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Juniperus navicularis - a study of Portuguese population structure and fitness

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Abstract. *Juniperus navicularis* Gand. is a dioecious endemic conifer that constitutes the understory of seaside pine forests in Portugal, areas currently threatened by increasing urban expansion. The aim of this study is to assess the conservation status of previously known populations of this species located on its core area of distribution. The study was performed in south-west coast of Portugal. Three populations varying in size and pine density were analyzed. Number of individuals, population density, spatial distribution and individual characteristics of junipers were estimated. Female cone, seed characteristics and seed viability were also evaluated. Results suggest that *J. navicularis* populations are vulnerable because seminal recruitment is scarce, what may lead to a reduction of genetic variability due solely to vegetative propagation. This vulnerability seems to be strongly determined by climatic constraints toward increasing aridity. Ratio between male and female shrubs did not differ from 1:1 in any population. Deviations from 1:1 between mature and non-mature plants were found in all populations, denoting population ageing. Very low seed viability was observed. A major part of described *Juniperus navicularis* populations have disappeared through direct habitat loss to urban development, loss of fitness in drier and warmer locations and low seed viability. This study is the first to address *J. navicularis* conservation, and represents a valuable first step toward this species preservation.

Keywords: clonal growth; *Cupressaceae*; endemic endangered species; fragmented population; population structure; seed viability.

[es] Estudio de la estructura y del valor adaptativo de poblaciones portuguesas de *Juniperus navicularis*

Resumen. *Juniperus navicularis* Gand. es una conífera dioica endémica de Portugal, que constituye el sotobosque de bosques de pinos junto al mar en áreas actualmente amenazadas por la creciente expansión urbana. El objetivo de este estudio es evaluar el estado de conservación de las poblaciones de esta especie en las regiones costeras del suroeste de Portugal. Se escogieron tres poblaciones debido a su diferente dimensión de fragmentación y a la densidad de pinos. Se analizaron el número de individuos, la densidad de la población; la distribución espacial, las características individuales de los arbustos, el cono femenino, las características y la viabilidad de las semillas. Los resultados sugieren que las poblaciones de *J. navicularis* son vulnerables debido al escaso reclutamiento seminal, lo que puede conducir a una reducción de la variabilidad genética. Esta vulnerabilidad parece ser determinada en gran medida por las limitaciones climáticas recientes con un incremento de la aridez en el área estudiada. Ninguna de las poblaciones analizadas muestra una relación entre arbustos masculinos y femeninos significativamente diferente de 1:1. Desviaciones a dicha proporción fueron detectadas en todas las poblaciones entre pies maduros y jóvenes, que parece indicar envejecimiento de las poblaciones. Además se observó una baja viabilidad de las semillas. Una gran parte de las poblaciones de *J. navicularis* descritos han desaparecido a través de la pérdida directa de hábitat con el desarrollo urbano, la pérdida de la aptitud en lugares más secos y cálidos y la baja viabilidad de las semillas. Este estudio es el primero en abordar la conservación de *J. navicularis*, y representa un valioso primer paso hacia esta preservación de esta especie.

Palabras clave: crecimiento clonal; *Cupressaceae*; especies endémicas amenazadas; poblaciones fragmentadas; estructura poblacional; viabilidad de semillas.

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Abbreviations: CA: coefficient of aggregation; DMS: Degrees, minutes, seconds; GIS: Geographical Information System; GPS: Global Positioning System; ICNF: Instituto da Conservação da Natureza e das Florestas; IGEO: Informação Geográfica; IUCN: International Union for Conservation of Nature; SAC: Special Area of Conservation;WGS84: World Geodetic System, 1984.

Introduction

Juniperus navicularis Gand. (= *J. oxycedrus* L. subsp. *transtagana* Franco), the Portuguese Prickly Juniper, is a dioecious shrub from *Cupressaceae* family (Franco, 1986) endemic to SW Iberian Peninsula. Spanish populations with a very different population ecology were recently described as being a geographic vicariant of the portuguese populations (Pérez Latorre, 2006), due to their genetic isolation since the Pleistocen (Boratynski & *al.*, 2014) *Juniperus navicularis* portuguese populations are threatened mainly by urban expansion and tourism development. *Juniperus navicularis* habitats were included under the Habitat Directive 92/43/CEE – Annex I, as a Priority Habitat 2250 "Coastal dunes with *Juniperus* spp." (European Commission, 1996; ICNF, 2008). Under the Portuguese law nº 49/2005 (Annex B-1), this habitat is also considered in a natural and semi-natural area of special interest, what allowed its inclusion in SAC's (Anonymous, 2005), but the species itself was not considered threatened (Dray, 1985). Beyond anthropogenic origin threats, the low germination rates of their seeds, a constraint shared with other *Juniper* species (Juan & *al.*, 2003), is another serious conservation issue for *J. navicularis.*

Along with irregular seed production, most junipers have germination limitations due to age of the juniper bush, insect predation and empty seeds (Thomas & *al.*, 2007; Verheyen & *al.*, 2009), and also to dormancy directly associated with the embryo, the seed coat impermeability or the presence of germination inhibitors (Juan & *al.*, 2006). These recruitment limitations might also be exacerbated by climate alterations, since more frequent warmer and drier summers can influence seed production and viability due to water deficit stress (García & *al.*, 1999; García & *al.*, 2000; Muñoz-Reinoso, 2003). According to IUCN Red List of Species, from the 1052 gymnosperm known species, 399 are considered threatened (38%), a number that more than doubled in the last 17 years (IUCN, 2014). Among these, 202 are conifers, representing ca. 33% of the 618 known conifers (Baillie & *al.*, 2004) one of the few taxonomic groups fully evaluated in terms of conservation status (Farjon & Page, 1999).

J. navicularis portuguese populations have a high ecological value, as they successfully occupy dry sandy soils with poor nutrient availability and, therefore, actively contribute for soil retention, nutrient and water cycle regulation in places and conditions where few species succeed (Neto & *al.*, 2004). They exert, therefore, a facilitation effect on associated flora whilst providing refuge for native fauna. Furthermore, communities formed by this species (*Daphno gnidi-Juniperetum navicularis* and *Junipero navicularis-Quercetum lusitanicae*) have higher species richness and conservation interest when compared with other Portuguese Juniper shrublands (Costa & *al.*, 2005). *J. navicularis* has also raised pharmacological interest, being researched for chemical composition of essential oils contained in its leaves (Adams, 1998; Velasco-Negueruela & *al.*, 2002) and berries (Cavaleiro & *al.*, 2003).

Despite its ecological value, Portuguese Prickly Juniper is a poorly known species, and essential information about its ecology is missing, hampering future restoration actions. The aim of this study is to provide data about population structure and fitness and to evaluate the conservation status of previously identified *J. navicularis* Portuguese populations in its core distribution area.

Materials and methods

Study species

Juniperus navicularis is a dioecious shrub up to 2m high with a dense canopy. Leaves are 4-12 x 1-1.5mm with two parallel white bands of stomata on the adaxial side. Female individuals bear 7-10 mm sphere to conic-shaped berry-like seed cones, green at first, reddish coral in full maturity (Franco, 1986).

In Portugal, this species constitutes the understory of *Pinus pinea* L. and/or *P. pinaster* Aiton pinewoods in the Pliocene low altitude (0-80m) consolidated dunes of SW Portugal coastal regions, namely those belonging to the Sadense Superdistrict biogeographical area (Rivas-Martínez & *al.*, 1990), its core distribution area (Franco & Rocha, 1982). The community named after this species belongs to the edafo-xerophytic formations of the psammophile sub-humid to dry thermo-mediterranean bioclimatic area of Portugal's Southwest coast.

Reassessing identified populations

Historical populations were identified from available bibliography on the species (e.g. Adams, 1998; Velasco-Negueruela & *al.*, 2002; Cavaleiro & *al.*, 2003) and records from Portuguese herbariums (from Universities of Algarve, Aveiro, Coimbra, Porto, Trás-os-Montes e Alto Douro, Instituto Superior de Agronomia and Jardim Botânico de Lisboa) and their current existence reassessed. Recent records of *J. navicularis* from the national herbariums presented GPS data allowing direct use of their coordinates. Populations from older records, without GPS data, were confirmed by a visit to the mentioned location and visual inspection on potential areas of occurrence of *Pinus pinea* and/or *Pinus pinaster*, two species associated with *J. navicularis*. DMS coordinates were obtained through WGS84 Datum.

We used GIS in ArcView 3.2 to estimate potential locations of *Juniperus navicularis* ocorrence (Figure 1). This was done by superposing lithological, hydrological and hypsometrical data (Agência Portuguesa do Ambiente, 2014) appropriate for this species according to Franco & Rocha Afonso (1982) and to Soil Occupation Charts (IGEO, 95). This was aimed at reducing search effort for potential *J. navicularis* Portuguese populations by eliminating areas without suitable habitat including urban areas and areas without sandy soils originating from the Pleistocene era. This map is currently the most updated map available for potential distribution of *J. navicularis* Portuguese populations.

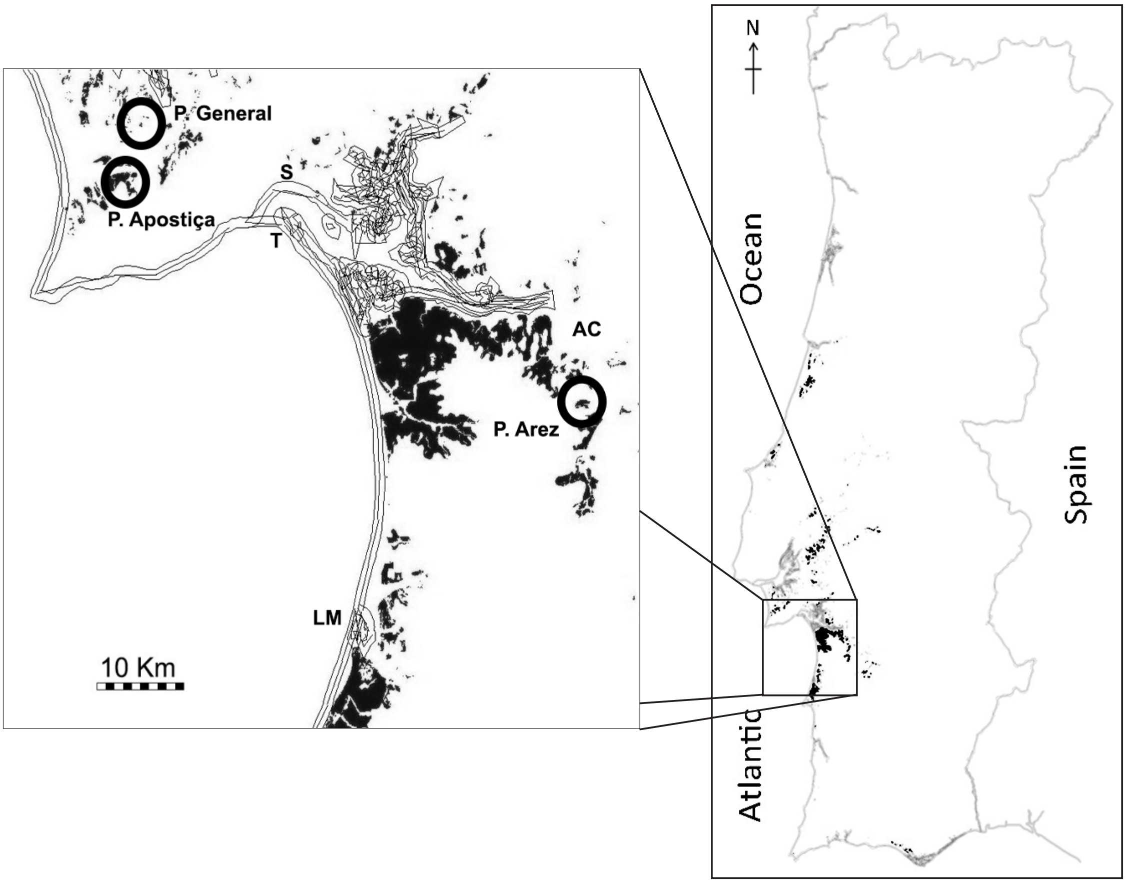
Study populations

To study the conservation status of *Juniperus navicularis*, three populations were selected within the species optimal distribution area (Figure 1) in accordance to different habitat fragmentation status due to human intervention and pine forest density and storey composition. Two large populations isolated from human occupation, one under a dense *Pinus pinaster* cover (Pinhal da Apostiça) and the other under a sparse *Pinus pinea* cover (Pinhal de Arez), and a small population (Pinhal do General) in the middle of two heavily constructed areas, named hereafter as population A, B and C, respectively (Table 1).

Table 1. Study area characteristics and *Juniperus navicularis* populations characterization: density and morphological parameters (mean values ± SE). One-way ANOVA for plants distance and height with LSD post-hoc test; Kruskal-Wallis with LSD post-hoc test applied to ranks for density and canopy area. Different letters in the same line indicate significant differences between populations mean values or mean ranks ($P<0.05$).

	Population A	**Population B**	**Population C**
Occupied area (ha)	82.51	59.37	12.61
Dominant tree	*Pinus pinaster*	*Pinus pinea*	*Pinus pinea*
Tree density (trees.ha^{-1})	56	29	17
Annual mean rainfall (mm)	616.64	516.26	676.12
Annual mean temperature (ºC)	16.15	16.29	15.81
Density (shrubs.ha^{-1}) (n=10)	4750 ± 593.8^{a}	4688 ± 668.8^{a}	2813 ± 362.5^{b}
Height of shrubs (m) (n=30)	0.49 ± 0.05^{a}	0.68 ± 0.05^{b}	0.63 ± 0.05^{b}
Canopy area (m^{2}) (n=30)	0.13 ± 0.03^{a}	0.39 ± 0.06^{b}	0.12 ± 0.02^{a}
Volume (m^{3}) (n=30)	0.10 ± 0.03^{a}	0.33 ± 0.07^{b}	0.09 ± 0.02^{a}

Figure 1. Black background areas represent potential distribution of *Juniperus navicularis* in Portugal, calculated through GIS tools (right). Close-up on the three studied areas (P. Apostiça; P. Arez and P. General, corresponding to population A, B and C, respectively) and nearest localities (S – Setúbal; AC – Alcácer-do-Sal; T – Troia; LM – Lagoa de Melides).



Population measurements were made in 2009, using distance sampling techniques adapted from “point centered quarter” and “nearest neighbour” methods (Allphin & *al.*, 2007) along a randomly established transect of 100 m × 4 m with a south-north orientation. Within each transect, ten 4 m × 4 m plots were evenly marked along its length.

Inside each of the ten plots, the following data were recorded: (a) number of individuals; (b) distance from the south-easternmost corner of the plot to the nearest individual, then to its first neighbour and finally from this to its nearest neighbour in order to access spatial distribution; (c) individual characteristics of each of these shrubs (sex, height, maximum canopy diameter and its perpendicular, age class). Canopy area was calculated using ellipse formula (π × largest diameter × perpendicular diameter). To estimate plant size we used shrub volume rather than height or canopy area because this measure is less sensitive to variations in plant structure.

Individuals were classified into three groups according to sex: female or male individuals when reproductive structures were present, and non-matures when reproductive structures were absent. Age structure was determined by assigning each individual to one of two age classes, based on reproductive structures presence and shrub height (García & *al.*, 1999; Allphin & *al.*, 2007): juveniles, plants with height inferior to 1m and without reproductive structures; and adults, plants higher than 0.50 m and displaying evident flowering apparatus.

Female cone and seed characteristics

After a thorough measurement of cone morphological parameters (Table 3), we realized that each population has their own cone characteristics, with cones from C population significantly smaller and lighter than those from the other two populations.

From each population 200 female cones were randomly analysed regarding the presence of apparently viable seeds. The observed percentages of female cones with at least one apparently viable seed inside were 28, 17 and 25% in populations A, B and C, respectively. These percentages differed significantly among populations (X^2 = 7.23, df = 2, P=0.027) with population B presenting a lower percentage of apparently viable cones when compared with the other populations. The apparently viable seeds obtained in each population were afterward subjected to Tetrazolium test and only 14, 7 and 21%, respectively, contained viable embryos. The proportion of viable embryos differed significantly among populations (X^2 = 6.219, df = 2, P=0.045), with population C presenting higher percentage of viable seeds when compared with population B. Extending these results to the total number of seeds examined made us realize that only 2, 1 and 3% of the seeds were viable in populations A, B and C, respectively. No apparent association between cones size, weight and number of apparently viable seeds was found.

Data analysis

One-way ANOVA followed by Fisher's Least Significant Difference (LSD) post-hoc test was employed to investigate differences among populations for mean shrub height. Due to the unbalanced and non-normality nature of the data, differences in shrub densities, canopy areas and in their volume among populations and sex (male, female and juveniles) were evaluated with Kruskal-Wallis test followed by LSD post-hoc test applied to ranks (Conover, 1999). These last tests were also used when testing for differences in both cone vertical and horizontal diameter and cone weight among populations. Differences in height, canopy area and volume between male and female shrubs in each population were evaluated with Mann-Whitney-Wilcoxon test (Conover, 1999).

Spatial distribution of plants was assessed by calculating their coefficient of aggregation (CA) according to Hopkins & Skellam (1954) and Welden & *al.* (1990), the most adequate method since only distances from the southeasternmost corner of the plot to the nearest plant and from this plant to its first neighbour were made. This coefficient employs both point-to-plant and plant-to-plant distances to access departures from random distributions of individuals.

Chi-square test was used to compare the number of male and female plants based on an expected 1:1 ratio and also to compare the proportion of viable female cones and viable seeds among populations.

All tests were performed in PASW Statistics (V.18, SPSS Inc., Chicago, IL) and conclusions of significance were based on $P < 0.05$.

Results

Reassessing identified populations

Some herbaria records were more than 100 years old and the populations represented by those records were destroyed by urban expansion. Most of the historical populations located in Setúbal Peninsula have already disappeared or subsist in an extremely fragmented condition, mostly due to urbanization. In Tróia Peninsula the presence of many populations could not be confirmed due to recent forest understory clearing operations. To the East of Tróia, larger populations were confirmed in Mata de Valverde and Arez (38°19'60.00"N; 8°32'0.00"W and 38°18'3.52"N; 8°29'16.98"W, respectively). The northern-most identified population is located near Figueira da Foz (40° 6'21.68"N; 8°51'12.78"W) and the southern-most in the dunes of Praia do Monte Velho (38° 4'51.48"N; 8°48'38.69"W). With a small number of individuals, this population was composed uniquely by dwarf individuals, probably due to the sea spray or to the proximity to its southern geographical range or both, while Figueira da Foz individuals have been producing less and less seed cones in the last years (pers. com. from University of Coimbra Herbarium curator).

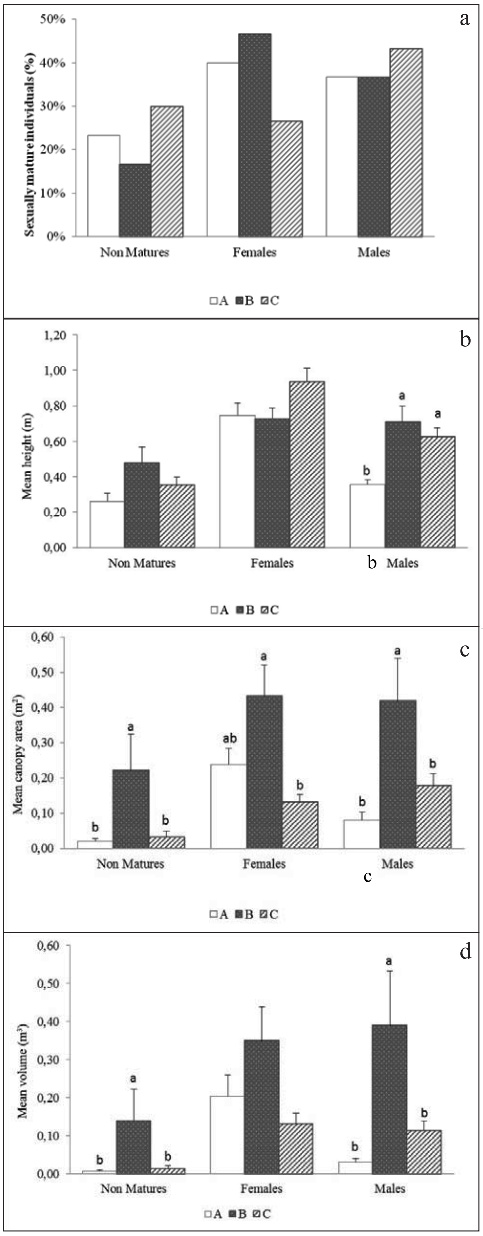
Comparing populations

Population A occupies the largest area and has the biggest pine tree density, followed by populations B and C. Juniper shrub density also increased from population A to C (Table 1). Shrubs were randomly distributed in populations A and C (CA=1.06; P-value=0.893 and CA=2.09; P-value=0.1063, respectively), while in population B they have an aggregated distribution (CA= 10.37; P-value<0.0001).

Individuals from populations B and C were higher than individuals from population A, but individual canopy area and volume were significantly larger in population B (Table 1).

When populations are compared taking individuals sex into account we verified that population B had the smallest proportion of non-mature shrubs, but these had the largest canopy and, consequently, with the highest volume (Figure 2a, b, c and d, respectively). Non-mature and female shrubs height was not significantly different between populations (Figure 2b). Canopy area and volume of non-mature and males of population B are significantly bigger than those of the other populations (Figures 2c and d). Canopy area of female shrubs from population B is significantly higher than that of population C.

Figure 2. Population composition (a), height (b), canopy area (c) and plant volume (d) according to sexual maturity, for each population studied. Different letters within the same sexual category mean significant differences between mean heights or mean ranks of volumes or canopy areas at $p<0.05$, after Kruskal-Wallis test; the absence of letters means that no significant differences were found.



Females were significantly higher than males in population A and C (0.75 m and 0.36 m in population A; 0.94 and 0.63 m in population C, respectively), while in B population no significant height differences between male and female shrubs were found. More significant differences were found only in population A, where canopy area and volume of males (0.08 m^2 and 0.03 m^3) were smaller than those of females (0.24 m^2 and 0.18 m^3, respectively).

Around 39% of the shrubs were males, 38% females and 23% non-mature. Ratio male:female did not differ in any population from the null hypothesis of a 1:1 (Table 2). The number of non-mature individuals was similar among populations. The mature individuals are significantly more than the non-mature individuals in all populations (Table 2).

Figure 2. Age structure relationship and distribution of *J. navicularis* according to sexual differentiation in each of the three studied sites. χ^2 applied to the number of mature and non-mature plants vs. number of male and female plants and calculated on an expected 1:1 ratio of mature to non-mature plants and male to female plants, respectively; *=P < 0.05; n.s. = not significant.

Population	Total	Non-mature	Mature	χ^2	Males	Females	χ^2
A	30	7	23	8.53^*	11	12	$0.04^{n.s.}$
B	30	5	25	13.33^*	11	14	$0.36^{n.s.}$
C	30	9	21	4.80^*	13	8	$1.19^{n.s.}$

Female cone and seed characteristics

Cone characteristics differed among populations (Table 3), with cones from population C being significantly smaller and lighter compared with the other two populations, especially population B that had the biggest volume and cone weight.

The observed percentages of female cones with at least one apparently viable seed inside were 28, 17 and 25% in populations A, B and C, respectively. These percentages differed significantly among populations (χ^2 = 7.23, df = 2, P=0.027) with population B presenting a lower percentage of apparently viable cones when compared with the other populations. The apparently viable seeds obtained in each population were afterward subjected to tetrazolium test and only 14, 7 and 21%, respectively, contained viable embryos. The proportion of viable embryos differed significantly among populations (χ^2 = 6.219, df = 2, P=0.045), with population C presenting higher percentage of viable seeds than population B. When the total number of seeds is considered only 2, 1 and 3% of the seeds were viable in populations A, B and C, respectively. No apparent association between cones size, weight and number of seeds was found.

Table 3. Cones morphological parameters (mean±SE) per population. Different letters in the same column indicate significant differences between mean ranks (n=500, p < 0.05). Kruskal-Wallis test with post-hoc LSD test applied to ranks.

Population area	Height (mm)	Width (mm)	Weight (g)	Seeds/cone
A (82.50 ha)	8.30±0.029 ^{a}	7.73±0.035 ^{b}	0.28±0.003 ^{b}	2.00±0.029 ^{b}
B (59.4 ha)	8.05±0.033 ^{b}	8.44±0.040 ^{a}	0.35±0.004 ^{a}	1.61±0.030 ^{a}
C (12.6 ha)	7.19±0.025 ^{c}	6.36±0.028 ^{c}	0.17±0.002 ^{c}	1.58±0.029 ^{a}

Discussion

Populations identified in herbaria records and in scientific papers were verified *in situ*. Most herbaria records dated from late nineteenth- and early twentieth-century and the majority of the historical *J. navicularis* populations are now part of the urban matrix and others face destruction due to direct or indirect anthropogenic interference. Together with fragmentation by human occupation, clearing of pinewoods (*J. navicularis* main habitats) with non-selective techniques to prevent fire are the main threats to the slow growing populations of *J. navicularis* (ICNF, 2008).

Juniperus navicularis is a dioecious species without secondary sexual characters to help identify plant gender in the absence of reproductive structures and, therefore, females or males are not identifiable until they reach sexual maturity and flowering begins. In our study, females were higher than males, or at least equal sized (in population B). Also, considering that shrub height was taken as an approximate measure of shrub age, higher shrubs identified are supposedly older. This could mean that females have a delayed flowering compared to males, that is, males flower younger than females (because they can do so with a minor resource investment) and are, therefore, smaller in size, as also found by Gao (2009). Regarding *Juniperus virginiana* L., Van Haverbeke & Read (1976) reported that male cones can develop on 4-yr-old trees, whereas female cone develops only in trees with no less than 10 yr. Falinski (1986) states that males of *Populus tremula* first flowering period occurred earlier than that of females – males flower around the age of thirteen years but females did not flower until they were older and bigger, with taller and thicker trunks. According to the Differential Reproductive Cost hypothesis (see Obeso, 2002 for a thorough revision), in woody dioecious species females reproductive costs are generally higher than those of males, because they have to support the additional costs of producing fruit. This can cause a lower female vegetative growth rate, a delay in flowering or even a reduced female survival rates. Testing this hypothesis requires an accurate determination of both female and male shrubs age and it may be the aim of future work on this species.

Population B is different from the other two populations: both adult and juvenile shrubs of this population have wider canopies while male and juvenile shrubs are the highest and the more voluminous. Additionally, unlike in the other populations, females are not higher than males but equal sized, despite their preferential location under pine canopies (pers. obs. Anabela Belo), what might enhance their performance and reduce stress levels (Bierzychudek & Eckhart, 1988) like excessive radiation. Also contrasting with the other two populations, spatial distribution of population B is aggregated. This kind of distribution is more frequent in unfavourable environments, because this spatial pattern facilitates the recruitment and the development of juveniles through the creation of microhabitat conditions (Montesinos & *al.*, 2007), namely the reduction on incoming solar radiation and water evaporation (Castro & *al.*, 2004). This nursing behaviour, enhanced by the wider canopies of population B shrubs, is particularly important in semi-arid environments where survival greatly depends on water availability and has been observed in other *Juniperus* species (Verdú & *al.*, 2004; Montesinos & *al.*, 2007). All above mentioned differences may be due to the fact that population B is located in a drier and warmer area than the other two studied populations, and where, additionally, the soil is sandier and loose. This makes water retention in soil more difficult and *J. navicularis* survival harsher, in spite of its ability to live in such environments (Neto & *al.*, 2004). We believe that females of population B don’t surpass males because their growth in stressful conditions like aridity increase, is reduced relative to males (Dudley, 2006; Iszkulo & *al.*, 2009).

Male:female ratio was not significantly different from the hypothetic 1:1 ratio, which means that there aren’t sex related bias in any of the three populations, at least at the considered spatial scale. The age structure of populations provides information on their regeneration process and conservation status (García & *al.*, 1999). The small proportion of juveniles found for all populations indicates population ageing, and this represents a serious recruitment limitation (Muñoz-Reinoso, 2003). Juvenile proportions varied between 17 and 30%, but an undetermined number of juveniles are vegetatively linked to nearby adult plants in spite of having been counted as single individuals, because they were located in a quite broadened neighbourhood that often reach up to 5 m and further away from the main shrub (pers. obs. allowed by excavating soil around some juveniles). Finding that part of the juveniles is not of seminal origin raises further concern regarding populations genetic impoverishment, even though *Juniperus* spp. are considered to be plants capable of surviving for long periods under unfavourable conditions (García & *al.*, 1999; García & Zamora, 2003; El-Bana & *al.*, 2010).

Junipers produce a large proportion of apparently viable but empty seeds (Thomas & *al.*, 2007), but proportions of viable seeds obtained in this study are lower than those found for *J. communis* in Iberian Peninsula (García & *al.*, 2000) or for the endangered *J. oxycedrus* ssp. *macrocarpa* (Juan & *al.*, 2003, 2006) and very low when compared to similar studies with other junipers (Rumeu & *al.*, 2009). Some junipers like *Juniperus communis* L. (Verheyen & *al.*, 2009) or *Juniperus sabina* L. (Wesche & *al.*, 2005) are threatened due to a lack of sexual reproduction caused by poor seed viability. The results obtained for *J. navicularis* suggest that the conservation of this species is endangered because populations are maintained mainly by vegetative reproduction. In fact, when the number of viable seeds per population is analyzed, we verified that viability of seeds varies in accordance with non-matures proportion and that they both vary positively with annual mean rainfall and negatively with annual mean temperature. This seems to indicate that climatic constraints toward increasing aridity might determine *Juniperus navicularis* survival through reduction of seeds viability as found for other junipers (García & *al.*, 2000; Wesche & *al.*, 2005; Otto & *al.*, 2012).

In summary, a major part of *Juniperus navicularis* populations recorded in the past have disappeared through direct habitat loss to urban development. This is still a major threat to this species. Seed viability is lower at drier and warmer locations, suggesting that this species distribution will be reduced with climate change. Male:female ratio deviations were not found in the studied populations but the small fraction of juveniles detected is a sign of population ageing (Muñoz-Reinoso, 2003). Furthermore, a part of those juveniles seems to result from clonal growth. Therefore, quantitative information on *Juniperus navicularis* population structure and natural regeneration is needed. Nevertheless, based on this first study, we can already advise that conservation measures to prevent further destruction of populations, especially in the more arid locations, should be taken in order to preserve this important species.

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References

Adams, R.P. 1998. The leaf essential oils and chemotaxonomy of Juniperus sect. Juniperus. Biochem. Syst. Ecol. 26(6): 637-645.

Agência Portuguesa do Ambiente. 2014. Atlas do Ambiente. (http://sniamb.apambiente.pt; last access 22 April 2014).

Allphin, L., Hunt, A.F. & Anderson, V.J. 2007. Genetic diversity and low reproductive success in isolated populations of Utah juniper, Juniperus osteosperma (Cupressaceae). West N. Am. Nat. 67(3): 323337.

Anonymous. 2005. Decreto-Lei nº 49/2005 de 24-02-2005, ANEXO B-I. Tipos de habitats naturais de interesse comunitário cuja conservação exige a designação de ZEC. Pp1670-1708. (http://dre.pt/pdf1s/2005/02/039A00/16701708.pdf; last access 22 April 2014).

Baillie, J.E.M., Hilton-Taylor, C. & Stuart S.N. (Eds.). 2004. 2004 IUCN Red list of threatened species. A Global Species Assessment. IUCN, Gland, Switzerland and Cambridge. (https://portals.iucn.org/library/sites/library/files/documents/RL-2004-001.pdf; last access 22 April 2014).

Bierzychudek, P. & Eckhart V. 1988. Spatial segregation of the sexes of dioecious plants. Am. Nat. 132: 34-43.

Boratynski, A., Wachowiak, W., Dering, M., Boratynska, K., Sekiewicz, K., Sobierajska, K., Jasinska, A.K., Klinko, M., Montserrat, J.M., Romo, A., Ok, T. & Didukh, Y. 2014. The biogeography and genetic relationships of Juniperus oxycedrus and related taxa from the Mediterranean and Macaronesian regions. Bot. J. Linn. Soc. 174: 637-653.

Castro, J., Zamora, R., Hódar, J.A., Gómez, J.M. & Gómez-Aparicio, L. 2004. Benefits of using shrubs as nurse plants for reforestation in Mediterranean mountains: a 4-year study. Restor. Ecol. 12(3): 352-358.

Cavaleiro, C., Salgueiro, L.R., da Cunha, A.P., Figueiredo, A.C., Barroso, J.G., Bighelli, A. & Casanova, J. 2003. Composition and variability of the essential oils of the leaves and berries from *Juniperus navicularis*. Biochem. Syst. Ecol. 31(2): 193-201.

Conover, W.J. 1999. Practical nonparametric statistics, Third edition. John Wiley & Sons, New York. 592 pp.

Costa, J.C., Ribeiro, S., Alves, J., Neto, C., Capelo, J., Rego, F. & Espírito-Santo, M.D. 2005. Diversidade das formações de Juniperus spp. em Portugal Continental. XX Jornadas Internacionales de fitosociologia, Málaga. Pp. 117-118.

Dray, A.M. 1985. Plantas a proteger em Portugal Continental. Serv. Nal. Parq. Res. Conserv. Nat., Lisboa. 56 pp.

Dudley, L.S. 2006. Ecological correlates of secondary dimorphism in Salix glauca (Salicaceae). Am. J. Bot. 93: 1775-1783.

El-Bana, M., Shaltout, K., Khalafallah, A. & Mosallam H. 2010. Ecological status of the Mediterranean Juniperus phoenicea L. relicts in the desert mountains of North Sinai, Egypt. Flora 205(3): 171-178.

European Commission. 1996. Interpretation manual of European Union habitats. Ver. Eur 15. Dg XI, Envir. Nucl. Saf. Civil Prot., Brussels. 107 pp.

Falinski, J.B. 1986. Vegetation dynamics in temperate lowland primeval forests. Ecol. Stud. Bialowieza For. Junk, Dordrecht. 548 pp.

Farjon, A. & Page, C.N. (Eds.). 1999. Conifers. Status survey and conservation action plan. IUCN/SSC Conifer Specialist Group. IUCN, Gland, Switzerland and Cambridge. (http://data.iucn.org/dbtw-wpd/edocs/1999-024.pdf; last access 22 April 2014).

Franco, J.A. 1986. Juniperus L. In: Castroviejo, S., Laínz, M., López-González, G., Montserrat, P., Munõz-Garmendia, F., Paiva, J., Villar, L. (Eds.). Flora Iberica vol. I. Pp 181-188. R. Jard. Bot., CSIC, Madrid.

Franco, J.A. & Rocha Afonso, M.L. 1982. Distribuição de pteridófitos e gimnospérmicas em Portugal (Continental). SNPRCN (Lisboa). Col. Parq. Nat. 14(1): 305-307.

Gao, P., Kang, M., Wang, J., Ye, Q. & Huang, H. 2009. Neither biased sex ratio nor spatial segregation of the sexes in the subtropical dioecious tree Eurycorymbus cavaleriei (Sapindaceae). J. Integr. Plant Biol. 51: 604-613.

García, D. & Zamora, R. 2003. Persistence, multiple demographic strategies and conservation in long-lived Mediterranean plants. J. Veg. Sci. 14: 921-926.

García, D., Zamora, R., Gómez, J.M., Jordano, P. & Hódar, J.A. 2000. Geographical variation in seed production, predation and abortion in Juniperus communis throughout its range in Europe. J. Ecol. 88: 436-446.

García, D., Zamora, R., Hódar, J.A. & Gomez, J.M. 1999. Age structure of Juniperus communis L. in the Iberian Peninsula: conservation of remnant populations in Mediterranean mountains. Biol. Conserv. 87(2): 215-220.

Hopkins, B. & Skellam, J.G. 1954. A new method for determining the type of distribution of plant individuals. Ann. Bot. London 18(2): 213-227.

I.C.N.F. 2008. Plano sectorial da Rede Natura 2000: habitats naturais. Dunas litorais com Juniperus spp., Ficha de habitat 2250. (http://www.icnf.pt/portal/naturaclas/rn2000/resource/rn-plan-set/hab/hab-2250; last access 22 April 201).

I.G.E.O. 1995. Usos do solo. COS'90M. (http://62.48.187.113:82/COS/; last access 22 April 2014).

Iszkulo, G., Jasinska, A.K., Giertych, M.J. & Boratynski A. 2009. Do secondary sexual dimorphism and female intolerance to drought influence the sex ratio and extinction risk of Taxus baccata?. Plant Ecol. 200(2): 229-240.

I.U.C.N. 2014. IUCN Red list of threatened species. Ver. 2014.2. (http://www.iucnredlist.org; last access 22 April 2014).

Juan, R., Pastor, J., Fernández, I. & Diosdado, J.C. 2003 Relationships between mature cone traits and seed viability in Juniperus oxycedrus L. subsp. macrocarpa (Sm.) Ball (Cupressaceae). Acta Biol. Cracov. Bot. 45(2): 69-78.

Juan, R., Pastor, J., Fernández, I. & Diosdado, J.C. 2006. Seedling emergence in the Endangered Juniperus oxycedrus subsp. macrocarpa (Sm.) Ball in Southwest Spain. Acta Biol. Cracov. Bot. 48(2): 49-58.

Montesinos, D., Verdú, M. & García-Fayos, P. 2007. Moms are better nurses than dads: sex biased self-facilitation in a dioecious juniper tree. J. Veg. Sci. 18: 271-280.

Muñoz-Reinoso, J.C. 2003. Juniperus oxycedrus ssp. macrocarpa in SW Spain: Ecology and conservation problems. J. Coast. Conserv. 9(2):113-122.

Neto, C., Capelo, J. & Costa, J.C. 2004. De Vegetatio Lusitana Notae III. 8. Comunidades vegetais dos solos arenosos podzolizados do Sado e Costa da Galé. Uma interpretação fitossociológica dos dados paleoecológicos e geomorfológicos. Silva Lus. 12(2): 256-262.

Obeso, J.R. 2002. The costs of reproduction in plants. New Phytol. 155(3): 321-348.

Otto, R., Barone, R., Delgado, J.D., Arévalo, J.R. Garzón-Machado, V., Cabrera-Rodríguez, F. & Fernández-Palacios, J.M. 2012. Diversity and distribution of the last remnants of endemic juniper woodlands on Tenerife, Canary Islands. Biodiv. Conserv. 21(7): 1811-1834.

Pérez Latorre, A.V. 2006. Juniperus oxycedrus L. subsp. transtagana Franco en la provincia de Málaga. Lagascalia 26: 148.

Rivas-Martínez, S., Lousã, M., Díaz, T.E., Fernández González, F. & Costa, J.C. 1990 La vegetación del sur de Portugal (Sado, Alentejo y Algarve). Itinera Geobot 3: 5-126.

Rumeu, B., Nogales, M., Elias, R.B., Padilla, D.P., Resendes, T., Rodríguez, A., Valdés, F. & Dias, E. 2009. Contrasting phenology and female cone characteristics of the two Macaronesian island endemic cedars (Juniperus cedrus and J. brevifolia). Eur. J. For. Res. 128(6): 567-574.

Thomas, P.A., El-Barghathi, M. & Polwart, A. 2007. Biological flora of the British isles: Juniperus communis L. J. Ecol. 95(6): 1404-1440.

Van Haverbeke, D.F. & Read, R.A. 1976. Genetics of eastern redcedar. United States Dep. Agric. For. Serv. Research Paper WO-32. 17 pp.

Velasco-Negueruela, A., Pérez-Alonso, M.J., Palá-Paúl, J., Íñigo, A. & López, G. 2002. Leaf essential oils analysis of Juniperus navicularis Gand. Bot. Compl. 26: 85-91.

Verdú, M., Villar-Salvador, P. & García-Fayos, P. 2004. Gender effects on the post-facilitation performance of two dioecious *Juniperus* species. Funct. Ecol. 18(1): 87-93.

Verheyen, K., Adriaenssens, S., Gruwez, R., Michalczyk, I.M., Ward, L.K., Rosseel, Y., Van den Broeck, A. & García, D. 2009. Juniperus communis: victim of the combined action of climate warming and nitrogen deposition?. Plant Biol. 11(s1): 49-59.

Welden, C.W., Slauson, W.L. & Ward, R.T. 1990. Spatial pattern and interference in pinon-juniper woodlands of northwest Colorado. Great Basin Nat. 50(4): 313-319.

Wesche, K., Ronnenberg, K. & Hensen, I. 2005. Lack of sexual reproduction within mountain steppe populations of the clonal shrub Juniperus sabina L. in semi-arid southern Mongolia. J. Arid Environ. 63: 390-405.