

## ***Carex*, subgenus *Carex* (Cyperaceae) – A phylogenetic approach using ITS sequences**

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**Abstract.** To evaluate the sectional classification in *Carex*, subgenus *Carex*, the ITS region of 117 species belonging to 32 sections was analyzed with Neighbor Joining (NJ) and Markov chain Monte Carlo (MCMC) methods. In our analyses (1) species of subgenus *Indocarex* appear as a statistically well supported group within subgenus *Carex*. (2) The representatives of sections *Vesicariae*, *Hirtae*, *Pseudocypereae*, *Ceratocystis*, *Spirostachyae*, *Bicolores*, *Paniceae*, *Trachychlaenae*, *Scirpinae*, *Atratae* and *Albae* group in statistically supported clades with higher support in MCMC than in NJ. (3) *C. rariflora* clusters with representatives of section *Limosae*, however only weakly supported. (4) Taxa of section *Phacocystis* are divided in two statistically supported subclusters that are closely related to a core group of section *Hymenochlaenae*. (5) Species of sections *Montanae*, *Pachystylae*, *Digitatae*, *Phacocystis*, *Rhomboidales*, *Careyanae* and *Frigidae* are segregated into two or more clusters each. (6) Five species of section *Frigidae* cluster together, whereas the seven others are in scattered positions. Based on these results, delimitation of sections is discussed.

**Key words:** Bayesian analysis, *Carex*, ITS, molecular phylogeny, systematics.

The genus *Carex* L. is widespread mainly in the northern hemisphere with approximately

2000 species (Reznicek 1990). In a worldwide taxonomic survey of the genus Kükenthal (1909) confirmed the subdivision of the genus *Carex* into the subgenera (Eu-) *Carex*, *Indocarex*, *Vignea* and *Primocarex*. Subgenus *Carex* comprises some three quarters of the species (Kükenthal 1909, Mackenzie 1931–1935, Chater 1980, Ball 1990). They have a worldwide distribution, with most of them occurring in the northern hemisphere. The inclusion of subgenus *Indocarex*, often assumed as not clearly separable from subgenus *Carex* (Koyama 1962, Reznicek 1990), would considerably enlarge the geographic distribution of subgenus *Carex* to the subtropics and tropics of East Asia and Central America. Kükenthal (1909) distinguished 48 sections and subsections within subgenus *Carex*. Since then, many additional species have been described but no new classification on a worldwide scale has been proposed.

Recent molecular phylogenetic studies in Cyperaceae using the *rbcL* gene (Muasya et al. 1998), chloroplast DNA sequences (Yen and Olmstead 2000), and ITS data (Roalson et al. 2001, Starr et al. 1999, Waterway and Olmstead 1998) have given new insight in the relationships between the

genera of Cariceae. Mainly species of section *Acrocystis* have been analyzed by Roalson et al. (2001). That study focused on the North American species and has shown the potential of the ITS region for phylogenetic interpretations on sectional level. We studied 62 species mainly from northern Europe; furthermore, sequences of 55 species derived from GenBank were included in this work (see Table 1). Thus, our molecular analyses comprise 117 species of 32 sections in total. Section delimitations will be discussed in detail and compared with the classical concept proposed by Kükenthal (1909).

## Materials and methods

**Plant collection and DNA extraction.** The analyzed *Carex* species are listed in Table 1. Assignment of sections and subsections corresponds mainly to the concept of Kükenthal (1909). Total genomic DNA was isolated from fresh or dried leaf tissue either by crushing the plant material in liquid nitrogen or by shaking the samples for 3 min at 30 Hz (Mixer Mill MM 300, Retsch, Haan, Germany). DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) was used following the manufacturer's protocol.

**PCR and sequencing.** The ITS region (about 700 bp) localized between the 18S and the 28S rRNA genes, was amplified with the primer pair ITS1 (Hsiao et al. 1995) or ITS5, respectively, and ITS4 (White et al. 1990). Amplification parameters were as described in Starr et al. (1999). We adjusted the annealing temperature to 54 °C for ITS5 and 51 °C for ITS1, the annealing time to 55 s, and the extension time to 3 min. The product was purified with QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). The dsDNA obtained was sequenced directly on both strands using the ABI PRISM Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems) on an automated sequencer (ABI 373A, PE Applied Biosystems). The sequences of both strands were combined and proof read with Sequencher™ 4.1 software (Gene Codes Corp., Michigan).

All sequences reported in this study have been deposited in GenBank (see Table 1).

The alignment contained 638 nucleotide sites. After removing ambiguously aligned positions

(221–237, 416–436, 588–595), 592 sites remained for analyses with 246 variable sites (ITS1: 141, 5.8S: 6, ITS2: 99). The ingroup alone contained 238 variable sites. The alignment is available upon request.

**Phylogenetic analysis.** DNA sequences were aligned using Clustal X (Jeanmougin et al. 1998). Some manual corrections were done in Se-AL v2.0a7b (Rambaut 2001) to improve ambiguously aligned positions.

The likelihood ratio test as implemented in Modeltest 3.0 (Posada and Crandall 1998) selected GTR + I + G (Swofford et al. 1996) as DNA substitution model (details below).

A Bayesian method of phylogenetic inference using a Metropolis-coupled Markov chain Monte Carlo (MCMC) approach was carried out as implemented in the program MrBayes (Huelsenbeck and Ronquist 2001) with GTR + I + G as substitution model. Four incrementally heated simultaneous Monte Carlo Markov chains were run over 2 000 000 generations. Trees were sampled every 100th generation, resulting in an overall sampling of 20 000 trees. To obtain estimates for the *a posteriori* probabilities, a 50% majority rule consensus tree was computed from the trees sampled after the process had reached stationarity (burnin = 2000). This Bayesian approach of phylogenetic analysis was repeated eight times, always using random starting trees and random starting values for the model parameters to test the reproducibility of the results. Branch lengths were estimated under the maximum likelihood criterion and the same substitution model in PAUP 4.0b10 (Swofford 2002).

Neighbor joining analysis (Saitou and Nei 1987) was done with PAUP 4.0b10 (Swofford 2002) using genetic distances according to GTR + I + G as substitution model with the following settings: base frequencies A = 0.154109, C = 0.307645, G = 0.366090, T = 0.172156; rate matrix AC = 1.11373, AG = 3.97984, AT = 0.93190, CG = 0.32777, CT = 6.41726, GT = 1.00000; proportion of invariant nucleotide sites = 0.495368 and gamma distribution shape parameter = 0.729428. Support for internal nodes was estimated by 1000 neighbor joining bootstrap replicates under the same model settings.

The unrooted phylograms from neighbor joining and MCMC analyses were rooted with three *Carex* species of subgenus *Vignea* that clustered together with high bootstrap support.

**Table 1.** Species analyzed in this study

Species	Section and subsection <sup>a</sup>	Chromos. no. (2n) <sup>b</sup>	Locality/Voucher <sup>c</sup>	GenBank accession no.
<i>C. acuta</i> L.	( <i>Phacocystis</i> Dumort.)		USSR, Siberia*	AF284992
<i>C. acutiformis</i> Ehrh. <sup>a</sup>	<i>Paludosae</i> Fries	78	Germany, FO 7501	AY278300
<i>C. acutiformis</i> <sup>b</sup>			USSR, Kazakh SSR*	AF284993
<i>C. alba</i> Scop.	<i>Albae</i> Asch. & Graebn.	54	Switzerland, HMH 2857	AY278259
<i>C. alma</i> Bailey	<i>Multiflorae</i> Kunth		USA, California*	AF285025
<i>C. angarae</i> Steud.	<i>Atratae</i> Kunth		USSR, Magadan Oblast*	AF284980
<i>C. angustata</i> Boott	( <i>Phacocystis</i> Dumort.)	66, 68	USA, Idaho*	AF285015
<i>C. antoniensis</i> A. Chev.	( <i>Pseudocypereae</i> Tuck.)		Cape Verde Islands, Santo Antao*	AF285041
<i>C. aquatilis</i> Wahlenb. <sup>a</sup>	( <i>Phacocystis</i> Dumort.)	72, 74, 76, 79–80, 84	Canada, Salt Plains, TUB	AY278302
<i>C. aquatilis</i> <sup>b</sup>			Finland, HeRB 4634	AY278301
<i>C. atrata</i> L.	<i>Atratae</i> Kunth	54	Sweden, HMH 2758	AY278263
<i>C. atrofusca</i> Schkuhr	<i>Frigidae</i> Fries, <i>Fuliginosae</i> Tuck.	36, 38, 40	Sweden, HMH 2652	AY278313
<i>C. aurea</i> Nutt.	( <i>Bicolores</i> Tuck.)	52	USA, California*	AF285062
<i>C. austroalpina</i> Becherer	<i>Frigidae</i> Fries, <i>Ferrugineae</i> Tuck.	40	Italy, HeRB 4252	AY278276
<i>C. bella</i> Bailey	<i>Atratae</i> Kunth	40	USA, New Mexico*	AF284966
<i>C. bicolor</i> All.	<i>Acutae</i> Fries, <i>Bicolores</i> Tuck.	50, 52	Switzerland, FO 11601	AY278283
<i>C. bigelowii</i> Torr. ex Schwein.	( <i>Phacocystis</i> Dumort.)	68, 70, 71	Finland, HeRB 4626	AY278303
<i>C. brachystachys</i> Schrank (& Moll)	<i>Frigidae</i> Fries, <i>Curvicolles</i> Kük.	40	Spain, Pyrenees, HeRB 5336	AY278277
<i>C. brevicollis</i> DC.	<i>Rhomboidales</i> Kük.	56	USSR, Moldavian SSR*	AF285011
<i>C. brunnea</i> Thunb.	( <i>Graciles</i> Tuck.)	62	China, Guizhou*	AF285003
<i>C. buxbaumii</i> Wahlenb.	<i>Atratae</i> Kunth	74, 106	Germany, HMH 1896	AY278262
<i>C. canescens</i> L.	<i>Canescentes</i> Fries		USSR, Siberia*	AF284990
<i>C. capillaris</i> L.	( <i>Capillares</i> Asch. & Graebner)	54	Sweden, HMH 2651	AY278256
<i>C. castanea</i> Wahlenb.	<i>Hymenochlaenae</i> Drejer, <i>Longirostres</i> Kük.	44, 64?	USA, Vermont*	AF285058
<i>C. communis</i> Bailey	<i>Montanae</i> Fries	28	Canada, Quebec*	AF284976
<i>C. concinnoides</i> Mack.	( <i>Digitatae</i> Fries)		USA, Oregon*	AF284965
<i>C. debilis</i> Michx.	<i>Hymenochlaenae</i> Drejer, <i>Debiles</i> Carey	52, 54, 56	USA, Texas*	AF285029
<i>C. demissa</i> Hornem.	( <i>Ceratocystis</i> Dumort.)		Germany, HeRB 2761	AY278307
<i>C. digitalis</i> Willd.	<i>Careyanae</i> Tuck.	48	USA, North Carolina*	AF285035
<i>C. digitata</i> L.	<i>Digitatae</i> Fries, <i>Eu-Digitatae</i> Kük.	48, 50, 52	Germany, WM 364	AY278267
<i>C. distans</i> L.	<i>Spirostachyae</i> Drejer	68?, 70–72, 74	Germany, HMH 1854	AY278312
<i>C. donnell-smithii</i> Bailey	<i>Fecundae</i> Kük.		Mexico, Chiapas*	AF285005
<i>C. eburnea</i> Boott	<i>Albae</i> Asch. & Graebn.	54	Canada, British Columbia*	AF285000
<i>C. elata</i> All.	( <i>Phacocystis</i> Dumort.)	74, 76	Germany, HeRB 4177	AY278255

**Table 1** (continued)

Species	Section and subsection <sup>a</sup>	Chromos. no. (2n) <sup>b</sup>	Locality/Voucher <sup>c</sup>	GenBank accession no.
<i>C. eleusinoides</i> Turcz.	( <i>Phacocystis</i> Dumort.)	ca. 60, 84	USSR, Buryatskaya ASSR*	AF285006
<i>C. ericetorum</i> Pollich	<i>Montanae</i> Fries	30	Germany, HeRB 3929	AY278281
<i>C. exsiccata</i> Bailey	<i>Physocarpae</i> Drejer, <i>Vesicariae</i> Tuck.		Canada, British Columbia*	AF285055
<i>C. extensa</i> Good.	<i>Spirostachyae</i> Drejer	60	Germany, HeRB 1557	AY278311
<i>C. falcata</i> Turcz.	<i>Paniceae</i> Tuck.		USSR, Magadan Oblast*	AF285016
<i>C. ferruginea</i> Scop.	<i>Frigidae</i> Fries, <i>Ferrugineae</i> Tuck.	40	France, HMH 2082	AY278275
<i>C. filicina</i> Nees	<i>Indicae</i> Tuck., <i>Gracilirostres</i> Kük.	44, 48	China, Sichuan*	AF284981
<i>C. firma</i> Host	<i>Frigidae</i> Fries, <i>Ferrugineae</i> Tuck.	34	Germany, HMH 1180	AY278279
<i>C. flacca</i> Schreb.	<i>Trachychlaenae</i> Drejer	76, 90?	Germany, HMH 2993	AY278274
<i>C. flava</i> L.	( <i>Ceratocystis</i> Dumort.)	60, 62	Germany, HMH 1869	AY278310
<i>C. frigida</i> All.	<i>Frigidae</i> Fries, <i>Fuliginosae</i> Tuck.	56	Switzerland HeRB 6360	AY278291
<i>C. fuliginosa</i> Schkuhr	<i>Frigidae</i> Fries, <i>Fuliginosae</i> Tuck.	40	Sweden, HMH 2729	AY278254
<i>C. gigas</i> (Holm) Mack.	<i>Scirpinae</i> Tuck.	58	USA, California*	AF285027
<i>C. globularis</i> L.	<i>Pachystylae</i> Kük.		USSR, Magadan Oblast*	AF285049
<i>C. gracillima</i> Schwein.	<i>Hymenochlaenae</i> Drejer, <i>Gracillimae</i> Carey	50, 52, 54	USA, Vermont*	AF285054
<i>C. grioletii</i> Roemer	<i>Pachystylae</i> Kük.	48	USSR, SFSR, Sochi*	AF285048
<i>C. hirta</i> L.	<i>Hirtae</i> Tuck.	112, (114?)	Germany, HMH 513	AY278296
<i>C. hispida</i> Willd. ex Schkuhr	<i>Trachychlaenae</i> Drejer	42	Italy, FO 9266	AY278272
<i>C. hostiana</i> DC.	( <i>Ceratocystis</i> Dumort.)	56	France, HMH 2140	AY278309
<i>C. humilis</i> Leysser	<i>Digitatae</i> Fries, <i>Eu-Digitatae</i> Kük.	36	Germany, WM 360	AY278260
<i>C. kitaibeliana</i> Degen ex Becherer	( <i>Frigidae</i> Fries)	36	Bosnia, FO 16810	AY278258
<i>C. lanceolata</i> Boott	<i>Digitatae</i> Fries, <i>Eu-Digitatae</i> Kük.	68–80	Japan, Kanagawa Pref.*	AF285009
<i>C. lasiocarpa</i> Ehrh.	<i>Hirtae</i> Tuck.	(56?), 76, 77	Sweden, HMH 2788	AY278297
<i>C. laxiflora</i> Lam.	<i>Careyanae</i> Tuck.	40?	USA, Texas*	AF284964
<i>C. lemmonii</i> Boott	( <i>Ferrugineae</i> Tuck.)		USA, California*	AF284971
<i>C. lepidocarpa</i> Tausch	( <i>Ceratocystis</i> Dumort.)		Germany, HeRB 1511	AY278293
<i>C. leucodonta</i> Holm	<i>Montanae</i> Fries		USA, Arizona*	AF284973
<i>C. limosa</i> L.	<i>Limosae</i> Tuck.	64	Austria, FO 21960	AY278298
<i>C. liparocarpos</i> Gaudin	<i>Lamprochlaenae</i> Drejer	38	Italy, HeRB 4258	AY278261
<i>C. lupulina</i> Muehlenb. ex Willd.	<i>Physocarpae</i> Drejer, <i>Lupulinae</i> Tuck.	56	USA, Texas*	AF284963
<i>C. lurida</i> Wahlenb.	<i>Physocarpae</i> Drejer, <i>Tentaculatae</i> Tuck.	64, 66	USA, North Carolina*	AF284962

**Table 1** (continued)

Species	Section and subsection <sup>a</sup>	Chromos. no. (2n) <sup>b</sup>	Locality/Voucher <sup>c</sup>	GenBank accession no.
<i>C. luzulina</i> Olney	<i>Frigidae</i> Fries, <i>Fuliginosae</i> Tuck.		USA, Washington, FO 30632	AY278252
<i>C. mairii</i> Cosson & Germ.	<i>Spirostachyae</i> Drejer	68, 70	France, FO 9499b	AY278253
<i>C. mandshurica</i> Meinsh.	<i>Pachystylae</i> Kük.		Korea, Kangwon Province*	AF285045
<i>C. microdonta</i> Torr. & Hook.	( <i>Granulares</i> O. Lang)	64	USA, Texas*	AF285052
<i>C. mira</i> Kük.	<i>Frigidae</i> Fries, <i>Mucronatae</i> Nyman	42	Korea, Kangwon Prov.*	AF285046
<i>C. montana</i> L.	<i>Montanae</i> Fries	38	Germany, HMH 1421	AY278271
<i>C. mucronata</i> All.	<i>Frigidae</i> Fries, <i>Mucronatae</i> Nyman	36	Italy, HMH 2240	AY278257
<i>C. nigra</i> (L.) Reich. ssp. <i>juncella</i>	( <i>Phacocystis</i> Dumort.)	84	Germany, FO 29072	AY278304
<i>C. norvegica</i> Retz. ssp. <i>media</i>	<i>Atratae</i> Kunth	56	Switzerland, HMH 3004	AY278264
<i>C. olbiensis</i> Jordan	<i>Careyanae</i> Tuck.	46	Italy, FO 9331	AY278282
<i>C. ornithopoda</i> Willd.	<i>Digitatae</i> Fries, <i>Eu-Digitatae</i> Kük.	54	Germany, HMH 2964	AY278269
<i>C. ornithopodioides</i> Hausm.	<i>Digitatae</i> Fries, <i>Eu-Digitatae</i> Kük.	54, 56	Austria, HeRB 6362	AY278268
<i>C. otrubae</i> Podp.	<i>Stenorhynchae</i> Holm		USSR, Crimea*	AF284996
<i>C. oxyandra</i> Kudo	( <i>Montanae</i> Fries)	18, 20, 24	Japan*	AF285061
<i>C. pallescens</i> L.	<i>Pachystylae</i> Kük.	64, 66, 70?	France, HMH 2105	AY278299
<i>C. panicea</i> L.	<i>Paniceae</i> Tuck.	32	Germany, HMH 1779	AY278284
<i>C. parviflora</i> Host	<i>Atratae</i> Kunth	54	Switzerland, HeRB 6361	AY278265
<i>C. paupercula</i> Michx.	( <i>Limosae</i> Tuck.)	58, 60	Finland, HeRB 4652	AY278292
<i>C. pedunculata</i> Muehlenb.	<i>Digitatae</i> Fries, <i>Eu-Digitatae</i> Kük.	26	USA, Michigan*	AF284969
<i>C. pellita</i> Willd.	<i>Hirtae</i> Tuck.	78, 81, 82	USA, California*	AF285031
<i>C. pennsylvanica</i> Lam.	<i>Montanae</i> Fries	36	USA, Michigan*	AF284977
<i>C. picta</i> Steud.	<i>Digitatae</i> Fries, <i>Eu-Digitatae</i> Kük.	32	USA, Alabama*	AF285020
<i>C. pilosa</i> Scop.	<i>Rhomboidales</i> Kük.	44	Germany, HeRB 4598	AY278286
<i>C. pilulifera</i> L.	<i>Montanae</i> Fries	18	Denmark, HMH 1934	AY278280
<i>C. polystachya</i> Swartz ex Wahlenb.	<i>Indicae</i> Tuck., <i>Turgidulae</i> Kük		Brazil, Federal District*	AF285014
<i>C. prasina</i> Wahlenb.	<i>Hymenochlaenae</i> Drejer, <i>Gracillimae</i> Carey		USA, North Carolina*	AF285043
<i>C. pseudocyperus</i> L.	<i>Pseudocypereae</i> Tuck.	66	Germany, HMH 2991	AY278295
<i>C. rariflora</i> (Wahlenb.) Sm.	<i>Limosae</i> Tuck.	52, 54	Norway, HeRB 6359	AY278305
<i>C. raynoldsii</i> Dew.	<i>Atratae</i> Kunth	58	USA, Idaho, FO 30938	AY278266
<i>C. rossii</i> Boott	<i>Montanae</i> Fries	36	USA, Oregon*	AF284972
<i>C. rostrata</i> Stokes	<i>Physocarpae</i> Drejer, <i>Vesicariae</i> Tuck.	60?, 76	Germany, HMH 1827	AY278294
<i>C. rugosperma</i> Mack.	<i>Montanae</i> Fries	32?	USA, Pennsylvania*	AF284978

**Table 1** (continued)

Species	Section and subsection <sup>a</sup>	Chromos. no. (2n) <sup>b</sup>	Locality/Voucher <sup>c</sup>	GenBank accession no.
<i>C. saxatilis</i> L.	<i>Physocarpae</i> Drejer, <i>Vesicariae</i> Tuck.	80	Sweden, HMH 2574	AY278288
<i>C. schottii</i> Dew.	( <i>Phacocystis</i> Dumort.)		USA, California*	AF285037
<i>C. scirpoidea</i> Michx. ssp. <i>scirpoidea</i>	<i>Scirpinae</i> Tuck.	62, 64, 68	USA, Utah*	AF285050
<i>C. scopulorum</i> Holm var. <i>bracteosa</i>	( <i>Phacocystis</i> Dumort.)	72, 76, 80	USA, Oregon*	AF285059
<i>C. sempervirens</i> Vill.	<i>Frigidae</i> Fries, <i>Ferrugineae</i> Tuck.	30, 32, 34	Germany, HMH 1156	AY278278
<i>C. serrulata</i> Biv.	( <i>Trachychlaenae</i> Drejer)		Italy, FO 32901	AY278273
<i>C. spissa</i> Bailey	<i>Trachychlaenae</i> Drejer		USA, California*	AF285040
<i>C. sylvatica</i> Huds.	<i>Hymenochlaenae</i> Drejer, <i>Longirostres</i> Kük.	58	Germany, WM 2015	AY278306
<i>C. tomentosa</i> L. <sup>a</sup>	<i>Pachystylae</i> Kük.	48	Germany, HMH 1777	AY278287
<i>C. tomentosa</i> <sup>b</sup>			USSR, Kazakhstan*	AF285047
<i>C. torreyi</i> Tuck.	<i>Pachystylae</i> Kük.	58	USA, Wyoming*	AF285051
<i>C. umbrosa</i> Host <sup>a</sup>	<i>Mitratae</i> Kük., <i>Eu-Mitratae</i> Kük.	66	Germany, HMH 2253	AY278270
<i>C. umbrosa</i> <sup>b</sup> ssp. <i>sabynensis</i>		60	USSR, Siberia*	AF285042
<i>C. vaginata</i> Tausch	<i>Paniceae</i> Tuck.	30, 32	Sweden, HMH 2684	AY278285
<i>C. vesicaria</i> L.	<i>Physocarpae</i> Drejer, <i>Vesicariae</i> Tuck.	82, 86	Germany, HMH 1782	AY278289
<i>C. viridula</i> Michx. <sup>a</sup>	( <i>Ceratocystis</i> Dumort.)	ca. 72	Canada, NWT, TUB	AY278308
<i>C. viridula</i> <sup>b</sup>			Switzerland, HMH 1856	AY278290
<i>C. wahuensis</i> C. A. Mey. ssp. <i>robusta</i>	<i>Rhomboidales</i> Kük.	48? 62	Japan, Shizuoka Pref.*	AF285023
<i>C. whitneyi</i> Olney	<i>Hymenochlaenae</i> Drejer, <i>Pubescentes</i> Kük.		USA, Nevada*	AF285053
<i>C. wiluica</i> Meinsh. ex Maack	( <i>Phacocystis</i> Dumort.)	ca. 50	USSR, Siberia*	AF285010

\* Origin of sequence: Roalson et al. 2001

<sup>a</sup> Sections and subsections mainly follow the concept of Kükenthal (1909); others in brackets

<sup>b</sup> Chromosome counts compiled from original literature (Cayouette and Morisset 1986; Crins and Ball 1988; Davies 1956; Dietrich 1964, 1967, 1972; Dunlop 1997; Dunlop and Crow 1999; Faulkner 1972, 1973; Favarger 1965; Halkka et al. 1992; Heilborn 1922, 1924, 1928, 1939; Jörgensen et al. 1958; Kjellqvist and Löve 1963; Löve and Löve 1981, 1982; Löve et al. 1957; Löve and Solbrig 1964; Luceño 1993; Martens 1939; McClintock and Waterway 1994; Moore and Calder 1964; Murín and Májovsky 1976; Naczi 1999; Nishikawa et al. 1984; Reese 1953; Schmid 1983; Standley 1985; Tanaka 1942a, 1942b, 1949; Wahl 1940; Whitkus 1981) or fide Chater (1980), Roalson et al. (2001) and FNA (2002)

<sup>c</sup> Acronyms of herbaria and collections: HeRB: R. Berndt (private collection); HMH: M. Hendrichs (private collection) FO: F. Oberwinkler (private collection); WM: W. Maier (private collection); TUB: Herbarium Tubingense

## Results

The different runs of the performed Bayesian phylogenetic analysis yielded consistent

results. Stationarity of the Markov chains was reached after approximately 200 000 generations of trees, i.e. after 2000 trees had been

sampled. Thus, we discarded the first 2000 trees and included 18 000 sampled trees in the 50% majority rule consensus tree of each run. One of these is given in Fig. 1. The phylogram obtained by the NJ analysis is shown in Fig. 2.

Our analyses include 117 species belonging to 32 sections in subgenus *Carex* (see Table 1), in which the sections *Capillares*, *Graciles*, *Granulares*, *Fecundae*, *Lamprochlaenae*, *Mitratatae* and *Paludosae*, are represented by one species only. In general, the tree topology of the MCMC analysis correlates with that of the NJ analysis (compare Fig. 1 with Fig. 2).

Rooted with three species of subgenus *Vignea*, the members of subgenus *Carex* appear as a highly supported lineage. Furthermore, in both analyses the supported sectional clusters contain the same representatives. Thus, in both analyses the representatives of subgenus *Indocarex* appear as a statistically well supported group within subgenus *Carex*. The representatives of sections *Vesicariae*, *Hirtae*, *Pseudocypereae*, *Ceratocystis*, *Spirostachyae*, *Bicolores*, *Paniceae*, *Trachychlaenae*, *Scirpinae*, *Atratae* and *Albae* form statistically supported clades. *C. rariflora* clusters together with the other representatives of section *Limosae*, however only weakly supported. In general, statistical support for these groups is higher in the MCMC topology than in the NJ topology. In both analyses the representatives of sections *Montanae*, *Pachystylae*, *Digitatae*, *Phacocystis*, *Rhomboidales*, *Careyanae* and *Frigidae* fall into two or more clusters. Furthermore, in both analyses five species of section *Frigidae* cluster together paraphyletically, referred to as section *Ferrugineae* in our dendrograms. Seven other representatives of section *Frigidae* included in our analyses are scattered throughout the trees. In both analyses the representatives of section *Phacocystis* fall into two statistically supported subclusters. They are closely related to a core group of section *Hymenochlaenae*.

While the ITS region is useful in defining sections within subgenus *Carex*, this region does not provide enough phylogenetic

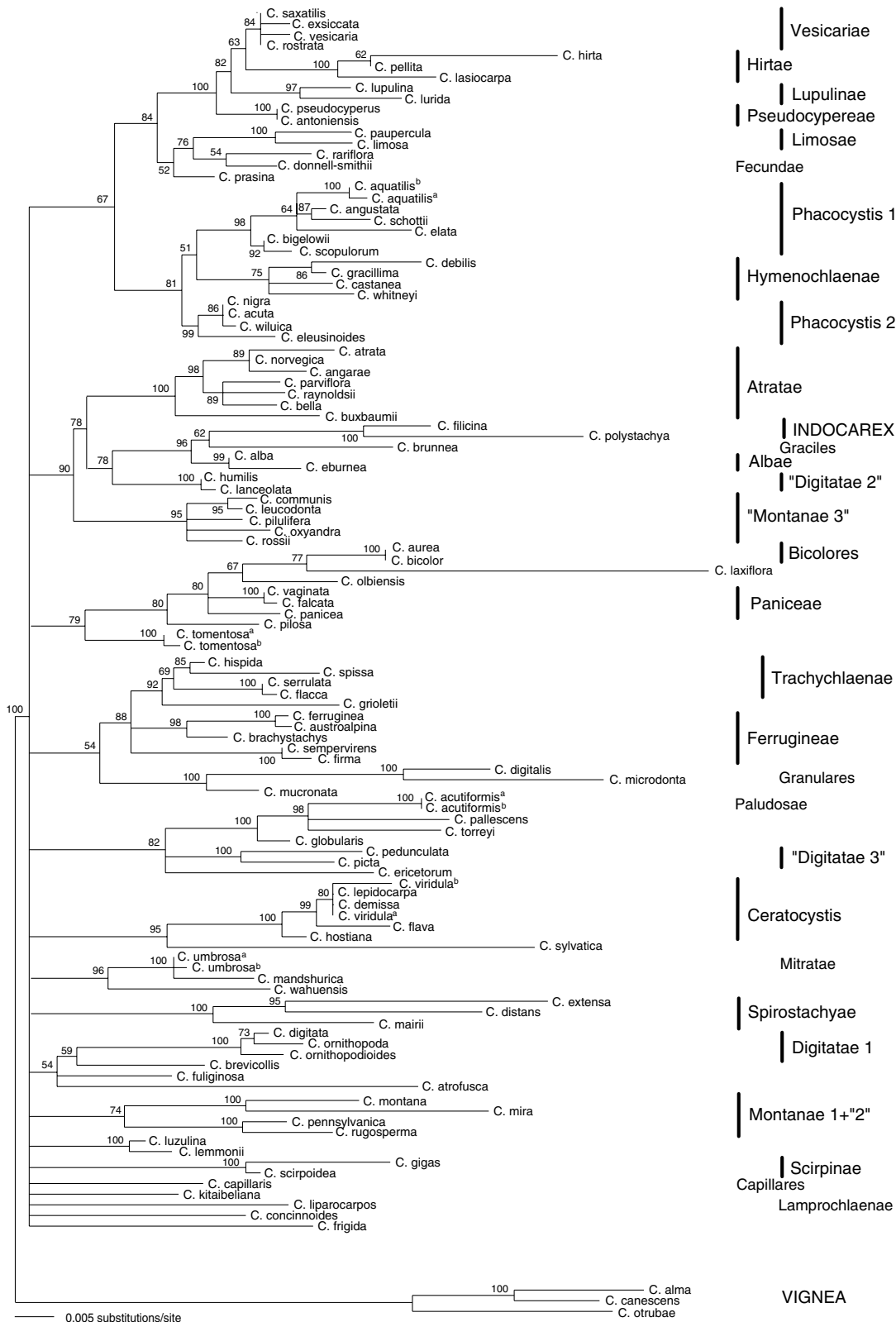
information to fully resolve relationships among sections in subgenus *Carex*.

A tendency towards lower chromosome numbers in more derived groups (Roalson et al. 2001) is not supported by our analyses (see below). Chromosome numbers of the species studied are listed in Table 1, giving the chromosome counts available in literature.

## Discussion

The discussion of the sections mainly follows their order of appearance in Fig. 1.

Section *Vesicariae* is represented in our analyses by four species. The three-stigmatic *C. vesicaria* and *C. rostrata* are very common in Central Europe. Both are adapted to wet habitats, the latter probably with a preference to acid soil conditions. *C. saxatilis*, very common in Northern European tundra vegetation, usually has two stigmas, rarely three. These taxa cluster together with the three-stigmatic *C. exsiccata*, which is adapted to the same wet and marshy habitats in Pacific North America. Species delimitation causes considerable difficulties in this group. *C. saxatilis* was treated as a subspecies of *C. vesicaria* by Kükenthal (1909), but was also regarded as a distinct species (e.g. Mackenzie 1931–1935, Chater 1980, Reznicek and Ford 2002). *C. exsiccata* was interpreted as variety of *C. vesicaria* by several authors (e.g. Boott 1867, Kükenthal 1909) but also as a separate species (e.g. Bailey 1889, Mackenzie 1931–1935, Reznicek and Ford 2002). Our ITS data can contribute to the understanding of the species concept by basepair(bp)-differences. *C. vesicaria* and *C. rostrata* differ in three bp over the total length of 638 bp, the difference between *C. vesicaria* and *C. saxatilis* is only one bp. *C. exsiccata* differs from *C. vesicaria* and *C. rostrata* in four bp. Therefore it can be concluded that *C. exsiccata* is separate from *C. vesicaria* as well as *C. rostrata*. A very close relationship of *C. saxatilis* and *C. vesicaria* has to be assumed, although our specimens are morphologically easily distinguishable: *C. saxatilis* has more compact and very dark





pistillate spikes, is normally smaller, and has mostly two stigmas. Many potential hybrids between *C. vesicaria* and *C. saxatilis* have been reported (Chater 1980; Ford et al. 1993, Cayouette and Catling 1992), named as *C. grahamii* Boott on the British Isles, *C. stenolepis* Less. in Southern Scandinavia and *C. mainensis* Porter ex Britton in North America. They all share the same ecology of wet and marshy, more calcareous grounds. As was shown for the short-beaked taxa of section *Vesicariae*, including *C. saxatilis*, allele frequencies of isozymes can contribute to the problem of species delimitation (Ford et al. 1991).

The representatives of section *Hirtae*, *C. hirta*, *C. lasiocarpa* and *C. pellita*, are related to the former section. The American *C. pellita* is more closely related to the European *C. hirta* than to *C. lasiocarpa* from Sweden. This result is not congruent with the chromosome counts giving the maximal count in the whole genus *Carex* of  $2n=112$  (Heilborn 1924, Davies 1956, Dietrich 1972) for the type species *C. hirta* (Egorova 1971). A significantly lower chromosome number is reported for the American *C. pellita* (Löve and Löve 1981, McClintock and Waterway 1994, Wahl 1940), and exactly the half number for *C. lasiocarpa*. (Reese 1953, McClintock and Waterway 1994). As was shown in section *Capillares* (Löve et al. 1957) the differentiation of chromosomes in classes based on length can give useful information to derivation, even if the simple chromosome count seems to be confusing.

The close affinity of section *Lupulinae* to *Vesicariae* has never been doubted. Kükenthal regarded both sections as subsections within the section *Physocarpae* (Kükenthal 1909);

Mackenzie (1931–1935) also points out a close relationship. Our data support the separation of section *Lupulinae* (e.g. Mackenzie 1931–1935, Fernald 1950) with *C. lupulina* and also *C. lurida* included.

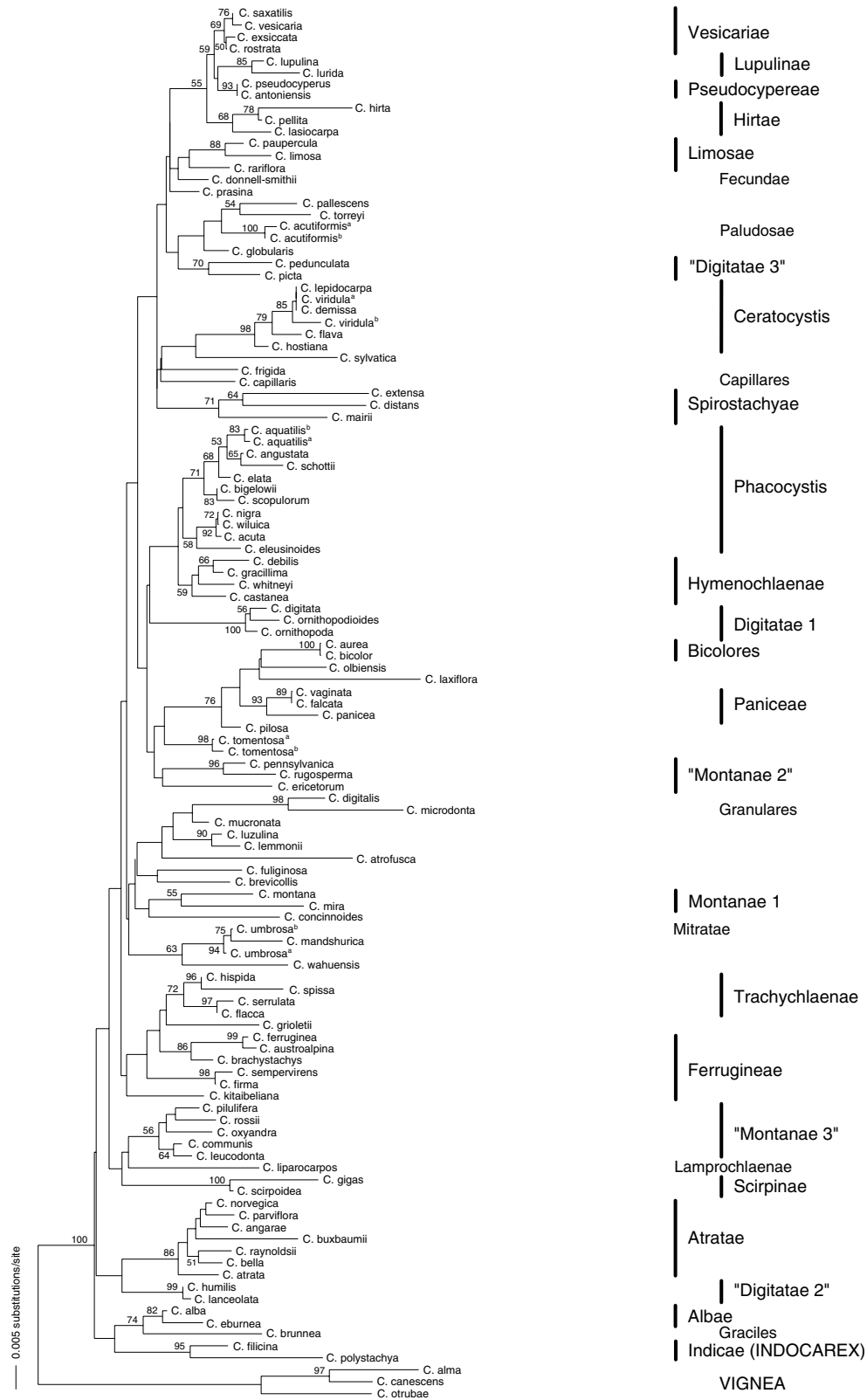
The two analyzed species of section *Pseudocypereae*, *C. pseudocyperus* collected in Germany and *C. antoniensis* from the Cape Verde Islands, share identical ITS sequence. A closer affinity of sections *Vesicariae* and *Lupulinae* and section *Pseudocypereae* is supported by NJ analysis, corresponding with the classical concept (Kükenthal 1909, Mackenzie 1931–1935).

A closer relationship of sections *Vesicariae*, *Lupulinae*, *Hirtae* and *Pseudocypereae* is indicated by our analyses (*a posteriori* probability 100%, bootstrap value 56%). This was suggested by various authors (e.g. Mackenzie 1931–1935, Reznicek 1990) mainly due to the persistent style in most species. It is also supported by anatomical data of transverse sections of leaf and culms (Shepherd 1976) and by hybridization patterns. The hybrids of *C. vesicaria* and *C. rostrata* with *C. pseudocyperus* are common in Europe, the hybrids with *C. lasiocarpa* and *C. hirta* are rarely found (Kükenthal 1909, Chater 1980). From North America a hybrid between *C. lurida* and *C. rostrata* is known (Cayouette and Catling 1992). These frequent hybridizations result from the highly similar ecological preferences of wet to marshy and usually calcareous habitats of all species within this group.

Section *Limosae* is a morphologically very homogeneous section of small species with characteristic pale-brown sheaths and a dense yellowish indumentum on the roots. These



**Fig. 1.** Bayesian inference of phylogenetic relationships within the subgenus *Carex*. Metropolis-coupled Markov chain Monte Carlo analysis of an alignment of nuclear sequences from the ITS region using the general time reversible model of DNA substitution with gamma distributed substitution rates, estimation of invariant sites, random starting trees, and random starting parameter values. Majority rule consensus tree from 18 000 trees that were sampled after the process had reached stationarity. The topology was rooted with *Carex alma*, *C. canescens* and *C. otrubae* (subgenus *Viginea*). The numbers on branches are estimates of *a posteriori* probabilities. Branch lengths were estimated using Maximum Likelihood settings and are scaled in terms of expected numbers of nucleotide substitutions per site. The sectional concept applied mainly corresponds to Kükenthal (1909) and Chater (1980). In section *Hymenochlaenae* only the core group is indicated



species share similar ecological preferences of very wet and open peat. In our analyses *C. paupercula* and *C. limosa* cluster together with good support. *C. rariflora*, morphologically and ecologically very similar to the latter species, is in most analyses connected to section *Limosae* but never with good support. According to our data, section *Limosae* is not closely related to section *Atratae*, as was proposed by Crins (1990) on morphological grounds.

*C. donnell-smithii* is the only representative of section *Fecundae* in our analysis. The position in the phylograms, indicated by NJ and MCMC analysis, has to be verified by additional sampling, including taxa from Central and South America.

*C. prasina* has been included in section *Hymenochlaenae* (Kükenthal 1909) and was considered closely related to *C. gracillima* (Kükenthal 1909, Mackenzie 1931–1935). Our molecular data do not support this interpretation.

Section *Phacocystis* comprises distigmatic species within subgenus *Carex*. The species are adapted to marshy and wet habitats and usually grow in populations with high numbers of individuals mainly in the northern temperate hemisphere. Kükenthal (1909) divided the section in seven subsections, Mackenzie (1931–1935) accepted six of them. Species delimitation is difficult in this section (Hjelmqvist and Nyholm 1947; Sylvén 1963; Hylander 1966; Faulkner 1972, 1973; Cayouette and Morisset 1986). For many species a hybrid origin was presumed with the hybrids even staying fertile (Lepage 1956, Dutilly et al. 1958, Faulkner 1973, Cayouette and Morisset 1985, Cayouette and Catling 1992).

The eleven species of section *Phacocystis*, analyzed in this study, form two well

supported sister clades. A first group comprises *C. aquatilis*, *C. angustata*, *C. schottii*, *C. elata*, closely related to *C. bigelowii* and *C. scopulorum* as members of Kükenthal's (1909) subsection *Rigidae*. The second group contains *C. eleusinoides* as sister group of *C. acuta*, *C. wiluica*, and *C. nigra*. An adequate phylogenetic treatment of this section would require worldwide sampling. Only in case of *C. angustata* and *C. schottii* the geographical distribution correlates strictly with the molecular grouping. For *C. aquatilis* we compared a Canadian and a European specimen. Their ITS sequences differ in only 1 bp – this similarity again proves the wide distribution ranges of northern hemispheric species. Our results for section *Phacocystis* support the subsectional grouping of the taxa proposed by Faulkner (1973) on the basis of hybridization experiments. However, the groups proposed by Standley (Standley 1987, 1989, 1990) after clustering analysis of different anatomical characters and cytological data are not fully supported.

The former subsection *Bicolores*, comprising distigmatic species, was often treated as a section of its own (e.g. Tuckerman 1843, Mackenzie 1931–1935, Ball 2002a). This separation is supported by our data (see below).

Section *Hymenochlaenae* clusters together with the two *Phacocystis*-groups in both analyses. This species-rich section is traditionally divided into four groups for North America (Mackenzie 1931–1935, Waterway 2002) or in six subsections by Kükenthal (1909). We studied eight species and found a core group including the North American species *C. debilis*, *C. gracillima*, *C. castanea*, and *C. whitneyi*. Another American species, *C. prasina*, is not closely related to this clade

**Fig. 2.** ITS phylogram of the subgenus *Carex* obtained by neighbor joining analysis using the GTR+I+G substitution model. The topology was rooted with *Carex alma*, *C. canescens* and *C. otrubae* (subgenus *Vigneae*). Percentage bootstrap values of 1 000 replicates are given at each furcation. Values smaller than 50% are not shown. Branch lengths are scaled in terms of expected numbers of nucleotide substitutions per site. The sectional concept applied mainly corresponds to Kükenthal (1909) and Chater (1980), respectively. In section *Hymenochlaenae* only the core group is indicated

(Roalson et al. 2001). The treatment of *C. whitney* in section *Longicaules* (comp. Mackenzie 1931–1935, Egorova 1999, Mastroguseppe 2002) is not supported by ITS data. Unexpectedly, *C. sylvatica* and *C. capillaris* neither cluster together nor with any other member of section *Hymenochlaenae*. This is in contrast to the broad sectional concept of Kükenthal (1909), who treated *C. capillaris* in subsection *Capillares*. Later authors (e.g. Mackenzie 1931–1935, Egorova 1999, Ball 2002b) separated both on sectional rank, which is supported by molecular data. Thus, our molecular data support the interpretation that section *Hymenochlaenae* sensu Drejer (1844) is heterogeneous as was proposed earlier considering morphological data (e.g. Ascherson and Graebner 1902–1904, Mackenzie 1931–1935), and recently by phylogenetic hypotheses based on ITS and cpDNA sequences (Waterway and Olmstead 1998, Roalson et al. 2001). Chromosome counts were not applicable for a better resolution of the species-rich *Hymenochlaenae* (Heilborn 1924, Davies 1956, Löve et al. 1957, Moore and Calder 1964, Dietrich 1972).

The seven species of the homogeneous section *Atratae* included in our analyses form a well supported clade in both analyses. The section is well characterized by mostly bisexual terminal spikes and the dark-colored scales and perigynia. In the MCMC analysis, *C. atrata* is closely connected to *C. norvegica* and *C. angarae*. These two species differ in 5 bp in our alignment. *C. parviflora*, found in Switzerland, seems closely related to *C. raynoldsii* from Idaho and *C. bella* from New Mexico. *C. buxbaumii* clusters within section *Atratae* but has no closer relationship to any species in the analyses. It also differs considerably in chromosome number (Heilborn 1924, Löve and Löve 1981; compare Table 1).

The two members of subgenus *Indocarex* integrated in our analyses, *C. polystachya* and *C. filicina* of section *Indicae*, cluster together with high support. The Chinese *C. brunnea*, as the only member of distigmatic section *Graciles*, clusters together with section *Indicae*

(Fig. 1) or section *Albae* (Fig. 2) respectively (comp. Roalson et al. 2001). To clarify the position of section *Indicae*, sampling of other species of subgenus *Indocarex* and of Asian species of related sections is required.

The two members of section *Albae*, *C. alba* and *C. eburnea*, cluster together with high support in all analyses. Kükenthal (1909) considered these taxa to be identical, however they can be easily separated by seven different bp in ITS sequences.

MCMC analysis reveals high support for a close relationship of section *Indicae*, subgenus *Indocarex*, to section *Albae*. Thus, subgenus *Indocarex* cannot be separated from subgenus *Carex* as a subgenus of its own (e.g. Raymond 1959, Koyama 1962), as was shown previously by Starr et al. (1999) and Roalson et al. (2001) and is supported by chloroplast DNA data (Yen and Olmstead 2000).

Section *Digitatae* is divided into three distinct groups in the ITS hypotheses. The European species *C. digitata*, *C. ornithopoda*, and *C. ornithopodioides* strongly cluster together (*Digitatae* 1) underlining morphological similarities. A second clade (“*Digitatae* 2”) combines *C. humilis* with *C. lanceolata*, again with high support. The sequence of Japanese *C. lanceolata* is with 1 bp difference nearly identical to *C. humilis*.

In some analyses the American *C. concinnoides*, characterized by a square achene and four stigmas per pistil, groups with the European *Digitatae* 1, but without sufficient support.

*C. pedunculata* clusters together with the dioecious *C. picta* in both analyses (“*Digitatae* 3”; *a posteriori* probability 100%, bootstrap value 72%). Traditionally, *C. picta* was placed in section *Pictae*, subgenus *Primocarex*. Kükenthal (1909) already gives a hint to the closer relationship of *C. picta* and *C. pedunculata* by referring to *C. baltzellii* as a member of section *Digitatae*: “Species subgeneris *Eucarex* ab hac derivata est *C. Baltzellii* Chapm.” (Kükenthal 1909, p. 82). This was followed by later authors (e.g. Mackenzie 1931–1935, Martens 1939, Ball 2002c) and can be

confirmed by molecular data (comp. Roalson et al. 2001). Chromosome numbers of *C. picta* ( $2n=32$ ) (Löve and Löve 1981) and *C. pedunculata* ( $2n=26$ ) (Löve and Löve 1981) are low compared to the zygotic numbers of other species of section *Digitatae*. Chromosome numbers of *C. digitata* (Davies 1956), *C. ornithopoda* and *C. ornithopodioides* are very similar to each other with an average diploid number of 52 (Heilborn 1924, Dietrich 1972). Chromosome numbers in “*Digitatae* 2” differ very much: *C. humilis* with zygotic number of 36 (Tanaka 1942b, Dietrich 1972, Murín and Májovsky 1976) and *C. lanceolata* with a range of counts from 68 to 80 (in Roalson et al. 2001).

Section *Montanae* appears non-monophyletic, too (Fig. 1). One group, designated “*Montanae* 3” comprises *C. communis* and *C. leucodonta* together with *C. rossii*, *C. pilulifera* and *C. oxyranda*. A second group (“*Montanae* 2”), not closely related to the first one, consists of *C. pennsylvanica*, *C. rugosperma* and *C. ericetorum*. The latter species does not cluster in this group in MCMC analysis, though in both groups European specimens are mixed together with North American or Asian species. Thus, a geographical separation is not supported. The namegiving *C. montana* (*Montanae* 1) clusters together with *C. mira*, and appears on a common branch with the former group in MCMC analysis. *C. mira*, integrated in section *Frigidae* by Kükenthal (1909), was classified as a member of section *Montanae* by Ohwi (1936), which is verified by our molecular data. The non-monophyletic nature of section *Montanae* recently was explained in great detail by Roalson et al. (2001) and is therefore not discussed here.

Two species of distigmatic section *Bicolores* were studied in our analysis: *C. bicolor* collected in Switzerland and *C. aurea* from California. Tuckerman (1843) separated section *Bicolores* from section *Phacocystis* (Kükenthal 1909). *C. eleusinoides*, ascribed to section *Bicolores* by Kükenthal (1909), was treated as member of section *Phacocystis* by Ohwi (1936), which is supported by our data.

Mackenzie (1931–1935) accepted this classification because of special characteristics of perigynia and distribution of sexes in the spikes. The ITS sequences of *C. bicolor* and *C. aurea* differ only in 1 bp. Also chromosome numbers are apparently identical (Davies 1956, Dietrich 1972, Faulkner 1972, Löve and Löve 1981). A larger sampling, including Californian species *C. hassei* and *C. garberi*, is required to clarify species delimitation of *C. aurea* and *C. bicolor*.

In our phylograms two species of section *Careyanae*, *C. laxiflora* from Texas and *C. olbiensis* from Italy cluster between section *Bicolores* and *Paniceae*. Another species, *C. digitalis*, traditionally classified in section *Careyanae*, clusters with *C. microdonta*, the only representative of section *Granulares* integrated in our analyses. A close relationship of section *Careyanae* and section *Paniceae* was often postulated in previous studies based on identical morphological characters, like perigynium structure and sheathing lowest bract (e.g. Carey 1848, Kükenthal 1909, Mackenzie 1931–1935, Koyama 1962). Based on foliar flavonoids, Manhart (1986) demonstrated that the North American species of the broadly defined section *Careyanae* can be separated in two subgroups. This was supported by detailed investigations in macro- and micromorphology (Bryson 1980, Naczi 1997) and by molecular data (Starr et al. 1999).

Section *Paniceae* forms a well supported clade in NJ analysis (Fig. 2). The Russian *C. falcata* is closely related to *C. vaginata* from Swedish Lapland. The difference of only one bp in ITS sequences raises the question how to distinguish these species. In contrast, *C. panicea* is well distinguished from *C. vaginata* in its ITS sequence by 11 bp exchanges. The chromosome number of the species within this section is  $2n=32$  (Heilborn 1922, 1924; Dietrich 1972; Löve and Löve 1981). The close relationship of section *Bicolores* and section *Panicea* was already proposed by Kükenthal (1909) and Mackenzie (1931–1935) and is supported by our molecular data.

Traditionally, *C. pilosa* is considered to belong to section *Rhomboidales*. In our phylograms the latter species always clusters within section *Paniceae*. Two other species of section *Rhomboidales*, *C. brevicollis* and *C. wahuensis*, do not cluster with *C. pilosa* in both molecular trees. In the MCMC analysis, *Carex wahuensis* is well supported in a cluster together with *C. umbrosa* and *C. mandshurica*. There is no support for the position of *C. brevicollis*. Additional sampling in this section with many species in East Asia is urgently required for critically reviewing Koyamas (1962) enlarged sectional concept.

The two specimens of *C. tomentosa* from Germany and Kazakhstan differ in only 1 bp in the ITS sequence. Their position in the tree is discussed below together with other members of section *Pachystylae*.

The analyzed species of section *Trachychlaenae* cluster together in both molecular trees. *C. flacca* is the most common sedge in Central Europe lacking clearcut ecological or altitudinal preferences. A mediterranean taxon, *C. serrulata*, has been classified as subspecies by Kükenthal (1909). The cytological similarities (Heilborn 1924, Davies 1956, Kjellqvist and Löve 1963, Dietrich 1972, Löve and Löve 1981) are underlined by our ITS data with only 1 bp difference within a total of 638 bp. The Mediterranean *C. hispida* is a sister taxon of *C. spissa* from California. The separation of these two species in section *Hispidae*, as proposed by Mackenzie (1931–1935), is at least not contrary to the molecular result. In both analyses, *C. grioletii*, a species of the heterogeneous section *Pachystylae*, clusters close to section *Trachychlaenae*.

Molecular phylograms reveal the section *Frigidae* to be non-monophyletic. The species ascribed to subsection *Ferrugineae* by Kükenthal (1909) cluster in two closely related groups together with section *Trachychlaenae* in both analyses. A first group comprises *C. ferruginea*, *C. austroalpina*, and *C. brachystachys*. These species are characterized by perigynia with prominent nerves and anthers with only slightly serrated terminal tips. The diploid

chromosome number is 40 (Dietrich 1967). The second group of *Ferrugineae*, including *C. sempervirens* and *C. firma*, can be characterized morphologically by a perigynium surface without conspicuous nerves and anthers terminating with a strongly serrated crown-like structure (Dietrich 1967). These morphological features and identical chromosome sets ( $2n = 36$ ) are shared by *C. kitaibeliana* and *C. mucronata* (Dietrich 1967). *C. kitaibeliana* clusters together with the *Ferrugineae* group in the NJ tree, but without support. In the MCMC phylogram, *C. kitaibeliana* appears as an isolated taxon within *Carex*. In both molecular trees, *C. mucronata* clusters together with *C. digitalis* and *C. microdonta*, however only weakly supported.

Species included in section *Frigidae* subsection *Fuliginosae* by Kükenthal (1909), *C. fuliginosa*, *C. luzulina*, *C. atrofusca*, and *C. frigida*, are scattered in both trees without any supported position. Only the American species *C. luzulina* clusters together with *C. lemmonii*, with significant support, as discussed below. However, they are not closely associated to the core group of this section, which consists only of European species. *C. fuliginosa* grows in tussocks, but resembles morphologically *C. atrata*, which forms long-creeping rootstocks. Both share bisexual terminal spikes with female flowers inserted above basal male flowers. In both trees there is no closer connection to section *Atratae*.

*C. atrofusca* was often doubted to be a member of section *Frigidae* (e.g. Christ 1885, Dietrich 1967). Dietrich (1967) suggested a closer relationship to section *Atratae*. In our analyses, however, the position of *C. atrofusca* is not resolved at all.

Also *C. frigida*, distinguished by bidentate beak and a chromosome number of  $2n = 56$  (Davies 1956, Dietrich 1967), does not appear within the core group of section *Frigidae*.

The next cluster in the MCMC tree comprises three species of section *Pachystylae*, *C. pallescens*, *C. torreyi* and *C. globularis*, surprisingly clustering always together with *C. acutiformis* as the only representative of

section *Paludosae*. *C. tomentosa*, another member of section *Pachystylae* in the traditional classification, does not cluster together with this group. Its unsupported position in the NJ tree is close to section *Paniceae*. This position is confirmed by the MCMC analysis. The two other members of section *Pachystylae* treated in our analysis, *C. mandshurica* and *C. grioletii*, do not appear within the core group. *C. mandshurica* is grouped with *C. umbrosa* in both the NJ and MCMC tree, and *C. grioletii* is close to section *Trachychlaenae* in both trees. Nevertheless, section *Pachystylae* sensu Kükenthal (1909) is proven to be non-monophyletic by our analyses.

Section *Ceratocystis* forms an optimally supported cluster in all analyses, with *C. flava* and *C. hostiana* connected to a core group comprising *C. viridula*, *C. lepidocarpa* and *C. demissa*. ITS sequences allow to distinguish *C. flava* from *C. hostiana* and the complex of taxa grouped around *C. viridula*. This corresponds with chromosome numbers, i.e. an aneuploid series from the diploid numbers 56 for *C. hostiana* (Davies 1956, Heilborn 1924) to 60 for *C. flava* (Heilborn 1939, Crins and Ball 1988, Halkka et al. 1992) and  $2n = 68, 70$  within a group including *C. demissa* and *C. lepidocarpa* (Heilborn 1928, Dietrich 1964, Halkka et al. 1992). Our data support the proposal of Schmid (1983) to rank *C. lepidocarpa* and *C. demissa* as subspecies of *C. viridula*. Crins and Ball (1989) accepted this taxonomy for the American species. The *C. flava* complex is one of the most difficult and actually well-studied groups (Schmid 1981, 1982, 1983, 1986; Crins and Ball 1988, 1989; Crins 1990; Bruederle and Jensen 1991; Halkka et al. 1992; Pykälä and Toivonen 1994; Hedrén and Prentice 1996). Therefore, we included sequences of five additional specimens of the *C. flava* agg. in our analyses. However, since ITS sequences yielded no apparent resolution these data are not presented. Analysis of a more variable region may probably supply better results.

The grouping of *C. sylvatica* with section *Ceratocystis* in both analyses underlines the

revealed heterogeneity of section *Hymenochlaenae*.

*C. umbrosa* is the only species of section *Mitratae* in our analyses. The specimen from Siberia, identified as subspecies *sabynensis* by Murray et al. (in Roalson et al. 2001) clusters closer to *C. mandshurica* than to *C. umbrosa* from Germany (Fig. 2). In contrast to Kükenthal (1909), who accepted only subspecies rank, Ohwi (1936) separated *C. sabynensis* as a distinct species, which is supported by NJ analysis.

Three species of section *Spirostachyae* form a well supported cluster. *C. extensa* and *C. distans* are closely related and always grouped together with *C. mairii*. Section *Spirostachyae* has often been united with section *Ceratocystis* to one section *Extensae* Fries (e.g. Fries 1835, Drejer 1844, Bailey 1889, Holm 1903, Mackenzie 1931–1935, Kreczetovicz 1935). The separation into two sections was favored by patterns of flavonoid compounds (Harborne 1971), by character compatibility analyses and multivariate statistical analyses of morphological data (Crins and Ball 1988). It is also in agreement with our molecular data.

*C. lemmonii* and *C. luzulina* both belong to a small group of species endemic to the mountains of western North America. *C. lemmonii* was included by Kükenthal (1909) in section *Spirostachyae*, but considered as a member of section *Frigidae* by Mackenzie (1931–1935). Interestingly, it clusters together with *C. luzulina* of section *Frigidae*, but shows no close relationship to the core group of the section. Accordingly, it would be at least not contrary to our molecular results, to restrict section *Spirostachyae* and also subsection *Ferrugineae* of section *Frigidae* to European species.

The dioecious section *Scirpinae* was included in subgenus *Primocarex* by some authors (e.g. Kükenthal 1909, Chater 1980), but also transferred to subgenus *Carex* (e.g. Bailey 1889, Mackenzie 1931–1935) based on recent support by molecular phylograms (Roalson et al. 2001). *C. gigas* and *C. scirpoidea*, two morphologically and cytologically slightly different American species (Löve and Solbrig 1964, Dunlop 1997,

Dunlop and Crow 1999) cluster together with statistical support. However, connections to any other section are not resolved.

*C. liparocarpos*, the only species of section *Lamprochlaenae* in our study, is placed in an isolated position in the MCMC tree.

In summary, the ITS region does not provide enough phylogenetic information to fully resolve the relationships between sections within subgenus *Carex*. Nevertheless, conclusions on some major affinities within adequately sampled sections and about defining these sections can be inferred. Certainly, a comprehensive interpretation of sectional limits within subgenus *Carex* requires additional data and therefore no taxonomic conclusions are drawn in this study.

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