



Affinities in C₃ *Cyperus* lineages (Cyperaceae) revealed using molecular phylogenetic data and carbon isotope analysis

ISABEL LARRIDON^{1*}, MARC REYNDERS¹, WIM HUYGH¹, KENNETH BAUTERS¹, KOBEKE VAN DE PUTTE², A. MUTHAMA MUASYA³, PASCAL BOECKX⁴, DAVID A. SIMPSON⁵, ALEXANDER VRIJDAGHS⁶ and PAUL GOETGHEBEUR¹

¹Ghent University, Department of Biology, Research Group Spermatophytes, K. L. Ledeganckstraat 35, 9000 Gent, Belgium

²Ghent University, Department of Biology, Research Group Mycology, K. L. Ledeganckstraat 35, 9000 Gent, Belgium

³University of Cape Town, Botany Department, Rondebosch 7700, South Africa

⁴Ghent University, Faculty of Bioscience Engineering, Laboratory of Applied Physical Chemistry – ISOFYS, Coupure 653, 9000 Gent, Belgium

⁵Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, UK

⁶K.U.Leuven, Institute of Botany and Microbiology, Laboratory of Plant Systematics, Kasteelpark Arenberg 31, 3001 Leuven, Belgium

Received 22 November 2010; revised 9 February 2011; accepted for publication 18 May 2011

Maximum likelihood and Bayesian analyses of nrDNA (ETS1f) and plastid DNA (*rpl32-trnL*, *trnH-psbA*) sequence data are presented for ‘C₃ *Cyperus*’ (Cyperaceae). The term ‘C₃ *Cyperus*’ indicates all species of *Cyperus s.l.* that use C₃ photosynthesis linked with eucyberoid vegetative anatomy. Sampling comprises 77 specimens of 61 different taxa, representing nearly all previously recognized subdivisions of C₃ *Cyperus* and the segregate genera *Courtoisina*, *Kyllingiella* and *Oxycaryum*. According to our results, the *Cyperus* clade is divided in six well-supported clades. The first of these clades (clade 1) forms three subclades largely corresponding to *Cyperus* sections *Haspani*, *Incurvi* and *Diffusi*. Clade 2 comprises the entirely New World *C.* section *Luzuloidei sensu* Denton (1978). Clade 3 is a highly diverse clade including two subclades: clade 3a, *C.* sections *Pseudanosporum* and *Anosporum* plus the segregate genera *Courtoisina* and *Oxycaryum*; and clade 3b, *C.* section *Fusci*. Clade 4 corresponds to *C.* section *Alternifolii* and clade 5 to *C.* section *Leucocephali* plus the segregate genus *Kyllingiella*. The sixth clade is a well-supported monophyletic clade encompassing all C₄ *Cyperus s.l.* species (‘C₄ *Cyperus*’). This study establishes a phylogenetic framework for future studies. © 2011 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2011, 167, 19–46.

ADDITIONAL KEYWORDS: Bayesian inference – Cyperaceae – Cyperoideae – molecular phylogeny – photosynthetic pathway – systematics.

INTRODUCTION

Cyperaceae have an almost cosmopolitan distribution (Govaerts *et al.*, 2007). Sedges did not only evolve a large diversity of genera and species in the tropics and subtropics, but they are also often dominantly

present in the vegetation of temperate and arctic regions (*Carex* L., c. 1800 spp.; Govaerts *et al.*, 2011). Because of their ecological significance in wetlands and the important role of some Cyperaceae in the food chain of dry grasslands, knowledge of the biodiversity and evolution of this plant family is very valuable. Cyperaceae are grass-like plants, often with complex compound inflorescences, in which many adaptations

*Corresponding author. E-mail: isabel.larridon@ugent.be

such as reductions and contractions have occurred, complicating evolutionary reconstruction and classification. This has not only led to uncertain interpretations of the inflorescence and flowers (homology problems, e.g. Bruhl, 1991; Muasya *et al.*, 2009b; Vrijdaghs *et al.*, 2009, 2010), but also to conflicting classification systems (e.g. Clarke, 1908; Kükenthal, 1935–1936; Kern, 1974; Haines & Lye, 1983). Prior to the era of molecular phylogenetics, classifications based on cladistic analyses of matrices of morphological and anatomical character states were presented (Goetghebeur, 1986; Bruhl, 1995). Since then, molecular phylogenetic research has resulted in new insights into Cyperaceae. Molecular phylogenetic studies at family level have been largely based on plastid DNA: sequence data for *rbcL* (e.g. Muasya *et al.*, 1998; Simpson *et al.*, 2007) or *rbcL* and *trnL-F* (the *trnL* intron and the *trnL-trnF* intergenic spacer) (Muasya, Simpson & Chase, 2002; Muasya *et al.*, 2009a). The latest molecular phylogenetic studies of Cyperaceae (Simpson *et al.*, 2003, 2007; Muasya *et al.*, 2009a) recognized only two subfamilies, Cyperoideae and Mapanioideae, which are easily distinguished by the structure of their reproductive units.

Tribe Cypereae Nees (Cyperoideae) is defined as including all taxa sharing the *Cyperus*-type embryo and the similar *Ficinia*-type embryo (Van der Veken, 1965; Goetghebeur, 1998; Muasya *et al.*, 2009a, b). Based on molecular data (Muasya, Simpson & Chase, 2002; Muasya *et al.*, 2009a), two clades are recognized in Cypereae. The *Ficinia* Schrad. clade is predominantly characterized by spikelets with spirally arranged glumes and includes *Scirpoides* Ség., *Dracoscirpoides* Muasya (Muasya *et al.*, 2009a, 2011), *Hellmuthia* Steud., *Isolepis* R.Br. and *Ficinia* (Muasya & de Lange, 2010). The *Cyperus* L. clade usually has spikelets with distichously arranged glumes.

The generic limits in the *Cyperus* clade (*c.* 950 spp.) are notoriously controversial (Muasya *et al.*, 2009b). When using the classification of Goetghebeur (1998), the *Cyperus* clade comprises a paraphyletic *Cyperus* s.s. as the core genus, in which 13 segregate genera are nested. These segregate genera (*Alinula* J.Raynal, *Androtrichum* (Brongn.) Brongn., *Ascolepis* Nees ex Steud., *Courtoisina* Soják, *Kyllinga* Rottb., *Kyllingiella* R.W.Haines & Lye, *Lipocarpa* R.Br., *Oxycaryum* Nees, *Pycrus* P.Beauv., *Queenslandiella* Domin, *Remirea* Aubl., *Sphaerocyperus* Lye and *Volkiella* Merxm. & Czech) were created because their species diverged significantly from typical *Cyperus* with respect to vegetative, floral and anatomical characters. Each of these segregates is circumscribed by a combination of morphological characters, including inflorescence and spikelet morphology, unit of

dispersal, nutlet orientation and photosynthetic pathway (Bruhl & Wilson, 2007; Muasya *et al.*, 2009b; Vrijdaghs *et al.*, 2011).

The presence of Kranz anatomy, linked with C₄ photosynthesis, is an important character in classifying taxa within the *Cyperus* clade. This character has been used in the classification of *Cyperus* since Rikli (1895), long before the discovery of the C₄ photosynthetic pathway. As already indicated by Raynal (1973) and Goetghebeur (1989), later authors such as Soros & Bruhl (2000), Muasya *et al.* (2002), Bruhl & Wilson (2007) and Besnard *et al.* (2009) confirmed that the C₄ photosynthetic pathway arose only once in Cypereae, although it arose at least four separate times in Cyperaceae. In the *Cyperus* clade, C₃ photosynthesis is characterized by the presence of the eucyperoid anatomy type (plesiomorphic), whereas C₄ photosynthesis is linked with the chlorocyperoid anatomy type. Bruhl, Stone & Hattersley (1987) and Bruhl & Perry (1995) clarified the chlorocyperoid anatomy. According to Besnard *et al.* (2009), the first appearance of C₄ photosynthesis in Cyperaceae probably occurred between 19.6 ± 4.9 and 10.1 ± 3.6 Ma in *Bulbostylis* DC., with the other C₄ appearances occurring during the last 12 Ma, making C₄ sedges generally younger than C₄ grasses (Christin *et al.*, 2008a, b). Although sedges generally occupy wetter habitats than grasses and commonly occur in wetlands and marshes, many C₄ and a considerable number of C₃ sedges occupy seasonally dry habitats. The C₄ pathway, which raises the water-use efficiency compared with the C₃ photosynthesis type (Sage, 2004), might have contributed to the colonization of drier habitats (Besnard *et al.*, 2009). However, water limitation might not have been the most important factor for the success of C₄ sedges. See Pyankov *et al.* (2010) for a discussion of C₄ photosynthesis in European representatives of Cyperaceae and other families. Li, Wedin & Tieszen (1999) and Stock, Chuba & Verboom (2004) argued that fire resistance, optimized nitrogen uptake and resistance to chemical stress (salt and heavy metals) and higher levels of irradiance were probably more important. Figure 1 represents some of the morphological and habitat diversity of C₃ *Cyperus* lineages.

As discussed in more detail in Larridon *et al.* (2011), *Cyperus* is most commonly divided into two units, determined by the character states of an anatomical and of an inflorescence character set. As mentioned above, the vegetative anatomy in *Cyperus* is either eucyperoid or chlorocyperoid. Based on this character, Rikli (1895) divided *Cyperus* into two separate genera: *Eucyperus* and *Chlorocyperus*. The prefix *Eu-*, which suggests the inclusion of the type species of *Cyperus* in this group, should have led Britton (1907) to use one of Linnaeus' (1753) original C₃ *Cyperus* spp. as the type of *Cyperus*. However, he

chose *Cyperus esculentus* L. instead (Huygh *et al.*, 2010), a species with chlorocyperoid anatomy. The inflorescence in *Cyperus* is either composed of digitately clustered spikelets or is an anthela composed of spikes of spikelets. Clarke (1893) divided *Cyperus* into two subgenera based on these two inflorescence types: *Pycnostachys* and *Choristachys*. As demonstrated by Goetghebeur (1989), the two systems (based on anatomy type or inflorescence type) are not completely congruent.

In the present study, molecular phylogenetic data and results of carbon isotope analysis of the *Cyperus* clade were analysed with the specific objectives of: (1) elucidating phylogenetic relationships focusing on the C₃ *Cyperus* lineages in order to establish a phylogenetic framework for future studies of the *Cyperus* clade; (2) testing whether infrageneric taxa in *Cyperus* (e.g. Kükenthal, 1935–1936; Denton, 1978) are monophyletic; and (3) characterizing which photosynthetic system is used in previously unstudied taxa. For the accepted names of the taxa treated in this paper, we refer to the *World Checklist of Cyperaceae* (Govaerts *et al.*, 2007, 2011). The classification used is that of Goetghebeur (1998). A paper documenting the necessary nomenclatural/taxonomical changes based on the results presented in this paper and further supported by morphological, embryological, ontogenetic and anatomical data will be published elsewhere (Larridon *et al.*, in press). The final objective of the research on *Cyperus* carried out by the authors is to recircumscribe the genus so that it is monophyletic and to create a new infrageneric classification of the genus supported by both molecular and morphological data.

MATERIAL AND METHODS

SAMPLING

Seventy-seven samples of 60 different taxa were used for this study. The samples with species names, voucher information, origin and GenBank accession numbers for the sequences are given in Table 1. Three sequences were used from a previous unpublished study (GenBank accession numbers GU135417, GU135444, GU135397; J. R. Abbott, K. M. Neubig, W. M. Whitten & N. H. Williams, unpubl. data). The other sequences were all newly generated for this study. Taxa within *Cyperus* were selected to represent a broad morphological and geographical range and to include a wide range of the traditionally recognized sections, subgenera and segregate genera (C₃ *Cyperus*: *Courtoisina*, *Kyllingiella* and *Oxycaryum*; C₄ *Cyperus*: *Alinula*, *Ascolepis*, *Lipocarpha*, *Kyllinga*, *Pycneus*, *Queenslandiella*, *Remirea* and *Sphaerocyperus*). As this study assesses relationships above the

rank of species, multiple species samples and infraspecific taxa were generally not used. The outgroup taxa were selected based on the family-wide analysis of plastid *rbcL* and *trnL-F* sequences by Muasya *et al.* (2009a).

Taxonomic details for most taxa mentioned (such as author, place and date of publication, synonyms, distribution) follow Govaerts *et al.* (2007, 2011). More detailed information on the nomenclature of generic and subdivisional names of the *Cyperus* clade is given in Huygh *et al.* (2010), Larridon *et al.* (2011) and Reynders *et al.* (2011).

DNA EXTRACTION

Samples were either of wild origin, mostly collected during recent field expeditions (silica-dried), or sampled from plants cultivated at the Ghent University Botanical Garden. Additional dried leaf samples were selected from herbarium specimens (GENT). Total DNA was extracted from 100 mg fresh or 20 mg dried material using the GenElute™ Plant Genomic DNA Miniprep Kit (Sigma-Aldrich, USA) or the DNeasy Plant Mini Kit (Qiagen, Germantown, USA) following the manufacturers' protocols. The material was first ground using a mortar and pestle with the addition of the extraction buffers and a knifepoint of sterilized sand. Additional DNA samples were provided by the DNA Bank at the Royal Botanic Gardens, Kew (UK).

MARKERS

Two non-coding plastid DNA markers were used in this study: the *rpl32-trnL* intergenic spacer of the small single-copy region of the plastid genome (Shaw *et al.*, 2007) and the *trnH-psbA* intergenic spacer (e.g. Kress *et al.*, 2005; Shaw *et al.*, 2005, 2007; Dragon & Barrington, 2009). To compare the information held in the plastid and nuclear genomes, sequences of a nuclear region were also produced. Also, molecular phylogenetic studies solely based on plastid markers (e.g. Muasya *et al.*, 2002, 2009a; Simpson *et al.*, 2007) give insufficient resolution at lower taxonomic levels, indicating that a marker with a more rapid rate of evolution was needed to resolve the relationships in the *Cyperus* clade. Although Álvarez & Wendel (2003) rightly indicated the challenges of using non-single-copy or low-copy nuclear markers, we chose to use a fragment of the external transcribed spacer 1 (ETS1f). We selected the ETS1f marker, not only because it displays a rapid rate of evolution compared with most plastid loci, but also because we found it can be readily amplified and sequenced even from poorly preserved plant material. The relative poor quality of DNA extracted from herbarium specimens

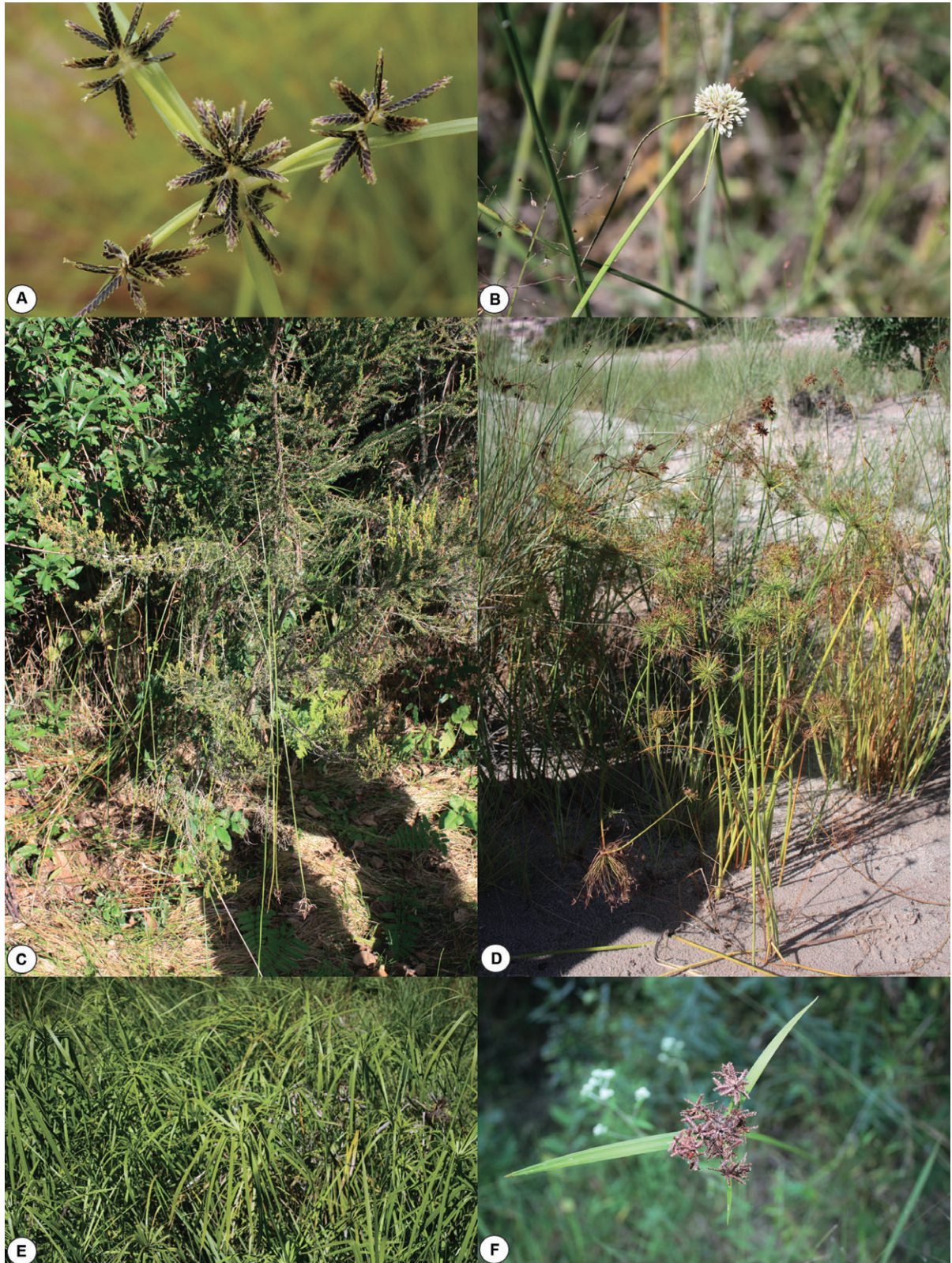


Figure 1. Diversity of *C₃* *Cyperus* morphologies and habitats. A. *Cyperus fuscus* with anthelate inflorescence with clearly distichously glume arrangement growing in the Ghent University Botanical Garden. B. *Cyperus pulchellus* with whitish capitate inflorescence growing seasonally wet area near Mahajanga, Madagascar. C. *Cyperus debilissimus* with extremely elongated culms growing up and hanging down from the vegetation at edge of the forest in Andringitra National Park, Madagascar. D. *Cyperus prolifer* with rays arranged so inflorescence is spherical growing with a creeping rhizome in wet sand at Cirque Rouge near Mahajanga, Madagascar. E. *Cyperus alternifolius* with many, almost equally sized involucre bracts growing on a shaded riverbank near Mahajanga, Madagascar. F. *Cyperus betafensis* with anthelate inflorescence and well-developed involucre bracts growing at the edge of the forest in Andringitra National Park, Madagascar. Photographs (A) by M. Reynders and (B-F) by W. Huygh.

prevents effective use of single-copy nuclear genes. ETS1f, like the internal transcribed spacer (ITS) part of the nuclear ribosomal DNA (nrDNA), has already proved useful in resolving phylogenetic relationships in Cyperaceae, e.g. *Uncinia* Pers. (Starr, Harris & Simpson, 2003), *Schoenoplectus* (Rchb.) Palla (Yano & Hoshino, 2005), *Fimbristylis* Vahl (Yano & Hoshino, 2007) and *Carex* (Dragon & Barrington, 2009).

PCR AMPLIFICATION, SEQUENCING AND ALIGNMENTS

PCR amplification was performed using buffer solutions and Taq polymerase from Qiagen. Reactions were carried out using a Gradient Mastercycler (Eppendorf Inc., Hamburg, Germany). Amplification of *rpl32-trnL* was performed following the protocol and using the primers of Shaw *et al.* (2007) for *rpl32-F*: (5'-CAGTTCCAAAAACGTACTTC-3'); and *trnL*^(UAG): (5'-CTGCTTCCTAAGAGCAGCGT-3'). Amplification of *trnH-psbA* was performed following the protocol of Shaw *et al.* (2005) and using adapted primers of Wanke (S. Wanke, unpubl. data) *Pe-trnH*: (5'-ATTCACAATCCACTGCCTTGAT-3'); and *Pe-psbA*: (5'-AATGCACACAACCTCCCTCTA-3'). Amplification of ETS1f was performed following the protocol and using the primers of Starr *et al.* (2003) for ETS1f: ETS-F (5'-CTGTGGCGTCGCATGAGTTG-3') and 18S-R (5'-AGACAAGCATATGACTACTGGCAGG-3'). The PCR products were electrophoresed on 1% agarose gels in 1 × Tris-acetate-EDTA (TAE) buffer (pH 8.0) and stained with ethidium bromide to confirm a single product.

Sequencing was performed using the same primers used in the PCR reactions. Sequencing was run on an Applied Biosystems ABI 3130XL Genetic Analyser (Life Technologies, CA, USA). The software Sequencher™ v4.8 (GeneCodes Corporation, Ann Arbor, MI, USA) was used to assemble forward and reverse sequences into contigs, inspect ABI chromatograms and edit nucleotides where needed. The sequences were aligned manually in PhyDE 0.995 (Müller, Müller & Quandt, 2008). To eliminate ambiguously aligned positions in the alignment as objectively as possible, the online program Gblocks v0.91b (Castresana, 2000) was used. The program was run with

settings allowing for smaller blocks, gaps within these blocks and less strict flanking positions. Alignments are available from the first author by request.

DATA ANALYSIS

Phylogenetic hypotheses were produced using maximum likelihood (ML) and Bayesian inferences (BI). All analyses were first performed on the single marker data sets (*ETS1f*, *rpl32-trnL*, *trnH-psbA*). As no conflicting clades with a significant confidence value (i.e. with bootstrap / PP support) were revealed, a combined data set was constructed and analysed. The latter was subdivided into three partitions, corresponding to the single markers. The program RAxML v7.0.3 (Stamatakis, 2006) was used to execute the Rapid Bootstrapping algorithm for 500 replicates, combined with an ML search, using the GTRCAT model (Stamatakis, Hoover & Rougemont, 2008). Model parameters were optimized for each partition when analysing the combined data set.

Following Van de Putte *et al.* (2010), two Bayesian phylogenetic (BI) analyses were carried out in MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003). For the first analysis, MrModeltest v2.3 (Nylander, 2004) was used to determine the model that best fits the data, applying Akaike's information criterion. For the combined data set, a model was determined for each partition. This method is referred to as the BI-MrModeltest method. For the second analysis, a single general time-reversible model with rate variation across sites and a proportion of invariable sites was used. Rates and all model parameters were unlinked between all partitions of the combined data set. This method is referred to as the BI-GTR+I+ Γ method.

Two independent, parallel runs of one cold and three heated chains were run for 10 million generations each. Trees and parameter estimates were saved every 1000th generation. Convergence, associated likelihood values, effective sample size values and burn-in values of the different runs were verified with Tracer v1.4.1 (Rambaut & Drummond, 2007). Calculation of the consensus tree and the posterior

Table 1. List of the samples used in the molecular study with species names, voucher information (* leaf sample courtesy of the collector/A. M. Muasya), origin and GenBank accession numbers for the sequences

Taxon	Voucher	Origin	ETS1f	<i>trnH-psbA</i>	<i>rpl32-trnL</i>
<i>Alinula paradoxa</i> (Cherm.) Goetgh. & Vorster	Reid 1027 (GENT)	South Africa	HQ705964	–	HQ705894
<i>Ascolepis capensis</i> (Kunth) Ridl.	Hess 52/1760 (GENT)	Angola	HQ705957	–	HQ705887
<i>Courtoisina assimilis</i> (Steud.) Maquet	Faden et al. 96/119 (K*; Muasya et al., 2002)	Tanzania	HQ705939	HQ705812	HQ705872
<i>Courtoisina cyperoides</i> (Roxb.) Soják	Faden et al. 96/456 (K*)	Tanzania	HQ705940	HQ705813	–
<i>Courtoisina cyperoides</i> (Roxb.) Soják	Larridon et al. 2010-0261 (GENT)	Madagascar	HQ705941	HQ705814	HQ705873
<i>Cyperus ajax</i> C.B. Clarke	Malaisse & Goetghebeur 130 (GENT)	DR Congo	HQ705916	HQ705794	HQ705852
<i>Cyperus albostrigatus</i> Schrad.	Reid 726 (GENT)	South Africa	HQ705915	HQ705793	HQ705851
<i>Cyperus alternifolius</i> L.	Goetghebeur 11516 (GENT)	BG Ghent	HQ705948	HQ705818	HQ705878
<i>Cyperus balfourii</i> C.B. Clarke	Dorr 2744 (GENT)	Madagascar	HQ705917	HQ705795	HQ705853
<i>Cyperus betafensis</i> Cherm.	Larridon et al. 2010-0326 (GENT)	Madagascar	HQ705918	–	–
<i>Cyperus betafensis</i> Cherm.	Larridon et al. 2010-0200 (GENT)	Madagascar	HQ705919	HQ705796	HQ705854
<i>Cyperus buchholzii</i> Boeck.	Viane 1327 (GENT)	Ivory Coast	HQ705921	HQ705798	–
<i>Cyperus buchholzii</i> Boeck.	Reynders et al. 090307/03 (GENT)	Cameroon, BG Ghent	HQ705922	HQ705799	HQ705856
<i>Cyperus chamaecephalus</i> Cherm.	Beentje 4774 (K – DNA Bank 29378)	Madagascar	HQ705930	–	–
<i>Cyperus cuspidatus</i> Kunth	Jongkind & Nieuwhuis 2847 (GENT)	Ghana	HQ705954	HQ705823	HQ705884
<i>Cyperus debilissimus</i> Baker	Larridon et al. 2010-0103 (GENT)	Madagascar	HQ705933	HQ705808	HQ705866
<i>Cyperus debilissimus</i> Baker	Larridon et al. 2010-0154 (GENT)	Madagascar	HQ705932	HQ705807	HQ705865
<i>Cyperus debilissimus</i> Baker	Larridon et al. 2010-0282 (GENT)	Madagascar	–	HQ705806	HQ705864
<i>Cyperus denudatus</i> L.f.	Muasya & Knox 1021 (EA)	Kenya	HQ705926	HQ705802	HQ705859
<i>Cyperus dichrostachyus</i> Hochst. ex A.Rich.	Muasya & Knox 976 (EA; Muasya et al., 2002)	Kenya	HQ705944	–	HQ705877
<i>Cyperus difformis</i> L.	Reynders & Sabulao 66 (GENT)	Philippines	HQ705945	HQ705817	–
<i>Cyperus distinctus</i> Steud.	Carter 9237 (GENT)	USA	HQ705907	HQ705788	HQ705844
<i>Cyperus elegans</i> L.	Goetghebeur 5601 (GENT)	Cuba	HQ705959	HQ705827	HQ705889
<i>Cyperus entrerianus</i> Boeck.	Zardini 29789 (GENT)	Paraguay	HQ705908	HQ705789	HQ705845
<i>Cyperus eragrostis</i> Lam.	Bryson 16965 (GENT)	USA	HQ705903	–	HQ705840
<i>Cyperus eragrostis</i> Lam.	Van der Veken 12823 (GENT)	France	HQ705904	HQ705785	HQ705841

Table 1. Continued

Taxon	Voucher	Origin	ETS1f	<i>trnH-psbA</i>	<i>rpl32-trnL</i>
<i>Cyperus eragrostis</i> Lam.	Goetghebeur 11494 (GENT)	BG Basel, BG Ghent	HQ705905	HQ705786	HQ705842
<i>Cyperus esculentus</i> L.	Goetghebeur 11303 (GENT)	BG Nantes, BG Ghent	HQ705960	HQ705828	HQ705890
<i>Cyperus foliaceus</i> C.B. Clarke	Larridon et al. 2010-0034 (GENT)	Madagascar	HQ705925	HQ705801	HQ705858
<i>Cyperus friburgensis</i> Boeck.	Montes 1799 (K – DNA Bank 29366)	Argentina	–	HQ705791	HQ705849
<i>Cyperus friburgensis</i> Boeck.	Goetghebeur 5869 (GENT)	BG Ghent	HQ705914	HQ705792	HQ705850
<i>Cyperus friburgensis</i> Boeck.	Øllgaard 74763 (GENT)	Ecuador	HQ705913	–	–
<i>Cyperus fuscus</i> L.	de Retz 67715 (GENT)	France	HQ705946	–	–
<i>Cyperus gardneri</i> Nees	Schessl 3316 (GENT)	Brazil	HQ705943	–	HQ705876
<i>Cyperus haspan</i> L.	Muasya & Muthama 1269 (EA)	Kenya	HQ705927	HQ705803	HQ705860
<i>Cyperus kerstenii</i> Boeck.	Muasya 984 (EA, K; Muasya et al., 2002)	Kenya	HQ705961	HQ705829	HQ705891
<i>Cyperus leptocladus</i> Kunth	Reid 902 (GENT)	South Africa	HQ705923	–	–
<i>Cyperus luzulae</i> (L.) Retz.	Goetghebeur 5868 (GENT)	BG Ghent	HQ705909	–	–
<i>Cyperus luzulae</i> (L.) Retz.	Van den Eynden 213 (GENT)	Ecuador	HQ705910	–	HQ705846
<i>Cyperus marginatus</i> Thunb.	Larridon et al. 2009-0076 (GENT)	Kenya	HQ705949	HQ705819	HQ705879
<i>Cyperus molliglumis</i> Cherm.	Larridon et al. 2010-0225 (GENT)	Madagascar	HQ705931	–	HQ705863
<i>Cyperus ochraceus</i> Vahl	Viane 681 (GENT)	Venezuela	HQ705911	–	HQ705847
<i>Cyperus papyrus</i> L.	Goetghebeur 5866 (GENT)	BG Ghent	HQ705962	HQ705830	HQ705892
<i>Cyperus pectinatus</i> Vahl	De Wolf 92-86 (GENT)	Senegal	HQ705935	–	HQ705868
<i>Cyperus pectinatus</i> Vahl	Larridon et al. 2010-0265 (GENT)	Madagascar	HQ705936	HQ705810	HQ705869
<i>Cyperus phaeolepis</i> Cherm.	Phillipson 1647 (GENT)	Madagascar	HQ705950	–	HQ705880
<i>Cyperus plantaginifolius</i> var. <i>minor</i> Cherm.	Larridon et al. 2010-0069 (GENT)	Madagascar	HQ705934	HQ705809	HQ705867
<i>Cyperus platystylis</i> R.Br.	Chantaranonthai et al. 814 (K – DNA Bank 29382)	Thailand	HQ705937	–	HQ705870
<i>Cyperus prolifer</i> Lam.	Larridon et al. 2010-0003 (GENT)	Madagascar	HQ705928	HQ705804	HQ705861
<i>Cyperus purpureoviridis</i> Lye	Muasya & Knox 964 (EA)	Tanzania	HQ705929	HQ705805	HQ705862
<i>Cyperus pseudovegetus</i> Steud.	Carter 6152 (GENT)	USA	HQ705912	HQ705790	HQ705848
<i>Cyperus reduncus</i> Hochst. ex Boeck.	Malaisse & Goetghebeur 1171 (GENT)	DR Congo	HQ705938	HQ705811	HQ705871
<i>Cyperus renschii</i> Boeck.	Mwachala et al. 446 (EA)	Kenya	HQ705924	HQ705800	HQ705857
<i>Cyperus rotundus</i> L.	Shaw 890 (K*)	Hong Kong (China)	HQ705963	HQ705831	HQ705893

Table 1. Continued

Taxon	Voucher	Origin	ETS1f	<i>trnH-psbA</i>	<i>rpl32-trnL</i>
<i>Cyperus schomburgkianus</i> Nees	<i>de Michel 2000</i> (GENT)	Bolivia	–	HQ705821	HQ705882
<i>Cyperus</i> sp. (<i>Diffusi</i>)	<i>Larridon et al. 2010-0215</i> (GENT)	Madagascar	HQ705920	HQ705797	HQ705855
<i>Cyperus submicrolepis</i> Kük.	<i>Laegaard 17222</i> (GENT)	Senegal	HQ705947	–	–
<i>Cyperus surinamensis</i> Rottb.	<i>Jansen-Jacobs 521</i> (GENT)	Guyana	HQ705906	HQ705787	HQ705843
<i>Cyperus textilis</i> Thunb.	<i>Goetghebeur 11517</i> (GENT)	BG Ghent	HQ705951	HQ705820	HQ705881
<i>Cyperus waterloti</i> Cherm.	<i>Larridon et al. 2010-0010</i> (GENT)	Madagascar	HQ705955	HQ705824	HQ705885
<i>Cyperus waterloti</i> Cherm.	<i>Larridon et al. 2010-0043</i> (GENT)	Madagascar	HQ705956	HQ705825	HQ705886
<i>Ficinia gracilis</i> Schrad.	<i>Muasya 2713</i> (BOL)	South Africa	HQ705902	HQ705784	HQ705839
<i>Isolepis fluitans</i> (L.) R.Br.	<i>Muasya & Knox 3195</i> (EA)	Kenya	HQ705901	HQ705783	HQ705838
<i>Kyllinga nemoralis</i> (J.R.Forst. & G.Forst.) Dandy ex Hutch. & Dalziel	<i>Goetghebeur 11518</i> (GENT)	Philippines, BG Ghent	HQ705965	HQ705832	HQ705895
<i>Kyllingiella microcephala</i> (Steud.) R.W.Haines & Lye	<i>Muasya & Muthama 1262</i> (EA)	Kenya	HQ705952	–	–
<i>Kyllingiella polyphylla</i> (A.Rich.) Lye	<i>Muasya & Muthama 1247</i> (EA)	Kenya	HQ705953	HQ705822	HQ705883
<i>Lipocarpa chinensis</i> (Osbeck) J.Kern	<i>Reynders & Sabulao 26</i> (GENT)	Philippines	HQ705958	HQ705826	HQ705888
<i>Oxycaryum cubense</i> (Poepp. & Kunth) Palla	<i>Mwachala 340</i> (EA)	Kenya	HQ705942	HQ705815	HQ705874
<i>Oxycaryum cubense</i> (Poepp. & Kunth) Palla	<i>Zardini 18398</i> (GENT)	Paraguay	–	HQ705816	HQ705875
<i>Pycnus polystachyos</i> (Rottb.) P.Beauv.	<i>Goetghebeur 11519</i> (GENT)	South Africa, BG Ghent	HQ705966	HQ705833	HQ705896
<i>Queenslandiella hyalina</i> (Vahl) Ballard	<i>Muasya 2490</i> (EA)	Kenya	HQ705967	HQ705834	HQ705897
<i>Remirea maritima</i> Aubl.	<i>Faden et al. 96/48</i> (K*; Muasya et al., 2002)	Tanzania	HQ705968	HQ705835	HQ705898
<i>Scirpoides holoschoenus</i> (L.) Soják	<i>Goetghebeur 11520</i> (GENT)	BG Porto, BG Ghent	HQ705900	HQ705782	HQ705837
<i>Sphaerocyperus erinaceus</i> (Ridl.) Lye	<i>Faden et al. 96/358</i> (K*; Muasya et al., 2002)	Tanzania	HQ705969	HQ705836	HQ705899

probability (PP) of clades was based upon the trees sampled after the chains converged. Trees were drawn using FigTree v1.3.1 and Adobe Photoshop CS3.

CARBON ISOTOPE ANALYSIS

Carbon isotope analysis ($\delta^{13}\text{C}$) was performed on 65 species to confirm their photosynthesis type. The

measurements of ^{13}C natural abundance in plant samples were performed using an elemental analyser (ANCA-SL, SerCon, UK) coupled to an isotope ratio mass spectrometer (20–20, SerCon, UK). The measured $^{13}\text{C}/^{12}\text{C}$ ratios are expressed as $\delta^{13}\text{C}$ values (‰) relative to the Vienna Pee Dee Belemnite (VPDB) standard:

$$\delta^{13}\text{C} = \left(\frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right) \times 1000$$

R_{sample} and R_{standard} refer to the $^{13}\text{C}/^{12}\text{C}$ ratio in the sample and the standard, respectively. The working standard for the measurements was wheat flour with a $\delta^{13}\text{C}$ value of $-27.01 \pm 0.04\text{‰}$ (certified by Iso-Analytical, UK). All analyses were performed in duplicate.

RESULTS

SEQUENCE ALIGNMENTS

After alignment and application of Gblocks, the ETS1f alignment included 70 sequences of 400 bases, the *rpl32-trnL* alignment 63 sequences of 819 bases and the *trnH-psbA* alignment 58 sequences of 787 bases. The concatenated data set included 77 sequences and the Gblocks program retained 61% or 2006 characters of the original alignment. Most excluded regions came from the ETS1f region.

PHYLOGENETIC ANALYSIS

The three single-locus ML analyses revealed nearly identical topologies and bootstrap values. As expected, the clades supported by single-locus analyses received even greater support in the multi-locus ML analysis. In the various analyses, only minor conflicts concerning the position of the species in the *C₄* *Cyperus* clade were detected, but most nodes in this clade are not supported. Also, in the ETS1f single locus ML tree, *Cyperus* section *Luzuloidei* branches off as a separate clade, whereas in all other analyses, section *Luzuloidei* (clade 2; Fig. 2) forms a clade sister to a clade with *C.* sections *Pseudanosporum* and *Anosporum*, the segregate genera *Courtoisina* and *Oxycaryum* and *C.* section *Fusci* (clade 3a and clade 3b; Fig. 2).

The three single-locus BI analyses did not differ significantly in tree topologies. The BI-MrModeltest and BI-GTR+I+ Γ methods also revealed the same topologies, but branches received slightly higher support in the BI-GTR+I+ Γ analysis. The multiple-locus BI topologies did not differ from the multiple-locus ML tree, except in *C₄* *Cyperus*, as mentioned above for the ML analyses. Evaluation of the multiple-locus BI analyses output shows that the

two runs of the BI-MrModeltest analysis converged on similar log likelihood (–17080) and parameter values. The burn-in value for both runs was determined at 1.5 million generations. The two runs of the BI-GTR+I+ Γ analysis also converged on similar log likelihood (–16947 to –16948) and parameter values. The burn-in value for the BI-GTR+I+ Γ method was determined at 2.5 million generations. The effective sample size (ESS) for the likelihood value of the combined runs was slightly higher for the BI-GTR+I+ Γ analysis. The latter consisted of 1455.91 uncorrelated samples, whereas the BI-MrModeltest analysis comprised 1282.38 uncorrelated samples.

Figure 2 shows the 50% majority consensus multiple-locus BI-GTR+I+ Γ tree with the associated PP values and the bootstrap values of the multiple-locus ML tree. Only bootstrap values above 75% and posterior probabilities above 0.85 are shown.

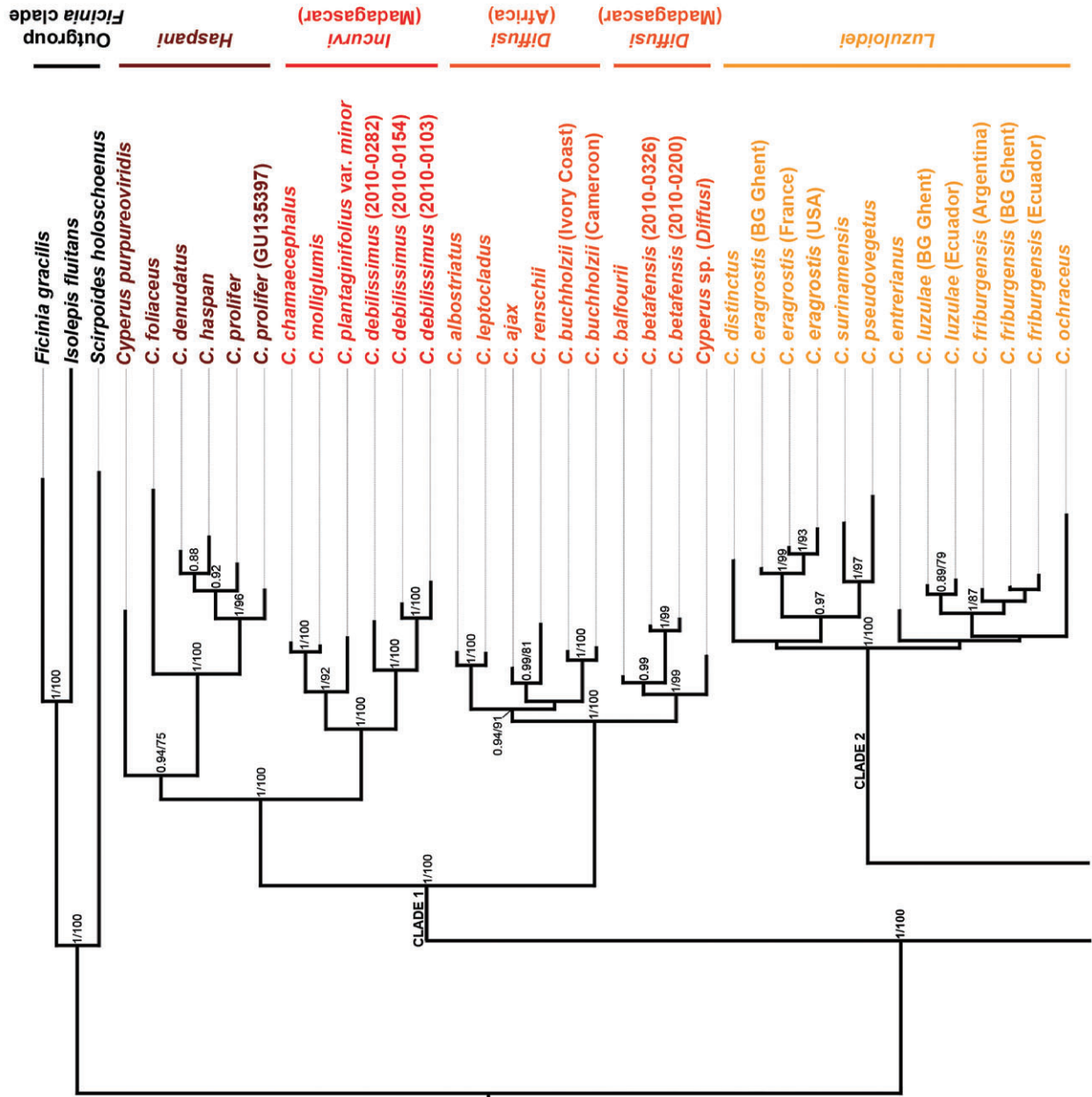
CARBON ISOTOPE ANALYSIS

Table 2 lists all confirmed *C₃* *Cyperus* spp. and some of the *C₄* *Cyperus* spp. used in the molecular study. The data listed in this table were either obtained through carbon isotope analysis ($\delta^{13}\text{C}$) performed on 65 species at Ghent University or taken from the literature (Bruhl & Wilson, 2007). For 15 species, the photosynthesis type was confirmed for the first time overall, and for an additional eight species [indicated in Table 2 by an asterisk (*)] this was confirmed for the first time using carbon isotope analysis. Out of a total of 98 species of the *c.* 187 *C₃* *Cyperus* species, the photosynthesis type is now confirmed (52%). Two sections as circumscribed by Kükenthal (1936) prove heterogeneous in this respect: '*Cyperus* section *Glutinosi*' and '*C.* section *Dichostylis*'.

DISCUSSION

GENERIC CIRCUMSCRIPTION AND OUTGROUP RELATIONSHIPS

The monograph of *Cyperus* by Kükenthal (1935–1936) offers an abundant source of hypotheses concerning groups of related species (Table 3). Nevertheless, not all taxa of the genus were explicitly placed within specific sections, and the generic circumscription of *Cyperus* and its allies has been adapted since Kükenthal's (1935–1936) revision. Also, it provides few clues as to how the taxa relate to each other or to possible outgroups. Previous molecular phylogenetic studies (Muasya *et al.*, 1998, 2000, 2002, 2009a; Simpson *et al.*, 2007), defined the closest related group within Cyperaceae as the *Ficinia* clade and place several segregate genera in the *Cyperus* clade. However, these studies do not



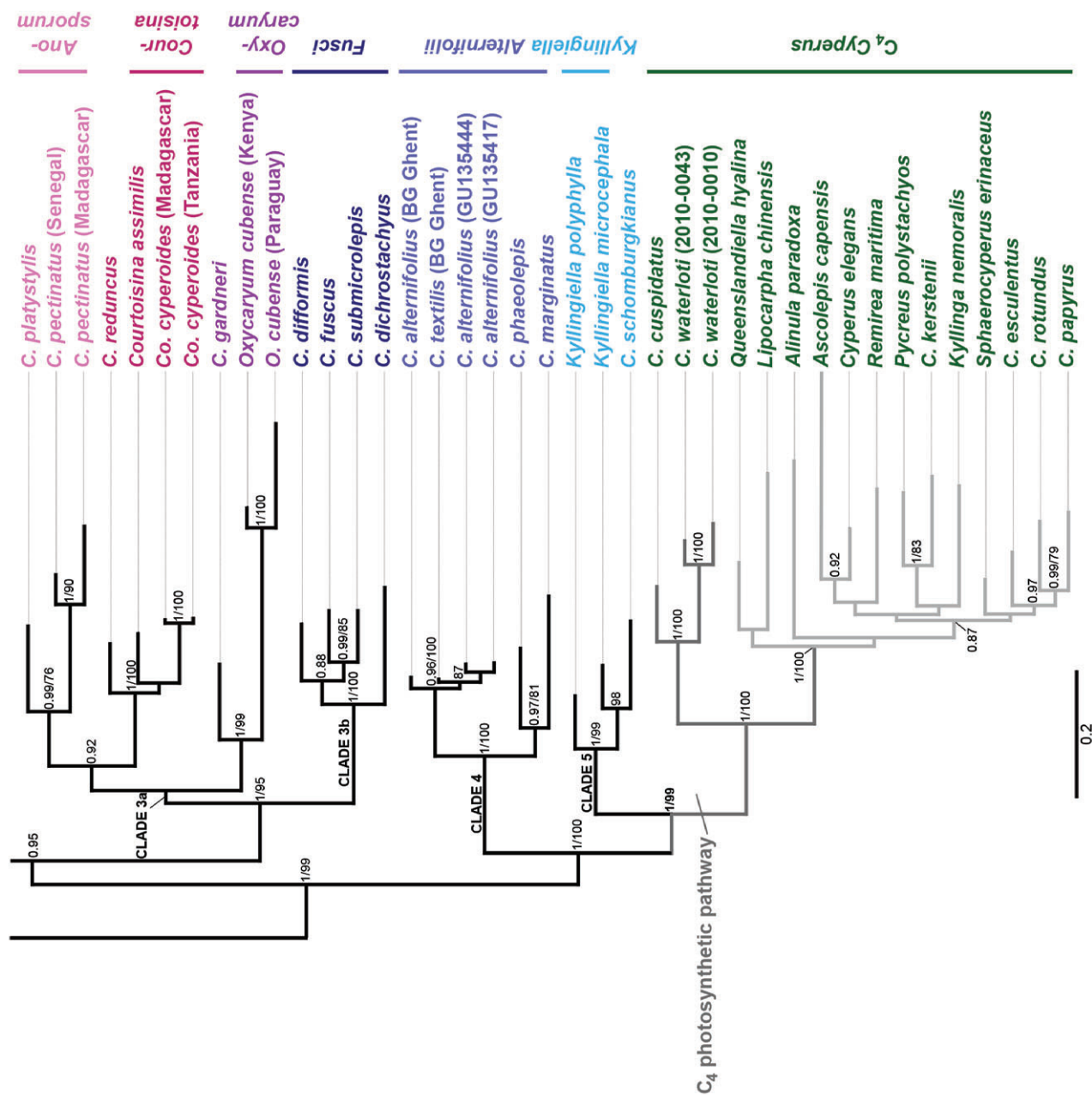


Figure 2. 50% majority consensus multiple-locus BI-GTR+I+Γ tree with the associated PP values and the bootstrap values of the multiple-locus ML tree. Only bootstrap values above 75% and posterior probabilities above 85% are shown.

Table 2. List of all confirmed C₃ *Cyperus* species and some of the C₄ *Cyperus* species used in the molecular study. The data listed in this table were either obtained through carbon isotope analysis ($\delta^{13}\text{C}$) performed on 65 species at the Ghent University or taken from literature (Bruhl & Wilson, 2007). Of fifteen species, the photosynthesis type was confirmed for the first time overall, and, of an additional eight species (*), this was performed for the first time using carbon isotope analysis. The species included in *Cyperus* section *Glutinosi* by Kükenthal (1936) are in bold.

Species	C ₃ /C ₄	References ($\delta^{13}\text{C}$: value, voucher)
<i>Ficinia gracilis</i> Schrad.	C ₃	IL ($\delta^{13}\text{C}$: -27.80, <i>Muasya 2365</i> GENT); Bruhl & Wilson, (2007)
<i>Isolepis fluitans</i> (L.) R.Br.	C ₃	IL ($\delta^{13}\text{C}$: -30.44, <i>Leten s.n.</i> GENT); Bruhl & Wilson, (2007)
<i>Scirpoides holoschoenus</i> (L.) Soják	C ₃	IL ($\delta^{13}\text{C}$: -26.40, <i>Goetghebeur 5246</i> GENT); Bruhl & Wilson, (2007)
<i>Androtrichum trigynum</i> (Spreng.) H.Pfeiff.	C ₃	IL ($\delta^{13}\text{C}$: -27.44, <i>Goetghebeur 4764</i> GENT); Bruhl & Wilson, (2007)
<i>Courtoisina assimilis</i> (Steud.) Maquet	C ₃	IL ($\delta^{13}\text{C}$: -28.14, <i>Van der Veken 9037</i> GENT); Bruhl & Wilson, (2007)
<i>Courtoisina cyperoides</i> (Roxb.) Soják	C ₃	IL ($\delta^{13}\text{C}$: -27.85, <i>Coppejans 693</i> GENT); Bruhl & Wilson, (2007)
<i>Kyllingiella microcephala</i> (Steud.) R.W.Haines & Lye	C ₃	Bruhl & Wilson, (2007)
<i>Kyllingiella polyphylla</i> (A.Rich.) Lye	C ₃	IL ($\delta^{13}\text{C}$: -31.38, <i>Kornas 755</i> GENT); Bruhl & Wilson, (2007)
<i>Kyllingiella simpsonii</i> Muasya	C ₃	IL ($\delta^{13}\text{C}$: -25.40, <i>Ole Sayalel 5320</i> EA)
<i>Oxycaryum cubense</i> (Poepp. & Kunth) Palla	C ₃	IL ($\delta^{13}\text{C}$: -30.06, <i>Zardini 18347</i> GENT); Bruhl & Wilson, (2007)
<i>Cyperus acuminatus</i> Torr. & Hook.	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus ajax</i> C.B.Clarke	C ₃	IL ($\delta^{13}\text{C}$: -28.05, <i>Hess 50/176</i> GENT); Bruhl & Wilson, (2007)
<i>Cyperus albostriatus</i> Schrad.	C ₃	IL ($\delta^{13}\text{C}$: -30.20, <i>Reid 726</i> GENT); Bruhl & Wilson, (2007)
<i>Cyperus alternifolius</i> L.	C ₃	IL ($\delta^{13}\text{C}$: -28.75, <i>Harley 22939</i> GENT); Bruhl & Wilson, (2007)
<i>Cyperus aquatilis</i> R.Br.	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus balfourii</i> C.B.Clarke	C ₃	IL ($\delta^{13}\text{C}$: -32.70, <i>Dorr 2744</i> GENT)
<i>Cyperus baronii</i> C.B.Clarke	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus buchholzii</i> Boeck.	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus burkartii</i> Guaglianone	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus canus</i> J.Presl & C.Presl	C ₃	IL ($\delta^{13}\text{C}$: -28.93, <i>Lopez 29</i> GENT)
<i>Cyperus cephalotes</i> Vahl	C ₃	IL ($\delta^{13}\text{C}$: -29.56, <i>Heckman 116</i> K); Bruhl & Wilson, (2007)
<i>Cyperus chalaranthus</i> J.Presl & C.Presl	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus chamaecephalus</i> Cherm.	C ₃	IL ($\delta^{13}\text{C}$: -35.34, <i>Schatz 2789</i> GENT, $\delta^{13}\text{C}$: -34.35, <i>Beentje 4774</i> K)
<i>Cyperus colymbetes</i> Kotschy & Peyr	C ₃	IL ($\delta^{13}\text{C}$: -26.30, <i>Denny 1283</i> GENT)
<i>Cyperus concinnus</i> R.Br.	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus constanzae</i> Urb.	C ₃	IL ($\delta^{13}\text{C}$: -29.19, <i>Ekman 6879</i> K)
<i>Cyperus debilissimus</i> Baker	C ₃	IL ($\delta^{13}\text{C}$: -29.54, <i>Messmer 895</i> K)
<i>Cyperus deciduus</i> Boeck.	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus dentatus</i> Torr.	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus denudatus</i> L.f.	C ₃	IL ($\delta^{13}\text{C}$: -27.26, <i>Hess 51/53</i> GENT); Bruhl & Wilson, (2007)
<i>Cyperus dereilema</i> Steud.	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus dichrostachyus</i> Hochst. ex A.Rich.	C ₃	IL ($\delta^{13}\text{C}$: -26.80, <i>Viane 2614</i> GENT); Bruhl & Wilson, (2007)
<i>Cyperus difformis</i> L.	C ₃	IL ($\delta^{13}\text{C}$: -29.44, <i>Hess 52/158</i> GENT); Bruhl & Wilson, (2007)
<i>Cyperus diffusus</i> Vahl	C ₃	IL ($\delta^{13}\text{C}$: -32.04, <i>Viane 1327</i> GENT)
<i>Cyperus disjunctus</i> C.B.Clarke	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus distinctus</i> Steud.	C ₃	IL * ($\delta^{13}\text{C}$: -28.82, <i>Carter 9237</i> GENT); Bruhl & Wilson, (2007)
<i>Cyperus drummondii</i> Torr. & Hook.	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus entrerianus</i> Boeck.	C ₃	IL ($\delta^{13}\text{C}$: -27.87, <i>Zardini 29789</i> GENT); Bruhl & Wilson, (2007)
<i>Cyperus eragrostis</i> Lam.	C ₃	IL ($\delta^{13}\text{C}$: -29.59, <i>Bryson 16965</i> GENT); Bruhl & Wilson, (2007)
<i>Cyperus fertilis</i> Boeck.	C ₃	IL ($\delta^{13}\text{C}$: -37.13, <i>Van der Veken 8940</i> GENT); Bruhl & Wilson, (2007)
<i>Cyperus fischerianus</i> A.Rich.	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus flaccidus</i> R.Br.	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus foliaceus</i> C.B.Clarke	C ₃	Bruhl & Wilson, (2007)

Table 2. Continued

Species	C ₃ /C ₄	References ($\delta^{13}\text{C}$: value, voucher)
<i>Cyperus friburgensis</i> Boeck.	C ₃	IL ($\delta^{13}\text{C}$: -34.30, <i>Øllgaard 74763</i> GENT)
<i>Cyperus fuscus</i> L.	C ₃	IL ($\delta^{13}\text{C}$: -28.52, <i>De Retz 67715</i> GENT); Bruhl & Wilson, (2007)
<i>Cyperus gardneri</i> Nees	C ₃	IL * ($\delta^{13}\text{C}$: -31.33, <i>Schessl 3316</i> GENT); Bruhl & Wilson, (2007)
<i>Cyperus glaucophyllus</i> Boeck.	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus gracilis</i> R.Br.	C ₃	IL ($\delta^{13}\text{C}$: -30.33, <i>Bruhl 5</i> GENT); Bruhl & Wilson, (2007)
<i>Cyperus gymnocalous</i> Steud.	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus haspan</i> L.	C ₃	IL ($\delta^{13}\text{C}$: -25.66, <i>Viane 908</i> GENT); Bruhl & Wilson, (2007)
<i>Cyperus hieronymi</i> Boeck.	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus humilis</i> Kunth	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus incomtus</i> Kunth	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus intricatus</i> Schrad. ex Schult.	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus kipasensis</i> Cherm.	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus laevis</i> R.Br.	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus laxus</i> Lam.	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus lecontei</i> Torr. ex Steud.	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus leptocladus</i> Kunth	C ₃	IL ($\delta^{13}\text{C}$: -27.84, <i>Reid 902</i> GENT); Bruhl & Wilson, (2007)
<i>Cyperus leucocephalus</i> Retz	C ₃	IL * ($\delta^{13}\text{C}$: -30.38, <i>Chantaranonthai 1630</i> K); Bruhl & Wilson, (2007)
<i>Cyperus limosus</i> Maxim.	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus luzulae</i> (L.) Retz	C ₃	IL ($\delta^{13}\text{C}$: -28.78, <i>Schessl 85/1-4</i> GENT); Bruhl & Wilson, (2007)
<i>Cyperus mapanioides</i> C.B.Clarke	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus marginatus</i> Thunb.	C ₃	IL ($\delta^{13}\text{C}$: -26.91, <i>Hess 51/16</i> GENT); Bruhl & Wilson, (2007)
<i>Cyperus megalanthus</i> (Kük.) G.C.Tucker	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus michoacanensis</i> Britton	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus microglumis</i> D.A.Simpson	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus miliifolius</i> Poepp. & Kunth	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus mirus</i> C.B.Clarke	C ₃	IL ($\delta^{13}\text{C}$: -31.16, <i>Boorman 229</i> GENT)
<i>Cyperus nayaritensis</i> Tucker	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus ochraceus</i> Vahl	C ₃	IL ($\delta^{13}\text{C}$: -30.19, <i>Viane 681</i> GENT)
<i>Cyperus palianparaiensis</i> Govindarajalu	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus pectinatus</i> Vahl	C ₃	IL ($\delta^{13}\text{C}$: -26.76, <i>De Wolf 92/86</i> GENT); Bruhl & Wilson, (2007)
<i>Cyperus perennis</i> (M.E.Jones) O'Neill	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus phaeolepis</i> Cherm.	C ₃	IL ($\delta^{13}\text{C}$: -29.80, <i>Phillipson 1647</i> GENT)
<i>Cyperus platycaulis</i> Baker	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus platystylis</i> R.Br.	C ₃	IL ($\delta^{13}\text{C}$: -27.35, <i>Chantaranonthai et al. 814</i> K); Bruhl & Wilson, (2007)
<i>Cyperus prolifer</i> Lam.	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus pseudoleptocladus</i> Kük.	C ₃	IL ($\delta^{13}\text{C}$: -26.02, <i>Reid 1816</i> GENT); Bruhl & Wilson, (2007)
<i>Cyperus pseudovegetus</i> Steud.	C ₃	IL ($\delta^{13}\text{C}$: -29.68, <i>Carter 6152</i> GENT); Bruhl & Wilson, (2007)
<i>Cyperus pulchellus</i> R.Br.	C ₃	IL ($\delta^{13}\text{C}$: -27.32, <i>De Smet 77/23</i> GENT); Bruhl & Wilson, (2007)
<i>Cyperus pulcher</i> Thunb.	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus reduncus</i> Hochst. ex Boeck.	C ₃	IL * ($\delta^{13}\text{C}$: -29.74, <i>Audru 5375</i> P); Bruhl & Wilson, (2007)
<i>Cyperus reflexus</i> Vahl	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus renschii</i> Boeck.	C ₃	IL ($\delta^{13}\text{C}$: -26.54, <i>Hess 52/779</i> GENT); Bruhl & Wilson, (2007)
<i>Cyperus schomburgkianus</i> Nees	C ₃	IL * ($\delta^{13}\text{C}$: -24.03, <i>Schessl 177/2</i> GENT); Bruhl & Wilson, (2007)
<i>Cyperus sciaphilus</i> Cherm.	C ₃	IL ($\delta^{13}\text{C}$: -33.91, <i>Lam & Meeuse 5816</i> K)
<i>Cyperus seslerioides</i> Kunth	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus sexangularis</i> Nees	C ₃	IL ($\delta^{13}\text{C}$: -26.24, <i>Lambinon 82/85</i> GENT)
<i>Cyperus sphaerospermus</i> Schrad.	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus stradbrokeensis</i> Domin	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus submicrolepis</i> Kük.	C ₃	IL * ($\delta^{13}\text{C}$: -28.75, <i>Viane 961</i> GENT); Bruhl & Wilson, (2007)

Table 2. *Continued*

Species	C ₃ /C ₄	References ($\delta^{13}\text{C}$: value, voucher)
<i>Cyperus surinamensis</i> Rottb.	C ₃	IL ($\delta^{13}\text{C}$: -28.68, <i>Jansen-Jacobs 521 GENT</i>); Bruhl & Wilson, (2007)
<i>Cyperus sylvestris</i> Ridl.	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus tenerrimus</i> J.Presl & C.Presl	C ₃	IL * ($\delta^{13}\text{C}$: -31.13, <i>Davidse 35095 GENT</i>); Bruhl & Wilson, (2007)
<i>Cyperus tenuispica</i> Steud.	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus textilis</i> Thunb.	C ₃	IL * ($\delta^{13}\text{C}$: -24.26, <i>Bohnen 7744 GENT</i>); Bruhl & Wilson, (2007)
<i>Cyperus trinervis</i> R.Br.	C ₃	IL ($\delta^{13}\text{C}$: -27.73, <i>Wilson 8565 GENT</i>)
<i>Cyperus uncinulatus</i> Schrad. ex Nees	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus vaginatus</i> R. Br.	C ₃	IL ($\delta^{13}\text{C}$: -28.64, <i>Wilson 905 K</i>); Bruhl & Wilson, (2007)
<i>Cyperus virens</i> Michx. var. <i>minarum</i> (Boeck.) Denton	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus virens</i> Michx. var. <i>montanus</i> (Boeck.) Denton	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus virens</i> Michx., as <i>C. virens</i> var. <i>virens</i>	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus xerophilus</i> Cherm.	C ₃	Bruhl & Wilson, (2007)
<i>Cyperus elegans</i> L.	C ₄	IL ($\delta^{13}\text{C}$: -11.46, <i>Goetghebeur 5601 GENT</i>); Bruhl & Wilson, (2007)
<i>Cyperus esculentus</i> L.	C ₄	IL ($\delta^{13}\text{C}$: -12.83, <i>Madsen 5310 GENT</i>); Bruhl & Wilson, (2007)
<i>Cyperus lacunosus</i> Griseb.	C ₄	Bruhl & Wilson, (2007)
<i>Cyperus oxylepis</i> Nees ex Steud.	C ₄	IL ($\delta^{13}\text{C}$: -11.63, <i>Carter 9061 GENT</i>); Bruhl & Wilson, (2007)
<i>Cyperus papyrus</i> L.	C ₄	IL ($\delta^{13}\text{C}$: -11.12, <i>Hess 50/88 GENT</i>); Bruhl & Wilson, (2007)
<i>Cyperus trachysanthos</i> Hook. & Arn.	C ₄	IL ($\delta^{13}\text{C}$: -12.47, <i>St. John 23599 K</i>); Bruhl & Wilson, (2007)
<i>Ascolepis capensis</i> (Kunth) Ridl.	C ₄	IL ($\delta^{13}\text{C}$: -10.77, <i>Traore 33 GENT</i>); Bruhl & Wilson, (2007)
<i>Lipocarpa chinensis</i> (Osbeck) J.Kern	C ₄	IL ($\delta^{13}\text{C}$: -10.35, <i>Malaisse & Goetghebeur 790 GENT</i>); Bruhl & Wilson, (2007)
<i>Pycurus polystachyos</i> (Rottb.) P.Beauv.	C ₄	IL ($\delta^{13}\text{C}$: -10.60, <i>Reekmans 6441 GENT</i>); Bruhl & Wilson, (2007)
<i>Queenslandiella hyalina</i> (Vahl) Ballard	C ₄	IL ($\delta^{13}\text{C}$: -12.93, <i>Mwachala 269 GENT</i>); Bruhl & Wilson, (2007)
<i>Remireia maritima</i> Aubl.	C ₄	IL ($\delta^{13}\text{C}$: -11.56, <i>MacDougal 3449 GENT</i>); Bruhl & Wilson, (2007)

answer questions concerning the affinities between the taxa within the *Cyperus* clade. In this study, the outgroup was chosen to represent various lineages in the *Ficinia* clade.

As the C₄ photosynthetic pathway arose only once in the *Cyperus* clade (e.g. Soros & Bruhl, 2000; Besnard *et al.*, 2009), we can recognize at least two main infrageneric groups in *Cyperus*: (1) an eucyperoid subgenus, uniting plants without the Kranz syndrome and with an inflorescence generally composed of digitately clustered spikelets (or contracted to a head-like inflorescence); and (2) a chlorocyperoid subgenus, uniting plants with a chlorocyperoid anatomy-type (Kranz syndrome) and an inflorescence composed of spikes of spikelets or condensed spikes. Using the data gathered in a previous study of the nomenclature of the *Cyperus* clade (Huygh *et al.*, 2010; Larridon *et al.*, 2011; Reynders *et al.*, 2011), the two subgenera have been named respectively: *Cyperus* subgenus *Anosporum* (Nees) C.B. Clarke (1884) and

subgenus *Cyperus*. Informally, we use the names C₃ *Cyperus* and C₄ *Cyperus* for these subgenera, respectively.

RELATIONSHIPS IN C₃ *CYPERUS*

The *Cyperus* clade (Fig. 2) is sister to the *Ficinia* clade and consists of a paraphyletic C₃ *Cyperus*, in which a well-supported monophyletic clade is nested, encompassing the species of the *Cyperus* clade that use C₄ photosynthesis (C₄ *Cyperus*). Table 3 shows the infrageneric classification of C₃ *Cyperus* species according to Kükenthal (1935–1936), with the species listed using their currently accepted names (Govaerts *et al.*, 2011). In contrast, Table 4 presents the preliminary subdivisional delimitations in C₃ *Cyperus* based on the results of the molecular phylogenetic hypothesis obtained in this study. The clades (or their subclades) in C₃ *Cyperus* (Fig. 2) largely concur with previously recognized taxa (*Cyperus* sections and sub-

Table 3. Infrageneric classification of *C₃* *Cyperus* species according to Kükenthal (1935–1936). The current synonymy for species names largely follows Govaerts *et al.* (2011)

<i>Cyperus</i> subgenus <i>Eucyperus</i> pars <i>Pycnostachys</i>		
Section	Species	Remarks
<i>Glutinosi</i>	<i>Cyperus constanzae</i> Urb.	
	<i>Cyperus elegans</i> L.	C ₄ <i>Cyperus</i>
	<i>Cyperus gardneri</i> Nees	
	<i>Cyperus lacunosus</i> Griseb.	C ₄ <i>Cyperus</i>
	<i>Cyperus oxylepis</i> Nees	C ₄ <i>Cyperus</i>
<i>Luzuloidei</i>	<i>Cyperus trachysanthos</i> Hook. & Arn.	C ₄ <i>Cyperus</i>
	<i>Cyperus acuminatus</i> Torr. & Hook.	
	<i>Cyperus altsonii</i> Kük.	
	<i>Cyperus celluloso-reticulatus</i> Boeck.	
	<i>Cyperus columbiensis</i> Palla	
	<i>Cyperus distinctus</i> Steud.	
	<i>Cyperus drummondii</i> Torr. & Hook.	
	<i>Cyperus entrerianus</i> Boeck.	
	<i>Cyperus eragrostis</i> Lam.	
	<i>Cyperus hieronymi</i> Boeck.	
	<i>Cyperus incomtus</i> Kunth	
	<i>Cyperus intricatus</i> Schrad. ex Schult.	
	<i>Cyperus luzulae</i> (L.) Retz.	
	<i>Cyperus megalanthus</i> (Kük.) G.C.Tucker	
	<i>Cyperus ochraceus</i> Vahl	
	<i>Cyperus pseudovegetus</i> Steud.	
	<i>Cyperus reflexus</i> Vahl	
	<i>Cyperus sordidus</i> J.Presl & C.Presl	
	<i>Cyperus surinamensis</i> Rottb.	
	<i>Cyperus virens</i> Michx.	
	<i>Cyperus xanthostachyus</i> Steud.	
	<i>Cyperus boeckeleri</i> Phil.	Unplaced name
<i>Cyperus elytropiptos</i> Steud.	Unplaced name	
<i>Pseudanosporum</i> <i>Vaginati</i>	<i>Cyperus platystylis</i> R.Br.	
	<i>Cyperus alternifolius</i> L.	
	<i>Cyperus canus</i> J.Presl & C.Presl	
	<i>Cyperus debilissimus</i> Baker	
	<i>Cyperus dioicus</i> I.M.Johnst.	
	<i>Cyperus gymnocaulos</i> Steud.	
	<i>Cyperus marginatus</i> Thunb.	
	<i>Cyperus phaeolepis</i> Cherm.	
	<i>Cyperus sexangularis</i> Nees	
	<i>Cyperus textilis</i> Thunb.	
	<i>Cyperus vaginatus</i> R.Br.	
	<i>Cyperus limiticola</i> Larridon & Reynders	Not seen by Kükenthal
	<i>Diffusi</i>	<i>Cyperus ajax</i> C.B.Clarke
<i>Cyperus albopurpureus</i> Cherm.		
<i>Cyperus albostrigatus</i> Schrad.		
<i>Cyperus balfourii</i> C.B.Clarke		
<i>Cyperus baronii</i> C.B.Clarke		
<i>Cyperus buchholzii</i> Boeck.		
<i>Cyperus chalaranthus</i> J.Presl & C.Presl		
<i>Cyperus chorisanthos</i> C.B.Clarke		
<i>Cyperus derreilema</i> Steud.		
<i>Cyperus diffusus</i> Vahl		
<i>Cyperus felipponei</i> Kük.		
<i>Cyperus fischerianus</i> Schimp. ex A.Rich.		
<i>Cyperus friburgensis</i> Boeck.		

Table 3. *Continued**Cyperus* subgenus *Eucyperus* pars *Pycnostachys*

Section	Species	Remarks
	<i>Cyperus glaucophyllus</i> Boeck.	
	<i>Cyperus helferi</i> Boeck.	
	<i>Cyperus kurzii</i> C.B.Clarke	
	<i>Cyperus laxus</i> Lam.	
	<i>Cyperus leptocladus</i> Kunth	
	<i>Cyperus longifolius</i> Poir.	
	<i>Cyperus multispicatus</i> Boeck.	
	<i>Cyperus nemoralis</i> Cherm.	
	<i>Cyperus pseudoleptocladus</i> Kük.	
	<i>Cyperus radians</i> Nees et Meyen ex Kunth	
	<i>Cyperus renschii</i> Boeck.	
	<i>Cyperus sylvestris</i> Ridl.	
	<i>Cyperus tabina</i> Steud. ex Boeck.	
	<i>Cyperus thorelii</i> E.G.Camus	
	<i>Cyperus trialatus</i> (Boeck.) J.Kern	
	<i>Cyperus xerophilus</i> Cherm.	
<i>Incurvi</i>	<i>Cyperus duclouxii</i> E.G.Camus	Not seen by Kükenthal (1936)
	<i>Cyperus anisitsii</i> Kük.	
	<i>Cyperus ankaizinensis</i> Cherm.	
	<i>Cyperus betafensis</i> Cherm.	
	<i>Cyperus chamaecephalus</i> Cherm.	
	<i>Cyperus consors</i> C.B.Clarke	
	<i>Cyperus dichromenaeformis</i> Kunth	
	<i>Cyperus disjunctus</i> C.B.Clarke	
	<i>Cyperus fertilis</i> Boeck.	
	<i>Cyperus filipes</i> Benth.	
	<i>Cyperus grandisimplex</i> C.B.Clarke	
	<i>Cyperus hoppiifolius</i> Uittien	
	<i>Cyperus hylophilus</i> Cherm.	
	<i>Cyperus inops</i> C.B.Clarke	
	<i>Cyperus longistylus</i> Kük.	
	<i>Cyperus mapanioides</i> C.B.Clarke	
	<i>Cyperus miliifolius</i> Poepp. & Kunth	
	<i>Cyperus molliglumis</i> Cherm.	
	<i>Cyperus neoguineensis</i> Kük.	
	<i>Cyperus pandanophyllum</i> C.B.Clarke	
	<i>Cyperus pearcei</i> C.B.Clarke	
	<i>Cyperus pedunculatus</i> F.Muell.	
	<i>Cyperus plantaginifolius</i> Cherm.	
	<i>Cyperus rufostriatus</i> C.B.Clarke ex Cherm.	
	<i>Cyperus sciaphilus</i> Cherm.	
	<i>Cyperus simplex</i> Kunth	
	<i>Cyperus subpapuanus</i> Kük.	
	<i>Cyperus tetraphyllus</i> R.Br.	
<i>Fusci</i>	<i>Cyperus dichrostachyus</i> Hochst. ex A.Rich.	
	<i>Cyperus difformis</i> L.	
	<i>Cyperus fuscus</i> L.	
	<i>Cyperus haematocephalus</i> Boeck. ex C.B.Clarke	
	<i>Cyperus pulcher</i> Thunb.	
	<i>Cyperus pulcherrimus</i> Willd. ex Kunth	
	<i>Cyperus reduncus</i> Hochst ex Boeck.	
	<i>Cyperus silletensis</i> Nees	
	<i>Cyperus submicrolepis</i> Kük.	
	<i>Cyperus trailii</i> C.B.Clarke	

Table 3. Continued

Cyperus subgenus <i>Eucyperus</i> pars <i>Pycnostachys</i>		
Section	Species	Remarks
<i>Haspani</i>	<i>Cyperus unicolor</i> Boeck.	
	<i>Cyperus soongoricus</i> Kar. & Kir.	
	<i>Cyperus commixtus</i> Kük.	
	<i>Cyperus concinnus</i> R.Br.	
	<i>Cyperus dentatus</i> Torr.	
	<i>Cyperus denudatus</i> L.f.	
	<i>Cyperus foliaceus</i> C.B.Clarke	
	<i>Cyperus haspan</i> L.	
	<i>Cyperus lecontei</i> Torr.	
	<i>Cyperus pendulus</i> Cherm.	
	<i>Cyperus pinetorum</i> Britton	
	<i>Cyperus platycaulis</i> Baker	
	<i>Cyperus prolifer</i> Lam.	
	<i>Cyperus sphaerospermus</i> Schrad.	
	<i>Cyperus subaequalis</i> Baker	
<i>Cyperus tenuispica</i> Steud.		
<i>Leucocephali</i>	<i>Cyperus hipasensis</i> Cherm.	Not seen by Kükenthal (1936)
	<i>Cyperus leucocephalus</i> Retz.	
	<i>Cyperus michoacanensis</i> Britton ex C.B.Clarke	
<i>Anosporum</i>	<i>Cyperus pulchellus</i> R.Br.	
	<i>Cyperus schomburgkianus</i> Nees	
	<i>Cyperus tenerrimus</i> J.Presl & C.Presl.	
<i>Graciles</i>	<i>Cyperus cephalotes</i> Vahl	
	<i>Cyperus colymbetes</i> Kotschy et Peyr.	
<i>Dichostylis</i>	<i>Cyperus pectinatus</i> Vahl	
	<i>Cyperus aquatilis</i> R.Br.	
	<i>Cyperus breviculmis</i> R.Br.	
	<i>Cyperus debilis</i> R.Br.	
	<i>Cyperus flaccidus</i> R.Br.	
	<i>Cyperus gracilis</i> R.Br.	
	<i>Cyperus laevis</i> R.Br.	
	<i>Cyperus mirus</i> C.B.Clarke	
	<i>Cyperus trichodes</i> Griseb.	Unlikely distribution
	<i>Cyperus trinervis</i> R.Br.	
<i>Dichostylis</i>	<i>Isolepis levynsiana</i> Muasya & D.A.Simpson	Isolepis
	<i>Isolepis leucoloma</i> (Nees) C.Archer	Isolepis
	<i>Cyperus humilis</i> Kunth	
	<i>Cyperus meeboldii</i> Kük.	C ₄ Cyperus
	<i>Cyperus michelianus</i> (L.) Link	C ₄ Cyperus
	<i>Cyperus seslerioides</i> Kunth	
	<i>Cyperus tweediei</i> C.B.Clarke	
<i>Cyperus uncinulatus</i> Schrad. ex Nees		
<i>Cyperus hilairenus</i> Steud.	Uncertain (Kükenthal, 1936)	
<i>Cyperus</i> subgenus <i>Juncellus</i>		
<i>Minuti</i>	<i>Juncellus minutus</i> C.B.Clarke	Unplaced name
<i>Cyperus</i> subgenus <i>Mariscus</i>		
<i>Decidui</i>	<i>Cyperus deciduus</i> Boeck.	
<i>Aristati</i>	<i>Courtoisina assimilis</i> (Steud.) Maquet	
	<i>Courtoisina cyperoides</i> (Roxb.) Soják	

Table 4. Preliminary subdivisional delimitations in *C₃ Cyperus* based on the presented results. The current synonymy for species names largely follows Govaerts *et al.* (2011). The distribution data were queried from Govaerts *et al.* (2011). Type species underlined

Taxon	Species	Distribution	
<i>Courtoisina</i>	<i>Courtoisina assimilis</i> (Steud.) Maquet	Ethiopia to S Africa, Madagascar	
	<i>Courtoisina cyperoides</i> (Roxb.) Soják	Chad to S Africa, Madagascar, Himalaya to Indo-China	
ex <i>Fusci</i>	<i>Cyperus reduncus</i> Hochst. ex Boeck.	W Trop. Africa to Uganda	
<i>Kyllingiella</i>	<i>Kyllingiella microcephala</i> (Steud.) R.W.Haines & Lye	Tropical and S Africa, Indian Subcontinent	
	<i>Kyllingiella polyphylla</i> (A.Rich.) Lye	Ethiopia to E Tropical. Africa	
	<i>Kyllingiella simpsonii</i> Muasya	Tanzania to Zambia	
	<i>Kyllingiella ugandensis</i> R.W.Haines & Lye	E Tropical Africa	
<i>Oxycaryum</i>	<i>Oxycaryum cubense</i> (Poepp. & Kunth) Palla	Tropical and Subtropical Africa, tropical and Subtropical America	
ex <i>Elegantes</i>	<i>Cyperus gardneri</i> Nees	Cuba, SE Mexico to NE Argentina	
<i>Cyperus</i> section <i>Alternifolii</i>	<i>Cyperus alternifolius</i> L.	Ethiopia to Mozambique, W Indian Ocean, Arabian Pen.	
	<i>Cyperus canus</i> J.Presl & C.Presl	Mexico to Colombia	
	<i>Cyperus dioicus</i> I.M.Johnst.	Mexico (Baja California Sur)	
	<i>Cyperus gymnocalos</i> Steud.	Australia	
	<i>Cyperus marginatus</i> Thunb.	Kenya to S Africa	
	<i>Cyperus phaeolepis</i> Cherm.	Madagascar	
	<i>Cyperus sexangularis</i> Nees	S Tropical and S Africa	
	<i>Cyperus textilis</i> Thunb.	S Africa	
	<i>Cyperus vaginatus</i> R.Br.	Australia	
	<i>Cyperus cephalotes</i> Vahl	Tropical Asia to NE Australia	
<i>Cyperus</i> section <i>Anosporum</i>	<i>Cyperus colymbetes</i> Kotschy & Peyr	Sudan to Mozambique, Madagascar	
	<i>Cyperus pectinatus</i> Vahl	Tropical and S Africa, Madagascar	
<i>Cyperus</i> section <i>Diffusi</i>	<i>Cyperus afromontanus</i> Lye	E Tropical Africa	
	<i>Cyperus ajax</i> C.B.Clarke	W Central Tropical Africa to Malawi	
	<i>Cyperus albopurpureus</i> Cherm.	E Madagascar	
	<i>Cyperus albostrigatus</i> Schrad.	S Tropical and S Africa	
	ex <i>Incurvi</i>	<i>Cyperus ankaizinensis</i> Cherm.	N Central Madagascar
	<i>Cyperus balfourii</i> C.B.Clarke	W Indian Ocean	
	<i>Cyperus baronii</i> C.B.Clarke	Tropical Africa, W Indian Ocean	
	ex <i>Incurvi</i>	<i>Cyperus betafensis</i> Cherm.	Central Madagascar
	<i>Cyperus breedlovei</i> G.C.Tucker	Mexico (Chiapas)	
	<i>Cyperus buchholzii</i> Boeck.	Tropical Africa.	
	<i>Cyperus burkartii</i> Guagl.	Argentina (Misiones)	
	<i>Cyperus chalaranthus</i> J. Presl & C. Presl	W South America to Paraguay	
	<i>Cyperus chorisanthos</i> C.B. Clarke	Mexico to Central America	
	<i>Cyperus cinereobrunneus</i> Kük.	Papua New Guinea	
	<i>Cyperus costaricensis</i> Gómez-Laur.	Costa Rica to Panama	
	<i>Cyperus davidsei</i> G.C.Tucker	Brazil (Bahia)	
<i>Cyperus derreilema</i> Steud.	Ethiopia to Malawi		
<i>Cyperus diffusus</i> Vahl	Tropical and Subtropical Asia to Queensland		
<i>Cyperus duclouxii</i> Camus	S Central China		
<i>Cyperus felipponei</i> Kük.	S Venezuela to NE Argentina		
<i>Cyperus fischerianus</i> Schimp. ex A.Rich.	Ethiopia to Malawi		
<i>Cyperus glaucophyllus</i> Boeck.	Kenya to Malawi		
<i>Cyperus helferi</i> Boeck.	Indo-China		
<i>Cyperus kurzii</i> C.B.Clarke	Andaman Island		
<i>Cyperus laxus</i> Lam.	Mexico to Tropical America		
<i>Cyperus leptocladus</i> Kunth	S Africa		

Table 4. Continued

Taxon	Species	Distribution
	<i>Cyperus longifolius</i> Poir.	W Indian Ocean
	<i>Cyperus matudae</i> G.C.Tucker	SE Mexico (Chiapas)
	<i>Cyperus meistostylus</i> S.T. Blake	New Guinea
	<i>Cyperus multispicatus</i> Boeck.	Assam to W Jawa
	<i>Cyperus muniziae</i> G.C.Tucker	Brazil (São Paulo)
	<i>Cyperus nemoralis</i> Cherm.	Central Madagascar
	<i>Cyperus nyererei</i> Lye	S Tanzania
	<i>Cyperus pseudoleptocladus</i> Kük.	Tropical and S Africa
	<i>Cyperus pseudopetirolatus</i> G.C. Tucker	Brazil (Bahia)
	<i>Cyperus renschii</i> Boeck.	Tropical Africa, Comoros
	<i>Cyperus rupicolus</i> S.T.Blake	Queensland to NE New South Wales
	<i>Cyperus sylvestris</i> Ridl.	Tropical Africa
	<i>Cyperus tabina</i> Steud. ex Boeck.	Central and S Tropical America
	<i>Cyperus thorelii</i> Camus	N Vietnam
	<i>Cyperus trialatus</i> (Boeck.) J.Kern	S China to W Malaysia
	<i>Cyperus turrialbanus</i> Gómez-Laur.	Costa Rica to Panama
	<i>Cyperus xerophilus</i> Cherm.	Central Madagascar
Cyperus section Fusci	<i>Cyperus dichrostachyus</i> Hochst. ex A.Rich.	Tropical and S Africa, Madagascar
	<i>Cyperus difformis</i> L.	Tropical and Subtropical Old World
	<i>Cyperus fuscus</i> L.	Macaronesia, Europe, Mediterranean to China
	<i>Cyperus heamotocephalus</i> Boeck. ex C.B.Clarke	S Africa
	<i>Cyperus pulcher</i> Thunb.	Cape Province to KwaZulu-Natal
	<i>Cyperus pulcherrimus</i> Willd. ex Kunth	Tropical Asia
	<i>Cyperus silletensis</i> Nees	Assam to Vietnam
	<i>Cyperus soongoricus</i> Kar. & Kir.	E Kazakhstan
	<i>Cyperus submicrolepis</i> Kük.	Tropical Africa
	<i>Cyperus traillii</i> C.B.Clarke	N South America to N Brazil
	<i>Cyperus unicolor</i> Boeck.	S Venezuela to Argentina
Cyperus section Graciles	<i>Cyperus aquatilis</i> R.Br.	New Guinea to N and E Australia
	<i>Cyperus breviculmis</i> R.Br.	N Australia
	<i>Cyperus cristulatus</i> S.T.Blake	N Australia
	<i>Cyperus enervis</i> R.Br.	Queensland, NE New South Wales
	<i>Cyperus flaccidus</i> R.Br.	N and E Australia
	<i>Cyperus gracilis</i> R.Br.	New Caledonia, E Australia
	<i>Cyperus laevis</i> R.Br.	E Australia
	<i>Cyperus mirus</i> C.B.Clarke	E Australia
	<i>Cyperus sculptus</i> S.T.Blake	Queensland to NE New South Wales
	<i>Cyperus stradbrogensis</i> Domin	Queensland, NE New South Wales
	<i>Cyperus trinervis</i> R.Br.	N and E Australia
Cyperus section Haspani	<i>Cyperus afroalpinus</i> Lye	Zaire to Kenya
	<i>Cyperus commixtus</i> Kük.	N Somalia
	<i>Cyperus concinnus</i> R.Br.	N and E Australia
	<i>Cyperus deciduus</i> Boeck.	Zaire to S Africa
	<i>Cyperus dentatus</i> Torr.	SE Canada to E USA
	<i>Cyperus denudatus</i> L.f.	Tropical and S Africa to N Australia
	<i>Cyperus foliaceus</i> C.B.Clarke	Tropical Africa
	<i>Cyperus graciliculmis</i> Lye	Tanzania (Mt Mnyera)
	<i>Cyperus haspan</i> L.	Tropical and Subtropical
	<i>Cyperus kasamensis</i> Podlech	Zambia
	<i>Cyperus kipasiensis</i> Cherm.	Angola to W Tanzania
	<i>Cyperus lecontei</i> Torr. ex Steud.	SE USA

Table 4. Continued

Taxon	Species	Distribution
ex <i>Diffusi</i>	<i>Cyperus microumbellatus</i> Lye	Kenya (Shimba Hills)
	<i>Cyperus pendulus</i> Cherm.	Madagascar
	<i>Cyperus pinetorum</i> Britton	Cuba (I. de la Juventud)
	<i>Cyperus platycaulis</i> Baker	Chad to KwaZulu-Natal, Madagascar
	<i>Cyperus prolifer</i> Lam.	Somalia to S Africa, W Indian Ocean
	<i>Cyperus purpureoviridis</i> Lye	Tanzania (Nguru Mts)
	<i>Cyperus sensilis</i> Baijnath	KwaZulu-Natal
	<i>Cyperus sphaerospermus</i> Schrad.	Mozambique to S Africa
	<i>Cyperus subaequalis</i> Baker	Madagascar
	<i>Cyperus tenuispica</i> Steud.	Tropical and Subtropical Old World to Central Asia
	<i>Cyperus vandervekenii</i> Reynders, Dhooghe & Goetgh.	Rwanda
Cyperus section <i>Incurvi</i>	<i>Cyperus almensis</i> D.A.Simpson	Brazil (Bahia: Pico das Almas)
ex <i>Alternifolii</i>	<i>Cyperus anisitsii</i> Kük.	Paraguay
	<i>Cyperus chamaecephalus</i> Cherm.	E Madagascar
	<i>Cyperus chinsalensis</i> Podlech	S Tanzania to Zambia
	<i>Cyperus consors</i> C.B.Clarke	SE and S Brazil
	<i>Cyperus debilissimus</i> Baker	Central Madagascar
	<i>Cyperus dichromenaeformis</i> Kunth	SE Brazil
	<i>Cyperus disjunctus</i> C.B.Clarke	E Australia
	<i>Cyperus fertilis</i> Boeck.	W Tropical Africa to Angola
	<i>Cyperus filipes</i> Benth.	New South Wales
	<i>Cyperus grandisimplex</i> C.B.Clarke	S Venezuela to Paraguay
	<i>Cyperus hoppüifolius</i> Uittien	N South America, N Peru
	<i>Cyperus hylophilus</i> Cherm.	E Madagascar
	<i>Cyperus inops</i> C.B.Clarke	S Brazil
	<i>Cyperus longistylus</i> Kük.	Solomon Is.
	<i>Cyperus lundellii</i> O'Neill	Mexico to Guatemala
	<i>Cyperus mapanioides</i> C.B.Clarke	Tropical Africa
	<i>Cyperus miliifolius</i> Poepp. & Kunth	Central and S Tropical America
	<i>Cyperus molliglumis</i> Cherm.	Central Madagascar
	<i>Cyperus multinervatus</i> Bosser	Madagascar
	<i>Cyperus neoguinensis</i> Kük.	New Guinea
	<i>Cyperus pandanophyllum</i> C.B.Clarke	E Madagascar
	<i>Cyperus pearcei</i> C.B.Clarke	Peru to Bolivia
	<i>Cyperus pedunculatus</i> F.Muell.	New Guinea to N. Australia
	<i>Cyperus plantaginifolius</i> Cherm.	Madagascar
	<i>Cyperus rufostriatus</i> C.B.Clarke ex Cherm.	E Madagascar
	<i>Cyperus sciaphilus</i> Cherm.	E Madagascar
	<i>Cyperus simplex</i> Kunth	S Mexico to Tropical America
<i>Cyperus subcastaneus</i> D.A.Simpson	Brazil (Bahia to Minas Gerais)	
<i>Cyperus semifertilis</i> S.T.Blake	Queensland	
<i>Cyperus subpapuanus</i> Kük.	Papua New Guinea	
<i>Cyperus tetrphyllus</i> R.Br.	E Australia	
Cyperus section <i>Leucocephali</i>	<i>Cyperus androhibensis</i> D.A.Simpson	NW Madagascar
	<i>Cyperus brumadoi</i> D.A.Simpson	Brazil (Bahia: Pico das Almas)
	<i>Cyperus leucocephalus</i> Retz.	Indian Subcontinent, Indo-China
	<i>Cyperus michoacanensis</i> Britton ex C.B.Clarke	SW Mexico (Sierra de Manantlán)
	<i>Cyperus microglumis</i> D.A.Simpson	Central Somalia
	<i>Cyperus nayaritensis</i> G.C.Tucker	SW Mexico

Table 4. Continued

Taxon	Species	Distribution
Cyperus section Luzuloidei	<i>Cyperus pulchellus</i> R.Br.	Tropical Old World
	<i>Cyperus schomburgkianus</i> Nees	N South America to Brazil
	<i>Cyperus tenerrimus</i> J.Presl & C.Presl	Mexico to Bolivia
	<i>Cyperus acuminatus</i> Torr. & Hook.	USA to NE Mexico
	<i>Cyperus altsonii</i> Kük.	Guyana
	<i>Cyperus celluloso-reticulatus</i> Boeck.	Venezuela, Bolivia, S Brazil
	<i>Cyperus columbiensis</i> Palla	Colombia
	<i>Cyperus distinctus</i> Steud.	SE USA, Bahamas
	<i>Cyperus drummondii</i> Torr. & Hook.	SE USA to Tropical America
	<i>Cyperus entrerianus</i> Boeck.	Mexico to N Argentina, Caribbean
ex <i>Diffusi</i>	<i>Cyperus eragrostis</i> Lam.	Easter Island, America
	<i>Cyperus friburgensis</i> Boeck.	S Tropical America
	<i>Cyperus hieronymi</i> Boeck.	Paraguay to N Argentina
	<i>Cyperus incomtus</i> Kunth	Peru to N Argentina
	<i>Cyperus intricatus</i> Schrad. ex Schult.	Costa Rica to N Argentina
	<i>Cyperus luzulae</i> (L.) Retz.	Mexico to Tropical America
	<i>Cyperus megalanthus</i> (Kük.) G.C.Tucker	Mexico to Central America
	<i>Cyperus ochraceus</i> Vahl	Tropical and Subtropical America
	<i>Cyperus pseudovegetus</i> Steud.	Central and E USA
	<i>Cyperus reflexus</i> Vahl	Oklahoma to Mexico, Costa Rica to Argentina
Cyperus section Pseudanosporum	<i>Cyperus sordidus</i> J.Presl & C.Presl	W Mexico
	<i>Cyperus surinamensis</i> Rottb.	Tropical and Subtropical America
	<i>Cyperus virens</i> Michx.	Tropical and Subtropical America
	<i>Cyperus xanthostachyus</i> Steud.	S South America
Cyperus section Radiantes	<i>Cyperus platystylis</i> R.Br.	Tropical and Subtropical Asia, Australia
Cyperus section Radiantes	<i>Cyperus radians</i> Nees & Meyen ex Kunth	SE China to W Malesia
Affinity unknown (C₃)		
ex <i>Dichostylis</i> '	<i>Cyperus humilis</i> Kunth	Mexico to Tropical America
ex <i>Dichostylis</i> '	<i>Cyperus seslerioides</i> Kunth	S USA to N Argentina
ex <i>Dichostylis</i> '	<i>Cyperus uncinulatus</i> Schrad. ex Nees	S Mexico to Tropical America
ex <i>Elegantes</i>	<i>Cyperus constanzae</i> Urb.	Caribbean
ex <i>Minuti</i>	<i>Cyperus limosus</i> Maxim.	Russian Far East, China to Vietnam
	<i>Cyperus palianparaiensis</i> Govind.	India
	<i>Cyperus perennis</i> (M.E.Jones) O'Neill	NW Mexico
Affinity unknown (photosynthesis type not confirmed)		
ex <i>Alternifolii</i>	<i>Cyperus limiticola</i> Larridon & Reynders	Central Madagascar
ex <i>Incurvi</i>	<i>Cyperus marojejyensis</i> Bosser	Madagascar
ex <i>Graciles</i>	<i>Cyperus trichodes</i> Griseb.	Jamaica
ex <i>Dichostylis</i> '	<i>Cyperus tweediei</i> C.B.Clarke	Argentina (Tucumán)
(related to <i>C. uncinulatus</i> ?)	<i>Cyperus arsenei</i> O'Neill & Ben.Ayers	Mexico
(related to <i>C. uncinulatus</i> ?)	<i>Cyperus hilairenus</i> Steud.	SE Brazil
(related to <i>C. uncinulatus</i> ?)	<i>Cyperus microbrunneus</i> G.C.Tucker	S Mexico to Central America
Unplaced name	<i>Cyperus boeckeleri</i> Phil.	Chili
Unplaced name	<i>Cyperus elyptiptos</i> Steud.	?

genera or segregate genera; e.g. Clarke, 1908; Kükenthal, 1935–1936; Haines & Lye, 1983). *Androt-richum*, which was indicated as the earliest branching taxon of the *Cyperus* clade (Muasya *et al.*, 2009a), was not included in this study because of the lack of material suitable for DNA extraction.

Clade 1

Clade 1 (Fig. 2) encompasses the three sections *Diffusi*, *Haspani* and *Incurvi*, of which *Diffusi* and *Incurvi* have pantropical distributions. *Cyperus* section *Diffusi* has important radiations of species in South America, Africa, Madagascar and Asia and *C.* section *Incurvi* has important radiations in South America, Madagascar and Oceania (Table 4). Species from both sections mostly occur in shaded forest habitats. *Cyperus* section *Haspani* has a mainly African distribution (Table 4), but also includes pantropical species (e.g. *C. haspan* L.). The three sections form clearly delineated subclades in clade 1.

Species of section *Haspani* (23 spp.; Table 4) can be annuals or more often perennials, usually with poorly developed leaf blades, and an inflorescence of digitate clusters of three to seven spikelets. Kükenthal (1936) included 17 species in this section (of which three were unseen by him) corresponding to 15 currently accepted species (Table 3). Kükenthal (1936) placed *C. deciduus* Boeck. in *C.* section *Decidui* Kük. of his subgenus *Mariscus* because of its deciduous spikelets. However, he noted the similarity in habit with *C. denudatus* L.f and *C. haspan*. Since Kükenthal's monograph of *Cyperus* (Kükenthal, 1936), several new species have been described in this section. In this study (Fig. 2), five species of section *Haspani* are included in the molecular study: *C. denudatus*, *C. foliaceus* C.B. Clarke, *C. haspan*, *C. prolifer* Lam. (Fig. 1D) and *C. purpureoviridis* Lye. This last species was described by Lye (1983) without clear indication of its affinities. However, in Haines & Lye (1983) *C. purpureoviridis* is listed among species belonging to section *Diffusi*. In the molecular phylogenetic hypothesis presented here (Fig. 2), the species clusters in section *Haspani*. The morphology of this species falls between sections *Diffusi* and *Haspani*, but a clear *Haspani* character is the well-developed creeping rhizome with relatively long internodes as in, for example, *C. denudatus* and *C. prolifer*.

Cyperus section *Incurvi* (32 species; Table 4) includes a number of remarkable species. The section is characterized by having obtuse or shortly mucronate incurved glumes, which articulate at their saccate (pouched) and persistent base (Kükenthal, 1936). Kükenthal (1936) described this section and originally included 31 species (of which three were unseen by him). These correspond to 27 currently recognized species (Table 3). Since then

new species have been described and several species have been placed in synonymy. Four Malagasy species (five accessions) of *C.* section *Incurvi sensu* Kükenthal (1936) were included in our molecular study (Fig. 2): *C. betafensis* Cherm. (Fig. 1F), *C. chamaecephalus* Cherm. (Gautier *et al.*, 2010), *C. molliglumis* Cherm. and *C. plantaginifolius* Cherm. var. *minor* Cherm.

Kükenthal (1936) included the strange Malagasy endemic *C. debilissimus* Baker (Fig. 1C) in his *C.* section *Vaginati* [= *C.* section *Alternifolii*] based on the reduction of the leaves to leaf sheaths. Chermeson (1937) thought this species deserved its own section (*C.* section *Debilissimi* Cherm., nom. nud.; Larridon *et al.*, 2011). However, our results place it with the other Malagasy species of *Incurvi* included in this study. *Cyperus betafensis* clusters with section *Diffusi*. *Cyperus betafensis* (Fig. 1F) differs in its habit from the other Malagasy *Incurvi* and Chermeson (1937) placed it in *C.* section *Diffusi*, together with *C. ankaizinensis* Cherm. At the same time, he placed the other endemic Malagasy *Incurvi* species in *C.* section *Pandanophylli* Cherm., nom. nud. (Larridon *et al.*, 2011), based on their capitate inflorescences contrasting with the anthelate inflorescences of *C. ankaizinensis* and *C. betafensis*. The rainforest-dwelling, Malagasy endemics of *C.* section *Incurvi* (*C. chamaecephalus*, *Cyperus hylophilus* Cherm., *C. molliglumis*, *C. multinervatus* Bosser, *C. pandanophyllum* Cherm., *C. plantaginifolius* Cherm., *C. rufostriatus* C.B. Clarke ex Cherm. and *C. sciophilus* Cherm.) are generally characterized by their broad leaves, purple leaf sheaths and long bracts much overtopping the capitate inflorescence. Their habit is rather unusual for the genus (Simpson, 1992; Gautier *et al.*, 2010). Bosser (1955) also included *C. marojejyensis* Bosser in this group, but here we consider this relationship not well enough established. In our opinion, *C.* section *Incurvi sensu* Kükenthal (1936) is heterogeneous; the molecular phylogenetic hypothesis confirms it is polyphyletic. The absence from this study of the South American and Australasian species of the section, including the type (*C. disjunctus* C.B. Clarke; Larridon *et al.*, 2011), makes it impossible to interpret the delimitations of the section correctly at this stage and to evaluate whether the Malagasy forest species should be considered as a separate group, as Chermeson (1937) indicated.

Cyperus section *Diffusi* (43 species; Table 4) is generally characterized by a perennial habit, well-developed leaves often with purplish leaf sheaths, large unequal bracts, and frequently a large, rather diffuse inflorescence of digitate clusters of one to six spikelets. Kükenthal (1936) recognized 26 species in section *Diffusi* (of which two were unseen by him)

agreeing with 30 currently accepted species (Table 3). Few of the species included by Kükenthal (1936) were South American. However, later studies revealed numerous overlooked species in the Neotropics (Gómez-Laurito, 1978; Gómez & Gómez-Laurito, 1982; Tucker, 1986, 2007; Guaglianone, 1990). *Cyperus diffusus* Vahl, interpreted by Kükenthal (1936) as a pantropical species, is now divided into several taxa: *C. laxus* Lam. (South America), *C. buchholzii* Boeck. (Africa) and *C. diffusus* s.s. (Asia). In this study (Fig. 2), seven species (10 accessions) of section *Diffusi sensu* Kükenthal (1936) from Africa (including Madagascar) are included (*C. ajax* C.B. Clarke, *C. albostratus* Cherm., *C. balfourii* C.B. Clarke, *C. buchholzii*, *C. leptocladus* Kunth and *C. renschii* Boeck.) and three samples of the South American species *C. friburgensis* Boeck. However, the last species consistently appears in the clade corresponding to *C.* section *Luzuloidei sensu* Denton (1978), making section *Diffusi sensu* Kükenthal (1936) polyphyletic. Consequently, *C. friburgensis* needs to be excluded from section *Diffusi*. As mentioned above, the Malagasy *C. betafensis* (Fig. 1F) does not belong to section *Incurvi* (cf. Kükenthal, 1936) but to section *Diffusi* (cf. Chermeson, 1937). The two Malagasy species included (*C. balfouri* and *C. betafensis*) appear more closely related to each other than to species of section *Diffusi* occurring in mainland Africa.

Clade 2

An entirely New World clade including *C.* section *Luzuloidei sensu* Denton (1978) ('Luzulae group') is sister to clade 3. This clade is a good example of a radiation of species in the New World. Of the 21 species in section *Luzuloidei* (Table 4), Denton (1978) included ten species in her 'Luzulae group', i.e. *C. acuminatus* Torr. & Hook., *C. distinctus* Steud., *C. eragrostis* Lam., *C. intricatus* Schrad. ex Schult., *C. luzulae* (L.) Retz., *C. ochraceus* Vahl, *C. pseudovegetus* Steud., *C. reflexus* Vahl, *C. surinamensis* Rottb. and *C. virens* Michx. She also included three more currently accepted species (Govaerts *et al.*, 2011): *C. entrerianus* Boeck. in synonymy with *C. luzulae*, *C. drummondii* Torr. & Hook., as a variety of *C. virens*, and *C. megalanthus* (Kük.) G.C. Tucker, as a variety of *C. pseudovegetus*. Furthermore, Denton (1978) excluded six species previously included in section *Luzuloidei* by Kükenthal (1936; Table 3), i.e. *C. altsonii* Kük., *C. celluloreticulatus* Boeck., *C. columbiensis* Palla, *C. hieronymi* Boeck., *C. incomtus* Kunth and *C. xanthostachyus* Steud., and omitted one more species (*C. sordidus* J. Presl & C. Presl). In our molecular phylogenetic analysis, ten samples of the *Luzulae* group were included, encompassing seven of its 13

currently accepted species. For two of the species, more than one accession was included: (1) for *C. eragrostis*, a specimen was used collected in the USA where it occurs as a native plant, another specimen was collected in France where the species is naturalized, and a specimen from the Ghent University Botanical Garden was also used; (2) for *C. luzulae*, two specimens were used, one of which was collected in the wild, and the other cultivated in the Ghent University Botanical Garden.

We can conclude here that Denton's *Luzulae* group (Denton, 1978) forms a natural group of species. However, the relationships between the species in this group are not well resolved in the phylogenetic tree (Fig. 2). Because no species excluded by Denton (1978) were included in this study, we cannot comment on the monophyly of section *Luzuloidei sensu* Kükenthal (1936). The species *C. friburgensis*, previously included in section *Diffusi* (e.g. Kükenthal, 1936; Tucker, 2007) clusters in section *Luzuloidei*. We included several different specimens of *C. friburgensis* in the phylogenetic study to ensure its inclusion in section *Luzuloidei* was not erroneous.

Clade 3

Clade 3 is highly diverse, as it not only includes several previously recognized sections, i.e. *Anosporum*, *Pseudanosporum* and *Fusci*, but also includes the segregate genera *Courtoisina* and *Oxycaryum*. This clade can be divided into two major subclades, clade 3a and clade 3b. *Anosporum*, *Courtoisina* and *Oxycaryum* have all been or are still recognized at generic level because of their highly unusual morphological characteristics. *Anosporum* and *Oxycaryum* are characterized by their (floating) aquatic lifeform and corky nutlets. *Oxycaryum* is also characterized by its spirally arranged glumes. *Courtoisina* is characterized by its yellowish green colour, flattened spikelets which disarticulate as a unit when mature, leaving the spikelet bract and prophyll behind, often conspicuously winged glumes, and linear-lanceolate to linear-oblong nutlets. A further paper will focus in more detail on the *C*₃ *Cyperus* segregate genera and the necessary nomenclatural/taxonomic changes to include these taxa into a monophyletic genus *Cyperus* (Larridon *et al.*, in press).

In this study (Fig. 2), an African and an American specimen of *Oxycaryum cubense* (Poepp. & Kunth) Palla are included in the phylogenetic analysis. *Cyperus gardneri* Nees, a Neotropical species, which Kükenthal (1936) placed in the mainly *C*₄ *Cyperus* section *Elegantes* (as '*C.* section *Glutinosi*'), clusters together with *Oxycaryum*. Analysis of the photosynthesis type used by the species in *C.* section *Elegantes* shows that, of the six species included by Kükenthal (1936; Table 3), two use *C*₃ photosynthesis (Table 2 in

bold, see below). The limited material available of the other proven C_3 species of *C.* section *Elegantes*, *C. constanzae* Urb., did not yield DNA fit for analysis.

For the genus *Courtoisina*, we included *Courtoisina assimilis* (Steud.) Maquet and an African and a Malagasy sample of *Courtoisina cyperoides* (Roxb.) Soják. Kükenthal (1936) placed the two species of *Courtoisina* in *Cyperus* subgenus *Mariscus* based on its deciduous spikelets, but *Mariscus* has been shown to be polyphyletic (e.g. Lye, 1992). A *Cyperus* species previously classified in section *Fusci* (*Cyperus reduncus* Hochst. ex Boeck.; Kükenthal, 1936; Table 3) clusters with the two known *Courtoisina* spp. The other taxa clustering in this subclade belong to *Cyperus* section *Anosporum* (*Cyperus pectinatus* Vahl) and section *Pseudanosporum* (*Cyperus platystylis* R.Br.).

Clade 3b corresponds to *C.* section *Fusci* (11 spp.; Table 4). This relatively small section is characterized by its generally annual habit (all species except *C. dichrostachyus* and *C. pulcher*), often reduced number of anthers (one or two, rather than three), and frequently small glumes and nutlets. Kükenthal (1936) included 11 species in his key of the section and mentioned a twelfth species (*C. soongoricus* Kar. & Kir.) as unknown to him (Table 3). In this study (Fig. 2), four species are included in the phylogenetic analysis (*C. dichrostachyus* Hochst. ex A.Rich., *C. difformis* L., *C. fuscus* L. (Fig. 1A) and *C. submicrolepis* Kük.). We exclude *C. reduncus* from section *Fusci* as it clusters with *Courtoisina* (see above).

Clade 4

The sampled species of *C.* section *Alternifolii* form a clade, which is sister to the clade including *C.* section *Leucocephali* and the genus *Kyllingiella*, and C_4 *Cyperus* (Fig. 2). This section is typified by the umbrella sedge (*C. alternifolius* L.), a well-known ornamental (Fig. 1E). Section *Alternifolii* has a southern hemisphere distribution, occurring in Australia, South America (including dioecious species), Madagascar and southern Africa, where the diversity of the section is noticeably higher (Table 4). The section comprises nine species (Table 4) and is characterized by its leafless culms, and many, almost equally sized involucre bracts. *Cyperus alternifolius* ssp. *flabelliformis* Kük. is sometimes recognized as the separate species, *C. involucreatus* Rottb., but here we treat *C. alternifolius* in the broad sense.

Kükenthal (1936) included *C. debilissimus* in his key to this section (as '*C.* section *Vaginati*'), and mentioned *C. paucispiculatus* Cherm. [= *C. limiticola* Larridon & Reynders] as unknown to him (Table 3). We included *C. alternifolius*, *C. marginatus* Thunb., *C. phaeolepis* Cherm., *C. textilis* Thunb. and *C. debilissimus* in the phylogenetic analysis. As mentioned above, *C. debilissimus* clusters with the Malagasy

species of section *Incurvi*. The affinities of *C. limiticola* are as yet unknown (Larridon, Reynders & Goetghebeur, 2008). In our results, *C. textilis* clusters in between the three included *C. alternifolius* accessions (Fig. 2). As noted in Gordon-Gray (1995), two Southern African species are closely related to *C. alternifolius*, i.e. *C. sexangularis* Nees and *C. textilis* Thunb., and there is a need to carefully establish the differences, in particular between typical *C. alternifolius* and *C. textilis*.

Clade 5

Clade 5 is formed by *Cyperus* section *Leucocephali* and the segregate genus *Kyllingiella* (Fig. 2). The close relationship of section *Leucocephali* and *Kyllingiella* is reflected in a marked resemblance in habit (small grass-like plants with a pale-coloured capitate inflorescence; see Fig. 1B) and in their preference for wet open grasslands. However, in *Kyllingiella* spp. the glumes are spirally arranged. In 1990, Simpson revised section *Leucocephali*, including seven species. Since then, two new species have been described. In this study, a specimen of *C. schomburgkianus* Nees from Bolivia is included. This specimen (Beck 25586) is a first record for the species from Bolivia. Simpson (1990) remarked on the disjunct distribution of this section across the tropics that might have implications for its monophyly. However, the recent discoveries of new species, and its intricate relationship with *Kyllingiella*, rather indicate a lack of knowledge of this group. A formal taxonomic revision with the inclusion of *Kyllingiella* in *Cyperus* will be published elsewhere (Larridon *et al.*, in press).

RELATIONSHIPS WITH C_4 CYPERUS

A well-supported monophyletic clade encompassing the C_4 *Cyperus* s.l. species (C_4 *Cyperus*; characterized by C_4 photosynthesis linked with chlorocyperoid vegetative anatomy) forms the sixth clade of *Cyperus*. The C_4 species *C. cuspidatus* Kunth (Bruhl & Wilson, 2007: chlorocyperoid anatomy, carbon isotope reading typical for C_4) and its Malagasy sister species *C. waterloti* Cherm. (synonymized with *C. cuspidatus* in Govaerts *et al.*, 2011), consistently form a clade outside the main C_4 *Cyperus* clade (Fig. 2; Muasya *et al.*, 2009a). Kükenthal (1936) placed *C. cuspidatus* in *C.* section *Amabilis* of his 'subgenus *Cyperus* pars *Pycnostachys*' (see above). The relationships in the main C_4 *Cyperus* clade are not well resolved in this study. A molecular phylogenetic study focusing on C_4 *Cyperus* is underway (W. Huygh, M. Reynders, I. Larridon, K. Bauters, A.M. Muasya, D.A. Simpson, P. Goetghebeur, unpubl. data).

CARBON ISOTOPE ANALYSIS

Carbon isotope analysis ($\delta^{13}\text{C}$) was performed on 65 species to confirm their photosynthetic pathway (Table 2). In this study, emphasis was put on *C.* section *Elegantes*. This section was considered to belong in the group with *C*₄ photosynthesis, but has inflorescences with digitate spikelet clusters (Kükenthal's (1935–1936) pars *Pycnostachys*; see above). *Cyperus* section *Elegantes* (Clarke, 1883) is the correct name for Kükenthal's (1936) section *Glutinosi* (Larridon *et al.*, 2011). This section includes *C. elegans* L. (the type species), *C. constanzae*, *C. gardneri*, *C. lacunosus* Griseb., *C. oxylepis* Nees and *C. trachysanthos* Hook. & Arn. (Table 3). The carbon isotope analysis conducted during this study confirmed previous studies (e.g. Bruhl & Wilson, 2007), indicating that *C.* section *Elegantes* is heterogeneous. *Cyperus constanzae* and *C. gardneri* have carbon isotope readings typical for *C*₃, whereas *C. elegans*, *C. lacunosus*, *C. oxylepis* and *C. trachysanthos* have carbon isotope readings typical for *C*₄.

Such heterogeneity can also be shown in other previously recognized sections using the data assembled by Bruhl & Wilson (2007), e.g. *C.* section *Dichostylis sensu* Kükenthal (1936). As well as two confirmed *C*₄ species, i.e. *C. meeboldii* Kük. and *C. michelianus* (L.) Delile, Kükenthal (1936) also included several confirmed *C*₃ species, namely *C. uncinulatus* Schrad. ex Nees, *C. seslerioides* Kunth and *C. humilis* Kunth. *Cyperus andinus* Palla ex Kük., another species included by Kükenthal (1936), is now considered to be a heterotypic synonym of *C. seslerioides* (Govaerts *et al.*, 2011). The photosynthesis type of the last species included in *C.* section *Dichostylis sensu* Kükenthal (1936: 310), *C. tweediei* C.B. Clarke, has not yet been confirmed. However, in the protologue of this species, Clarke (1908: 4) clearly stated its affinity to *C. uncinulatus* and *C. humilis*, so we can tentatively identify it as a *C*₃ *Cyperus* species. Since the publication of Kükenthal's monograph of *Cyperus* (Kükenthal, 1935–1936), two more species have been described which are placed in the vicinity of *C. uncinulatus*, i.e. *C. arsenei* O'Neill & Ben.Ayers and *C. microbrunneus* G.C.Tucker. The phylogenetic position of this group of species in *C*₃ *Cyperus* could not yet be verified.

Cyperus section *Graciles* is another group that includes confirmed *C*₃ *Cyperus* species. This section was not included in the molecular study because the specimens present in the GENT herbarium did not yield any useable DNA. Kükenthal (1936) included eight species in *C.* section *Graciles*, corresponding with 11 accepted species names (Table 3). Blake (1939) published a revision of *C.* section *Graciles* representing a more natural circumscription of this

section. After correspondence, Kükenthal (1943) accepted Blake's opinions. Three species included by Kükenthal (1936) in section *Graciles* were no longer included by Blake (1939). Two of these, *C. tenellus* L.f and *C. leucoloma* Nees, have since been moved to the genus *Isolepis*, as *I. levynsiana* Muasya & D.A.Simpson and *I. leucoloma* (Nees) C.Archer, respectively (Archer, 1998; Muasya *et al.*, 2002; Muasya, Simpson & Smets, 2006, 2007). A third species included in section *Graciles* by Kükenthal (1936), *C. trichodes* Griseb., was excluded most probably based on its highly unlikely distribution in Jamaica (Table 4); all other species of section *Graciles* are limited to Australia (Oceania). Furthermore, Blake (1939) had a quite different view of the synonymy and rank of some of the taxa included in section *Graciles* (see Table 4). For seven of the 11 species now included in section *Graciles* (Table 4), the photosynthesis type is confirmed as *C*₃ (Table 2; Bruhl & Wilson, 2007).

Cyperus radians Nees & Meyen ex Kunth was included by Kükenthal (1936) in section *Diffusi*, although Suringar (1898) placed it in its own section, *Radiantes*, an opinion which was shared by Kern (1974). Although generally considered as a eucyperoid species, no studies (carbon isotope analysis/molecular study) have as yet been performed to test this view.

CONCLUSIONS

From the maximum likelihood and Bayesian analyses of nrDNA (ETS1f) and plastid DNA (*rpl32-trnL* and *trnH-psbA*) sequence data presented here, we conclude that the *Cyperus* clade consists of a paraphyletic group with eucyperoid anatomy using *C*₃ photosynthesis (*C*₃ *Cyperus*; clades 1–5) and a well-supported monophyletic clade with chlorocyperoid anatomy using *C*₄ photosynthesis (*C*₄ *Cyperus*). In *C*₃ *Cyperus*, five major clades are recognizable. Clade 1 can be divided in three subclades largely corresponding to *Cyperus* sections *Haspani*, *Incurvi* and *Diffusi*. The other major clades respectively correspond to: clade 2, an entirely New World *C.* section *Luzuloidei sensu* Denton (1978); clade 3, a highly diverse clade including two subclades: clade 3a, sections *Pseudanosporum* and *Anosporum* and the segregate genera *Courtoisina* and *Oxycaryum*, and clade 3b, *C.* section *Fusci*; clade 4, *C.* section *Alternifolii*; and clade 5, *C.* section *Leucocephali* and the segregate genus *Kyllingiiella*. This study establishes a phylogenetic framework for future studies in the diverse *Cyperus* clade.

ACKNOWLEDGEMENTS

We thank Pieter Asselman for his helpful suggestions with the lab work, Andy Vierstraete for performing the

sequence reactions (Ghent University, Belgium) and Lazlo Csiba (Royal Botanic Gardens, Kew) for the extraction of DNA from the specimens in the Kew Herbarium (Kew DNA Bank). We thank the Department of Environment and Natural Resources (DENR Region 8), for providing a collecting permit for Cyperaceae in the Philippines, and the Ministry of Scientific Research and Innovation for providing a collecting permit for Cameroon (No. 082/MINRESI/B00/C00/C10/C12), and the general secretariat of the AETFAT congress 2007 and the staff of the National Herbarium in Yaoundé and Limbe Botanic Gardens for their field assistance. We are grateful for the invitation of the East African Herbarium (National Museums of Kenya, Nairobi) and the Kenya Wildlife Service for the permission to access and to collect sedges in the protected areas of Kenya and for their help with organizing the expedition. The ANGAP Madagascar National Parks authority, the general secretariat of the AETFAT congress 2010 and the staff of the MBG office in Antananarivo are acknowledged for their support in securing collecting permits (No. 082/10/MEF/SG/DGF/DCB. SAP/SLRSE – I. Larridon) for Cyperaceae in Madagascar and their help with organizing the expedition.

FUNDING

This work was supported by research grants of the Special Research Fund (BO5622, BO7418, BOF, Ghent University, Belgium) and the Department of Biology, Ghent University, Belgium. The field expeditions were financed through travel grants of the Research Foundation–Flanders (FWO) and the Leopold III-Fund, and with support of the Department of Biology, Ghent University, Belgium.

REFERENCES

- Álvarez I, Wendel JF. 2003.** Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution* **29**: 417–434.
- Archer C. 1998.** A new combination in *Isolepis*. *Bothalia* **28**: 41–42.
- Besnard G, Muasya AM, Russier F, Roalson EH, Salamin N, Christin P-A. 2009.** Phylogenomics of C₄ photosynthesis in sedges (Cyperaceae): multiple appearances and genetic convergence. *Molecular Biology and Evolution* **26**: 1909–1919.
- Blake ST. 1939.** (publ. 1940). Notes on Australian Cyperaceae 3. *Proceedings of the Royal Society of Queensland* **51**: 32–50.
- Bosser J. 1955.** Cyperacées nouvelles de Madagascar. *Naturaliste Malgache* **7**: 119–121.
- Britton NL. 1907.** The sedges of Jamaica (Cyperaceae). *Bulletin of the Department of Agriculture Jamaica* **5** (Suppl. 1): 1–19.
- Bruhl JJ. 1991.** Comparative development of some taxonomically critical floral/inflorescence features in Cyperaceae. *Australian Journal of Botany* **39**: 119–127.
- Bruhl JJ. 1995.** Sedge genera of the world: relationships and a new classification of the Cyperaceae. *Australian Systematic Botany* **8**: 125–305.
- Bruhl JJ, Perry S. 1995.** Photosynthetic pathway-related ultrastructure of C₃, C₄ and C₃-like C₃-C₄ intermediate sedges (Cyperaceae), with special reference to *Eleocharis*. *Australian Journal of Plant Physiology* **22**: 1–10.
- Bruhl JJ, Stone NE, Hattersley PW. 1987.** C₄ acid decarboxylation enzymes and anatomy in sedges (Cyperaceae): first record of NAD-malic enzyme species. *Australian Journal of Plant Physiology* **14**: 719–728.
- Bruhl JJ, Wilson KA. 2007.** Towards a comprehensive survey of C₃ and C₄ photosynthetic pathways in Cyperaceae. In: Columbus JT, Friar EA, Porter JM, Prince LM, Simpson MG, eds. *Monocots III/grasses IV. Aliso* 23. Claremont, CA: Rancho Santa Ana Botanic Garden, 99–148.
- Castresana J. 2000.** Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* **17**: 540–552.
- Chermeson H. 1937.** *Cypéracées*. In: Humbert H, ed. *Flore de Madagascar*. 29e Fam. Tananarive: Imprimerie Officielle, 1–335.
- Christin PA, Besnard G, Samaritani E, Duvall MR, Hodkinson TR, Savolainen V, Salamin N. 2008a.** Oligocene CO₂ decline promoted C₄ photosynthesis in grasses. *Current Biology* **18**: 37–43.
- Christin PA, Salamin N, Muasya AM, Roalson EH, Russier F, Besnard G. 2008b.** Evolutionary switch and genetic convergence on *rbcL* following the evolution of C₄ photosynthesis. *Molecular Biology and Evolution* **25**: 2361–2368.
- Clarke CB. 1883.** Cyperaceae. In: Baker JG, ed. Contributions to the Flora of Madagascar 3. *Journal of the Linnean Society, Botany* **20**: 279–299.
- Clarke CB. 1884.** On the Indian species of *Cyperus*; with remarks on some others that specially well illustrate the subdivisions of the genus. *Journal of the Linnean Society, Botany* **21**: 1–202.
- Clarke CB. 1893.** *Cyperaceae*. In: Hooker JD, ed. *Flora of British India* 6, 19. London: L. Reeve & Co., 585–672.
- Clarke CB. 1908.** New genera and species of Cyperaceae. *Kew Bulletin of Miscellaneous Information, Additional Series* **8**: 1–196.
- Denton MF. 1978.** A taxonomic treatment of the *Luzulae* group of *Cyperus*. *Contributions from the University of Michigan Herbarium* **11**: 197–271.
- Dragon JA, Barrington DS. 2009.** Systematics of the *Carex aquatilis* and *C. lenticularis* lineages: geographically and ecologically divergent sister clades of *Carex* section *Phacocystis* (Cyperaceae). *American Journal of Botany* **96**: 1896–1906.
- Gautier L, Nusbaumer L, Larridon I, Callmander MW. 2010.** Distribution of *Cyperus chamaecephalus* Cherm., a forest undergrowth species with inconspicuous inflorescences. *Candollea* **65**: 364–367.

- Goetghebeur P. 1986.** *Genera Cyperacearum. Een bijdrage tot de kennis van de morfologie, systematiek en fylogenesen van de Cyperaceae-genera.* PhD Thesis, Ghent University, Belgium.
- Goetghebeur P. 1989.** Studies in Cyperaceae 9. Problems in the lectotypification and infrageneric taxonomy of *Cyperus*. *Bulletin de la Société Royale de Botanique de Belgique* **122**: 103–114.
- Goetghebeur P. 1998.** Cyperaceae. In: Kubitzki K, ed. *The families and genera of vascular plants 4. Flowering plants – monocotyledons.* Berlin: Springer-Verlag, 141–190.
- Gómez LD, Gómez-Laurito J. 1982.** Plantae mesoamericanae novae 7. *Phytologia* **52**: 227–229.
- Gómez-Laurito J. 1978.** De Cyperacearum costaricensium notitate. *Cyperus. Brenesia* **14–15**: 357–359.
- Gordon-Gray KD. 1995.** Cyperaceae in Natal. In: Leistner OA, Momberg BA, eds. *Strelitzia* 2. Pretoria: National Botanical Institute, 1–218.
- Govaerts R, Simpson DA, Goetghebeur P, Wilson KL, Egorova T, Bruhl J. 2007.** *World checklist of cyperaceae.* Sedges. Kew: Kew Publishing, Royal Botanic Gardens, xiii + 765 p.
- Govaerts R, Simpson DA, Goetghebeur P, Wilson KL, Egorova T, Bruhl J. 2011.** *World checklist of cyperaceae.* Kew: The Board of Trustees of the Royal Botanic Gardens, Published on the Internet; available at <http://www.kew.org/wcsp/monocots/> accessed 25.03.2011.
- Guaglianone ER. 1990.** Une especie nueva de *Cyperus* (Cyperaceae) de Misiones, Argentina. *Darwiniana* **30**: 233–236.
- Haines RW, Lye KA. 1983.** *The sedges and rushes of East Africa.* Nairobi: East African National History Society.
- Huygh W, Larridon I, Reynders M, Muasya AM, Govaerts R, Simpson DA, Goetghebeur P. 2010.** Nomenclature and typification of names of genera and subdivisions of genera in *Cypereae* (Cyperaceae): 1. Names of genera in the *Cyperus* clade. *Taxon* **59**: 1883–1890.
- Kern JH. 1974.** Cyperaceae. In: van Steenis CGGJ, ed. *Flora malesiana*, ser. 1. Den Haag: Junk, 107–187.
- Kress WJ, Wurdack KJ, Zimmer EA, Weigt LA, Janzen DH. 2005.** Use of DNA barcodes to identify flowering plants. *Proceedings of the National Academy of Sciences of the United States of America* **102**: 8369–8374.
- Kükenthal G. 1935–1936.** Cyperaceae–Scirpoideae–Cypereae. In: Engler A, ed. *Das pflanzenreich* 4 (20) [Heft 101]. Berlin: Engelmann, 1–671.
- Kükenthal G. 1943.** Neue oder nicht genügend bekannte Cyperaceen. *Mitteilungen des Thüringischen Botanischen Vereins* n.f., **50**: 1–13.
- Larridon I, Huygh W, Reynders M, Muasya AM, Govaerts R, Simpson DA, Goetghebeur P. Accepted.** Nomenclature and typification of names of genera and subdivisions of genera in *Cypereae* (Cyperaceae): 2. Names of subdivisions in *Cyperus*. *Taxon* **60**: 868–884.
- Larridon I, Reynders M, Goetghebeur P. 2008.** *Cyperus limiticola*, a new name for a Madagascan *Cyperus* (Cyperaceae). *Novon* **18**: 187–188.
- Larridon I, Reynders M, Huygh W, Bauters K, Vrijdaghs A, Leroux O, Muasya AM, Goetghebeur P. In press.** Taxonomic changes in C₃ *Cyperus* (Cyperaceae) supported by molecular phylogenetic data, morphology, embryology, ontogeny and anatomy. *Plant Ecology and Evolution*.
- Li M-R, Wedin DA, Tieszen LL. 1999.** C₃ and C₄ photosynthesis in *Cyperus* (Cyperaceae) in temperate eastern North America. *Canadian Journal of Botany* **77**: 18–209.
- Linnaeus C. 1753.** *Species plantarum.* Stockholm.
- Lye KA. 1983.** Studies in African Cyperaceae 25. New taxa and combinations in *Cyperus* L. *Nordic Journal of Botany* **3**: 213–232.
- Lye KA. 1992.** The history of the genus *Mariscus*. *Lidia* **3**: 37–72.
- Muasya AM, Bruhl JJ, Simpson DA, Culham A, Chase MW. 2000.** Suprageneric phylogeny of Cyperaceae: a combined analysis. In: Wilson KL, Morrison DA, eds. *Monocots: systematics and evolution.* Melbourne: CSIRO Publishing, 593–601.
- Muasya AM, de Lange PJ. 2010.** *Ficinia spiralis* (Cyperaceae) a new genus and combination for *Desmoschoenus spiralis*. *New Zealand Journal of Botany* **48**: 31–39.
- Muasya AM, Reynders M, Goetghebeur P, Simpson DA, Vrijdaghs A. 2011.** *Dracoscirpoides* (Cyperaceae) – a new genus from Southern Africa, its taxonomy and floral ontogeny. *South African Journal of Botany*. doi:10.1016/j.sajb.2011.05.011.
- Muasya AM, Simpson DA, Chase MW. 2002.** Phylogenetic relationships in *Cyperus* s.l. (Cyperaceae) inferred from plastid DNA sequence data. *Botanical Journal of the Linnean Society* **138**: 145–153.
- Muasya AM, Simpson DA, Chase MW, Culham A. 1998.** An assessment of suprageneric phylogeny in Cyperaceae using *rbcL* DNA sequences. *Plant Systematics and Evolution* **211**: 257–271.
- Muasya AM, Simpson DA, Smets E. 2006.** *Isolepis tenella*, a new combination in Cyperaceae. *Novon* **16**: 89–90.
- Muasya AM, Simpson DA, Smets E. 2007.** *Isolepis levynsiana*, a new name for *Cyperus tenellus* (Cyperaceae). *Novon* **17**: 59.
- Muasya AM, Simpson DA, Verboom GA, Goetghebeur P, Naczi RFC, Chase MW, Smets E. 2009a.** Phylogeny of Cyperaceae based on DNA sequence data: current progress and future prospects. *Botanical Review* **75**: 2–21.
- Muasya AM, Vrijdaghs A, Simpson DA, Chase MW, Goetghebeur P, Smets E. 2009b.** What is a genus in Cypereae: phylogeny, character homology assessment and generic circumscription in Cypereae. *Botanical Review* **75**: 52–66.
- Müller J, Müller K, Quandt D. 2008.** PhyDE – phylogenetic data editor, version 0.995. Available at <http://phyde.de/>
- Nylander JAA. 2004.** *Mmodeltest v2.* Program distributed by the author. Uppsala: Evolutionary Biology Centre, Uppsala University.
- Pyanokov VI, Ziegler H, Akhani H, Deigle C, Lüttge U. 2010.** European plants with C₄ photosynthesis: geographical and taxonomic distribution and relations to climate parameters. *Botanical Journal of the Linnean Society* **163**: 283–304.

- Rambaut A, Drummond AJ. 2007. Tracer v1.4. Available at <http://beast.bio.ed.ac.uk/Tracer>
- Raynal J. 1973. Notes cypérologiques 19. Contribution à la classification de la sous-famille des Cyperoideae. *Adansonia* sér. 2, **13**: 145–171.
- Reynders M, Huygh W, Larridon I, Muasya AM, Govaerts R, Simpson DA, Goetghebeur P. Accepted. Nomenclature and typification of names of genera and subdivisions of genera in Cyperaceae (Cyperaceae): 3. Names of subdivisions in segregate genera of *Cyperus*. *Taxon* **60**: 885–895.
- Rikli M. 1895. Beiträge zur vergleichenden Anatomie der Cyperaceen mit besonderer Berücksichtigung der inneren Parenchymscheide. *Jahrbücher für Wissenschaftliche Botanik* **27**: 485–580.
- Ronquist F, Huelsenbeck JP. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.
- Sage RF. 2004. The evolution of C₄ photosynthesis. *New Phytologist* **161**: 341–370.
- Shaw J, Lickey EB, Beck JT, Farmer SB, Liu W, Miller J, Siripun KC, Winder CT, Schilling EE, Small RL. 2005. The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* **92**: 142–166.
- Shaw J, Lickey EB, Schilling EE, Small RL. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *American Journal of Botany* **94**: 275–288.
- Simpson DA. 1990. A revision of *Cyperus* section *Leucocephali*. *Kew Bulletin* **45**: 485–501.
- Simpson DA. 1992. A new species of *Cyperus* and a reassessment of *Cyperus rufostriatus* (Cyperaceae) from Madagascar. Notes on Madagascar Cyperaceae 1. *Kew Bulletin* **47**: 745–751.
- Simpson DA, Furness CA, Hodkinson TR, Muasya AM, Chase MW. 2003. Phylogenetic relationships in Cyperaceae subfamily Mapanioideae inferred from pollen and plastid DNA sequence data. *American Journal of Botany* **90**: 1071–1086.
- Simpson DA, Muasya AM, Alves M, Bruhl JJ, Dhooge S, Chase MW, Furness CA, Ghamkhar K, Goetghebeur P, Hodkinson TR, Marchant AD, Nieuborg R, Reznicek AA, Roalson EH, Smets E, Starr JR, Thomas WW, Wilson KL, Zhang X. 2007. Phylogeny of *Cyperaceae* based on DNA sequence data – a new *rbcL* analysis. In: Columbus JT, Friar EA, Porter JM, Prince LM, Simpson MG, eds. *Monocots III/grasses IV. Aliso 23*. Claremont, CA: Rancho Santa Ana Botanic Garden, 72–83.
- Soros CL, Bruhl JJ. 2000. Multiple evolutionary origins of C₄ photosynthesis in the Cyperaceae. In: Wilson KL, Morrison DA, eds. *Monocots: systematics and evolution*. Melbourne: CSIRO, 629–636.
- Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**: 2688–2690.
- Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* **57**: 758–771.
- Starr JR, Harris SA, Simpson DA. 2003. Potential of the 5' and 3' ends of the intergenic spacer (IGS) of rDNA in the cyperaceae: new sequences for lower-level phylogenies in sedges with an example from *Uncinia* Pers. *International Journal of Plant Sciences* **164**: 213–227.
- Stock WD, Chuba DK, Verboom GA. 2004. Distribution of South African C₃ and C₄ species of Cyperaceae in relation to climate and phylogeny. *Austral Ecology* **29**: 313–319.
- Suringar JV. 1898. *Het geslacht Cyperus*. Leeuwarden: Hugo Suringar.
- Tucker GC. 1986. New mesoamerican species of *Cyperus* (Cyperaceae). *Rhodora* **88**: 503–513.
- Tucker GC. 2007. Systematics of *Cyperus* L. section *Diffusi* Kunth (Cyperaceae) in the neotropics. *A Botânica no Brasil: Pesquisa, Ensino e Políticas Públicas Ambientais [58 Congresso Nacional de Botânica]* 311–314.
- Van de Putte K, Nuytinck J, Stubbe D, Le HT, Verbeke A. 2010. *Lactarius volemus* sensu lato (Russulales) from northern Thailand: morphological and phylogenetic species concepts explored. *Fungal Diversity* **45**: 99–130.
- Van der Veken P. 1965. Contribution à l'embryographie systématique 1 des *Cyperaceae-Cyeroideae*. *Bulletin du Jardin botanique de l'État à Bruxelles* **35**: 285–354.
- Vrijdaghs A, Muasya AM, Goetghebeur P, Caris P, Nagels A, Smets E. 2009. A floral ontogenetic approach to questions of homology within the Cyperoideae (Cyperaceae). *Botanical Review* **75**: 30–51.
- Vrijdaghs A, Reynders M, Larridon I, Muasya AM, Smets E, Goetghebeur P. 2010. Spikelet structure and development in Cyperoideae (Cyperaceae): a monopodial general model based on ontogenetic evidence. *Annals of Botany* **105**: 555–571.
- Vrijdaghs A, Reynders M, Muasya AM, Larridon I, Goetghebeur P, Smets E. 2011. Spikelet and floral morphology and development in *Cyperus* and *Pycneus* (Cyperaceae). *Plant Ecology and Evolution* **144**: 44–63.
- Yano O, Hoshino T. 2005. Molecular phylogeny and chromosomal evolution of Japanese *Schoenoplectus* (Cyperaceae), based on ITS and ETS1f sequences. *Acta Phytotaxonomica et Geobotanica* **56**: 183–195.
- Yano O, Hoshino T. 2007. Phylogenetic relationships and chromosomal evolution of Japanese *Fimbristylis* (Cyperaceae) using nrDNA ITS and ETS1f sequence data. *Acta Phytotaxonomica et Geobotanica* **57**: 205–217.