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# Fine Structure of the Optic Cusion in the Asteroid *Nepanthia belcheri*

**P. E. Penn* and C. G. Alexander**

School of Biological Sciences, James Cook University of North Queensland; Townsville, Queensland 4811, Australia

## Abstract

The optic cushion of *Nepanthia belcheri* (Perrier) is a prominent pigmented sense organ situated on the oral surface below the terminal tentacle. The distal region contains up to 170 optic cups, whilst proximally are numerous pyriform glandular cells traversed by supporting fibres. The outer margin of the optic cup is formed by alternating pigmented and photoreceptor cells. The pigmented cells contain numerous densely staining granules of scarlet pigment. The distal ends of the photoreceptors are elaborated into many long microvilli regularly arranged about a modified cilium. There is a clear circumciliary space delimiting the cilium from the microvilli.

## Introduction

The optic cushion, a specialisation of ectoneural tissue of the radial nerve, is considered by Reese (1966) to be the only prominent sense organ found in the class Asteroidea. This pigmented structure, first reported by Vahl in 1780 (quoted by Smith, 1937), is situated at the distal end of each arm on the oral surface below the terminal tentacle.

The role of the optic cushion in photoreception is uncertain because of inconsistencies in phototactic responses reported by previous investigators as reviewed by Hyman (1955) and Yoshida (1966). Yoshida and Ohtsuki (1966, 1968) presented evidence from reflex action spectra and phototactic behaviour in *Asterias amurensis* that the optic cushion may act in conjunction with a diffuse dermal sensitivity. However, Castilla (1971) demonstrated that in *A. rubens* the response varied according to the adaptive state of the seastar, such as physiological condition and previous exposure to illumination.

Hartline *et al.* (1952) provided the only electrophysiological evidence demonstrating the responsiveness to light by the optic cushion.

Smith (1937) demonstrated that the optic cushion of *Marthasterias glacialis* contained numerous cone-shaped invaginations of the superficial ectoderm termed optic cups. Since this work, fine structural observations on four genera of asteroids (Eakin and Westfall, 1962; Eakin, 1963; Vaupel von Harnack, 1963) suggested that the optic cups were made up of both pigmented and sensory cells and that the sensory cells were extended distally into an array of disordered microvilli projecting into the optic cup lumen from the base of a modified cilium.

Ultrastructural examination of the optic cup in *Nepanthia belcheri* reveals an exception to the pattern of ciliary development proposed by Eakin (1963) for echinoderm photoreceptor organisation.

## Materials and Methods

Specimens of *Nepanthia belcheri* (Perrier) were collected from the intertidal zone of Rowes Bay, Townsville, Australia, and maintained in tanks of circulating sea water. Optic cushions were removed and fixed immediately in 2.5% glutaraldehyde in 0.2 M cacodylate buffered sea water, pH 7.4, at 25.5 °C for 40 min before post-fixation with 1% osmium tetroxide in a similar buffer for the same time and at the same temperature. Following a wash in sea water for 30 min, the tissues were dehydrated in graded ethanols before infiltration and embedding in epoxy resin following the method of Spurr (1969). Sections 1 μm thick for light microscopy were stained in 1% toluidine blue in 1% borax. For electron microscopy, silver-grey sections were stained with uranyl acetate and lead citrate and examined in a Siemens Elmiskop 102 electron microscope.

*Present address: College of Medicine, Texas A&M University, Olin E. Teague Research Center, College Station, Texas 77843, USA

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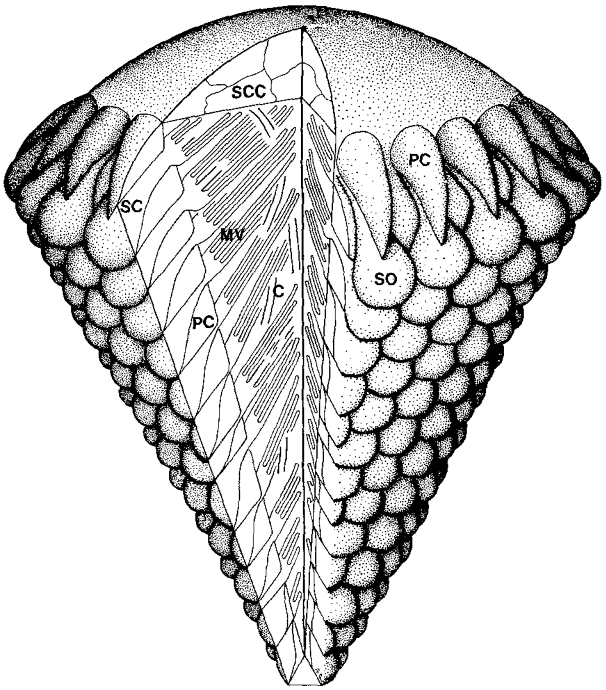


**Fig. 1.** *Nepanthia belcheri.* Three-dimensional diagrammatic representation of an optic cup revealed by optical and electron microscopy. C: cilium; MV: microvilli; PC: pigment cell; SC: sensory cell; SCC: subcuticular space; SO: soma region of sensory cell

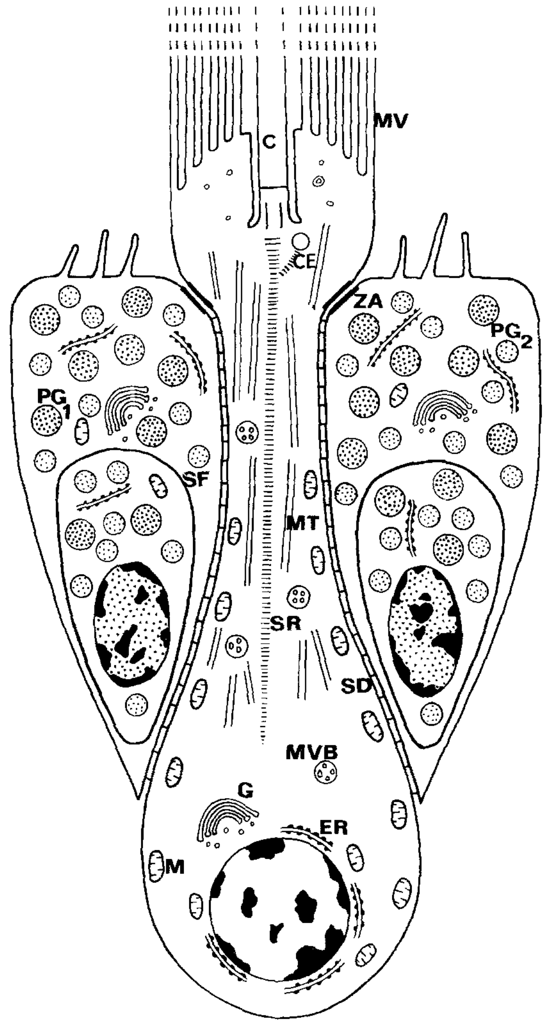


**Fig. 2.** *Nepanthia belcheri.* Diagrammatic representation of sensory cell and pigment cell. C: cilium; CE: centriole; ER: rough endoplasmic reticulum; G: Golgi apparatus; M: mitochondria; MT: microtubule; MV: microvilli; MVB: multivesicular body; $PG_1$: large pigment granule; $PG_2$: small pigment granule; SD: septate desmosome; SF: supporting fibre; SR: striated rootlet; ZA: zonula adherens

## Observations

The optic cushion of *Nepanthia belcheri* is a conspicuous saddle-shaped, red pigmented body approximately 0.37 mm in length x 0.21 mm at its broad distal end.

Optical longitudinal sections show that the cushion is divided into two distinct regions. The proximal area, some 10 to 15% of the total volume, contains many pyriform glandular cells with basal nuclei and cytoplasm filled with membrane-bound spheres. These cells, averaging 13 x 6 μm, occur singly or in groups and are traversed by supporting fibres resembling those described in the radial nerves of both asteroids and echinoids (Kawaguti, 1965; Kawaguti *et al.*, 1965; Cobb, 1970). The remainder of the cushion is characterised by 150 to 170 optic cups, which are regularly arranged in rows across the surface of the cushion through almost 180°.

Reconstruction from serial optical sections show that the conical optic cups are embedded in the cushion with the base directed to the outer surface. The cup is approximately 25 μm across the base and 55 μm from the base to the apex. The outer margin of the cup is formed from alternating pigment and sensory cells, of which there are approximately 150 of each type (Fig. 1). Details of the sensory and pigment cells are diagrammatically shown in Fig. 2.

Microvilli and cilia originating from the distal end of the sensory cells fill the lumen of the cup, which is capped at the outer surface by an arch of epithelial or subcuticular cells.

### Pigment Cells

The wall of the optic cup is formed from the truncated bases of cone-shaped pigment cells, which appear roughly triangular in optical and thin sections. Microvilli containing fine filaments and electron-dense granules attached to the peripheral cell membrane project into the lumen for a short distance. The tapering apices of these cells curve to lie parallel to the long axis of the optic cup in a similar fashion to that described in *Marthasterias glacialis* (Smith, 1937).

These cells are connected to each other by extensive septate desmosomes. The ellipsoid nucleus, which typically contains densely stained chromatin material, lies in the apical region of the cell and is often invested by intracellular fibres (Fig. 3: *3*). Such fibres, lying parallel to the long axis of the cell, were also observed by Engster

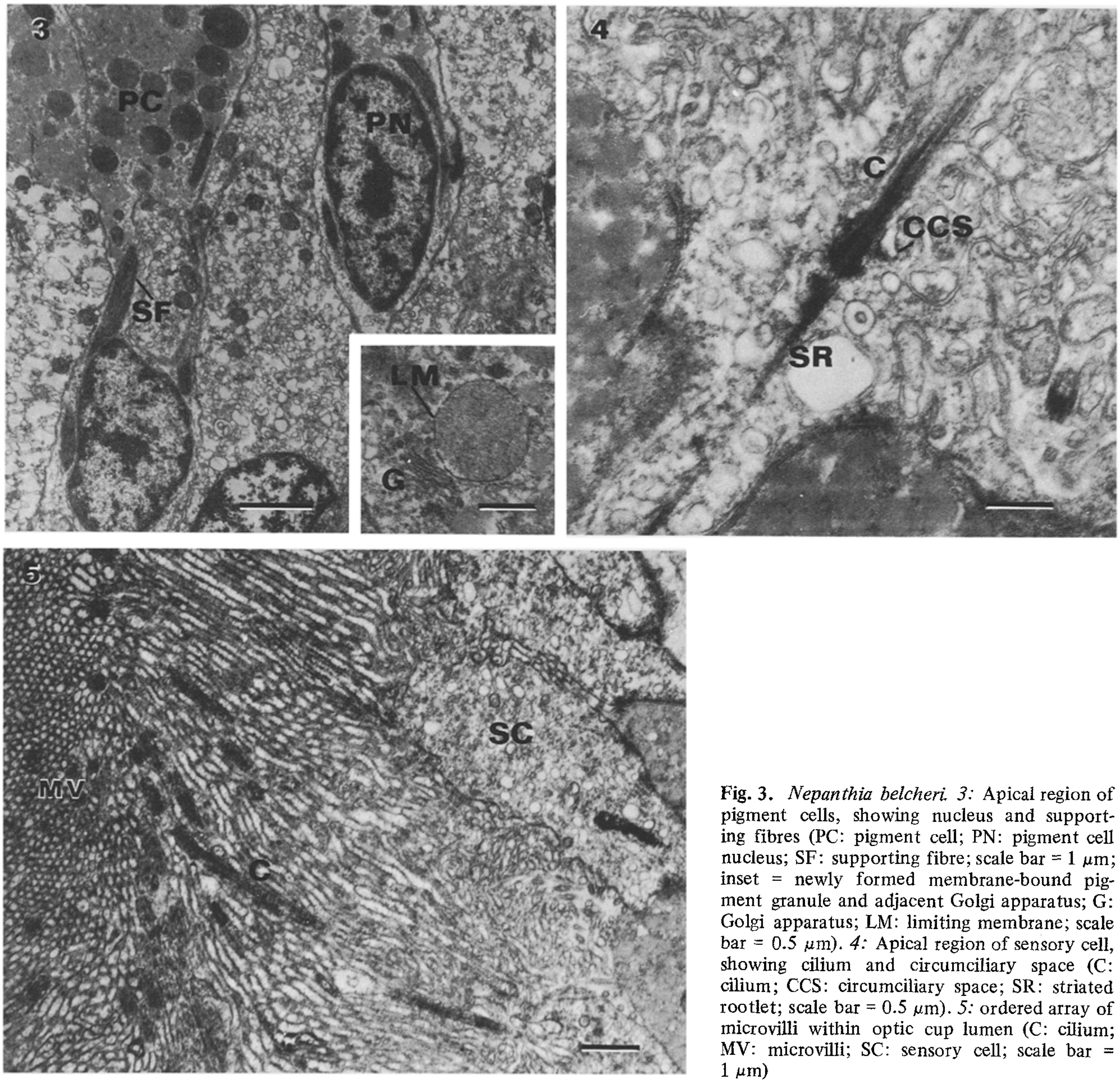


**Fig. 3.** *Nepanthia belcheri. 3:* Apical region of pigment cells, showing nucleus and supporting fibres (PC: pigment cell; PN: pigment cell nucleus; SF: supporting fibre; scale bar = 1 μm; inset = newly formed membrane-bound pigment granule and adjacent Golgi apparatus; G: Golgi apparatus; LM: limiting membrane; scale bar = 0.5 μm). *4:* Apical region of sensory cell, showing cilium and circumciliary space (C: cilium; CCS: circumciliary space; SR: striated rootlet; scale bar = 0.5 μm). *5:* ordered array of microvilli within optic cup lumen (C: cilium; MV: microvilli; SC: sensory cell; scale bar = 1 μm)

and Brown (1972) in what they described as vacuolated cells in the tube feet of *Astropecten* sp.

Much of the cytoplasm is packed with a large number of pigment granules. The smaller (0.36 μm in diameter) more numerous granules contain amorphous, weakly osmiophilic material, whilst the larger granules (0.74 μm in diameter) are made up of granules of strongly osmiophilic material. The larger granules are associated with and probably produced by a well developed supranuclear Golgi apparatus as seen in Fig. 3: *3* (inset), which shows an apparent newly formed pigment granule with a distinct limiting membrane.

These pigment granules give the optic cup and the whole optic cushion its characteristic brilliant scarlet colour, which, according to Millott and Vevers (1955), is a complex of β-carotene and astaxanthin. In addition to the pigment-containing granules, which account for most of the cytoplasm, there are free ribosomes, mitochondria and rough endoplasmic reticulum.

## Photoreceptor Cells

The sensory photoreceptor cells alternate with and intrude between adjacent pigment cells (Figs. 1 and 2). The cell is very elongated, measuring on average 20 x 6 μm at the level of the nucleus and is delimited into 3 morphologically distinct regions: the apex, shaft and soma.

## The Apex

The bulbous apex, which is attached to the lumenal margin of the pigment cup by a zonula adherens, is characterised by a prominent modified cilium. Although similar to that mentioned by Eakin (1963) from three different species of asteroids, the axonemal configuration tends to vary even within a single cup. Some axonemes contain the 9 + 0 configuration whilst others have the 8 + 1 arrangement.

The ciliary shaft is sunk into the apex of the photoreceptor cell and is clearly delimited by a circumciliary space (Fig. 3: *4*). The cilium extends towards the centre of the lumen, where it projects upwards towards the base of the optic cup. It does not penetrate the base to the exterior but spreads out radially beneath the subcuticular cells (Fig. 1).

The distal segment is characterised by numerous microvilli with granulated cytoplasm. Unlike echinoderm photoreceptors previously described, the sensory cell microvilli of *Nepanthia belcheri* are not randomly orientated within the optic cup lumen but are obviously arranged very regularly. Fig. 3: *5* shows microvilli in approximately longitudinal section close to their origin, but immediately beyond this point they are in transverse section. This indicates that close to their origin they bend through 90° to lie parallel to the long axis of the cup and that different microvilli groups interdigitate closely, forming a regular pattern similar to the arrangement of microvilli in the arthropod rhabdom.

The cytoplasm adjacent to the ciliary base contains numerous free ribosomes, smooth vesicles and microtubules. The microtubules and the ciliary rootlet extend into the shaft.

## The Shaft

This accounts for approximately 50% of the total cell length. It contains long microtubules extending from the apex, orientated parallel to its length. The ciliary rootlet extends for the entire shaft length and penetrates the cell soma. Very often the ciliary rootlet has a side branch, which reflexes to an accessory centriole situated in the apex.

The shaft contains multivesicular bodies and many large elongated mitochondria. The shaft region is firmly attached to the adjacent pigment cells by septate desmosomes.

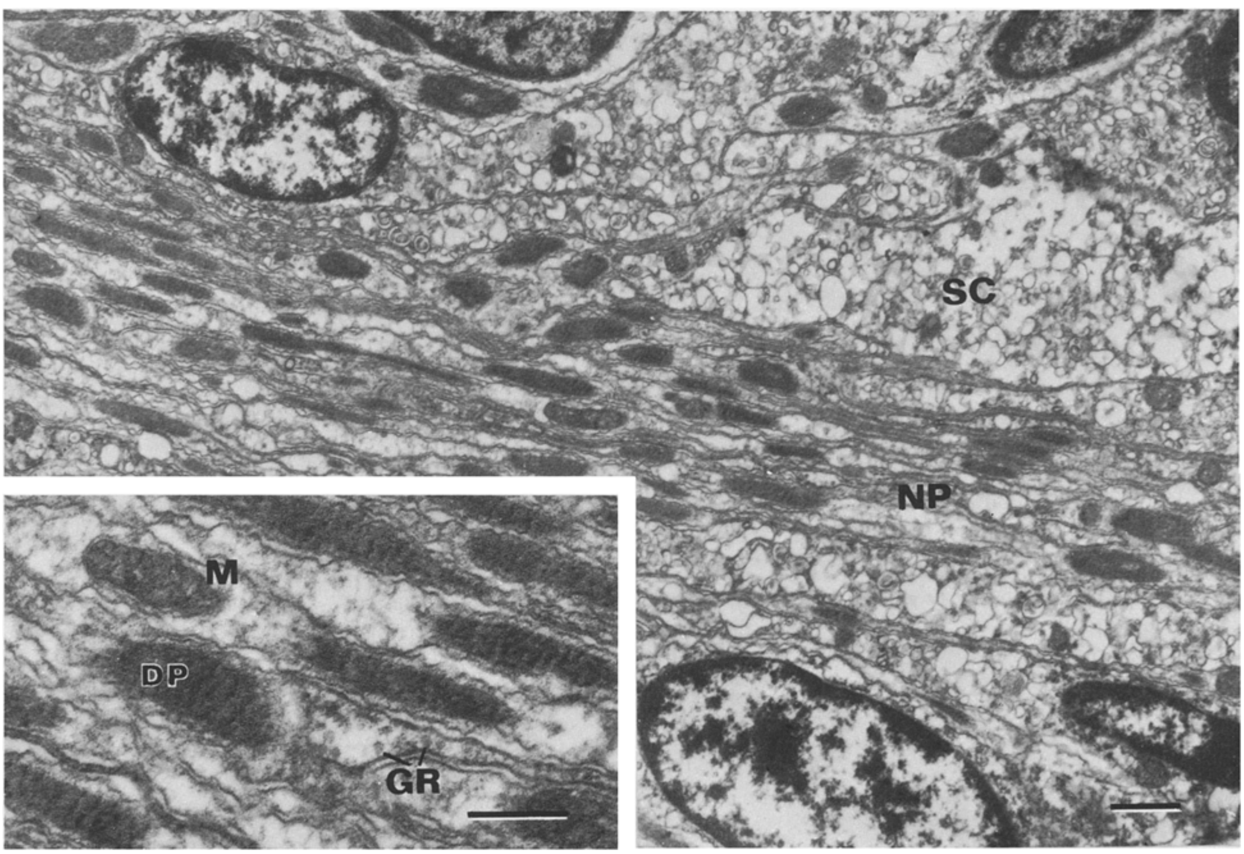


**Fig. 4.** *Nepanthia belcheri*. Closely packed unmyelinated fibres of nerve plexus containing dense processes; NP: nerve plexus; SC: sensory cell (scale bar = 1 μm). Inset: Nerve plexus showing dense processes and vesicles at high resolution; DP: dense processes; GR: granular vesicles; M: mitochondria (scale bar = 0.5 μm)

The Soma

The cell nucleus is spherical and many micrographs show mitochondria closely associated with it. In other cases, however, mitochondria are situated peripherally. Some sections reveal rough endoplasmic reticulum packed around the nucleus together with a prominent supranuclear Golgi apparatus.

Closely associated with the soma region is a nerve plexus, which lies at the level of each sensory cell. Nervous connections are present between adjacent sensory cells of the same optic cup and between adjacent optic cups.

The Nerve Plexus

The nerve plexus is composed of closely packed, unmyelinated fibres (Fig. 4). Each fibre is about 0.30 to 0.37 μm in diameter and contains dense processes, elongated mitochondria and granular vesicles, which are approximately 60 to 70 nm in diameter (Fig. 4, inset). Many sections also reveal the presence of clear centred vesicles and neurofilaments.

The fibres of the plexus enter the radial nerve at the level of the optic cup apex and intrude between the axons of the ectoneural plexus. Synaptic junctions were not observed between the two nerve types.

## Discussion

Complex sense organs are not an obvious feature of the echinoderms. The only type of organ so far described in these animals is the optic cushion. Echinoderms are notoriously difficult to investigate electrophysiologically, and most workers have relied on structural examinations and to some extent behavioural observations.

Echinoderms are sensitive to light in that the integument is itself photosensitive (Yoshida, 1966). The complex arrangement of optic cups within the optic cushion obviously must enhance this facility. The individual pigment cells, which collectively form the optic cup, provide support and serve to orientate the sensory cell endings within the cup lumen. The pigment cells allow light to impinge on the receptor cells from certain directions only. Furthermore, the reflecting capacity of the pigment ensures that maximal reception of light entering the cup is achieved. The cups are so arranged that between them they can receive light from an angular arc of almost 180°, which probably enables the seaster to respond differentially to the direction of light.

Detailed microscopical examination has shown nervous connections between cups, so it is implied that integration of sensory activity can occur. Similarly, there is neural communication between the arms of the seastar (Smith, 1965). Further evidence of this was demonstrated when a point light source impinging on the sensory cushion of a moving specimen of *Acanthaster planci* caused the arm to lift away from the light source very rapidly, and at least two arms on either side of the stimulated arm did like wise (Alexander, unpublished observations).

Past observations on the optic cushion of a number of asteroid species (*Asterias rubens*, Vaupel von Harnack, 1963; and *Leptasterias pusilla, Henricia leviuscula, Patria minata*, Eakin, 1963), demonstrated that echinoderms possess a ciliary type of photoreceptor in that the light-sensitive units are derived from and are morphologically associated with the base of a modified cilium. In the typical ciliary type of photoreceptor, the cilia possess considerable membranous elaborations which are expressed either as saccules, lamellae, discs, tubules or villi (Eakin, 1972). The microvilli, which occupy the optic cups of these asteroids, resemble those found in the hydromedusan *Polyorchis penicillatus* (Eakin and Westfall, 1962) in that they display a disorganised arrangement.

The microvilli of *Nepanthia belcheri* originate from the apical plasma membrane of the sensory cells and are not derived from the cilia. The cilia, which contain the typical axonemal configuration of sensory cilia (9 + 0 or 8 + 1), are isolated from the microvilli by a distinct circumciliary space and are not externally elaborated. This resembles the rhabdomeric arrangement reported in two species of Onychophora (Eakin and Westfall, 1964) and in the sipunculid *Phascolosoma agassizii* (Hermans and Eakin, 1969). Rudimentary cilia have been described in the rhabdomeric photoreceptors of the pulmonate snail *Helix aspersa* (Eakin, 1966; Eakin and Brandenburger, 1967) and in the ocelli of some nereid polychaetes (Dorsett and Hyde, 1968).

The arrangement of microvilli within the lumen is regular, and clearly resembles the arrangement of the light-sensitive region of the arthropod eye, the rhabdom. Although any dioptric capability is open to question, the conical shape suggests a role in light intensity discrimination. There is a gradual decrease in the number of sensory cells as well as absorptive surface area within the lumen from the base to the apex of the cup. It can be deduced from this arrangement that light of low intensity would stimulate those cells closest to the light source, whereas light of a higher intensity could penetrate deeper into the cone and activate more sensory units. In this way, the seastar can appreciate the quantity of light and readjust its position in the environment.

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