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Population differences in morphological and anatomical traits of *Pinus mugo* Turra needles from the Polish part of the Tatra Mountains

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Abstract. The main aim of this study was to describe the variation between the populations of the dwarf mountain pine *Pinus mugo* Turra based on the morphological and anatomical traits of their needles, and to investigate the relationship between the observed variation and environmental conditions (altitude and substrate).Two-year-old needles were collected from 180 individuals of six populations of *P. mugo* growing in the Tatra Mts. Two populations were classified as dense, located at 1360–1450 m altitude, and the remaining four formed loose clusters and were situated at 1500–1650 m altitude. Four of the populations are growing on granite and two on a limestone substrate. The natural variation of 10 morpho-anatomical and 3 synthetic needle traits was measured. In addition to descriptive statistics, the analyses of variance (ANOVA) with a Tukey test and principal component analysis were computed. We also estimated Pearson correlation coefficients for the examined needle traits and altitude as well as substrate. Our results indicate that the *P. mugo* populations differ significantly with regard to the investigated traits for which the Trzydniowiański Wierch population was the most distinct. The observed pattern of variability is largely caused by differences in stomatal traits and these features are positive correlated with altitude. Additionally, populations growing on granite have larger values for most of the examined traits compared to populations growing on limestone.

Keywords: dwarf mountain pine, Tatra Mts., variability, needle, altitude, substrate

1. Introduction

In Poland, *Pinus mugo* Turra (dwarf mountain pine) occurs in the Tatra Mountains and Babia Góra massif, in the subalpine belt called the dwarf mountain pine belt, as well as in some lower parts of the mountains, but only within the oligotrophic peat bogs.

Both zones are characteristic of nutrient deficiencies, and therefore, they are inaccessible to many tree species. Dwarf mountain pine is classified as a pioneer species (Piękoś-Mirkowa et al. 1996), as it occupies the areas remote to other woody species. Large tolerance to prevailing habitat conditions makes the dwarf mountain pine exceptionally adaptable, so it grows both on alkaline soils (limestones, dolomites) and acidic soils (granites, peat bogs) (Christensen 1987a).

There are hardly any data available, as compared to those on *Pinus sylvestris*, describing the morphological variability of *P. mugo* from different sites of its occurrence (e.g., Bobowicz, Krzakowa 1986, Bączkiewicz, Głowacki 1997, 2005, Bączkiewicz et al. 2005, Boratyńska et al. 2015). Most of the studies on dwarf mountain pine have referred to the natural hybrids of *P. mugo* and *P. sylvestris* (e.g., Staszkiewicz 1993; Bączkiewicz 1995; Boratyńska, Bobowicz 2000, 2001) – by and large focusing on the species' differences and not comparing the variability of populations from different growth environments. As has been shown in previous studies on conifers, the morphological differences are the response to various environmental conditions (Lukáčik, Repáč 1992; Kmeť et al. 1994; Bączkiewicz et al. 2005; Urbaniak 1998; Urbaniak et al. 2003, Pawlaczyk et al. 2010). In the case of dwarf mountain pine, these differences may be caused

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by natural geographic barriers that occur in the mountains, leading to isolation of populations, or else – climatic factors related to the gradient of altitude. So far, it has not been evidently determined if the morphological differences are caused due to ecological factors or are related to the climatic conditions associated with the height above sea level.

The aim of present study was to compare the levels of morphological and anatomical variability in the needles of *P. mugo* growing on different substrates (limestone, granite), with respect to the gradient of elevation above sea level.

2. Material and methods

Plant material

The study material was the needles of two-year-old dwarf mountain pine (*Pinus mugo*), collected from six populations growing in the Tatra Mts. (Fig.1). The two populations tested were located at the altitude of 1360 m–1450 m above sea level (valleys: Wielka Świstówka and Chochołowska Wyżnia), which is below the *P. mugo* altitudinal zone. Four more populations tested were at the height of 1500-1650 m a.s.l., that is, within the species altitudinal zone (in the valleys: Jarząbcza, Chochołowska Wyżnia, Długi Upłaz ridge and Trzydniowiański Wierch peak). The plants tested grew on limestone (1360 m–1450 m a.s.l.) and granite rocks (1500 m–1650 m ASL).

The samples were collected randomly from 30 specimens from each population in the designated transects at every 20 m. The method of harvesting the plant material was the same as described in detail by Boratyńska and Bobowicz (2000, 2001) and Boratyńska and Boratyński (2003). The collected needles were stored in 70% ethanol until the analyses began. There were 10 analysed needles for each specimen. In total, 1800 needles from 180 specimens were examined.

Biometric methods

Each needle was examined with regard to 10 morphological and anatomical traits as well as 3 conversion traits (Table 1). In order to measure the anatomical traits of the needles, cross-cut sections were prepared at halfway down the length of the needle. The cross-cut sections obtained were fixed by immersing them in polyvinyl alcohol. Olympus camera images were taken under a light microscope and needle traits were measured using Cell B software (Olympus Corporation).

Statistical methods

The obtained biometric data was statistically analysed using Statistica v. 12.0 (StatSoft PL). The calculated measures were: the arithmetic mean, the minimum and maximum values, standard deviation and coefficient of variation (Ferguson, Takane 2009) and coefficients of correlation between traits and altitude (Łomnicki 2014). Furthermore, one-way analysis of variance (ANOVA) with Tukey's test, Student's t-test (Triola 1998), principal component analysis (PCA) were performed, the Mahalanobis distances were determined and a dendrogram was constructed using the Euclidean distance. Prior to performing the calculations, the normality of the distribution of tested traits in individual populations was tested with Shapiro-Wilk test and Levene's homogeneity of variance. The affirmative answer allowed for parametric tests.

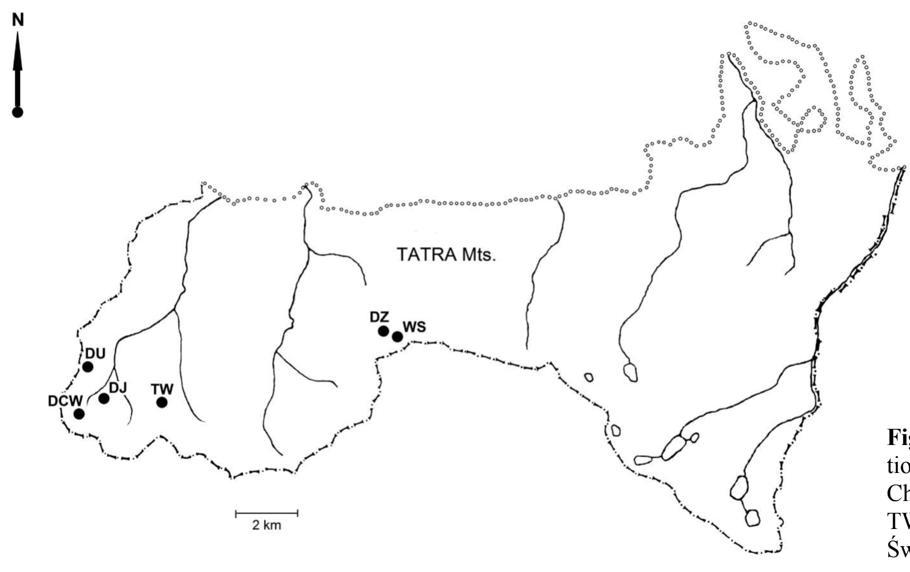

Figure 1. The localities of six studied populations in the Tatra Mts. Symbols: DCW – Dolina Chochołowska Wyżnia, DJ – Dolina Jarząbcza, TW – Trzydniowiański Wierch, WS – Wielka Świstówka, DU – Długi Upłaz, DZ – Dziurawe

Table 1. Studied needle traits of *Pinus mugo*

No.	Traits
1.	Number of stomata rows on convex (abaxial) side of needle
2.	Number of stomata rows on flat (adaxial) side of needle
3.	Stomata row index (trait 1/2)
4.	Mean number of stomata in a 2-mm-long section of convex (abaxial) side of needle
5.	Mean number of stomata in a 2-mm-long section of flat (adaxial) side of needle
6.	Number of resin canals on cross-section
7.	Height of epidermal cells in µm
8.	Width of epidermal cell in µm
9.	Needle width on cross-sections in µm
10.	Needle height on cross-sections in µm
11.	Distance between vascular bundles, in µm
12.	Needle height to needle width ratio (trait 10/9)
13.	Marcet's coefficient (trait 9 x 11 /10) (Marcet 1967)

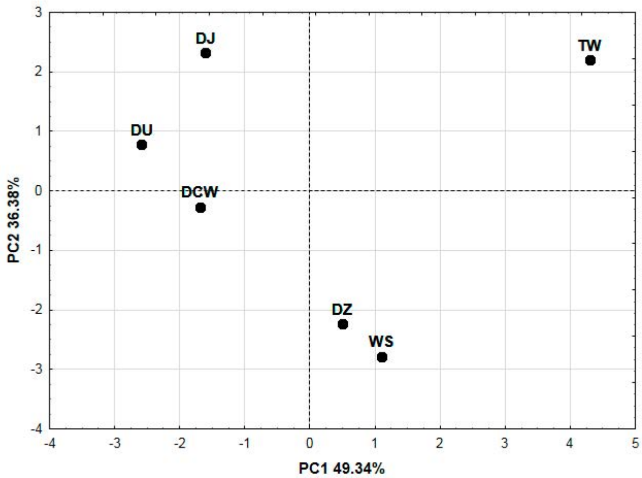

Figure 2. Principal component analysis (PCA) for six studied populations

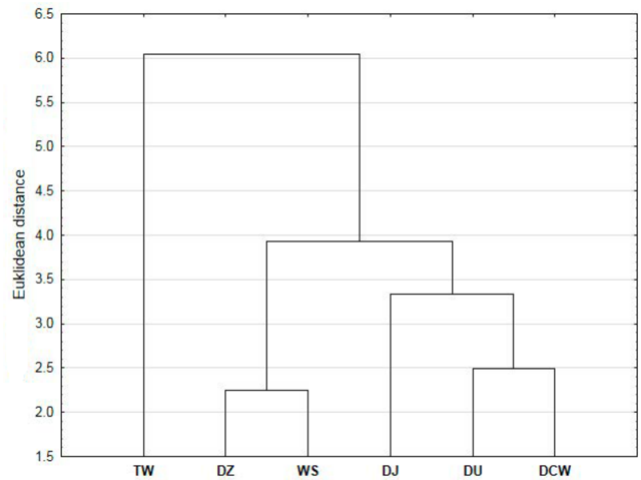

Figure 3. Dendrogram of six studied populations of dwarf mountain pine constructed based on the nearest neighborhood method

3. Results and Discussion

By analysing the differences between the populations, it was concluded that the greatest distinctiveness was shown by the dwarf mountain pine population growing on the slope of Trzydniowiański Wierch (TW). This was confirmed by the calculated Mahalanobis distances between populations, PCA results (Fig. 2) and the dendrogram based on Euclidean shortest distances (Fig. 3). TW population grows on granite bedrock, at an altitude of 1600 m–1650 m a.s.l. Of the 13 examined traits, 6 had the highest means as compared to those obtained in other populations tested, whereas the means obtained for 4 traits were comparatively the lowest (Table 2). The first group (the highest means) includes traits describing the stomatal density in the needles, that is, the number of stomata rows on the flat and convex sides of the needle (#1 and #2), and the number of stomata on 2 mm long flat or convex sides of the needle (#4 and #5). The obtained values were higher than the results of the authors who studied the dwarf mountain pine in the Western Tatras (Boratyńska 2002), and higher than those obtained in the Ukrainian populations (Boratyńska, Pashkevich 2001). In comparison to the rest of the populations examined in the present study, the above traits showed the highest maximum values (16, 15, 24.6 and 24.6) as well as the minimum values (8, 7, 19.6 and 18), and were characterized by a small variability (Table 2). In addition to the first group, there belonged the trait: the number of resin channels on needle cross-section (#6); unlike the traits described above, this one was characterized by a large variability (Table 2). The second group of traits, formed due to the fact that TW population showed the lowest mean values with regard to these traits, when compared with rest of the dwarf mountain pine populations tested, was the quotient of the stomata rows (stomata row index #3), that is, the ratio of the numbers of rows observed on the needle convex and flat parts (#1/#2). In TW population, the index mean was 1.1, indicating that the numbers of the rows on both sides of the needle are analogous, whereas in the rest of populations tested, the number of stomata rows was significantly higher on the convex side of the needle (Table 2). The next two traits with low values, observed in TW population, were the height and width of the epidermal cells (#7 and #8). In this case, the needle epidermis was thin and built of fine cells (20.84 µm and 11.80 µm, respectively). The distance between the vascular bundles (#11) in TW population was also the smallest and ranged from 0.470 mm to 0.651mm (on average – 0.562 mm), but not significantly different from other populations (Table 2). To sum up, as compared to the other studied dwarf mountain pine populations, the population from Trzydniowiański Wierch (TW) was characterized by the largest number of stomata in the needles and the thinnest needle epidermis.

Dwarf mountain pine population growing at an altitude of 1400 m in the Chochołowska Wyżna Valley (DCW), was the most different from TW population. The Mahalanobis distance between these two populations was 11.94. In contrast to TW population, in DCW population, the number of stomata on the convex and flat needle sides (#1, #2, #4, #5) was one of the lowest. On the other hand, when compared to TW, needle epidermis (#7 and #8) in DCW was much thicker (Table 2). DCW population showed the greatest similarity to dwarf mountain pine population growing at 1500 m a.s.l. – on the slope of Długi Upłaz ridge (DU) (Figs. 2 and 3). DCW and DU populations were characterized by a relatively small number of stomata on both sides of the needle (#1, #2, #4, #5), the significant difference between the number of stomata rows on the convex and flat (#3) sides, broad epidermal cells (#8) and high (thick) needles (#10, #11, #12) (Table 2). Similar to the TW population, DCW and DU populations grew on granite bedrock, but DU population grew over 100 m higher than DCW and 100 m lower than TW, which could influence the manner of development of some traits.

As proved by PCA results (Fig. 2) and presented in the dendrogram (Fig. 3), of all the dwarf mountain pine populations examined, the populations from Wielka Świstówka (WS) and Dziurawe (DZ) were most similar. The Mahalanobis distance between WS and DZ was 2.21. Both populations grew on limestone bedrock, close to each other geographically (Fig. 1), but at different altitudes (WS at 1360 m–1450 m and DZ at 1600 m–1630 m). In WS and DZ populations, the variability of majority of traits (#1, #2, #4, #5, #6, #7, #8, #12 and #13) was at similar levels (Table 2). Both populations were characterized by a low number of stomata on both sides of the needles. WS population showed the lowest number of stomata on the convex side of the needle (#1) (8.51), as compared to the rest of populations studied and DZ population, the lowest number of stomata on 2 mm long needle flat side (#5) (17.68) (Table 2). DZ and WS populations were also characterized by the lowest number of resin channels in cross-section (#6; 3.43 and 3.49 respectively), flat needles (#12; 0.044 mm and 0.041 mm, respectively), and they also showed low values of Marcet's coefficient (#13; 0.076 and 0.070, respectively) (Table 2). Flat needles in dwarf mountain pine population growing on limestone is the trait noted also by Bączkiewicz et al. (2005). However, in the latter study, unlike in the populations described in the present paper, the needles from *P. mugo* population growing on limestone bedrock had more stomata, when compared to the needles from population growing on granite bedrock. Similarity of the two investigated populations (WS and DZ) could be influenced not only by similar ecological conditions, but also by the small geographic distance that favoured the exchange of genes between these populations.

The last studied population was from the Jarząbcza Valley (DJ) growing on granite bedrock at an altitude of 1530 m–1580 m above sea level and it showed: the largest needle width on cross-section (#9; 1.3624 mm), the highest Marcet's coefficient (#13; 0.173) and the widest and tallest cells of the epidermis (#7 and #8; 25.95 µm and 15.24 µm, respectively)(Table 2). The epidermal cells of this population were larger than those of the remaining populations tested, and slightly larger than those of the Ukrainian population (on average 22.51 ± 14.29 µm) (Boratyńska, Pashkevich 2001), but more flattened than those observed in the Western Tatras (27.45 µm) (Boratyńska 2002). On the dendrogram, the DJ population is intermediate between the populations growing on limestone (DZ, WS) and granite (DU and DCW) (Figs. 2 and 3). The similarity of DJ population to those growing on limestone bedrock was due to 3 traits: the number of stomata rows on the convex and flat sides of the needle (#1, #2), and the number of stomata within 2 mm long convex side of the needle (#4). DJ similarity to DU and DCW populations was attributable to 5 traits: the number of stomata on 2 mm long flat side of the needle (#5), the number of resin channels on cross-section (#6), the width of epidermal cells (#8), the width of needle on cross-section (#9) and the needle height on cross-section (#10).

Table 2. Descriptive statistics of 13 needle traits for studied six populations of dwarf mountain pine. Symbols: M – arithmetic mean, Min – minimal value, Max – maximal value, SD – standard deviation, V% – coefficient of variability

Trait	Population	**M**	**Min**	**Max**	**SD**	**V%**	Trait	Population	**M**	**Min**	**Max**	**SD**	**V%**
1	DZ	9.52	5.0	12.0	1.24	13.01	**8**	DZ	12.31	7.2	19.1	2.06	16.70
	WS	8.51	5.0	12.0	1.29	15.17		WS	12.50	4.8	19.1	2.43	19.47
	DJ	10.66	7.0	14.0	1.07	10.06		DJ	15.24	9.6	23.9	2.43	15.93
	DU	9.45	6.0	12.0	1.38	14.61		DU	14.10	9.6	19.1	2.00	14.15
	TW	12.83	8.0	16.0	1.25	9.76		TW	11.80	7.2	16.7	2.06	17.48
	DCW	8.88	6.0	11.0	1.10	12.40		DCW	14.17	9.6	19.1	2.14	15.18
2	DZ	6.94	5.0	11.0	1.09	15.67	**9**	DZ	1.2934	1.056	1.632	0.10	7.85
	WS	6.66	4.0	9.0	1.10	16.51		WS	1.2347	0.960	1.670	0.11	8.96
	DJ	8.50	6.0	11.0	1.12	13.17		DJ	1.3624	1.152	1.670	0.10	7.46
	DU	7.03	5.0	11.0	1.31	18.56		DU	1.3317	1.018	1.632	0.14	10.43
	TW	11.51	7.0	15.0	1.35	11.69		TW	1.3062	1.075	1.594	0.11	8.10
	DCW	6.53	5.0	10.0	1.01	15.50		DCW	1.2989	1.018	1.651	0.11	8.43
3	DZ	1.40	0.8	4.1	0.27	19.15	**10**	DZ	0.7398	0.595	0.979	0.07	8.91
	WS	1.29	0.8	2.0	0.20	15.15		WS	0.7151	0.576	0.902	0.08	10.50
	DJ	1.25	1.0	1.7	0.16	12.57		DJ	0.7779	0.557	0.941	0.07	8.52
	DU	1.36	1.0	2.0	0.18	13.38		DU	0.7942	0.595	1.344	0.08	10.31
	TW	1.12	0.9	1.4	0.09	8.24		TW	0.7336	0.576	0.922	0.06	8.44
	DCW	1.37	0.9	2.0	0.19	13.94		DCW	0.7610	0.614	0.998	0.06	8.23
4	DZ	18.44	14.0	21.6	1.45	7.87	**11**	DZ	0.5725	0.494	0.705	0.03	5.83
	WS	19.12	15.3	22.0	1.43	7.49		WS	0.5799	0.436	0.811	0.04	7.56
	DJ	18.24	15.3	21.6	0.97	5.30		DJ	0.5717	0.468	0.746	0.04	6.58
	DU	16.99	14.3	20.0	1.21	7.11		DU	0.5980	0.481	0.875	0.04	7.05
	TW	21.57	19.6	24.6	0.94	4.37		TW	0.5625	0.470	0.651	0.03	6.09
	DCW	17.18	14.6	20.6	1.16	6.76		DCW	0.5869	0.494	0.717	0.03	5.65
5	DZ	17.68	13.0	20.6	1.25	7.08	**12**	DZ	0.044	0.02	0.07	0.01	28.97
	WS	18.16	14.6	21.3	1.43	7.87		WS	0.041	0.00	0.09	0.02	40.86
	DJ	18.53	16.0	21.6	1.03	5.57		DJ	0.098	0.02	0.20	0.03	30.48
	DU	18.35	15.3	21.6	1.35	7.37		DU	0.101	0.03	0.21	0.04	34.84
	TW	20.89	18.0	24.6	0.88	4.22		TW	0.058	0.02	0.53	0.03	56.18
	DCW	18.38	15.6	21.6	1.24	6.77		DCW	0.095	0.02	0.22	0.04	38.06
6	DZ	3.43	1.0	6.0	0.91	26.63	**13**	DZ	0.076	0.03	0.15	0.02	29.69
	WS	3.49	1.0	6.0	0.79	22.66		WS	0.070	0.01	0.16	0.03	41.49
	DJ	3.89	3.0	6.0	0.73	18.82		DJ	0.173	0.04	0.35	0.05	31.56
	DU	3.90	2.0	7.0	0.77	19.68		DU	0.171	0.05	0.39	0.06	37.38
	TW	4.13	2.0	7.0	1.00	24.32		TW	0.104	0.03	0.86	0.06	53.51
	DCW	4.11	3.0	7.0	0.67	16.40		DCW	0.095	0.02	0.22	0.04	38.06
7	DZ	22.24	14.3	31.0	3.13	14.06							
	WS	22.73	11.9	33.4	3.32	14.59							
	DJ	25.95	14.3	38.2	4.18	16.10							
	DU	23.23	11.9	33.4	3.34	14.36							
	TW	20.84	14.3	28.7	2.80	13.44							
	DCW	23.94	14.3	35.8	3.12	13.05							

Based on the above description, it can be concluded that the studied populations are characterized by a high degree of distinctiveness. This is confirmed by the calculated Mahalanobis distances, which are significantly important between all the studied populations (p <0.001), as well as the results of ANOVA and Tukey's test.

The results of Tukey's test show which populations differ in terms of each of the 13 needle traits tested. And so, in terms of the number of stomata on the convex side of the needle (#1), all populations differ from each other except for the population from the Chochołowska Wyżna Valley (DCW), which is no different from those from Wielka Świstówka (WS), Długi Upłaz (DU) and Dziurawe (DZ). Additionally, the DU population is not different than the DZ population. The numbers of stomata rows on the flat side of the needle (#2) are most distinctive in the dwarf mountain pine populations from Trzydniowiański Wierch (TW) and the Jarząbcza Valley (DJ), when compared to the rest of the populations tested (these show no differences in terms of trait #2). TW and DJ populations show the highest values of traits #1 and #2 (Table 2). With regard to the stomata row index (#3), almost all populations differ from each other, except for DCW population, which is not different from DU and DZ populations as well as WS population, which is no different from DJ population. The number of stomata per 2 mm long convex side of the needle (#4) differentiates nearly all populations, except for DU, DZW, DZ and DJ. With respect to the number of stomata per 2 mm long flat side of the needle (#5), TW population is most different from other tested populations. TW population shows the highest value of trait #5. Also, DZ population is different from DCW, DJ and DU populations in terms of this feature. In DZ population, the smallest number of stomata are observed on a 2 mm long flat side of the needle (Table 2). The numbers of resin channels in the cross-section (#6) are different only in WS and DZ populations, when compared to DCW population. The lowest numbers of resin channels are observed in the needles of WS and DZ populations (Table 2). The height of the epidermal cell (#7) distinguishes DJ from the rest of the populations, as well as TW population from DCW, WS and DU, and DZ from DCW. DJ population have the highest epidermal cells, and the lowest are observed in TW population (Table 2). At the same time, DJ population is distinctive with the widest cells of the epidermis (#8), as compared to the rest of the populations, not being different only from DCW population. In terms of trait #8, no differences are found between DZ population and TW and WS populations as well as between WS population and TW population, and between DCW and DU as well. Minor disparities apply to the width of the needle on cross-section (#9) as well, which distinguishes only WS from DU and DJ populations. Dwarf mountain pine needles in WS population are the narrowest when compared to other tested populations (Table 2). The height of the needle on cross-section (#10) differs only DU from DZ, TW and WS and WS from DCW and DJ. The needles are the highest in DU population, and those in WS population are the lowest (Table 2). The distance between the vascular bundles (#11) is the highest in DU population (Table 2), which distinguishes this population from the rest, except

Table 3. Result of Turkey test between six populations of dwarf mountain pine for each needles trait separately. *** – $p< 0.001$, ** – $p< 0.01$, * – $p< 0.05$, ns – $p> 0.05$

[1]	DCW	DJ	TW	WS	DU	DZ
DCW						
DJ	***					
TW	***	***				
WS	ns	***	***			
DU	ns	***	***	***		
DZ	ns	***	***	***	ns	

[2]	DCW	DJ	TW	WS	DU	DZ
DCW						
DJ	***					
TW	***	***				
WS	ns	***	***			
DU	ns	***	***	ns		
DZ	ns	***	***	ns	ns	

[3]	DCW	DJ	TW	WS	DU	DZ
DCW						
DJ	***					
TW	***	***				
WS	**	ns	***			
DU	ns	***	***	*		
DZ	ns	***	***	***		

[4]	DCW	DJ	TW	WS	DU	DZ
DCW						
DJ	***					
TW	***	***				
WS	***	***	***			
DU	ns	***	***	***		
DZ	***	ns	***	*	***	

[5]

	DCW	DJ	TW	WS	DU	DZ
DCW						
DJ	ns					
TW	***	***				
WS	ns	ns	***			
DU	ns	ns	***	ns		
DZ	**	**	***	ns	*	

[6]

	DCW	DJ	TW	WS	DU	DZ
DCW						
DJ	ns					
TW	ns	ns				
WS	**	ns	**			
DU	ns	ns	ns	ns		
DZ	***	ns	***	ns	ns	

[7]

	DCW	DJ	TW	WS	DU	DZ
DCW						
DJ	*					
TW	***	***				
WS	ns	***	*			
DU	ns	***	***	ns		
DZ	**	***	ns	ns	ns	

[8]

	DCW	DJ	TW	WS	DU	DZ
DCW						
DJ	ns					
TW	***	***				
WS	***	***	ns			
DU	ns	*	***	***		
DZ	***	***	ns	ns	***	

[9]

	DCW	DJ	TW	WS	DU	DZ
DCW						
DJ	ns					
TW	ns	ns				
WS	ns	***	ns			
DU	ns	ns	ns	**		
DZ	ns	ns	ns	ns	ns	

[10]

	DCW	DJ	TW	WS	DU	DZ
DCW						
DJ	ns					
TW	ns	ns				
WS	*	**	ns			
DU	ns	ns	**	***		
DZ	ns	ns	ns	ns	**	

[11]

	DCW	DJ	TW	WS	DU	DZ
DCW						
DJ	ns					
TW	***	ns				
WS	ns	ns	*			
DU	ns	***	***	*		
DZ	ns	ns	ns	ns	***	

[12]

	DCW	DJ	TW	WS	DU	DZ
DCW						
DJ	ns					
TW	***	***				
WS	***	***	*			
DU	ns	ns	***	***		
DZ	***	***	ns	ns	***	

[13]

	DCW	DJ	TW	WS	DU	DZ
DCW						
DJ	***					
TW	ns	***				
WS	ns	***	**			
DU	***	ns	***	***		
DZ	ns	***	*	ns	***	

Table 4. Pearson correlation coefficients between 13 needle traits and altitude and substrata where growing studied populations of dwarf mountain pine. ** – *p*< 0.01, * – *p*< 0.05

Trait	Correlation with altitude		Correlation with substrata	
	r	*p*	*r*	*p*
1	0.7232	0.104	0.4711	0.346
2	0.6107	0.198	0.4283	0.397
3	-0.2711	0.603	-0.3348	0.517
4	0.4075	0.423	-0.0887	0.867
5	0.4070	0.423	0.5110	0.300
6	0.1432	0.787	0.9401**	0.005
7	-0.3183	0.539	0.3009	0.562
8	-0.2601	0.619	0.5457	0.263
9	0.4883	0.326	0.7346	0.096
10	0.0900	0.865	0.6859	0.133
11	-0.5817	0.226	0.1465	0.782
12	-0.0951	0.858	0.8352*	0.038
13	0.1175	0.825	0.7041	0.118

for DCW population. Moreover, this feature is also different in TW population when compared to DCW and WS populations. The ratio of the needle height to width (#12) differentiates the populations tested, except for DZ and TW and WS; DCW and DJ and DU, as well as DU and DJ. Likewise, Marcet's coefficient (#13) does not differentiate DCW from TW, WS and DZ populations, DZ population from WS population and DU from DJ.

The analysis of variance (ANOVA) shows that traits associated with stomata had the highest effect on variability between the tested populations, that is, the number of stomata rows on the flat side of the needle (#2, F = 204.99; $p < 0.001$), the number of stomata on 2 mm long convex side of the needle (#4, F = 121.02; $p < 0.001$), and the number of stomata rows on the convex side of the needle (#1, F = 110.21, $p < 0.001$). It should be noted that these traits showed positive, medium and high correlations with the altitude (Table 4): for #2 - $r = 0.61$ ($p = 0.198$), for #4 - $r = 0.41$ ($p = 0.426$), and #1 - $r = 0.72$ (p = 0.104). This means that with the increasing height above sea level, the number of stomata in the needles increases, which influences the respiration intensity as adaptation to high altitude conditions, by improving the ability of individuals to endure.

Two more traits pertaining to stomata were much associated with the differences between dwarf mountain pine populations, that is, stomata row index (#3, F=47.04; $p<0.001$) and the number of stomata on 2 mm long flat side of the needle (#5, F = 52.57; $p < 0.001$). Other traits concerning needle shape were the proportion of needle height to width (#12, F = 47.15; $p < 0.001$) as well as Marcet's coefficient (#13, F = 49.18; $p < 0.001$). The coefficients of variation for traits #12 and #13 were very high – $V = 53.23\%$ and $V = 54.19\%$, respectively (Table 2). The authors (Boratyńska, Pashkevich 2001; Boratyńska 2002, Bączkiewicz et al. 2005) of other studies on the *P. mugo* complex point to Marcet's coefficient is very variable same as the number of resin channels (#6) and the distance between vascular bundles (#11). In the present study, the coefficient of variation for trait #6 ranged from 16.40 for DCW to 26.63 for DZ, whereas for feature #11, it was low and ranged from 5.63 (DCW) to 7.56 (WS). Features #12 and #13 were not correlated with the altitude ($r = -0.09$ and $r = -0.2$, respectively), but correlated with bedrock type: #12, $r = 0.83$ ($p = 0.038$) and #13, r = 0.70 ($p = 0.118$) (Table 4). The variability observed was influenced by the number of resin channels on cross-section (#6), the needle width on cross-section (#9) and the needle height on cross-section (#10). All these were correlated with the type of bedrock (#6: $r = 0.94$, $p = 0.005$, #9: $r = 0.73$ $p = 0.096$ and #10: $r = 0.69$ $p = 0.133$) (Table 4). The traits achieved higher values in dwarf mountain pine populations growing on the granite bedrock, when compared to those on limestone bedrock (Table 5). A similar set of traits (#1, #3, #8, #9, #12 and #13) influencing *P. mugo* population variability was described by Bączkiewicz et al. (2005), in a study comparing 3 populations of *P. mugo* from 3 different sites. The studied populations, growing on limestone (locality: Gładkie Upłaziańskie), peat bog (Polana Waksmundzka) and on granite (Czarny Staw Gąsienicowy), differed significantly from each other. The most different from the rest was the population growing on peat bog, which had the largest needles as a result of the highest moisture content in its growing substrate, when compared to the other sites tested. In the population growing on limestone, the needles were more flattened than those in the rest of the populations tested, and this was also observed in the present study. Other traits, that is, needle width on cross-section (#9) and needle height at cross-section (#10), as well as height-to-width ratio (#12), achieved higher values in populations growing on granite bedrock (Table 5). In the study by Bączkiewicz et al. (2005), the population growing on limestone rocks showed higher numbers of stomata as compared to the population growing on granite bedrock (with the lowest stomata numbers). Similar, but not the same results were obtained in the present study. The populations growing on limestone bedrock showed higher values for stomata row index (#3) and the number of stomata on 2 mm long convex side of the needle (#4), but fewer rows of stomata on the convex and flat side of the needle (#1, #2) and smaller stomata numbers on 2 mm long flat side of the needle (#5) (Table 5). The number of stomata rows on the convex side of the needle (#1) is often specified as the discriminating and differentiating trait for *P. sylvestris* (e.g., Bobowicz and Korczyk, 1994; Urbaniak 2003).

Table 5. The result of Student t-test between dwarf mountain pine populations which growing on granite (G) and limestone (W) substrata. *** – $p< 0.001$, ns – $p> 0.05$

Trait	Mean for G	Mean for W	Student's t-test value	*p*-value
1	10.484	9.018	6.23	***
2	8.419	6.802	5.91	***
3	1.275	1.343	-3.56	***
4	18.505	18.782	-1.02	ns
5	19.054	17.917	5.90	***
6	4.012	3.460	5.30	***
7	21.278	22.487	-1.83	ns
8	16.125	12.408	6.09	***
9	1.325	1.264	3.79	***
10	0.767	0.727	3.92	***
11	0.580	0.576	0.91	ns
12	0.088	0.042	10.92	***
13	0.136	0.073	8.56	***

In other studies describing the *P. mugo* variability expressed by the morphological and anatomical features of needles, it was found that the traits differentiating the population at the most were: needle length (not investigated in this study) (Bączkiewicz 1995), the width and width-height ratio in epidermal cells (Boratyńska et al. 2004) and the width (#9) and height (#10) of the needle on cross-section (Boratyńska et al. 2004). In the present study, 2 populations differed from rest of the tested populations with regard to needle width and height (achieving the highest values), namely, from the Jarząbcza Valley (DJ), and especially from the Długi Upłaz (DU). These two populations grow on granite bedrock and on similar (intermediate) elevations above sea level. In addition, the above traits were strongly correlated. In the studied populations, Pearson's correlation coefficient between the needle width (#9) and the height (#10) ranged from 0.85 to 0.91, and was statistically significant (p <0.001). Similar results were obtained by Boratyńska and Bobowicz (2000) on *Pinus uncinata* from the Pyrenees, as well as by Boratyńska (2002) on *P. mugo* from the Western Tatras. In the work of Bączkiewicz et al. (2005) concerning *P. mugo* from the Tatras, such a relationship was not detected. The height and width of pine needles from the Western Tatras examined by Boratyńska (2002) were greater than those obtained in this study, but are similar to the values for these two traits observed in the Ukrainian dwarf mountain pine populations (Boratyńska, Pashkevich 2001).

Comparing the *P. mugo* populations growing on granite and limestone bedrocks as a whole (table 5), there can be seen that only the values of 3 traits from 13 are higher in the populations on limestone bedrock. These are stomata row index (#3), the number of stomata on 2 mm long convex side of the needle (#4) and the height of epidermal cells (#7). However, based on the Student's t-test, it can be concluded that for traits #4 and #7, the differences observed were not statistically significant (Table 5). With regard to the remaining 10 examined traits, the differences between populations growing on granite and limestone were statistically significant (p <0.001), except for the distance between vascular bundles (#11). This trait was characterized by the lowest variability, when compared to all the examined traits (V ranged from 5.65% to 7.56%) (Table 2), which is not confirmed by other authors who studied *P. mugo* from the Western Tatras and Ukraine (Boratyńska, Pashkevich 2001; Boratyńska 2002).

4. Conclusions

1. The studied dwarf mountain pine populations differed significantly with regard to the analysed traits.

2. The most distinctive was the population from the Trzydniowiański Wierch, growing on the granite bedrock, at the altitude of 1600 m–1650 m a.s.l. It was characterized by the highest number and the highest density of stomata, as well as the smallest epidermal cells.

3. The most similar to each other were populations from Wielka Świstówka and Dziurawe, which grew on limestone bedrock in close proximity to each other. The similarity of these populations may be influenced by site conditions and, above all, the flow of genes between the populations.

4. The populations growing on granite bedrock exhibited higher values of 10 out of the 13 traits analysed. The observed variability of the studied populations may, however, be more influenced by the altitude of their occurrence than the bedrock type on which they grow. Populations growing at higher altitudes were characterized by the higher values of traits associated with stomata. These features may be an expression of adaptation of plants to high altitudes.

Conflict of interest

The Authors declare no conflict of interest.

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Authors' contribution

EMP – research concept, statistical analysis, development of the results, manuscript writing; AB – concept of research, collection of biological material, trait measurements, development of the results, manuscript writing; PW – preparation of results, literature review, manuscript writing; MC – preparation of the results, literature review, manuscript writing; PG – trait measurements, development of the results; KBC – research concept, collection of biological material, development of the results, manuscript writing.

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