Protoplasma 131, 23–31 (1986)

PROTOPLASMA



# A Comparative Study of Periplast Structure in *Cryptomonas cryophila* and *C. ovata* (*Cryptophyceae*)

S. J. BRETT * and R. WETHERBEE

School of Botany, University of Melbourne

Received June 18, 1985
Accepted November 1, 1985

## Summary

Freeze-fracture followed by deep-etch was used with transmission electron microscopy to characterize and compare the periplasts of two cryptomonads, *Cryptomonas ovata* and *C. cryophila*. The periplast of *C. ovata* consists of a dense surface mat of granular/fibrillar material overlying a series of polygonal plates attached to the undersurface of the plasma membrane (PM) at their upturned edges. Fracture faces of the PM reveal a highly stable substructure with distinct patterns of intra-membrane particles (IMPs) associated with the underlying plates; a role for the PM in plate development is indicated. The surface periplast component of *C. cryophila* exhibits a cover of morphologically complex, overlapping heptagonal scales (termed rosette scales) in addition to elongate fibrils. The arrangement of IMPs within the PM is predominantly random and the inner periplast component consists of a sheet with regular pores where ejectisomes are located. The sheet does not appear closely associated with the PM. The combination of features exhibited by the periplast of *C. cryophila* warrants its inclusion as a new type within the *Cryptophyceae*.

*Keywords:* *Cryptomonas;* *Cryptophyceae;* Freeze-fracture/etch; Periplast; Scales.

## 1. Introduction

The *Cryptophyceae* is a well-defined class of phytoflagellates common in both marine and freshwater habitats. Cells are characterized by a number of features (see WETHERBEE *et al.* 1986) including a periplast consisting of an inner periplast component (IPC) and an additional surface periplast component (SPC) external to the PM. The arrangement and positioning of the IPC and the nature of the SPC varies between taxa. The differences have resulted in the definition of at least two major periplast types within the group, and the suggestion that periplast structure may be of taxonomic importance (SANTORE 1977).

In *Hemiselmis* (SANTORE 1977, 1982 a) and *Chroomonas* (DODGE 1969, GANTT 1971, SANTORE 1977, 1982 b) internal and external plate-like structures of similar dimensions and thickness are closely appressed to the PM. The plate elements of *Hemiselmis* and *Chroomonas* have been described as rectangular, although a recent study (WETHERBEE *et al.* 1986) demonstrates that the periplast of *Hemiselmis brunnescens* is composed of hexagonal plates. The rectangular plates of *Chroomonas* are arranged in precise rows, and vesicle bound ejectile organelles known as ejectisomes are generally located at the anterior corners of each plate.

In *Cryptomonas* (LUCAS 1970, FAUST 1974, HIBBERD *et al.* 1971, SANTORE 1977) and *Pyrenomonas* (ANITA *et al.* 1973, OAKLEY and DODGE 1976, HAUSMANN and WALZ 1979, SANTORE 1977, 1982 b, 1984), the only supportive plates are reported to be those of the IPC. These polygonal or rectangular internal plates make contact with the PM only at their upturned edges, and ejectisomes are located at the plate corners. Fibrillar material and minute hexagonal scales have been reported on the cell surface (SANTORE 1983).

The use of deep-etch in the present study has enabled an accurate visualization of the complex SPC that is often difficult to observe using thin sectioning techniques alone. In *C. cryophila*, the SPC consists of a complex layer of heptagonal "rosette" scales while the SPC of *C. ovata* is composed of a mat of fine striated fibrils.

* Correspondence and Reprints: School of Botany, University of Melbourne, Parkville, Vic. 3052, Australia

Freeze-fracture/etch, in combination with standard thin sectioning techniques has allowed detailed observation of the periplasts of both *C. cryophila* and *C. ovata*, and has enabled examination of the relationships between the components which make up this complex system.

## 2. Materials and Methods

*C. ovata* was originally obtained from the University of Texas Culture Collection (UTEX 385) and maintained in BBM (BISCHOFF and BOLD 1963) plus 5% soil extract at 20 °C under a light/dark cycle of 16–8 hours. *C. cryophila*, collected from the Weddel Sea (antarctic) by Dr. G. I. MCFADDEN, was grown in f/2-enriched seawater medium (GUILLARD and RYTHER 1962) under constant illumination and a constant temperature of −2 °C. All cells were harvested during the logarithmic growth phase. The methods used in this study are described in the accompanying paper (WETHERBEE *et al.* 1986).

## 3. Results

Cells of *C. cryophila* are 14 μm in length, 8 μm in width and maintain the characteristic asymmetric cryptomonad cell shape, with two pleuronematic flagella emerging from an anterio-ventral vestibulum (Fig. 1). The organisms also possess a furrow progressing along the cell surface for approximately 3 μm. Scanning electron micrographs reveal fibrillar material on a relatively smooth cell surface, although the structure of the fibrils is difficult to ascertain with this technique (Fig. 2). In all peripheral regions except the gullet, thin sections reveal a layer of electron dense material which underlies the PM at a distance of 25 nm (Figs. 3 and 4) and follows its contours exactly. The IPC is regularly interrupted by ejectisomes contained in membrane-bound vesicles. The SPC of *C. cryophila* is difficult to visualize in thin section, generally appearing as circular electron dense structures or amorphous granular material. However, observation of rotary and unidirectionally shadowed freeze-etched replicas of unfixed cells (Figs. 6–8) has allowed detailed examination of the SPC.

The most prominent surface feature is a layer of morphologically complex rosette scales. Each heptagonal scale is approximately 120–140 nm in diameter and consists of a central axis from which seven spokes radiate to a perimeter of apparently interwoven fibrillar material (Fig. 6). The rosette scales are arranged in an overlapping array and are generally randomly oriented, forming a dense mat across the entire cell surface.

In addition to the scalar component, fibrils 10 nm wide and up to 1.5 μm in length are seen originating from the cell surface in etched replicas and positively stained wholemounts (Figs. 6, 8, and 9). The fibrillar material does not adhere closely to the cell surface, and ranges from randomly oriented, isolated fibrils to dense mats which make other surface features indistinguishable. These fibrils are not of bacterial origin as they are observed on cells isolated from axenic culture.

The distribution of IMPs in the PM is predominantly random in both the protoplasmic and exocellular fractures faces (Figs. 10 and 11). However, dense aggregates of IMPs do occur in regions of the PM which are associated with cytoplasmic ejectisome vesicles (Fig. 11). In these areas, IMPs are located adjacent to the regularly spaced ejectisome docking sites. Here, the plane of fracture may pass into the cytoplasm, leaving the PS adhering to the EF, and often exposing the ejectisome vesicle membrane (Figs. 12–14).

The IPC has not been observed in freeze-etched preparations, although thin sections reveal that this component is a continuous layer regularly interrupted by

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Figs. 1–14. *C. cryophila*

Fig. 1. Light micrograph (Nomarski) (scale bar = 4 μm)

Fig. 2. Scanning electron micrograph showing fibrillar material on cell surface (scale bar = 2 μm)

Figs. 3 and 4. Thin sections of periplast region

Fig. 3. Continuous IPC underlying PM (large arrows). Granular SPC (small arrows) can be seen on the cell surface (scale bar = 300 nm)

Fig. 4. Thin section showing IPC regularly interrupted by ejectisomes (*E*) which closely associate with the PM (arrows) (scale bar = 300nm)

Fig. 5. Glancing section of cell periphery. Ejectisomes (*E*) are arranged in a highly ordered diagonal array (scale bar = 500 nm).

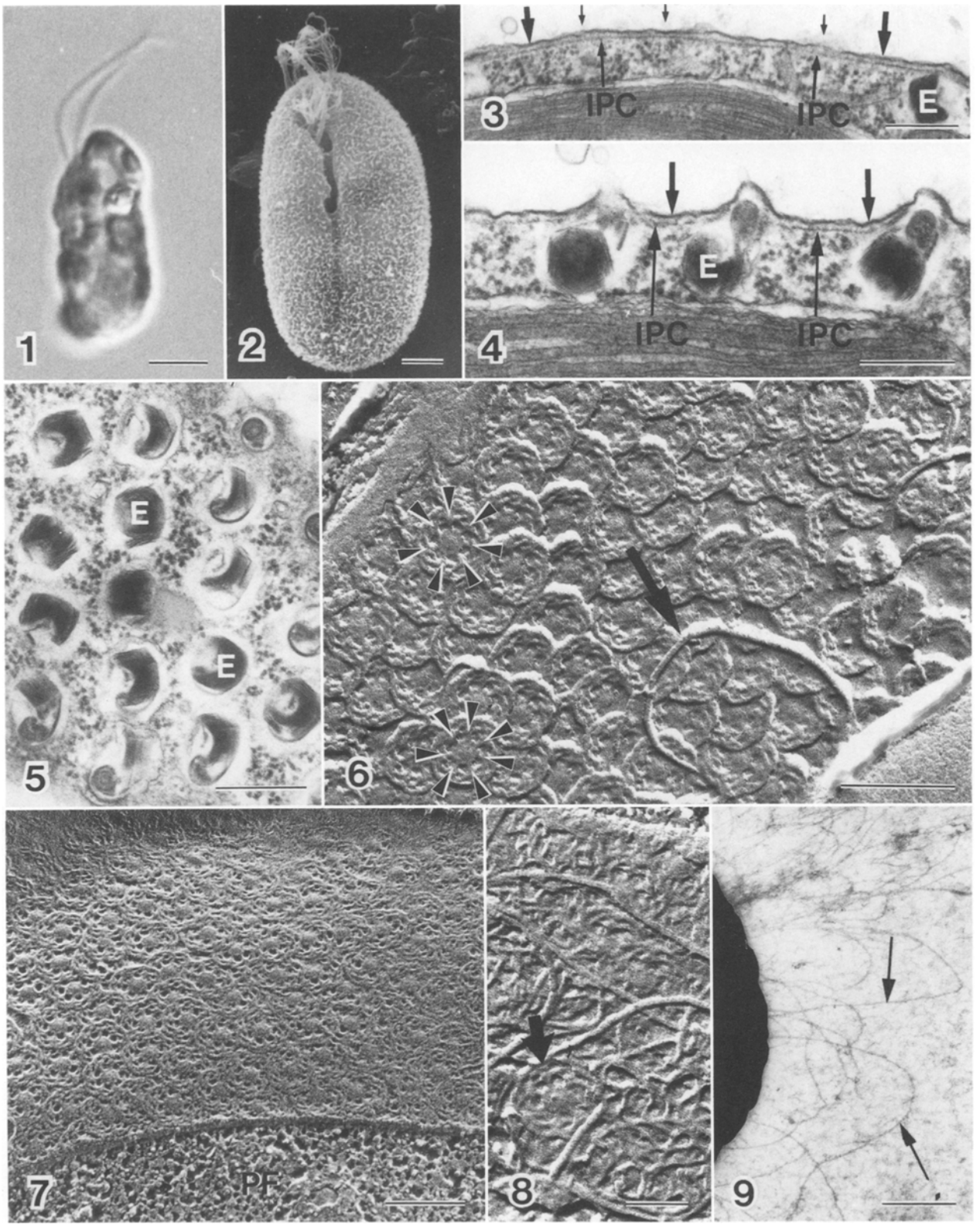
Figs. 6–8. Freeze-etched replicas of cell surface

Fig. 6. Unidirectionally shadowed replica. Fibrils (large arrow) and an overlapping array of rosette scales, each with a heptagonal symmetry (small arrows) (scale bar = 200 nm)

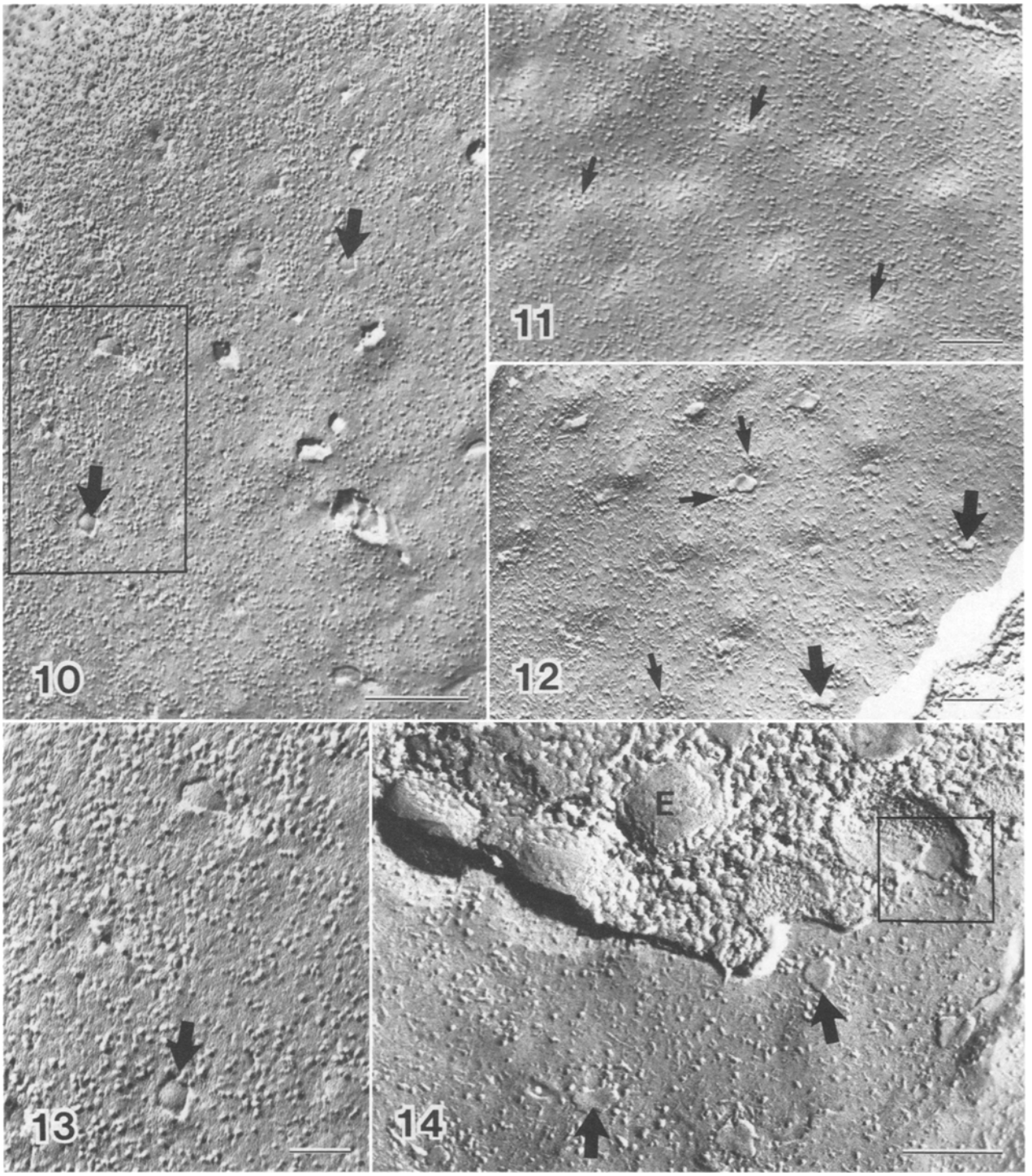
Fig. 7. Rotary shadowed replica of cell surface showing the complement of overlapping rosette scales (scale bar = 200 nm)

Fig. 8. Unidirectionally shadowed replica showing the surface scales (arrow) obscured by fibrillar material (scale bar = 100 nm)

Fig. 9. Positive stained wholemount showing fibrils originating from the cell surface (scale bar = 300 nm)




Figs. 1–9




Figs. 10–14. Unidirectionally shadowed freeze-fracture replicas of the PM

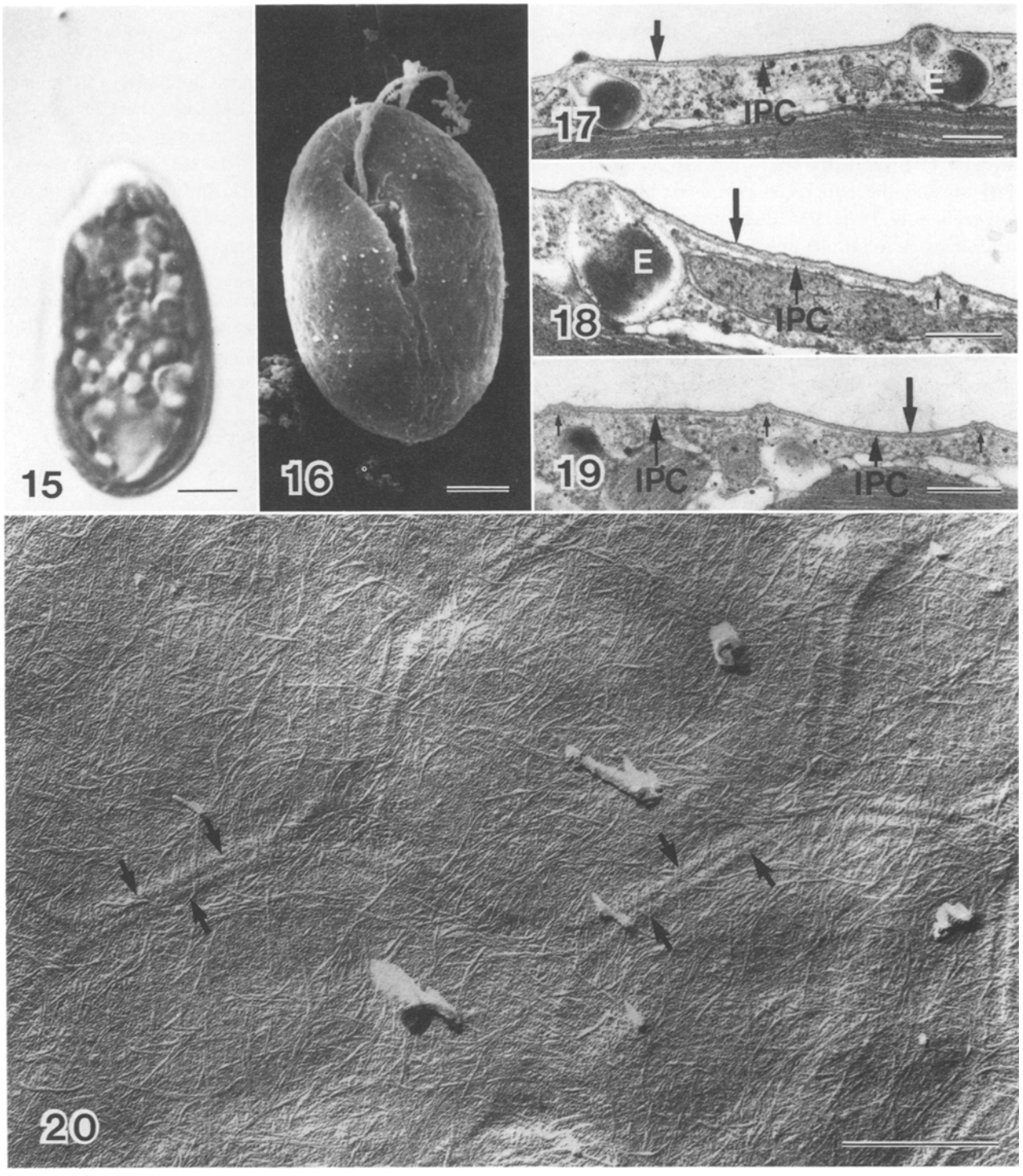
Fig. 10. PF of PM showing the generally randomly distributed IMPs. In the regions where ejectisome vesicles associate with the PM the plane of fracture has occasionally passed into the cytoplasm (arrows). For enlargement of boxed region see Fig. 13 (scale bar = 300 nm)

Fig. 11. EF showing aggregations of IMPs (arrows) which overly the region of PM/ejectisome vesicle association (scale bar = 200 nm)

Fig. 12. EF showing aggregations of IMPs (small arrows) in the region of PM/ejectisome vesicle association. In these areas the plane of fracture has passed from the PM into the cytoplasm (large arrows) (scale bar = 200 nm)

Fig. 13. PF of PM showing fracture plane passing into cytoplasm (arrow) and exposing the ejectisome vesicle (scale bar = 100 nm)

Fig. 14. EF of PM showing fracture plane passing into cytoplasm (arrows) and ejectisome vesicle (boxed region) (scale bar = 200 nm)




Figs. 15–24. *C. ovata*

Fig. 15. Light micrograph (Nomarski) (scale bar = 2 μm)

Fig. 16 Scanning electron micrograph showing apparently naked cell surface (scale bar = 2 μm)

Figs. 17–19. Thin sections of periplast region

Fig. 17. Plates of the IPC underlying the PM (arrow). Ejectisomes are located at the plate corners and are closely associated with the PM (scale bar = 200 nm)

Fig. 18. Thin section showing edges of IPC upturned and contacting the PM (large arrow) at the junction of two cytoplasmic plates (small arrow) (scale bar = 300 nm)

Fig. 19. Thin section showing plate junctions (small arrows) (scale bar = 300 nm)

Fig. 20. Unidirectionally shadowed freeze-etched replica. Fine striated fibrils originate from granular regions on the surface, and a series of polygonally arranged ridges (arrows) is visible (scale bar = 300 nm)

ejectisomes (Figs. 3 and 4). The ejectisomes are arranged in a highly ordered diagonal array (Fig. 5), and must pass through pores in the IPC to make contact with the PM. While the IPC follows the contours of the PM around the entire cell periphery, direct contact between it and the PM does not occur. This layer appears to maintain its position relative to the PM via close association with the ejectisome vesicles which pass through it.

Mature cells of *C. ovata* are 11 μm in length and 9 μm in width (Fig. 15), with the general cell form being similar to that of *C. cryophila*; the cells maintain a distinct ventral furrow/gullet system and rounded posterior. Scanning electron micrographs suggest that the cell is naked (Fig. 16) and that the important periplast support components are possibly intracellular. In thin sections (Figs. 17–19), the IPC appears to comprise a system of discrete plates underlying the PM and regularly interrupted by ejectisomes. Fine granular material is sometimes observed adhering to the cell surface (Fig. 19), although in thin sections the nature of the SPC remains indistinct.

Freeze-etch has allowed detailed examination of the surface of *C. ovata*, and indicates that the SPC is markedly different to that of *C. cryophila* (Fig. 20). Its most distinctive feature is a dense mat of randomly oriented fibrillar material that closely adheres to the cell surface. The fibrils are approximately 6 nm in width and range up to 200 nm in length. A series of ridges outlining a polygonal pattern is also a prominent feature of the surface topography in freeze-etched preparations (Fig. 20). The ridges reflect the position of ordered rows of IMPs within the PM (Figs. 21, 23, and 24). These IMPs possibly represent attachment sites between the PM and the upturned edges of the polygonal cytoplasmic periplast plates. Freeze-fracture shows that the substructure of the PM is composed of discrete IMP domains. Within regions of the PM overlying the cytoplasmic plates and bounded by the plate attachment sites, IMPs are arranged into ordered rows exhibiting a periodicity of 20 nm. However, particle distribution between plate areas is markedly different to that in regions associated with the cytoplasmic plates. IMPs appear randomly distributed with dense aggregates found only at plate corners where ejectisome vesicles are associated with the PM. At these sites, the plane of fracture often leaves the PM and passes to the cytoplasm or surface.

In actively dividing cells, the size and shape of the cytoplasmic plates changes markedly (Figs. 21 and 22). Extra ejectisomes are inserted along the plate perimeters and new plate borders, as indicated by attachment IMPs, develop across the PM from these regions. The production of these IMP sutures appears to coincide with the rearrangement of the IPC during division.

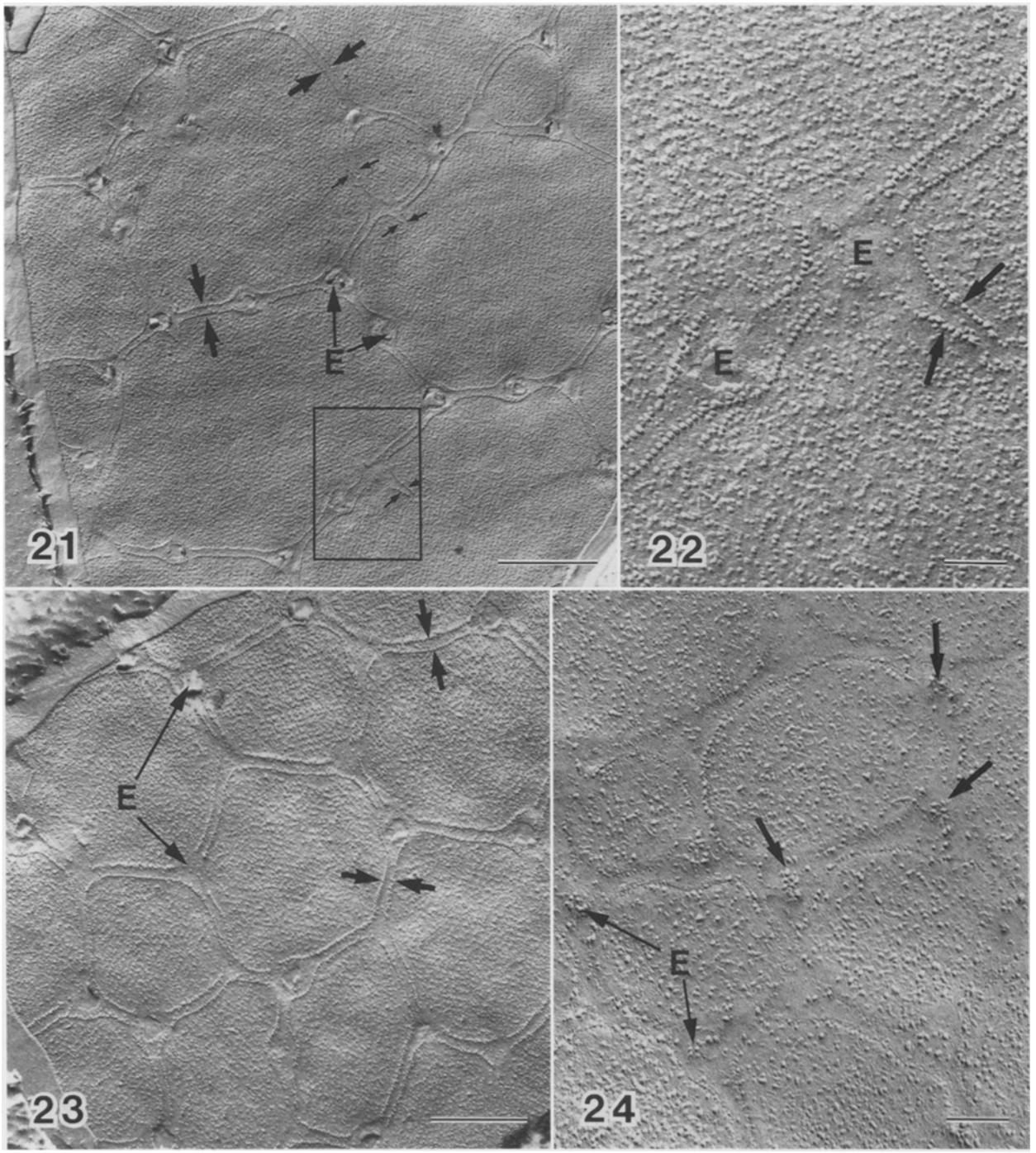
Thin-sectioned material also shows the close relationship between the cytoplasmic plates and the PM (Figs. 17–19). The polygonal plates of the IPC are positioned beneath the PM at a distance of 12 nm. The plate edges are characteristically upturned where they associate with the PM, causing the membrane profile to be raised at the attachment sites and slightly depressed in the space between adjacent plates. In mature cells, ejectisomes are located at the plate corners, and although the ejectisome vesicles do not contact plate material, they are closely associated with the PM.

## 4. Discussion

The cryptomonad periplast functions, in the absence of a microtubular cytoskeleton, to maintain the shape of these cells. Cytoplasmic support components (*e.g.*, plates or a periplast sheet) interact with the PM and extracellular structures to form a complex system which is present in all peripheral regions except the gullet. The evidence presented here indicates that at least two distinct periplast types exist within the presently constituted genus *Cryptomonas* and that the periplast of the antarctic flagellate *C. cryophila* is unlike any previously reported within the *Cryptophyceae*.

The periplast of the type species *C. ovata* consists of a prominent IPC of rigid polygonal plates with ejectisomes located at the plate corners (FAUST 1974). The IPC of *C. cryophila* is not composed of discrete units, but rather a continuous layer of electron dense material forming a sheet which generally follows the contours of the PM. This sheet does not contact the PM, and is regularly interrupted by ejectisomes ordered into a diagonal array. The IPC of *C. cryophila* bears some similarity to that reported for *Chilomonas paramecium* (GRIM and STAEHELIN 1984) although the periplast system of this organism has not been fully described.

Perhaps the most notable difference between the two periplast types lies in the morphology of the SPC. Although both organisms possess an outer fibrillar component, the surface of *C. cryophila* is dominated by a layer of overlapping rosette scales. Scales were first reported on cryptomonad flagella (PENNICK 1981) using freeze-etched replicas. At present there is disagreement about the exact morphology of the scale. SANTORE (1983), from thin sections and shadow-cast whole-




Figs. 21–24. Unidirectionally shadowed freeze-fracture replicas of the PM

Fig. 21. PF of dividing cell. The membrane is arranged into a series of highly organized domains. Areas of PM overlying the cytoplasmic plates have a highly ordered IMP distribution and are bounded by plate attachment IMPs (large arrows). Regions of PM/ejectisome vesicle association (*E*) are located along the plate edges and IMP sutures (small arrows) are seen passing across the membrane domains. For enlargement of boxed area see Fig. 22 (scale bar = 1 μm)

Fig. 22. PF showing IMP suture (arrows) developing across membrane (scale bar = 100 nm)

Fig. 23. PF of mature cell showing plate attachment IMPs (arrows) and highly ordered membrane overlying plates. In region of PM/ejectisome vesicle association (*E*) the fracture plane passes either into the cytoplasm or to the cell surface (scale bar = 500 nm)

Fig. 24. EF showing membrane between plate regions is relatively free of IMPs except in the region of PM/ejectisome vesicle association (*E*) (scale bar = 200 nm)

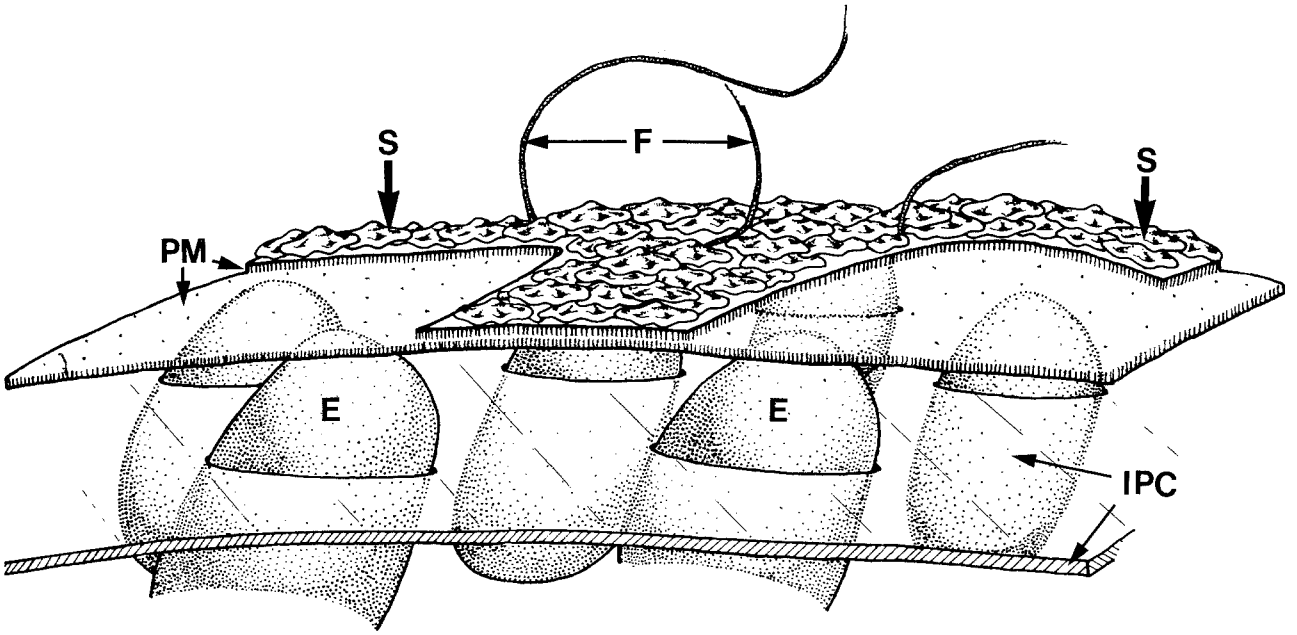



Fig. 25. Schematic representation of the periplast of *C. cryophila*. Ejectisome vesicles (*E*) pass through regularly arranged pores in the IPC to associate with the PM. The surface is covered with overlapping heptagonal scales (*S*) and random fibrils (*F*)

mounts, reported minute body scales (only 12 to 14 nm in diameter) on the surface of several cryptomonads. He suggests the method used by PENNICK would make the scales indistinct, and proposed that the scale symmetry is actually hexagonal. Our findings with *C. cryophila* and other cryptomonad species (HILL and WETHERBEE 1986, WETHERBEE *et al.* 1986) demonstrate that scales possessing a heptagonal symmetry are common in the cryptomonads. However, we have not examined the same organisms as SANTORE and therefore an additional hexagonal scale type may be present on these species.

Deep-etches demonstrate that the surface fibrillar material differs between the two species. Positive-stained wholemounts of elongate fibrils on the surface of *C. cryophila* indicate that these do not adhere to the cell but pass into the surrounding environment. These fibrils appear to originate from the PM, although the dense complement of rosette scales obscuring the surface makes their exact origin unclear. In comparison, the fibrils of *C. ovata* are closely associated with a granular surface and may result from PM mediated development. The thin fibrillar mat does not appear to obscure any additional surface components (*e.g.*, plates), as details of the underlying PM substructure are visible in freeze-etched surfaces.

Fracture-faces of the PM of *C. ovata* reveal discrete, highly ordered domains suggesting a high level of inherent stability within this membrane. The size and shape of the underlying periplast plates is reflected by rows of IMPs (also visible from the surface) which associate with the polygonal plate perimeters. These IMPs apparently serve to connect the plates to the PM, forming a cohesive periplast system.

The distinct areas of PM overlying the cytoplasmic plates also contain IMPs organized into highly ordered rows. The arrangement and periodicity of these IMP rows corresponds to the striated lattice pattern observed by FAUST (1974) in isolated periplast plates of *C. ovata* var. *palustris*. The similarity suggests a close relationship between the PM and IPC. It is possible that membrane microarchitecture is associated with plate development, and that the formation of cytoplasmic plates is mediated via the PM.

The relationship between membrane substructure and function is best observed in dividing cells of *C. ovata*, where dynamic membrane reorganization appears to be occurring. The positioning of additional ejectisomes and the development of IMP sutures across the membrane domains indicates major activity in the underlying periplast component. It is possible that this rearrangement reflects division of the periplast plates, which must subsequently grow and reorient to provide cytoskeletal support for the new daughter cells. Examination of the fracture-faces suggests that the orientation and redistribution of IMPs may direct division,

development and positioning of such internal periplast components as the ejectisomes and plates.

In contrast to the inherently ordered system of IMPs in *C. ovata*, their distribution within the PM of *C. cryophila* is predominantly random. The absence of IMP specializations indicates that the underlying protoplasmic sheet is not as closely associated (structurally or developmentally) with the PM as the periplast plates of *C. ovata*. However, ejectisome vesicles, which pass through pores in the sheet, are in close contact with the PM. In the absence of direct interaction between the protoplasmic sheet and the PM, it is possible that the sheet is able to position itself below the PM and maintain cell shape via association with the peripheral ejectisome vesicles.

Membrane modifications in the area of PM/ejectisome vesicle association are consistent in both species. Dense aggregations of IMPs, similar to those observed in the PM of *Chilomonas paramecium* (GRIM and STAEHELIN 1984), are found adjacent to regions of PM/ejectisome interaction. However, the PM directly overlying the ejectisome vesicles is often devoid of IMPs, and in this region the plane of fracture may pass out of the PM and into the cytoplasm. A similar spatial relationship is also observed in fracture-faces of the ejectisome vesicle. The resistance to fracture within both the PM and ejectisome vesicles suggests a highly modified membrane substructure, and provides further evidence for the close functional relationship between the two membrane systems which must fuse on the discharge of ejectisomes.

The entire periplast system of *C. cryophila*, comprising a protoplasmic sheet with pores for diagonally arranged ejectisomes, a simple continuous plasmalemma and surface structures consisting of elongate fibrils and overlapping heptagonal scales, has not been previously reported within the *Cryptophyceae* and warrants inclusion as a new periplast type within the group.

## Acknowledgements

The first author was supported by an Australian Commonwealth Postgraduate Research Award during this study. The second author was funded by the Australian Research Grants Scheme. We wish to thank Dr. G. I. MCFADDEN for assistance in the preparation of freeze-fractured material.

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