

SEQUENTIAL VALVE DEVELOPMENT IN THE MONORAPHID DIATOM *ACHNANTHES MINUTISSIMA* VAR. *SAPROPHILA*

Shigeki Mayama & Hiromu Kobayasi

*Department of Biology, Tokyo Gakuzei University,
Koganei-shi, Tokyo 184, Japan*

Valve development has been examined in *Achnanthes minutissima* var. *saprophila* H. Kobayasi & Mayama. The formation of the raphid valve (RV) proceeds in the same sequence as has previously been described for *Navicula*. Early development of the araphid valve (AV) is remarkably similar to that of the RV, in that a primary central rib and primordial central nodule are initiated, followed by the development of secondary arms from the centre and the recurring of the primary rib at the poles. Therefore raphe slits appear both at the centre and poles during the early development of the AV. However, thin fin-like depositions arise along the primary central rib between the secondary arms extending out from the centre and the centripetal extensions of the primary central rib recurring from the poles. The fins then fuse with the secondary arms and the in-turned primary central rib, so that the initially-formed raphe slits do not develop any further and are filled in: an axial area devoid of a raphe is thus formed. The valve ontogeny of this taxon suggests secondary reduction from the biraphid *Naviculae*.

INTRODUCTION

As reviewed by Andrews (1981) there have been two views on the evolution of the monoraphid diatom, *Achnanthes*. One is the traditional idea that *Achnanthes* was derived from araphid diatoms and the other is that it was derived from biraphid diatoms. Andrews advocated the latter on the basis of morphological comparisons between raphe, vestigial raphe and helictoglossa. Loss of the raphe in one valve was also suggested by Mann (1984), following observations on the related genus *Cocconeis* (Mann 1982).

Boyle *et al.* (1984) examined valve morphogenesis in *Achnanthes coarctata* (Bréb. in W. Smith) Grun. and demonstrated that the developing araphid valve (AV) bears a raphe slit at least near the valve centre in the earliest stages, as in the raphid valve (RV). However, the development of the valve was not fully elucidated, because their studies were mainly based on cross sections.

Observations of developing whole valves are necessary to clarify the sequence of valve development, but such studies have been scarce in pennate diatoms. Chiappino & Volcani (1977) and Schmid (1979) have presented detailed accounts but their scheme applies only to naviculoid diatoms. We now describe the sequential valve development of *Achnanthes minutissima* var. *saprophila* H. Kobayasi & Mayama by examining whole valves at various developmental stages. From this we attempt to infer the phylogeny of *Achnanthes*.

Achnanthes minutissima var. *saprophila* was described originally from severely polluted rivers in the vicinity of Tokyo (Kobayasi & Mayama 1982). Valve morphology of this taxon closely resembles the nominate variety but the variety can be distinguished easily with electron microscopy. Moreover, it is noteworthy that var. *saprophila* has a high tolerance to organic pollution and often occurs as a dominant taxon even in polysaprobic waters (Mayama & Kobayasi 1984, Kobayasi *et al.* 1985).

MATERIAL AND METHODS

The unialgal cultures used for this study were originally obtained from material attached to a submerged stone in the Minamiasa-kawa (Minamiasa river). They were first established on NN-1

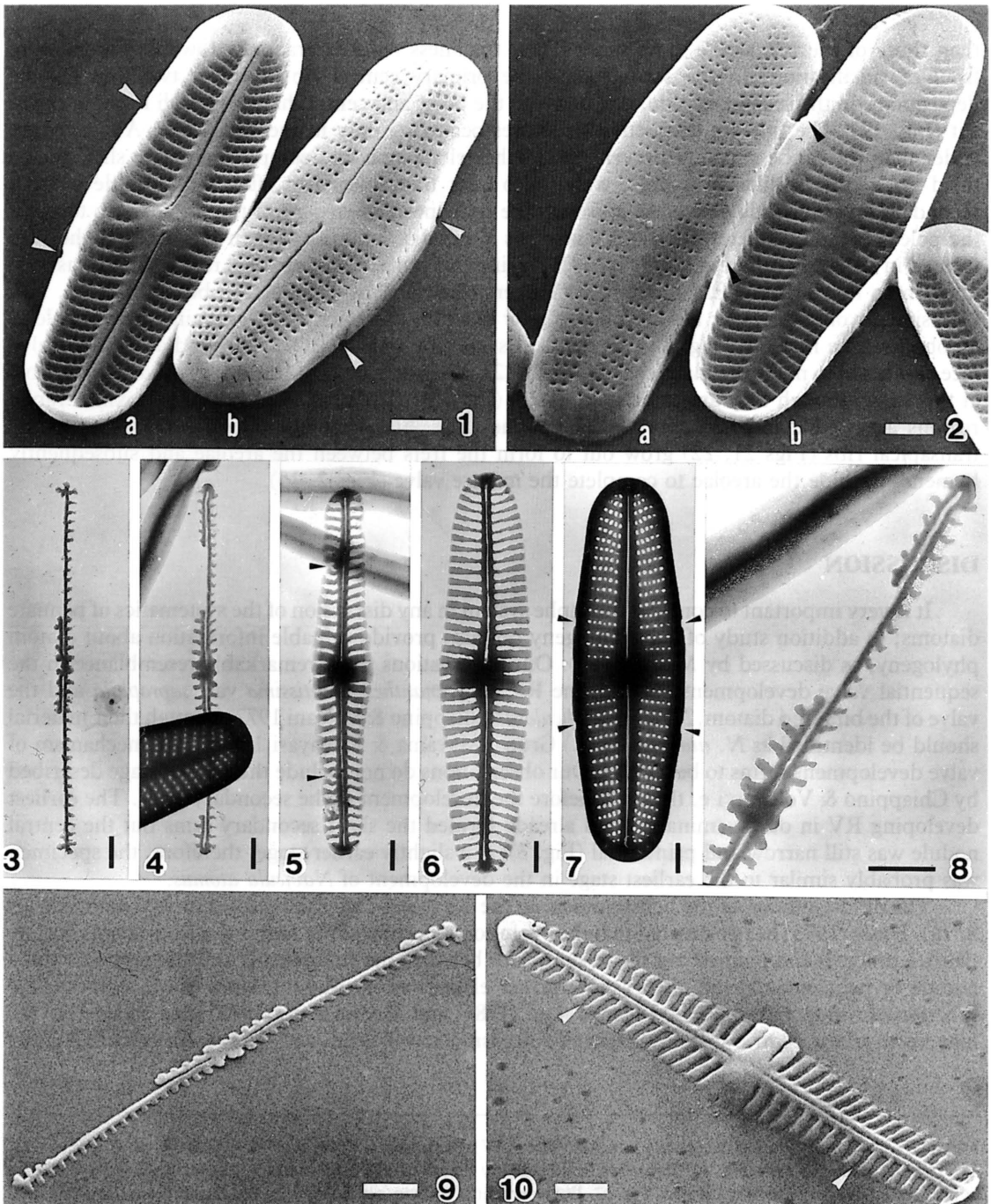
medium with 1.5% agar, but subsequently grown in an agar-water biphasic medium (Ooshima 1975). Cultures were maintained at 20°C under fluorescent light of ca. 2,000 lux on a 12:12 (L:D) photoperiod. Cells scraped off the agar surface were gently boiled in concentrated sulphuric acid for 30 min. After several washes in distilled water, a drop containing the suspended silicified elements was placed on a formvar-coated grid for transmission electron microscopy (TEM), or on a cover glass, followed by gold-palladium coating, for scanning electron microscopy (SEM). JEOL 7 (TEM), JEOL 100CX-II (TEM) and JEOL F15 (SEM) instruments were used in examinations.

OBSERVATIONS

In the original description of *Achnanthes minutissima* var. *saprophila*, we presented LM and TEM photographs (Kobayasi & Mayama 1982); here we present SEM photographs of both the mature RV (Fig. 1) and the AV (Fig. 2). The valve face of the RV is almost flat, slightly concave along the apical axis, and surrounded by a marginal hyaline rim between the valve face and mantle (Fig. 1b). In the AV the appearance of valve face is the same as in the RV except for the convexity along the apical axis (Fig. 2a). The raphe slits are straight (Fig. 1). Externally the central raphe endings are curved to the same side and terminate in slightly dilated central pores, while the terminal fissures are narrow and straight (Fig. 1b). Internally, the valve bears a small mounded central area; on either side of this are the raphe endings, which are slightly deflected in opposite directions, terminating in small helictoglossae (Fig. 1a). In the AV, the axial area is broader and slightly lanceolate: this is one of the characteristics of this variety (Figs 2, 15). The striae consist of rectangular areolae elongated in the apical direction throughout the RV (Figs 1, 7). Each areola in the RV and the AV is occluded internally by a hymen (Figs 1, 2). The external apertures of the mantle areolae are slit-like and orientated parallel to the perivalvar axis. In both RV and AV the mantle edge bears four small chip-like cut-outs. Figs 1 & 2 do not show all the cuts because of the angle of the specimen but all four can be seen in the TEM photograph (Fig. 7, arrow heads); one lies nearly mid-way between the valve centre and the Voigt fault in each quadrant of the valve.

Achnanthes minutissima var. *saprophila* initiates the development of the raphid valve with deposition of the primary central rib and the central nodule, as described for *Navicula pelliculosa* by Chiappino & Volcani (1977). At the earliest stage observed, the developing valve already consists of a full-length central rib with very short transapical ribs (Fig. 3). The half of the central nodule lying to the primary side of the central rib is already silicified, but the secondary side of the nodule is not, even though short secondary arms have already been initiated. In the next stage the primary central rib curves round at the poles (Figs 4, 8, 9); this stage corresponds to the second stage of Chiappino & Volcani (1977). By now, both sides of the central nodule have been completed and the secondary arms are extending. The developing raphe is not plicate but a straight slit (Fig. 8). In the third stage, the reflexed primary rib and the secondary arms approach each other and ultimately fuse with each other to complete the raphe system. The second and third stages are accompanied by the continued growth of the transapical ribs, and Voigt discontinuities are formed where the primary rib and secondary arms meet, as described by Mann (1981) (Fig. 5). During the next stage, the transapical ribs grow towards the valve margin (Figs 6, 10), the axial ribs and central nodule thicken, and the helictoglossae are generated. After this stage the frets between the transapical ribs arise to delimit the areolae, which are subsequently occluded by the hymenes; then the RV is complete (Figs 1, 7). We could not detect any relationship between the four small chip-like cuts in the mantle edge of the mature valve and valve development.

The earliest development observed in the formation of the araphid valve is shown in Figs 11, 16 & 17. Again we find the reflexing of the ends of the primary central rib found in *Navicula pelliculosa* (Chiappino & Volcani 1977), and during the second stage of RV formation (Figs 4, 8). The central nodule is evident and short secondary arms are also present. As is clear from Fig. 11, there is a space between the primary central rib and the secondary arms, so that the raphe slits are temporarily recognizable on both sides of the valve centre and also near the poles. The transapical ribs extending from the primary central rib and the secondary arms are short, but they are wider



Figs 1-10. *Achmanthes minutissima* var. *saprophila*. Scale bars = 1 μ m. Figs 1, 2, 9 & 10, SEM; Figs 3-8, TEM. Fig. 1. Mature raphid valves (RV) with small chip-like cuts (arrow heads). a. Internal view showing helictoglossae and areolae. b. External view of concave valve. Fig. 2. Mature araphid valves (AV) with small chip-like cuts (arrow heads). a. External view of concave valve. b. Internal view showing smooth surface of axial area. Figs 3-7. Sequence of RV development. Fig. 3. Development of primary central rib and primordial central nodule with short secondary arms. Fig. 4. Development of central nodule, secondary arms and recurring of primary central rib at the poles. Fig. 5. Meeting and fusion of the primary central rib and the secondary arms; formation of Voigt faults (arrow heads) and development of transapical ribs. Fig. 6. Transapical ribs developing over the valve face. Fig. 7. Mature valve showing four small chip-like cuts on the mantle edge (arrow heads). Fig. 8. Enlargement of Fig. 4, showing the formation of simple, clear raphe slits. Fig. 9. Internal view of the developing valve at the same stage as in Fig. 4. Fig. 10. Internal view of the developing valve at the same stage as in Fig. 5, showing helictoglossa and Voigt fault (arrow heads).

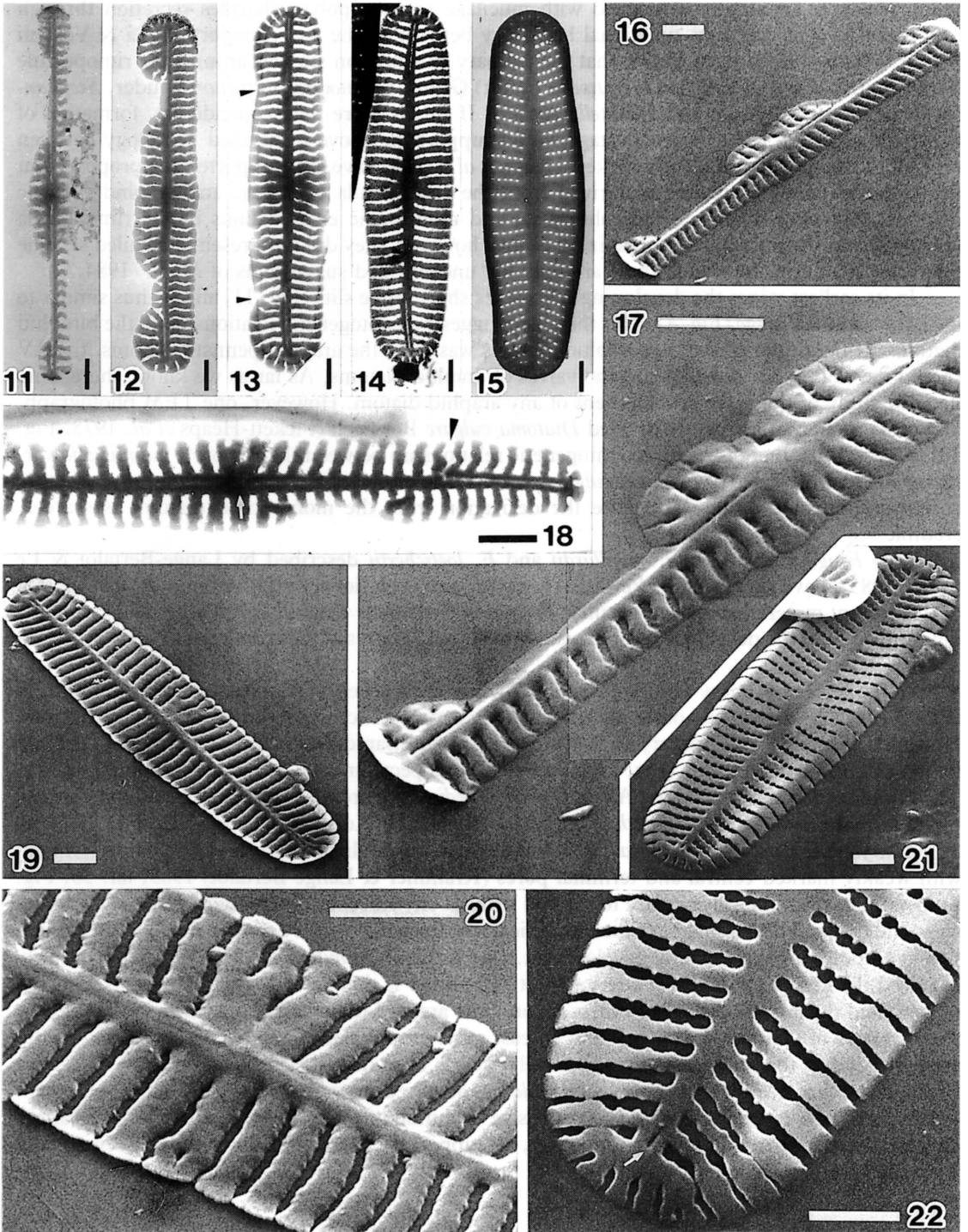
than those of the RV at the same stage. Their thickness reduces gradually towards the growing front of silicification. In the second stage the primary central rib produces two thin fin-like depositions on the secondary side, one between the polar and central halves of each forming raphe slit (Fig. 17). The fins are distinctive structures occurring only in the developing AV; they are wider than the raphe slits and prevent the further elongation and completion of the slits. In the third stage, the fin-like structures, which also bear transapical ribs, fuse with the developing secondary arms towards the centre and with the centripetal extensions of the primary rib towards the poles (Fig. 12). Therefore, the AV also has Voigt discontinuities, as in the RV, but they are formed in a slightly different way (Fig. 13). During this process the proximal raphe slits are usually filled in so that in the TEM they are no longer penetrated by the electron beam (Figs 12, 13). Occasionally only the outer fissure is completely filled in, the inner fissure remaining detectable as an obscure line in TEM (Fig. 18). In the next stage, the transapical ribs expand across the valve face and begin to make the mantle (Figs 14, 19-22). The polar parts of the raphe slits are still visible at this stage, though they are variable in length (Figs 14, 19, 21, 22), whereas the proximal raphe regions are visible in SEM as a vestigial raphe (Fig. 20). Then the lateral projections on the transapical ribs (Figs 21, 22) grow out to form the frets between the areolae and subsequently hymenes occlude the areolae to complete the mature valve (Figs 2, 15).

DISCUSSION

It is very important to consider the raphe system in any discussion of the systematics of pennate diatoms; in addition study of valve ontogeny can also provide valuable information about diatom phylogeny, as discussed by Mann (1984). Our observations show remarkable resemblance in the sequential valve development between the RV of *Achnanthes minutissima* var. *saprophila* and the valve of the biraphid diatom, *Navicula pelliculosa* (Chiappino & Volcani 1977), though their material should be identified as *N. atomus* (Kütz.) Grun. (Mayama & Kobayasi 1988). The mechanism of valve development seems to be similar. Our observations do not include the earliest stage described by Chiappino & Volcani, i.e. the stage before the development of the secondary arms. The earliest developing RV in our examination had already formed the short secondary arms but the central nodule was still narrow and primordial (Fig. 3); at a slightly earlier stage, therefore, the specimen was probably similar to the earliest stage in the development of *Navicula atomus*.

The differentiation of the helictoglossae in the RV begins after the second stage (compare Figs 9, 10). Hasle (1973) has postulated that the evolutionary origin of the raphe was from a rimoportule (labiate process) and pointed out the resemblance between the rimoportula and the raphe terminal nodule or helictoglossa; this theory was further elaborated by Mann (1984). With regard to the function of rimoportulae, Pickett-Heaps *et al.* (1986) and Medlin *et al.* (1986) have referred to the movement of the centric diatoms, *Odontella sinensis* (Grev.) Grun. and *Actinocyclus subtilis* (Greg.)

Figs 11-22. *A. minutissima* var. *saprophila*, araphid valves (AV). Scale bars = 1 μ m. Figs 16, 17, 19-22, SEM; Figs 11-15, 18, TEM. AV development. Fig. 11. Recurring of the primary central rib at the poles and formation of the secondary arms at the centre, with short transapical ribs. Fig. 12. Development of primary central rib, fin-like depositions, secondary arms and transapical ribs. Fig. 13. Formation of Voigt fault (arrow heads). Fig. 14. Transapical ribs extending to the valve margin and the raphe slits remaining in the terminal area. Fig. 15. Mature AV. Fig. 16. Internal view of specimen in the same stage with Fig. 11. Fig. 17. Enlargement of Fig. 16 showing thin fin-like deposition between the forward extensions of primary central ribs and the secondary arms with transapical ribs. Fig. 18. Developing valve showing the Voigt fault (arrow heads), the distal short slit and the proximal raphe lightly filled-in with silica. Note the pale, obscure lines visible on both sides of the central nodule (arrow). The transapical ribs are still flexible and parts of them seem to have been deformed in preparation. Fig. 19. Internal view of the developing valve showing the short raphes in the terminal areas. Fig. 20. Enlargement of Fig. 19, showing the central vestigial raphe branches. Fig. 21. External view of the developing valve. Fig. 22. The raphe just before the completion of its filling in and obliteration (arrow) and initiation of fret formation between transapical ribs.



Ralfs, which is apparently associated with mucilage or mucopolysaccharides secretion through rimoportulae. This suggests functional homology between raphe and rimoportula. Li & Volcani (1985a, 1985b, 1985c) have shown that the primary silicification site lies around the rimoportule in the centric diatoms, *Ditylum brightwellii* (West) Grun., *Chaetoceros rostratum* Lauder, *Stephanopyxis turris* (Grev.) Ralfs and *Odontella sinensis*. If this is more than coincidental, formation of helictoglossa of the RV at an early stage would support the above-mentioned homology between raphe and rimoportula. Recently Pickett-Heaps *et al.* (1988) have also supported the proposal that the raphe of pennate diatoms is derived from the rimoportula of centric and araphid pennate diatoms, following functional, morphological and ontogenetic examinations of the rimoportula apparatus and the microtubule-organizing centre; however, they did not present a detailed scheme for raphe evolution, for which there are only the unconfirmed suggestions of Mann (1984).

In its earliest stages the developing AV makes short raphe slits (Fig. 11) and is thus similar to the equivalent RV stage (Fig. 4). This therefore suggests an ontogenetic relationship to the biraphid diatoms. If the origin of the monoraphid diatoms was from the araphid pennate diatoms, the AV should develop in the same way as the valves of araphid diatoms. As far as we know, there is no detailed study of the valve development of any araphid diatom. However, one TEM photograph, of a cross section of a recently divided *Diatoma vulgare* Bory cell (Pickett-Heaps *et al.* 1975, Fig. 30), suggests that there is no direct ontogenetic relationship between the AV of *A. minutissima* var. *saprophila* and the valve of *Diatoma vulgare*, because the cross section does not show any structure corresponding to a raphe. Therefore the derivation from the monoraphid diatoms is scarcely credible.

Two *Fragilaria* species, *F. maillardii* and *F. loetschertii* described by Lange-Bertalot & Le Cohu (1985), have short raphe-like slits. However, any evolutionary relationship between the AV of the present material and these peculiar *Fragilaria* specimens without apical pore field cannot be determined until the development of the valve and other features of these unusual *Fragilaria* species have been examined.

In the RV the primary central rib and secondary arms develop the transapical ribs unilaterally (Fig. 5), as in *N. atomus* and other raphid diatoms examined in cross section by many investigators. However, in the AV the primary central rib develops transapical ribs bilaterally, by the differentiation of fin-like depositions (Fig. 12). This process is peculiar even in the monoraphid diatoms. The raphe seen in the developing AV of *Cocconeis pediculus* Ehrenb. (Mann 1982) and the vestigial raphe in mature valves of *Achnanthes*, e.g. *A. convergens* H. Kobayasi (Kobayasi *et al.* 1986, Fig. 52), *A. kryophila* Hust., *A. austriaca* var. *helvetica* Hust. (unpublished data) and *A. bioretii* Germain, with weakly marked central and terminal pores (Krammer & Lange-Bertalot 1985, taf. 30, Fig. 7), continues from the valve centre to the end without any siliceous obstruction; hence they do not show the bilateral development of the transapical ribs from the primary central rib. We can suggest two hypotheses for this bilateral development. One is that *A. minutissima* var. *saprophila* has newly acquired the ability for bilateral development. The other is that an ancestral feature has recurred or persisted during the evolution of this taxon. The araphid diatoms are generally believed to be the ancestors of the raphid diatoms, and at least some groups possessing a narrow pattern- centre seem to develop the transapical ribs bilaterally, as has been described by Mann (1984). It cannot be ruled out that *A. minutissima* var. *saprophila* has acquired the capacity for bilateral development as the result of a reversion during the regressive evolution of the raphe system from the biraphid diatoms. From our studies on valve ontogeny, we support the idea that *Achnanthes* has been derived from the biraphid diatoms.

ACKNOWLEDGMENTS

We are thankful to Dr. F.E. Round and Dr. D.G. Mann for reading the manuscript critically and for correcting the language.

REFERENCES

- ANDREWS, G.W. (1981). *Achnanthes linkei* and the origin of monoraphid diatoms. *Bacillaria*, **4**, 29-40
- BOYLE, J.A., PICKETT-HEAPS, J.D. & CZARNECKI, D.B. (1984). Valve morphogenesis in the pennate diatom *Achnanthes coarctata*. *Journal of Phycology*, **20**, 563-573
- CHIAPPINO, M.L. & VOLCANI, B.E. (1977). Studies on the biochemistry and fine structure of silica shell formation in diatoms. VII. Sequential cell wall development in the pennate *Navicula pelliculosa*. *Protoplasma*, **93**, 205-21.
- HASLE, G.R. (1973). The 'mucilage pore' of pennate diatoms. *Nova Hedwigia, Beiheft*, **45**, 167-194
- KOBAYASI, H. & MAYAMA, S. (1982). Most pollution-tolerant diatoms of severely polluted rivers in the vicinity of Tokyo. *Japanese Journal of Phycology*, **30**, 188-196
- KOBAYASI, H., MAYAMA, S., ASAI, K. & NAKAMURA, S. (1985). Occurrence of diatoms collected from variously polluted rivers in Tokyo and its vicinity, with special reference to the correlation between relative frequency and BOD. *Bulletin of Tokyo Gakugei University, Sect. 4*, **37**, 21-46
- KOBAYASI, H., NAGUMO, T. & MAYAMA, S. (1986). Observations on the two rheophilic species of the genus *Achnanthes* (Bacillariophyceae), *A. convergens* H. Kob. and *A. japonica* H. Kob. *Diatom*, **2**, 83-93
- KRAMMER, K. & LANGE-BERTALOT, H. (1985). Naviculaceae. Neue und wenig bekannte Taxa, neue Kombinationen und Synonyme sowie Bemerkungen zu einigen Gattungen. *Bibliotheca Diatomologica*, **9**, 1-210
- LANGE-BERTALOT, H. & LE COHU, R. (1985). Raphe like vestiges in the pennate diatom suborder Araphidineae? *Annales de Limnologie*, **21**, 213-220
- LI, C.-W. & VOLCANI, B.E. (1985a). Studies on the biochemistry and fine structure of silica shell formation in diatoms. VIII. Morphogenesis of the cell wall in a centric diatom, *Ditylum brightwellii*. *Protoplasma*, **124**, 10-29
- LI, C.-W. & VOLCANI, B.E. (1985b). Studies on the biochemistry and fine structure of silica shell formation in diatoms. IX. Sequential valve formation in a centric diatom, *Chaetoceros rostratum*. *Protoplasma*, **124**, 30-41
- LI, C.-W. & VOLCANI, B.E. (1985c). Studies on the biochemistry and fine structure of silica shell formation in diatoms. X. Morphogenesis of the labiate process in centric diatoms. *Protoplasma*, **124**, 147-156
- MANN, D.G. (1981). A note on valve formation and homology in the diatom genus *Cymbella*. *Annals of Botany*, **47**, 267-269
- MANN, D.G. (1982). Structure, life history and systematics of *Rhoicosphenia* (Bacillariophyta). I. The vegetative cell of *Rh. curvata*. *Journal of Phycology*, **18**, 162-176
- MANN, D.G. (1984). An ontogenetic approach to diatom systematics. In: *Proceedings of the Seventh International Diatom Symposium*, (D. G. Mann, ed.), 113-144. O. Koeltz, Koenigstein
- MAYAMA, S. & KOBAYASI, H. (1984). The separated distribution of the two varieties of *Achnanthes minutissima* Kuetz. according to the degree of river water pollution. *Japanese Journal of Limnology*, **45**, 304-312
- MAYAMA, S. & KOBAYASI, H. (1988). Morphological variations in *Navicula atomus* (Kütz.) Grun. In: *Proceedings of the Ninth International Diatom Symposium* (F. E. Round, ed.), 427-435. Biopress, Bristol & Koeltz Scientific Books, Koenigstein
- MEDLIN, L.K., CRAWFORD, R.M. & ANDERSEN, R.A. (1986). Histochemical and ultrastructural evidence for the function of the labiate process in the movement of centric diatoms. *British Phycological Journal*, **21**, 297-301
- OOSHIMA, K. (1975). The agar-water biphasic culture medium for obtaining clonal cultures of microscopic algae. *Bulletin of the Nippon Dental College, General Education*, **1975**, 275-286
- PICKETT-HEAPS, J.D., McDONALD, K.L. & TIPPIT, D.H. (1975). Cell division in the pennate diatom *Diatoma vulgare*. *Protoplasma*, **86**, 205-242
- PICKETT-HEAPS, J.D., HILL, D.R.A. & WETHERBEE, R. (1986). Cellular movement in the centric diatom *Odontella sinensis*. *Journal of Phycology*, **22**, 334-339
- PICKETT-HEAPS, J.D., WETHERBEE, R. & HILL, D.R.A. (1988). Cell division and morphogenesis of the labiate process in the centric diatom *Ditylum brightwellii*. *Protoplasma*, **143**, 139-149
- SCHMID, A. -M. (1979). The development of structure in the shells of diatoms. *Nova Hedwigia, Beiheft* **64**, 219-236