

Morphological variations in *Navicula atomus* (Kütz.) Grun.

by

S. Mayama and H. Kobayasi

Department of Biology, Tokyo Gakugei University,
Koganei-shi, Tokyo 184, Japan

Abstract: The slide containing the original specimens of *Amphora atomus* Kütz. was examined at the British Museum. On the basis of the observations, the specimens identified as *Navicula atomus* (Kütz.) Grun., which were collected from various locations in Japan and Germany, were examined using electron microscopy. These specimens showed a wide range of variation of the valve dimensions and the striation density. However there was no fine structural differences of the frustules though they seem to be separated into two types using light microscopy by a superficial discontinuity introduced by the optics. The detailed analyses of 14 randomly selected populations revealed that there was no recognizable discontinuity among the valves of these populations concerning the length, breadth, striation density and axial area breadth. The populations occurred with high relative frequency in rivers from clear waters to severely polluted ones and they did not seem to be separated ecologically. We consider that all these variants are conspecific at least concerning Japanese populations.

Though Kobayasi *et al.* (1985) have already commented briefly on the identity of *Navicula atomus* (Kütz.) Grun. and *Navicula permitis* Hust. on the basis of light microscopical observations of the type slide, further information on the circumscription of *N. atomus* is presented in this paper based on the detailed observations of *N. atomus* group collected from Japanese and German rivers and soils in Japan. Use was made of both scanning and transmission electron microscopy.

Key-words: diatoms, ultrastructure, taxonomy, indicator species, *Navicula*.

Introduction

As is obvious from many early studies, the description and figures of *Amphora atomus* Kütz. are not particularly informative and the understanding of the circumscription of *Navicula atomus* differs among diatomists (e.g. Lund 1946, Hustedt 1962, Lange-Bertalot & Bonik 1976, Archibald 1983, etc.). These authors seem to have built up their concept of the species based mainly on descriptions originating from Grunow (1860) or Van Heurck's Types de Synopsis des Diatomées de Belgique No. 149 and without examination of either Kützing's original slide or original material.

Specimens identifiable as *Navicula atomus* (Kütz.) Grun. have been found in many rivers polluted to varying degrees in Japan (Kobayasi *et al.* 1985) and its ecological importance for assessment of water quality has been evaluated (Kobayasi & Mayama 1982). Thus, access to Kützing's original type slide became essential to us.

Fortunately a slide which appeared to have been made from original material was found in the herbarium of the British Museum (Natural History) (BM). On the species card headed *Amphora atomus* in the Kützing collection in the BM, two sources are indicated, i.e., Nordhausen no. 24 and 475; these are samples collected from the type locality cited in the original description, although the slide number had not been indicated. The slide labelled '*Eunotia amphioxys* Ehr. Nordhausen, Kützing 24' was provided through the kindness of Ms P.A. Sims. In the slide (BM 17826), specimens which coincide well with Kützing's original description were found and we propose to designate it as the lectotype of *A. atomus*.

Material and Methods

The samples examined were collected from the following locations: Aono-gawa (Aono River) 1.5 km above its mouth, Shizuoka Pref. 30 May 1976, Temp. 19.0(°C), Salinity 6(‰), DO 6.4 (mgO₂·l⁻¹), Elec. Cond. 9,100 (μS·cm⁻¹ at 25°C), Sample No. K-40; Aono-gawa at Kamigamo, Shizuoka Pref. 30 May 1976, Temp. 19.5, pH 7.2, E.Cond. 105, Sample No. K-44; Ikeshiro-gawa at Minewa, Shizuoka Pref. 30 May 1976, Temp. 17.5, pH 7.5, DO 8.1, E.Cond. 90, Sample No. K-54; Aono-gawa at Kawaino, Shizuoka Pref. 5 December 1977, Temp. 13.3, pH 8.2, E.Cond. 116, Sample No. K-372; Tama-gawa at Sannose, Yamanashi Pref. 23 October 1978, Temp. 7.0, pH 7.1, DO 11.6, E.Cond. 20, Sample No. K-491; soil beside the Natural Science Hall of Tokyo Gakugei Univ. 14 April 1980, pH 7.2, Sample No. K-829; soil of the farm in the campus of the Tokyo Gakugei Univ. 9 May 1980, Sample No. K-832; soil behind the house of student's circles of the Tokyo Gakugei Univ. 9 May 1980, Sample No. K-846; Yachi-gawa above its confluence with Tamagawa, Tokyo. 21 July 1980, Temp. 26.5, pH 7.4, DO 8.0, BOD 3.7 (mg O₂·l⁻¹), E.Cond. 280, Sample No. K-914; soil of the farm of the Tokyo Gakugei Univ. 23 October 1980, pH 7.2, Sample No. K-923; Minamiasa-kawa above the Higashiyokoyama Bridge, Tokyo. 23 May 1981, Temp. 24.0, E.Cond. 148, Sample No. K-1092; Minamiasa-kawa below the Higashiyokoyama Bridge, Tokyo. 17 October 1981, Temp. 17.5, pH 7.2, DO 8.7, BOD 7.5, E.Cond. 270, Sample No. K-1247; Ohguri-gawa at the Houon Bridge, Tokyo. 4 December 1981, Temp. 10.5, pH 7.4, DO 10.1, BOD 6.3, E.Cond. 352, Sample No. K-1421; Shingashi-gawa at the Shimo Bridge, Tokyo. 23 March 1982, Temp. 12.4, pH 7.2, DO 0.8, BOD 18, E.Cond. 656, Sample No. K-1437; Hohno-gawa below its confluence with the Mozato-gawa, Nara Pref. 26 April 1983, Temp. 20.8, E.Cond. 180, BOD 2.8, Sample No. K-1634; Tsurumi-gawa at the Asoh Bridge, Tokyo. 1 March 1983, Temp. 9.5, pH 7.5, DO 9.8, BOD 14, E.Cond. 354, Sample No. K-1656; River Main at Trennfeld, Germany. 27 August 1980, Temp. 18.0, pH 8.9, Sample No. K-2782; River Neckar at Heidelberg, Germany. 27 August 1980, Temp. 20.0, pH 7.5, Sample No. K-2787. The samples from rivers were all epilithic. The soil diatoms were collected from undersides of coverglasses which were placed on the moistened soil for seven days at 15°C. This method has the advantage that living cells can be gathered effectually as mentioned by Lund (1945). The cleaning method for all samples are described by Mayama & Kobayasi (1984).

SEM and TEM observations were made using JEOL F15 and JEOL 7 electron microscopes respectively. Transapical striae density is given after conversion into a value per 10 μm; striae were counted along the margin (5 μm) near the middle of

this small diatom (Anonymous 1975). The breadth of the axial area was measured at the mid-portion between the central nodule and the terminal nodule.

Results

Specimens found in the lectotype slide of *Amphora atomus* (BM 17826) revealed a wide range of striation density – 18-28 in 10 μm . Valves were 8-10 μm in length, 3.5-4.5 μm in breadth (Figs 1-4). In the case of densely striated specimens, the striae are invisible without an oblique bright field illumination. Our specimens identified as *Navicula atomus* can be divided broadly into two types. One is a larger valve, having coarser striae which are distinct using a bright field illumination (Figs 5-10) and the other is comparatively small, having closer striae which can be seen only by using oblique illumination (Figs 11-16). Photomicrographs taken from living cells of the larger type (Figs 17-19) and smaller type (Figs 20,21) agree well with the original figures of *A. atomus* which seem to be drawn from living specimens with protoplast, though we cannot obtain the correct dimensions of the original figures due to the lack of any indication of the magnification in the original text.

No fine structural differences have been found between the two types of typical populations, K-1437 (Figs 22,24,26) and K-1656 (Figs 23,25,27); the former specimens possess coarser while the latter possess closer striae. Both specimens show that externally the valve face is smooth and the raphe branches are slightly curved and the terminal fissure and the expanded central raphe endings bend in the opposite directions (Figs 22,23). Internally both have a somewhat thickened axial area accompanied by terminal nodules and the central nodule (Figs 24,25). Each poroid is occluded by a rica near the outer surface (Figs 22,23,24,27). The ricae of both types of valves bear perforations of the same type and density. The perforations are regularly scattered, and 9-10 in 0.1 μm (Figs 39,40). The mature cingulum consists of three bands in both specimens (Figs 26,27).

Using light microscopy for small diatoms like *N. atomus*, difficulty always occurs in the measurement of the dimensions and the striae count. Therefore, in the present study these valves are measured from the TEM photographs taken from 14 populations. As seen in Table 1, there are no recognizable discontinuities among the valves of these populations in the length, breadth, striation density and axial area breadth (Table 1). The values obtained from each population range from 5.9-10.0 μm for length, 2.6-4.4 μm for breadth, 18-40 striae in 10 μm and 0.36-0.99 μm in axial area breadth. In addition, the length of the central nodule and the average poroid density in the striae are 0.50-0.89 μm in length and 34-70 poroids in 10 μm , respectively. There is a tendency within these populations that the length, the breadth, the axial area breadth and the central nodule length are positively correlated, while the striation density and poroid density are negatively correlated against the dimensions (Figs 28-38). The negative correlation between length and striae density seems to be especially remarkable (Text fig.1). Besides the above-mentioned features, others such as the angles formed between striae and the apical axis, the number of the inserted short central striae and the shape of the central area are also variable; however we could not find any correlation between these and the above-mentioned features.

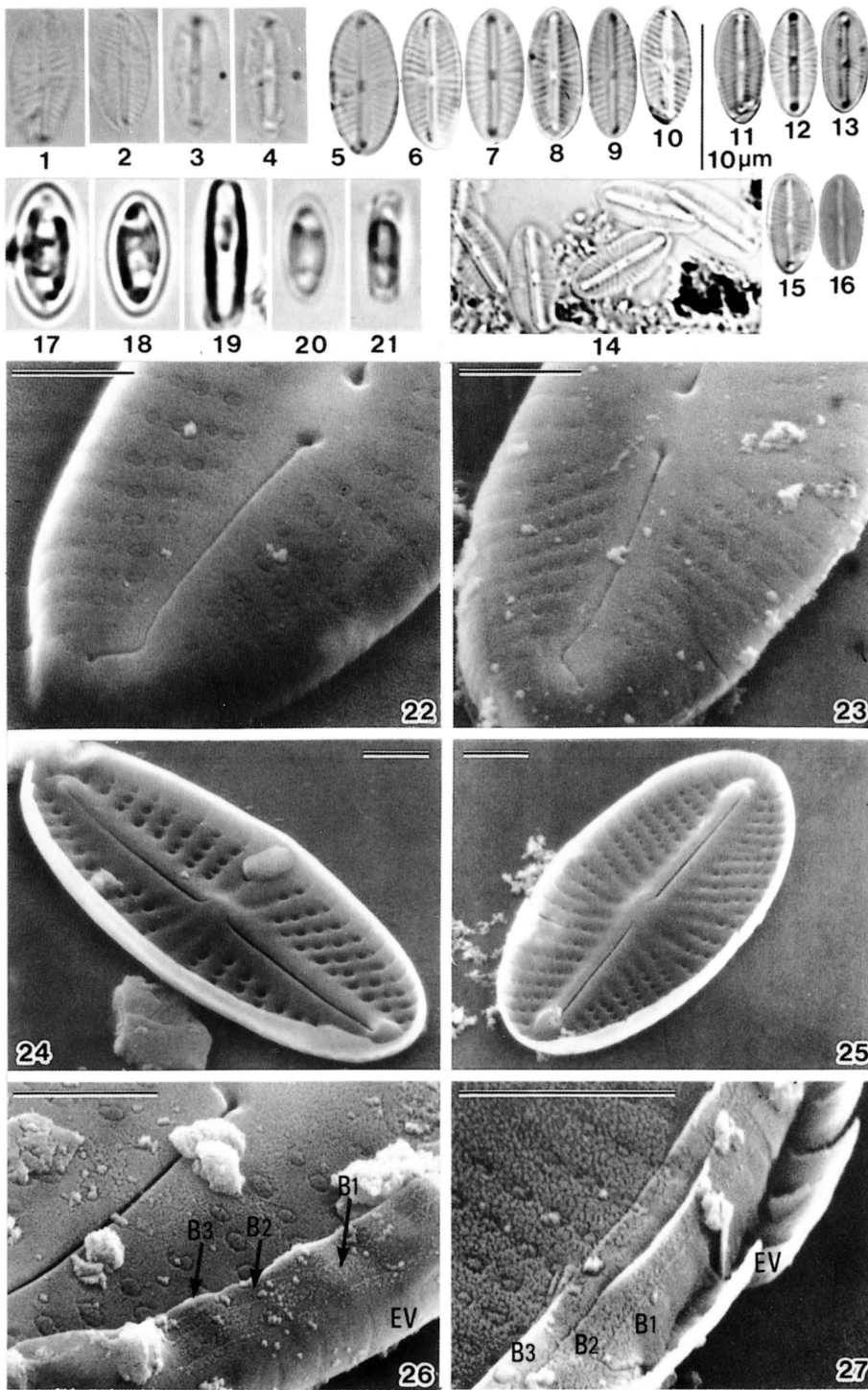


Table 1. Summary of the dimensions and striation densities of the specimens observed using TEM in 14 populations.

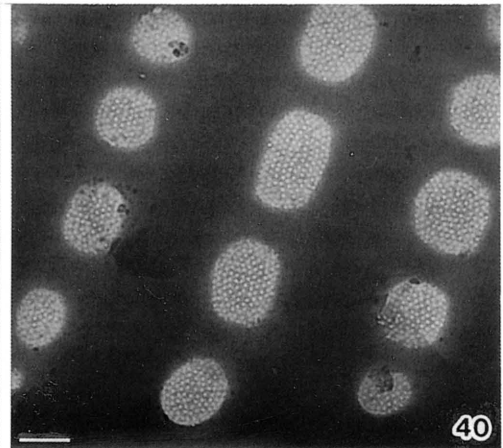
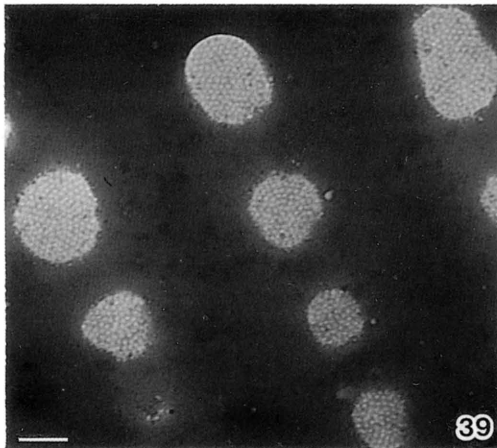
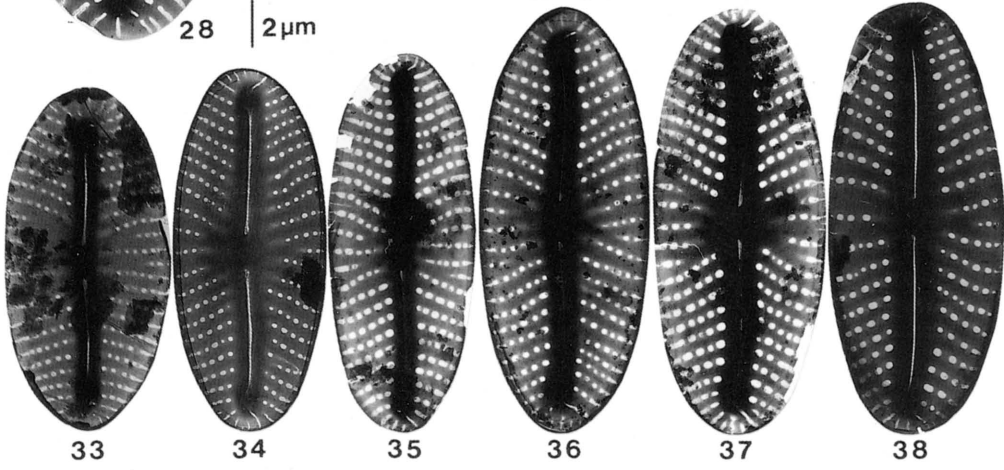
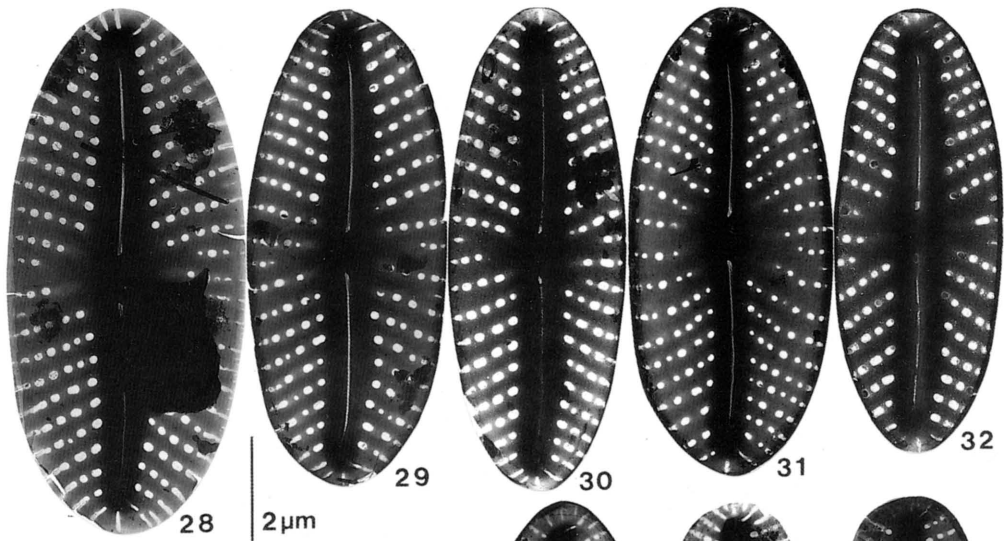
Sample no.	Length (μm)	Breadth (μm)	Striae in 10 μm	Breadth of axial area (μm)	Valves observed
K- 44	6.1- 7.5	2.7-3.5	30-34	0.41-0.50	16
K- 54	6.2- 7.3	2.7-3.0	32-38	0.46-0.47	6
K- 491	6.4- 6.9	2.8-2.9	33-37	0.46-0.55	6
K- 829	6.6-10.0	2.8-4.4	19-31	0.44-0.93	66
K- 832	8.4- 7.0	3.1-3.5	21-26	0.75-0.93	5
K- 914	6.4- 7.8	2.7-3.1	29-33	0.45-0.63	19
K- 923	6.8- 9.0	2.9-3.8	19-33	0.49-0.94	14
K-1092	6.9- 7.8	2.6-3.5	29-36	0.44-0.61	21
K-1247	7.0- 8.4	2.9-4.1	26-36	0.40-0.54	19
K-1421	7.2- 7.8	3.4-3.6	26-32	0.53-0.57	5
K-1437	7.0- 9.8	3.2-4.4	18-26	0.61-0.99	35
K-1656	6.8- 7.4	2.7-3.4	29-33	0.50-0.63	18
K-2782	6.5- 8.3	2.7-3.4	25-36	0.39-0.59	14
K-2787	5.9- 7.1	2.6-3.2	32-40	0.36-0.50	25
Range	5.9-10.0	2.6-4.4	18-40	0.36-0.99	Total 269

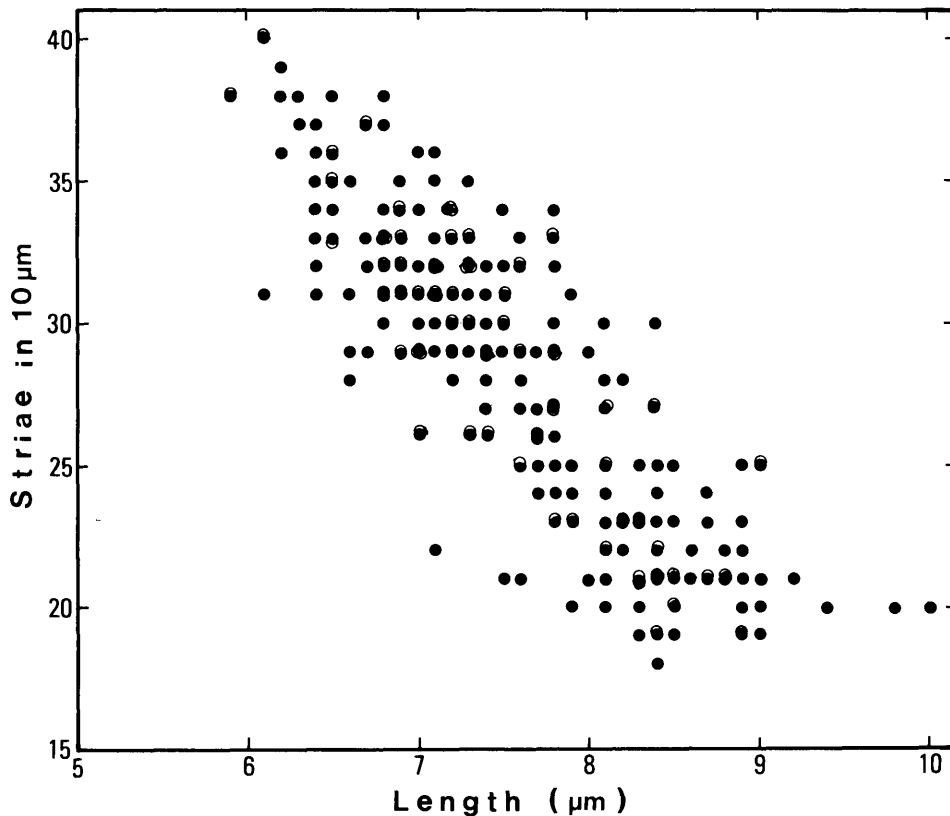
Discussion

It is quite likely that Kützing also used Paris lignes for the measurement of dimensions of the diatoms in his 'Die Kieselschaligen Bacillarien order Diatomeen' according to Schoeman & Archibald (1977). If this is true, the valve length of *Amphora atomus* as originally described corresponds to 10.25 μm in the metric system. However, this valve is larger than those of the specimens found in the lectotype slide (BM 17826). In the TEM observations, Reimann *et al.* (1966) revealed a mucilaginous sheath surrounding the walls of this species, which was misidentified as *Navicula pelliculosa* in the paper. This zone is the same as the capsule accumulating around

Plate 1

Figs 1-27. *Navicula atomus* (Kütz.) Grun. Figs 1-4. Nordhausen, Germany, Kützing Coll., BM 17826, desig. Lectotype. Figs 3, 4 are the same specimens but the latter was taken using an oblique bright field illumination (OI). Figs 5, 7. Shingashi-gawa, K-1437. Fig.6. Hohno-gawa, K-1634. Fig.8. Soil, K-846. Fig.9. Soil, K-829. Fig.10. Soil, K-923. Figs 11, 12 and 14. Tsurumi-gawa, K-1656, OI. Fig.13. Aonogawa, K-40, OI. Fig.15. Minamiasa-kawa, K-1092, OI. Fig.16. River Neckar, Germany, K-2787. Striae are not resolved despite using OI. Figs 17-19. Raw specimens from Shingashi-gawa, K-1437. Figs 20, 21. Raw specimens from Tsurumi-gawa, K-1656. Figs 22, 23. Exterior view of valve. Fig.22. Shingashi-gawa, K-1437 and Fig.21 Tsurumi-gawa, K-1656. Figs 24, 25. Internal view of valve. Fig.24. Shingashi-gawa, K-1437 and Fig.25 Tsurumi-gawa, K-1656. Figs 26, 27. Epitheca composed of epivalve (EV), a valvocopula (B1) and two bands (B2, B3). Fig.26. Shingashi-gawa, K-1437, Fig.27. Tsurumi-gawa, K-1656. (Figs. 22-27. Scale bars = 1 μm).





Text fig.1. Plot of valve length versus striation density on the specimens recorded in Table 1. Each dot represents one specimen.

the cells as observed under light microscopy by Lewin (1955). Judging from the original figures of the protoplast, Kützing's figures are clearly drawn and measured from living material. Consequently the valve length expressed by Kützing is a little larger than that of actual valves.

Navicula pseudatomus described as a soil diatom by Lund (1946) was synonymized with *Navicula atomus* by Hustedt (1962). The fact that our specimens from soils are involved in the same series of the variation as the population from the rivers supports Hustedt's opinion.

The smaller specimens with denser striae agree well with the TEM photographs presented by Lange-Bertalot & Bonik (1976) and Germain (1981), though their specimens were identified as *Navicula permitis* Hust. Archibald (1983) gave details

Plate 2

Figs 28-40. *Navicula atomus* (Kütz.) Grun. Figs 28,29,31,38. Shingashi-gawa, K-1437. Fig.30. Soil, K-829. Fig.32. Soil, K-923. Fig.33. River Neckar, K-2787. Fig.34. Tsurumi-gawa, K-1656. Fig.35. River Main, Germany, K-2782. Fig.36. Aono-gawa, K-372. Fig.37. Tama-gawa, K-5829. Figs 39, 40. Pore occlusions with perforations of a regular scatter type (Scale bars = 0.1 µm). Fig.39 specimen with coarser striae, Tsurumi-gawa, K-1437 and Fig.40 specimen with denser striae, Aono-gawa, K-44.

of type specimens of *N. permitis* and referred to many specimens which seemed to be conspecific with *N. permitis*. Our small specimens also coincide with these. However, the valve structure of *N. permitis* is the same as that of *N. atomus* and only the valve dimensions and the striation density differ between the two taxa; there is a continuity from the small to the large and the dense to the coarse. The girdle bands of *N. permitis* are examined from cultured material by Chiappino & Volcani (1977), (Strain no. 688, Indiana University Culture Collection). This strain had been misidentified as *Navicula pelliculosa* (see Archibald 1983). The epicingulum at interphase was shown to be composed of three bands. The same set of epibands is observed not only in the specimens of 'permitis' type but also in the larger specimens with coarser striae. Okuno's electron micrograph (1979, pl.879) identified as *N. permitis* by Lange-Bertalot & Bonik (1976) has a perforation density of the rita of about 10 in 0.1 μm , and our specimens of both types also conform to this measure. Therefore specimens with such similar structure should not be divided into two species by superficial observations based on optical observations.

Recently Krammer & Lange-Bertalot (1985) have proposed a new combination, *Navicula atomus* var. *permitis* (Hust.) Lange-Bertalot, based on the isolated occurrence of the variety. Variation in occurrence of infraspecific taxa has been recognized in the two varieties of *Achnanthes minutissima* Kütz. which are distributed according to the degree of river water pollution (Mayama & Kobayasi 1984). However, the populations of 'permitis' type and the larger ones with coarser striae did not show any such ecological separation in the rivers in Tokyo and its vicinity. They occurred widely and sometimes abundantly in rivers in which water qualities were estimated to be I/II to IV (Kobayasi *et al.* 1985). Therefore it seems unreasonable to separate the Japanese populations into two taxa based on ecological data.

Acknowledgments

We are grateful to Mr. T.B.B. Paddock and Ms P.A. Sims of the British Museum (Natural History), London for their kind help in access to slide collections, and to Ms F. Itoh of the Tokyo Gakugei Univ. for her preparation of soil diatoms. Also we would like to thank Dr. F.R. Schoeman of the National Institute for Water Research, Pretoria and Dr. R. Jahn, Freie Universität Berlin for their kind suggestions and information to the Kützing's measurements.

References

- ANONYMOUS (1975). Proposals for a standardization of diatom terminology and diagnoses. *Nova Hedwigia Beih.* **53**, 323-354.
- ARCHIBALD, R.E.M. (1983). The diatoms of the Sundays and Great Fish Rivers in the Eastern Cape Province of South Africa. *Bibl. Diatomol.* **1**, 1-362. pl. 1-34.
- CHIAPPINO, M.L. & VOLCANI, B.E. (1977). Studies of the biochemistry and fine structure of silica shell formation in diatoms. VII. Sequential cell wall development in the pennate *Navicula pelliculosa*. *Protoplasma* **93**, 205-221.
- GERMAIN, H. (1981). *Flore des Diatomées eaux douces et saumâtres*. Boubée, Paris.
- GRUNOW, A. (1860). Über neue order ungenugend gekannte Algen. Erste Folge, Diatomaceen, Familie Naviculaceen. *Verh. kais.-kön. zool.-bot. Ges. Wien* **10**, 503-582, pl. 3-7.
- HUSTEDT, F. (1962). Die Kieselalgen Deutschlands, Österreichs und der Schweiz unter Berücksichtigung der übrigen Länder Europas sowie der angrenzenden Meeresgebiete. In L. Rabenhorst (ed.), *Kryptogamen-Flora von Deutschland, Österreich und der Schweiz*. **7**, Teil 3, Lief 3, Akad. Verlag., Leipzig.
- KOBAYASI, H. & MAYAMA, S. (1982). Most pollution-tolerant diatoms of severely polluted rivers in the vicinity of Tokyo. *Jap. J. Phycol.*, **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 BODs. *Bull. Tokyo Gakugei Univ. Sect. 4*, **37**, 21-46.

- KRAMMER, K. & LANGE-BERTALOT, H. (1985). Naviculaceae, neue und wenig bekannte Taxa, neue Kombinationen und Synonyme sowie Bemerkungen zu einige Gattungen. *Bibl.Diatomol.*, **9**, 1-230.
- LANGE-BERTALOT, H. & BONIK, K. (1976). Massenentwicklung bisher seltener und unbekannter Diatomeen als Indikator starker Abwasserbelastung in Flüssen. *Arch.Hydrobiol.Suppl.* **49**, 303-332.
- LEWIN, J.C. (1955). The capsule of the diatom *Navicula pelliculosa*. *J.gen.Microbiol.* **13**, 162-169.
- LUND, J.W. (1945). Observations on soil algae. I. The ecology, size and taxonomy of British soil diatoms, part 1. *New Phytologist* **44**, 196-219.
- LUND, J.W. (1946). Observations on soil algae. I. The ecology, size and taxonomy of British soil diatoms, part 2. *New Phytologist* **45**, 56-110.
- MAYAMA, S. & KOBAYASI, H. (1984). The separated distribution of the two varieties of *Achnanthes minutissima* Kuetz. according to the degree of river water pollution. *Jap.J.Limnol.* **45**, 304-312.
- OKUNO, H. (1974). Freshwater diatoms. In: *Diatomeenschalen im elektronen-mikroskopischen Bild*. (J.-G.Helmcke, W.Krieger & J.Gerloff, eds.). Teil IX, J. Cramer, Vaduz.
- REIMANN, B.E., LEWIN, J.C. & VOLCANI, B.E. (1966). Studies on the biochemistry and fine structure of silica shell formation in diatoms. II. The structure of the cell wall of *Navicula pelliculosa* (Bréb.) Hilse. *J.Phycol.* **2**, 74-84.
- SCHOEMAN, F.R. & ARCHIBALD, R.E.M. (1977). The diatom flora of Southern Africa. No. 3. Explanatory Notes (2). *CSIR special report WAT 50*. CSIR, Pretoria.