

THE VARIABILITY OF NATURAL POPULATIONS OF *BRACHYPODIUM PINNATUM* AND *B. SYLVATICUM* BASED ON MORPHOLOGICAL FEATURES

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ABSTRACT

The morphological diversity in 25 populations of two native species of *Brachypodium* occurring in Poland, was studied. Principal component analysis and cluster analysis of morphometric data segregate specimens or populations of *Brachypodium* into two groups, corresponding to the two species. In most of morphological characters, *B. pinnatum* and *B. sylvaticum* are similar to each other. However, in terms of characteristics, such as the hairiness of culms and leaf-sheaths both can also vary much within a single population. In comparison with *B. pinnatum*, *B. sylvaticum* always has pubescent abaxial palea surfaces. Apparent differences were not detected between the two ecotypes of *B. pinnatum* named "Grassland" and "Woodland".

KEY WORDS: *Brachypodium pinnatum*, *B. sylvaticum*, morphological variability, Poaceae, Poland.

INTRODUCTION

Brachypodium Beauv., one of the oldest genera of the family Poaceae, commonly known as False-brome with regard to its brome-like appearance, includes grasses with a simple spike-like racemose inflorescence and spikelets that are not strongly flattened and often have awned lemmas. The common name of False-brome indicates that *Brachypodium* species are often misidentified or mistaken for a brome (*Bromus*). According to Schippmann (1991), there are currently nine species and subspecies in the genus *Brachypodium*, which occur in temperate areas of Europe. The two commonest species, the only ones that occur in Poland (Mirek et al. 2002), are Chalk False brome *Brachypodium pinnatum* (L.) P. Beauv. and Slender False-brome *B. sylvaticum* (Huds.) P. Beauv. The Eurosiberian *B. pinnatum*, a long-lived extensively rhizomatous perennial grass, is a characteristic species of the Festuco-Brometea class. It is a frequent, locally abundant and dominant grass species of semi-natural chalk xerothermic grassland communities of the lowlands and submontane regions in Poland, although it is also a frequent member of chalk scrub, wood margins and open woodlands. The woodland *B. sylvaticum*, a perennial bunchgrass, is indigenous to Europe, temperate Asia and northwest Africa. It is a characteristic species of the Querco-Fagetea class and is most commonly found in Poland in lowland, submontane and lower montane woods, copses, along hedgerows, and in other shady places, but may grow in grassland and along roadsides in originally

woodland areas (Hultén and Fries 1986; Zając and Zając 2001; Matuszkiewicz 2002; Mirek and Piękoś-Mirkowa 2002). Both can occur in close proximity, for instance in thermophilous oak forests.

B. pinnatum and *B. sylvaticum* overlap with one another in most of their morphological characters (Smith 1980). Therefore, Saint-Yves (1934) earlier recognised only one species – *B. pinnatum* (L.) Beauv. including *B. sylvaticum* Hudson (Beauv.) as a variety, var. *sylvaticum* St-Y. According to Hubbard (1968), *B. pinnatum* is distinguished from *B. sylvaticum* by its form of growth, presence of rhizomes, usually hairless culms and leaf-sheath, stiffer racemes and especially by the shorter-awned lemmas. According to Johnson (2004) the most distinctive feature in identifying *B. sylvaticum* in the USA is the single row of ciliate-pilose hairs fringing the leaf blade and similar hairs covering the leaf-sheath and accentuated at the collar.

B. pinnatum and *B. sylvaticum* are species complexes that have different variants or cytotypes in Europe. These variants have very similar morphological characters, the same chromosome base numbers ($x=7$ and 9), and similar seed-protein electrophoretic band pattern (Smith 1980; Khan 1984; Schippman 1991). *B. pinnatum* (L.) P. Beauv. occurs in Europe as two forms, which are recognised as species (*B. pinnatum* (L.) P. Beauv., *B. rupestre* (Host) Roemer and Schultes; Smith 1980, Schippman 1991, Khan 1992, Shi et al. 1993, Catalán and Olmstead 2000), subspecies of *B. pinnatum* (subsp. *pinnatum*, and subsp. *rupestre* (Host) Schübler and Martens, Smith 1980), or varieties of

TABLE 1. The range for the 13 quantitative characters and the results of ANOVA. Differences between species were considered significant at the level of $p < 0.05$.

Character	F (p<0.05)	<i>B. pinnatum</i> range	<i>B. sylvaticum</i> (for S-20) range
*ratio: length of lemma awn/length of lemma	8171.17	0.22-0.63	0.72-1.45
*length of lemma awn [mm]	7280.67	2.0-5.9	6.7-14.0 (11.0-16.0)
**length of lemma awn [mm]	7242.50	0.0-2.6	4.0-10.0
*number of nerves on lower glume	764.06	3-7	4-8
*length of lower glume [mm]	591.02	3.3-8.0	5.5-9.8
length of pedicel of fourth spikelet [mm]	568.00	0.9-4.2	0.5-1.5
*length of upper glume [mm]	388.04	5.0-9.8	6.5-11.3
width of penultimate leaf [mm]	264.99	2.3-10.0	5.0-12.0
number of florets in the fourth spikelet	229.19	6-19	4-16
length of penultimate leaf ligule [mm]	208.68	0.8-3.7	1.4-3.5 (3.1-5.5)
*length of upper glume awn [mm]	165.37	0.0-1.0	0.0-2.0
number of spikelets per raceme	163.86	6-16	5-10
length of penultimate leaf [mm]	29.5	5.2-37.0	10.1-34.0

* measured at the fourth floret in the fourth spikelet

** measured at the first floret in the fourth spikelet

TABLE 2. Descriptions, abbreviations and attributes of qualitative characters and their frequencies in *Brachypodium pinnatum* and *B. sylvaticum*. p = characters used in PCA.

Description of character	Abbreviation of character [attributes]	Frequency	
		<i>B. pinnatum</i> (N=320)	<i>B. sylvaticum</i> (N=275)
culm: hairiness of upper internodes	[hairless/hairy]	300/20	243/32
culm: hairiness of middle internodes	[hairless/hairy]	235/85	214/61
p culm: hairiness of lower internodes	[hairless/hairy]	124/196	149/125
p uppermost leaves: hairiness of sheaths	[hairless/only margins hairy/hairy]	43/158/119	22/171/82
p middle leaves: hairiness of sheaths	[hairless/only margins hairy/hairy]	30/109/181	10/120/145
p lowest leaves: hairiness of sheaths	[hairless/only margins hairy/hairy]	23/81/216	5/95/174
p penultimate* leaf: hairiness on adaxial side	[hairless/hairy]	30/289	5/267
penultimate* leaf: hairiness on abaxial side	[hairless/hairy]	153/166	165/107
p margins of penultimate* leaf: hairiness	[hairless/hairy]	43/276	156/118
p penultimate* leaf: shape of ligule	[truncate/obtuse]	295/25	44/231
middle raceme: hairiness of 4th internode from below	[hairless/hairy]	18/302	34/241
lower glume: hairiness on abaxial surface**	[hairless/hairy]	16/304	43/232
upper glume: hairiness on abaxial surface**	[hairless/hairy]	17/303	42/233
lemma: hairiness on abaxial surface of lemma**	[hairless/hairy]	5/315	6/269
p palea: pubescence on abaxial surface of palea**	[glabrous/short-ciliate hairs]	229/91	3/272

* penultimate = second from the top

** estimate at fourth floret in fourth spikelet from

PCA was used to detect groups of specimens and to estimate the contribution of each variable to the analysis. PCA was based on correlation matrices of the whole set of quantitative characters (Table 1), and eight qualitative characters (Table 2). Cluster analysis was used to assess the groupings among *Brachypodium* populations based on 12 quantitative characters, excluding penultimate leaf length (Table 1). Standardised data were used to compute the distance matrix based on average taxonomic distance, and this was subjected to the unweighted pair-group method, arithmetic average (UPGMA) clustering algorithm.

RESULTS

Qualitative characters

All individuals of the *Brachypodium* species examined have hairy culms at and near their nodes, hairy spikelets and floret pedicels. Most of the *Brachypodium* specimens studied have hairy: adaxial (upper) side of the penultimate

leaf blade, raceme internodes, abaxial (lower) surface of the lemma, abaxial surface of lower and upper glume, and sheath margin. About half of the lowest and middle leaf-sheaths are covered with long scattered or dense hairs. Lowest sheaths of most of *Brachypodium* individuals are more likely to be hairy than upper leaf-sheaths. Most of the *Brachypodium* plants examined have hairless culm internodes, though the lowest ones are hairy frequently in *B. pinnatum* individuals (Table 2).

Almost all *B. sylvaticum* individuals have a short-ciliate abaxial surface of palea (272 of 275) and most of *B. sylvaticum* plants have obtuse ligules (222 of 275). These characters are not species-specific, because the part of *B. pinnatum* individuals also have these attributes (Table 2). The penultimate leaves of most *B. pinnatum* individuals usually (85%) have hairy margins, whereas only 40% of *B. sylvaticum* plants have the same attribute (Table 2).

Twelve of 15 individuals of *B. sylvaticum* (S-20) accessed from the Świetlista Dąbrowa reserve have shorter-ciliate abaxial surface of palea and all individuals from the two

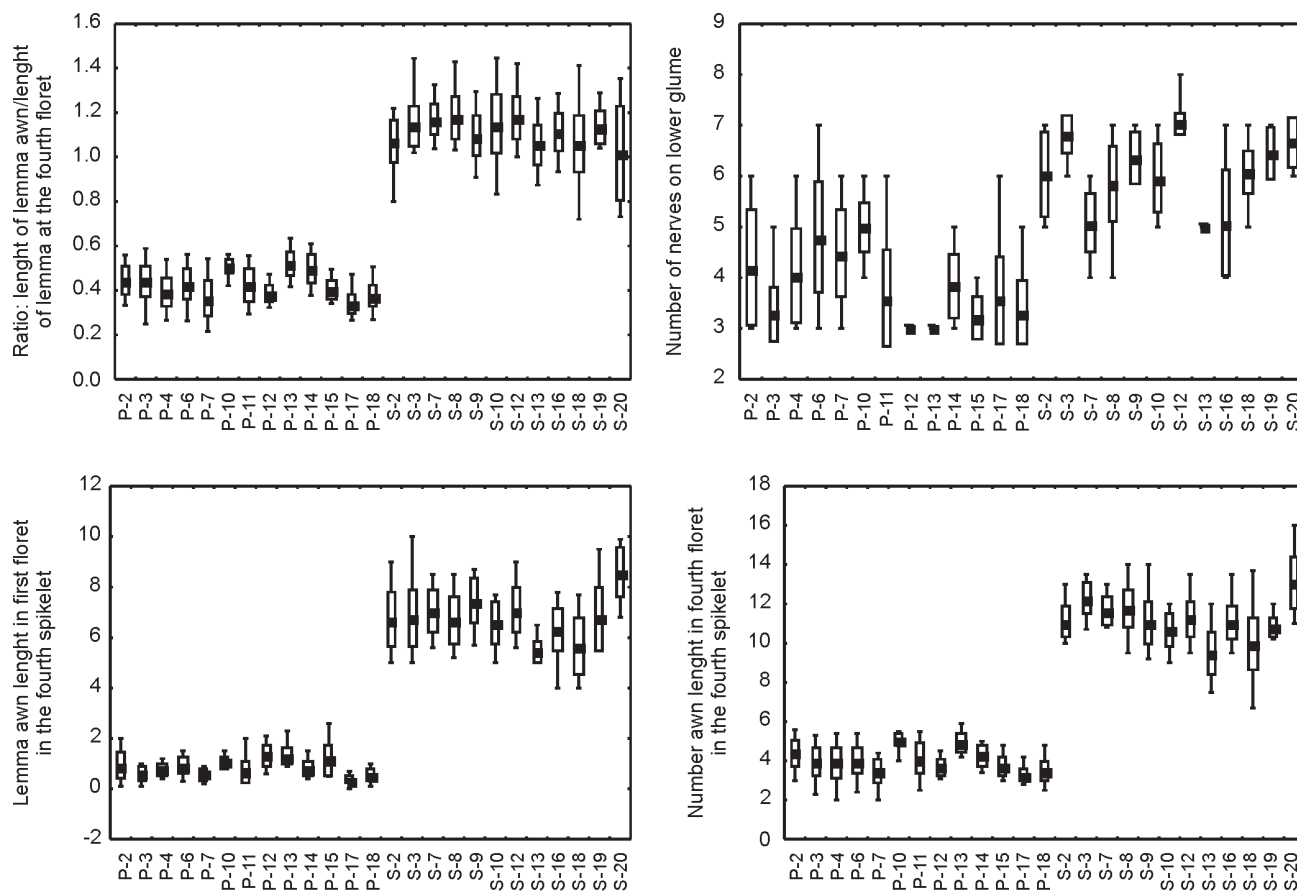


Fig. 2. Box-and-whisker plots of selected spikelet characters for *B. pinnatum* (based on 320 specimens) and *B. sylvaticum* (based on 265 specimens). The mean (dots), range of standard deviations (box) and minimum/maximum (whisker) indication.

population samples of *B. pinnatum* (P-12, P-13) from this locality also share this attribute (Table 2). What is of interest, is that the penultimate leaves of all *B. pinnatum* as well as *B. sylvaticum* individuals from the Świetlista Dąbrowa have margins fringed with hairs.

Quantitative characters

The results of ANOVA analysis showed a significant variation ($P < 0.05$) between species of *Brachypodium* for the morphological characters (Table 1). However, the most useful characteristic for discriminating between the studied species of *Brachypodium* is lemma awn length. The lateral lemma awn length (at the fourth floret in the fourth spikelet) in *B. pinnatum* ($N=320$) is less than 6 mm, whereas *B. sylvaticum* ($N=265$) has a lateral lemma awn of 6.5–14 mm in length (Table 1, Fig. 2). The remaining quantitative characters overlapped between Polish species of *Brachypodium*.

There was no apparent difference between two ecotypes of *B. pinnatum* named “Grassland” and “Woodland”. The examination of the variation in traits for these two ecotypes revealed that plants of the “Woodland” ecotype are similar to the woodland *B. sylvaticum* as regards the length of the penultimate leaves, and have an intermediate appearance regarding the width of penultimate leaves and the number of spikelets in the raceme (Fig. 3).

The UPGMA dendrogram for the *Brachypodium* accessions (Fig. 4) shows two major clusters. The first major cluster contains four sub-clusters of *B. pinnatum* accessions. Two of these are most numerous, the first containing accessions of *B. pinnatum* from grassland habitats and the

other, accessions of *B. pinnatum* from woodland habitats. Both ecotypes of *B. pinnatum* appear to be very similar and are placed very close to each other in a single cluster. The second cluster contains accessions of *B. sylvaticum* at some distance. It is interesting to note that the accession of *B. sylvaticum* (S-20) from the Świetlista Dąbrowa reserve is distinct. The examination of trait ranges for this accession revealed that S-20 is unusual compared to other *B. sylvaticum* populations in having longer penultimate leaf ligules, longer lemma awn (at the fourth floret in the fourth spikelet) (Table 1) and abundant pubescence of leaves, leaf-sheaths, culms, and spikelets. The outlying values for these traits are likely to have resulted in the separation of this population into unique cluster.

There are some morphological differences between plants of different habitats within *B. pinnatum*, however those differences are too inconsistent and imprecise to allow for the recognition of two intraspecific taxa within *B. pinnatum*. One would expect that under such circumstances, a significant degree of morphological diversification would have occurred, making it possible to visually distinguish plants of different habitats.

Quantitative and qualitative characters

Output from the PCA (Fig. 5) showed the pattern of morphological variation of Polish *Brachypodium* accessions based on 21 morphological characters (13 quantitative and eight qualitative) (Tables 1 and 2). PCA axis 1 is most highly influenced by (presented in decreasing order of loadings): (1) length of the lemma awn at the fourth floret, (2)

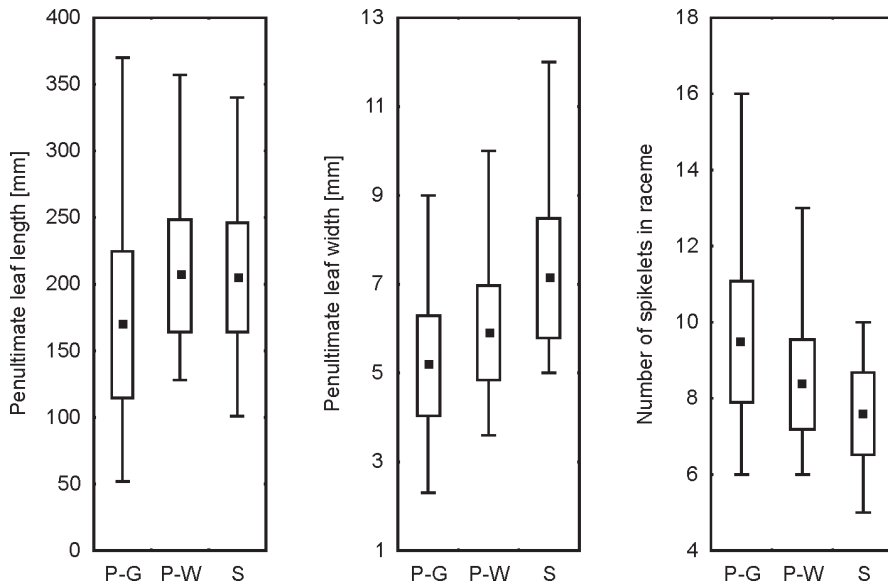


Fig. 3. Variation of three morphological characters for two ecotypes of *B. pinnatum*, named "Grassland" (P-G; N = 200) and "Woodland" (P-W; N = 120), and *B. sylvaticum* (S; N = 265). Dots represent the mean, the mean ± 1 SD (box), and the minimum/maximum (whisker).

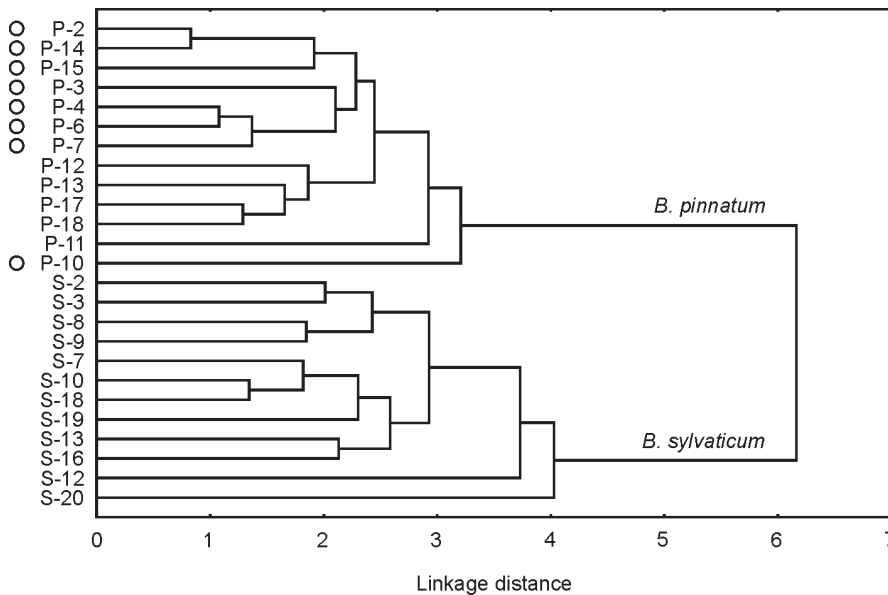


Fig. 4. UPGMA phenogram of 25 accessions of *Brachypodium* species from Poland based on Euclidean distances computed among accession means of quantitative morphological traits listed in Table 1. Accessions of "Woodland" ecotype of *B. pinnatum* clustered closely together, with accessions of "Grassland" ecotype (marked with circle).

length of the lemma awn at the first floret, (3) ratio: length of lemma awn/length of lemma at the fourth floret, (4) length of the lower glume, (5) number of nerves on the lower glume, (6) the shape of the ligule, (7) hairiness of the abaxial surface of palea, (8) length of the upper glume. In this axis the traits with the most important contribution were related to lemma awn length in the first and fourth floret at the fourth spikelet. PCA axis 2 by: (1) hairiness of the middle leaf-sheaths, (2) hairiness of the lowest leaf-sheaths, (3) hairiness of the lowest culms, (4) hairiness of the abaxial surface of the penultimate leaves, (5) hairiness of the uppermost leaf-sheaths. The second principal component is interpreted as a factor of hairiness. Two distinct groups are evident. One group consists of *B. pinnatum*, and the other includes *B. sylvaticum* (Fig. 5). Both species show the same pattern of variation as regards the hairiness of leaf-sheaths, culms, and penultimate leaves. Most specimens of *B. pinnatum* and *B. sylvaticum* have more or less hairy penultimate leaves, leaf-sheaths, lower culm-internodes, and spikelets.

DISCUSSION

Differences and similarities between species

The results revealed that *B. sylvaticum* differs from *B. pinnatum* by the following combination of characters: (1) longer awn of the lemma of the fourth flower (6.7-14.0 mm vs. 2.0-5.9), (2) always short-ciliate hairy vs. glabrous palea abaxial surfaces, (3) the racemes, which tend to have less spikelets (5-10 vs. 6-16), (4) the lower glumes, which tend to have more nerves ((4)5-7-nerved vs. 3-5 (6)-nerved), (5) the penultimate leaves, which tend to be wider (5-10 mm vs. 2-8), (6) longer lower glumes (5.5-10 mm vs. 3-8). The most remarkable differences between *B. pinnatum* and *B. sylvaticum* are the length of the lemma awn, its form habit, presence of rhizomes and stiffer racemes. Awn length is the best single character for identifying the two studied species (Table 1, Fig. 6).

B. pinnatum, according to the author's observations, is similar to *B. sylvaticum* as regards: (1) the length of the culm and the number of culm nodes, (2) the length of the

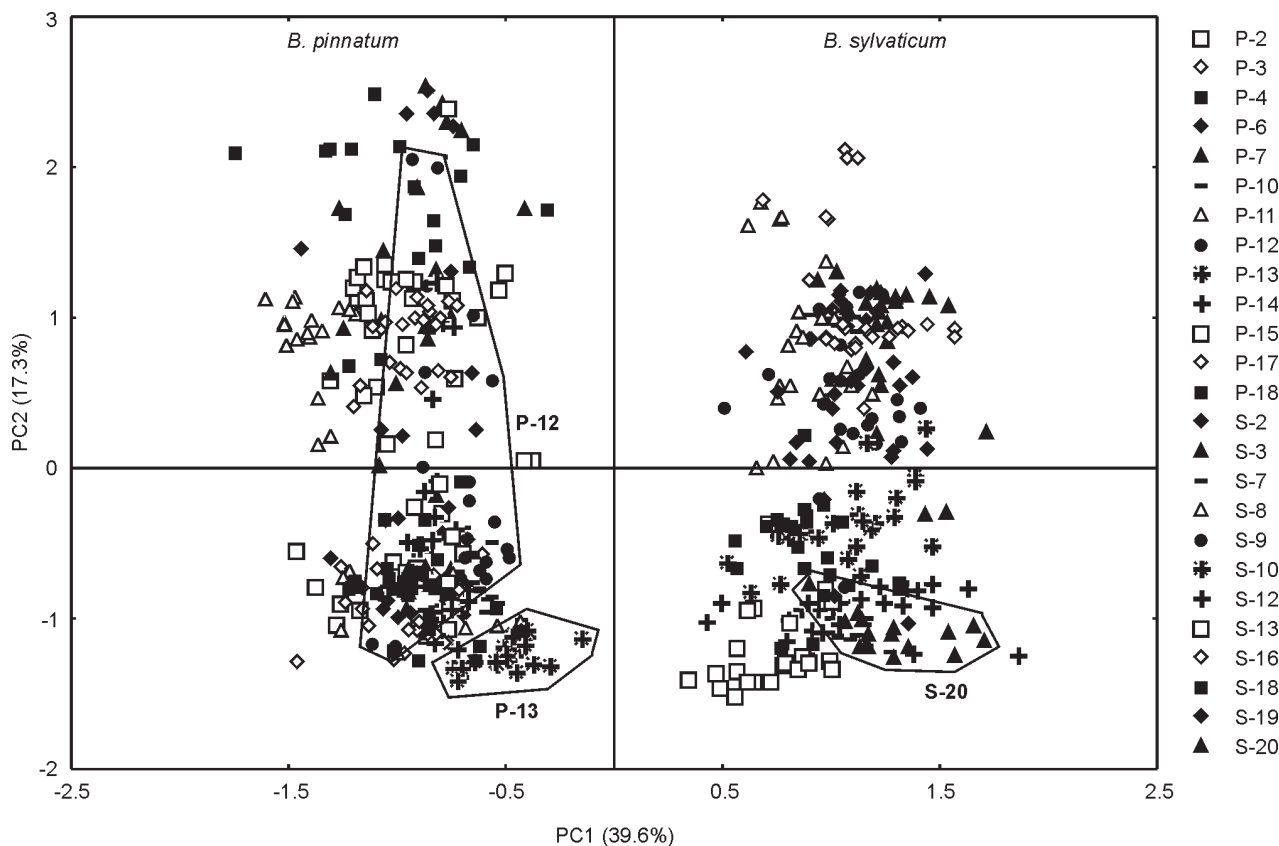


Fig. 5. Principal components analysis (PCA) – scatter diagram of individual specimens of *Brachypodium pinnatum* (P) and *B. sylvaticum* (S) along PC1 and PC2, based on 12 selected, quantitative characters (Table 1, excluding penultimate leaf length) and eight qualitative (Table 2) characters.

spike-like raceme, (3) the length of the spikelet and internode between the third and fourth spikelet, (4) the hairiness of culm nodes, (5) hairiness of leaves and leaf-sheaths (6) hairiness of culm internodes, and (7) hairiness of glumes and lemmas. The morphological variation for most culm and spikelet characters is continuous with a high degree of overlap across both studied species of *Brachypodium*.

According to Hubbard (1968), *B. pinnatum* is distinguished from *B. sylvaticum* by its usually hairless culm and leaf-sheath. However, according to my observations, the pattern of variation within both the Polish *Brachypodium* species is very similar as regards the culm and leaf-sheath pubescence. When analysing leaf-sheaths, those on the lower half of the culm, 90% and 63% of individuals of *B. pinnatum* and *B. sylvaticum* respectively, have hairy leaf-sheaths. Leaf-sheath pubescence, especially on the middle and lower part of the culm, has a dominant character in both native species of *Brachypodium* in Poland. However, glabrousness is a dominant characteristic on the upper half of the culm, 63% and 71% of individuals of *B. pinnatum* and *B. sylvaticum*, respectively, have glabrous uppermost leaf-sheaths (Table 2). Both studied species of *Brachypodium* consist of many forms as regards the hairiness of leaf-sheaths and leaves, from almost glabrous types, through a multitude of intermediate forms, to abundant hairy types. Within both species almost glabrous types are rather rare, most frequent are largely hairy individuals or plants of intermediate appearance as regards the hairiness. A certain pattern of variation can be found as regards the hairiness of leaf margins, but this characteristic is not specific to any of both *Brachypodium* species. This has been confirmed by

Stace's (1991) opinion that pubescence is generally of a very limited value.

The disagreement between the results presented here and those obtained by Hubbard (1968), is explained by the fact that two different species were compared. Hubbard's concept of *B. pinnatum* included *B. rupestre*, which is much more common than *B. pinnatum* in Britain (Stace, pers. comm.). The distinction between *B. pinnatum* and *B. rupestre* was not determined until after the publication of the New Atlas (Preston et al. 2002) and as such, precious little mapping data are available (Cheffings and Farrell 2005). This confusion also explains the erroneous statement by Stace (1975) that hybrids between *B. pinnatum* and *B. sylvaticum* occur in several European countries. Such plants are almost certainly *B. pinnatum*, which appear to be more or less intermediate between *B. sylvaticum* and *B. rupestre*, but have the same chromosome number as the latter, and are fertile (Stace, pers. comm.). Nevertheless, wild hybrids should be expected also in Poland.

Ecotypes of B. pinnatum

It is interesting that ecological segregation among accessions of *B. pinnatum* was detected based on morphological characters. Furthermore, the author's analyses indicate that *B. pinnatum* cannot be subdivided in any meaningful way. The results of both CA and PCA can only be interpreted to support the recognition of a single species in *B. pinnatum*. The degree of morphological similarity among the populations is too high to justify the recognition of more than one species, while the lack of pronounced gaps in the pattern of variation renders recognition impractical (Fig. 5).

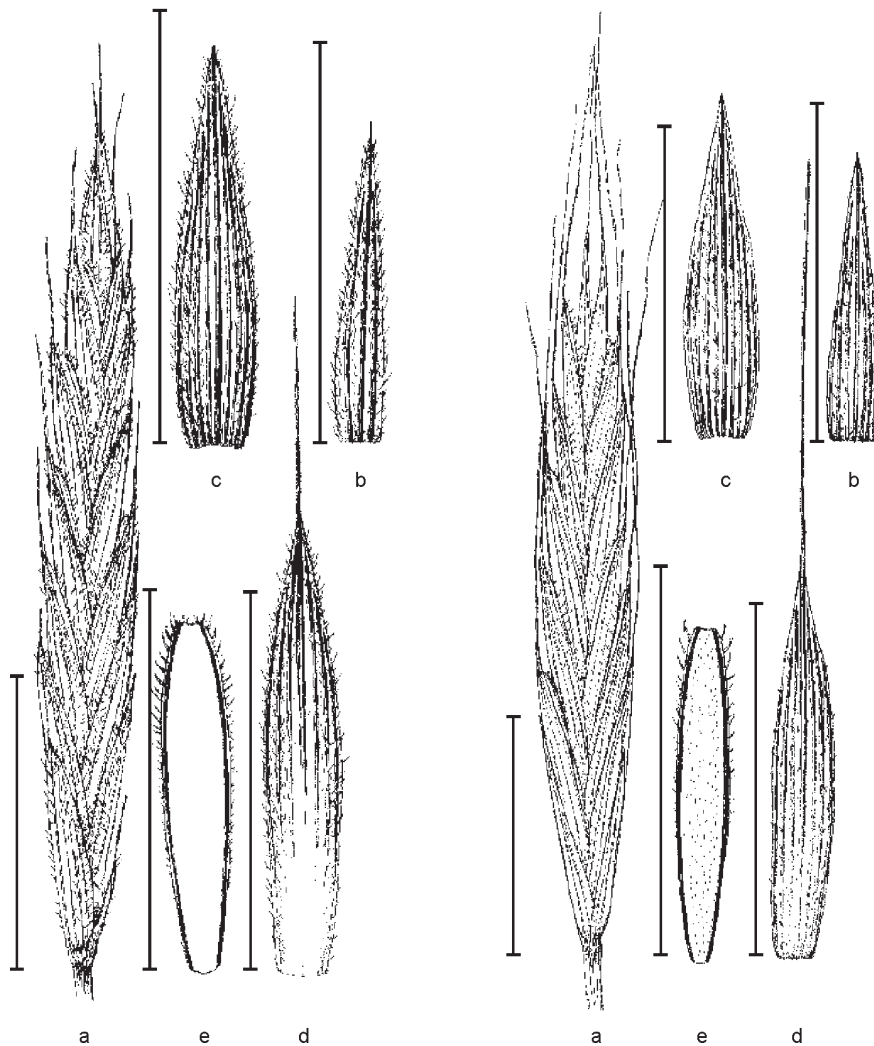


Fig. 6. Spikelet (a), lower glume (b), upper glume (c), lemma (d), palea (e) of *Brachypodium pinnatum* (on the left) and *B. sylvaticum* (on the right). Scale bar = 1 cm.

Hybridisation

Despite the apparent morphological similarity of three populations in the Świetlista Dąbrowa reserve (P-12, P-13, and S-20), plants from this area do not form a uniform group with respect to other populations of *B. pinnatum* and *B. sylvaticum* (Table 1, Figs 2 and 3). It is interesting, however, that all individuals of *B. sylvaticum* from the Świetlista Dąbrowa reserve (S-20) have longer lemma awns and leaf ligules than other *B. sylvaticum* individuals (Table 1) and three of the 15 individuals have a glabrous abaxial surface of palea, such as most individuals of *B. pinnatum*. *B. pinnatum* individuals from the P-13 accession have a slightly longer lemma awn than other *B. pinnatum* plants and have profusely hairy culms, leaves, leaf-sheaths, and spikelets (Fig. 3). Both *Brachypodium* species grow there in close proximity, which renders hybridisation possible. Thus, the area sampled for specimens P-12, P-13 and P-20 (Table 1, Figs 2 and 3) may represent a hybrid zone. Hybrid zones may confound morphological characters alone; therefore, other markers – preferably chromosome count – would be needed in the further assessment of these populations. I cannot suggest any taxonomic status for the False-brome in the Świetlista Dąbrowa reserve at this time and continue to refer them to *B. pinnatum* (individuals from the P-12 and P-13 accessions) and *B. sylvaticum* (S-20), respective-

ly. Further investigation and confirmatory chromosome count in regions where both *Brachypodium* species sympatrically appear, are needed to throw light on this interesting issue.

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APPENDIX

Appendix 1. Sampled populations of *Brachypodium pinnatum* (P) and *B. sylvaticum* (S) collected by B. Paszko. The number of plants measured in each population sample is given in brackets.

B. pinnatum

P-2: (25) Kraków vicinity, Ojcowski National Park, Pieskowa Skała, steep escarpment, N 50°13', E 19°49'; **P-3:** (25) Kraków vicinity, Hebdów, steep escarpment alongside the Vistula River, N 50°08', E 20°24'; **P-4:** (25) Kielce vicinity, Góry Pińczowskie reserve, calcareous xerothermic grassland, N 50°32', E 20°30'; **P-6:** (25) Iłża, xerothermic grassland, N 51°10', E 21°15'; **P-7:** (25) Sanok vicinity, Trecza, a steep escarpment alongside the San River, N 49°36', E 22°12'; **P-10:** (25) Częstochowa vicinity, Olsztyn, castle hill, calcareous semi-natural dry grassland, N 50°45', E 19°16'; **P-11:** (25) Sieradz vicinity, Półboru reserve, thermophilous oak forest (Potentillo albae-Quercetum), N 51°36', E 18°49'; **P-12:** (25), **P-13:** (20) Łódź vicinity, Krośniewice, Świetlista Dąbrowa reserve, thermophilous oak forest, N 52°17', E 19°12'; **P-14:** (25) Bydgoszcz vicinity, Plutowo, Zbocza Plutowskie reserve in the valley of the Lower Vistula River, xerothermic grassland (*Adonido-Brachypodietum*), N 53°16', E 18°23'; **P-15:** (25) Gdańsk vicinity, Małkowo, roadside, N 54°22', E 18°21'; **P-17:** (25) Poznań vicinity, Dziewicza Góra reserve, mixed forest edge, N 52°28', E 17°00'; **P-18:** (25) Wrocław vicinity, Ślęza Mt., the Tapała pass, mixed forest edge, N 50°50', E 16°42'.

B. sylvaticum

S-2: (25) Sanok vicinity, Ustrzyki Dolne, quarry near roadside, N 49°19', E 22°38'; **S-3:** (25) Sanok vicinity, Baligród, roadside in forest, N 49°21', E 22°15'; **S-7:** (25) Nowy Targ vicinity, Little Pieniny Mts., Homole Gorge, N 49°24', E 20°33'; **S-8:** (25) Nowy Targ vicinity, Gorce Mts., Knurowska Pass, roadside, N 49°30', E 20°11'; **S-9:** (25) Beskid Śląski Range, Barania Góra Mountain, mixed forest near the Bystra River, N 49°37', E 19°02'; **S-10:** (25) Kłodzko vicinity, Polanica Zdrój, forest near the road to Szczytna, N 50°24', E 16°29'; **S-12:** (25) Katowice vicinity, NE Trzebinia: between Lgota and Myślachowice, roadside, N 50°11', E 19°31'; **S-13:** (25) Białystok vicinity, Białowieża National Park, Hajnówka, forest near the roadside, N 52°43', E 23°37'; **S-16:** (25) Suwałki vicinity, Szwalk, forest between Szwalk Wielki Lake and the Szwalk Mały Lake, N 54°06', E 22°15'; **S-18:** (24) Grudziądz vicinity, Hawa, forest near roadside, N 53°36', E 19°31'; **S-19:** (11) Poznań vicinity, Mosina, Wielkopolski National Park, mixed forest, N 52°16', E 16°49'; **S-20:** (15) Łódź vicinity, Krośniewice, Świetlista Dąbrowa Reserve, thermophilous oak forest (Potentillo albae-Quercetum), N 52°17', E 19°12'.