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A Study on the Fine Structure of the Marine Diatoms of Korean Coastal Waters - Genus *Thalassiosira* 3

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A study on the fine structure of the marine diatom *Thalassiosira* has been carried out during the periods from January 2007 to March 2008 in Korean coastal waters. As the third series of the *Thalassiosira* species, a fine structure, description, distribution and taxonomic remarks of the six *Thalassiosira* species were observed by means of light microscope and scanning electron microscope. The critical features of *Thalassiosira* species were a shape of external tubes of marginal strutted processes and labiate process. Six species showed each different shape of external tubes, marginal strutted processes and labiate process. The shape of external tube was divided into five types: T shape of *Thalassiosira curviseriata*, small-rounded shape of *T. lundiana*, double-layer form and flame shape of *T. nordenskioeldii*, tulip shape of *T. punctigera* and tooth-shape of *T. tenera*. This external character may be able to key character for positive identification of the *Thalassiosira* species. Of these *Thalassiosira lundiana*, *T. minuscula* and *T. tenera* were new records for Korean coastal waters.

Key Words: external tube, fine structure, Korea, labiate process, strutted process, *Thalassiosira*

INTRODUCTION

The genus *Thalassiosira* was erected by Cleve (1873), and the description was emended by Hasle (1973). Various authors have mentioned that the number of species of the genus *Thalassiosira* may exceed 100 taxa (Round *et al.* 1990; Hasle and Syversten 1996). Regional investigations, some of them including descriptions of new species, have been published in a high number since the first transmission electron microscopy (TEM) examinations started to appear in the 1950s (Helmcke and Krieger 1953, 1954) and the first examinations taking with SEM in the 1960s (Hasle 1968). Attempts have been made to impose structure on this genus which seems to increase in number of species parallel to the number of localities investigated. This figure and the morphological complexity of the genus make positive species identification difficult. The most important characters for species identification are the feature of strutted and labiate processes on the valve face (Hasle 1968, 1973). Hasle and Syvertsen (1996) redefined the original subgroups as proposed by Hasle (1968). Makarova (1988) suggested a system that is based on the pattern of the areolae for dividing the genus into smaller groups. However, a number of studies have shown that areolation is often influenced by environmental factors (Hasle 1978; Hallegraeff 1984; Syvertsen and Hasle 1984).

Lee and Cho (1985) listed up a total of 18 taxa of *Thalassiosira* species in Korean coastal waters. These species have been merely limited to the list of inventory. Lee and Yoo (1986, 1987) have studied a fine structure of the genus *Thalassiosira* from Korean coastal waters and reported 6 *Thalassiosira* species as new records for Korean coastal waters. Lee (1995) have listed up a total of 761 taxa including 653 species, 89 varieties, 10 forma and 3 others of phytoplankton diatoms in the Korean coastal waters. Of these, the genus *Thalassiosira* species have been recognized 25 species.

The present study was carried out as a series of the third of the fine structure, description, distribution and taxonomic remarks of the six *Thalassiosira* species by light microscope and scanning electron microscope observations in Korean coastal waters.

MATERIALS AND METHODS

Material figured were collected in the Korean coastal waters during the periods from July 2006 to March 2008. Table 1 shows the sampling locations. Fixed materials

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Table 1. Collection data of *Thalassiosira* species in Korean costal waters

Species	Date	Locality	Latitude (N)	Longitude (E)
Thalassiosira curviserita	Sep. 28, 2007	Ulsan Bay	35° 28' 46''	129° 23' 45''
	Feb. 24, 2008	Incheon coast	37° 22' 20''	126° 32' 13''
	Mar. 23, 2008	Dangin coast	36° 26' 35''	126° 33' 41''
T. lundiana	Sep. 28, 2007	Ulsan Bay	35° 28' 46''	129° 23' 45''
	Mar. 4, 2008			
T. minuscula	Jan. 18, 2007	Taean coast	36° 24' 09''	126° 24' 06''
	Jan. 13, 2007	Tongyeong coast	34° 46' 12''	128° 23' 61''
T. nordenskioeldii	Sep. 28, 2007	Ulsan Bay	35° 28' 46''	129° 23' 45''
	Mar. 23, 2008	Dagin coast	36° 26' 35''	126° 33' 41''
T. punctigera	Jan. 13, 2007	Tongyeong coast	34° 46' 12''	128° 23' 61''
T. tenera	Sep. 28, 2007	Ulsan Bay	35° 28' 46''	129° 23' 45''
	Mar. 23, 2008	Seocheon coast	36° 10' 26''	126° 31' 30''

were collected with 20 μm mesh nets in vertical towing and were in situ fixed with 5% neutralized formalin. (Hasle and Fryxell 1970) and mounted in pleurax for Light Microscope and on aluminum stubs for Scanning Electron Microscope. Materials was coated with gold-palladium and examined with SEM (JSM-5600LV, Japan). Terminology is that suggested by the Working Party on Diatom Terminology (Anonymous 1975).

RESULTS AND DISCUSSION

The present study was adopted Simonsen's (1979) systematic system based on ideas of phylogeny as follows;

Class Bacillariophyceae Haeckel 1878
Order Centrales Hustedt 1930
Suborder Coscinodiscineae Simonsen 1979
Family Thalassiosiraceae Lebour 1930, emend. Hasle 1973
Genus *Thalassiosira* Cleve 1873
Thalassiosira curviseriata Takano 1981
T. lundiana Fryxell 1975
T. minuscula Krasske 1941
T. nordenskioeldii Cleve 1873
T. punctigera Hasle 1983
T. tenera Proschkina-Lavrenko 1961

A total of six species of the genus *Thalassiosira* have been identified during the present study; *Thalassiosira curviseriata, T. lundiana, T. minuscula, T. nordenskioeldii, T. punctigera* and *T. tenera.* Of these *Thalassiosira lundiana, T. minuscula* and *T. tenera* were new records for Korean coastal waters.

Description of the species

Thalassiosira curviseriata Takano (Pl. I, Figs 1-6)

Takano 1981, p. 34, Figs 26-38; Hallegraeff 1984, p. 498, Fig. 8; Takano 1983, sheet 123, Figs A-H; Hoppenrath *et al.* 2007, p. 276, Figs 13-15.

Cells elliptical to octagonal in the girdle view. Cells 8-10 μm in diameter. Valve areolae with radial rows. Number of areolae 20 to 25 in 10 μm. Valve face covered with siliceous granules. Marginal hyaline developed very well. One or two central strutted processes next to an annulus. One marginal ring of 4 to 7 conspicuous winged strutted processes showing two wings per process diverging into 2-3 branches. One slitted-labiate process present adjacent to a marginal strutted process and also external tube present. In the internal view, central strutted process composed with 3 satellite pores, whereas marginal strutted process with 4 satellite pores.

Distribution: *Thalassiosira curviseriata* was known to cosmopolitan species excluding polar regions (Hoppenrath *et al.* 2007). In Korea, *T. curvieriata* was previously reported one time in Jinhae bay (Han *et al.* 1994). The present study was recorded three times in September 28, 2007 in Ulsan Bay, February 24, 2008 in Incheon coastal water and March 23, 2008 in Dangjin coastal water.

Taxonomic remarks

Takano (1981) have distinguished *Thalassiosira curviseriata* from *T. tealata* Proshkina-Lavrenko by the shape of external tube of marginal strutted processes. The tube was "T" shape and as similar species *T. tealata* also have same shape of external tube. Takano (1980a, 1981) have mentioned that marginal strutted process of *T. curviseriata* have two wings diverging into 2-3 branches, whereas in *T. tealata* the long wings each have a single slender tip.

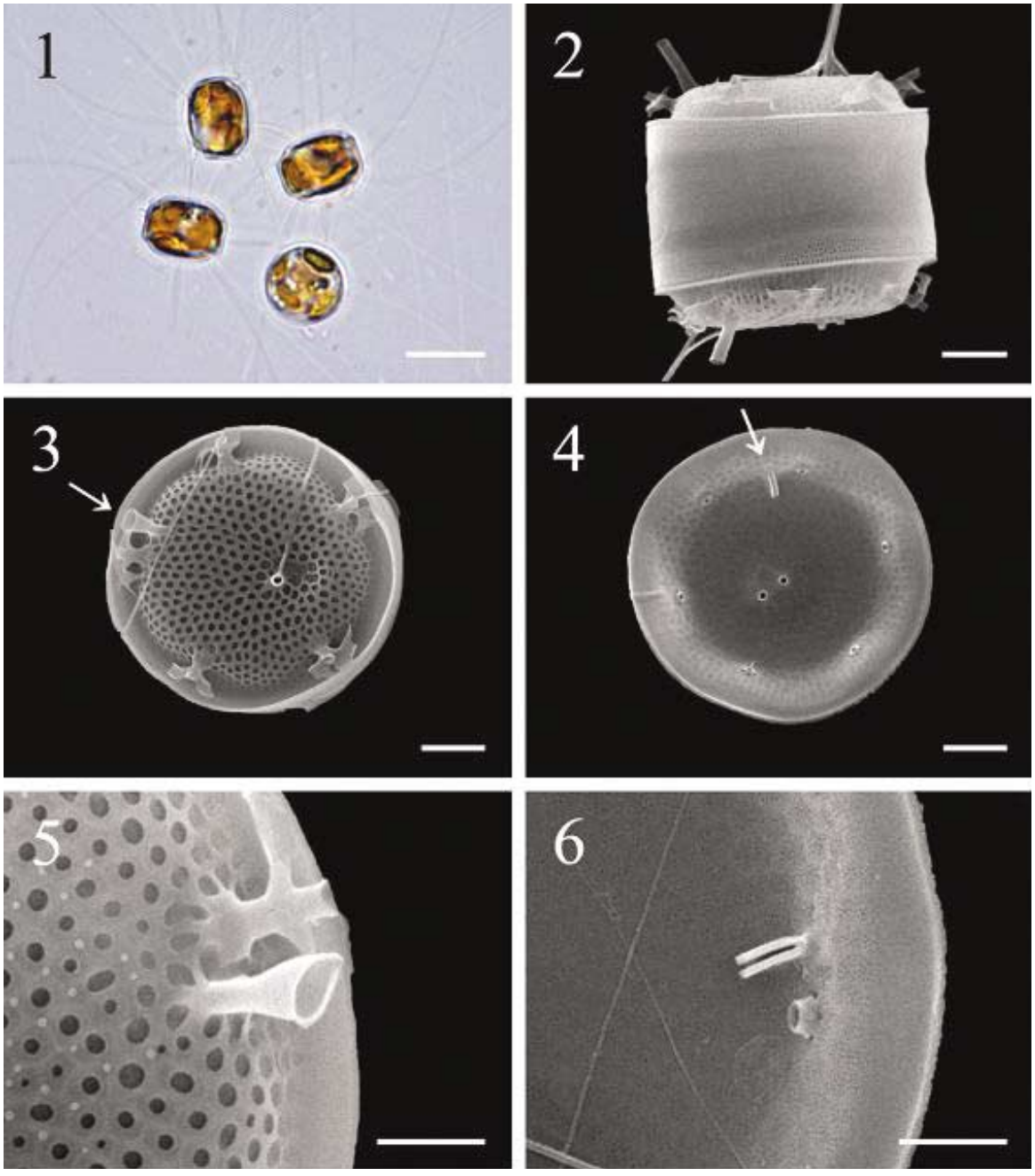


Plate I. *Thalassiosira curviseriata*, LM and SEM. **Fig. 1.** LM. **Fig. 2.** External girdle view. **Fig. 3.** External valve view. Arrow indicates the external tube of labiate process. **Fig. 4.** Internal valve view. Arrow indicates the labiate process. **Fig. 5.** External tube of the labiate process with a marginal strutted process. **Fig. 6.** Focusing on the labiate process and a marginal strutted process. Scale bar: 10 μm (Fig. 1), 2 μm (Figs 2-4), 1 μm (Figs 5, 6).

Hoppenrath *et al.* (2007) mentioned that simply the morphological variability of two species requires reinvestigation with morphometric and genetic analyses of clonal culture.

***Thalassiosira lundiana* Fryxell (Pl. II, Figs 7-12)**

Fryxell 1975, p. 362-363, Pl. 3, 4, Figs 12-24; Takano 1979a, sheet 14, Figs A-F; Mahood *et al.* 1986, p. 137, Figs 38-41; Hoppenrath *et al.* 2007, p. 279, Figs 31-32.

Cells slightly convex in the girdle view. Cells 10-36 μm in diameter. Valve with fine areolae arranged in sectors, fasciculated and marginal striae. Number of areolae 17 to 36 in 10 μm. One central strutted processes next to an annulus, and composed with 3 or 4 satellite pores. One marginal ring of strutted processes showing zigzag form arrangements. Several or many strutted processes scat-

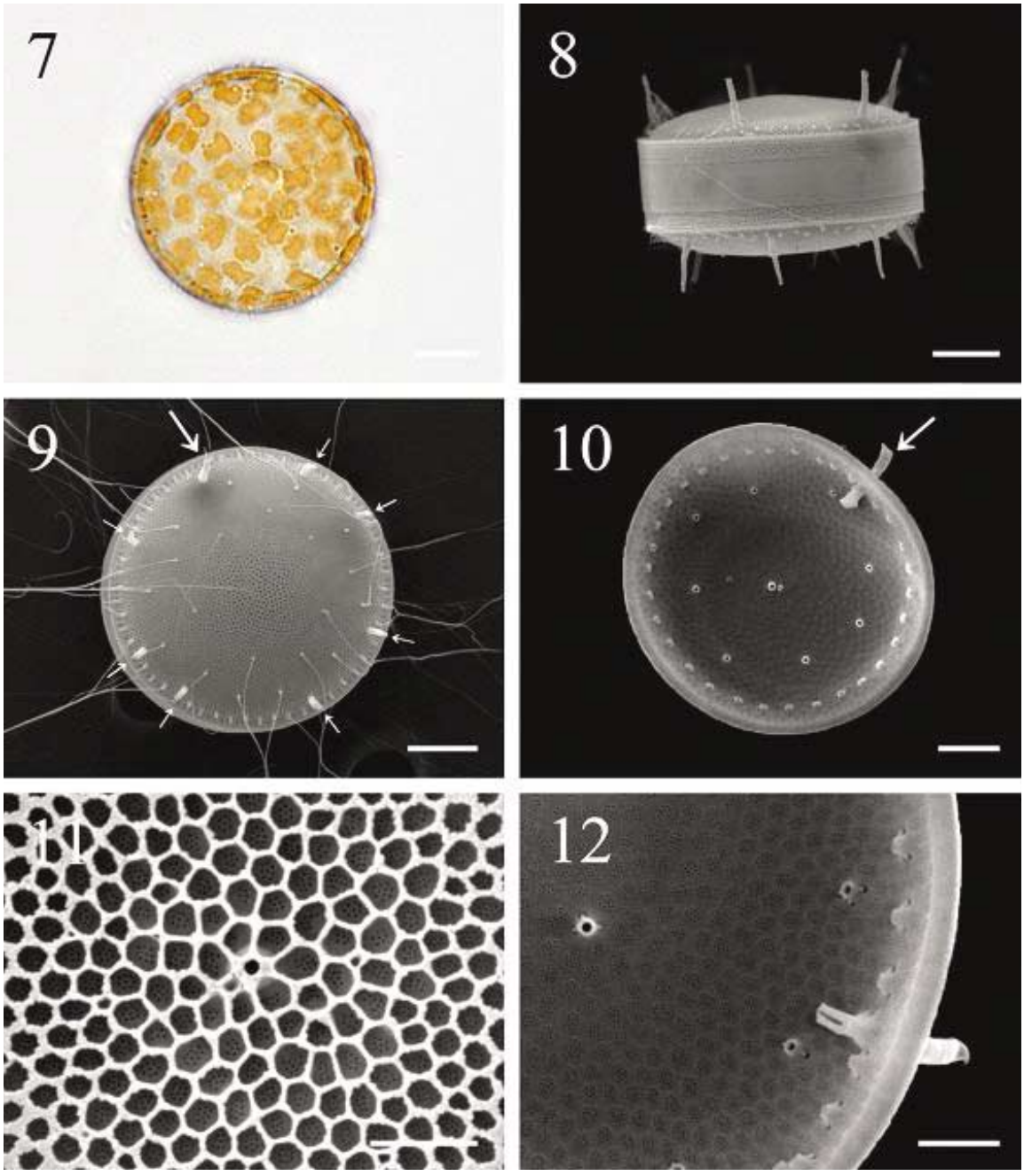


Plate II. *Thalassiosira lundiana*, LM and SEM. **Fig. 7.** LM. Fig. 8. External girdle view. **Fig. 9.** External valve view. Large arrow indicates the external tube of labiate process and small arrows occluded processes. **Fig. 10.** Internal valve view. Arrow indicates the labiate process. **Fig. 11.** Central strutted process and areolation. **Fig. 12.** Marginal labiate process and one marginal ring of strutted processes. Scale bar: 10 μm (Fig. 7), 5 μm (Figs 8, 9), 2 μm (Fig. 10), 1 μm (Figs 11, 12).

tered on the valve face. One submarginal ring of occluded processes present. One external tube of labiate process positioned between marginal strutted processes. In the internal view, marginal and valve strutted process composed with 2 or 3 satellite pores.

Distribution: Fryxell (1975) reported that *Thalassiosira lundiana* found in the Atlantic Ocean, off West Africa and off South Africa, as well as in the Pacific Ocean at Rocky Point, Mexico, in the Gulf of California, and the northern Pacific. She has been isolated this species near the mouth of the Missisippi River ranging from 21 to 38 , it can be assumed euryhaline species. *Thalassiosira lundiana* was unknown to the Korean coastal waters till up to present. Our samples were collected in July 28, 2007 and March 4, 2008 in Ulsan Bay. *T. lundiana* was reported for the first time in Korean coastal waters.

Taxonomic remarks

Fryxell (1975) mentioned that occluded processes of this species are a striking feature. Occluded processes feature is closely related to *T. punctigera* (Castracane) Hasle and *T. licea* Fryxell. However, this process may or not be only present in the three species. And she also mentioned that *T. lundiana* has strutted processes scattered on the valve face, but clear patterns are not evident. The present study was observed that small-sized *T. lundiana* has submarginal ring of strutted processes instead of scattered strutted processes. This species have similar with the external tube of marginal strutted processes of *T. rotula.*

***Thalassiosira minuscula* Krasske (Pl. III, Figs 13-18)**

Krasske 1941, p. 262, Pl. 5, Figs 4-6; Simonsen 1974, p. 10; Hasle 1976a, p. 104, Figs 6-10; Rivera 1981, p. 95 Figs 246-262; Hallegraeff 1984, p. 497, Figs 4a-b; Hernández-Becerril and Tapis-Peña 1995, p. 550, Figs 49-60; Aké-Castillo *et al.* 1999, p. 494, Fig. 20; Hoppenrath *et al.* 2007, p. 279, Figs 38-40.

Synonym: *Thalassiosira monoporocyclus* Hasle 1972, p. 129, Figs 49-60.

Chloroplast discoidal, fully, peripheral in LM. Cell shape convex in the girdle view. Cells 10-36 μm in diameter. Valve face with fine areolae arranged in rows from parallel to radial. Areolae 17 to 36 in 10 μm in the whole valve. One central strutted process and one marginal ring of strutted processes. One labiate process located between marginal strutted process. External tube of labiate process invisible, but large and slitted large-sized labiate process in the internal view. In the internal view, all strutted processes composed with 4 satellite pores.

Distribution: *Thalassiosira minuscula* was known to warm water species (Hasle 1972, 1976a). *T. minuscula* was found in January 18, 2007 in Taean coast and January 13, 2007 in Tongyeong coast and recorded new to Korea.

Taxonomic remarks

Thalassiosira minuscula was not showed the external tube. Hasle and Syvertsen (1996) mentioned that due to the lack of external process tubes and the location of the labiate process at some distance from the valve margin. All *Thalassiosira* species were divided into two group A and B by Hasle and Syvertsen (1996). *T. minuscula* could just as well be placed into group B as in group A. Unlike species of group B, *T. minuscula* has short internal tubes, a feature shared with *T. subtilis*, *T. diporocyclus*, and *T. fragilis*, all appearing in mucilage colonies.

***Thalassiosira nordenskioeldii* Cleve (Pl. IV, Figs 19-26)**

Cleve 1873, p. 7, Pl. Fig. 1; Hustedt 1930, p. 321, Fig. 157; Cupp 1943, p. 46, Fig. 8; Hendey 1964, p. 85, Pl. 1, Fig. 8; Hasle 1976b, p. 323, Figs 4-7; Hasle 1978, p. 79, Figs 1, 5-20; Takano 1979b, sheet 9, Figs A-F; Hoppenrath *et al.* 2007, p. 281, Figs 41-43.

Cells octagonal in the girdle view. Cells 11-41 μm in diameter. Diameters three times wider than pervalvar axis. Valve with hexagonal areolae in sectors. Number of areolae 4 to 5 in 10 μm. One central strutted process next to an annulus. One marginal ring of prominent strutted processes with long external tubes and about 3 in 10 μm. Marginal strutted processes with double layers. Out layer flame form at the top. One large tube-like labiate process located in the marginal ring of strutted processes. In internal view, all strutted processes composed with 4 satellite pores.

Distribution: *Thalassiosira nordenskioeldii* was found to about 50°N in the eastern and to about 40°N in the western Atlantic coastal waters and to about 35°N in the Pacific Ocean (Hasle 1976b). *T. nordenskioeldii* was reportded in cold to temperate waters (Hasle and Syversten 1996). In Korean coastal waters, *T. nordenskioeldii* was recorded 18 times till the year of 1990's (Lee 1995). The present study was recorded in September 28, 2007 and March 23, 2008 in Taean and Dangjin coastal waters, respectively.

Taxonomic remarks

Thalassiosira nordenskioeldii is type species of the genus *Thalassiosira.* Hasle (1978) mentioned that in spite of useful information already available, they feel that the morphology of *T. nordenskioeldii* as the type species of the genus deserves a detailed discussion. And she characterized *T. nordenskioeldii* by valve face structure, areolae pattern, position of marginal strutted processes and labiate process, but she didn`t mentioned about shape of the marginal strutted processes. Hasle and Fryxell (1977) mentioned that external tubes of marginal strutted processes were described many terms as "skirt", "collar". Our *T. nordenskioeldii* specimens were also observed double layers tube shape and we described the shape as "flame".

***Thalassiosira punctigera* Hasle (Pl. V, Figs 27-34)**

Castracane 1886, p. 167, Pl. 3, Fig. 1; Makarova 1970, p. 13; Fryxell 1978, p. 133, Figs 9-20; Takano 1980b, sheet 32, Figs A-H; Hasle 1983, Figs 1-45; Makarova 1988, p. 67, Pl. 38, Figs 1-10.

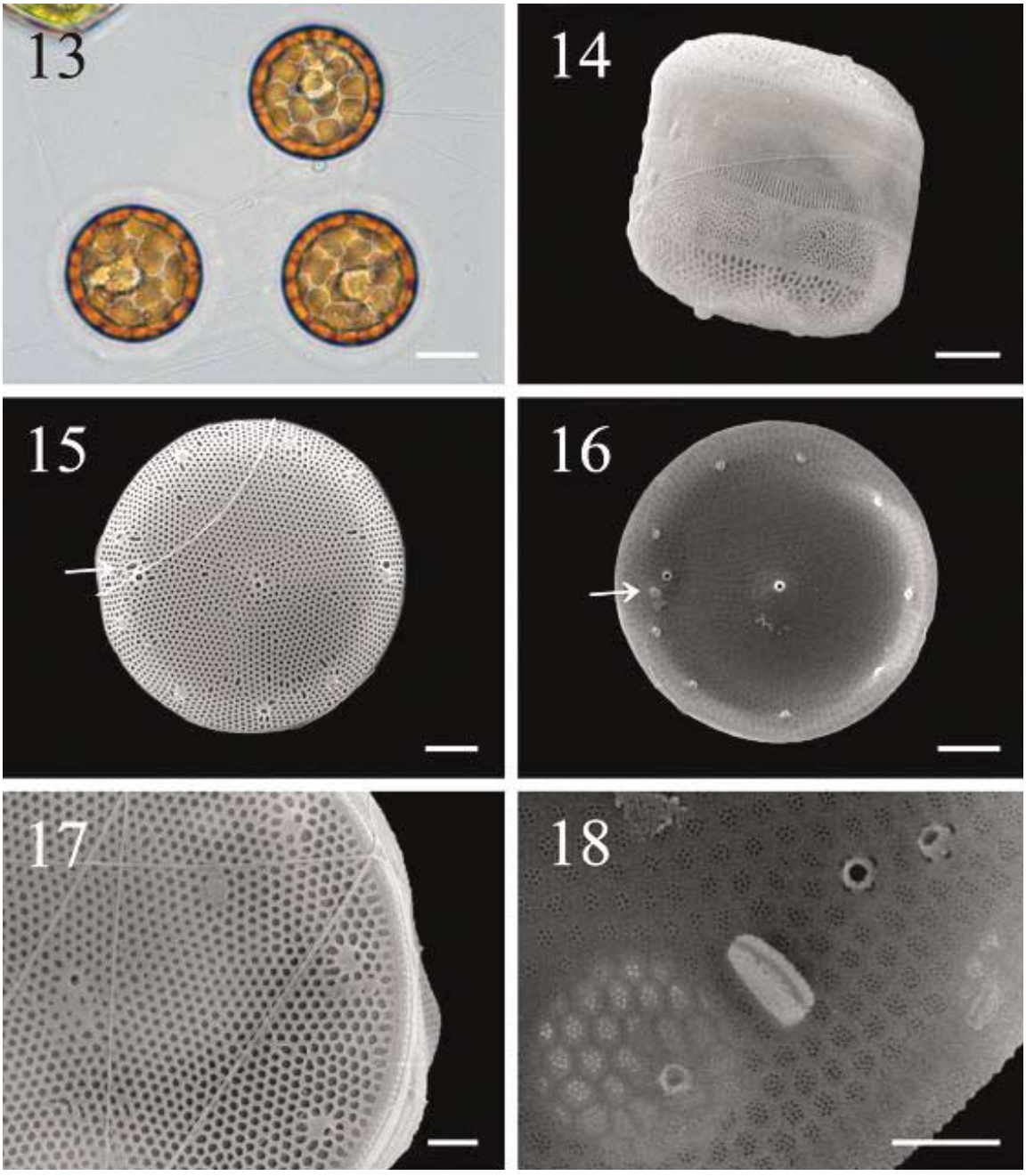


Plate III. *Thalassiosira minuscula*, LM and SEM. **Fig. 13.** LM. **Fig. 14.** External girdle view. **Fig. 15.** External valve view showing the labiate process (arrow). **Fig. 16.** Internal valve view showing the labiate process (arrow) and a marginal strutted processes. **Fig. 17.** Central and marginal strutted processes and areolation. **Fig. 18.** Focusing on the labiate process and one sub-marginal strutted process with four satellite pores. Scale bar: 10 μm (Fig. 13), 2 μm (Figs 14-16), 1 μm (Figs 17-18).

Basionym: *Ethmodiscus punctiger* Castracane

Synonym: *Ethmodiscus punctiger* Castracane; *Coscinodiscus punctiger* (Castracane) Peragallo; *Coscinodiscus verecundus* Mann; *Coscinodiscus angstii* Gran; *Thalassiosira japonica* Kiselev; *Thalassiosira angstii* (Gran) Makarova.

Cells slightly convex in the girdle view. Cells 62-74 μm in diameter. Valve with fine fasciculated areolation and ribbed margin. Areolae 20 to 23 in 10 μm. One central strutted process in the valve center. One marginal ring of strutted processes showing an external tulip-shaped. One ring of occuluded processes located ring of marginal strutted processes inside in the external view. In the internal view, one ring of widely spaced, variable in number of large occluded marginal processes could be present. One external tube of labiate process just positioned inside the marginal ring. In internal view, margin-

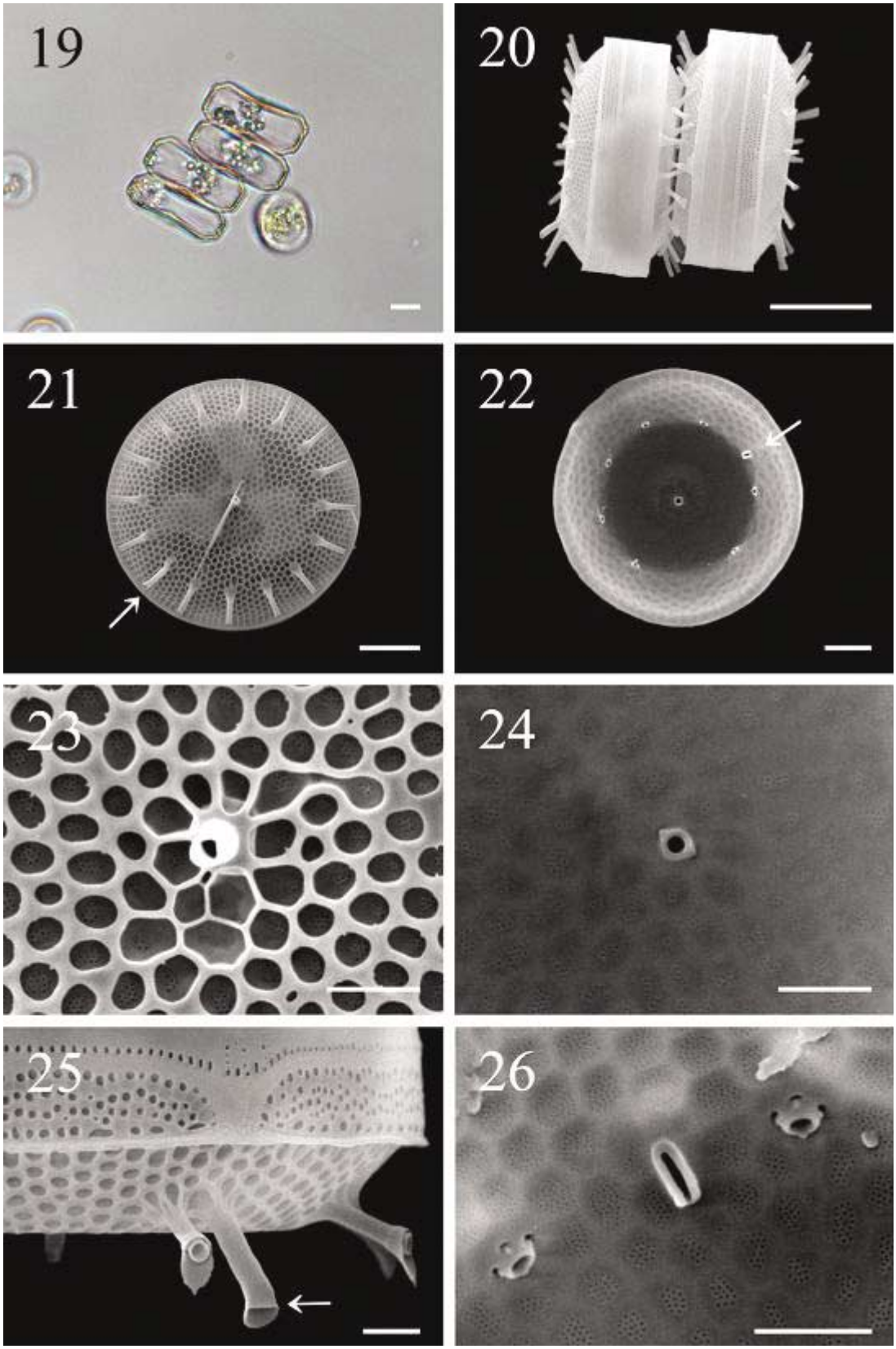


Plate IV. *Thalassiosira nordenskioeldii*, LM and SEM. **Fig. 19.** Cell colony formation in LM. **Fig. 20.** Two cells in the external girdle view. **Fig. 21.** External valve view showing the external tube of labiate process (arrow). **Fig. 22.** Internal valve view showing the labiate process (arrow) and marginal strutted processes. **Fig. 23.** Central strutted process and areolation. **Fig. 24.** Central strutted process with two satellite pores. **Fig. 25.** External tube of labiate process (arrow) and long marginal strutted process. **Fig. 26.** Focusing on labiate process and marginal strutted processes. Scale bar: 10 μm (Figs 19-21), 2 μm (Fig. 22), 1 μm (Figs 23-26).

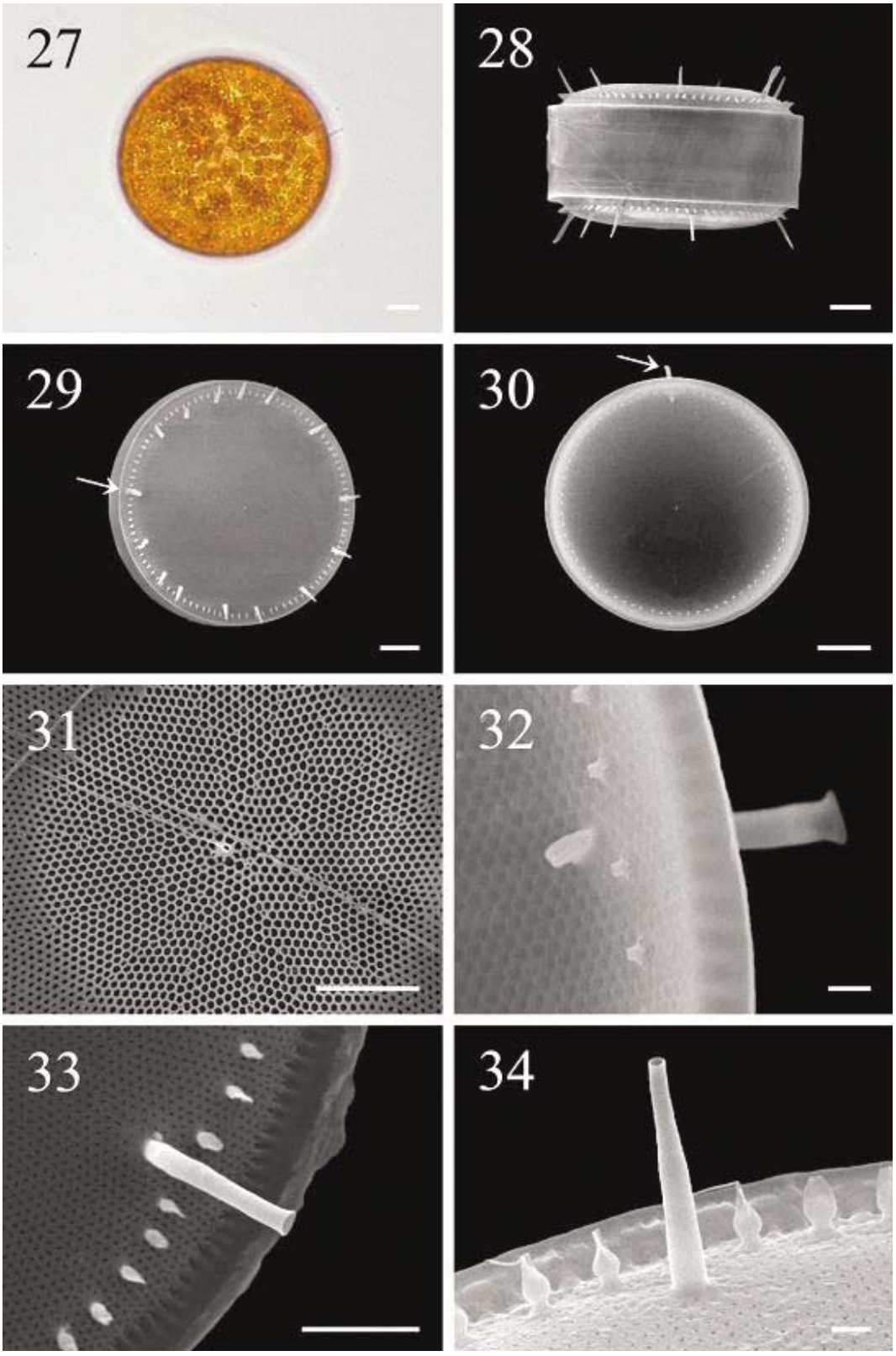


Plate V. *Thalassiosira punctigera*, LM and SEM. **Fig. 27.** LM. **Fig. 28.** External girdle view showing the external tube of labiate process (arrow) and occluded processes. **Fig. 29.** External valve view showing the external tube of labiate process (arrow), occluded processes and marginal strutted processes. **Fig. 30.** Internal valve view showing the labiate process and marginal strutted processes. **Fig. 31.** Central strutted process and radial areolation of valve face. **Fig. 32.** Focusing on labiate process. **Fig. 33.** External tube of the labiate process and a marginal strutted processes. **Fig. 34.** Occluded process and jar-shaped marginal strutted processes. Scale bar: 10 μm (Figs 27-30), 5 μm (Figs 31, 33), 1 μm (Figs 32, 34).

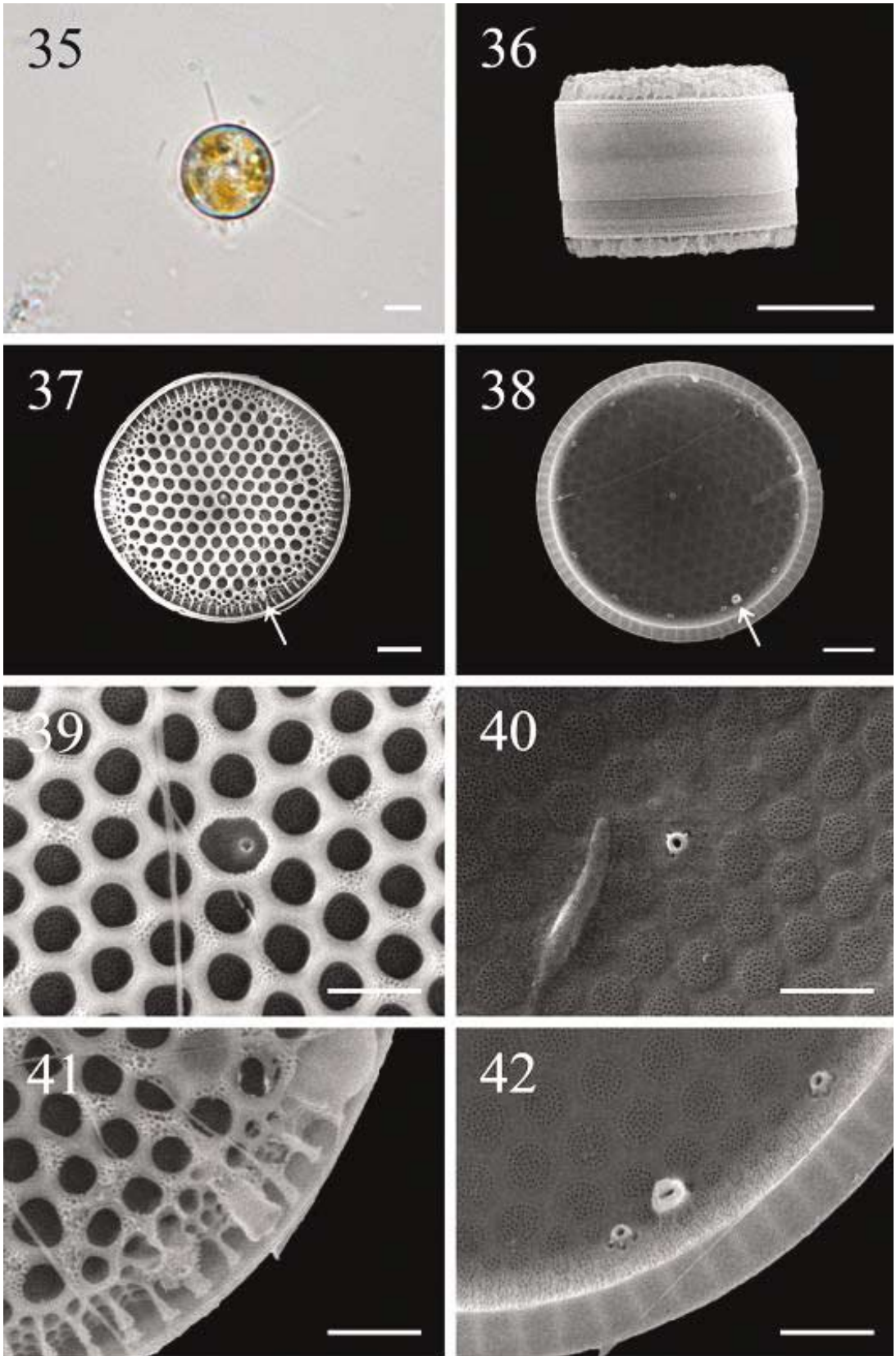
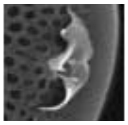
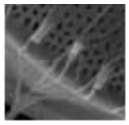
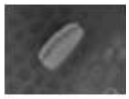
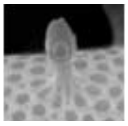
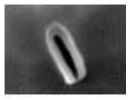
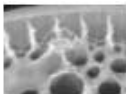
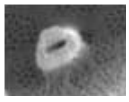


Plate VI. *Thalassiosira tenera*, LM and SEM. **Fig. 35.** Small living cell in LM. **Fig. 36.** External girdle view. **Fig. 37.** External valve view showing areolation and the external tube of labiate process (arrow). **Fig. 38.** Internal valve view showing the labiate process (arrow) and marginal strutted processes. **Fig. 39.** Central strutted process and areola situation. **Fig. 40.** Central strutted process with three satellite pores. **Fig. 41.** External tube of labiate process and marginal strutted process. **Fig. 42.** Focusing on labiates process and marginal strutted processes. Scale bar: 5 μm (Fig. 35), 2 μm (Figs 36, 38), 1 μm (Figs 37, 39-42).

Table 2. Morphological character of *Thalassiosira* species from Aug. 2006 to Mar. 2008 in Korean costal waters

species	Diameter (μm)	Areolae in 10 μm		Number & features of strutted process		Labiate process feature		Occluded process
		Valve face	Valve margina	valve face	Marginal ring	External tube	Internal form	
Thalassiosira curviseriata Takano	8-10 (n=5)	20-25	35-40	One or two central		Present		None
T. lundiana Fryxell	10-36 (n=49)	17-36	17-36	One central, Many sub-central scattered		Present		Many in the margin
T. minuscula Krasske	11-14 (n=13)	40-50	40-50	One central		None[1] or Present[2]		None
T. nordenskioeldii Cleve	11-41 (n=12)	11-22	14-18	One central		Present		None
T. punctigera (Castracane) Hasle	62-74 (n=33)	20-23	20-23	One central		Present		Many in the margin
T. tenera Proshkina-Lavrenko	9-12 (n=9)	15-20	40-45	One central		Present		None

[1] Present study, [2] Hoppenrath *et al.* 2007

al strutted processes composed with 4 satellite pores and little extension.

Distribution: *Thalassiosira punctigera* was known to wide distribution; warm to temperate waters (Hasle 1983). In Korean coatal waters, *T. punctigera* was recorded 11 times till the year of 1990's (Lee 1995), and the present study was collected in January 13, 2007 in Tongyeong coastal waters.

Taxonomic remarks

Hasle (1983) suggested that 8 taxa reduced to synonym with *Thalassiosira punctigera* (Castracane) comb. nov., an extremely variable species as to size and valve structure. Occluded process may be also able to striking feature of *Thalassiosira punctigera*, however occluded processes may or may not be present in this species. *T. punctigera* have specific shape of external long tube. Marginal strutted processes was tulip-shaped and this character is striking feature of *T. punctigera*.

***Thalassiosira tenera* Proschkina-Lavrenko (Pl. VI, Figs 35-42)**

Proschkina-Lavrenko 1961, p. 33, Pl. 1, Figs 1-4, Pl. 2, Figs 5-7; Hasle and Fryxell 1977, p. 28, Pl. 12 and 13, Figs 54-65; Takano 1980c, sheet 33, Figs A-F; Harris *et al.* 1995, p. 121, Figs 6 and 24.

Cells rectangular in the girdle view. Cells 9-12 μm in diameter. Valve with straight and/or linear areolation with relatively coarse hexagonal areolae. Number of areolae 15-20 in 10 μm. One central strutted process positioned in one central areolae and surrounded by a central areola slightly larger than the other areolae. One marginal ring of closely standing tooth-shaped strutted processes. One labiate processes in the marginal ring directly next to a strutted one. In the internal view, central strutted process composed with 3 satellite pores, whereas marginal strutted processes with 4 satellite pores. Labiate process size smaller than other *Thalassiosira* species in the present study.

Distribution: *Thalassiosira tenera* was known to cosmopolitan species by Hasle and Fryxell (1977). Takano (1980b) reported the distribution from Yokohama coastal

waters to several estuarine of Japan. Our specimens were found in December 20, 1998 in Jinding Bay and March 23, 2008 in Seocheon coastal water and new to Korea.

Taxonomic remarks

Thalassiosira tenera and *T. exigua* are very similar species. Hasle and Fryxell (1977) have pointed out that the differences between these species are the smaller diameter of the former as well as the structure of the valvocopula. Aké-Castillo *et al.* (1999) have pointed out that the differences are the marginal costae, cribra structure, satellite pores. However, Hasle and Fryxell (1977) have mentioned that the satellite pores are variable. The present study showed that the shape of external tube of marginal strutted process was enclosed with siliceous. Marginal strutted process was tooth-shaped in the external view.

Since phytoplankton studies in the coastal waters of the Korean Peninsula were begun in the early of 1930's, most papers published on the phytoplankton diatoms ecology to be focused on the spatial and temporal distribution. The systematics and taxonomy of the marine phytoplankton diatoms were not many papers. Lee and Yoo (1986, 1987) have studied for the first time the fine structure, description, ecology and taxonomic remarks of the genus *Thalassiosira* 10 species by means of light microscope and scanning electron microscope in the Korean coastal waters. Although thereafter many papers on the taxonomy were published in earnest; pennate diatoms (Choi and Noh 1987), six *Cyclotella* species (Lee and Lee 1988), 2 *Coscinodiscus* species (Lee 1989; Lee *et al.* 1992), *Roperia tesselata* (Lee and Lee 1990), the family Hemicadiscaceae (Lee *et al.* 1992), *Pseudohimantidium pacificum* (Lee *et al.* 1993), there were not rich papers in Korea.

Thalassiosira, with its more than 100 species, is one of the most biggest planktonic genus in marine environment (Round *et al.* 1990; Hasle and Syversten 1996). Also, the genus is most throughly examined in the world. By reason of a small-sized cells of *Thalassiosira*, there were no active works except Lee and Yoo (1986, 1987) in Korea. It is absolutely necessary for excellent studies of the *Thalassiosira* species to need scanning electron microscope.

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