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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. & HÅK.

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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. & HÅK. Jap. J. Phycol. 33: 293-300.

Specimens which can be identified either as *Stephanodiscus minutullus* or as *S. parvus* have been found in some fresh and brackish waters such as Hime-numa, Oo-numa, Hachiro-gata and Nakagawa in Japan. Comparative studies on the valve structure of these specimens using mainly a scanning electron microscope reveal the fact that *S. minutullus* and *S. parvus* are conspecific. The central elevation or depression of the valves varies continuously from flat to strongly undulate surfaces.

Key Index Words: *Centric diatom*; *fine structure*; *plankton*; Stephanodiscus minutullus; Stephanodiscus parvus.

Specimens which can be identified either as *Stephanodiscus minutullus* (KÜTZ.) ROUND or as *S. parvus* STOERM. & HÅK. often co-occurred in the materials collected from fresh and brackish waters in Japan. They are small and circular in valve view, being 6-10 (up to 15) μm in diameter. The valve faces observed vary from flat or almost flat ones to strongly concentrically undulate ones, though it is often very difficult to ascertain surface undulations in the case of smaller specimens.

The type material from the Lünneburger Heide of *Cyclotella minutula* KÜTZ. (1844) was examined by ROUND (1981) using mainly scanning electron microscope (SEM). It was transfered as an separate species to the genus *Stephanodiscus* by ROUND, although it had been included in *S. astraea* as var. *minutula* (KÜTZ.) GRUN. in VAN HEURCH (1880-1883) and later in *S. rotula* as var. *minutula* by ROSS and SIMS (1978). This taxon is strongly characterized by the valves with conspicuous central elevation or depression as seen in ROUND's Figs. 19-23 besides other features.

On the other hand, *Stephanodiscus parvus* STOERM. & HÅK. (1984) is the youngest taxon which was newly named based on the original materials from which invalid GRUNOW's *S. hantzschii* form. *parva* was named only in his note book. This name was published later by CLEVE and MÖLLER (1877-1882, slides Nos. 265, 266, examined by GRUNOW from Ceyssal, Puy-de-Dôme. France.) As seen in Figs 5-8 of STOERMER and HÅKANSSON (1984), this taxon is character-

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ized by the flat or almost flat valve faces. However when we kept an eye on the features other than the undulations of the valve face, *S. minutullus* and *S. parvus* seemed to be quitei dentical in valve structure. It has been suggested by HÅKANSSON that "the difference between *S. parvus* and *S. minutullus* can sometimes be very difficult, because it is not always possible to see if the valve is flat or not." (pers. letter to H. KOBAYASI on 6th Feb. 1985). In order to clarify the relationship between these two taxa, comparative studies were carried out.

Materials and Methods

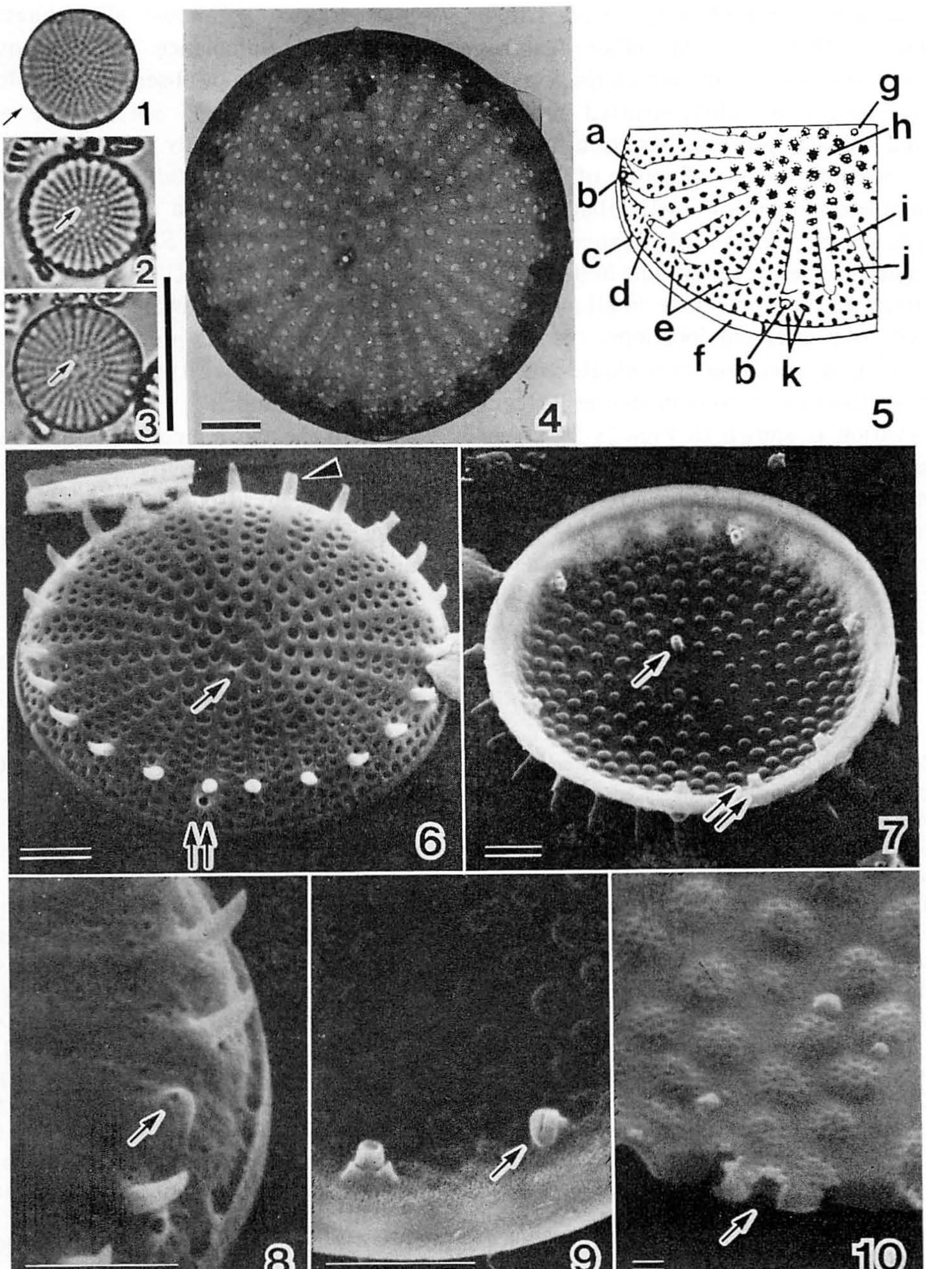
Materials used were collected from the following locations 1) Bottom mud in small lake Hime-numa (pH. 7.7, wt. 15°C, salinity 0‰) in Rishiri Island, northern Hokkaido on 25th Aug. 1984, K-1950. 2) Bottom mud in brackish lake Oo-numa (pH. 8.5, wt. 17°C, salinity 9‰), northern Hokkaido on 25th Aug. 1984, K-1954. 3) Plankton in lagoon Hachiro-gata (pH. 7.5, wt. 12.8°C, salinity 56.8 mgCl/*l*), Akita Prefecture on 4th Oct. 1985, N-1005. 4) Plankton in the estuary of the Naka-gawa (pH. 7.0, wt. 14°C, salinity 18‰), at the Shiodome Bridge on 9th Nov. N-935.

Materials were cleaned by ultraviolet radiation which was very effective in cleaning without destroying the weakly silicified structure, or cleaned with sulfuric acid and potassium dichromate, followed by washing with distilled water. SEM and TEM observations were made using a JEOL F15 and a JEOL 100B respectively.

Results

Observations of the specimens that can be identified either as *Stephanodiscus parvus* or as *S. minutullus* using a light microscope showed fully similar appearance with regard to the valve view. Valves are seen almost flat in the most abundant size, being 7-10 μm in diameter (Figs 1-3, 11, 12) except the extremely larger ones, up to 15 μm in diameter in that the centric undulation of the valve face is always evident (Figs. 14, 15). However, as shown in Fig. 13, the undulation of the valve face is clearly noticed in small specimens in a oblique view. Facicles are a single row at the center and obscurely biseriate at the periphery of the valve and the central region appears to have randomly arranged puncta. A short spine occurs at the end of nearly every interfascicle and by downward focusing, it is possible to see that the marginal strutted process occurs as a dot every 3rd to 6th interfascicle just below the spines (Figs 1, 3, 11, 14). At the center of the valve, the rosette-like structure is inconspicuous. This structure is called rosette or rosette-like structure by LOWE and CRANG (1972), STOERMER and HÅKANSSON (1983), annulus by STOSCH von (1977), and pattern center by MANN (1984), as seen in *Stephanodiscus invisitatus* HOHN & HELL. (KOBAYASI and INOUE, 1985) and *S. hantzschii* form. *tenuis* (HUST.) HÅK. et STOERM. (1984) (KOBASYAI *et al.* 1985). The central strutted process is also less conspicuous at the same level focusing with the striae of the fascicles due to its depth.

Valves with a flat face are shown in Figs 1-10, and the diagramatic representation of the features is shown in Fig. 5. The marginal spines occur at the end of each interfascicle and the marginal strutted processes are placed very close to the spines they subtend (Figs 5b, 6). The valve mantle is very shallow. Each stria, a row of pores, on the mantle is composed of three to four pores and only one or two pores just beneath the marginal strutted process. It reaches to the valve edge as an extention of each stria forming fascicles (Fig. 4). The pores surrounding each marginal strutted process and spine are arranged radially forming an asteroid mark (Fig. 6 double arrow). This arrangement is conspicuous in the moderately or lightly silicified valves (Fig. 6) but obscured in the heavily silicified ones (Fig. 8). The structure of valve varies markedly depending on the degree of silicification. In the lightly silicified specimens the exterior opening of each pore is more or less broad (Figs 6, 10 arrow). In the more heavily silicfied ones, it is reduced in size and tends to be partially occluded externally (Fig. 8). Each areola of both types of valves is occluded by the domed cribrum internally (Figs. 9, 10). A single strutted process with two struts is generally seen towards the center of the valve (Figs 4, 7 arrow). A single marginal labiate process occurs on a interfascicle in the nearly opposite direction to the central strutted process, at nearly the same level with the marginal ring of spines (Figs 7 double arrow, 9 arrow). The external opening of the single marginal labiate process is tubular in moderately silicified valves (Fig.



6 arrow head) and is variously modified spine shape in heavily silicified valves (Fig. 8 arrow). They are all open in place of a marginal spine and the longitudinal slit of the process is situating obliquely to the radial axis in a different degree internally (Figs 7, 9).

Valves with an undulate face are shown in Figs 11-22. As seen in the figuers, undulate valves seem to be more heavily silicified than flat ones. However, the other features such as the fascicles, interfascicles, central strutted process, marginal strutted process, marginal labiate process, striation on the mantle, and the areolar structure of the pore are completely identical with that of flat valves as shown in Figs 1-10. It appears to be a continuous series of gradations in degree of undulation of the valve face in all materials collected from different locations. In order to visualize a variation in a single population, a series of gradations in degree of central elevation is shown in Figs 23-28, from flat valve (Fig. 23) to slightly (Figs 24, 25), moderately (Figs 26, 27) and strongly undulate valves (Fig. 28). In these figures variation in silicification can also be seen from a moderately silicified valve (Fig. 23) to the most heavily silicified one (Fig. 28).

Discussion

Small and tiny diatoms, with circular valves, of the genus *Stephanodiscus* are distributed worldwide in rivers, lakes, lagoons and reservoirs and often constitute the major component of the plankton (ROUND 1981). However, there is serious confusion in identification among these species mainly due to their co-occurrence at almost all habitats, morphological resemblance and inadequacy of the light microscope observations. Moreover, almost all of these species are strongly polymorphic as already pointed out by ROUND (1981) on *S. minutullus*, STOERMER and HÅKANSSON (1984) on *S. parvus*, KOBAYASI and INOUE (1985) on *S. invisitatus*, HÅKANSSON and STOERMER (1984) on *S. hantzschii*, KOBAYASI *et al.* (1985) on *S. hantzschii*, form. *tenuis*. In the present studies both flat and undulate valve types co-occurred in all materials examined.

Due to the above mentioned disadvantages, taxonomy of the group has strongly been left behind until observations of the type materials using mainly scanning electron microscope were carried out in detail especially with regard to some important classical species (ROUND 1981, STOERMER and HÅKANSSON 1984, HÅKANSSON and STOERMER 1984). However, there seems to still remain minor

Plate 1. Figs 1-10. *Stephanodiscus minutullus* (KÜTZ.) ROUND with flat or almost flat face. Hime-numa unless otherwise noted. Fig. 1. Valve view in lower focus showing marginal strutted process and the marginal labiate process (arrow) LM ×2000. Figs 2, 3. Valve views in upper and lower focus of the same specimen showing the central strutted process appeared as black and white dots (arrows) LM ×2000 (bar=10 μm). Fig. 4. Valve view of heavily silicified valve showing the interfascicular thickening and a central strutted process with two struts TEM ×7000 (bar=1 μm). Fig. 5. Diagramatic representation of the exterior valve structure. a. marginal spines b. marginal strutted processes, c. vertical slit-like marking of the flange, d. outer opening of the labiate process, e. areolar rows on the valve mantle, f. flange, g. central strutted process, h. pattern center, i. interfascicles, j. fascicles, k. asteroid arrangement of pores. Fig. 6. Outside view of valve showing central strutted process (arrow), marginal strutted process, external opening of the labiate process (arrow head) and pores forming asteroid mark (double arrow) SEM ×9300 (bar=1 μm). Fig. 7. Inner view of valve showing the central strutted process with two struts (arrow), and marginal labiate process (double arrow). Oo-numa. SEM ×7500 (bar=1 μm). Fig. 8. Enlargement of valve margin showing the outer opening of the labiate process (arrow) SEM ×20000 (bar=1 μm). Fig. 9. Enlargement of inner valve margin showing the marginal strutted process with three struts and labiate process (arrow). SEM ×20000 (bar=1 μm). Fig. 10. Inner view of broken valve showing the areola with broad exterior opening (arrow). SEM ×40000 (bar=0.1 μm).

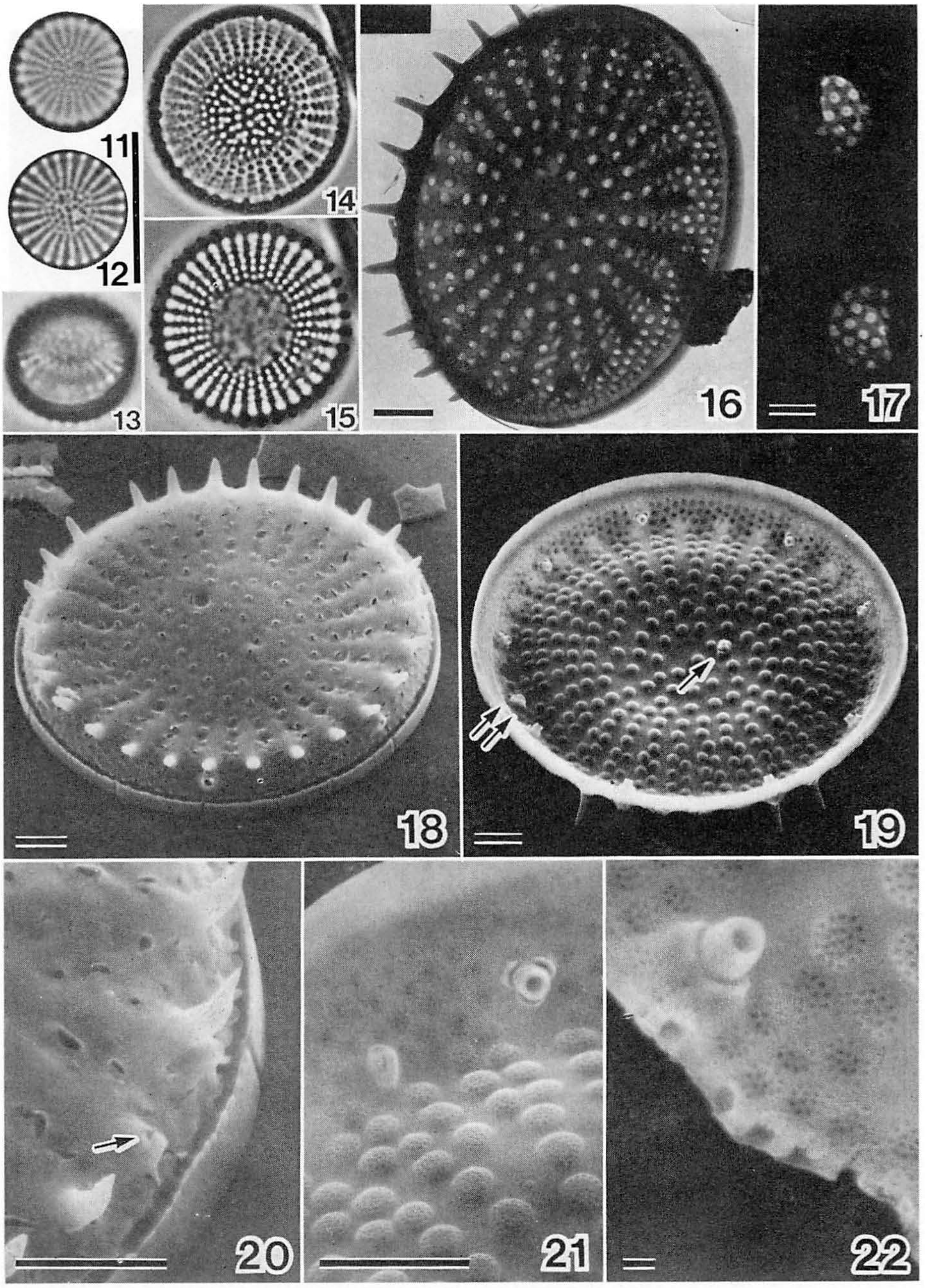


Plate 2. Figs 11–22. *Stephanodiscus minutullus* (KÜTZ.) ROUND with undulate face. Himenuma. Figs 11, 12. Valve view. LM ×2000 (bar=10 μm). Fig. 13. Oblique view of frustle. LM ×2000. Figs 14, 15. Valve view in lower and upper focus of the same valve. LM ×2000. Fig. 16. Oblique view of heavily silicified valve. TEM ×8000 (bar=1 μm). Fig. 17. Enlargement of domed cribrum occluding areola, with perforations in a hexagonal array. TEM ×60000 (bar=0.1 μm). Fig. 18. Outside view of heavily silicified and slightly undulate valve. SEM ×6800 (bar=1 μm). Fig. 19. Inside view of valve showing the central strutted process with two struts (arrow) and marginal labiate process (double arrow). SEM ×6300 (bar=1 μm). Fig. 20. Enlargement of valve margin showing the outer opening of the labiate process (arrow). SEM ×20000 (bar=1 μm). Fig. 21. Enlargement of inner valve margin showing the marginal strutted process with three struts and the labiate process. SEM ×20000 (bar=1 μm). Fig. 22. Inner view of broken valve margin showing the areolae occluded by flaps externally and the domed cribra internally. SEM ×40000 (bar=0.1 μm).

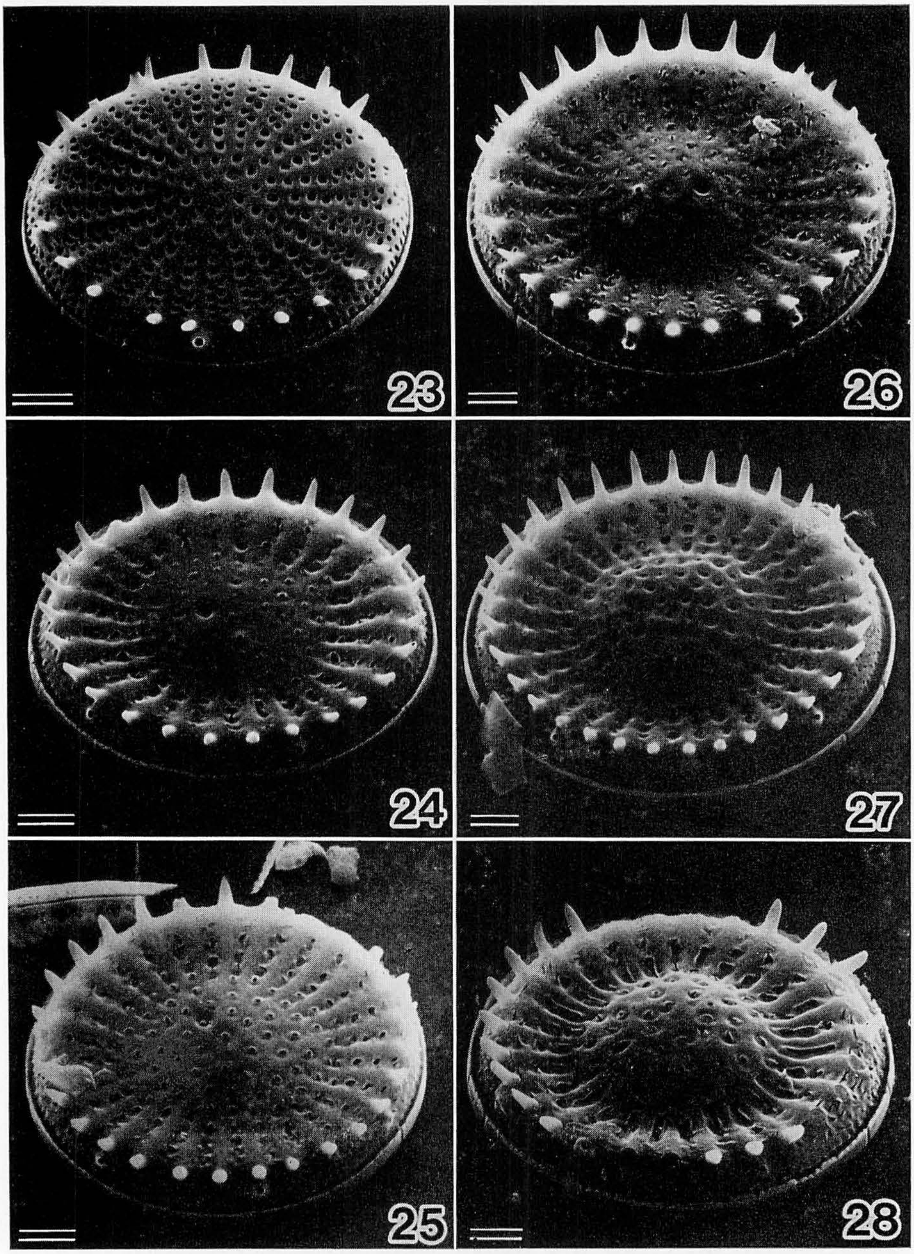


Plate 3. Figs 23–28. A series of gradations in degree of central elevations of *Stephanodiscus minutullus* (KÜTZ.) ROUND. Hime-numa bar=1 μm. Fig. 23. Flat and mediately silicified valve. SEM ×7800. Figs. 24, 25. Slightly undulate valves. SEM ×7500. Figs 26, 27. Moderately undulate valves. SEM ×6600 and SEM ×6200. Fig. 28. Strongly undulate and heavily silicified valve. SEM ×7000.

confusions. In the three light microscopical photographs of STOERMER and HÅKANSSON (1984) taken from the type materials of *S. parvus*, Fig. 1 seems to be a specimen with an undulate face and Fig. 2 also seems to be *S. invisitatus*. Only Fig. 3 seems to be *S. parvus*. Careful observations on scanning electron microphotographs lead us to confirm the above assumptions. Valves of their Fig. 4 and 11 may be identified as *S.*

invisitatus mainly from the finer structure and the relatively broad mantle. Fig. 9 may be identified as *S. minutullus* from its conspicuous central elevation and Fig. 10 also can be identified as *S. hantzschii* form. *tenuis* from the lack of the central strutted process and the finely striated broad mantle. A little difference can be noticed between ROUND's (1981) description and our observations of the Japanese specimens about the number of the struts surrounding the interior tube of the marginal strutted process. ROUND stated in the legend of his Fig. 24, that "there are two open tubes on either side of the central tube of the fultoportule." However, we could not find any specimens possessing two struts. The type materials from Lünneburger Heide examined by ROUND have been strongly eroded and protrusions such as exterior and interior tubes and struts of the marginal strutted process, marginal spines and domed cribra occluding areola internally are all missing. The strutted process shown in ROUND's Fig. 24 seems to us to be surrounded by three struts, two visible and a hidden underside one, judging from the upside location of the two struts on the two corners of a triangle. The other features of the type specimens are quite identical with those of our specimens.

Thus, our specimens as well as *S. parvus* and *S. minutullus* are all considered to be conspecific with each other; then this taxon would have to taken following synonymy:

Stephanodiscus minutullus (KÜTZ.) ROUND. Arch. Protistenk. **24**: 462, *f.* 19–24. 1981.

Basionym: *Cyclotella minutula* KÜTZ. Bacillarien 50. *pl.* 2, *f.* 3. 1844.

Synonyms: *Stephanodiscus perforatus* GENKEL et KUZMIN Bot. Zhr. **63**: 1310. *pl.* 3, *f.* 1–9. 1978.

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Stephanodiscus astraea var. *minutula* (KÜTZ.) GRUN. in V. HEURCH, Syn. Diat. Belg. *pl.* 95, *f.* 7–8. 1882.

Stephanodiscus rotula var. *minutula* (KÜTZ.) ROSS & SIMS Bacillaria **1**: 152. 1978.

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小林 弘*・小林秀明*・出井雅彦：日本産小形ステファノディスクス属（ケイソウ類）の微細構造と分類 3. *Stephanodiscus minutullus* (KÜTZ.) ROUND と *S. parvus* STOERM. & HÅK. の同時出現**

本邦の淡水または汽水の湖沼や河川には，*Stephanocliscus minutullus* とも，また，*S. parvus* とも同定できる小形の種類が同時に出現する。そのため，おもに SEM による比較を行ったところ，長野県涌池，利尻島姫沼，稚内大沼，八郎潟，東京都中川潮止橋から得た，どの試料のものも，殻面の凹凸以外の諸形質については全く違いがなく，殻面の凹凸も，平坦なものから，殻の中心部の盛り上り，または落ち込みが強いものまで，切れ目なく連続して見られた。従って，この二つの分類群はまとめて一つの分類群とするのが妥当と思われる。(* 184 小金井市貫井北町 4-1-1 東京学芸大学生物学教室，**305 茨城県新治郡桜村天王台 1-1-1 筑波大学生物科学系)

第9回 国際現生・化石珪藻シンポジウムのお知らせ

第9回国際現生・化石珪藻シンポジウム (Ninth International Symposium on Living and Fossil Diatoms) が，61年 (1986) 8月24日から29日まで，英国のブリストル大学で開催されます。

会期1週間のうち，研究発表に4日間を充て，発表は質疑応答を含めて 20分，他にポスター発表も予定され，残りはエクスカーションその他に充てられる予定。

このシンポジウムの講演申し込み，詳しい日程，宿泊等の申し込みは 2nd サーキュラーに掲載される予定ですので，2nd サーキュラーの入手を希望される方は，下記へ申し込まれるとよいと思います。

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