# STRUCTURE AND GENERAL DISTRIBUTION OF FREE NEUROMASTS IN THE BLACK GOBY, *GOBIUS NIGER*

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(Figs. 1–5)

Nomarski, binocular and scanning electron microscopy were used to map the distribution and structure of free neuromast papillae in the black goby (*Gobius niger* (L.)). Neuromast papillae are arranged over the head in rows and columns as in most goby species. The papillae are fingerlike structures projecting from the surface of the head with neuromast organs on the tip of each projection. A viscous, rather than gelatinous, cupula envelops the upper portion of the papillae, thus covering the neuromast.

All neuromasts contain a number of hair cells of opposing polarities that make up a sensory plate. In every instance the neuromast sits on a diamond-shaped tissue stud or supportive plate. The individual polarities of hair cells give each row or column of free neuromasts an 'overall polarity' that is perpendicular to its axial orientation on the fishes head, viz., dorso-ventral in the horizontal rows and antero-posterior in the vertical columns. Where lines of organs become oblique, the overall polarity remains perpendicular to the direction the row or column has turned.

An exception to the above observations is found in one row of free neuromasts, the mandibular row. Here the size and shape of the papillae and cupulae differ from others seen. Most notably, however, the overall polarity of the row is along its length in an antero-posterior direction. This and other observations have led to the suggestion that the mandibular row is functionally different from the standard row and columns of neuromasts found on the head of *G. niger*. Furthermore, it is possible that this row may represent an adapted paedomorphic row of head canal neuromasts.

## INTRODUCTION

This paper on *Gobius niger* describes the ultrastructure of free neuromast papillae of both standard and mandibular types, emphasizing the polarity of the hair cells in each row or column. Evidence to suggest a functional difference between mandibular and standard neuromasts is reviewed, together with an hypothesis concerning the developmental origin of the mandibular row, relevant to the evolution of lateral line organs in general.

Much work has been done on the distribution of free neuromasts in gobies from a systematic viewpoint, but little is known of their fine structure. Neuromasts form a network of rows and columns over much of the head and provide the fish with information on local and general water movements. The network possibly coordinates accurate localization of small water disturbances, caused by swimming prey for example. Head canals are also present, making up an incomplete system with presumably some functional difference from the free neuromasts.

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An unusual row of free neuromasts occurs in the mandibular region and has been called the internal mandibular row by Miller & El-Tawil (1974). Here it is simply called the mandibular row and is sufficiently different in form from 'standard' free neuromasts to suggest a functional difference.

## METHODS

Specimens of *G. niger* were caught in bottom nets trawled near Plymouth and St Andrews.

### Scanning Electron Microscopy (SEM)

Fish were immediately killed by an overdose of the anaesthetic $MS_{222}$ and the heads removed and fixed in 5 % glutaraldehyde in cacodylate buffer. The skin was removed from the head, washed in buffer and transferred into 2 % osmium tetroxide in cacodylate buffer. After dehydrating in acetone, critical point drying and sputter coating with gold, portions of skin were examined in Plymouth by a JEOL JSM 35C SEM. Some portions of skin were treated in an ultrasonic bath at 80 kHz for 3–10 s to remove remnants of cupulae and leave cilia intact (Blaxter, Gray & Best, 1983). Ultrasonic treatment was continued to 15 seconds to remove cilia from the hair cells. This left clearly visible cilium stubs in the membrane of the hair cells.

### Neuromast mapping by binocular microscopy

Fish heads were put directly into 2 % osmium tetroxide in cacodylate buffer for 1 h, after which only the mucus layer and cupulae were stained. On peeling off the mucus the cupula of each organ was clearly visible against the unstained skin of the head.

### Papilla size and shape

The size and shape of neuromast papillae were revealed by using a Nomarski microscope to examine portions of skin taken from the head of the fish soon after death. Information gathered in such a way was backed up to some extent by SEM and binocular observations.

## RESULTS

### Distribution

The distribution of free-standing neuromast papillae was mapped after having fixed and stained a complete head in osmium tetroxide for one hour. The outer mucoid layer and cupulae were stained black by this brief exposure to osmium tetroxide, so, on peeling away the mucus, the cupulae were clearly visible, standing out on the neuromast papillae against the pale unstained skin. The position of the remaining head canals was also noted by dissection.

*G. niger* shows the characteristic row and column arrangement of free neuromasts found in most members of the suborder Gobioidei. Rows run horizontally in an approximately antero-posterior direction, while columns run approximately dorso-ventrally in a vertical direction. Both rows and columns are found spreading over the head from the snout as far back as the pectoral fins.

They are concentrated in the sub-orbital and pre-orbital regions, where in the latter of these two areas the papillae are often found in small clusters rather than rows or columns. A certain amount of variation is found in free neuromast arrangement between individuals, Fig. 1 being an exact drawing of one such individual. Such plasticity in patterns of free neuromasts within a species is well reported in *Pomatoschistus minutus* (L.) (Webb, 1980).

A row of unusual free neuromast papillae runs from almost the tip of the lower jaw round the pre-operculum up to the ventral opening of the remaining

pre-opercular head canal. In this position it apparently exactly corresponds to the mandibular part of the preoperculo-mandibular canal found in percoid fish and one primitive species of goby, *Rhyacichthys aspro* (L.) (Miller, 1973). This row of papillae is about 50 % broader than, the standard papillae, often shorter, and they possess broader, flatter cupulae (exact measurements shown in Fig. 2). They run along a mandibular groove or depression of varying width and depth (Fig. 1). Their cupulae frequently failed to stain using the method described to map free neuromast papillae, suggesting that they may not protrude much above the mucus secreted by the fish that tends to fill the groove. It may, however, be that on death the fish release extra mucus, giving a false impression of the thickness of the mucoid layer in life.
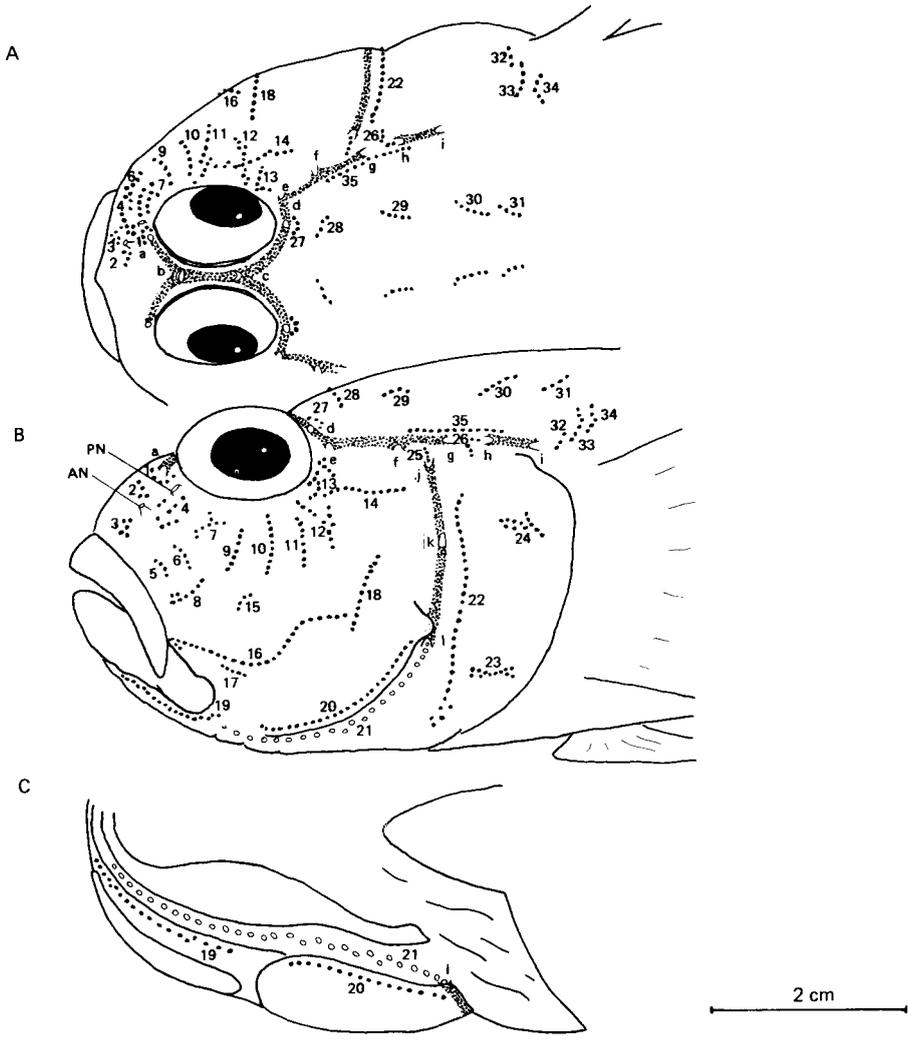


Fig. 1. Rows and columns of sensory papillae together with remaining canal systems of *G. niger*. (A) Dorsal view of head. (B) Lateral view, (C) Ventral view. M is the mandibular or internal mandibular row; AN, PN, anterior and posterior nostrils.

## Free neuromast papilla structure

The overall structure of standard and mandibular neuromast papillae of *G. niger* is shown in Fig. 2. The standard papillae are fairly elongate, forming fingerlike projections from the surface of the skin. A few of these finger-like papillae are bent over and lie parallel to the fish's head, the cupulae making contact with the surface of the head. Mandibular papillae are similar in shape, though more squat in appearance. The cupulae are viscous, rather than gelatinous, appearing rather like the viscid secretion of the sundew (*Drosera* sp.) as it adheres to and surrounds the tip of the sundew tentacles.
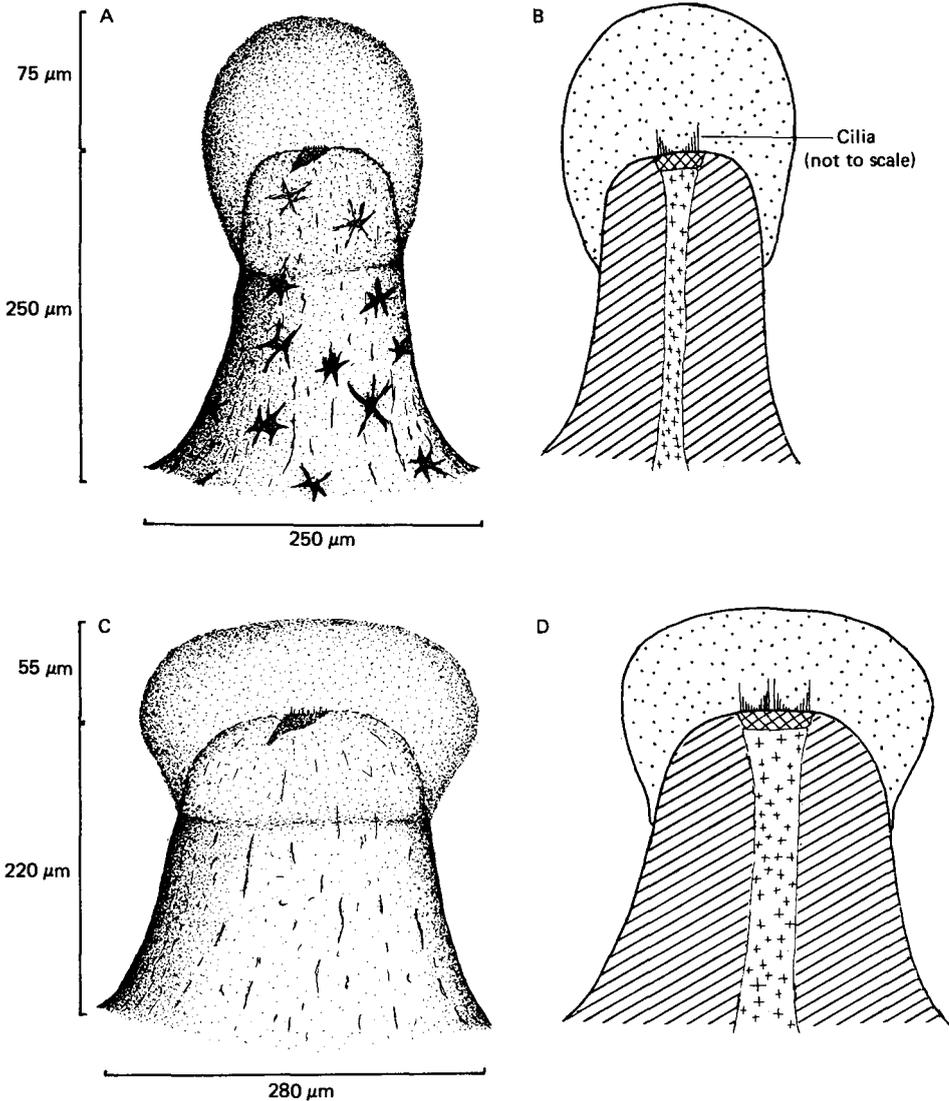
Fig. 2. The structure of standard (A), (B) and mandibular (C), (D) sensory papillae and cupulae. (B) and (D) are diagramatic cross sections of the papillae. ▨, Cupula; ▨, neuromast; ⊞, nerve; ▨, papilla.

Kuiper (1967) has suggested in his study of *Acerina cernua* (L.) that cupulae of teleost fish project into the zone of mucus at the surface of the fish. However, the long papillae of *G. niger* probably protrude beyond this layer and bring the cupulae into direct contact with the water. This is suggested by the staining of the cupulae of standard free neuromasts by osmium tetroxide as they protrude through the mucus layer.

These observations suggest a functional difference between the two types of free neuromasts found on *G. niger*, a view strengthened by observations related in the next two sections.

### Ultrastructure of free neuromasts and hair cell polarity

Free-standing neuromasts on *G. niger* are sited on the end of papillae, so lie about a quarter of a millimetre above the skin surface. SEM not only helped in revealing the size and form of these structures but clearly demonstrated the presence of hair cells in the neuromasts on their tip. The hair cells were examined in two conditions, (a) with the cilia present and 'cleaned' by a short exposure to ultrasound 3–10 s at 80 kHz (Fig. 3). This removed any bits of cupulae adhering to the cilia, (b) with the cilia themselves removed by a slightly longer exposure to ultrasound, 15 s at 80 kHz, in order to reveal the polarity of the hair cells (Fig. 4).

### Cilia present

The free neuromast organs of *G. niger* consist of an elongated ellipsoid of hair cells making up the sensory plate (Figs. 4, 5). This is held within a connective tissue bolster or stud, a group of supportive cells forming a smooth approximately diamond-shaped region round the hair cells (Pfüller, 1914). This basal diamond of tissue is characteristic of all goby species I have examined. Cells within this bolster are almost certainly responsible for secreting much of the cupulae (Fig. 5). The sensory plate within its tissue bolster is positioned on top of each papilla, the surface of which is covered by a highly sculptured epithelium (Fig. 3 C).

Each hair cell possesses a single kinocilium, usually 10–12 $\mu$m long and 22–28 stereocilia varying from 1–2 $\mu$m long (Fig. 3). This is the case in both standard neuromasts and the unusual mandibular row. The mandibular sensory plate contains on average more hair cells, 100–110 as opposed to 45–50 found in the standard neuromast. Thus the area covered by the mandibular neuromast is greater, being on average 13 × 28 $\mu$m, more than twice that of the 10 × 16 $\mu$m of the standard free neuromast sensory plate. This might suggest that these neuromasts are more sensitive to water movement than are their standard free neuromast counterparts.

### Cilia removed – hair cell, sensory plate and overall polarity

Once the neuromast has been denuded of its cilia, the polarity of each hair cell can be clearly seen. This is shown by the orientation of the kinocilium in relation
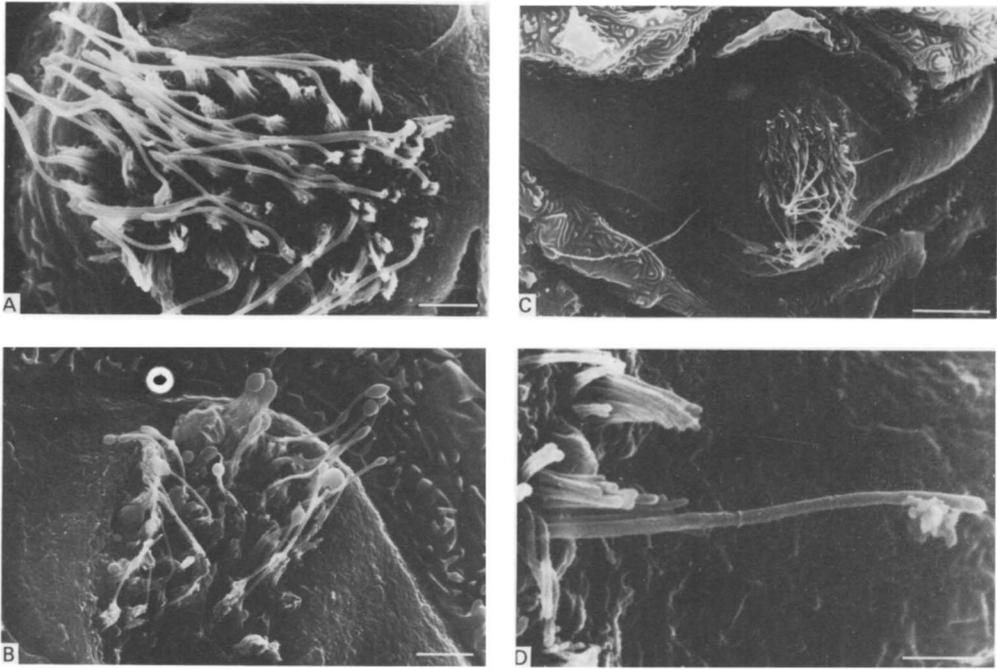
Fig. 3. Details of sensory plates and cilia cleaned by a short exposure to ultrasound. (A) A standard neuromast sensory area showing kinocilia with characteristic end swellings and shorter stereocilia. Scale line, 2 $\mu$m. (B) Kinocilia with interesting artifactual swellings which often occur. Scale line, 3 $\mu$m. (C) Shows highly sculptured epithelium surrounding the supportive stud. Scale line, 10 $\mu$m. (D) The cilia of an individual hair cell, the kinocilium has broken half-way along its length. Scale line, 1 $\mu$m.

to the stereocilia (Dijkgraaf, 1963; Flock, 1965). The hair cell is only excited if the bundle of cilia is displaced, by cupular movement, in the direction of the kinocilium. The technique of removing cilia in an ultrasonic 'cleaning' bath leaves easily visible hair stubs where each stereocilium and the kinocilium enter the hair cell. This elegantly shows the ciliary arrangement on each cell, the orientation of cells to each other, and their overall position on the sensory surface (Fig. 4).

Each free neuromast contains a mixture of hair cells of opposing polarity with adjacent cells usually being opposites. Here, as in other neuromasts examined (Flock, 1965), opposed cells appear in approximately equal numbers on the sensory surface giving it what may be called a 'general polarity' in its longer axis (Figs. 4 A, 5 A).

On examination of the rows and columns on the head of G. niger, a clear pattern of sensory plate general polarities emerges. Their orientation within a line of neuromast papillae gives each row or column an 'overall polarity' (Fig. 5). In all columns the general polarity of the sensory plate is in the antero-posterior axis so hair cells give their greatest response to fore and aft water movements perpendicular to the orientation of the column. All rows, with the exception of the mandibular row, have hair cells which are dorso-ventral in polarity, they show
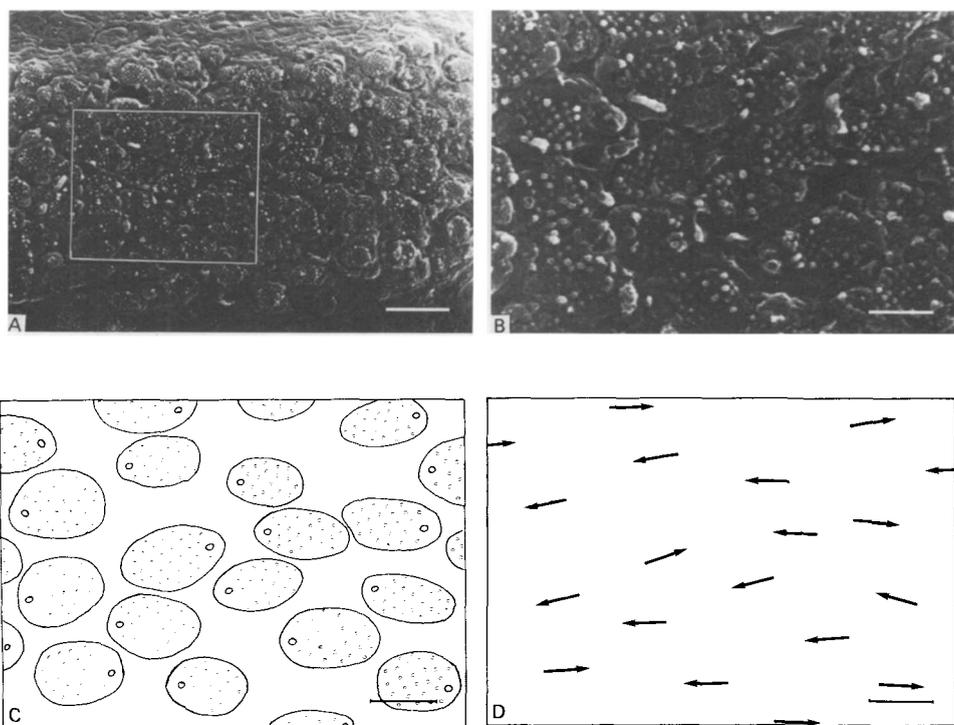
Fig. 4. Free neuromast from the mandibular row. (A) Low-power SEM picture of a complete sensory plate showing approximately 100 hair cells. Scale line 3 $\mu$m. (B) Higher power of inset from (A) with 18 hair cells shown. Scale line 1 $\mu$m. (C) A direct drawing of (B) to clarify the position of kinocilia and stereocilia stubs on the hair cells. From this a polarity map can be drawn (D) with each arrow pointing from stereocilium to kinocilium. Thus the 'general polarity' of the complete sensory surface is in its long axis. Scale line: C, D, 1 $\mu$m.

their greatest response to water movements in the up and down direction, which is again perpendicular to the orientation of the row. Where rows or columns become obliquely orientated (such as the middle portion of row 16 (Fig. 1)) the polarity remains perpendicular to the direction in which each section of the line of organs is running.

The mandibular row is exceptional, both in its form and its hair cell orientation (Fig. 5). The hair cells of this row give their greatest response to water moving along the line of the row, in the fore and aft direction. This is more evidence to suggest that the mandibular free neuromasts have a different function to the standard free neuromasts.

## DISCUSSION

Free-standing neuromasts form a network of rows and columns over the head of *G. niger*. Each line of neuromasts responds best to water movements perpendicular to the direction in which they run. This is due to the orientation of hair cells within each sensory plate. Any goby swimming forward or facing
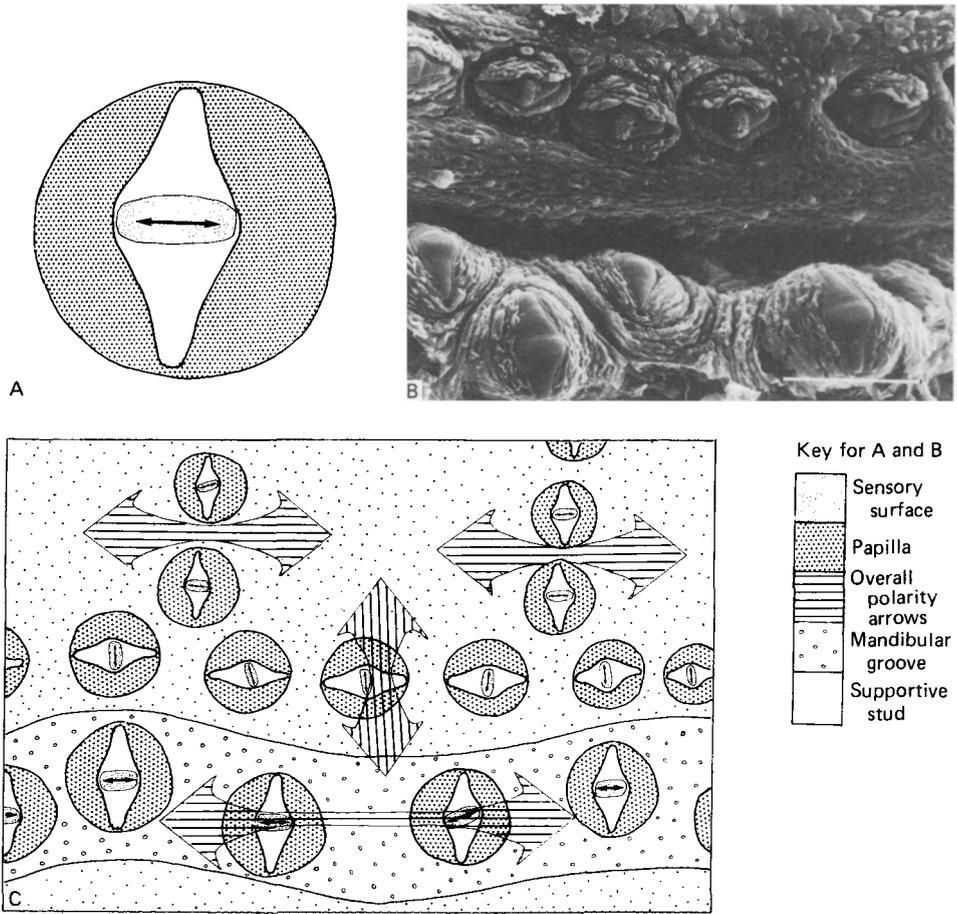
Fig. 5. The difference in mandibular and standard free neuromasts. (A) A diagrammatic organ with the general polarity of the sensory surface shown by the double-headed arrow. Scale line, 30 $\mu$m. (B) SEM of the internal mandibular row within its groove (below) and a standard row (above). The tissue shrinkage can be clearly seen. Scale line, 120 $\mu$m. (C) A diagram of an imaginary area of the head with standard row and columns and the mandibular row. Again general polarity of each neuromast is shown by double-headed arrows. Overall polarity of each row or column shown by large arrows. Note unusual overall polarity of mandibular row. Scale line, 100 $\mu$m.

into a current will probably register the relative water movement mainly in its dorso-ventral columns of free neuromasts. It is interesting to note that most species of gobies possess more columns than rows of neuromasts, suggesting that they gather more information about fore and aft water movements than any other. A predominance of fore and aft orientated free neuromasts would be predicted, whether to measure the fish's motion through the water, currents or disturbances in the environment (Denton & Gray, 1982). This orientation is also compatible with obstacle avoidance (Dijkgraaf, 1963). Such a network of sensors spread over the head of a a fish could be used in precise pinpointing of local water disturbances. Using this ability gobies may accurately locate prey such as swimming plankton.

The differences in structure and orientation between mandibular and standard neuromasts suggests that they may serve slightly different purposes. The location and orientation of the mandibular neuromasts may imply that these unusual organs may be an adapted paedomorphic line of head canal organs. In some fish, such as the herring, *Clupea harengus* (L.), as head canals develop, existing larval free-standing organs are incorporated into the canal system (Blaxter *et al.* 1983). The larvae of the gold fish, *Carassius auratus* (L.), have mandibular supraorbital and infraorbital lines of free-standing organs (Iwai, 1967). These may become incorporated into the head canals of these regions in the adult fish or simply regress as the canals develop. I believe it likely that the mandibular free neuromasts in *G. niger* represent a row of organs that would have been incorporated in the mandibular canal had their developmental state not remained larval.

The evidence for functional divergence and paedomorphic development in the mandibular row is summarized below. This row seems to be a half-way stage between free-standing organ and canal organ, in both a functional and developmental sense. It should be emphasized that structural evidence of the kind presented here, however detailed, can only give limited indications of possible function.

(i) The papillae and cupulae of the mandibular neuromasts are broader and more squat in appearance. The sensory plates possess on average more than twice as many hair cells as do the standard free neuromasts, perhaps making them more sensitive. This certainly suggests some difference in function and may be indicative of canal-like organs in which the cupula tends to be more like a flat plate over the neuromast.

(ii) The mandibular papillae are situated in a groove that runs from the tip of the lower jaw round to behind the pre-operculum. This groove was always found to be well filled with mucoid secretion so at least some of the cupulae would be covered or partially covered in mucus. (The depth and width of the groove varies along its length.) Conversely the standard neuromast papillae project their cupulae beyond the mucoid layer.

Again these observations suggest that mandibular and standard free neuromasts serve different purposes. If these organs are paedomorphic they may only begin to sink into the skin and then have their development towards becoming complete canals arrested. Thus they appear as they do in life as a partially sunk, uncovered set of 'canal' organs. Had they developed fully, they would rest in a bony groove in the mandibular bone, in fact they are situated slightly above this bony depression, again suggesting arrested development.

(iii) The mandibular row as outlined above, in fact occupies the exact position of the mandibular canal found in some 'primitive' gobies, for instance *Rhyacichthys aspro* (Miller, 1973). (The primitiveness of canals in gobies is discussed below.) This canal when present runs round the pre-operculum and joins the pre-opercular canal, giving the complete system the name of pre-operculo-mandibular canal. In *G. niger* and other gobies studied, the mandibular row seems

to run into the ventral opening of the pre-opercular canal, which still exists in most gobies.

This strongly supports the ideas as to the origins of this group of free neuromasts.

(iv) The most striking observation is that the polarity of the hair cells in the mandibular row is fore and aft, along the length of the row. This is unusual for a row of free-standing neuromasts as they generally transduce movement perpendicular to their axis and suggests a functional difference for this unique row of organs. Canal neuromasts give their greatest response to water movement along the axis of their line as in a canal, water movement is restricted to this direction by the structure of the canal itself. This observation, therefore, reinforces those given so far that point to the mandibular row being adapted from a canal of neuromasts.

The mandibular row almost certainly serves a different function to other free neuromasts found. If it also represents a modified head canal then it is by no means just a canal that has remained unclosed. The number of papillae in the row is more than would be predicted for a canal of equal length. Indeed, the very fact that papillae exist to support the neuromasts shows a considerable change from the canal condition. It also has to be noted that although there are other absent head canals in these and other gobies they are not apparently represented by corresponding arrangements of free neuromasts.

Thus the special features of the mandibular row described here can have no definite implication relating to the evolution and development of canal and free-standing organs in general. Miller (1973) has suggested that, in gobies at least, the possession of extensive head canals (as found in their suggested percoid ancestors) is a primitive feature. Lowenstein (1957) on the other hand is 'tempted to speculate' in a broader way that free-standing organs precede canal organs, though fossil evidence suggests otherwise (Stensio, 1926; White, 1935).

Although this investigation has dealt solely with *G. niger* I have examined other species of goby. To date all species examined – *Pomatoschistus minutus*, *Pomatoschistus microps* (L.) and *Gobius paganellus* (L.) – have shown similar ultrastructure and orientation of free neuromasts. This includes the diamond-shaped tissue stud supporting each neuromast. There are, however, some marked differences in neuromast papillae shape, cupular shape and of course general distribution. These differences and the possible significance of such interspecific variables will provide material for future work of a more comparative nature.

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