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GLOBAL DIVERSITY OF TWO WIDESPREAD, COLONY-FORMING DIATOMS OF THE MARINE PLANKTON, *CHAETOCEROS SOCIALIS* (SYN. *C. RADIANS*) AND *CHAETOCEROS GELIDUS* SP. NOV.[1]

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Marine phytoplankton samples containing diatoms of the *Chaetoceros socialis* group were collected from Thailand, China, Denmark, and Greenland, and cells were isolated into culture for light and electron microscopy and DNA sequencing of D1–D3 of the LSU rDNA. Species of this lineage are characterized by three short and one long setae extending from each cell, the long setae from several cells joining into a common center to form large colonies, which are sometimes visible with the naked eye. Phylogenetic analyses including sequences from other parts of the world revealed segregation into three groups. Most sequences fell into two large clades, one comprising material from cold waters, whereas the other contained material from warmer waters. Strain CCMP 172 from the Strait of Georgia, Washington State, USA, formed a separate group. The warm-water species included Chinese and Thai material and therefore probably also material from the type locality of *C. socialis*, Hong Kong. It is characterized by all setae being covered by spines and the setae extending from the valve at some distance from the margin. In the resting spores, both valves are ornamented with spines. The cold-water material is characterized by three spiny and one mostly smooth long setae, and the setae extend from the valve near the margin. Both valves of the resting spore are smooth. This material is described as *C. gelidus* sp. nov. *C. radians*, described from the Baltic in 1894, is considered a synonym of *C. socialis*. CCMP172 is in many ways intermediate and probably constitutes a separate species. The published evidence on this globally distributed and sometimes bloom-forming group of species indicates higher species diversity than presently thought.

Key index words: **Arctic; biogeography; *Chaetoceros gelidus* sp. nov.; *Chaetoceros radians*; *Chaetoceros socialis*; diatom; distribution; global; phylogeny**

***Abbreviations*: BLAST, basic local alignment search tool; CTAB, cetyltrimethyl ammonium bromide**

Chaetoceros socialis Lauder 1864 is a well-known diatom of the marine phytoplankton, reported from polar to tropical areas (e.g., Ostenfeld 1913, Rines and Hargraves 1988). It is often numerous and may form blooms (e.g., Chiloë, Chile: Clément and Lembeye 1993). According to Ostenfeld (1913) it is neritic and occurs not far from the coast or from sea ice. Along the northern Norwegian coast it is at times the most abundant species in spring (Degerlund and Eilertsen 2010), and in arctic areas it is one of the most common diatoms, which may form extensive blooms. Thus, in Baffin Bay it is one of the two dominant groups of diatoms in summer, reaching 30 million cells $\cdot$ L^{-1} in July 1998 and constituting up to 91% of the total phytoplankton cells in moored sediment traps (Booth et al. 2002). Intense and long-term blooms have been reported to affect the appetite of cultured fish (Clément and

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Lembeye 1993) and a negative impact of blooms has also been reported on growth of seaweed laver (*Porphyra*) in Japan (Takano in Fukuyo et al. 1990). Since the description of the very similar *C. radians* Schütt 1895, uncertainty developed regarding the difference between *C. socialis* and *C. radians*, with some authors treating them as varieties of *C. socialis* (from Proschkina-Lavrenko 1953, 1963 onwards). Presently most researchers consider the two species/varieties to differ only in the morphology of the resting spores, those of *C. socialis* being smooth and those of *C. radians* covered with spines (e.g., Rines and Hargraves 1988, Jensen and Moestrup 1998). This concept goes back to Ostenfeld (1913) who worked extensively on the two taxa and concluded that they could be distinguished only when resting spores were present. He therefore urged caution regarding records of the two species. Recently, Degerlund et al. (2012) published on a number of strains referred to *C. socialis* and concluded that based on spore morphology, physiology, and molecular data, the clones fell into two groups, a northern group from NE Atlantic/the Arctic and a southern group from the Tyrrhenian Sea. We have during our ongoing project on the taxonomy and phylogeny of *Chaetoceros* (e.g., Chamnansinp 2012) had access to material and cultures from both arctic, temperate and tropical waters, including Thailand and China (near the original description of *C. socialis* from Hong Kong), Denmark (near the original description of *C. radians* from the Baltic), and Greenland, and this has allowed a detailed study comprising both light- and electron microscopy and molecular sequencing of geographically widely separated strains. These strains also fell into two groups, which were characterized by morphology, type of resting spore, biogeography, and molecular signature. We conclude that the material constitutes two separate species and discuss the associated taxonomic and nomenclatural issues.

MATERIAL AND METHODS

Material was collected from four areas: Thailand, China, Denmark, and Greenland (Table S1 in the Supporting Information). Some cells were preserved in Lugol's solution or in formaldehyde, others were isolated into monoclonal culture in their country of origin, before being transported to and maintained at University of Copenhagen, Denmark. Cultures were established by single-cell or single-chain pipetting and maintained in L1-medium at a salinity of 31 (Guillard and Hargraves 1993). All cultures were incubated in a 16:8 light: dark (L:D) cycle, the illumination provided by cool fluorescent lamps. Cultures from Thailand and China were incubated at 22°C–26°C and cultures from Denmark and Greenland at 4 ± 1°C. For DNA sequencing, culture aliquots were concentrated and frozen.

Microscopy. For light microscope observations and photomicrographs, we used a Zeiss AxioCam HRc light microscope (Zeiss, Oberkochen, Germany), equipped with Nomarski interference contrast. For TEM and SEM, fixed samples were rinsed thrice in distilled water and left to sediment for 10 h in each change. The material was then diluted and dried onto formvar-coated copper grids for examination in a JEOL-1010 transmission electron microscope (TEM; Jeol, Tokyo, Japan). For SEM, fixed and rinsed material was filtered onto Isopore™ membrane filters, pore size 8 μm (Millipore, Billerica, MA, USA) and dehydrated in an ethanol series, 10 min in each change of 30, 50, 70, and 96% ethanol, followed by 15 min in 99.9% ethanol and 30 min in absolute ethanol. They were critical-point-dried in a BAL-TEC CPD 030 critical point drier (Balzer, Liechtenstein). The filters were attached to stubs with double-sticky carbon tape (12 mm diam., Agar Scientific, Essex, UK) and sputter coated for 100 s with gold-palladium in a JEOL JFC-2300HR coating unit (Jeol) before examination in a JEOL JSM-6335F scanning electron microscope (Jeol). Statistical analyses were performed using the XL-ToolBox addition for Excel.

Molecular data acquisition. DNA extraction was performed using a modified cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1987, Lundholm et al. 2003). PCR amplification of D1–D3 of the LSU rDNA was done using forward primer D1R-F (Scholin et al. 1994) and reverse primer D3B-R (Nunn et al. 1996) following Lundholm et al. (2002). The PCR products were purified using QIAquick PCR Purification Kit (Qiagen, Venlo, Netherlands) and sent to Macrogen (http://dna.macrogen.com) for sequencing using the same primers as for PCR.

Alignment and phylogenetic analyses. All sequences of *C. socialis* available from Genbank were included in the alignment. Sequences were initially aligned using Clustal W (Thompson et al. 1994) and subsequently edited manually in Bioedit (Hall 1999). Sequences of *C. costatus* and *C. fallax* were used as outgroup taxa according to initial analyses of a larger alignment of available *Chaetoceros* taxa from Genbank. All base pair positions were included in the analyses. Except for Bayesian, all analyses were performed using PAUP* version 4.0b.8 (Swofford 2003). Maximum parsimony (MP) analyses were done using heuristic searches with random addition of sequences (1,000 replicates) and a branch-swapping algorithm of TBR (tree-bisection reconnection). Gaps were treated as missing data and characters treated as multistate and unordered. The alignment included 54 parsimony-informative sites. Distance analyses were performed by neighbor joining (NJ; 1,000 replicates), using the general time reversible (GTR) model. The optimal model for the maximum likelihood (ML) analyses was found according to the Akaike Information Criterion with a 99% level of significance in Modeltest version 3.7 (Posada and Crandall 1998). ML analyses were performed by heuristic searches with 100 random addition replicates and the TBR branch-swapping algorithm. One thousand bootstrap replicates were performed in MP and NJ and 100 in ML. Bayesian analyses were performed in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). The analyses using four chains were run for 1,200,000 generations. The temperature was set to 0.1. Sample frequency was set to 100 and the number of burn-in generations was 3,000. A basic local alignment search tool (BLAST) search was used to explore whether additional sequences found would extend the geographic distribution of the taxa.

RESULTS

Morphological studies. The three morphological types examined by us will be described separately:

1. *Chaetoceros gelidus* sp. nov. Figures 1 and 2, Figure S1–S3 in the Supporting Information; Table 1, Table S2 in the Supporting Information

Synonym: Chaetoceros sociale var. *congesta* Meunier 1913, p. 46.

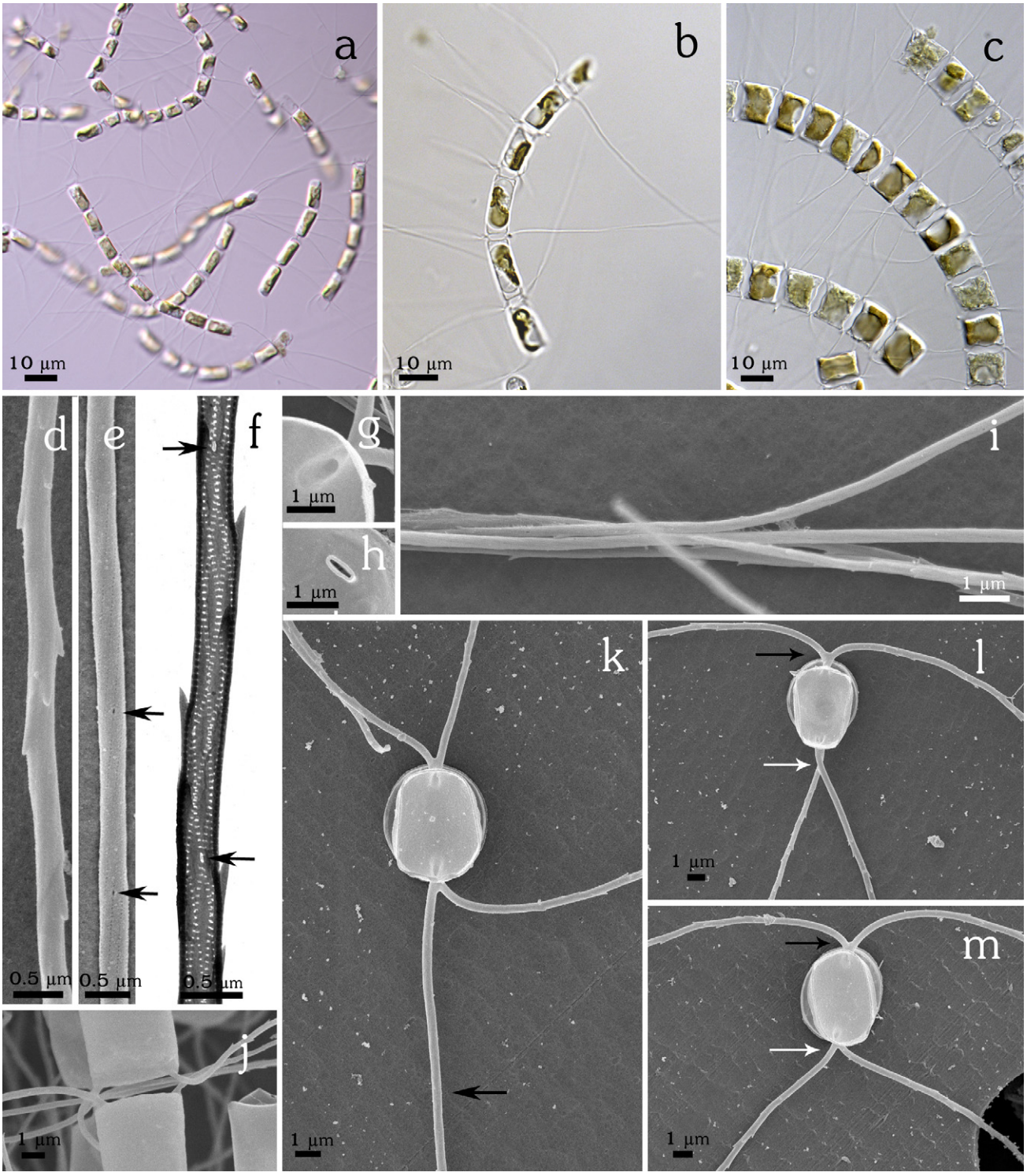

FIG. 1. *Chaetoceros gelidus* sp. nov. Cultures from Denmark (a, b, and d–m) and Greenland (c). (a–c) Culture D8, holotype (a, b, and h–j), D2 (d–f and k–m), P5G8 (c), D11 (g). (a–c) Nomarski Interference Contrast LM; (d, e, and g–m) SEM; (f) TEM. (a) Whole colony. (b) Single chain in girdle view, cells are longer than wide. (c) Cells from Greenland are more or less squared, often wider than long. (d) Spines on short seta. (e) Smooth long seta, the larger pores are visible (arrows). (f) Spines on short seta, large pores (arrows) and the system of poroids are all visible. (g) Internal view of valve showing base of seta near the valve margin. (h) Valve seen from the outside, showing unusual position of rimoportula. (i) Long setae are usually smooth except where they join each other. (j) Windows between adjacent cells are very narrow and the basal parts of the setae short. (k) Intercalary cell from colony showing long, smooth seta on the left, below (arrow), and three shorter spiny setae (Group II). (l) Single cell with four short spiny setae, those on top (black arrow) are opposite while those below (white arrow) cross-over at the base. (m) Single cell with four spiny setae extending in four directions. (l, m) Setae diverging 30–95° (white arrow) or 100–180° (black arrow).

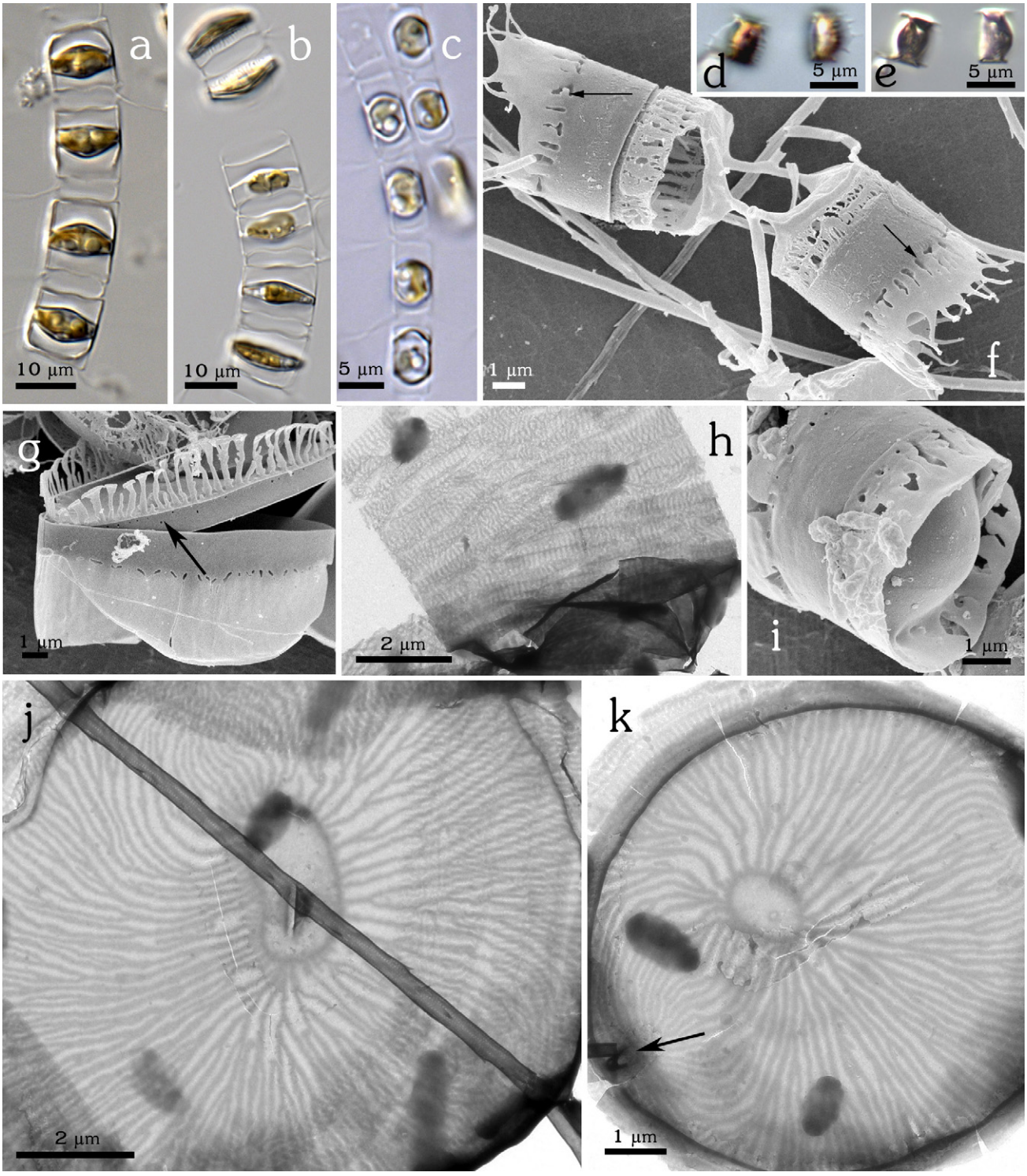

FIG. 2. *Chaetoceros gelidus* sp. nov. Cultures from Greenland (a, b, and g) and Denmark (c–f, h–k). (a–c). Cultures: P4D2 (a), P2F1E (b), D2 (c, h–k), D11 (d–f), P2F1E (g). (a–e) Nomarski interference contrast LM; (f, g, and i) SEM; (h, j, and k) TEM. (a, b) Resting spores located centrally in the mother cells, some immature spores are present in b. (c) Longer, smooth resting spores. (d, e) Resting spores in different focal levels showing both smooth valve surface and crest of projections. (f) Two resting spores of opposite polarity, the primary valves with smooth mantle to which is attached rim of processes, secondary valve rim of more or less fused processes. (g) Secondary valve seen from the inside, a row of punctae is present near the base of the mantle (arrow); the valve is enclosed in primary valve with ring of fused processes. (h) Girdle bands. (i) Resting spore, both primary and secondary valve with crest of fused projections, the convex valve face of the resting spore is smooth. (j, k) Valves with radial pattern of dichotomously branching costae from the annulus, in (j) with rimoportula slit. The costae converge toward the point of emergence of the setae near the valve margin. (k) The setae emerge near the margin of the valve (arrow), the costae converge toward the point of emergence of the setae.

TABLE 1. Morphometric data of *Chaetoceros gelidus* sp. nov., *C. socialis* in total based on four and nine strains, respectively (See Table S2). All measurements were based on 20 cells. The values indicate mean ± SD, with minimum and maximum values in brackets.

Code	Species	Apical axis (μm)	Pervalvar axis (μm)	Windows (μm)	Pervalvar axis with basal part (μm)
In total	*C. gelidus*	7.1 ± 2.7 (3.6–16.9)	9.7 ± 1.8 (5.0–14.7)	2.0 ± 0.6 (0.7–3.3)	11.5 ± 1.9 (7.8–16.9)
In total	*C. socialis*	6.8 ± 1.5 (4.3–11.5)	9.1 ± 1.6 (5.1–12.9)	5.1 ± 1.0 (3.1–6.9)	13.1 ± 2.1 (8.3–17.5)
CCMP 172	*Chaetoceros* sp.	5.7 ± 1.5 (3.8–8.8)	10.6 ± 1.9 (7.7–14.2)	2.3 ± 0.5 (1.4–3.3)	12.8 ± 1.9 (10.0–15.6)

Diagnosis. Curved chains of cells forming a more or less spherical colony, the cells included in a mucilaginous matrix. Cells in girdle view quadrangular, longer than wide or wider than long. Window between cells narrow with a wide hexagonal shape, often with central constrictions. Valves broadly elliptical to circular, with central annulus and costae radiating from the annulus. Setae extend from just within the corners of the cell and cross-over at the chain edge. The three short setae with spirally arranged sharp spines and spiral rows of poroids, the single long seta smooth with poroids, and with short spines where it joins the long setae from other cells. Terminal valve with rimoportula near the center of the valve. Girdle bands ornamented with parallel costae and two rows of scattered poroids. Apical cell axis is 7.1 ± 2.7 (3.6–16.9) μm long, pervalvar axis 9.7 ± 1.8 (5.0–14.7) μm long, pervalvar axis including the basal part of the setae 11.5 ± 1.9 (7.8–16.9) μm long, and length of window in the pervalvar axis 2.0 ± 0.6 (0.7–3.3) μm. Resting spores biconvex, one side usually more convex than the other, located near the middle of the mother cell, both valve faces smooth. The mantle on both primary and secondary valve with crest of more or less fused projections and a ring of perforations near the base. One chloroplast per cell. The marker sequenced was D1–D3 of LSU rDNA, GenBank accession number KF219703.

Holotype. Figure 1a, of culture D8, a fixed sample of the culture deposited at the Natural History Museum of Denmark, University of Copenhagen (CAT2482).

Isotype. Fixed sample of culture D12 deposited in the Natural History Museum of Denmark, University of Copenhagen (CAT2483).

Type locality. Skovshoved Harbour, The Sound (Øresund), Denmark.

Etymology. From gelus (Lat.): ice, the species occurring in cold water.

Detailed description: Curved chains of cells form a more or less spherical colony, with the cells included in a mucilaginous matrix (Fig. 1, a–c). Single cells were also observed. Cells are quadrangular in girdle view and longer than wide or wider than long (Fig. 1, a–c). The window between cells (aperture) is narrow and of a wide hexagonal shape (Figs. 1, b, c, j; 2, a and b), often with central constrictions (Figs. 1, c and 1; 2, a and b). Setae are delicate, one typically longer than the other three, and the long setae from several cells join into a common point (Brunel Group VI, Brunel 1972, Fig. 1a). Setae extend from just within the corners of the valve (Fig. 1, g, j–m), and cross-over at the chain edge. The three short setae have spirally arranged sharp spines and spiral rows of poroids (Fig. 1f), while the single long seta is smooth with spirally arranged poroids, and only with short spines where it joins the long setae from other cells (Fig. 1, d and i). All setae have large solitary pores (Fig. 1, e and f). The setae of the chains fall into two morphological types. In Type I, with only short setae, the setae of one pair diverge 30–95° from each other and sometimes cross-over proximally (Fig. 1, l and m, white arrow) whereas the setae of the other pair diverge 100–180° from each other (Fig. 1, l and m, black arrow); in Type II, with one long seta, setae of one pair diverge 50–90° (mostly 60) from each other (Brunel Group VI, states 30–50°). Setae of the other pair diverge about 90° from each other; one seta curving backwards while the other is long and directed straight toward the center of the colony (Fig. 1k, arrow). The terminal valve has a short elongate rimoportula slit near the valve center (Fig. 2j). It was seen once near the margin (Fig. 1h), but this may be a culture artifact. Rimoportulae are absent on intercalary valves (Fig. 2k). Valves are broadly elliptical to circular with a pattern of dichotomously branching costae radiating from a central annulus and from the two insertion points of the setae (Fig. 2, j and k). The area within the annulus is hyaline without costae. The shape of annulus is variable, from elliptical to very elongate or more irregular, generally of relatively smooth outline (Fig. S1, f–h).

Girdle bands are ornamented with parallel costae and two rows of scattered poroids (Fig. 2h).The apical cell axis is 3.6–16.9 μm long, the pervalvar axis 5.0–14.7 μm long, the pervalvar axis including the basal part of the setae 7.8–16.9 μm long, and the length of window in the pervalvar axis 0.7–3.3 μm (Table 1; Fig. S3). Resting spores are more or less biconvex (Fig. 2, a–c), one side usually more convex than the other, and located near the middle of the mother cell, with both valve faces being smooth (Fig. 2, e and i). The mantle on both primary and secondary valve has a crest of more or less fused projections (Fig. 2, d, f–h), and a ring of perforations near the base (Fig. 2, f and g). One chloroplast per cell.

Geographic distribution (localities where the resting spores have been illustrated or described). Denmark, April and December (present study), February, March (Ostenfeld 1913, Sundström 1973); Greenland, April and June (present study), July and August (Ostenfeld 1913); other parts of the Arctic (Ostenfeld 1913, probably also Degerlund and Eilertsen 2010 but cyst structure not given); the Norwegian coast (e.g., Evensen and Hasle 1975); the Faeroes (Ostenfeld 1913); the Bristol Channel, the English Channel, the North Sea, Iceland, the Murmansk Sea, February in the Irish Sea (Mangin 1913, Ostenfeld 1913, Lebour 1930); Kieler Föhrde (Busch 1916); France (Peragallo and Peragallo 1897-1908), Narragansett Bay (Hargraves 1979, Rines and Hargraves 1988), Gulf of California (Cupp and Allen 1938, Cupp 1943), Japan (Takano in Fukuyo et al. 1990), Beaufort Sea and Arctic Sea (from BLAST search). The optimum temperature of occurrence is reported to be 4°C–6°C (present study) and 1.9°C–3.0°C for Scandinavia by Cleve-Euler (1951).

Note. In material from Denmark the cells were usually longer than wide (Figs. 1, a and b; 2c), and valves of the resting spores were equally convex (Fig. 2c). In material from Greenland, however, cells were usually wider than long (Figs. 1c and 2b) and one valve in the resting spores sometimes less convex than the other or occasionally almost flat (Fig. 2e).

2. *Chaetoceros socialis* Lauder 1864, p. 77, pl. 8, fig 1. Figures 3 and 4, Figures S1–S3; Table S2

Synonyms: *C. radians* Schütt 1895, p. 41, *C. socialis* f. *autumnalis* Proschkina-Lavrenko 1953, p. 51, (invalid name), *C. socialis* f. *vernalis* Proschkina-Lavrenko 1953, p. 51, *C. socialis* f. *radians* (Schütt) Proschkina-Lavrenko 1963, p. 113.

Illustrated in the TEM by Evensen and Hasle (1975) (figs 40 and 41), as *C. radians*.

Epitype: Fixed sample of the culture YL1 deposited at the Natural History Museum of Denmark, University of Copenhagen (CAT2484), collected Daya Bay, Guangdon Coast, South China Sea, March 15, 2009.

Emended description (based on the epitype): Curved chains of cells, forming a more or less spherical colony. Single cells were also observed. Cells quadrangular, mostly wider than long in girdle view, occasionally longer than wide (Fig. 3, a–c). Windows large, of wide hexagonal shape, often with slight central constrictions (Fig. 3, b, c, g, and h). Setae emerge from the valve face at a distance of 0.20–0.25 times the cell width (the apical axis) from the valve margin (Figs. 3, d, g, and h; 4, e–h). One of the setae is typically longer than the others and join the long setae from other cells (Fig. 3, a and m; Fig. S2). Cells with four short setae are also observed (Fig. S2). All setae are covered with spirally arranged spines and poroids (Fig. 3, e, f, and l) and large, elongate, solitary pores (Fig. 3l). The orientation of the setae in the chains separate the material into two types, as in *C. gelidus* (Fig. 3, k and m). The valve face is narrowly to broadly elliptical or oval (Fig. 3, d and k), with radial, more or less wavy, dichotomously branching costae arising from a central annulus (Fig. 4, e and f). The annulus is irregularly elliptical (Fig. S3, a–e; Fig. 4, e and f), generally with a wavy outline. Terminal valve with an elongate rimoportula slit with short external tube (Fig. 3, i and j) situated centrally on the valve, intercalary valves without rimoportulae (Fig. 4, e and f). Several girdle bands with numerous costae and scattered, minute poroids (Fig. 4, i and j). Apical cell axis is 4.3–11.5 μm long (Table 1; Fig. S3), pervalvar axis 5.1–12.9 μm long, pervalvar axis with basal part of the setae 8.3–17.5 μm long, and length of window in the pervalvar axis 3.1–6.9 μm (Table 1). Resting spores biconvex, one side sometimes less convex than the other, with a small number of spines on each valve face (visible in the LM; Fig. 4, a–d). One chloroplast per cell. The marker sequenced was D1–D3 of LSU rDNA, GenBank accession number KF219701.

Geographic distribution (except for Hong Kong, only localities where the resting spores are illustrated). Hong Kong (Lauder 1864), Korea (Lee et al. 2013, identified in BLAST search), the Baltic (Schütt 1895), Mannai Island, Thailand, October–December (present study), Daya Bay, China, March (present study), Denmark, August (present study, Jensen and Moestrup 1998), the North Sea (Peragallo and Peragallo 1897-1908), Gulf of Naples, Italy (Kooistra et al. 2010), Atlantic coast of USA, Narragansett Bay, Chesapeake Bay (Evensen and Hasle 1975, Hargraves 1979), Sea of Azov more or less the year-round (Proschkina-Lavrenko 1963), Black Sea (Proschkina-Lavrenko 1953), Buenos Aires coastal waters (Sunesen et al. 2008).

3. Clone CCMP 172 (EF423466) from the Strait of Georgia, Washington State, USA Figure 5; Table 1

Curved chains of cells, forming more or less spherical colonies. Cells are quadrangular in girdle view (Fig. 5, a and b). Solitary cells also observed (Fig. 5c). Windows usually hexagonal, sometimes narrow, often with small central invagination (Fig. 5b), sometimes flat (Fig. 5a). Setae delicate, circular in transverse section, mostly with long basal parts extending from within the corners of the valve (Fig. 5, g–i). Three shorter setae with spiral rows of poroids and strong spines, with large solitary pores in the proximal part of each seta (Fig. 5, e and f). Long setae smooth proximally with large solitary pores, more distally with spiral rows of small poroids and tiny spines (Fig. 5, d and e). Setae extend from the cell in Brunel II and VI conformation. Valve face is elliptical to circular, with dichotomously branching costae extending from a central annulus (Fig. 5j). Terminal valves with distinct central tube-like rimoportula (Fig. 5i). Numerous girdle bands with transverse costae (Fig. 5i). Apical cell axis is 3.8–8.8 μm long (Fig. S3), pervalvar axis 7.7–14.2 μm long, per-

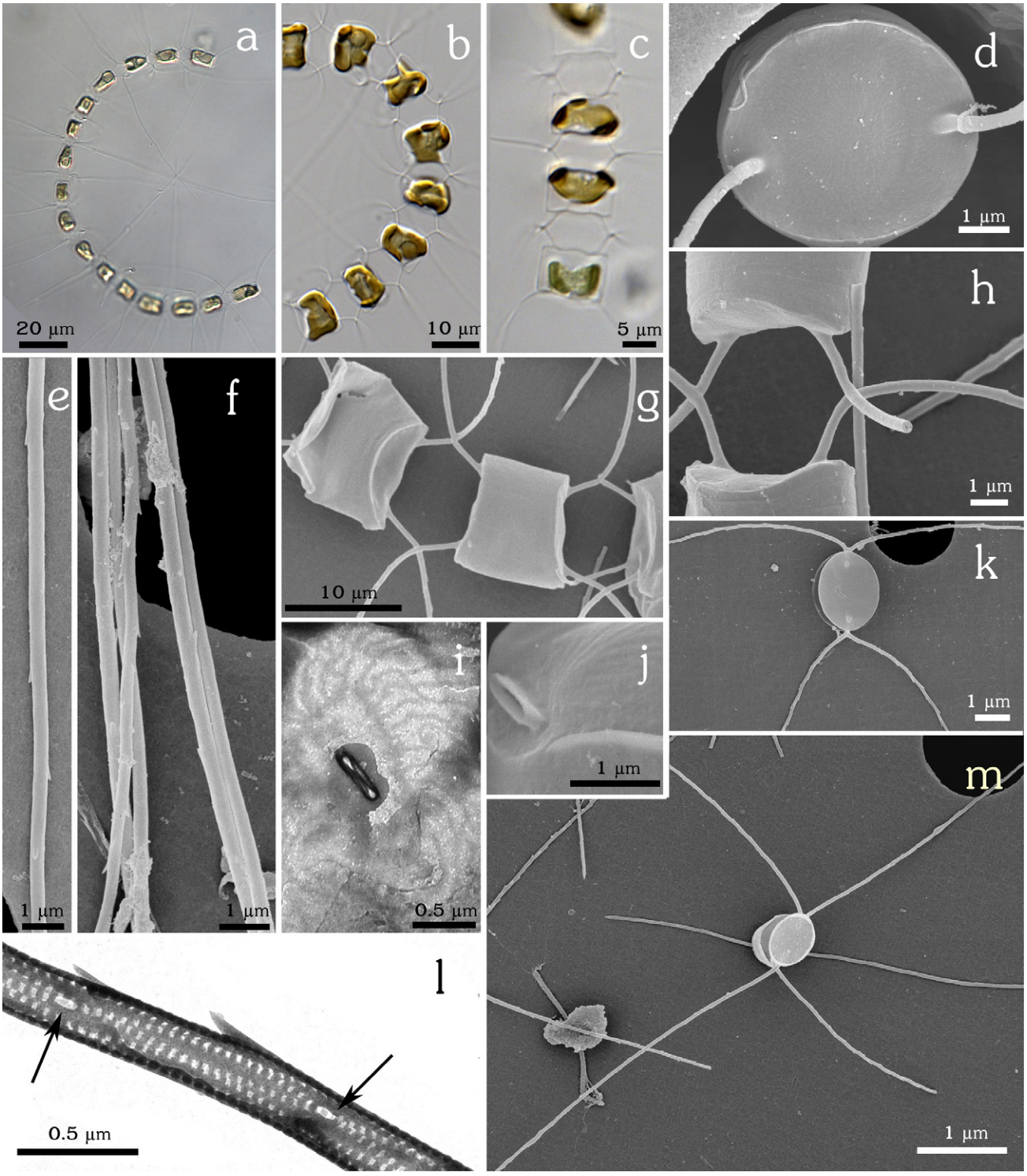

FIG. 3. *Chaetoceros socialis*. Cultures from Thailand (a, c–m) and China (b). Cultures: YL1 (a, b), MR27 (c, l), No.1 (d–k, m). (a–c) Nomarski interference LM; (d–h, j, k, and m) SEM; (i, l) TEM. (a) Part of whole colony, showing the long setae fusing centrally in the colony. (b, c) The large, hexagonal apertures at higher magnification. (d) Valve face showing ovoid shape of the valve and the emergence of the setae well within the valve margin. Costae are barely visible. (e). Long seta with minute spines. (f). Long setae with minute spines on the distal part of the seta where it joins up with long setae from other cells. (g, h) Cells showing large hexagonal apertures and setae arising from the valve face at some distance from the valve rim. (i) Rimoportula slit. (j) Rimoportula seen from the outside of the cell showing short tube-like structure. (k) Two sibling valves with four short setae. (l) Spines, large elongate pores (arrows), and the system of poroids all visible. (m) Two sibling valves from colony, the long seta is on the left, below (Group II).

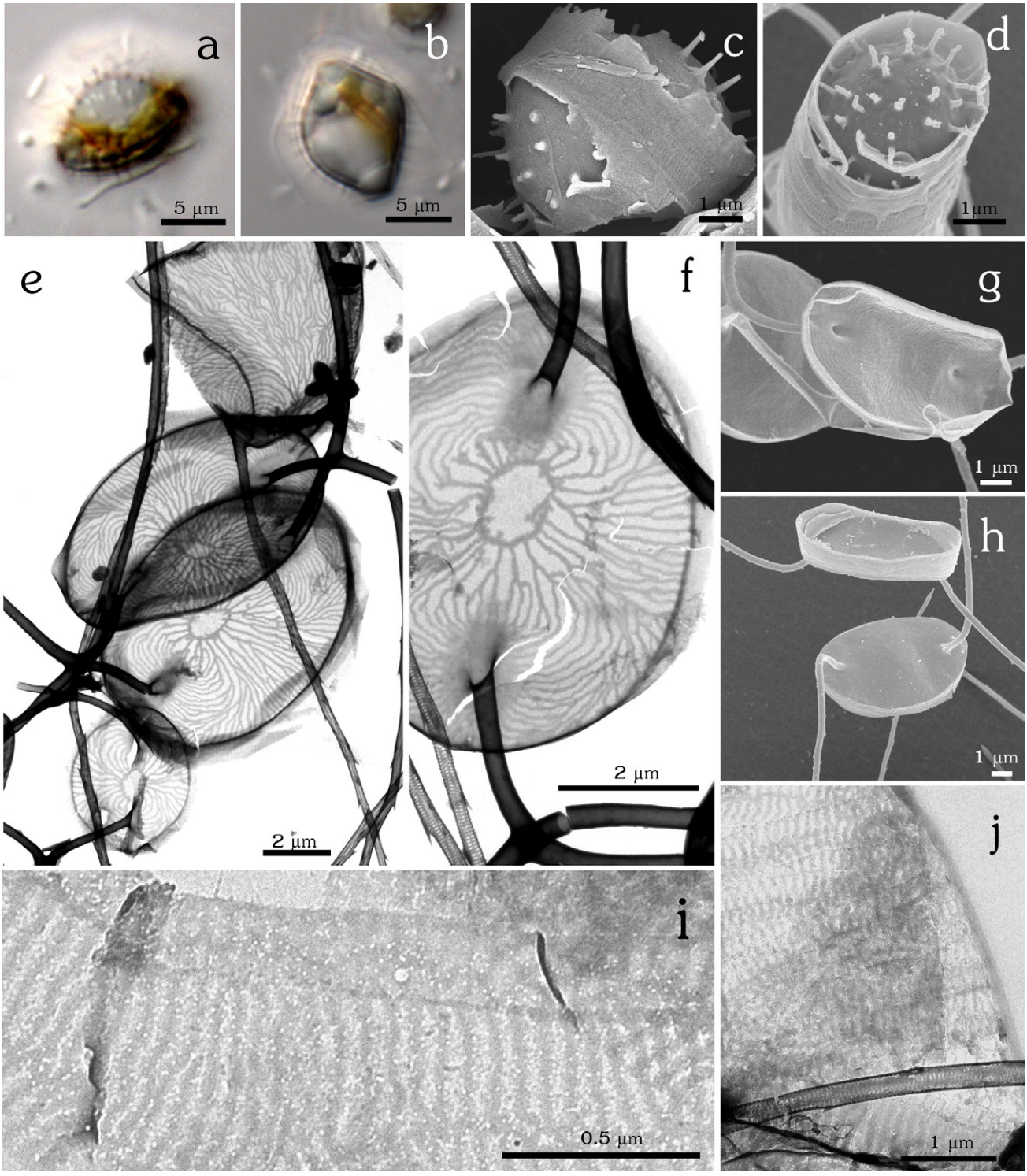

FIG. 4. *Chaetoceros socialis*. (a, b) cultures from China; (c–j) cultures from Thailand. Cultures: YL1 (a, b), No. 1 (c, d, g–j), MR7 (e, f). (a, b) Nomarski interference LM; (c, d, g, and h) SEM; (e, f, i, and j) TEM (a, b) Resting spores of slightly different shape, with short spines on both valve faces. (c, d) Resting spores seen in SEM. (e, f) The valve face with radial costae extending from the annulus and from the insertion point of the two setae some distance from the edge of the valve. (g) Emergence of the setae as seen from within the cell; (h) setae seen from the outside of the cell. (i, j) Girdle bands with costae and numerous tiny perforations between costae.

valvar axis including the basal part of the setae 10.0–15.6 µm long, and the length of the window in the pervalvar axis is 1.4–3.3 µm ($n = 15$). One chloroplast per cell. The marker sequenced was D1–D3 of LSU rDNA, GenBank accession number EF423466.

Resting spores not observed.

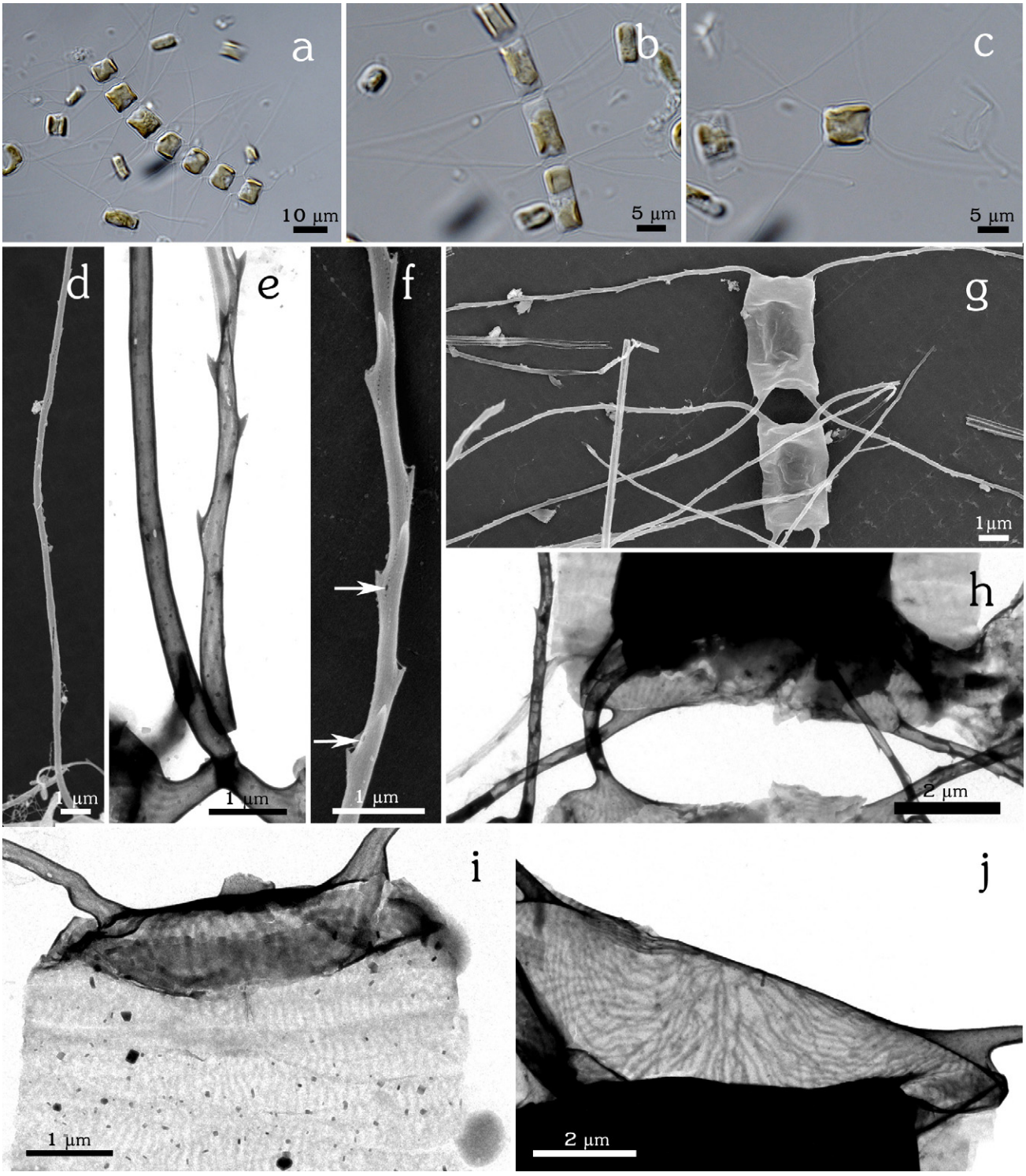

FIG. 5. Strain CCMP 172 from Strait of Georgia. (a–c) Nomarski interference LM; (d, f, and g) SEM; (e, h–j) TEM. (a) Single filament in girdle view. (b) Cells are longer than wide. (c) Single cell. (d) The long seta is smooth proximally. (e) Proximal parts of the two types of setae. (f) Spines on seta. (g) Orientation of setae as in Brunel Group II. (h) Attachment of setae to valves. (i) Terminal valve showing rimoportula in girdle view. (j) Detail of terminal valve showing radiating costae.

Geographic distribution. Strait of Georgia, Washington State, USA.

Phylogenetic analyses. All phylogenetic analyses gave a similar tree topology (Fig. 6). The cold-water strains from Greenland, Norway, Barents Sea, and the cold seasons in Denmark (December and April) clustered in one highly supported clade. Another highly supported clade comprised the strains from

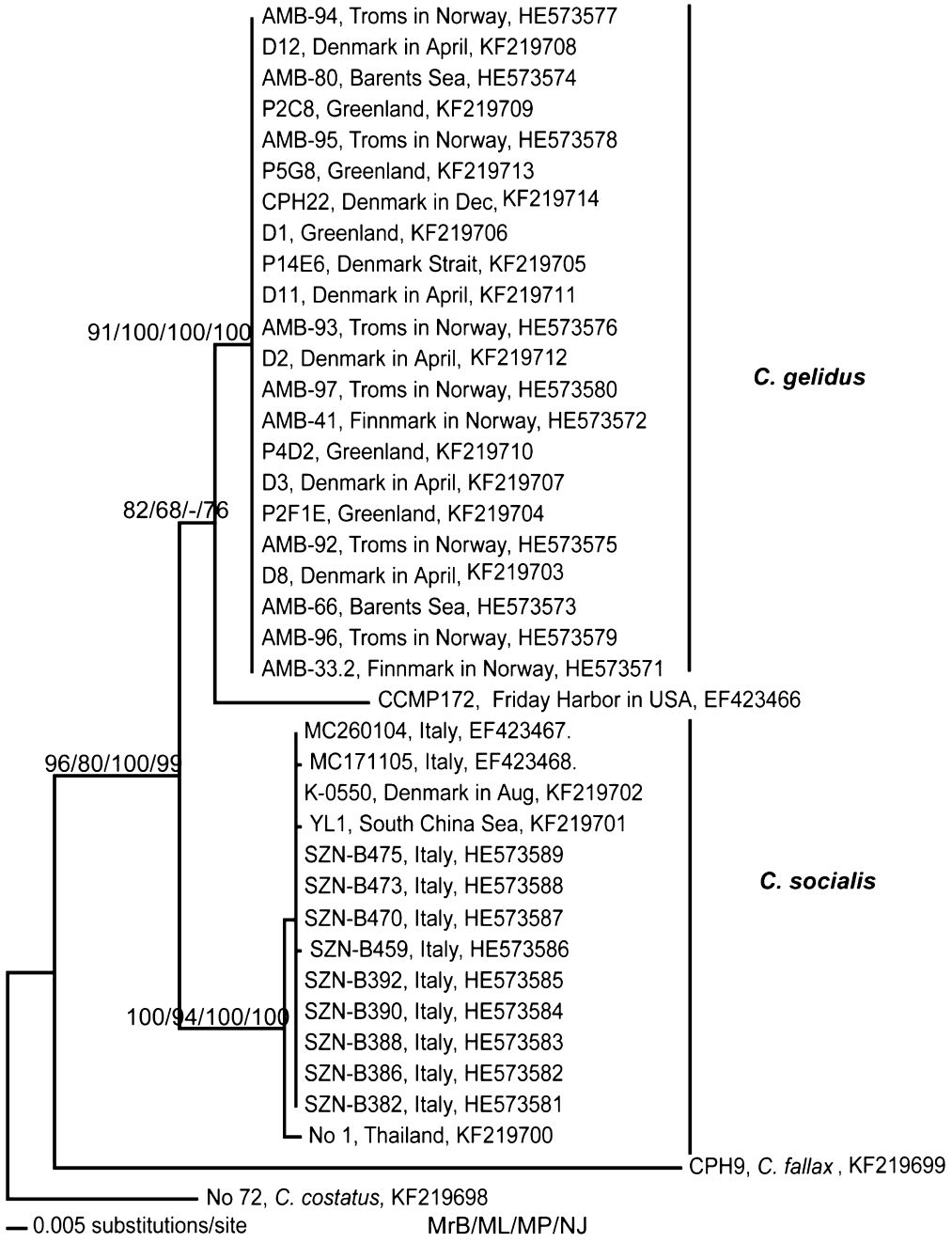

FIG. 6. Phylogenetic tree from maximum likelihood analysis. Posterior probabilities (as percentages) and bootstrap support on the branches show the support from Bayesian/maximum likelihood/maximum parsimony/neighbor-joining analyses.

warmer waters in Italy, Thailand, China, and the warm season (August) in Denmark. A single strain (CCMP 172) from Friday Harbor clustered between the two major clades.

We found the two species to differ in 22 base pair positions. Two of these included deletion/insertion events, 17 were transitions (four of A-G, 13 of C-T) and three were transversions (two of T-G, one of T-A). In strain CCMP 172 (EF423466), 15 of these positions were similar only to *C. gelidus* and six positions similar only to *C. socialis*. This strain had 22 unique base pair positions, i.e., positions that differed from both *C. socialis* and *C. gelidus*. All sequences of *C. gelidus* were completely identical, except for a deletion in one position for a single strain. For *C. socialis,* the sequences were also identical except for eight single base pair substitutions (C↔T, G↔A, and T↔G), each occurring in one strain.

Morphological statistics. One-way ANOVA analyses of the morphological measurements showed that the characters window size ($F_{1,195} = 39.67$, $P < 0.01$) and

pervalvar axis with basal parts ($F_{1,195} = 543.11$, $P < 0.01$) (see Table S2; Fig. S3) were significantly larger in *C. socialis* than in *C. gelidus*, whereas the size of the apical and the pervalvar axis was not significantly different at the 0.01 level. Because the pervalvar axis is not different in the two species, the basal parts must differ in size. This is expected, as they make up part of the windows which also differ in size.

DISCUSSION

Taxonomic history. *C. socialis* is one of the best-known diatoms of the marine plankton. It was first described from warm waters in Hong Kong by Lauder (1864), whose figure we reproduce as Figure S4a in the Supporting Information. Lauder's description is brief: "Filaments slender, aggregated, embedded in gelatine, with wavy, spirally dotted awns [now known as setae], some of which are more elongated, and converge to a common center. Hong Kong" (Lauder 1864, p. 77). Lauder subsequently mentions that the frustules are quadrate, with an awn from a little within each angle, one of them more elongated. Many frustules were found, however, in which the awns were not thus connected. In side view the cells were oval. Lauder did not provide any information about cell dimensions, nor did he give any ecological information.

Thirty-one years later, Schütt (1895) described *C. radians* sp. nov. from the Baltic (reproduced in this study as Fig. S4b) but, somewhat surprisingly, did not compare it to *C. socialis*, in spite of its close similarity to Lauder's species. This is the more surprising because Schütt mentions Lauder's article when discussing other species of *Chaetoceros*. Schütt described the setae to arise from within each corner ("in einiger Entfernung vom Schalenrande entspringend"; Schütt 1895, p. 41). The windows were high, "geigenförmig" (i.e., violin shaped), with shallow central inflations. Cells were slightly wider than long in girdle view (width 10 μm, width:length ratio 1:0.8), with right-angled corners in side view. Bases of setae were 3–4 μm long, curved, directed obliquely outwards, the distal part (i.e., after cross-over) filamentous, straight or slightly curved, transversely opposite. Cells contained a single large, plate-like chloroplast, located along the girdle. Resting spores were short, nearly spherical, one valve more curved than the other and both valves covered with short spines. Ecological data were not provided, and the time of the year the material was collected was not mentioned.

For many years the two species were considered separate taxa, based on the presence of spines on resting spores of *C. radians* and their absence in *C. socialis* spores, a difference first mentioned and illustrated by Cleve the year following the description of *C. radians* (Cleve 1896) and subsequently accepted by many researchers (e.g., Peragallo and Peragallo 1897-1908, Okamura 1907, Ostenfeld 1913, Busch 1916, Lebour 1930, Cupp and Allen 1938, Cupp 1943, Cleve-Euler 1951, Sundström 1973, Rines and Hargraves 1988, Fukuyo et al. 1990, Jensen and Moestrup 1998). However, such a difference is not mentioned in the original descriptions of the two species, Lauder does not mention the existence of resting spores in *C. socialis*. Cleve was uncertain about the identity of his material from Baffin Bay and Davis Strait in the Arctic. He found no spines on the resting spores, and this probably induced him to conclude that his material was different from Schütt's *C. radians*; thus, he concluded that it was identical to Lauder's *C. socialis*. Shortly afterward, Ostenfeld (1913) described *C. socialis* as an arctic species which in temperate areas occurs in spring. Ostenfeld also commented on the unusual observation of a species described from the warm water of Hong Kong occurring in arctic waters. Following Cleve's identification, Gran (1897) reported *C. socialis* from the North Atlantic and, based on the apparent wide geographic distribution, concluded that the species was "probably cosmopolitan" (Gran 1897, p. 26). On the other hand, he found *C. radians* (from the Baltic and Oslo Fiord), to differ by the presence of spines on the primary valve (!) of the resting spore. Half a century later, Proschkina-Lavrenko (1953, 1963) merged the two species and described and illustrated spines on the resting spores of *both* taxa, which in 1963 she referred to as *C. socialis* var. *socialis* 1963 (initially as *C. socialis* f. *autumnalis* Proschkina-Lavrenko 1953) and *C. socialis* f. *radians* Proschkina-Lavrenko 1963 (initially as *C. socialis* f. *vernalis* Proschkina-Lavrenko 1953). How Proschkina-Lavrenko distinguished between the two taxa is not clear. As the resting spores of both varieties were drawn with identical spines on both valves (reproduced as Fig. S4c), we consider them to belong to the same species (*C. socialis*). Ostenfeld (1913) found smooth resting spores in spring in temperate areas (i.e., *C. gelidus*) and spiny resting spores in autumn (i.e., *C. socialis*). We also found *C. gelidus* in Danish waters in winter, and *C. socialis* in summer.

Observations and conclusions based on new material. When this study was begun, we expected to confirm the present use of the names *C. socialis* and *C. radians*. However, it soon became clear from the phylogenetic studies that present usage could not be sustained. In the phylogenetic trees, two distinct entities appeared, one from cold water in Denmark (April, December), Greenland, the North-East Atlantic and Norway (April), Arctic Sea and Beaufort Sea and the other from warm water in Denmark (August), Thailand, Korea, Italy, and China. The material from warm water agreed well with *C. radians* as described by Schütt (1895) from the Baltic, whereas the cold-water material was different. However, the original description of *C. socialis* being from warm water led us to search for information on the seawater temperature in Hong Kong, which has been

reported to be no lower than 15°C–17°C in winter (Yin 2002, Xu et al. 2010). Considering that the material separated into two phylogenetic clades which correlated with different temperatures, it became likely that the cold-water material was different from *C. socialis*. Lauder's description may be interpreted to indicate that his material was either a separate, so far undescribed species, or that it is identical to *C. radians*. Considering also that we found only one species in our material from China and Thailand, this most likely represents Lauder's *C. socialis*. However, it had spines on the resting cysts as described by Schütt in *C. radians*. We therefore conclude that the two taxa are conspecific and should be given the oldest name, *C. socialis*. In consequence, the cold-water taxon is described here as a new species, *C. gelidus* sp. nov.

As mentioned above, *C. gelidus* differs from *C. socialis* not only in the structure of the resting spore. *C. gelidus* differs in the long setae being smooth except where they join the long setae from other cells, in the statistically significant more narrow windows between the cells (Fig. S3), and in the emergence of the setae near the margin of the cells. In *C. socialis*, the long setae are covered with spines throughout, the windows are very large, and the setae arise well within the valve margin. Regarding resting spores, both valves of *C. socialis* are covered with a small number of short spines. In *C. gelidus* the valve faces of the resting spores are smooth, but the valve mantles have a distinct crest (sensu Ishii et al. 2011) which may sometimes break up into spine-like structures, thus superficially resembling the resting spores of *C. socialis*. The crest was illustrated first by Evensen and Hasle (1975) (fig. 36, as *C. socialis*). The two taxa are well separated in the phylogenetic trees. Based on the available morphological and molecular evidence, we conclude that they represent separate species.

More species in the socialis *group* The two species have undoubtedly been mixed up in the literature As mentioned above, all cells illustrated by Proschkina-Lavrenko from the Sea of Azov belong to *C. socialis* because of the spines on the resting spores.

The cells described and illustrated by Sunesen et al. (2008) from autumn waters in coastal areas near Buenos Aires agree with *C. socialis* in having large hexagonal windows between the cells and in the setae originating well within the rim of the colony. However, resting spores from Argentina occurred in pairs joined by setae, and the primary valve was observed with centrally located processes, whereas the secondary valve was smooth. The authors found considerable variation in resting spore morphology, with "convex valves or with one valve convex and the other more flattened, both smooth or ornamented with spines" (Sunesen et al. 2008, p, 320). This raises some doubt about its identity, and it would be interesting to examine material from Buenos Aires and from the Argentinian Sea with molecular methods.

That more species may be found in this group of *Chaetoceros* was indicated by Hargraves (1979) who found different types of spores from both Narragansett Bay and the coast of Peru. In those from Narragansett Bay both valves were ornamented with circular or ovoid pores with a thickened rim. In the Peruvian material, the convex primary valve showed a system of radiating raised slots whereas the secondary valve was concave with a raised central portion.

The findings of *C. socialis* reported in the literature often give somewhat contradictory information. Thus, Takano (in Fukuyo et al. 1990) illustrated material identified as *C. socialis* from Japan and the illustrations agree well with this species. However, Takano mentions that resting spores had smooth walls as in *C. gelidus*. The same problem applies to Cupp (1943) whose drawings show typical *C. socialis* but who reported that resting spores from southern California were smooth. Cupp found *C. socialis* abundantly in March–April and again in late June–August, perhaps corresponding to both *C. socialis* and *C. gelidus* being present. In the distribution records above, we have interpreted reports of *C. socialis* with smooth resting spores as representing *C. gelidus*. rather than *C. socialis*.

Strain CCMP 172 from the west coast of North America is presently a taxonomic problem. In morphological characteristics such as the place of insertion of the setae, size of windows (Fig. S3), as well as position in the phylogenetic trees it occupies an intermediate position between *C. gelidus* and *C. socialis*. It may represent a third species of the *socialis* group. However, our culture did not produce any resting spores nor have such spores been observed by other researchers. We therefore consider it premature to describe it as a separate species.

Shevchenko et al. (2006) (fig. 127) illustrated a resting spore in mixed material from Peter the Great Bay, Sea of Japan, whose valves were covered with numerous spines. Its identity is not clear and it should be brought into culture and examined further. It may represent yet another species.

Varieties of C. socialis. Three varieties of *C. socialis* were described by Meunier (1910, 1913). They were described with smooth resting cysts and therefore belong to *C. gelidus*:

1 var. *congesta* Meunier 1913, p. 46, which according to Meunier is 'la forme vegetative par excellence de l'espèce' (Meunier 1913, p. 46), in other words the nominal variety of *C. socialis* (now *C. gelidus*).
2 var. *solitaria* Meunier 1910, p. 250, which is characterized by having solitary cells with four almost equal setae; corresponding to single cells of *C. gelidus*.
3 var. *flabelliformis* Meunier 1913, p. 46, which differs in having solitary rather than chain-forming cells, in which one seta is longer than the others and in which the long setae join in a common point. We have no new information on this variety.

Mangin (1913, p. 201), when describing *C. socialis* from the English Channel (with smooth spores; i.e.,

C. gelidus), mentions that among the numerous colonies examined by him, two size classes occurred, some colonies having cells that were twice the size of those in other colonies. Mangin found no colonies with intermediate-sized cells and thus named the two types, f. *major* and f. *minor*. He did not provide any cell size measurements and his observations therefore have to be confirmed. The cells may simply represent different populations of the same species or different cohorts of the same population.

Observations and conclusions based on new material. When this study was being written up for publication, two articles on functional diversity in cryptic species of *C. socialis* appeared (Degerlund et al. 2012, Huseby et al. 2012). The authors cultivated monoclonal strains from the North Atlantic/Arctic and the Mediterranean (Tyrrhenian) Sea at temperatures between 2.5°C and 13°C and examined morphological, phylogenetic, and physiological traits. The authors found unequivocal phylogenetic divergence between northern and southern strains, differences in spore morphology "despite the morphological similarity in vegetative form," and a functional partition between the northern and southern strains. The northern strains grew well at 2.5°C and 8°C ($\mu = 0.38$ and 0.42), more slowly at 13°C ($\mu = 0.31$), whereas the southern strains grew slowly at 2.5°C and 8°C ($\mu = 0.22$ and 0.25), and twice as fast as the latter at 13°C ($\mu = 0.51$). Our studies are in agreement with these very detailed and careful studies, which extend the studies reported in this study, notably by including physiological experiments. The authors conclude that *C. radians* should probably be reinstated as a name for the southern strains but refrained from doing this due to lack of a thorough phylogeographical investigation. Considering that material from a much wider geographic area has now been included, the conclusions of Degerlund et al. (2012) have been strengthened further and the new studies allow us to describe the northern and southern material as separate species. However, the name *C. radians* should not be reinstated but considered a synonym of *C. socialis*, for reasons discussed above. Also, in the tree of Degerlund et al. (2012) the strain CCMP172 forms a group of its own, occupying a sister group relation to the northern species, thus indicating it to be a cryptic or, more likely, a separate species. Considering, e.g., the morphological diversity of the resting spores of *C. socialis* described by Hargraves (1979) from Narragansett Bay and the coast of Peru, it appears likely that the species diversity within this very distinct group of *Chaetoceros* is somewhat higher than presently thought.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Figure S1. Shape of annulus in *Chaetoceros socialis* (a–e) and *C. gelidus* sp. nov. (f–h). All scale bars indicate 1 μm.

Figure S2. *Chaetoceros socialis*, mixed sample from Thailand, showing two types of setae in the same chain of cells: (a) sibling cells with four short setae, (b) sibling cells with three short and one long seta. SEM.

Figure S3. Graph showing length of apical axis, pervalvar axis, pervalvar axis with basal parts, and window size in CCMP 172, *Chaetoceros socialis* and *C. gelidus* sp. nov. Each box plot shows the median, the 25 and 75 percentiles (box) and the 10 and 90 percentiles (error bar). Please note that for window size, the *y*-axis is shown to the right. For the other characters, the *y*-axis is shown to the left.

Figure S4. (a) The original drawing of *Chaetoceros socialis* by Lauder (1864). (b) The original drawing of *C. radians* by Schütt (1895). (c) Drawings of resting spores of *C. socialis* var. *socialis* (4-7) and *C. socialis* f. *radians* (12-15) by Proschkina-Lavrenko (1963).

Table S1. List of cultures of *Chaetoceros socialis, C gelidus* sp. nov. and outgroup taxa established in this study, with strain designation, location, and date of sampling.

Table S2. Morphometric data of *Chaetoceros gelidus* sp. nov. and *C. socialis*. All measurements were based on 20 cells. The values indicate mean ± SD, with minimum and maximum values in brackets.