


MICROMORPHOLOGY OF A SMALL DINOFLAGELLATE PROROCENTRUM MARIAE-LEBOURIAE (PARKE & BALLATINE) COMB. NOV.1,2,3

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SUMMARY

The surface structures of the bivalvate dinoflagellate Procentrum mariae-lebouriae are described in detail. It has an almost spheroidal shape in face view, a compressed saucer-shape in side view, with a distinct striated band at the edge of the cell. Its surface is covered with small spines in a regular pattern, with 150 nm distance between pairs. The spines are 100-120 nm wide and 200-300 nm long. There are 600-700 spines on each valve. At the anterior cell end, one of the valves has a V-shaped depression which contains a specialized structure accommodating the 2 flagellar pores. The flagellar pores are enclosed by 8 small, thick plates held together and to the valves by sutures. The flagellar pore area consists of 2 distinct structures: an apical collar possessing a curved forked plate and a larger structure composed of an unbranched plate. There are 2 flagellar canals located between the flagellar pore plates. Beneath each flagellar canal lies a row of 11 microtubules. A row of microtubules forming a microtubular cylinder is situated adjacent to the oblong flagellar canal near a simple pusule. The microtubular cylinder encircles electron dense bodies. The bases of the longitudinal and transverse flagella appear to lie at an angle to each other. The above features are illustrated with transmission and scanning electron micrographs.

INTRODUCTION

The fine structure of the surface of the bivalvate dinoflagellate genera Procentrum and Euxiniaella appears to have a highly characteristic and critical taxonomic value in algal classification (1,2,7,13-17). Light microscopy has not provided sufficient information because of the small size of these organisms. Electron microscopic examination of several species indicated distinct differences in architecture, size, ornamentation of the valves, and the organization of the apical flagellar pore area. Small spines were reported on the surface of the valve of E. mariae-lebouriae (2,3,5,8,15), smooth cell surface of P. micans Ehrenb. (3). Later, Dodge (5) extended the description of the valve surfaces to P. balticum and P. obtusidens; both were covered with evenly spaced small spines. Dodge (7) also described the flagellar pore structure of P. mariae-lebouriae, composed of a number of small thick plates.

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Hulbert (12) recognized the difficulty in separating small Exuviaella and Prorocentrum species, which were collected from the natural environment, using the light microscope, because of their extensive variations in cell shape, size, and the presence and absence of anterior spine. As the ultrastructural information on Prorocentrum and Exuviaella species increased, their identity as distinct and separate genera was not warranted. Abé (1) and Dodge & Bibby (7) recognized this, and proposed that the 2 genera should be merged into the prior genus Prorocentrum Ehrenberg. They defined the most unique features of the combined genera: the distinct parallel orientation of the flagella (7), the presence of a simple sack pusule (6), and the characteristic apical flagellar pore structure (1,7,13). The above characteristics are regarded as unique features for Prorocentrum species and distinguish them from other algae. The current investigation revealed that parallel orientation of the flagella might not be universal for this genus.

The above-described criteria were used to identify the organism responsible for a dense bloom in the Rhode River arm of the Chesapeake Bay. In this study light, transmission, and scanning electron microscopes were used to examine the structure of this small flagellate identified as P. mariae-lebouriae (Parke & Ballantine) comb. nov. This investigation revealed that the apical pore area of this organism is more complex than recognized previously and provides additional information on this small bivalvate dinoflagellate.

 MATERIALS AND METHODS Bay water samples were collected from 1 m below surface and fixed immediately in 4% glutaraldehyde in 0.1 M phosphate buffer (pH 6.8). Cell dimensions were determined by measuring the length and width of 100 cells using an eyepiece micrometer with brightfield optics of a Carl Zeiss light microscope at a magnification of X 1000. Light micrographs were taken with the Nomarski Interference Filter with the above microscope using Kodak High Contrast Copy film.

Fixed cell suspensions were prepared for electron microscopy. Cells were postfixed with 1% aqueous osmium tetroxide and embedded in epon as described previously (12). Thin sections were stained with lead citrate or uranyl acetate and examined in a Philips 300 electron microscope.

Negative staining was carried out in distilled water. One drop of cell suspension was mixed with a drop of 2% phosphotungstic acid buffered at pH 7.0 and applied to Formvar-coated grids. Trichocyst band spacing was determined with an ocular micrometer using 10 different prints at ×96,000.

Replicas were prepared of glutaraldehyde fixed cells as described by Gantt (10). After several washings in distilled water, cells were applied to freshly cleaved mica, shadowed in a Denton Vacuum Evaporator (Denton Vacuum Inc., Cherry Hill, N.J.). Replicas were floated on distilled water and then transferred to alkaline citric acid for 60 min to remove organic material. This was followed by 2 distilled water rinses. The replicas were picked up on Formvar-coated grids and were examined in the electron microscope.

Longitudinal sections of 20 randomly selected cells were photographed at a magnification of ×35,500. The width and length of the 2 apical plate structures were measured on the photographs, and the average measurements determined.

Fixed cells used for scanning electron microscopy were also postfixed with 1% osmium tetroxide for 15 min and washed several times in distilled water. A small drop of cell suspension was placed directly on a stub previously lightly coated with acetone-solubilized adhesive from 3M double-stick Scotch tape. Specimens on stubs were plunged directly into liquid nitrogen and quickly frozen. Specimens were dried in Pearse tissue dryer (Edwards High Vacuum Ltd.) to complete dryness as reported previously (18). Specimens have been examined with a Cambridge Stereoscan II Scanning electron microscope (Engis Equipment Company) using 10 kv accelerating voltage and a 200-μ aperture.

OBSERVATIONS

The cell shape of P. mariae-lebouriae as seen through light microscope equipped with Nomarski Interference filter and with the electron microscope is illustrated in Fig. 1 2, 3, and 4, respectively. Prorocentrum mariae-lebouriae is almost spherical in face view; it is strongly compressed and saucer-shaped in side view; it has a very distinct striated band at the edge of the cell; and its surface is covered with small projections in a regular pattern.

Fig. 1-3. The almost spherical cell outline of P. mariae-lebouriae is evident in light micrographs taken with Nomarski Interference filter. Fig. 1. The surface of the organism is covered with small projections and ridges are exhibited at the cell periphery. The 2 flagella are not visible in this illustration. ×1500.

Fig. 2, 3. The separated bivalves have the same outline as of Fig. 1. The striated band at the edge of each valve shows a regular periodicity and appears thicker relative to the whole valve, and Fig. 3 has a V-shaped depression at the anterior cell end, into which the flagellar pore structures are fitted. ×1500.

Fig. 4. Strongly compressed and flattened shape of P. mariae-lebouriae is pictured in this scanning electron micrograph. The surface of the cell has an evenly distributed pattern, composed of raised bulges mark the tiny spines. A clear view of the cell margin is seen on one of the saucer-shaped organism. Amorphous material adhered to their surface of the cell is debris from the natural environment. ×3600.

Fig. 5. The anterior end of the cells of P. mariae-lebouriae has apical plates, 2 flagellar pores, tiny spines, and trichocyst pores (arrows) on the valve surface. The apical collar (c) frames the flagellar pore in the rear. A second structure, the apical spine (a), additionally protrudes from the apical plates, near the periphery of the second flagellar pore. ×11,000.

Fig. 6. In thin section the V-shaped flagellar pore area is composed of 8 small thick plates variously sized and shaped, enclosing the circular and oblong flagellar pores. The 8 small plates are held together and to the valves by tightly fitted sutures. The 8 plates have been observed in various sections, but not all of them shown here. ×35,000.

Fig. 7. The flagellar pore area in longitudinal section is shown. The 2 flagellar canals are located between the apical plates (p). Beneath each flagellar canal (fe) lie a row of 11 microtubules (arrows). ×35,000.
The projections are composed of spines covering the cell surface revealed in negatively stained preparation (PTA) and platinum-carbon replicas (Fig. 8, 9). These spines are evenly distributed over the cell surface with 450 nm distance between pairs. The spines are about 100-120 nm wide and 200-300 nm long. There are about 600-700 spines on each valve. Numerous trichocyst pores are also found scattered through out the surface of the valve (Fig. 5, 8, 11). Data concerning surface features of the valve of *E. mariae-lebouriae* has been reported previously (3,15) and comparison is made in Table 1 with the new information on similar features of *P. mariae-lebouriae*. The data reported previously differ from ours as follows: cell size of *P. mariae-lebouriae* is larger than of *E. mariae-lebouriae*; diameter of true pores are narrower and more numerous per valve; length and width of spines are shorter and they occur more frequently on the valve surface; trichocyst band spacing found at shorter intervals.

The cell of *P. mariae-lebouriae* possesses 2 saucer-shaped valves with a smooth inner surface (Fig. 2, 3). The above observations were confirmed in scanning electron micrographs at higher magnifications (Fig. 5, 13, 14). The larger one, the oblong flagellar canal (a) (Fig. 5), was found adjacent to the oblong flagellar pore (O) shown in Fig. 13, and appears to be aligned straight upward (Fig. 14). In thin sections the apical tooth is solid with a length of 900–1000 nm and width of 200–300 nm (Fig. 12). We confirmed the erected position of the apical tooth by taking stereo pictures at an 8° tilt with the scanning electron microscope (not shown).

The second apical plate observed at the flagellar pore area has a slightly curved flared appearance (c) in scanning electron micrographs (Fig. 5, 11). Based upon the shape of the above plate, we designated it the apical collar (c). The apical collar is found next to the circular flagellar pore (C) seen in Fig. 13. In thin sections the same plate is a slightly curved forked plate with a double spine (Fig. 10, 11). This forked plate has a length of 850–870 nm and a width of 80–85 nm (Fig. 10). A similar forked plate has been observed in thin sections with a tubular or double spine in another specimen of *P. mariae-lebouriae*, and described as an apical plate or spine by Dodge & Bibby (7).

In Fig. 7 the flagellar pore area is shown in longitudinal section. There are 2 flagellar canals located between the flagellar pore plates. Beneath each flagellar canal is a row of 11 microtubules. In addition, a row of microtubules forming a microtubular cylinder is situated adjacent to the oblong flagellar canal (Fig. 12) near a simple pusule (Fig. 16). This microtubular cylinder consists of an array of 40 microtubules shown in Fig. 12 and 15 and the tubules encompass electron dense bodies. In Fig. 16 the microtubular cylinder runs parallel beneath the valve, beginning at one end of the pusule and encompassing fibrous bodies and mitochondria. The pusule is constructed of un-

### Table 1. Comparison of cell size, surface structure, and trichocysts of *Prorocentrum mariae-lebouriae* and *E. mariae-lebouriae*.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Cell size (μm)</th>
<th>True pores</th>
<th>Spines on thecal surface</th>
<th>Trichocyst band spacing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Diameter</td>
<td>Length (nm)</td>
<td>Width (nm)</td>
</tr>
<tr>
<td><em>E. mariae-lebouriae</em> (14–17) × (11–15)</td>
<td>Not observed</td>
<td>-</td>
<td>600</td>
<td>200-250</td>
</tr>
<tr>
<td><em>P. mariae-lebouriae</em> (18–20) × (16–17)</td>
<td>20 200-250</td>
<td>500</td>
<td>300-500</td>
<td>150</td>
</tr>
</tbody>
</table>

One valve is thickened at the anterior end of the cell and has a V-shaped depression (Fig. 8) into which the flagellar pore structures are fitted (Fig. 6). The flagellar pores are unequal in size, one is circular the other is oblong in shape (Fig. 6). As is revealed in transverse section, the flagellar pores are enclosed by 8 thick, small plates of unequal size held together and to the valve by tightly fitted sutures. The complete set of 8 plates has been observed in numerous thin sections.

The flagellar pore area is surrounded by 2 large apical plates seen in Fig. 5 and designated, because of their shape, as the apical tooth (a) and the apical collar (c). Both plates differ from the small surface spines of the valves and from each other in shape, size, and position. The special features of the above apical plates are illustrated in numerous scanning electron micrographs taken from various angles and magnifications (Fig. 5, 13, 14). The larger one, the apical tooth (a) (Fig. 5), was found adjacent to the oblong flagellar pore (O) shown in Fig. 13, and appears to be aligned straight upward (Fig. 14). In thin sections the apical tooth is solid with a length of 900–1000 nm and width of 200–300 nm (Fig. 12). We confirmed the erected position of the apical tooth by taking stereo pictures at an 8° tilt with the scanning electron microscope (not shown).

The second apical plate observed at the flagellar pore area has a slightly curved flared appearance (c) in scanning electron micrographs (Fig. 5, 11). Based upon the shape of the above plate, we designated it the apical collar (c). The apical collar is found next to the circular flagellar pore (C) seen in Fig. 13. In thin sections the same plate is a slightly curved forked plate with a double spine (Fig. 10, 11). This forked plate has a length of 850–870 nm and a width of 80–85 nm (Fig. 10). A similar forked plate has been observed in thin sections with a tubular or double spine in another specimen of *P. mariae-lebouriae*, and described as an apical plate or spine by Dodge & Bibby (7).
Fig. 8. The surface of the valves is covered by tiny spines, evenly distributed among trichocyst pores (arrows) shown in a negatively stained (PTA) electron micrograph. Due to the absence of the outer membrane the spines are very pointed and 2 of them are broken. × 95,000.

Fig. 9. The evenly spaced spines on the valve shown in platinum-carbon replica at lower magnification. Here the outer membrane covers the valve surface and the spines have a blunted shape which is their normal appearance. × 35,000.

Fig. 10. Longitudinal section showing the apical collar of the flagellar pore area. This is a forked, slightly curved, solid plate. Indications of the outer membrane are shown (arrows). The position of the flagellar bases is seen in the cytoplasm (f). A glancing section through a portion of one of the flagella displays the arrangement of axonemes. Transverse section through the second flagellum may indicate that the flagellar bases lie at an angle to each other. Chloroplast (Ch) is also present. × 35,000.

Fig. 11. The apical collar (c) surrounded by the outer membrane shown in this scanning electron micrograph. Evenly distributed spines and trichocyst pores (arrows) dominate the cell surface. × 12,000.
dulating double membrane walls and vesicles positioned close to the cell vacuole.

The position of the longitudinal and transverse flagellum inserted into the cytoplasm is shown in Fig. 10. The position of one flagellum suggests that it may emerge from the cell through the circular flagellar pore. Additionally, a glancing section through the length of the flagellum displays the arrangement of axonemes. A transverse section through the second flagellum indicates that the flagellar bases lie at an angle to each other.

Other ultrastructural features of *E. mariae-lebouriae* and *P. mariae-lebouriae* previously described (3-7,9,15) appear to be similar than those observed of *P. mariae-lebouriae* causing a dense bloom in the Rhode River. The above organisms have 2 large multilobed chloroplasts and large pyrenoids situated midway between the base and apex, one at each side of the cell. The pyrenoids occur in the swollen part of the chloroplast, transversely with several pairs of chloroplast lamellae. The pyrenoid matrix consists of rows of particles forming a paracrystalline structure described previously by Dodge & Crawford (8). No starch was found to be associated with the pyrenoids, but starch granules were found within the cytoplasm. The chloroplast thylakoids occur in threes and run parallel across the chloroplast (Fig. 12). The nucleus is situated in the posterior end of the cell. It is a typical mesocaryotic nucleus, spherical in shape, with numerous chromosomes situated within the granular nuclear matrix (3). The golgi bodies, the mitochondria, and trichocysts are all typical in structure and need not be described in detail.

**DISCUSSION**

The electron microscope is necessary to define ultrastructural features of the genera of *Prorocentrum* and *Exuviallella* (2-5,8,13). Examinations of several species by the above investigators indicate that there is no sharp distinction between the 2 genera. Many features of these organisms are regarded as typical of the *Dinophyceae* (1,5,13), but others, such as the size and ornamentation of the valves, the organization of the apical flagellar pore area, and the flagellar structures, are not.

In this study, using various microscopical techniques, it was possible to show that the bivalvate surface of *P. mariae-lebouriae* collected from the Rhode River estuary is covered by small spines. The same type of spine, of different dimensions, already has been observed in specimens of *E. mariae-lebouriae* and *P. mariae-lebouriae* and in species of *P. balticum* and *P. obtusiids*, but not in all *Prorocentrum* species examined (3,5,7).

Scanning electron microscopy revealed additional information on the features of the flagellar pore area of *P. mariae-lebouriae*, which was found more complex than previously described of other specimens of *P. mariae-lebouriae* (7,13). The use of the term apical collar to describe the flared plate was chosen because of the structural and architectural characteristics revealed with the scanning electron microscope. A similar structure of different dimensions has been described as a winged spine for *P. micans* (3).

A second structure, a single spine, also fringes the oblong flagellar pore of *P. mariae-lebouriae*. Presumably this large and wider structure has been observed with the light microscope, identified as an apical tooth or spine of other *Prorocentrum* and *Exuviallella* species by Hulburt (12), Martin (14), and Pavillard (16). The double-spine structure observed with the electron microscope by Dodge & Bibby (7) is also designated as an apical spine. In our preparations both structures are present on the same organism. This may be unique for *P. mariae-lebouriae* found in the Rhode River, but it could also be more widespread in other *Prorocentrum* species and not observed so far. The existence of the 8 thick plates of the flagellar pore area makes it a more complex feature of *P. mariae-lebouriae* described in the present investigation than in other specimens of *P. mariae-lebouriae*. Only 4 or 6 plates were identified previously in the latter organism (7).

Location of the longitudinal and transverse

![Fig. 12. Longitudinal section through the apical flagellar pore area reveals a large straight structure, a single spine, protruding from the apical plates (p). The thickened value has a uniform dense granular appearance. The flagellar canal (fc) is adjacent to a row of microtubular cylinder (mc), fibrous bodies (f), and mitochondria (m). The chloroplast lamellae (Ch) consist of 3 thylakoids running parallel across the chloroplast. Cell vacuole (V) consisting of electron dense material lie between the microtubular cylinder (mc) and the chloroplast. × 35,000.](image-url)

![Fig. 13. Intimate arrangement of the apical flagellar pore area between the valves shown in a scanning electron micrograph. The large single spine shown in Fig. 12 and 13 is the same structure positioned around the oblong flagellar pore (O). The circular flagellar pore (G) and apical collar complete this structure. × 22,000.](image-url)

![Fig. 14. The single spine, observed from another angle, surrounded by numerous spines of the valve surface seen in a scanning electron micrograph. × 24,000.](image-url)

![Fig. 15. Transverse section of a microtubular cylinder (mc) consists of 40 microtubules and encircles electron dense bodies (arrow). It is positioned beneath the valve adjacent to a cell vacuole (V) and fibrous bodies (b). × 61,000.](image-url)

![Fig. 16. Longitudinal section of the microtubular cylinder (mc) runs parallel to the valve, beginning at one end by the simple pusule (P), adjacent to the flagellar canal (fc), mitochondria (m), and fibrous bodies (b). The simple pusule is constructed of double-membrane walls and vesicles. × 346,000.](image-url)
flagella has been observed in thin sections. The bases of the 2 flagella appear to lie at an angle to each other in P. mariae-lebouriae. This observation is different from the flagellar structure of P. triestinum, the only other organism examined in detail (7). The flagellar system shows some diversity of organization in dinoflagellates examined (5). The observations of the flagellar structures of Prorocentrum species are obviously too incomplete for any generalization.

Although only a few Prorocentrum species have been studied, it is believed that the apical flagellar pore area will be unique to this group as are the surface structures of the bivalves and the flagellar structure. It is expected that the above ultrastructural features, while essentially similar, will vary according to the species and may become useful in taxonomic characterization.

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