

The deep-sea teleost cornea: a comparative study of gadiform fishes

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Summary. The corneal structure of three deep-sea species of teleosts (Gadiformes, Teleostei) from different depths (250-4000 m) and photic zones are examined at the level of the light and electron microscopes. Each species shows a similar but complex arrangement of layers with a cornea split into dermal and scleral components. The dermal cornea comprises an epithelium overlying a basement membrane and a dermal stroma with sutures and occasional keratocytes. *Nezumia aequalis* is the only species to possess a Bowman's layer, although it is not well-developed. The scleral cornea is separated from the dermal cornea by a mucoid layer and, in contrast to shallow-water species, is divided into three main layers; an anterior scleral stroma, a middle or iridescent layer and a posterior scleral stroma. The iridescent layer of collagen and intercalated cells or cellular processes is bounded by a layer of cells and the posterior scleral stroma overlies a Descemet's membrane and an endothelium. In the relatively shallow-water *Microgadus proximus*, the keratocytes of the dermal stroma, the cells of the iridescent layer and the endothelial cells all contain aligned endoplasmic reticulum, which may elicit an iridescent reflex. No alignment of the endoplasmic reticulum was found in *N. aequalis* or *Coryphanoides (Nematonurus) armatus*. The relative differences between shallow-water and deep-sea corneas are discussed in relation to the constraints of light, depth and temperature.

Key words: Cornea, Ultrastructure, Deep-sea, Iridescent layer, Spectacle, Fishes

Introduction

The cornea of shallow-water teleosts has undergone extensive selection incorporating a number of unique structural adaptations setting them apart from almost all other vertebrate corneas. Specialisations such as

spectacles (Hein, 1913; Walls, 1942), corneal filters (Moreland and Lythgoe, 1968; Appleby and Muntz, 1979; Heinermann, 1984; Kondrashev et al., 1986), iridescent layers (Locket, 1972; Lythgoe, 1975, 1976), annular ligaments (Tripathi, 1974; Collin and Collin, 1996), autochthonous layers (Walls, 1942; Collin and Collin, 1988), sutural fibres (Smelser, 1962; Fisher and Zudunaisky, 1977), mucoid layers (Walls, 1942; Tripathi, 1974) and epithelial goblet cells (Collin and Collin, 1996) are features of a range of shallow-water species from a diverse range of habitats.

In contrast, the deep-sea teleost cornea has received relatively little attention. Although primarily thought to act as a protective goggle, the cornea of deep-sea teleosts must also overcome the physical constraints of temperature and pressure, while maintaining a clear optical pathway for the transmission of both low levels of sunlight and bioluminescent emissions. Most deep-sea teleosts survive between 2 and 5 °C at depths where the pressure may attain over 400 atmospheres. Although light levels are high in the upper euphotic zone, levels fall markedly to a depth of 1000 m, beyond which not even single quanta of sunlight penetrate (Denton, 1990).

Previous studies on the eyes of deep-sea fishes have identified a number of corneal specialisations. These include corneal projections (Percy et al., 1965) or pearly accessory corneal bodies (Whitehead et al., 1989) adjacent to the secondary globe in the eye of *Bathylchnops exilis* (Opisthoproctidae), which may increase the monocular visual field. Similarly, the lens pad in the scopolarchid, *Scopelarchus guntheri*, and the optical fold in the evermannellid, *Evermannella indica*, are both thought to extend the monocular visual field, allowing light to strike the ventro-lateral aspect of the lens and a specialised region of the accessory retina by light guiding and geometrical optics, respectively (Locket, 1971, 1977; Collin et al., 1997).

With the exception of the few species mentioned above, little is known of the basic arrangement of layers in the corneas of deep-sea teleosts, although the tendency for a split cornea has been noted by a few authors. In the tripod fish, *Bathypterois longipes* (Bathypteroidae) and the sea snail, *Careproctus*

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kermadecensis (Liparididae), the dermal and scleral corneas are split with the dermal cornea continuous with the skin and the scleral cornea continuous with the scleral eyecup (Munk, 1964a). Munk (1966) also noted that in the mesopelagic *Nansenia groenlandica*, the inner surface of the scleral cornea is covered by Descemet's membrane, a thin endothelium and a thick annular ligament in the irido-corneal angle. Even where the eyes are thought to be degenerate (i.e. in the angler fishes *Cryptopsaras cosei* and *Ceratius holboelli*, Ceratiidae), the cornea is well developed with dermal and scleral components separated by loose connective tissue (Munk, 1964b), although the scleral stroma is thought to be thin.

In this study, we have examined three species of gadiform fishes from three different photic zones; the Pacific tomcod, *Microgadus proximus* (Gadidae) from the well-lit waters of the euphotic zone, the rat-tail, *Nezumia aequalis* (Macrouridae) from the twilight or mesopelagic zone and the armoured grenadier, *Coryphanoides (Nematonurus) armatus* (Macrouridae) from the dark benthopelagic zone where sunlight fails to penetrate. This detailed ultrastructural study, provides a comparative analysis of the structure and arrangement of the cornea in response to the constraints of light, temperature and pressure.

Materials and methods

Four specimens of each of the Pacific tomcod, *Microgadus proximus* (Gadidae, Gadiformes), the rattail, *Nezumia aequalis* (Macrouridae, Gadiformes) and the armoured grenadier, *Coryphanoides (Nematonurus) armatus* (Macrouridae, Gadiformes) were used in this study. Specimens of *M. proximus* were collected in Puget Sound, San Juan Island, Washington (USA) and specimens of *N. aequalis* and *C. (N.) armatus* were collected on a scientific expedition on board the RRS 'Challenger' in 1992 (Cruise No. 94) in the vicinity of 49-51 °N (latitude) and 110-150 °W (longitude) over the Globan Spur and Porcupine Sea Bight. All specimens were captured with semi-balloon otter trawling nets (OTSB 14) at depths between 100 and 4,000 m.

For all specimens, sampling was carried out according to the ethical guidelines of the National Health and Medical Research Council of Australia. Most animals were dead when the trawl was brought on deck but some animals were killed by immersion in an overdose of tricaine methane sulphonate (MS 222, 1:2,000) in seawater, after which the eyes were excised. The eyes of all specimens were intact with intraocular pressure maintained. However, due to the trawling techniques employed, superficial damage to the cornea of some specimens was unavoidable (observed histologically as a loss of the outer epithelial cell layers). Enucleated eyes were immersion fixed in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1M sodium cacodylate buffer (pH, 7.4) for between 12 and 24 hours, after which the posterior globes were removed and the corneae prepared for electron microscopy.

Corneas were post-fixed in 2% osmium tetroxide with 1.5% potassium ferrocyanide in 0.1M sodium cacodylate buffer (the reduced osmium method of Collin and Allansmith, 1977, which is a slight modification of the osmium potassium ferrocyanide method of Dvorak et al., 1972). Tissue was then dehydrated in acetone and embedded in resin (Polybed/812, Polysciences Inc). Thick (1 µm) sections were stained with Richardson's stain and examined by light microscopy. Thin sections were stained with lead citrate and uranyl acetate and examined on a Siemens Elmiskop 1A or an Hitachi H500 transmission electron microscope.

Measurements were taken from photographic enlargements using a graticule and magnifying glass. Photographs were taken on either 35 mm Kodak Technical Pan film (rated at 50 ASA, light microscopy) or Kodak 4489 electron microscope film.

Results

The general structure and arrangement of the corneas in the three species examined is shown in Fig. 1. Detailed measurements of each layer and its component structures for each species are also provided (Table 1). The overall corneal structure of all three species is similar. The corneas of species from three photic zones (namely the euphotic, the mesopelagic and the benthopelagic zones) showed evidence of an epithelium. However, as a result of the method of collection, all except one cornea (*Microgadus proximus*) were devoid of a complete epithelium. Hence, the thickness of only one species could be estimated.

All three species have a well-developed epithelial basement membrane, although that of *Coryphanoides (Nematonurus) armatus* is particularly thick (1.8 µm). Anchoring fibrils are evident in all species and are particularly prominent in *M. proximus* and *C. (N.) armatus* (Fig. 2A, B, D). Only *Nezumia aequalis* has a thin Bowman's layer (0.2 µm in thickness) of randomly arranged collagen fibrils (Fig. 2C).

There are three separate collections of collagen fibril lamellae forming three stromas (Fig. 1). The central thickness of these stromas varies considerably (Table 1) with the dermal stroma (or spectacle) being thickest (up to 0.18 mm), followed by the anterior scleral stroma (30 to 53 µm) and the posterior scleral stroma (2.7 to 6 µm). The central and peripheral stromal thickness is similar for each species except for the anterior scleral stroma of *M. proximus*, which is approximately 30 µm thick centrally and 40 µm peripherally. All stromas are composed primarily of collagen fibrils arranged in lamellae in which the fibrils are parallel and at right angles to those in adjacent lamellae. Except for the anterior scleral stroma of *M. proximus*, all have collagen fibrils passing antero-posteriorly (sutural fibres) between lamellae (Figs. 2B, C, 3A, B).

The collagen fibrils of the dermal and scleral stromas in all species are of similar diameter (around 25 nm, range 20 to 27 nm) and with similar macro-

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Table 1. Summary of corneal measurements in the three species examined. Where a range is quoted there is considerable intra- as well as inter-specimen variation. When tissue appeared distorted or where insufficient readings were available for calculations of standard deviation, an approximate measurement is given (-). ER: endoplasmic reticulum; OP: only peripheral; Post, posterior; RDC: removed during capture; °: the thickness of the mucoid layer may be artificially large due to the loose attachment between the dermal and scleral corneas. The recorded depth ranges quoted are from Grzimek (1984)¹, Merrett et al., (1991a,b)^{2,3}.

	<i>Microgadus proximus</i>	<i>Nezumia aequalis</i>	<i>Coryphanoides (Nematonorus) armatus</i>
Family	Gadidae	Macrouridae	Macrouridae
Size (SL) (mm)	240-260	280-285	430-535
Depth range (m)	34-250 ¹	445-1512 ^{2,3}	2172-4850 ^{2,3}
Maximum depth dredged (m)	100	730	4000
Epithelium (number of layers)	6 layers	RDC	RDC
Basement membrane (μm)	~0.30	~0.35	1.80 \pm 0.28
Bowman's layer (μm)	No	~0.2	No
Dermal stroma			
Thickness (μm)	\geq 70	~140	~180
Sutures (anterior)	Yes	Yes	Yes
Collagen fibril diameter (nm)	26 \pm 4	20 \pm 3	27 \pm 6
Collagen fibril periodicity (nm)	~6	~6	~6
Keratocytes	Few	Few (OP)	Few
Aligned endoplasmic reticulum	Yes	No	No
Mucoid layer			
Thickness (μm)	~60	~500°	~200°
Collagen fibrils present	Yes	Yes	Yes
Anterior scleral stroma			
Central thickness (μm)	~30	~50	~53
Peripheral thickness (μm)	~40	~50	~53
Sutures	No	Yes	Yes
Collagen fibril diameter (nm)	22 \pm 5	25 \pm 3	22 \pm 6
Collagen fibril periodicity (nm)	~6	~6	~6
Keratocytes	No	Few (Post)	Few
Iridescent layer			
Central thickness (μm)	~2.3	~5	~4
Peripheral thickness (μm)	~23	~20	~8
Collagen present	Yes	Yes	Yes
Aligned endoplasmic reticulum	Yes	No	No
Posterior scleral stroma			
Thickness (μm)	~6	~2.8	2.7 \pm 0.4
Sutures	Yes	Yes	Yes
Collagen fibril diameter (nm)	22 \pm 5	20 \pm 3	21 \pm 2
Keratocytes	No	No	No
Descemet's membrane thickness (μm)	0.10 \pm 0.02	0.20-0.50	0.23 \pm 0.03
Endothelium			
Thickness (μm)	2-3	2-3	1.5-2.5
Aligned endoplasmic reticulum	Yes	No	No

periodicities (6 nm). As with other teleost species, there are a few scattered cells (keratocytes) present in the dermal stroma, although in *N. aequalis* they appear to be only in the peripheral cornea. Cells are not found in the anterior and posterior scleral stromas in any of the three species. In *M. proximus*, the endoplasmic reticulum of the keratocytes in the dermal stroma is aligned in layers parallel to the corneal surface (Fig. 3A, B). There is no alignment in the other two species.

Between the dermal stroma and the anterior scleral stroma of all three species is a mucoid layer. This measures up to 0.5 mm in *N. aequalis* and 0.2 mm in *C. (N.) armatus*. However, because of the nature of the tissue, this layer may have become enlarged during

histological preparation. In all three species, the mucoid layer consists of loose tissue with thin cells (and cell processes) and occasional small bundles of collagen fibrils (Fig. 4).

The anterior scleral stromas of all three species are similar in structure and dimensions. There are multiple lamellae of collagen fibrils (22 to 25 nm in diameter and 6 nm macro-periodicity) but no cells (Fig. 4D). Sutural fibres are present in *N. aequalis* and *C. (N.) armatus* but were not seen in *M. proximus*.

Between the anterior and posterior scleral stromas of all three species is an iridescent layer, which is thin in the centre (2.3 to 5 μm) and thicker in the periphery (up to 23 μm , Table 1). Centrally, it is primarily cellular,

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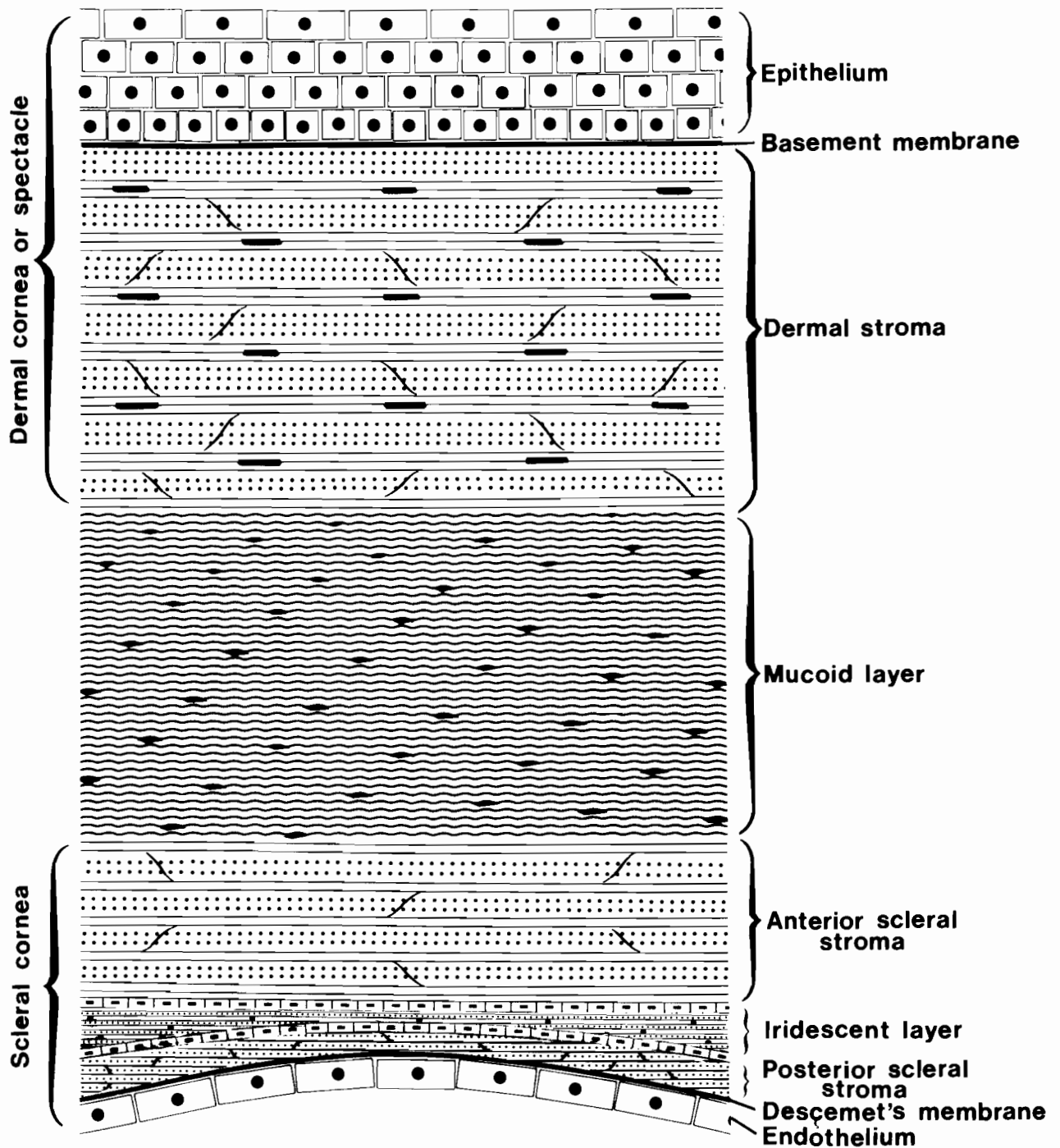
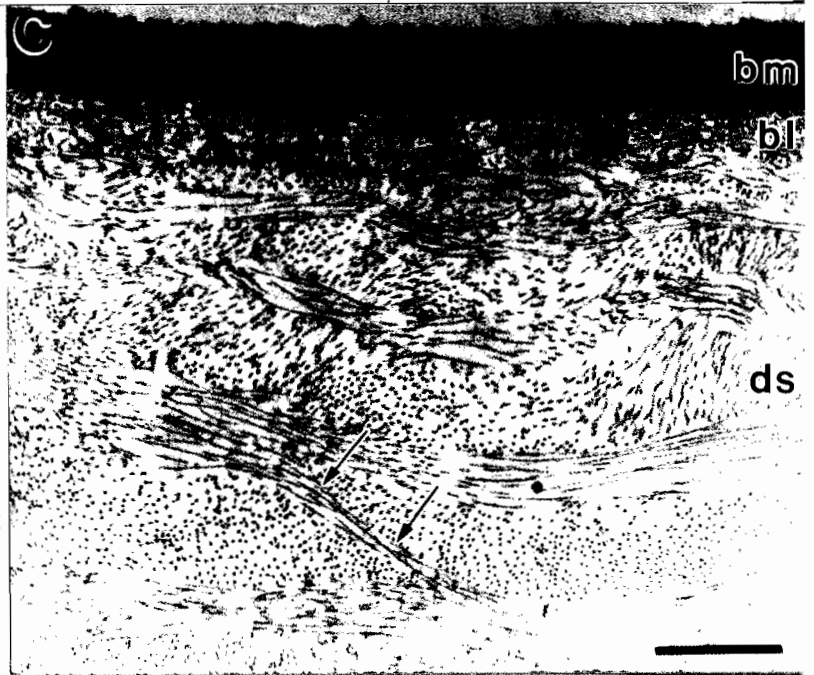
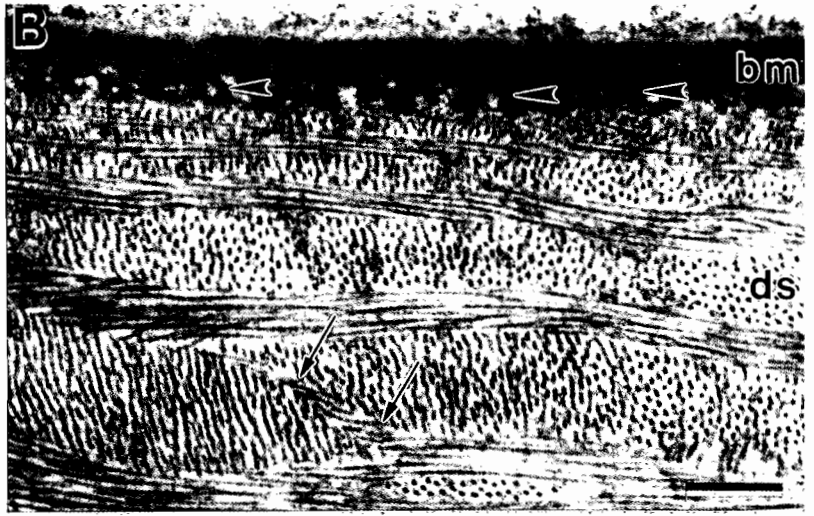
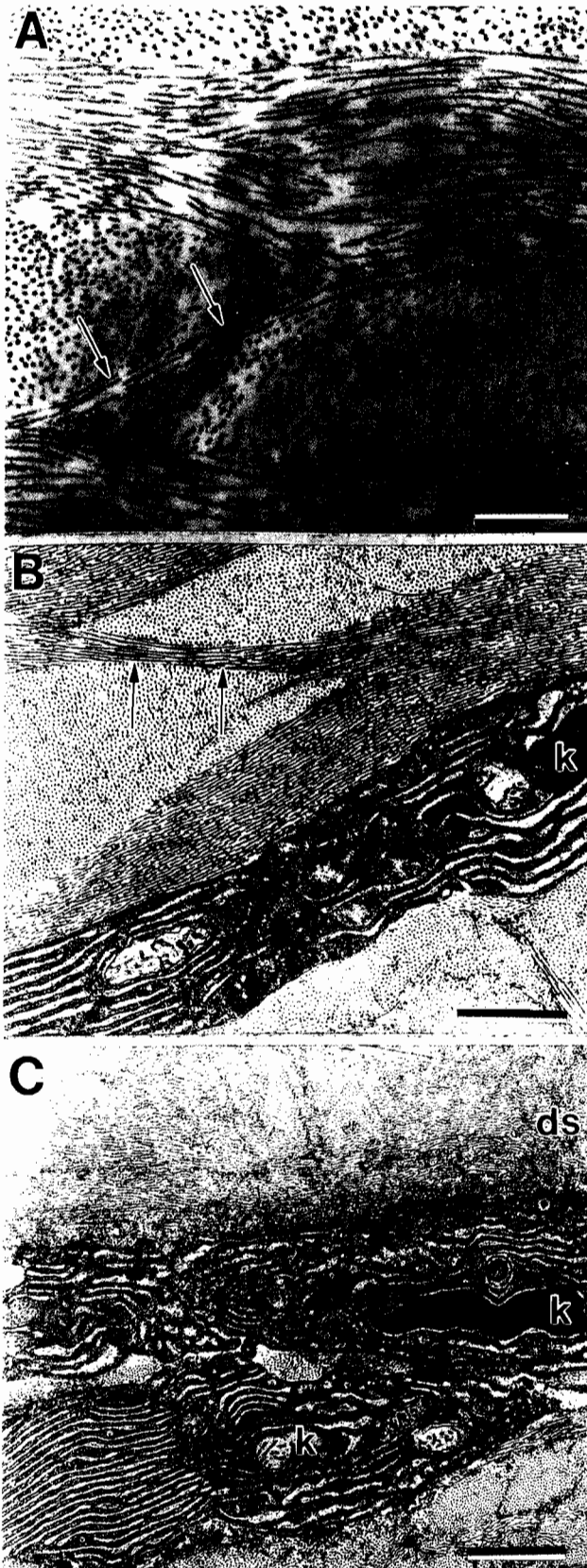


Fig. 1. Schematic drawing of the central region of the gadiform cornea in transverse section showing the arrangement of layers and their approximate relative size although some species show some differences (see Table 1). Incident light strikes the epithelium. The oblique lines within the dermal, anterior scleral and posterior scleral stromas depict sutural fibres traversing collagen lamellae. The dark bars represent keratocytes within the lamellae of the dermal stroma and cells within the mucoïd layer. The iridescent layer is bordered by a cellular monolayer and the dark rectangles represent nuclei. All layers and structures are not represented in all species.

Fig. 2. A. Light micrograph of a transverse section of the central cornea of *Coryphanoides (Nematonurus) armatus* showing the arrangement of layers. Note the absence of an epithelium which was removed during capture. The large and small arrowheads depict the iridescent layer and endothelium, respectively. B-D. Electron micrographs of the basement membrane (bm) and dermal stroma (ds) in the corneas of *Microgadus proximus* (B), *Nezumia aequalis* (C) and *C. (N.) armatus* (D). Note the prominent anchoring fibrils (arrowheads) and sutures (arrows). ass: anterior scleral stroma; ml: mucoïd layer; pss: posterior scleral stroma. Bars: A, 50µm; B-D, 0.5µm.





consisting of only two or three layers of cells, without collagen in *M. proximus* or with thin bands of collagen in *N. aequalis* and *C. (N.) armatus*. In the peripheral cornea of *N. aequalis*, there are approximately 17 layers of cells interspersed with collagen fibrils (Fig. 5B). However, in *C. (N.) armatus* (8 μm) and particularly *M. proximus* (23 μm), the thicker peripheral iridescent layer is composed primarily of collagen fibrils with a few cells. In *N. aequalis* and *C. (N.) armatus*, the cells of the iridescent layer are thin and flat. However, in the central cornea of *M. proximus*, the endoplasmic reticulum is arranged parallel to the corneal surface (Fig. 5C). In the peripheral regions, the endoplasmic reticulum is sometimes arranged in a circular pattern (Fig. 5D). This was not observed in the other two species.

In the posterior cornea of all three species is a well-formed Descemet's membrane (0.1 to 0.23 μm thick, Fig. 6) and a single layer of cells or endothelium (2 to 3 μm thick). Again, as observed in the keratocytes and the cells of the iridescent layer, the endoplasmic reticulum of the corneal endothelial cells of *M. proximus* is aligned parallel with the corneal surface (Fig. 6A). An annular ligament was not observed in the irido-corneal angle in any of the three species.

Discussion

The three gadiform species examined are all benthic scavengers which inhabit the cold, deep water of the Pacific (*Microgadus proximus*) and Atlantic (*Nezumia aequalis* and *Coryphanoides [Nematonurus] armatus*) oceans. The Pacific tomcod, *M. proximus* (Gadidae) inhabits the lower euphotic zone to depths of 250 m where light levels have diminished to one thirtieth of their surface intensity. The benthopelagic *N. aequalis* (Macrouridae) lives between 445 and 1,512 m (Merrett et al., 1991a,b), where only low levels of sunlight penetrate 10-9 W m^{-2} compared to 1 kW m^{-2} at the surface (Denton, 1990). The armoured grenadier, *C. (N.) armatus* (Macrouridae), one of the predominant species found below 2,200 m (with a maximum limit of 4,850 m, Merrett et al., 1991a,b; Table 1), lives below the penetration limits of sunlight (approximately 1,000m, Denton, 1990). All three species are closely related but each inhabits a different photic zone of the deep-sea.

The most remarkable feature of the gadiform cornea is the number of subdivisions or layers which contain collagen. Each cornea is divided into three stromas; a dermal stroma and two scleral stromas. Between the two scleral stromas is an iridescent layer comprising a series of cells or cellular processes also separated by collagen (Fig. 5). The propensity of the cornea to split into dermal (continuous with the underlying epithelium of the conjunctiva and skin) and scleral (continuous with the

Fig. 3. A. Electron micrograph of the sutural fibres (arrows) in the dermal stroma of *N. aequalis*. B, C. Sutural fibres (arrows in B) and aligned endoplasmic reticulum within keratocytes (k) in the dermal stroma (ds) of *M. proximus*. Bars: A,B, 0.5 μm ; C, 1.0 μm .

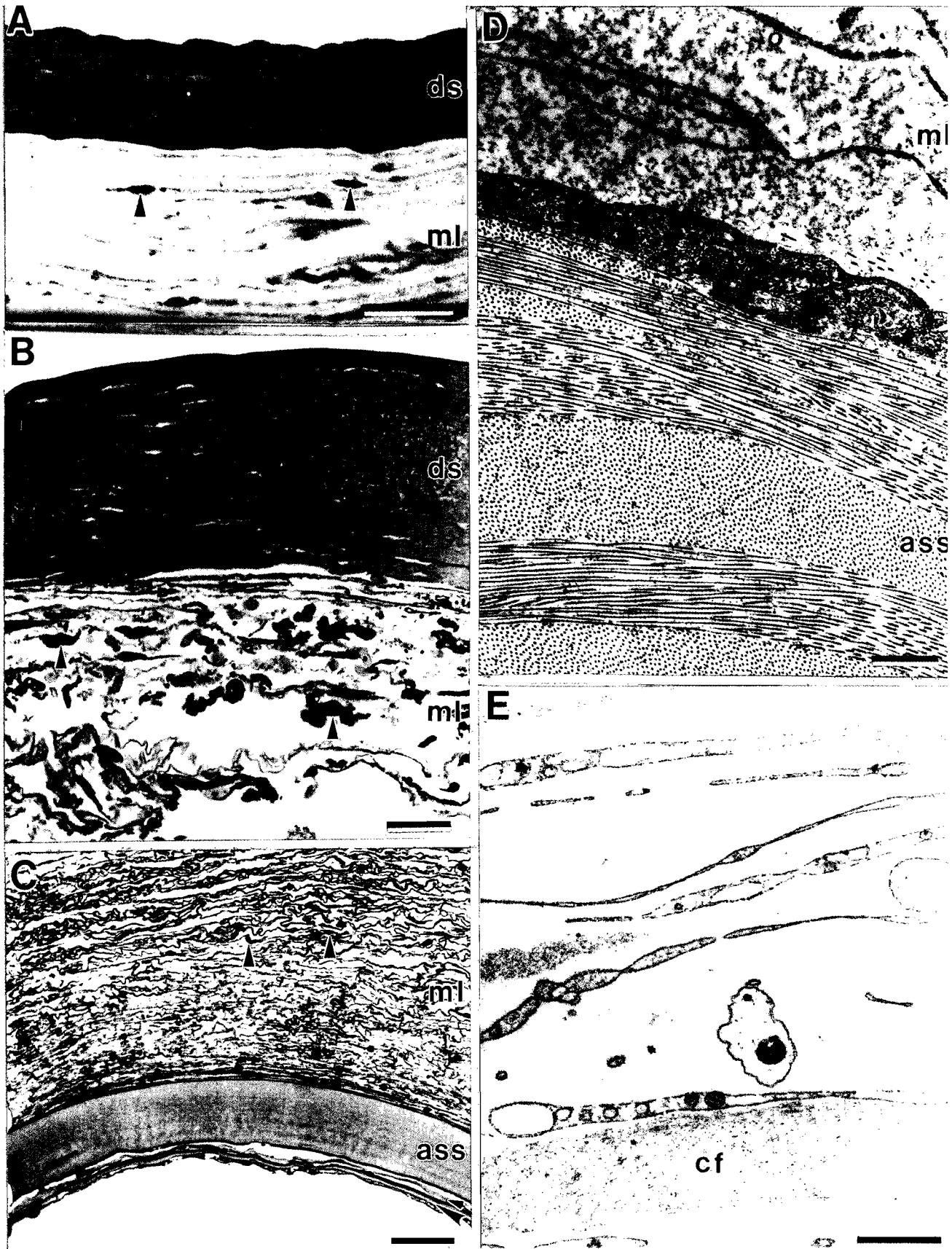


Fig. 4. **A, B.** Light micrographs of the dermal stroma (ds) and mucoid layer (ml) in *M. proximus* (A) and *N. aequalis* (B). **C.** Light micrograph of the mucoid layer (ml) and the anterior scleral stroma (ass) in *C. (N.) armatus*. The arrowheads within the mucoid layer depict cell nuclei. The large and small arrowheads in C depict the posterior scleral stroma and the iridescent layer, respectively. **D.** Electron micrograph of the transition between the mucoid layer and the anterior scleral stroma in *M. proximus*. **E.** High power electron micrograph of the cellular processes and collagen fibrils (cf) in the cornea of *C. (N.) armatus*. Bars: A, 75 μ m; B, 40 μ m; C, 50 μ m; D, 1.0 μ m; E, 1.5 μ m.

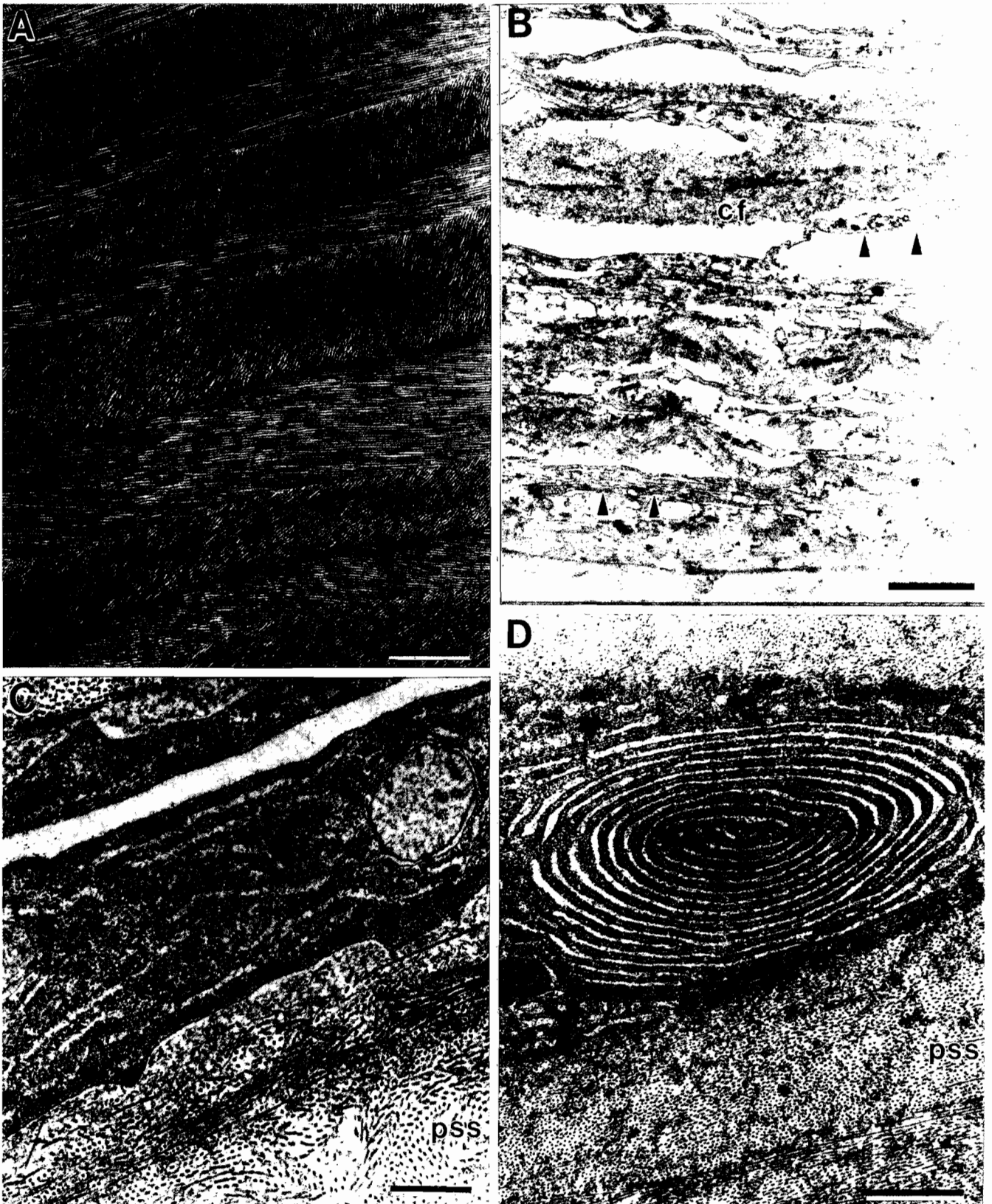
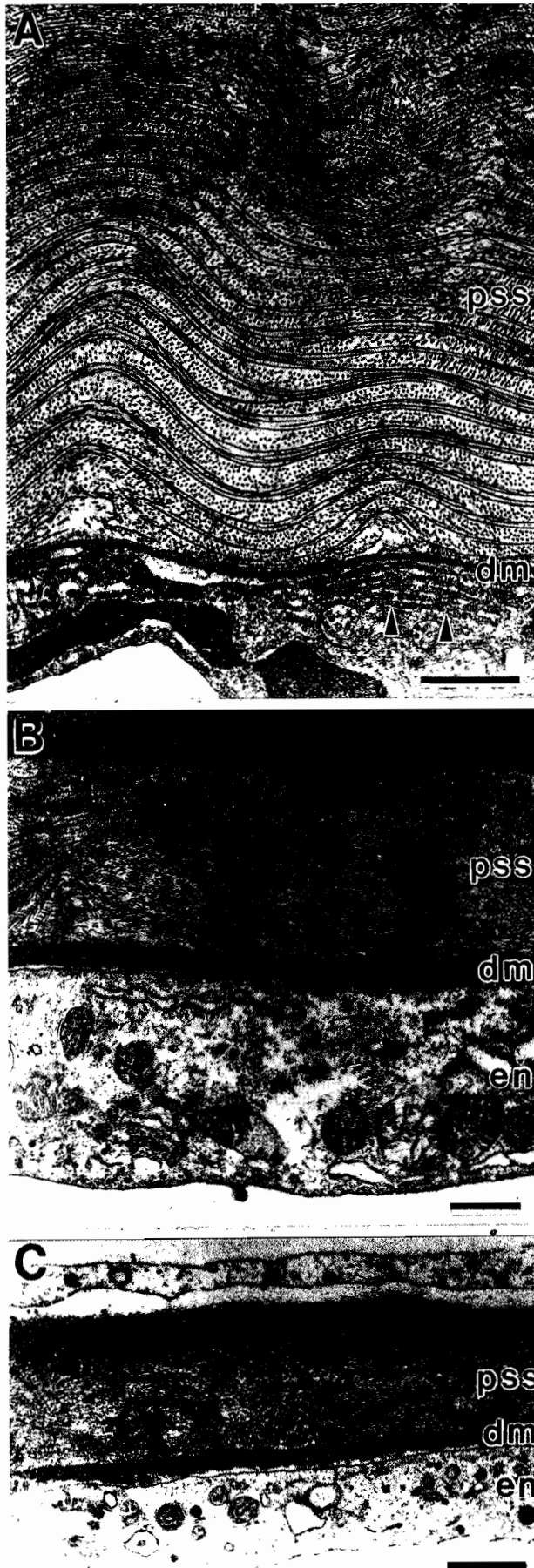


Fig. 5. A. Electron micrograph of the anterior scleral stroma of *M. proximus* showing the orientation of collagen lamellae. B. The cellular processes (arrowheads) and collagen fibrils (cf) in the iridescent layer of *N. aequalis*. C, D. The iridescent layer of *M. proximus* showing the parallel (C) or circular (D) arrangement of endoplasmic reticulum in central and peripheral cornea, respectively. pss: posterior scleral stroma. Bars: A, 1.5 μ m; B, 2.0 μ m; C, 0.5 μ m; D, 2.0 μ m.



sclera surrounding the globe) components has previously been found in a number of amphibious and benthic shallow-water species (Walls, 1942) but the subdivision of the scleral cornea seems to be unique to the deep-sea gadiforms. Whether these divisions are structurally and biochemically different, possessing different types of collagen and different hydration properties, as found for the dermal and scleral stromas of the shallow-water whiting, *Merlangus merlangus* (Moczar and Moczar, 1972), or simply represent one scleral stroma separated by cells and an iridescent layer is unknown. The increased corneal thickness produced by the intercalation of these various layers may incur some advantages including providing support, an effective hydration barrier and suitable interference patterns.

Even after allowing for differences in specimen size, the total corneal thickness and the thickness of the dermal and anterior scleral stromas is greater in species inhabiting deeper water (i.e. 70 μm and 180 μm in the dermal stromas of *M. proximus* and *C. (N.) armatus*, respectively, Table 1). This may provide additional strength or reflect a need to incorporate more layers of collagen with different structural and biochemical properties, for the maintenance of corneal transparency at low temperatures (Fisher and Zadunaisky, 1977). By way of comparison with shallow-water fishes, the thickness of the dermal stroma is 4.5 μm in *Limnichthyes fasciatus* (Collin and Collin, 1988), 16 μm in the mosquitofish, *Gambusia affinis* (Lantzing and Wright, 1982) and 23 μm in the pipefish, *Corythoichthyes paxtoni* (Collin and Collin, 1995).

The thin layer of randomly arranged collagen fibrils or Bowman's layer is only found in *N. aequalis*. Its function in teleosts (or any vertebrate) is unknown but Edelhauser and Siegesmund (1968) suggest that "Bowman's membrane" may be an adaptation to an aquatic environment and may act as a corneal barrier to sodium and water movement. Due to the interruption in the regularity of the orientation of the collagen fibrils, we suggest that Bowman's layer may also provide some additional strength and inhibit splitting, however its presence in a number of disparate vertebrate groups (see review in Collin and Collin, 1993) including humans, provides few insights into its function or phylogeny.

The thickness of the collagen fibrils throughout the corneas of all three species were relatively constant in the range 20 to 27 μm . This is similar to findings for mammals (23 to 26 nm, Craig and Parry, 1981), but slightly greater than for bony fish (16 to 18 nm, Craig and Parry, 1981) using techniques similar to those employed in this study. More recently, Craig and Parry (1989) claimed that using low temperature preparative techniques enhanced the preservation of collagen fibril

Fig. 6. Electron micrographs of the posterior scleral stroma (pss), Descemet's membrane (dm) and the endothelium (en) in *M. proximus* (A), *N. aequalis* (B) and *C. (N.) armatus* (C). Note the alignment of the endoplasmic reticulum (arrowheads) within the endothelial cells of *M. proximus*. Bars: A, B, 1.0 μm ; C, 2.0 μm .

structure and suggested that the "in vivo" corneal collagen fibril diameters should be revised from 17 nm to 25.5 nm for bony fishes and from 25 nm to 36 nm for all other classes of vertebrates.

Sutures or sutural fibres were observed in the three stromas of each species (except the anterior scleral stroma of *M. proximus*). These bundles of 1 to 5 collagen fibrils traverse from one stromal lamella across another in which the fibrils are at right angles and join the next lamella (Figs. 1B,C, 2A,B). Similar structures have been described in the salamanderfish, *Lepidogalaxias salamandroides* (Collin and Collin, 1996), the sea lamprey, *Petromyzon marinus* (Van Horn et al., 1969; Pederson et al., 1971), various species of cartilaginous fishes (Keller and Pouliquen, 1988; Conrad et al., 1994) and in the developing chick cornea (Hay and Revel, 1969; Bee et al., 1988). However, these sutures appear to be different from those described here, consisting of bundles of scattered microfibrils which terminate in triangular insertions and traverse more than one stromal lamellae almost at right angles. It is unknown whether these two types of sutures are functionally different. In the clearnose skate, *Raja eglanteria*, the sutural fibres form as the basal layer of epithelial cells sends strands of collagenous fibrillar material into the underlying stroma (Conrad et al., 1981; 1994). The functions of sutural fibres have been investigated in elasmobranchs and teleosts and they are thought to inhibit swelling of the cornea when it is immersed in fresh or distilled water (Smelser, 1962; Edelhauser et al., 1981; Wheaton and Edelhauser, 1983; Menasche et al., 1988). It is unlikely that the osmotic environment of the deep-sea species examined would change appreciably, so the sutural fibres are more likely to provide the structural links necessary to inhibit the cornea from swelling in extreme cold (Fisher and Zadunaisky, 1977). These three species are exposed to deep-sea temperatures between 2 and 5 °C. At these temperatures, the teleost cornea has been found to swell and lose transparency, becoming opaque (Smelser, 1962; Fisher and Zadunaisky, 1977). In contrast, the elasmobranch cornea fails to swell under the same conditions, relying on sutural fibres to anchor the stromal lamellae. Therefore, the presence of sutural fibres in the corneas of these three deep-sea species, may be an adaptation for maintaining transparency by inhibiting stromal swelling.

All three species possess a thick mucoid layer between the dermal and scleral corneas. This collection or loose aggregation of thin cells and cellular processes with occasional bundles of collagen is thick but an accurate assessment was not possible given the affinity of the dermal and scleral components of the cornea to separate. This propensity of the cornea to split is common to many teleost corneas and may allow movement of the globe beneath the protective dermal cornea or spectacle. The appearance of this mucoid layer seems to differ among the three species but appears similar to the loose connective tissue zone of the sea

lamprey, *Petromyzon marinus* (Dickson et al., 1982) and the eel, *Anguilla anguilla* (Walls, 1942) and the loose episcleral lamellar tissue in various other species of teleosts (Tripathi, 1974). In a few rare examples, this zone may also comprise an intraconjunctival space (i.e. in the milkfish, *Chanos chanos*, Nicol, 1989), a granular zone (i.e. in the pipefish, *Corythoichthyes paxtoni*, Collin and Collin, 1995) or even a refractive autochthonous layer (i.e. in the sandlance, *Limnichthyes fasciatus*, Collin and Collin, 1988; Pettigrew and Collin, 1995).

All three gadiform fishes possess what appears to be an iridescent layer between the anterior and posterior scleral stromas. This is the first study to find corneal iridescence in any members of the class Gadiformes. Therefore, together with the sargassum fish, *Histrio histrio* (Batrachoideiformes; Lythgoe, 1975) and the deep-sea anglerfish, *Lophius piscatorius* (Lophiiformes; unpublished data) which inhabits depths up to 800 m, the presence of corneal iridescent multilayers are now well represented in the Paracanthopterygii although more species must be examined in order to establish phylogenetic trends. These findings also conform to the current theory that suggests that only benthic or benthopelagic (demersal) species possess iridescent corneas but refutes any suggestion that iridescence is restricted to diurnal species above 400 m (Lythgoe, 1976).

The iridescent layer is similar in each species and comprises cells or cellular processes alternating with rows of collagen fibrils. Although appearing similar to that of the rabbitfish, *Siganus vulpinus* (Type 2a), its appearance is more reminiscent of the Type 3 iridescent layer of Lythgoe (1975) in both structure and position. One major difference between these deep-sea species and those previously described is that the cellular processes are oriented parallel to the endothelial surface rather than obliquely as is found in shallow-water fishes. In shallow-water, the oblique orientation of a series of reflecting plates causes interference often producing a coloured reflection (Lythgoe, 1971; Locket, 1972), which may act as a coloured reflector, a transmission filter, a polarising filter or as a sunshade (Lythgoe, 1976). In these deep-sea species the function of the iridescent layer is unknown. Although it is possible that these stacks of cellular processes may produce interference by filtering unwanted wavelengths of light from entering the eye at specific angles, especially in the relatively shallow *M. proximus*, a more plausible function may be for the suppression of reflection. The orientation of the cellular processes parallel to the surface of the epithelium (Fig. 1) may produce destructive interference on the optic axis depending on the spacing, thickness and refractive index of each layer. This type of interference may provide some advantage in camouflaging the thick multilayered cornea (and therefore the eye) in the twilight zone of dim downwelling sunlight (*M. proximus* and *N. aequalis*) or during bright flashes of bioluminescent emissions below 1,000

m (*C. (N.) armatus*).

The alignment of the endoplasmic reticulum (ER) in the endothelium, within the keratocytes of the dermal stroma and within the cells beneath the iridescent layer in *M. proximus* is unique because this suggests that there is more than one structure within the cornea that may produce interference. These regularly-spaced ER appear similar to the aligned ER in the iridescent cornea of the serranid, *Nemanthias carberryi* (Locket, 1972; Type 2b of Lythgoe, 1975) which produces a blue-green iridescent reflex with a wavelength around 546 nm (Locket, 1972). Although no iridescent reflex was observed in the preserved cornea of *M. proximus*, assuming that the spacing of the lamellae of the ER is $1/4 \lambda$ thick (Huxley, 1968), where λ is the wavelength of the preferentially reflected light, the iridescent reflex in *M. proximus* would be blue and approximately 350 nm (calculated from the lamellae spacing and assuming 11% shrinkage during histological processing). Therefore, there are two putative mechanisms for producing iridescent interference in *M. proximus*; an iridescent layer possibly used for the suppression of reflection and the alignment of ER which may reduce intraocular flare, at least in the upper limits of its depth range.

The posterior scleral stroma of all three gadiform fishes may represent the "autochthonous layer" described by Walls (1942) although it is composed of collagen fibres without keratocytes and does not appear to thicken peripherally. However, the posterior scleral stroma is located immediately behind the iridescent layer (composed of cells and collagen fibres) which is more extensive in the peripheral region and, together, these two layers may represent the autochthonous layer.

In summary, the interspecific differences in corneal structure may reflect functional adaptations of the visual system for life in different photic zones of the deep-sea. The reported differences in corneal thickness, the presence of sutural fibres and the putative interference produced by iridescent multilayered structures may all contribute to maximising the chances of survival at these inhospitable depths. However, many other cellular processes must also alter in order to survive the extremely high pressures and low temperatures. Membrane fluidity and the percentage of unsaturated fatty acids in the membrane lipids of the brain and ocular tissues also occurs in *N. aequalis* and *C. (N.) armatus* (Cossins and Macdonald, 1989). Further research of the biochemical, enzymatic and membrane properties of the corneal tissue of deep-sea teleosts will undoubtedly elucidate the mechanisms which give rise to at least some of the structural adaptations observed (Somero, 1992).

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