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Fine structure of the marine pennate diatom *Entomoneis decussata* (Grun.) comb. nov.

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The fine structure of *Entomoneis decussata* collected from Japanese marine habitats has been examined using mainly electron microscopy to clarify stable features of this taxon. Through the reproduction process in clonal cultures, valves of both maximum and minimum size were produced and then compared. The taxon has the following morphological features recognizable to be stable and characteristic: 1) Decussating wing costae with many linking fibulae: 2) Arcuate junction line formed by a row of basal fibulae fusing with each other into H or Y shape in places: 3) Spines on the costae of the valve body: 4) Density of striae, being 22–26 in 10 μm: 5) Perforations arranged in a line of the hymenate areolar occlusion of the valve: 6) Externally raised interareolar costae and numerous warts on the surface of the band.

Key Index Words: clonal culture—Entomoneis—Entomoneis decussata—*fine structure—marine diatom.*

The species, *Entomoneis decussata*, was originally described by Grunow (Cleve and Grunow 1880) as *Amphiprora decussata*. In the original description, the valve dimensions of 63–65 μm long, 21–24 striae in 10 μm, and the presence of a keel with decussate lines were given, but the figures were not given. However, Grunow's figure was presented in Van Heurck's (1880) atlas. The figure which measured 64 μm long and about 22 striae in 10 μm seems to coincide well with the original description. The taxon was later included in *Amphiprora gigantea* Grun. as one of its varieties by Cleve (1894) based on only the similarity in its decussate puncta on the wings. Although there had been a few records of this taxon in literature, Poulin *et al.* (1987) recently reported the taxon under a new name combination, *E. gigantea* var. *decussata*.

Our light (LM) and electron microscopical (SEM and TEM) examinations of this taxon collected from two Japanese marine habitats and the clonal cultures obtained showed that the taxon should be better classified as a separate species.

A new nomenclatural combination to the genus *Entomoneis* is proposed.

Material and methods

Materials were collected from the bottom mud of the coast of Shimabara-wan (Shimabara Bay), Misumi-cho, Kumamoto Pref., on October 12, 1986 (OS-381) and from a culture tank of abalones in the Yamagata Prefectural Fisheries Experimental Station, Tsuruoka City, Yamagata Pref., on January 20, 1988 (OS-425). Some individuals from the latter were isolated into clonal cultures and grown in Erlenmeyer flasks containing a modified PES medium (Osada and Kobayasi 1990). The cultures were maintained at 18°C under fluorescent light of about 2000 lux with a 14/10 light-dark cycle. When the culture materials were transferred to a fresh medium about three months later from the first inoculation, auxospore formation was induced (KE-1554-3).

To obtain cleaned specimens all materials

were treated by the method in KOBAYASI *et al.* (1985). The specimens were embedded in Pleurax for light microscopy (LM). For SEM, the specimens were dried naturally or critical point drying and then coated with platinum-palladium using a HITACHI E-102. The specimens for TEM were placed on formvar-coated copper grids. SEM and TEM observations were made using a HITACHI S-800 and a JEOL 1200EX respectively.

The terminology used is that suggested by ANONYMOUS (1975), ROSS *et al.* (1979) and PADDOCK and SIMS (1977, 1981).

Observations and discussion

The frustules are panduriform in outline constricted deeply on both sides of the valve centre from a girdle view, because of the strong bilobate keel of the valve. The keel is divided into two wings by a lower central nodule (Figs. 1, 3, 5, 6, 22). In the valve view, the valve is linear-lanceolate with acute ends and the keel is sigmoid (Fig. 2). In LM, the valve body and the wing are separated clearly by an obvious junction line. These two parts have clearly different structures from each other. The wing is composed of peripheral hyaline region with the raphe and the region with decussating rows of small puncta, while the valve body bears transapical striae throughout. The valves are 40-66 μm long and about 7-12 μm wide, and have 22-26 striae in 10 μm. The junction lines are arcuate. The above features of our specimens agree fairly well with both GRUNOW's original description (CLEVE and GRUNOW 1880. p. 63) and his illustration in VAN HEURCK (1880. pl. 22. f. 13). However, our specimens seem to be different from POULIN *et al.*'s (1987) *E. gigantea* var. *decussata* which has short striae along the raphe canal in their light micrographs.

In one clonal culture (KE-1554-3) containing auxospores, mother valves of 26-30 μm long (Fig. 5) and large auxospores or valves which attain a maximum size of 71-87 μm long (Fig. 6) by the sexual reproduction process are observed. The LM structures of the valve body and the wing, i.e. the striae density and the shape of the junction line, are not observed to vary so markedly among specimens in the field materials nor between field and cultured specimens. The valve length of this species is considered to be at least in a range from 26 to 87 μm.

In SEM and TEM, the costae continue from the valve edge to the raphe canal at the distal margin of the wing (Figs. 4, 7, 8). The wing costae on the two walls forming a wing are decussate in the girdle view (Fig. 4). The wing costae on one half of the keel are arranged obliquely and in parallel slanting toward the apex, while on the other wing of the same keel almost all the costae are arranged also slanting in the same direction as the former but change their direction radially only at the apex (Figs. 7, 8). Many small spines are on the valve body costae externally but not on the wing costae (Figs. 7, 8, 13, 18). Each intercosta forming a stria also continues from the valve margin to the raphe canal across the junction line, and has two rows of areolae and randomly distributed external small spines (Figs. 9, 10). The areolae of the valve body are round, 160-175 in 10 μm, while those of the wing are extremely elongated, 40-90 in 10 μm. Each of the areolae bears an externally swollen pore occlusion in the same manner as that of *E. alata* var. *japonica* (OSADA and KOBAYASI 1985) and of *E. paludosa* (OSADA and KOBAYASI 1990) (Figs. 10, 11). However, the pore occlusion of this species is a hymen with perforations forming lines arranged in parallel (Figs. 10-12). Such an arrangement of the perforations is clearly different from those of *E. alata* (MANN 1981), *E. alata* var. *japonica* (OSADA and KOBAYASI 1985) and *E. paludosa* (OSADA and KOBAYASI 1990), and also can be distinguished from the parallel array type described by KOBAYASI and NAGUMO (1985).

In broken valves, the small puncta on the wing seen in LM are the fibulae linking costae on opposite walls of the wing (Figs. 18, 19). The outermost fibulae, raphe fibulae, are arranged in a row and separate proximally the

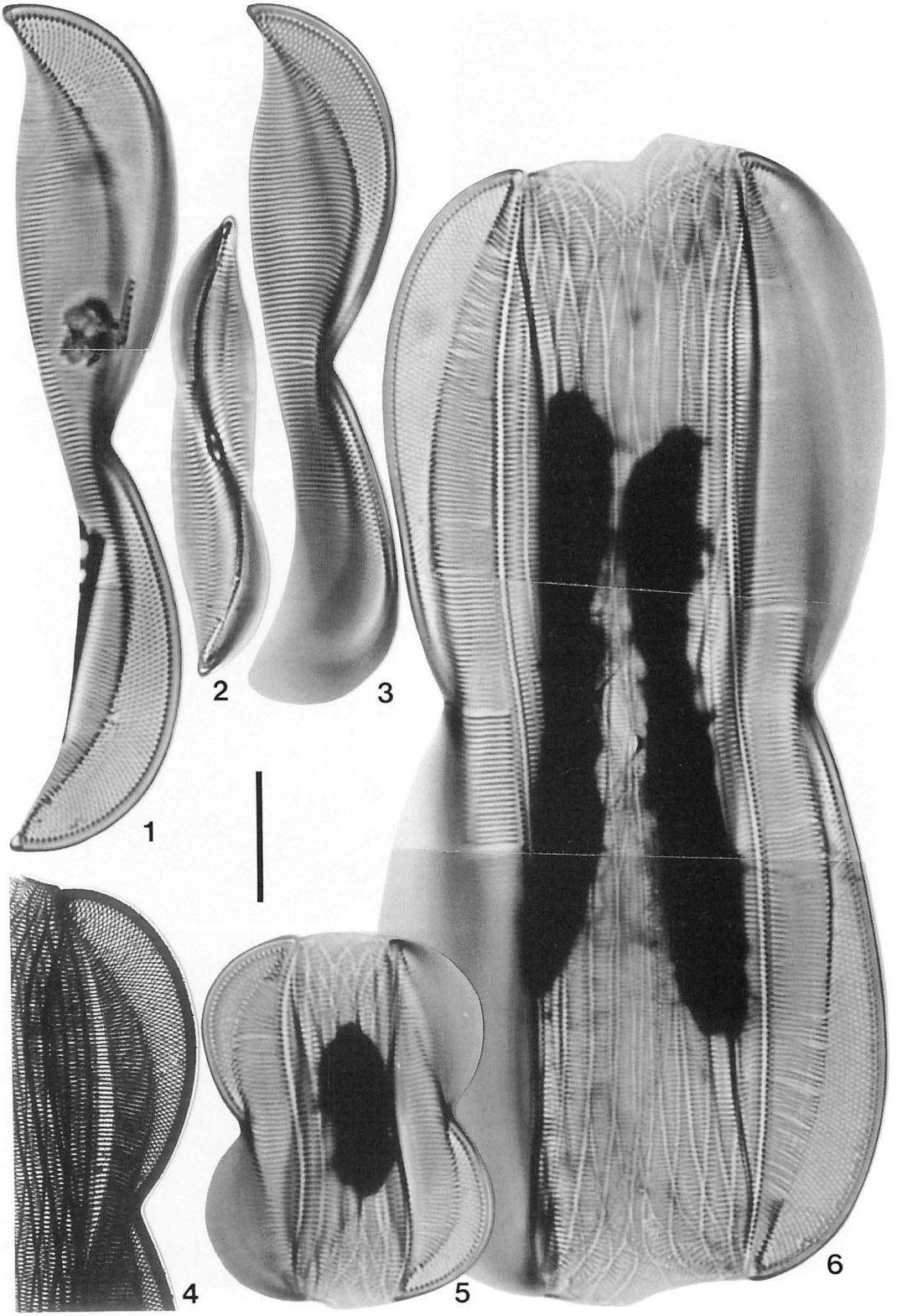

Plate 1.

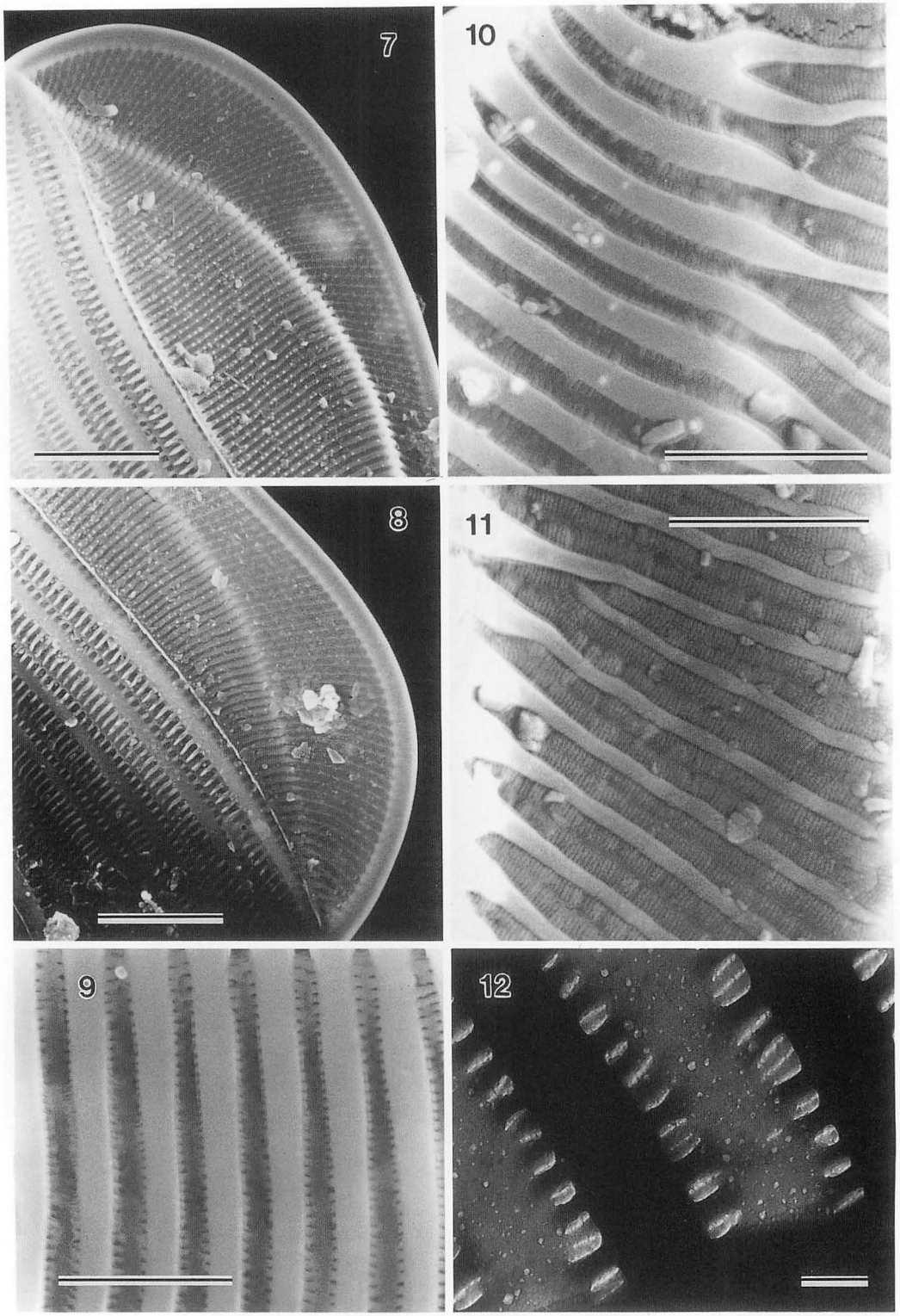

Plate 2.

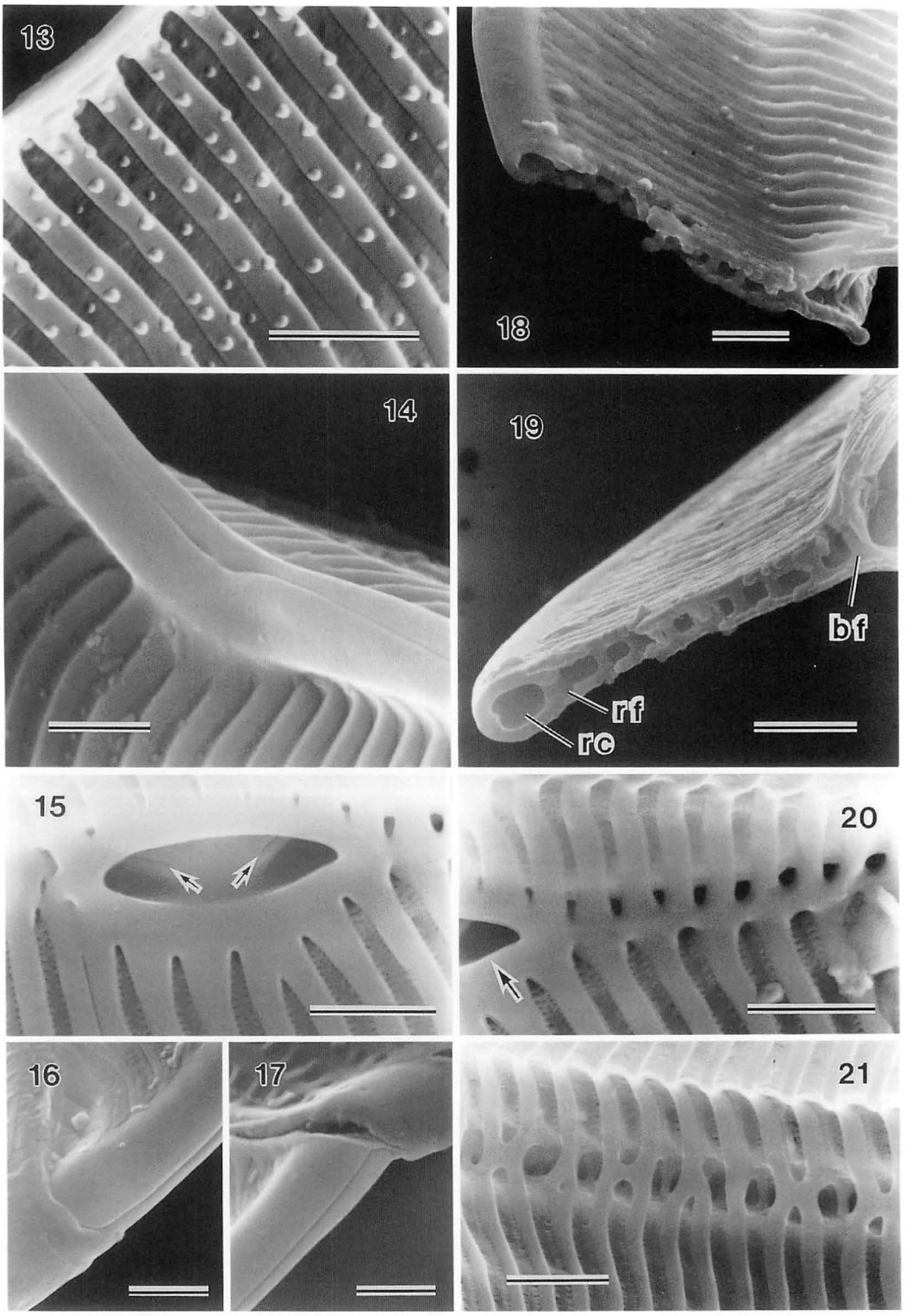

Plate 3.

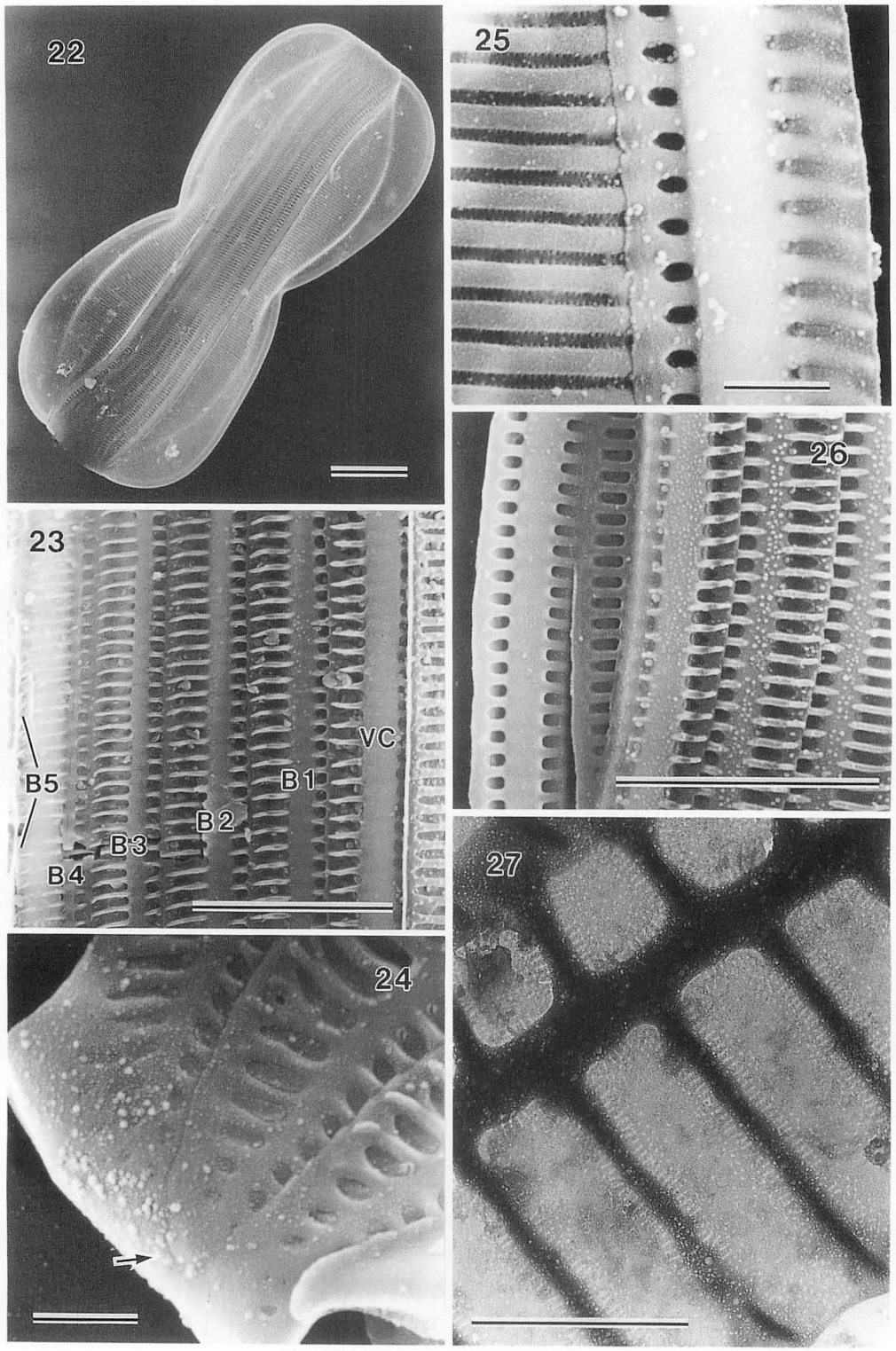

Plate 4.

cavity of the raphe canal. The inmost row of fibulae, the basal fibulae, divides the wing from the valve body (Fig. 19). The neighboring two basal fibulae frequently fuse at their midpoints (Fig. 21). These lateral fusions of the fibulae are continuous and form a fishbone-like structure near the central nodule (Fig. 20). Though the fusion structure is not the same, the fusion of basal fibulae was seen in *E. pseudoduplex* Osada & H. Kob. (1990). It seems that the fusion of basal fibulae is closely related to the forms with decussating wing costae.

The raphe fissure is extremely narrow throughout in comparison with the broad external wall of the raphe canal. Each of the central endings of the raphe fissures terminates in a slightly dilated central pore externally (Fig. 14), but terminates more simply on the inside (Fig. 15). The terminal fissures curve in opposite directions at both ends of a valve (Figs. 16, 17).

The cingulum is composed of at least five to six open bands; one valvocopula and four to five bands. They open and close alternately at each pole of the frustule (Fig. 24) and all have similar structure. Each band has two rows of areolae on the pars exterior and has a smooth edge on the pars interior even in the valvocopula (Figs. 25, 26). The band areolae forming the advalvar row are elliptical or almost circular in the valvocopula, but those of the abvalvar row are elongated (Figs. 23, 26). Each band areola is occluded externally by a hymen with marginal linear perforations arranged in a parallel array (KOBAYASI and NAGUMO 1985) and randomly scattered central ones (Fig. 27). The interareolar costae are markedly raised and numerous warts are on the band surface especially on that be-

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Plate 1. *Entomoneis decussata*. Scale bar=10 μm.
Fig. 1. Girdle view of valve. Shimabara-wan. OS-381. Fig. 2. Valve view. KE-1554-3. Fig. 3. Girdle view of valve. Yamagata Prefectural Fisheries Experimental Station. OS-425. Fig. 4. Girdle view of a frustule corner. Shimabara-wan. OS-381. TEM. Fig. 5. Frustule in girdle view just before sexual reproduction, 28 μm in length. KE-1554-3. Fig. 6. Frustule attained maximum size just after auxospore formation, 87 μm in length. KE-1554-3.

Plate 2. *Entomoneis decussata*. Shimabara-wan. Scale bars: Figs. 7, 8=5 μm, Figs. 9–11=1 μm, Fig. 12=0.1 μm. Fig. 7. External girdle view of a frustule corner showing the winged keel (wing). The wing costae are arranged obliquely and in parallel slanting toward the valve centre except those arranged radially near the apex. A row of small spines is on the valve costae. Fig. 8. The other corner of the same winged keel as shown in Fig. 7, showing the wing costae all arranged obliquely and in parallel. Fig. 9. Internal valve showing the inner openings of areolae arranged in two rows in each intercosta. Fig. 10. External valve body showing broad valve costae and domed pore occlusions of areolae in each intercosta. Fig. 11. External wing showing the narrow wing costae and elongated pore occlusions with perforations arranged in parallel. Fig. 12. Pore occlusions of areolae on the valve body showing hymenes each with perforations forming lateral lines and arranged in parallel. TEM.

Plate 3. *Entomoneis decussata*. Shimabara-wan. Scale bars=1 μm.
Fig. 13. External surface of the valve margin showing the small spines on the valve costae and on the intercostae. Fig. 14. Oblique view of the central nodule showing the smooth external surface of the raphe canal elevated from the valve body, and the central raphe endings which terminate in slightly dilated central pores. Fig. 15. Internal view of the central nodule showing the inner opening of the raphe canal, and the central raphe endings which terminate simply (arrows). Figs. 16, 17. Both external apices of the same valve showing terminal fissures curving in opposite directions. Fig. 18. External broken valve showing the smooth surface of the raphe canal and the wing costae. Fig. 19. Broken end of Fig. 18 at a different angle showing the raphe canal (rc), linking fibulae between opposing wing costae, the raphe fibula (rf), and the basal fibula (bf) dividing the valve body and the wing. Fig. 20. Internal valve centre showing the central opening of the raphe canal (arrow), and the basal fibulae centrally fused into a fishbone-like structure. Fig. 21. Internal basal fibulae fused into H or Y shape at location more distal from the valve centre.

Plate 4. *Entomoneis decussata*. Shimabara-wan. Scale bars: Fig. 22=10 μm, Figs. 23, 26=5 μm, Figs. 24, 25=1 μm, Fig. 27=0.5 μm. Fig. 22. External girdle view of a whole frustule. Fig. 23. Enlargement of the frustule shown in Fig. 22, showing the epicingulum composed of six bands; one valvocopula (VC), five bands (B1, B2, B3, B4, B5). Fig. 24. Oblique view of one end of the broken theca showing the open end (arrow) of the band between two bands each with a closed apex. Fig. 25. Internal valve margin and a valvocopula with its smooth advalvar edge. The advalvar areolar row of the band is composed of shorter areolae and those of the abvalvar row are composed of more elongated ones. Fig. 26. Advalvar cingulum end showing the internal and external surfaces. Warts are on the external surface of the bands. Fig. 27. Pore occlusions of both the short and the elongate areolae of a band showing linear perforations arranged in parallel near the margins and randomly scattered ones toward the centre. TEM.

tween two areolar rows. The internal surface of the bands is flat (Fig. 26). These features of the cingulum are extremely similar to those of *E. pseudoduplex*, but clearly different from those of *E. alata* var. *japonica, E. paludosa* and *E. punctulata* mainly in the presence of the raised interareolar costae. This species seems to be closely related to *E. pseudoduplex*.

The above-mentioned fine structural features are common in both small and large frustules before and after sexual reproduction. Consequently, it is considered that the following features are stable and characteristic of this species: 1) Decussating wing costae with many fibulae: 2) Arcuate junction line formed by the row of basal fibulae fused randomly to each other: 3) Spines on the costae of the valve body: 4) Density of striae, being 22-26 in 10 μm: 5) Perforations arranged in lines in the hymenes which close valve areolae: 6) Externally raised interareolar costae and numerous warts on the surface of the bands.

Nomenclatural treatment

Entomoneis decussata (Grun.) comb. nov.

Basionym: *Amphiprora decussata* Grun. In Cleve and Grunow. Kongl. Sven. Vet. Akad. Handl. **17**(2): 63. 1880.

Synonym: *Amphiprora gigantea* Grun. var. *decussata* (Grun.) Cl. Kongl. Sven. Vet. Akad. Handl. **26**(2): 18. 1894.

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長田敬五*・小林　弘**：海産羽状珪藻 *Entomoneis decussata* (GRUN.) comb. nov. の微細構造

島原湾沿岸および山形県のアワビ養殖場から得た天然個体とクローン培養株に基づいて，*Entomoneis decussata* の微細構造に関する詳細な観察を行った。その結果，本分類群は，1）翼は多くの間板を伴う交差型の肋骨と比較的太い縦溝管を持つ，2）弓形の縫合線は隣同士で時折融合する基部間板の一列によって形成される，3）殻本体は比較的高い密度の条線と肋骨上に多数の小刺を持つ，4）殻の胞紋は1－3本の線状の小孔列を持つ薄皮によって閉塞される，5）各殻帯片の胞紋列の胞紋間肋骨は強く外側に隆起する，などのかなり安定した形質によって特徴づけられることが明かとなった。（*951 新潟市浜浦町1-8　日本歯科大学新潟歯学部生物学教室；**184 東京都小金井市本町3-8-9-813　東京珪藻研究所）