

## Phylogenetic relationships in *Carex*, subgenus *Vignea* (Cyperaceae), based on ITS sequences

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**Abstract.** To evaluate the sectional classification in *Carex*, subgenus *Vignea*, the ITS region of 58 species of 20 sections was analyzed with Neighbor Joining (NJ) and Markov chain Monte Carlo (MCMC) methods. Sections *Dioicae*, *Physodeae* and *Ovales* are found to be monophyletic, with *C. bohémica* well integrated in the section *Ovales*. Section *Heleonastes* turns out to be monophyletic, if *C. canescens* is treated separately in section *Canescentes*. Section *Elongatae* is monophyletic, but *C. remota* is placed in section *Remotae* and *C. bromoides* in section *Deweyanae*. In both analyses, six representatives of section *Arenariae* cluster together in a terminal group, whereas *C. disticha*, *C. repens* and *C. siccata* form a basal cluster. *C. maritima*, as the only member of section *Incurvae*, shares this basal position. *C. chordorrhiza* is ascribed to section *Chordorrhizeae* and not ascribed to the paraphyletic section *Divisae*. *C. vulpina* and *C. otrubae* are assigned to section *Vulpinae* and separated from the heterogeneous section *Stenorhynchae*. The other members of sections *Divisae*, *Muehlenbergianae*, *Multiflorae* and *Stenorhynchae* are scattered throughout the trees. The representatives of section *Foetidae* are dispersed in both analyses, section *Paniculatae* appears to be non-monophyletic in the molecular results as well.

The subgenus appears subdivided in at least four larger subgroups in all analyses. Whereas these

subgroups are strongly supported, the relationships between these subgroups remain only poorly resolved.

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

*Carex* subgenus *Vignea* (Beauv.) Nees is morphologically characterized by the lack of a prophyll surrounding the base of the axis of partial inflorescence (cladoprophyll), mostly bisexual spikes and usually 2, rarely 3 stigmas. It has been regarded as a natural lineage within the genus *Carex* L. since the early systematic studies in the 19th century (e.g. Schweinitz 1825, Dumortier 1827, Fries 1835, Kunth 1837, Tuckerman 1843, Drejer 1844). Nearly a quarter of the species of *Carex* are members of subgenus *Vignea*. In North America it comprises about one third of the recognized *Carex* species, in Eurasia estimations reach from 25 to 30% (Ball 1990, Egorova 1966). In the worldwide monograph of Georg Kükenthal (1909) subgenus *Vignea* contains 20 sections with 140 species in his broad species concept. Since then, number of species has at least tripled, due to a narrower

species concept and to new findings especially outside Europe. For North America, 168 species in 17 sections are described (Ball and Reznicek 2002), the Flora of Russia and adjacent countries (Egorova 1999) covers 320 species in 25 sections.

For subgenus *Carex*, the potential of the ITS region for phylogenetic interpretations on the sectional level was recently demonstrated (Roalson et al. 2001, Hendrichs et al. 2004). For subgenus *Vignea*, the available molecular data are scarce. Recent molecular studies focused mainly on family and subfamily relationships (Muasya et al. 1998, Yen and Olmstead 2000) or on species of subgenus *Carex* (Waterway and Olmstead 1998, Starr et al. 1999, Roalson et al. 2001). For better understanding of sectional circumscriptions in subgenus *Vignea* we analyzed 58 species mainly from northern Europe including sequences of 12 species derived from GenBank (see Table 1).

## Materials and methods

**Plant collection and DNA extraction.** The analyzed species of *Carex* 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 with a micro pestle 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 (ITS1, 5.8 S, ITS2; about 700 bp) was amplified with the primer pair ITS1 (Hsiao et al. 1995), ITS5 and ITS4 (White et al. 1990) or ITS5i and ITS4i (Roalson et al. 2001). PCR was performed as described in Hendrichs et al. (2004). The PCR 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 and LICOR 4000). The

sequences of both strands were combined and proof-read with Sequencher™ 4.1 software (Gene Codes Corp., Michigan) and BioEdit (Hall 1999). The sequences reported in this study have been deposited in GenBank (see Table 1). The alignment contained 614 nucleotide sites. After removing ambiguously aligned positions (220–233), 600 sites remained for analyses with 234 variable sites (ITS1: 129, 5.8S: 8, ITS2: 97). The ingroup alone contained 220 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). The likelihood ratio test as implemented in Modeltest 3.0 (Posada and Crandall 1998) selected TrN + G (Swofford et al. 1996) as 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 computer program MrBayes (Huelßenbeck and Ronquist 2001) with GTR + I + G (Swofford et al. 1996) 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 those trees that were sampled after the process had reached stationarity (burnin=2000). This Bayesian approach of phylogenetic analysis was repeated five 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 using 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 estimated under the maximum likelihood criterion and TrN + G as substitution model with the following settings: base frequencies A=0.235075, C=0.273445, G=0.281677, T=0.209804; rate matrix AC=1.00000, AG=2.25212, AT=1.00000, CG=1.00000, CT=6.34211, GT=1.00000; gamma distribution shape parameter=0.357885. Support for internal nodes was estimated by 1000 neighbor joining bootstrap replicates under the

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

Species	Locality/Voucher <sup>†</sup>	GenBank accession no.
<i>Carex alma</i> L. H. Bailey	USA, California*	AF285025
<i>Carex appropinquata</i> Schum.	Germany; HMH 3358	AY280549
<i>Carex arenaria</i> L.	Germany; SMC 126	AY280529
<i>Carex athrostachya</i> Olney	USA, Washington; JM 132	AY280539
<i>Carex bicknellii</i> Britton	USA, Michigan*	AF285039
<i>Carex bohémica</i> Schreb.	Germany; SMC 75	AY280532
<i>Carex bonplandii</i> Kunth	Costa Rica; HeRB 6108	AY280563
<i>Carex brizoides</i> L.	Germany; HMH 503	AY280546
<i>Carex bromoides</i> Schkuhr	USA, Tennessee; JE	AY280534
<i>Carex brunnescens</i> (Pers.) Poir.	Sweden; HMH 2776	AY280567
<i>Carex canariensis</i> Kük.	Spain; HMH 2938	AY280558
<i>Carex canescens</i> L. <sup>a</sup>	USSR, Siberia*	AF284990
<i>Carex canescens</i> L. <sup>b</sup>	France; HMH 2282	AY280550
<i>Carex chordorrhiza</i> Ehrh.	Sweden; HMH 2804	AY280568
<i>Carex curvata</i> Knaf	Germany; SMC 131	AY280530
<i>Carex davalliana</i> Smith	Germany; HMH 1413	AY280542
<i>Carex densa</i> L. H. Bailey	USA, Washington; JM 161	AY280538
<i>Carex diandra</i> Schrank	Denmark; HMH 1931	AY280551
<i>Carex dioica</i> L.	Germany; HeRB 2185	AY280543
<i>Carex disticha</i> Lam.	Germany; FO 8396	AY280571
<i>Carex divisa</i> Huds.	France; FO 18408	AY280552
<i>Carex divulsa</i> Stokes	Germany; HMH 1792	AY280553
<i>Carex duriuscula</i> C.A. Mey.	Canada,**	AF027436
<i>Carex echinata</i> Murray	Denmark; HMH 1920	AY280559
<i>Carex elongata</i> L.	Germany; FO 9351	AY280560
<i>Carex foetida</i> All.	France; HMH 2292	AY280544
<i>Carex fracta</i> Mack.	USA, California*	AF285030
<i>Carex heleonastes</i> Ehrh.	Germany; HMH 2946	AY280566
<i>Carex hoodii</i> Boott	USA, Washington; JM 129	AY280537
<i>Carex jonesii</i> L. H. Bailey	USA, California*	AF285038
<i>Carex lachenalii</i> Schkuhr	Sweden; HMH 2692	AY280564
<i>Carex leavenworthii</i> Dewey	USA, Texas*	AF285033
<i>Carex leersii</i> F.W. Schultz	France; HeRB 3031	AY280554
<i>Carex ligerica</i> Gay	Germany; SMC 120	AY280531
<i>Carex loliacea</i> L.	Sweden; HMH 2955	AY280565
<i>Carex macloviana</i> d'Urv.	Sweden; HMH 2957	AY280562
<i>Carex macrocephala</i> Willd.	USA, Oregon*	AF285017
<i>Carex macrorrhiza</i> Boeck.	Argentina, Santa Cruz*	AF285018
<i>Carex maritima</i> Gunn.	Switzerland; JM 146	AY280570
<i>Carex muricata</i> L.	Estonia, Muhu Island*	AF285036
<i>Carex muskingumensis</i> Schw.	Canada; SMC 181	AY280541
<i>Carex otrubae</i> Podp.	Germany; HMH 1776	AY280556
<i>Carex ovalis</i> Good.	Germany; HMH 1780	AY280561
<i>Carex paniculata</i> L.	Switzerland; HMH 2865	AY280557
<i>Carex parallela</i> (Laest.) Sommerf.	Sweden; HMH 2780	AY280545
<i>Carex pinetorum</i> Liebm.	Bolivia; LPB	AY280540

**Table 1** (continued)

Species	Locality/Voucher <sup>†</sup>	GenBank accession no.
<i>Carex praecox</i> Schreb.	Germany; SMC 149	AY280527
<i>Carex pseudobrizoides</i> Clavaud	Germany; SMC 97	AY280526
<i>Carex remota</i> L.	Germany; HMH 3354	AY280548
<i>Carex repens</i> Bell.	Poland; SMC 93	AY280528
<i>Carex siccata</i> Dewey	USA, Washington; JM 125	AY280536
<i>Carex spicata</i> Huds.	Germany; HeRB 624	AY280555
<i>Carex stenophylla</i> Wahlenb.	Spain; JM 9772	AY280535
<i>Carex stipata</i> Muehlenb.	USA, Wisconsin; JE	AY280533
<i>Carex vallicola</i> Dewey	USA, Wyoming; FO 31030	AY280569
<i>Carex vernacula</i> L. H. Bailey	USA, Oregon*	AF285022
<i>Carex vulpina</i> L.	Germany; HMH 3359	AY280547
<i>Carex vulpinoidea</i> Michx.	USA, Texas*	AF284968
<i>Kobresia capillifolia</i> (Decne.) C. B. Clarke	China, Xinjiang*	AF284984
<i>Kobresia myosuroides</i> (Vill.) Fiori & Paol.	USSR, Siberia*	AF284985
<i>Kobresia sibirica</i> (Turcz. ex Ledeb.) Boeck.	USSR, Siberia*	AF284986

<sup>†</sup> Acronyms of herbaria and collections: GOET: Herbarium University Göttingen; HeRB: R. Berndt (private collection); HMH: M. Hendrichs (private collection); FO: F. Oberwinkler (private collection); JE: Herbarium Haussknecht, Jena; JM: J. Müller (private collection); LPB: Herbarium Nacional de Bolivia; SMC: S. Michalski (private collection); TUB: Herbarium Tubingense; WM: W. Maier (private collection);

\* Origin of sequence: Roalson et al. 2001

\*\* Origin of sequence: Starr et al. 1999

same model settings. The unrooted phylograms from neighbor joining and MCMC analyses were rooted with three species of the genus *Kobresia* Willd. from GenBank.

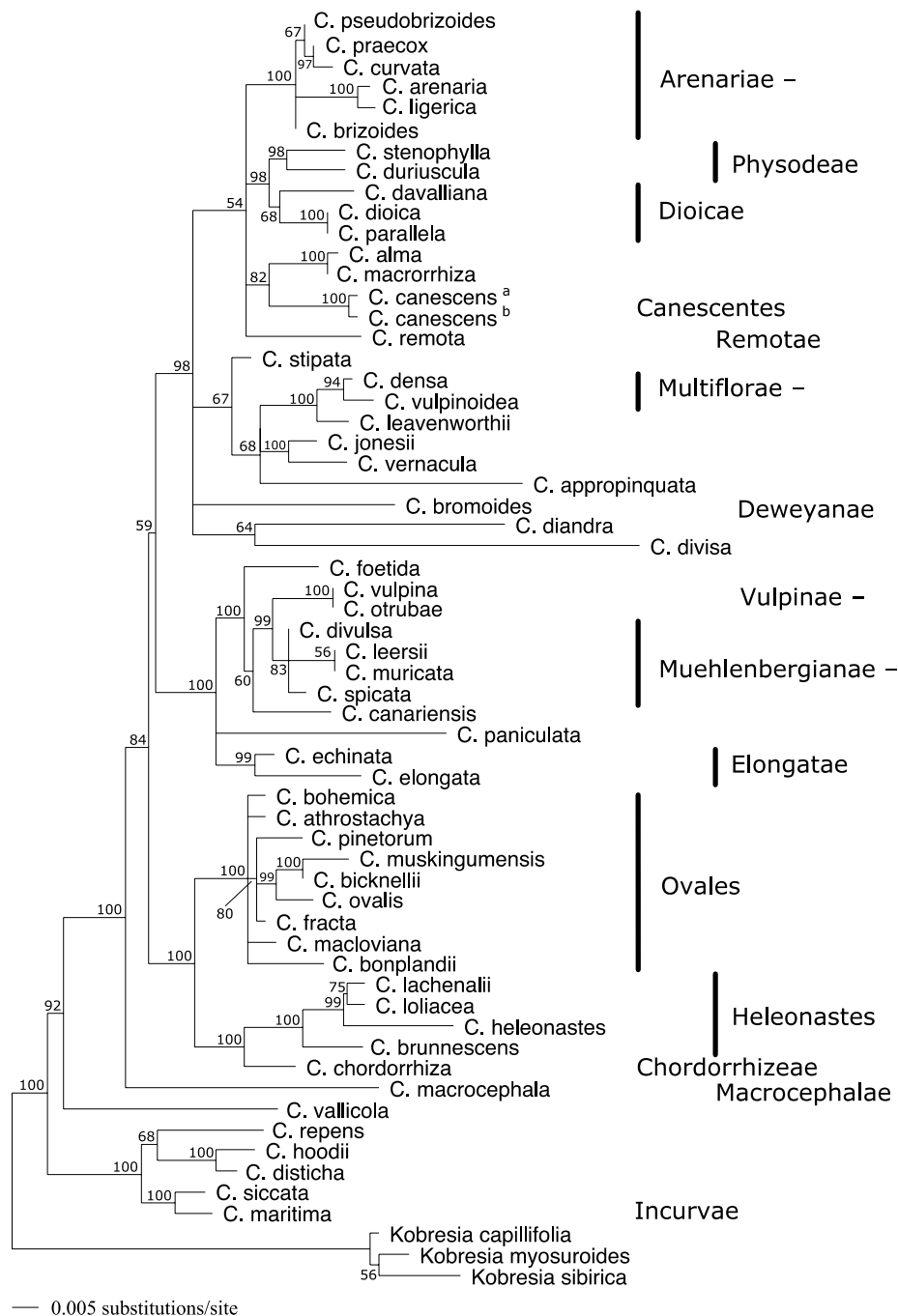
## Results

The different runs of 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 them is given in Fig. 1. The phylogram obtained by the NJ analysis is shown in Fig. 2. In both analyses the supported clusters contain the same species, and the tree topology of the MCMC analysis correlates with that of the NJ analysis. In general, statistical support is higher in the MCMC topology than in the

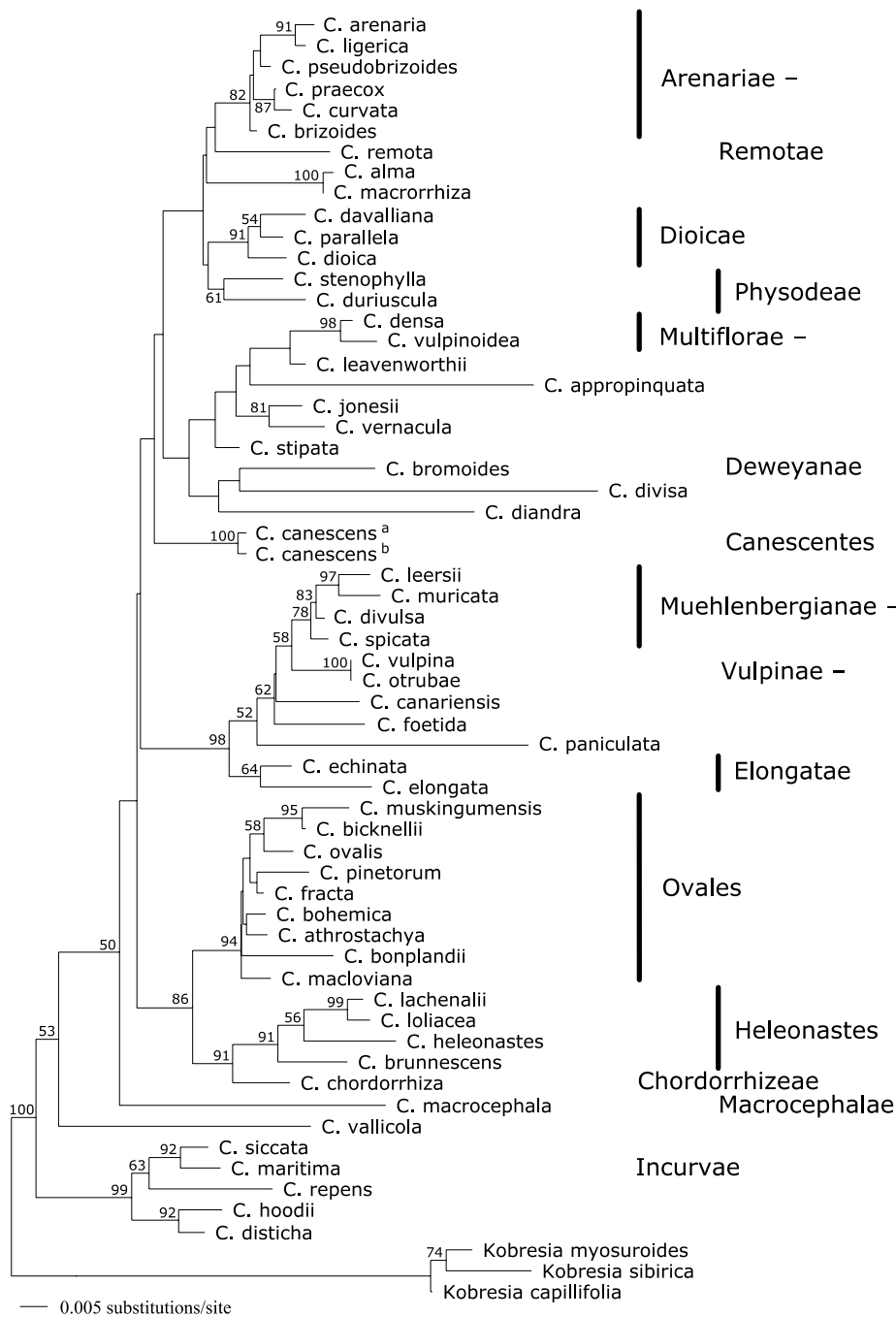
NJ topology (compare Fig. 1 with Fig. 2). Parsimony analyses revealed consistent results, but with lower statistical significance (Hendrichs et al. 2003a).

Rooted with three species of the genus *Kobresia*, the members of subgenus *Vignea* group as a highly supported monophyletic lineage. The use of *Kobresia* as outgroup for our analyses is justified through the results of molecular investigations in the relationship of the genus *Carex* (comp. Yen and Olmstead 2000, Roalson et al. 2001, Hendrichs et al. 2003b).

The sectional delimitations within subgenus *Vignea* have been of great difficulty and constant rearrangement since the early approaches. Of the 20 traditionally accepted sections represented in our analyses only sections *Dioicae*, *Physodeae* and *Ovales* are found to be monophyletic. *C. bohémica*, often placed in section *Schellhammeria*, appears fully integrated in section *Ovales*. Section



**Fig. 1.** Bayesian inference of phylogenetic relationships within *Carex* subgenus *Vignea*. 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 and estimation of variant sites. 50% majority rule consensus tree from 18 000 trees that were sampled after the process had reached stationarity. The topology was rooted with three species of the genus *Kobresia*. The numbers on branches are estimates of *a posteriori* probabilities. Branch lengths were estimated using Maximum Likelihood and are scaled in terms of expected numbers of nucleotide substitutions per site. The groups marked with “-” do not include all analyzed members of the specified section



**Fig. 2.** ITS phylogram of *Carex* subgenus *Vignea* obtained by neighbor joining analysis using TrN + G as substitution model (parameters are given in the text). The topology was rooted with three species of the genus *Kobresia*. Percentage bootstrap values of 1000 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. Groups marked with “-” do not include all analyzed members of the specified section

*Macrocephalae* is represented in our analyses only by *C. macrocephala*. Section *Heleonastes* is found to be monophyletic, if *C. canescens* is

treated in separated section *Canescentes*. Section *Elongatae* is marked monophyletic, whereby *C. remota* is placed in section

*Remotae* and *C. bromoides* is referred to section *Deweyanae*. These two sections are represented by one species each in our dendrograms. In both analyses, six representatives of section *Arenaria* constitute a well supported group. *C. disticha*, *C. repens* and *C. siccata*, traditionally treated in section *Arenariae*, fall into a basal cluster. *C. maritima*, as the only member of section *Incurvae*, shares this basal position in our dendrograms. *C. chordorrhiza* is referred to section *Chordorrhizeae* and is not treated in the paraphyletic section *Divisae*. *C. vulpina* and *C. otrubae* are assigned to section *Vulpinae* and separated from the heterogeneous section *Stenorhynchae*. Circumscription of sections *Divisae*, *Muehlenbergiana*, *Multiflorae* and *Stenorhynchae* was always regarded as very difficult and artificial in larger parts (e.g. Kükenthal 1909, Ball and Reznicek 2002; comp. Table 2). The members of these sections are mainly scattered throughout the trees. If two or three species cluster together, these are labeled in our dendrograms as core-groups, marked with a “–” to indicate that not all analyzed members of the specified section are included. The representatives of section *Foetidae* are dispersed in both analyses. According to our analyses, section *Paniculatae* appears to be non-monophyletic as well, even though well-defined by gross morphology.

The ITS region is useful in defining sections within subgenus *Vignea* and reveals at least four larger subgroups comprising different sections. In a first subgroup species traditionally ascribed to section *Arenariae* cluster together with *C. hoodii* and *C. maritima*. A second well supported cluster includes members of sections *Ovales* and *Heleonastes* together with *C. chordorrhiza*. Species of sections *Muehlenbergiana*, *Vulpinae* and *Elongatae* form a third well supported subgroup with *C. canariensis*, *C. foetida* and *C. paniculata* included. The largest subgroup comprises species of sections *Arenariae*, *Physodae*, *Dioicae* and *Multiflorae* together with *C. remota*, *C. alma*, *C. macrorrhiza*, *C. leavenworthii*, *C. appropinquata*, *C. jonesii*, *C. vernacula*, *C. stipata*, *C. bromoides*, *C. divisa*,

*C. diandra* and *C. canescens*. However, the relationships between these subgroups are only weakly supported.

Chromosome numbers of the species studied are listed in Table 2, giving the chromosome counts available in literature.

## Discussion

The sections and species discussed are arranged in order of position in Fig. 1, starting at the base of the dendrogram.

**Sections *Incurvae*, *Macrocephalae* and related species.** A highly supported group in both analyses (*a posteriori* probability 100%, bootstrap value 99%) comprises *C. hoodii*, *C. maritima* and three species ascribed to section *Arenariae* by Kükenthal (1909): *C. siccata*, *C. repens* and *C. disticha*. *Carex siccata* forms a monophyletic group with *C. maritima* which was placed in the monotypic section *Incurvae* by Kükenthal (1909). *C. disticha* is originally an Eurasian endemic, but introduced to North America. It shows closer affinity to *C. hoodii*, which Kükenthal (1909) ascribed to the large and inhomogeneous section *Muehlenbergiana*. These two subgroups are highly supported (*a posteriori* probability 100%, bootstrap value 92%). Although different sectional classifications for *C. maritima* and *C. disticha* have been proposed (e.g. Egorova 1966, Hylander 1966, Chater 1980, Ball and Reznicek 2002), the group as a whole is not congruent with any existing sectional concept. *C. repens* is a local endemic species with three disjunct areals in Central Europe, a hybrid origin was sometimes suggested (e.g. Chater 1980). It differs morphologically from *C. arenaria* only by a greater number of spikes and the absence of a scale-like prophyll at the base of spikes. In molecular dendrograms, *C. arenaria* and other members of section *Arenariae* cluster together as a group at the greatest possible distance in a terminal branch. Interestingly, this group as a whole is characterized by a specific indel in alignment-position 10 and a characteristic sequence pattern in positions 120–123.

**Table 2.** Species, sections, and chromosome numbers. The classification mostly follows Kükenthal (1909)

Species	Section	Chromos. no. (2n)*
<i>Carex alma</i> L. H. Bailey	<i>Multiflorae</i> Kunth	
<i>Carex appropinquata</i> Schum.	<i>Paniculatae</i> Kunth	64
<i>Carex arenaria</i> L.	<i>Arenariae</i> Kunth	56, 58, 64
<i>Carex athrostachya</i> Olney	<i>Ovales</i> Kunth	68
<i>Carex bicknellii</i> Britton	<i>Ovales</i> Kunth	76, 78
<i>Carex bohémica</i> Schreb.	<i>Schellhammeria</i> (Moench) Kunth	62, 64, 80
<i>Carex bonplandii</i> Kunth	<i>Ovales</i> Kunth	
<i>Carex brizoides</i> L.	<i>Arenariae</i> Kunth	58
<i>Carex bromoides</i> Schkuhr	<i>Deweyanae</i> Tuck.	64, 66, 68
<i>Carex brunnescens</i> (Pers.) Poir.	<i>Heleonastes</i> Kunth	56
<i>Carex canariensis</i> Kük.	<i>Muehlenbergianae</i> Tuck.	58
<i>Carex canescens</i> L. <sup>a</sup>	<i>Canescentes</i> Fries	54, 56
<i>Carex chordorrhiza</i> Ehrh.	<i>Chordorrhizae</i> Fries	62
<i>Carex curvata</i> Knaf	<i>Arenariae</i> Kunth	58
<i>Carex davalliana</i> Smith	<i>Dioicae</i> Tuck.	46
<i>Carex densa</i> L. H. Bailey	<i>Multiflorae</i> Kunth	
<i>Carex diandra</i> Schrank	<i>Paniculatae</i> Kunth	50, 54, 60
<i>Carex dioica</i> L.	<i>Dioicae</i> Tuck.	52
<i>Carex disticha</i> Lam.	<i>Arenariae</i> Kunth	62
<i>Carex divisa</i> Huds.	<i>Divisae</i> Christ	58, 60, 62
<i>Carex divulsa</i> Stokes	<i>Muehlenbergianae</i> Tuck.	56, 58
<i>Carex duriuscula</i> C.A. Mey.	<i>Physodeae</i> Christ ex Kük.	52
<i>Carex echinata</i> Murray	<i>Elongatae</i> Kunth	56, 58
<i>Carex elongata</i> L.	<i>Elongatae</i> Kunth	56
<i>Carex foetida</i> All.	<i>Foetidae</i> Tuck.	58
<i>Carex fracta</i> Mack.	<i>Ovales</i> Kunth	
<i>Carex heleonastes</i> Ehrh.	<i>Heleonastes</i> Kunth	56
<i>Carex hoodii</i> Boott	<i>Muehlenbergianae</i> Tuck.	58, 60
<i>Carex jonesii</i> L. H. Bailey	<i>Stenorhynchae</i> Holm	
<i>Carex lachenalii</i> Schkuhr	<i>Heleonastes</i> Kunth	58, 62, 64, 74
<i>Carex leavenworthii</i> Dewey	<i>Muehlenbergianae</i> Tuck.	
<i>Carex leersii</i> F.W. Schultz	<i>Muehlenbergianae</i> Tuck.	58
<i>Carex ligerica</i> Gay	<i>Arenariae</i> Kunth	58
<i>Carex loliacea</i> L.	<i>Heleonastes</i> Kunth	54
<i>Carex macloviana</i> d'Urv.	<i>Ovales</i> Kunth	82, 86
<i>Carex macrocephala</i> Willd.	<i>Macrocephalae</i> Kük.	74
<i>Carex macrorrhiza</i> Boeck.	<i>Divisae</i> Christ	
<i>Carex maritima</i> Gunn.	<i>Incurvae</i> Kük.	60
<i>Carex muricata</i> L.	<i>Muehlenbergianae</i> Tuck.	56, 58
<i>Carex muskingumensis</i> Schw.	<i>Ovales</i> Kunth	80
<i>Carex otrubae</i> Podp.	<i>Vulpinae</i> (Carey) Christ	58, 60
<i>Carex ovalis</i> Good.	<i>Ovales</i> Kunth	62, 64, 66, 68
<i>Carex paniculata</i> L.	<i>Paniculatae</i> Kunth	60, 62, 64
<i>Carex parallela</i> (Laest.) Sommerf.	<i>Dioicae</i> Tuck.	43, 44
<i>Carex pinetorum</i> Liebm.	<i>Ovales</i> Kunth	
<i>Carex praecox</i> Schreb.	<i>Arenariae</i> Kunth	58
<i>Carex pseudobrizoides</i> Clavaud	<i>Arenariae</i> Kunth	
<i>Carex remota</i> L.	<i>Remotae</i> Aschers.	62



**Table 2** (continued)

Species	Section	Chromos. no. (2n)*
<i>Carex repens</i> Bell.	<i>Arenariae</i> Kunth	70
<i>Carex siccata</i> Dewey	<i>Arenariae</i> Kunth	70
<i>Carex spicata</i> Huds.	<i>Muehlenbergianae</i> Tuck.	58, 60
<i>Carex stenophylla</i> Wahlenb.	<i>Physodeae</i> Christ ex Kük.	60
<i>Carex stipata</i> Muehlenb.	<i>Stenorhynchae</i> Th. Holm	48, 52
<i>Carex vallicola</i> Dewey	<i>Muehlenbergianae</i> Tuck.	
<i>Carex vernacula</i> L. H. Bailey	<i>Foetidae</i> Tuck.	
<i>Carex vulpina</i> L.	<i>Vulpinae</i> (Carey) Christ	68
<i>Carex vulpinoidea</i> Michx.	<i>Multiflorae</i> Kunth	52, 54

\* Chromosome counts compiled from: Böcher 1938; Dalgaard 1991; Davies 1956; Delay 1971; Dietrich 1972; Heilborn 1922, 1924, 1928, 1939; Kjellqvist and Löve 1963; Löve and Löve 1981; Moore and Calder 1964; Moore and Chater 1971; Murin and Májovsky 1976; Naczi 1999; Rothrock and Reznicek 1996; Tanaka 1942a, 1942b, 1948; Whitkus 1981, 1991 and fide FNA 2002

It is worth mentioning that Kükenthal (1909) arranged the monotypic section *Incurvae* at the beginning of the sections in subgenus *Vignea*, unfortunately without any explanation. The separation from section *Physodeae* and section *Foetidae* is limited to the contour of spikes and the number of spikelets.

In our trees, *C. vallicola* and *C. macrocephala* appear as members of subgenus *Vignea*, each in an isolated position. *C. vallicola*, distributed in pacific North America, is traditionally ascribed to section *Muehlenbergianae*. This heterogeneous section is divided into almost six different parts in our dendrograms. However, a core group can be detected and is discussed below. *C. macrocephala* is the only three-stigmatic species integrated in our analyses. The isolated position of this species supports the concept of section *Macrocephalae* (e.g. Kükenthal 1909, Mackenzie 1931–1935, Ohwi 1936, Egorova 1999, Mastrogiuseppe 2002). On the other hand it can be concluded that *C. macrocephala* and presumably also its East Asian sister taxon *C. kobomugi* Ohwi are true members of subgenus *Vignea*. These species can not be separated based on the presence of three stigmata as Kreczetovicz (1935) proposed by establishing the new subgenus *Megalocranion* Kreczetovicz.

**Sections Chordorrhizeae, Heleonastes and Ouales.** With the exception of *C. chordorrhiza*,

this group as a whole is characterized by inflorescences with gynaeandrous spikes.

Members of section *Heleonastes* cluster together, except *C. canescens*, which therefore is referred to as a single member of section *Canescentes* in our dendrograms. *C. lachenalii*, known from Europe and North America (as *C. bipartita* All.) occurs as sister taxon to Eurasian *C. loliacea*, both sharing subarctic distribution. Kükenthal (1909) ascribed *C. loliacea* to section *Tenuiflorae*, the sister-section of *Canescentes*. Later authors (e.g. Mackenzie 1931–1935, Kreczetovicz 1935, Hylander 1966) transferred this species to section *Heleonastes* in a broader sense, comprising sections *Canescentes* and *Tenuiflorae*. *C. heleonastes*, the name-giving species, is connected to them at short distance. Also *C. brunnescens* can be integrated into this highly supported group (*a posteriori* probability 100%, bootstrap value 91%). Surprisingly, the striking morphological similarity between *C. brunnescens* and *C. canescens* is not reflected by molecular data.

*Carex chordorrhiza* is characterized by a unique gross-morphology with long-creeping overground tillers and preference of very moist to wet habitats. It was ascribed to section *Divisae* by most caricologists (e.g. Kükenthal 1909, Mackenzie 1931–1935, Ohwi 1936, Chater 1980, Egorova 1999) and placed in section

*Acroarrhenae*, subsection *Foetidae* by Bailey (1886). Fries (1845) established the section *Chordorrhizeae*, which was accepted by Mackenzie (1931–35) and Reznicek and Catling (2002) and is used in our dendrograms. The closer relationship to the core group of *Heleonastes* is well supported in both analyses (*a posteriori* probability 100%, bootstrap value 99%).

Species of section *Ovales* are distributed mainly in North and South America, from the Arctic to Patagonia, with the greatest diversity in the mountains of the western United States (Reznicek 1993). Kükenthal (1909) listed 21 species in this section, combining many similar taxa as subspecies and varieties under a comprehensive species name. Mackenzie (1931–35) favored a narrow species concept and listed 73 taxa for North America, Mastrogioseppe et al. (2002) accepted 72 species. In contrast to other sections, the circumscription of section *Ovales* is rather precise: cespitose growth, spikelets gynaeandrous, perigynia flattened, more or less broadly winged. However, many transitions exist and species-delimitation in this section is therefore rather difficult (Reznicek 1993). Section *Schellhammeria* is separated from section *Ovales* only by the smaller perigynium. Therefore the position of *C. bohémica* within section *Ovales* could be expected and was shown by Roalson et al. (2001) in a smaller species sampling. Bailey (1886) and Mackenzie (1931–35) placed the American sister taxon *C. synchnocephala* Carey in section *Ovales*. The densely capitate inflorescence and the protruding leafy lowermost bracts of *C. bohémica* can also be found in species of section *Ovales*: *C. athrostachya* is characterized by a leafy basal bract.

The analyzed members of section *Ovales* form a very homogeneous group with high support in both dendrograms (*a posteriori* probability 100%, bootstrap value 94%). The name-giving Eurasian species *C. ovalis* is naturalized in North America and many other parts of the world. It clusters together with *C. bicknellii* and *C. muskingumensis* with high

support. *C. fracta* and *C. pinetorum* appear closely related to this core group of *Ovales* in both dendrograms.

The South American species *C. bonplandii* with scarcely winged perigynium was treated as member of section *Elongatae* by Kükenthal (1909). It was referred to section *Ovales* by American authors (e.g. Bailey 1886, Mackenzie 1931–1935). *C. pinetorum* also has a weakly winged perigynium in comparison with *C. athrostachya*. Many transitions between wing-structures can be found in section *Ovales* (Reznicek 1993).

A specimen of *C. macloviana* from Swedish Lapland was studied. It clusters well in section *Ovales*, but the relationship to other species of the section remains unresolved. The disjunct distribution of *C. macloviana* and many closely related species in North America (Whitkus and Packer 1984, Whitkus 1988, Mastrogioseppe et al. 2002) would be worth detailed investigations.

**Sections *Elongatae*, *Muehlenbergianae* and *Vulpinae*.** The molecularly highly supported cluster (*a posteriori* probability 100%, bootstrap value 98%) comprises species of different sections.

*Carex elongata* and *C. echinata* cluster together with high support, thus showing the difficult separation of section *Elongatae* from section *Muehlenbergianae*; the position of *C. remota* is discussed below. According to the molecular data, *C. elongata* is not closely related to section *Canescentes*, as was already assumed by Russian authors (Kreczetovicz 1935, Egorova 1966). The achene epidermis, densely covered with nodular protrusions of the many silica bodies (Toivonen and Timonen 1976), shows striking similarity between *C. elongata* and *C. echinata*. This morphology strongly supports the grouping based on molecular data.

*Carex paniculata* is widely distributed in the northern hemisphere, Australia and New Zealand. It is one of the conspicuous sedges growing in large tussocks. Although it appears to be very similar to *C. appropinquata* and *C. diandra* in gross morphology and in

chromosome number, the three species, classically included in section *Paniculatae*, appear at separate positions in the molecular trees.

The core group of section *Muehlenbergiana*, although not highly supported (*a posteriori* probability 83%, bootstrap value 78%), comprises 4 very similar species mainly distributed in Europe. In many floras it is referred to as aggregate of *C. muricata*. *C. divulsa* was introduced in North America and other parts of the world. The distinction to *C. leersii* is not easy, therefore the latter is often treated as subspecies of *C. divulsa* (e.g. Chater 1980, Sebald 1998). According to ITS data, these two species are well separated. *C. leersii*, collected in France, seems closely related to *C. muricata* from Estonia. *C. spicata*, morphologically characterized by the swollen spongy basal part of the perigynium, is clearly separated in our dendrograms. It is distributed in Eurasia and North Africa and was also introduced to North America. To clarify the circumscription of the fairly diverse section *Muehlenbergiana* a larger sampling, especially of North American species, is required.

In both analyses, section *Vulpinae* is a sister group of the core cluster of section *Muehlenbergiana*. The two species studied, *C. vulpina*, the True fox sedge and *C. otrubae*, the False fox sedge, share identical ITS sequences. Thus, the morphologically closely related taxa cannot be distinguished by ITS data.

*Carex canariensis*, a local endemic of the Canary Islands described by Kükenthal (1900) and referred to section *Muehlenbergiana*, is a close relative to the group. Kükenthal (1900) postulated differences to *C. paniculata*, which is supported by ITS data.

*Carex foetida*, the name-giving species of section *Foetidae*, is distributed in the central and western Alps and the Pyrenees. It is adapted to moist meadows covered by snow for long periods. A basal position in subgenus *Vignea*, as was supposed by Kükenthal (1909), is not supported by molecular results.

The group including *C. canariensis*, *C. foetida* and the core-groups of sections *Muehlenbergiana* and *Vulpinae* is supported in

MCMC analysis with 100%, in NJ with only 62%. It is characterized by an almost stable chromosome number of  $2n=58$ . A single chromosome count of *C. vulpina* ( $2n=68$ ) has to be verified. Also species of section *Arenariae* form a highly supported lineage in molecular trees and share a stable chromosome number of  $2n=58$ .

Regarding the highly supported larger cluster (*a posteriori* probability 100%, bootstrap value 98%), including *C. paniculata* and section *Elongatae*, chromosome numbers are rather homogeneous reaching from 56 to 58, in *C. paniculata* up to 62.

**Sections *Deweyanae*, *Multiflorae*, *Remotae*, *Canescentes*, *Dioicae*, *Physodeae* and *Arenariae*.** The terminal group appears well supported in MCMC analysis. In NJ analysis *C. canescens* is placed distantly and the whole cluster is not supported. The unexpected position of *C. canescens* is discussed below.

*Carex divisa* and *C. diandra* cluster together with *C. bromoides* at a basal position in this terminal group. *C. divisa* seems closely related to *C. diandra* in MCMC analysis, whereas the NJ dendrogram reveals a closer relationship to *C. bromoides*, both positions without significant support.

In its gross morphology *C. diandra* is a slightly atypical member of section *Paniculatae*, normally not growing in dense tussocks. The perigynium is significantly smaller than in other *Paniculatae*-species. There are also micromorphological differences in achene epidermis structures (Toivonen and Timonen 1976). In our molecular tree *C. paniculata* and *C. appropinquata* are separated and belong to different clades.

The next group is weakly supported in MCMC analysis (*a posteriori* probability 67%). It comprises mainly North American species of different traditional sections, indicating that the separation of sections *Muehlenbergiana*, *Stenorhynchae* and *Multiflorae* might be artificial.

*Carex stipata* is distributed in North America and East Asia and is treated in different sections in the regional floras (e.g.

Kükenthal 1909, Mackenzie 1931–1935, Ohwi 1936). The only European species in this group is *C. appropinquata*. It is a typical member of section *Paniculatae*. Gross morphology and ultrastructure of achene epidermis (Toivonen and Timonen 1976) suggest a closer relationship to *C. paniculata*. However, this is not supported by ITS data. The three members of section *Paniculatae* integrated in our analyses do not appear closely related to each other; yet the unsupported positions in the ITS dendrograms do not allow conclusive groupings.

*Carex densa* and *C. vulpinoidea* cluster together, but they are clearly separated from *C. alma*, the third species of section *Multiflorae* in our analyses. *C. vulpinoidea* originated from temperate North America and is naturalized in parts of Europe.

*Carex leavenworthii* is one of nine species of the inhomogeneous section *Muehlenbergianae* studied in our analyses. Kükenthal (1909) separated section *Muehlenbergianae* from section *Bracteosae* on the basis of rhizome development and the basal structure of the perigynium. Mackenzie (1931–35) united both sections to one species-rich section *Bracteosae*. Ball (2002) considered section *Bracteosae* to be endemic to Central and South America and accepted sections *Phaestoglochin* and *Stellulatae* for North America, which were established by Egorova (1966, 1999) for the Flora of Russia. None of the proposed classifications corresponds with our present molecular results. Therefore only the core group of *Muehlenbergianae* is labeled in our dendrograms (see above).

The interpretation of *C. vernacula* as an American variety of the European *C. foetida* (e.g. Kükenthal 1909, Reznicek 2002) is not supported by ITS data. *C. vernacula* and *C. foetida* appear in distant positions in both dendrograms. The closer relationship between *C. vernacula* and *C. jonesii* is well supported in both analyses (*a posteriori* probability 100%, bootstrap value 81%).

*Carex canescens* is represented in our analyses by a Siberian and a French specimen

differing in only 1 bp in ITS sequence. The distant position of *C. canescens* to other members of section *Heleonastes* in molecular trees is surprising and was never recognized in other caricological studies. *C. canescens* is known to hybridize easily with species of nearly all sections in subgenus *Vigneae* (Hylander 1966, Flatberg 1972, Toivonen 1981). This extreme hybridization potential is shared only by *C. remota* and *C. echinata* and is still not understood. Morphological structures of the perigynium and the achene epidermis, (Toivonen and Timonen 1976) cannot be used to explain the exceptional position of *C. canescens* revealed by ITS data either.

Kükenthal (1909) placed *C. remota* in section *Elongatae*, together with *C. elongata* and *C. echinata*, the latter erroneously named *C. stellulata* Good. (Kükenthal 1911). Microscopic structures of the achene epidermis in *C. remota* differ considerably from those of other members of section *Elongatae* (Toivonen and Timonen 1976), thus supporting our molecular result. *C. remota* is positioned differently in both analyses, but it has no close relationship to members of sections *Elongatae* and *Canescentes*, contrary to what had been postulated by Egorova (1966). We therefore separate sections *Remotae* and *Elongatae* in our dendrograms. *C. remota* is distributed from northern Africa to northern Europe up to 63° North. It is known as one of the most intensively hybridizing *Carex* species. *C. remota* hybridizes not only with *C. arenaria*, *C. brizoides*, and *C. canescens*, but also with *C. appropinquata*, *C. otrubae*, *C. divulsa*, *C. spicata*, *C. paniculata*, *C. echinata*, *C. elongata*, and *C. ovalis* (Kükenthal 1909, Nannfeldt 1977, Toivonen 1981, Jermy et al. 1982, Sebald 1998).

*Carex alma*, traditionally placed in section *Multiflorae*, and *C. macrorrhiza*, ascribed to section *Divisae*, cluster together with high support. In MCMC analysis these taxa occur in sister position to *C. canescens*.

*Carex duriuscula* was treated as the Siberian variety of *C. stenophylla* by Kükenthal (1909). In our analyses, these two species are

well separated by long branches, but show no closer relationship to any other member of section *Divisae*; therefore we labeled this well supported group as section *Physodeae* (Chater 1980, Egorova 1999). It occurs in sister position to the three European members of section *Dioicae*, *C. davalliana*, *C. dioica* and *C. parallela*.

Kükenthal (1909) placed section *Dioicae* in subgenus *Primocarex*. The vast majority of caricologists (e.g. Bailey 1889, Heilborn 1922, Kreczetowicz 1935, Ohwi 1936, Nelmes 1952, Egorova 1966, Toivonen and Timonen 1976, Nannfeldt 1977, Chater 1980, Cochrane 2002) included section *Dioicae* in subgenus *Vignea*, which is supported by our data. The terminal spike can consequently be interpreted as a reduced composite spike with only one-dimensional lateral branches. The common hybridization with species of subgenus *Vignea* (*C. canescens*, *C. maritima*, *C. lachenalii*, *C. echinata*) was already mentioned by Kükenthal (1909). Additional and independent support for the integration of section *Dioicae* in subgenus *Vignea* is lent through the relationship of *Carex*-specific parasitic smut fungi (e.g. Nannfeldt 1968, 1977). In MCMC analysis, *C. dioica* and *C. parallela* appear closely related, in the NJ tree a closer relationship of *C. davalliana* to *C. parallela* is indicated, as it is also by chromosome numbers (comp. Table 2).

The terminal cluster comprises species ascribed to section *Arenariae* with Eurasian distribution. This group includes *C. arenaria* as name-giving species and is supported in both analyses (*a posteriori* probability 100%, bootstrap value 82%). A heterogeneous assemblage of species, traditionally placed in *Arenariae*, was discussed above and is apparently not belonging to the core group as revealed by our data. We concentrate in the following on the well supported terminal *Arenariae*-group. Within this clade, only *C. arenaria* and *C. ligerica* appear closely related in both analyses. *C. arenaria* is well adapted to sandy habitats, distributed mainly in the coastal areas of temperate Europe. We studied a

specimen from inland sands of eastern Germany. The group as a whole is also characterized by almost similar chromosome numbers of  $2n = 58$  (*C. arenaria* up to 64).

*Carex praecox* and *C. brizoides* are well characterized species, distributed in southern and continental Europe except the northern regions. Taxonomically *C. curvata* has been treated differently in European floras. Parent (1974), Chater (1980) and Sebald (1998) considered it as a common hybrid of *C. praecox* and *C. brizoides*. Kükenthal (1892, 1909) and Schultze-Motel (1967–1980) treated it as a subspecies of *C. praecox*. Molecular analyses reveal a closer relationship to *C. praecox* than to *C. brizoides*, without giving a clue to the origin of the taxon. *C. pseudobrizoides* was treated as ‘species incertae sedis’ by Kükenthal (1909). Chater (1980) presumed identity with *C. reichenbachiana* Bonnet; Luceño (1994) postulated identity with *C. brizoides*. The phylogenetic position of *C. pseudobrizoides* is not congruent in the two dendrograms; a close relationship with *C. praecox*, as suggested by MCMC analysis, is only weakly supported.

An interesting morphological character of the *Arenariae*-group is the existence of a scale-like prophyll at the basis of spikes. It appears to be absent in all other species of subgenus *Vignea* (Kükenthal 1909, Egorova 1999).

**General aspects of the subgenus *Vignea*.** Subgenus *Vignea* was considered to be a natural group within genus *Carex* by most caricologists. This morphological concept was recently confirmed by ITS and chloroplast DNA sequence data (Starr et al. 1999, Yen and Olmstead 2000, Roalson et al. 2001).

The delimitation of sections within this subgenus is very difficult and remains artificial in most parts. However, our molecular results allow better sectional circumscriptions and understanding for the species studied. Thus, the relationships between sections are well resolved by ITS sequences, revealing at least four larger subgroups, which are highly supported in all analyses (see above).

Chromosome numbers (Table 2) appear rather homogeneous in subgenus *Vignea*, not

indicating whether fission or fusion of polycentric chromosomes was predominant. It is noticeable that two groups revealed by molecular analyses might be characterized by their chromosome numbers. The well supported group including *C. canariensis*, *C. foetida* and the core groups of sections *Muehlenbergianae* and *Vulpinae* and also the terminal cluster including members of section *Arenariae* share a chromosome number of  $2n = 58$ .

Although the composition of some sections has become clearer in the course of the present study, no taxonomic conclusions are drawn. As was argued for subgenus *Carex* (Hendrichs et al. 2004), a worldwide approach is necessary to understand the natural groups within the subgenus *Vignea*.

Even after 200 years of intensive work in the genus *Carex*, we are far from understanding the natural delimitations of sections within the well-defined subgenus *Vignea*. Due to the limited number of species studied so far answer only a few problems could be solved and many open questions remain. Nevertheless, the presented dendrograms can give at least new stimulation for detailed investigations in morphological characters of the species. Ultrastructural data of achene epidermis (Toivonen and Timonen 1976, Starr and Ford 2001) and microscopic features of leaf anatomy (Shepherd 1976) have shown some potential and their exploration should be resumed.

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