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Gonium sociale (Volvocales, Chlorophyta) from Antarctica

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Detailed accounts of *Gonium sociale* (Dujardin) Warming originating from Antarctica were obtained, based on cultured materials isolated from a meltwater pool near Great Wall Station on King George Island. The alga exhibited vegetative colonies, which were essentially the same as those of *G. sociale* previously reported from non-antarctic regions, except for its somewhat larger size. In addition, the effects of temperature on the growth of the antarctic plant were studied at 5–25°C, in comparison with those of a Japanese strain of *G. sociale*. The antarctic strain was able to grow normally at 5, 10 and 15°C, but showed abnormal colonies at 20°C and did not grow at 25°C. In contrast, the Japanese strain produced normal vegetative colonies at 5–25°C. This is the first report on identification of antarctic colonial Volvocales at the species level.

Key Index Words: *Antarctica—Chlorophyta—culture*—Gonium sociale—*morphology—Volvocales.*

The occurrence of the colonial Volvocales in Antarctica has been reported by Thomas (1965) for *Pandorina* sp. and by Parker *et al.* (1972) for *Gonium* sp. However, their studies were not based on cultured materials, and detailed accounts and identification at the species level are lacking for these algae.

During the "Japanese-Chinese co-operative study on terrestrial biology in King George Island" (Ohtani and Nakatsubo 1992), one of the authors (S.O.) found a colonial green flagellate growing in a meltwater pool near Great Wall Station. Unialgal cultures of this alga were established from the water sample and detailed accounts were obtained. Vegetative morphology observed by light microscopy clearly indicated that the organism is referable to *Gonium sociale* (Dujardin) Warming. In addition, the effects of temperature on growth of this Antarctic alga were studied, in comparison with those of a Japanese *G. sociale* strain. Morphological details and the effects of temperature on growth of *G. sociale* originating from Antarctica are described in this report.

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Materials and Methods

Water samples were collected in a meltwater pool near Great Wall Station on King George Island in December 1990. The pool was about 1 m in diameter and 20–30 cm in depth. The water was at 5.5°C and pH 10.6, and its conductivity was 845 μS/cm. During November to December of 1990, all the water in the pool often became frozen. Unialgal cultures were established by streaking the diluted sample on a Bold Basal Medium (BBM) (Nichols 1973) agar (1.5%) plate. For observation, the cultures were grown in screw-cap tubes containing 12 ml of AF-6 medium (Kato 1982), with 40 ml/l of distilled water substituted for soil-water medium (Starr and Zeikus 1987). The cultures were maintained at 15°C under an irradiance of 5000 lux, with a 14-h daylength provided by cool-white fluorescent lamps. For growth experiments, 1 ml of an actively growing culture (*c.* 1×10^4 colonies/ml) at

15°C was inoculated into 12 ml of the new growth medium. The inocula were then placed at 5, 10, 15, 20 or 25°C, under the same illumination as described above. After seven days, growth of the colonies was detected with a stereomicroscope. A strain of *G. sociale* var. *sociale* originating from Japan (Nozaki 1986a, b) was also used and treated as described above. Light microscopy was carried out using a Nikon LUR-Ke microscope equipped with phase optics or a Leitz Orthoplan Microscope with Nomarski interference optics.

Results and Discussion

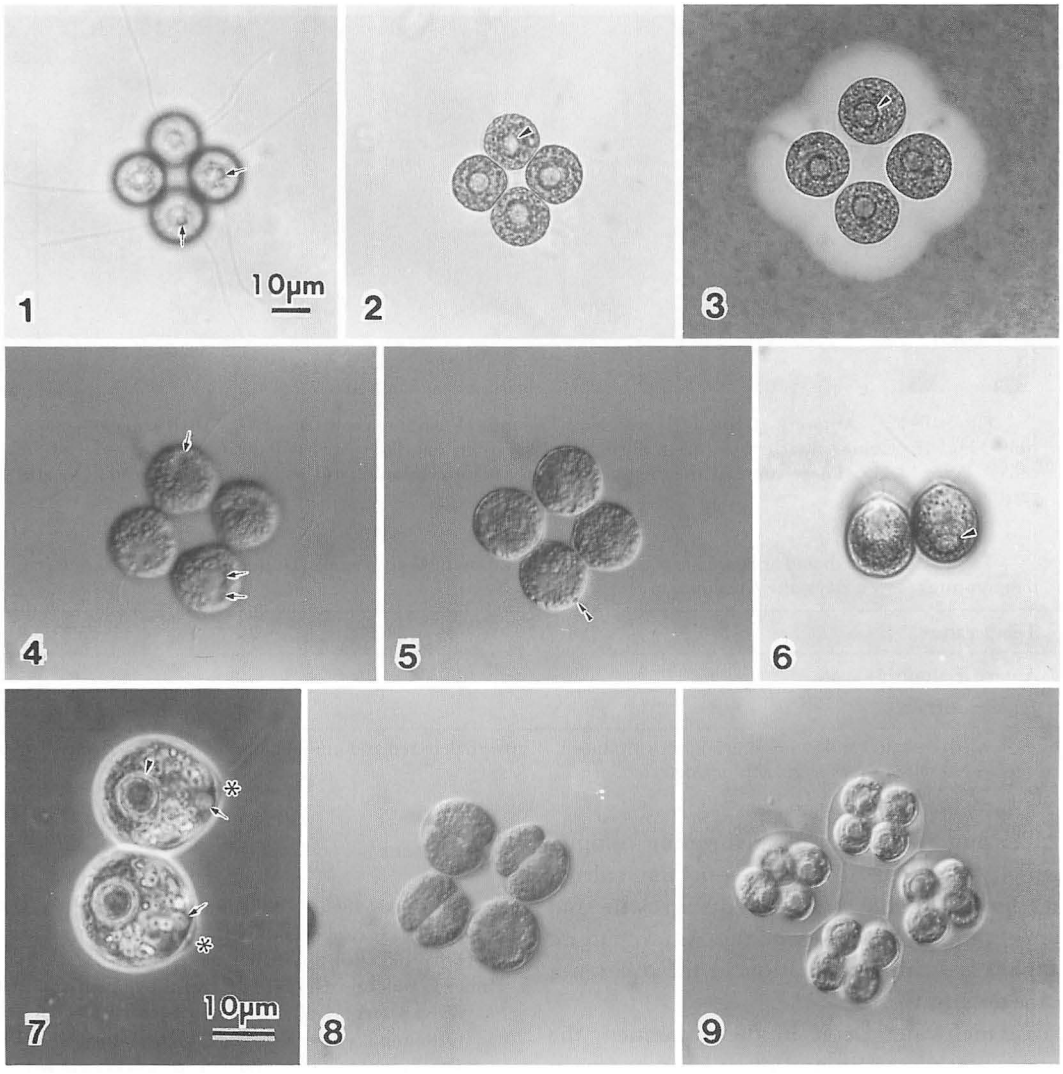
Vegetative colonies of the antarctic plant were square in shape (Figs. 1-5), measuring up to 50 μm in diameter, and each generally contained four cells, which were placed in the four corners of the square and oriented their anterior-posterior axes toward nearly the same direction (Fig. 6). The whole colony was embedded in a watery gelatinous matrix, which could be recognized clearly in an ink preparation (Fig. 3).

The cells were ovoid or nearly spherical in shape, up to 20 μm wide, and each had two equal flagella, a stigma (Fig. 5), two contractile vacuoles at the base of the filagella (Figs. 1, 4, 7) and a massive cup-shaped chloroplast. The chloroplast usually had a single large pyrenoid in the bottom (Figs. 2, 3, 6, 7). Each protoplast was enclosed by a gelatinous sheath, which exhibited a broad papilla at the base of the flagella (Fig. 7). The constitutive cells were connected by the two protuberances of each gelatinous sheath, forming a square fenestration in the center of the colony (Fig. 5).

In asexual reproduction, each protoplast within the gelatinous sheath conducted two longitudinal divisions (Figs. 8, 9). After the divisions, each daughter protoplast produced two equal flagella and developed a stigma and a single basal pyrenoid in the chloroplast. The newly formed daughter colonies measured 22-25 μm in diameter. During the daughter colony formation, the parental gelatinous sheath became expanded and the daguther colony remained for some time within the expanded sheaths (Fig. 9). The daughter colonies were then gradually liberated from their parental sheaths.

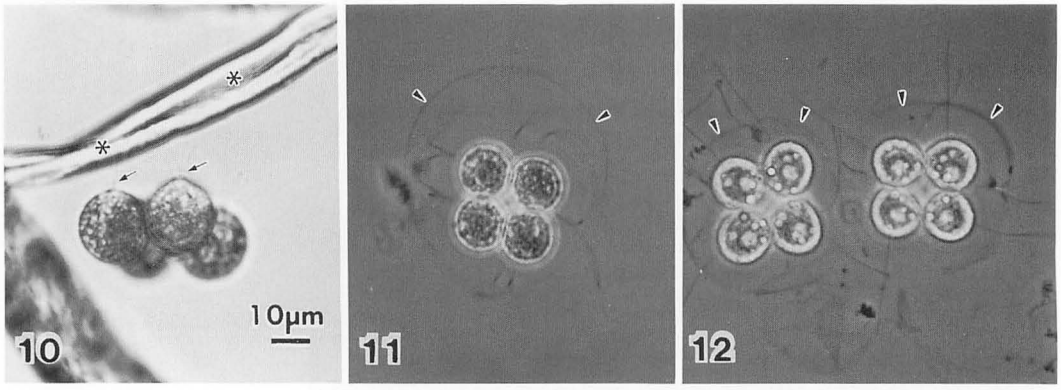
The vegetative morphology of the present organism agreed well with that of *G. sociale* collected in non-antarctic regions (Stein 1959, Huber-Pestalozzi 1961, Nozaki 1986b). Stein (1959) observed two varieties of this species, var. *sociale* and var. sacculum Stein, on the basis of her cultured materials. In lacking a "sac" (mother cellular sheath) in vegetative colonies (Fig. 6), the present antarctic alga could be assigned to *G. sociale* var. *sociale*. However, the antarctic alga produced vegetative colonies of somewhat larger size. According to Stein (1959) and Huber-Pestalozzi (1961), colonies and cells of *G. sociale* var. *sociale* measure 20-48 μm in diameter and 6-16 μm in width, respectively. Nozaki (1986b) reported the maximum diameter of the colonies of *G. sociale* var. *sociale* originating from Japan to be 32 μm. However, Hansgirg (1888) reported a larger form of *G. sociale* as *G. sociale* var. *majus* Hansgirg, which was collected in Czechoslovakia in November. The cells of this variety were 15-18 μm wide, but rarely up to 21 μm. Therefore, the present antarctic alga may be referable to this variety, which has been previously collected only once in winter (Stein 1959).

When the cultures were grown at 5, 10 or 15°C, they produced vegetative colonies which were always swimming actively. They were spread throughout the culture medium, except for some colonies gathering near the surface of the liquid by phototaxis. However, the colonies gathered and attached to the inner surface of the glass of the culture tube when they were grown at 20°C. When such colonies were observed after shaking the culture tube by hand for preparation, two types of colonies were recognized. The morphology of the first type was essentially the same as that of the normal motile colonies at low temperature. However, the motility of such colonies was very low and the colonies



Figs. 1–9. Antarctic strain of *Gonium sociale* (Dujardin) Warming. Arrow head indicates pyrenoid. Figs. 1–3, 6. Bright field. Figs. 4–6, 8, 9. Nomarski interference contrast. Fig. 7. Phase contrast. Fig. 1. Surface view of vegetative colony grown at 5°C, showing contractile vacuoles (arrows). Fig. 2. Optical section of colony in Fig. 1. Fig. 3. Colony observed in ink preparation (10°C). Note encompassing gelatinous matrix. Fig. 4. Surface view of colony grown at 5°C, showing contractile vacuoles (arrows). Fig. 5. Optical section of colony in Fig. 4. Double arrow heads indicates stimga. Fig. 6. Lateral view of colony grown at 5°C. Fig. 7. Cells showing anterior papilla (asterisk) of gelatinous sheaths. Arrow indicates contractile vacuole. Figs. 8, 9. Asexual reproduction (10°C). Fig. 8. Two-celled stage. Fig. 9. Newly formed daguther colonies within parental colony. Scale in Fig. 1 applies to Figs. 2–6, 8, 9.

became attached to the substratum with their flagella. Such behavior was clearly observed when the materials were mounted with cotton fibrils (Fig. 10). The second type includes fairly mature colonies, which measured up to 35 μm in diameter and remained within their expanded parental gelatinous sheaths with their long flagella retained within the sheaths (Fig. 11). These flagella often projected through the parental sheaths (Fig. 12). Such colonies were also immobile. Growth was not detected in the antarctic alga at 25°C. On the other hand, the Japanese strain of *G. sociale* was able to grow at 5, 10, 15, 20 and



Figs. 10–12. Antarctic strain of *Gonium sociale* (Dujardin) Warming grown at 20°C. All at same magnification. Fig. 10. Colony attached to cotton fibril (asterisks) by its flagella. Arrows indicate flagellar bases of the cells. Figs. 11, 12. Phase-contrast micrographs of fairly mature colonies still within parental cellular sheaths (arrow-heads).

Table 1. Growth and appearance of colonies in two strains of *Gonium sociale* (Dujardin) Warming at different temperatures, seven days after inoculation.

Temperature	5°C	10°C	15°C	20°C	25°C
Antarctic strain	+	+	+	#	*
Japanese strain	+	+	+	+	+

+ growth detected and swimming colonies produced; # growth detected and immobile colonies attached to the inner surface of the glass tube; * growth not detected.

25°C and exhibited only swimming colonies which were spread throughout the culture medium. Table 1 represents growth and appearance of colonies of the Antarctic and Japanese strains in relation to the difference of temperature.

In meltwater pools in the antarctica, the water was very cold and often became frozen (see Materials and Methods). It therefore seems likely that the growth of non-motile colonies at 20°C in the antarctic strain may result from its reaction to the unusual high temperatures for it.

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野崎久義*・大谷修司**：南極産の *Gonium sociale*（緑藻・オオヒゲマワリ目）

南極産の *Gonium sociale* (Dujardin) Warming をキングジョージ島の長城基地付近の水溜りより分離・培養し，その詳細を得た。本藻の栄養群体の形態は南極以外の場所から今までに報告された *G. sociale* と基本的に一致したが，ややそのサイズが大きかった。この南極産の株の温度による増殖を調べたところ，5度から15度では正常な生育をしたが，20度に於いては異常な非遊泳の群体を作り，25度では生育を示さなかった。一方，日本産の *G. sociale* の株は，5度から25度に於いて，正常な遊泳群体を作った。本報告は南極産の群体性オオヒゲマワリ目に於ける最初の種レベルの同定である。（*305 茨城県つくば市小野川16–2　国立環境研究所生物圏環境部環境微生物研究室，**173 東京都板橋区加賀1–9–10　国立極地研究所　（現）690 島根県松江市西川津町1060　島根大学教育学部生物学研究室）