Desiccation tolerance, longevity and storage at sub-zero temperatures of uni- and multicellular spores of the moss family Orthotrichaceae (Bryophyta)

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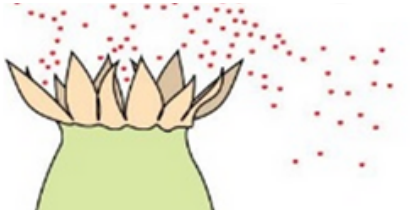
TABLE OF CONTENTS

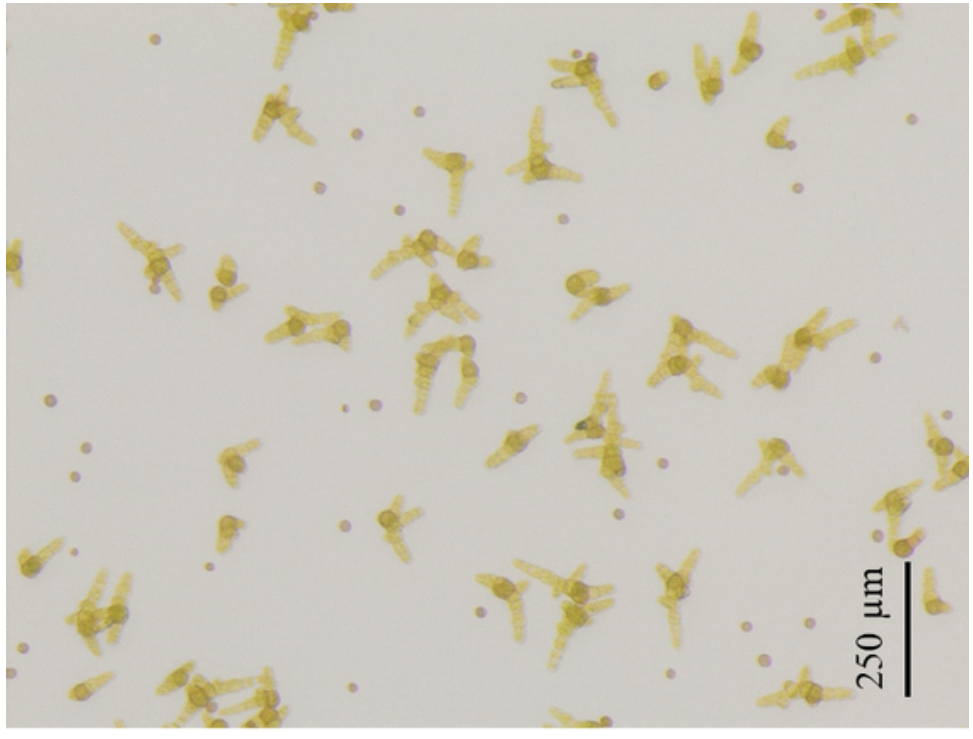
INTRODUCTION

Bryophyte spores



- Sexual reproductive structures of bryophytes.
- Generally unicellular.
- Less commonly, there are also **multicellular spores**, originating from "**endosporic germination**".
- It originates very large spores of various shapes.
- In mosses, frequent in some families, occasional in others ⟶ **Orthotrichaceae.**

Unicellular vs. multicellular spores



Germinating **unicellular** spores of *Lewinskya tasmanica*

Germianted **multicellular** spore of *Ulota membranata*

Desiccation tolerance, longevity and sub-zero temperature tolerance

Unicellular spores

- Desiccation tolerant.
- Relatively long-lived in dry state.

Multicellular spores

- Desiccation tolerance **(DT) unkown.**
  - **Hypoth. 1:** poikilohydric DT gametophyte ⟶ DT spore.
  - **Hypoth. 2:** DT lost during endosporic germination.

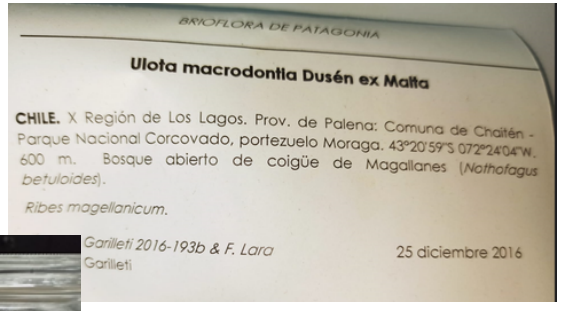
- Scarce development of *ex situ* conservation techniques of bryophyte spores.
- Understanding **sub-zero temperature tolerance** (alongside DT and longevity) is the first step.

OBJECTIVES

1. To determine the **viability** of uni- and multicellular spores preserved in herbarium sheets for years.
2. To characterise the **desiccation tolerance** and **longevity** of uni- and multicellular spores in the dry state.
3. To characterise the **tolerance** to **sub-zero temperatures** of these spores in order to obtain keys for their *ex situ* conservation.

MATERIAL AND METHODS

- **Germination** of spores at 20 ºC on Knop Medium solidified with 1% agar.


BRYOFLORA DE PATAGONIA

Ulota macrodontia Dusén ex Malta

CHILE. X Región de Los Lagos. Prov. de Palena: Comuna de Chaitén - Parque Nacional Corcovado, portezuelo Moraga. 43°20'59"S 072°24'04"W. 600 m. Bosque abierto de coigüe de Magallanes (*Nothofagus betuloides*).

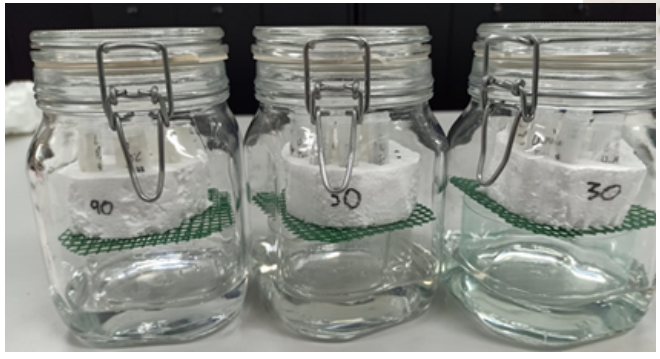
Ribes magellanicum.

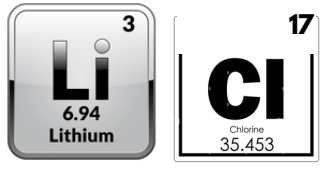
Garilleti 2016-193b & F. Lara
Garilleti

25 diciembre 2016

- **Herbarium sheets** (4 - 7 years).

- **Dry storage**.





- **Sub-zero storage**: spores equilibrated at different RH were stored at -20 and -80 ºC freezers.

RESULTS

Viability of *Ulota* spp. spores preserved in herbarium sheets

- Spores from the studied species were **not viable** after 4 - 7 years of conservation under herbarium conditions.

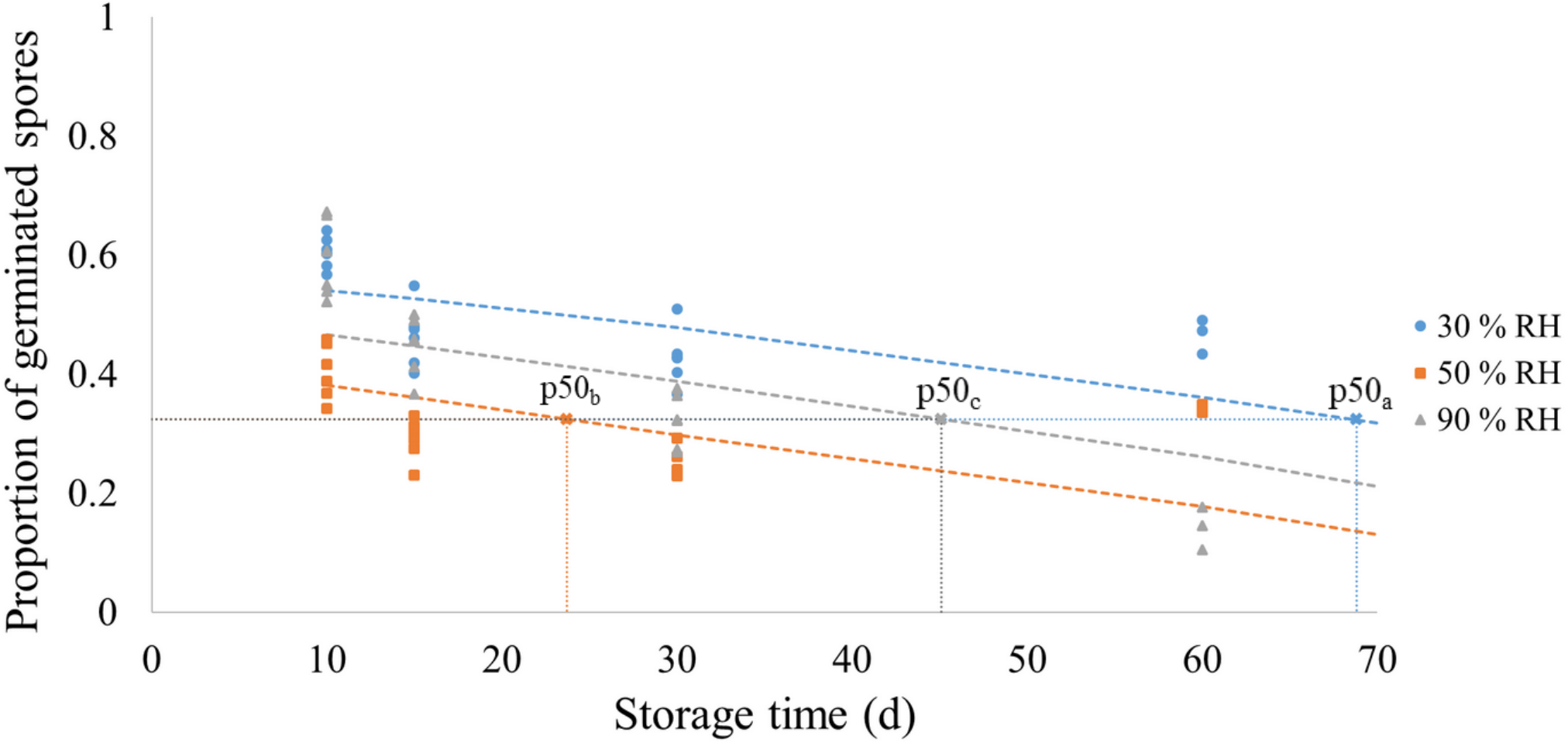
Desiccation tolerance and longevity in dry stage of the *Ulota membranata* and *Lewinskya tasmanica* spores

- Spores of both species germinated after 30 days of dry storage ⟶ **Desiccation tolerant.**
- **Significant loss of viability** over time and RH treatments?
  - **Yes**: *Lewinskya tasmanica* ⟶ GLM
  - **No**: *Ulota membranata*
    - ANOVA + Tuckey-B

Ulota membranata	10 (d)	15 (d)	30 (d)
30 % RH	68.3 % (A)	-	49.6 % (BC)
50 % RH	15.0 % (C)	46.1 % (B)	44.9 % (B)

GLM analysis: calculation of p50

Lewinskya tasmanica



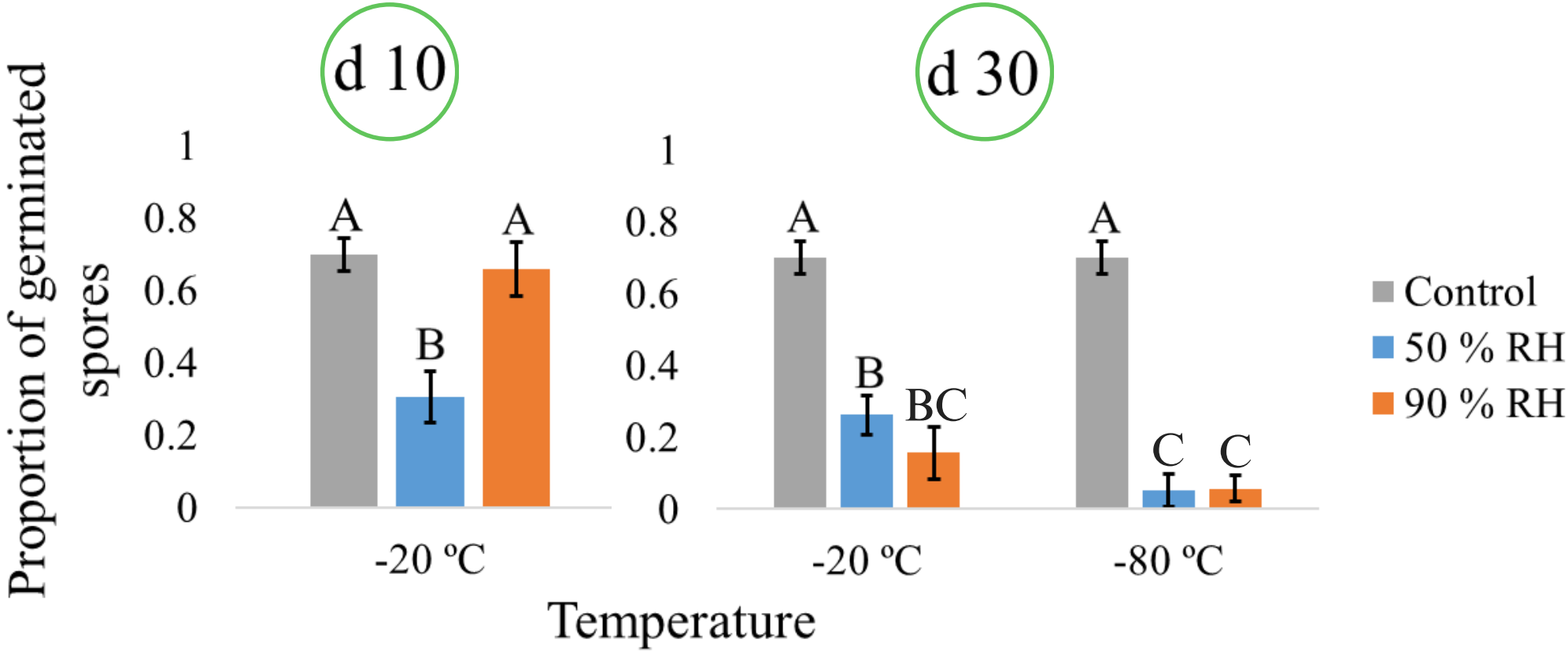
Comparative longevity

p50 (d)			
Species	**30 % RH**	**50 % RH**	**90 % RH**
Lewinskya tasmanica	68.8^{a} [44, 175]	23.7^{b} [-4, 51]	45.1^{c} [25, 103]
Ulota membranata	Ns	Ns	-
Equisetum hyemale *	5.9 ± 0.1	7.3 ± 0.1	-
Osmunda regalis *	39 ± 1	31 ± 1	-
Matteuccia struthiopteris *	119 ± 1	-	-
Dicksonia macrocarpa *	175 ± 2	143 ± 1	-
Polystichum aculeatum *	872 ± 30	993 ± 29	-
Pteris vittata *	4,781 ± 199	4,650 ± 180	-

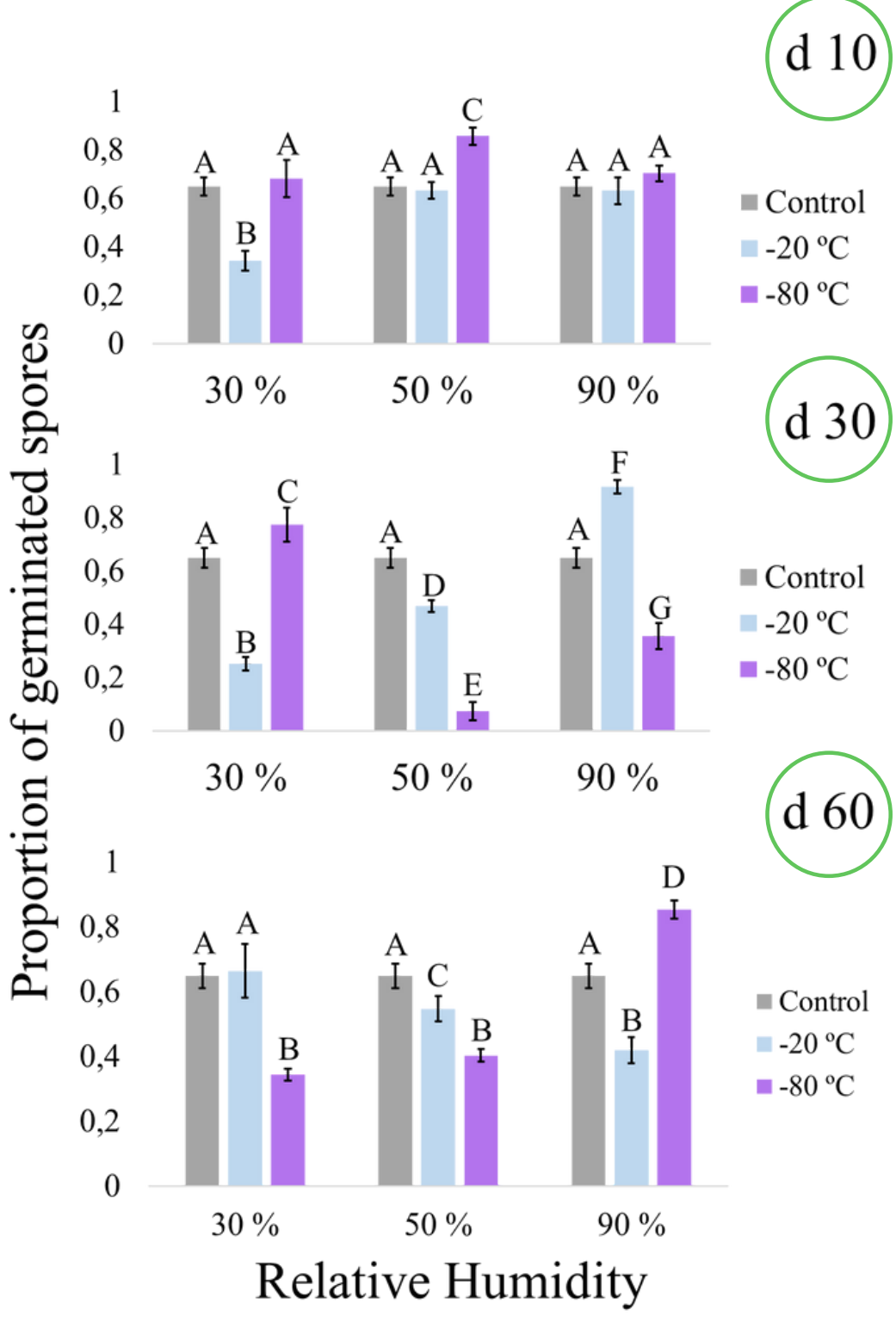
* Ballesteros, D., Hill, L. M., Lynch, R. T., Pritchard, H. W., & Walters, C. (2019). Longevity of preserved germplasm: the temperature dependency of aging reactions in glassy matrices of dried fern spores. *Plant and Cell Physiology, 60*(2), 376–392.

Sub-zero temperature tolerance in spores of *Ulota membranata* and *Lewinskya tasmanica*

Ulota membranata



Lewinskya tasmanica



DISCUSSION

Viability of *Ulota spp*. spores preserved in herbarium sheets

- The longevity of the spores of the studied species is less than 4 - 7 years under herbarium conditions.
  - **Less long-lived** than spores of other bryophytes studied by Malta (1921, 1922) and other species of the family Orthotrichaceae studied by the work team.
- Spores from sheets of these ages are not suitable for germination studies.

Desiccation tolerance and longevity in dry stage of the *Ulota membranata* and *Lewinskya tasmanica* spores

- Both unicellular spores of *L. tasmanica* and multicellular spores of *U. membranata* are **desiccation tolerant**.
  - Endosporic germination does not entail loss of DT.
- In ***U. membranata*** no significant drops in germination were detected in the probit analysis ⟶ **Longer lived?**
- ***L. tasmanica*** spores aged the least in the driest conditions, followed by the wettest.
  - Dry conditions: vitrification of cytoplasm.
  - Wet conditions: cell repair.
    - Similar longevity to chlorophyllous fern spores.

Sub-zero temperature tolerance in spores of *Ulota membranata* and *Lewinskya tasmanica*

- Both unicellular spores of *L. tasmanica* and multicellular spores of *U. membranata* **tolerate freezing** if their water content is low.
- Also if the water content is high.
  - Greatest damage in *U. membranata*.
  - Best condition for *L. tasmanica*.
- Multicellular spores of ***U. membranata*** **presented more freezing damage** than unicellular spores of *L. tasmanica*.
- More research is needed to determine optimal *ex situ* conservation conditions.

CONCLUSIONS

1. Spores of the species studied present **short-longevity**, comparable to **chlorophyllous fern spores**.

   Spores of ***U. membranata*** **appear to be longer-lived** than *L. tasmanica* spores.

2. Uni- and multicellular spores are desiccation tolerant. **Endosporic germination ≠ loss of DT.**

3. Both uni- and multicellular spores **tolerate storage at sub-zero temperatures**.

   Multicellular spores present **more freezing damage**. Unicellular spores were better preserved with pre-equilibration at 90 % RH.

Thank you for your time

Any questions?