Cyperaceae tribe Cypereae: phylogenetic relationships and evolutionary patterns of diagnostic characters

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ABSTRACT

Phylogenetic relationships in *Cyperus* and allied genera in the tribe Cypereae (family Cyperaceae) have been reconstructed using parsimony and Bayesian analyses of a combined data matrix, which consisted of plastid DNA (*rbcL, trnL-F* intergenic spacer, and *rps16*), nuclear ribosomal DNA (internal transcribed spacer ITS) sequences and morphological data. From both analyses, tribe Cypereae were resolved into a very strongly supported clade (Posterior probability =98%; Bootstrap = 100%) characterised by the *Cyperus* -type of embryo and the absence of perianth segments. *Cyperus* sensu stricto is not monophyletic as currently resolved as several cyperoid genera are embedded within it. The Cyperoid clade splits into two distinct clades which are diagnosed by the Eucyperoid (C_3) and Chlorocyperoid (C_4) anatomy. The evolution of key morphological characters used to diagnose genera is discussed and genera whose classification needs to be revisited are highlighted.

INTRODUCTION

Cyperaceae (commonly known as the sedges) are the third largest family of monocotyledons (with about 104 genera and 5000 species (Goetghebeur 1998) with considerable economic and conservation importance. They are dominant components of many wetland ecosystems and are reliable indicators of habitat deterioration in such systems (Simpson *et al.* 2003). On the other hand, several sedges are troublesome weeds on arable land. These include: *Cyperus rotundus* L. (the world's worst weed-commonly called purple nutsedge (Goetghebeur 1998)); *Cyperus rigidifolius* Steud. (common in cultivated areas in South Africa, Kenya and Uganda); *Cyperus ustulatus* A. Rich (a pasture weed in New Zealand); and *Cyperus radians* (Nees & Mey.) Kunth. (common in waste places and aquatic biotopes in India, south and eastern China and Malaysia) (Simpson & Inglis 2001). Nevertheless, several sedges are useful as food, animal fodder, medicine, material and as environmental protectors (Table 1).

Catergory	**Taxon**	**Uses**	**Country where used**
Food	*Cyperus subumbellatus* Kük.	Rhizomes aromatic and used for food flavoring	W. Indies
	Cyperus usitatus Burch.	Tubers eaten raw, roasted or boiled	Namibia, South Africa
Animal fodder	*Carex egglestonii* Mack. *Cyperus alterniflorus* L. *Cyperus jeminicus* Rottb.	livestock fodder	USA Australia Senegal, Sudan
Medicinal	*Kyllinga triceps* Rottb. *Cyperus renschii* Boeck. *Fimbristylis squarrosa* Vahl.	skin problems e.g. itching circulatory system disorders treats sore throat	India Tanzania Nepal
Material	*Cyperus papyrus* L.	Making paper, fibre boards, roofing, boats, sleeping mats etc	Various countries
	Cyperus rotundus L.	Culm bases used for incense and perfumery	Tanzania
Environment	*Carex exserta* Marck.	revegetators: restoration of vegetation cover using sod plugs	USA
	Cyperus arenarius Retz.	dune stabilization	India

Table 1. Some examples of sedges of economic importance, adapted from Simpson & Inglis (2001)

The genus *Cyperus* L. is the second largest in the family Cyperaceae (Ssegawa *et al.* 2004) consisting of up to 600 species (Muasya et *al.* 2002). It is widely distributed, with the largest concentration of taxa occurring in the tropics (Muasya et *al.* 2002; Ssegawa *et al.* 2004). This genus is associated with lots of controversy with regards to its circumscription or infrageneric classification which has led to some treating it as one large genus with several subgenera, (e.g. Kukenthal 1936 and Haines & Lye 1983) while others have split it into various genera (e.g. Goetghebeur 1986; Bruhl 1995 and Goetghebeur 1998 -Table.2). In addition, a number of genera (*Lipocarpha* R.Br., *Ascolepis* (Nees) Steud., *Volkiella* Merxm. & Czech., *Kyllinga* Rottb., *Kyllingiella* (A.Rich) Lye, *Oxycaryum* Nees., *Remirea* Aubl., *and Sphaerocyperus* (Ridl.) Lye.) in tribe Cypereae are segregated from *Cyperus* on the basis of few characters.

Differences in opinion regarding the classification of the Cypereae arise from the morphology and anatomy of its member genera. Zhang *et al.* (2004) highlighted that the branching pattern of spikelets is an important character in determining systematic arrangements within Cyperaceae. They noted that the spikelets, however, are extremely contracted, leading to different interpretations of spikelet morphology in Cyperaceae and consequently to different classifications. In terms of anatomy, Muasya *et al.* (2002) noted that the presence of Kranz anatomy, correlated with C_4 photosynthesis (Carolin *et al.* 1977), has been used in the classification of subgenera in *Cyperus*. However, they noted that some of the species showing either C_3 or C_4 anatomy are indistinguishable on gross morphology and this raises questions about the switch from C_3 to C_4 anatomy either having evolved repeatedly with no morphological divergence or only once with subsequent convergence in the morphology of unrelated species.

The use of morphology and anatomy alone to infer phylogenetic relationships in the Cypereae does not provide an unambiguous resolution of its classification. Plastid DNA sequence data have been used to infer angiosperm phylogenetic relationships at suprageneric levels, for example, the *rbcL* gene (e.g. Chase *et al.*, 1993, 1995; Muasya *et al.*, 1998), *rps16* intron (e.g. Oxelman *et al.,* 1997), *trnL* intron and *trnL-F* intergenic spacer (e.g. Taberlet, *et al.*, 1991). Similarly, in Cypereae, plastid DNA sequence data (*rbcL, rps16, trnL-F*) have been used to study the tribe (e.g. Muasya *et al.*, 2001; 2002; in press). However, none of these studies have incorporated regions from nuclear DNA yet this might help reveal some important information that will lead to a better resolution of the phylogeny of the whole tribe.

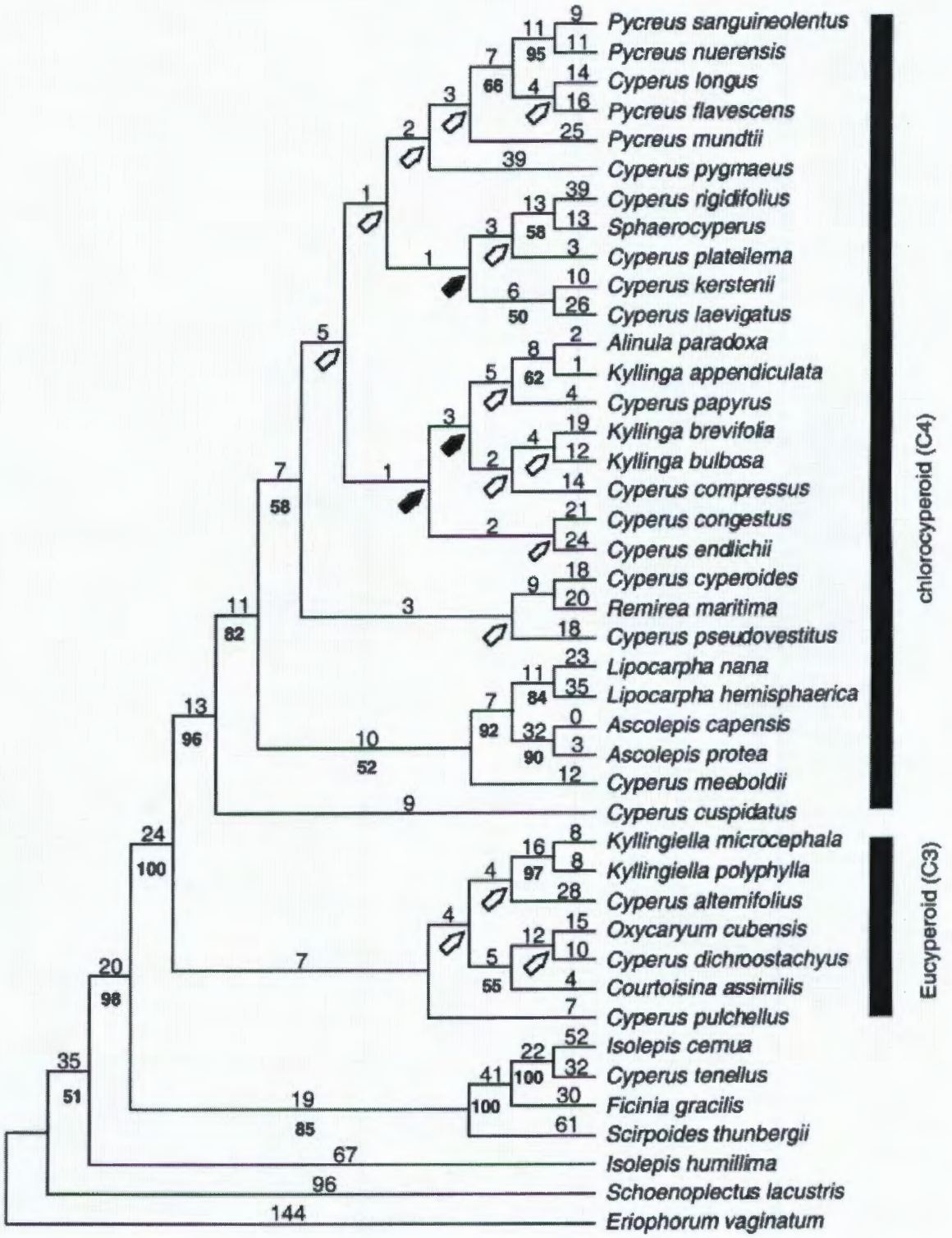
Kükenthal (1936)	Haines & Lye (1983)	Goetghebeur (1986)	Bruhl (1995)	Goetghebeur (1998)
CYPERUS	*CYPERUS*	*CYPERUS*	*CYPERUS*	*CYPERUS*
subgen. *Eucyperus*	subgen. *Anosporum*	subgen. *Anosporum*		subgen. *Anosporum*
	subgen. *Cyperus*	subgen. *Cyperus*		subgen. *Cyperus*
	subgen. *Protocyperus*			
	subgen. *Sorostachys*			
	subgen. *Xerocyperus*			
	subgen. *Courtoisia*	*COURTOISINA*	*COURTOISINA*	*COURTOISINA*
subgen. *Juncellus*			*JUNCELLUS*	
subgen. *Kyllinga*	subgen. *Kyllinga*	*KYLLINGA*	*KYLLINGA*	*KYLLINGA*
subgen. *Mariscus*	subgen. *Aristomariscus*	*MARISCUS*	*MARISCUS*	*CYPERUS*
	subgen. *Bulbocaulis*		*MONANDRUS*	subgen. *Cyperus*
	subgen. *Bulbomariscus*			
	subgen. *Fimbricyperus*			
subgen. *Pycreus*	subgen. *Pycreus*	*PYCREUS*	*PYCREUS*	*PYCREUS*
	subgen. *Queenslandiella*	*QUEENSLANDIELLA*	*QUEENSLANDIELLA*	*QUEENSLANDIELLA*
subgen. *Torulinium*		*TORULINIUM*	*TORULINIUM*	*CYPERUS*
				subgen. *Cyperus*

Table 2. Some classifications of *Cyperus*

Adapted from: Muasya *et al.* (2002)

Muasya *et al.* (2001) studied the phylogeny of *Cyperus* and allied genera in Cyperaceae tribe Cyperaea using parsimony analysis of a combined matrix of plastid DNA (*rbcL, trnL* intron and *trnL-F* intergenic spacer) sequences and morphology. From their study, the tribe Cypereaea was resolved into a strongly supported clade defined by the *Cyperus*-type embryo and absence of perianth segments. However, *Cyperus sensu stricto* was not monophyletic. It was observed that several cyperoid genera (e.g. *Ascolepis, Courtoisina Kyllinga, Kyllingiella Lipocarpha, Oxycarium, Pycreus, Remirea* and *Sphaerocyperus*) were embedded within it.

Further work by Muasya *et al.* (2002) using four DNA sequence regions: the *rbcL* gene and the non coding regions- *rps16* intron, *trnL* intron and *trnL-F* spacer with point substitutions, length variations and insertions or deletions (indels). Their results showed that subgeneric classification of *Cyperus s.s.* into subgenera *Cyperus* and *Anosporum* based on the presence of Kranz anatomy (i.e. chlorocyperoid vs. eucyperoid) was supported by DNA sequence data. Nevertheless, there were still several genera (e.g. *Lipocarpha, Ascolepis, Kyllingiella and Oxycaryum*) in Cypereae, recognized by one or few morphological autapomorphies, embedded within *Cyperus s.l.* Therefore, the circumscription of *Cyperus* s.s. is paraphyletic as shown in the phylogenetic tree in Figure 1.



Adapted from: Muasya *et al.* (2002)

Figure 1. Phylogenetic tree showing relationships among *Cyperus s.l.* Arrows mark the clades not present in the strict consensus tree of the Fitch (open arrows) and both Fitch and SW (solid arrows) analyses. Numbers above the branches are inferred substitutions (ACCTRAN optimization); Fitch bootstrap percentages are shown below the branches. Vertical bars show the vegetative anatomy in the taxa.

Effectively, the work that has been done so far with regards to the classification of the Cypereae does not provide a clear solution as to whether *Cyperus sensu lato* should be regarded as a large genus with several subgenera embedded in it or to simply split it into several genera as in Table 1.

Having recognized the unresolved classification of the genera in tribe Cypereae this study was set out to try and resolve the phylogeny and test the hypothesis of the monophyly of *Cyperus sensu lato*. The hypothesis was that *Cyperus sensu lato* is monophyletic. This was based on the concept of monophyly of genera. If *Cyperus* is monophyletic then it can be accepted as one large genus, but if it is not, perhaps the idea of splitting it into several genera is the way to go. The aim of the study was is to build on the work by Muasya *et al.* (2002) to reconstruct the phylogeny of *Cyperus sensu lato* and its allied genera, based on DNA (nuclear and plastid) and morphological data. Our objectives were to: infer phylogenetic relationships in the tribe Cypereae using DNA (nuclear and plastid) and to code morphological data; to evaluate evolution of key diagnostic characters; and to revise the classification of the tribe Cypereae.

MATERIALS AND METHODS

DNA Extraction and amplification

A total of 70 taxa (including an out-group *Eriophorum vaginatum*) were studied and these are shown in Table 3. Plant samples were provided by Dr. A. M Muasya as silica gel dried specimens although a few were herbarium specimens. DNA was extracted using the CTAB method of Doyle & Doyle (1987) with modifications where necessary for example, the DNA was allowed to precipitate in isopropanol for three to four days. This seemed to improve the quantity of DNA. Standard PCR methods were used for DNA amplification. Primers used for the nuclear region were internal transcribed spacers ITS4, ITS5 (White *et al.*, 1990), and ITS L. The PCR reactions were performed in 30 μl volumes consisting of 18.6 μl sterile distilled water, 3 μl of 10x DNA polymerase buffer, 3 μl of $MgCl_2$ (50 mM), 1 μl each of the forward and reverse primers (10 μM), 1.2 μl of dNTP (10 mM), 0.2 μl of *Taq* DNA polymerase and 2 μl of template dna. The reaction was done on an applied Biosystems GeneAmp 2700 thermal cycler (Applied Biosystems, Foster City, CA, USA). The program had an initial denaturation phase of 2 minutes at 94°C, followed by 30 cycles of 60 seconds at 94 °C, 60 seconds at 52 °C and 2 minutes at 72 °C. The final extension phase of 7 minutes was done at 72 °C. The PCR products were run on 1% agarose gel mixed with ethidium bromide to visualize how they had amplified.

PCR products which had amplified successfully were sent to MacroGen (http://www.macrogen.com) in Korea for sequencing using the same primers used for amplification. For some of the taxa which did not

Table 3. Taxa analysed in the combined matrix.

Taxon	Herbaria & voucher	*rbcL*	*rps16*	*trnL-F*	ITS
Abildgaardia_ovata (Burm.F.)Kral	Kenya: Muasya *et al*. 684(EA, K)	Y12985		AJ295754	AB250638
Actinoscirpus_grossus(L.f.)Goetgh.& D.A. Simpson	Malaysia:Simpson 2660 (K)	Y12953		AJ295765	MD
Alinula_lipocarphoides (Kuk)J. Ranal	Kenya: Muasya :2592 (EA)			EF178608	MD
Alinula_paradoxa Goetgh.& Voster	Tanzania: Faden et al. 96/29 (K)	AJ278290		AJ295756	
Androtrichum_giganteum (Kunth)H.Pfeiff.	Argentina: Tressens et al.4292(K)	EF178546			MD
Androtrichum_trigynum(Spreng.)H. Pfeiff	Argentina: Goetghebeur4764 (GENT)	EF178547			
Ascolepis_capensis (Kunth)Ridl.	Kenya: Muasya: 1009 (EA, K)	Y13003	AF449518	AJ295757	MD
Ascolepis_protea Welw.	Congo: Fay 2700 (K)	Y13002			
Bolboschoenus_maritimus (L.)Palla	Botswana:Smith: 2452 (K)	Y12996		AJ295767	MD
Bolboshoenus_nobilis (Ridl.)Goetgh.& D.A.Simpson	S. Africa: Leistner 144 (K)	Y12995			
Courtosina_assimilis (Steud.)Maquet	Tanzania: Faden et al. 96/119 (K)	AY40590	AY449519	AY40595	
Cyperus_compressus L.	Thailand: Muasya:1375 (K)	AF449506	AF449521	AF449555	
Cyperus_cuspidatus Kunth.	Thailand: Muasya:1374 (K)	AF449508	AF449523	AF449557	
Cyperus_cyperoides (L.)Kuntze	Muasya 1277 (K)	AF449509	AF449524	AF449558	AB61665
Cyperus_dichroostachyus A.Rich	Muasya 976 (EA, K)	Y12965	AF449525		
Cyperus_endilchii Kük.	Muasya 695 (K)	AF449510	AF449526	AF449559	
Cyperus_involucratus Rottb.	Madagascar: Kew acc.6136603	Y12967	AF445920	AJ295758	AY242052
Cyperus_laevigatus L.	Kenya: Muasya 1041(EA)	Y13017	AF449527	AY040596	
Cyperus_papyrus L.	Chad: Hepper 4213(K)	Y12966	AF449531	AJ295759	AY242048
Cyperus_plateilima (Steud.)Kük.	Muasya 969 (EA, K)	AF449512	AF449532	AF449561	
Cyperus_pulchellus R.Br	Thailand: Muasya:1377 (K)	AY40591		AY040599	MD
Cyperus_pygmaeus Rottb.	Kenya: Muasya 1133 (K)	AJ404698	AF449534	AJ295760	
Eriophorum_vaginatum L.	Poland: Beyer et al. 2 (K)	Y12951	AF449553	AJ295769	MD
Ficinia_bergiana Kunth.	S. Africa: Muasya:2337 (BOL)	EF200588		AJ295753	MD
Ficinia_distans C.B.Clarke	S. Africa: Muasya:2283 (BOL)	EF178548		EF178594	
Ficinia_esterhuyseniae Muasya	S. Africa: Muasya:2312 (BOL)	EF178549	EF078975	EF178590	MD
Ficinia_gracilis Schrad.	Tanzania:Faden et al. 96/433	EF178550		EF178534	MD
Ficinia_nodosa(Rottb.)Goetgh.,Muasya &D.A.Simpson	Australia:Stind: 21216 (K)	Y12984	EF174386	AJ295793	DQ385568
Ficinia_radiata (Lf.)Kunth	S. Africa: Muasya: 2310 (K)	EF200589	EF078976		MD
Ficinia_rigida Levyns	S. Africa: Muasya: 2319 (K)	EF178557	EF174387	EF178602	MD
Ficinia_trichodes (Schrad.)Benth. & Hook.F.	S. Africa: Muasya: 2328 (K)	EF178558	EF174388	EF178603	MD
Fimbristylis_dichotoma L.	Kenya: Muasya: 1006 (EA, K)	Y13008		AJ29755	AB250630
Fuirena_sp.	Brazil: Thomas et al. 10404 (NY)	Y12970			MD
Hellmuthia_membranacea (Thunb.)R.W. Haines & Lye	269 (K);Muasya: 1145 (K)	Y13000	EF174389	AJ295815	MD
Isolepis_cernua_(Vahl.)Roem.& Schlut var._cernua	Britain: Muasya:1058 (K)	Y13014	AF449538	AJ295575	DQ385576
Isolepis_fluitans (L.)R.Br.	Kenya: Muasya: 1057 (K)	Y12961	EF174390	AJ295780	DQ355579
Isolepis_humillima (Benth.) K.L. Wilson	Australia: Thomas et al. 622 (BRI)	AJ404728	AF449539	AJ295784	
Isolepis_hystrix (Thunb.)Nees	S. Africa: Muasya: 1150 (K)	AJ404711		AJ295785	MD
Isolepis_levynsiana Muasya & D.A. Simpson	S. Africa: Muasya: 1151 (K)	AF449514	AF449514	AF449575	

Isolepis_marginata (Thunb.)A.Dietr.	Australia:Convey et al.17452 (K)	AJ404714	EF174391	AJ295790	
Isolepis_setacea (L.)R.Br.	Kenya: Muasya: 1059 (K)	Y12962	EF174392	AJ295799	AY242053
Isolepis_tenuissima (Nees)Kunth	S. Africa: Muasya: 2369 (K)	AY725947			MD
Isolepis_venustula Kunth	S. Africa: Muasya: 1189 (K)	AJ404724		AJ295804	MD
Kyllinga_appendiculata K. Schum.	Kenya: Muasya: 1050 (EA, K)	Y13007	AF449542	AJ295761	
Kyllinga_brevifolia Rottb.	Australia:Coveny et al.17459 (K)	AF449515	AF449543	AF449576	
Kyllinga_bulbosa P. Beauv	Kenya: Muasya: 1020 (EA, K)	Y12979	AF449544	AY040601	
Kyllingiella_microcephala (Steud.)R.W.Haines & Lye	Zimbabwe: Muasya et al. 1118 (K)	AY040592	AF449540	AJ295807	
Kyllingiella_polyphylla (A.Rich.)Lye	Tanzania: Wingfield 497(K)	Y113013	AF449541	AJ295515	MD
Lipocarpha_hemisphaerica (Roth.)Goetgh.	Thailand: Muasya: 1217 (K)	AF449516	AF449546	AF449565	MD
Lipocarpha_nana (A. Rich.)J.Raynal	Kenya: Muasya: 972 (EA, K)	Y12990	AF449545	AJ295762	
Oxycaryum_cubense (Poepp.& Kunth)E.Palla	Zambia: Richards 13318 (K)	Y13006		AY040602	
Pycreus_flavescens (L.)Rchb.	Kenya: Muasya: 1022 (EA, K)	Y13005	AF449547	AJ295763	MD
Pycreus_nuerensis (Boeck.) S.S.Hooper	Tanzania: Muasya: 940 (EA, K)	Y13004	AF449549	AY040603	
Pycreus_sanguinoletus (Vahl.)Nees	Coveny et al. 17461			AF449567	AB261671
Queenslandiella_hyalina (Vahl)Ballard	Kenya: Mwachala 296 (EA)	AY725953			
Remirea_maritima Aubl.	Tanzania Faden et al. 96/48	AY040593	AF449550	AY040604	MD
Schoenoplectus_articulatus (L.)Lye	Tanzania: Muasya: 947 (EA, K)	Y12987			MD
Schoenoplectus_corymbosus J.Raynal	Kenya: Muasya: 1004 (EA)	EF178570		EF178607	MD
Schoenoplectus_lacustris (L.)Palla	Britain: Muasya:1043 (K)	Y12943	AF449554	AJ295809	
Schoenoplectus_litoralis (Schrad.)Palla	Hong Kong: Shaw: 883 (K)				AY506753
Scirpoides_holoschoenus(L.)Soják	S. Africa: Acocks s.n. (K).	Y12994	AY344153	AJ295811	MD
Scirpoides_thunbergii (Schrad.)Soják	S. Africa: Muasya: 1205 (K)	AJ404727	AF449551	AJ295812	MD
Scirpus_ancistrochaetus Schuyler	USA: Nasci 7544 (DOV)	EF178578	EF174395		
Scirpus_falsus C.B. Clarke	S. Africa: Hilliard 13609 (GENT)	EF178559	EF174393		MD
Scirpus_ficinioides Kunth	S. Africa: Hilliard 16095 (GENT)	EF178560	EF174394		MD
Scirpus_sylvaticus L.	HBUG/86-0541 (GENT)	EF178586	EF174396		
Sphaerocyperus_erinaceous (Ridl.) Lye	Tanzania: Faden et al. 96/338 (K)	AJ404699	AF449552	AJ295764	
Volkiella_disticha Merxm. & Czech	Namibia: Muller et al. 4245 (K)	EF178561			

The numbers indicated in the DNA regions are Gen bank accession numbers for the sequences of those taxa. Blank spaces represent missing sequences and MD indicates sequences done as part of the current study which have not yet been submitted to Genbank.

amplify, sequences were downloaded from Genbank. Plastid DNA sequences (*rbcL, rps16* and *trnL-F*) were provided by Muasya from the 2002 study (Table 3). Alignment of sequences was done using ClustalW alignment in BioEdit and also in McClade. Scoring of morphological characters was done by checking from published papers and monographs.These were; Haines & Lye (1983); Gordon-Gray (1995); Goetghebeur (1998); Muasya *et al.* (in press) and Bruhl & Wilson (in press). The morphological characters studied are shown in Table 4.

Data analysis

The complete aligned matrix had 4054 characters, consisting of *trnL-F* (1150), *rbcL* (1406), *rps*16 intron (902), ITS (583), and morphological characters (10). For all DNA characters, gaps were coded as missing. Data analysis was carried out in two steps: (A) the partial data set analysis: the ITS matrix of 43 out of the 70 taxa was analysed separately and in a combined matrix, (B) combined data set including plastid DNA sequences, nuclear dna sequences and morphological data for all the 70 taxa studied. Phylogenetic relationships were analyzed using the parsimony algorithm of PAUP* version 4.0b10 (Swofford, 2002) and MrBayes Version 3.12 (Huelsenbeck & Ronquist, 2003). For parsimony, the Heuristic tree search was done (using unordered, equal weights; Fitch, 1971). 1000 random replicates with tree bisection-reconnection (TBR) swapping were done and only ten trees were held at each replicate. To evaluate the support at the nodes bootstrap analysis was done for 1000 replicates (Felsenstein, 1985). Characters were sampled using equal weighting (Fitch, 1971). Trees were built using simple taxon addition with TBR branch swapping, retaining groups with frequencies greater than 50 % in the final bootstrap consensus tree.

Bayesian inference of phylogeny with posterior probabilities (PP) as measures of support was done using Mr Bayes. This was done for the combined matrix (i.e. plastid, nuclear DNA and morphological data matrix). The model of molecular evolution for all the gene regions sampled was GTR+I + G. The choice of this model was based on the observation by Huelsenbeck & Rannala (2004) that the accuracy of a Bayesian model suffers more in response to under parameterization than over parameterization. Default priors of Mr Bayes were used. For each analysis, two simultaneous runs were done (starting from random trees). The number of chains for each run was set to be four (with three heated and one cold chain) and the temperature was set at 0.3. Markov chains were sampled every 100^{th} generation. Analyses were run until the average standard deviation of the split frequencies approached 0.01, indicating that the two runs converged onto a stationary distribution. The analysis was run twice; each for $1x10^6$ generations. To check for stationarity, the log-likelihoods were ploted against the generation time, and this also gave an idea of the number of trees to discard (i.e. trees sampled during the burn in

Table 4. Morphological characters studied.

	A	B	C	D	E	F	G	H	I	J
Abildgaardia ovata	0	0	1	0	0	0	1	0	2	0
Actinoscirpus grossus	0	0	0	0	0	0	0	1	1	0
Alinula lipocarphoides	1	1	?	1	0	0	1	0	0	1
Alinula paradoxa	1	1	?	1	0	0	1	0	0	0
Androtrichum giganteum	0	0	1	0	0	0	0	0	0	0
Androtrichum trigynum	0	0	1	0	0	0	0	0	0	0
Ascolepis capensis	0	1	?	1	0	0	1	0	0	0
Ascolepis protea	0	1	?	1	0	0	1	0	0	0
Bolboschoenus maritimus	0	0	0	0	0	0	0	1	1	0
Bolboshoenus nobilis	0	0	0	0	0	0	0	1	1	0
Courtosina assimilis	1	0	1	1	0	0	0	0	0	0
Cyperus compressus	1	0	1	0	0	0	1	0	0	0
Cyperus cuspidatus	1	0	1	0	0	0	1	0	0	0
Cyperus cyperoides	0	0	1	1	0	0	1	0	0	0
Cyperus dichroostachyus	0	0	1	0	0	0	0	0	0	0
Cyperus endilchii	0	0	1	0	0	0	?	0	0	0
Cyperus involucratus	0	0	1	0	0	0	0	0	0	0
Cyperus laevigatus	0	0	1	0	0	1	1	0	0	0
Cyperus papyrus	0	0	1	0	0	0	1	0	0	0
Cyperus plateilima	0	0	1	0	0	0	1	0	0	0
Cyperus pulchellus	0	0	1	0	0	0	0	0	0	0
Cyperus pygmaeus	1	0	1	0	0	0	1	0	0	0
Desmoschoenus spiralis	0	0	0	0	0	0	0	0	0	1
Eleocharis marginulata	0	0	0	0	0	0	0	1	4	0
Eriophorum vaginatum	0	0	0	0	0	0	0	1	0	0
Ficinia bergiana	0	0	0	0	0	0	?	0	0	1
Ficinia distans	0	0	1	0	0	0	0	0	0	1
Ficinia esterhuyseniae	0	0	0	0	0	0	?	0	0	1
Ficinia gracilis	0	0	0	0	0	0	0	0	0	1
Ficinia nodosa	0	0	0	0	0	0	0	0	0	1
Ficinia radiata	0	0	0	0	0	0	0	0	0	1
Ficinia rigida	0	0	0	0	0	0	?	0	0	0
Ficinia trichodes	0	0	0	0	0	0	?	0	0	1
Fimbristylis dichotoma	0	0	0	0	0	1	1	0	2	0
Fuirena sp.	0	0	0	0	0	0	?	0	1	0
Hellmuthia membranacea	0	0	0	0	0	0	0	0	0	0
Isolepis cernua var. cernua	1	0	0	0	0	0	0	0	0	0
Isolepis fluitans	1	0	0	0	0	1	0	0	0	0
Isolepis humillima	1	0	0	0	0	0	0	0	?	0
Isolepis hystrix	1	0	0	0	0	0	0	0	0	0
Isolepis levynsiana	1	0	1	0	0	0	0	0	0	0
Isolepis marginata	1	0	0	0	0	0	0	0	0	1
Isolepis setacea	1	0	0	0	0	0	0	0	0	0
Isolepis tenuissima	1	0	0	0	0	0	0	0	0	0
Isolepis venustula	1	0	0	0	0	0	0	0	0	0
Kyllinga appendiculata	0	0	1	1	1	1	1	0	0	0
Kyllinga brevifolia	0	0	1	1	1	1	1	0	0	0
Kyllinga bulbosa	0	0	1	1	1	1	1	0	0	0
Kyllingiella microcephala	0	0	0	0	0	0	0	0	0	0
Kyllingiella polyphylla	0	0	0	0	0	0	0	0	0	0
Lipocarpha hemisphaerica	1	1	?	0	0	1	1	0	0	0
Lipocarpha nana	1	1	?	0	0	0	1	0	0	0
Oxycaryum cubense	0	0	0	0	0	1	0	0	0	0
Pycreus flavescens	1	0	1	0	1	1	1	0	0	0
Pycreus nuerensis	0	0	1	0	1	1	1	0	0	0
Pycreus sanguinoletus	0	0	1	0	1	1	1	0	0	0
Queenslandiella hyalina	1	0	1	1	1	1	1	0	0	0
Remirea maritima	0	0	1	1	0	0	1	0	0	0
Schoenoplectiella articulata	1	0	0	0	0	0	0	0	1	0
Schoenoplectus corymbosus	0	0	0	0	0	0	0	1	1	0
Schoenoplectus lacustris	1	0	0	0	0	0	0	1	1	0
Schoenoplectus litoralis	0	0	0	0	0	1	0	1	1	0
Scirpoides holoschoenus	0	0	0	0	0	0	0	0	0	0
Scirpoides thunbergii	0	0	0	0	0	0	0	0	0	0
Scirpus ancistrochaetus	0	0	0	0	0	0	0	1	3	0
Scirpus falsus	0	0	0	0	0	0	?	1	?	0
Scirpus ficinioides	0	0	0	0	0	0	?	1	?	0
Scirpus sylvaticus	0	0	0	0	0	0	0	1	3	0
Sphaerocyperus erinaceous	0	0	1	1	0	0	1	0	0	0
Volkiella disticha	0	0	1	?	0	0	1	0	0	0

A: HABIT(0 = Perennial, 1 = annual); **B**: Number of florets per spikelet (0 = many, 1 = one);
C: Glume arrangement (0 =spiral, 1 = distichous, ? = not applicable);
D: Dispersal unit (0 = nutlet, 1 = spikelet); **E**: Nutlet orientation (0 = dorsiventral, 1 = lateral);
F: Number of style branches (0 = three, 1 = two); **G**: Photosynthetic pathway (0 = C3, 1 = C4, ? = unknown);
H: Perianth segments (0 = absent, 1 = present); **I**: Embryo type (0 = Cyperus, 1 = Schoenoplectus, 2 = Fimbristylis, 3 = Scirpus, 4 = Eleocharis, ? = Unknown); **J**: Gynophore (0 = absent, 1 = present)

period). Trees sampled from the 'burn in' phase were discarded from the analysis before calculating the posterior probabilities (PP). Tracing of morphological character evolution was done using maximum likelihood in Mesquite (Maddison & Maddison, 2006) based on the parsimony tree.

RESULTS

Phylogeny

For the parsimony analysis, the combined matrix had 2506 constant characters, 656 variable characters (which were parsimony un-informative) and 823 parsimony informative characters. Gaps were treated as missing. The analysis gave 91 equally parsimonious trees of length 3622. The consistency index (CI) was 0.579 and the retention index (RI) was 0.684. One of the trees is presented in Figure 2, showing the branch lengths above the lines and the bootstrap values below the lines. From the Bayesian analysis, out of 10000 trees generated, 2000 trees were discarded and the posterior probabilities were calculated on the remaining 8000 trees. 50% majority rule was used for the posterior probabilities. One of the Bayesian trees obtained is shown in Figure 3, showing the posterior probabilities in percentages above the lines. For both the parsimony and the Bayesian analysis, the trees were rooted using *Eriophorum vaginatum* as the outgroup. The two trees (Fig 2. and Fig 3.) show similar topologies, with a few differences in the placement of some taxa. For descriptions of the main features of the phylogeny, I will refer to the Bayesian tree (i.e. Figure 3.).

As Figure 3 shows, the results of the combined matrix show all taxa belonging to tribe Cypereae except *Isolepis humilima* forming a very strongly supported clade (PP = 98%). *Isolepis humillima* is embedded within the *Schoenoplectus* clade (PP = 100%) as a sister taxon to *Schoenoplectiella articulata*. The *Cyperus* clade (PP = 98%) is a sister clade to the *Isolepis-Ficinia-Scirpoides* clade. The *Cyperus* clade is further split, into a C_3 grade (including *Androtrichum, Courtoisina, Kyllingiella* and *Oxycarium*) and a C_4 clade (including *Lipocarpha, Kyllinga, Alinula, Pycreus, Ascolepis, Sphaerocyperus, Remirea, Volkiella,* and *Queenslandiella*). Within the C_4 *Cyperus* there is *Volkiella disticha* which has a C_3 Photosynthetic pathway (according to Bruhl & Wilson, in press). *Cyperus* as resolved in this phylogeny is not monophyletic. Within the chlorocyperoid clade, is embedded taxa in the genera *Alinula, Lipocarpha, Ascolepis, Pycreus, Remirea, Sphaerocyperus, Kyllinga, Quenslandiella, and Volkiella. Cyperus dichroostachyus* (a C_3 Cyperus) is embedded within a clade consisting of *Androtrichum, Courtoissina and Oxycarium*, which also have a C_3 photosynthetic pathway and are forming a sister clade to the C_4 *Cyperus* clade. *Queenslandiella* is embedded within *Kyllinga*; a strongly supported clade (PP = 91%).

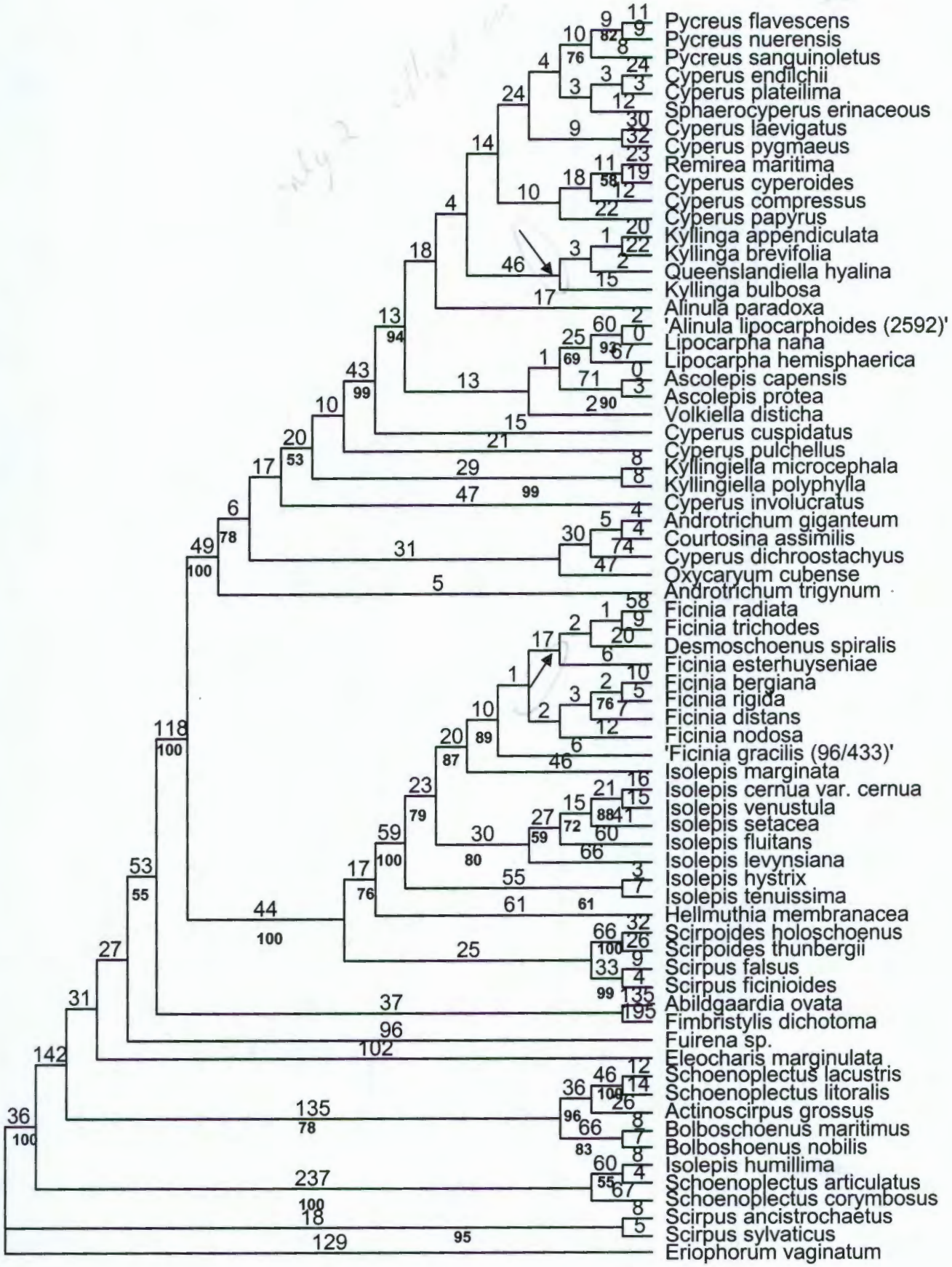


Figure 2. One of the trees obtained from analysis of the combined matrix using parsimony, showing relationships among the Cypereae. The arrows show nodes which collapse in the strict consensus tree.numbers above branches are branch lengths and Numbers below branches are bootstrap percentage.

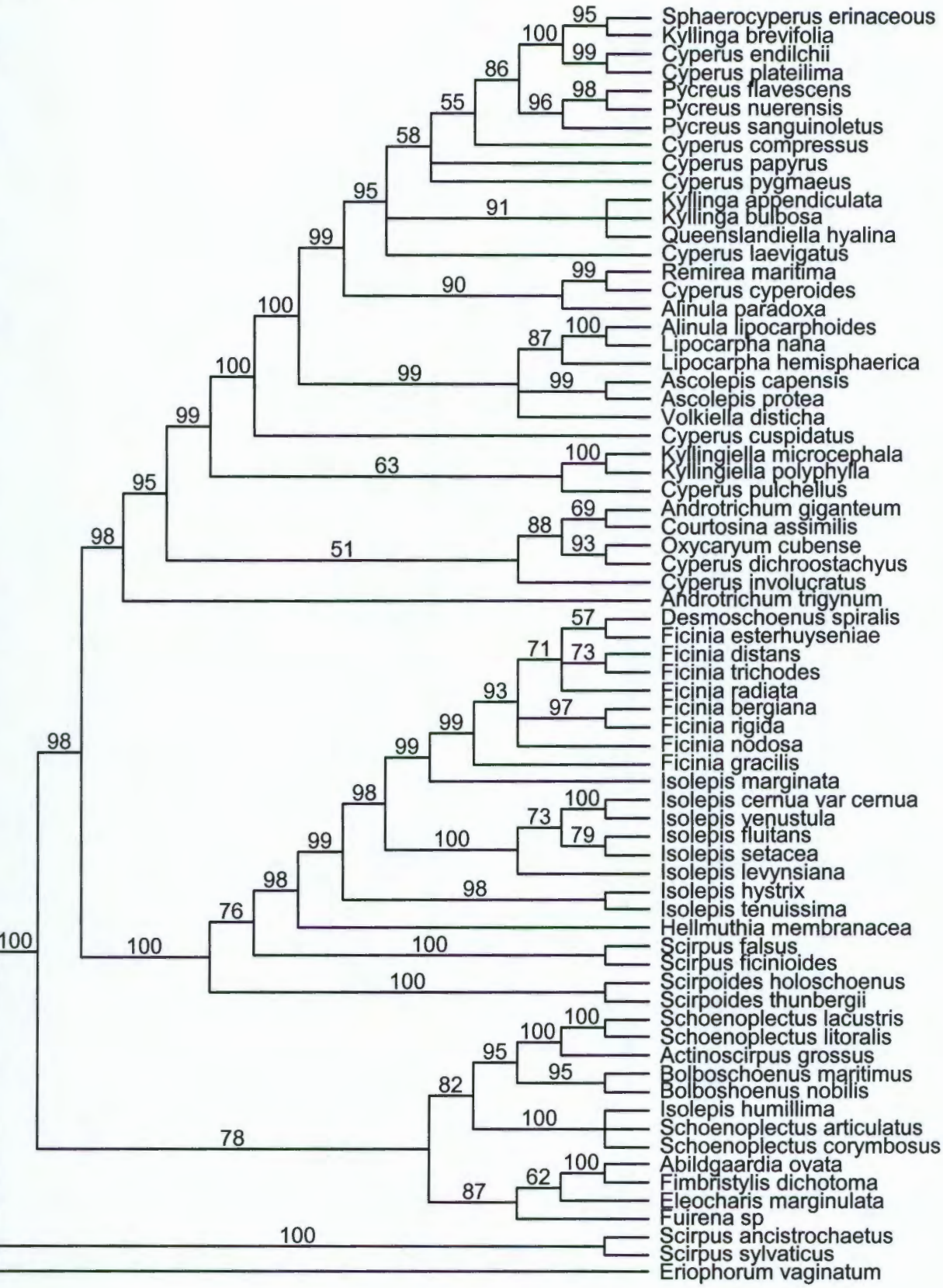


Figure 3. The majority rule consensus tree obtained from Bayesian analysis of the combined matrix, showing relationships among Cypereae. The numbers on top of branches are the posterior probabilities.

Isolepis marginata is embedded within the *Ficinia* clade which is sister to the rest of *Isolepis*. *Scirpus falsus* and *Scirpus ficinoides* are embedded within the *Isolepis- Ficinia- Scirpoides* clade while *Scirpus ancistrochaetus* and *Scirpus sylvaticus* are resolved as sister to the outgroup (*Eriophorum vaginatum*)

Morphological characters

The morphological characters studied and how they vary in the different taxa are as shown in Table 4. Ancestral trait reconstructions for these characters are shown in Figure 4. Reconstruction showed that the perennial habit is the ancestral state in the Cypereae (probability = 0.99) and annual habit has arisen independently multiple times [Figure 4 (a)]. In terms of the number of florets per spikelet, having many florets is the ancestral state (probability = 0.99) and having only one floret per spikelet is a unique character of the *Alinula- Lipocarpha- Ascolepis* clade, including *Alinula paradoxa* [Figure 4 (b)]. In terms of glume arrangement, the spiral state is ancestral (probability = 0.94) and the distichous state has arisen predominantly in the *Cyperus* clade [Figure 4 (c)]. The *Ficinia- Isolepis- Scirpoides* clade has retained the ancestral state of having a spirally arranged glume with some members of *Ficinia* and *Isolepis* having distichous glumes (e.g. *Ficinia distans* and *Isolepis levynsiana*). For dispersal unit, the ancestral state is shedding a nutlet (probability = 0.99) and it has been retained in most genera. The derived character of shedding the whole spikelet has arisen in the *Cyperus* clade but there is a switch back to the ancestral state observed in some members of this clade such as *Cyperus* and *Pycreus,* which could also be interpreted as convergent independent evolution [Figure 4 (d)]. Lateral nutlet orientation has arisen only twice: in *Pycreus* and *Kyllinga*; [Figure 4 (e)] otherwise the other genera have retained the ancestral state (dorsiventral, probability = 0.99). The ancestral state in terms of style branching is three (probability = 0.99), and the possession of two style branches has arisen independently many times within the phylogeny [Figure 4 (f)].

The photosynthetic pathway is predominantly C_3 (which is the ancestral state, probability = 0.99) and C_4 has arisen twice: in the *Cyperus* clade and the *Fimbristylis* clade [Figure 4 (g)]. However, *Volkiella* is embedded within the C_4 *Cyperus* clade which could represent a switch from C_4 to C_3. The absence of perianth segment which is the ancestral state (probability = 0.99), is shared by all members of tribe Cypereae (but presence of perianth segments arises independently in *Scirpus falsus* and *Scirpus ficinoides*) and the presence of perianth segments has evolved independently multiple times in other lineages [Figure 4 (h)]. In terms of embryo type, the *Cyperus* type is ancestral to the Cypereae (probability = 0.99). The *Eleocharis* embryo appears as having arisen from the *Schoenoplectus* type of embryo. *Isolepis humilima* whose embryo type has not yet been defined is embedded within the *Schoenoplectus* clade [Figure 4 (i)]. The absence of a gynophore is the ancestral state (probability =

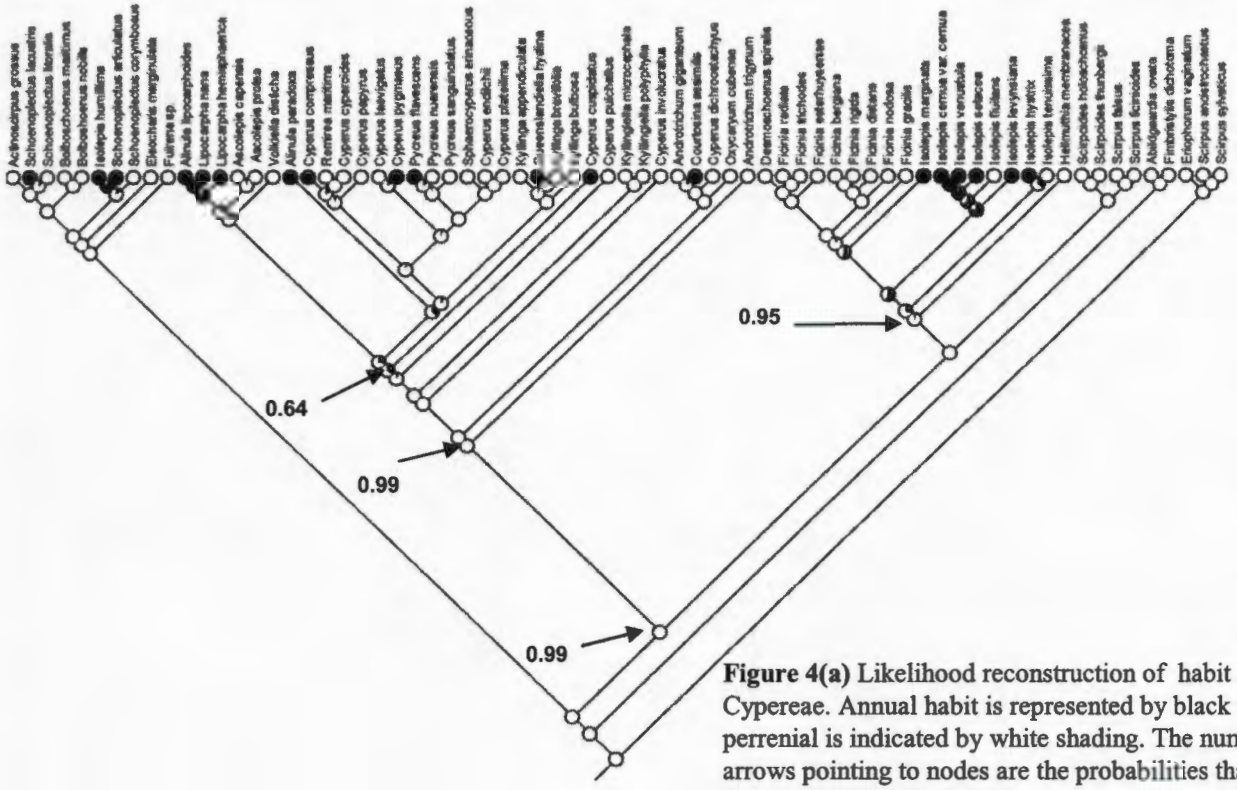


Figure 4(a) Likelihood reconstruction of habit in Cypereae. Annual habit is represented by black while perrenial is indicated by white shading. The numbers with arrows pointing to nodes are the probabilities that the the ancestor at that node was perennial.

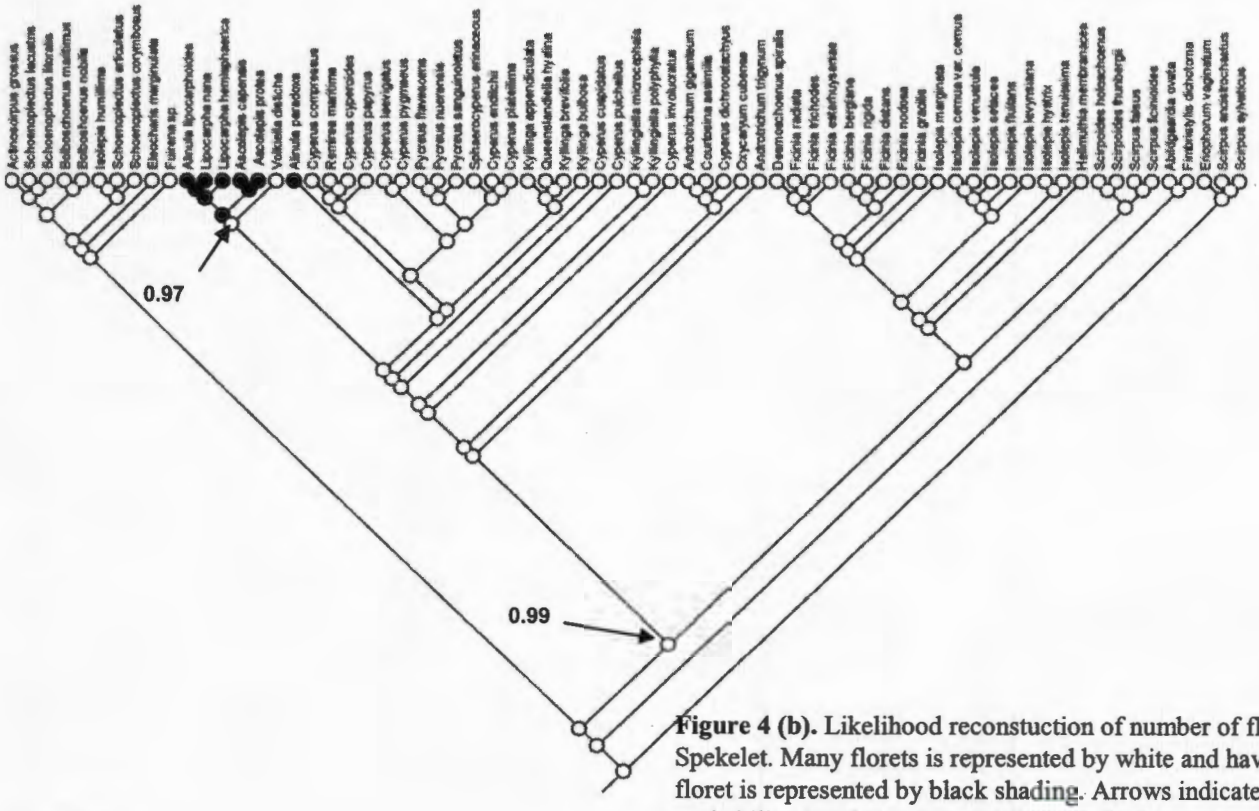


Figure 4 (b). Likelihood reconstuction of number of florets per Spekelet. Many florets is represented by white and having one floret is represented by black shading. Arrows indicate the probability that the ancestor at the node had many florets.

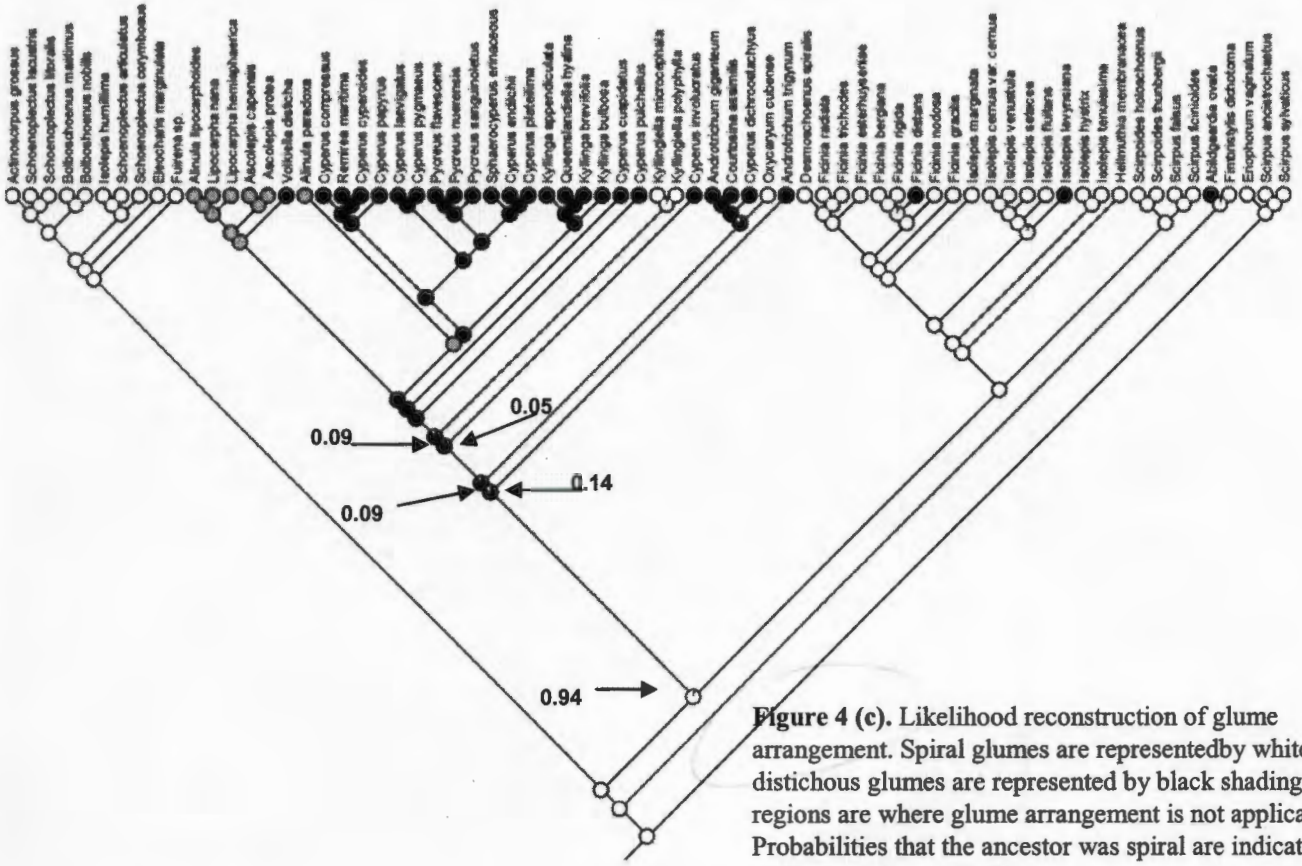


Figure 4 (c). Likelihood reconstruction of glume arrangement. Spiral glumes are representedby white and distichous glumes are represented by black shading. Grey regions are where glume arrangement is not applicable. Probabilities that the ancestor was spiral are indicated by arrows pointing at nodes.

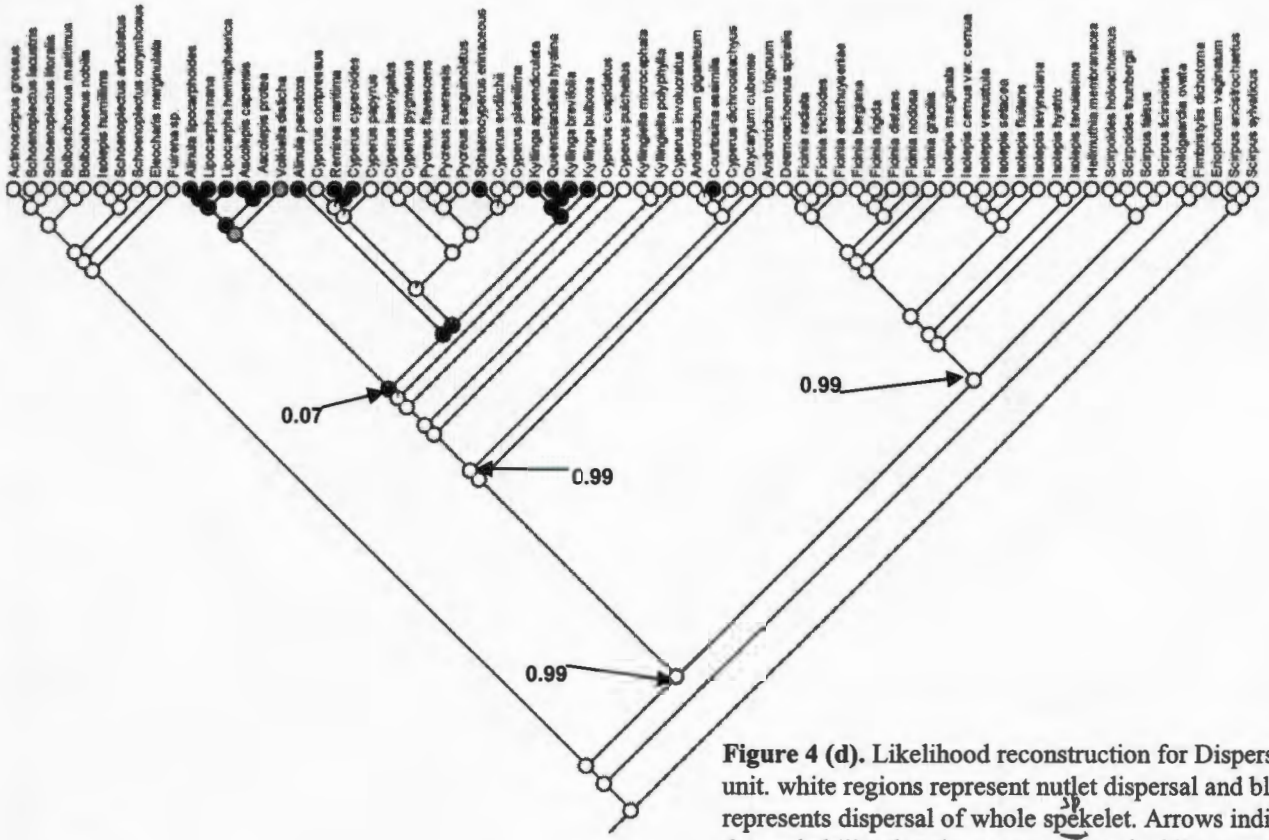


Figure 4 (d). Likelihood reconstruction for Dispersal unit. white regions represent nutlet dispersal and black represents dispersal of whole spekelet. Arrows indicate the probability that the ancestor was shedding a nutlet.

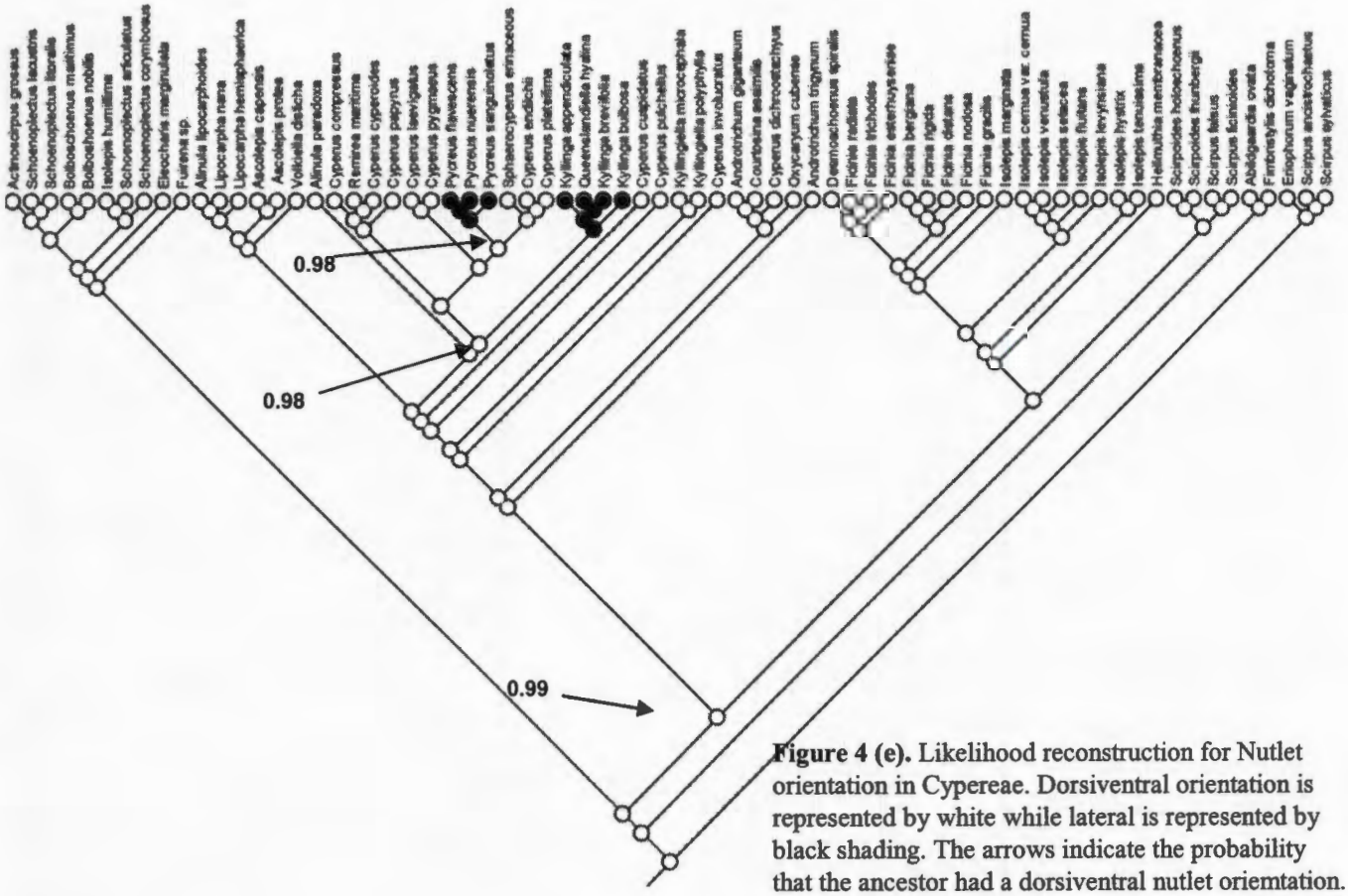


Figure 4 (e). Likelihood reconstruction for Nutlet orientation in Cypereae. Dorsiventral orientation is represented by white while lateral is represented by black shading. The arrows indicate the probability that the ancestor had a dorsiventral nutlet oriemtation.

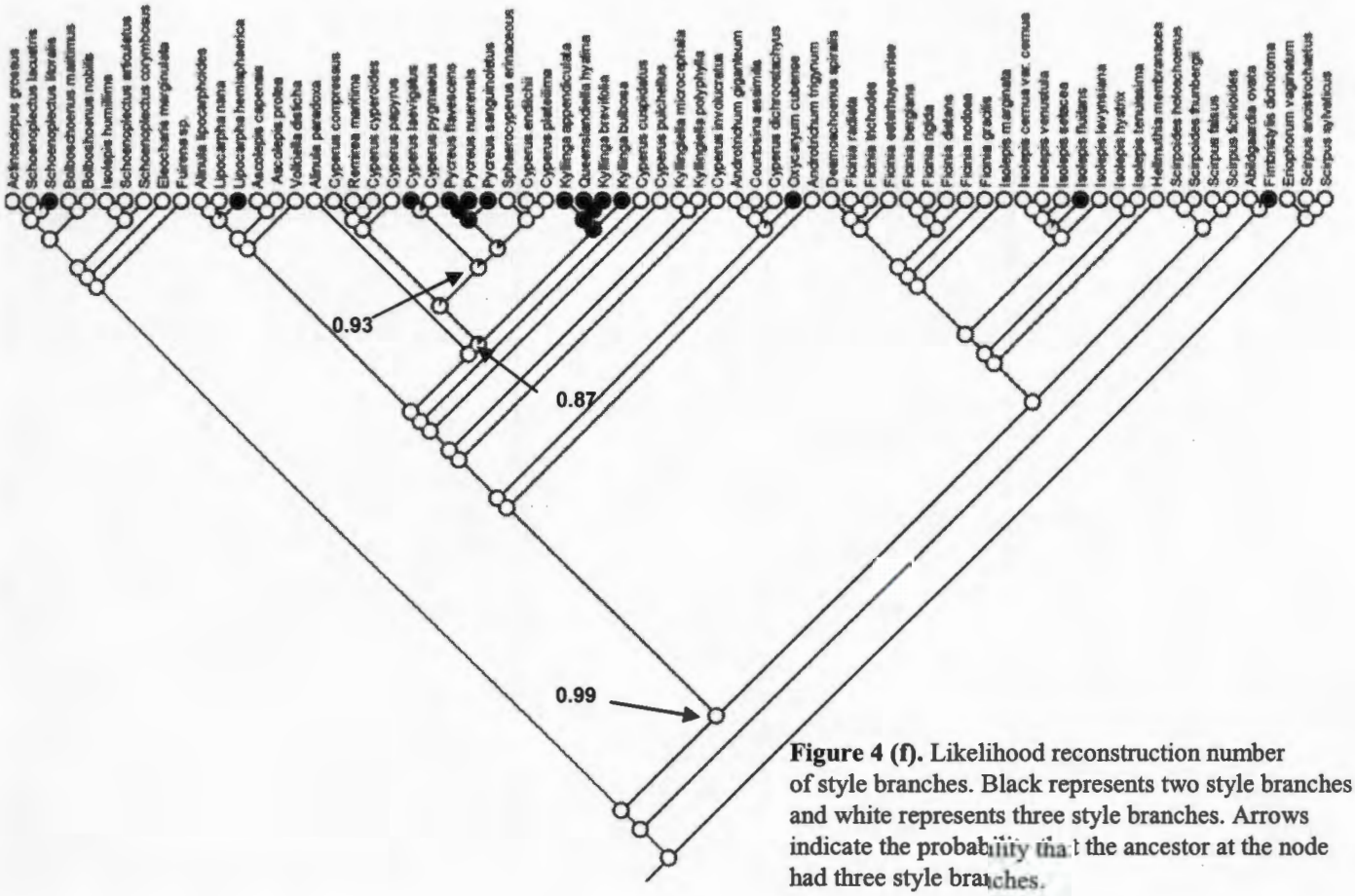


Figure 4 (f). Likelihood reconstruction number of style branches. Black represents two style branches and white represents three style branches. Arrows indicate the probability that the ancestor at the node had three style branches.

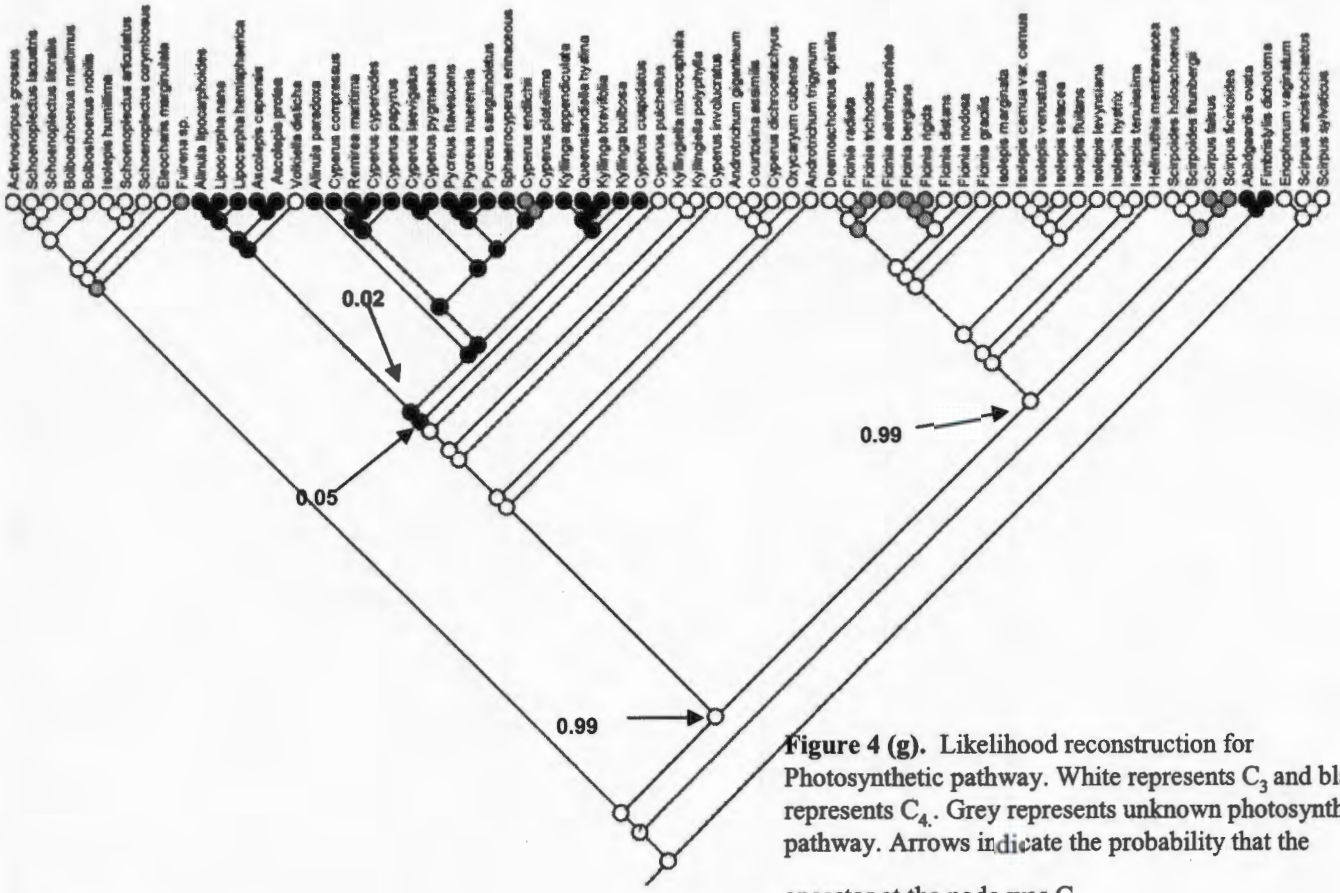


Figure 4 (g). Likelihood reconstruction for Photosynthetic pathway. White represents C_3 and black represents C_4. Grey represents unknown photosynthetic pathway. Arrows indicate the probability that the ancestor at the node was C_3.

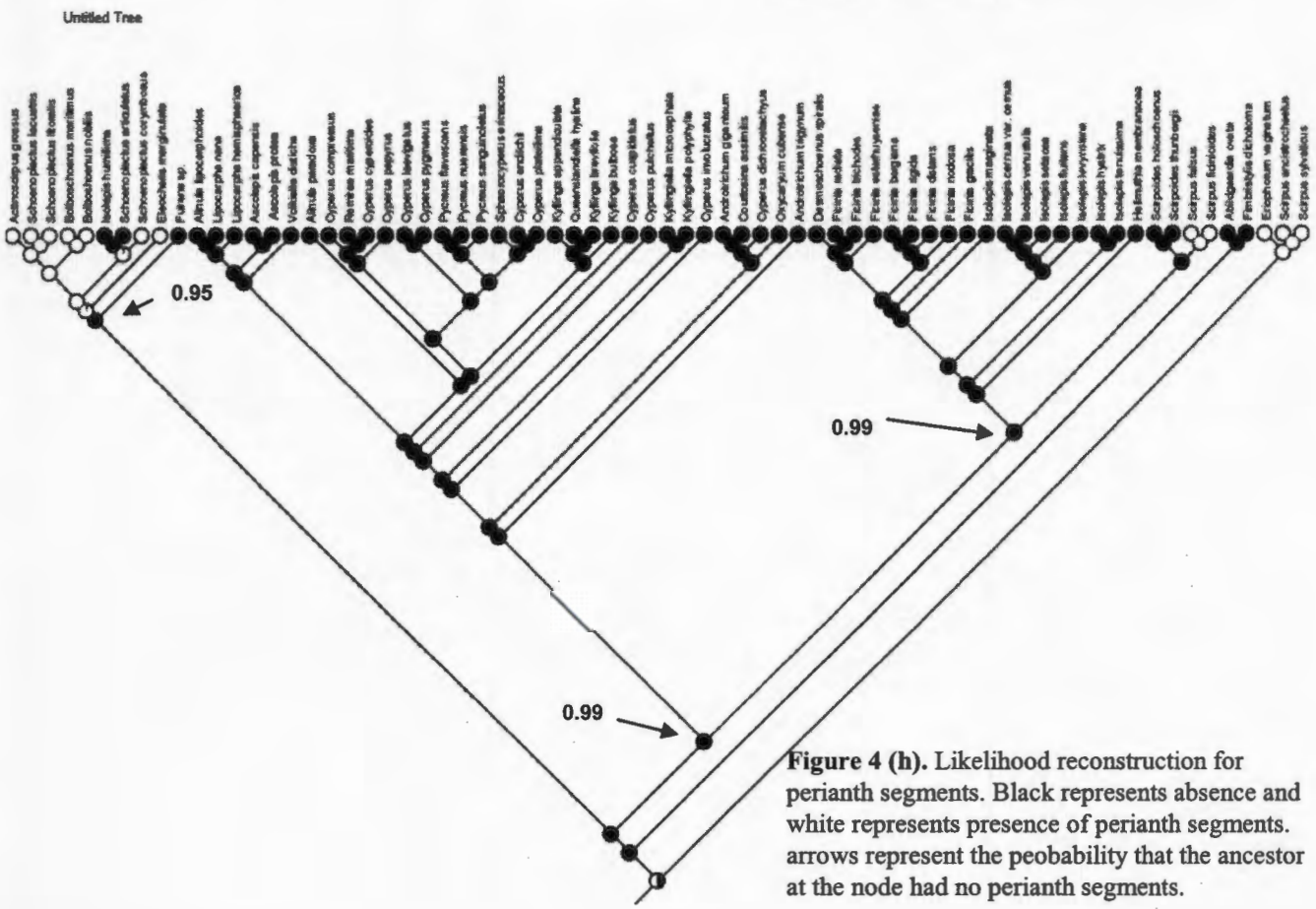


Figure 4 (h). Likelihood reconstruction for perianth segments. Black represents absence and white represents presence of perianth segments. arrows represent the peobability that the ancestor at the node had no perianth segments.

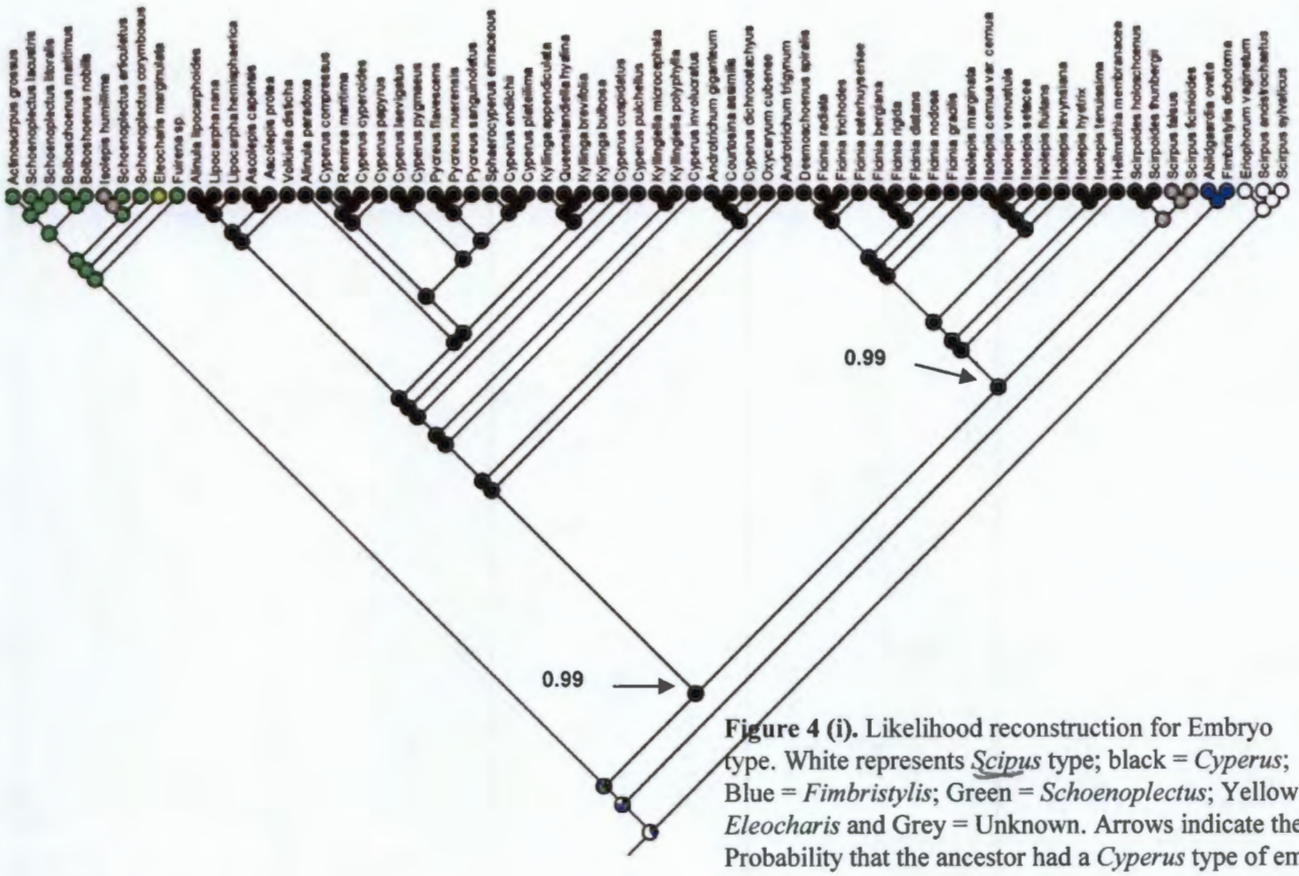


Figure 4 (i). Likelihood reconstruction for Embryo type. White represents *Scipus* type; black = *Cyperus*; Blue = *Fimbristylis*; Green = *Schoenoplectus*; Yellow = *Eleocharis* and Grey = Unknown. Arrows indicate the Probability that the ancestor had a *Cyperus* type of embryo.

Untitled Tree

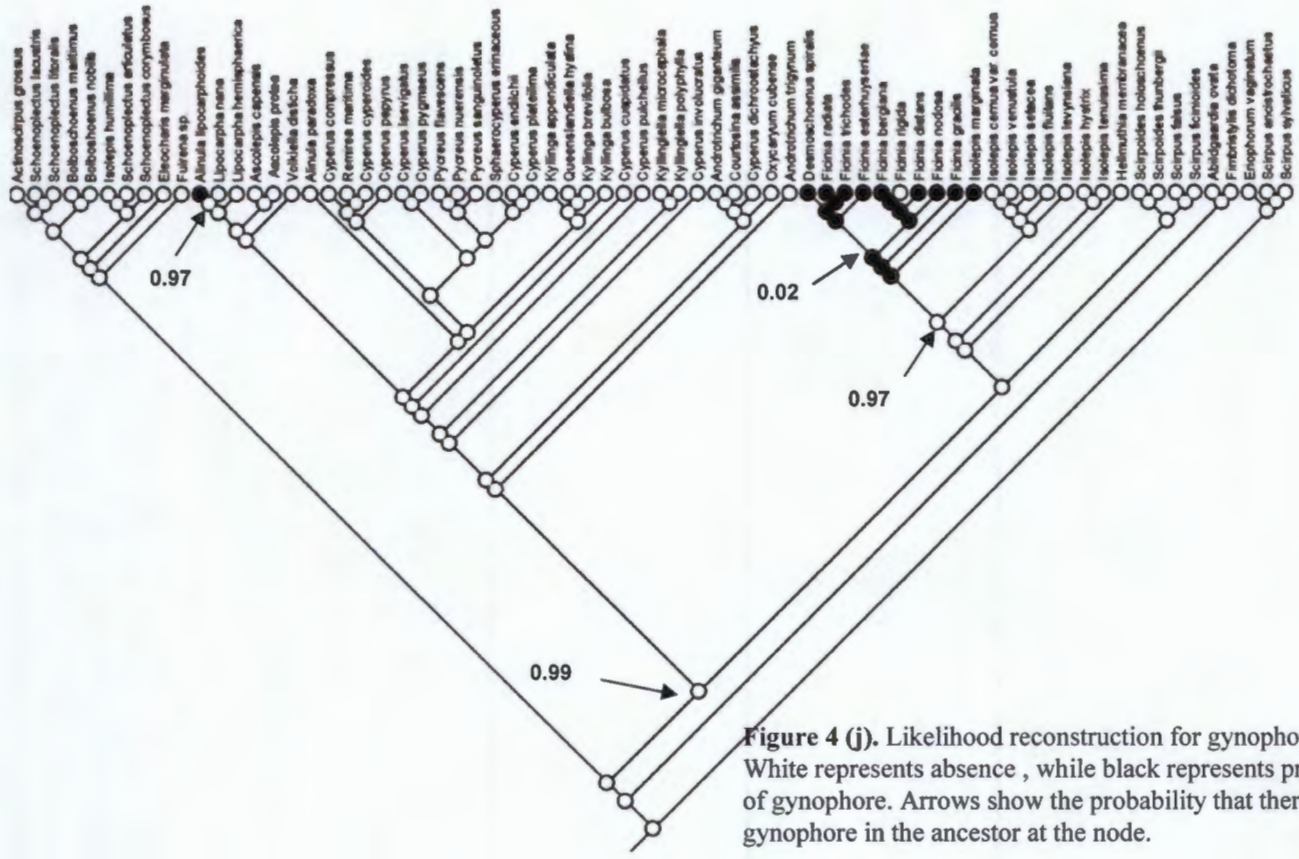


Figure 4 (j). Likelihood reconstruction for gynophore. White represents absence , while black represents presence of gynophore. Arrows show the probability that there was n gynophore in the ancestor at the node.

Untitled Tree

0.99) and the presence of a gynophore has arisen mainly in the *Ficinia* clade but *Ficinia distans* shows a secondary loss of this character state. *Alinula lipocarphoides* has independently evolved the presence of a gynophore [Figure 4 (j)].

DISCUSSION

Phylogenetic relationships

The strict consensus tree (parsimony analysis) and the majority rule consensus tree (from Bayesian analysis) obtained from this study show a similar topology of the phylogeny of the Cypereae as other studies using chloroplast DNA data (Muasya *et al.* 1998; 2001; 2002). This is in spite of the fact that for this study nuclear DNA as well as morphological characters were included in the matrix. (Nevertheless, the nuclear DNA used for this study did not amplify well for all the C_4 *Cyperus* and therefore there were no nuclear DNA sequences for some taxa in our analysis). Our tree (Figure 3) shows three distinct clades, namely, the *Ficinia-Isolepis-Scirpoides* clade; the *Cyperus sl.* clade; and the *Schoenoplectus* clade. The *Ficinia-Isolepis-Scirpoides* clade is sister to the *Cyperus* clade. This is as expected because they are members of the same tribe (Cypereae). In my discussion I will focus only on the members of the tribe Cypereae, as defined by Goetghebeur (1998) to include all taxa that share the presence of *Cyperus* type of embryo.

Isolepis- Ficinia- Scirpoides clade

This clade is characterized by spirally arranged glumes with the exception of a few taxa (e.g. *Ficinia distans* and *Isolepis levynsiana*) whose glume arrangement is distichous. All genera in this clade share the ficinioid morphology, e.g. tufted perennials, spiral glume arrangement, and have a centre of diversity in the Cape floristic region of South Africa (Goetghebeur, 1998; Muasya & Simpson, 2002; Muasya, 2005). The genera in this clade are differentiated as follows: *Ficinia* is diagnosed by the presence of a gynophore and ligule (This also includes *Desmoschoenus*.); *Helmuthia* has two or three scales in the lower florets while, *Isolepis* includes predominantly annual species with spiral glume arrangement and *Scirpoides* has perennial growth form and spiral glume arrangement (Muasya *et al.* in press).

However, there are some overlaps in morphological characters which cause the delimitation of genera difficult. For example, *Desmoschoenus* is embedded in the *Ficinia* clade (Fig. 2 & Fig. 3). This monotypic genus which has morphological features typical of *Ficinia* (Goetghebeur, 1998) occurs in New Zealand in the same coastal habitat as *Ficinia nodosa* (Muasya *et al.*, in press) yet it is till being treated as a separate genus from *Ficinia*. Furthermore, *Isolepis marginata,* an annual species which has

a gynophore is resolved as being closely related to *Ficinia* from DNA studies in this study (Fig. 2 & Fig. 3) and other previous studies (Muasya *et al.* 2001; 2002; in press). The two South African species, *Scirpus falsus* and *Scirpus ficinoides*, which have the gross morphology of *Ficinia* but have an additional character of having perianth segments, (Gordon-Gray, 1995) have been placed in *Scirpus* despite that typical *Scirpus* have paniculate inflorescenses and nodded culms. This study resolved these taxa as sister to *Scirpoides* (Fig. 2 & Fig. 3) while the other *Scirpus* (*S. ancistrochaetus* and *S. sylvaticus)* were more related to the outgroup, which suggests that they are not closely related to *Scirpus*. This suggests that either *Scirpus falsus* and *Scirpus ficinoides* need to be put into a new genus of their own or the generic circumscrirption of one of the current genera e.g. *Scirpoides* should be expanded to accommodate them.

The *Cyperus* clade

Several genera are embedded in this clade and their circumscription is defined by a range of morphological characters such as spikelet morphology, dispersal unit and nutlet orientation (Table 4). These genera are normally grouped according to their photosynthetic pathway as C_3 and C_4 (e.g. Muasya *et al.* 2002) but, there are few observable gross morphological characters to separate the species of *Cyperus sensu stricto* with the two kinds of anatomy (Muasya *et al.,* in press).

Just like in the *Isolepis- Ficinia- Scirpoides* clade, there are some problems with the circumscription of genera in the *Cyperus* clade. In this study, two species of *Androtrichum* were included, but their resolution in the phylogeny does not show them as sister taxa as expected (Fig. 2 & Fig. 3). This was also observed by (Muasya *et al.* in press). The genus is diagnosed by the presence of elongated stamen filaments that are persistent and are dispersed with the nutlet. The lack of a close link between these taxa suggests that the filaments could be a result of parallel evolution for dispersal considering that both taxa occur in swampy coastal dunes, otherwise the two taxa do not show common ancestry. In fact according to Goetghebeur (1998) *Androtrichum giganteum* is not convincingly an *Androtrichum*.

In the C_4 Cyperus clade are embedded a number of monotypic genera or genera with a few species (*Queenslandiella, Sphaerocyperus, Remirea, Volkiella,* and *Alinula*) which are separated from the larger genera by a combination of characters. For example, *Kyllinga*, *Pycreus* and *Queenslandiella* have laterally flattened nutlets. This study resolved *Queenslandiella* within the *Kyllinga* clade (Fig. 2 & Fig. 3), which suggests that this genus needs to be sunk into *Kyllinga* especially because its morphology resembles that of *Kyllinga.* However, it has anthellate inflorescence, while *Kyllinga* has capitate inflorescence, and it is annual while a majority of *Kyllinga* species are perennial. Therefore, describing it

as a *Kyllinga* might not be appropriate. The genus *Alinula* also shows the need for reclassification. Its two species considered in this study are not resolved as sister taxa. *Alinula lipocarphoides* as the name suggests, is resolved as sister to *Lipocarpha nana* with a very strong posterior probability (100%) while, *Alinula paradoxa* is resolved as sister to another clade. *Alinula lipocarphoides* has only one floret per spikelet, a character only possessed by *Lipocarpha* and *Ascolepis*. It also has a gynophore, a character which is considered unique to *Ficinia*. In contrast, *Alinula paradoxa* has no gynophore, which suggests that it is not closely related to *Alinula lipocarphoides*.

Morphological character evolution and homology

Habit

Habit in this study refers to whether the taxon is annual or perennial [Fig 4(a)]. This character has been used to distinguish *Ficinia* which are annuals from *Isolepis* which are perennials. It has also been used in *Schoenoplectus* whereby the perennials have been placed in *Schoenoplectus* and the annuals in *Schoenoplectiella.* However, as highlighted in the discussion of phylogenetic relationships, this character has caused some classification problems; with *Isolepis marginata* and *Desmoschoenus spirallis*. Furthermore, considering that the annual habit has evolved multiple times independently [Fig 4(a)] reduces the value of habit as a diagnostic character. This shows that it is not a unique character of a particular lineage and therefore cannot be used to unambiguously segregate genera in the Cypereae.

Number of florets per spikelet

This character is shared by members of *Lipocarpha* and *Ascolepis* which are sister clades but, it is also present in *Alinula lipocarphoides* and *Alinula paradoxa* [Figure 4 (b)] perhaps through convergent evolution. Since this character has not arisen anywhere else in tribe Cypereae, it is a useful character for distinguishing these three genera from the rest of the tribe.

Glume arrangement

Distichous glume arrangement has arisen predominantly in the Cyperoid clade [Figure 4 (c)], but there has been a reversal into the spiral glume arrangement in *Kyllingiella* and *Oxycaryum* which have a spiral glume arrangement and thus distinguishes them from the rest of the C_3 *Cyperus*. Otherwise, all other genera share the ancestral state of having a spirally arranged glume. Because of this, this character may be useful only within the *Cyperus* clade unless taken with a combination of other characters. Even though most *Ficinia* have spiral glumes, *Ficinia distans* has a distichous glume. The same applies to the *Isolepis* clade where *Isolepis levysiana* has distichous glume contrary to the rest of *Isolepis*. The recent

transfer of *Isolepis levynsiana* and *Isolepis leucoloma* to *Isolepis* (Archer, 1998; Muasya *et al.* 2007) which had previously been described as *Cyperus* because of their distichous glumes is supported by DNA data from the current study and that of Wiswedel (2006).

Dispersal unit

Evolution of the derived state (dispersing the whole spikelet) has arisen many times within the C_4 *Cyperus* clade and had a switch back to the ancestral state (shedding a nutlet) in some members of the clade. [Figure 4 (d)]

Nutlet orientation

All genera have their nulet oriented dorsiventrally but only *Pycreus, Kyllinga and Queenslandiella* have evolved lateral nutlet orientation[Figure 4 (e)]. As discussed earlier, *Queenslandiella* is embedded within the *Kyllinga.* Given its morphology and considering that it has a character state which is unique to only *Kyllinga* and *Pycreus*, I think it should be recognized as a *Kyllinga.*

Style branching.

As already noted, the ancestral state in terms of style branching in the Cypereae is three. Having two style branches is the derived state which has arisen multiple times independently within the tribe Cypereae[Figure 4 (f)]. This makes style branching to be of little value as a diagnostic character.

Photosynthetic pathway

The ancestral state in the Cypereea is C_3 [Figure 4 (g)]. Multiple independent origins of Kranz anatomy are recorded in several lineages including *Rhynchospora, Eleocharis, Fimbristylis* and *Cyperus* (Muasya *et al.*, 2002; Goetghebeur, 1998; Bruhl & Wilson, in press). Among the Cyperaea, Kranz anatomy has evolved once among the *Cyperus* clade. However there is an unexpected occurrence of C_3 anatomy in *Volkiella*, a taxon currently classified among the C_4 clade. This is the first case in Cypereae where a reversal from C_4 to C_3, is demonstrated. A study by Stock *et al.* (2004) showed that for members of the Cyperaceae, the ecological advantages (high water use efficiency, high nitrogen use efficiency, reduced photorespiration and effectiveness at low carbon dioxide concentrations) conferred by the C_4 pathway in Poaceae do not correlate with the distribution of Cyperaceae in temperate Southern Africa. Considering that *Volkiella* is tropical in distribution, this supports their assertion that the C_4 pathway is not universally beneficial to all taxa but may be a consequence of interactions between the properties of the C_4 pathway and the specific evolutionary history of the group.

Perianth segments

The absence of perianth segments is one character that has been used to define members of tribe Cypereae and this is supported by the results of this study as shown in Figure 4 (h). However, *Scirpus falsus* and *Scirpus ficinoides* do not have this character, which further emphasizes that their classification needs to be revised.

Embryo type

The tribe Cypereae is characterized by having the *Cyperus*-type of embryo [Fig 4 (i)]. The embryo type of *Scirpus falsus* and *S. ficinoides* are not known as yet. From this study, the Cypereae have been resolved to share this common character. However, due to the nature of embryos, they are subject to individual interpretation and therefore are not a good character for segregating genera. For example, *Isolepis humillima* placed in *Isolepis* due to the presence of a spiral glume arrangement has been interpreted to have an embryo similar to the *Scirpoides* (Wilson, 1981). This taxon according to this study (Fig. 2 & Fig. 3) and others (e.g. Muasya *et al.* in press; Wiswedel, 2006) is placed among the clade having a *Schoenoplectus*- type of embryo.

Gynophore

The gynophore is formed by the development of the hypogynous stalk characterized by a lobed cup that envelopes the basal part of the nutlet (Vrijdaghs *et al.* (2005). This structure has evolved predominantly in the *Ficinia* clade and it varies in size and shape between species. However, some *Ficinia* species lack a gynophore while on the other hand some *Isolepis* (e.g. *Isolepis marginata*) have a rudimentary gynophore (Muasya *et al.* in press). It is also present in *Alinula lipocarphoides*, a taxon previously described in *Ficinia* and later transferred to *Alinula* (Kukenthal, 1936; Raynal 1977) here resolved in the *Cyperus* clade as sister to *Lipocarpha*. Considering that *Desmoschoenus* which is resolved as embedded within the *Ficinia* clade and has a gynophore, and also having noted earlier that it has similar morphology and habitat with other *Ficinia*, I see no reason why it should not be recognized as a *Ficinia*. Nevertheless, the value of the gynophore as a diagnostic feature of *Ficinia* is reduced when considering that it has arisen elsewhere in tribe Cypereae.

CONCLUSIONS

This is the first study in which the phylogeny of tribe Cypereae has been reconstructed based on DNA (nuclear and plastid) and morphology using both parsimony and Bayesian analysis. The results showed all members of the tribe resolved in a strongly supported clade defined by the *Cyperus* type of embryo

and the absence of perianth segments. However, *Cyperus sensu stricto.* is not monophyletic. These findings are similar to those of previous studies. As highlighted earlier, the classification of *Cyperus sensu lato* is associated with many different opinions, with some recognizing *Cyperus* as a large genus with several subgenera (e.g. Kukenthal, 1936; Haines & Lye, 1983) and some splitting it into several genera (e.g. Bruhl, 1995; Goetghebeur, 1998). The present study shows *Cyperus sensu stricto* to be paraphyletic. I therefore see recognizing *Cyperus* as a large genus with the various segregate genera as subgenera within *Cyperus* as the way towards a monophyly. Nevertheless, considering that this makes *Cyperus* a very large genus, which may reduce taxonomic clarity, perhaps the classification by Goetghebeur (1998) (see Table 1) is by far the most convenient classification until all phylogenetic relationships have been fully resolved.

This study has highlighted some problems with the circumscription of genera in the Cypereae and it has made it clear that the classification needs to be revised. However, I still feel that more sampling is needed in order to have a more thorough resolution of the relationships between the genera. The incorporation of nuclear DNA and morphology has shown the potential to give a better resolution than previous studies and this suggests that further analyses with greater sampling will yield more useful results to guide in the classification. Therefore we still need to explore more DNA regions and given that most of the C_4 *Cyperus* did not have ITS sequences incorporated into the matrix, this area still needs to be explored as well as other nuclear regions such as ETS.

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