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AFLP-based differentiation in north Atlantic species of *Carex* sect. *Phacocystis*

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A total of 59 plants from 30 populations of 15 species of *Carex* sect. *Phacocystis* (including *Carex bicolor* as outgroup) from eastern Canada and northern Europe were investigated for genetic differentiation of taxa using AFLP. Seven species were studied with material from both Europe and America, three species were investigated with North American material only and five species with European material only. The neighbour joining analysis (NJ) indicates that *Carex bicolor* may not belong to section *Phacocystis*, while all other investigated species clearly belong to this section. The sorting of the species according to NJ and UPGMA is mostly in accordance with accepted taxonomy, but with the exceptions that the American *C. bigelowii* ssp. *bigelowii* may be specifically distinct from European *C. bigelowii* ssp. *rigida*, and *C. stans* should perhaps not be considered a subspecies or variety of *C. aquatilis*, but either as a separate species or as a hybrid between *C. aquatilis* and *C. bigelowii*. North American *C. aquatilis* is heterogenic and may contain more than one species.

Carex sect. *Phacocystis* Dumort. (syn. sect. *Acutae*) is one of the most difficult groups of this giant genus of more than 2000 species (Ball and Reznicek 2002). The section is mainly distributed in wet or humid habitats in the northern cold and temperate regions with relatively few species reaching hot regions and the Southern Hemisphere. Many species have an amphi-Atlantic or circumpolar distribution (Hultén 1958, 1964). In Europe the section contains 16 species (Chater 1980), in North America 31 species, including 18 species from eastern North America (Standley et al. 2002), and in northern Asia 38 species (Schishkin 1935, Egorova 1999). Kükenthal (1909) in his world monograph of the genus *Carex* includes 79 species of section *Acutae* subdivided into 7 subsections. Since the distinguishing characters between many species are so minute and obscure, and since many species frequently hybridize producing partly fertile hybrids, the number of species recognized in the various areas much depends on whether the author is a lumper or splitter. The section is very much in need of a world revision. Our pilot research using few samples of many species is intended to indicate general relationships and which are the most urgent species or species complexes to be further investigated.

In recent years new attempts have been made to resolve sectional classifications of *Carex* subgenus *Carex* (Roalson et al. 2001, Hendrichs et al. 2004, Waterway and Starr 2007). However, most attempts on infrasectional classifications are of much earlier date before the advent of DNA techniques (Kükenthal 1909, Mackenzie 1935a, 1935b, Sylvén 1963). The species examined in this study were placed in section *Acutae* by Kükenthal (1909) in his worldwide monograph of *Carex*, and he divided the section into seven subsections including subsection *Bicolores* containing *C. bicolor* All., *C. rufina* Drejer and *C. eleusinoides* Kunth. *C. bicolor* has since been removed from the section (Standley et al. 2002). Mackenzie (1935a, 1935b) in his classification divided the species of *Acutae* into two sections *Acutae* and *Cryptocarpae*. However, Standley (1990) and Standley et al. (2002) merged the two sections, which are now referred to as section *Phacocystis*. Faulkner (1973) used hybridisation experiments dealing with European species of the section and a similar classification to that of Sylvén (1963) except that *C. aquatilis* was placed in subsection *Rigidae*. The problem on which species to include in which subsection and whether subsections should be accepted in sect. *Phacocystis* is still not fully resolved.

Species of the genus *Carex* lack localized centromeres and chromosome number changes as a result of fission and fusion events rather than polyploidy; this results in extreme chromosomal variability (Hipp et al. 2006, Hipp 2007). Chromosome number varies within species as well, and some species, e.g. *C. aquatilis* and *C. nigra*, exibit a wide range in chromosome number (Table 1). The investigated species of section *Phacocystis* exhibit an agmatoploid chromosome series ranging from $2n = 66$ (in the American species *C. gynandra* and *C. torta*) to $2n = 86$ (in *C. rufina*).

In recent years a number of molecular studies in the genus *Carex* have been published analysing nuclear ribosomal genes, e.g. ITS nrDNA region (Escudero et al. 2008) or combining this with noncoding chloroplast DNA

Table 1. The chromosome numbers of the investigated *Carex* species. The numbers from Europe are from Heilborn (1924), Løve and Løve (1944, 1956), Harling (1945), Hjelmqvist and Nyholm (1947), Davies (1956), Sylvén (1963), Knaben and Engelskjøn (1967), Faulkner (1972, 1973), Elven in Lid and Lid (2005). The numbers given above from America (including Greenland) are from Bøcher (1938), Jørgensen et al. (1958), Løve and Løve (1966), Løve and Ritchie (1966), Cayouette and Morisset (1986b), Standley (1987), Cayouette and Catling (1992), Standley et al. (2002).

	Europe	North America
C. acuta	2n = 83, 84, 85	
C. aquatilis	2n = 76, 77	2n = 72, 74–77, 79–80
C. bicolor	2n = 52	2n = ca 52
C. bigelowii	2n = 68, 69, 70, 71	2n = 68–71
C. cespitosa	2n = 80 (78-80)	
C. elata	2n = 74–78 (80)	
C. gynandra		2n = 66, 68
C. nigra	2n = 83, 84, 85	2n = 83, 84, 85
C. paleacea	2n = 71, 72, 73	2n = 71, 72, 73
C. rufina	2n = 86	
C. stans	2n = 76, 80	2n = 76
C. torta		2n = 66
C. subspathacea	2n = 78–84	2n = 78, 80–83
C. trinervis	2n = ca 84	

analysis (Roalson et al. 2001, Waterway and Starr 2007). None of these efforts have been entirely successful in separating closely related species, e.g. Escudero et al. (2008) were not able to separate *Carex binervis* Sm. from *C. laevigata* Sm., nor *C. diluta* M. Bieb. and *C. idaea* Greuter, Matthäs & H. Risse from *C. distans* L., nor *C. lepidocarpa* Tausch, *C. viridula* Michx. and *C. demissa* Hornem. from *C. flava* L.

We therefore selected the amplified fragment length polymorphism (AFLP) fingerprinting method for this research, since this method (like SSRs) more easily separates closely related species and individuals (Jones et al. 1997, Belaj et al. 2003). The advantages of the AFLP technique are that it reveals a large number of fragments at one time and gives highly reproducible results, and AFLP is less prone to homology problems than are other anonymous DNA fragment methods (Sasanuma et al. 2004). A disadvantage of AFLP is that it is a dominant marker and hence information on heterozygosity of an individual at a locus is difficult to detect.

Use of AFLP to classify genetic variation in the genus *Carex* is very recent (viz. Choler et al. 2004, Arens et al. 2005, Ford et al. 2006, Hipp et al. 2006, Schönswetter et al. 2006, Vollan et al. 2006, Luo et al. 2007). Although sequencing is generally preferred for phylogenetic reconstruction, Albertson et al. (1999) found AFLP a better method to reconstruct phylogeny when hybridization results in potentially misleading phylogenetic signal in single-marker studies. Since this work deals with a group of taxa subject to frequent hybridization, we believe AFLP fingerprinting is an ideal method to obtain both reliable fine-scale differentiation of taxa as well as an acceptable phylogenetic construction. The major practical disadvantage of the method is that it requires fresh or recently collected material, and we were thus not able to sample from the large Asian continent where many species of *Carex* section *Phacocystis* are widespread.

Material and methods

Sampling procedure

During 2003–2006 the authors collected plants from five Canadian provinces and from many different Scandinavian localities.

The youngest leaves of individual plants (assumed to be free of fungal endophytes) were collected in the field and dried using silica gel. Two to five samples were collected from each population ensuring spatial separation of at least 3–5 m between the samples. From each population at least one herbarium sample was collected and presented to professional herbaria (herb. O for all collections, herb. DAO or NFLD for Canadian collections) for future storage.

Material

We have investigated a total of 16 taxa (Table 2); for six taxa we used plants from both Europe and America (mostly amphi-Atlantic species; Hultén 1958), viz. *C. aquatilis* Wahlenb. ssp. *aquatilis*, *C. bigelowii* Schweinitz ssp. *bigelowii*, *C. nigra* (L.) Reich., *C. paleacea* Wahlenb., *C. subspathacea* and *C. bicolor* All., the latter species here included as an outgroup as it is not usually included in *Carex* sect. *Phacocystis* (Ball 2002). Four taxa investigated are known from North America (including Greenland) only, viz. *C. gynandra* Schweinitz, *C. stans* Drej., *C. torta* Boott and a taxon at present named *C. aquatilis* Wahlenb. taxon B. The six taxa investigated from European material only are *C. acuta* L., *C. bigelowii* Schweinitz ssp. *rigida* Sch.-Motel, *C. cespitosa* L., *C. elata* All., C. *rufina* Drej. and *C. trinervis* Degl.

We have included all north European and most north Atlantic species of *Carex* sect. *Phacocystis*, but excluded the hybrids or species with supposedly post-glacial hybrid origin (Cayouette and Morisset 1985, 1986a), viz. *C. halophila* Nyl., *C. lyngbyei* Horn., *C. recta* Boott, *C. salina* Wahlenb. and *C. vacillans* Drej., because these will need more detailed studies. We have also excluded the two North American species *C. crinita* Lam. and *C. mitchelliana* M. A. Curtis, which are very close allies to the investigated *C. gynandra* Schweinitz (Standley 1983, Bruederle and Fairbrothers 1986). Also excluded is the *Carex stricta* complex with three American species in the North Atlantic region, viz. *C. haydenii* Dewey, *C. emoryi* Dewey and *C. stricta* Lam. (Standley 1989, Standley et al. 2002), none of which are closely related to European species. We are also lacking material from the American species *C. lenticularis* Michx.

DNA extraction and AFLP procedure

DNA was isolated from ca 20 mg of leaf tissue dried in silica gel, this was done according to the protocol outlined in the DNeasy Plant Mini Kit QIAGEN. Elution in the final step was accomplished using a total of 80 µl of AE buffer instead of the recommended 200 µl. DNA quality and quantity was then checked on a 0.8% agarose gel. DNA from two plants (selected on the basis of DNA quality) from each of the 25 populations was used for the AFLP

Table 2. Origin and site description of the AFLP fingerprinted populations of the investigated species of *Carex* section *Phacocystis*.

Species and population	Country	Region (Province)	Locality	Coordinates	Altitude (m)	Number	Herb
C. acuta L.							
Ac300	Norway	Oppland	Ringebu, Elstad	61°30′N, 10°10′E	183	28574	O
Ac391	Finland	Ostrobottnia bor	southwest of Tervola	66°03′N, 24°45′E	40	28694	O
C. aquatilis Wahlenb. subsp. *aquatilis*							
AqA395	Finland	Lapponia kem.	Inari	68°54′N, 27°01′E	118	28698	O
AqA753	Canada	Labrador	Forteau River bridge	51°30′N, 56°57′W	0.5	30056	NFLD,O
C. aquatilis Wahlenb. taxon B							
AqB776	Canada	Newfoundland	L'Anse au Meadow	51°36′N, 55°31′W	1	30081	NFLD,O
AqB777	Canada	Newfoundland	L'Anse au Meadow	51°36′N, 55°31′W	1	30082	DAO,NFLD,O
C. bicolor All.							
Bic325	Norway	Hedmark	Folldal, Tunheim	62°10′N, 10°13′E	650	28616	O
Bic779	Canada	Newfoundland	North Boat Harbour	51°31′N, 56°08′W	1	30084	NFLD,O
C. bigelowii Schwein. subsp. *bigelowii*							
BigB750	Canada	Quebec	Labrador, Blanc-Sablon	51°25′N, 57°09′W	80	30053	NFLD,O
BigB255	Norway	Nordland	Fauske, Sulitjelma	67°07′N, 16°06′E	180	28527	O
C. bigelowii Schwein. subsp. *rigida* Schultze-Motel							
BigR321	Norway	Hedmark	Alvdal, Tronden	62°10′N, 10°42′E	1290	28611	O
C. cespitosa L.							
Ces66	Norway	Akershus	Ås, Pollevatn	59°44′N, 10°45′E	1	28354	O
Ces390	Finland	Ostrobottnia bor	southwest of Tervola	66°03′N, 24°45′E	40	28693	O
C. elata All.							
Ela107	Norway	Akershus	Asker, Semsvatn	59°51′N, 10°26′E	145	28354	O
Ela564	Sweden	Närke	Karlskoga	59°17′N, 14°42′E	150	29215	O
C. gynandra Schwein.							
Gyn733	Canada	Newfoundland	Avalon Peninsula, South Dildo	47°31′N, 53°33′W	1	30035	NFLD,O
Gyn806	Canada	New Brunswick	Queens county, Young Cove	45°57′N, 65°50′W	100	30115	DAO,O
C. nigra (L.) Reichard							
Nig402	Norway	Finnmark	Sør-Varanger, Svanvik	69°30′N, 30°05′E	23	28706	O
Nig734	Canada	Newfoundland	Avalon Peninsula, South Dildo	47°31′N, 53°33′W	1	30036	DAO,NFLD,O
C. paleacea Wahlenb.							
Pal278	Norway	Nordland	Lurøy, Aas	66°24′N, 13°08′E	0.1	28551	O
Pal826	Canada	Quebec	Gaspe, Baies-des-Capucins	49°02′N, 66°51′W	0.1	30136	O
C. rufina Drejer							
Ruf377	Norway	Vest Agder	Sirdal, Suleskar	59°01′N, 07°00′E	920	28680	O
Ruf440	Norway	Møre og Romsdal	Stranda, Geiranger	62°02′N, 07°17′E	1050	28769	O
C. stans Drejer							
Sta878	Greenland	Qeqertarsuaq	Blæsedalen	69°19′N, 53°28′W	3	30084	O,TROM
C. subspathacea Wormsk.							
Sub397	Norway	Finnmark	Sør-Varanger, Neidenfjorden	69°43′N, 29°39′E	0.2	28700	O
Sub767	Canada	Newfoundland	Labrador, N of Mary's Harbour	49°02′N, 66°51′W	0.1	30084	NFLD,O
C. torta Boott							
Tor813	Canada	New Brunswick	Victoria, Two Brooks	47°04′N, 67°18′W	150	30122	DAO,O
Tor860	Canada	Nova Scotia	Cape Breton Isl., Indian Brook	46°22′N, 60°32′W	5	30170	DAO,O
C. trinervis Degl.							
Tri590	Denmark	Jylland	Riebe, Fanø	55°23′N, 08°25′E	3	29312	O
Tri592	Denmark	Jylland	Riebe, Fanø	55°23′N, 08°25′E	2	29314	O

fingerprinting. The AFLP analysis followed that of Vos et al. (1995). In short 400 ng of DNA was digested with *Eco*RI and *Mse*I restriction endonucleases in 1 × RL buffer, 10 mM Tris acetate, 5 mM DTT and incubated at 37°C for 2 h. Adapters fitting EcoRI and MseI cutting sites were ligated to the ends of the restriction fragments by adding an adapter ligation solution plus T_4 DNA ligase and reactions were then incubated at 37°C for three hours. Preselective amplification of restriction fragments was performed using primers E01 (GACTGCGTACCAATTCA) and M01 (GATGAGTCCTGAGTAAA) following the PCR conditions outlined by Vos et al. 1995, viz. 30 cycles at 94°C for 30 s, 56°C for 1 min, 72°C for 1 min. The preamplifications were diluted between 20 × and 30 × with deionised water depending on product concentration and used as a template for selective amplification with *Eco*RI and *Mse*I primers with three selective nucleotides. The four combinations used for fingerprinting were E42/M33, E42/M40, E41/M32, E36/M36. The primer sequences read from the 5′ to the 3′ end are: E36* GACTG CGTACCAATTCACC, E41* GACTGCGTACCAATTC AGG, E42* GACTGCGTACCAATTCAGT, M32 GATG AGTCCTGAGTAAAAC, M33 GATGAGTCCTGAGTA AAAG, M36 GATGAGTCCTGAGTAAACC, M40 GAT GAGTCCTGAGTAAGGC (*radioactively labelled). The 20 µl PCR mix (30 ng Mprimer, 5 ng ^{33}P ATP labelled E-primer, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.5 U Taq polymerase) was run on a GeneAmp PCR system 9700 thermocycler, one cycle of 94°C for 30 s, 65°C for 30 s, 72°C for 1 min followed by 11 cycles of 0.7°C lower annealing temperature of each cycle and 24 cycles of 94°C for 30 s, completed by a final extension at 72°C for 10 min. Thereafter 20 µl loading dye (containing 99% formamide, 10 mM EDTA and 0.1% each of xylene cyanol FF and bromophenol blue) was added to the reaction mix at the end of the PCR run. After denaturing, 3 µl of this mixture was loaded on a 5% denaturing polyachrylamide gel (PAGE). The PAGE run was performed at 80W with 1 × TBE buffer in the upper buffer chamber and 2 × TBE in the lower chamber. The gel was fixed in 10% glacial acetic acid and dried. The bands were made visible by exposing X-ray films (Kodak BioMax MR) for 1–3 days depending on the signal strength. The different PAGE runs (each loaded with 60 reactions and two flanking lanes with a 30–330 bp AFLP ladder from invitrogen) were all scored. The presence (1) or absence (0) of bands was scored to produce a data matrix that was used for statistical analyses.

Statistical analyses

Pairwise genetic distance (Nei and Li 1979) was calculated using Treecon software (Van de Peer and De Wachter 1994) to generate both the NJ (neighbour joining, Saitou and Nei 1987) and UPGMA trees. Trees were rooted using the individuals of *C. bicolor* and support for all branches was determined using bootstrap analysis (1000 replicates), which was categorized as poor (<55%), weak (55–64%), moderate (65–74%), good (75–84%), very good (85–94%) and strong (95–100%) according to Ford et al. (2006).

Results and discussion

Each of the four AFLP primer combinations gave more than 100 polymorphic bands, and when the primer combinations were merged the resulting data set comprises 487 AFLP bands (i.e. 141 AFLPs with primer combination E42/M33, 106 with E41/M32, 133 with E42/M40 and 107 with E36/M36); 478 bands were polymorphic, nine bands monomorphic. *Carex trinervis* had the highest number of bands (234) and *C. rufina* the lowest (130). The average number of bands per taxon was 183.9, and the average number of bands per individual was 148.5.

We obtained five infrasectional clusters in a neighbour joining analysis (Fig. 1) as well as in an unweighted pair group method analysis (Fig. 2). These clusters do not entirely correspond to any previous known formal infrasectional classifications of section *Phacocystis* (Kükenthal 1909, Sylvén 1963, Faulkner 1973, Egorova 1999, Standley et al. 2002). However, the grouping agrees with the different taxonomists at certain degrees as elaborated below.

In the NJ analysis (Fig. 1) the species in cluster 1 are classified in subsections *Vulgares* and *Cespitosae* by Faulkner (1973), who unlike Kükenthal (1909) and Sylvén (1963) did not include *C. aquatilis* in the same subsection as *C. nigra*. However *C. trinervis* and *C. rufina* were not used in Faulkner's analysis. This cluster is composed of C. *acuta, C. trinervis, C. elata, C. nigra* and *C. cespitosa* and is believed to form a natural group (Luceño and Aedo 1994).

The second group comprises *C. rufina* only and could perhaps be included in group 1 as a somewhat less related species, but *C. rufina* is more prominently separated from this group in the UPGMA analysis (Fig. 2). It has a slightly higher chromosome number (2n = 86) than the other species of section *Phacocystis* investigated (2n = 66–85; Table 1).

The third group is in agreement with earlier work based on morphological, chromosomal and interfertility data (Standley 1990), which indicate that *C. aquatilis* and *C. bigelowii* are very closely related and should be included in the same subsection. *Carex paleacea* and *C. subspathacea* have also nearly always been placed near each other (Kükenthal 1909, Sylvén 1963, Faulkner 1973, Egorova 1999), and sometimes in a separate section *Crytocarpae* (Mackenzie 1935b). Although these two species are undoubtedly closely related, our study does not support them as a distinct section or subsection, since they are also closely joined to *C. aquatilis* and *C. bigelowii* ssp. *rigida*.

The fourth group includes the two American species *C. gynandra* and *C. torta* only, and because they are the best separated group in the UPGMA analysis (Fig. 2) they may merit being included in a separate subsection. Although *C. gynandra* has the long awned glumes of *C. paleacea* they are in our opinion not very closely related. But note that Kükenthal (1909) places *C. gynandra* in subsection *Cryptocarpae* as *C. crinita* Lam. var. *gynandra* and *C. torta* in section *Praelongae*, but he also describes their hybrid.

In the UPGMA analysis (Fig. 2) the five infrasectional clusters in section *Phacocystis* are in even greater agreement with literature on morphology of the species than those of the NJ analysis. We have defined the UPGMA clustering by cutting the dendrogram at genetic distance value of 0.4. The

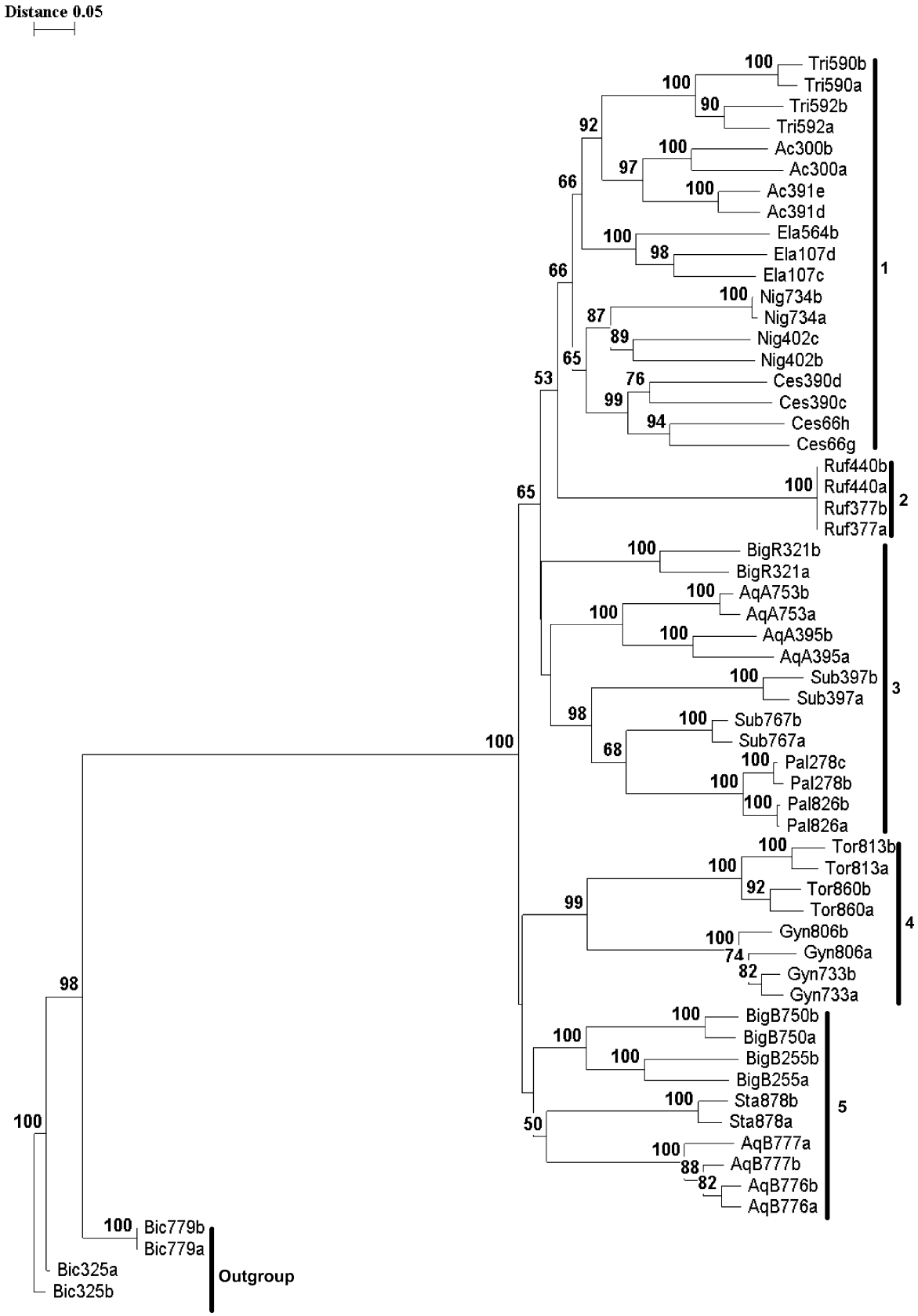

Fig. 1. Neighbour joining (NJ) analysis for *Carex* section *Phacocystis*. Abbreviations of species (from below upwards): Bic = *C. bicolor*; AqB = *C. aquatilis* taxon B; Sta = *C. stans*; BigB = *C. bigelowii* ssp. *bigelowii*; Gyn = *C. gynandra*; Tor = *C. torta*; Pal = *C. paleacea*; Sub = *C. subspathacea*; AqA = *C. aquatilis* ssp. *aquatilis*; BigR = *C. bigelowii* ssp. *rigida*; Ruf = *C. rufina*; Ces = *C. cespitosa*; Nig = *C. nigra*; Ela = *C. elata*; Ac = *C. acuta*; Tri = *C. trinervis*. For population numbers see Table 2.

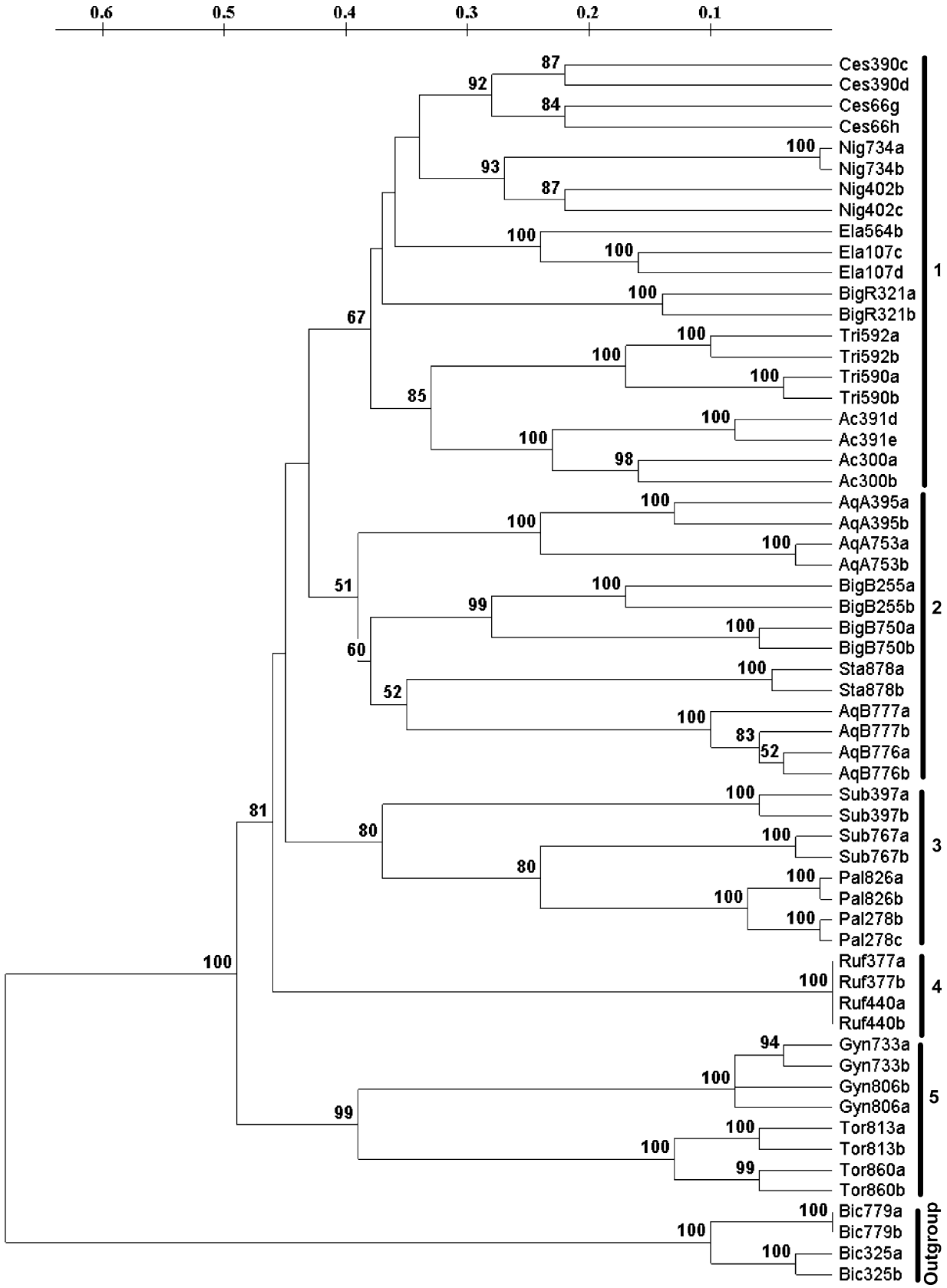

Fig. 2. Unweighted pair group method analysis (UPGMA) for *Carex* section *Phacocystis*. Abbreviations of species (from below upwards): Bic = *C. bicolor*; Tor = *C. torta*; Gyn = *C. gynandra*; Ruf = *C. rufina*; Pal = *C. paleacea*; Sub = *C. subspathacea*; AqB = *C. aquatilis* taxon B; Sta = *C. stans*; BigB = *C. bigelowii* ssp. *bigelowii*; AqA = *C. aquatilis* ssp. *aquatilis*; Ac = *C. acuta*; Tri = *C. trinervis*; BigR = *C. bigelowii* ssp. *rigida*; Ela = *C. elata*; Nig = *C. nigra*; Ces = *C. cespitosa*. For population numbers see Table 2.

only fundamental differences between the NJ and UPGMA analyses are that in the UPGMA analysis the European *C. bigelowii* subsp. *rigida* is included in the first cluster containing the European group of species, and that the two American forms of *C. aquatilis* are included in the same cluster. Also subsection *Cryptocarpae* comes out as better separated from subsection *Rigida* in the UPGMA analysis.

Carex bicolor All.

This species was used as an outgroup in our analysis (Fig. 1, 2) because according to Ball (2002) *C. bicolor* belongs to *Carex* sect. *Bicolores*, a small section of four species, and not to sect. *Phacocystis*. *Carex bicolor* is very clearly separated from the other investigated species and is in a strongly supported (BS = 100) lineage. *Carex bicolor* has a lower chromosome number (2n = 52) than the other species investigated (2n = 66–86, Table 1). Mackenzie (1935a) included a fifth species, *C. rufina*, in this section. We obtained a total number of 148 AFLP bands for *C. bicolor* with an average of 135 (131, 131, 136, 142), which is the second lowest. Our UPGMA and NJ analyses data indicate that *C. bicolor* may not belong to section *Phacocystis*, but that *C. rufina* does belong here. Ball (2002) maintains that the four species in sect. *Bicolores* (i.e. *C. bicolor*, *C. garberi* Fernald, *C. hassei* L. H. Bailey and *C. aurea* Nutt.) form a morphological continuum with only weak discontinuities, that putative hybrids between the species appear to be fertile and difficult to recognise, and that intersectional hybrids involving members of section *Bicolores* are uncommon.

Carex rufina Drejer

This species is most unusual in that the four investigated plants from two Norwegian sites separated by 350 km of high mountains showed no genetic variation using four different primers (Fig. 1, 2). We obtained a total number of 130 AFLP bands for this species with an average of 130 (as all plants had the same bands), the lowest of all investigated taxa. This can only be explained by the two populations having survived the last glaciation as one population experiencing bottleneck conditions either during glaciation or during the immigration period (Abbott and Brochmann 2003). New investigations using material from more sites both from Europe and America is needed to throw more light on its genetic variation and immigration history.

However, our results show that *C. rufina* is not a high alpine or arctic variant of *C. nigra* or *C. bigelowii*, which could have been the case according to gross morphology. According to our UPGMA and NJ analyses data *C. rufina* is not particularly closely related to any of the investigated taxa. Our results also support that *C. rufina* belongs to section *Phacocystis* although placed by Mackenzie (1935a) in sect. *Bicolores*. According to Standley et al. (2002) *C. rufina* is closely related to *C. eleusinoides* Kunth and *C. lenticularis* Michx. This statement can only be confirmed or rejected by including samples of all the three relevant species in a new molecular analysis.

Carex nigra (L.) Reichard, including *C. juncella* (Fr.) Th. Fr.

In the NJ analysis *C. nigra* is in a fairly supported cluster (BS = 68) together with C. *acuta, C. trinervis, C. elata* and *C. cespitosa*, four species known from the European side of the Atlantic only. We obtained a total number of 183 AFLP bands for this species with an average of 132 (124, 128, 138, 138), which is near average number.

Plants of *Carex nigra* are variable in height and degree of development of rhizomes and stolons, but are not separable into distinct taxa. The cluster analysis shows that there is some considerable genetic distance between the populations from Norway and Canada. The shorter branches of the populations from Canada in comparison to those from Norway seem to be in agreement with Chater's (1980) statement that there is much less morphologic or ecotypic variation in plants from North America than in Europe. In North America, *C. nigra* hybridizes with *C. stricta*, *C. aquatilis*, *C. paleacea*, *C. subspathacea*, and *C. salina*. It is a member of the *C. acuta* subgroup, based on the veined, stipitate perigynia distended by the base of the achenes (Standley et al. 2002).

Carex cespitosa L.

This is a characteristic species not known to America. We obtained a total number of 214 AFLP bands for this species with an average of 146 (144, 144, 147, 148), which is among the highest numbers. Our analyses (Fig. 1, 2) show *C. cespitosa* to be genetically most similar to *C. nigra* s.l. Both the UPGMA and NJ analyses show that the populations from Norway and Finland had about the same amount of genetic variation. In most parts of Europe *C. cespitosa* is easily recognized, but towards the north and east it is more polymorphic, and Egorova (1999) recognizes two additional closely related species from Russia, viz. *C. schmidtii* Meinsh. and *C. minuta* Franch. *C. cespitosa* is believed to hybridize with *C. acuta, C. aquatilis, C. nigra*, *C. elata* and *C. schmidtii* (Egorova 1999, Elven in Lid and Lid 2005), but some of the hybrid records are doubtful.

Carex elata All.

This is a very tussocky species not known from America. We obtained a total number of 214 AFLP bands for this species with an average of 129 (100, 142, 145), which is among the highest total numbers. Our analyses (Fig. 1, 2) show *C. elata* to be genetically most similar to *C. acuta* and *C. trinervis*, but also similar to *C. nigra* s.l. and *C. cespitosa*. The populations from Sweden and Norway are well separated, but the Swedish population was sampled late in the year and did not give good result. This species hybridizes mainly with *C. acuta* and *C. nigra* (Egorova 1999) and is found in a major cluster containing both these two species, but with closer relationship to *C. acuta*. In most parts of Europe *C. elata* is not very variable morphologically, but towards the east it is more polymorphic, and Jalas and Hirvelä (1964) and Egorova (1999) recognize an additional subspecies from Finland and Russia, viz. ssp. *omskiana* (Meinsh.) Jalas. Future molecular work should

study both these taxa and include material of ssp. *elata* from the type locality in Italy.

Carex acuta L.

This is another species not known to America. We obtained a total number of 223 AFLP bands for this species with an average of 168 (156, 163, 175, 176), which is the next highest number. Our analyses (Fig. 1, 2) show *C. acuta* to be genetically most similar to *C. trinervis* and less so to *C. elata*, *C. nigra* and *C. cespitosa*. This species generates partly fertile hybrids with particularly *C. nigra*, *C. aquatilis* and *C. elata* (Egorova 1999, Elven in Lid and Lid 2005). More molecular work is needed to clarify the taxonomic position of *C. acuta* versus *C. aquatilis* and their hybrid in northern Scandinavia.

Carex trinervis Degl.

This is a rare dune-species not known from America. It is found scattered along the west European Atlantic coasts from Portugal to Denmark (Foley 2005). We obtained a total number of 234 AFLP bands for this species with an average of 192 (177, 191, 196, 203), which is the highest number in our investigated species. As this species is very similar to *C. nigra*, with which it hybridizes, it is surprising that the species both in our NJ and UPGMA analyses (Fig. 1, 2) comes out as closer to *C. acuta* than to *C. nigra*. Our data seems to indicate that *C. trinervis* is a good species.

Carex bigelowii Torr. ex Schwein. ssp. *bigelowii* and *C. bigelowii* ssp. *rigida* W. Schultze-Motel

Carex bigelowii is a highly variable species with four subspecies recognized in Europe (Chater 1980) and two subspecies in North America (Standley et al. 2002). The North American populations can be divided into an eastern and a western taxon (ssp. *bigelowii* and spp. *lugens* respectively), which cannot always be distinguished morphologically (Standley et al. 2002). For genetic variation along the arctic Eurasian coast see Stenström et al. (2001). We obtained a total number of 223 AFLP bands for ssp. *bigelowii* (143, 151, 152, 177 with average 156), and 165 AFLP bands for ssp. *rigida* (145, 148 with average 147). In our analysis the populations from Europe (ssp. *rigida*) and America (ssp. *bigelowii*) fall in two different clusters both in the UPGMA and NJ analyses. This may indicate that ssp. *rigida* could be considered a separate species, but due to the weakly supported main clades in our analyses many more populations particularly from Siberia (and including all subspecific taxa), must be investigated for obtaining reliable results.

Tall plants of ssp. *bigelowii* may be confused with *C. aquatilis* (including *C. stans*, i.e. *C. aquatilis* var. *minor* in Standley et al. 2002), but *C. bigelowii* can be distinguished by the short bracts, hypostomic leaves, and dull achenes. These two taxa have been reported to hybridize (Duman and Kryszczuk 1958), while ssp. *rigida* and *C. aquatilis* have been artificially hybridized by Faulkner (1973), who found that these two taxa were very closely related. Although the Nordic population of *C. bigelowii* ssp. *bigelowii* is morphologically distinct from ssp. *rigida*, the Canadian population of ssp. *bigelowii* studied is less distinct. Detailed morphological as well as more extensive molecular research is needed to solve their taxonomic position and whether they are sibling taxa or not.

Carex aquatilis Wahlenb.

This wetland species is very widespread throughout the northern cold and temperate regions. We obtained a total number of 172 AFLP bands for this species with an average of 130 (117, 121, 139, 144). In our NJ analysis *C. aquatilis* occurs in the same cluster as *C. paleacea*, while in the UPGMA analysis the saline species *C. paleacea* and *C. subspathacea* form a distinct separate cluster. *Carex aquatilis* is most similar to *C. stans* and *C. bigelowii*. Further work should focus on this relationship and its many hybrids, e.g. with *C. acuta* and *C. paleacea* (Cayouette and Morisset 1986b). Also, more research is needed with regards to its recognized American varieties.

Carex aquatilis taxon B.

Although this taxon from Newfoundland is considered as typical *C. aquatilis* by American botanists, it is morphologically as well as genetically atypical and should be further studied. We obtained a total number of 194 AFLP bands for this taxon with an average of 175.5 (166, 173, 180, 183), which is the next highest average number. It seems to be most closely related to *C. bigelowii* subsp. *bigelowii*, while typical *C. aquatilis* is more closely related to *C. bigelowii* subsp. *rigida*.

Carex stans Drejer

This species is either considered a good species (Egorova et al. 1966), as a subspecies (Hultén 1964, Egorova 1999, Elven in Lid and Lid 2005) or variety (Standley et al. 2002) of *C. aquatilis*, or as a hybrid between *C. aquatilis* and *C. bigelowii* (Nilsson 1986). We obtained a total number of 182 AFLP bands for this species with an average of 173 (165, 181). The Greenland population is the true *C. stans*. The investigated Norwegian collection has been named alternatively *C. stans* and C. cf. *bigelowii*. It is morphologically very similar to *C. stans* from Greenland, but genetically it clusters with Canadian *C. bigelowii* and is probably *C. bigelowii* subsp. *bigelowii*, which may be specifically distinct from ssp. *rigida*. Future work will prove or disprove whether *C. stans* is a good species, and whether it occurs outside Greenland.

Carex paleacea Schreber ex Wahlenb.

The cluster analyses reveal that populations from Norway and those from America have about the same genetic variation. We obtained a total number of 161 AFLP bands for this species with an average of 138.5 (136, 136, 139, 143). According to the UPGMA analysis only *C. rufina* (above) and the American populations of *C. nigra* and

C. bigelowii had almost as low genetic diversity. One plant of this species was found to be self compatible by Faulkner (1973), and it seems *C. paleacea* is structurally adapted for this, because its inflorescence is often much shorter than and concealed by the leaves. *Carex paleacea* is genetically similar to *C. subspathacea* despite the many morphological differences between these two species. Many *C. paleacea* hybrids or hybrid backcrosses are given specific names, e.g. *C. salina* Wahlenb., *C. vacillans* Drejer, *C. recta* Boott and *C. halophila* Nyl. More molecular data are needed from more regions for both *C. paleacea* and its hybrids to assess their molecular diversity more fully.

Carex subspathacea Wormsk.

Carex subspathacea is the species enduring the highest salinity in sect. *Phacocystis*, and with *C. rufina*, is the smallest species of the section. Larger plants with one to two staminate spikes and some bisexual spikes have been found in America, but their perigynium and achene characteristics are typical of *C. subspathacea* (Standley et al. 2002). We obtained a total number of 175 AFLP bands for this species with an average of 132 (112, 123, 144, 150). In our analyses *C. subspathacea* is found in a well supported cluster together with *C. paleacea*, but the Canadian population investigated is more similar to *C. paleacea* than the European population.

Carex torta Boott

Carex torta is a strongly tussocky early flowering river shore species in eastern North America (Standley et al. 2002). We obtained a total number of 183 AFLP bands for this species with an average of 156 (153, 156, 156, 160). It is a distinct species and in our NJ and UPGMA it forms a clade with *C. gynandra*, which is not morphologically similar. In fact Kükenthal (1909) did not include *C. torta* in sect. *Phacocystis* (*Acuta*).

Carex gynandra Schwein.

Carex gynandra is another species native to eastern North America (Standley 1983, Bruederle and Fairbrothers 1986, Standley et al. 2002). We obtained a total number of 161 AFLP bands for this species with an average of 143 (131, 142, 147, 151). In our NJ and UPGMA analyses the two populations group together with another endemic North American species, viz. *C. torta*, but these two species are not morphologically similar. Further DNA studies should be undertaken including material of the two very similar species *C. crinita* Lam. and *C. mitchelliana* M. A. Curtis.

Conclusion

All investigated species were clearly separated using the AFLP technique. The NJ indicates that *C. bicolor* may not belong to section *Phacocystis* (and thus confirming the views of Ball 2002), while all other investigated species clearly belong to this section. However, the NJ does not give strong evidence for a subsectional division of the section as the major clades are not strongly supported. The sorting of the species according to NJ and UPGMA is mostly in accordance with accepted taxonomy, but with the exceptions that the American *C. bigelowii* may be specifically distinct from European *C. bigelowii* ssp. *rigida*, and *C. stans* should perhaps not be considered a subspecies of *C. aquatilis*, but either as a separate species or as a hybrid between *C. aquatilis* and *C. bigelowii*. North American *C. aquatilis* is heterogenic and should be further studied. However more work is needed as regards *C. bigelowii* and the relationship between *C. stans* and *C. aquatilis*.

Faulkner (1973) found that *C. aquatilis* was interfertile with *C. bigelowii* ssp. *rigida* and therefore classified them in the same subsection. Our results agree with this as seen in the NJ tree where *C. bigelowii* subsp. *rigida* from Norway is in the same subcluster as *C. aquatilis*, while the UPGMA tree shows these two species in the same cluster. The results also show that *C. bigelowii* as well as *C. aquatilis* are genetically very variable. Our results are supported by Standley (1986, 1990), who documented anatomical heterogeneity in *C. aquatilis*, since some plants of this species had epistomatic leaves and others amphistomatic leaves.

Our results are in agreement with Faulkner's (1973) grouping of the European species in this section and the merging of the two subsections *Vulgares* and *Cespitosae* and perhaps maintaining subsections *Rigidae* and *Cryptocarpae*. However, the species forming the most distinct group in Fig. 2 are *C. torta* and *C. gynandra*, two species which are morphologically different, and they consequently form the group, which first have to be accepted as a subsection or new section, but at present we think sect. *Phacocystis* should not be split.

Although not including all North Atlantic species of section *Phacocystis* our work adds considerable new knowledge on the relationships of the species in this section. It is the first study using AFLP markers to study the relationship between many species of *Carex* sect. *Phacocystis*.

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