Differentiation of Scots pine (*Pinus sylvestris* L.) populations in the Tatra Mountains based on needle morphological traits

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Abstract: We investigated the variation of five needle morphological traits of the Scots pine (*Pinus sylvestris* L.) in four populations from the Tatra Mts. The greatest variation was found in needle length, number of stomatal rows, both on the adaxial and abaxial side of leaf, whereas the number of stomata per 2 mm of the needle length, for both sides of the leaf, showed only small intrapopulational variation. The results obtained by discriminant analysis show the distinct character of Scots pine populations from the Tatra National Park, particularly from the 'Filipczańskie Skałki' locality. 'Filipczańskie Skałki' is a small, isolated population differing in the number of stomata and stomatal rows.

Key words: *Pinus sylvestris* L., populations, needle morphological variation, interpopulational differentiations, Tatra Mts., discriminant analysis, cluster analysis

1. Introduction

The Scots pine (*Pinus sylvestris* L.) is a species of a broad ecological amplitude, which allowed it to colonize vast and diverse habitats in the territory of Eurasia (Boratyński 1993; Obmiński 1970; Przybylski 1993). In the lowlands of Poland, where Scots pine has its continuous distribution area, it is a very common species and represents about 70% of forest tree species (Białobok 1970). The situation changes in the southern part of Poland, where the distribution area of the Scots pine becomes irregular and sometimes takes the form of isolated islands. In the mountainous areas *P. sylvestris* often forms specific forest communities, which are scattered in dry places or in nutrient-poor habitats where the competition from more shade-demanding or shade-tolerant species is less. Such a situation is observed in the Tatra Mts., where the Scots pine is found in spatially isolated populations usually covering hard south facing accessible rocks. In such habitats the Scots pine wins the competition with the spruce for nutrients and the sun and forms relict Scots pine populations built of several dozen or several hundred trees (Łysek 1974).

The history of the species greatly affects the currently observed picture of the differentiation between mountainous populations of the Scots pine (Staszkiewicz 1993). In the glacial period the Scots pine migrated to the southern part of Europe from which it returned to the north in the warmer interglacial times. These oscillations of the Scots pine distribution area contributed to the rise of a population characterized by new and different proprieties (Staszkiewicz 1993). Nowadays, it is claimed that at least some of the mountain populations of *P. sylvestris* which grow in the Tatra Mts. have a relict character and are dated back to the last interglacial period (Staszkiewicz 1993). However, Obidowicz (1996) excludes the possibility of the survival of any trees in the Tatra Mts. at that period. In this case Tatra mountain populations of the Scots pine would be the late-glacial-holocene relicts that created the main formation in the interstadial Alleröd (11.800-11.000) (Obidowicz 1996).

The purpose of this research was to characterize the representative populations of the Scots pine from the Tatra Mts. on the base of needle morphological traits. We also wanted to broaden the knowledge about the differentiation of *P. sylvestris*, especially from a particularly interesting area of border range, which promotes the emergence of interpopulational differences (Mayr 1963; Nevo *et al.* 1997).

2. Material and methods

Scots pine needles used in this research were collected, in October 2003, from the following four populations located in the Tatra National Park (TNP): 1 – Łysa Skała (1080-1119 m a.s.l.), 2 – Filipczańskie Skałki (1150-1200), 3 – Siwarowe Pańskie (1250-1300) and 4 – Wielkie Koryciska (1120-1130) (Fig. 1). Trees from the above stands are found in the highest parts or on mountain-sides exposed to the south. Populations: 1 – Łysa Skała, from the eastern part of the TNP, and 4 – Wielkie Koryciska, from its western part, are separated by a distance of ca. 20 km.
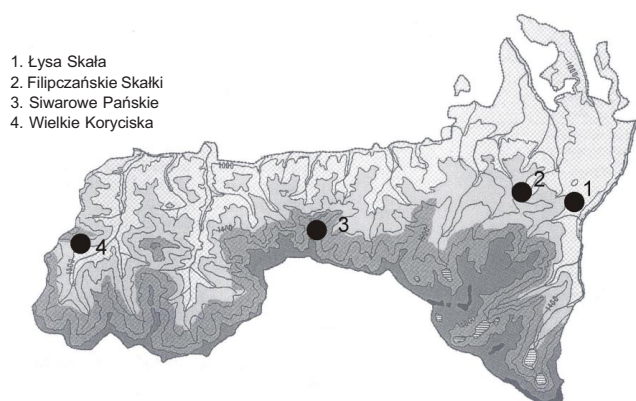

1. Łysa Skała
2. Filipczańskie Skałki
3. Siwarowe Pańskie
4. Wielkie Koryciska

Fig. 1. Location of the investigated *Pinus sylvestris* populations on the territory of the Tatra National Park

Long shoots were cut, if possible, from the highest and sunniest parts of each tree and subsequently, ten 2-years old needles were taken from each tree for biometrical analysis. Each needle was analyzed separately in regard to the following five morphological traits: 1 – needle length (mm), 2 – number of stomatal rows on the flat side of a needle, 3 – number of stomata per 2 mm of needle length on the flat side, 4 – number of stomatal rows on the convex side of a needle, 5 – number of stomata per 2 mm of the needle length on the convex side. The data obtained for 15 individuals in each population were statistically analyzed. The biometric analysis of morphological traits 2-5 was conducted in the middle of each needle.

The following statistical analyses were conducted on biometrically averaged values for each individual: mean, minimum and maximum value, range, variation coefficient, skewness and curtosis; correlation coefficient of traits. Moreover discriminant analysis and agglomerative grouping using the method of the neighbourhood based on Euclidean distances were used to present the scattering of the populations in the space of the first three discriminant variables and their similarities on the basis of dendrograms. All the statistical calculations were done using STATISTICA computer program.

3. Results and discussion

The basic statistical characteristics of the five analyzed morphological needle traits which include mean, minimum and maximum value, range, variation coefficient, skewness and curtosis are shown in Table 1.

Table 1. Basic statistical characteristics of five analyzed morphological needle traits

Populations	Traits	Mean	Minimum	Maximum	Range	Standard deviation	Variation coefficients [%]	Skewness	Curtosis
1. Filipczańskie Skałki	1	34.40	23.30	46.60	41.04	6.41	18.62	0.05	-0.46
	2	8.54	5.70	11.50	2.79	1.67	19.55	0.37	-0.75
	3	22.53	20.30	24.10	1.16	1.08	4.78	-0.71	-0.32
	4	8.43	5.80	11.00	2.27	1.51	17.87	0.05	-1.01
	5	21.94	19.60	23.50	1.27	1.13	5.14	-0.42	-0.44
2. Łysa Skała	1	35.33	28.10	45.60	27.24	5.22	14.77	0.54	0.13
	2	7.48	5.40	9.50	1.10	1.05	14.01	-0.06	0.15
	3	22.43	19.90	25.60	2.10	1.45	6.47	0.70	0.83
	4	8.74	7.30	10.80	1.08	1.04	11.91	0.73	0.26
	5	21.52	19.50	24.40	1.66	1.29	5.98	0.77	0.37
3. Siwarowe Pańskie	1	36.59	27.90	52.00	48.88	6.99	19.11	1.00	0.09
	2	7.53	6.20	10.20	1.15	1.07	14.26	0.95	1.30
	3	22.19	20.40	23.70	0.90	0.95	4.29	-0.12	-0.79
	4	8.79	7.20	12.10	1.65	1.29	14.64	1.23	1.97
	5	21.92	19.40	24.10	1.56	1.25	5.70	-0.43	0.77
4. Wielkie Koryciska	1	33.01	24.60	39.80	19.75	4.44	13.46	-0.32	-0.60
	2	7.55	5.90	10.40	1.04	1.02	13.52	1.46	3.93
	3	22.17	19.00	24.30	2.03	1.42	6.42	-0.37	0.22
	4	8.65	6.60	10.40	1.36	1.16	13.46	0.01	-0.81
	5	21.24	19.50	24.30	1.54	1.24	5.84	0.99	1.45

Explanations: 1 – needle length; 2 – number of stomatal rows on the flat (adaxial) side of the needle; 3 – number of stomata per 2 mm of needle lenght on the flat (adaxial) side; 4 – number of stomatal rows on the convex (abaxial) side of the needle; 5 – number of stomata per 2 mm of needle lenght on the convex (abaxial) side

As was to be expected, the most stable morphological traits were the number of stomata per 2 mm of the needle length on the flat (no. 3) and convex side (no. 5), showing the lowest variation coefficients ranging from 4.29 to 6.47%. Moreover these traits indicate high values of correlation coefficients (Table 2). The stability of these traits and their low susceptibility to environmental modifications were proven in the research of Żelawski & Niwiński (1966). Furthermore, comparison of numerous *P. sylvestris* populations from enormous distribution area in Europe and Asia, and also populations which grow in extreme and contrasting environments, give very similar results (Urbaniak 1998; Urbaniak *et al.* 2003; Woźniak *et al.* 2005). Therefore, these morphological traits seem to be very useful in describing differentiation among Scots pine populations (Urbaniak 1998).

Table 2. Correlation matrix between five morphological traits

	2	3	4	5
1 – Łysa Skała				
1	0.02	-0.11	0.13	-0.35
2		-0.26	0.70*	-0.25
3			-0.36	0.86*
4				-0.43
2 – Filipczańskie Skałki				
1	0.53*	0.42	0.74*	0.48
2		0.22	0.89*	0.3
3			0.19	0.87*
4				0.28
3 – Siwarowe Pańskie				
1	0.3	-0.57*	0.31	-0.42
2		0.02	0.83*	0.09
3			-0.07	0.76*
4				-0.03
4 – Wielkie Koryciska				
1	0.27	0.22	0.45	-0.12
2		0.42	0.66*	0.23
3			0.28	0.74*
4				0.07

Explanations: * – significant value at the level of 0.05; other see Table 1

The rest of the morphological traits is more variable. The most variable one is needle length, which is indicated by a high variation coefficient (Table 1). The needle length is a very plastic feature, but according to Zajączkowski (1949) the Scots pine from the Tatra Mts. maintain their typical values of that morphological trait of needle; even if these populations are planted in garden experiments away from their natural stands. In analyzed populations the mean values of needle length range from 33.01 to 36.59 mm, which correspond to the report mentioned by Zajączkowski (1949).

However, it is worth paying attention to the different values of curtosis, for trait 2 – number of stomatal rows on the flat side of the needle that is a measure of the concentration of trait value around the mean. The value of curtosis for population 3 – Siwarowe Pańskie is 1.30 and for population 4 – Wielkie Koryciska even 3.93, which proves a high concentration of the measured values around the means. On the contrary, negative values of curtosis for population 1 – Filipczańskie Skałki and almost equalling 0 (zero) values for population 2 – Łysa Skała indicate a more flattened distribution. That differentiation can be a result of the contrasting environments of Filipczańskie Skałki and Łysa Skała leading, consequently, to the emergence of a wider range of variability of trait 2.

Reciprocal similarities among the investigated populations are a separate issue. In order to compare all studied populations, discriminant analysis and cluster analysis with the shortest Euclidean distances were conducted. The calculations were done in two ways i.e. taking into account the whole set of traits (1-5) and omitting one of them – needle length. Figures 2-5 showed the position of each population in the space of the first three variables and relationships among the populations within the confines of dendrograms.

Cluster analysis conducted on the basis of four morphological needle traits showed that the populations with the greatest similarities are 1 – Łysa Skała, 4 – Wielkie Koryciska and 3 – Siwarowe Pańskie, whereas the small, isolated population 2 – Filipczańskie Skałki has a more distinctive nature (Fig. 2).
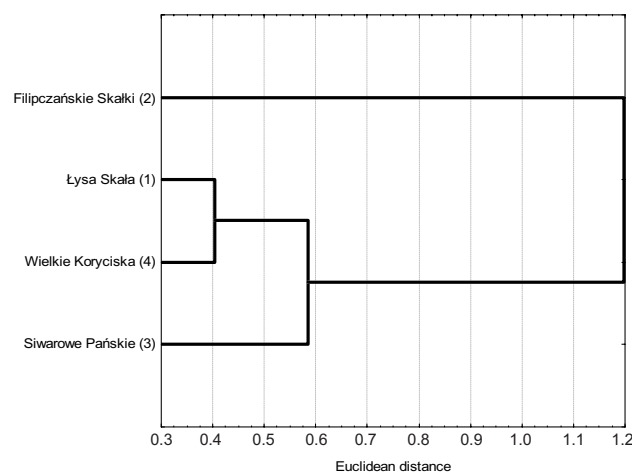
Fig. 2. Dendrogram of population grouping based on four morphological traits (2-5)

The discriminant analysis based on four morphological traits was presented in the space of the first three variables U_1, U_2, U_3 and shows separates of populations 2 – Filipczańskie Skałki and 3 – Siwarowe Pańskie. On the other hand, the two most geographically distant populations 1 – Łysa Skała and 4 – Wielkie Koryciska are closely related on the plane of the first and second discriminant variables (U_1, U_2), but differ from each

other in regard to the third discriminant variable (U_3) (Fig. 3). Trait 2 – number of stomatal rows on the flat side of a needle has the biggest influence on the distinction of populations presented in respect to the first discriminant variable. Trait 5 – number of stomata per 2 mm of the needle length on the convex side, exerts a severe influence on the second discriminant variable, whereas the third discriminant variable is mostly affected by traits 3 – number of stomata per 2 mm of the needle length on the flat side, and 5 – number of stomata per 2 mm of the needle length on the convex side (0.8%).
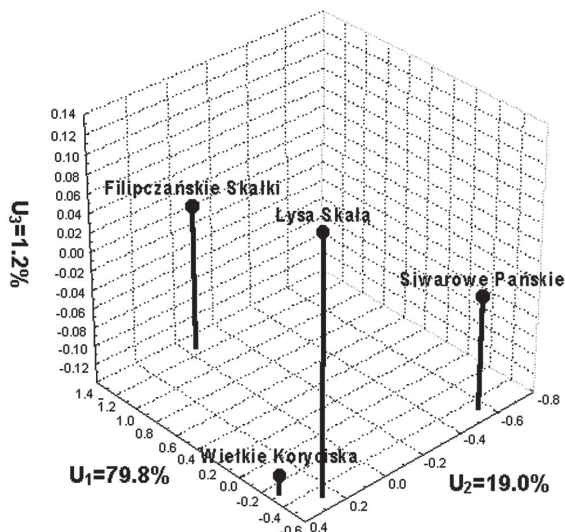

Fig. 3. Result of discriminant analysis based on four needle traits (2-5) plotted along the first three discriminant variables

In the case of the discriminant analysis based on all five morphological needle traits, populations 1 – Łysa Skała and 3 – Siwarowe Pańskie showed the most signi-

ficant differences, whereas populations 2 – Filipczańskie Skałki and 4 – Wielkie Koryciska bear a similarity in the space of the first two discriminant variables (U_1, U_2), but differ from each other in the value of the third discriminant variable (U_3) (Fig. 4). Traits: 2 – number of stomatal rows on the flat side of a needle, 5 – number of stomata per 2 mm of the needle length on the convex side, 1 – needle length; and 3 – number of stomata per 2 mm of the needle length on the flat side, mostly influence the population distribution on the plane of the first three discriminant variables.
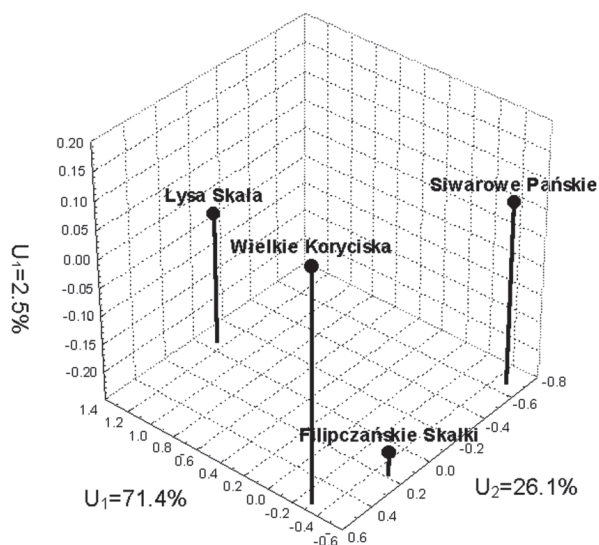
The dendrogram constructed on the grounds of cluster analysis took into consideration all five morphological needle traits and showed the greatest similarity among populations 3 – Siwarowe Pańskie, 1 – Łysa Skała and 2 – Filipczańskie Skałki; population 4 – Wielkie Koryciska differed significantly from the rest (Fig. 5).
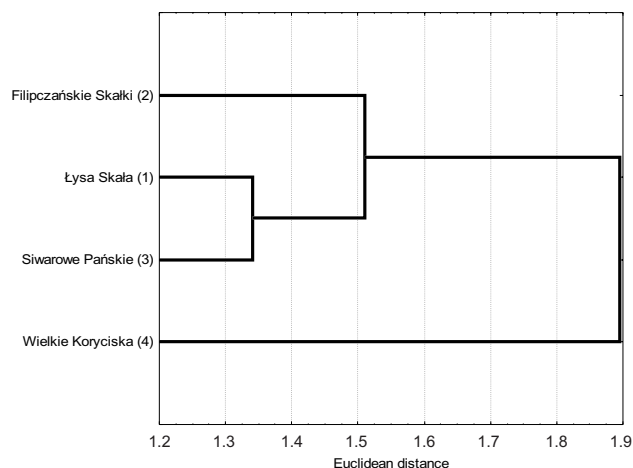

Fig. 5. Dendrogram of population grouping based on five morphological traits (1-5)

4. Conclusion

The main result of our study is consistent with previous research work by Staszkiewicz (1961, 1993). Based on the morphological variability of cones, his research proved the existence of two different types of Scots pine populations in the Tatra Mts.: slightly changed *polonica* in Łysa Skała and *meridionalis* in Wielkie Koryciska.

Observed pattern of differentiation of scots pine (*Pinus sylvestris* L.) from TNP is a result of several factors from which the history of the species and influence of various selection processes in different areas of TNP seem to be the most important.

Fig. 4. Result of discriminant analysis based on five needle traits (1-5) plotted along the first three discriminant variables

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