

## SCANNING ELECTRON MICROSCOPY (SEM) AS A TOOL IN STUDYING SPECIES VARIATIONS IN THE Dinoflagellate *Protoperidinium*

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**ABSTRACT.** Two species of the dinoflagellate *Protoperidinium* (order: Peridiniales), viz. *Protoperidinium pellucidum* BERGH 1881 and *P. punctulatum* (PAULSEN) BALECH, when viewed under the SEM showed variations in the ornamentations of the thecal plates which were not clearly observed under the light microscope. *P. punctulatum* is characterized by a finely punctured thecal surface without antapical spines, a 4-sided first apical plate (1') and a 5-sided second intercalary plate. *P. pellucidum* shows randomly distributed trichocyst pores on the thecal surface, two antapical spines with list, a 5-sided first apical plate (1') and a 6-sided second intercalary plate (2a). These characters were found to be important in the classification of *Protoperidinium* to the species level.

**KEYWORDS.** Classification, taxonomy of dinoflagellates, thecal plate.

## INTRODUCTION

Electron microscopy has been used in dinoflagellate studies since 1952, with research concentrating on the ultrastructural morphology of the cells such as the structural and functional of the individual organelles, rather than morphological descriptions (Gaudsmith and Dawes, 1972). Consequently, after dinoflagellates were recognized as the main causative organisms of most red tides (Jothy, 1984), scanning electron microscopy (SEM) was fully used for a variety of objectives. SEM, for example, was used to determine morphological variations between species (Faust and Balech, 1993), as well as to confirm taxonomic identifications at the species level (Faust, 1994). In some species, the morphological variations such as the thecal plate morphology (ornamentation of the thecal plate, shape, size and number of value pores), architecture of the periflagellar area and intercalary band are the most important features. All these characters which can only be observed under SEM were used by Faust, (1994) to redescribed three benthic dinoflagellates. In this study, thecal plate morphology of 2 species of *Protoperidinium* viewed under the SEM are described.



## MATERIAL AND METHOD

Dinoflagellate samples were taken from the shellfish growing area, Sebatu and Sungai Rambai, Malacca located from latitudes N 02° 05' 42.8" to N 02° 05' 57.2" and from longitudes E 102° 28' 56.5" to E 102° 29' 26.1". Samples were concentrated using a 20 m mesh plankton net and were preserved with 2% formalin. Taxonomic identification of thecate species were ascertained under light microscope with differential interference contrast. For SEM studies, samples were processed and dehydrated under series of acetone with concentrations of 35%, 50%, 70%, 90% and 95%. Samples then were pipetted on aluminium foil coated with albumin before coated with gold using a Polaron SEM Coating Unit E5100.

## RESULTS

From the dinoflagellate samples, 2 species of *Protoperidinium* viz. *Protoperidinium punctulatum* and *Protoperidinium pellucidum* were observed using both light and scanning electron microscopy to see the morphological differences between the species. Under the SEM, *P. punctulatum* showed different thecal plate ornamentation compared to *P. pellucidum*. *P. punctulatum*, which is spherical in shape has a finely punctured thecal surface, without antapical spine, a 4-sided first apical plate (1') and a 5-sided second intercalary plate (2a) (Figure 1A-B). *P. pellucidum* has a striae marking on the thecal surface, two pointed antapical spines ornamented with list, randomly distributed trichocyst pores on the thecal surface, a 6-sided first apical plate (1'), a 6-sided second intercalary plate (2a) and cell spherical in shape (Figure 2A-B). All these characters were not visible under the light microscope.

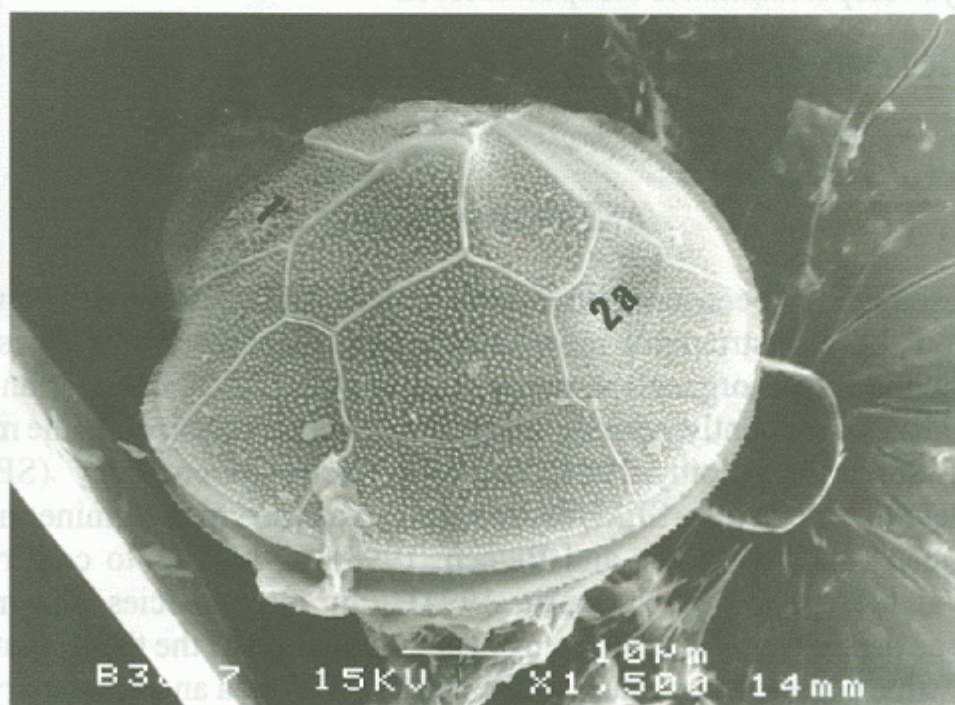


Figure 1A. Scanning electron micrograph of *Protoperidinium punctulatum* (epitheca view) showing finely punctured thecal surface, 4-sided first apical plate (1') and 5-sided second intercalary plate (2a)



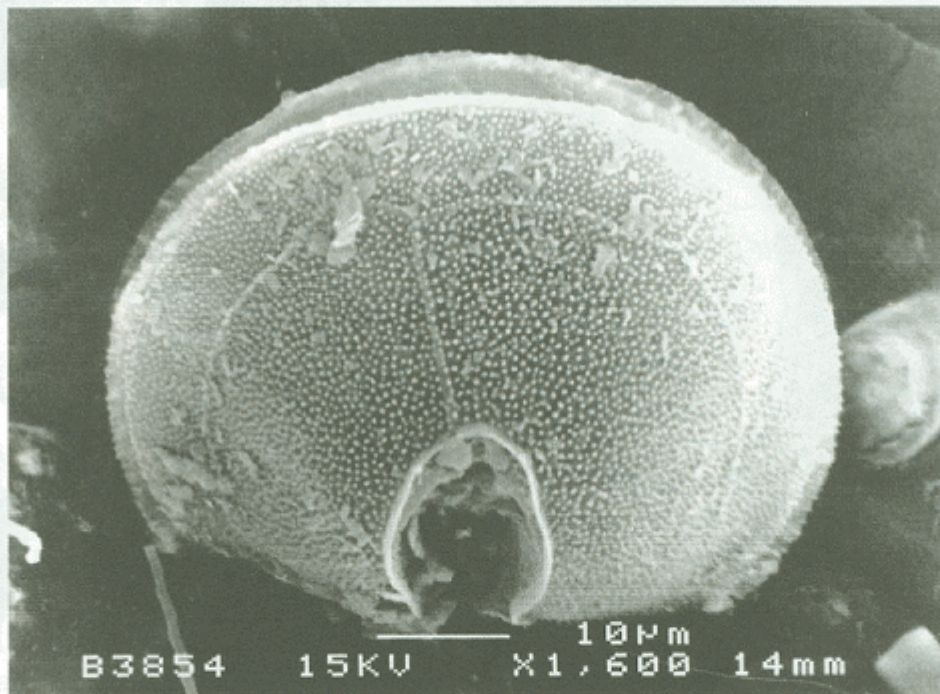


Figure 1B. Scanning electron micrograph of *Protoperidinium punctulatum* showing finely punctured thecal surface. Hypotheca view

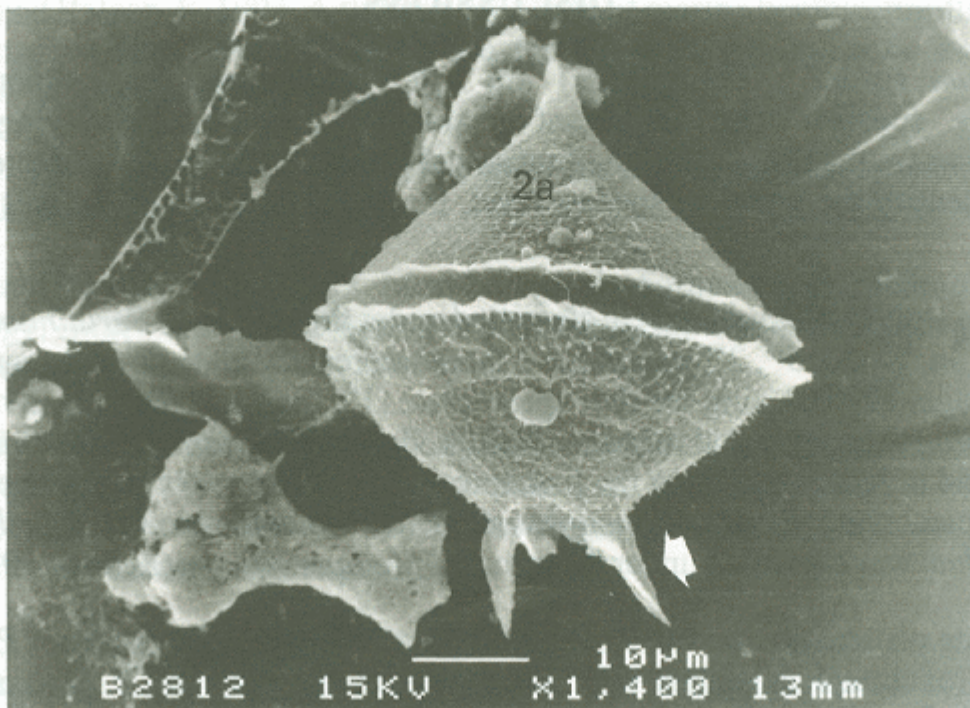


Figure 2A. Scanning electron micrograph of *Protoperidinium punctulatum*. Dorsal view showing two antapical spines ornamented with list (arrow) and -sided second intercalary plate (2a).



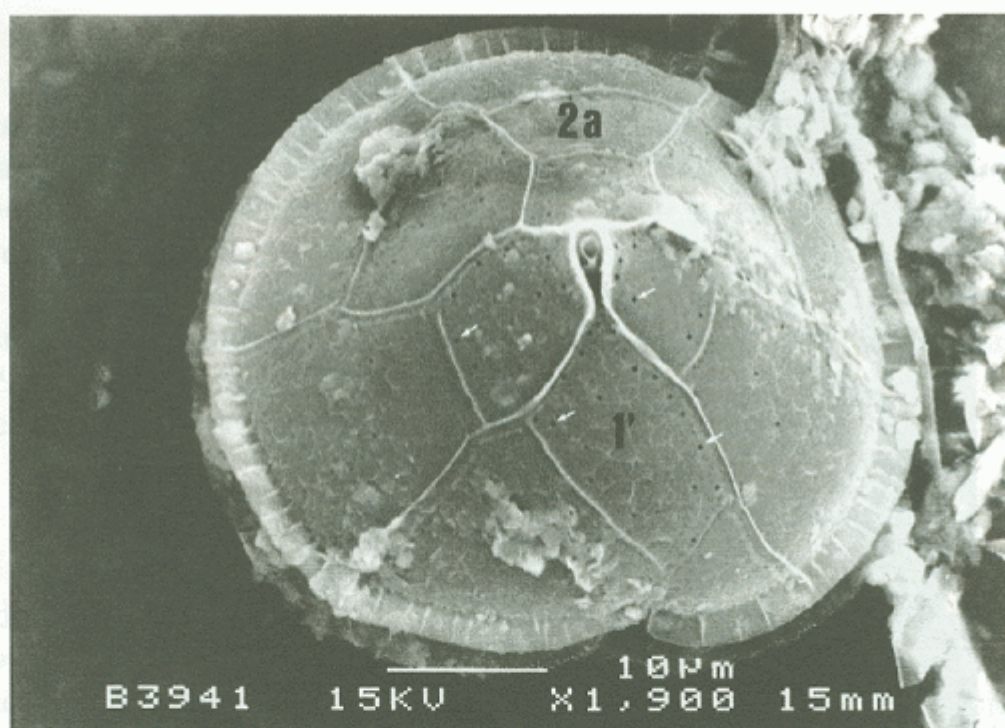


Figure 2B. Scanning electron micrograph of *Protoperidinium punctulatum*. Epitheca view showing striae marking on the thecal surface, 5-sided first apical plate (1'), 6-sided second intercalary plate (2a) and trichocyst pores (arrows).

## DISCUSSIONS

*Protoperidinium* (order : Peridinales) are often larger in size compared to other dinoflagellates species. The important characters used in identifying the genus *Protoperidinium* are the presence of the first apical plate (1') and the second intercalary plate (2a). But to identify the genus to species level, the shape and size of the cell are the main characters.

*P. punctulatum* and *P. pellucidum* were classified under the subgenus *Peridinium* with a plate formula of 4', 3a, 7'', 3c, 5''', 2''' which have three intercalary plate or are known as the true peridinea (Dodge, 1985). Using the first apical plate (1'), both species were divided into two groups i.e. *Orthoperidinium* and *Metaperidinium*. In *Orthoperidinium*, the first apical plate is four sided and in *Metaperidinium* the first apical plate is five or six sided. Therefore, based on their first apical plate, *P. punctulatum* was classified under the groups of *Orthoperidinium* and *P. pellucidum* was classified under the group of *Metaperidinium* (Lebour, 1925).

The thecal plate ornamentation of *P. punctulatum* are clearly different from *P. pellucidum*. The presence of antapical spines with list at the thecal plate of *P. pellucidum* clearly differentiates it from *P. punctulatum*. But the antapical list which ornaments the antapical spines is a very delicate structure. The list may be easily damaged during preservation, handling and SEM processing which involves a series of harsh chemicals. For example, long thin spines attached at the thecal plates of *Microcanthodinium setiferum* was loss in SEM processing, which made the species being placed in the genus *Paleophalacroma* (Dodge,



1995). This study showed the importance of the SEM in the taxonomy of *Proto-peridinium*, where the cells were not damaged during the processing of samples and the differentiation between the 2 species were made possible.

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