CLINICAL AND EXPERIMENTAL

OPTOMETRY

The head and eye of the sandlance, Limnichthyes fasciatus–a field emission scanning electron microscopy study

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Background: The anterior surface of the cornea is an important component in retinal image formation. In mammals, the anterior cell surface is covered with numerous microvilli but in fish there are microplicae. However, there is little else known about the corneal surface of teleosts.
Methods: The cornea, conjunctiva and skin of the head of the sandlance, *Limnichthyes fasciatus*, a small teleost found off the coast of Australia, were examined using field emission scanning electron microscopy.
Results: The central corneal surface has an epithelial cell density of 21,475 ± 4,750 cells per mm² while in the periphery the cell density is less (14,785 ± 3,630 cells per mm²). The central and peripheral epithelial cells have a dense pattern of microplicae, around 135 nm in width and many microns in length. There are no craters in the cell surface but holes or pits around 1.6 μm in diameter are present at 10 to 15 per cent of junctions where three surface cells meet in the peripheral cornea. The cells of the conjunctiva, nose and skin of the head also have microplicae, the patterns of which show similarities to those of the cornea. Some nasal cells are partially covered with a material, possibly mucus.
Conclusion: The microplical pattern found on the cornea of the sandlance is similar but not identical to that seen in other teleosts. The pattern found on the conjunctiva and scales of the head is also similar, although these structures show a lesser cell density and a greater microplical separation than the central cornea. These changes may be associated with differences in cell function.
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Key words: conjunctiva, cornea, corneal craters, epithelium, fish, microplicae

In humans and non-aquatic vertebrates, the anterior surface of the cornea provides the major refractive component of the eye. However, without a smooth optical surface provided by an adequate tear film a sharp retinal image cannot be formed. Stability of the tear film is aided by the presence of microvilli and/or microplicae. These increase the surface area of the superficial epithelial cells permitting the adsorption of more tear mucin onto the corneal cell surface.

There have been very few scanning electron microscopy (SEM) studies of the cornea of teleosts[1-5] and none using field emission SEM. Some teleosts have an intricate pattern of microplicae on the surface of the cornea.[1-3,5]

The sandlance or tommy fish, *Limnichthyes fasciatus*, is a small fish 15 to 30 mm long, found in the coastal waters of Australia. The surface cells of the cornea are 'covered with a distinct complex pattern of microplicae'.[2] A similar pattern of slightly thicker ridges covers the conjunctiva.[2] However, this study[2] was primarily a transmission electron microscopic investigation of the structure of the cornea with only a minimal description of the ocular surface.

In order to understand better the nature and role of the surface microplicae in teleost vision, we investigated and com-

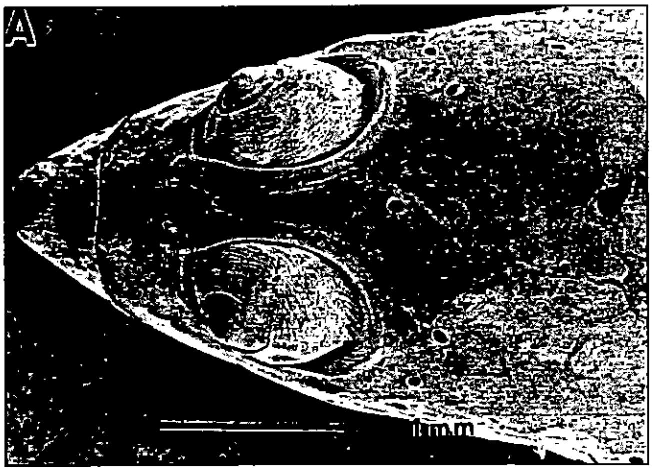

Figure 1a. Field emission scanning electron micrograph of the head of the sandlance

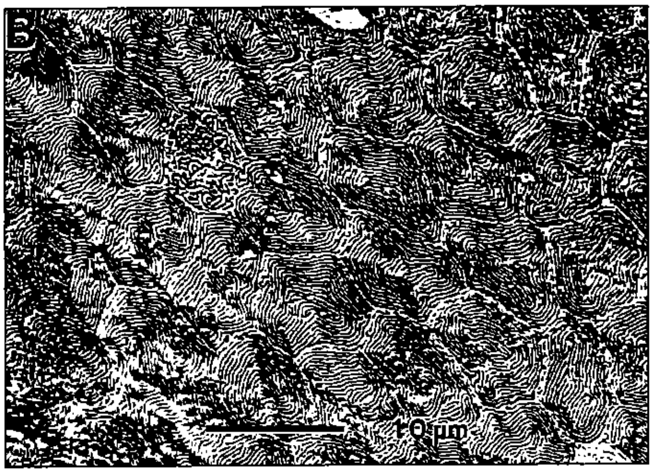

Figure 1b. Electron micrograph of the central corneal epithelium of the sandlance showing the dense pattern of microplicae

pared the surface morphology of the cornea, the conjunctiva and the facial tissues of the sandlance.

MATERIALS AND METHODS

Three specimens of the sandlance, *Limnichthyes fasciatus*, were collected on SCUBA at Rottnest Island off the south-eastern coast of Western Australia under permit with a fine mesh net. They were anaesthetised and killed using tricaine methane sulphonate (MS222). The specimens were placed in Karnovsky's fixative at pH 7.2 and the heads were carefully dissected from the body of the fish. Tissues were rinsed in 0.1 sodium cacodylate buffer.

Post fixation was in 0.1 per cent osmium tetroxide in 0.1 per cent cacodylate buffer. Specimens were dehydrated in graded alcohols and then dried in a Polaron critical point dryer and mounted onto 10 mm aluminium stubs with double-sided tape. Here specimens were oriented to display the dorsal side of the head.

The mounted specimens were coated with 12 to 15 nm of gold-palladium in a Polaron Sputter coater and placed in an oven at 40° C overnight before being examined.

The corneal surfaces were examined using a JOEL FSEM (field emission scanning electron microscope) model 6300F at an accelerating voltage of 3 kv. Results were recorded on 35 mm film and digital images were downloaded onto a Power Macintosh for examination and printing.

RESULTS

A field emission scanning electron micrograph of the head of the sandlance is shown in Figure 1a.

Cornea

The superficial corneal epithelial cells had an average density (Table 1) of around 18,180 ± 5,560 cells/mm^2 (n = 40). In the centre of the cornea (Figure 1b) the cells are smaller and the density is greater (21,475 ± 4,750 cells/mm^2) than in the periphery (14,785 ± 3,630 cells/mm^2).

The cells are irregular in shape and each has a dense pattern of microplicae. In the centre these are approximately 136 ± 11 nm wide and many microns in length (Figure 2a). The spaces between the microplicae are 119 ± 23 nm. The microplical pattern is similar for each cell but not identical, some six to 10 ridges being arranged around the periphery of the cell and parallel with the cell border. No holes, pits or craters are found in the surface of these central cells or between them.

In the periphery of the cornea (Figure 2b), where the cells are larger, the

	Cell density (cells/mm^2)	Microplical width (nm)	Mcroplical separation (nm)
Central corneal epithelium	21,475 ± 4,750	136 ± 11	119 ± 23
Peripheral corneal epithelium	14,785 ± 3,630	134 ± 41	273 ± 40
Conjunctival epithelium	6,310 ± 598	189 ± 39	336 ± 132
Facial epithelium	6,875 ± 1,037	136 ± 13	339 ± 118

Table 1.

Figure 2a. High power electron micrograph of the central corneal epithelium of the sandlance showing the microplicae

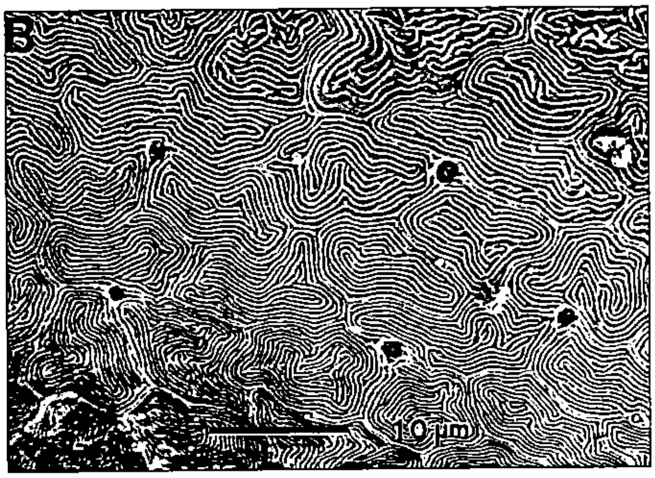

Figure 2b. Electron micrograph of the peripheral corneal epithelium of the sandlance. The cells are larger than those of the central cornea (Figure 2). Holes or pits are present at some of the triple cell junctions

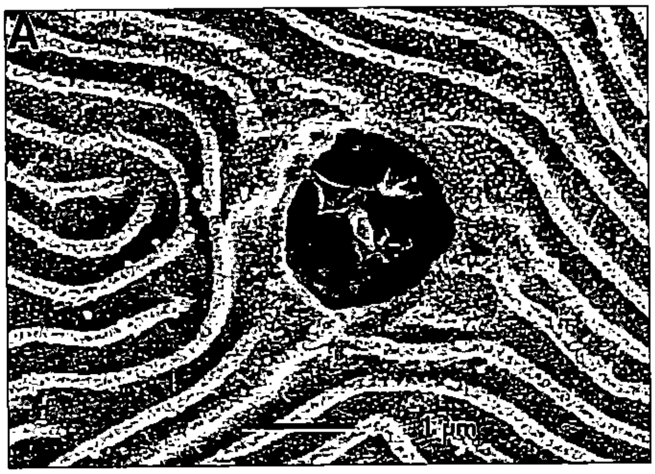

Figure 3a. High power micrograph of the microplicae and a pit at the junction of three epithelial cells in the periphery of the cornea of the sandlance

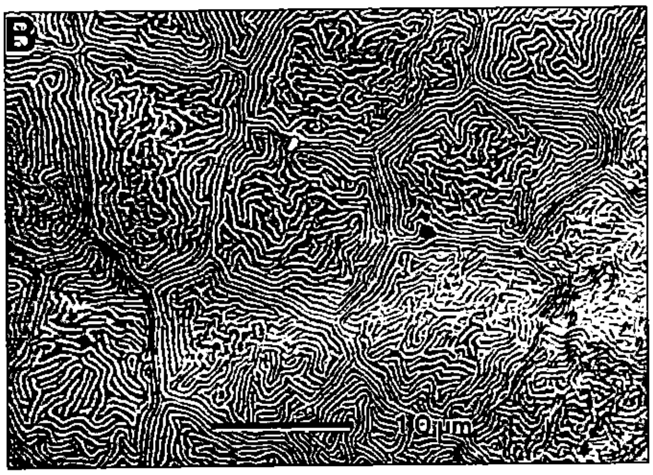

Figure 3b. Electron micrograph of the conjunctival epithelial cells of the sandlance. The microplicae are less regular than those of the corneal epithelial cells and there is one hole at the junction of two epithelial cells

microplicae are similar (134 ± 41 nm) but there are larger separations between the ridges (273 ± 40 nm). Again, there are no pits or craters in the surface of the cells. However, at the junctions where three surface cells meet, there are occasional (approximately 10 to 15 per cent) holes. These are round, approximately 1.6 µm in diameter and frequently there is some tissue or structure in the floor of the hole or pit (Figure 3a).

Conjunctiva

In the bulbar conjunctiva, the surface epithelial cells are larger than those of the peripheral cornea (6,310 ± 598 cells/mm^2). The microplicae are slightly wider (189 ± 39 nm) with a slightly larger separation than in the peripheral cornea (336 ± 132 nm). The microplical pattern (Figure 3b) differs in that it is far less regular than in either the central or peripheral corneal cells. Pits or craters are not present on the cell surface or at the junctions between cells as in the peripheral cornea. However, on rare occasions there are small holes (approximately 1 µm in diameter) between the cells but not at the triple cell junctions.

Scales

The cells or scales (Figure 4a) over the surface of the head of the sandlance are similar in size (6,875 ± 1,037 cells/mm^2)

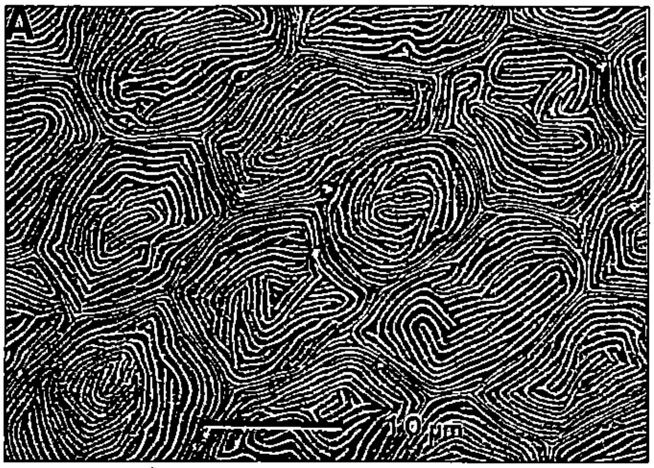

Figure 4a. Micrograph of the surface scales in the region of the head of the sandlance behind the eyes. The microplicae are long, straight and more like those of the cornea than those of the conjunctiva

Figure 4b. High power micrograph of the surface of the scales shown in Figure 7. A hole is present at the junction of three cells

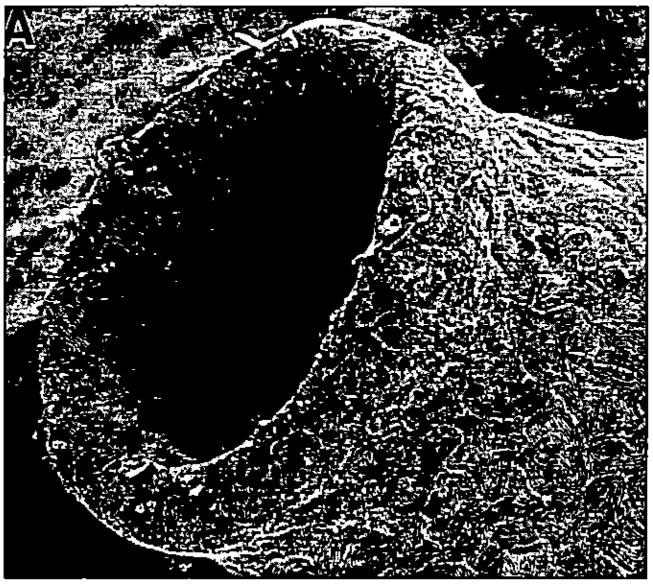

Figure 5a. Micrograph of one of the nasal openings on the head of the sandlance. Cells with microplicae similar to those of the cornea are present both inside and outside the opening

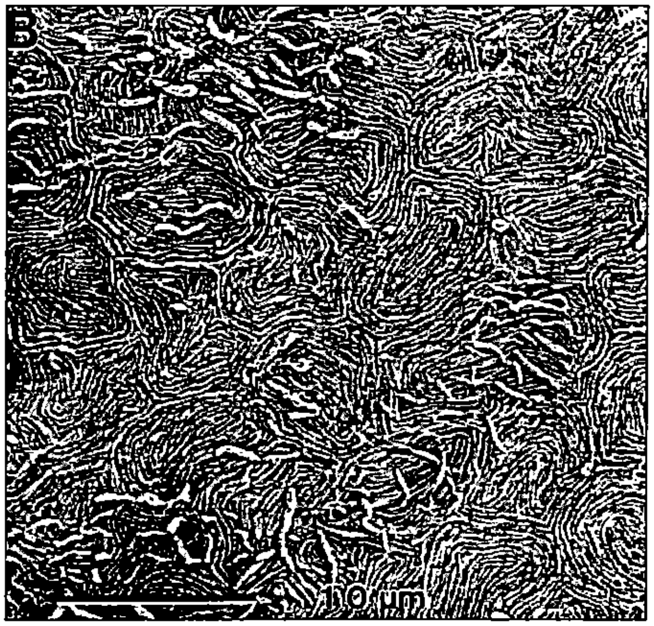

Figure 5b. Electron micrograph of the surface in the region of the nose of the sandlance. The cells and the pattern of microplicae are partially obscured by the presence of material over the surface of the cells

and shape to those of the conjunctiva. The width of the microplicae (136 ± 13 nm) is similar to the corneal cells, although the separation is more variable and is slightly greater (339 ± 118 nm). The surface pattern is similar to that of the peripheral cornea with long straight microplicae but considerably different from that of the conjunctiva. Small pits are occasionally seen at the triple cell junctions (Figure 4b).

Nares

Cells or scales with a similar pattern of microplicae are also present both inside and outside the nasal openings (Figure 5a). In some areas, the cells of the nasal openings are covered with what may be the remains of a coating of mucus (Figure 5b). A similar material was not present over the ocular surface, although such material may have been washed away during capture and processing.

DISCUSSION

The mean central corneal epithelial cell density of the sandlance (21,475 cells/mm^2) is similar to the only other marine teleost reported, *viz.* the Australian salamanderfish, *Lepidogalaxias salamandroides*[5] (21,880 cells/mm^2). However, this is markedly different from the figures reported for mammals (for example, a species of rabbit,[6] 2,800 cells/mm^2) or for the fresh water small rainbow trout, *Oncorhynchus mykiss*,[4] for which the epithelial cell density may be 2,309 or 3,565 cells/mm^2 depending on the osmolarity of the fixative used. As the full effects of fixation, dehydration and critical point drying are not fully understood, these estimates of epithelial cell density and other tissue measurements in this study are not exact. Allowances must be made for considerable tissue shrinkage.

The variation in cell density between the centre (21,475 ± 4,750 cells/mm^2) and the periphery (14,785 ± 3,630 cells/mm^2) of the cornea has not been reported previously and may indicate a transition of the epithelial cells from the central cornea to the conjunctiva (6,310 ± 598 cells/mm^2) and the periocular tissues (6,785 ± 1037 cells/mm^2).

The corneal epithelial surface of mammals, including a species of rabbit,[7] a rat,[7] a dog,[7] a cat,[7] a monkey[7] and humans[8] are covered with microvilli. However, microvilli were not observed on the sandlance cornea or conjunctiva but were replaced by prominent patterns of ridges or microplicae covering the superficial cells.

The pattern of the microplicae seen in the sandlance shows similarities to those found in other teleosts including the salamanderfish, *Lepidogalaxias salamandroides*;[5] the scup, *Stenotomus chrysops*;[1] the flounder, *Paralichthy dentatus*;[1] the northern sea robin, *Prionotus carolinus*;[1] the toadfish, *Opsanus tau*;[1] and the bluefish, *Pomatomus saltatrix*.[1] However, it is less similar to the Florida garfish,[3] *Lepisosteus platyrhincus*, in which the microplicae are less pronounced, although this may be due to differences in fixation and processing and/or the type of scanning electron microscope used. Despite the similarities in the pattern of microplicae in the different fish, Harding and colleagues[1] claim that there appears to be a pattern which is species specific.

The width of the corneal microplicae, 134 to 136 nm, is similar although slightly less than that reported for other teleosts, *viz.* 200 nm,[1,4] 250 nm[1] or 120 to 250 nm.[3] The increased separation between the microplicae in the peripheral cornea has not been reported previously. As with the epithelial cell densities, there appears to be a transition from the central cornea to the conjunctiva. The separation of the microplicae is less in the central cells (119 ± 23 nm), greater in the peripheral corneal cells (273 ± 40 nm) and greatest in the conjunctival epithelium (336 ± 132 nm) and scales (339 ± 118 nm). This transition may be associated with the greater nutritional needs of the non-vascularised cornea compared with the vascularised conjunctiva. With closer microplicae, there is a greater epithelial surface area, which should assist in the processes of diffusion and active transport,[1] which are essential for the maintenance of healthy corneal epithelium.

Holes, pits or craters in the surface epithelial cells have been described in the cornea[7,9,11] or conjunctiva[12] of the rabbit, the human cornea[8] and the cornea of the Florida garfish, *Lepisosteus platyhrincus*.[3] In the rabbit, craters may be found in up to 72.5 per cent of the surface cells.[9] They may be up to 10 μm in diameter[3,7,11] and typically have an encircling collar.[9,10,12] Hoffman[10] suggests that these craters develop only when the cell reaches the surface, they may have a metabolic function.[10] These craters were not observed in the sandlance. Their significance is unknown.[9]

The holes described in the peripheral cornea, conjunctiva and scales of the sandlance differ from those in the garfish, *Lepisosteus platyhrincus*,[3] and those described in some mammalian species.[7,9,12] They do not have an encircling collar and they occur between the cells rather than on the superficial cell surface. The only published report of holes or pits at the triple cell junctions appears to be in the salamanderfish, *Lepidogalaxias salamandroides*.[5] Although they were mentioned, their structure was not detailed.

This is the first report that demonstrates the similarity between the pattern of microplicae on the teleost scales and on the corneal epithelial cells. Microplicae arranged in a pattern somewhat resembling that of the teleost cornea have been described on the mantle cells of the neuroblasts in three teleosts *Eigenmannia lineata*,[13] *Oreochromis aureus*[14] and *Cichlasoma nigrofasciatum*.[14] However, the whole surface of the neuroblast in these species is covered by a gelatinous cupula.[13]

In mammals, the microvilli appear to play a role in the maintenance and stability of the tear film,[15] by increasing the surface area of the plasma membrane allowing more mucin to be adsorbed.[7] In the aquatic environment, there is no tear film as seen in some mammalian species. However, relatively viscous secretions have been observed on the cornea of two elasmobranchs, the dogfish, *Mustelus canis*, and the skate, *Raja erinacea*.[1] Therefore, as teleosts and elasmobranchs both lack eyelids to help protect the surface of the cornea, a coating material, the composition of which is unknown, may be particularly important.[1] A similar coating material was observed on the nasal epithelium of the sandlance but not on the cornea. It may have been lost from the cornea during processing. This is in contrast to previous studies of the sandlance[2] and salamanderfish,[5] in which the epithelial cells were covered with diffuse debris, which may have represented the remnants of a mucin layer. Such a layer on the cornea may fulfil an optical role by providing a smooth refractive surface.[1]

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