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Present and potential distribution of the endangered tree *Prunus lusitanica* subsp. *azorica*: Implications in conservation

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Abstract
Prunus lusitanica subsp. *azorica* is an endangered tree endemic to the Azores Archipelago, considered as top priority for conservation. It is currently found in São Miguel, Terceira, São Jorge, Pico, Faial, and Flores islands (i.e. in 6 out of the nine islands) occurring at altitudes above 500 m, mainly in craters and deep narrow ravines, or scattered in hyper-humid native forest. In this paper we evaluate the conservation status of this taxon based on its present abundance and distribution. We also estimate its potential distribution using Ecological Niche Factor Analysis and discuss conservation measures. The present area of occurrence encompasses 20.5 km^2 with around 91 to 200 mature individuals. The islands of Faial, São Jorge and Terceira show the most depauperate populations and in Flores only one individual was found. According to the IUCN criteria *P. lusitanica* subsp. *azorica* should be considered as critically endangered [B1ab(iii)+2ab(iii); C2a(i)], considering that the extant areas of occupancy are small, there is a severe fragmentation of the populations and a continuing decline of the available area and quality of the habitat, unless active conservation measures are taken. Modelling results showed that *P. lusitanica* subsp. *azorica* is more likely to occur at relatively high elevations with relatively low temperature, high relative humidity and small annual variation, when compared to the average conditions in the Azores; it is associated with narrow conditions of superficial water flow accumulation and altitude as compared to the range of conditions available in the Azores. Potentially, the current distribution of *Prunus lusitanica* subsp. *azorica* could increase considerably in several islands. In addition to habitat restoration measures, a conservation plan could include programs of vegetative and seed propagation to reinforce the most depauperate populations and eventually actions to expand *P. lusitanica* subsp. *azorica* to potentially suitable locations, while preserving its genetic variability and identity.

1. Introduction

It has been estimated that about 25% of vascular plant species known today could disappear within 20 years (Given, 1994). Furthermore, about 60,000 out of 287,655 species of plants known in the world are facing the threat of the extinction. The 2012 IUCN Red List of Threatened Species contains assessments for almost 70,000 species, of which about 40,000 include spatial data (IUCN, 2012). Of the 20,000 species of vascular plants in Europe, 1826 were evaluated and 467 identified as endangered (Bilz *et al.*, 2011). Recognizing the critical situation of the vegetation worldwide a Global Strategy for Plant Conservation (GSPC) was established worldwide and adopted by the Convention on Biological Diversity (CBD, 2002a). The GSPC has set targets and objectives for the period reporting to 2011-2020 (CBD, 2010a) and has demanded "an assessment of the conservation status of all known plant species, as far as possible, to guide conservation actions".

The fauna and flora of oceanic islands have special features unparalleled in other terrestrial ecosystems, being of great relevance to studies on biodiversity (Hortal *et al.*, 2005). Many island species are unique, have a considerable importance in terms of conservation and are especially vulnerable endemic species that occupy small native forest fragments, while under invasive species pressure (Martin *et al.*, 2010; Silva *et al.*, 2008). Although islands comprise only some 5% of the Earth's land surface, about one quarter of all known extant vascular plant species are endemic to islands (Kreft *et al.*, 2008). Indices of vascular plant diversity are markedly higher for islands than for continental areas (Kier *et al.*, 2009), and 20 of the 34 biodiversity hotspots defined by Conservation International (Myers *et al.*, 2000; Biodiversityhotspots.org, 2013) are islands, or have an important insular component. Caujapé-Castells *et al.* (2010) estimated that between 3,500 and 6,800 of the estimated 70,000 insular endemic plant species worldwide might be endangered (CR+EN) of which between 2,000 and 2,800 would be critically endangered (CR).

The total number of terrestrial endemic species from the Azores is 411 however, when compared to the neighboring Macaronesian archipelagos (Madeira and Canaries), the Azorean terrestrial fauna and flora is characterized by a lower percentage of endemism (only 7%, which contrasts with nearly 20% for Madeira and 30% for the Canary Islands; Borges *et al.*, 2010). The vascular plants comprise 1,110 taxa (14%) and are an important component of the currently known Azorean species diversity. Of these, 73 (6.6%) taxa are endemic to the Azores (Borges *et al.*, 2010; Carine and Schaefer, 2010; Silva *et al.*, 2010). Fifty percent of the Azorean vascular endemic plants are considered as a priority in terms of future conservation actions, 36 being included in the Top 100 Azores (Cardoso *et al.*, 2008). Those species mainly occur in natural forests (laurel and juniper) in native scrubland and in coastal areas, the main threats including habitat degradation, expansion of agricultural fields and of production forest and competition with invasive species (Martín *et al.*, 2008). Several of these endemic species have important ecological roles (Cardoso *et al.*, 2008) and according to Corvelo (2010), after applying the criteria defined by the IUCN, 7 (10%) endemic species should be considered as critically endangered (CR), 20 (28%) as endangered (EN), 18 (25%) as vulnerable, 17 (24%) as near threatened, 4 (5%) as of least concern, 5 (7%) with insufficient data, and 1 (1%) as extinct.

Prunus lusitanica L. subsp. *azorica* (Mouill.) Franco commonly known as Azorean cherry or ginja-do-mato, is an endangered tree endemic to the Azores, with an ecological and ornamental interest. It is important as a laurel forest component, particularly at medium altitude (Silva *et al.*, 2009), and as a food source for the endangered bird *Pyrrhula murina* (Arosa *et al.*, 2009). Currently found in São Miguel, Terceira, São Jorge, Pico, Faial and Flores islands (Silva *et al.*, 2010), this species has become very rare and recently, only scattered individuals were found in Faial and Pico, while in Flores only one individual is known to exist. Considered as one of the Top 100 priority taxa for conservation in Macaronesia (Martín *et al.*, 2008), *P. lusitanica* subsp. *azorica* was listed as Rare (R) on the 1997 IUCN Red List of Threatened Plants (Walter and Gillett, 1998); in 1998 Oldfield *et al.* (1998) listed this taxa as Endangered (EN B1+2ce) in the World List of Threatened Trees; in the IUCN Red List 2011, the population size was estimated to be less than 250 mature individuals and it was considered as Endangered (EN D); it is protected under the Habitats Directive (Annex II) and the Berne Convention (Annex I). *Prunus lusitanica* subsp. *azorica* is usually a small tree or shrub, rarely above 4 m tall. However, in sheltered locations (i.e. water stream margins) it is able to grow up to more than 10 m high. It occurs at altitudes above 500 m, mainly in craters and deep narrow ravines, or scattered in hyper-humid native forest (Silva *et al.*, 2009). Recent studies devoted to *P. lusitanica* subsp. *azorica* targeted vegetative and seed propagation (Moreira *et al.*, 2009; 2012), and population genetics (Moreira *et al.*, 2013; García-Verdugo *et al.*, 2013).

However, to design effective conservation strategies or recovery plans, information on plant distribution, population number, population size and on the ecological constraints to species establishment are considered as fundamental (Brigham *et al.*, 2003). Geographic Information Systems (GIS) provide opportunities to carry out spatial analyses of genetic diversity patterns identified with markers (Kozak *et al.*, 2008). GIS are also an acknowledged tool to prioritize areas for conservation of plant genetic resources (Guarino *et al.*, 2002). Moreover, results obtained using GIS can be presented in a clear way on maps, which facilitates the incorporation of these findings into the formulation of conservation strategies and the implementation of conservation measures (Jarvis *et al.*, 2010). Assessing the spatial distribution of rare and endangered species is essential for efficient conservation management (Margoluis and Salafsky, 1998; Stem *et al*., 2005). Species distribution modelling tools have become increasingly popular in ecology and are being widely used in many ecological applications (Elith *et al*., 2006; Peterson *et al*., 2006). These models establish relationships between occurrences of the species and the biophysical and environmental conditions in the study area. A variety of species distribution modelling methods are available to predict potential suitable habitat for a species (Guisan and Zimmermann, 2000; Guisan and Thuiller, 2005; Elith *et al*., 2006; Guisan *et al*., 2007a,b; Wisz *et al*., 2008). The goal of habitat suitability modelling is to identify areas of habitat suitable for the persistence of a species based on a set of variables describing environmental conditions (Franklin, 1995; Guisan and Zimmermann, 2000; Hirzel and Le Lay, 2008). The ecological niche factor analysis (ENFA) implemented in the software BIOMAPPER by Hirzel *et al*. (2002), is a profile based approach which compares the conditions prevailing on sites with proved species presence with those found across the entire study area. It has been successfully used to model invasive and native species in the Azores (Costa *et al*., 2012; Costa *et al*., 2013a).

This study aims i) to evaluate the present distribution and abundance of *P. lusitanica* subsp. *azorica* in the Azores; ii) to re-evaluate its conservation status, according to IUCN criteria, based on the new data recently made available; iii) to determine the environmental factors that might limit its distribution; iv) to estimate its potential distribution; and v) to assess the possibilities of reforestation. Based on previous knowledge about this species, we expect to find that *P. lusitanica* subsp. *azorica* has a somewhat different ecological niche than the Azorean average environmental conditions and some degree of specialization (e.g. wet, sheltered locations). Based on the results of this research and on the work recently performed on propagation and population genetics, conservation guidelines for the establishment of a future recovery plan are discussed.

2. Materials and Methods

2.1. Study Site

The archipelago of the Azores (36º35'–39º43'N, 24º45'–31º17'W), scattered across 615 km on a WNW–ESE alignment, covering a total of 2352 km^2, comprises nine volcanic islands in three main groups: western (Flores and Corvo islands), central (Terceira, São Jorge, Pico, Faial, and Graciosa islands), and eastern (São Miguel and Santa Maria islands). The topography of the islands is characterized by large catchments, ravines, and seasonal water streams, with maximum elevation ranging from 450 m in Graciosa to 2351 m in Pico, with several islands peaking near 1000 m. The Azores climate can be classified as mesothermal (average annual temperature of 17.5ºC) humid, being strongly oceanic, with small temperature changes, high precipitation and relative humidity values (Silva *et al*., 2008). The long distance to the nearest mainland (Europe, 1300 km), and their low geological age and the homogeneous oceanic climate of the islands, with low thermal amplitude and high relative humidity and rainfall throughout the year, partly explain the lower number of endemic species compared with the neighbouring archipelagos of Madeira and the Canary Islands (Carine and Schaefer, 2010). Several natural plant communities, however, existed prior to human settlement, including coastal and wetland vegetation, meadows, and various types of scrubland and forest (Dias, 1996). Subsequently, the increasing fragmentation of natural plant communities associated with the expansion of monoculture landscapes for pastures and forests has drastically altered the biodiversity in many areas of the archipelago, leading to the expansion of non-indigenous and invasive species (Silva and Smith 2006; Silva *et al*. 2008). About 24% of the Azores territory is presently under legal protection (Cardoso, 2008), including different types of areas for conservation. These include the UNESCO Biosphere Reserves, Ramsar sites, Natura 2000 sites (Gil, 2006) and more recently, Island Natural Parks (Calado *et al*., 2009).

2.2. Species Data

Prunus lusitanica subsp. *azorica* presence data were obtained from field surveys: (i) from a random sampling performed in 2001 (described in Silva and Smith, 2006); and (ii) from collecting trips for the AZB Herbarium DNA Bank conducted in 2007, 2008 and 2010.

The 2001 field survey is included in ATLANTIS, which is a regional species database of 500 m grid-based spatial incidence information for about 5000 species (Borges *et al*., (2010; The Azorean Biodiversity Portal, 2013). We matched the data sources so that both refer to a 500 m spatial resolution. As a result, we used 82 occurrences recorded across the Azores (Figure 1).

2.3. Conservation Status

The conservation status of *P. lusitanica* subsp. *azorica* was revised according to version 3.1. IUCN Red List Categories and Criteria (2001). We based our analysis mainly on the area of occurrence, estimated numbers of populations and of individuals and on the degree of population fragmentation. Long term monitoring data are not available for this species, so recent declines are difficult to quantify. We also estimated the proportion of the area of occurrence located inside Island Natural Parks, and thus under some type of legal protection.

2.4. Ecogeographical Variables

We used ecogeographical variables (EGV) of climate, topography and land cover. Climatic variables were selected from the CIELO Model developed by Azevedo (1996). In CIELO Model, a raster GIS environment with 100 m spatial resolution is used to model local scale climate variables relying on limited available data from synoptic coastal meteorological stations. More information on the CIELO Model is available in Azevedo *et al*. (1999) or through the

CLIMAAT project in Azevedo (2003). We submitted the annual average of the minimum, maximum, mean and range values of temperature, relative humidity and precipitation to principal component analyses (PCA) since most were highly correlated. We held the first two components for temperature (TPC1-2) and relative humidity (RHPC1-2) and the first one for precipitation (PPC1), explaining 99.67, 99.98 and 90% of the original variables' variance, respectively. For topography, we used a digital elevation model (ALT) available in the CIELO Model database and derived new information from it with a GIS (ESRI® ArcGIS^{TM} 9.3): slope (SLP), summer hillshade (HS), winter hillshade (HW), flow accumulation (FLOW) and curvature (CURV). Hillshade is a simulation of the lighting conditions on the surface dictated by the topography and the position of the Sun. We considered either the summer or the winter solstices. Flow accumulation is the accumulated water flow from all cells flowing into each downslope cell, considering the surface as impervious. Curvature is the second derivate of the surface, thus finding flat, convex and concave areas. Finally, land cover data was also obtained from the CIELO Model database. The land cover classes were sorted in the following order to define an ordinal land cover variable (OLC), from "like forest" to "unlike forest": (1) forest, (2) natural vegetation, (3) pastureland, (4) agriculture, (5) barren/bare areas and (6) urban/industrial areas. All the EGV were resampled to 500 m to match the presence data.

2.5. Modelling

The ecological-niche factor analysis (ENFA) implemented in the Biomapper 4.0 software (Hirzel *et al.*, 2007) was used to estimate the potential distribution of *P. lusitanica* subsp. *azorica.* ENFA compares the distribution of locations where the focal species was identified to a reference set in the multidimensional space of the EGV previously selected to run the analysis. From this comparison, the so-called global marginality and specialization coefficients are calculated. The former is interpreted as the difference between the conditions used by the focal species and the average conditions available in the study area. Most often it ranges from zero to one and a large value means the species lives in a very particular habitat. The specialization describes the narrowness of the species niche and any value exceeding unity indicates some form of specialization. In mathematical terms, the comparison is analogous to a PCA, but here the first factor is calculated so that it accounts for all the marginality of the species and the following factors so as to maximize the specialization not explained in the first factor. Consequently, the resulting ENFA factors have an ecological meaning and enables for modelling the species habitat suitability (HS) for the whole study area through an *environmental envelope* technique. This technique is conceptually very close to the niche theory and it consists in delineating, in the space of the EGV used, the hypersurface that circumscribes all suitable conditions for the species. As a result, HS is expressed in a continuous raster map of HS scores ranging from 0 to 100. These HS scores do not equal probabilities of species presence but relative likelihood of species presence (for a full description of the ENFA, see details in Hirzel *et al.*, 2002; Hirzel and Arlettaz, 2003; Hirzel *et al.*, 2006). The evaluation of the results relied on the continuous Boyce Curve (Hirzel *et al.*, 2006). This is a threshold-independent method based on presence only data, estimating how much the modelling results differ from random expectation, which is assumed to be a measure of the quality of the model. A good HS model yields a monotonically increasing curve. This method is applied within an ordinary *k*-fold cross-validation procedure, thus defining averaged curves and dispersal measures. Finally, the continuous Boyce curve was also used to reclassify continuous HS scores into discrete classes of suitability as this is more honest and practical (Liu *et al.*, 2005). Therefore, three classes of suitability were created following guidelines from Hirzel *et al*. (2006) and Costa *et al*. (2013a).

3. Results

Currently, *P. lusitanica* subsp. *azorica* is present on six islands (Flores, Pico, Faial, Terceira, São Jorge and São Miguel), distributed by nine very fragmented populations, and occupying a relatively small area (Table 1), with ca. 91 to 200 mature individuals sampled. The area of occurrence included 82 cells (500 x 500 m) corresponding to a total of 20.5 km^2 assuming total coverage of those cells by *P. lusitanica* subsp. *azorica*. This is however not expected, since the populations are very small, and thus the area of occupation is much smaller than the area of occurrence.

Table 1. *Distribution of Prunus lusitanica subsp. azorica in the Azores. Extent of occurrence in terms of the number of occupied cells (500 x 500 m), the corresponding area in km^2 and the number of individuals sampled, for the Azores and inside Island Natural Parks (INP). For INP the '%' was calculated relative to the total area occupied per island or in the archipelago.*

Island	Azores			INP		
	Cells	km^2	# Trees	Cells	km^2	%
Corvo	0	0	0	0	0	0
Faial	17	4.25	6	2	0.50	12
Flores	5	1.25	1	0	0	0
Graciosa	0	0	0	0	0	0
Pico	22	5.5	18	18	4.50	82
Santa Maria	0	0	0	0	0	0
São Jorge	1	0.25	9	0	0	0
São Miguel	4	1	46	3	0.75	75
Terceira	33	8.25	11	26	6.50	79
Total	82	20.50	91	49	12.25	63

The area of occurrence within INP, 12.3 km^2, corresponded to 59.8% of the total. On the islands of São Jorge and Flores the whole area of occurrence fell outside the parks boundaries. In São Miguel, Terceira and Pico, the areas within INP ranged from 75 to 82% of the total. However, only 12% of the area occupied by the existing populations on Faial Island is included in the INP.

According to the IUCN criteria a taxon is Critically

Endangered when the best available evidence indicates that it meets any of the criteria A to E for that category, and it is therefore considered to be facing an extremely high risk of extinction in the wild.

Based on the data collected, the proposed conservation status for *P. lusitanica* subsp. *azorica* is CR [B1ab(iii)+2ab(iii); C2a(i)]: i) Extent of occurrence estimated to be less than 100 km^2 (B1), estimates indicating severely fragmented or known to exist at only a single location (a), and continuing decline, observed, inferred or projected, in area, extent and/or quality of habitat (biii); Area of occupancy estimated to be less than 10 km^2 (B2), and estimates indicating severely fragmented or known to exist at only a single location (a), and continuing decline, observed, inferred or projected, in area, extent and/or quality of habitat (biii); Population size estimated to number fewer than 250 mature individuals and a continuing decline, observed, projected, or inferred, in numbers of mature individuals (C2) with no subpopulation estimated to contain more than 50 mature individuals (ai).

Regarding modelling, the global marginality coefficient calculated by the ENFA was very high (1.078) and the specialization coefficient was high (2.663). These results show that *P. lusitanica* subsp. *azorica* occurs in sites with environmental conditions which differ from the average environmental conditions of the archipelago, and also a limited niche breadth, considering the multidimensional space of EGV and presence data set used. The importance and role played by each EGV is revealed by the correlations between the EGV and the ENFA factors (Table 2).

The most important variables determining *P. lusitanica* subsp. *azorica* distribution are more correlated with the marginality factor: altitude, relative humidity and temperature. The positive symbols for the altitude, and the principal component of the temperature indicate that *P. lusitanica* subsp. *azorica* is more likely to occur at sites with relatively high elevation, low temperature and small temperature variations.

The negative symbols for the first principal component of relative humidity indicate that the species is more likely to occur in areas characterized by high values of relative humidity with low annual variation, while the positive symbols for the second component indicate high values for the minimum relative humidity. Also, negative symbols for the first principal component of rainfall indicate preference for locations with high rainfall. In terms of specialization, the highest coefficients for factors 2 and 3 were obtained for flow accumulation and altitude, corresponding to a narrow niche relative to the range of values available in the Azores for those EGV. Regarding altitude, the preferred values are middle altitudes (400-700 m), while for flow accumulation the values tend to be smaller than average (i.e. sites with large slopes and thus low water accumulation, but also at plateaus or hill tops). Based on these results, ENFA modelled the potential distribution for *P. lusitanica* subsp. *azorica* throughout the Azores (Figure 1). We divided the potential habitat of *P. lusitanica* subsp. *azorica* into suitable and not suitable based on the continuous Boyce curve (Figure 2). Around 18% of the total area of the archipelago is suitable as habitat for this species. On the island of São Jorge around 25% of the total area is potentially suitable. The islands of Faial, Pico, São Miguel, Flores, Terceira and São Jorge include between 16 and 25% of the area as suitable habitat. On the islands of Corvo, Graciosa and Santa Maria 94-100% of the available area corresponds to unsuitable habitat (Table 3).

Table 2. *Correlations between the EGV and the ENFA factors. Factor 1 is the marginality factor, which explains 100% of the marginality and 28% of the specialization. Factors 2 and 3 are specialization factors, which explain the remaining specialization in decreasing amounts (in brackets). Only the first three factors are shown for practical reasons. (+) Species was found in locations with higher values than average; (-) Means the reverse. A larger number of symbols indicates higher correlation. (*) Species was found occupying a narrower range of values than available. The larger the number of asterisks, the narrower the range. No symbol - very low correlation.*

EGV	Factor1 (28%)	Factor2 (26%)	Factor3 (11%)
ALT	+++++	***	****
RHPC1	- - - -		*
TPC1	++++	*	**
PPC1	- - - -		*
OLC	- - - -	*	*
RHPC2	++	**	***
SLP	++	***	***
TPC2	- -	*	
FLOW	-	********	
HW	+		**
CURV	+	*	
HS	-		**

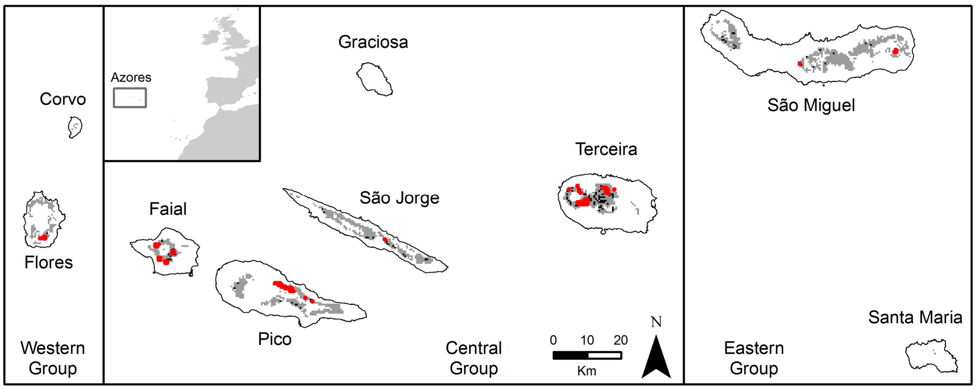

Fig. 1. *Distribution of Prunus lusitanica subsp. azorica in the Azores islands. Presently known distribution (red dots) obtained by field sampling, and*

potential distribution estimated using ENFA - highly suitable area (black), moderately suitable area (grey), and area not suitable (white).

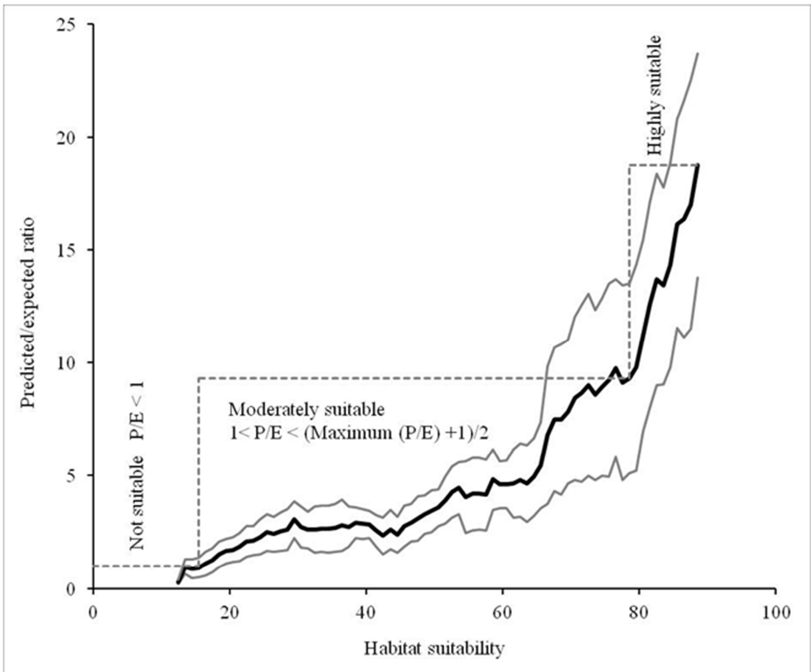

Fig. 2. *Average continuous Boyce curve and the confidence intervals at 95% confidence level. The X-axis is the habitat suitability value (HS) calculated by the model. The Y axis is the ratio between the values of occurrences of Prunus lusitanica subsp. azorica predicted by the model (P) and the expected value if the distribution of occurrences was random (E). As higher the P/E ratio, better is the model. The continuous Boyce curve was the basis for reclassifying the model to discrete classes of habitat suitability: 0- 16, not suitable; 16 - 78, moderately suitable, 78-100, highly suitable*

Table 3. *Area potentially suitable (moderately suitable or highly suitable) and not suitable for the occurrence of Prunus lusitanica subsp. azorica in the Azores. Results of the ENFA. Number of 500 x 500 m cells, area in km² and percentage of the island surface. Habitat suitability defined according to the shape of the continuous Boyce curve (Figure 2).*

Island	Not suitable			Moderately suitable			Highly suitable			Suitable			Island	
	Cells	km^2	%	Cells	km^2	%	Cells	km^2	%	Cells	km^2	%	Cells	km^2
Corvo	81	20.3	94.2	5	1.3	5.8	0	0	0	5	1.3	5.8	86	21.5
Faial	632	158.0	83.9	106	26.5	14.0	15.0	3.8	2.0	121	30.3	16.1	753	188.3
Flores	496	124.0	79.7	124	31.0	19.9	2.0	0.5	0.3	126	31.5	20.3	622	155.5
Graciosa	274	68.5	100.0	0	0	0	0	0	0	0	0	0	274	68.5
Pico	1565	391.3	82.5	312	78.0	16.4	20.0	5.0	1.1	332	83.0	17.5	1897	474.3
Santa Maria	438	109.5	99.5	2	0.5	0.5	0	0	0	2	0.5	0.5	440	110.0
São Jorge	818	204.5	74.8	262	65.5	23.9	14.0	3.5	1.3	276	69.0	25.2	1094	273.5
São Miguel	2586	646.5	82.0	549	137.3	17.4	17.0	4.3	0.5	566	141.5	18	3152	788.0
Terceira	1354	338.5	79.6	276	69.0	16.2	70.0	17.5	4.1	346	86.5	20.4	1700	425.0
Total	8244	2061.0	82.3	1636	409.0	16.3	138.0	34.5	1.4	1774	443.5	17.7	10018	2504.5

4. Discussion

The current distribution of *P. lusitanica* subsp. *azorica* corresponds to nine fragmented populations with very few individuals, occupying a relatively small area. According to Martin *et al*. (2008) the number of individuals ranges from 50 to 250 and the distribution area is in continuous decline, which is in accordance with the data obtained in our survey. The conjunction of these factors might compromise the survival of the species in the medium to long term. One of the main objectives of a conservation program is to define a minimum viable population (MVP; Shaffer 1981) and its value indicates which populations are in need of conservation measures in order to ensure long term survival (Pavlik, 1996). MVP depends on several factors such as breeding system, ecology, and propagation ability, and can be highly variable depending on the species (Mace and Lande, 1991; Given, 1994; Nantel *et al*., 1996; Brook *et al*., 2006; Flather *et al.,* 2011). Franklin (1980) proposed the 50/500 rule that became a popular guiding principle in conservation genetics for assessing MVP. Franklin suggested that the effective population size (N_e) in the short term should not be <50 and in the long term should not be <500. Fifty was deemed desirable to reduce the likelihood of extinction in the short term because of harmful effects of inbreeding depression on demography and the long-term '500' rule was based on the theoretical N_e required to balance the loss of additive genetic variation per generation (Franklin,1980; Frankel *et al*.,1981). Below this size, populations will decline and fall into the extinction vortices described by Gilpin and Soule (1986). Based on the above general guidelines, the estimated number of mature *P. lusitanica* subsp. *azorica* individuals is probably below a minimum level capable of avoiding extinction. This is further complicated since individual island populations include even lower numbers of mature individuals.

The previous classification of *P. lusitanica* subsp. *azorica* as EN (B1+2ce) (Oldfield *et al*., 1998), implied extent of occurrence less than 5,000 km^2 or area of occupancy less than 500 km^2 (B), and evidence indicating the species to be: 1) severely fragmented or known to exist at no more than five locations; 2) continuing decline, inferred, observed or projected area, extent and/or quality of habitat (c) and number of mature individuals (e). The species so far had not been officially assessed by the IUCN criteria version 3.1 (2001). However, Corvelo (2010) suggested a classification of EN [B2ab(i,ii,iii,iv,v); C2a(i)]. Our surveys confirmed that populations are severely fragmented. Additionally, according to our results, the extent of occurrence should be estimated to be less than 100 km^2 and the area of occupancy should be estimated to be less than 10 km^2. Also, ca 88% of the populations include much less than 50 mature individuals. Considering all this factors, the classification of *P. lusitanica* subsp. *azorica* should be changed to Critically Endangered (CR). In proposing this classification we are also assuming that if no conservation measures are taken, the quality and area of the habitat will continue to decline due to expansion of invasive species inside INP (Costa *et al.*, 2013b), and due to human disturbance outside INP (Marcelino *et al.*, 2013).

Regarding modelling using ENFA, we found very high marginality and high specialization values. This could indicate limited ability to adapt to different ecological conditions. By measuring the departure of the ecological niche from the average available habitat, the marginality identifies the preference of the population or species for specific conditions of the environment, among the whole set of possibilities; and the specialization appears as a consequence of the narrowness of the niche on some environmental variables (Basille *et al*., 2008). The results showed that *P. lusitanica* subsp. *azorica* is preferentially found at sites located at middle elevation with relatively low temperature, high relative humidity and rainfall. These areas usually corresponded to laurel-juniper forest (Dias *et al*., 2005).

The modelling results also indicate that *P. lusitanica* subsp. *azorica* is not at equilibrium with the environment because its present distribution does not match its whole potential distribution. This means that this species can potentially spread to areas not yet colonized, or inversely, that its present distribution is only a small sample of its past distribution. As suggested for *Morella faya* (Costa *et al*., 2012), considerable changes in land use associated to human activities might lead to the situation where some areas with suitable ecological conditions may no longer be occupied by a native species, resulting in a characterization by ENFA as a specialist plant. This reinforces the possibility of a past decline both in the extent of occurrence and in the area of occupancy.

Considering our findings a recovery plan should thus be designed for *P. lusitanica* subsp. *azorica* taking into account the several topics which we will next address in detail.

Management of threats

Habitat loss has been, and still is, the greatest threat to biodiversity (Brooks *et al*. 2002; Hanski, 2005; Groom *et al*., 2006). In the Azores, human activities, particularly changes in land use, the extension of agricultural fields (mainly pastures) and of production forest, resulted in the complete disappearance of the previously large forests found at low and medium altitude, dominated by large trees of *Juniperus brevifolia, Ilex perado* ssp. *azorica*, *P. lusitanica* ssp. *azorica*, *Picconia azorica* and *Laurus azorica* (Martins, 1993; Dias, 1996; Silva, 2001; Martín *et al*., 2008), while human disturbance resulted in changes in community composition affecting the remains of native plant communities (Marcelino *et al*., 2013). Human activity is considered as one of the major causes of genetic erosion and extinction of species on islands through over-exploitation, habitat destruction and degradation, and exotic species introduction (Olson, 1989; Francisco-Ortega *et al*., 2000; Kingston and Waldren, 2003). Accordingly, the decrease in the fragmentation of native habitats in the Azores has been considered as a fundamental strategy in the conservation and management of plant and invertebrates species considered at risk (Hortal *et al*., 2005). The best strategy for in situ conservation of the genetic diversity of island plants is preservation of natural habitats (Francisco-Ortega *et al*., 2000). Presently, the remaining natural habitats in the Azores are legally protected within INP, thus further habitat destruction is not probable, but effective measures for habitat preservation are welcome, namely those avoiding or minimizing disturbance of sensitive areas by touristic and recreational use (Martín *et al*., 2008). However, since ca. 37% of the area of occurrence of *P. lusitanica* subsp. *azorica* is not covered by INP, the individuals on those areas are more exposed to threats such as habitat loss and degradation, biological invasions and human activities, since it was found that clear differences in species composition are associated with different degrees of anthropogenic disturbance in the Azores (Marcelino *et al*., 2013). Therefore, those individuals located outside INP lack any effective protection measures and thus are more vulnerable to human intervention. Considering the extreme rarity of *P. lusitanica* subsp. *azorica*, efforts should be undertaken by the regional administration (environmental and forestry services) in order to locate and preserve those individuals, what might demand awareness campaigns directed to land users (Martin *et al*., 2008). Also, natural disturbance associated with storms and gales, by causing landslides, are natural risks that may aggravate the situation of the species, due to the loss of individuals or to the rapid colonization of forest openings by invasive species.

In the Azores islands, more than 60% of the vascular flora corresponds to non-indigenous species (Silva and Smith, 2004; 2006). This threat is particularly serious in island ecosystems, (Caujapé-Castells *et al*., 2010; Kueffer *et al*., 2010; Martin *et al*., 2010). Several plants are now considered as serious threats to the conservation of the endemic flora of the Azores and to native plant communities (Silva *et al*., 2008), including in INP (Costa *et al*., 2013b). The continuous expansion of some invasive plants like *Hedychium gadnerianum*, *Pittosporum undulatum* and *Hydrangea macrophylla*, is threatening several fragments of native vegetation (Silva *et al*., 2008). Thus, even in the most preserved communities, invasive plants are present. Therefore, monitoring and effective control measures are needed. According to Costa *et al*. (2013a) modelling of invasive plant distributions in the Azores is feasible and could thus be used as a tool to predict and better manage plant invasions. However, other measures should be implemented for an effective control of invasive species (see Silva et *al*. 2008): early detection and eradication; risk analysis to select priority target species; education and training campaigns, since the participation of the public is crucial to control and prevent biological invasions. See Costa *et al*. (2013b) for examples of control actions devoted to invasive plants in the Azores.

Human activities have enhanced genetic migration by eliminating many of the previous ecological and geographical barriers separating populations and species (Francisco-Ortega *et al*., 2000). This could be further exacerbated by uncontrolled exchange of plant material between islands, endangering the genetic identity of the *P. lusitanica* subsp. *azorica* populations (Moreira *et al*., 2013; García-Verdugo *et al*., 2013). Thus, all recovery programs should take into consideration the available information about the genetic structure of the populations.

Augmentation strategies and maintenance of genetic variability

When it comes to conservation measures for threatened or endangered species, one of the first steps to take is to make ex situ cultivation (Baudet, 2002). Vegetative propagation using cuttings and air layering was shown to be very effective for *P. lusitanica* subsp. *azorica* (Moreira *et al*., 2009). Since the number of individuals is relatively low, clones of each individual might be obtained by vegetative propagation and preserved in a germplasm bank. At each population, however, seed propagation should be used instead to increase population numbers while maintaining genetic variability. The main objective of propagation is to potentiate populations in situ, or to return series of individuals germinated and developed ex situ, in order to ensure their survival in nature (Baudet, 2002). Moreira *et al*. (2012) obtained more than 90% of germination ex situ with *P. lusitanica* subsp. *azorica* seeds, with a seedling survival of 100%, allowing its propagation in large quantities in forestation projects while still preserving the genetic diversity. Although Azorean populations exhibit a relatively low level of genetic variability and differentiation, a substantial separation from *P. lusitanica* subspecies was found (García-Verdugo *et al*., 2013; Moreira *et al*., 2013). Thus, preserving the identity and genetic diversity of the Azorean populations is crucial. As such, the introduction of *P. lusitanica* individuals from the mainland or other Macaronesian archipelagos should be avoided at all cost, since it might reduce the genetic differentiation of the Azorean species as a result of hybridization events. The genetic diversity of a species confers biological effectiveness and allows it to adapt to different environmental conditions, so it is a biological resource that must be conserved in order to the long term maintenance of the species (Baudet, 2002). Genetic diversity is one of the most important attributes to any population; environments are constantly changing, and genetic diversity is necessary if populations are to continuously evolve and to adapt to new situations (Freeland *et al*., 2011). Knowing the distribution of diversity within and among populations is important for conservation because it provides useful guidelines for the preservation of genetic diversity (Hamrick *et al*., 1991; Hamrick and Godt, 1996). Considering that for *P. lusitanica* subsp. *azorica* more than 90% of the genetic variation was found within populations (Moreira *et al*., 2013), arbitrary translocation of material between Azorean populations risks further reducing their degree of differentiation. Instead, seed breeding programs using diaspores originating from the same populations should be favoured, eventually incorporating isolated individuals located in neighbouring locations, as these are most likely remnant individuals of a wider population. In cases where only one individual persists, as is the case of Flores, vegetative propagation should be used to avoid extinction of the local genotype. The present study showed that it is possible to expand the occurrence area of *P. lusitanica* subsp. *azorica* in several islands to a total of suitable habitat more than 400 km^2. Thus, there is a considerable potential for using this species in reforestation projects linked to biodiversity preservation or to the establishment of buffer areas surrounding important resources (e.g. water lines, lakes).

5. Concluding Remarks

All the scientific information presently available on *P. lusitanica* subsp. *azorica* could be used as a basis to design an objective and feasible recovery plan. Otherwise, decline in population extent/numbers but also in genetic diversity might continue in the future, particularly if propagation programs do not take into consideration the available knowledge on the genetic diversity and structure of the populations, or ignore the areas potentially suitable for this endemic tree.

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