



*Krystyna Boratyńska, Marcin Hinca*

## Morphological characteristic of *Pinus sylvestris* L. in the southernmost, isolated locality in the Sierra de Baza (S Spain) as expressed in the needle characters

Received: 10 July 2003, Accepted: 1 September 2003

**Abstract:** Two year-old needles of *Pinus sylvestris* were collected from 32 individuals in the Sierra de Baza (Spain). The needles were analysed in respect to 15 morphological and anatomical characters. Data obtained were subject of multivariate statistical analyses. The most stable characters appear to be needle thickness/width ratio, thickness and width of epidermal cells. The most variable characters include the distance between vascular bundles and Marcet's coefficient. Intrapopulation variation is low.

**Additional key words:** Plant variation, Scots pine, biometrics, multivariate analysis

**Addresses:** K. Boratyńska, Polish Academy of Sciences, Institute of Dendrology, Parkowa 5, 62-035 Kórnik, Poland, e-mail: borkrys@man.poznan.pl  
M. Hinca, August Cieszkowski Agricultural University of Poznań, Faculty of Horticulture, Dąbrowskiego 159, 60-594 Poznań, Poland

### Introduction

In the Iberian Peninsula, Scots pine (*Pinus sylvestris* L.) occurs mainly in the Pyrenees and in the central part of the Peninsula. Besides, it grows also in the Sierra de Gerês and in Sierra Nevada and Sierra de Baza (Fig. 1). In the last, isolated populations Scots pine has survived since the Tertiary (Mirov 1967, Tobolski and Hanower 1971).

Scots pine is believed to have at least three climatic varieties (climatypes) in the Iberian Peninsula: (1) var. *iberica* Svob., in the mountains of central Spain; (2) var. *pyreneica* Svob., in the Pyrenees; and (3) var. *nevadensis* Chist., in Sierra Nevada and in the Sierra de Baza in southern Spain (Svoboda 1953). In the Pyrenees, within var. *pyreneica*, two subvarieties can be

distinguished: eastern and western. The eastern subvariety is close to Scots pine from Catalonia, described by Gausen (1960), while the western is similar to the typical var. *pyreneica* Svob. (Nicolas and Gandullo 1969). Most probably, the material from Sierra de Baza can be assigned to var. *nevadensis*. Results of genetic research suggest that this population is clearly distinct from populations found in central and northern Spain (Pardos et al. 1990, Prus-Głowacki and Stephan 1994).

The aim of this study was to assess the variability of Scots pine needles from the relict locality in Sierra de Baza in southern Spain. In this population, no such an extensive biometric analysis of needles was conducted before.



Fig. 1. Range of *Pinus sylvestris* on the Iberian Peninsula (after Galán Cela, Gamarra Gamarra, García Vinas 1998)

## Material and methods

The isolated population of Scots pine in the mountain range Sierra de Baza in Granada (S Spain) is located at the 37°22' N latitude. Two-year-old needles were collected in 2001 from 32 trees growing close to the summit of Baza Mt., at an altitude of about 2000 m (Fig. 1). A total of 320 needles (10 from each tree) were analysed in this study.

The biometric analysis involved 11 directly measured characters (morphological 1–5, anatomical 6–11), and 4 calculated characters (12–15) (Table 1). The set of characters and the method of measurement were based on earlier biometric studies of pine needles (Szweykowski 1969; Bobowicz 1990, Boratyńska and Bobowicz 2000, 2001).

Additionally, two observed anatomical characters were taken into account. They are concerned with the development of sclerenchyma cells in needles (Szweykowski 1969, Bobowicz 1990). On the basis of the character of cells surrounding resin canals and between two vascular bundles, each individual can be assigned to the typical *P. sylvestris*, *P. mugo* or an intermediate form. The first character describes the cells surrounding resin canals, in three variants: (A) sclerenchyma fibres with very thick, colourless walls and narrow lumen, typical for *P. sylvestris*; (B) intermediate cells; and (C) cells with average wall thickness and wide lumen, yellowish, typical for *P. mugo* (Fig. 2). The second character describes the cells located between vascular bundles, in four variants: (A) large groups of sclerenchyma fibres, with very thick, colourless walls and very narrow lumen, typical for *P. sylvestris*; (B) sclerenchyma fibres in small groups or single; (C) cells with average wall thickness, no sclerenchyma fibres; (D) cells with thin or only slightly thickened walls, lumen very distinct, typical for *P. mugo* (Fig. 3). Contributions of the various cell types were estimated as percentages.

Data were analysed statistically with use of the package STATISTICA PL for Windows, 1–5. To characterize the studied population, the arithmetic mean, standard deviation, coefficient of variation were calculated, and the minimum and maximum value of each character were found. Pearson's coefficients of correlation between characters and their significance were determined on the basis of mean values of the characters (Łomnicki 2000). For measured characters, cluster analysis was conducted with the method of single linkage, using Euclidean distances, and a dendrogram was constructed (Marek 1989). Finally, a discriminant analysis (Krzyśko 1990) was performed on the basis of measured characters. It was used to

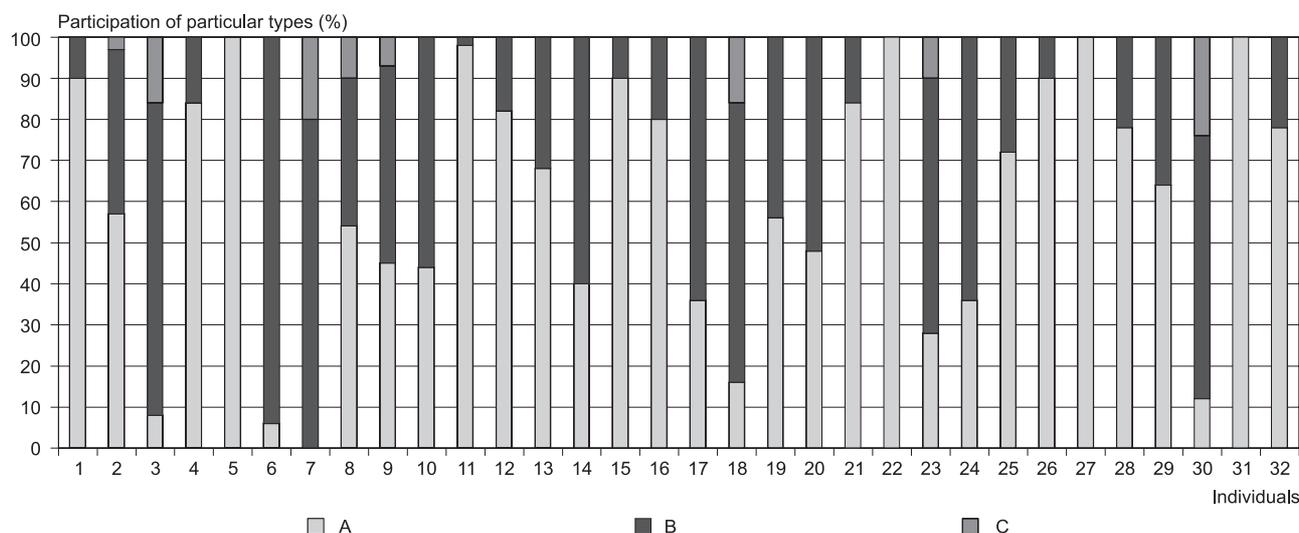


Fig. 2. Type of sclerenchyma cells around the resin canals for 32 trees of *P. sylvestris* from the Sierra de Baza: A – fibre like cells with very thick cell walls, lumen small; B – cells intermediate; C – no fibre like cells, cells walls medium thick, lumen distinct

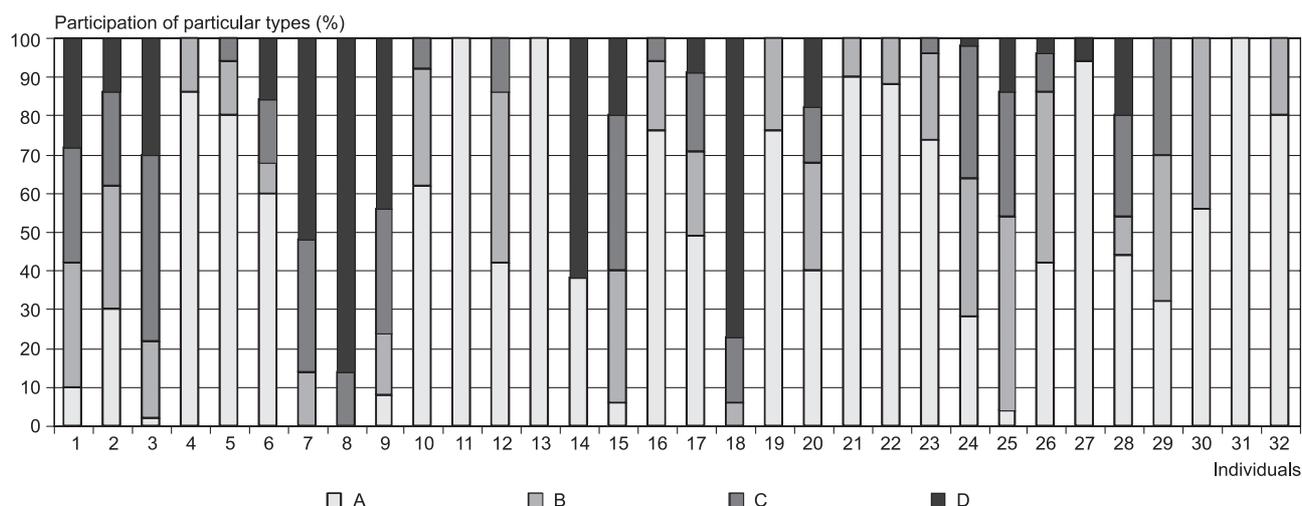


Fig. 3. Type of cells between vascular bundles for 32 trees of *P. sylvestris* from the Sierra de Baza: A – fibre like cells with very thick walls, lumen very distinct; B – fibre like cells singularly scattered or only small groups; C – fibre like cells lacking, cell walls of medium thickness, lumen distinct; D – fibre like cells lacking, cell walls thin, lumen very distinct

show the intrapopulational variability in the space of discriminant variables and to assess the discriminant value of the measured characters.

## Results

Needles of Scots pine from Sierra de Baza are relatively short, thick and wide, and have a thick epidermis (Table 1). Variation coefficients of individual characters ranged from slightly over 4% to nearly 28%. As many as 9 characters can be regarded as stable, because they are characterized by low values of this coefficient (Fig. 8). The lowest variation (4.4%) was recorded for the needle thickness/width ratio (character 14). The most variable characters are: the number of resin canals and distance between two vascular bundles, and the Marcet's coefficient.

The cells surrounding resin canals (Fig. 2) in 61% of individuals are typical for *P. sylvestris*, with thick walls and a narrow lumen. Thin-walled cells with a wide lumen, typical for *P. mugo*, were found only in 3% of trees, while the other 36% had the intermediate type of cells.

The cells located between vascular bundles (Fig. 3) were typical for *P. sylvestris* in 70% of individuals, typical for *P. mugo* in 16%, and intermediate in 14%.

More than half of the analysed correlations between characters are significant (Table 2). The strongest correlation ( $r = 0.95$ ) was observed between numbers of stomata on the convex and flat sides of the needle (characters 4 and 5, respectively). Very strong correlations were noted also between numbers of stomata rows on the convex and flat sides of the

Table 1. Statistical descriptions of the analysed 15 characters of *P. sylvestrica* from Sierra de Baza

Characters	Arithmetic mean	Minimum	Maximum	Range	Standard deviation
1. Needle length (mm)	39.28	26.00	59.00	33.00	6.24029
2. Number of stomatal rows on convex (abaxial) side of needle	11.63	7.00	17.00	10.00	2.18358
3. Number of stomatal rows on flat (adaxial) side of needle	10.39	6.00	16.00	10.00	1.96535
4. Number of stomata on 2 mm long section of needle, on convex (abaxial) side	24.61	18.33	32.66	14.33	2.54660
5. Number of stomata on 2 mm long section of needle, on flat (adaxial) side	24.33	17.66	32.66	15.00	2.45434
6. Number of resin canals	10.04	5.00	19.00	14.00	2.15429
7. Needle width ( $\mu\text{m}$ )	1648.15	1253.75	2040.00	786.25	163.91595
8. Needle thickness ( $\mu\text{m}$ )	825.82	637.50	998.75	361.25	76.77233
9. Distance between vascular bundles ( $\mu\text{m}$ )	208.24	59.94	382.92	322.98	59.95376
10. Thickness of epidermal cells ( $\mu\text{m}$ )	34.19	26.64	43.29	16.65	2.85032
11. Width of epidermal cells ( $\mu\text{m}$ )	16.37	10.82	22.20	11.38	1.54740
12. Marcet's coefficient (=traits 9 $\times$ 7/8)	419.45	118.26	818.10	699.84	134.74244
13. Stomatal rows ratio (=traits 2/3)	1.13	0.69	1.67	0.97	0.17179
14. Needle thickness/width ratio (=traits 8/7)	0.50	0.41	0.65	0.24	0.03137
15. Width/thickness ratio of epidermal cells (=traits 11/10)	0.48	0.36	0.65	0.29	0.04670

Table 2. Correlation coefficients between characters of *P. sylvestris* from Sierra de Baza: \* –significance at level  $p=0.05$ ; \*\* –significance at level  $p=0.01$  (character number as in Table 1)

2	0.21										
3	0.14	0.90**									
4	-0.11	0.55**	0.60**								
5	-0.05	0.51**	0.52**	0.95**							
6	0.12	0.56**	0.50**	0.46**	0.47**						
7	0.18	0.78**	0.75**	0.43*	0.42*	0.59**					
8	0.31	0.62**	0.58**	0.28	0.27	0.54**	0.88**				
9	0.17	0.63**	0.52**	0.26	0.33	0.57**	0.75**	0.53**			
10	0.21	0.24	0.14	-0.02	-0.03	-0.02	0.37*	0.39*	0.36*		
11	0.11	0.13	0.01	-0.17	-0.19	0.12	0.19	0.14	0.06	0.40*	
Characters	1	2	3	4	5	6	7	8	9	10	

needle ( $r=0.90$ ), as well as between needle width and thickness ( $r = 0.88$ ).

Results of cluster analysis attest to a low variability of the sample (Fig. 4). Only trees 14 and 23 slightly deviate from the others. Tree 14 is characterized by larger needles (44.7 mm long, 1.8 mm wide, 0.95 mm thick), and large numbers of stomata rows on both sides of the needle (13.2 and 13.3 on average). By contrast, needles of tree 23 are short (38.10 mm), but relatively thick (0.9 mm) and wide (1.8 mm), with large numbers of stomata on both sides (28.0 and 28.4 per 2-mm fragment of the needle).

The discrimination analysis indicate that the main characters that discriminate individual trees are: needle length (trait 1), number of resin canals (trait 6), distance between vascular bundles (trait 9), and nee-

dle thickness (trait 8). These characters have the lowest Wilk's lambda values (0.1633, 0.2970, 0.4302, and 0.4919, respectively), which attest to their discrimination strength. Contributions of all other characters to the discrimination between trees are lower but also statistically significant.

The first three canonical variables  $U_1$ ,  $U_2$  and  $U_3$ , account for over 76% of intrapopulation variation of the analysed sample:  $U_1$  for 36%,  $U_2$  for nearly 27%, and  $U_3$  for nearly 13%. The intrapopulation variation of the studied individuals in the space of the first three variables is small (Fig. 5). The first discriminant variable,  $U_1$ , is affected mainly by needle width and thickness (traits 7 and 8), and number of resin canals (trait 6). The same characters are the most correlated

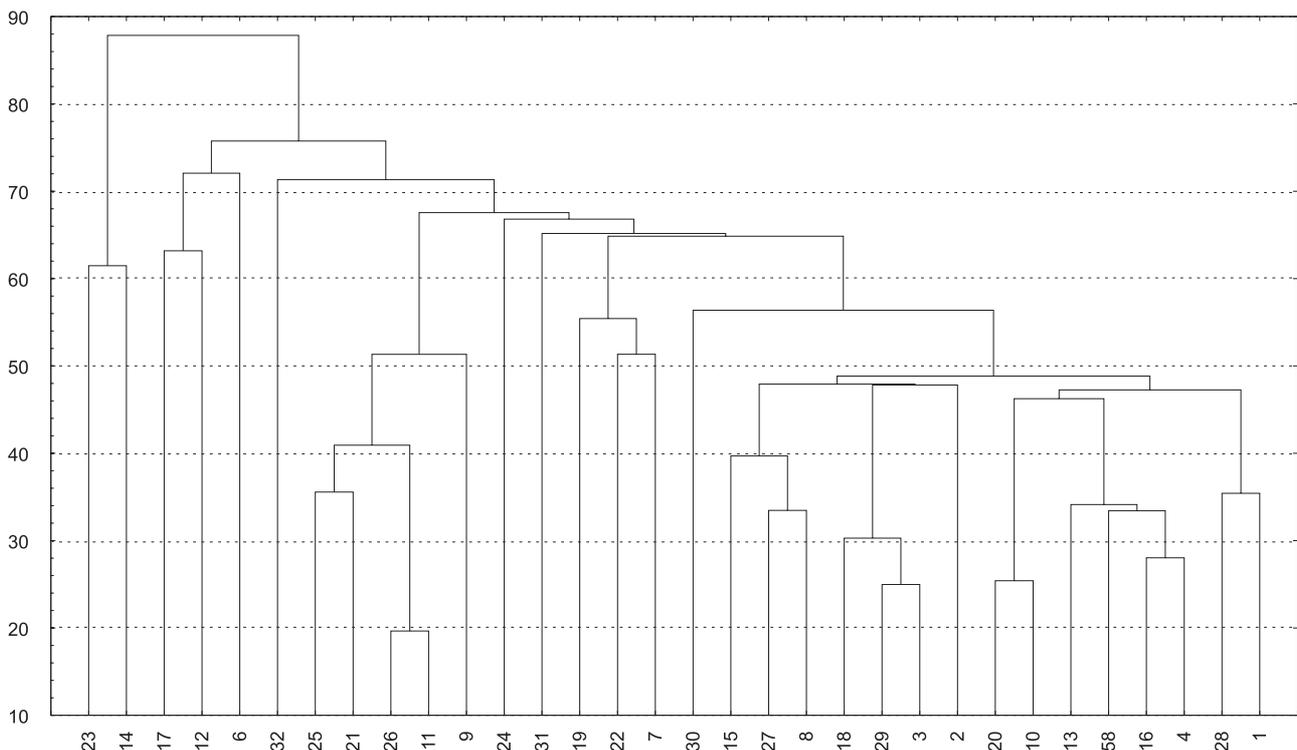


Fig. 4. Dendrogram for 32 trees of *P. sylvestris* from the Sierra de Baza

Table 3. Determination coefficients between discriminant variables  $U_1$ ,  $U_2$ ,  $U_3$  and 11 characters of needles of *P. sylvestris* (character number as in Table 1)

Characters	$U_1$	$U_2$	$U_3$
1	2.36	18.88	0.86
2	5.98	0.04	0.16
3	5.87	0.14	0.20
4	2.83	1.55	0.55
5	3.76	1.74	0.99
6	8.72	0.43	3.23
7	14.74	0.02	2.26
8	11.22	0.60	1.46
9	5.71	0.04	0.49
10	0.14	0.09	0.40
11	0.01	0.03	0.02

also with  $U_3$ . By contrast,  $U_2$  is defined mainly by needle length (trait 1) (Table 3).

## Discussion and conclusions

*Pinus sylvestris* was collected from Sierra Nevada in 1861 by D. Pedro del Campo and described as var. *nevadensis* by Christ two years later (Laguna 1883; Willkomm 1893). In variety description it was stressed that it differs from typical Scots pine by shorter, wider, more rigid and bluish-white pruinose needles, with reddish, sub-sessile macrostrobils and grayish, dull cones with eminent apophyses. The analyzed population of *Pinus sylvestris* from Sierra de Baza is very close to those of Sierra Nevada, but much more numerous. Morphologically both of them are similar and are included into var. *nevadensis*.

Our biometrical studies of needles sampled in Sierra de Baza confirmed differences between typical *Pinus sylvestris* and var. *nevadensis*, pointed out in description of var. *nevadensis*. When compare to data from Poland reported by Bobowicz and Korczyk (2000), the most significant differences were found in the needle length, while in the needle width are not so great (Figs. 6 and 7). It shall be stressed, that needles in Spain and Poland were collected from very old trees of similar age.

Variation of analyzed characters in sample from Sierra de Baza was different, but three of them, the width/thickness ratio, the width and the height of epidermis cell characterized with very small variation coefficient, 4.4, 4.7 and 4.9%, respectively. The similar results were found in the samples from Poland (Bobowicz and Korczyk 2000) (Fig. 8). The other stable characters are numbers of stomata on both, ad- and abaxial sides of the needle (variation coefficient 8.2 and 8.5%, respectively), however, not so stable, as Urbaniak (1998) found in the populations from Central and North Europe. The only slight variation of

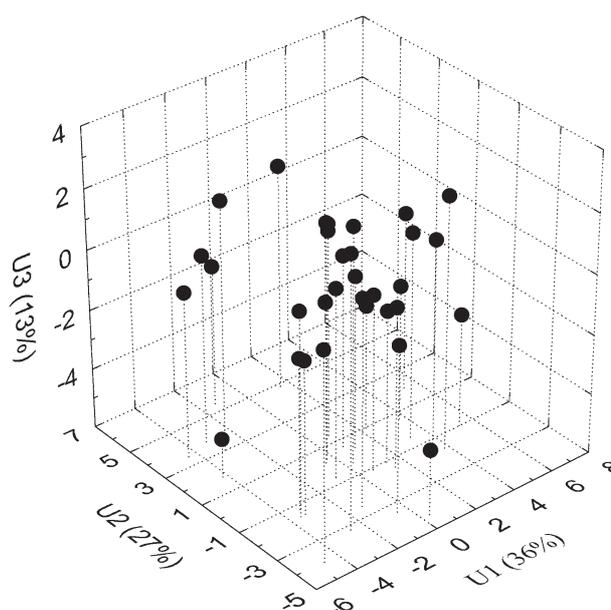


Fig. 5. Result of the discriminant analysis for 32 trees of *P. sylvestris* from the Sierra de Baza plotted along the three first discriminant variables  $U_1$ ,  $U_2$  and  $U_3$

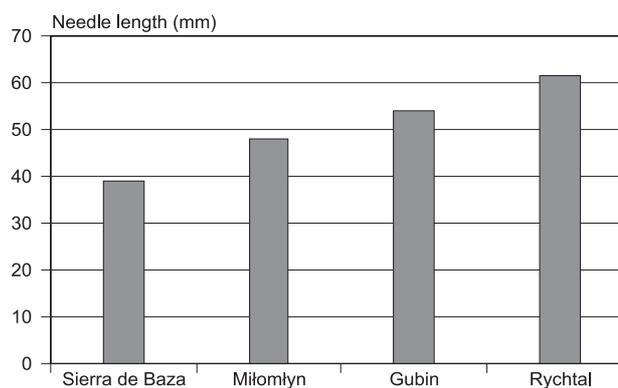


Fig. 6. Compared average needle lengths for 4 samples of *P. sylvestris* from the Sierra de Baza and from Poland

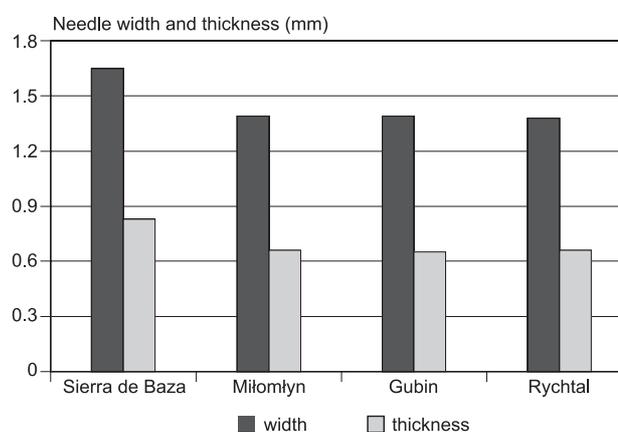


Fig. 7. Compared average needle widths and thickness for 4 samples of *P. sylvestris* from the Sierra de Baza and from Poland

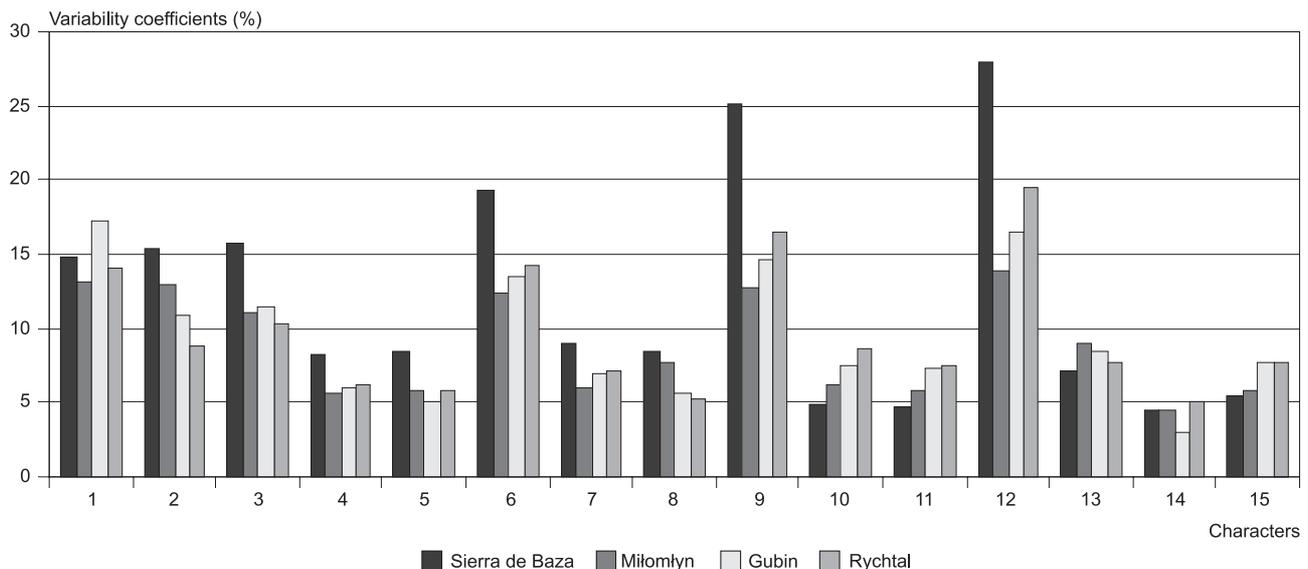


Fig. 8. Variability coefficients for 4 samples of *P. sylvestris* from the Sierra de Baza and from Poland; 1–15 trait numbers as in Table 1

these two characters was also found by Bobowicz and Korczyk (1994).

The most variable characters of the needles sampled in Sierra de Baza appeared the distance between the vascular bundles, the Marcet's coefficient and number of resin canals, with variation coefficient 19, 24 and 28%, respectively. These characters were more variable in the Central and North European *Pinus sylvestris* populations (Urbaniak 1998) but less variable in populations from Poland (Bobowicz and Korczyk 1994). The most variable characters, as needle length and number of resin canals, are a source of specimens differentiation, however, the intrapopulation variation of the studied population is generally low.

In conclusion it can be stressed, that sample of *Pinus sylvestris* from Sierra de Baza differ significantly from all other compared samples mostly in the needle length, this, however, should be checked in detailed study on greater material from the Iberian Peninsula.

## References

- Bobowicz A.M. 1990. Mieszaniec *Pinus mugo* Turra × *Pinus sylvestris* L. z rezerwatu „Bór na Czerwonym” w Kotlinie Nowotarskiej. Uniwersytet im. A. Mickiewicza w Poznaniu, ser. Biologia 40. Poznań.
- Bobowicz A.M., Korczyk 1994. Interpopulational variability of *Pinus sylvestris* L. in eight polish localities expressed in morphological and anatomical traits of needles. Acta Societatis Botanicorum Poloniae 63 (1): 67–76.
- Bobowicz A.M., Korczyk A.F. 2000. Variability of needle morphology traits in old-growth scots pine trees from the Białowieża Primeval Forest as compared to the variability in eight polish populations. Biological Bulletin of Poznań 37(1): 17–31.
- Boratyńska K., Bobowicz A.M. 2000. Variability of *Pinus uncinata* Ramond ex DC as expressed in needle traits. Dendrobiology 45: 7–16.
- Boratyńska K., Bobowicz A.M. 2001. *Pinus uncinata* Ramont taxonomy based on needle characters. Plant Systematics and Evolution 227:183–194.
- Galán Cela P., Gamarra Gamarra R., Garcia Viñas J.I. 1998. Árboles y Arbustos de la Península Ibérica e Islas Baleares. Madrid, 684 pp.
- Gausen H. 1960. Les Gymnospermes actuelles et fossiles, Travaux du Laboratoire Forestal de Toulouse, 4. 272 pp.
- Krzyśko M. 1990. Analiza dyskryminacyjna. Wyd. Naukowo-Techniczne Warszawa, 155 pp.
- Laguna D.M. 1883. Flora Forestal Española, 1 (reprint Xunta de Galicia 1993). Madrid, 459 pp.
- Łomnicki A. 2000. Wprowadzenie do statystyki dla przyrodników. PWN Warszawa.
- Marek T. 1989. Analiza skupień w badaniach empirycznych. PWN Warszawa, 169 pp.
- Nicolas A., Gandullo J.M. 1969. Ecología de los pinares españoles. II. *Pinus sylvestris*. Ministerio de Agricultura. Madrid, 303 pp.
- Mirov N.T. 1967. The Genus *Pinus*. The Ronald Press Company. New York, 602 pp.
- Pardos J.A., Lange W., Weissmann G. 1990. Morphological and chemical aspects of *Pinus sylvestris* L. from Spain. Holzforschung 44: 143–146.
- Prus-Głowacki W., Stephan B.R. 1994. Genetic variation of *Pinus sylvestris* from Spain in relation to other European populations. Silvae Genetica 43: 7–14.
- Svoboda P. 1953. Lesní dřeviny a jejich porosty, 1. Státní Zemědělské Nakladatelství, Praha, 411 pp.

- Szweykowski J. 1969. The variability of *Pinus mugo* Turra in Poland. Bulletin de la Société des Amis des Lettres de Poznań, ser. D. 10: 39–54.
- Tobolski J., Hanover J.W. 1971. Genetic variation in the monoterpenes of Scotch pine. Forest Sciences 17: 293–299.
- Urbaniak L. 1998. Zróżnicowanie geograficzne sosny zwyczajnej (*Pinus sylvestris* L) z terenu Eurazji na podstawie cech anatomicznych i morfologicznych igieł. Wydawnictwo Naukowe PAN, Poznań, 141 pp.
- Willkomm M. 1893. Supplementum Prodromi Florae Hispanicae (reprint 1972). Stuttgart, 370 pp.

