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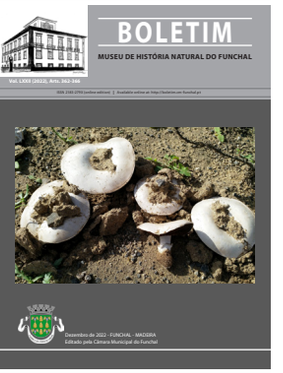

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Conservation actions in *Polystichum drepanum* (Sw.) C. Presl, a critically endangered fern from the island of Madeira

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With 1 figure

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ABSTRACT: *In vitro* culture techniques, using spores, were used to propagate the critically endangered fern *Polystichum drepanum*, in order to reinforce its known natural populations. In 1998 and 2010, a total of 194 plants of *P. drepanum* were reintroduced at Ribeira do Inferno, a historical habitat of this species and the source of the spores used in this research. In 2021, we observed that, only two of those plants had survived, without any indication of natural regeneration. Reintroduction failures and new reintroduction actions are discussed herein in order to promote the long-term conservation of this species.

Key words: *in vitro* propagation, critically endangered, fern, *Polystichum drepanum*, Madeira Island.

RESUMO: A técnica de cultura *in vitro* foi utilizada para propagar, através dos esporos, o feto, criticamente ameaçado de extinção, *Polystichum drepanum*, a fim de reforçar as suas populações naturais conhecidas. Em 1998 e 2010, um total de 194 plantas de *P. drepanum* foram reintroduzidas na Ribeira do Inferno, habitat histórico desta espécie e origem dos esporos utilizados neste trabalho. Em 2021 observamos que apenas duas dessas plantas sobreviveram, sem indícios de regeneração natural. Falhas na reintrodução e novas ações de reintrodução são discutidas a fim de promover a conservação desta espécie a longo prazo.

Palavras-chave: propagação *in vitro*, criticamente em perigo, feto, *Polystichum drepanum*, ilha da Madeira.

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INTRODUCTION

The word *Polystichum* is derived from the Greek word *polys*, which means many, and *stichos*, meaning line, in reference to the many rows of spores that the various species of *Polystichum* have (Jonhson & Smith, 1931). The genus *Polystichum* comprises between 160 and 200 species (Roux, 2005). In Macaronesia (for the purposes of this paper, considered as encompassing the archipelagos of Azores, Madeira, Selvagens, Canary Islands and Cape Verde), this genus is represented by five *taxa*: *Polystichum drepanum* (Sw.) C. Presl, *Polystichum falcinellum* (Sw.) C. Presl, *Polystichum* x *maderense* J. Y. Johnson, all endemic to the island of Madeira (Eriksson *et al*., 1979; Vieira, 1992; Hansen & Sunding, 1993; Press & Short, 1994; Jardim & Francisco, 2000; Jardim & Sequeira, 2008); *Polystichum setiferum* (Forssk.) T. Moore ex Woynar, with a more widespread distribution, occuring in Britain, Europe and North Africa (Roux, 2005) and which is native to the island of Madeira (Press & Short, 1994; Jardim & Sequeira, 2008), as well to the archipelago of the Azores (all islands; Silva *et al*., 2010) and the archipelago of the Canary Islands (El Hierro, La Palma, La Gomera, Tenerife and Gran Canaria, according to Acebes Ginovés *et al*., 2010); *Polystichum aculeatum* (L.) Roth, is native to the archipelago of the Canary Islands (La Palma, La Gomera and Tenerife, according to Acebes Ginovés *et al*., 2010). The archipelago of Cape Verde does not have any representative taxon from this genus (Sánchez-Pinto *et al*., 2005). There was another endemic *taxon*, formerly included under *Polystichum*, (*P. webbianum*) that now is treated as *Arachniodes webbiana* (A. Braun) Schelpe (Manton *et al*., 1987).

In the XIX century, Lowe (1851), under the specific name of *Aspidium drepanum* Sw, saw "abundant wild specimens" of this fern on the island of Madeira. Now, *P. drepanum* it is reportedly one of the rarest species worldwide (Christenhusz *et al*., 2017), having suffered at the hands of over-zealous collectors (Benl, 1971).

According to the IUCN Red List of Threatened Species and other authors, *Polystichum drepanum* is listed as Critically Endangered with 40-50 mature individuals (Jardim *et al*., 2006; Christenhusz *et al*., 2017; García Criado *et al*., 2017). This species is also listed in the top 100 Management Priority Species for the European archipelagoes of Azores, Madeira and the Canary Islands, taking into account both their protection priority and management feasibility (Jardim *et al*., 2008). According to Jardim & Sequeira (2008), *P. drepanum* is a protected and prioritary species under the Bern Convention and Habitats Directive (Annexes II and IV), restricted to the *Ocotea* laurel forest (*Laurissilva do Til*), an habitat within the protected area of Madeira Natural Park and the European Commission legislation, through the Natura 2000 network, under the designation of "Laurissilva da Madeira". *P. drepanum* has an extremely fragmented distribution in the valleys and slopes of the north coast of the island of Madeira, where it is confined to ravines in shady, wet places under laurel forest.

MATERIAL AND METHODS

1. *In vitro* propagation – Madeira Botanical Garden (MBG) propagated this species by sowing spores using i*n vitro* techniques adapted from Chá-Chá *et al*. (2005).

1. 1. Spores – Fronds with ripe sporangia were collected from live plants of MBG (Fig. 1A), and preserved in paper bags, at room temperature, until sterilization. After a few days in the bag, the sporangia opened and spores are dispersed inside it.

1. 2. Sterile conditions – Petri dishes and goblets were sterilized for 4 h at 160 °C. Culture medium, water, eppendorfs, and micropippete tips were sterilized at 120 °C (1 atm.) for 20 min. All sowing of the spores, on the culture medium, were made at sterile conditions in a laminar horizontal flow hood.

1. 3. Spores disinfection – Spores were surface disinfected for 5 min. in 5% commercial sodium hypochlorite solution in a sterilized eppendorf, then washed 3 times in sterile, distilled water (disinfection solution and water were separated from spores by centrifugation at 13,000 rpm).

1. 4. Spores transfer to medium – Disinfected spores were resuspended in sterile distilled water; 100 µl of this solution, containing spores, were transferred to 13.5x3 cm glass flasks with 10 ml of culture medium, using a micropipette.

1. 5. Spores culture – Spores were sown in MS medium (Murashige & Skoog, 1962) supplemented with sucrose at 20 g/l. The pH was adjusted to 5.5, before adding 6.0 g/l of agar. Culture medium was prepared from stock solution MS major salts (10x), MS minor salts (100x), MS vitamins (500x). Spores were kept in culture chambers, equipped with temperature and light control, at 20 °C ± 1 °C, under a 16 h/day photoperiod.

1. 6. Sporophyte induction – After spores had developed into prothallus [gametophyte stage], sterilized water was added to the glass flasks to promote fertilization and sporophyte growth.

1. 7. Acclimatization – *In vitro* sporophyte plants were washed in water to remove the agar, transferred to pots containing a substrate consisting on peat and perlite (50/50 mix) and transferred to a green house at room temperature.

2. Reintroduction – After growth of sporophyte, plants in the green house with 3-year growth were reintroduced into natural habitat, with each individual fern planted in isolation.

RESULTS

In 1998, the MBG, the former Madeira Natural Park (now, IFCN - *Instituto das Florestas e Conservação da Natureza, IP-RAM*) and the Conservatoire Botanique National de Brest (CBNB) drew up an integrated conservation programme for *P. drepanum* which included *in situ* and *ex situ* actions, namely population reintroduction, monitoring of new populations, *in vitro* propagation, and live population maintenance in MBG.

CBNB have propagated *P. drepanum* by spores, since 1979 from one adult specimen taken from Ribeira do Inferno on the north coast of the island of Madeira (the exact location is kept confidential due to plant security reasons) (Lesouëf, 1999) and in July 1998 sent a total of 120 plants propagated from this one specimen, to the island of Madeira. Seventy of them were reintroduced into the original locality (Ribeira do Inferno) and the rest were planted in the MBG and around the Forestry House at Encumeada (Lesouëf, 1999).

MBG propagated species growing at MBG using *in vitro* propagation by spores techniques described above in the materials and methods. It was found that spores started to germinate after 22 days in culture (Fig. 1B) and after two months, the prothallus [gametophyte stage] were evident in the glass flasks (Fig. 1C). After six months (Fig. 1D), water was added to the glass flasks, to promote sporophyte growth (Fig. 1E). In April 2010, MBG took 124 of their *in vitro* propagated plants and reintroduced them into Ribeira do Inferno.

In total therefore, 194 plants were reintroduced into Ribeira do Inferno between July 1998 and April 2010. In 2021, a survey of the areas where these two reintroductions had taken place recorded only two surviving reintroduced plants and noted that there was no indication of natural regeneration.

DISCUSSION

Conservation of threatened species combines both *ex situ* and *in situ* measures within an integrated conservation programme. Conservation *in situ* (*e.g*., natural area protection and management and threat abatement) must always be the preferred policy to protect species, however, in some cases human intervention and *ex situ* conservation (*e.g*., botanical gardens, seed or DNA banks, *in vitro* culture, reintroduction, etc.) is necessary to support long-term conservation.

P. drepanum is a tetraploid (n = 82) fern (Manton *et al*., 1987). Polyploid species are expected to self-fertilize (termed 'selfing') more often than diploids, because genome duplication initially mitigates the effects of genetic load. Because selfing enables a single individual to reproduce in a new location, this ability to self-fertilize may often be an important component of population establishment following long-distance dispersal (Flinn, 2006). The rarity of *P. drepanum*, however, despite being a polyploidy fern, implies it is not efficient at selfing or long-distance dispersal and colonization and conservation programmes should consider this.

Reintroduction is a technique to restore or establish plant populations to enable them to become self-sustaining in natural habitats (Akeroyd & Jackson, 1995). It is the intentional movement and release of an organism inside its indigenous range from which it has disappeared (IUCN/SSC, 2013). According to IUCN/SSC (2013), "Conservation translocations consist of (i) reinforcement and reintroduction within a species' indigenous range, and (ii) conservation introductions, comprising assisted colonisation and ecological replacement, outside indigenous range". IUCN/SSC (2013) goes on to note that "It is increasingly recognised that, while species conservation remains a priority for conserving biodiversity, reintroduction needs to be undertaken in the context of the conservation and restoration of habitats and ecosystem services".

In vitro methods of propagation can be used to help preserve endangered plants (Fay, 1992; Fernandes *et al*., 1999; Maryam *et al*., 2014), reintroducing the propagated specimens to reinforce known natural populations. The *in vitro* technique, described in this paper, has proved to be effective in obtaining plants from spores for *P. drepanum* but the survival rate of reintroduced plants has not been shown to be effective in reinforcing known natural populations.

Fig. 1 – *In vitro* germination of spores and acclimatization of *P. drepanum*: **A** – Spore source plant at MBG; **B** – Spores germination; **C**, **D** – Prothallus (gametophyte stage); **E** – Beginning of sporophyte; **F** – Plant acclimatization in green house (photos taken by the first author and published in Gouveia *et al.* (2010).

The process of plant reintroduction combines the art and science of horticulture, ecology, and evolution (Maschinski & Albrecht, 2017). Reintroduction of native species has become increasingly important in conservation worldwide, however, many projects illustrate that various plant species seem to be particularly difficult to reintroduce and are considered as a relatively high-risk, high-cost activity (Godefroid *et al*., 2011). Although early efforts to reintroduce plants into the wild often suffered from failure (Falk *et al*., 1996), practitioners have since refined the practice of reintroduction and shared their experiences of success and failure for the sake of improving future practice (Maschinski & Haskins, 2012).

A number of factors can be considered to improve plant reintroductions, namely: propagule material type (*e.g*. spore, sporophyte, etc.); number of individuals reintroduced, provenance of material introduced, and demographic status of source population, introduction method and management of out-planting sites (Godefroid *et al*., 2011). In determining sites for plant reintroductions, the focus must be placed on defining the former distribution borders and historical habitat of species (Gorbunov *et al*., 2008), but should satisfy the same conditions (Akeroyd & Jackson, 1995; Gorbunov *et al*., 2008; IUCN/SSC, 2013; Soorae, 2013).

Considering possible shortcomings in the two reintroductions of *P. drepanum* in 1998 and 2010, it could help to define what could be improved for outcomes that are more successful. Godefroid *et al*. (2011) suggests that reproductive failure and genetic decline in reintroduced populations is likely to accelerate in subsequent generations. The fact that all specimens reintroduced at Ribeira do Inferno in 1998 and 2010 originated from one single founding individual, may be considered sub-optimal and it is recommended that future reintroductions ensure the specimens are propagated from different natural origins.

Future reintroductions should also consider establishing populations with greater than 50 plants, with relatively high planting densities (Maschinski & Haskins, 2012). Additionally, since this species has a global population that is fragmented and isolated, with less than 50 individuals in total, a review or study its genetic information to inform future reintroductions, may help improve the process (Maschinski & Albrecht, 2017).

Overall it is noted that reintroduction as a means of restoring or increasing the viability of plant populations is not an easy exercise. The whole procedure may take many years, is time consuming and is expensive (Akeroyd & Jackson, 1995). Setting clearly defined success criteria, ensuring sufficient monitoring over the years and having adequate documentation, may all help improve the design of future reintroductions (Godefroid *et al*., 2011). This paper helps document reintroductions of *P. drepanum* and make some observations and recommendations. It is hoped that this might facilitate improved success of future reintroductions of this species.

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REFERENCES

ACEBES GINOVÉS, J. R., M. C. LEÓN ARENCIBIA, M. L. RODRÍGUEZ NAVARRO, M. DEL ARCO AGUILAR, A. GARCÍA GALLO, P. L. PÉREZ de PAZ, O. RODRÍGUEZ DELGADO, V. E. MARTÍN OSORIO & W. WILDPRET de la TORRE:

2010. Pteridophyta & Spermatophyta. In: *Lista de especies silvestres de Canarias (hongos, plantas y animales terrestres)* (eds.: M. Arechavaleta, S. Rodríguez, N. Zurita & A. García (coord.)), pp. 119-172. Gobierno de Canarias.

AKEROYD, J. & P. K. JACKSON:

1995. *A Handbook for Botanic Gardens on the Reintroduction of Plants to the Wild*. Botanic Gardens Conservation International.

BENL, G.:

1971. Fern hunting in Madeira. *The British Fern Gazette*, **10** (4): 165-174.

CHÁ-CHÁ, R., F. FERNANDES & A. ROMANO:

2005. *In vitro* spore germination of *Polystichum drepanum*, a threatened fern from Madeira Island. *Journal of Horticultural Science and Biotechnology*, **80** (6): 741-745. DOI: 10.1080/14620316.2005.11512008.

CHRISTENHUSZ, M., R. BENTO ELIAS, R. DYER, Y. IVANENKO, G. ROUHAN, F. RUMSEY & H. VÄRE:

2017. *Polystichum drepanum*. The IUCN Red List of Threatened Species 2017: e.T162178A85427290. https://dx.doi.org/10.2305/IUCN.UK.2017-2.RLTS.T162178A85427290.en.

(Last accessed on 03 February 2022).

ERIKSSON, O., A. HANSEN & P. SUNDING:

1979. *Flora of Macaronesia, checklist of vascular plants*. Part I. Revised Edition. 93 pp.

FALK, D. A., C. MILLAR & P. OLWELL:

1996. *Restoring Diversity: Strategies for Reintroduction of Endangered Plants*. Island Press, Washington.

FAY, M. F.:

1992. Conservation of Rare and Endangered Plants Using *in vitro* Methods. *In Vitro Cellular & Developmental Biology. Plant*, Vol. **28** P, no. 1, pp. 1-4.

FERNANDES, F. M., S. S. PAIS & M. C. NEVES:

1999. The preservation of *Goodyera macrophylla* Lowe by *in vitro* germinatiom. *Boletim do Museu Municipal do Funchal*, **51** (295): 43-52.

FLINN, K. M.:

2006. Reproductive biology of three fern species may contribute to differential colonization success in post-agricultural forest. *American Journal of Botany*, **93**: 1289-1294.

GARCÍA CRIADO, M., H. VÄRE, A. NIETO, R. BENTO ELIAS, R. DYER, Y. IVANENKO, D. IVANOVA, R. LANSDOWN, J. A. MOLINA, G. ROUHAN, F. RUMSEY, A. TROIA, J. VRBA & M. J. M. CHRISTENHUSZ:

2017. *European Red List of Lycopods and Ferns*. Brussels. Belgium. IUCN. iv + 59 pp.

GODEFROID, S., C. PIAZZA, G. ROSSI, S. BUORD, A.-D. STEVENS, R. AGURAIUJA, C. COWELL, C. W. WEEKLEY, G. VOGG, J. M. IRIONDO, I. JOHNSON, B. DIXON, D. GORDON, S. MAGNANON, B. VALENTIN, K. BJUREKE, R. KOOPMAN, M. VICENS, M. VIREVAIRE & T. VANDERBORGHT:

2011. How successful are plant species reintroductions? *Biological Conservation*, **144**: 672-682.

GORBUNOV, Y. N., D. S. DZYBOV, Z. E. KUZMIN & I. A. SMIRNOV:

2008. *Methodological Recommendations for Botanic Gardens on the Reintroduction of Rare and threatened Plants*. Botanic Gardens Conservation International.

GOUVEIA, L., J. A. CARVALHO, F. FERNANDES & C. LOBO (Eds.):

2010. *50 Anos – Jardim Botânico da Madeira Eng.º Rui Vieira*. Direção Regional de Florestas. Secretaria Regional do Ambiente e dos Recursos Naturais. 133 pp.

HANSEN, A. & P. SUNDING:

1993. Flora of Macaronesia. Checklist of vascular plants. 4th revised edition, *Sommerfeltia*, **17**: 295 pp.

IUCN/SSC:

2013. *Guidelines for Reintroductions and Other Conservation Translocations*. Version 1.0. Gland, Switzerland: IUCN Species Survival Commission, viiii + 57 pp.

JARDIM, R. & D. FRANCISCO:

2000. *Flora Endémica da Madeira*. Múchia Publicações.

JARDIM, R., F. FERNANDES & J. CARVALHO:

2006. *Fauna e Flora da Madeira. Espécies endémicas ameaçadas: vertebrados e flora vascular.* Governo Regional da Madeira. DRAamb.

2008. As cem espécies ameaçadas prioritárias em termos de gestão na região europeia biogeográfica da Macaronésia. In: *Top 100: As cem espécies ameaçadas prioritárias em termos de gestão na região europeia biogeográfica da Macaronésia* (eds.: J. L. Martin, M. Arechavaleta, P. A. V. Borges & B. Faria). Consejería de Médio Ambiente y Ordenación Territorial, Gobieno de Canarias, pp. 161-365.

JARDIM, R. & M. M. SEQUEIRA:

2008. As plantas vasculares (Pteridophyta e Spermatophyta) dos arquipélagos da Madeira e das Selvagens. In: *Listagem dos fungos, flora e fauna terrestre dos arquipélagos da Madeira e Selvagens* (eds.: P. A. V. Borges, C. Abreu, A. M. F. Aguiar, P. Carvalho, R. Jardim, I. Melo, P. Oliveira, C. Sérgio, A. R. M. Serrano & P. Vieira), pp. 157-208. Direcção Regional do Ambiente da Madeira and Universidade dos Açores. Funchal and Angra do Heroísmo.

JONHSON, A. T. & A. SMITH H:

1931. *Plant Names Simplified: Their Pronunciation, Derivation & Meaning*. 2nd revised edition. The Hamlyn Publishing Group Ltd. Sixth impression, 1972. Landsman Bookshop LTD. Buckenhill, Bromyard, Herefordshire.

LESOUËF, J.-Y.:

1999. *Mission de sauvetage des éléments les plus menacés de la flore de Madère et réintroduction de deux espèces*. Conservatoire Botanique National de Brest. 12 p.

LOWE, R. T.:

1851. *Primitiae et Novitiae Faunae et Flore Maderae et Portus Sancti*. London: John Van Voorst, Paternoster Row. pp. 523.

MANTON, I, J. D. LOVIS, G. VIDA & M. GIBBY:

1987. Cytology of the fern flora of Madeira. In: *Botany series. Bulletin of the British Museum (Natural History)*, Vol. **15**, pp. 1-388.

MARYAM, A., R. TARIQ, S. CHUADHARY, R. AZMAT, S. JAVED & S. KHANAM:

2014. A review: Role of tissue culture (*in-vitro*) techniques in the conservation of rare and endangered species. *Pacific Journal of Life Sciences*, Vol. **2** (2): 93-103.

MASCHINSKI, J., K. E. HASKINS (Eds.):

2012. *Plant Reintroduction in a Changing Climate: Promises and Perils*. Island Press, Washington DC.

MASCHINSKI, J. & M. A. ALBRECHT:

2017. Center for plant conservation's best practice guidelines for the reintroduction of rare plants. *Plant diversity*, **39**: 390-395.

MURASHIGE, T. & F. SKOOG:

1962. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiol Plantarum*, **15**: 473-497.

PRESS, J. R. & M. J. SHORT (Eds.):

1994. *Flora of Madeira*. HMSO. The Natural History Museum. London.

ROUX, J. P.:

2005. The fern genus *Polystichum* (Pteropsida: Dryopteridaceae) in Macaronesia). *Systematics and Biodiversity*, **2** (2): 147-161.

SÁNCHEZ-PINTO, L., M. L. RODRÍGUEZ, S. RODRÍGUEZ, K. MARTÍN, A. CABRERA & M. C. MARRERO:

2005. Spermatophyta. In: *Lista preliminar de especies silvestres de Cabo Verde (hongos, plantas y animales terrestres* (eds.: M. Arechavaleta, N. Zurita, M. C. Marrero & J. L. Martín). Consejería de Medio Ambiente y Ordenación Territorial, Gobierno de Canarias, pp. 40-57.

SILVA, L., M. MOURA, H. SCHAEFER, F. RUMSEY & E. F. DIAS:
2010. List of vascular plants (Tracheobionta). In: *A list of the terrestrial and marine biota from the Azores* (eds.: P. A. V. Borges, A. Costa, R. Cunha, R. Gabriel, V. Gonçalves, A. F. Martins, I. Melo, M. Parente, P. Raposeiro, P. Rodrigues, R. S. Santos, L. Silva, P. Vieira & V. Vieira), pp. 117-146, Princípia, Cascais, 432 pp.

SOORAE, P. S. (Ed.):
2013. *Global Re-introduction Perspectives: 2013. Further case studies from around the globe*. Gland, Switzerland: IUCN/ SSC Re-introduction Specialist Group and Abu Dhabi, UAE: Environment Agency-Abu Dhabi. xiv + 282 pp.

VIEIRA, R.:
1992. Flora da Madeira. *O Interesse das Plantas Endémicas Macaronésicas*. Colecção Natureza e Paisagem, no. 11. Serviço Nacional de Parques, Reservas e Conservação da Natureza. Lisboa.