

# Millennial timescale regeneration in a moss from Antarctica

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Mosses, dominant elements in the vegetation of polar and alpine regions, have well-developed stress tolerance features permitting cryptobiosis. However, direct regeneration after longer periods of cryptobiosis has been demonstrated only from herbarium and frozen material preserved for 20 years at most [1]. Recent field observations of new moss growth on the surface of small moss clumps re-exposed from a cold-based glacier after about 400 years of ice cover have been accompanied by regeneration in culture from homogenised material [2], but there are no reported instances of regrowth occurring directly from older preserved material.

Here, we show unprecedented millennial-scale survival and viability deep within an Antarctic moss bank preserved in permafrost. Regrowth was observed from existing shoots or rhizoids at various depths to the base of a 138 cm core of the moss *Chorisodontium aciphyllum* (Hook. f. & Wilson) Broth. obtained from an actively growing bank extending into the permafrost on Signy Island, maritime Antarctic. Gametophyte material adjacent to regrowth at 110 cm depth was radio-carbon dated to 1533–1697 cal years BP. Bank-forming mosses, combining good structural preservation and incorporation into permafrost, provide the potential for even longer periods of viability.

The re-exposure of moss communities covered during periods of ice and snowfield advance has been reported from various Antarctic, Arctic, alpine and even tropical high montane locations. However, such reports generally state or assume that the plant material is non-viable [2,3]. Mosses have well-developed abilities to survive environmental extremes in the short term, in particular relating to desiccation and freezing. More generally, cryptobiosis (e.g. tolerance of freezing, desiccation, anhydrobiosis) is well documented in the invertebrates, plants and microbes of

polar and other extreme environments [4]. However, the potential timescales of survival have largely not been examined, and evidence is only available confirming durations of up to about two decades [1,5]. Microbial viability in permafrost or ice over tens of thousands of years (and possibly much longer) has been described (e.g. [6]), but no previous reports on entire plants or other multicellular organisms over such timescales exist.

Moss banks up to three metres deep are a striking feature of the vegetation in the maritime Antarctic [3] (Supplemental Figure S1B). The banks are permanently frozen below the active layer. Cores from banks at various locations in this region have been radio-carbon dated, with the oldest basal <sup>14</sup>C dates obtained being 4801 ± 300 yr BP in a 1.25 m deep bank on Signy Island (South Orkney Islands) and 5350 ± 60 yr BP in a 1.5 m deep bank on Elephant Island (South Shetland Islands) [3,7]. These banks show continued growth at the surface while retaining good preservation of shoot and cell morphological features throughout their profile, which becomes progressively incorporated in the permafrost. Thus, there is potential for regeneration from much greater depths than the growing surface, as recently reported from the Canadian Arctic [2].

A core was therefore obtained from Signy Island in the maritime Antarctic and returned to the University of Reading for further study (see Supplemental Experimental Procedures). *C. aciphyllum* was the only species present at the surface of the core, and the only distinct gametophyte within the core material. The core down to 121 cm consisted primarily of clearly-defined gametophyte shoots in a vertical orientation, with no evidence visible of any hiatus or discontinuity in growth. The surface 1–3 cm green layer was underlain by an unfrozen light brown band that extended to 23 cm (Figure S1C). Within the permafrost, the core was coloured very dark brown to almost black (Figure S1D). The shoots at all depths consisted of intact stems bearing complete leaves and occasional rhizoids. The cell walls were intact in all leaves examined microscopically. The basal section of the core, from 121–138 cm, consisted primarily of dark brown micro-schist and small quartz particles permeated by moss rhizoids.

Strong regeneration occurred from gametophytes near the surface of the core to a depth of 30 cm. Four new shoots of *C. aciphyllum* also developed on the fresh-cut core face at 110 cm depth. Work with forceps and a dissecting microscope indicated that these were firmly attached to the existing gametophytes within the core. Growth also developed from rhizoids at 121–138 cm depth, including new shoots of the liverwort *Cephaloziella* sp. (most likely *C. varians*, the most common representative of this genus recorded on Signy Island). The length of time required before growth became visible increased with depth, being 27 days at 7 cm, 40 days at 30 cm and 55 days at 110 cm. However, the new growth in the basal layer at 121–138 cm required only 22 days to become visible. Microscopic examination of the new growth above 30 cm and at 110 cm depths suggested that this arose as branches from existing gametophytes (Figure 1A). The growth that occurred in the basal layer (Figure 1B) consisted of green protonema growing from dark brown rhizoids, subsequently giving rise to shoots (Figure 1C,D).

Gametophytes adjacent to the new growth at 110 cm were carefully removed, and AMS radio-carbon dated (Oxford University Radiocarbon Accelerator Unit, sample ref. OxA-14562). The conventional radiocarbon age obtained was 1754 ± 32 years BP. After calibration in CALIB 6.01 using the shcal04.14c calibration data set [8] this gave a 2-σ calibrated age of 1533–1697 cal years BP.

Moss gametophytes may persist physically over very long periods in permafrost with little structural change, as is the case here. By comparison with recent studies from Signy Island [9], regeneration from shallow depths in our core represents an age of 50–150 years, which is still older than the maximum previously published records from whole moss plants. Crucially, our study provides the first confirmation of regeneration directly from gametophytes deep within a moss bank that have been preserved in permafrost for a period of at least 1530 years. These gametophytes would have already been several decades to a small number of centuries in age when first incorporated in the permafrost layer. The likelihood of the regrowth observed being due to contamination with more recent viable spores or other propagules is extremely low,

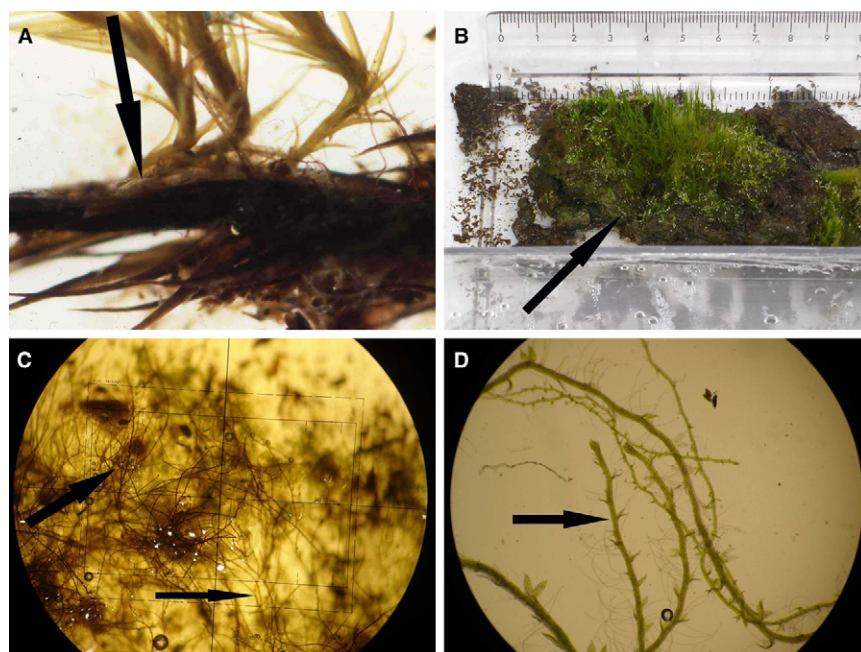


Figure 1. Moss regrowth at different depths in the core.

(A) Site of new growth emerging from existing gametophytes on the fresh-cut face of the core at 110 cm depth (arrow). (B) Extensive regrowth of *Chorisodontium aciphyllum* from the basal section of the core at 121–138 cm depth. (C) Regrowth from the basal section of the core, showing preserved brown rhizoids (thick arrow) and new protonemal growth (thin arrow). (D) New shoots of the liverwort *Cephaloziella* sp.

given the extent of permafrost within the moss bank cored, growth taking place on the fresh-cut surfaces of the core sections, and that sporophytes of *C. aciphyllum* are unknown in the maritime Antarctic. Indeed, a wide range of other mosses are known to produce mature sporophytes on Signy Island and, thus, if germination from preserved spores was to take place in a core such as this, then the presence of many other species rather than *C. aciphyllum* amongst the new growing material would be predicted.

In moss banks such as the one we describe, the generally slow decomposition typical of mosses in comparison with vascular plants may act to enhance the probability of viable material surviving long enough to become incorporated in the permafrost.

Other than the current study, *in situ* regrowth after prolonged preservation has not been demonstrated in a bryophyte or indeed any plant. The only analogous studies available have employed either plate culture of homogenised moss tissue obtained from the surface of recently re-exposed moss clumps [2], or extraction of genetic material from fruiting tissue of the flowering plant *Silene stenophylla*

obtained from Siberian permafrost, followed by cloning and culture on nutrient media [10]. The radiocarbon date obtained here therefore considerably extends the potential timescale for viability demonstrated for entire bryophytes. Further, the potential clearly exists for much longer survival — although viability between successive interglacials would require a period of at least tens of thousands of years — for instance, where a moss bank profile already preserved through permafrost formation is subsequently over-run by an advancing cold-based glacier (*i.e.* in the absence of glacial scouring). Such a possibility provides an entirely new survival mechanism and a refugium for a major element of the polar terrestrial biota.

#### Supplemental Information

Supplemental Information includes one figure, supplemental experimental procedures and supplemental references, and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2014.01.053>.

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