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Observations on *Cymbella mexicana* (Ehrenb.) Cleve var. *mexicana* (Bacillariophyceae) with special reference to the band structure

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Abstract

Cymbella mexicana (Ehrenb.) Cleve var. *mexicana* was collected from Tama-gawa (Tama River), Tokyo. The fine structure of the species was examined by scanning electron microscopy (SEM), with special reference to colony formation and band morphology. A mature cingulum of this species is usually composed of four open bands. The correlation between the position of the second band (B2) of the cingulum and the attaching pole of the frustule is discussed.

Introduction

Cymbella mexicana was described by Ehrenberg as *Cocconema mexicana* from fossil material (Ehrenberg, 1844). In Japan, *C. mexicana* has hitherto been reported only from stagnant waters (Watanabe, 1990; Mizuno & Saito, 1990), but not from running waters. However, recently we found *C. mexicana* forming colonies on submerged rocks in Tama-gawa (Tama River). On a previous survey of diatoms at the same sampling point we did not observe this species (Mayama & Kobayasi, 1982).

Though the band structure has been employed as a useful criterion in pennate diatom taxonomy (Williams 1985, 1986; Krammer, 1982; Kobayasi & Kobori, 1990), good accounts of diatom girdles are still very scarce. Thus, it seems necessary that more evidence is accumulated.

Krammer (1982) observed the fine structure of many *Cymbella* species, including *C. mexicana*, using SEM. He also described the band structure in some species (Krammer, 1981), but did not refer in detail to *C. mexicana*. In the present study, the band structure of *C. mexicana* is described in detail, and the correlation between the position of the second band (B2) of the cingulum and the attaching pole of the frustule is discussed.

Material and methods

Specimens were collected from the surfaces of the stones and rocks about 30 m downstream from the Hamura Intake Dam of the Tama-gawa (Tama River), Tokyo on 18 December 1991 (K-6977: sample number) and under Nagata-bashi (Nagata Bridge) of the Tama-gawa, Tokyo, on 13 August 1991 (K-6976) and 14 June 1992 (K-6978).

After observation with a light microscope (LM), living cells were isolated and prepared for SEM observation, either by direct drying or by freeze-drying. Otherwise, cells were cleaned by

the acid treatment described by Mayama & Kobayasi (1991). Specimens for the SEM were coated with gold-palladium using JEOL JFC-1100 and observed with JEOL F15.

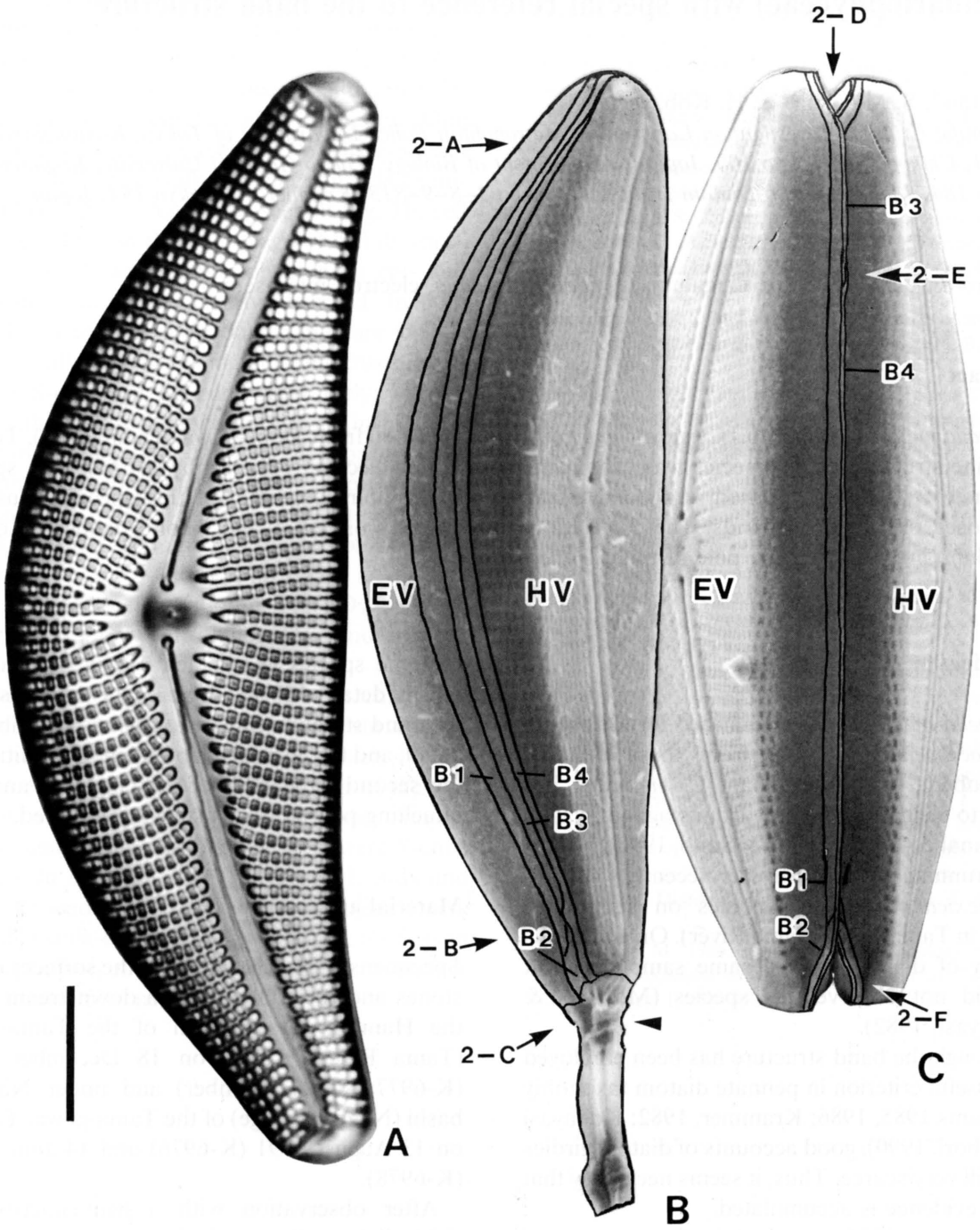

Fig. 1. C. mexicana. A. Whole valve. LM. B. Dorsal view of a whole cell with its stalk, SEM. C. Ventral view of a whole cell, SEM. Scale bar = 10 μm. EV = epivalve, HV = hypovalve, B1 = first band, B2 = second band, B3 = third band, B4 = fourth band. 2-(A-F) = portions enlarged in Fig. 2A-F.

Results and discussion

Valves of *C. mexicana* are mostly characterized by a large isolated punctum in the middle, between the central pores of the raphe (Fig. 1A). The cells have a single chloroplast (Fig. 3A, B). They are solitary or produce mucilage stalks and form branching colonies. The stalk is colourless but an obscure longitudinal line is visible along the centre with the LM (Fig. 3A). In the specimens prepared by freeze-drying (Fig. 1B), however, this central line is recognizable as a single groove.

External dorsal and ventral views of frustules are shown in Fig. 1B, C, with the outlines of valves and bands traced in for clarity. The bands are narrow ventrally and wide dorsally.

Freeze-dried specimens show that the stalk consists of many mucilaginous strands (Fig. 2C). In the advalvar portion of the stalk, a noticeable aggregation of mucilage was observed (Figs 1B, 2B, C, arrow heads) in contrast to the directly dried specimens which appeared smooth (Fig. 3C-G). Daniel *et al.* (1987) examined the mucilage stalks in some marine diatoms morphologically and cytochemically and called a structure like this a "collar" in *Achnanthes longipes* Agardh. The location of the collar is similar to that of the mucilage aggregation of *C. mexicana*, but the latter differs a little in shape. Only cytochemical analysis can clarify whether the collars are homologous.

Two types of the *Cymbella* girdle have been distinguished by Krammer (1981), namely, the "leptoceros" type and the "cocconema" type. The "leptoceros" cingulum is composed of two bands, a valvocopula and a pleura, although each band is itself composed of two segments. The "cocconema" cingulum is composed of three bands, one valvocopula and two pleurae; only the valvocopula is composed of two segments.

Using the same kinds of interpretation as Krammer (1981), it would be possible to say that the mature cingulum of *C. mexicana* is composed of three bands, one of which is segmented. In this sense, the species belongs to the Krammer's "cocconema" type. However, the valvocopula of *C. mexicana* is fully identical to that of the "leptoceros" type.

In the present studies, we recognize each segment described by Krammer as an independent band and refer to the bands forming the cingulum as B1, B2,..... in turn, outwards from the valve.

The cingulum of *C. mexicana* is composed of four bands (Fig. 1B-C), though we have observed a specimen with five bands. We have examined more than 50 thecae but found a theca with five bands only once and, even in this case, the opposing theca (hypotheca) of the same frustule had four bands (Fig. 3C).

The first band (B1), attaching to the valve mantle, extends from one pole toward the other pole, but its ventral and dorsal ends do not reach the other pole. The rest of the mantle edge is filled by the short second band (B2). This arrangement of B1 and B2 is completely identical to Krammer's segmented valvocopula of the "leptoceros" type (Krammer, 1981). The closed end of the third band (B3) is located at the same pole as that of B1. The ventral arm of B3 is short and usually does not extend for more than one third of the valve length from its closed end. The dorsal arm of B3 is longer than the ventral arm and extends as far along as B1. This structure nearly corresponds to one segment of Krammer's "pleura" in the "leptoceros" type of cingulum (Krammer, 1981). The closed end of the fourth band (B4) is located at the same pole as that of B2. The dorsal arm of the B4 extends from the closed end almost to the opposite pole. The ventral arm of the B4 is usually shorter than the dorsal arm but extends over at least two thirds of the valve length from its closed end (Fig. 1B, C). This structure corresponds to the Krammer's "pleura" in the "cocconema" type of cingulum (Krammer, 1981).

B1, B3 and B4 bear a transverse row of pores on the advalvar edge of the pars exterior (Fig. 3C, E, F). These pores are lacking around the poles. The pores of each band are similar to each other in shape and arrangement, and these are about 20 in 10 μm. Pores are lacking in B2 (Figs 2F, 3C, E-G).

In the case of this species, attachment is associated with the secretion of extracellular mucilagi-

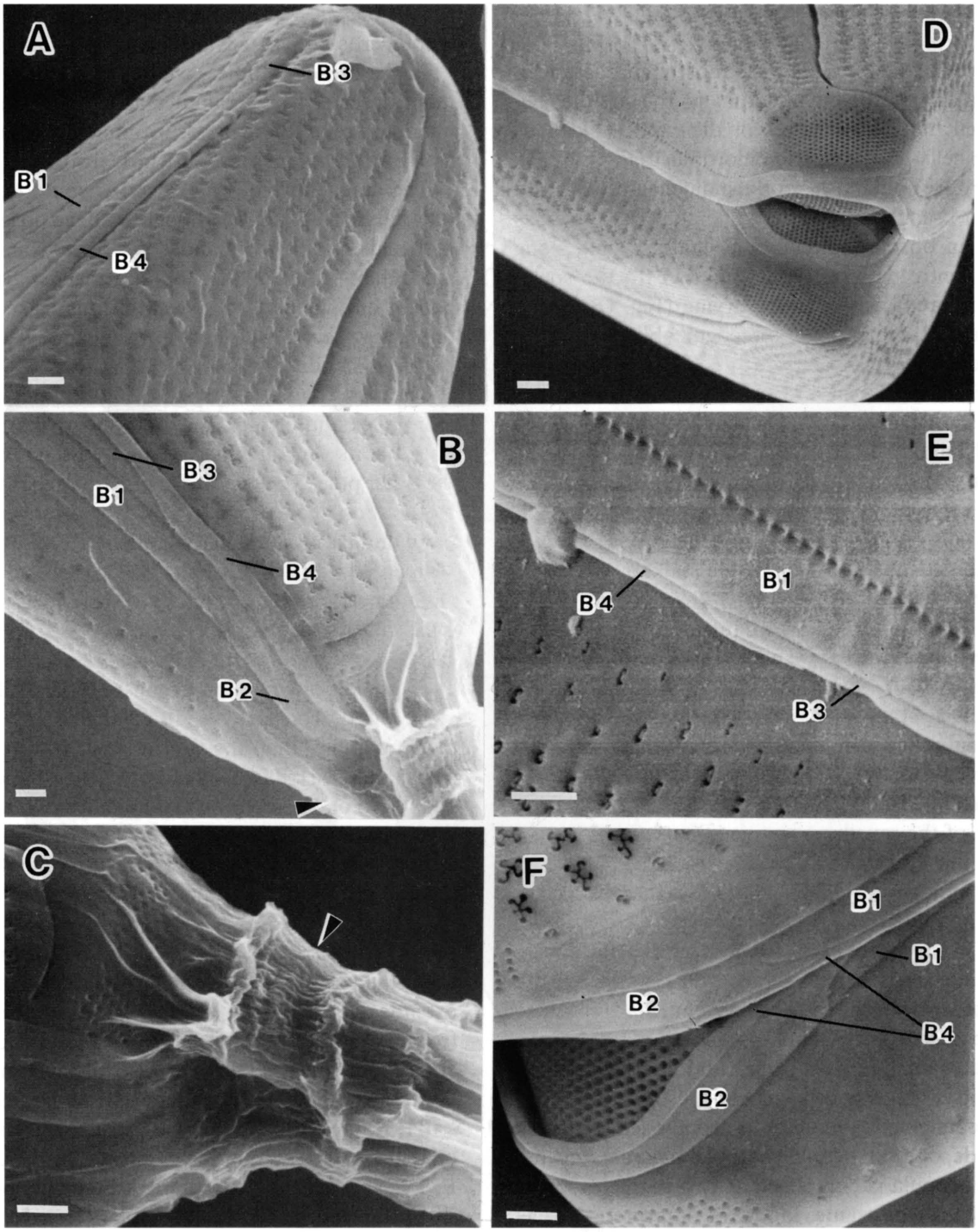

Fig. 2. C. mexicana. A. Distal end of the frustule in Fig. $1B_{2\text{-}A}$, showing the L-end. SEM. B. Proximal end of the frustule in Fig. $1B_{2\text{-}B}$, showing the S-end. SEM. C. Stalk-cell junction of the frustule in Fig. $1B_{2\text{-}C}$, showing the collar-like aggregation of mucilage. SEM. D. Upper end of the frustule in Fig. $1C_{2\text{-}D}$, showing the LL-end. SEM. E. Overlapping portion of the B3 and B4 of the frustule in Fig. $1C_{2\text{-}E}$. SEM. F. Lower end of the frustule in Fig. $1C_{2\text{-}F}$, showing the SS-end. SEM. Scale bars = 1 μm. B1 = first band, B2 = second band, B3 = third band, B4 = fourth band.

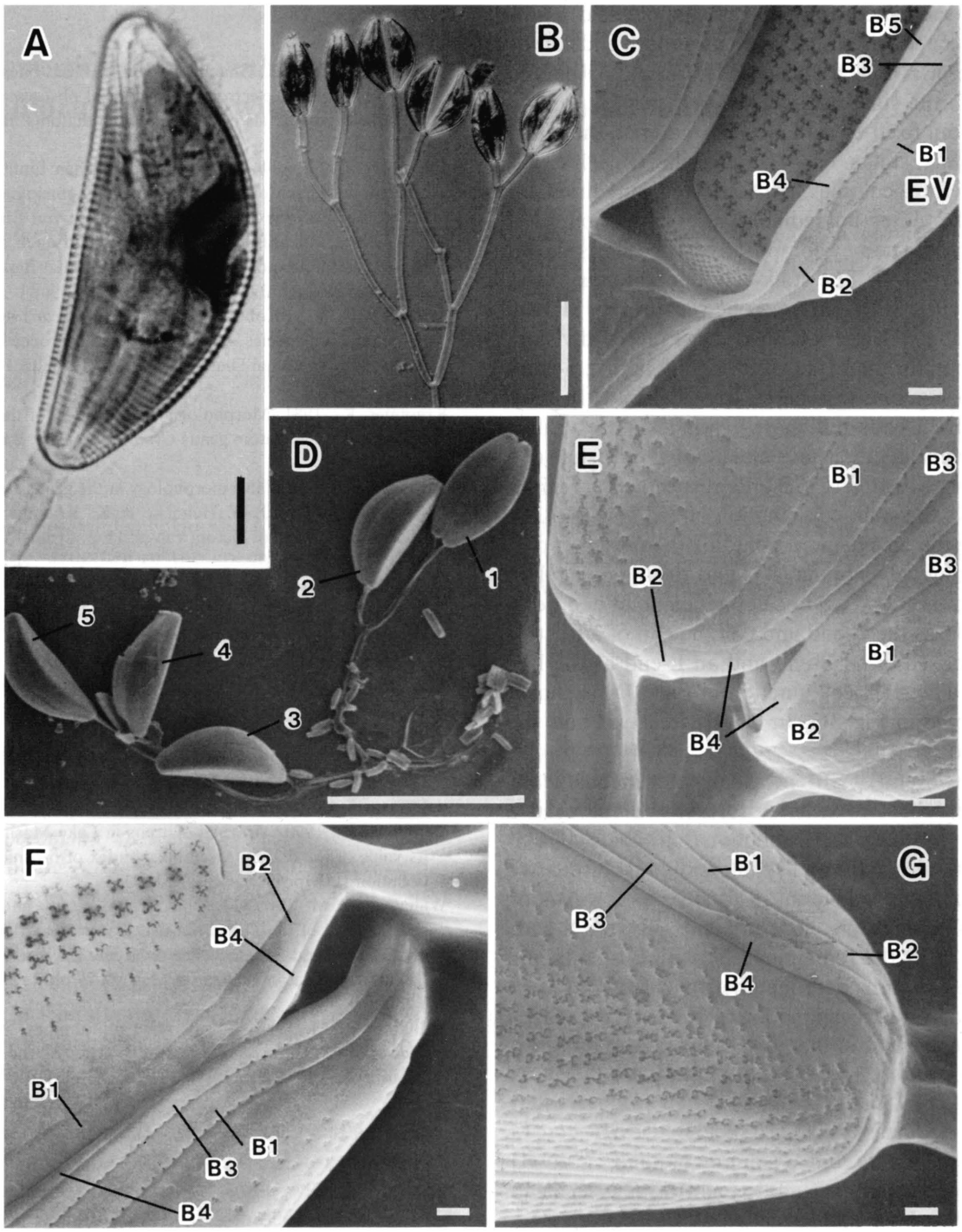

Fig. 3. C. mexicana. A. Living cell with a stalk. LM. B. Cells with dichotomously branched stalks. LM. C. Proximal ends with SS-end showing the epicingulum composed of five bands and the hypocingulum composed of four bands. EV = epivalve. SEM. D. Colony composed of five cells. SEM. E. Proximal end of cell 1 of the Fig. 3D colony, showing the SS-end. SEM. F. Proximal end of the cell 5 of the Fig. 3D colony, showing the LS-end. SEM. G. Proximal end of the cell 3 of the Fig. 3D colony, showing the S-end. SEM. Scale bars = 1 μm (Figs C, E-G), 10 μm (Figs A, D) and 100 μm (Fig. B). B1 = first band, B2 = second band, B3 = third band, B4 = fourth band, B5 = fifth band.

nous substances from one apical pore field. However, since this structure exists at both ends of the valve, it is very difficult to determine the pole to which the stalk invariably attached.

From fixed or acid cleaned material, the mucilaginous stalks easily come out (Fig. 1A, C). Therefore, colonies were selected and dried for SEM studies immediately after collection. One example, a colony composed of five cells, is shown in Fig. 3D-G. The stalk-cell junction was examined (Fig. $3D_{1-5}$). As can be seen in Fig. 3E, cell 1 in Fig. 3D is in the final stage of cell division, so that the band constitution of both epi- and hypocingulum can be clearly observed. In both cingulum ends the short bands (B2) are visible. We will call this type of cingulum and frustule end as the S-end and SS-end respectively. Fig. 3F shows the stalk-cell junction of cell 5 in Fig. 3D. Here the epicingulum contains the closed end of the long valvocopula (B1) and the hypocingulum contains B2. We will call the cingulum end bearing B1 as the L-end and call the end of a frustule composed of an S-end and an L-end as the LS-end. The stalk-cell junction in Fig. $3D_3$ is shown enlarged in Fig. 3G. In this frustule end only the S-end is visible.

The S-end or SS-end seems to be mechanically more robust to exterior forces. We surveyed a total of 38 stalk-cell junctions, in which 47 cingulum ends were analyzable (only one of the two cingula was visible in 29 frustule ends). Among the 47 analyzable cingulum ends 38 were S-ends (77%). In the remaining 23%, nine L-ends and three LS-ends were included. No LL-ends were found in stalk-cell junctions. However, the reason or mechanism of the appearance of the L-ends is still uncertain to us.

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