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Ontogeny of the head of the Pacific hagfish (*Eptatretus stouti*, Myxinoidea): development of the lateral line system

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SUMMARY

The head of adult hagfishes (jawless craniates, Myxinoidea) of the family Eptatretidae displays a number of skin grooves of uncertain origin. These grooves have been homologized to the neuromast lines of other craniates, and they are innervated by two ganglionated cranial nerves that have been interpreted as lateral line nerves. The grooves do not, however, contain the compound receptors that are typical of a lateral line (i.e. neuromasts or electroreceptors), and both their development and function have remained enigmatic. To elucidate the embryonic origin of the grooves (which should develop from placodes if they are homologues of the lateral line system), embryos of Pacific hagfish were examined by means of three-dimensional reconstructions from serial sections. Because of the scarcity of specimens of embryonic hagfishes, only two embryos were reconstructed, but these reconstructions clearly show that a number of placodes and placodal derivatives (i.e. sensory ridges, receptor primordia, and cranial ganglia) occur in the head of embryonic eptatretid hagfishes. Some of these placodes correspond to the lens and epibranchial placodes of other craniates, but there are also three other placodes which represent possible homologues of lateral line placodes. The topology of the placodes in this latter group corresponds to the topology of the grooves of adult hagfishes, and we therefore reach three conclusions: (i) that an embryonic lateral line system is present in hagfishes; (ii) that the grooves of adult hagfishes in all probability derive from lateral line placodes; and (iii) that the presence of lateral line placodes is a primitive character of craniates.

1. INTRODUCTION

The lateral line system of craniates develops from a series of embryonic placodes. Gnathostomes (jawed craniates) have up to six lateral line placodes, each of which gives rise to two different sets of structures: a group of epidermal lateral line organs, typically containing compound elements (neuromasts) that act as mechanoreceptors; and a cranial nerve and sensory ganglion that innervate these receptors (Northcutt 1992*a*, *b*; Northcutt *et al.* 1994). Placode formation begins with a change in the appearance of the cells of the inner ectodermal layer, which transform from a flat or cuboidal layer into a columnar layer of cells. Shortly afterwards, the basement membrane beneath the placode is disrupted, and cells migrate from the placode into the underlying mesenchyme. Eventually, these cells will form a sensory ganglion that later innervates the neuromasts arising from its parent placode. In the head, the placodes then elongate and form sensory ridges in which the mechanoreceptive neuromasts develop and, in many species, the electroreceptive organs. In the case of the lateral lines on the trunk of bony fishes and amphibians, which arise from a placode initially located in the occipital region of the head, the placodal cells actively migrate to their target regions, where they differentiate into receptors (Metcalfe *et al.* 1986; Smith *et al.* 1990). Finally, ectodermal folds, which arise parallel to the sensory ridge, may enclose the neuromasts in grooves; in cartilaginous and bony fishes the folds may even fuse above the neuromasts to form a system of epithelial canals that open to the surface between adjacent neuromasts (Northcutt *et al.* 1994).

Relatively little is known about the organization of the lateral line system in hagfishes (jawless craniates, order Myxinoidea), although the details of this organization are critical for reconstructing the phylogenetic relationships of early craniates (Janvier & Blieck 1979; Aldridge *et al.* 1993; Forey & Janvier 1993). The order Myxinoidea consists of two families: Eptatretidae and Myxinidae. Considerably more information is available for eptatretid hagfishes than for myxinids.

In the Pacific hagfish, *Eptatretus stouti*, and most eptatretid hagfishes examined, the head has a varying number of short skin-grooves in the pre- and postocular region. The preocular set of grooves is oriented horizontally, and the postocular set consists of two subsets: dorsal grooves oriented transversely; and

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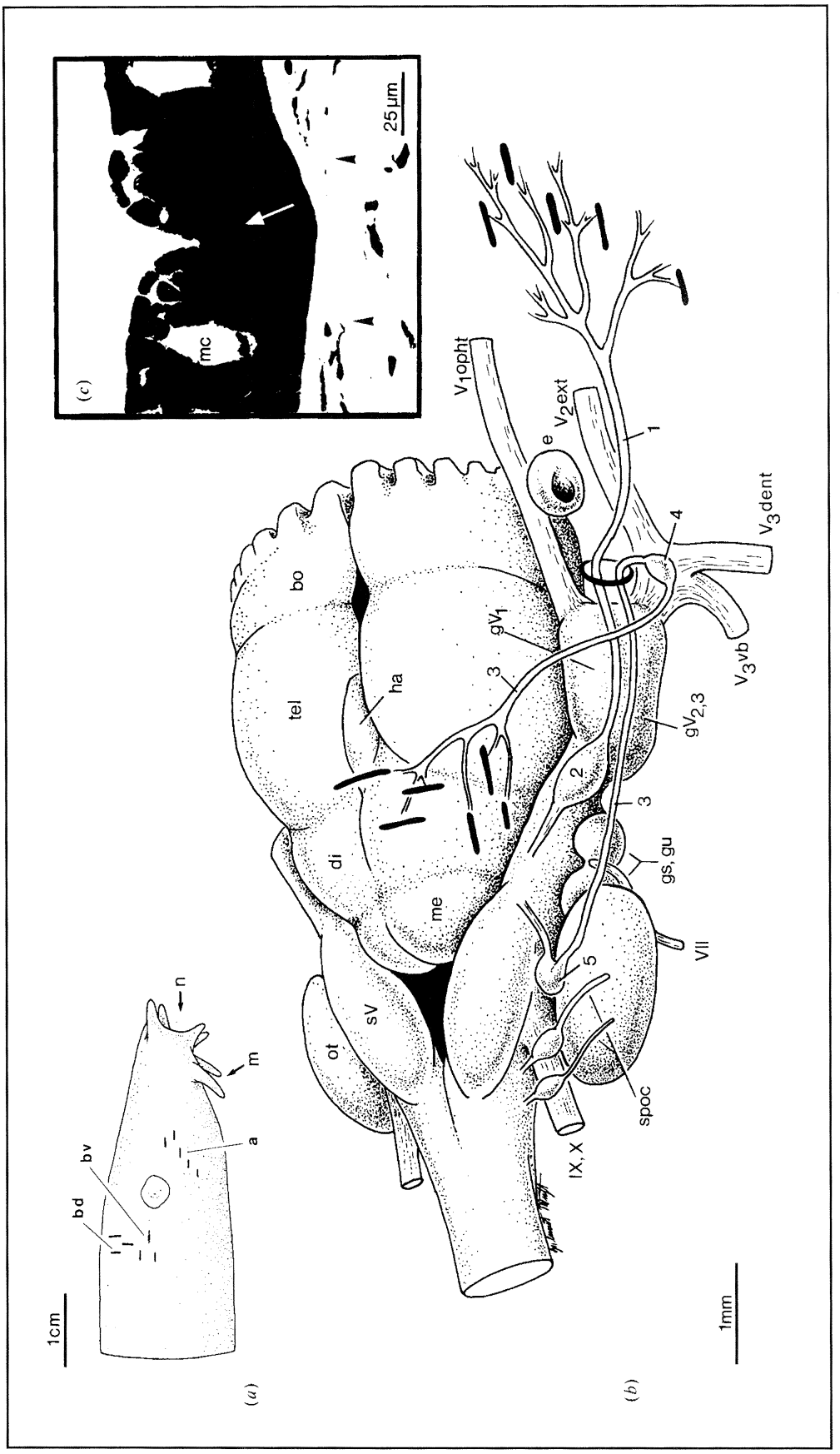

Figure 1. Head, brain and skin grooves of an adult Pacific hagfish, *Eptatretus stouti*. In this figure and in all subsequent drawings of heads and brains, anterior is to the right. (*a*) Lateral view of the head. The position of skin grooves is indicated by black lines. Note that the postocular set of grooves consists of two subsets: dorsal grooves (bd) with transverse orientation and ventral grooves (bv) with horizontal orientation. Arrows labelled n and m point to mouth and nasal openings, respectively. (*b*) A dorsolateral view of the brain, showing the roots and ganglia of the major cranial nerves and the course of the nervi laterales a and b. The skin grooves are again symbolized by black lines. The semicircle posterior to the eye marks the point where the nervi laterales pierce the general body fascia and enter the subcutaneous blood sinus. Note that the nervus lateralis b has two ganglia: a proximal ganglion (5) medial to the otic capsule and a distal ganglion (4) posterior to the eye. The diagram is based on descriptive and experimental data from Worthington 1905; Kishida *et al.* 1987; Braun *et al.* 1993; and unpublished observations. (*c*) Semi-thin plastic section (5 µm) through one of the grooves. Compound receptors are lacking, but there is a specialized type of cell (see white arrow, cell with intensely staining nucleus) which may be a single-celled receptor. Arrowheads beneath the groove point to a gap in the pigment layer of the dermis. This gap may be responsible for the macroscopic appearance of the grooves, which are visible as white stripes on the brownish skin.

ventral grooves oriented horizontally (see figure 1*a*). As early as 1907, Ayers and Worthington suggested that these grooves might represent the skin component of a lateral line system. They had observed an intraepidermal canal system and neuromasts that were associated with the grooves, which reminded them of a typical lateral line system. It has since become clear, however, that the canals and the neuromasts observed by Ayers and Worthington were histological artefacts. Neither an electron microscopical investigation (Fernholm 1985), nor our own observations of semithin sections through the grooves (see figure 1*c*), has provided any evidence for the presence of either intraepidermal canals or any kind of compound receptor. Nevertheless, other recent studies have shown a dense innervation of these grooves by two ganglionated cranial nerves (Braun *et al.* 1993, see figure 1*b*), and the central projection of these nerves is remarkably similar to projections of typical lateral line nerves (Kishida *et al.* 1987). Julia Worthington first described these nerves (Worthington 1905), and we now use a modified version of her terminology. The anterior nerve, which possesses a single ganglion slightly dorsal and caudal to the trigeminal ganglion, is called nervus lateralis a; it supplies the preocular set of grooves (see figure 1*b*). The more posterior nerve, nervus lateralis b, curves around the otic capsule, then courses rostrally towards the eye. Slightly posterior to the eye, it pierces the general body fascia, together with nervus lateralis a, but then turns caudally and dorsally to innervate the postocular set of grooves. Notably, nervus lateralis b carries two ganglia, a proximal ganglion medial to the otic capsule and a distal ganglion immediately posterior to the eye, above the general body fascia (unpublished observations, see figure 1*b*). This raises the possibility that nervus lateralis b and its ganglia originate from two separate placodes.

Members of the second family of hagfishes, the Myxinidae, show no trace of the skin-grooves, although descriptions of the brain and cranial nerves (Jansen 1930; Lindström 1949; Peters 1963) indicate that *Myxine glutinosa* appears to possess a homologue of nervus lateralis a, called nervus buccalis in this species.

There is very little information on the embryology of the lateral line system in hagfishes, and much of the existing information is contradictory. If the entire system is homologous to the lateral line system in other craniates, it should develop from placodes in a sequence comparable to that described above in gnathostomes. In his pivotal study of the development of *Eptatretus stouti*, however, Dean (1899, p. 273) found 'no obvious evidence that an embryonic series of end organs is present comparable to a distinct lateral line', although von Kupffer (1900), studying the same species, observed three lateral line placodes and three sets of 'Knospen' (buds), which he interpreted as neuromast primordia arising from those placodes. He also claimed that these buds were innervated by rami of the trigeminal, facial, and vagal nerves, but his description does not match that of nervus lateralis a and nervus lateralis b reported by Worthington (1905) to innervate the grooves in adult eptatretid hagfishes. These conflicting observations prompted us to reinvestigate the embryology of the lateral line system in the Pacific hagfish (*Eptatretus stouti*) by means of three-dimensional reconstructions from serial sections.

2. LIST OF ABBREVIATIONS

numerical:

1	nervus lateralis a
2	ganglion of nervus lateralis a
3	nervus lateralis b
4	distal ganglion of nervus lateralis b
5	proximal ganglion of nervus lateralis b
V_1opht	trigeminal nerve, ophthalmic branch
V_2ext	trigeminal nerve, external branch
V_3dent	trigeminal nerve, dental branch
V_3vb	trigeminal nerve, velobuccal branch
VII	facial nerve
VIII	octaval nerve
IX, X	glossopharyngeal, vagal nerves

alphabetical:

a	the preocular set of skin grooves and their embryonic precursors
ad	anterodorsal lateral line placode
au	auricles of medulla oblongata
av	anteroventral lateral line placode
bd	the dorsal, transversely oriented set of postocular skin grooves and their embryonic precursors
bo	olfactory bulb
bs	subcutaneous blood sinus
bv	the ventral, horizontally oriented set of postocular skin grooves and their embryonic precursors
ca	cartilage
de	dermis
di	diencephalon
e	eye
end	external nasal duct
fe	facial epibranchial placode
fm	future mouth opening
fn	future nasal opening
ge	glossopharyngeal epibranchial placode
gf	general body fascia
glX	glosspharyngeal epibranchial ganglion
gp	gill plate
gs	saccular ganglion
gu	utricular ganglion
gV_1	ganglion of the ophthalmic branch of the trigeminal nerve
$gV_{2,3}$	ganglion of the external, dental, and velobuccal branches of the trigeminal nerve
gVII	facial epibranchial ganglion
ha	habenula
i/inf	infundibulum
l	lens placode
m	mouth opening
mi	middle lateral line placode
mc	mucous cell
me	mesencephalon
mo	medulla oblongata
n	nasal opening
nc	nasal cavity
np	nasopharyngeal duct

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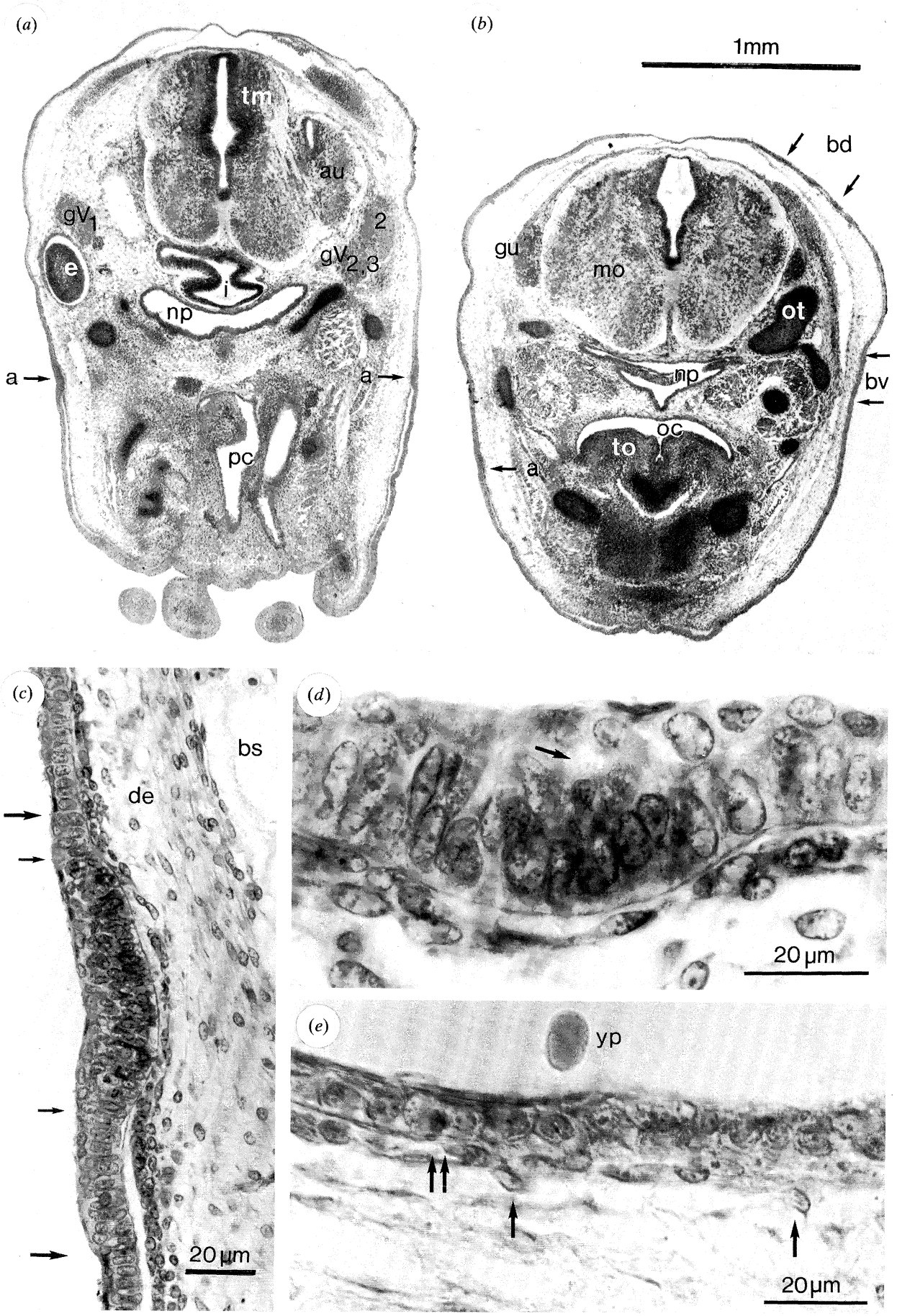

Figure 2. For description see opposite.

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o	otic lateral line placode
oc	oral cavity
ot	otic vesicle/organ
pc	pharyngeal cavity
pd	dorsal subdivision of posterior lateral line placode
pe	future pigment epithelium
pm	medial subdivision of posterior lateral line placode
pv	ventral subdivision of posterior lateral line placode
ret	future retina
s	supratemporal lateral line placode
sc	spinal cord
spoc	spinooccipital nerve(s)
sV	sensory nucleus of trigeminal nerve
tel	telencephalon
tm	mesencephalic tectum
to	tongue apparatus
vo	optic ventricle
yp	yolk platelet
ys	yolk sac

3. MATERIALS AND METHODS

The data presented in this paper were obtained from a historic collection of embryonic *Eptatretus stouti*. We will briefly review the history and current status of this collection, then describe the method used for three-dimensional reconstructions and give estimates of the age of the embryos examined, and finally, discuss problems of interpretation and terminology.

(*a*) *The collection of embryos*

Data on the embryology of hagfishes are very difficult to obtain. Currently, the vast majority of known specimens of hagfish embryos are in the 'Dean–Conel' collection; embryos of *Eptatretus stouti* (family Eptatretidae, old genus name: *Bdellostoma*) that were collected in the Monterey Bay in 1896 and 1930 (Dean *et al.* 1897; Conel 1929, 1931, 1942). Information on the embryology of the second family of hagfishes, the myxinids, is even more limited: only three myxinid embryos have ever been found (Holmgren 1946; Fernholm 1969). The unique 'Dean–Conel' collection originated in 1896, when an expedition from Columbia University spent a summer in Pacific Grove, California, on Monterey Bay. Bashford Dean, one of the heads of the expedition, found a Chinese fisherman, Ah Tack Lee, who knew the location of a spawning ground in Monterey Bay and had managed to collect a few embryos there. For the rest of that summer, Bashford Dean hired the entire Chinese fishing community in the Monterey area, and 'the dozen or more fishing boats of the Chinese village were impressed into zoölogical service.' (Dean *et al.* 1897). By dredging, they managed to collect 150 fertilized eggs from a depth of 12 fathoms (approximately 22 m). These embryos formed the basis for Dean's early description of myxinoid development (Dean 1899), and they were also the source of data for papers by Stockard (1906*a*, *b*, 1907) on various developmental aspects and monographs by Conel (1929, 1931) on brain development in myxinoids. Only two years later, in 1898, Carl von Kupffer sent a student named Doflein to the same location to collect embryos for his own laboratory in Munich (Doflein 1898). Doflein managed to obtain 'eine schöne Collection von Embryonen' ('a nice collection of embryos', no numbers given) which he took home to Munich. These embryos formed the basis of Carl von Kupffer's account of myxinoid development (1900). Unfortunately, this collection of embryos has disappeared (Conel 1931).

It was more than thirty years before the next set of embryos was secured from Monterey Bay. In the summer of 1930, LeRoy Conel collected an unspecified number of embryos (Conel 1931, 1942) and incorporated them into the collection. To our knowledge, no additional embryos of *Eptatretus* have ever been collected.

The collection remained essentially in storage until the 1980s, when Aubrey Gorbman and co-workers investigated adenohypophyseal and stomodeal development (Gorbman 1983; Gorbman & Tamarin 1985). When we became aware of the Dean–Conel embryos, they had become part of the Minot embryological collection at the Warren Anatomical Museum (Harvard Medical School, Boston, Massachusetts). The Dean–Conel collection, which consists of histological preparations of approximately 150 embryos, is currently located at the Museum of Comparative Zoology (Harvard University, Cambridge, Massachusetts).

Unfortunately, little is known about the fixation and histological processing of the embryos. Conel (1929) mentions that Dean had stored about thirty embryos in 80% (by volume) alcohol since 1896, before they were finally embedded (probably in paraffin) and cut (between 1926 and 1929), but it is not clear whether the embryos had also been fixed in alcohol. With regard to the embryos that he himself collected in 1931, Conel (1931, 1942) also gives no details

Figure 2. Photomicrographs of transverse sections from embryo number 2357. The scale bar in the upper right corner applies to (*a*) and (*b*). (*a*) A section through the anterior part of the head at the level of the posterior pole of the eye on the left side. Arrows labelled 'a' point to focal ectodermal specializations. (*b*) A section through the posterior part of the head at the level of the anterior pole of the otic capsule on the right side. Arrows labelled 'a' and 'bd' point to focal ectodermal specializations. The arrows labelled 'bv' point to an ectodermal region ventral to the otic capsule; this region does not seem to differ from the general ectoderm at this magnification, but a specialization becomes evident at higher power (see (*e*)). (*c*) Higher power view of the ectodermal region labelled 'a' on the left side of frame (*a*). The large arrows mark a thickened ectodermal region that could be a late sensory ridge. Within that region, the small arrows mark an area that contains densely packed cells with intensely staining nuclei. An inward bulging of the ectoderm can also be seen underneath the area and may thus represent a large developing neuromast in longitudinal section. (*d*) Ectodermal region ventral to the eye in a section somewhat anterior to (*a*) and (*c*). A cluster of cells with intensely staining nuclei form a bud. Underneath the bud, the ectoderm protrudes towards the dermis, and there is a well developed apical cavity (arrow) above the apical poles of the cells with intensely staining nuclei. This structure might be a developing neuromast in transverse section. (*e*) Ectodermal region ventral to the otic capsule, showing the region labelled 'bv' in frame (*b*), in the section directly adjacent to the one shown in (*b*). General ectoderm is visible in the region to the left of the double arrows; to the right of those arrows the basement membrane disappears, the ectoderm shows a slight increase in thickness, and single cells (arrows) appear to migrate from the ectoderm into the underlying mesenchyme. This structure may therefore be a placode that gives rise to ganglion cells.

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concerning the fixation. Most of the series were cut in the transverse plane at 8 µm or 10 µm, and the vast majority of the series were stained with alum–cochineal and orange G (basophilic structures appear in red, azidophilic structures in blue). In most of the series, this stain has faded almost entirely, rendering them practically useless for detailed microscopical investigations.

(*b*) *Histological analysis, staging of embryos, and three-dimensional reconstructions*

The entire Dean–Conel collection was screened, and histological series from two embryos, identified as number 2337 and number 2357 were chosen for three-dimensional reconstruction. In contrast to most other series, the sections of these two series were still unfaded and nearly complete; only a few individual sections were torn or distorted.

The older embryo, number 2357, (histological sections shown in figures 2 and 3, reconstruction in figure 4) had been cut at 10 µm in the transverse plane. Judging from the total number of sections in the series, the embryo must have been 22–23 mm long prior to sectioning (length at hatching is approximately 45 mm (Dean 1899)), but it had probably shrunk during fixation and embedding. Based on this information and the fact that histogenesis was complete (see, for example, figure 3*b*, where well-differentiated pieces of cartilage can be identified), we estimated this embryo to be in the second third of its post-gastrulational development; it is a 'late embryo' according to Dean's (1899, p. 255) staging system (after the completion of gastrulation, Dean distinguished 'early', 'late', and 'latest' embryos). Every third section through the head (55 sections altogether) was drawn with the aid of a camera lucida.

The younger embryo, number 2337, (histological sections shown in figures 5 and 6, reconstructions in figures 7 and 8) had also been cut at 10 µm in the transverse plane, and, judging from the total number of sections in the series, it was about 12 mm to 13 mm long prior to sectioning. Organogenesis was completed in this embryo, however the tissue differentiation, particularly with respect to mesodermal/mesenchymal derivatives, had just begun. Thus, somites were still present in the trunk region, and the head was filled with large amounts of undifferentiated mesenchyme (see figures 5 and 6). We therefore estimated this embryo to be in the first third of its post-gastrulational development; it is an 'early embryo' according to Dean's (1899) staging system. Twenty-two sections at varying distances through the head of this embryo were drawn with the aid of a camera lucida.

The drawings were aligned manually, and contours of numerous structures were digitized and entered into a VIDAS image analysis system (KONTRON, Eching, F.R.G.): head, brain and eyes, ventricles, cranial nerves and ganglia, pharyngeal and nasal cavities, otic capsule, and epidermal placodes and their derivatives. A black and white example of an originally coloured, computer-generated reconstruction is shown in figure 7. Reconstructions like this one were used as templates to prepare the ink-drawings as shown in figures 4 and 8.

(*c*) *Terminology*

As noted in §1, there is some confusion regarding the terminology applied to the entire lateralis system of myxinoids. We use a modified version of Worthington's (1905) terminology, because her terms avoid the possibility of further confusion with a newly proposed nomenclature for lateral line nerves, ganglia, and placodes in other craniates (see Northcutt 1992*b*; Northcutt *et al.* 1994). We thus use the letter 'a' to refer to the preocular ectodermal grooves connected with nervus lateralis a (see figure 1), and we also use this letter to identify the embryonic ectodermal structures that we interpret as the precursors of those grooves in adults. Similarly, the letter 'b' is used to identify the postocular grooves connected with nervus lateralis b; since two subsets of postocular grooves can be distinguished (see above), we use the letters 'bd' (b/dorsal) and 'bv' (b/ventral) to denote the grooves in adults as well as the embryonic structures that we believe antedate them.

4. RESULTS

Figure 1 illustrates the lateral line system in an adult eptatretid hagfish, as a reference. We will first describe the older embryo (number 2357), followed by the younger specimen (number 2337). The following text is mainly a description of the reconstructions shown in figures 4 and 8, respectively, so repeated references to those figures will not be made.

(*a*) *Embryo number 2357* (*figures 2 and 3, reconstruction in figure 4*)

The head has not yet acquired its adult shape. The future external openings of mouth (fm) and nasal duct (fn) are still closed and directed ventrally instead of anteriorly, as in adults (compare with figure 1). Apart from these conspicuous features, the nasal and pharyngeal structures are very similar to the condition seen in adults: the nasal cavity (nc) is connected to the (future) tip of the head by a long external nasal duct (end) and communicates with the pharynx (pc) by way of the nasopharyngeal duct (np). The oral cavity (oc) shows two pronounced impressions caused by the tongue apparatus (to) which projects from the floor of the mouth into the oral cavity (see figure 2*b*). In the brain, the formation of the major cytoarchitectural units is almost complete, but the occlusion of the ventricular spaces that is typical of adult hagfish brains has not yet occurred (see figures 2*a*, *b*). All the major cranial ganglia that occur in adults can be identified on the basis of their relative position. The ophthalmic (V_1opht), the external (V_2ext, corresponding to the maxillary nerve of other craniates), and the velobuccal/dental (V_3vb and V_3dent, corresponding to the mandibular nerve) branches of the trigeminal nerve and their respective ganglia (gV_1 and $gV_{2,3}$) can be clearly identified (see also figures 2*a* and 3*b*; in the reconstruction shown in figure 4, the ophthalmic ganglion [gV_1] is hidden behind the eye). The facial nerve (VII) carries two small but distinct peripheral ganglia immediately ventral to the otic capsule (ot). Two spino-occipital nerves (spoc) and the combined root of the vagal-glossopharyngeal complex (IX, X) are present in the postotic region.

The ganglion of nervus lateralis a (labelled '2') can be found immediately dorsal and caudal to the $V_{2,3}$ ganglionic complex ($gV_{2,3}$). This ganglion is connected with the brain by a nerve (labelled '1') that traverses

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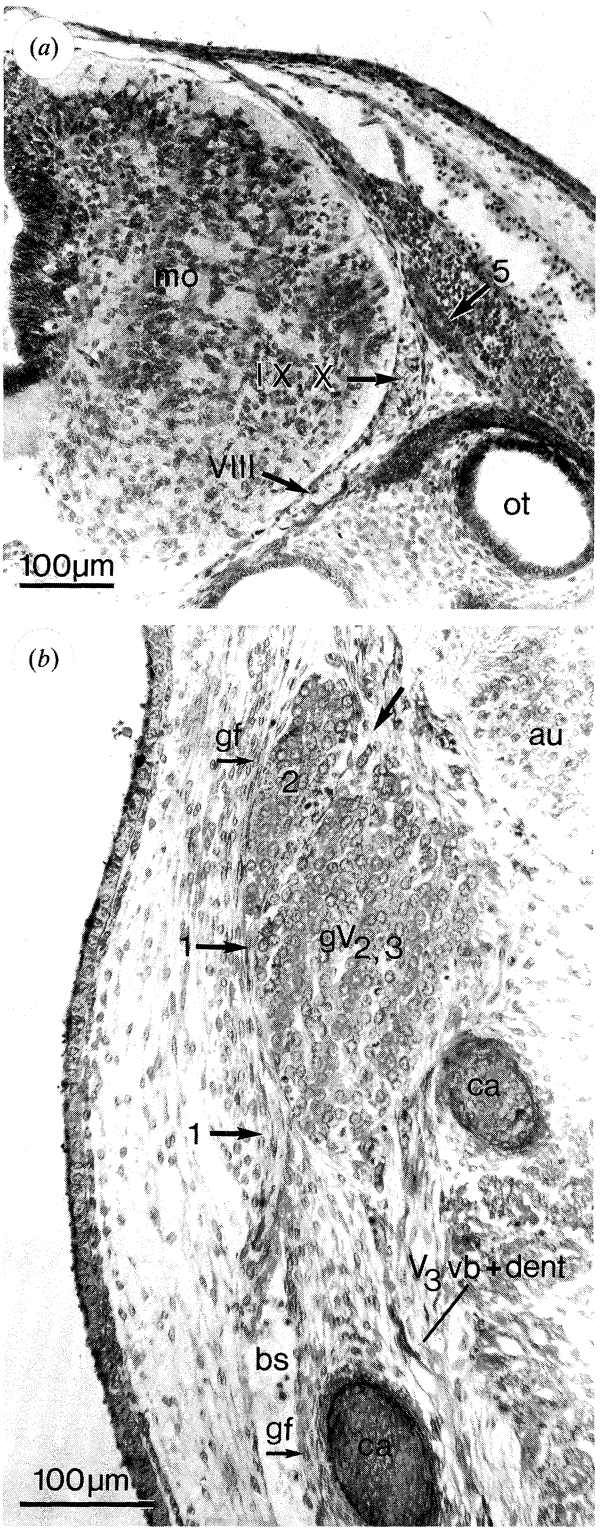

Figure 3. Photomicrographs of transverse sections from embryo number 2357. (*a*) Section through the posterior part of the head at a level midway through the otic capsule. Arrow labelled '5' points to the developing proximal ganglion of nervus lateralis b medial to the otic capsule. (*b*) Section through the anterior part of the head, slightly posterior to the eye at a level through the ganglionic complex of the trigeminus. Note the developing ganglion of nervus lateralis a (labelled '2') dorsal and lateral to the trigeminal ganglion. Also note the thin sheath of connective tissue (unlabelled arrow) that separates the ganglia. Arrows labelled '1' point to nervus lateralis a, which is directed towards the infraocular skin.

the anterior pole of the utricular ganglion (gu), and it also emits a peripheral nerve which projects towards the infraocular skin region (see figure 3*b*). We could not trace this nerve into the skin, because it was relatively thin, and the staining made it very difficult to distinguish thin nerves from surrounding connective tissue (see figure 3*b*). The infraocular epidermis displays a number of conspicuous focal specializations, which are represented by dark spots in the reconstruction (labelled 'a', see also figure 2*a*). Histologically, the general ectoderm of the anterior head consists of a two-layered epithelium with a flat external layer, a columnar internal layer, and a well developed basal lamina (for examples see the regions above and below the large arrows in figure 2*c*). In the foci, however, the thickness and the number of cell strata of the inner ectodermal layer are increased (see, for example, the region between the large arrows in figure 2*c*). Within these regions of increased epidermal thickness, we observed structures that consist of more densely packed cells (see, for example, the region between small arrows in figure 2*c*). At higher magnification and in transverse sections (see figure 2*d*), the structures appear as buds, consisting of relatively small cells with intensely stained nuclei and larger cells with pale nuclei. In some cases, as seen in figure 2*d*, a distinct apical cavity is visible in the ectoderm, directly above the bud. The basal lamina beneath these structures is generally intact.

The proximal ganglion of nervus lateralis b (labelled '5') was identified on the basis of its relative position medial to the otic capsule (ot), midway between the saccular ganglion (gs) and the first spino-occipital ganglion (spoc, compare figures 1 and 5, see also figure 3*a*), wedged in between the otic capsule (ot) and the developing connective tissue sheath surrounding the brain. We could not trace the proximal or peripheral neurites arising from that ganglion (i.e., the nervus lateralis b), though we assume that they are present. The distal ganglion of nervus lateralis b could not be found. In the postocular region, two sets of focal ectodermal specializations could be identified: dorsal and slightly anterior to the otic capsule, a set of buds conforms to the description of the infraocular buds given above (labelled 'bd', see also figure 2*b*); more ventrally and also slightly anterior to the otic capsule (ot), we observed another region of specialized ectoderm (labelled 'bv', see also figure 2*d*). The general ectoderm surrounding the specialized region consists of a bilayered epithelium with a flat external layer; in contrast to the general ectoderm in the more anterior regions of the head, the internal layer is not columnar but cuboidal (see, for example, the region left of double arrows in figure 2*e*). The specialized region is characterized by the absence of a light-microscopically visible basal lamina and a slight increase in the thickness of the inner ectodermal layer (see the region right of double arrows in figure 2*e*). Single cells, distinct from the surrounding mesenchyme were visible immediately subjacent to the focal specialization (indicated by single arrows in figure 2*e*). These cells might be migratory cells arising by focal ingression from the epidermal placode.

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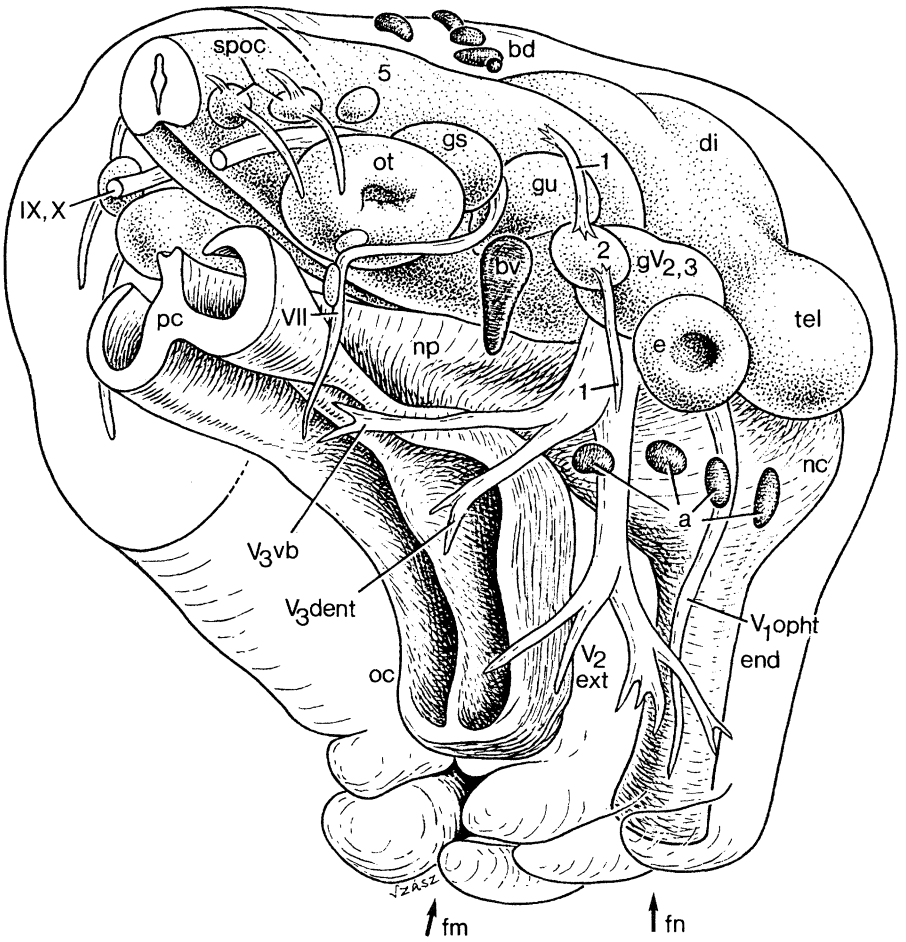

Figure 4. Three-dimensional reconstruction of the head of embryo number 2357, from a perspective posterior, lateral, and somewhat ventral to the right side of the head. The arrows labelled fm and fn point to the future external openings of mouth and nose, respectively. Blackened areas in the ectoderm represent the positions of focal ectodermal specializations. This figure is described in detail in the text.

(*b*) *Embryo number 2337* (*figures 5 and 6*; *reconstructions in figures 7 and 8*)

In this embryo, the yolk-sac (ys) is still very extensive, and the posterior regions of the head are embedded in a groove of that sac (see figure 5*b*, *c*). More anteriorly, the head has begun to lift from the yolk (see figure 5*a*). In the preotic region, the sections are slightly distorted (see figure 5*a*), probably an artifact of fixation, but as this distortion affects all sections to the same degree, it did not hinder the reconstruction.

The head is characterized by the presence of a very dense, cell-rich mesenchyme, denser ventrally than dorsally (see figure 5*a*–*c*). This mesenchyme displays local condensations, for example around the otic vesicle (ot, figure 5*b*), but the differentiation of muscle, cartilage, and connective tissues has not yet occurred. Cranial nerves and ganglia, however, have already formed (see below). The embryo displays paired nasal cavities (nc, see also figure 5*a*) which do not communicate with the exterior or with the pharyngeal cavity (pc). The oral cavity (oc) shows a complicated pattern of diverticuli and fenestrae; more posteriorly, the pharyngeal cavity (pc) displays a series of diverticuli that represent the first (facial) to fourth branchial pouch (see also figure 5*b*, *c*). Even more posteriorly, a collar-like gill-plate (gp) occurs laterally adjacent to the pharynx (pc), and a number of developing branchial pouches can be seen within that plate (see figure 5*c*). Please note that this complicated system of pouches is not included in the reconstruction. Major subdivisions of the brain can be recognized; the differentiation of the white matter in the marginal zone and the migration of neurons into that zone have just begun (see figure 6*c*). The eye (e) is in a late eye-cup stage; a small optic ventricle (vo) is still present in the eye (figure 6*d*) but has disappeared from the optic nerve. The major cranial ganglia have formed, and some of the cranial nerves can also be identified. The trigeminal complex consists of the ganglion of the ophthalmic branch (gV_1) and the ganglion of the external, dental, and velobuccal branches ($gV_{2,3}$, see also figure 5*a*) of the trigeminal nerve. The ophthalmic branch (V_1opht, see also figure 6*a*) and the massive combined root of the external/dental/velobuccal branches (V_2ext and V_3dent + vb) can also be identified. The saccular (gs) and utricular (gu) ganglia of the eighth nerve are present, and the facial nerve (VII) can be traced from the brain to its epibranchial ganglion (gVII), which is located beneath the otic capsule and immediately dorsal to the first branchial pouch. The roots of the vagal (X), the glossopharyngeal (IX), and spino-occipital (spoc) nerves can

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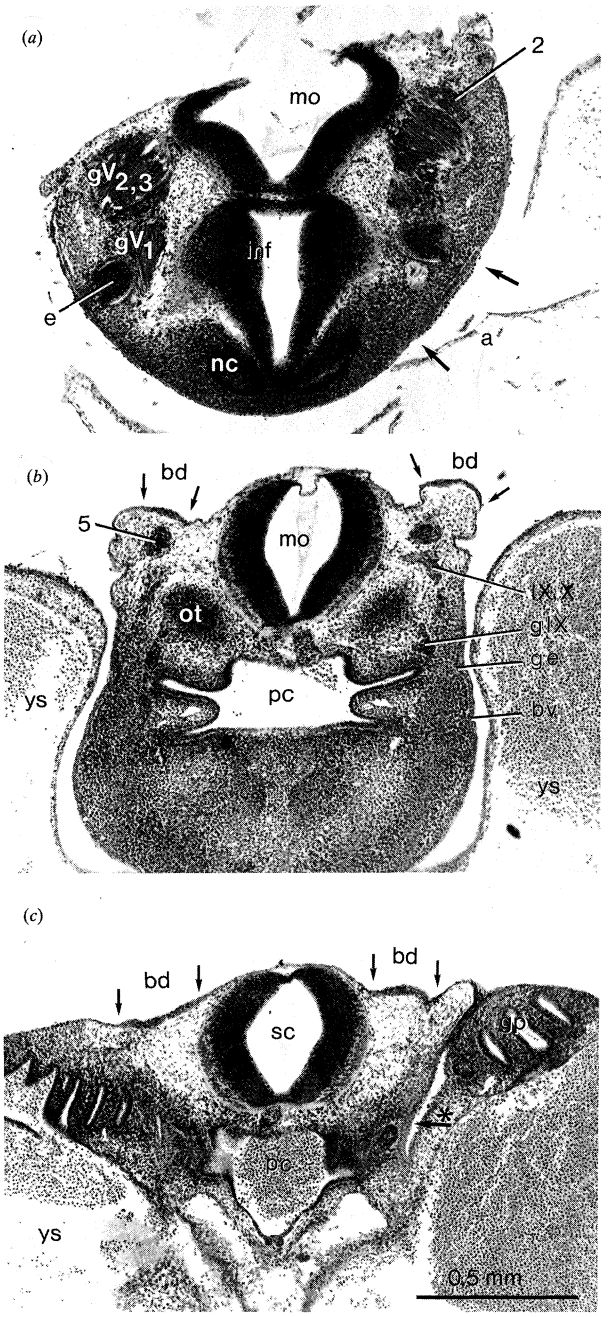

Figure 5. Low-power photomicrographs of transverse sections from embryo number 2337. The scale bar in the lower right corner applies to all frames. (*a*) Section through the anterior part of the head at the level of the eye-cups on both sides. Arrows labelled 'a' point to an ectodermal specialization ventral to the eye, which is shown at higher magnification in figure 6*a*. The ganglion of nervus lateralis a is visible as a distinct unit (labelled '2') dorsal to the trigeminal complex. (*b*) Section through the otic region, at the level of the posterior pole of the otic capsule. Focal ectodermal specializations labelled 'bd' are visible dorsal to the otic capsule; directly underneath those specializations, the proximal ganglion of nervus lateralis b (labelled '5') can be seen. These structures are shown at higher magnification in figure 6*c*. On the right side the glossopharnygeal epibranchial placode (ge) can be seen as a thickening in the ectoderm laterally adjacent to the second (glossopharyngeal) branchial pouch; a condensation of cells dorsal to that pouch (gIX) represents the glossopharyngeal epibranchial ganglion. In an adjacent section, a string of cells connects the ganglion and the placode. Another ectodermal specialization, labelled 'bv', is barely visible at this magnification ventral to the glossopharyngeal epibranchial placode. This structure is shown at higher power in figure 6*b*. (*c*) Section through the region of the gill plate. Ectodermal specializations labelled 'bd' are visible laterally adjacent to the neural tube. On the right side, there is a deep fold in the ectoderm which separates the gill plate from the side walls of the developing head. In the region that is marked by an arrow and an asterisk, i.e. the region lateral to the third branchial pouch, the ectoderm contains additional specializations that may be part of a series of vagal epibranchial placodes. There is no distinct basal lamina in this region; however, the underlying mesenchyme is too dense, and the staining differences between ectodermal and mesenchymal cells too small, to allow a clear-cut delineation of placodes.

be identified, however, we could not identify the sensory ganglia of the spinooccipital nerves.

A number of focal ectodermal specializations can be observed in the head. The general ectoderm consists of a two-layered epithelium with a cuboidal inner layer and a flat outer layer (for examples, see the regions above the upper arrow and below the lower arrow in figure 6*a*), and the basal lamina is barely visible. In the ectoderm immediately adjacent to the developing eye (e), a distinct cone-like projection of ectodermal cells (l) is directed towards the eye-cup (see figure 6*d*) and thus represents the lens placode (even though adult hagfishes do not possess a lens, see §5). The ectoderm lateral to the first (facial) and second (glossopharyngeal) pharyngeal pouches is also thickened (labelled 'fe' and 'ge', see also figure 5*b*); in the case of the glossopharyngeal pouch, a string of cells connects the thickening to a developing ganglion (glX) dorsal to the pouch itself (the ganglion and the thickening, but not the connecting string of cells, are visible in figure 5*b*). It is possible that other thickenings and ganglia occur lateral to the remaining pharyngeal pouches, but we were unable to definitely identify them, as it was impossible to differentiate between ectodermal and mesenchymal cells in those regions (see arrow with asterisk in figure 5*c*).

The proximal ganglion of nervus lateralis a (labelled '2') could be identified based on its position between the $V_{2,3}$ ganglion ($gV_{2,3}$) and the utricular ganglion (gu, see also figure 5*a*). The cells in that ganglion have already established contact with the alar plate of the medulla, and a short peripheral root of nervus lateralis a (labelled '1') can also be found. In the skin ventral to the eye (e) and the lens placode (l) i.e. in the infraocular region but still rostral to the future oral cleft, there is another region of specialized ectoderm (labelled 'a'; region between arrows in figures 5*a* and 6*a*) where the cells of the inner ectodermal layer are columnar in shape and arranged in two to three layers.

The proximal ganglion of nervus lateralis b (labelled '5', see also figures 5*b* and 6*c*) lies dorsal to the otic capsule (ot), caudal to the saccular ganglion (gs), and rostral to the combined root of the glossopharyngeal/vagal nerves (IX, X). We could see the proximal neurites of the ganglion cells enter the alar plate of the medulla, however neither the peripheral neurites nor the peripheral ganglion of nervus lateralis b could be detected. A large epidermal specialization is present in

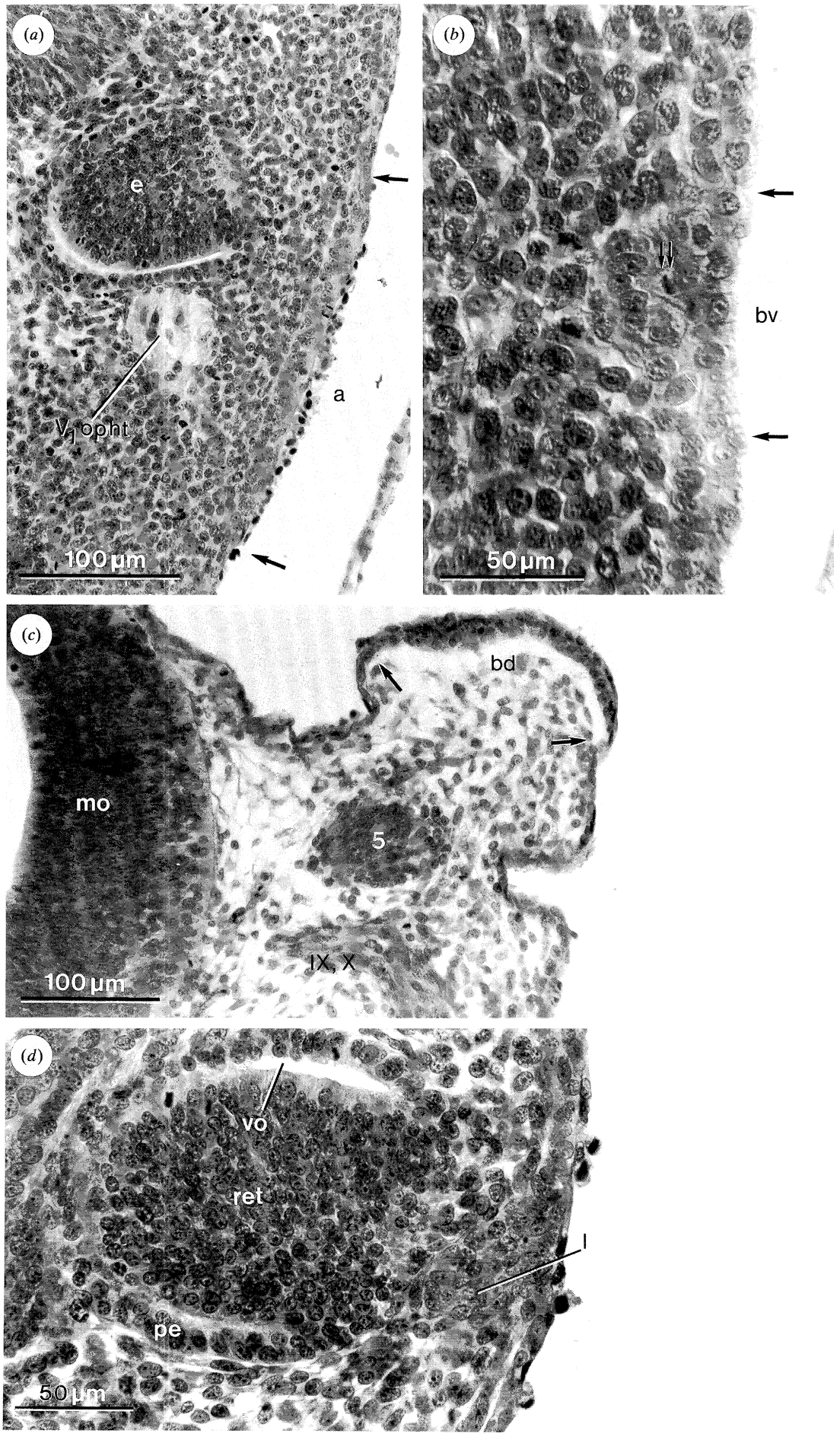

Figure 6. For description see opposite.

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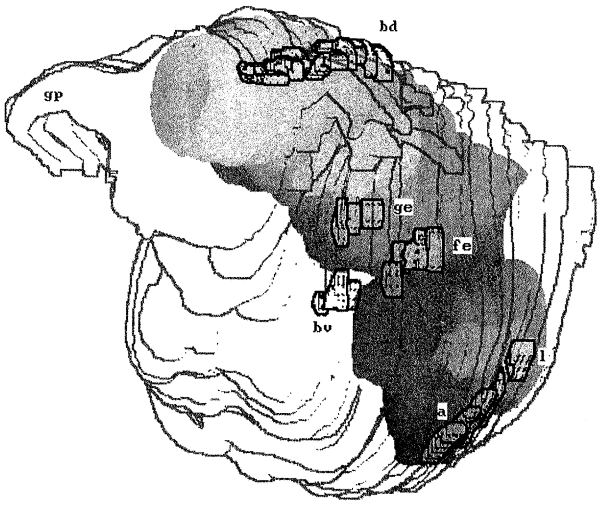

Figure 7. Example of a computer-generated three-dimensional reconstruction of the head of embryo number 2337. This figure is a computer screen print-out, from a perspective posterior and lateral to the right side of the head. Computer-generated images like this one were used as templates for the ink-drawings in figures 4 and 8. Grey lines outline the head, and the neural tube is visible as a shaded grey structure in the background. The highlighted areas in the ectoderm represent the positions of focal ectodermal specializations. The outlines of many structures present in the data base used for the reconstruction (pharynx, cranial nerves and ganglia etc., see subsequent figure 8) are not shown in this print-out. The programme allows all structures to be displayed simultaneously by different colours on the screen, but this is not possible in a black and white print-out.

the region dorsal and posterior to the otic capsule (labelled 'bd'; see also figures 5*b*, *c* and 6*c*); again, this region differs from the surrounding general ectoderm in having an increased thickness of the inner ectodermal layer (region between arrows in figure 6*c*). Finally, there is another specialization in the ectoderm ventral to the developing pharyngeal pouches, about halfway between the second (glossopharyngeal) and third branchial pouch (labelled 'bv'; see also figures 5*b* and 6*b*). In this region, a cone-like group of ectodermal cells, containing occasional mitotic figures, projects into the underlying mesenchyme (double arrows in figure 6*b*).

5. DISCUSSION

In the first part of the discussion, we will deal with the development and phylogenetic significance of the lens placode. The second part will focus on the developmental fate and the homology of lateral line placodes in hagfishes. Finally, in the third part, we will put our observations on the lateral line system into an evolutionary context.

(*a*) *The lens placode*

The presence of a lens placode in early embryos was first noticed by Price (1896), von Kupffer (1900), and Stockard (1907). The eyes of late embryos and adult hagfishes do not possess lenses, however, and they also lack an iris, external eye muscles, and the accompanying nerves. How does one interpret this peculiar combination of embryonic and adult characters? It would be obvious to speculate that it is a degenerative or apomorphic character combination i.e. that the ancestors of modern hagfishes possessed lenses which were lost in their descendants. Under these circumstances, the lens placode may be retained due to the inductive forces of the early eye-cup onto the ectoderm. Alternatively, this condition might be primitive or plesiomorphic, i.e. lens placodes that do not develop into lenses predate lens placodes that do phylogenetically. The latter assumption may seem unlikely, but it is supported by two lines of evidence. First, the function of the lens placode is not only to produce a lens; the lens placode itself may also serve as an inductor for normal development of the retinal pigment epithelium, chorioidea, and sclera (Tripathi *et al.* 1991). In eptatretid hagfishes, the lens placode may also participate in the formation of the 'cornea' i.e. the opaque, unpigmented patch of skin that covers the eye. As seen in figure 1*c* and mentioned in its legend, there are no pigment cells underneath the skin grooves of adult hagfishes, and the skin grooves presumably also derive from placodes (see §5*b*). Therefore, one of the functions (and perhaps even the initial and plesiomorphic function) of the lens placode in hagfishes may be the prevention of skin pigmentation above the eye-cup. Thus, the inductive role of the lens could predate its visual, light-refractive role in phylogeny. Secondly, there are well-documented cases of degenerative evolution of the eyes among caecilians (limbless, burrowing amphibians). In these animals, the lens is one of the last characters to disappear, and it is lacking only in species with extrmely small eyes that are hidden under layers of muscle and bone (Wake 1985). Species that possess eyes comparable to those of *Eptatretus*, with respect to subcutaneous location and relative size, inevitably possess lenses. Similarly, the eyes of mole-rats, which also show varying degrees of degeneration, retain their lenses even in cases of extreme reduction of

Figure 6. High-power photomicrographs of transverse sections through embryo number 2337. (*a*) Section through the right eye-cup, a detail from the same section shown in figure 5*a*. Note the ectodermal specialization between arrows labelled 'a'. The ectoderm is thickened because the inner layer is multistratified and columnar, thus resembling a sensory ridge. The black granules covering the ridge are yolk platelets. (*b*) Detail from the section depicted in figure 5*b*, showing an ectodermal thickening ('bv') that occurs ventral to the glossopharyngeal epibranchial placode. A cone-like arrangement of ectodermal cells can easily be distinguished from the dense mesenchyme; the small double arrows point to a mitotic figure within that cone. This structure probably is an early placode. (*c*) Detail from the section in figure 5*c*, showing the ectodermal specialization labelled 'bd', which resembles a sensory ridge, and the adjacent proximal ganglion of nervus lateralis b (labelled '5'). (*d*) Detail from a section slightly anterior to that depicted in figure 5*a*, showing the eye-cup and the distinct lens placode (l).

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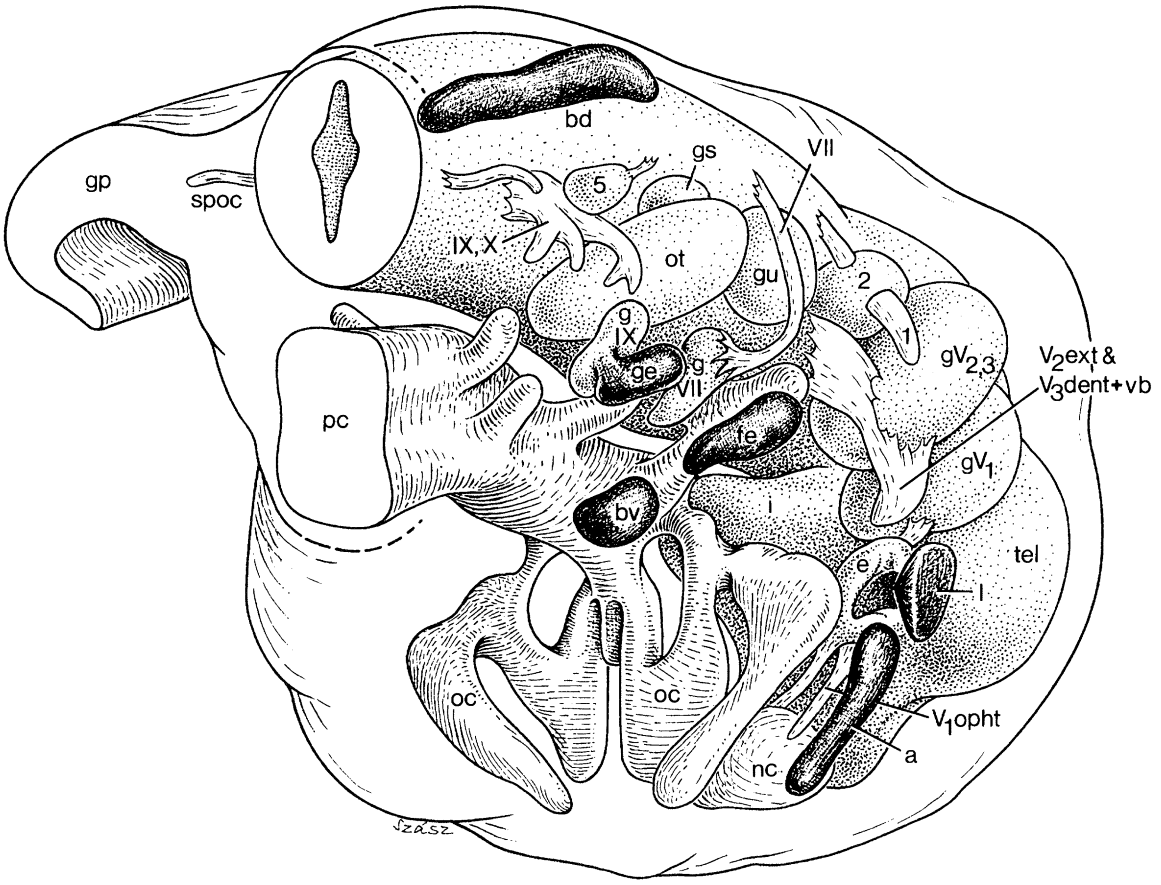

Figure 8. Three-dimensional reconstruction of the head of embryo number 2337. Orientation is the same as in figure 7. Blackened areas represent the positions of focal ectodermal specializations. This figure is described in detail in the text.

the eyes (Hanke 1900; Sanyal *et al.* 1990). Thus, by analogy, one would not expect the complete reduction of the lens in *Eptatretus* if this were a case of degenerative evolution. (For additional arguments, both for and against, see Fernholm 1975; Wicht & Northcutt 1990).

Unfortunately, the fossil record is of no help with respect to the evolution of the lens. The only known fossil hagfish (*Myxinikela siroka* from the Upper Carboniferous (Bardack 1991)) has eyes that are comparable in size to the eyes of modern eptatretid hagfishes, but there is no evidence for either presence or absence of a lens. Similarly, the presence or absence of a lens cannot be determined for the recently discovered fossil remnants of the conodont *Clydagnathus sp.* (from the Carboniferous) which is presumed to have been closely related to modern myxinoids and which appears to have possessed very large eyes (Aldridge *et al.* 1993). Heterostracans, another group of fossil jawless fishes related to hagfishes (Janvier & Blieck 1979) did not possess, in all likelihood, external eye-muscles (Janvier 1975), and this may indicate that the absence of these muscles is a primitive feature for craniates. Again, the heterostracan fossils yield no information about the lens.

Similar problems occur in interpreting the developmental and adult states of the lateral line system (see §5*b*). Ultimately, the choice between the competing hypotheses (degenerative evolution *versus* retention of primitive characters) should be based on an out-group comparison with the sister-group of craniates (Hennig 1966); unfortunately, the members of the sister-group (the cephalochordates, see §5*b*) do not display any of the characters under consideration. Therefore, to determine which hypothesis is most likely to be valid, we need more information on the embryology of the visual system in hagfishes plus, with luck, a well preserved fossil in which the presence or absence of a lens can be established.

(*b*) *Development and homology of the lateral line system of* Eptatretus stouti

The scarcity of embryonic material, and the poor state of preservation of many of the embryos in the Dean–Conel collection, preclude complete documentation of the development of the placodal systems in general and the lateral line system in particular. Thus, some deductive reasoning is needed to reconstruct the ontogeny of the lateral line system. Our deductions are based on the assumption that the developmental sequence of head lateral line placodes described for gnathostomes (Northcutt 1992*a*; Northcutt *et al.* 1994; see first paragraph of §1 for a summary) also applies to other craniate taxa, including hagfishes. This assumption also implies that the focal ectodermal specializations in hagfishes are homologous to placodes, or their derivatives, in other craniates. A number of arguments support the validity of these assumptions.

1. The focal ectodermal specializations seen in hagfishes resemble placodes, or their derivatives, in

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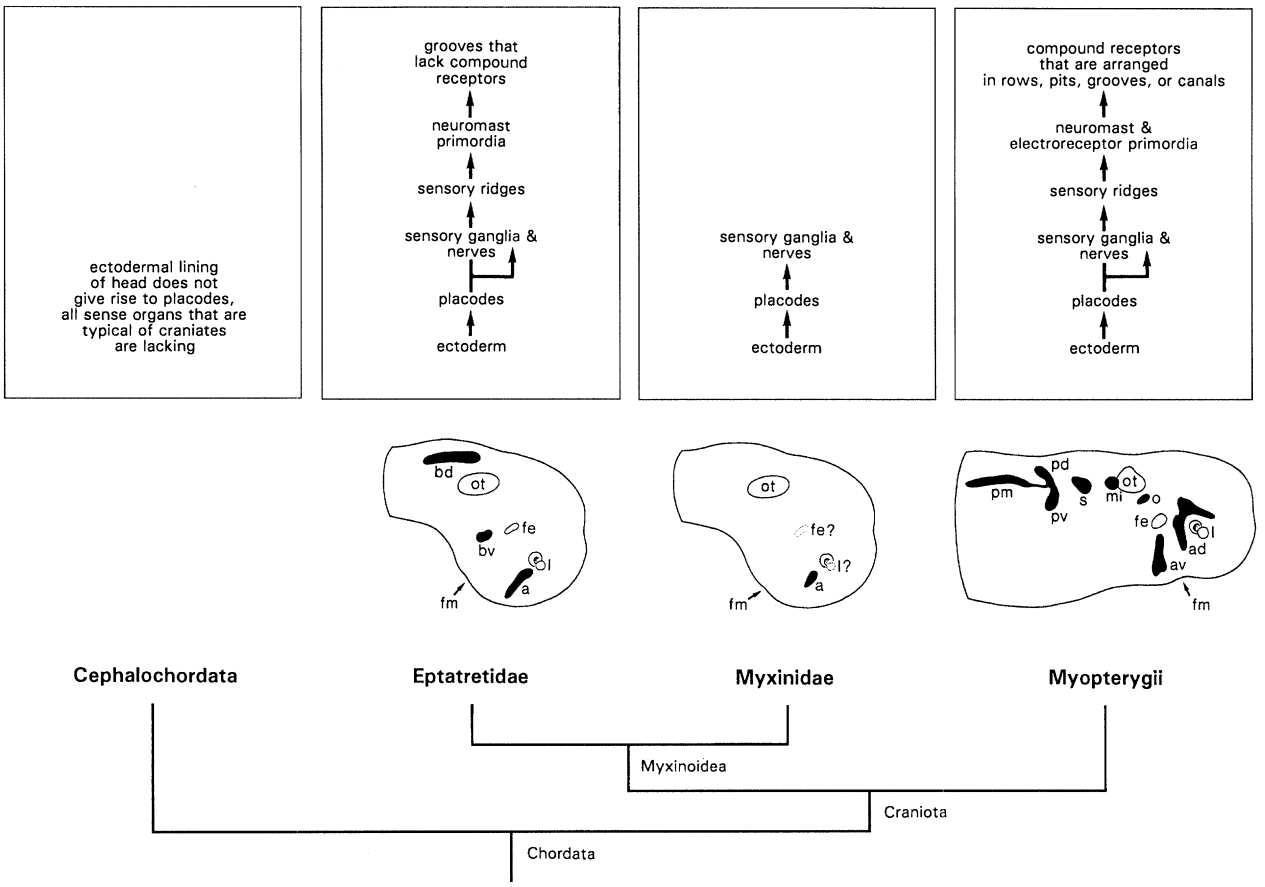

Figure 9. A cladogram (see at the bottom) of the relationships of the major Recent chordate taxa, based on the cladistic analyses of Løvtrup (1977), Janvier & Blieck (1979), Janvier (1981), Jefferies (1986) and Aldridge *et al.* (1993). Note that the taxon Myopterygii, which comprises lampreys and gnathostomes, includes all Recent craniates except hagfishes. The middle part of the figure shows developing heads, in lateral view, of the taxa named underneath. The positions of lateral line placodes/sensory ridges are symbolized by black areas. The positions of the otic capsule, the facial epibranchial placode, the lens placode, and the position of the future mouth are also indicated. Cephalochordates do not display any ectodermal specializations during development (Willey 1894), thus a diagrammatic view of their embryonic 'head' has not been included. The pattern shown for eptatretid hagfishes is described in the text. The pattern shown for myxinid hagfishes is somewhat hypothetical but can be deduced from observations of adult and embryonic specimens of *Myxine glutinosa* (see §5). The facial epibranchial and lens placodes are shown stippled, because it is not clear whether they occur in Myxinidae. The placodal pattern shown for myopterygians is presumably plesiomorphic for that group (Northcutt 1992*a*; Northcutt *et al.* 1994) and has been projected onto the outlines of a urodele amphibian embryo. It should be noted that the otic lateral line placode does not occur in urodeles; it is depicted here since it appears to be a primitive component of the placodal pattern in myopterygians. The boxes on top of the figure contain descriptions of the developmental fate of lateral line placodes in the individual taxa. Again, the data for eptatretid hagfishes stem from the present paper, the data for Myxinidae are deduced (see §5), and the description of the myopterygian sequence is from Northcutt (1992*a*) and Northcutt *et al.* (1994).

other craniates histologically, particularly with respect to the thickening of the inner ectodermal layer.

2. The ectodermal specializations appear in positions that are typical for placodes, or their derivatives, in the heads of other craniates. This is particularly evident for the lens placode (see above), but it is also evident for the structures above the pharyngeal pouches in embryo number 2337, which correspond to epibranchial placodes in all respects. Similarly, the ectodermal specializations labelled 'a', 'bd', and 'bv' are interpreted as lateral line placodes, since they occur in positions comparable to those of lateral line placodes in other craniates (see below).

3. As evidenced by the glossopharyngeal placode in embryo number 2337, which appears to give rise to a ganglion, ectodermal specializations in hagfishes are likely sources of neuronal material, as is true in other craniates. Similarly, the bv-placode in embryo number 2357 appears to be a source of migrating, potentially ganglion-forming cells.

4. In embryo number 2357, promordia of sensory structures can be seen to develop within regions that display ectodermal specializations. Again, this feature is typical of placodal development in the heads of other craniates, where sensory primordia develop from elongated sensory ridges, which, in turn, arise from placodes (Northcutt *et al.* 1994).

Based on these observations and assumptions, the following developmental sequence for the lateral line system of hagfishes can be deduced. The nervus lateralis a and its ganglion may arise from a placode located posterior and ventral to the eye. This placode

probably then transforms into a sensory ridge (labelled 'a' in embryo number 2337), which gives rise to the neuromast primordia in the infraocular region (as seen in embryo number 2357). Later, due to the shift of the external openings of the mouth and nasal duct from a ventral to an anterior position, the primordia are displaced dorsally and anteriorly and may thus be the precursors of the preocular set of grooves. Judging from the position of the sensory ridge, and from the topography of the nerve and ganglion in adult hagfishes, the entire complex of nervus lateralis a may be homologous to the derivatives of the ventral arm of the anterodorsal placode (Northcutt *et al.* 1994) in other craniates (see figure 9 for a comparison of the placodal pattern in hagfishes and myopterygians). Similarly, a placode dorsal to the otic capsule may give rise to the proximal ganglion of the nervus lateralis b. It may then transform into the elongated sensory ridge labelled 'bd' in embryo number 2337. This ridge could give rise to the supraotic neuromast primordia, as seen in embryo number 2357. These neuromast promordia, again, could be the precursors of the dorsal, transversely oriented set of postocular grooves in adults. It is difficult to recognize a definite homologue of this placode in other craniates, but the midele or supratemporal placodes (see Northcutt 1992*a*, *b*; Northcutt *et al.* 1994) are potential candidates (see figure 9). The small placode ('bv') that occurs ventral to the epibranchial placodes in embryo number 2337 appears to develop later than the other lateral line placodes. If our interpretation is correct, ganglion cells migrate from this placode in the late (number 2357) embryo stage, at which time the other placodes have long since disappeared, having given rise to ganglia, transformed into sensory ridges, and begun to form neuromast primordia. Judging from its position, the bv-placode could be the precursor of the ventral, longitudinally oriented set of postocular grooves. The relative delay in the development of this placode may be the reason for the presence of a *distal* ganglion in nervus lateralis b, as at the time the ganglion cells are formed, the connective tissue in the head has already lost its mesenchymal character, and the general body fascia is relatively well developed (see figure 3*b*). It is possible that the migrating ganglion cells cannot cross this connective tissue barrier and need to remain in a distal position. Based on the initial position of the placode, ventral to the epibranchial series of placodes, it is a possible homologue of the anteroventral placode (see Northcutt 1992*a*, *b*; Northcutt *et al.* 1994) in other craniates (see figure 9). If this interpretation is valid, this would also imply that nervus lateralis b is not a natural unit, as it arises from two different placodes and supplies two receptive fields that also have a dual embryonic origin.

Our interpretations are in accord with the early observations of von Kupffer (1900) who also reported three lateral line placodes in similar positions; they do not, however, confirm von Kupffer's claim with regard to the innervation of the receptor primordia that derive from these placodes. Von Kupffer claimed that these receptor primordia were innervated by rami of the trigeminal, facial, and vagal nerves. Although we could not actually trace the peripheral branches of any of the lateral line nerves to the receptor primordia, we found that the sensory ganglia associated with those nerves develop independently from those of other cranial nerves, and the ganglia and nerves maintain that independence until adulthood. Thus, our data emphasize the concept of lateral line nerves as structures that are primarily independent from other cranial nerves and should be regarded as a set of nerves in their own right (also see Cole 1896; Worthington 1905; Northcutt 1989; Song & Northcutt 1991; Northcutt 1992*a*, *b*; Northcutt *et al.* 1994).

There is one potential error in our interpretation that needs to be addressed; it relates to the transformation of the embryonic neuromast primordia into the adult skin grooves, a transformation we did not actually observe. The oldest embryos in the Dean–Conel collection display neuromast primordia; the youngest adults (about 100 mm long) we have seen display skin grooves. We have tried to fill other gaps in information by deduction from known developmental sequences in other taxa, however neuromast-groove transformations do not occur in other craniates. Our notion that the neuromast primordia in hagfishes transform into the skin grooves is thus conjectural but is, nevertheless, supported by topological correspondence between the embryonic structures and the adult grooves and the fact that they are innervated by cranial nerves that resemble lateral line nerves in many respects (Kishida *et al.* 1987; Braun *et al.* 1993).

(*c*) *Functional and phylogenetic problems*

The function of the entire lateral line system of *Eptatretus* remains enigmatic. There is no electrophysiological or anatomical evidence for electroreception (Bullock *et al.* 1983), and the fact that the adult grooves do not contain neuromasts seems to rule out a 'traditional' (i.e. neuromast-based) mechanoreceptive role. Yet the grooves are innervated (Braun *et al.* 1993), and instead of compound neuromasts, they may contain single-celled (mechano-?) receptors. Further speculation on the function of the grooves should await more extensive investigations, however.

In order to establish the phylogenetic polarity of the character sequence in *Eptatretus*, it is necessary to perform out-group comparisons among the major chordate taxa (see figure 9). In this context, it should be noted that adults of the second family of hagfishes, the Myxinidae, do not possess skin grooves. Only three late embryos of Myxinidae (*Myxine glutinosa*) have ever been found (Holmgren 1946; Fernholm 1969). The youngest of these embryos is comparable to our late embryo (number 2357), and Holmgren (1946) did not observe neuromast primordia in its skin. Nevertheless, *Myxine glutinosa* does possess at least one cranial nerve that may correspond to a lateral line nerve, the so-called nervus buccalis (Jansen 1930; Lindström 1949), which is homologous to nervus lateralis a in *Eptatretus stouti*. It is therefore plausible to assume that this nerve and its ganglion also develop from a placode similar to the 'a' placode in eptatretid hagfishes, but this placode apparently does not give rise to any ectodermal specializations in the adults.

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An out-group comparison (see figure 9) of the two myxinoid taxa (i.e. Myxinidae and Eptatretidae) with their sister group (the Myopterygii, i.e. lampreys and gnathostomes) suggests a truncation of the developmental sequence in Myxinidae, thus the sequence seen in Eptatretidae appears to be the plesiomorphic one for hagfishes in general.

Which sequence is plesiomorphic for craniates (i.e. hagfishes, lampreys, and gnathostomes), however? Unfortunately, the members of the sister group of craniates, the cephalochordates, do not display any of these characters (Willey 1894), nor do more remotely related taxa such as urochordates and enteropneusts, thus, an out-group analysis cannot be applied. Since hypotheses regarding ancestral ontogenies can be derived only from ontogenetic data from extant taxa, there are only two possibilities: either the developmental sequence seen in eptatretid hagfishes, or the sequence seen in myopterygians, represents the plesiomorphic craniate pattern. We think that the latter case is more likely for three reasons.

1. As discussed above, comparison of different families of hagfishes reveals a trend towards truncation of lateral line development in myxinid hagfishes, compared to that in eptatretid hagfishes. This trend might also affect the taxon Myxinoidea, as a whole, and degenerative evolution of the lateral line system would have resulted.

2. This interpretation is further supported by the fact that there is considerable variation in the number and shape of the grooves in eptatretid hagfishes (Braun *et al*. 1993). This variation may result from the absence of selective pressures that would otherwise stabilize the system and may also, thus reflect degenerative evolution.

3. It is difficult to understand why primordia of compound receptors should form and later disintegrate in any case other than degenerative evolution, although, like the lens placode, neuromast primordia may have functions (e.g. inductive ones) other than to become neuromasts, and these functions may be phylogenetically older than their sensory functions.

4. The fossil evidence from heterostracans, osteostracans, anaspids, and placoderms (summarized in Northcutt 1992*a*) indicates that the presence of typical lateral line receptors and, in some cases, even canals, is an ancient craniate character which arose prior to the origin of gnathostomes.

In summary, the following conclusions can be drawn from the present data: (i) the presence of a lateral line system is plesiomorphic for hagfishes and myopterygians, i.e. this system was present in the last common ancestor of all craniates; (ii) a developmental sequence that leads from placodes, *via* production of ganglion cells, to sensory ridges and to the formation of primordia of compound receptors is primitive for craniates; and (iii) with regard to the number of placodes, the presence of at least three lateral line placodes is primitive for craniates. As it is likely that the evolution of the lateral line system in hagfishes has been characterized by degeneration, even more placodes may have been present in the common ancestor of craniates.

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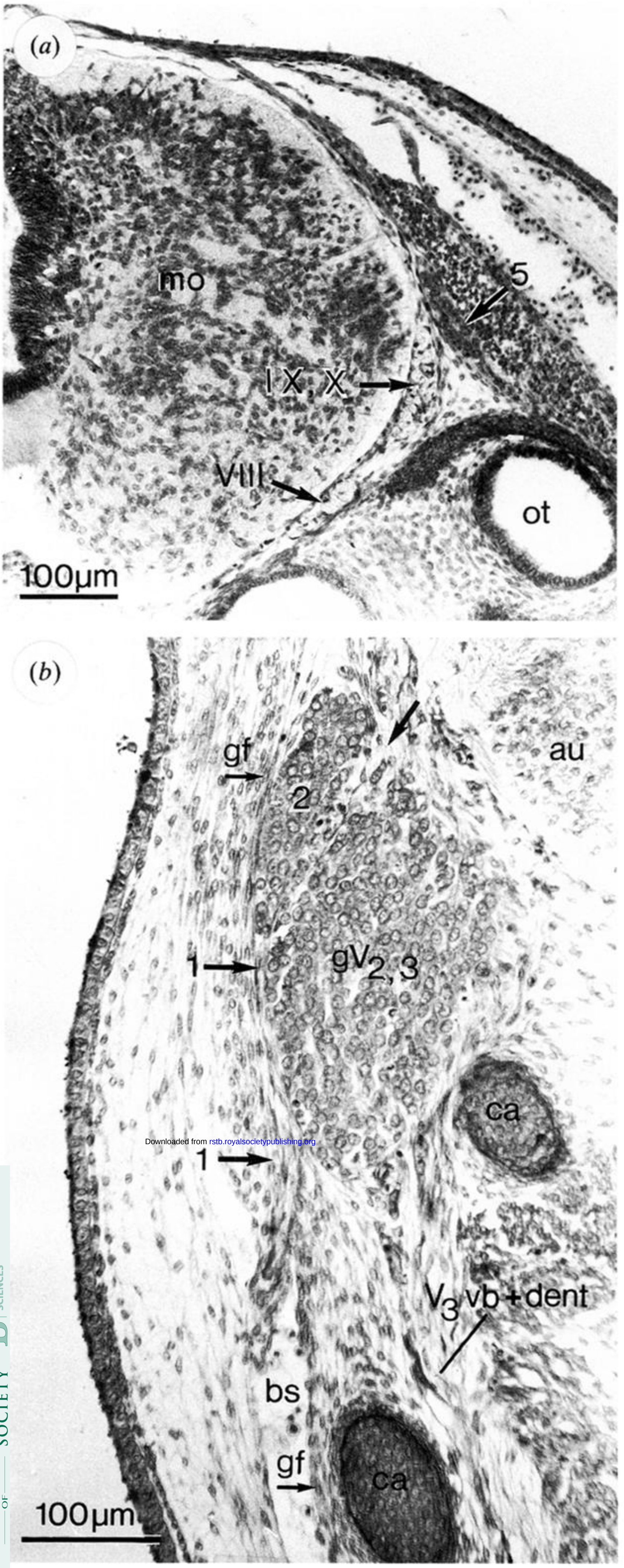
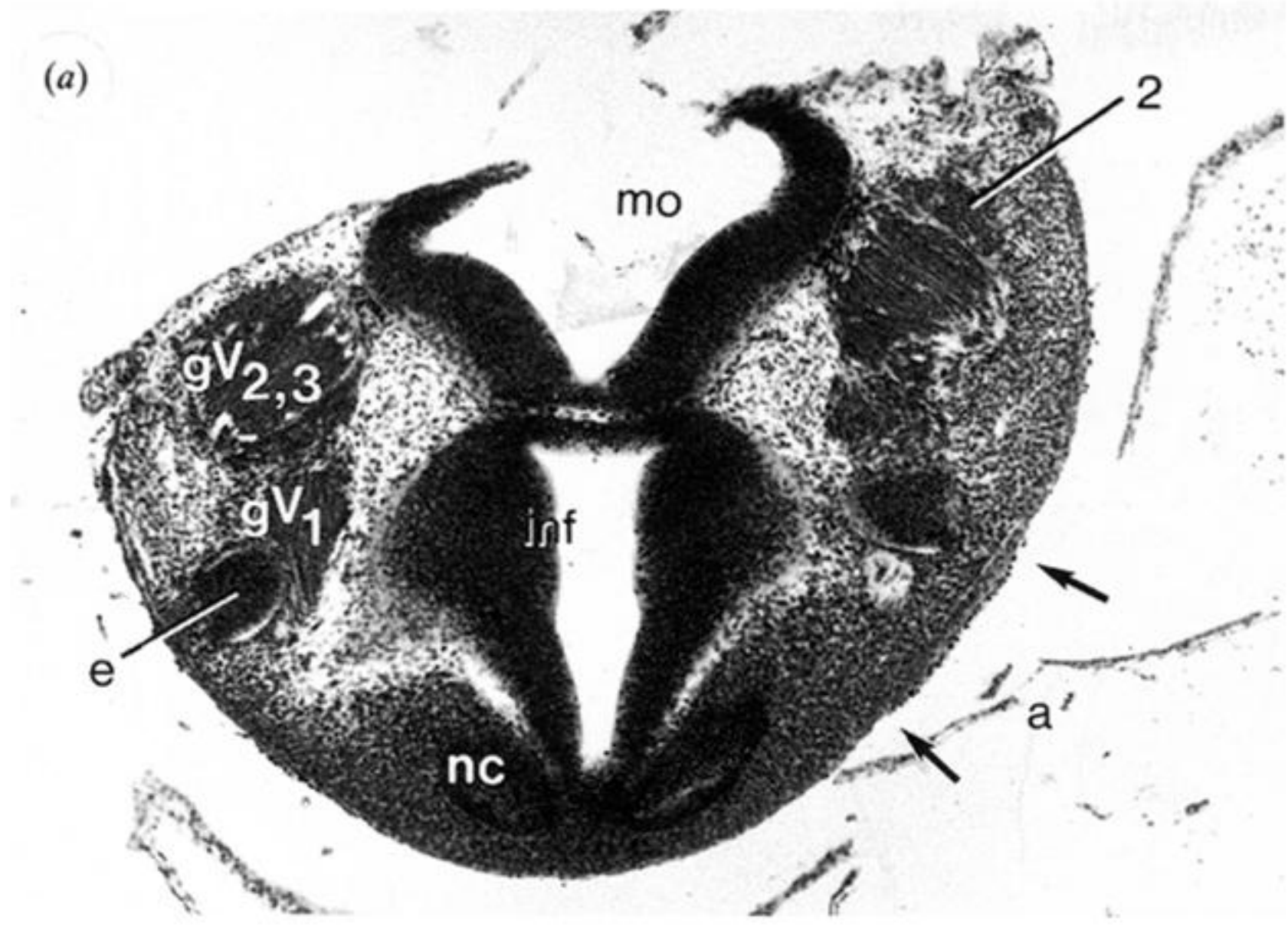
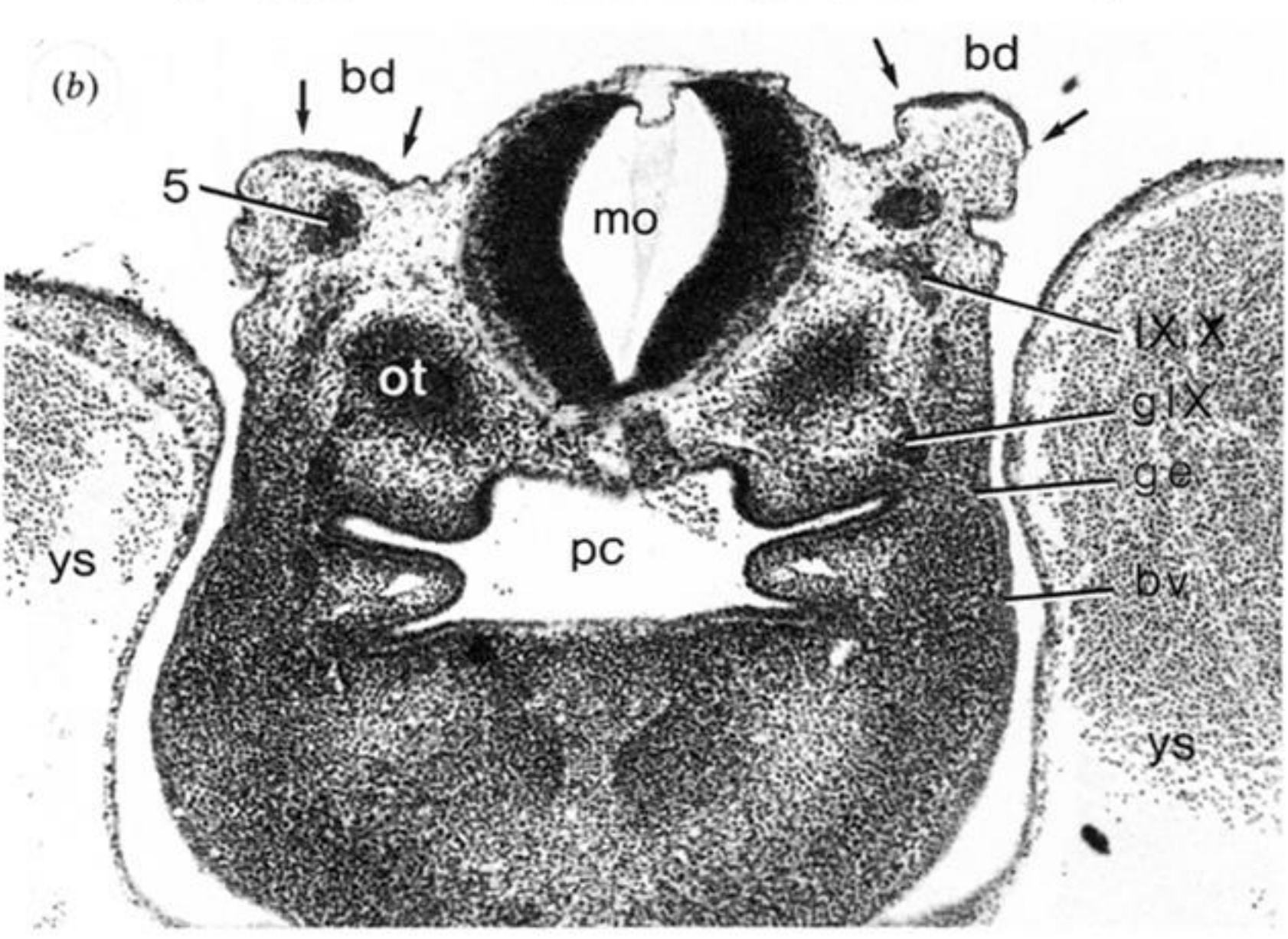
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Figure 2. For description see opposite.



igure 3. Photomicrographs of transverse sections from nbryo number 2357. (*a*) Section through the posterior part f the head at a level midway through the otic capsule. rrow labelled '5' points to the developing proximal anglion of nervus lateralis b medial to the otic capsule. (*b*) ection through the anterior part of the head, slightly osterior to the eye at a level through the ganglionic complex f the trigeminus. Note the developing ganglion of nervus teralis a (labelled '2') dorsal and lateral to the trigeminal anglion. Also note the thin sheath of connective tissue nlabelled arrow) that separates the ganglia. Arrows belled '1' point to nervus lateralis a, which is directed wards the infraocular skin.





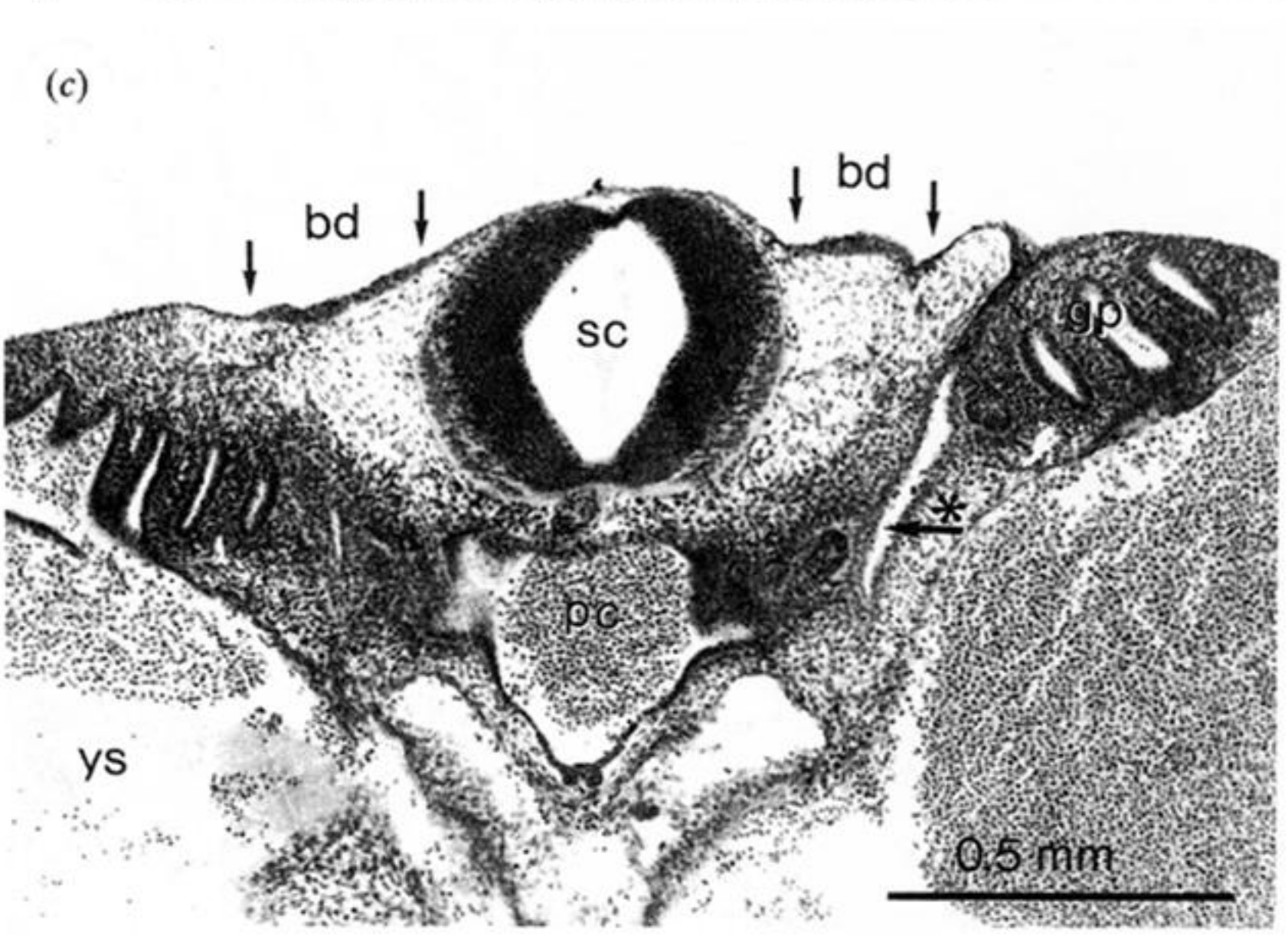


Figure 5. Low-power photomicrographs of transverse sections from embryo number 2337. The scale bar in the lower right corner applies to all frames. (*a*) Section through the anterior part of the head at the level of the eye-cups on both sides. Arrows labelled 'a' point to an ectodermal specialization ventral to the eye, which is shown at higher magnification in figure 6*a*. The ganglion of nervus lateralis a is visible as a distinct unit (labelled '2') dorsal to the trigeminal complex. (*b*) Section through the otic region, at the level of the posterior pole of the otic capsule. Focal ectodermal specializations labelled 'bd' are visible dorsal to the otic capsule; directly underneath those specializations, the proximal ganglion of nervus lateralis b (labelled '5') can be seen. These structures are shown at higher magnification in figure 6*b*. On the right side the glossopharnygeal epibranchial placode (ge) can be seen as a thickening in the ectoderm ventrolaterally adjacent to the second (glossopharyngeal) branchial pouch; a condensation of cells dorsal to that pouch (gIX) represents the glossopharyngeal epibranchial ganglion. In an adjacent section, a string of cells connects the ganglion and the placode. Another ectodermal specialization, labelled 'bv', is barely visible at this magnification ventral to the glossopharyngeal epibranchial placode. This structure is shown at higher power in figure 6*b*. (*c*) Section through the region of the gill plate. Ectodermal specializations labelled 'bd' are visible laterally adjacent to the neural tube. On the right side, there is a deep fold in the ectoderm which separates the gill plate from the side walls of the developing head. In the region that is marked by an arrow and an asterisk, i.e. the region lateral to the third branchial pouch, the ectoderm contains additional specializations that may be part of a series of vagal epibranchial placodes. There is no distinct basal lamina in this region; however, the underlying mesenchyme is too dense, and the staining differences between ectodermal and mesenchymal cells too small, to allow a clear-cut delineation of placodes.

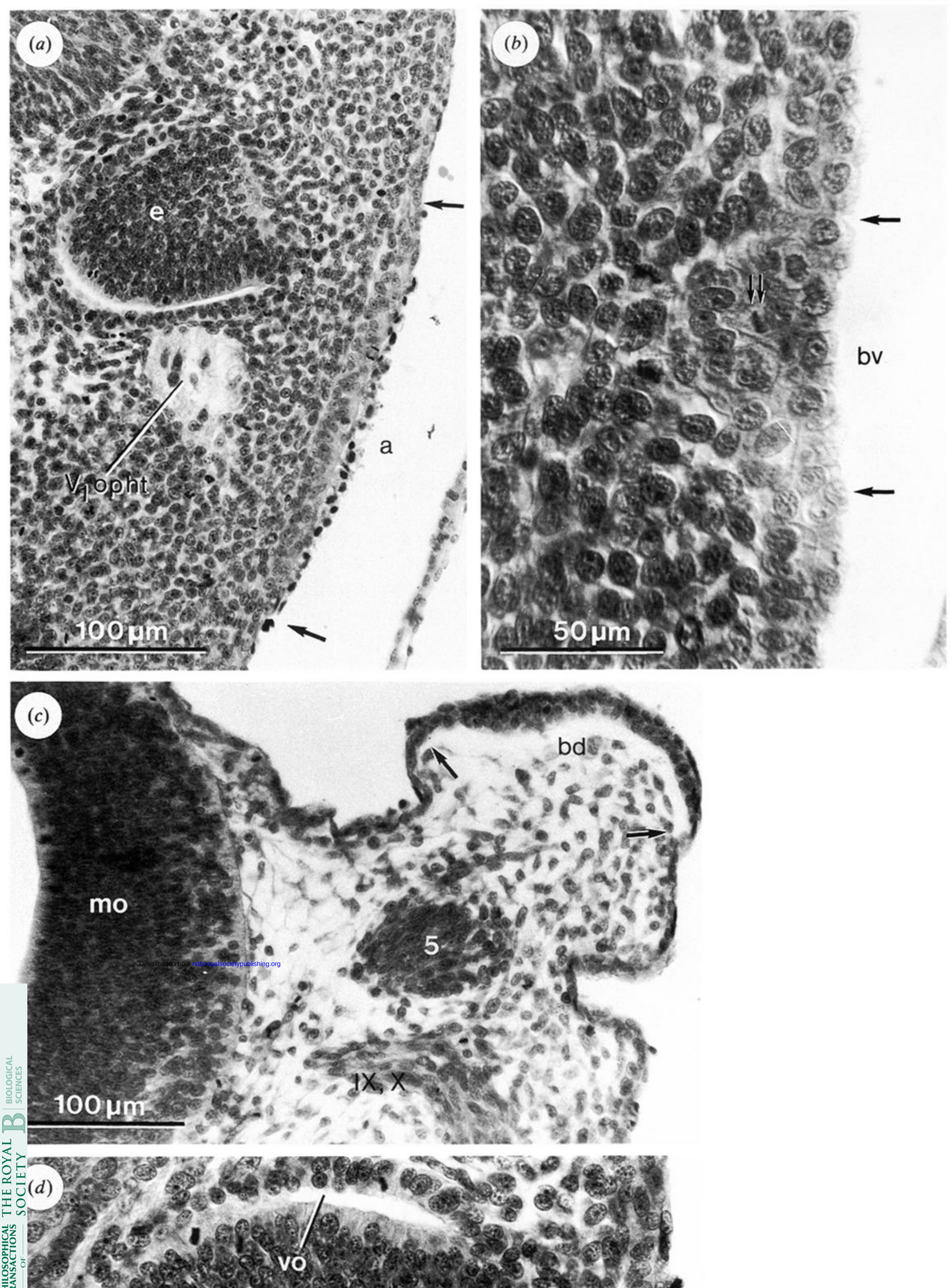
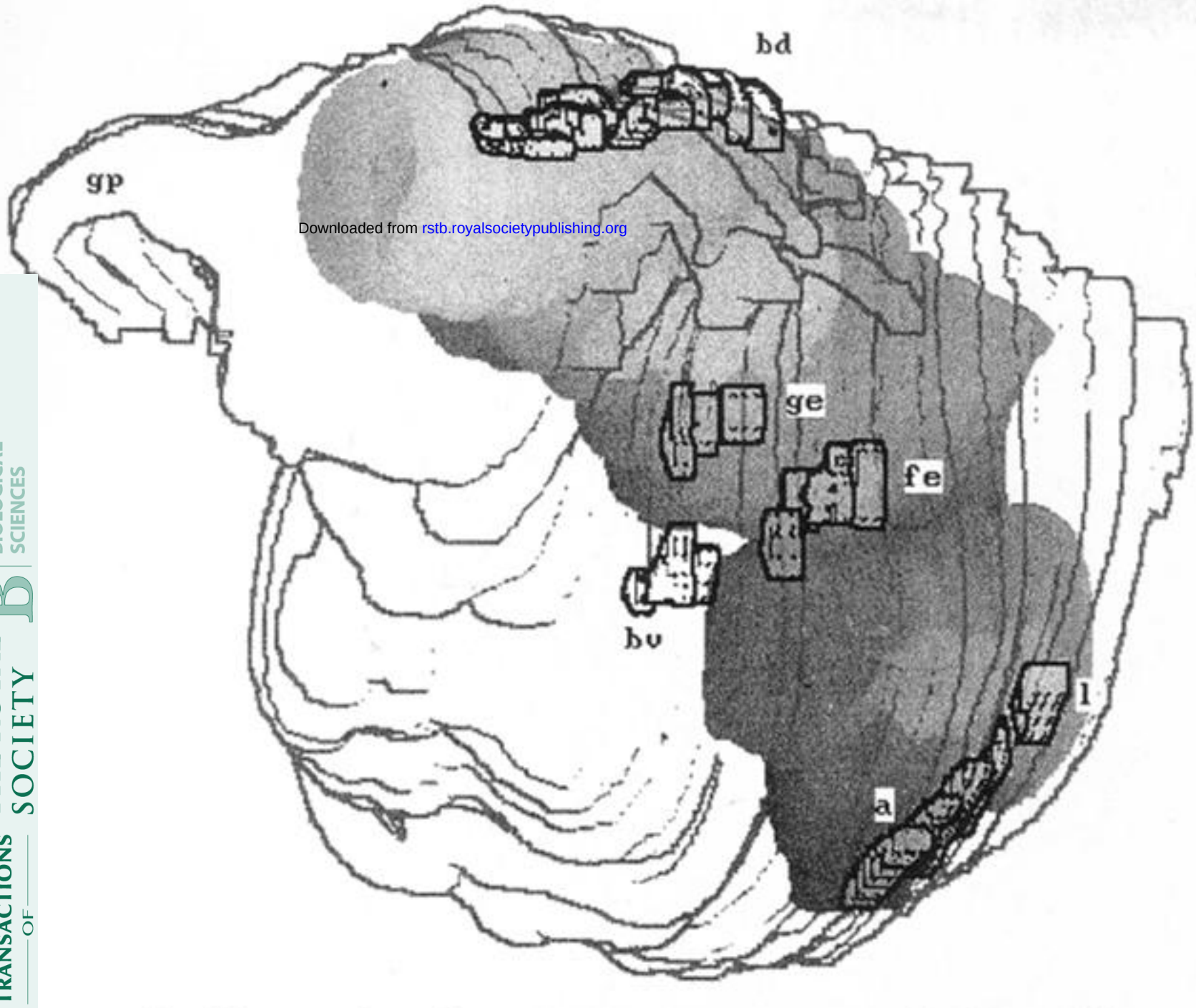


Figure 6. For description see opposite.



gure 7. Example of a computer-generated three-dimen-onal reconstruction of the head of embryo number 2337. his figure is a computer screen print-out, from a perspective osterior and lateral to the right side of the head. Computer-nerated images like this one were used as templates for the k-drawings in figures 4 and 8. Grey lines outline the head, id the neural tube is visible as a shaded grey structure in the ackground. The highlighted areas in the ectoderm represent e positions of focal ectodermal specializations. The outlines many structures present in the data base used for the construction (pharynx, cranial nerves and ganglia etc., see bsequent figure 8) are not shown in this print-out. The rogramme allows all structures to be displayed simul-neously by different colours on the screen, but this is not ossible in a black and white print-out.