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Morphological *versus* molecular markers to describe variability in *Juniperus excelsa* subsp. *excelsa* (Cupressaceae)

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ABSTRACT

Background and aims: *Juniperus excelsa* M.-Bieb. is a major forest element in the mountains of the eastern part of Mediterranean and sub-Mediterranean regions. This study comprises the first morphological investigation covering a large part of the geographical range of *J. excelsa* and aims to verify the congruency between the morphological results and molecular results of a previous study.

Methodology: We studied 14 populations sampled from Greece, Cyprus, Ukraine, Turkey and Lebanon, from which 11 have previously been investigated using molecular markers. Three hundred and ninety four individuals of *J. excelsa* were examined using nine biometric features characterising cones, seeds and shoots; and eight derived ratios. Statistical analyses were conducted in order to evaluate the intra and interpopulation morphological variability.

Principal results: The level of intra-population variability observed did not show any geographic trends. The total variation mostly depended on the ratios of cone diameter/seed width and seed width/seed length.

The discrimination analysis, the Ward agglomeration method and barrier analysis results showed a separation into three main clusters of the sampled populations. These results confirmed, in part, the geographic differentiation revealed by molecular markers with a lower level of differentiation and a less clear geographic pattern. The most differentiated populations using both markers corresponded to old, isolated populations in the high altitudes of Lebanon (>2000 m). Moreover a separation of the northern Turkish population from the southern Turkish populations was observed using both markers.

Conclusions: Morphological variation together with genetic and biogeographic studies together make an effective good tool for detecting relict plant populations and also populations subjected to more intensive selection.

INTRODUCTION

Juniperus excelsa M.-Bieb (Grecian Juniper), is an arborescent juniper that can reach 20-25 m in height (Farjon 2005; Schulz *et al.* 2005; Adams 2008; Farjon 2010). It is slow growing, monoecious or dioecious, and wind pollinated (Farjon 2005; Adams 2008), with seeds dispersed by gravity or at longer distances by birds and small mammals (Jordano 1992; Santos *et al.* 1999). It is a pioneer species, light demanding, with a high resistance to severe drought, cold conditions and shallow, degraded soils (Zohary 1973; Browicz 1982; Mayer and Aksoy 1986; Quézel and Médail 2003; Magyari *et al.* 2008; Ozkan *et al.* 2010).

Juniperus excelsa is divided into two subspecies based on morphological data (Farjon 2005; Farjon 2010): *J. excelsa* subsp. *excelsa*, covering mountain and sub-mountain areas from the Balkan Peninsula in the west, through Anatolia, Syria and Lebanon to Crimea in the north and Iran in the east (Jalas and Suominen 1973; Browicz 1982; Greuter *et al.* 1984; Boratynski *et al.* 1992; Christensen 1997; Farjon 2005; Farjon 2010) (Fig. 1); and *J. excelsa* subsp. *polycarpos* (K.Koch) Takht., found further to the east with a Transcaucasian-Central-Asian distribution. Adams (2008), based on RAPD molecular markers, considers these two taxa as separate species, *J. excelsa* and *J. polycarpos* respectively.

Juniperus excelsa subsp. *excelsa* is a major mountain forest element in the East Mediterranean Basin and sub-Mediterranean region. It colonizes sites that vary from sub-humid to the adjacent semi-arid steppe zone of the Mediterranean region. The altitudinal range of *J. excelsa* subsp. *excelsa* is very wide. In the Anatolian peninsula, it is mainly found at elevations between 1000 and 1300 m and in

Lebanon between 1600 and 1800 m in the western and eastern slope of Mount Lebanon (Quézel 1973; Abi-Saleh *et al.* 1976; Akman *et al.* 1979; Quézel and Médail 2003). It forms the tree line in the East Mediterranean Basin with old, sparse populations reaching elevations of 2100 m in Greece and some individuals can be found at elevations of 2700-2800 m in the Taurus (Quézel 1973; Abi-Saleh *et al.* 1976; Akman *et al.* 1979; Browicz 1982; Barbero *et al.* 1994).

The regions of the contemporary occurrence of *J. excelsa* subsp. *excelsa* are situated around the Pleistocene refugial areas of the tertiary floras in the

East Mediterranean Basin (Comes 2004; Tzedakis 2004; Weiss and Ferrand 2007a; Weiss and Ferrand 2007b; Médail and Diadema 2009). It seems possible that the Grecian juniper survived the glacial periods of the Pleistocene in places close to its present localities. Unfortunately, the pollen of junipers was not determined to the species level in palynological studies (e.g. (Elenga *et al.* 2000; Eastwood 2004; Tzedakis 2004). This makes a direct analysis of species migration during the Pleistocene/Holocene temperature oscillations impossible. In spite of that, the occurrence of the species during the Last Glacial Maximum (LGM) was confirmed by macro-fossils from eastern parts of the Balkan Peninsula (Magyari *et al.* 2008). This could reflect a certain level of stability in the eastern Mediterranean Basin during Pleistocene climatic oscillations that favoured the conservation of a high level of genetic and probably also morphological diversity of tree species (Fady-Welterlen 2005; BouDagher-Kharrat *et al.* 2007; Fady *et al.* 2008; Fady and Conord 2010; Douaihy *et al.* 2011).

Morphological data are important in the apprehension of life cycles, geographic and ecologic distributions, evolution, conservation status, as well as species delimitation (Kaplan 2001). However, with the rapid rise and advancement of molecular techniques, the role of the morphological data in phylogenetic studies was put into question and has raised an ongoing scientific debate (Jenner 2004; Lee 2004; Stuessy *et al.* 2003; Wiens 2004; Wortley and Scotland 2006). The phenotypic variation of plants does not always follow the genetic pattern of variation and diversity of plant populations. The lack of congruence between morphological and genetic diversity was reported (e.g. Smissen and Heenan (2010), Ayele *et al.* (2011)). On the other hand, partial congruence in the geographic patterns of genetic and phenotypic diversity was described several times (e.g. (Jang *et al.* 2005; Ruisi *et al.* 2011)). As it is also the case for juniper taxa like *J. thurifera* (Gauquelin *et al.* 1988; Barbero *et al.* 1994; Adams *et al.* 2003; Romo and Boratyinski 2007; Terrab *et al.* 2008) and *J. pheonicia* (Adams 2008; Boratynski *et al.* 2009).

The morphological variability of *J. excelsa* across its large geographical range has not been extensively studied. The previous studies were always done on a limited number of populations (Barbero *et al.* 1994; Christensen 1997; Mazur *et al.* 2004; Farjon 2005; Marcysiak *et al.* 2007). Mazur *et al.* 2004, showed that the multivariate differences between two populations of *J. excelsa* from Crimea and one from the Balkan Peninsula correlated with geographical distance.

A recent genetic study on a large geographical range of *J. excelsa* subsp. *excelsa* based on nuclear microsatellites (Douaihy *et al.* 2011) showed a high level of

genetic diversity within this taxon, with a clear clustering of populations into three centres. The most differentiated populations corresponded to old vestigial stands found at the tree line (>2000 m) in Lebanon. The lower altitude Lebanese populations clustered together separated from the populations from Turkey, Cyprus, Greece and Ukraine (Crimea).

The main aims of the present study are 1) to present a first extensive morphological investigation of *J. excelsa* subsp*. excelsa* in the East Mediterranean Basin and 2) verify the hypothesis that *J. excelsa* subsp*. excelsa* has an intra-specific differentiation at the morphological level that correspond to the differentiation described using molecular markers.

MATERIALS AND METHODS

Plant material and characters studied

Plant material was sampled from 14 populations from Greece, Cyprus, Ukraine (Crimea), Turkey (Anatolia) and Lebanon (Fig. 1). The samples of cones and twigs from the last ramification were collected only from arborescent adults baring seed cones and were gathered separately from the southern parts of the individuals, at a height of about 1.0-5.0 m above ground level, as described by Mazur *et al.* (2010). 394 individuals of *J. excelsa* were examined. Around 30 individuals per population were examined except for the Lebanese population LB3, where only 18 individuals with cones were found (Table 1). Each individual from every population tested was represented by 10 cones and 10 pieces of twigs. Some individuals in the analyzed populations had less than 10 twigs.

Nine morphological characters and eight ratios were examined (Table 2) in order to assess the variation within and between populations. The measurements of the shoots were performed under a stereoscope microscope of ×8 magnification with a scaled ocular (LN and ST,) as describe by Marcysiak *et al.* (2007). Cone dimensions measurements (CL and CD, Table 2) were measured using a numerical calliper (0.01mm) and the numbers of cone scale rows and of cone scales (CSR and CSN) were counted manually. Seed dimensions (SL and SW) were measured automatically using WinSEEDLE software and the number of seeds in a cone (SN) was counted manually.

Statistical analysis

The symmetry and unimodality of the distribution frequency of the measured character were verified using Shapiro-Wilks' *W*-test to assess the possibility of conducting a statistical analysis (Tabachnik and Fidell 1996; Zar 1999; Sokal and Rohlf 2003). The main statistics (arithmetic means, standard deviation, variation coefficient) were calculated for the particular characters for individuals and populations. The correlations between the measured characters were verified using Pearson's correlation coefficient to avoid the most redundant ones. Tukey's *T*-test was performed to verify the influence of particular characters on the differentiation between individuals within populations and between populations. The level of intra-population variation was assessed using the Ward's agglomeration on the shortest Euclidean distances among individuals (Zar 1999; Sokal and Rohlf 2003).

The discrimination analysis was performed to identify the discriminate power of each character and to determine the interpopulation variation (Tabachnik and Fidell

1996; Sokal and Rohlf 2003). A dendrogram of the closest Euclidean distances on the basis of the unweighted pair-group method using arithmetic averages (UPGMA) was constructed to check the affinities revealed in the discrimination analysis (Zar 1999; Sokal and Rohlf 2003). The discrimination analysis was calculated on the characters obtained from the ratios, except for the ratio of cone diameter to the number of cone scale rows (CD/CSR), because of the stable characteristic of cone scale rows (CSR). All the above mentioned statistical analyses were performed using STATISTICA 8 (StatSoft Poland).

The geographic distances between the populations were calculated using MapInfo (Pitney Bowes Software Inc.). The geographic structure of the populations was further analysed using Monmonier's maximum difference algorithm, implemented in BARRIER 2.2 software (Manni *et al.* 2004). This analysis reveals discontinuities in morphological differentiation in relation to the populations' geographical arrangement. The barriers can be interpreted as breaks between adjacent populations in their morphological construction.

A K-mean analysis was performed on the calculated characters. This analysis reveals the number of K-clusters, which optimally illustrates the differentiation between populations. A classification matrix was constructed to show the percentage of individuals from each population that could be properly included in particular groups and to assess the number of individuals that fell into another K-group (Sneath and Sokal 1973).

RESULTS

Evaluation of characters

The distribution frequency of the examined characters was normal or only slightly left- or right-biased (data not shown), which enabled further statistical analyses.

The variation coefficients of the characters at the population level varied between 1.5 and 33% (Table 3). The average values of cone length (CL) and cone diameter (CD) were approximately equal to 9 mm. These two characters varied from 6.3 to 13.5 mm (CV=9%) and from 6.1 to 14.4 mm (CV=10%), respectively. The average value of the ratio CL/CD was almost equal to 1 and varied between 0.72 and 1.28 (CV=6%). The largest cones were found in the populations from southern Lebanon and Crimea (samples LB5, LB6 and CR2, respectively), while the smallest ones were from the high altitude population in Lebanon (sample LB3) (Table 3). The majority of the cones measured had four scale rows (CSR) and only few cones (20% of the trees from TU2) had six scale rows. The cone scale number (CSN) varied between 4 and 10 (CV=10%) and averaged at 6.00±0.57. The mean number of seeds per cone (SN) was close to 6, with cones containing 1 to 13 seeds (CV=19%) (Table 2). As for the cones, the highest value of SN was found in the sample from southern Lebanon (sample LB5) and the lowest value was found in the sample from the high mountains of this country (sample LB3). The seeds had a mean length (SL) and width (SW) of 4.75±0.35 mm and 2.89±0.28 mm, respectively. Seed length varied between 3.08 and 6.5 mm (CV=7%) and SW between 1.8 and 4.87 mm (CV=10%). The mean ratio SL/SW was 1.66±0.14 with CV=8%. The seed dimensions did not show the same geographic trend as for the cone dimensions: the smallest seeds were found in CR1 and LB1 (Table 3). The

ratios CD/SN and SL/SW had a high level of variation (CV=22% and 26%, respectively). The ratios of the cone dimension over the seed dimension averaged at 3.32±0.39 (CD/SW, CV=12%).

The average number of leaves per the 5 mm apical section of the ultimate lateral branchlet (LN) was 22.73±2.91 and varied between 12 and 36 (CV=13%) (Table 2). The average value of the thickness of the last ramification shoot with leaves (ST) was 0.72±0.07 mm and varied between 0.3 and 1.05 mm (CV=6%). All of the populations had almost the same mean ST except for LB3 and LB4, which had a higher mean of 0.8 mm (Table 3). The ratios ST/LN, SW/SN and CD/SN had the highest levels of variation.

Cone length and cone diameter (CL and CD) are highly and significantly positively correlated (R^2 = 0.98, P<0.01) as well as seed length (SL) and seed width (SW) (R^2 = 0.74, P<0.01) (Table 4). The two features of the cone dimensions (CL and CD) significantly affected the number of seeds per cone (SN) (R^2 = 0.77, P<0.01 and R^2 = 0.8, P<0.01, respectively). Likewise, cone length and diameter (CL, CD) were positively correlated with the number of cone scales (CSN) (R^2 = 0.83, P<0.01 and R^2 = 0.86, P<0.01, respectively). We obtained a high but less significant correlation coefficient (P<0.05) between the number of seeds per cone (SN) and the number of cone scales (CSN). This correlation was most likely derived from the higher correlations noted earlier between these two characters and the cone dimension features.

The Tukey's post-hoc test (Table S1) showed that the highest number of statistically significant differences between the populations was found for the ratio

of cone diameter/seed width (CD/SW), the ratio of cone diameter/number of cone scale rows (CD/CSR) and the cone diameter (CD) and cone length (CL). The ratio of cone length/cone diameter (CL/CD), cone diameter/number of seeds per cone (CD/SN) and the seed length (SL) were significantly different between only few populations, but mainly between the Turkish population (sample TU2) and some of the others. On the other hand, seed width (SW) show significant differences only between Crimean and Turkish populations (CR2 and TU2). Moreover, the thickness of the last ramification shoot with leaves (ST), the number of leaves (LN) and the ratio of cone diameter/number of cone scale rows (CD/CSR) only differed significantly (P<0.01) between two or three of the sampled populations.

All of the calculated characters used in this study significantly discriminated between the samples at the level of P<0.01. The discriminatory powers of the characters were very close, with values of partial Wilks' λ varying between 0.8 and 0.9 (Table 5).

Intra-population variation

Sampling within each population was relatively uniform, with comparative numbers of individuals (Table 1). The within-population differentiation using Ward agglomeration method was generally found at similar levels, with the first split into two groups of individuals at a distance between 11 and 17 (Figure S1). We note that the smallest distances observed for this split are observed in the most fragmented and margin populations, such as LB3, CY, CR1 and GR. The maximum separation distances of individuals within populations varied between 6 and 9 and were observed for individual 22 from TU3, 13 from CY and 3 from GR.

Inter-population variation

In the discrimination analyses using the calculated characters (without CD/CSR), all of the individuals were grouped together, except for the high mountain Lebanese population (LB3) and the Cypriote population (CY) (data not shown). The discrimination analysis at the population level shown in Figure 2 reveals a clustering of the populations. According to the variable U_1, which explained about 53% of the total variation and mostly depended on the ratio of cone diameter/seed width (Table 6), the high altitude Lebanese population of Wadi El Njass (LB3) was separated from the other Lebanese populations (LB1, LB2, LB5 and LB6). The second high altitude Lebanese population from Jbab el Homr (LB4) was dislocated at a middle distance in between. The populations most closely grouped were from Qammouaa, Barqa and Afqa in Lebanon (LB1, LB5 and LB6, respectively). All of the other compared populations formed one group, although more dispersed.

According to the variable U_2, which was responsible for about 20% of the total variation and mostly depended on the ratio between seed length and width (SL/SW), all the populations except for three of the Turkish populations and the Greek one (TU1, TU2, TU3 and GR, respectively) formed one group. However, the separation of the high altitude Lebanese population (LB3), and the populations from Cyprus and one from Turkey, can be recognized in the space between the second and third discrimination variables U_2 and U_3. These two variables also differentiated between all Lebanese and Turkish, and Greek and Crimean populations (Fig. S2, Table 6).

According to the cluster analysis by the Ward method, the sampled populations could be divided into three main sub-clusters (Fig. 3). The first included the high

altitude population from Lebanon (LB3) with the coastal population from Crimea (CR1) and the population from Cyprus (CY). The second cluster grouped the southern Turkish populations (TU2, TU3 and TU4) with the population from Greece (GR). The third cluster included all of the other populations from Lebanon (LB1, LB2, LB4, LB5 and LB6) with the northern Turkish population (TU1) and the mountain population from Crimea (CR2).

The K-grouping method also revealed the most probable split of the populations into three groups (Fig. 1). The classification matrix (Table 7) showed that the level of congruence was the highest for the Lebanese high altitude, the Cypriote, and the coastal Crimean populations (LB3, CY and CR1, respectively), where 67, 63 and 57% of the individuals, respectively, were included in the correct population group (Table 7).

The first three barriers revealed by Monmonier's maximum difference algorithm applied by using BARRIER 2.2 software confirmed the separation of the high mountain Lebanese population from all of the others and in the second turn the different characteristics of the Cypriote population (Fig. 1).

DISCUSSION

Variation of characters

The values of the *J. excelsa* subsp. *excelsa* characters placed them directly into the characteristics of the taxon and did not differ drastically from the data reported in published taxonomical work and basic floras on samples from Bulgaria, Greece,

Turkey and Crimea (Coode and Cullen 1965; Barbero *et al.* 1994; Christensen 1997; Mazur *et al.* 2004; Farjon 2005; Marcysiak *et al.* 2007) (Table S2).

We found that the values of the dimensional characters of the cones and seeds were positively correlated at a statistically significant level (P<0.01). A high correlation between the cone dimensions (CL and CD) as well as between the cone and seed dimensions was also observed in previous studies on *Juniperus* species (Mazur *et al.* 2004; Klimko *et al.* 2004; ; Klimko *et al.* 2007; Marcysiak *et al.* 2007; Mazur *et al.* 2010). On the other hand, we obtained a much higher level of correlation of seed number with the cone dimensions (CL and CD) and with the CSN comparing with the previous studies.

The characters which significantly differentiated between the largest number of populations were CD/SW, CD, CL and SN (Fig. 2). The latter two differed between the high altitude population from Lebanon (LB3) and the other populations. The smallest cones found in that population, which had CL and CD below 7 mm and the lowest number of seeds, with an SN of 4.2 on average, can be interpreted as resulting from 1) the harsher environmental conditions at the altitudinal line of the species. But we note that the mean cone dimensions in the other high altitude population from the eastern side of Mount Lebanon (LB4) were as big as in the middle altitude populations. 2) the marginal position of this population in the species range as it was observed in other taxa (Yamada and Miyamura 2005). However, the biggest cones were found also in the marginal populations from southern Lebanon and Crimea (samples LB5, LB6 and CR2, respectively). or 3) a genetic differentiation of this population.

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The ontogenesis of *J. excelsa* seed cones has not been studied in detail. Our results on the mature cones indicate that typical dimerous cones strongly prevailed in all compared populations. The trimerous cones were found sporadically in three populations from Turkey. This pattern of cone arrangement is also typical for *J. phoenicea* (Schulz *et al.* 2003; Mazur *et al.* 2010) and *J. oxycedrus* (Klimko *et al.* 2007). The number of seeds per cone (SN) reported for *J. excelsa* subsp. *excelsa* ranged between 4 and 6, and sometimes from 2 to 8 (Table S2). We obtained an average value of SN close to 6, but ranged from 1 to 13 in particular populations. An average seed number higher than 6 for some populations (Table 3) suggest a more frequent occurrence of two whorls of the seed scales, one with two ovules and the second with one ovule on each scale, or alternatively, three ovules on one dimerous whorl of seed scales, as it was described for other junipers (Schulz *et al.* 2003). The two unexpected seed cones containing 13 seeds, found in populations from Greece and Turkey (GR and TU2, respectively), were dimerous. This suggests at least three whorls of fertile cone scales with at least one of them bearing three ovules.

Intra-population variability

The level of differentiation between individuals within populations evaluated using the Ward agglomeration analysis (Figure S1) was generally similar, except for the populations from the margins of the species geographic range, such as GR, CY and CR1 and also LB2 and LB3. The lower level of individual differentiation within these populations can be explained as resulting from 1) the long-lasting geographic isolation and adaptation to the local environmental conditions in Lebanese (LB2 and LB3) and Cypriote (CY) populations, 2) the lower number of individuals

representing Lebanese population LB3, or 3) the possible origin from the low number of founders, such as in the case of samples GR and CR1. The latter two populations were also characterized by a lower level of observed heterozygosity than the other populations (Douaihy *et al.* 2011), which could be a trace of an ancient founder effect.

We did not find connection between genetic and morphological levels of variation comparing the genetic heterozygosity with variation coefficients of studied morphological characteristics of particular populations. This confirms the independence of the genetic and morphological markers, as reported earlier for several vascular species (Smissen and Heenan 2010; Ayele *et al.* 2011).

Inter-population variability

The morphological multivariate differentiation of the studied populations of *J. excelsa* subsp. *excelsa* did not show a clear geographical pattern. The clustering of populations based on molecular data (Douaihy *et al.* 2011) had a clearer geographical pattern with a strong clustering of the middle altitude – 1000 to 1900m - Lebanese populations (LB1, LB2, LB5 and LB6) separated from the Turkish, Greek, Cypus and Crimean populations that were grouped together (compare Fig. 2 in our paper with Douaihy *et al.* (2011): Fig. 3). Nevertheless some main congruencies are found between the two studies.

The discrimination, clustering and barrier analyses results based on morphological data (Figs 2, 3 and 1, respectively) showed a high differentiation of the high altitude Lebanese population (LB3). This same population along with another high altitude Lebanese population from Aarsal (not included in this study) were found to be the

most differentiated populations by Douaihy *et al.* (2011). Moreover the separation of the northern Turkish (TU1) from the southern Turkish populations obtained by the Ward dendrogram based on the morphological data was also observed based on molecular data (Douaihy *et al.* 2011: Figs. 2, 3, and 4).

The high level of morphological differences between geographically close populations in the Lebanese mountains could be interpreted as resulting from different abiotic conditions. However, genetic data also showed a separation of the high altitude populations (Douaihy et al. *2011*). Hence these differences could have resulted from a lack of gene flow and/or 4) a different population history during glacial and postglacial migrations.

The junipers, as wind-pollinated plants, are expected to produce large amounts of pollen grain and could be successfully pollinated from long distances. Long distance pollination plays a major role in reducing the differences among tree species populations (Hamrick *et al.* 1992; Fady-Welterlen 2005; Fady *et al.* 2008). The pollen grains of the Juniper species are relatively small and characterized by slow setting velocity (Huntley and Birks 1983; Moore *et al.* 1991), but their production is in fact not too high. The dispersion of *J. excelsa* pollen grains in northern Iran was found to be significantly smaller than those of wind-pollinated broadleaved trees, as *Alnus*, *Carpinus*, *Quercus* or *Zelkova* and restricted to the areas close to juniper woodlands (Djamali *et al.* 2009).

The similarity between Turkish, Crimean and Greek populations is very interesting (Fig. 2). The inclusion of CR1 and CR2 in the Turkish group of populations could have resulted from a common origin, which seems to be very possible when the

history of the biota around the Black Sea during glaciation is taken into account. The level of the Black Sea was much lower during the glacial period than it is at present (Yena *et al.* 2004; Yena *et al.* 2005), making much more possible plant migrations along the coast from Anatolia to Crimea and vice versa.

The situation of the Greek population, marginal in the group of Turkish-Crimean populations (Fig. 2), could also have resulted from another paleohistory event. The presence of *J. excelsa* in the eastern Mediterranean Basin during the last glacial maximum (Magyari *et al.* 2008) indicates possible migrations between the Anatolian and Balkan peninsulas during the glacial periods of the Pleistocene, and can explain the affinity between Turkish and Greek populations of this species. The different ways of migration to the Crimean and Balkan peninsulas can be a reason for the slightly higher differences between the populations of *J. excelsa* from these two centres, which also confirm the previous finding (Mazur *et al.* 2004), and is partly consistent with the results of genetic analyses (Douaihy *et al.* 2011). On the other hand, the significant differences among populations coupled to a lack of clear geographic pattern of differentiation was described for other juniper species: *Juniperus seravschanica* Kom. (Sultangaziev *et al.* 2010), *Juniperus oxycedrus* L. subsp. *oxycedrus* from the Balkan Peninsula (Brus *et al.* 2011) and for *J. excelsa* subsp. *excelsa* in the Lake district of the central Anatolia on the cone diameter and number of seeds (Yücedağ *et al.* 2010). The lack of a clear geographical clustering in these studies was explained by a restricted sampling area as well by a recent fragmentation that did not allow the appearance of a geographical structuring of the morphological traits.

The Cypriote population of *J. excelsa* subsp. *excelsa* morphologically differed from the geographically closest Turkish and Lebanese populations but was genetically similar to the populations from Turkey, Crimea and Balkan Peninsula (Douaihy et al., 2011). This observation could have resulted from a variation in the environmental conditions or could suggest a selection process with a more rapid phenotypic than genetic differentiation in plants (McKay and Latta 2002). Interestingly this was observed in *Cedrus* genus, were the Cyprus population and the Turkish populations, phenotypically distinct, were found to be genetically very close (BouDagher-Kharrat *et al.* 2007). We can similarly explain the morphological differences between core and marginal populations, as Greek, Crimean and Lebanese.

CONCLUSIONS and FORWARD LOOK

The results of the multivariate biometrical analyses follow at least partly the genetic differentiation of *J. excelsa* subsp. *excelsa*, indicating also the selection processes and/or variability in response to environmental differences in some number of localities, frequently marginal ones. It is concluded that examination of the morphological variation should be treated, together with genetic and biogeographic studies, as an effective tool for detecting relict plant populations and also populations with more intensive selection, where it is important to conserve the morphological characteristics. Finally, it should be stressed that plant populations with high levels of morphological diversity are key to establishing adequate strategies of biodiversity protection   crucial for conservation in the Mediterranean region. This is especially important in the case of relict, southern-most populations

of *J. excelsa* in the mountains of Lebanon and in Cyprus, but also the northern-most ones in the Crimea and on the Balkan Peninsula.

ADDITIONAL INFORMATION

The following [**ADDITIONAL INFORMATION**] is available in the on-line version of this article:

File 1. Table. Results of the Tukey T-test for 17 characters of 14 samples of *Juniperus excelsa*; * significance at level p=0.05; ** significance at level p= 0.01 (populations acronym as in Table 1; characters as in Table 2)

File 2. Figure. Intra-population variability of 14 populations of *Juniperus excelsa* subsp. *excelsa* analysed using the Ward agglomeration on the shortest Euclidean distances among individuals (populations acronym as in Table 1).

File 3. Figure. Discriminate analysis results based on the three main variables (U1, U and U3).

File 4. Table. The morphological variation between two populations of *J. excelsa* subsp. *excelsa* from the Crimea and one from the Balkan Peninsula.

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CONTRIBUTIONS BY AUTHORS

All the authors contributed to a similar extent overall.

CONFLICTS OF INTEREST

No conflicts of interest.

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FIGURE LEGENDS

Fig. 1 Interpopulation diversity of *Juniperus excelsa* in the East Mediterranean Basin according to the K-mean and Barrier results. The geographic positions of the sampled populations are indicated on a global distribution map of the taxa [1,2,3] (acronyms as in Table 1) . The K-mean analysis assigned the populations into 3 groups (1-3). The three main barriers (a-c) as obtained by Barrier 2.2 are also shown.[1]Jalas and Suominen 1976; [2]Browicz and Zieliński 1982; [3]Boratyński *et al.* 1992.

Fig.2 Discrimination analysis results for *Juniperus excelsa*. The results obtained by the two main discriminant variables (U1, U2) based on seven ratios are shown (acronyms as in Table 1).

Fig.3 Dendrogram constructed by Ward method of cluster analysis on the Euclidean distances between samples of *Juniperus excelsa* (acronyms as in Table 1).
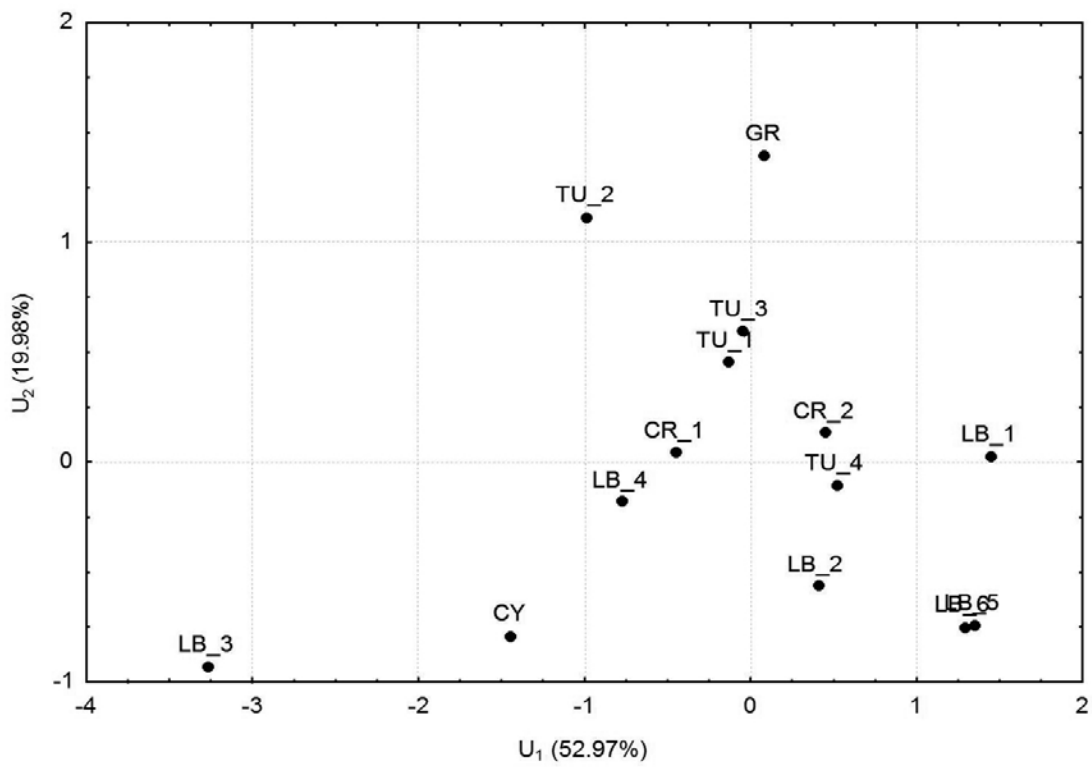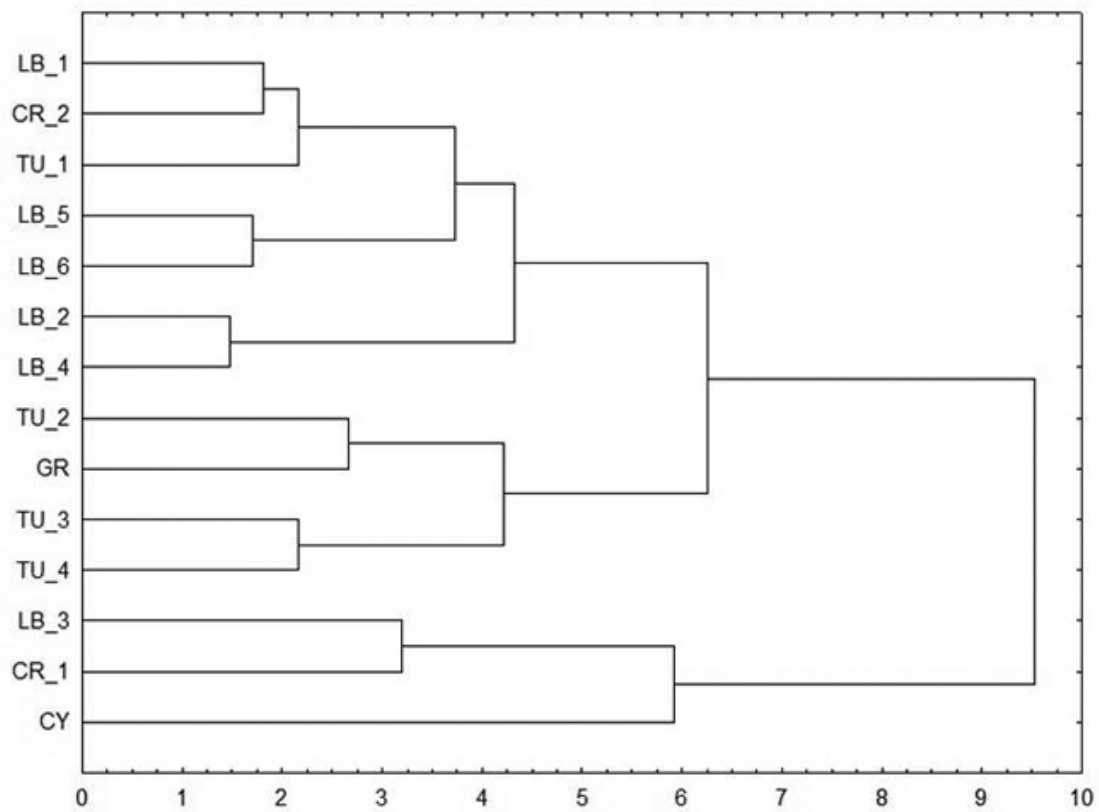
Table 1. Sampled populations of *Juniperus excelsa* subsp. *excelsa*.

Code	Country	Locality	Longitude	Latitude	Altitude (m)
LB1	Lebanon	Qammouaa	N34°29'34"	E36°15'14"	1450-1800
LB2		Danniyeh	N34°23'17"	E36°05'60"	1600-1850
LB3		Wadi El Njass	N34°19'49"	E36°03'16"	1870-2300
LB4		Jbab El Homr	N34°20'16"	E36°12'18"	1860-2061
LB5		Barqa	N34°11'48"	E36°08'15"	1600-2200
LB6		Afqa	N34°04'25"	E35°54'20"	1100-1600
TU1	Turkey	Ilgaz-Tosya	N40°53'04"	E33°42'24"	850
TU2		Eğirdir	N38°08'12"	E30°46'42"	950
TU3		Göltarla	N36°34'56"	E29°58'29"	1100
TU4		Akçali Dağlari	N36°19'36"	E33°00'44"	1200
GR	Greece	Askion Oros	N40°15'58"	E21°37'26"	1000
CY	Cyprus	Troodos Oros	N34°55'20"	E33°05'55"	1500
CR1	Ukraine	Crimea-Mys Aja	N44°25'18"	E33°39'57"	35
CR2		Crimea-Kolkhoznoe	N44°29'00"	E33°49'54"	500

Table 2. Average value with standard deviation (SD), minima, maxima and variation coefficients of analyzed characters of cones, seeds and leaves of *Juniperus excelsa* subsp. *excelsa*. N – Number of measurements for particular character. * The scales of cones of junipers are decussate or exceptionally ternate, alternately arranged and forming 4 or 6 rows, respectively.

Code	Character	Mean± SD	Minimum	Maximum	Variation coefficient	N
CSR	Number of cone scale rows*	4.00±0.06	4	6	1.50	3957
CL	Length of cone [mm]	9.19±0.83	6.3	13.5	9.03	3956
CD	Diameter of cone [mm]	9.46±0.98	6.1	14.4	10.36	3956
CSN	Cone scale number	6.00±0.57	4	10	9.50	3956
SN	Number of seeds	5.64±1.07	1	13	18.97	3923
SL	Length of seed [mm]	4.75±0.35	3.08	6.5	7.37	3914
SW	Width of seed [mm]	2.89±0.28	1.8	4.87	9.69	3914
LN	Number of leaves per 5 mm apical section of ultimate lateral branchlet	22.73±2.91	12	36	12.80	3287
ST	Thickness of the last raminification shoot with leaves	0.72±0.07	0,3	1.05	9.72	3286
CL/CD	Ratio of length of cone / diameter of cone	0.97±0.06	0,72	1.28	6.19	3956
CD/CSR	Ratio of diameter of cone / number of cone scale rows	2.36±0.25	1.44	3.61	10.59	3956
CSN/CL	Ratio of cone scale number / length of cone	0.66±0.06	0.37	1.17	9.09	3956
SL/SW	Ratio of mean length of seed / mean width of seed	1.66±0.14	1.08	2.46	8.43	3914
CD/SN	Ratio of diameter of cone / number of seeds	1.84±0.40	0.61	10.09	21.74	3922
SW/SN	Ratio of mean width of seed / number of seeds	0.57±0.15	0.19	4.46	26.32	3913
CD/SW	Ratio of diameter of cone / mean width of seed	3.32±0.39	1.9	5.58	11.75	3913
ST/LN	Ratio of thickness of the last raminification shoot widh leaves / number of leaves on the 5 mm of the last ramification shoot	0.03±0.01	0.01	0.07	33.33	3286

Table 3. Descriptive statistics of the measured morphological traits at the population level. N – number of individuals representing population; M- average value; CV – variation coefficient (%).

Population	N	CSR		CL (mm)		CD (mm)		CSN		SN		SL (mm)		SW (mm)		LN		ST (mm)	
		M	CV	M	CV	M	CV	M	CV	M	CV	M	CV	M	CV	M	CV	M	CV
LB1	30	4.00	0.00	9.46	7.36	10.04	8.85	5.92	8.44	6.16	19.50	4.54	7.48	2.82	10.45	21.78	11.28	0.74	7.33
LB2	30	4.00	0.00	9.45	7.12	9.63	6.37	6.19	5.88	5.40	15.49	4.74	7.13	2.79	10.76	21.45	8.64	0.71	7.58
LB3	18	4.00	0.00	7.80	7.41	7.70	7.07	5.34	7.81	4.23	21.50	4.78	7.44	2.93	7.73	25.10	13.19	0.78	5.59
LB4	27	4.00	0.00	8.93	7.24	9.25	7.99	5.97	4.33	4.92	16.87	4.90	6.05	2.91	8.67	25.84	12.29	0.82	7.82
LB5	30	4.00	0.00	10.10	7.12	10.48	7.90	6.01	3.59	6.28	17.70	4.93	7.68	2.88	11.27	22.49	11.96	0.75	5.77
LB6	30	4.00	0.00	9.61	7.04	9.83	7.55	6.01	9.40	6.16	16.44	4.57	7.57	2.69	10.26	22.23	11.42	0.70	8.67
TU1	31	4.01	0.90	8.93	7.40	9.27	9.64	5.65	7.11	5.45	14.10	4.70	5.77	2.95	6.94	22.76	11.22	0.74	10.16
TU2	30	4.05	5.93	8.81	7.96	8.97	7.73	6.02	14.16	6.00	17.64	4.86	6.49	3.08	7.86	21.32	13.23	0.67	8.28
TU3	30	4.01	0.91	9.08	7.36	9.49	6.61	6.22	6.45	5.73	12.11	4.76	7.44	2.95	7.60	23.23	8.06	0.70	6.96
TU4	29	4.01	0.93	9.41	7.16	9.90	6.62	6.39	11.17	6.22	12.26	4.82	6.75	2.81	7.78	23.53	8.87	0.66	8.53
GR	32	4.00	0.00	9.09	8.69	9.59	9.48	6.23	11.79	5.99	12.29	4.69	5.65	3.02	8.27	20.25	10.89	0.72	7.00
CY	30	4.00	0.00	8.72	6.67	8.46	6.99	5.55	9.63	5.46	16.25	4.86	6.98	2.77	10.14	24.11	12.69	0.70	6.91
CR1	30	4.00	0.00	8.75	5.85	8.84	7.47	5.91	5.39	4.60	18.18	4.47	7.73	2.84	7.66	22.63	13.82	0.69	9.30
CR2	30	4.00	0.00	9.87	7.02	10.23	6.48	6.27	7.13	5.84	17.21	4.97	5.68	3.07	8.02	23.04	11.33	0.71	6.41

Table 4. Correlation coeficients between 9 characters of *Juniperus excelsa* subsp. *excelsa* from all populations sampled; character acronyms as in Table 2. * Significance at level p<0.05, ** Significance at level p<0.01

Characters	CSR	CL	CD	CSN	SN	SL	SW	LN
CL	-0.64*							
CD	-0.55*	0.98**						
CSN	-0.6*	0.83**	0.86**					
SN	-0.16	0.77**	0.8**	0.56*				
SL	-0.63*	0.53	0.43	0.4	0.24			
SW	-0.56*	0.38	0.36	0.38	-0.01	0.74**		
LN	-0.23	-0.13	-0.17	0.02	-0.32	0.58*	0.52	
ST	-0.39	0.01	0.04	0.04	-0.36	0.32	0.52	0.53

Table 5. Discriminant power testing for the calculated characters of *Juniperus excelsa* subsp. *excelsa.*

Character	Partial Wilks' lambda	*P*
Ratio of length of cone / diameter of cone (CL/CD)	0.904	0.000
Ratio of cone scale number / length of cone (CSN/CL)	0.848	0.000
Ratio of mean length of seed / mean width of seed (SL/SW)	0.741	0.000
Ratio of diameter of cone / number of seeds (CD/SN)	0.840	0.000
Ratio of mean width of seed / number of seeds (SW/SN)	0.817	0.000
Ratio of diameter of cone / mean width of seed (CD/SW)	0.800	0.000
Ratio of thickenses of the last raminification shoot width leaves / number of leaves on the 5 mm of the last ramification shoot (ST/LN)	0.892	0.000

Table 6.The coefficients of determination between discrimination variables and analysed characters of *Juniperus excelsa* subsp. *excelsa* (character acronyms as in Table 2).

	U_1	U_2	U_3
CL/CD	3,65	1,74	0,07
CSN/CL	3,08	3,33	0,51
SL/SW	0,13	**7,40**	1,82
CD/SN	0,42	0,72	0,57
SW/SN	7,74	0,16	1,35
CD/SW	**32,89**	3,78	0,35
ST/LN	0,42	0,45	**3,45**

Table 7. Classification matrix for individuals of *Juniperus excelsa* subsp. *excelsa* as a result of a K-means cluster analysis for calculated characters; acronyms as in Table 1. * percentage of individuals, which were correctly classified to the population; A- number of analysed individuals; N- total number of individuals.

Population	Level of conformability (%)*	Number of individuals classified to the populations													
		A/N	LB1	LB2	LB3	LB4	LB5	LB6	TU1	TU2	TU3	TU4	GR	CY	CR1	CR2
LB1	31	29/30	9	3	0	0	2	4	2	0	0	0	6	0	2	1
LB2	33	30/30	2	10	0	2	0	3	0	0	1	3	1	0	4	4
LB3	**67**	18/18	0	0	**12**	0	0	0	0	0	0	0	0	6	0	0
LB4	36	25/27	1	1	1	9	0	0	1	1	0	3	1	3	2	2
LB5	36	28/30	2	4	0	0	10	7	0	0	0	1	0	0	0	4
LB6	47	30/30	2	3	0	0	4	14	0	0	3	1	0	1	1	1
TU1	38	29/31	4	0	1	1	2	0	11	2	2	1	1	2	2	0
TU2	33	24/30	0	0	0	0	0	2	6	8	5	0	1	1	0	1
TU3	23	30/30	2	1	0	2	0	1	2	2	7	3	4	1	1	4
TU4	41	29/29	0	1	0	0	2	2	1	0	7	12	0	1	0	3
GR	38	29/32	0	1	1	1	0	0	4	1	3	1	11	0	2	4
CY	**63**	27/30	0	1	1	1	0	1	0	3	0	0	0	**17**	2	1
CR1	**57**	30/30	0	2	0	2	0	0	0	3	3	1	0	1	**17**	1
CR2	37	30/30	1	2	0	1	5	0	3	0	2	2	2	0	1	11