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Interpopulational variability of *Pinus uncinata* Ramond ex DC. in Lam. & DC. (Pinaceae) cone characters

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Abstract: The characteristic of *Pinus uncinata* on the basis of biometrical analyses of 16 cone features was done. The research was based on 8 samples representing 6 populations, consisting of 50 cones each, collected in the Pyrenees, within the natural range of the species. All the investigated cone features were moderately differentiated, with the variation coefficient ranging from 8 to 30%. The particular populations differ significantly from each other statistically according to several features examined. The samples collected in the same population also vary significantly. The differences between populations, however, do not enable their division into groups, which supports their origination from the same Pleistocene refugia.

Additional key words: plant variation, biogeography, plant taxonomy, numerical taxonomy

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Introduction

Mountain pine – *Pinus uncinata* Ramond ex DC. in Lam. & DC. belongs to the genus *Pinus*, the most species-rich one within the family *Pinaceae* (Farjon 1998). It occurs in the mountains of the Western Europe, mainly in the Pyrenees, but also in Sierra Cebollera and Sierra de Gudar, in Massif Central and in the Western (Central) Alps (Amaral F. 1986; Christensen 1987b; Jalas and Suominen 1973). In the Alps the taxon meets dwarf mountain pine – *Pinus mugo* Turra, which has a wide range: from the Alps to the southern Carpathians and the mountains of the Balkans (Meusel et al. 1965; Jalas and Suominen 1973; Czopik 1976; Staszkiwicz and Tyszkiewicz 1976; Sokolov et al. 1977). Such a distribution of *Pinus mugo* suggests its possible dispersal from various Pleistocene refugia (Reinig 1937, after Kornaś and Medwecka-Kornaś 2002).

The morphological differentiation of *Pinus uncinata* has so far not been the subject of investigation. Among studies dealing with the *Pinus mugo* complex, including *Pinus uncinata*, there are historical monographic studies by Wilkomm from 1861 and Müller from 1887 (Christensen 1987b). Christensen (1987b) conducted the morphometric analysis of the *Pinus mugo* complex and its natural hybridization with *Pinus sylvestris*, but this was based mainly on herbarium material. Boratyńska and Bobowicz (2000) in their study of the variability of *Pinus uncinata* based on needle traits showed small and statistically non-significant differences among the investigated trees.

Thus, the aim of the present work is to characterise the morphological variation of *Pinus uncinata* on the basis of cone features, considered to be important from the taxonomic point of view (Bobowicz 1988; Christensen 1987a, b; Staszkiwicz and Tyszkiewicz

1976), and by any patterns, to substantiate migrations during Holocene.

Materials and methods

Because of the great variability of individuals (Holubičková 1965; Staszkiwicz and Tyszkiewicz 1976), the research was based on the samples representing populations. The samples were collected in the Pyrenees, outside the range of *Pinus mugo*, to exclude the influence of this taxon (Table 1). Two populations were sampled twice, in consecutive years, to detect year to year variation.

Each population was represented by 25 standing trees. 8–10 cones were collected from each tree, at the height of 2–3 m, from the sunny, southern, including

south-western and south-eastern side of the tree-crown. The cones were dried and seeds were taken out, atypical and damaged cones were excluded. Then 2 cones per tree were randomly selected. A few populations were sampled from the ground, by collection of 2 cones from under 25 trees. Eventually, each population was characterised on the basis of measurements of 50 cones, according to Staszkiwicz (1961).

The cone characters (Table 2) were selected in such way as to have the possibility of comparing the results with the data from earlier publications on *Pinus sylvestris*, *Pinus uliginosa* (= *Pinus rotundata*) and *Pinus mugo* (Staszkiwicz 1961, 1963, 1968, 1993; Staszkiwicz and Tyszkiewicz 1969, 1976; Szweykowski and Bobowicz 1977; Bobowicz et al. 1983, Bobowicz

Table 1. Location of studied populations of *Pinus uncinata* (1–8)

Number of sample	Location	Acronym	Longitude E	Latitude N	Altitude [m]
1	Spain, Central Pyrenees, Vall de Benasque	UNC 01	0°39'50"	42°41'20"	2000
2	Spain, Central Pyrenees, Port de la Bonaiqua	UNC 02	0°00'00"	42°38'10"	2100
3	Andorra, East Pyrenees, Coma Pedrosa	UNC 03	1°27'20"	42°34'50"	2100–2200
4	Andorra, East Pyrenees, Vall de Ransol	UNC 04	1°38'15"	42°37'00"	1800–1900
5	Andorra, East Pyrenees, Vall de Ransol	UNC 05	1°38'15"	42°37'00"	1800–1900
6	Spain, East Pyrenees, Vall de Nuria	UNC 06	2°11'10"	42°22'10"	2100–2300
7	Spain, East Pyrenees, Vall de Nuria,	UNC 07	2°11'10"	42°22'10"	2100–2300
8	France, East Pyrenees, Col de Jau	UNC 08	2°14'50"	42°42'00"	1500

Table 2. Cone characters analysed

No	Character	Accuracy and measure
1	Cone length	1 mm
2	Cone width	1mm
3	Cone scales number	
4	Apophyse of cone scale length	0.1 mm
5	Apophyse of cone scale width	0.1 mm
6	Apophyse of cone scale thickness	0.1 mm
7	Distance between umbo and scale top	0.1mm
8	Diameter of cone top	1 mm
9	Diameter of cone at midpoint between top and maximal diameter	1 mm
10	Measurement of convex cone side from stalk to top	1 mm
11	Measurement of concave cone side from stalk to top	1 mm
12	Ratio of cone length/width (1/2)	–
13	Ratio of cone length/number of scales (1/3)	–
14	Ratio of apophyse of cone scale length/width (4/5)	–
15	Ratio of apophyse of cone scale length/thickness (4/6)	–
16	Cone assymetry (ratio of convex/concave cone measurements, 10/11)	–

1988; Bobowicz and Korczyk 1990). Additionally, the characters which enabled the estimation of the cone assymetry (10, 11, 16) and the measurement of the length of an apophyse protuberance (7), a feature important for distinguishing *Pinus uncinata*, were analyzed. 11 measured and 5 other calculated cone features were included in the examination (Table 2, Fig. 1).

The cones were soaked to get them closed for measurements in that condition. Measurement of the cone length, width, two diameters and the circumference of the cone (characters: 1, 2, 8, 9, 10, 11) (Fig. 1) were conducted in such a state. The scale characters were measured on the scales taken off from the convex side and maximal diameter of the cone. Cone scales were counted on the opened cones, including even the smallest ones.

The data obtained were analyzed statistically, with the Statistica for Windows 1–5. The arithmetical means, standard deviations and variation coefficient were calculated for each sample and for the whole taxon, and the minimal and maximal values of characters were found. The unimodality of frequency distribution of all the examined characters values was checked. Associations between characters were tested with the Pearson's correlation coefficient (Łomnicki 2000).

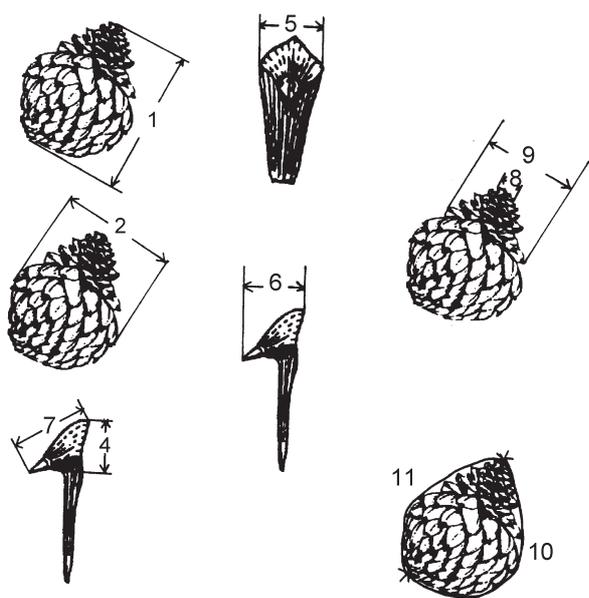


Fig. 1. Method of cone characters measurement (characters numbers according to Table 2)

Every population was compared to each other with t-Student's test according to the characters examined (Morrison 1990).

To show the interpopulational variability, the discriminant analysis was conducted (Zar 1999). The analysis was based on measured characters only.

Results

The frequency distributions of examined *Pinus uncinata* cones features values are unimodal or very close to unimodal (Fig. 2), indicating subjects investigated belong to the same taxon.

Cones of the examined *Pinus uncinata* populations are moderately differentiated. They are rather large, with the average length (character 1) 51.0 mm and the average width (character 2) 29.0 mm but these values range from 25 to 72 mm and from 17 to 40

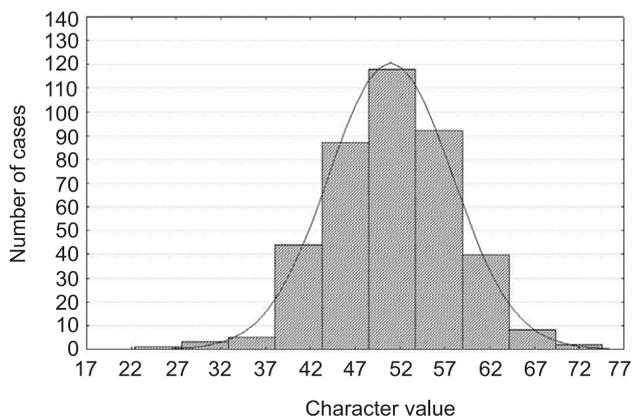


Fig. 2. Frequency distribution of character 1 (length of cone) values for 400 cones

mm, respectively (Table 3). The cone lengths average for the samples range from 47.2 to 55.1 mm and the cone widths from 26.9 to 31.4 mm. The variation coefficient of the above described features are 14 and 12%, respectively. Cones are elongated, their length is on average 1.76 time larger than their width and vary between 1.67 – 1.91 in particular populations. It is the most stable of the cone features (character 12), with the variation coefficient about 10%. The large difference between the cone diameter at the top (character 8) – on an average 7.7 mm, and the diameter measured in the midpoint between top and maximal diameter of a cone (character 9) – on an average 23.0 mm indicates that the cone shape is more conical than ovoid. These features are very variable, however, especially the smaller diameter, which ranges in the samples from 6.8 to 9.2 mm (the variation coefficient 25%). The asymmetry of the base of a cone is an important character. It was calculated as the ratio of convex to concave side of a cone (character 16= character 10/character 11). The mean value for the taxon is 1.51, with the variation coefficient about 13% (average values for samples vary from 1.37 to 1.63). The number of cone scales (character 3) range from 52 to 164 (variation coefficient 16%), the average value for the whole taxon is 109.4, for samples: 100.5 to 120.3. The apophyses of cone scales are relatively large, with mean length (character 4) of 8.56 mm and width (character 5) 9.17 mm. The range of both features is from 5.4 to 12.7 mm and average values in samples vary from 8.10 to 9.27 mm for the length and from 8.32 to 9.76 mm for the width of cone scales. Thick scales with long hook-shaped apophyse protuberances are very characteristic feature of *Pinus uncinata* cones. The cone scales thickness (character 6) is a variable feature (variation coefficient 23%) and range from 3.1 to 11.8 mm. The mean value for the taxon is 6, 14 mm, and for samples: 5.35 to 7.04 mm. The distance between umbo and scale top (the length of the apophyse protuberance) is a little more stable (variation coefficient 19%) and range from 4.4 to 12.7 mm, its average value for the whole taxon is 8.59 and for samples: 7.59 to 9.79. The most variable feature is the ratio of cone scale length/thickness (character 15), influenced by the character 6. Its variation coefficient is 26%, the mean value – 1.46, the averages of the samples: 1.26 to 1.65.

There are a few significant correlations between the analysed cone characters (Table 4). The most frequent are the correlations of the cone length (character 1), the cone width (character 2), the two parts of the circumference of the cone (characters 10, 11) and the diameter measured in the midpoint from the top to maximal diameter (character 9). The strong connection of the cone length with the two parts of the cone circumference, as well as of the cone width with the bigger diameter (character 9) is obvious. In addi-

Table 3. Statistical characteristics of features of *Pinus uncinata* cones

Statistical characteristics	Samples	Characters															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Mean	UNC 01	51.4	28.5	108.1	8.74	8.90	5.45	7.74	6.8	21.7	76.4	52.2	1.81	0.48	0.99	1.65	1.47
	UNC 02	51.2	26.9	103.9	8.75	8.92	6.08	8.60	7.5	21.7	78.7	48.7	1.91	0.50	0.98	1.50	1.63
	UNC 03	47.3	28.2	100.5	8.35	8.32	6.04	8.37	7.2	21.4	72.3	49.6	1.47	0.48	1.02	1.43	1.47
	UNC 04	54.8	31.3	120.3	8.40	9.56	6.76	9.64	8.0	26.0	83.8	56.3	1.75	0.46	0.89	1.27	1.49
	UNC 05	50.4	29.2	101.6	9.27	9.34	6.40	9.09	6.8	23.9	77.4	50.1	1.73	0.50	1.00	1.51	1.57
	UNC 06	55.1	31.4	116.8	8.59	9.76	7.04	9.79	9.2	25.6	84.4	55.3	1.76	0.48	0.89	1.26	1.53
	UNC 07	50.3	28.2	112.8	8.29	9.16	6.03	7.93	8.7	22.6	72.9	53.4	1.79	0.45	0.91	1.44	1.37
	UNC 08	47.2	28.0	111.7	8.10	9.33	5.35	7.59	7.4	21.4	72.3	48.0	1.69	0.43	0.88	1.62	1.52
All samples	51.0	29.0	109.0	8.56	9.17	6.14	8.59	7.7	23.0	77.3	51.7	1.76	0.47	0.94	1.46	1.51	
Minimum	UNC 01	41	20	81	5.4	6.8	3.5	4.5	4	13	61	40	1.4	0.3	0.7	1.0	1.2
	UNC 02	39	19	78	6.6	7.5	3.7	6.1	3	16	58	34	1.5	0.4	0.7	0.9	1.3
	UNC 03	25	18	52	5.5	5.4	3.4	4.4	4	12	41	27	1.3	0.3	0.6	0.7	1.0
	UNC 04	43	27	95	5.9	7.8	4.5	7.0	5	22	68	42	1.4	0.3	0.5	0.9	1.3
	UNC 05	40	24	76	7.6	7.7	4.2	6.4	3	18	60	36	1.3	0.4	0.7	0.9	1.2
	UNC 06	45	25	83	6.2	7.5	3.8	6.1	6	20	71	43	1.4	0.3	0.7	0.8	1.2
	UNC 07	40	22	73	5.8	7.5	3.5	4.9	6	18	59	40	1.5	0.3	0.7	0.7	1.1
	UNC 08	35	17	63	5.9	7.2	3.1	4.4	5	14	55	31	1.4	0.3	0.7	0.7	1.2
All samples	25	17	52	5.4	5.4	3.1	4.4	3	12	41	27	1.31	0.3	0.5	0.7	1.0	
Maximum	UNC 01	71	35	164	12.7	12.7	8.3	11.2	9	31	98	77	2.4	0.7	1.5	2.8	1.9
	UNC 02	72	36	136	11.6	11.7	8.3	11.7	15	31	106	64	2.3	0.7	1.4	2.3	2.1
	UNC 03	67	36	159	11.0	11.0	9.1	10.9	13	29	95	74	2.1	0.6	1.4	2.1	2.1
	UNC 04	67	36	146	12.1	12.4	8.4	12.4	10	31	96	66	2.0	0.6	1.5	1.9	1.8
	UNC 05	64	35	132	11.4	12.0	11.2	11.2	12	30	89	68	2.0	0.7	1.3	2.5	2.2
	UNC 06	65	37	146	12.3	11.5	10.0	12.7	18	33	103	69	2.3	0.7	1.3	3.0	1.9
	UNC 07	62	40	152	10.2	11.8	11.8	11.6	11	30	91	70	2.1	0.6	1.2	2.6	1.9
	UNC 08	58	38	149	11.3	11.6	9.7	12.0	11	32	87	58	2.2	0.6	1.3	3.2	2.6
All samples	72	40	164	12.7	12.7	11.8	12.7	18	33	106	77	2.4	0.7	1.5	3.2	2.6	
Standard deviation	UNC 01	7.14	3.61	16.68	1.65	1.28	1.08	1.61	1.29	3.54	10.32	7.31	0.19	0.09	0.20	0.40	0.16
	UNC 02	7.22	3.55	13.57	1.07	0.86	1.31	1.37	2.35	3.55	10.31	7.86	0.17	0.07	0.11	0.32	0.19
	UNC 03	8.80	4.21	20.45	1.34	1.19	1.27	1.50	2.03	3.98	12.67	8.78	0.13	0.79	0.17	0.31	0.19
	UNC 04	5.65	2.37	13.23	1.39	1.08	1.03	1.40	1.16	2.11	7.37	5.13	0.14	0.06	0.19	0.27	0.13
	UNC 05	5.50	2.39	12.33	0.98	0.91	1.39	1.11	1.94	2.70	6.93	6.95	0.15	0.07	0.14	0.34	0.22
	UNC 06	4.72	2.37	15.24	1.28	0.88	1.26	1.51	2.13	2.28	6.90	5.26	0.17	0.07	0.15	0.34	0.14

Statistical characteristics	Characters															
	Samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
UNC 07	5.57	3.20	14.74	1.12	0.82	1.44	1.37	1.36	2.58	8.11	6.06	0.14	0.06	0.12	0.36	0.16
UNC 08	5.40	3.78	19.41	1.28	1.06	1.50	1.65	1.26	3.42	8.30	6.19	0.16	0.07	0.15	0.50	0.21
All samples	6.91	3.56	17.18	1.31	1.10	1.39	1.63	1.91	3.54	10.06	7.34	0.17	0.07	0.16	0.38	0.19
Variation	13.88	12.67	15.42	18.90	14.35	19.88	20.78	19.02	16.30	13.51	13.98	10.22	18.17	19.89	24.31	11.12
coefficient	14.13	13.19	13.07	12.25	9.67	21.52	15.97	31.33	16.39	13.11	16.12	8.80	15.02	11.53	21.16	11.70
UNC 03	18.61	14.95	20.36	16.00	14.35	21.01	17.91	27.98	18.58	17.51	17.71	7.82	16.52	17.11	21.62	12.81
UNC 04	10.31	7.59	11.00	16.51	11.34	15.17	14.53	14.58	8.13	8.79	9.11	7.80	12.08	21.67	21.22	8.67
UNC 05	10.90	8.16	12.14	10.52	9.79	21.66	12.19	28.50	11.30	8.95	13.86	8.84	13.82	13.98	22.36	13.81
UNC 06	8.57	7.56	13.06	14.91	9.00	17.95	15.41	23.12	8.91	8.17	9.51	9.51	15.24	16.40	27.24	9.29
UNC 07	11.08	11.33	13.07	13.49	8.96	23.86	17.30	15.76	11.41	11.13	11.35	7.56	13.80	13.31	25.24	11.65
UNC 08	11.44	13.48	17.37	15.75	11.41	28.02	21.70	16.99	16.01	11.49	12.89	9.37	16.13	17.09	30.56	13.91
All samples	13.56	12.30	15.71	15.27	11.98	22.67	19.02	24.83	15.39	13.02	14.20	9.61	15.81	17.42	26.11	12.59

tion, the cone length is significantly correlated with the larger diameter and with the cone width. The apophyses features show fewer significant correlations. The strongest is the connection of the apophyse width (character 6) with the length of the apophyse protuberance (character 7). The two above mentioned apophyse features are strongly correlated with the larger diameter (character 9) and also with the cone width (character 2). The apophyse width (character 5) has similar associations, with characters 2 and 9. The scale number (character 3) is correlated with the apophyse width (character 5) and the measurement of the concave side of a cone (character 11) only, while the apophyse length (character 4) and the diameter of the cone top (character 8) do not have any significant correlations.

There are many statistically significant differences between populations and all the characters examined are important for this variability (Table 5). What is interesting, the significant differences between samples collected in the same populations (samples 4–5, 6–7) are also numerous.

The analysis of discriminant function of 11 measured cone characters indicated that all characters had very weak influence on population differentiation, because their Wilk's lambda component is close to 1. The first three discriminant variables explain almost 75% of the whole differentiation (Table 6). In the space between them, the points representing samples' averages appear to be a little dispersed (Fig. 3). On the other hand, in the analysis of all the cones representing examined populations, the objects form one concentration and any groups of cones belonging to particular samples cannot be distinguished (Fig. 4).

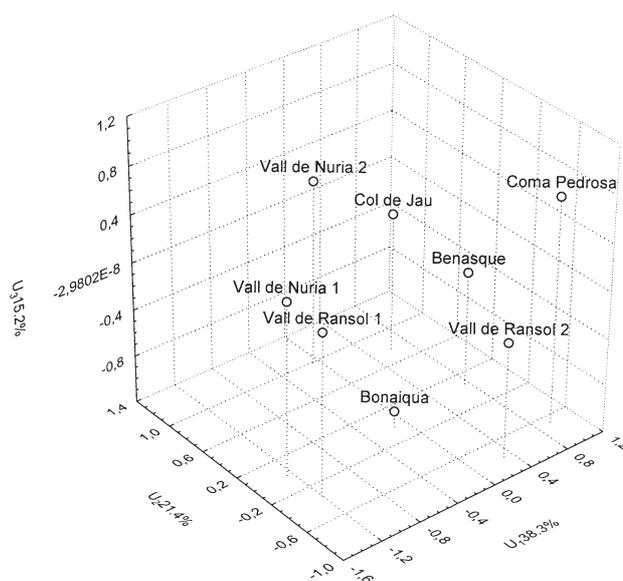


Fig. 3. Result of the discriminant analysis based on cone characters 1–11 plotted along the three first discriminant variables U_1 , U_2 and U_3 , for samples' averages

the taxon is bigger, though it was usually analysed for *Pinus mugo* treated as the complex species, comprising typical *Pinus uncinata* (Christensen 1987b).

The differentiation of *Pinus sylvestris* cones, the other species closely related to *Pinus uncinata*, is much greater, but the range of this taxon is much more extensive, too (Staszkiwicz 1961, 1968, 1993). Interpopulational variability of Scots Pine cones in Poland was statistically analysed by Bobowicz et al. (1983). Results of this study showed the significant variability of populations in different regions of Poland and confirmed the heterogeneity of Scots Pine.

The variability of *Pinus uncinata* cone characters is slightly larger than that of needle characters (Boratyńska and Bobowicz 2000). There are also more statistically significant differences between populations for cones than for needle traits. But generally *Pinus uncinata* is moderately variable, on the basis of cones and needles characters.

The small differences between populations support the theory that all of them originate from the same Pleistocene refugium. This thesis is also supported by rather weak differences in the needle characters (Boratyńska and Bobowicz 2001) and in the isoenzymes (Lewandowski et al. 2000).

Conclusions

The analysis show that the investigated populations of *Pinus uncinata* differ a little, as far as cone features are considered. However, the differences are not large enough to distinguish and describe various types of cones. It can be stated that all investigated populations appear to come from the same Pleistocene refugium.

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