

氷雪性緑藻 *Chloromonas* 属の日本産 1 未記載種

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An undescribed species of snow-inhabiting *Chloromonas* (Volvocales, Chlorophyceae) from Japan

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The snowpacks in mountainous areas as well as the snowfields in polar regions are sometimes stained green, red or other colors during the snow-melting season. The phenomena are called “colored snow” and principally caused by blooms of cold-adapted microalgae. Within green snow, motile vegetative cells classified as the species of *Chloromonas* (Volvocales, Chlorophyceae), a unicellular green biflagellate genus, are generally dominant (Hoham 1980; Hoham & Duval 2001). On the other hand, nonmotile cells which frequently accumulate carotenoid pigments within the cell usually dominate in red snow; most of the cells are identified as the zygotes of snow-inhabiting species of *Chloromonas* according to the previous taxonomic studies on the basis of continuous observation of field-collected materials (e.g. Hoham & Mullet 1977). Recently, several snow-inhabiting species of *Chloromonas* were delineated based on comparative morphological analyses and molecular data of cultured materials of vegetative cells (Matsuzaki et al. 2014). However, the species lack the information of zygotes since induction of zygote formation is difficult in culture (Hoham et al. 2006). Besides, actual species of field-collected zygotes of snow *Chloromonas* have been almost unclear because of the difficulty of not only inducing vegetative cell production via germination but also molecular identification of the zygotes (Muramoto et al. 2008; Remias et al. 2010). Very recently, Matsuzaki et al. (2015) established the method for molecular identification of such zygotes and revealed that one Japanese lineage of spindle-shaped zygotes was conspecific with the strains of *C. miwae* originating from Japan. Therefore, further taxonomic studies are required to delineate natural species of snow-inhabiting *Chloromonas*, on the basis of the use of both cultured and field-collected materials.

Here, we reported an undescribed species of snow-inhabiting *Chloromonas* based on the culture strain and field-collected samples from Japan. The vegetative cell of the species was morphologically distinguished from those of all previously described species of snow *Chloromonas* in having an ovoid cell shape and an eyespot in the chloroplast. Our multigene phylogenetic analyses and the comparison of genetic differences of a rapidly evolving DNA region demonstrated that the spindle-shaped zygotes collected from Mt. Hakkoda, Aomori, Japan and Mt. Tateyama, Toyama, Japan were considered conspecific with the undescribed species. The present molecular phylogeny also showed that the species was sister to *C. miwae*, which produces spindle-shaped zygotes (Matsuzaki et al. 2015). Although the zygote morphologies of the sister species were seemingly similar to each other under light microscope, field-emission scanning electron microscopy (FE-SEM) exhibited that the zygotes of the undescribed species could be distinguished from those of *C. miwae* in differences of the arrangement of flanges developing on the zygote wall. The result indicates that detailed morphological characteristics of zygotes provided by FE-SEM might be useful for species identification of snow-inhabiting *Chloromonas*.

山岳地域の残雪や極域の雪原が融雪期に緑や赤などに色付く「彩雪」現象は、寒冷適応した微細藻類がブルームを形成することで主に引き起こされる。一般的に、緑色の彩雪には2本の鞭毛をもつ単細胞性緑藻クロロモナス (*Chloromonas*) の種に分類される遊泳栄養細胞が優占する (Hoham 1980; Hoham & Duval 2001)。一方、赤色の彩雪には通常、細胞内にしばしばカロテノイド色素を蓄積する不動細胞が優占する。それらの多くは野外サンプルの継続的な観察に基づく分類学的研究 (e.g. Hoham & Mullet 1977) に従い、氷雪性クロロモナスの接合子と同定されている。近年、栄養細胞の培養株を用いた比較形態観察と分子データに基づき、数種の氷雪性クロロモナスが識別された (Matsuzaki et al. 2014)。しかしながら、培養株を用いて実験的に接合子の形成を誘導することは難しく (Hoham et al. 2006)、それらの種は接合子の情報を欠く。加えて、彩雪中の接合子は発芽誘導も分子同定も

困難なため (Muramoto et al. 2008; Remias et al. 2010)、種の実体はほとんど不明なままである。Matsuzaki et al. (2015) は最近、そのような接合子の分子同定法を確立し、日本産の紡錘形の接合子の 1 系統と *C. miwae* の日本産培養株が同一種であることを明らかにした。従って、氷雪性クロロモナスの種を正確に把握するために、培養株と野外サンプルの両方を用いた更なる分類学的研究が必要である。

本発表では、日本産の培養株と野外サンプルに基づき、氷雪性クロロモナスの 1 未記載種を報告する。本種は、栄養細胞が卵形で葉緑体に眼点をもつ点で、既知の氷雪性クロロモナスと形態的に区別された。複数遺伝子分子系統、および DNA の高進化速度領域における遺伝的差異の比較から、青森県八甲田山と富山県立山から採集された紡錘形の接合子が本未記載種のもと考えられた。また、分子系統の結果、本種は紡錘形の接合子を形成する *C. miwae* (Matsuzaki et al. 2015) の姉妹系統に位置した。それら姉妹種の接合子は光学顕微鏡下では一見類似するが、電界放出型走査電子顕微鏡 (FE-SEM) 観察の結果、接合子表面にみられる翼の配向の違いで未記載種と *C. miwae* の接合子を識別できると考えられた。従って、FE-SEM 観察による接合子の詳細な形態データは、本生物群の種同定に有用であることが示唆された。

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