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Fine structure and taxonomy of the small and tiny *Stephanodiscus* (Bacillariophyceae) species in Japan 5. *S. delicatus* GENKEL and the characters useful in identifying five small species[1)]

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Five small *Stephanodiscus* taxa, *S. invisitatus* HOHN & HELL., *S. costatilimbus* H. KOB., *S. hantzschii* GRUN. f. *tenuis* (HUST.) HÅK. & STOERM., *S. delicatus* GENK., *S. minutullus* (KÜTZ.) CL. & MÖLL. (incl. *S. parvus* STOERM. & HÅK.) have hitherto been found in Japan not only by light microscopy but also by scanning and transmission electron microscopy. Among these species, four species were reported in this series of papers. Presently, the fifth one, *S. delicatus* is examined in detail by using transmission electron microscope (TEM) and scanning electron microscope (SEM).

S. delicatus is characterized by the fascicles composed of two to three densely arranged rows of areolae, insertion of short fascicles not reaching the centre between every two to five longer fascicles, and the variable shape of the external opening of the labiate process.

The characters useful in identifying the above five small species are listed. These include the shape of the mantle costa, the shape and position of the external opening of the labiate process and the number of the struts of the central and marginal strutted processes. These characters are quite constant within each species.

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

As emphasized by HÅKANSSON et al. (1986), the identification of the small and tiny *Stephanodiscus* species whose valves are less than 15 μm in diameter has been especially difficult by the lack of taxonomic precision and confusion concerning the nomenclature of some small but widely distributed and ecologically important species.

However, the types or authentic slides and materials of some species which were very often incorrectly identified and which caused confusion in the literature, have been examined precisely one after another (ROUND 1981; HÅKANSSON & STOERMER 1984 a, b; STOERMER & HÅKANSSON 1984). Consequently, the five Japanese small species which had hitherto been found by the authors and their co-workers have been clarified and the four of them including one new species, *S. costatilimbus* H. KOB., were reported (KOBAYASI & INOUE 1985, KOBAYASI et al. 1985 a, b. KOBAYASI & KOBAYASHI 1986).

In the present paper, the fifth one, *S. delicatus* GENKEL, is examined in detail by SEM and TEM and the characteristics useful in identifying the five small *Stephanodiscus* species are discussed.

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Materials and Methods

Materials used here were collected from the following three localities. (1) Plankton from a small fresh water lake, Waku-ike (pH 8.6, wt. 19.9°C), Nagano Prefecture, on Sept. 21, 1972, sample number K–2118. (2) Plankton from lagoon Hachiro-gata (pH 7.5, wt. 12.8°C, salinity 17.2‰), Akita Prefecture, on Oct. 4, 1985. N–1005. (3) Plankton from the estuary of the Naka River (pH 7.0, wt. 14°C, salinity 18‰) at the Siodome Bridge, Tokyo, on Nov. 9, 1984, N–935. In addition to the above materials, the following samples were used for the observations and the occurrence check. (4) Bottom mud from Inogashira Pond (pH 9.5, wt. 26°C, salinity 0‰, conductivity 146 $\mu S \cdot cm^{-1}$), Tokyo, on Sept. 13, 1983, K–1737. (5) Bottom mud from a small lake, Hime-numa (pH 7.7, wt. 15°C, salinity 0‰) in Rishiri Island, northern Hokkaido, on Aug. 25, 1984, K–1950. (6) Bottom mud from brackish lake Oo-numa (pH 8.5, wt. 17°C, salinity 9‰), northern Hokkaido, on Aug. 25, 1984, K–1954. (7) Epiphytic in brackish river, Hinuma-gawa (pH 9.6, wt. 20°C, salinity 3‰, conductivity 4800 $\mu S \cdot cm^{-1}$) Ibaragi Prefecture, Central Japan, on Apr. 17, 1985, K–2612.

Methods of cleaning, washing, preparing samples for light and electron microscopy are previously reported (Kobayasi et al. 1985b).

Results

Under light microscope (LM), specimens are circular with strongly elevated or depressed central region. In our material, valve diameters are in a range of 6 to 14 μm. The number of striae is 14–18 in 10 μm measured along the valve margin. These values coincide well with that of the original description, though the Japanese specimens are a little larger than those from Rybinski Reservoirs, USSR (Genkel, 1985). The striation in a fascicle is so delicate that the punctae forming striae are unresolvable both in central and marginal regions. A single central strutted process, slightly excentric, can occasionally be distinguished (Figs 2–4, arrowed) and the central elevation or depression is so strong in most valves in our specimens that the central structure usually is out of focus when the marginal striae are in focus (Figs 1, 3, 4).

In contrast to the invisible marginal spines, the marginal strutted processes are visible as a dot occurring on every 3rd to 6th interfascicles in almost all valves observed.

In SEM observations, those characteristics observed by LM are well comfirmed and these are in agreement with the SEM photographs presented by Genkel (1985) except the degree of central undulation. Frustules and the diagramatic representation of the features are shown in Figs 5–10. The central elevation and depression of our specimens are more conspicuous in exterior views than that shown by Genkel (1985). However, the same specimens with small and less prominent central elevation or depression as shown in Genkel's paper (1985, Figs 2, 3) are also observed in our materials.

The interfascicles are generally elevated in the external view. The elevation is less conspicuous in the heavily silicified valves as shown in Figs 9, 10. These two photographs are hypo- and epi-valves of the same frustule taken from different angles. In the weakly silicified valve with central depression, the elevation of the interfascicles is more prominent than those of the heavily silicified valve. In the latter, the exterior surface is almost smooth and the smaller exterior openings of the areolae are visible (Figs 10, also 18–22).

The spines are often slightly bent upward and occur on the end of every interfascicle. The fasciculate organization of the areolae on the valve mantle is lost below the spine insertion and the asteroid pore ring surrounding spines and marginal strutted processes is seen in general (Figs 7–8, 14, 17–

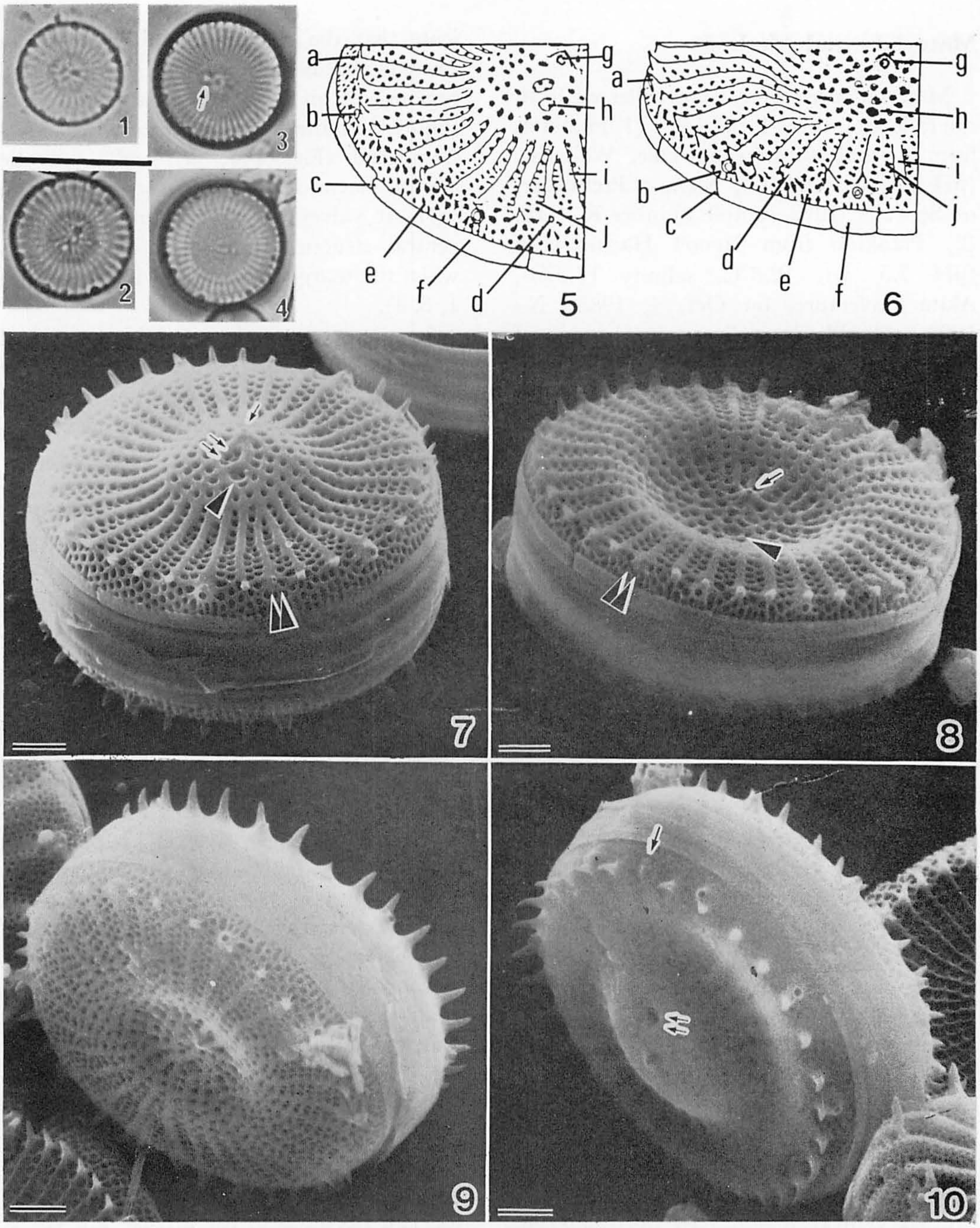

Plate 1. *Stephanodiscus delicatus* GENK. (Waku-ike). Figs 1–4. Whole valves showing the central strutted process (arrow) and marginal strutted processes. LM × 2000 (bar = 10 μm). Figs 5, 6. Diagrammatic representation of the valves with central elevation and depression, a. marginal spine, b. marginal strutted process, c. vertical slit-like marking of the flange, d. external opening of the labiate process, e. areolar rows on the valve mantle, f. flange, g. central strutted process, h. impression of the central strutted process of the sibling valve, i. interfascicle, j. fascicle. Figs 7, 8. Exterior valves with central elevation and depression showing the central strutted process (arrow), pattern centre (double arrow), impression of the central strutted process of the sibling valve (arrow head) and exterior opening of the marginal labiate process (double arrow head) SEM × 7500 (bar = 1 μm). Figs 9, 10. Epitheca with a central depression and hypotheca with a central elevation of the same frustule showing exterior opening of the labiate process (arrow) and impression of the central strutted process of the sibling valve (double arrow) SEM × 8000 (bar = 1 μm).

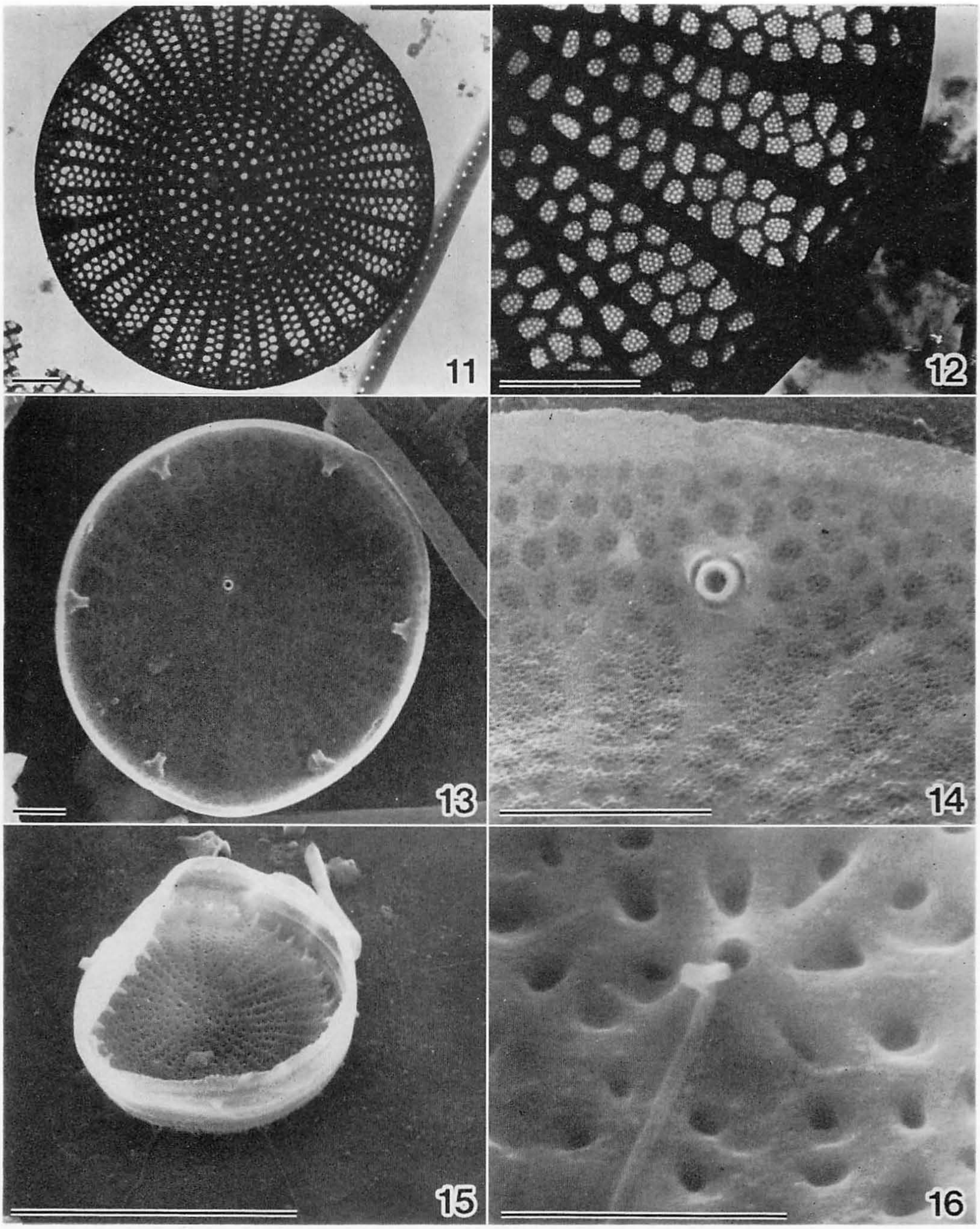

Plate 2. *Stephanodiscus delicatus* GENK. (Figs 11–14. Waku-ike, Figs 15, 16. Hachiro-gata) Fig. 11. Valve view. TEM×7000 (bar=1 μm). Fig. 12. Enlargement of the valve margin showing the densely arranged pores and pore occlusions with regularly scattered perforations. TEM×20000 (bar=0.1 μm). Fig. 13. Interior view of whole valve showing the central strutted process with two arc type buttresses and a labium placed parallel to the radial axis of the valve. Fig. 14. Enlargement of the interior valve margin showing the marginal strutted process with unequal arc type buttresses. SEM×30000 (bar=0.1 μm). Fig. 15. Whole uncleaned frustule showing chitan threads extruding from marginal strutted processes. SEM×4000 (bar=10 μm). Fig. 16. Chitan thread extruding from the central strutted process. SEM×40000 (bar=0.1 μm).

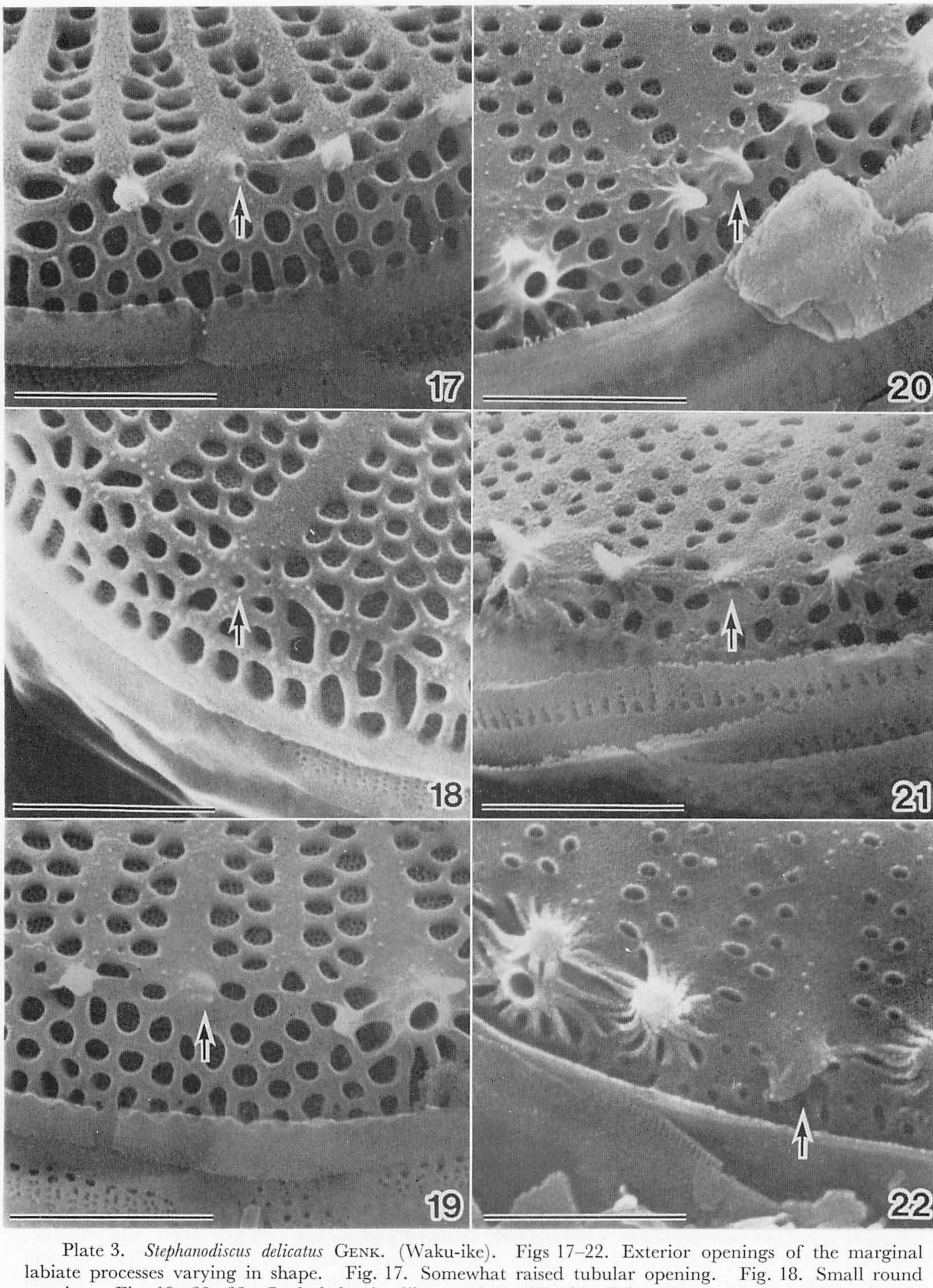

Plate 3. *Stephanodiscus delicatus* GENK. (Waku-ike). Figs 17–22. Exterior openings of the marginal labiate processes varying in shape. Fig. 17. Somewhat raised tubular opening. Fig. 18. Small round opening. Figs 19, 20, 22. Occluded spine-like opening. Fig. 21. Spine-like projection with underside opening. SEM × 30000 (bar = 0.1 μm).

22) as seen in *Stephanodiscus minutullus* (KOBAYASI et al. 1985b, Figs 5, 6).

In the interior views (Figs 13, 14), areolae on the valve face have domed cribra but those of the valve mantle have flat cribra like a windowpane. In the exterior views, the central strutted process is apparent as a slightly raised opening (Figs 7, 8, arrow). The impression of the central strutted process of the sibling valve occurs opposite the opening of the central strutted process as a prominent depression (Fig. 7, arrow head). Both central strutted process and central impression are more prominent on the heavily silicified valves (Fig. 10 double arrow). The areolae forming fascicles are arranged densely in the marginal zone of the valve, the number being about 50 in 10 μm at the margin. This arrangement is clearly seen in TEM photographs (Figs 11, 12).

The central strutted process has two struts interiorly (ROUND 1981) (Fig. 13) and the marginal one has three struts, two of which are more developed than the one facing the valve face (Fig. 14). The chitan threads (MCLACHAN et al. 1965) extruding from the central and marginal strutted process can be noticed on the uncleaned frustules (Figs 15, 16).

A single labiate process occurs at the junction of the valve face/mantle and at almost the same level as the spine insertion (Figs 17–22, arrowed). However, some are slightly higher (Figs 17, 19, 20) and some are slightly lower than the spine level (Figs 18, 22). The outer shape of the labiate process is variable, being a somewhat raised tube (Fig. 17), or a sunken hole smaller than the exterior opening of the areolae (Fig. 18), like a spine with an underside opening (Fig. 21) and fully occluded (Figs 19, 20, 22). Though the occluded external labiate process has rarely been pointed out by HÅKANSSON et al. (1986), this is also quite new to us. The labium is parallel to the radial axis (Fig. 13) and occurs on the end of a interfascicle on the side nearly opposite the central strutted process (Figs 8, 10, 13).

Discussion

Comparative studies of the five small *Stephanodiscus* species in Japan using SEM and TEM show both similarities and differences in specific characters between the taxa, even though their gross appearances under the light microscope are similar to each other.

Those characters which are useful in identifying the taxa are listed in Table I. All taxa listed are small and with their valve diameter in a range of 5–16 μm.

S. invisitatus HOHN & HELL. (KOBAYASI & INOUE 1985) and *S. costatilimbus* H. KOB. (KOBAYASI & KOBAYASHI 1986) are quite similar in both LM and SEM views but can be clearly distinguished only by the shape of costae on the valve mantle and the position of the exterior opening of the marginal labiate process. The former has costae branched in V-shape or fork-shape and the latter has linear ones as an elongation of the interfascicles. There are many features that can be detected by LM when once they have been recognized by SEM (REICHARDT 1986). However, clear distinction between *S. invisitatus* and *S. costatilimbus* with LM is very difficult except the very rare case presented in our previous paper (KOBAYASI & KOBAYASHI 1986, Figs 1, 2).

The interfascicles often elongate to the strutted processes on the mantle as seen in many *Stephanodiscus* species such as *S. alpinus* HUST (HÅKANSSON and STOERMER 1984 b), *S. aegypticus* EHR. (HÅKANSSON and LOCKER 1981), *S. niagarae* EHR. (ROUND 1981, 1982b, THERIOT and STOERMER 1981). It is very rare that the interfascicle elongation reaches to the valve edge beyond the marginal strutted process, as seen in *S. incognitus* KUZMIN et GENKEL (GENKEL and KUZUMIN 1978). This is an important generic and specific character of the genus *Cyclostephanos* ROUND (1982a). The mantle costae of *S. invisitatus* and *S. costatilimbus* are slightly elevated interiorly but not so visual-

Table I Characteristics of five *Stephanodiscus* species

Characters / Species	Valve diam. (μm)	Central elevation or depression	Fascicles: Number in 10 μm at margin	Fascicles: Marginal pore rows	Elevation of Interfascicles (IF): Exterior	Elevation of Interfascicles (IF): Interior	Strutted processes: Central	Strutted processes: Marginal	Valve mantle: Depth (Pore number between spines and valve edge)	Valve mantle: Costae	Labiate process: Number & placement of labium to radial axis	Labiate process: Shape and place of external opening
S. invisitatus HOHN & HELL.	5–14	without	14–20	2	with	without to with (thin valves)	one with two arc	on every 4–7th IF with 2 arc	3–4	V or fork	parallel	slightly raised small round pore, on a branch of V or fork marginal costa, lower than marginal strutted process level
S. costatilimbus H. KOB	7–11	without	14	2–3	with	with	one with two arc	on every 3–8th IF with 2 arc	5–6	linear	parallel	small pore on linear marginal costa, between spine and marginal strutted process levels
S. hantzschii GRUN. f. *tenuis* (HUST.) HÅK. & STOERM.	7–16	without	8–16	2–4	without to with (thin valves)	without	without	on every 3–4th IF with 3 arc	5–7	without	crosswise or oblique	tubular, replaced with spine
S. delicatus GENKEL	6–14	with	14–18	2–3	without to with (thin valves)	without	one with two arc	on every 3–6th IF with 3 arc (incl. 1 small)	2–3	without	parallel	variable in shape, some are occluded, replaced with spine, upper than spine level
S. minutullus (KÜTZ.) CL. & MÖLL. (incl. *S. parvus* STOERM. & HÅK.)	6–10	without to with	10–13	2–3	with	without	one with two arc	on every 3–6th IF with 3 arc	2–3	without	oblique	tubular but variable in shape, replaced with spine

ly prominent, even though they reached to the valve edge, and this is a reason why we placed these two species under *Stephanodiscus*.

S. hantzschii GRUN. f. *tenuis* (HUST.) HÅK. & STOERM. (KOBAYASI et al. 1985a) has a flat valve face similar to the above two species. This species is distingished by the absence of a central strutted process and by the absence of a costal structure on the valve mantle. Valve face strutted processes are considered to be an important diagnostic structure in the genus *Stephanodiscus*. Their absence or presence was used to separate *S. parvus* and *S. hantzschii* by STOERMER and HÅKANSSON (1984) and HÅKANSSON and STOERMER (1984a).

S. delicatus GENKEL is characterized by having the central elevation or depression, the delicately areolate structure and a central strutted process of the valve. Although it resembles *S. minutullus* (KÜTZ.) CL. & MÖLL. in some features detectable with SEM, it can be distinguished with ease by its unresolvably fine punctation of the fascicles with LM. As listed in Table I, the striae number as measured along the valve margin of *S. delicatus* observed is clearly greater than that of *S. minutullus*. Each marginal strutted process is surrounded by three arc-shaped buttresses (ROUND 1981, 1982b) in both species, but the one facing the valve face is smaller than the other two in *S. delicatus*.

As clearly shown in our previous paper (KOBAYASI et al. 1985b) with Plate 3 showing a series of gradations in the degree of central valve elevation of *S. minutullus*, specimens collected from Hime-numa, a small fresh-water lake in Hokkaido, are characterized by having both flat and undulate valves. Consequently it is sometimes very difficult to make a distinction between this species and *S. hantzschii* f. *tenuis* where these species co-occurred. Careful focussing of the valve at different focus levels in the way suggested by REICHARDT (1986) may resolve this problem in finding a central strutted process changing from white to black dot under LM (KOBAYASI et al. 1985b, Figs 2, 3).

Small centric diatoms are abundant and ecologically important in many fresh-water ecosystems (HÅKANSSON et al. 1986), but the difficulty of identification of these small taxa, especially that of *Stephanodiscus* having the polymorphic nature of the valves (HÅKANSSON and STOERMER 1984a, THERIOT and STOERMER 1981, KOBAYASI and INOUE 1985, KOBAYASI et al. 1985a, 1985b), has been one of the serious problems to the workers in the field. The occurrence in Japanese waters of the five species whose identities were ascertained by SEM are shown in Table II. All species occurred both in fresh and brackish waters except *S. costatilimbus* which was quite scarce in the original material. In a brackish lagoon, Hachiro-gata, all taxa were found in a small bottle of plankton sample. Therefore the exsistence of slightly different, but

Table II Occurrence of five *Stephanodiscus* species

Localities / Species	Inogasira Pond fresh	Hime-numa Lake fresh	Waku Pond fresh	Hachiro Lagoon brackish	Oo-numa Lake brackish	Hinuma River brackish	Naka River brackish
S. invisitatus		r	r	++			r
S. costatilimbus				r r			
S. hantzschii f. *tenuis*	+		+++	r		+	+
S. delicatus		+	++	r r		+	
S. minutullus	++	+++		r	r	++	+

+++ =abundant, ++ =frequent, + =common, r=rare, rr=very rare.

overlapping ecological features of these taxa are significant. After correctly distinguishing each of them, further work will be necessary to define their ecological ranges.

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小林　弘*，小林秀明：日本産小型ステファノデスクス属（ケイソウ類）の微細構造と分類　5．*Stephanodiscus delicatus* GENKEL と小型種5種類の同定に有用な形質**

本邦の淡水および汽水の湖沼や河川に出現する4種類の小型 *Stephanodiscus* 属珪藻，*S. invisitatus*, *S. hantzschii* f. *tenuis*, *S. minutullus*, *S. costatilimbus* については，すでに本誌上に報告を行った。今回は *S. delicatus* について，おもに SEM によって諸形質を明らかにした。この種類は *S. minutullus* に似ているが，構造がより微細である点を特徴としている。

なお，今までのところ上述の5種類が本邦に見られた本属のすべてであるが，これらを識別するのに役立つ形質の比較を行い，併せて，本邦での出現状態を SEM によって確認しながら調べた。(*184　小金井市貫井北町4-1-1　東京学芸大学生物学教室，**108　東京都港区三田2-17-23　慶応義塾女子高等学校)