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Intra-specific differentiation of *Juniperus phoenicea* in the western Mediterranean region revealed in morphological multivariate analysis

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Abstract: The biometrical comparison of nine populations of *Juniperus phoenicea* from the western part of the species geographic range was the aim of the present study. Seven features of the cones and seeds, two of the shoots and leaves and eight proportions were studied biometrically and analysed using statistical methods. Two of analyzed populations, supposed to be representatives of *J. phoenicea* subsp. *phoenicea*, are closely related each other. The seven other populations representing *J. phoenicea* subsp. *turbinata*, appeared much more variable and differed each other at higher level. The results confirm the biochemical and genetic differentiation of the species, however, it can partly be an effect of smaller number of compared populations of subsp. *phoenicea*. The individuals included into each of distinguished subspecies are different and only a few individuals of subsp. *turbinata* from the Atlantic coast in Portugal were found to be joined into the group of subsp. *phoenicea*. The differences between populations of subsp. *turbinata* are higher than between those of subsp. *phoenicea*. The most distant population of the subsp. *turbinata*, from Cabo Rizuto in Italy appears also the most different from all the other. It results probably from the isolation for a longer time.

Additional key words: plant variation, taxonomy, biogeography, Cupressaceae, biometry

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Introduction

Juniperus phoenicea L. is mostly a shrub or a small, polycormic tree to about 8–12 m tall. It is a light-demanding, relatively resistant to a dry climate and characterize with high pioneer properties (Quezel and Pesson 1980; Quezel and Barbero 1981; Auclair

1996; Charco 2001; Quézel and Médail 2003). Their geographic range covers the Mediterranean region from the Canary Islands, the Atlas mountains in Africa and the Atlantic coast of Portugal in the West, to Jordan and Saudi Arabia in the East (Fig. 1), but the main part of area is centred on the Iberian Peninsula and in the North West Africa (Jalas and Suomi-

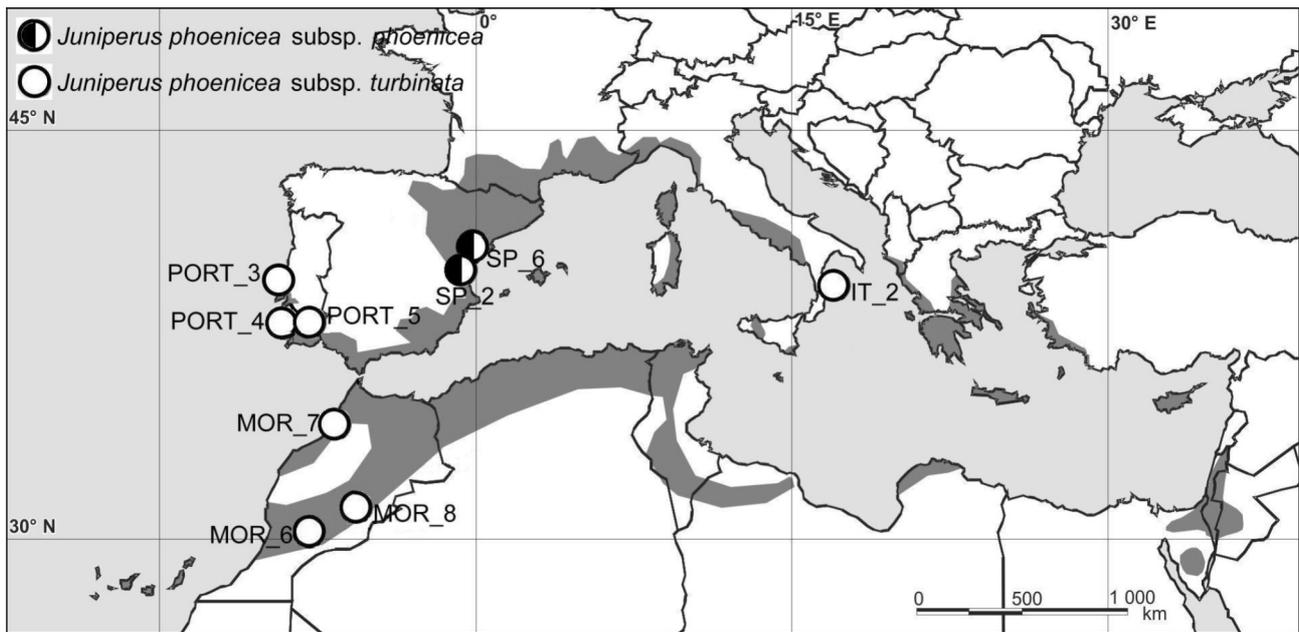


Fig. 1. Range of *Juniperus phoenicea* (after: Jalas and Suominen 1973; Quezel and Pesson 1980; Browicz 1982; Boratyński et al. 1992; Charco 2001; Farjon 2005)

nen 1973; Quezel and Pesson 1980; Quezel and Barbero 1981; Browicz 1982; Kerfoot and Lavranos 1984; Greuter et al. 1984; Amaral Franco 1986; Christensen 1997; Charco 2001; Farjon 2005).

The regions of contemporary occurrence of *J. phoenicea* are situated directly inside or around the Pleistocene refugial areas of the tertiary floras (Comes 2004; Weiss and Ferrand 2007a, b; Médail and Diadema 2009). For pioneer character of the species, it probably did not occur inside the refugia, but on their peripheral sites.

It seems to be possible, that Phoenician juniper survived the glacial periods of Pleistocene in the places close to their present localities. Occurrence of the species during Late Glacial Maximum was confirmed only in South East Spain by macroscopic palaeo-findings (Uzquiano and Arnaz 1997). Unfortunately, the pollen of *Junipers* are not determined to the species (eg. Huntley 1988; Elenga et al. 2000; Carrión 2002; Eastwood 2004; Tzedakis 2004; González-Sampériz et al. 2005). In spite of that, it is recognized the positive reaction of *J. phoenicea* to the more arid and warmer periods during last 14–15 thousand of years and its suppression by more humid-demanding, predominantly broad-leaved trees (Uzquiano and Arnaz 1997; Carrión et al. 2001a, b, 2003, 2004).

The studies on genetic, biochemical and morphological differentiation of *J. phoenicea* confirmed possibility of two main centres of the species in the Iberian Peninsula, connected strongly with their present taxonomic division onto two subspecies *J. phoenicea* subsp. *phoenicea* and *J. phoenicea* subsp. *turbinata*, which migrated from these regions across Mediterranean Europe and North Africa (Lebreton 1983; Le-

breton and Thivend 1981; Lebreton and Rivera 1989; Adams et al. 1996; Boratyński et al. 2009).

The existence of two subspecies of *J. phoenicea* on the Iberian Peninsula and NW Africa was firstly documented on the biochemical characteristics and later confirmed in biometrical studies (Lebreton 1983; Mazur et al. 2003), the random amplified polymorphic DNA (RAPD) (Adams et al. 2002) and by isozyme examinations (Boratyński et al. 2009). The morphological examination of herbarium samples revealed a much more frequent presence and a larger range of the subsp. *phoenicea* than subsp. *turbinata* (Farjon 2005: 340) than those described by Lebreton and Rivera (1989) and Boratyński et al. (2009). The third subspecies has been described from the Canary Islands, but lately is included into synonyms of *J. phoenicea* subsp. *turbinata* (Farjon 2005).

Distinguishing the two subspecies of *J. phoenicea* (Amaral Franco 1986; Lebreton and Rivera 1989) within the area of the western Mediterranean region can be interpreted as indicating of the two centres of the species or its ancestor, isolated against gene flow for a long period of time. This, and differences between west- and east-Mediterranean populations of subsp. *turbinata* was also detected using RAPD (Adams et al. 2002), inter simple sequences repeat (ISSR) (Meloni et al. 2006) and isoenzymes (Boratyński et al. 2009).

The aim of the present study was verification of the hypothesis, that *J. phoenicea* has an intra-specific differentiation at the morphological level in the West Mediterranean region, that corresponds to the species differentiation described on the basis of biochemical, RAPD and isoenzymatic studies.

Material and methods

The material for this study was sampled in Spain, Portugal, Italy and Morocco (Table 1), in the areas of or close to refugia of quaternary floras (Médail and Diadema 2009). The samples of cones and short parts of shoots of the last ramification were gathered separately from the southern parts of individuals, at a height of about 1.0–2.5 m above ground level. The 269 individuals of *J. phoenicea* were examined, each represented by 10 cones and 10 pieces of twigs and leaves. The measurements were performed on dry material, referring to the previously conducted studies (Mazur et al. 2003, 2004; Marcysiak et al. 2007). The nine characters of cones, seeds and shoots and leaves and eight proportions were examined (Table 2).

The measurements and evaluation of the data were taken under the stereoscope microscope with a scaled ocular. The obtained results of measurements were statistically analyzed with *STATISTICA 8* (*StatSoft Polska*) and *GenALEx 6.1* (*Genetic Analysis in Excel*, Peakall and Smouse 2006). The symmetry and unimodality of frequency distribution of measured character values were verified to assess the possibility of conducting statistical analysis (Zar 1999; Łomnicki 2003; Sokal and Rohlf 2003). The main statistics (arithmetic means, standard deviation, variation coefficient) were calculated to determine the values of particular characters for populations, subspecies and species (Zar 1999; Łomnicki 2003). The interactions between particular

measured characters (characters 1–9) were checked with Pearson's correlation coefficient to avoid the most closely correlated and possibly redundant ones (Zar 1999). The Tukey's *T*-test was used to determine the impact of individual characteristics on the diversity of the populations (Zar 1999; Sokal and Rohlf 2003).

The discrimination analysis was performed to identify the discriminant power of each character, to determine the interspecific differences and the inter-population variation (Marek 1989; Morrison 1990; Sokal and Rohlf 2003). Dendrograms of the Euclidean distances by the Ward method between populations were constructed to check their affinities revealed in discriminating analysis (Karoński and Caliński 1973; Sokal and Rohlf 2003). Both analyses: discrimination and cluster were made on the characters obtained from the ratio (characters 10–17). Student's test was carried out to determine which of the 17 analyzed characters influence on the differentiation of *Juniperus phoenicea* on two subspecies (Łomnicki 2003).

To assess the relationship between the two distance matrices: geographical and biometric, accordance with the procedure presented by Smouse et al. (1986, 1992, by Peakall and Smouse 2006), the Mantel test was used (Mantel 1967). The correlation coefficients were examined whether there is a statistical correlation between the Mahalanobis distances obtained for the analysis of biometric data and geographical distance calculated from geographic coordinates using *MapInfo* software.

Table 1. Studied populations of *Juniperus phoenicea*

No.	Acronym	Locality	Longitude and latitude	Altitude [m]	Number of specimens
1	MOR_6	Morocco, High Atlas, AitLekak, near Tadmamt, on the N-E from Oukaimeden	N 31°15'45" W 07°50'00"	1600	30
2	MOR_7	Morocco, sandy coast near Kenitra	N 34°14'16" W 06°38'55"	20	30
3	MOR_8	Morocco, High Atlas, Agouti	N 31°38'20" W 06°29'35"	2000	29
4	PORT_3	Portugal, Peniche, on the S-E from Playa de Consolação	N 39°19'40" W 09°21'32"	10	30
5	PORT_4	Portugal, Cabo de Espichel	N 38°24'48" W 09°12'33"	100–150	30
6	PORT_5	Portugal, 1 km on the S-E from Troy (Setubal)	N 38°26'55" W 08°50'25"	25	30
7	SP_2	Spain, Aragon (Maestrazgo), Teruel, Sierra de Noguera, on the E from Rubielos de Mora	N 40°10'38" W 00°37'17"	1100	30
8	SP_6	Spain, Sierra de Valdeancha, Cintorres and Portella de Morella, on the S-W from Morella	N 40°33'15" W 00°14'48"	1100	30
9	IT_2	Italy, Cabo Rizutto, on the S from Crotone	N 38°53'55" E 17°05'54"	10	30

Table 2. Analyzed characters of cones, seeds and shoots with means \pm standard deviation and results of Tukey's T-test showing how many times the average value of particular characters substantially affect on the differentiation between 9 populations of *Juniperus phoenicea*

No.	Character	Mean \pm SD									Tukey's T-test	
		MOR 6	MOR 7	MOR 8	PORT 3	PORT 4	PORT 5	SP 2	SP 6	IT 2	T	
1	Number of recta (4 or 6)	4.23 \pm 0.6431	4.08 \pm 0.3926	4.30 \pm 0.7120	4.06 \pm 0.3423	4.32 \pm 0.7344	4.20 \pm 0.6010	4.44 \pm 0.8299	4.69 \pm 0.9512	4.18 \pm 0.5733	9	
2	Length of cone	10.55 \pm 1.1870	10.07 \pm 1.0675	10.85 \pm 1.2229	10.01 \pm 0.8592	8.95 \pm 0.9069	9.25 \pm 0.9507	7.83 \pm 0.9555	8.04 \pm 0.9820	9.93 \pm 1.0612	27	
3	Diameter of cone	9.68 \pm 1.1105	9.98 \pm 1.1200	10.62 \pm 1.0070	10.04 \pm 0.8222	9.10 \pm 0.8293	9.16 \pm 0.8433	8.45 \pm 0.9588	8.92 \pm 1.0593	8.56 \pm 0.8779	24	
4	Cone scale number	9.68 \pm 1.6850	8.24 \pm 0.9454	9.31 \pm 1.7839	8.57 \pm 0.9958	9.25 \pm 1.4385	8.80 \pm 1.3144	8.72 \pm 1.7141	9.53 \pm 1.9686	8.72 \pm 1.2782	14	
5	Number of seeds	7.14 \pm 1.7027	6.09 \pm 1.0238	7.52 \pm 1.8827	5.71 \pm 0.9852	5.71 \pm 1.2400	6.08 \pm 1.2645	7.63 \pm 1.8193	8.55 \pm 2.5328	5.33 \pm 1.3835	23	
6	Length of seed	5.22 \pm 0.7396	5.67 \pm 0.6407	5.65 \pm 0.5588	4.90 \pm 0.4144	4.62 \pm 0.5050	4.86 \pm 0.4235	3.90 \pm 0.3917	3.96 \pm 0.3874	5.13 \pm 0.6047	29	
7	Width of seed	2.81 \pm 0.5344	3.02 \pm 0.4545	2.88 \pm 0.3420	2.57 \pm 0.2779	2.62 \pm 0.3355	2.48 \pm 0.2395	2.18 \pm 0.2301	2.27 \pm 0.2710	2.64 \pm 0.3615	27	
8	Number of leaves per 5-mm section of ultimate lateral branchlet	21.18 \pm 3.7693	21.84 \pm 3.6357	23.86 \pm 3.6857	24.67 \pm 2.8722	25.13 \pm 3.6399	24.23 \pm 3.4717	25.36 \pm 3.1871	26.33 \pm 3.4808	22.05 \pm 2.3558	19	
9	Thickness of the ultimate lateral branchlet and leaves	0.81 \pm 0.1144	0.74 \pm 0.1140	0.71 \pm 0.0982	0.75 \pm 0.0807	0.86 \pm 0.0902	0.74 \pm 0.0818	0.78 \pm 0.1025	0.74 \pm 0.1027	0.74 \pm 0.0884	15	
10	Ratio of cone length/diameter (2/3)	1.10 \pm 0.1054	1.01 \pm 0.0812	1.02 \pm 0.0859	1.00 \pm 0.0545	0.98 \pm 0.0490	1.01 \pm 0.0680	0.93 \pm 0.0451	0.90 \pm 0.0478	1.17 \pm 0.1221	25	
11	Ratio of seed length/width (6/7)	1.91 \pm 0.4044	1.90 \pm 0.2515	1.98 \pm 0.2527	1.92 \pm 0.2214	1.78 \pm 0.2185	1.97 \pm 0.2165	1.80 \pm 0.2003	1.76 \pm 0.2019	1.97 \pm 0.2668	16	
12	Ratio of cone diameter/number of seeds (3/5)	1.42 \pm 0.3240	1.68 \pm 0.3049	1.50 \pm 0.4014	1.80 \pm 0.3132	1.66 \pm 0.3354	1.56 \pm 0.2834	1.16 \pm 0.2795	1.11 \pm 0.2772	1.71 \pm 0.4807	22	
13	Ratio of cone diameter/seed width (3/7)	3.56 \pm 0.7689	3.35 \pm 0.4712	3.73 \pm 0.5406	3.94 \pm 0.4332	3.51 \pm 0.4606	3.72 \pm 0.4414	3.90 \pm 0.4547	3.96 \pm 0.5787	3.28 \pm 0.4315	17	
14	Ratio of thickness of branchlet/number of leaves (9/8)	0.04 \pm 0.0088	0.04 \pm 0.0091	0.03 \pm 0.0070	0.03 \pm 0.0052	0.04 \pm 0.0072	0.03 \pm 0.0062	0.03 \pm 0.0064	0.03 \pm 0.0063	0.03 \pm 0.0061	19	
15	Ratio of cone diameter/number of recta (3/1)	2.32 \pm 0.3539	2.46 \pm 0.3127	2.52 \pm 0.3751	2.48 \pm 0.2465	2.14 \pm 0.2914	2.21 \pm 0.2772	1.95 \pm 0.3224	1.95 \pm 0.3263	2.07 \pm 0.2595	25	
16	Ratio of cone length/number of leaves (2/8)	0.52 \pm 0.1134	0.47 \pm 0.0982	0.47 \pm 0.0995	0.41 \pm 0.0604	0.36 \pm 0.0651	0.39 \pm 0.0742	0.31 \pm 0.0637	0.31 \pm 0.0535	0.46 \pm 0.0760	25	
17	Ratio of cone scale number/ cone length (4/2)	0.93 \pm 0.1811	0.82 \pm 0.1054	0.86 \pm 0.1693	0.86 \pm 0.1126	1.04 \pm 0.1548	0.96 \pm 0.1553	1.12 \pm 0.2140	1.19 \pm 0.2133	0.89 \pm 0.1437	24	

Results

The characters, except the number of recta (character 1) and the cone scale number (character 4), have a unimodal or very close to unimodal frequency distributions. This enables the further statistical analyses.

The values of variation coefficient of the characters differed only slightly between subspecies and particular populations. The seeds size (character 6 and 7) and characteristics of shoots (character 8 and 9) are more variables for *J. phoenicea* subsp. *turbinata*, while the numbers of recta (character 1), cone scales (character 4) and seeds (character 5) are strongly variables for *J. phoenicea* subsp. *phoenicea*. Generally, the most stable features for all populations are the diameter of cone (character 3), with the variation coefficient ranging from 8.19% (PORT 3) to 11.47% (MOR 6) and the thickness of the ultimate lateral branchlet and leaves (character 9), with the variation coefficient between 10.51% (PORT 4) and 15.38% (MOR 7). The most variable character of each population is the number of seeds (character 5) with the variation coefficient ranging from 16.80% (MOR 6) to 29.61% (SP 6).

Correlations between measured characters (Table 3) shows, that most of them are correlated statistically significantly at level $p = 0.01$. The most positively correlated are characteristics of cones and seeds: the length of cone (character 2) with the diameter of cone (character 3) and with the length of seeds (character

6), the number of recta (character 1) with the cone scale number (character 4) and the length of seed (character 6) with the width of seed (character 7). Generally, the values of the traits describing leaves and branchlets (characters 8 and 9) did not correlate or only slightly correlate with traits of cones and seeds.

In particular populations the correlations between characters are similar. It is interesting that correlation between the cone scale number (character 4) with the number of seeds (character 5) are significant in each population.

All analyzed characters differentiate statistically significant ($p = 0.05$) 9 populations of *J. phoenicea*. The most important are the length and width of seed (character 6 and 7) and the length of cone (character 2) (Table 2 – Tukey’s *T*-test).

The Mantel test showed a statistically insignificant relationship ($p = 0.1$) between the Mahalanobis and geographical distances (Fig. 2). The average correlation coefficient was $R_{xy} = 0.365$. The graph shows only 13.31% explained variance. The relationship with the logarithmic function (Rousset 1997) explains more – 22.79% of the explained variance.

The analysis of discriminating function for 9 populations showed that the ratio of thickness of branchlet/number of leaves (character 14), the ratio of cone length/number of leaves (character 16) and the ratio of cone diameter/seed width (character 13) were discriminating among individuals and populations at

Table 3. Correlation coefficient between 9 measured characters of *Juniperus phoenicea* (character numbers as in Table 2; * – significance level $p = 0.05$; ** – significance level $p = 0.01$)

Character	1	2	3	4	5	6	7	8
2	-0.02							
3	0.13**	0.75**						
4	0.72**	0.18**	0.24**					
5	0.38**	0.08**	0.28**	0.41**				
6	-0.10**	0.74**	0.56**	0.02	-0.13**			
7	-0.09**	0.54**	0.47**	0.04*	-0.12**	0.65**		
8	0.07**	-0.32**	-0.11**	0.01	0.10**	-0.32**	-0.21**	
9	0.01	-0.06**	-0.04	0.07**	0.00	-0.07**	0.00	-0.17**

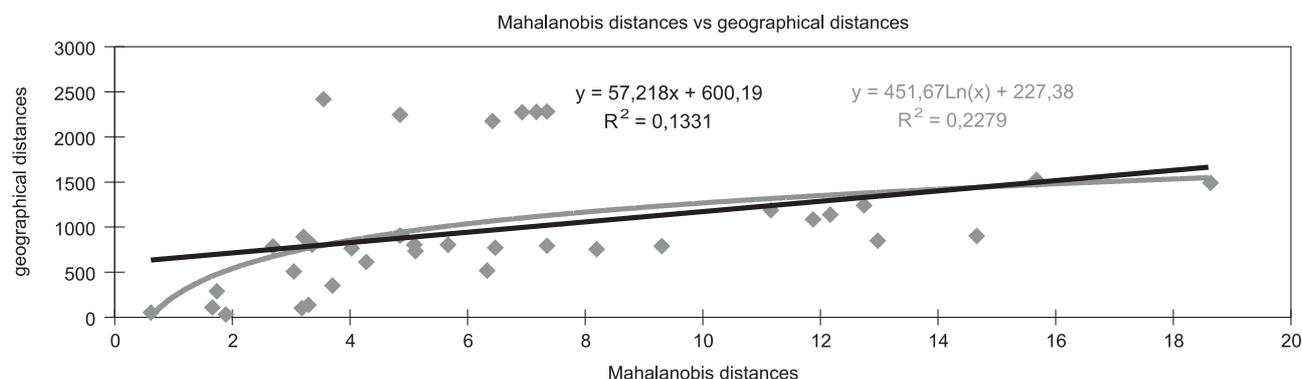


Fig. 2. Result of the Mantel test based on Mahalanobis distances and geographical distances, showing correlation between them for 9 populations of *J. phoenicea*

Table 4. Discriminant power testing for 8 calculated characters of *Juniperus phoenicea* (p – levels of significance)

Character		Wilks' lambda	p
10	Ratio of cone length/diameter	0.75	0.000000
11	Ratio of seed length/width	0.65	0.000000
12	Ratio of cone diameter/number of seeds	0.57	0.000000
13	Ratio of cone diameter/seed width	0.52	0.000000
14	Ratio of thickness of branchlet/number of leaves	0.47	0.000000
15	Ratio of cone diameter/number of recta	0.65	0.000000
16	Ratio of cone length/number of leaves	0.51	0.000000
17	Ratio of cone scale number/cone length	0.75	0.000000

highest level, with the partial Wilks' lambda values of 0.47, 0.51 and 0.52, respectively (Table 4). All the analyzed features influenced statistically significant on the discrimination between groups. The first discriminant variable (U_1), covering almost 57% of the total variability, was determined mostly by the ratio of cone scale number/cone length (character 17). The second discriminant variable (U_2) covering more than 19% of total variability was determined by the ratio of cone length/diameter (character 10). The third discriminant variable (U_3), covering more than 9% of total variability, was determined mostly by the ratio of cone diameter/number of seeds (character 12). The graph made between U_1 and U_2 shows that all individuals from 9 populations form two separate groups (Fig. 3). The individuals from Spain populations form

smaller and more homogeneous group, distinct from the other of the first discriminant variable. Variations between individuals of the second separating group were checked on the graph made between U_2 and U_3 . In the space between them, average values calculated for the 7 populations form 3 overlapping groups, associated with their origin (Fig. 4). Moroccan attempts seem to be the most distinct to population from Italy (IT 2). Between this two groups are populations from Portugal.

The closest Euclidean distances between mean values for 9 analyzed populations agglomerated them in two main groups (Fig. 5). The first one consist samples from Morocco, Italy and Portugal included into subsp. *turbinata*, the second includes two populations from Spain gathered as subsp. *phoenicea*. Student test

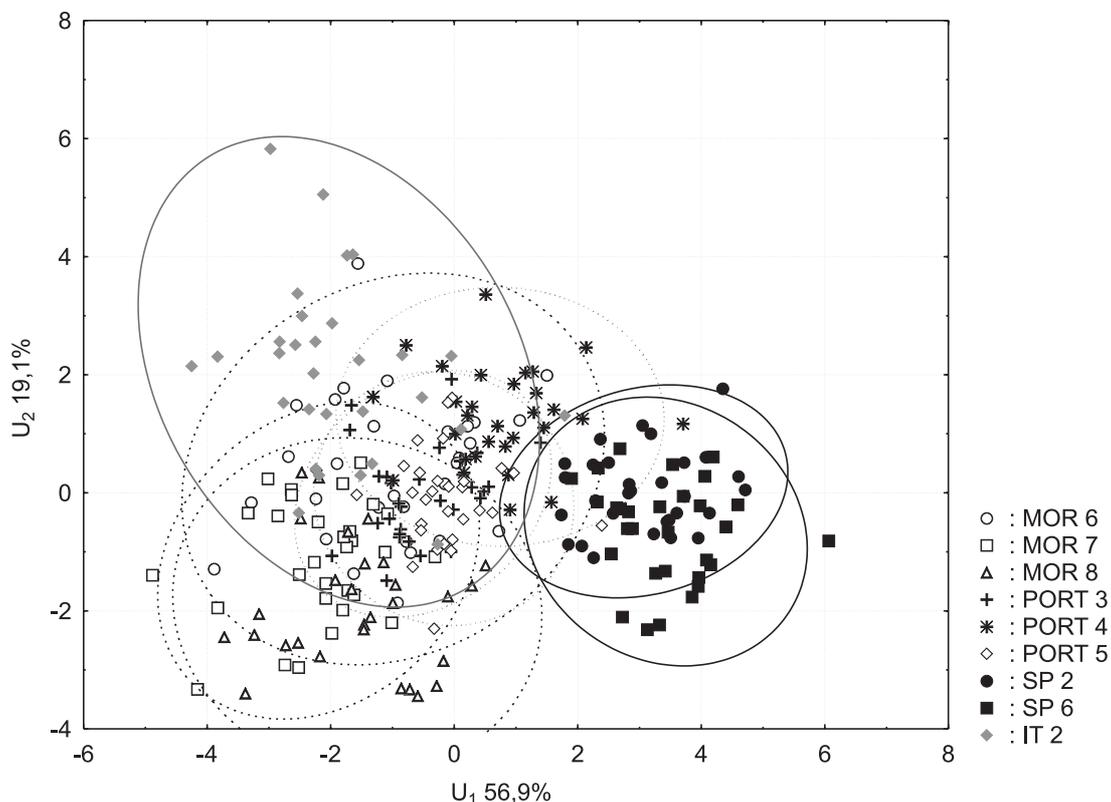


Fig. 3. Result of the discriminant analysis based on 8 characters obtained from the ratio (characters 10–17) of individuals of *J. phoenicea*, on the plane of the two first discriminant variables, which accounted 76% of the total variation

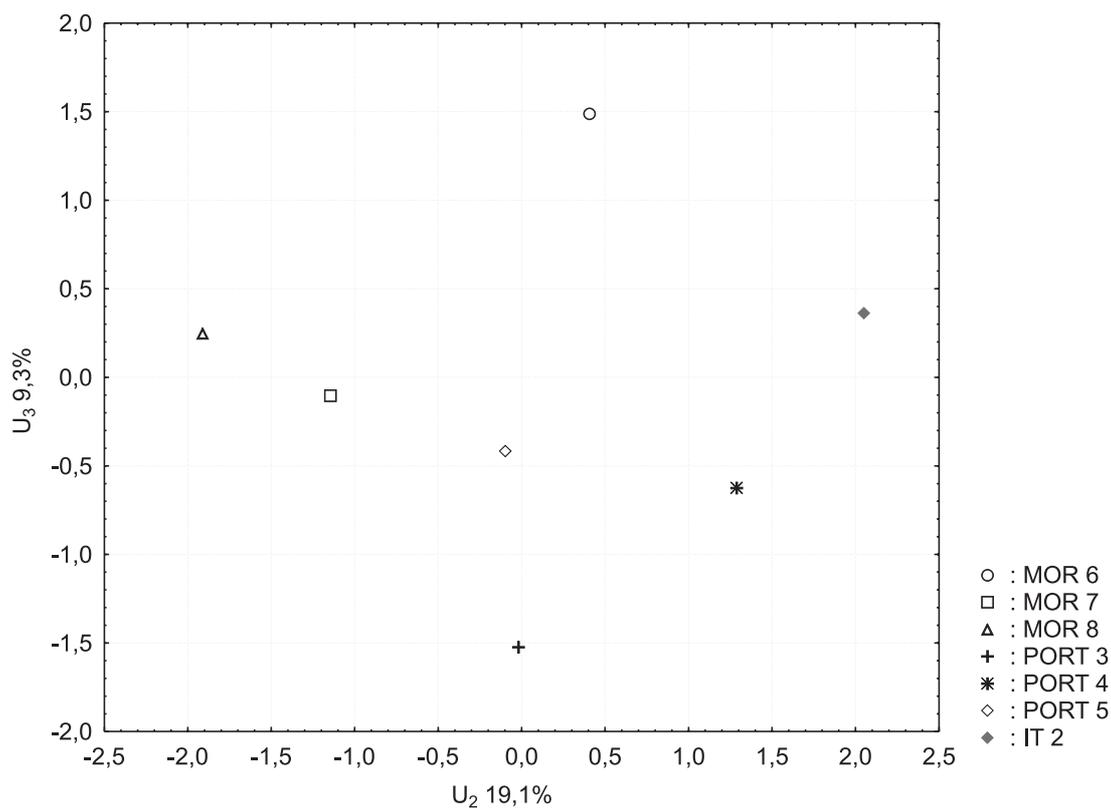


Fig. 4. Result of the discriminant analysis based on 8 characters obtained from the ratio (characters 10–17) of mean value of 7 populations of *J. phoenicea* subsp. *turbinata*, on the plane of the second and third discriminant variables, which accounted 28,4% of the total variation

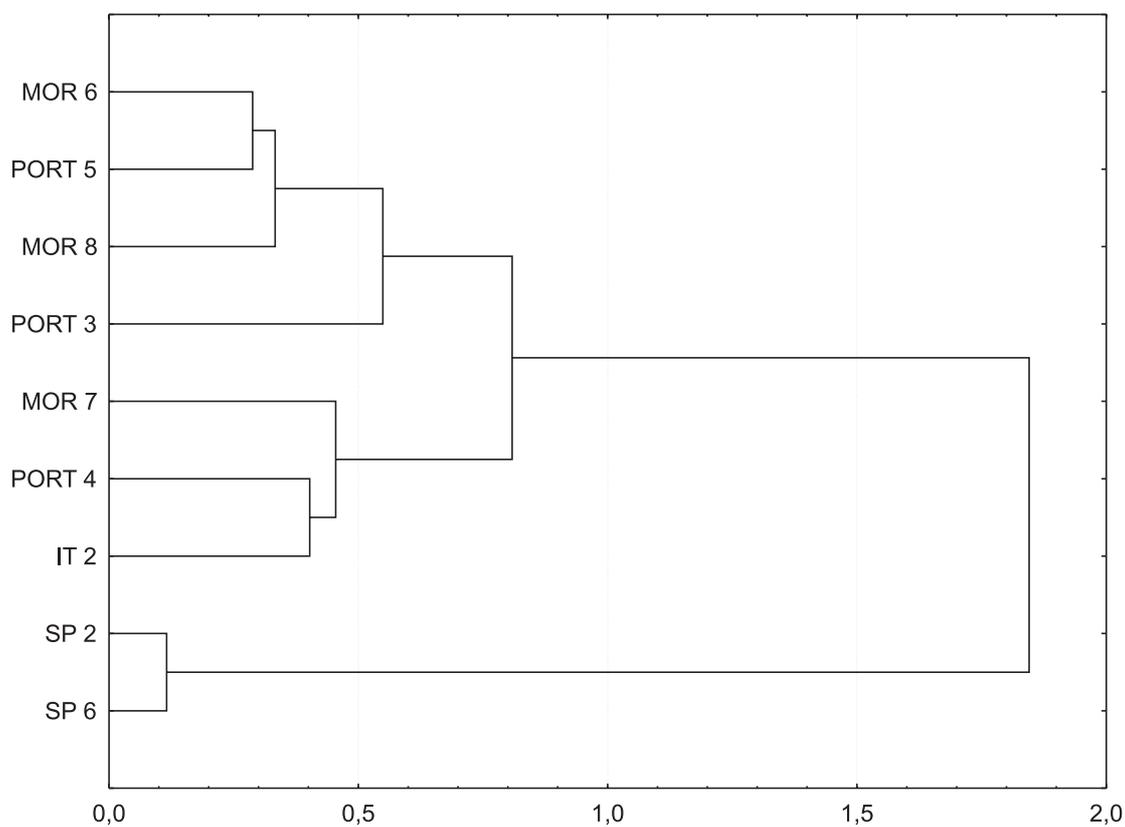


Fig. 5. Dendrogram constructed on the shortest Euclidean distances according to Ward's method, showing connections among 9 populations of *J. phoenicea*

Table 5. Results of Student's test for mean values of investigated characters (1–17) in the *Juniperus phoenicea* subsp. *phoenicea* and subsp. *turbinata* (SD – standard deviation, p – levels of significance)

Character	Mean ± SD subsp. <i>turbinata</i>	Mean ± SD subsp. <i>phoenicea</i>	t	p
Number of recta (4 or 6)	4.20 ± 0.5938	4.56 ± 0.9004	-11.7826	0.000000
Length of cone	9.94 ± 1.2118	7.94 ± 0.9742	37.2399	0.000000
Diameter of cone	9.58 ± 1.1481	8.68 ± 1.0369	17.3308	0.000000
Cone scale number	8.94 ± 1.4513	9.12 ± 1.8879	-2.5827	0.009856
Number of seeds	6.22 ± 1.5715	8.09 ± 2.2509	-23.1444	0.000000
Length of seed	5.15 ± 0.6745	3.93 ± 0.3904	42.2957	0.000000
Width of seed	2.72 ± 0.4150	2.23 ± 0.2558	27.6015	0.000000
Number of leaves per 5-mm section of ultimate lateral branchlet	23.29 ± 3.6651	25.85 ± 3.3698	-15.3306	0.000000
Thickness of the ultimate lateral branchlet and leaves	0.76 ± 0.1070	0.76 ± 0.1043	0.0791	0.936940
Ratio of cone length/diameter (2/3)	1.04 ± 0.1040	0.91 ± 0.0479	29.0531	0.000000
Ratio of seed length/width (6/7)	1.92 ± 0.2762	1.78 ± 0.2023	11.4341	0.000000
Ratio of cone diameter/number of seeds (3/5)	1.62 ± 0.3747	1.14 ± 0.2792	29.1167	0.000000
Ratio of cone diameter/seed width (3/7)	3.58 ± 0.5606	3.93 ± 0.5209	-13.7095	0.000000
Ratio of thickness of branchlet/number of leaves (9/8)	0.03 ± 0.0077	0.03 ± 0.0065	10.2434	0.000000
Ratio of cone diameter/number of recta (3/1)	2.31 ± 0.3469	1.95 ± 0.3241	22.9107	0.000000
Ratio of cone length/number of leaves (2/8)	0.44 ± 0.0984	0.31 ± 0.0588	30.0474	0.000000
Ratio of cone scale number/ cone length (4/2)	0.91 ± 0.1626	1.15 ± 0.2160	-30.1340	0.000000

showed that all the characters, except one – the thickness of the branchlet and leaves (character 9), statistically significantly differentiate two subspecies at the level of significance $p < 0.01$ (Table 5).

Discussion

The biometrical analysis of 9 populations of *Juniperus phoenicea* from the western part of the range: Iberian Peninsula, North Africa and Italia shows important taxonomic distances between them. Médail and Diadema (2009) distinguished 3 types of Pleistocene refugia in the Mediterranean region, 1) the most spatially extended areas located on medium altitudes (400–800 m a.s.l.), less dry and less cold than adjacent areas, 2) more spatially limited but relatively common in the deep gorges and closed valleys characterized by higher humidity and higher temperatures and 3) in low-level valley bottoms, on the coastal plains and wetlands, in humid and warm places. About 20 of the 52 refugia of Tertiary floras were described from Spain, Portugal, Italy and Morocco – the areas of our study (compare Table 1 with Médail and Diadema 2009, Fig. 1: 1336). It shall be expected that occurrence of *J. phoenicea* in different types of refugia during cold periods of Pleistocene had a significant impact on reported differences between 9 analyzed populations (Table 2 – Tukey's T-test) and between two subspecies (Figs 3 and 5, Table 5).

The discrimination analysis and cluster analysis showed clear differences between populations from Spain (SP 2 and SP 6) and populations from Portugal,

Italy and Morocco. These results confirm the morphological, biochemical and genetic differences between *J. phoenicea* subsp. *phoenicea* and subsp. *turbinata* (Lebreton and Thievend 1981; Lebreton 1983; Lebreton and Rivera 1989; Lewandowski et al. 2000; Adams et al. 2002; Mazur et al. 2003; Meloni et al. 2006; Boratyński et al. 2009) and allow to distinguish them. On the basis of the Student test it was found that this diversity is influenced by characters of cones, seeds and leaves. As in previous studies by the other authors (Amaral Franco 1986; Mazur et al. 2003; Farjon 2005) this study also showed that the cones and seeds of the subspecies *J. phoenicea* subsp. *phoenicea* are smaller, with more flattened shape and the diameter (character 3) larger than the length (character 2). Cones of *J. phoenicea* subsp. *turbinata* are more spherical and slightly elongated with a length (character 2) larger than the diameter (character 3).

Mantel test showed no statistically significant relationship between Mahalanobis distances and geographical distances among the compared 9 populations of *J. phoenicea* (Fig. 2), differently than it has been demonstrated for other organisms, including humans (Manni et al. 2004; Boattini et al. 2007; Kuehn et al. 2007; Brown and Stepien 2009; Dzialuk et al. 2009). Relatively low percentage of explained variance in the Mantel test can be explained by relatively low diversity of abiotic environmental conditions, particularly in the coastal zone of the Mediterranean, and thus the lack of diversity of environmentally-oriented processes of evolution within even the most geographically distant populations. It may also

be a result of a single, rapid colonization from an initial population, for example during the Messinian Salinity Crisis, which was the period when the Mediterranean Sea evaporated, partly or completely, during the Messinian period of the Miocene epoch, 5.96 million years ago (Bocquet et al. 1978; Verdu et al. 2003; Kovar-Eder et al. 2006; Favre et al. 2007) and the subsequent evolution of isolated, at least partially, populations to restore the Mediterranean Sea.

In spite of lack of geographical pattern of species variation, the easternmost population from examined of *Juniperus phoenicea* subsp. *turbinata* from Italy is different from the others (Figs 3 and 4). It can indicate the longer isolation by distance and accumulation of differences, as in case of isoenzymatic study (Boratyński et al. 2009). The differentiation of *J. phoenicea* subsp. *turbinata* indicate also differences between Portugal and Moroccan populations (Fig. 4), which indicate the role of Gibraltar Strain in differentiation of the species, similarly as in case of *Juniperus thurifera* (Romo and Boratyński 2007; Terrab et al. 2008) and of *Abies pinsapo* and *A. marocana* (Terrab et al. 2007).

In conclusion it can be assumed that biometrical analysis confirmed the older data on intraspecific structure of *Juniperus phoenicea* and indicated a role of the Gibraltar Strain in the species differentiation.

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