

# Morphological differentiation supports the genetic pattern of the geographic structure of *Juniperus thurifera* (Cupressaceae)

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Received: 9 August 2012 / Accepted: 21 January 2013 / Published online: 17 February 2013  
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**Abstract** *Juniperus thurifera* is an important component of woodland communities of dry sites within the West Mediterranean region and is characterised by a strongly disjunctive geographic range. Two subspecies were recognised, subsp. *thurifera* in Europe and subsp. *africana* in Africa. The aim of the study was the comparison of phenetic diversity to the pattern of AFLP geographic differentiation of the species described in the literature. The examination of phenetic diversity was based on the biometrical analysis of 17 populations using 12 morphological characters of cone and seed. The differences among populations were analysed using Student's *t* test, analysis of discrimination, UPGMA agglomeration and hierarchical analysis of variance. The majority of morphological characters differentiated at a statistically significant level between populations and between *J. thurifera* subsp. *thurifera* and subsp. *africana*. Three groups of populations were detected using multivariate statistical analyses. The first, well separated, is subsp. *africana*, while the following two concern subsp. *thurifera*.

The morphological differentiation of populations appeared similar to that described on the AFLP. The Gibraltar Strait appeared to be the most important barrier.

**Keywords** Biometry · Geographic differentiation · *Juniperus thurifera* · Numerical taxonomy · Plant morphology · Plant variation

## Introduction

*Juniperus thurifera* L. is a tree-like juniper of the section *Sabina* (Mill.) Spach. (Farjon 1993, 2005; Adams 2011), occurring in the western Mediterranean region (Fig. 1), where it grows in the continental climate conditions of the mountain systems (Achhal et al. 1980; Fromard and Gauquelin 1993; Romo and Boratyński 2005; Gastón González 2006). The present range of *J. thurifera* covers several, more or less isolated, areas in the Iberian Peninsula, Pyrenees, Alps and Corsica in Europe; and the Middle, High and Anti-Atlas and Aurès in North Africa (Jalas and Suominen 1973; Barbero et al. 1988; Lathuillière 1994; Gamisans et al. 1994; Gauquelin et al. 1999; Quézel and Médail 2003; Farjon 2005; Romo and Boratyński 2005).

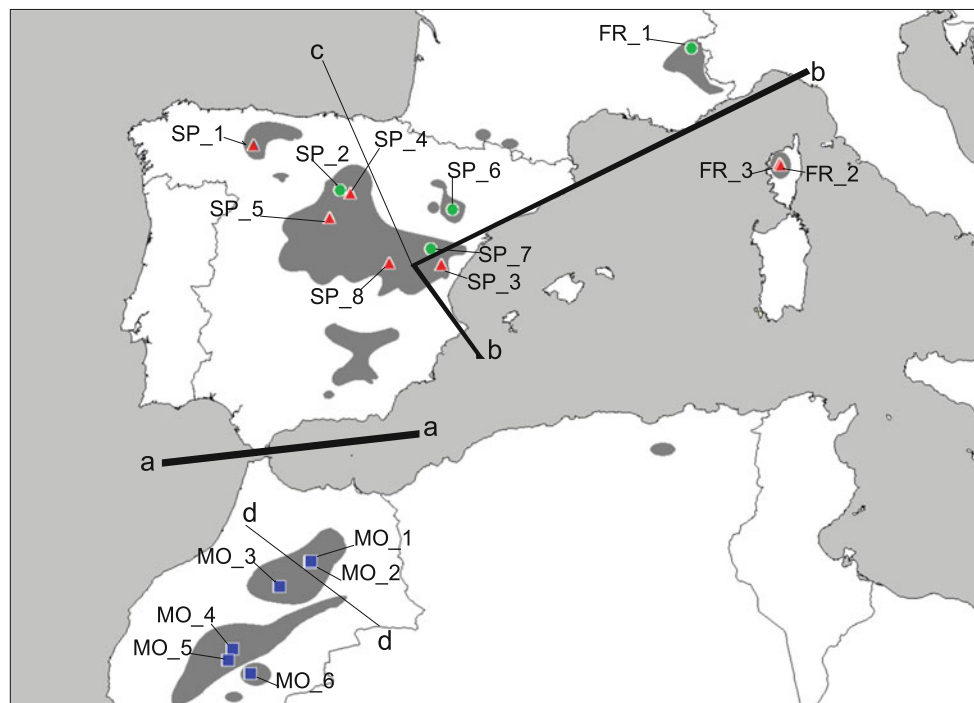
The genetic variation of *J. thurifera*, tested on the random amplified polymorphic DNA (RAPD) by Jiménez et al. (2003), suggests that the species migrated from the European mountain systems southward and westward towards Corsica, the Iberian Peninsula and then the Atlas Mountains in N Africa. The African populations, the most isolated from the others, appeared to be the most distant genetically and morphologically (Adams et al. 2003; Jiménez et al. 2003; Romo and Boratyński 2007; Terrab et al. 2008). The morphological differences between European and African individuals of *J. thurifera* were the

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**Fig. 1** Distribution of *Juniperus thurifera* (compiled from: Jalas and Suominen 1973; Romo and Boratyński 2005; Gastón González 2006) and location of sampled populations (acronyms as in Table 1): subsp. *thurifera*: filled triangle Iberian–Corsican group, filled circle Alpine–Iberian Group, filled square subsp. *africana*: morphological boundaries (a–d) obtained with Monmonier’s maximum difference

reason for the distinguishing of subsp. *africana* (Maire) Romo et Boratyński, which differs from *J. thurifera* subsp. *thurifera* by its smaller cone diameter, lower number of seeds per cone and shorter leaf scales (Romo and Boratyński 2007). Differences between subsp. *thurifera* and *africana* have also been found in the biochemical composition (Gauquelin et al. 1988, 1999; Lebreton 1990; Akkad et al. 2001) and genetic structure (Adams et al. 2001, 2003; Jiménez et al. 2003; Terrab et al. 2008). The latter study revealed strong differences between European and African populations of *J. thurifera*, and confirmed subsp. *africana* as a separate taxon. The analysis of European populations showed two different groups, the first including populations from the Alps, Pyrenees, northern part of the Iberian Peninsula and Corsica, and the second overlapping those from the central part of the Iberian Peninsula (Terrab et al. 2008). The biometrical examinations of four European populations (Mazur et al. 2005; Marcysiak et al. 2007) revealed the great differences among them, especially between one from the Alps and three from the Iberian Peninsula (Mazur et al. 2005). Taking into account the last result we hypothesised that *J. thurifera*’s phenetic diversity pattern should be similar to AFLP geographic differentiation and is mainly a consequence of the history of the species. *J. thurifera* is well adapted to the dry environments

algorithm of Euclidean distances among populations revealed by Barrier: a between African and European, b between Corsican and Iberian, c between Iberian and north-Iberian–Alpine, and d between north and southern Middle Atlas populations; the thickness of barriers is related to its importance

and occurs on the sites inaccessible or only weakly accessible for other trees (Quézel and Médail 2003). The adaptation by selection as an important source of phenotypic differentiation of that species cannot be excluded however, but probably had similar influence in various parts of its geographic range (Terrab et al. 2008).

The aim of the present study was (1) to determine morphological dissimilarities between populations by performing a biometrical comparison of morphological characters of female cones, seeds and shoots, and (2) to evaluate to what extent the multivariate biometrical analysis would resemble the pattern of variation and differentiation described by Jiménez et al. (2003) and Terrab et al. (2008) based on RAPDs and AFLP analyses, respectively.

## Materials and methods

### Plant material and measuring procedures

The material for the study was based on 17 populations of *J. thurifera* (Table 1), which cover almost the entire species area of natural distribution (Fig. 1). Populations were represented by 16–36 individuals (27 in average) except for MO\_02 (see Table 1), where only eight individuals have

been sampled due to lack of cones on more junipers in that locality. The 441 individuals were biometrically examined, each represented by 10 adult cones and 10 young shoots with leaves, all collected from the sunny part of the crown. However, a few individuals were represented by smaller numbers, because of lack of sufficient number of cones or destruction of shoots. The cone scales (CSN) were counted after cone soaking. The seeds were taken off the soaked cones and dried after measuring. The thickness of the ultimate lateral branchlet with leaves (ST) was measured following methods described by Marcysiak et al. (2007). The measurement was performed on dry material. The populations were compared on the basis of six characters of cones and seeds, two of shoots and leaves, and four proportions (Table 2).

### Data analysis

All data were standardised before analysis to avoid the possible influence of variation resulting from various types of

used characters. The STATISTICA (StatSoft) procedures were performed. The frequency distribution of measured character values was verified using the Shapiro–Wilk’s test to assess the symmetry and unimodality and, consequently, the possibility of conducting the multivariate statistical analyses (Sokal and Rohlf 1997; Zar 1999). The homoscedasticity of data was checked using Brown–Forsythe test as implemented by STATISTICA (StatSoft) to assess the possibility of use of parametric statistic tests (Sokal and Rohlf 1997).

The main statistics such as arithmetic means, standard deviations and variation coefficient were calculated for each population in order to determine the range of their variation. The interactions between characters were checked with Pearson’s correlation coefficient to detect possible redundant variables. A stepwise discrimination analysis was performed to: (1) definitively eliminate the possible redundant variables, (2) identify the discrimination power of particular characters, and (3) determine the intra- and inter-population variation (Tabachnik and Fidell 2007; Sokal and Rohlf 1997).

**Table 1** Studied populations of *Juniperus thurifera*; italicized (FR\_01 and SP\_02, 06 and 07) samples used in previous studies (Mazur et al. 2005; Marcysiak et al. 2007)

Taxon	Locality	Acronym	Number of specimens	Geographic coordinates	Altitude (m)
Subsp. <i>thurifera</i>	<i>France, Hautes Alpes, St. Crépin</i>	<i>FR_01</i>	36	<i>N44°56' E06°30'</i>	1500
	France, Corse, Niolo, Monte Cinto	FR_02	21	N42°22'05'' E8°57'47''	900
	France, Corse, Calacuccia, Golo Valley	FR_03	26	N42°20'31'' E9°00'11''	600
	Spain, León, Montes de la Luna	SP_01	31	N 42°47'01'' W 05°45'18''	1050
	<i>Spain, Santo Domingo de Silos</i>	<i>SP_02</i>	27	<i>N41°48' W03°20'</i>	1100
	Spain, Teruel, Sierra de Gúdar	SP_03	17	N40°08'17'' W00°30'32''	1000
	Spain, Soria, Sierra de Cabreja, slopes above Ucero	SP_04	30	N 41°43' W 03°03'	1150
	Spain, Segovia, Siguero, SW slopes above the Rio Duratón	SP_05	30	N 41°11' W 03°37'	1100
	<i>Spain, Zaragoza: Montes de la Retuerta de Pina, W of Bujaraloz</i>	<i>SP_06</i>	20	<i>N41°22' W00°12'</i>	400
	<i>Spain, Sierra de Noguerauelas, Rubielos de Mora</i>	<i>SP_07</i>	32	<i>N40°30' W00°48'</i>	900
Spain, Cuenca, Serranía de Cuenca, between La Toba and Buenache de la Sierra	SP_08	30	N 40°10' W 01°58'	1300	
Subsp. <i>africana</i>	Morocco, Middle Atlas, Jbel Bou Iblane, E of Talzemt,	MO_01	31	N 33°36' W 04°10'	2200
	Morocco, Middle Atlas, Jbel Bou Iblane, Pass on the road NE from Marmucha, E of Talzemt,	MO_02	8	N 33°38' W 04°09'	2400
	Morocco, Middle Atlas, Aguelmame Sidi-Ali	MO_03	16	N33°04'42'' W05°01'30''	2100
	Morocco, High Atlas, Jbel Azourki, below Tizi-n-Ilissi, SE slopes above Iglaouane	MO_04	34	N 31°42' W 06°20'	2400
	Morocco, High Atlas, Slopes above Tessaout (Toufrine)	MO_05	36	N 31°27' W 06°28'	2500
	Morocco, Anti Atlas, Jbel Sarhro, Jbel Taush S of Tiouit	MO_06	16	N31°08'54'' W 5°50'09''	2450

**Table 2** Average values, minima, maxima and variation coefficients of analysed characters of cones, seeds and sprouts with leaves of *Juniperus thurifera*

Acronym	Character	Subsp. <i>thurifera</i> (European populations)						C. subsp. <i>africana</i> (Moroccan populations)						p values of t and Kruskal–Wallis tests (for CSN, SN and SN/CSN) between pairs of groups			Discrimination power testing among populations				
		A Alpine–Iberian populations (FR_1, SP_2, SP_6, SP_7)			B Iberian–Corsican populations (FR_2, FR_3, SP_1, SP_3–5, SP_8)			N	Mean	Min.	Max.	V	A/B	A/C	B/C						
		N	Mean	Min.	Max.	V	N									Mean		Min.	Max.	V	Partial Wilk's $\lambda$
CL	Length of cone (mm)	1,150	8.07	5.2	11.8	10.97	1,850	8.11	5.6	13.7	11.26	1,399	7.42	5.0	10.0	12.42	0.223	0.000	0.000	0.698	0.000
CD	Diameter of cone (mm)	1,150	8.42	5.8	12.3	11.11	1,850	8.27	4.6	12.5	11.80	1,399	6.59	4.3	10.1	14.79	0.000	0.000	0.000	0.636	0.000
CSN	Cone scale number	1,150	8.01	6	12	12.51	1,850	6.07	4	8	4.12	1,399	5.49	2	8	16.40	0.000	0.000	0.000	0.765	0.000
SN	Number of seeds	1,144	3.71	1	8	31.81	1,842	3.22	1	10	38.22	1,386	1.33	1	4	41.42	0.000	0.000	0.000	0.796	0.000
SL	Length of seed (mm)	1,145	4.92	2.4	7.5	13.15	1,840	5.29	3.3	7.6	10.45	1,386	5.56	2.8	7.6	12.17	0.000	0.000	0.000	0.843	0.000
SW	Width of seed (mm)	1,145	3.67	1.5	6.1	18.99	1,838	3.91	1.3	6.8	15.83	1,386	4.75	2.1	7.6	16.86	0.000	0.000	0.000	0.972	0.742
LN	Number of leaves per 5 mm apical section of ultimate lateral branchlet	1,114	19.39	10	34	17.98	1,850	20.71	8	42	18.31	1,410	18.86	10	36	23.75	0.000	0.015	0.000	0.447	0.000
ST	Thickness of the last ramification shoot with leaves	1,114	0.82	0.4	1.4	12.86	1,844	0.69	0.3	1.2	15.36	1,410	0.68	0.4	1.0	14.77	0.000	0.000	0.091	0.595	0.000
CL/CD	Ratio of length of cone/diameter of cone	1,150	0.96	0.77	1.22	5.75	1,850	0.98	0.75	1.72	7.77	1,399	1.171	0.76	2.35	16.56	0.000	0.000	0.000	0.354	0.000
SL/SW	Ratio of length of seed/width of seed	1,145	1.37	0.11	3.13	16.75	1,836	1.38	0.65	3.38	15.49	1,386	1.192	0.69	2.12	14.47	0.183	0.000	0.000	0.941	0.061
CD/SN	Ratio of diameter of cone/number of seeds	1,144	2.53	0.9	8.45	35.42	1,842	3.07	0.88	9.81	46.64	1,386	5.54	1.53	9.75	30.07	0.000	0.000	0.000	0.819	0.000
SN/CSN	Ratio of seed number/cone scale number	1,144	0.47	0.13	1.00	32.47	1,842	0.53	0.13	1.67	35.18	1,386	0.25	0.125	1.00	43.09	0.000	0.000	0.000	0.818	0.000

A, B and C indicate the three groups of populations, as distinguished in the analysis of discrimination and agglomeration. The last two columns show the level of differences between pairwise comparisons of the three population groups assumed and the discrimination power test, respectively. Values in italics indicate the mean values of characters of distinguished groups of populations. N number of measurements for particular character; owing to the lower than 10 sprouts, cones and/or seeds for some number of individuals in analysed populations, the real number of measurements were somewhat smaller than the assumed; Min. minimal value of character, Max. maximal value of character, V variation coefficient, p level of statistic significance of differences between mean values of characters

The significance of differences between mathematic means of characters for two groups of populations of subsp. *thurifera* and populations of subspecies *africana* were verified using *t* test for independent samples. The Kruskal–Wallis test for the traits with biased distribution was performed to attest the statistical significance of the differences (Sokal and Rohlf 1997; Zar 1999).

Dendrograms of the Euclidean distances of nearest neighbourhood among populations were constructed according to Ward's method, to verify their affinities and taxonomic relations, revealed in discrimination analysis (Sokal and Rohlf 1997). Dendrograms on the Mahalanobis distances was analysed to examine the similarities among populations and taxa (Sokal and Rohlf 1997). STATISTICA software (StatSoft) was used in all the above-mentioned analyses.

To evaluate the correlation between geographic and morphological multicharacter differences among populations, a Mantel test (Mantel 1967) was performed on the matrices of Euclidean and Mahalanobis distances and the geographic distances (Manni et al. 2004), using GenAIEX software. Geographic distances were counted on the geographic coordinates using Map Info 9.5 (Pitney Bowes). To define the barriers within the network of populations, the Monmonier's algorithm applied on a Delanaunay triangulation was used, as implemented by software BARRIER 2.2 (Manni et al. 2004).

The hierarchical analysis of variance was performed to determine which characters differentiated between subspecies and between three groups of populations detected by the discrimination analysis (Sokal and Rohlf 1997), as implemented by JMP software (SAS Institute).

## Results

### Variation and correlation of characters

The characters examined, except for CSN and SN, have a unimodal and normal or very close to normal frequency distribution, which enables us to use multivariate statistical methods of comparison and further analyses. The CSN and SN data were transformed into % frequencies and arcsined before the further, multivariate analyses.

The original data of all characters except CL, CD and CL/CD have a non homoscedastic character, which prevented direct utilisation of parametric analyses, but after standardisation have a homogenous level of variation.

The average values of characters were specific for every population at some degree, but with frequency distributions overlapping in groups of populations (Table 2). The values of variation coefficient of the measured characters differed slightly among three groups of populations, while SN was

very variable, showing high differences (Table 2). The most stable appeared to be CL and SL.

Correlations between measured characters were slightly different for each sample of *J. thurifera*, but generally, the dimensional characters of cones and seeds (CL, CD, SL and SW) positively correlate to each other at a statistically significant level ( $p \leq 0.01$ ) in all samples. The SN also positively correlated to CL and CD ( $p \leq 0.01$ ). The characters of cones and seeds are correlated at a much lower level with characters of needles and branchlets (LN and ST).

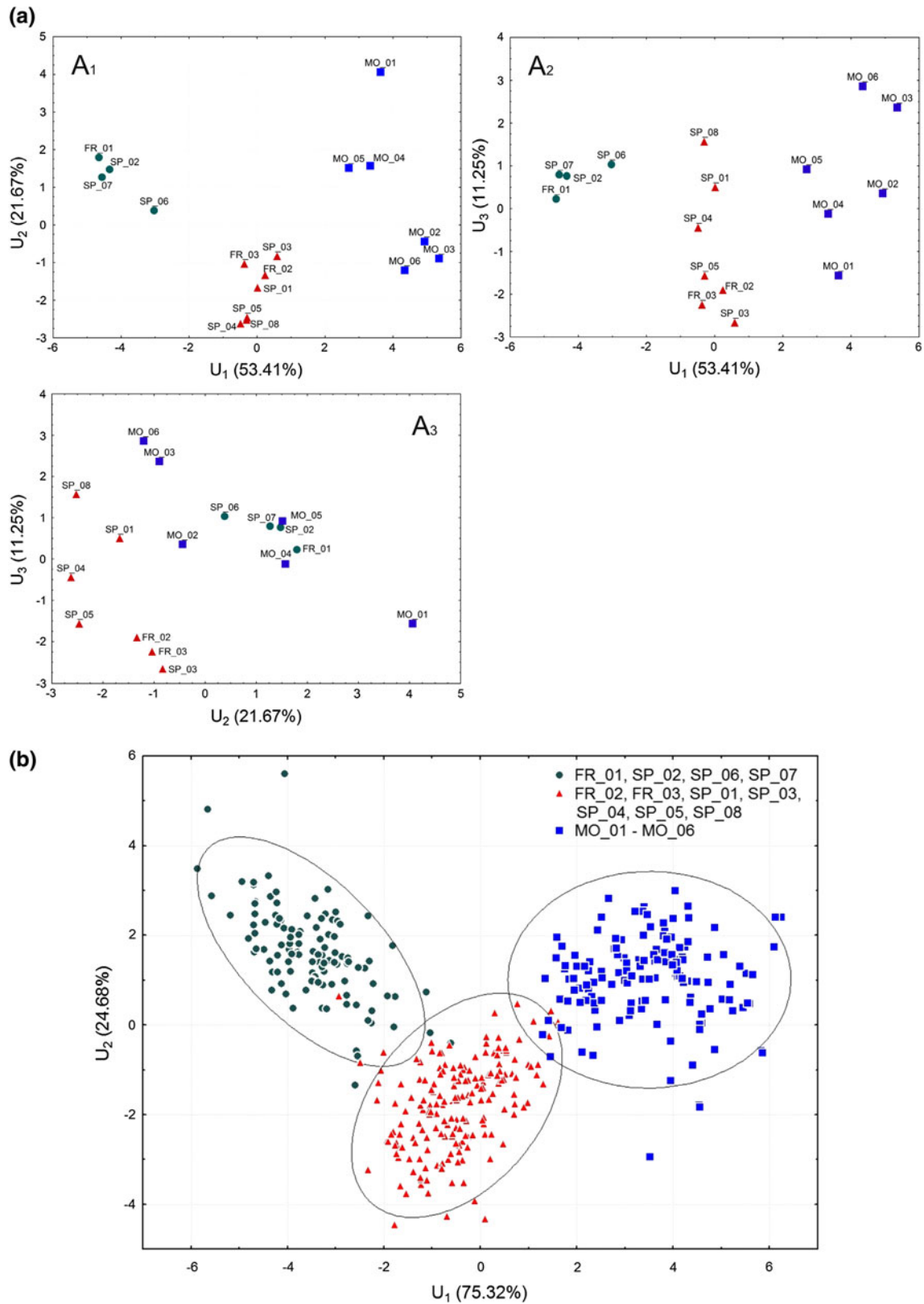
### Multivariate differentiation of populations

All characters except those of SW and SL/SW had a statistically significant value for discrimination among populations of *J. thurifera* (Table 2). The greatest discriminating power had the ratio of CL/CD and LN, with a partial Wilks lambda values of about 0.35 and 0.45, respectively (Table 2). The first three discrimination variables covered 86.3 % of the total variation. On the plane of the two first discrimination variables  $U_1$  and  $U_2$ , responsible for about 79 % of the variation, compared populations formed three well-separated agglomerations (Fig. 2A<sub>1</sub>), visible also on the plane between  $U_1$  and  $U_3$  (Fig. 2A<sub>2</sub>). The populations from the Middle, High and Anti-Atlas in Africa form the most distinct group, determined by the first variable, which in the greatest degree depends on CSN, SN, ratio CD/SN and CL/CD. The African populations, however, did not form a compact group, but were differentiated along the variables  $U_2$  and  $U_3$  (Fig. 2A<sub>3</sub>).

The two remaining groups of populations are also determined in the first place by  $U_1$ , and one of them has a compact, the other a dispersed character influenced by  $U_2$  and  $U_3$  (Fig. 2A<sub>3</sub>). The isolation of four Alpine–Iberian populations (FR\_01, SP\_02, SP\_06 and SP\_07), which form a second group on the discrimination scatter plot, is very high. The third group is made up of Iberian–Corsican populations (FR\_02, FR\_03, SP\_01, SP\_03–05 and SP\_08) and occupies the position between the Moroccan and Alpine–Iberian populations (Fig. 2A<sub>1</sub>). These two groups differ from each other at a statistically significant level in all respects of except for the CL and SL/SW. Also, both groups of populations representing subsp. *thurifera* differed from subsp. *africana* statistically significantly as regards of the whole set of characters except ST, which in Iberian–Corsican populations and African populations was almost the same (Table 2). The Iberian–Corsican populations have mean values of characters predominantly intermediate between subsp. *africana* and Alpine–Iberian populations (e.g. characters CD, CSN, SL, SW).

The individuals of each particular group of populations hardly penetrated others (Fig. 2b). The proper ordination of individuals into particular populations was nearly 65 % on





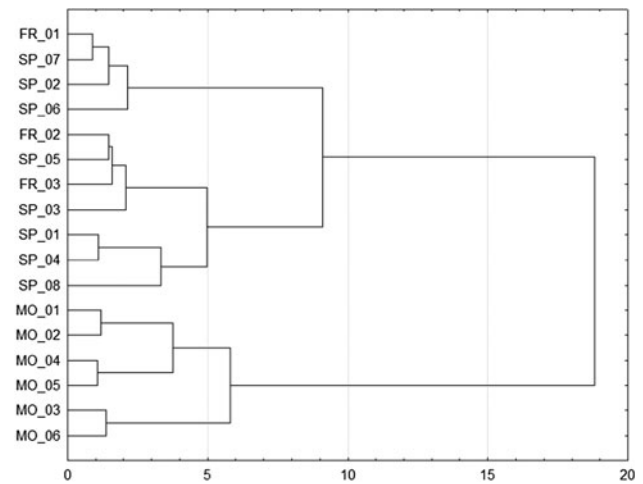
**Fig. 2** Results of discrimination analysis for *Juniperus thurifera*: **a** according to the discrimination variables  $U_1$ ,  $U_2$ ,  $U_3$  for populations; **b** for individuals according to discrimination variables  $U_1$  and  $U_2$  (acronyms of populations as in Table 1)

average, and varied from 37.5 % in population SP\_07 to 88.2 % in SP\_02. Ordination to each of the three distinguished groups of populations, however, has generally a much higher success rate: 99.3 % for Moroccan, 98.9 % for the second (FR\_01, SP\_02, SP\_06 and SP\_07) and 97.8 % for the third (FR\_02, FR\_03, SP\_01, SP\_03–05 and SP\_08). Such a high level of the fitness of ordination of individuals to the three distinguished groups of populations indicates the very high probability of proper inclusion of practically every single individual (Fig. 2b). The distribution of individuals on the plane of the two first discrimination variables, which account for 100 % of variation, shows that the first of them distinguishes completely the Alpine–Iberian group from all the others. The Central Iberian and Corsican group are distinguished by both discrimination variables; however, the first is responsible for a three times higher percentage of the total variation. The first discrimination variable relies on the full set of variables, but mostly from CSN, SN, CD/SN, CD, SN/CSN, and the second discrimination variable correlated additionally with ST.

On the graph showing results of the cluster analysis on the basis of the closest Euclidean distances, the populations also formed three separate groups. Those separated at the highest level are from Africa, then two subgroups are formed in an identical way to that of the discrimination analysis (Fig. 3). The shortest Mahalanobis distances connected samples directly at a similar level to that found in the cluster on the Euclidean distances and discrimination analyses (data not shown).

#### Differences between subspecies and populations in particular characters

The mean values of characters calculated separately from data of European and African individuals, comprehended as representing *J. thurifera* subsp. *thurifera* and subsp. *africana*, respectively, all differed at a statistically significant level (Table 2). In spite of that, the ranges of distribution of particular character values of subsp. *thurifera* overlapped those of subsp. *africana* at least partly (Table 2). Even SN, attaining an average value in the Alpine–Iberian populations of 3.71, the Iberian–Corsican 3.22 and in the African ones 1.33, ranged from 1 to 10 and 1 to 4, in Europe and Africa, respectively. Regardless, every African population (*J. thurifera* subsp. *africana*) differed from every other one in the mean values of SN as a result of the Tukey's and Kruskal–Wallis' tests among groups of populations. The next character, which differed European from most of the African populations, was SW. The European populations have smaller seeds, with SW ranging between 1.3 and 6.8 mm, 3.67 mm on average of Alpine–Iberian and 3.91 mm of Iberian–Corsican



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

populations, while African between 2.1 and 7.6 mm, 4.75 mm on average. Most African populations also differed from European ones by having smaller cones (CL and CD). The northernmost Moroccan ones (MO 1 and MO 2) showed a smaller number of statistically significant differences than observed between other African and European populations (Table 3).

#### Geographic pattern of variation

The results of the Mantel test conducted on the Euclidean and the Mahalanobis distances among populations indicated statistically significant relationships to the geographic spatial distance. Correlations for Euclidean and Mahalanobis distance are  $r = 0.523$ ,  $p < 0.001$  and  $r = 0.411$ ,  $p < 0.001$ , respectively. The geographic discontinuities among populations detected in the Barrier 2.2 analysis indicated the high importance of the Gibraltar Straight on species differentiation (Fig. 1a). The second and third barriers separate between the Alpine and NE Iberian populations (FR\_1, SP\_6 and SP\_7), Corsican and E Iberian (FR\_2, FR\_3 and SP\_3) and all the other Iberian ones (Fig. 1b, c). The fourth barrier separates the two northernmost populations sampled in the Middle Atlas from the rest of the analysed subsp. *africana* populations (Fig. 1d).

The result of the hierarchical analysis of variance showed that the African populations differed from the European at a statistically significant level as regards most characters, as likewise between three groups of populations detected during analyses of discrimination and agglomeration on the Euclidean distances (Table 4). The only exceptions are LN in both cases and ST between European and African populations.

**Table 3** Numbers of characters differed between pairs of populations of *Juniperus thurifera* in the Tukey's *t* test: straight numbers at  $p \leq 0.01$ , italicized at  $p \leq 0.05$ ; bold values are crossing values from subsp. *africana* and subsp. *thurifera*

	FR_01	FR_02	FR_03	SP_01	SP_02	SP_03	SP_04	SP_05	SP_06	SP_07	SP_08	MO_01	MO_02	MO_03	MO_04	MO_05
FR_02	2, 3															
FR_03	2, 3															
SP_01	6, 6	1, 2	3, 4													
SP_02	1, 1	3, 3	3, 4	5, 5												
SP_03	2, 3			2, 4	3, 1											
SP_04	4, 4	1, 2	1, 1	3, 3	4, 4	1										
SP_05	3, 4	1, 1	1, 1	4, 4	3, 3	1, 2	1, 1									
SP_06	3, 3	3, 3	3, 3	3, 4	1, 1	2, 3	2, 3	3, 3								
SP_07		3, 4	3, 3	6, 7		4, 5	4, 4	4, 4	1, 1							
SP_08	4, 4	2, 2	4, 4	2, 3	4, 2	3, 4	4, 5	4, 4	7, 7	6, 7						
MO_01	<b>10, 10</b>	<b>8, 9</b>	<b>8, 8</b>	<b>9, 10</b>	<b>11, 11</b>	7, 7	<b>8, 8</b>	<b>9, 9</b>	<b>10, 10</b>	<b>10, 10</b>	<b>9, 10</b>					
MO_02	7, 7	<b>5, 5</b>	<b>6, 7</b>	<b>4, 6</b>	<b>7, 8</b>	7, 7	<b>6, 7</b>	<b>6, 6</b>	<b>9, 9</b>	<b>7, 8</b>	<b>7, 9</b>	2, 2				
MO_03	<b>9, 10</b>	<b>8, 8</b>	<b>9, 11</b>	7, 8	<b>10, 10</b>	9, 9	<b>9, 10</b>	<b>10, 10</b>	<b>9, 9</b>	<b>11, 11</b>	<b>8, 8</b>	5, 5	1, 2			
MO_04	<b>11, 11</b>	<b>5, 5</b>	<b>8, 9</b>	<b>8, 9</b>	<b>10, 11</b>	7, 8	<b>9, 11</b>	<b>9, 9</b>	<b>11, 11</b>	<b>11, 11</b>	<b>9, 9</b>	3, 4	1, 1	2, 2		
MO_05	<b>10, 10</b>	<b>6, 8</b>	<b>7, 7</b>	7, 7	<b>9, 10</b>	7, 8	<b>8, 8</b>	<b>8, 8</b>	<b>11, 11</b>	<b>11, 11</b>	<b>9, 9</b>	2, 3	1, 1	2, 2	2, 2	
MO_06	<b>9, 9</b>	<b>8, 8</b>	<b>9, 9</b>	<b>6, 6</b>	<b>8, 9</b>	<b>8, 8</b>	<b>10, 10</b>	<b>9, 10</b>	<b>6, 8</b>	<b>10, 11</b>	<b>6, 6</b>	5, 6	1, 1	1, 1	4, 4	2, 2

## Discussion

### Evaluation of characters

Average values of particular characters of cone, seed and sprout with leaves of *J. thurifera* found during study is generally similar to the data reported earlier (do Amaral Franco 1986; Marcysiak et al. 2007; Romo and Boratyński 2007), but reveal broader ranges of distribution. The marginal values of particular characters found in our study, taking into account the numbers of tested individuals can, with high probability, be treated as real minima and maxima, while data published earlier resulted from only a few measurements on the herbarium materials (do Amaral Franco 1986) or only few characters (Barbero et al. 1994). Such kind of data was also collected and compared for *J. thurifera* subsp. *thurifera* and subsp. *africana* (Romo and Boratyński 2007).

The CL, CD and SN have been recognised as differentiating between *J. thurifera* subsp. *thurifera* and subsp. *africana* (Gauquelin et al. 1988; Lebreton 1990; Romo and Boratyński 2007). We confirmed this, stressing a very high probability of correct distinguishing of herbarium materials of subsp. *thurifera* from subsp. *africana* using only CD and SN.

The leaf-scale length, which attains about 2.0–2.5 mm and 1.0–1.5 mm in subsp. *thurifera* and subsp. *africana*, respectively, was also recognised as differentiating between them (Romo and Boratyński 2007). However, the latter difference did not correspond well with LN, as found in our study. The average values of LN for subsp. *thurifera* and subsp. *africana* were 20.21 and 18.86, respectively. The leaves of *J. thurifera* are decussate and form four rows, 5.01 and 4.71 leaves of a particular row 5 mm long on the sprout of subsp. *thurifera* and *africana*, respectively. Consequently, visible parts of the leaves are 1.01 and 0.94 mm for the apical 5 mm of lateral branchlets of subsp. *thurifera* and subsp. *africana*, respectively. These data confirm differences between subspecies but at a much smaller level than shown by Romo and Boratyński (2007) on the basis of data from Flora Iberica for subsp. *thurifera* (do Amaral Franco 1986). It can result from measurements of different parts of sprouts. We tested only apical parts of the lateral branchlets, while do Amaral Franco (1986) in all probability used other parts of the sprouts, which have larger leaves. The other analysed characters also differentiated significantly between subsp. *thurifera* and subsp. *africana* (Table 2).

### Geographic structure of morphological differentiation

Morphological differentiation of *J. thurifera* coincides with Terrab et al. (2008) suggestion, that the Strait of Gibraltar functioned as the principal barrier between European and



**Table 4** Analysis of hierarchical variance between *Juniperus thurifera* groups of populations for morphological characters (acronyms as in Table 2) data for characters differentiating groups at a statistically significant level only

Character	Component	df	Sum of squares	Variance component	Total variance (%)	p
Subsp. <i>thurifera</i> (European populations) versus subsp. <i>africana</i> (African populations)						
CL	Between groups	1	522.805	0.329	26.978	0.0002
	Between populations	15	421.587	0.092	7.563	<0.0001
	Within populations	424	1,945.480	0.422	34.566	0.0000
CD	Between groups	1	2,080.890	1.350	56.774	<0.0001
	Between populations	15	584.860	0.133	5.583	<0.0001
	Within populations	424	2,196.390	0.477	20.075	0.0000
CSN	Between groups	1	1,811.670	1.082	45.279	0.0051
	Between populations	15	3,298.440	0.855	35.783	<0.0001
	Within populations	424	855.381	0.174	7.297	<0.0001
SN	Between groups	1	3,200.400	2.121	63.759	<0.0001
	Between populations	15	423.273	0.095	2.848	<0.0001
	Within populations	424	1,809.730	0.354	10.648	<0.0001
SL	Between groups	1	130.021	0.078	14.962	0.0057
	Between populations	15	243.128	0.058	11.000	<0.0001
	Within populations	424	703.010	0.143	27.249	<0.0001
SW	Between groups	1	687.673	0.451	45.684	<0.0001
	Between populations	15	235.605	0.054	5.469	<0.0001
	Within populations	424	880.814	0.179	18.153	<0.0001
Between three groups <i>Juniperus thurifera</i> populations detected in discrimination analysis						
CL	Between groups	2	523.212	0.186	17.127	0.0024
	Between populations	14	420.241	0.101	9.285	<0.0001
	Within populations	424	1,945.480	0.422	38.858	0.0000
CD	Between groups	2	2,100.430	0.806	43.754	<0.0001
	Between populations	14	569.692	0.141	7.646	<0.0001
	Within populations	424	2,196.390	0.477	25.919	0.0000
CSN	Between groups	2	4,314.550	1.685	73.160	<0.0001
	Between populations	14	613.423	0.166	7.194	<0.0001
	Within populations	424	855.381	0.174	7.570	<0.0001
SN	Between groups	2	3,373.840	1.340	53.460	<0.0001
	Between populations	14	255.871	0.056	2.235	<0.0001
	Within populations	424	1,809.730	0.354	14.128	<0.0001
SL	Between groups	2	225.805	0.083	16.338	0.0011
	Between populations	14	149.435	0.036	7.121	<0.0001
	Within populations	424	703.010	0.143	28.170	<0.0001
SW	Between groups	2	731.353	0.283	34.851	<0.0001
	Between populations	14	195.338	0.047	5.844	<0.0001
	Within populations	424	880.814	0.179	22.039	<0.0001
ST	Between groups	2	13.764	0.005	29.297	0.0004
	Between populations	14	7.285	0.002	11.046	<0.0001
	Within populations	424	13.052	0.002	12.713	<0.0001

African populations of the species. These authors pointed out a lack of gene flow between Moroccan and Iberian populations after glacial periods (Terrab et al. 2008). Our result is generally in accordance with the separate

taxonomic position of the Moroccan populations (Gauquelin et al. 1988; Barbero et al. 1994; Adams et al. 2003; Jiménez et al. 2003; Romo and Boratyński 2005), treated as subspecies *africana* (Romo and Boratyński 2007) or

ultimately proposed as *maroccana* (till now *nomen nudum*) (Vela and Gauquelin 2011). The more recently published results of the studies on the genetic differentiation of conifers across the Strait of Gibraltar confirmed a high level of differences (Terrab et al. 2007; Jaramillo-Correa et al. 2010; Dzialuk et al. 2011), however, some taxa did not follow this rule (Jaramillo-Correa et al. 2010). Species other than coniferous plants also revealed a high level of genetic differences between European and North African populations (Lumaret et al. 2002; Ortiz et al. 2007), but generally several patterns of species differentiation and gene flow across the Strait of Gibraltar have been described and both high and relatively low levels of differences were reported (Rodríguez-Sánchez et al. 2008). The significant differences between European and Moroccan populations of *J. thurifera*, found during our study in the majority of tested morphological characteristics, indicated that patterns of genetic and morphological variation of this species are convergent.

The biometrical differentiation of European populations of *J. thurifera* into two groups with an almost majority of well-distinguished individuals (Fig. 2b) only partly follows the pattern of genetic variation revealed by AFLP analysis (Terrab et al. 2008). It can be explained by the origin of part of our material from other populations and other individuals than those used by Terrab et al. (2008). In spite of this, we have to point out some similarities between genetic and morphological patterns of differentiation between populations representing the same regions. The first of them is the different multivariate character of populations from Corsica and the Iberian Peninsula found by Terrab et al. (l.c.) and observed in our study (compare our Figs. 1, 2a, 3 with Terrab et al. 2008: Figs. 1, 2, 3). Terrab et al. (2008) explained this type of connection between populations as resulting from the early Holocene migration from the Iberian Peninsula to Corsica, similarly to the case of several other plant species. The rejection of an origin of the Corsican population from much more remote times, when the microplate of contemporary Corsica and Sardinia separated from the Iberian microplate in the early Oligocene (Bellon et al. 1977; Rosenbaum et al. 2002) is supported by the low divergence between Corsican and Iberian populations. The divergence time of *J. thurifera* determined as before 10 Mya (Mao et al. 2010) also indicates the most recent migration between the Iberian Peninsula and Corsica.

The second example of similarity of our results and AFLP differentiation concerned Alpine and north Iberian populations. Unfortunately, we examined only one population from the Alps, but its biometric characteristics placed it into the clade of north- and central-Iberian populations (Fig. 3).

The reasons for detected morphological differentiation are, as also in the case of genetic variation, the history and isolation of particular populations in the fragmented habitats (Saunders et al. 1991; Lindenmayer and Fischer 2006). The success of gene flow between *J. thurifera* stands by pollen and seed was strongly reduced with increased distance (Rhanem 2010; Olano et al. 2012). The fragmentation of habitats suitable for *J. thurifera* (Gauquelin et al. 2003; Romo and Boratyński 2005; Gastón González 2006; DeSoto et al. 2010; Rhanem 2010; Olano et al. 2012) has altered not only genetic structure, but also phenotypic variation of the species (Santos et al. 1999; compare e.g. Douaihy et al. 2011, 2012). The pattern of morphological differentiation of compared populations can be interpreted as support for the hypothesis of migration of the species (or its ancestor) from the North and colonisation of the Iberian Peninsula and Africa during Miocene climate cooling (Barbero et al. 1994; Jiménez et al. 2003). However, the hypothesis that Iberian populations were divided into two morphologically different types, which then settled in the Alps and Corsica (Terrab et al. 2008) cannot be decisively excluded without more detailed investigation.

## Conclusion

The morphological polymorphism of the compared populations of *J. thurifera* supports the subspecific taxonomic position of Moroccan populations of the species. The geographic differentiation of the morphological characteristics resembles those received with the AFLP and RAPDs (Jiménez et al. 2003; Terrab et al. 2008). The most eminent differences between European populations from the Western Alps and the Ebro Basin with its northern peripheries on the one side, and from Corsica and the central part of the Iberian Peninsula, on the other side, support another geographic origin, suggested by Terrab et al. (2008). The strong geographic differentiation between the two continents and among populations within Europe and Africa suggest rather local management of the *J. thurifera* woodlands, without seed exchange between the separated parts of the species' geographic range.

**Acknowledgments** We would like to thank Amelia Lewandowska for technical help, Samuel Pyke and Proof-Reading Service for linguistic verification of the previous version of the manuscript. Funding the work was partly sponsored by the Institute of Dendrology of the Polish Academy of Sciences. The collection of material was made possible due to the bilateral cooperation of the Polish Academy of Sciences with the Spanish National Research Council "Consejo Superior de Investigaciones Científicas".

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