Valuable taxonomic characters in the valve mantle and girdle of some *Eunotia* species

Shigeki Mayama[1]

Dedicated to Prof. Dr. Dr. h.c. Horst Lange-Bertalot on the occasion of his 65th birthday

Abstract

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Throughout their life cycle the stability of some characters of the valve mantle and girdle were examined by scanning electron microscopy of cultures and field populations of some *Eunotia* species, i.e., *E. arcus* var. *arcus*, *E. arcus* var. *bidens*, *E. indica*, *E. multiplastidica* and *E. tropica*. Areola numbers in two areas of the mantle proved to be stable and useful taxonomic criteria. One is the areola number in the area between the juncture of valve face and mantle and the middle of raphe branch on the mantle, and the other is the number of areolae between the middle of raphe branch on the mantle and the mantle margin. Epitheca depth is also a stable and valuable character regardless of the number of the bands. Moreover, the shape of epibands can be also considered as a useful character.

Introduction

Taxonomic criteria of pennate diatoms have usually been recorded from valve view in the light microscope, although some characteristics in girdle view have been used, e.g., septa. The scanning electron microscope (SEM) has clarified fine structures not only of the valve but also of the girdle; some authors have indicated taxonomic significance in the girdle bands as well as the valve (Krammer 1981, Williams 1985, 1986, Williams & Round 1986, 1987, Kobayasi & Kobori 1990).

The basic structure of the frustule in *Eunotia* is different from most other pennate genera, as most of the raphe is located on the valve mantle. In addition, the frustule in the girdle view is much wider than that in the valve view. Therefore, the valve mantle and girdle structures of *Eunotia* may be valuable as a potential source of information. However, their use has been neglected in the taxonomy of this genus.

Prior to this study, 30 *Eunotia* taxa had been observed, and useful characteristics were surveyed regarding the length and the areola number in certain areas of the mantle and girdle (unpublished data). However, to be adopted as taxonomic criteria, it is necessary to examine the stability of the structures throughout the life cycle. Although the valve shape of *Eunotia* is extremely changeable, e. g., *Eunotia formica* Ehrenberg (Geitler 1932),

[1] Department of Biology, Tokyo Gakugei University, Koganei-shi, Tokyo 184, Japan
e-mail: mayama@u-gakugei.ac.jp

Eunotia arcus Ehrenberg (Mayama & Kobayasi 1991, Mayama 1995a), *Eunotia multiplastidica* Mayama (Mayama 1992), and *Eunotia tropica* Hustedt (Mayama 1995b), in the valve face the areola density and the location of the pattern center are stable throughout the life cycle (Mayama & Kobayasi 1991, Mayama 1992).

In this study, three taxonomic characteristics were evaluated throughout the life cycle using SEM from cultured populations, which underwent both sexual and asexual phases. In addition, evaluations were performed on field populations composed of cells of various sizes, including auxospores and initial valves, and also on strains cultured for a long period.

Material and Methods

The materials examined are listed as follows:

(1) *Eunotia arcus* Ehrenberg var. *arcus*: a fossil sample from Cleve's collection 247, Degernas in Sweden, housed in the Swedish Museum of Natural History, Stockholm, H. Kobayasi's personal collection number = K-6686 (Fig. 1). This sample contains initial cells and various sized vegetative cells generated during the life cycle.

(2) *Eunotia arcus* var. *bidens* Grunow: an epiphytic sample from Saino-ko (Saino Pond), Tochigi Pref., Japan, K-5865 (Fig. 2). This sample already contained auxospores and initial cells in the field population.

(3) *Eunotia bidens* Ehrenberg: an epipelic sample from a bog in Nyugasa-yama (Nyugasa Mt.), Nagano Pref., Japan, K- 6958 (Fig. 3). A clone culture (strain number A29) was established from K-6958 and maintained for 18 months, but auxosporulation did not occur.

(4) *Eunotia bigibba* Kützing: a sample from submerged dead leaves in a small puddle, Okayama Shinrin Park, Okayama Pref., Japan, K-6946 (Fig. 4). A unialgal culture (A25) was established from K-6946 and maintained for 6 months without auxosporulation.

(5) *Eunotia biseriatoides* H. Kobayasi et al.: a sample from moss sprayed by falling water, Kumaoshi Falls, Okayama Shinrin Park, Okayama Pref., Japan, K-6492 (Fig. 5). A unialgal culture was established from this sample and maintained for 6 months without auxosporulation.

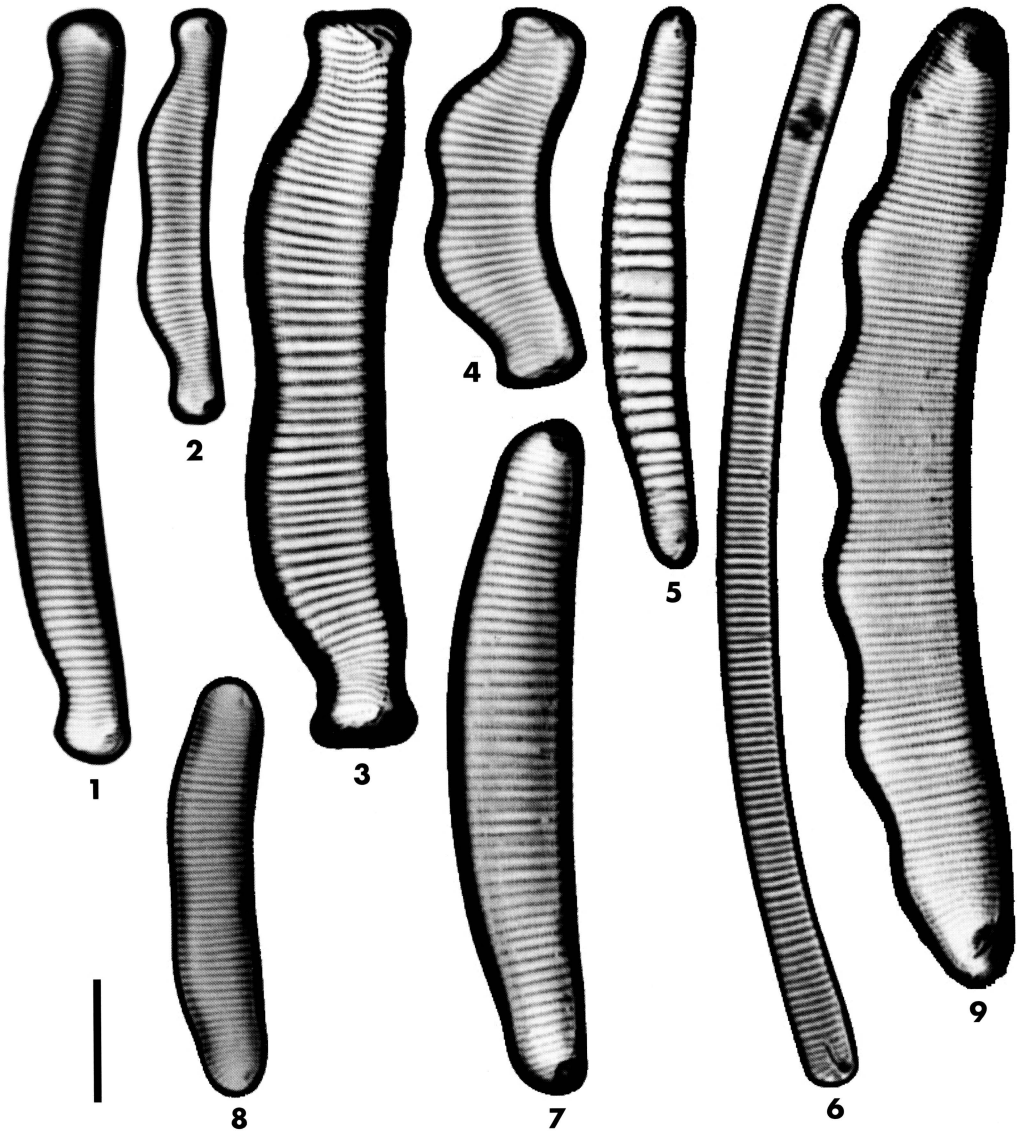
(6) *Eunotia curvata* var. *linearis* (Okuno) H. Kobayasi et al.: an epiphytic sample from Suge-numa (Suge Pond), Gunma Pref., Japan, K-6525 (Fig. 6). A clone culture (A01) was established from K-6525 and maintained for 18 months without auxospore formation. The valve length had reduced from 155 μm (max.) to 25 μm (min.) during this period.

(7) *Eunotia indica* Grunow: a sample from a goldfish tank used for a personal hobby, Koganei-shi, Tokyo, Japan, S. Mayama's personal collection number = M-0129 (Fig. 7).

(8) *Eunotia multiplastidica* Mayama: a sample from wet moss in Sainokawa, Ehime Pref., Japan, K-6921. A unialgal culture (A22) was established from K-6921 (Fig. 8). After 7-14 days, the population of the culture began sexual reproduction.

(9) *Eunotia tropica* Hustedt: an epipelic sample from Fuse-tameike (Fuse Reservoir), Shiga Pref., Japan, K-6476 (Fig. 9). A unialgal culture was established from K-6476. After 14-21 days, the population of the culture began sexual reproduction.

The culture medium used was Bold's Basal Medium (Bischoff & Bold 1963), to which was added 50 mg $NaSiO_3H{\cdot}9H_2O$ per liter, and diluted with distilled water to one-fifth strength, adjusted to pH 6.8. The cultures were maintained at 20°C under cool white fluorescent light of 1500-2500 lux on a 12:12 (L:D) photoperiod. The samples were cleaned by ultraviolet radiation as given in Mayama & Kobayasi (1986). Almost all the frustules of *Eunotia* could remain completely intact with this cleaning method, so that it was useful for external observations of the valve and frustule. Cleaned and dried specimens were coated with gold-palladium on cover-slips. Jeol F15 was used for the SEM observations.



Figs 1-9. *Eunotia* species observed in this study.
Fig. 1. *E. arcus* var. *arcus*. Fig. 2. *E. arcus* var. *bidens*. Fig. 3. *E. bidens*. Fig. 4. *E. bigibba*. Fig. 5. *E. biseriatoides*. Fig. 6. *E. curvata* var. *linearis*. Fig. 7. *E. indica*. Fig. 8. *E. multiplastidica*. Fig. 9. *E. tropica*. Scale bar = 10 μm.

Results

Definitions with restricted meanings are necessary for some terms in order to explain new criteria. One is the juncture of valve face and mantle. This term has already been defined for *E. multiplastidica* (Mayama 1992), and this definition can be adapted to all *Eunotia* species. As the juncture of valve face and mantle is defined to mean just a line, the meaning of the valve face and the valve mantle are also confined. Namely, the valve face is defined as the plane visible only from top view (Fig. 10A), even if the margin of the valve face is somewhat bevelled (Fig. 10B) or rounded (Fig. 10C). Consequently, the valve mantle is defined as a plane perpendicular to a valve plane without any rounded or bevelled area.

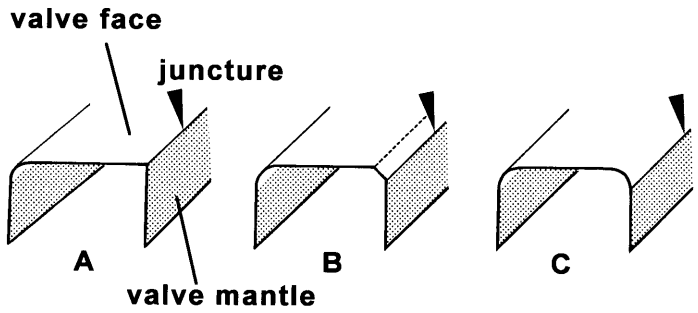


Fig. 10. Diagrammatic drawings defining the locations of the valve face, the valve mantle and the juncture of valve face and mantle (arrow head) in various valve sections. A: Valve face connecting with the mantle at a right angle in one side of the valve. B: Valve face with bevelled margin on one side. C: Valve face with rounded margin on both sides.

It is not easy to measure the length of a particular area of the valve mantle, even using SEM. In this study, the areola numbers were applied as a parameter to express dimensions in the valve mantle, and their stability was assessed in two limited areas. One is the areola number in the area between the juncture of valve face and mantle and the middle of raphe branch on the mantle (Fig. 11), and the other is that of the area between the middle of the raphe branch on the mantle and the mantle margin. When two striae consisting of different numbers of areolae were present in these limited areas, the average was used. The areola number counted in these two areas showed noticeable stability in the cultured population of *E. tropica* (Fig. 12A), which had completed its life cycle via sexual reproduction. The shortest valve was a gametangial mother cell 49 μm long, and the longest one was a first vegetative cell 189 μm long. The post-initial cell was more than three times as long as the gametangial mother cell, so that the range of length was very wide. However, the areola number between the juncture of valve face and mantle

and the middle of raphe branch on the mantle was always 3 or 4 and the number between the middle of raphe branch on the mantle and the mantle margin was 6-8 throughout the life cycle.

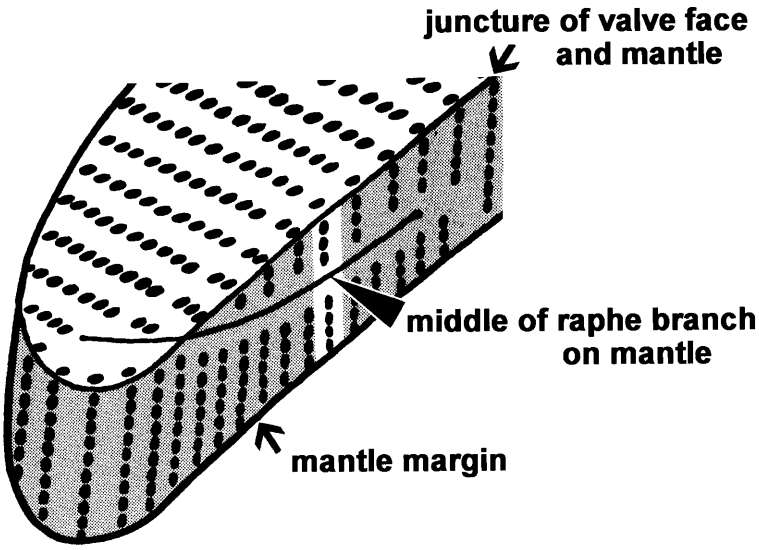


Fig. 11. Diagrammatic drawing showing the location of the juncture of valve face and mantle, the middle of raphe branch on the mantle and the mantle margin.

E. arcus also showed stability in the areola number between the juncture of valve face and mantle and the middle of raphe branch on the mantle, which was 1.5–2.5 (rarely 3) throughout the life cycle (Fig. 12B), but the number between the middle of raphe branch on the mantle and the mantle margin was variable, i.e., 7–11 areolae. Stability was also found in the cultured population of *E. curvata* var. *linearis* (Fig. 12C). In this species, there were no areolae between the juncture of valve face and mantle and the middle of raphe branch on the mantle, and 1-2 areolae were observed between the middle of raphe branch on the mantle and the mantle margin.

The areola numbers were also examined in other limited areas, i.e., the section between the juncture of the valve face and mantle and the central raphe ending and the section between the central raphe ending and the mantle margin. However, I found no stability in the areola number in these areas, because the areolae around the central raphe ending were often filled by excessive silica deposition and hence disappeared.

As is summarized by Round et al. (1990), the girdle width changes during the cell cycle. It is narrowest just after cell division, but widest just before cell division. However, the depth of the epitheca does not change throughout the cell cycle, because new bands can only be added to a hypotheca.

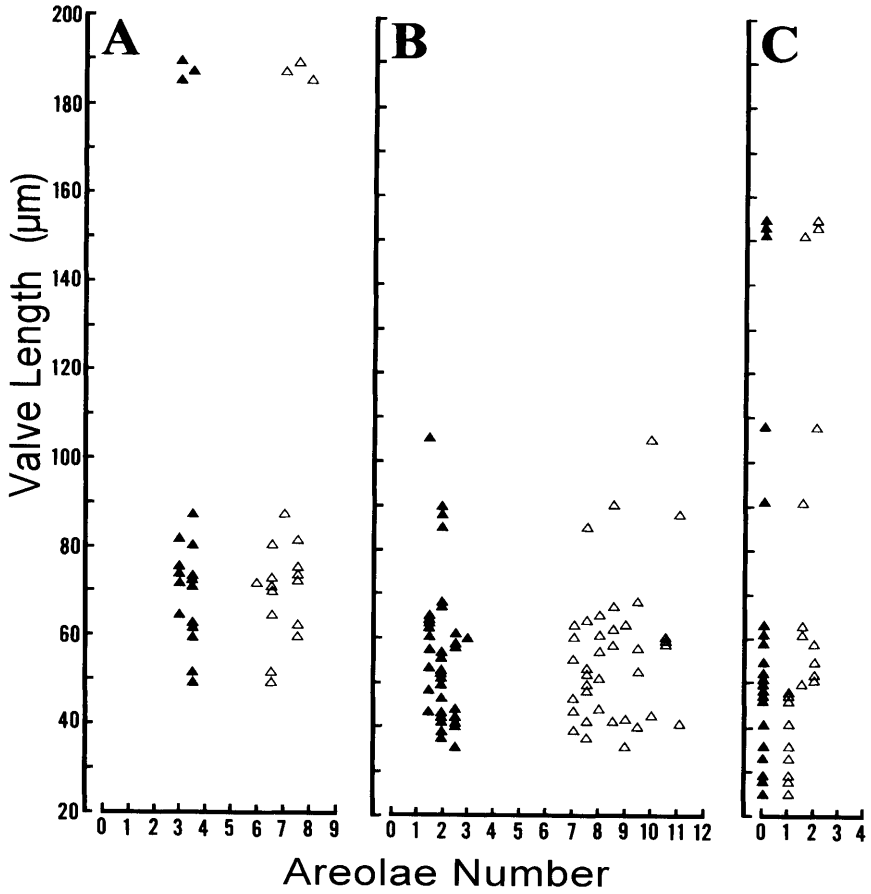


Fig. 12. Correlation between the valve length and the number of areolae showing the stability of the latter. A: *E. tropica*. B: *E. arcus* var. *arcus*. C: *E. curvata* var. *linearis*. Open triangle: areola number between the juncture of valve face and mantle and the middle of raphe branch on the mantle. Closed triangle: areola number between the middle of raphe branch on the mantle and the mantle margin.

The relationships between the valve length and the epitheca depth in the populations of four different species are shown in Fig. 13. As the depth was usually different between the apices and the center (Figs 14-21), the average of both measurements was plotted. The longest vegetative valve of *E. indica* was formed from an initial cell and 107 μm long. The minimum frustule length was 27 μm and the gametangial mother cells appeared to be similar in length. Though the difference between the maximum and minimum valve length is 80 μm, the difference between the maximum and mimimum epitheca depth was only 3 μm.

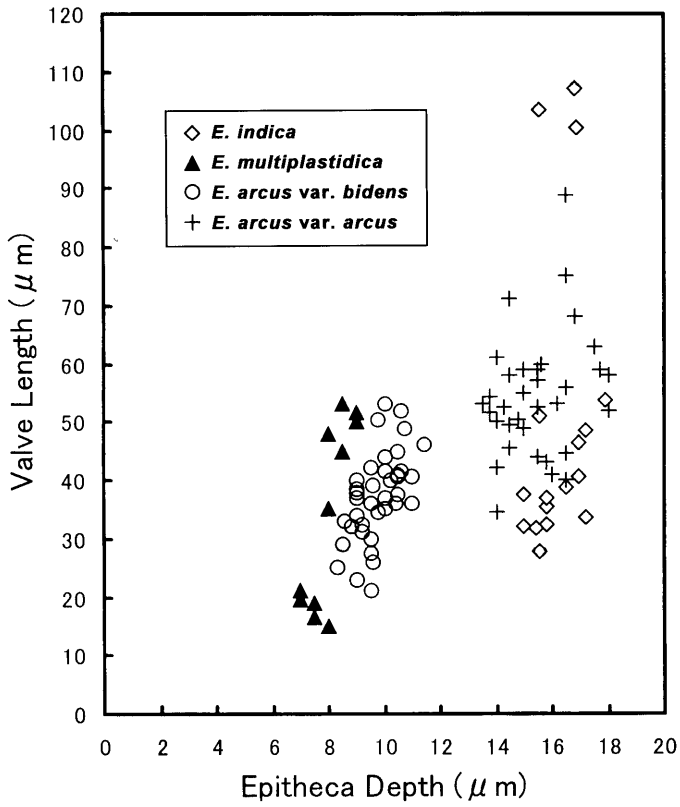
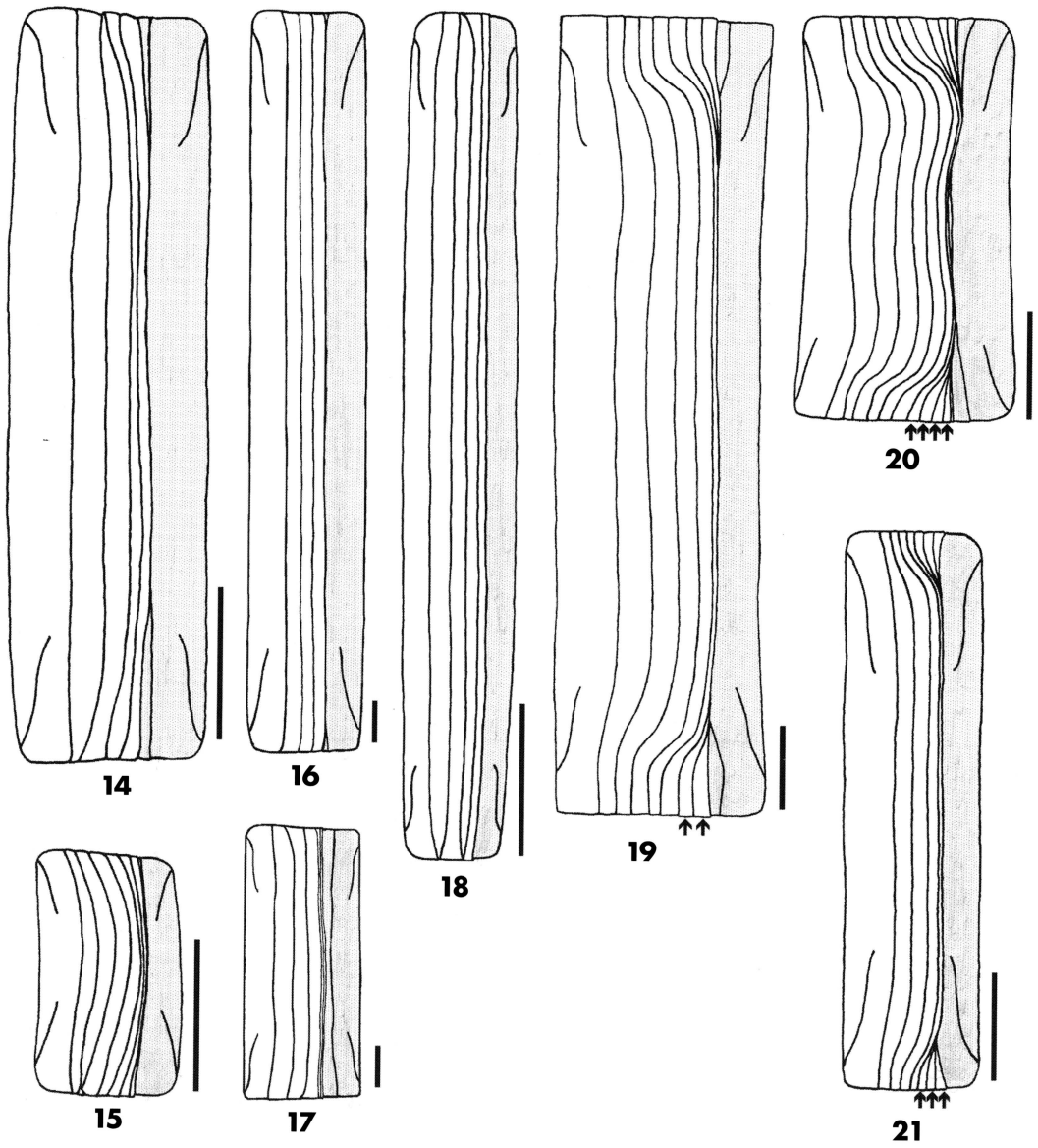


Fig. 13. Correlation between the valve length and the epitheca depth showing the stability of the latter in four *Eunotia* taxa.

Such stability in the epitheca depth had been reported in other *Eunotia* taxa (Mayama & Kobayasi 1991, Mayama 1992). These are shown in Fig. 13 along with the present results. The population of *E. multiplastidica* was cultured and underwent both sexual and vegetative phases, and *E. arcus* var. *arcus* and *E. arcus* var. *bidens* were field populations containing initial valves. In these species the differences between the maximum and minimum epitheca depth were 2-4 μm, but this variation seemed too small in comparison with the changes of the valve lengths.

The number of girdle bands was variable in the population of each taxon, namely, 4 or 5 in *E. indica* (n = 22), 4-8 in *E. multiplastidica* (n = 24), 4 or 5 in *E. arcus* var. *bidens* (n = 38), and 4-6 in *E. arcus* var. *arcus* (n = 68). There was a tendency for shorter valves to have many bands in the taxa *E. indica* and *E. multiplastidica*. Conversely, there was a tendency for longer valves to have many bands in the taxa *E. arcus* var. *arcus* and *E. arcus* var. *bidens*. However, these were only tendencies and there was no firm relationship between the valve length and the number of girdle bands.



Figs 14-21. The frustules traced from SEM photographs showing epitheca (white) and hypotheca (grey) in girdle view. Figs 14-18. The epicingula are composed of bands arranged almost in parallel throughout the valve length. Figs 14, 15. *E. multiplastidica*. Fig. 14. Post-initial cell. Fig. 15. Gametangial mother cell. Figs 16, 17. *E. tropica*. Fig. 17. Post-initial cell. Fig. 18. Gametangial mother cell. Fig. 18. *E. curvata* var. *linearis*. Figs 19-21. The epicingula are composed by integrating the main bands with reduced bands (arrows). Fig. 19. *E. bidens*. Fig. 20. *E. bigibba*. Fig. 21. *E. biseriatoides*.
Scale bars = 10 μm.

Among successive bands of epitheca, the further the band was located from the valve, the more narrow the width of the girdle band became; the epitheca depth is hardly affected by the number of girdle bands.

The epicingulum of *Eunotia* is usually more than half as wide as the epitheca and has a conspicuous structure (Figs 14-21). In the 30 *Eunotia* species I have observed, the epicingula were classified into two types (unpublished data). One type is composed of bands arranged almost in parallel throughout the valve length, e.g., in *E. multiplastidica*, *E. tropica* and *E. curvata* var. *linearis* (Figs 14-18, respectively). The other type is composed of integrating main bands, visible throughout the entire valve length, with reduced bands, visible at the frustule ends, e.g., *E. bidens*, *E. bigibba* and *E. biseriatoides* (Figs 19-21, respectively). The main bands prominently curve toward the valve near the apices, but the reduced bands may not really be short bands but full-length bands identical to the main ones, as very narrow band-like "strings" were often seen along the edge of the outermost main band (Fig. 20). Stability was examined in this shape of the epiband.

A post-initial cell and a gametangial mother cell of *E. multiplastidica* indicate that its characteristic state, in which all bands are arranged almost in parallel throughout the valve length (Figs 14, 15), is maintained throughout the life cycle. However, the number of bands and the width of each band are variable. In the post-initial cell, *E. tropica* showed epibands arranged in parallel from one apex to the other (Fig. 16). This feature was seen also in the gametangial mother cell of this species (Fig. 17). A stability similar to this type of epiband was also observed in *E. curvata* var. *linearis* cultured for 18 months (Fig. 18).

The stability of the other type of epibands was evaluated for *E. bidens*, *E. bigibba*, and *E. biseriatoides* (Figs 19-21, respectively) which were cultured for 18, 6 and 6 months respectively. During these periods, inducement of sexual reproduction was attempted in each strain by a change of old to fresh medium or by mixing of two clones, but without success. However, the same characteristic state of the epibands also appeared in the younger reduced cells.

Discussion

Fine structures of pennate diatoms have usually been studied from field material. Wahrer (1981) and Steinman & Sheath (1984) observed fine structures in cultured *Eunotia*, but their populations did not bear any auxospores. However, observation of populations bearing the auxospore is important to understand the absolute stability of the fine structure. Mann (1984) compared the fine structures between pre-auxospore cells and post- initial cells in *Rhoicosphenia curvata* (Kützing) Grunow which showed no change in the basic construction of the frustule. However, he did not refer to any frustule dimensions. Cohn et al. (1989) and Mayama (1992) have compared fine structures between pre-auxospore cells and post-initial cells of *Navicula cuspidata* and *E. multiplastidica* respectively, and noticed stability of areola density in the valve face.

In this study, not the areola density but the areola number in a certain area of the valve mantle was measured. Measurements of the absolute length around the raphe in the mantle is not easy because it can be measured only in specimens showing the ventral side of the mantle on the specimen stage of the SEM. However, the number of areolae can be easily counted not only in such specimens but also in specimens showing either the outer or inner valve face on the specimen stage, if the stage is tilted. Therefore, the areola number is a more practical measurement in the mantle. The number of areolae counted between the juncture of valve face and mantle and the middle of the raphe branch on the mantle are stable and seem to be good taxonomic criteria.

There is little information about the epitheca depth in cultured pennate diatoms, though there are some observations about the pervalvar depth of the frustule in centric diatoms, e.g., von Stosch & Drebes (1964) and von Stosch et al. (1973). The measured valve length and the epitheca depth in Geitler's drawings of *E. formica*, which had been cultured for ca. six months (Geitler 1932), were 30.7–32 μm and 14.7–15.3 μm respectively at the time the culture was started, and 16.0–18.7 μm and 13.3–14.7 μm at the end of the culture. The difference between the minimum and the maximum epitheca depth was only 2 μm. Geitler's culture showed a stability of the epitheca depth similar to our results. The stability of the epitheca depth was also recognized in two populations of the same species collected from different localities (Mayama & Kobayasi 1991), so that the epitheca depth seems to be stable within a species and is another useful criterion for the differentiation of *Eunotia* species.

Williams (1986) and Williams & Round (1986, 1987) used the characteristics of the girdle bands as a generic criteria for the *Synedra* and *Fragilaria* complex. Krammer (1981) used them for the classification of the infrageneric groups of *Cymbella*. Moreover, they were described as useful specific criteria in some genera, e.g., *Diatoma*, *Meridion* (Williams 1985), *Entomoneis* (Osada & Kobayasi 1990), *Nitzschia* (Kobayasi & Kobori 1990), *Cymbella* (Terao et al. 1993) and *Eunotia* (Mayama 1997). These studies indicate that the girdle bands are as informative as the valve face. However, the variability of these structure has not usually been discussed. The present study confirms its stability throughout the life cycle in some species having the bands arranged in parallel throughout the valve length. The stability of the epicingulum, characterized by integrating the main bands with the reduced bands, was confirmed only in the limited period of the asexual phase. This type of epicingulum was widely seen in field populations including cells of various sizes in certain species, e. g., *E. bidens*, *E. bigibba*, *E. biseriatoides* (Figs 19-21, respectively), *E. arcus* var. *arcus* (Mayama & Kobayasi 1991) and *Eunotia pseudovalida* Mayama (Mayama 1997), and it was considered to be one of the important criteria for distinguishing *E. biseriatoides* and *Eunotia sparsistriata* Mayama (Mayama 1993) as well as *Eunotia pseudovalida* Mayama and *Eunotia valida* Hustedt (Mayama 1997). An evaluation using a population in which sexual reproduction has taken place should be done to confirm the usefulness of this criterion.

It is interesting for the phylogeny of *Eunotia* that two types of band structures were found. However, other characteristics which correspond to the distribution of the band

types, were found neither in valve structure nor plastids. The band structure similar to the integrating type is also seen in some species outside the *Eunotia* species, e. g. *Diatoma mesodon* (Ehrenberg) Kützing (Krammer & Lange-Bertalot 1991). This fact may imply that the different types of girdle structure are associated with the ecology of these species. Further studies are needed to understand the relationship between the band structures and the autecology of these species.

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