

CRYOCHYTRID: are fungi the missing links in high Arctic freshwater food webs?

Sara M. E. Rassner^{1,2}, Arwyn Edwards¹, Ottavia Cavalli¹ and Tristram D. L. Irvine-Fynn²

¹*Institute of Biological, Environmental and Rural Sciences,
Aberystwyth University, Aberystwyth, UK*

²*Department of Geography and Earth Sciences, Aberystwyth University, Aberystwyth, UK*

Glaciers provide a multitude of habitats for microbes and thriving microbial communities can be found in cryoconite holes on the ice surface, in particular. Cryoconite holes are water-filled pockets created by preferential melting of the ice around granular microbe–mineral aggregates, or cryoconite, which harbour cyanobacteria, fungi, heterotrophic bacteria, microeukaryotes and meiofauna.

Previous work has highlighted that fungi can constitute a large proportion of the heterotrophs in cryoconite and in our study (*CRYOCHYTRID*), we focus on a group of less well-known fungi that may have a key role in the cryoconite food web. The chytrids comprise basal true fungi with motile zoospores and are found in a wide range of aquatic and soil habitats. Chytrids are commonly saprotrophic or parasitic, and although both types would fulfil important functions in cryoconite food webs, the extent of either group in glacial ecosystems is largely unknown. Therefore, we aimed to characterise chytrid diversity on glaciers and evaluate their importance in cryoconite food webs.

As reported in *FBA News Autumn/Winter 2013*, we carried out three weeks of fieldwork on Svalbard in July/August 2013, where we sampled a large number of cryoconite holes on several of the glaciers around our base at NERC Research Station in Ny-Ålesund. Since our return to Aberystwyth, we have been busy processing the collected samples, as well as setting up and tending to chytrid cultures. Here, we present some of our preliminary findings.

Early last autumn, we set up cultures with the aim to isolate and identify the chytrids present in our cryoconite samples. After several months of cold-room incubation, we began to see some growth of both fungi and potential hosts (e.g. cyanobacteria and algae). The task of maintaining the, by now, vast number of cultures is being continued by 1st year PhD student Ottavia Cavalli, who will carry on the work of isolating chytrid–host pairs as part of her PhD.

The work to identify hosts and sites of chytrid infection using microscopy is still on-going, but we have been able to get an idea of the extent of fungi in cryoconite, as well as identify some of the substrates used by cryoconite chytrids. When cryoconite granules are stained with calcofluor white (a chitin and cellulose stain) and observed under UV light, the networks of fungal hyphae extending throughout the granules are evident (Fig. 1). By using baits, such as pollen grains, we have been able to draw out a subset of chytrids from cryoconite into pure cultures (Fig. 2).

In addition to the more traditional methods of culturing and microscopy, we also used high-throughput sequencing of

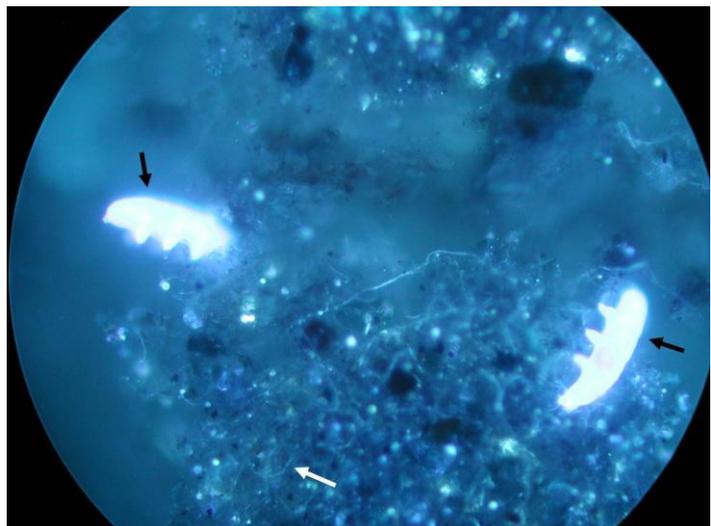


Figure 1. Fungal hyphae (white arrow) visible in cryoconite stained with calcofluor white (a chitin and cellulose stain) under UV light. Two tardigrades (black arrows) were also captured (200x magnification).

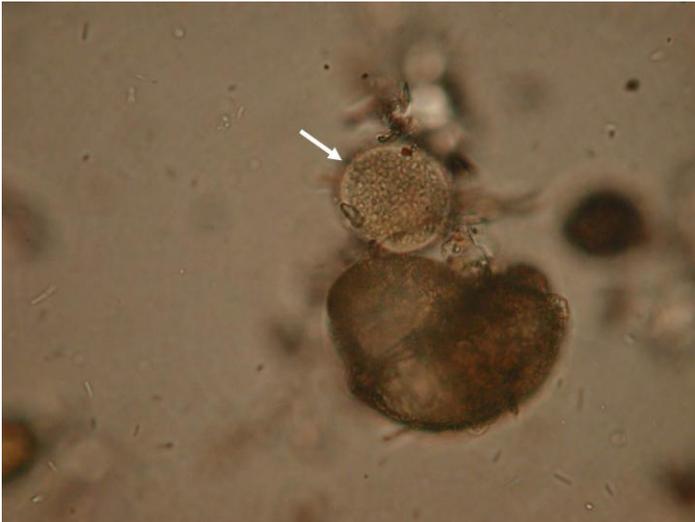


Figure 2. Chytrid (arrow) growing on pine pollen after baiting of cryoconite (400x magnification).

the large subunit ribosomal DNA to describe the fungal diversity of cryoconite. Initial data show that unlike the relative abundance of most other fungi, which remained roughly similar between different glaciers, the relative abundances of the three chytrid phyla (Chytridiomycota, Blastocladiomycota and Neocallimastigomycota) varied significantly between glaciers (PERMANOVA, pseudo-F = 4.79, $p(\text{perm})= 0.0001$). Chytridiomycota was much better represented in cryoconite than were the other two phyla and is likely influencing the fungal community composition in these habitats. The relative abundance of Chytridiomycota was higher on two of the glaciers (Austre Brøggerbreen and Midtre Lovénbreen) (Fig. 3A). We also found that Austre Brøggerbreen and Midtre Lovénbreen were more similar to each other than to other glaciers when comparing the fungal community structures of different glaciers (Fig. 3B). These results suggest that the Chytridiomycota strongly influence the fungal community structure in cryoconite.

With the results from on-going experiments, we will be able to elucidate the roles of chytrids in these glacial ecosystems.

Acknowledgements

The authors would like to thank Mr James Wake for support in the field, and Dr Andrew Detheridge and Dr Toby Wilkinson for assistance with high-throughput sequencing and bioinformatics. This work was funded by the Hugh Cary Gilson memorial award 2013 and a NERC New Investigator grant to AE (NE/K000942/1).

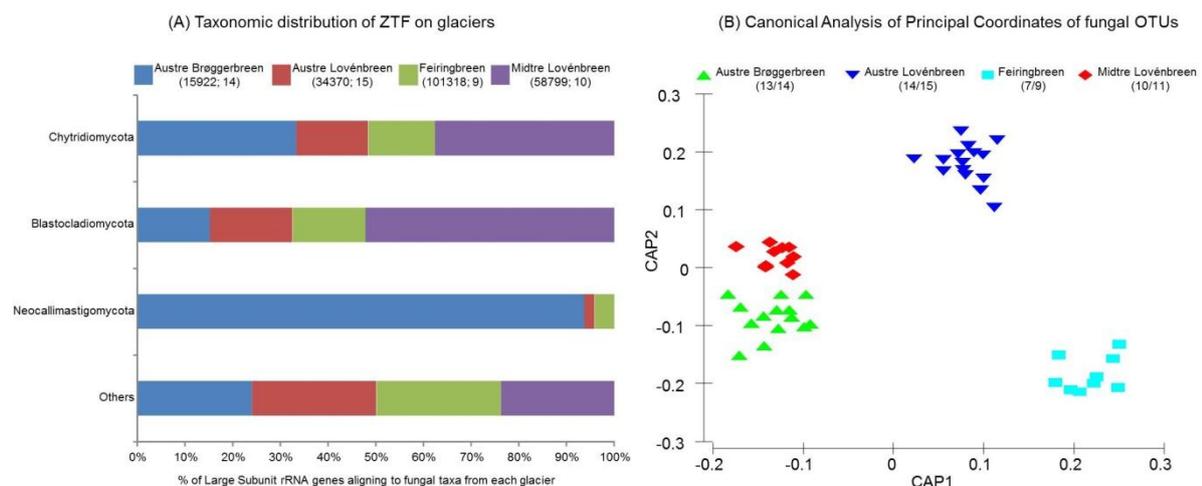


Figure 3. Fungal diversity on four Svalbard glaciers, as revealed by large subunit of ribosomal DNA amplicon sequencing. (A) Distribution of zoosporic true fungi (ZTF) and other fungi; parenthetical numbers indicate average number of sequencing reads per sample aligning to fungi and number of samples per glacier, respectively. (B) Model of fungal community structure according to glacier; parenthetical numbers indicate number of samples correctly allocated to group in cross-validation leave-one-out analysis.