Observations of *Eunotia arcus* Ehr., type species of the genus *Eunotia* (Bacillariophyceae)

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Mayama, S. and Kobayasi, H. 1991. Observations of *Eunotia arcus* Ehr., type species of the genus *Eunotia* (Bacillariophyceae). Jpn. J. Phycol. **39**: 131–141.

Morphological studies of *Eunotia arcus* Ehr. var. *arcus*, type species of the genus, have been carried out with electron microscopy using the classical material collected from Degernäs (the type locality) and the Japanese material. The former material contains various sizes of valves including both initial and first division valves. The following characteristics of the post-initial thecae of this taxon are stable: external and internal surface structure; striae density; arrangement of the pattern center to the valve face and mantle juncture; raphe placement; location of labiate process; areola structure; areolae density; epitheca depth. As for the epitheca depth, a detailed examination comparing this taxon and *Eunotia arcus* var. *bidens* Grun. has been carried out. Special attention has been paid to the areola structure and the structure of initial valves.

Key Index Words: areola—diatom—epitheca—Eunotia—Eunotia arcus—fine structure—initial valve—topotype material.

Eunotia arcus Ehr. var. *arcus* known to be a cosmopolitan species and occurring in slightly acidic to circumneutral water, is the type species of the genus designated by Boyer (1927). Though many taxonomists and ecologists have described this species, no observation of the type specimen has been made yet. As is seen in the other *Eunotia* species, this species also shows a wide variation in valve size and shape. This seems to be one of the main reasons that many infraspecific taxa, 6 forms and 24 varieties (according to VanLandingham 1969), have been made.

We have found topotype material of *E. arcus* in the Swedish Museum and have examined it light microscopically, and this material has been strongly assumed to be taken from the same lump as examined by Ehrenberg (1837) (Mayama and Kobayasi 1990). In this material, post-initial valves in various sizes and shapes are contained in addition to the initial valves. This is a valuable material to study a whole variation of valves during the life cycle of this species. In the present study, the fine structure of the topotype specimens is observed in comparison with the Japanese ones and the stable characteristics of the species are discussed.

Materials and Methods

The material examined is listed as follows: (1) Topotype material: diatomite from Cleve's collection 247, Degernäs in Sweden, graft, housed in the Swedish Museum of Natural History, Stockholm, (our sample number, K-6686).
(2) Recent materials from Japan: an epipelic sample from Sugenuma Pond, Gunma Pref., on 17 June 1986, K-5865; an epiphytic sample from Saino-ko Pond, Tochigi Pref., on 17 June 1986, K-5879.

These materials were boiled with sulfuric acid and potassium permanganate to remove organic matter, or were suspended with hydrogen peroxide and then cleaned softly by ultraviolet radiation so as not to take the frustule apart. After one or the other treatment specimens were washed in distilled water. For SEM observations specimens

were dried on glass coverslips which were then fixed to metal stubs. They were coated with gold-palladium using JEOL JFC-1100 and observed with JEOL F15. Specimens for TEM were placed on formvar-coated copper grids and observed with JEOL 100B.

Observations and Discussion

Specimens from the topotype material show various valve shapes and sizes (Fig. 1A). The longest valve is an initial valve and 115 µm (Figs. 1A with asterisk, 2, 4). The reason why we can recognize this specimen as an initial valve of *Eunotia arcus* is that we have found an initial valve paired with a first division valve (first vegetative valve) of *E. arcus* (Figs. 3, 5). The initial valve is rounded in the cross section and the valve face cannot be distinguished from the valve mantle. The ventral side is concave and the dorsal side is convex, and they are parallel throughout the valve. The valve ends have a semidome-like form and they have no construction of the dorsal side as seen in typical vegetative valves (Figs. 6, 7, 12, 13, 22, 25).

There has been no SEM observation of the initial valve of *Eunotia*, and even in the other raphid diatoms few observations of the fine structure have been carried out (Krammer 1982, *Cymbella silesiaca*; Mann 1982, 1984a, *Rhoicosphenia curvata, Gomphonema intricatum, Cymbella* sp., *Cocconeis pediculus*; Cohn *et al.* 1989, *Navicula cuspidata*). These initial valves are reported to have a rounded section as in *E. arcus*. Geitler (1951) observed initial cells in the specimens identified as *E. arcus*, and despite light microscopy, his description of the initial valve agrees well with our SEM observations. However, our specimen does not completely conform his description and illustrations of a bent girdle view. We have observed such a bend in *Eunotia tropica, Eunotia* sp. and *Actinella brasiliensis* (unpublished data) but have not recognized such a bend in the present specimen. Cohn *et al.* (1989) have observed that the central area is not rounded but depressed in both initial epi- and hypovalves of *Navicula cuspidata*. Mann (1984b) has
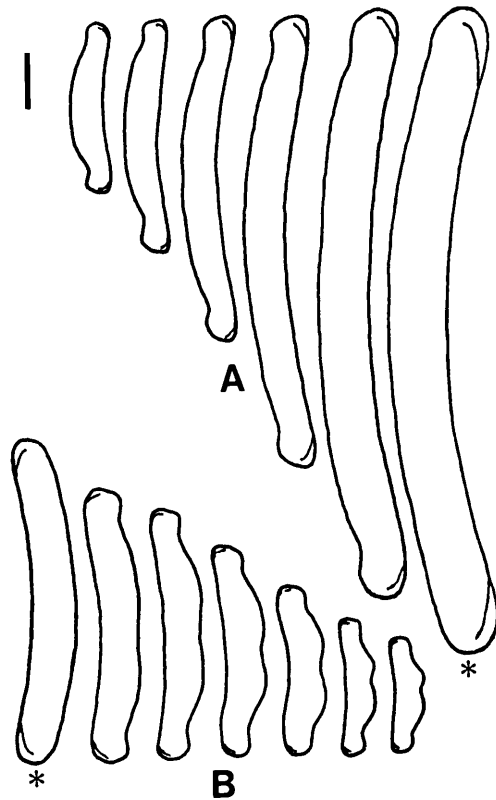

Fig. 1. Variations of valve size and outline seen in two populations of *Eunotia arcus*. The valves with asterisks are initial valves. A: *E. arcus* var. *arcus*. Topotype material, B: *E. arcus* var. *bidens*. K–5879. Scale bar = 10 µm.

reported the central constriction of the initial epivalve in the girdle view in *Neidium affine*. However, our specimen has neither such a depression nor constriction at the centre. Cohn *et al.* (1989) have suggested the accumulation of mucilage secreted from the central raphe endings as one of the reasons for this depression, however, our initial valve without such a depression has no central raphe endings at the center.

Mann (1984a) has pointed out a wide hyaline marginal strip characteristic of their initial epivalves in the four species, *Rhoicosphenia curvata, Gomphonema intricatum, Cymbella* sp. and *Cocconeis pediculus*. However, our specimen does not have such a structure.

In the initial valve, a pattern centre, or a sternum, runs between both apical raphe endings (Fig. 2) which are located at the centre of
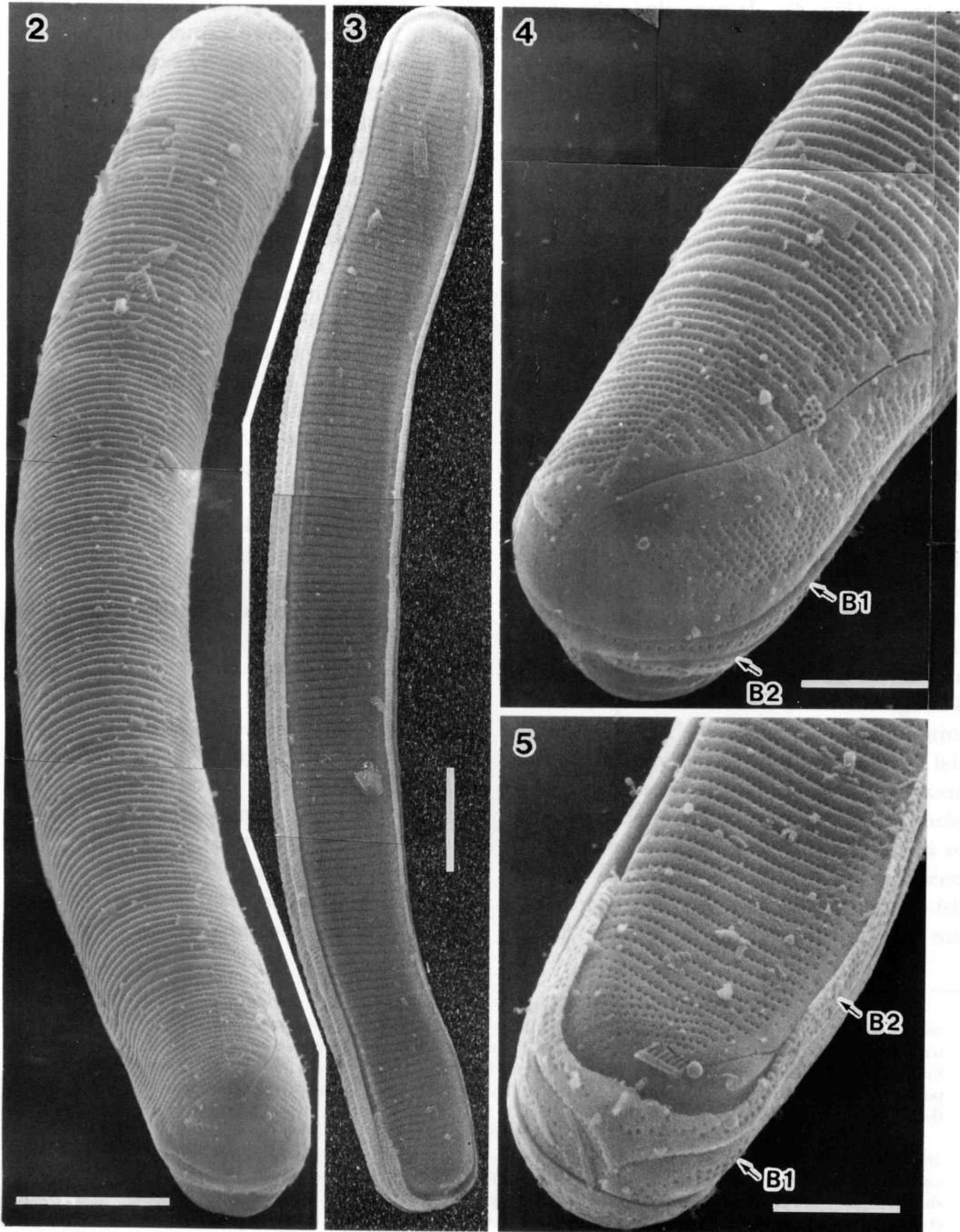
Figs. 2–5. *Eunotia arcus* var. *arcus*. Scale bars = 10 μm (Figs. 1, 2) or 5 μm (Figs. 4, 5) Fig. 2. Oblique external view of initial cell with rounded section. Topotype material. Fig. 3. External view of a first division valve with flat valve face. Topotype material. Fig. 4. Detail of Fig. 1 showing the initial epivalve with two bands (B1, valvocopula and B2). Fig. 5. Detail of Fig. 2 in oblique view. A first division valve is seen inside the initial hypotheca with two bands (B1 and B2).

the apices (Fig. 4). It runs along the apical axis near the valve ends (Figs. 1, 3) but moves away from the apical axis gradually, and its location is most eccentric at the valve centre on the ventral side (Fig. 2). This characteristic placement is only seen in the initial valve and is not in the vegetative valves (Figs. 3, 5-11, 25), however, this placement of the pattern center may imply some clues explaining a systematic relationship to the araphid diatoms such as postulated by Simonsen (1979) and Mann (1984c). The initial epivalve of *E. arcus* drawn by Geitler (1951) has the pattern center interrupted in the valve centre, but this discrepancy seems to be caused by the arched valve face and the observation with a light microscope.

The polar raphe fissure ends at the midpoint of the valve breadth, somewhat distant from the apical margin (Figs. 2, 4). The raphe branch extends down smoothly into the central ending because of the rounded valve face, and terminates in slightly dilated pores as seen in the vegetative cells (Figs. 4, 8, 9). Though Cohn *et al.* (1989) have represented a raised area surrounding the raphe in the initial valve of *Navicula cuspidata*, such a raised area does not appear in our specimens. The placement of the pattern center and the raphe as seen in the initial valve of *E. arcus*, is observed in some other *Eunotia* species (unpublished data), and perhaps these placements are a common feature in the genus. There-fore, *Eunotia shweickerdtii* Cholnoky (1954), which has the pattern center and the polar raphe endings on the apical axis of the valve, can be considered to be an initial valve of some other species.

From the pattern centre, areolae are arranged transversely forming rows towards the dorsal and ventral sides (Figs. 2, 4). An exact measurement of the areolae density is not easy because of the curvature of the initial valve face but its value converted in 10 μm is approximately 36. Because the external opening of the pore is located lower than the level of the interstria, the stria is seen as a shallow furrow. The striae density is about 13 in 10 μm in the main valve body, but is becomes denser near the ends, being about 16 in 10 μm. The striae extending downwards from the raphe branch are a little different in their arrangement (Fig. 4). They are a little denser, being about 18 in 10 μm.

The cingulum of the initial epivalve observed is composed of two bands, the valvocopula (B1) and the second band (B2) (Fig. 4). These bands are very narrow in comparison with those of the normal vegetative valves (Figs. 22-25). The bands have a longitudinal row of short striae on the pars exterior. The striae in B1 are composed of two to three pores along most of the band but have only pore at each end, and the number of pores composing striae is reduced in B2 (Fig. 4). Each band has one open end but at alternate
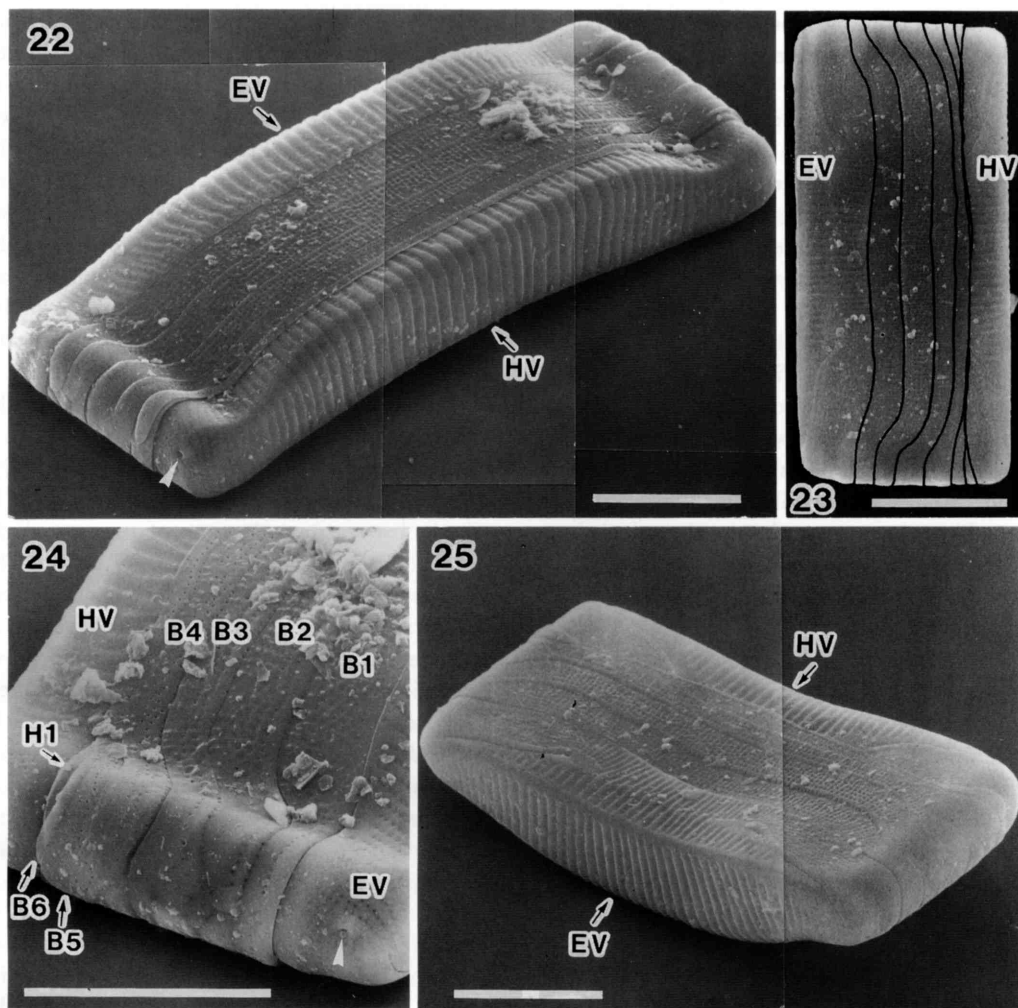
Figs. 6-11. *Eunotia arcus* var. *arcus*. Scale bars = 10 μm (Figs. 6, 7) or 5 μm (Figs. 8-11). Fig. 6. External view of a vegetative valve with rounded apices. K-5865. Fig. 7. External view of a vegetative valve with obliquely truncated apices. Topotype material. Fig. 8. Detail of Fig. 7 showing the whole raphe branch in the valve end. Fig. 9. Detail of Fig. 6 showing the whole raphe branch in the valve end. Fig. 10. Detail of Fig. 7 showing the polar raphe ending on the mid-line of the valve. Fig. 11. Detail of Fig. 6 showing the polar raphe ending on the mid-line of the valve.

Figs. 12-21. *Eunotia arcus* var. *arcus*. Scale bars = 10 μm (Figs. 12, 13), 2 μm (Figs. 14, 15), or 0.2 μm (Figs. 16-20). Fig. 12. Internal vegetative valve. Labiate process is located on the bisecting line of the apex in the valve mantle. Topotype material. Fig. 13. Internal vegetative valve. The labiate process is located at a point shifted slightly toward the dorsal side from the bisecting line. K-5865. Fig. 14. Detail of Fig. 12 in oblique view showing a large polar nodule (= helictoglossa) and a thickened hyaline area extending from the central raphe ending toward the valve centre. Fig. 15. Detail of Fig. 13 in oblique view showing the placement of the inner fissure and the thickened hyaline area extending from the central raphe ending toward the valve centre. Fig. 16. External view of valve areolae with the pore occlusions. Some of them are broken. Note the groove between the edge of the aperture and the occlusion. Fig. 17. Broken central valve showing the areolae each with occlusions set on a narrow ledge located slightly inward of the external aperture. Fig. 18. External view of cingulum areolae showing the shallow groove between the edge of the aperture and the pore occlusion. Note the minute openings of the areola open into the groove (arrow heads). Fig. 19. Internal view of valve areolae showing a simple aperture. Fig. 20. Pore occlusions without any perforations. TEM. Fig. 21. Diagram of the areola in section.
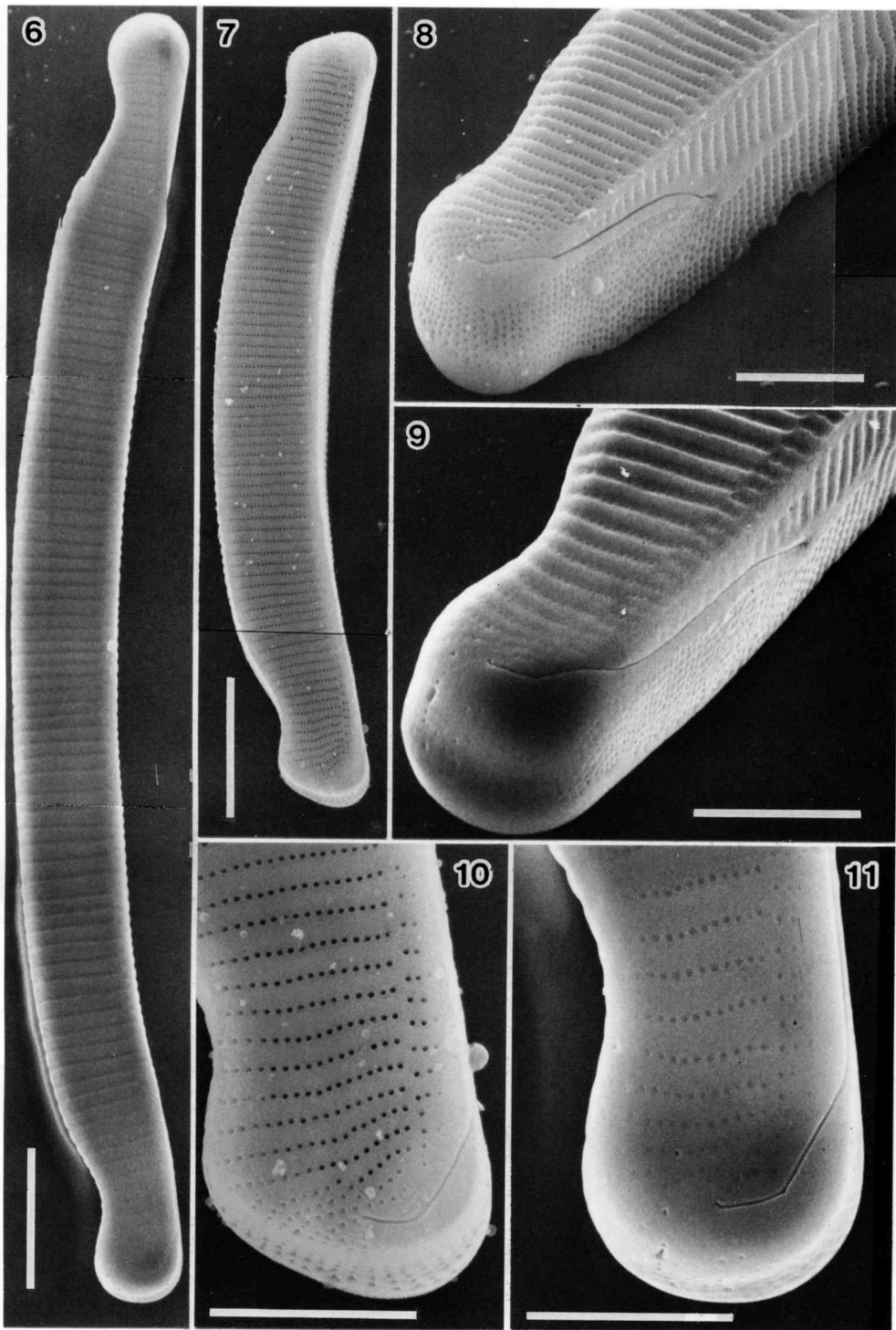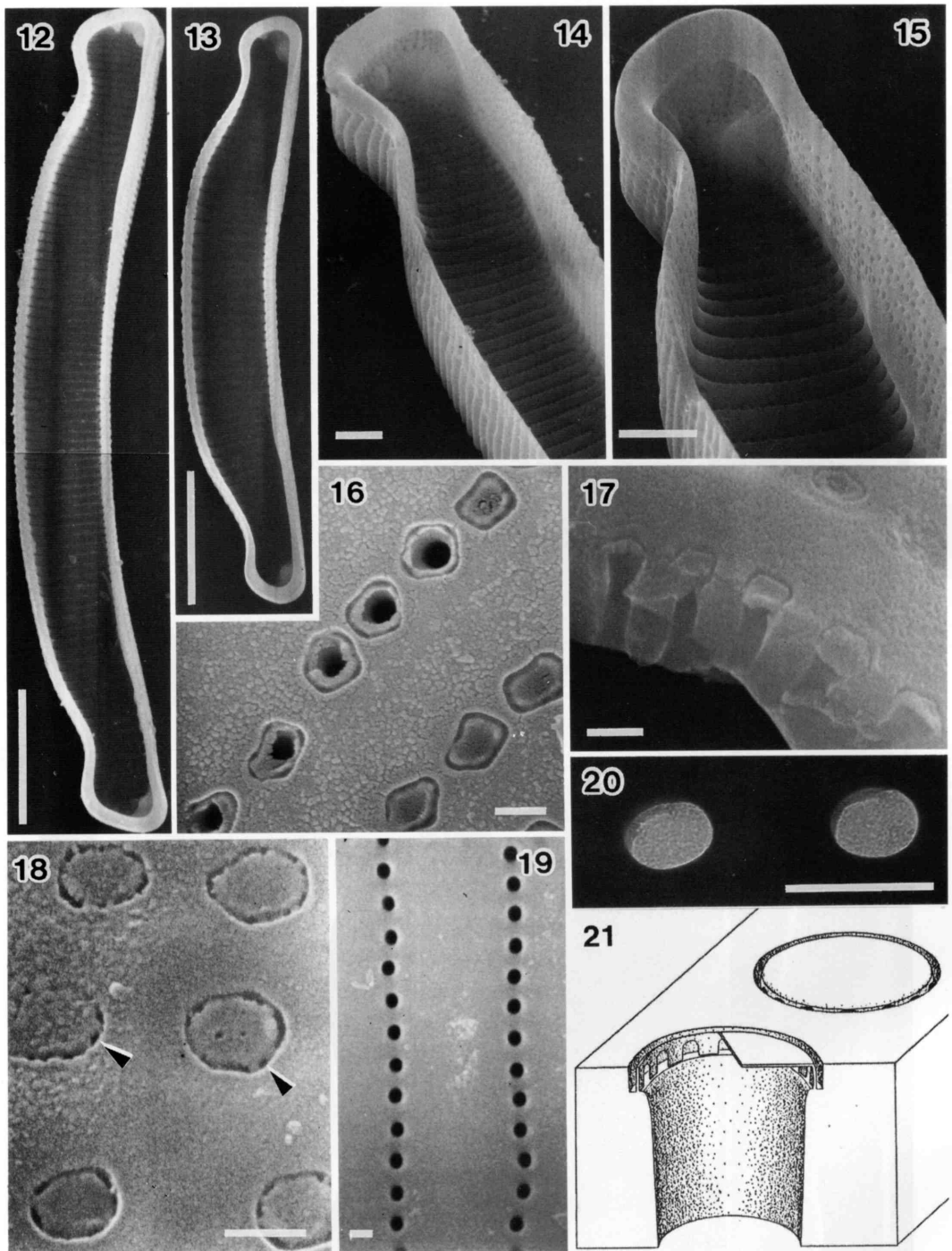
Figs. 22–25. *Eunotia arcus* var. *arcus*. Scale bars = 10 μm. EV = epivalve; HV = hypovalve; B1–B6 = first epiband (epivalvocopula) to sixth epiband; H1 = first hypoband. Fig. 22. Oblique view of a whole frustule from the dorsal side. Note the location of the outer opening of the labiate process (arrow head). Fig. 23. External view of a frustule from the ventral side showing the epitheca composed of an epivalve and six epibands. Fig. 24. Detailed figure of the far end of the frustule in Fig. 22. Note the closed end of B1, B3 and B5 located at the valve end with the labiate process (arrow head). Fig. 25. Oblique ventral view of the whole frustule in Fig. 23.

ends from each other. The cingulum of the initial hypovalve is also composed of two bands, but they are broader than those of the initial epivalves. The striae on the bands are composed of three to four pores along most of the band but reduced in number at the ends (Fig. 5). In the initial cells of *Rhoicosphenia curvata*, Mann (1984a) also observed broader bands in the hypocingulum than those of the epicingulum. Geitler (1951) has mentioned nothing about the band width in the initial

cells, but found 2—4 bands in the epicingulum and 4 bands in the hypocingulum.

The post-initial valves are 29–114 μm long in the topotype material and 22–101 μm long in the Japanese material. These ranges overlap those described in the life cycle of *E. arcus* (Geitler 1951, 13.5–95.2 μm long), though our ranges are generally higher.

The vegetative valves from the topotype material are presented in Figs. 3, 5, 7-9, 12, 14 and those from Japan are in Figs. 6, 9, 11,

13, 15. In the topotype material, the longest is the first division valve formed inside the initial valve (Figs. 3, 5). This valve has a flat face and can be clearly distinguished from the initial valve. Since the first division valve has parallel sides and rounded apices and has no constriction in the dorsal side near the apices, its outline is quite different from that of the shorter vegetative valves which are considered typical shape of *E. arcus* (Figs. 6, 7), and rather resembles that of the initial valve (Fig. 2). Comparing Figs. 3, 6 and 7, it is evident that the degree of dorsal constriction and the obliquity of the valve ends increase as the valve length becomes shorter. On the other hand, Steinman and Sheath (1984) have observed just the reverse changes in apical shape in cultured *Eunotia pectinalis* var. *minor*. In their case, the constriction disappears as the valves get shorter.

The placement of the raphe and pattern center are very stable regardless of valve size or habitat. The pattern center runs between both apices, but its terminating points are different from those of the initial epivalve. In the vegetative valve, the pattern center terminates approximately at the midpoint between the polar raphe ending and the ventral valve margin (Figs. 5, 8-11). This point corresponds to the location of the internal helictoglossa (Figs. 14, 15). Just a short distance from the terminating points, the pattern center approaches the ventral margin and runs along the length of the main valve body (Figs. 3, 6, 7). In the main valve body, the pattern center does not unite with the valve margin, or the juncture of the valve face and mantle. These are always one or two areolae between them. However, the union of the pattern center and the juncture was rarely observed in small specimens and only in the stretch between both central raphe endings.

The polar ending of the outer raphe fissure always terminates on the mid-line of the valve (Figs. 5, 10, 9). This location is the same as that of the initial valve (Fig. 4), but the fissure in the valve face is very short and immediately turns down into the valve mantle. The fissure in the valve mantle is longer

than that in the valve face. The central ending of the outer fissure forms a slightly dilated depression. Internally the polar ending of the raphe fissure terminates in the well developed helictoglossa (Figs. 12-15). Each raphe branch is surrounded by an obvious hyaline area (Figs. 8, 9, 14, 15). This area characteristically extends beyond the central raphe ending. The extension tapers towards the valve center externally and thickens markedly, internally. Our observations of the raphe correspond well with those of Wahrer (1981).

The striae consist of areolae as seen in the initial valve. The areolae are arranged in a shallow furrow both externally and internally. However, the furrow is detectable only in tilted observations because of its very slight depth (Figs. 8-11, 14, 15, 16, 17, 19). The areolae density in the valve face is stable, ca. 35-38 in 10 μm. There is no difference in the areolar density between the initial valve (ca. 36 in 10 μm) and the post-initial valves. Our examination supports the idea in *Navicula cuspidata* by Cohn et al. (1989) that the transapical spacing between pores could be a precise genetically controlled taxonomic indicator. In the main valve body, the striae density is 11-14 in 10 μm in the Swedish specimens, and 9-14 in 10 μm in the Japanese specimens. Cohn et al. (1989) observed finer striation more clearly in their post-initial valves than in the initial valves, but the vegetative valves from Sweden observed by us have a similar striae density as our initial valve (13 in 10 μm). In the culture of *Eunotia pectinalis*, Steinman and Sheath (1984) have stated that as time passed, the valve length decreased and striae density increased. Moreover, Mayama and Kobayasi (1988) have indicated the increase of the striae density according to the decrease of the valve length in *Navicula atomus*. However, we could not recognize any such remarkable tendencies in *E. arcus*.

Each valve has one labiate process located on the bisecting line of the apex in the valve mantle (Figs. 12, 14) or at a point shifted slightly toward the dorsal side from the bisecting line (Figs. 13, 15). Wahrer (1981) con-

cluded that the labiate process location is one of the most stable characteristics in the *Eunotia* species and he set up four placements of the labiate process in the apex. The location assigned to this species by him is the B placement (slightly above the midline). However, the labiate process is variable in placement as seen in Figs. 12–15. When the inside valve is arranged as in Figs. 12, 13, *i.e.* with the dorsal side to the left and the ventral side to the right, the labiate process is always located on the top side. This situation was first observed by Moss *et al.* (1978) and a more detailed explanation was given later by Wahrer (1981).

Because the topotype material is diatomite, all specimens are eroded to some extent so that they lack pore occlusion of the areola. Therefore, the complete areola structure has been observed only with living materials from Japan. The areolae are found in both valves (Figs. 16, 17, 19, 20) and bands (Fig. 18). The shape of the external aperture varies from circular to rectangular (Figs. 16–18). A groove is observed between the edge of the aperture and the pore occlusion. The area of the external aperture is larger than that of the circular internal aperture (Figs. 17, 20). The pore occlusion has no perforations as seen in the hymenate pore occlusion (Fig. 19) nor flaps as in the volate pore occlusion described by Mann (1981). The entire form of the occlusion is like a shallow Petri dish, which has small openings not in the ceiling but in the wall (Fig. 21). This occlusion is set on a narrow ledge placed slightly inward of the external aperture. The opening of the occlusion appears as a slit in the band because of the very shallow groove running between the edge of the aperture and the pore occlusion (Fig. 18).

The epicingulum consists of four to six open bands. The open end of B1, the valvocopula, is placed at the apical side lacking the labiate process and the closed end is at the other side (Figs. 22, 24) and this band orientation confirms the observations of Wahrer (1981). In each successive band, this orientation alternates in turn. As the location of the band
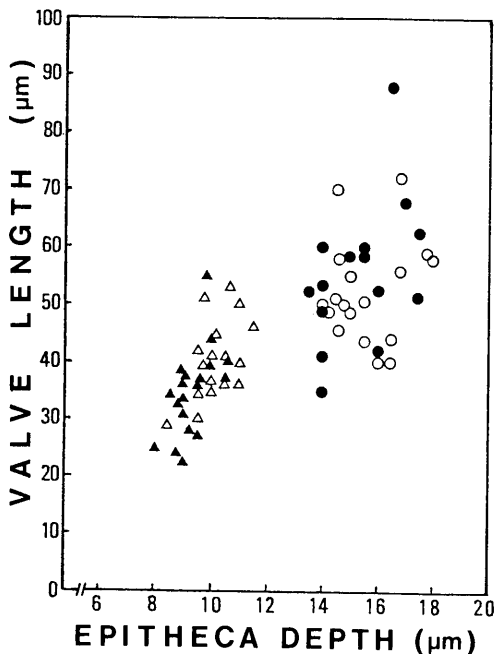

Fig. 26. Plot of valve length versus epitheca depth in *Eunotia arcus* var. *arcus* (●: topotype material, ○: K-5865) and *E. arcus* var. bidens (▲: K-5865, △: K-5879).

becomes further from the valve, the band width becomes narrower. Therefore, bands 5 and 6 are barely visible in the middle portion, but they are clearly visible at the widened band ends (Figs. 23, 25). Cingula with five bands are abundant in both Swedish and Japanese materials. The frequency for the former is 25% (four bands), 65% (five bands) and 10% (six bands) among 20 frustules examined, and in the latter it is 18% (four bands), 60% (five bands) and 22% (six bands) among 50 frustules examined. There is no firm relationship between the valve length and the band number, but there is a tendency for longer valves to have many bands.

The relationship between valve length and epitheca depth is shown in Fig. 26. Because the edge of the outermost band is not straight (Fig. 23), we have measured the depth at both the apices and the center and plotted the average. The epitheca depth is 13.7–17.5 μm in the Swedish material (closed circle) and this range agrees with that of the Japanese

materials of 14.2–18.0 μm (open circle).

To be emphasized is that the epitheca depth is a reliable taxonomic characteristic. This parameter is confirmed to be stable by us in *E. arcus* var. *arcus* (Fig. 26, circles) and var. *bidens* (Fig. 26, triangles). As is seen in Fig. 26, changes in the epitheca depth are markedly slight when compared with those in the valve length. There is a slight tendency for the epitheca depth to become shorter as the valve length decreases. This tendency is recognized in the nominate variety also, but it is more obvious in var. *bidens*. The ranges of valve length partly overlap in var. *arcus* and var. *bidens*. The longer valve of var. *bidens* is rather similar in appearance to the shorter valves of var. *arcus* because of their less undulated dorsal side (Figs. 1A, 1B). However, the difference in epitheca depth clearly classifies them into two taxa (Fig. 26).

Acknowledgements

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真山茂樹*・小林　弘**：羽状珪藻 *Eunotia* の属のタイプ種である *Eunotia arcus* Ehr.
var. *arcus* の観察

　スウェーデン自然史博物館所蔵の P. T. Cleve のコレクションの中の *Eunotia arcus* var. *arcus* の同地基準標本試料を，走査型および透過型電子顕微鏡を用いて観察した。この試料には初生殻を含む，さまざまな大きさと形の殻が含まれており，本種の無性生殖期における安定した形質を探ることができた。それらは，殻の外・内表面構造，条線密度，パターンセンターと殻面殻套接合線の位置関係，縦溝の位置，唇状突起の位置，胞紋構造，胞紋密度，上半被殻の深さであり，これらの形質は本邦産の個体群においても安定した形質であった。(*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，**184 東京都小金井市本町3-8-9-813　東京珪藻研究所)