

THE HYBRID ORIGIN
OF *CALAMAGROSTIS* × *GRACILESCENS* (POACEAE)
IN POLAND INFERRED FROM MORPHOLOGY AND AFLP DATA

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(Received: March 23, 2009. Accepted: January 18, 2010)

ABSTRACT

The morphology of *Calamagrostis canescens* and *C. stricta* recorded in the Małopolska Upland (the vicinity of Zbijów Mały, ca. 10 km northeast of the town of Skarżysko-Kamienna, Central Poland) was examined due to intermediate individuals found in sympatric populations of these species. Both putative parents as well as individuals that appeared hybrid-like were found in an extensive, wet hay-meadow. Various vegetative and reproductive characteristics were studied to identify hybrids. Interestingly, Polish accessions of *C. ×gracilescens* exhibited some degree of morphological intermediacy but resembled *C. stricta* in spikelet morphology. Branching of the mid-culm, the number of nodes per culm, callus hair length and relative callus hair length are the best characters to distinguish the Polish *C. ×gracilescens*. AFLP analysis proved to be suitable for detecting recent hybridization events between *C. canescens* and *C. stricta*. Analysis of the Bayesian clustering analysis showed that *C. ×gracilescens* were subjected to gene flow from the *C. canescens* gene pool as well as from the *C. stricta* gene pool.

KEY WORDS: *Calamagrostis ×gracilescens*, *C. canescens*, *C. stricta*, hybrid, habitat conditions, morphology, AFLP markers, gene flow.

INTRODUCTION

With approximately 270 species, *Calamagrostis* Adans. sensu Clayton and Renvoize (1986) is one of the largest genera in the family Poaceae. As is the case with many grass genera, an important feature of *Calamagrostis* is the prevalence of interspecific hybrids (Nygren 1962). There are at least 15 hybrids given by Conert (1989) in Central Europe and 20 listed by Tzvelev (1976) in the former USSR within the genus *Calamagrostis* alone. The evidence for hybrids is usually derived from studies on their morphology and reproductive biology (Nygren 1962; Scholz 1964; Crackles 1994, 1995). *Calamagrostis* hybrids vary considerably in their frequency and distribution. Some are found at numerous localities across Europe, whereas others are restricted to single locations.

The hybrid between *Calamagrostis canescens* (Weber) Roth and *C. stricta* (Timm) Koeler [= *C. neglecta* (Ehrh.) Gaertn.] was for a long time given the rank of a species

and was named *C. gracilescens* Blytt, a name which also included a number of apomictic derivatives (Nygren 1946, page 205). At present it is known as *C. ×gracilescens* (Blytt) Blytt. It is a very rare hybrid in Central Europe and the largest concentrations of its localities have been reported from southern Sweden and Finland (Palmgren 1933-1934; Nygren 1962; Hämet-Ahti et al. 1998; Karlsson and Christofferson 2007). Single localities of this hybrid taxon are known from the Netherlands, Britain and Norway (Holmberg 1922; Corporaal 1984; Crackles 1994, 1995). In Britain, *C. ×gracilescens* has the status of a rare species (Preston et al. 2002; Cheffings 2004) and is classified as vulnerable by Cheffings et al. (2005).

Populations of *Calamagrostis canescens* and *C. stricta* are known in the Małopolska Upland (Central Poland), where they are distributed throughout the region. The two species have usually been delimited from each other based on spikelet traits, especially the length of callus hairs surrounding the florets, length of lemma awn and position of

awn insertion on the lemma, as well as culm traits such as the number of culm nodes, culm branching and the structure of the upper leaf surface. Typical specimens can be classified appropriately using these characters. *C. canescens* and *C. stricta* occasionally occur sympatrically. At Zbijów Mały village (Małopolska Upland), plants having certain morphological features intermediate between *C. canescens* and *C. stricta* were present together with the two species and were believed to be their hybrid: *Calamagrostis* \times *gracilescens* (Blytt) Blytt (its occurrence in Poland was mentioned by Nobis (2007) and Paszko and Nobis (2007)).

The aim of this paper is to evaluate characters used to distinguish a hybrid between *C. canescens* and *C. stricta*. A population of the putative hybrid *C.* \times *gracilescens* is examined to test the current view that this taxon arose from hybridization between *C. canescens* and *C. stricta*. The structure of phenotypic variation in morphological characters is therefore analyzed to investigate the relationship between the hybrids and their putative parents. It should help to exclude characters that were considered discriminatory by previous authors but display significant low-scale variation. In this study, an AFLP marker system has been used with the objective of clarifying the taxonomic status of *C.* \times *gracilescens* compared to its putative parental species.

MATERIAL AND METHODS

Specimens

Field observations were carried out in the years 2002–2007. Detailed investigations were performed in early July 2004 and repeated in the seasons of 2005, 2006, and 2007. Phytosociological relevés were performed using either the Braun-Blanquet method or floristic checklists compiled at the sites where *C.* \times *gracilescens* plants and other accompanying plants formed homogenous patches of vegetation (Nobis 2007). The nomenclature follows relevant authors: vascular plant species – Mirek et al. (2002), mosses – Ochyra et al. (2003); the names of syntaxa are given after Matuszkiewicz (2001). The herbarium material is deposited in the herbarium of the Institute of Botany, Polish Academy of Sciences (KRAM) and in the herbarium of the Institute of Botany, Jagiellonian University (KRA) in Kraków.

Analysis of morphological characters

Morphological characters that were investigated are those that commonly separate the two parental species. They included the following culm characters: culm length, panicle length, leaf length and width (at the second node from the top), presence of long white hairs on upper leaf surfaces (0 – not hairy, 1 – sparse hairs, 2 – hairy), number of culm nodes, culm branching (at the second node from the top), length of the basal branch of the panicle, peduncle length, as well as the following spikelet characters: lower and upper glume length and width (LGL, LGW, UGL, UGW), lemma and palea length (LL, PL), callus hair length (CHL), rachilla extension length (RL) (if absent, code: 0), rachilla extension length with hairs (RHL), awn length (AL) and awn insertion (AWNINS) (measured as the length from the base of the lemma to the point of awn insertion). Characters were scored for 124 plants collected in Zbijów Mały. Measurements of the length and width of the leaf blade were done by taking the second leaf from the

top of the culm. One well-developed spikelet per individual was used for measurement of spikelet characters. Measurements of spikelet characters were taken with a stereo microscope (Nikon SMZ-10A) with $\times 20$.

Data were analyzed using *Statistica* ver. 8.0 for Windows (StatSoft 2007). Differences in the means of three groups (two parental species and one hybrid population) for morphological characters were examined using one-way analysis of variance (ANOVA). Principal Component Analysis (PCA on the correlation matrix) was applied to examine morphological variation patterns in *C.* \times *gracilescens* and its putative parents.

Amplified fragment length polymorphism (AFLP)

Fifty three leaf samples of *C.* \times *gracilescens*, *C. canescens*, and *C. stricta* from Poland, Czech Republic, Sweden, Finland, and Iceland were collected in silica gel prior to the AFLP analysis. Nineteen accessions of *C.* \times *gracilescens* were taken into AFLP analysis. Eight Polish accessions came from the locality of Zbijów Mały (Central Poland). Eleven Swedish accessions were descended from 3 localities: seven from Kiaholmen Island, three from Hunna village (both in the vicinity of Lake Skatelövfjorden in Småland Prov.), and one from the Singö Island (Upland Prov.). Eighteen accessions of *C. canescens* from Poland, Czech Republic, Sweden, and Finland, and 16 of *C. stricta* from Poland, Sweden, and Iceland were taken into account for comparison (see Appendix 1). Total genomic DNA was extracted from 20 mg of dried leaf tissue following DNeasy Plant Mini Kit protocol supplied by the manufacturer (Qiagen). AFLP analysis was carried out according to Vos et al. (1995) with minor modification. Pre-amplification and selective amplification were performed as described by Vos et al. (1995). Three primer pairs (E+AGT/M+CAG, E+ATG/M+CGC, and E+ATT/M+CTA) were chosen for selective PCR based on a preliminary survey of 12 primer pairs. The resulting PCR products were separated with the internal size standard GeneScan 500Rox (Applied Biosystems) in an automated DNA sequencer ABI 3100-Avant (Applied Biosystems) and analysed using GeneMapper software packages, version 4.0 (Applied Biosystems). AFLP bands ranging from 50 to 500 base pairs were scored.

Unweighted pair group method with arithmetic mean (UPGMA) was applied with restriction sites similarity coefficient method as described by Nei and Li (1979) using Treecon for Windows, version 1.3b (Van de Peer & De Wachter 1994). Internal support for groupings was assessed using the bootstrap procedure of Felsenstein (1985) with 2000 replications. Pairwise fixation index (F_{ST}) between pairs of taxa was assessed using Arlequin ver. 3.0 (Excoffier et al. 2005). Principal components analysis (PCA on the variance-covariance matrix) was used as to explore the main patterns of genetic variation of *C.* \times *gracilescens* in comparison with its putative parental species. These analyses were conducted using *Statistica* ver. 8.0 for Windows (StatSoft 2007). Usually, the PCA of the covariance matrix is meaningful only if the variables are expressed in the same units. Results of PCA were consistent with Principal Coordinates Analysis performed using Euclidean distances. The gene flow between the two putative parental species of *C.* \times *gracilescens* was investigated using the Bayesian clustering approach implemented in STRUCTURE (version 2.3; Pritchard et al. 2009).

RESULTS

Locality

The collection site is situated ca. 0.5 km NW of the Zbijów Mały village (ATPOL grid squares: EE47 00, based on cartogram units 2.5×2.5 km, Zając 1978) and SE of the Zbijów Mały – Stary Mirów road (Fig. 1), in hay-meadows along a water-course (right-hand side affluent of the Iżanka river). Its geographic coordinates are 51°10'29.2'' N/21°02'15.5'' E and it is located at an altitude of ca. 190 m. Zbijów Mały is situated ca. 10 km NW of the town of Skarżysko-Kamienna (NE part of the Małopolska Upland, Central Poland).

Share in plant communities

Calamagrostis canescens is a species characteristic of forest communities belonging to the class *Alnetea glutinosae* (Matuszkiewicz 2001); it grows, however, in extensive wet hay-meadows, in a fen and by a water-course (to the left of the road connecting Mirów and Zbijów Mały) in Zbijów Mały. At this locality, *C. canescens* forms its own, local community, characterized by a marked domination of *C. canescens* (forming beds) and a small coverage of other plant species. *C. canescens* was also observed on the edge of a drainage ditch and on the margins of a mixed forest.

C. stricta is a species characteristic of peatlands belonging to the class *Scheuzerio-Caricetea nigrae*. It grows mainly in drainage ditches or near them at the present locality. Its coverage is extensive in some parts of the fen (Nobis and Piwowarczyk 2004). *C. stricta* has the highest share in the communities examined and its coverage ranges between + and 5 (Table 1).

C. ×gracilescens grows mainly in wet hay-meadows (phytosociological relevé no. 6) belonging to the class *Molinio-Arrhenetheretea*, especially the order *Molinietales*. It was also observed in their surroundings: in a fen representing the class *Scheuzerio-Caricetea nigrae* and in drainage ditches (nearly forming beds in places). Therefore, a great

participation of rush species, especially of the *Magnocaricion* alliance, and fen species is noted in the plant communities documented phytosociologically. The taxon grows almost exclusively close to its parents, either both or one of them.

Morphological analysis

C. stricta, *C. canescens* and several other interesting specimens were collected during field studies in the vicinity of Zbijów Mały. While they appeared to be similar to *C. stricta* at first glance, the specimens turned out to resemble *C. canescens* upon closer examination; the two species, however, are completely different in their external appearance. Culms of *C. canescens* plants from Zbijów Mały are usually branched in the middle part of the stem and 5-7 noded. The short awn, up to 0.65 mm long, is apical, rarely inserted below the lemma apex. The lower glume is 4.5-6.4 times longer than wide. The lemma is ringed at the base with white callus hairs, 3.0-4.8 mm long, longer than lemma length. Leaves are limp, scarcely ribbed and usually with white long hairs on the upper leaf surface, these being about 1 mm long (Table 2). Culms of *C. stricta* collected in Zbijów Mały are always unbranched in the middle part of the stem and 2-5 noded. The lemma bears a fine straight awn on the back, 1.5-2.5 mm long. The point of awn insertion is between lower one-fifth and two-fifth above the base. The lower glume is 2.6-4.1 times longer than wide. The lemma is ringed at the base with white callus hairs, 1.6-2.75 mm long, up to 0.9 long as the lemma. The upper leaf surface of *C. neglecta* is prominently vein-ridged and scabrous. Range values calculated for the best characters to differentiate the species are presented in Table 2.

The intermediate nature of the *Calamagrostis canescens* × *C. stricta* hybrids recorded in Zbijów Mały prompted a more extensive survey of morphological characters of the populations. *Calamagrostis canescens*, *C. stricta* and *C. ×gracilescens* from Zbijów Mały did not differ significantly ($P > 0.05$) according to seven characters: panicle length,

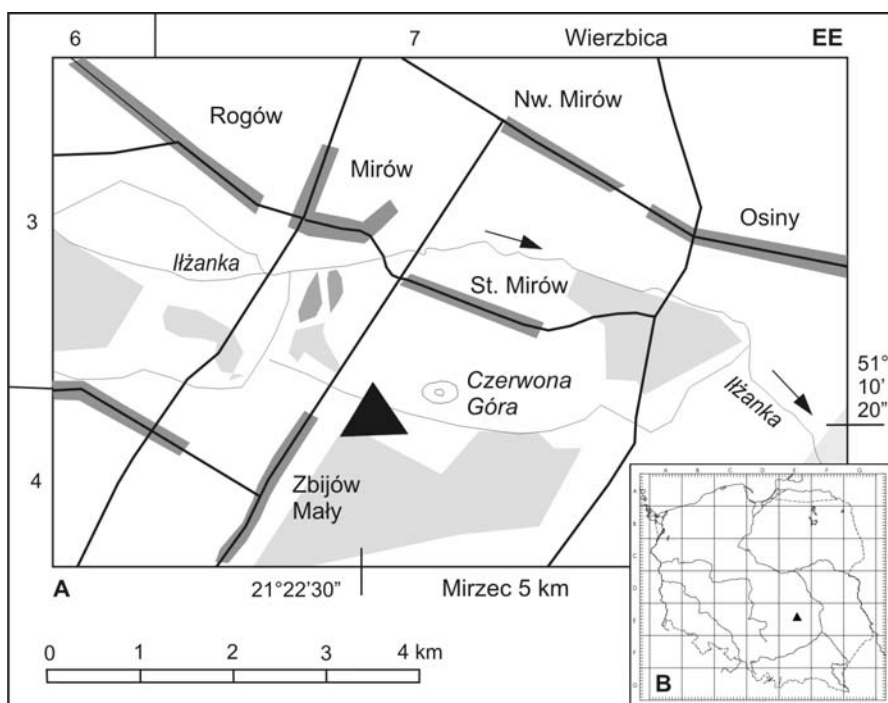


Fig. 1. The locality of *Calamagrostis ×gracilescens* located nearby Zbijów Mały village (Central Poland) – ▲.

TABLE 1. Plant communities with *Calamagrostis* × *gracilescens* (Blytt) Blytt and its parental species.

Number of relevé	1	2	3	4	5	6	C
Date	06.07.04	06.07.04	06.07.04	29.06.06	29.06.06	05.07.05	n
Cover of herb layer c (%)	100	100	95	95	90	100	s
Cover of mosses layer d (%)	exiguously	exiguously	exiguously	5	exiguously	30	t
Area of relevé (m ²)	150	100	100	50	100	70	a
Altitude a.s.l.	190	190	190	190	190	190	n
Number of species	25	26	28	30	24	26	c
<i>Calamagrostis</i> × <i>gracilescens</i>	.	.	+	.	+	5	III
Cl. Scheuzerio-Caricetea nigrae							
<i>Calamagrostis neglecta</i>	5	5	4	4	5	.	V
<i>Comarum palustre</i>	2	1	+	1	1	+	V
<i>Carex nigra</i>	+	+	II
<i>Stellaria palustris</i>	+	.	+	.	.	.	II
<i>Ranunculus flammula</i>	.	.	.	2	.	+	II
Cl. Molinio-Arrhenatheretea							
<i>Poa pratensis</i>	+	+	+	2	+	.	V
<i>Ranunculus acer</i>	+	+	+	+	+	.	V
<i>Rumex acetosa</i>	+	+	+	+	+	.	V
<i>Cardamine pratensis</i>	.	+	+	1	+	+	V
<i>Festuca rubra</i>	+	+	+	.	.	+	IV
<i>Poa trivialis</i>	.	.	+	+	.	1	III
<i>Plantago lanceolata</i>	.	+	.	.	.	+	II
O. Molinietales and characteristic of *its associations							
* <i>Caltha palustris</i>	+	+	1	1	+	1	V
<i>Equisetum palustre</i>	1	+	+	1	+	1	V
<i>Deschampsia caespitosa</i>	1	1	+	3	2	2	V
* <i>Juncus effusus</i>	1	+	+	1	+	+	V
<i>Galium uliginosum</i>	1	+	+	1	+	.	V
<i>Lotus uliginosus</i>	+	+	+	.	+	+	V
* <i>Lythrum salicaria</i>	r	.	1	1	+	.	IV
<i>Cirsium palustre</i>	+	.	+	.	+	.	IV
* <i>Lysimachia vulgaris</i>	+	+	+	.	.	.	III
<i>Climacium dendroides</i>	.	.	.	+	.	1	II
Cl. Phragmitetea							
<i>Galium palustre</i>	+	+	+	2	1	1	V
<i>Poa palustris</i>	+	.	+	2	1	+	V
<i>Carex rostrata</i>	.	.	+	1	2	1	IV
<i>Carex gracilis</i>	.	+	+	.	.	.	II
<i>Carex paradoxa</i>	.	+	I
Cl. Alnetea glutinosae							
<i>Calamagrostis canescens</i>	+	.	3	1	.	+	IV

Sporadic: Cl. Scheuzerio-Caricetea nigrae: *Agrostis canina* 3, *Menyanthes trifoliata* 6, *Veronica scutellata* 4; Cl. Molinio-Arrhenatheretea: *Festuca pratensis* 4; O. Molinietales: *Lychnis flos-cuculi* 5, *Lysimachia vulgaris* 5(1), *Myosotis palustris* 4, *Scirpus sylvaticus* 2; Other: *Agrostis capillaris* 5(1), 6; *Anthoxanthum odoratum* 2, 6(1); *Brachythecium* sp. 1(1); 2(1); 3, 5(1), 6; *Caliergonella cuspidata* 1(1), 2(1), 3(+), 4(1), 5, 6(2); *Epilobium palustre* 4, 6; *Euchrychium* sp. 1, 2, 3, 4, 6; *Holcus lanatus* 6(1); *Mentha* sp. 4; *Plagiomnium* sp. 1, 2, 3, 4; *Plantago intermedia* 4; *Ranunculus repens* 4(1); *Salix cinerea* (c) 1.

leaf length, ligule length, peduncle length, lemma length, palea length and hairiness of the upper leaf surface (Table 2). These characters were excluded from PCA analysis (Fig. 2). Other characters selected for examination were shown to differ significantly ($P < 0.001$) among the three groups of taxa and a few of them could distinguish the hybrid taxon.

C. xgracilescens is very similar to *C. stricta* and significantly differs from it only with regard to four characters: (1) number of culm nodes, (2) callus hair length, (3) callus hair length/lemma length ratio, and (4) mid-culm branching. Low F-values in ANOVA indicate a greater similarity between *C. xgracilescens* and *C. canescens* in relation to the first three characters. *C. xgracilescens* considerably differs from *C. canescens* by all of the characters studied. However, the differences are very small in the case of many characters, the F-value is lower than 50 and therefore the differences are not of any taxonomical significance. Some

intermediate characters were detected: callus hair length and the callus hair length/lemma length ratio. The number of culm nodes in *C. xgracilescens* overlaps with both parental species (Table 2). The hybrid expressed the upper leaf surface characteristic of *C. stricta*. Adaxial surfaces of the leaves in the hybrid and *C. stricta* are prominently ribbed, with wide grooves, scabrous and hairless or sparsely hairy compared to leaf surfaces weakly ribbed and hairy or non-hairy in *C. canescens*.

As the PCA of these morphological measurements shows, 79% of the variation could be explained by two principal components. A graph of these showed that *C. canescens* and *C. stricta* formed two distinct clouds (Fig. 2). Individuals of Polish accessions of *C. xgracilescens* are closer to the cloud of data points of *C. stricta*, partly overlapping with it. Swedish accessions of *C. xgracilescens* are located more in the center, close to an average of the scores of the parent species. The first principal component expres-

TABLE 2. Morphological variation among the hybrid and its parents, *C. canescens* and *C. stricta* for 21 characters from plants collected at Zbijów Mały near Skarżysko-Kamienna (Central Poland). N – number of individuals studied. Characters indicated by asterisk (*) were included in a PCA analysis.

Character	Species	<i>C. canescens</i> N = 31 Mean Min-max	<i>C. ×gracilescens</i> N = 30 Mean Min-max	<i>C. stricta</i> N = 63 Mean Min-max
culm length [cm]		116.9 96.5-137.0	99.7 82.0-139.0	101.9 71.5-135.0
number of culm nodes*		6.52 5-7	5.17 3-7	3.46 3-5
panicle length [cm]		16.3 13.0-20.0	16.0 10.3-28.5	15.2 10.0-25.0
length of bottom branch of the panicle [cm]		6.4 4.2-8.0	5.3 2.2-9.2	4.4 2.2-9.2
peduncle length [cm]		41.5 30.3-53.0	42.9 27.3-65.0	49.1 23-75.7
leaf length [cm]		21.8 16.7-31.0	21.3 16.2-29.8	19.1 13.5-29.0
leaf width [cm]*		5.3 4.3-7.5	4.1 3.1-5.5	3.7 2.8-5.3
upper leaf surface		<i>canescens</i> -like	<i>stricta</i> -like	<i>stricta</i> -like
ligule length [mm]		2.7 1.0-5.0	3.0 1.6-5.5	2.2 0.7-6.2
branching of culm*		87%	53%	0%
presence of long white hairs on the upper leaf surfaces		hairless – 3 single hairs – 6 hairy – 22	hairless – 0 single hairs – 2 hairy – 28	hairless – 23 single hairs – 2 hairy – 38
lower glume length [mm]*		4.7 3.85-5.50	3.7 3.1-4.4	3.6 2.9-4.6
lower glume width [mm]*		0.89 0.75-1.05	1.08 0.80-1.25	1.10 0.90-1.35
lemma length [mm]		2.81 2.25-3.2	2.89 2.3-3.4	2.79 2.35-3.35
awn insertion*		2.49 1.60-3.00	0.78 0.5-1.2	0.80 0.5-1.15
lower glume length/lower glume width*		5.28 4.53-6.4	3.47 2.96-4.56	3.34 2.56-4.10
callus-hairs length [mm]*		3.78 3.0-4.75	2.84 2.0-3.55	2.05 1.65-2.75
callus hairs length/lemma length*		1.35 1.03-1.64	0.98 0.76-1.16	0.73 0.58-0.88
rachilla length [mm]*		absent or up to 0.35	0.53 0.25-0.85	0.74 0.50-1.4
relative lemma awn insertion*		0.89 0.6-1	0.27 0.19-0.49	0.29 0.18-0.39
awn length [mm]*		0.44 0.25-0.65	1.76 1.05-2.25	1.97 1.45-2.5

sed 69% of the total variance and is explained by all the characters which were used in the PCA. Strongly negative

(<-0.70) and strongly positive loadings (>+0.60) indicate that all these characters take part in the differentiation of

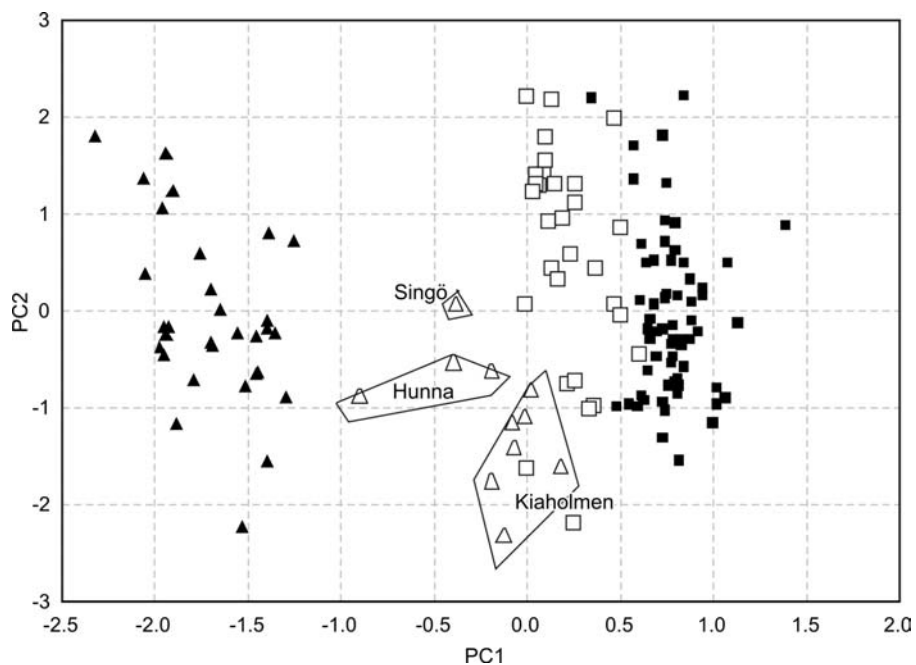


Fig. 2. Two-dimensional ordination diagram of PCA analysis (on the correlation matrix) based on 17 morphological characters. Taxa are indicated as follows: *C. canescens* (▲ solid triangles), *C. stricta* (■ solid squares), Polish accessions of *C. xgracilescens* (□ open squares), Swedish accessions of *C. xgracilescens* (△ open triangles).

the two parental species of *C. xgracilescens*. The second principal component explaining 11% of the total variance does not have loadings higher than ± 0.70 , but has two cross-loadings (with culm branching and lower glume width).

AFLP analysis

In the 53 studied accessions of *Calamagrostis xgracilescens* and its parental species, three primer combinations amplified a total of 563 fragments, 551 of which were polymorphic (Table 3). Of those, 457 were shared by two or more accessions, and thus were potentially informative, whereas 94 were either unique to a single accession or present in all except one. The selective primer pair combinations varied in their ability to detect AFLP variation across *Calamagrostis* accessions with numbers of detected polymorphic fragments ranging from 120 (E+ATT/M+CTA) to 248 (E+ATG/M+CGC). However, the percentage of potentially informative fragments (out of the total number) generated by each selective primer combination was stable be-

tween 80.0% (E+ATT/M+CTA) and 81.9% (E+ATG/M+CGC). A significant number of shared AFLP fragments was found in this study: 24 between *C. xgracilescens* and *C. canescens*, 16 between *C. xgracilescens* and *C. stricta*, and 14 between *C. canescens* and *C. stricta* (Table 3). Species-specific AFLP markers (i.e., bands that are present in all individuals of one species and none of the other) were found. Three *C. canescens*-specific and four *C. stricta*-specific markers were detected. For these seven bands the putative hybrids had usually high band frequencies. None of completely hybrid-specific AFLP markers were noticed.

Relying on the 551 polymorphic AFLP markers, a dendrogram was constructed based on Nei Li's genetic distance using UPGMA methods (Fig. 3). Studied specimens formed three major clusters, which corresponded with three studied taxa of *Calamagrostis*. *C. canescens* accessions showed the highest number of shared fragments with *C. xgracilescens* accessions (Table 3), and formed a cluster with them (Fig. 3). Bootstrap support values for branches, which included *C. xgracilescens* and *C. stricta* were mode-

TABLE 3. Characteristics of fragment variation generated by three primer combinations in the AFLP analysis of 53 accessions of *Calamagrostis xgracilescens* and its putative parents. Fragments were scored for each accession within a readable range of 50-500 bp.

Characteristics / primer combinations	<i>Calamagrostis xgracilescens</i> and its parental species				
	E+AGT / M+CAG	E+ATG / M+CGC	E+ATT / M+CTA	Total	Mean
Total fragments	195	248	120	563	187.7
No. present in all accessions	7	3	2	12	4
No. unique to one or present in all accessions except one	30	42	22	94	31.3
No. present in two or more accessions	158	203	96	457	152.3
Potentially informative fragments (%)	81.0	81.9	80.0	81.2	81.0
No. shared between <i>C. canescens</i> and <i>C. stricta</i>	8	4	2	14	4.7
No. shared between <i>C. xgracilescens</i> and <i>C. stricta</i>	10	3	3	16	5.3
No. shared between <i>C. canescens</i> and <i>C. xgracilescens</i>	11	9	4	24	8

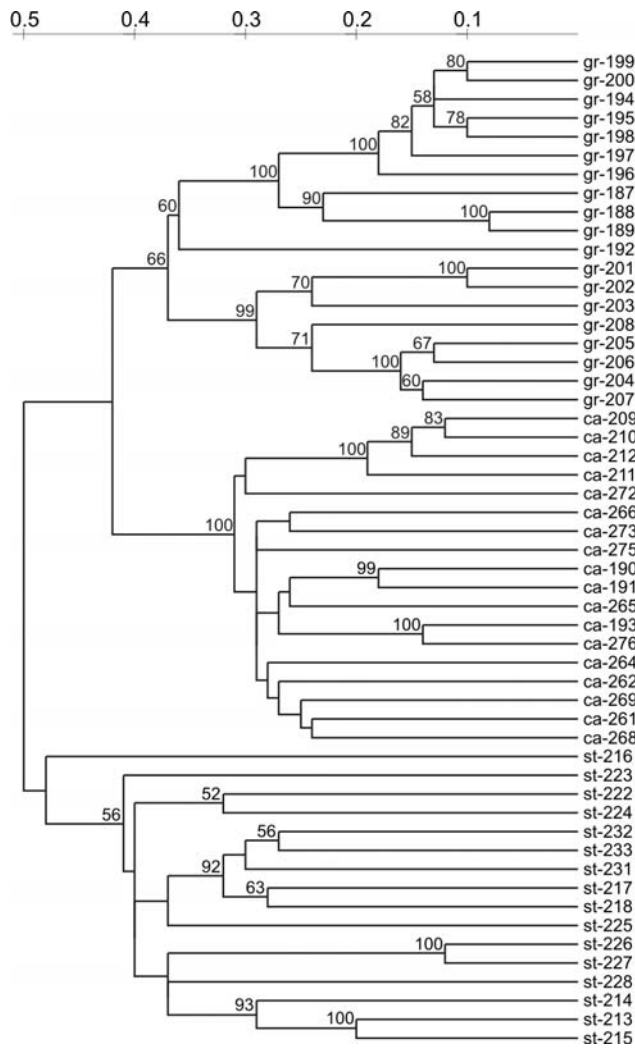


Fig. 3. UPGMA dendrogram of *C. xgracilescens* and its putative parental species *C. canescens* and *C. stricta* based on based on Nei-Li's genetic distance for 551 AFLP fragments. Bootstrap support values above 50% are shown. *Calamagrostis* samples codes see Appendix 1.

rately low, 66% and 56%, respectively. Branch, which comprehended accessions of *C. canescens* were very well

supported (100%) (Fig. 3). Two subclusters were possible to be distinguished within *C. xgracilescens* cluster. The first one included eleven Swedish accessions of *C. xgracilescens* from Kiaholmen Island (gr-194–gr-200), Hunna village (gr-187–gr-189), and Singö Island (gr-192). The first two Swedish localities are situated close to each other at Lake Skatelöv fjorden (Småland Prov., Alvesta kommun). The latter subcluster comprised eight Polish accessions from the locality Zbijów Mały village (gr-201–gr-208).

High levels of pairwise fixation index (F_{ST}) (0.196–0.386) were observed among the three genetic clusters therefore supporting their existence. The highest value of F_{ST} (0.386) was detected for *C. canescens* and *C. stricta*, relatively lower value was assessed for *C. xgracilescens* and *C. canescens* (0.290), and for *C. xgracilescens* and *C. stricta* (0.196).

There were 551 polymorphic markers used in PCA analysis. The first PCA axis accounted for 19.6% of the variation while the second for 9.1%. A plot of the first two axes resulted in spatial separation of two species along the first and the second axis. The hybrids are distributed within the area between the two species, however Polish accessions are more concentrated towards individuals of *C. stricta* (Fig. 4).

We ran STRUCTURE for 100,000 MCMC steps after a burn-in of 10,000 iterations. The admixture model and performed clustering without population information data, and assumed independent allele frequencies were used (Pritchard et al. 2009). The objective of this analysis was to test whether samples of *C. xgracilescens* can be classified within or between the putative parental species. Initial simulations in Structure were performed assuming genetic cluster numbers from $K = 2-5$. While $K = 2$ resulted in two discrete genetic clusters with the parental samples being placed firmly in either cluster, an increase in K to three, four or five displayed relatively higher ln likelihood and also higher variance of ln likelihood.

We concluded that the existence of two genetic clusters best explained the data. Out of 53 samples, 18 samples of *C. canescens* had high membership value (mean $Q = 0.997$) to *C. canescens* gene pool, 16 samples of *C. stricta*

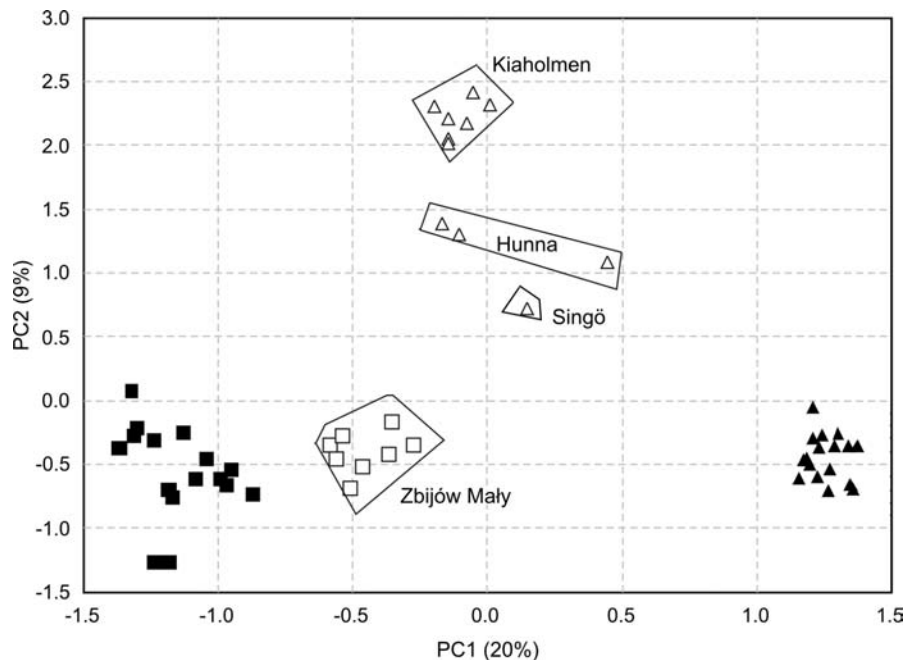


Fig. 4. PCA analysis (on the variance-covariance matrix) of AFLP fragments for *C. xgracilescens* and its putative parents: *C. canescens* (▲ solid triangles), *C. stricta* (■ solid squares), Polish accessions of *C. xgracilescens* (□ open squares), Swedish accessions of *C. xgracilescens* (△ open triangles). Different Swedish accessions of *C. xgracilescens* are shown using polygons. Percentage of the total variance explained by two principal components is given.

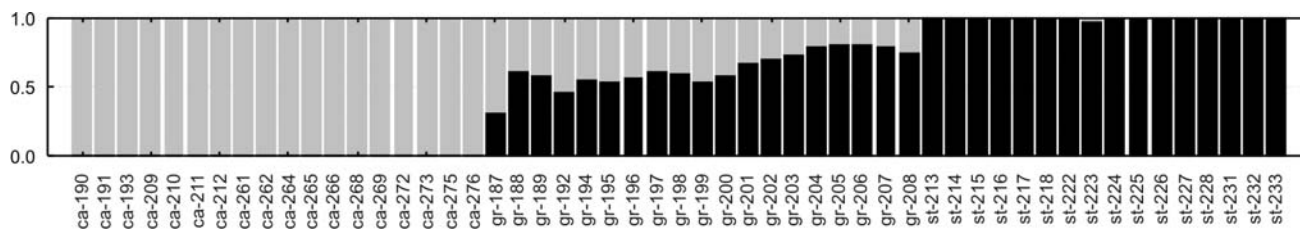


Fig. 5. Coefficients of estimated ancestry per individual (Q) and grouped by taxa. Each individual is represented by a single vertical line broken into two coloured segments, with lengths proportional the individual's ancestry fraction from each of the two biological gene pool: *C. canescens* (gray) and *C. stricta* (black).

fit into *C. stricta* gene pool (mean $Q = 0.994$). Samples of *C. xgracilescens* had higher membership value to *C. stricta* gene pool, (mean $Q = 0.63$, SD = 0.13) and lower to *C. canescens* gene pool (mean $Q = 0.37$, SD = 0.13) (Fig. 5, Table 4).

TABLE 4. Proportion of estimated ancestry (Q) of 53 samples of *C. xgracilescens* and its putative parents. In brackets the number of studied samples of each taxa.

Taxa	Q <i>C. canescens</i> pool Min-max (average)	Q <i>C. stricta</i> pool Min-max (average)
<i>C. canescens</i> (18)	0.996-0.998 (0.997)	0.002-0.004 (0.003)
<i>C. xgracilescens</i> (19)	0.194-0.689 (0.374)	0.311-0.806 (0.626)
<i>C. stricta</i> (16)	0.001-0.037 (0.006)	0.963-0.999 (0.994)

Description of the Zbijów Mały

Calamagrostis xgracilescens population with reference to some diagnostic features of its parental species

The description of *C. xgracilescens* is based on 30 plants examined in the analysis.

Perennial, rhizomatous, forming beds. Culms reaching a height of 82-139 cm, 3-7-noded, peduncle 27-65 cm long (a feature of *C. canescens* that frequently occurs in the hybrids is culm branching at basal and middle nodes, 16 out of the 30 specimens examined). Leaves green, blade 16-30 cm long, 3-5.5 mm wide, surface scabrous (rough to the touch), with long white hairs on upper leaf surface as in *C. canescens*, ligule 1.6-5.5 mm long. Panicle *C. canescens*-like in length, 10-28 cm long. Basal branch of panicle longer than in *C. stricta* 2.2-9.2 cm, average 5.3 cm long. Glumes similar to those of *C. stricta* in length to slightly longer, lower glume 3.1-4.4 mm long, 1-veined, upper glume 2.9-4.3 mm long, 3-veined, two lateral nerves very short, and similar to those of *C. stricta* in width, 0.8-1.25 mm wide. Lemma 2.3-3.4 mm, average 2.9 mm. Callus hairs intermediate in length, 2.0-3.6 mm long, usually more or less equal the floret in length, 0.8-1.2 length of lemma. Awn length and insertion as in *C. stricta*. Awn dorsal, straight, 1.0-2.3 mm long, arising 0.2-0.5 way up back of lemma. Palea 1.4-2.4 mm long. Spikelet axis (rachilla) prolonged as a minute bristle as in *C. stricta*, 0.3-0.9 mm long, pilose.

DISCUSSION

The phenetic analysis of morphological characters examined in this study allows genetic affinities among the taxa to be estimated quantitatively with greater accuracy. *Calamagrostis*

canescens is morphologically clearly distinct from *C. stricta* at the locality in Zbijów Mały. The most striking observation is that *C. xgracilescens* is not phenotypically intermediate. However, recent studies by Rieseberg (1995) have shown that morphological characters of hybrids are not always intermediate between those of the parental species and hybrids are actually a mosaic of parental, intermediate and extreme characters. Thus, some traits are more likely to reveal hybrid ancestry than others (Rieseberg 1995). Extreme characters were not detected in *C. xgracilescens*. It was evident that *C. xgracilescens* is closer to *C. stricta* for the majority of the morphological characters examined in the analysis. A smaller set of characters (mid-culm branching, callus hair length, relative callus hair length and number of culm nodes) indicated a closer affinity between the hybrid and *C. canescens*.

The occurrence of hybrid populations at 3-4 sites as beds 4-5 m in diameter and some smaller tufts suggests that they are a result of single hybridization events and persist through vegetative reproduction. The ability of *C. xgracilescens* to reproduce in nature considerably increases the number of biotypes in the complex. Like *C. canescens* and *C. stricta*, *C. xgracilescens* has the power to form stolones and can also reproduce vegetatively. Therefore, the number of seed-producing panicles increases annually in the perennial hybrid.

Natural hybrids of *C. xgracilescens* have been described from Sweden (Nygren 1962), Germany (Scholz 1964) and Great Britain (Crackles 1994, 1995). However, partly or totally different sets of characters differentiated the hybrid in each case. According to Hämet-Ahti et al. (1998) *C. xgracilescens* is a polymorphic taxon. Nygren (1962) used callus hair length, length of glumes, structure of the upper leaf surface, culm branching, presence of long hairs on the upper leaf surface and number of culm nodes. Nygren (1962) claimed that two *stricta*-like characters, the structure of the upper leaf surface and the number of culm nodes, as well as one *canescens*-like character, culm branching, are dominant characters. Leaf hairiness, a *canescens*-like character, is less dominant in *C. xgracilescens* according to Nygren (1962).

Scholz (1964) described *C. xgracilescens* as plants with characters intermediate between *C. canescens* and *C. stricta* and believed that they are similar to *C. villosa* in spikelet morphology. Two diagnostic characters of *C. xgracilescens* were found by Scholz (1964) in the vegetative habit. The first is the leaf surface which is of the *stricta*-type and the second is culm branching which is of the *canescens*-type according to him. *C. xgracilescens* may be distinguished from *C. villosa* by the upper leaf surface: *C. villosa* does not have any ribs on the upper leaf surface.

Interestingly, Crackles (1994, 1995) used totally different sets of characters to study morphological variation of hybrids between *C. canescens* and *C. stricta*. The following groups of characters were chosen by him to study the morphological variation of hybrids occurring along the Leven Canal, SE Yorks.: culm-related (width at 2nd node, culm rough near panicle, hairiness of bottom leaf sheath), panicle-related (length and width, length of basal branch of panicle) and spikelet-related (glume length and width, callus hairs length, awn length, awn insertion, ligule length). Two diagnostic characters (culm branching and structure of the upper leaf surface), selected by Nygren (1962) and Scholz (1964), were omitted in his studies. Crackles (1994) delimits morphological variation of *C. stricta* on the basis of culm height (up to 95 cm), panicle length (up to 14 cm) and length of the bottom branch of the panicle (up to 4 cm). These traits were found to have a great discriminatory power between the two species studied by Crackles (1994, 1995). Such a discrepancy might be caused by slight differences in the environmental conditions experienced by the populations analyzed by him and those analyzed in the present study.

The pattern of morphological variation of *C. stricta* throughout its entire distribution area is unknown. According to Conert (1989), *C. stricta* is a rather invariable species in Europe. However, according to Tzvelev (1976), five subspecies of the widespread and circumboreal *C. neglecta* (Ehrh.) Gaertn. s. lato occur in the former USSR: subsp. *neglecta*, subsp. *inexpansa* (A. Gray) C.W. Greene, subsp. *stricta* (Timm) Tzvel., subsp. *micrantha* (Kearney) Tzvel., and subsp. *groenlandica* (Schrank) Matuszk. In North America, Marr et al. (2007) distinguished two subspecies: *C. stricta* subsp. *stricta* and *C. stricta* subsp. *inexpansa*. Matuszkiewicz (1948) listed two forms within *C. neglecta*: f. *laxa* Griseb. (panicle with long branches) and f. *interrupta* Prah. (panicle branches very short and panicle interrupted) without, however, giving actual numerical values. According to many authors (Matuszkiewicz 1948; Falkowski 1982; Szafer et al. 1988; Conert 1989; Hubbard 1968; Crackles 1994; Aiken et al. 1999; Marr et al. 2007) who describe the variation of *C. stricta* s. stricto, its height is nearly always delimited up to 100 cm, length of panicle up to 15 cm, seldom up to 20 cm, and length of basal lateral branches of panicle up to 4 cm. However, the height of 53% of individuals of *C. stricta* collected in Zbijów Mały is greater than 100 cm. Some have also a very long panicle (up to 25 cm) and long basal lateral branches of the panicle (up to 9.2 cm). Variation in culm and panicle length and the length of the bottom panicle branch needs detailed evaluation between and within *C. canescens* and *C. stricta*. These characters do not seem to differ between the two parental species of *C. ×gracilescens*. However, further studies with greater numbers of plants are required.

Plants from controlled reciprocal crosses between *C. canescens* and *C. stricta* were produced by Nygren (1962). F₁ plants as well as the second generation can be morphologically distinguished by two *C. canescens*-like characters (culm branching, presence of long white hairs on the upper leaf surface) and two *C. stricta*-like characters (structure of upper leaf surface, number of culm nodes). According to Nygren (1962), hairiness of the upper leaf surface varies considerably in several pure species and hybrids of *Calamagrostis* in different years. Natural hybrids from Sweden

fit very well with F₁ plants on the basis of callus hairs and glume length, structure of the upper leaf surface and type of ligule, but differ in having an intermediate number of culm nodes (4) and in culm branching (36% have a branched culm) (Nygren 1962). According to Nygren (1962), the node number normally varies between 1-3 in *C. neglecta*, while *C. canescens* has between 4-6 nodes. Natural hybrids of *C. ×gracilescens* detected in Poland resemble Swedish hybrids in callus hair length and the structure of the upper leaf surface.

The occurrence of *Calamagrostis ×gracilescens* in Poland in the area of sympatry of their putative parental species, *C. canescens* and *C. stricta*, was confirmed based on morphology, gene flow indicated by AFLP markers, and information collected during field studies. The Polish hybrid accessions from Zbijów Mały exemplifies the difficulty of detecting hybridization based entirely on morphological characters. Morphological characters typically have an unknown but presumably complicated genetic basis and a nonheritable component that is difficult to estimate. These natural hybrids are not easy to found in natural conditions. However, amongst several hybrids within the genus *Calamagrostis*, *C. ×gracilescens* is the most common one, especially on the Scandinavian Peninsula.

ACKNOWLEDGEMENTS

This study was financially supported by the State Committee for Scientific Research of Poland, grant no. 2 P04G 04930. Beata Paszko would like to thank all people which were helpful during field studies. Fieldwork in Sweden and Finland has been made all the more memorable by the companionship of many of her friends: Lennart Stenberg (Stockholm, Sweden), Lennart Persson (Jönköping, Sweden), Bengt Nilsson (Sölvesborg, Sweden), and Jouko Sipari (Tampere, Finland).

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APPENDIX 1

List of *Calamagrostis* samples studied in AFLP analysis. Vouchers were deposited in KRAM.

Calamagrostis ×gracilescens (Blytt) Blytt (19 specimens)
POLAND: gr-201, gr-202, gr-203 – Zbijów Mały, 51°10'28.2" N/021°01'55.1" E, 2007-06-17, *leg. B. Paszko*; gr-204, gr-205 – Zbijów Mały, 51°10'28.8" N/021°01'58.8" E, 2007-06-17, *leg. B. Paszko*; gr-206, gr-207, gr-208 – Zbijów Mały, 51°10'28.9" N/021°01'53.1" E, 2007-06-17, *leg. B. Paszko*; **SWEDEN:** gr-187, gr-188, gr-189 – Hunna, W shore of Lake Skatelövfjorden, 56°43'03.04" N/014°35'25.27" E, 2008-06-25, *leg. B. Paszko & B. Nilsson*; gr-192 – Singö Island, ca. 3 km N of Singö, 60°11'47.0" N/018°44'11.9" E, 2007-07-31, *leg. B.*

Paszko & L. Stenberg; gr-194, gr-195, gr-196, gr-197 – W of Vrankunge, Kiaholmen Island in Lake Skatelövfjorden, 56°42'59.03" N/014°36'55.13" E, 2008-06-25, *leg. B. Paszko, B. Nilsson & L. Persson*; gr-198, gr-199, gr-200 – W of Vrankunge, Kiaholmen Island in Lake Skatelövfjorden, 56°42'56.08" N/014°36'44.05" E, 2008-06-25, *leg. B. Paszko, B. Nilsson & L. Persson*

Calamagrostis stricta (Timm) Koeler (16 specimens)
POLAND: st-213, st-214, st-215 – Zbijów Mały, 51°10'29.3" N/021°01'52.7" E, 2007-06-17, *leg. B. Pa-*

szko; **ICELAND:** st-216, st-217 – Lonkot, close to the beach, 66°00'20.7" N [66°00.345 N]/019°24'07.8" W [019°24.130 W], 2007-07-23, *leg. L. Persson*; st-218 – Skútustaðöir, by the small Stakhólstjörn Lake located near the big Mývatn Lake, 66°34'05.0" N/017°01'55.0" W, 2007-07-25, *leg. L. Persson*; **Sweden:** st-222, st-223, st-224 – S of Lake Hammarsjön, 58°02'24.98" N/013°50'13.34" E, 2008-06-29, *leg. B. Paszko & L. Persson*; st-225 – Blädinge village, shore of Lake Salen, 56°50'55.66" N/014°31'56.90" E, 2008-06-26, *leg. B. Paszko & L. Persson*; st-226, st-227 – Bronholm, 57°19'32.15" N/014°27'56.65" E, 2008-06-26, *leg. B. Paszko & L. Persson*; st-228 – Skämershult, Lake Möllesjön, 56°23'20.63" N/014°27'45.24" E, 2008-06-23, *leg. B. Paszko & B. Nilsson*; st-231 – ca. 1.5 km W of Jarhois, 66°56'43.47" N/023°48'28.38" E, 2007-07-13, *leg. B. Paszko, L. Stenberg & J. Sipari*; st-232 – ca. 14 km SW of Täreändö, ca. 8 km NW of Aho, 66°59'43.30" N/022°34'25.84" E, 2007-07-14, *leg. B. Paszko, L. Stenberg & J. Sipari*; st-233 – ca 8 km E of Lillselet, 66°33'59.79" N/022°52'45.58" E, 2007-07-16, *leg. B. Paszko, L. Stenberg & J. Sipari*

Calamagrostis canescens (Wigg.) Roth (18 specimens)
CZECH REPUBLIC: ca-273 – W of Svetnov, 49°37'05.15" N/015°56'52.29" E, 2007-07-03, *leg. B. Paszko*; **FINLAND:** ca-272 – Lammiupää, 61°09'37.67" N/027°33'15.19" E, 2008-07-20, *leg. B. Paszko & J. Sipari*; **POLAND:** ca-209, ca-210 – Zbijów Mały, 51°10'28.9" N/021°01'53.1" E, 2007-06-17, *leg. B. Paszko*; ca-211, ca-212 – Zbijów Mały, 51°10'27.7"/021°01'54.8" E, 2007-06-17, *leg. B. Paszko*; **SWEDEN:** ca-190, ca-191 – Hunna, shore of Lake Skatelövfjorden, 56°43'03.04" N/014°35'25.27" E, 2008-06-25, *leg. B. Paszko & B. Nilsson*; ca-193, ca-275, ca-276 – Singö Island, ca. 3 km N of Singö, 60°11'47.0" N/018°44'11.9" E, 2007-07-31, *leg. B. Paszko & L. Stenberg*; ca-261, ca-262 – between Bergkvara and Gemla by, 56°52'51.66" N/014°41'46.71" E, 2008-06-26, *leg. B. Paszko & L. Persson*; ca-264 – W of Kullen, 56°20'15.02" N/013°19'21.65" E, 2008-06-23, *leg. B. Paszko & B. Nilsson*; ca-265 – N of Ynde, 56°05'00.11" N/014°33'26.09" E, 2008-06-24, *leg. B. Paszko & B. Nilsson*; ca-266 – W of Vrankunge, Kiaholmen Island in Lake Skatelövfjorden, 56°42'56.44" N/014°36'52.56" E, 2008-06-25, *leg. B. Paszko, B. Nilsson & L. Persson*; ca-268 – Västra Fagerhult, 57°46'07.84" N/014°41'33.18" E, 2008-06-28, *leg. B. Paszko & L. Persson*; ca-269 – Stockholm, S side of Lake Brunnsvikens, 59°19'46.81" N/018°07'22.34" E, 2008-07-01, *leg. B. Paszko & P. S. Lindberg*