

Ex situ Conservation of Bryophytes

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Importance of Bryophytes



- Bryophytes are plant pioneers and are ubiquitous in the terrestrial environment
- They play important roles in ecosystem function

UK Bryophytes

The UK has an exceptional bryophyte flora (c.600 moss and c. 300 liverwort species) but many are restricted to a single or handful of sites and are vulnerable to catastrophic events



Buxbaumia viridis - only 2 sites in Scotland

UK Biodiversity Action Plan

- UK Government response to Rio convention
- Lists Species Action Plans for
13 liverworts,
51 mosses
(95 vascular plants)



Ditrichum cornubicum
(Cornish Path Moss)

UK endemic, known from 1
locality, Endangered World RDB
species

Ex Situ Conservation Project for UK Bryophytes

A collaborative project to develop and evaluate experimental protocols for the collection, *in vitro* propagation and cryogenic storage of threatened UK bryophytes



Orthodontium gracile in vitro

Advantages of *ex situ* conservation

- Maintain basal storage collection representing genetic diversity
- Supply material for re-establishment trials minimising sampling from wild populations
- Provide source of uncontaminated DNA for genetic studies
- Resource for auto-ecological research where material limited in the wild

Collection Protocols developed for:

Desiccation tolerant mosses

Desiccation intolerant mosses and
leafy liverworts

Thalloid liverworts and hornworts

Collection emphasis

- Prevent damage to *in situ* populations (eg no gaps in colonies, safeguarding against invasive species)
- Ensure genetic diversity represented
- Collection details(site, substrate, grid reference, donor, verifier)

Why aseptic culture ?

- Sterile (aseptic) culture essential to prevent cross contamination of samples stored in liquid Nitrogen
- Novel methods of sterilisation using NaDCC (Sodium dichloroisocyanurate) without addition of detergents



Sterilisation Protocols

- Sporophytes (1% NaDCC 3-6 mins)
- Leafy gametophores (0.5% NaDCC 2-5 mins)
- Gemmae (0.1% or 0.5% NaDCC 2-8 mins)
- Thallus tissue (0.01% NaDCC 10-20 mins)

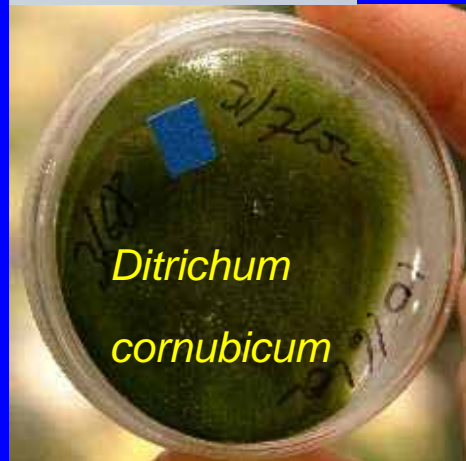
5 UK BAP species in Axenic Culture



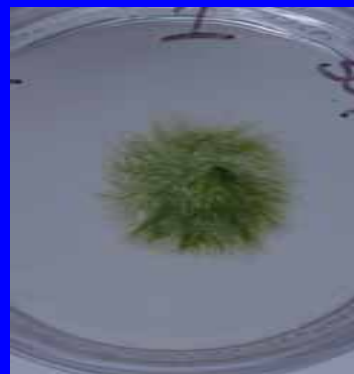
*Ditrichum
plumbicola*



Orthodontium gracile



*Ditrichum
cornubicum*



Leptodontium



Bartramia stricta

Propagation

Gametophytic material grown on 1/4 , 1/2 MS or Knops minimal medium without sucrose and using Gelrite as gelling agent



Cryopreservation

- Storage in liquid nitrogen (-196°C) secures conservation collections on a long term basis
- Suspension of cellular metabolic activities minimises genetic drift
- Cost and space efficient



Cryopreservation of Bryophytes

Gametophytic material is cryopreserved as sporophytes are not always available



O.gracile re-growing after cryopreservation

Cryopreservation Protocol

- Encapsulation in alginate strips
- Pre-treatment with ABA and sucrose
- Dehydration
- immersion and storage in liquid nitrogen

Survival of *D. cornubicum* after freezing

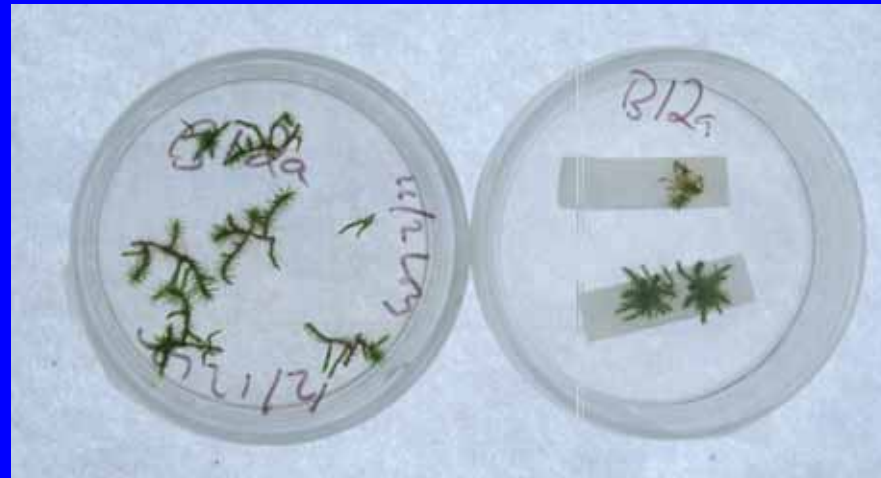
Survival after Cryostorage

Survival after cryopreservation appears to be linked to desiccation tolerance and is being assessed for a wide number of species

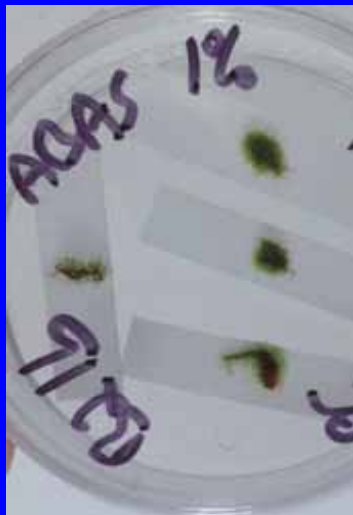
Five UK BAP species in cryostorage



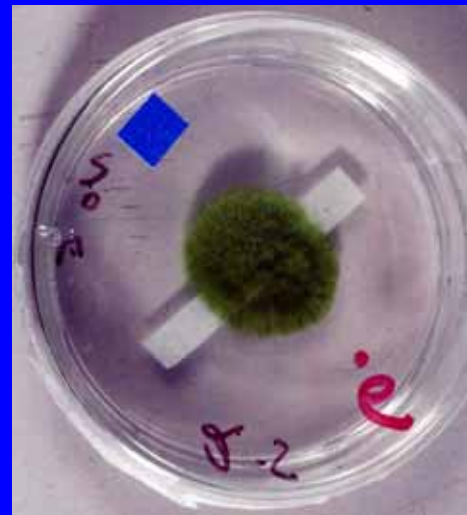
Orthodontium gracile



Bartramia stricta



Ditrichum plumbicola



Ditrichum cornubicum



Leptodontium gemmescens

Cryo - exchange

- Cryopreserved samples of *Ditrichum cornubicum* shipped to Kings Park and Botanic Garden, Western Australia as part of cryo-exchange project
- Potential for duplicate gene-banks

Collaborative Research

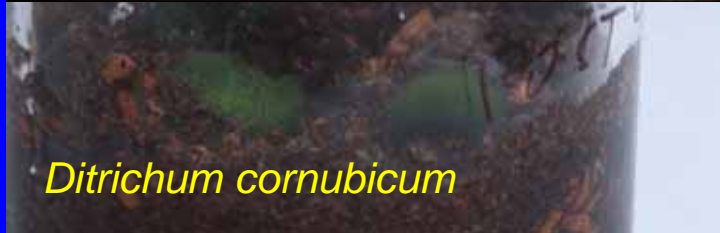
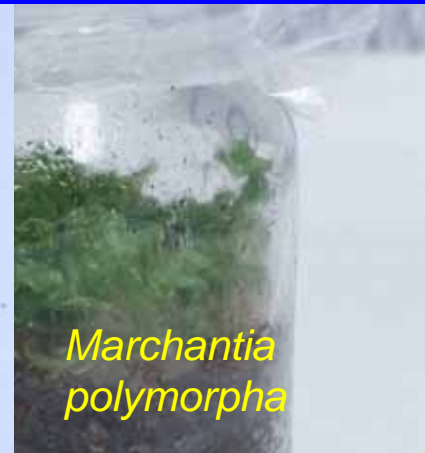
- Material supplied to Queen Mary University to determine effect of cryopreservation on bryophyte tissue as part of joint NERC Studentship project

Weaning

Gradual acclimatisation of plants to:

- Lower humidity
- Increased airflow
- Variable temperatures
- Lower nutrient availability

Four species weaned onto natural substrates



Next Steps

- Increase number of priority species in the *ex situ* collection
- Investigate methods for re-establishment using material propagated and stored in the laboratory

Reintroduction

- “Intentional movement of an organism into part of its native range from which it has disappeared or become extirpated in historic times as a result of human activities or natural catastrophe” (IUCN 1987)
- The ultimate goal of the *ex situ* project

In situ management of Kew's estates

Wakehurst Place,
Kew's garden in West
Sussex has a SSSI of
UK and European
significance for its
bryophyte
assemblages on
sandstone outcrops .



Threatened Plant Appeal



Sorbus domestica

Jasminum leptophyllum

Orthodontium gracile

Echium wilpottii

Lotus bertelottii

Lysimachia minoricensis

Hibiscus liliiflorus

Centaurea cyanus

Hyophorbe lagenicaulis

Bromus interruptus



Orthodontium gracile

Material of *O. gracile*, a critically endangered species, is being bulked up for genetic analysis, to determine the genetic variability of the species from 16 sites around the UK



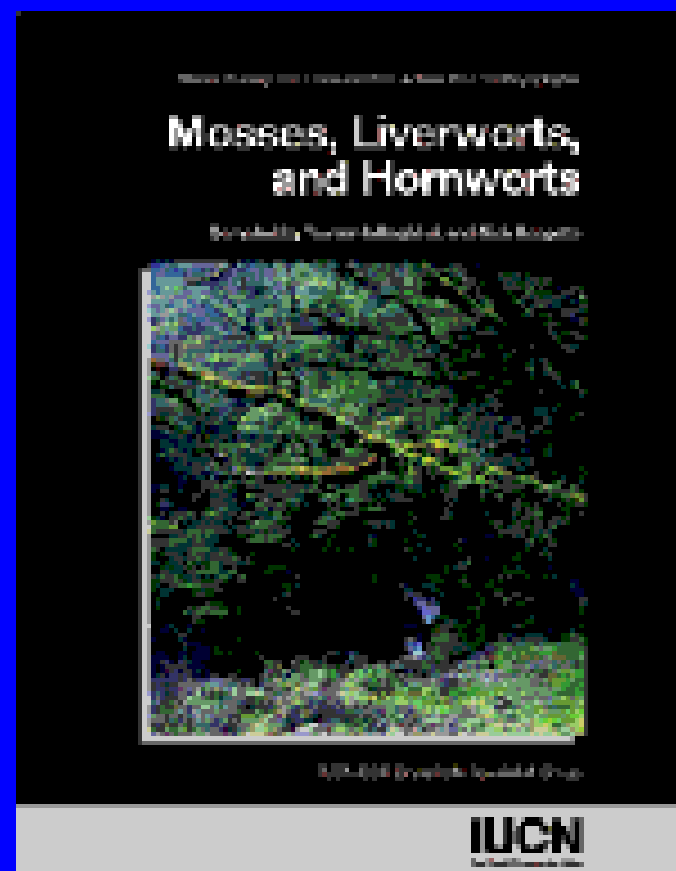
Francis Rose Reserve at Wakehurst

- A nature reserve dedicated to mosses, liverworts, lichens and filmy ferns
- Use of reserve as testing ground for re-establishment trials



Re- introduction Guidelines

- IUCN SSC Reintroduction Specialist Group and Bryophyte Specialist Group
- Joint Re-introduction Guidelines to be developed



Global Strategy for Plant Conservation - targets

3. Development of models with protocols for plant conservation and sustainable use, based on research and practical experience;
8. 60 per cent of threatened plant species in accessible ex situ collections, preferably in the country of origin, and 10 per cent of them included in recovery and restoration programmes

Plant Diversity Challenge

- The UK's response to the Global strategy for Plant Conservation
- Considerable progress towards Target 8 particularly for vascular plants
- Ongoing actions include developing methodologies for the *ex situ* conservation and re-introduction of bryophytes

European Plant Conservation Strategy

- 2.6 At least 12 priority species of bryophytes brought into *ex situ* conservation and methodology promoted internationally
- Working with **European Committee for Conservation of Bryophytes**



Species Currently in Collection

Species	Status	World red list	European red list ECCB (1995)	British red list Church et al (2001)
<i>Aplodon wormskjoldii</i>	Sterile			Critically Endangered
<i>Bartramia stricta</i>	Sterile Cryo			Critically Endangered
<i>Cyclodictyon laetevirens</i>	Sterile		Rare	Endangered
<i>Ditrichum cornubicum</i>	Sterile Cryo	Critically Endangered	Endangered	Endangered
<i>Ditrichum plumbicola</i>	Sterile		Vulnerable	Near threatened
<i>Leptodontium gemmascens</i>	Sterile Cryo		Rare	Vulnerable
<i>Orthodontium gracile</i>	Sterile Cryo		Endangered	Vulnerable
<i>Orthotricum obtusifolium</i>	Non-sterile			Endangered
<i>Seligeria carnicolica</i>	Sterile			Critically Endangered
<i>Weissia multcapsularis</i>	Sterile		Endangered	Endangered
<i>Weissia rostellata</i>	Sterile		Rare	Near threatened
<i>Zygodon forsteri</i>	Sterile		Vulnerable	Endangered
<i>Zygodon gracilis</i>	Sterile		Vulnerable	Endangered

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