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Variability of needle characters of *Pinus mugo* Turra populations in the Karkonosze Mountains in Poland

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Abstract: The basic aim of the work was to widen the knowledge about variation of *Pinus mugo* in the Karkonosze (Giant) Mountains and verification of provenance of several, probably planted populations on that basis. Seven populations were sampled, four on hardly accessible precipices of glacial cirques as natural, and three on the topical, mostly plane or only slightly inclined parts of the mountains as potentially alien ones. The variation of the needles was biometrically analyzed in respect of 23 morphological and anatomical characters. Measurement data were subject of multivariate statistical analyses. Results call attention on rather continuous variability of *P. mugo* in the Karkonosze Mts. The differences among samples were found as relatively low and no direct connections have been detected between variation and provenance from precipices versus plane sites.

Additional key words: mountain pine, morphology, anatomy, plant variation, plant conservation

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Introduction

Pinus mugo Turra is sub-Alpine plant, which form a specific plant communities from above upper forest line, but also appears sometimes in lower, mostly peat-bog locations (Jalas and Suominen 1973; Gostyńska-Jukaszewska and Zieliński 1976). The *Pinetum mugo* association from *Piniom mughi* alliance is typical for the subalpine vegetation belt of the highest mountain massifs throughout the mountains of central Europe (Pawłowski and Walas 1949; Beldie 1967; Malinovsky 1980; Sanda et al. 1992; Jirašek 1996; Matuszkiewicz 2001, Chytrý and Tichý 2003; Poldini et al. 2004; Tsaryk et al. 2006).

Sudetes stands appoint northern border of species occurrence (Jalas and Suominen 1973). *P. mugo* forms a thicket in the Karkonosze (Giant) Mountains. It forms there the most extend thickets of all the Sudetes, at elevations between 1250 and 1450 m, described as association *Pinetum mugo sudeticum* (Matuszkiewicz and Matuszkiewicz 1975; Matuszkiewicz 2001). This community covers area of subalpine storey, independently of site conditions, exhibition and inclination (Boratyński 1994). *P. mugo* in Karkonosze Mts. occurs also on dispersed localities on summits of rock or on peat-bog within forest layer. The lowest locality of the species has been reported from 850 m from peat-bog near Jakuszyce and the highest from 1550 m on the north-east slopes of Śnieżka in Poland (Boratyński 1994) and 1560 m on southern slops in Czech Republic (Skalický 1983).

The variation of *P. mugo* in the Karkonosze has not been an object of extended research. Only two populations, namely from Równia below Śnieżka and from

Łabski Szczyt were compared with populations from other than Sudetes populations of the species (Szweykowski 1969, Staszkiewicz and Tyszkiewicz 1976, Boratyńska and Boratyński 2003; Boratyńska et al. 2003, 2004, 2005; Boratyńska and Boratyński 2007; Marcysiak and Boratyński 2007).

Populations from Równia below Śnieżka and Łabski Szczyt have mark separateness in the majority of theses studies, that's why the basic aim of the present work was to widen of knowledge about variability of *Pinus mugo* in the Karkonosze Mountains.

The upper parts of the Karkonosze Mountains have been utilized as pastures in the historical times and *P. mugo* thickets have been destroyed on the large areas, except of precipices of the glacial cirques. The pasturing activity has stopped at the end of XIX century and *P. mugo* recovered at least partly its area, and has been planted in some places. Several populations on the planes or slightly inclined slopes at the topic parts of the mountains can be of such origin and even of seeds from out of Karkonosze. We expect that alien populations if any exist in the Karkonosze, will be different in morphological and anatomical characters of the needles from the native ones. This problem is important for the native gene conservation in the Karkonosze National Park.

Materials and methods

Needles of *P. mugo* were collected from seven populations in the Karkonosze Mts. Three of them, Równia below Śnieżka (GM 1), slopes between Łabski Szczyt and Szrenica (GM 2) and Śląskie Kamienie (GM 7) have been sampled at the topical parts of the mountains, while other four populations from steep slopes the glacial cirques (Table 1). The sampling has been performed according to methods described before (Boratyńska 2004; Boratyńska et al. 2005).

Two-year-old needles were analyzed in respect of morphological and anatomical traits (Table 2) generally accepted as diagnostic and distinguishing of *P. mugo* from *P. sylvestris* (Szweykowski 1969, Staszkiewicz and Tyszkiewicz 1976; Szweykowski and Bobowicz 1977; Bobowicz and Krzakowa 1986, 1988; Boratyńska and Bobowicz 2000, 2001; Boratyńska and Paszkiewicz 2001; Boratyńska 2002, 2004). Detailed descriptions of the methods of measurement can be found in earlier works (Boratyńska and Bobowicz 2001; Boratyńska et al. 2004).

The basic characteristics of traits were calculated for every individual within sample and for every sample. Student t-test has been used to verify the statistical significance of differences between populations. The cluster analysis and minimum spanning tree has been constructed on the basis of the closest Euclidean distances among individuals within samples and then among samples to verify the total species differentiation.

Relations among populations were illustrated using discriminant analysis. The aim of this analysis was to show the nearest-neighbourhood clusters of individuals in first two discriminates variables and to establish discriminant value of traits. Relations among all populations were also analyzed using the minimum spanning tree constructed on squares of minimal Mahalanobis distances. Multivariable analyses were based on the synthetic and simple characters not included to synthetics ones; the direct measurements have been omit (Łomnicki 2001; Watała 2002; Stanisz 2007 a, b). The STATISTICA 7.0 for Windows software (StatSoft) was used in the statistical analyses.

Results

Populations from Karkonosze are different at respect of several characters (Fig. 1). The most variable and differentiating among compared samples at statistically significant level is a length of needle (trait 1). The longest needles were observed for population from Kocioł Małego Stawu (GM 4) and from Czarny Kocioł Jagniątkowski (GM 5), with average 45 and 46 mm respectively. The sample Wielki Kocioł Śnieżny (GM 6) belongs also to long-needle populations. The shortest needles characterize population from Równia below Śnieżka (GM 1) and population from slopes between Łabski Szczyt and Szrenica (GM 2), with average about 38 and 35 mm, respectively. The samples from Śląskie Kamienie (GM 7) and Śnieżka slopes (GM 3) have also short needles (Table 3).

Table 1. Characteristics of studied populations of *Pinus mugo*

No.	Akronim	Localization	Longitude	Latitude	Altitude n.p.m. (m)	Number of tested specimens
1	GM 1	Równia below Śnieżka	15°47'41"	50°44'44"	1400–1420	30
2	GM 2	Between Łabski Szczyt and Szrenica	15°33'15"	50°47'40"	1350–1450	32
3	GM 3	Śnieżka above Kocioł Łomniczki	15°47'50"	50°44'40"	1300–1500	31
4	GM 4	Kocioł Małego Stawu near Samotnia	15°47'34"	50°44'41"	1350–1400	31
5	GM 5	Czarny Kocioł Jagniątkowski	15°35'30"	50°47'05"	1300–1400	33
6	GM 6	Wielki Kocioł Śnieżny	15°34'00"	50°46'55"	1400–1450	32
7	GM 7	Śląskie Kamienie	15°36'10"	50°46'40"	1410–1420	32

T-test showed that researched populations on statistically significant level differentiate also number of stomatal rows from both part of needles (traits 2 and 3), thickness of needle (trait 10), number of layers of sclerenchyma cells above vascular bundles (trait 16), Marcet's coefficient (trait 17) and shape of needle (trait 19). The number of stomata on convex (abaxial) and flat (adaxial) sides of needle (traits 2 and 3, respectively) distinguishes population from slopes between Łabski Szczyt and Szrenica (GM 2), and also but less significantly, population from Śląskie Kamienie (GM 7). In case of first of mentioned populations, also the number of resin canals on adaxial side of needle (trait 7) differ statistically significant level it from the others, except for those from the Równia below Śnieżka (GM 1). The needle thickness (trait 10) differs population from Śląskie Kamienie (GM 7) from all the others on statistically significant level. The number of layers of sclerenchyma cells above the vascular bundles (trait 16) differs at statistically significant level population from Równia (GM 1) from all the others. The width of needle (trait 9) and measurements of epidermis cells (traits 12–14) differentiates researched populations in small degree (Fig. 1).

Any significant differences have been observed for the number of stomata on both sides of needle (traits 4 and 5), quotient of stomatal rows (trait 18), shape of epidermis and hypodermis cells (traits 20 and 21), percent participation of fibre-like cells between vascular bundles and cells with thin walls and large lumens (traits 22 type A and type D) and percent participation of cells with thin walls and intermediate cells around resin canals (trait 23 type B and type C). These traits have approximated values at all analysed populations (Fig. 1).

The shortest and statistically insignificant (1,37; p= 0,3302) Mahalanobis' distance units populations from Wielki Kocioł Śnieżny (GM 6) and Śląskie Kamienie (GM 7). On the border of statistical significance is Mahalanobis' distance between populations Wielki Kocioł Śnieżny (GM 6) and Kocioł Małego Stawu (GM 4) (2,46; p=0,0123). The shortest Mahalanobis' distances among all the other populations are strongly statistically significant (Fig. 2).

The discriminant analysis shows that length of needle (trait 1), shape of needle (trait 19), type of cells between vascular bundles (trait 22 types B, C, D) expect of frequency of fibre-like cells (trait 22 type A), types of cells around resin canals (trait 23 types A, C, B), the shape of epidermis and hypodermis cells (traits 20 and 21, respectively) differentiate among populations at the highest level. The number of resin canals (trait 8), width of epidermis cells (trait 12), ratio of stomatal rows (trait 18), and frequency of fibre-like cells between vascular bundles (trait 22 type A) did not differentiate among researched populations.

The first discriminant variable (U_1) is responsible for almost 50% information of the total variation and depends mostly on length of needle (trait 1) and shape of needle (trait 19) (Table 4). The second variable (U_2) bears almost 22% of information about variability and has been determined mostly by shape of the needle and number of layers of sclerenchyma cells over floem on vascular bundles (traits 19 and 16, respectively).

On diagram of discriminant analysis individuals from all verified populations created one group and ranges of variability of these populations are similar. However, it is possible to notice that most distant are populations from Łabski Szczyt (GM 2), and in more restricted range also from Równia below Śnieżka (GM 1), Czarny Kocioł Jagniątkowski (GM 5) and Kocioł Małego Stawu (GM 4) (Fig. 3). This image first

Table 2. Needle characters analyzed

No.	Characters
1	Needle length [mm]
2	Number of stomatal rows on convex (abaxial) side of needle
3	Number of stomatal rows on flat (adaxial) side of needle
4	Number of stomata on 2 mm long section of needle, on convex side
5	Number of stomata on 2 mm long section of needle, on flat side
6	Number of resin canals on convex side of needle
7	Number of resin canals on flat side of needle
8	Total number of canals
9	Width of needle [μm]
10	Thickness of needle [μm]
11	Distance between the vascular bundles [μm]
12	Width of epidermis cells [μm]
13	Thickness of epidermis cells with hypodermis cells [μm]
14	Thickness of epidermis cells [μm]
15	Thickness of hypodermis cells [μm]
16	Number of layers of sclerenchyma cells above vascular bundles
17	Marcet's coefficient (character 11*9/10)
18	Stomatal rows ratio (character 2/3)
19	Needle thickness/width ratio (character 10/9)
20	Width of epidermis cells with hypodermis/thickness of epidermis cells (character 12/13)
21	Width of epidermis cells /thickness of epidermis cells (character 12/14)
22	Character of cells between vascular bundles [in %]
	Type A: fibre-like cells
	Type B: intermediate type, semi-fibrous cells
	Type C: intermediate type, fibre like elements lacking
	Type D: cells with thin walls and large lumens
23	Character of cells around the resin canals [in %]
	Type A: fibre-like cells
	Type B: intermediate type
	Type C: cells with thin walls and large lumens

1

	GM 1	GM 2	GM 3	GM 4	GM 5	GM 6
GM 2	x					
GM 3	x	xx				
GM 4	x	xx	xx			
GM 5	x	xx	x			
GM 6	x	xx	xx			
GM 7		xx		xx	x	x

2

	GM 1	GM 2	GM 3	GM 4	GM 5	GM 6
GM 2	x					
GM 3		xx				
GM 4		x				
GM 5		x	x			
GM 6		xx				
GM 7	x			x		x

3

	GM 1	GM 2	GM 3	GM 4	GM 5	GM 6
GM 2						
GM 3		x				
GM 4		xx				
GM 5		xx				
GM 6		xx				
GM 7			x	x	x	x

6

	GM 1	GM 2	GM 3	GM 4	GM 5	GM 6
GM 2						
GM 3						
GM 4	x		xx			
GM 5			x			
GM 6			x			
GM 7				x		

7

	GM 1	GM 2	GM 3	GM 4	GM 5	GM 6
GM 2						
GM 3		x				
GM 4		xx				
GM 5		xx				
GM 6		x				
GM 7						

13

	GM 1	GM 2	GM 3	GM 4	GM 5	GM 6
GM 2						
GM 3						
GM 4	x					
GM 5						
GM 6						
GM 7						

14

	GM 1	GM 2	GM 3	GM 4	GM 5	GM 6
GM 2						
GM 3						
GM 4	x					
GM 5						
GM 6						
GM 7						

15

	GM 1	GM 2	GM 3	GM 4	GM 5	GM 6
GM 2	x					
GM 3		xx				
GM 4						
GM 5	x	xx				
GM 6					x	
GM 7		xx				

16

	GM 1	GM 2	GM 3	GM 4	GM 5	GM 6
GM 2	xx					
GM 3	x					
GM 4	xx					
GM 5	xx					
GM 6	xx					
GM 7	xx		x			

17

	GM 1	GM 2	GM 3	GM 4	GM 5	GM 6
GM 2						
GM 3						
GM 4						
GM 5	x	xx	x	xx		
GM 6					xx	
GM 7					x	

8

GM 2						
GM 3						
GM 4	x	x	xx			
GM 5			x			
GM 6						
GM 7				x		
	GM 1	GM 2	GM 3	GM 4	GM 5	GM 6

9

GM 2						
GM 3						
GM 4						
GM 5			xx			
GM 6						
Gm 7			xx			
	GM 1	GM 2	GM 3	GM 4	GM 5	GM 6

10

GM 2						
GM 3		xx				
GM 4						
GM 5						
GM 6						
GM 7	x	x	xx	xx	xx	xx
	GM 1	GM 2	GM 3	GM 4	GM 5	GM 6

11

GM 2						
GM 3						
GM 4						
GM 5	x	x				
GM 6			x	xx	x	
GM 7					x	
	GM 1	GM 2	GM 3	GM 4	GM 5	GM 6

12

GM 2						
GM 3						
GM 4	x		x			
GM 5						
GM 6						
GM 7						
	GM 1	GM 2	GM 3	GM 4	GM 5	GM 6

19

GM 2						
GM 3		xx				
GM 4		x				
GM 5	xx	xx	xx	xx		
GM 6					xx	
GM 7					xx	
	GM 1	GM 2	GM 3	GM 4	GM 5	GM 6

22 typ B

GM 2						
GM 3						
GM 4						
GM 5	x		x			
GM 6		xx		x	xx	
GM 7					x	
	GM 1	GM 2	GM 3	GM 4	GM 5	GM 6

22 typ C

GM 2						
GM 3		x				
GM 4						
GM 5	x		xx			
GM 6		xx			xx	
GM 7					x	
	GM 1	GM 2	GM 3	GM 4	GM 5	GM 6

23 typ A

GM 2						
GM 3		x				
GM 4	x	x				
GM 5						
GM 6				x		
GM 7						
	GM 1	GM 2	GM 3	GM 4	GM 5	GM 6

Fig. 1. Result of Student's t-test for analysed characters from 7 samples of *Pinus mugo* (character numbers as in Table 2)

Table 3. Average values of needle traits of 7 analysed populations of *Pinus mugo* (populations name as in Table 1 and characters number as in Table 2)

Characters	GM 1	GM 2	GM 3	GM 4	GM 5	GM 6	GM 7
1	37.72	34.82	40.91	46.14	45.05	44.04	40.86
2	8.57	9.36	8.76	8.73	8.78	8.73	9.29
3	6.49	6.84	6.33	6.27	6.28	6.32	6.75
4	18.82	18.80	19.26	19.23	19.16	19.31	19.28
5	19.14	18.74	19.18	19.41	19.27	19.15	19.12
6	3.16	3.16	2.99	3.48	3.30	3.30	3.12
7	0.78	0.63	0.82	0.87	0.89	0.81	0.79
8	3.94	3.78	3.81	4.35	4.19	4.11	3.92
9	1396.64	1389.99	1374.50	1390.96	1436.89	1389.99	1428.31
10	829.86	838.46	808.36	824.31	818.14	826.63	858.12
11	84.00	85.41	84.30	77.23	99.69	83.18	85.55
12	15.74	15.20	15.77	15.24	15.47	15.56	15.87
13	42.85	40.67	41.84	41.14	41.75	42.49	42.20
14	30.31	28.07	29.53	28.54	29.77	29.66	29.77
15	12.55	12.60	12.32	12.60	11.99	12.83	12.43
16	1.16	0.74	0.85	0.70	0.73	0.71	0.55
17	142.42	51.38	144.54	45.62	176.74	140.92	51.15
18	1.35	1.40	1.41	1.43	1.43	1.41	1.40
19	0.60	0.60	0.59	0.59	0.57	0.60	0.60
20	0.37	0.37	0.38	0.37	0.38	0.37	0.38
21	0.53	0.55	0.54	0.54	0.53	0.53	0.54
22 type A	0.10	0.88	5.39	1.48	1.67	0.78	0.75
22 type B	2.50	26.91	36.19	32.74	20.67	48.41	35.97
22 type C	32.40	72.22	56.48	65.77	77.67	50.81	62.66
22 type D	64.97	0.53	0.00	0.00	0.21	0.81	0.03
23 type A	1.87	5.94	0.23	0.00	2.64	3.19	1.59
23 type B	36.10	21.53	27.48	22.87	30.03	23.78	20.75
23 type C	62.00	72.00	70.35	76.48	67.12	72.22	77.31

of all defines first discriminant variable (U_1). Rest of populations on diagram of discriminant analysis occupies center stages of the scatter plot. In respect of the second discriminant variable (U_2) the researched populations differ in very small range.

Dendrogram indicate on certain disparity of researched populations because they created two groups (Fig. 4), with population from Równia below Śnieżka (GM 1) and from Łabski Szczyt (GM 2) in one and all other in the second of them.

Discussion

The population of *P. mugo* from Równia below Śnieżka has exerted certain separateness from all other populations of the species from Poland in respect of the traits of needles (Szweykowski 1969). The populations from Równia and Łabski Szczyt in research based on traits of needles and cones have exerted greater similarity to population from Tatra Mts than to West Alp and Abruzzi Mountains (Boratyńska et al. 2004, 2005; Boratyńska and Boratyński 2007; Marcysiak and Boratyński 2007). In research concerning only traits of cones population from slopes between Łabski Szczyt and Szrenica is distinguish from researched Carpathians' populations (Staszkiewicz and Tyszkiewicz 1976). But in traits of needles populations from Równia below Śnieżka and from Łabski Szczyt relatively to Eastern Carpathians' populations, have exerted also observable significant differences (Boratyńska and Paszkiewicz 2001). The highest frequency of three-needles dwarf shoots were observed on Równia below Śnieżka, among more than 30 compared populations (Boratyńska and Boratyński 2003, 2006). It remembers population from Tatra and Seaside Alps in this respect (Boratyńska and Boratyński 2003, 2007).

Results of the present comparisons, despite important differences between populations in average values of several traits, indicate continuous variability *P. mugo* in the Karkonosze Mts. It shows lack of deep differences in based analysis on individuals of all popula-

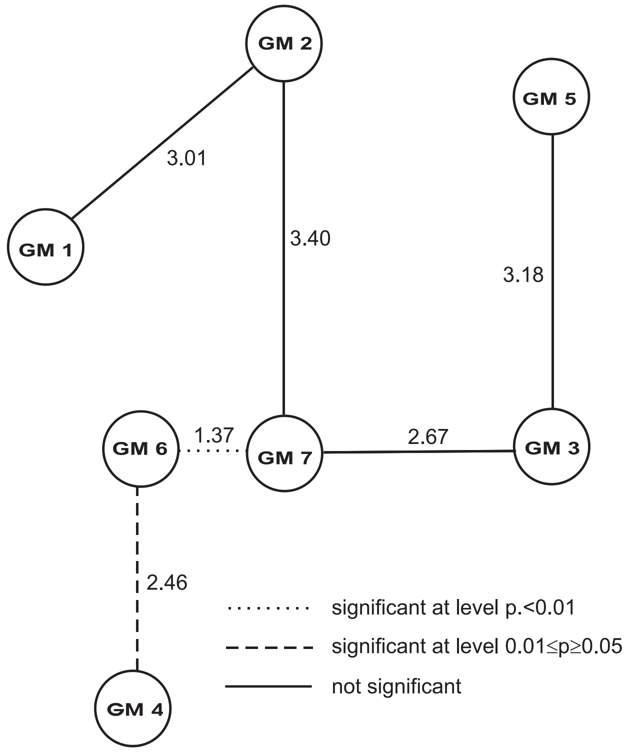

Fig. 2. Minimum spanning tree of *Pinus mugo* constructed on the basis of the squares of minimal Mahalanobis distances (acronyms as in Table 1)

Table 4. The coefficients of determination between discrimination variables and analyseed characters (number of characters as in Table 2)

Characters	U_1	U_2
1	19.72	1.27
4	0.69	0.27
5	0.96	0,00
8	2.19	0.21
12	0.66	0.05
16	0.35	2.23
17	0.46	1.58
18	0.80	0.16
19	6.76	4.19
20	0.04	0,00
21	0.03	0.16
23 type A	0.21	1.40
23 type B	0.12	1.07
23 type C	0.05	0.64
22 type A	0.62	0.05
22 type B	2.54	0.18
22 type C	0.01	1.17
22 type D	0.01	0.89

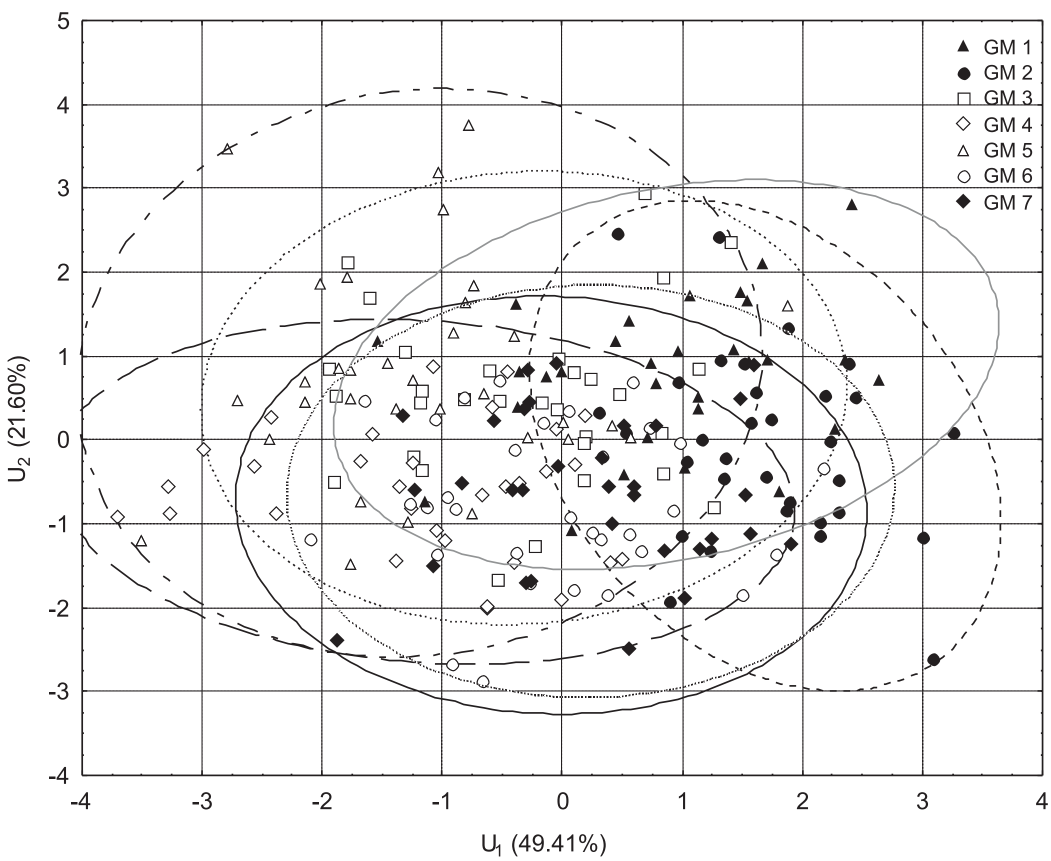

Fig. 3. Result of discriminant analysis based on the needle characters of 7 samples of *Pinus mugo* plotted along the two first discriminating variables U_1 and U_2 (acronyms as in Table 1)

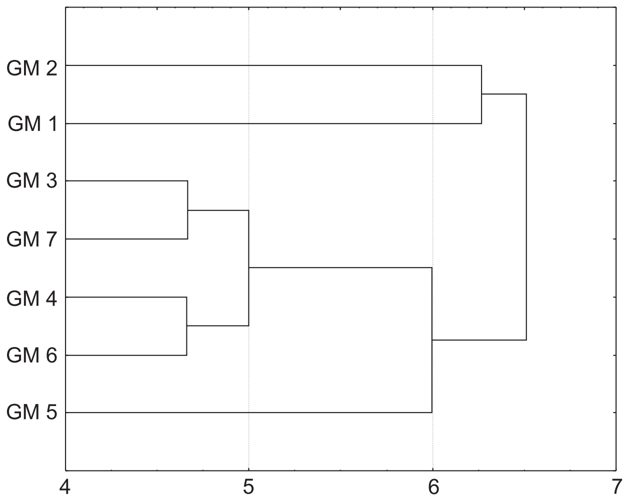

Fig. 4. Dendrogram of 7 samples of *Pinus mugo* constructed on the basis of the weighted pair-group method using arithmetic average (WPGMA) of the matrix of distances (acronyms as in Table 1)

tions. Discovered differences in this context can have an adaptation character or result from the spatial isolation and small population size, just as it has been said for *P. mugo* in Tatra Mts. (Bączkiewicz et al. 2005, Prus-Głowacki et al. 2005).

Studied morphological and anatomical characters of needles did not detect alien populations in the Karkonosze Mts. We can conclude, that populations of *P. mugo* from the topic, more or less plane part of the mountains, when even planted, come from the local material. This, however, shall be verified in the molecular study.

Several characters (traits 4, 5, 18, 20, 21, 22 and 23, see Table 2) did not differentiate among compared populations at statistically significant level and can be treated as typical for *P. mugo* from the Karkonosze Mts.

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