MORPHOLOGICAL VARIABILITY OF *JUNIPERUS PHOENICEA* (CUPRESSACEAE) FROM THREE DISTANT LOCALITIES ON IBERIAN PENINSULA

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ABSTRACT

The aim of the present study was biometrical comparison of three Iberian populations of *Juniperus phoenicea*, represented by the subsp. *turbinata* and subsp. *phoenicea*. Eight features of the cones and seeds, two of the shoots and leaves were studied.

The biometrical analysis of three distant populations of *J. phoenicea* shows great taxonomic distances among them. Two of them, representatives of *J. phoenicea* subsp. *turbinata*, are closer related each other than to the third, which represents *J. phoenicea* subsp. *phoenicea*. These results confirm the genetic differentiation of the taxons and also the biochemical and morphologic division of them. Nevertheless, the distances between particular populations are so great that more resemble the distances between species than between subspecies.

KEY WORDS: statistical analysis, biometry, juniper, cone, morphological differentiation, variability.

INTRODUCTION

Juniperus phoenicea L. is a small tree, normally to 6-8 (11) m tall, or sometimes only a shrub, with scaly leaves and brown cones (Fig. 1-3). It occurs in southern Europe, south-west Asia and northern Africa (Fig. 4), but most frequently in the western part of the Mediterranean region. The species occupies costal dunes, maritime or somewhat inland slopes, but enters the mountain regions in the western part of their range, up to altitudes of 2400 m in Morocco and 1800 m in Spain. It grows in various types of Mediterranean forests and sometimes also forms pure stands (Quezel and Pessn 1980; Quezel and Barbero 1981; Browicz 1982; Quezel et al. 1992).

Juniperus phoenicea belongs to the section *Sabina* of the genus *Juniperus*. It is a variable species characterized by a great morphological and biochemical differentiation, which was the reason for distinguishing three subspecies (Greuter et al. 1984; Amaral Franco 1986; Lebreton and Rivera 1989).

Juniperus *phoenicea* L. Sp. Pl. 1039 (1953)
– subsp. *phoenicea*
– subsp. *turbinata* (Guss.) Nyman, Consp. Fl. Eur. 676 (1881)
Syn.: *J. lycia* L. Sp. Pl. 1039 (1753)
J. turbinata Guss. Fl. Sicul. Syn. 2: 634 (1845)
J. oophora G. Kunze, In: Flora (Regensburg) 29: 637 (1846)

Fig. 1. *Juniperus phoenicea* tree about 9 m high; Spain, dunes in Matalascañas (Photo A. Boratyński).

Fig. 2. Branch of *Juniperus phoenicea* with cones; Portugal, Cabo de Espichel (Photo A. Boratyński).

– subsp. *mediterranea* Lebreton et Thievend In: Naturalia Monspeliensia, Ser. Bot., 47: 8 (1981) (the most probably identical with subsp. *turbinata*).

In spite of the large morphological and biochemical variation described, *Juniperus phoenicea* has not been intensively studied. The only known biometric comparison of cones (Lebreton and Rivera 1989) was based on the material from single specimens sampled in many dispersed localities, mainly from west-Mediterranean regions. The results confirm the biochemical differences between the studied individuals and were the basis of morphological characteristics of the subsp. *mediterranea* and subsp. *phoenicea* (Lebreton and Thievend 1981; Lebreton 1983; Lebreton and Rivera 1989). The characters studied by them (weight, length and thickness of the cones and number of seeds) are very important from the taxonomic point of view. The other ones, as the shoots arrangement and leaves characters, are also considered to be important in distinguishing subspecies (Amaral Franco 1986).

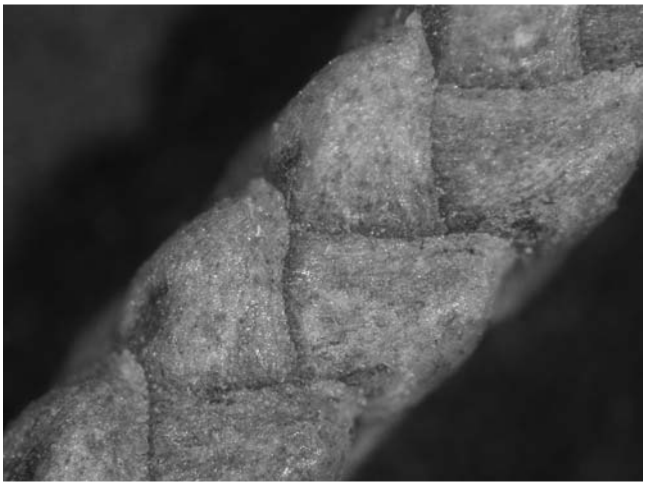

Fig. 3. Scaly-like leaves of *Juniperus phoenicea* (Photo D. Tomaszewski).

Generally, the typical subspecies (subsp. *phoenicea*) is characterized by denser habit, more compact crowns, and more obtuse leaves and globose cones of 8-10 mm in diameter. The subsp. *turbinata* have more elongated and not so dense crowns, more acute leaves and ovoid cones about 12-14 mm in diameter (Amaral Franco 1986). The typical subspecies is distributed evenly in the area of the Iberian Peninsula, while the subsp. *turbinata* colonizes only maritime regions, mostly coastal dunes or maritime rocky slopes (Amaral Franco, l.c.).

The aim of the present study was biometrical comparison of three populations of the species. Two of them are represented by the subsp. *turbinata,* probably identical with subsp. *mediterranea* described by Lebreton and Rivera (1989), and one by the typical subsp. *phoenicea*. The first particular aim was to verify if the maritime populations consist of individuals representing only subsp. *turbinata* or also specimens morphologically resembling typical subspecies. The contrary hypothesis, concerning the population of subsp. *phoenicea* from the continental part of Spain was examined too. Then the comparison of the studied subspecies was provided to define its morphological characters.

MATERIAL AND METHODS

The material for the present study has been collected on the Iberian Peninsula in September 1999 and comprises two subspecies distinguished there (Table 1). The samples of cones and small fragments of branches were gathered separately from every individual and then dried. The samples were collected from the sunny, mostly southern parts of the crowns (including the south-western and south-eastern expositions), from the height of 1.0-2.5 m. The measure-

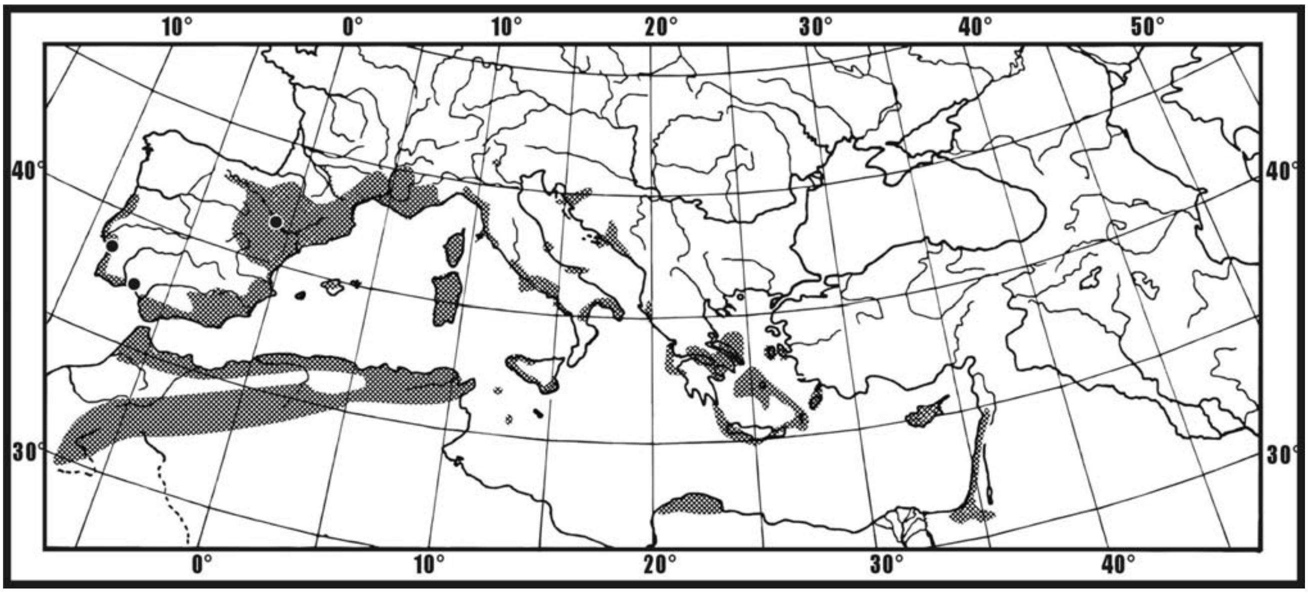

Fig. 4. Range of *Juniperus phoenicea* (drown by P. Kosiński after maps and data of Quezel and Santa 1962; Jalas and Suominen 1973; Fenaroli and Gambi 1976; Browicz 1982; Boratyński et al. 1992; Quezel et al. 1992).

TABLE 1. Localization of studied populations of *Juniperus phoenicea*.

No	Locality	Subspecies	Spec. number	Longitude	Latitude	Altitude
1	Spain, Adalucía, Matalascañas, forests on the dunes	*turbinata* (Guss.) Nyman	28	6° 31'W	37° 00'N	10-20 m
2	Portugal, Estremadura, Cabo de Espichel, coastal slopes	*turbinata* (Guss.) Nyman	27	9° 46'W	38° 30'N	40-50 m
3	Spain, Aragón, Nueno (Huesca), Salto de Roldán, near Santa Eulalia de la Peña	*phoenicea*	27	0° 25'W	42° 17'N	1000 m

ment was done in 2001 on dry material. The cones, shoots and seeds were measured with accuracy to 0.1 mm. The populations were characterized on the basis of 27-28 individuals. Ten ripen cones and ten short fragments of one-year old shoots of the last ramification were sampled from the 89 individuals, and consequently, a total of 890 values of each of the characters examined were analysed statistically. Each individual was analysed separately, and later the three local samples and two subspecies were analysed together. Eight features of the cones and seeds, two of the shoots and leaves and four proportions were studied (Table 2).

The measurements and evaluation of the data were taken under the stereoscope microscope of 8× magnification with a scaled ocular. The data obtained were statistically analysed with the STATISTICA 5.1 software. Arithmetic means, standard deviations and variation coefficients were calculated for every feature. The interaction between particular characters was tested using the Pearson's linear correlation coefficient. A discrimination analysis was performed and the position of the specimens was examined along with the first discriminant variables inside particular populations studied to check their homogeneity.

The distribution of individuals in the two main variables were tested to verify the distance among them, among the populations studied and between the subspecies compared. The dendrogram of the shortest Euclidean distances among the populations studied were constructed (Marek 1989; Morrison 1990; Underwood 1997, Moczko et al. 1998).

RESULTS

Mean values, ranges, standard deviations and variation coefficients are presented for every sample separately and for all individuals combined (Table 3). The greatest differences between mean values of particular features of the compared populations are observed in the number of recta (character 1) and number of seeds per cone (character 6).

TABLE 2. Analysed characters of the cones, seeds, shoots and leaves.

No	Characters	Accuracy and measure
1	Number of recta (4 or 6)	Specimen
2	Length of cone	0.1 mm
3	Width of cone	0.1 mm
4	Thickness of cone	0.1 mm
5	Cone scale number	Specimen
6	Number of seeds	Specimen
7	Length of seed	0.1 mm
8	Width of seed	0.1 mm
9	Number of leaves on the 5 mm of the last ramification shoot	Specimen
10	Thickness of the last ramification shoot with leaves	0.1 mm
11	Ratio of cone length/wide (2/3)	
12	Ratio of cone length/thickness (2/4)	
13	Ratio of seed length/width (7/8)	
14	Ratio of cone width/number of seeds (3/6)	

TABLE 3. Statistical descriptions of the analysed 14 characters of *J. phoenicea* (numbers 1-14' full names in Table 2).

Calculated characteristics	Samples	Characters													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Mean	Matalascañas	4.10	10.14	9.95	9.31	9.84	5.96	5.54	2.90	25.07	0.90	1.02	1.09	1.93	1.72
	Cabo de Espichel	4.23	8.58	8.76	8.26	10.16	4.97	5.29	3.07	25.53	1.04	0.98	1.04	1.74	1.85
	Salto de Roldán	4.51	8.32	9.11	8.62	10.50	8.05	4.11	2.66	27.74	1.04	0.91	0.97	1.59	1.19
	All samples	4.28	9.04	9.29	8.75	10.16	6.39	4.98	2.87	26.13	0.99	0.97	1.03	1.76	1.58
Minimum	Matalascañas	4.0	7.3	7.6	6.4	6.0	3.0	2.9	2.0	16.0	0.7	0.88	0.94	0.47	1.01
	Cabo de Espichel	4.0	6.1	6.2	5.8	8.0	2.0	3.2	2.0	2.4	0.7	0.84	0.88	1.20	0.93
	Salto de Roldán	4.0	5.9	6.5	6.2	8.0	3.0	2.5	1.3	18.0	0.8	0.80	0.83	1.00	0.65
	All samples	4.0	5.9	6.2	5.8	6.0	2.0	2.5	1.3	2.4	0.7	0.80	0.83	0.47	0.65
Maximum	Matalascañas	6.0	13.8	13.5	12.2	15.0	10.0	7.1	6.2	36.0	1.1	1.23	1.30	2.82	2.95
	Cabo de Espichel	6.0	10.6	10.7	10.3	15.0	10.0	7.5	4.6	34.0	1.4	1.23	1.32	2.45	4.50
	Salto de Roldán	6.0	11.5	12.0	11.2	18.0	13.0	5.4	5.1	36.0	1.4	1.06	1.18	3.46	3.10
	All samples	6.0	13.8	13.5	12.2	18.0	13.0	7.5	6.2	36.0	1.4	1.23	1.32	3.46	4.50
Standard deviation	Matalascañas	0.4367	0.9350	0.8888	0.9120	1.4891	1.2072	0.5625	0.3780	3.8155	0.0864	0.0662	0.0758	0.2733	0.3174
	Cabo de Espichel	0.6362	0.7922	0.8198	0.7978	1.7975	1.1976	0.6229	0.4200	3.8376	0.1102	0.0708	0.0704	0.2511	0.4953
	Salto de Roldán	0.8720	0.9725	0.9345	0.8892	2.3314	1.9492	0.5141	0.5354	3.4502	0.1032	0.0548	0.0576	0.3029	0.2867
	All samples	0.690	1.217	1.016	0.972	1.915	1.963	0.847	0.478	3.880	0.121	0.078	0.087	0.312	0.467
Variation coefficient	Matalascañas	10.65	9.23	8.94	9.80	15.14	20.25	10.15	13.02	15.22	9.62	6.49	6.94	14.13	18.41
	Cabo de Espichel	15.05	9.23	9.37	9.66	17.70	24.08	11.78	13.68	15.03	10.64	7.20	6.76	14.40	26.73
	Salto de Roldán	19.36	11.69	10.26	10.31	22.21	24.20	12.52	20.15	12.44	9.89	5.99	5.97	19.05	24.12
	All samples	16.13	13.46	10.93	11.11	18.86	30.72	16.99	16.65	14.85	12.17	8.04	8.37	17.70	29.60

The most variable feature was the number of seeds in a cone (character 6) with a variation coefficient of more than 30% in all three populations, but about 20-24% in particular populations. The most stable characters appear to be the shape of a cone measured as ratio of length/width and length/thickness (characters 11 and 12), which attain a variation coefficient of about 8% for all populations together, but vary at 6-7% when examined separately (Table 3).

The correlation between many features is statistically significant at level $p>0.01$. The numbers of correlations and the correlated characters are different in the particular populations and in all populations together (compare Table 4-7). Some characters in one population are strongly correlated, while in others the correlations are slight and insignificant. It indicates that differentiation of specimens is different within each sampled population.

The most strongly correlated features are the length (character 2), width (character 3) and thickness (character 4) of a cone. Also the number of recta (character 1) correlates with number of cone scales (character 5), number of seeds in a cone (character 6) and cone dimensions with number of cone scales (characters 2-5).

The number of leaves on the 5 mm of shoot (character 9) and thickness of shoot with leaves (character 10) are inversely more or less strongly correlated with the features of the cone and seeds (Tables 4-7), but not in every population studied.

The homogeneity of samples was tested with analysis of discriminant function for every sampled population separately. The intra-population variations of individuals in the samples from Matalascañas and Cabo de Espichel were weak (Fig. 5 and 6). The greatest discriminating power in the population from Matalascañas has the cone scale number (character 5) and the seed length/width ratio (character 13) characterized by the Wilks' lambda value of 0.354 and 0.44, respectively. The first main variable (U_1) includes ±61% of the whole variability and is deter-

TABLE 4. Correlation coefficients between characters of *Juniperus phoenicea* from Matalascañas. * – significance at level p = 0.05; ** – significance at level p = 0.01 (character numbers as in Table 2).

2	0.04								
3	0.02	0.81**							
4	0.05	0.79**	0.96**						
5	0.40*	0.04	0.24	0.25					
6	0.20	0.21	0.32	0.39*	0.33				
7	0.20	0.79**	0.71**	0.61**	-0.02	-0.08			
8	-0.19	0.28	0.40*	0.38*	-0.10	0.00	0.19		
9	-0.12	-0.44*	-0.27	-0.24	-0.17	-0.13	-0.25	-0.27	
10	0.18	-0.06	-0.05	-0.13	0.21	0.20	0.03	-0.42*	0.04
Characters	1	2	3	4	5	6	7	8	9

TABLE 5. Correlation coefficients between characters of *Juniperus phoenicea* from Cabo de Espichel. * – significance at level p = 0.05; ** – significance at level p = 0.01 (character numbers as in Table 2).

2	-0.03								
3	0.13	0.71**							
4	0.17	0.77**	0.98**						
5	0.73**	0.33	0.27	0.35					
6	0.21	0.21	0.34	0.35	0.38				
7	0.02	0.67**	0.66**	0.69**	0.35	0.23			
8	-0.04	0.49**	0.46*	0.50**	0.20	-0.30	0.43*		
9	-0.05	-0.41*	-0.48*	-0.50**	-0.33	-0.08	-0.39*	-0.43*	
10	-0.17	0.16	0.14	0.11	0.09	0.05	0.24	0.12	-0.24
Characters	1	2	3	4	5	6	7	8	9

TABLE 6. Correlation coefficients between characters of *Juniperus phoenicea* from Salto de Roldán. * – significance at level p = 0.05; ** – significance at level p = 0.01 (character numbers as in Table 2).

2	0.22								
3	0.24	0.90**							
4	0.22	0.91**	0.99**						
5	0.65**	0.59**	0.59**	0.57**					
6	0.17	0.49**	0.53**	0.55**	0.52**				
7	0.27	0.78**	0.70**	0.72**	0.44*	0.12			
8	0.30	0.13	0.13	0.17	0.09	-0.17	0.39*		
9	-0.35	-0.08	-0.17	-0.17	-0.06	0.06	-0.29	-0.43*	
10	0.32	0.28	0.39*	0.32	0.41*	0.01	0.36	0.12	-0.42*
Characters	1	2	3	4	5	6	7	8	9

TABLE 7. Correlation coefficients between characters of *Juniperus phoenicea* from all populations sampled. * – significance at level p = 0.05; ** – significance at level p = 0.01 (character numbers as in Table 2).

2	-0.30**								
3	-0.07	0.84**							
4	-0.03	0.81**	0.98**						
5	0.68**	-0.02	0.15	0.19					
6	0.45**	-0.11	0.21	0.26*	0.41**				
7	-0.41**	0.73**	0.47**	0.43**	-0.13	-0.61**			
8	-0.15	0.23*	0.12	0.14	-0.03	-0.54**	0.54**		
9	0.11	-0.43**	-0.31**	-0.29**	0.00	0.34**	-0.55**	-0.50**	
10	0.37**	-0.53**	-0.34**	-0.33**	0.37**	0.17	-0.36**	-0.04	0.09
Characters	1	2	3	4	5	6	7	8	9

mined mostly by cone scale number (character 5), while the second main variable (U_2) covers 29% of the variation and is determined by seed length/width ratio (character 13).

The greatest discriminating power in the population from Cabo de Espichel has the cone length/width ratio (character 11) and cone width (character 3), with Wilks' lambda 0.396 and 0.463, respectively. The two first main variables are responsible for 100% of variation (Fig. 6). The first main variable (U_1) is influenced mostly by cone length/wide ratio (character 11) and the second variable (U_2) by width of cone (character 3).

The population from Salto de Roldán has a quite different type of variation. The two first main variables cover only 49% of variation. For this reason it was analysed in the space of the first three main variables, which are responsible for 62% of variation (Fig. 7). All individuals form one group. The first main variable (U_1) is determined mostly by number of leaves (character 9), cone width (character 3) and cone thickness (character 4); the second main variable (U_2) also by character 3, 4 and 9, and the third main variable (U_3) by cone length/wide ratio (character 11).

The analysis of discriminating function for all three samples together showed that the most important in the distinguishing of individuals from particular populations were the length of seeds (character 7) (particular Wilks' lambda value of 0.55), the thickness of the last ramification shoot

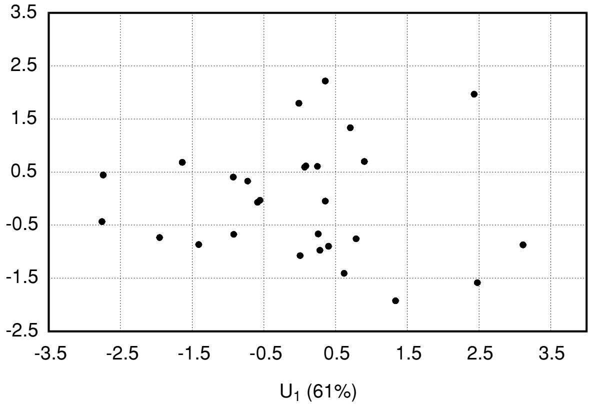

Fig. 5. Result of discriminant analysis based on 14 characters of cone and needle of *Juniperus phoenicea* subsp. *turbinata* from Matalascañas plotted along the two first discriminant variables U_1 and U_2, which accounted for 90% of the total variation.

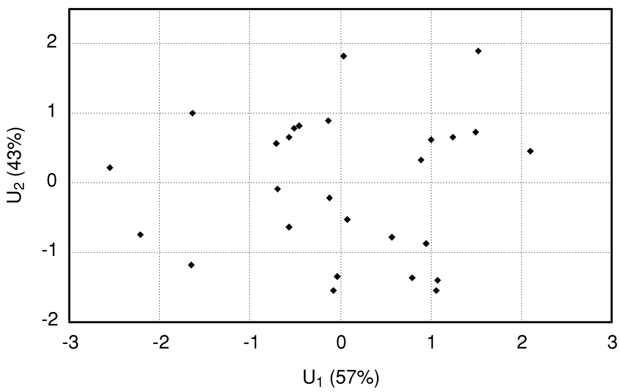

Fig. 6. Result of discriminant analysis based on 14 characters of cone and needle of *Juniperus phoenicea* subsp. *turbinata* from Cabo de Espichel plotted along the two first discriminant variables U_1 and U_2, which accounted for 100% of the total variation.

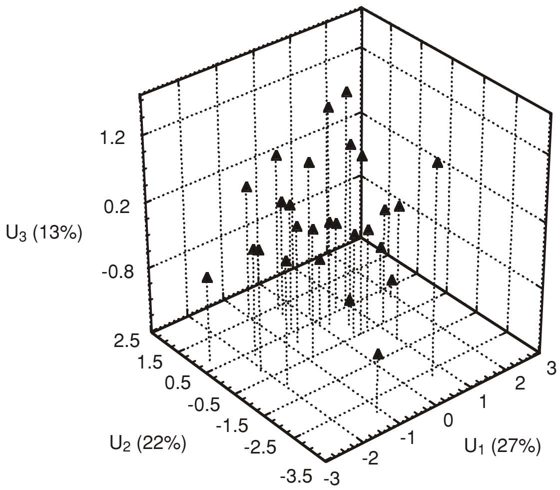

Fig. 7. Result of discriminant analysis based on 14 characters of cone and needle of *Juniperus phoenicea* subsp. *phoenicea* from Salto de Roldán plotted along the three first discriminant variables U_1, U_2, and U_3 which accounted for 62% of the total variation.

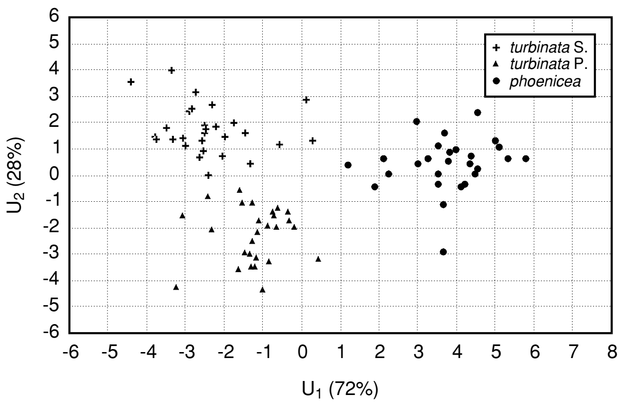

Fig. 8. Result of discriminant analysis based on 14 characters of cones and needles of the two populations of *Juniperus phoenicea* subsp. *turbinata* from Matalascañas (●) and Cabo de Espichel (◆) and 1 population of *Juniperus phoenicea* subsp. *phoenicea* from Salto de Roldán (▲) plotted along the two first discriminant variables U_1 and U_2, which accounted for 100% of the total variation (S – Spain, P – Portugal).

with leaves (character 10 with particular Wilks' lambda 0.67) and length of cone (character 2 with particular Wilks' lambda 0.68). All specimens sampled from particular populations form three distinguished groups (Fig. 8) in the space of two main variables responsible for 100% of whole variation. The first main variable (U_1) is determined mostly by characters 7, 6, 14, 11 and 2, and U_2 by characters 10, 2 and 6.

The population from Salto de Roldán, with *Juniperus phoenicea* subsp. *phoenicea*, is the most distinct one and forms a separate group. This population differs more strongly from the two others in the distance determined by the first main variable (U_1), responsible for 72% of variation. The populations of *J. phoenicea* subsp. *turbinata* are much more weakly differentiated, mostly in the distance determined by the second main variable (U_2) responsible for 28% of total variation. In all three populations single specimens are of intermediate character (Fig. 8).

The closest Euclidean distances between particular specimens agglomerate them in three main groups (Fig. 9). The first one consists of conglomeration of subgroups which include specimens from the two populations of *Juniperus phoenicea* subsp. *turbinata* with 4 specimens from subsp. *phoenicea*. The second group is divided into 2 large subgroups, the first one consists of individuals from the population from Salto de Roldán (*Juniperus phoenicea* subsp. *phoenicea*) with 2 specimens from the population from Matalascañas (*J. phoenicea* subsp. *turbinata*). The second subgroup includes specimens from Matalascañas and Cabo de Espichel (*J. phoenicea* subsp. *turbinata*). It can be concluded that the two populations collected as representing subsp. *turbinata* are heterogenic and consist of more or less distant groups of very closely related individuals. The specimens from the population from Salto de Roldán (*J. phoenicea* subsp. *phoenicea*) are more closely related and almost all form one large group.

The agglomeration on the basis of mean values for 3 analysed populations (Fig. 10) shows closer relations between those representing *J. phoenicea* subsp. *turbinata* than between *J. phoenicea* subsp. *phoenicea*.

DISCUSSION AND CONCLUSIONS

The biometrical analysis of three distant populations of *Juniperus phoenicea* from the Iberian Peninsula shows gre-

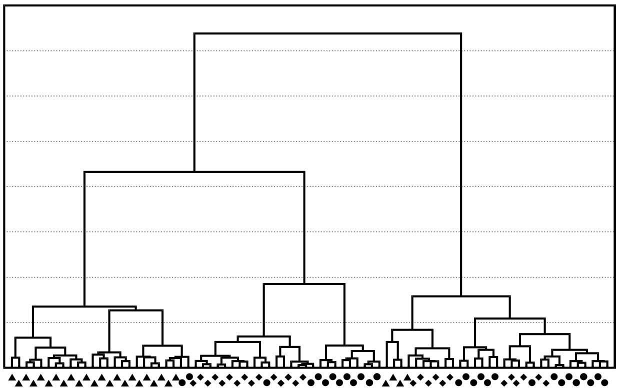

Fig. 9. Dendrograms of all individuals of *Juniperus phoenicea* subsp. *turbinata* from Matalascañas (●) and Cabo de Espichel (◆) and of *Juniperus phoenicea* subsp. *phoenicea* from Salto de Roldán (▲) constructed according to Ward Method.

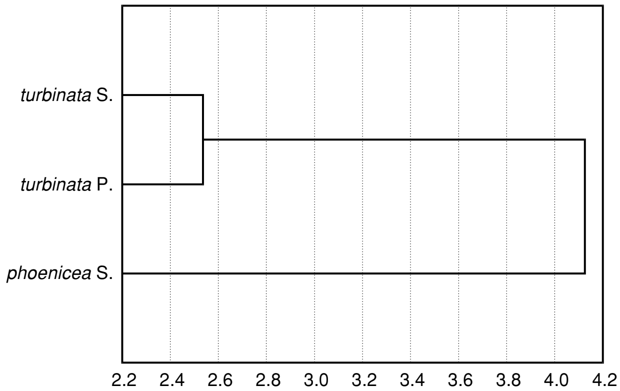

Fig. 10. Dendrograms of populations of *Juniperus phoenicea* subsp. *turbinata* and *Juniperus phoenicea* subsp. *phoenicea* constructed according to Single Linkage Method (S – Spain, P – Portugal).

at taxonomic distances among them. The differentiation done on the basis of the 10 measured and 4 additional calculated features is manifested in large Euclidean distances between all analysed populations. Two of them, representatives of *J. phoenicea* subsp. *turbinata*, are closer related each other than to the third, which represents *J. phoenicea* subsp. *phoenicea*. These results confirm the genetic differentiation of the taxons (Lewandowski et al. 2000) and also the biochemical and morphologic division of them (Lebreton and Thievend 1981; Lebreton 1983; Lebreton and Rivera 1989). Nevertheless, the distances between particular populations are so great that they more resemble the distances between species than between subspecies.

The great differences between particular populations are manifested by the majority of individuals representing them. Only single specimens from subsp. *turbinata* were found to be joined into the group of subsp. *phoenicea* (Fig. 9). Also only 4 individuals from the population of subsp. *phoenicea* fall into the group of subsp. *turbinata*. It shows a different origin or a long isolation of the two subspecies.

The differences between two populations of subsp. *turbinata* are also manifested on the high level (Fig. 10), however, many individuals from the two populations examined are of intermediate character. It can also be explained by isolation for a long time, but probably the same origin.

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ZMIENNOŚĆ MORFOLOGICZNA *JUNIPERUS PHOENICEA* (CUPRESSACEAE) Z TRZECH ODLEGŁYCH POPULACJI NA PÓŁWYSPIE IBERYJSKIM

STRESZCZENIE

Celem przedstawionych badań było porównanie cech biometrycznych trzech iberyjskich populacji *Juniperus phoenicea* reprezentowanych przez subsp. *turbinata* i subsp. *phoenicea*. Przebadano osiem cech szyszkojagód i nasion i dwie dotyczące pędów i liści.

Analiza biometryczna trzech populacji *J. phoenicea* wskazuje na istnienie znacznej odległości taksonomicznej między nimi. Dwie z nich, reprezentujące *J. phoenicea* subsp. *turbinata*, są bardziej zbliżone do siebie niż do trzeciej populacji, której osobniki należą do *J. phoenicea* subsp. *phoenicea*. Wyniki te potwierdzają nie tylko genetyczne zróżnicowanie tych taksonów, ale także ich biochemiczną i morfologiczną odrębność. Odległości pomiędzy poszczególnymi populacjami są tak duże, że bardziej przypominają relacje między gatunkami niż podgatunkami.

Znaczne różnice pomiędzy populacjami wykazuje większość osobników je tworzących. Jedynie pojedyncze egzemplarze subsp. *turbinata* zaliczono do grupy subsp. *phoenicea*. Tylko cztery osobniki z populacji subsp. *phoenicea* należą do taksonu subsp. *turbinata*. Wskazuje to na różne pochodzenie i długą izolację tych dwóch podgatunków.

Różnice między populacjami subsp. *turbinata* są istotne, jednakże wiele z przebadanych osobników z tych populacji ma charakter pośredni. Można to wyjaśnić w ten sposób, iż wprawdzie były one izolowane od dawna, ale jednocześnie mają wspólne pochodzenie.

SŁOWA KLUCZOWE: analiza statystyczna, biometria, jałowiec, szyszkojagoda, zróżnicowanie morfologiczne, zmienność.