



# Towards a new classification of the giant paraphyletic genus *Cyperus* (Cyperaceae): phylogenetic relationships and generic delimitation in $C_4$ *Cyperus*

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Maximum likelihood and Bayesian inference analyses of nuclear ribosomal DNA (ETS1f) and plastid DNA (*rpl32-trnL*, *trnH-psbA*) sequence data are presented for ‘ $C_4$  *Cyperus*’ (Cyperaceae). The term ‘ $C_4$  *Cyperus*’ encompasses all species of *Cyperus s.l.* that use  $C_4$  photosynthesis linked with chlorocyperoid vegetative anatomy. Sampling comprises 107 specimens of 104 different taxa, including many of the subdivisions of  $C_4$  *Cyperus s.s.* and all  $C_4$  segregate genera (*Alinula*, *Ascolepis*, *Kyllinga*, *Lipocarpha*, *Pycreus*, *Queenslandiella*, *Remirea*, *Sphaerocyperus* and *Volkiella*). According to our results,  $C_4$  *Cyperus* is a well-supported monophyletic clade nested in  $C_3$  *Cyperus*. Despite the lack of resolution along the backbone of the  $C_4$  *Cyperus* clade and for some internal branches, several well-supported clades can be distinguished. The first clade in  $C_4$  *Cyperus* is formed by *Cyperus cuspidatus* and *C. waterloti*. Other recognizable and well-supported clades correspond to segregate genera, i.e. *Ascolepis*, *Lipocarpha* including *Volkiella*, and *Kyllinga*. Species of  $C_4$  *Cyperus s.s.* form a core grade in which the  $C_4$  segregate genera are embedded. *Pycreus*, the largest segregate genus composed of *c.* 120 species, is not monophyletic as it includes several  $C_4$  species of *Cyperus s.s.* This study establishes a phylogenetic framework for revising the classification and character evolution in *Cyperus s.l.* © 2013 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2013, **172**, 106–126.

**ADDITIONAL KEYWORDS:** Cyperaceae – Cyperoideae – molecular phylogeny – paraphyly – species radiation – systematics.

## INTRODUCTION

Cyperaceae (the sedge family) has an almost cosmopolitan distribution and plays a dominant role in wetland vegetation. The many reductions and convergences in the inflorescences of Cyperaceae have impeded evolutionary reconstruction (homology questions, e.g. Bruhl, 1991; Vrijdaghs *et al.*, 2009, 2010; Muasya *et al.*, 2009b) and classification (e.g. Clarke, 1908; Kükenthal, 1935–36; Kern, 1974; Haines & Lye, 1983; Bruhl, 1995; Goetghebeur, 1998). Based on recent molecular phylogenetic studies, Cyperaceae

consists of two main clades, corresponding to subfamilies Cyperoideae and Mapanioideae (Simpson *et al.*, 2003, 2007; Muasya *et al.*, 2009a). In Cyperoideae, two clades stand out because of their extraordinary species diversity: (1) the clade corresponding to the predominantly temperate tribe Cariceae (*c.* 1950 spp.); and (2) the clade corresponding to the mainly tropical tribe Cypereae (*c.* 1120 spp.). Together, they cover nearly three-fifths of the species diversity in Cyperaceae (Govaerts *et al.*, 2012).

Recent molecular phylogenetic studies of Cyperaceae (Simpson *et al.*, 2003, 2007; Muasya *et al.*, 2009a) have shown Cypereae *sensu* Goetghebeur (1998) to be monophyletic, but the generic delimita-

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**Table 1.** The genera in Cyperae currently accepted by Govaerts *et al.* (2012), plus the recently published genus *Dracoscirpoides* (Muasya *et al.*, 2012) and the recent phylogenetic novelty *Erioscirpus* (Yano *et al.*, 2012). The segregate genera using the C<sub>4</sub> photosynthetic pathway are underlined. The taxa indicated by an asterisk were recently included in *Cyperus* (Larridon *et al.*, 2011b)

Cypereae		
<i>Ficinia</i> clade	?	<i>Cyperus</i> clade
<i>Dracoscirpoides</i> Muasya (3 spp.)	<i>Androtrichum</i> (Brongn.) Brongn.	<i>Alinula</i> J.Raynal (4 spp.)
<i>Erioscirpus</i> Palla (2 spp.)		<i>Ascolepis</i> Nees ex Steud., (22 spp.)
<i>Hellmuthia</i> Steud. (1 sp.)		<i>Courtoisina</i> Soják (2 spp.)*
<i>Ficinia</i> Schrad. (75 spp.)		<i>Kyllinga</i> Rottb. (74 spp.)
<i>Isolepis</i> R.Br. (76 spp.)		<i>Kyllingiella</i> R.W.Haines & Lye (4 spp.)*
<i>Scirpoides</i> Ség. (4 spp.)		<i>Lipocarpa</i> R.Br. (36 spp.)
		<i>Oxycaryum</i> Nees (1 sp.)*
		<i>Pycneus</i> P.Beauv. (114 spp.)
		<i>Queenslandiella</i> Domin (1 sp.)
		<i>Remirea</i> Aubl. (1 sp.)
		<i>Sphaerocyperus</i> Lye (1 sp.)
		<i>Volkiella</i> Merxm. & Czech (1 sp.)

tions in Cyperae remain controversial (Muasya *et al.*, 2009b). In the past, members of Cyperae were circumscribed as having spikelets with distichous glumes and reduced, perianthless flowers (e.g. Kükenthal, 1935–36). However, neither the distichy of the glumes nor the absence of a perianth can be regarded as phylogenetically informative characters (e.g. Vrijdaghs *et al.*, 2006; Muasya *et al.*, 2009a, b, in press). Currently, members of Cyperae are circumscribed by the presence of a *Cyperus*-type embryo or the similar *Ficinia*-type embryo (Van der Veken, 1965; Goetghebeur, 1998; Muasya *et al.*, 2009a, b). The presence of various combinations of characters (e.g. reduced flowers, reduced and/or contracted inflorescences) and convergent morphologies has led to the misinterpretation of the relationships of many lineages of Cyperae. A number of taxa (belonging especially to *Erioscirpus* Palla, *Ficinia* Schrad, *Hellmuthia* Steud., *Isolepis* R.Br., *Kyllingiella* R.W.Haines & Lye, *Oxycaryum* Nees, *Scirpoides* Ség.) have been allocated to various tribes in Cyperaceae, including Scirpeae, Rhynchosporae, Hypolytreae and Schoeneae (e.g. Kunth, 1837; Nees von Esenbeck, 1842; Steudel, 1854–55; Clarke, 1908). However, extensive anatomical (Kranz anatomy), embryographical and molecular phylogenetic studies (e.g. Van der Veken, 1965; Goetghebeur, 1986, 1998; Bruhl, 1995; Muasya *et al.*, 2001a, 2009a, b; Muasya, Simpson & Chase, 2002; Simpson *et al.*, 2003, 2007; Larridon *et al.*, 2011a, b; Yano *et al.*, 2012) have revealed that these genera are closely related to *Cyperus* L. Consequently, the reinterpretation of the morphological characters of these genera in the context of Cyperae is required.

On the basis of molecular phylogenetic studies (e.g. Simpson *et al.*, 2007; Muasya *et al.*, 2009a), two clades are recognized in Cyperae: (1) the *Ficinia* clade; and (2) the *Cyperus* clade. The first, smaller clade (c. 160 spp.) consists of several genera with a mainly southern African distribution, a ficinoid habit (hemicryptophytes, culm scapose, inflorescence capitate and appearing pseudolateral with main involucre bract being stem-like) and mostly spiral glumes. The basalmost branches include species with perianth parts (*Dracoscirpoides* Muasya, *Erioscirpus*, *Hellmuthia*; Vrijdaghs *et al.*, 2006; Muasya *et al.*, 2012; Yano *et al.*, 2012). Prior to the embryographical study of Van der Veken (1965), most of these genera had been classified in or near *Scirpus* L.

The second, larger, pantropical clade (c. 950 spp.), with mostly distichous glumes, comprises a paraphyletic *Cyperus* s.s. as the core genus (c. 700 spp.), in which at least 12 segregate genera are nested (Goetghebeur, 1998; Govaerts *et al.*, 2012; see Table 1). The branch leading to *Androtrichum* (Brongn.) Brongn. (two species) appears to be at the base of the *Cyperus* clade (Muasya *et al.*, 2002, in press), but this needs further confirmation. Although molecular phylogenetic studies have revealed that all of these genera are nested in *Cyperus* (e.g. Muasya *et al.*, 2002; Larridon *et al.*, 2011a), there has been considerable discussion about whether to include these taxa in *Cyperus*. Contemporary treatments either recognize the segregate genera as separate from *Cyperus* (e.g. Bruhl, 1995; Goetghebeur, 1998; Govaerts *et al.*, 2007, 2012) or merge them into

*Cyperus* at an infrageneric rank (e.g. Kükenthal, 1935–36; Haines & Lye, 1983; Lye, 1997).

The *Cyperus* clade includes a grade of branches characterized by C<sub>3</sub> photosynthesis (C<sub>3</sub> *Cyperus*, c. 190 spp.), which were well resolved in a combined analysis of ETS1f, *trnH-psbA* and *rpl32-trnL* (Larridon *et al.*, 2011a). In C<sub>3</sub> *Cyperus*, most sections of the classification according to Kükenthal (1935–36) were confirmed. Larridon *et al.* (2011b) included the C<sub>3</sub> segregates *Courtoisina* Soják, *Oxycaryum* and *Kyllingiella* in *Cyperus*, supported by molecular data, combined with morphology, embryography, ontogeny and anatomy.

Nested in C<sub>3</sub> *Cyperus* is a highly diverse clade (C<sub>4</sub> *Cyperus*, c. 760 spp.) with the C<sub>4</sub> photosynthetic pathway as a synapomorphy (e.g. Muasya, Simpson & Chase, 2001b; Muasya *et al.*, 2002, 2009a, in press; Besnard *et al.*, 2009; Larridon *et al.*, 2011a). The nine C<sub>4</sub> segregate genera represent c. 30% of diversity in the C<sub>4</sub> *Cyperus* clade. Figure 1 shows some of the morphological diversity of C<sub>4</sub> *Cyperus* lineages. They are generally considered to be well-delimited entities (e.g. Goetghebeur, 1998) and are circumscribed by a combination of morphological characters, including inflorescence and spikelet morphology, unit of dispersal and nutlet orientation (e.g. Muasya *et al.*, 2009b; Vrijdaghs *et al.*, 2011; Reynders *et al.*, 2012; Figure 2). However, the mutual relationships of the taxa in C<sub>4</sub> *Cyperus* still need to be determined.

#### PARAPHYLY AND MODERN CLASSIFICATION STRATEGIES

With the advancement of molecular phylogenetic research, species relationships and evolutionary patterns in giant genera provide new and valuable opportunities to study evolutionary processes. Often, these giant genera appear to contain derived lineages that have, up to now, been considered as separate genera (e.g. *Acacia* Mill., Miller & Bayer, 2001; *Carex* L., Starr & Ford, 2009; *Croton* L., Berry *et al.*, 2005; *Euphorbia* L., Steinmann & Porter, 2002; *Salvia* L., Walker *et al.*, 2004). The development of new classifications, encompassing the concept of monophyly for these large paraphyletic entities and their segregate genera, has been highly challenging. Three main strategies can be implemented: (1) splitting; (2) accepting paraphyletic taxa; and (3) lumping. Splitting paraphyletic taxa into a large number of small genera has been proposed for a number of large genera (e.g. *Acacia*; Maslin, Miller & Seigler, 2003). The decision on where to split needs to be based on a well-resolved phylogenetic hypothesis, and there are challenges to identifying diagnostic characters for the segregate entities and controversies about name application (*Acacia*; e.g. Moore *et al.*, 2010, 2011;

Smith & Figueiredo, 2011; Thiele *et al.*, 2011). A second, less popular, strategy is a classification in which various segregate genera are upheld which are themselves monophyletic, but remain part of a paraphyletically circumscribed giant genus. The use of paraphyletic genera has been defended by some authors (e.g. Brummitt, 1996; Brummitt & Sosef, 1998), but has been strongly opposed by others (e.g. Nelson, Murphy & Ladiges, 2003). The third and most popular strategy when dealing with paraphyletic giant genera is the lumping of all the segregates into a broader circumscribed genus (e.g. in *Euphorbia*; Steinmann & Porter, 2002). A negative consequence of lumping is that it can become difficult to describe clearly the giant genus as a whole.

#### OBJECTIVES

In the present study, molecular phylogenetic data of the *Cyperus* clade were analysed: (1) to determine the mutual relationships of the taxa (i.e. genera, sections, species) included in C<sub>4</sub> *Cyperus*; (2) to test whether the segregate genera and infrageneric taxa in C<sub>4</sub> *Cyperus* (Kükenthal, 1935–36; Govaerts *et al.*, 2012) are monophyletic; and (3) to examine the most suitable classification strategy for C<sub>4</sub> *Cyperus*. Papers documenting the necessary nomenclatural/taxonomical changes based on the results presented in this article and more detailed studies of several of the larger C<sub>4</sub> segregates will be published elsewhere. This study is part of a larger research project aimed at recircumscribing *Cyperus* as a monophyletic unit and at creating a new infrageneric classification of the genus supported by both molecular and morphological data.

#### MATERIAL AND METHODS

One hundred and seven samples from 104 different taxa were used for this study. Sixty-seven sequences from 23 species were used from a previous study (Larridon *et al.*, 2011a). The other 213 sequences from 81 different taxa were newly generated for this study. The samples with species names, voucher information, origin and GenBank accession numbers for the sequences are given in Table 2. 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. 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 on the basis of the results of previous molecular phylogenetic analyses of Cypereae by Muasya *et al.* (2002, 2009a) and Larridon *et al.* (2011a). Taxonomic information for

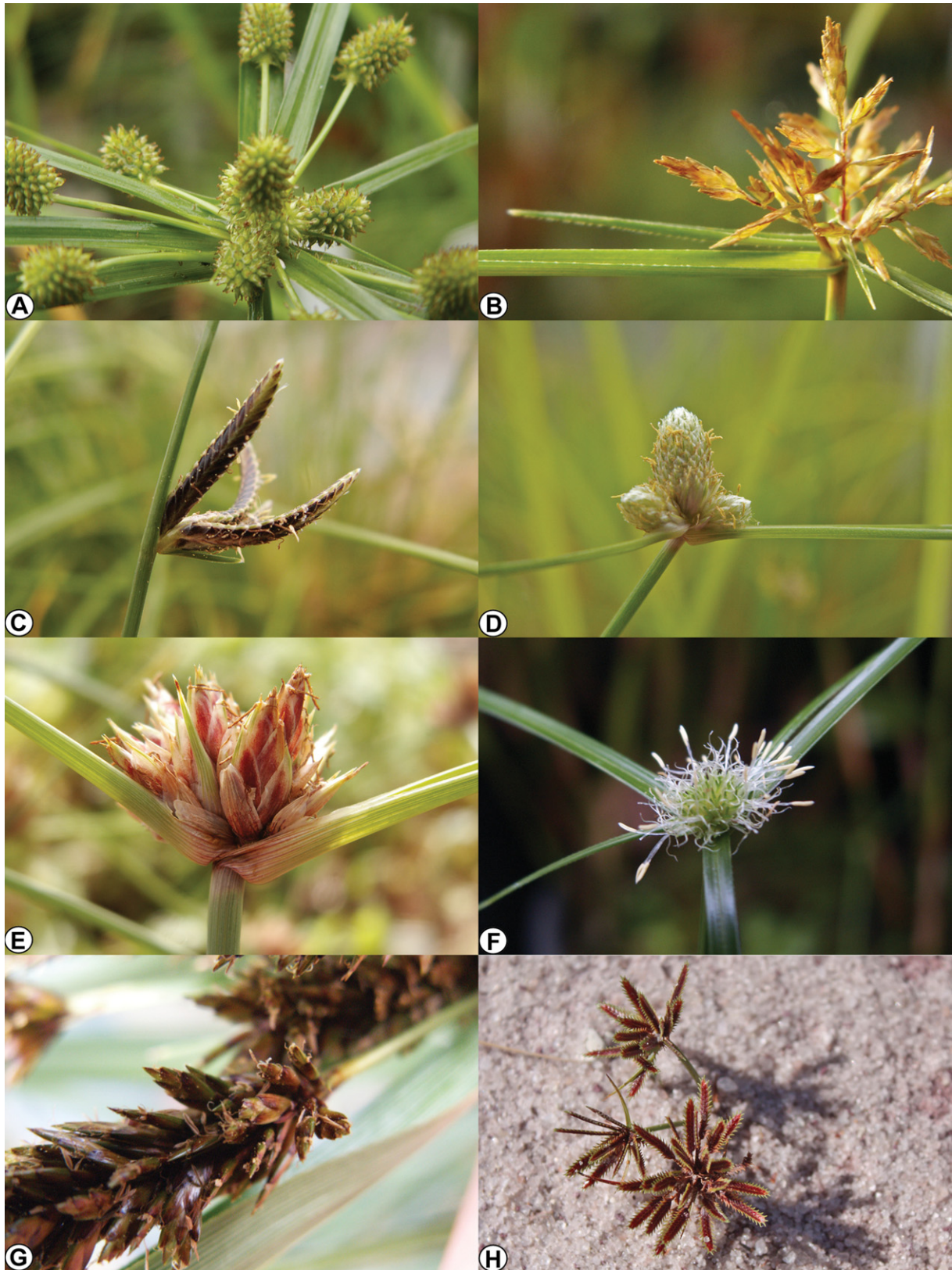
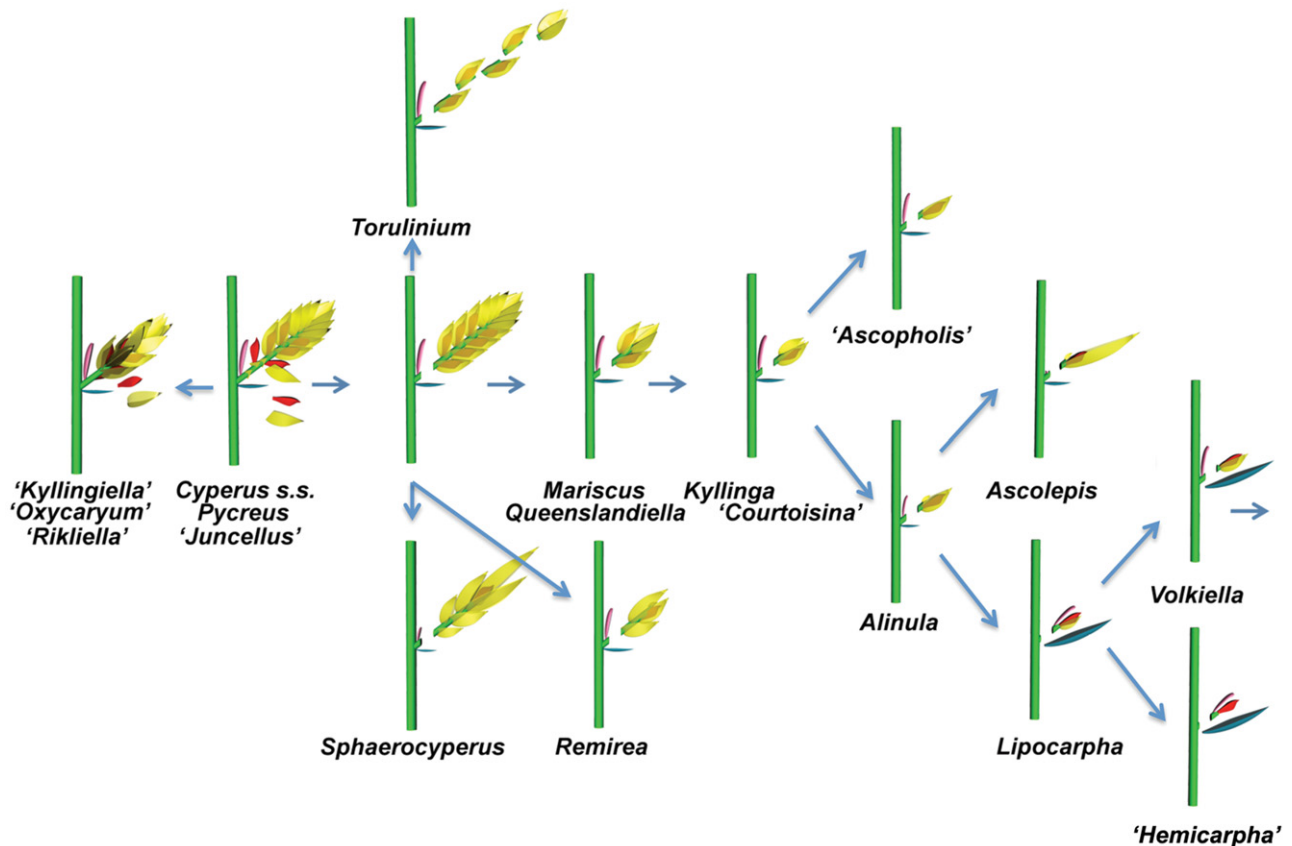


Figure 1. See caption on next page.

**Figure 1.** Morphological diversity in  $C_4$  *Cyperus*. A, *Cyperus cyperoides* (L.) Kuntze with strongly contracted spikes of spikelets in an antherlate inflorescence. B, *Pycurus polystachyos* (Rottb.) P.Beauv. with spikes of spikelets in an antherlate inflorescence. C, *Cyperus laevigatus* L. with a reduced inflorescence consisting of only a few sessile spikelets in a pseudolateral inflorescence. D, *Lipocarpa chinensis* (Osbeck) J.Kern with three sessile pseudospikelets. E, *Cyperus capitatus* with a capitulate inflorescence. F, *Kyllinga polyphylla* Willd. ex Kunth with a capitulate inflorescence of reduced, deciduous spikelets. G, *Cyperus ustulatus* A.Rich. with contracted spikes of spikelets in an antherlate inflorescence. H, *Cyperus waterloti* Cherm. with an inflorescence of digitately clustered spikelets. Photographs A–G taken by M. Reynders in the Ghent University Botanical Garden, H taken by W. Huygh at Cirque Rouge near Mahajanga, Madagascar.



**Figure 2.** Three-dimensional reconstruction of the spikelet evolution in the *Cyperus* clade. The illustrations were drawn in Rhinoceros 3D® (Mc Neel, Seattle, WA, USA) by M. Reynders. The basic *Cyperus* spikelet with distichous glumes developed several times independently into lineages with spiral glumes. In addition, deciduous spikelets originated several times and, from there, different reduction lineages can be identified resulting in single-flowered spikelets. In the extreme situation, the bracts subtending the spikelets behave like glumes bearing the strongly reduced spikelets. Difficult interpretation of the latter resulted in the classification of these taxa among various Cyperaceae tribes before their affinity with *Cyperus* had been resolved.

most taxa mentioned (such as author, place and date of publication, synonyms, distribution) follows Govaerts *et al.* (2007, 2012). The molecular phylogenetic hypothesis obtained was compared with the classification of Kükenthal (1935–36). Detailed information on the nomenclature of generic and subdivisional names of the *Cyperus* clade (including the synonymy of the names used by Kükenthal) is given in Huygh *et al.* (2010), Larridon *et al.* (2011c) and Reynders *et al.* (2011).

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, BR). The DNA extraction protocol, markers (ETS1f, *rpl32-trnL* and *trnH-psbA*) and material and methods for polymerase chain reaction (PCR) amplification and sequencing and obtaining alignments used in this study follow Larridon *et al.* (2011a).

**Table 2.** 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 (herbarium)	Origin	ETS1f	<i>trnH-psbA</i>	<i>rpl32-trnL</i>
<i>Alinula paradoxa</i> (Cherm.) Goethg. & Vorster	Reid 1027 (GENT)	South Africa	HQ705964	–	HQ705894
<i>Ascolepis brasiliensis</i> (Kunth) Benth. ex C.B. Clarke	Larridon et al. 2010-0304 (GENT)	Madagascar	HE993954	HE993894	HE993685
<i>Ascolepis eriocauloides</i> (Steud.) Nees ex Steud.	De Wilde s.n. (BR)	Congo	HE993955	HE993895	–
<i>Ascolepis hemisphaerica</i> Peter ex Goethg.	Reekmans 6729 (GENT)	Burundi	HE993956	–	–
<i>Ascolepis protea</i> Welw.	Malaisse & Kisimba 695 (GENT)	Congo	HE993957	HE993896	HE993686
<i>Ascolepis pusilla</i> Ridl.	Malaisse & Goetghebeur 846 (GENT)	Congo	HE993958	HE993897	–
<i>Cyperus alopecuroides</i> Rottb.	Hess 52/1581 (GENT)	Angola	HE993959	HE993898	HE993687
<i>Cyperus alternifolius</i> L.	Goetghebeur 11516 (GENT)	BG Ghent	HQ705948	HQ705818	HQ705878
<i>Cyperus aterrimus</i> Hochst. ex Steud.	Muasya & Ramdhani 2722 (BOL)	South Africa	HE993960	HE993899	HE993688
<i>Cyperus bulbosus</i> Vahl	Laegaard et al. 17024 (GENT)	Senegal	HE993961	HE993900	HE993689
<i>Cyperus capitatus</i> Vand.	Goetghebeur 10744 (GENT)	BG Ghent	HE993962	HE993901	HE993690
<i>Cyperus compressus</i> L.	Reynders & Sabulao 15 (GENT)	Philippines	HE993963	HE993902	HE993691
<i>Cyperus congestus</i> Vahl	Goetghebeur 11988 (GENT)	BG Ghent	HE993964	HE993903	HE993692
<i>Cyperus croceus</i> Vahl	Rostad s.n. GENT	USA	HE993965	HE993904	–
<i>Cyperus cuspidatus</i> Kunth	Jongkind & Nieuwhuis 2847 (GENT)	Ghana	HQ705954	HQ705823	HQ705884
<i>Cyperus dives</i> Delile	Muasya et al. 2529 (EA)	Kenya	HE993966	HE993905	HE993693
<i>Cyperus dubius</i> Rottb.	Muasya & Muthama 1251 (EA)	Kenya	HE993967	–	HE993694
<i>Cyperus elegans</i> L.	Goetghebeur 5601 (GENT)	Cuba	HQ705959	HQ705827	HQ705889
<i>Cyperus endlichii</i> Kük.	Muasya & Knox 954 (EA)	Tanzania	HE993968	–	–
<i>Cyperus esculentus</i> L.	Goetghebeur 11303 (GENT)	BG Nantes, BG Ghent	HQ705960	HQ705828	HQ705890
<i>Cyperus filiculmis</i> Vahl	Carter 4355 (GENT)	Florida	HE993969	HE993906	HE993695
<i>Cyperus fulgens</i> C.B. Clarke	Goetghebeur 4329 (GENT)	South Africa	HE993970	HE993907	HE993696
<i>Cyperus haspan</i> L.	Muasya & Muthama 1269 (EA)	Kenya	HQ705927	HQ705803	HQ705803
<i>Cyperus impubes</i> Steud. var. <i>fallax</i> (Cherm.) Kük.	Dhondt 9 (GENT)	Madagascar	HE993971	–	HE993697
<i>Cyperus iria</i> L.	Desmet 77/13 (GENT)	Burkina Faso	HE993972	HE993908	HE993698
<i>Cyperus javanicus</i> Houltt.	Reynders & Sabulao 60 (GENT)	Philippines	HE993973	–	HE993699
<i>Cyperus kerstenii</i> Boeck.	Muasya 984 (EA, K; Muasya et al., 2002)	Kenya	HQ705961	HQ705829	HQ705891
<i>Cyperus laevigatus</i> L. 053	Goetghebeur 10201 (GENT)	Morocco, BG Ghent	HE993975	HE993910	–
<i>Cyperus laevigatus</i> L. 138	Larridon et al. 2009-0033 (GENT)	Kenya	HE993974	HE993909	HE993700
<i>Cyperus laevigatus</i> L. 142	Goetghebeur 10202 (GENT)	Morocco, BG Ghent	HE993976	HE993911	HE993701
<i>Cyperus longus</i> L.	Farjon 217 (GENT)	Netherlands	HE993977	HE993912	HE993702
<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 meeboldii</i> Kük.	Kilian & Lobin 6848 (GENT)	Somalia	HE993978	HE993913	HE993703
<i>Cyperus meyenianus</i> Kunth	Fosberg 47227 (GENT)	Hawaii	HE993979	HE993914	HE993704

**Table 2.** *Continued*

Taxon	Voucher (herbarium)	Origin	ETS1f	<i>trnH-psbA</i>	<i>rpl32-trnL</i>
<i>Cyperus papyrus</i> L.	Goetghebeur 5866 (GENT)	BG Ghent	HQ705962	HQ705830	HQ705892
<i>Cyperus pectinatus</i> Vahl	Larridon <i>et al.</i> 2010-0265 (GENT)	Madagascar	HQ705936	HQ705810	HQ705869
<i>Cyperus pustulatus</i> Vahl	Porembski 624 (GENT)	Ivory Coast	HE993980	HE993915	HE993705
<i>Cyperus rigidifolius</i> Steud.	Samain 2005-001 (GENT)	Kenya	HE993981	HE993916	HE993706
<i>Cyperus rotundus</i> L.	Shaw 890 (K*)	Hong Kong (China)	HQ705963	HQ705831	HQ705893
<i>Cyperus rubiginosus</i> Hook.f.	Unknown <i>s.n.</i> (GENT)	Ecuador	HE993982	HE993917	HE993707
<i>Cyperus rupestris</i> Kunth	Laegaard 15909 (GENT)	Zimbabwe	HE993983	HE993918	HE993708
<i>Cyperus</i> sp.	Goetghebeur 5965 (GENT)	BG Ghent	HE993985	HE993920	HE993710
<i>Cyperus sphacelatus</i> Rottb.	Goetghebeur 4908 (GENT)	Cameroon	HE993984	HE993919	HE993709
<i>Cyperus spiralis</i> Larridon	Muasya & Muthama 1247 (EA)	Kenya	HQ705953	HQ705822	HQ705883
<i>Cyperus strigosus</i> L.	BG 20051035G (GENT)	BG Poznan, BG Ghent	HE993986	HE993921	HE993711
<i>Cyperus waterloti</i> Cherm.	Larridon <i>et al.</i> 2010-0010 (GENT)	Madagascar	HQ705955	HQ705824	HQ705885
<i>Cyperus waterloti</i> Cherm.	Larridon <i>et al.</i> 2010-0043 (GENT)	Madagascar	HQ705956	HQ705825	HQ705886
<i>Ficinia gracilis</i> Schrad.	Muasya 2713 (BOL)	South Africa	HQ705902	HQ705784	HQ705839
<i>Isolepis fluitans</i> (L.) R.Br.	Muasya & Knox 3195 (EA)	Kenya	HQ705901	HQ705783	HQ705838
<i>Kyllinga alata</i> Nees	Acocks 22902 (BR)	South Africa	HE993987	–	HE993712
<i>Kyllinga brevifolia</i> Rottb.	Reynders and Sabulao 68 (GENT)	Philippines, BG Ghent	HE993988	HE993922	HE993713
<i>Kyllinga bulbosa</i> P.Beauv.	Goetghebeur 11989 (GENT)	BG Ghent	HE993989	–	HE993714
<i>Kyllinga chlorotropis</i> Steud.	Muasya & Gerhke 2606 (EA)	Kenya	HE993990	HE993923	HE993715
<i>Kyllinga nemoralis</i> (J.R.Forst. & G.Forst.) Dandy ex Hutch. & Dalziel	Goetghebeur 11518 (GENT)	Philippines, BG Ghent	HQ705965	HQ705832	HQ705895
<i>Kyllinga odorata</i> Vahl	Strong 3485 (GENT)	USA	HE993991	HE993924	HE993716
<i>Kyllinga polyphylla</i> Willd. ex Kunth	Beeckman Z35 (GENT)	Congo	HE993992	HE993925	HE993717
<i>Kyllinga pulchella</i> Kunth	Muasya & Knox 991 (EA)	Kenya	–	HE993926	HE993718
<i>Lipocarpa albiceps</i> Ridl.	Hess 52/195 (GENT)	Angola	HE994025	HE993944	HE993748
<i>Lipocarpa chinensis</i> (Osbeck) J.Kern	Reynders & Sabulao 26A (GENT)	Philippines	HE994029	HE993948	HE993752
<i>Lipocarpa comosa</i> J.Raynal	Mincier 1027 (GENT)	Zambia	HE994028	HE993947	HE993751
<i>Lipocarpa filiformis</i> (Vahl) Kunth	Vanden Berghen 7913a (GENT)	Senegal	HE994030	HE993949	HE993753
<i>Lipocarpa kernii</i> (Raymond) Goetgh.	Laegaard 21195 (GENT)	Burkina Faso	HE994026	HE993945	HE993749
<i>Lipocarpa micrantha</i> (Vahl) G.C.Tucker	Luceño 186 (GENT)	Brazil	HE994032	HE993951	–
<i>Lipocarpa nana</i> (A.Rich.) Cherm.	Larridon <i>et al.</i> 2010-0041A (GENT)	Madagascar	HE994031	HE993950	HE993754
<i>Lipocarpa rehmannii</i> (Ridl.) Goetgh.	Larridon <i>et al.</i> 2010-0320 (GENT)	Madagascar	HE994027	HE993946	HE993750
<i>Lipocarpa salzmaniana</i> Steud.	Luceño 28 (GENT)	Brazil	HE994033	HE993952	–
<i>Pycnus africanus</i> (S.S.Hooper) Reynders	Leeuwenberg 8527 (GENT)	Congo	HE993994	HE993927	–
<i>Pycnus alleizettei</i> Cherm.	Larridon <i>et al.</i> 2010-0299 (GENT)	Madagascar	HE993993	–	HE993719
<i>Pycnus bipartitus</i> (Torr.) C.B.Clarke	Goetghebeur 11990 (GENT)	BG Ghent	HE993995	HE993928	HE993720

Table 2. Continued

Taxon	Voucher (herbarium)	Origin	ETS1f	<i>trnH-psbA</i>	<i>rpl32-trnL</i>
<i>Pycurus capillifolius</i> (A.Rich.) C.B.Clarke	Muasya & Knox 999 (EA)	Kenya	HE993996	–	HE993721
<i>Pycurus cataractarum</i> C.B.Clarke	De Wilde 1452 (GENT)	Cameroon	HE993997	–	HE993722
<i>Pycurus elegantulus</i> (Steud.) C.B.Clarke	Unknown 348 (GENT)	Kenya	HE993998	HE993929	HE993723
<i>Pycurus fibrillosus</i> (Kük.) Cherm.	Schmitz 7479 (GENT)	Congo	HE994005	–	HE993729
<i>Pycurus flavescens</i> (L.) P.Beauv. ex Rchb.	Goetghebeur 10224 (GENT)	BG Ghent	HE993999	HE993930	HE993724
<i>Pycurus flavescens</i> (L.) P.Beauv. ex Rchb. subsp. <i>microglumis</i> Lye	Malaisse & Goetghebeur 390 (GENT)	Congo	HE994000	HE993931	–
<i>Pycurus flavidus</i> (Retz.) T.Koyama (Py021)	Reynders & Sabulao 45 (GENT)	Philippines	HE994001	HE993932	HE993726
<i>Pycurus gracillimus</i> Chiov.	Lewalle 2112 (GENT)	Burundi	HE994002	HE993933	–
<i>Pycurus intactus</i> (Vahl) J.Raynal	Reid 609 (GENT)	South Africa	HE994003	–	HE993727
<i>Cyperus</i> ‘ <i>Pycurus</i> ’ <i>juncelliformis</i> Peter & Kük.	Malaisse & Goetghebeur 409 (GENT)	Congo	HE994004	–	HE993728
<i>Pycurus longistolon</i> (Peter & Kük.) Napper	Muasya & Knox 1027 (EA)	Kenya	HE994006	HE993934	HE993730
<i>Pycurus macranthus</i> (Boeck.) C.B.Clarke	Edwards 1038 (GENT)	South Africa	HE994007	–	HE993731
<i>Pycurus macrostachyos</i> (Lam.) J.Raynal	Muasya with Kirika, Obunyali & Musili 2471 (EA)	Kenya	HE994008	HE993935	HE993732
<i>Pycurus megapotamicus</i> (A.Dietr.) Nees	Goetghebeur 4826 (GENT)	Argentina	HE994009	–	HE993733
<i>Pycurus melanacme</i> Nelmes	Richards 8409 (GENT)	Congo	HE994010	HE993936	–
<i>Pycurus melas</i> (Ridl.) C.B.Clarke	Robinson 3478 (GENT)	Zambia	HE994011	–	HE993734
<i>Pycurus micromelas</i> Lye	Robinson 2310 (GENT)	Zambia	HE994012	–	HE993735
<i>Pycurus mundtii</i> Nees	Muasya & Knox 1018 (EA)	Kenya	HE994013	HE993937	HE993736
<i>Pycurus nigricans</i> (Steud.) C.B.Clarke	Unknown 368 (GENT)	Kenya	HE994014	–	HE993737
<i>Pycurus nuerensis</i> (Boeck.) S.S.Hooper	Muasya & Knox 940 (EA)	Tanzania	HE994015	HE993938	HE993738
<i>Pycurus pauper</i> (Hochst. ex A.Rich.) C.B.Clarke	Milne-Redhead & Taylor 9184 (GENT)	Tanzania	HE994016	–	HE993739
<i>Pycurus pelophilus</i> (Ridl.) C.B.Clarke	Muasya & Muthama 1263 (EA)	Kenya	HE994017	HE993939	HE993740
<i>Pycurus polystachyos</i> (Rottb.) P.Beauv.	Goetghebeur 11519 (GENT)	South Africa, BG Ghent	HQ705966	HQ705833	HQ705896
<i>Pycurus polystachyos</i> (Rottb.) P.Beauv. subsp. <i>holocericeus</i> (Link) T.Koyama	Reynders and Sabulao 64 (GENT)	BG Ghent	–	–	HE993741
<i>Pycurus pumilus</i> (L.) Nees	Muasya & Muthama 1264 (EA)	Kenya	HE994018	–	HE993742
<i>Pycurus reductus</i> Cherm. 017	Dhondt 11 (GENT)	Congo	HE994020	HE993940	HE993744
<i>Pycurus reductus</i> Cherm. 046	Larridon et al. 2010-0161 (GENT)	Madagascar	HE994019	–	HE993743
<i>Pycurus rehmannianus</i> C.B.Clarke	Muasya & Knox 1022 (EA)	Kenya	–	HE993941	HE993725



**Table 2.** *Continued*

Taxon	Voucher (herbarium)	Origin	ETS1f	<i>trnH-psbA</i>	<i>rpl32-trnL</i>
<i>Pycreus rhizomatosus</i> C.B.Clarke	<i>Gereau &amp; Dumetz 3259</i> (GENT)	Madagascar	HE994021	HE993942	–
<i>Pycreus sanguinolentus</i> (Vahl) Nees	<i>Kwika &amp; Mundi 21</i> (GENT)	Kenya	HE994022	–	HE993745
<i>Pycreus smithianus</i> (Ridl.) C.B.Clarke	<i>Reekmans 7531</i> (GENT)	Burundi	HE994023	HE993943	HE993746
<i>Pycreus xantholepis</i> Nelmes	<i>Reekmans 9809</i> (GENT)	Burundi	HE994024	–	HE993747
<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
<i>Volkiella disticha</i> Merxm. & Czech	<i>Müller &amp; Giess 493</i> (GENT)	Namibia	HE994034	HE993953	HE993755

Alignments are available from the first author on request.

Phylogenetic hypotheses were produced using maximum likelihood (ML) and Bayesian inference (BI) analyses. All analyses were first performed on the single-marker datasets (ETS1f, *rpl32-trnL*, *trnH-psbA*). As no conflicting clades with a significant confidence value were revealed, a combined dataset was constructed and analysed. The latter was subdivided into three partitions, corresponding to the single markers. The program RAxML v7.2.8 (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 dataset.

Bayesian phylogenetic (BI) analyses were carried out in MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003). For the analysis, MrModeltest v2.3 (Nylander, 2004) was used to determine the model that best fitted the data, applying the Akaike Information Criterion. For the combined dataset, a model was determined for each partition. This method is referred to as the BI method. Four independent, parallel runs of one cold and three heated chains were run for 30 million generations each. Trees and parameter estimates were saved every 1000 generations. The analyses were run on a high-performance computer at Ghent University (Stevin Supercomputer Infrastructure, ICT Department). Convergence, associated likelihood values, effective sample size (ESS) values and burn-in values of the different runs were verified with Tracer v1.5 (Rambaut & Drummond, 2007). Calcula-

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

## RESULTS

### SEQUENCE ALIGNMENTS

After alignment and application of Gblocks v0.91b (Castresana, 2000), the ETS1f alignment included 105 sequences of 953 bases, the *rpl32-trnL* alignment 94 sequences of 1334 bases and the *trnH-psbA* alignment 81 sequences of 1364 bases. The concatenated dataset included 108 sequences and the Gblocks program retained 57%, or 2101 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 greater support in the multi-locus ML analysis. In the various analyses, only minor conflicts concerning the position of some *C<sub>4</sub> Cyperus* spp. in the backbone of the *C<sub>4</sub> Cyperus* clade were detected. Most nodes in the backbone of this clade had little or no support.

The three single-locus BI analyses did not differ significantly in tree topologies. The multi-locus BI topologies did not differ from the multi-locus ML tree, except for some of the *C<sub>4</sub> Cyperus* spp. in the main polytomy, as mentioned above for the ML analyses.

Evaluation of the multi-locus BI analysis output showed that the four runs converged on similar log likelihood (−23 908) and parameter values. The burn-in value for all runs was determined at three million generations. ESS for the likelihood value of the combined runs consisted of 1397.38 uncorrelated samples.

Figure 3 shows the 50% majority consensus multi-locus BI tree with the associated PP values and the bootstrap values of the multi-locus ML tree. Only bootstrap values above 75% and PPs above 0.85 are shown.

## DISCUSSION

### AFFINITIES WITH C<sub>3</sub> CYPERUS

In Cyperaceae, the *Cyperus* clade is sister to the *Ficinia* clade, here used as outgroup represented by species of *Scirpoides*, *Isolepis* and *Ficinia* (Fig. 3). The *Cyperus* clade is strongly supported as monophyletic, but includes several lineages which are currently recognized at the generic level. As in Larridon *et al.* (2011a), C<sub>3</sub> *Cyperus* spp. form a grade at the base of *Cyperus* (Fig. 3; Table 3). The clade sister to the C<sub>4</sub> *Cyperus* clade is formed by *Cyperus* section *Leucocephali* Cherm. ex Kük. *sensu* Larridon *et al.* (2011b) (Fig. 3). Although the species of this section use C<sub>3</sub> photosynthesis (e.g. Bruhl & Wilson, 2007; Larridon *et al.*, 2011a), they occur in open grassland habitats which are generally dominated by species using C<sub>4</sub> photosynthesis. This suggests that the species of *Cyperus* section *Leucocephali* have characters (e.g. geophytic hemicryptophytes, resprouting immediately at the start of the wet season and dying back on onset of the dry season, photosynthesis at high temperatures and irradiation) which make them fitter to survive in these habitats than most other C<sub>3</sub> *Cyperus* spp.

### C<sub>4</sub> CYPERUS RADIATION

Our molecular phylogenetic hypothesis shows very short branch lengths for most of the C<sub>4</sub> *Cyperus* clade when compared with the C<sub>3</sub> *Cyperus* grade and the deepest nodes of the C<sub>4</sub> *Cyperus* clade, suggesting a rapid diversification of the clade. Endress (2011: 370) wrote: 'Many structural innovations originated in several clades [of angiosperms] and in special cases could become key innovations, which likely were hotspots of diversification'. The evolution of C<sub>4</sub> photosynthesis in Cyperaceae can be considered as a key innovation, being the cause of a burst of speciation as a result of: (1) increased fitness in drier habitats (Besnard *et al.*, 2009); (2) optimized nitrogen uptake; and (3) improved resistance to higher irradiance, fire and chemical stress caused by salt and heavy metals

(Li, Wedin & Tieszen, 1999; Stock, Chuba & Verboom, 2004). Based on our results and on literature and herbarium data on the distribution of species, we hypothesize that the evolution of the C<sub>4</sub> photosynthetic pathway in Cyperaceae occurred in East Africa. This region, particularly present-day Tanzania, is the centre of diversity for C<sub>4</sub> *Cyperus* spp. In addition, all segregate lineages and most sections are represented in the East African flora. Outside Africa, the *Cyperus* clade is either represented by widespread species or by taxa which evolved locally as a result of smaller radiations originating from dispersal events.

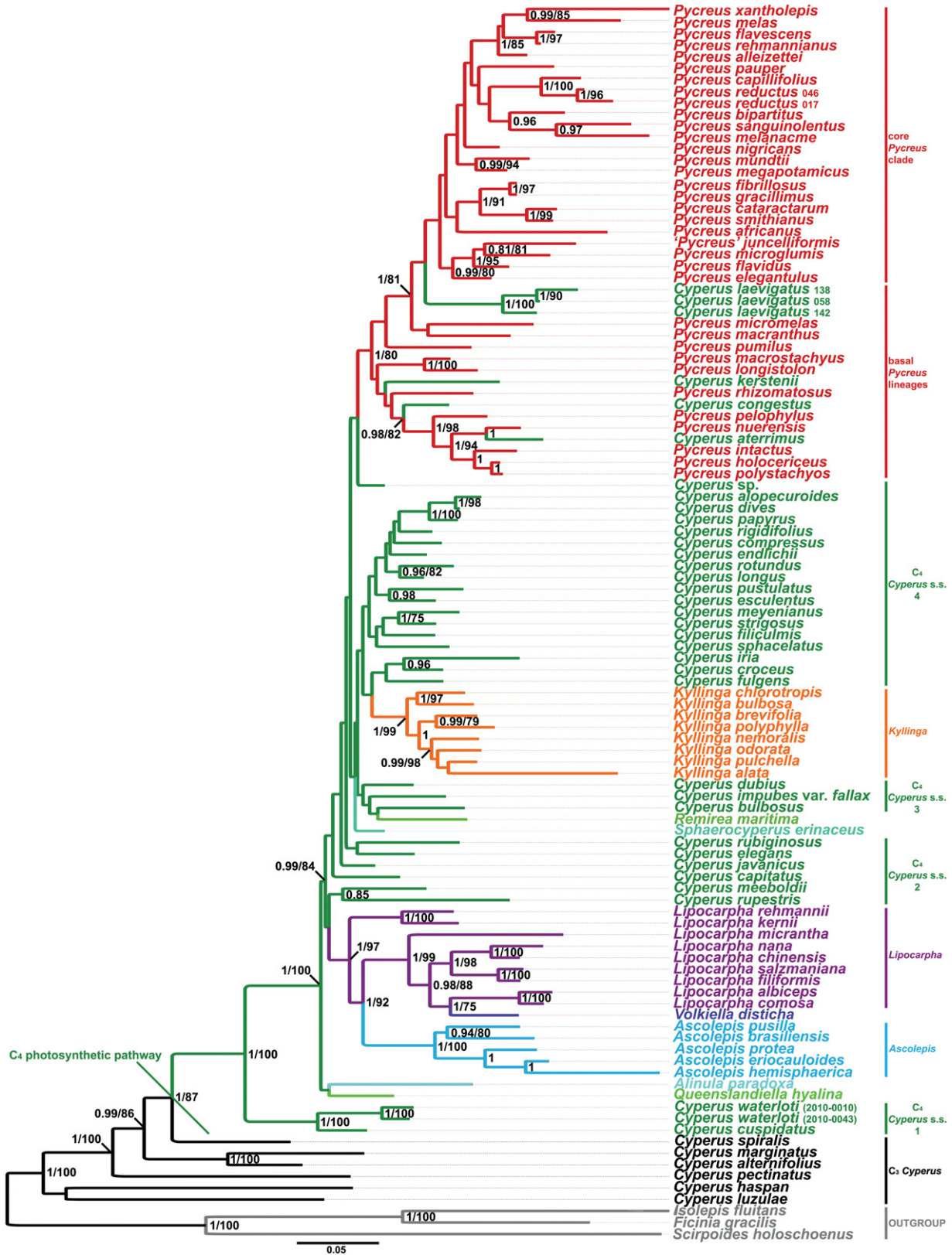
### AFFINITIES IN C<sub>4</sub> CYPERUS

#### *The basal nodes*

Several early branches of the C<sub>4</sub> *Cyperus* clade are strongly supported (Fig. 3). The first subclade, also retrieved in previous studies (e.g. Muasya *et al.*, 2002, in press; Larridon *et al.*, 2011a), is represented by *Cyperus cuspidatus* Kunth (and its Malagasy relative *C. waterlotii* Cherm.). Kükenthal (1935–36) placed the species of this clade in *Cyperus* section *Amabiles* C.B. Clarke. Although homogeneous, this section is only held together by characters which probably represent the plesiomorphic condition in C<sub>4</sub> *Cyperus*, such as spikelets arranged in digitate clusters (as in many C<sub>3</sub> *Cyperus* spp. vs. generally spikes of spikelets in C<sub>4</sub> *Cyperus*) and multi-nerved glumes with an excurrent mucro. Species of *Cyperus* sections *Amabiles*, *Aristati* Nees and *Rupestres* C.B. Clarke show similar characters. After the *C. cuspidatus* clade, the next branches of our molecular phylogenetic hypothesis include species of the segregates *Alinula* J. Raynal, *Ascolepis* Nees, *Lipocarpha* R.Br., *Queenslandiella* Domin and *Volkiella* Merxm. & Czech, and of *Cyperus* section *Rupestres* (i.e. *C. rupestris* Kunth and *C. meeboldii* Kük.). The relationship between the two species of *Cyperus* section *Rupestres* is strongly supported in our analysis. Taxonomically, this section is well circumscribed by several synapomorphies, such as swollen stem bases and a tendency to reduced flowers, each with a single stigma branch and a single stamen. The exact position of its corresponding clade remains to be confirmed, but its position among the early branches of the C<sub>4</sub> *Cyperus* clade seems acceptable.

#### *Queenslandiella*

The monotypic *Queenslandiella* is currently recognized as a separate genus, based on its laterally flattened, dimerous gynoecea and its deciduous spikelets. *Queenslandiella* has multi-nerved glumes with an excurrent mucro, suggesting that it is an early branching lineage of C<sub>4</sub> *Cyperus* (Fig. 3). When dried, it has a strong curry odour, a character it shares with



**Figure 3.** Phylogenetic hypothesis for the *Cyperus* clade: 50% majority consensus multi-locus Bayesian inference (BI) tree with the associated posterior probability (PP) values and the bootstrap values of the multi-locus maximum likelihood (ML) tree. Only bootstrap values > 75% and posterior probabilities > 85% are shown.

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**Table 3.** C<sub>3</sub> *Cyperus* species included in the phylogeny and the sections they represent

Species	Section
<i>Cyperus haspan</i>	<i>Cyperus</i> section <i>Haspani</i> (Kunth) C.B. Clarke
<i>Cyperus luzulae</i>	<i>Cyperus</i> section <i>Luzuloidei</i> (Kunth) C.B. Clarke
<i>Cyperus pectinatus</i>	<i>Cyperus</i> section <i>Anosporum</i> (Nees) Pax
<i>Cyperus alternifolius</i> and <i>C. marginatus</i>	<i>Cyperus</i> section <i>Alternifolii</i> (Kunth) C.B. Clarke
<i>Cyperus spiralis</i>	<i>Cyperus</i> section <i>Leucocephali</i> Cherm. ex Kük.

*C. squarrosus* L., another species showing many of the presumed plesiomorphic characters of the clade. *Cyperus squarrosus* falls among the basal nodes in an internal transcribed spacer (ITS) analysis of *Cyperus* (C.S. Reid, Louisiana Department of Wildlife and Fisheries, Baton Rouge, unpubl. data).

#### *Alinula*

According to the current circumscription, *Alinula* includes four species (Goetghebeur, 1998; Govaerts *et al.*, 2012). Only one species, *Alinula paradoxa* (Cherm.) Goetgh. & Vorster, is included in this study (Fig. 3). From a morphological point of view, this species differs significantly from the other three. In our opinion, the current circumscription of *Alinula* does not represent a natural group. Haines & Lye (1983), who treated *Alinula* as a subgenus in *Cyperus*, included *A. paradoxa* in *Cyperus* subgenus *Fimbricyperus* Lye separate from the other *Alinula* spp. In our molecular phylogenetic hypothesis, *A. paradoxa* clusters among the early branches of the C<sub>4</sub> *Cyperus* clade. More research is needed to reveal its exact relationships. *Alinula lipocarphioides* (Kük.) J.Raynal has been shown to be closer to *Lipocarpha* (Muasya *et al.*, 2009a, in press).

#### *Ascolepis–Lipocarpha* clade

A well-supported clade in our molecular phylogenetic hypothesis includes the genera *Ascolepis*, *Lipocarpha* and *Volkiella* (Fig. 3), which are all characterized by strongly reduced deciduous spikelets grouped into pseudospikelets (spikes of spikelets). Our results confirm that *Ascolepis* and *Lipocarpha* are closely related, as already observed by Muasya *et al.* (2002). Their relatively early branching position in C<sub>4</sub> *Cyperus* is corroborated by the presence of a small, weakly differentiated *Cyperus*-type embryo, which is also common in C<sub>3</sub> *Cyperus* and in the early branches of the *Ficinia* clade (M. Reynders, Ghent

University, Gent, unpubl. data). *Lipocarpha* appears to be paraphyletic, including *Ascolepis* and *Volkiella* (Fig. 3). The first diverging branch is formed by *Lipocarpha kernii* (Raymond) Goetgh. and *L. rehmannii* (Ridl.) Goetgh. (Fig. 3), formerly placed in a separate genus *Rikliella* J.Raynal. Although these species strongly resemble *Lipocarpha*, prophyll and glumes have not been observed around the flower. Therefore, Goetghebeur & Van den Borre (1989) interpreted *Rikliella* as a highly evolved lineage of *Lipocarpha*. However, on the basis of our phylogenetic trees, it is unclear whether the partial inflorescences should be interpreted as pseudospikelets or as true spikelets with spiral glumes (which occur in at least three other lineages of the *Cyperus* clade; Muasya *et al.*, in press). Sister to this clade is a clade comprising *Ascolepis* and *Lipocarpha s.s.* (Fig. 3). *Ascolepis* spikelets are characterized by a single large glume subtending a flower and the loss of the spikelet prophyll. In *Lipocarpha s.s.*, the first branching clade is formed by *L. micrantha* (Vahl) G.C.Tucker (Fig. 3), which is characterized by a reduction of the glume. This clade is followed by the rest of *Lipocarpha s.s.*, which also includes the monotypic *Volkiella* (Fig. 3). *Volkiella* possesses both a spikelet prophyll and a glume, and is included in a subclade with *L. albiceps* Ridl. and *L. comosa* J. Raynal (Fig. 3). These two *Lipocarpha* spp. are characterized by a well-developed, firm and often dark-coloured prophyll which falls off the rachis separately from the flower and its glume. In other *Lipocarpha* spp., the prophyll is hyaline and falls off together with the nutlet and glume. *Volkiella* shares the more rigid prophyll with the two above-mentioned species. *Volkiella disticha* Merxm. & Czech is, in many aspects, a special, highly derived species differing from *Lipocarpha* by the distichous arrangement of the spikelets on the rachis. A more elaborate study of *Lipocarpha*, integrating molecular phylogeny and morphology, will be presented in another paper (K. Bauters *et al.*, Ghent University, Gent, unpubl. data).

#### *The hard polytomy*

The vast majority of C<sub>4</sub> *Cyperus* spp. are included in an unresolved polytomy (Fig. 3), which can also be found in all previous molecular phylogenetic studies (e.g. Muasya *et al.*, 2002, 2009a, b). As it has not been possible to resolve this polytomy, even when using fast mutating plastid and nuclear markers, additional markers need to be tested, as well as other techniques based on next-generation sequencing (e.g. Harrison & Kidner, 2011). However, in our molecular phylogenetic study, several subclades and the relationships between some taxa are strongly supported (Fig. 3). These taxa are discussed below.

**Table 4.** Comparison between the noncore *Pycurus* species and the core *Pycurus* clade (with the exception of *Cyperus laevigatus*)

Character	Noncore species	Core <i>Pycurus</i> clade
Glumes	Multi-nerved	Midrib with only three nerves
Mucro	Usually present, excurrent	Not present or rarely shortly excurrent
Anthela	Well-developed with long and narrow spikelets, often with second-order branches	Often condensed or reduced, especially in therophytic species
Nutlet epidermal cells	Isodiametric	Isodiametric to strongly elongate
Ecology	Mostly opportunistic and lowland concentrated	Often very specialized
Distribution and habitat	Widespread and common on roadsides and rice fields	Narrow distribution, occurring in high-altitude bogs, salt marshes, floating on open water, etc.

#### *C<sub>4</sub> Cyperus s.s.*

One subclade of *C<sub>4</sub> Cyperus s.s.* which is strongly supported in our molecular phylogenetic hypothesis (Fig. 3) contains species belonging to *Cyperus* sections *Papyrus* (Willd.) Thouars (*C. papyrus* L., *C. dives* Delile, *C. alopecuroides* Rottb.) and *Rotundi* C.B. Clarke (*C. rotundus* L., *C. longus* L., *C. endlichii* Kük., *C. rigidifolius* Steud.). These species are all characterized by a narrowly to broadly winged rachilla with deciduous or persistent wings. Several other sections which are not represented in the current analysis, i.e. *Cyperus* sections *Brevifoliati* C.B. Clarke, *Exaltati* (Kunth) C.B. Clarke and *Fastigiatii* Kük., share these characters. *Cyperus compressus* L. (*Cyperus* section *Compressi* Nees) also clusters in this clade (Fig. 3).

#### *Kyllinga*

*Kyllinga* Rottb. forms a strongly supported monophyletic clade (Fig. 3). There is weak support for the *Kyllinga* clade as sister to a clade including *C. iria* L., *C. croceus* Vahl and *C. fulgens* C.B. Clarke. *Kyllinga* is delimited by the combination of a head-like inflorescence, deciduous spikelets and laterally flattened gynoecea. Three subclades can be recognized in the current molecular phylogenetic hypothesis (Fig. 3). A detailed molecular phylogenetic study of *Kyllinga*, including amplified fragment length polymorphism (AFLP) data, is being prepared (W. Huygh *et al.*, Ghent University, Gent unpubl. data).

#### *Remirea* and *Sphaerocyperus*

The monotypic genera *Remirea* Aubl. and *Sphaerocyperus* Lye remain unresolved in *C<sub>4</sub> Cyperus* (Fig. 3). Both taxa are characterized by a series of empty scales below the flower-bearing glume. For this reason, affinities with Schoeneae or Rhynchosporae have been suggested (Fenzl, 1836: 144; Bentham, 1883: 1038; Ridley, 1884: 165; Pax, 1888: 116; Baillon, 1894: 377; Clarke, 1901–02: 267; Kükenthal, 1944: 200–209). In addition, *Remirea* has corky rachilla internodes.

#### *Pycurus*

*Pycurus* P.Beauv. is here retrieved as a paraphyletic entity including several *Cyperus* spp. (Fig. 3). In *Pycurus*, relationships are poorly resolved, although good resolution is obtained for some smaller clades of related species. Furthermore, one large clade is well supported and contains the majority of the sections and species in addition to *C. laevigatus* L. (Fig. 3). This clade is referred to as the 'core *Pycurus* clade'.

The *Pycurus* species which are not included in the core *Pycurus* clade all belong to four of Kükenthal's (1935–36) sections, namely *Cyperus* section *Albomarginati* Kük., *Cyperus* section *Lancei* Kük., nom. superfl., *Cyperus* section *Polystachyi* (C.B. Clarke) Kük., nom. illeg., *Cyperus* section *Pumili* Kük. and *Cyperus* section *Rhizomatosi* Kük. Their mutual relationships remain unresolved, but their position outside the core *Pycurus* clade can be justified as the species in these sections possess plesiomorphic characters in contrast with the species in the core *Pycurus* clade (Table 4).

Among the early branching lineages, two smaller clades are well supported (Fig. 3). *Pycurus longistolon* (Peter & Kük.) Napper and *P. macrostachyos* (Lam.) J. Raynal are strongly supported together. Kükenthal (1935–36) classified *P. longistolon* in *Cyperus* section *Lancei*, nom. superfl., a section which appears to be artificial as the species only share rather large and dark glumes. *Pycurus macrostachyos* was included in *Cyperus* section *Albomarginati* [as *C. albomarginatus* (Mart. & Schrad. ex Nees) Steud.]. The inclusion of *P. longistolon* in *Cyperus* section *Albomarginati* seems to be appropriate in view of the overall habit of the plants (except for the stolons), the large dimensions of the spikelets, glumes and nutlets, and the wide, hyaline glume margins. However, the last character is less conspicuous than in *P. macrostachyos*.

Another well-resolved subclade corresponds to *Cyperus* section *Polystachyi*, nom. illeg., and is characterized by typically elongated nutlets and a winged rachilla. *Pycurus pelophilus* C.B. Clarke is an excep-

tion in having broad nutlets. Nevertheless, it was placed in this section by Kükenthal (1935–36) and this relationship is confirmed here (Fig. 3).

The strongly supported inclusion of *C. aterrimus* (Fig. 3) in the early branching lineages of *Pycreus* is noteworthy as this species has triangular nutlets, a different inflorescence and overall larger dimensions of the glumes and nutlets compared with *Pycreus*. *Cyperus aterrimus* Hochst. ex Steud. is strongly supported as sister to *P. nuerensis* (Boeckeler) S.S.Hooper, which it resembles in its growth form, dark-coloured inflorescence and Afromontane distribution. *Cyperus kerstenii* Boeckeler and *C. congestus* Vahl also appear to be associated with the early branching *Pycreus* lineages, although without support. We found no morphological characteristics to support this relationship, especially as both species have deciduous glumes, a character which does not occur in *Pycreus*. The presence of species with triangular nutlets in *Pycreus* suggests a reversion of the dimerization of the gynoecium. Recently, Vrijdaghs (2006) and Reynders *et al.* (2012) showed that gynoecia in Cyperoideae originate from an annular primordium on which stigma primordia originate. This offers more flexibility for the positioning of stigma branches with respect to the restrictions previously assumed based on the anatomical studies by Blaser (1941a, b).

The core *Pycreus* clade only includes *Pycreus* spp., except for *C. juncelliformis* Peter & Kük. and *C. laevigatus*. *Cyperus juncelliformis* is a true *Pycreus*, but its name has never been combined into *Pycreus*. Therefore, its name is mentioned as '*Pycreus*' *juncelliformis* in Figure 3. The association of *C. laevigatus* with the core *Pycreus* clade seems to be strong. It was verified by including three separate samples of *C. laevigatus*, and this relationship also occurred in the analyses of the three markers separately (M. Reynders, Ghent University, Gent, unpubl. data). Whereas *Pycreus* is characterized by laterally flattened dimerous gynoecia, *C. laevigatus* has dorsiventrally flattened dimerous gynoecia. This might represent either an intermediate state between a trimerous *Cyperus* ancestor and *Pycreus* or a derived state from a *Pycreus* ancestor. Moreover, the vascularization pattern in the rachilla of *C. laevigatus* differs from the pattern in rachillas of several *Pycreus* spp. studied by Vrijdaghs *et al.* (2011). Shared characters of *C. laevigatus* and *Pycreus* are the rather glossy glumes and their ecology.

ETS1f sequences of the species in the core *Pycreus* clade (except *C. laevigatus*) show a large duplication of 140 bp, which is a strong additional argument that this represents a natural group. In the core *Pycreus* clade, several species clusters are resolved

(Fig. 3). *Pycreus flavidus* (Retz.) T.Koyama clusters with '*Pycreus*' *juncelliformis*, corresponding to Kükenthal's (1935–36) *Cyperus* section *Globosi* (C.B.Clarke) Kük. The inclusion of *P. niger* (Ruiz & Pav.) Cufod. is morphologically supported by the similar nutlets and the shape of the glumes. In contrast, the inclusion of *P. flavescens* (L.) P.Beauv. ex Rchb. ssp. *microglumis* Lye is remarkable and needs further investigation. Morphologically, the species cluster of *P. capillifolius* (A.Rich.) C.B.Clarke and *P. reductus* Cherm. shows resemblances to *Cyperus* section *Globosi*, but this relationship remains unresolved in the current study. In addition, species of *Cyperus* section *Sulcati* Kük., nom. illeg., are distributed between two clades, although the species of this section all share peculiar glumes with a furrow on both sides. *Pycreus sanguinolentus* (Vahl) Nees and *P. bipartitus* (Torr.) C.B.Clarke are smaller representatives of this section, whereas *P. mundtii* Nees and *P. megapotamicus* (A.Dietr.) Nees are taller plants with long culms with spaced leaves that form floating mats on open water. The clustering of *P. melanacme* Nelmes with this section needs further investigation, as this is, in many ways, a rather distinct therophytic species.

*Pycreus africanus* (S.S.Hooper) Reynders, *P. smithianus* (Ridl.) C.B.Clarke, *P. cataractarum* C.B.Clarke, *P. fibrillosus* (Kük.) Cherm. and *P. gracillimus* Chiov. form a well-resolved clade. *Pycreus africanus* belongs to *Pycreus* section *Tuberculati* Cherm. (Reynders & Goetghebeur, 2010). *Pycreus smithianus* and *P. cataractarum* share many characters, such as a contracted inflorescence, straight rachilla, bright white glumes and a Guineo-Congolese distribution, with a preference for habitats by running water. Kükenthal (1935–36) included both species in *Cyperus* section *Propinqui* (C.B.Clarke) Kük. *Pycreus fibrillosus* and *P. gracillimus* both have a plant base covered with fibrous remains of old leaf sheaths, an inflorescence reduced to only a few spikelets, a flexuous rachilla and a Zambesian distribution in Afromontane habitats. These species were placed in *Cyperus* section *Propinqui* and *Cyperus* section *Latespicati* Kük., respectively, by Kükenthal (1935–36) based on their pale vs. dark glumes. As this character seems to depend on altitude (many species of *Cyperus s.l.* growing above 2000 m have dark-coloured glumes), it is not considered as reliable for sectional delimitation. Therefore, these two sections are likely to be polyphyletic.

A final strongly supported clade contains species belonging to *Cyperus* section *Latespicati* (*P. alleizettei* Cherm.) and *Cyperus* section *Flavescentes* Kük. (*P. flavescens*, *P. rehmanianus* C.B.Clarke) *sensu* Kükenthal (1935–36). *Pycreus xantholepis* Nelmes, a tall therophyte, shares its yellow glume colour and

nutlet shape with *P. alleizettei* and *P. flavescens*. The inclusion of *P. melas* (Ridl.) C.B. Clarke [Cyperus section *Globosi*] needs further investigation, as this species is morphologically distinct.

#### RE-EVALUATION OF THE GENERIC STATUS OF THE SEGREGATE LINEAGES

In this section of the paper, we re-evaluate the generic status of the segregate lineages based on the currently available knowledge about these taxa. This is a combination of morphological, anatomical, ontogenetic and embryographical data, and the results of previous and current molecular phylogenetic studies.

##### *Alinula*

Goetghebeur & Vorster (1988) included four species in this genus. A species from eastern Africa was originally described as *Ficinia lipocarphioides* Kük. based on the presence of a hypogynous disc around the base of the fruit. However, after studying its inflorescence morphology and chlorocyperoid anatomy, Raynal (1973) hypothesized that the species was intermediate between *Ascolepis* and *Mariscus* Vahl, and eventually placed it in a new genus *Alinula* (Raynal, 1977). Three more species were added to *Alinula*, after a complex taxonomical trajectory (e.g. Goetghebeur, 1977; Goetghebeur & Vorster, 1988; Haines & Lye, 1983). In our opinion, the current circumscription of *Alinula* does not represent a natural group, although *A. lipocarphioides*, *A. malawica* (J. Raynal) Goetgh. & Vorster and *A. peteri* (Kük.) Goetgh. & Vorster show clear morphological affinities, such as the presence of pseudospikelets. However, as pseudospikelets also occur in other, more distantly related taxa of Cyperaceae (e.g. *Ascolepis* and *Lipocarpha*), their presence is, in our opinion, insufficient for generic delimitation. Moreover, *A. lipocarphioides* has been shown to be nested in the *Lipocarpha* clade (Muasya *et al.*, in press).

##### *Ascolepis*

The head-like inflorescence of *Ascolepis* consists of clusters of single-flowered spikelets, sometimes with a rudimentary second glume. Typically, the spikelet prophyll does not develop, but the only glume subtending the single flower is always well developed and larger than the bract which subtends the spikelet. In other species, the glume encloses the flower completely and wings are often developed, possibly for wind dispersal. In other species, the glume is strongly elongated and/or brightly coloured, which gives the inflorescence heads an Asteraceae-like appearance (e.g. as in *Ascolepis protea* Welw.), suggesting insect pollination. Raynal (1973) postulated the origin of *Ascolepis* from a mariscoid ancestor. However,

Goetghebeur (1980) argued that, although glume and nutlet are shed together in *Ascolepis*, the rachilla remains fixed on the rachis in contrast with *Mariscus*. *Mariscus* was an artificial genus grouping together members of Cyperaceae with deciduous spikelets. Our results concur with Muasya *et al.* (2002) in resolving *Ascolepis* and *Lipocarpha* as sister taxa. Morphological differentiation in these two taxa shows that similar functional inflorescences originated in both groups using different organs (e.g. *As. protea* vs. *L. comosa*). In *Lipocarpha*, the spikelet bract is strongly developed, whereas the glume subtending the flower is reduced. In *Ascolepis*, the spikelet bract is rudimentary, whereas the glume subtending the flower is strongly developed. Because of the morphological diversity of the inflorescence, rachilla and glumes among the different subgroups in *Ascolepis*, Goetghebeur (1986) considered the possibility that *Ascolepis* is a complex of convergent lineages which developed a similar inflorescence Bauplan. A more thorough molecular investigation of *Ascolepis* is needed to test the monophyly of this taxon.

##### *Kyllinga*

*Kyllinga* is characterized by the combination of laterally flattened gynoecea, deciduous spikelets with a reduced number of flowers and capitate inflorescences. The close relationship of *Kyllinga* with *Cyperus* has always been acknowledged, and various authors have treated *Kyllinga* at the subgeneric level in *Cyperus* (e.g. Kükenthal, 1935–36; Haines & Lye, 1983). However, *Kyllinga* has always been considered as a homogeneous, natural entity, as illustrated by the fact that several authors have maintained *Kyllinga* as a separate genus whilst lumping *Mariscus*, *Pycneus*, *Torulinium* Desv. ex Ham. and *Juncellus* C.B. Clarke in *Cyperus* (Lye, 1972, 1982; Tucker, 1983). The monophyly of *Kyllinga* is confirmed by our results, where it is retrieved as a strongly supported clade (Fig. 3). As (1) *Kyllinga* is nested in C<sub>4</sub> *Cyperus*, (2) capitate inflorescences with reduced, deciduous spikelets (i.e. pseudospikelets) are encountered in various lineages in C<sub>4</sub> *Cyperus*, such as *Cyperus* section *Bulbocaulis* (C.B. Clarke) Kük., *Ascolepis*, *Lipocarpha* and *Remirea*, and (3) laterally flattened gynoecea also occur in *Pycneus* and *Queenslandiella*, which are not immediately related, there are, in our opinion, no sufficient arguments to warrant generic status for *Kyllinga*.

##### *Lipocarpha*

*Lipocarpha* spp. generally have a highly specialized inflorescence consisting of a spike of highly reduced spikelets, with each spikelet, subtended by a bract, containing an abaxial prophyll and an adaxial glume subtending the flower. A few *Lipocarpha* spp. have

lost the glume subtending the flower, although some rudiments of it remain visible (Goetghebeur & Van den Borre, 1989). These species were originally classified in a separate genus *Hemicarpha*, based on the reduction of the glume and the presence of a pseudolateral inflorescence (Nees von Esenbeck, 1834). As the type species of *Hemicarpha* Nees, *H. isolepis* Nees [accepted name: *L. hemisphaerica* (Roth) Goetgh.], does not show this reduction, *Hemicarpha* was synonymized with *Lipocarpha* (Goetghebeur & Van den Borre, 1989). *Lipocarpha micrantha*, which belongs to this group, is sister to all other *Lipocarpha* spp. studied, including *Volkiella*.

Haines & Lye (1971, 1983) and Goetghebeur & Van den Borre (1989) considered *Rikliella* to represent a final reduction step of a *Lipocarpha* spikelet, in which the spikelet prophyll and glume subtending the flower are lost, resulting in a perfect pseudospikelet with flowers in the axil of the spikelet bracts. *Hemicarpha* was indicated as the transitional stage between *Lipocarpha* and *Rikliella*. *Hemicarpha* and *Rikliella* are no longer recognized at the generic level (Goetghebeur & Van den Borre, 1989; Govaerts *et al.*, 2012). Our results place the two species of *Rikliella* (*L. rehmannii* and *L. kernii*) on a separate, strongly supported branch, and not as a specialized lineage of *Lipocarpha*. This questions previous interpretations of its inflorescence Bauplan.

As in *Alinula*, *Ascolepis* and *Kyllinga*, we do not consider the presence of pseudospikelets sufficient to warrant generic status for *Lipocarpha*. Furthermore, in this study, *Lipocarpha* is found to be paraphyletic, containing *Ascolepis* and *Volkiella*. A more detailed study of *Lipocarpha* and *Rikliella* will be published elsewhere (K. Bauters *et al.*, Ghent University, Gent, unpubl. data).

### *Pycreus*

*Pycreus* is the largest segregate genus in C<sub>4</sub> *Cyperus*. Furthermore, it is morphologically and ecologically diverse. The close relationship between *Cyperus* and *Pycreus* has never been doubted, as *Pycreus* only differs from *Cyperus* s.s. in its laterally flattened gynoecia. These gynoecia also occur in *Kyllinga* and *Queenslandiella*, which, in contrast with *Pycreus*, also have deciduous spikelets. The generic status of these taxa has always been controversial, and their status strongly correlated with the taxonomic value granted to laterally flattened gynoecia. From our results, it is evident that taxa with laterally flattened gynoecia are not sister groups, and *Kyllinga* is strongly supported as a separate entity. Therefore, we can conclude that there have been multiple independent origins of lateral gynoecia in Cyperaceae.

Our current molecular phylogenetic study includes species representing all 13 sections of Kükenthal

(1935–36). Although relationships between the different sections remain poorly resolved, several patterns require further attention. *Pycreus* is not monophyletic, as species that Kükenthal (1935–36) included in *Cyperus* sections *Albomarginati*, *Polystachyi*, nom. illeg., *Pumili* and *Rhizomatosi* are found in the main C<sub>4</sub> *Cyperus* polytomy (Fig. 3). Many species of these sections share several plesiomorphic characters which also occur in C<sub>4</sub> *Cyperus*, whereas species in the core *Pycreus* clade show more evolved character states (see Table 4). As in *Kyllinga*, we do not consider laterally flattened gynoecia sufficient to maintain *Pycreus* at the generic level, especially as it was resolved as polyphyletic in the present study.

### *Queenslandiella*

*Queenslandiella* is a third taxon nested in the C<sub>4</sub> *Cyperus* polytomy, which is characterized by laterally flattened gynoecia. It shares the open inflorescence with *Pycreus* (which is the plesiomorphic condition in C<sub>4</sub> *Cyperus*). However, it has most often been considered to be related to *Kyllinga*, with which it shares deciduous spikelets, and keeled and multi-nerved glumes (Chermezon, 1919; Ballard, 1932, 1933; Koyama, 1976). The species has always been placed in or near *Cyperus*. However, even when included in *Cyperus*, it was most often retained in its own section or subgenus (Kern, 1974; Govindarajalu, 1975; Haines & Lye, 1983).

As with the other specialized, short-lived and monotypic segregate lineages, *Queenslandiella* has also accumulated many peculiar characters which isolate it from the other C<sub>4</sub> *Cyperus* taxa. These characters include the large proportions of glumes and nutlets compared with most other *Cyperus* spp., vegetative anatomy (Govindarajalu, 1975) and embryo type (Van der Veken, 1965). Several *Cyperus* spp. have been considered to be closely related to *Queenslandiella*, including *C. soyauxii* Boeckeler, which has similar deciduous spikelets with similar glumes and a similar embryo (Kükenthal, 1936; Van der Veken, 1965), but trimerous pistils (Goetghebeur, 1986). Lye (1983) described *C. micromariscus* Lye, which is only known from its type collection in Tanzania. This plant also has an open inflorescence with deciduous spikelets and laterally flattened pistils comparable with *Queenslandiella*, but differs in the small glumes and nutlets and different habit. Therefore, Lye (1983) assumed a different origin of this species and placed it in its own *Cyperus* subgenus *Micromariscus* Lye (Haines & Lye, 1983). The relationship of *Queenslandiella* to both *C. soyauxii* and *C. micromariscus* needs further confirmation. As for the segregates above, we do not consider the specialized characters of *Queenslandiella* sufficient to warrant recognition at the generic level.



*Remirea*

*Remirea* is another monotypic entity with special adaptations to its coastal habitat. It is characterized by a capitate inflorescence with deciduous spikelets. Each spikelet contains a few empty glumes at the base and a corky rachilla which envelops the fruit. For these reasons, it had been classified among Rhynchosporeae (Fenzl, 1836; Bentham, 1883; Pax, 1888; Baillon, 1894; Clarke, 1901–02; Kükenthal, 1944; Haines & Lye, 1983). However, Nees von Esenbeck (1834) had already placed *Remirea* correctly in Cyperae. After Kunth (1837) gave a correct interpretation of the spikelet, this opinion was followed by Chermezon (1922), Kern (1958, 1974), Oteng-Yeboah (1975), Hooper (1983) and Goetghebeur (1986, 1998).

*Remirea* is nested in the main  $C_4$  *Cyperus* polytomy, similar to *Sphaerocyperus* (Fig. 3), which also has empty glumes in the lower part of the spikelets. The relationship between these two taxa remains unclear. However, we do not believe empty glumes at the base of the spikelets to be sufficient as a generic character considering that other links with  $C_4$  *Cyperus* are clear. The corky rachilla is also observed in *C. odoratus* L. (formerly in the genus *Torulanium*), a species with multiple flowers in which the rachilla breaks up into individual segments. The affinity between *Remirea* and *C. odoratus* needs further investigation.

*Sphaerocyperus*

The deciduous spikelets of the monotypic *Sphaerocyperus* have six or seven distichously arranged glumes, only one of which bears a maturing nutlet. The sole species has variously been placed in *Actinoschoenus* Benth., *Cyperus*, *Schoenus* L. and *Rhynchospora* Vahl before it was described as a separate genus *Sphaerocyperus* (Lye, 1972). Like *Remirea*, we consider the empty glumes as insufficient to retain this taxon as a separate genus nested in a paraphyletic *Cyperus* with which it shares clear morphological affinities.

*Volkiella*

*Volkiella* is a rare monotypic taxon from southwestern Africa (mainly Namibia), and can be seen as an extremely specialized lineage adapted to psammophytic habitats. When described, *Volkiella* was considered to be intermediate between *Cyperus* and *Lipocarpha* (Merxmüller & Czech, 1953). The relationship with *Lipocarpha* was explained by the similar presence of the two ‘floral scales’ (‘hypogynen Skalen’), for which the correct interpretation is not yet clear, but the relationship with *Cyperus* was assumed on the basis of the distichous placement of the ‘Glumae’, which are, in fact, the spikelet bracts and thus not homologues of the glumes in *Cyperus* and other sedges. This initial interpretation was followed by Van der Veken (1965) and Reynal (1973), but

was later correctly interpreted by Goetghebeur (1986, 1998). As in several other lineages, such as *Ascolepis*, *Lipocarpha* and *Alinula*, *Volkiella* shows highly derived pseudospikelets with a Bauplan comparable with that of *Lipocarpha*, possessing a spikelet bract, a spikelet prophyll, a proximal glume subtending the single flower and a spikelet bract larger than the glume. Peculiarly, in *Volkiella*, the spikelets are distichously arranged on the spike axis, whereas this position is spiral in all other  $C_4$  *Cyperus* spp. Although *Volkiella* shows an abundance of autapomorphic, derived characters which isolate it from all other  $C_4$  *Cyperus* spp., it is nested in *Lipocarpha* and should thus be sunk into *Cyperus* together with *Lipocarpha*.

BASIS FOR A MODERN CLASSIFICATION OF *CYPERUS*

From the current and previous molecular phylogenetic analyses, it is evident that the classification of Goetghebeur (1998) in Cyperae can no longer be upheld without accepting paraphyletic genera. Although most of the segregate genera are morphologically well circumscribed, the rapid diversification of the *Cyperus* clade has resulted in several nested paraphyletic entities (e.g. the genus *Volkiella* is nested in the genus *Lipocarpha*, which is nested in the group of  $C_4$  *Cyperus* spp. formerly known as *Mariscus*, and  $C_4$  *Cyperus* is, in turn, nested in  $C_3$  *Cyperus*). Moreover, most morphological characteristics used for the delimitation of the different genera related to *Cyperus* appear to have a high level of homoplasy in the *Cyperus* clade (e.g. spiral glumes, dorsiventrally flattened dimerous pistils, deciduous spikelets, pseudospikelets; Fig. 2). Subsequently, different combinations of the same sets of these morphological characters have been used to circumscribe most taxa.

Larridon *et al.* (2011a, b) placed the  $C_3$  segregate genera in  $C_3$  *Cyperus* based on a well-resolved phylogenetic hypothesis combined with morphological, embryographical, ontogenetic and anatomical data. In that study, a classification for the *Cyperus* clade was suggested in which two subgenera were recognized. Although *Cyperus* subgenus *Anosporum* (Nees) C.B. Clarke ( $C_3$  *Cyperus*) is currently circumscribed as a paraphyletic entity (Larridon *et al.*, 2011a, b), the single origin of the  $C_4$  photosynthetic pathway, a clear apomorphy for the  $C_4$  *Cyperus* clade, forms a sufficiently strong argument for the use of an evolutionary approach restricted to the subgeneric level in *Cyperus*. For the lower level classification, a cladistic approach was followed in circumscribing only monophyletic sections and, subsequently, the segregate genera will be included in existing or new sections in *Cyperus*.

This classification can be extended to include the different taxa of the C<sub>4</sub> *Cyperus* clade (*Cyperus* subgenus *Cyperus*). However, as most segregate genera are nested in a hard polytomy with many species from different sections of *Cyperus* s.s. and as the lower level relationships in several segregate genera are poorly resolved, it is currently premature to build a new sectional classification for the largest part of *Cyperus* subgenus *Cyperus*. A joint international effort will be necessary to expand the current phylogenetic studies with more DNA markers and taxa. This will then serve as a basis for the growing modern classification of the giant genus *Cyperus*.

### CONCLUSIONS

From the data presented here, we conclude that the *Cyperus* clade consists of a paraphyletic C<sub>3</sub> *Cyperus* and a well-supported monophyletic C<sub>4</sub> *Cyperus* clade. Nine segregate genera are nested in C<sub>4</sub> *Cyperus*, i.e. *Alinula*, *Ascolepis*, *Lipocarpha*, *Kyllinga*, *Pycneus*, *Queenslandiella*, *Remirea*, *Sphaerocyperus* and *Volkia*, most of which are monophyletic. Because they are nested in the *Cyperus* clade, and as a consequence of the multiple origins of the characters used to circumscribe them, we suggest that all nine C<sub>4</sub> *Cyperus* segregate genera should be included in a more broadly circumscribed *Cyperus*. This study establishes a phylogenetic framework for future studies of the different C<sub>4</sub> *Cyperus* sections and segregates, and for the taxonomic inclusion of the C<sub>4</sub> segregate genera into *Cyperus* s.l.

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