Molecular and morphological evidence for a new species from South Africa: Carex rainbowii (Cyperaceae)

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Carex rainbowii (Cary cineae, Cyperaceae), a new species from the Drakensberg mountains (KwaZulu-Natal province), is described and illustrated. It was found in the shady understory of the Afromontane forest in the Cathedral Peak area. An additional, nearby population was also identified based on previously collected herbarium material. Morphological and molecular (cpDNA 5′trnK intron and nrDNA ITS and ETS sequences) data were used to evaluate the taxonomic status of these populations and shed light on their systematic placement. Our data strongly support their taxonomic identity and inclusion in Carex sect. Sylvaticae. The new species can be readily distinguished from other related taxa mainly by the frequently androgynecandrous terminal spike, dense female spikes, hyaline glumes, as well as by some quantitative features. This finding implies a considerable biogeographic disjunction from the mainly Eurasian-North African range of the remaining species of sect. Sylvaticae, a pattern also found in the related sections Ceratocystis, Rhy nchocystis and Spirostachyae. Comments are provided on previous misidentifications of C. rainbowii as the closely related Carex sylvatica. Data pertinent to the conservation status of the species are provided.

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1. Introduction

With over 2000 species (Reznicek, 1990), Carex L. (Cyperaceae, Cary cineae) is the most diverse angiosperm genus of Earth’s temperate zone (Escudero et al., 2012). It displays the highest species diversity in cold and temperate areas of the Northern Hemisphere, with 527 species recorded in China, 480 in North America (Brach and Song, 2006) and 231 species in Europe and the Mediterranean region (Jiménez-Mejías and Luceño, 2011). Despite the genus being more diverse in northern temperate areas, at least 81 species have been reported from Sub-Saharan Africa and Madagascar (Gehrke, 2011). Among the four traditionally recognized subgenera within Carex (Carex, Psyllophora (Degl.) Peterm., Vignea (P. Beauv. ex T. Lestib.) Peterm. and Vigneastræ (Tuck.) KüK; Kükenthal, 1909; Egorova, 1999), subgenus Carex (c. 1400 species) is the largest and also the most species rich in Sub-Saharan Africa and Madagascar, with 34 species recognized to date (Gehrke, 2011). Eighteen species from this subgenus are currently known in South Africa, 11 of which grow in KwaZulu-Natal (Govaerts et al., 2012).

Within subgenus Carex, section Sylvaticae Rouy has been frequently subsumed within a widely-circumscribed section Hymenochlaenæae (Drejer) L.H. Bailey (Kükenthal, 1909). Despite section Hymenochlaenæae being split up into several sections by Mackenzie (1935), many authors have followed Kükenthal’s criteria until recent times (e.g. Reznicek, 1986; Waterway, 1990). Egorova (1999) circumscribed section Sylvaticae and considered that some American species from Hymenochlaenæae, like Carex debilis Michx., could be transferred to Sylvaticae. Subsequently, the first ITS phylogeny by Hendrichs et al. (2004), later confirmed by Waterway and Starr (2007) and Waterway et al. (2009) revealed the high polyphyly of section Hymenochlaenæae. Specifically, Carex sylvatica was allied to sections Rhyncho cystis Dumort., Ceratocystis Dumort. and Spirostachyæae Drejer ex L.H. Bailey, whereas representatives of section Hymenochlaenæae were distantly related. Under Egorova’s (1999) circumscription, section Sylvaticae would comprise eight species distributed through Eurasia and North Africa, namely: C. sylvatica subsp. sylvatica (Europe to Iran and NW Africa) and subsp. paui (Sennens) A. Bolòs & O. Bolòs (W Mediterranean), Carex algeriensis Nelmes (Algérie), Carex arnelli Christ. (Russia, from Europe to Far East), Carex bostrychostigma Maxim. (E Asia), Carex hondoensis Ohwi (Japan), Carex hypenaura V.I. Krecz. (Transcaucasus), Carex latifrons V.I. Krecz. (Anatolia to W Caucasus) and Carex strigosæ Huds. (Europe to N Iran). Subsequently, C. latifrons and C. algeriensis were included within C. sylvatica (= C. sylvatica subsp. latifrons (V.I. Krecz.) Ō. Nilsson and C. sylvatica subsp. paui (Sennens) A. Bolòs & O. Bolòs, respectively; see Jiménez-Mejías and Luceño, 2011).

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Finally, Carex cretica Gradst. & J. Kern, an endemic to Crete, was recently added to the section (Escudero and Luceño, 2009).

Taxonomy of Cyperaceae in South Africa has remained an active field of research during the last years, with several new species and even a genus very recently described (i.e. Muasya et al., 2011, 2012a, b), although most of them belong to tribe Cyperaeae, which is more diversified than Cariceae in this region, and includes some species-rich genera like Cyperus L. and Ficinia Schrad. In a recent field campaign in the Drakensberg mountains (November 2011), we found a population of Carex subgenus Carex whose morphology was apparently similar to that displayed by some members of Carex sect. Spirostachyaeeae (especially the Tropical African Carex fischeri Schkuhr, Carex clavata Thunb., Carex ecklonii Nees and Carex burchelliana Boeckeler; Escudero and Luceño, 2009). However, a closer examination revealed the absence of red crystalline bodies in the utricile epidermis and antligule, and a smooth utricile beak, indicating a closer relationship to section Sylvaticae than to Spirostachyaeeae. The presence of section Sylvaticae in South Africa was previously noted by Gehrke (2011) based on a specimen collected in KwaZulu-Natal in 1987 by C. Reid (n. 1370; PRE 762278) (‘Estcourt district’, Ntabamhlope [uThukela district]) and identified as C. sylvatica. Another specimen collected in the same place in 1944 (‘Weenen district’, Ntabamhlope [uThukela district]) by J.P.H. Acocks (n. 10784; PRE 109769; available at JSTOR) was also identified as C. sylvatica by C. Archer. An additional population from Eastern Cape (Hogsback) has been reported by C. Archer (pers. comm.). This species has been considered as probably introduced in South Africa (SANBI, 2012; C. Archer, pers. obs.).

The aim of this work is to perform a detailed morphological and molecular study of these taxonomically problematic individuals to evaluate their taxonomic placement and relationships within Carex.

2. Material and methods

2.1. Morphological study

In our morphological study, we considered 24 quantitative and 23 qualitative characters, based on the diagnostic characters for the taxonomy of section Sylvaticae (Egorova, 1999; Luceño, 2008) and related sections (Ceratocystis, Luceño and Jiménez-Mejías, 2008; Rhynchochystis, Luceño, 2008; Spirostachyaeeae, Escudero and Luceño, 2011). Eleven specimens from the same population (Rainbow Gorge, Cathedral Peak area, KwaZulu-Natal, South Africa; see 3.3) as well as two previously collected specimens from an additional, nearby population (uThukela district; Acocks n. 10784, PRE 109769; available at JSTOR) for both localities were identified and considered for the morphological study. Measurements were made as in our previous taxonomic studies of Carex (i.e. Escudero and Luceño, 2011; Martin-Bravo et al., 2012).

2.2. Molecular study

We tested the phylogenetic position of the problematic South African individuals by including: 1) two samples of the South African problematic population from Cathedral Peak area, 2) two species representing sect. Sylvaticae: C. sylvatica (two samples each in the nrDNA and the cpDNA phylogeny) and C. cretica (two samples in the nrDNA phylogeny and one sample in the cpDNA phylogeny) and 3) sequences from two samples representing two species for each of the related sections (Waterway and Starr, 2007): Carex flava L. and Carex viridula Michx. (sect. Ceratocystis), Carex distans L. and Carex punctata Gaudin (sect. Spirostachyaeeae), and Carex pendula Huds. and Carex bequaertii De Wild. (sect. Rhynchochystis). Carex rostata Stokes (sect. Vescariaeeae) and Carex melanostachya M. Bieber (sect. Tumidaeeae) were included as the outgroup for the cpDNA analyses and Carex michauxiana Boeckeler (sect. Rostraleeae) and Carex foliculata L. (sect. Rostraleeae) for the nrDNA analyses. We sequenced and analysed one cpDNA (5′trnK intron) and two nrDNA (ITS and ETS) regions, which have been successfully used in molecular systematic studies of the Cariceae (i.e. Escudero and Luceño, 2009; Waterway and Starr, 2007; Jiménez-Mejías et al., 2012). All sequences were downloaded from GenBank except for those from the problematic population as well as four 5′trnK intron (C. sylvatica, C. pendula, C. bequaertii) and one ETS sequences (C. bequaertii) which were PCR amplified and sequenced for this study (see Appendix A). Procedures for DNA extraction, amplification and sequencing followed those in Escudero and Luceño (2009) for ITS and 5′trnK intron, and in Waterway and Starr (2007) for ETS. We performed Maximum Parsimony and Bayesian Inference phylogenetic analyses as outlined in Martin-Bravo et al. (2007) and Escudero et al. (2008), respectively, for the ITS, ETS and 5′trnK datasets individually. Topologies retrieved from the ITS and ETS matrices were congruent (results not shown) and both matrices were therefore combined and analysed. The simplest models of nucleotide evolution that best fit the data for each studied DNA region were HKY for 5′trnK intron, GTR + G for ETS and ITS-1, HKY + G for ITS2, and K80 for 5.8S region. Informative indels were coded as a fifth binary character state and analysed with the F81 model of sequence evolution as specified in MrBayes manual (Ronquist and Huelsenbeck, 2003). We also obtained an additional measurement of statistical branch support by performing a Maximum Parsimony fast bootstrap analysis with 1000 replicates as implemented in PAUP (Swofford, 2002).

3. Results and discussion

3.1. Morphological study

The plants from the two KwaZulu-Natal problematic populations (uThukela district; Cathedral Peak area and Ntabamhlope) were compared against the species of sect. Sylvaticae (Egorova, 1999; Luceño, 2008). Several morphological features did not match the morphology of any known species of section Sylvaticae (Table 1). In particular, these plants frequently display androgynoeandrous upper spikes (a previously unknown feature in the section), dense female spikes, sometimes ramified at the base, and hyaline female glumes, which readily allow their distinction from other species in the section.

3.2. Molecular study

The majority rule consensus trees obtained from the Bayesian Inference (Fig. 1) yielded more resolved, but congruent topologies, with respect to the strict consensus trees retrieved from the Maximum Parsimony analyses (not shown). The sectional phylogenetic relationships depicted by the cpDNA (5′trnK intron) and nrDNA (ITS-ETS) sequences analysed were significantly different (Fig. 1A, B). Thus, sections Sylvaticae and Spirostachyaeeae appear as sister groups in the cpDNA analyses (0.97 PP, 57% BS; Fig. 1A), whereas Sylvaticae is supported as sister to Rhynchochystis in the nrDNA tree (1.0 PP, 89% BS; Fig. 1B). Therefore, nuclear-plastid sequences were not combined but analysed separately. Both the plastid and nuclear analyses support the inclusion of the studied population from Cathedral Peak area within sect. Sylvaticae, represented by two samples of C. sylvatica and one sample of C. cretica in the cpDNA tree (Fig. 1A) and by two samples each of C. sylvatica and C. cretica in the nrDNA tree (Fig. 1B). Interestingly, the South African population appears as sister to the European–Northern African C. sylvatica–C. cretica clade in the nrDNA tree (1.0 PP, 98% BS; Fig. 1B), while unresolved in the plastid tree (Fig. 1A). The lower resolution and support for the monophyly of sect. Sylviaeae in the cpDNA tree (0.91 PP, 56% BS) than in the nrDNA tree (1.0 PP, 98% BS) are due to the lower number of informative characters in the plastid than in the nuclear matrix (12 vs. 95, excluding the outgroup and the coded indels). Overall, these molecular results support the morphological findings and suggest the
taxonomic identity of the studied South African populations and their sectional circumscription within sect. *Sylvaticae*.

### 3.3. Description of new species

Although apparently close to *C. sylvatica*, *C. rainbowii* is not morphologically more similar to *C. sylvatica* than to other species from sect. *Sylvaticae* (Table 1). In addition, phylogenetic trees (Fig. 1) do not clearly indicate that *C. rainbowii* is closer to *C. sylvatica* than to the other sampled *Sylvaticae* species (in fact, in the nrDNA tree *C. sylvatica* is closer to *C. cretica* – although with low support – than to *C. rainbowii*; Fig. 1B). In our opinion, despite the small amount of studied material, the congruent and distinct set of clear-cut morphological characters (Table 1), together with the evidence from the molecular phylogenetic analyses (Fig. 1), warrants formal taxonomic recognition at the species level, which leads us to propose the following new species.

#### 3.3.1. C. rainbowii Luceño, Jim. Mejías, M. Escudero & Martín-Bravo, sp. nov. (Fig. 2)

Similar to *C. sylvatica*, from which it differs mainly by its frequently androgynecandrous upper spike, the dense female spikes and the hyaline female glumes.

*Type*: South Africa. KwaZulu-Natal Province, uThukela district (2829): Cathedral Peak area, Rainbow Gorge, shady understory of a montane forest dominated by *Blechnum giganteum* (Nees) Schltdl. The habitat in the paratype area appears to be similar, with plants found in damp and shady places in a forest. c. 1500–1700 m.

#### 3.3.2. Ecology

In the holotype population, plants were growing in the shady understory of a montane forest dominated by *Podocarpus latifolia* (Thunb.) Mirb. and *Carissa bispinosa* Desf. Other accompanying observed species were *Celtis africana* Burm. f., *Carex spicato-paniculata* C. B. Clarke, *Schoenoxylothium lehmannii* (Nees) Steud., *Dietes iridioides* (L.) Kattl and *Blechnum giganteum* Schltdl. The habitat in the paratype population appears to be similar, with plants found in damp and shady places in a forest. c. 1500–1700 m.

#### 3.3.2.1. Distribution (Fig. 3)

South Africa, KwaZulu-Natal (NAT; Brummit, 2001), known only from two collecting sites in the Drakensberg Mountains (uThukela district): Cathedral Peak area and Ntabamhlope.

#### 3.3.2.2. Conservation

Because the species is currently known from only two collecting sites in South Africa, we hereby summarize the available information for assessing the conservation status of *C. rainbowii*, based on IUCN Red List categories, criteria, and guidelines (*IUCN, 2001, 2011*). The known collecting sites would correspond to only two subpopulations (*IUCN, 2011*) in an extremely reduced area of occupancy (AOO) of 8 km² (grid size 4 km²). We could not precisely estimate the number of locations (1–2) due to no information available about threats affecting subpopulations (*IUCN, 2001*). However, this geographic range and number of locations (maximum two) would classify the species as “Endangered” under criteria B2, if appropriate knowledge of subcriteria b and c were available. Unfortunately, no information is available for these subcriteria, which refer to a verifiable continuing decline (subcriteria b) or extreme fluctuation (subcriterion c) in any of the extent of occurrence, AOO, area, extent, and/or quality of the habitat; number of locations; and number of mature individuals (*IUCN, 2001, 2011*). Alternatively, it could be classified as “Vulnerable” under...
criterion D2, because the AOO and the number of locations are smaller than 20 km² and fewer than five, respectively. However, this criterion requires that a plausible future threat for the species survival is identified. However, because the species can be locally frequent (ca. 100 plants seen in the holotype population, Martín-Bravo and Luceño, pers.obs.; “frequent” and “locally fairly common” in the paratype population, Acocks and Reid, in sched., respectively), and at least the Rainbow gorge population is situated on protected land (Ukhahlamba Drakensberg park), no clear future threat is currently apparent as required by criterion D2. To sum up, the necessary information for a complete conservation assessment was insufficient to meet some of the criteria and subcriteria required by the IUCN to qualify the species under the different threatened categories (Critically Endangered, Endangered, Vulnerable). Therefore, the species should be classified as “Data Deficient” at the present time, although it is likely that it would deserve protection under the IUCN guidelines if more information was obtained. An additional population of this species could be present in the Eastern Cape province (Hogsback; C. Archer, pers. comm.), which would considerably increase the extent of occurrence (EOO) of this species [IUCN, 2001, 2011].

3.3.2.2. Biogeographic observations. The fact that the paratype population (Ntabamhlope) of *C. rainbowii* had been previously misidentified as *C. sylvatica* (Gehrke, 2011), indicates that *C. sylvatica* should be.
excluded from the checklist of alien flora of South Africa (SANBI, 2012). The presence of this new species in South Africa implies a hitherto unknown disjunction with respect to the mainly Eurasian-North African distribution of the remaining species of section Sylvaticae. Interestingly, this biogeographic pattern has also been found in several of the related Carex sections. In section Spirostachyae, ancestral range reconstruction analyses and estimations of diversification times indicate that long-distance dispersal events from Eurasia may have been the origin of the two South African endemic species (C. ecklonii and C. burchelliana; Escudero et al., 2009; reviewed in Martín-Bravo and Escudero, 2012). For the origin of the C. aethiopica–C. clavata complex, both long-distance dispersal from Eurasia and ecological vicariance with its East Tropical African congeners have been proposed (Gehrke and Linder, 2009; Escudero et al., 2009). In section Ceratocystis, the Drakensberg endemic Carex monotropa Nees is likely the result of a North to South Hemisphere migration (Jiménez-Mejías et al., 2012). Finally, section

Fig. 2. Analytical drawing of the holotype material of Carex rainbowii. A. plant; B. ligule; C. androgynecandrous spike; D. female spike; E. male glume; F. female glume; G. utricle; H. achene (South Africa, KwaZulu-Natal, uThukela district, Cathedral Peak area, Rainbow Gorge, S. Martín-Bravo 1205MB11 & M. Luceño, UPOS). — Drawing by Rodrigo Tavera.
Rhynchocystis also shows a similar pattern of disjunction, with its species displaying a Euro-North African-Western Asian (C. pendula, Carex microcarpa) or Sub-Saharan (Carex moosi, C. bequaertii, C. penduliformis) range. Comparative biogeographic studies of these groups are needed to uncover if these shared patterns are reflecting common evolutionary histories.

3.3.2.3. Additional specimens studied (paratypes). South Africa: KwaZulu-Natal province, ‘Weenen district’ [uThukela district] (2929): Ntabamhlope, near police post, damp place in forest, 5500′, 19 Nov 1944, J.P.H. Acocks 10784 (PRE, photo!); Idem, ‘Estcourt district’ [uThukela district] (2929): near Ntabamhlope, c. 2 km on road to Kamberg from White Mountain Resort, small forest patch, along stream bed in forest, 30 Jan 1987, C. Reid 1370 (PRE, photo!; GENT, J, NH, iso.).

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C. bequaertii (KC122381*, KC122389*),  M. Escudero & Martín-Bravo (KC122380*, KC122388*), Gaudin (DQ384182, AY757659),

References

Egorova, T.V., 1999. The sedges (Carex L.) of Russia and adjacent states (within the limits of the former USSR). St. Petersburg State Chemical-Pharmaceutical Academy, St. Petersburg (Missouri Botanical Garden Press, St. Louis, MO).