Phytotaxa 550 (2): 159–170
https://www.mapress.com/pt/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

https://doi.org/10.11646/phytotaxa.550.2.5

Carex ×*favratii* (Cyperaceae), new record for Romania and evidence of its hybrid origin

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Abstract

The article presents a find of *Carex* ×*favratii* (*Carex echinata* × *C. paniculata*) in Romania, whose genetic relationships with its putative parents was tested using AFLP and microsatellite data. The putative hybrid was genetically intermediate between the parental taxa, i.e. *C. echinata* and *C. paniculata*. A morphological analysis shows ten intermediary characters, six ones closer to *C. echinata*, five closer to *C. paniculata*, and three unique, whereas the perigynium and achene characters could not be evaluated due to hybrid sterility (with exception of the type of perigynium). The morphological features supported by molecular markers strongly support the hybrid nature of the collected plants. The results provide evidence that a hybrid between these species really occurs in nature.

Keywords: *Carex*, hybridisation, morphological features, AFLP, microsatellite markers

Introduction

The extensive genus *Carex* L. (1753: 972) with ca 2000 species and worldwide distribution (Roalson *et al.* 2021) is a plant group which may be suitable for the study of hybridisation. Hybridisation is an important phenomenon, which occurs more frequently within some sections than between sections of *Carex* (Wronska-Pilarek *et al*. 2010) and complicates its already difficult taxonomic classification. Many interspecific hybrids have been described worldwide (Cayouette & Catling 1992 for North America). For example, Koopman (2011) mentioned 300 *Carex* hybrids in Europe. However, its taxonomic identity must be critically assessed in the future. Most hybridisation events in *Carex* belong to just a few sections: *Ceratocystis* Dumort. (1827: 147), *Phacocystis* Dumort. (1827: 146) and *Vesicariae* Meinsh. (1901: 366) in subgenus *Carex*, *Canescentes* Fr. (1845: 72), *Heleoglochin* Dumort. (1827: 146) and *Vulpinae* (Carey 1848: 541) H. Christ (1885: 18) in subgenus *Vignea* (P. Beauv. ex T. Lestib.) Peterm. (Cayouette & Catling 1992, Wieclaw & Wilhelm 2014, Roalson *et al.* 2021). Chromosomal changes in *Carex* can become stabilised through backcrossing or selfing, which could result in the establishment of a new, fully fertile cytotype or chromosomal race, followed by the formation of reproductive barriers, resulting in intraspecific variability and *Carex* speciation (Nordenskiöld 1963, Whitkus 1988, Hipp *et al*. 2009, Escudero *et al*. 2010).

Many cases of interspecific hybridisation in the genus *Carex* have been detected by means of molecular markers (Ford *et al*. 1993, Smith & Waterway 2008, Volkova *et al*. 2008, Korpelainen *et al*. 2010). On the other hand, since many publications on *Carex* hybridisation only rely on morphological markers (Catling 1993, Blackstock & Ashton 2010, Wieclaw & Koopman 2013, Bergeron & Pellerin 2014, Wieclaw & Wilhelm 2014), the rate of hybridisation could be overestimated. This fact was described by Řepka *et al*. (2014) for the putative hybrid between *C. flacca* Schreber (1771: 178) and *C. tomentosa* L. (1767: 123), and by Escudero *et al*. (2014) on the example of section *Ovales* (Kunth) Christ.

In 2009, we found a plant morphologically suspected of hybridisation between species belonging to different sections of subgenus *Vignea* and growing together with their potential parent species [*Carex echinata* Murray (1773: 76), sect. *Stellulatae* (Kunth 1837: 399) Christ (1885: 17) and *C. paniculata* L. (1755: 32), sect. *Heleoglochin*]. The plant was conspicuous by its morphological intermediacy and the exceptional structure of its inflorescence, which is

Accepted by Pedro Jimenez-Mejias: 6 Jun. 2022; published: 14 Jun. 2022

not formed in any of the potential parent species. The habitat was a small spring dominated by *Scirpus sylvaticus* L. (1753: 51) in the valley of the river Jium de Vest, near the town of Uricani in SW Romania. The first and last indication of the existence of this hybrid was directly found in the protologue of *Carex ×favratii* Christ (1889: 173), under the species combination "*C. grypos* × *paniculata*" (Christ 1889). The author of the protologue specifies the find of the hybrid in the upper part of the alpine valley by the village of Ulrichen, Valais Canton, Switzerland, at an altitude of 1800 m (coll. L. Favrat). The diagnosis of the hybrid speaks of intermediate morphological traits between the parents, with the rhizome closer to *C. paniculata*, the stem as short and slender as in *C. echinata*, leaf morphology and width being intermediate, inflorescence being a reduced panicle with the sex distribution of flowers in the pseudospikelets (i.e. false spikelet in *Carex,* see Vegetti 2002, Jiménes-Mejías *et al.* 2016) as in *C. paniculata*, scale colour closer to *C. paniculata*, but scale length in relation to utricle length as in *C. echinata*; mature utricles being intermediate between species with beak teeth similar to *C. echinata* (Christ 1889). This hybrid had not yet been found in the overlap of the range of the two species elsewhere than in Switzerland and had been identified according to morphological traits.

The aim of our research was to study an individual showing intermediate morphological traits between the possible hybrid and its putative parents using morphological markers, AFLP and microsatellite data, and to demonstrate that this hybrid really does arise in nature.

Material and methods

Plant material and DNA extraction

For the analysis, plant material from putative parent species *C. echinata* and *C. paniculata* was used (four populations from each species, one sample from one individual) and their putative hybrid (1 population/one sample). Detailed information on the origin of the samples is given in Table 1.

TABLE 1. List of studied taxa and their origin.

Taxon	Locality	Geographical cooordinates
Carex echinata	Hohentauern, A	47°26'22.980"N, 14°25'14.100"E
	Hutě pod Třemšínem, CZ	49°34'40.532"N, 13°48'0.660"E
	Bansko, BG	41°45'13.667"N, 23°24'52.420"E
	Campu lui Neag, Uricani, RO	45°16'42.540"N, 22°55'13.289"E
Carex paniculata	Prein an der Rax, A	47°40'41.160"N, 15°42'50.700"E
	Lendak, SK	49°12'52.012"N, 20°22'36.181"E
	Vidnava, CZ	50°22'54.185"N, 17°12'2.148"E
	Stankovany, SK	49°9'18.291"N, 19°9'0.939"E
Carex ×favratii	Campu lui Neag, Uricani, RO	45°16'43.695"N, 22°55'19.932"E

Total genomic DNA was isolated from young leaf tissue using the DNeasy Plant Mini Kit (Qiagen, Netherlands, Venlo).

Morphological characters

One sterile plant suspected of hybridisation was found at a locality near the town of Uricani, SW Romania (see Table 1) and documented by a herbarium specimen (Jun 19, 2009, coll. R. Řepka, herbarium BRNL). Morphological and genetic traits of only one plant could be studied and compared with its parents. A set of morphological characters of the presumed parents was obtained from material from 25 localities for *C. echinata* and 25 localities for *C. paniculata* in Europe deposited in the BRNM herbarium (abbrev. by Holmgren *et al.* 1998; see Appendix 1). In the hybrid plant and the presumed parents, 12 qualitative morphological characters (root surface, root colour, basal leaf sheaths, cross-sectional shape of upper part of stem, roughness of stem, leaf top, type of inflorescence, shape of spikelet, gender arrangement within pseudospikelet, shape and colour of pistillate scale, shape of perigynium) and 12 quantitative characters (root diameter, stem length, diameter of lower and upper part of stem, length of lower stem leaves, length and width of leaves of sterile shoots, height of stem leaf ligule, inflorescence length, ratio of length of lower inflorescence bract to inflorescence length, number of pseudospikelets, pseudospikelet size, see Table 2) were measured. Characters on the perigynium and achene could not be compared with the presumed parent species because they *appear* only

rarely, drying immature and do not develop further in the hybrid. All the features were observed or studied using a ruler and a binocular microscope.

TABLE 2. Comparison of morphological characters of *Carex ×favratii* and its parents (**bold**: qualitative characters). Legend of evaluation: inter—intermediate character, echin—character close to *C. echinata*, panic—character close to *C. paniculata*.

Character	*Carex echinata*	*Carex ×favratii*	*Carex paniculata*	Evaluation
root diameter (mm)	(0.3–)0.4–0.8(–1.1)	0.6–1.3	1.6–3.0	inter
root surface	glabrous	glabrous	with whitish or grey hairs (glabrous when old)	echin
root colour	light straw yellow when young, light brown or grey-brown when older	light brown	light grey with a touch of pink	echin
basal leaf sheaths	scale-like, whitish or grey-brown, rarely brown, glossy or matte, not disintegrating	scale-like, stiff, dark brown, non-disintegrating or more often very slightly disintegrating into narrow strips	scale-like, stiff, dark brown to black-brown, not disintegrating or then very slightly disintegrating into narrow strips	panic
stem length (cm)	(9–)13–42(–62)	37–45	(30–)60–140(–165)	inter
diameter of lower part of stem (mm)	1.0–2.8	4.0–4.8	4.5–8.0	panic
diameter of upper part of stem (mm)	0.5–0,9	0.7–1.2	1.5–2.5	inter
cross-sectional shape of upper part of stem	bluntly triangular to round	triangular	sharp triangular	inter
roughness of upper part of stem	smooth or somewhat rough	smooth, rough on the edges	roughly spiny on the edges	inter
length of lower stem leaves (cm)	6–15	2.6–4.0	5–15	unique
leaf top	elongated tip	suddenly tapered tip	suddenly tapered tip	panic
length of leaves of sterile shoots (cm)	10–68	26–35	60–90(–100)	echin
width of leaves of sterile shoots	(1.4–)1.8–3.9(–4.0)	1.4–3.9	(2.5–)3.0–6.5(–7.5)	echin
height of stem leaf ligule (mm)	1.0–2.4	1.8–2.5	1–3	inter
inflorescence length (mm)	(10–)14–30(–35)	19–29	(30–)43–120(–155)	echin
lower bract of inflorescence	missing to rare (but then exceeding inflorescence)	missing to rare (but then exceeding lower spikelet)	absent or rare (but then shortly bristly not exceeding inflorescence)	inter
number of pseudospikelets	(2–)3–6(–7)	21–46	(25–)40–105(–180)	inter
type of inflorescence	spike, unbranched	panicula-like, constricted and clustered	branched, paniculate, rarely a spike	unique
shape of pseudospikelet	spherical or ovoid	elongated on the base surrounded by shorter ovoid spikelets	ovoid, spherical at maturity	unique
size of pseudospikelet (mm)	5–10 x 4–8	3.5–6.2 x 1.5–2.3	4–5 x 4–5	inter
arrangement of gender in pseudospikelet	gynandrous	androgynous or sterile	androgynous	panic
shape of pistillate scale	ovoid, pointed	ovoid, pointed	ovoid, blunt or pointed, distinctly keeled	panic
colour of pistillate scale	light brown, with a narrow green central stripe	light brown, with a wide chamfered edge and a light central stripe	brown or dark brown, broadly or narrowly lined in the upper half, with a light central stripe	inter
shape of perigynium	ovate-lanceolate, plano-convex with 1 mm beak narowly winged	sterile, but their shape corresponding with *C. echinata*	ovoid to inversely cordate, obtusely trigonous, beak 1,0-1,5 mm broadly winged	echin

Molecular markers

AFLP protocol

The AFLP reactions were performed according to Vos *et al.* (1995). Restriction and ligation were performed simultaneously: 0.5 µg genomic DNA was incubated for 3 h at 37 °C in 20-µl reaction volume with 2.5 *Eco*RI (New England Biolabs, USA, Massachusetts, Ipswich), 2.5 U *Mse*I (New England Biolabs), 15 µg of BSA, 50 pmol *Mse*I adapter (Vos *et al*. 1995), 5 pmol *Eco*RI adapter (Vos *et al*. 1995), 80 NEB U of T4 DNA ligase (New England Biolabs), 1x CutSmart™ Buffer (1x NEB2 Buffer for *Eco*RI), and 1 mM ATP. The reaction mixture was then diluted 20-fold in $TE_{0.1}$ buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

The preamplification reaction mixture was composed of 0.5 U *Taq* DNA polymerase (Qiagen), 2.5 pmol of each primer (*Eco*RI+A; *Mse*I+C), 1x PCR Buffer (Qiagen), 0.2 mM dNTPs and 4 µl of the diluted mixture from the first reaction in a 20-µl reaction volume. The reaction profile was as follows: 2 min at 72 °C followed by 20 cycles of 20 s at 94 °C, 30 s at 56 °C, 2 min at 72 °C, and finished with one 30-min cycle at 60 °C. The preamplification reaction was diluted 10-fold in $TE_{0.1}$ buffer.

The selective amplification was performed using three different *Mse*I primers with three selective nucleotides (*Mse*I+CGG/CCA/CCC). The second primer (*Eco*RI+ACG) used in the selective amplification was fluorescently labelled at the 5'-end (6-FAM, NED, PET). The reaction mixture had a total volume of 20 µl and contained 0.5 U *Taq* DNA polymerase (Qiagen), 0.2 mM dNTPs, 2 pmol *Asc*I/*Eco*RI/*Sbf*I primer, 5 pmol *Mse*I primer, 4 µl of the diluted mixture from the second reaction, and 1x PCR buffer (Qiagen). The reaction profile was as follows: 2 min at 94 °C, followed by 10 cycles of 20 s at 94 °C, 30 s at 66 °C, 2 min at 72 °C, with the annealing temperature decreased by 1 °C (Stringent condition 1 by 0.7 °C) in each cycle, followed by 20 cycles of 20 s at 94 °C, 30 s at 56 °C (Stringent condition 1 at 59 °C), 2 min at 72 °C; and finally 30 min at 60 °C.

SSR (microsatellites) protocol

For microsatellite analysis, seven microsatellite loci were used: Cko1-9, Cko1-11, Cko1-47, Cko2-112, Cko2-139 according to the original paper by Ohsako & Yamane (2007) and CM01, CM35 after King & Roalson (2009) with forward primers fluorescently labelled (6-FAM, NED, PET, VIC). PCR reaction was performed in a 20-µl reaction mixture, containing 0.5 U MYTaq DNA Polymerase (Bioline, USA, Taunton), 1x reaction buffer Bioline), 500 nM of each primer and ~15 ng of DNA filled in with dH_2O to 20 µl; amplification conditions: 94 °C for 5 min, 30 cycles of 94 °C for 15 s, 52 °C for15 s, 72 °C for 40 s and final extension of 72 °C for 7 min for the microsatellite loci of Ohsako & Yamane (2007). For the microsatellite loci of King & Roalson (2009), reaction and amplification conditions were as follows: a 20-µl reaction mixture contained 1 U of MYTaq DNA Polymerase (Bioline), 1x reaction buffer (Bioline), 500 nM of each primer and ~15 ng of DNA filled in with dH_2O to 20 µl; amplification conditions: 95 °C for 3 min, 30 cycles of 95 °C for 30 s, 51 °C for 30 s, 72 °C for 15 s and final extension of 72 °C for 30 min.

Data analysis

AFLP and microsatellite fragments were separated electrophoretically on an ABI PRISM 3730XL automated sequencer (Applied Biosystems, USA, California, Foster City) using the GeneScan-500 LIZ internal standard (Applied Biosystems). GeneMapper V4.1 (Applied Biosystems) was used to characterise the fragments.

The genetic diversity between taxon pairs was calculated as Nei Genetic distance and Analysis of molecular variance (AMOVA) calculating PHI_{pt} based on 999 permutations which were performed using GenAlEx 6.5 (Peakall & Smouse 2012) for both data sets.

To present the genetic differentiation of combinations of parental taxa and putative hybrids graphically, principal coordinate analysis (PCoA) was used also employing GenAlEx 6.5. The analysis was made separately for AFLP and microsatellite datasets, and individuals were ordered according to the first two axes.

Results

Morphological characters

The presumed hybrid grew right next to both parent species and was apparently an intermediate morphotype. Morphological analysis showed that ten characters were intermediate, six closer to *C. echinata,* five closer to *C. paniculata*, while three were unique. We found only one unique quantitative trait (e.g. not present in either parent

species): length of lower stem leaves. However, two interesting unique features are qualitative (type of inflorescence and shape of spikelets). The inflorescence is arranged in an exceptional way: it is shortened and has elongated spikelets in the lower part, which are surrounded by shorter ovoid spikelets at their base. This is reminiscent of the reduced and strongly crowded inflorescence of *C. paniculata* with reduced branches (only 1–2 lower clusters of spikelets are located on the short branch) which are composed of elongated sterile spikelets. The spikelets have a different size and shape than those in both parents due its sterility. Another unique feature of the hybrid is the presence of small sterile spikelets inserted on the base of bisexual ones which are typical in the subgenus *Vignea*. The colour and shape of all scales are similar to *C. paniculata*, but they are light brown with a wide whitish membranous margin, thus resembling *C. echinata*. Surprisingly, although the inflorescence is more similar to *C. paniculata*, the morphotype of immature perigynia is identical to that in *C. echinata*. The perigynium in the spikelets of the hybrid is developed very rarely (5–12 per one inflorescence, their size being 2.8–3.1 × 0.5–0.6 mm), drying immature and does not develop further. Achenes are completely missing and the hybrid is completely sterile (Table 2).

Molecular markers

We examined combinations of parental species and the putative hybrid in this study using AFLP and microsatellite markers. In *Carex ×favratii* we detected 163 AFLP loci, 139 polymorphic and 6 specific to the putative hybrid. The putative hybrid sample shared 25 loci with *C. paniculata* and 42 with *C. echinata.* In microsatellite data we analysed five loci, where one to six alleles were detected (Table 3). There was no specific allele to the putative hybrid and the genotype pattern had one allele from each parent.

The results of the Analysis of Molecular Variance (AMOVA) for the studied parental taxa and their putative hybrid are listed in Table 4. The percentages of molecular variance show the contribution of within- and between-population variability of the parental species and their putative hybrid for microsatellite and AFLP datasets. The combined overall genetic diversity was higher between populations of parental species and their putative hybrid than within them. On the other hand, the contribution of within-population variability of the parental species and their putative hybrid was also appreciable.

TABLE 3. Allelic composition of studied *Carex* taxa and their putative hybrid. The bold font indicates DNA fragments which are unique to each parental taxon and are shared with the hybrid.

Taxon	SSR locus				
	CM01	Cko1-9	Cko2-139	Cko2-112	CM35
C. echinata	231, **233**	168, 189, 215, 219, **221**	210, 231, 258, 261, **263**	**231**	**192**
C. paniculata	**217**, 222, 224	**213**	**255**	**206**, 227	**192**
C. ×favratii	**217, 233**	**213, 221**	**255, 263**	**206, 231**	**192**

TABLE 4. Molecular variance in AFLP and SSR within and among populations of the putative hybrid and its parental species.

Taxon	Molecular variance (%)			
	within populations		among populations	
	AFLP	SSR	AFLP	SSR
C. echinata, C. paniculata, C. ×favratii	19	49	81	51

TABLE 5. Nei's Genetic Distances for taxon pairs.

Taxon pair		Nei's Genetic Distances	
		AFLP	SSR
C. echinata	*C. paniculata*	0.754	1.268
C. echinata	*C. ×favratii*	0.372	0.416
C. ×favratii	*C. paniculata*	0.563	0.293

Nei's Genetic Distances between taxon pairs rendered a considerable range of values (Table 5). They were significantly higher for microsatellite data than for AFLP data when comparing parental taxa *C. echinata* and *C.*

paniculata. We see contradictory results between AFLP and microsatellites: the hybrid is placed closer to one or the other parental taxon, but Nei's Genetic Distances are always larger when comparing *C. echinata* with *C. paniculata* than in combinations where each of the parental taxa is compared to a hybrid.

The graphical pattern of genetic relationships between all individuals of the four combinations is represented in PCoA plots separately for AFLP and microsatellite data (Fig. 1). The plots depict the position of these individuals in relation to each other based on molecular data characterising their genotypes. The first axis, showing the greatest variability (28.4%), divides the AFLP data of *C. echinata* and the putative hybrid into a common space, while the data of *C. paniculata* are clearly separated from that of the remaining taxa. However, the second axis with residual variability (9.0%) separates the data of *C. echinata* and the putative hybrid. The PCoA analysis thus demonstrates that the AFLP data indicate a closer affinity of the putative hybrid to one of the parental species, i.e. *C. echinata*. The multivariate space with microsatellite data clearly shows a separation of the data of all three taxa according to the first axis (36.1% of variability), whereas the hybrid individual occupies an intermediate position between two separated clusters of the parental species. The second axis (29.6% of variability) only divided *C. echinata* samples into 2 groups according to different genotypes (see Fig. 1).

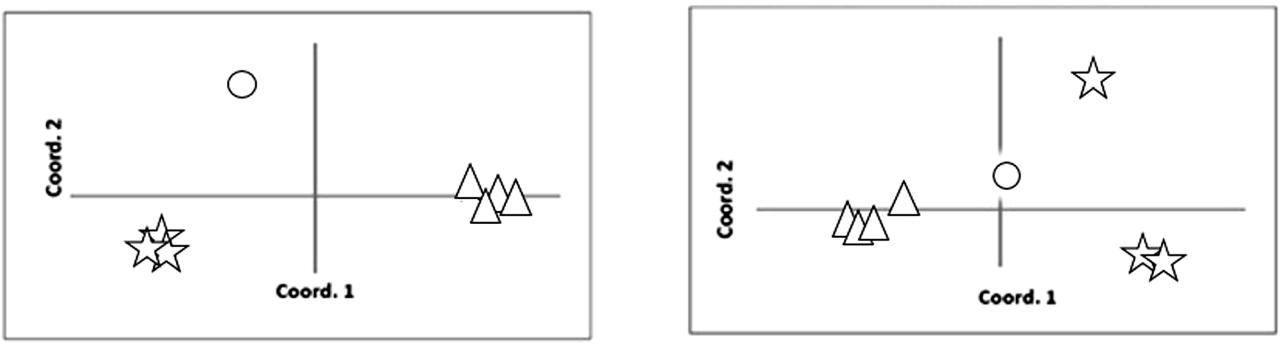

FIGURE 1. PCoA plots depicting genotype differentiation between putative hybrid and parental species samples. Left: AFLP data, right: SSR (microsatellite) data. Legend: *Carex echinata* (asterisk), *C.* ×*favratii* (circle), *C. paniculata* (triangle).

Discussion

In this study, we investigated the causes of morphological intermediary of putative hybrid *Carex* ×*favratii* and its parent species (*C. echinata* and *C. paniculata*), in which the hybrid status was confirmed by both AFLP and microsatellites markers. The utility of the AFLP method for detection of *Carex* hybrids and introgression has been demonstrated by several studies (Hipp & Rothrock 2007, Nakamatte & Lye 2007, Ford *et al.* 2012, Escudero *et al.* 2014), however, these authors did not address an intersectional hybrid as in our study. In addition, also Volkova *et al.* (2008) and Smith & Waterway (2008) used AFLP to confirm hybridisation and introgression of *Carex* taxa, in *C. salina* Wahlenb. (1803: 165) and taxa of *C. complanata* agg., respectively. However, in our study, despite the predominant number of AFLP loci, the results of microsatellites for molecular detection of the hybrid appear to be more conclusive (see Fig. 1 and Table 5). The importance of combining morphological features with microsatellites and ISSR markers to determine a hybrid status was also mentioned by Korpelainen *et al*. (2010), who dealt with hybridisation between *C. aquatilis* Wahlenb. (1803: 165) and *C. paleacea* Schreb. ex Wahlenb. (1803: 164), confirming these species to be parents of the hybridogenous species *C. recta* Boott (1839: 220).

In the case of *Carex* ×*favratii*, its intermediate morphology, which is not influenced by the environment or the variability of morphological features of one or the other parent, correlates with the molecular markers. Although both parent species are members of the same subgenus, they belong to different sections (Henrichs *et al.* 2004). Their hybrid is probably so rare in nature that it is not documented in herbaria and there is only one mention of its existence in the literature, and that exclusively in the protologue (Christ 1889). In *Carex*, it cannot be assumed that higher species relatedness has an effect on hybridisation, as hybrids simply do not develop in several sections, even though some species have sympatric areas and co-occur in their habitats. In this study, the parent species of *C.* ×*favratii* have sympatric distribution ranges, but they rarely meet in joint habitats. *Carex echinata* grows most frequently in acidic moss-rich fens, transitional mires and wet meadows, preferring acidic, oligotrophic or mesotrophic soils, as well as organic soils with an acidic soil reaction. In contrast, *C. paniculata* grows mainly in fen meadows and tall

sedge communities. It is often found around meadow springs, stream banks, rarely in willow and alder carrs. *Carex paniculata* usually grows on gley soils rich in nutrients and with a neutral to slightly alkaline pH, which are often formed on carbonate bedrock (Grulich & Řepka in Kaplan *et al*. 2016, 2017).

FIGURE 2. Habitus of the herbarium specimen of *Carex* ×*favratii* (deposited in BRNL herbarium).

Unique characters may indicate the hybrid origin of a plant in the field and in culture (Rieseberg 1995). The special arrangement of the inflorescence in *Carex ×favratii* was remarkable and we consider it a unique character: it is more reminiscent of *C. paniculata*, but the branches of the panicle are reduced and the elongated pseudospikelets with light brown scales and wide whitish membranous margins are very crowded, especially in the lower part of the inflorescence. Christ (1889: 166) describes a similar feature: „Spica atrato-brunnea late pyramidata 4 centim. longa paniculata, ramis abbreviatis…" [Inflorescence a dark brown panicle, broadly pyramidal, 4 cm long, with short branches…]. Significant unique characters which we found in *C. ×favratii* include the presence of sterile pseudospikelets on the base of predominantly bisexual ones, length of lower stem leaves (shorter than indicated in the protologue, see Table 2). However, although the pseudospikelets are sterile and the perigynia are formed very rarely and remain undeveloped and dry, the final shape of the pseudospikelets has thus become unique. This was measured and its exceptional shape is qualitatively expressed in Table 2. On the contrary, Christ (1889) mentioned characters on the perigynium, which leads us to the idea that the described plant of *Carex ×favratii* was not sterile.

The morphological similarity of closely related species could affect the confirmation of putative hybrids, since intraspecific variability may be explained incorrectly as the result of a hybridisation event (Jiménez-Mejías *et al*. 2011). A similar observation was also demonstrated by Řepka *et al*. (2014), who investigated four samples of a putative hybrid between *C. flacca* and *C. tomentosa*. They gave the impression to be morphologically intermediate, but based on molecular data samples (ITS, AFLP, *trnL*-F) they were found to be inseparable from *C. flacca*. Such findings may indicate greater intraspecific morphological variability than has been observed to date. The observed variances do not have to be consequences of interspecific hybridisation but may also result from genotypic differentiation or phenotypic plasticity (Sultan 1993). As detected in many studies, some species are able to produces various functionally appropriate phenotypes in different environments (Stenström *et al*. 2001, 2002, Košnar *et al*. 2012, Bugg *et al*. 2013, Abudureheman *et al*. 2014). In sedges, phenotype plasticity can arise due to changes or fluctuations in environment (Heathcote *et al.* 1987, Abudureheman *et al*. 2014) or geographic isolation of populations (Urbanek 1998, Stenström *et al.* 2001, 2002). A further case is the presence of another taxon, as found by Yu *et al.* (2006) on the example of *Carex sempervirens* Vill. (1787: 214) tufts overgrown with other similar species to form a genetically variable cluster.

At the site close to the town of Uricani we found a single tussock of a sterile hybrid in vegetative state not reproducing generatively. The existence of other hybrid plants is likely, but they probable originate very rarely. Our morphological observations, as well as the overall results of molecular analysis in this study support the hypothesis that *Carex echinata* and *C. paniculata* actually hybridise in nature.

Conclusions

The paper informs about the rediscovery of the hybrid *Carex ×favratii* in nature after 120 years, which according to the results of molecular markers is indeed the product of hybridisation between *C. echinata* and *C. paniculata*. It is one of the few *Carex* hybrids described so far, in which hybrid origin has been demonstrated by molecular markers. At the same time, it is one of those which probably originates in nature only rarely due to different ecological requirements of the parents. Evidence for other hybrids so far described solely by morphology may be severely limited in the future by their rarity or due to them being overlooking in nature. The lifespan of tuft morphotypes, which are limited to a few individuals, can be ephemeral due to sterility.

Acknowledgements

We thank Petra Veselá for technical assistance in the laboratory and we are very grateful to Jan W. Jongepier for language improvement.

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APPENDIX 1. List of studied specimens of *Carex echinata* and *C. paniculata* for morphological comparison.

Carex echinata

AUSTRIA:—Ad silvarum margines prope Seitenstetten, s. a., *Strasser* (BRNM). –
Tirol, um Seefeld bei Scharnitz, 1913, *F. Teuber* (BRNM).

BOHEMIA:—Police nad Metují, in vallis Kovářova rokle, 1932, *K. Krčan* (BRNM).—Holetín, Torfwiesen bei oppidum Hlinsko, 1869, *J. Freyn* (BRNM).—distr. Hradec Králové, Kuks, Betlém, 1983, *E. Uhlířová* (BRNM)

FINLAND:—Loppi, S of Lake Sorsamo, 1989, *P. Alanko* (BRNM).

HUNGARY:—In palude exsiccato Nagy-Lásló montis Jolymos prope opp. Gyöngyör, 1874, *V. Borbás* (BRNM).—Com. Abaúj-Torna, in pratis turfosis paludosis vallis Monokpatak prope Kassa, 1909, *L. Thaisz* (BRNM).

MACEDONIA:—Mavrovi Anovi, spring slopes above the lake, 1976, *L. Pokluda* (BRNM).

MORAVIA:—Žďár nad Sázavou, um Grossen Saaren Teich, 1911, *F. Teuber* (BRNM).—Leipnik in Mähren: Sumpfwiesen am Bohuslawek, 1913, *F. Petrak* (BRNM).—Bojkovice- Bzová: bog part of the slope meadows to the village of Starý Hrozenkov, 1924, *S. Staněk* (BRNM).—Gundersdorf [village of Guntramovice, distr. Opava], 1907, *F. Böhm* (BRNM).—Dubňany, low sedge meadows near the village, 1946, *S. Staněk* (BRNM).—Vracov-Osmek, peat clearing in the Dúbrava forest, 1946, *S. Staněk* (BRNM).—Beskydy Mts, in sphagnetis piceetosis ad vicum Bílý kříž, 1946, *H. Zavřel* (BRNM).—Hrubý Jeseník Mts., in sphagnetis Moosbruch ad Reihwiesen, 1898, *F. Teuber* (BRNM).

POLAND:—Flora Vratislaviensis: in schattigen Sphagnetis des Einganges der Lissaer Wald bei der Schaferei, 1882, *Uechtritz* (BRNM).

ROMANIA:—Thordaer Alpen: Hochsümpfe am Muntje le Mare, 1871, *J. Freyn* (BRNM).

SLOVAKIA:—Slanské vrchy hills, Zlatá Baňa, meadow on the hill (summit 870 m), 1985, *K. Sutorý* (BRNM).—Nízké Tatry Mts, Prostredná dolina valley, peat meadows near the village of Kyslá, 1973, *J. Dvořák* (BRNM).—Nízké Tatry Mts, Liptovský Ján, spring in the Bystrá valley, 1958, *L. Pokluda* (BRNM).

SUISSE:—Canton Schwyz, „Hessenmoos“ inter Einsiedeln and Bennau, 1981, *A. Charpin* & *P. Geissler* (BRNM).

UKRAINE:—Stovna, 1930, *J. Buček* (BRNM).—Tuří Polana, Šrpata vallis, in sphagneto, 1932, *J. Buček* (BRNM).

Carex paniculata

AUSTRIA:—Tümpel bei Gutenstein, 1920, *F. Teuber* (BRNM).—Tirol, zwischen Huben und Kals bei Lienz, 1901, *F. Teuber* (BRNM).—Nieder-Oesterreich, Wien, Sumpfwiesen bei Moosbrunn,1867, *J. Freyn* (BRNM).

BOSNIEN: Travnik, Sumpfstelle, 1890, *Brandis* (BRNM).

BOHEMIA:—Chotěboř, Podmoklany, peat meadows on the N slope of the Čerhovy hill, 1990, *R. Řepka* (BRNM).—Jestřebí, wet peat meadow near the Konvalinkový vrch hill, 1991, *R. Řepka* (BRNM).—Osečná, banks of a small pond in front of Lázně Kundratice, 1991, *R. Řepka* (BRNM).—Trhová Kamenice, Zubří, peat meadow in valley SE of the village, 1985, *R. Řepka* (BRNM).—Svitavy, pond shore at the NW margin of the town, 1998, *P. Lustyk* (BRNM).—Šumava Mts., village of Přední Zvonková, Kyselovský les nature reserve, 1996, *P. Lustyk* (BRNM).

FRANCE:—Pyrenees orientales: Ax-les-Thermes, in pratis subalpinis humidis ad „Col du Pradel“, 1993, *F. Černoch* (BRNM).

ITALY:—Regione Del Veneto: Dolomiti: village of La Valle Agordina, Passo Duran, 2014, *P. Batoušek* (BRNM).

MORAVIA:—Olomouc, meadows near the village of Grygov, 1913, *J. Otruba* (BRNM).—Střebětice near Hulín, 1910, *F. Gogela* (BRNM).—Korytná, marsh by the Korytnice stream below Kadlečková forest, 1924, *S. Staněk* (BRNM).—Bučovice: bog meadows in the Svatá valley, 1946, *J. Šmarda* (BRNM).—Vyškov: locis humidis ad Opatovice, 1949, *V. Skřivánek* (BRNM).—Popůvky near Brno, peat meadow in the valley of stream 1 km W of the village, 1984, *R. Řepka* (BRNM).—Opava: meadows near the village of Štáblovice, 1951, *J. Šmarda* (BRNM).

ROMANIA:—Muntii Rodnei, jugum montis prope saltum Pasul Prislop, 1986, *K. Sutorý* (BRNM).

SLOVAKIA:—Jablonica: meadows near the village of Cerové-Lieskové, 1950, *M. Součková* (BRNM).—Kralovany: travertine spring near the village of Stankovany, 1951, *J. Šmarda* (BRNM).—Velká Fatra Mts, Stredná Revúca: in stream valley W of the village, 1953, *L. Pokluda* (BRNM).—Strážovská hornatina Mts, Velké Košecké Podhradie, spring, 1985, *K. Sutorý* (BRNM).—distr. Bardejov, Sveržov, marsh N of the village, 1960, *V. Pospíšil* (BRNM).—Orava, Oravská Polhora, wetland between the road and the Dlhá voda stream, 2015, *P. Batoušek* (BRNM).