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POPULATION VARIABILITY OF SCOTS PINE (*PINUS SYLVESTRIS* L.) IN TURKEY ACCORDING TO THE NEEDLE MORPHOLOGY

VARIJABILNOST POPULACIJA OBIČNOG BORA (*PINUS SYLVESTRIS* L.) U TURSKOJ PREMA MORFOLOŠKIM OBILJEŽJIMA IGLICA

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SUMMARY

In the present study, needle variation of Scots pine (*Pinus sylvestris* L., Pinaceae) populations in Turkey was investigated. From selected eight populations, a total of 1314 needles belonging to 206 trees were examined. Four morphological needle traits were measured and analyzed to describe the population diversity and differentiation. Analyzed morphological traits showed significant variability. The trees within populations differ significantly in all analyzed needle characteristics, while the differences between populations were significant for the three of four studied characteristics. Present findings revealed that needle length, needle width and the ratio of needle length to needle width showed clinal variation in response to altitudinal gradients. Populations from higher altitudes were characterized with the smaller and wider needles as compared to the populations from lower altitudes. The results of this study could be valuable baseline data for the development of more efficient management plans for this forest tree species.

KEY WORDS: Scots pine, population variability, needle characteristics, morphometric analysis, clinal variation

INTRODUCTION
UVOD

Scots pine (*Pinus sylvestris* L., family Pinaceae) is one of the most important timber and forest tree species globally (Koprowski *et al.* 2012). It has a very wide distribution in Europe and Asia due to its high degree of ecological tolerance (Alemdağ 1967; Pehlivan 2010). The tree is tolerant to poor soils, drought, and frost. It is a pioneer species, able to colonize nutrient-poor soils in disturbed areas (Mátyás *et al.* 2004; Houston Durrant *et al.* 2016). Scots pine frequently grows in large single species stands in altitudes ranging from sea level up to 2600 MASL. However, across its huge range it may also be found in mixed stands with most of the boreal tree species of Europe and Asia.

Scots pine is the third-most dominant conifer tree species in Turkey (Kandemir and Mataracı 2018). Its distribution in Turkey extends from Pınarbaşı to Ayancık in the longitudinal, and Orhaneli to Kağızman in the latitudinal directions (Saatçioğlu 1944; Kayacık 1954; Pamay 1962). The geographical limits of the southeastern distribution of Scots pine have been reported in the Pınarbaşı district of the Kayseri province of Turkey (Demirci 2006; Pehlivan 2010). *P.*

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sylvestris grows from sea level (along the Black Sea) to 3125 MASL in the Allahuekber Mountains of Eastern Anatolia (Eliçin 1971).

Wide geographical distribution resulted with considerable morphological and genetic diversity within *P. sylvestris* (Wright and Bull 1963; Pravdin 1969; Ruby 1967; Mirov 1967; Szmidt 1984; Wang *et al.* 1991; Androsiuk *et al.* 2011; Jasińska *et al.* 2014; Dering *et al.* 2017). Variation observed in its needle and cone characteristics resulted in the description of several subspecies and varieties (Gaussen 1960; Mirov 1967; Farjon 2008; Jasińska *et al.* 2014). Farjon (2008) reported the existence of three varieties of *P. sylvestris*: var. *sylvestris*, var. *mongolica* Litv., and var. *hamata* Steven. According to Farjon (2008), the distribution range of the var. *sylvestris* and var. *hamata* extend through Turkey. In addition, Kandemir and Mataracı (2018) described a new variety, var. *elicinii* Kandemir and Mataracı, from Turkey mainly based on needle length and color.

Jasińska *et al.* (2014) detected morphological differences between the East and West Anatolian populations of *P. sylvestris*. Similar results were also reported by Bilgen and Kaya (2007). The isolation of the eastern from western Anatolian populations was explained by the mountain ranges known as the "Anatolian diagonal". In addition, Jasińska *et al.* (2014) stated that morphological pattern of diversity in Anatolian populations of the Scots pine may also be a result of: (1) another origin source - the western populations from the Balkans and the eastern ones from the Caucasus; and (2) different rates of evolution in the two regions. Furthermore, Dering *et al.* (2017) revealed strong spatial genetic structure within the Scots pine range, involving four distinct groups well related to the LPG refugial areas previously defined for this species (Naydenov *et al.* 2007; Pyhäjärvi *et al.* 2008; Sinclair *et al.* 1998, 1999). Authors revealed that two most spatially restricted groups of populations correspond to Scots pine refugia located on the Iberian and Asia Minor Peninsulas. Those populations represent the valuable relict genetic resources that are of high conservation priority (Naydenov *et al.* 2007; Pyhäjärvi *et al.* 2008; Dering *et al.* 2017).

The aim of the present study is to assess needle size variation among Scots pine populations of Turkey and identify relationships with respect to altitude.

MATERIAL AND METHODS
MATERIJAL I METODE

Samples for morphometric analysis were collected from eight natural Scots pine populations in Turkey (Table 1). Needles were sampled from 14 to 31 trees per population, and each individual tree was represented by 5 to 10 needles from well-grown shoots. In total 1314 needles belonging to 206 individuals were analyzed. The following traits were included in the analysis: needle length, needle width, needle length/needle width ratio, and sheath length.

Minimal and maximal values of characteristics were determined, and arithmetical means, standard deviation and variation coefficients were calculated and analyzed for each population. Analysis of variance (ANOVA) was performed to determine the statistically significant differences between populations and between trees within populations.

The relationship between average values of morphological needle traits and altitude (e.g. Krauze-Michalska and Boratyńska 2013; Poljak et al. 2015, 2018) were tested using Spearman's coefficient (Sokal and Rohlf 2012).

Multivariate statistical methods were used to identify the population differentiation (McGarigal *et al.* 2000; Zebec *et al.* 2010; Poljak *et al.* 2012, 2018): cluster analysis and discriminant analysis. The conducted cluster analysis resulted in a hierarchical tree, where the unweighted pair-group method with arithmetic mean (UPGMA) was used to join

Table 1. Sampled populations.
Tablica 1. Uzorkovane populacije.

Populations Populacije	Total number of trees Ukupan broj stabala	Nadmorska visina (m) Altitude (m)	Geographical region of Turkey (Kantarcı 2005) Geografska regija Turske (Kantarcı 2005)	Habitat zones (Kantarcı 2005) Stanišne zone (Kantarcı 2005)
Ardahan-Yalnızçam (AY)	30	1850-2300	Eastern Anatolia	Habitat zone of the Kars
Artvin-Arhavi, Hopa (AH)	14	0-600	Black Sea	Habitat zone of the Rize - Kaçkar Mountains, Rize-Hopa Sub-Region
Trabzon-Sürmene (TS)	15	0-450	Black Sea	Habitat zone of the Trabzon Mountains
Giresun-Espiye (GE)	30	1600-2200	Black Sea	Habitat zone of the Canik - Giresun Mountains
Kastamonu-Taşköprü (KT)	30	1200-1800	Black Sea	Habitat zone of the Mountainous area
Bolu – Aladağ (BA)	31	1200-1800	Black Sea	
Ankara- Çamlıdere (AC)	26	1400-2000	Central Anatolia	Habitat zone of behind the Western Black Sea Region
Eskişehir-Çatacık (EC)	30	1200-1800	Central Anatolia	Habitat zone of the West Central Anatolia

Table 2. Descriptive statistical parameters for measured morphological traits. Needle length (NL), needle width (NW), ratio of needle length to width (NL/NW), sheath length (SL), standard deviations (SD), coefficients of variability (CV).

Tablica 2. Deskriptivni statistički parametri za mjerene morfološke značajke. Dužina iglice (NL), širina iglice (NW), omjer dužine i širine iglice (NL/NW), dužina rukavca (SL), standardna devijacija (SD), koeficijent varijabilnosti (CV).

Trait Značajka	Statistical parameters Deskriptivni pokazatelji	Populations Populacije							
		AY	AH	TS	GE	KT	BA	AC	EC
NL	mean	5.41	7.09	7.59	4.49	4.74	4.25	4.34	5.13
	max	7.70	9.50	13.20	7.45	11.10	6.40	6.40	7.10
	min	3.50	5.50	4.10	2.10	2.55	2.40	1.85	3.40
	SD	2.97	2.82	6.43	3.78	6.05	2.82	3.22	2.62
	CV (%)	54.90	39.77	84.72	84.19	127.64	66.35	74.19	51.07
NW	mean	1.56	1.42	1.37	1.64	1.32	1.43	1.38	1.30
	max	2.04	1.94	1.73	2.26	1.82	1.83	2.07	1.84
	min	1.27	1.24	1.18	1.13	1.06	1.14	1.07	0.93
	SD	.54	.49	.39	.80	.54	.49	.71	.64
	CV (%)	34.62	34.51	28.47	48.78	40.91	34.27	51.45	49.23
NL/NW	mean	3.45	5.00	5.57	2.71	3.55	2.97	3.14	3.95
	max	4.83	6.44	6.93	3.91	6.97	4.36	4.52	6.23
	min	2.38	3.35	2.83	1.47	2.24	1.65	1.54	2.73
	SD	1.73	2.18	2.90	1.73	3.34	1.92	2.11	2.47
	CV (%)	50.14	43.60	52.06	63.84	94.08	64.65	67.20	62.53
SL	mean	4.83	5.22	4.95	5.32	4.91	4.79	4.61	4.96
	max	15.42	9.45	8.36	12.81	9.24	11.03	11.62	10.58
	min	1.82	2.85	2.03	2.14	2.12	1.25	1.52	2.17
	SD	9.62	4.67	4.48	7.54	5.03	6.92	7.14	5.95
	CV (%)	199.17	89.46	90.51	141.73	102.44	144.47	154.88	119.96

the clusters, and the Euclidean distance to define the distance between the studied populations. For the discriminant analysis, standardized data were used. The plot was constructed by two discriminant functions showing analyzed individuals 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 3x (Hammer et al. 2001) statistical programs.

RESULTS
REZULTATI

Average values of needle characteristics of the 206 trees belonging to eight natural Scots pine populations from Turkey are given in Table 2. The highest mean values for needle length were observed in the two eastern populations TS and AH. In contrast, the shortest needles were observed in the AÇ, GE and BA populations, respectively. Furthermore, the longest sheaths were observed in AH and GE populations, and the widest needles in AY and GE populations. In addition, the highest values for the ratio of needle length to needle width were observed in the populations AH and TS, and the lowest for the population GE.

As expected, strong correlations between needle morphological traits were observed. Almost all measured needle traits correlated with each other at a statistically significant level. Using Spearman's correlation coefficient, a highly positive correlation was found between altitude and needle width. On the other hand, needle length and the ratio of needle length to needle width were highly negatively correlated with the altitude.

The ANOVA revealed significant differences with respect to needle properties among the eight populations examined, with the exception of sheath length, which did not significantly differ among tested populations. The trees within populations differ significantly for all studied variables.

The structure of the eight Scots pine populations was inferred by the cluster analysis. The results are presented with the hierarchical tree (Figure 1), where the unweighted pair-group method with arithmetic mean (UPGMA) was used to join the clusters. The results clearly indicated that studied populations can be divided into three distinct sub-clusters. The first sub-cluster consisted only of GE population. The second sub-cluster consisted of AH and TS populations. Finally, the third sub-cluster consisted of the

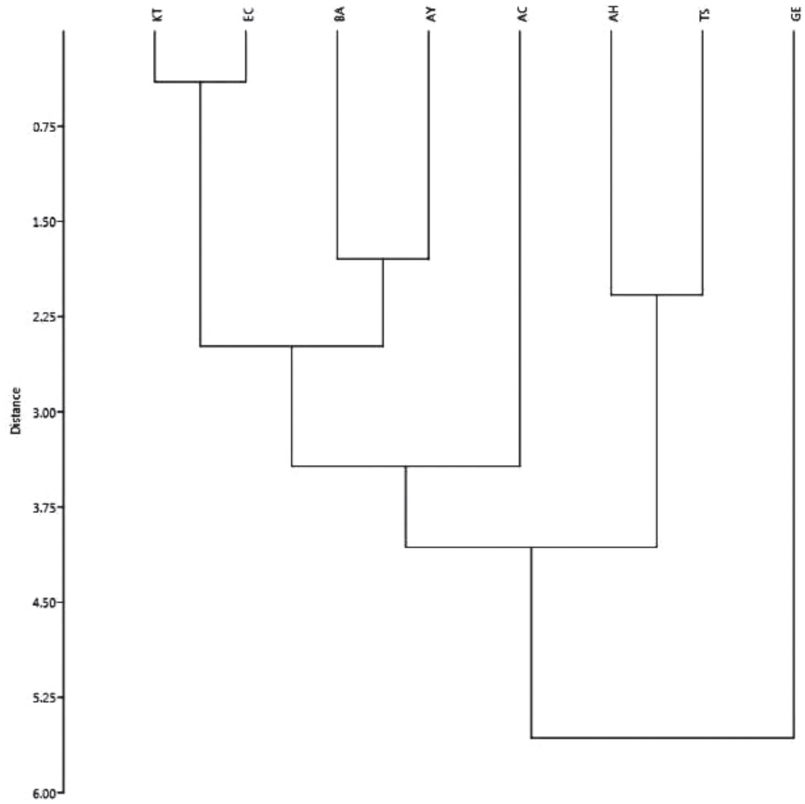

Figure 1. Horizontal hierarchical tree diagram.
Slika 1. Horizontalno hijerarhijsko stablo.

remaining five populations: AC, AY, KT, EC, BA. As seen in Figure 1, the most similar populations were KT and EC, and the most distinct population was GE.

The results of the discriminant analysis are presented in two-dimensional plot in the Figure 2. The first discriminant function explained 65.2% of the total variation, and

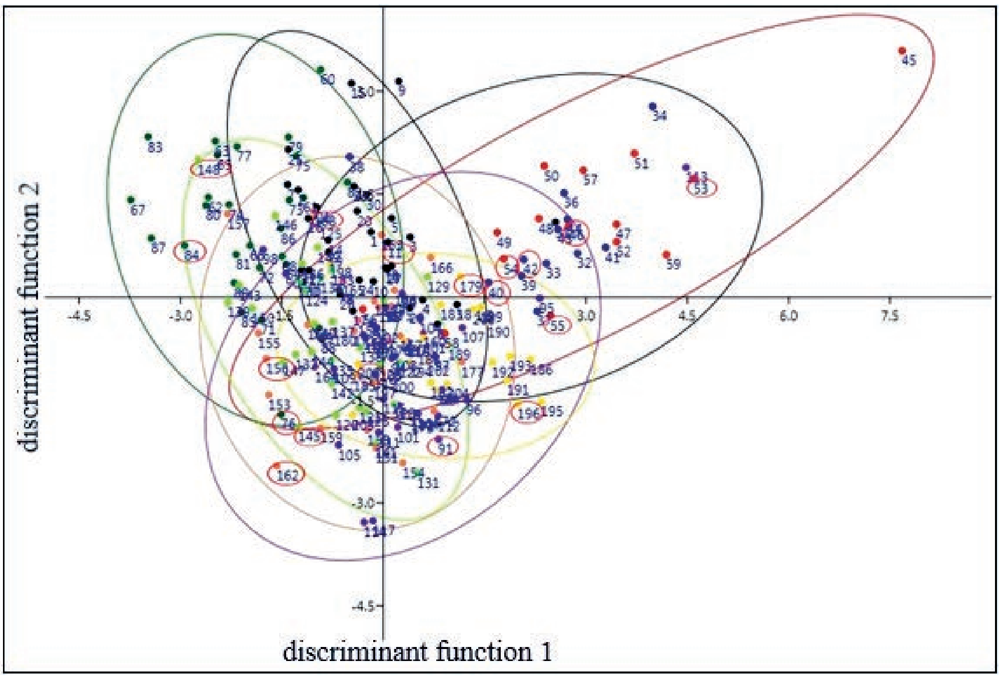

Figure 2. Scatterplot of the canonical scores of researched populations for the first two discriminant functions. Black: AY, Blue: AH, Red: TS, Dark green: GE, Blue violet: KT, Chartreuse: BA, Coral: AC, Gold: EC.
Slika 2. Projekcija kanoničkih vrijednosti istraživanih populacija u prostor prvih dviju diskriminantnih funkcija. Crna: AY; Plava: AH; Crvena: TS; Tamnozelena: GE; Plavo-ljubičasta: KT; Chartreuse: BA; Koraljno-crvena: AC; Zlatna: EC.

the second discriminant function explained 29.0%. The discriminant analysis showed that the trees from eight natural populations of Scots pine in Turkey cannot be clearly separated.

DISCUSSION AND CONCLUSIONS
RASPRAVA I ZAKLJUČCI

The conducted research established significant variability of the morphological characteristics of Scots pine populations in Turkey. Statistically significant differences between the trees within and between populations were confirmed for all studied characteristics, except for the sheath length. In general, the morphological traits of needles appeared to be a useful tool for estimating pine species variability (Irvine *et al*. 1998; Niinemets *et al*. 2001; Pensa *et al*. 2004; Pravdin 1969; Żelawski and Niwiński 1966; Paule 1971; Urbaniak *et al*. 2003; Androsiuk *et al*. 2011; Jasińska *et al*. 2014; Poljak *et al*. 2020).

It is well known that phenotypic differences among populations are often a result of the environmental distances between populations (Dewoody *et al*. 2015; Poljak *et al*. 2012, 2014, 2018; Zebec *et al*. 2014). This is because the distribution of plant species depends highly on their competitive abilities to respond to environmental factors (Schoettle and Rochelle 2000). In some cases, morphological variability can be related to the altitude (Friend and Woodward 1990; Schoettle and Rochelle 2000; Poljak *et al*. 2015, 2018, 2020; Zebec *et al*. 2015). Specific gradient i.e. changes in morphological variability related to the change in altitude have been reported for Scots pine populations in Turkey (Turna and Güney 2009) and Croatia (Poljak *et al*. 2020). The mentioned authors stated that populations from lower altitudes had smaller cones as compared to the populations from higher altitudes. The present study revealed that needle length, needle width and the ratio of needle length to needle width significantly vary depending on altitude. In general, we reveled that populations from lower elevations were characterized with lager needles than the populations from higher altitudes. This may be related to the capacity of trees to adapt to environmental variation, which causes morphological changes in plant species and also facilitates the successful survival of plants subjected to new environmental conditions (Abrams 1990; Ellsworth and Reich 1992; Kubiske and Abrams 1992; Lei and Lechowicz 1997; Teklehaimanot *et al*. 1998; Aranda *et al*. 2001). Similarly, needle length of *P. roxburghii* Sarg. from the northwestern Indian Himalayas significantly correlated with altitude (Tiwari *et al*. 2013). Furthermore, differences in the morphological and anatomical properties of cones, needles and seeds along altitudinal and longitudinal gradients were reported in four populations of *P. brutia* Ten. by Dangasuk and Panetsos (2004). In addition, Xu *et al*. (2016) noted that needle length and the ratio of needle length to fascicle sheath length showed clinal variation in response to latitudinal and altitudinal gradients in *P. yunnanensis* Franch.

The results of the cluster and discriminant analysis did not confirmed divergence between the populations from different habitat zones from Turkey. Moreover, microclimatic effects that depended on existing geological structures, even when very short distances are considered, can result with significant interpopulation variability of Scots pine populations in Turkey (Ergül Bozkurt 2017). According to Kantarcı (2005), vicinities in which Bolu, Kastamonu, Ankara and Eskişehir are found share similar ecological conditions. However, samples collected from these localities were not distinctly separated from the rest of the samples examined via multivariate statistical analysis.

According to reports within the Flora of Turkey and the East Aegean Islands (Davis *et al*. 1984), only *P. sylvestris* var. *hamata* is naturally distributed in Turkey. However, Farjon (2008) noted that *P. sylvestris* var. *sylvestris* and *P. sylvestris* var. *hamata* are naturally distributed in Turkey. In addition, Kandemir and Mataracı (2018), described a new variety of *P. sylvestris*, var. *elicinii* Kandemir and Mataracı, based on needle length from a Sürmene-Çamburnu population (TS). However, average, minimum, and maximum values of needle length of the TS population are closely related to Artvin-Arhavi (AH) and Kastamonu-Taşköprü (KT) populations. Additionally, present findings inferred from multivariate statistical analysis did not support the separation of TS population. In general, our result does not support the validity of different subspecies and variates of Scots pine in Turkey. In addition, Jasińska *et al*. (2014) reported that the morphological needle and cone characteristics of *P. sylvestris* varied among the populations of Iberia, Anatolia, the Balkans, and Crimea. Nevertheless, their results did not confirm the existence of *P. sylvestris* subsp. *sylvestris* and *P. sylvestris* subsp. *hamata*.

We observed significant phenotypic differentiation of studied populations of *P. sylvestris* in Turkey. Those populations represent the valuable relict genetic resources that are of high conservation priority. To confirm the conclusions reached on the variability of the Scots pine populations obtained by morphometric methods, the research also needs to be extended to molecular methods.

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SAŽETAK

U radu je istražena varijabilnost prirodnih populacija običnoga bora (*Pinus sylvestris* L., Pinaceae) u Turskoj s obzirom na morfologiju iglica. U istraživanje je ukupno uključeno osam populacija, 206 stabala i 1314 iglica. Kako bi se utvrdila raznolikost i strukturiranost populacija izmjerene su i analizirane četiri značajke iglica. Istraživanjem je utvrđena značajna varijabilnost te da se istraživane populacija, kao i stabla unutar populacija, statistički značajno razlikuju. Izuzetak čini značajka dužina rukavca za koju nisu potvrđene razlike na međupopulacijskoj razini. Dobiveni rezultati također upućuju na to da značajke dužina i širina iglice te odnos dužine i širine iglice pokazuju klinalnu varijabilnost s obzirom na nadmorsku visinu. Populacije s viših nadmorskih visina odlikovale su se kraćim i debljim iglicama u odnosu na populacije s nižih nadmorskih visina. Općenito, rezultati ovog istraživanja mogu poslužiti kao vrijedna osnova za određivanje i razvijanje smjernica za učinkovitije planove gospodarenja ovom važnom šumskom vrstom drveća.

KLJUČNE RIJEČI: obični bor, populacijska varijabilnost, svojstva iglica, morfometrijska analiza, klinalna varijabilnost