



Effect of environment on distributions of rock ferns in the Mediterranean climate: The case of the genus *Asplenium* in Montenegro



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ABSTRACT

The aim of this study was to explore the way in which environmental aspects influence distributions of rock flora of the Mediterranean basin, using the genus *Asplenium* as a study system. Using the most comprehensive dataset to date built with the data from museum collections and those obtained in the course of a field survey, we identified patterns of species richness, carried out multivariate analysis, and fitted generalised linear models to the presence/pseudo-absence and abundance data of the three wide-spread taxa. We complemented the information on climatic preferences of the *Asplenium* ferns by phytosociological data.

Based on their distributions in the ecological space defined by temperature- and precipitation-related variables, the *Asplenium* ferns known for Montenegro were assigned to the three groups: coastal species, generalist, and highland species. Within these groups, no further niche differentiation could be detected given the available environmental factors. Phytosociological information from herbarium specimens was consistent with the results of the multivariate ecological analysis. Non-climatic environmental factors were limiting the distributions of the *Asplenium* ferns on the country-size scale, but were not statistically significant in separating niches among the species. Our results showed a significant impact of water deficit on the distributions of the two morphologically distinct and locally abundant species; these species can be recommended as potential indicators of the increasing water deficit stress in the European Mediterranean climate. Species richness patterns of the spleenworts of Montenegro suggested that the whole country was important for maintaining the diversity of this group of plants.

This study demonstrates the value of natural history collections combined with field data to inform ecological research. For the first time, a numerical analysis of the data concerning distribution and abundance of the most common and wide-spread European ferns of the genus *Asplenium* in an area with the typical Mediterranean climate was carried out.

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1. Introduction

The pteridological flora of the Mediterranean basin consists of ca. 100 taxa (Pichi-Sermolli, 1979) and makes an important contribution in terms of species diversity to the vascular flora of this globally important hot-spot of biodiversity (Comes, 2004). Despite

that the Mediterranean Region has been recognised as a key area for the long-term survival of the species and genetic diversity of ferns (Vogel et al., 1999), pteridophytes are currently underrepresented in ecological studies focused on the vegetation of Southern Europe. As a result, only a limited number of research reports on the subject has been published so far (e.g. Birks, 1976; Bona et al., 2007; Marquez et al., 1997; Prelli and Boudrie, 2002; Saiz and Lobo, 2008).

The genus *Asplenium* L. represented in Europe by ca. 50 taxa (Tutin et al., 1993; Vogel et al., 1999) is an essential component of the Mediterranean fern flora. This is largely due to the preferences of the majority of the European *Asplenium* species for limestone

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outcrops (Vogel et al., 1999) common in the region. Some aspects of ecology (life forms, associations with other species and plant communities) of the common species of *Asplenium* have been studied in detail (Database of Insects and their Food Plants, <http://www.brc.ac.uk/dbif/hosts.aspx>; Didukh, 2000, 2011; Prelli and Boudrie, 2002; Rašomavičius, 2007; Sârbu et al., 2013; Soster, 2001; Vashka and Bezsmertna, 2012). However, the knowledge about spleenworts' relationships with environmental factors remains limited (Troia et al., 2012). Anecdotal evidence suggests that species of *Asplenium* can have contrasting environmental preferences, despite their largely similar growth form and tendency to occupy similar substrates. For example, *A. ceterach* is known for its high drought tolerance (Zivkovic et al., 2010) which allows the species to grow in full sun, while *A. scolopendrium* prefers deeper, more protected crevices, and also occurs in forests (Reichstein, 1984). Whether the presumed differences in species' environmental niches can be adequately quantified given the available data on species distributions remains questionable (Bystriakova et al., 2012).

The aim of this research was to identify the main environmental gradients separating species niches and limiting their distributions at the country-size geographic scale using the case of 10 species of the genus *Asplenium* distributed in Montenegro. Despite a relatively small area of just under 14,000 km², the terrain of Montenegro ranges from sea level to 2500 m asl. (Bulić et al., 2011). Over two thirds of the terrain are dominated by karst formations (Radulovic and Radulovic, 1997). A combination of the distinct climatic gradients with calcium rich substrate found in abundance in a relatively small area makes Montenegro an ideal location for studying the ecology of spleenworts. Ecological descriptions of the *Asplenium* species of Montenegro – mostly in the form of brief characteristics of substrate preferences – are available from floristic literature (Bubanja, 2004; Bulić, 2008; Čurović et al., 2011; Hadžiablahović, 2010; Karaman, 1997; Menković et al., 2011; Mrdak et al., 2011; Pančić, 1875; Petrović, 2004, 2005, 2011; Pulević, 2005; Rohlena, 1942; Steč1ević, 2001, 2002; UNEP Vienna, 2010; Vladimirov et al., 2006 Čurović et al., 2011; Hadžiablahović, 2010; Karaman, 1997; Menković et al., 2011; Mrdak et al., 2011; Pančić, 1875; Petrović, 2004, 2005; Petrović, 2011 Pulević, 2005; Rohlena, 1942; Steč1ević, 2001 Steč1ević, 2002 UNEP Vienna, 2010; Vladimirov et al., 2006). However, the wealth of information displayed on the labels of herbarium specimens appeared to be largely unreported. In the course of our project we captured not only collection localities available from herbarium specimens, but also phytosociological characteristics of habitats where species were collected. We complemented the data from herbarium collections and literature on species abundance data obtained in the course of a field survey covering the most fern species rich habitats of Montenegro.

Specifically we were asking the following questions: (1) what are the environmental gradients separating niches among *Asplenium* species in Montenegro? (2) Do phytosociological characteristics of species obtained from literature, herbarium specimens and field records support the results of the niche analysis? (3) What environmental factors control distributions of *Asplenium* species on the country-size geographic scale?

2. Material and methods

2.1. Distribution data

According to the available literature, the ferns of Montenegro comprise 40 species from 16 genera, of which 12 belong to the genus *Asplenium* (Rohlena, 1942; Pulević, 2005; Stešević and Berg, 2015). We used two distinct datasets to conduct our study: Dataset 1 consisted of presence-only distribution records (483 unique localities in total) of 10 species of *Asplenium* (Table 1 and

Table 1
Species of the genus *Asplenium* in Montenegro selected for the study.

Species and authorities	Acronym	Number of records (Dataset 1 + Dataset 2)
<i>A. adiantum-nigrum</i> L. complex	aspadi	85 (78 + 7)
<i>A. ceterach</i> L. complex	aspctet	187 (118 + 69)
<i>A. fissum</i> Kit ex Willd.	aspfis	56 (50 + 6)
<i>A. lepidum</i> C. Presl	asplep	6 (6 + 0)
<i>A. petrarcae</i> DC.	asppet	1 (1 + 0)
<i>A. ruta-muraria</i> L.	asprut	87 (55 + 32)
<i>A. scolopendrium</i> L.	aspsco	17 (17 + 0)
<i>A. septentrionale</i> (L.) Hoffm.	aspsep	7 (6 + 2)
<i>A. trichomanes</i> L. complex	asptri	181 (125 + 56)
<i>A. viride</i> Huds.	aspvir	33 (27 + 6)

Table S1) obtained by georeferencing specimen information held by seven herbaria (Table S2); Dataset 2 consisted of 131 presence/true absence records of seven species (Fig. S1) and was assembled in the course of fieldwork carried out in 2013. As part of Dataset 2, 30 plots were selected so that they were at least 1 km apart from each other and from the georeferenced locations of Dataset 1. In each plot with the size of 5 m × 5 m, the numbers of viable individuals of all identified species of *Asplenium* were recorded. Here we treat *A. adiantum-nigrum* in its wider taxonomic sense by including *A. cuneifolium* and *A. onopteris* (Tutin et al., 1993). The collected specimens were deposited in the Herbarium of the Natural History Museum, London, UK (BM); Herbarium of the Natural History Museum of Montenegro, Podgorica, Montenegro; and Herbarium O.V. Fomin Botanical Garden of the National Taras Shevchenko University of Kyiv (KWHU).

2.2. Phytosociological characteristics

All phytosociological data (i.e. descriptions of the plant communities where species were collected from) available from herbarium specimens, field records and literature (Bubanja, 2004; Bulić, 2008; Chytrý, 2009; Čurović et al., 2011; Hadžiablahović, 2010; Karaman, 1997; Menković et al., 2011; Mrdak et al., 2011; Pančić, 1875; Petrović, 2004, 2005, 2011; Pulević, 2005; Rohlena, 1942; Stešević, 2001, 2002; UNEP Vienna, 2010; Vladimirov et al., 2006 Pulević, 2005; Rohlena, 1942; Stešević, 2001, 2002; UNEP Vienna, 2010; Vladimirov et al., 2006) were combined in a single database (Table S3). The format of the available data on plant communities was heterogeneous, because in the past botanists of the former Yugoslavia often used Eastern European vegetation classification (Aleksandrova, 1969), while current research on the Balkan vegetation is based on principles and methods of the Zürich-Montpellier School (Braun-Blanquet, 1964). For the purposes of the present study, the collected information was generalised and the names of the plant communities were used in accordance with the Braun-Blanquet's system (Braun-Blanquet, 1964) widely accepted in Europe (Mucina, 1997; Rodwell et al., 2002). Comparison with the data from Spain (Carmona et al., 1997), the Czech Republic (Chytrý, 2009), Poland (Świerkosz, 2004), Romania (Sârbu et al., 2013), and the Ukraine (Didukh, 2000; Solomakha, 2008) provided re-assurance that the resulting cenological characteristics of *Asplenium* species of Montenegro were thorough and comprehensive.

2.3. Data analysis

All statistical analyses were performed in R version 3.1.2 (The R Foundation for Statistical Computing, 2014) and ArcGIS 10.2.2 (Esri Inc., 2014). A species richness map was generated by assigning each collection locality (533 unique records from Datasets 1 and 2 combined) to a ca. 100 km² grid cell and calculating the number of individual species in each grid cell.

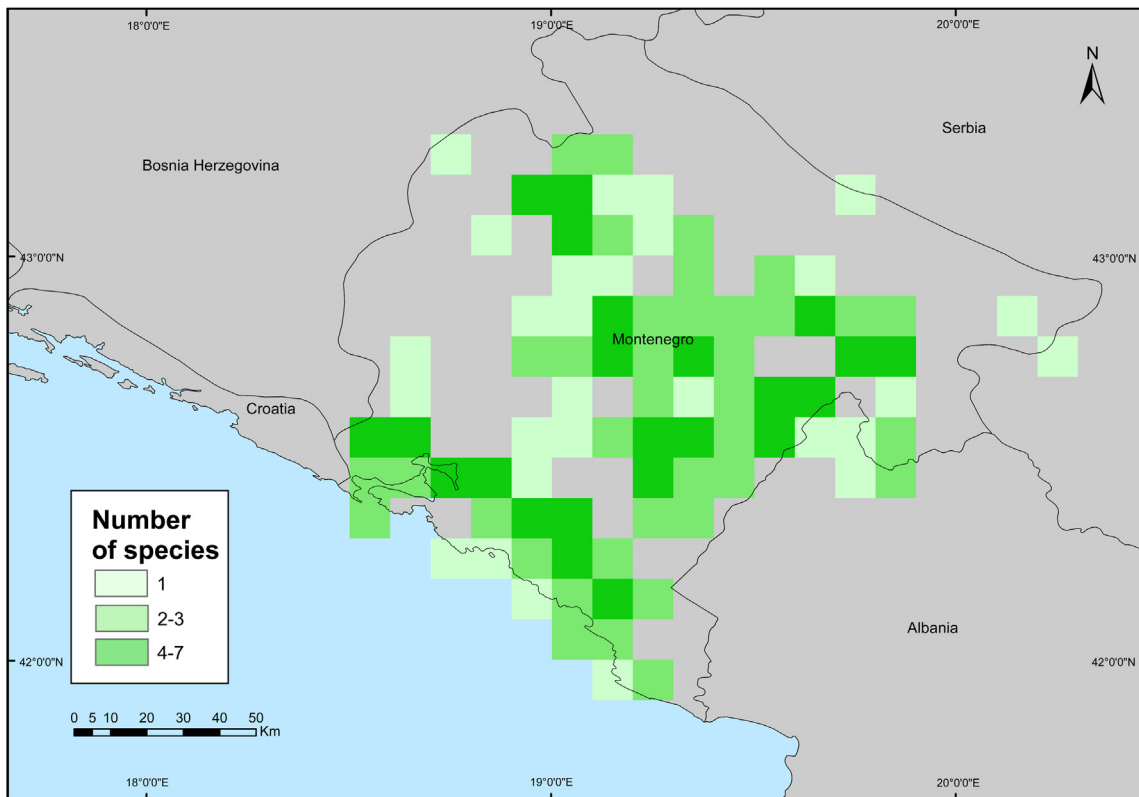


Fig. 1. Species richness of the genus *Asplenium* in Montenegro. Darker colours of green represent higher numbers (maximum of seven) of co-occurring species in ca 100 km² grid cells.

To account for variability within the dataset due to climatic factors, we used 19 Bioclim variables representing the current climate (Hijmans et al., 2005), mean annual potential evapotranspiration (PET), actual evapotranspiration (AET), and annual water deficit (WDEF, defined as PET–AET) variables downloaded from www.cgiar.org (last accessed 15.04.15.). To assign distribution records to various land use types, we used five categories following CORINE Land Cover 2006 classification (<http://www.eea.europa.eu/legal/copyright>, last accessed 15.04.15.): artificial surfaces; agricultural areas; forests; scrub; and open spaces. The type of dominant parental material at species' locations was identified from the European Soil Database (Panagos et al., 2012) using the following three categories: sandstone (including flysch); limestone; and unconsolidated deposits (including glacio-fluvial deposits). To characterize surface features of the landscape, we used the value of slope (in degrees) calculated from the altitude variable with the resolution of 30-arc seconds (ca 1 km).

In the absence of an *a priori* knowledge of species ecology, we used all environmental variables described above and collection localities from Datasets 1 and 2 combined to perform canonical correspondence analysis (CCA) implemented in the R package “vegan” with the default settings. To identify a set of variables making significant contribution to explaining variation within the dataset, we used function “step” in R with the default settings. Because contributions of variables describing substrate, topography and land cover were not statistically significant (the results are not presented here), we carried out outlying mean index (OMI) analysis (Doledec et al., 2000) using a set of climatic variables (i.e. 19 Bioclim variables, PET, AET and WDEF). This is a multivariate analysis designed to study niche separation along environmental gradients; unlike other multivariate techniques (e.g. CCA or RDA), this type of community analysis does not make *a priori* assumptions about the shape of response curves, which could be either linear or unimodal

(Doledec et al., 2000; Thuiller et al., 2004). To find out whether the resulting positions of the species' niches were significantly different from each other, we calculated distance matrixes using the observed and randomly permuted values. The permutation test (function “as.rundtest” implemented in the R package “ade4”) was repeated 1000 times and the corresponding *P*-values extracted. If the *P*-value was 0.05 or smaller, the observed distance between the positions of any two species was considered as significantly different from random (Thuiller et al., 2004).

For the three co-occurring wide-spread taxa, the *A. ceterach* complex, *A. ruta-muraria* and *A. trichomanes* complex we fitted generalised linear models (GLM) with binomial errors (implemented in R package “stats”) to Dataset 1. No attempt was made to separate subspecies recognized within these species complexes. The locations with the target species records were classified as “presences” and those where at least one other species of *Asplenium* was recorded, but not the target one, were classified as “pseudo-absences”. Because the Bioclim variables were highly correlated with each other, in the regression analysis we used two variables, water deficit, quantified as the amount of water by which potential evapotranspiration exceeds actual evapotranspiration, and temperature seasonality, defined as standard deviation of the monthly mean temperatures expressed as a percentage of the mean of those temperatures (i.e. the annual mean). Correlation between these two variables was low: for Dataset 1, Spearman correlation coefficient was 0.4; and for Dataset 2, 0.17. The variables describing substrate, topography and land cover were converted to factors. The results of the regression analysis of Dataset 1 were compared with those obtained from GLM with Poisson errors fitted to the abundance data (interpreted as count data) from Dataset 2.

The taxonomy mainly follows the treatment provided in Flora Europea (Tutin et al., 1993). To enable integration of information on distribution of taxa without checking all vouchers, several species

complexes were recognized in a broad sense. Those were the *A. adiantum-nigrum* complex, besides the tetraploid *A. adiantum-nigrum* also including the diploids *A. cuneifolium* and *A. onoperis*; *A. ceterach* complex including diploid, tetraploid and perhaps also hexaploid karyotypes; and the *A. trichomanes* complex including diploid and tetraploid karyotypes such as the diploid subspecies *trichomanes*, diploid subspecies *inexactans*, tetraploid subspecies *quadri-valens*, and tetraploid subspecies *pachyrachis*. Diploid and tetraploid karyotypes are also known from *A. ruta-muraria* while all other recorded species comprise only a single karyotype in the flora of Montenegro.

3. Results

3.1. Phytosociological characteristics

In Montenegro, species of the genus *Asplenium* occurred in nine classes of vegetation types (Table S3). The majority of species occurred in three to four vegetation classes with the exception of *A. septentrionale* and *A. lepidum* found in one class only, and *A. trichomanes* found in six classes. All species were found in the class *Asplenieta trichomanis* where plants typically experience various types of stress including water and nutrient shortage and large temperature fluctuations.

The roles of *Asplenium* species in these vegetation classes (as described in the available literature) were different: most of the taxa were diagnostic species for some classes (*Asplenieta trichomanis*, *Adiantetea*, *Thlaspietea rotundifolii*), while they rarely occurred in other classes. In particular, *Asplenium adiantum-nigrum*, *A. ceterach*, *A. lepidum*, *A. petrarchae*, *A. ruta-muraria*, *A. scolopendrium*, *A. septentrionale*, *A. trichomanes* and *A. viride* were diagnostic species for *Asplenieta trichomanis*; *A. scolopendrium* was a diagnostic species for *Adiantetea*; and *A. fissum* for *Thlaspietea rotundifolii*.

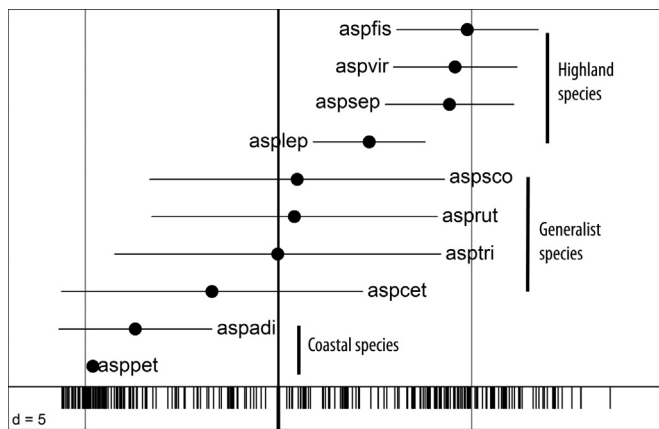


Fig. 2. Species niches' projections on the first axis of the ordination diagram defined by temperature and rainfall decreasing from left to right, and summer rainfall increasing in the same direction. For instance, *A. petrarchae* (asppt, in the bottom-left corner of the left chart) occupies the warmest sites with high annual rainfall, but experiencing summer drought. *A. fissum* (aspfis, in the top right corner of the left chart) occupies the coldest locations with the lowest amount of rain, mostly falling in summer. Horizontal bars indicate niche breadth (two standard deviations from the mean), centres of gravity of the individual species are shown as dots, the vertical black line corresponds to the average (mean) of the axis; vertical bars at the bottom of the diagram show the positions of collection localities (expressed as standard deviations from the mean) along the x-axis, all vertical lines are five standard deviations apart. Species acronyms are as in Table 1.

Table 2

Results of the Outlying Mean Index (OMI) analysis (Doledec et al., 2000) designed to study niche separation along environmental gradients (in our case those were 19 Bioclim variables, PET, AET and WDEF). Decomposed variability (total inertia) of the niche of species *S*: (1) an index of marginality (M_i), which is the deviation of the average position of species *S* from the centre of gravity, or the origin of the PCA axes, which corresponds to the overall mean habitat; (2) an index of tolerance (T_m), or niche breadth; and (3) a residual tolerance (T_r), or the variance of the niche that has not been taken into account by the marginality axis. The total inertia of a species *S*, $I_t(S)$ is given by: $I_t(S) = M_i(S) + T_m(S) + T_r(S)$. Species acronyms as in Table 1. The generalist species, i.e. those with the niches positioned close to the overall mean habitat, are shown in bold. For the detailed description of the method, see Doledec et al. (2000).

Species	Total inertia	M_i	T_m	T_r
asptri	20.938	0.022	2.583	18.333
asprut	16.130	0.270	6.703	9.157
aspsco	17.602	0.335	10.128	7.139
aspct	22.074	3.102	15.306	3.665
aspsep	14.256	10.825	0.311	3.120
aspdi	21.888	14.555	4.225	3.108
aspsep	23.930	20.087	2.270	1.573
aspvir	25.341	21.061	2.518	1.762
aspfis	28.976	24.373	3.442	1.161
asppt	26.954	26.954	0.000	0.000

3.2. Species richness and environmental niches of *Asplenium* species

The results presented in this section are based on the analysis of the Datasets 1 and 2 combined. The highest number of co-occurring species in a ca. 100 km² grid cell was seven (Fig. 1). Visual examination of the *Asplenium* species richness patterns in Montenegro did not reveal any apparent spatial structure, correlation with environmental gradients or a single centre of species diversity.

Only climate-related variables were making significant contribution to separation of species' niches. In the hypothetical climatic space defined by selected variables, the species of *Asplenium* were clearly separated into three distinctive groups (Fig. 2). The composition of these groups was also confirmed by results of the multivariate analysis. Thus, the species positioned closely to the overall mean habitat (*A. scolopendrium*, *A. ruta-muraria*, *A. trichomanes* and *A. ceterach*) also appeared to be generalists with relatively wide climatic niches (Table 2). The remaining species (*A. lepidum*, *A. fissum*, *A. septentrionale* and *A. viride*) found in colder climate, with a less pronounced seasonality in rainfall distribution, were clearly separated from *A. adiantum-nigrum* based on the results of the permutation tests (Table 3). *Asplenium adiantum-nigrum* was grouped together with *A. petrarchae* (represented by one collection only) based on predominantly coastal distributions of these taxa in the warmest habitats characterised by the typical Mediterranean climate. The visual representation of species' niches in the two-dimensional space is provided in Fig. 3.

Table 3

Results of the permutation test for significance of differences in niche parameters of the five species (species acronyms as in Table 1). The niche of *A. adiantum-nigrum* (aspdi, in bold) is significantly different from the niches of the remaining highland species.

	aspfis	aspsep	aspvir	aspdi
aspdi	0.001	0.001	0.001	0.001
aspfis	0	0.086	0.601	0.678
aspsep	0	0	0.355	0.179
aspvir	0	0	0	0.837

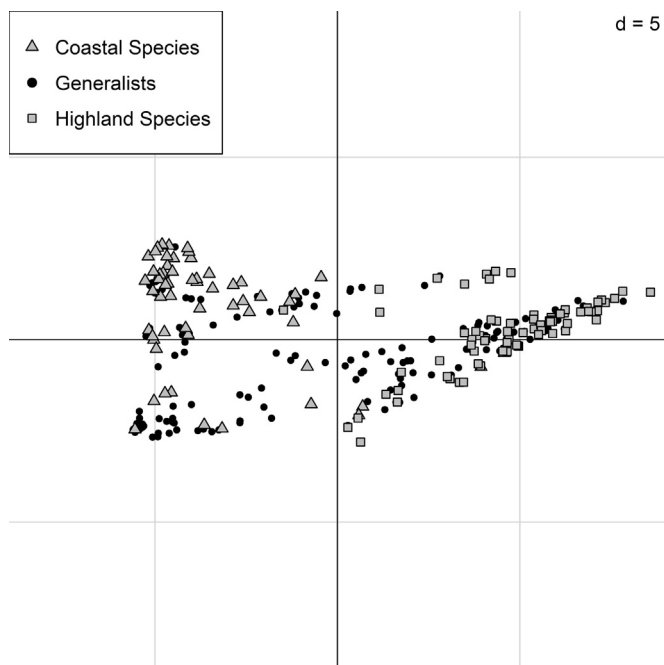


Fig. 3. Climatic niches in a two-dimensional hypothetical space defined by selected climatic variables. The first axis is defined by temperature and rainfall decreasing from left to right, with summer rainfall increasing in the same direction. The second axis is defined by temperature seasonality and temperature annual range increasing from top to bottom. Collection localities shown as triangles for coastal species: *A. adiantum-nigrum* complex (aspadi) and *A. petrarchae* (represented by one collection only); as bullet points, for generalists: *A. ceterach* complex (aspcet), *A. ruta-muraria* (asprut), *A. scolopendrium* (aspsco) and *A. trichomanes* complex (asptri); as squares, for highland species: *A. fissum* (aspsfis), *A. lepidum* (asplep), *A. septentrionale* (aspsep) and *A. viride* (aspvir). The grid mesh size is five standard deviations.

Table 4
Coefficients and significance values of generalised linear models (family “binomial”) fitted to Dataset 1 (presence/pseudo-absence records).

Parameter	Estimate	Standard error	z value	Pr (> z)
<i>A. ceterach</i> /intercept	−0.617	0.120	−5.129	<0.0001
Temperature seasonality (BIO4)	0.230	0.131	1.753	0.0796
Water deficit	0.505	0.137	3.703	0.0002
Slope	−0.397	0.134	−2.955	0.0031
<i>A. ruta-muraria</i> /intercept	−2.930	0.459	−6.384	<0.0001
Limestone	1.950	0.484	4.028	<0.0001
Open spaces	−1.597	0.618	−2.583	0.0098
<i>A. trichomanes</i> /intercept	0.163	0.330	0.493	0.6223
Limestone	−0.722	0.355	−2.035	0.0418
Sandstone	−0.673	0.419	−1.607	0.1080

3.3. Correlations of species distributions with environmental variables

The amount of variation explained by the regression models was low for all species when presence/pseudo-absence data from the Dataset 1 were analysed using a generalised linear model; less than 10% for *A. ceterach* complex and *A. ruta-muraria*, and less than 1% in the case of *A. trichomanes* complex. In the case of *A. ceterach* complex, temperature seasonality and water deficit were positively correlated with species' presence, while the effect of slope was negative. The distributions of *A. ruta-muraria* and *A. trichomanes* complex were not significantly related to any of the climatic variables; however, the dominant parental material and type of land cover (in the case of *A. ruta-muraria*) made significant contribution to the models (Table 4).

Table 5
Coefficients and significance values of generalised linear models (family “poisson”) fitted to Dataset 2 (abundance records).

Parameter	Estimate	Standard error	z value	Pr (> z)
<i>A. ceterach</i> /intercept	1.942	0.090	21.560	<0.0001
Temperature seasonality (BIO4)	−0.273	0.054	−5.079	<0.0001
Water deficit	1.003	0.092	10.891	<0.0001
<i>A. ruta-muraria</i> /intercept	0.923	0.182	5.073	<0.0001
Water deficit	−1.057	0.155	−6.804	<0.0001
Forest	−0.665	0.247	−2.690	0.0071
<i>A. trichomanes</i> /intercept	1.765	0.147	12.003	<0.0001
Temperature seasonality (BIO4)	−0.473	0.133	−3.554	0.0004
Water deficit	0.230	0.135	1.697	0.0896
Sandstone	−0.829	0.356	−2.330	0.0198
Slope	1.024	0.078	13.058	<0.0001

When Dataset 2 was considered, the amount of variation explained by regression models fitted to the abundance records was higher for all species: 49.3% in the case of *A. ceterach* complex; 46.2% in the case of *A. ruta-muraria*, and 61.1% in the case of *A. trichomanes* complex. The relationship between water deficit and species abundance was positive in the case of *A. ceterach* complex, and negative in the case of *A. ruta-muraria* complex (Table 5). The model for *A. trichomanes* complex had the largest number of variables, including slope and type of dominant parental material.

4. Discussion

4.1. Ecological niche differentiation among *Asplenium* species

Vegetation type, substrate and terrain did not play a role in separating species' niches in environmental space. However, the combined effect of climatic variables successfully identified three broad ecological groups. The four species (*A. scolopendrium*, *A. ruta-muraria*, *A. trichomanes* and *A. ceterach*) with largely overlapping and relatively wide climatic niches positioned close to the overall mean habitat were identified as generalists. The group consisting of *A. adiantum-nigrum* and *A. petrarchae* was associated with the coastal areas characterised by the typical Mediterranean climate, with low temperature fluctuations around the year, but uneven distribution of precipitation. Although *A. petrarchae* was represented in the study by a single collection locality, its preference for stable temperature and ability to tolerate periods of drought revealed by the ecological niche analysis was consistent with previous reports (Marquez et al., 1997). The climatic niche of *A. adiantum-nigrum* had its centre in the area of Mediterranean climate with the highest temperatures and seasonal precipitation; however, several collection localities were found well outside the core distribution. Such heterogeneity of the niche poses questions about either the taxonomy of this species, which in the present study was treated in its broad sense, or the impact of allopolyploidy on niche limits (see Vogel et al., 1996). The role of polyploidy in the definition of niche limits may also be important for understanding the wide environmental ranges of the “generalists”, of which three out of four are actually species complexes including diploids and polyploids (Vogel et al., 1996, 1999; Pinter et al., 2002; Trewick et al., 2002). The group consisting of the remaining four species (*A. lepidum*, *A. fissum*, *A. septentrionale* and *A. viride*) was associated with the highlands of the central and northern part of the country; this group was characterised by relatively narrow climatic niches.

According to our results, the two utilized approaches (environmental niche modelling and phytosociological approach) were largely complementary. All studied species were recorded in *Asplenietea trichomanis* vegetation class, which is typical of rocky cliff

crevices and stone walls in Europe, North Africa, Middle East, Arctic islands and Greenland. In these habitats plants experience various forms of stress (nutrient limitation, water shortage, temperature fluctuations), and as a result of reduced competition weak colonizers such as *Asplenium* species have a better chance to establish and proliferate. The species identified by ecological niche modelling as generalists were found in the largest number of vegetation classes (maximum of six in the case of *A. trichomanes*) ranging from rocky habitats and alpine grasslands to temperate grasslands, steppe, woodlands and shrublands. The group of ferns associated with coastal habitats, in addition to *Asplenietea trichomanis* vegetation class, was recorded in temperate woodlands only. The group of highland species was confined to montane habitats, with a single species (*A. fissum*) also recorded in temperate woodlands. Presence of *A. ceterach* and *A. trichomanes* in grassland and forest should be regarded as an exception, because these vegetation types do not provide the optimum ecological conditions for the species, the survival of which depends on the existence of microhabitats characterized by specific substrates and water availability. The fact that our two rather different approaches, environmental niche modelling and phytosociological approach, yielded essentially similar results gives credibility to our inferences.

4.2. Environmental constraints on distribution of generalist species

The species identified by multivariate analysis as generalists (*A. scolopendrium*, *A. ruta-muraria*, *A. trichomanes* and *A. ceterach*) appeared to be less dependent on climate compared with coastal and highland species as shown by their relatively wide climatic tolerance and their close position to the overall mean of the habitat (cross point of the horizontal and vertical axes in Fig. 3). To test the relationships between all available environmental variables and presence/pseudo-absence data for the three species identified as generalists (*A. ruta-muraria*, *A. trichomanes* and *A. ceterach*) we used generalised linear models. The low amount of explained variation (typically less than 10%) confirmed that the distribution of the selected species was poorly explained by the environmental parameters given the available data. When abundance data (Dataset 2) were used, the amount of explained variation increased substantially (up to 46–61%). Thus the seemingly low dependence on climate appeared to be an artefact of incomplete sampling and/or inability to obtain true absence (as opposed to pseudo-absence) data. In addition to the climatic variables, characteristics of substrate, topography and land use type were making significant contribution to all models. Although the contribution of these variables was not consistent across the models (i.e. different variables were significant depending on the type of the data used), in most cases the relationships of these variables with species' distribution data appeared to be ecologically meaningful. For instance, the negative relationship with slope in the case of *A. ceterach* (presence/pseudo-absence dataset only) can be attributed to the high drought tolerance of the species which enables colonisation of hotter and drier habitats at lower elevations. Due to the lack of the true presence/absence data, the relationships between environmental variables and species distributions recovered by our study are likely to reflect differences between the species' habitats rather than between those species and the general environment of the study area.

In the abundance models, the effect of water deficit on species' distributions was significant and positive (i.e. species' abundance increased with the increased water deficit stress) in the case of *A. ceterach* and negative (i.e. species' abundance decreased with the increased water deficit stress) in the case of *A. ruta-muraria*. Water deficit effectively integrates the combined effects of solar radiation, evapotranspiration, and air temperature on watershed

conditions given available soil moisture derived from precipitation. In a Mediterranean climate, climatic water deficit can also be thought of as a surrogate for water demand based on irrigation needs, and changes in climatic water deficit effectively quantify the supplemental amount of water needed to maintain current vegetation cover, whether natural vegetation or agricultural crops (Stephenson, 1998). The Mediterranean basin has been identified as one of the world's regions most vulnerable to climatic and anthropogenic changes (Doblas-Miranda et al., 2014), with one of the major threats to the current vegetation in the region being increasing drought stress (Milano et al., 2013). Because the abundance of the two species, *A. ceterach* and *A. ruta-muraria*, included in the present study was shown to be closely related to water deficit, these species can be considered as potential indicators of climate change. Moreover, they are locally plentiful and easily recognised due to their distinct morphology; counts of their abundance can be easily obtained by anyone with basic training in taxonomy. We therefore argue that it is worthwhile to consider plot counts of *A. ceterach* and *A. ruta-muraria* as an alternative way of monitoring changes in water availability.

It is worth mentioning that the abundance data were collected in a small part of the species' distribution range in only 30 plots; yet the explanatory power of the regression models fitted with the abundance data increased substantially compared with those fitted with the presence/pseudo-absence data collected over a wider geographic extent. Our results corroborate the findings of Howard et al. (2014), who achieved large improvements in the ability to predict species distributions when abundance data were used.

5. Conclusions

Intrinsic limitations of datasets sourced from biological collections, including difficulty to assemble, inaccurate, incomplete, and difficult to interpret or absent information about collection localities, taxonomic errors, and incomplete and selective sampling have been discussed in detail elsewhere (Boakes et al., 2010; Graham et al., 2004; Ponder et al., 2001; Pyke and Ehrlich, 2010). When small rock ferns are used in ecological research as a study system, additional difficulties arise from potential failure to capture environmental preferences of the species, due to low resolution of predictors or their inability to describe important environmental properties of micro-niches, as well as from inability to account for potential effects of breeding system on the properties of the environmental niche (Bystriakova et al., 2012; De Groot et al., 2012; Wubs et al., 2010) and the requirements of gametophyte (Testo and Watkins, 2013). Although the basis of the present study is formed by herbarium collections prone to the limitations described above, we show that the ecological relationships resulting from the analysis of the available distribution data were largely consistent with anecdotal evidence from literature and phytosociological information; these relationships were also supported by the results of the analysis of detailed species abundance data obtained from field-work.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.flora.2015.07.003>

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