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Differences among three populations of *Pinus uliginosa* and their relation to *P. sylvestris* as expressed by the needle characters

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Abstract: The results of morphological and anatomical studies on *Pinus uliginosa* needles from newly-discovered localities of this species in Węglowiec (Bory Dolnośląskie) are presented. The data obtained were compared to similar published material on *P. uliginosa* needles from its loco classico in Batorów and from the lowland locality in Węgliniec Nature Reserve, and to *P. sylvestris* growing nearby. In terms of needle structure, all three *P. uliginosa* populations were slightly different. The samples analyzed differed markedly from the sample of *P. sylvestris*, which contradicts the hypothesis that Scots pine has had a significant influence on the gene pool of *P. uliginosa*.

Additional key words: plant variability, peat-bog pine, Scots pine, biometric analysis, statistical analysis

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Introduction

Taxonomically, the peat-bog pine (*Pinus uliginosa* Neumann) belongs to the most controversial gymnosperms in Europe. It was described more than 170 years ago (Neumann 1837, Wimmer 1837), but its origin and systematic rank is still disputed. *P. uliginosa* belongs to the subsection *Sylvestres* of the genus *Pinus* and is closely related to dwarf mountain pine (*P. mugo* Turra) and mountain pine (*P. uncinata* Ramond), which grow in the mountains of Central Europe and in the Pyrenees and West Alps, respectively. These two species form more or less frequent intermediate forms and so they are sometimes treated as subspecies of *P. mugo* sensu lato (Christensen 1987; Businský and Kischner 2006). *P. uliginosa* is often treated as a hybrid form of *P. mugo* and *P. uncinata* (e.g. Holubičkova 1965), a marginal population of the latter taxon (Krzakowa et al. 1984), a hybrid of *P. mugo* and *P. sylvestris* L. (Staszkiewicz, Tyszkiewicz 1972; Bobowicz 1990; Staszkiewicz 2001), or sometimes as a synonymed of *P. rotundata* Link (Skalická 1988; Businský 1998). These four hypotheses have neither been confirmed nor rejected in the most recent investigations at the genetic and molecular level (Siedlewska and Prus-Głowacki 1995, Prus-Głowacki et al. 1998, Lewandowski et al. 2000, Wachowiak et al. 2005).

Morphologically, *P. mugo*, *P. uncinata, P. rotundata* and *P. uliginosa* form one complex, and peat-bog pine is intermediate between *P. mugo* and *P. uncinata*, being closer to one in some characters, and closer to the other in other characters (Businsky and Kirschner 2006, Marcysiak and Boratyński 2007, Boratyńska and Boratyński 2007). *P. uliginosa* also has a similar, intermediate character in genetic comparisons (Lewandowski et al. 2000, Lewandowski et al. 2002). The intermediate position of peat-bog pine between *P. mugo* and *P. uncinata* in spite of their geographical vicariance (compare maps 169 and 170 in Jalas and Suominen 1973), may be due to the history of their formation and migration during the Pleistocene.

Independently of its systematic position, *Pinus uliginosa* is a rare Central European taxon, known from the Eastern parts of the Alps and from the Sudetes (Businsky and Kirschner 2006). It is a medium-tall, light-demanding tree that survives only in places such as peat-bogs or rocky areas, where faster and higher growing trees do not outcompete it (Boratyński 1978, 1994). In Poland it is known from a few localities in the south-western part of the country, mostly in the Sudetes. It is a protected species with most of its stands occurring in Nature Reserves (Staszkiewicz 2001). It also survives at the classical locality on the Large Batorów Peatland in the Stołowe Mountains (Neumann 1837; Gołąb 1999). The species is recognized as endangered because of the disappearance of suitable sites in its geographic range (Staszkiewicz 2001). On the lowland it has been reported as spontaneous in the Bory Dolnośląskie region around Węgliniec, but by the middle of the XX century it was only found in the "Torfowisko pod Węglińcem" Nature Reserve (Piotrowska 1958, Boratyński 1994). Lately it has also been found in the Węglowiec Forest, close to the Nature Reserve (Danielewicz and Zieliński 2000). It covers a smaller area here but the peat-bog pine population consists of more individuals in better health.

Morphological and anatomical investigations of *Pinus uliginosa* from Poland and the Czech Republic showed that the population from Węgliniec differs from the others (Staszkiewicz, Tyszkiewicz 1972, Boratyńska et al. 2003). This was interpreted as being a result of more intensive hybridization between *P. uliginosa* and *P. sylvestris* in Węgliniec because of the large areas covered by the latter species around the Nature Reserve in which the population of *P. uliginosa* is conserved. However, the expected gene flow from Scots pine toward the peat-bog pine has not been confirmed (Lewandowski et al. 2002, 2005, Wachowiak et al. 2005), in spite of the lack of phenological isolation between these pines (Boratyński et al. 2003).

Every locality of *Pinus uliginosa* deserves to be scientifically verified and conserved because of the unrecognized character and rarity of the taxon. Analyses of the morphological and anatomical characters of the needle are useful tools for a comparative study of pines from the *P. mugo* complex, as found in previous investigations (Szweykowski 1969, Staszkiewicz and Tyszkiewicz 1972, Boratyńska et al. 2003, Boratyńska and Boratyński 2007). The main aim of the present study was to compare the needle traits of *P. uliginosa* from Węglowiec to those of a nearby population of the species from the Nature Reserve in Węgliniec and from its classical locality in Batorów, as well as to *P. sylvestris* from the nearest population. The comparative study between populations of the species from Węgliniec and Węglowiec was expected to be useful for determination of their origin and natural state.

Material and methods

A new locality of *Pinus uliginosa* was found in the territory of the Węgliniec Forest Office, at Węglowiec. The species covers an area of 0.64 ha close to the Czerna Mała river bank. The distribution of specimens of *P. uliginosa* on the peat-bog indicates that it could have occurred over a much larger area that has since dried out and become covered with *P. sylvestris* plantation (Danielewicz and Zieliński 2000). The locality in Węglowiec is about 3 km from the "Torfowisko pod Węglińcem" Nature Reserve. In total, 181 individuals of *P. uliginosa* have been found in Węglowiec, among them 87 in healthy condition (Danielewicz and Zieliński 2000).

Material for the present study was gathered from 33 individuals that were randomly dispersed over the area occupied by the peat-bog pine. Data from previous studies on *P. uliginosa* from the Nature Reserve Peat-Bog near Węgliniec and from Batorów Peat-Bog in the Stołowe Mountains, and *P. sylvestris* in the vicinity of Węgliniec, were used as comparative material (Table 1). Ten dwarf shoots were collected from two-year-old shoots, from well-insolated parts of the tree crown. One needle from every dwarf shoot was

Table 1. Localization of studied populations of. *P. uliginosa* and *P. sylvestris*

Taxon	Localization	Acronym	Longitude	Latitude	Altitude (m) n.p.m.	Number of specimens
Pinus uliginosa	Forestry management Węgliniec, forestry Węglowiec (Lover Silesia Forest)	ULG_1	15°12' E	51°19' N	170	33
	Reserve "Torfowisko pod Węglińcem" near Węgliniec (Lover Silesia Forest)	ULG_2	15°14' E	51°18' N	190	52
	Large Batorów Peatland (Stołowe Mts.)	ULG_3	11°16' E	47°29' N	750	50
Pinus sylvestris	Forestry management Węgliniec, forestry Węgliniec (Lover Silesia Forest)	SYLV	15°14' E	51°18' N	190	34

examined morphologically and anatomically. Five morphological and eleven anatomical characters and five proportions were used to describe needle variation (Table 2). The characters were selected based on previous biometric investigations on the pine needles (Szweykowski 1969, Boratyńska and Bobowicz 2001, Boratyńska et al. 2008). The measurement procedures and equipment used were similar to those described by Boratyńska and Bobowicz (2001) and Boratyńska and Boratyński (2007). The number of layers of sclerenchymatic cells above the vascular bundles inside the circum-vascular envelope were also described according to the following scheme: 0 – without sclerenchyma cell layer; 0.5 – incomplete sclerenchyma cell layer; 1–3 – sclerenchyma cell layer composed of 1, 2 or 3 rows of cells, respectively (Table 2). The number of rows of sclerenchyma cells above the vascular bundles, and the width of the epidermal cells, the width of the hypodermal cells, and the width/thickness ratio of epidermal cells (characters 21, 12, 13 and 18, respectively), were not utilized in comparisons because they were not assessed in the previous study.

All calculations were performed on the results of the measurements. Every individual of the verified population of *P. uliginosa* from Węglowiec (ULG_1) was described by the average values of characters calculated from ten needles. Percentages, such as frequencies of sclerenchyma cells (characters 19 and 20), were arcsine transformed ($p' = arcsin \sqrt{p}$) before statistical analyses (Watała 2002). The discriminant power of characters was calculated to evaluate their influence on differentiation of the population. Differences among particular trees were examined using Student's *t*-test. Discriminant analysis calculated on the synthetic and direct not included into synthetic characters was performed and ordination methods were used to check the range of variation within 33 tested trees.

The distance of the population of *P. uliginosa* from Węglowiec (ULG_1) to the two other populations of this species and to *P. sylvestris* was tested using average values of characters for every sample. The discri-

Table 2. Major statistical characteristics of the 21 needle characters analysed of *Pinus uliginosa* from Węglowiec

No	Characters	Arithmetical means	Minimum	Maximum	Standard deviation	Variability coefficient
1	Needle length (mm)	48.45	37.20	71.4	8.62	17.80
2	Number of stomatal rows on abaxial side of needle	9.94	7.50	12.8	1.42	14.29
3	Number of stomatal rows on adaxial side of needle	6.82	4.80	9.5	1.19	17.45
4	Number of stomata on 2 mm long section of needle on abaxial side	18.97	16.00	21.6	1.33	7.00
5	Number of stomata on 2 mm long section of needle on adaxial side	18.66	16.50	20.77	1.13	6.04
6	Number of resin canals	5.33	1.70	9.4	1.85	34.74
7	Needle width (μm)	1325.19	1082.40	1560.94	111.86	8.44
8	Needle thickness (μm)	794.47	685.16	901.11	60.71	7.64
9	Distance between vascular bundles (μm)	134.33	60.35	231.55	37.47	27.89
10	Thickness of epidermal cells (μm)	17.06	15.22	19.80	1.25	7.36
11	Width of epidermal with hypodermal cells (μm)	41.47	35.52	48.98	3.80	9.16
12	Width of epidermal cells (μm)	30.00	24.82	36.14	3.25	10.83
13	Width of hypodermal cells (μm)	11.50	9.42	14.27	1.18	10.23
14	Marcet's coefficient (=traits 9*7/8)	227.38	101.73	447.80	73.70	32.41
15	Stomatal rows ratio (=traits 2/3)	1.50	1.26	1.96	0.15	10.10
16	Needle thickness/width ratio (=traits 8/7)	0.60	0.52	0.69	0.04	6.10
17	Width/thickness ratio of epidermal with hypodermal cells (=traits 11/10)	0.42	0.31	0.52	0.04	9.93
18	Width/thickness ratio of epidermal cells (=traits 12/10)	0.58	0.42	0.73	0.06	11.11
19	Character of cells around the resin canals (in %):					
	typ A: fibre-like cells	76.95	47.00	94.00	11.82	15.36
	typ B: intermediate cells	22.70	6.00	46.00	11.09	48.86
	typ C: cells with thin walls and large lumens	0.62	0.00	8.00	1.69	272.23
20	Character of cells between vascular bundles (in %):					
	typ A: fibre-like cells	14.00	0.00	88.00	22.28	159.16
	typ B: intermediate, semi-fibrous cells	17.67	0.00	52.00	14.22	80.50
	typ C: intermediate cells	36.17	0.00	75.00	18.75	51.85
	typ D: cells with thin walls and large lumens	32.16	0.00	100.00	27.62	85.87
21	Number the sclerenchyma layer over the vascular bundles	1.44	0.39	2.25	0.46	31.63

minant power of particular characters was calculated and Student's *t*-test for independent samples was performed to determine the characters that caused the differences between populations and taxa. The relationships among populations were tested using multivariate statistical analyses, such as analysis of discriminant function and conglomeration. All characters were standardized before analyses (Łomnicki 2000, Watała 2002, Stanisz 2007). Statistica PL for Windows software was used for analyses and calculations.

Results

Intra-population variation of *Pinus uliginosa* in Węglowiec

The average values of analyzed characters and their statistical characteristics are presented in Table 2. The coefficients of variation calculated as the average for particular characters of all individuals in the peat-bog pine at Węglowiec, were relatively small for most characters except for type of sclerenchyma cell (Table 2). The number of stomata on the adaxial and abaxial sides of the needle (character 4 and 5) was the most stable, with a variation coefficient of 7.00 and 6.04%, respectively. The width and height of the needle, width and height of the epidermis cell, the shape of the epidermis cell and needle cross-section (characters 7, 8, 10, 11, 17 and 16, respectively) were also relatively stable.

The type of cells around resin canals, particularly the frequency of cells with a large lumen and thin walls (character 19C) was the most variable with a coefficients of variation above 270%. Only somewhat less variable was the type of cells between vascular bundles (characters 20A, 20B, 20C and 20D), with a coefficients of variation varying between 51 and 160%. The number of resin canals, the distance between vascular bundles, Marcet's coefficient and the number of rows of sclerenchyma cells above vascular bundles (characters 6, 9, 14 and 21, respectively) were also variable (Table 2).

The highest number of statistically significant ($p=0.01$) differences between individuals occurred in the length and width of the needle, and the number of resin canals and thickness of the needle (characters 1, 7, 6 and 8, respectively) (Table 3). There was a lack or a relatively low number of statistically significant differences between individuals in terms of the type of sclerenchyma cells around resin canals, the width of the hypodermal cell, the ratio of stomata rows and the thickness of the epidermis cell (characters 19C, 13, 15 and 10, respectively).

Analysis of the discriminating power of the characters (Table 4) showed that most individuals significantly differed from each other in all characters except frequency of fibre-like and intermediate cells around resin canals and frequency of intermediate, semi-fibrous cells between vascular bundles (characters 19A and 19B, and 20B, respectively). The most discriminating characters among individuals of *P. uliginosa* in the Węglowiec population were the length of the needle, the number of resin canals and the number of sclerenchyma layers over the vascular bundle (characters 1, 6 and 21, respectively, Table 4).

On the scatter-plot of discrimination between the first two discriminating variables, which are responsible for about 50% of the total variation in the sample (Fig. 1), individuals form a sufficiently compact group except for N° 5, 11, 13, 23 and 33, which are marginal and slightly distant. These trees were discriminated by the length of the needle (character 1) (Table 5). Individuals N° 11 and 13 had the longest needles (more than 70 mm), while N° 5, 23 and 33 had the shortest (about 40 mm) needles. The length of the needle (character 1) mostly determined the first discriminating variable U_1, responsible for about 29% of the total variation. The second discriminating

Table 3. Differentiation of *Pinus uliginosa* population from Węglowiec in 21 characters of needles calculated using Student's-t test at significance level p=0.01 and p=0.05 (character numbers as in Table 2)

Traits	XX p=0,01		X p=0,05	
	Number differences	%	Number differences	%
1	368	69.70	408	77.28
2	267	50.57	336	63.64
3	256	48.48	321	60.79
4	210	39.77	291	55.11
5	198	37.50	269	50.95
6	314	59.47	357	67.61
7	333	63.07	398	75.38
8	315	59.66	365	69.11
9	230	43.56	299	56.63
10	66	12.50	148	28.03
11	210	39.77	292	55.32
12	189	35.80	278	52.66
13	25	4.73	91	17.23
14	243	46.02	319	60.41
15	57	10.80	132	25,00
16	239	45.27	310	58.72
17	107	20.27	168	31.82
18	112	21.21	187	35.41
19A	152	28.79	242	45.84
19B	149	28.22	233	44.13
19C	0	0.00	25	4.73
20A	186	35.23	239	45.27
20B	194	36.74	273	51.70
20C	178	33.71	249	47.16
20D	247	46.78	322	60.98
21	269	50.95	330	62.50

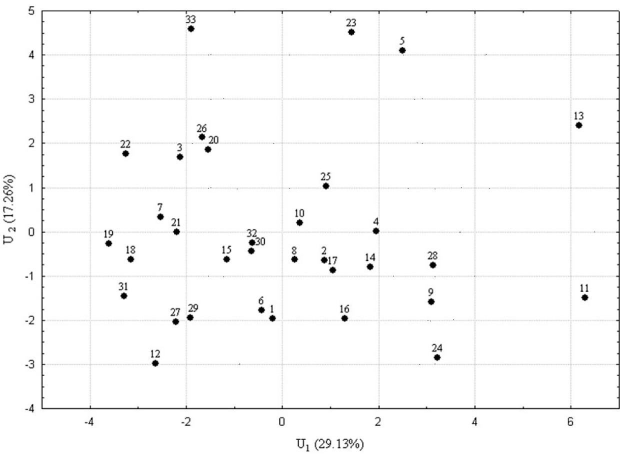

Fig. 1. Result of discriminant analysis for *Pinus uliginosa* from Węglowiec (ULG_1) plotted along the two first discriminant variables U_1 and U_2, which accounted for 46.39% of the total variation; 1–33 – tree numbers

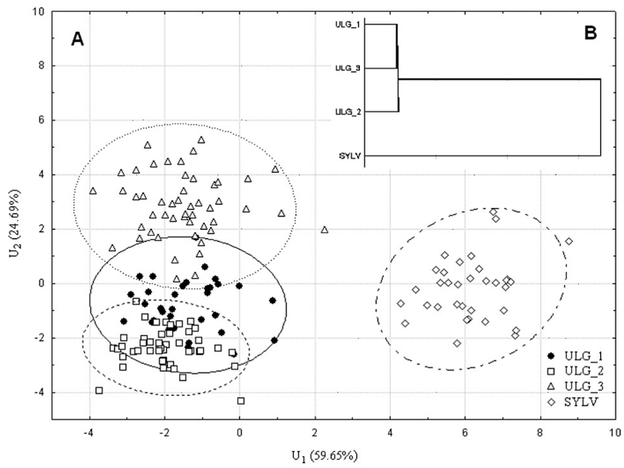

Fig. 2. Results of discriminant analysis (A) and dendrogram constructed on the shortest Euclidean distances (B) for *Pinus uliginosa* (ULG_1, ULG_2 and ULG_3) and *P. sylvestris* (SYLV) (population acronyms as in Table 1)

variable U_2, responsible for about 17% of the variation, was determined mostly by the frequency of fibre-like sclerenchyma cells between vascular bundles and the number of resin canals (characters 20A and 6, respectively). The somewhat isolated individuals N° 33, 23, 5 and 13 had a high number of resin canals and 42–88% of fibre-like sclerenchyma cells, while the average for the population was about 14%.

Relationships among populations of *Pinus uliginosa* and *P. sylvestris*

The average values of particular characters of the population of *Pinus uliginosa* from Węglowiec (ULG_1) were compared with data from the species populations from Węgliniec and Batorów (ULG_2 and ULG_3, respectively) and from *P. sylvestris* (Table 1 and 6). All three populations of *P. uliginosa* differed from *P. sylvestris* at a statistically significant level in every character except the number of stomata rows on the ad- and abaxial sides of the needle and the thickness of the epidermis cell (characters 2, 3 and 10, respectively). *P. uliginosa* from Węgliniec (ULG_2) had the same number of stomatal rows as *P. sylvestris*. The thickness of the epidermis cell was the same in *P. uliginosa* from Węgliniec (ULG_2) and Batorów (ULG_3) (Table 6).

Table 4. Discriminant power testing for the characters of *P. uliginosa* from Węglowiec (character numbers as in Table 2)

Traits	F statistics	p value
1	46.52	0.0000
4	5.80	0.0000
5	4.43	0.0000
6	18.50	0.0000
13	4.14	0.0000
14	8.19	0.0000
15	2.78	0.0000
16	7.87	0.0000
17	3.57	0.0000
18	4.60	0.0000
19A	1.22	0.2017
19B	1.00	0.4699
19C	1.84	0.0052
20A	10.72	0.0000
20B	0.00	1.0000
20C	3.42	0.0000
20D	7.43	0.0000
21	12.30	0.0000

Table 5. The determination coefficients between discriminant variables U_1 and U_2 and characters of needles of *Pinus uliginosa* from Węglowiec (character numbers as in Table 2)

Traits	U_1 (29.13%)	U_2 (17.26%)
1	24.20	0.01
4	0.00	0.58
5	0.01	0.53
6	0.00	6.42
13	0.01	0.23
14	0.08	1.03
15	0.27	0.09
16	0.99	0.06
17	0.00	0.00
18	0.01	0.04
19A	0.12	0.01
19B	0.11	0.02
19C	0.00	0.00
20A	0.37	8.01
20B	0.00	0.00
20C	0.01	1.37
20D	0.01	0.44
21	0.66	0.03

Table 6. Result of Student's t-test for analyzed characters of needles of *Pinus uliginosa* (ULG_1, ULG_2 and ULG_3) and *P. sylvestris* (SYLV): x – value significant at p=0.05, xx – value significant p=0.01 (population acronyms as in Table 1 and character numbers as in Table 2

Trait		ULG_1	ULG_2	ULG_3
Trait 1	ULG_2			
	ULG_3	xx	xx	
	SYLV	x	x	xx
Trait 2	ULG_2			
	ULG_3	xx		
	SYLV	x		x
Trait 3	ULG_2	x		
	ULG_3	xx	xx	
	SYLV	xx		xx
Trait 4	ULG_2			
	ULG_3			
	SYLV	xx	xx	xx
Trait 5	ULG_2	xx		
	ULG_3	xx	xx	
	SYLV	xx	xx	xx
Trait 6	ULG_2			
	ULG_3	xx	xx	
	SYLV	xx	xx	xx
Trait 7	ULG_2			
	ULG_3	xx	xx	
	SYLV	x	x	xx
Trait 8	ULG_2			
	ULG_3	xx	xx	
	SYLV	xx	xx	xx
Trai 9	ULG_2			
	ULG_3	xx	xx	
	SYLV	xx	xx	xx
Trait 10	ULG_2	xx		
	ULG_3	xx		
	SYLV	xx		
Trait 11	ULG_2	xx		
	ULG_3	xx	xx	
	SYLV	xx	xx	xx
Trait 14	ULG_2			
	ULG_3	xx	xx	
	SYLV	xx	xx	xx
Trait 15	ULG_2	xx		
	ULG_3	xx		
	SYLV	xx	xx	xx
Trait 16	ULG_2			
	ULG_3			
	SYLV	xx	xx	xx
Trait 17	ULG_2	x		
	ULG_3	xx	xx	
	SYLV	xx	xx	xx
Trait 19A	ULG_2	xx		
	ULG_3	xx		
	SYLV	xx	xx	xx
Trait 19B	ULG_2	xx		
	ULG_3	xx	x	
	SYLV	xx	xx	xx
Trait 19C	ULG_2	xx		
	ULG_3	x	xx	
	SYLV	x	xx	xx
Trait 20A	ULG_2			
	ULG_3			
	SYLV	xx	xx	xx
Trait 20B	ULG_2			
	ULG_3			
	SYLV	xx	xx	xx
Trait 20C	ULG_2			
	ULG_3			
	SYLV	xx	xx	xx
Trait 20D	ULG_2			
	ULG_3			
	SYLV	xx	xx	xx

Table 7. Discriminant power testing for the analysed characters of *Pinus uliginosa* (including ULG_1, ULG_2 and ULG_3) and *P. sylvestris* (character numbers as in Table 2)

Traits	F statistics	p value
1	16.98	0.0000
4	3.34	0.0210
5	3.43	0.0189
6	3.52	0.0166
14	0.46	0.7140
15	69.97	0.0000
16	6.20	0.0005
17	56.41	0.0000
19A	50.30	0.0000
19B	0.00	1.0000
19C	2.97	0.0340
20A	7.28	0.0001
20B	1.01	0.3915
20C	0.46	0.7089
20D	0.34	0.7938

Table 8. The determination coefficients between discriminant variables U_1 and U_2 and analized characters of *Pinus uliginosa* (including ULG_1, ULG_2 and ULG_3) and *P. sylvestris* (character numbers as in Table 2)

Traits	U_1 (59.65%)	U_2 (24,69%)
1	1.45	5.67
4	6.88	0.02
5	5.48	1.35
6	6.13	1.30
15	1.19	10.46
16	9.30	0.13
17	15.37	5.54
19A	10.00	0.39
19C	0.59	0.38
20A	10.32	0.04
20B	0.42	0.00

The various characters differed between populations of *P. uliginosa*. The population from Batorów (ULG_3) differed from Węglowiec (ULG_1) by the highest number of characters (Table 6). The only common characters for the ULG_1 and ULG_3 samples were the number of stomatal rows on the abaxial side of the needle, the stomatal rows ratio, the needle thickness/width ratio and the frequencies of types of sclerenchyma cells between vascular bundles (characters 4, 15, 16 and 20, respectively). The population of *P. uliginosa* from Węgliniec (ULG_2) differed significantly from that of Węglowiec by a lower number of characters (Table 6). The greatest differences between these two populations concerned the number of stomata on the adaxial side of the needle, the thickness and width of the epidermis cell, the stomatal rows ratio, the width/thickness ratio of the epidermis and hypodermis and the frequencies of particular types of sclerenchyma cells around the resin canals (characters 5, 10, 11, 15, 17 and 19, respectively).

The discrimination analysis revealed that centroids of all populations of *P. uliginosa* were distant from the centroid of the population of *P. sylvestris* (Fig. 2A, Table 7). The difference was determined by the first discriminating variable (U_1), which was responsible for about 60% of the variation. This variable is connected mainly with the shape of the epidermis cell and the frequency of fibre-like cells in the sclerenchyma around the resin canals and between vascular bundles (characters 17, 20A and 19A, respectively) (Table 7 and 8).

Differences between populations of *P. uliginosa* were not as great and were determined by the second discriminating variable (U_2), which was responsible for about 25% of the variation. This variable is connected primarily with the stomatal rows ratio, the shape of the epidermis cell and the length of the needle (characters 15, 17 and 1, respectively) (Table 8). Characters not quoted in Table 7 had very low or no discriminant power. The population of *P. uliginosa* from Węglowiec (ULG_1) had an intermediate character between populations from the Torfowisko pod Węglińcem Nature Reserve and from the Batorów peat-bog (ULG_2 and ULG_3, respectively).

The large differences between *P. sylvestris* and *P. uliginosa* and the similarities among the three populations of the latter taxon were also revealed by analysis of conglomerations (Fig. 2B).

Differences between individuals within the populations of *P. uliginosa* varied between populations. The population from Batorów (ULG 3) had the highest degree of differentiation, while that from Węgliniec Nature Reserve had the lowest (Fig. 2A).

Discussion

Conditions in the locality of *P. uliginosa* at Węglowiec indicate that it is the remains of a larger population of this taxon, as suggested by Danielewicz and Zieliński (2000). It is surrounded by large areas of *P. sylvestris* forest, as in the Węgliniec Nature Reserve.

The differences between *Pinus sylvestris* and *P. uliginosa* in terms of the needle characters are significant and have been discussed previously (e.g. Staszkiewicz and Tyszkiewicz 1972, Boratyńska et al. 2003). The most important are the various numbers of resin canals, the differing thickness of the epidermis cell and whole needle, the various values of Marcet coefficient and the numbers of stomata on both sides of the needle. The *P. sylvestris* needle cross-section contains 9–10 resin canals, while *P. uliginosa* has 3–5 (Staszkiewicz, Tyszkiewicz 1972, Boratyńska et al. 2003, Boratyńska et al. 2008). The thickness of the epidermis cell of *P. sylvestris* is about 20–25 μm and in *P. uliginosa* it is about 28–36 μm on average (Bobowicz and Korczyk 1994, Boratyńska et al. 2003). The needle of *P. sylvestris* is flatter than that of *P. uliginosa*, with an average ratio of height/width (character 16) of 0.51, while in *P. uliginosa* the ratio is 0.60. The needle of *P. sylvestris* generally has a higher Marcet coefficient and a higher number of stomata on both sides than the needle of *P. uliginosa* (Bobowicz and Korczyk 1994, Boratyńska et al. 2003, Boratyńska et al. 2008). Another important character is the very high frequency of fibre-like sclerenchyma cells between vascular bundles in and around the resin canals in the needle of *P. sylvestris*, while in *P. uliginosa* these are significantly rarer (Boratyńska and Boratyński 2007, Boratyńska et al. 2008). The same differences were also found in the needles of *P. uliginosa* at Węglowiec.

P. sylvestris and *P. uliginosa* also show significant differences in their biochemical composition and genetic structure (Prus-Głowacki and Szweykowski 1979, Siedlewska and Prus-Głowacki 1995, Lewandowski et al. 2000).

As discussed above, the three populations of *P. uliginosa* differed from *P. sylvestris* in many characters. Szweykowski (1969) reported that the population of this species from Węgliniec differed from the population at Batorów in the length of the needle, the area of the needle cross-section, the thickness of the epidermis and in the dimensions of the mesophyll cell. Staszkiewicz and Tyszkiewicz (1972) showed that the number of resin canals, number of stomata and Marcet coefficient of the population of *P. uliginosa* from Batorów were more similar to these characters in *P. mugo*, while in Węgliniec they were intermediate between *P. mugo* and *P. sylvestris*. It was also reported that there were more numerous cells with thin walls and large lumens around resin canals in individuals from Węgliniec than from Batorów (Szweykowski 1969, Boratyńska et al. 2003). More fibre-like cells and a lower number of medium-thin walled cells were found in the population from Węglowiec. The cells between vascular bundles in the needles of *P. uliginosa* from Węgliniec were reported as having predominantly thick and medium-thick walls (Szweykowski 1969) or thin and medium-thin walls (Boratyńska et al. 2003, Boratyńska and Boratyński 2007). The population at Batorów, was reported to have cells with medium-thick and medium-thin walls dominating between vascular bundles (Szweykowski 1969) or similar frequencies of each type (Boratyńska et al. 2003, Boratyńska and Boratyński 2007). At Węglowiec cells with thin and medium-thin walls predominated, as at Węgliniec (Boratyńska et al. 2003, Boratyńska and Boratyński 2007).

The ranges of variation in the three populations of *P. uliginosa* were also slightly different. The most variable population was from Węgliniec, and the lowest variation coefficients were found within the population from Batorów. The smaller number of individuals from Węgliniec (Danielewicz and Zieliński 2000) and Batorów (Gołąb 1999) did not influence the observed morphological differentiation (Boratyńska et al. 2003).

The variation coefficients of every character in particular populations of *P. uliginosa* differed (Table 2, Boratyńska et al. 2003), but showed similar tendencies. Nevertheless, there were 9 characters with variation coefficients below 10% at Węgliniec, 8 at Batorów and 8 at Węglowiec. In every population the lowest variation coefficients were found for the number of stomata (characters 4 and 5), width and thickness of the needle (characters 7 and 8), thickness and width of epidermal cells (characters 10 and 11), needle thickness/width ratio (character 16) and width/thickness ratio of epidermal and hypodermal cells (character 17).

At the same time, several characters had high variation coefficients. The characters with variation coefficients above 30% were the number of resin canals (character 6), distance between vascular bundles (character 9) and Marcet's coefficient (character 14). Extremely high variation coefficients were found for frequencies of sclerenchyma cells around resin canals and between vascular bundles (Boratyńska and Boratyński 2007). The frequency of cells with thin walls around resin canals had a variation coefficient of 140% at Batorów, 209% at Węgliniec (authors' unpublished data), and 272% at Węglowiec (Table 2). These extremely high variation coefficients resulted from the very low average frequency of thin-wall cells around resin canals (see Table 2). Nevertheless, the variation coefficients of frequencies of other types of cell around resin canals and between vascular bundles were very high (Boratyńska and Boratyński 2007). The variation coefficients in the population of *P. uliginosa* from Węglowiec were comparable with the other populations of this species.

Conclusion

The newly found population of peat-bog pine at Węglowiec differs in terms of needle morphology and anatomy from *P. sylvestris* and groups with previously described populations of *P. uliginosa* from the lowland locality of Węgliniec Nature Reserve and from the mountain reserve of Large Batorów Peatland.

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