



The systematic position of the enigmatic rare South African endemic *Carex acocksii*: Its relevance on the biogeography and evolution of *Carex* sect. *Schoenoxiphium* (Cyperaceae)

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ABSTRACT

Carex acocksii is a sedge species known only from two populations in the Karoo of the Northern Cape in South Africa. It was described from a single locality (Hantam Mts., Calvinia) more than twenty years ago, and tentatively ascribed to section *Petraea* because of its unispicate inflorescence and utricles with membranaceous-papyraceous walls. However, its systematic relationships have remained largely unexplored. We perform molecular, morphological and biogeographic studies in order to elucidate the systematic relationships and origin of this poorly known species. A phylogenetic reconstruction based on two nuclear (ITS, ETS) and two plastid (*matK*, *rps16*) markers strongly supports that *C. acocksii* belongs to *Carex* sect. *Schoenoxiphium* (former genus *Schoenoxiphium*), a lineage with its center of diversity in South Africa. However, *C. acocksii* displays a remarkable molecular (i.e. long phylogenetic branch), morphological (i.e. unispicate inflorescence and poorly veined, weak utricles walls), geographical (isolated populations placed more than 100 kms from any other native *Carex* species) and ecological (the only *Carex* species known exclusively from the Great Karoo) differentiation with respect to the remainder of sect. *Schoenoxiphium*. The section has been inferred to have originated 15 Mya in the Drakensberg, and to have speciated after dispersal to other areas at least three times (*C. chermesonii* was not included in this study), one of these cases being *C. acocksii*. These features highlight the evolutionary singularity and conservation importance of this species, especially in the context of South African flora. We reassessed the conservation status of *C. acocksii* at a global scale under IUCN categories and criteria, resulting in the proposal of the Critically Endangered category for the species.

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1. Introduction

With over 2000 species, *Carex* L. is one of the three most diverse angiosperm genera in the world (WCSP, 2020). The genus has been reported to have originated in southeastern Asia, from where it expanded and colonized the rest of the globe, with a remarkable diversity in the Northern Hemisphere. The Southern Hemisphere regions are comparatively poor in number of *Carex* species and have been colonized several times by different lineages. However, the Austral species often belong to endemic and locally diversified lineages (Martín-Bravo et al., 2019). This is the case of *Carex* in South Africa, for which species have steadily been described (Archer and Balkwill, 1997; Martín-Bravo et al., 2013; Márquez-Corro et al., 2017). There

are about 37 *Carex* species in South Africa (WCSP, 2020), of which half belong to *Carex* sect. *Schoenoxiphium* Baillon.

Section *Schoenoxiphium* is composed of 18 species distributed from southwestern to eastern mainland Africa, a few of them reaching Madagascar and also marginally the SW Arabian Peninsula (Villaverde et al., 2017). This group was considered separate genus until its combination under *Carex* (Global *Carex* Group -GCG-, 2015) and reevaluation as a section (Villaverde et al., 2017). This new treatment was supported by recent molecular works (Gehrke et al., 2010; Starr et al., 2015; GCG, 2015, 2016; Villaverde et al., 2017). Yet, new species are still being described in this group (Márquez-Corro et al., 2017), which depicts that sect. *Schoenoxiphium* is much in need of revision. While the taxonomy of *Carex* sect. *Schoenoxiphium* has been studied for over a century (see Kükenenthal, 1909), hybridization and ongoing speciation processes have greatly hindered species delimitation (Gehrke et al., 2010). Thus, studies using a multidisciplinary approach

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are needed for further comprehension of the section in general and of *C. acocksii* in particular.

Carex acocksii C.Archer was described by Archer and Balkwill (1997) and named after J.H.P. Acocks (1911–1979), a South African botanist and collector. It is a small to medium size sedge with a combination of several features that makes it unique among *Carex*: inflorescence reduced to a single terminal spike, utricles with papyraceous-membranaceous walls, and habitat restricted to the drylands of the Upper Great Karoo. Few species of *Carex* display utricles with weak utricule walls, and certainly even less are able to grow in arid or semi-arid environments (see treatments in Kükenthal, 1909; Ball and Reznicek, 2002; Egorova, 1999; Dai et al., 2010, among many others). Upon examination of its morphological characters, Archer and Balkwill (1997) concluded that the unispicate inflorescence and the papyraceous utricule walls of *C. acocksii* pointed to affinities with sect. *Petraea* Lang, according to Kükenthal's (1909) world monograph. The authors of the species also discussed the affinities of *C. acocksii* with other African unispicate *Carex* belonging to other sections (*C. peregrina* Link, *C. monostachya* A. Rich. and *C. runsoroensis* K. Schum.) and concluded that, based on the deviant morphology of *C. acocksii*, it was probably distantly related to them. *Carex acocksii* has a very limited distribution range, with the two only known populations found in the Northern Cape province of South Africa. Biogeographically, they are included in the Extra Cape Floristic Region, which embraces the territories considered part of the Greater Cape Floristic Region, but not the core Cape Floristic Region itself, of which the flora has been comparatively much more studied (Snijman, 2013). The Extra Cape Floristic Region is divided into eight ecogeographical units, of which *C. acocksii* is entirely endemic to the Western Mountain Karoo unit (Snijman, 2013). This unit harbors 170 endemic plants out of a total of 1237 species (13.7%), ranking second in endemism in the Extra Cape Floristic Region (Snijman, 2013). Specifically, the populations are located in the Hantam Plateau Dolerite Renosterveld and the Roggeveld Shale Renosterveld (Fig. 1), which are

known to constitute a centre of plant endemism in South Africa (Hantam–Roggeveld Centre of Plant Endemism or “HRC”; Born et al., 2007; Clark et al., 2011a; Snijman, 2013). *Carex acocksii* inhabits dolerite outcrops summits, between 1450–1600 m above sea level (a.s.l.), which provide special habitats, such as seasonal wetlands (Snijman, 2013). Thus, these peaks behave as islands of more mesic conditions, with mean rainfall 270 mm per year and annual mean temperature of 13°C (based on the *C. acocksii* populations, 291 mm and 14.4°C for the whole Karoo Renosterveld; Mucina and Rutherford, 2006), embedded within the arid Succulent Karoo Biome, with hotter, drier conditions (Snijman, 2013; Fig. 1). *Carex acocksii* occurs in habitats with sandy/rocky dolerite soils, usually near ephemeral watercourses but also somewhat far from them, growing under shrubs (Archer and Balkwill, 1997; Archer and Muasya, 2013; Clark et al., 2011a). Floristic checklists of nearby, southern regions with similar habitats such as the Tankwa Karoo National Park do not record *C. acocksii* (Steyn et al., 2013), and its presence is unlikely eastwards, since it has not been recorded in the near, though more arid, Nuweveld Mountains (Clark et al., 2011b).

Here, we perform a multidisciplinary reappraisal of the South African endemic *C. acocksii*, spanning molecular phylogenetics, biogeographical analysis and a morphological study. We also provide new insights on the morphological evolution and biogeography of sect. *Schoenoxiphium*. We perform a reassessment of the conservation status of *C. acocksii*, since it presents a very limited range of distribution and a very small number of known populations.

2. Materials and methods

2.1. Sampling

The authors JIMC and ML visited the type location in Hantam Mountains (South Africa, Northern Cape, Hantam Municipality;

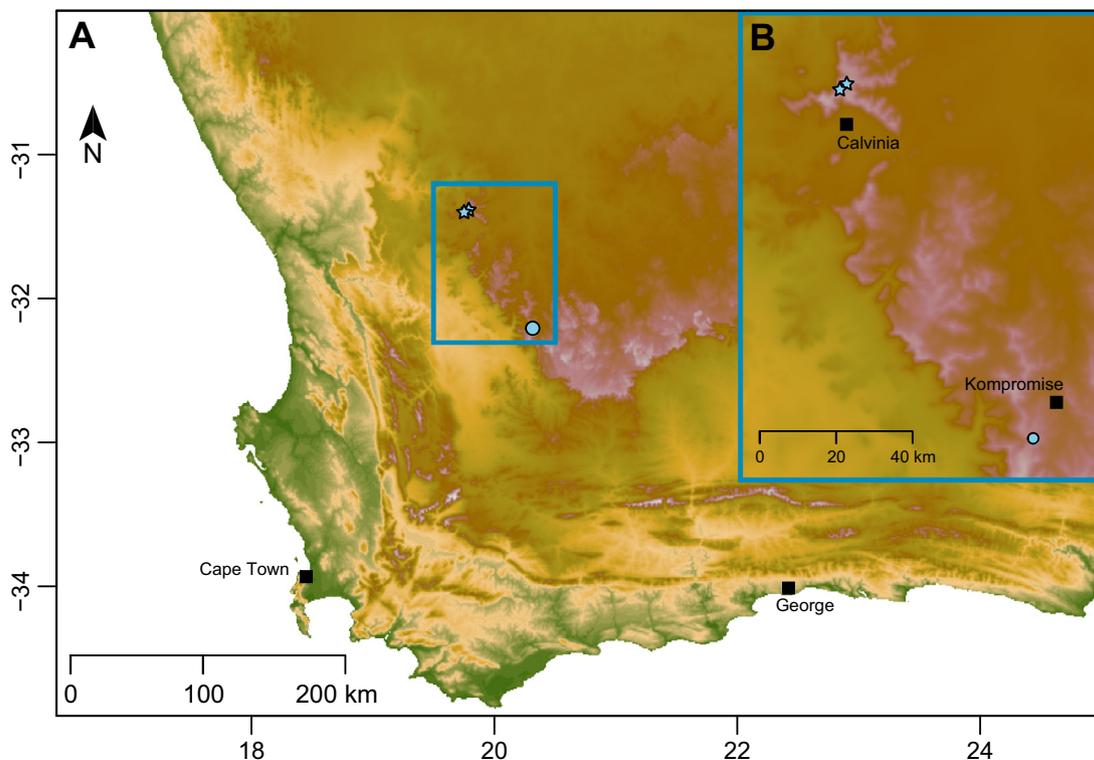


Fig. 1. (A) Map displaying the locations of the populations of *Carex acocksii* (dots and stars). (B) Inset showing the placement of the two populations. Note that the two known occurrences of *C. acocksii* are separated more than 100 km. Stars represent individuals sampled for molecular analysis.

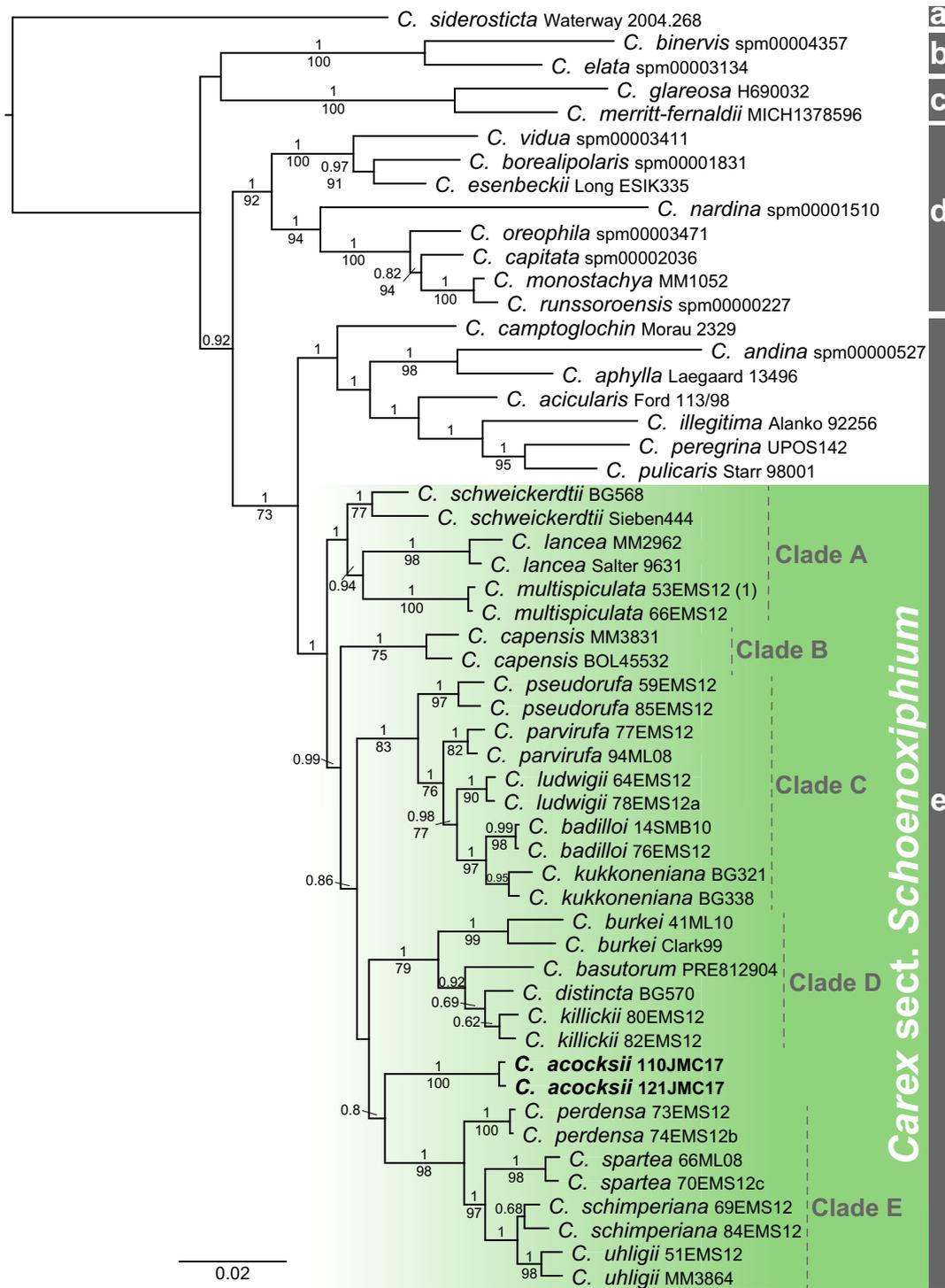


Fig. 2. Maximum a posteriori (MAP) phylogeny estimate of the combined nrDNA-cpDNA matrix (ETS, ITS, *rps16*, *matK*). The numbers above the branches are posterior probabilities (>0.80 pp) for the clades and below the branches, maximum likelihood bootstrap support (>70% BS). Clades within sect. *Schoenoxiphium* are indicated with dashed lines, and named according to Villaverde et al. (2017). Major *Carex* lineages are indicated with a grey bar in the right: (a) subg. *Siderosticta* (b) subg. *Carex*, (c) subg. *Vignea*, (d) subg. *Euthyceras*, (e) subg. *Psyllophorae*. The scale bar depicts substitutions per site.

Fig. 1) during the late spring of 2017, and collected specimens were deposited at UPOS, PRE and NBG (acronyms according to Thiers, 2019). Mostly parts of individuals were collected in order to minimize the negative impact in the population. Moreover, individuals from a population of *C. acocksii* were collected by the author NAH in early summer of 2013 in the Roggeveld Mountains (Northern Cape, Karoo Hoogland Municipality; Fig. 1) and

deposited at NBG. Both populations were recorded by Archer and Muasya (2013).

Only the most recent material of *C. acocksii*, from the type population, could be included in the molecular analysis. Preliminary BLAST tests using the *Carex* barcode region ITS (nrDNA; see below) revealed the putative affinity of *C. acocksii* with species of sect. *Schoenoxiphium* and other closely related groups (Gehrke

et al., 2010; GCG, 2016; Villaverde et al., 2017; Martín-Bravo et al., 2019). Accordingly, all known species of sect. *Schoenoxiphium* were included (see below), with exception of *C. chermeszonii*, as well as representatives of the most closely related groups (those conforming to what currently we conceive as subg. *Psyllophorae*; Villaverde et al., under review; see Fig. 2). In addition, a number of additional species from the main clades of the genus were sampled as outgroup (Appendix A).

2.2. Extraction, PCR amplification and sequencing

To evaluate the placement of *C. acocksii* among sect. *Schoenoxiphium* species, we amplified and sequenced a total of four DNA regions (Appendix A) that have been previously used to elucidate the phylogenetic relationships within this lineage (Villaverde et al., 2017): two corresponding to nuclear ribosomal DNA regions (nrDNA: ITS and ETS) and the remaining two to plastid DNA regions (cpDNA: *rps16* intron and part of the *matK* gene). Leaf material was extracted using DNeasy Plant Mini Kit (Qiagen, Valencia, California) and PCR amplification was performed according to protocols and primers in Villaverde et al. (2017). PCR products of *C. acocksii* were Sanger-sequenced by Macrogen (Amsterdam, Netherlands). All remaining sequences were downloaded from GenBank (<http://ncbi.nlm.nih.gov/genbank>, see Appendix A). Sequences were edited and aligned using Muscle v. 3.8.425 (Edgar, 2004) and the alignment manually revised using Geneious v. 11.1.2 (Biomatters, Auckland, New Zealand) to reduce homoplasy due to point mutations.

2.3. Phylogenetic analyses

To elucidate the phylogenetic placement of *C. acocksii*, we individually analyzed and compared the nuclear (54 accessions, 1370 bp aligned length) vs. plastid (52 accessions, 1785 bp aligned length) matrices and also combined them (54 accessions, 3155 bp aligned length, Appendices A–B). We reconstructed the phylogenetic relationships among species using Bayesian Inference as implemented in revBayes v. 1.0.10 (Höhna et al., 2016). This software is more flexible than other phylogeny-construction programs, because it allows adjusting different parameters to give selective weights during iterations. The script is detailed in the supplementary material. To sum up, GTR+ Γ +I was used as substitution model, and 7 runs of Markov chains with 5000 generations were run, sampling every generation and with a previous tuning burn-in of 500 generations. Moreover, maximum likelihood was estimated with RAxML 8.2.9 (Stamatakis, 2014) with 10000 bootstrap runs and a GTR+ Γ substitution model.

2.4. Morphological study

The most important morphological characters (e.g. leaf width, branching order, utricle) for the taxonomy of *Carex* sect. *Schoenoxiphium* were examined (Kukkonen, 1983; Gordon-Gray, 1995). Herbarium material of *C. acocksii* was studied from its two populations (19 specimens, deposited at BM, GENT, K, MO, NBG, P, PRE, S, TCD, UPOS; see "Specimens examined"). Leaf width was measured as a qualitative ordinal character categorized in three leaf types: filiform (up to ca. 1 mm), narrow (ca. 1–5 mm) and broad (>5 mm). Branching order as a measure of inflorescence complexity (see Jiménez-Mejías et al., 2016) was also considered as qualitative ordinal. The minimum value of one (last branching order) consists of unispicate inflorescences: thus, the utricles (pseudospikelets), which are the last branching unit in a *Carex* inflorescence, are placed on an unbranched main axis, without any other additional branches (paracladia). The values grow higher as the inflorescence further complicates by adding branches before reaching the utricle (last branching unit): second-to-last for racemose inflorescences 1-time branched, and third and fourth-to-last for paniculate inflorescences with additional ramifications (see Figs. 3 and 4).

Ancestral state reconstruction was estimated using the most common category in each species, with the R function 'make.simmap' in *phytools* v. 0.6–60 (Revell, 2012). We checked for the best model (equal, symmetrical, meristic and all-rates-different models; 'fitDiscrete' function in *geiger* package v. 2.0.6.2, Harmon et al., 2008) and summarized 100000 stochastic character maps (simmap trees) by MCMC sampling the posterior probability distribution.

2.5. Biogeographical analysis

The phylogeny resulting from the molecular analysis was dated using BEAST (Drummond et al., 2012) and calibrated with a fossil (*C. colwellensis*) as crown node of the genus and a secondary calibration for the crown node of the subg. *Psyllophorae* (Martín-Bravo et al., 2019). Standard deviation for subg. *Psyllophorae* calibration was set to BEAUti default value of 1, as ages obtained by Martín-Bravo et al. (2019) did not include error estimation. Six chains of 1000000 length were run, sampling trees every 1000, and burn-in was set to 10%. Ancestral geographic ranges reconstruction analysis was performed on a pruned phylogeny of the section with one tip per species -and a few outgroup species- with the R package *BioGeoBEARS* (Matzke, 2014), under dispersal-extinction-cladogenesis (DEC) model. We considered seven areas for the section *Schoenoxiphium*, attending either to the vegetation types (i.e. fynbos and karoo; Mucina and Rutherford, 2006) or the delimited regions the species inhabit (e.g. Madagascar, Drakensberg mountains, Ethiopian Highlands and the southwestern region of the Arabian Peninsula; Sayre et al., 2013). The remaining two areas include Western Palaearctic and South America, where the sister group of the section is distributed (Martín-Bravo et al., 2019).

2.6. Conservation assessment

We took into account previous assessments of the species performed at the national level (Victor, 2002; Archer and Victor, 2006; Raimondo et al., 2009), which has consistently resulted in the category Vulnerable (VU). We reevaluated the conservation status of *C. acocksii* at global level following criteria, categories and guidelines from IUCN (2012, 2017). Area of occupancy (AOO) and extent of occurrence (EOO) were calculated using GeoCAT tool (Bachman et al., 2011).

3. Results

3.1. Molecular phylogenetics

Our analyses were fully congruent with previous phylogenetic reconstructions of *Carex* sect. *Schoenoxiphium* (Villaverde et al., 2017). Monophyly of *Carex* sect. *Schoenoxiphium* was supported in all analyses, but received strong support only for the plastid and combined DNA analyses (1 pp/81% BS, Fig. 2). The five main clades (A–E) retrieved in Villaverde et al. (2017) were also well supported in the combined analyses, although relationships between some species remain unclear.

All the phylogenetic reconstructions conducted, irrespective of the markers analyzed, clearly retrieved *C. acocksii* nested within *Carex* sect. *Schoenoxiphium*. However, the sister phylogenetic relationships of *C. acocksii* within the section were not clearly resolved, and varied among the different reconstructions (Fig. 2, Appendix C). Low support was obtained for its phylogenetic placement in the nuclear and plastid analyses, while the combined analysis suggested that *C. acocksii* was sister to species in clade E (Fig. 2), but with low support (0.80 pp/50% BS). Overall, separate plastid and nuclear phylogenies retrieved only a few highly supported clades, the rest of accessions being unsupported as in a polytomy. Thus, there was minimal well-supported incongruency between the nuclear and plastid regions. For instance, the clade E retrieved strong support in nuclear and plastid phylogenies, but the latter inferred *C. perdensa* as sister of *C. schimperiana* and *C. uhligii* with

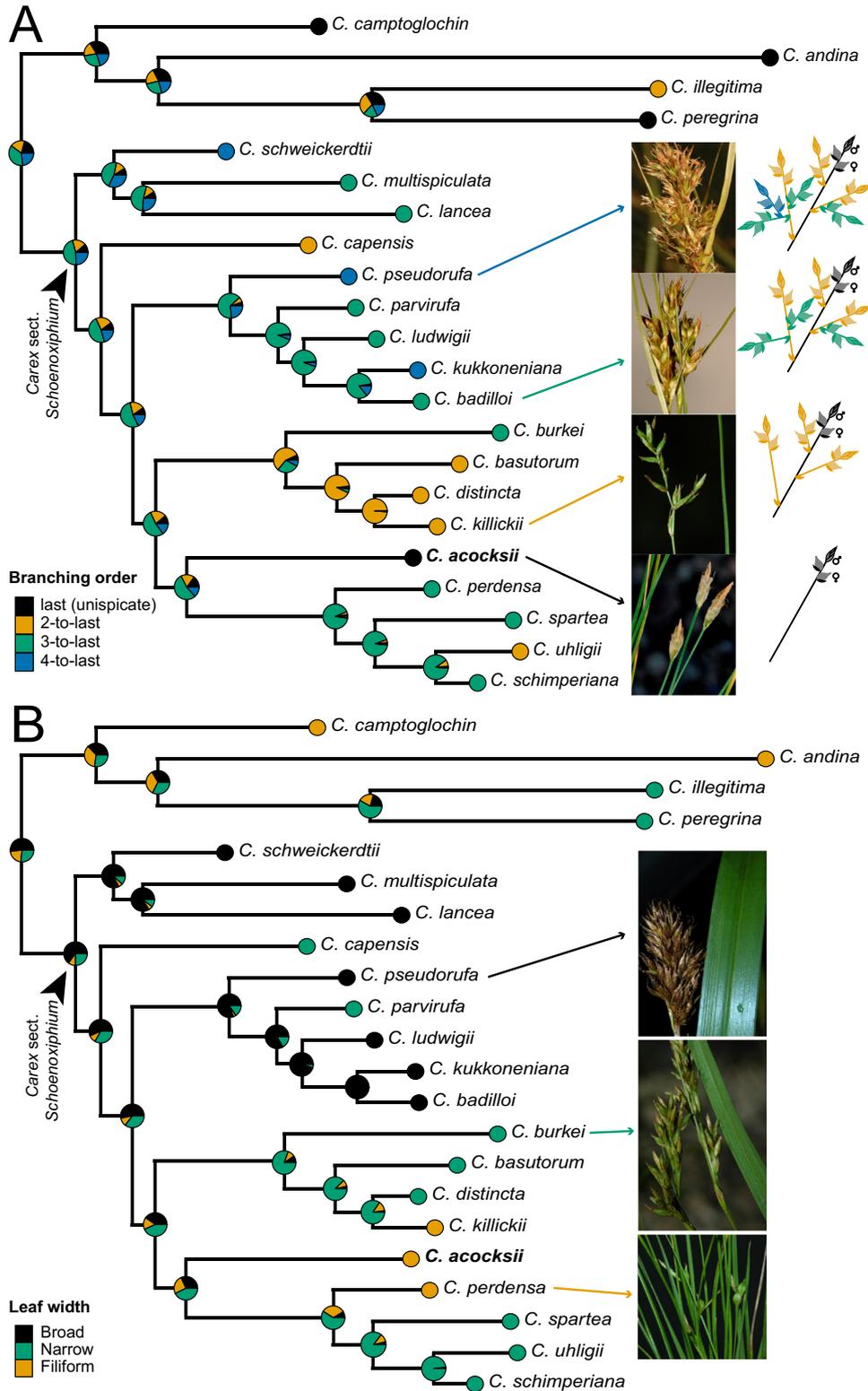


Figure 3. Ancestral state reconstruction of (A) most common inflorescence branching pattern, and (B) leaf width for *Carex* sect. *Schoenoxiphium* species using SIMMAP. Posterior probabilities are represented with pie charts on each node. A schematic illustration and a representative photo of the different types of inflorescence and leaves are indicated alongside the species. Note that the image of *C. pseudorufa* only includes the first partial inflorescence.

a moderate support whilst the nuclear inferred *C. spartea* as the sister species with strong support. Moreover, the remaining clades do not provide much support beyond the species level, as most of the inner nodes were not supported (that is, below a combination of 0.80 pp and 75% BS; Appendix C).

3.2. Morphological and biogeographical studies

Reconstructions of ancestral characters (Figs. 3 and 4) and the biogeographical analysis (Fig. 5) emphasize the singular morphology and distribution of this species within the section. The dated

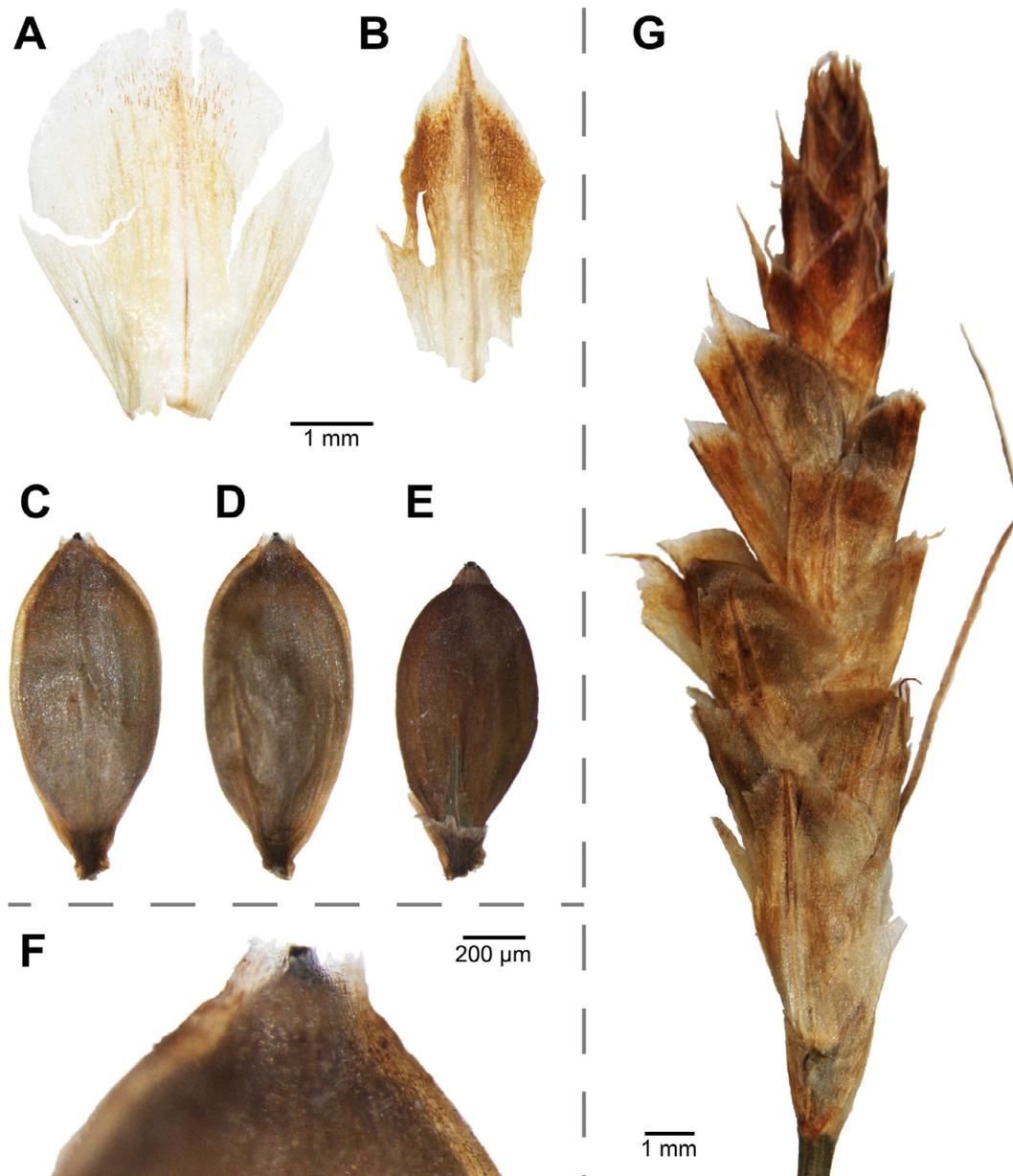


Figure 4. *Carex acocksii* (A–F, 110JMC17; G, 121JMC17). (A) Pistillate glume; (B) staminate glume; (C) utricle, abaxial side; (D) utricle, adaxial side; (E) nutlet with rachilla; (F) detail of utricle tip, note that the nutlet is visible below the papyraceous-membranaceous scarious utricle wall; (G) androgynous unispicate inflorescence.

biogeographical analysis (Fig. 5) places the most probable origin of sect. *Schoenoxiphium* in the Drakensberg ca. 15 Mya, where the group seems to have been resident for all or most its evolutionary history. Our analysis reveals at least three outwards dispersal events from the ancestral area of Drakensberg mountains followed by speciation: two to the afro-montane forest and the fynbos ecosystems for *C. lancea* and *C. capensis* (note that the category Fynbos in Fig. 5 includes surrounding afro-montane forests), and a third to the Great Karoo (i.e. Karoo Renosterveld type) for *C. acocksii*.

There are a total of three subpopulations of *C. acocksii*, that add up to 12 km² of area of occupancy (AOO; 2 km² grid) and a total extent of occurrence (EOO) of 55.2 km². According to the IUCN guideline (IUCN, 2012), a subpopulation is considered as any distinct group of individuals between which there is little demographic or genetic exchange. Here we considered three subpopulations taking into account geographic distance alone (2 km² grid), as no detailed population genetic or demographic study has been carried out. Nonetheless, considering two or three subpopulations is irrelevant, as this

would be of importance only for criterion C (IUCN, 2012), which is not applicable to *C. acocksii* with the available data.

4. Discussion

4.1. Evolutionary history of *Carex acocksii*

Section *Schoenoxiphium* has been considered to date to be composed of 18 accepted species (GCG 2015; Márquez-Corro et al., 2017; Villaverde et al., 2017), grouped in five well-supported clades (Gehrke et al., 2010; Villaverde et al., 2017, see Fig. 2). *Carex acocksii* displays a relatively long phylogenetic branch, indicative of a remarkable molecular differentiation with its relatives. Although it is clearly included in the section, further phylogenetic studies are needed to better clarify the sister group of *C. acocksii*. High-throughput sequencing techniques providing more loci, such as restriction-site associated DNA sequencing (RAD-seq) are known to provide satisfactory results in *Carex* (Massatti et al., 2016; Maguilla et al., 2017).

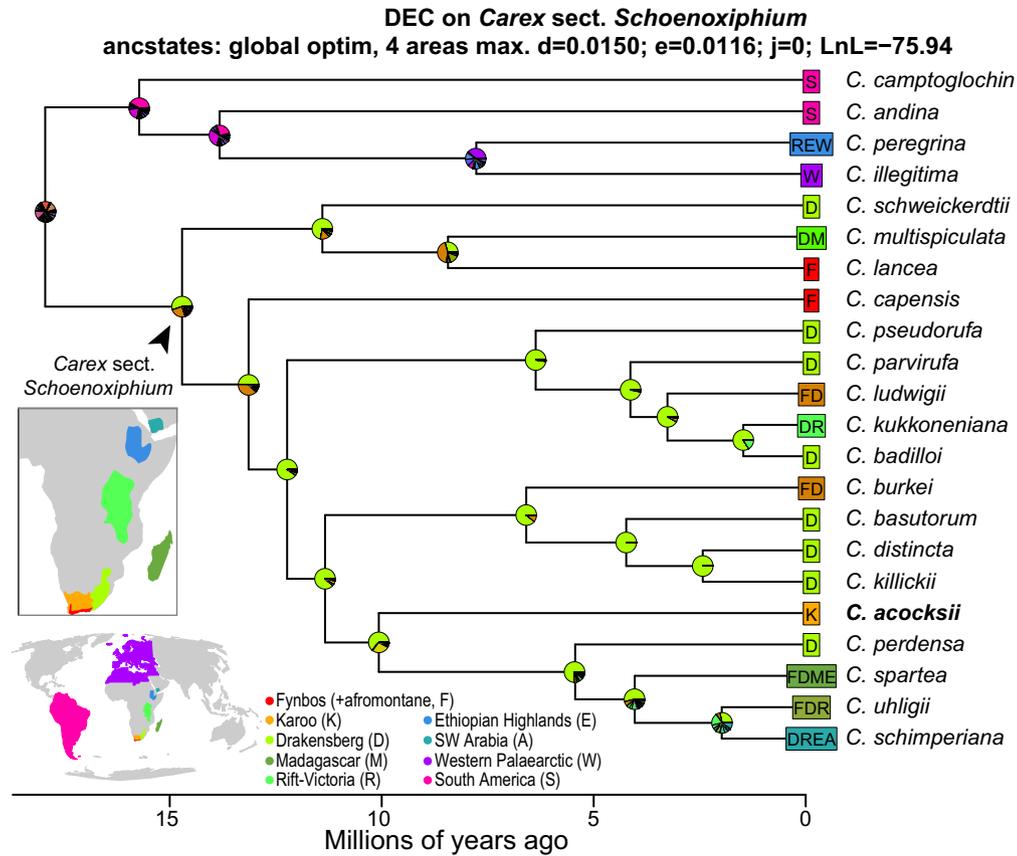


Figure 5. Ancestral distribution ranges inferred by DEC model in BioGeoBEARS. Labels indicate the area of each species and the ancestral areas as follows. F: Fynbos (+ Afromontane forests). K: Great Karoo (including part of Succulent Karoo, Nama Karoo and Karoo Renosterveld). D: Drakensberg Mountains. M: Madagascar. R: Rift-Victoria. E: Ethiopian Highlands. A: South-western Arabia. W: Western Palaeartic. S: South America.

The section *Schoenoxiphium* apparently started to diversify about 15 Mya (Middle Miocene) after a dispersal from the Western Palaeartic (Martín-Bravo et al., 2019; Fig. 5), although additional sampling of the most closely related groups would be desirable to confirm the geographical origin of sect. *Schoenoxiphium*'s ancestors. Secondary diversification events of the section would have taken place in concert with the uplift of the Drakensberg mountain range at the Mio-Pliocene boundary (5.5 Mya) as has been suggested for other lineages of South African plants (e.g. Bentley et al., 2014).

Species delimitation within sect. *Schoenoxiphium* is challenging (Villaverde et al., 2017) due to the lack of a comprehensive revision since Kükenthal's (1909) and Kukkonen's revision (1983). The most recent account by Gordon-Gray (1995), focused on species inhabiting KwaZulu-Natal. *Carex acocksii* presents distinctive morphological characters when compared to the remaining species of the section regarding inflorescence configuration, and utricle and leaf morphology.

Carex acocksii presents unique characteristics that increase the morphological diversity found in the section. Regarding the inflorescence, the ancestral reconstruction indicates that sect. *Schoenoxiphium* ancestor probably displayed a paniculate (3-to-last order branched) inflorescence, a morphology that seems to be plesiomorphic and to have been maintained along the inner nodes of the phylogeny (Fig. 3). Further complications or simplifications of it (as in *C. acocksii*) seem to have taken place several times across sect. *Schoenoxiphium* history. *C. acocksii* is the only species that consistently displays unispicate inflorescences (Fig. 4). Although some individuals of *C. killickii* may sometimes bear a single terminal androgynous spike, the spikes in both species differ greatly (see images in Fig. 3). *Carex acocksii* has a denser spike, but also presents a rachilla that does not elongate beyond the maximum width of the achene and is entirely contained within the utricle. On the contrary, *C. killickii* usually tends

to present a rachilla protruding the utricle mouth and that often branches or bears a male spike (Kukkonen, 1983, Kükenthal, 1909).

The morphology of leaves also seems to respond a plesiomorphic-homoplastic pattern in sect. *Schoenoxiphium*. The ancestor probably displayed broad leaves and these became narrower several times during the evolution of the lineage. There seems to be a relationship between leaf width (Fig. 3) and habitat, with narrower leaves apparently linked to drier, open habitats (e.g. some species in clade E, authors pers. obs.) vs. broader leaves in species inhabiting mesic or wet grasslands or forest understories (e.g. some species in clade C, authors pers. obs.).

Regarding the utricle morphology, *C. acocksii* is unique among sect. *Schoenoxiphium* members. While all the species of the section are very conspicuously veined on the utricle wall, the utricle veins of *C. acocksii* are neither conspicuous nor that numerous. Moreover, while the remaining sect. *Schoenoxiphium* species bear herbaceous or subcoriaceous utricles, the utricle walls of *C. acocksii* are very weak, scariose and papyraceous-membranaceous. This morphological feature clearly differentiates *C. acocksii* from all other members of the group, and points to a closer relationship with other groups of *Carex*, on the basis of morphology. This clearly explains the original placement of *C. acocksii* among the members of sect. *Petraea* by Archer and Balkwill (1997), as these plants also develop utricles with weak walls. Papyraceous-membranaceous utricles are certainly rare among *Carex* species; apart from sect. *Petraea*, they are also found in the former genus *Kobresia*, and in the sections *Filifoliae* (Tuck.) Mack. and *Physodeae* Kük. (Kükenthal, 1909; Egorova, 1999; Ball and Reznicek, 2002; Dai et al., 2010). Interestingly, all these groups, as in *C. acocksii*, inhabit arid, sandy or rocky environments, which may suggest a relationship between habitat dryness and that particular type of utricle, perhaps also with functional implications.

4.2. Biogeography

Carex sect. *Schoenoxiphium* started to diversify little after its origin in the Drakensberg area ca. 15 Mya (Fig. 5), and dispersed beyond its area of origin several times. This is the case for *C. acocksii*, *C. lancea* and *C. capensis*. However, whereas *C. lancea* and *C. capensis* have a distributions in the fynbos and surrounding vegetation types (Villaverde et al., 2017), adjacent to the Drakensberg area (including most of the Eastern Cape mountains, Fig. 5), *C. acocksii* is the only species in the section with such a disjunct distribution and disparity of habitat. In addition, we should also consider *C. chermesonii*, an unsampled species which is reportedly only in the northern part of Madagascar, which would account for an additional fourth dispersal with speciation event. Seven other species have wide distribution ranges that include the Drakensberg and other areas. The clade formed by *C. schimperiana*, *C. spartea* and *C. uhligii* seems to have expanded repeatedly from the Drakensberg to the Cape Region and Tropical E Africa, reaching regions as far as the Arabian Peninsula (*C. schimperiana*). Madagascar would have been colonized at least twice, once by *C. spartea* and another time by *C. multispiculata*, with *C. chermesonii* still to be included in the phylogeny.

Remarkably, *C. acocksii* is the only native *Carex* species recorded not only for the Western Mountain Karoo unit, but for the whole Extra Cape Floristic Region (*C. divisa* Huds. is also recorded, but it has been reported as introduced, probably from Europe; Archer and Muasya, 2013). The absence of other *Carex* species in this vast territory reflects the scarcity of suitable habitats for *Carex* and underlines the biogeographical and ecological singularity of *C. acocksii*. The closest populations of *Carex* sect. *Schoenoxiphium* are situated more than 100 km away, in the core Cape Floristic Region (Villaverde et al., 2017), which constitutes a considerable disjunction. Biogeographic connections have been postulated for the Western Mountain Karoo and the Drakensberg Mountains in Lesotho and KwaZulu-Natal (Snijman, 2013), which is particularly interesting given that the Drakensberg is the centre of diversity and endemism of *Carex* sect. *Schoenoxiphium* (Gehrke et al., 2010; Márquez-Corro et al., 2017; Villaverde et al., 2017; see Fig. 5). There are environmental similarities between both areas regarding climate and soils (Snijman, 2013). The biogeographic connections are reflected in the presence of shared, disjunct species, as well as closely related or sister species in the Western Mountain Karoo and the Drakensberg Alpine Centre (Snijman, 2013). It is also noticeable that the open habitat in which *C. acocksii* occurs is, more similar to that of the majority of the species in clades D and E (Fig. 2; the only exception is *C. uhligii*, typical of forest understories –JIMC and ML pers. obs.–). Clades D and E are primarily distributed through the Drakensberg Mountains, which reinforces the suggested connection between this region and the Western Mountain Karoo (Fig. 5).

4.3. Conservation assessment

As described above, this species is confined to the Hantam–Roggeveld dolerite summit areas, and its presence is unlikely to extend further east (Clark et al., 2011b) or north. Given the current predictions of climatic change into a drier and warmer trend, together with the southwards advance of the desert, the future of these mesic patches surrounded by more arid territories could be at risk (Clark et al., 2011a). Since this habitat occupies the highest ground in the region, species inhabiting it would have no possibility of escaping the warming climate by moving to higher elevations. Indeed, extinction of species from this habitat has already been suggested (Clark et al., 2011a), as it is highly dependent on the winter rains that are likely to decrease with the global climate crisis. The vulnerability of this unique habitat with high endemism is exacerbated by the absence of legal protection (Clark et al., 2011a), as overgrazing is evident by livestock in the two locations where it occurs. Apparently, shrubs are presumed to act as protection against livestock grazing. The subpopulations are not dense, with individuals appearing sparsely, up to 30 individuals per hectare. Therefore,

unfortunately, it is highly likely for this habitat to deteriorate quickly in the years to come. The species was previously assessed as Vulnerable (VU; Victor, 2002; Archer and Victor, 2006; Raimondo et al., 2009) at the national level, based on the known occurrence at that time of only the type location, which was considered threatened by livestock overgrazing and potentially by natural disasters (criterion D2; IUCN, 2012). Nonetheless, taking into account the distribution range and the above-mentioned threats to the future persistence of the habitat of the species and its impact on the two known locations (three subpopulations), we propose raising the current conservation status of the species from VU to Critically Endangered (CR). The two known locations cover an area of occupancy (AOO) of 12 km² (2 × 2 km grid size; IUCN, 2017). This allows the application of the criterion B2 for the EN category (threshold of 500 km²; IUCN, 2012). The criterion B1, related to the extent of occurrence (EOO) defines a total area of 55.2 km² based on the few collections of the species. This criterion places *C. acocksii* in CR due to its EOO below 100 km² (IUCN, 2012). However, we believe that this result is biased by the few specimens collected to date. We think that it could easily surpass the threshold with few more occurrences, as the species seems to follow a patchy distribution within its habitat, with disjunct subpopulations separated by extensions of more arid, unsuitable habitats. Thus, we find that the application of the EN category for the criterion B1 is likely to be fulfilled for *C. acocksii* once further prospecting are made. However, at present, the distribution is severely fragmented, as there are only two known locations for the species, fulfilling the sub-criterion a (IUCN, 2012). Finally, according to previous and own field observations regarding livestock overgrazing and to the potential impact of ongoing climate change (Clark et al., 2011a; see above), the known populations are facing a decline in the following conditions: (i) extent of occurrence, (ii) area of occupancy, (iii) area, extent and/or quality of the habitat, and the number of (v) individuals (sub-criterion b(i,ii,iii,v); IUCN, 2012). Therefore, with the current available data, we propose the category CR B1ab(i,ii,iii,v). The CR conservation category would be applicable at both the national and the global levels, since the species is endemic to South Africa. Nonetheless, further field prospecting would be desirable to census the number of individuals, monitor habitat quality, and search for additional populations in similar habitats. Inclusion of *C. acocksii* in national and global red data lists (with this updated category) as well as in conservation legislation, to enforce legal protection, together with the protection of the area where populations occur, seem to be essential conservation measures to safeguard the species' future.

5. Conclusions

Our study helps to elucidate the systematic placement of the rare endemic *Carex acocksii* within sect. *Schoenoxiphium*. While the phylogenetic relationships with the other members of the section remain unclear, our study highlights the remarkable molecular, morphological, and ecological differentiation of this species with respect to its closest relatives. It also displays an intriguing biogeographic pattern, involving a considerable disjunction not only with the other species in sect. *Schoenoxiphium*, but with all *Carex* species. *Carex acocksii* constitutes one of at least three dispersal events outside the ancestral distribution areas of sect. *Schoenoxiphium* (Drakensberg mountain range) followed by speciation. It is the only *Carex* species occupying the Western Mountain Karoo area in the Great Karoo. All this could be pointing to a long period of evolution driven by geographic isolation. Our conservation assessment has resulted in the proposal of the critically endangered category, since the two known locations could be sensitive to different threats, especially climatic change, causing increased aridity, and livestock overgrazing. Our study highlights the high biological conservation value of this species, in view of its phylogenetic, ecological and morphological singularity within *Carex*, and the need for its urgent conservation prioritization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A.

Voucher and GenBank accession data for material of *Carex* sect. *Schoenoxiphium* and the *Carex* species used as outgroup for phylogenetic reconstructions. Studied specimens of *Carex acocksii* appear below the table

Appendix B.

Characteristics of the matrices for each locus and the combined regions used for phylogenetic reconstructions

Appendix C.

Nuclear ribosomal DNA (ETS and ITS) and plastid DNA (*rps16* and *matK*) trees resulted from phylogenetic reconstruction of *Carex* sect. *Schoenoxiphium*

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