

NEW INSIGHTS INTO THE SYSTEMATICS OF THE SCHOENOXIPHIMUM CLADE (*CAREX*, CYPERACEAE)

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Editor: Erika Edwards

Premise of research. The Schoenoxiphium clade (*Carex*, Cyperaceae) exhibits a high species diversity in South Africa and a complex taxonomy. Previous phylogenetic studies did not resolve the species relationships within the Schoenoxiphium clade due to the lack of informative characters in DNA markers used. Our aim is to resolve the species relationships within the Schoenoxiphium clade by adding information from more markers and more samples to information from previous studies.

Methodology. We sampled 19 out of 20 recognized species in the former genus *Schoenoxiphium*. Four DNA regions (two nuclear ribosomal: internal transcribed spacer, external transcribed spacer; two plastid: *matK* and *rps16*) were sequenced for 134 samples. Phylogenetic reconstruction was performed using Bayesian inference and maximum likelihood analyses.

Pivotal results. The monophyly of the Schoenoxiphium clade was confirmed. Five main clades with strong support were retrieved in congruence with a previous phylogeny. Although species relationships within these clades are still partially unresolved, our phylogeny highlights the need for the description of at least two new species in this group.

Conclusions. The monophyly of the former genus *Schoenoxiphium*, taken together with its morphological synapomorphies and the recent phylogenetic studies and subsequent recircumscription of the genus *Carex* to include all nested genera, advises the consideration of this clade as a section (*Carex* sect. *Schoenoxiphium* Baillon). Additional investigations based on genomic sequencing are needed to fully resolve the species relationships within each of the five main clades.

Keywords: phylogeny, species complex, ITS, ETS, taxonomy.

Introduction

Carex L. (Cyperaceae), with ca. 2000 species (Goetghebeur et al. 1998; Global Carex Group 2015), has an almost cosmopolitan distribution and a broad habitat range. Traditionally, the genus was included as one of five genera in the tribe Cariceae Kunth ex Dumort., together with *Cymophyllus* Mack. ex Britton & A.Br. (monotypic), *Kobresia* Willd. (ca. 60 spp.), *Schoenoxiphium* Nees (ca. 20 spp.), and *Uncinia* Pers. (ca. 70 spp.). Although tribe Cariceae forms a monophyletic group nested in the subfamily Cyperoideae, *Carex* is paraphyletic, because it includes the remaining genera considered within the tribe (Waterway and Starr 2007; Muasya et al. 2008). The Global Carex Group (2015) therefore suggested a new classifi-

cation resulting in a broader circumscription of a monophyletic genus *Carex* that includes all other members of the tribe.

Phylogenetic work has shown that this broader circumscribed *Carex* comprises three strongly supported clades (Siderostictae, Vignea, and Core Carex) and a weak to moderately supported clade, the Caricoid clade, which includes most unispicate and a few multispicate *Carex* species as well as the remaining genera traditionally considered within the tribe (*Cymophyllus*, *Kobresia*, *Schoenoxiphium*, and *Uncinia*; Starr et al. 1999; Yen and Olmstead 2000; Roalson et al. 2001; Waterway and Starr 2007; Waterway et al. 2009). Within the Caricoid clade, two well-supported lineages are found: the Schoenoxiphium clade, including the former genus *Schoenoxiphium* plus two small clades of *Carex* species (one containing sections *Junciformes* and *Aciculares* and the other grouping *C. distachya* and allies; Gehrke et al. 2010; Global Carex Group 2016), and another comprising the polyphyletic genus *Kobresia*, the monophyletic genus *Uncinia*, and several clades with mostly unispicate *Carex* species (Waterway et al. 2009). Morphologically, most species in the Schoenoxiphium clade present compound inflorescences

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(but unispicate in sections *Junciformes* and *Aciculares*, *C. macrostyla*, *C. peregrina*, *C. pulicaris*, and rarely in some individuals of a few species in the former genus *Schoenoxiphium*), flattened rachilla mostly present and well developed (ciliate or aculeolate in at least some individuals of all species in the former genus *Schoenoxiphium*), usually leading to a male spike or a spike of spikelets, perigynia closed (utricles) to wide-mouthed (utricles with male spikes protruding or utriculiform cladophylls; Jiménez-Mejías et al. 2016), and three stigmas. Although this group is more or less morphologically well circumscribed (Global Carex Group 2015), its species boundaries are still weakly studied, and the previous published studies (Levyns 1945; Kukkonen 1983; Timonen 1989; Gordon-Gray 1995; Gehrke et al. 2010) included only a partial species sampling. The former genus *Schoenoxiphium*, a monophyletic group nested in the Schoenoxiphium clade, has about 20 species, with its center of diversity in the southeastern parts of Africa, with a few species extending their distribution to southwestern Africa, eastern Africa, Madagascar, and the Arabian Peninsula (fig. 1; Gehrke et al. 2010). Moreover, recent phylogenetic analyses have suggested a complex evolutionary history of the group due to hybridization events and/or lineage sorting (Gehrke et al. 2010). In this article, our objective is to investigate the phylogenetic relationships within the former genus *Schoenoxiphium* to (1) increase sampling, including all extant recognized species, or nearly so; (2) represent the full distribution range of species; (3) increase DNA region sampling; (4) improve the knowledge of species boundaries; and (5) resolve the phylogenetic backbone of the former genus *Schoenoxiphium*.

Material and Methods

Taxon Sampling

We have sampled 19 out of 20 of the species as recognized for the former genus *Schoenoxiphium* by Gordon-Gray (1995) and Kukkonen (1983, 1986; see table 1), including two previously nonsampled species (*Carex schimperiana* Boeck. and *C. kukkoneniana* Luceño & Martín-Bravo) by Gehrke et al. (2010), as well as an expanded population sampling of the other previously sampled species. The only species that was not included in the analyses is *C. chermesonii* Luceño & Martín-Bravo (= *Schoenoxiphium gracile* Cherm.), because we were unable to obtain herbarium or field samples.

Outgroup taxa were selected on the basis of previous work (Gehrke et al. 2010) and included the following: *C. andina*, *C. camptoglochis*, and *C. transandina* in the Andina clade and *C. distachya*, *C. macrostyla*, *C. peregrina*, *C. pulicaris*, and *C. oedipostyla* in the Distachya clade. These species formed a monophyletic group in the cited work.

DNA Extractions and Sequencing

DNA extractions and sequencing were done following Gehrke et al. (2010). We amplified the internal transcribed spacer (ITS) region (ITS-A, ITS-4; White et al. 1990; Blattner 1999), external transcribed spacer (ETS) region (ETS-1f, 18S-R; Starr et al. 2003), a portion of the *matK* gene using primers *matK* 2.1F

and *matK* 5R (Ford et al. 2009), and *rps16* intron using primers *rps16F*–*rps16R* (Shaw et al. 2005). Therefore, two additional DNA markers were studied (ETS and *matK*), different from those used by Gehrke et al. (2010; ITS, *trnL-F*, *rps16*), to try to get a better resolution of the phylogeny. Sequences were edited, automatically aligned with MUSCLE, version 3.8.31 (Edgar 2004), and manually adjusted using the program Geneious, version 6.1.7 (Biomatters, Auckland, New Zealand).

Phylogenetic Analyses

We used NCBI GenBank accessions and newly obtained sequences, for a total of 116 ITS sequences, 35 ETS, 46 *matK*, and 87 *rps16* (see appendix). All loci were analyzed independently and in combination (albeit the missing data) using maximum likelihood (ML) and Bayesian inference (BI). ML analyses were performed using RAxML, version 7.2.6 (Stamatakis 2006), with general time reversible with gamma-distributed rate variation among sites (GTR+G) and node support assessed with 10,000 bootstrap (BS) replicates. BI analyses were executed in MrBayes, version 3.2.1 (Huelsenbeck and Ronquist 2001). The most appropriate nucleotide substitution model for each partition was chosen using the Akaike information criterion in jModeltest (Posada 2008). The Markov chain Monte Carlo search was run for five million generations with a tree sampled every 1000 generations and two simultaneous analyses started from different random trees (Nruns = 2), each with four Markov chains (Nchains = 4). The first 20% of the trees were discarded from each run as the burn-in, as they were sampled before convergence to the optimal mean log likelihood plateau as retrieved from BI analyses results as implemented in Geneious, version 6.1.7. A Bayesian majority-rule consensus tree was calculated in MrBayes with posterior probability (PP) values as a measure for clade support. Trees were edited using Figtree, version 1.3.1 (<http://tree.bio.ed.ac.uk/software/figtree/>). Gehrke et al. (2010) showed that there is a recombinant nuclear ITS region (ITS2) shared by all species in the *C. ludwigii* (= *S. rufum*) clade and by *C. camptoglochis*. Therefore, we repeated our analyses excluding the ITS2 region of the *C. ludwigii* clade. Clades were considered strongly supported when PP was >0.95 and when BS was >70.

Results

Phylogenetic Reconstruction

The combined matrix of both nuclear and plastid DNA regions consisted in 134 samples with 2960 sites (see appendix). Selected nucleotide substitution models were GTR+G, K80+I, and GTR+G for ITS1, 5.8s, and ITS2, respectively; they were GTR+G for ETS and *matK* and GTR+I+G for *rps16* (appendix). Although few topological changes were found on the individual ITS gene trees when removing the ITS2 region of the *Carex ludwigii* clade, those changes in topology were not supported. Consequently, the analyses of the concatenation of all four DNA regions reported exactly the same topology with irrelevant changes in clades support.

Monophyly of former genus *Schoenoxiphium* was strongly supported in BI and ML analyses (1 PP; 92% BS; fig. 2). Sister to former genus *Schoenoxiphium* was the *C. andina* clade. The

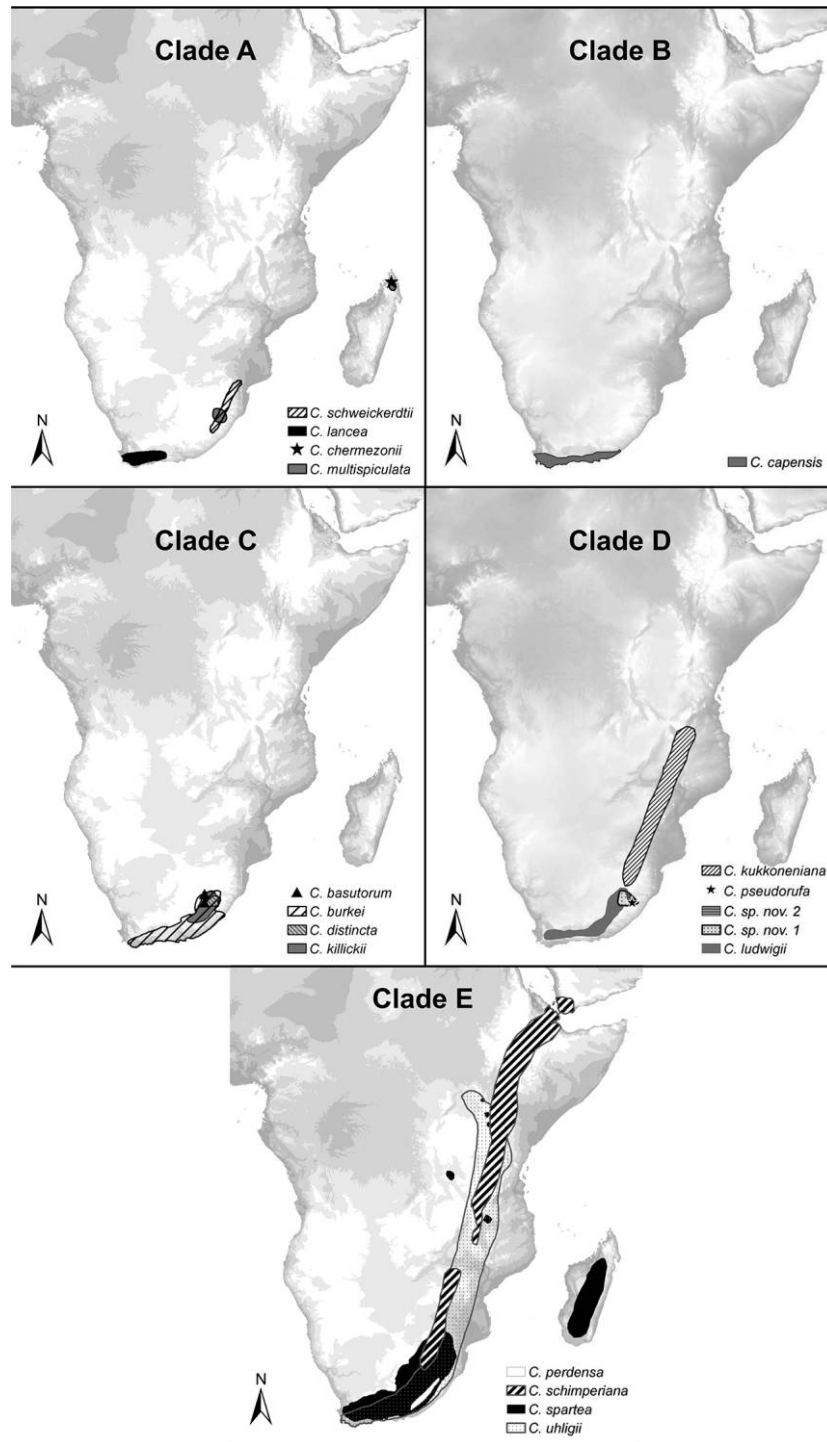


Fig. 1 Approximate distribution of species on each of the main clades in the former genus *Schoenoxiphium*. Based on Kukkonen (1983), field trips, and herbarium specimens examined. Elevation data are distributed by the Land Processes Distributed Active Archive Center, located at US Geological Survey Earth Resources Observation and Science Center, Sioux Falls, South Dakota (<http://lpdaac.usgs.gov>).

main phylogenetic structure of former genus *Schoenoxiphium* was well resolved, with five strongly supported main lineages.

Clade A (0.99 PP; 85% BS; figs. 1, 2), sister to the remaining species of former genus *Schoenoxiphium*, was composed of three strongly supported monophyletic species: *C. schweickerdtii* (1 PP;

99% BS), *C. lancea* (1 PP; 100% BS), and *C. multispiculata* (1 PP; 100% BS). *Carex capensis* is retrieved as monophyletic in a strongly supported clade (clade B; 1 PP; 100% BS), sister to clades C, D, and E (figs. 1, 2). Clade C (1 PP; 99% BS; figs. 1, 2), sister to clades D and E, is formed by two lineages: one com-

Table 1

Taxonomic Treatments of the Former Genus *Schoenoxiphium*

Gordon-Gray (1995)	New circumscription in <i>Carex</i> (Global Carex Group 2015)
<i>S. altum</i> Kukkonen ^a	<i>C. capensis</i> Thunb.
<i>S. ecklonii</i> Nees ^a	<i>C. capensis</i> Thunb.
<i>S. basatorum</i> Turrill	<i>C. basatorum</i> (Turrill) Luceño & Martín-Bravo
<i>S. bracteosum</i> Kukkonen	<i>C. schimperiana</i> Boeck.
<i>S. schimperianum</i> (Boeckeler) C.B. Clarke	<i>C. schimperiana</i> Boeck.
<i>S. buchananii</i> C.B. Clarke	<i>C. kukkoneniana</i> Luceño & Martín-Bravo
<i>S. burkei</i> C.B. Clarke	<i>C. burkei</i> (C.B. Clarke) Luceño & Martín-Bravo
<i>S. burttii</i> Kukkonen	<i>C. pseudorufa</i> Luceño & Martín-Bravo
<i>S. caricoides</i> C.B. Clarke	<i>C. spartea</i> Wahlenb.
<i>S. sparteam</i> (Wahlenb.) C.B. Clarke	<i>C. spartea</i> Wahlenb.
<i>S. distinctum</i> Kukkonen	<i>C. distincta</i> (Kukkonen) Luceño & Martín-Bravo
<i>S. filiforme</i> Kük.	<i>C. killickii</i> Nelmes
<i>S. strictum</i> Kukkonen	<i>C. killickii</i> Nelmes
<i>S. gracile</i> Cherm. ^a	<i>C. chermesonii</i> Luceño & Martín-Bravo
<i>S. lanceum</i> (Thunb.) Kük. ^b	<i>C. lancea</i> (Thunb.) Baill.
<i>S. lehmanii</i> (Nees) Steud.	<i>C. uhligii</i> K. Schum. ex C.B. Clarke
<i>S. ludwigii</i> Hochst.	<i>C. ludwigii</i> (Hochst.) Luceño & Martín-Bravo
<i>S. rufum</i> Nees	<i>C. ludwigii</i> (Hochst.) Luceño & Martín-Bravo
<i>S. madagascariense</i> Cherm.	<i>C. multispiculata</i> Luceño & Martín-Bravo
<i>S. perdensum</i> Kukkonen	<i>C. perdensa</i> (Kukkonen) Luceño & Martín-Bravo
<i>S. schweickerdtii</i> Merxm. & Podlech	<i>C. schweickerdtii</i> (Merxm. & Podlech) Luceño & Martín-Bravo

^a Kukkonen (1986).^b Kukkonen (1983).

prises samples of *C. burkei* (1 PP; 93% BS), and the other comprises samples of *C. burkei* as well as samples of *C. basatorum*, *C. distincta*, and *C. killickii* (1 PP; 93% BS). *Carex burkei* appears as polyphyletic, whereas *C. distincta* and *C. killickii* are retrieved as paraphyletic. The single sample of *C. basatorum* included in our analyses does not allow us to test for the monophyly of the species. Clade D (1 PP; 99% BS; figs. 1, 2), sister to clade E, is constituted by the monophyletic *C. pseudorufa* (1 PP; 100% BS) and another monophyletic clade comprising two tentative new species (hereafter *Carex* sp. nov. 1 and *Carex* sp. nov. 2; Márquez-Corro et al., forthcoming), *C. kukkoneniana*, *C. ludwigii*, and an unidentified species from Cape region similar to *C. ludwigii* (*Carex* sp.; fig. 2). Clade E (1 PP; 98% BS) retrieved a monophyletic *C. perdensa* and a paraphyletic lineage constituted by *C. schimperiana*, *C. spartea*, and *C. uhligii* (figs. 1, 2). Sister group relationships of all clades were strongly supported except for clades D and E.

Discussion

Our results show that former genus *Schoenoxiphium* is monophyletic and is sister to the clade containing sections *Aciculares* and *Junciformes* (fig. 2), confirming with strong support previous analyses (Waterway and Starr 2007; Starr and Ford 2009; Gehrke et al. 2010; Global Carex Group 2016). The species of sections *Aciculares* and *Junciformes* are distributed in South America and New Zealand, whereas former genus *Schoenoxiphium* has its center of diversity in South Africa. Phylogenetic relationships among species in the *Schoenoxiphium* clade herein reported are generally congruent with those obtained by Gehrke et al. (2010) based on ITS, *rps16*, and *trnL-F*. The former genus *Schoenoxiphium* has been recircumscribed within genus *Carex*

(Global Carex Group 2015; table 1). This group of species can be defined by a set of characters showed by most individuals: (i) compound inflorescences; (ii) scabrous or ciliate flattened raquilla, frequently leading to a male spike or a spike of spikelets, perigynia closed (utricles) to wide-mouthed (utricles with protruding male spikes or utriculiform cladophylls; Jiménez-Mejías et al. 2016); and (iii) occasional occurrence of bisexual flowers (Gehrke et al. 2012). These morphological features of the former genus *Schoenoxiphium* and its strongly supported monophyly (fig. 2) deserve, in our opinion, taxonomic recognition at the sectional level (see below).

Monophyly of Species within Section Schoenoxiphium

In the strongly supported monophyletic clade A, we found species composed by big-sized plants: *Carex schweickerdtii*, *C. lancea*, and *C. multispiculata* (see table 1; fig. 2). All of them constitute well-supported monophyletic species. We suspect that the only unsampled species in our study, *C. chermesonii*, known only from the type locality in Madagascar (fig. 1), could be phylogenetically related to this group on the basis of morphological observations of the type specimen (M. Luceño, personal observation).

Carex capensis is retrieved as monophyletic in clade B (fig. 2). It displays a wide morphological variability, which has allowed the traditional recognition of two closely related species under the former genus *Schoenoxiphium*: *S. ecklonii* and *S. altum* (Kukkonen 1986). However, we have found that these species are not monophyletic and that diagnostic characters between them are inconstant (i.e., morphological intermediates are frequent; M. Luceño, personal observation). Therefore, we support the unification of both species, as has been stated by the

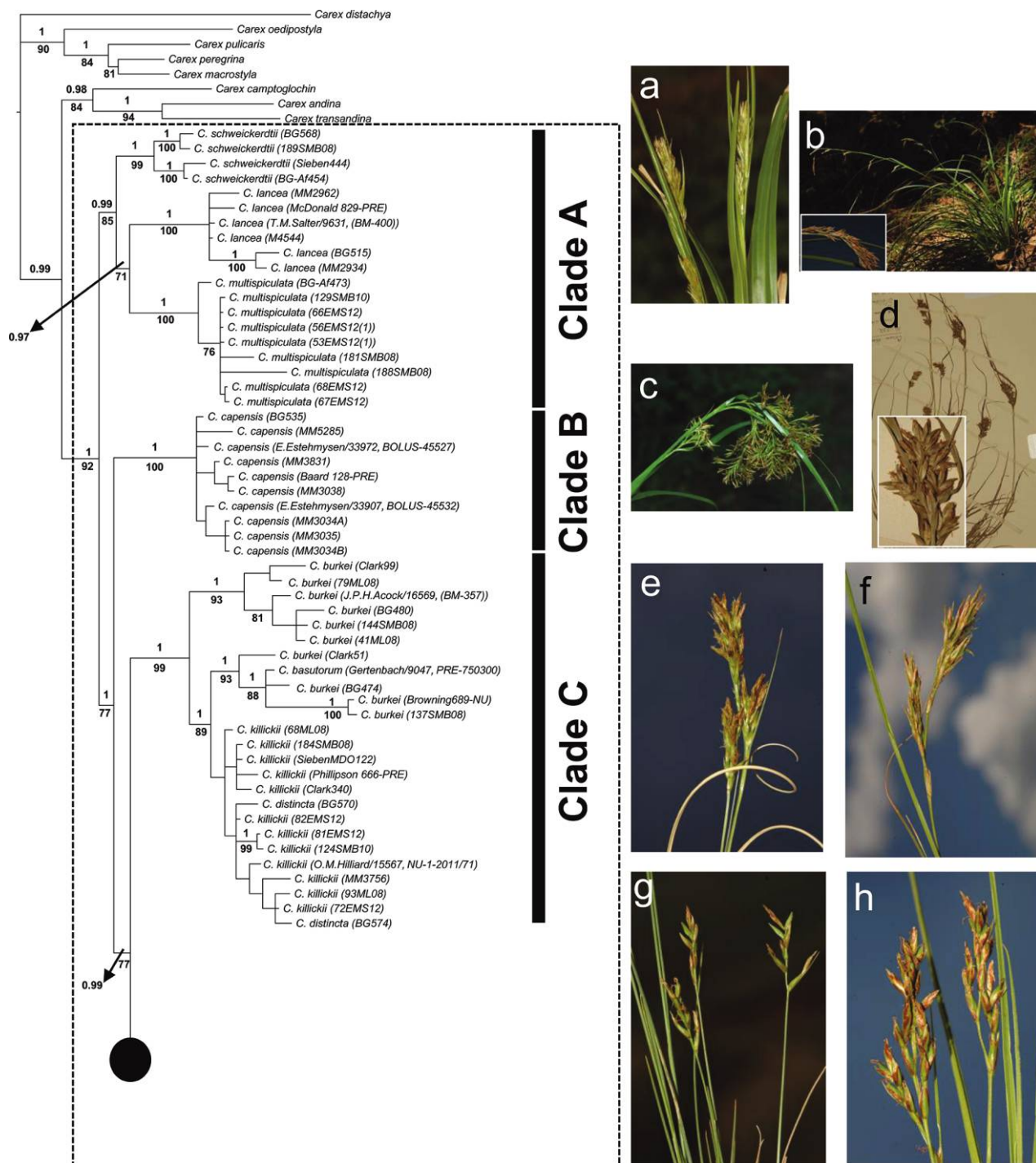


Fig. 2 Majority-rule consensus tree obtained in the Bayesian inference analysis of the combined matrix including ETS, ITS, *matK*, and *rps16* DNA regions. Posterior probabilities (only if >0.9) are shown above branches, and maximum likelihood bootstrap support (if >70%) is shown below branches. Dashed line indicates circumscription of the *Carex* section *Schoenoxiphium*. Lateral bars indicate names of the clades. Photographs of species correspond to species in clade A (a, *C. schweickerdtii*; b, *C. lancea*; and c, *C. multispiculata*), clade B (d, *C. capensis*), clade C (e, *C. burkei*; f, *C. basatorum*; g, *C. killickii*; and h, *C. distincta*), clade D (i, *C. pseudorufa*; j, *C. ludwigii*; k, *C. kukkoneniana*; l, *Carex* sp. nov. 2; and m, *Carex* sp. nov. 1), and clade E (n, *C. perdensa*; o, *C. spartea*; p, *C. schimperiana*; and q, *C. ubligii*).

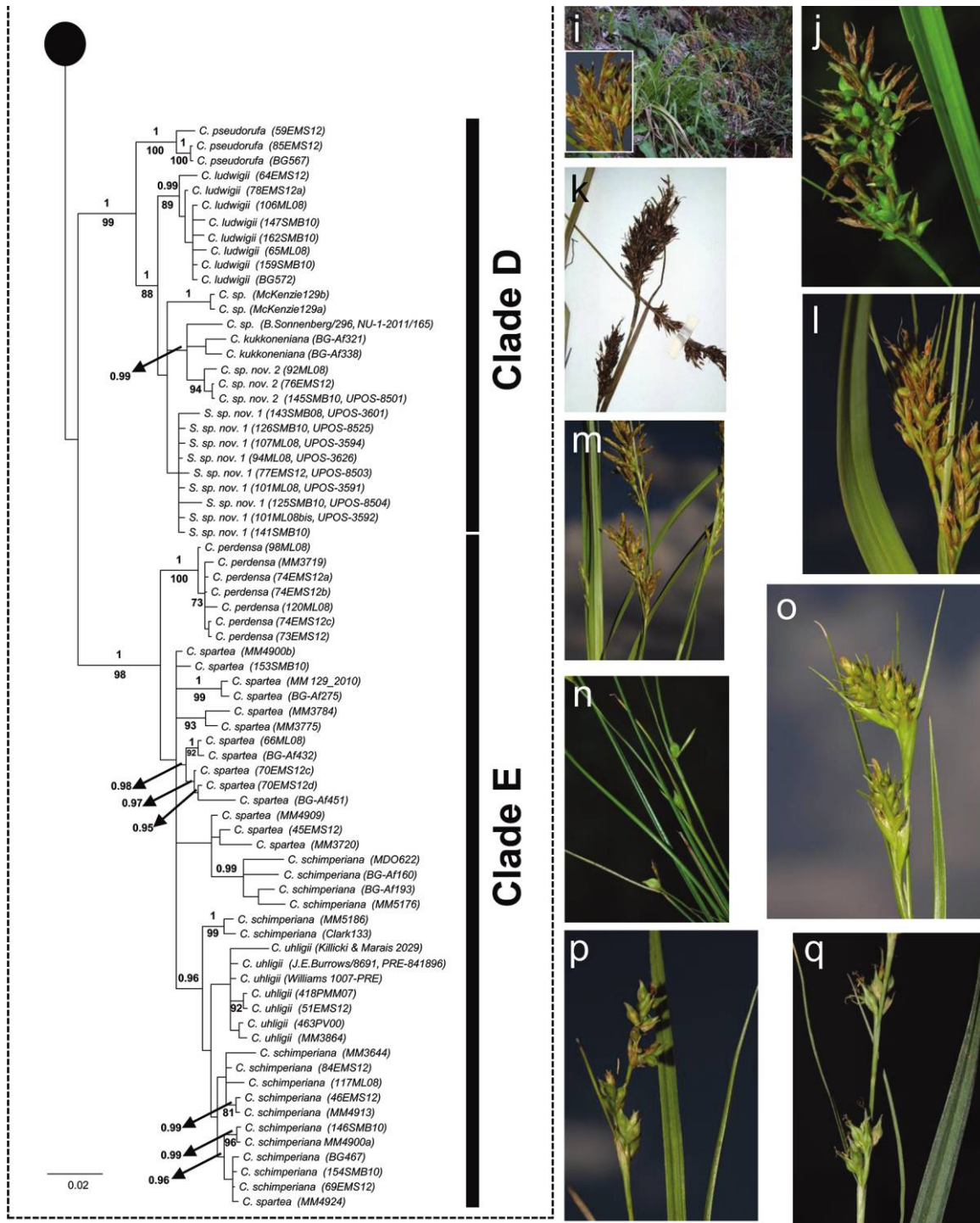


Fig. 2 (Continued)

Africa, Cape Region, KY322166/KY322253/KY322367/KY322300; E. Estehmynsen 33972 (BOLUS-45527), South Africa, Cape Region, —/KY322265/—/—; M. Muasya et al. MM3034A, South Africa (UPOS) KY322167/—/—/KY322315; M. Muasya et al. MM3034B, South Africa (UPOS), KY322169/—/—/KY322338; M. Muasya et al. MM3035, South Africa (UPOS), KY322168/—/—/KY322318; M. Muasya et al. MM3038 (UPOS), South Africa, Cape Region, GU176182/—/—/—; M. Muasya et al. MM3831, South Africa (UPOS), KY322165/—/—/KY322386/KY322341; M. Muasya et al. MM5285, South Africa (UPOS), —/—/—/KY322323. *Carex distachya* Desf., M. Escudero et al. 65ME06 (UPOS-2266), Turkey, Manisa, GU176156/—/—/—/GU176257. *Carex distincta* (Kukkonen) Luceño & Martín-Bravo, B. Gehrke & M. Pirie BG574 (UPOS-4390), South Africa, Kwaazulu-Natal, KY322188/—/—/—; B. Gehrke & M. Pirie BG570 (UPOS-4391), South Africa, Kwaazulu-Natal, KY322181/—/—/—. *Carex killickii* Nelmes, E. Maguilla et al. 72EMS12 (UPOS), South Africa, Kwaazulu-Natal, KY322187/—/—/—/KY322385/KY322340; E. Maguilla et al. 80EMS12 (UPOS), South Africa, Kwaazulu-Natal, KY322183/KY322266/KY322383/KY322332; E. Maguilla et al. 82EMS12 (UPOS), South Africa, Kwaazulu-Natal, KY322182/KY322255/KY322369/KY322303; M. Luceño et al. 68ML08 (UPOS), South Africa, Free State, KY322175/—/—/—; M. Luceño et al. 93ML08 (UPOS), South Africa, Kwaazulu-Natal, KY322186/—/—/—; M. Muasya et al. MM3756, South Africa (UPOS), KY322185/—/—/—/KY322291; Phillipson et al. 666 (PRE), South Africa, Eastern Cape, AY242020/—/—/—; Sieben, MDO122, South Africa (UPOS), KY322179/—/—/—; S. Martín-Bravo et al. 124SMB10 (UPOS), South Africa, Free State, KY322184/—/—/—; S. Martín-Bravo et al. 184SMB08 (UPOS), South Africa, Kwaazulu-Natal, KY322176/—/—/—; V.R. Clark & T. Te Water Naude Clark340 (UPOS), South Africa, Cape Region, KY322180/—/—/—/KY322309; O.M.Hilliard, 15567, South Africa, Bamboo Mt., (NU-1-2011/71), —/—/—/KY352040. *Carex kukkoneniana* Luceño & Martín-Bravo, B. Gehrke & H.I. Patel BG-Af321 (Z-39364), Malawi, Northern Region, KY322220/—/—/—/KY322362/—; F. Mbago et al. BG-Af338 (Z-39363), Tanzania, Iringa District, EU288650/—/—/—. *Carex lancea* (Thunb.) Baill., B. Gehrke et al. BG515 (BOL), South Africa, Western Cape, GU176185/—/—/—; McDonald 829 (PRE), South Africa, Cape Province, AY242028/—/—/—; Muasya et al. MM2934, South Africa (UPOS), KY322161/—/—/—/KY322307; M. Muasya et al. MM2962, South Africa (UPOS), KY322158/—/—/—/KY322389/KY322343; M. Muasya et al. MM4544, South Africa (UPOS), KY322160/—/—/—/KY322393/—; T.M. Salter 9631 (BM), South Africa, Cape Region, KY322159/KY322273/KY322392/KY322348. *Carex ludwigii* (Hochst.) Luceño & Martín-Bravo, B. Gehrke & M. Pirie BG572 (UPOS-4393), South Africa, Kwazulu-Natal, KY322197/—/—/—/KY322290; E. Maguilla et al. 64EMS12 (UPOS), South Africa, Kwaazulu-Natal, KY322192/KY322246/KY322357/KY322280; E. Maguilla et al. 78EMS12a (UPOS), South Africa, Kwaazulu-Natal, KY322193/—/—/—/KY322358/KY322281; M. Luceño et al. 65ML08 (UPOS-3616), South Africa, South Africa, Free State, KY322195/—/—/—; M. Luceño et al. 106ML08 (UPOS), South Africa, Kwazulu-Natal, KY322194/—/—/—/KY322287; S. Martín-Bravo et al. 147SMB10 (UPOS), South Africa, Kwazulu-Natal, —/—/—/KY322289; S. Martín-Bravo et al. 159SMB10 (UPOS), South Africa, Kwazulu-Natal, KY322196/—/—/—/KY322288; S. Martín-Bravo et al. 162SMB10 (UPOS), South Africa, Kwaazulu-Natal, —/—/—/KY322282. *Carex macrostyla* Lapeyr., J.A. Alejandre & M.J. Escalante s.n. (UPOS-2667), Spain, Burgos, GU176157/—/—/—/GU176258. *Carex multispiculata* Luceño & Martín-Bravo, B. Gehrke et al. BG-Af473 (Z-39370), South Africa, Kwaazulu-Natal, KY322162/—/—/—/KY322336; E. 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