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VARIATION IN NEEDLE ANATOMY OF SCOTS PINE (*PINUS SYLVESTRIS* L.) POPULATIONS ACCORDING TO HABITAT AND ALTITUDINAL ZONES IN TURKIYE

VARIJACIJE U ANATOMIJI IGLICA OBIČNOG BORA (*Pinus sylvestris* L.) S OBZIROM NA STANIŠTA I NADMORSKU VISINU U TURSKOJ

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SUMMARY

In this study, eight Scots pine populations from Turkiye were studied to explore the influence of different habitats and altitudinal zones on the needle anatomical traits. A total of 496 needles belonging to 64 individuals were examined using light microscopy with the aim to score variability of sixteen needle anatomical traits. Variance analysis showed significant differences in needle thickness, needle width, resin canal number, resin canal diameter, central cylinder width, central cylinder thickness, endodermis cell number, endodermis width and endodermis thickness of eight populations depending on habitat zones. However, only resin canal diameter, endodermis width and endodermis thickness differ significantly in examined populations depending on altitudinal gradients. Cluster analysis showed the greatest similarities between the Bolu-Aladağ and Ardahan-Yalnızçam populations, and the most distinguishable population was the Giresun-Espiye population based on the anatomical characteristics of the needles. Although principal component analysis showed that needle width, central cylinder width, needle thickness, and central cylinder thickness had the greatest influence on the delimitation of Scots pine populations distributed in Turkiye, discrimination analysis did not separate the examined populations depending on the anatomical characteristics of the needles.

KEY WORDS: Anatolia, altitude, needle anatomy, *Pinus sylvestris*, variation

INTRODUCTION
UVOD

Scots pine (*Pinus sylvestris* L., Pinaceae) occupies large areas in relatively dry regions within the Mediterranean basin, from the Iberian Peninsula to Turkiye (Martínez-Vilalta *et al.* 2009). It is the third most widespread conifer tree species in Turkiye after *Pinus brutia* Ten. and *Pinus nigra* J. F. Arnold (Davis *et al.* 1984; Kandemir and Mataracı 2018).

Pinus sylvestris naturally spreads in different habitat zones of three geographical regions in Turkiye. The distribution of plant species mostly depends on competitive abilities and environmental factors (Friend and Woodward 1990; Scho-

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ettle and Rochelle 2000). The plant communities, plant physiology and morphology, gene ecology, life history characteristics are adversely affected by the altitude-related theory of biological phenomenon (Körner 2007; Klimes 2003; Hoch and Körner 2003; Reisch *et al.* 2005). Needle morphology is often affected by the ecosystem's characteristics (Tiwari et al. 2013). However, Taleshi et al. (2013) reported that there are no significant differences based on leaf morphological properties among tree oak population distributed in Zagros (Iran) depending on altitudinal gradient. Change in leaf anatomy is another important mode of adaptation of plants (slow evolutionary process) and acclimation (shorter-term adjustment) to new environmental conditions (Kivimäenpää *et al.* 2017). Anatomical changes in pine needles have been observed in connection with changes in light conditions and the content of nutrients in the soil (Niinemets *et al.* 2001). Nikoli**ć** et al. (2016) reported that morpho-anatomical needle properties supported geographic delimitation of distant populations of *Pinus heldreichii* Christ distributed in Montenegro and Serbia. Boratyńska et al. (2008) investigated the effect of tree age (seedlings, saplings and adult trees) on needle morphology and anatomy of *Pinus uliginosa* Neumann. The results showed that needles of all three *P. uliginosa* generations differ significantly among each other.

Scots pine is an undemanding species and grows both on fertile and infertile soils (Mandre 2003). Ergül Bozkurt *et al.* (2021) reported that needle length, needle width and the ratio of needle length to needle width of Scots pine distributed in Turkiye showed variation in response to altitudinal gradients. However, there is no study on variability of the needle anatomy depending on habitat zones and altitudinal gradients of Scots pine distributed in Turkiye. To fill this gap, the present study aims to provide a comprehensive analysis of the influence of habitat zones and altitudinal boundaries on the needle anatomical characteristics of Scots pine distributed in Turkiye.

MATERIAL AND METHODS
MATERIJALI I METODE RADA

Needle samples for anatomical analysis were collected from eight natural Scots pine populations (Figure 1) selected according to habitat zones of Kantarcı (2005) in the year of 2013 and 2014. All samples were fixed in FAA (5 parts stock formalin 5 parts glacial acetic acid, 90 parts 70% ethanol) for 24 h and stored in 70% ethanol as suggested by ***Ö***zban and ***Özden*** (1991).

Five to 10 needles were sampled from six to 12 trees per population (Table 1). In total 496 needles belonging to 64 individuals from eight populations were used for anatomical investigation. In order to determine the anatomical needle variation within selected populations, all samples were firstly grouped in habitat zones according to Kantarcı (2005) and following altitudinal limits: 0-300 m, 300-600 m, 1000-1300 m, 1300-1600 m, 1600-1900 m, 1900-2100 m and 2100-2400 m (Table 1). The following anatomical traits were analysed: needle thickness (NT), needle width

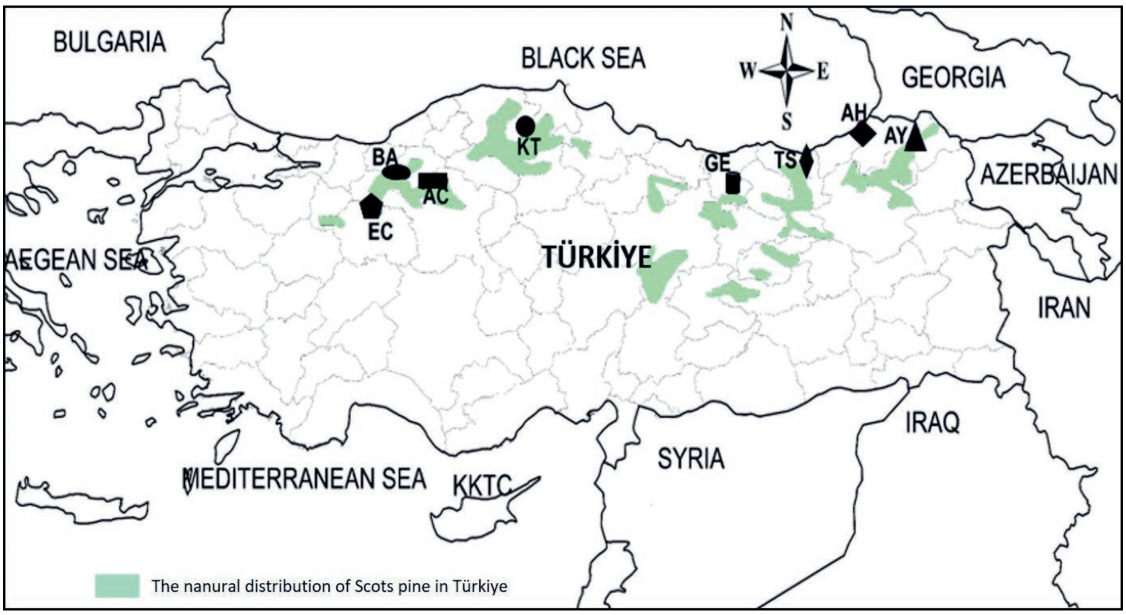

Figure 1. The distribution map of the Scots pine populations in Turkiye: ▲ – Ardahan-Yalnızçam (AY), ◆ – Artvin-Arhavi, Hopa (AH), ♦ – Trabzon-Sürmene (TS), ▮ – Giresun-Espiye (GE), ● – Kastamonu-Taşköprü (KT), ⬬ – Bolu-Aladağ (BA), ▬ – Ankara-Çamlıdere (AC), ⬟ – Eskişehir-Çatacık (EC) (Adapted from Anonymous 2009).

Slika 1. Karta rasprostranjenosti bijelog bora u Turskoj: Ardahan-Yalnızçam (AY), Artvin-Arhavi, Hopa (AH), Trabzon-Sürmene (TS), Giresun-Espiye, Kastamonu-Taşköprü, Bolu-Aladağ, Ankara-Çamlıdere, Eskişehir-Çatacık (Preuzeto iz Anonymous 2009).

Table 1. Eight analysed Scots pine stands distributed in Turkiye
Tablica 1. Ispitanih osam sastojina običnog bora raspoređenih u Turskoj

Populations (Acronym) Populacija	Individuals Jedinke	Altitudinal range (m) Visinske granice	Geographical region of Turkiye (Avcı, 2014) Geografsko područje	Habitat zones (Kantarcı (2005) Stanište
Ardahan-Yalnızçam (AY)	10	1900–2100 2100–2400	Eastern Anatolia	Habitat zone of the Kars
Artvin-Arhavi, Hopa (AH)	6	0–300 300–600	Black Sea	Habitat zone of the Rize – Kaçkar Mountains, Rize-Hopa Sub-Region
Trabzon-Sürmene (TS)	7	0–300 300–600	Black Sea	Habitat zone of the Trabzon Mountains
Giresun-Espiye (GE)	12	1600–1900 1900–2100 2100–2400	Black Sea	Habitat zone of the Canik – Giresun Mountains
Kastamonu-Taşköprü (KT)	9	1000–1300 1300–1600 1600–1900	Black Sea	Habitat zone of the Mountainous area
Bolu – Aladağ (BA)	6	1300–1600 1600–1900	Black Sea	
Ankara- Çamlıdere (AC)	7	1300–1600 1600–1900	Central Anatolia	Habitat zone of behind the Western Black Sea Region
Eskişehir-Çatacık (EC)	7	1000–1300 1300–1600	Central Anatolia	Habitat zone of the West Central Anatolia

(NW), cuticle thickness (CT), epidermis width (EW), epidermis thickness (ET), sclerenchyma width (SW), sclerenchyma thickness (ST), mesophiyll thickness (MT), resin canal number (CN), resin canal cell number (CCN), resin canal diameter (CD), central cylinder width (CW), central cylinder thickness (CCT), endodermis cell number (ECN), endodermis width (ENW) and endodermis thickness (ENT).

Cross sections of a needle (20–25 μm) were taken by a freezing microtome using a small part of the needle plade. All sections were stained with hematoxylin and fast green for 30 min and mounted in entellan to create permanent slides (Vardar 1987). Well stained sections were examined with an Olympus BX51 light microscope (LM). Sixteen anatomical traits were measured (µm) using a BX51 LM equipped with the Bs200Pro analysis system software. All measurements and observations were checked at least three or four times from sections taken from selected specimens of examined populations. A raw data matrix generated by the measurements of sixteen traits was used for statistical analysis. Minimal and maximal values of characters were determined, and arithmetical means, standard deviation and variation coefficients were calculated and analysed for each population and elevation limits (Table 2, Table 3). Analysis of variance (ANOVA) was performed to determine the differences between populations and between trees within populations. The relationship between average values of anatomical traits and altitude were tested using Spearman's coefficient (Sokal and Rohlf 2012). Multivariate statistical methods (cluster analysis and discriminant analysis) were used to identify structure of investigated populations. The cluster analysis resulted in a hierarchical tree, where the unweighted pair-group method with arithmetic mean (UPGMA) was used to join the clusters, and the Euclidean distance to define the distance between the studied populations. Principal component analysis was used to identify the best discriminating components and the best anatomical traits allowed the grouping of the investigated populations. Standardized data were used for the principal component analysis. The plot was constructed by two components (DF-1 and DF-2) showing analysed individuals (trees) and populations. The above statistical analyses were conducted using the SPSS Statistics 23.0 (Nie *et al.* 1975; IBM Corp 2015), SYNTAX 2000 (Podani 2001), and Past 3 (Hammer *et al.* 2001) statistical programs.

RESULTS
REZULTATI

Descriptive statistics of needle anatomical traits of the 64 trees belonging to eight natural Scots pine populations from Turkiye are given in Table 2. The highest mean values for NT, NW, EW, ET, MT, CW, CCT, ECN and ENT were observed in GE population (Figure 2). In contrast, the lowest mean values for NT, NW, ET, CCN, CW and CCT were observed in the EC and KT populations (Figure 2). Almost all measured needle anatomical traits correlated with each other at a statistically significant level. Using Spearman's correlation coefficient (rs), a highly positive correlation was found between altitude and the mesophile thickness of (rs: 0.83).

As a result of the ANOVA, significant differences in needle anatomical traits among the examined eight populations

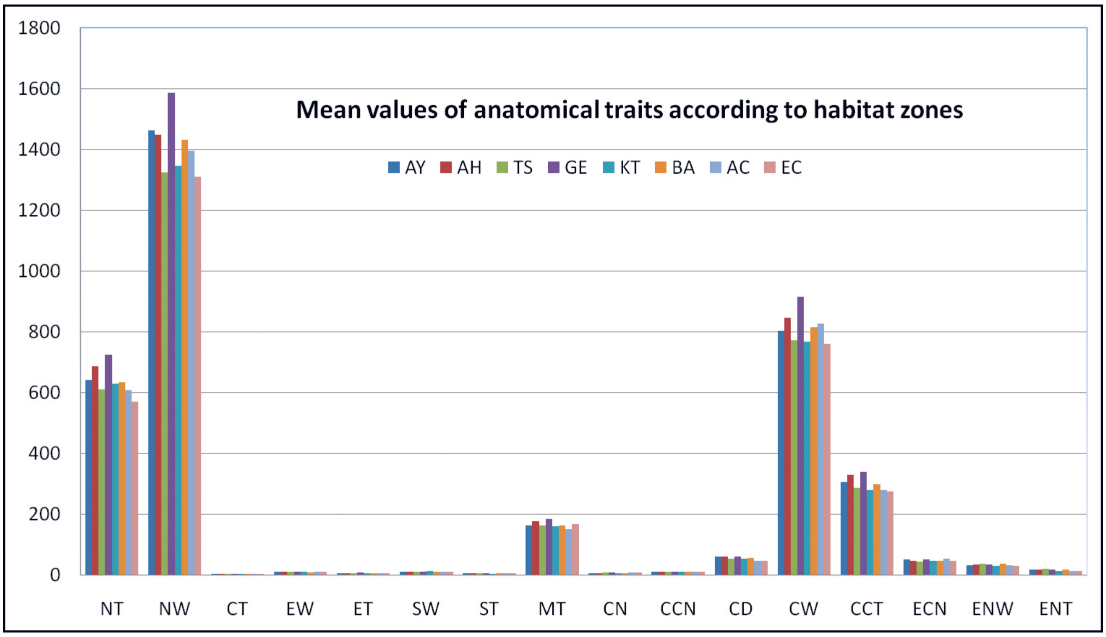

Figure 2. Mean valus of needle anatomical traits according to habitat zones of Kantarcı (2005).
Slika 2. Srednje vrijednosti anatomskih svojstava iglica prema zonama staništa u Kantarcu (2005)

Table 2. Descriptive statistical parameters of anatomical needle traits according to habitat zones (Kantarcı, 2005)
Tablica 2. Deskriptivni statistički parametri za anatomska svojstva iglica (μm) prema zonama staništa (Kantarcı, 2005)

Traits	Descriptive statistics	AY	AH	TS	GE	KT	BA	AC	EC
NT	Min	529.89	576.19	488.27	613.51	503.55	552.61	487.30	448.92
	Max	788.77	786.96	658.88	827.17	748.77	718.41	756.07	628.68
	Mean	642.43	687.53	611.87	725.65	628.91	633.63	607.44	570.20
	SD	79.39	74.35	60.40	83.44	71.97	66.52	89.54	61.35
	CV (%)	12.35	10.81	9.87	11.49	11.44	10.49	14.74	10.75
NW	Min	1134.19	1327.82	1196.29	1317.41	1092.70	1258.44	1176.37	1112.54
	Max	1719.75	1688.84	1402.41	1832.72	1537.39	1626.58	1627.72	1465.60
	Mean	1461.41	1448.79	1323.29	1586.69	1344.89	1431.47	1395.20	1309.25
	SD	179.60	132.17	79.40	149.69	144.30	121.87	171.47	122.86
	CV (%)	12.28	9.12	6.00	9.43	10.72	8.51	12.28	9.38
CT	Min	2.76	3.20	2.24	3.37	3.03	3.20	3.83	3.12
	Max	4.83	4.64	4.82	6.00	5.25	4.85	5.31	5.01
	Mean	3.84	3.79	3.61	4.34	4.35	4.03	4.32	4.12
	SD	0.69	0.56	0.81	0.77	0.72	0.57	0.48	0.74
	CV (%)	17.96	14.77	22.43	17.74	16.55	14.14	11.11	17.96
EW	Min	7.49	7.08	8.61	7.21	9.97	9.34	7.84	5.80
	Max	14.20	14.53	13.65	16.20	14.93	11.12	18.14	21.77
	Mean	11.36	11.83	10.96	12.27	12.23	10.23	11.19	11.49
	SD	1.98	2.78	1.86	2.74	1.61	0.86	3.24	5.11
	CV (%)	17.42	23.49	16.97	22.33	13.16	8.40	28.95	44.47
ET	Min	4.95	5.29	5.98	4.23	5.71	6.80	5.21	4.10
	Max	9.70	10.08	8.36	11.55	9.88	9.10	10.60	12.27
	Mean	6.97	7.48	7.09	8.10	7.33	7.71	7.74	6.62
	SD	1.43	1.61	1.00	2.14	1.36	0.92	1.76	2.72
	CV (%)	20.52	21.52	14.10	26.42	18.55	11.93	22.74	41.09
SW	Min	10.26	9.84	8.15	8.87	10.53	9.22	9.68	8.71
	Max	14.86	15.08	14.79	17.18	16.76	16.46	15.89	15.33
	Mean	12.62	11.29	11.83	12.32	12.72	12.32	11.21	11.79
	SD	1.59	1.93	2.37	2.70	1.99	2.47	2.20	2.21
	CV (%)	12.60	17.09	20.03	21.92	15.64	20.05	19.63	18.74

Traits	Descriptive statistics	AY	AH	TS	GE	KT	BA	AC	EC
ST	Min	4.40	4.65	5.01	3.35	3.87	4.18	4.51	3.56
	Max	7.65	7.40	6.64	9.28	6.76	8.11	7.44	9.28
	Mean	6.36	5.89	6.07	5.81	5.20	6.27	5.57	5.61
	SD	1.01	0.91	0.58	1.58	1.03	1.42	1.07	1.89
	CV (%)	15.88	15.45	9.56	27.19	19.81	22.65	19.21	33.69
MT	Min	107.98	125.09	127.35	138.60	132.50	141.70	119.54	140.37
	Max	211.46	200.55	220.73	230.20	193.08	203.70	190.88	192.32
	Mean	164.35	179.12	162.53	183.97	161.20	164.16	152.58	167.81
	SD	30.76	27.51	31.16	24.60	22.01	26.53	26.70	16.76
	CV (%)	18.72	15.36	19.17	13.37	13.65	16.16	17.50	9.99
CN	Min	3.75	7.25	5.00	6.25	3.00	5.50	7.00	6.25
	Max	7.50	8.75	10.75	10.75	10.00	11.50	11.00	10.50
	Mean	6.21	7.75	8.03	8.06	7.11	7.50	9.09	8.42
	SD	1.11	0.54	2.13	1.53	2.03	2.12	1.47	1.84
	CV (%)	17.87	6.97	26.53	18.98	28.55	28.27	16.17	21.85
CCN	Min	8.29	10.00	9.17	9.64	9.00	9.25	9.17	8.91
	Max	14.83	15.25	14.30	14.83	13.44	14.50	13.45	11.60
	Mean	12.56	12.25	11.59	11.93	11.18	11.18	11.38	10.29
	SD	1.81	1.97	1.81	1.56	1.39	2.36	1.67	0.87
	CV (%)	14.41	16.08	15.62	13.08	12.43	21.11	14.67	8.45
CD	Min	44.50	46.78	46.83	45.02	38.58	41.70	22.07	39.76
	Max	80.18	73.88	63.67	75.32	69.72	72.70	63.92	60.23
	Mean	62.51	61.45	54.31	61.59	54.96	56.33	46.68	46.84
	SD	12.09	9.83	6.38	9.00	10.30	13.83	13.27	6.88
	CV (%)	19.34	16.00	11.75	14.61	18.74	24.55	28.43	14.69
CW	Min	616.59	725.04	678.30	773.38	506.28	700.67	615.09	633.89
	Max	943.62	1054.10	889.69	1102.05	940.03	887.77	1017.71	815.37
	Mean	803.81	846.56	772.95	916.35	766.96	814.85	826.31	761.75
	SD	124.67	113.91	72.84	92.62	128.60	66.71	130.92	63.38
	CV (%)	15.51	13.46	9.42	10.11	16.77	8.19	15.84	8.32
CCT	Min	261.96	285.26	238.41	275.87	241.66	251.58	205.51	240.60
	Max	348.94	381.36	324.57	392.97	317.17	344.26	368.74	311.36
	Mean	306.21	330.31	288.29	338.55	280.40	299.19	281.08	275.36
	SD	33.27	34.10	32.74	38.97	29.31	35.55	54.35	22.75
	CV (%)	10.87	10.32	11.36	11.51	10.45	11.88	19.34	8.26
ECN	Min	42.25	42.00	41.50	41.50	32.75	39.00	45.50	40.25
	Max	59.00	58.00	54.75	61.00	54.50	51.75	64.25	50.75
	Mean	51.20	47.29	45.42	51.87	46.31	48.08	54.28	46.50
	SD	5.23	5.68	4.63	5.53	6.88	4.56	7.46	4.08
	CV (%)	10.21	12.01	10.19	10.66	14.86	9.48	13.74	8.77
ENW	Min	25.80	33.00	32.18	27.68	22.65	30.40	26.24	26.92
	Max	39.73	40.90	43.05	43.97	38.77	42.38	43.63	34.17
	Mean	32.28	36.42	37.64	36.35	29.93	36.86	33.43	30.78
	SD	4.61	3.33	3.39	4.63	4.20	4.25	5.34	3.05
	CV (%)	14.28	9.14	9.01	12.74	14.03	11.53	15.97	9.91
ENT	Min	12.87	14.08	16.77	14.94	12.06	14.58	12.05	11.62
	Max	24.38	19.89	25.31	23.81	19.58	24.51	16.97	16.72
	Mean	18.39	17.72	20.11	18.89	14.14	19.29	14.30	14.47
	SD	3.46	2.09	3.08	3.31	2.44	3.80	2.03	1.77
	CV (%)	18.81	11.79	15.32	17.52	17.26	19.70	14.20	12.23

selected according to habitat zones were found for NT, NW, CN, CD, CW, CCT, ECN, ENW and ENT characters (Table 3). However, no significant differences were found for CT, EW, ET, SW, ST, MT and CCN values.

According to Duncan's test results, in terms of needle width, AH, AY and BA were in the same group, TS, KT, AC and EÇ populations were in the same group and GE was in a different group. In terms of needle thickness, AY, TS, KT,

Table 3. Statistically significant results of variance analysis depending on habitat zones (P< 0.05*)
Tablica 3. Rezultati analize varijance ovisno o stanišnim zonama (Vrijednosti P<0,05* smatraju se statistički značajnima)

Needle traits Značajke iglica	Populations Populacija	İndividuals (n) Jedinke	Mean±Standard Deviation Srednja vrijednost±standardna devijacija	F	Significance Level (P) Nivo različitosti
NW	AY	10	1461,41±179,60 a,b	3,835	,002*
	AH	6	1448,80±132,17 a,b		
	TS	7	1323,29±79,40 a		
	GE	12	1586,70±149,70 b		
	KT	9	1344,90±144,30 a		
	BA	6	1431,47±121,88 a,b		
	AÇ	7	1395,20±171,48 a		
	EÇ	7	1309,25±122,86 a		
	Total	64	1425,53±165,74		
NT	AY	10	642,43±79,39 a,b	3,753	,002*
	AH	6	687,53±74,35 b,c		
	TS	7	611,87±60,40 a,b		
	GE	12	725,65±83,44 c		
	KT	9	628,91±71,97 a,b		
	BA	6	633,63±66,52 a,b		
	AÇ	7	607,44±89,54 a,b		
	EÇ	7	570,20±61,35 a		
	Total	64	644,47±85,99		
CN	AY	10	6,21±1,11 a	2,322	,037*
	AH	6	7,75±0,54 a,b,c		
	TS	7	8,03±2,13 a,b,c		
	GE	12	8,06±1,53 a,b,c		
	KT	9	7,11±2,03 a,b		
	BA	6	7,50±2,12 a,b,c		
	AÇ	7	9,09±1,47 c		
	EÇ	7	8,42±1,84 b,c		
	Total	64	7,70±1,78		
CD	AY	10	62,51±12,09 b	2,891	,012*
	AH	6	61,45±9,83 b		
	TS	7	54,31±6,38 a,b		
	GE	12	61,59±9,00 b		
	KT	9	54,96±10,30 a,b		
	BA	6	56,33±13,83 a,b		
	AÇ	7	46,68±13,27 a		
	EÇ	7	46,84±6,88 a		
	Total	64	56,25±11,46		
CCT	AY	10	306,21±33,27 a,b	3,796	,002*
	AH	6	330,31±34,10 b		
	TS	7	288,29±32,74 a		
	GE	12	338,55±38,97 b		
	KT	9	280,40±29,31 a		
	BA	6	299,19±35,55 a,b		
	AÇ	7	281,08±54,35 a		
	EÇ	7	275,36±22,75 a		
	Total	64	302,16±41,31		
CW	AY	10	803,81±124,67 a,b	2,393	,032*
	AH	6	846,56±113,91 a,b		
	TS	7	772,95±72,84 a		
	GE	12	916,35±92,62 b		
	KT	9	766,96±128,60 a		
	BA	6	814,85±66,71 a,b		
	AÇ	7	826,31±130,92 a,b		
	EÇ	7	761,75±63,38 a		
	Total	64	819,26±112,38		

Needle traits Značajke iglica	Populations Populacija	İndividuals (n) Jedinke	Mean±Standard Deviation Srednja vrijednost±standardna devijacija	F	Significance Level (P) Nivo različitosti
ECN	AY	10	51,20±5,23 a,b	2,508	,026*
	AH	6	47,29±5,68 a		
	TS	7	45,42±4,63 a		
	GE	12	51,87±5,53 a,b		
	KT	9	46,31±6,88 a		
	BA	6	48,08±4,56 a,b		
	AÇ	7	54,28±7,46 b		
	EÇ	7	46,50±4,08 a		
	Total	64	49,17±6,11		
ENT	AY	10	18,39±3,46 b	5,614	,000*
	AH	6	17,72±2,09 b		
	TS	7	20,11±3,08 b		
	GE	12	18,89±3,31 b		
	KT	9	14,14±2,44 a		
	BA	6	19,29±3,80 b		
	AÇ	7	14,30±2,03 a		
	EÇ	7	14,47±1,77 a		
	Total	64	17,22±3,57		
ENW	AY	10	32,28±4,61 a,b	3,924	,002*
	AH	6	36,42±3,33 b,c		
	TS	7	37,64±3,39 c		
	GE	12	36,35±4,63 b,c		
	KT	9	29,93±4,20 a		
	BA	6	36,86±4,25 b,c		
	AÇ	7	33,43±5,34 a,b,c		
	EÇ	7	30,78±3,05 a		
	Total	64	34,08±4,89		

Letters (a,b,c) showed the significant differences ($P \leq 0.05$) among populations.

BA and AÇ were in the same group. In terms of resin canal number, AH, TS, GE and BA were in the same group. There were 3 different groups in terms of central cylinder width. First was AY, AH, BA, AÇ, second was TS, KT, EÇ, third was GE.

ANOVA resulted in significant differences in CD, ENW and ENT needle anatomical characters among the seven elevation limits (Table 4). However, no significant results were found for NT, NW, CT, EW, ET, SW, ST, MT, CN, CCN, CW, CCT and ECN characters.

The structure of the 64 individuals and eight populations of Scots pine inferred by the cluster analysis (UPGMA) are presented with the hierarchical tree (Figure 3). These results clearly show that trees collected from the same habitat zones are not clustered together however they (64 individuals) are divided into more than seven distinct subclusters belonging to different habitat zones (Figure 3A). However, the structure of eight Scots pine populations inferred by the cluster analysis clearly indicated that studied populations can be divided into three distinct subclusters (Figure 3B). The first subcluster only consisted of GE population and the second subcluster consisted of TS, KT and EC populations. Finally, the third sub-cluster consisted of the remaining five populations (BA, AY, AC and AH). As seen in Figure 2B, the most similar populations were BA and AY, and the most distinct population was GE.

The results of the discriminant analysis are presented in two-dimensional plot in the Figure 3. The first discriminant function (DF-1) explained 90.03% of the total variation, and the second discriminant function (DF-2) explained 5.15%. The discriminant analysis showed that the sixty-four trees from eight natural populations of Scots pine in Turkiye can not be clearly separated based on needle anatomical properties. PCA also verified that some anatomical traits are more important in delimiting the investigated taxa (Letters at the end of bars at Figure 4). According to Figure 4, the needle anatomical traits contributing most to separation of examined population are NW, CW, NT and CCT which is also among the significant traits revealed by ANOVA.

DISCUSSION AND CONCLUSION
RASPRAVA I ZAKLJUČCI

Altitude and climate have a decisive influence on the distribution of plant taxa. The altitude is one of the most im-

Table 4. Statistically significant results of the Variance analysis depending on altitude (P< 0.05*)
Tablica 4. Rezultati analize varijance ovisno o nadmorskoj visini (vrijednosti P<0,05* smatrane su statistički značajnim)

Needle Characters Karakteristike iglica	Altitude (asl.) Visina	İndividuals (n) Jedinke	Mean±Standard Deviation Srednja vrijednost±standardna devijacija	F	Significance Level (P) Nivo različitosti
CD	0-300	7	57,6543±9,26066 a,b,c	2,889	,016*
	300-600	6	57,5533±8,70441 a,b,c		
	1000-1300	8	48,0150±8,42505 a		
	1300-1600	13	51,3592±12,73242 a,b		
	1600-1900	14	55,9364±11,38879 a,b,c		
	1900-2100	8	60,0086±8,05295 b,c		
	2100-2300	8	67,0900±11,19489 c		
	Total	64	56,2592±11,46764		
ENT	0-300	7	7,0557±0,82609 c	3,434	,006*
	300-600	6	7,5317±1,71918 b,c		
	1000-1300	8	6,6790±1,29544 a		
	1300-1600	13	7,4708±2,11700 a,b		
	1600-1900	14	8,0214±1,67281 b,c		
	1900-2100	8	7,0753±1,52559 b,c		
	2100-2300	8	7,5700±2,26304 b,c		
	Total	64	7,4155±1,71150		
ENW	0-300	7	11,5043±2,23267 c	2,705	,022*
	300-600	6	11,2050±2,52793 c		
	1000-1300	8	11,1162±2,14756 a		
	1300-1600	13	11,8654±3,58906 a,b		
	1600-1900	14	11,6979±2,99878 b,c		
	1900-2100	8	11,6380±2,71304 a,b,c		
	2100-2300	8	11,4063±1,94554 a,b,c		
	Total	64	11,5479±2,66579		

Letters (a,b,c) showed the significant differences (P ≤ 0.05) among population.

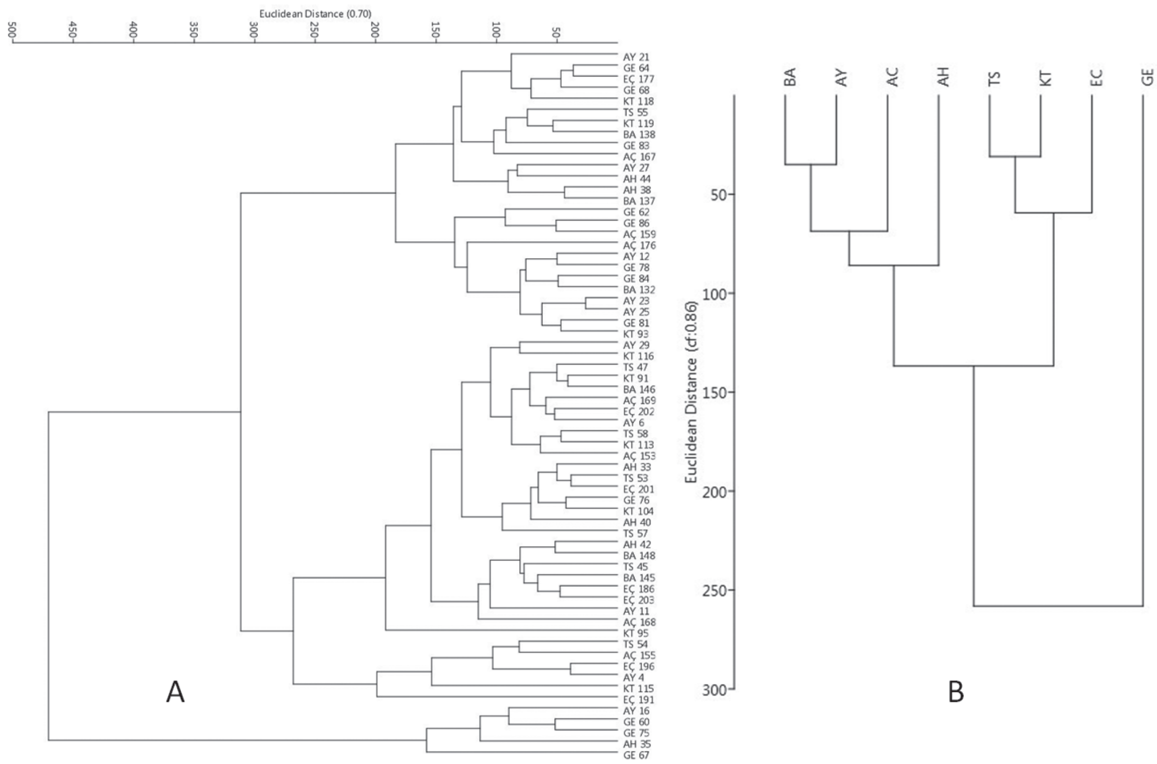

Figure 3. Horizontal hierarchical tree diagram of trees (A) and populations (B) based on needle anatomical traits (cf: cophenetic correlation coefficient). The letters before the sample number at Figure 3A (AY, AH, TS, GE, KT, BA, AC, EC) indicate the population from which the tree was collected.
Slika 3. Horizontalni hijerarhijski dijagram stabala (A) i populacija (B) na temelju anatomskih svojstava iglica (usp.: kofenetski korelacijski koeficijent).

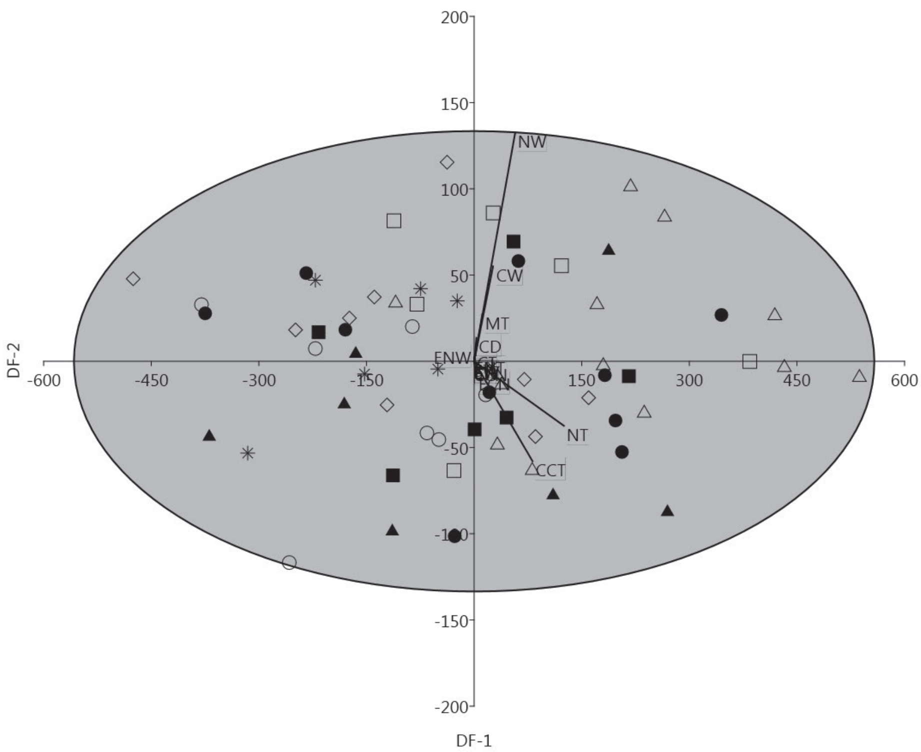

Figure 4. Scatterplot of the principal component scores of investigated populations for the first two discriminant functions (Dot: AY, Square: AH, Star: TS, Triangle: GE, Diamond: KT, Filled square: BA, Filled triangle: AC, Circle: EC, NW: needle width, CW: central cylinder width, NT: needle thickness and CCT: central cylinder thickness)

Slika 4. Dijagram raspršenosti rezultata glavne komponente istraživanih populacija za prve dvije diskriminantne funkcije (točka: AY, kvadrat: AH, zvijezda: TS, trokut: GE, dijamant: KT, popuni kvadrat: BA, popuni trokut: AC, krug : EC)

portant factors, and is revealed various climate types. Vegetation can be changed by climatic differences in a very short distances (Duran and Günek 2010; Randin et al. 2009). As a result of this, some morphological and anatomical changes occur on the plants (Husain 2016). For instance, Hengxiao *et al.* (1999) report the changes of needle anatomy of *Pinus yunnanensis* Franch depending on the altitude. In this study, epidermal thickness and resin canal diameter showed statistically significant differences in upper (2000 asl.), middle (1850 asl.) and lower altitude (1700 asl.). Meicenheimer *et al.* (2008) examined the *Pinus nigra* Arnold and *P. resinosa* Ait. taxa in terms of needle anatomy. The authors determined the epidermal cell thickness varied depending on altitude. Tiwari *et al.* (2013) determined that the anatomical traits of needles are affected by environmental factors and they were directly correlated with altitude. In addition, they pointed out that the number of resin channels and the location of resin channels vary significantly when going from a low altitude to a high altitude. Similarly, the present study showed that the needles from different altitude levels of the research areas (0-300 asl., 300-600 asl., 1000-1300 asl., 1300-1600 asl., 1600-1900 asl., 1900-2100 asl., 2100-2400 asl.) had significant differences according to anatomical characters (resin canal diameter, endodermis thickness, and endodermis width) depending on altitude.

Previous studies reported the morphological differences depending on the habitat conditions which was supported by anatomical studies (Niinemets and Lukjanova 2003; Lukjanova and Mandre 2006). Donahue and Upton (1996) investigated the *Pinus greggii* Engelm., in terms of needle anatomy and determined the significant differences between the populations of the colder northern part of Mexico and the southern part of the country, depending on the climate and altitude. Nikolić *et al.* (2016) determined the significant statistical differences on needle anatomy and morphology according to habitat, as a result of their morphological and anatomical studies on the needles of *Pinus heldreichii* in the regions of Montenegro and Serbia. Their study also statistically determined that the resin canal diameter and the width of endodermis were affected by both altitudinal and climatic difference. Bączkiewicz *et al.* (2005) examined the *Pinus mugo* Turra species in terms of thirteen quantitative needle characteristics (resin canal number, leaf thickness and width, feature of epidermal cell etc.) and they found that the populations were significantly different from each other, but the variation within the populations was low and similar, regardless of habitat types. Luomala *et al.* (2005) found in their studies about the needle anatomy of Scots pine that adaptations developed in the anatomical structure of the needle according to the habitat. The needle surface area and the needle width are affected by different habitat conditions and different altitudes (Soudani *et al.* 2002; Schoettle and Rochelle 2000; Xiao 2003). Similarly, this study suported the influence of habitat and elevation limits on the needle anatomical properties in Scots pine populations distributed in Turkiye (Table 4).

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SAŽETAK

U ovoj studiji proučavano je osam populacija običnog bora iz Turske, kako bi se istražio utjecaj zona staništa I admorske visine na anatomske značajke iglica. Svjetlosnim mikroskopom promatrano je ukupno 496 iglica uzorkovanih sa 64 stabla, s ciljem utvrđivanja varijabilnosti dieciséis anatomskih karakteristika. Analiza varijance pokazuje da postoje značajne razlike u debljini iglica, ***širini*** iglica, broju smolnih kanala, promjeru smolnih kanala, ***širini*** središnjeg cilindra, debljini središnjeg cilindra, broju stanica endoderme, ***širini*** endoderme i debljini endoderme u osam populacija ovisno o zonama staništa. Međutim, jedino se promjer smolnog kanala, ***širina*** endodermisa i debljina endodermisa značajno razlikuju u ispitivanoj populaciji, ovisno o visinskim gradijentima. Klasterska analiza pokazala je najveće sličnosti između populacija Bolu-Aladağ i Ardahan-Yalnızçam, a najistaknutija populacija bila je populacija Giresun-Espiye na temelju anatomskih značajki iglica. Iako je analiza glavnih komponenti pokazala da ***širina*** iglice, ***širina*** središnjeg cilindra, debljina iglice i debljina središnjeg cilindra imaju najveći utjecaj na razlikovanje populacija običnog bora rasprostranjenih u Turskoj, diskriminantnom analizom ispitivane populacije nisu razdvojene uzimajući u obzir anatomske značajke iglica.

KLJUČNE RIJEČI: Anatolija, nadmorska visina, anatomija iglice, *Pinus sylv*estris, varijabilnost.