Musschia isambertoi a Desertas Islands endemism on the edge of extinction

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INTRODUCTION

The Madeiran endemic genus *Musschia* Dum. includes three endemic species *M. aurea* (L.f.) Dum., *M. wollastonii* Lowe and *M. isambertoi* M. Seq., R. Jardim, M. Silva & L. Carvalho. This last taxon was described in 2007 from 2 locations in Deserta Grande. The largest known population corresponds to the holoptype locality, "Portugal, Madeira: Ilhas Desertas, Deserta Grande, perto da Fajã Pequena, Porto das Moças, 16-V-2006, M. Silva, L. Carvalho, C. Viveiros & P. Gouveia 868 (MA 751556)" (Fig 1).

"Porto das Moças" population is known to be the largest by far (possibly less than 5 individuals were ever found outside the range of this location). Images taken in 2006 show age complex population with several fully flowering/fructifying plants and other mature but not flowering plants along with many younger and seedlings (Fig. 3 and 4). Nevertheless the species was classified by Menezes de Sequeira and collaborators (2007) as Critically Endangered (CR, C2a(i,ii); D), mainly due to "the scarce number of populations and the reduced occupancy and occurrence (…) and also due to the grazing effects through the introduction of goats". Feral goats in Deserta Grande are a very well-known threat to local flora (Fig. 2). *M. isambertoi* is a monocarpic plant with greenish flowers that are pollinated by endemic lizards (although other pollinators could probably occur) (Fig. 3).

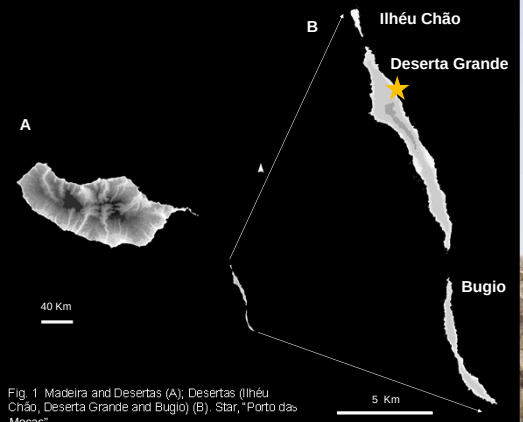

Fig. 1 Madeira and Desertas (A); Desertas (Ilhéu Chão, Deserta Grande and Bugio) (B). Star, "Porto das Moças"

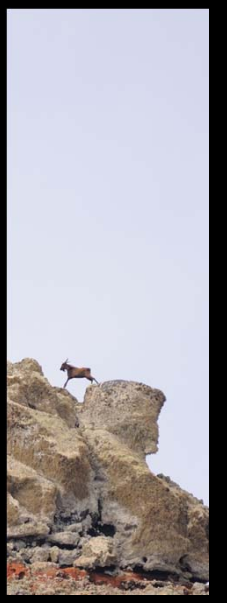

Fig. 2 Deserta Grande, June 2018. Feral goat, above Porto das Moças

MATERIAL AND METHODS

Field work (June 2018) took place in "Porto das Moças", Deserta Grande, the *locus typicus* where in 2006 many plants were detected with a high variation of age/size between individuals, and with both fully flowering and non-flowering adult plants as well as seedlings, corresponding to a healthy population (Fig. 3 and 4). The present study included counting of individuals, age/size and (non-destructive) leaf sampling (for molecular studies), as well as a floristic inventory.

Total genomic DNA was extracted from silica gel dried leaves using the method of Pich & Shubert (1993) with minor modifications. DNA solutions were quantified by ImageJ program and diluted to 10 ng μL^{-1}. ISSR assays were performed as described before (Gouveia *et al.* 2014), using three primers from the University of British Colombia, UBC 888, UBC 889 and UBC 890. Amplification products were resolved by electrophoresis on 1.5% agarose gels, in 1x TAE buffer containing 0.5 $\mu g\ mL^{-1}$ ethidium bromide and photographed under ultraviolet light (DigiGenius, Syngene, UK).

RESULTS AND DISCUSSION

Ecological data and direct herbivory evidence suggest that perennial plants are subjected to elimination during the short summer season by the feral goat population.

The non-chasmophyte habitats, i.e. mesic conditions of *M. isambertoi* habitat, observed in Porto das Moças, corresponds to approximately 1500 square meters. Floristic/synecological data suggest a shift to annual/ biennial plant taxa domination, and direct evidence of herbivory was detected (Fig. 5 and 6). Table 1 resumes the floristic data, it is clear (1) the absence of phanerophytes, (2) almost absence of chamaephytes (except chasmophytes), (3) the reduced number of hemicryptopytes, (4) the domination of therophytes. These results and the fact that the scarce chamaephytes present are all represented by young plants support the view of a permanent factor that prevents successional dynamics.

Only 10 *M. isambertoi* plants were detected, all corresponding to young plants no more than 15 cm high, all presenting the same size and number of leaves (4 to 8), no adult plants were seen and no remains of adult plants were also found (Fig. 7 and 8).

The threat factor that can be observed is the presence of feral goats (Fig. 2) and evidence of herbivory was observed directly and by community shift to annual plant domination (Fig. 6).

ISSR results clearly show that all plants sampled (all plants present) are absolutely genetically similar (Fig. 9 and 10). Therefore presumably being the offspring of one plant. *Musschia isambertoi* being monocarpic the parent plant is not expected to survive seed production, however no remains of any adult plants were found during field work.

The presence of young plants (1 year?) is coherent with a pioneer plant community dominated by annuals where perennials (hemicriptophytes or chamaephytes) are grazed yearly during summer drought. Therefore, both age and lack of genetic diversity support a continuous seed recruitment on a limited seed bank of *M. isambertoi* originated from one single parental plant.

Results clearly suggest that *Musschia isambertoi* is on the edge of extinction. Urgent conservation measures should include fresh leaf material collection for tissue culture, moving some seedlings into conservation gardens, an urgent fencing of the population and finally the elimination of feral goats from the Deserta Grande, a process long initiated (1996, LIFE95 NAT/P/000125, 383,467.00 €) but stopped due to wrong conservation policies of the former Services of the Natural Park of Madeira.

Figure 7. Detail of a young (1 year?, pl. 4) plant of *Musschia isambertoi*, Porto das Moças (Deserta Grande), June 2018.

Figure 8. Detail of a young (1 year?, pl. 6) plant of *Musschia isambertoi*, Porto das Moças (Deserta Grande), June 2018.

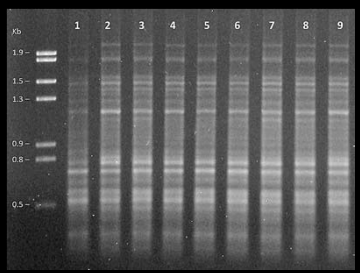

Figure 9. ISSR patterns detected in *M. isambertoi* using primer UBC 889. Lanes 1 to 9 corresponds to plant number.

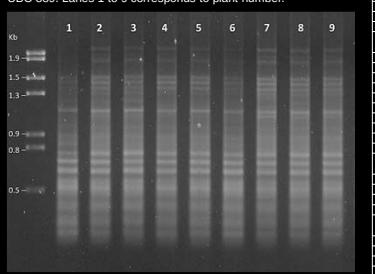

Figure 10. ISSR patterns detected in *M. isambertoi* using primer UBC 888. Lanes 1 to 9 corresponds to plant number.

Fig. 3 Desertas, Porto das Moças, May 2006. Complex population with young and adult plants, and a flowering individual of *Musschia isambertoi*. Details of flower and lizard pollination.

Fig. 4 Desertas, Porto das Moças, May 2006. Complex population with young and adult plants of *Musschia isambertoi*. Co-dominance of chamaephytes, *Phyllis nobla* and *Tolpis succulenta* (taken above Fig. 3).

Fig. 5 Desertas, Porto das Moças, June 2018. General aspect of the site, it is clear the lack of plant cover.

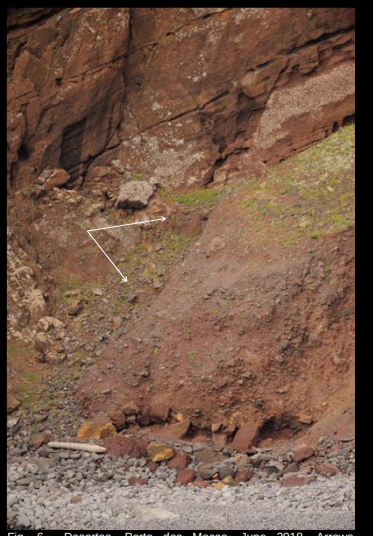

Fig. 6 Desertas, Porto das Moças, June 2018. Arrows correspond to the limits of Fig. 3 taken in 2006. It is noticeable the lack of perennials and the absence of any adult *M. isambertoi*.

Table 1. Floristic diversity in the plant community of *M. isambertoi*, Porto das Moças. June 2018. Raunkiaer- Raunkiaer Life Forms (T – Therophyte, H – Hemicriptophyte, C – Chamaephyte). Colonization Status (END – Madeiran archipelago endemic, END D – Desertas Endemism, N – Native, MAC – Macaronesian endemic, I – Introduced). Br.-Bl. Index - Braun-Blanquet Abundance-Dominance Index

Taxa	Raunkiaer	Native status	Br.-Bl. Index
Aeonium glandulosum (Aiton) Webb & Berthel.	H	END	(+)
Aichryson villosum (Aiton) Webb & Berthel.	T	END	(r)
Ammi majus L.	T	N	2b
Andryala glandulosa Lam.	T	END	(+)
Brachypodium distachyum (L.) P. Beauv.	T	N	+
Briza maxima L.	T	N	+
Bromus madritensis L.	T	N	+
Calendula maderensis DC.	H	END	1
Cotula australis (Sieber ex Spreng.) Hook.f.	T	I	+
Chenopodium murale L.	T	N	+
Crithmum maritimum L.	C	N	+
Fumaria bastardii Boreau	T	N	1
Holcus lanatus L.	T	N	R
Lagurus ovatus L.	T	N	+
Matthiola maderensis Lowe	H	END	1
Melilotus indicus (L.) All.	T	N	1
Mercurialis ambigua L.f.	T	N	+
Micromeria thymoides (Sol. ex Lowe) Webb & Berthel.	C	END	(+)
Monizia edulis Lowe	C	END	.
Musschia aurea Dumort.	C	END	(+)
Musschia isambertoi M.Seq., R.Jardim, Magda Silva & L.Carvalho	C	END-D	R
Nicotiana tabacum L.	T?	I	R
Papaver somniferum L. subsp. somniferum	T	I	1
Parietaria debilis G. Forst.	T	N	+
Phyllis nobla L.	C	MAC	+
Plantago coronopus L.	H	N	1
Plantago maderensis Decne.	C	MAC	R
Polycarpon tetraphyllum (L.) L. subsp. tetraphyllum	T	N	+
Polypogon maritimus Willd.	T	N	+
Reseda luteola L.	T	NP	R
Senecio incrassatus Lowe	T	MAC	R
Rumex bucephalophorus L. subsp. canariensis (Steinh.) Rech.f.	T	MAC	+
Silene gallica L.	T	N	1
Silene uniflora Roth	C	N	R
Sinapidendron sempervivifolium Menezes	C	END-D	R
Sonchus oleraceus L.	T	NP	+
Sonchus ustulatus Lowe subsp. imbricatus (Lowe) R.Jardim & M. Seq.	C	END	(r)
Stachys ocymastrum (L.) Briq.	T	N	2a
Tolpis succulenta (Dryand.) Lowe	C	MAC	+
Trifolium scabrum L.	T	N	+
Urospermum picroides (L.) Scop. ex F.W. Schmidt	T	N	+
Urtica portosanctana Press	T	END	+
Wahlenbergia lobelioides (L.f.) Link subsp. lobelioides	T	MAC	r

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