

CYTOPLASMIC MASSES PRESERVED IN EARLY HOLOCENE DIATOMS: A POSSIBLE TAPHONOMIC PROCESS AND ITS PALEO-ECOLOGICAL IMPLICATIONS¹

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In Lake Suigetsu, central Japan, greenish/light-brown granules identified as cytoplasmic masses had been preserved in siliceous cell walls of freshwater diatoms in annual layers of lacustrine muds since the early Holocene. The lacustrine muds consisted of alternating dark-colored (rich in diatom valves, clay, and organic matter) and light-colored (mainly diatom valves) laminae. The greenish/light-brown granules were predominately preserved in frustules of the genus *Aulacoseira* preserved in the dark-colored laminae. The dark-colored laminae were inferred to have formed annually under stratified water caused by surface water warming in summer that caused the formation of an organic-rich anoxic layer on the lake bottom that favored granule preservation. The good preservation of cytoplasmic masses in dark-colored laminae suggested a cause for diatom assemblage periodicity, a phenomenon that was commonly noted in temperate lakes: the cells containing these masses could be potential seed stocks for subsequent spring blooms. Frustules of the most abundant granule-containing species, *Aulacoseira nipponica* (Skvortzow) Tuji, in the dark-colored laminae of the Early Holocene muds were abundant in the overlying light-colored laminae, suggesting that these species reproduced abundantly in springtime yielding a massive diatom bloom.

Key index words: anoxic lacustrine muds; *Aulacoseira* species; cytoplasmic masses; diatoms; early Holocene; laminae

Abbreviations: AMS, accelerator mass spectrometry; DAPI, 4'-6 diamino-2-phenylindole; NIH, National Institutes of Health

Freshwater planktonic diatoms belonging to the genus *Aulacoseira* usually contain two to six discoid chloroplasts per cell. These chloroplasts expand during the vegetative phase and contract during the resting phase. Sicko-Goad et al. (1986) observed the rejuvenation processes of resting *Melosira granulata* Ehrenberg (= *Aulacoseira granulata* (Ehr.) Simonsen) cells obtained from the anoxic sediments of Lake Douglas, Michigan. They reported the sequence of cytological changes that occurs in the rejuvenation process, and showed that the resting phase cells contained a shrunken cytoplasmic mass. Incubation of *Aulacoseira subarctica* (O. Müller) Haworth in darkness revealed that a resting phase induced under low illumination ($<1.2 \mu\text{mol} \cdot \text{photons} \cdot \text{m}^{-1} \cdot \text{s}^{-1}$) was accompanied by shrinkage and rounding of the chloroplasts (Gibson and Fitzsimons 1990).

In frustules of *Aulacoseira* species preserved in annual lacustrine mud layers deposited during the early Holocene (approximately 6.5 cal kyr BP), we found greenish/light-brown granules that are morphologically similar to the “cytoplasmic masses” in Sicko-Goad et al. (1986) and to the “rounded chloroplasts” in Gibson and Fitzsimons (1990). In a piston core of the

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postglacial lacustrine sediments of Lake Suigetsu (35°35'N, 135°53'E) on Honshu Island, Japan, a continuous section with well-preserved laminae was found to be rich in these light-brown granules. Based on a comparison of the granules found in the muds with cytoplasmic masses from the cells of living *Aulacoseira* species collected from the anoxic sediments of a modern lake, we suggest that the granules in the muds are shrunken cytoplasmic masses that have been preserved for approximately 6500 years, and discuss the taphonomic and paleo-ecological implications of this finding. The role of resting stages in annual diatom cycles has been examined in modern marine and freshwater environments with various results (McQuoid and Hobson 1996). This paper presents a geologic record of annual diatom cycles that may help to elucidate the role of resting stages in the seasonal succession of diatom assemblages in temperate lakes.

MATERIALS AND METHODS

Lake Suigetsu, one of five lakes in a river system that discharges north into the Japan Sea, has an area of 4.16 km². The lake consists of a simple primary basin with a maximum depth of 33.7 m (Kawakami et al. 1996).

SG-2 core. Piston core SG-2 (35° 34'.51 N, 135° 52'.57 E, 11 m long, collected at a water depth of 33 m) consists predominantly of laminated sediment (Kitagawa et al. 1995, Kawakami et al. 1996). Alternating light- and dark-colored laminae are typically one to several millimeters thick. A well-preserved section containing 12 laminae was used for detailed analysis (Fig. 1a). The section, 11.66 mm thick, is about 80 cm above the K-Ah tuff (about 7325 years before 1995; Fukusawa 1995). The sediment accumulation rate for nearby core SG-1, used to estimate the rate for site SG-2, averages 0.94 mm/year (Itota et al. 1993) or 0.91 mm/year (Fukusawa et al. 1995). Therefore, this section was inferred to have been deposited around 6.5 cal kyr BP.

A high-quality color digital image from the laminated section was converted to a gray-scale image. A 50-pixel-wide representative cross-section was extracted from the image, and a power-spectrum estimate of a light-color intensity transect was obtained using NIH Image version 1.57. Sediment from the laminated section was sequentially peeled from the flat core face with 4-cm lengths of Scotch adhesive tape (3M, St. Paul, MN, USA). The tape was mounted on a glass slide with immersion oil and covered with a 24 × 60 mm cover slip.

Counts of diatom valves were made on 220 × 220 μm² areas of representative laminae using a microscope equipped with differential interference contrast optics (Axioplan, Carl Zeiss, Jena, Germany). Relative species abundances were determined by counting the diatoms in each square in the 11.66 mm long transect at ×640 magnification. A total of 13,589 specimens were counted in 53 view areas, which contained 108–471 specimens per area. The presence or absence of greenish/light-brown granules within each frustule of *Aulacoseira* species was also recorded.

To identify any long-term change in both the preservation of greenish/light-brown granules in *Aulacoseira* frustules and the composition of the *Aulacoseira* species, lamina-by-lamina counts were performed on 235 laminae of core SG-2 (792–771.7 cm below the core top; horizon G in Fig. 1a). The counts were made on 220 × 220 μm² areas of representative laminae using a transmitted light microscope, and about 1.5 areas per lamina were examined. A total of 44,934 specimens were counted in 359 view areas, which contained 83–206 specimens

per area. Preservation state was classified as good (granules were found in 60%–90% of the frustules), moderate (30%–60%), poor (5%–30%), or dissolved (<5%). Relative species abundances were determined by counting *Aulacoseira* species in each square.

Mud samples from eight horizons in core SG-2 (arrows A–F, H, and I in Fig. 1a, Table 1) were studied to determine the presence or absence of greenish/light-brown granules within *Aulacoseira* frustules. The counting procedure and the presence/absence determination for granules were identical to the methods described above for the 12-lamina section.

For taxonomy on *Aulacoseira* species, samples used in SEM observations were prepared by concentrating materials on a 0.45 μm millipore filter (type HA, Millipore Corp., Bedford, MA, USA). Dried filters were mounted onto SEM stubs and coated with gold. The slides for LM observations were prepared as in Tanimura (1999). Taxonomic references are provided in Appendix 1.

Deoxyribonucleic acid content of the greenish/light-brown granules in *Aulacoseira* frustules from the section was tested by staining with 4'-6 diamino-2-phenylindole (DAPI) (Sigma Inc., St. Louis, MO, USA). For each of the 12 laminae, total carbon contents were measured at Tokyo Metropolitan University using a carbon determination device (LECO c-200, LECO Corporation, St. Joseph, MI, USA) and a high-frequency combustion method, with iron and copper chips as accelerator in the ceramic pot.

Rejuvenation experiment on Aulacoseira species from Lake Fukami. Under a transmitted light microscope, the greenish/light-brown granules from *Aulacoseira* frustules in core SG-2 were compared with cytoplasmic masses in the frustules of living *Aulacoseira* species collected from surface sediments of Lake Fukami (35°19'N, 137°49'E, perimeter: 670 m, area: 22,000 m², average depth: 5 m, maximum depth: 9.3 m; Ishihara et al. 2003). Anoxic sediment is deposited in this lake owing to the intense stratification that develops each summer. Before the comparison, a rejuvenation experiment was performed using cells of *Aulacoseira* species from the anoxic sediments of Lake Fukami to confirm that the cells were in the resting phase. The *Aulacoseira* cells were inoculated into distilled water and incubated in a growth chamber for 2 weeks. Cultures were maintained at 15° C and 20° C, under a 12:12 light:dark regime under 2.5 μmol photons · m⁻² · s⁻¹ light intensity.

RESULTS

Cyclic alternations. The well-preserved lamination in core SG-2 consisted of alternating 0.1–59.5-mm-thick layers of clay-rich (dark-colored) and clay-poor (light-colored) mud (Fig. 1a). Some of the thick, dark-colored layers were “speckled beds” (Chang and Grimm 1999), which were not used in the analyses. The clay-rich laminae also contained plant fragments, pollen, sponge spicules, and chrysophycean cysts, whereas the clay-poor ones consisted mainly of diatom valves (Fig. 1b, c). The lithologic changes from light- to dark-colored laminae were generally gradual (Fig. 1d). Thin laminae consisting of valves of *Synedra* species locally separated a light-colored lamina from the overlying dark layer (Fig. 1e).

A power-spectrum estimate of the representative laminae revealed six layers with low values (Fig. 2b), which corresponded to both dark-colored laminae and laminae with better preservation of the greenish/light-brown granules in the frustules (Fig. 2, a and d). In

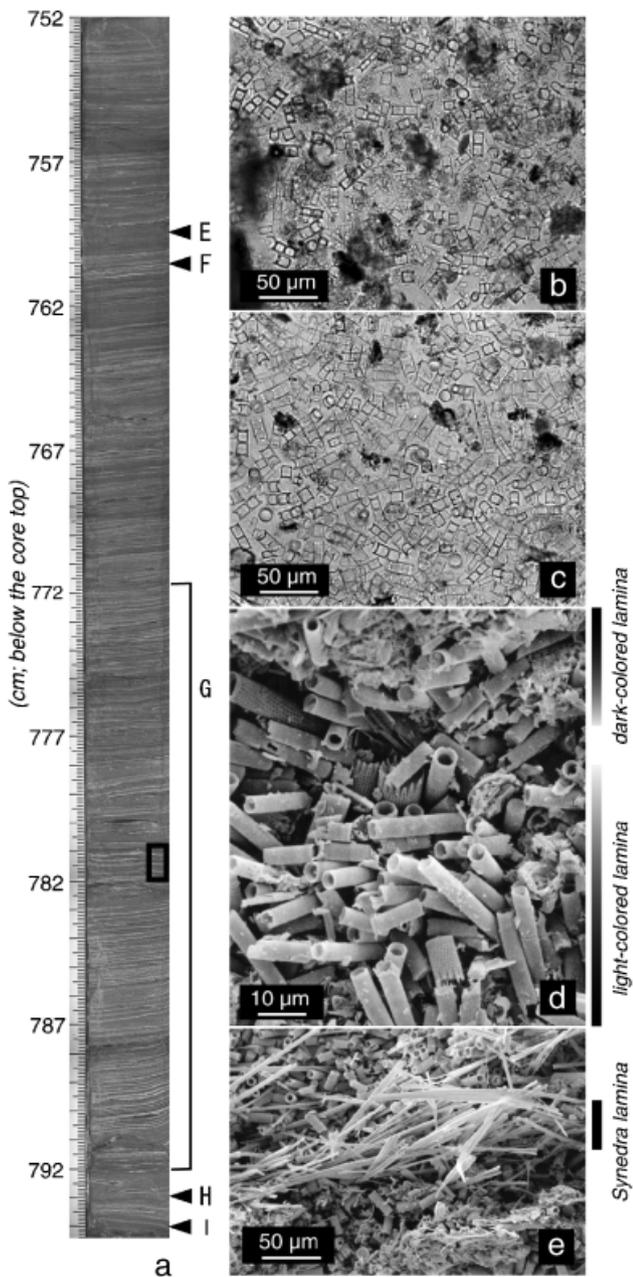


FIG. 1. Photograph of core SG-2 and light microscopy (LM) and SEM images of laminae. (a) Photograph of core SG-2 (752–794.5 cm below the core top). The rectangle encloses the 12 laminae examined in the detailed analysis (Fig. 2). Four arrows and one line (E–I) indicate the horizons for which floral analyses were performed. (b) and (c) LM photomicrographs of sediment peeled from the core face using adhesive tape (b, dark-colored lamina, c, light-colored lamina). (d) SEM images of the laminae showing gradual changes in content from light-colored (mainly *Aulacoseira ambigua* valves) to dark-colored (rich in diatom valves, clay, and organic matter) laminae. (e) A thin monospecific lamina of *Synedra* sp. between light- and dark-colored laminae.

both light- and dark-colored laminae, granules were better preserved in frustules of *A. ambigua* (Grun.) Simonsen, as compared with in those of *A. nipponica* and *A. subarctica* (Fig. 2d).

TABLE 1. Greenish/light-brown granules in frustules of *Aulacoseira* species.

Sample name Centimeters below the core top	A 338		B 456		C 553		D 654		E 759.5		F 760.5		H 793		I 794	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
<i>A. ambigua</i>	37	36	0	40	48	5	78	5	9	21	54	15	30			
<i>A. granulata</i>	41	16	5	<1	<1	2	46	<1	<1	<1	<1	1	1			
<i>A. nipponica</i>	0	93	6	15	26	84	32	84	5	73	22	79	6			
<i>A. subarctica</i>	21	26	2	13	13	8	32	10	6	4	13	4	6			
Other <i>Aulacoseira</i> species	<1	<1	0	1	1	<1	<1	1	1	1	1	<1	<1			

a, percent abundances of *Aulacoseira* species in the total *Aulacoseira* species counts.
 b, percent abundances of frustules of *Aulacoseira* species in which greenish/light-brown granules are preserved.

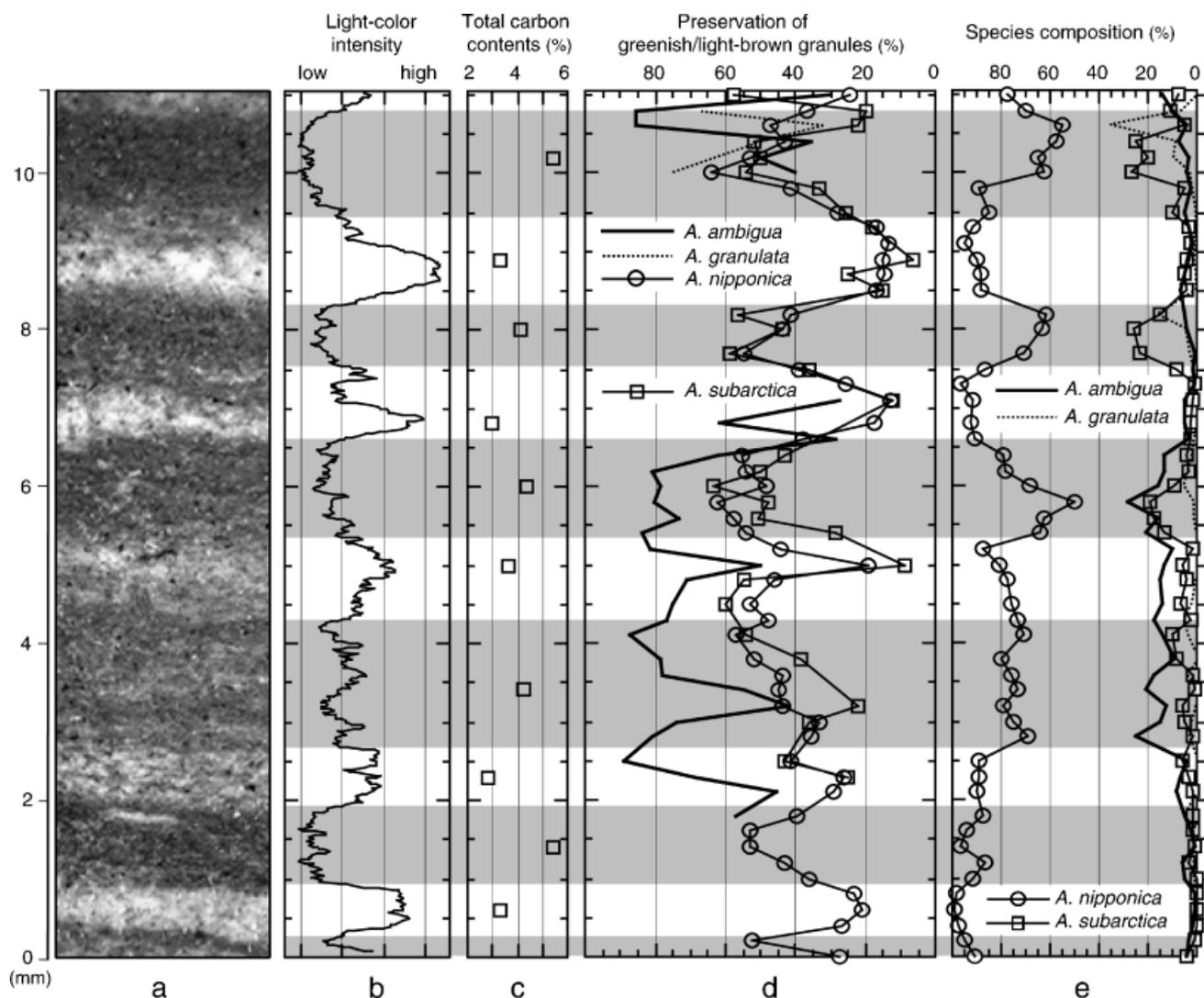


FIG. 2. Results of image and floral analyses on the 12 laminae (rectangle in Fig. 1a). (a) Photograph of the 12 laminae. (b) Power-spectrum estimate of a light-color intensity transect across the laminae. (c) Stratigraphic change in total carbon content. (d) Stratigraphic change in percent abundance of frustules of *Aulacoseira* species in which greenish/light-brown granules are preserved. (e) Stratigraphic change in percent abundance of *Aulacoseira* species in the total *Aulacoseira* species counts. Shaded zones indicate the dark-colored laminae.

All 12 laminae were dominated by *Aulacoseira* species: *A. ambigua*, *A. granulata*, *A. nipponica*, and *A. subarctica* (Fig. 2e). Together, these species represented approximately 95% of the diatom flora. Species of *Cyclotella*, *Stephanodiscus* (Kato et al. 2003, 2004), and other freshwater forms made up the remaining 5%, except for the thin layers of *Synedra* that formed monospecific laminae.

In the lamina-by-lamina counts, the following trends were evident: (1) the greenish/light-brown granules were predominantly preserved in dark-colored laminae; (2) *A. nipponica* are generally dominant in light-colored laminae, including the section containing the 12 laminae studied; and (3) *A. ambigua* and *A. subarctica* were relatively abundant in the section's dark-colored laminae (Fig. 3).

No granules stained with DAPI. The total carbon content measurements revealed that dark-colored laminae had slightly higher total organic carbon contents

(around 5%) than light-colored ones (up to 4%) (Fig. 2c). In the eight mud samples from elsewhere in the core, greenish/light-brown granules were preserved in frustules of *A. ambigua*, *A. granulata*, *A. nipponica*, and *A. subarctica* (Table 1).

Resting cells of Aulacoseira species. Two types of cytoplasmic masses were found in resting cells of *Aulacoseira* species from Lake Fukami: discoid masses (two to six per cell, greenish/light-brown in color, about 2 μm in diameter, commonly with a depression in the middle; Fig. 4a), and smaller, partly broken particles (two to six per cell, greenish brown in color, less than 1 μm in diameter). After 14 days in culture, about 67% of the cells with the former type of masses rejuvenated (Fig. 4b).

The greenish/light-brown granules in frustules of *Aulacoseira* species from core SG-2 were discoid, usually two to six per cell, about 2 μm in diameter, and

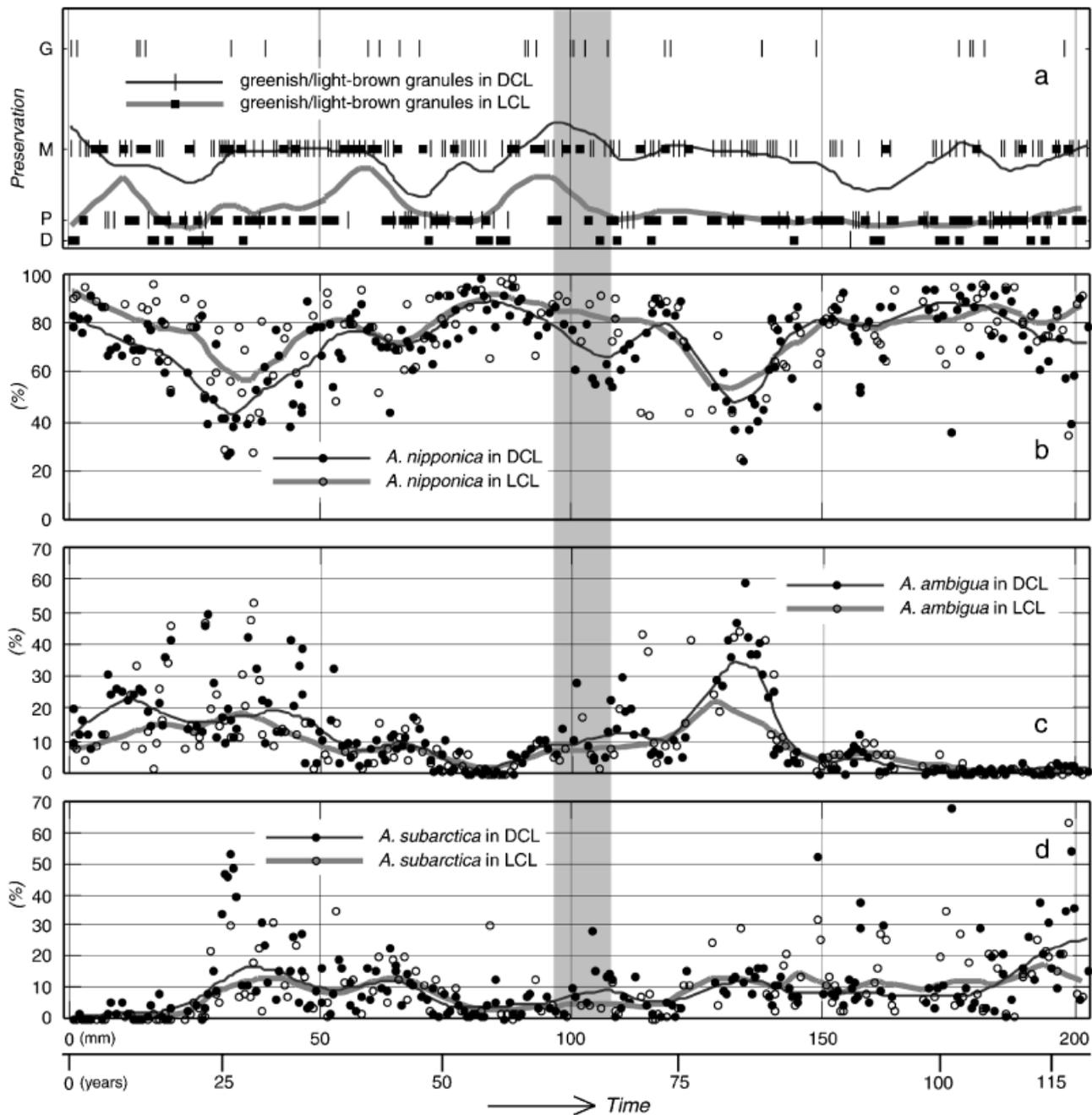


FIG. 3. Results of floral analyses on the 235 laminae (line G in Fig. 1a). (a) Preservation of greenish/light-brown granules in frustules of *Aulacoseira* species (G, good; M, moderate; P, poor; D, dissolved). (b–d) Stratigraphic changes in percent abundances of *Aulacoseira* species in the total *Aulacoseira* species counts. Calculated ages were dated by varve chronology. “Speckled beds” (Chang and Grimm 1999) were not included in the dating. Shaded zone indicates the 12 laminae studied in the detailed analysis. (DCL, dark-colored laminae; LCL, light-colored laminae).

commonly had one or several depressions (Fig. 4, d–h). Frustules containing larger granules similar to the expanded cytoplasmic masses in frustules of living *Aulacoseira* species were not found in laminae of core SG-2.

DISCUSSION

Greenish/light-brown granules. Cells of *Aulacoseira* species collected from the modern anoxic sediments

from Lake Fukami rejuvenated within 2 weeks of the onset of incubation, indicating that these cells were in a resting phase. Based on a comparison of the morphology of cytoplasmic masses in the resting cells from Lake Fukami and that of greenish/light-brown granules in *Aulacoseira* frustules from core SG-2, the latter were identified as contracted cytoplasmic masses in resting phase cells. The shape (discoid, with a depression) and number of cytoplasmic masses (2–6)

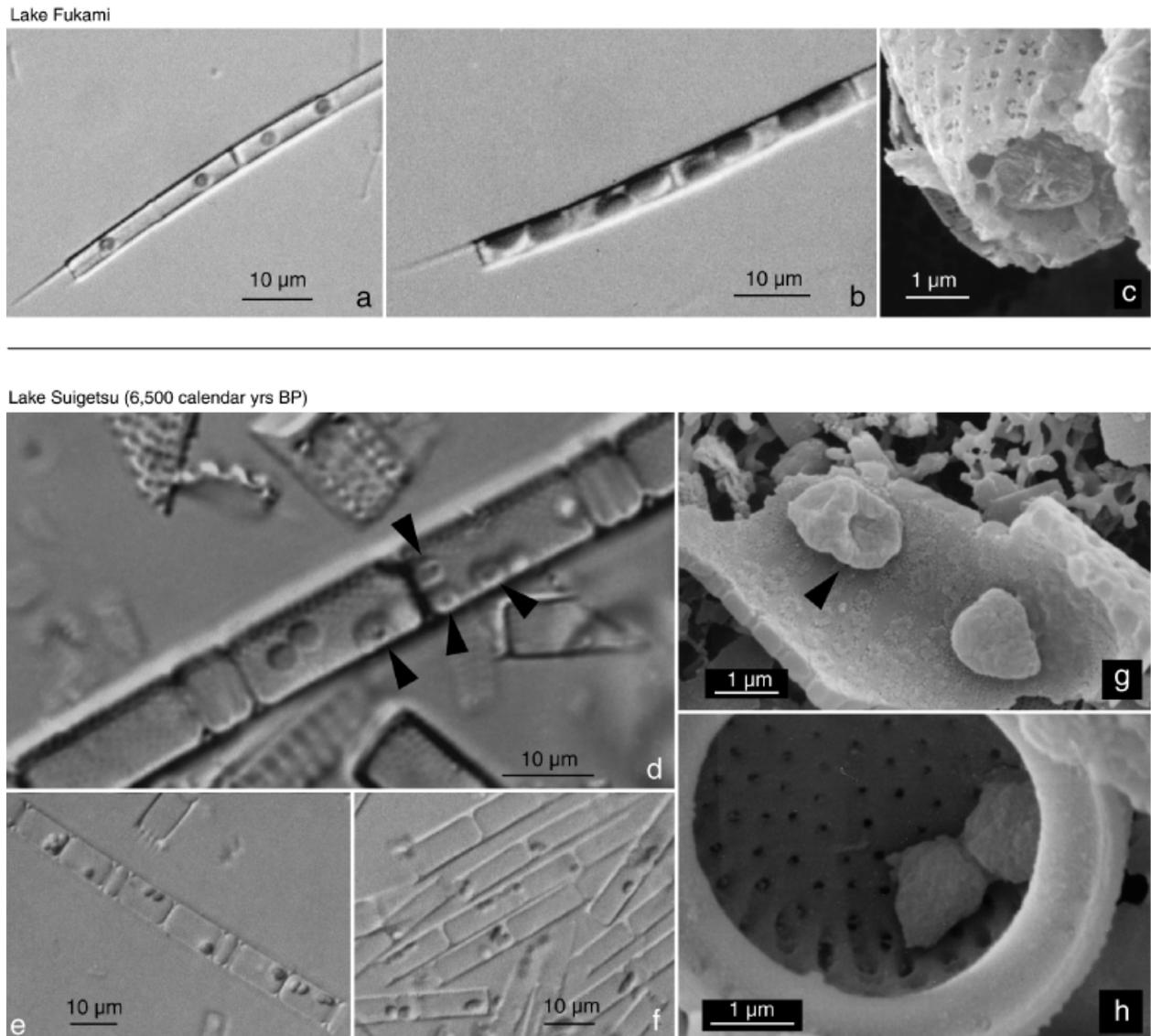


FIG. 4. Light microscope (LM) and SEM photomicrographs of cytoplasmic masses and greenish/light-brown granules. (a) Cells of living *Aulacoseira granulata* in the resting phase, Lake Fukami (LM). Each cytoplasmic mass has a depression in its middle. (b) *A. granulata* cells after rejuvenation, showing expanded cytoplasmic masses, Lake Fukami (LM). (c) A cytoplasmic mass in an *A. granulata* valve, Lake Fukami (SEM). (d–h) Greenish/light-brown granules preserved in *Aulacoseira* frustules, core SG-2 (d–f, granules preserved in *A. ambigua* frustules (LM); g, granules in *A. ambigua* (?) valve (SEM); h, granules in *A. subarctica* valve (SEM)). Arrows indicate granules with central depressions.

in the resting cells were similar to the granules seen in diatom frustules from the lacustrine muds of core SG-2. Sicko-Goad et al. (1986) divided the rejuvenation process of *Aulacoseira* species into three stages based on cytoplasmic expansion: condensed, partly expanded, and fully expanded. Cytoplasmic masses in frustules of *Aulacoseira* species from core SG-2 were classified as condensed (i.e. they contained small, dark brown cytoplasmic masses), because they had a discoid shape similar to that reported in Sicko-Goad et al. (1986). Given that resting-phase cells have condensed cytoplasmic masses (Sicko-Goad et al. 1986), the cells with the greenish/light-brown granules in

core SG-2 were probably in the resting phase just before burial in anoxic mud.

The result of the DAPI staining experiment suggests that the greenish/light-brown granules in *Aulacoseira* frustules from core SG-2 probably contain no undamaged DNA, and that the granules are not modern bacterial cells.

Sedimentologic and taphonomic processes. Lake Fukami is likely a good modern analog for Early Holocene Lake Suigetsu. In Lake Fukami, pronounced stratification develops annually in summer, causing the formation of an anoxic nepheloid layer on the lake bottom (Saijo et al. 1981). Lake stratification

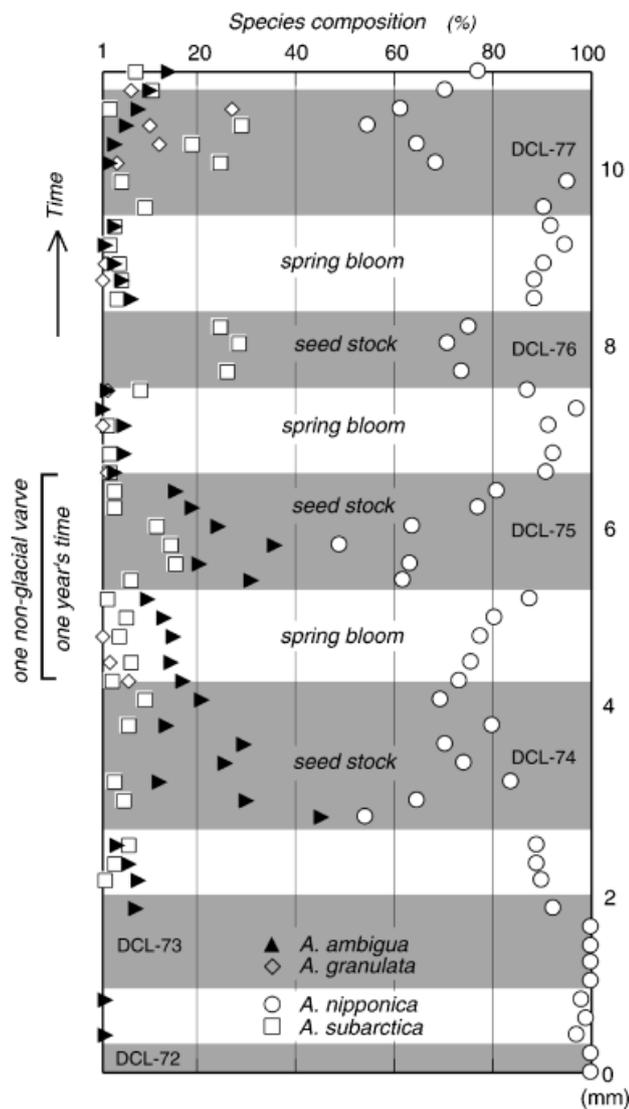


FIG. 5. Changes in percent abundance of *Aulacoseira* species in the 12 laminae studied. Based on a count of *Aulacoseira* frustules in which cytoplasmic masses are preserved, percent abundance of *Aulacoseira* species for each dark-colored lamina was determined. Percent abundances of *Aulacoseira* species in each light-colored lamina were determined by percent abundances of *Aulacoseira* species in each total *Aulacoseira* species count. Shaded zones indicate dark-colored laminae and white zones indicate light-colored laminae (DCL-72 to -77: dark-colored laminae).

owing to surface water warming during summer prevents oxygenation of the deeper parts of the water column, and an organic-rich anoxic layer is formed. Each organic-rich annual sediment layer becomes a dark-colored lamina upon burial, so that the annual cycle of water stratification results in alternating light- and dark-colored laminae. Ishihara et al. (2003) correlated changes in diatom assemblages with alternating lamina colors, and found that light- and dark-colored laminae represent an annual couplet.

The mud in Lake Suigetsu also consists of light- and dark-colored laminae, with similar contents and sedimentary structures to those in Lake Fukami. Fukusawa (1995) examined thinly laminated parts of core SG-2 and identified a relationship between AMS ^{14}C ages and calculated ages dated by varve chronology, yielding the conclusion that the thinly laminated parts represented non-glacial varves. Each springtime diatom bloom was recorded as a light-colored lamina in the lower part of a single varve, whereas diatoms that reproduced from early summer (rainy season) to autumn were recorded as a dark-colored lamina in the upper half of each varve. An increased influx of river water transporting clay minerals to the lake bottom in the rainy season likely caused the high-clastic content of dark-colored laminae. This conclusion, together with the similarity in sedimentary content and structures of both lakes, suggests that an organic-rich anoxic layer on the lake bottom also prevailed in Lake Suigetsu during summers in the Early Holocene. This inference is supported by the identification of siderite (FeCO_3) in the dark-colored laminae from thinly laminated parts of core SG-2 using an X-ray diffractometer JDX3536 (JEOL, Akishima, Japan) (Fukusawa 1995, Kawakami et al. 1996). Allison (1988) studied the depositional conditions required to preserve organism soft parts as pyrite, phosphate, and carbonate, and concluded that carbonate preservation requires organic-rich sediments and rapid (catastrophic) burial.

The organic-rich anoxic layer could be suitable for preserving cytoplasmic masses in *Aulacoseira* frustules on the lake bottom. Anoxic lake-bottom conditions probably prevented the decomposition of the diatoms' organic matter and silica frustules by aerobic bacteria (Bidle and Azam 1999). The absence or poor preservation of cytoplasmic masses in the light-colored laminae is consistent with this inference. Before the onset of water stratification in the spring, the lake bottom is likely oxygen rich, permitting aerobic bacteria to decompose organic matter, including the cytoplasmic masses in *Aulacoseira* frustules.

Paleoecological implications. Some *Aulacoseira* (= *Melosira*) species have been found to survive in sediments for one to decades (Nipkow 1950, Lund 1954, Stockner and Lund 1970). Because resting cells can survive in the dark at cool temperatures, McQuoid and Hobson (1995) proposed that resting cells are over-wintering forms that provide seed stocks for subsequent spring blooms. In Lake Suigetsu, the cytoplasmic masses of *Aulacoseira* species are abundantly preserved in dark-colored laminae that formed in the summer months, whereas few are preserved in the light-colored laminae that represent spring blooms. This cyclic alternation in the preservation of cytoplasmic masses in the lake sediment suggests that the diatoms in dark-colored laminae had the potential to act as seed stocks for the annual cycle of diatom assemblages.

If this inference is correct, the seed stocks of *A. nipponica* rejuvenated each spring, and contributed to the

formation of each light-colored lamina (Fig. 5). In dark-colored laminae (e.g., DCL-74, -75, and -76), percent abundances of *A. nipponica* among the frustules containing greenish/light-brown granules averaged 71.1, 66.1, and 73.4%, respectively, and the species' abundances in the overlying light-colored laminae (between DCL-74 and -75, DCL-75 and -76, and DCL-76 and -77) averaged 79.0, 91.7, and 90.9%, respectively. This evidence indicates that *A. nipponica* successfully reproduced in the spring blooms. In regard to long-term changes in species composition, this species also dominated in light-colored laminae for about 115 years, with the exception of three short intervals. Adequate mixing of the water column in winter to early spring owing to a monsoon wind might have elevated the species' resting cells in the production zone by resuspending the topmost dark-colored layer or the dark-colored and the underlying layers, allowing the resting cells to rejuvenate.

The granule-containing *A. ambigua* and *A. subarctica* were common in DCL -74 and -75, and in DCL -75 and -76, respectively. However, the two species were rare in the overlying light-colored laminae (Fig. 5). There are two possible explanations for this. First, springtime lake water factors such as temperature or nutrient concentration might have been suitable for rejuvenation of *A. nipponica*, but not for the two species. Second, the two species may have reproduced mainly in the autumn months then settled to the anoxic lake bottom where cytoplasmic masses could be well preserved. The latter inference is consistent with the facts that the granules were generally better preserved in frustules of *A. ambigua* than in *A. nipponica* (Fig. 2d), and *A. ambigua* generally dominated in dark-colored laminae in the long-term changes in species composition (Fig. 3c).

Chains of *Synedra* sp., seen at boundaries between dark- and light-colored laminae, were commonly locked together, suggesting that this taxon reproduced abundantly in a short period in late spring to early summer, were partly intertwined during life, and sank rapidly to the bottom.

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APPENDIX 1

TAXONOMIC REFERENCES WITH NOTES

- Aulacoseira ambigua* (Grun.) Simonsen, R., 1979: *Bacillaria*, 2: 56. (Fig. 6, a and b)

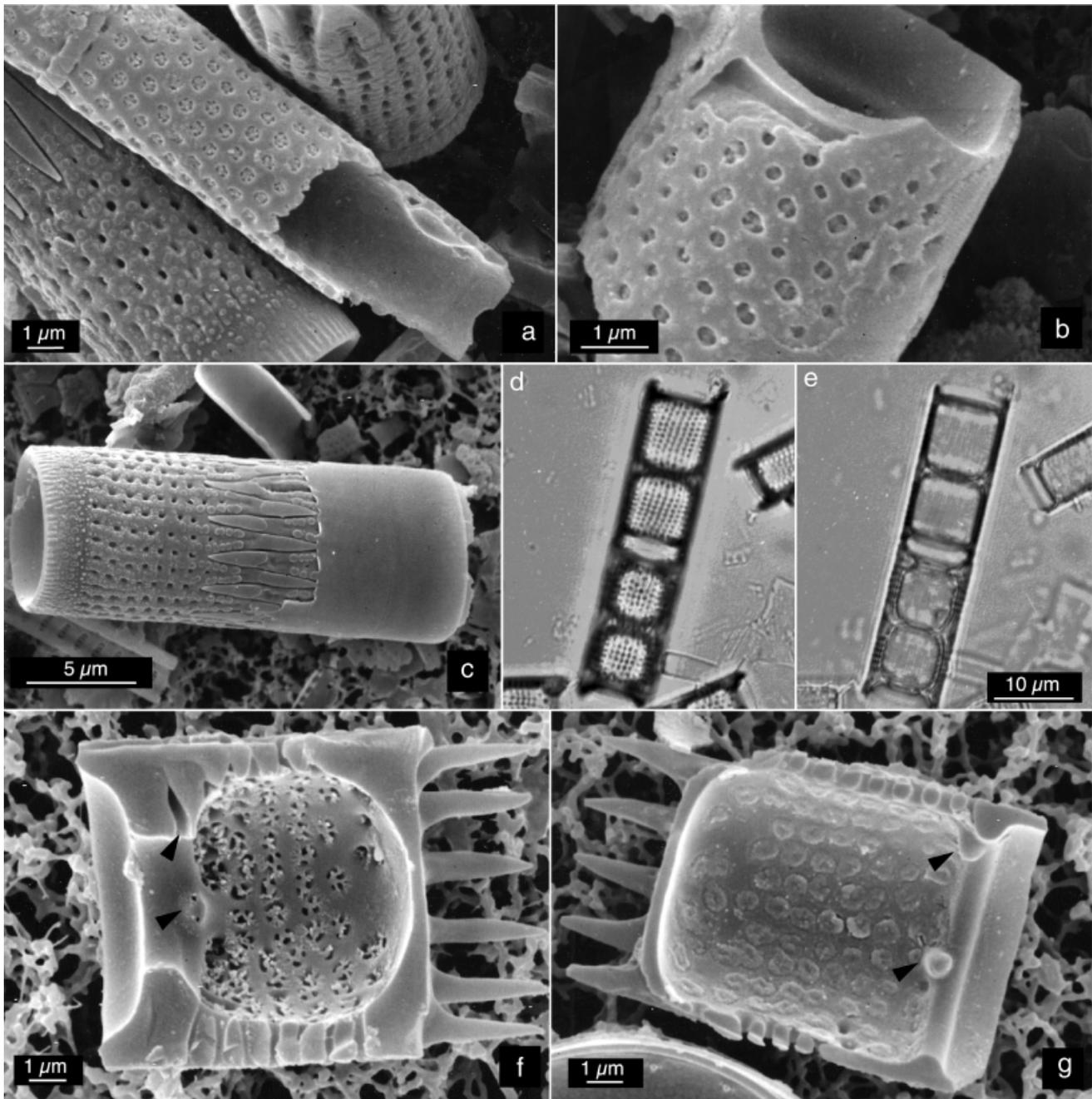


FIG. 6. Light microscope (LM) and SEM images of *Aulacoseira* species. (a and b) *A. ambigua* (SEM). (c) *A. nipponica* (SEM). Two intact valves making a colony with large linking spines. (d and e) *A. nipponica* (LM). A colony consisting of two thinner (upper half) and two thicker valves. (f and g) *A. nipponica* (SEM). A thicker valve with open cribra and two rimoportulae on the sulcus (arrows), and a thinner valve with occlusive cribra and two rimoportulae on the sulcus (arrows).

Melosira crenulata var. *ambigua* Grunow in van Heurck, H., 1882: Synopsis des diatomées de Belgique, pl. 88, Figs. 12–14.

Aulacoseira granulata (Ehr.) Simonsen, R., 1979, Bacillaria, 2: 58. (Fig. 7, a–e)

Gaillonella granulata Ehrenberg, C. G., 1843 in Abh. Königl. Akad. Wiss. Berlin: 127

This highly variable taxon has at least two morphotypes, a thicker and a thinner one (Fig. 7, a, b and c, d, respectively).

Aulacoseira nipponica (Skvortzow) Tuji, A., 2002, Phycological Research, 50: 315. (Fig. 6, c–g)

Based on comparisons of valve morphology between *Melosira solida* Eulenstein (manuscript, Grunow in van Heurck, 1882, pl. 86, Figs. 36–39, from Carcon in California) and *M. solida* sensu Skvortzow (1936, p. 255, pl. 1, Figs. 3–6, 17 and 24, from Lake Biwa), Tuji (2002) concluded that these were different species. He transferred a variety of *M. solida* sensu Skvortzow, *M. solida* var. *nipponica* Skvortzow (1936, p. 255, pl. 1, Figs.

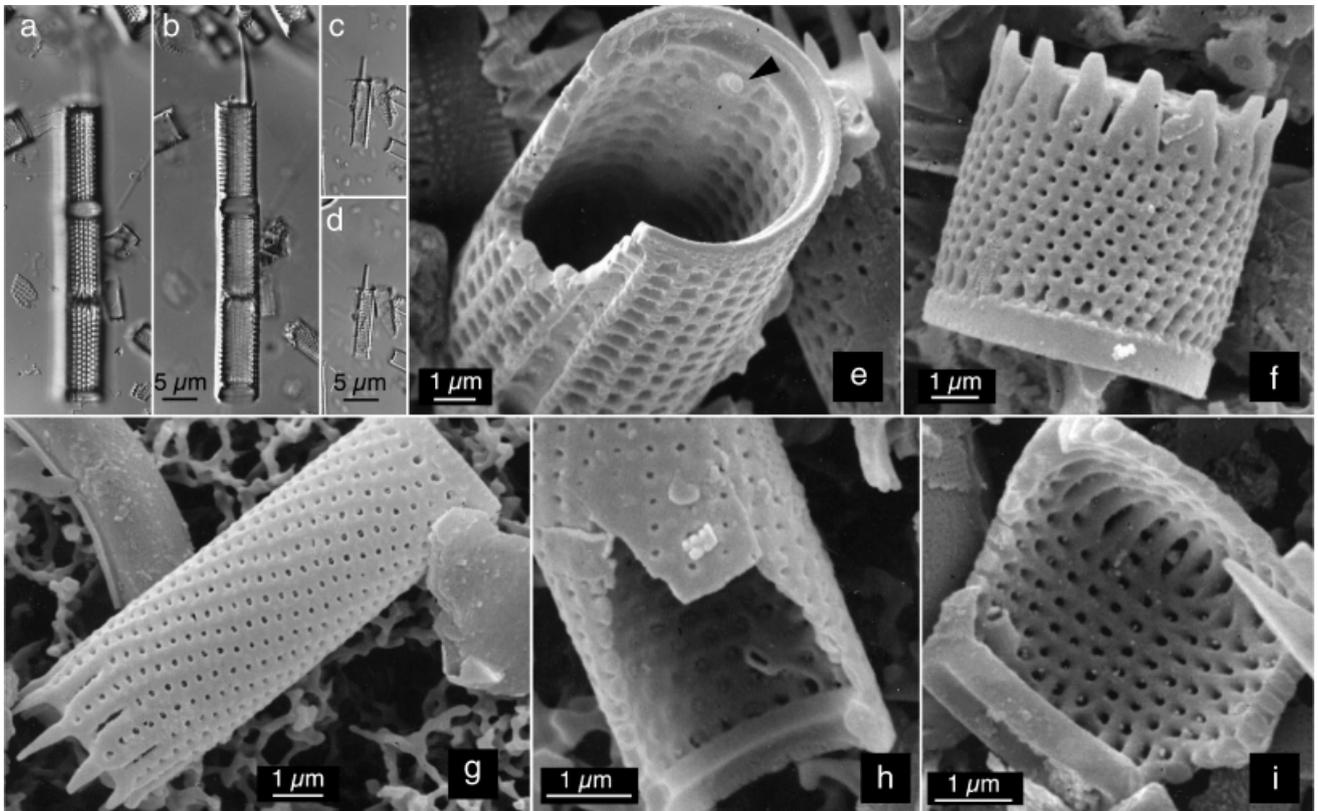


FIG. 7. Light microscope (LM) and SEM images of *Aulacoseira* species. *A. granulata* (LM). A colony consisting of three thicker valves (a, b) and a thinner valve. (e) *A. granulata* (SEM). A thicker valve with one rimoportula near the sulcus (arrow). (f–i) *A. subarctica* (SEM). A shorter (f) and a taller (g) valve. (h) A taller valve with one rimoportula located near, but not on, the sulcus. (i) A shorter valve with one rimoportula.

1–2, and 21), to species rank *Aulacoseira nipponica* (Skvortzow) Tuji, and placed *M. solida* var. *solida* sensu Skvortzow (1936) synonymous with *A. nipponica*.

In core SG-2, two types of valves were found (thicker and thinner valves), which together form colonies (Fig. 6, d and e). In transmitted light, thicker valves are safely assigned to *A. nipponica*. Tuji (2002) and Tuji and Houki (2001) did not illustrate the thinner valve of this species. In this study's floral analyses, thinner valves were also included under *A. nipponica*.

Aulacoseira subarctica (O. Müller) Haworth, E., 1988, *Diatom Research*, 5: 195. (Fig. 7, f–i)

Melosira italica subsp. *subarctica* Müller, O., 1906, *Jahrb. Wiss. Botanik* 43: 78, pl. 2, Figs. 7–11.

Tuji and Houki (2004) examined an *A. subarctica* species complex using type materials and reported that the rimoportulae of the species are located either near or distant from, but not on, the sulcus. They also suggested that the position of rimoportulae relative to the sulcus was very stable in *A. subarctica* specimens from a single locality. Rimoportulae of *A. subarctica* specimens from core SG-2 were located near the sulcus (Fig. 7, h and i). Tuji and Houki (2004) found the species to be hyaline on the valve face, whereas some specimens examined here with SEM had areolae on the valve face (Figs. 4h and 7i), as shown in Gibson et al. (2003).