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# A light and electron microscopic study of the benthic diatom *Diploneis marginestriata* HUST. (Bacillariophyceae)

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*Diploneis marginestriata* was collected from Japan and Finland. Specimens from Finland were very similar to *D. oculata* in valve shape, dimensions and density of striae. The two species were difficult to distinguish using LM. However, SEM and TEM observations showed that these two species are clearly different in the valve structure. The valves of *D. marginestriata* from both countries, Japan and Finland, were basically the same. They shared two characteristic features: elongated flaps protruding from the transapical costae close to the interior occlusion of the alveolus, and openings at the center of the longitudinal canals. These are new structures recognized for this genus.

*Key Index Words:* diatom—Diploneis marginestriata—*fine structure.*

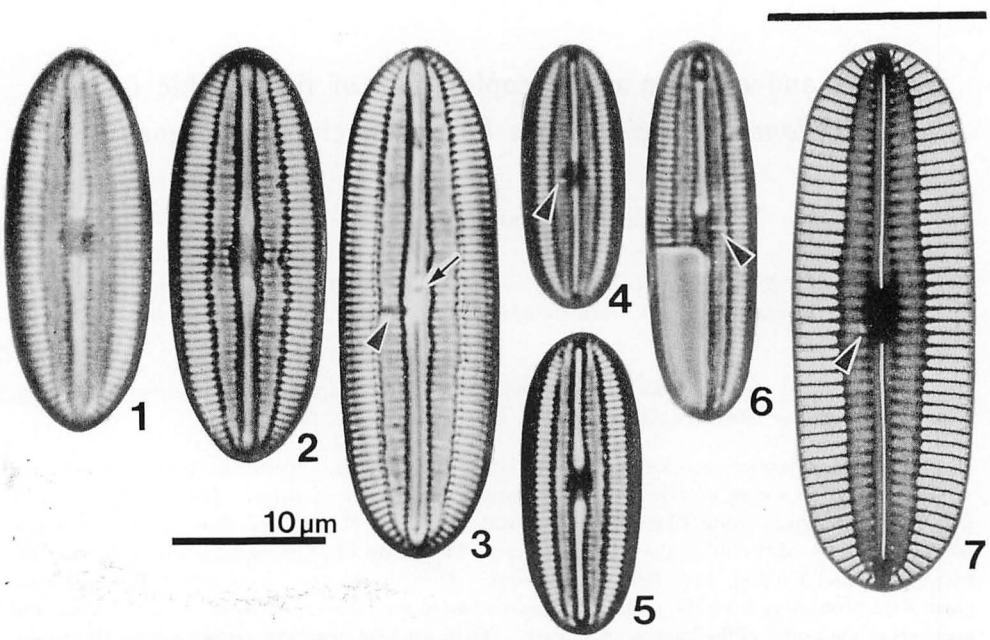
In order to clarify taxonomic relationships between species composing genus *Diploneis* as well as between genera of the Pennales, the fine structure of each taxon has been studied in detail by us (IDEI and KOBAYASI 1986, 1988). *D. marginestriata* HUST. was originally described by HUSTEDT (1922) based on specimens collected from Lunz Untersee in Austria. Distribution of this taxon is not rare (HUSTEDT 1937), but in Japan it is restricted to a few lakes. *D. marginestriata* is very similar to *D. oculata* (BRÉB.) CL. (IDEI and KOBAYASI 1986) and *D. peterseni* HUST., but this species has a broader longitudinal canal. However, when these taxa occur in the same sample, it is difficult to distinguish them using only light microscopy (LM).

Recently, KRAMMER and LANGE-BERTALOT (1986) presented microphotographs as the holotype (Lunz Untersee Coll. HUST. 02/72), but later SIMONSEN (1987) presented photographs of the lectotype specimens (Lunz Untersee Coll. HUST. 2/6) for there are two slides with marked specimens. We have also examined syntype slides (Lunz Untersee 02/72, 04/74) at Brehmerhaven.

HUSTEDT (1927) reported this taxon from Aoki Lake, Japan. We have also collected it from this lake. In addition to this material, we have examined some Finnish specimens using LM, transmission electron microscopy (TEM) and scanning electron microscopy (SEM), and found some characteristic morphological features which have not been reported so far for the genus *Diploneis*.

## Materials and Methods

Specimens were collected from the following locations and dates. 1, 2. Sediment in Aoki-ko (Aoki Lake), Nagano Prefecture, Japan (K-5605, on 19 March, 1974; K-2825, on 3 June, 1985). 3. Sediment in Nojiri-ko (Nojiri Lake), Nagano Prefecture, Japan (K-2845, on 4 June, 1985). 4,5. Sediments in Pää-järvi, Finland (K-6324, on 11 August, 1986; K-6331, on 12 August, 1986). 6. Sediment in Isolampi, Finland (K-6345, 11 August, 1986).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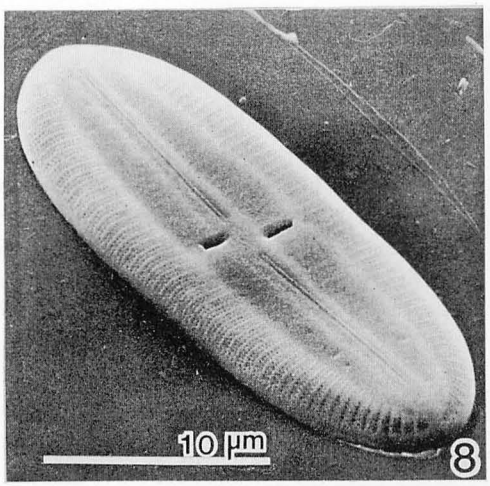
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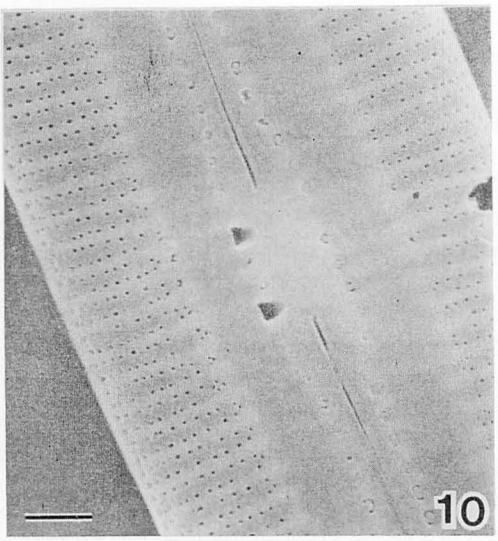
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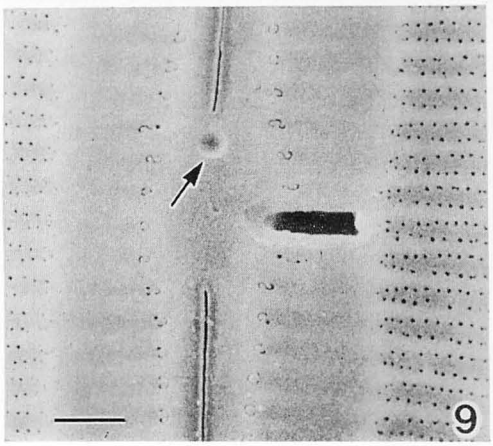
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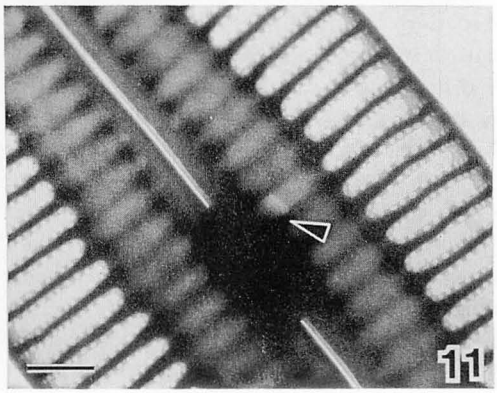
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Specimens were cleaned by ultraviolet radiation and then washed in distilled water. For light microscopy, the cleaned specimens were embedded in Pleurax. Specimens for SEM were prepared by air drying on a glass coverslip and then fixing to a metal stub with silver paste. They were coated with gold-palladium and observed using a JEOL-F15 field-emission SEM. Material for TEM was placed on formvar-coated 50-mesh copper grids and observed using a JEOL-100C.

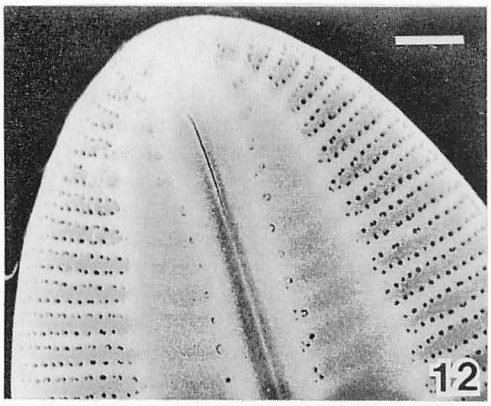
The terminology used is that suggested by the Warking Party on Diatom Terminology (Anonymous 1975, Ross *et al.* 1979).

## Observations

Valves of Japanese specimens are 28–48 $\mu$m long, 9–11 $\mu$m wide and 16–18 striae in 10 $\mu$m (Figs 2, 3). This range differs from the original description by HUSTEDT (20–35 $\mu$m long, 9–13 $\mu$m wide and about 20 striae in 10 $\mu$m) (1922), the Japanese specimens being longer but generally more narrow. A type specimen (Coll. HUST. Lunz. Untersee 02/72) (Fig. 1, 25 $\mu$m long, 9 $\mu$m wide, 18 striae in 10 $\mu$m), has similar basic taxonomic features to the Japanese specimens in having broad longitudinal canals and the same striae density. Four of the five specimens selected by SIMONSEN (1987. Pl. 94, figs 8–12) as lectotype (Coll. HUSTEDT Slide 2/6, from Lunz N. Oest. U.S. st. 5. 11 m) were measured to have 16 or 17 striae in 10 $\mu$m, and the fifth, the smallest, had 22 striae in 10 $\mu$m. The photographs of the specimens taken from syntype slides (Coll. HUSTEDT 04/74 Untersee Lunz st. 5. 12 m and 02/72 Lunz Untersee st. 4. 15 m) by us showed 16–18 striae in 10 $\mu$m. While, valves of Finnish specimens are small, 17–25 $\mu$m long and 6.5–7 $\mu$m wide. Transapical striae are denser, 22 in 10 $\mu$m (Figs 4–7). However, as given below the fine structural features of both Japanese and Finnish specimens are basically the same.

In specimens from both countries, some valves have one or two distinct short striae at the central portion of the longitudinal canal (Figs 3, 4, 6 arrowheads). These short striae were not detected in the type specimens observed by us (Fig. 1) and KRAMMER and LANGE-BERTALOT (1986) as well as in the lectotype photomicrographs taken by SIMONSEN (1987). This structure seems to be only detectable in the specimens mounted by high refractable medium, Pleurax, and by very careful forcusing (Figs 3, 4, 6). However, they were seen in all valves when observed by TEM or SEM (Fig. 7 arrowhead, Figs 8, 9, 10, Fig. 11 arrowhead). SEM observation makes it clear that these short striae are openings on the outer wall of the longitudinal canal. The openings are different in both shape and position in specimens from Japan and Finland. Valves in the Japanese specimens have rectangular openings which fully traverse the longitudinal canal at one or both sides (Figs 8, 9). Finnish specimens have one or two triangular openings along the raphe side of the canal (Fig. 10, arrowhead in Fig. 11). These openings are never situated at the center but either side of the transapical axis of the valve. A

Figs 1–11. *Diploneis marginestriata*. Figs 1–6 are light micrographs. Figs 8–10 are scanning electron micrographs. Figs 7 and 11 are transmission electron micrographs. Figs 2, 3, 8, 9. Japanese specimens. Figs 4–7, 10, 11. Finnish specimens. Fig. 1. A type specimen, Lunz Untersee, Coll. HUST. 02/72. Fig. 2. Specimen to show that canal openings are not visible by LM. Fig. 3. Specimen with a canal opening (arrowhead) and a circular depression (arrow). Fig. 4. Specimen with a canal opening (arrowhead). Fig. 5. Specimen to show that canal openings are not visible by LM. Fig. 6. Specimen with a canal opening (arrowhead). Fig. 7. Specimen with a canal opening (arrowhead). ×3000. Fig. 8. Exernal surface, whole valve with two rectangular openings. Fig. 9. External surface, valve with a rectangular opening and a circular depression (arrow). Fig. 10. External surface, valve with two triangular openings. Fig. 11. Valve with a triangular opening (arrowhead). (bar in Fig 7 =10 $\mu$m, bars in Figs 9–11 =1 $\mu$m)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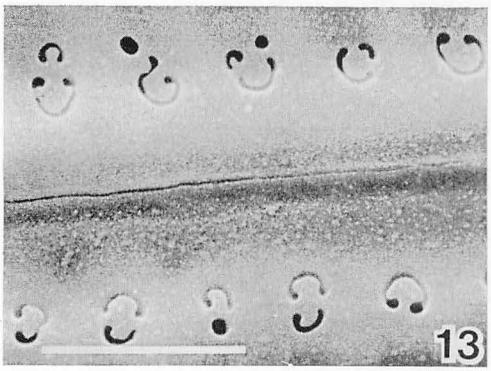
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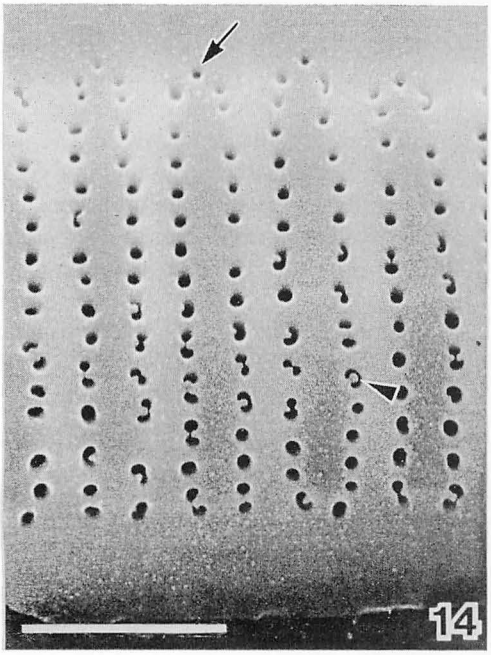
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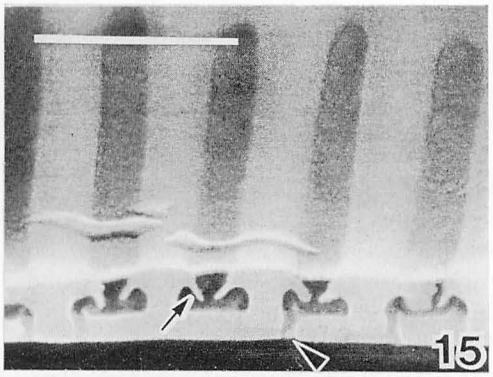
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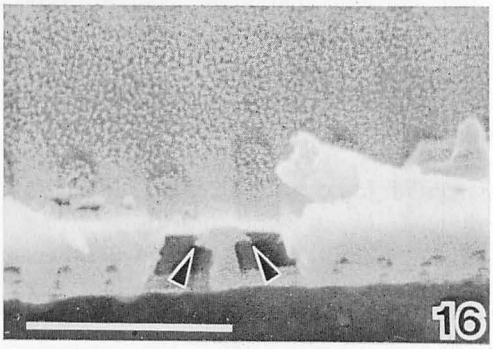
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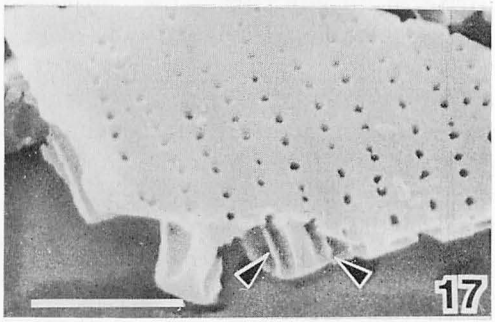
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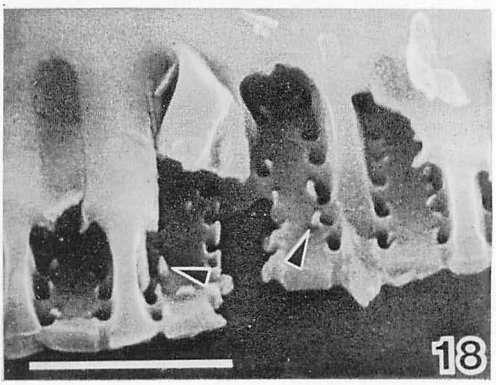
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single opening is seen on either or both canals in Japanese specimens, while one or two openings are seen on either canal in Finnish specimens. We have never found a valve with two openings on either canal in Japanese specimens and one with openings on both canals in Finnish specimens. However, the differences of the number, position and shape of these openings cannot be considered for taxonomic criteria to distinguish Japanese and Finnish specimens.

The valve face is almost flat and the mantle is relatively shallow (Fig. 8). The raphe is a straight slit externally (Figs 8–12) similar to *D. oculata* and *D. minuta* (IDEI and KOBAYASI 1986). In some Japanese specimens one or two circular depressions were observed in the central nodule (Figs 3, 9 arrows). The proximal ends of the raphe branches are straight without forming central pores (Figs 8–11). Along both sides of the raphe a longitudinal row of poroids is present (Figs 8–10, 12, 13). These poroids surround the central and the terminal nodules (Figs 9, 10, 12). Each poroid is occluded by a flap which is attached to the wall of the pore at one or two points (Fig. 13).

The valve has alveolate striae. Externally, each alveolus is occluded by an areolated wall and internally by a thin siliceous layer with perforations arranged in an hexagonal array (MANN 1981) (Fig. 19). These areolae are of a simple poroid type, and are arranged along the transapical costae (Figs 14–18). Each alveolus has two rows of poroids, with a single poroid at the axial side (Fig. 14 arrow). Some poroids have a single volate occlusion with a reniform openings externally (Figs 14, 15 arrowheads).

Internally there is a small projection at the side of each poroid (Fig. 18 arrowheads). Peculiar components of the alveolus are elongated flaps which obliquely extend from the transapical alveolar walls, close to the interior occlusion of the alveolus, to the alveolus lumen (Fig. 15 arrow, Figs 16, 17 arrowheads). The distal edge of the elongated flap is not smooth, it seems to have irregular notches (Fig. 19 arrowhead). These elongated flaps are present in specimens from both countries, though they differ in degree of extension (Figs 15, 16).

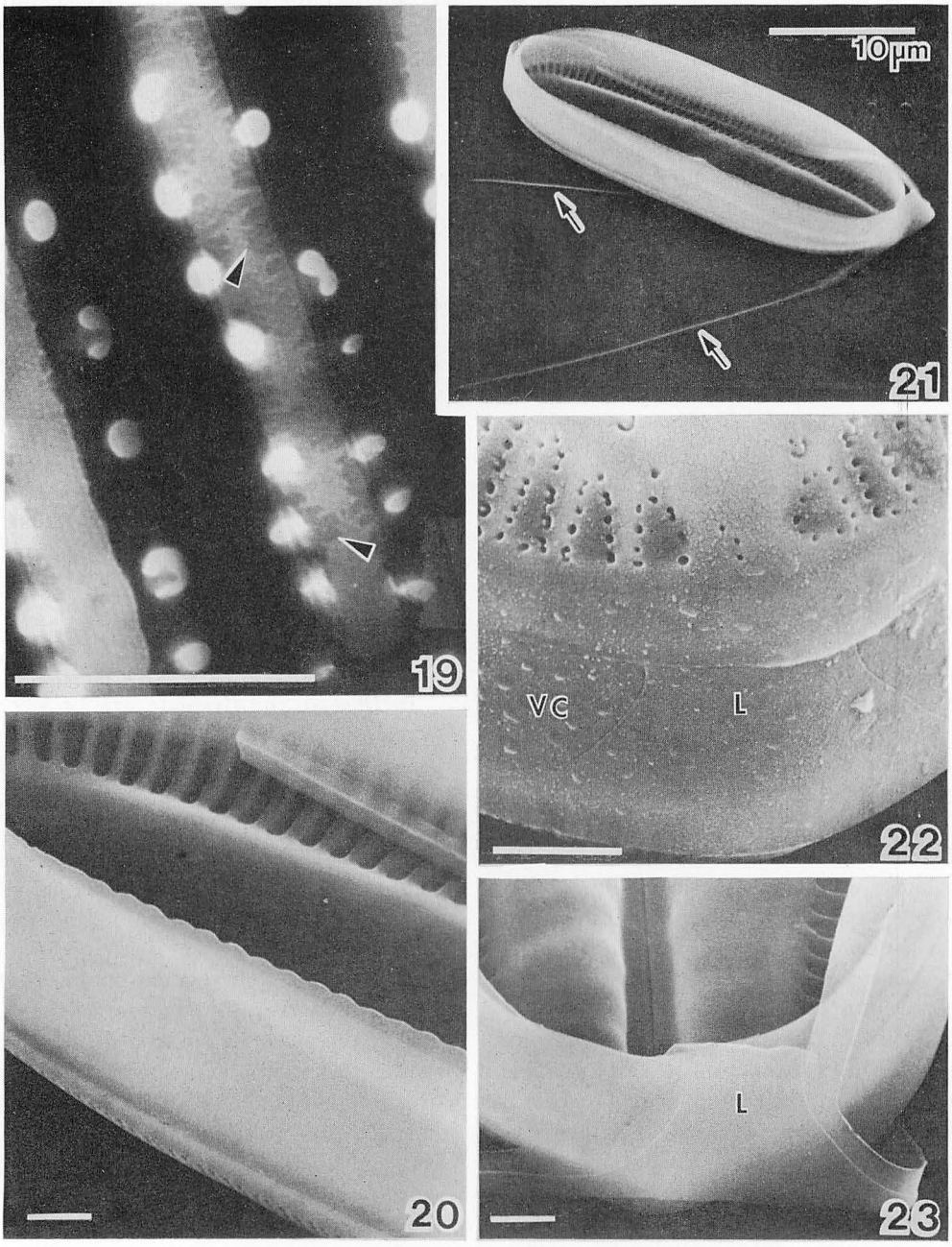
The cinglum is composed of two bands, one valvocopula and one pleura (Figs 20–22). The valvocopula is an open band with a serrated advalvar edge (Fig. 20). The pleura is a filamentous open band except it has a broad mid-portion with a ligula (Figs 21–23). The decoration on the bands is absent as in *D. oculata* and *D. parma* (IDEI and KOBAYASI 1986, 1988).

## Discussion

In *Diploneis* the alveolate stria or alveolus (Cox and Ross 1981) is closed by outer and inner walls/occlusions. In all *Diploneis* species observed (HELMCKE & KRIEGER 1962, 1963; OKUNO 1964, 1970; GERLOFF & HELMCKE 1975; GERMAIN 1979; SIMS & PADDOCK 1979; IDEI & KOBAYASI 1986, 1988) the alveolus is occluded internally by a thin siliceous layer with perforations arranged in an hexagonal array (MANN 1981). However, the structure of the outer wall of the alveolate striae is different from species to species. Their form in longitudinal section of the valve is also different.

In *D. marginestriata,* the valves have two characteristic features. The first is the presence of elongated flaps which extend from the costae close to the interior occlusion of

Figs 12–18. *Diploneis marginestriata*. All Figures except Fig. 16, Japanese specimens. All Figures are SEM. Fig. 12. External surface, valve pole with a straight raphe. Fig. 13. External surface, detail of longitudinal rows of poroids along the raphe. Fig. 14. External surface, detail of alveolus with two rows of poroids, with a single poroid at the axial side (arrow). Fig. 15. Internal surface, broken edge of valve shows elongated flaps (arrow) of alveolus and flap (arrowhead) of poroid. Fig. 16. Inrernal surface, broken edge of valve with elongated flaps (arrowheads), Finnish specimen. Fig. 17. External surface, broken valve with elongated flaps (arrowheads). Fig. 18. Internal surface, broken valve with small projections (arrowheads) at the side of the poroids. (all bar= 1 $\mu$m)




Figs 19–23. *Diploneis marginestriata*. All Figures, are of Japanese specimens. Fig. 19 is a transmission electron micrograph, the others are scanning electron micrographs. Fig. 19. Hymenate occlusion and elongate flaps (arrowheads) of alveolus. Fig. 20. Serrate adyalvar edge of a valvocopula without epivalve, from outside. Fig. 21. Frustule without epivalve showing a valvocopula and a fallen pleura of the epivalve. Arms of the pleura are visible (arrows). Fig. 22. External surface, pole of frustule with the open ends of the valvocopula (VC) and a ligula (L) of the pleura. Fig. 23. Enlargiment of the part of the pleura with a ligula (L). (all bars except in Fig. 21=1 $\mu$m)

the alveolus. The shape of the transapiacl costae is fungiformis in longitudinal section of the valve (Fig. 15). In Finnish specimens this structure is not so conspicuous (Fig. 16). This is a newly described component of the alveolate striae for this genus, and has not been described for other genera. The second characteristic feature is the presence of rectangular- or triangular-shaped openings of the outer wall of the longitudinal canal. *D. crabro* (SIMS & PADDOCK 1979) also has a regular series of unoccluded pores in the inner wall of the canal, but they are different from that of *D. marginestriata*. These openings cannot be considered to be artificial products or made by erosion, because the outer wall of the canal is the most thickened portion of the valve and they are restricted to the center of the canal. They were present in all valves from the four localities. Finnish specimens of *D. marginestriata* resembled *D. oculata* in shape, size of the valve and density of striae (IDEI and KOBAYASI 1986), and separation of these two species without SEM and TEM observations is difficult. These structures, peculiar to *D. marginestriata,* are not present in the specimens reported as *D. marginestriata* by GERMAIN (1979), which appear to be large specimens of *D. oculata*.

## Acknowledgements

Special thanks to Dr. R. SIMONSEN of the Institut für Meeresforschung, Bremerhaven for access to the Institute slide collection and Mr. M. LIUKKONEN of the Lammi Biological Station, University of Helsinki for his kind help in collecting materials. We also thank Prof. M. CHIHARA of the University of Tsukuba for encouragement and advice, and Dr. R.W. RIDGE of the University of Tsukuba for his critical appraisal of the manuscript and helpful comments.

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出井雅彦*・小林　弘**：底生珪藻 ***Diploneis marginestriata*** **HUST.** の光顕及び電顕による研究

本邦及びフィンランドより得た *D. marginestriata* について光顕及び電顕を用い観察した。フィンランドのものは小型で *D. oculata* に非常に類似していたが，微細構造の点では両国産のものは基本的に同一であった。本種には少なくとも本属では全く知られていない二つの特徴的な構造が見られた。一つは長胞内に張り出した長いフラップであり，もう一つは縦走管の外壁の開口である。(*305 茨城県つくば市天王台1-1-1　筑波大学生物科学系．**184 東京都小金井市貫井北町4-1-1　東京学芸大学生物学教室)

## ニュース

### 1. 第4回国際植物バイオシステマティックス・シンポジウム

第4回国際植物バイオシステマティックス・シンポジウム（4th International Symposium of Plant Biosystematics）は，1989年7月10〜14日に京都市・京都会館（予定）で開催されることになりました。

この会議は，植物の種生物学およびバイオシステマティックス（種分類学）に関する進化的研究の成果を発表・討論するため，3年ごとに開催される国際会議であります。主要議題として，1)Biology and Evolution of Weeds and Crops。2)Molecular Approach in Plant Biosystematics。3)Population Biology and Life History Evolution。4)Biosystematics in Lower Plant Groups，が挙げられており，藻類に関する講演も予定されています。

このシンポジウムに関する詳しい問合わせ先：
〒606 京都市左京区北白川追分町　京都大学理学部植物学教室　河野昭一

### 2. 第5回国際微生物生態学シンポジウム

第5回国際微生物生態学シンポジウム（The Fifth International Symposium on Microbial Ecology）は，1989年8月27日－9月1日に京都市・国立京都国際会館で開催されます。

この会議は，生態系における微生物の存在のメカニズムとその機能の発現を正しく把握するための研究の成果を発表・討論するために，3年ごとに開催される国際会議であります。　今回の会議では，微生物の持つ多様な生化学的能力の種々の分野での利用，新遺伝子資源としての可能性，人工微生物の自然界での挙動の問題などとともに，藻類の異常発生も主要題目の一つとして取り挙げられています。

このシンポジウムに関する詳しい問合わせ先：
〒164 東京都中野区南台1-15-1　東京大学海洋研究所　清水　潮。

(中原紘之，〒625　京都府舞鶴市長浜　京都大学農学部附属水産実験所)