Jap. J. Phycol. (Sôrui) 34 : 87-93. June 20, 1986

Observations on the valve structure of fresh water *Diploneis* (Bacillariophyceae), *D. oculata* (BRÉB.) CLEVE and *D. minuta* PETERSEN

Masahiko IDEI* and Hiromu KOBAYASI**

**Institute of Biological Sciences, University of Tsukuba, Sakura-mura, Ibaraki, 305 Japan*
***Department of Biology, Tokyo Gakugei University, Koganei-shi, Tokyo, 184 Japan*

IDEI, M. and KOBAYASI, H. 1986. Observations on the valve structure of fresh water *Diploneis*, (Bacillariophyceae), *D. oculata* (BRÉB.) CL. and *D. minuta* PET. Jap. J. Phycol. 34 : 87-93.

Two *Diploneis* species with a sinuous slit on the exterior occlusion of the alveolus are identified on the basis of authentic specimens and examined using TEM and SEM. The alveolus of these species is occluded externally by a vola with a sinuous slit and internally by a hymen (a very thin perforated layer), bearing perforations in a hexagonal array. The cingulum is composed of two bands, one valvocopula, which is a broad open band, and a narrow pleura.

Key Index Words: *Diatoms*; Diploneis oculata; Diploneis minuta; *fine structure*; *sinuous slit*.

The fine structure of valves of the genus *Diploneis* has been mainly observed using transmission electron microscopy (TEM) (HELMCKE and KRIEGER 1962, GEISSLER *et al.* 1963, OKUNO 1964, 1970, GERMAIN 1979, 1981), although a few but noteworthy works have used scanning electron microscopy (SEM) (GERLOFF and HELMCKE 1975, SIMS and PADDOCK 1979, SCHOEMAN and ASHTON 1982).

Morphological characteristics useful for the taxonomy of the genus are more clearly visible with SEM, because almost all *Diploneis* valves have strongly silicified longitudinal canals and complex alveoli, the structure of which is hardly detectable by TEM.

The valve structure of *D. oculata* and *D. minuta* has already been examined with TEM by GERMAIN (1979, 1981). One of the peculiar features of these species, namely, the presence of a sinuous line on the alveolus, has been clarified, but whether the sinuous line is situated on the inner or outer side of the alveolus is still unclear. In this paper, further investigation using SEM makes clear a three-dimensional structure of the sinuous line and other features such as open valvocopula and pleura.

Materials and Methods

Specimens used for SEM observation of *Diploneis oculata* were collected from the following locations. 1) Bottom mud in Aoki-ko (Aoki Lake), Nagano Prefecture on 19 March 1974, K-3094. 2) Bottom mud in Yamanaka-ko (Yamanaka Lake), Yamanashi Prefecture on 22 Feb. 1984, K-1811. 3) Bottom mud in an irrigation pond without name near Ueda City, Nagano Prefecture in 5 May 1978, K-2924. 4) Bottom mud in an irrigation pond without name near Soma City, Fukushima Prefecture on 16 June 1984, K-1941.

The specimens of *D. minuta* were collected from moss on a rock beside Yōro Fall, Chiba Prefecture on 16 Dec. 1979, N-1006 (K-3172).

Methods of cleaning, washing, and preparing objects for light and electron microscopy are in KOBAYASI *et al.* (1985).

Results and Discussion

Diploneis oculata (BRÉB.) CLEVE (1894, p. 92). Figs 1-5, 9-20.

This species is found in various lakes and ponds in Japan, but the cells in each sample are usually very scarce. We found it in a considerable amount only in the sample collected from an irrigation pond near Sōma City, Fukushima Prefecture.

Valves of our specimens are 16-34 μm in length, 7-8 μ in width. Transapical striae number about 18 in 10 μm at the center and up to 20 in 10 μm at the poles. The valves are longer than those described for European specimens. CLEVE (1894) gave a length range of 15-20 μm, HUSTEDT (1930, 1937) and PATRICK & REIMER (1966) gave 10-20 μm and GERMAIN (1979) gave 8-12 μm. Our measurement of specimens from the following European collections showed a length range of 14-20 μm: V. Heurck Type Slide (No. 106, *Navicula oculata* Bréb. Bruxelles, Belgique) housed in the Naturhistorisches Museum, Wien (Fig. 1): Kützing collection in the British Museum (BM 18861) (Fig. 2); a collection from Lunzer Untersee, Austria (K-2090) (Fig. 3).

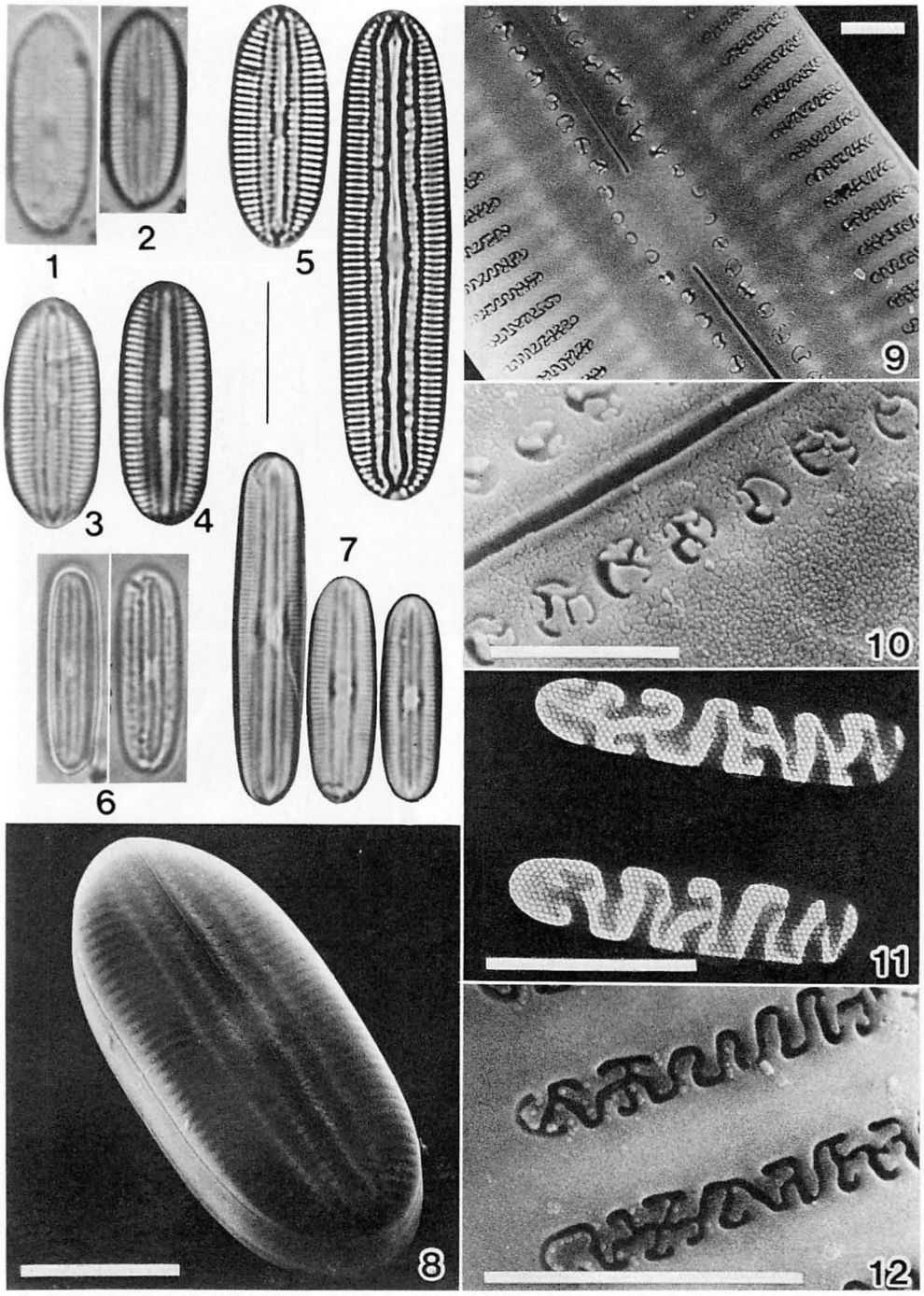
The striae of our specimens are coarser than those of European specimens, being 19 in 10 μm in Fig. 4 and 18 in 10 μm in Fig. 5. On the other hand, as seen in Figs 1-3, striae of the European specimens measure up to 22 in 10 μm. However, the fine structure of our specimens observed with SEM and TEM appeared to be identical with that of European specimens (GERMAIN 1979) and thus the Japanese specimens were identified as *D. oculata*.

In SEM, the valve face is almost flat and the mantle is relatively shallow (Fig. 8). Externally the raphe is a narrow slit. The proximal ends of the raphe branches are straight without forming a central pore. The distal ends are also straight and terminate some distance from the valve margin. Along both sides of the axial area, a longitudinal row of poroids extends the whole length of the valve. Each row expands slightly to the outside at the center and conspicuously at the terminal nodule (Figs 8, 9, 18 arrow). Each poroid penetrates obliquely the outer wall from the raphe side to the axial edge of the longitudinal canal lumen (Fig. 15 arrow). Those poroids that are arranged on either side of the central nodule are occluded by a single round flap (Fig. 9), while the remaining poroids are occluded externally by a vola and are uniformly larger (Fig. 10).

The internal fissures of the raphe are enclosed in a slightly raised rib lying between two prominent longitudinal canals, their proximal ends terminating up against a raised central nodule (Figs 15, 19; cf. SIMS and PADDOCK 1979, SCHOEMAN and ASHTON 1982). The costae run from the outer edges of the two longitudinal canals to the valve margin (Figs 13-15). The intercostal spaces consist of elongated alveoli (Figs 15, 17), each occluded externally by an elongated vola (Figs 9, 11, 12) and internally by a thin siliceous layer with perforations arranged in a hexagonal array (Figs 11, 15-17) (MANN 1981).

The area with volate occlusions occupies about half the valve width (Figs 8, 9, 18), but the alveoli extend beyond the occlusion

Plate 1. Figs 1-5. L.M. *Diploneis oculata* (BRÉB.) CL. bar=10 μm. Fig. 1. Kützing's Coll., BM 18861, Paris 1708. ×2,000. Fig. 2. Grunow's Coll., V.H. Type Slide No. 106, Bruxelles, Belgique. ×2,000. Fig. 3. Lunzer Untersee, Austria. K-2090. ×2,000. Fig. 4. A pond, Ueda City, Nagano Pref. K-2924. ×2,000. Fig. 5. A pond, Sōma City, Fukushima Pref. K-1941. ×2,000. Figs 6, 7. *Diploneis minuta* PET. bar=10 μm. Fig. 6. Isotype specimens. Hustedt's Coll., 04/59, moss, Eyvindará, Iceland,



Pet. 25. ×2,000. Fig. 7. On moss, Yōro Fall, Chiba Pref. N-1006. (=K-3172). ×2,000. Figs 8-12. *Diploneis oculata* (BRÉB.) CL. Fig. 8. Frustule from outside. A pond, Sōma City. SEM. ×4,500 (bar =5 μm). Fig. 9. Center enlarged, from outside. Aoki Lake Nagano Pref. K-3094. SEM. ×9,000 (bar =1 μm). Fig. 10. Axial row of poroids enlarged, from outside. Aoki Lake. SEM. ×27,000 (bar=1 μm). Fig. 11. Alveoli enlarged. A pond, Sōma City. TEM. ×60,000 (bar=0.5 μm). Fig. 12. Alveoli with a sinuous slit enlarged, from outside. A pond, Sōma City. SEM. ×45,000 (bar=1 μm).

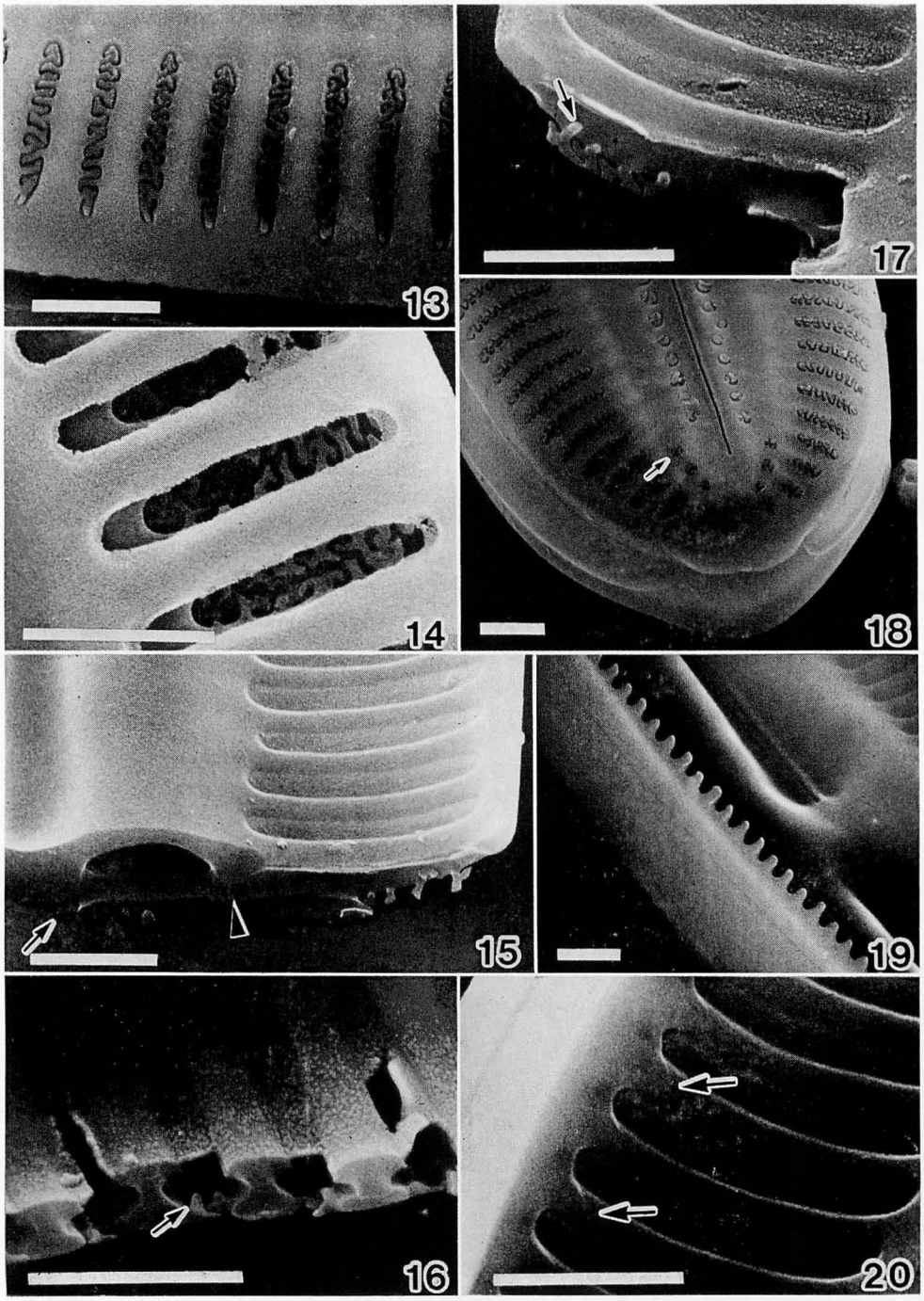


Plate 2. Figs 13-20. *Diploneis oculata* (BRÉB.) CL. A pond, Sōma City, Fukushima Pref. K-1941. SEM. Scale bar =1 μm. Fig. 13. Alveoli enlarged, from outside. ×18,000. Fig. 14. Alveoli enlarged, from inside. ×27,000. Fig. 15. Broken valve, valve center, from inside. ×18,000. Fig. 16. Cut ends of the transapical costae enlarged, contral valve, from inside. ×30,000. Fig. 17. Broken alveolus enlarged, from inside. ×27,000. Fig. 18. Valve pole enlarged, from outside. ×9,000. Fig. 19. Valvocopula without epivalve, enlarged, from outside. ×9,000. Fig. 20. Valve with valvocopula enlarged, from inside. ×27,000.

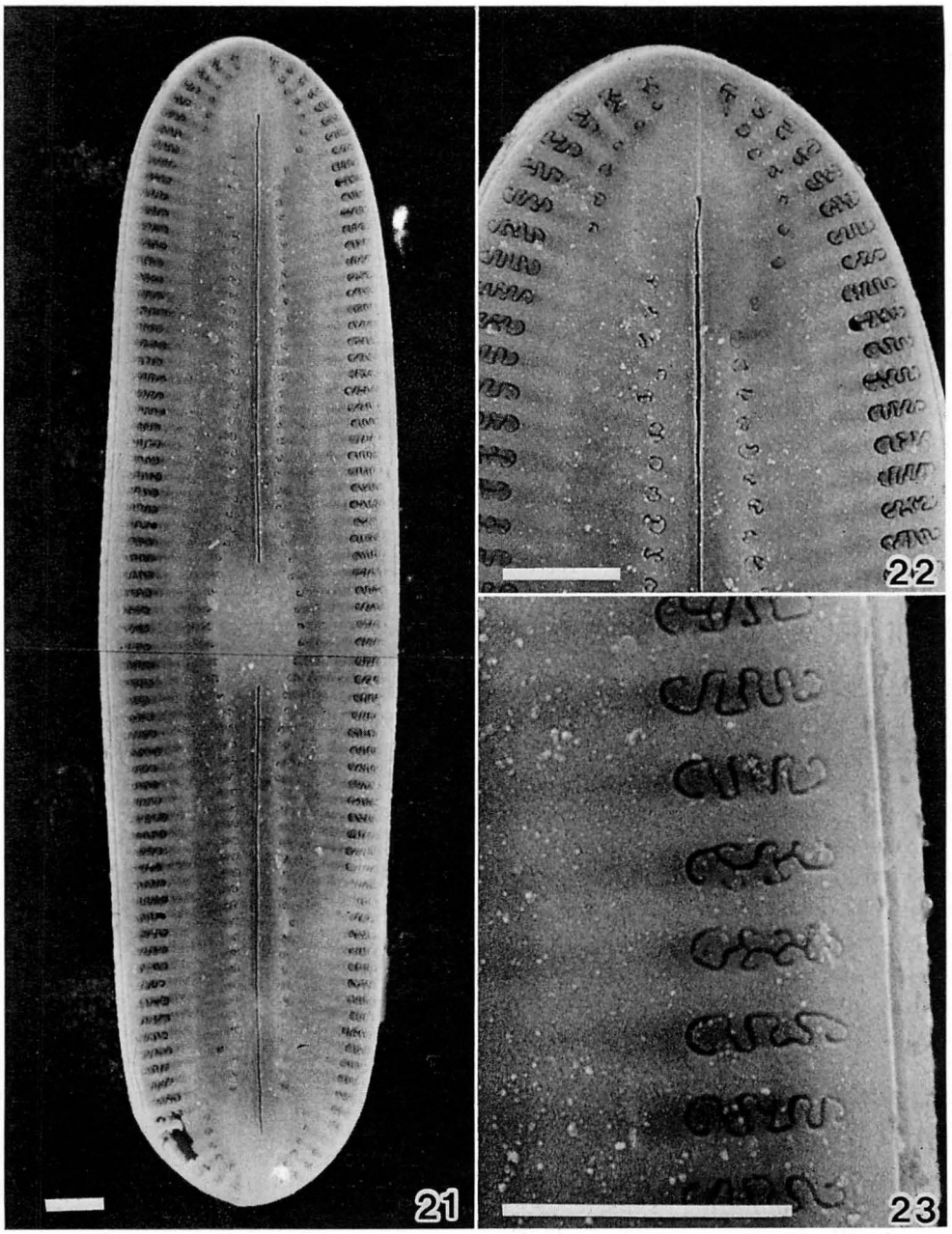


Plate 3. Figs 21-23. *Diploneis minuta* PET. On moss, Yōro Fall, Chiba Pref. N-1006 (=K-3172). SEM. Scale bar=1 μm. Fig. 21. Valve view, from outside. ×8,000. Fig. 22. Valve pole enlarged, from outside. ×16,000. Fig. 23. Alveoli enlarged, valve center from outside. ×40,000.

a short distance toward the axis (Figs 14, 15). Each alveolus opens laterally into the longitudinal canal through a tube-like opening (Fig. 15 arrow head) and opens externally through a sinuous slit with partial branching (Figs 11, 12). This structure was termed "sinuous line" by GERMAIN (1979).

The sinuous slit is formed as an interspace between variously shaped flaps extending from transapical costae. Each marginal flap is acutely elongate to the margin of the valve (Fig. 13). Internally, spine-like projections extend from each flap (Figs 16, 17 arrows). This feature seems to be peculiar to this species for it has hitherto appeared neither in the literature nor in our own observations.

Transapical costae are broader than alveoli in both inner and outer views (Figs 12, 14, 16) and are strongly constricted in the middle as clearly seen in the longitudinal section of the valve (Fig. 16).

The form of a cut end of a transapical costa seems to be an important taxonomic criterion, especially for this genus, although the fact has not been noticed by diatomists.

The cingulum is composed of two bands, one valvocopula and one pleura. The valvocopura is a broad open band (Fig. 18) with a smooth abvalvar edge and a serrated advalvar edge (Figs 18, 19) consisting of numerous small projections. Each projection lies on the internal surface of a transapical costa (Fig. 20). The pleura is a narrow open band except the broad mid-portion with a ligula which fits the opposing pole of the valve (Fig. 18).

Diploneis minuta PETERSEN
(1928, p. 381. fig. 6) Figs 6, 7, 21-23.

This species has only been found in a sample collected from moss on a wet rock in the spray zone of Yōro Fall, Chiba Prefecture. Valves observed are 11-25 μm in length, 4.5-5 μm in width. Valves of our specimens are longer than those of originally, described. It seems probable from PETERSEN's original description that he saw only one or two specimens, as the dimensions were given as 13 μm long and 4.4 μm broad. In 1937, HUSTEDT expanded the reported dimensions of this species to 13-18 μm long and 3.5-4 μm broad, presumably based on his reexamination of a slide prepared from the original material by the original author, for we have been able to see a slide No. 04/59 labeled "Iceland Eyvindará, Pet. 25" in the HUSTEDT Collection, Bremerhaven. This slide may be considered an isotype slide and the photomicrograph taken from this slide (Fig. 6) shows an isotype specimen. The striae density of our specimens is 28-32 in 10 μm, somewhat lower than that given for European specimens by HUSTEDT (1937) and GERMAIN (1979), namely, 32-35 in 10 μm. (Striae density was not given in the original description.)

Because of a scarcity of specimens in the sample, we could not get an adequate number of valves for a thorough SEM study. The fine structure that we were able to observe, however, is identical to that reported by GERMAIN (1979). The fine structure of *D. minuta* is similar basically to that of *D. oculata*. The valve face is almost flat and the raphe is a narrow straight slit externally. Both proximal and terminal endings are not dilated or curved (Figs 21, 22). The row of poroids bordering the axial area is almost straight and runs parallel to the longitudinal axis except the portions by the side of the central and terminal nodules (Figs 21, 22). The poroids are of a similar size and are occluded externally by mostly a round or reniform single flap.

The volate occlusion of the alveoli in *D. minuta* is relatively shorter than that of *D. oculata*, occupying only about one third of the valve width. On the other hand, the hyaline area between the axial row of poroids and the marginal row of occlusions of the alveoli is very broad, being about half of the valve width (Figs 21, 22). The sinuous slit on the volate occlusion is simple in shape. The slit is narrow and rarely branched (Fig. 23).

Acknowledgements

Especial thanks are due to Dr. REIMER SIMONSEN of the Institute für Meeresforschung, Bremerhaven, Dr. UWE PASSAUER of the Naturhistorisches Museum, Wien and Mr. T. B. B. PADDOCK of the British Museum (Natural History), London for access to slide collections. We also thanks to Prof. Chihara for helpful encouragement and advice and to Dr. PAUL C. SILVA of the University of California, Berkeley for reading and commenting on the nomenclatural problems and language problems, and Mr. T. NAGUMO, Mr. S. MAYAMA and Miss J. OZAWA for providing samples and useful assistance.

References

CLEVE, P. T. 1894. Synopsis of the naviculoid diatoms. Kongl. Sv. Vet. Akad. Handl. **26**(2) : 1-194.

GEISSLER, U., GERLOFF, J., HELMCKE, J.-C., KRIEGER, W. and REIMANN, B. 1975. *In* HELMCKE, J.-G. and KRIEGER, W. [ed.], Diatomeenschalen im electronenmikroskopishen Bild. Teil IV. J. Cramer, Weinheim.

GERLOFF, J. and HELMCKE, J.-G. 1975. Der fine Bau der Schalen von *D. papula* (A. S.) Cl., *D. smithii* (BRÉB.) CL. and *D. parca* (A. S.) BOY. Willdenowia **7**: 539-563.

GERMAIN, H. 1979. Detalis of structure in three small freshwater *Diploneis*. Nova Hedwigia Beih. **64**: 207-217.

GERMAIN, H. 1981. Flore des diatomées eaux douces et saumâtres. Boubée, Paris.

HELMCKE, J.-G. and KRIEGER, W. 1962. Diatomeenschalen im electronenmikroskopishen Bild. Teil II. J. Cramer, Weinheim.

HUSTEDT, F. 1930. Bacillariophyta. *In* PASCHER, A. [ed.], Süsswasser-Flora Mittereuropas. ed. 2. no. 10. Gustav Fischer, Jena.

HUSTEDT, F. 1937. Die Kieselalgen Deutschlands, Österreichs und der Schweiz. *In* RABENHORST, L. [ed.], Kryptogamen-Flora Deutschland, Österreich und der Schweiz. **7**(2) : 578-718.

KOBAYASI, H., KOBAYASHI, H. and IDEI, M. 1985. Fine structure and taxonomy of the small and tiny *Stephanodiscus* (Bacillariophyceae) species in Japan 3. Co-occurrence of *Stephanodiscus minutullus* (KÜTZ.) ROUND and *S. parvus* STOERM & HÄK. Jap. J. Phycol. **33**: 293-300.

OKUNO, H. 1964. Fossil diatoms. *In* HELMCKE, J.-G. and KRIEGER, W. [ed.], Diatomeenschalen im electronenmikroskopischen Bild. Teil V. J. Cramer, Weinheim.

OKUNO, H. 1970. Marine diatoms. *In* HELMCKE, J. G. and KRIEGER, W. [ed.] Diatomeenschalen im electronenmikroskopischen Bild. Teil VII. J. Cramer, Lehre.

MANN, B. G. 1981. Sieve and flaps: siliceous minutiae in the pores of raphid diatoms. pp. 279-300. *In* ROSS, R. [ed.], Proceeding of the sixth symposium on recent and fossil diatoms. Otto Koeltz, Koenigstein.

PATRICK, R. and REIMER, C. W. 1966. The diatoms of the United States. 1. Acad Nat. Sci. Philadelphia, Philadelphia.

PETERSEN, J. B. 1928. The aerial algae of Iceland. The Botany of Iceland. **2**(2) : 325-447. J. Frimodt, København.

SCHOEMAN, F. R. and ASHTON, P. J. 1981. The diatom flora in the vicinity of the Pretoria Salt Pan, Transvaal, Republic of South Africa I. Nova Hedwigia Beih. **73**: 21-54.

SIMS, P. A. and PADDOCK, T. B. B. 1979. Observations and comments on some prominent morphological feature of naviculoid genera. Nova Hedwigia Beih. **64**: 169-191.

出井雅彦*・小林　弘：淡水産デイプロナイス属（ケイソウ類），*D. oculata* (BRÉB.) CL. と *D. minuta* PET. の殻構造**

Grunow, Kützing, Hustedt のコレクション中の標本に基づいて同定した2種類のディプロナイス属ケイソウ，*D. oculata* と *D. minuta* を SEM と TEM を用い観察した。これらの種類の長胞はその外側を曲がりくねったスリットをもつ肉趾状師板によって閉ざされ，また，内側は六角整列をした小孔をもつ薄皮によって閉ざされていた。殻帯は幅の広い接殻帯片と幅の狭い連結帯片の2枚からなり，また，*D. oculata* の横走肋骨の断面は鼓状であった。(*305 茨城県新治郡桜村天王台 1-1-1 筑波大学生物科学系，**184 小金井市貫井北町 4-1-1 東京学芸大学生物学教室)