ESCAPE – UNIVERSITY OF HELSINKI

Action C.5 Milestone

Bryophyte ex situ conservation scheme

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Monitoring *Meesia longiseta* reintroduction site in 2016. Photo: Sanna Laaka-Lindberg

An ex situ conservation scheme is presented on the basis of compiled results of the ESCAPE project as a model for bryophyte conservation.

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Introduction

Ex situ conservation is a species conservation method used as a compliment to the primary conservation tool *in situ* conservation, a process of protecting an endangered species in its natural habitat. *Ex situ* conservation is targeted to species in most serious threat, especially when the conservation measures in nature are not adequate for species survival. There are basically two different types of *ex situ* conservation tools: 1) tools aimed at storing and securing the species and its genetic variation e.g. on national or even on wider (global) level, and 2) tools aimed at increasing species survival ability in nature. The ultimate goal of *ex situ* conservation is to provide support for the survival of species in their natural environments.

Conservation of biodiversity is a continuous and long-term assignment, so the decisions on *ex situ* conservation to a species need to be made on a solid basis. This involves thorough investigation on conservation priorities, background knowledge of the biology and ecology of the species to be conserved, and the feasibility of the conservation plan. In the ESCAPE project, a priority list was compiled for vascular plants (see Ryttäri 2013), but no such list is made for bryophytes. As bryophyte *ex situ* is all new in Finland method development and testing was initiated on three threatened species. These three focal bryophytes representing different habitats and ecological demands were selected on the basis of the recommendations from the Finnish Bryophyte Expert Group (Finnish Environment Institute SYKE), a group responsible for the evaluation of the status of threatened bryophytes in Finland. The selected species include two mosses *Meesia longiseta* and *Tortula cernua*, and a thalloid liverwort *Mannia fragrans*. The IUCN threat categories (Rassi ym. 2010), number of known localities and ecological demands of these species are shown in Table 1.

Development of bryophyte *ex situ* conservation methods was selected as one of the major innovative goals of the ESCAPE project. A scheme of, or rather, a model for bryophyte *ex situ* conservation is presented here. A more detailed guide-book on the bryophyte *ex situ* conservation methods will be published (in Finnish) separately as a deliverable product of the ESCAPE project.

Table 1. Threat category (Rassi et al. 2010), conservation status in Europe (Hodgetts 2015), number of known localities and ecological demands and habitats in Finland (Syrjänen 2009a, 2009b, Virtanen 2009) based on records in the literature.

Species	IUCN threat category in Finland (2010)	European Red list candidate 2015/Directive species	Number of known localities (2009)	Ecological demands
Mannia fragrans	EN	X / -	9	calcareous/rich stones and soil, dry leas
Meesia longiseta	EN	X / X	101, of which 35 recent, 10 in Southern part of Finland	marsh, rich fen
Tortula cernua	CR	X / -	3	man-made calcareous substrates on walls, lime kilns and waste pits

Bryophyte ex situ conservation scheme

The bryophyte *ex situ* conservation scheme is shown in a flow chart (Fig. 1) below. On the basis of the experience gathered in ESCAPE project we suggest to follow a stepwise process, which may be adjusted to the local situation and specific regulations, if needed. The steps and alternatives in the process are described with examples on the ESCAPE focal bryophyte species.

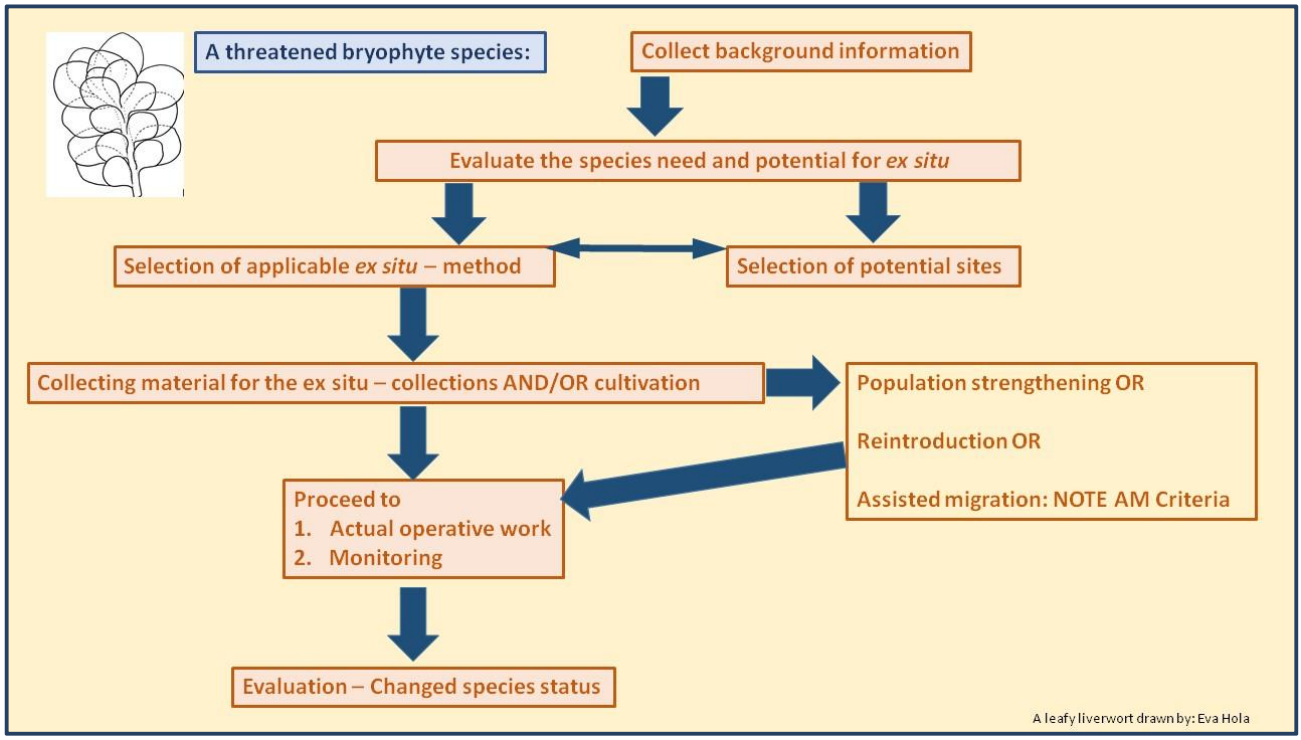

Figure 1. A flow chart illustrating the bryophyte ex situ conservation scheme designed for planning and decision making of the action procedure.

A. Species and site selection, and background information

For applying *ex situ* methods for bryophyte species conservation, the first step is to evaluate the need and potential of the species for ex situ. Serious decline and high risk of destruction of existing populations, are among the arguments advocating ex situ as a conservation tool. Thus, candidates for bryophyte *ex situ* are among the species in high threat categories and occurring in most threatened habitat types.

The selection of applicable *ex situ* method follows the species evaluation. Practically all species are valuable enough for inclusion in *ex situ* collections. For species with seriously declining populations, reintroductions and/or population strengthening may be applicable (see below). Selection of potential sites is necessary for all activities in nature, even for collecting material for *ex situ* collections. The sites for reintroductions and population strengthening need to fulfil the species´ environmental requirements. Especially for reintroduction sites, the time from the last records before the species has been declared as extinct/disappeared from the site cannot be too long. The habitat need to be as intact as possible, with no evident changes in its conditions. For collecting material for *ex situ* purposes, the source populations need to be strong enough so that risk of damaging the potential for growth and reproduction is minimal. In ESCAPE project, a recommended maximal sample of bryophyte shoots and sporophytes was set on only 10% of the population.

In setting priorities for *ex situ* conservation, most of the rare bryophytes lack background information such as that obtained for vascular plants (Ryttäri 2013). Therefore, collecting background information in the literature is important as a starting point, studies on existing natural populations on population dynamics, reproductive capacity, information of genetic diversity, and dispersal abilities are of essential for achieving successful *ex situ* actions.

B. Permissions

In Finland, all bryophyte collecting requires landowner´s permission, which is an important step before starting *ex situ* conservation activities even with species not protected by law. Bryophytes which are mentioned in Habitat Directive Annex II, protected by law or so called specifically protected species of high threat classes need exemption for all activities from collecting, translocating, export and field activities. Additionally, exemption is required also for *ex situ* actions in protected areas.

C. Selecting the *ex situ* - conservation method

C. 1 One of the most critical issues of *ex situ* conservation is to keep high levels of genetic diversity. Most threatened species have lower genetic diversity than related, non-threatened species with larger population sizes (Frankham et al. 2010), so practically, all genetic resources of the threatened species are valuable enough for storing in *ex situ* collections. Bryophyte propagules do not always stay alive as dried specimens similarly as vascular plant seeds. However, some species may be adapted to natural spore/propagule banks, and may, therefore be able to survive dried as well (see e.g. Black & Prichard 2002).

C.1.1 Bryophyte tissues have been shown to have high potential to survive deep-frozen even in nature. Protocol for tissue culture and cryopreservation in liquid nitrogen tanks have been developed already for many bryophyte species (e.g. Sabovljević et al. 2014), including those of *Meesia longiseta* and *Tortula cernua* in the ESCAPE project. Success of micropropagation (tissue culture) of bryophytes depends on species and the growing conditions. In the ESCAPE – project, testing *in vitro* growing of green shoots collected directly from nature appeared challenging, as surface sterilization of the whole shoots was impossible as molds and other fungi and epiphytic algae easily contaminated the cultures. However, the shoot fragments of the thalloid liverwort *Mannia fragrans* demonstrated their ability to grow on turf in green house conditions. Sometimes the solution may be the most obvious! For ESCAPE focal mosses, growing from surface sterilized spores appeared as the most reliable method of *in vitro* propagation.

Micropropagation precedes the cryopreservation, and the handling of bryophyte tissues needed before cryostorage varies among species. In some cases chemical and other treatments are unnecessary for survival in cryostorage, but some species need e.g. addition of sugar on the growing medium before the cryostorage. Often the protocol for one species is applicable to its close relatives. Thus, for saving the rare species natural populations as intact as possible, using their common relatives in testing the methods is highly recommended (Fig. 2).

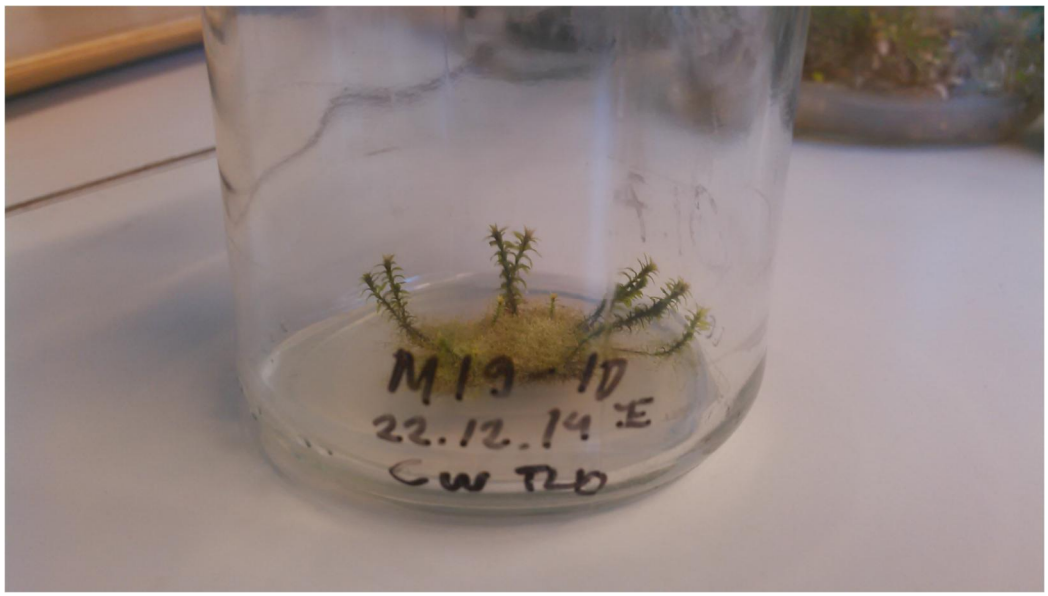

Figure 2. Shoots of *Meesia triquetra*, a cryopreservation test species in *in vitro* cultivation in the Botanical Garden cryolaboratory of University of Oulu. Photo: Peetu Rytkönen

C.1.2 Outdoor *ex situ* collection of threatened native bryophytes has been initiated with ESCAPE – project focal species *Tortula cernua* and *Meesia longiseta* in a bryophyte-lichen garden in Kaisaniemi Botanical Garden in Helsinki in 2016 (see Launis et al. 2016). *T. cernua* has been planted on an experimental moss roof in the botanical garden among other species sprayed with a moss-paint technique on roof surface. Small tufts of *Tortula* were also clued on a terrace wall with egg white. *Ex situ* propagated *Meesia* have been stored in botanical garden outdoor collection in turf pots (Fig. 3) and these will later be planted in the bryophyte garden mire species collection.

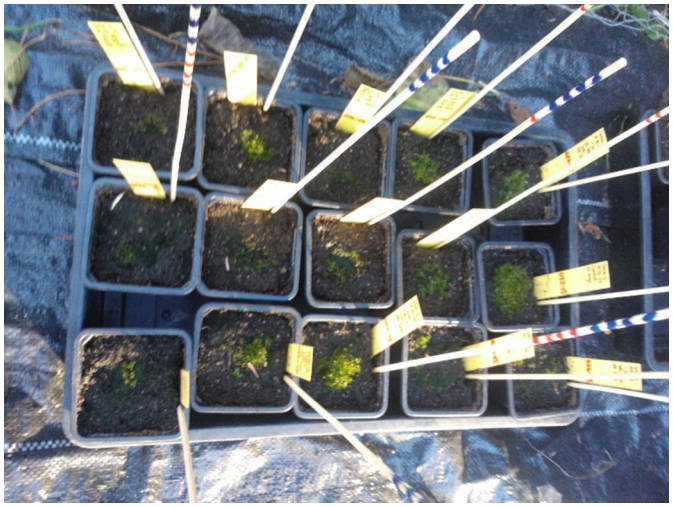

Figure 3. *Ex situ* cultivated *Meesia longiseta* cushions in turf pots at Kaisaniemi Botanical Garden. Photo: Sanna Laaka-Lindberg

C.2 Bryophyte population strengthening may bring solution for declining populations also in protected areas such as nature conservation areas and national parks, where the primary *in situ* conservation is not adequate e.g. as a consequence of reproductive restrictions and poor ability to move between habitat patches. In *ex situ* cultivation it is preferable to use shoots of the same or nearby origin of the population to be strengthened. In some cases source material may be collected from other populations, especially if the target population is too small for collecting. Population strengthening by shoots from different population may even be profitable as it may prevent inbreeding depression. Genetic scoring of the variability within and between populations is recommended. In the ESCAPE project, both alternatives are tested: *ex situ* cultivated shoots originating from the same population is tested on *Mannia fragrans* (Fig. 4), and from different origin on *Tortula cernua*.

Figure 4. Background study of the original site of *Mannia fragrans* in Lammi in 2016. Photo: Sanna Laaka-Lindberg

C.3 Bryophyte reintroductions start with evaluating the situation of populations in the selected region, e.g. within a country or geographical entity. In the ESCAPE project the situation of *Meesia longiseta* was evaluated as seriously declined. Thus a site in Southern Finland from where the species had been recorded only ten years ago but disappeared since, was visited and the expert opinion on the site vegetation and hydrological situation scored in connection to applying the permission for reintroduction. Source material for reintroduction of *Meesia* was collected from the only known close-by site with sporophytes, but also in a large population in the North where the species has still relatively better hold. The experimental reintroduction was designed to

include many different angles to the reintroduction methodology: it included two different origins, *ex situ* cultivated shoots which had gone through the cryopreservation and as control shoots which had not. Additionally, the treatments included testing the effects of different protocols in micropropagation and cryopreservation and different planting times (Fig. 5) in order to get basis for further recommendations. The multiple treatments require an adequate number of repetitions. A permission for reintroduction was granted only for this one site, ending in a case study design. It will, however, produce valuable information on the conditions and methods required in future reintroductions.

Figure 5. A set of *Meesia longiseta* test plots were planted in spring 2016 at the reintroduction site. Photo: Sanna Laaka-Lindberg

C.4 Assisted migration (AM) is thus far neither applied in species conservation in general nor on bryophytes in Finland. The concept of AM includes species translocation from its original distribution area and habitat to an area and habitat type considered, as a consequence of climate change, potentially suitable for the species in the future. Forecasting and selecting the suitable future distribution areas is based on climate change modelling. Suitability of AM for a species is tested by applying specified AM criteria (Fig. 6, see also Hällfors 2013). In ESCAPE these criteria will be tested and discussed on the three focal bryophyte species.

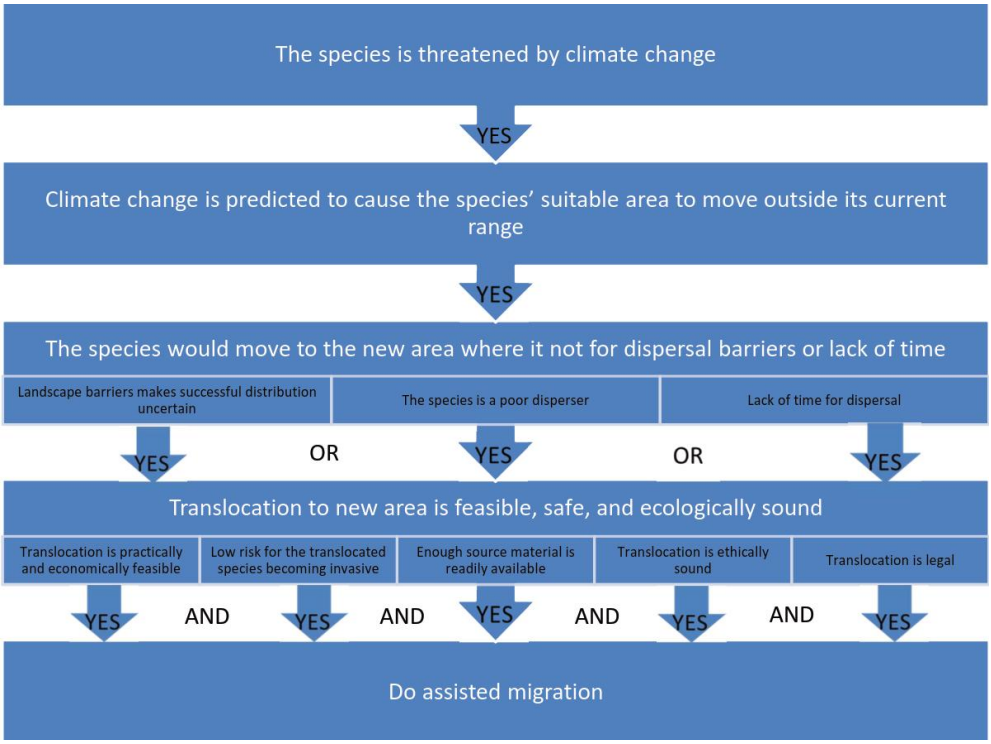

Figure 6. Formal application of criteria for suitability of assisted migration as an *ex situ* conservation tool for a species, based on Hällfors 2013.

D. Monitoring

Assessing the success of *ex situ* conservation activities requires testing the vitality of living collections and monitoring of the field activities. Regular testing of vitality of e.g. cryopreserved bryophyte tissues is required for comparison of different preparation protocols but also for preventing loss of deep-frozen tissues. Modifications may be required for bryophyte growing conditions in living collections in botanic gardens, requiring also annual monitoring and prevention of e.g. litter accumulation or other disturbances.

Monitoring the bryophyte patch number and size, shoot density and reproduction in population strengthening and reintroduction sites is important in assessing the success of these activities. In the ESCAPE project, monitoring time was rather short, from half a year to two years, showing however well the success of the colonization phase of the transplanted individuals, but the proper establishment and long-term success of population strengthening and reintroduction can be seen only after longer period monitoring for several years or even more. Monitoring is required not only for evaluating the success of the conservation activities as such, but also the effects on the associated species and vegetation in the sites, not the least to prevent possibly harmful effects on other species.

Figure 7. Monitoring *Meesia longiseta* in autumn 2016. Photo: Sanna Laaka-Lindberg

E. Applications and future of bryophyte ex situ conservation

Applications of *ex situ* or similar activities have already been used often, however, lacking a systematic scheme. Such activities have mostly focused on vascular plants, but in restoration of habitats the role of mosses, especially *Sphagnum* has been important on peatlands, but also liverworts have been already tested for restoring specific habitats (Flagmeier 2016). Experiments on mire restauration both with and without *Sphagnum* have led to a conclusion that the peat mosses are often crucial for successful restoration efforts (e.g. Ferland & Rochefort 1997). Techniques of peatland restoration have become more or less standardized during the last few decades. Potentially, in restoring rich fens, knowledge on reintroduction of such bryophytes as *Scorpidium scorpioides* (see Kooijman et al. 1994) and *Meesia longiseta* may open potential for bringing back the rich bryophyte flora of such habitats. Other habitat types which may need similar *ex situ* efforts in species reintroductions are e.g. old growth forests, naturalized brooks and springs.

New species conservation techniques such as "ecosystem hotels" have been applied in vascular plant conservation. Species have been dug up as complete plants from their original localities and evacuated temporarily to another site in order to be brought back after the construction work at the original site is finished. This type of *ex situ* species conservation method may have a potential in bryophyte conservation as well, and is worth considering even though no experience on bryophytes is yet available.

Furthermore, for display in public parks and in landscaping has been mostly utilizing vascular plants, but in future also bryophytes including the threatened species may be planted especially in urban areas e.g. on moss roofs.

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