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Differential Freeze–Thaw Tolerance of Alpine Bryophytes and Lichens in the Glacial Retreat Zone of Haba Snow Mountain: Links to Partitiviridae Carriage

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Abstract. Climate warming has intensified diurnal freeze–thaw cycles (FTCs) in alpine glacial retreat zones, where pioneer cryptogams establish. We compared the cold hardiness of two mosses (Bryum argenteum, Polytrichum strictum) and two lichens (Cladonia sp., Usnea sp.) collected on Haba Snow Mountain, and asked whether cryptic viruses contribute. Over 30 days of multi-factorial simulation we tracked morphology, extracellular leachate pH, and relative electrical conductivity (REC). Under wet-cold stress the mosses degraded progressively—browning, increasingly acidic leachate, and REC rising above 70%—whereas the lichens held morphology, pH, and REC at control levels. CF-11 purification and sequencing detected Partitiviridae double-stranded RNA in 75.0% of the lichens but in none of the mosses, so viral carriage tracks freeze–thaw tolerance at the phenotypic level. We read the lichen edge as layered symbiosis plus possible mycovirus regulation, and add a viral-ecological route to understanding high-altitude persistence.

Keywords: Alpine cryptogams, Freeze-thaw cycles, Partitiviridae, Relative electrical conductivity, Viral ecology

1. Introduction

Alpine glacial retreat zones, such as the Haba Snow Mountain ecotone, are among the most fragile habitats on Earth, with thin, poorly buffered, fast-draining soils [1]. Here pioneer cryptogams—bryophytes and lichens—drive soil formation, nutrient cycling, and primary production [1, 2]. Climate warming has thinned winter snow cover and stripped the sub-nivean insulation that once shielded these communities, exposing them to diurnal freeze–thaw cycles at unprecedented frequency [2, 3]. How far these pioneers can be pushed therefore matters for predicting alpine biodiversity.

Repeated sub-zero stress injures non-acclimated vegetation through intracellular ice crystallisation, oxidative damage, and lipid-phase changes, so membranes lose competence and leak electrolytes [3, 4]. Mosses and lichens both tolerate desiccation at baseline [5-9], but under fluctuating FTCs they diverge; fast sub-zero cooling is known to injure sensitive bryophyte tissue when under-dried or before cold-acclimation genes engage [5, 6]. What is missing is a direct,

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controlled comparison of moss and lichen membrane integrity—leachate pH and REC—from the same sites [4, 7].

Environmental virology now reads plant and fungal stress tolerance as partly brokered by cryptic endosymbionts [10, 11]. Mycoviruses, notably the double-stranded RNA (dsRNA) *Partitiviridae*, can act as mutualists that adjust host transcription and raise stress tolerance [12, 13], and in the clearest case a virus is required for host thermal tolerance [13]. Lichens—obligate mycobiont–photobiont partnerships with a dense internal microbiota [14]—likely harbour mycoviruses [15], and metagenomics has confirmed viral diversity that follows elevation gradients [16]. Whether such viromes are group-specific, and whether carriage relates to membrane stability under alpine freeze–thaw, remain untested. We therefore profiled latent dsRNA viromes alongside membrane physiology in co-occurring mosses and lichens to (1) resolve how the two groups differ under FTCs, and (2) test whether *Partitiviridae* carriage correlates with cold hardiness.

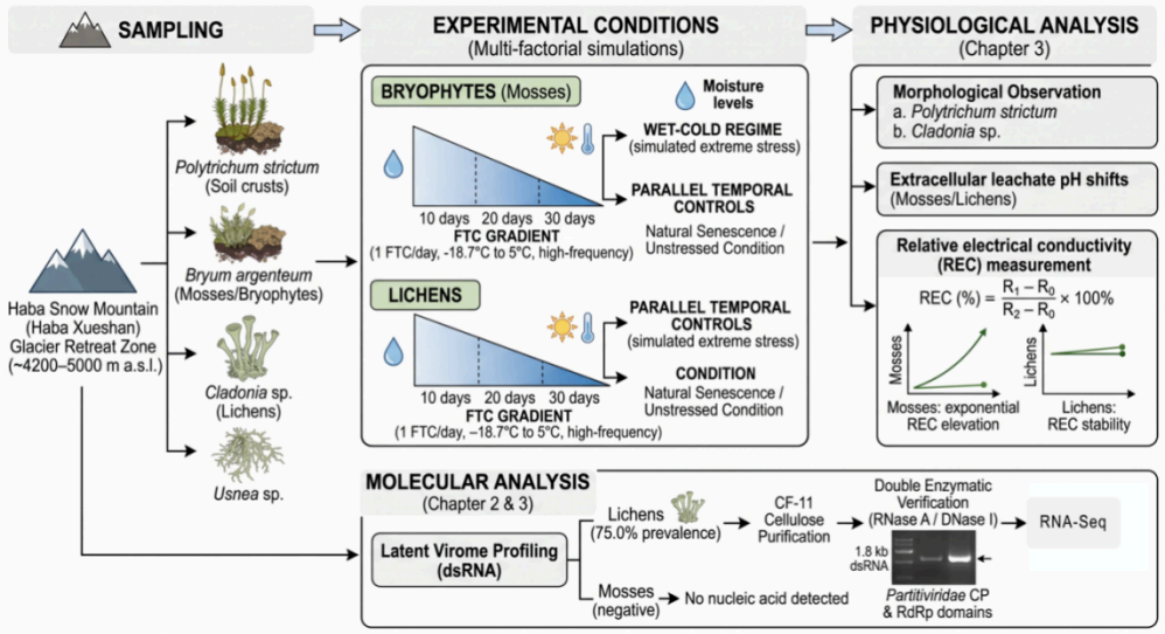


Figure 1. Schematic overview of the experimental design and multi-factorial freeze-thaw simulations

2. Materials and methods

2.1. Plant material collection

Four dominant cryptogams were collected from scree slopes of the glacial retreat zone on the north slope of Haba Snow Mountain (5000–5300 m a.s.l.): two mosses (*Bryum argenteum*, *Polytrichum strictum*) and two lichens (*Cladonia* sp., *Usnea* sp.). In total 180 visually uniform, undamaged individuals (45 per species) were stored at 4 °C and transported to the laboratory within 24 h.

2.2. Experimental design of freeze-thaw stress

A multi-factorial design crossed five environmental regimes (dry-cold −18.7 °C; dry-warm 24.1 °C; wet-cold −18.7 °C, fully hydrated; wet-warm 24.1 °C; control 20 °C) with three stress durations (10, 20, 30 d) and the four species (Table 1), with three biological replicates per combination. Fully hydrated samples were soaked in deionised water for 2 h and blotted; extreme treatments applied

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one freeze–thaw cycle per day (12 h freezing / 12 h thawing), while controls stayed at 20 °C to account for natural senescence.

Table 1. Factors and levels of the multi-factorial freeze-thaw stress experimental design

Level	Factor A: Environmental Regime	Factor B: Stress Duration	Factor C: Cryptogam Species
1	Dry-cold (-18.7°C, naturally dry)	10	*Polytrichum strictum*
2	Dry-warm (24.1°C, naturally dry)	20	*Bryum argenteum*
3	Wet-cold (-18.7°C, fully hydrated)	30	*Cladonia* sp.
4	Wet-warm (24.1°C, fully hydrated)	-	*Usnea* sp.
5	Control (20°C, natural state)	-	-

2.3. Morphological and physiological assessments

Morphological change (chlorosis, browning, shrinkage) was photographed dynamically and compared against the parallel controls. At each duration, samples were immersed in 5 mL deionised water for 10 min and leachate pH was measured with a digital pH meter (±0.01). Relative electrical conductivity (REC) was measured on the wet-cold subset (n = 3 per species per duration) following Whitlow et al [4].: ≈0.1 g tissue was incubated in 10 mL deionised water for 24 h (R_1), boiled at 100 °C for 20 min (R_2), and REC (%) = $(R_1 - R_0)/(R_2 - R_0) \times 100$, with R_0 the blank conductivity.

2.4. dsRNA extraction and sequencing

A subset of 52 samples (24 mosses; 28 lichens) was used for viral detection. Total nucleic acids were phenol/chloroform-extracted and dsRNA was purified on CF-11 cellulose in 16% ethanol [17], washed to remove DNA and ssRNA, and eluted. Aliquots were treated with RNase A (degrades ssRNA), DNase I, or left untreated and resolved on 1% agarose; the verified band was quantified, reverse-transcribed, and sequenced on Illumina MiSeq, with reads aligned to NCBI GenBank for taxonomic identification.

2.5. Statistical analysis

Data are mean ± SD. Normality (Shapiro–Wilk) and variance homogeneity (Levene) were checked; extreme treatments were compared with controls by two-sample t-tests, and regime/duration effects by two-way ANOVA with Tukey HSD. Viral prevalence was compared with Pearson's χ^2 test (SPSS 26.0; significance $P < 0.05$).

DOI: 10.54254/2753-8818/2027.39004

3. Results

3.1. Morphological responses to freeze-thaw stress

Controls (20 °C) showed no visible change over 30 d, so natural senescence did not confound treatment effects. Lichens kept colour and shape through every regime and duration. Mosses degraded progressively, worst under wet-cold: only faint chlorosis after 10 d, spreading chlorosis and shrinkage by day 20, and clear chlorosis, necrotic browning, and brittleness in all treated mosses by day 30.

3.2. Physiological responses

3.2.1. Inherent group differences

Lichen leachate was always more acidic than moss leachate ($P < 0.01$): baseline pH 5.40 ± 0.22 (*Cladonia* sp.) and 5.37 ± 0.24 (*Usnea* sp.) versus 6.13 ± 0.29 (*B. argenteum*) and 6.19 ± 0.27 (*P. strictum*) (Figure 2).

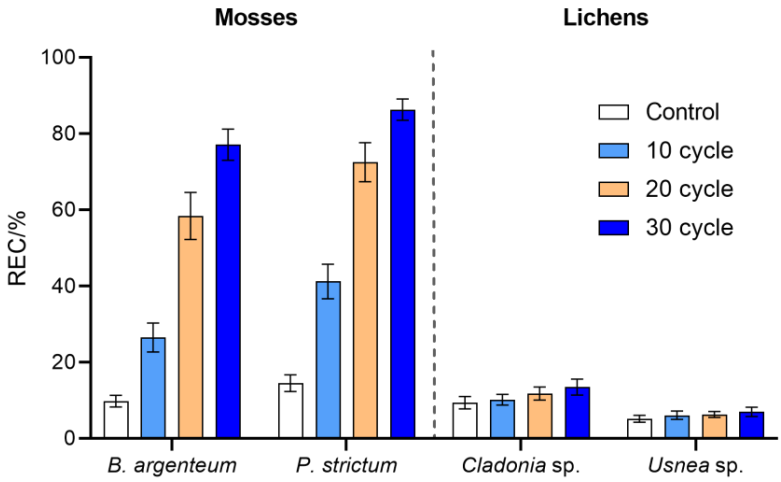


Figure 2. Extracellular leachate pH shifts of alpine cryptogams under multi-factorial freeze-thaw simulations

3.2.2. Stress-induced membrane disruption in mosses

Moss membranes failed in proportion to stress duration. Both freezing regimes acidified leachate (*P. strictum* 6.51 → 5.87, *B. argenteum* 6.36 → 5.90; $P < 0.001$), and wet-cold REC climbed from ≈10–15% in controls to >70% by day 30 ($P < 0.001$), indicating irreversible membrane lysis.

3.2.3. Physiological buffering stability in lichens

Lichens held steady: freezing did not move their pH ($P = 0.20–0.40$) and REC stayed below 15% after 30 wet-cold days, matching controls.

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3.2.4. ANOVA of environmental factors

Moisture alone did not affect pH; temperature (freezing) was the sole driver of moss degradation ($F = 156.7$, $P < 0.001$) with no interactions, while lichens showed no significant main effects or interactions.

3.3. Identification of dsRNA viruses in cryptogams

3.3.1. Enzymatic verification and viral prevalence

After RNase A/DNase I digestion a ~1.8 kb band persisted in 75.0% (21/28) of lichens (15 *Cladonia* sp., 6 *Usnea* sp.; Figure 3) but in none of the 24 mosses ($\chi^2 = 38.7$, $P < 0.001$); controls behaved as expected.

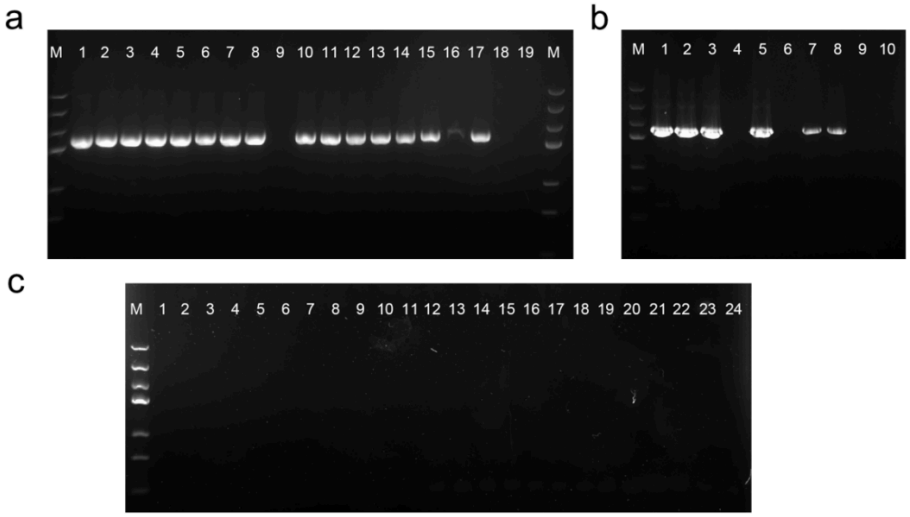


Figure 3. Detection of dsRNA viral segments in alpine cryptogams by agarose gel electrophoresis. (a) Cladonia sp., (b) Usnea sp., most showing the ~1.8 kb dsRNA band; (c) moss extracts, with no band. Lane M, size marker; numbers, biological replicates

3.3.2. Nucleic acid quantification and taxonomic identification

Extracts ran 15.3–28.6 ng/μL (A260/A280 1.85–1.92). Sequencing placed the segment in the *Partitiviridae* (91% identity to high-altitude fungal dsRNA viruses), encoding an RNA-dependent RNA polymerase (RdRp) and capsid protein (CP).

3.4. Correlation between membrane stability and dsRNA virus carriage

The 21 dsRNA-positive lichens kept pH and REC steady across durations, matching controls (Figure 4); the seven virus-free lichens were also stable but more variable. All 24 mosses (all virus-free) acidified and spiked in REC. Viral carriage thus tracks freeze–thaw tolerance, but this observational association—likely reflecting the fungal–algal structure plus possible mycovirus help—requires causal testing.

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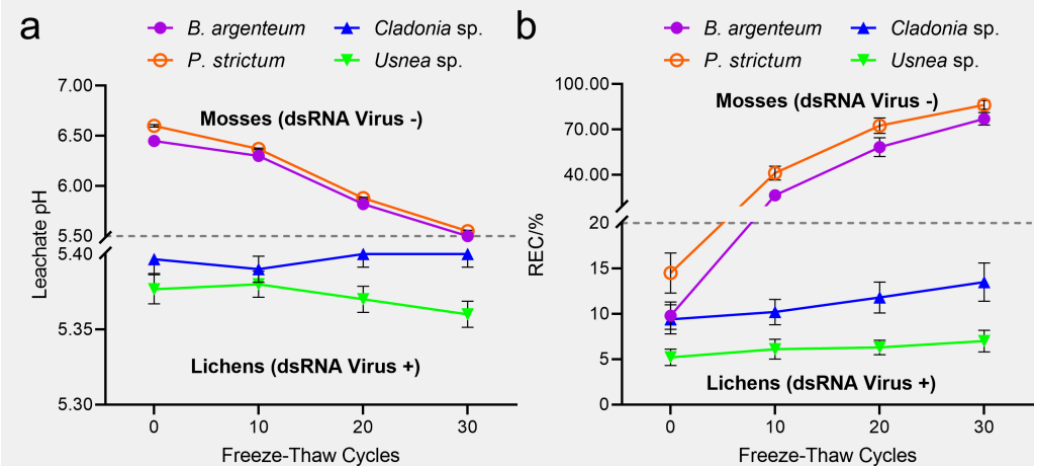


Figure 4. Dynamic shifts in (a) extracellular leachate pH and (b) relative electrical conductivity (REC) across prolonged freeze–thaw cycles and their correlation with dsRNA viral carriage

4. Discussion

4.1. Group specificity and universality of freeze-thaw response

How pioneer cryptogams survive repeated FTCs at extreme altitude remains open [1, 2]. Running both groups through one design separated their strategies cleanly. Mosses acidified and spiked in REC under wet-cold, as the cryobiological account predicts [3, 4]: rapid equilibrium freezing in water-saturated non-vascular tissue lets ice shear the plasma membrane [3], matching lethal intracellular freezing in fast-cooled sensitive bryophytes [5, 6] and faster electrolyte leakage in alpine mosses as winters warm [7, 8]. Lichens instead buffered pH and REC at control levels—stronger than trans-Himalayan epiphytic communities under comparable shifts [7].

4.2. Partitiviridae and a possible mechanism

A solid chain of evidence—CF-11 purification, double-enzymatic verification, and sequencing—places the ~1.8 kb segment in the *Partitiviridae* [17], closing the gap gel electrophoresis alone leaves. A 75.0% carriage rate fits the view that lichen-inhabiting fungi are mycovirus reservoirs [11, 15, 16]. Mycoviruses act as symbionts that can improve host fitness under stress [10, 12, 13]; the conserved RdRp and CP [18] could up-regulate antioxidant enzymes (SOD, CAT) to clear freeze-induced reactive oxygen species and stabilise membranes [19-21]. Lichen hardiness likely rests chiefly on the layered fungal–algal cortex that buffers ice-crystal stress [14], with the virus an added layer—not proof of causation.

4.3. Research limitations and future directions

Limitations: the 30-day simulation is short beside year-round natural cycles, and the causal link is untested; lichen ice-nucleating agents [22] further complicate attributing resilience to the microbiota. Next steps are virus curing/transfection in isogenic strains, cold-stress transcriptomics, and broader species and altitudinal surveys.

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5. Conclusions

Under simulated extremes, lichens retained morphology, pH, and REC across all freeze–thaw durations, whereas mosses degraded with time, driven mainly by freezing. *Partitiviridae* dsRNA occurred in 75.0% of lichens and no mosses—a group-specific virome that tracks freeze–thaw tolerance. Lichen resilience most likely reflects their fungal–algal structure, with mycoviruses a possible further aid; both now warrant causal testing. These results link cryptogamic viromes to extreme-environment adaptation and add a viral-ecological angle to high-altitude persistence.

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