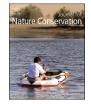


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# Population decline in the Critically Endangered *Musschia isambertoi* (Campanulaceae) endemic to Desertas Islands (Madeira Archipelago) calls for urgent conservation management

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M. Menezes de Sequeira<sup>a, b, \*</sup>, R. Jardim<sup>a</sup>, M. Gouveia<sup>a, b</sup>, C.A. Góis-Marques<sup>a, c, d</sup>, W.M. M. Eddie<sup>e</sup>

<sup>a</sup> Madeira Botanical Group (GBM), Faculty of Life Sciences, University of Madeira, Campus da Penteada, 9000-390, Funchal, Portugal

<sup>b</sup> InBio, Research Network in Biodiversity and Evolutionary Biology, CIBIO-Azores, Pólo dos Açores, 9501-801, Ponta Delgada, Azores, Portugal

<sup>c</sup> Departamento de Geologia, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016, Lisboa, Portugal

<sup>d</sup> Instituto Dom Luiz (IDL), Laboratório Associado, Universidade de Lisboa, Campo Grande, 1749-016, Lisboa, Portugal

e 20 (2F2) Gosford Place, Edinburgh, EH6 4BH, Scotland, United Kingdom

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## ABSTRACT

Macaronesian islands display a high degree of plant endemicity and correspond to some of the most threatened biodiversity hotspots due to several human driven impacts such as habitat loss, invasive species, overexploitation or climate change. The conservation status of the largest of the two known population of Musschia isambertoi M. Seq., R. Jardim, Magda Silva and L. Carvalho (Campanulaceae) was assessed recently. Field work was done in June 2018 in "Porto das Moças" Deserta Grande (Madeira Archipelago, Portugal), the locus typicus, where in 2006 close to 30 plants were detected with a high variation of age/size between individuals comprising both fully flowering and non-flowering adult plants as well as seedlings, and thus corresponding to a healthy population. This study assesses population size, age and plant size, and genetic, as well as floristic diversity. The total area of the site corresponds to approximately 1500 square meters. Ten individuals were detected, all corresponding to young seedlings not exceeding 15 cm high, and exhibiting approximately the same size and numbers of leaves (4-8). No adult plants or remains of adult plants were seen. Ecological data and direct herbivory evidence suggest that perennial plants are subjected to elimination during the short summer by the feral goat population. Molecular data based on ISSR markers show that the nine sampled individuals (out of ten individuals found) are genetically identical, possibly resulting from one parental plant. Results clearly suggest that Musschia isambertoi is very close to extinction. Urgent conservation measures are imperative and should include the immediate fencing of the population followed by the elimination of feral goats from Deserta Grande. The elimination of feral goats was initiated in 1996 (LIFE95 NAT/P/000125, 383,467.00 €) but stopped due to inappropriate conservation policies of the former Services of the Natural Park of Madeira.

#### 1. Introduction

The Macaronesian archipelagos, due to their high degree of plant diversity and endemicity are included in the Mediterranean Basin hotspot (Myers, Mittermeier, Mittermeier, Fonseca, & Kent, 2000), being also among the most threatened due to several human driven impacts (e. g. Caujapé-Castells et al., 2010; Médail & Quézel, 1997). Historical documents refer to an early and rapid destruction of the vegetation in the Madeira Archipelago, which comprises Madeira Island, Porto Santo Island and Desertas Islands. The main drivers of the elimination of the pristine forest were: wheat and other cereals cultivation (early 15th century), sugar cane plantation and production (15th and 16th centuries), followed by the expansion of agriculture and tree felling for wood exploitation (Menezes de Sequeira, Jardim, & Capelo, 2007; Moore, 2009, 2010). Simultaneously, the introduction of non-native herbivores such as goats (*Capra hircus*), pigs (*Sus scrofa*) and rabbits (*Oryctolagus cuniculus*) (e.g. for the introduction of rabbits in Porto Santo Island see Rocha et al. (2017) and references therein), was the main driver of landscape change from as early as the 15th century.

Invasive alien species are the most significant drivers of species

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<sup>\*</sup> Corresponding author at: Madeira Botanical Group (GBM), Faculty of Life Sciences, University of Madeira, Campus da Penteada, 9000-390, Funchal, Portugal. *E-mail address:* sequeira@uma.pt (M. Menezes de Sequeira).

extinctions on islands (e.g. Elton, 1958, for a revision see Reaser et al., 2007, and references therein). Impacts of alien species include not only direct effects, but also changes in biogeochemical cycles, and several authors refer to indirect effects (e.g. Traveset & Richardson, 2006, on mutualism-disruption, spreading of diseases, etc.). Herbivory in insular ecosystems has long been recognized as destructive on the flora and vegetation (Mueller-Dombois & Spatz, 1975 or Caujapé-Castells et al., 2010; Courchamp, Chapuis, & Pascal, 2003 and Keitt et al., 2011).

The effects of alien herbivores (namely feral goats) on island plant biodiversity as the main drivers of species extinctions are well known (e. g. Brooks et al., 2002; Donlan, Tershy, & Croll, 2002; Campbell & Donlan, 2005; Courchamp et al., 2003; Garzón-Machado et al., 2010; Keitt et al., 2011; Mooney & Cleland, 2001; Traveset & Richardson, 2006; Vitousek, Mooney, Lubchencho, & Melillo, 1997; Wilcove, Rothstein, Dubow, Phillips, & Losos, 1998).

In the Madeira Archipelago several introduced herbivores such as rabbits, goats and pigs became feral (Menezes de Sequeira, Jardim, & Capelo, 2007). The arrival of these invasive species occurred in the 15th century soon after or simultaneously with the first human settlers. In the Portuguese settlements that occurred in most Atlantic islands, the same common behaviour was followed, and included the introduction of mammals, later used as food supply during long ship voyages (e.g. Chynoweth, Litton, Lepczyk, Hess, & Cordell, 2013; Grove, 1995). According to the accounts given by Diogo Gomes, goats were introduced in the Selvagens possibly before 1460 by order of the Infante D. Henrique (Pereira, 1899; Zino & Biscoito, 1994). In any case the introduction of goats in the Desertas was even earlier, due to the proximity to Madeira Island and to the references, by Gomes Eanes de Azurara in his "Chronica do Descobrimento de Guiné" (1452-1453) and Valentim Fernandes in the 1508 manuscript "Das Ilhas do Mar Oceano" (Baião, 1940), to the orders given by the Infante D. Henrique (1394–1460) to introduce goats in the Desertas Islands. The presence of goats in large numbers in the Desertas Islands in the 16th century is referred to by Nicols Nichols (1583) and by Arditi (1567), the latter author even describes the presence of shepherds, and apart from goats, other animals such as cows, sheep, mules, etc.

The genus *Capra* is restricted to the old World, possibly originating in Central Asia (Pilgrim, 1947) with a rapid radiation during the Plio-Pleistocene (Hartl, Burger, Willing, & Suchentrunk, 1990; Manceau, Després, Bouvet, & Taberlet, 1999; Pilgrim, 1947). The domestic goat (*Capra hircus*) is included on the list of the 100 worst invading species on the planet (Global Invasive Species Database, 2020; Lowe, Browne, Boudjelas, & De Poorter, 2000).

The Madeiran endemic genus *Musschia* Dum. includes three species *M. aurea* (L.f.) Dum., *M. wollastonii* Lowe and *M. isambertoi* M. Seq., R. Jardim, Magda Silva & L. Carvalho (Fig. 1d). This last taxon was described in 2007 (Menezes de Sequeira, Jardim, Silva, & Carvalho, 2007) from two localities in Deserta Grande (Madeira Archipelago). *Musschia isambertoi* is a monocarpic plant with greenish flowers (Fig. 2b) that are visited, and possibly pollinated, by endemic lizards (Fig. 2c). A recent study using molecular markers clearly supports the segregation of *M. isambertoi* (Menezes, Romeiras, Menezes de Sequeira, & Moura, 2018).

The largest known population of *Musschia isambertoi* corresponds to the holotype locality, "Portugal, Madeira: Ilhas Desertas, Deserta Grande, perto da Fajā Pequena, Porto das Moças, 16-V-2006, M. Silva, L. Carvalho, C. Viveiros and P. Gouveia 868 (MA 751556)" (Fig. 1a, b, c). "Porto das Moças" (possibly less than five individuals were ever found outside the range of this location in one single location). Images taken in 2006 show an age-complex population with several fully flowering/ fructifying plants and other mature plants not in flower along with many younger individuals and seedlings (Fig. 2a, d). Nevertheless, the species was classified by Menezes de Sequeira, Jardim, Silva and Carvalho (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".

In order to re-evaluate the conservation status of Musschia isambertoi,

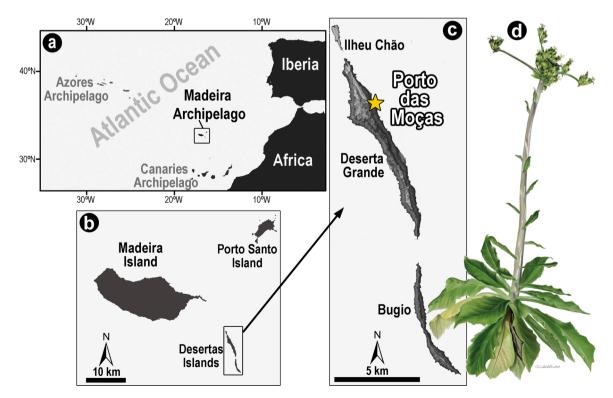


Fig. 1. Geographical location of Madeira Archipelago and Desertas Islands. **a**, Location of Madeira archipelago. **b**, Madeira archipelago with the location of Desertas Islands. **c**, Desertas Islands with the indication of the Porto das Moças (star), the *locus typicus* of *Musschia isambertoi*. **d**, Illustration of a flowering *M. isambertoi* by Juan Castillo (compare with Fig. 2a).



Fig. 2. Porto das Moças (Deserta Grande) in May 2006. a, Flowering individual of *Musschia isambertoi*. b, Details of flower. c, Lizard (*Lacerta dugesii mauli*) pollination. d, complex population with young and adult plants of *Musschia isambertoi*. Co-dominance of chamaephytes, *Phyllis nobla* and *Tolpis succulenta*.

this study assesses population dimension, age, plant size and genetic diversity, as well as floristic diversity, therefore evaluating possible changes in species dominance, life form dominance change or percentage of endemics versus native non-endemic or non-native plant taxa.

## 2. Material and methods

Field work (7 June 2018) took place at "Porto das Moças", Deserta Grande island, the *locus typicus* where on May 16th, 2006 many plants were detected with a high variation of age/size between individuals (Fig. 2a, d).

## 2.1. Site description

The Madeira archipelago comprises three groups of islands, Porto Santo (about 18 Mya old), Madeira and Desertas (>7 Mya old), that share a common geological origin (see Ramalho et al., 2015 and references therein). To the southeast the Madeira archipelago is continued by the Desertas sub-archipelago composed by three small islands. The northernmost Ilhéu Chão (ca.  $0.4 \text{ km}^2$ ,  $1.6 \text{ km} \log 0.5 \text{ km}$  wide), is the smallest rising only 98 m above sea level. The largest, Deserta Grande (ca.  $10 \text{ km}^2$ ,  $11.7 \text{ km} \log and 1.9 \text{ km}$  wide), has a maximum elevation of 479 m above sea level. Finally, Bugio (ca.  $3 \text{ km}^2$ ,  $7.5 \text{ km} \log 0.7 \text{ km}$ 

wide), the southern island, and the most orographically complex, has a maximum elevation of 388 m above sea level. According to the model proposed for Madeira Natural Potential Vegetation (Capelo, Menezes de Sequeira, Jardim, & Costa, 2004) Desertas islands should have possessed a microforest of Madeiran oleaster (Olea maderensis) (0-200 m a.s.l.), a microforest dominated by Sideroxylon marmulano (200-300 m a.s.l.) and a mediterranean laurel forest dominated by Apollonias barbujana (300-800 m a.s.l.). The Desertas' flora includes many known floristic elements of these two communities (Table 1, based on Jardim & Menezes de Sequeira, 2008), nowadays only growing in crevices of deep ravines. No native mammal terrestrial herbivores were present on the archipelago of Madeira and, as described for Madeira island and Porto Santo, deforestation rate must have been fast and complete in the Desertas, due to the early introduction of several herbivores (a common practice in the discovery see Grove, 1995 and references above) and the reported use for agriculture (Silva & Menezes, 1946; Menezes de Sequeira, Jardim, & Capelo, 2007). Rabbits were eradicated from the Desertas (Bell, 2001) but goats are still present. Musschia isambertoi was found growing near to seashore, at the base of the unstable cliff of Porto das Moças, within a colluvial deposit mainly composed of boulders with poor soil development.

#### Table 1

List of Native and Madeiran Endemic or Macaronesian Endemic phanerophytes or chamaephytes taxa reported for the Desertas Islands based on Jardim and Menezes de Sequeira (2008). Native Status: N, native not endemic; END, Madeira archipelago or Desertas Endemic (\*); MAC, Macaronesian Endemic. Raunkiaer Life Form: P, Phanerophytes; C, Chamaephytes.

	_		
Family	Taxa	Native	Raunkiaer
		status	Life Form
Apiaceae	Crithmum maritimum L.	Ν	С
Apiaceae	Monizia edulis Lowe	END	C
Asteraceae	Argyranthemum haematomma	END	Р
	(Lowe) Lowe		-
Asteraceae	Artemisia argentea L' Hér.	END	Р
Asteraceae	Carlina salicifolia (L.f.) Cav.	MAC	C
Asteraceae	Helichrysum melaleucum Rchb. in	END	C
risteruccue	Holl subsp. melaleucum	LIND	G
Asteraceae	Phagnalon lowei DC.	END	С
Asteraceae	Phagnalon saxatile (L.) Cass.	N	C
Asteraceae	Sonchus ustulatus Lowe subsp.	END	C
Asteraceae	imbricatus (Lowe) R.Jardim & M.	LIND	C
	Seq.		
Asteraceae	Tolpis succulenta (Dryand.) Lowe	MAC	С
	Echium nervosum Dryand.	END	Р
Boraginaceae	-		P C
Brassicaceae	Crambe fruticosa L.f. subsp.	END	L.
D	fruticosa	PND*	0
Brassicaceae	Sinapidendron sempervivifolium	END*	С
a 1	Menezes		
Campanulaceae	Musschia aurea (L.f.) Dumort.	END	C
Campanulaceae	Musschia isambertoi M. Seq., R.	END*	С
	Jardim, Magda Silva & L.		
	Carvalho		
Caryophyllaceae	Silene uniflora Roth	N	С
Celastraceae	Maytenus umbellata (R. Br.) Mabb.	END	Р
Chenopodiaceae	Suaeda vera Forssk. ex J,F. Gmel	N	Р
Chenopodiaceae	Bassia tomentosa (Lowe) Maire &	N	С
	Weiller		
Convolvulaceae	Convolvulus massonii F. Dietr.	END	Р
Crassulaceae	Aeonium glutinosum (Aiton) Webb	END	С
	and Berthel.		
Crassulaceae	Sedum nudum Aiton	END	С
Ephedraceae	Ephedra fragilis Desf.	N	Р
Euphorbiaceae	Euphorbia piscatoria Aiton	END	Р
Fabaceae	Lotus argyrodes R.P. Murray	END	С
Fabaceae	Teline paivae (Lowe) P.E. Gibbs &	END	Р
	Dingwall		
Globulariaceae	Globularia salicina Lam.	MAC	Р
Lamiaceae	Micromeria thymoides (Sol. ex	END	С
	Lowe) Webb and Berthel. subsp.		
	thymoides		
Lamiaceae	Sideritis candicans Aiton var.	END	С
	crassifolia Lowe		
Lamiaceae	Teucrium heterophyllum L'Hér.	END	С
	subsp. heterophyllum		
Lauraceae	Apollonias barbujana (Cav.)	MAC	Р
	Bornm.		
Liliaceae	Asparagus umbellatus Link subsp.	END	С
	lowei (Kunth) Valdés		-
Liliaceae	Semele androgyna (L.) Kunth	MAC	Р
Myrsinaceae	Heberdenia excelsa (Aiton) Banks	MAC	Р
	ex DC.		-
Oleaceae	Jasminum odoratissimum L.	MAC	Р
Oleaceae	Olea maderensis (Lowe) Rivas	END	P
Orcaccac	Mart. & del Arco	LIND	1
Diantoginggoog	Plantago maderensis Decne.	MAC	С
Plantaginaceae Rosaceae	Chamaemeles coriacea Lindl.	END	P
			P
Rosaceae	Rubus ulmifolius Schott Phyllis nobla L.	N MAC	P
Rubiaceae Rubiaceae		MAC	
Rublaceae	Rubia fruticosa Aiton subsp.	MAC	С
Dute eee -	fruticosa Buta abalanancia I	N	C
Rutaceae	Ruta chalepensis L.	N	C
Sapotaceae	Sideroxylon mirmulans R. Br.	END	Р

2.2. Study species (based on Menezes de Sequeira, Jardim, Silva, & Carvalho, 2007)

Tall rosetted monocarpic unbranched plant, up to 2 m. Young leaves

(1st year) up to 6 cm, adult leaves up to 40 cm, with indistinct petiole, herbaceous, shallowly undulated, with bi-serrate margins, becoming senescent but attached to the stout stem, plants producing 4–5 leaves yearly, flowering after 4–5 years growth. Inflorescence up to 150 cm long, unbranched except for the terminal part, bracteate (bracts gradually shorter towards the top); flowers densely crowded, green, yellowish with reddish-brown tones. Sepals green with reddish brown apex and veins, sometimes yellowish towards the base. Corolla bright green, yellowish towards the base, lobes narrowly triangular-lanceolate, acuminate, pollinated by lizards (*Lacerta dugesii mauli*, = *Teira dugesii mauli*).

## 2.3. Population age structure

*Musschia isambertoi* population age structure in 2006 and 2018 was compared assuming, the following age categories, seedlings (plants with no adult leaves, i.e. large leaves ca. 7 cm), young adult plants (with adult leaves but no evidence of senescent leaves), mature adult plants (with adult leaves and clear evidence of senescent leaves), flowering plants. The 2006 plants were counted using several pictures taken during the original visit (Suppl. Mat. Images 1–4), seedlings must have been underestimated due to the fact that they are small and grow amid other plants and between rocks, whereas the 2018 data correspond to direct observation.

## 2.4. Floristic diversity

A list of all vascular plant taxa was prepared based on plants identified in the field, collected, and further identified based on Press and Short (1994). Nomenclature and native status follow Jardim and Menezes de Sequeira (2008). Plant specimens corresponding to all collected plant taxa are kept at the herbarium of the University of Madeira (UMad). Abundance-dominance for each plant taxa was registered using the coefficients defined by Braun-Blanquet (1964), percent cover was obtained following van der Maarel (1979).

## 2.5. Genetic diversity

To characterize genetic diversity within the population, total genomic DNA was extracted from silica gel dried leaves using the method of Pich and Schubert (1993) with minor modifications. The extracted DNA was evaluated by loading 1  $\mu$ L on a 0.8 % agarose gel containing ethidium bromide. Gels were digitally acquired under UV light (DigiGenius, Syngene, UK). Densitometric analysis of gel lanes were performed through ImageJ software (http://rsbweb.nih.gov/ij/) after which DNA solutions were diluted to 10 ng  $\mu$ L<sup>-1</sup>.

Inter Simple Sequence Repeats (ISSR) assays were performed as described before (Gouveia, Gonçalves, Benedito, & Menezes de Sequeira, 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<sup>-1</sup> ethidium bromide and photographed under ultraviolet light (DigiGenius, Syngene, UK).

## 3. Results and discussion

Only ten *Musschia isambertoi* plants were found, corresponding to a coeval population of young plants less than 15 cm high, with the number of leaves ranging from 4 to 8 (Fig. 3d, e), these plants can be considered as seedlings or, at most, young plants in their second year of growth. Neither adult plants nor remains of adult plants were found in the study area (Fig. 3b, c). Based on the images taken in 2006 and on field work in 2018 the population status can be compared for both years by the number of seedlings, adult plants (with or without senescent leaves) and flowering plants as shown in Fig. 4. These results stress the lack of age structure in 2018 population.

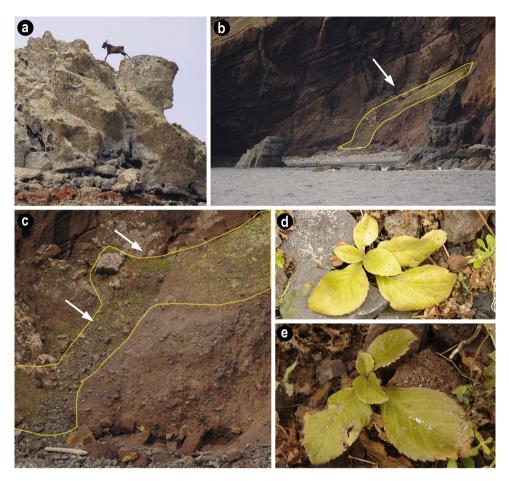
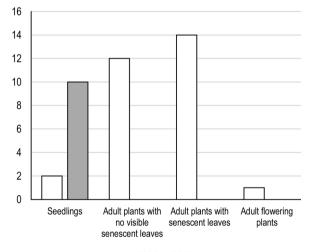


Fig. 3. Porto das Moças (Deserta Grande) in June 2018. **a**, Feral goat, above Porto das Moças. **b**, General aspect of the site, it is clear the lack of plant cover and **c**, correspond to the limits of Fig. 2 taken in 2006 (arrows) where it is noticeable the lack of perennials and the absence of any adult *Musschia isambertoi* (line corresponds to the study area). **d** and **e**, examples of seedlings of *M. isambertoi* found in Porto das Moças.



□2006 □2018

Fig. 4. Age structure of *Musschia isambertoi* population at Porto das Moças, in 2006 and 2018.

Although Menezes de Sequeira, Jardim, Silva and Carvalho (2007) refer to a second population based on herbarium data (MADM 485, 1992) this population was not found (in a 2016 survey conducted by the IFCN, for the study later published by Menezes et al. (2018)), it is presumably extirpated, in any case we were unable to observe the location during 2018 field work and therefore we have calculated both the EOO  $(8 \text{ km}^2)$  and AOO  $(8 \text{ km}^2)$ , if based in  $100 \times 100 \text{ m}$  then AOO  $0.03 \text{ km}^2)$  assuming two populations still occur, therefore without any changes regarding the evaluation by Menezes de Sequeira, Jardim, Silva and Carvalho (2007).

ISSR results clearly show that all plants sampled (9 out of 10, destructive sampling was avoided) are genetically identical (Fig. 5a, b) and therefore are presumably the offspring of one self-pollinated plant. *Musschia isambertoi* being an example of a monocarpic plant with insular woodiness, mature plants are not expected to survive seed production. However, no remains of any adult plants were found during fieldwork, possibly indicating that several years have passed since the last seed setting event.

Ecological data and direct herbivory evidence suggest that perennial plants are subjected to elimination during the short summer season by the feral goat population. Musschia isambertoi habitat is a nonchasmophyte habitat that corresponds to the mesic conditions observed in Porto das Moças. Table 2 summarises the floristic/synecological data (see also Fig. 3b, c), which clearly shows: (1) the absence of phanerophytes, (2) near absence of chamaephytes (except chasmophytes; see Table 2), (3) the reduced number of hemicryptophytes, (4) the prevalence of therophytes. From a total of 24 chamaephytes and 19 phanerophytes referred to the Desertas Islands, as natives or endemics (Table 1), only eight chamaephytes were detected (Table 2), corresponding to a very low frequency and cover (less than 5 %, Fig. 6). These results and the fact that the low number of chamaephytes observed corresponded to young plants support the occurrence of a limiting factor blocking succession. Cubas et al. (2019) refer to the high palatability of island endemic plants, which could also be the case for Musschia plants

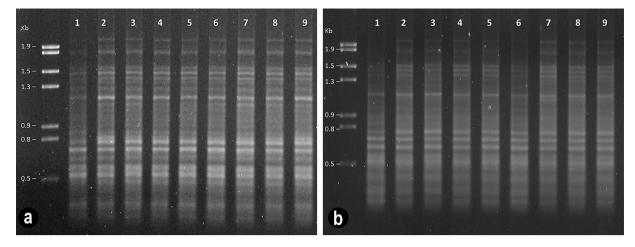


Fig. 5. Inter Simple Sequence Repeats (ISSR) patterns detected in Musschia isambertoi using **a**, primer UBC 889 and **b**, primer UBC 888 (B). Lanes 1 to 9 corresponds to plant number.

(all species of Campanulaceae are readily eaten by goats in Greece – Eddie, W.M.M., personal communication). However, in heavily grazed and degraded ecosystems with Mediterranean bioclimate (Mesquita, Capelo, & Sousa, 2004), annual plants tend to complete their life cycle before July, therefore the only summer green plants would be chamaephytes and phanerophytes, even if they correspond to young plants, as is the case for *M. isambertoi* and another Desertas islands endemic, *Sinapidendron sempervivifolium* also observed in the studied area.

Feral goats in Deserta Grande are a very well-known threat to local flora and their control was the aim of several conservation projects (LIFE95 NAT/P/000125, see also Silva & Menezes, 1946; Bell, 2001 and IFCN, 2017), in fact the actual landscape corresponds to highly disturbed plant communities with almost no tree or shrub cover.

During field work, goats (a small herd, one male and two females) were seen in cliffs above the site at approximately 1 km (Fig. 3a). Although accessibility from the cliffs seems to be complex, reports of goats at the site and evidence of herbivory observed directly (damage on *Tolpis succulenta, Sinapidendron sempervivifolium* and *Musschia aurea*, Suppl. Mat. Image 5), in addition to a predominance of seedlings of perennial plants including *Musschia isambertoi* (Fig. 3d, e), support goats as the main drivers of ecological shift and plant diversity loss.

Donlan et al. (2002) refer to a differential effect of herbivory on perennial/endemic plant taxa, which is the case for *Musschia isambertoi*. However, the preference could simply be the result of phenology *versus* foraging by goats throughout the year, and in fact early October/November rains result in a fast production of biomass by therophytes, which, together with hemicryptophytes, must constitute the most important part of the goats' diet, until late spring. However, these earlystage (pioneer) plant communities result in extreme food shortage during late spring, summer and early autumn. Therefore, chamaephytes and in fact all perennial plants must be under high herbivore pressure during, at least, late spring to autumn. Palatability could in this case play a secondary role, at least during drought months (June to September), the only plants keeping green leaves and stems being endemic perennials.

The early introduction of goats in the Desertas Islands (Arditi, 1567; Baião, 1940; Azurara, 1452-1453; Nicols Nichols, 1583), certainly led to a massive landscape change such as the one reported for Porto Santo (historical data for Porto Santo support the presence of a primeval forest similar to the one observed in Madeira). Deforestation led to loss of the primeval forest cover possibly at a very fast rate as described for all Madeira archipelago islands (Menezes de Sequeira, Jardim, & Capelo, 2007).

In the Canary Islands Garzón-Machado et al. (2010) tested the effects of herbivory by several feral mammals (barbary sheep, *Ammotragus*  *lervia*, goat, and European rabbit (*Oryctolagus cuniculus*) and concluded that herbivory was responsible for the extremely low diversity patterns found in key ecosystems.

Several authors correlate the evolution of secondary woodiness in island endemics as related to the absence of herbivory (e.g. Carlquist, 1974; Dulin & Kirchoff, 2010). The detection of, exclusively, Musschia isambertoi young plants (presumably seedlings) is coherent with a pioneer plant community dominated by annuals where perennials (hemicryptophytes or chamaephytes) are grazed yearly during summer drought. The shift towards a therophyte dominated community can also be confirmed by the observation of Fig. 2d (2006), where dominant plants are all chamaephytes such as Phyllis nobla, Sonchus ustulatus subsp. imbricatus and Tolpis succulenta. The 2006 plant community corresponds to a local variant of Crithmo-Helichrysetum oboconicae (see Capelo et al., 2004), very dynamic due to the slope and proximity to the sea. However, in the upper part of the study area (milder slope, less rock cover and deeper soil) could correspond to the Euphorbietum piscatoriae shrub community. In the 2018 observations it is clear that domination shifted towards annual plants such as Ammi majus, Calendula maderensis, Fumaria bastardii, Melilotus indicus, Papaver somniferum subsp. somniferum, Plantago coronopus and Silene gallica (Table 2, Fig. 6). Similar communities can be found in the extreme east of Madeira at Ponta de S. Lourenço corresponding to areas highly disturbed and grazed. This shift to annual plant dominance, as well as a reduction in cover and diversity of endemics (Fig. 7), is a common pattern in plant communities affected by herbivores, namely goats (e.g. Mueller-Dombois & Spatz, 1975).

Therefore, both the ecological shift, and the age and lack of genetic diversity of *Musschia isambertoi*, support continuous seed recruitment on a limited seed bank that probably originated from a single parental plant. Other perennial chamaephytes formerly dominant were also observed as small young plants resembling the observations on *Musschia isambertoi*. The feeding on seedlings is reported by <u>Mueller-Dombois and Spatz (1975)</u> and explains the lack of adult flowering chamaephytes (or even phanerophytes).

Results clearly suggest that *Musschia isambertoi*, on the edge of extinction in 2007, was almost effectively extinct in nature during our field work that led to this current publication. In any case imperative conservation measures are needed and should include: 1. The immediate fencing of the population site; 2. Fresh leaf material collection for tissue culture; 3. The elimination of feral goats from the Deserta Grande; 4. Translocation of some seedlings into conservation gardens. The translocation of *M. isambertoi* to both the Bugio and ilhéu Chão islands is admissible, although there are no records, actual or historical, of the presence of *M. isambertoi* in these islets.

Koutsovoulou, Daws, and Thanos (2014) report the successful

#### Table 2

Floristic diversity in the plant community of *Musschia isambertoi*, Porto das Moças. June 2018. Collector Number, MS-M. Sequeira collector number (plants kept in the UMad herbarium); Raunkiaer-Raunkiaer Life Forms (T – Therophyte, H – Hemicryptophyte, C – Chamaephyte). Native Status (END – Madeiran archipelago endemic, END\* – Desertas Endemism, N – Native, NP – probable native, MAC – Macaronesian endemic, I – Introduced). Br.-Bl. Index - Braun-Blanquet Abundance-Dominance Index.

Blanquet Abundance Dominian	ee maem			
Таха	Collector Number	Raunkiaer	Native status	BrBl. Index
Ammi majus L.	MS8575	Т	Ν	2b
Stachys ocymastrum (L.) Brig.	MS8594	T	N	20 2a
Calendula maderensis DC.	MS8579	Ĥ	END	1
Fumaria bastardii Boreau	MS8593	Т	Ν	1
Matthiola maderensis Lowe	_	н	END	1
Melilotus indicus (L.) All.	MS8577	Т	N	1
Papaver somniferum L. subsp.	MS8600	Т	I	1
somniferum	1100000	1	-	-
Plantago coronopus L.	MS8585	Н	Ν	1
Silene gallica L.	MS8574	Т	N	1
Brachypodium distachyum (L.)	MS8570	Т	N	+
P. Beauv.	1000070	1	IN IN	1
Briza maxima L.	MS8603	Т	N	+
Bromus madritensis L.	MS8597	Т	Ν	+
Cotula australis (Sieber ex	MS8594	Т	I	+
Spreng.) Hook.f.				
Chenopodium murale L.	MS8588	Т	N	+
Crithmum maritimum L.	MS8589	С	N	+
Holcus lanatus L.	MS8604	Т	Ν	r
Lagurus ovatus L.	MS8596	Т	Ν	+
Mercurialis ambigua L.f.	MS8583	Т	Ν	+
Monizia edulis Lowe	MS8602	C	END	r
Musschia isambertoi M.Seq., R.	MS8610	Č	END*	r
Jardim, Magda Silva & L. Carvalho		-		-
Nicotiana tabacum L.	_	Т	I	r
Parietaria debilis G. Forst.	MS8686	Т	Ν	+
Phyllis nobla L.	MS8601	С	MAC	+
Plantago maderensis Decne.	MS8590	C	MAC	r
Polycarpon tetraphyllum (L.) L.	MS8580	T	N	+
subsp. <i>tetraphyllum</i>	MCOFOO	T	N	
Polypogon maritimus Willd.	MS8582	Т	N	+
Reseda luteola L.	MS8573	Т	NP	r
Senecio incrassatus Lowe	MS8587	T	MAC	r
Rumex bucephalophorus L. subsp. canariensis (Steinh.) Rech.f.	MS8581	Т	MAC	+
Silene uniflora Roth	MS8571	С	Ν	r
Sinapidendron sempervivifolium Menezes	MS8608	С	END*	r
Sonchus oleraceus L.	MS8578	Т	NP	+
Tolpis succulenta (Dryand.)	MS8607	С	MAC	+
Lowe Trifolium scabrum L.	MS8576	т	N	
Urospermum picroides (L.)	MS8592	T	N	+ +
Scop. ex F.W. Schmidt				Ŧ
Urtica portosanctana Press	MS8572	Т	END	+
Wahlenbergia lobelioides (L.f.) Link subsp. lobelioides	MS8391	Т	MAC	r
Other plants observed as chas	monhytes, clo	ose to the same	oled area	
Aeonium glandulosum (Aiton)	MS8605	H	END	+
Webb & Berthel.				
Aichryson villosum (Aiton) Webb & Berthel.	MS8606	Т	END	r
Andryala glandulosa Lam.	MS8605	Т	END	+
Micromeria thymoides (Sol. ex	MS8595	С	END	+
Lowe) Webb & Berthel.				
Musschia aurea Dumort.	MS8609	С	END	+
Sonchus ustulatus Lowe subsp.	MS8611	С	END	r
<i>imbricatus</i> (Lowe) R.Jardim & M.Seq				

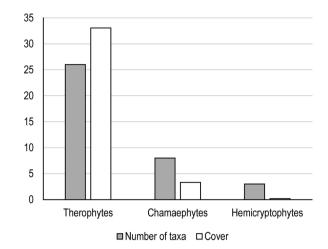
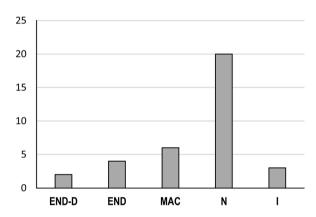


Fig. 6. Life form cover and number of plant taxa, *Musschia isambertoi* site at Porto das Moças, 2018.



**Fig. 7.** Native status of vascular plant taxa in the plant community of *Musschia isambertoi*, Porto das Moças. June 2018. END – Madeiran archipelago endemic, END D – Desertas Endemism, N – Native, MAC – Macaronesian endemic, I – Introduced.

germination of *Musschia aurea* seeds. In fact, this plant has long been cultivated in gardens (see Mesquita, Castel-Branco, & Menezes de Sequeira, 2020), so possibly *M. isambertoi* seeds will have a similar germination pattern. In the seedbank of the Madeira Botanical Garden (JBM) there are, presumably, still seeds obtained from one collection in July 2008 (by C. Nóbrega). Although several (14) plants were obtained (at JBM) in 2010 from seed collection in 2008, none flowered. These seeds (if viable) as well as those obtained from the fenced population or from plants obtained from tissue culture, could be used in the translocation process.

The elimination of feral goats from Deserta Grande is a process long initiated (1996, LIFE95 NAT/P/000125, 383,467.00  $\notin$ ) but stopped, in 2002, due to inappropriate conservation policies of the former Services of the Natural Park of Madeira. Recent reports (IFCN, 2017) on the number of goats eliminated by year can be used as an estimate both of densities and effort on the elimination. 140 goats were eliminated in 2014 but the number rose to 667 in 2016 before actions were suspended. Therefore, the number of goats must have quickly risen since 2002 until 2014/2016 when there was possibly a stabilization followed by an uncontrolled new increase.

Campbell and Donlan (2005) on their revision of goat eradication (see also Global Invasive Species Database, 2020) report that the eradication from large areas (>150 km<sup>2</sup>) is scarce (it would be the case of Madeira Island with close to 736 km<sup>2</sup>) but Deserta Grande ( $10 \text{ km}^2$ ) falls in the range of success reported by these authors (islands with less than

150 km<sup>2</sup>). The eradication of rabbits was completed with success from Deserta Grande (Bell, 2001), but the attempt to eradicate feral goats failed, although the project final report states the opposite (https://ec. europa.eu/environment/life/project/Projects/index.cfm?fuseaction=se arch.dspPage&n\_proj\_id=38). The failure to eradicate goats from Deserta Grande corresponds to the public opposition cases reported by Genovesi (2005) in his revision of the eradication of invasive species in Europe, including the eradication in Parco Naturale di Portofino in Italy. In fact recent attempts to mitigate the effect of feral goats in Deserta Grande by the IFCN have faced the opposition of some political and animal rights groups (e.g. https://funchalnoticias.net/2018/10/19/pa n-madeira-insurge-se-contra-o-abate-a-tiro-de-cabras-nas-ilhas-desertas / or https://www.radiocalheta.pt/manifestacao-contra-exterminio-dascabras-nas-desertas-surpreende-partida-do-rali). In Madeira, local laws allow for goat eradication; there was public funding (EU) but there was no public awareness of the procedures undertaken. Moreover, it was the permitted filming of the eradication programme (issued in the News in RTP, the Portuguese National Television, https://arquivos.rtp.pt/conteu dos/operacao-cabra/), sanctioned by the Services of the Natural Park of Madeira, that caused some public outcry due to the methods ("Judas goats" were being used). The suspension of the programme when only a few goats were left resulted in a massive resurgence of the goat population and therefore was a misuse of EC funding.

As referred by Keitt et al. (2011), the eradication of invasive vertebrates has proven to be a crucial and highly beneficial action on several islands. In the Madeira archipelago the eradication of both goats and rabbits from the Bugio islet (Fig. 1) was clearly a success. But in other islets, and in Porto Santo, these actions not only failed to achieve their objectives, and in the case of Porto Santo, the reintroduction of rabbits was actually promoted by the local authorities (IFCN, 2019).

Keitt et al. (2011) describe a long history of eradication attempts in islands starting in 1673 and report very high success rate in goat eradication (95.2 %) in a total of almost 170 attempts. As stated by Simberloff et al. (2013), biological invasions should be dealt with in a time frame from prevention to early detection and finally management (including eradication). These strategies result in distinct management costs that increase in time. For Deserta Grande the total cost of the actions, although unknown, can be estimated ranging from 500,000 to 1, 000,000 euros (including an EC LIFE project). The recent opening of both Deserta Grande and Selvagem Grande to tourism (Regional Legislative Decree 15/2017/M, https://dre.pt/application/conteudo/107477153) should raise increased concerns on the introduction of alien species.

Goat eradication has proved to be an important conservation tool leading to ecosystem restoration (e.g., Courchamp et al., 2003; Campbell & Donlan, 2005; Genovesi, 2005; Garzón-Machado et al., 2010; Keitt et al., 2011), in addition to clearly benefitting the biodiversity of islands (Campbell & Donlan, 2005; see Keitt et al., 2011, for a review on eradication programs; see Schweizer, Jones, & Holmes (2015), for a review of results on biodiversity).

As proposed by Campbell and Donlan (2005) or Genovesi (2005) and supported by Garzón-Machado et al. (2010) on the Canary Islands, the eradication of feral goats from island ecosystems should be a routine procedure. Our results strongly support eradication procedures being re-started in Deserta Grande and, the immediate fencing of the known largest population must be implemented immediately, in order to prevent herbivory on an already small, coeval and genetically homogenous population of the rarest Madeiran endemic plant species. These actions should be accompanied by vegetation monitoring in order to closely follow succession and early detection of any possible invasions by non-native plants. Due to the usual discontinuous and limited available funding, complete eradication must be implemented instead of limited control actions, as suggested by Cruz, Donlan, Campbell, and Carrion (2005). Campbell, Donlan, Cruz, and Carrion (2004) refer to the financial costs of incomplete eradication attempts. Although they refer to Pinta Island (Galapagos) and to monitoring actions that assumed (wrongly) that the goats had been eradicated, this case can be compared with the attempt to eradicate goats from Deserta Grande. Limited control actions have led *Musschia isambertoi* to the edge of extinction, or possible extinction, and the island's vegetation to a lower diversity state where pioneer communities dominate most of the landscape.

### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jnc.2021.125955.

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