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The corneal surface of aquatic vertebrates: microstructures with optical and nutritional function?

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The anterior surface of the mammalian cornea plays an important role in maintaining a smooth optical interface and consequently a sharp retinal image. The smooth surface is produced by a tear film, which adheres to a variety of microprojections, which increase the cell surface area, improve the absorbance of oxygen and nutrients and aid in the movement of metabolic products across the outer cell membrane. However, little is known of the structural adaptations and tear film support provided in other vertebrates from different environments. Using field emission scanning electron microscopy, this study examines the density and surface structure of corneal epithelial cells in representative species of the classes Cephalaspidomorphi, Chondrichthyes, Osteichthyes, Amphibia, Reptilia, Aves and Mammalia, including some Marsupialia. Variations in cell density and the structure and occurrence of microholes, microridges, microplicae and microvilli are described with respect to the demands placed upon the cornea in different aquatic environments such as marine and freshwater. A progressive decrease in epithelial cell density occurs from marine (e.g. 29 348 cells mm^{-2} in the Dover sole *Microstomius pacificus*) to estuarine or freshwater (e.g. 5999 cells mm^{-2} in the black bream *Acanthopagrus butcheri*) to terrestrial (e.g. 2126 cells mm^{-2} in the Australian koala *Phascolarctos cinereus*) vertebrates, indicating the reduction in osmotic stress across the corneal surface. The microholes found in the Southern Hemisphere lampreys, namely the pouched lamprey (*Geotria australis*) and the shorthead lamprey (*Mordacia mordax*) represent openings for the release of mucus, which may protect the cornea from abrasion during their burrowing phase. Characteristic of marine teleosts, fingerprint-like patterns of corneal microridges are a ubiquitous feature, covering many types of sensory epithelia (including the olfactory epithelium and the oral mucosa). Like microplicae and microvilli, microridges stabilize the tear film to maintain a smooth optical surface and increase the surface area of the epithelium, assisting in diffusion and active transport. The clear interspecific differences in corneal surface structure suggest an adaptive plasticity in the composition and stabilization of the corneal tear film in various aquatic environments.

Keywords: cornea; corneal surface; epithelium; microvilli; microprojections

1. INTRODUCTION

In air, the major refracting component of the vertebrate eye is the curved air–tear interface. The curvature of the tear film reflects the shape of the anterior corneal surface, where the mucus layer of the tear film is adsorbed onto the epithelial cell membranes. In water, the anterior corneal surface contributes little to the total refractive power of the eye, due to the small difference between the refractive indexes of the tear film and the epithelial cells ($n = 1.365$, Sivak 1982) and that of salt water ($n = 1.340$, Jerlov 1976) or freshwater ($n = 1.333$, Horváth & Varjú 1995).

The presence of finger-like microprojections (microvilli) and randomly directed curved and branched membranous folds up to 2 μm long (microplicae), which increase the surface area of the superficial corneal epithelial cells in most terrestrial vertebrates, is thought to improve the stability of the tear film. However, the surface of most, if not all, aquatic corneas is covered with a mucous coating. In bony fishes, this is stabilized by a series of membranous microridges, which are relatively straight corneal surface microprojections, have few branches, may be up to about 20 μm in length and generally run parallel to each other and to the cell margins.

In this study, we used field emission scanning electron microscopy to examine the corneal surfaces of 35 vertebrates from the following classes, Cephalaspidomorphi (two species), Chondrichthyes (four), Osteichthyes (13), Amphibia (two), Reptilia (three), Aves (seven) and Mammalia (four, including two Marsupialia) (table 1). Tissues were prepared using glutaraldehyde fixation, osmium tetroxide post-fixation and dehydration with alcohol. Following these procedures, there was little evidence of a layer of mucus. From electron micrographs, which were mostly of the central cornea, we analysed the

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Table 1. *Species examined and corneal epithelial cell densities measured*

class	species	common name	cell density (cells mm^{-2})
Cephalaspidomorphi	*Geotria australis*	pouched lamprey[b]	5511 ± 2025
Cephalaspidomorphi	*Mordacia mordax*	shorthead lamprey[b]	11 041 ± 2752
Chondrichthyes	*Galeocerdo cuvier*	tiger shark[b]	8286 ± 3212
Chondrichthyes	*Squalis acanthias*	spiny dogfish[a]	4176 ± 2470
Chondrichthyes	*Dalatias licha*	black shark[a]	14 492 ± 4056
Chondrichthyes	*Hydrolagus colliei*	ratfish[a]	not measured
Osteichthyes	*Acanthopagrus butcheri*	black bream[b]	5999 ± 1976
Osteichthyes	*Neoceratodus forsteri*	Australian lungfish[b]	7843 ± 2214
Osteichthyes	*Hippocampus angustus*	West Australian seahorse[a]	14 186 ± 2385
Osteichthyes	*Hippoglossoides elassodon*	flathead sole[a]	28 860 ± 9214
Osteichthyes	*Lepidogalaxias salamandroides*	salamanderfish[d]	21 880 ± 4200
Osteichthyes	*Torquigener pleurogramma*	blowfish[b]	not measured
Osteichthyes	*Aldrichetta forsteri*	yellow-eye mullet[b]	10 943 ± 4538
Osteichthyes	*Platycephalus endrachtensis*	bar-tailed flathead[b]	11 171 ± 5740
Osteichthyes	*Amniabata caudavittatus*	yellow-tail trumpeter[b]	13 453 ± 1385
Osteichthyes	*Nematalosa vlaminghi*	Perth herring[b]	19 639 ± 4010
Osteichthyes	*Myoxocephalus polyacanthocephalus*	great sculpin[a]	22 615 ± 4800
Osteichthyes	*Polypera greenii*	lobefin snailfish[a]	28 229 ± 9334
Osteichthyes	*Microstomius pacificus*	Dover sole[a]	29 348 ± 12 917
Amphibia	*Ambystoma mexicanum*	salamander (axolotl)[c]	2918 ± 1753
Amphibia	*Xenopus laevis*	African clawed toad[c]	7095 ± 3470
Reptilia	*Crocodilus porosus*	crocodile[c]	2283 ± 824
Reptilia	*Ctenophorus ornatus*	ornate lizard[c]	5095 ± 1746
Reptilia	*Caretta caretta*	loggerhead turtle[c]	4191 ± 1672
Aves	*Phoenicopterus chilensis*	Chilean flamingo	5944 ± 2042
Aves	*Eolophus roseicapillus*	Australian galah	11 561 ± 6175
Aves	*Struthio camelus*	South African ostrich	7658 ± 2397
Aves	*Phoenicopterus ruber*	Caribbean flamingo	8674 ± 3034
Aves	*Dromaius novaehollandiae*	emu	5250 ± 2076
Aves	*Eudyptala minor*	fairy penguin[a]	not measured
Aves	*Bubo strix*	barred owl	5351 ± 1325
Mammalia	*Pseudorca crassidens*	false killer whale[c]	2211 ± 794
Mammalia	*Ovis aries*	sheep	not measured
Mammalia	*Sminthopsis crassicaudata*	fat-tailed dunnart	9785 ± 9900
Mammalia	*Phascolarctos cinereus*	Australian koala	2126 ± 713

[a] Marine species.
[b] Estuarine or freshwater species.
[c] Grouped with terrestrial and aerial species for statistical analysis.
[d] Classified with the marine species because it lives in high osmolality freshwater ponds (Christensen 1982).

size and shape of the surface epithelial cells and the nature of the surface epithelial microprojections in the context of their function in different (predominantly aquatic) environments.

2. EPITHELIAL CELL DENSITIES

The corneal surfaces in all of the species studied is formed of essentially straight-sided pentagonal and hexagonal epithelial cells. Two exceptions are the pouched lamprey *Geotria australis*, which possess some oval cells among the predominantly polygonal cells (figure 1*a*) and the Australian koala *Phascolarctos cinereus*, in which the polygonal cells sometimes appear rounded.

There was great variation in the corneal surface epithelial cell densities measured in this study (table 1). The cell densities range from 2126 ± 713 cell mm^{-2} in the Australian koala *P. cinereus* (a marsupial) to 29 348 ± 12 917 cells mm^{-2} in the Dover sole *Microstomius pacificus* (a bony fish). Analysis of these and other published results shows a significant difference ($p = 0.000018$) between the corneal epithelial cell densities of the aquatic species (17 602 ± 9604 cells mm^{-2}) compared with the aerial and terrestrial vertebrates (3755 ± 2067 cells mm^{-2}), where the latter group included two species of amphibians and the false killer whale *Pseudorca crassidens*.

An examination of the environments of the aquatic species indicates that marine species (22 553 ± 8878 cells mm^{-2}) have a significantly ($p = 0.0034$) higher epithelial cell density than species that live in either freshwater or in both salt water and freshwater (estuarine) environments (10 529 ± 5341 cells mm^{-2}). Thus, there appears to be a progressive lowering of the corneal epithelial cell density from marine vertebrates through the estuarine and freshwater species to the aerial and terrestrial vertebrates. The lowest densities occur among the mammals, including the false killer whale *P. crassidens*.

In addition to giving more strength to the corneal surface, the greater epithelial cell densities in the salt water vertebrates may permit more active transport of

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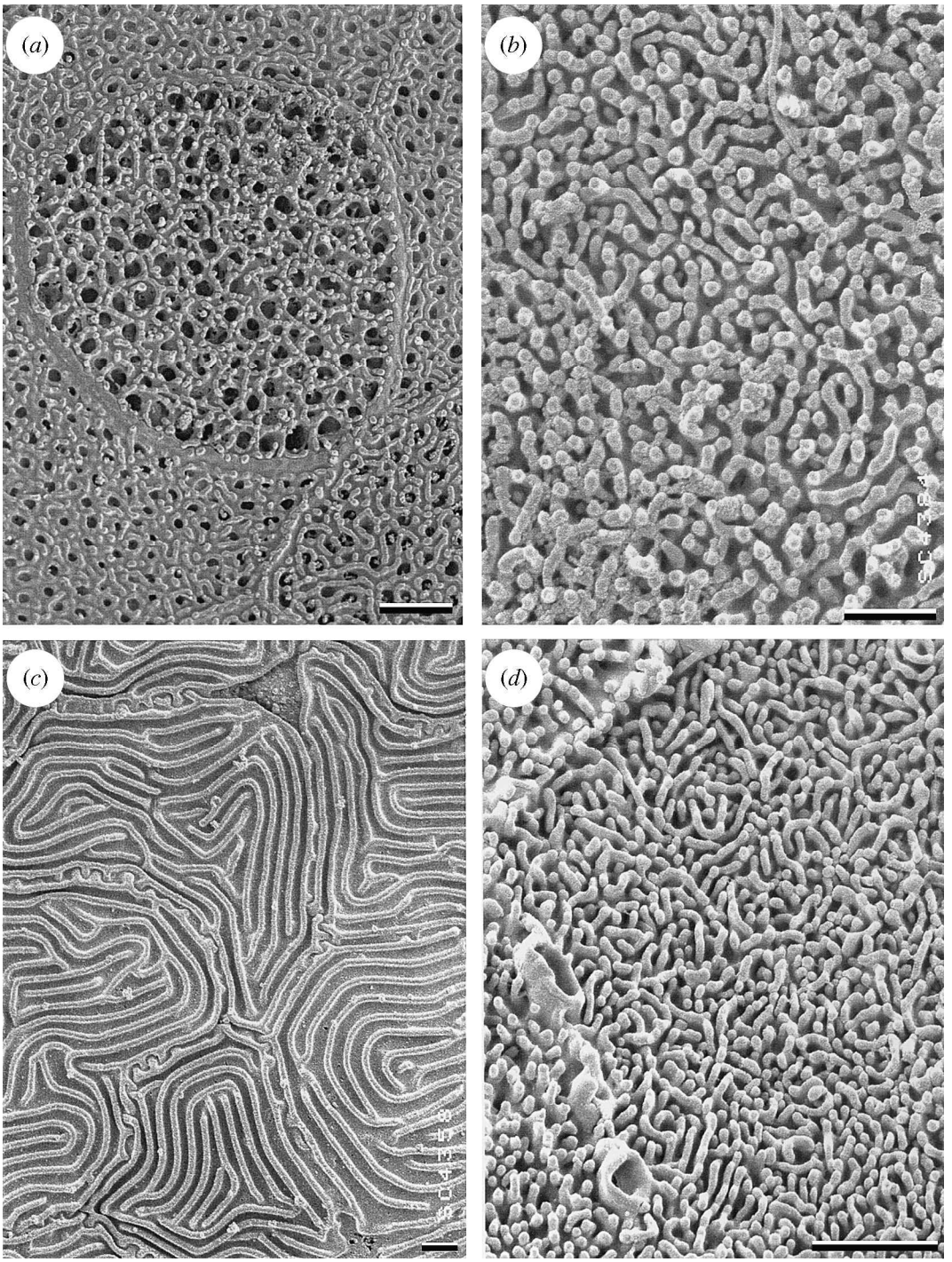

Figure 1. (*a*) The corneal surface of the pouched lamprey *Geotria australis*, showing the presence of a round to oval cell in the midst of otherwise pentagonal and hexagonal cells. There are numerous small microplicae but the predominant feature is the presence of many relatively large (395 ± 15 nm) microholes, through which mucous granules can be expelled. (*b*) Micrograph of the corneal surface of the black shark *Dalatias licha*, showing the dense pattern of microvilli with some microplicae. (*c*) The corneal surface of the blowfish *Torquigener pleurogramma*, showing the long, sometimes continuous microridges that lie parallel to each other and to the cell border. An unusual feature of this bony fish, compared with all others so far described, is the presence of the extensions or nodules on the side of the microridges adjacent to the cell borders. (*d*) Numerous microvilli on the corneal surface of the Australian lungfish *Neoceratodus forsteri*. At the cell borders are larger microprojections, some of which have hollowed centres. The function of these latter microprojections is unknown. Scale bars, 1 μm.

salts and water from the cornea to maintain an appropriate level of dehydration of the stroma to ensure a clear, transparent cornea. Similarly, the lower densities in non-aquatic vertebrates assist in the evaporation of water from the cornea and therefore aid deturgescence.

Differences between the cell densities of central and peripheral corneas are known to occur in some species. In both the rabbit (Doughty & Fong 1992) and the sandlance *Limnichthyes fasciatus* (Collin & Collin 1997), the corneal epithelial cells are larger in the periphery than in

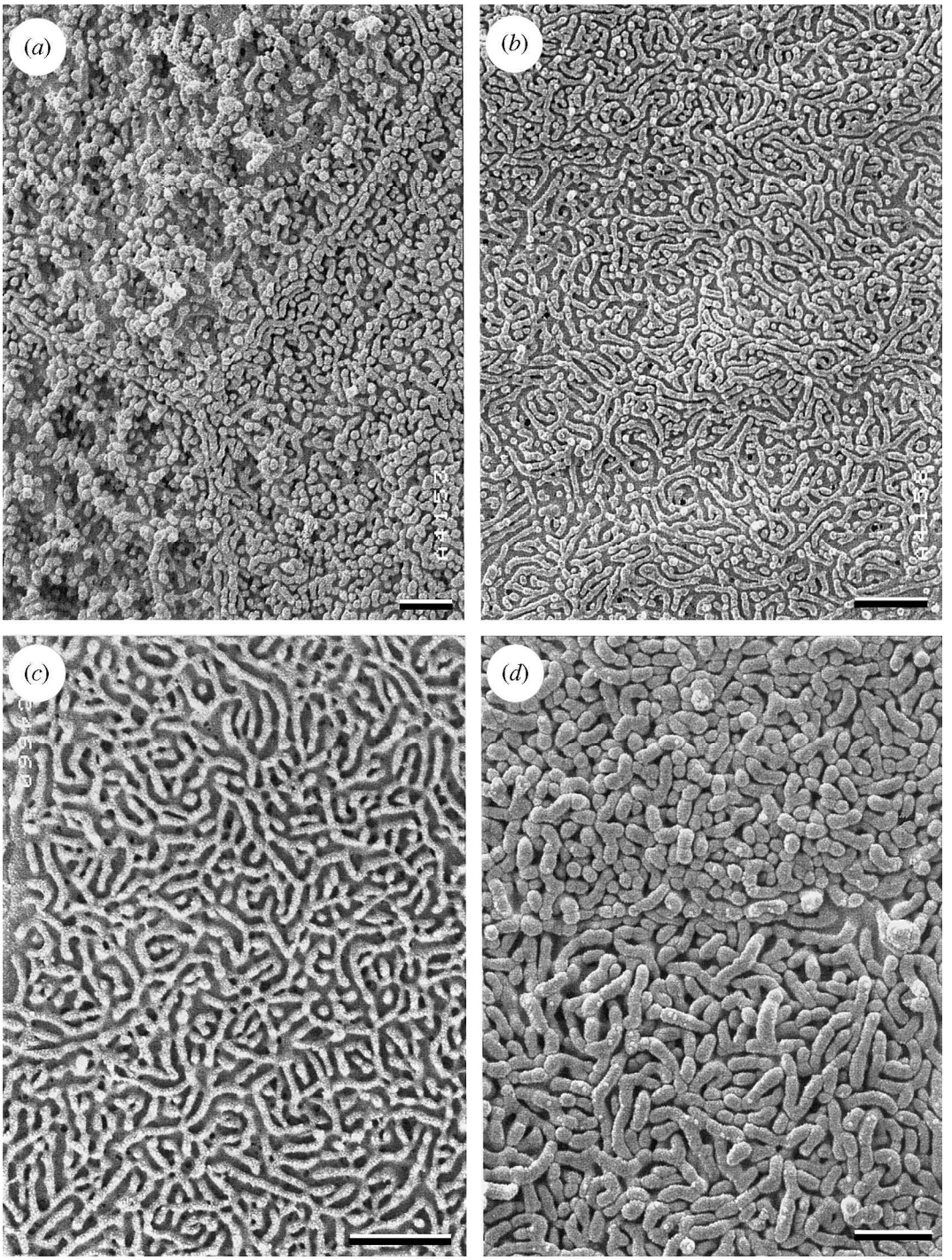

Figure 2. (*a*) The corneal surface of the loggerhead turtle *Caretta caretta*, showing the dense carpet of microvilli, some of which appear to be aggregated into clumps. (*b*) Micrograph of the corneal surface of the ornate lizard *Ctenophorus ornatus*, with its pattern of short microridges that appear to be randomly arranged. (*c*) While most other birds have microvilli, the fairy penguin *Eudyptula minor* has a pattern of microridges that are shorter than those of bony fishes and randomly arranged. (*d*) The dense pattern of microvilli on the corneal surface of the emu *Dromaius novaehollandiae*. This pattern appears to be typical of non-aquatic birds. Scale bars, 1 μm.

the centre. However, these differences were not investigated in this study.

3. SURFACE MICROPROJECTIONS

Scanning electron micrographs showing the appearance of the corneal surface of various vertebrate species are shown in figures 1 and 2. The corneal surfaces of most mammals, birds, reptiles and amphibians are covered with small membrane-bound projections in the form of finger-like microvilli (80–120 nm in diameter, Collin & Collin 2000) and microplicae. The corneal surface of the cartilaginous fishes studied also show the presence of microvilli and microplicae. The microvilli on the surface of the cornea of the black shark *Dalatias licha* are shown in figure 1*b*.

In contrast, the corneal surfaces of most bony fishes possess no microvilli and are covered with microridges (120–227 nm in width, Collin & Collin 2000), which are often continuous, frequently orientated parallel to each other and to the cell border, and never cross from one cell to another (Collin & Collin 2000) (figure 1*c*). Following comparisons of a number of bony fishes, the patterns of microridges, although similar, appear to be different and possibly species specific. A somewhat unusual feature of the microridge pattern is found in the blowfish *Torquigener pleurogramma* (figure 1*c*), where there are small extensions or nodules on the outer side of the microridges adjacent to the cell border. To date, the only bony fish found to possess microvilli, instead of microridges, is the Australian lungfish *Neoceratodus forsteri* (figure 1*d*).

Although microridges are a characteristic of the corneal surface of bony fishes, there are some species of reptiles that also possess microridges. The ornate lizard *Ctenophorus ornatus* has a pattern of microridges (figure 2*b*) that are both narrower (96 ± 13 nm) and shorter than those found in bony fishes. In addition, the microridges are arranged in an apparently random pattern, rather than aligned parallel to each other and to the cell border. Among other members of the Reptilia, the corneas of the crocodile *Crocodilus porosus* and the loggerhead turtle *Caretta caretta* are covered with microvilli (figure 2*a*).

The corneal surfaces of most mammals and birds, including the emu (figure 2*d*), are covered with microvilli and/or microplicae. However, there are also numerous microridges in the central corneal surface of the Australian koala *Phascolarctos cinereus* and in the partially marine aquatic fairy penguin *Eudyptula minor* (figure 2*c*), which closely resemble those of the ornate lizard *C. ornatus* (figure 2*b*).

4. THE ROLE OF SURFACE MICROPROJECTIONS

In humans and other mammals, several functions have been suggested for the microvilli and microplicae. They increase the surface area of the superficial epithelial cells, which aids the absorption of oxygen (Beuerman & Pedrosa 1996) and other nutrients into the cells and assists the movement of metabolic products from the cells. In addition, they stabilize the tear film, which is essential for clear vision in an aerial or terrestrial environment (Pfister 1973).

In aquatic vertebrates, the role of the microvilli and microridges is less clear. A stable tear film is not essential in the production of a clear retinal image due to the minimal refractive index difference between the cornea and the surrounding water. However, stabilizing the mucous layer on the corneal surface may enable it to act as a protective coating, which is particularly important in those species, such as the salamanderfish *Lepidogalaxias salamandroides*, that burrow into the sand. Although the cell surface area of microridges is less than that of microvilli, microridges will still result in a significant increase in cell surface area and therefore assist in nutrition and waste removal. Actin filaments play a role in the formation of microridges (Uehara *et al.* 1991) and, together with the structure of the longer parallel microridges, will provide rigidity and plasticity to the surface epithelial cells (Uehara *et al.* 1991; Zeiske *et al.* 1992). Highlighting the ubiquitous need for either structural support or nutritional exchange in an aquatic environment, microridges have also been described in epithelia associated with sensory structures. These include the conjunctival and nasal epithelia of the sandlance (Collin & Collin 1997) and the olfactory epithelium, oral mucosa, neuromasts of the lateral line receptors, electroreceptors, chemosensory cells and taste buds in a range of teleosts (reviewed in Collin & Collin 2000).

5. SURFACE MICROHOLES

In two species of Southern Hemisphere lampreys, microholes are present in the corneal surface (figure 1*a*). These holes are quite large (395 ± 15 nm in the pouched lamprey *Geotria australis* and 245 ± 177 nm in the shorthead lamprey *Mordacia mordax*) and represent openings for the release of mucous granules, which may form a coating over the cornea to protect it against abrasion during burrowing. Microholes (324 ± 76 nm) similar to those observed in the lampreys are also present in the premetamorphic salamander (axolotl) *Ambystoma mexicanum*. These holes also permit the release of mucous granules onto the corneal surface (H. B. Collin and S. P. Collin, unpublished data).

Goblet cells are present in the corneal epithelium of the salamanderfish *L. salamandroides* (Collin & Collin 1996). As their habitat of small ponds dries out in the summer months, *L. salamandroides* burrows into the mud and aestivates, inducing the release of mucus from the goblet cells, which covers the surface of the cornea. Holes in the corneal surface have also been found in the bar-tailed flathead *Platycephalus endrachtensis* (Collin & Collin 2000) and the sandlance *Limnichthyes fasciatus* (Collin & Collin 1997), two species which are partially buried when lying in wait for prey with eyes protruding. However, these are *ca.* 2 μm in diameter, are present only in the peripheral cornea and are situated at the junctions of three and sometimes four cells.

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