Ultrastructure and organisation of the retina and pigment epithelium in the cutlips minnow, *Exoglossum maxillingua* (Cyprinidae, Teleostei)

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Summary. The structure of the light- and dark-adapted retina, pigment epithelium and choriocapillaris of the cutlips minnow, Exoglossum maxillingua (Cyprinidae, Teleostei) is examined by light and electron microscopy. A pronounced vitreal vascularisation overlies the inner retina where the blood vessel walls, the inner limiting membrane and the Müller cell endfeet are all closely apposed. The thick Müller cell processes divide the inner plexiform layer and nerve fibre layer into discrete compartments. The ganglion cells do not form fascicles and lie within both the ganglion cell and inner plexiform layers. The inner nuclear layer consists of amacrine, bipolar, Müller cell somata and two rows of horizontal cells. The photoreceptor terminals comprise either multiple (3-5 in cone pedicles) or single (rod spherules) synaptic ribbons. These photoreceptor terminals form either a triad (rods and cones) or a quadrad (cones) arrangement of contact with the invaginating processes of the inner nuclear layer cells. The horizontal cell processes of the cone photoreceptor terminals reveal spinule formation in the light-adapted condition. Five photoreceptor types are classified using morphological criteria; triple cones, unequal double cones, large single cones, small single cones and rods. The ratio of rods to cones is approximately 7:1. All photoreceptor types show retinomotor responses. Only the cones possess accessory outer segments but both rods (8-11) and cones (15-19) possess calycal processes. The retinal pigment epithelium displays retinomotor responses where pigment granules within fine apical processes move vitread to mask the rods in the light. The cells of the retinal pigment epithelium are joined by various types of junctions and contain numerous phagosomes, mitochondria and polysomes. Bruch's membrane or the complexus basalis is trilaminate with two types of collagen fibrils comprising the central layer. The endothelia of the blood vessels of the choriocapillaris, facing Bruch's membrane, are fenestrated. Two to three

layers of melanocytes interspersed between large thinwalled capillaries and several layers of collagen fibrils comprise the choriocapillaris.

Key words: Retina, Fish, Photoreceptors, Retinal pigment epithelium, Müller cells, Electron microscopy

Introduction

Members of the family Cyprinidae are thought to be one of the most successful freshwater groups in North America with over 1,500 species described thus far. With this impressive radiation, one may expect interspecific variation in the visual system since members of this group have been recorded from silty backwaters with little penetration of light to fast flowing streams which are always clear and well lit. This ecological diversity is also reflected in the morphology of the brain in various species (Kotrschal and Junger, 1988) where the relative volumes of primary sensory lobes have changed in response to the sensory needs of the animal (Brandstätter and Kotrschal, 1990). It is also known that many cyprinid species show pronounced retinomotor responses (Ali and Wagner, 1975) and possess retinal regions of higher density of both photoreceptors (Mednick et al., 1988; Zaunreiter et al., 1991) and ganglion cells (Mednick and Springer, 1988; Collin and Ali, 1994). These zones of high cell density are thought to indicate zones of acute vision, further emphasizing the important role vision plays in the survival of some cyprinids.

Developmental plasticity has been investigated in this group and large ontogenetic changes in photopic and scotopic sensitivity take place in addition to changes in visual acuity according to lifestyle (Zaunreiter et al., 1991). Therefore, this group, aided by the ease in which these fish can be captured and reared, is rapidly becoming the preferred model for the investigation of visual processing. Some cyprinid species, especially the goldfish, *Carassius auratus* have been used in studies on optic nerve regeneration (Grafstein, 1983), retinal

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development (Johns, 1981), optic axon organisation (Springer and Mednick, 1986) and ganglion cell classification (Hitchcock and Easter, 1986).

Morphological examination of the retina of cyprinids has received much attention at the level of the light microscope (see the comparative studies of Ali and Wagner, 1975; Ali and Anctil, 1976; Zaunreiter and Kotrschal, 1989; Zaunreiter et al., 1991) but few studies have concentrated on retinal morphology at the level of the electron microscope (Engström, 1960; Engström and Rosstorp, 1963; Wang, 1968; Collin et al., 1996).

The cutlips minnow, Exoglossum maxillingua (Cyprinidae) is a common species throughout northeastern United States and Canada (Scott and Crossman, 1973). This slow-moving, bottom-dweller prefers clear, warm, gravelly streams without excessive plant growth. Due to its dependence on a silt-free environment, this species is also a good indicator of water quality. Previous studies have concentrated on reproductive strategies (Hankinson, 1922; Van Duzer, 1939), diet (Haase and Haase, 1975; Johnson, 1981), life history (Pappantoniou et al., 1984a,b) and development (Fuiman and Loos, 1978). Investigations relating to vision and the visual environment of this species are restricted to correlations between the photic environment and the visual pigment porphyropsin (Heinermann and Ali, 1985).

This study provides detailed morphological information on the vitreal vascularisation, the retinal cells including the various types of photoreceptors present, the retinal pigment epithelium and the choroid. The proportion of rods to cones and the extent of retinomotor responses for each type of photoreceptor cell also provides a better understanding of the available behavioural data. As more ultrastructural data becomes available, the interspecific variability in retinal morphology may also enable the phylogeny of specific visual characters to be traced throughout this large and diverse group.

Materials and methods

Five adult individuals of the cutlips minnow, *Exoglossum maxillingua* (Mitchill), 92-117 mm total length, were collected from Lake Cromwell, near the Biological Research Station of the Université de Montréal, near St. Hippolyte, Quebec, Canada. Collections were made between December and February when the lake was covered by approximately 1 metre of ice. To immerse the fish traps, a number of holes (0.3 m in diameter) were bored using a diesel powered auger and a steel crowbar. Fish traps, each consisting of a plastic basket (0.25 m long and 0.15 m wide) with a funnel-shaped opening at each end, were filled with bread and lowered to lie on the bottom (1-3 m in depth) and left overnight. The following day, accumulated ice formation was removed and the traps retrieved. Each specimen was then transferred into an aerated tank and transported, via snowmobile, to the laboratories of the Biology Station.

Fish were either light-adapted for two hours (two animals) or dark-adapted for three to four hours (three animals), before being sacrificed with an overdose of tricaine methane sulphonate (MS222, 1:2,000). All procedures were carried out according to the ethical guidelines of the National Health and Medical Research Council of Australia. The eyes were excised and following the removal of the cornea, lens and vitreous, each eyecup was fixed in either 4% paraformaldehyde in 0.1M phosphate buffer (for light microscopy) or 4% glutaraldehyde in 0.067M sodium cacodylate buffer (for electron microscopy) overnight. For light microscopic examination, small pieces cut from various regions of the retina were washed in phosphate buffer and embedded in Historesin (Reichert-Jung). One to two micron sections were cut 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). For electron microscopic examination, retinal pieces were 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 or stained with paraphenylenediamine and examined using phase-contrast microscopy. Selected thin sections were then prepared for transmission electron microscopy and stained with lead citrate and uranyl acetate and examined on either a Siemens Elmiskop 1A (School of Optometry, University of New South Wales) or an Hitachi H500 (Department of Marine Biology, University of California San Diego) electron microscope.

Measurements were made on enlargements of electron micrographs using a magnifier and graticule and are quoted as mean and standard deviations. 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 retina of the cutlips minnow, *Exoglossum*

Fig. 1. A.Transverse section (1 μm) of the dark-adapted retina of the cutlips minnow, *Exoglossum maxillingua.* **B.** Transverse section of the inner retina showing the close apposition of a vitreal blood vessel (vv) to the inner limiting membrane. Note also the prominent processes of the Müller cells (m) which pierce the nerve fibre layer. **C.** Electron micrograph of the somata in the inner nuclear layer. Cell types are distinguished on the basis of nuclear staining, size and shape. a: amacrine cell; b: bipolar cell; g: ganglion cell; gcl: ganglion cell layer; inl: inner nuclear layer; ipl: inner plexiform layer; nfl: nerve fibre layer; onl: outer nuclear layer; opl: outer plexiform layer; pl: photoreceptor layer. Scale bars: 20 μm (A), 10 μm (B), 3 μm (C).



maxillingua is approximately 265 μ m thick with its inner surface covered by a complex arrangement of vitreal blood vessels which branch to form capillaries (Fig. 1A,B). These vitreal blood vessels lie within an indentation of the retinal surface and closely appose the inner limiting membrane (ILM) where the convoluted endfeet of the Müller cell processes also form a close association (Fig. 1B). The Müller cells are a prominent feature of this retina with their thick processes dividing the inner plexiform (43 μ m in thickness) and nerve fibre (30 μ m in thickness) layers to surround the unmyelinated ganglion cell axons at fairly regular intervals (Fig. 1B).

Ganglion cells (4-8 µm in diameter) are predominantly located within the ganglion cell layer but are also dispersed throughout the inner plexiform layer (IPL). The cytoplasm of these cells contain extensive profiles of rough endoplasmic reticula organised into Nissl bodies, mitochondria, Golgi apparati and lysosomes. The IPL comprises a complex arrangement of axonal connections between the dendrites of inner nuclear cells and the ganglion cells. Amacrine (5-15 µm in diameter, distinguished by their vitread location and the presence of a basophilic bar or nuclear fold), bipolar (3-6 µm in diameter, distinguished by their sclerad location, smaller size and dark nuclear staining), Müller cells (identified by the presence of ascending and descending collaterals) and two rows of horizontal cells (7-10 µm in diameter, distinguished by their sclerad location) form a thick inner nuclear layer (INL, 37 µm in transverse section, Fig. 1C).

The pre- and post-synaptic terminals of bipolar, horizontal and photoreceptor cells form the outer plexiform layer (OPL, 15 µm in thickness). The junctions between these neurons are complex and are of two types; synaptic ribbons and surface contacts. Typically rod spherules possess a single synaptic ribbon $(1.03\pm0.41 \text{ }\mu\text{m} \text{ in length}, \text{ n}=21 \text{ and } 0.05\pm0.008 \text{ }\mu\text{m} \text{ in}$ width, n=20) when viewed in transverse section, which projects from the juncture of two lateral processes and a single central process (triad, Fig. 2A,B). At the base of each synaptic ribbon, an arciform density lies surrounded by a complex arrangement of invaginating processes. Numerous surface contacts appear as aggregations of amorphous material of both the pre- and post-synaptic cytoplasm along the inner surfaces of the cell membranes within the triad (Fig. 2B). Synaptic vesicles (35-50 nm in diameter) are aligned along the length of the synaptic ribbons and are of similar dimensions in both rod and cone terminals.

Cone pedicles possess 3 to 5 synaptic ribbons when

viewed in transverse section, which join two lateral processes and either a single or double central process to form a triad or quadrad, respectively (Fig. 2C). Cone synaptic ribbons are $0.82\pm0.33 \ \mu\text{m}$ in length (n=40) and $0.02\pm0.003 \ \mu\text{m}$ in width (n=40). Similar to rod spherules, cone pedicles possess surface contacts around their basal arciform density and along the inner surfaces of the lateral processes (Fig. 2D,E). In the light-adapted retina, numerous spinules are observed on the inner membrane surface of the lateral horizontal cell processes. These appear as membrane densities which are either continuous or form interrupted clumps of an osmiophilic substance (Fig. 2D). Spinules are only found in light-adapted retina and are restricted to the invaginating lateral processes of the cone pedicles.

Typically up to five layers of fine Müller cell microvilli surround both rod and cone photoreceptor terminals in the OPL (Fig. 2C), but it is not uncommon to find stacks of microvilli immediately vitread of the outer nuclear layer (ONL) up to 40 layers in thickness. These longitudinally oriented stacks of microvilli are up to 4.5 μ m in length (sclerad to vitread in transverse section) and 5.5 μ m in width, with each microvillus approximately 40 nm in diameter, maintaining an intermembrane space of approximately 60 nm (Fig. 3A,B). At the extremities of these stacks, the fine microvilli give rise to larger diameter processes which turn perpendicularly to traverse the retina and encapsulate the rod and cone nuclei before piercing the outer limiting membrane (OLM).

The outer nuclear layer (20 μ m in thickness) consists of three to four layers of rod nuclei vitread to the OLM and a single layer of lightly staining cone nuclei predominantly sclerad to the OLM. At this level, the Müller cell microvilli are larger in diameter and envelope each nucleus before finally reaching the inner segments of the photoreceptors. At the OLM, the Müller cell processes form junctions, of the zonula adhaerens type, with the rod and cone myoids.

The photoreceptors are of five basic types; triple cones, unequal double cones, large and small single cones and rods (Figs. 3C,D). The criteria for differentiating between rods and cones include, the shape of the outer segment, mitochondrial staining of the inner segment, the presence of incisures, photoreceptor size, retinomotor responses and nuclear staining. Triple cones were observed only rarely and appeared as three closely apposing cones, one central, principal cone and two smaller accessory cones, all joined along their inner segments (Fig. 3D). Unequal double cones comprise a large principal cone ($62\pm3 \mu m$ in length in light-adapted

Fig. 2. Ultrastructure of the photoreceptor terminals. **A.** Rod spherule (RS) showing a single synaptic ribbon (arrowheads) in a light-adapted retina. **B.** Two rods spherules each with a single ribbon synapse typically projecting from three basal processes in the dark-adapted retina. Note also the surface contacts (arrowheads). **C.** A cone pedicle (CP) with four ribbon synapses projecting from a complex arrangement of neuronal processes (np). Müller cell microvilli (m) surround the photoreceptor terminal. **D.** Three ribbon synapses at the base of a cone pedicle projecting from either three (triad) or four (quadrad) neuronal processes. Spinules (arrowheads) appear on the inner surface of neuronal cell membranes in the light-adapted state. **E.** Ribbon synapse showing the alignment of vesicles along the ribbon, the arciform density (arrowed) at the base of the ribbon and the lateral surface contacts. Scale bars: 0.3 μm (A), 1 μm (B), 1 μm (C), 0.5 μm (D), 0.3 μm (E).





Fig. 3. A. Transverse section of a multi-layered stack of Müller cell microvilli (mm) at the level of the photoreceptor terminals. **B.** Tangential section of a small collection of Müller cell microvilli (mm) packed into the extracellular space between the photoreceptor terminals. **C, D.** Light micrographs of the various types of photoreceptors observed in a dark-adapted retina. ac: accessory cone; ls: large single cone; my: myoid region of the unequal double cone; olm: outer limiting membrane; onl: outer nuclear layer; pc: principal cone; ptv: photoreceptor terminal vesicles; r: rod; ss: small single cone; tc: triple cone. Scale bars: 0.3 μm (A,B), 20 μm (C,D).

Fig. 4. Cone photoreceptors. A. Unequal double cone consisting of a principal (pc) and an accessory (ac) cone in the dark-adapted state. B. The accessory outer segment (aos) of a principal cone. Note the connecting cilia (arrowed) at the base of the accessory outer segment. C. The accessory outer segment of a large single cone. D. High magnification of part of a principal cone. E. Large single cone in the dark-adapted state. cp: calycal process; is: inner segment; m: mitochondrion; my: myoid; os: outer segment. Scale bars: 4 µm (A), 2 µm (B), 1.5 µm (C), 1 µm (D), 1.5 µm (E).



and $71\pm 2 \mu m$ in dark-adapted state, n=25) and a smaller accessory cone (46±3 µm in length in light-adapted and 59±6 µm in dark-adapted state, n=25; Figs. 3C, 4A). Both photoreceptors of the double cone are closely apposed at the level of their inner segments where cisternae lie just beneath the surface of both outer membranes. As in all cone types, the inner segments of the double cones are densely packed with large mitochondria sclerad and smaller mitochondria vitread (Fig. 4A). The large $(50\pm3 \text{ }\mu\text{m} \text{ in length in light-adapted})$ and 69 ± 4 µm in dark adapted state, n=25; Figs. 3D, 4C,D) and small (19±2 µm in length in light-adapted and 35±2 µm in dark-adapted state, n=25; Fig. 3D) single cones have long slender outer segments, relatively lightly staining ellipsoids and a less obvious gradation in mitochondrial size. All cone types possess between 15 and 19 calycal processes which surround a tapering outer segment comprising a series of membranous discs (14 nm thick, maintaining an interdisc space of 8 nm, Fig. 4D,E) which are enveloped within another membranous sheath more vitread. Each cone has an accessory outer segment emanating from the sclerad region of the ellipsoid which runs the length of the main outer segment. These elongated accessory segments are joined by connecting cilia at their bases, are up to 2 µm in diameter and are devoid of discs and other organelles (Fig. 4B,C).

Rods are the most abundant photoreceptor type (rod to cone ratio is approximately 7:1) which display strong retinomotor responses. In both the light- and darkadapted states, the rods are aligned in two irregular rows or banks, the first row vitread (93±2 µm in length in light-adapted and 66±2 µm in dark-adapted state, n=25) to the second (150 \pm 4 µm in length in light-adapted and 132 ± 6 µm in dark-adapted state, n=25) although the alignment is not as obvious in the light-adapted condition (Fig. 3C,D). Devoid of an accessory outer segment, the ellipsoids of rods give rise to a maximum of 11 calycal processes which closely appose the outer segment membrane for a distance of approximately 11 µm. In some instances, the calycal processes may thicken and create indentations in the rod outer segments. The rod mitochondria are all of similar dimensions and therefore not graded in either size or density. The outer segments of the rods are long and cylindrical and possess stacks of membranous discs enclosed in a cell membrane. The rod discs (each 15 nm thick, maintaining an interdisc space of 35 nm), in contrast to those of cones, are often interrupted by up to 5 incisures (Fig. 5A,B). These are longitudinal seams in the outer segment where the discs are pinched off, leaving a small space. The incisures are not regularly

spaced and may also run obliquely.

In transverse section, a connecting cilium comprises nine pairs of microtubules, which project from a basal body in the sclerad region of the rod ellipsoid, connected to the cilium outer membrane by way of cross links (Figs. 5C,D). Some microtubules also join the vitread discs of the rod outer segments (Fig. 5C).

The array of cone photoreceptors, when viewed in tangential section, are arranged in a row mosaic. The junction of each component of the unequal double cones all lie in parallel with the small and long single cones filling alternating positions within the mosaic (Fig. 6). Therefore, both types of single cones form a square mosaic within the array. The rods do not appear to be arranged in a regular pattern. No attempt was made to analyse the photoreceptor mosaic in different regions of the retina.

The retinal pigment epithelium (RPE) is also capable of photomechanical changes. In the light, two different populations of membrane-bound osmiophilic granules (granules, circular in cross section and therefore most probably spherical, 0.49±0.13 µm in longest dimension, n=60) and long cylindrical granules (1.23±0.32 µm in diameter, n=60) migrate vitread to surround the photoreceptors. The cuboidal cells of the RPE are between 10 and 16 µm in diameter, where they abut Bruch's membrane (Fig. 7A), and are joined by junctions of either the zonula adhaerens, zonula occludens or desmosome type (Fig. 7B). The nucleolus is prominent in these uninucleate cells as are large numbers of mitochondria and numerous aggregations of ribosomes (polysomes). Phagosomes of shed outer segments, pigment granules, lipid droplets, Golgi apparati and smooth and rough endoplasmic reticula (especially in peripheral retina) are also common (Fig. 7B,C).

Bruch's membrane (2.3 µm in thickness) is trilaminate and comprises the basal lamina of the RPE cells, the basal lamina of the choriocapillaris and a central layer of collagen fibrils (Fig. 7D). The collagen fibrils are of two types: the vitread layer (1.2 µm in thickness) consisting of fibrils 40 nm in diameter and the sclerad layer (0.8 µm in thickness) consisting of fibrils 24 nm in diameter. There is no central elastic layer. The basal surface of the RPE cells is smooth with infrequent infoldings while the endothelial cells of the choriocapillaris are moderately fenestrated. The choroid (16 µm in thickness) comprises two to three layers of melanocytes containing melanosomes (0.5-1.0 µm in diameter) interspersed between large and small thinwalled capillaries within an extracellular matrix containing collagen fibrils (Fig. 7C,E). Endothelial cells (with peripherally located concentrations of hetero-

Fig. 5. Rod photoreceptors. **A.** Transverse section of a rod in the dark-adapted state showing a longitudinal incisure (arrowheads) in the discs of the outer segment (osd). **B.** Tangential section of two rod outer segment discs. Note the length of penetration of the incisures (arrowheads). **C.** At the base of the outer segment (os) the rod incisures (arrowheads) follow the path of a microtubule of the connecting cilium (cc). Two microtubules appear to make contact with the vitread region of discs. The cilium projects from a basal body (bb) embedded in the sclerad region of the inner segment. **D.** Tangential section of a connecting cilium consisting of nine pairs of microtubules (arrowed) surrounded by the mitochondria (m) of the inner segment. cp: calycal process; g: pigment granule. Scale bars: 0.7 µm (A), 1 µm (B), 0.5 µm (C), 0.3 µm (D).



chromatin within their nuclei) line the lumen of each capillary. This species possesses a horseshoe-shaped choroidal body which is highly vascularised and surrounds the optic nerve head (Fig. 7E).

Discussion

The extensive system of vitreal blood vessels overlying the ILM ensures that the inner retina receives the nutrition it requires and together with the choroidal circulation, maintains a high oxygen tension throughout the entire retina (Wittenberg and Wittenberg, 1962). The lack of a falciform process signifies the complete closure of the embryonic fissure. A radial pattern of vitreal blood vessels emanating from the hyaloid artery which enters the retina with the optic nerve, has been described for seven other cyprinid species (Hanyu, 1959; Kohbara et al., 1987). The dual vascular supply in teleosts (vitreal and choroidal) and mammals (retinal and choroidal) is thought to be more effective than simply a choroidal blood supply (Kohbara et al., 1987).

The OPL of *E. maxillingua* contains both cone pedicles and rod spherules which surround the invaginating processes of bipolar and horizontal cell processes. Although neither of these specific cell types was pre-labelled in this study, previous reports in other cyprinids find the horizontal cells contact a single functional photoreceptor type; either rods or cones. In contrast, bipolar cells form synaptic interactions with both rods and cones. This has been found for both goldfish (Stell and Lightfoot, 1975) and catfish (Hidaka et al., 1986). The triad arrangement of two lateral processes (from horizontal cells) surrounding a central process (from a bipolar cell) is a common feature of fish, amphibians, birds and mammals (Dowling, 1968). The lateral processes of goldfish cone pedicles are the terminal dendrites of three types of horizontal cells (H1-H3) and the lateral processes of the rod spherules are the terminal dendrites of the fourth and most vitread horizontal cell (H4 or RH). These cells have been characterised by correlating their electrophysiological responses to stimuli of different intensities and wavelengths with morphology (Stell and Lightfoot, 1975; Weiler, 1978). Quantitative analysis of the connectivity of cones with functionally-identified horizontal cells has also been investigated by Downing and Djamgoz (1989) in roach, Rutilus rutilus at the level of the electron microscope. The finding of a fourth process (to form a quadrad) at the basal region of cone pedicles in E. maxillingua has been found in the creek chub, Semotilus atromaculatus (Collin et al., 1996) and may constitute the process of another type of bipolar cell (Rodieck, 1973). The presence of surface contacts at the bases of the photoreceptor terminals has been found in animals as diverse as the velvet cichlid, Astronotus ocellatus (Braekevelt, 1992a) and a primate (Kolb, 1970).

The large stacks of Müller cell microvilli vitread to



Fig. 6. A. Light micrograph of a tangential section of the retina showing the loosely-regular array of the inner segments of the principal cones (x) of the unequal double cones of *E. maxillingua*. The darkly staining profiles are rod outer segments. **B.** Row mosaic of the double cones at the more vitread level of the cell nuclei showing the positions of the small (small arrow) and large (large arrow) single cones in the array. The arrowhead depicts a triple cone. Scale bars: 10 μm (A), 10 μm (B).

Fig. 7. A. Light micrograph of a transverse section of the dark-adapted retina showing the photoreceptor layer (pl), retinal pigment epithelium (rpe) and the choriocapillaris (cc). **B.** Electron micrograph of the cuboidal cells of the light-adapted retinal pigment epithelium and Bruch's membrane (B). Note the various types of junctions. **C.** A large diameter capillary of the choriocapillaris overlying Bruch's membrane in the dark-adapted retina. **D.** High magnification of Bruch's membrane comprising the basal lamina of the capillaries of the choriocapillaris (arrowheads), the basal lamina of the retinal pigment epithelium and two layers of collagen fibrils depicted by arrows. **E.** The choroidal body (cb) overlying the choriocapillaris. Dense aggregations of collagen fibrils (*) are part of the fibre system that supports the choriocapillaris. d: desmosome-like junction; g: pigment granule; m: mitochondrion; N: nucleolus within nucleus; p: phagosome; pe: pericyte; rb: red blood cell; ros: rod outer segment; za: zonula adhaerens; zo: zonula occludens. Scale bars: 25 µm (A), 2 µm (B), 3 µm (C), 0.5 µm (D).





and surrounding the photoreceptor terminals, increase the surface area and may enable a more efficient diffusion of nutrients. Although these large stacks have not been reported previously, the smaller columns of microvilli which surround the photoreceptor terminals and project sclerad to pierce the OLM, have been described in chicks (Prada et al., 1989) and the Florida garfish, Lepisosteus platyrhincus (Collin and Collin, 1993). In Trachinus vipera (Kunz et al., 1985) and the coldwater nototheniid, Trematomus bernacchii (Meyer-Rochow and Klyne, 1982) similar "parallel running fibres and neurotubules" were found in association with the "lateral fins" of the cone inner segments. Due to the large number of Müller cell microvilli and the increased thickness of the primary Müller cell processes throughout the retina of this species, these cells probably play an important role in the distribution of nutritive substances from the inner retina to the choroid.

Moore et al. (1950), Engström (1960, 1963), Engström and Rosstorp (1963) and Ali and Wagner (1975) characterised up to five types of photoreceptor cells in fourteen species of cyprinids using light microscopy. However, seven other cyprinid species did not possess triple cones (see review by Ali and Wagner, 1975). Quadruple cones have only been reported in Phoxinus laevis (Lyall, 1957; Engström, 1963). The finding that all the various photoreceptor types in E. maxillingua display retinomotor responses is in contrast to some members of the family Cyprinidae. In the creek chub, Semotilus atromaculatus (Collin et al., 1996), Leuciscus rutilus (Engström and Rosstorp, 1963) and Ericymba buccata (Moore et al., 1950), the short single cones either do not display any retinomotor responses or possess a "high photomechanical threshold". In L. rutilus and E. buccata, the principal and accessory cones also lengthen differentially in the dark-adapted state (Moore et al., 1950; Engström and Rosstorp, 1963). Generally, retinomotor activity allows the rods and cones to alternately occupy a maximal area adjacent to the ONL for light absorption. This response is aided by the sclerad migration of pigment granules within the pigment epithelium in the dark. Since retinomotor movements are known to occur at low light intensities (Nicol, 1989), this suggests that the marked changes in the positions of the photoreceptors in E. maxillingua may reflect a high sensitivity in dark and light adaptation between the photopic and scotopic states. The inability of the short single cones to migrate sclerad in the dark and the failure of one of the double cone components to migrate in these species, may provide a distinct advantage for crepuscular vision.

Spectrophotometric examination of the visual pigment in the rods of *E. maxillingua* show that this species possesses a single scotopic visual pigment based on vitamin A_2 (Heinermann and Ali, 1985). This porphyropsin absorbs maximally at one of the longest wavelengths reported for scotopic visual pigments and is well matched to the wavelength of the ambient light at a depth of 0.5 metres. It is hypothesised that *E*.

maxillingua feeds primarily during periods of low light intensity where its principal food source of chironomid insect larvae (Pappantoniou et al., 1984a,b) would appear darker against the floor of the lake or appear as a dark silhouette against the background spacelight (Heinermann and Ali, 1985). The rod to cone ratio of 7:1 would indicate that scotopic vision may be important to this species.

Accessory outer segments attached to the sclerad region of the inner segment of the photoreceptors via connecting cilia have been reported previously in both rods and cones (Engström, 1961; Yacob et al., 1977) or only in cones (Engström, 1963; Fineran and Nicol, 1974; Munk, 1977; Braekevelt, 1992a; Collin et al., 1996). Their origin and function is unknown but theories include a reservoir for high energy metabolites (Fineran and Nicol, 1974) and the maintenance of any photoreceptor mosaic that is present during retinomotor responses (Braekevelt, 1992a) by filling the extracellular space (Wagner and Ali, 1978).

The rod outer segments of *E. maxillingua* are comprised of stacks of membranous discs which are often interrupted by longitudinal incisures. Although these incisures sometimes follow the underlying connecting cilia and form connections with the vitread discs of the outer segment (Fig. 5C), it is unknown whether these supportive structures induce their formation. Primarily found in rod outer segments in a variety of vertebrates including teleosts (Collin et al., 1996), reptiles (Dunn, 1973), birds (Cohen, 1963) and mammals (Kroll and Machemer, 1968), incisures may also be found in cones (Braekevelt, 1983). Presumably the function of the incisures is to aid in the diffusion of substances to and from the disc membranes.

The structure of the connecting cilia with nine pairs of microtubules surrounding a clear centre in the rods of *E. maxillingua* is typical of non-motile sensory cilia. In the vitread region of the rod outer segment, where the microtubules of the connecting cilium appear to make contact with the discs (Fig. 5C), the presence of actin has been demonstrated in the pinfish (Chaitin and Burnside, 1989).

The row mosaic formed by the unequal double cones has previously been found in a number of other cyprinids [i.e. the minnow, Phoxinus laevis (Lyall, 1957), the roach, Rutilus rutilus and the bream, Abramis brama (Engström, 1960; Zaunreiter et al., 1991) and the creek chub, Semotilus atromaculatus (Collin et al., 1996)]. No regular mosaic can be found in the carp, Cyprinus carpio, the asp, Aspius aspius, the sabre carp, Pelecus cultratus or the rudd, Scardinius erythrophthalmus (Lyall, 1957; Zaunreiter et al., 1991). The arrangement of unequal double cones in the goldfish, Carassius auratus, however, is a square pattern with four double cones surrounding a single cone (Engström, 1960). A regular mosaic may be an efficient packing method of increasing the number of photoreceptors in the retina but it is also thought to be associated with high acuity vision (Fernald, 1982) and

the arrangement of double cones used as a basis for polarization vision (Cameron and Pugh, 1991).

The RPE of E. maxillingua also shows marked photomechanical changes with its apical processes and pigment granules migrating vitread in the light-adapted state to mask the rods. Due to the relatively thick photoreceptor layer (52% of the retinal thickness) and the moderately high rod to cone ratio, complete masking may not be possible or even necessary given the spectral tuning to the rod visual pigments to the ambient downwelling light (Heinermann and Ali, 1985) within its chosen environment (Heinermann and Ali, 1988). The RPE appears similar to other teleosts (Braekevelt, 1982, 1992b; Collin et al., 1996) with numerous mitochondria, phagosomes, lysosomes and Golgi apparati. The mitochondria are of heterogeneous shapes and are the centres of oxidative phosphorylation and the production of adenosine triphosphate (ATP, Zinn and Benjamin-Henkind, 1979). The presence of lysosomes is also typical and aids in the degradation of outer segment discs following phagocytosis (Young, 1978). The Golgi apparatus secretes substances necessary for the formation of the membranous sheaths surrounding the photoreceptors (Zinn and Benjamin-Henkind, 1979).

The cuboidal cells of the RPE in *E. maxillingua* are attached via various types of junctions all of which form a seal between adjacent epithelial cells, permitting selective exchange between the choroidal circulation and the photoreceptors. In addition, in *E. maxillingua* there are numerous desmosomes which are not present in the RPE of all vertebrates (e.g. chicks, Owaribe et al., 1988) and, in conjunction with the zonula adhaerens, act simply as strong adhesions.

Bruch's membrane is trilaminate in this species which is typical for teleosts (Braekevelt, 1992b) but contrasts the pentalaminate structure of elasmobranchs (Braekevelt, 1994) and mammals (Braekevelt and Hollenberg, 1970) which includes a central elastic layer which is continuous from the optic nerve head to the ciliary body. The endothelium of the blood vessels adjacent to Bruch's membrane is fenestrated facilitating the passage of substances into and the bi-products out of the RPE (Casley-Smith, 1981). The choroidal body functions as a counter-current system to elevate the oxygen tension in the retina (Copeland, 1974) and may also act as a source of biochemical exchange (Barnett, 1951).

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