



The seashore sedges of the Russian Kola Peninsula: How many species?

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Abstract

A necessary basis for environmental protection is thorough knowledge of the biodiversity to be protected. Setting conservation priorities in taxonomically complex groups is an especially difficult task. The seashore sedges of the *Carex salina* group (Cyperaceae) of the Russian White and Barents Seas form important parts of the coastal ecosystems and include species listed as rare and endangered. However, their taxonomy is poorly understood and supposed to be blurred by hybridization, also including the closely related *C. aquatilis* and possibly other species of sect. *Phacocystis* (*C. bigelowii*, *C. nigra* s. lat.). We address the taxonomic situation in the *C. salina* group in the Kola Peninsula with emphasis on taxa of supposed hybrid origin (*C. salina* and *C. recta* coll.). We analyzed 92 plants from 28 sites for 101 amplified fragment length polymorphisms (AFLPs) and 10 morphological characters. The plants referred to three supposedly “pure” species (*C. aquatilis*, *C. paleacea* and *C. subspathacea*) formed different extreme parts of the morphological and molecular variation. These species could be discriminated by a combination of morphological characters. The two taxa of proposed hybrid origin had extremely variable morphology and could not be clearly distinguished from each other or from the supposedly “pure” species. Our results demonstrate extensive gene flow between all taxa, suggesting that the entire *C. salina* group including *C. aquatilis* acts as a single large biological species.

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Introduction

A necessary basis for environmental protection is thorough knowledge of the biodiversity to be protected. Such knowledge must be based on taxonomic

investigations. It is, however, often problematic to distinguish closely related species belonging to “species complexes” or “critical groups”. Setting conservation priorities in such taxonomically complex groups is especially difficult, in particular if hybridization is involved (Rieseberg and Gerber, 1995).

The sedges from the so-called “*Carex salina* group” form an important part of the circumpolar coastal ecosystems, but belong to one such critical group of taxa. Egorova (1999) recognized this group as a separate

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section, *Temnemis* (Raf.) V.I.Krecz. The taxonomy of this section is controversial; different authors have accepted from six (Elven et al., 2005) to 15 (Egorova, 1999) species. Elven et al. (2005) suggested that all species of this section belong to a single large “biological” species, bridged by extensive gene flow. In addition, vast hybridization between sect. *Temnemis* and some mire and wetland species from the closely related section *Phacocystis* Dumort. (for example with *C. aquatilis* Wahl. or *C. nigra* (L.) Reichard) has also been suspected. This led Standley et al. (2002) to merge these two sections into the single section *Phacocystis* s.l.

In northwestern Russia, four species are traditionally accepted in the *C. salina* group (summarized in Egorova, 1999): *C. paleacea* Schreb. ex Wahlenb., *C. recta* Boott, *C. salina* Wahlenb. and *C. subspathacea* Wormsk. All of them are classified as belonging to the rare species of Karelia (Kravchenko et al., 2000), and *C. paleacea* and *C. salina* are listed as endangered in the Red Book of the Murmansk region (Konstantinova et al., 2003).

The taxonomy of the *C. salina* group has been well-studied in North America. These studies included meiosis in experimental hybrids, pollen fertility, and morphology (Cayouette and Morisset, 1985), and allozyme variation and foliar anatomy (Standley, 1990). *Carex paleacea* and *C. subspathacea* appeared to be the most well-defined (“pure”) species, whereas *C. salina* was concluded to represent a stabilized hybrid between them. *Carex recta* turned out to be the most problematic taxon. In North America, it was hypothesized to consist of several entities derived from more or less stabilized crosses between one species from section *Temnemis* and one from section *Phacocystis*, namely *C. paleacea* and *C. aquatilis* (*C. recta* s. str.; Cayouette and Morisset, 1985; Standley, 1990).

However, morphological studies in northwestern Europe have led to an alternative hypothesis on the origin of *C. salina* and *C. recta*. Egorova (1999) suggested that the Russian material referred to *C. salina* is derived from *C. recta* × *subspathacea*. There are also suggestions that a part of the northwestern European material referred to *C. recta* could be derived from *C. nigra* × *paleacea* (*C. vacillans* Drejer ex Hartman, Faulkner, 1973) or *C. aquatilis* × *salina* (*C. halophila* sensu auct. norv., non F.Nyl., Elven et al., 2005). Thus, it is possible that the taxonomic situation, including delimitation, characterization, and origin of the taxa, may be different in Europe and North America. More detailed investigations of European material are therefore needed.

It is clear that the conservation policy for rare species strictly depends on their taxonomic status. For example, in the case of species with recent hybrid origin, the best

way can be to protect the parental species. However, clarification of the taxonomic status and testing of hybrid hypotheses are difficult without using molecular markers. This is especially true for the group under consideration because common evidence of hybrid origin such as low pollen fertility and intermediate morphology can hardly be used in *Carex* (Cayouette and Morisset, 1985; Egorova, 1999; Faulkner, 1972; Standley, 1990). For example, high phenotypic plasticity was reported in all members of the group based on common garden experiments (Cayouette and Morisset, 1985; Faulkner, 1972), and the pollen fertility has been shown to be very variable even in supposedly “pure” species of this group (Egorova, 1999).

Here we address the taxonomic situation in the *C. salina* group in the Kola peninsula with emphasis on the taxa of supposed hybrid origin (*C. salina* and *C. recta* coll.). We use amplified fragment length polymorphism (AFLP), a high resolution molecular method which is efficient for identifying interspecific hybrids (e.g., Kapralov et al., 2006). We combined molecular analysis with morphological analysis, an approach shown to be a powerful tool for testing hypotheses in complex low-level taxonomy (Hansen et al., 2000; Shipunov et al., 2004).

Materials and Methods

Sampling

In 2005 and 2006 a total of 92 individuals were sampled from 28 sites, ranging from one to five individuals from each site (Fig. 1; Table 1). Several taxa co-occurred in some of the sites. Because the plants are rhizomatous and capable to form large clones, the plants were collected 5 m apart to minimize resampling of the same clone. The plants were selected to represent the morphological variation observed at each site. Leaf samples from most plants were dried separately in silica gel for DNA analysis, and the remains of each plant was pressed as a voucher for morphological analysis. The voucher specimens were later determined by R. Elven, and his tentative determinations are used throughout this paper. The plants were referred to *C. salina*, *C. recta* coll., *C. subspathacea* and *C. paleacea*, and three putative hybrid combinations (Table 1). Thus, our sample included all European Russian species of section *Temnemis* sensu Egorova, 1999. In addition, we collected *C. aquatilis* from section *Phacocystis* (sensu Egorova, 1999) because it has been suggested to be involved in extensive hybridization with the *C. salina* group. We also included a collection of *C. subspathacea* from the Svalbard archipelago. Voucher specimens from each site are deposited at the Botanical Museum in Oslo (O).

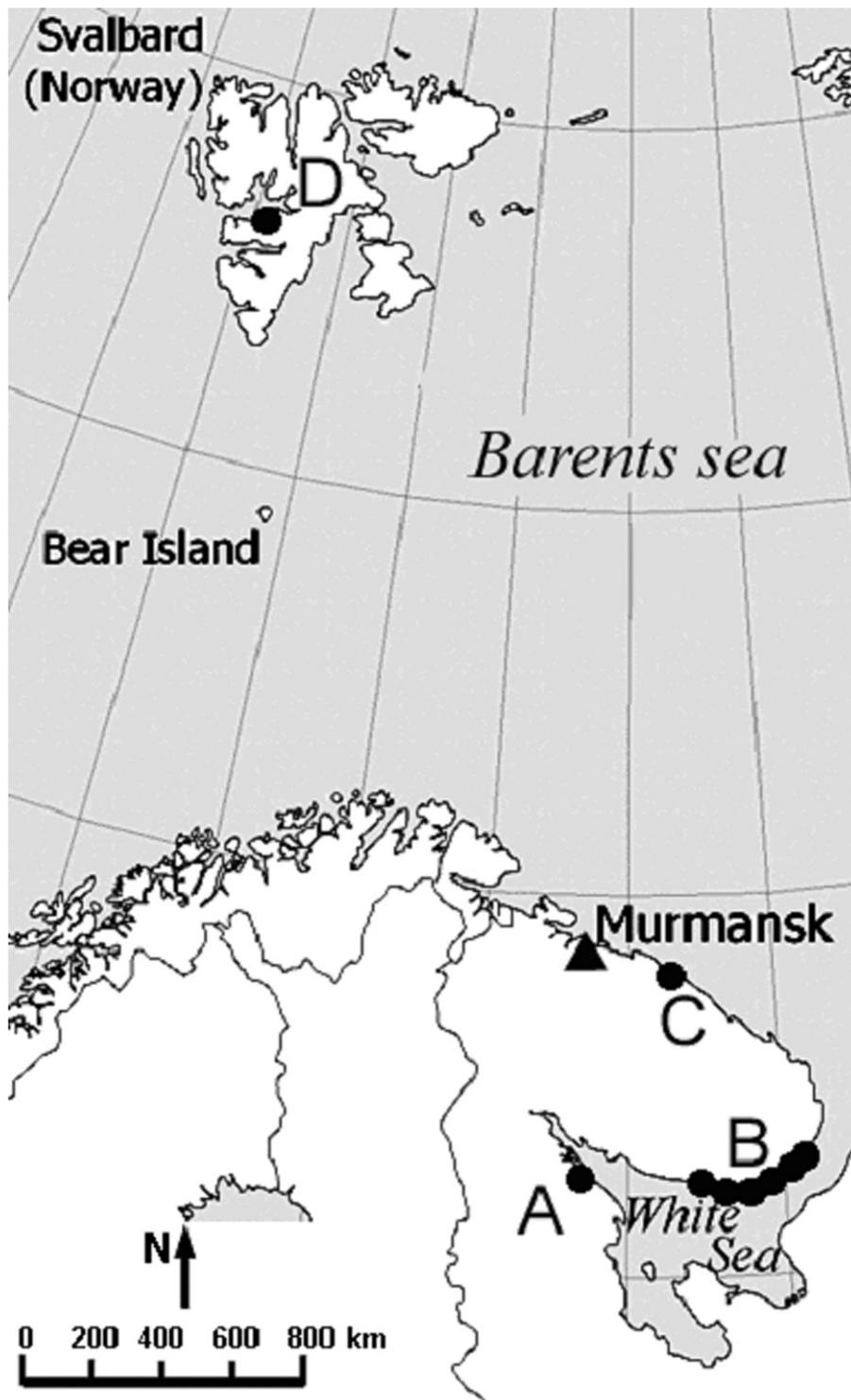


Fig. 1. The four geographic regions with the investigated sites of *Carex* (cf. Table 1). A – Karelia, Loukhi region, shore of the White Sea, Kandalaksha Gulf, Russia, B – Terskij seashore, Kola Peninsula, between the rivers Varzuga and Ponoj, Russia, C – Murmansk region, Severomorsk district, Kola Peninsula, Barents Sea shore, Russia, D – Spitsbergen, Svalbard, Norway.

DNA isolation and AFLP

DNA was extracted from dried leaf material using the CTAB method following Schönswetter et al. (2002), except that the samples were frozen for 15 min at -80°C

after adding CTAB buffer. We used silica gel samples from all but five of the sites. For these five sites, silica gel samples were not available and we used leaves taken from the voucher specimens (Table 1); we found no significant differences between the results of AFLP

Table 1. Collection data for *Carex* samples analysed in this study. Tentative determinations were made by R. Elven prior to the molecular analysis

Site no.	Geographic origin (cf. Fig. 1)	Date	Collectors ^a	No. of plants	Taxon ^b
<i>A: Karelia, Loukhi region, shore of the White Sea, Kandalaksha Gulf, Russia</i>					
53	Sidorovyh Ostrova Arch., Cherepakha Isl., seashore, 66° 21' N, 33° 52' E	04.08.05	DM, AV	1	<i>C. paleacea</i>
73	Krasnaya Bay, between littoral zone and birch forest, 66° 26' N, 33° 35' E	11.08.05	OK, PB	1 ^c	<i>C. paleacea</i>
54	Sidorov Isl., NE shore, 66° 21' N, 33° 49' E	04.08.05	DM, AV	5	<i>C. salina</i>
55	Chupa Bay, Cape Ivanov Navolok, seashore, 300 m SE of the Ivan'kov Isl., 66° 20' N, 33° 40' E	05.08.05	DM, AV	2	<i>C. salina</i>
56	Chupa Bay, Bol'shoj Gorelyhj Isl., SE shore, 66° 18' N, 33° 36' E	07.08.05	DM, AV	1	<i>C. aquatilis</i>
71	Kem-Ludskij Arch., Asafij Isl., central bay of the S shore, 66° 25' N, 33° 50' E	10.08.05	AV, AS	3	<i>C. recta</i>
75	Chupa Bay, 100 m E of the vil. Nizhnyaya Pulonga, seashore	12.08.05	EA	4	<i>C. recta</i>
61	Chupa Bay, 3 km SE of the vil. Nizhnyaya Pulonga, seashore, 66° 17' N, 33° 13' E	04.08.05	OK	4	<i>C. halophila</i>
57	Chupa Bay, Bol'shoj Gorelyhj Isl., E shore, 66° 19' N, 33° 36' E	07.08.05	DM, AV	2	<i>C. aquatilis</i> × <i>salina</i>
60	Medvezh'ya Bay, Pryaostrov Isl., 66° 21' N, 33° 36' E	09.08.05	OK, LM	1 ^c	<i>C. aquatilis</i> × <i>salina</i>
62	Chupa Bay, 3 km SE of the vil. Nizhnyaya Pulonga, seashore, 66° 17' N, 33° 13' E	04.08.05	EA	5 ^c	<i>C. aquatilis</i> × <i>salina</i>
64	Chupa Bay, central part of the vil. Nizhnyaya Pulonga, seashore, 66° 18' N, 33° 17' E	04.08.05	OK, EA	1 ^c	<i>C. aquatilis</i> × <i>salina</i>
67	Chupa Bay, 2.5 km E of the vil. Nizhnyaya Pulonga, seashore, 66° 18' N, 33° 20' E	06.08.05	OK	1 ^c	<i>C. aquatilis</i> × <i>salina</i>
59	Chupa Bay, Bol'shoj Gorelyhj Isl., E shore, 66° 19' N, 33° 39' E	07.08.05	DM, AV	1	<i>C. aquatilis</i> × <i>salina</i>
<i>B: Terskij seashore (Kola peninsula, shore of the White Sea between rivers Varzuga and Ponoj), Russia</i>					
310	NW part of Bol'shaya Bab'ya Bay, 24 km SW of the vil. Sosnovka, coastal marsh, 66° 23, 4' N, 40° 18, 5' E	26.08.06	PV, LA, YK	5	<i>C. salina</i>
308	W shore of the riv. Bol'shaya Kumzhevaya, 300 m upriver, coastal marsh, 66° 12, 5' N, 39° 43, 7' E	23.08.06	PV, LA, YK	5	<i>C. recta</i>
311	Stream estuary, 1.5 km SWS of the vil. Sosnovka, coastal marsh, 66° 29, 6' N, 40° 74, 3' E	28.08.06	PV, LA, YK	3	<i>C. subspathacea</i>
303	Sandy shore of stream, 5.5 km ESE of the riv. Varzuga estuary, 66° 15, 1' N, 37° 04, 2' E	13.08.06	PV, LA, YK	4	<i>C. aquatilis</i> + <i>C. recta</i>
306	Stream estuary 1.5 km E of the riv. Strel'na estuary, coastal marsh, 66° 03, 7' N, 38° 41, 5' E	18.08.06	PV, LA, YK	5	<i>C. aquatilis</i> + <i>C. recta</i>
304	Sandy shore of stream, 5 km ESE of the riv. Varzuga estuary, 66° 15, 25' N, 37° 03, 54' E	13.08.06	PV, LA, YK	5	<i>C. aquatilis</i> + <i>C. paleacea</i> × <i>recta</i>
305	E shore of stream, 2 km NW of the vil. Tetrino, coastal marsh, 66° 04, 2' N, 38° 12, 5' E	17.08.06	PV, LA, YK	5	<i>C. aquatilis</i>
307	S shore of former river-bed of the riv. Chapomka, 3 km E of the vil. Chapoma, coastal marsh, 66° 06, 5' N, 38° 55, 7' E	19.08.06	PV, LA, YK	5	<i>C. salina</i> + <i>C. halophila</i>
309	W shore of the riv. Pulonga, 500 m upriver, coastal marsh, 66° 15, 7' N, 39° 59, 0' E	24.08.06	PV, LA, YK	3	<i>C. salina</i> + <i>C. nigra</i> × <i>subspathacea</i>
<i>C: Murmansk region, Severomorsk district, Kola Peninsula, Barents Sea shore, Russia</i>					
51	W shore of Gavrilovskaya Bay, 10 km NE of the vil. Dal'niye Zelentsy, 69° 04' N, 35° 22' E	27.07.05	OK, DM, PV	3	<i>C. salina</i> + <i>C. nigra</i> × <i>subspathacea</i>
52	E shore of Gavrilovskaya Bay, 10 km NE of the vil. Dal'niye Zelentsy, 69° 04' N, 35° 22' E	27.07.05	OK, DM, PV	1	<i>C. halophila</i>

Table 1. (continued)

Site no.	Geographic origin (cf. Fig. 1)	Date	Collectors ^a	No. of plants	Taxon ^b
301	Gavrilovskije Ostrova Arch., isl. Bol'shoj Gavrilovskij, 6 km NE of the vil. Dal'niye Zelentsy, wet rocks, 69° 10, 3' N, 35° 57, 0' E	06.08.06	PV, LA, DS	5	<i>C. aquatilis</i> × <i>salina</i>
302	Gavrilovskije Ostrova Arch., isl. Bol'shoj Gavrilovskij, 6 km NE of the vil. Dal'niye Zelentsy, stream shore, 69° 10, 3' N, 35° 57, 0' E	06.08.06	PV	5	<i>C. aquatilis</i> × <i>salina</i>
<i>D: Svalbard, Spitsbergen, Norway</i>					
312	Bolternosa Mtn., Adventdalen valley, wet marsh, 78° 20' N, 17° 00' E	07.08.06	KW, TP	5	<i>C. subspathacea</i>

^aCollectors: AS, Shipunov, A.; AV, Vedinin, A; DM, Mordvinkin, D; DS, Sukhova, D; EA, Al'tshuler, E; KW, Westergaard, K; LA, Abramova, L; ML, Lyovina, M; OK, Kudina, O; PB, Burchits, P; PV, Volkova, P; TP, Presto, T.; YS, Kosenko, Ya; VL, Loginov, V; VV, Vasenina, V.

^bSeveral taxa occurred together in some sites.

^cDNA was extracted from pressed plants (tested for DNA quality; see text).

analysis of these two types of samples taken from the same individual plants (see also below). We extracted 19 randomly selected plants twice. These duplicates, as well as negative controls, were included to test for reproducibility and contamination (cf. Bonin et al., 2004).

AFLP analysis was performed according to Gaudeul et al. (2000), except that the reaction volumes in the polymerase chain reaction (PCR) were reduced by 50% and that the preselective PCR-products were diluted ten times. Twenty-four primer pair combinations were tested on eight plants representing several taxa. AFLP profiles with many polymorphic markers and well separated fragments were selected. A second primer test was carried out using five primer pair combinations, chosen from the first primer test, on 32 plants from several taxa. Finally, three of the primer pair combinations were chosen, which produced a manageable number of fragments that were well separated. The final AFLP analysis was carried out with the following primer pair combinations: *EcoRI* AGA (6FAM)-*MseI* CTG, *EcoRI* ATC (VIC)-*MseI* CTA, and *EcoRI* AAC (NED)-*MseI* CAT. For each individual, 2.0 µl 6-FAM, 2.0 µl VIC and 3.0 µl NED labeled selective PCR products were mixed with 11.7 µl formamide and 0.3 µl GeneScan ROX 500 size standard and run on an ABI 3100 sequencer (Applied Biosystems).

Raw data were analyzed using the ABI prism GeneScan version 3.7 analysis software (Applied Biosystems) and imported for scoring into Genographer (version 1.6. available at <http://hordeum.oscs.montana.edu/genographer/>). Fragments in the size range of 70–500 bp were scored as present or absent. The average reproducibility, calculated as the average proportion of correctly reproduced bands over all replicates (Bonin et al., 2004), was 97% for the three primer pair combinations. There was no significant

difference in average proportion of correctly reproduced bands over herbarium and silica dried samples taken from the same plant (12 pairs of samples tested) and over all replicates (t-test: $p = 0.6$).

Morphometry

We initially explored the variation in a number of characters that previously had been used to separate the taxa (Altshuler and Shipunov, 2005). A final set of eight primary and two derived characters were used for the analysis (Table 2). The morphological measurements were performed on the field-collected material.

Data analyses

Principal coordinate analysis (PCO) was used to visualize pair-wise similarities between the AFLP multi-locus phenotypes, using both the simple matching and Dice similarity coefficients. The Dice coefficient takes into account only similarity in presence of fragments, while the simple matching coefficient takes into account both presence and absence of fragments. PCO analyses were executed in the R 2.1.1 environment for statistical computing (R Development Core Team, 2004).

Analyses of molecular variance (AMOVA) were computed with the software Arlequin 3.0.1 (Schneider et al., 1997) to quantify genetic differentiation at different hierarchical levels. The significance levels of the variances were estimated in a permutation test with 10 000 permutations.

The taxonomical structure was also examined by genetic mixture analysis using the software STRUCTURE 2.1 (Pritchard et al., 2000) with a model-based clustering method using Markov Chain Monte Carlo

Table 2. Morphological variation in the *Carex salina* group based on the tentative determinations given in Table 1

Character	<i>C. aquatilis</i> (N = 10/15)				<i>C. paleacea</i> (N = 1/2)				<i>C. subspathacea</i> (N = 3/3)				<i>C. salina</i> (N = 19/24)				<i>C. recta coll.</i> (N = 13/17)				<i>C. aquatilis</i> × <i>salina</i>							
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Karelia (N = 6/9)		Murmansk region (N = 8/9)					
																					Mean	SD	Min	Max	Mean	SD	Min	Max
Vegetative shoot length, cm (a)	37.0	13.00	26	71	39.0	–	39	39	24.7	3.60	21	28	36.2	13.90	18	61	43.1	15.40	23	83	62.7	24.50	27	100	70.4	15.50	41	90
Generative shoot length, cm (b)	39.0	13.00	21	66	50.8	26.52	32	70	17.8	3.44	14	20	32.0	15.23	12	66	45.2	13.71	24	71	43.3	15.50	22	65	70.2	20.36	41	111
a: b	0.97	0.130	0.8	1.3	1.20	–	1.2	1.2	1.40	0.083	1.3	1.5	1.37	0.379	0.8	2.6	0.98	0.245	0.5	1.4	1.20	0.404	0.7	1.7	1.03	0.213	0.8	1.5
Leaf width, mm (upper leaf)	4.0	0.72	3	6	5.5	0.71	5	6	2.7	0.58	2	3	3.5	1.05	2	5	4.0	1.26	2	7	4.1	0.73	3	5	5.3	1.00	4	7
Male spike length, mm (upper spike)	28	6.50	17	40	19.5	2.12	18	21	12.7	1.15	12	14	25.0	6.01	14	37	29.6	7.12	20	47	22.9	3.49	17	27	35.7	10.76	17	50
Peduncle length, mm (lowest female spike) (c)	8.9	8.08	0	30	46.0	50.91	10	52	0.0	0.00	0	0	15.4	10.01	2	47	13.7	7.56	0	28	10.3	4.60	2	19	43.9	8.85	30	55
Female spike length, mm (lowest spike) (d)	39	15.16	16	70	35.5	13.44	26	45	9.0	1.00	8	10	27.0	7.50	12	38	41.2	10.59	23	57	31.9	9.57	16	52	51.9	9.91	42	71
c: d	0.22	0.165	0.0	0.6	1.10	1.017	0.4	1.8	0.00	0.000	0.0	0.0	0.57	0.310	0.1	1.4	0.34	0.188	0.0	0.8	0.32	0.113	0.1	0.5	0.85	0.156	0.6	1.1
Glume length, mm ^a	1.9	0.62	1	3	2.3	0.99	2	3	2.0	0.00	2	2	2.9	0.69	2	5	2.9	0.91	2	5	2.9	0.73	2	4	3.1	1.10	1	5
Awn length, mm ^a	0.0	0.13	0	1	5.8	3.89	3	9	0.0	0.00	0	0	0.9	0.87	0	3	1.0	0.95	0	3	0.3	0.37	0	1	1.2	1.30	0	3

Only plants used for AFLP analysis were included. N – number of plants measured (the first number concerns vegetative shoot length, vegetative shoot length: generative shoot length; the second number (after slash) concerns all other characters). SD – standard deviation.

^ameasured in the middle of the lowest female spike; glume length exclusive awn.

estimation. In this program it is possible to identify the optimal number of groups (K) by comparing the likelihood of the data estimated in different runs for different numbers of groups. Individuals are assigned (probabilistically) to one of the clusters defined by allele frequencies at each locus. Our data were analyzed at the Bioportal, University of Oslo (<http://www.bioportal.uio.no>), with K ranging from one to 10, 10 replicate runs for each K and a burn-in period of 2×10^5 and 10^6 iterations. The no admixture model and uncorrelated allele frequencies were assumed for the analysis. Similarity coefficients among pairs of Structure runs were calculated according to Rosenberg et al. (2002) using the R-script AFLPdat (Ehrich, 2006).

We performed multilocus assignment tests using AFLPOP (Duchesne and Bernatches, 2002) to test the hypotheses on hybrid origins of plants referred to *C. salina*, *C. recta* coll. and *C. salina* \times *C. aquatilis*. In AFLPOP the differences in frequencies at polymorphic loci are used to assign a specimen to its most probable species or simulated F₁ and F₂ hybrids or backcrosses (see Kapralov et al., 2006 for details). Marker frequencies of zero were replaced by $1/(\text{sample size} + 1)$.

Converting of the output of Genographer to an AFLP data matrix for PCO and generating the input files for Arlequin and STRUCTURE were made with the R-script AFLPdat (Ehrich, 2006).

Results

AFLP variation

The AFLP analysis provided 101 polymorphic markers. The PCO plot based on the Dice similarity coefficient (Fig. 2) showed a continuum with the three supposedly “pure” species (*C. aquatilis*, *C. subspathacea* and *C. paleacea*) at its extreme parts, forming a triangle. Interestingly, the geographically distant Svalbard sample of *C. subspathacea* were placed close together with the Murmansk region plants of this species. The plants referred to *C. salina* were, with three exceptions, positioned between *C. subspathacea* and *C. paleacea*. The plants referred to *C. recta* coll. occupied central positions in the plot but in general intermediately between *C. aquatilis* and *C. paleacea*, and three plants referred to *C. paleacea* \times *recta* were situated between their putative parents. Four plants supposed to represent *C. nigra* \times *subspathacea* were positioned between those referred to *C. aquatilis* and those referred to *C. subspathacea*. Finally, the plants referred to *C. aquatilis* \times *salina* were divided into two distinctly separated groups corresponding to different geographic origins: those from Karelia were placed between *C. aquatilis* and *C. subspathacea* while those from Murmansk region were situated more or less between

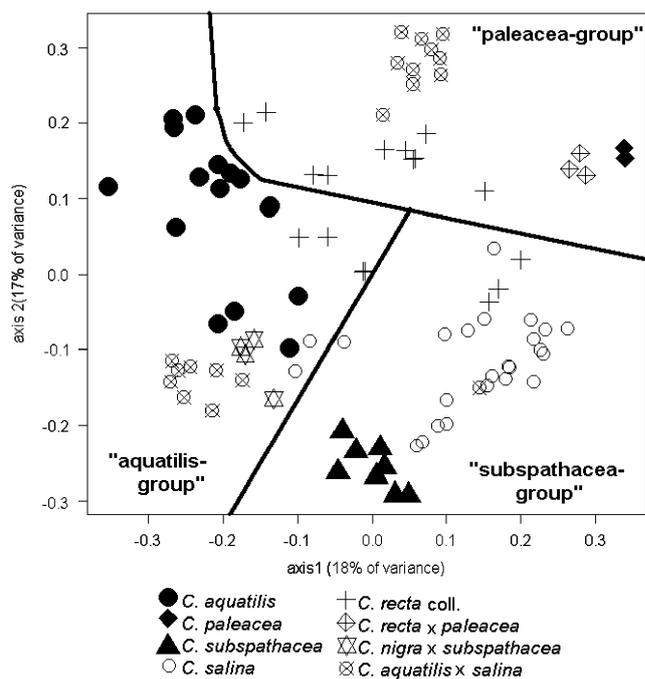


Fig. 2. Principal coordinate analysis (PCO) of all individuals from the 28 collection sites of *Carex* based on Dice similarity between AFLP multilocus phenotypes. The variation is divided into three parts according to the STRUCTURE analysis. The three supposedly “pure” species are shown with filled symbols.

C. aquatilis and *C. paleacea*. The PCO plot based on the simple matching similarity coefficient showed a very similar structure (not shown).

The STRUCTURE analysis clearly revealed three groups, hereafter named after the three supposedly “pure” species (Figs. 2 and 3). The “paleacea-group” included all plants referred to *C. paleacea*, *C. paleacea* \times *recta*, and *C. aquatilis* \times *salina* from Murmansk region, as well as some of the plants referred to *C. recta* coll. The “subspathacea-group” consisted of all plants referred to *C. subspathacea* and *C. salina* as well as some plants referred to *C. recta* coll. The “aquatilis-group” included all plants referred to *C. aquatilis*, *C. nigra* \times *subspathacea*, *C. aquatilis* \times *salina* from Karelia and the remaining plants referred to *C. recta* coll. Thus, the plants referred to *C. recta* coll. were divided among all three STRUCTURE groups.

In the AMOVA analyses (Table 3), the largest proportion of the genetic variation was found within the tentative taxa (78%) and within the three supposedly “pure” species (61%). There was also a much larger proportion of the genetic variation within the three STRUCTURE groups (78%) than among them. The proportion of the genetic variation found among the collection sites was almost the same as that within them (57%).

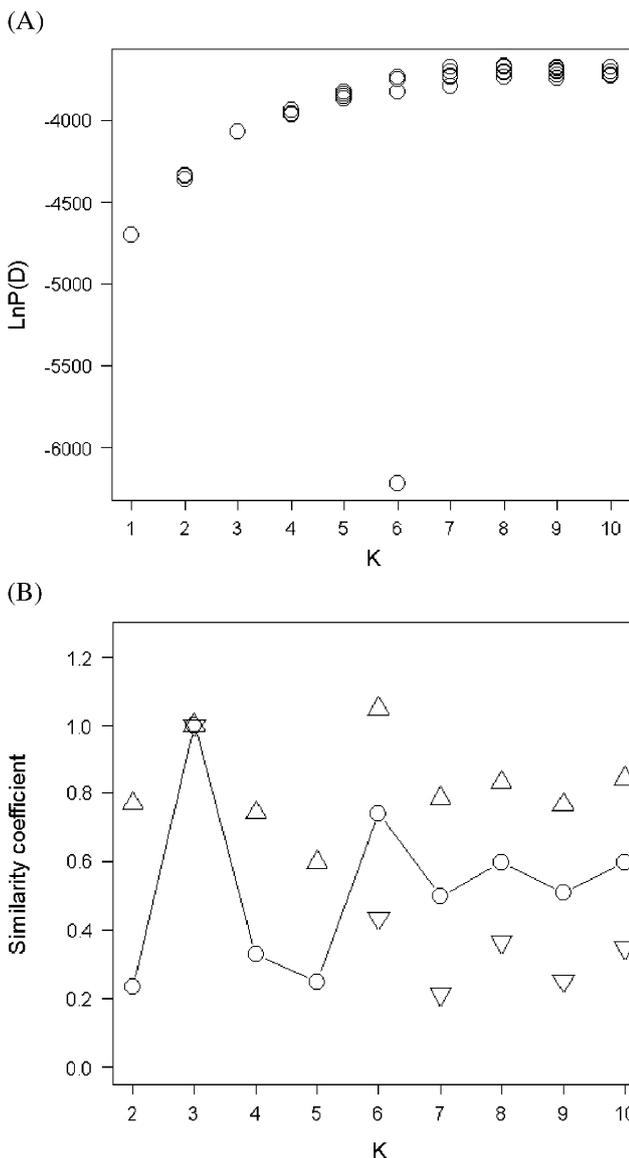


Fig. 3. STRUCTURE analysis of the AFLP data for *Carex*. (a) Estimated likelihood for values of K ranging from 1 to 10. (b) Similarity coefficients of the results from different runs of STRUCTURE calculated according to Rosenberg et al. (2002). Dots represent the average similarity coefficient for the pairwise comparisons among 10 runs and triangles show the standard deviation.

In the assignment tests, an AFLP phenotype was assigned to a group if the likelihood was ten times higher for belonging to this group than to any other group, otherwise the AFLP phenotype was not assigned. The plants referred to *C. recta* coll. were divided into three groups according to the STRUCTURE results (Figs. 2 and 4) before conducting the tests. The first assignment test was performed to find the most likely parents of these three groups. The plants belonging to the “paleacea-group” were either assigned to the simulated *C. aquatilis* × *paleacea* F_1 hybrid (two plants) or to

Table 3. Analyses of molecular variance (AMOVA) of the AFLP data for the *Carex salina* group

Source of variation	% of total variance
Among all taxa	22.3
Within all taxa	77.7
Among supposedly “pure” species (<i>C. aquatilis</i> , <i>C. subspathacea</i> , <i>C. paleacea</i>)	38.8
Within supposedly “pure” species	61.2
Among three STRUCTURE groups	22.0
Within three STRUCTURE groups	78.0
Among collection sites	42.8
Within collection sites	57.2

p -values were <0.001 in all cases (estimated with 10 000 permutations).

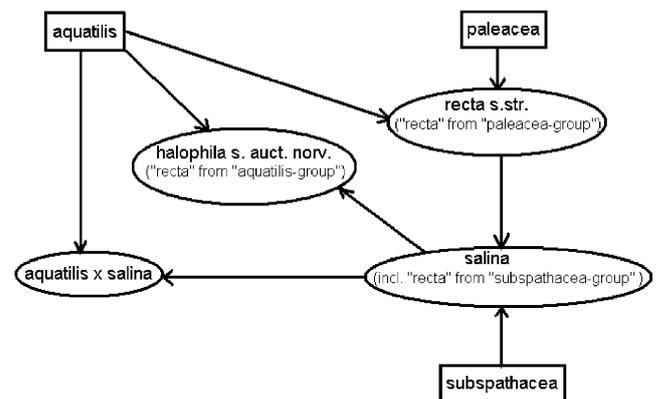


Fig. 4. A tentative hypothesis on the relationships in *C. salina* group based on AFLPOP analysis of the AFLP data. Arrows indicate hybrid origin. Supposedly “pure” species are shown in rectangles, inferred hybrid combinations in ovals.

C. salina (one plant; five plants were not assigned). The plants belonging to the “subspathacea-group” were assigned to *C. salina* (two plants; two plants were not assigned). All plants belonging to the “*aquatilis*-group” were assigned to the simulated *C. aquatilis* × *salina* F_1 hybrid (three plants).

The second assignment test was conducted to find the most likely parents of the plants referred to *C. salina*. The majority of these plants were assigned to the simulated *C. recta* from the “*paleacea*-group” × *C. subspathacea* F_1 hybrid (seven plants; nine plants were not assigned). In the last test for the putative *C. aquatilis* × *C. salina* hybrid only two Karelian plants (the other five plants were not allocated) and the majority of plants from the Murmansk region (six plants; three plants were not allocated) were assigned to the simulated F_1 hybrid between *C. aquatilis* and *C. salina*.

Morphological variation

The three supposedly “pure” species (*C. aquatilis*, *C. subspathacea* and *C. paleacea*) could be discriminated by a combination of morphological characters (Table 2). The plants referred to *C. paleacea* could be separated from the other two species by presence of long awns on the glumes (mean 5.8 mm vs. absent in the other taxa) and by its much longer peduncles of the female spikes (46 vs. 0–9 mm). The plants referred to *C. subspathacea* could be separated from those referred to *C. aquatilis* mainly by size characters such as the length of the generative shoots (178 ± 34 vs. 387 ± 130 mm, respectively), the width of the upper leaf (2.7 ± 0.6 vs. 4.0 ± 0.7 mm) and the length of the lowest female spike (9 ± 1 vs. 39 ± 15 mm).

The two taxa of proposed hybrid origin (*C. salina* and *C. recta* coll.) had extremely variable morphology, and it was not possible to distinguish them from each other or from the supposedly “pure” species with certainty (Table 2). It was neither possible to distinguish the three genetic groups of plants of tentative *C. recta* coll. found in the STRUCTURE analysis, nor plants tentatively referred to *C. recta* s.str. and *C. halophila*.

The two genetic groups identified within *C. aquatilis* × *salina* were only poorly separated morphologically (Table 2). For example, the Karelian plants were somewhat smaller than those from Murmansk (the length of the generative shoot was 433 ± 155 vs. 702 ± 204 mm, respectively) and had shorter peduncles of the female spikes (10 ± 5 vs. 44 ± 9 mm).

Discussion

The plants tentatively referred to the three supposedly “pure” species (*C. aquatilis*, *C. paleacea* and *C. subspathacea*) formed different extreme parts of the morphological and molecular variation; all other plants analyzed were more or less intermediate between them. This result provides strong evidence for extensive gene flow, supporting the hypothesis that the entire *C. salina* group including *C. aquatilis* acts as a single large biological species in the Kola Peninsula (Elven et al., 2005).

The results of the STRUCTURE analysis and the assignment tests suggest that what is named *C. recta* coll. is heterogeneous and taxonomically polyphyletic (Fig. 4). Our data provide support both for the American (Cayouette and Morisset, 1985; Standley, 1990) hypothesis on the origin of *C. recta* (i.e., derived from the cross *C. aquatilis* × *paleacea*, named *C. recta* s.str.) and the European (Elven et al., 2005) hypothesis (i.e., from the cross *C. aquatilis* × *salina*, named *C. halophila*). In addition, some of the plants we initially referred to as *C. recta* coll. belonged to the “subspatha-

cea-group” in the STRUCTURE analysis of the genetic data, and turned out to be genetically very similar to the plants referred to *C. salina*. However, these three groups of *C. recta* coll. could not be distinguished based on our morphological analysis and do not correspond to the tentative separation between *C. recta* s.str. and *C. halophila*.

The plants we tentatively referred to as *C. salina* seem to represent a hybrid swarm between *C. subspathacea* and *C. recta* s.str., thus supporting the European hypothesis on the origin of *C. salina* (Egorova, 1999). However, the difference between the American hypothesis (suggesting that *C. salina* originated from *C. paleacea* × *subspathacea*, see e.g. Standley, 1990) and the European hypotheses is rather subtle, taking into account the supposed origin of *C. recta* s.str. (see above). We therefore conclude that more extended sampling is needed to clarify the origin of the European *C. salina*.

The plants referred to *C. salina* were as variable morphologically as those referred to *C. recta* s.str. and *C. halophila*. Egorova (1999) did not find any sharp morphological boundary between *C. salina*, *C. recta* and *C. subspathacea* in the territory of the former USSR. This was confirmed by our analysis of the Karelian plants, which were intermediate between *C. salina* and *C. subspathacea* in morphology.

The taxonomic situation in the *C. salina* group in NW Europe seems to be even more complex than that described for North America (Cayouette and Morisset, 1985; Standley, 1990). Firstly, there is sympatric existence of at least two differently named entities of hybrid origin within *C. recta* coll. that cannot be reliably distinguished by morphology. Secondly, a stabilized part of *C. salina* (which obviously is a taxon of hybrid origin) has probably acted as a parent for another entity of hybrid origin (*C. halophila*). Moreover, there are probably many instances of backcrosses and hybrids at different levels of stabilization, making the identification of separate plants in this aggregate almost impossible. The morphological and molecular heterogeneity of plants from Karelia and Murmansk that were referred to *C. salina* × *C. aquatilis* adds to this complexity. It should be noticed that more extensive sampling than we had in this study would likely have revealed even more complex patterns, including additional taxa of unclear status (e.g. *C. ramenskii* from the Russian Far East). In our opinion, the difference between the taxonomic situations in the investigated group in NW Europe vs. North America reflects the different agricultural and glacial histories of these regions rather than a disagreement on species concepts between European and American systematists (cf. Elven et al., 2005).

Our data for *C. aquatilis* (sect. *Phacocystis* sensu Egorova, 1999) as well as recent phylogenetic inference based on nuclear and chloroplast DNA sequencing

(J. Dragon, unpublished) do not support exclusion of *C. aquatilis* from sect. *Temnemis* sensu Egorova (1999), but agree with the wide concept of sect. *Phacocystis* adopted by Standley et al. (2002) and the PAF checklist (Elven et al., 2005). Interestingly, the morphology of the chromosomes of *C. aquatilis* is more similar to that in sect. *Temnemis* (for example to *C. paleacea*) than to other species of sect. *Phacocystis* sensu Egorova (Faulkner, 1973).

High levels of genetic variability within taxa, as shown in our data, are typical for some rare plant species (Bruederle, 1999). It has been suggested that rare species may evolve means of maintaining high levels of genetic variability, such as increased outcrossing (Rabinowitz, 1981), which is especially pronounced in the group under consideration where all species appear to hybridize freely.

We argue that conservation efforts in the *C. salina* group in the Kola peninsula should be focused on the two rare “pure” species of the Karelia and Murmansk region – *C. paleacea* and *C. subspathacea* (cf. Konstantinova et al., 2003; Kravchenko et al., 2000). The rarity of these two species in the Kola peninsula is confirmed by our own observations: we have observed *C. subspathacea* only once and *C. paleacea* not at all along 200 km of coastline (vil. Ust'-Varzuga – vil. Sosnovka), although there are several specimens of both *C. subspathacea* and *C. paleacea* from this region in the herbaria of Moscow State University (MW) and Komarov Botanical Institute (LE). These species can be easily recognized morphologically (see for example Egorova, 1999), which is important for successful realization of conservation programmes. On the other hand, any attempts to preserve more unstable taxa or hybrid swarms such as *C. salina*, which currently is listed in the Red Book of the Murmansk region (Konstantinova et al., 2003), will probably be fruitless.

In order to conserve genetic variability, it is necessary to maximize the number of populations preserved due to significant within-species (i.e. among-population) component of diversity (Bruederle, 1999). The other important way is to preserve the habitats where parental species (in our case *C. paleacea*, *C. subspathacea* and *C. aquatilis*) co-occur and hybrids could originate and become established, as a way to preserve the evolutionary process (Pillon et al., 2006). The contact zones between mires (the typical habitat for *C. aquatilis*) and seashores (the typical habitat for *C. paleacea* and *C. subspathacea*) serve as such habitats.

The most endangered region of the Kola peninsula due to increased human pressure on the seashores is the part of the White Sea shore between the rivers Varzuga and Ponoj. However, the suggestion to establish this shoreline as the National Park “Terskij bereg”, proposed in 1994, was rejected in 1996 mainly according

to the administration's and locals' opinion. Thus, nowadays in the absence of this National Park we see no real opportunities for the realization of the conservation programmes for the *C. salina* group in the Kola peninsula under conditions of constantly increasing recreation and industry pressure on the seashores.

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References

- Altshuler, E.P., Shipunov, A.B., 2005. Morfologicheskaja izmenchivost' osok iz gruppy *Carex salina* Wahl. (Cyperaceae) na poberezh'je Belogo morja. Vestnik VOOB 10, 1–6 (in Russian).
- Bonin, A., Bellemain, E., Eidesen, P.B., Pompanon, F., Brochmann, C., Taberlet, P., 2004. How to track and assess genotyping errors in population genetics studies. Mol. Ecol. 13, 3261–3273.
- Bruederle, L.P., 1999. Genetic differentiation of geographically marginal populations in *Carex mitchelliana* (Cyperaceae): implications for conservation. J. Torrey Bot. Soc. 126, 1–8.
- Cayouette, J., Morisset, P., 1985. Chromosome studies on natural hybrids between maritime species of *Carex* (sections *Phacocystis* and *Cryptocarpae*) in northeastern North America, and their taxonomic implications. Can. J. Bot. 63, 1957–1982.
- Duchesne, P., Bernatches, L., 2002. AFLPOP: a computer program for simulated and real population allocation using AFLP markers. Mol. Ecol. Notes 2, 380–383.
- Egorova, T.V., 1999. The sedges (*Carex* L.) of Russia and adjacent states (within the limits of the former USSR).- St.-Petersburg State Chemical-Pharmaceutical Academy. St.-Petersburg & Missouri Botanical Garden Press, St. Louis.
- Ehrich, D., 2006. AFLPdat: a collection of R functions for convenient handling of AFLP data. Mol. Ecol. Notes 6, 603–604.
- Elven, R., Murray, D.F., Razzhivin, V.Y., Yurtsev, B.A., 2005. Checklist of the Pan-Arctic Flora (PAF). Vascular Plants, Draft Version. University of Oslo, Oslo.

- Gaudeul, M., Taberlet, P., Till-Bottraud, I., 2000. Genetic diversity in an endangered alpine plant, *Eryngium alpinum* L. (Apiaceae), inferred from amplified length polymorphism markers. *Mol. Ecol.* 9, 1625–1637.
- Hansen, K.T., Elven, R., Brochmann, C., 2000. Molecules and morphology in concert: tests of some hypotheses in arctic *Potentilla* (Rosaceae). *Am. J. Bot.* 87, 1466–1479.
- Kapralov, M.V., Gabrielsen, T.M., Sarapultsev, I.E., Brochmann, C., 2006. Genetic enrichment of the arctic clonal plant *Saxifraga cernua* at its southern periphery via the alpine sexual *Saxifraga sibirica*. *Mol. Ecol.* 15, 3401–3411.
- Konstantinova, N.A., Korjakin, A.S., Makarova, O.A., 2003. Krasnaja Kniga Murmanskij oblasti. Knizhnoe izdatelstvo, Murmansk (in Russian).
- Kravchenko, A.V., Gnatjuk, E.P., Kuznetsov, O.L., 2000. Rasprostraneniye i vstrechaemost' sosudistykh rastenij floristicheskikh rajonov Karelii. Petrozavodsk (in Russian).
- Faulkner, J.S., 1972. Chromosome studies on *Carex* sect. *Acutae* in NW Europe. *Bot. J. Linn. Soc.* 65, 271–301.
- Faulkner, J.S., 1973. Experimental hybridisation of NW European species in *Carex* sect. *Acutae* (Cyperaceae). *Bot. J. Linn. Soc.* 67, 233–253.
- Pillon, Y., Fay, M.F., Shipunov, A.B., Chase, M.W., 2006. Species diversity versus phylogenetic diversity: a practical study in the taxonomically difficult genus *Dactylorhiza* (Orchidaceae). *Biol. Conserv.* 129, 4–13.
- Pritchard, J.K., Stephens, M., Donnelly, P.J., 2000. Inference of population structure using multilocus genotype data. *Genetics* 155, 945–959.
- R Development Core Team, 2004. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.
- Rabinowitz, D., 1981. Seven forms of rarity. In: Synge, H. (Ed.), *The Biological Aspects of Rare Plant Conservation*. Wiley, New York, pp. 205–217.
- Rieseberg, L.H., Gerber, D., 1995. Hybridization in the Catalina Island mountain mahogany (*Cercocarpus traskiae*): RAPD evidence. *Conserv. Biol.* 9, 199–203.
- Rosenberg, N.A., Pritchard, J.K., Weber, J.L., Cann, H.M., Kidd, K.K., Zhivotovsky, L.A., Feldman, M.V., 2002. Genetic structure of human populations. *Science* 298, 2381–2385.
- Schneider, S., Kueffer, J., Roessli, D., Excoffier, L., 1997. Arlequin 2.000: a software for population genetic analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.
- Shipunov, A.B., Fay, M.F., Pillon, Y., Bateman, R.M., Chase, M.W., 2004. *Dactylorhiza* (Orchidaceae) in European Russia: combined molecular and morphological analysis. *Am. J. Bot.* 91, 1419–1427.
- Schönswetter, P., Tribsch, A., Barfuss, M., Niklfeld, H., 2002. Several Pleistocene refugia detected in the high alpine plant *Phyteuma globulariifolium* Sternb. et Hoppe (Campanulaceae) in the European Alps. *Mol. Ecol.* 11, 2637–2647.
- Standley, L., 1990. Allozyme evidence for the hybrid origin of the maritime species *Carex salina* and *Carex recta* (Cyperaceae) in Eastern North America. *Syst. Bot.* 15, 182–191.
- Standley, L.A., Cayouette, J., Bruederle, L., 2002. *Carex* Linnaeus sect. *Phacocystis* Dumortier//Flora of North America 23. University Press, New York, NY, pp. 379–401.