



## Research Article

## SPECIAL ISSUE: Physiology and Ecology of Halophytes—Plants Living in Salt-Rich Environments

## Habitat specificity of a threatened and endemic, cliff-dwelling halophyte

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**Received:** 31 March 2014; **Accepted:** 2 June 2014; **Published:** 18 June 2014

**Guest Editor:** Adele Muscolo

**Citation:** Caperta AD, Espírito-Santo MD, Silva V, Ferreira A, Paes AP, Róis AS, Costa JC, Arsénio P. 2014. Habitat specificity of a threatened and endemic, cliff-dwelling halophyte. *AoB PLANTS* 6: plu032; doi:10.1093/aobpla/plu032

**Abstract.** Coastal areas and other saline environments are major contributors to regional and global biodiversity patterns. In these environments, rapidly changing gradients require highly specialized plants like halophytes. In European coastal cliff-tops, rocky and sandy seashores, and saltmarshes, typical halophytes from the genus *Limonium* are commonly found. Among them, the aneuploid tetraploid ( $2n = 4x = 35, 36, 37$ ) *Limonium multiflorum*, endemic to the west coast of Portugal, is an interesting case study for investigating the ecology and conservation of a halophyte agamospermic species. Although it is listed in the IUCN red list of threatened species, information on its population size or rarity, as well as its ecology, in some respects is still unknown. Field surveys in the largest known population were performed (Raso cape, Portugal) in order to determine habitat requirements and conservation status. A total of 88 quadrats were monitored, 43 of which contained at least one *L. multiflorum* individual. For each sampled quadrat, four abiotic and four biotic variables as well as two spatially derived variables were recorded. Principal component analysis and cluster analysis showed narrow habitat specificity for this species which appeared to be intolerant to competition with invasive alien plants. We conclude that *in situ* conservation in a local 'hotspot' of this rare and vulnerable species emerges as a priority in order to ensure that biodiversity is not lost.

**Keywords:** Agamospermic species; cliff-dwelling species; conservation; habitat specificity; halophyte; *Limonium*.

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## Introduction

Habitat assessment is a fundamental requirement for species conservation. Attempts to set plant conservation priorities revealed the need to consider attributes such as species ecological specificity, geographical rarity and rate of threat (Dominguez Lozano et al. 2003; Pärtel et al. 2005). Furthermore, a study on plant life history traits in rare versus common taxa also showed that infrequent species exhibited a narrower geographical range and more habitat specialization than their common relatives (Farnsworth 2007). Thus, knowledge on habitat requirements of a rare species is crucial, in particular in the case of agamospermic species (defined by an asexual breeding system) (Dominguez Lozano et al. 2003), to help establish the best conservation methods.

In Europe, coastal vegetation presents lower diversity values when compared with other world regions (Mucina 2013) but European dry coastal terrestrial habitats, which include maritime rocks, sea-cliffs and coastal slopes, show higher values in terms of plant diversity (van der Maarel 1993; van der Maarel and van der Maarel-Versluys 1996). Among the best represented flowering plant families of these habitats are the *Plumbaginaceae* with nearly 90 % of coastal species, including most members from the genus *Limonium* (Kubitzki 1993; van der Maarel and van der Maarel-Versluys 1996). This cosmopolitan halophytic genus comprises annual and perennial species found on cliff-tops, rocky and sandy seashores, and salt-marshes (Erben 1993; Kubitzki 1993). These habitats are an important source of endemics (van der Maarel and van der Maarel-Versluys 1996), and nearly 37 % of all typical littoral species are considered threatened either because they have an extremely local distribution or because they are in decline as a result of negative human impacts in coastal areas (van der Maarel and van der Maarel-Versluys 1996).

Knowledge of habitat requirements of threatened populations from rare species selected for conservation is crucial for assuring their viability (Simberloff 1988; Brussard 1991; Schemske et al. 1994; Heywood and Iriondo 2003), since the establishment and expansion of a species is dependent on growth under favourable ecological conditions (e.g. Baumberger et al. 2012). Increasing a species' survival prospects through reintroduction or reinforcement (increase population size and diversity) of native species (Akeroyd and Wyse Jackson 1995), and knowing ecological processes in combination with demographic and genetic processes and breeding systems is therefore essential (Godefroid et al. 2011). However, objective data for documenting habitat preferences of rare plant species are relatively scarce, in particular those on ocean-exposed high cliff-tops subjected to

salt spray transported inland by wind (Salisbury 1952; Maun 2004; Frederiksen et al. 2006).

The Portuguese coast is known for the richness of its flora due to a singular biogeographic position (Braun-Blanquet et al. 1972; Asensi et al. 1993; van der Maarel and van der Maarel-Versluys 1996). About 35 % of all Portuguese Natura 2000 habitats consist of coastal habitats, including Atlantic ocean-exposed cliff-tops (Costa et al. 2007; Martins et al. 2013). These locations present the original flora and vegetation on limestone, sandstone and marly clay areas, and include the rare and endemic cliff-dwelling *Limonium multiflorum* (Erben 1978, 1993; Costa et al. 2000; Espírito-Santo et al. 2012). This species is listed in Annex II of Habitats Directive (Council Directive 92/43/EEC 1992) and in the IUCN red list of threatened species (IUCN 2013), and most of its known populations are located in Portuguese NATURA 2000 Sites of Community Importance (EC 2013). Although *L. multiflorum* is considered a Portuguese crop wild relative (Magos-Brehm et al. 2008) and has an assessed conservation status of 'Least Concern' in the IUCN red list (IUCN 2013), information on its ecological preferences, essential for recovery plans, especially population restoration, population augmentation or population reintroduction is lacking.

In the present study we assessed habitat requirements for *L. multiflorum*. Two main questions were addressed: (i) What are the main abiotic and biotic variables favourable for its persistence? (ii) What is the vegetation cover and respective species composition associated with its presence?

## Methods

### Study species

*Limonium multiflorum* ( $2n = 4x = 35, 36, 37$ ) (Erben 1993; Róis et al. 2012) is a perennial species endemic to a 120-km-long shoreline stretch in western Portugal (ICNF 2013) (Fig. 1) (Espírito-Santo et al. 2012). It is mainly found in NATURA 2000 habitat 1240 *Vegetated sea cliffs of the Mediterranean coasts with endemic Limonium spp.* and habitat 1330 *Atlantic salt meadows* (APA 2011). Along the western coast, populations are found on different cliffs separated by unsuitable habitats such as acid rock cliffs or steep slopes (mostly granite and syenite), sandy beaches or pinewoods. It occurs in small populations from <10 individuals to 1000 flowering plants on average (Caperta et al. 2013; Róis et al. 2013). Seedling emergence is mainly observed in autumn, following dispersal of seeds in summer. However, a persistent seed bank is not formed in such a thin soil and only a small amount of seeds originate seedlings (A.D.C. and A.S.R.,

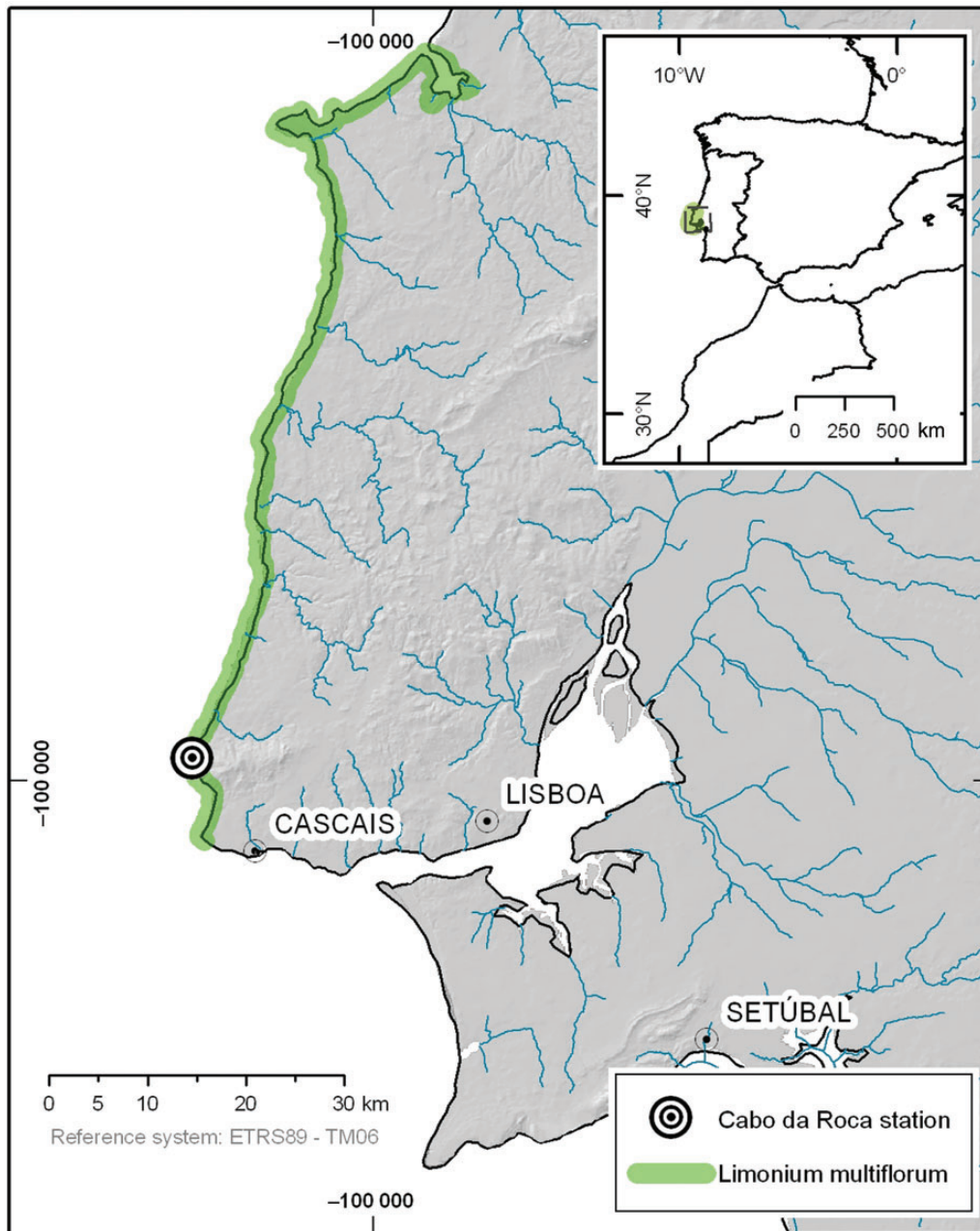


Figure 1. Map of *L. multiflorum* distribution in the western coast of Portugal.

unpubl. res.). Plants then grow as vegetative rosettes for several years on cliff-tops and in nearby rocky areas with a high degree of exposure to salt spray and salt-laden winds from the Atlantic Ocean (Caperta et al. 2013). Flowering mainly occurs in spring and summer (April to July), although in some years, it is also observed in November and February (A.D.C. and A.S.R., unpubl. res.).

### Study site

Our study was conducted in Raso cape, a broad promontory found in the west of Lisbon (municipality of Cascais,

district of Lisbon, Portugal; mean location coordinates are 38°42'34"N and 9°29'12"W) (Fig. 2). Presently, the largest known population of *L. multiflorum* (about 1000 individuals) is found at this site, within an area of ~0.6 km<sup>2</sup>, inside a Site of Community Importance (SCI Sintra/Cascais PTCO0008) for the Mediterranean biogeographical region. This cape consists of gently folded limestone, forming a rocky shoreline with low cliffs and numerous deep incisions along fault lines or abraded mylonitic rocks (Scheffers and Kelletat 2005). In terms of biogeographical typology this site is included in the



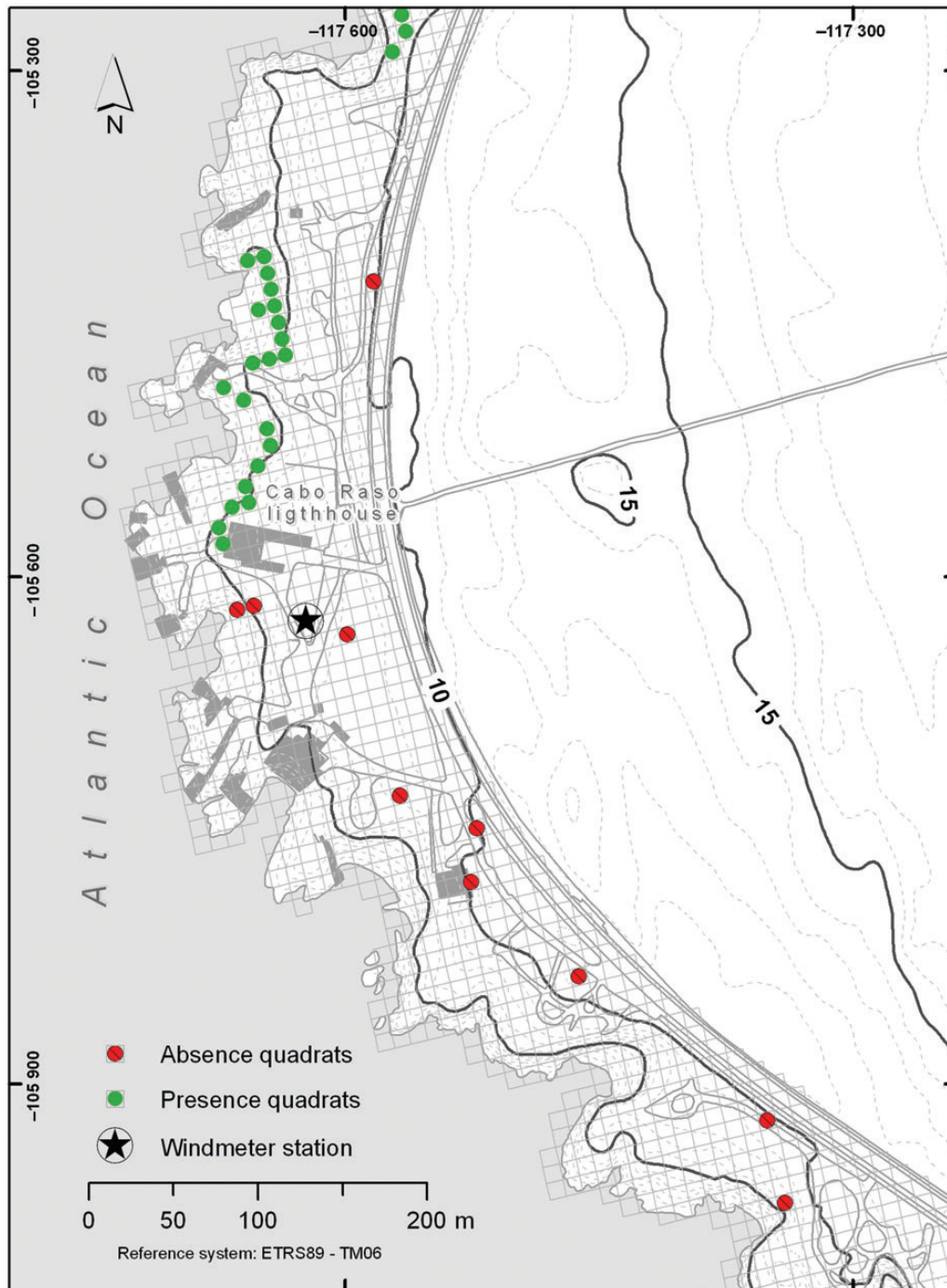


Figure 2. Schematic drawing of the quadrat sampling of *L. multiflorum* in the coast of Raso cape.

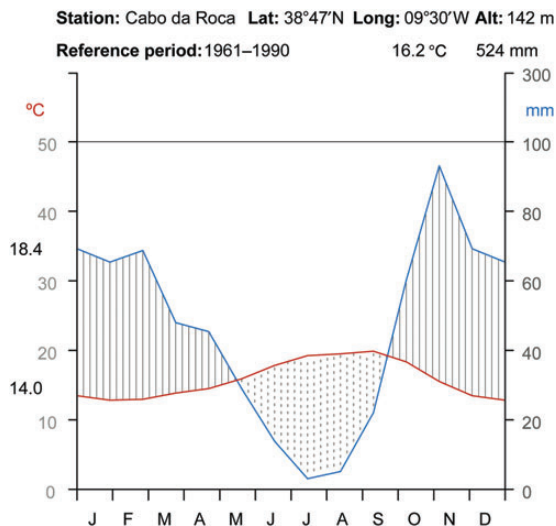
Olissiponean District, Dividing Portuguese Sector, Sadensean-Dividing Portuguese Subprovince and Coastal Lusitan-Andalusian Province (Costa et al. 1998a; Rivas-Martínez 2007). The region's climate is well represented by the thermopluviometric diagram of Cabo da Roca station (Fig. 3), which presents a typical hyperoceanic Mediterranean climate pattern, as indicated by the low variation of average monthly temperatures and the

existence of more than two consecutive dry months in the summer period. Another noteworthy feature of the region's climate is the high frequency of relatively strong winds coming from the northern and northwestern sectors (Fig. 4). Bioclimatically speaking (*sensu* Rivas-Martínez 2007), most of this region falls in the upper thermomediterranean thermotype and in the lower sub-humid to upper dry ombrotypes (Mesquita and Sousa

2009; Monteiro-Henriques 2010). Here, the predominant plant community is *Limonietum multiflori-virgati* (Costa et al. 1998b, 2012) which integrates *L. multiflorum*, *L. virgatum*, *Dactylis marina*, *Plantago coronopus* and *Crithmum maritimum*, among other species. This area encompasses different human occupations, namely buildings and other built structures like seafood tanks, off-road motorized driving and tourism which contribute to population fragmentation.

**Sampling design.** Field surveys used a grid of 10 × 10 m quadrats which was overlaid over the aerial photographs of the site and later transferred to the site using global positioning system (GPS) receivers. A total of 88 quadrats were surveyed, 43 of which contained at least one *L. multiflorum* individual (presence quadrat) and the remaining 45 were randomly selected quadrats

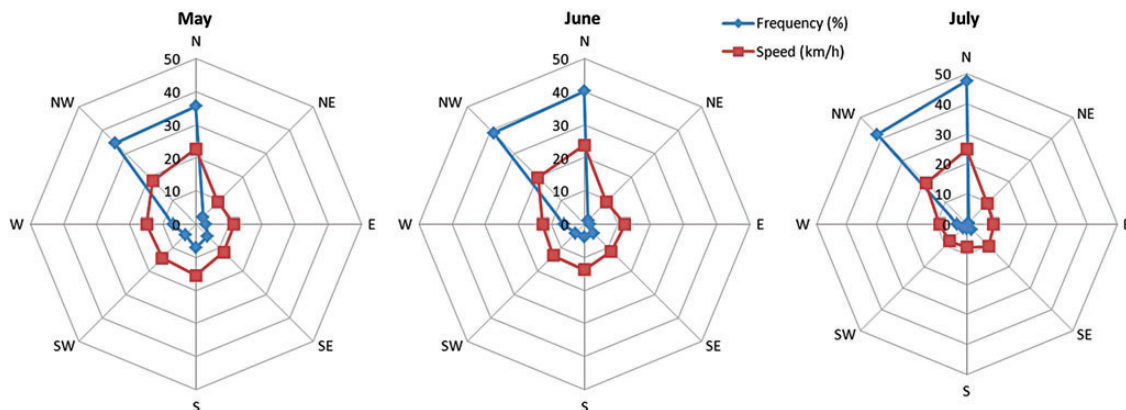
containing no individuals from this species (absence quadrat) (Fig. 2). The random selection procedure was performed using R software's 'sample' routine (R Core Team 2013). In quadrats where *L. multiflorum* was present censuses were carried out from April to June 2013. For each sampled quadrat four abiotic variables, namely rock formation (RockForm), cobble (Cobble), coarse sand (CoarseSd) and fine sand (FineSand) (all expressed in %), were recorded. Four biotic variables related to vegetation cover (Coverage) as well as invasive non-native species (INNS) cover, dead organic matter (lignified or not) (DOM) cover and litter (Residues) cover were also estimated (expressed in %) in each quadrat. To facilitate data collection, each quadrat was then sub-divided into sub-quadrats of 1 m<sup>2</sup>, and the percent coverage value obtained for the sub-quadrats of the same 100 m<sup>2</sup> quadrat were averaged. Two spatial variables, distance from coastline (Dist\_coa) and mean quadrat slope (Mean\_slo), were also derived using the ArcGIS<sup>®</sup> 10.0 software, by ESRI.



**Figure 3.** Thermopluviometric diagram based on the weather station in the coast of Raso cape. The red line corresponds to mean monthly temperature (°C) and the blue line corresponds to mean monthly precipitation (mm).

### Data analysis

To define *L. multiflorum* habitat requirements we performed a principal component analysis (PCA) on the resulting data matrix using abiotic, biotic and spatially derived variables. In order to discriminate the vegetation structure and composition in the monitored quadrats, a cluster analysis using Euclidean distance and Ward clustering method (Ward 1963) was performed using the set of quadrats containing *L. multiflorum*. To ensure that the group of quadrats (clusters) had the same influence from the environmental variables, Kruskal–Wallis tests of variance were performed (Sokal and Rohlf 1997; Zar 2010). To complete habitat characterization of *L. multiflorum*, the most frequent and abundant plant species within each cluster was defined as described in Baumberger et al. (2012). The mean coverage of each species within each group was also calculated. All statistical analyses were



**Figure 4.** Wind regime diagrams based on the weather station in the coast of Raso cape during *L. multiflorum* flowering peak (May–July).

conducted using the STATISTICA software (StatSoft V10). Bioclimatic and biogeographic nomenclature followed the proposals of Rivas-Martínez (2007). Taxonomic nomenclature followed Menezes de Sequeira et al. (2011).

## Results

### Influence of environmental variables on the presence/absence of *L. multiflorum*

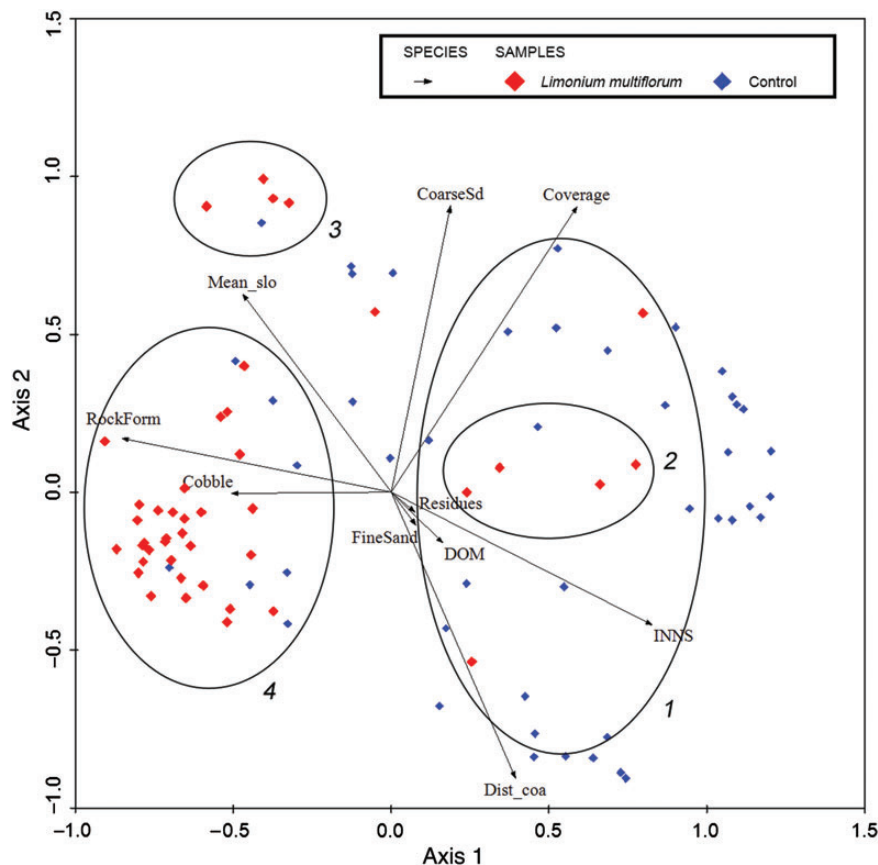
On the western coast most *L. multiflorum* populations occur at inaccessible cliff sites and/or show very small size (Róis et al. 2013). Under these circumstances, this study was conducted at the best accessible site and on the largest known population for this species, in Raso cape. Study site rocks were bare of vegetation up to ~7–8 m asl, due to strong exposure to Atlantic storms and waves over the years.

Of the 88 quadrats sampled, only 43 contained at least one *L. multiflorum* individual (presence) while control quadrats did not show any individual from this species (absence). Considering the presence quadrats, *L. multiflorum* mean coverage was very low (about

0.67 %). Only one of the variables measured in each quadrat fitted a normal distribution (variable ‘Mean\_slo’), and the other nine failed to do so, even after a logarithmic transformation. Therefore, the remaining analyses were performed using the original (untransformed) values. Remarkably, visual inspection of PCA revealed that in the first two represented ordinations, quadrats showing the presence of *L. multiflorum* individuals were highly correlated with rock formation (variable ‘RockForm’) and non-correlated with high coverage (variable ‘vegetation cover’) (Fig. 5). Considering the first two principal components of PCA, the percentage of accumulated variance was 64.4 % (Fig. 5). The first axis accounted for 44.8 % of the variance and was explained by vegetation cover, while the second axis accounted for 19.7 % of the variance explained by distance from coast (variable ‘Dist\_coa’).

### Influence of environmental variables in *L. multiflorum* persistence

A cluster analysis was performed using the dataset of quadrats showing *L. multiflorum* presence. The data



**Figure 5.** Two first axes of PCA based on *L. multiflorum* presence and absence quadrats. Red symbols mean that *L. multiflorum* was recorded whereas blue symbols signify that it was absent. Axis 1 represents 44.8 % of variation, and Axis 2, 19.7 %. CoarseSd, coarse sand; Cobble, cobble; Coverage, vegetation cover; Dist\_coa, distance from coast; DOM, dead organic matter cover; FineSand, fine sand; INNS, invasive non-native species cover; Mean\_slo, mean slope; Residues, litter cover; RockForm, rock formation.





**Table 2.** Mean coverage (%) of the most frequent species in each of the four environmental groups (clusters) based on the presence data of PCA.

Floristic list		Mean coverage (%)			
		1	2	3	4
Psammophilous	<i>Armeria welwitschii</i> Boiss.	1.17	2.34	9.67	0.03
	<i>Inula crithmoides</i> L.	0.00	0.17	0.12	1.33
	<i>Herniaria maritima</i> Link	0.00	0.67	0.00	0.07
	<i>Lobularia maritima</i> (L.) Desv. subsp. <i>maritima</i>	0.00	0.00	0.20	0.00
	<i>Parapholis incurva</i> (L.) C.E.Hubb.	0.50	0.00	21.33	0.70
	<i>Helichrysum italicum</i> (Roth) G.Don subsp. <i>picardi</i> (Boiss. & Reut.) Franco	0.33	0.00	0.17	0.03
	<i>Andryala arenaria</i> (DC.) Boiss. & Reut. subsp. <i>arenaria</i>	0.17	0.33	0.58	0.06
	<i>Otanthus maritimus</i> (L.) Hoffmanns. & Link	0.00	2.75	0.00	0.02
	<i>Elymus farctus</i> (Viv.) Runemark ex Melderis subsp. <i>boreo-atlanticus</i> (Simonet & Guin.) Melderis	3.33	0.77	1.00	0.98
	<i>Lotus creticus</i> L.	0.00	0.01	0.25	0.40
	<i>Medicago minima</i> (L.) L.	0.00	0.50	1.00	0.03
	<i>Crucianella maritima</i> L.	0.13	0.00	0.00	0.03
	<i>Cakile maritima</i> Scop.	0.00	0.00	0.00	0.01
	<i>Dactylis smithii</i> Link subsp. <i>marina</i> (Borrill) Parker	0.02	0.01	0.25	0.09
	Chamosphilous	<i>Limonium ovalifolium</i> (Poir.) Kuntze	0.00	0.00	1.33
<i>Limonium virgatum</i> (Willd.) Fourr.		0.17	0.00	0.07	0.07
<i>Plantago coronopus</i> L.		0.02	0.17	0.75	0.07
<i>Limonium multiflorum</i> Erben		0.03	0.05	0.11	0.84
<i>Frankenia laevis</i> L.		0.83	1.67	1.00	1.03
<i>Crithmum maritimum</i> L.		0.30	3.67	0.23	0.66
<i>Catapodium marinum</i> (L.) C.E.Hubb.		0.00	0.00	0.00	0.03
Nitrophilous	<i>Carpobrotus edulis</i> (L.) N.E.Br.	73.00	33.00	0.00	0.88
	<i>Beta maritima</i> L.	0.08	0.33	0.50	0.28
	<i>Daucus halophilus</i> Brot.	0.00	0.00	0.00	0.01
	<i>Leontodon taraxacoides</i> (Vill.) Mérat subsp. <i>taraxacoides</i>	0.00	0.00	0.00	0.01
	<i>Parapholis filiformis</i> (Roth) C.E.Hubb.	0.50	0.00	0.00	0.06
	<i>Polypogon maritimus</i> Willd.	0.00	0.00	0.00	0.02

‘INNS’ percentages were greater than those in the other groups, Group 2 differed in terms of fine sand percentages (variable ‘FineSand’).

Vegetation composition also varied between clusters. Cluster 1 was dominated by *Carpobrotus edulis* with a coverage percentage >65 % whereas Cluster 2 presented a moderate coverage of *C. edulis* (<35 %). In this cluster significant mean coverage was observed for psammophilous species like the perennials *Armeria welwitschii* and *Elymus farctus* subsp. *boreo-atlanticus*. As for Cluster 3, significant frequencies of the perennial *Limonium ovalifolium* and the therophyte *Parapholis incurva* were revealed. Finally, Cluster 4 which had the greatest coverage percentage of *L. multiflorum* (0.835 %) of all clusters appeared to be associated with chasmophytic species such as *C. maritimum*, and other perennials like *Frankenia laevis* and *Inula crithmoides*.

## Discussion

### *L. multiflorum* displays narrow habitat specificity

In this study we show that *L. multiflorum* grows on sea-cliffs preferentially up to 50 m asl in karstic crevices within exposed rocks more or less filled with substrate, or on shallow soil above the rock strata and on scree slopes. The presence of *L. multiflorum* was favoured by the presence of rock formation, cobble, low percentage of vegetation coverage, low frequency of invasive non-native species and closest distance to coast. In fact, as pointed out by Warming (1909), *L. multiflorum*’s preference for salt-rich rock crevices seems to be a strategy to use such locations as refuges, thus avoiding competition with many other coastal plants that are unable to colonize such habitats. Furthermore, as it happens in endemic *Limonium* spp., populations tend to be fragmented and



usually show low population sizes as a consequence of many social and economic activities like urbanization, tourism and traffic (Rodríguez et al. 2003; Palop-Esteban et al. 2007; Suárez-García et al. 2009; Khan et al. 2012; Róis et al. 2013) on their habitats. This is also the case of *L. multiflorum* (ICNF 2013), in particular, in the Raso cape population (A.F. and V.S., unpubl. res.).

Vegetation composition significantly differs among the four floristically defined groups. Cluster 1 is dominated by the alien plant *C. edulis* with *E. farctus* subsp. *boreo-atlanticus*'s due to nitrophilous coarse sand dunes corresponding to *Elytrigietum junceo-boreoatlanticae* association (Costa et al. 2012). The presence of *L. multiflorum* in this cluster can be explained by 5 % of rock outcrop, uncovered with sand. Cluster 2 includes the farthest quadrats from the sea where rock depressions filled up with fine sand and cobble appear. In this cluster *A. welwitschii*, *F. laevis* and *L. multiflorum* predominate. This cluster presents a poor floristic composition, as it happens in the previous cluster, due to invasion of *C. edulis*, possibly related with the proximity to roads (higher nitrification). Cluster 3 is typically formed by species of rocky substrate, with a high slope and direct influence of wind loaded with salt. Here *L. ovalifolium*, *C. maritimum*, *A. welwitschii*, *F. laevis*, *L. multiflorum*, *P. coronopus* form the perennial community *Limonieta multifloro-virgati* (Costa et al. 1998b, 2012). In their clearings *P. incurva*, *Medicago minima*, *Andryala arenaria* form the poor, annual association *Parapholida incurvae*–*Catapodietum marini*. Also, the presence of *Lotus creticus* is due to coarse sand. Cluster 4 also exhibits species that constitute the *Limonieta multifloro-virgati* association (Costa et al. 1998b, 2012), namely *C. maritimum*, *L. multiflorum*, *I. crithmoides*, *F. laevis*, *P. coronopus*. The referred species are at their ecological optimum in this rocky sea-cliff cluster although a small percentage of coarse sand explains the presence of *E. farctus* subsp. *boreo-atlanticus* and *L. creticus*. Plant survival in this ecological context is difficult due to low availability of fresh water, low level of essential nutrients and the abrasive action of strong winds laden with salt and increasing conditions of extreme dryness (Costa et al. 1998b). To overcome this some plants respond with adaptations, such as adopting the hemicyptophyte or therophyte life forms, presenting succulent leaves and/or salt glands in the leaves (Warming 1909; Costa 2001; Grigore et al. 2014).

### ***L. multiflorum* appears to be intolerant to competition with invasive species**

Our results show that both native and non-native vegetation cover are negatively correlated with *L. multiflorum* presence, emphasizing its preferences for sites where competition with other species is low. Among the species

having a greater negative effect in their persistence is the exotic, invasive, crawling *C. edulis*. This species commonly invades coastal habitats in Mediterranean Europe forming mantles on maritime rocks, cliffs and sand dunes (D'Antonio 1990; Traveset et al. 2008; Pyšek et al. 2013) competing directly with native plant species, suppressing the growth and establishment of other plants (D'Antonio and Mahall 1991; Vilà et al. 2006), altering certain soil parameters (Vilà et al. 2006; Conser and Connor 2009) or creating litter accumulation on the soil surface (Molinari et al. 2007). Although *C. edulis* seems to develop well on rocky substrates at sites where seagulls' (*Larus* sp.) nutrient-rich droppings are frequent (M.D.E.-S., unpubl. res.), in our study it seems to compete mainly with species showing preference for sand substrates. Remarkably, some littoral species show strong resilience to *C. edulis* invasion, like the narrow endemic cliff-species *Armeria pseudoarmeria* (Pinto-Ricardo et al. 2010) or other *Limonium* spp. (Snogerup 1971; Suehs et al. 2003; Campos et al. 2004).

### **Conservation issues**

For the effective protection of the rare and endemic *L. multiflorum* not only ecological data but also life story, demography and genetic data should be taken into account to ensure that maximum genetic variation is preserved. In this context, one of the factors to be considered in conserving *L. multiflorum* is related with chromosome polymorphisms since euploid and aneuploid cytotypes occur within populations (Róis et al. 2012). Although, it was first described as an unbalanced aneuploid tetraploid ( $2n = 4x = 35$ ) (Erben 1978), both unbalanced and balanced tetraploid cytotypes ( $2n = 4x = 35, 36, 37$ ) and diverse karyological polymorphisms were found within and among populations, with greater variability in the Raso cape population (Róis et al. 2012). This has also been observed in other *Limonium* taxa (e.g. Castro and Rosseló 2007), and even in a variety of genera from different plant families (e.g. *Brassicaceae*, Böcher 1954; *Campanulaceae*, James 1965; *Onagraceae*, Bloom 1974; *Plumbaginaceae*, Erben 1978; *Poaceae*, Sieber and Murray 1981; *Ranunculaceae*, Borchers-Kolb 1983). These cytological differences should be considered to ensure that maximum genetic variation is preserved. Furthermore, the resultant combination of incompatible cytotypes could result in the failure of reintroduced plants to reproduce and may bring reproductive instability to augmented populations (Young and Murray 2000; Ennos et al. 2005; Severns and Liston 2008).

Noticeably, male sterility and gynodioecism are found in plants from experimental collections (Róis et al. 2012) and field plants (Heike Sprenger and A.D.C., unpubl. res.). However, these plants are capable of producing large

numbers of viable seeds with variable ploidy levels, as revealed by flow cytometry seed screening (Róis et al. 2012) and reproduce through apomixis (A.S.R. and A.D.C., unpubl. res.). This form of uniparental reproduction is found in ‘taxonomically complex groups’ like in other *Limonium* spp. (Erben 1978; Palacios et al. 2000; Lledó et al. 2005) and also in dandelions (*Taraxacum* spp.; van Dijk 2003), blackberries (*Rubus fruticosus* agg.; Salvini et al. 2006) and in *Ranunculus* spp. (Hörandl et al. 2009), which generates a diverse mixture of related individuals (Ennos et al. 2005).

While some *L. multiflorum* populations are located in natural parks (Parque Natural Sintra-Cascais) and other areas under legal protection (e.g. Site of Community Importance—SCI Sintra/Cascais PTC00008; SCI Peniche/Sta Cruz PTC00056), in the past decades no information on population size along its distribution range has been available. However, we hypothesized that population decline might have occurred due to increasing urban development in the coastal areas and the impact of tourism. For instance, in Raso cape, sightseeing tourists, sports fishing and other socio-economic activities, together with competition associated with invasion by non-native species are cumulatively leading to population decline (V.S., A.F. and A.D.C., unpubl. res.). Notably, methylation-sensitive amplified polymorphism (MSAP) data from *L. multiflorum* natural populations reveal low levels of genetic/epigenetic diversity (Róis et al. 2013). Altogether these data mean that none of the populations analysed could restore the genotypic diversity observed in any other *L. multiflorum* population. Hence, habitat protection emerges as the top priority to prevent population extinction of the narrow-specialist *L. multiflorum*. For the conservation of this species, management actions such as those referred by Laguna et al. (2013), namely limiting access to populations, for example, the experimental exclusion of herbivores by means of fences to protect populations of *L. dufourii* in ‘Marsh dels Moros’ (Spain) and to perform selective removal and control of invasive species (Laguna et al. 2013), are required. Other technical solutions could be population reinforcements, reintroduction or establishment of new populations, the goal of which is the introduction of new specimens. In the case of *L. multiflorum*, attempts to create new populations through experimental actions like sowing seeds directly *in situ* did not prove to be successful (A.D.C. and V.S., unpubl. res.) but population reinforcements by planting newly produced specimens using seed stock that originated from the same natural populations (e.g. Raso cape) appears to give better results (Caperta et al. 2013). Complementary *ex situ* conservation actions for this species such as collection and storage of seeds preserved in João do Amaral Franco Seed Bank (Ajuda

Botanical Garden) have also been conducted (Caperta et al. 2013) with the hope that the genetic diversity contained in these collections could be representative of natural populations. Thus, to conserve *L. multiflorum*, it is preferable to develop small, localized experimental actions as Laguna et al. (2013) pointed out. In fact, only moderate success is reported for reintroduction projects due, for example, to insufficient long-term monitoring following reintroduction and/or lack of understanding of the underlying reasons for decline in existing plant populations (e.g. Godefroid et al. 2011).

## Conclusions

According to Rabinowitz’ scheme (1981), in which species were classified into categories according to their geographic range, habitat specificity and local population size, *L. multiflorum* can be classified as a ‘classic rarity’ since it presents both narrow geographic range and narrow habitat specificity, thus being considered a restricted endemic. Furthermore, species’ populations observed throughout its entire range consistently present small sizes and low levels of genetic diversity as revealed by population genetic and epigenetic studies using MSAP markers (Róis et al. 2013). Taking the above into consideration, as well as diverse social-economic impacts exerted on its populations, we considered that *L. multiflorum* deserves a status of ‘Vulnerable’ in the IUCN red list.

As already pointed out for other ‘taxonomically complex groups’ (Ennos et al. 2005), conservation of rare and endemic *L. multiflorum* is best achieved by facilitating evolutionary interactions among its members that generate and maintain their taxonomic biodiversity.

## Sources of Funding

Our work was funded by Fundação para a Ciência e Tecnologia (FCT) (Portugal) (PEST-OE/AGR/UI0240/2011). A.D.C. (Researcher, CBAA/ISA) and A.S.R. (SFRH/BD/62542/2009 grant) were supported by FCT.

## Contributions by the Authors

A.D.C. and P.A. designed and coordinated the study. M.D.E.-S. coordinated the fieldwork and A.D.C., M.D.E.-S., A.P.P. and A.S.R. performed the fieldwork. A.F. and V.S. processed the raw data and carried out statistical analysis. J.C.C. and M.D.E.-S. performed the phytosociological analysis. A.D.C. and P.A. drafted the manuscript. All authors read and approved the manuscript.

## Conflicts of Interest Statement

None declared.

## Acknowledgements

We thank Manuela Rodrigues (Volunteer), Ana Cortinhas (CBAA/ISA) and Vanessa Mendonça (CBAA/ISA) for help in field work, Jorge Cadima (ISA) for help in defining the experimental design, and the anonymous reviewers for all the helpful comments and suggestions. We thank the Instituto da Conservação da Natureza e das Florestas (ICNF) for providing permissions for field work.

## Literature Cited

- Akeroyd J, Wyse Jackson P. 1995. *A handbook for botanic gardens for reintroduction of plants to the wild*. Richmond: BGCI.
- APA [The Environmental Protection Agency of Portugal]. 2011. *Guide for the assessment of imminent threats and environmental damages*. Lisboa: The Ministry of Agriculture, the Sea, The Environment and Territorial Planning.
- Asensi A, Diez Garretas B, van der Maarel E. 1993. Dry coastal ecosystems of Portugal. In: van der Maarel E, ed. *Dry coastal ecosystems: polar regions and Europe. Ecosystems of the World 2A*. Amsterdam: Elsevier, 342–347.
- Baumberger T, Affre L, Torre F, Vidal E, Dumas PJ, Tatoni T. 2012. Plant community changes as ecological indicator of seabird colonies' impacts on Mediterranean Islands. *Ecological Indicators* **15**: 76–84.
- Bloom WL. 1974. Origin of reciprocal translocations and their effects in *Clarkia speciosa*. *Chromosoma* **49**:61–76.
- Böcher T. 1954. Experimental taxonomic studies in the *Arabis holboellii* and allied species. *Biologiske Skrifter* **14**:1–74.
- Borchers-Kolb E. 1983. Ranunculus sect. Auricomus in Bayern und den angrenzenden Gebieten. I. Allgemeiner Teil. *Mitteilungen der Botanischen Staatssammlung München* **19**:363–429.
- Braun-Blanquet J, Rozeira A, Pinto da Silva AR. 1972. Résultats de deux excursions géobotaniques à travers le Portugal septentrional et moyen IV. Esquisse sur la végétation dunale. *Agronomia Lusitana* **33**:217–234.
- Brussard PF. 1991. The role of ecology in biological conservation. *Ecological Applications* **1**:6–12.
- Campos JA, Herrera M, Biurrun I, Loidi J. 2004. The role of alien plants in the natural coastal vegetation in central-northern Spain. *Biodiversity and Conservation* **13**:2275–2293.
- Caperta AD, Paes AP, Espírito-Santo MD, Silva V, Ferreira A, Saraiva S. 2013. Arribas da faixa costeira de Cascais. Projecto de Recuperação de Habitats. *El Botânico* **7**:69–71.
- Castro M, Rosseló JA. 2007. Karyology of *Limonium* (Plumbaginaceae) species from the Balearic Islands and the western Iberian Peninsula. *Botanical Journal of the Linnean Society* **155**: 257–272.
- Conser C, Connor EF. 2009. Assessing the residual effects of *Carprobrotus edulis* invasion, implications for restoration. *Biological Invasions* **11**:349–358.
- Costa JC. 2001. Tipos de Vegetação e adaptações das plantas do litoral de Portugal continental. In: Moreira ME, Moura A, Granja H, Noronha F, eds. *Homenagem (In Honorium) Professor Doutor Gaspar Soares de Carvalho*. Braga: Universidade do Minho, 283–299.
- Costa JC, Aguiar C, Capelo JH, Lousã M, Neto C. 1998a. Biogeografia de Portugal Continental. *Quercetia* **0**:5–56.
- Costa JC, Capelo J, Lousã M, Espírito-Santo MD. 1998b. Sintaxonomia da vegetação halocasmofítica das falésias marítimas portuguesas (Crithmo-Staticetea Br.-Bl. 1947). *Itinera Geobotanica* **11**:227–247.
- Costa JC, Capelo J, Aguiar C, Neto C, Lousã M, Espírito-Santo MD. 2000. An overview of the Pegano harmalae-Salsoletea vermiculatae Br.-Bl. and. O. Bolòs 1958, vegetation class in continental Portugal. *Colloques Phytosociologiques* **27**:81–93.
- Costa JC, Monteiro-Henriques T, Neto C, Arsénio P, Aguiar C. 2007. The application of the Habitats Directive in Portugal. *Fitosociologia* **44**(Suppl.1):23–28.
- Costa JC, Neto C, Aguiar C, Capelo J, Espírito-Santo MD, Honrado J, Pinto-Gomes C, Monteiro-Henriques T, Menezes de Sequeira M, Lousã M. 2012. Vascular plant communities in Portugal (Continental, the Azores and Madeira). *Global Geobotany* **2**: 1–180.
- Council Directive 92/43/EEC. 21 May 1992. On the conservation of natural habitats and of wild fauna and flora. *Official Journal of the European Union L* **206**:7–50.
- D'Antonio CM. 1990. Seed production and dispersal in the non-native, invasive succulent *Carprobrotus edulis* (Aizoaceae) in coastal strand communities of central California. *Journal of Applied Ecology* **27**:693–702.
- D'Antonio CM, Mahall BE. 1991. Root profile and competition between the invasive, exotic perennial, *Carprobrotus edulis* and two native species in California coastal shrubs. *American Journal of Botany* **78**:885–894.
- Dominguez Lozano F, Moreno Saiz JC, Sainz Ollero H. 2003. Rarity and threat relationships in the conservation planning of Iberian flora. *Biodiversity and Conservation* **12**:1861–1882.
- EC. 2013. European Commission: seventh updated list of sites of community importance for the Mediterranean biogeographical region. *Official Journal of the European Union L* **350**:101–198.
- Ennos RA, French GC, Hollingsworth PM. 2005. Conserving taxonomic complexity. *Trends in Ecology and Evolution* **20**:164–168.
- Erben M. 1978. Die gattung *Limonium* im südwestmediterranen Raum. *Mitteilungen der Botanischen Staatssammlung München* **14**:361–631.
- Erben M. 1993. *Limonium* Miller. In: Castroviejo S, Aedo C, Cirujano S, Lainz M, Montserrat P, Morales R, Muñoz Garmendia F, Navarro C, Paiva J, Soriano C, eds. *Flora Iberica, Vol. 3. Plumbaginaceae (partim)–Capparaceae*. Madrid: Real Jardín Botánico, CSIC, 2–143.
- Espírito-Santo MD, Alves HN, Caperta AD, Moreira I. 2012. Plantas endémicas do litoral de Portugal Continental. In: Monteiro A, Gomes da Silva F, Jorge R, eds. *Gestão e Conservação da Flora e da Vegetação de Portugal e da África Lusófona. In Honorium do Professor Catedrático Emérito Ilídio Rosário dos Santos Moreira*. Lisboa: ISA Press, 267–302.
- Farnsworth EJ. 2007. Plant life history traits of rare versus frequent plant taxa of sandplains: implications for research and management trials. *Biological Conservation* **136**:44–52.
- Frederiksen L, Kollmann J, Vestergaard P, Bruun HH. 2006. A multivariate approach to plant community distribution in the coastal dune zonation of NW Denmark. *Phytocoenologia* **36**:321–342.
- Godefroid S, Piazza C, Rossi G, Buord S, Stevens AD, Agurajua R, Cowell C, Weekley CW, Vogg G, Iriondo J, Johnson I, Dixon B, Gordon D, Magnanon S, Valentin B, Bjureke K, Koopman R, Vicens M, Virevaire M, Vanderborgh T. 2011. How successful are plant species reintroductions? *Biodiversity and Conservation* **144**:672–682.



- Grigore M-N, Ivanescu L, Toma C. 2014. *Halophytes: an integrative anatomical study*. New York: Springer.
- Heywood VH, Iriondo JM. 2003. Plant conservation: old problems, new perspectives. *Biological Conservation* **113**:321–335.
- Hörandl E, Greilhuber J, Klimova K, Paun O, Temsch E, Emadzade K, Hodálová I. 2009. Reticulate evolution and taxonomic concepts in the *Ranunculus auricomus* complex (Ranunculaceae): insights from morphological, karyological and molecular data. *Taxon* **58**: 1194–1215.
- ICNF. 2013. *Relatório Nacional de Aplicação da Directiva Habitats (2007–2012)*. Lisboa: Instituto da Conservação da Natureza e das Florestas.
- IUCN. 2013. *IUCN red list of threatened species*. Version 2013.2. <http://www.iucnredlist.org> (5 February 2014).
- James SH. 1965. Complex hybridity in *Isotoma petraea*. 1. The occurrence of interchange heterozygosity, autogamy and a balanced lethal system. *Heredity* **20**:341–353.
- Khan Z, Santpere G, Traveset A. 2012. Breeding system and ecological traits of the critically endangered endemic plant *Limonium barceloi* (Gil and Llorens) (Plumbaginaceae). *Plant Systematics and Evolution* **298**:1101–1110.
- Kubitzki K. 1993. Plumbaginaceae. In: Kubitzki K, Rohwer JG, Bittrich V, eds. *The families and genera of vascular plants*, Vol. 2. Berlin: Springer, 523–530.
- Laguna E, Deltoro V, Ferrer PP, Navarro A, Ferrando I, Escribá MC, Albert FJ. 2013. Relevant plant recovery programmes. Conservation management of plant micro-reserves and ecological restoration. In: Kadis C, Thanos CA, Laguna E, eds. *Plant micro-reserves: from theory to practice. Experiences gained from EU LIFE and other related projects*. Athens: Utopia Publishing, 127–140.
- Lledó MD, Crespo MB, Fay MF, Chase MW. 2005. Molecular phylogenetics of *Limonium* and related genera (Plumbaginaceae): biogeographical and systematic implications. *American Journal of Botany* **92**:1189–1198.
- Magos-Brehm J, Maxted N, Ford-Lloyd BV, Martins-Loução MA. 2008. National inventories of crop wild relatives and wild harvested plants: case-study for Portugal. *Genetic Resources and Crop Evolution* **55**:779–796.
- Martins MC, Neto C, Costa JC. 2013. The meaning of mainland Portugal beaches and dunes' psammophilic plant communities: a contribution to tourism management and nature conservation. *Journal of Coastal Conservation* **17**:279–299.
- Maun MA. 2004. Burial of plants as a selective force in sand dunes. In: Martínez ML, Psuty NP, eds. *Coastal dunes. Ecology and conservation*. Heidelberg: Springer, 119–135.
- Menezes de Sequeira M, Espírito-Santo MD, Aguiar C, Capelo J, Honrado J, coords. 2011. *Checklist da Flora de Portugal (Continental, Açores e Madeira)*. Lisboa: ALFA [Associação Lusitana de Fitossociologia].
- Mesquita S, Sousa AJ. 2009. Bioclimatic mapping using geostatistical approaches: application to mainland Portugal. *International Journal of Climatology* **29**:2156–2170.
- Molinari HBC, Marur CJ, Daros E, Campos MKF, Carvalho JFRP, Filho JCB, Pereira LFPP, Vieira LGE. 2007. Evaluation of the stress-inducible production of proline in transgenic sugarcane (*Saccharum* spp): osmotic adjustment, chlorophyll fluorescence and oxidative stress. *Physiologia Plantarum* **130**: 218–229.
- Monteiro-Henriques T. 2010. *Fitossociologia e Paisagem da Bacia Hidrográfica do Rio Paiva/Landscape and phytosociology of the Paiva River's hydrographical basin*. PhD Thesis, Universidade Técnica de Lisboa, Instituto Superior de Agronomia, Lisboa.
- Mucina L. 2013. Europe, ecosystems of. In: Levin SA, ed. *Encyclopedia of biodiversity*. Waltham, MA: Academic Press, 333–346.
- Palacios C, Rosselló JA, González-Candelas F. 2000. Study of the evolutionary relationships among *Limonium* species (Plumbaginaceae) using nuclear and cytoplasmic molecular markers. *Molecular Phylogenetics and Evolution* **14**:232–249.
- Palop-Esteban M, Segarra-Moragues JG, González-Candelas F. 2007. Historical and biological determinants of genetic diversity in the highly endemic triploid sea lavender *Limonium dufourii* (Plumbaginaceae). *Molecular Ecology* **16**:3814–3827.
- Pärtel M, Kalamees R, Reier U, Tuvi E-L, Roosaluuste E, Vellak A, Zobel M. 2005. Grouping and prioritization of vascular plant species for conservation: combining natural rarity and management need. *Biological Conservation* **123**:271–278.
- Pinto-Ricardo C, Espírito-Santo MD, Carrasquinho I, Veloso C, Ricardo CP. 2010. Impact of the invasive *Carpobrotus edulis* on *Armeria pseudarmeria* at Cabo da Roca. *Proceedings of the XIII OPTIMA Meeting*, 85.
- Pyšek P, Genovesi P, Pergl J, Monaco A, Wild J. 2013. Plant invasions of protected areas in Europe: an old continent facing new problems. In: Foxcroft LC, Pyšek P, Richardson DM, Genovesi P, eds. *Plant invasions in protected areas: patterns, problems and challenges, invading nature*, Vol. 7, Springer Series in Invasion Ecology, Dordrecht.
- R Core Team. 2013. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. <http://www.R-project.org>.
- Rabinowitz D. 1981. Seven forms of rarity. In: Synge H, eds. *The biological aspects of rare plant conservation*. New York: John Wiley & Sons Ltd, 205–217.
- Rivas-Martínez S. 2007. Mapa de series, geoseries y geopermaseries de vegetación de España. Memoria del mapa de vegetación potencial de España. *Itinera Geobotanica* **17**:5–436.
- Rodríguez S, Palop ML, Palacios C, González-Candelas F. 2003. Molecular and morphological differentiation in *Limonium dufourii* (Plumbaginaceae), an endangered Mediterranean plant. *Conservation Genetics* **4**:383–391.
- Róis AS, Teixeira G, Sharbel TF, Fuchs J, Martins S, Espírito-Santo MD, Caperta AD. 2012. Male fertility versus sterility, cytotype, and DNA quantitative variation in seed production in diploid and tetraploid sea lavenders (*Limonium* sp., Plumbaginaceae) reveal diversity in reproduction modes. *Sexual Plant Reproduction* **25**: 305–318.
- Róis AS, López CMR, Cortinhas A, Erben M, Espírito-Santo MD, Wilkinson M, Caperta AD. 2013. Epigenetic rather than genetic factors may explain phenotypic divergence between coastal populations of diploid and tetraploid (*Limonium* sp.; Plumbaginaceae) in Portugal. *BMC Plant Biology* **13**:205.
- Salisbury E. 1952. *Downs and dunes. Their plant life and environment*. London: G. Bell and Sons.
- Salvini D, Fineschi S, Pastorelli R, Sebastiani F, Vendramin G. 2006. Absence of geographic structure in European populations of *Rubus fruticosus* L. complex using chloroplast DNA microsatellites. *Journal of the American Society for Horticultural Science* **131**:616–621.
- Scheffers A, Kelletat D. 2005. Tsunami relics on the coastal landscape west of Lisbon, Portugal. *International Journal of the Tsunami Society* **1**:3–16.



- Schemske DW, Husband BC, Ruckelshaus MH, Goodwillie C, Parker I, Bishop JG. 1994. Evaluating approaches to the conservation of rare and endangered plants. *Ecology* **75**:584–606.
- Severns PM, Liston A. 2008. Intraspecific chromosome number variation: a neglected threat to the conservation of rare species. *Conservation Biology* **22**:1641–1647.
- Sieber VK, Murray BG. 1981. Structural and numerical chromosomal polymorphism in natural populations of *Alopecurus* L. (Poaceae). *Plant Systematics and Evolution* **139**:121–136.
- Simberloff D. 1988. The contribution of population and community to conservation science. *Annual Review of Ecology and Systematics* **19**:473–511.
- Snogerup S. 1971. Evolutionary and plant geographical aspects of chasmophytic communities. In: Davis PH, Harper PC, Hedge IC, eds. *Plant life of South West Asia*. Aberdeen: Botanical Society of Edinburgh UK, 157–170.
- Sokal RR, Rohlf FJ. 1997. *Biometry: the principles and practice of statistics in biological research*, 3rd edn. New York: WH Freeman and Co.
- Suárez-García C, de Paz JP, Febles R, Caujapé-Castells J. 2009. Genetic diversity and floral dimorphism in *Limonium dendroides* (Plumbaginaceae), a woody *Canarian* species on the way of extinction. *Plant Systematics and Evolution* **280**:105–117.
- Suehs CM, Médail F, Affre L. 2003. Invasion by South African *Carpobrotus* (Aizoaceae) taxa in the Mediterranean Basin: the effects of insularity on plant reproductive systems. In: Child L, Brock JH, eds. *Plant invasions: ecological threats and management solutions*. Leiden: Backhuys Publishers, 247–263.
- Traveset A, Brundu G, Carta L, Mprezetou I, Lambdon P, Manca M, Médail F, Moragues E, Rodríguez-Pérez J, Siamantziouras AD, Suehs CM, Troumbis AY, Vilà M, Hulme PE. 2008. Consistent performance of invasive species within and among islands of the Mediterranean basin. *Biological Invasions* **10**:847–858.
- van der Maarel E. 1993. *Dry coastal ecosystems: polar regions and Europe*. The Netherlands: Elsevier Science Publishers.
- van der Maarel E, van der Maarel-Versluys M. 1996. Distribution and conservation status of littoral vascular plant species along the European coasts. *Journal of Coastal Conservation* **2**: 73–92.
- van Dijk PJ. 2003. Ecological and evolutionary opportunities of apomixis: insights from *Taraxacum* and *Chondrilla*. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **358**:1113–1121.
- Vilà M, Tessier M, Suehs CM, Brundu G, Carta L, Galanidis A, Lambdon P, Manca M, Médail F, Moragues E, Traveset A, Troumbis AY, Hulme PE. 2006. Local and regional assessment of the impacts of plant invaders on vegetation structure and soil properties of Mediterranean islands. *Journal of Biogeography* **33**:853–861.
- Ward JH. 1963. Hierarchical grouping to optimize an objective function. *Journal of the American Statistical Association* **58**: 236–244.
- Warming E. 1909. *Oecology of plants: an introduction to the study of plant-communities*. Oxford: Clarendon Press.
- Young AG, Murray BG. 2000. Genetic bottlenecks and dysgenic gene flow into re-established populations of the grassland daisy, *Rutidosis leptorrhynchoides*. *Australian Journal of Botany* **48**: 409–416.
- Zar JH. 2010. *Biostatistical analysis*. Prentice-Hall: Upper Saddle River.