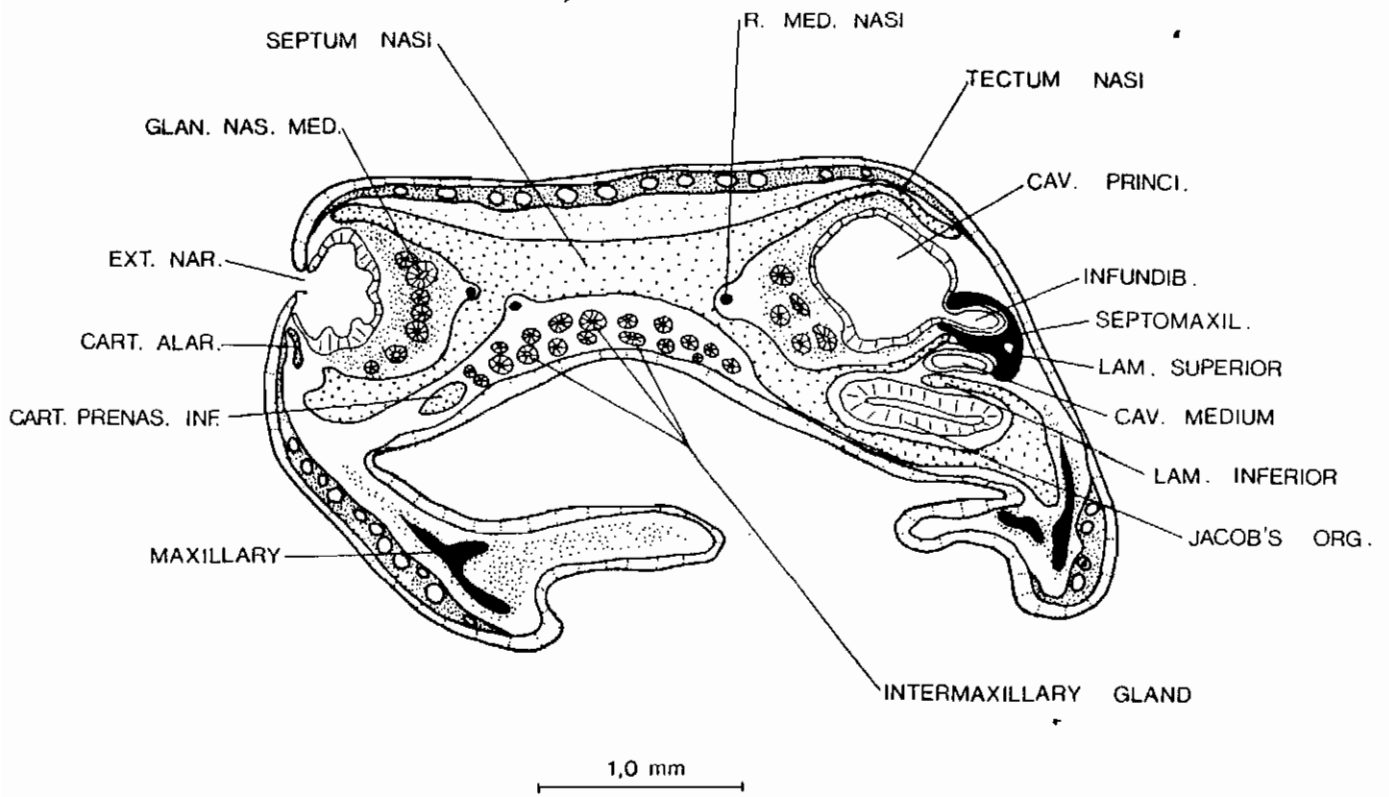


FIG. 24 STAGE 5 - CROSS-SECTION THROUGH REGION OF THE SEPTOMAXILLARY .

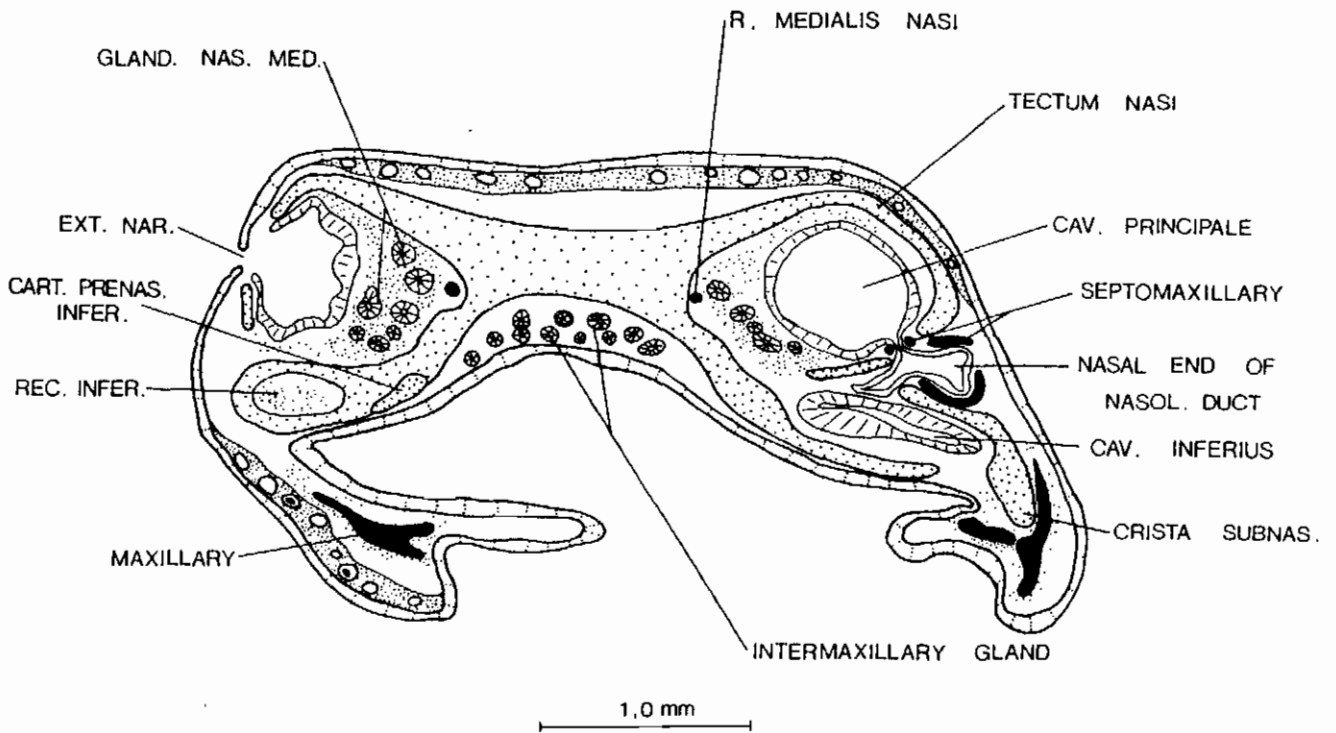


FIG. 25 STAGE 5 - CROSS-SECTION THROUGH THE NASAL END OF NASOLACRIMAL DUCT .

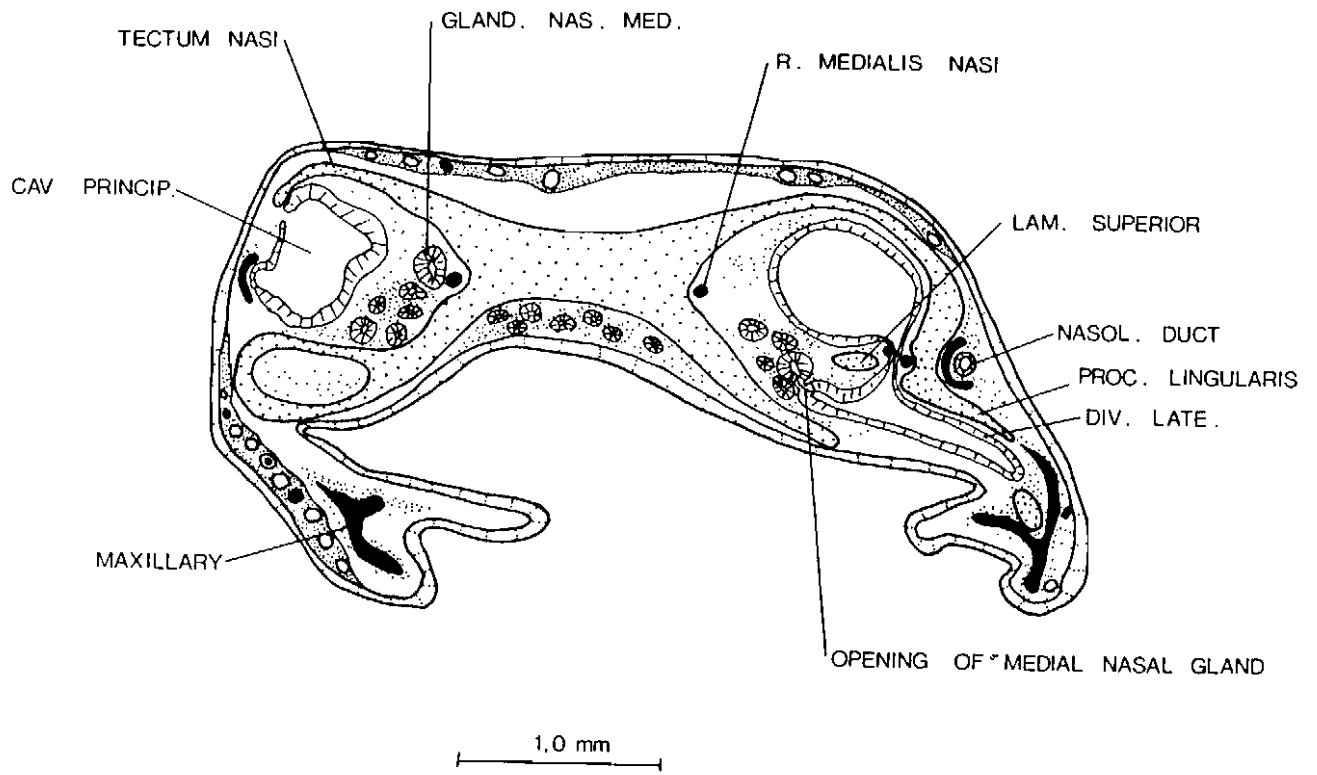


FIG. 26 STAGE 5 - CROSS-SECTION THROUGH OPENING OF MEDIAL NASAL GLAND.

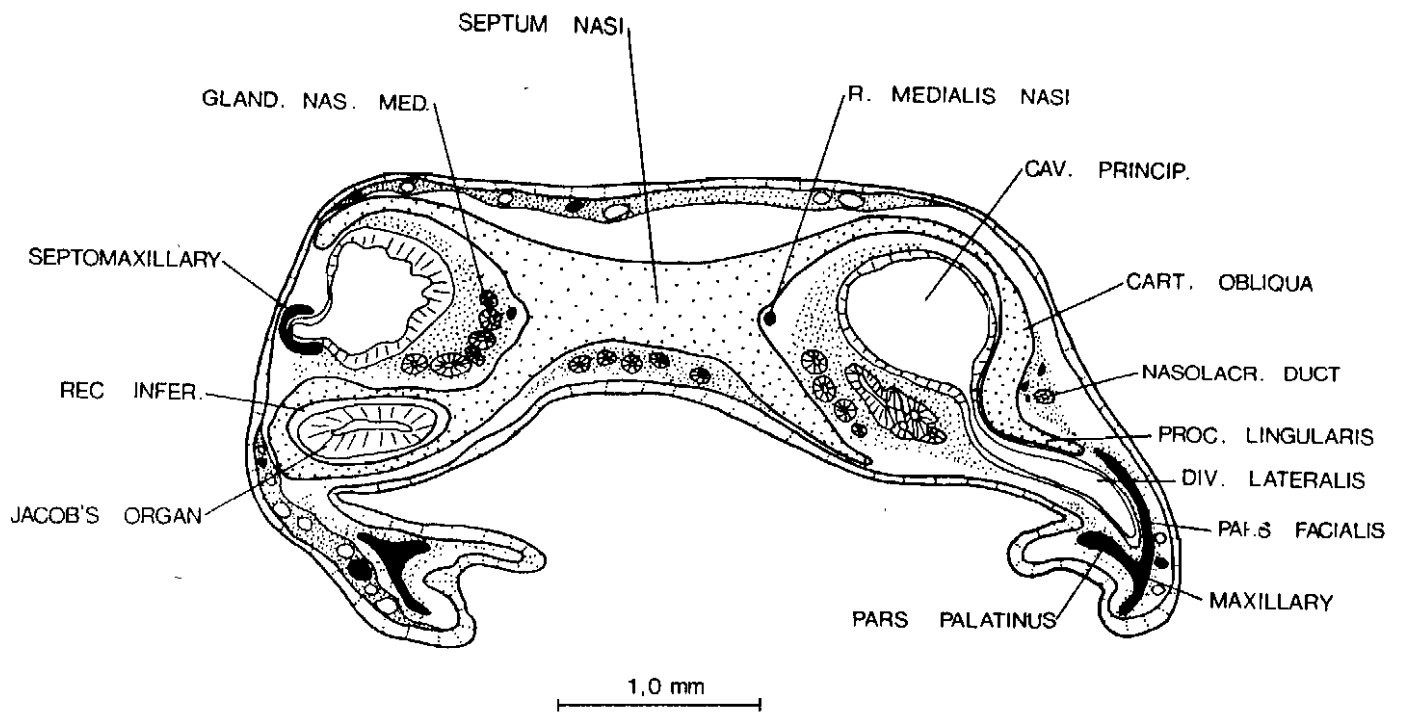


FIG. 27 STAGE 5 - CROSS-SECTION THROUGH REGION OF DIVERTICULUM LATERALE.

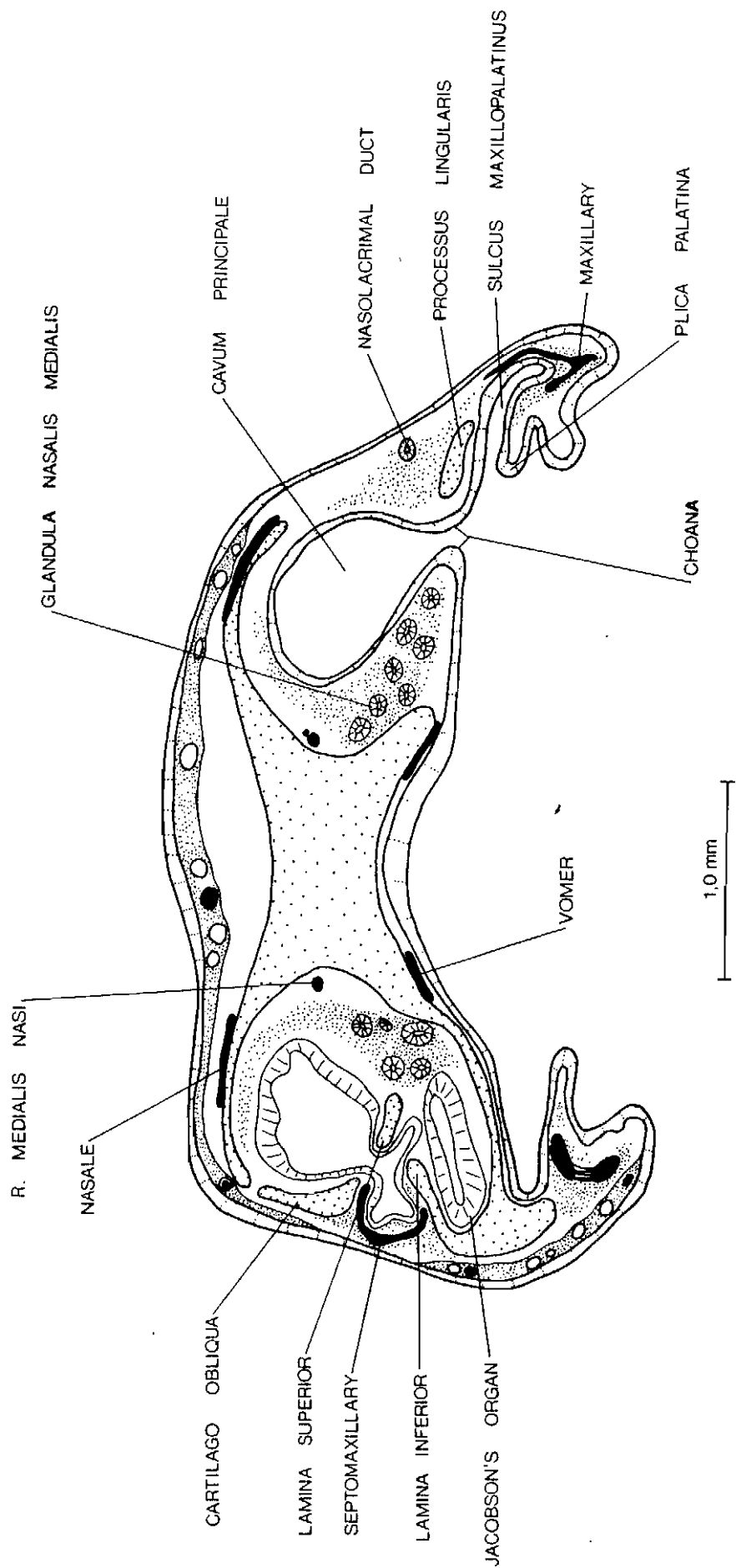


FIG. 28 STAGE 5 - CROSS-SECTION THROUGH THE CHOANAL REGION

DISCUSSION

Diverticulum Principale

The diverticulum principale is the largest of the three nasal sacs in anurans. In the youngest larval stage studied, no differentiation of the nasal sac into the characteristic three accessory sacs has taken place. However, an evagination associated with the nasal sac, namely the Jacobson's organ can be seen where it is situated ventrally to the main nasal sac. In this young stage, the diverticulum principale takes on a more or less elongated form. Ventromedially it has an evagination which opens into the preoral buccal cavity through the choana. The larger part of the diverticulum principale lies anterolaterally to the olfactory foramen. No olfactory epithelium is present in the wall of the diverticulum principale. Differentiation of the indifferent epithelium has not taken place yet.

The external naris opens anterolaterally from a projecting nasal tube. The opening of the external naris is valvular and therefore the naris can be closed. The choana or the internal nasal opening, which opens into the preoral buccal cavity, also is valvular and can be closed. However, a muscular mechanism is wanting, therefore the closure of the

choana would be merely mechanical. These observations can be brought into correlation with the aquatic life of the larva (Pearlman, 1934).

In a later stage of development the main nasal sac is more elongated and the nasal tube on which the external naris opens, longer. The nasal sac still has a ventromedial evagination, which extends medially and opens into the preoral buccal cavity by means of the choana. The choana is slit-like and no muscles are present in the choanal flap. The wall of the nasal sac, except for the lateral part, consists of ill-differentiated olfactory epithelium. Already the three basic layers of cells can be recognised. Olfactory hair form a dense layer on the inner surface of the olfactory epithelium.

In the next stage of development the nasal sac shows no signs of differentiation either. However it looks as if the sac is relatively shorter. The slit-like choana is still situated far posteriorly and the ventromedial evagination has become smaller.

The subsequent stage of development studied, just prior to metamorphosis, shows that some differentiation has taken place. As is evident from sagittal sections and reconstructions, the diverticulum laterale and diverticulum

medium have appeared. The main nasal sac has shortened considerably and now for the first time the three parts of the nasal sac are discernable. The diverticulum principale is situated anterodorsally, with the diverticulum medium, which is quite minute, posteroventrally to it. The nasal sac has extended laterally, forming a diverticulum laterale. It can be accepted that metamorphosis has begun to take place, coinciding with the flattening of the rest of the head. The olfactory epithelium of the main nasal sac has differentiated into the three typical layers of basal, supporting and olfactory cells as described by Gaupp (1896). The thickness of the olfactory epithelium ranges from relatively thick in the anterodorsal part to thin in the posterodorsal and posteroventral parts. In this stage a characteristic feature of terrestrial vertebrates, the nasolacrimal duct, appears. Medially the cavum principale is confluent with the cavum inferius, while laterally the two cavities are separate.

In the juvenile stage, the differentiation of the nasal sac into its characteristic form is completed. No anterior nasal tube is present and the external naris opens directly into the diverticulum principale. The nasal sac has shortened further and the choana has shifted to a more anterior position. The choana itself is not slit-like anymore and has widened considerably.

Diverticulum Medium

The diverticulum medium originates in quite a late stage of development. It is only in the stage prior to metamorphosis, where it is seen for the first time, originating in the vicinity of the anlage of the nasolacrimal duct. In Stage 4 the diverticulum medium is seen being wedged in between the diverticulum principale and the diverticulum mediale or Jacobson's organ. It is minute in appearance and there is a marked difference between its epithelium and the epithelium of the rest of the nasal sac.

The epithelium of the diverticulum medium has a squamous-like appearance with a layer of "Flimmerepithel" (Gaupp, 1896). It is noteworthy that the epithelium of the diverticulum corresponds to that of the nasolacrimal duct.

Diverticulum Mediale (Jacobson's Organ)

The diverticulum mediale or Jacobson's organ originates as a pouch-like evagination ventrally to the main nasal sac. From the earliest stage, the wall of the organ is lined with olfactory epithelium like that of the rest of

the nasal sac. As the nasal sac takes on its elongated form, the connection of the Jacobson's organ with the main nasal sac shifts medially, while the organ itself becomes displaced laterally. The Jacobson's organ enlarges during the development of the tadpole. The anterior end has a sac-like appearance, while the posterior end flattens out into a ventrolateral-orientated groove. In the juvenile stage the Jacobson's organ comes to lie ventrolaterally to the diverticulum principale, having shifted from a position ventrally to the main nasal sac. The Jacobson's organ now is a separate olfactory organ and the typical olfactory epithelium with which its walls is lined, ensures that chemical testing of the contents of the oral cavity is possible.

In view of the fact that the larva of Ascaphus leads an aquatic life until emergence upon land, the olfactory medium is water. This implies that there would be a flow of olfactory medium (water) through the nasal sac. However, because of the valvular choanal flaps, preventing a flow from the oral cavity to the cavity of the nasal sac, there would be a one-way flow from the outside through the external naris, via the nasal sac, into the oral cavity (Helling, 1938). Therefore, no perception of odours and testing of oral cavity contents by the Jacobson's organ would be possible.

During metamorphosis the choanal flaps disappear leaving a wide choana. Therefore, at this stage it appears as if the Jacobson's organ for the first time is in a position to test the contents of the mouth cavity.

According to the theory of Schmalhausen (1968) on the origin of the Jacobson's organ, the nasal sacs of the first vertebrates to emerge onto land, retained water in the ventral parts. This created an extra olfactory region for perceiving odours from the outside as well as from the oral cavity, through the choana. According to Schmalhausen, this ventral part of the nasal sac became specialized as the Jacobson's organ.

Taking into account Jurgens' (1971) observations on the migration of the Jacobson's organ in urodeles and anurans, some evolutionary tendencies show up. Jurgens (1971) states that the Jacobson's organ in lower urodeles tends to migrate from a position ventrally to the main nasal sac, to a ventrolateral position in higher urodeles like Triturus alpestris. He further states that in lower anurans the Jacobson's organ lies in a ventrolateral position. In anurans there is a tendency for the Jacobson's organ to migrate anteromedially to lie anteriorly between the septum nasi and the diverticulum principale in more advanced anurans like Hyla regilla. Helling (1938) also states

that there is a tendency for the Jacobson's organ in anurans to migrate from a position ventrolaterally to the nasal sac to a position medially between the septum nasi and diverticulum principale.

If the theory of the monophyletic origin of the Amphibia is accepted, the anurans and urodeles originated from the crossopterygian fishes in the late-Devonian/early-Carboniferous (Romer & Parsons, 1977). It is also generally accepted that the urodeles are more primitive than the anurans. These opinions concerning the migration of Jacobson's organ during phylogeny, is corroborated by the development of the organ in Ascaphus.

Diverticulum Laterale

No diverticulum laterale is present in the early stages of development. Just prior to metamorphosis the main nasal sac extends laterally to form the blind-ending diverticulum laterale. Respiratory epithelium with "Flimmern" lines its ventral wall.

In the juvenile stage the diverticulum laterale is fully developed, lying posterolaterally to the diverticulum principale and the Jacobson's organ. It is into the medial part of the diverticulum laterale that the choana

opens. Posterior to the choana, the diverticulum laterale flattens out, forming the sulcus maxillopalatinus, which reaches far posteriorly.

Nasolacrimal Duct

The subcutaneous anlage of the duct is observable for the first time in the stage prior to metamorphosis. It originates laterally to the main nasal sac, which at this stage also begins to differentiate into its characteristic form. During later development, the anlage extends anteriorly to come into contact with the nasal sac; posteriorly it reaches the lower eyelid. On its course anteromedially, the anterior end of the duct is almost surrounded by the septomaxillary. The nasolacrimal duct is connected to the diverticulum principale via the diverticulum medium. As to the question whether the diverticulum medium is a part of the main nasal sac or of the nasolacrimal duct, the fact that there is a marked difference between the epithelia of the diverticulum principale and that of the diverticulum medium, is quite clear. On the other hand, the similarity of the epithelia of the diverticulum medium and that of the nasolacrimal duct, leads us to deduce that the diverticulum medium could have originated from the anterior end of the nasolacrimal duct.

On the origin of the nasolacrimal duct, much have been written and hypothesized. Today there are two main theories as put forth by Jarvik (1942) and Schmalhausen (1968). One school believes, as put forth by Jarvik (1942), that the nasolacrimal duct had an intracapsular origin. The duct then migrated to the outside of the nasal capsule after extensive reduction of the original capsule side-wall. Jarvik (1980) favours the idea that the posterior nasal tube of the osteolepiform crossopterygians developed into the nasolacrimal duct. The side-wall of the anuran nasal capsule was extensively reduced, the fenestra endonarina posterior dematerialized and through this opening the posterior nasal tube emerged.

The other school believes, as put forth by Schmalhausen (1968), that the nasolacrimal duct did not originate intracapsular, but had a dual origin laterally to the original side-wall. For the dual origin Schmalhausen suggests a connection of the posterior nasal tube with part of the infraorbital seismosensory system. This theory is favourable and takes cognizance to the fact of the relation of the anlage of the nasolacrimal duct to the anlage of the seismosensory system and its independence of the olfactory sac (Schmalhausen, 1968). There also is a secondary connection of the nasolacrimal duct with the anterior naris and the olfactory sac. Another

fact that supports this theory, is that the dermal bones which in primitive fishes are associated with the canals of the infraorbital seismosensory system, are probably homologous to the septomaxillary and lacrimal, which in tetrapods are closely associated with the nasolacrimal duct (Jarvik, 1942; Schmalhausen, 1968).

In fishes like Amia and Polypterus the nasal sac has two openings, namely the anterior and the posterior nostrils. In tetrapods a third opening, the choana, is present and opens into the oral cavity. Schmalhausen (1968) is of the opinion that in the Osteolepiformes, as in primitive Stegocephalia (Ichthyostega, Megalocephalus), the anterior and the posterior nostrils have shifted down to the edge of the mouth. The possibility of a connection between the anterior and posterior nostrils by means of the seismosensory system would have been likely. With the division of the posterior nostril into two, an outer duct and an inner choana would have been formed. The outer posterior nostril shifted to the eye, while its duct remained connected to the nasal sac through the seismosensory canal. Upon emergence onto the land, the new function of carrying excess liquid from the eye was possible for the nasolacrimal duct.

Dorsolateral Appendix of Main Nasal Sac

In his paper Tsui (1946 b), gives a detailed description of the development of the lateral appendix of the olfactory sac in Rana nigromaculata. There are some histological differences between the epithelia of the lateral appendix and the rest of the nasal sac. He states that the appendix undergoes development till the larva has reached a certain length. After this, the appendix rapidly degenerates and soon disappears. Tsui also states that the relative size of the lumen to the rest of the appendix is small, and that the degeneration of the appendix coincides with the appearance of the Jacobson's organ. He then makes the assumption that the lateral appendix is the fore-runner to the Jacobson's organ.

In Ascaphus there also is a dorsolateral appendix present. However, it is very small and practically no lumen is present (Fig. 8). The degeneration of the appendix takes place during early embrionic stages. This appendix in Ascaphus is most probably homologous to the appendix in Rana. The author, however, does not agree with the view of Tsui (1946 b) that the lateral appendix is a fore-runner to the Jacobson's organ, because in the early developmental stages in Ascaphus, both structures are present.

Nerves of the Ethmoidal Region

The nervus olfactorius enters the nasal capsule through the olfactory foramen, which pierces the thick anterolateral wall of the brain capsule. The ramus ophthalmicus profundus, while still running through the orbital region, divides into a medial and a lateral branch. The medial one, the ramus medialis nasi, enters the nasal capsule through the foramen orbitonasale mediale. In the region of the olfactory foramen, it gives off a lateral branch, the ramus externus narium. This branch carries on anteriorly and leaves the nasal capsule through the foramen frontale. The ramus medialis nasi follows a course close to the septum nasi and leaves the nasal capsule through the fenestra nasobasalis.

The lateral branch in the orbital region is termed the ramus lateralis nasi proper (Jurgens, 1971). This branch reaches anterolaterally and enters the nasal capsule through the foramen orbitonasale laterale. After entering the nasal capsule, the ramus lateralis nasi proper extends anteroventrally, medially to the cartilago obliqua, to innervate the skin in the region of the anterior naris.

Jurgens (1971) states in his work that in all the forms he investigated, except in Ascapus, the profundus branch

divides within the nasal capsule into a ramus medialis nasi and a ramus lateralis nasi, with the branching off of the ramus externus narium far anteriorly. In Ascaphus, however, he describes a more caudal branching off for the ramus externus narium and an apparent lacking of a ramus lateralis nasi. He continues to describe for Ascaphus, Barbourula, Discoglossus, Bombina, Scaphiopus and Hypopachus "a well developed nerve branching off from the ramus ophthalmicus profundus in the orbit." (Jurgens, 1971 p. 67). He terms it the ramus lateralis nasi proper and homologizes it with the ramus lateralis nasi in Urodela. Jarvik (1980) does not accept this homologization and tries to ignore "this rather inconsiderable anatomical detail" (Jarvik, 1980 p. 221). Obviously Jurgens' observations furnish additional evidence to corroborate the theory of a monophyletic origin for the anurans and urodeles.

In urodeles the ramus lateralis nasi is a well-developed lateral branch of the ramus ophthalmicus profundus, branching from the latter while still in the orbital region. It also, like the ramus lateralis nasi proper in anurans, enters the nasal capsule through the foramen or pitonasale laterale and continues anteroventrally, terminating in the region of the anterior naris.

In view of the fact that this branch in urodeles lies in the same position as in anurans, the homologization of

these two nerves is not quite such an "inconsiderable anatomical detail". Accepting the monophyletic origin of amphibians, the homologization of the two nerves becomes an important link between the two groups.

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* Not seen in original.