THE STRUCTURE OF THE LATERAL PREMAXILLARY SPINES OF THE ANTARCTIC FISH *MURAENOLEPIS MICROPS* LÖNNBERG, 1905 (GADIFORMES)

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ABSTRACT. The lateral premaxillary spines appear in young *Muraenolepis microps*, 16–20 mm in length, in a single wave of development rostrodorsally to caudoventrally, disappearing by the time the fish reach 70 mm in length. The developmental stages and mature structure of the spines parallel those of teeth. However the mature spines are larger, more robust and curved than teeth. The spines, along with teeth, are part of the dermal skeleton of the fish. Spines may function as defensive structures against unknown predators in the small young fish.

INTRODUCTION

Muraenolepididae is a family of Gadiformes which inhabits the waters of the Southern Hemisphere. It comprises one genus, *Muraenolepis*, and four species (Fischer and Hureau, 1985).

There are few descriptions of early stages of development of Muraenolepididae. North and White (1982) sketch a *Muraenolepis microps* post-larva, 16.6 mm in length and Efremenko (1983) describes and draws 3 post-larvae, 9.5, 20.5, and 26.5 mm, respectively. Only Fahay and Markle (1984) in a line drawing of a 32.5-mm juvenile *Muraenolepis* sp., show the presence of about 6 lateral premaxillary spines. Noting that although North and White (1982) and Efremenko (1983) failed to describe or illustrate the spines in larvae (or adults), Fahay and Markle (1984) 'consider them a unique and diagnostic larval specialization of the family'.

Although the Gadiformes include eight families, most of which have world-wide distribution (Fahay and Markle, 1984), only in the Muraenolepidae, mainly restricted to the Antarctic seas, are the transitory premaxillary spines found. No descriptions of structure or function have ever been given.

Early observations indicated a similarity in structure between the externally located spines and the teeth, hard structures on the biting margins of the jaws. The present study examines and compares the histological structure of the lateral premaxillary spines and premaxillary teeth of *M. microps*. Some suggestions regarding possible function(s) are presented.

MATERIAL AND METHODS

Seven specimens of *M. microps* (Table I), including five post-larvae (defined by Hureau, 1982, as young stages without yolk-sac) 16–20 mm in length and two juveniles (defined by Hureau, 1982, as individuals with a full complement of fin rays and most adult characters), 45 and 52 mm in length, were collected from the epipelagic zone of the coastal waters around South Georgia by members of the British Antarctic Survey. The fish were fixed intact in 10% formalin in sea-water and forwarded to us for study.

The heads were removed and washed in running tap water for 24 h, except for fish 4 (Table I), in which the left and right premaxillae and adjacent tissue were dissected and decalcified in EDTA, prior to washing in tap water. The tissues were dehydrated

Table I. Length of fish, plane of sectioning of tissue and number of spines

Fish no.	*Length (mm)*	*Plane of sectioning*	*Number of spines*	
			Dissecting microscope	*Histological sections*
1	16	Sagittal	4	4
2	18	Cross	4	4
3	45	Right half, cross	6	6
		Left half, sagittal	6	6
4	52	Premaxilla, left longitudinal	3	3
		Premaxilla, right longitudinal	1	1
5	20	Sagittal	0	6
6	18	Cross	0	4
7	18	Cross	0	5

to 95% alcohol, infiltrated and embedded in 2-butoxyethanol methacrylate pla[illegible] (Polysciences Inc., Warrington, Pa.). The tissue blocks were sectioned serially on a JB-4 Porter Blum microtome using glass knives.

The plastic, in which tissues from fishes 1–4 were embedded, failed to polymerize normally, although plastic in which trunk regions of the same fish were embedded polymerized as expected. To obtain sufficient hardening of the blocks to allow sectioning, the blocks needed several months of drying; even so, it was impossible to cut satisfactory serial sections. Histological examination of the head revealed no multicellular glands which might have been secreting enzymes which prevented hardening of the plastic. However, the heads of fishes 5, 6 and 7 were placed in saturated aqueous mercuric chloride to destroy any enzymes, which might be present, for 0.5 h before processing. Hardening of the plastic proceeded normally and serial sections were obtained.

Sections were stained mainly with Haematoxylin and Eosin-Orange G (H&E) (Humason, 1972). For fishes 5, 6 and 7, tracings of the serial sections were made in order to build up a three-dimensional understanding of the morphology of the spines.

Table I provides some pertinent information on the fish examined.

Results

Gross morphology of spines

Lateral premaxillary spines were visible when fishes 1–4 were examined under the dissecting microscope (Table I). Fig. 1 illustrates the six spines seen on fish 3. The spines extend perpendicularly at their bases from the surface of the head in the region of the premaxillae and then backward and inward towards the sides of the head. Spines are conical in shape, slightly curved and whitish in colour. With gentle prodding the spines could be moved slightly.

No spines were visible under the dissecting microscope in post-larval fishes 5, 6 and 7 although they were seen in various stages of development in the histological sections. In the post-larval fishes in which spines were seen, the spines varied in length with the longest spines being found more rostrally and dorsal and the shortest more caudally and ventral. In fish 2, four spines were found on each side of the mouth, the most rostral measuring 0.5 mm in length, the next 0.3 mm and the two most caudal seen as small barely observable protuberances. Juvenile fish 3 (Fig. 1) had six pairs

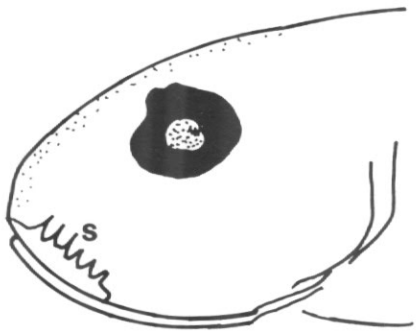


Fig. 1. Left side of head of fish 3 showing six spines (S) decreasing in length rostrodorsally to caudoventrally. × 5.

of spines, the largest located rostrally, measuring 0.8 mm in length and the others decreasing in length more caudally. In juvenile fish 4 (52 mm), the three observable spines were each about 0.8 mm in length and were located ventrally.

The spines are bilaterally symmetrically arranged on the left and right premaxillae (Figs 2, 2a, 2b), except for juvenile fish 4 in which three spines were seen on the left and one on the right.

Examination of the erupted teeth shows that the teeth are conical and not curved. Except for four fang-like teeth lying at the lateral rostral 'corners' of the upper and lower jaw, mature teeth are smaller than mature spines.

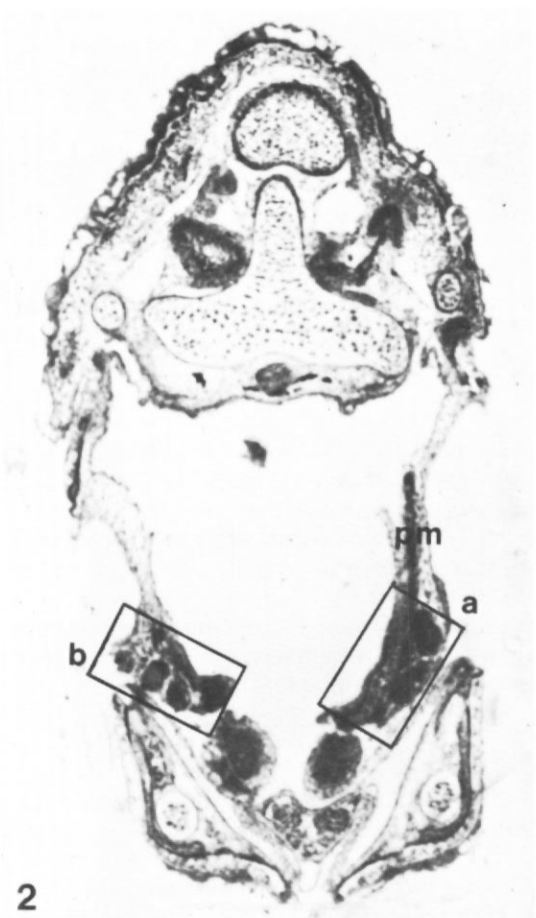
Histology of mature premaxillary spines and teeth

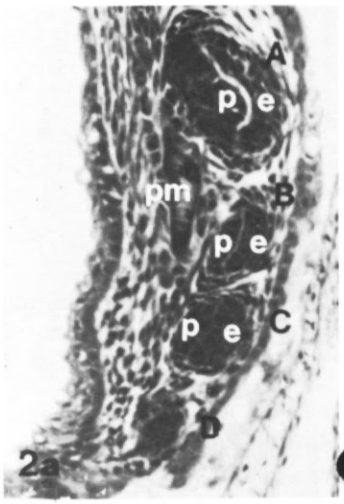
Early observations indicated that the structure of the spines closely resembled that of teeth. Consequently the conventional terms used for tooth morphology have been applied to the spine.

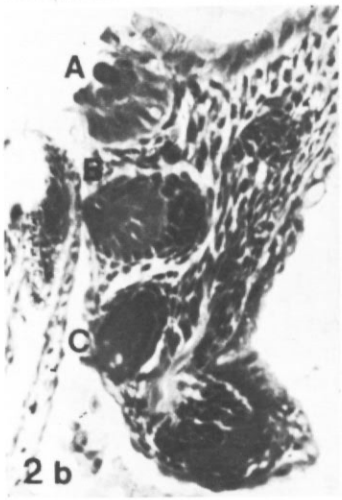
A mature spine and tooth from fish 4 are seen in Figs 3 and 4 respectively. The bulk of the spine is composed of a calcified matrix (Figs 3, 5, 6); the body of the spine is dentine and is covered at its apex by a cap of calcified material, here considered to be enameloid (Fig. 6). A thin rim of uncalcified dentine matrix (Figs 5, 6) lies between the calcified dentine and the pulpal columnar cells, the odontoblasts. Blood vessels, foamy cells and mesenchymatous cells are also present in the pulp cavity (Figs 5,

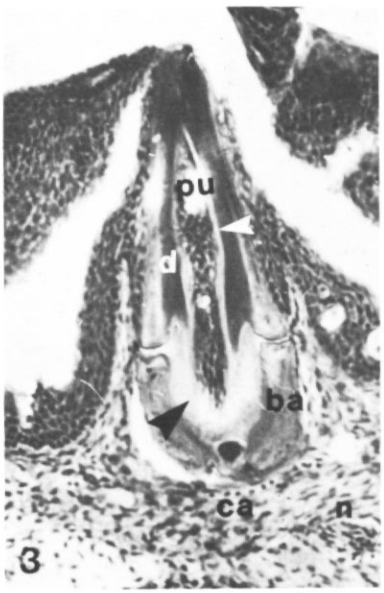
The proximal end of the spine articulates with the bone of attachment (Figs 3, 5) which in turn connects to the premaxilla. The pulp of the spine extends into the bone of attachment. From the internal surface of the bone of attachment, two sets of collagen fibres connect with the spine; one substantial sheath of long fibres extends to the internal surface of the dentine and a second set connects to the external surface of the base of the spine.

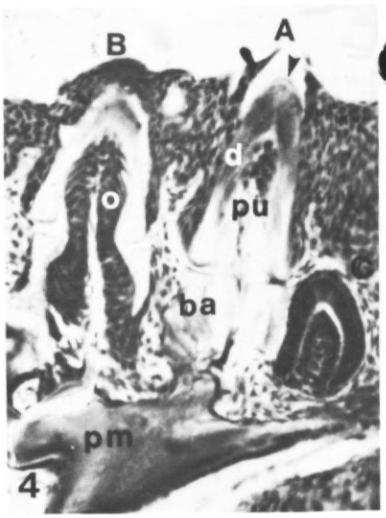
Although spines may not be observable with the dissecting microscope, they may appear to have erupted when examined in histological section. Figs 7 and 8 show two erupted spines of fish 5 at two different planes of section. Spine A cut in cross section (Fig. 7) shows calcified dentine, and the thin inner rim of uncalcified dentine surrounding the pulpal cells; in Fig. 8, the spine lies free of epithelium. Spine B is cut tangentially and shows little calcification of the dentine and the pulp cavity packed with cells (Fig. 7); in Fig. 8, the curved free tip of the spine is heavily mineralized.











Figs 2–4. For legend see opposite.

The mature tooth (Fig. 4) consists of the calcified material, dentine and the enameloid cap, and the pulpal cavity extending into the bone of attachment. The bone of attachment, connected by collagen fibres to the tooth, is confluent with the premaxilla. Although similar in structure, mature spines are larger than mature teeth. Developing teeth are often seen at the bases of mature teeth; no replacement germs or buds are ever seen adjacent to spines.

Stages in development of spines and teeth

Because developmental stages of premaxillary spines and teeth (Figs 9–13) show great similarity, the conventional terms for developing teeth will be used for developing spines. Primordia of the teeth include the bud, cap and bell stages followed by maturation and eruption.

Examination of histological sections confirmed that spines on either side of the head may be at different stages of development with the later (older) stages being more rostral (Figs 2a, b). Further, the first spines appear before the first teeth. Later both spines and teeth are seen in various stages of development.

The bud stage of a spine primordium consists of a rounded mass of ectodermal cells extending into the underlying mesenchyma. The epithelial bud then becomes invaginated by a condensed aggregation of mesenchyme; this is the cap stage of development (Figs 9, 10, 13). The epithelial portion of the spine primordium is termed the dental epithelial organ and it retains a connection to the surface epithelium by an epithelial cord (Figs 9, 13); the condensed mesenchyme forms the dental papilla. As development of the dental epithelial organ proceeds, a layer of outer dental epithelium can be distinguished from an inner dental epithelium (Figs 10, 11, 13). The developing spines are associated with the developing premaxillae (Figs 2a, 10, 11, 13).

Continued invagination of the dental epithelial organ gives rise to the bell stage of the spine primordia (Fig. 11). The cells of the outer dental epithelium become cuboidal and those of the inner dental epithelium columnar. The cells of the dental papilla adjacent to the inner dental epithelium increase in height and are referred to as the odontoblasts. Similar structures are seen in a tooth primordium in the bell stage (Fig. 12). However, the tissues of the tooth primordia always appear more highly organized than those of the spine primordia. In the developing tooth, the outer and

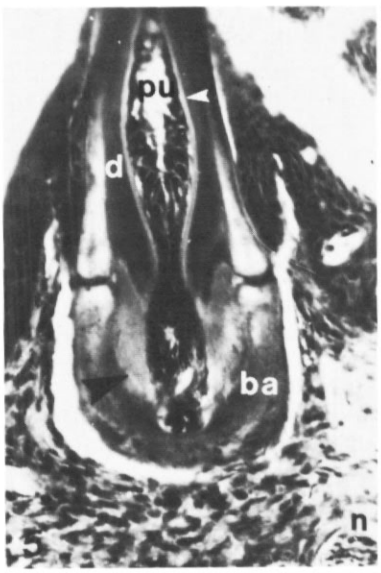
Fig. 2. Cross-section of head of fish 7. The developing spines (in boxes a, b) are located on the aboral surfaces of the right and left premaxillae (pm). ×65.

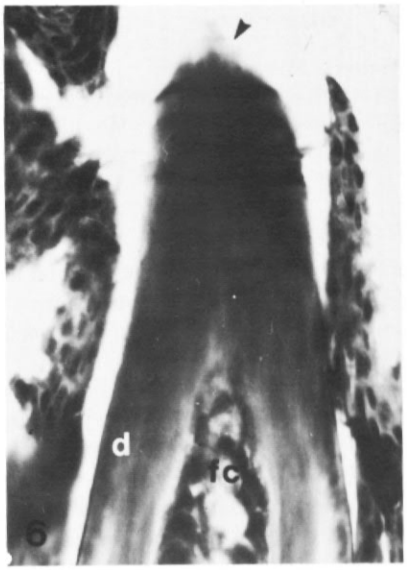
Fig. 2a. Four developing spines (A–D) on premaxilla (pm). Spines A, B and C show the dental epithelial organ (e) and the dental papilla (p). ×230

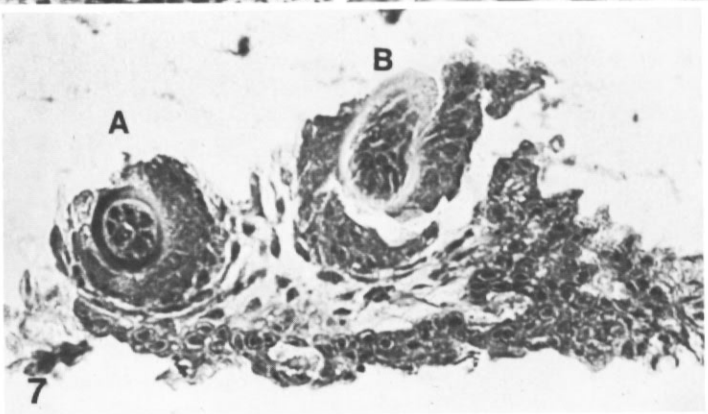
Fig. 2b. Three spines (A–C) tangentially sectioned, Spine A shows mineralized tip (arrow). ×230

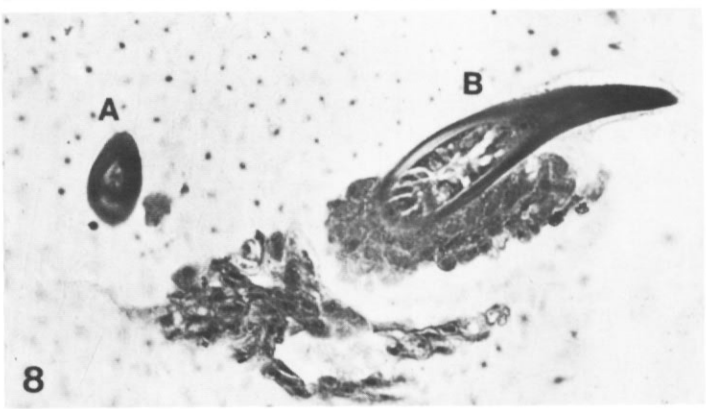
Fig. 3. A mature spine from fish 4 showing the mineralized dentine (d), the thin rim of unmineralized predentine (small arrow) surrounding the pulp cavity (pu). The bone of attachment (ba) is connected by an extensive collagen sheath (large arrow) to the spine. In the underlying connective tissue, a capillary (ca) and nerve (n) are found. ×180.

Fig. 4. Three teeth from fish 4. A mature tooth (A) shows mineralized dentine (d) capped by enameloid (arrow) and pulp (pu). The bone of attachment (ba) is confluent with the premaxilla (pm). Tooth B is in an earlier stage of development and shows the large odontoblasts (o) in the pulpal cavity. A tooth primordium (C) is in the bell stage of development. ×180.









Figs 5–8. For legend see opposite.

inner dental epithelia and the odontoblasts of the dental papilla were clearly distinguishable (Fig. 12). A layer of pale staining slightly vacuolated material (enameloid or predentine) appears between the dental papilla and the inner dental epithelium in both developing spines and teeth (Figs 11, 13 and 12). The odontoblasts of the spine continue to produce a calcifiable matrix which mineralizes to form dentine except for a thin rim adjacent to the pulp cavity (Figs 3, 5, 6, 7, 8). The pulp cavity does not extend into the highly calcified tip of the spine (Figs 2b, 3, 6, 8).

No stages in the formation of the bone of attachment of spines were found. However during later stages of maturation of teeth, the bone of attachment forms at the base of the tooth and then grows towards the premaxilla.

Discussion

The only previous report of the presence of lateral premaxillary spines in *M. microps* is that of Fahay and Markle (1984) in a 32.5-mm juvenile. There is no information on the time of first appearance, the maximal number, or the time of disappearance of the spines.

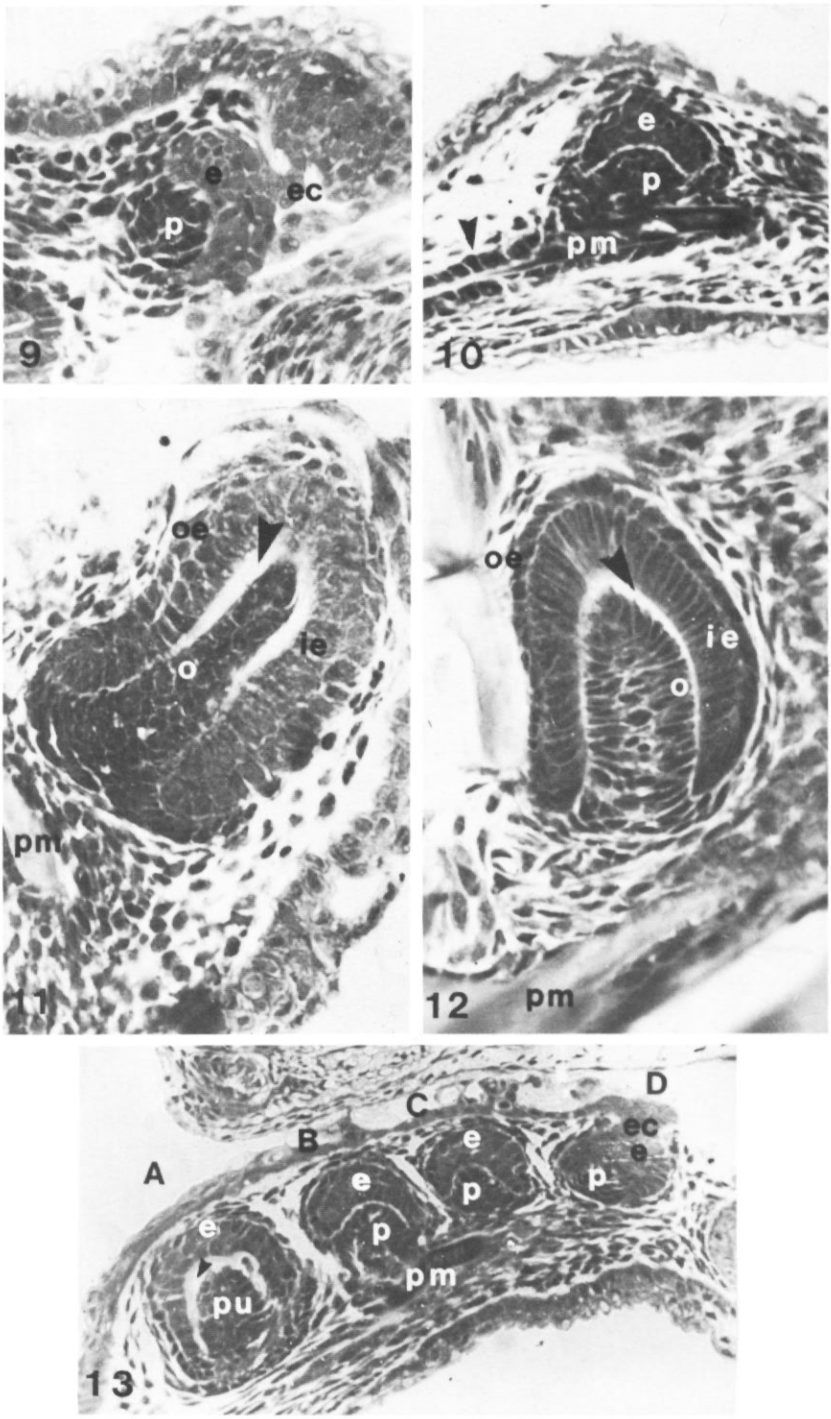
In *M. microps*, the spines erupt when the post-larvae have reached a length of 16–20 mm, in a single wave extending rostrodorsally to caudoventrally. As the fish matures to the juvenile stage, several pairs of large prominent spines may be present. The spines disappear in the same order as they appear. No replacement spines develop.

The dermal skeleton of vertebrates gives rise to a variety of structures, including teeth, scales, denticles, dermal bone and fin rays. The hard tissues formed are enamel/enameloid, dentine, cellular and acellular bone (Schaeffer, 1977). All components of the dermal skeleton are considered to represent a single morphogenetic system in which the epithelial component is of ectodermal origin and the mesenchymatous component derived from neural crest (neuroectoderm) (Schaeffer, 1977). During the ontogeny of vertebrate teeth, the following stages are recognized: dental lamina, a thickening of the basal epithelium; tooth bud, a rounded swelling from the dental lamina; cap stage, in which an epithelial dental organ with an outer and inner enamel epithelium partially encloses a dental papilla of mesenchymatous tissue; growth through the bell stage to maturity and eruption of the tooth. The inner dental epithelial cells give rise to ameloblasts which in vertebrates form enamel or contribute to enameloid; the peripheral cells of the dental papilla form odontoblasts which produce dentine. The structure and development of teeth in fishes has been described by several authors, including Gaunt and Miles (1967), Berkovitz (1977), Fink (1981), Huysseune (1983), Meinke and Thomsom (1983); although there are some differences, the general pattern of tooth development is similar to that of reptiles and mammals.

Fig. 5. Same spine as in Fig. 3 but in different plane of section. Dentine (d), predentine (white arrow), pulp cavity (pu), bone of attachment (ba), the collagen sheath (black arrow) connecting the spine and bone of attachment and a section of nerve (n) are seen. × 230.

Fig. 6. Tip of the same spine as in Figs 3 and 5. Enameloid (arrow) covers the tip of dentine (d). Foamy cells (fc) are present in the pulp. × 775.

Figs 7 & 8. Apical regions of two spines from fish 5 at different planes of section. Spine A is cut in cross-section. Spine B is cut tangentially: the curving of the spine is illustrated in Fig. 8. Both spines have erupted. × 300.



Figs 9–13. For legend see opposite.

The present study indicates that tooth development in *M. microps* is similar to that described for other fishes

Stages comparable to those of tooth development are seen during the development of the premaxillary spines. The similarities between premaxillary spines and teeth in morphology and development presented here indicate that spines can be considered part of the dermal skeleton in *M. microps*.

The mature spines and teeth in *M. microps*, like vertebrate teeth in general, are composed of dentine surrounding a pulp cavity containing a variety of cell types, capped by a thin layer of enameloid/enamel. The bone of attachment is confluent with the premaxillary bone and is connected to the spine or tooth by a substantial layer of collagen fibres extending from the cavity of the bone to the inner and outer surfaces of the spine or tooth.

The identity of the surface cap of spines and teeth of *M. microps* is unknown. Poole (1967) used the term 'enameloid' to distinguish the hypermineralized surface layer of teeth in fish and amphibians from the true enamel seen in reptilian and mammalian teeth. Meinke and Thomsom (1983) consider that during development, enameloid matrix is produced mainly by the ectomesenchymal odontoblasts before dentine genesis begins; on the other hand, enamel is deposited by ameloblasts of ectodermal origin only after dentine formation has commenced. Recently, Prostak and Skobe (1986), on the basis of their ultrastructural studies on cichlid teeth, concluded that enameloid collagen is formed by the ameloblasts, i.e. is of ectodermal origin. However, there are some fish in whose teeth enamel is reliably reported (Smith, 1978; Kemp, 1985). Morphological features such as the presence of dentinal tubules in enameloid and their absence in enamel (Poole, 1971) and the positive birefringence with polarized light of enamel due to vertical orientation of hydroxyapatite crystals in enamel and its absence in enameloid (Schmidt and Keil, 1971) have been used to distinguish enameloid from enamel. Careful examination of the cap of spines and teeth in this study shows the presence of fine striations, which may represent dentinal tubules; if this is so then the tissue is probably enameloid. However, Meinke and Thomsom (1983) point out that the methods of Poole (1971) and Schmidt and Keil (1971) cannot reliably distinguish enameloid from enamel.

Fig. 9. Cap stage of development of spine from fish 7. The dental epithelial organ (e), attached to the surface epithelium by the epithelial cord (ec), is slightly invaginated by the dental papilla (p). × 400.

Fig. 10. Later cap stage of development of spine from fish 7. The dental epithelial organ (e) is composed of two indistinct layers and is invaginated by the dental papilla (p). The developing premaxilla (pm) is seen with its associated osteoblasts (arrow). × 285.

Fig. 11. Developing spine from fish 6. The outer dental epithelial layer of cuboidal cells (oe) and inner dental epithelial layer of columnar cells (ie) are distinguished. Between the odontoblasts (o) and inner dental epithelial layer lies pre-enameloid or predentine (arrow). A portion of developing premaxilla (pm) is seen. × 525.

Fig. 12. Developing tooth from fish 4 (tooth C of Fig. 4) showing the outer dental epithelial cells (oe), inner dental epithelial cells (ie), odontoblasts (o), pre-enameloid or predentine (arrow) the premaxilla (pm) is seen. × 525.

Fig. 13. Four developing spines shown with the premaxilla (pm) from fish 7. Spine A is the most mature, spine D the least. The two layers of the dental epithelial organ (e) are seen in spines A, B and C. In spine D, the epithelial organ (e) is connected to the surface epithelium by the epithelial cord (ec). Pulp (pu) and unmineralized matrix (arrow) are found in spine A. The dental papillae (p) invaginate the epithelial organs in spines B, C and D. × 275.

Fink (1981) describes the forms of tooth attachment found in actinopterygians based on the degree of mineralization of the collagen between the tooth base and the bone of attachment. While primitive actinopterygians possess teeth ankylosed to the bone (Fink's Type 1), the predominant attachment of teeth in teleosts is through a thin ring of unmineralized collagen between the tooth and bone (Fink's Type 2) allowing some tooth movement. Attachments of both teeth and spines in *M. microps* correspond to the typical teleost pattern, i.e. are Type 2. Some movement of spines could be determined grossly.

Although the premaxillary spines and teeth in *M. microps* show similarities in development and the same basic morphology, can spines be considered as misplaced teeth? Ørvig (1977) points out the similarities between true teeth and odontodes (dermal teeth or denticles) in their development from a single mesenchymal papilla and epithelial organ; dentine and enameloid (usually) are found in both. However, he distinguishes between teeth and odontodes on the basis of their positions, functions, modes and places of formation, size and gross morphology. He defines teeth as hard structures found on the biting margins of jaws which function in capture, crushing etc. of food while odontodes, found anywhere on the dermal skeleton, show a vari of functions such as defence, protection of neuromasts, sieving of food, etc. During development, odontodes typically show no submergence of the epithelial organ; however, if the odontode is tooth-like in shape, the epithelial organ resembles the submerged epithelial dental organ seen in tooth development. No replacement germs are found during odontode development. Generally, odontodes are firmly attached to the supporting bone; occasionally they may be attached by ligaments. Although odontodes are found in many fossil and some extant fishes they are rarely seen in teleosts except for some dermal dental structures of the oral cavity and pharynx. Ørvig (1977) writes that although it may be difficult to distinguish between teeth and odontodes, they are fundamentally similar components of the dermal skeleton.

Thus, we believe that the premaxillary spines, clearly part of the dermal skeleton, fulfil the criteria of odontodes in that they are found outside the biting margins of the jaws and probably are not involved with food capture, they develop as do some tooth-like odontodes, they are attached to bone by collagen fibres as some odontodes, and their hard tissues are dentine and probably enameloid.

The functions of these transitory lateral premaxillary odontodes are unknown. Their possibilities for slight movement suggest that they may serve a tactile function in sensing direction or orientation as water moves over them. It is hard to conceive of the spines acting as filters or for food capture. Large nerve bundles do pass close to the base of the spine, but we cannot say from these studies that nerve fibres pa directly to the spine.

The spines begin to form prior to development of teeth on the premaxillae. When mature they are larger, longer and more robust than mature teeth. Because of their position, their curved, pointed morphology and early appearance, they may function in the small larvae as defensive structures against unknown predators. The maturing fish lose the non-replaced spines but by this time may be large enough that they no longer 'need' the spines for protection.

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References

BERKOVITZ, B. K. B. 1977. The order of tooth development and eruption in the rainbow trout (*Salmo gairdneri*). *Journal of Experimental Zoology*, **201**, 221–6.

EFREMENKO, V. N. 1983. Atlas of fish larvae of the Southern Ocean. *Cybium* **7**, 1–74.

FAHAY, M. P. *and* MARKLE, D. F. 1984. Gadiformes: development and relationships. ASIH Special Publication No. 1.

FINK, W. L. 1981. Ontogeny and phylogeny of tooth attachment modes in actinopterygian fishes. *Journal of Morphology*, **167**, 167–84.

FISCHER, W. *and* HUREAU, J. C. (*ed.*). 1985. FAO species identification sheets for fishery purposes. Food and Agriculture Organization of the United Nations, Rome.

GAUNT, W. A. *and* MILES, A. E. W. 1967. Fundamental aspects of tooth morphogenesis. (*In* MILES, A. E. W. *ed. Structural and chemical organization of teeth.* New York, Academic Press, 151–97.)

HUMASON, G. L. 1972. *Animal tissue techniques*. 3rd ed. San Francisco, W. H. Freeman and Co.

HUREAU, J. C. (*ed.*). 1982. Methods for studying early life history stages of Antarctic fishes. *Cybium*, **6**, 3–11.

HUYSSEUNE, A. 1983. Observations on tooth development and implantation in the upper pharyngeal jaws in *Astatotilapia elegans* (Teleostei; Cichlidae). *Journal of Morphology*, **175**, 217–34.

[illegible]MP, N. E. 1985. Ameloblastic secretion and calcification of the enamel layer in shark teeth. *Journal of Morphology*, **184**, 215–30.

MEINKE, D. K. *and* THOMSOM, K. S. 1983. The distribution and significance of enamel and enameloid in the dermal skeleton of osteolepiform rhipidistian fishes. *Paleobiology*, **9**, 138–49.

NORTH, A. W. *and* WHITE, M. G. 1982. Key to fish postlarvae from the Scotia Sea, Antarctica. *Cybium*, **6**, 13–32.

ØRVIG, T. 1977. A survey of odontodes ('dermal teeth') from developmental, structural, functional, and phyletic points of view. (*In* ANDREWS, S. M., MILES, R. S. *and* WALKER, A. D. *eds. Problems in vertebrate evolution*. Linnaean Society Symposium Series Number 4. Academic Press, pp. 53–75.)

POOLE, D. F. G. 1967. Phylogeny of tooth tissues: enameloid and enamel in recent vertebrates, with a note on the history of cementum. (*In* MILES, A. E. W. *ed. Structural and chemical organization of teeth*. New York and London, Academic Press.

POOLE, D. F. G. 1971. An introduction to the phylogeny of calcified tissues. (*In* DAHLBERG, A. A. *ed. Dental morphology and evolution*. Chicago, Univ. Chicago Press. (*In* MEINKE, D. K. *and* K. S. THOMSOM, 1983.)

PROSTAK, K. *and* SKOBE, Z. 1986. Ultrastructure of the dental epithelium and odontoblasts during enameloid matrix deposition in cichlid teeth. *Journal of Morphology*, **187**, 159–72.

SCHAEFFER, B. 1977. The dermal skeleton in fishes. (*In* ANDREWS, S. M., MILES, R. S. *and* WALKER, A. D. *eds. Problems in vertebrate evolution*. Linnaean Society Symposium Series Number 4. Academic Press, pp. 25–52.)

SCHMIDT, W. J. *and* KEIL, A. 1971. *Polarizing microscopy of normal and diseased dental tissues in man and other vertebrates*. Oxford, Pergamon.

SMITH, M. M. 1978. Enamel in the oral teeth of *Latimeria chalumnae* (Pisces: Actinistia): a scanning electron microscope study. *Journal of Zoology* (London), **185**, 355–69.