

## Morphology of *Eunotia multiplastidica* sp. nov. (Bacillariophyceae) Examined throughout the Life Cycle

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A moss diatom, *Eunotia multiplastidica* was newly described from Ehime Pref, Japan. This species is peculiar among the *Eunotia* species because of its possession of many discoid chloroplasts in a protoplast. In culture, sexual reproduction occurred and the elongated initial cells and the post-initial cells were collected. The detailed observation of the initial valve was carried out using SEM and, simultaneously, a comparative morphology examining vegetative valves both before and after sexual reproduction was also done. Though the initial valve was rounded in cross section, it shared many structures with the vegetative valves. The stable characteristics in the vegetative valves throughout the life cycle were the location of the pattern center and raphe, the striae density, the structure of the areola, the areola density, the number and location of the labiate process and the epitheca depth.

**Key Words:** diatom, *Eunotia*, fine structure, initial valve, life cycle, taxonomy

### INTRODUCTION

Morphological variations of the *Eunotia* species have been described by many authors in field materials. Some authors examined the variations using cultured materials (Wahrer, 1981; Steinman and Sheath 1984). However, these materials lacked the stage of sexual reproduction. Studies aimed at the morphological stability or changes throughout the life cycle have been very scarce, though Geitler (1932) examined the changes of the valve shape and length of *Eunotia formica* throughout the life cycle. Mayama and Kobayasi (1991) studied the stable structures in *Eunotia arcus* Ehr. var. *arcus* using SEM, but their material was not a cultured population but a fossil population including initial valves.

There have been few studies of the chloroplast number and shape in *Eunotia*. It seems that many diatomists have not been interested in the chloroplast in taxonomy at all or that they have considered all the species of the genus to have two plate

like chloroplasts as described by Hustedt (1932) and Patrick and Reimer (1966). The only one exception has been Geitler (1958, 1959, 1973), who reported the species with many chloroplasts, i.e. *Eunotia pectinalis* var. *polyplastidica* and *E. robusta* var. *tetraodon*.

In this study, *Eunotia multiplastidica* sp. nov. was described and its valve morphology before and after sexual reproduction was examined using specimens cultured.

### MATERIALS AND METHODS

Wet moss was obtained from Sainokawa, Ehime Pref., Japan, on 9 October 1988 (Sample number: K-6921). The moss sample was put in a test tube with a small amount of water and then the diatoms were detached from the moss surface by firmly squeezing a pipette repeatedly. A drop of diatom suspension was pre-incubated in a medium in a Petri dish for 7 days, and then cells were isolated using capillary pipettes and maintained as a unialgal culture (Culture number: A22). The medium I

used was Bold's Basal Medium (Bischoff and Bold, 1963), to which was added 50 mg  $\text{Na}_2\text{SiO}_3 \cdot \text{H}_2\text{O}$  per liter, and diluted with distilled water to one-fifth strength, adjusted to pH 6.8. The culture was maintained at 20°C under cool white fluorescent light of 1,500-2,500 lux on a 12:12 (L:D) photoperiod.

The cleaning methods for the diatom sample are described by Mayama and Kobayasi (1986) and Nagumo and Kobayasi (1990). Cleaned and dried diatom samples were mounted on slides using Naphrax for light microscopy and were coated with gold-palladium on cover-slips for scanning electron microscopy. JEOL F15 was used for SEM observations at an accelerated voltage of 15 KV.

## DESCRIPTION

### *Eunotia multiplastidica* sp. nov. (Figs. 1-30)

Frustula rectangular in aspectu cingulari. Valvae margine ventrali leviter concava, margine dorsali convexa et lecta in media parte valvae longioris, apicibus leviter protractis et rotundatis, circa 9-46  $\mu\text{m}$  longae, 6-8  $\mu\text{m}$  latae. Striae transapicales parallelae, 14-17 in 10  $\mu\text{m}$ . Chloroplasti discoidi vel elliptici et multi, circa 10 per celluam.

Holotype: H.K.T.-100. this slide will be housed in the Nat. Sci. Mus. Tokyo (TNS).

Type locality: Sainokawa, Ehime Pref., Japan.

Frustules rectangular in girdle view. Ventral margin of the valve slightly concave. Dorsal margin of the valve convex but straight in the center of the longer valves. Ends of the valve slightly protracted and rounded, about 9-46  $\mu\text{m}$  long and 6-8  $\mu\text{m}$  wide. Transapical striae parallel, 14-17 in 10  $\mu\text{m}$ . Chloroplasts discoid or ellipsoid, and numerous, about 10 per cell.

## RESULTS

**LM observations:** The cells of *Eunotia multiplastidica* were about 15-20  $\mu\text{m}$  long with 10 discoid chloroplasts at the beginning of culture (Figs. 1, 4). After 7-14 days, the population of the culture began sexual reproduction in which a single auxospore was formed from two gametangial mother cells (Fig. 3). The length range of the mother cells

is 13-17  $\mu\text{m}$  and the shorter (Fig. 5) or longer specimens outside of these ranges did not become gametangial cells. All chloroplasts in each gametangial mother cell migrated into a zygote. Completely elongated auxospores were three to four times longer than the gametangial mother cells. After initial valve was formed beneath the auxospore casing, a series of vegetative valves was formed by ordinary cell division and along this course of asexual reproduction the size became reduced and the shape changed gradually (Figs. 6-8). The initial cell contained 20 discoid chloroplasts but post-initial cells contained 10 chloroplasts, the same as in the cells before sexual reproduction (Fig. 2).

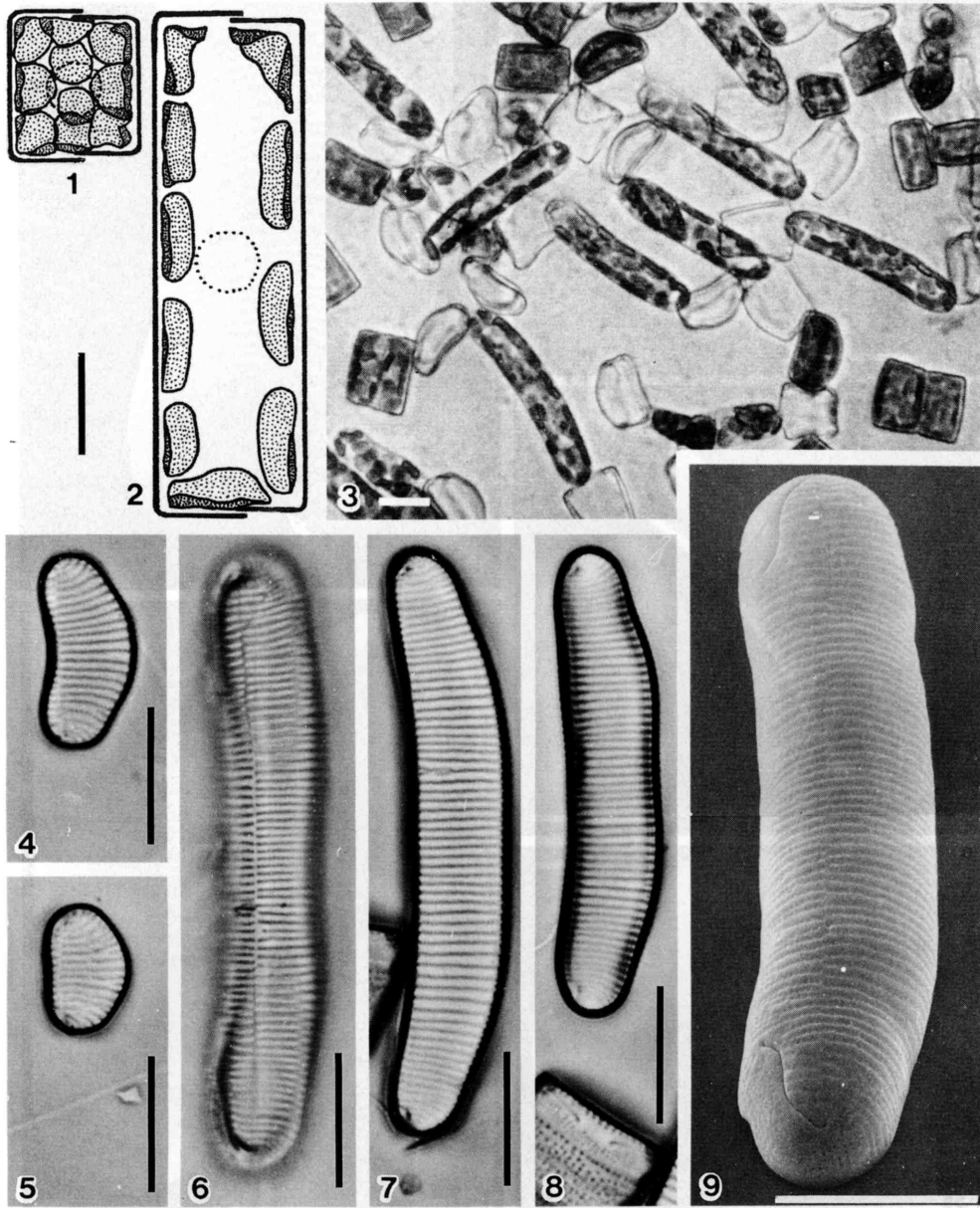
**SEM observations:** Initial valves produced after auxospore formation in the culture are presented in Figs. 9-15. The valve is rounded in cross section and the valve face can not be distinguished from the valve mantle. The valve ends have a semidome-like form. Rounded indentations in the margin have been found in some valves (Fig. 10, arrows). These indentations have always been formed within the raphe branch stretches.

The pattern center of the initial valve runs almost straight between the external polar raphe endings along the apical axis in the ventral side (Figs. 6, 9-11).

Externally, the raphe branches are sigmoid. The terminal raphe endings are located on the apical axis and slightly curved toward the valve center (Figs. 9, 11). The external central raphe endings are slightly inflated (Fig. 9).

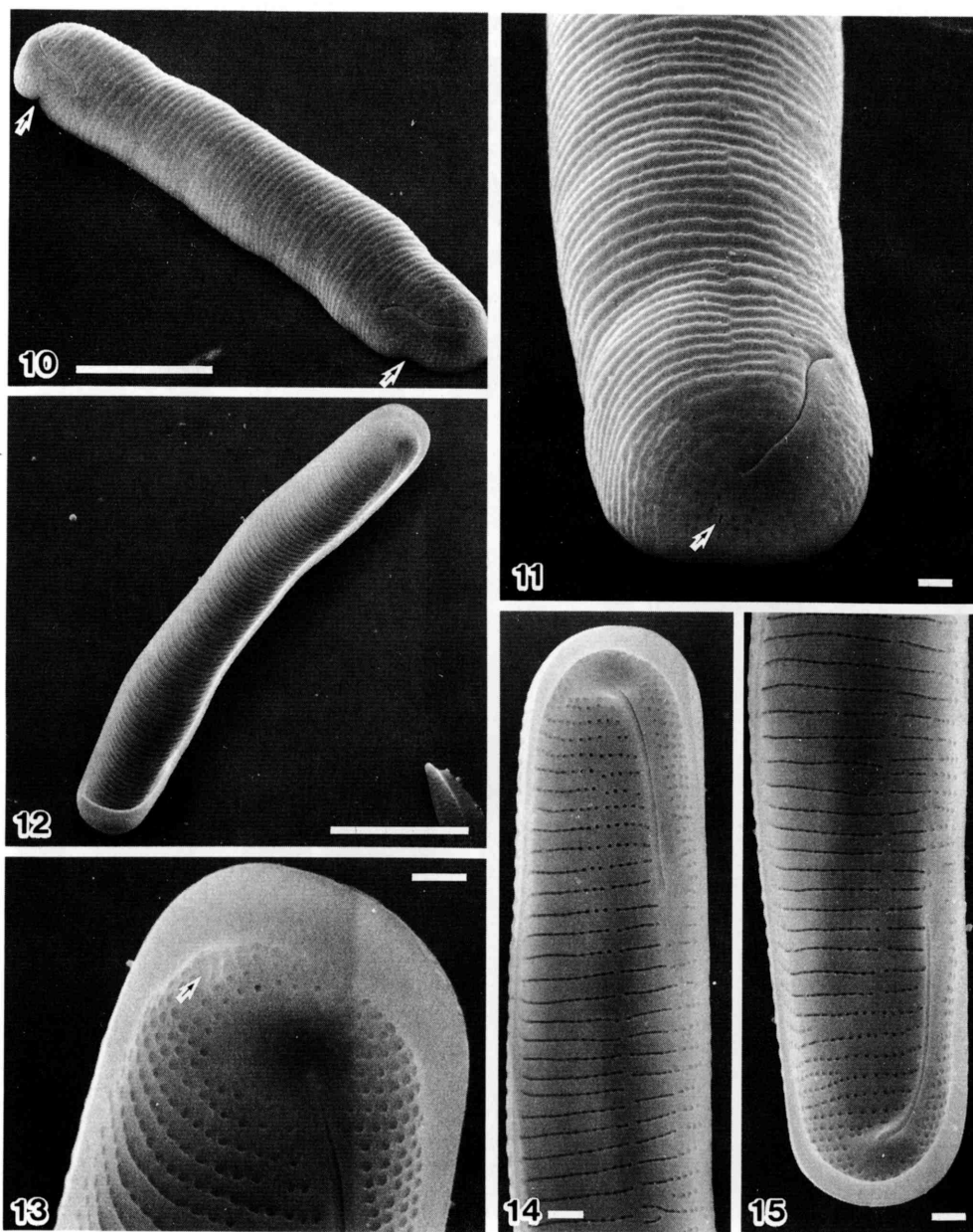
The interstriae are arranged regularly in almost all parts of the valve, though they become slightly denser toward the end. Striae are composed of single row of areolae. Near the center of the valve, the areolae in each stria are about 38 in 10  $\mu\text{m}$ .

Internally, the initial valve has a labiate process in one apex, which is always on the top side, when the valve inside is oriented as in Fig. 12. Though the labiate process is too small to be detected in the front view (Fig. 14), it can be seen in the oblique view (Fig. 13, arrow), and the location is in the dorsal side of the apex. The outer opening of the labiate process is a short slit and is clearly differentiated from the areolar openings with occlusions (Fig.



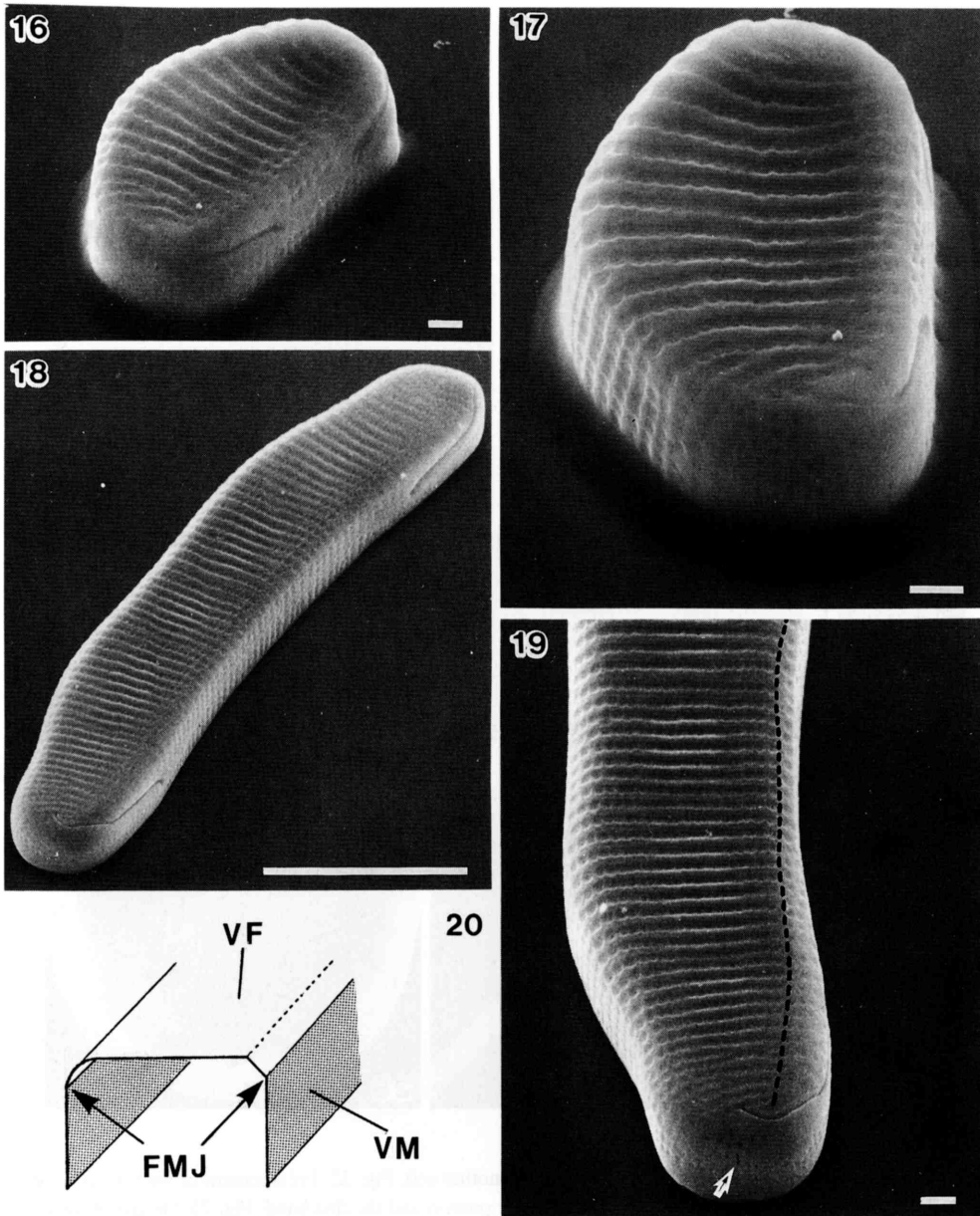
**Figs. 1-9.** *Eunotia multiplastidica* sp. nov.

Fig. 1. Gametangial mother cell with 10 chloroplasts. Fig. 2. Post-initial cell with 10 chloroplasts. Fig. 3. Auxospore formation in a unialgal culture. Figs. 4-8. Variation of the valve size and shape during a life cycle. LM. Figs. 4, 5. Valves produced before sexual reproduction. Fig. 4. Valve of gametangial mother cell. Fig. 5. Valve of the cell smaller than the gametangial mother cells. Figs. 6-8. Valves produced after sexual reproduction. Fig. 6. Initial valve with an almost straight pattern center. Figs. 7, 8. Post-initial valves. Fig. 8. Holotype specimen. H.K.T.-100. Fig. 9. External oblique view of an initial valve showing a rounded valve face and sigmoid raphe branches. SEM.



**Figs. 10-15.** *Eunotia multiplastidica* sp. nov.

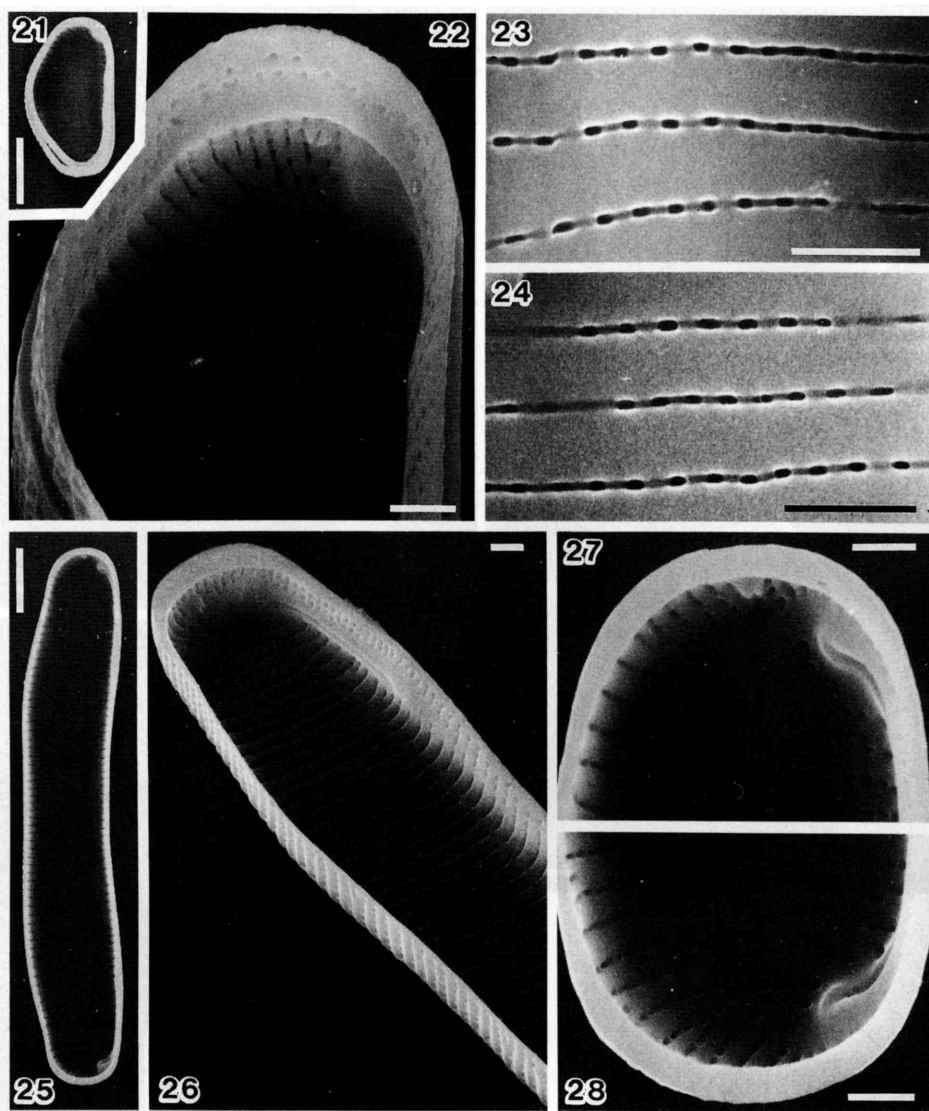
Fig. 10. External oblique view of an initial valve from the ventral side showing raphe branches and the indentations beneath them (arrows). Fig. 11. Top end of the valve in Fig. 9. showing the outer opening of the labiate process (arrow). Fig. 12. Internal oblique view of an initial valve. Fig. 13. Details of the top end of the valve in Fig. 12 in oblique view showing the labiate process (arrow). Figs. 14,15. Enlargement of both ends of the valve in Fig. 12 showing the pattern center running along the apical axis. Scale bars = 1  $\mu$ m (Figs. 11, 13-15) or 10  $\mu$ m (Figs. 10, 12).



**Figs. 16-19.** *Eunotia multiplastidica* sp. nov.

Figs. 16, 17. External oblique view of a gametangial valve from different angles showing the pattern center and polar raphe endings. Fig. 18. External oblique view of a post-initial valve. Fig. 19. Details of the bottom end of the valve in Fig. 18 showing the outer opening of the labiate process (arrow), the polar raphe ending, the rounded margin of the valve face and the location of the pattern center (broken line). Scale bars = 1  $\mu\text{m}$  (Figs. 16, 17, 19) or 10  $\mu\text{m}$  (Fig. 18).

**Fig. 20.** Diagrammatic drawings defining the locations of valve face (VF), valve mantle (VM) and valve face and mantle juncture (FMJ).



**Figs. 21-28.** *Eunotia multiplastidica* sp. nov.

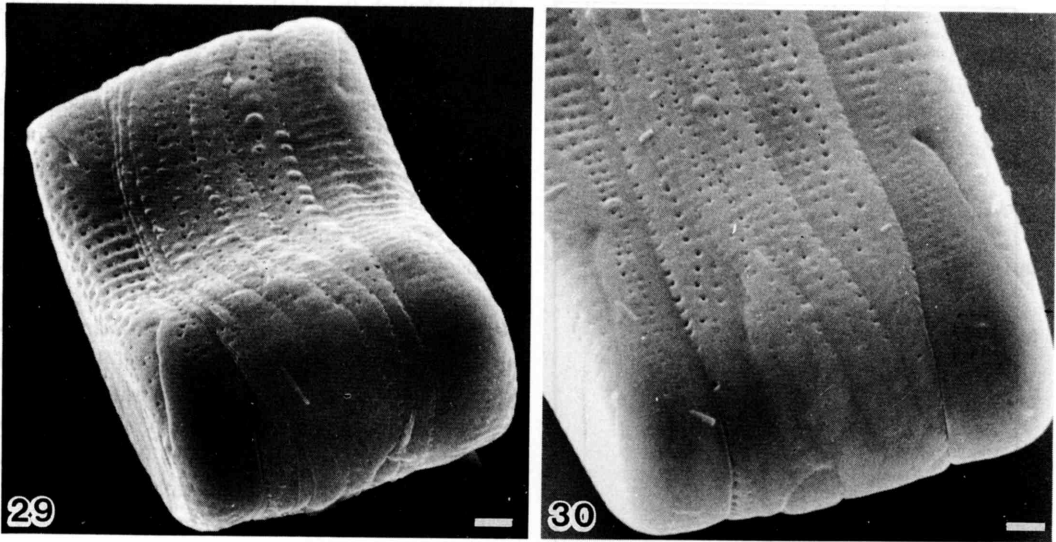
Fig. 21. Internal view of a whole valve of gametangial mother cell. Fig. 22. Enlargement of the top end of the valve in Fig. 21 in oblique view showing helictoglossa, labiate process and the first band. Fig. 23. Details of the valve center in Fig. 21 showing areolar openings aligned in narrow ditches. Fig. 24. Details of the valve center in Fig. 25 showing areolar openings aligned in narrow ditches. Fig. 25. Internal view of a whole valve of post-initial cell. Fig. 26. Enlargement of the top end of the valve in Fig. 25 showing the slightly thickened hyaline area surrounding the raphe branch. Figs. 27, 28. Enlargement of both ends of the valve in Fig. 25 showing the helictoglossae and the labiate process in one apex. Scale bars = 1  $\mu\text{m}$  (Figs. 22-24, 26-28) or 10  $\mu\text{m}$  (Figs. 21, 25).

11, arrow).

The helictoglossae of the initial valve are located on the apical axis a little distance from the apices. The raphe branches starting from the helictoglossae are slightly curved, and the areas surrounding them

are slightly raised (Figs. 12-15).

Externally, each of the areolae of the initial valve is occluded by a thin siliceous layer (Figs. 9, 11). Internally, the openings are arranged in the narrow ditches in the main body, though the ditches disap-



Figs. 29, 30. *Eunotia multiplastidica* sp. nov.

Fig. 29. Oblique ventral view of gametangial frustule showing the epicingulum composed of seven open bands with alternating open and closed ends. Fig. 30. Details of the frustule end of the post-initial cell showing the epicingulum composed of four open bands with alternating open and closed ends. Scale bars = 1  $\mu$ m.

pear at the end (Figs. 12-15).

The valves of gametangial mother cells (Figs. 16, 17, 21-23, 29) and those of post-initial cells produced after sexual reproduction (Figs. 18, 19, 24-28, 30) are presented to compare the fine structure. They are representatives of shorter and longer specimens and show which structure is stable and which one is variable throughout the life cycle.

Externally, the valve faces are flat in the main part but rounded or slightly bent near the valve face and mantle juncture (FMJ) in both ventral and dorsal sides in both gametangial and post-initial valves (Figs. 16-19). Here, I propose new definitions of this term or FMJ with restricted meanings to express the siliceous structures. I restrict this term to mean just a line, so that the meanings of the valve face and the valve mantle are also confined (Fig. 20). Namely the valve face is expressed as the plane visible only from a front view, even if the margin of the face is sometimes rounded or obtusely bent. The valve mantle is confined to a meaning of a plane perpendicular to a valve plane without any rounded or obtusely bent area.

Each terminal of the pattern center is located at a point slightly off the external polar raphe ending toward the ventral side. The pattern center runs

parallel to the FMJ, because there are single or double transapical rows of areolae between them (Figs. 16-19).

The external polar raphe endings are located on the apical axis in the post-initial valves but the locations are shifted slightly toward the ventral side in the gametangial valves (compare Figs. 16, 17 and Figs. 18, 19). Both the polar and central endings are slightly inflated. The raphe branches in the mantle are almost straight and the central endings do not bend strongly toward the mantle margin (Figs. 16-18) as seen in the initial valves (Figs. 9-11).

The interstriae are linear costa and there is no bifurcation in the valve faces of the longer valves but the shorter valves occasionally have some bifurcations on the ventral side of the face because of the insertion of the stria from the mantle margin (Figs. 4, 5, 17). Areolae composing striae are 34-38 in 10  $\mu$ m and the number of areolae in the initial valve is also within this range.

Each valve has a labiate process in the center of the apical mantle (Figs. 22, 26, 27). When the valve insides are oriented as in Figs. 21, 25, namely with the dorsal side to the left and the ventral side to the right, the labiate process is located on the top and

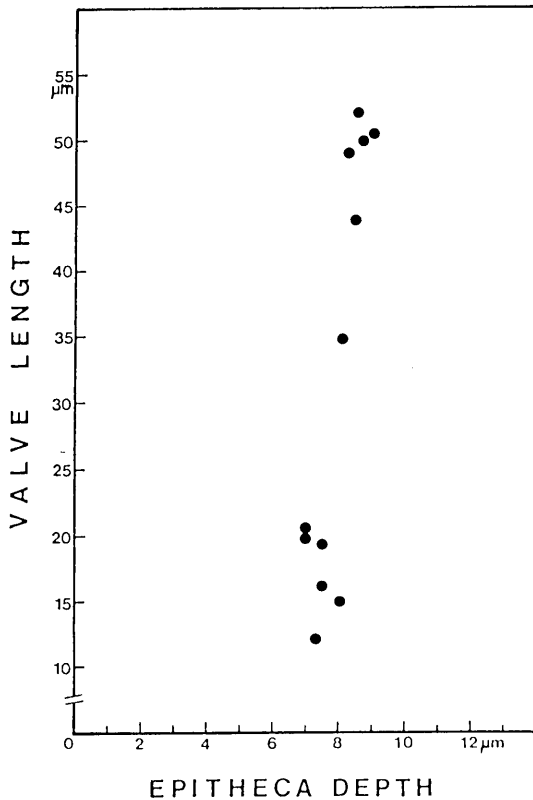


Fig. 31. Correlation between the valve length and the epitheca depth showing the stability of the latter throughout the life cycle of *Eunotia multiplastidica* sp. nov. in culture.

the bottom has no labiate process (Figs. 27, 28). The outer opening of the labiate process is a short slit (Fig. 19).

The helictoglossae are located midway between the labiate process and the ventral mantle. They are situated on the FMJ in the gametangial valves (Figs. 21, 22), but in the longer valves, it is on the valve face close to the FMJ (Figs. 25, 27, 28). Therefore, the raphe and terminal nodules of the shorter valves can not be observed in light microscopy (Figs. 4, 5).

Areolae have occlusions at the outer openings in both shorter and longer valves (Figs. 17, 19). The level of the opening is slightly lower than that of the interstriae. The inner openings are arranged in the narrow ditch in the main body (Figs. 23, 24) as seen in *Eunotia sparsistriata* Mayama (Mayama,

1993), though the ditches become obscure at the ends (Figs. 22, 26).

The epicingulum consists of 4-8 open bands, with the open and closed ends arranged alternately (Figs. 29, 30). The closed end of the first band is attached to the theca end with the labiate process. The bands after the 4th are very narrow and they can be seen well only at the end of the frustule. The epitheca depth described by Mayama and Kobayasi (1991) is 7-9 μm throughout the life cycle (Fig. 31). As the edge of the outermost band is not straight (Figs. 29, 30), I have measured the depth at both the apices and the center and plotted the average. The shorter valves tend to become slightly shallower than the longer valves in the epitheca depth, though the former tends to have more bands than those of the longer.

## DISCUSSION

Hustedt (in Schmidt, 1933) originally described *Eunotia similis* from Java. The valve shape is similar to *E. multiplastidica*, but he neither gave dimensions nor referred to chloroplasts. My research of the lectotype slide (Hustedt's collection, L3/52, Java, Gunung Lawn, Quelle, and Moos, BRM) designated by Simonsen (1987) confirms that the valve width of *E. similis* is 4-5 μm and narrower than of *E. multiplastidica*, and the ventral and the dorsal sides are parallel even in the smaller valves in the former species.

This species resembles the longer valves of *Eunotia arcus* var. *bidens* Grun. Mayama and Kobayasi (1991) reported the epitheca depth of the former to be 8-12 μm. It is a little deeper than that of *E. multiplastidica*. But both ranges of the epitheca depth partly overlap. This species is also similar to some forms of *Eunotia pectinalis* var. *minor* Kütz. However, *E. multiplastidica* can be clearly distinguished from these two similar species, because this species has many chloroplasts.

In the sexual reproduction of *Eunotia multiplastidica*, a single auxospore is produced from two gametangial mother cells. This mode of sexual reproduction is the same as some *Eunotia* species, i.e. *E. arcus*, *E. flexuosa* and *E. pectinalis* var. *polyplastidica*, described by Geitler (1951a, b, 1958, 1973). In these reports, he observed also the behav-



ior of chloroplasts accompanying sexual reproduction. The behavior of chloroplasts in *E. multiplastidica* is similar to that of *E. flexuosa* in the fact that all chloroplasts contained in the gametangial mother cell are used for the auxospore formation. The feature of the cell having many discoid chloroplasts is stable throughout the life cycle of *E. multiplastidica* and seems to be an important characteristic of this species. The sexual reproduction of this species will be discussed more fully in a future publication.

The fine structure of the initial valve has been studied in *Eunotia arcus* var. *arcus* by Mayama and Kobayasi (1991). The morphology of the initial valve of *E. multiplastidica* is almost the same as *E. arcus*. A little difference is that *E. multiplastidica* has slight constrictions of both ventral and dorsal sides near the valve ends but *E. arcus* has no constriction. In the latter species, both sides are parallel throughout the valve length. As seen in *E. arcus*, the striae density (17 in 10  $\mu\text{m}$ ) and the areola density in the initial valves are also within the ranges of the vegetative valves in *E. multiplastidica*. In this study, some initial valves have indentations in the valve margin within the raphe branch stretches (Fig. 10, arrows). The shape of the indentations seems to correspond to the indentations as seen in the later stages of the valve development of *Achnanthes minutissima* var. *saprophila* H. Kobayasi and Mayama (Mayama and Kobayasi 1989, Figs. 5, arrows, 12, 13, arrows). The valve development of *Eunotia* is still unclear, however, if it can be supposed that formation of the raphe branch of *Eunotia* is similar to the formation of the raphe branch of *Navicula* and *Achnanthes*, the indentation on the margin beneath the raphe branch seems to be homologous to the Voigt fault formed during the valve development of the biraphid (Chiappino and Volcani, 1977; Schmid 1979) and the monoraphid (Mayama and Kobayasi, 1989) diatoms.

The structures of the initial valve exterior and interior are quite similar to those of ordinary vegetative valves except for the locations of the pattern center and the raphe branches. Mayama and Kobayasi (1991) compared the structures between both initial and post-initial valve exteriors in *Eunotia arcus* and described a resemblance of those

structures. The structural resemblance between the initial valve and the ordinary vegetative valve is also likely to be found in the other *Eunotia* species not examined yet. This resemblance seems to be valuable for an analysis of the morphological valve variation occurring in the life cycle, because the initial valve can be identified in comparison with the post-initial valves without observation of auxospore formation.

The stable characteristics throughout the life cycle examined in *Eunotia multiplastidica* are the location of the pattern center and raphe, the striae density, the external and internal structures of the areola opening, the areola density, the number and location of the labiate process, the epitheca depth and the shape and number of chloroplasts. The stability of these structures agrees well with the results reported in *Eunotia arcus* by Mayama and Kobayasi (1991), though the two materials they used were living field material without initial cell and also fossil material with initial valve. The valve shape of the *Eunotia* species is extremely variable, however, the structures mentioned above are available for the taxonomic characteristics in *E. multiplastidica* and *E. arcus*. Further evaluation of these characteristics is expected in other *Eunotia* species.

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