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Fine structure of the brackish water pennate diatom *Entomoneis alata* (EHR.) EHR. var. *japonica* (CL.) comb. nov.

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Observations of the fine structure of a brackish water diatom *Entomoneis alata* var. *japonica* collected from a few localities were mainly carried out using scanning and transmission electron microscopy. In addition to this, some individuals were isolated from samples collected from the estuary of Tama-gawa (Tama River) for clonal cultures, and structural variations in the clonal cultures were examined in order to distinguish stable features of this diatom. The following morphological features are apparently stable and characteristic of this taxon: 1) bisinuous junction-line, 2) wing with many fibulae on each wing-costa, 3) cingulum composed of five open bands, a valvocopula, three copulae and a pleura, 4) poroid areola closed at the external surface by a hymenate pore occlusion with regularly scattered perforations.

Key Index Words: Brackish diatom; clonal culture; Entomoneis; Entomoneis alata *var.* japonica; *fine structure.*

The genus *Entomoneis* was first described by EHRENBERG in 1845 based on a different interpretation from that of his *Amphiprora*, and typified by his *Navicula alata*. Nevertheless, a group of diatoms having a quite characteristic and conspicuous features similar to that of the type species has been called *Amphiprora*, although the type species of *Amphiprora* has already been transferred to other genus. On the other hand, REIMER (in PATRICK and REIMER 1975) have stated about so doubtful availability of the generic name, *Amphiprora*, and proposed that *Entomoneis*, should be used instead.

In some species of the genus *Entomoneis*, valves characterized mainly by the well developed sigmoid wing have been shown in electron microscopical studies of HELMCKE and KRIEGER (1962), OKUNO (1970), GERLOFF and HELMCKE (1977) and PLANCKE and BAILLEUX (1976). Recently, the wing structure of the genus and allied genera has considerably ellucidated by the comprehensive works of PADDOCK and SIMS (1977, 1981).

Entomoneis alata var. *japonica* was originally described from Japan as *Amphiprora alata* var. *japonica* by CLEVE (1894). Since then, only a few records on this diatom have appeared in the literature, even in Japan. However, we have samples containing a considerable number of individuals belonging to this taxon from several brackish waters. In order to clarify stable characteristics of this diatom, some individuals were isolated from fresh samples for clonal cultures.

The morphological terminology mainly employed in this investigation is that proposed by ROSS *et al.* (1979) and PADDOCK and SIMS (1981).

Materials and Methods

Materials were collected from the mud surface of the tideland of Obuchi-numa (Obuchi Bog), Aomori Prefecture on May 2, 1980 and the estuary of Tama-gawa (Tama River), Kanagawa Prefecture on June 16, 1980. According to Pringsheim's pippette-washing method, clonal cultures were introduced from the samples collected from the estuary of Tama-gawa.

The cultures were carried out in a series of test tubes containing the agar-water biphasic culture medium (OOSHIMA 1975). The medium used as a liquid medium for the liquid phase was made by adding Chu no. 10 (CHU 1942) to f-medium (GUILLARD and RYTHER 1962) to obtain a final salinity of 15-25‰. The same medium with 0.6% agar was used as an agar medium for the solid phase. All the cultures were maintained at 18°C under fluorescent lights of ca. 3000 lux intensity with a 12/12 hr light-dark cycle. Consequently, the cultures, K. E-116, K. E-213 and K. E-343, were established.

Both materials, field and culture, were cleaned by the Patrick's method (PATRICK and REIMER 1966) but some of the materials were treated with potassium persulphate which is a mild oxidizing agent proposed by MA and JEFFREY (1978) for the observation of fragile girdle structures.

For light microscopy Pleurax was used as a mountant. For SEM observation the cleaned material was dried on glass coverslips ($5\times6\ mm^2$) and coated with platinum-palladium. Likewise, some of the specimens broken by a small knife were prepared for SEM observation. SEM observations were made with a Hitachi S-500 and a JSM F-15. For TEM, the specimens were dried up on grids coated with formvar and observed with a Hitachi H-500.

In order to obtain thin sections, cultured specimens were fixed for 1 hr in 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) and washed with the same buffer and then postfixed in 1% osmium tetroxide for 1 hr. They were washed with distilled water and embedded in 1% purified agar. Small blocks of the agar were then dehydrated slowly in ethanol and embedded in Spurr's resin. Thin sections were cut on a diamond knife. Sections were stained with uranyl acetate and lead citrate.

Results and Discussion

Frustules of this variety are strongly bilobate in girdle view because of their well developed bilobed wing (Figs 1, 6). The wing is strongly sigmoid in valve view (Fig 8) and deeply sunk at the central nodule (Figs 2, 3, 5, 7). In valve view, the valve is linear-lanceolate with acuminate ends. Valves in the field materials (Figs 1-3) are 75-150 μm long and 20-40 μm wide and the striae density on the valve face is 11-12 in 10 μm. The junction line observed in girdle view is clearly bisinuous on each side of the central nodule. In addition to these features, the other features agree quite well with Cleve's description (CLEVE 1894).

In the clonal cultures, valves in the 5-week-old cultures (K. E-343) showed the size and features similar to those of the field materials (Figs 7, 8), however, most of the valves obtained from a continued 28 weeks culture (K. E-213) showed a considerable decrease in size, being 55-80 μm in length and 18-30 μm in width (Figs 5, 6). Such smaller valves are occasionally observed in the field materials (Fig. 3). Therefore, the valve dimensions of this variety seem to vary within a wide range of 55-150 μm in length and 18-40 μm in width. In contrast, the striae density and the wing shape did not vary so markedly with the decrease of the valve size. The junction line described as one of the important characteristics of this variety by CLEVE (1894) retained bisinuous shape in all valves obtained from cultures (Figs 5-8) and field (Fig 3). Although, the distal sinuosity of the bisinuous junction line showed marked variation in shape, the proximal one kept its constant shape (Figs 3, 5, 6).

In SEM observations, it is evident that the valve has indeed a prominent bilobed wing (Figs 9, 10). Most of costae continue from the valve body to the top of the wing across the junction line (Figs 9–13). Some of these costae bifurcate more frequently in the wing than in the valve body (Figs 9, 10, 12, 13). Intercostae viewed as striae under the light microscope are composed of two rows of pores (Figs 11–14) in the same manner with that of *Amphiprora alata* (GERLOFF and HELMCKE 1977). Each pore is a poroid areola closed by a domed occlusion at the external surface (Figs 13, 14). As seen in the TEM micrographs of the pore occlusion and its cross section, pore occlusions are of uniform thickness (Fig. 28) and are perforated by more or less elongate holes with a dimension of 6–10 nm (Fig. 27). This form of pore occlusion agreed well with that of hymenate pore occlusion proposed by MANN (1981). The type of arrangement of the perforations is the regular scatter in contrast to the centric array of the *Entomoneis alata* (MANN Fig. 19, 1981).

In each wing of the valves, linear series of dots as distinctly illustrated in Cleve's figure (CLEVE 1894) are clearly seen in the light microscope (Figs 1–3, 5, 6). REIMER (in PATRICK and REIMER 1975) have described the dots as one of the important characteristics distinguishing *E. alata* from *E. paludosa*. However, our SEM observations of the external surface of the wing could not reveal any structure except two rows of areolae constituting each stria (Figs 12–14). However, in the observation of broken valves, there are many fibulae inside the wing (Fig. 12). The wing is traversed by the fibulae arranged along on each wing-costa, so that a fibula of the wing may be observed to be a dot in the light microscope. The fibulae row on a wing costa seem to be homologous to the perforated fibulate plate of *Amphiprora* sp. (PADDOCK and SIMS 1977).

PADDOCK and SIMS (1981) have suggested that there may be no obvious morphological distinction between the fibulae immediately adjacent to the raphe canal and the rest of the fibulae within the wing, but termed the former "primary or raphe fibulae" and the latter "secondary or keel fibulae". Moreover, they have applied the term "basal fibulae" to the fibulae arranged longitudinally along the base of the wing. In *E. alata*, so far as it appeared on the SEM micrographs of GERLOFF and HELMCKE (in HELMCKE and KRIEGER 1977), the basal fibulae are lacking from some costae. In contrast, basal fibulae in this taxon occur regularly on all costae and are situated on the border between wing and valve body (Figs 11, 12). Consequently, it is proved that the junction line obvious in the light microscopical observations is a row of basal fibulae. The perforated fibulate plate could be observed particularly near the junction line (Fig. 13).

Although the presence of a canal raphe in some *Amphiprora* species has already been pointed out by PADDOCK and SIMS (1977), this diatom also possesses the canal raphe along the apex of the keel. The raphe canal is a cavity separated by a row of raphe fibulae (Fig. 14). The raphe fissure is a plicate type as clearly seen in a transapical section across the raphe (Fig. 14). At each valve apex the raphe fissure terminates internally in well developed helictoglossa (Figs 9, 11). Terminal fissures curve in opposite directions at both ends of a valve (Figs 25, 26).

In the girdle view, a number of linear series of short dashes arranged parallel to the apical axis have been illustrated for *E. alata* (REIMER, in PATRICK and REIMER 1975). However, any comments on the girdle of this taxon have not been found even in the original description (CLEVE 1894). In the present study, short dashes similar to those of *E. alata* are observed in the girdle region in the light microscope, and are 20–22 in 10 μm (Fig. 4). According to the SEM and TEM observations, it is proved that each of these dashes is a poroid areola with an elongate or round inner aperture (Figs 16, 17, 22). Each areola is occluded by the hymen (MANN 1981) with randomly arranged

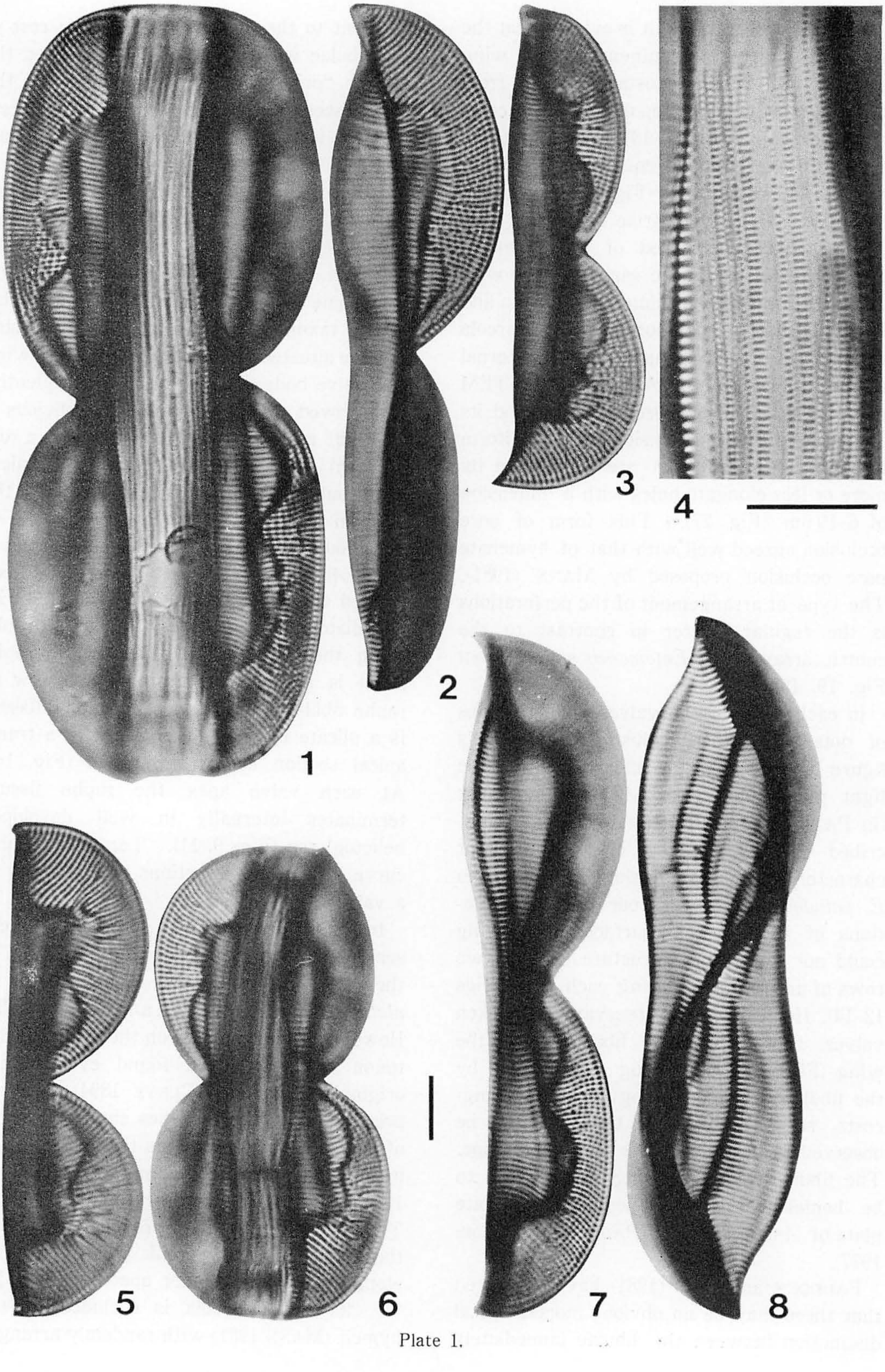

Plate 1.

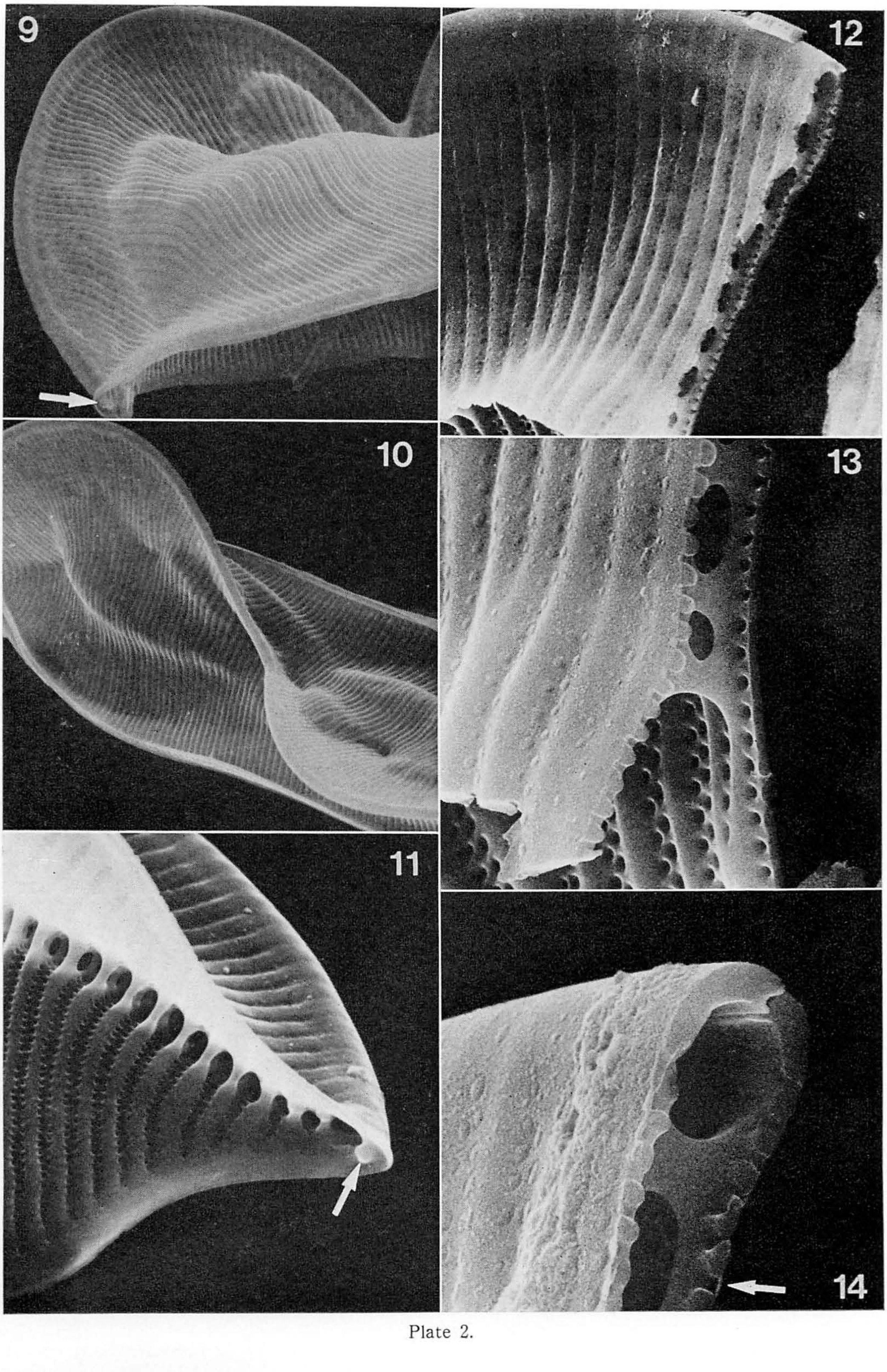

Plate 2.

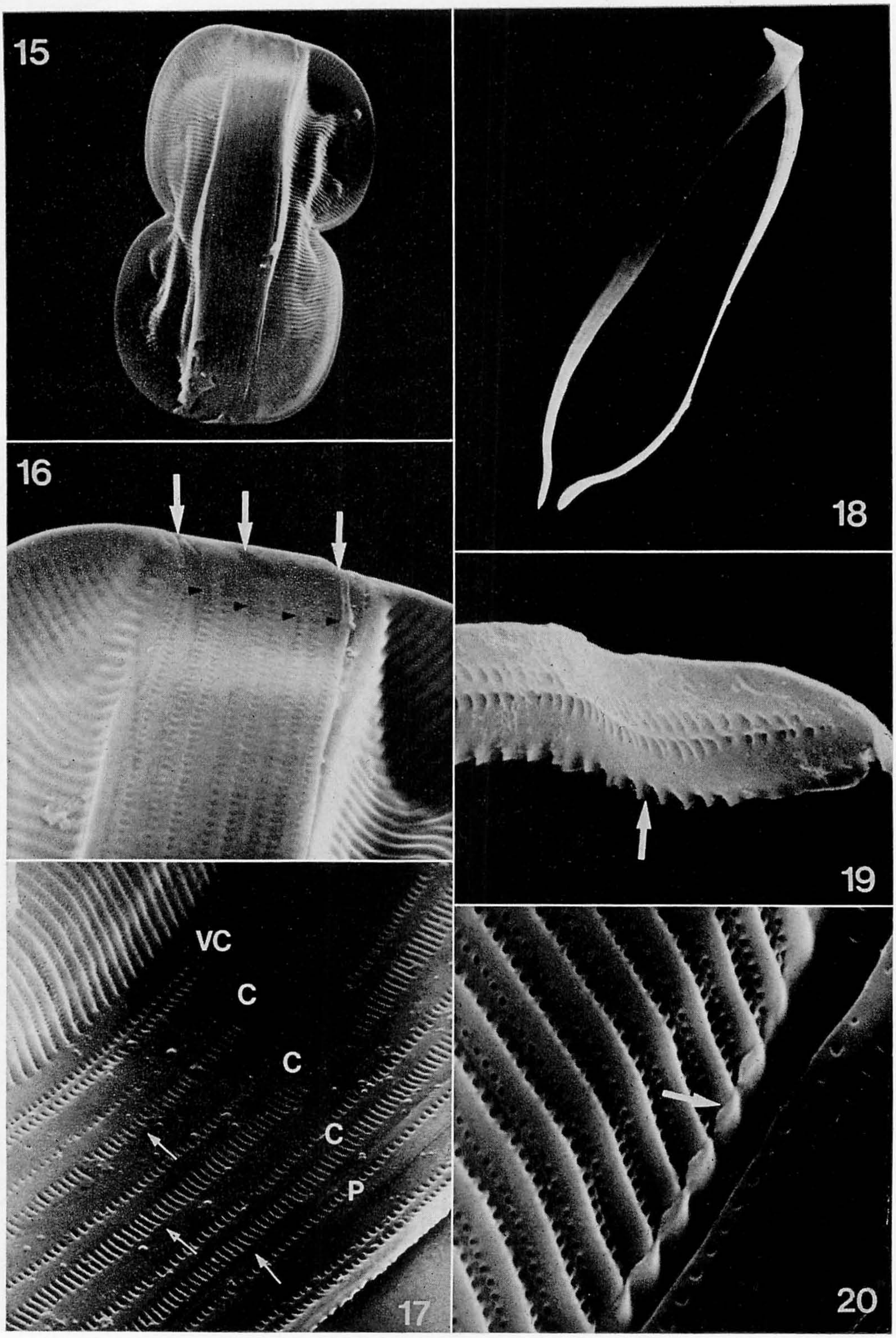

Plate 3.

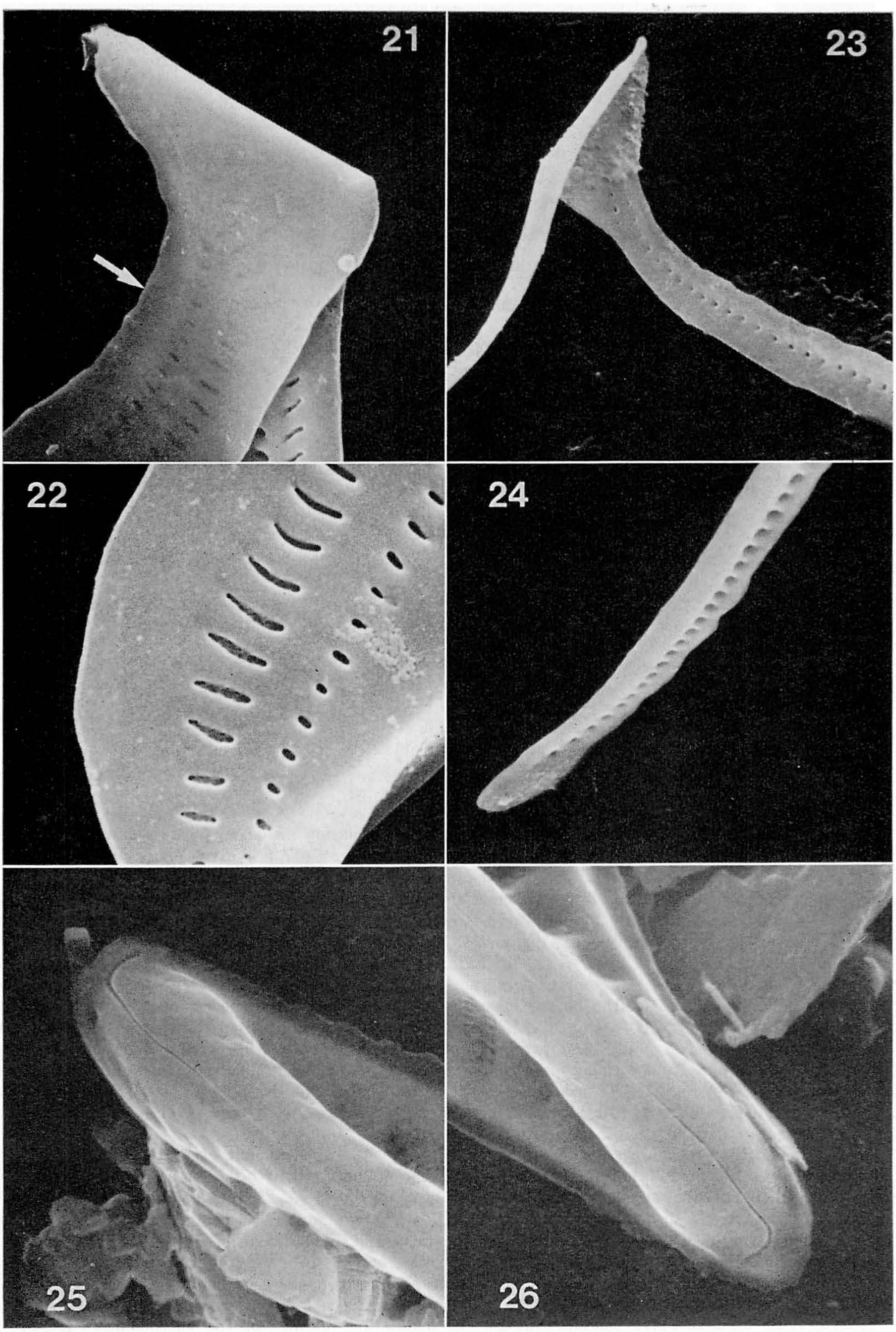

Plate 4.

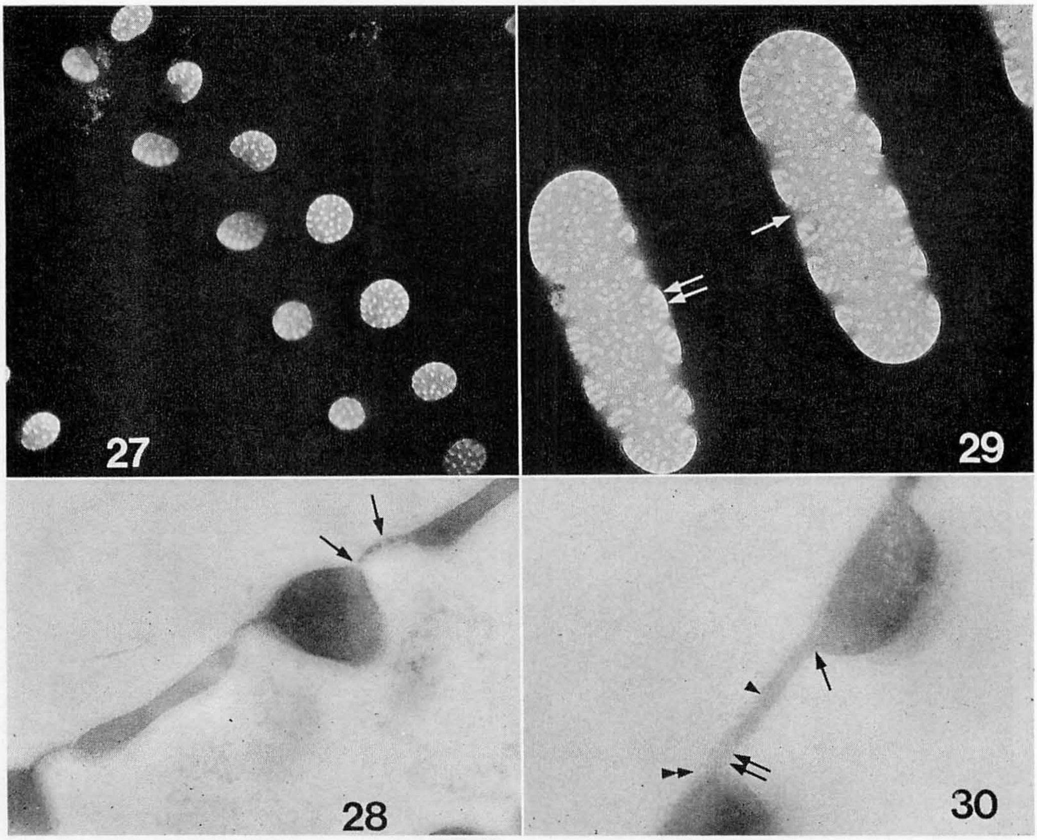

Plate 5.

Plate 1. Figs. 1-8. Light micrographs showing the variation in size. Scale bars=10 μm; 1-3. Specimens in nature; 5, 6. Specimens in 28-week-old cultures; 4, 7, 8. Valves and middle portion in girdle view in 5-week-old cultures, 7. Girdle view showing bilobed wing and bisinuous junction line on each side of the central nodule, 8. Valve view showing sigmoid wing.

Plate 2. Fig. 9. Oblique view of half valve showing the prominent wing with a keel at its top, bisinuosity occurred on the border between wing and valve body and a helictoglossa (arrow) on the internal surface of the valve apex. ×2300. Fig. 10. External view of valve showing a sigmoid wing and costae continuous from the valve edge to the wing top. ×1500. Fig. 11. Internal view of valve showing a row of basal fibulae placed on the border between the valve body and the wing and well developed helictoglossa (arrow). ×6000. Fig. 12. Broken valve showing many fibulae on each costa and a few bifurcated costae. ×6800. Fig. 13. Lower part of the valve shown in Fig. 12. showing the structure of perforated fibulate plate and striae composed of two rows of poroid areolae with external domed pore occlusions. ×17000. Fig. 14. Transapical section of wing showing the plicate raphe fissure at the top, the raphe canal separated by the raphe fibula, and poroid areolae with pore occlusions (arrow) on the external surface. ×25000.

Plate 3. Fig. 15. Frustule in girdle view. ×900. Fig. 16. Enlargement of Fig. 15 showing epicingulum composed of five bands distinguishable by imbrication lines (arrow heads), and open apices (arrows) and closed apices of the bands arranged alternately. ×2800. Fig. 17. Internal view of hypocingulum composed of five bands showing a valvocopula (VC) with two rows of areolae nearly equal in size, three copulae (C) with two rows composed of short and elongate areolae and a pleura (P) with a single row of areolae. Note the smooth edges of pars interior of the copulae and pleura (arrows). ×3000. Fig. 18. Whole copula showing a tweezers-like open band. ×780. Fig. 19. Internal view of valvocopula showing two rows of areolae and undulate pars interior (arrow). ×10000. Fig. 20. Internal oblique view of valve margin showing the undulation (arrow) of the valvocopula edge overlapping valve costae and striae composed of two rows of areolae. ×12000.

round holes in its thin area and radially arranged linear holes around the margin and lying near the external surface (Figs 29, 30).

The girdle region usually occupies about 1/3-1/2 of the breadth of the frustule in girdle view, and the epicingulum overlaps most of the hypocingulum (Figs 15-17). Each cingulum is composed of five bands, and each band is open at one apex (Figs 16-18). These open bands are classified into three types, a valvocopula, three copulae and a pleura, based on the structure of pars interior and the arrangement of areolae. The valvocopula has two rows of round or more or less elongate areolae nearly equal in size (Figs 17, 19) and its pars interior is undulate except a short region near the closed apex (Fig. 19). Each undulation is observed to fit closely over the inner surface of the valve costa (Fig. 20). Such a juncture between the valvocopula and the valve seems to be similar to that of *Rhabdonema arcuatum* reported in detail by POCOCK and COX (1982), except the difference in the degree of development of the pars interior. Each copula also has two rows of areolae, but each row could be distinguished by the size of areola. The abvalvar row is composed of more elongate areolae and the advalvar one is composed of short areolae (Figs 17, 21, 22). The pars interior of the copula has a smooth edge (Figs 21, 22). The fifth band in each cingulum, pleura, is observed to have basically a single row of areolae except the short region near the closed apex where two rows of areolae are present (Figs 23, 24). The opening of each band alternates between the apices of the frustules (Fig. 16). The closed apex in each band has an extension well developed in the advalvar direction (Figs 16, 18, 21, 23). The extension seems to be homologous to a ligula as seen in the bands of centric diatoms such as *Aulacosira italica* (KOBAYASI and NOZAWA 1982).

According to the present investigation using culture methods, it is considered that the following characteristics are apparently stable in *Entomoneis alata* var. *japonica*: 1) the junction line is bisinuos from the valve center to the apex. 2) the wing has numerous fibulae in its major part and has a raphe canal along its free edge. 3) the mature cingulum is composed of five open bands, i.e., a valvocopula, three copulae and a pleura. 4) poroid areolae on both valve and cingulum are closed at the external surface by pore occlusions. 5) the pore occlusion of the valve pore is a hymen with regularly scattered perforations and that of cingulum is also a hymen with scattered round perforations in its thin area and with radially arranged linear perforations along its margin.

As mentioned at the beginning of this paper, if Reimer's opinion is correct, thent his taxon should have the following combination as its correct and valid name.

Entomoneis alata (Ehr.) Ehr. var. *japonica* (Cl.) comb. nov.

Amphiprora alata Kuetz. var. *japonica* Cl. Kong. Sven. Vet. Akad. Handle **26**(2): 16. *pl. 1. f. 2.* 1984.

Plate 4. Fig. 21. Closed apex of the copula showing two rows of areolae and well developed extention and smooth edge of the pars interior (arrow). ×6200. Fig. 22. Internal view of the open end of the copula showing the long and short pores forming rows. ×14000. Fig. 23. Closed apex of the pleura showing well developed extention and a single row of round pores. ×6000. Fig. 24. Oblique view of open apex of the pleura showing a single row of pores and smooth edge. ×10000. Figs. 25, 26. External view of both apices of the same valve showing terminal fissures curved in opposite directions. ×12000.

Plate 5. Fig. 27. Hymenate pore occlusions with regularly scattered perforations. TEM ×56000. Fig. 28. Longitudinal thin section of wing showing pores occluded at the external surface by the domed hymen of uniform thickness and perforations (arrows). TEM ×84000. Fig. 29. Hymenate pore occlusions on a band showing round holes in the thin area and radially arranged linear holes along its margin. Note the thickened portion (arrow) and thin marginal portion with radially arranged linear holes (double arrow). TEM ×63000. Fig. 30. Thin section of the band parallel to the valvar plane showing thickened (arrow) and thin marginal portion (double arrow), round holes (arrow head), and a linear hole (double arrow head) of the hymen. TEM ×112000.

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長田敬五*・小林　弘**: 汽水産羽状珪藻 *Entomoneis alata* (EHR.) EHR. var. *japonica* (CL.) comb. nov. の微細構造

青森県小畝沼，神奈川県多摩川の汽水域から得た個体，および多摩川から採取したものから得たクローン培養を，主に TEM と SEM によって観察した。この分類群では，1) 翼と殻本体との間の 3 の字形の縫合線，2) 翼の間条線上に多数の間板をもつ翼構造，3) 1枚の接殻帯片，3枚の中間帯片，および1枚の連結帯片の計5枚の殻帯片からなる殻帯，4) 規則的散在型の小孔をもつ薄皮で外側を閉塞された胞紋の構造，の諸形質は天然の試料でも，また，培養の個体群の中でも，殻の変異と関係なく，極めて安定していた。(*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 東京都小金井市貫井北町 4-1-1 東京学芸大学生物学教室)