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Pinus uliginosa from Czarne Bagno peat-bog (Sudetes) compared morphologically to related *Pinus* species

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Abstract: *Pinus uliginosa* is an interesting taxon from the *Pinus mugo* complex with controversial systematic position and specific characteristics, intermediate among *P. mugo*, *P. uncinata* and *P. sylvestris*. The peat-bog pine is rare and protected in Poland. All its' known populations have a relict character and are slightly different from each other.
The aim of the present study was comparison of the individuals from the Czarne Bagno of the "Torfowisko pod Zieleńcem" Nature Reserve (Sudetes), determined in the field on the basis of morphological characteristics as *Pinus uliginosa*, with four samples of this taxon from the northern limits of its range in Poland and Germany and with *Pinus sylvestris*, *P. mugo* and *P. uncinata*, to verify morphological and taxonomic relations between them.
The material collected from 30 individuals determined as *P. uliginosa*, was closest to populations of *P. uliginosa* from the Bory Dolnośląskie, and to *P. mugo* from the Tatra Mts., concerning the needle characters. The cone characteristics of *P. uliginosa* individuals from the Czarne Bagno appeared similar to all other of that taxon. In spite of that, the cone characters first of all differentiate *P. uliginosa* from *P. sylvestris*, *P. mugo* and *P. uncinata*. The combination of needle and cone morphological characters are a good tool to distinguish *P. sylvestris*, *P. uncinata*, *P. mugo* and *P. uliginosa* with a very high probability.

Additional key words: plant variation, peat-bog pine, Scots pine, dwarf mountain pine, mountain pine

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Introduction

Pinus uliginosa Neumann, morphologically intermediate between *P. uncinata* Ramond and *P. mugo* Turra, is included into *P. mugo* complex (Christensen 1987; Businský 1999). Peat-bog pine was described from the Stołowe Mountains as *P. uncinata* (Neumann 1837; Wimmer 1837). In the following studies, it was classified on a different taxonomic level, lately as a hybrid swarm of *P. mugo* and *P. sylvestris* (Szweykowski 1969; Staszkiewicz and Tyszkiewicz 1972, 1976; Prus-Głowacki and Szweykowski 1979), the nothosubspecies of *P. mugo*, originating from hybridization of *P. mugo* sensu stricto with *P. uncinata*

(Christensen 1987), *P. rotundata* Link (Businský 1999), *P. uliginosa* Neumann (Danielewicz and Zieliński 2000; Boratyńska 2004), or *P. uncinata* subsp. *uliginosa* (Neumann) Businský (Businský 2008; Businský and Kirschner 2010). Because the systematic and nomenclatural status of this taxon is still unclear, in this article we use the name *Pinus uliginosa*. It is a rare tree, which in Poland reaches its northern limit of the geographic range. All localities of the species are under protection and in some of them *P. uliginosa* is endangered, mostly because of the lack of the regeneration. Another possible threat may be a gene flow from *P. sylvestris* to *P. uliginosa*, but the intensity of this process has lately been reported as very low (Lewandowski et al. 2002; Wachowiak et al. 2005a,b) or even of a reverse direction, from species of *P. mugo* complex to *P. sylvestris* (Wachowiak 2003; Jasińska et al. 2010).

By now, several populations of *P. uliginosa* from Poland and the Czech Republic have been compared biometrically on the basis of cone characters (Staszkiewicz and Tyszkiewicz 1972). The morphological characteristics of three extant populations of *P. uliginosa* from Poland were done during last decade, using needle and cone characters (Boratyńska et al. 2003; Marcysiak et al. 2003; Boratyńska and Boratyński 2007). The most numerous population of the species from "Torfowisko pod Zieleńcem" Nature Reserve in the Bystrzyckie Mountains has not been analyzed so far using multivariate statistical methods. What is more, within the reserve, a number of *Pinus mugo* specimens grow together with *P. uliginosa* and *P. sylvestris*. The latter species grows in the part surrounding the peat-bog, making this area a very interesting experimental system to study the potential reciprocal gene flow among the mentioned pine taxa, potentially resulting in their morphological affinities (Boratyński et al. 2003). However, the analyses based on cpDNA showed separateness of *P. uliginosa*, *P. mugo* and *P. sylvestris* on Zieleniec peat-bog (Wachowiak and Prus-Głowacki 2008).

The aim of the present study is a verification of morphological relations among: 1) *Pinus uliginosa* populations from its northern geographic range, 2) populations of *P. uliginosa* and closely related *P. uncinata*, *P. mugo* and *P. sylvestris*, using the needle and cone characteristics.

Methods

Study area

"Torfowisko pod Zieleńcem" Nature Reserve is situated in the Bystrzyckie Mountains, at the altitude of about 750–760 m and covers an area of 157 hectares (Fig. 1). It was established in 1954 to conserve the peat-bog with one of the only three localities of *Betula nana* L. in Poland (Regulation of the Minister of Forestry and Wood Industry 1954). The peat-bog was formed in the early Holocene, about 9000 years ago and has a sequence of the plants occurrence typical for central Europe, with pollen of *P. sylvestris*-type dominating in the early stages of the peat-bog (Madeyska 1989). It shall be underlined, that the first pine-type pollens in the peat-bog belonged most probably to *P. mugo/P. uliginosa*, but not to *P. sylvestris*, as only mountain pines were able to survive the last glaciation close to the Bystrzyckie Mountains and, consequently, settled there before *P. sylvestris* (Obidowicz 1996; Jankovská 2001, 2008; Jankovská and Pokorny 2008; Birks and Willis 2008). Currently, *P. uliginosa*, *P. mugo* and *P. sylvestris* grow on the peat-bog and, interestingly, the species rather do not form the putative hybrid swarm, as could be expected (Wachowiak et al. 2005a, b; Wachowiak and Prus-Głowacki 2008).

Material collection

For the present study, the material was collected in the northern area of Czarne Bagno, which lies in the southern part of "Torfowisko pod Zieleńcem" Nature Reserve (Fig. 1). Ten dwarf shoots, with normally developed needles, and 1–2 cones were collected from each of 30 individuals, morphologically identified as *P. uliginosa*. They were mid-high, mono- or polycormic trees with upright stem covered with dark, fissured

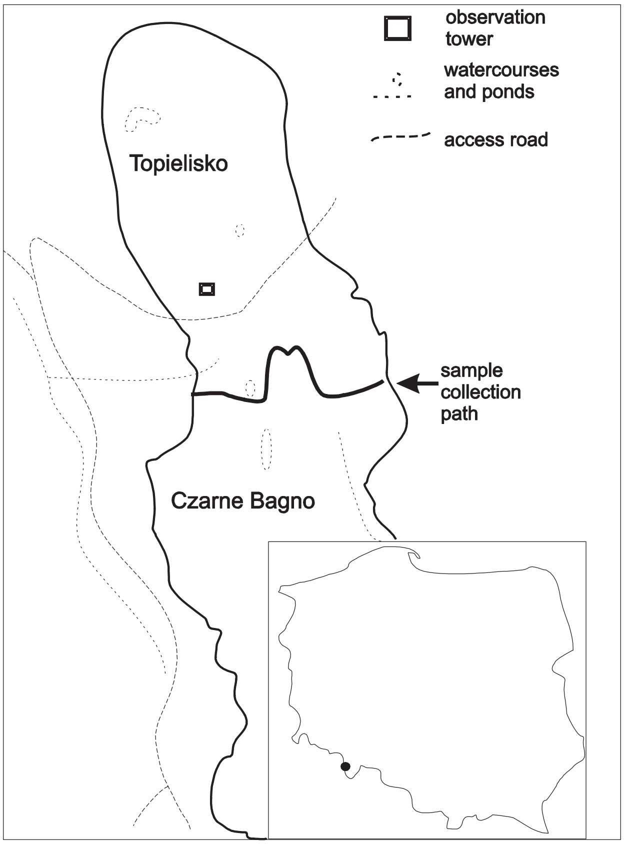

Fig. 1. Location the Nature Reserve "Torfowisko pod Zieleńcem"

bark, having the dense crown with dark sprouts, the dark green needles and at least slightly asymmetric cones.

Identically investigated material of *P. uliginosa, P. mugo*, *P. uncinata* and *P. sylvestris* was used for comparison (Table 1).

Characters studied

Characters of needles and cones diagnostic for identifying and distinguishing between taxa of the *P. mugo* complex were used (Szweykowski 1969; Staszkiewicz and Tyszkiewicz 1972; Christensen 1987; Boratyńska and Bobowicz 2000; Businský 2008; Boratyńska and Boratyński 2007, Marcysiak and Boratyński 2007).

In particular, the needle measurements methods and characters studied were based on Boratyńska and Bobowicz (2000) and Boratyńska and Boratyński (2007). The length of needles was measured immediately after the collection and then the needles were conserved in the 70% ethanol. The other needle characters were taken from the central part of the needle as described in Boratyńska and Bobowicz (2000), Boratyńska et al. (2003) and Boratyńska and Boratyński (2007) (Table 2 and 3).

Cones were characterized on the basis of 16 characters, according to the methods described by Marcysiak et al. (2003), Marcysiak (2004), and Marcysiak and Boratyński (2007) (Table 5).

Statistical analyses

The statistical calculations and comparisons of populations and taxa were conducted separately for needles and cones, because of the different nature of the data. The needle analyses were based on the average values of characters of individuals, calculated from 10 needles for every specimen, and each population was characterized on the base of 30 individuals. As far as cones are concerned, every population was characterized using a 50 cones sample without distinguishing individuals (Marcysiak 2004; Marcysiak and Boratyński 2007).

The unimodality of characters of needles were verified before calculations. The values of particular types of sclerenchyma (characters 16 and 17), presented in percentages, were arcsine transformed and then all the characters were standardized before the further statistical analyses to avoid a possible influence of different values and units of particular traits. The one-way ANOVA was used to verify the statistical significance of differences between average values of characters for populations. Tukey's post-hoc analysis (HSD) was performed to find out characters differentiating statistically significantly between populations

Table 1. Location of studied populations of *Pinus uliginosa*, *P. mugo*, *P. uncinata* and *P. sylvestris*

Taxon	Locality	Acronym	Geographic coordinates	Altitude (m)	Subject	Source
P. mugo	Poland, Karkonosze Mts., Czarny Kocioł Jagniątkowski	M_1	50°47'N 15°35'E	1350	needles cones	Sobierajska and Boratyńska 2008; Sobierajska et al. 2010
	Poland, Tatry Mts., Dolina Pięciu Stawów	M_2	49°13'N 20°03'E	1700	needles cones	Boratyńska et al. 2010; unpublished data of cones
P. uliginosa	Poland, Bystrzyckie Mts., Nature Reserve Torfowisko Zieleniec, Czarne Bagno	UL_1	49°13'N 20°03'E	700	needles cones	unpublished data
	Poland, Stołowe Mts., Large Batorów Peatland	UL_2	50°28'N 16°15'E	750	needles	Boratyńska et al. 2003
	Poland, Bory Dolnośląskie, Nature Reserve Węgliniec	UL_3	51°18'N 15°14'E	190	needles cones	Boratyńska et al. 2003; Marcysiak et al. 2003
	Poland, Bory Dolnośląskie, forestry Węglowiec	UL_4	51°19'N 15°12'E	170	needles	Boratyńska and Lewandowska 2009
	Germany, Bayerische Alpen, Mittelwalde	UL_5	47°29'N 11°16'E	850	needles cones	unpublished data
P. uncinata	Andorra, E Pyrenees, Vall de Ransol	UN	42°38'N 1°37'E	2000	needles cones	Boratyńska et al. 2004; Marcysiak and Boratyński 2007
P. sylvestris	Poland, Góry Stołowe, Szczeliniec Wielki Mt.	S_1	50°29'N 16°17'E	900	needles	Boratyńska et al. 2003
	Poland, Bory Dolnośląskie, forestry Węgliniec	S_2	51°18'N 15°14'E	170	needles	Boratyńska et al. 2003
	Poland, Bory Tucholskie, Krówka	S_3	50°29'N 16°17'E	90	cones	Marcysiak and Boratyński 2007
	Andorra, E Pyrenees, St. Miguel d'Engolasters	S_4	42°31'N 1°33'E	1400	cones	Boratyńska et al. 2009

and compared taxa. For needle traits of 16 and 17, with percentage values, the Kruskal-Wallis' test was used. Principal Component Analysis (PCA) was performed to verify the relationship between traits and the two principal variables, and to show relations between compared populations and taxa. The nested ANOVA was performed to assess the distribution of the variation of particular characters among taxa and populations within taxa.

STATISTICA PL 9 (StatSoft) was utilised in the calculations.

Results

Needles

The analysed populations and taxa differed significantly with regard to the all needle characters (1–15) according to the ANOVA. The greatest differences were found in the thickness of epidermis, the shape of epidermis cells and the shape of needle cross-section (characters 10, 15 and 14, respectively). The smallest, but still statistically significant differences concerned the number of stomata rows on the abaxial side of needle (character 2).

The analysed population of *P. uliginosa* from "Torfowisko pod Zieleńcem Nature Reserve" (UL_1) differed at statistically significant level in respect of at least three characters from all the others of that taxon (Table 2). The populations from Węglowiec and "Węgliniec Nature Reserve" (UL_4 and UL_3, respectively) were the most similar to it, while the populations representing Batorów peat-bog in the Stołowe Mountains (UL_2) and Mittelwalde peat-bog in the northern Alps (UL_5) were different with the respect of the most of the needle characters. Surprisingly, *P. uliginosa* from Czarne Bagno was more similar to the population of *P. uncinata* from the Pyrenees (UN) than to other populations of *P. uliginosa* (Table 2). A lot of needle characters did not differentiate at a significant level between population of *P. uliginosa* from Czarne Bagno and compared populations of *P. mugo* from the Sudetes and the Tatras, while most of them distinguished significantly *P. uliginosa* from *P. sylvestris* (Table 2).

In respect of the percentages of four types of the sclerenchyma cells between the vascular bundles (character 16), populations of *P. uliginosa* were similar to each other, as well as to *P. mugo* and *P. uncinata* samples, but differed significantly from *P. sylvestris* (Table 3). Some differences at a statistically significant level were found; however, between the population of *P. uliginosa* from Zieleniec and from Węglowiec (UL_4) and between *P. mugo* and *P. uncinata* (Table 3). Generally, the taxa from *P. mugo* complex showed rather inconspicuous differences in the percentages of various three types of the sclerenchyma cells around the resin canals (characters 17), while they differed significantly from *P. sylvestris* (Table 3).

The variation is divided into 9 variables in the Principal Component Analysis (PCA), but only the four first of them are statistically significant and resolve 87% of the whole variation of the needle characteristics. On the plane of the two first components, which

Table 2. Differentiation population from Torfowisko pod Zieleńcem and populations of *Pinus uliginosa*, *P. mugo*, *P. sylvestris* and *P. uncinata* in 15 characters of needles calculated using Tukey's test (RIR); × – significance at level p=0.05, ×× – significance at level p=0.01 (character numbers as in Table 2, population acronyms as in Table 1)

No.	Character	Population								
		M_1	M_2	UL_2	UL_3	UL_4	UL_5	UN	S_1	S_2
1	Needle length (mm)			××			×	××	××	
2	Number of stomatal rows on abaxial side of needle	××		××	××		××	×		××
3	Number of stomatal rows on adaxial side of needle	××		××			××		××	
4	Number of stomata on 2 mm long section of needle on abaxial side						××		××	××
5	Number of stomata on 2 mm long section of needle on adaxial side			××			××		××	××
6	Number of resin canals				××	××			××	××
7	Needle width (μm)			××	××	××	××	××		××
8	Needle thickness (μm)	××		××	××	××	××		××	××
9	Distance between vascular bundles (μm)	××	××	××			××		××	××
10	Thickness of epidermal cells (μm)		××	××	××		×		××	××
11	Width of epidermal cells (μm)	××	××	××	××				××	××
12	Marcet's coefficient (characters 9*7/8)		××	××			××		××	××
13	Stomatal rows ratio (characters 2/3)		××		××				××	××
14	Needle thickness/width ratio (characters 8/7)	××					××	××	××	××
15	Width/thickness ratio of epidermal cells (characters 11/10)		××	××	××		××		××	××

Table 3. Differences between the population from Czarne Bagno and *Pinus uliginosa*, *P. mugo*, *P. sylvestris* and *P. uncinata* populations, calculated for frequencies of types of sclerenchyma cells between vascular bundles and surrounding resin canals in the needles using Kruskal-Wallis test; ×× – significance at level p=0.01, × – p=0.05 (population acronyms as in Table 1)

No.	Character	Population								
		M_1	M_2	UL_2	UL_3	UL_4	UL_5	UN	S_1	S_2
1	Fibre-like cells between vascular bundles (character 16 type A)								××	××
2	Intermediate, semi-fibrous cells between vascular bundles (character 16 type B)							××		
3	Intermediate cells between vascular bundles (character 16 type C)		×						××	
4	Cells between vascular bundles with thin walls and large lumens (character 16 type D)							×	××	××
5	Fibre-like cells around resin canals (character 17 type A)				×	××			××	××
6	Intermediate cells around resin canals (character 17 type B)	××	××			××			××	××
7	Cells around resin canals with thin walls and large lumens (character 17 type C)		×			××			××	××

cover nearly 70% of the total variation, all the compared populations form two groups (Fig. 2). The first of them is formed by two populations of *P. sylvestris*, and the second, by *P. uliginosa*, *P. mugo* and *P. uncinata* samples. The population from “Torfowisko pod Zieleńcem Nature Reserve" is the closest to two populations of *P. uliginosa* from the Bory Dolnośląskie (UL_3 and UL_4), as determined by the first principal variable, responsible for 53% of the total variation (Fig. 1), which is the most closely positively correlated to the presence of fibre-like cells between vascular bundles, the number of resin canals, Marcet’s coefficient and the number of stomata on the abaxial side (characters 16A, 6, 12 and 4, respectively), and negatively to the ratio of the needle thickness/width and the presence of the thin-wall cells between the vascular bundles (characters 14, 16C and 16D). The second principal variable is responsible for less than 17% of the total variation and is determined mostly by the needle width (characters 7). It is noteworthy that the two first principal variables resolve 70% of the total variation of such characters as the number of resin canals, the thickness and the shape of the needle cross-section, the Marcet’s coefficient and the per-

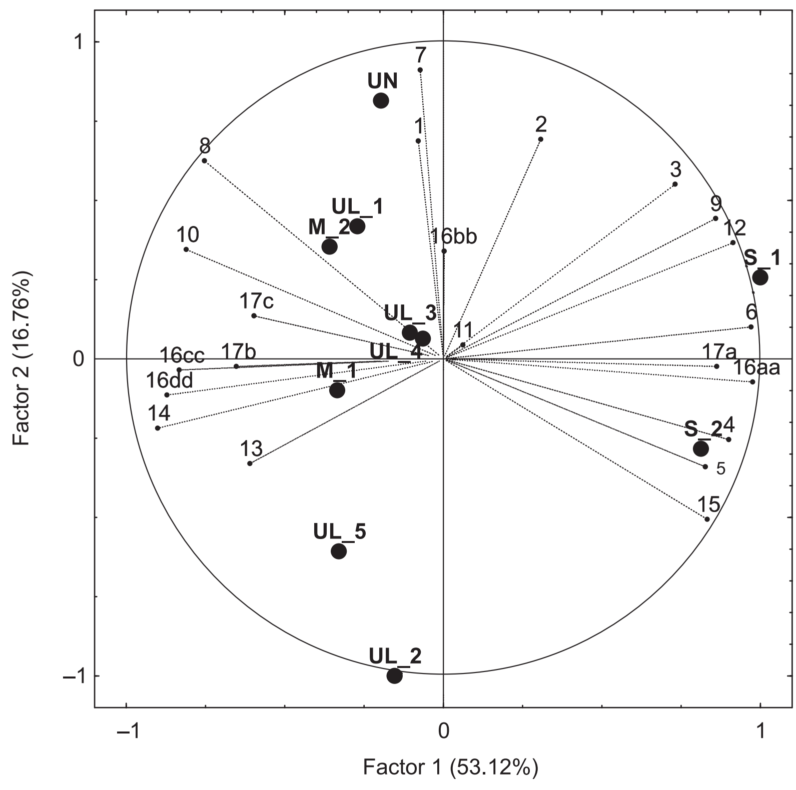

Fig. 2. Graph of the principal component analysis (PCA) bi-plot for 10 populations and 22 needle characters (acronym of populations as in Table 1, number of characters as in Tables 2 and 3)

centage of the fibre-like sclerenchyma cells between the vascular bundles (characters 6, 8, 15, 12 and 16, respectively).

The two first principal variables indicates the close connection of *P. uliginosa* from Czarne Bagno to *P. mugo* from the Tatra mountains (M_2). These two populations did not differ in respect of the number of stomata, the number of the resin canals, the needle width, the thickness of epidermal cells, the ratio of the width/thickness of the epidermal cells and the types of the sclerenchyma cells between the vascular bundles as well as the types of the sclerenchyma cells surrounding the resin canals (characters 4, 5, 6, 7, 10, 15 and 16A, 16B, 16C, 17A, 17C, respectively).

With the help of the mentioned above characters, the needles of *P. sylvestris* can be easily distinguished from needles of *P. mugo*, *P. uncinata* and *P. uliginosa*. The significantly higher number of the fibre-like cells occur around the resin canals of the needle of *P. sylvestris* (approximately 60–80%) when compared to the needles of the *P. mugo* complex. The number of resin canals in *P. sylvestris* needles is also about twice

Table. 4. Hierarchical analysis of variance based on the needle traits (character numbers as in Tables 2 and 3)

No. of charac-ters	Variance component	Percent of total	MS	DF	F ratio	Prob > F
4	between taxa	64.41	341	3	23.56	0.0009
	between populations of species	5.43	15	6	7.50	<.0001
	residual	30.17	2	344	.	.
5	between taxa	51.13	277	3	7.31	0.0195
	between populations of species	17.07	41	6	20.40	<.0001
	residual	31.81	2	344	.	.
6	between taxa	70.71	735	3	21.20	0.0013
	between populations of species	7.11	38	6	12.59	<.0001
	residual	22.18	3	344	.	.
9	between taxa	50.15	155319	3	7.58	0.0178
	between populations of species	15.93	22621	6	17.97	<.0001
	residual	33.92	1259	344	.	.
12	between taxa	62.33	910094	3	11.74	0.0062
	between populations of species	12.14	85589	6	18.17	<.0001
	residual	25.54	4709	344	.	.
14	between taxa	72.50	0.22	3	16.35	0.0026
	between populations of species	9.98	0.02	6	21.57	<.0001
	residual	17.52	0.01	344	.	.
15	between taxa	69.10	0.80	3	8.90	0.0124
	between populations of species	19.19	0.10	6	60.20	<.0001
	residual	11.71	0.01	343	.	.
16A	between taxa	79.95	70146	3	69.55	<.0001
	between populations of species	1.98	1016	6	4.47	0.0002
	residual	18.08	227	312	.	.
16B	between taxa	53.89	15565	3	31.01	0.0005
	between populations of species	2.54	505	6	2.85	0.0101
	residual	43.57	177	312	.	.
16C	between taxa	33.61	11915	3	8.17	0.0152
	between populations of species	8.41	1471	6	5.61	<.0001
	residual	57.99	262	312	.	.
16D	between taxa	43.43	31047	3	14.91	0.0034
	between populations of species	5.19	2099	6	4.21	0.0004
	residual	51.38	498	312	.	.
17C	between taxa	62.89	38736	3	10.56	0.0083
	between populations of species	13.63	3705	6	19.46	<.0001
	residual	23.48	190	312	.	.

higher (10–11 in average), than in the needles of *P. mugo* and related taxa.

The nested ANOVA showed that every characteristic of the needle differentiated between populations of every taxon at statistically significant level, while between taxa differentiated at significant level 12 of them (Table 4). The percent of variation between taxa was much higher than between populations within taxa for all characteristics differentiating significantly between taxa.

Cones

All the analysed character of cones differed between populations and taxa at the statistically significant level. The most discriminant were the cone diameter in the mid distance between the cone apex and the maximal cone diameter, and the distance between umbo and the cone scale apex (characters 9 and 7, respectively). The smallest, but still statistically significant differences, concerned the ratio of the length and width of apophysis and the ratio of the length to the maximal cone diameter (characters 14 and 12, respectively).

Population of *P. uliginosa* from Czarne Bagno (UL_1) were the most similar to the Alpine population (UL_5) (Table 5). A higher number of the statistically significant differences was found between peat-bog pine from Czarne Bagno and the remaining two populations of the taxon, and the population of from Węgliniec was the most different among them (UL_3). The Czarne Bagno and Węgliniec populations did not differ only with regard to the cone scale number (character 3) and the ratio of the length and width of the cone scale apophysis (character 14), but the latter did not differentiate among all the populations of *P. uliginosa*. Population of *P. uliginosa* from Czarne Bagno showed significant differences to *P. mugo* and *P. uncinata* samples, but lower level of differences to *P. sylvestris*.

The cone variation is described by 7 canonical variables, but 4 of them are statistically significant and resolve 96% of the total variation. On the graph presenting the results of the PCA analysis, the big distance of *P. uncinata* (UN) from all the others is remarkable. The plot is mostly determined by the first canonical variable, resolving almost 60% of the total variation (Fig. 3). This variable correlate positively at the highest level with the cone circumferences, the distance between umbo and the scale top, the cone apex diameter, the maximal cone diameter and the cone length (characters 10, 7, 9, 2 and 1, respectively). The second canonical variable, responsible for nearly 20% of the total cone variation, positively correlates to the ratio of the cone apophysis length to width (character 14). In the space between the two first canonical variables, all the populations of *P. uliginosa* formed one group, together with the sample of *P. sylvestris* from the Pyrenees (S_4). The second population of *P. sylvestris* (S_3) and the populations of

Table 5. Differences between the population from Czarne Bagno and *Pinus uliginosa*, *P. mugo*, *P. sylvestris* and *P. uncinata* populations, calculated for 16 cone characters using Tukey's Tukey's test (RIR); × – significance at level p=0.05, ×× – significance at level p=0.01 (population acronyms as in Table 1)

No.	Character	Population						
		M_1	M_2	UL_3	UL_5	UN	S_3	S_4
1	Length of cone (mm)	××	××	×		××	××	××
2	Maximal diameter of cone (mm)	××	×	××		××		
3	Cone scale number (item)	××			××	××	×	×
4	Length of apophyse (mm)	××	××	××		××	××	××
5	Width of apophyse (mm)	××		×		××		××
6	Thickness of apophyse (mm)	××		××		××		
7	Distance between umbo and scale top (mm)	××	××	××		××	××	××
8	Diameter of cone top	××	××	××	××	××		××
9	Diameter of cone in the middle distance between top and maximal diameter (mm)	××	××	××	××	××		
10	Measurement of convex side of cone from stalk to the top (mm)	××	××	××	××	××	×	
11	Measurement of concave side of cone from stalk to the top (mm)	××	××	××	××	××		
12	Ratio of cone length/maximal diameter (character 1/2)			××			××	××
13	Ratio of cone length/number of scales (characters 1/3)	××	××	××	××		××	
14	Ratio of apophyse length/width (characters 4/5)	××	××			××	××	
15	Ratio of apophyse length/thickness (characters 4/6)		××	××		××	×	××
16	Cone asymmetry (ratio of convex/concave cone measurements (characters 10/11)	××	××	××		××	××	

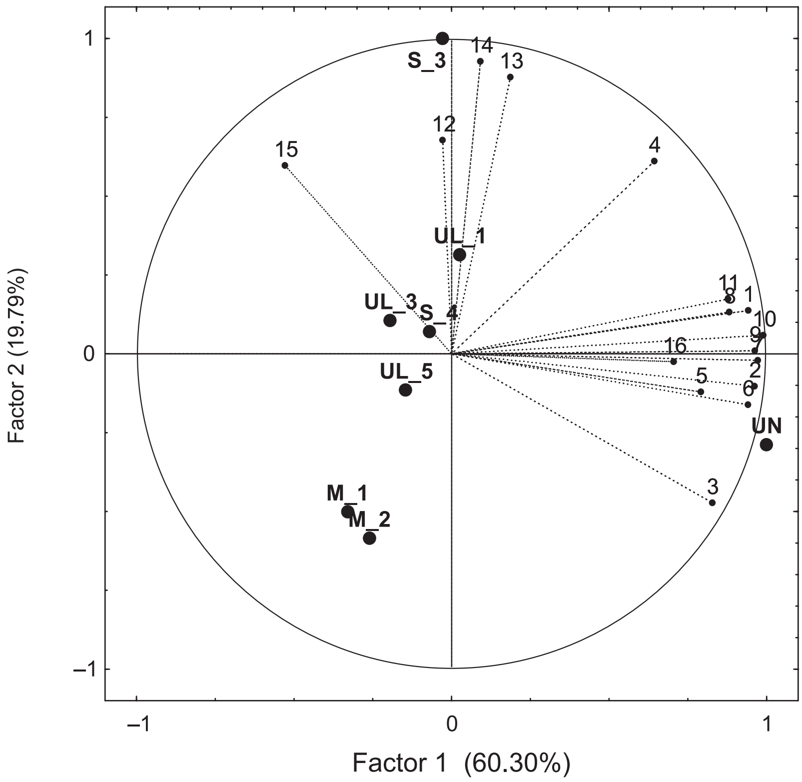

Fig. 3. Graph of the principal component analysis (PCA) bi-plot for 8 populations and 16 cone characters (acronym of populations as in Table 1, number of characters as in Table 5)

Table. 6. Hierarchical analysis of variance based on the cone traits (character numbers as in Table 5)

No of characters	Variance component	Percent of total	MS	DF	F ratio	Prob > F
1	between taxa	76.39	5901	3	64.85	0.0009
	between populations of species	1.67	871	4	3.89	0.0043
	residual	21.94	22	311	.	.
2	between taxa	72.72	1806	3	12.18	0.0178
	between populations of species	11.68	140	4	29.37	<.0001
	residual	15.59	4	311	.	.
3	between taxa	66.24	24463	3	10.95	0.0215
	between populations of species	11.82	2125	4	21.39	<.0001
	residual	21.95	99	311	.	.
6	between taxa	80.84	201.47	3	28.64	0.0038
	between populations of species	5.08	6.70	4	14.65	<.0001
	residual	14.08	0.46	311	.	.
7	between taxa	86.89	454.57	3	55.13	0.0011
	between populations of species	2.72	7.86	4	10.92	<,0001
	residual	10.39	0.72	311	.	.
9	between taxa	75.28	1820.80	3	11.00	0.0212
	between populations of species	13.70	157.22	4	48.09	<,0001
	residual	11.02	3.27	311	.	.
10	between taxa	80.36	16317	3	28.95	0.0037
	between populations of species	4.97	536	4	13.83	<,0001
	residual	14.67	38	311	.	.
16	between taxa	55.62	1.65	3	7.60	0.0400
	between populations of species	14.91	0.21	4	20.16	<,0001
	residual	29.47	0.01	311	.	.

P. mugo lay in the greater distance from *P. uliginosa* with regard to the cone characters.

The nested ANOVA showed that every cone characteristic differentiated between populations of every taxon at statistically significant level, while 8 of the differentiated between taxa (Table 6). The percent of variation between taxa was much higher than between populations within taxa for all characteristics differentiating significantly between taxa, similarly as in case of the needles.

Discussion

The compared material sampled as *Pinus uliginosa* on "Torfowisko pod Zieleńcem Nature Reserve" has morphological and anatomical characteristics similar to this described for this taxon from its several other localities (Boratyńska 2004; Boratyńska and Boratyński 2007; Boratyńska and Lewandowska 2009; Boratyńska et al. 2003, 2009, 2010). This confirms a possibility of determination of individuals in the field on the basis of the macro-morphological characters, as tree-like form of growth, dense crown, dark bark on the trunk and sprouts and dark, densely arranged needles. Such individuals generally have needle form typical for *P. uliginosa* from other Sudetan localities of that taxon. The cone characters of *P. uliginosa* were found more similar to *P. mugo* and/or *P. sylvestris*, than to *P. uncinata* (Marcysiak et al. 2003). The same relations were found in the present study. Generally, in respect of the needle characters *P. uliginosa* population from Czarne Bagno was the most close to *P. mugo* and in respect of cones to *P. sylvestris*.

The frequent opinions that co-occurrence of *Pinus sylvestris* and taxa of *P. mugo* complex leads directly to the formation of the putative hybrid swarm, as a result of an uncontrolled gene flow between them (eg. Amaral Franco 1986; Christensen 1987; Staszkiewicz and Tyszkiewicz 1969, 1972; Bobowicz 1990; Siedlewska 1994; Prus-Głowacki and Stephan 1998; Businský and Kirschner 2010), were partly confirmed by the results of the biometrical cones analyses, showing intermediate character of *P. uliginosa* compared to *P. sylvestris* and *P. mugo* (Szweykowski 1969; Staszkiewicz and Tyszkiewicz 1969, 1972; Szweykowski and Bobowicz 1977), as well as by the biochemical studies (Prus-Głowacki et al. 1978, 1981; Prus-Głowacki and Szweykowski 1983). However, the isoenzymes (Lewandowski et al. 2002) and *cp*DNA analyses (Wachowiak et al. 2000; Wachowiak et al. 2005a, b) showed that the hybrids occurrence is rather restricted. The examinations of the plastid and mitochondrial DNA, paternally and motherly inherited, respectively, confirm only one way of the gene flow, from taxa of *P. mugo* complex to *P. sylvestris* (Wachowiak and Prus-Głowacki 2008), in spite of the possibility of the reverse pollination (Boratyński et al. 2003). Thank to that, the existence of isolated small populations of *P. uliginosa*, retaining the morphological characteristics of the taxa, is possible, as it is observed in the Węgliniec and Węglowiec, which are surrounded by the extensive forests of *P. sylvestris* (Lewandowski et al. 2002; Boratyńska et al. 2003; Wachowiak et al. 2005a; Kormuťák at al. 2005).

The formation of hybrids within the *P. mugo* complex is another problem. The possible and very probable origin of *P. uliginosa* is by the hybridization between *P. mugo* and *P. uncinata* (Christensen 1987). The intermediate characteristics of needles of the latter taxa can be interpreted as confirmation of this thesis (Boratyńska et al. 2010). The DNA analyses of *P. mugo*, *P. uliginosa* and *P. sylvestris* from "Torfowisko pod Zieleńcem Nature Reserve" showed, however, rather a restricted gene flow among these three taxa (Wachowiak and Prus-Głowacki 2008). Despite the frequent occurrence of specimens of intermediate phenotype, they were seldom identified as hybrids with the genetic methods. The isoenzymatic polymorphism of expected hybrid of *P. sylvestris* and *P. mugo/P. sylvestris* on Zieleniec peat-bog showed high level of differentiation of compared groups of specimens (Siedlewska 1994), which indicates the heterogenity of tested material.

The genetic markers make now possible the precise distinguishing of *P. sylvestris* from *P. mugo/P. uliginosa* complex, but not in the case of *P. mugo*, *P. uliginosa* and *P. uncinata* (Wachowiak 2003; Heuertz et al. 2010). Thus, the determination whether *P. mugo* or *P. uliginosa* participated in the formation of hybrids of these taxa with *P. sylvestris* in "Torfowisko pod Zieleńcem Nature Reserve" is impossible.

The individuals morphologically resembling *P. uliginosa* can be also easily distinguished from *P. sylvestris* considering the needle characters (Boratyńska et al. 2003; Boratyńska at al. 2010). The biometrical studies of individuals determined in the field on the basis of morphological characteristics typical for *P. uliginosa*, the mono- or polycormic small trees with a dense crown form, very dark bark of the stems and branches and dark green needles, appeared generally resembling other populations of *P. uliginosa*, however, some of them were similar to *P. mugo*.

As far as the needle characters are concerned, the individuals from "Torfowisko pod Zieleńcem Nature Reserve" in the present study are similar to the individuals from other four populations of that taxon. Described before as the most different within the taxon sample of *P. uliginosa* from the Stołowe Mountains (Boratyńska et al. 2003), was confirmed to be the most distant from the others *P. uliginosa* populations in the present study. Surprisingly, the relations among compared taxa are quite reverse, when the cone characters are concerned. The cones of *P. uliginosa* resemble more the cones of *P. mugo* and *P. sylvestris* than of *P. uncinata*, as could be expected, being based on the former findings (Staszkiewicz and Tyszkiewicz 1972; Marcysiak et al. 2003; Marcysiak and Boratyński 2007).

It can be concluded that needle characters are a good tool to distinguish *P. sylvestris* from taxa representing *P. mugo* complex, but there are no powerful morphological markers, which allow distinguishing between *P. mugo*, *P. uncinata* and *P. uliginosa*. The only distinctive differences among the latter taxa were found in the thickness and the shape of the epidermic cells and in the shape of the needle cross-section, as it was pointed out earlier (Boratyńska et al. 2003; Boratyńska and Lewandowska 2009). Reversely, the cone characters, allow distinguishing *P. uncinata* from *P. sylvestris*, *P. mugo* and *P. uliginosa*. Combination of those two sets of characters is a good tool to distinguish correctly *P. sylvestris* and *P. uncinata* and also *P. mugo* and *P. uliginosa* with a very high probability.

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