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Anatomical and morphological variability of needles of *Pinus mugo* Turra on different substrata in the Tatra Mountains

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Abstract: *Pinus mugo* is an extremely tolerant species in relation to habitat and has a great ability to adapt to various environmental conditions. The aim of this study was to compare the level of morphological and anatomical variation of needles of *P. mugo* growing on different substrata (limestone, granite and peat bog). The material was collected in the Tatra Mts. from three natural populations: Gładkie Upłaziańskie slope, Gąsienicowa Valley (E slope near Czarny Staw Gąsienicowy) and Polana Waksmundzka peat bog. Thirteen quantitative traits were examined. The results indicate that the populations significantly differed from each other, while variation within populations was low and on a similar level, irrespective of the kind of habitat.

Key words: *Pinus mugo*, dwarf mountain pine, variation, biometry, quantitative traits

INTRODUCTION

In the Tatra Mountains, dwarf mountain pine (*Pinus mugo* Turra) is found at altitudes between 1400 m and 1800 m (MEDWECKA-KORNAŚ 1959). It occurs occasionally also on peat bogs at the lower altitudes of its range. A wide ecological amplitude concerning both water and soil relations is the main factor due to which *P. mugo* is considered to be a pioneer species colonizing the areas inaccessible to other woody plants (PIĘKOŚ-MIRKOWA & MIREK 1996). There are relatively few papers (in comparison to *P. sylvestris*) describing the morphological variation of dwarf mountain pine (SZWEYKOWSKI et al. 1976, BOBOWICZ et al. 1983, BOBOWICZ & KRZAKOWA 1986, BĄCZKIEWICZ & PRUS-GŁOWACKI 1997, BORATYŃSKA 2002, STRUŽKOVÁ 2002), and some data have become available on the occasion of studying variation of natural hybrids of *P. mugo* and *P. sylvestris* (STASZKIEWICZ & TYSZKIEWICZ 1969, SZWEYKOWSKI 1969, UZUNOVA & YURUKOV 1986, YURUKOV &

TASHEV 1992, STASZKIEWICZ 1993, BĄCZKIEWICZ 1995, BORATYŃSKA & BOBOWICZ 2000, 2001). *P. mugo*, morphologically rather variable (SZWEYKOWSKI et al. 1976, SZWEYKOWSKI & BOBOWICZ 1977, MUSIL 1977, BOBOWICZ et al. 1983, BOBOWICZ & KRZAKOWA 1986, BOBOWICZ 1993, BĄCZKIEWICZ & PRUS-GŁOWACKI 1997, BORATYŃSKA 2002, STRUŽKOVÁ 2002), is an extremely tolerant species in relation to habitat (CHRISTENSEN 1987) and has a great ability to adapt to environmental conditions (BUGAŁA 1991). It grows on both alkaline (limestones, dolomites) and acid substrata (granites, acid peat bogs) (CHRISTENSEN 1987). It can be found on dry sandy sites as well as on very wet peat bogs. The occurrence of *P. mugo* in different natural habitats raises a question: is there a relationship between morphological variation of *P. mugo* and habitat? Morphological modification in response to varying environmental conditions in conifers has been confirmed by, for example, VIDÂKIN (1981), LUKACIK & REPAC (1992), KMET at al. (1994), URBANIAK (1998) and URBANIAK et al. (2003).

The aim of this paper was to compare the level of morphological and anatomical variation of needles of *P. mugo* growing on different substrata (limestone, granite and peat bogs).

MATERIALS AND METHODS

The material used in this study included *P. mugo* needles collected in the Tatra Mts. from three natural populations: Gładkie Upłaziańskie slope (GU), Gąsienicowa Valley (E slope near Czarny Staw Gąsienicowy – DG), and Polana Waksmundzka peat bog (PW). These populations occurred in natural locations of *P. mugo* and grew on three different types of substrata: the GU population on limestone, PW on peat, and DG on granite. Some details of these sites are presented in Table 1. From each population, 30 samples were collected every 15 m along a transect and then preserved in 70% ethanol. Next, from each sample, 10 pairs of two-year-old needles were examined. In total, 900 pairs of needles were investigated: one of each pair in respect of morphology and the other in respect of anatomy.

Table 1. Main characteristics of the studied populations of *P. mugo* in the Tatra Mts.

Population	Site	Latitude (N)	Longitude (E)	Altitude (m)	Substratum
PW	Polana Waksmundzka peat bog	49°16’30”	20°03’30”	1380	peat
GU	Gładkie Upłaziańskie (E slope of Upłaziańska Kopa)	49°15’30”	19°53’30”	1550–1750	limestone
DG	Gąsienicowa Valley (E slope near Czarny Staw Gąsienicowy)	49°14’00”	20°01’30”	1630–1700	granite

Table 2. List of studied traits of *P. mugo*

No.	Traits
1	Number of stomatal rows on abaxial (convex) side of needle
2	Number of stomatal rows on adaxial (flat) side of needle
3	Stomatal row index (value of trait 1 divided by value of trait 2)
4	Mean number of stomata in a 2-mm-long section of abaxial side of needle
5	Mean number of stomata in a 2-mm-long section of adaxial side of needle
6	Number of resin canals
7	Thickness of epidermis on adaxial side of needle, in µm
8	Mean width of epidermal cells, measured as average width of 3 neighbouring cells, in µm
9	Needle thickness, measured on cross-sections, in µm
10	Needle width, measured on cross-sections, in µm
11	Needle thickness to width ratio (value of trait 10 divided by value of trait 9)
12	Distance between vascular bundles, in µm
13	Marcet's coefficient (values of traits 9 × 12 divided by value of trait 10 (MARCET 1967)

Thirteen quantitative traits were assessed (Table 2). The data obtained were analysed statistically with STATISTICA 5.5 for Windows. Descriptive statistics (means, standard deviations, minima and maxima), Pearson's correlations between all traits, and coefficients of variation, were computed to evaluate the range of variation of anatomical and morphological traits. Multivariate analysis of variance (MANOVA), the standard method of discriminant analysis, and complete linkage method of cluster analysis based on Euclidian distances, were performed to examine variation within each investigated population as well as relationships between the populations. In order to check the significance of Mahalanobis distances, the *F* statistic was used. To test whether there are differences in morphological and anatomical traits between the studied populations, and to see whether these differences could be related to substratum type, two-level nested ANOVA (mixed model) was performed. The random factor B (ramet) was nested within the fixed factor A (site) according to the equation

$$y_{\mathrm{ijk}} = \mu + s_{\mathrm{i}} + T_{j(i)} + \varepsilon_{ijk}$$

where y_{ijk} = the observed character, μ = general mean of the experiment, s_i = impact of the i^{th} site, $T_{j(i)}$ = impact of the j^{th} ramet within i^{th} site, and ε_{ijk} = impact of the k^{th} needle within j^{th} ramet and i^{th} site (MORRISON 1990, SOKAL & ROHLF 1997, TRIOLA 1998, ŁOMNICKI 2000). As estimated according to the equation

$$\varphi = \sqrt{\frac{n'\delta^2}{2k's^2}}$$

(ZAR 1999), the minimum sample size $N = 30$ per population allows to detect 10–15% differences between population means with a specified power $1\text{-}\beta \geq 0.90$ at significance level $\alpha = 0.05$. Three data matrices obtained by successive computing of mean values from original measurements were used in the analyses: (1) matrix of

Table 3. Descriptive statistics (mean, standard deviation SD, minimum and maximum) of 13 quantitative traits of the studied populations of *P. mugo* in the Tatra Mts.

Trait	GU		DG		PW	
no.	mean±SD (min-max)	V%	mean±SD (min-max)	V%	mean±SD (min-max)	V%
1	10.33±1.81 (6.00-15.00)	17.87	8.24±1.38 (6.00-12.00)	16.77	9.33±1.66 (5.00-16.00)	17.77
2	6.20±1.28(3.00-12.00)	20.60	6.66±0.97 (5.00-9.00)	14.51	6.61±1.35 (4.00-11.00)	20.42
3	1.72±0.37 (0.75-3.33)	21.83	1.25±0.18 (0.86-1.83)	14.65	1.45±0.31 (0.75-2.67)	21.23
4	19.05±1.59 (12.33-23.33)	8.35	18.18±1.32 (16.00-22.67)	7.27	18.63±1.87 (13.30-23.70)	10.00
5	18.66±1.78 (14.00-23.67)	9.55	17.73±1.33 (14.33-21.33)	7.57	18.33±1.89 (10.30-23.70)	10.34
6	3.53±0.78 (1.00-6.00)	22.10	4.20±0.82 (2.00-8.00)	20.10	4.12±0.77 (3.00-6.00)	18.71
7	28.28±5.08 (17.50-45.00)	17.96	23.81±2.85 (16.72-35.82)	11.98	25.94±3.63 (15.00-37.50)	14.00
8	16.51±2.53 (10.00-21.67)	15.32	14.03±1,80 (9.55-21.49)	12.83	16.13±1.81 (10.80-21.70)	11.25
9	1299.90±109.54 (1012.0-1584.0)	8.43	1279.90±85.10 (999.9-1538.3)	6.65	1435.20±98.26 (1232.0-1804.0)	6.85
10	726.37±64.18 (462.0-880.0)	8.84	738.52±75.72 (596.1-1326.8)	10.25	828.56±64.09 (660.0-990.0)	7.74
11	0.56±0.06 (0.40-0.68)	10.71	0.58±0.03 (0.49-1.08)	5.17	0.58±0.03 (0.44-0.67)	5.98
12	73.49±26.76 (20.00-155.00)	36.41	73.80±25.12 (0.00-145.67)	34.05	105.9±29.31 (38.00-183.00)	27.67
13	132.14±49.67 (34.55-278.09)	37.59	129.30±47.39 (0.00-291.34)	36.65	184.55±54.69 (67.06-336.32)	29.63

original data $N = 900$ (90 ramets × 10 needles); (2) matrix of means for ramets ($N = 90$); and (3) matrix of means for populations ($N = 3$).

RESULTS

Descriptive statistics and coefficients of variation of all examined traits computed for each population are given in Table 3. Six of the studied traits (1, 3, 4, 5, 7, 8) had the highest mean values in the GU population, while their lowest mean values were found in the DG population. The PW population was characterized by the highest mean values of traits 9, 10, 11, 12, 13. Traits 11, 4, 5, 9, 10 exhibited the smallest variation (V<11%), whereas traits 13 and 12 showed the highest variation (V >25%) in all studied populations, reaching V=37.59% for character 13 in the GU population. The DG population was the least variable and had the lowest variation coefficients for most of the studied traits, whereas the GU population was the most variable in the examined traits and its V values were usually the highest (Table 3).

Interrelations between the traits were analysed using three matrices of Pearson's correlation coefficients. The highest percent of statistically significant correlations ($\alpha = 0.01$) was 42.31% in the GU population, whereas in the DG and the PW populations the percent of statistically significant correlations was lower: 32.05% and 29.49%, respectively. All studied populations had the highest positive correlation ($r \geq 0.98$**) between traits 12 and 13. Only 9 (11.54%) of all significant correlations

Table. 4. The F statistics and results of the Scheffé test for all traits jointly and for each trait separately for particular populations (GU, DG and PW) of *P. mugo*, ** p ≤ 0.01, *** p ≤ 0.001

Population / Traits	GU		DG		PW	
All traits df = 377, 3112	F= 6.30***		F = 4.09***		F = 5.67***	
Particular traits df = 29, 270	F	Scheffé test [1]	F	Scheffé test	F	Scheffé test
1	6.44***	1	8.03***	2	6.79***	1
2	5.75***	1	3.78***	1	4.52***	1
3	4.03***	1	3.51***	1	2.24***	1
4	6.61***	1	5.39***	1	5.91***	1
5	7.97***	1	8.17***	4	5.58***	1
6	9.56***	10	13.67***	37	10.15***	23
7	5.88***	1	2.32***	1	6.89***	2
8	2.80***	1	1.80**	1	2.59***	1
9	49.32***	157	15.05***	39	18.36***	49
10	32.75***	110	8.64***	7	17.66***	53
11	4.73***	1	3.76***	1	10.07***	15
12	10.31***	15	6.82***	1	17.32***	48
13	10.18***	12	7.19***	3	17.83***	49

[1]Number of statistically significant differences between pairs of individual ramets within particular populations per all 435 possible pairs

Table 5. Mean squares (MS) and *F* statistics of 13 quantitative traits of the studied populations of *P. mugo* in nested ANOVA: * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001

Source of variation	df	Nesting	1		2		3		4		5		6		7	
			MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Population	2	A	328.87	28.59***	19.52	3.82*	16.72	69.52***	3961X	5.63**	66.60	5.36**	40.17	11.08***	1497.40	25.30***
Individuals	87	B (A)	11.50	6.93***	5.11	4.78***	0.24	3.30***	10.30	5.99***	12.40	6.96***	3.63	11.26***	59.20	5.36***
Error	810		1.66		1.07		0.07		1.70		1.80		0.32		11.00	

Source of variation	df	Nesting	8		9		10		11		12		13	
			MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Population	2	A	536.00	62.53***	2.14	28.80***	0.94	32.84***	0.035	5.36**	104157.0	25.22***	290350.0	19.96***
Individuals	87	B (A)	8.60	2.37***	0.07	24.18***	0.03	13.59***	0.007	4.93***	4131.0	11.14***	14545	11.35***
Error	810		3.60		0.003		0.002		0.001		371.0		1282	

Table. 6. The multiple comparison Scheffé test in nested ANOVA for 13 quantitative traits of the studied populations of *P. mugo* – probabilities of statistically significant differences between the studied populations

Trait no.	GU-DG	GU-PW	DG-PW
1	0.0000	0.0000	0.0000
2	0.0000	0.0000	0.8584
3	0.0000	0.0000	0.0000
4	0.0003	0.0001	0.0000
5	0.0103	0.0000	0.0000
6	0.0000	0.0000	0.2259
7	0.0000	0.0000	0.0000
8	0.0000	0.0545	0.0000
9	0.0000	0.0000	0.0000
10	0.0052	0.0000	0.0000
11	0.0000	0.0000	0.9991
12	0.9813	0.0000	0.0000
13	0.6226	0.0000	0.0000

had $r \geq 0.50$** (between traits 1 and 2; 1 and 3; 2 and 3; 4 and 5; 9 and 10; 9 and 12; 9 and 13; 10 and 11; 12 and 13). Moreover, traits 9 and 10 correlated with most of other traits.

According to the results of multivariate analysis of variance (MANOVA), the general hypotheses H_0 stating that all ramets in each examined population (GU, DG and PW) are identical in respect of all traits taken together were rejected. The highest morphological and anatomical differences between ramets occurred in the GU population ($F = 6.30$*), the DG population was the most homogeneous ($F = 4.09$*), while the PW population exhibited an intermediate level of variation ($F = 5.67$*). Univariate ANOVA on each of the variables, followed by multiple comparisons (Scheffé test) to test the differences between pairs of means for each variable indicate the greatest influence of traits 9 and 10 on rejecting the above-mentioned general null hypothesis H_0. The highest differentiation of ramets in respect of these traits was recorded in the GU population, where the most statistically significant differences between all possible pairs of ramets were observed (Table 4). The greatest mean value of Mahalanobis' distance, equal to 1.35 (computed for each studied population from distances between each pair of ramets), was found in the GU population and confirms its highest within-population variation.

A two-level nested multivariate analysis of variance (MANOVA) shows lower differences between ramets within the studied populations ($F_{1131,\ 1062.466} = 1.95$, $p \leq 0.05$) than between the populations ($F_{26,\ 156} = 17.36$, $p \leq 0.001$). A nested ANOVA for each of the 13 morphological and anatomical traits indicates the main effect of traits: 3, 8, 10, 1, 7, 12 and 13 on rejection of the general hypothesis (Table 5). The Scheffé test showed that the PW population differed statistically significantly in 12 traits from the GU and in 10 traits from the DG populations, whereas the last two populations differed from each other in 11 traits (Table 6).

The distribution of ramets in the space of the first two discriminant axes U_1, U_2 indicates distinctness of the PW population along the first axis (Fig. 1). A partial

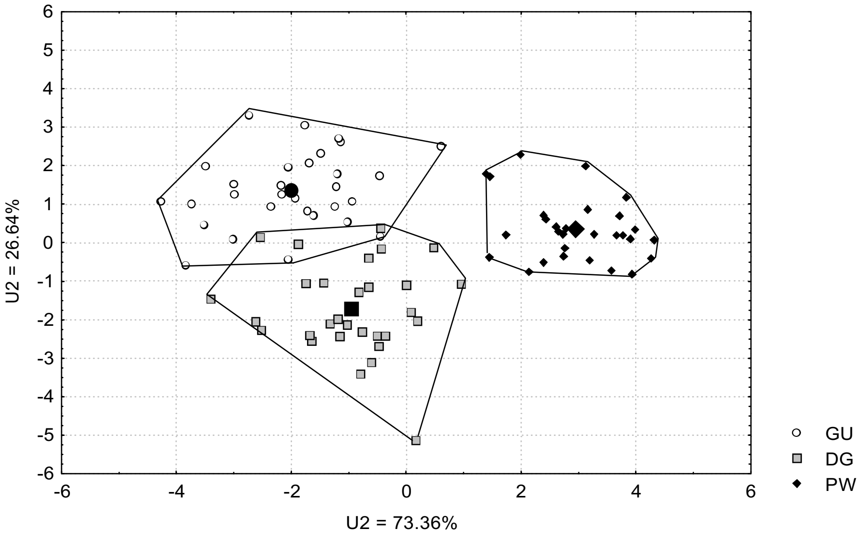

Fig. 1. Scatter diagram of all ramets with centroids of the studied populations (GU, DG, PW) of *Pinus mugo* in the first two discriminant axes (U_1, U_2)

separation was also observed between the GU and the DG populations along the second axis. However, it was not very clear, because of a partial overlap between their variation ranges. Moreover, these populations are spread out along the first axis, indicating their larger within-population variation concerning traits correlated with this axis than variation of the PW population. The greatest Mahalanobis' distance equal to 3.54* was found between the PW and the GU populations, whereas the

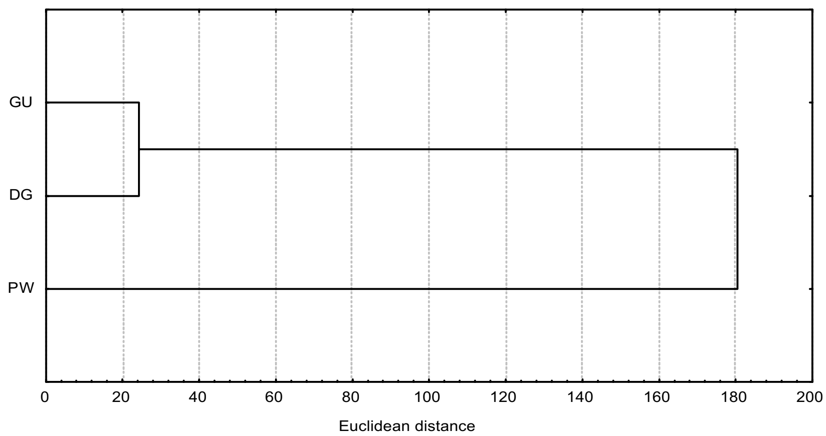

Fig. 2. Dendrogram of the studied populations (GU, DG and PW) of *Pinus mugo* constructed on the basis of the shortest Euclidean distances according to single linkage method, using the set of 13 traits

Table. 7. Correlation coefficients between 13 traits and discriminant axes in the studied populations of *P. mugo* in the Tatra Mts.

Trait no.	Discriminant axes I	II
1	0.18	-0.35
2	-0.16	0.10
3	0.38	-0.53
4	0.18	0.12
5	0.27	-0.01
6	-0.26	0.17
7	0.20	-0.33
8	-0.06	-0.55
9	-0.56	-0.16
10	-0.65	-0.09
11	-0.20	0.11
12	-0.56	-0.11
13	-0.49	-0.12
% of total variance	73.36%	26.64%
% of cumul. variance	73.36%	100%

shortest 2.22* between the GU and the DG populations. Traits 10, 9, 12, and 13 were most strongly correlated with the first discriminant axis, while traits 8 and 3 were correlated with the second axis, so these traits were the most important in separating the examined populations (Table 7). The cluster analysis based on the shortest Euclidean distance showed distinctive morphological and anatomical traits of the PW population (Fig. 2).

DISCUSSION

Three populations of *P. mugo* growing in the Tatra Mountains in different habitats – on limestone, granite and peat – were compared in this study. Needle morphology and anatomy varied significantly both within and between the populations. The *F* value for variation between the populations (*F* = 66.41**) was one order of magnitude higher than those for variation within the studied populations (*F* = 4.09* for DG, 5.67* for PW, and 6.30* for GU).

Traits 1, 3, 8, 9, 10, 12 and 13 contributed most significantly to the morphological and anatomical differences between the studied populations (Tables 3 and 6). Trait 1 (number of stomatal rows on the abaxial side of the needle) is also often listed as a distinguishing character between populations of *P. sylvestris*, the most closely related species to *P. mugo* (Bobowicz 1984, Bobowicz & Korczyk 1994, 2000, Urbaniak et al. 2003).

The studied populations differed in respect to examined traits (Table 3). The most variable traits were: distance between vascular bundles (trait 12) and Marcet's coefficient (trait 13) (mean variability coefficients for all three populations: *V* = 32.04 % and *V* = 33.61%, respectively). The values of these traits in the population growing on limestone (GU) were the highest and those in the population growing on peat (PW) were the lowest. Bobowicz & Krzakowa (1986, 1988) as well as Boratyńska (2002) also found the highest variability of these traits of *P. mugo* and in both studies the variation coefficient exceeded 20%. This indicates that these traits of needles belong to the most variable traits in *P. mugo*. Those authors noted that the ratio of needle thickness to width (trait 11) was the least variable. This was confirmed in our study, i.e. this character also appeared to be the least variable (V = 5.7% to 10.2%).

The correlation coefficients calculated between all the traits indicated that needle traits were generally not strongly correlated with one another, as for most traits $r \leq 0.5$. Statistically significant correlations were the most frequent in the GU population, growing on limestone (42% of correlations were statistically significant), while the least frequent on peat (29%). In this study the most variable traits were simultaneously the most correlated with each other: the distance between vascular bundles and Marcet's coefficient, as correlation coefficient between these traits was r ≥ 0.98 ** in all populations. Similar results are reported by Bobowicz & Krzakowa (1986, 1988) and Boratyńska (2002). Those authors also found high correlations between the number of stomatal rows on both sides of the needle (traits 2 and 3) and between needle thickness and width (traits 9 and 10) but in the present study the correlation coefficients are lower ($r \geq 0.59$ and $r \geq 0.79$, respectively).

Variation within populations was on a similar level irrespective of the kind of habitat, but the populations significantly differed from one another (Tables 4 and 5, Fig. 1). The most distinct in the group of the studied populations was the PW population growing on the peat bog, which is visible on the scatter diagram (Fig. 1) and dendrogram (Fig. 2). This population is characterized by the largest needles, which may result from the higher wetness of the peat bog in comparison to the other habitats. Needles of the GU population, growing on limestone, are more flattened (traits 11, 10, 9) than in the remaining populations. They also generally have more stomata (traits 1, 3, 4, 5) than needles from the populations growing on granite, in which values of these traits were the lowest (Table 3).

The observed differences between the populations can be due to a modifying influence of the environment upon plants or they may have a genetic basis (as a result of a selecting pressure of the environment, preferring the development of individuals with the best-adapted traits). In this study the population from the peat bog (PW) was the most distinct (Figs. 1 and 2). The same conclusion in respect to morphological needle traits was drawn by Urbaniak et al. (2003) about *P. sylvestris*. The relationships between different substrata and genetic variation of Scots pine have been described in few genetic papers. Siedlecka & Prus-Głowacki (1995) revealed a lower genetic variation of peat bog populations compared with the populations from other habitats (granite and limestone). More general conclusions concerning correlation of genotypes and phenotypes with the environment require further studies.

CONCLUSIONS

1. The studied populations significantly differed from one another and the most distinct was the population from the peat bog.
2. Variation between populations was about 10 times higher than within populations.

3. Variability coefficients and *F* statistics indicate that the DG population from granite was the least variable, whereas the GU population from limestone was the most variable.
4. The correlation coefficients calculated for all the traits indicated that most needle traits were poorly correlated with one another.
5. Needle thickness and width, distance between vascular bundles and Marcet's coefficient contributed most significantly to the anatomical and morphological differences between needles of the studied populations.

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