

Conservation of genetic diversity in Mediterranean endemic species: *Arenaria balearica* (Caryophyllaceae)

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Background – Biodiversity loss is a problem that needs to be urgently addressed, particularly with the uncertainties of climate change. Current conservation policies principally focus on endangered species but they often give little consideration to the evolutionary processes, genetic diversity, or the rarity of non-endangered species. Endemic species occurring in rocky habitats that are undergoing exceptional habitat loss appear to be one of the most important candidates for conservation. The aim is to establish *in situ* and *ex situ* conservation recommendations for the Mediterranean endemic species *Arenaria balearica*.

Material and methods – *Arenaria balearica* is a species endemic to the Mediterranean with a disjunct distribution range throughout Majorca, Corsica, Sardinia, and other small Tyrrhenian islands. A combination of molecular techniques (AFLPs and plastid DNA) was employed to determine genetic diversity and rarity across populations and to calculate the Relevant Genetic Units for Conservation (RGUCs). Moreover, Species Distribution Models (SDMs) were developed to assess the potential current distribution and the expected situation under future climatic scenarios.

Key results – To preserve the genetic diversity and rarity of the species, *in situ* conservation is proposed for six populations as RGUCs. Moreover, as the RGUCs can only account for a part of the phylogeographic signal, *ex situ* conservation is also suggested for some additional populations. According to the results, the habitat suitability in the 2050 scenario is limited and suitable areas for *A. balearica* could have disappeared by 2070. Therefore, the persistence of the species could be in danger in a short period of time and conservation planning becomes necessary.

Keywords – *Arenaria balearica*; conservation; Mediterranean endemism; RGUCs; species distribution models.

INTRODUCTION

The basis of biological diversity is found at the genetic level regardless of the conservation status of a species, i.e., endangered vs. non-endangered (Convention on Biological Diversity, Brazil 1992). It is the genetic diversity that deserves to be preserved (IUCN 1993). Genetic diversity and rarity levels in widely distributed species are in many cases similar to those of endangered congeneric species (Gitzendanner & Soltis 2000). Although conservation efforts often focus on

the preservation of the endangered species in an attempt to avoid species extinction, the diversity and rarity conservation of non-endangered endemic species is also important to ensure their long-term survival as they hold the evolutionary and adaptive potential of the species (Spielman et al. 2004; Frankham 2005; O’Grady et al. 2006; Kahilainen et al. 2014). Furthermore, conservation measures are more likely to be successful when identifying declining species before they are threatened by immediate extinction (Jansen et al. 2019). Knowledge of the genetic diversity can facilitate

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making decisions regarding both *in situ* and *ex situ* conservation methods (Haig 1998; Pérez-Collazos et al. 2008). Moreover, the reconstruction of the phylogeographic patterns of species can show how interactions between evolutionary and ecological processes can influence diversity at multiple scales (Webb et al. 2002). The rapid environmental changes force us to look beyond taxonomic diversity and begin to consider the evolutionary and functional diversity (Pollock et al. 2017). All these considerations favour the dynamic conservation of plant species and populations (Volis & Blecher 2010; Heywood 2014), ensuring the survival of the species and its monitoring in a changing world (Markert et al. 2010).

Arenaria balearica L. is distributed throughout the Tyrrhenian islands of Majorca, Corsica, Sardinia (fig. 1), including some populations in Tavolara, La Maddalena, Caprera, and Asinara islands, and in the Tuscan Archipelago, particularly in Montecristo and Capraia islands (Diana Corrias 1981). The species distribution pattern shows a notable relict character (Bolós & Molinier 1958) and has traditionally been considered a Mediterranean paleoendemic in the broad sense of the term (Favarger & Contandriopoulos 1961) and a disjunct endemism by Thompson (2005). The paleohistoric traits described in previous studies (Bobo-Pinilla et al. 2016) reinforce the importance of genetic conservation as a way to preserve evolutionary units. This species occupies a rare microhabitat on rock walls and cliffs, which are already spatially fragmented and offer limited surface area for plant development. These habitats are listed in the Habitat Directive (8210 - Calcareous rocky slopes with chasmophytic vegetation) and described in the aforementioned directive as habitats with a great regional diversity and hosting many plant

species with a restricted range (Fornós et al. 2009). Moreover, these habitats are considered to serve as refugia for flora subjected to water stress over long periods (Piñar Fuentes et al. 2017). Even though these habitats are not directly affected by human activity, these areas are suspected to suffer dramatic changes under climate change scenarios (e.g., Giorgi & Lionello 2008). It is therefore necessary to propose an approach for preserving the genetic diversity of the species that occur there.

Several estimators have been proposed for quantitatively and qualitatively selecting populations deserving conservation priority: Evolutionary Significant Units (Ryder 1986), Management Units (Moritz 1994), Operational Conservation Units (Doadrio et al. 1996), Fundamental Geographic and Evolutionary Units (Riddle & Hafner 1999), and Functional Conservation Units (Maes et al. 2004), among others. Recently, Pérez-Collazos et al. (2008), introduced the concept of Relevant Genetic Units for Conservation (RGUCs). This genetic-based approach considers both common and rare alleles as suitable parameters to estimate the minimum number of conservation units that should be targeted for an adequate representation of the total genetic variability of a species, and to select within a species those populations that hold higher levels of diversity and/or rarity. This method is based on the idea that rare alleles are essential in conservation because they represent unique evolutionary products that could provide the species with the ability to adapt to environmental changes (Bengtsson et al. 1995; Pérez-Collazos et al. 2008; Lopez et al. 2009; Shaw & Etterson 2012).

The selection of RGUCs has been successfully used to propose sampling strategies for species such as *Boleum*

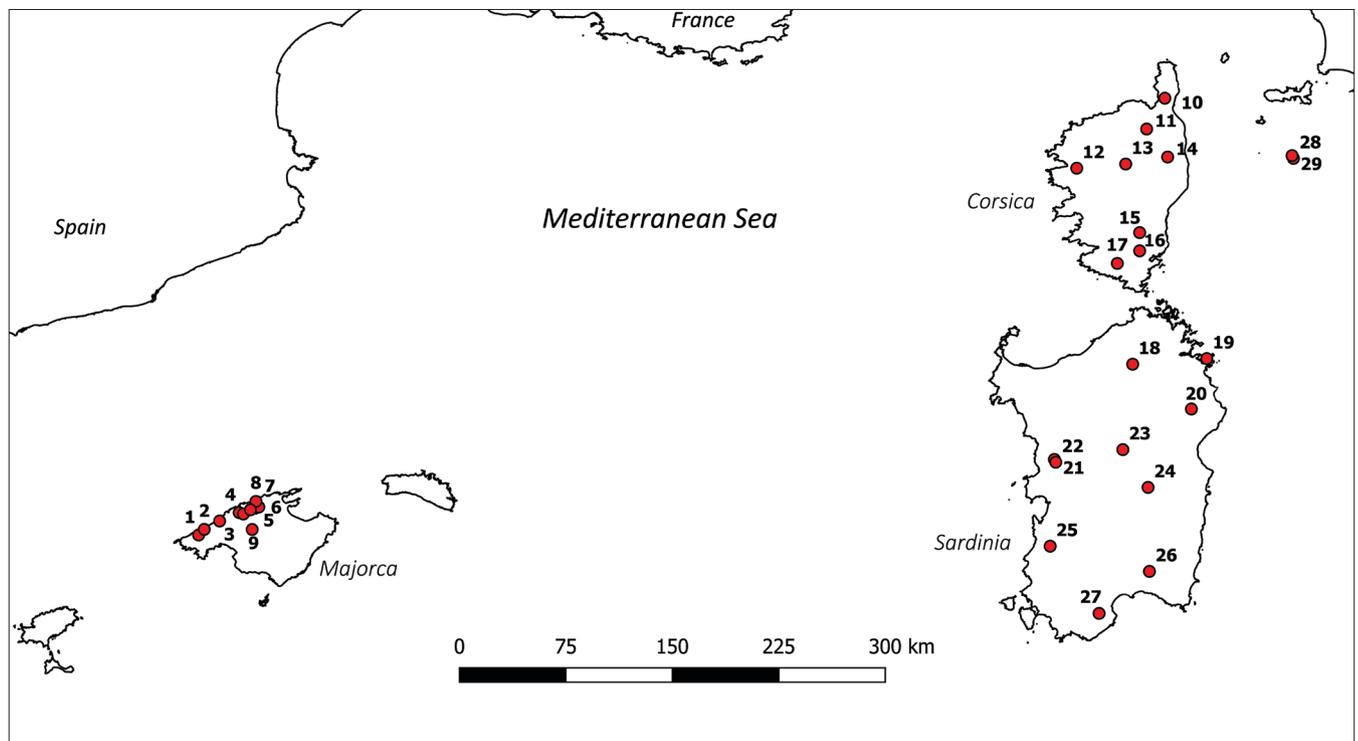


Figure 1 – Sample locations of *Arenaria balearica* in the Mediterranean. Numbers correspond to the population numbers in table 1. Map created with QGIS v.2.18 (QGIS Development Team 2016).

asperum Desv. (85.10% of the rare AFLP bands were captured by sampling 50% of the populations; Pérez-Collazos et al. 2008), *Borderea pyrenaica* Miégev. (97.7% of the rare alleles were captured by sampling five out of 12 populations; Segarra-Moragues & Catalán 2010), and *Astragalus edulis* Bunge (six populations were sampled from a total of 17; Peñas et al. 2016).

Climate change has a widespread impact on plant populations, therefore understanding how plants respond to this change is essential to our efforts to conserve them (Christmas et al. 2016). Climate vulnerability, species sensitivity, adaptive capacity, and exposure to change are all important factors to consider in conservation planning (Dawson et al. 2011). Species distribution models (SDMs) use information about the distribution of the species and the associated ecological data to estimate habitat suitability of species. Model projections under future scenarios allow the assessment of areas that will be most affected by climate change, which could compromise the survival of the species (Elith & Leathwick 2009; Guisan et al. 2013). Moreover, considering changes in suitable area may also help to understand additional biogeographical patterns and processes (Price & Wagner 2011), for example, relict species distribution or adaptation to microclimate conditions.

The aim of this work is the design of conservation strategies for the Mediterranean endemic species *Arenaria balearica*. To achieve this goal, we propose to: (1) assess the number of populations that should be preserved in order to establish a representative percentage of the total genetic variation of *A. balearica*; (2) identify which populations should be prioritized to better represent the genetic singularity, paleogeographic history, and geographic variability for both *ex situ* and *in situ* conservation; and (3) evaluate climate change over the distribution range of *A. balearica*.

MATERIAL AND METHODS

Species

Arenaria balearica is an herbaceous perennial plant whose branched stems and small leaves form low, compact, evergreen, moss-like dense mats, preferentially on cool, moist soils in shaded rocky places (chomophyte) (Diana Corrias 1981; López González 1990). Although there is no available data on the reproductive biology of this species, its slender, short, upright stems bear white, actinomorphic flowers suggesting that this plant is most probably pollinated by both wind and nocturnal insects. It is a diploid species with a chromosome number of $2n = 18$ (Diana Corrias 1981; López González 1990).

Genetic data

Genetic data (AFLP and plastid DNA) was taken from Bobo-Pinilla et al. (2016). These data were taken from a total of 226 plants belonging to 29 different populations: 9 from Majorca (MAJ), 8 from Corsica (COR), 10 from Sardinia (SAR), and the other 2 from Montecristo and Capraia. The sampling covers the distribution range of the species (table 1, fig. 1; see also Diana Corrias 1981). Due to the small size of

the populations, a variable number of individuals were collected (1–16). Voucher specimens were deposited at the herbaria of the University of Salamanca (SALA) and the University of Granada (GDA) in Spain, and the herbarium of the University of Cagliari (CAG) in Italy.

The AFLP data include 213 individuals from 23 populations: 2 populations from MAJ, 2 from COR, and the 2 from Montecristo and Capraia were not evaluated as only 1 or 2 individuals were sampled in each one of these locations. The R package *AFLPdat* (Ehrich 2006) was used to calculate Nei's gene diversity index (Nei 1987) for each population as well as the frequency down-weighted marker values (DW; Schönswetter et al. 2005) to represent the diversity and rarity, respectively. Moreover, to take the uneven sampling into account, AFLPDIV v.1.1 (first described in Coart et al. 2005) was used to compute the band richness (Br) and the percentage of polymorphic loci (PLP) with standardized sample sizes. This measure of genetic diversity can be interpreted as an analogue of allelic richness (Coart et al. 2005).

The plastid regions *trnL-trnF* (Taberlet et al. 1991), *psbA-30*, *trnK-matK*, and *rps16* (Shaw et al. 2005) analysed by Bobo-Pinilla et al. (2016) were used to reconstruct the phylogeographic signal of the species. This dataset includes a total of 196 plants from the same 29 populations (table 1).

Relevant Genetic Units for Conservation (RGUCs)

The evaluation of RGUCs is based on AFLP data and relies on the combination of two parameters accounting for the population structure and the probability of the loss of rare alleles. In summary, the values of the probability of rare-allele loss are compared to those of the degree of inter-population subdivision (Caujapé-Castells & Pedrola-Monfort 2004; Pérez-Collazos et al. 2008).

First, the modified equation $P = 1 - F_{ST}^n$ (Segarra-Moragues & Catalán 2010) was used to estimate the total number of populations that should be targeted (fig. 2) according to Ceska et al. (1997). In this equation, n is the number of populations to be sampled in order to represent a given proportion (P) of the genetic diversity within a population, whereas the F_{ST} for our case study was obtained with the software ARLEQUIN v.3.5.1.2 (Excoffier & Lischer 2010). P -value was established at 99.9% to include the total genetic diversity.

Secondly, rare AFLP bands (those with an overall frequency lower than 10% and present in less than 20% of the populations; supplementary file 1) were selected using the mean frequencies of these bands and their associated probabilities of loss. Additionally, the probability that a sample size of N populations fails to include an allele with population frequency p was calculated (Caujapé-Castells & Pedrola-Monfort 2004; Pérez-Collazos et al. 2008). To calculate the probabilities of loss, the expression $L = (1 - p)^{2N}$ (Bengtsson et al. 1995) was used, where p is the allele frequency and N is the number of populations in which a rare allele is present (Pérez-Collazos et al. 2008). For each rare allele, the observed and expected probabilities of loss were calculated (L_o and L_e , respectively), where L_o derives from a direct estimation and L_e is calculated attributing to p the mean allele frequency over all populations considered (supplementary file 1). The negative natural logarithms ($-\text{Log } L_o$ and $-\text{Log}$

Table 1 – Sampling locations of *Arenaria balearica* and their characteristics in the three geographical regions Majorca (MAJ), Corsica (COR), and Sardinia (SAR).

The AFLP derived diversity and rarity descriptors, and the cpDNA haplotypes are obtained from Bobo et al. (2016). N_{aflp} , number of samples per population included in the AFLP analysis; Nei's GD, Nei's diversity index; DW, frequency down-weighted marker values; Br[4], band richness after rarefaction to $N = 4$; PLP1%, percent polymorphic loci based on band phenotypes after standardization to $N = 4$ with 1% threshold; H_{cp} , haplotypes; Bold indicates higher interest for conservation. Protection status within a protected area and their names; SAC, Special Area of Conservation (Natura 2000 Network); The level of conservation management depends on the type of protection in each country.

N°	Geo	Sampling locality	Long./Lat.	N_{AFLP}	Nei's GD	DW	Br[4]	PLP1%	H_{cp} (N° indivs)	Status	Name of the Protected Area
1	MAJ	SP; Majorca, Estellencs, Puig de Galatzó	2.48°/39.63°	11	0.096	5.872	1.179	0.31	I (1); II (11)	Protected	Paraje Natural Serra de Tramuntana
2	MAJ	SP; Majorca, Banyalbufar, Mola de Plàncies	2.52°/39.67°	10	0.098	4.491	1.185	0.32	II (9)	Protected	Paraje Natural Serra de Tramuntana
3	MAJ	SP; Majorca, Valldemossa, Puig des Teix	2.63°/39.73°	10	0.119	6.775	1.221	0.36	I (9)	Protected	Paraje Natural Serra de Tramuntana
4	MAJ	SP; Majorca, Escorca, Puig Major	2.77°/39.79°	–	–	–	–	–	I (2)	Protected	Paraje Natural Serra de Tramuntana
5	MAJ	SP; Majorca, Escorca, Tossals	2.80°/39.78°	10	0.110	5.625	1.207	0.36	I (9)	Protected	Paraje Natural Serra de Tramuntana
6	MAJ	SP; Majorca, Escorca, Clot d'Albarca	2.88°/39.82°	–	–	–	–	–	I (1)	Protected	Paraje Natural Serra de Tramuntana
7	MAJ	SP; Majorca, Escorca, Puig Tomir	2.91°/39.83°	10	0.189	14.83	1.346	0.52	I (7)	Protected	Paraje Natural Serra de Tramuntana
8	MAJ	SP; Majorca, Escorca, Puig Caragoler	2.89°/39.87°	8	0.095	7.083	1.181	0.29	I (7)	Protected	Paraje Natural Serra de Tramuntana
9	MAJ	SP; Majorca, Escorca, Puig d'en Galileu	2.85°/39.81°	9	0.119	5.653	1.223	0.37	I (4)	Unprotected	–
10	COR	FR; Corsica, Cap Corse, Commune d'Olimeta	9.69°/42.75°	–	–	–	–	–	I (1)	Unprotected	–
11	COR	FR; Corsica, Massif de Monte Astu	9.26°/42.53°	–	–	–	–	–	I (1)	Protected	Massif du Tenda et forêt de Stella (SAC)
12	COR	FR; Corsica, Gorges de Spelunca, Le Sentier de la Spilonca	8.76°/42.25°	10	0.187	10.881	1.307	0.48	I (4); V (1); XII (1); XVI (3)	Protected	Parc Naturel Régional de Corse
13	COR	FR; Corsica, Valle de la Restonica	9.11°/42.28°	8	0.182	13.227	1.329	0.37	I (7); XIII (1)	Protected	Parc Naturel Régional de Corse
14	COR	FR; Corsica, Valle de'Alesani, Quercetto	9.41°/42.33°	10	0.163	9.794	1.296	0.44	I (10)	Protected	Parc Naturel Régional de Corse
15	COR	FR; Corsica, Col de Bavella	9.21°/41.79°	9	0.169	8.681	1.247	0.35	I (4); XI (1); XIV (4)	Protected	Parc Naturel Régional de Corse
16	COR	FR; Corsica, La Cascade de Piscia di Ghjadu	9.21°/41.66°	10	0.194	13.182	1.279	0.42	I (9); XV (1)	Protected	Parc Naturel Régional de Corse
17	COR	FR; Corsica, Gianuccio	9.05°/41.57°	4	0.200	12.906	1.252	0.39	XIV (3)	Unprotected	–
18	SAR	IT; Sardinia, Tempio Pausania, Monte Limbara	9.16°/40.85°	10	0.167	8.518	1.193	0.40	IV (2); V (8)	Protected	Monte Limbara (SAC)
19	SAR	IT; Sardinia, Olbia, Tavolara	9.69°/40.89°	5	0.179	13.208	1.329	0.47	I (5)	Protected	Area Marina Protetta Tavolara Punta Coda Cavallo
20	SAR	IT; Sardinia, Lula, Punta Turuddò - Monte Albo	9.58°/40.53°	9	0.161	8.894	1.24	0.39	VII (10)	Protected	Monte Albo (SAC)
21	SAR	IT; Sardinia, Cuglieri, La Madonnina	8.60°/40.17°	8	0.135	7.506	1.229	0.38	I (6)	Unprotected	–
22	SAR	IT; Sardinia, Santu Lussurgiu, Zorzia - Monte Urriugu	8.61°/40.15°	9	0.151	6.808	1.347	0.56	I (7)	Unprotected	–

Table 1 (continued) – Sampling locations of *Arenaria balearica* and their characteristics in the three geographical regions Majorca (MAJ), Corsica (COR), and Sardinia (SAR). The AFLP derived diversity and rarity descriptors, and the cpDNA haplotypes are obtained from Bobo et al. (2016). N_{aflp} , number of samples per population included in the AFLP analysis; Nei's GD, Nei's diversity index; DW, frequency down-weighted marker values; Br[4], band richness after rarefaction to $N = 4$; PLP1%, percent polymorphic loci based on band phenotypes after standardization to $N = 4$ with 1% threshold; H_{sp} , haplotypes; Bold indicates higher interest for conservation. Protection status within a protected area and their names; SAC, Special Area of Conservation (Natura 2000 Network); The level of conservation management depends on the type of protection in each country.

N°	Geo	Sampling locality	Long./Lat.	N_{AFLP}	Nei's GD	DW	Br[4]	PLP1%	H_{sp} (N° indivs)	Status	Name of the Protected Area
23	SAR	IT; Sardinia, Oliena, Monte Corradi	9.09°/40.24°	9	0.136	6.936	1.333	0.46	I (1); VIII (7)	Unprotected	–
24	SAR	IT; Sardinia, Desulo, Taccu di Giurini	9.27°/39.97°	16	0.103	5.664	1.299	0.46	VI (10); IX (1)	Protected	Parco Nazionale del Gennargentu
25	SAR	IT; Sardinia, Guspini, Montevecchio	8.57°/39.55°	8	0.179	9.914	1.310	0.45	1 (9)	Unprotected	–
26	SAR	IT; Sardinia, Burcei, Rio Niu Crobu - Monte Serpeddi	9.28°/39.37°	10	0.129	6.700	1.357	0.54	1 (7)	Unprotected	–
27	SAR	IT; Sardinia, Villa S. Pietro, Rio Is Canargius - Monte Nieddu	8.92°/39.07°	10	0.123	6.744	1.372	0.37	I (7); X (2)	Unprotected	–
28	–	IT; Tuscan Archipelago, Montecristo, Collo a fundo	10.31°/42.32°	–	–	–	–	–	1 (1)	Protected	Riserva Naturale Isola di Montecristo
29	–	IT; Tuscan Archipelago, Montecristo, Grotta del Santo	10.30°/42.34°	–	–	–	–	–	1 (2)	Protected	Riserva Naturale Isola di Montecristo

L_e) of those values were plotted (y-axis) against the mean frequency of each rare allele (x-axis) and used to calculate the respective linear regressions (fig. 3). The representative R-value (which indicates the proportion of rare alleles captured by sampling only one population) was calculated as the quotient between the slope of the expected regression line and the slope of the observed regression line (m_o and m_e , respectively), i.e., $R = m_o(-\text{Log } L_e)/m_e(-\text{Log } L_e)$ (Bengtsson et al. 1995; Caujapé-Castells & Pedrola-Monfort 2004; Pérez-Collazos et al. 2008; Segarra-Moragues & Catalán 2010) (fig. 3).

Plant genetic diversity is spatially structured at different scales, e.g., geographical areas, populations, or among neighbouring individuals (Engelhardt et al. 2014) as a result of environmental influences, life-history traits, and the demographic history of the species. In this case, after the results obtained by Bobo-Pinilla et al. (2016), the main islands were chosen as geographic units, as they encompass the main genetic diversity of *A. balearica*. Moreover, the three islands belong to three different countries with their own environmental legislation. The Preferred Sampling Area (PSA hereafter) was calculated taking into account the number of populations and individuals each holding one of the rare alleles (supplementary file 1). Regarding the PSA percentages and the R-values of the three geographical areas, the optimal proportion of the populations to be sampled in each area was calculated. For each PSA, the populations were chosen regarding the Nei's gene diversity index and the DW index, to cope with the maximum amount of diversity and rarity (fig. 2A).

In addition, the haplotype diversity (Bobo-Pinilla et al. 2016), the conservation status of the populations (protected versus unprotected), and the protection level of the different areas (table 1) were taken into account in order to establish an *ex situ* conservation that encompasses the total diversity (fig. 2B).

Distribution modelling

To model the current climatic suitability of *A. balearica*, the Bioclim climatic layers available at www.worldclim.com (v.1.4; 1960–1990; 30 arc-seconds grid cell resolution) were used (Hijmans et al. 2005). Correlation analysis among bioclimatic variables was performed to select non-correlated variables. The variance inflation factor (VIF) values (Marquardt 1970) were used to test multicollinearity through the “vif” function of the *HH* R package (Heiberger 2015) and all variables with a value > 5 were discarded. A stepwise variable selection based on the Akaike Information Criterion (AIC) was then used until only three variables were left, this was done following the rule of one predictor for ten occurrences (Harrell et al. 1984) to avoid over-parametrization and multicollinearity among the climatic data. The climatic features that were selected are the mean diurnal temperature range (bio2), mean temperature of wettest quarter (bio8), and precipitation of warmest quarter (bio18).

Random forest models (Breiman 2001) generated by the R package *randomForest* were used to model the habitat suitability of the species as they seem to be useful to predict rare species distribution (Mi et al. 2017) as it avoids overfit-

ting issues (Breiman 2001). The area under the curve (AUC), the Kappa statistic, and the overall accuracy were used to evaluate the models (Swets 1988; Monserud & Leemans 1992; Elith 2002). The models with an AUC above 0.75, a Kappa above 0.25, and an overall accuracy over 0.75 were retained. These were combined with a leave-one-out strategy (jackknife) to compensate for the low number of presence records (Pearson et al. 2006). The best model was selected using these criteria. The variable importance is measured by looking at the deterioration of the predictive ability of the model when each predictor is replaced one by one by random noise. The resulting deterioration is a measure of predictor importance (percentage increase in mean square error, %IncMSE), higher %IncMSE indicates greater variable importance (e.g., Vincenzi et al. 2011). The future distributions (MIROC models CMIP5_2050 and CMIP5_2070) were generated by projecting the best model onto future scenarios using the R package *raster* (Hijmans et al. 2005). The representative concentration pathway 2.6 (RCP 2.6) and 4.5 (RCP 4.5) were used as the least aggressive scenarios.

RESULTS

Genetic diversity in *Arenaria balearica*

According to the AFLP results, the genetic diversity at the species level was 0.159; levels of genetic diversity within populations ranged from a maximum of 0.2 (population 17

in central Sardinia) to a minimum of 0.095 (population 8 in Majorca). The DW ranged from 4.49 (population 2) to 14.83 (population 7), with both populations growing in Majorca (table 1).

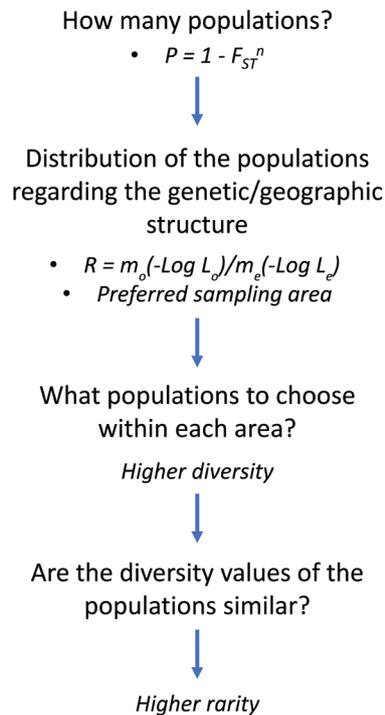
On average, the populations from Sardinia showed the highest percentage of polymorphic loci (PLP = 0.44, using the 1% polymorphism criterion), with population 22 showing the highest value (0.56), followed by the populations of Corsica (0.40) and Majorca (0.36). The same pattern has been found for the Br values.

Regarding the haplotype diversity, 16 haplotypes were found (table 1; Bobo-Pinilla et al. 2016). The species showed a single major haplotype (present in 24 out of the 29 populations studied), which occurred on all the islands (and the only haplotype on Tavolara and Montecristo). In addition, there were 15 less common derived haplotypes: two haplotypes (II and III) from MAJ, seven haplotypes from COR (XI, XII, XIII, XIV, XV, and XVI), and six haplotypes from SAR (IV, VI, VII, VIII, IX, and X). Only one haplotype is shared between two areas (haplotype V between COR and SAR).

Selection of RGUCs

Only five populations are needed ($n = 4.27$) to cover 99.9% of the genetic diversity (fig. 2). From a total of 1792 bands, 474 alleles met the rarity requirements (table 2). Of these, 49 were exclusive to MAJ (Majorca), 63 were exclusive to COR (Corsica), and 88 were exclusive to SAR (Sardinia).

A) *In situ* conservation proposal



B) *Ex situ* conservation proposal

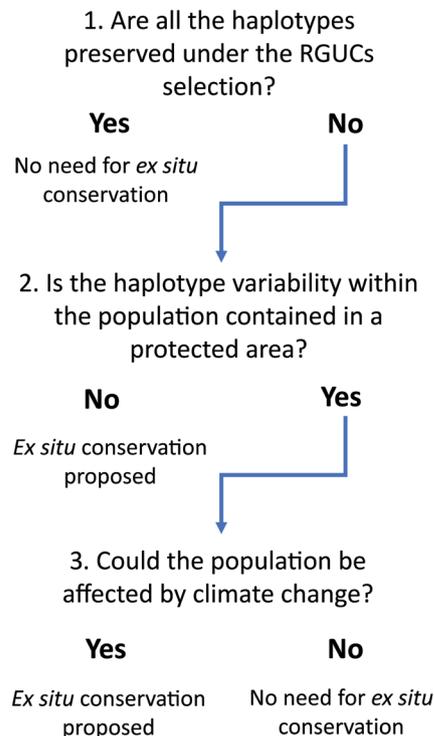


Figure 2 – Flow chart of the steps followed in the selection of the populations to be preserved. **A.** *In situ* conservation method (RGUCs selection); P, proportion of the genetic diversity; L_o and L_e observed and expected probabilities of loss of each allele; R, proportion of rare alleles captured by sampling only one population. **B.** *Ex situ* conservation proposal.

Table 2 – Rare AFLP alleles distribution and RGUCs calculation values.

Rare alleles are considered those with an overall frequency lower than 10% and present in less than 20% of the populations. Preferred sampling area (PSA); R-value (indicates the proportion of rare alleles captured by sampling only one population); n (number of populations to be sampled in order to represent a given proportion of the genetic diversity).

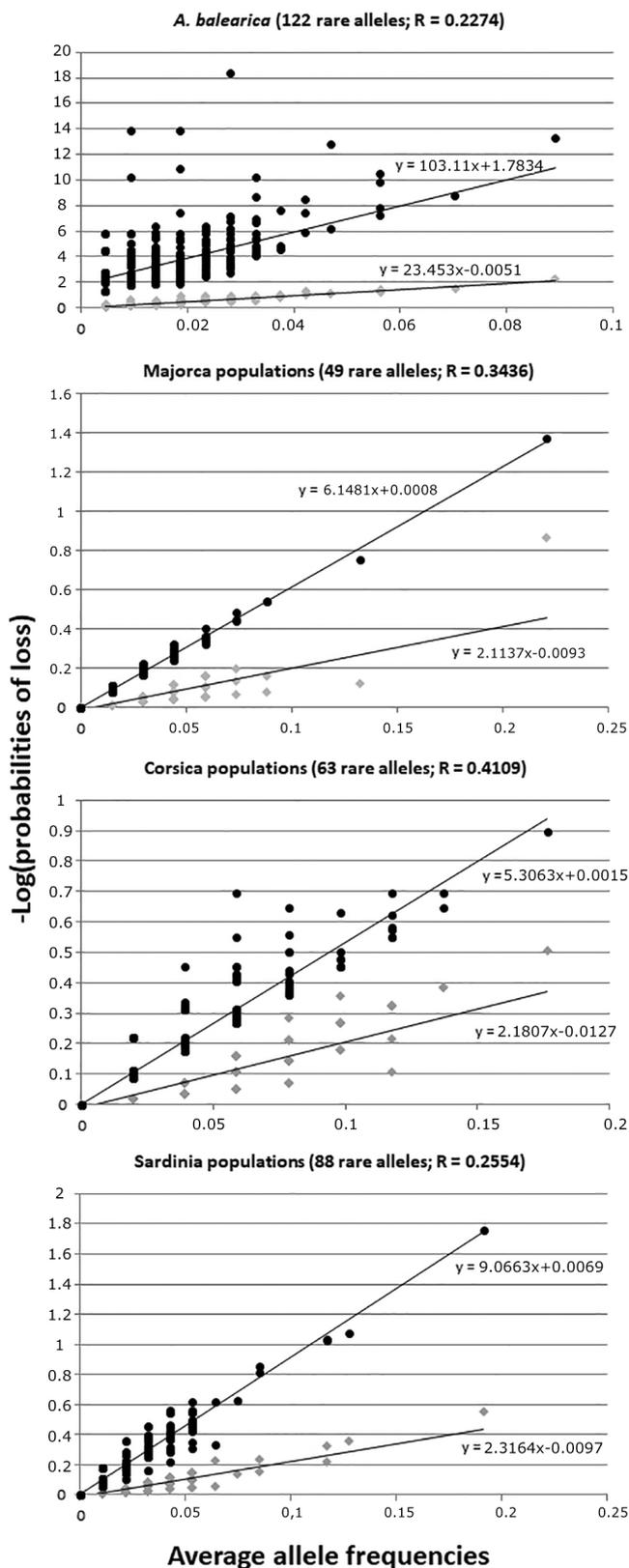
	Full range	Majorca	Corsica	Sardinia
Total n° of AFLP alleles	1792	–	–	–
N° of rare alleles (shared between areas)	474	49	63	88
% of rare alleles	26.45	20.70	23.10	28.40
N° of rare alleles (by PSA)	–	99	138	161
% of rare alleles (by PSA)	–	24.80	34.30	40
R-value (%)	22.74	34.36	41.09	25.54
Optimal proportion	–	0.30	0.38	0.33
n	4.27	1.26	1.61	1.40
n (integer)	–	2	2	2

These rare alleles represented 20.7%, 23.1%, and 28.4% of the total AFLP set, respectively. Taking into account the rare alleles shared by two islands (fig. 4), the highest rate is shown by COR and SAR (87 alleles shared); MAJ shares 65 rare alleles with SAR and only 52 with COR. Considering the whole AFLP set, the proportion of rare alleles captured by choosing only one population of the entire range of the species (i.e., R-value) was 22.74%. Considering the three geographic areas independently, R-values of 34.36% (MAJ), 41.09% (COR), and 25.54% (SAR) were obtained (table 2 and fig. 4).

Based on the island distribution of these rare alleles, and after choosing the PSA for each of them, a total of 99 alleles were assigned to MAJ, 138 to COR, and 161 to SAR (table 2 and fig. 4). The optimal proportion of the populations to be sampled within each geographical area was: 0.29 (MAJ), 0.38 (COR), and 0.33 (SAR). For Majorca, 1.26 populations were targeted; for Corsica, 1.61; and for Sardinia, 1.4 populations (table 2 and fig. 2).

Many *A. balearica* populations are inside some type of Protected Area (i.e., Natural Park, National Park, etc.; table 1) with management categories according to their conser-

► **Figure 3** – Regression lines of the average allele frequency (x-axis) with $-\log(L_0)$ (grey diamonds) and $-\log(L_1)$ (black circles) for the alleles that fulfilled the criteria stated before over the three geographical regions (Majorca, Corsica, and Sardinia) of *Arenaria balearica* and over the full set of rare alleles. The quotient between the slopes of the expected and the observed regression lines indicates the percentage of rare bands captured when sampling a single population.



vation objectives (Dudley 2013) within the three countries. Therefore, most of the populations on MAJ are located inside a protected area (Paraje Natural Serra de Tramuntana), and only population 9 from Majorca is located outside this area. This is also the case in the COR area where all populations except for populations 10 and 17 are under protection (Parc Naturel Régional de Corse and Special Area of Conservation Massif du Tenda et forêt de Stella). Sardinia, however, contains only four populations under protection (i.e., population 15 in the Area Marina Protetta Tavolara - Punta Coda Cavallo, population 20 in the Parco Nazionale del Genargentu, population 18 in the Special Area of Conservation Monte Limbara, and population 20 in the Special Area of Conservation Monte Albo; table 1).

Distribution modelling

The model corresponding to the potential present distribution of the species (fig. 5A) showed high predictive accuracy (AUC = 0.824; Kappa = 0.346; accuracy = 0.883). The currently known distribution of the species mostly coincided with that predicted by the model. From the three bioclimatic variables used in the analyses, bio18 (precipitation of the warmest quarter) showed the highest explanatory power (%IncMSE = 30.9), followed by bio8 (mean temperature of the wettest quarter; %IncMSE = 21.1) and bio2 (mean di-

urnal temperature range; %IncMSE = 2.4). Majorca showed the smallest area with suitable habitat for the species.

The projection of the present distribution model over the future (CMIP5_2050 RCP 2.6) climatic layers shows very few areas with similar climatic characteristics to the current ones (fig. 5B). Regarding the CMIP5_2070 RCP 2.6, CMIP5_2050 RCP 2.6, and CMIP5_2070 RCP 4.5 climatic layers, the model found no similar climatic areas to the current ones.

DISCUSSION

Genetic diversity of *A. balearica*

The AFLP analysis revealed low levels of genetic diversity (Nei's GD = 0.159), which together with the rarity levels found in *A. balearica*, is evidence of a genetic deterioration of the species. This is in contrast with other research regarding palaeoendemic Mediterranean species that found higher genetic diversity levels (e.g., *Petagnaea gussonei* (Spreng.) Rauschert, De Castro et al. 2013), while low genetic diversity is generally related to founder events of neoendemics (Fernández-Mazuecos et al. 2014).

While many endangered endemic species show higher values of genetic diversity (*Flagellaria guineensis*

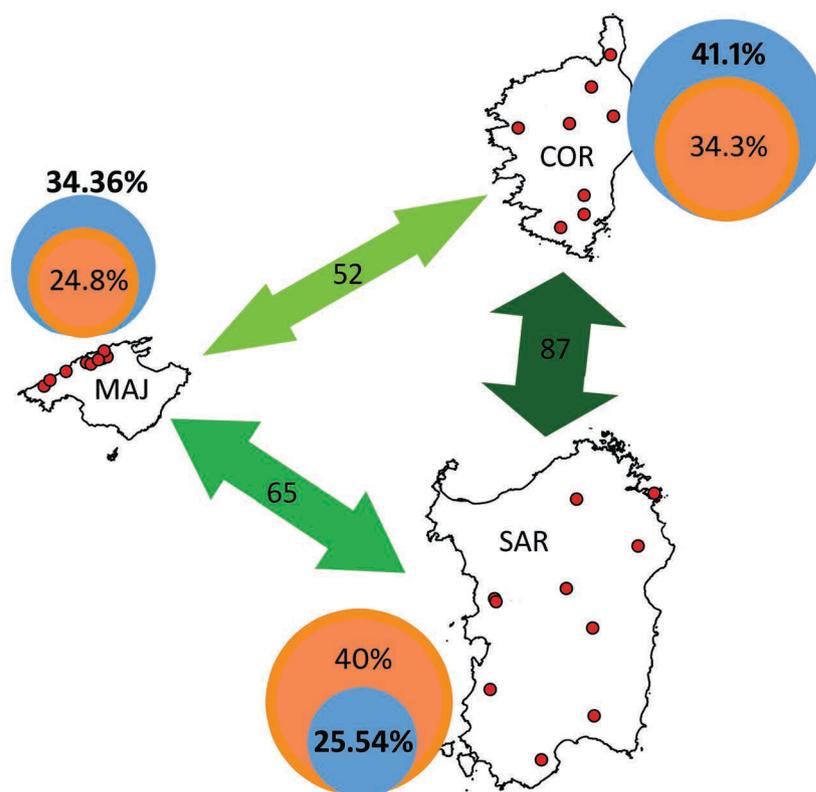


Figure 4 – Rare AFLP alleles and R-value distribution in the three geographical regions (Majorca, Corsica, and Sardinia). Green arrows indicate the number of rare alleles shared between areas; orange circles represent the proportion of rare alleles chosen by PSA; blue circles represent the R-value (proportion of rare alleles captured by sampling only one population by area).

Schumacher, Hamrick et al. 1991; *Carthamus balearicus* (J.J.Rodr.) Greuter, Vilatersana et al. 2007 (as *Femeniasia balearica*)), the genetic diversity of *A. balearicus* is similar to values found in extremely narrow endemic species (*Ligusticum lucidum* subsp. *huteri* (Porta) O.Bolòs, López-Pujol et al. 2013 (as *Coristospermum huteri*); *Staphisagria picta* subsp. *requienii* (DC.) B.Bock, Orellana et al. 2009 (as *Delphinium requienii*); *Astragalus cremnophylax* Barneby, Travis et al. 1996; *Agrostis barceloi* L.Sáez & Rosselló, Massó et al. 2016). Long-term isolation, both geographical and ecological, and inbreeding over long periods may explain these levels of diversity. This is also supported by the haplotype network of the species (Bobo-Pinilla et al. 2016): the star-

like topology suggests that the main haplotype holds the ancestral signal, while the secondary haplotypes indicate isolation and genetic drift.

Protecting the genetic diversity and evolutionary potential of a species could reduce the probability of extinction (Frankham 2005) as it is closely linked to the ability of a species to respond to environmental changes (Huenneke 1991).

Ex situ and in situ conservation proposal

The identification of the populations that represent the diversity and rarity of the species is essential to develop appropriate conservation guidelines (Ryder 1986; Ciofi & Bruford

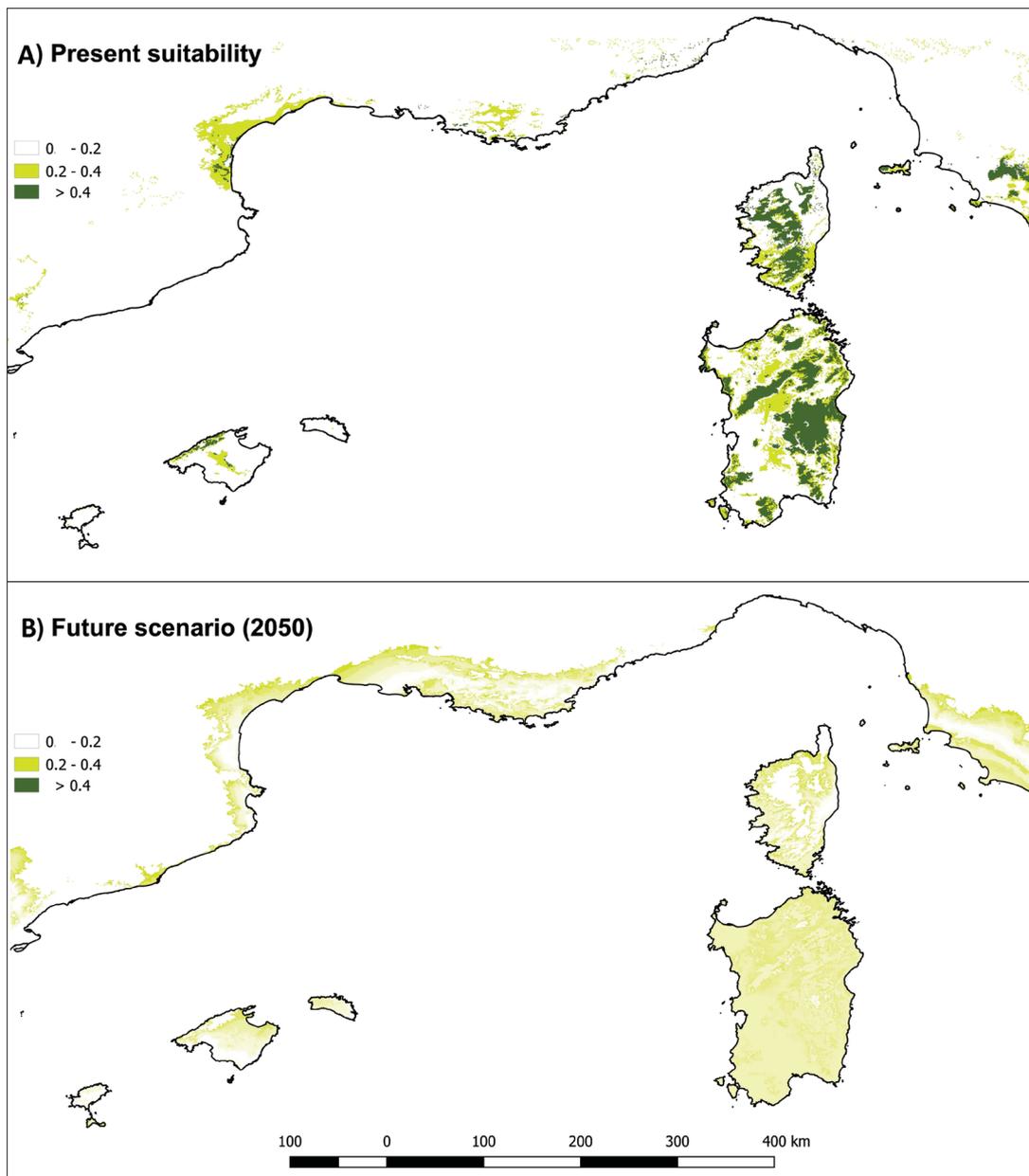


Figure 5 – Maps showing the result of the distribution modelling (maps created with R, packages *randomForest* and *raster*, see Material and methods). The habitat suitability is represented by green tones (dark green = high, light green = medium/low). **A.** Current habitat suitability. **B.** Habitat suitability under the future scenario CMIP5_2050 representative concentration pathway 2.6.

1999). The RGUCs sampling strategy permitted us to decide which and how many populations to choose for conservation purposes (Pérez-Collazos et al. 2008; Peñas et al. 2016). Based on the AFLP data and the island distribution of the rare alleles, 1.26 populations were targeted from Majorca, 1.61 from Corsica, and 1.4 from Sardinia (table 2). This number should be set to 2 as the real number of populations to be protected in each area. This selection should cope with the high genetic conservation standards needed.

Genetic diversity should be the priority when choosing the populations, but rarity should not be overlooked. With respect to plastid variation, the preservation of all haplotypes should be a primary goal since this variation represents the paleo-phylogeographic signal of the species (Rumeu et al. 2014). The currently limited suitable area for the species in Majorca implies a greater risk of loss of genetic diversity and this is consistent with lower numbers of rare alleles combined with the low variation of plastid haplotypes (Bobo-Pinilla et al. 2016).

Regarding Majorca, populations 7 and 9 should be considered as of conservation priority because they harbour higher levels of diversity and rarity (table 1 and fig. 2). Population 12 from Corsica also holds higher diversity and rarity and should therefore be considered for conservation; the same applies to population 17, which has the highest diversity level within the species (and rarity values similar to the other populations). In Sardinian populations, the diversity estimates are especially high in populations 19 and 25 and consequently these populations are proposed as of conservation priority.

With respect to the conservation of the haplotypes, all haplotypes from Majorca and Corsica are located in protected areas, however, *ex situ* conservation is the best option to preserve the plastid diversity for populations 1 from Majorca and 13, 15, and 16 from Corsica. The populations located in Sardinia are least protected, with only four known populations under protection (populations 18, 19, 20, and 24). Additionally, the populations 18, 20, 23, and 27 are also recommended for *ex situ* conservation as they hold different haplotypes (table 1, fig. 2).

Facing climate change

Given our results, the current climatic conditions characterizing the distribution area of *Arenaria balearica* are expected to severely change in a future scenario, being a potential threat for the species' survival (fig. 5). The species distribution model is performed with macroclimate information and, in this specific case, microclimate features could be important drivers of habitat suitability as well. The most explanatory variable of the model, precipitation of the warmest quarter (bio18), indicates the need of the species for specific humidity conditions, especially in the warmer months, which seems to be directly linked to the microclimatic conditions of humidity of these areas, as has been detected by other authors as well (Piñar Fuentes et al. 2017).

The Mediterranean area is considered one of the regions that will face the most extreme climate changes worldwide (e.g., Giorgi & Lionello 2008; Médail 2017). The Mediterranean basin requires research to determine the populations

and areas that should be the focus of conservation efforts. Plant species linked to wet and rocky habitats (as *A. balearica*) may be the most vulnerable (Blondel & Médail 2009) as these peculiar habitats are regarded as long-term stability sites with a reduced spatial scale (e.g., Médail & Diadema 2009; Harrison & Noss 2017). In most cases, this could imply the reduction of the plant's ability to adapt in a climate-change scenario. The areas having these types of habitats are not interconnected and the dispersal capacity of many of the resident plants is severely limited due to specialization (Fernández-Mazuecos et al. 2014).

Plant populations can respond to climate change in three different ways: migration, *in situ* adaptation, or extirpation (Christmas et al. 2016). To avoid disappearance (extirpation *sensu* Christmas et al. 2016), the species should be able to migrate and/or adapt locally. There is a high degree of habitat diversity in Mediterranean-type ecosystems, which may represent an 'ecological insurance' that allows species to migrate locally to more favourable ecological niches (Médail 2017). However, successful migration via natural dispersal of *A. balearica* would be highly unlikely due to the extreme specificity of the habitat and the low availability of niches. The probability of adaptation *in situ* is also uncertain, although the response and capacity to adapt to future climatic scenarios needs to be evaluated experimentally.

Giving the characteristics of the species and the results obtained, it seems adequate to establish an *ex situ* conservation proposal in order to be prepared for possible disturbances that could reduce the population sizes. Firstly, the populations that are not part of the RGUCs selection must be considered for *ex situ* conservation if those areas are under the threat of significant climate change (fig. 2); for instance, seed banks can be used to guarantee the survival and long-term maintenance of genetic diversity (Bacchetta et al. 2008). Moreover, the low levels of genetic diversity also sustain the proposals of reinforcement of impoverished populations and assisted colonization of potentially suitable areas. Assisted migration can be implemented by selecting seeds of appropriate origin (Aitken & Whitlock 2013), but the risk of outbreeding depression needs to be considered (Breed et al. 2013). Assisted colonization in areas outside the current distribution areas can be considered, i.e., translocation to places identified as adequate through modelling (Schwartz 2012). Human intervention might therefore be required through well-planned and carefully managed conservation and restoration activities (Christmas et al. 2016), but for this, a thorough knowledge of the species is essential.

Complementary microclimate studies over this chomophyte communities are recommended to identify the specific drivers of habitat suitability and to help the selection of conservation areas for *A. balearica*. Our work may contribute to different possible conservation solutions for *A. balearica*, since we have evaluated the suitability of the habitat under future climatic scenarios, and identified the populations that best represent the genetic rarity and diversity, in order to preserve the phylogeographic signal and the evolutionary potential of the species.

SUPPLEMENTARY FILE

One supplementary file is associated with this paper:

Supplementary file 1: Probabilities of loss of 474 rare AFLP alleles when all populations of *Arenaria balearica* are considered as one single management unit and preferred sampling area.

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