

# Effect of geographic range discontinuity on taxonomic differentiation of *Abies cilicica*

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## Abstract

Three populations of *Abies cilicica* subsp. *isaurica* and four of *A. cilicica* subsp. *cilicica* were analyzed using 35 morphological and anatomical needle characters with the implementation of multivariate statistical methods to verify the differences between subspecies. Moreover, the possible geographic differentiation of *A. cilicica* subsp. *cilicica* populations from the East Taurus and Lebanon Mountains was examined. *Abies cilicica* subsp. *isaurica* has been distinguished from *A. cilicica* subsp. *cilicica* by its glabrous young shoots and resinous buds. We detected that needles of *A. cilicica* subsp. *isaurica* are longer, broader and thicker, with a higher number of stomata rows, and larger cells of the epidermis, hypodermis and endodermis than *A. cilicica* subsp. *cilicica*. Additionally, *A. cilicica* subsp. *isaurica* needles have frequently rounded to obtuse-acute apex and resinous canals positioned more centrally inside the mesophyll than needles of *A. cilicica* subsp. *cilicica*. This indicates that a set of most of the tested needle characters can be used to distinguish the subspecies; however, any of characters enable that when used separately. Morphological and anatomical distinctiveness between these two taxa justify their recognition at the subspecies rank. Additionally, the populations of *A. cilicica* subsp. *cilicica* from the East Taurus and Lebanon are morphologically different. This geographic differentiation of populations is congruent with results provided by genetic analyses of nuclear microsatellites markers (nSSR).

**Keywords:** biogeography; biometrics; Cilician fir; East Mediterranean region; multivariate analyses; plant diversity; plant variation

## Introduction

The observed geographic ranges of species are historically determined and have been formed together with the species evolution [1]. The Mediterranean region history has been altered with geological events and climate changes. The land movements connected with regression of Tethys [2–5], the Messinian “salt crisis” [6] and climate cooling during late Tertiary and Quaternary, with the Pleistocene climate oscillations [7–9] had a significant imprint on the plant evolution and migrations. These processes also concerned the oro-Mediterranean plant species [10], which evolved together with the formation of mountain ridges [8].

The East Mediterranean mountain systems have been formed mostly during Miocene [3,4]. Expansion of ancestors

of the genus *Abies* is connected with this process [11]. The ancestor of contemporary *A. cilicica* (Antoine & Kotschy) Carrière probably appeared during Oligocene and Miocene [12] and settled first at Taurid and then also at Lebanese mountains [11]. It also had a somewhat broader geographic range during Miocene–Pliocene than at present ([11] and Fig. 2 and Fig. 3 therein). The Pliocene climate cooling and Pleistocene climate oscillations were the reasons for the fragmentation of the geographic range of *A. cilicica*, including its divergence and the formation of the subspecies *A. cilicica* subsp. *isaurica* Cullen & Coode in the West Taurus [13]. The development of the Taurids in Anatolia and at the Lebanese mountains allowed *A. cilicica* to persist in these regions during Pleistocene. The species could migrate up during hot and down during cold periods [7,8]. However, the isolation of the mountain massifs and, more importantly to *A. cilicica*, the climate aridity during cold periods [14], has led to the reduction and strong fragmentation of the geographic range of the species. The early Holocene distribution

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of the genus *Abies* in the Mediterranean region was more abundant than at present [15]. The increased aridity during late Holocene together with extensive deforestation from the millennia concerning this region [16,17] formed the bases of the further fragmentation of oro-Mediterranean tree species, including the Cilician fir [18–21]. Finally, the historically broader geographic range of *A. cilicica* has been reduced [22] and the species is currently at risk of extinction due to aridity in its lower localities [21]. It was recognized as a near threatened species in Turkey, Syria and Lebanon [18,20,23]. It grows in the areas assumed to be glacial refugia of the Tertiary flora [24] in several dozen mountains isolated from each other [20,25].

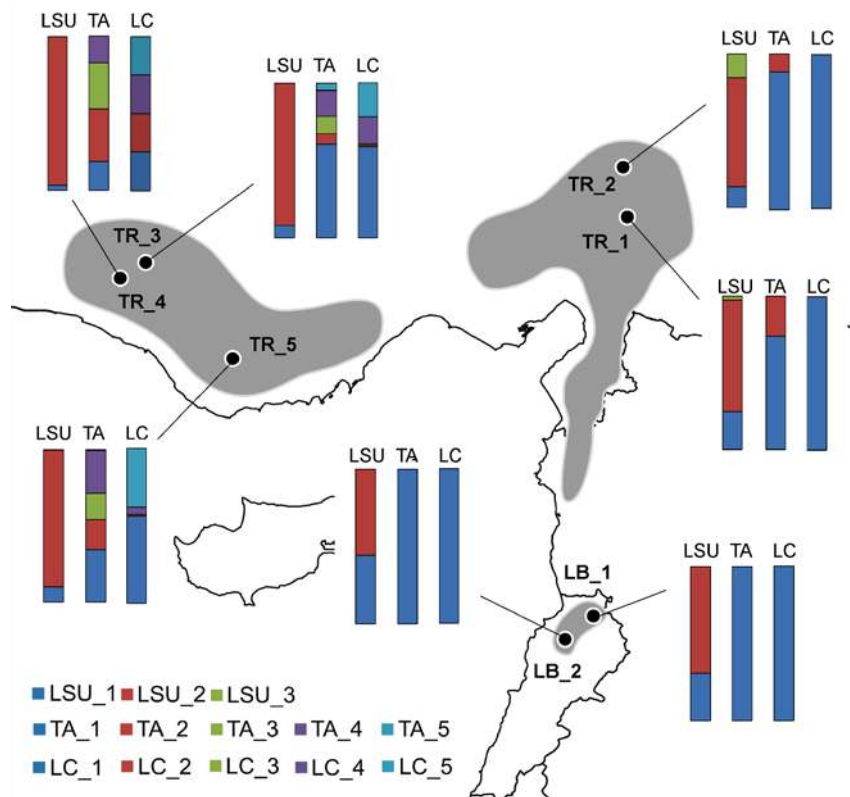
The spatial isolation between the West (Isaurian) Taurus and East Taurus is assumed to be one of the reasons for the differentiation of *A. cilicica* into eastern *A. cilicica* subsp. *cilicica* and western *A. cilicica* subsp. *isaurica*, with pubescent versus glabrous young shoots, respectively [26–28]. These two subspecies were clearly distinguished using nSSR markers [13]. The disjunctive character of occurrence of the Cilician fir and genetic differentiation between populations from the West and East Taurus and Lebanon Mountains also suggests morphological and anatomical differences between them. Similar geographic pattern of phenotypic structure has been described for *Juniperus excelsa* M. Bieb. using morphological characters of cones and sprouts [29] and for *Cedrus libani* A. Rich. using morphological and anatomical characters of needles [30].

Thus, we hypothesized that (i) the long lasting spatial isolation between West Taurus and East Taurus and Lebanon mountains caused not only genetic but also morphological and anatomical differences between *A. cilicica* subsp. *cilicica* and *A. cilicica* subsp. *isaurica*, and (ii) the isolation between mountain massif within the geographical range of *A. cilicica* subsp. *cilicica* also involved phenotypic differences between populations from these distant regions. In the present study we verified these hypotheses applying biometric analyses of morphological and anatomical needle characters. Most of the characters used in our study are applied for the first time and were not considered before in the subspecies descriptions (except of L, MW, SW and ST; see [31]), nor for evidence of phenotypic differentiation in the geographical space.

## Material and methods

### Studied species

*Abies cilicica* is a large tree, attaining a height of 30–35(–42) m and diameter of 1(–2) m at 1.3 m above ground [22,27,32]. It grows in the mountains of the East Mediterranean region, in Turkey in the West and East Taurus and in the Amanos, in Syria on the Jebel Ansariye and in Lebanon on the J. Ammoua and the J. Ehden [18,19,25] (Fig. 1). In Turkey, *A. cilicica* occurs between 1150 m and the timberline at about 2000 m on the north facing slopes, and between 1450 and 2000 m on the south facing slopes of the Taurus,



**Fig. 1** Distribution of *Abies cilicica* s. l. [25], location of analyzed populations (acronyms as in Tab. 1) and the differentiation of LSU – percentage of needles with stomata on the upper (adaxial) side of the needle (LSU\_1 – without stomata, LSU\_2 – stomata at apical part of needle, LSU\_3 – stomata at apical and central part of needle), TA – percentage of needles with different apex form (TA\_1 – indented, TA\_2 – rounded, TA\_3 – obtuse, TA\_4 – obtuse-acute, TA\_5 – acute), LC – percentage of needles with position of resin canals (LC\_1 – marginal lower, LC\_2 – marginal central, LC\_3 – marginal upper, LC\_4 – mesophyll lower, LC\_5 – mesophyll central).

with optimal conditions between 1200 and 1800 m, mostly in the valleys [20,22]. The species forms pure, shady forests or mixed forests with *Cedrus libani*, and also with *Pinus nigra* J.F. Arnold subsp. *pallasiana* (Lamb.) Holmboe in the West Taurus [20,22,33]. *Juniperus excelsa*, *J. foetidissima* Willd. and *J. drupacea* Labill. frequently enter the Cilician fir forests and even replace *A. cilicica* when overexploited and/or overgrazed [33].

#### Plant material

The needles were collected from seven natural populations of *A. cilicica*, four representing *A. cilicica* subsp. *cilicica* from the East Taurus Mountains in Turkey and the Lebanon Mountains, and three representing *A. cilicica* subsp. *isaurica* from West Taurus in Turkey (Tab. 1). Thirty cone-bearing individuals, separated by a distance of about 50 m, were sampled from each population, with the exception of the LB\_2, where only 12 individuals could be sampled. Studied individuals were ascribed to the subspecies based on morphology of the young shoots [26,27] and molecular identification [13]. Ten needles from the central part of a two-year-old shoot increment were collected from each individual, from the sunny, predominantly south-facing parts of the tree crown, at a height of about 2.0 to 5.0 m above ground level. Plant material was conserved in 70% alcohol and kept there until further preparation and measurements. In total, 192 individuals were examined, represented by 1920 needles.

Five needles from each individual were used to analyze morphology, and another five to measure anatomical characters from needle cross-sections (Tab. 2). The set of biometric characters, methods of preparation and measurements were based on previous investigations of West Mediterranean firs [34] and Turkish firs [31], and supplemented by characters of stomata occurring on the upper side of needles (Tab. 2). The CH was estimated in the following scale: discontinuous layer of single cells – 0.5; continuous layer of single cells – 1; continuous layer of single cells with additional discontinuous cell layer – 1.5.

#### Statistical treatment

The normality of the frequency distribution of each character was verified using the Shapiro–Wilk *W*-test, and the homoscedasticity of the variance of the measured data using the Brown–Forsythe test. The evaluated characters (LSU, TA and LC) data were converted to percentages and arcsine transformed. Values of all characters were standardized before multivariate statistical analyses [35]. The Pearson correlation between characters was verified to avoid the most redundant ones, with  $|r| > 0.9$ .

A *t*-test (measured and ratio characters) and the Mann–Whitney *U*-test (evaluated characters) for independent samples were used to evaluate the significance of differences between the subspecies of *A. cilicica* and between Turkish and Lebanese populations of *A. cilicica* subsp. *cilicica*. Tukey's honest significant differences (HSD) post-hoc test and Kruskal–Wallis test for the characters with biased distribution were performed on average values of characters for individuals to test the significance of differences between populations, and, consequently, between subspecies and regions.

A forward stepwise discrimination analysis (FSDA) was performed to identify the discrimination power of each character, to eliminate the closely redundant ones and to detect the relationships between populations, and consequently between subspecies and regions. A set of cluster analyses on the shortest Euclidean distances and Mahalanobis' distances (after Ward's, UPGMA, WPGMA) were applied to verify the relationships between populations between taxa and regions. Afterwards, it was verified again using discrimination analysis, to detect fit differentiation of particular individuals from the populations representing each of the groups [35]. The statistical analyses were carried out using STATISTICA v. 9 (StatSoft PL).

The Mantel test [36] was implemented to verify the relationships between Euclidean distances among populations and the geographic distances. Geographic distances were retrieved from the geographic coordinates, using MapInfo 9.5 (Pitney Bowes). The significance of the correlation was tested with 9999 random permutations. PopTools v.3.2 software [37] was used in the calculations.

**Tab. 1** Geographic and climatic data for studied populations of *Abies cilicica*.

Taxon	Location	N	Code	Herbarium voucher	Longitude E (°)	Latitude N (°)	Altitude (m)	Climate data	
								AMT (°)	APR (mm)
subsp. <i>cilicica</i>	Turkey, Central Taurus, Başkonuş	30	TR_1		36.5847	37.5700	1300	10.96	688
	Turkey, Central Taurus, Goksun	30	TR_2		36.5553	37.9556	1475	8.58	604
	Lebanon, Ammoua (Aakkar)	30	LB_1		36.2611	34.4956	1565	11.26	823
	Lebanon, Ehden	12	LB_2	KOR 47198	35.9920	34.3075	1565	12.43	1067
subsp. <i>isaurica</i>	Turkey, West Taurus, Seydişehir	30	TR_3	KOR 47351	32.0094	37.2236	1700	9.40	665
	Turkey, West Taurus, Akseki	30	TR_4	KOR 11201	31.7583	37.1033	1400	10.40	738
	Turkey, West Taurus, Kazanci	30	TR_5	KOR 47335	32.8353	36.4820	1430	10.52	722

N – number of individuals sampled; AMT – annual mean temperature; APR – annual average precipitation.

**Tab. 2** Analyzed needle traits of *Abies cilicica*: mean values (*M*), variation coefficients (*V*), significance differences (*P*) of subspecies and populations within *A. cilicica* subsp. *cilicica* tested using *t*-test (measured and ratio traits) and the Mann–Whitney *U*-test (evaluated traits); discrimination among populations power of characters as shown by Wilks' partial  $\lambda$ ; *P* – significance of  $\lambda$ .

Character	I	Acronym	<i>A. cilicica</i>						<i>A. cilicica</i> subsp. <i>cilicica</i>						Discrimination	
			subsp. <i>cilicica</i>			subsp. <i>isaurica</i>			Turkey			Lebanon			$\lambda$	<i>P</i>
			<i>M</i>	<i>V</i>	<i>P</i>	<i>M</i>	<i>V</i>	<i>P</i>	<i>M</i>	<i>V</i>	<i>P</i>	<i>M</i>	<i>V</i>	<i>P</i>		
2	3	4	5	6	7	8	9	10	11	12	13	14				
<b>Measured</b>																
Needle area (mm <sup>2</sup> )	A	28.88	26.94	37.65	21.70	0.000	28.64	24.31	29.12	29.56	0.288	0.861	0.000			
Needle perimeter (mm)	P	45.54	20.60	50.69	18.79	0.002	45.80	19.14	45.27	22.07	0.683	0.956	0.282			
Needle length (mm)	L	21.38	20.12	23.48	15.75	0.003	21.25	19.13	21.51	21.11	0.341	0.970	0.539			
Needle maximum width (mm)	MW	1.52	9.38	1.85	8.88	0.000	1.53	9.38	1.50	9.37	0.859	0.976	0.672			
Needle width in 95% of its length (mm)	W_95	1.13	10.74	1.34	11.82	0.000	1.13	10.82	1.13	10.66	0.281	0.978	0.720			
Needle width in 50% of its length (mm)	W_50	1.50	14.88	1.76	10.15	0.000	1.56	18.40	1.44	11.36	0.192	0.915	0.022			
Distance from the basis to the needle maximum width (mm)	BD	10.34	25.81	11.82	21.05	0.001	10.39	25.20	10.30	26.42	0.493	0.969	0.531			
Number of stomata rows on abaxial needle surface at the central part of needle	NRL	11.61	11.84	15.14	11.70	0.000	11.94	13.17	11.27	10.50	0.239	0.944	0.143			
Number of stomata on the 1 mm of central part of abaxial side of needle surface	NSL	10.43	5.96	11.91	7.40	0.000	10.21	7.30	10.66	4.63	0.000	0.549	0.000			
Needle width on the cross-section ( $\mu\text{m}$ )	SW	1552.85	8.42	1895.75	8.47	0.000	1554.54	8.93	1551.16	7.92	0.510	0.769	0.000			
Needle thickness on the cross-section ( $\mu\text{m}$ )	ST	662.25	11.04	820.39	10.50	0.000	693.69	10.55	630.82	11.53	0.156	0.779	0.000			
Width of endodermis tube ( $\mu\text{m}$ )	VCW	410.97	10.29	488.31	10.46	0.000	410.31	10.62	411.64	9.97	0.408	0.854	0.000			
Height of endodermis tube ( $\mu\text{m}$ )	VCT	274.90	9.63	324.71	9.08	0.000	278.02	10.12	271.77	9.13	0.836	0.918	0.027			
Number of mesophyll palisade layers	NML	1.52	14.67	1.70	13.73	0.001	1.58	12.11	1.47	17.23	0.849	0.926	0.046			
Thickness of the one mesophyll palisade layers ( $\mu\text{m}$ )	MT	80.83	11.03	81.66	9.20	0.924	82.80	10.13	78.86	11.94	0.249	0.976	0.668			
Distance between vascular bundles ( $\mu\text{m}$ )	DV	27.97	33.59	26.77	39.07	0.784	27.51	33.60	28.43	33.58	0.672	0.927	0.049			
Width of epidermal cell ( $\mu\text{m}$ )	EW	20.20	7.50	22.22	6.76	0.000	20.80	7.82	19.60	7.19	0.016	0.957	0.293			
Height of epidermal cell ( $\mu\text{m}$ )	EH	19.78	8.52	21.86	7.96	0.000	20.23	8.81	19.32	8.23	0.571	0.868	0.000			
Width of hypodermal cell ( $\mu\text{m}$ )	HW	16.91	7.21	18.83	8.41	0.000	17.33	7.95	16.49	6.47	0.007	0.861	0.000			
Height of hypodermal cell ( $\mu\text{m}$ )	HH	17.22	6.84	18.30	7.75	0.000	17.10	7.04	17.34	6.64	0.087	0.884	0.002			
Width of resin canal ( $\mu\text{m}$ )	WC	90.68	15.65	109.11	18.09	0.000	90.18	13.54	91.18	17.75	0.749	0.907	0.013			
Height of resin canal ( $\mu\text{m}$ )	HC	86.10	16.12	102.25	16.46	0.000	83.96	14.01	88.24	18.22	0.550	0.876	0.001			
Number of resin canals	NC	2.00	0.00	2.00	1.27	0.288	2.00	0.00	2.00	0.00	0.997	0.971	0.549			
Continuity of hypodermis	CH	0.81	23.07	0.88	41.33	0.694	1.00	24.07	0.63	22.08	0.000	0.828	0.000			
<b>Ratio</b>																
Shape of needle in cross-section (SW/ST)	NS	2.39	8.05	2.33	8.05	0.691	2.27	7.07	2.50	9.02	0.002	0.731	0.000			
Shape of endodermis in cross-section (VCW/VCT)	VCS	1.50	4.49	1.51	5.42	0.145	1.48	5.31	1.52	3.67	0.100	0.915	0.022			

Tab. 1 (continued)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Ratio of needle width/endodermis width (SW/VCW)		RW_1	3.80	5.98	3.90	5.85	0.006	3.82	6.08	3.79	5.88	0.341	0.866	0.000
Ratio of needle thickness/endodermis thickness (ST/VCT)		RW_2	2.40	5.27	2.53	4.70	0.000	2.49	4.97	2.32	5.57	0.000	0.920	0.031
Ratio of needle maximum width /needle width in 50% length (MW/W_50)		RW_3	1.03	5.74	1.05	4.51	0.035	1.02	8.04	1.05	3.44	0.108	0.913	0.018
Ratio of needle width in 95% length/needle width in 50% length (W_95/W_50)		RW_4	0.77	9.77	0.74	9.17	0.037	0.74	11.67	0.79	7.88	0.003	0.933	0.073
Location of the maximum width of the needle (MW/L*100%)		LMW	7.39	16.93	8.10	13.94	0.000	7.51	17.71	7.27	16.14	0.286	0.988	0.921
Marcel's coefficient (DV/SW*ST)		MC	11.71	33.40	11.40	36.28	0.728	12.12	33.78	11.31	33.01	0.144	0.931	0.064
Shape of epidermal cell in cross-section (EW/EH)		ES	1.03	7.00	1.03	6.94	0.906	1.04	7.30	1.03	6.70	0.064	0.954	0.246
Shape of hypodermal cell in cross-section (HW/HH)		HS	0.99	8.37	1.04	8.65	0.001	1.02	10.10	0.96	6.63	0.000	0.895	0.005
Shape of resin canal in cross-section (WC/HC)		CS	1.06	5.43	1.07	7.17	0.529	1.08	4.93	1.04	5.93	0.003	0.946	0.156
<b>Evaluated</b>														
Location of stomata on the upper (adaxial) surface of needle (%)		lack	LSU_1	29.04	125.29	7.19	322.95	0.000	19.48	154.45	38.59	96.13	0.025	0.946
		apical	LSU_2	66.73	53.07	92.81	20.66	0.000	72.01	46.61	61.41	59.52	0.311	0.971
		central and apical	LSU_3	4.24	136.01	0	0	0.238	8.48	272.02	0	0	0.188	0.943
Needle apex (%)		indented	TA_1	90.46	21.37	36.54	112.56	0.000	80.93	42.75	100.00	0	0.019	0.878
		rounded	TA_2	9.54	99.49	20.33	200.87	0.093	19.07	198.99	0	0	0.019	0.946
		obtuse	TA_3	0	0	20.27	188.16	0.000	0	0	0	0	0.997	0.975
		obtuse-acute	TA_4	0	0	21.08	154.31	0.000	0	0	0	0	0.997	0.941
		acute	TA_5	0	0	1.78	321.35	0.688	0	0	0	0	0.997	0.938
Resin canal position (%)		marginal lower	LC_1	100.00	0	43.21	104.49	0.000	100.00	0	100.00	0	0.997	0.710
		marginal central	LC_2	0	0	0.67	365.15	0.789	0	0	0	0	0.997	0.924
		marginal upper	LC_3	0	0	0.22	182.57	0.894	0	0	0	0	0.997	0.964
		mesophyllum lower	LC_4	0	0	10.19	208.03	0.001	0	0	0	0	0.997	0.939
		mesophyllum central	LC_5	0	0	45.48	107.37	0.000	0	0	0	0	0.997	0.923

## Results

### Variation and correlation of characters

The distribution of most of the characters was unimodal and normal or very close to normal. The evaluated characters LSU, TA and LC were the only exceptions. The latter data were arcsine-transformed and assumed to have a close-to-normal distribution, which allowed the application of multivariate tests. The data after transformation and standardization were homoscedastic or close to, which allowed the assessment of parametric tests.

The needle dimensional characteristics (A, P, and L) correlated positively with each other at very high significant level ( $r = 0.95$ ,  $P < 0.01$ ). The anatomical characters of the needle ST, SW, VCT and VCW, as well as WC and HC correlated significantly with each other at a similar level. The level of correlation was slightly different for each population, but generally the same pattern of relationships between measured characters was found. From the groups of the most closely correlated and thus redundant characters, only single ones were used for the multivariate analyses. The forward stepwise analysis of discrimination (FSDA) reduced the set of characters and only 22 from 48 previously measured/evaluated ones were the basis of the discrimination and clustering, which described the differentiation between populations, subspecies and regions. The fourteen needle characteristics discriminated between populations of *A. cilicica* s. l. at a significant level ( $P < 0.01$ ; Tab. 2), but P, MT, NC, MC, LSU\_1, LSU\_2, TA\_3, LC\_3 and LC\_45 were excluded from the dataset in the FSDA. The highest discriminant power had NSL, LC\_1, RW\_1 and A with values of partial Wilks'  $\lambda$  of 0.620, 0.811, 0.836 and 0.844, respectively.

The particular characters differed in the value of variation coefficients. NC was the most stable trait, completely without variation in several populations and  $V = 0.4\%$  on average. Among the other characters, NSL, EW and HH had average values of  $V \approx 7\%$ . Apex forms (TA), position of resin canals (LC) and location of stomata on the upper side of the needle (LSU) were the most variable. Among the measured characters, A, P, L, BD, DV and CH had  $V$  between 20 and 40% (Tab. 2).

### Phenotypic distinctiveness of subspecies

The average values of characters appeared to some degree to be specific for particular populations, but with generally overlapping frequency distribution between populations. Most of the analyzed needle characters differentiated between *A. cilicica* subsp. *cilicica* and *A. cilicica* subsp. *isaurica* at a statistically significant level (Tab. 2). The average values of needle characters were higher in populations classified as *A. cilicica* subsp. *isaurica* than in populations of *A. cilicica* subsp. *cilicica*, with the only exceptions being DV and NC. The ratio characters differentiated subspecies to a lesser extent, with only RW\_1, RW\_2, LMW and HS being significant at  $P < 0.01$  (Tab. 2).

According to the Mann–Whitney  $U$ -test, significant differences ( $P < 0.01$ ) between subspecies were observed in the TA\_1 and LC\_1 (Tab. 2). The HSD Tukey's test also revealed that the majority of morphological and anatomical characters differed at a significant level ( $P \leq 0.01$ ) between sampled

populations of *A. cilicica* subsp. *cilicica* (TR\_1, TR\_2, LB\_1 and LB\_2) and *A. cilicica* subsp. *isaurica* (TR\_3–5; Tab. 3). The characters DV, NC, LSU\_3, TA\_3, TA\_5, LC\_2, LC\_3 and LC\_4 were the only biometric characters that did not differ significantly between samples according to the results of the Tukey's test.

Based on the first three discriminant variables of FSDA,  $U_1$ ,  $U_2$ , and  $U_3$ , which explained 86% of the total variation, the analyzed populations formed three groups. The first group was composed of *A. cilicica* subsp. *isaurica* populations (TR\_3, TR\_4 and TR\_5), while the other two were of *A. cilicica* subsp. *cilicica* (Fig. 2a–c). The first discrimination variable ( $U_1$ ), which covered 60% of the total variation, was determined mostly by LC\_1, NSL and NRL, the second ( $U_2$ ), which covered 15% of the variation, was determined first of all by NML, NS and RW\_2, while the third ( $U_3$ ), which covered 11% of the variation, was determined by RW\_2, CH and TA\_1. The analyzed populations were discriminated by  $U_1$  at the subspecies level (Fig. 2a), while further grouping of the populations of *A. cilicica* subsp. *cilicica* was mostly determined by  $U_3$  (Fig. 2b and Fig. 2c).

Afterwards, we verified how particular individuals from the populations representing each of subspecies fitted this differentiation. Again, we used FSDA with the characters: NC, MC, CS, LSU\_1, LSU\_2, TA\_3, TA\_4, TA\_5, LC\_2, LC\_3 and LC\_4 excluded from the model. From the remaining characteristics, 11 discriminated between individuals at a significant level. NSL, LC\_1, CH and RW\_1 had the highest discrimination power, with partial Wilks'  $\lambda$  values: 0.800, 0.846, 0.883 and 0.907, respectively. The total variation was divided between the first two discriminant variables, where  $U_1$  covered more than 81%. It was determined first of all by NRL, LC\_1, NSL, TA\_1, LC\_5 and A. The second discrimination variable  $U_2$  was determined mostly by CH, RW\_2 and HS. The individuals formed three groups on the dispersion diagram (Fig. 2d). The populations representing *A. cilicica* subsp. *isaurica* (TR\_3, TR\_4 and TR\_5) formed a coherent group with only one individual outside of the 95% confidence interval, but included six individuals from *A. cilicica* subsp. *cilicica* (Fig. 2d). In summary, 95% of individuals of *A. cilicica* subsp. *isaurica* were correctly classified to the subspecies.

The cluster analysis on the shortest Euclidean distances according to Ward's method, divided all of the samples into two main groups. The populations assigned to *A. cilicica* subsp. *cilicica* formed the first cluster, while the populations classified as *A. cilicica* subsp. *isaurica* (TR\_3, TR\_4, and TR\_5) comprised the second one (Fig. 3). Similar patterns of differences between *A. cilicica* subsp. *cilicica* and *A. cilicica* subsp. *isaurica* populations were detected using UPGMA and WPGMA cluster analyses on the Euclidean distances and analyses on Mahalanobis distances (data not shown).

### Variation within subspecies

All populations of *A. cilicica* subsp. *cilicica* had a marginal-lower type of resin canal position (LC\_1), while two types of resin canal positions were observed at a similar frequency in *A. cilicica* subsp. *isaurica*, namely marginal-lower (TR\_3 and TR\_5) and mesophyll-central (TR\_5; Fig. 1). This subspecies was quite homogenous in terms of the location of stomata

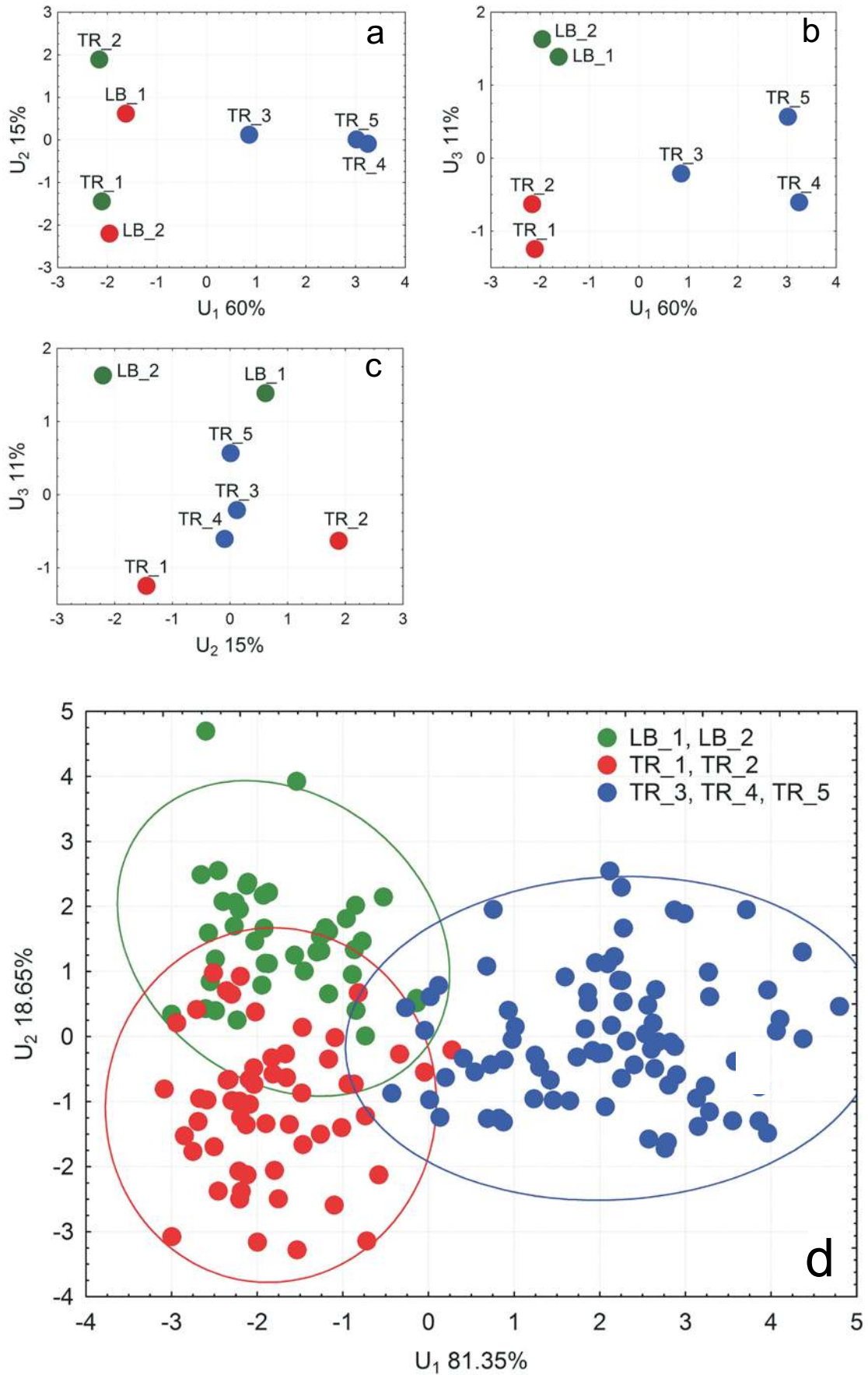
**Tab. 3** Tukey's and Kruskal–Wallis tests results for characters differentiating at  $P < 0.01$  (bold) and  $P < 0.05$  (italic) between analyzed populations (characters acronyms as in Tab. 2; populations acronyms as in Tab. 1).

	TR_1	TR_2	TR_3	TR_4	TR_5	LB_1
TR_2	A, MW, W_95, W_50, <i>NRL</i> , SW, ST, VCW, VCT, NML, MT, EW, EH, HH, HW, NS, RW_1, RW_2, RW_3, ES					
TR_3	A, MW, W_95, W_50, <i>BD</i> , <i>NRL</i> , NSL, SW, ST, VCW, VCT, NML, EW, EH, WC, HH, HW, HC, VCS, RW_2, ES, LC_1	MW, <i>NRL</i> , NSL, SW, VCS, NS, RW_3, LC_1				
TR_4	A, MW, W_95, W_50, <i>NRL</i> , NSL, SW, ST, VCW, VCT, NML, EW, EH, WC, HH, HW, HC, NS, RW_2, LMW, ES, LSU_2, TA_1, TA_3, LC_1, LC_5	MW, W_95, <i>NRL</i> , NSL, SW, ST, VCW, VCT, RW_3, LSU_2, TA_1, LC_1, LC_5	<i>NRL</i> , NSL, ST, VCT, TA_1, LC_1, LC_5			
TR_5	A, P, L, MW, W_95, W_50, <i>BD</i> , <i>NRL</i> , NSL, SW, ST, VCW, VCT, NML, EW, EH, WC, HH, HW, HC, TA_1, TA_4, LC_1, LC_5	A, MW, <i>NRL</i> , NSL, SW, EH, WC, HC, NS, RW_1, RW_2, RW_3, TA_4, TA_1, LC_1	NSL, WC, HC, VCS, MC	ST, EW, EH, HC, NS, RW_1, RW_2, LC_5		
LB_1	A, MW, W_95, SW, ST, VCW, VCT, NML, EH, EH, HH, HS, ES	W_50, ST, EW, EH, HW, NS, CH, RW_2, RW_3, RW_4, HS	MW, W_50, <i>NRL</i> , SW, ST, VCW, EW, WC, HW, HC, VCS, RW_2, RW_4, HS, TA_1, LC_1	MW, W_95, W_50, <i>NRL</i> , NSL, SW, ST, VCW, VCT, EW, EH, WC, HW, HC, RW_2, LMW, HS, LSU_1, LSU_2, TA_1, TA_2, TA_3, LC_1, LC_5	A, MW, W_95, W_50, <i>NRL</i> , NSL, SW, ST, VCW, VCT, EW, WC, HW, HC, RW_1, RW_4, HS, TA_1, TA_4, LC_1	
LB_2	VCS, NS, CH, RW_2	MW, W_95, W_50, <i>NRL</i> , SW, ST, VCW, VCT, NML, MT, EW, EH, HW, NS, CH, RW_2, RW_3, CS	A, MW, W_95, W_50, <i>NRL</i> , SW, ST, VCW, VCT, NML, EW, EH, HH, HW, NS, CH, RW_2, LSU_1, LSU_2	A, MW, W_95, W_50, <i>NRL</i> , NSL, SW, ST, VCW, VCT, NML, MT, EW, EH, HH, HW, NS, CH, RW_2, CS, LSU_1, LSU_2, TA_1, LC_1, LC_5	A, P, L, MW, W_95, W_50, <i>BD</i> , <i>NRL</i> , NSL, SW, ST, VCW, VCT, NML, EW, EH, WC, HW, HC, NS, CH, RW_2, TA_1, LC_1	ST, VCT, NML, EH, NS, RW_2, ES

at the upper needle surface (LSU), while *A. cilicica* subsp. *cilicica* was more variable in this aspect (Fig. 1). Individuals of *A. cilicica* subsp. *cilicica* had indented (TA\_1), or rounded (TA\_2) types of needle apex, 90.5% and 9.5%, respectively, while in *A. cilicica* subsp. *isaurica* all of the types were observed, with prominent percentages of obtuse (TA\_3) and acute (TA\_4) types (Fig. 1).

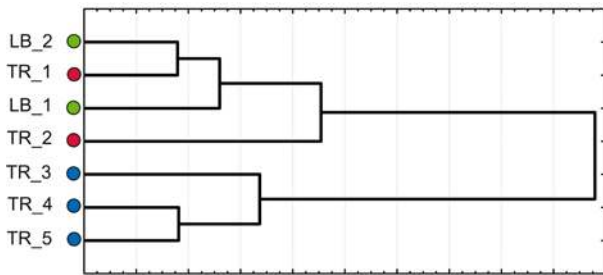
The Mantel test detected positive and significant correlations between the Euclidean distance and geographic distances for populations ( $r^2 = 0.54$ ,  $P = 0.012$ ). The multivariate differences were found not only between subspecies, but also between populations of *A. cilicica* subsp. *cilicica* from the East Taurus (TR\_1 and TR\_2) and the Lebanon mountains (LB\_1 and LB\_2; Fig. 2a–c). The latter two groups of populations were determined mostly by the U<sub>3</sub> variable

(Fig. 2b and Fig. 2c). We used FSDA to verify how particular individuals of *A. cilicica* subsp. *cilicica* from the East Taurus and the Lebanon mountains fit the two geographic groups described. The FSDA detected that the compared individuals formed two partly intermingled groups on the dispersion diagram (Fig. 2d). Three individuals from the East Taurus and another three individuals from the Lebanon Mountains fall into the 95% confidential interval of *A. cilicica* subsp. *isaurica*. The individuals of *A. cilicica* subsp. *cilicica* from the Eastern Taurus formed a separate group from that representing the Lebanon Mountains; however, about 30% of the East Taurus individuals entered the Lebanese group at a 95% confidential interval (Fig. 2d). The correct classification of the Lebanese versus East Taurus individuals were at the level of 93% and 86%, respectively.



**Fig. 2** Results of discrimination analysis of *Abies cilicica*: for populations (a–c), for individuals (d) in three groups: West Taurus (TR\_3, TR\_4 and TR\_5), Lebanon (LB\_1, LB\_2) and East Taurus (TR\_1 and TR\_2; acronyms as in Tab. 1), with 95% confidence intervals for each group.





**Fig. 3** Dendrogram constructed on Euclidean shortest distances after the Ward's method between populations of *Abies cilicica* from Lebanon (LB\_1–2 – green circle), the East Taurus (TR\_1–2 – red circle) and the West Taurus (TR\_3–5 – blue circle); the population codes as in Tab. 1.

The populations of *A. cilicica* subsp. *cilicica* from East Taurus and the Lebanon Mountains differed with respect to the type of needle apex. In the Lebanese populations only the indented type (TA\_1) was observed, while in those from the East Taurus 20% of individuals had rounded (TA\_2) type of needle apex (Fig. 1). A significant level of statistical differences ( $P < 0.01$ ) was also observed between the Turkish and Lebanese *A. cilicica* subsp. *cilicica* populations for number of stomata (NSL), width of hypodermal cells (HW), shape of needle cross-section (NS), shape of hypodermis cells (HS) and shape of resin canal cross-section (CS; Tab. 2). The geographic differentiation of *A. cilicica* subsp. *cilicica*, however, has not been confirmed using the agglomeration method (Fig. 3).

## Discussion

### Needle characteristics variation

Data on the morphological and anatomical variation of the needle characteristics of *A. cilicica* were scarce, with only the length and width of needle (L and MW) and sometimes the needle apex type (TA) reported. This results in a low level of differences between the Mediterranean taxa of the genus *Abies* on the needle characters known to date [38,39]. It is commonly known and generally accepted that cones are essential to correctly determine the *Abies* taxa (e.g., [27,28,40–42]). This rule was also confirmed in the only known biometric study of the Turkish firs, but some differences between Turkish fir species in the needle characters were also described [31]. Comparing these data with our findings, it should be stressed that we found higher average values of needle width and height on the cross-section preparation (SW and ST, respectively) and diameter of resin canals (WC and HC) than reported by Bağcı and Babaç [31]. The differences between our data and that of Bağcı and Babaç [31] might be a result of different preparation and measurement procedures used in both studies and the higher number of individuals tested in our study. The comparison of Bağcı and Babaç [31] and other accessible data with our results also stresses similarities in the data concerning the length and width of needles (Tab. 4).

Our data are based on the examination of a large number of individuals and thus shall be considered as bearing not only the real values of the examined characters, but also ranges of variation. Our results fill the gap in data and provide a broad set of needle characteristics of *A. cilicica*. We expect that some of them could also be used in palaeobotanical studies. *Abies* needles were detected several times in the Tertiary and Quaternary deposits and many of them have not been determined to the species level (e.g., [12,40,43]).

### Intraspecific differentiation

Our study is the first where the differences between *A. cilicica* subsp. *cilicica* and *A. cilicica* subsp. *isaurica* are studied using wide spectrum of the needle characters [only L and MW (e.g., [28,41,42,44,45]), ST, SW and WC/HC were investigated before [31]]. The biometric analyses reveal that the most of examined needle characteristics are suitable to distinguish *A. cilicica* subsp. *cilicica* from *A. cilicica* subsp. *isaurica* at a significant level (Tab. 2).

**Tab. 4** Comparison of data values of *Abies cilicica* needle characters known from the literature and received in the study (bold).

Character	Value	Remarks	Source of data
L	2.5–4.0 cm		[41]
	2.5–4.0 cm		[28]
	1.5–4.0 cm		[45]
	2.45 cm	subsp. <i>cilicica</i>	[31]
	2.10 cm	subsp. <i>isaurica</i>	[31]
	<b>2.16 (1.1–4.1) cm</b>	subsp. <i>cilicica</i>	
	<b>2.35 (1.3–4.0) cm</b>	subsp. <i>isaurica</i>	
MW	1.86 mm	subsp. <i>cilicica</i>	[31]
	1.88 mm	subsp. <i>isaurica</i>	[31]
	1.5–1.8 mm		[41]
	1.5–1.8 mm		[28]
	<b>1.53 (1.1–2.1) mm</b>	subsp. <i>cilicica</i>	
SW	<b>1.85 (1.4–2.7) mm</b>	subsp. <i>isaurica</i>	
	1.36 mm	subsp. <i>cilicica</i>	[31]
	1.42 mm	subsp. <i>isaurica</i>	[31]
	<b>1.56 (1.1–2.1) mm</b>	subsp. <i>cilicica</i>	
ST	<b>1.89 (1.4–2.3) mm</b>	subsp. <i>isaurica</i>	
	485 $\mu$ m	subsp. <i>cilicica</i>	[31]
	479 $\mu$ m	subsp. <i>isaurica</i>	[31]
(WC+HC)/2	<b>676 (427–1040) <math>\mu</math>m</b>	subsp. <i>cilicica</i>	
	<b>818 (573–1120) <math>\mu</math>m</b>	subsp. <i>isaurica</i>	
	55 $\mu$ m	subsp. <i>cilicica</i>	[31]
	43 $\mu$ m	subsp. <i>isaurica</i>	[31]
	<b>87 (45–154) <math>\mu</math>m</b>	subsp. <i>cilicica</i>	
	<b>105 (39–210) <math>\mu</math>m</b>	subsp. <i>isaurica</i>	

The differences between average values of the most of verified characters found in our study justify the taxonomic position of *A. cilicica* subsp. *isaurica* when compared with typical *A. cilicica* subsp. *cilicica*. Generally, the needles of *A. cilicica* subsp. *cilicica* are smaller than those of *A. cilicica* subsp. *isaurica* (compare characters A, P, L, MW; Tab. 2), have a smaller endodermis tube (SW and ST), slighter epidermis and hypodermis cells (EW, EH, HW, HH), lower values of resin canal width and height (WC, HC) and lower numbers of stomata rows and stomata (NRL and NSL). *Abies cilicica* subsp. *isaurica* could be distinguished using a set of these characters and the evaluated ones, which are types of location of stomata on the adaxial needle side (LSU), the needle apex type (TA) and position of resin canals (LC; Fig. 1). The average values of measured characters of *A. cilicica* subsp. *isaurica* are about 20–30% higher than detected for *A. cilicica* subsp. *cilicica*, which has not been described until now [26,27,31,32]. However, none of the mentioned characters allows distinction between subspecies solely, as the distribution ranges of the characters that may be used for distinguishing between subspecies overlap to some degree.

#### Geographic pattern of differentiation

The Mantel test result suggests an important role of spatial isolation in shaping the inter-population differentiation of the phenotypic characters. The pattern of intraspecific morphological and anatomical differentiation of *A. cilicica* s. l. documented in the present study based on the needle characteristics appeared similar to those described using nuclear microsatellite markers (compare Fig. 1–Fig. 3 and Fig. 1, Fig. 2 in [13]). On the other hand, the geographic differentiation among populations of *A. cilicica* subsp. *cilicica* was less evident in phenotypic characters than in molecular markers. This result is somehow in contrary with molecular evidence, because the genetic differences between Lebanese and Turkish populations of *A. cilicica* subsp. *cilicica* were even at a higher level than between subspecies (see [13] and Fig. 2 therein).

The pattern and significant level of genetic differentiation found between populations of *A. cilicica* subsp. *isaurica* and *A. cilicica* subsp. *cilicica* as well as between populations of the latter from the Lebanon Mountains versus East Taurus were interpreted as a result of a long-lasting isolation [13].

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#### Authors' contributions

The following declarations about authors' contributions to the research have been made: field studies and material collection: AB, KB, AKJ, TO, MBDK; laboratory works: AKJ, KB, KS, GI; data analysis and interpretation: AKJ, DT, KB, KS, GI; bibliography studies: AB, AKJ, KS, MBDK, TO; writing the manuscript: AKJ, AB, TO, DT, GI, KS, MBDK.

#### Competing interests

No competing interests have been declared.

The spatial isolation and climate changes during glacial and interglacial periods of the Pleistocene [46,47] caused only local, vertical migrations in the mountains of the Mediterranean region [47,48], which reduced the possibility of gene exchange by seeds and pollen between populations and, consequently, were the reason for differentiation or even speciation processes (e.g., [49–51]). This also concerns the *A. cilicica* (or its ancestor) populations in the Taurids and Lebanese mountain systems [11]. A similar pattern of morphological differentiation to that mentioned above was detected in *Juniperus drupacea* [52], *J. excelsa* subsp. *excelsa* [29] and *Cedrus libani* [30]. All three species co-occur with *A. cilicica* [22,25,32,53]. Interestingly, the geographic differentiation on the morphological and/or anatomical characteristics of each of these three taxa resembled geographic structure on the genetic markers. Congruent genetic and phenotypic patterns of differences between the West Taurus, East Taurus and Lebanon Mountains populations were detected in *Cedrus libani* [30,54] and *Juniperus excelsa* [55], and differences between Lebanese and Turkish populations were found in *J. excelsa* [29,55]. This could indicate a more universal character of differentiation that resulted from the species history and ancient demographic processes for the oro-East-Mediterranean tree species.

#### Conclusion

The populations sampled as *A. cilicica* subsp. *cilicica* and *A. cilicica* subsp. *isaurica* could be clearly distinguished, but only using the set of morphological and anatomical characters of the needles. No one single needles character allowed distinguishing between them without any doubt. The differences were also detected between Lebanese and East Taurus populations of the typical subspecies *A. cilicica* subsp. *cilicica*. The geographic pattern of differentiation among populations based on the morphological and anatomical needle characters resembles those received with the nSSR markers [13]. The geographic differentiation between both subspecies and among populations in the East Taurus and Lebanon Mountains, detected using both nSSR markers and phenotypic characters, suggests local management of the *A. cilicica* woodlands, without seed exchange between the regions.

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