

Morphological differences and chromosome numbers in *Puccinellia distans* and *P. limosa* populations from Central Europe

Morfológické rozdíly a počty chromosomů u středoevropských populací *Puccinellia distans* a *P. limosa*

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Twenty five populations of *Puccinellia* from Czech Republic (Bohemia and Moravia), Slovakia and Hungary were examined to verify the exact distribution of *P. distans* and *P. limosa* in south Moravia and south Slovakia. Determination of the studied species was based on morphometric analysis (PCA and discriminant analysis). Chromosome numbers were counted in each population. Morphometric analysis divided *Puccinellia* populations in two groups. Populations from Bohemia and Moravia correspond to *P. distans* while those from Slovakia and Hungary correspond to *P. limosa*. Exterior palea length and anther length are the best characters for calculation of a simple discriminant function to distinguish between the investigated species. The distribution pattern found corresponds well to the results of the determination of older herbarium material from the studied localities. Two ploidy levels were ascertained in the studied populations, i.e. (i) all plants of *P. distans* and those of *P. limosa* from Slovakia and western part of Hungary were hexaploids ($2n = 42$), while (ii) *P. limosa* from the eastern part of Hungary were tetraploids ($2n = 28$). Morphological differences between both cytotypes of *P. limosa* were tested.

Key words: *Puccinellia distans*, *P. limosa*, morphology, chromosome numbers, morphometric analysis, discriminant function, Central Europe

Introduction

The genus *Puccinellia* (*Poaceae*) is represented by 5 species in Central Europe: *P. maritima* (Hudson) Parl., *P. capillaris* (Liljeband) Jansen, *P. distans* (Jacq.) Parl., *P. limosa* (Schur) Holmb. and *P. peisonis* (Beck) Jav. (Conert 1998). Three of them, i.e. *P. distans*, *P. limosa* and *P. peisonis*, usually referred to as *Puccinellia distans* group (see Engelmaier 1982), are closely related species growing in saline habitats.

P. distans is a facultative halophyte growing in salt marshes, ruderal places or dumps. It has been spreading from salt marshes to secondary habitats (roadsides, vicinity of railway stations or factories) since 1950, when salt began to be used for winter treatment of roads. Recently, trends to expand can be observed and the species occurs as a ruderal plant in the whole Europe with the exception of its southwestern part and Svalbard islands (Conert 1998).

P. limosa is an obligate halophyte growing exclusively in salt marshes. It occurs in the Pannonian lowland, reaching the Black Sea coast on the northwest. An isolated occurrence was reported from Germany (Conert 1998).

P. peisonis is an obligate halophyte, which seems to be an endemic species of the Pannonian Lowland. It was reported from the vicinity of the Neusiedler See lake and from

the region between the Danube and Tisza rivers (Austria and Hungary, see Conert 1998). *P. peisonis* differs from the other members of the group by very narrow and thick leaves (Conert 1998: Fig. 216). It is rather distinct in habit and distribution, hence relatively easy to distinguish from both *P. distans* and *P. limosa*. However, the precise determination and exact distribution of the latter two species in the Czech and Slovak Republics deserves attention.

Because of the retreat of natural saline habitats of *P. distans* within the area studied (in the Czech and Slovak Republics), the species is more frequently found in human-made, ruderal places. The only species growing in salt marshes of south Moravia and south Slovakia is thus *P. limosa* (Vicherek 1973). Maglocký & Svobodová (1999) and Danihelka & Grulich (1996) reported on the occurrence of *P. limosa* from salt marshes in the Dyje river basin (south Moravia). However, Dostál (1989) and Conert (1998) referred to *P. limosa* growing only in salt marshes of south Slovakia and did not mention south-Moravian sites. Our preliminary investigation showed that populations from south Moravia correspond morphologically to *P. distans*. Morphological features of *Puccinellia* populations from the salt marsh Kamenín in south Slovakia resemble those of *P. limosa* from Hungary. Interestingly enough, the chromosome numbers that we found in both south Slovakian plants (which we considered *P. limosa*) and Prague plants (considered *P. distans*) were $2n = 42$. Nevertheless, only $2n = 28$ has been reported for both *P. limosa* and *P. peisonis* up to now (Conert 1998).

Three ploidy levels have been known for *P. distans* group in Central Europe, with hexaploids prevailing (Table 1). Tetraploids were reported from Rumania, Hungary, Germany and Austria, diploids from Bulgaria and Germany. A question thus arises: How many different taxa are involved in the *P. distans* group?

The aim of this study was to (1) find a tool to discriminate between *P. limosa* and *P. distans*, (2) compare morphological differences and chromosome numbers of selected Central European populations of these two species, and (3) clarify their distribution in south Moravia and south Slovakia.

Material and methods

Plants sampled

Morphometric analyses were based on 584 individual plants from the Czech Republic (Bohemia, south Moravia), south Slovakia and Hungary. Sampled plants were grown from seed collected in 1995 and 1996 from 25 field populations (Appendix 1, Fig. 1). The samples included plants from both salt marshes and ruderal populations, except of *P. distans* from Bohemia where only ruderal populations were used, because plants reported to occur in natural habitat at former saline site at the Srpské louky meadows (Sládek 1993) have not been found. Similarly, most natural salt marshes in south Moravia have been destroyed by human activities. The selection of localities in Moravia and south Slovakia was based on those given by Vicherek (1973) but only three salt marshes with presumed natural occurrence of *Puccinellia* (Dobré Pole, Nový Přerov and Lanžhot near Kyjovka stream) could have been found. All but one Hungarian samples originate from natural salt marshes.

Table 1. – List of published chromosome numbers in *Puccinellia* species in Central Europe. Nomenclature corresponding to Conert (1998) is given in brackets. Entries followed by an asterisk represent non-determined taxa of *Puccinellia distans* group

Species.	2n	Locality	Source
<i>Atropis distans</i> Griseb. *	28	München	Stählin 1929
<i>P. distans</i> *	28	Romania (without locality)	Tarnavski 1938
<i>Puccinellia distans</i> (L.) Parl. subsp. <i>limosa</i> (Schur) Jáv. [= <i>P. limosa</i> (Schur) Holmb.]	28	Hungary (without locality)	Pólya 1948
<i>P. distans</i> (L.) Parl. s. str. [= <i>P. distans</i> (Jacq.) Parl.]	42	Hungary: Tihany peninsula of the Balaton lake	Felföldy 1949
<i>P. limosa</i> (Schur.) Jáv. [= <i>P. limosa</i> (Schur) Holmb.]	28	Hungary: Tihany peninsula of the Balaton lake	Felföldy & Felföldy 1949
<i>P. distans</i> (L.) Parl. *	42	Bulgaria: District Sofia and District Svishtov	Kozuharov & Kuzmanov 1968
<i>P. distans limosa</i> (Schur) Holm.	14	Bulgaria: District Varna	Kozuharov & Kuzmanov 1968
<i>P. distans</i> (Jacq.) Parl.	42	Braunschweig: 1. Barnstorf. N of Ührde; 2. near Jerxheim	Dersch 1974
<i>P. distans</i> s.l. *	14	Braunschweig: 1. Barnstorf. N of Ührde; 2. between Barnstorf and Watenstedt; 3. near Jerxheim	Dersch 1974
<i>P. peisonis</i> (Beck) Jáv.	28	Neusiedler See: S of Podersdorf	Dersch 1974
<i>P. distans</i> (Jacq.) Parl.	28	Neusiedler See	Engelmaier 1982
<i>P. limosa</i> (Schur) Holmb.	28	Neusiedler See	Engelmaier 1982
<i>P. peisonis</i> (Beck.) Jáv.	28	Neusiedler See	Engelmaier 1982
<i>P. distans</i> (L.) Parl. [likely = <i>P. distans</i> (Jacq.) Parl.]	42	Slovakia: Podunajská nížina lowland, Bratislava	Murín & Feráková 1978
<i>P. distans</i> (L.) Parl. [likely = <i>P. distans</i> (Jacq.) Parl.]	42	Slovakia: Devínska Kobyla Hill, Devín	Váchová & Feráková 1978

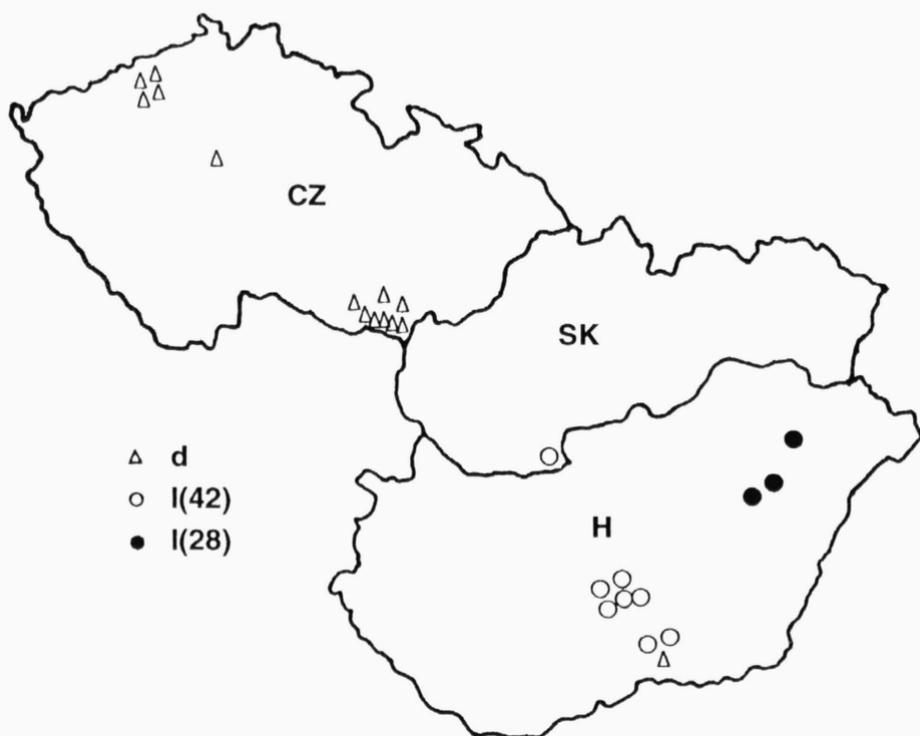


Fig. 1. – Distribution of localities with sampled populations of *Puccinellia* representatives. d = *P. distans*, l (42) = *P. limosa* 2n = 42, and l (28) = *P. limosa* 2n = 28 (see Appendix 1 for details).

For each population, 25 seedlings were planted in 8 × 8 cm pots. To avoid undesirable hybridization, only original plants grown from the seed collected in the field were used. In 1996–1997, number of tillers and maximum culm length were recorded in flowering plants, the panicles were harvested and subsequent measurements were carried out on dried plant material.

To verify the discriminant function and clarify the field distribution of *Puccinellia* taxa, 76 specimens were used deposited at PR under the following catalogue numbers: P4S 692/123–125, 127–130, 132–135, 137, 140, 143–144, 149, 190–191, 193–195, 197–200, 202–207, 209–214, 216, 233–234, 250–254, 280–283, 296, 299–302, 304–305, 310, 314–315, 317, 322–323, 327–328, 330, 332, 334, 338–341.

Characters examined

The following quantitative morphological characters (used by Adler et al. 1994, Conert 1998) were measured on dried material: number of branches of the lowest panicle node; length of the shortest branch of the lowest panicle node; length of the four-floret spikelet (if merely spikelets with more than four florets were present, measurements were taken up to the upper tip of the fourth floret); exterior palea length of the lowest floret of the same four-floret spikelet; and length of anther in the same spikelet. These characters, together

with the data on maximum culm length and number of tillers, which were recorded on living plants in the experimental garden, were used for determination of the studied species.

Among other characters distinguishing between *P. distans* and *P. limosa*, lamina shape (flat to channelled vs. involute – Adler 1994) and presence of papils on its lower side (Adler 1994, Conert 1998) are given by some authors. However, these characters were found to be rather equivocal so that they could not be used for determination of both species. It seems that lamina shape depends on the physiological state of a plant (water contents). Neither we were able to determine clear differences in the presence of papils. For that reason, these characteristics were not considered for analyses.

Chromosome numbers

To count chromosomes, root tips of cultivated plants or of young seedlings were pre-treated with p-dichlorobenzene, fixed in alcohol-acetic acid 3:1 mixture and stained with lacto-propionic-orceine.

Statistics

PCA ordination of sampled populations based on all measured characters was carried out using CANOCO software (ter Braak & Šmilauer 1998) to verify the possibility of using morphological characters to distinguish between *P. distans* and *P. limosa*. Two basic groups of populations were defined on the basis of PCA results, hence the standard discriminant analysis was used to test all measured characters and populations together. Backward stepwise method of discriminant analysis was used to find the classification rules. Statistica™ software was used for statistical analyses (StatSoft 1998).

Results

Cytological analysis of *Puccinellia* populations studied confirmed the chromosome number $2n = 42$ in *P. distans* as well as in *P. limosa* from south Slovakia and western Hungary. Only three populations of *P. limosa* from east Hungary showed $2n = 28$ (Appendix 1, Fig. 1). Morphological data for *P. distans* and both ploidy levels of *P. limosa* are given in Table 2.

PCA indicated that the 25 populations studied can be divided in two groups corresponding to the species *P. distans* and *P. limosa* (Fig. 2). Characters distinguishing these two groups (spikelet length, anther length, maximum culm length, number of branches of the lowest panicle node, and palea length) are mutually correlated.

Standard discriminant analysis of 7 investigated characters, using *P. distans* and both ploidy levels of *P. limosa* (see Appendix 1), yielded two groups. The group corresponding to *P. distans* included all Bohemian and Moravian populations while the other, corresponding to *P. limosa*, was formed by Slovakian and Hungarian populations belonging to both ploidy levels (Fig. 3). There was only one exception, represented by a single Hungarian *P. distans* population from a ruderal site (Appendix 1, no. 17) which appeared among the *P. distans* group confirming hence the taxonomical pattern regardless of geographical location. By using all 7 characteristics, 97.0% of individuals were correctly classified as *P. distans* or *P. limosa* (Table 3). Attempts to discriminate between all three taxon/ploidy

Table 2. – Mean values and ranges of individual characters for *P. distans*, *P. limosa* 2n = 42 and *P. limosa* 2n = 28.

Species	Character	n	Mean	s.d.	Min. – max.	Range (99% confidence)
<i>P. distans</i>	number of tillers	358	15.82	6.59	4.00–40.00	14.91–16.72
	number of branches	358	3.87	1.29	1.00– 7.00	3.69– 4.05
	branch length (mm)	358	18.16	9.62	3.00–54.00	16.84–19.48
	culm length (cm)	358	28.44	10.54	7.00–72.00	26.99–29.88
	spikelet length (mm)	326	4.00	0.36	2.96– 4.87	3.95– 4.05
	palea length (mm)	330	1.99	0.17	1.56– 2.44	1.97– 2.02
	anther length (mm)	356	0.75	0.11	0.52– 1.04	0.74– 0.77
<i>P. limosa</i> 2n = 42	number of tillers	157	20.73	10.68	3.00–53.00	18.51–22.96
	number of branches	155	4.59	1.22	1.00– 8.00	4.34– 4.85
	branch length (mm)	155	14.57	11.16	5.00–59.00	12.23–16.91
	culm length (cm)	157	54.63	11.26	20.00–83.00	52.29–56.97
	spikelet length (mm)	149	4.95	0.37	3.83– 5.74	4.88– 5.03
	palea length (mm)	149	2.50	0.19	2.09– 2.96	2.46– 2.54
	anther length (mm)	155	1.30	0.21	0.78– 1.83	1.26– 1.34
<i>P. limosa</i> 2n = 28	number of tillers	69	22.75	10.2	4.00–53.00	19.5 –26.01
	number of branches	69	4.23	1.24	2.00– 7.00	3.84– 4.63
	branch length (mm)	69	13.26	6.98	4.00–42.00	11.03–15.49
	culm length (cm)	69	44.75	8.65	13.00–60.00	42.00–47.51
	spikelet length (mm)	69	4.53	0.45	3.65– 5.48	4.38– 4.67
	palea length (mm)	69	2.27	0.22	1.83– 2.78	2.20– 2.34
	anther length (mm)	69	1.24	0.20	0.78– 1.74	1.18– 1.30

Table 3. – Efficiency of a discriminant function based on all 7 characters, 5 characters (exterior palea length, anther length, number of branches at the lowest panicle node, shortest branch length at the lowest panicle node, and maximum culm length) and 2 characters (exterior palea length and anther length) in distinguishing *Puccinellia limosa* from *P. distans*. Rows: observed classification; columns: predicted classifications.

Characters (no):		7	
	correct (%)	<i>P. distans</i>	<i>P. limosa</i>
<i>P. distans</i>	99.1	323	3
<i>P. limosa</i>	93.9	13	201
Total	97.0	336	204
Characters (no):		5	
	correct (%)	<i>P. distans</i>	<i>P. limosa</i>
<i>P. distans</i>	99.4	328	2
<i>P. limosa</i>	93.9	13	201
Total	97.2	341	203
Characters (no):		2	
	correct (%)	<i>P. distans</i>	<i>P. limosa</i>
<i>P. distans</i>	99.1	327	3
<i>P. limosa</i>	91.2	19	197
Total	96.0	346	200

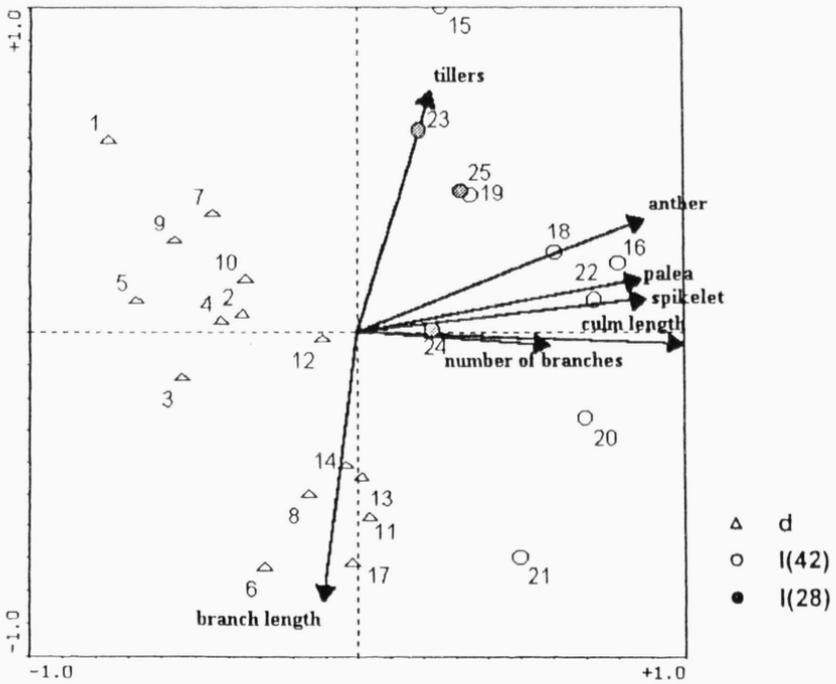


Fig. 2. – PCA ordination based on 7 characters and 25 populations of *Puccinellia*: d = *P. distans*, l(42) = *P. limosa* 2n = 42, and l(28) = *P. limosa* 2n = 28 (see Appendix 1 for details).

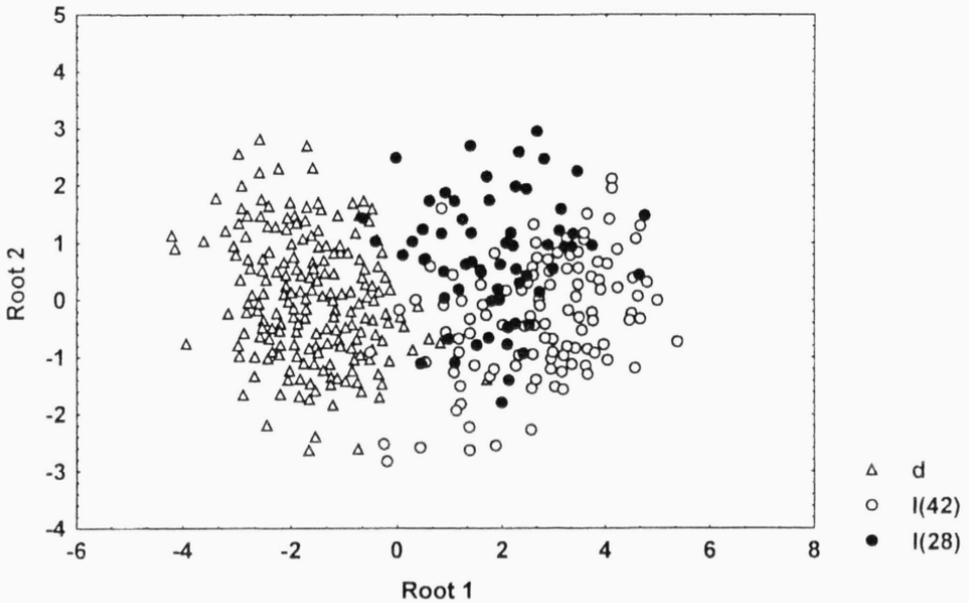


Fig. 3. – Discriminant analysis of 7 characters examined for all *Puccinellia* populations based on *P. distans* and both ploidy levels of *P. limosa*: d = *P. distans*, l(42) = *P. limosa* 2n = 42 and l(28) = *P. limosa* 2n = 28 (see Appendix 1 for details).

groups, represented by *P. distans* and both ploidy levels of *P. limosa*, always led to a considerable decrease in the number of correctly determined individuals of the respective two cytotypes of *P. limosa* (89.7% in $2n = 42$; 44.9% in $2n = 28$). Hence the discriminant function was only computed to distinguish between *P. distans* and *P. limosa*, without taking the two ploidy levels of the latter species into account.

Backward stepwise method of discriminant analysis revealed the most significant characters. If only 5 characters (i.e. maximum culm length, palea length, anther length, number of branches at the lowest panicle node, shortest branch of the lowest panicle node) are included, 97.2% of individuals were correctly determined (Table 3). Given that all populations of *Puccinellia* are cultivated under the same conditions, maximum culm length can also be employed as an important character (in the wild, it is affected by environmental factors). The remaining 2 characters (number of branches of the lowest panicle node and the shortest branch length of the lowest panicle node) were irrelevant for species determination because of their high between-population variation.

Palea length, panicle length, and anther length are the most important characters to distinguish *P. distans* from *P. limosa*. Spikelet length appears to be correlated with exterior palea length (see Fig. 2). The following discriminant function was therefore computed using palea length and anther length; it was able to classify correctly 96.0% of investigated individuals (Table 3):

$$H_d = 279.156 \text{ exterior palea length (mm)} + 35.782 \text{ anthers length (mm)} - 163.282$$

$$H_l = 302.15 \text{ exterior palea length (mm)} + 74.677 \text{ anther length (mm)} - 217.271$$

$$H_d > H_l - \textit{Puccinellia distans}$$

$$H_l > H_d - \textit{Puccinellia limosa}$$

Discussion

The present study showed that the length of spikelet, palea and anther are the most important morphological characters discriminating between *Puccinellia distans* and *P. limosa*. Critical values of spikelet length as determined by our study correspond better to the data of Conert (1998), whereas conclusions concerning the length of palea seem to correspond to both Conert (1998) and Adler (1994) (Table 4). Anther length was taken into consideration as discriminating character only by Adler (1994), who used it despite of a wide overlap between both species in this character. In our case, however, anther length is much better discriminating character because of it being considerably longer in *P. limosa*.

According to Adler (1994), the shortest branch of the lowest panicle node in *P. distans* is longer than in *P. limosa*. This was reflected by PCA analysis dividing *P. distans* populations in two groups. One group, with a short branch, is likely to represent autochthonous salt marsh populations, while the other, with longer branch, consists exclusively of plants from ruderal habitats which can be regarded as representing genetically allochthonous material (cf. Lembitz 1998). This hypothesis could explain some of the older records of *P. limosa* reported from south Moravian salt marshes; using the branch length as one of the key characters could have led to considering *P. distans* populations with a short branch as *P. limosa*. However, to reveal a detailed taxonomical relationship of the two groups of *P. distans*, cytogenetical studies behind the frame of the present work are needed.

Table 4. – Comparison of results with published ranges of values of the two measured characters in *P. distans* and *P. limosa*.

species	Flora Mitteleuropaea	Exkursions flora von Österreich	Our results		
	Conert 1998	Adler et al. 1994	n	mean (s.d.)	min. – max.
	Four floret spikelet length (mm)				
<i>P. distans</i>	3.5–4.5	3.6–3.9	326	4.0 (0.4)	3.0–4.9
<i>P. limosa</i>	4.0–5.5	(4.0) 4.2–5.0	218	4.8 (0.4)	3.7–5.7
	Exterior palea length (mm)				
<i>P. distans</i>	1.8–2.2	1.7–2.0	330	2.0 (0.2)	1.6–2.4
<i>P. limosa</i>	2.2–2.6	2.0–2.7	218	2.4 (0.2)	1.8–3.0
	Anther length (mm)				
<i>P. distans</i>	–	0.5–1.0	356	0.8 (0.1)	0.5–1.0
<i>P. limosa</i>	–	0.6–1.2	224	1.3 (0.2)	0.8–1.8

As concerns chromosome numbers, the most interesting result of our study is the record of two ploidy levels in *P. limosa*. Our finding of hexaploid populations ($2n = 42$) of *P. limosa* represents the first record of such ploidy level in this species. An attempt to correlate different ploidy levels with morphological differences within *P. limosa* yielded a wide overlap in morphological characters between both cytotypes (see Fig. 3). Hence a conclusion can be drawn that the morphological data used in the present study are suitable for determination of *P. distans* and *P. limosa* within the frame of contemporary taxonomic conception but individual cytotypes of *P. limosa*, despite of their possibly different taxonomic value, cannot be separated on morphological grounds.

Our results as well as data published so far (Tarnavski 1938, 1948, Pólya 1948, Conert 1998) indicate that tetraploids probably tend to occur in the eastern part of *P. limosa* distribution area while hexaploids are present in the western part of its distribution. There is one record of tetraploid population from NE Austria (Neusiedler See; Engelmaier 1982) but in this region, three species of *Puccinellia* occur and possible hybridization can be involved.

Populations of *Puccinellia* from Bohemia and Moravia and the ruderal population from Hungary correspond morphologically to *P. distans*, while those from south Slovakian and Hungarian salt marshes belong to *P. limosa*. This result is in agreement with distribution of *P. limosa* as given by Dostál (1982) and Conert (1998) but does not confirm the reported occurrence of *P. limosa* in south Moravia (Vicherek 1973, Danihelka & Grulich 1996, Čerňovský et al. 1999). Two explanations of the absence from the latter region can be given: (1) *P. limosa* never grew there and the reports on its occurrence from that area were due to incorrect determination of *P. distans*; (2) It reflects the recent process of disappearance of natural salt marshes. The study of old herbarium material, from south Moravian and south Slovakian salt marshes in particular, has shown that all available material from south Slovakian salt marshes (11 specimens from the localities Kamenný mlýn, Kamenné Ďarmoty and Kamenín) belonged to *P. limosa* and the specimens from non-saline localities of south Slovakia as well as the localities from salt marshes of south Moravia are *P. distans*. These results indicate that the former explanation is more probable.

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Souhrn

Tématem práce bylo srovnání morfologie a ploidie populací druhů *Puccinellia distans* a *P. limosa* z Čech, Moravy, Slovenska a Maďarska s cílem upřesnit rozšíření daných druhů a najít vhodné znaky k jejich rozlišení. K morfologickému srovnání byla použita morfometrická analýza (PCA a diskriminační analýza) 7 vybraných znaků. Zároveň byly pro všechny studované populace určeny počty chromozomů. Morfometrická analýza rozdělila studované populace do dvou skupin. Populace z Čech a Moravy odpovídaly *P. distans*, zatímco populace z jižního Slovenska a z Maďarska odpovídaly *P. limosa*. Z analýzy vyplynulo, že nejvhodnější rozlišovací znaky mezi oběma druhy jsou délka pluchy a délka prašníku. Tyto znaky zároveň umožnily stanovit jednoduchou diskriminační funkci k rozlišení obou druhů. Platnost získaných výsledků byla ověřena na dokladovém materiálu daných druhů ze studovaných lokalit, uloženém v herbáři Národního muzea.

V rámci studovaných populací byly podle počtu chromozomů stanoveny dvě ploidie. Všechny populace určené jako *P. distans* a populace určené jako *P. limosa* z Slovenska a západní části Maďarska byly hexaploidní ($2n = 42$), zatímco populace určené jako *P. limosa* z východní části Maďarska byly tetraploidní ($2n = 28$). Byly testovány případné morfologické rozdíly mezi oběma cytotypy *P. limosa*, ale ukázalo se, že je nelze na základě morfologických znaků spolehlivě rozlišit.

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Appendix 1. – Localities of seed collections.

Chromosome number $2n = 42$

Puccinellia distans.

Czech Republic (CZ), Moravia: **1.** Nový Přerov – north edge of the village, former salt meadow, at the wheel track on the path, 30 km SE of Znojmo, 180 m a.s.l. (48°48'N, 16°29'E). **2.** Moutnice – near the crossing of the roads to Brno, Velké Němčice and Blučina, entrance to the field, 18 km SE of Brno, 200 m a.s.l. (49°03'N, 16°44'E). **3.** Krumvíř – rubbish-heap on the north edge of the village, 31 km SE of Brno alt. 181 m (48°59'N, 16°55'E). **4.** Rakvice – margin of the former sand pit on the SE border of Rakvice village near the road to Podivín, 3 km NW of Podivín, 161 m a.s.l. (48°51'N, 16°49'E). **5.** Lanžhot – sand near the Kyjovka stream, between motorway, Kyjovka stream and railway, 7 km SE of Břeclav, 156 m a.s.l. (48°43'N, 16°59'E). **6.** Sedlec u Mikulova – former “old” salt marsh, wheel track on the path, 6 km SE of Mikulov, 175 m a.s.l. (48°46'N, 16°42'E). **7.** Želetice – road near the railway station, 12 km NE of Znojmo, 190 m a.s.l. (49°01'N, 17°00'E). **8.** Lanžhot – near the new custom house, building site, 10 km SE of Břeclav, 155 m a.s.l. (48°43'N, 17°00'E). **9.** Dobré pole – football pitch in the village, former salt marsh, 7 km NW of Mikulov (48°49'N, 16°32'E). Czech Republic (CZ), Bohemia: **10.** Sedlec u Mostu – in the village near the house no. 53, 2.5 km SE of Most, 220 m a.s.l. (50°29'N, 13°43'E). **11.** Zaječice – 1.6 km SW of the village, on the left side of the road near the turn to the former Židlochovice village, 5 km SE of Most, 235 m a.s.l. (50°28'N, 13°43'E). **12.** Volevčice – 0.4 km NW from the centre of the village, near the rail way crossing, wheel track on the path along rail way, 6.5 km S of Most, 213 m a.s.l. (50°26'N, 14°41'E). **13.** Bečov – 1.3 km NW of the village, path to the former Židlochovice village, near NW of foot of the Chlum hill, 5.5 km SE of Most alt. 235 m a.s.l. (50°27'N, 13°43'E). **14.** Hrnčiče – main crossing of the village (to Práhonice and Šeberov), sand silt near the road, 8 km SE of Prague, alt. 300 m a.s.l. (50°00'N, 14°31'E).

Puccinellia limosa

Slovak Republic (SK): **15.** Kamenín – salt marsh between the Kamenný mlýn and Kamenín villages, between road and rail way, 10 km NW of Štúrovo, alt. 115 m (47°52'N, 18°38'E). Hungary (H): **16.** Fehér – exposed bottom at the N border of the Kelemenszék lake, near the road from Dunaföldvár to Kécskemét about 45 km WSW of Kécskemét town (46°50'N, 19°12'E). **17.** This species was determined as *Puccinellia distans*. **18.** Kistelek – sand pit close to the railway station near Kistelek village by the road from Budapest to Szeged towns, about 30 km NW of Szeged (46°28'N, 19°58'E). **19.** Büdösszék – exposed bottom of the summer-drained salt lake Büdösszék, about 10 km NE of Kistelek village (46°31'N, 19°59'E). **20.** Kelemen – exposed bottom at the W border of the summer-drained salt lake Kelemenszék in the Kiskunsági National Park, about 7 km SW of Fülöpszálás village, 45 km WSW of Kécskemét town (46°50'N, 19°50'E). **21.** Bösztör – channel margin near the road bridge about 1 km N of the railway station Bösztör, 45 km W of the Kécskemét town (46°59'N, 19°12'E). **22.** Kis-Rét – exposed bottom of the Kis-Rét lake about 2 km SW of the railway station Szabadszállás, 45 km WSW of Kécskemét town (46°53'N, 19°12'E).

Chromosome number $2n = 28$

H u n g a r y (H): **23.** Kócsújfalu – field depression in the pasture N of the road from Tiszafüred to Debrecen, near the settlement Kócsújfalu, about 15 km ESE of Tiszafüred town (47°34'N, 21°00'E). **24.** Nyugati – field depression near the patch along the channel Nyugati – Fücsatoma, near the road from Tiszafüred town to Debrecen, near the turn to the Telekháza village. (47°38'N, 21°04'E). **25.** Tiszavasvári – exposed bottom of the lake N of the road from Tiszavasvári to Nyíregyháza, about 5 km E of Tiszavasvári town, alt.(47°58'N, 21°29'E).