Ex situ conservation of a critically endangered fern

CAROLINE CHONG[1,3,4,*], AMELIA STEVENS[1,3], ALASDAIR GRIGG[2,3], TOM NORTH[1,3], JOE McAULIFFE[1,3] AND LYDIA GUJA[1,3]

[1] Australian National Botanic Gardens, ACT.
[2] Christmas Island National Park, IO.
[3] Parks Australia, Department of Agriculture, Water and the Environment, ACT.
[4] Current address: Flora and Fauna Division, Northern Territory Government Department of Environment, Parks and Water Security, NT.
*Corresponding author: yfcaroline@gmail.com

Summary

Pneumatopteris truncata is listed as Critically Endangered under the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act). The Australian population occurs only on Christmas Island where fewer than 50 mature individuals are known and population size fluctuates significantly across years. *Ex situ* conservation was prioritised as a critical response to insure against loss of the wild population. Spore from wild subpopulations was collected and germination and propagation techniques were trialled. This work has established *ex situ* spore and plant collections (more than 100 plants), developed protocols for germination and propagation of this species and identified priority future research for threatened fern species.

Introduction

The fern *Pneumatopteris truncata*

Although *P. truncata* occurs across Indonesia and South East Asia, the Australian regional population is confined to Christmas Island, a geographically isolated island in the north-east Indian Ocean (off Western Australia). Here, the taxon is known as the Dales Waterfall fern and occurs as three subpopulations across two locations, including The Dales Ramsar site (Butcher and Hale 2010; Figure 1a). The large, erect fern has fronds to 120 cm and occurs only within closed forest where springs create permanently wet limestone habitat (Figure 1b).

Current and potential threats

The small population size (total number of mature individuals) provided the basis for listing the species as Critically Endangered in 2004 (Threatened Species Scientific Committee 2004; Butcher and Hale 2010). Surveys conducted throughout the late 1980s, 2002 and 2003 recorded only 45 mature individuals at two subpopulations (Holmes and Holmes 2002). Disturbances such as cyclone damage to canopy cover negatively impact the population.

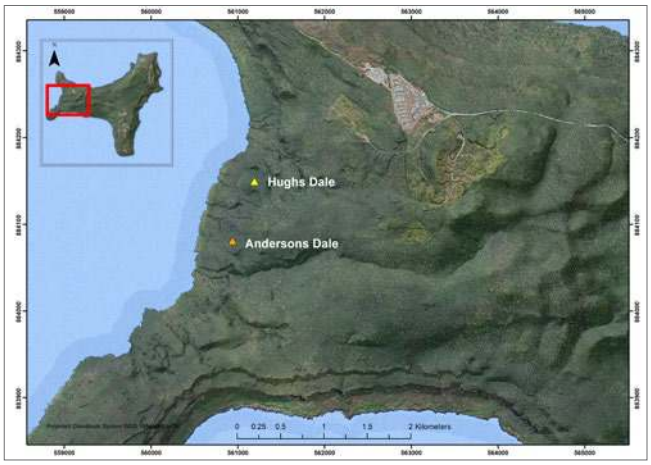

Figure 1a. Location of the Hughs Dale and Andersons Dale subpopulations of *Pneumatopteris truncata* on Christmas Island, Indian Ocean.

Figure 1b. *Pneumatopteris truncata* (foreground) in forest habitat. Photo: Alasdair Grigg, Christmas Island National Park staff

In situ management

Since 2010 Parks Australia has conducted annual subpopulation monitoring at Hughs Dale. Monitoring at this site has identified extreme fluctuations in the number of individuals present (juvenile and mature), ranging between 0 to 500 individuals across years with the proportion of juvenile plants typically exceeding 80% (A. Grigg, 2020). A comparison of the number of individuals per year with annual precipitation indicates that larger numbers of individuals may be positively associated with above-average rainfall and declines associated with dry years (Figure 2).

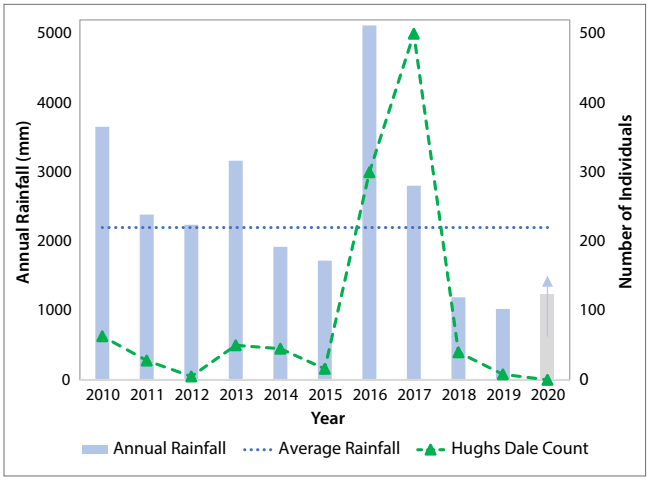

Figure 2. Number of individuals of *Pneumatopteris truncata* recorded at the Hughs Dale subpopulation from 2010 to 2020 (green line), annual rainfall (mm) (blue solid bars); average rainfall (mm) (blue dashed line).

Methods – *ex situ* conservation

Pneumatopteris truncata was prioritised for *ex situ* conservation on the basis of threats, observed population fluctuations and cost-benefit ranking (Di Fonzo *et al*. 2017).

Spore collection

Spore collecting occurred in March 2018, closely following the monsoon season. Collecting aimed to maximise genetic representativeness without placing stress on the population. Whole fronds greater than 1 m bearing mature sporangia were collected from 16 individuals and placed into large paper bags. Collected fronds were kept at ambient temperature for 2–3 days until spore release. Spore was then transferred into paper envelopes and kept separate as maternal lines. To facilitate spore drying, spore collections were maintained at ambient temperature and approximately 50% relative humidity (RH) for approximately 3 weeks before transport and storage in the dark at 5°C.

Germination trial

A germination trial was conducted eight months after spore collection at the Australian National Botanic Gardens (ANBG). Four germination media were tested: 1) milled sphagnum moss (saturated with purified water), 2) 70:30 mix of peat moss and sand (saturated with purified water), 3) 0.7% water agar (pH 6-7), 4) alkaline 0.7% water agar (pH 8.0, 1 M HEPES biological buffer). All media (150 mL per replicate) and containers (90 mm diameter plastic lidded containers) were sterilised in an autoclave (Tomy ES315) and water adjusted to establish a thin film of water on the media surface. Four replicates of 15 maternal lines on 4 media types were trialled with 2 mg of spore hand sown in each container and sealed with parafilm. Containers were stored in incubators at 25°C and a 12/12 hr light/dark photoperiod with light intensity maintained by LED strip lights at 970 lumens per metre.

Containers were monitored fortnightly for germination and contamination. Spore germination was recorded by placing a 1 cm^2 grid across each container and counting the number of grid cells containing green prothalli and/or sporophyte plants. The percentage of grid cells containing germinated spores at 10 weeks was arcsine transformed and analysed by ANOVA in Genstat (VSN International 2020).

Propagation and cultivation

Germinants on agar were transferred to sphagnum moss either 74 or 112 days after the germination trial began. Once all germinants were transferred to sphagnum moss, the plants were moved to a propagation house at approximately 20°C, 95% RH under a hood and on capillary matting. Container lids were incrementally removed, and holes pierced in the bottom of containers to introduce gas exchange and water flow gradually over two weeks to prevent shock. The sporophytes showed signs of nutrient deficiency (chlorosis), which was addressed with fortnightly applications of Hortico All Purpose Soluble fertiliser at a rate of 0.5 grams per 500ml of water (boiled and cooled). Between seven and nine months after the germination trial began, sporophytes were potted into 70 mm pots containing plugger 666 growing medium (Australian Growing Supplies) avoiding root disturbance. At this stage a single application of Multicrop Plant Starter at 24 mL per 9 litres of water was supplied.

Results

Germination trial

Germination was successful from eight-month-old spore from 15 maternal lines from two subpopulations (Figure 3a, 3b). Of the four media types, germination was only observed on water agar and sphagnum. Time to first observed germination (prothalli) was four weeks after sowing on 0.7% water agar, while germination on sphagnum moss was first observed at six weeks. Germination media significantly affected both the total germination ($P < 0.001$) and the rate of spore germination ($P < 0.001$; Table 1). Total spore germination varied with maternal line ($P = 0.003$). Contamination in some agar containers after six weeks killed some germinants, which resulted in reduced total germination (Figure 3a).

Propagation and cultivation

Although germination was generally five times higher on agar than sphagnum moss, subsequent transfer shock associated with movement to sphagnum growing medium caused many germinants to die. Germinants from both agar and sphagnum dishes were transferred to pots and grown at approximately 20°C and 95% RH where they grew equally as vigorously regardless of germination medium.

Propagation and cultivation efforts resulted in 52 plants from 3 maternal lines from the Hughs Dale subpopulation and 51 plants from 5 maternal lines from the Anderson Dale subpopulation, totalling 103 plants established and maintained at the Australian National Botanic Gardens (Figure 3c).

Discussion

We developed protocols for short-term storage, germination and propagation to establish an *ex situ* population and provide information to guide conservation and research efforts for *Pneumatopteris truncata*. Such *ex situ* techniques may be suitable to conserve other fern species. We found that *P. truncata* spore, if collected when mature and dried appropriately, can be stored at 5°C and less than 70% RH for at least eight months. Germination was greatest in spore sown on water agar, but relative survival was greatest from spore sown on sphagnum moss, potentially due to minimal root disturbance during transfer. Plants grow well in standard potting media and benefit from consistent water and nutrient availability. Cultivation conditions with temperatures between 20°C to 30°C and RH greater than 80% produced healthy growing plants.

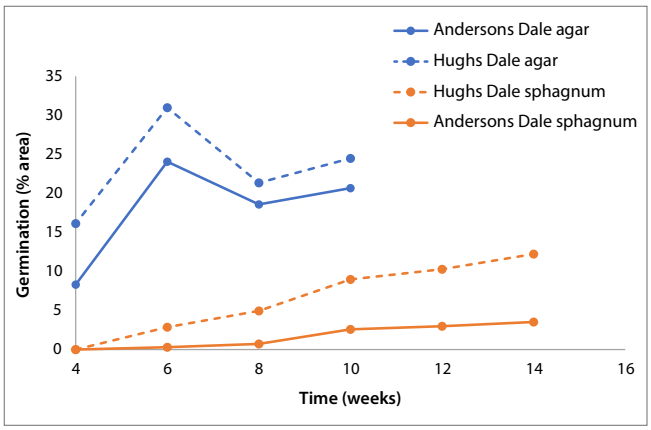

Figure 3a. Observed germination of *Pneumatopteris truncata* from two subpopulations (Andersons Dale, Hughs Dale) on water agar and sphagnum moss. X-axis values are time (number of weeks) since spore sowing. Germinants on agar were transferred to sphagnum from 6 weeks following observed germination.

Figure 3b. Successful growth of sporophyte plants at 11 weeks following transfer to sphagnum moss from agar and c. 5 months from observed germination. Photo: Fanny Karouta-Manasse

Figure 3c. Potted ferns of *Pneumatopteris truncata* growing at the Australian National Botanic Gardens nursery 9 months after spore germination. Photo: Fanny Karouta-Manasse

Table 1. Summary analysis of variance of *Pneumatopteris truncata* spore germination. Significant sources of variation in bold.

	Final % germination			Days to first germination		
Source of variation	d.f.	v.r.	F pr.	d.f.	v.r.	F pr.
Germination media	3	87.48	**< 0.001**	1	321.30	**< 0.001**
Maternal line	15	4.62	**0.003**	15	2.21	0.012

The living conservation collection of *P. truncata* plants at the ANBG may represent a greater than doubling the number of individuals in Australia once they reach reproductive maturity. These plants and spores secured at conservation facilities are useful resources to evaluate, under controlled conditions, the environmental requirements for growth and survival to inform potential future augmentation of wild populations.

Future trials at the Christmas Island nursery will aim to understand the environmental thresholds for plant growth and survival. Laboratory trials will aim to understand spore longevity and requirements for effective long term *ex situ* spore conservation, including through assessing spore desiccation tolerance and chilling sensitivity.

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