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The taxonomic position of *Juniperus phoenicea* s.l. (Cupressaceae) from Andorra, on the basis of morphological characters

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Abstract

The isolated populations of *Juniperus phoenicea* found in mountain areas of the Pyrenees have traditionally been assigned to subsp. *phoenicea*. However, the populations occurring in the southern limits of the taxon in the Maghreb have been assigned with little precision both to subsp. *phoenicea* and subsp. *turbinata*. In this context subsp. *turbinata* has traditionally been considered as a taxon belonging to the plant communities found growing on coastal sands.

In order to verify the taxonomic status of the Pyrenean populations, and to better understand the distribution of subsp. turbinata, a biometric study based on morphological characteristics has been carried out. This enables us to clarify the taxonomic position of the Andorran populations, and to elucidate the taxonomy of the remaining Iberian populations situated in mountain and coastal regions, as well as the coastal populations from the Italian peninsula and the coastal and inland Moroccan populations.

The resulting conclusions indicate that the Andorran populations should be included within subsp. *phoenicea* along with the other inland Iberian populations. Subsp. *turbinata*, on the basis of the studied samples, colonises the southern and western Iberian regions. In Morocco, however, all the studied populations – both along the coast and in the Atlas mountains – correspond to subsp. *turbinata*.

The high degree of morphological variation of the Andorran populations suggests the relict character of those isolated Pyrenean valley stands.

The biogeographical interest of those fragmentary populations is commented on. They are plants that grow in the limits of the taxon's area, and should be included in the priority strategies for conservation of mountain biodiversity.

Keywords: biometry, conservation, dendrograms, Euclidean distances, relictic population.

La posición taxonómica de *Juniperus phoenicea* s.l. (Cupressaceae) de Andorra sobre la base de caracteres morfológicos

Resumen

Las poblaciones aisladas de *Juniperus phoenicea* situadas en zonas montañosas de la cordillera pirenaica han sido tradicionalmente asignadas a la subsp. *phoenicea*. En cambio las poblaciones que se encuentran el límite sur del taxon en el Magreb han sido asignadas de forma poco precisa a la subsp. *phoenicea* o a la subsp. *turbinata*. En este escenario tradicionalmente se ha considerado la subsp. *turbinata* como un taxon propio de los comunidades vegetales de los arenales marítimos costeros. Para verificar el estatus taxonómico de las poblaciones pirenaicas, y conocer la distribución de la subsp. *turbinata* se ha llevado a cabo un estudio biométrico en base a los diferentes caracteres morfológicos. Esto permite verificar la posición taxonómica de las poblaciones de Andorra. Con el fin de dilucidar la taxonomía del resto de poblaciones ibéricas, situadas en zonas de montaña y litorales, e indagar en la taxonomia de las poblaciones litorales de la península itálica y las magrebíes del litoral y de las zonas montañosas del Atlas, se han esudiado diferentes poblaciones con esos origenes.

Los resultados obtenidos indican que las poblaciones Andorranas deben incluirse en la subsp. *phoenicea* lo mismo que las otras poblaciones ibéricas de zonas de montaña. La subsp. *turbinata*, en base al muestreo realizado, coloniza las comunidades litorales ibéricas e itálicas. Además todas las poblaciones estudiadas de las comunidades litorales y de las montañas del Atlas magrebí de Marruecos pertenecen a la subsp. *turbinata*.

El alto rango de variación morfológica de las poblaciones andorranas sugiere un carácter relíctico de estas poblaciones aisladas en valles pirenaicos.

Se comenta el interés biogeográfico de estas poblaciones fragmentadas de plantas que se encuentran en límite de área y que deberían integrarse en las estrategias prioritarias de conservación de la biodiversidad en áreas de montaña.

Palabras clave: biometría, conservación, dendrogramas, distancias Euclídeas, población relíctica.

La position taxonomique de *Juniperus phoenicea* s.l. (Cupressaceae) d'Andorre sur la base de caractères morphologiques

Résumé

Les populations isolées de *Juniperus phoenicea* situées dans les zones de montagne des Pyrénées ont été traditionnellement attribuées à la subsp. *phoenicia*, à l'exception des populations à la limite sud de l'aire du taxon dans les pays du Maghreb, qui ont elles été attribuées aux subsp. *phoenicia* ou *turbinata*. *Juniperus phoenicea* subsp. *turbinata* a été traditionnellement considérée comme caractéristique des communautés végétales de sables maritimes côtiers. Afin de vérifier le statut taxonomique des populations pyrénéennes et de connaître la répartition de la subsp. *turbinata*, nous avons mené une étude biométrique sur la base de caractères morphologiques, incluant des populations d'Andorre et autres populations ibériques, ainsi que des populations d'Afrique du Nord côtières et des montagnes de l'Atlas.

Les résultats de cette étude indiquent que les populations andorranes ainsi que les populations ibériques orientales des zones montagneuses devraient être incluses dans la subsp. *phoenicea*, alors que les populations du sud et de l'occident ibérique correspondent à la subsp. *turbinata*. Toutes les populations maghrébines étudiées appartiennent à la subsp. *turbinata*.

Le haut degré de variation morphologique des populations andorranes suggère le caractère relique de ces populations isolées dans les vallées pyrénéennes.

Nous soulignons l'intérêt biogéographique de ces populations fragmentées en limite d'aire de distribution, qui devraient être intégrées dans les priorités stratégiques pour la conservation de la biodiversité en zones de montagne.

Mots-clés : biométrie, conservation, dendrogrammes, distances euclidiennes, population relique.

I. Introduction

Juniperus phoenicea L. is a Mediterranean species with its centre of occurrence in the western part of the region (Fig. 1) (Jalas & Suominen 1973). Two subspecies: subsp. *phoenicea* and subsp. *turbinata* (Guss.) Arcang. are recognized within it (Greuter *et al.* 1984, Amaral Franco 1986, Farjon 2005). In the northern limit of distribution of *J. phoenicea* it colonises the southern slopes of the pre-pyrenean range and some stations in the axial Pyrenees. The isolated populations from Andorra and other pyrenean populations have usually been assigned to *J. phoenicea* subsp. *phoenicea*. In Andorra, *J. phoenicea* s.l., (Carrillo *et al.* 2008) is known from a few localities in the southern part of the Principality, between (980) 1 100 and 1 350 m above sea level.

Conversely, the populations of the southern range of distribution on the Atlas mountains have been assigned to both taxa described within *J. phoenicea* (Quézel & Pesson 1980, Dobignard & Chatelain 2010). The aim of the present study was the comparison of the morphological characteristics of the population of *J. phoenicea* from the SE slopes above Sant Julià-de-Lòria with populations of *J. phoenicea* subsp. *phoenicea* and subsp. *turbinata* from other regions, in order to verify the taxonomic status of the Andorran population of the species, and its level of morphological variation.

J. phoenicea is a pioneer, light demanding, drought tolerant bush or small tree, and an easy coloniser of open areas by means of its ornithochorous fleshy cones (Amaral Franco 1986, Browicz & Zieliński 1982, Charco 2001, Quézel & Médail 2003). From the biogeographical point of view, the Andorran localities of the species can have either a relict character and conserve a rather high level of morphological variation, or result from the most recent colonisation and, in that case, can exhibit a low level of variation, as a bottleneck effect (Boratyński *et al.* 2009).

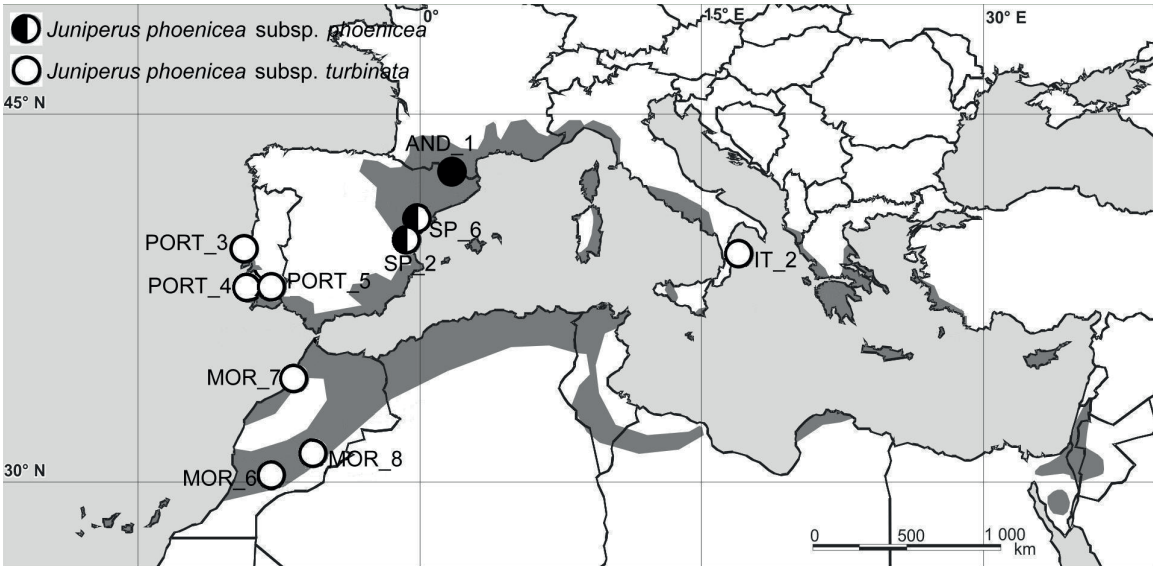

Fig. 1. Range of *Juniperus phoenicea* (after: Jalas and Suominen 1973; Quézel and Pesson 1980; Browicz & Zieliński 1982; Boratyński *et al.* 1992; Charco 2001; Farjon 2005 and own data) and location of studied populations (circles).

Acronymo	Locality	Longitude and latitude	Altitude [m]	Number of specimens
AND-1	Andorra, Coll de Jou above Sant Julià de Lòria	N 42o27'25" E 01o28'45"	1150	27
MOR-6	Morocco, High Atlas, AitLekak, near Tadmamt, to the N-E of Oukaimeden	N 31o15'45" W 07o50'00"	1600	30
MOR-7	Morocco, sandy coast near Kenitra	N 34o14'16" W 06o38'55"	20	30
MOR-8	Morocco, High Atlas, Agouti	N 31o38'20" W 06o29'35"	2000	29
PORT-3	Portugal, Peniche, to the S-E of Playa de Consolacão	N 39o19'40" W 09o21'32"	10	30
PORT-4	Portugal, Cabo de Espichel	N 38o24'48" W 09o12'33"	100-150	30
PORT-5	Portugal, 1 km to the S-E of Troya (Setubal)	N 38o26'55" W 08o50'25"	25	30
SP-2	Spain, Aragón (Maestrazgo), Teruel, Sierra de Nogueruela, to the E of Rubielos de Mora	N 40o10'38" W 00o37'17"	1100	30
SP-6	Spain, Sierra de Valdancha, Cintorres and Portella de Morella, to the S-W de Morella	N 40o33'15" W 00o14'48"	1100	30
IT-2	Italia, Cabo Rizutto, to the S of Crotone	N 38o53'55" E 17o05'54"	10	30

Table 1. Location of Andorran and compared populations of *Juniperus phoenicea.*

No	Measured characters	Mean ± SD Andorra	Mean ± SD subsp. *phoenicea*	t	p	Mean ± SD Andorra	Meean ± SD subsp. *turbinata*	t	p
1	Number of recta (4 o 6)	4,36±0,7723	4,56±0,9004	3,1693	0,002	4,36±0,7723	4,20±0,5938	-4,2029	0,000
2	Length of cone	7,95±0,8414	7,94±0,9742	-0,1522	0,879	7,95±0,8414	9,94±1,2118	26,2528	0,000
3	Diameter of cone	8,45±0,8570	8,68±1,0369	3,2733	0,001	8,45±0,8570	9,58±1,1481	15,7390	0,000
4	Cone scale number	9,29±1,4953	9,12±1,8879	-1,2724	0,204	9,29±1,4953	8,94±1,4513	-3,7382	0,000
5	Number of seeds	7,50±2,0162	8,09±2,2509	3,7125	0,000	7,50±2,0162	6,22±1,5715	-12,1391	0,000
6	Length of seed	4,16±0,3877	3,93±0,3904	-8,1388	0,000	4,16±0,3877	5,15±0,6745	23,5485	0,000
7	Width of seed	2,51±0,3221	2,23±0,2558	-13,8139	0,000	2,51±0,3221	2,72±0,4150	8,0480	0,000
8	Number of leaves per 5 mm section of ultimate lateral branchlet	25,39±3,6450	25,85±3,3698	1,8214	0,069	25,39±3,6450	23,29±3,6543	-8,8504	0,000
9	Thickness of the ultimate lateral branchlet and leaves	0,85±0,0915	0,76±0,1043	-12,3933	0,000	0,85±0,0915	0,76±0,1069	-13,3615	0,000
10	Ratio of cone length / diameter (2/3)	0,94±0,0607	0,91±0,0479	-7,1667	0,000	0,94±0,0607	1,04±0,1040	15,4053	0,000
11	Ratio of seed length / width (6/7)	1,68±0,2413	1,78±0,2023	6,0794	0,000	1,68±0,2413	1,92±0,2762	13,3009	0,000
12	Ratio of cone diameter / number de seeds (3/5)	1,20±0,3146	1,14±0,2792	-2,8615	0,004	1,20±0,3146	1,62±0,3747	17,5761	0,000
13	Ratio of cone diameter / seed width (3/7)	3,41±0,4789	3,93±0,5209	13,9931	0,000	3,41±0,4789	3,58±0,5606	4,7819	0,000
14	Ratio of thickness of branchlet / number of leaves (9/8)	0,03±0,0067	0,03±0,0065	-8,9120	0,000	0,03±0,0067	0,03±0,0076	-1,5600	0,119
15	Ratio of cone diameter / number of recta (3/1)	1,98±0,2992	1,95±0,3241	-1,0762	0,282	1,98±0,2992	2,31±0,3469	15,2868	0,000
16	Ratio of cone length / number of leaves (2/8)	0,32±0,0636	0,31±0,0588	-1,7470	0,081	0,32±0,0636	0,44±0,0977	19,4629	0,000
17	Ratio of cone scale number / cone length (4/2)	1,18±0,1973	1,15±0,2160	-1,4728	0,141	1,18±0,1973	0,91±0,1626	-24,8471	0,000

Table 2. Analyzed characters of galbuli (cones), seeds and shoots for 10 populations of *Juniperus phoenicea* and results of Student's t-test for mean values of investigated characters (1-17) between Andorran populations of *Juniperus phoenicea* (AND 1) compared with the average values of subsp. *phoenicea* (SP 2 and SP 6) and subsp. *turbinata* (MOR 6 - MOR 8, PORT 3 - PORT 5 Y 2) (SD = standard deviation, p = levels of significance, t = t-test result).

II. Material and methods

All the material for the study came from the ten populations of *J. phoenicea*, sampled in the Iberian peninsula in Europe and the Atlas Mountains in Africa, representing both subsp. *phoenicea* and subsp. *turbinata* (Table 1). The samples of cones and small pieces of lateral branches were gathered separately from each of about 27-30 individuals within each of 10 populations, from the sunny, mostly southern parts of their crowns at a height of 1.0-2.5 m. Each of the individuals was represented by 10 cones and branches (Mazur *et al.* 2003, Marcysiak *et al.* 2007, Mazur *et al.* 2012). Every population was described on the basis of five characters of the cones, three of the seeds, two of the shoots and leaves and eight synthetic, calculated as ratios of measured ones (Table 2).

III. Statistical treatment

The measurement and evaluation of the data were taken under the stereoscope microscope of 8 × magnification with a scaled ocular piece. Arithmetic means, standard deviations and variation coefficients were calculated for every feature. The interactions between particular characters were tested using the Pearson's liner correlation coefficient (Fig. 2). The discrimination analysis (Table 4) was performed and the position of the specimens was examined along with the first discriminant variables to find differences among the species. The dendrogram of the shortest Euclidean distances among the populations was constructed according to the Ward method to check their affinity. The multivariate analyses were conducted on the synthetic, recalculated characters and also the simple ones, not included in the previous analyses, using Statistica (StatSoft) software.

IV. Results

The greatest discriminating power among the compared populations was found in the thickness of the last ramification shoot with leaves (character 9). All specimens sampled from the 10 populations form two groups in the space of two main discriminant variables, which make up more than 75% of the whole variation (Fig. 2). The closest Euclidean distances agglomerated them also in two of the same main groups (Fig. 3). The two subspecies were recognized: subsp. *phoenicea* in a restricted area of the inland localities of northeastern Spain, and subsp. *turbinata*, broadly distributed across the coastal regions of Spain and Portugal in Europe, and coastal and mountain regions of Morocco in Africa. Surprisingly, the mountain populations from the High Atlas appeared similar to the coastal ones, representing subsp. *turbinata*.

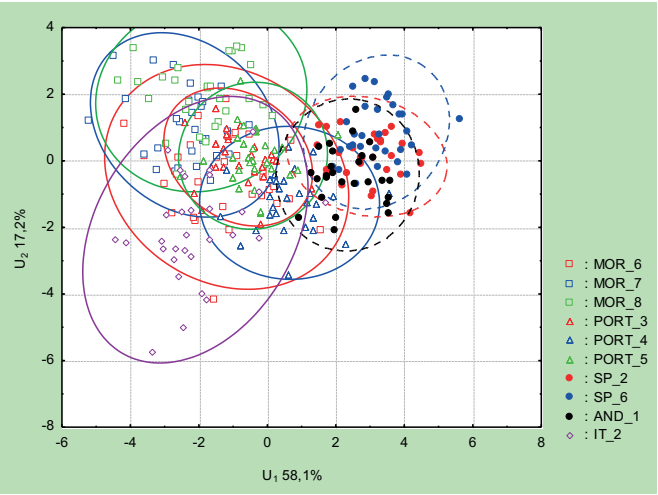

Fig. 2. Result of the discriminant analysis based on eight characters obtained from the ratio (characters 10-17) of individuals of *J. phoenicea*, on the plane of the two first discriminant variables, which accounted for 75,3% of the total variation.

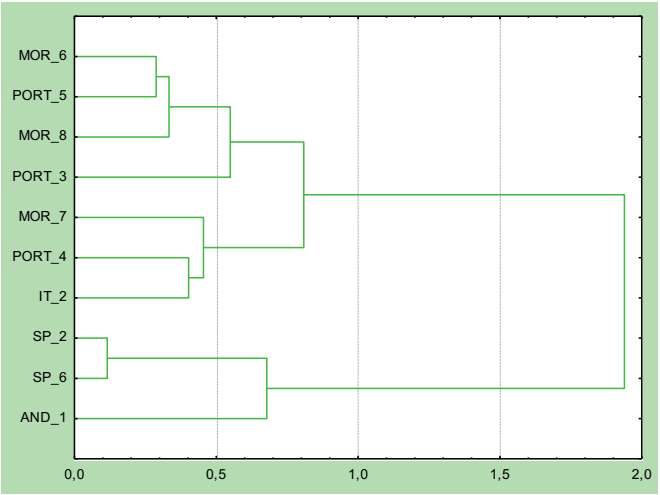

Fig. 3. Dendrograms constructed on the shortest Euclidean distances according to Ward's method, showing connections among 10 populations of *J. phoenicea.*

Character	1	2	3	4	5	6	7	8
2	-0,02							
3	0,12**	0,77**						
4	0,72**	0,16**	0,21**					
5	0,37**	0,06**	0,26**	0,40**				
6	-0,10**	0,75**	0,58**	-0,00	-0,14**			
7	-0,09**	0,51**	0,46**	0,02	-0,12**	0,63**		
8	0,08**	-0,32**	-0,13**	0,03	0,11**	-0,32**	-0,20**	
9	0,01	-0,13**	-0,09**	0,07**	0,03	-0,13**	-0,01	-0,12**

Table 3. Correlation coefficient between nine measured characters of *Juniperus phoenicea* (number of characters as in table 2, * = level of significance p = 0,05; ** = level of significance p = 0,01).

The Andorran population appeared correctly included within *J. phoenicea* subsp. *phoenicea*; however, many individuals revealed intermediate characters, resembling subsp. turbinata (Fig. 2). The morphological variation of *Juniperus phoenicea* subsp. *phoenicea* from Andorra, evaluated by comparison of variation coefficients of tested characters, revealed a similar level, as in other populations compared. As in other tested populations, the lowest level of variation was detected in the ratio of length to diameter of the cone. The correlations between many dimensional features were statistically significant at a level of p>0.01, with the highest connection of length and diameter of cones (Table 3).

V. Discussion

The differentiation in two groups between the analysed populations that represent *J. phoenicea* subsp. *phoenicea*, on the one hand, and *J. phoenicea* subsp. *turbinata* on the other, is congruent with recently published genetic studies (Boratyński *et al.* 2009, Adams *et al.* 2010) and the previous biometrical analyses (Mazur *et al.* 2003, 2010). At the same time, our study confirmed the taxonomic status of the Andorran population situated on the slopes above Sant-Julià-de-Lòria, which correspond to *J. phoenicea* subsp. *phoenicea*, and this, despite the fact that for some parameters the values given for branchlet thickness / number of leaves (character 14) approach subsp. *turbinata* rather than subsp. *Phoenicea*.

This could have been as a result of the population's adaptation to the high mountain conditions owing to the elimination of some of the more sensitive individuals. The same process could be responsible for the level of differentiation within the Andorran population of *J. phoenicea*. This heightened degree of morphological variation of the population situated on the slopes above Sant-Julià-de-Lòria (Mazur *et al.* 2010) in comparison with that of the other non-Andorran populations (Table 1) suggests, rather, a colonisation of an abundant population with several events of seed transport and the relict nature of the Andorran population rather than suggesting the result of a recent colonisation event. For the populations of *J. phoenicea* subsp. *phoenicea* in Andorra present biometrical and genetic peculiarities (Boratyński *et al.* 2009) and within them a high level of intrapopulational morphological differentiation can be found.

Genetically, the Andorran populations are more closely related to those of the south of France rather than those from the Iberian peninsula. Besides, and regarding the Iberian populations, they show a greater genetic affinity with those of the Iberian Range (Sistema Ibérico) than with those of the Ebro Depression (Boratyński *et al.* 2009). This scenario suggests that the Andorran populations have been subjected to a long period of isolation and a stronger connection with the populations situated in the surrounding mountain ranges rather than with the Ebro Valley populations.

This taxon, belonging as it does to a lineage of pre-mediterranean plants (Verdú *et al.* 2003), is pioneer, heliophile, stress-tolerant and a coloniser of open spaces, with ornithochoric dissemination of its galbuli or cones (Quézel & Médail 2003) and could have been present in the mediterranean region during the glacial periods towards the end of the Pleistocene (125-11,5 KyrBP) in various localities (Uzquiano & Arnanz 1997) in which it has remained since the conclusion of the Last Glacial Maximum (LGM) and the beginning of the Holocene (Carrión & Leroy 2010), above all in deforested areas subject to the only mildly continental climatic conditions (Carrión *et al.* 2003, 2004).

Bearing in mind the high degree of genetic and morphological variation, it is postulated that *J. phoenicea* survived the LGM (LGM: 18-21 ka BP) not far from the stations where the plant is at present known.

Although the Ebro Depression was a glacial refuge for Mediterranean trees during the Holocene and the early LGM (González-Sampériz *et al.* 2004, 2010), from this refuge the topography of the pre-Pyrenean ranges did not necessarily favour the latitudinal displacement towards the axial Pyrenees associated with the climatic variability of the Holocene of this dendrotaxon of pre-mediterranean origin (Verdú *et al.* 2003).

The genetic data available also support the difficulty of latitudinal displacement, since the Andorran population is genetically very different from the Ebro Valley one (Boratyński *et al.* 2009).

VI. Conclusions

The local area of these peculiar stands on the south-facing slope below the road from Sant Julià de Lòria to Fontaneda, above Riu Negre, deserves to be protected and conserved, considering its high level of morphological and genetic variability.

It can also be concluded that biometrical analyses can be considered a good tool, together with the relevant genetic and biogeographical studies, to distinguish the areas with persistent populations of relictic plants. It must be stressed

Character		Wilks' lambda	p
10	Ratio of cone length / diameter	0,65	0.000000
11	Ratio of seed length / width	0,74	0.000000
12	Ratio of cone diameter / number de seeds	0,65	0.000000
13	Ratio of cone diameter / seed width	0,55	0.000000
14	Ratio of thickness of branchlet / number of leaves	0,63	0.000000
15	Ratio of cone diameter / number of recta	0,75	0.000000
16	Ratio of cone length / number of leaves	0,68	0.000000
17	Ratio of cone scale number / cone length	0,81	0.000000

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

that the recognition of this type of plant population is very important for the proper development of strategies for biodiversity conservation in the mediterranean region.

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