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Ultrastructure and organisation of the cornea, lens and iris in the pipefish, *Corythoichthyes paxtoni* (Syngnathidae, Teleostei)

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Summary. The corneas of nine pipefish, *Corythoichthyes paxtoni* (Syngnathidae, Teleostei), five freshly fixed and four museum specimens, were examined using light and electron microscopy. In transverse section, the surface of the corneal epithelium is covered by a complex series of ridges or microplicae which extends over the conjunctiva. The cornea is considerably thicker in the centre (80 μm) than in the periphery (40 μm) and can be separated into two distinct zones. The anterior dermal cornea (23 μm) consists of two layers of epithelial cells, a thick basement membrane (0.75 μm) and numerous lamellae of collagen fibrils with a few scattered keratocytes. This layer is continuous with the conjunctiva which also contains two layers of epithelial cells and lamellae of collagen fibrils. In the juvenile, separating the two zones, is a lens-shaped (concavo-convex) region approximately 6 μm thick in the centre and about 175 μm in diameter containing a fine granular material. In the adult, this region contains both granular material and fibres. It overlies the posterior zone which consists of an anterior iridescent layer (21 μm thick) possessing numerous cell processes parallel with the corneal surface and a few collagen fibrils. The scleral cornea contains 33 lamellae of collagen fibrils without cells and a single layer of cells with several cell processes, similar in appearance to the anterior iridescent layer, which may represent a second or posterior iridescent layer. There is a thick (2 μm) Desçemet's membrane and a thin (1.5 μm) corneal endothelium. There is a spherical lens close to the posterior corneal surface and the iris contains guanine crystals anteriorly and pigment granules posteriorly.

Key words: Cornea, Ultrastructure, Fishes, Iridescent layer, Growth, The pipefish, *Corythoichthyes paxtoni*

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Introduction

The pipefish, *Corythoichthyes paxtoni* (Syngnathidae, Teleostei) is a slow swimmer that actively feeds near the sea floor on microscopic zooplankton in shallow, well lit reef areas. It feeds primarily in a vertical position by sucking in its prey through its elongated snout. This species enjoys a high degree of independent eye mobility and possesses a deep convexiclivate fovea (S.P. Collin and H.B. Collin, unpublished observation). Previous studies on the visual system of members of the family Syngnathidae have all concentrated on either the retinal structure (Carriére, 1885; Verrier, 1928; Engström, 1963; Wagner, 1972) or the presence (Slonaker, 1897; Kahmann, 1934, 1936) or absence (Easter, 1992) of a foveal pit. Reports of the cornea in this group are limited to light microscopic analyses of the cornea and irido-corneal angle in species of the family Syngnathidae by Rauther (1925), the propensity of the inner and outer layers of the cornea of some species of Syngnathid pipefishes to separate (Harman, 1900 cited by Rauther, 1925; Harms, 1914 cited by Rauther, 1925), which is disputed by Lauber (1901, cited by Rauther, 1925), who claimed that for *Hippocampus antiquoroum*, the subcutaneous connective tissue bundle separating the dermal and the scleral corneae was not easily distinguished. In addition, Nicol (1989) reported the presence of both mucous and pigmented cells in the cornea of *Syngnathus sp.* and Lythgoe (1976) found an iridescent layer in the cornea of *Aulostoma maculatus*, (Syngnathiformes/Solenichthyes, Carcasson, 1977).

The aquatic cornea is thought to provide little or no refractive power due to the small difference in refractive index between the corneal tissue and the surrounding seawater (Sivak, 1990). However, it still constitutes a protective cover for the eye and provides an optically smooth surface and a transparent window. In comparison with the mammalian cornea many specialisations have been reported. These include spectacles (Walls, 1942),

corneal filters (Appleby and Muntz, 1979; Heinermann, 1984; Kondrashev et al., 1986), iridescent layers (Locket, 1972; Lythgoe, 1975a, 1976), an «annular ligament» (Tripathi, 1974) and/or an autochthonous layer (Walls, 1942; Collin and Collin, 1988), all of which are thought to provide some visual advantage to the animal.

In this study, the cornea, lens and iris of the pipefish, *Corythoichthyes paxtoni* were investigated using light and electron microscopy to establish the morphology and organisation of the anterior segment and provide an evolutionary perspective in the complex development of the teleost cornea.

Materials and methods

Five juvenile individuals (8-10 cm in length) of the pipefish, *Corythoichthyes paxtoni* (Syngnathidae, Teleostei) were collected on Heron Island reef in the Capricorn Bunker group of islands at the southern tip of the Great Barrier Reef under permit by the Great Barrier Reef Marine Park Authority (GBRMPA) and maintained in large, aerated holding tanks at the Heron Island Research Station (University of Queensland). Four additional adult specimens were also supplied by Dr. Mark McGrouther from the Australiam Museum in Sydney, Australia.

The juvenile specimens were killed with an overdose of tricaine methane sulphonate (MS222; 1:2,000) under the ethical guidelines of the National Health and Medical Research Council of Australia. The eyes were excised and five were immersed in 4% glutaraldehyde in 0.067M sodium cacodylate buffer (pH 7.2), fixing the cornea, lens and retina *in situ* for light microscopic examination. The four adult specimens from the Australiam Museum had previously been fixed in 10% formaldehyde and stored in 70% ethyl alcohol. The fixed tissue was embedded in historesin (Reichert-Jung) and sectioned at one to two microns on an American Optical microtome using a steel knife. Sections were stained with either toluidine blue or Richardson's stain, dehydrated and coverslipped for analysis with a compound light microscope (Olympus, BH-2).

The corneae of the remaining five eyes from the juvenile specimens were also fixed in 4% glutaraldehyde in 0.067M sodium cacodylate buffer (pH 7.2) and post-fixed in 2% osmium tetroxide with 1.5% potassium ferrocyanide in 0.1M sodium cacodylate buffer (reduced osmium method of Collin and Allansmith, 1977, which is a slight modification of the osmium potassium ferricyanide 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 then prepared for transmission electron microscopy, stained with lead citrate and examined on a Siemen's Elmiskop 1A electron microscope.

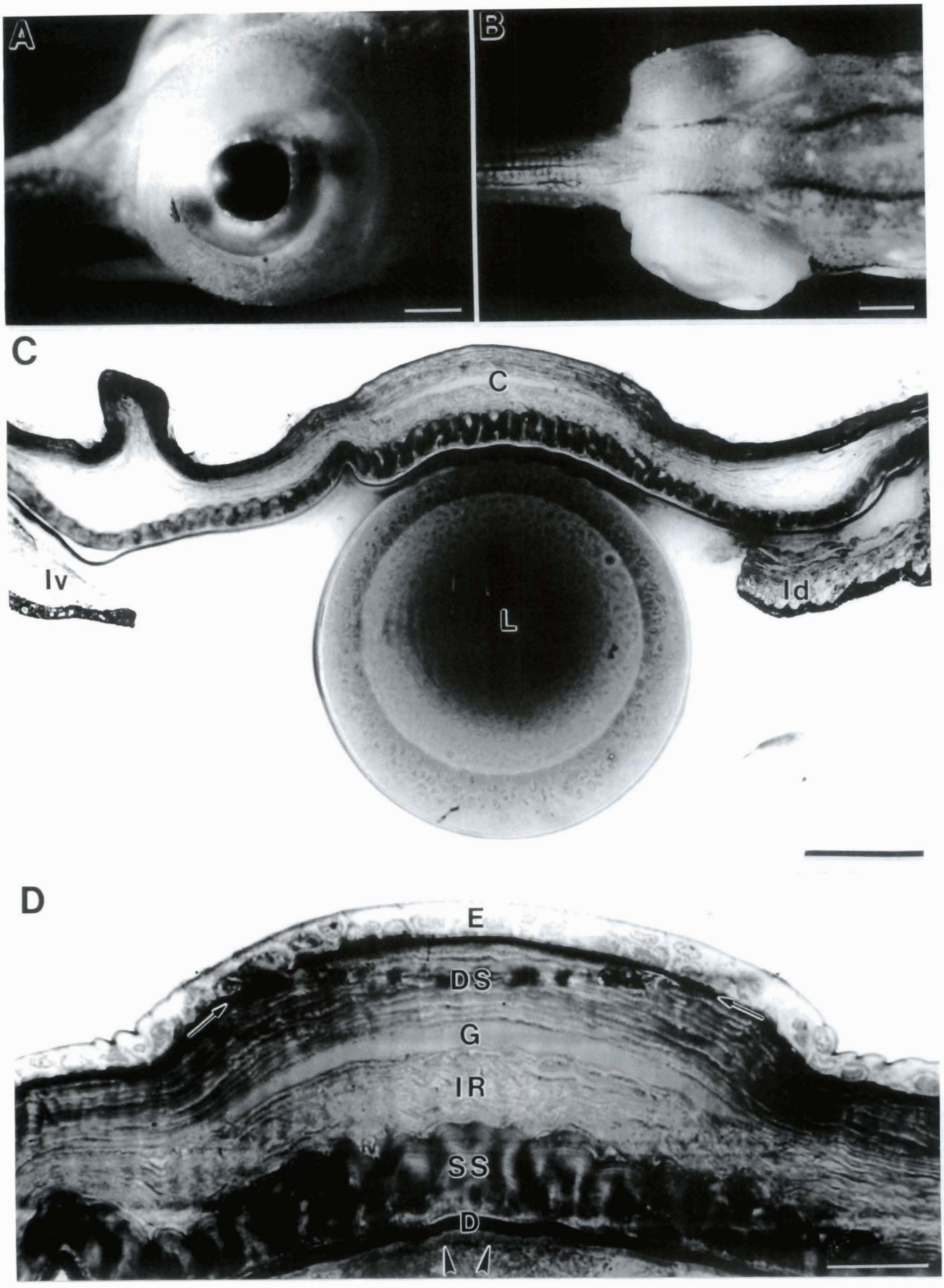
Measurements were made on enlargements of electron micrographs using a magnifier and graticule. Shrinkage (in linear dimensions) is known to be between 5 and 9% in tissue fixed in 4% glutaraldehyde and embedded in resin (Hayat, 1970). In order to minimise shrinkage, we post-fixed with osmium tetroxide, which causes swelling when used as a fixative and dehydrated our tissue in acetone, which is known to cause less shrinkage than ethanol (Hayat, 1970). Shrinkage has not been taken into account in the measurements reported which allows direct comparison with other previously published electron micrographs. Photographs were taken on either 35mm Kodak Technical Pan film (rated at 50 ASA, light microscopy) or Kodak 4489 electron microscopic film.

Results

The cornea of the juvenile pipefish (Fig. 1A,B) is considerably thicker in the centre (approximately 80 μm) than in the periphery (40 μm) (Fig. 1C,D). This is apparent in the young individuals since the distinct granular zone (see below) situated in central cornea increases in size and becomes fibrous forming a larger area of loosely arranged connective tissue dividing the dermal and scleral corneae in the adult.

There is a corneal epithelium 9 to 10 μm thick and consisting of two layers of cells. This bi-layered epithelium is continuous with that of the conjunctiva with a similar thickness. The large basal cells and the flatter superficial epithelial cells of the cornea are joined with numerous attachment devices including desmosomes and what might be zonulae adhaerentes. The surface of the corneal epithelium is covered by a complex series of projections or microplicae (Fig. 2A). These protrusions of the outer layer of epithelial cells are broad based and taper to a height of 0.14 μm, with a peak to peak separation of approximately 0.6-1.5 μm (Fig. 2A). Similar ridges are found on the conjunctival surface where they are larger (up to 0.62 μm in height) with a peak to peak separation (0.5-1.8 μm) and occur at the borders between adjacent epithelial cells (Fig. 2B,C).

Fig. 1. A. Lateral view of the head of the juvenile pipefish, *Corythoichthyes paxtoni* showing the eye and the slight dorso-temporal taper of its otherwise circular pupil. Bar= 1.5 mm. **B.** Dorsal view of the head of the pipefish illustrating the corneal curvature and circum-orbital fold in the conjunctiva of its two laterally placed eyes. Bar= 2.5 mm. **C.** Light micrograph of a transverse section close to the visual axis of the anterior segment showing the cornea (C) and closely apposing spherical lens (L). The corneal epithelium stained poorly and is not discernible in this micrograph. Note the ventral iris (Iv) is tapered relative to the dorsal iris (Id). Bar= 75 μm. **D.** Higher magnification of the cornea showing the upper dermal cornea separated from the lower scleral cornea by a lens-shaped (concavo-convex) zone of fine granular material (G). The dermal cornea comprises the epithelium (E), a basement membrane (arrows) and the dermal stroma (DS). The scleral cornea comprises a thick iridescent layer (IR), a scleral stroma (SS), Desçemet's membrane (D) and a thin endothelium (arrowheads). Bar= 25 μm.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Goblet cells are present in the conjunctival epithelium and the epidermis of the vestigial eyelid (Fig. 2B) but not in the corneal epithelium.

Below the corneal epithelium is a thick (0.75 μm) basement membrane, which becomes thinner (0.25 μm) beneath the conjunctiva and is separated from the epithelium by a clear zone (70 nm) (Fig. 2A,D).

The main body of the cornea can be divided into two regions. The anterior zone (about 23 μm thick) consists of numerous lamellae (up to 60) of collagen fibrils, with flattened cells (keratocytes) occurring between the lamellae (Fig. 2A). The keratocytes are separated by 3 to 6 lamellae. In the anterior of this zone, the lamellae are less well defined and are composed of only three layers of collagen fibrils with a fibril diameter of 17 to 20 nm. While the more posterior lamellae are thicker, a well defined Bowman's layer is not present. The fibrils of each thin lamella are approximately at right angles to those in adjacent lamellae. This portion of the cornea is continuous with the subepithelial conjunctival tissue and ultimately with the dermis and hence represents the dermal cornea or secondary spectacle described in some other benthic fishes.

The posterior zone has an anterior iridescent layer which is composed primarily of numerous bundles of long, thin, membrane-bound cell processes containing a few scattered organelles (Figs. 2E, 3A). The cell processes are parallel with the corneal surface and have a few thin lamellae of collagen fibrils scattered irregularly between the bundles.

Behind the iridescent layer is the scleral cornea which is approximately 19 μm thick and consists of 33 lamellae of parallel collagen fibrils, each lamella arranged with fibrils at right angles to those of its neighbours (Fig. 3B,C). The collagen fibrils are 22 to 24 nm in diameter, which is considerably smaller than those (up to 100 nm) of the non-corneal sclera, with which it is continuous. There are no cells or pigment interspersed between these lamellae or the collagen fibrils which is consistent with the finding of Nicol (1989) in a closely related species of pipefish, *Syngnathus sp.*

There is a prominent Desçemet's membrane (2 μm thick) extending to the periphery of the cornea (Figs. 1D, 4A). Anterior and parallel to this membrane and separating it from the scleral stroma is a continuous cellular monolayer. The non-nucleated part of these cells is formed into several thin membrane-bound layers (Fig. 4A,B). This resembles the anterior iridescent layer and could represent a second or posterior iridescent layer. The corneal endothelium, approximately 1.0-1.5 μm thick and comprised of a single layer of cells, is present behind Desçemet's membrane (Fig. 4A).

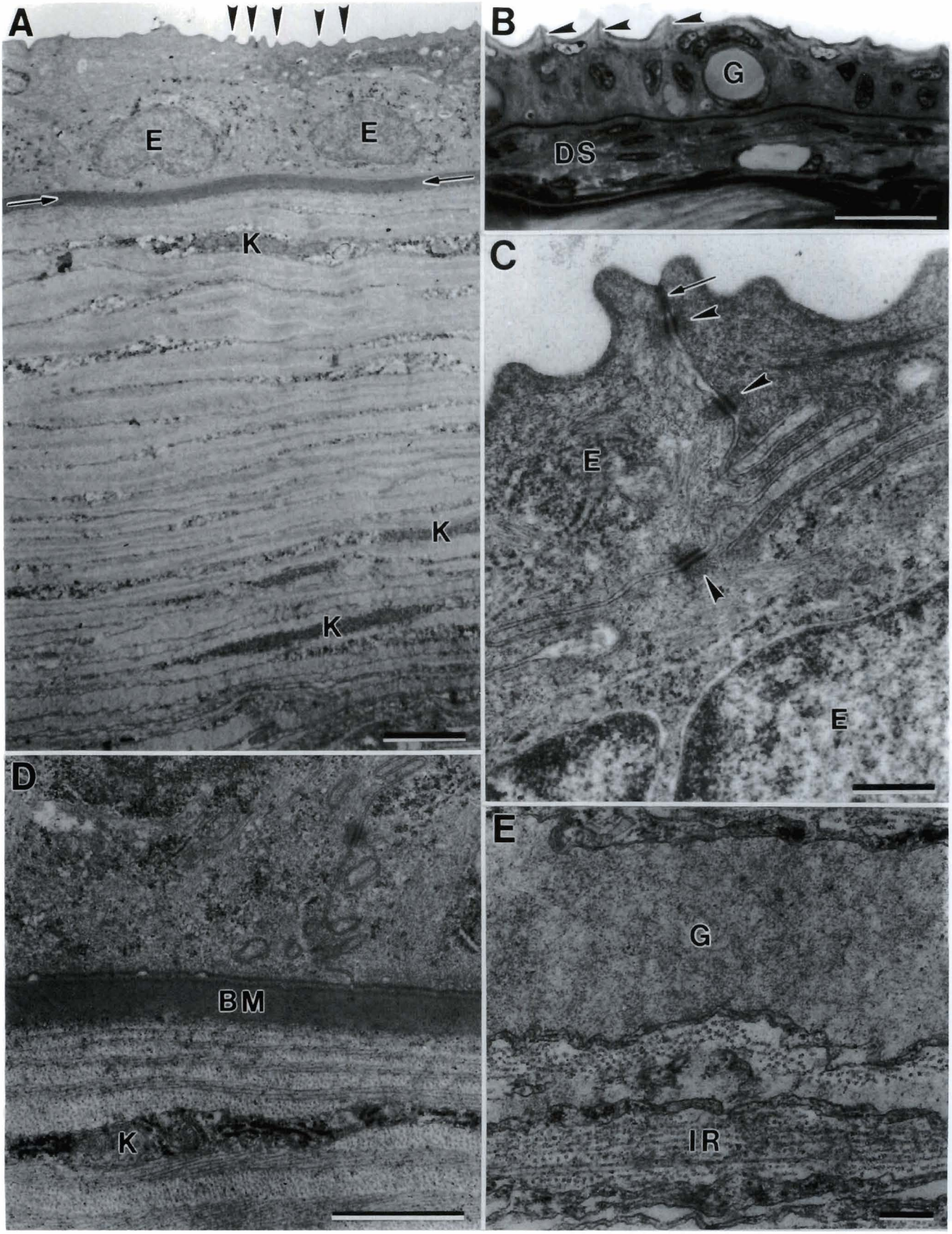
Between the dermal cornea and the iridescent layer in juveniles, is a lens-shaped (concavo-convex) zone, approximately 6 μm thick in the centre and about 175 μm in diameter (Fig. 1D). In juveniles, this structure is found only in the centre of the cornea, is lined with cell processes similar to those found in the iridescent layer and is filled with an amorphous or finely granular material (Fig. 2E). During growth, this granular zone increases in size and eventually separates the dermal cornea (or secondary spectacle) and the scleral cornea over their total diameter.

There is no autochthonous layer bordering Desçemet's membrane and no loose meshwork of cellulo-fibrous tissue, described as an «annular ligament» in various species by Tripathi (1974) , within the irido-corneal angle of *C. paxtoni.*

A spherical lens approximately 230 μm in diameter is positioned some 2 μm behind the central cornea and is separated from the corneal endothelium by a finely granular substance (Fig. 1C). There is an anterior lens epithelium, which extends just posterior to the equator. The lens is surrounded by a dense capsule, approximately 1.8 μm thick anteriorly and 1.4 μm posteriorly. The outer portion (0.7 μm thick) of the lens capsule is less dense and consists of granular material and fine fibres (Fig. 4C). Within the lens capsule is a concentric arrangement of cells surrounding an ill-defined lens nucleus. The lens is suspended dorsally by a suspensory ligament and ventrally by a ligament attached to the retractor lentis muscle which contains smooth muscle fibres with mitochondria and scattered pigment granules.

The pupil of the pipefish is circular except for a slight temporo-dorsal elongation. When viewed in transverse section, the iris tapers towards the ventral edge of the pupil, while the dorsal edge of the iris is thick and rounded (Figs. 1C, 5A,B). The posterior or retinal half of the iris consists of a thick epithelium containing pigment granules circular to ovoid in shape and 0.38 to 1.47 μm in diameter (Fig. 5A,B,D). This epithelium is continuous with the pigment granules of the inner layers of the retinal pigment epithelium. The pigmented epithelium is bordered by a basal lamina (Fig. 5D), which separates it from large collections of guanine crystals measuring up to 1.5 μm in diameter in the anterior or uveal component of the iris. The arrangement of guanine crystals appears random, forming loose aggregations among scattered cell nuclei (Fig. 5C), protrusions of cytoplasm, blood vessels and nerve bundles.

Fig. 2. A. Electron micrograph of the dermal cornea or secondary spectacle showing the fine ridges or microplicae (arrowheads) over the surface of the epithelial cells (E). The epithelium overlies a granular basement membrane (arrows) and a multi-layered (up to 60 lamellae) dermal stroma with scattered keratocytes (K). Bar= 5 μm. **B.** Light micrograph of a transverse section of the conjunctiva. Note the attachment devices (arrowheads) between adjacent epithelial cells. DS: dermal stroma; G: goblet cell. Bar= 20 μm. **C.** Electron micrograph of epithelial cells (E) in the conjunctiva showing three junctions resembling desmosomes (arrowheads) along their interdigitating cell membranes and what appears to be a zonula adhaerens at the cell surface (arrow). Bar= 0.5 μm. **D.** The thick basement membrane (BM) underlying the corneal epithelium and the thin anterior lamellae of collagen fibrils. K: keratocyte. Bar= 2.5 μm. **E.** Central cornea showing the thick granular zone (G) overlying the iridescent layer (IR). Bar= 0.25 μm.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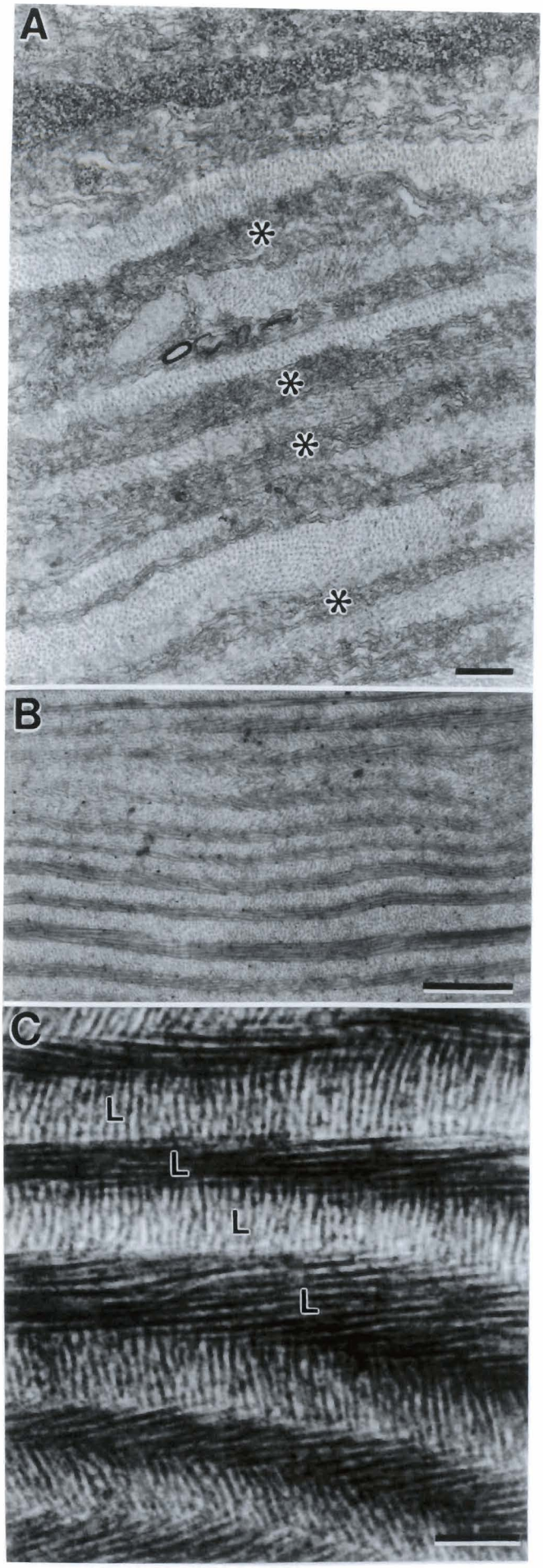


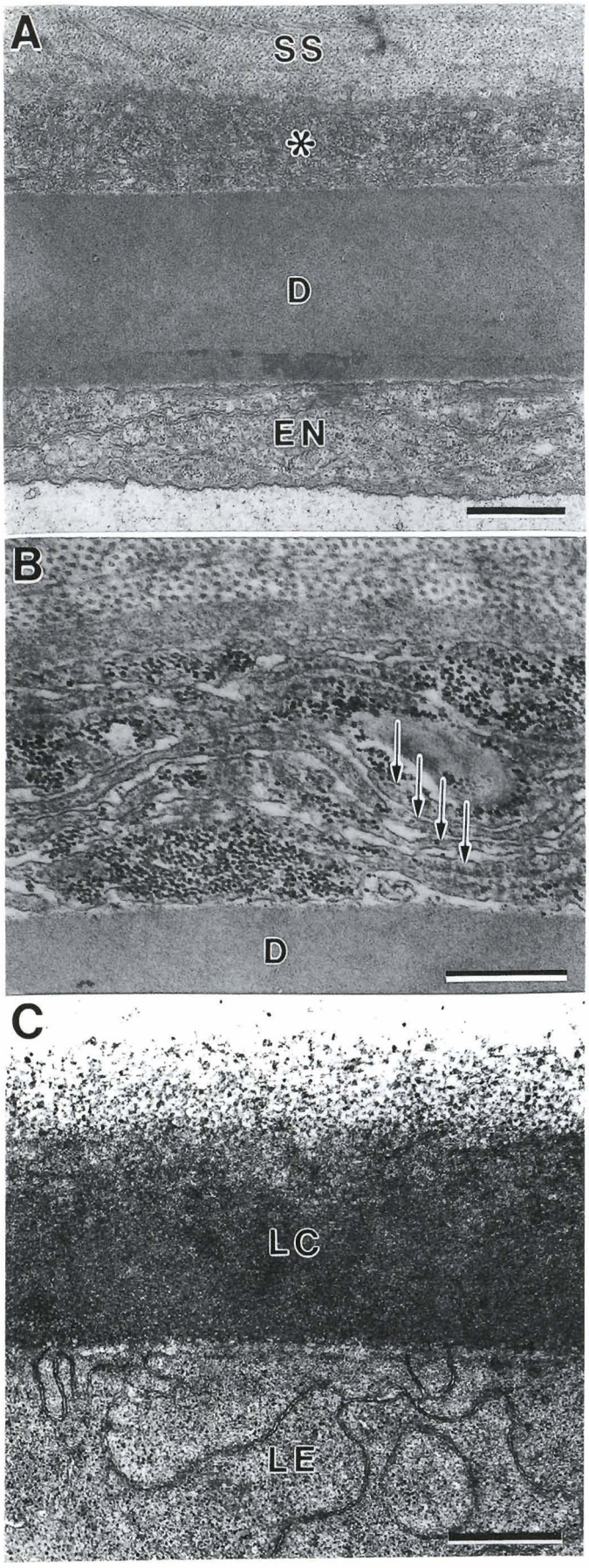
Discussion

The cornea of the pipefish, *Corythoichthyes paxtoni* is divided into distinct regions with the dermal cornea and the scleral cornea separated by a zone which is anterior to an iridescent layer and occupied by either granular material in the juveniles or loosely arranged connective tissue in the adults. The dermal cornea, or secondary spectacle, is composed of epithelial cells overlying numerous layers of tightly-packed collagen fibrils and is continuous with epithelial and collagen layers of the conjunctiva and skin. The scleral cornea is composed of numerous collagen lamellae, a Desçemet's membrane and an endothelium. The scleral cornea is continuous with the sclera of the globe.

The corneal thickness of the pipefish (80 μm in the centre) is similar to the urodele amphibian, *Triturus c. cristatus* (Margaritis et al., 1976) and appreciably thinner than the sandlance, *Limnichthyes fasciatus* (140 μm, Collin and Collin, 1988), the Florida garfish, *Lepisosteus platyrhincus* (240 μm, Collin and Collin, 1993), the dogfish, *Squalus acanthias* (250 μm, Goldman and Benedek, 1967) rabbits (329 μm, Maurice, 1969) and humans (530 μm, Maurice, 1969). This variation is in part due to the thickness of the epithelium. Only two layers of cells comprise the corneal epithelium of the pipefish, *C. paxtoni* (this study) and another pipefish, *Syngnathus sp.* within the same family (Nicol, 1989) while *L. fasciatus* (Collin and Collin, 1988) and *L. platyrhincus* (Collin and Collin, 1993) possess 5-6 and 8-10 layers of epithelial cells, respectively. The relative epithelial thickness varies greatly within the ray-finned fishes, e.g. the Florida garfish, *L. platyrhincus* and the brown trout, *Salmo trutta*, possess an epithelium which is 9% (Collin and Collin, 1993) and 40% (Edelhauser and Siegesmund, 1968) of their corneal thickness, respectivley.

Microplicae, as seen in transverse section of the pipefish cornea, appear to cover the surface of the cornea and are thought to stabilize the coating material, which is produced by the goblet cells of the conjunctiva, and maintain a smooth optical surface. Thought to be species specific (Harding et al., 1974; Collin and Collin, 1988), these patterned ridges may also increase the surface area of the epithelium aiding diffusion and active transport of salts and other solutes (Harding et al., 1974). Although the epithelium provides the most effective barrier to ionic movements between the aqueous humor and seawater (Candia et al., 1976), the relatively small size and spacing of the corneal microplicae in conjunction with a thin epithelium may indicate a reduced role in osmotic balance in this species.

Fig. 3. A. The iridescent layer comprising bundles (asterisks) of membrane-bound cell processes interspersed with collagen lamellae. Bar= 0.5 μm. **B.** Arrangement of collagen lamellae in the scleral stroma. Note the absence of keratocytes. Bar= 1 μm. **C.** Higher magnification of the scleral stroma in which parallel bundles of collagen fibrils are at right angles to those in adjacent lamellae. Bar= 0.25 μm.



The epithelial basement membrane of *C. paxtoni* is very thick and in view of the thin epithelium may help to provide a more effective barrier to the movement of substances in and out of the cornea. The basement membrane thickness of 750 nm in *C. paxtoni* compares with 40 nm in the Florida garfish, *L. platyrhincus* (Collin and Collin, 1993), 100 nm in rabbits (Margaritis et al., 1976) and 60 nm in humans (Dohlman, 1971).

The anterior dermal cornea of *C. paxtoni* does not have an obvious Bowman's layer, although the lamellae are very thin in the anterior stroma. In contrast, a true Bowman's layer with a random arrangement of collagen fibrils underlying the basement membrane of the epithelium is easily recognizable in the sea lamprey, *Petromyzon marinus* (Van Horn et al., 1969; Pederson et al., 1971), all elasmobranchs studied thus far (Keller and Pouliquen, 1988), the Florida garfish, *L. platyrhincus* (Collin and Collin, 1993) and in some species of teleosts (Lantzing and Wright, 1981; Shand, 1988) but not all (Collin and Collin, 1988). For those species possessing a Bowman's layer, its relative thickness varies markedly from less than 1% in the sea lamprey (Van Horn et al., 1969) to 1% in the garfish (Collin and Collin, 1993), 15% in elasmobranchs (Keller and Pouliquen, 1988) and 2.3% in humans (Dohlman, 1971). Thought to give additional strength to the anterior cornea, this random arrangement of collagen fibrils may also be an adaptation to an aquatic environment where, in combination with the epithelium it may act as a corneal barrier to sodium and water movement (Edelhauser and Siegesmund, 1968). The ontogeny and phylogeny of Bowman's layer is not well understood (Collin and Collin, 1993) and further research is necessary to explain its seemingly haphazard development throughout the vertebrate kingdom.

The central cornea of the juvenile pipefish is twice the thickness of the periphery, although this is not so apparent in the adult. Similar findings have been found in the trout, *Salmo fario* (Tripathi, 1974) and the sandlance, *L. fasciatus* (Collin and Collin, 1988) in which the central cornea is almost four times the thickness near the limbus. This is in contrast to the mosquitofish, *Gambusia affinis* (Lantzing and Wright, 1982), the blue-eye, *Pseudomugil signifer* (Lantzing and Wright, 1981), the garfish, *L. platyrhincus* (Collin and Collin, 1993) the salamander, *Triturus c. cristatus* (Margaritis et al., 1976), chicks (Renard et al., 1978), some mammals (Kayes and Holmberg, 1960; Hogan et al., 1971) and humans, where the peripheral cornea is

Fig. 4. A. The posterior of the scleral cornea showing the arrangement of layers. The scleral stroma (SS) overlies a monolayer of cells (asterisk), Desçemet's membrane (D) and the endothelium (EN). Bar= 1 μm. **B.** The cell layer depicted as an asterisk in A situated anterior to Desçemet's membrane (D) showing several layers formed by a series of thin membrane-bound cell extensions (arrows). The dark granules are glycogen. Bar= 0.5 μm. **C.** High magnification of the lens surface showing the lens capsule (LC) with less dense anterior portion. LE: lens epithelium. Bar= 1 μm.

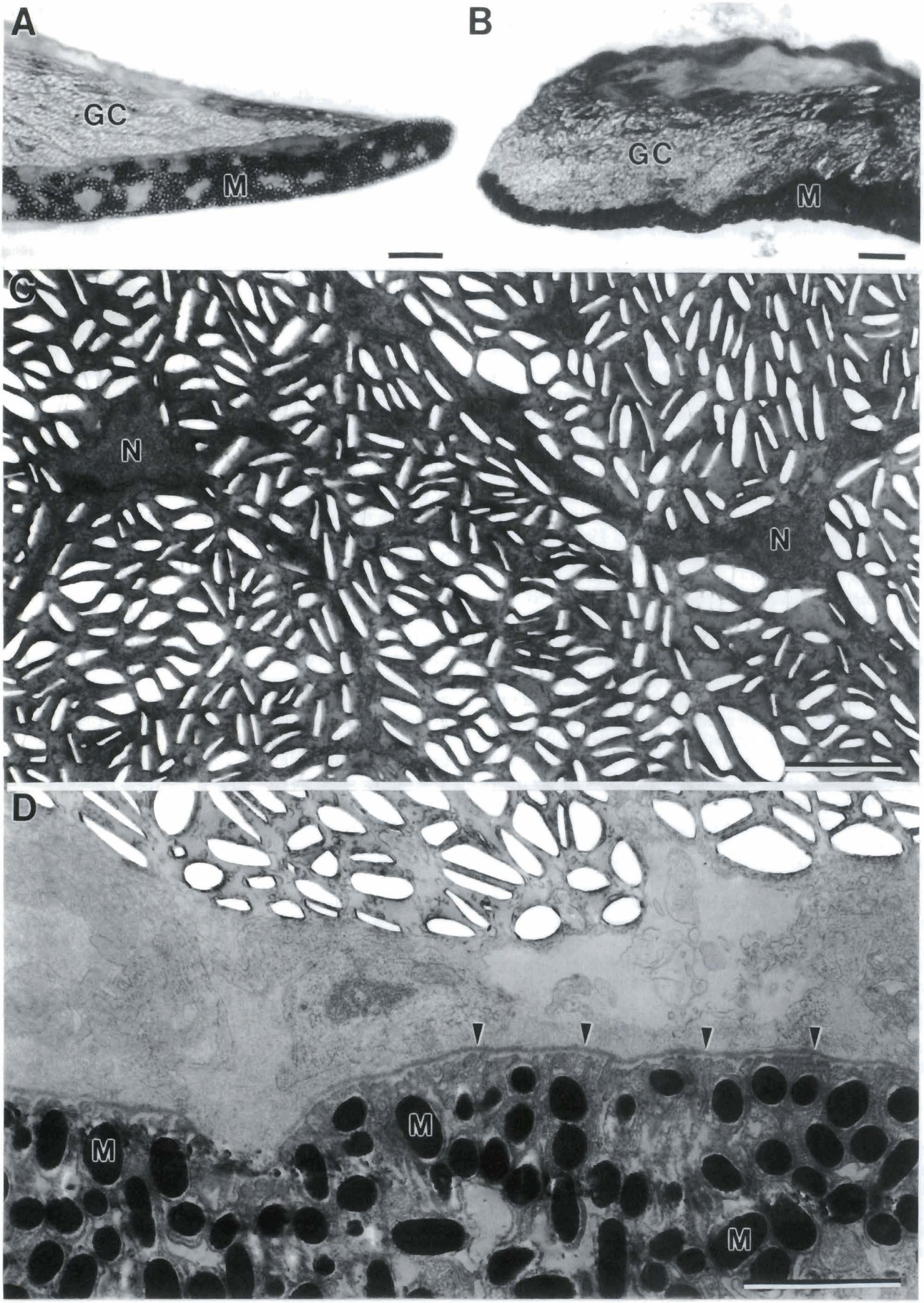
thicker than the centre.

The thick central cornea of *C. paxtoni* contains a lens-shaped inclusion of finely granular material. Other aquatic vertebrates also possess various corneal inclusions which include an autochthonous layer of large nucleated cells containing little cytoplasm and few organelles as in the sandlance, *L. fasciatus* (Collin and Collin, 1988), an intraconjunctival space as in the milkfish, *Chanos chanos* (Nicol, 1989) and the anchovy, *Engraulis sp.* (Hein, 1913), loose episcleral lamellar tissue as in various teleosts (Tripathi, 1974), loose connective tissue as in the eel, *Anguilla anguilla* (Walls, 1942) or mucoid tissue as in the sea lamprey, *Petromyzon marinus* (Van Horn et al., 1969) and *Syngnathus sp.* (Nicol, 1989) all of which increase the thickness of the central cornea. This lens-shaped area between the dermal and scleral corneae in *C. paxtoni* may be homologous to the thick layer of loose subcutaneous tissue between the dermal and scleral layers of lamellae in *Syngnathus typhle* described by Rauther (1925) or the mucoid or loose connective tissue zones of the lamprey, *Petromyzon marinus* (Dickson et al., 1982) and some fishes which develop a split cornea to enable movement of the scleral cornea behind the dermal cornea or secondary spectacle (Walls, 1942). The large size of this central granular zone in the juvenile pipefish and the propensity of the cornea to divide into dermal and scleral components during growth may reflect a higher degree of eye mobility beneath the protective spectacle in the adult. The central position of the granular zone in the juveniles may also result in an increase in the curvature and hence the refractive power of the anterior surface of the dermal cornea, although in seawater this effect is minimal.

This is the second report of an iridescent layer in the cornea of a species within the order Syngnathiformes (Solenichthyes). Described by Lythgoe (1976) to be type 3, the iridescent layer of the flutemouth, *Aulostomus maculatus* (Aulostomidae, Solenichthyes) consists of plates of protoplasm, probably whole cells, separated by an extracellular ground substance which is continuous with the ground substance surrounding the collagen fibrils in the stroma. However, although *C. paxtoni* is placed within the same order, the appearance of the main or anterior iridescent layer is quite different and resembles the type 1c of Lythgoe (1975a). This type of iridescent layer, comprising an ordered array of collagen fibrils interspersed between densely staining bands of material bounded by cell membrane, has not been reported previously outside the Scorpaeniformes group and appears similar to the iridescent layer of *Pholis gunellus* (Lythgoe, 1975a). The position of this anterior iridescent layer in *C. paxtoni* also differs from *A. maculatus*. It is situated between the central zone of granular material and the scleral corneal stroma in *C. paxtoni* while the protoplasmic plates of *A. maculatus* lie between the scleral corneal stroma and Desçemet's membrane (Lythoge, 1976). In this same position in *C. paxtoni* is a single layer of cells with multiple membrane-bound protoplasmic plates. This may represent a poorly developed or vestigial second iridescent layer. The presence of two iridescent layers in the one cornea has not been reported previously, and in fact the similarity of the two iridescent zones could indicate a similar origin or separation during development. At present, at least six morphologically distinct multi-layered arrangements have been reported in phylogenetically diverse groups of marine teleosts and, given further investigation, the structure and/or the position of the iridescent layer may be a useful trait in elucidating its phylogeny.

The function of the iridescent layer has been postulated to reduce intraocular flare thereby increasing visual range underwater without sacrificing sensitivity (Lythgoe, 1976). Other putative functions of this multi-layered stack include, birefringence, a coloured filter, a polarising filter, camouflage or display and the enhancement or suppression of reflection. The multi-layered iridescent layer of *C. paxtoni* is oriented parallel to the collagen lamellae of the scleral stroma and thereby is not perpendicular to the bright downwelling light as found in some other species. However, due to the inclusion of the concavo-convex layer of finely granular material in the central cornea, the orientation of the iridescent multistack forms an infinite number of different angles with the dermal stroma of the secondary spectacle and with the surface of the cornea. This arrangement is also found in the cornea of the sandlance, *L. fasciatus* (Collin and Collin, 1988). It is possible that the iridescent layer may not constitute a reflecting surface to reduce intraocular flare along the visual axis but acts as an anti-reflection device whereby light entering at normal incidence may show destructive interference, assuming the refractive indices of stroma and plates of the iridescent layer are sufficiently different (Lythgoe, 1975b). For light rays passing into the eye along the visual axis, this may be an advantage given the fact that light must be focussed into a temporal convexiclivate fovea (Collin and Collin, unpublished observation) and, as part of its natural behaviour, the pipefish frequently adopts a vertical posture with its eyes facing into bright downwelling light (Burton and Burton, 1975).

Fig. 5. Light micrographs of the ventral **(A)** and dorsal **(B)** regions of the iris. Note the differences in shape and relative thickness of the layers of guanine crystals (GC) and melanosomes (M). See Fig. 1C for orientation. Bars= 10 μm. **C.** Electron micrograph of the random arrangement of guanine crystals separated by scattered cell nuclei (N) and protrusions of cytoplasm. Bar= 1.5 μm. **D.** Posterior iris showing the basal lamina (arrowheads) of the epithelium containing pigment granules (M). Bar= 2.5 μm.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Desçemet's membrane is very thick (2μm) in *C. paxtoni* compared with 0.30 μm in the mosquitofish, *Gambusia affinis* (Lantzing and Wright, 1982) and 0.27 μm in the sandlance, *L. fasciatus* (Collin and Collin, 1988). In fact, Desçemet's membrane is only partially present in the Florida garfish, *L. platyrhincus* (Collin and Collin, 1993) and the trout, *Salmo fario* (Smelser and Chen, 1954) and is absent in the carp, *Cyprinus carpio* (Smelser and Chen, 1954) and the red gurnard, *Triglia cuculus* (Lythgoe, 1976). Using light microscopy Rauther (1925) found the thickness of the «stratum intimum», now referred to as Desçemet's membrane, to be 4 μm in *Syngnathus typhle*, a closely related species to *C. paxtoni*.

The thickness of the corneal endothelium in aquatic vertebrates varies greatly. An endothelial thickness of 1.5 μm in the pipefish is similar to that of the mosquitofish, *Gambusia affinis* (1.47 μm, Lantzing and Wright, 1982) but considerably greater than the lamprey, *Petromyzon marinus* (0.75 μm, Dickson et al., 1982) or the garfish, *L. platyrhincus*, (1.0 μm) (Collin and Collin, 1993). The endothelium appears to be replaced by a few glycogen-containing cells in the carp, *Cyprinus carpio* (Smelser and Chen, 1954) and is non-existent in elasmobranchs (Keller and Pouliquen, 1988).

The close proximity of the spherical lens to the scleral cornea in *C. paxtoni* is worthy of comment. Although the optical dimensions of the eye have not been investigated in living tissue, it is interesting to note that Slonaker (1897) found the lens in a similar position in the eye of the closely related pipefish, *Siphostoma fuscum* (suborder Syngnathoidei). This optical arrangement is reminiscent of the agnathan (Collin and Fritzsch, 1993) and avian (Schaeffel and Howland, 1987) eyes which rely on the multifocal lens properties and corneal accommodation, respectively, for maintaining the focus of light onto the retina. Presumably the axis of accommodation is in the rostro-caudal direction to aid in the focussing of light onto the deep convexiclivate fovea located in temporo-dorsal retina (Collin and Collin, unpublished observation). This hypothesis may be supported by the slight temporo-dorsal taper of the otherwise circular pupil. It is also interesting to note that in this region, the edge of the iris is rounded versus the tapered naso-ventral edge.

The conjuctiva and iris of *C. paxtoni* are camouflaged by a number of pigmented bands and spots, continuous with the disruptive colouration over the remainder of the head and body. This pigmentation fails to reach the cornea in *C. paxtoni* in contrast to *Hippocampus sp.* described by Lauber (1901, cited by Rauther, 1925). The iris also possesses densely-packed guanine crystals, similar to the iridophores in the conjuctiva and skin. These plates of guanine crystals are highly reflective and are thought to act as biological mirrors. In addition, this may help to camouflage the pupil in brightly lit water.

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