

The Biogeography of Green Algae Associated with Red Snow in Japan

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Red snow is a world-wide phenomenon which is the changes in the color of snow surface into red. Red snow is known to be caused by the bloom of green algae, mainly *Chlamydomonas nivalis* (Takeuchi et al., 2006). Because a snow surface with red color decreases its albedo, it implies that red snow accelerates snow melting (Thomas and Duval, 1995, Yallop et al. 2012). Increasing melting of ice should be a big problem because it would lead to the loss of habitats of polar animals and a cause a flood and water-level elevation.

Therefore, understanding the mechanism of the colonization and establishment of microbes associated with red snow is important in evaluating the risk of ice thawing. However, little is known about the mechanism of the determinants of their distribution. Two conflicting views exist in the mechanism of distribution of snow algae. First, snow algae is cosmopolitan with migrating widely through the atmosphere, which is implied by Baas-Becking hypothesis (Baas-Becking 1934). Another view is that the snow algae is endemic, which is an idea that snow algae keep resting in soil, and move up to snow surface from the soil when it is covered by snow (Hoham, 2001).

In this study, we collected red snow samples from the snowfields of Japanese mountains and conducted a phylogenetic analysis of the microbes in order to understand the biogeography of red snow algae. Red snow samples were collected from 14 Japanese mountains in June and July 2012 (Fig.1). We extracted eDNA from the samples using ISOPLANT2 (Nippongene, Japan). PCRs were performed for *rbcL* gene coding region (ca.700 ~ 800 bp). Since it was microscopically confirmed that microbes with red pigment were highly dominant in the samples (Fig.2), we assumed that direct sequence of DNA of microbes associated with red snow should be possible. However, the sample from Tateyama could not be analyzed because of its higher diversity which might cause the contamination in PCR process. PCR products were directly sequenced using the ABI3500 sequencing machine. Each sequence was BLASTed against DNA databanks to find a similar sequence. A multiple sequence alignment and a phylogenetic analysis were performed using MEGA 5 (Tamura, 2011). Phylogenetic trees were constructed by the most likelihood method using MEGA 5.

From these analyses, we obtained two main clades: Clade A and Clade B (Fig.3). Clade A consists of OTUs similar to *Chlamydomonas*, whereas Clade B consists of OTUs similar to *Chloromonas*. Clade B was exclusively observed in Tohoku area, whereas the clade A consists of samples from all over the sampling area. These results showed that red snow is caused by several species. Furthermore, the shallower branches of clade A suggest the recent divergence time of OTUs in this clade, while deeper branches in the clade B suggest the older divergence time. These findings might imply that the OTUs for the clade A have greater dispersal ability than those in the clade B. Finer genetic markers and analysis methods, such as clone library and single cell sequence, are needed for better understanding of the biogeography of the microbes.

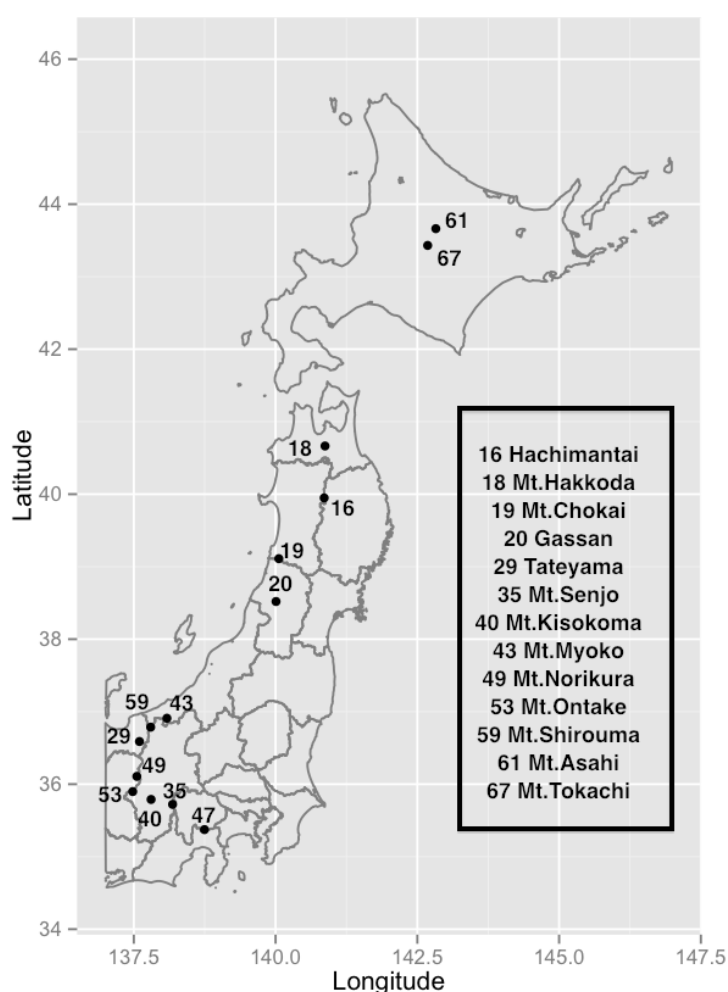


Fig.1 The map of sampling locations for red snows

References

Baas-Becking, LGM (1934) Geobiologie of Inleiding Tot de Milieukunde (Van Stockkum & Zoon, The Hague).
 Hoham RW, Duval B (2001) Microbial ecology of snow and freshwater ice with emphasis on snow algae. In: Jones HG, Pomeroy JW, Walker DA, Hoham RW (eds) Snow ecology. Cambridge University Press, New York, pp 168–228
 Takeuchi, N., Dial R, Kohshima R, Segawa T, and Uetake L (2006) Spatial distribution and abundance of red snow algae on the Harding Icefield, Alaska derived from a satellite image, *Geophys. Res. Lett.*, 33, L21502.
 Thomas WH and Duval B. (1995). Sierra Nevada, California, U.S.A., snow algae: snow albedo changes, algal-bacterial interrelationships, and ultraviolet radiation effects. *Arct Antarct Alp Res* 27: 389–399.
 Yallop ML et al. (2012). Photophysiology and albedo-changing potential of the ice algal community on the surface of the Greenland ice sheet. *The ISME Journal*. 10.1038/ismej.2012.107. 2012, 1-12. (online), <<http://dx.doi.org/10.1038/ismej.2012.107>>

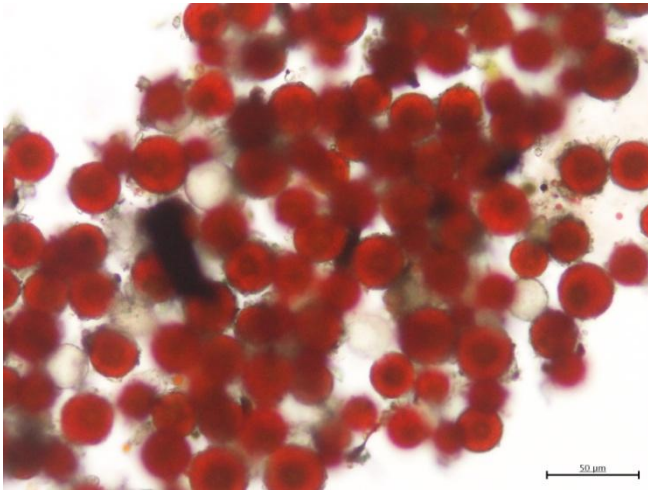


Fig.2 Microscopic view of red snow sample from Mt. Tokachi, Hokkaido, Japan. Scale bar = 50 um.

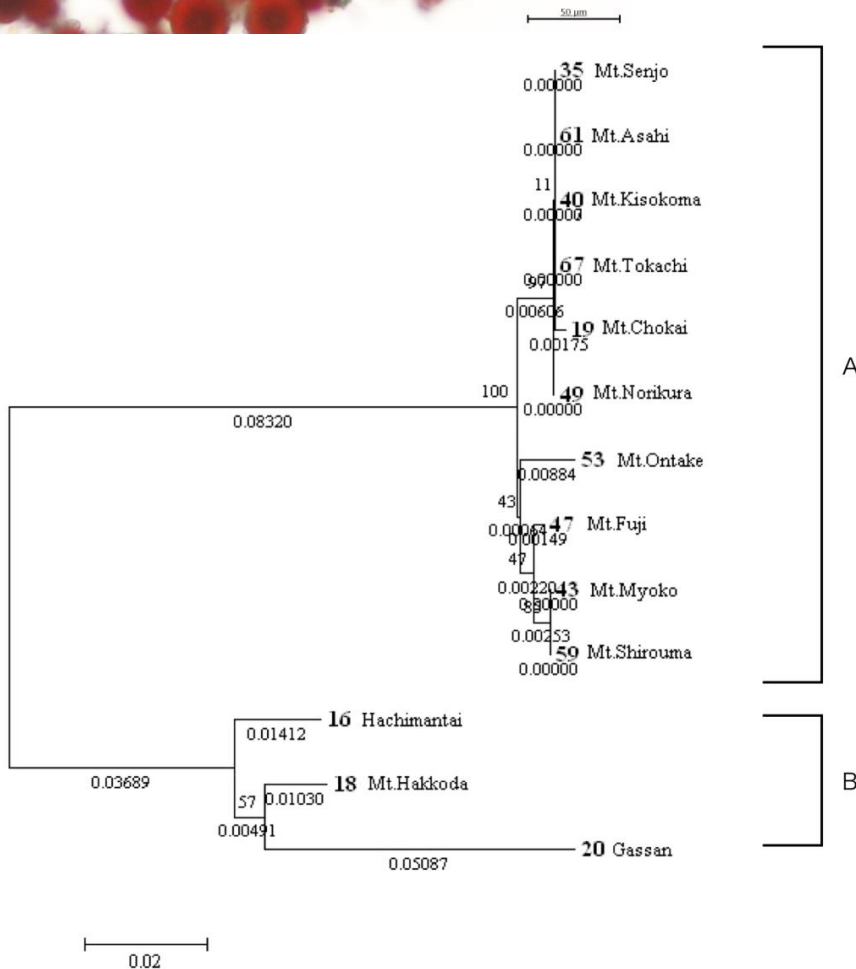


Fig.3 ML phylogram based on rbcL sequences (tentative). Results of BLAST show that OTUs in the clade A are similar to *Chlamydomonas*, and OTUs in the clade B are similar to *Chloromonas*. The clade A consists of samples from all over the sampling area, while the clade B consists of samples from the Tohoku region.