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Genetic diversity and conservation of the Madeira Island (Portugal) endemic *Geranium maderense* Yeo (Geraniaceae)

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ABSTRACT

Geranium maderense is a perennial, monocarpic herb, up to 200 cm tall, producing very showy inflorescences, very rare in the wild, although cultivated throughout the world as an ornamental plant. *G. maderense* is protected under the EC habitats directive (Annex *B-II, IV) and Bern Convention (Annex I) only found, so far, in two cliff-associated sites, and classified as a Critically Endangered species (IUCN). Using unmanned aerial vehicle (UAV; drone) technology, two new populations were detected, and a new distribution map is presented. Inter-simple sequence repeat markers were used to detect genetic diversity in natural populations and garden grown plants. Genetic diversity estimates revealed retention of genetic variation and weak genetic structure in *G. maderense* populations. Cluster and principal component analyses formed coherent groups, indicating that genetic diversity in the studied wild populations of *G. maderense* is heterogeneously distributed. Our results provide crucial information for the preservation of natural populations of *G. maderense*, and allow for the revaluation, following IUCN criteria as Endangered (EN).

1. Introduction

Madeira archipelago (760 Km^2) is located in the NE Atlantic, 795 km SW of mainland Portugal (Fig. 1), comprising the volcanic islands of Madeira (734 Km^2, 1861 m a.s.l.), Porto Santo and its six islets (42 km^2, 517 m a.s.l.), and the uninhabited Desertas (14.8 km^2, 479 m a.s.l.). Madeira Island (< 7 Mya, Ramalho et al., 2015), and the Desertas Islands (5.07 Mya, Mata et al., 2013), belong to the same volcanic complex as Madeira, being considerably younger than Porto Santo (close to 14 Mya) (Ramalho et al., 2015; Mata et al., 2013).

In Macaronesia, three genera of Geraniaceae occur *Geranium* L., *Erodium* L'Hér. and *Pelargonium* L'Hér. (in this case corresponding to neophytes). *Geranium* is a cosmopolite genus that includes 307 species, being the largest genus of the Geraniaceae and highly diverse in the mountains around the Mediterranean Basin, Caucasus, Himalayas, Andes and Mexico (Aedo, 2023). *Geranium* does not occur in Cape Verde (Sánchez-Pinto et al., 2005) and most Azorean taxa are considered neophytes (Silva et al., 2010), by contrary all *Geranium* taxa in the Madeira and Canary archipelagos are native, including the Canary Islands endemic *Geranium reuteri* Aedo & Muñoz Garm. and the Madeira Island endemics *Geranium maderense* Yeo, *Geranium palmatum* Cav. and *Geranium yeoi* Aedo & Muñoz Garm. (Acebes Ginovés et al., 2009; Jardim & Menezes de Sequeira, 2008). Table 1 lists the Macaronesian Geraniaceae based on Sánchez-Pinto et al., (2005), Jardim and Menezes (2008), Acebes Ginovés et al. (2009) and Silva et al. (2010), with additions and corrections based on Aedo (2023).

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All these endemic species of *Geranium* are classified in the sect. *Ruberta* Dumort. [subg. *Robertium* (Picard) Rouy]. The species in this section are annual or perennial herbs with predominantly actinomorphic flowers, rounded petals, with bicarinate claw, *Geranium*-type pollen, fruit of carpel-projection type, with reticulate mericarps, non-twisted rostrum and cotyledons with entire margins. The sect. *Ruberta* in addition to the four species endemic to the Macaronesian islands, includes three species endemic to the Mediterranean Basin and three taxa widespread throughout the Mediterranean Basin, central Europe, Asia and Africa (Aedo, 2017).

Geranium maderense is a perennial, monocarpic herb, up to 200 cm tall, with persistent leaf petioles, producing very showy inflorescences. According to Short (1994) *G. maderense* is "Restricted to a small number of sires in the mountains of central and northern Madeira, where it is locally abundant. Reported growing on moist, north-facing rocks. Also cultivated in gardens as an ornamental". In fact, although widely cultivated (Madeira and elsewhere), it is a protected species according to the EC habitats directive (Annex *B-II, IV) and Bern Convention (Annex I) and according to (Fernandes, 2011) a very rare plant in the wild, with fewer than 50 individuals, found only in two localities associated with sea cliffs classified as a Critically Endangered species.

Polyploidy appears to be an important pathway for evolution in some groups of *Geranium*, like sect. *Ruberta* (Yeo, 1973). Yeo (1969, 1973) indicated that *G. maderense* has $2n = 68$, as well as *G. palmatum*, while *G. reuteri* and *G. yeoi* have $2n = 128$. Widler-Kiefer and Yeo (1987) reported that the hybrid of *G. maderense* × *G. cataractarum* formed ca. 16 univalents and ca. 18 bivalents at the first division of meiosis, which strongly suggests an allopolyploid origin for *G. maderense*, with *G. cataractarum* Coss., a species from southeastern Spain and Morocco belonging to sect. *Ruberta*, and an unknown species as parents.

Geranium maderense is a tall and spectacular garden plant first described in the second part of the XX century by Yeo (1969) therefore evading the attention of previous botanists namely R. T. Lowe. Although without naming the new species this plant was first reported by Costa in 1934 (Pereira, 1967) "…nas rochas escarpadas do litoral das Achadas da Cruz…" i.e. found in sea cliffs in the northwestern part of the island, as "nova espécie" [new species] described in Portuguese and photographed but not named. It was never collected again in Achadas da Cruz until 2021 (see Fig. 1 and below). Yeo's (1969) formal description is based on a specimen collected by Major C.H.C. Pickering, a British resident in Madeira. This collector located the plant in Levada do Moiro, a mountainous location (1200 m a.s.l.) far from the coastal cliffs, but not far from the garden of "Casa do Lombo Moiro". Therefore, of doubtful native status, it was possibly a garden escape plant, as are those that sometimes occur around old houses or ruins, a sign of the past cultivation of this spectacular plant. José Gonçalves da Costa (1899–1967) was an important collector of Madeiran plants and author of several new taxa for the islands of Madeira and Porto Santo, the first author to collect and also to introduce *G. maderense* as garden plant at the Funchal Seminar (Pereira, 1967). J.G. Costa left Madeira abruptly in 1948 for

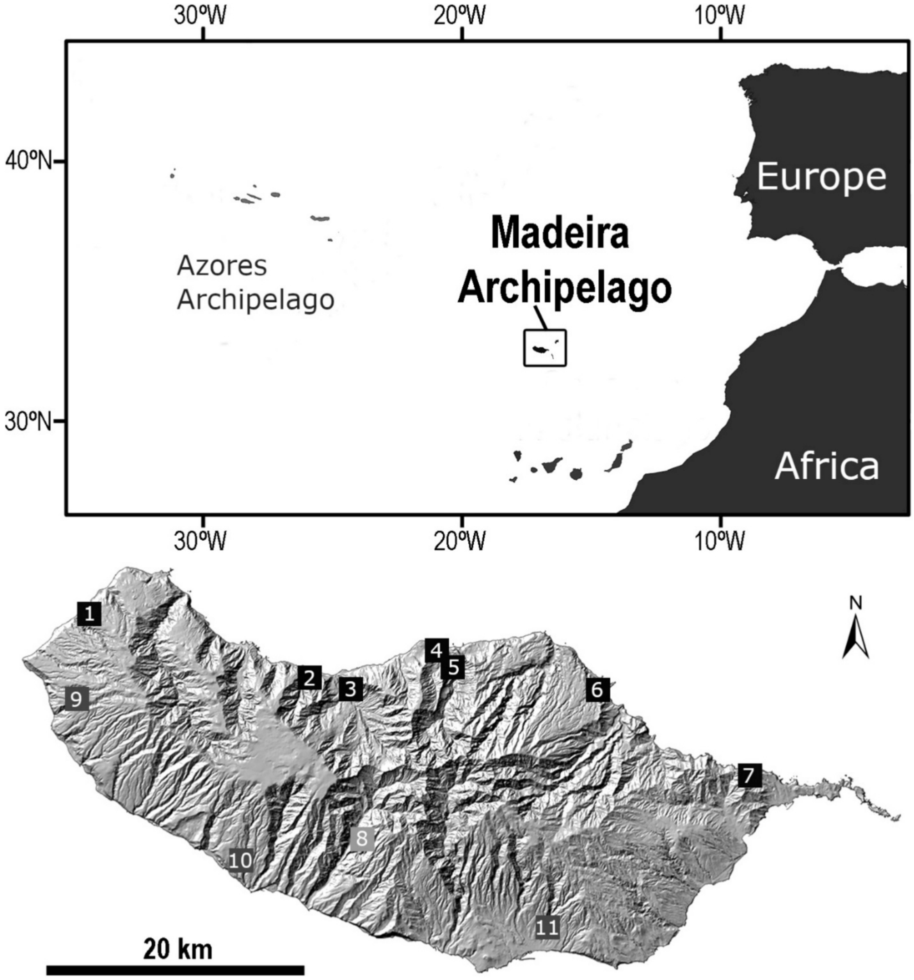

Fig. 1. Distribution map of *Geranium maderense*. Wild populations (1–8; 1, Porto Moniz: Achadas da Cruz; 2, São Vicente: Água d'Alto; 3, S. Vicente: M. Cardais; 4, S. Vicente: Pt. Delgada; 5, S. Vicente: Boaventura; 6, Santana: Pico da Boneca; 7, Porto da Cruz: Larano; 8, Ribeira Brava); sampled garden plants (9–11; 9, Calheta: Pt. Pargo; 10, Pt. Sol: Canhas; 11, Madeira Botanical Garden).

Table 1
Distribution and native status of Macaronesian Geraniaceae. Canary Islands (CAN), Azores archipelago (AZ), Madeira archipelago (MAD), Cape Verde archipelago (CV), native non endemic (N), native endemic (END), neophyte (I).

Taxa	Distribution and Native Status
Erodium aethiopicum (Lam.) Brumh. & Thell. [= *E. cicutarium* subsp. *bipinnatum*; = *E. cicutarium* subsp. *jacquinianum*	CAN(N)
Erodium botrys (Cav.) Bertol.	MAD(N), CAN(N)
Erodium brachycarpum (Godr.) Thell.	CAN(N)
Erodium chium (L.) Willd. [= *E. chium* ssp. *littoreum* (Léman) P.W. Ball]	MAD(N), CAN(N)
Erodium ciconium (L.) L'Hér.	CAN(N)
Erodium cicutarium (L.) L'Hér.	AZ(I) MAD(N), CAN(N)
Erodium laciniatum (Cav.) Willd. [= *E. hesperium* (Maire) Lindberg]	CAN(N)
Erodium malacoides (L.) L'Hér.	AZ(I), MAD(N), CAN(N), CV(I)
Erodium maritimum (L.) L'Hér.	CAN(N)
Erodium moschatum (L.) L'Hér.	AZ(I), MAD(N), CAN(N)
Erodium neuradifolium Delile ex Godr. [=*Erodium meynieri* Maire]	CAN(N)
Erodium salzmannii Delile	CAN(N)
Erodium touchyanum Delile ex Godr.	CAN(N)
Geranium dissectum L.	AZ(N), MAD(N), CAN(N)
Geranium lucidum L.	MAD(N)
Geranium maderense Yeo	MAD(END)
Geranium molle L.	AZ(N), MAD(N), CAN(N)
Geranium palmatum Cav.	MAD(END)
Geranium purpureum Vill.	AZ(N), MAD(N), CAN(N)
Geranium reuteri Aedo & Muñoz Garm.	CAN(END)
Geranium robertianum L.	AZ(N), MAD(N)
Geranium rotundifolium L.	AZ(N), MAD(N), CAN(N)
Geranium texanum (Trel.) A. Heller	AZ(I)
Geranium yeoi Aedo & Muñoz Garm.	MAD(END)
Pelargonium capitatum (L.) L'Hér. ex Aiton	CAN(I)
Pelargonium glutinosum (Jacq.) L'Hér.	MAD(I)
Pelargonium graveolens (Thunb.) L'Hér.	CAN(I)
Pelargonium inquinans (L.) L'Hér. ex Aiton	MAD(I), CAN(I)
Pelargonium odoratissimum (L.) L' Hér.	MAD(I)
Pelargonium peltatum (L.) L'Hér. ex Aiton	CAN(I)
Pelargonium quercifolium (L. f.) L'Hér.	CAN(I)
Pelargonium vitifolium (L.) L'Hér.	MAD(I)
Pelargonium zonale (L.) L'Hér. ex Aiton	CAN(I), CV(I)

Mozambique leaving unpublished taxa and some named but undescribed (see Jardim & Menezes de Sequeira, 2011). His collection of the putative new *Geranium* species possibly corresponds to one of his undescribed species. Eduardo Nunes Pereira (1887–1976) was also a catholic priest, and they certainly knew each other, as well as other catholic members deeply involved in the publication of Broteria (Menezes de Sequeira & Jesus, 2015).

Seeds of *G. maderense* can be easily obtained making it a common garden plant, also found in Botanic Gardens (a total of 87 gardens including 63 european, according to Botanic Gardens Conservation International, PlantSearch, https://tools.bgci.org/plant_search.php) and depicted on several websites. Being widely used in gardens, the presence of *G. maderense* in New Zealand, is probably a garden escape (Aedo, 2017).

Knowing the genetic diversity of rare and endemic species is crucial not only for a reliable assessment of biodiversity, but also for developing effective conservation strategies to preserve natural populations. Several approaches using molecular markers based on PCR techniques are used to assess genetic diversity. Among these techniques, inter-simple sequence repeat (ISSR) does not require prior knowledge of the DNA sequence and provides highly reproducible markers which has been widely used for the genetic diversity, population structure studies and species differentiation. Few investigations on genetic structure and diversity have been carried out with *Geranium* species except those from Iran (Bozchaloyi & Sheidai, 2018; Bozchaloyi, Sheidai, Keshavarzi, & Noormohammadi 2017a; 2017b) and Italy (Cardinali & Ceccarelli, 2024), but to our knowledge there are no similar studies using *Geranium* species from Macaronesia. The aims of this study were to: (1) present the native or probably native localities of *G. maderense*; (2) update population data to establish its conservation status; (3) evaluate the genetic diversity and structure through ISSR markers; and (4) identify the origin of plants grown in gardens.

2. Material and methods

2.1. Field work and sampling

The locations were surveyed based on the herbarium specimens studied (MADS, MADJ, UMad, C, MA, Annex I) and other locations cited by (Aedo, 2023), as well as historical references (Yeo, 1969; Pereira, 1967). Searches were performed by drone (2022, 2023) in several areas.

The number of adults, flowering or sterile plants was estimated by direct observation or by using drone-collected images.

Drone survey imagery was collected using the DJI Phantom 4 Pro – a consumer-grade drone featuring a 20-megapixel camera with one-inch RGB sensor. Manual flight operations allowed the pilot to assess diverse native cliff habitats while achieving the high-resolution necessary for the identification of small to medium-sized taxa. In most cases, the ground sampling distance (GSD) or pixel size of the images ranged from 1 – 1.5 mm. Each photograph captures approximately 40 sq. m of the cliff surface, and the GPS location was recorded in the file's metadata.

Identification and tagging of target species were conducted manually using Adobe Lightroom photo management software. A digital bounding box was placed around each plant of interest, this data was then collated within the user interface. Using this software, large image libraries can be classified, organized, filtered, and mapped to aid in future conservation efforts.

Field collections of *G. maderense* took place between 2019 and 2023 on all known locations (including those detected by drone if accessible).

At each location a leaf sample was collected from all accessible plants, climbing equipment was used for less accessible populations, also providing safety for plant collectors.

At least one voucher specimen was obtained from each population, and in most cases a voucher was obtained for each individual sampled. Vouchers were included in the R.T. Lowe Herbarium at the University of Madeira (UMad). Collecting numbers correspond to the populations and the letters to a single individual, i.e., MS10425 corresponds to the population of Porto da Cruz: Larano and "A-T" to 20 distinct individuals (Table 2). GPS coordinates were obtained for each individual using a Trimble TDC150 GPS.

Other leaf samples were obtained from the Botanical Garden of the University of Lisbon, the Botanical Garden of Madeira and two other private gardens in Madeira Island, in order to include samples of cultivated plant specimens. Leaf samples were also obtained from plants grown from seeds collected in the extirpated population of S. Vicente: Pt. Delgada (North of Madeira) stored in the Seed Bank of the Madeira Botanical Garden. Collected leaves were ground in liquid nitrogen and stored at −80 °C until use.

2.2. DNA extraction and ISSR amplification

Genomic DNA was extracted from fresh or frozen leaves using the NucleoSpin Plant II kit (Macherey-Nagel, GmbH & Co, KG) according to the instructions contained therein. DNA quality was observed by electrophoresis on 0.8 % agarose gel stained with ethidium bromide. The DNA concentration was measured by NanoDrop One Spectrophotometer (Thermo Fisher Scientific, Madison, USA) and normalized to 10 ng/μL in TE buffer.

Polymerase chain reaction (PCR) was carried out in a 10 μL reaction mixture containing 20 ng total DNA, 1 × MyTaq Plant PCR mix (Bioline, UK) and 0.3 μM oligo-deoxynucleotide primer (Gouveia et al., 2014).

Table 2
Geranium maderense population reference, location and population code, number of samples used in this study, number of plants counted and native status.

Reference	Location	Code	Samples	N° of Plants	Native Status
MS10552AD	1 Porto Moniz: Achadas da Cruz	PMAC	4	10	Wild, rediscovered
MS09916AD	2 S. Vicente: Água d'Alto	SVAA	4	10	Possible wild
MS09917AB	3 S. Vicente: M. Cardais	SVMC	2	2	Possible wild
MS10744AE	4 S. Vicente: Pt. Delgada	SVPD	5	0 (seedlings f. seeds)	Wild, extirpated
MS10550AB	5 S. Vicente: Boaventura	SVB	2	2	Possible Wild
MS12102AD	6 Santana: Pico da Boneca	SPB	4*	147	Wild, new population
MS10425AT	7 Porto da Cruz: Larano	PCL	20	112	Wild
Not accessed	8 Ribeira Brava	RB	0	16	Wild, new population
MS10551AD	9 Calheta: Pt. Pargo	CPP	4	n.a.	Garden plants
MS09918AB	10Pt. Sol: Canhas	PSC	2	n.a.	Garden plants
MS10713AD	11 Madeira Botanical Garden	MBG	4	n.a	Garden plants
MS10740AG	Lisbon University Botanical Garden	LUBG	6	n.a.	Garden plants

* The attempted to collect on the 543 m height ravines (almost vertical to the sea), where the main populations occur at approximately 440 m, would imply a rope descent (and vertical climb) of close to 100 m.

Initially, 14 primers were evaluated for amplification success, reproducibility of generated fragments and polymorphism levels. Following this study, six primers were selected for subsequent analyses (Table 3). The amplifications were carried out on a GeneAmp PCR System 2720 (Applied Biosystems, USA) as follows: initial denaturation at 94 °C for 3 min, 35 cycles of denaturation at 94 °C for 30 s, annealing temperature depending on the melting temperature of each primer (Table 3) for 30 s, polymerization at 72 °C for 2 min and a final extension step of 72 °C for 5 min. A negative control was included in each PCR reaction. PCR fragments were separated by electrophoresis on a 1.2 % agarose gel (10 cm long and 20 cm wide) stained with ethidium bromide in 1x TAE buffer. Loading buffer (3 µL) was added to each PCR reaction and 4 µL was loaded into each well. A molecular weight marker (Lambda DNA digested with *Eco*RI and *Hin*dIII) was loaded into the first and last well of each electrophoresis gel to provide a measure of fragment length. Gels were run at 87–88 V for 90 min. The gel documentation system-BL (Axygen®) was used to document gel images.

2.3. Data analysis

GeoCat (https://geocat.iucnredlist.org/) was used to estimate the occupancy and occurrence areas based on the georeferenced individuals.

In order to calculate distances between populations geographic coordinates were converted to metric coordinates UTM (using the converter of the "Centro de Informação geoespacial do Exército", available at https://www.igeoe.pt/coordenadas/trans.aspx). Linear distances were calculated by simple geometric inference.

Only distinct, unambiguous, and reproducible fragments were considered in replicated amplifications. The molecular weight of amplification products was calculated using TotalLab 1D software. For each ISSR reaction fragments in the size range 300 bp to 2.5 kb were scored and coded as present (1) or absent (0), to create a binary data matrix. The data matrix was used to calculate the percentage of polymorphic loci (% P), number of effective alleles (*Ne*), Nei's genetic diversity (*He*) and Shannon information index (*I*) as a measure of diversity using POPGENE software version 1.32 (Yeh et al., 1997). These parameters were used to estimate the genetic diversity for each population. POPGENE also provided genetic diversity within populations (*Hs*), total gene diversity (*Ht*), the coefficient of genetic differentiation among populations (*Gst*) and level of gene flow (*Nm*) between populations. Nei's unbiased genetic distance between population was calculated using the same program (Nei, 1978).

NTSYSpc (Numerical Taxonomy and Multivariate Analysis System) software version 2.2 W (Rohlf, 2023) was used for cluster analysis, using UPGMA (unweighted pair group method with arithmetic mean) and Dice coefficient to construct the dendrogram and perform a Principal Coordinate Analysis (PCoA).

Table 3
Details of ISSR primers used in this study, PCR annealing temperature (Tm), number of fragments per primer (NFP), number of polymorphic fragments (NPF) and percentage of polymorphic fragments (PPF). The IUB codes are as follows: Y = (C, T); B = (C, G, T); D = (A, G, T); H = (A, C, T); V = (A, C, G).

Name	Sequence 5′ → 3′	Tm (°C)	NFP	NPF	PPF
UBC807	$(AG)_8T$	52	9	6	67
UBC836	$(AG)_8YA$	51	11	7	64
UBC888	$BDB(CA)_7$	55	9	7	78
UBC889	$DBD(AC)_7$	55	14	12	86
UBC890	$HVH(GT)_7$	52	7	3	43
UBC891	$HVH(TG)_7$	55	6	4	67
Average			9.3	6.5	67.5
Total			56	39	

3. Results

3.1. Distribution, populations and number of individuals

Based on field collections and observations, as well as previously known populations (herbarium specimens and publications), an updated distribution map of *G. maderense* was produced. Fig. 1 shows the distribution of *G. maderense* populations and the collections carried out on Madeira Island. No plants were found at the *locus typicus* (Lombo do Mouro), a location that possibly corresponds to a secondary location resulting from the cultivation of previously collected seeds.

New data on the distribution of *G. maderense* includes two previously unknown populations detected using unmanned aerial vehicles (drone) technology (Fig. 1, 6 Santana: Pico da Boneca, MS12102A-D, and 8 Ribeira Brava, not collected), and one rediscovered wild population (1 Porto Moniz: Achadas da Cruz, MS10552A-D, Fig. 1). However, even using the drone it was not possible to locate the S. Vicente: Pt. Delgada population (although the population has been observed by Menezes de Sequeira in 2000).

Individual counts were obtained for all populations (Table 2). The population found at Ribeira Brava (Fig. 1. Reference 8) could not be sampled due to the steepness of the ravines where it was detected (Nyberg et al., 2024). This population seems to constitute an exception in terms of ecology (for being away from the sea).

Geranium maderense occurs in sea cliffs at 122 up to 497 m a.s.l., in shrubby plant communities with several other plants endemic to Madeira Island. The population found at Santana: Pico da Boneca (6) (Fig. 2) clearly corresponds to the best-preserved plant community and its floristic diversity closely matches the *Bystropogono punctati-Telinetum maderensis* Capelo, Costa, Lousã, Fontinha, Jardim, Sequeira & Rivas-Martínez (Costa et al., 2004; Mucina et al., 2016).

A total of 299 individuals were counted (fully flowering or sterile individuals), the new population at Santana: Pico da Boneca (6) (Table 2, Fig. 1 and Fig. 2) alone includes almost 49 % of all known individuals. If the doubtful wild populations (2, 3 and 5) were to be

Fig. 2. Drone image of the population (MS12102) of *Geranium maderense* at Pico da Boneca (Santana, Madeira Island). A. Several plants in full bloom (purple flowers) can be seen in the steep ravine. B. Detail of the plant community showing among others *G. maderense*, *Pericalis aurita*, *Helichrysum melaleucum*, *Sideritis candicans*, *Argyranthemum pinnatifidum* subsp. *succulentum*, *Hypericum glandulosum* and *Aeonium glutinosum*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

removed (see Table 2) the number of individuals would be reduced to 285.

Table 4 summarizes plant diversity, based on drone images, and their native status. Of the 30 plant taxa, 14 were endemic to the Madeira Island or archipelago and nine were Macaronesian endemics (shared with Canary Islands), five were non-endemic natives and only two detected taxa corresponded to non-native taxa.

3.2. Genetic diversity

A total of 57 *G. maderense* leaf samples were collected from seven wild populations (including three possibly wild and one extirpated wild) and four from garden collections (Madeira Botanical Garden, Lisbon University Botanical Garden and two private gardens) (Table 2).

Using six anchored ISSR primers, a total of 56 polymorphic DNA fragments were obtained, of which 39 (67.5 %) were polymorphic (Table 3). The UBC889 primer triggered the amplification of the largest number of polymorphic fragments (12), while the UBC890 primer the smallest (3), with 6.5 being the average of polymorphic fragments. Genetic variation estimates based on ISSR markers were relatively low regardless of population size; all populations presented a low percentage of polymorphic loci, with the highest values obtained for more isolated populations (PCL, Porto da Cruz: Larano and SPB, Santana: Pico da Boneca) (Table 5). At the species level, the percentage of polymorphic loci for populations in the wild was 67.9 % and 49.4 % for plants grown in gardens (Table 5). Considering populations in the wild, PCL population exhibited the highest levels of variability (PPL = 14.8 %, *He* = 0.044, *I* = 0.068), followed by the SPB population (P = 12.4, *He* = 0.034, *I* = 0.061), while for plants grown in gardens the highest estimates of genetic variation were obtained for Lisbon University Botanical Garden (LUBG, PPL = 19.8 %, *He* = 0.046, *I* = 0.076). Using all polymorphic loci, the effective number of alleles per locus in wild populations estimated *Ne* = 1.317 (ranged from 1.000 for SVB, S. Vicente: Boaventura, to 1.068 for PCL, Porto da Cruz: Larano), which was lower in garden-grown plants (*Ne* = 1.237). Assuming Hardy-Weinberg equilibrium, the mean Nei's gene diversity (*He*) was estimated to be 0.187 and 0.141 for wild populations and cultivated, respectively. The Shannon diversity index (*I*) as a measure of diversity within each population was low per population but revealed greater variability at species level (0.287 for wild populations and 0.218 for plants grown in gardens), suggesting high genetic differentiation between populations. Compared to the species level, genetic diversity parameters at the population level were lower (*He* = 0.023, *I* = 0.034) for populations in the wild, than for plants in gardens (*He* = 0.030, *I* = 0.046).

Genetic diversity within populations (Hs) showed low levels, but between populations (Ht) levels were much higher (Table 6). The

Table 4
Plant diversity associated to Pico da Boneca (Santana, Madeira Island) population (MS12102) of *Geranium maderense*.

Taxa	Native status
Aeonium glutinosum (Aiton) Webb & Berthel.	Macaronesian Endemic
Apollonias barbujana (Cav.) Bornm.	Macaronesian Endemic
Carlina salicifolia (L.f.) Cav.	Macaronesian Endemic
Dactylis smithii Link subsp. *hylodes* Parker	Macaronesian Endemic
Hypericum glandulosum Aiton	Macaronesian Endemic
Laurus novocanariensis Rivas Mart. & al.	Macaronesian Endemic
Phyllis nobla L.	Macaronesian Endemic
Semele androgyna (L.) Kunth	Macaronesian Endemic
Tolpis succulenta (Dryand.) Lowe	Macaronesian Endemic
Aeonium glandulosum (Aiton) Webb & Berthel.	Madeira Archipelago Endemic
Andryala glandulosa Lam. subsp. *glandulosa*	Madeira Archipelago Endemic
Convolvulus massonii F. Dietr.	Madeira Archipelago Endemic
Helichrysum melaleucum Rchb. subsp. *melaleucum*	Madeira Archipelago Endemic
Sideritis candicans Aiton	Madeira Archipelago Endemic
Sonchus ustulatus Lowe subsp. *ustulatus*	Madeira Archipelago Endemic
Argyranthemum pinnatifidum subsp. *succulentum* (Lowe) Humphries	Madeira Island Endemic
Bystropogon punctatus L'Hér.	Madeira Island Endemic
Erysimum bicolor (Hornem.) DC.	Madeira Island Endemic
Geranium maderense Yeo	Madeira Island Endemic
Pericallis aurita (L' Hér.) B. Nord.	Madeira Island Endemic
Sedum brissemoretii Raym.-Hamet	Madeira Island Endemic
Teline maderensis Webb & Berthel.	Madeira Island Endemic
Teucrium betonicum L'Hér.	Madeira Island Endemic
Adiantum reniforme L. subsp. *reniforme*	Native
Bituminaria bituminosa (L.) C.H. Stirt.	Native
Davallia canariensis (L.) Sm.	Native
Polypodium macaronesicum A.E. Bobrov	Native
Rubus ulmifolius Schott	Native
Ageratina adenophora (Spreng.) R.M. King & H. Rob.	Non-Native
Petroselinum crispum (Mill.) Fuss	Non-Native

Table 5
Estimates of genetic variation in *Geranium maderense* using ISSR markers. Number of polymorphic loci (NPL), percentage of polymorphic loci (PPL), effective number of alleles (Ne), Nei's gene diversity (*He*) and Shannon's information index (*I*). Standard deviation in parentheses. Population code as in Table 2.

Population	Sample sizes	NPL	PPL	Ne	*He*	*I*
In the wild						
SVAA	4	4	4.9	1.034 (0.158)	0.019 (0.088)	0.028 (0.128)
SVMC	2	2	2.5	1.017 (0.110)	0.010 (0.065)	0.015 (0.094)
PCL	20	12	14.8	1.068 (0.193)	0.044 (0.116)	0.068 (0.175)
SVB	2	0	0	1.000 (0.000)	0	0
PMAC	4	5	6.2	1.041 (0.178)	0.023 (0.096)	0.034 (0.139)
SVPD	5	4	4.9	1.042 (0.188)	0.022 (0.099)	0.032 (0.141)
SPB	4	10	12.4	1.065 (0.200)	0.040 (0.114)	0.061 (0.170)
Average		5.3	6.5	1.038 (0.147)	0.023 (0.083)	0.034 (0.121)
Species level	41	55	67.9	1.317 (0.373)	0.187 (0.195)	0.287 (0.272)
In gardens						
PSC	2	0	0	1.000 (0.000)	0	0
CPP	4	6	7.4	1.046 (0.177)	0.027 (0.101)	0.041 (0.148)
MBG	4	10	12.4	1.078 (0.234)	0.045 (0.128)	0.068 (0.187)
LUBG	6	16	19.8	1.068 (0.183)	0.046 (0.108)	0.076 (0.167)
Average		8	9.9	1.048 (0.149)	0.030 (0.084)	0.046 (0.126)
Species level	16	40	49.4	1.237 (0.344)	0.141 (0.189)	0.218 (0.267)

Table 6
Estimates of genetic diversity in *Geranium maderense*. Gene diversity within population (Hs), total gene diversity (Ht), genetic differentiation (Gst) and gene flow (Nm). Standard deviation in parentheses.

Population	Sample size	Hs	Ht	Gst	Nm
In the wild	41	0.023 (0.003)	0.194 (0.036)	0.883	0.066
In gardens	16	0.030 (0.003)	0.144 (0.037)	0.794	0.130

estimated coefficient of genetic differentiation (Gst) indicated that 88.3 % of the genetic variation was distributed among the wild populations, in broad agreement with the Shannon information index. The gene flow rate (Nm) was lower in wild populations (0.066) than in garden-grown plants (0.130), indicating differentiation among populations.

Nei's unbiased measures of genetic similarity among the populations studied ranged from 0.7187 (between PCL and SVMC) to 0.9318 (between LUBG and MBG), as shown in Table 7. Pairwise comparisons of wild populations revealed greater genetic dissimilarity for the Porto da Cruz: Larano (PCL) population. Considering the genetic similarity between wild populations and plants in gardens it was observed that the PMAC (Porto Moniz: Achadas da Cruz) population was closer to the CPP, Calheta: Pt. Pargo (0.9218) and PSC, Pt. Sol: Canhas (0.9171) populations.

Populations were clustered based on Nei's unbiased measures of genetic similarity using UPGMA (Fig. 3A). The clustering of the Botanical Gardens was expected since the provenance of the seeds could be common and ancient (see discussion). The S. Vicente populations also cluster very closely together, just as the eastern PCL and SPB populations also appear in a common western clade, suggesting a correlation between geographic origin and genetic diversity. To test for a possible correlation between location (geographical origin) and wild populations, a distance metric matrix between wild populations was calculated (Table 8) and a new matrix of Nei's unbiased genetic similarity measures (not shown) was calculated, excluding non-wild "populations" (garden grown plants) since their geographical origin obviously cannot be related to their genetic diversity.

Two dendrograms were then produced, the first (Fig. 3B) based on genetic similarity and the second based on metric distance (Fig. 3C), UPGMA was the clustering method for both. Although the correlation between the Mantel statistic Z correlation was low (0.259) the topography was similar except for the population of Porto Moniz: Achadas da Cruz (PMAC).

The ISSR data pattern for each individual was also used to produce a dendrogram based on the UPGMA and Dice coefficient (Fig. 4). The clustering is clear for each population (without exception), although the clustering does not show a clear geographic pattern. Both Botanical Gardens clustered together, again suggesting a common historical origin. Both Porto da Cruz: Larano and Santana: Pico da Boneca appear isolated and with clear segregation of plants.

3.3. IUCN threat category

Using the IUCN default cell width of 2 km, an Extent of Occurrence (EOO) of 290.760 km^2 and an Area of Occupancy (AOO) of 28.000 km^2 were estimated if all populations were to be considered. If the doubtful wild populations (see "Possible Wild" in Table 2) were excluded, both the EOO (287.892 km^2) and AOO (16.000 km2) drop significantly.

Meeting the criteria B1ab(i,ii,iii,iv,v) + 2ab(i,ii,iii,iv,v); C1 + 2a(i)

Table 7
Estimate values of Nei's unbiased measures of genetic similarity for G. maderense, based on Nei (1978).

	SVAA	SVMC	PCL	SVB	PMAC	SVPD	SPB	PSC	CPP	MBG	LUBG
SVAA	1	0.8808	0.7444	0.8410	0.7735	0.8384	0.8011	0.8160	0.7828	0.8303	0.7913
SVMC		1	0.7187	0.8329	0.8389	0.8343	0.8051	0.9074	0.8075	0.8533	0.8747
PCL			1	0.7214	0.7553	0.7875	0.8259	0.7214	0.7246	0.8269	0.7962
SVB				1	0.8011	0.8940	0.7514	0.7778	0.8720	0.8394	0.8297
PMAC					1	0.7911	0.7827	0.9171	0.9218	0.8771	0.8829
SVPD						1	0.7376	0.7690	0.8550	0.8395	0.8389
SPB							1	0.7588	0.7075	0.8641	0.8648
PSC								1	0.8949	0.8100	0.8216
CPP									1	0.8093	0.8129
MBG										1	0.9318
LUBG											1

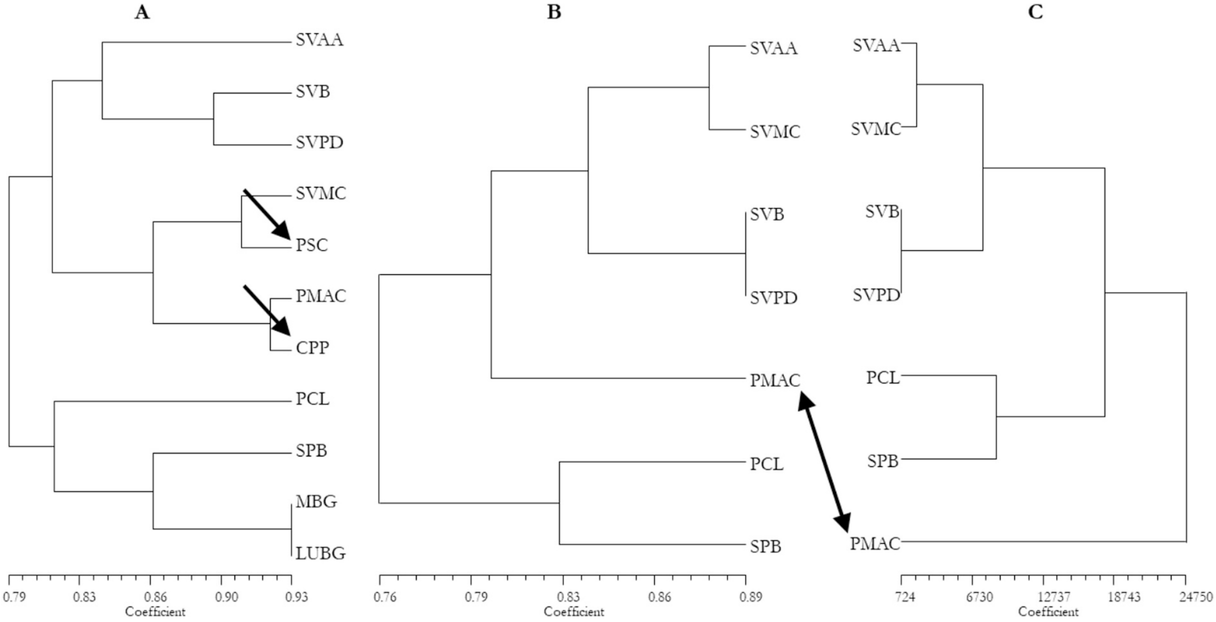

Fig. 3. A, Dendrogram of *Geranium maderense* populations using Nei's unbiased measures of genetic similarity using UPGMA, arrows show non-wild "populations" (garden grown plants). B, dendrogram using the same coefficient as in A, excluding non-wild "populations". C, Dendrogram based on metric distances between populations shown in Table 8. Arrow marks the non-coincidence of PMAC on dendrograms B and C.

Table 8
Geographic distance in meters between wild populations.

	SVAA	SVMC	SVB	PMAC	SVPD	PCL	SPB
SVAA	0						
SVMC	2040	0					
SVB	8571	6895	0				
PMAC	14,410	16,444	22,432	0			
SVPD	8344	6783	725	22,005	0		
PCL	26,307	24,310	18,447	40,657	18,999	0	
SPB	18,503	16,628	10,117	32,549	10,566	8820	0

G. maderense should be considered as Endangered (EN), instead of Critically Endangered (CR).

As defined by the IUCN (2012) the new populations found (Santana: Pico da Boneca and Ribeira Brava) cannot be considered as a real increase IUCN (2012) in the number of individuals, but rather the result of more detailed field work. Human usage (e.g. touristic trails) and overgrazing by feral rabbits are the main threats. Wildfires are also a major concern (the 2024 summer fires affected the Ribeira Brava population), as well as the genetic admixture due to the use as a garden plant possibly, resulting in outcrossing depression.

4. Discussion

Geranium maderense is restricted to sea ravines below 500 m a.s.l., mainly on the north side of Madeira Island, it is not a nemoral plant, contradicting the ecology and distribution given by some authors (Yeo, 1969; Short, 1994).

In this study two new populations of *G. maderense* were discovered. The first population in Santana (Pico da Boneca) was found within a well-preserved plant community and is currently the largest known population, although only four plants were sampled due to the steepness of the terrain. Considering that the plants were on nearly vertical sea cliffs, the use of a collecting drone would be a requirement to obtain a larger sample of this population (La Vigne et al., 2022). The second population, in Ribeira Brava, was detected after field work, post-flight of the drone, that is, during image analysis. The plants were on the crest of the Ribeira Brava valley, on one of the slopes, being the first population found in the southern part of Madeira Island and the first not to be associated with maritime ravines. A putative wider distribution can be assessed by using drones to search for new populations in remote, unexplored, and inaccessible locations. This unsampled population was affected by wildfires of the summer of 2024 and to date no specimens from this population have been collected, given the topography of the site. Plants of *G. maderense* were already in cultivation even before it was described by Yeo (1969) as stated by Pereira (1967) and supported by

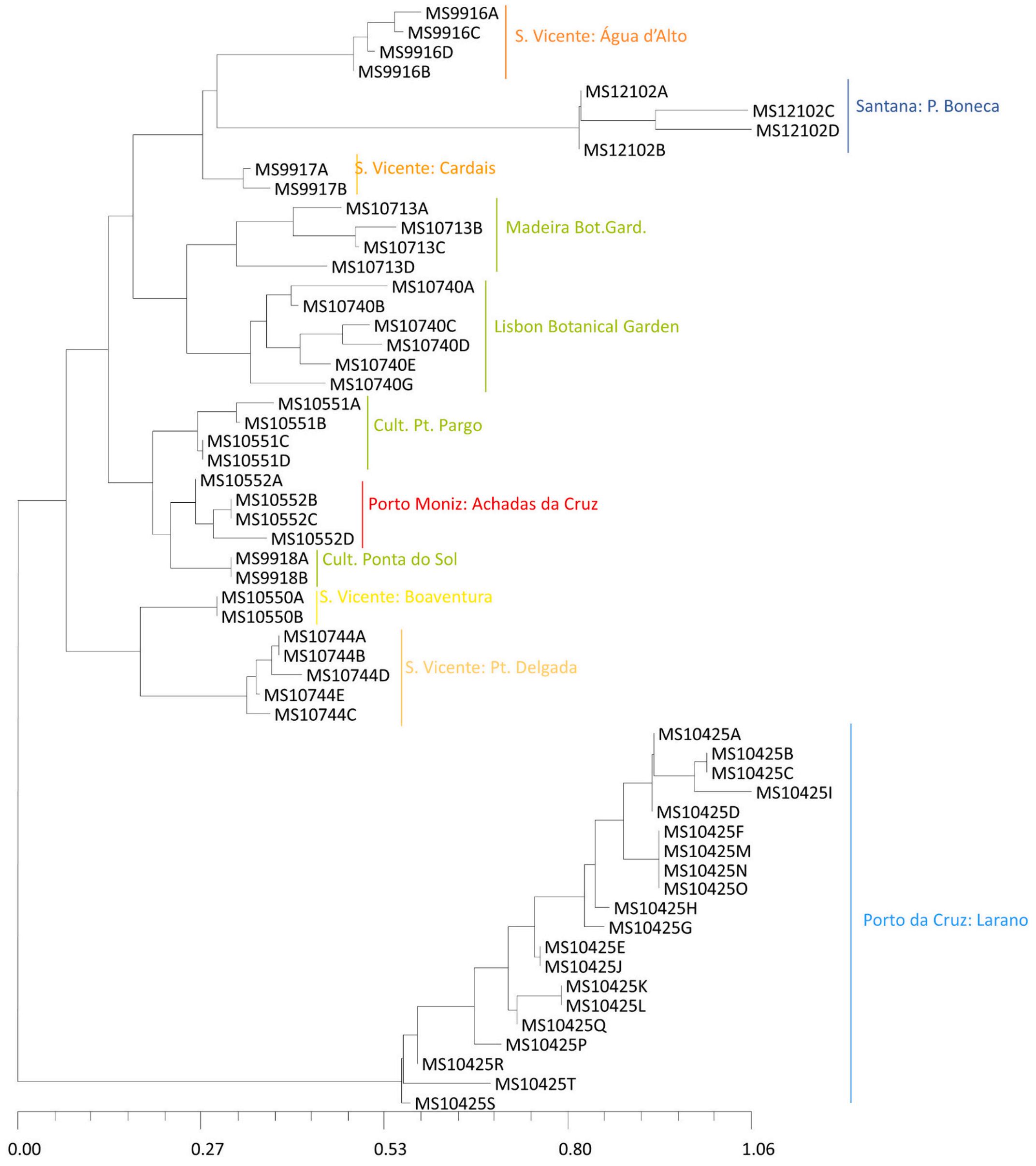

Fig. 4. Dendrogram of *Geranium maderense* populations using Nei coefficient and UPGMA. Colour codes correspond to East (blue and light blue), West (yellow to red), garden plants are shown in light green. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

labels of herbarium specimens at LISU (see Annex I). The western population of Porto Moniz: Achadas da Cruz corresponds to a rediscovery of the population recorded in 1934 and is undoubtedly the oldest location (see Annex I: specimens collected by Costa at MADS and MADM, and Pereira (1967). Using drones in Achadas da Cruz could greatly increase the number of plants counted, however, so far, it has been impossible to use the drone in this windy location.

The total number of plants detected corresponded to a six-fold increase in the number of individuals reported by Fernandes (2011) and its classification as CR was based on the underestimation of the number of individuals (less than 50 according to the author, 299 in this study). Certainly, small sterile plants may have escaped detection, but if included, the total number of plants in nature could be even larger. This study highlights the importance of combining herbarium specimens and label information, including locations (Eckert et al., 2024; Bebber et al., 2010; Nualart et al., 2017), detailed field work, as well as the use of drone surveys to detect rare plants in steep and complex landscapes (Nyberg et al., 2024; Wagner et al., 2024).

Molecular markers are useful tools for assessing genetic diversity in species. Genetic variability in both islands and archipelagos has been used to propose new species or subspecies through anagenetic or cladogenetic speciation (Takayama et al., 2015), the former common in oceanic islands (Stuessy et al., 2006) or to establish population genetic variation within and among populations a key factor for establishing conservation priorities and ecosystem management (Lopes et al., 2014; Gentili et al., 2015; Stuessy, 2020). A reduction in the level of genetic variation within and between populations is observed on oceanic islands, often attributed to the founder effect (Stuessy et al., 2014).

The population diversity parameters obtained in this study revealed low levels of genetic diversity within *G. maderense* populations compared to other *Geranium* taxa (Bozchaloyi & Sheidai, 2018; Bozchaloyi, Sheidai, Keshavarzi, & Noormohammadi 2017a; 2017b; Bozchaloyi, Sheidai, Keshavarzi, Noormohammadi, et al., 2017), using the same marker. Low genetic variability is common in island endemic species, regardless of the molecular marker used, e.g, *Armeria maderensis* (Piñeiro et al., 2009), *Picconia azorica* (Martins et al., 2013; Ferreira et al., 2011), *Aeonium nobile* and *A. davidbramwellii* (Harter et al., 2015), although it may not be widespread in island plant populations (García-Verdugo et al., 2015). On the other hand, genetic differentiation between wild populations was high (Gst = 0.883) and the level of gene flow was low (Nm = 0.066) suggesting the absence of gene flow between populations. Low genetic variation within populations of *G. maderense* and high genetic differentiation among populations may be related to founder effects considering the small number of populations and the reduced number of individuals within each population, in addition to their geographic isolation. *Geranium maderense* occurs in severely fragmented and widely separated populations, with an average distance of 16 km between them. These populations are found on cliffs, where the topography further enhances isolation, contributing to the observed low rates of gene flow between populations. Other factors affecting gene flow are linked to pollination and seed dispersion, as discussed below.

Geranium maderense produces a single inflorescence with thousands of insect-pollinated, protandrous flowers. Gueitonogamous pollination is likely the dominant mechanism, as seen in other *Geranium* species (Aedo, 2023). Pollinating insects tend to wander within the same inflorescence, visiting flowers at different states of maturity, which promotes selfing. Therefore, the pollination mechanisms support low gene flow between populations. Although no studies have been published on the specific pollination mechanisms, pollinators, or self-incompatibility of *G. maderense*, its long-lasting and gigantic inflorescence suggests that gueitonogamous pollination is advantageous when colonizing new habitats with scarce mating opportunities also described as a compensating adaptation for the lack of pollinators as part of an island syndrome (Baker, 1955).

Seed dispersal mechanism in *G. maderense* is referred by Yeo (1984) as "carpel-projection", where the mericarp containing the seed is projected away from the fruit. This is a short-distance dispersal mechanism. Despite producing thousands of mericarps, each containing one seed, the seeds are only projected a low distance, which does not promote significant gene flow.

Collecting plants from each population of *G. maderense* has always been a constant challenge, given the small number of individuals present and the topography of the terrain that would allow access to the plants. It can be assumed that the number of plants used per population in the current study may affect the genetic differentiation, since higher numbers may increase data quality (Nybom, 2004). However, this may not be the case since genetic parameters were similar for the populations of Porto da Cruz: Larano ($n = 20$) and Santana: Pico da Boneca ($n = 4$). According to Lewontin's Paradox, genetic diversity should correspond to effective population size. However, this relationship does not scale as expected. Despite significant differences in population sizes among species, the observed range of genetic diversity is much narrower than predicted. This discrepancy likely arises because genetic diversity is influenced by other factors such as geographical range size, life history traits, mutation rate, and demographic history (Ellegren & Galtier, 2016).

An important factor that contributes to the generation of genetic structure is the specific biological characteristics of the species, such as reproduction and seed dispersal systems. The reproductive biology of *G. maderense* remains poorly studied, although Yeo (1973) proposed light, short-tongued bees as pollinators and seeds dispersed by ejection. Nevertheless, we should consider autogamy as an important factor contributing to the low genetic variation and high genetic differentiation within this species, since outcrossing species tend to be more genetically diverse (Nybom, 2004). The characteristics of the Madeira Island landscape constitute in themselves a barrier to gene flow, which is another important factor that influences the genetic structure of natural populations (Manel et al., 2003; Parada-Díaz et al., 2022).

The ISSR patterns strongly support complete population segregation and a clear geographic pattern, where the Eastern population (Porto da Cruz: Larano) stands out from all others. The use of ISSR to determine molecular diversity and taxonomic relationships within *Geranium* taxa has been used by others (Bozchaloyi, Sheidai, Keshavarzi, & Noormohammadi, 2017a; Bozchaloyi & Sheidai, 2018) in species delimitation, effectively separating populations, and ecotypes. Genetic and geographic distances showed a similar pattern. Although plants from S. Vicente (SVAA, SVMC SVB) were considered doubtfully native, since they occur near agricultural fields, urban areas, and gardens, they showed a close genetic relationship with plants obtained from seeds from the nearby ancient population of Pt. Delgada (SVPD) (not detected using drones or observed for at least 10 years). These populations although considered doubtfully native, may be native or derived from seeds obtained locally and used in gardens by local people.

The population of Porto Moniz: Achadas da Cruz showed close relationships with the samples cultivated nearby to the west (Pt. Pargo and Pt. Sol). The plants sampled at the Lisbon Botanical Garden and the Madeira Botanical Garden were genetically similar, but their relationship with native populations is unclear. The 1951 specimen from Madeira in the LISU herbarium collect by Romariz (see LISU P43032, Annex I) may be linked to the first cultivation of *G. maderense* outside Madeira Island, i.e. is based on a plant grown from seed at the former Funchal seminary ("Cultivado no seminário e trazido da serra por G. Costa", translates as *Cultivated at the seminary and brought from the mountains by G. Costa*), therefore linked to the original specimens collected by J.G. Costa in 1934 deposited both in MADM and MADS (see Annex I), therefore possibly corresponding to the origin of the plants in the University of Lisbon Botanical Garden, as well as those in the Madeira Botanical Garden (founded 1960). If this is the case, both Lisbon and Madeira Botanical Garden plants could be linked to the original population of Achadas da Cruz, however other plants were also sent to Lisbon by M. Tavares (around 1968–1969) and there are records of seeds being sent back to Madeira from Lisbon (C. Garcia, Com. pers.).

The increase in samples number both from the new population and from the unsampled population in Ribeira Brava, the reinforcement of populations already collected, such as Santana: Pico da Boneca Boneca (where nearly 150 plants were counted but only 4 were sampled), and Achadas da Cruz could further contribute even more to the understanding of genetic patterns and population diversity.

The possibility that some populations (excluding Achadas da Cruz, Larano and Pico da Boneca) could result from seed release through careless reintroductions or garden escapade, i.e. that the observed pattern could just be the repeated founder effect of a few siblings of a single garden plant cannot be ruled out. In fact, several endemic plants have since long been introduced in several locations around Madeira Island (cf. *Musschia wollastonii* Lowe, *Marcetella maderensis* (Bornm.) Svent., *Monizia edulis* Lowe, *Andryala chritmifolia* Aiton*, Goodyera macrophyla* Lowe, among others).

5. Conclusions

The scarcity of populations and individuals is related to anthropogenic actions, including agricultural practices or the introduction of invasive species (e.g. rabbits and feral goats) that accelerate the loss and degradation of natural ecosystems. These processes lead to the elimination of species or to their presence exclusively on refuge locations, like cliffs and ravines. Several other endemic Madeiran plants are also restricted to refugia, namely *Cheirolophus massonianus* (Lowe) A. Hansen & Sunding, *Helichrysum monizii* Lowe, *Musschia aurea* (L.f.) Dumort., *Monizia edulis* Lowe, *Argyranthemum haematoma* (Lowe) Lowe, *Autonoe madeirensis* (Menezes) Speta and many others.

The use of unmanned aerial vehicles (drone) technology, combined with historical data and sampling using climbing techniques, are crucial for the study of rare species endemic to islands, being useful approaches for detecting individuals in known populations or for surveying unknown populations. This is the first study to detect molecular variation of *G. maderense* using ISSR markers, which revealed low genetic diversity within populations and high genetic differentiation in natural populations or in plants cultivated in gardens. This pattern may be related to founder effects, lack of gene flow or possibly autogamy. However, further research is needed to provide evidence for autogamy in *G. maderense*. Our results support the need to absolutely avoid using seeds of “unknown” origin in any reintroductions, and the need to regulate the use of this plant as a garden plant, carefully using distinct geographic origins. Given the small number of populations and their small size, and low genetic diversity, this species could be particularly susceptible to anthropogenic disturbance, landscape changes and habitat loss due to wildfires (e.g.), *G. maderense* should be considered a threatened species and, as such, population genetics should be integrated into any conservation management actions.

CRediT authorship contribution statement

M. Gouveia: Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis. **B. Nyberg:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Formal analysis. **C. Aedo:** Writing – review & editing, Writing – original draft, Investigation, Data curation. **C. Bairos:** Writing – review & editing, Writing – original draft, Investigation. **M. Menezes de Sequeira:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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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Annex I.

List of herbarium specimens

Finland: cultivated at the Kaisaniemi Botanical Garden, Helsinki, P. Havas, s.n., 4-IV-1989 (H1713891) (cultivated);

Portugal: Jardim botânico da UL, M. Sequeira, MS10740ABCDEG, 8-VII-2021 (UMad) (DNA, cultivated);

Portugal (Madeira): Achadas da Cruz, J.G. Costa, s.n., V-1934 (MADM); Achadas da Cruz, J.G.Costa, V-1934 (MADS1733) (collection year corrected, stated as 1955); Madeira: cult. Seminario de sementes. Cultivado no seminário e trazido da serra por G. Costa, existe estampado no Seminário, Romariz, 691, 11-VI-1951 (LISUP43032) (cultivated); Paul da Serra, C.H. Pickering, s.n., IV-1960 (KK000417202) (Holotype); Paul da Serra, C.H. Pickering, s.n., IV-1960 (KK000417203) (Holotype); cultivated from seeds collected on the Paul da Serra at the Quinta São Roque, C.H. Pickering, 22, 6-V-1966 (BMBM001048925) (cultivated); cultivated in Major C.H. Pickering’s garden, Funchal, A. Hansen, 494, 5-IV-1973 (C) (cultivated); Funchal; Jardim Botânico da Madeira, Rui Vieira, 8-I-1975 (MADJ4578) (cultivated); Jardín Botánico de Funchal, J. Güemes, s.n., 1998 (MA933407) (cultivated); levada de Casa do Lombo do Mouro, C. Navarro, 3396, 28-VI-2000 (MA654726) (cultivated); Calheta, Ponta do Pargo, R. Jardim, J. Carvalho, 22-III-2000 (MADJ9477) (cultivated); Funchal/Monte. Em frente ao Centro de Saúde do Monte, terreno baldio. Arbusto. Solo nem seco nem húmido. Alt. 0–700 m s.m., decl. 5°, exp. S, Mónica Aguiar, 64, 1-V-2000 (UMad13103) (cultivated); São Vicente – Miradouro, R. Gonçalves, P. Gouveia, 10-IV-2001 (MADJ9686); Calheta, Ponta do Pargo – Senhora do Amparo, R. Gonçalves, P. Gouveia, 17-IV-2001 (MADJ9684) (cultivated); Calheta – Achada de Cima, R. Gonçalves, P. Gouveia, 2-IV-2001 (MADJ9685) (cultivated); Calheta – Achada de Cima- levada da Central da Calheta, R. Gonçalves, P. Gouveia, 2-IV-2001 (MADJ9687) (cultivated); Calheta – Lombo do Doutor, R. Gonçalves, s.n., 2-IV-2001 (MADJ) (cultivated); Ponta do Sol – Canhas- Levada do Poiso, R. Gonçalves, P. Gouveia, 2-IV-2001 (MADJ9688) (cultivated); São Vicente, Olga Baeta, 29-V-2002 (MADJ12650); Rochas da Ponta Delgada, Paulo Gouveia, 8-V-2002 (MADJ10003); cultivated at Quinta de Santo Antonio, J.G. Quinn, J. Silva, s.n., 23-V-2002 (MADM) (cultivated); cultivated at Quinta de Santo Antonio, J.G. Quinn, J. Silva, s.n., 23-V-2002 (MADM) (cultivated); cultivated at Quinta de Santo Antonio, J.G. Quinn, J. Silva, s.n., 23-V-2002 (MADM) (cultivated); cultivated at Quinta de Santo Antonio, J.G. Quinn, J. Silva, s.n., 23-V-2002 (MADM) (cultivated); Canhas, cultivada en casa de M. Sequeira, L. Medina, LM2448, 25-VI-2003 (MA701994) (cultivated); Monte. Berma de estrada, terreno seco. Alt. 550 m s.m., decl. 0°., Maria João Faria & Sandra Capontes, 75, 14-V-2004 (UMad9759) (cultivated); Boaventura, Prazo Cerrao, entre el pueblo y el cementerio; talud seco, S. Castroviejo, M. Sequeira, SC18205, 21-X-2006 (MA775802); Boaventura, Prazo Cerrao, entre el pueblo y el cementerio; talud junto a la carretera, S. Castroviejo, al., SC18258, 25-III-2007 (MA757683) (cultivated); Santana, Ribeiro Frio, Edit Summer School 2010, 20-VII-2010 (MADJ14168) (cultivated); Encumeada, levada forestal, exp. N antes do segundo tunel no sentido este/oeste; laurisilva, M. Sequeira, C. Góis-Marques, MS7961, 29-V-2014 (MA933407) (cultivated); Funchal; Jardim Botânico da Madeira, Olga Baeta, 5-V-2015 (MADJ13936) (cultivated); Porto da Cruz, Larano, na escarpa da vereda Larano-Boca do Risco; *Globulario salicinae-Ericetum arboreae*, R. Jardim, M. Sequeria, MS6429, 6-V-2016 (MA910968); São Vicente-Cardais; na escarpa, habitat: *Euphorbietum piscatoriae*, população, duvidosamente nativa, R. Jardim, M. Sequeria, MS6432, 6-V-2016 (MA910966); São Vicente. Na estrada antiga numa ravina próximo do mar. Alt. 81 m s.m., decl. 0°, exp. S, Pedro Jardim & Tiago Andrade, PT59, 19-V-2018 (UMad5653); Porto da Cruz; Larano. Encosta rochosa, virado para o mar. Alt. 369 m s. m., decl. 80°, exp. N, Ana Goís & Marisa Gouveia, MG134, 9-V-2018 (UMad6149); Ribeira do Inferno, antes do túnel, ravina para o mar, abaixo da estrada., M. Sequeira, C. Bairos & M. Gouveia, MS9232, 17-IV-2020 (UMad21317); Maiata à Boca do Risco, meio da vereda., M. Sequeira, C. Bairos & M. Gouveia, MS9249, 20-IV-2020 (UMad21333); Achadas da Cruz, no topo da falésia sob o teleférico. Comunidade herbácea com várias espécies alóctones (dominada por *Arundo donax*)., M. Sequeira, C. Góis-Marques & M. Gouveia, MS10552E, 13-V-2021 (UMad21683) (DNA); Ponta do Pargo, Amparo. Jardim de uma casa abandonada na estrada regional., M. Sequeira, C. Góis-Marques & M. Gouveia, MS10551A, 13-V-2021 (UMad21684) (DNA, cultivated); Jardim Botânico da Madeira., M. Sequeira & M. Gouveia, MS10713ABCD, 17-VI-2021 (UMad22094) (DNA, cultivated); S. Vicente, estrada antiga para o Seixal, antes do primeiro túnel, na ravina para o mar. Com filmagem de drone., M. Sequeira, M. Gouveia & C. Góis Marques, MS9916A, 1-II-2021 (UMad22366) (DNA); S. Vicente, estrada antiga para o Seixal, antes do primeiro túnel, na ravina para o mar. Com filmagem de drone., M. Sequeira, M. Gouveia & C. Góis Marques, MS9916C, 1-II-2021 (UMad22367) (DNA); S. Vicente, estrada antiga para o Seixal, antes do primeiro túnel, na ravina para o mar. Com filmagem de drone., M. Sequeira, M. Gouveia & C. Góis Marques, MS9916D, 1-II-2021 (UMad22368) (DNA); S. Vicente, estrada antiga para o Seixal, antes do primeiro túnel, na ravina para o mar. Com filmagem de drone., M. Sequeira, M. Gouveia & C. Góis Marques, MS9916B, 1-II-2021 (UMad22370) (DNA); S. Vicente, miradouro dos

Cardais., M. Sequeira, C. Góis Marques & M. Gouveia, MS9917A, 1-II-2021 (UMad22369) (DNA); Boca do Risco., M. Sequeira, MS10425D, 22-IV-2021 (UMad21528) (DNA); Boca do Risco., M. Sequeira, MS10425E, 22-IV-2021 (UMad21529) (DNA); Boca do Risco., M. Sequeira, MS10425G, 22-IV-2021 (UMad21530) (DNA); Ponta Delgada, plantas obtidas a partir de sementes da população extinta., M. Sequeira, MS10744bis (ABCDE), 26-VII-2021 (UMad) (plants obtained from seeds); Boaventura, estrada para o cemitério. Na borda de um campo agrícola., M. Sequeira, C. Góis-Marques, T. Andrade, C. Gomes & M. Gouveia, MS10550B, 6-V-2021 (UMad21681) (DNA); Ribeiro Frio. Perto de estrada alcatroada, rodeado por *Arundo donax* L. e *Ageratina adenophora* (Spreng.) R.M.King & H.Rob. Alt. 158,1m s.m., decl. 116°, exp. SE, A.Cardoso, C.P.Aguiar, F.Spínola, R.Freitas & T.Ornelas, ACFRT62, 23-IV-2022 (UMad10060) (cultivated); Vereda do Larano, partindo da Maiata, depois da ponta com vista para a Ponta de S. Lourenço e para a Boca do Risco. Em acumulos de solo de pequenas derrocadas, com *Argyranthemum pinnatifidum* subsp. *succulentum*, *Pteridium aquilinum*, *Helychrisum melaleucum* etc. correspondentes a comunidades de *Euphorbietum piscatoriae*. Alt. 335 m m s.m., M. Sequeira & alumni, MS11050, 6-IV-2022 (UMad11459); Vereda do Larano, partindo da Maiata, depois da ponta com vista para a Ponta de S. Lourenço e para a Boca do Risco. Em acumulos de solo de pequenas derrocadas, com *Argyranthemum pinnatifidum* subsp. *succulentum*, *Pteridium aquilinum*, *Helychrisum melaleucum* etc. correspondentes a comunidades de *Euphorbietum piscatoriae*. Alt. 125 m s.m., M. Sequeira & alumni, MS11050, 7-V-2022 (UMad11469); Acima da saída de emergência da via expresso da Ponta dos Clérigos. Alt. m s.m., exp., M. Sequeira, C. Bairos & M. Gouveia, MS12102D, 22-IV-2023 (UMad22833) (DNA); Acima da saída de emergência da via expresso da Ponta dos Clérigos. Alt. m s.m., exp., M. Sequeira, C. Bairos & M. Gouveia, MS12102C, 22-IV-2023 (UMad22834) (DNA); Acima da saída de emergência da via expresso da Ponta dos Clérigos. Alt. m s.m., exp., M. Sequeira, C. Bairos & M. Gouveia, MS12102B, 22-IV-2023 (UMad22835) (DNA); Acima da saída de emergência da via expresso da Ponta dos Clérigos. Alt. m s.m., exp., M. Sequeira, C. Bairos & M. Gouveia, MS12102A, 22-IV-2023 (UMad22836) (DNA); Canhas. Alt. m s.m., exp., M. Sequeira & C. Bairos, MS12114, 24-IV-2023 (UMad22831) (cultivated); Vereda do Larano, Boca do Risco, depois do miradouro, espigão amarelo. Em acumulos do solo de pequenas derrocadas com *Argyranthemum pinnatifidum* subsp. succulentum, *Pteridium aquilinum*, *Helichrysum melaleucum* etc. correspondentes a comunidades de *Euphorbietum piscatoriae*., M. Sequeira, C. Bairos et Alumni, MS12252, 6-V-2023 (UMad16898); Porto da Cruz, Vereda do Larano, Boca do Risco. Em acúmulos de solo de pequenas derrocada com *Argyranthemum pinnatifidum* subsp. *succulentum*, *Pteridium aquillinum*, *Helychrisum melaleucum*, etc. Correspondentes a comunidades *Euphorbietum piscatoriae*. Alt. 341 m s.m., M. Sequeira & alumni, MS12744, 16-III-2024 (UMad20800); São Vicente, Estrada Antiga ER 101. Num talude com declive elevado e substrato húmido. Comunidade de herbáceas em ambiente exposto. Alt. 47 m s.m., G. Gomes & R. Nunes, RN038, 1-V-2024 (UMad20583).

South Africa: cultivated at Johannesburg, L.E. Davidson, 3845, 25-IX-1985 (FI) (cultivated).

Spain: cultivated at Coin, Spain, G. Kunkel, s.n., V-1980 (C) (cultivated).

Data availability

Data will be made available on request.

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