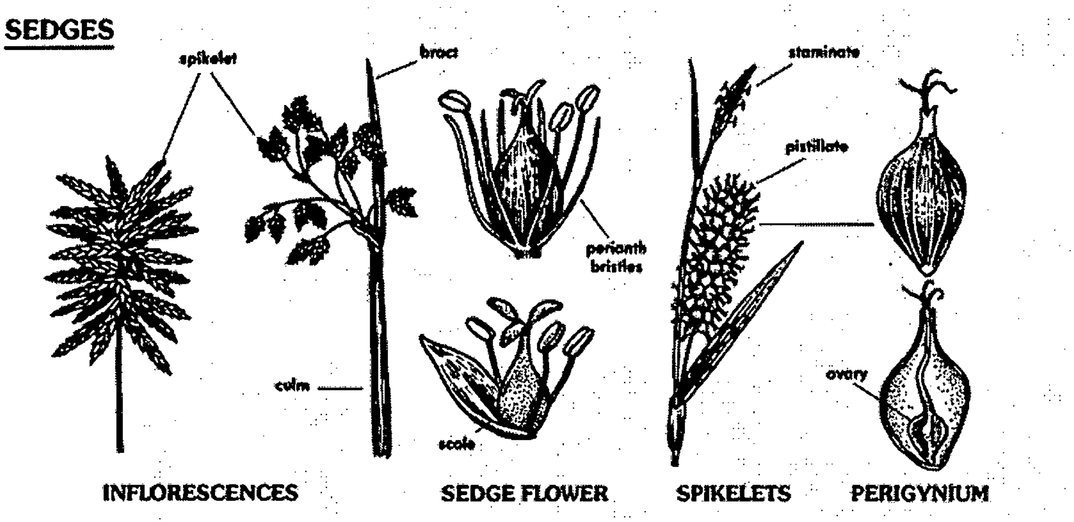
Classification and Evolutionary history in Cyperaceae.



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KD SKEL

Abstract:

of what group?

Do not start with 24. Twenty-four.

An analysis of 107 taxa representing 85 genera from 14 tribes, sequenced for the plastid *rbcL, trnL-F* (intron and intergenic spacer) and *rps16* intron is presented. 24 species were sequenced for the first time in the *rps16* intron region of the plastid genome in an attempt to further resolve the phylogenetic relationships within Cyperaceae. Cyperaceae are monophyletic and resolved into two subfamilies, the Mapanioideae and Cyperoideae. The overall topology is similar to those observed in other studies, with a few exceptions. Our study suggests that *Cladium* should be treated as a separate tribe, the Cladieae. Sclerieae is observed as sister to Schoeneae. *Fuirena* is observed here as sister to either Eleocharideae or Abildgaardieae whereas the rest of Fuireneae form a grade and are ? closer to Cypereae. Our robust phylogeny allowed us to conduct phylogenetic optimizations of habitat preferences and adaptive character states, which enabled us to investigate the evolutionary history of the family. Cyperaceae most probably evolved in permanently moist habitats in tropical regions. The Cyperaceae have since radiated into a number of other habitats, notably open grassland, woodland and savannah. Diversification into these habitats was likely to have been influenced or facilitated by morphological features such as a change in the number of functional stamens or a switch to an annual life history.

Introduction to Cyperaceae:

The Cyperaceae is the third largest monocotyledonous family, after Orchidaceae and Poaceae. It contains 109 genera and approximately 5500 species (Muasya *et al.* in press). The Cyperaceae have an almost cosmopolitan distribution only being absent from Antarctica (Goetghebeur 1998). The species occur in a variety of habitats, although they are mostly dominant in many wetland ecosystems (Muasya *et al.* in press and Simpson *et al.* 2003). About 35% of the genera are monotypic, 26% have two to five species while 6% of the genera have over 200 species (Muasya *et al.* in press). *Carex* (1757 species) and *Cyperus* (686 species) are the largest genera (Muasya *et al.* in press).

Classification of the Cyperaceae

No other refs here.

Historically family-level phylogenetic studies of the Cyperaceae have largely made use of morphological data (Muasya *et al.* in press). The determination of relationships within Cyperaceae on morphological grounds is difficult, largely due to the presence of highly reduced flowers and condensed inflorescences (Muasya *et al.* in press). In particular subfamily and tribal delimitation has been particularly inconsistent (Muasya *et al.* in press). For example Goetghebeur (1998) recognized four subfamilies: the Sclerioideae, Mapanioideae, Caricoideae and Cyperoideae. Bruhl (1995) on the other hand only recognized two subfamilies: the Caricoideae and the Cyperoideae. The two studies also differed in their tribal circumscription, as Bruhl (1995) recognized 12 tribes, while Goetghebeur (1998) recognized 14 tribes.

Over the last 10 years a number of molecular studies have improved our knowledge of the phylogeny of the family (e.g. Muasya *et al.* in press). DNA sequencing is assisting tremendously in building a better understanding of intra-familial relationships of the Cyperaceae (Simpson *et al.* 2003). The first family-wide DNA based phylogenetic tree was conducted by Muasya *et al.* (1998) using plastid DNA from the *rbcL* gene region. Subsequent broad suprageneric studies have so far sampled all subfamilies and tribes, although sampling effort is not evenly distributed among all tribes (Muasya *et al.* in press). Family-level studies have been based mainly on *rbcL* sequence data, which is a relatively slowly evolving plastid marker (Muasya *et al.* in press). Other plastid and nuclear regions have been used in tribal or subfamilial level studies (Muasya *et al.* in press). Studies of the subfamily Mapanioideae (e.g. Simpson *et al.* 2003) and a number of other tribes or genera have been based mainly upon the plastid regions *rps16* intron, *trnL* intron and *trnl-F* intergenic spacer (e.g. Verboom 2006). Slowly evolving plastid regions, such as *rbcL,* provide less resolution at the tribal or generic level than more rapidly evolving markers, such as *rps16* intron, although they are more easily aligned across a family (Muasya *et al.* in press). The choice of a slowly evolving marker in the majority of studies of Cyperaceae may account for poor resolution of the relationship between a number of clades, such as Fuireneae and Schoeneae (Muasya *et al.* in press).

Further, few studies have combined multiple molecular marker data sets or molecular and morphological data sets (e.g. Muasya *et al.* 2000a). One potential solution to poor resolution is to combine data from the same DNA regions (e.g. *rbcL, trnl-F* and *rps16*) for similar taxa (Muasya *et al.* in press). Muasya *et al.* (in press) also suggested that greater sampling effort is required to elucidate the phylogenetic relationships within certain ? trobes, such as Cryptangineae, Bisboeckelerieae, Fuireneae, Schoeneae and Sclerieae (Muasya *et al.* in press). In Fuireneae there is a need for greater resolution of the relationship between the *Schoenoplectus* and *Actinoschoenus* and the *Schoenoplectiella* groups, which have been shown to form a polytomy with Cypereae (Muasya *et al.* in press). The relationship between four clades (*Cladium, Gymnoschoenus, Gaustis* to *Didymiandrum* and the *Rhynchospora* clades) is unresolved and requires further investigation (Muasya *et al.* in press). Not clear what you try to do.

The evolutionary history of Cyperaceae

A robust phylogeny allows one to gain an understanding of the evolutionary history of morphological characters and ecological states of a taxon (e.g. Linder and Rudall 2005). Linder and Rudall (2005) conducted phylogenetic optimizations of habitat preferences and adaptive character states, which enabled them to investigate the factors that have influenced the evolution of the Poales. They included a number of morphological characters, such as the growth form, type of rooting system, the thickness of trichomes, photosynthetic pathway and the presence or absence of annual or perennial life history

(Linder and Rudall 2005). Their study included a single terminal for the Cyperaceae and thus a more extensive sampling effort is required to further evaluate the evolutionary history of the Cyperaceae.

There are a number of interesting and informative characters that one could include in an evaluation of the evolutionary history of the Cyperaceae (Goetghebeur 1998). The Cyperaceous flower is generally a bisexual trimerous structure with 3 + 3 perianth parts, 3 (+ 3) stamens and a 3-carpellate pistil with a single, basal, anatropous ovule, although numerous variations occur (Goetghebeur 1998). The number of stamens varies from 0 (in female flowers) to 1-2-3-6 or more (Goetghebeur 1998). Other traits relevant to the Cyperaceae include the structure of inflorescence, the sexuality of the flower – is it unisexual (dioecious) or bisexual (monoecious) – and the presence or absence of a perianth (Simpson et al. 2003). Goetghebeur (1998) reported that the perianth structure is extremely variable in Cyperaceae. Bruhl and Wilson (in press) provide an up to date review of the distribution of C_3 and C_4 photosynthetic pathways in Cyperaceae, the latter of which has been suggested to have evolved multiple times independently. Among the studied 107 genera 91 were consistently C_3, 11 were consistently C_4 and a few (5) contained species with both C_3 and C_4 (Bruhl and Wilson in press).

91 + 11 = 102

Simpson *et al.* (2003) investigated pollen development in the Mapanioideae and a few related taxa to infer pollen type. They reported finding at least two-types of pollen, the Mapanioid-type and "pseudomonad" pollen (Simpson *et al.* 2003). Simpson *et al.* (2003) also investigated the distribution of each pollen type within the family and found that the Mapanioid type is restricted to a few taxa in the tribe Hypolytreae, while the rest of the Cyperaceae had the pseudomonad type. The evolutionary significance of each type is not yet fully understood but there are some indications that pseudomonad pollen might be an adaptation to wind-pollination, as the shape is fairly streamlined. Simpson *et al.* (2003) reported that the Mapanioid pollen is stickier than pseudomonad pollen, which together with floral morphology and habit indicate that Mapanioid pollen may be an adaptation to animal dispersal. While most of the Cyperaceae is adapted to anemophily, a few species are known to be visited by pollen-gathering insects or have traits indicating potential pollination by insects (Simpson *et al.* 2003 and Goetghebeur 1998). It has even been suggested that entomophily may be linked to the forest habitat, as wind velocity is much reduced and alternative pollen vectors may be required (Goetghebeur 1998).

In an investigation of the evolutionary history of the Poales, Linder and Rudall (2005) included habitat ecology as one of the characters of interest. They reported that it is likely that ancestral Poales grew in marshy or wet habitats, although the extant groups of Poales still found in this habitat are mostly species poor (Linder and Rudall 2005). Species of a few genera, such as *Prionium* can still be found to dominate wetland habitats and most of the sedge families include at least a few species that prefer wetland habitats (Linder and Rudall 2005). Despite this it is uncertain whether these species represent reversals or the ancestral state within Cyperaceae (Linder and Rudall 2005). The majority of Cyperaceae species are known to have a perennial life history, while the annual species are restricted to a limited number of genera (Goetghebeur 1998). Despite this no study has investigated the likely state of the ancestral sedge.

Aims and objectives:

The aim of this study is to reconstruct a robust phylogeny of the family Cyperaceae, using three of the most commonly used plastid gene regions (*rbcL, trnl-F* and *rps16* intron) and morphological characters. Subsequently we will use this phylogeny to review the current suprageneric classification of the family. In particular we will attempt to provide greater resolution to the relationship between a number of poorly resolved clades, such as Cryptangineae, Bisboeckelerieae, Fuireneae, Schoeneae and Sclerieae by providing sequences of previously unsequenced taxa (table 1). Newly sequenced taxa will be added to sequence data lodged in GenBank from previous studies that have used the same plastid gene regions.

wrong [illegible]

A second objective is use the robust phylogeny to reconstruct the evolutionary pattern of key morphological and ecological characters. We aim to conduct character optimization analyses which will allow us to infer the likelihood of each state at specific nodes. This will allow us to evaluate what the ancestral state was likely to be and to trace the evolution of particular traits within and between clades.

Summary of research questions:

1) Are we able to provide greater resolution to poorly resolved nodes?

2) If we are able to provide greater resolution, is the current classification of the Cyperaceae in need of revision?

3) Can we trace the evolutionary history of specific characters within Cyperaceae?

4) Are we able to infer the likely state of the ancestral sedge using our phylogeny?

Methods:

A total of 107 taxa were included in this study. They consist of two outgroups and 105 Cyperaceae species. The outgroup taxa were a species from the Juncaceae, *Juncus effusus* L. and a species from the Thiuniaceae, *Prionium serratum* Drege (Muasya *et al.* in press). The 105 species of Cyperaceae were taken from 85 genera from the 14 tribes and four subfamilies recognised by Goetghebeur (1998). The complete table of taxa, together with the type of DNA material used in the analysis, can be seen in appendix 1.

DNA extraction and PCR:

Total DNA was extracted from 0.02-0.08g silica dried samples or from 0.05-0.1g fresh vegetative (leaves or culm) material collected in the field or from herbarium specimens. DNA was also obtained from stock held at Kew Gardens. DNA extraction, amplification and sequencing were performed according to published procedures (e.g.: Muasya *et al.* in press). Three plastid regions were amplified using polymerase chain reaction (PCR). The *rbcL* intron region of the plastid genome was amplified using the *rbcL74F*, *rbcL938R* and the *rbcL1368R* primers designed by Muasya *et al.* (in press). The *trnL* intron region of the plastid genome was amplified using *trn-c, trn-f, trn- d, trn-e* and *trnL-f* designed by Taberlet *et al.* (1991). The *rps16* intron region of the plastid genome was amplified using the primers *rpsF* and *rpsR2* designed by Oxelman *et al.* (1997).

The PCR reactions were performed in 30μl volumes consisting of 18.6μl of sterile water, 3μl of 10x DNA polymerase buffer, 1μl each of the forward and reverse primers (at 10μM), 1,2μl dNTP (10mM), 0,2μl of Taq DNA polymerase and 2μl of template DNA. The amplification was carried out on an applied Biosystems GeneAmp 2700 thermal cycler (Applied Biosystems, Foster City. CA, USA). The programme used had an initial denaturation phase of 2 minutes at 94°C, followed by 30-35 cycles of 60 seconds at 94°C, 60 seconds at 52°C and 2 minutes at 72°C, followed by a final extension phase of 7 minutes at 72°C. The PCR products were checked on a 1% agarose gel.
Successful products were subsequently sent to the Macrogen (http://www.macrogen.com) laboratories in Kumchum-ku in Seoul, Korea for cycle sequencing using the same

primers as were used in the PCR. Sequences were cleaned, assembled, edited and aligned both manually and using ClustalW in the BioEdit programme (Thompson *et al.* 1994). 25 rps16 sequences from taxa representing 25 genera were added to sequences downloaded from GenBank (www.genbank.com). cite all.

Morphological and ecological data:

Morphological and ecological data were obtained by reading through published literature (e.g. Goetghebeur 1998). Table 1 provides a list of the characters used in the analysis. These characters were scored at the genus-level, such that even though the tips of the branches were represented by species, the character states indicated the state present within each genus. This was done for two main reasons. Firstly many Cyperaceae genera are monotypic and secondly the available literature on specific character states was not extensive enough to allow for a species-level evolutionary character reconstruction.

Analysis:

The plastid sequence and the morphological and ecological data matrices were analysed in three separate ways. Firstly the individual plastid region matrices were analysed separately using parsimony reconstruction, secondly a parsimony analysis was conducted on the combined morphological and ecological and plastid sequence matrices and thirdly a Bayesian analysis was conducted on the combined morphological and plastid sequence matrices. ← How was this done?.

Parsimony analysis:

The parsimony analysis of the individual matrices was done to ensure that the separate data sets produced topologically similar results so that the data sets could be combined and produce a result with little conflict (Wiens 1998). Heuristic analyses were carried out using PAUP* (Swofford 2002). Searches were conducted under Fitch (1971) parsimony, TBR (tree-bissection-reconnection) branch swapping, and random taxon addition. The analysis was run for 1000 replicates, holding no more than 10 trees per replicate. The parsimony analysis of the combined data sets used a full heuristic search with 1 000 random addition replicates, holding a single tree at each step with SPR branch swapping and saving no more than 10 trees per replicate. A single strict consensus tree was kept and compared to the Bayesian output, to ensure that a similar topology was observed using both methods.

Bayesian analysis:

Final phylogenetic relationships were inferred using a Bayesian approach. The trees were rooted on *Juncus effusus* and *Prionium serrata*. ? Mr Bayes version 3.12 (Huelsenbeck and Ronquist 2003) was used to perform the Bayesian analysis of the combined data set. The most complex model – the GTR+I+G model – was used in the analysis. This was chosen as it has been found that the accuracy of a Bayesian model suffers more in response to under parameterisation than to over parameterisation (Huelsenbeck and Rannala 2004). In the analysis parameters were estimated separately for each of the gene regions using uniform prior probabilities. Each run consisted of four Markov chains, each chain had random starting seeds. One chain was cold while the other three were heated. The temperature parameter was set to 0.1 to improve mixing. ? The analysis was run for 10^6 generations and was sampled at every 100th generation, thus producing 10 000 sampled trees per run. To ascertain whether stationarity had occurred, a plot of the –log likelihoods against generation time was investigated and this was also used to determine the "burn-in" time. The average standard deviation of split frequencies between runs was also used to determine stationarity, and as they had dropped to a value less than 0.03 this indicated that the tree samples had become similar enough after 10^6 generations to be regarded as stationary. — refs for this is useful.

morphology?

Analysis of morphological and ecological data:

The final Bayesian tree was loaded into Mesquite Version 2.0 (Maddison and Maddison 2007). Ancestral state reconstructions were done by imposing character states onto the tree. Marginal probability reconstructions were calculated with model MKI (est.) reporting likelihoods as proportional likelihoods (PL).

Table 1: Showing the morphological and ecological characters used to infer the evolutionary history of the Cyperaceae (Goetghebeur 1998)

	State 1	**State 2**	**State 3**	**State 4**
Distribution:				
Tropical	Absent	Present		
Temperate	Absent	Present		
Habitat:				
Forest	Absent	Present		
Grassland, Woodland or Savannah	Absent	Present		
Inselberg	Absent	Present		
Mediterranean	Absent	Present		
Permanently moist (Stream/Marsh/Bog/Swamp)	Absent	Present		
Ecological character:				
Pollination mode	Anemophily	Zoophily		
Anatomical and Morphological Character:				
Flowers	Unisexual	Bisexual		
Photosynthetic pathway	C_3	C_4		
Life-form	Annual	Perennial ?		
Root structure	Rhizome	Other (e.g. culm)		
Number of functional stamens	1	2	3	4 or more
Pollen type	Tetrad	Pseudomonad	Mapanioid	

Results:

what is meant by this. woolly.

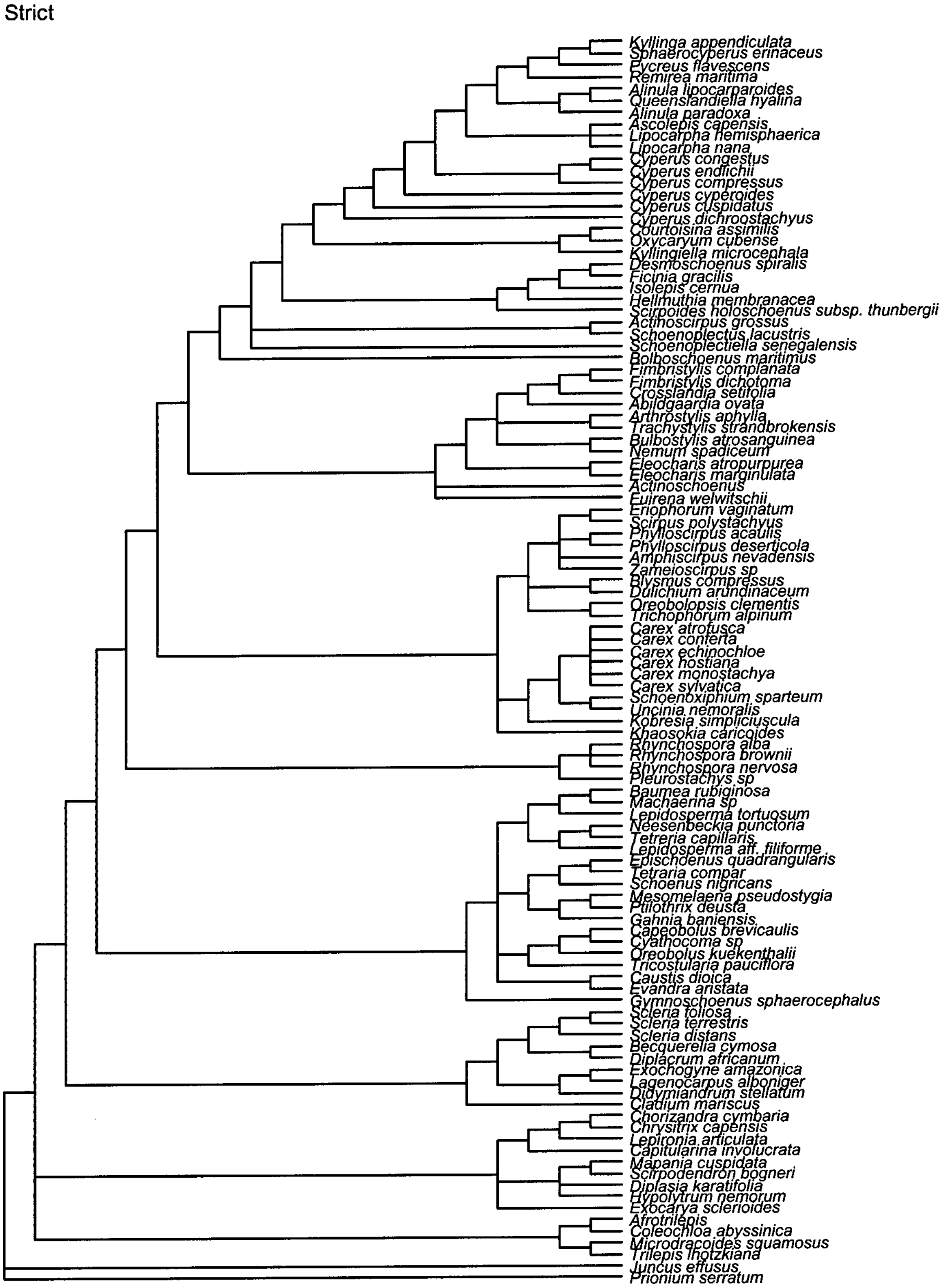
Out of 3263 characters, 661 were uninformative and 1056 were informative. 460 equally most parsimonious trees each of tree length 5212 were kept. The consistency index (CI) was 0.50, and the retention index was 0.703. The phylogenies produced by the Bayesian and the Parsimony analyses were similar in topology (Fig. 1 and Fig. 2).

Phylogenetic relationships among genera and tribes:

The Cyperaceae are resolved as monophyletic (Fig. 1 and Fig. 2). The Mapanioideae are shown to be sister to the rest of the Cyperaceae, with strong support (PP = 1.00; Fig. 2). Goetghebeur's (1998) Caricoideae (Cariceae in Fig. 2) and Sclerioideae (Sclerieae, Cryptangieae, Trilepideae and Bisboeckelerieae in Fig. 2) are embedded in Cyperoideae (Fig. 2).

Within the Mapanioideae there is strong support for Hypolytreae and Chrysitricheae as sister groups (PP = 1.00; Fig. 2). *Capitularina* (PP = 0.89) and *Exocarya* (PP = 0.94) are included in the Chrysitricheae (Fig. 2). There is strong support (PP = 1.00) for the monophyly of the Trilepideae with *Afrotrilepis* sister to *Coleochloa* (PP = 0.99; Fig. 2). There is strong support (PP = 1.00) for *Cladium* as sister to the rest of the Cyperaceae (Fig. 2). The next branching includes Sclerieae and Bisboeckelerieae as sister to the rest of the Cyperaceae (PP = 0.61; Fig. 2). There is weak support (PP = 0.68) for Schoeneae as sister to Cryptangieae (Fig. 2). There is strong support (PP = 1.00) for Rhynchosporeae as sister to the rest of the Cyperaceae (Fig. 2).

There is strong support (PP = 1.00) for a clade including *Khaosokia* to *Carex* being sister to the remaining Cyps (Fig. 2). *Khaosokia* branches out first and closest to Dulicheae (PP = 1.00) and this group is sister to a grade including Scirpeae and Cariceae (PP = 0.99; Fig. 2). Cariceae is shown to be embedded within Scirpeae (PP = 0.52; Fig. 2). The position of *Oreobolopsis* and *Trichophorum* makes Scirpeae paraphyletic with Cariceae (PP = 0.76; Fig. 2). Cariceae are found to be monophyletic (PP = 1.00; Fig. 2).



2

Fig. 1: Maximum parsimony strict consensus tree of Cyperaceae, showing the outgroup.

Fig. 2: Bayesian phylogeny of Cyperaceae based on a combined data set of three plastid gene regions (rbcL, trnl-F and rps16) showing the outgroup and Cyperaceae subfamilies and tribes. 2a) Shows the Hypolytreae and Chrysitricheae of the Mapanioideae and the Trilepideae, Cladieae (*), Bisboeckelereae (1), Sclerieae, Cryptangieae, and a section of Schoeneae of the Cyperoideae. 2b) Shows the rest of the Schoeneae, Rhynchosporeae, Dulicheae (2), and a section of Scirpeae. The presence of *Khaosokia* is indicated by #. 2c) Shows the rest of Scirpeae, Cariceae, Eleocharideae, Abildgaardieae, and a section of Fuireneae (*Fuirena* is indicated by +). 2d) Shows the rest of Fuireneae and Cypereae. Maximum likelihood (PP) values are shown at each node.

legend for individual clades/pages should be with figures

(Figures 3-15 can be found before the Appendix.)

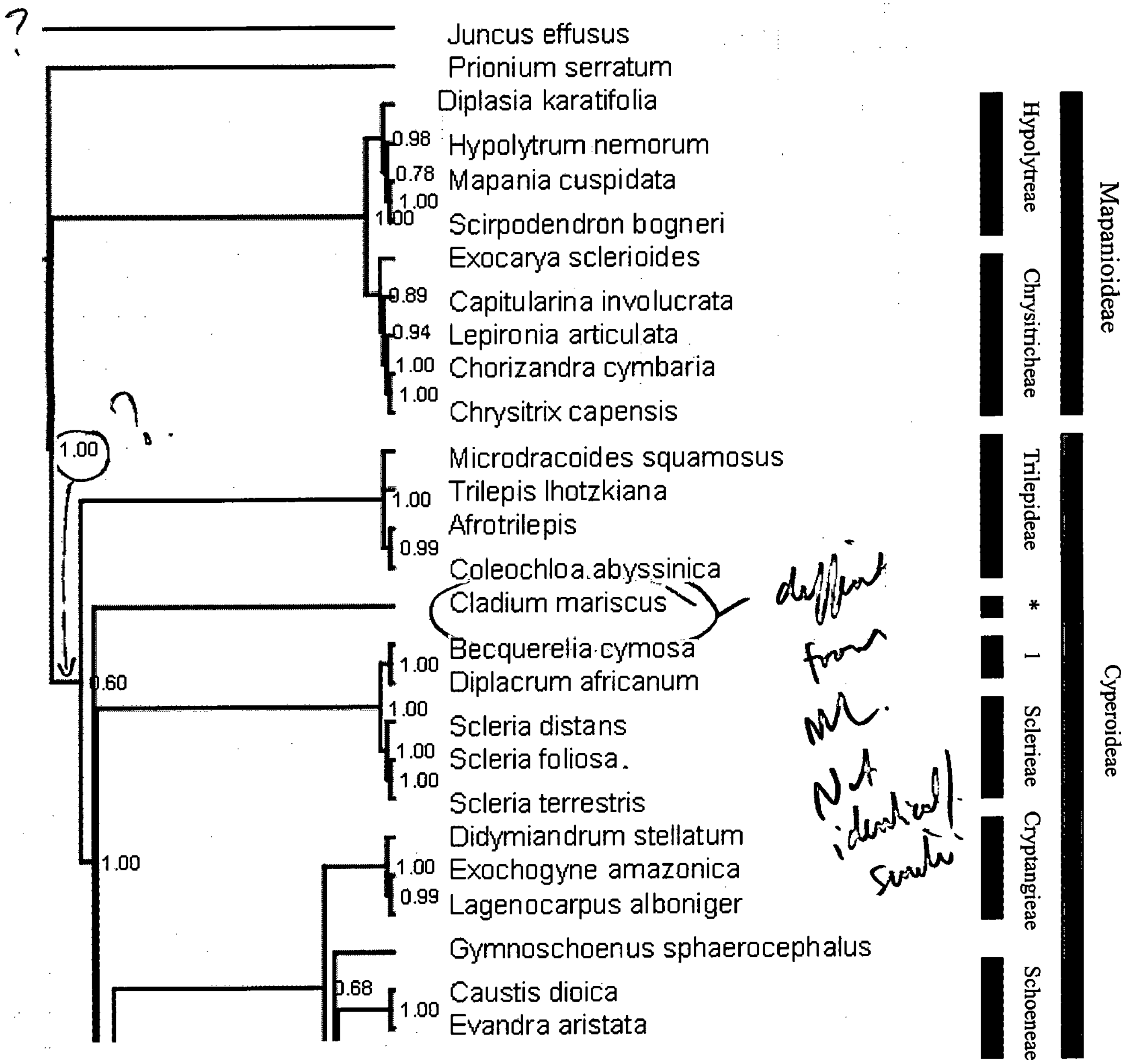

?
Juncus effusus
Prionium serratum
Diplasia karatifolia
0.98
Hypolytrum nemorum
0.78
Mapania cuspidata
1.00
1.00
Scirpodendron bogneri
Exocarya sclerioides
0.89
Capitularina involucrata
0.94
Lepironia articulata
1.00
Chorizandra cymbaria
1.00
Chrysitrix capensis
1.00
?.
Microdracoides squamosus
1.00
Trilepis lhotzkiana
Afrotrilepis
0.99
Coleochloa abyssinica
Cladium mariscus
Becquerelia cymosa
1.00
Diplacrum africanum
0.60
1.00
Scleria distans
1.00
Scleria foliosa.
1.00
Scleria terrestris
Didymiandrum stellatum
1.00
1.00
Exochogyne amazonica
0.99
Lagenocarpus alboniger
Gymnoschoenus sphaerocephalus
0.68
Caustis dioica
1.00
Evandra aristata
Hypolytreae
Chrysitricheae
Trilepideae
*
1
Sclerieae
Cryptangieae
Schoeneae
Mapanioideae
Cyperoideae
differs from ML.
Not identical/similar!

Fig. 2a.

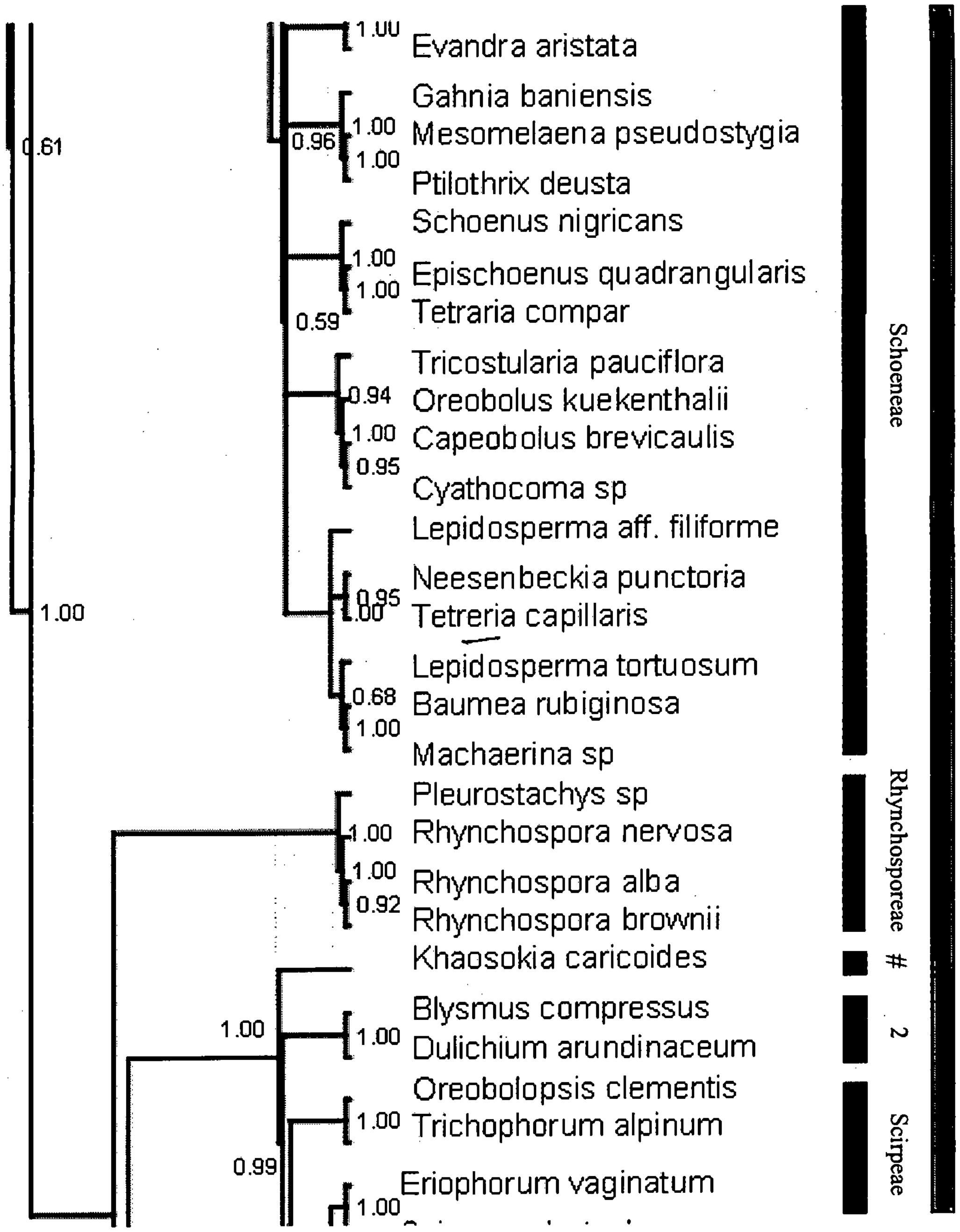

1.00
Evandra aristata
Gahnia baniensis
0.96
1.00
Mesomelaena pseudostygia
1.00
Ptilothrix deusta
0.61
Schoenus nigricans
1.00
Epischoenus quadrangularis
1.00
Tetraria compar
0.59
Tricostularia pauciflora
0.94
Oreobolus kuekenthalii
1.00
Capeobolus brevicaulis
0.95
Cyathocoma sp
Lepidosperma aff. filiforme
Neesenbeckia punctoria
0.95
1.00
Tetreria capillaris
1.00
Lepidosperma tortuosum
0.68
Baumea rubiginosa
1.00
Machaerina sp
Pleurostachys sp
1.00
Rhynchospora nervosa
1.00
Rhynchospora alba
0.92
Rhynchospora brownii
Khaosokia caricoides
Blysmus compressus
1.00
1.00
Dulichium arundinaceum
Oreobolopsis clementis
1.00
Trichophorum alpinum
0.99
Eriophorum vaginatum
1.00
Schoeneae
Rhynchosporeae
#
2
Scirpeae
Cyperoideae

Fig. 2b

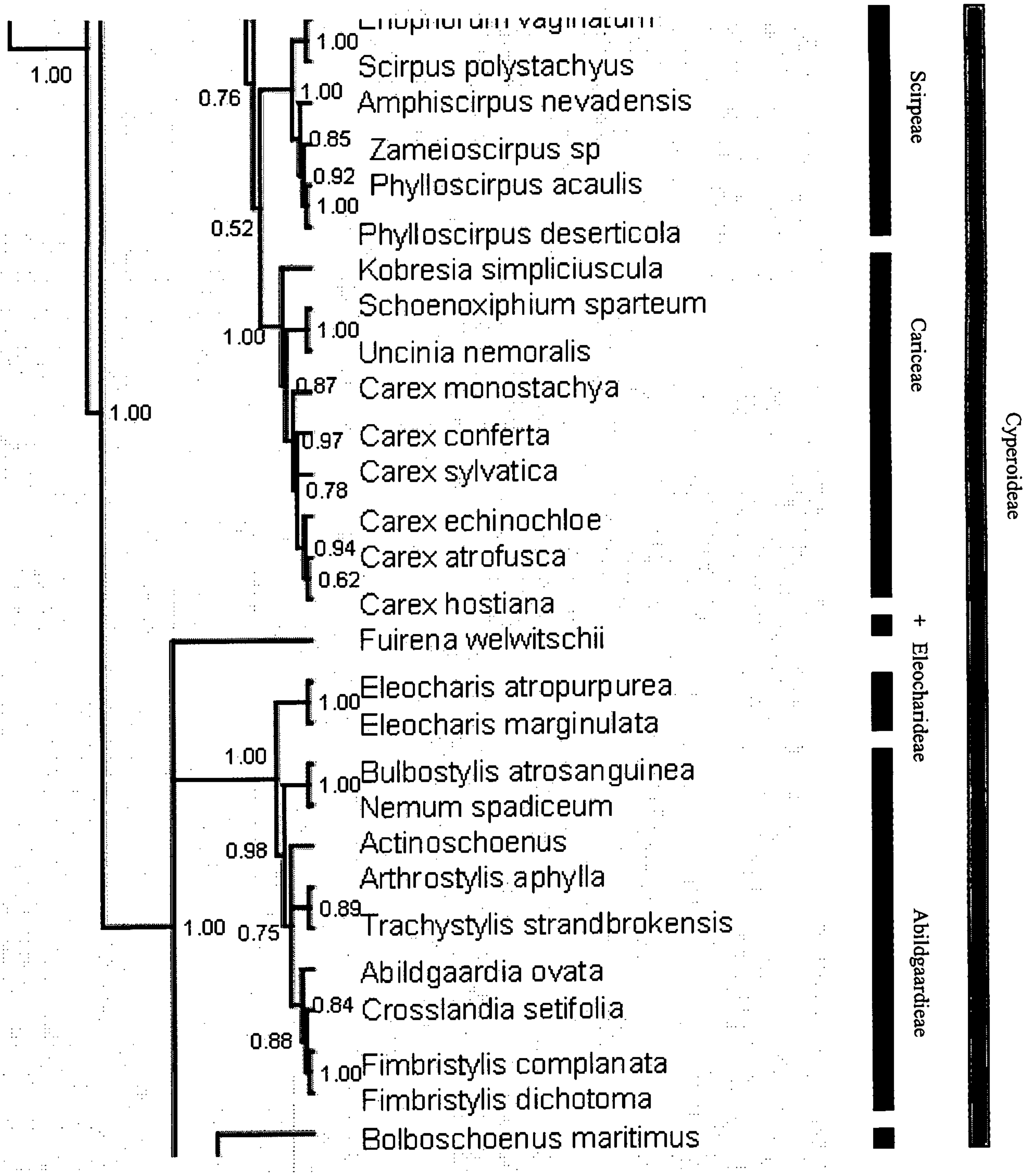

1.00
1.00
Scirpus polystachyus
1.00
0.76
Amphiscirpus nevadensis
0.85
Zameioscirpus sp
0.92
Phylloscirpus acaulis
1.00
0.52
Phylloscirpus deserticola
Kobresia simpliciuscula
Schoenoxiphium sparteum
1.00
1.00
Uncinia nemoralis
0.87
Carex monostachya
1.00
0.97
Carex conferta
Carex sylvatica
0.78
Carex echinochloe
0.94
Carex atrofusca
0.62
Carex hostiana
Fuirena welwitschii
1.00
Eleocharis atropurpurea
Eleocharis marginulata
1.00
1.00
Bulbostylis atrosanguinea
Nemum spadiceum
0.98
Actinoschoenus
Arthrostylis aphylla
0.89
1.00
0.75
Trachystylis strandbrokensis
Abildgaardia ovata
0.84
Crosslandia setifolia
0.88
1.00
Fimbristylis complanata
Fimbristylis dichotoma
Bolboschoenus maritimus
Scirpeae
Cariceae
+ Eleocharideae
Abildgaardieae
Cyperoideae

Fig. 2c

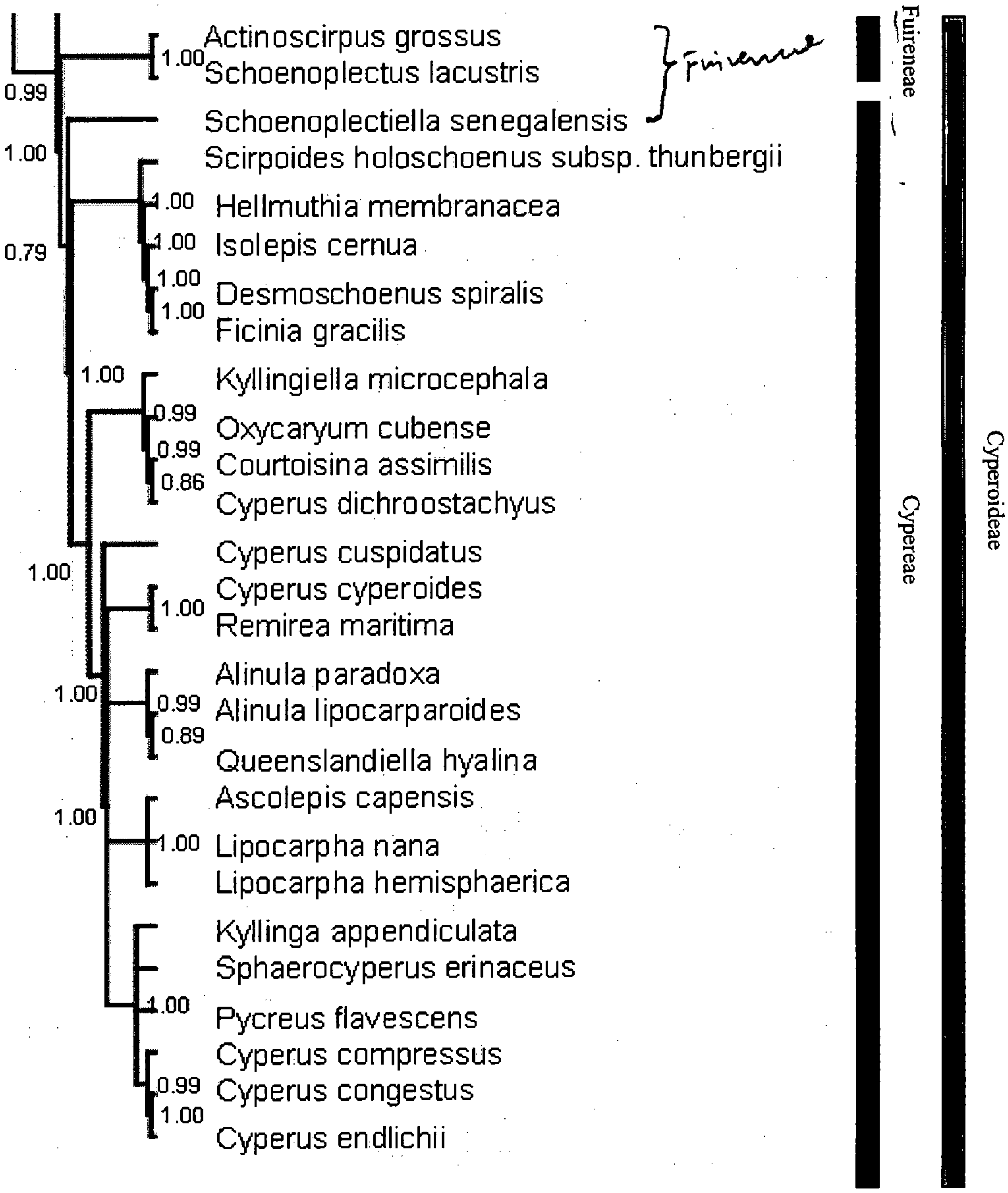

Actinoscirpus grossus
Schoenoplectus lacustris
1.00
0.99
Schoenoplectiella senegalensis
1.00
Scirpoides holoschoenus subsp. thunbergii
Hellmuthia membranacea
1.00
0.79
Isolepis cernua
1.00
Desmoschoenus spiralis
1.00
Ficinia gracilis
1.00
1.00
Kyllingiella microcephala
Oxycaryum cubense
0.99
Courtoisina assimilis
0.99
Cyperus dichroostachyus
0.86
1.00
Cyperus cuspidatus
Cyperus cyperoides
1.00
Remirea maritima
1.00
Alinula paradoxa
Alinula lipocarparoides
0.99
0.89
Queenslandiella hyalina
1.00
Ascolepis capensis
Lipocarpha nana
1.00
Lipocarpha hemisphaerica
Kyllinga appendiculata
Sphaerocyperus erinaceus
1.00
Pycreus flavescens
Cyperus compressus
Cyperus congestus
0.99
1.00
Cyperus endlichii
Fuireneae
Fuireneae
Cypereae
Cyperoideae

Fig. 2d

? ? terminology bad!

There is strong (PP = 1.00) for the Abildgaardieae clade including Eleocharideae and Fuireneae as sister to the rest of the Cyps (Fig. 2). Abildgaardieae and Eleocharideae are shown to be monophyletic with strong support (PP = 1.00) and as sister to each other (PP = 1.00; Fig. 2). *Actinoschoenus* is resolved into the Abildgaardieae (PP = 0.75; Fig. 2). Our results show that *Fuirena* cannot be resolved as sister to either Abildgaardieae or Eleocharideae, as these three clades form a trichotomy (Fig. 2). The rest of the Fuireneae, including *Bolboschoenus*, *Actonoscirpus*, *Schoenoplectus* and *Schoenoplectiella* are shown to be sister to Cypereae, although these genera display a grade topology (Fig. 2). There is strong support (PP = 1.00) for *Bolboschoenus* to be resolved as a separate clade, sister to the remainder of the Fuireneae (Fig. 2). There is also strong support (PP = 1.00) for *Actinoscirpus* and *Schoenoplectus* to be resolved as sister to *Schoenoplectiella* (Fig. 2). Cypereae, including *Schoenoplectiella*, form a monophyletic clade (PP = 0.79; Fig. 2). Within Cypereae there is shown to be strong support (PP = 1.00) for two main clades: a *Scirpoides – Ficinia* group and a *Kyllingiella – Cyperus endlichii* group (Fig. 2).

Distribution and Habitat:

The majority (85%) of the genera included in this study were found to have a tropical distribution (Fig. 3). Further it is likely (PL = 0.99) that the ancestral sedge had a tropical distribution (Fig. 3). A number of genera are shown to occupy habitats in temperate regions (Fig. 4).

It is shown that the ancestral sedge most likely occurred in a permanently moist habitat, such as a swamp, stream, bog or a marsh (PL = 0.54; Fig. 5). The Trilepideae and Abildgaardieae are shown to be the only two tribes that are predominantly not found in permanently moist habitats (PL = 0.60 and 0.77 respectively; Fig. 5). The majority (56%) of the genera were found to occur in grasslands, woodlands and savannahs (Fig. 6). While it is unlikely (PL = 0.09) that the ancestral sedge occurred in this type of habitat, there is a fairly high likelihood (PP = 0.53) that the ancestor to the Scirpeae, Cariceae, Dulicheae, Cypereae, Fuireneae, Eleocharideae and the Abildgaardieae; and the ancestors of Sclerieae and Bisboeckelerieae (PL = 0.63) occurred in grassland, woodland or savannah (Fig. 6). The Cariceae (PL = 0.87) and Mapanioideae (PL = 0.97) are shown to have a number of genera found within a forest habitat (Fig. 7). The ancestor of the Mapanioideae was likely (PL = 0.97) to have occurred within a forest environment (Fig. 7). The Trilepideae are shown to occur on Inselbergs (PL = 0.99; Fig. 8).

Pollination biology:

Our results show that the ancestral sedges were likely to be anemophilous (PL = 0.99; Fig. 10). Zoophily – or traits indicating pollination by insects and other invertebrates - is shown to have evolved at least 9 times independently within Cyperaceae (Fig. 10). Two genera in the Mapanioideae - *Hypolytrum* and *Mapania* – are shown to have evolved zoophilous traits independently (PL = 0.56; Fig. 10).

Life history:

The ancestral life-history state of the Cyperaceae is shown to have a high likelihood (PL = 0.97) of having been perennial (Fig. 11). Annualness is shown to have arisen multiple times independently within the Cyperaceae, in Sclerieae, Schoeneae, Rhynchosporeae, Abildgaardieae, Fuireneae and multiple times within Cypereae and Eleochareae (Fig. 11). The majority of Cyperaceae are perennial (Fig. 11).

Photosynthetic pathway:

The photosynthetic pathway in ancestral sedges was likely to be C_3 (PL = 0.99; Fig. 12). C_4 photosynthetic pathway is shown to have evolved at least four times independently, in four separate tribes: Rhynchosporeae, Eleocharideae, Abildgaardieae and Cypereae (Fig. 12).

~~Anatomical and~~ Morphological data:

There is strong likelihood (PL = 0.98) that the ancestral state within the Cyperaceae was to have bisexual flowers (Fig. 13). Presence of a unisexual flower is shown to have evolved multiple times independently within Cyperaceae (Fig. 13). There is strong support (PL > 0.97) showing that unisexual flowers have evolved independently from an ancestor with bisexual flowers in Trilepideae, Sclerieae, Cryptangieae, Cariceae, Cypereae and Eleocharideae (Fig. 13). Our results show that unisexual flowers appear to have evolved at least 10 times independently within the Cyperaceae (Fig. 13).

The ancestral sedge is shown to have three functional stamens (PL = 0.86; Fig. 14). The majority of species are shown to have three functional stamens (Fig. 14). The Mapanioideae are shown to have lost all but one of their functional stamens, with the exception of *Diplasia* which has more than four (Fig. 14).

Our results show that the two outgroups *Juncus* and *Prionium* have a tetrad pollen type (PL = 0.85; Fig. 15). It is shown to be highly likely (PL = 0.99) that the ancestral pollen type within the Cyperaceae was pseudomonad (Fig. 15). There is strong support (PL = 0.99) showing that the Mapanioid pollen type has arisen within the Hypolytreae (Fig. 15).

Discussion:

Classification:

This study supports the monophyly of the Cyperaceae reported by a number of studies (e.g. Muasya *et al.* in press). Goetghebeur's (1998) classification of the Mapanioideae according to their peculiar floral structure, where the lateral bisexual flowers are provided with a pair of usually larger, keeled, laminar hypogynous scales, is supported by our analysis. This study and other analyses of DNA data support the recognition of Mapanioideae as sister to all other Cyperaceae (Fig. 2; Muasya *et al.* in press). Our results provide evidence that the Sclerioideae and Caricoideae are paraphyletic, and consequently the recognition of Caricoideae and Sclerioideae as subfamilies separate from Cyperoideae is not supported by our findings. Goetghebeur (1998) recognized the Cyperoideae as having at least one (sometimes all) bisexual flower per spikelet and as lacking a mapanioid lateral pair of keeled hypogynous scales. Caricoideae ~~Sclerioid~~eae were recognized as having strictly unisexual flowers enclosed by a utricle (Goetghebeur 1998). Our data support the proposal for a revised classification of Cyperaceae into two subfamilies, Mapanioideae and Cyperoideae *sensu* Muasya *et al.* (in press).

This study broadly supports the tribal circumscriptions of Goetghebeur (1998) with a few modifications. Within the Mapanioideae Goetghebeur (1998) recognized two tribes, Hypolytreae and Chrysitricheae. Hypolytreae are characterized as large-leaved species, usually with many spikelets per inflorescence, and very poorly differentiated embryos. Chrysitricheae are characterised as having a much reduced vegetative apparatus and inflorescence, and a highly differentiated embryo. These two tribes are well supported by this study (Fig. 2) although the position of *Capitularina* and *Exocarya* differs from Goetghebeur (1998). Our study provides evidence that both genera should be included in the Chrysitricheae, a finding that is supported by Simpson et al. (2003) in a combined pollen and DNA data study. The position of the Trilepideae within the Cyperaceae is not well-resolved by this study (PP = 0.60; Fig. 2). It thus remains uncertain whether this tribe could be sister to the rest of the Cyperioideae *sensu* Muasya *et al.* (in press) or if Mapanioideae is the true sister clade to the Cyperioideae *sensu* Muasya *et al.* (in press). Goetghebeur (1998) characterized the Trilepideae as having a panicle composed of many dense spikes of many tiny spikelets with few distichous glumes. Goetghebeur (1998) reported that the perianth is similar to that of the Cryptangieae, in that it is usually formed by 3 fimbriate scales opposite the flat sides of the achene. This suggests that the Trilepideae could be more closely related to the rest of the Cyperioideae *sensu* Muasya *et al.* (in press). A greater sampling effort is required to be able to fully resolve this issue.

Previously *Cladium* was placed within Schoeneae due to a restricted number of bisexual flowers per spikelet and a fairly well-developed perianth (Goetghebeur 1998 and Bruhl 1995). This study provides evidence which suggests that *Cladium* should be treated as a separate tribe, Cladieae, which had been previously recognized (Bruhl 1995).

Goetghebeur (1998) recognized the Sclerieae as having bisexual or unisexual spikelets and an achene surrounded at the base by a hypogynium and a cupula. This circumscription is supported by our findings, which show a well-resolved and supported Sclerieae clade (Fig. 2). Goetghebeur (1998) recognized Bisboeckelereae as having (sometimes connate) empty glumes surrounding the apparently terminal female flower and male spikelets with the unusual structure of having glumes with a single stamen. Our study supports the recognition of Bisboeckelereae (PP = 1.00; Fig. 2). Bisboeckelereae and Sclerieae are resolved to be sister (PP = 1.00), an observation reported from previous studies (e.g. Muasya *et al.* in press). In this study this clade is sister to Schoeneae, unlike in Muasya *et al.* (in press) where this clade was embedded in Schoeneae.

The Cryptangieae were circumscribed by Goetghebeur (1998) as having unisexual spikelets, spirally arranged glumes and a perianth usually formed by 3-fimbriate scales opposite the flat sides of the achene. The monophyly of the Cyptangieae is found to be supported by our study (PP = 1.00; Fig. 2). Cryptangieae are poorly supported (PP = 0.68) as sister to Schoeneae (Fig. 2).

Goetghebeur (1998) circumscribed the Schoeneae as having a restricted number of bisexual flowers per spikelet often provided with a well-developed perianth and flowers that are included by the wings of adjacent glumes. However, Schoeneae *sensu* Goetghebeur (1998) is found to be heterogenous in this study (Fig. 2). This is supported by Muasya *et al.* (in press) which resolved four clades within Schoeneae. Thus our study supports the call for a need of further revision and possible division of the tribe made by Goetghebeur (1998) and Muasya *et al.* (in press). Our study also provides support for the recognition of *Gymnoschoenus* as a tribe separate from the rest of Schoeneae and for the recognition of Rhynchosporeae, proposed by Muasya *et al.* (in press).

There is strong support (PP = 1.00) for a clade comprising *Khaosokia* and members of Dulicheae, Scirpeae and Cariceae. This relationship has been reported by other studies (e.g. Muasya *et al.* 1998 and Muasya *et al.* 2000). Goetghebeur's (1998) classification of the Dulicheae as having a fertile spikelet prophyll bearing a bisexual flower appears to be well supported by our study (Fig. 2). Our results also suggest that *Khaosokia* could be included in a tribe separate from Cariceae and as sister to Dulicheae, a finding supported by Simpson et al. (2005). There is some indication that *Khaosokia* may be closely related to *Sumatroscirpus*, a sedge found in Sumatra. Goetghebeur (1998) recognised Scirpeae as having fertile spikelets with spirally arranged glumes and flowers with hypogynous scales. This circumscription is not upheld by this and other studies conducted with DNA data (e.g. Muasya *et al.* in press). Cariceae is found to be embedded within Scirpeae, as *Oreobolopsis* and *Trichophorum* are basal to both clades (PP = 0.76; Fig. 2). We propose that these two genera be included in a new tribe, which would resolve both Cariceae and Scirpeae as monophyletic. The monophyly of the Cariceae is supported by Goetghebeur (1998) which reported that the clade is easily recognized by the female flower being enclosed within a utricle.

The relatively close relationship between Fuireneae and Scirpeae shown by our analysis is supported by Goetghebeur (1998) which reported that both tribes have a similar floral morphology. Fuireneae are shown to have a grade topology, meaning that a number of taxa could be resolved into a number of separate tribes (Fig. 2). This could be as a result of inadequate sampling, although a likelier explanation, supported by Muasya *et al.* (in press) is that this section should be split into a number of separate tribes. Muasya *et al.* (in press) showed that Fuireneae could be split into several clades. *Fuirena* is observed here to be sister to either Eleocharideae or Abildgaardieae, whereas the rest of Fuireneae form a grade and are closer to Cypereae (PP = 1.00). Previous studies (e.g. Muasya *et al.* in press) have shown *Fuirena* to resolve apart from other Fuireneae raising questions as to the monophyly of the tribe. It is evident that Fuireneae needs further evaluation.

Goetghebeur (1998) recognized the Eleocharideae as having reduced vegetative apparatus, a fixed unispiculate inflorescence, a unique embryo type and a helophilous life-form. This tribe is well supported by our analysis as being monophyletic and sister to the Abildgaardieae (Fig. 2). The close relationship between Eleocharideae, Abildgaardieae and Fuireneae is supported by Goetghebeur (1998). Goetghebeur (1998) stated that the Eleocharideae shares a number of characters in common with Fuireneae, including a similar vegetative morphology, an embryo with broadened cotyledon and a bristle-like perianth. Eleocharideae were also reported to share the Kranz syndrome (having a green sheath around the vascular bundles), distichous glumes, a differentiated and thickened style base and moniliform stigmatic hairs (Goetghebeur 1998). Our analysis supports the proposal to include *Trachystylis* and *Arthrostylis* and *Actinoschoenus* in Abildgaardieae made by Muasya *et al.* (in press). Similar results were obtained by Ghamkar *et al.* (in press) based on plastid and nuclear ribosomal (ITS) data. The monophyly of the Cypereae is well-supported, in agreement with Goetghebeur (1998) which characterized Cypereae by the presence of the Cyperus-type embryo. *Hellmuthia*, a genus previously included in the Chrysitricheae, is resolved within the Cypereae, a finding supported by Muasya *et al.* (in press).

Distribution and habitat:

Our analysis provides evidence to support the hypothesis that the ancestral sedge grew in marshy or wet habitats in tropical regions (Fig. 5 and Fig. 3). A few of the tribes, such as the Chrysitricheae, are shown to occur predominantly in open swamps (Fig. 5). The Cyperaceae appear to have radiated into drier open habitats, such as grassland, woodland, savannahs and Mediterranean shrubland, while a few clades appear to have moved into closed forest habitats (Fig. 6, Fig. 9 and Fig. 7).

Life history:

The evolution of an annual life history is a remarkable adaptation to seasonal climates. Although annuals are relatively rare in monocots, Linder and Rudall (2005) reported that the strategy has evolved independently in seven families of the Poales, including Cyperaceae. Despite this Linder and Rudall (2005) found that annualness has not become the dominant strategy within any of these seven families. This is supported by our analysis which found that only a few genera contained annual species, while the majority of the genera were perennial (Fig. 11). Hu *et al.* (2003) found that in Poaceae the shift between the annual and perennial life histories appears to be controlled for by only two genes, which suggests that it may be a relatively simple evolutionary transition. The fairly high number of shifts within the Cyperaceae found in our analysis supports this conclusion (Fig. 11).

Anatomical and morphological data:

The ancestral photosynthetic pathway in the Cyperaceae is C_3, although the C_4 photosynthetic pathway system appears to have evolved multiple times (Fig. 12). There is some controversy surrounding the ecological advantages of the C_4 photosynthetic pathway. Stock et al. (2004) investigated the contribution of climatic factors and phylogenetic relationships affecting the geographical distribution of C_3 and C_4 genera of the Cyperaceae in South Africa. Their analysis showed that there is no simple relationship between Southern African C_3/C_4 species distributions and environmental factors. Similar results have also been reported from other regions of the world (Teeri et al. 1980; Ueno and Takeda 1992). Linder and Rudall (2005) reported that the parallel evolution of C_4 in grasses and sedges is probably an adaptation to changes in atmospheric CO_2 concentrations as opposed to other environmental factors such as seasonal drought. However, with a lack of precise estimate of dating, this hypothesis is difficult to test and further investigation is needed to resolve why this photosynthetic pathway has evolved multiple times within Cyperaceae.

Linder and Rudall (2005) reported that unisexual flowers are often associated with wind pollination and that the shift from bisexual to unisexual flowers has occurred numerous times during the evolution of Poales. Our analysis shows that this pattern is present within the Cyperaceae, as the ancestral sedge was likely to have had bisexual flowers and unisexual flowers are shown to have arisen multiple times independently (Fig. 13).

Faegri and Van der Pijl (1979) reported that increased pollen to ovule ratio is normally regarded as characteristic of wind-pollinated species and that this often correlates to an increase in stamen number. However Linder and Rudall (2005) showed that within Poales there have been numerous reductions in stamen number and that stamen number is remarkably labile in grasses. This led them to conclude that the number of functional stamens is an unlikely adaptive modification in wind-pollinated groups in which an increase in stamen number is expected, maximizing pollen output (Linder and Rudall 2005). Our data show that the number of functional stamens is also labile in the sedges, and that it is thus unlikely to be an adaptation to anemophily (Fig. 14).

Simpson et al. (2003) reported that members of the Hypolytreae possess "sticky" pollen in monads, which they suggested might be evidence for zoophily. Our analysis supports this observation as the Hypolytreae are found to have evolved zoophily and are shown to have Mapanioid pollen (Fig. 10 and Fig. 15). There also appears to be a correlation between ocurrance in a forest habitat and zoophily (Fig. 7 and Fig. 10). Both states were found within a number of the same genera in the Hypolytreae and Cariceae (Fig. 7 and Fig. 10). Goetghebeur (1998) reported that entomophily is probably linked to the forest habitat as wind velocity is reduced compared to an open habitat. This relationship does however require further investigation, at the specific level.

Conclusion:

There was a call from Muasya et al. (in press) for a need to study the same DNA regions – such as *rbcL, trnL-F* and *rps16* – for similar taxa such that different data sets could be combined. This study has attempted to achieve this. Cyperaceae are monophyletic and resolved into two subfamilies, the Mapanioideae and Cyperoideae. The overall topology of our analysis is similar to those observed in other studies, with a few exceptions. Our study suggests that *Cladium* should be treated as a separate tribe, the Cladieae. Sclerieae is observed as sister to as opposed to being embedded in Schoeneae. *Fuirena* is observed here as sister to either Eleocharideae or Abildgaardieae whereas the rest of Fuireneae form a grade and are closer to Cypereae. 24 species were sequenced for the first time in the *rps16* intron region of the plastid genome in an attempt to further resolve the phylogenetic relationships within Cyperaceae.

In addition we were able to reconstruct the evolutionary history of the family using a number of morphological and ecological characters. The ancestral sedge was shown to have bisexual flowers, 3 functional stamens, pseudomonad pollen and the C_3 photosynthetic pathway. Further, the ancestral sedge is likely to have been an anemophilous perennial plant which grew in marshy or wet tropical habitats.

Future prospects:

A complete investigation of the evolutionary history of the Cyperaceae would require dating the phylogeny. There are currently a number of both molecular and paleontological methods with which one could investigate the temporal framework of lineages or to estimate ages of clades (Magallon 2004). Bremer (2000) and Janssen and Bremer (2004) attempted to date the major monocot groups based both on fossil data and *rbcL* sequence data. It would be informative to be able to date the phylogeny of the Cyperaceae, as this would allow one to infer when a particular trait arose and thus to further investigate the evolutionary history of the family.

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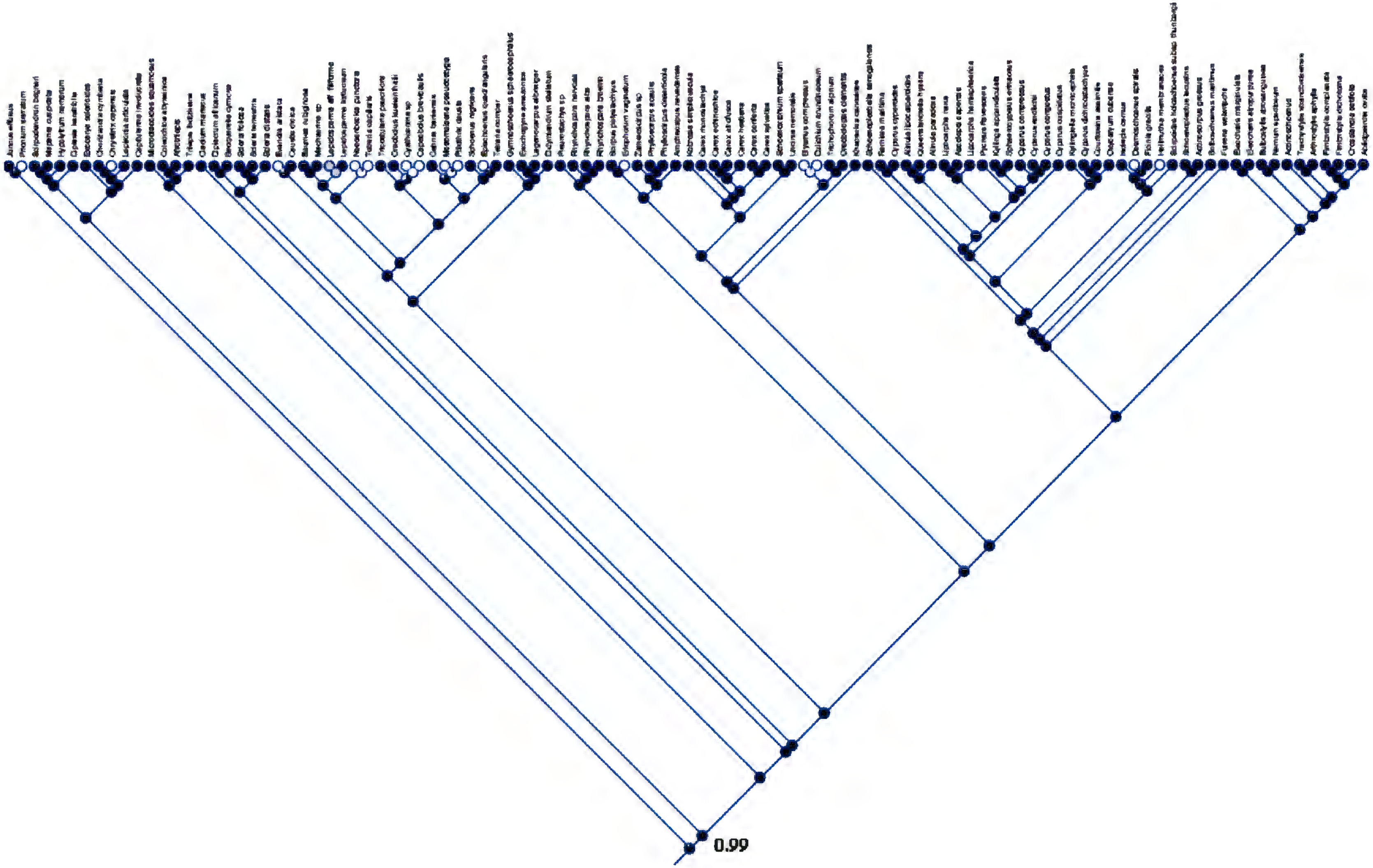

Fig. 3: Optimisation of genera with a tropical distribution (black circles), showing proportional likelihood values for important nodes.

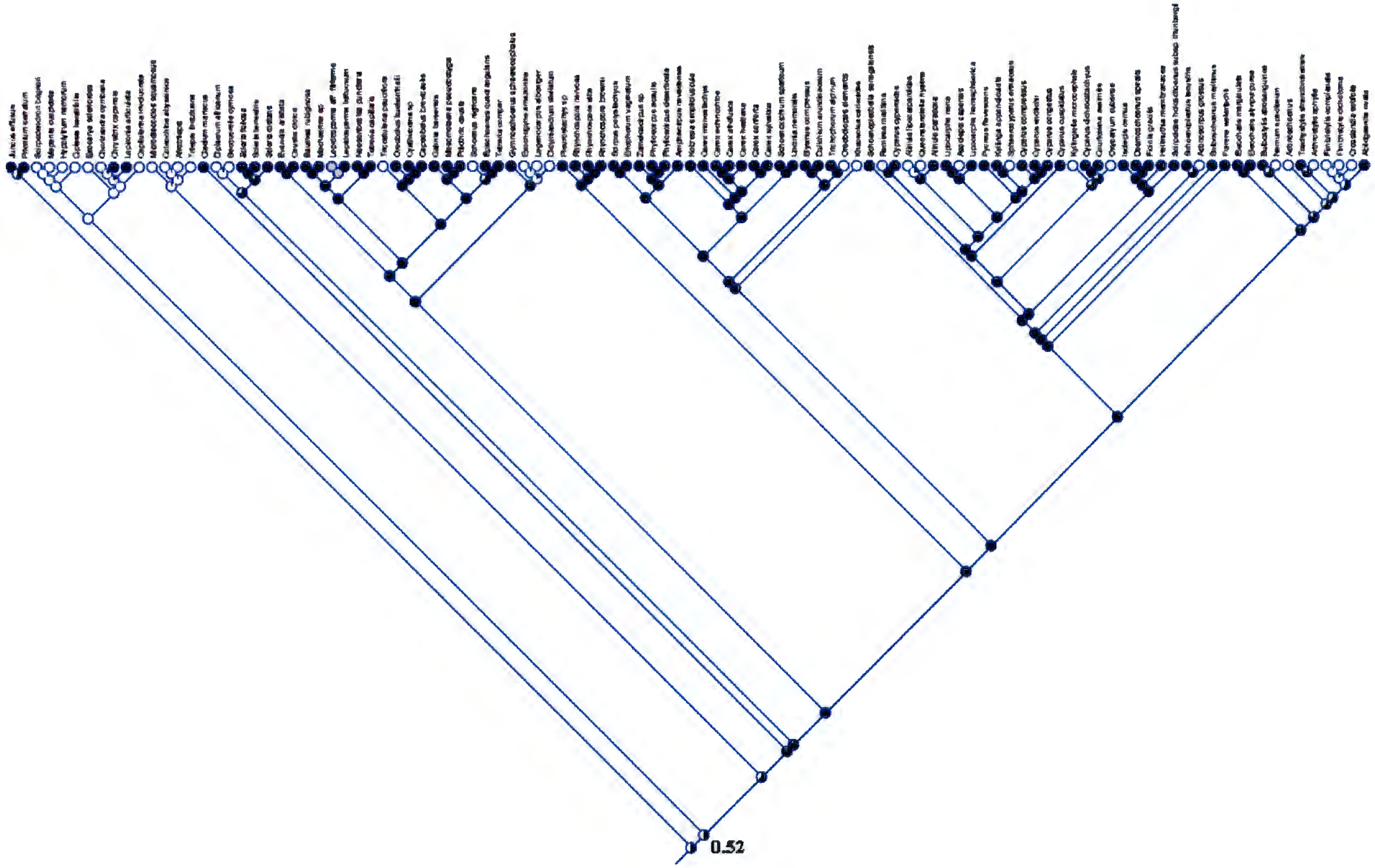

Fig. 4: Genera found in temperate regions (black circles), showing proportional likelihood values for important nodes.

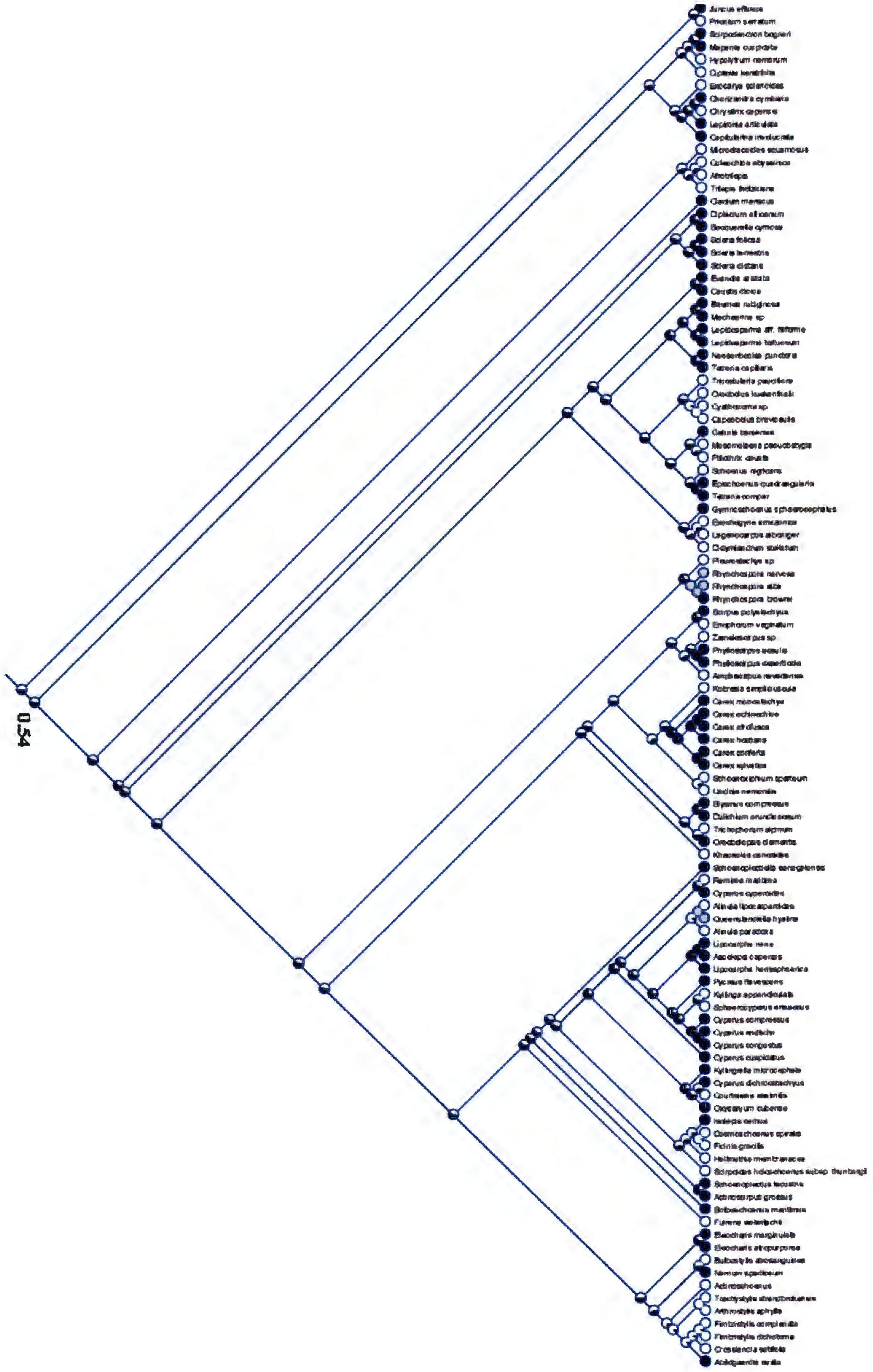

Fig. 5: Optimisation of genera occurring in permanently moist habitats, such as marshes, bogs, swamps or stream (black circles). Proportional likelihood values are shown for important nodes.

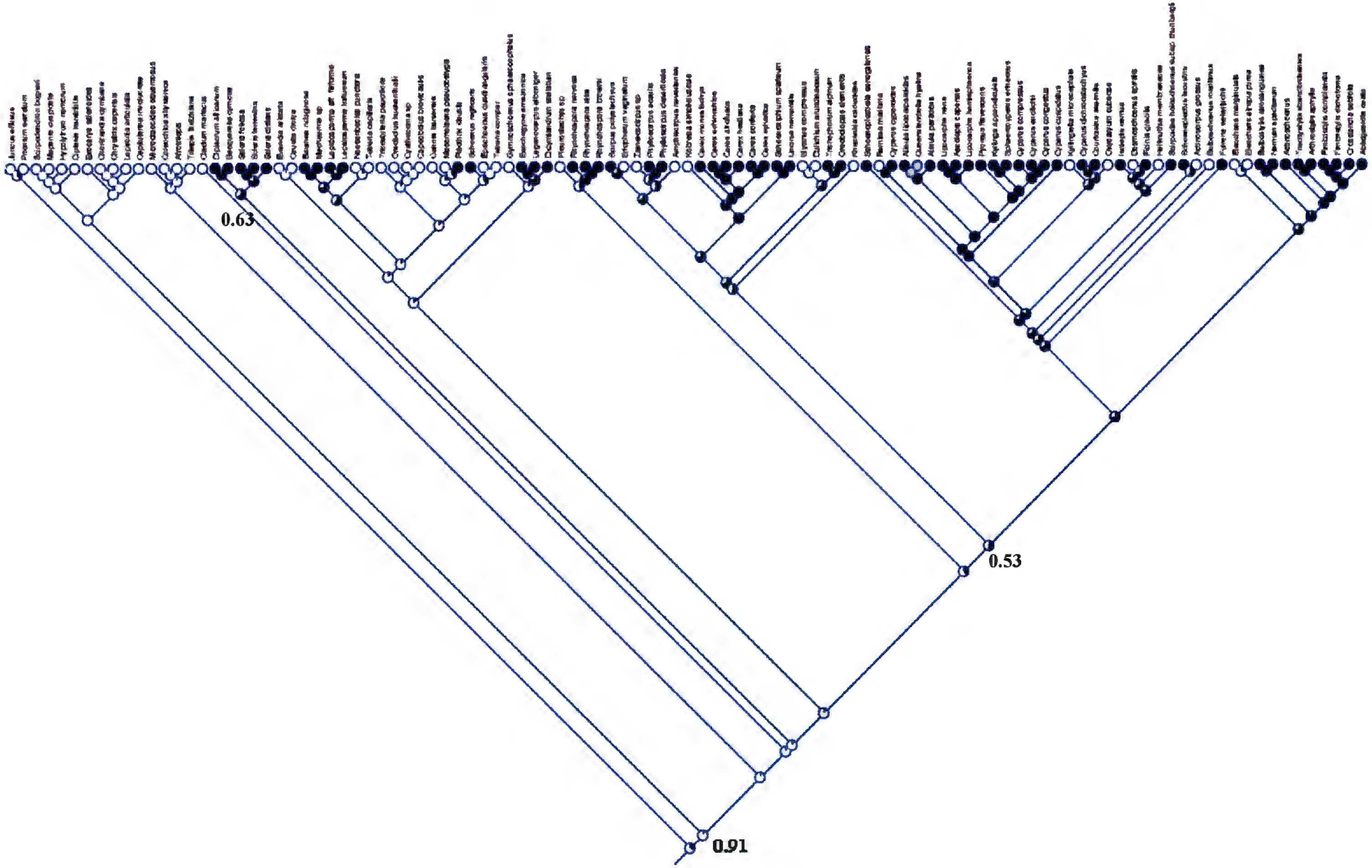

Fig. 6: Optimisation of genera occurring in open habitats, such as grassland, woodland or savannah (black circles). Proportional likelihood values are shown for important nodes.

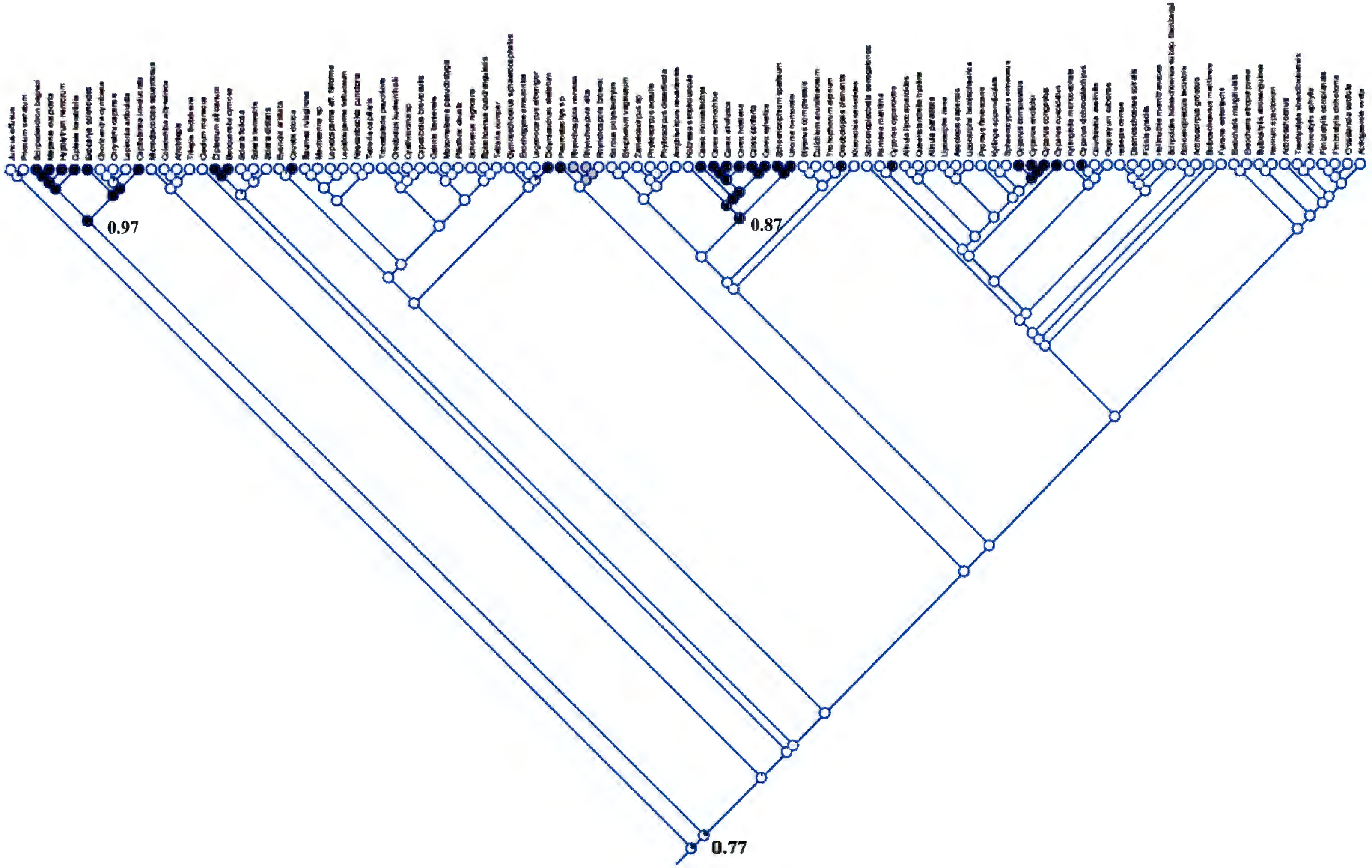

Fig. 7: Optimisation for genera occurring in closed forest habitat (black circles), showing proportional values for important nodes.

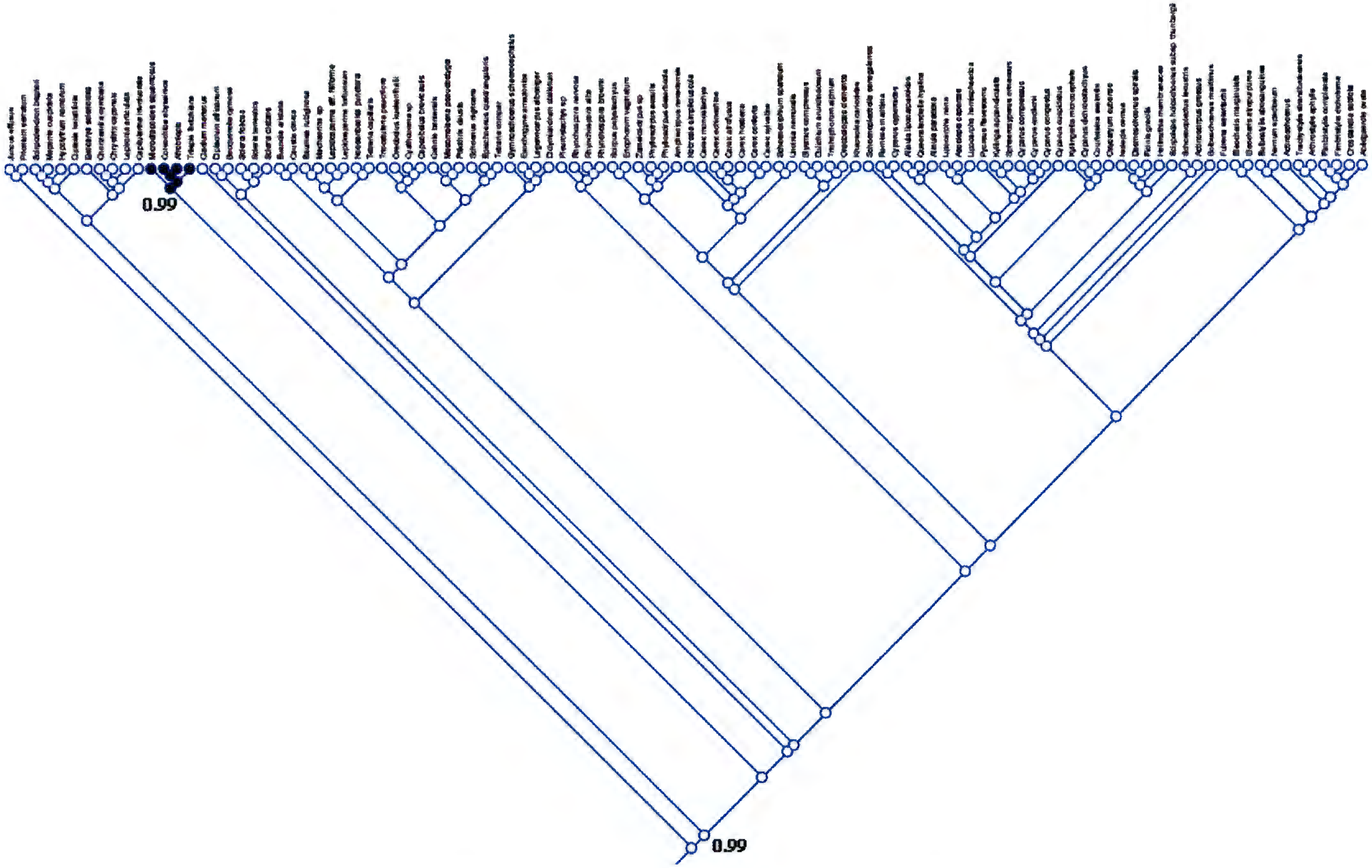

Fig. 8: Optimisation for genera occurring on Inselbergs (black circles), showing proportional likelihood values for important nodes.

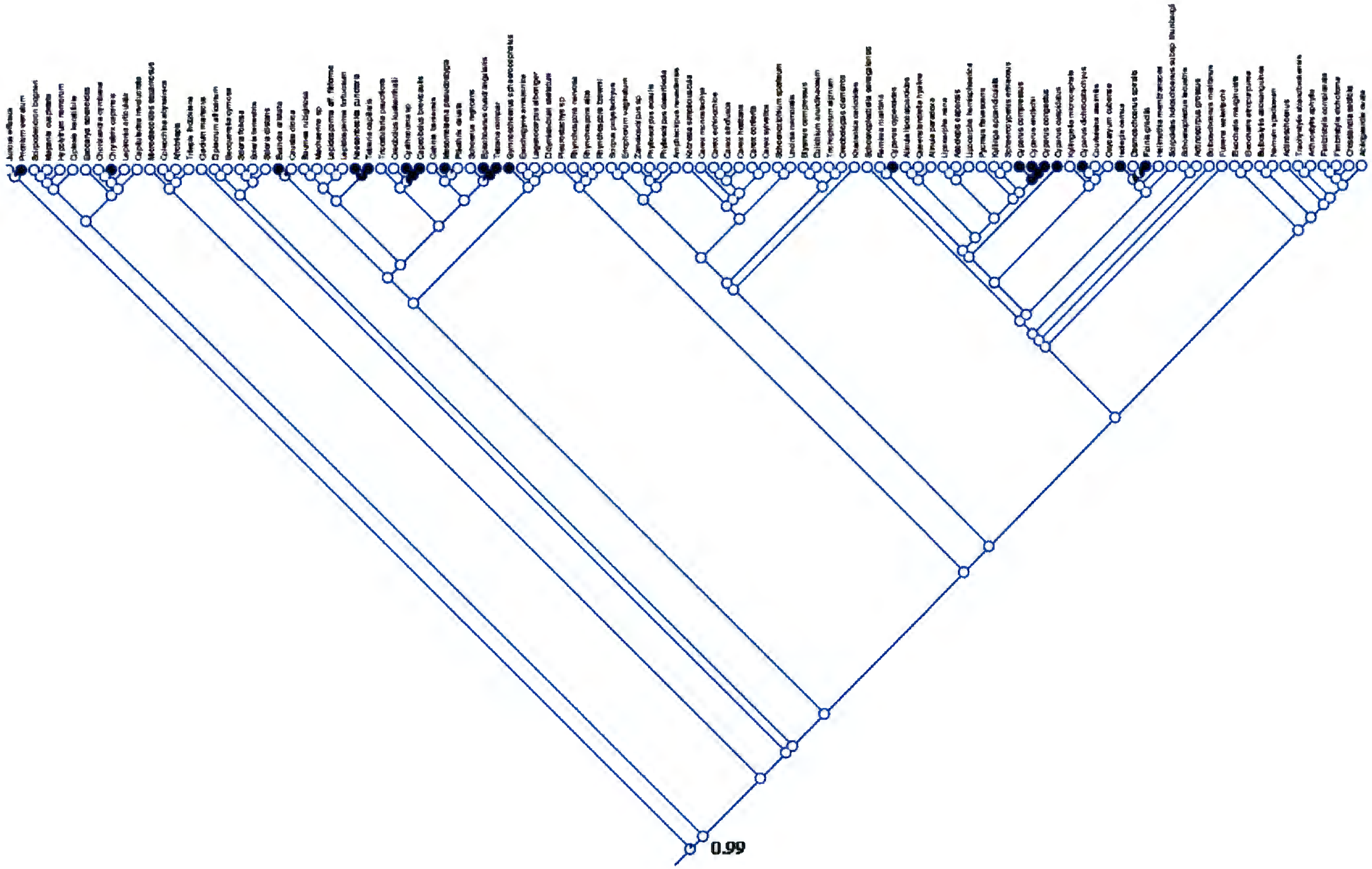

Fig. 9: Optimisation of genera occurring in open Mediterranean habitat (black circles) showing proportional likelihood values for important nodes.

not presented in results

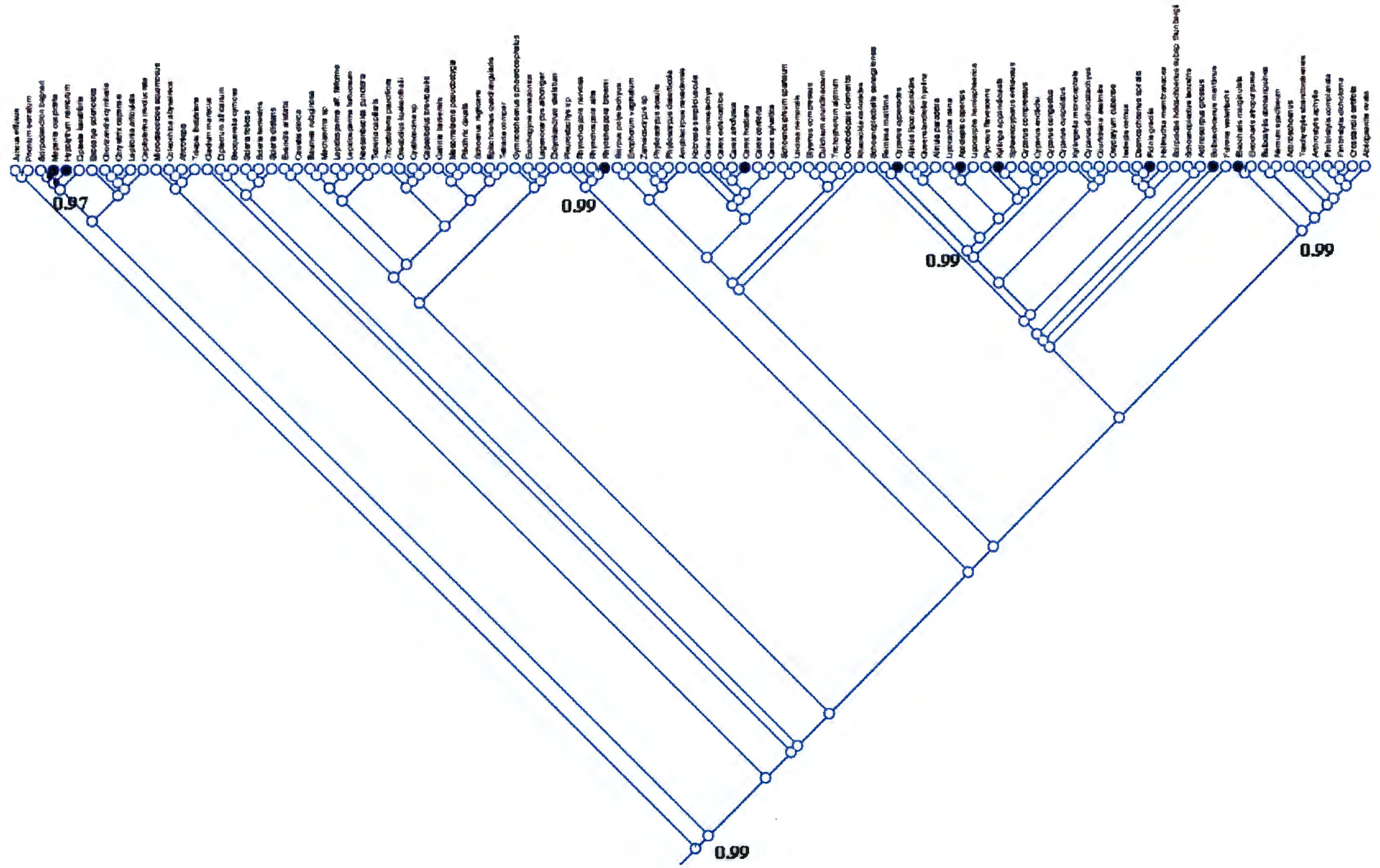

Fig. 10: Optimisation of pollination mode, showing proportional likelihood values for important nodes. Black circles indicate zoophily (or presence of traits indicating potential pollination by insects or other invertebrates); white circles indicate anemophily.

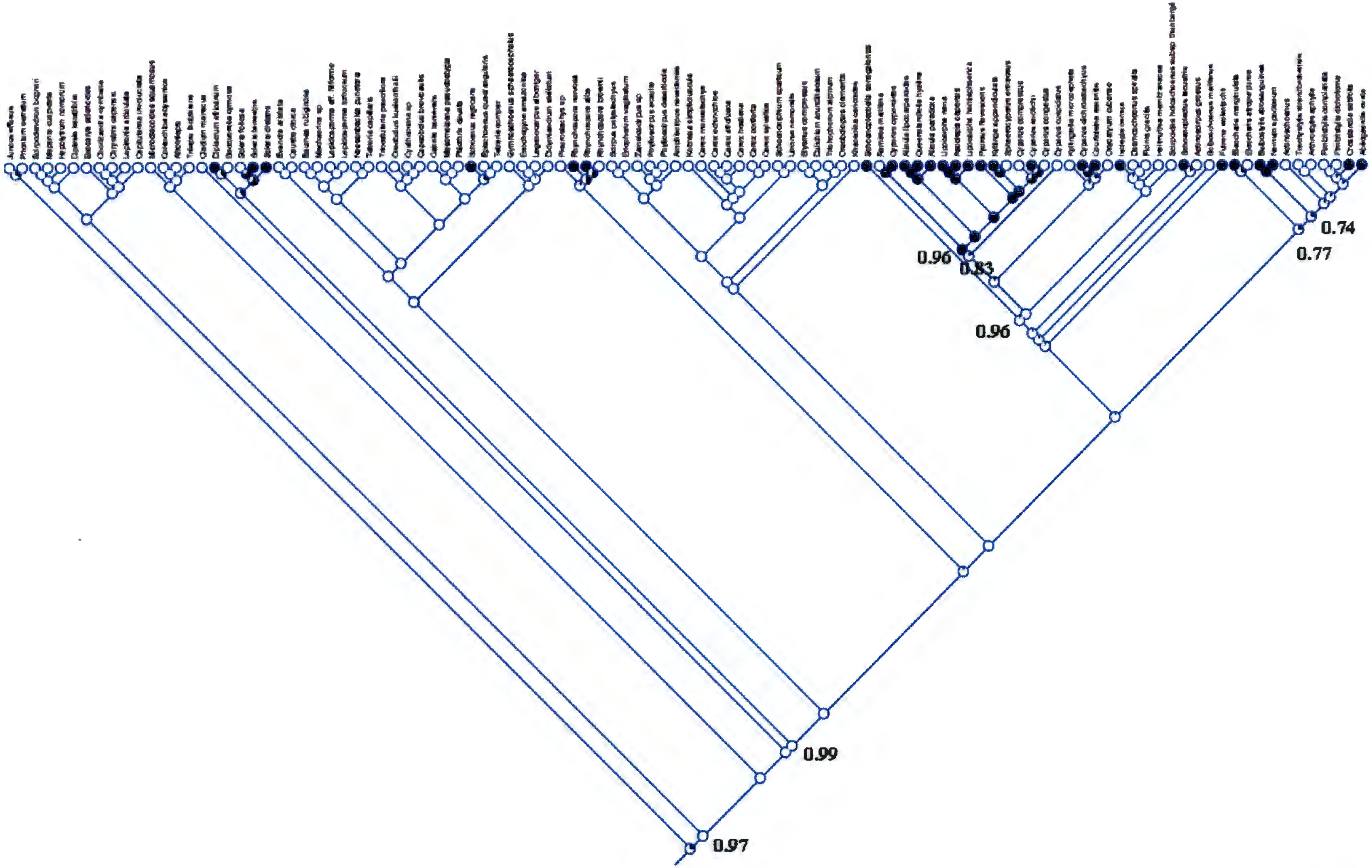

Fig. 11: Optimisation of life history in Cyperaceae and related taxa, showing proportional likelihood values for important nodes. Black circles indicate presence of annual life-form, white circles indicate presence of perennial life form.

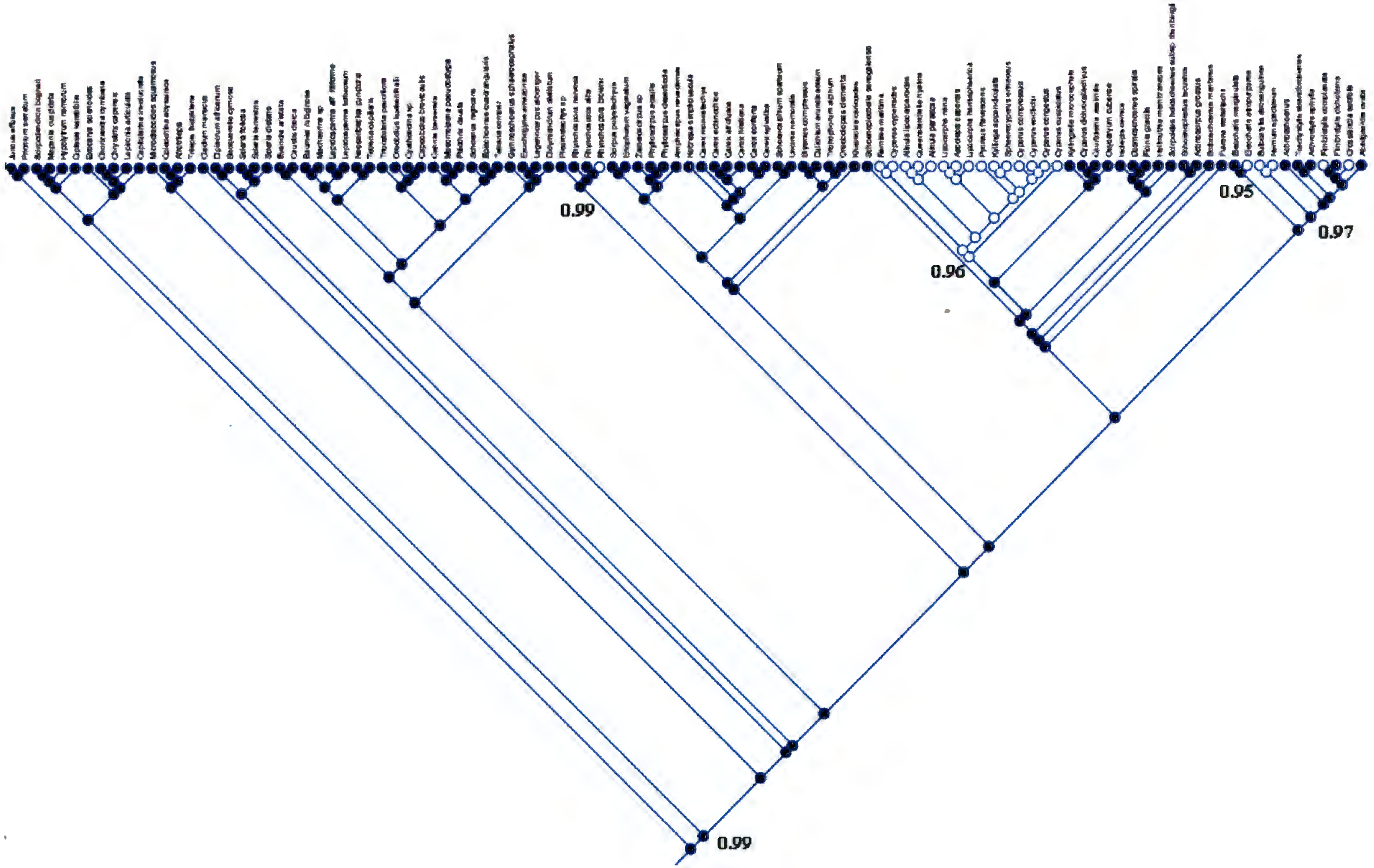

Fig. 12: Optimisation of C_3 (black circles) and C_4 (white circles) photosynthetic pathways in Cyperaceae and related taxa, showing proportional likelihood values for important nodes.

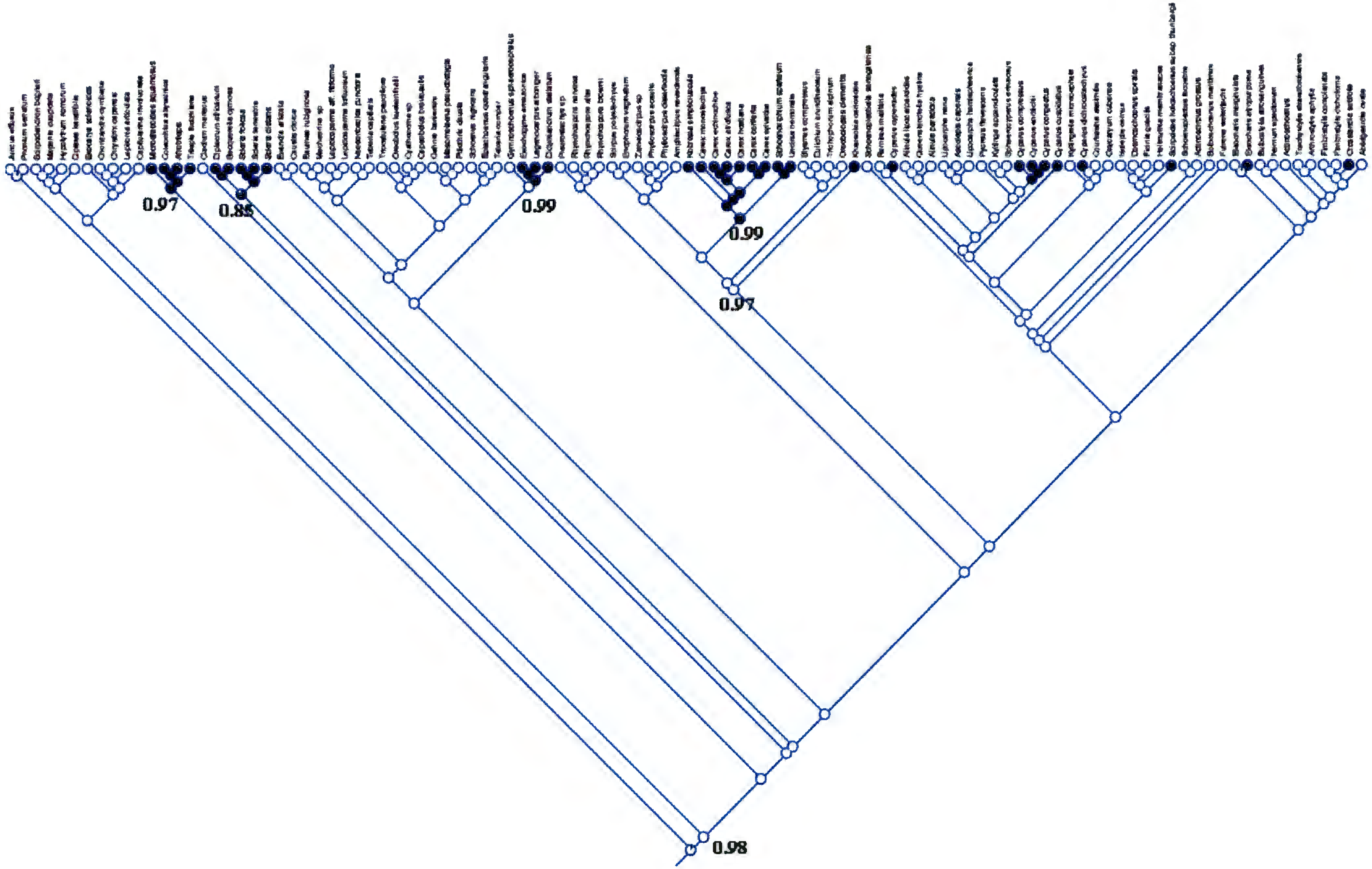

Fig. 13: Distribution of unisexual (black circles) and bisexual (open circles) in Cyperaceae, showing proportional likelihood values for important nodes.

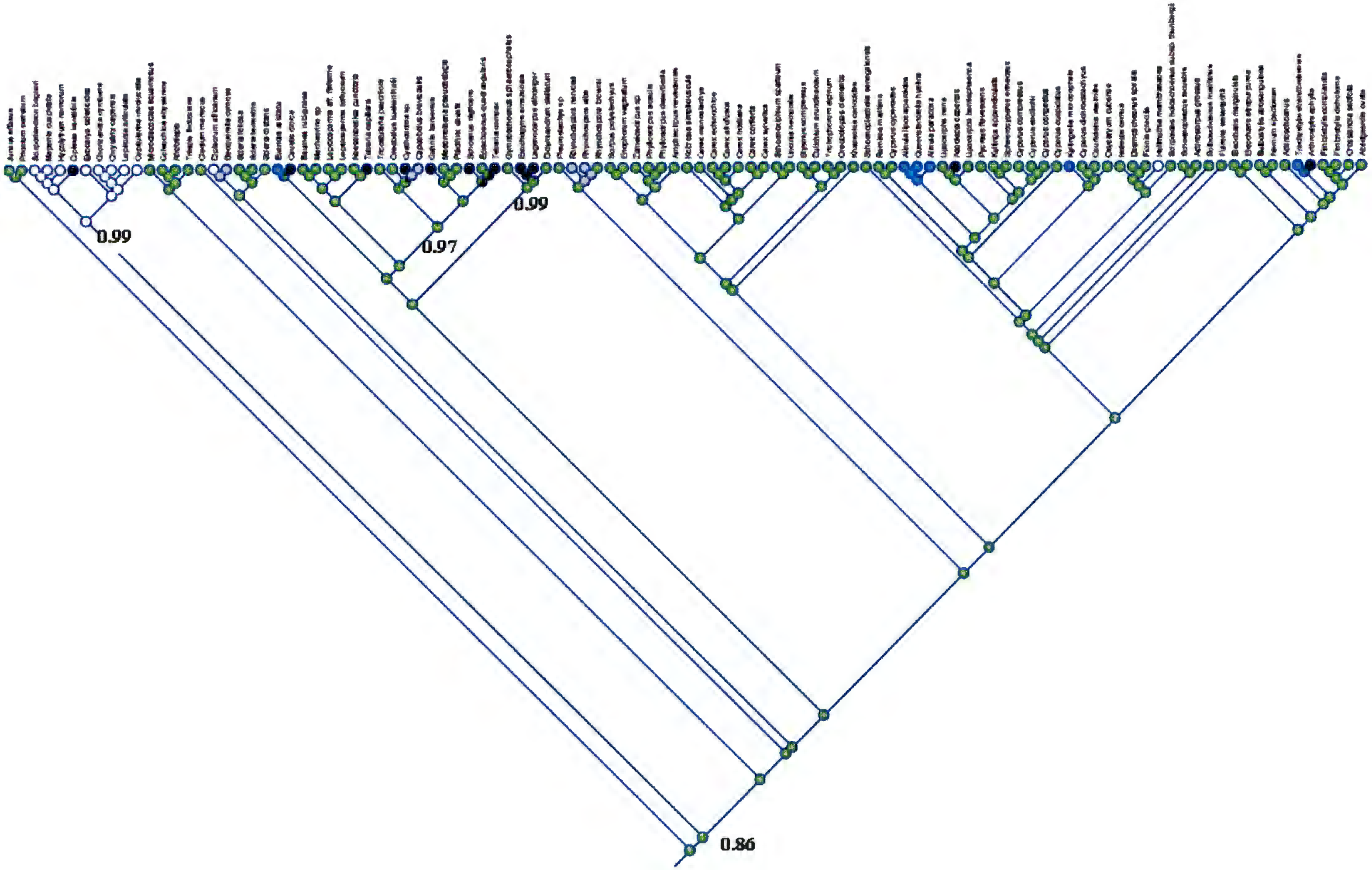

Fig. 14: Optimisation of the number of functional stamens, showing proportional likelihood values for important nodes. Green circles indicate presence of 3 functional stamens, Blue indicates presence of 2; Black indicates presence of 4 or more; white circles indicate presence of a single functional stamen.

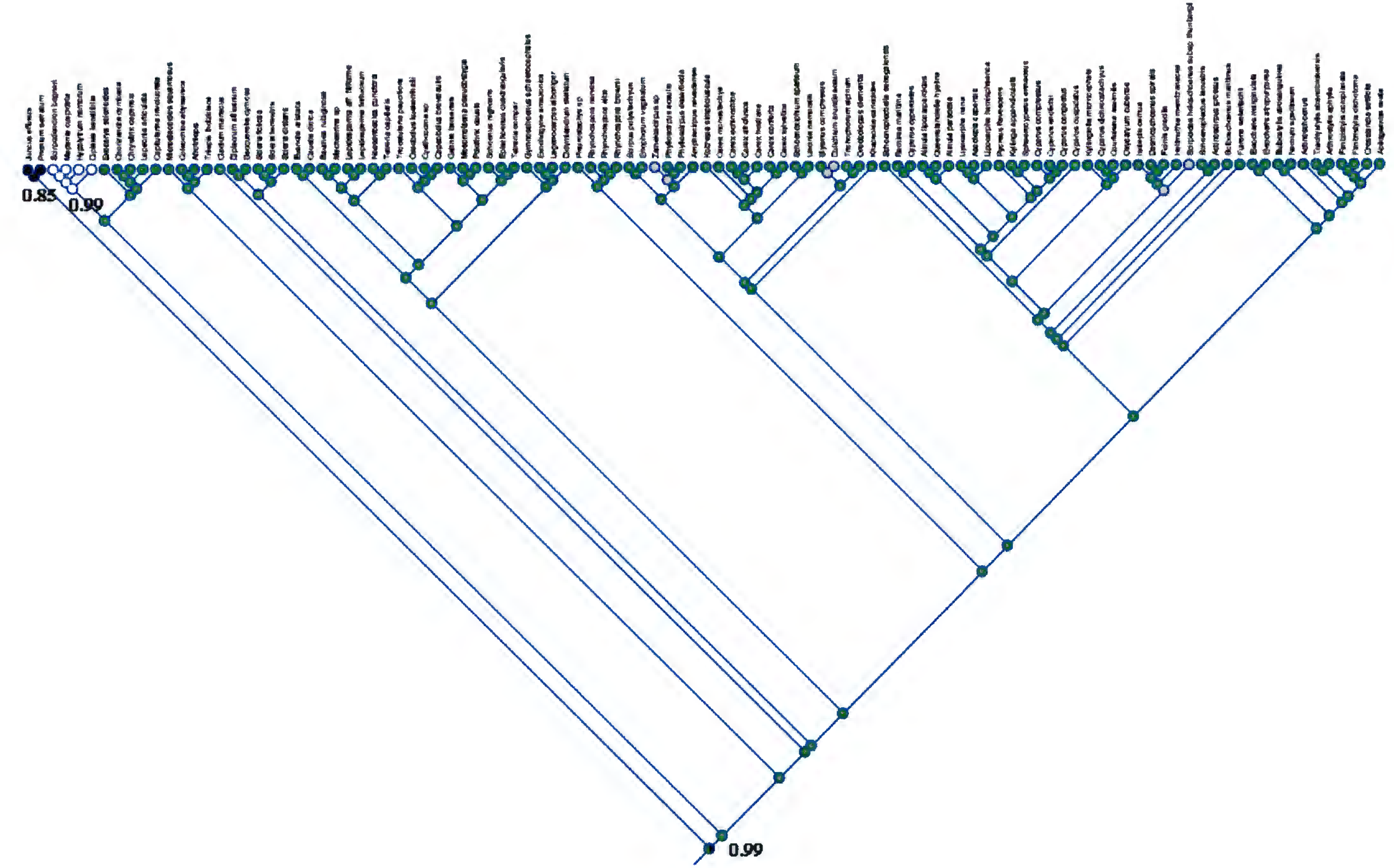

Fig. 15: Optimisation of pollen type in Cyperaceae and related taxa, showing proportional likelihood values for important nodes. Black circles indicate presence of tetrad; white circles indicate presence of Mapanioid type pollen and green circles indicate presence of pseudomonad type pollen. Grey circles indicate uncertainty.

Appendix 1: List of taxa used in this analysis, showing the voucher number and the Genbank accession numbers for each of the three plastid DNA regions.

Taxon	**Voucher**	**GenBank accession numbers**		
		rps16 intron	*rbcL*	*trnL-F OR intron/spacer*
I. Cyperoideae Suess.				
Abildgaardieae Lye				
Abildgaardia ovata (Burm.F.) Kral	Kenya: Muasya et al. 684 (EA, K)	This study (Skelton, R.)	Y12985	AJ295754
Actinoschoenus repens Raynal	Zambia: Robinson 3643 (K)	This study (Skelton, R.)	EF178537	
Arthrostylis aphylla R.Br.	Australia: Wilson 8249 (NSW)		AY725939	
Bulbostylis atrosanguinea (Boeck.) C.B.Clarke	Kenya: Muasya 1037 (EA, K)		Y12992	
Crosslandia setifolia W.Fitzg.	Australia: Wilson 10147 (K)		EF178538	EF178592
Fimbristylis complanata (Retz.) Link	Kenya: Muasya 1029 (EA, K)		Y13009	
Fimbristylis dichotoma (L.) Vahl	Kenya: Muasya 1006 (EA, K)		Y13008	AJ295755

Nemum spadiceum (Lam.) Desv. Ex Ham.	WEST AFRICA: Baldwin 9766 (K)		Y12945	
Trachystylis stradbrokensis (Domin) Kukenth.	Australia: Wilson 8175 (K)		EF178539	EF178591
Bisboeckelereae Pax ex L.T. Eiten				
Becquerelia cymosa Brongn.	Brazil: Thomas et al. 10284 (NY)	This study (Skelton, R.)	Y12948	
Diplacrum africanum C.B.Clarke	Tanzania: Vollensen 3967 (K)		AY725942	
Cariceae Kunth ex Dumort.				
Carex atrofusca		AM085614		
Carex conferta A.Rich.	Kenya: Muasya 1055 (K)	This study (Skelton, R.)	Y12999	
Carex echinochloe Kunze	Kenya: Muasya 1051 (K)	This study (Skelton, R.)	Y12997	AF191818
Carex hostiana DC.	Chase et al. (1993)		L12672	
Carex monostachya A.Rich.	Kenya: Muasya 1052 (K)		Y12998	
Carex sylvatica Huds.	Simpson et al. (2003)			AY344175

Kobresia simpliciuscula (Wahlenb.) Mackenzie	Plunkett et al (1995); Yen et al. (2000)		U49232	AF164948
Uncinia nemoralis K.L.Wilson	Australia: Wilson et al. 9533 (K)		AY725956	
Schoenoxiphium sparteum (Wahlenb.) C.B.Clarke	Kenya: Muasya 2566 (EA)		EF178543	
Uncertain tribe aff. Caricieae				
Khaosokia caricoides D.A.Simpson, Chayam. & J.Parn.	Thailand: Simpson et al. 1886 (K)		AY725948	EF178535
Cryptangieae Benth.				
Didymiandrum stellatum (Boeck.) Gilly	Venezuela: Liesner 23562 (GENT)	This study (Skelton, R.)	EF178544	
Exochogyne amazonica C.B.Clarke	Brazil: Aparecida da Silva 1986 (GENT)		EF178545	
Lagenocarpus alboniger (A.St.Hil.) C.B.Clarke	Brazil: Thomas 11111 (NY)		AY725949	
Cypereae Dumort.				
Alinula lipocarparoides		This study (Skelton, R.)		
Alinula paradoxa Goetgh. & Vorster	Tanzania: Faden et al. 96/29 (K)		AJ278290	AJ295756

Ascolepis capensis (Kunth) Ridl.	Kenya: Muasya 1009 (EA, K)	AF449518	Y13003	AJ295757
Courtoisina assimilis (Steud.) Maquet	Tanzania: Faden et al. 96/119 (K)	AF449519	AY40590	AY040595
Cyperus compressus L.	Thailand: Muasya 1375 (K)	AF449521	AF449506	AF449555/
Cyperus congestus Vahl	Australia: Coveny et al. 17492 (K)	AF449522	AF449507	AF449556/ AF449568
Cyperus cuspidatus Kunth.	Thailand: Muasya 1374 (K)	AF449523	AF449508	AF449557/ AF449569
Cyperus cyperoides (L.) Kuntze	Thailand: Muasya 1277 (K)	AF449524	AF449509	AF449558/ AF449570
Cyperus dichroostachyus A.Rich.	Kenya: Muasya 976 (EA, K)	AF449525	Y12965	/AF449571
Cyperus endlichii Kük.	Kenya: Muasya 695 (K)	AF449526	AF449510	AF449559/ 449572
Desmoschoenus spiralis Hook.f.	New Zealand: Ford 44/94 (NU)		AJ404701	AJ295753
Ficinia gracilis Schrad.	S. Africa: Muasya 2355	AF449537	EF178589	EF178595
Hellmuthia membranacea (Thunb.) R.W.Haines & Lye	S. Africa: Weerderman et al. 269 (K); Muasya 1145 (K)		Y13000	AJ295815
Isolepis cernua (Vahl) Roem. & Schult. var. *cernua*	Britain: Muasya 1058 (K)	This study (Skelton, R.)	Y13014	AJ295775
Kyllinga appendiculata K. Schum.	Kenya: Muasya 1050 (EA, K)	AF449542	Y13007	AJ295761

Kyllingiella microcephala (Steud.) R.W.Haines & Lye	Zimbabwe: Muasya et al. 1118 (K)	AF449540	AY040592	
Lipocarpha hemisphaerica (Roth.) Goetgh.	Thailand: Muasya 1217 (K)		AF449516	AJ295807
Lipocarpha nana (A.Rich.) J.Raynal	Kenya: Muasya 972 (EA, K)	AF449545	Y12990	AF449565/ AF449577
Oxycaryum cubense (Poepp. & Kunth) E.Palla	Zambia: Richards 13318 (K)	This study (Skelton, R.)	Y13006	AJ295762
Pycreus flavescens (L.) Rchb.	Kenya: Muasya 1022 (EA, K)	AF449547	Y13005	AY040602
Queenslandiella hyalina (Vahl) Ballard	Kenya: Mwachala 296 (EA)		AY725953	AJ295763
Remirea maritima Aubl.	Tanzania: Faden et al. 96/48 (K)	AF449550	AY040593	
Scirpoides holoschoenus (L.) Soják	S. Africa: Acocks s.n. (K)		Y12994	AY040604
Scirpoides thunbergii (Schrad.) Soják	S. Africa: Muasya 1205 (K)	AF449551	AJ404727	AJ295811
Scirpus polystachyus	Australia: Pullen 4091 K			AJ295812
Dulicheae Rchb. ex J. Schultze-Motel				
Blysmus compressus Panz.	Afghanistan: Dobson 221 (K)	This study	AJ404700	AJ295766

Schoenoplectus lacustris (L.) Palla	Britain: Muasya 1043 (K)	AF449554	Y12943	AJ295809
Rhynchosporeae				
Pleurostachys sp.	Brazil: Kallunki et al. 513 (NY)		Y12989	
Rhynchospora alba (L.) Vahl	Simpson et al. (2003)	AY344151		AY344174
Rhynchospora brownii Roem. et Schult.	S. Africa: Verboom 616 (BOL)	DQ058336	DQ058353	DQ058316
Rhynchospora nervosa (Vahl.) Boeck.	Brazil: Kallunki et al. 512 (NY)		Y12977	
Schoeneae Dumort.				
Baumea rubiginosa (Spreng.) Boeck.	Australia: Wilson et al. 9471 (K)	This study (Skelton, R.)	AY725940	
Capeobolus brevicaulis (C. B. Clarke) J. Browning	S. Africa: Verboom 646, BOL	DQ058324	DQ058343	DQ058303
Carpha alpina	Wardle et al. (2001); Zhang et al. (2004)		AF307909	AY230012
Caustis dioica R.Br.	Australia: Chase 2225 (K)	This study (Skelton, R.)	Y12976	
Cladium mariscus (L.) R. Br.	Locality unknown: MJC 292 (K)	DQ058319	DQ058338	DQ058298

Cyathocoma bachmannii (Kuk.) C.Archer	S. Africa: Browning 835 (GENT)	**DQ058325**	EF200590	EF178604
Cyathocoma hexandra (Nees) J. Browning	S. Africa: Verboom 648, BOL)		DQ058344	DQ058304
Epischoenus quadrangularis (Boeck.) C. B. Clarke	S. Africa: Verboom 636 (BOL)	**DQ058332**	DQ058349	DQ058311
Evandra aristata R.Br.	Australia: Wilson et al. 8974 (NSW)		AY725944	
Gahnia baniensis Benl.	Malaysia: Simpson 2737 (K)	**DQ058323**	DQ058342	DQ058302
Gymnoschoenus sphaerocephalus (R.Br.) Hook.f.	Australia: Wilson et al. 9463 (K);Zhang et al. (2004)	This study (Skelton, R.)	AY725945	AY230033
Lepidosperma aff. filiforme				
Lepidosperma tortuosum F.Muell.	Australia: Coveny et al. 17470 (K); Roalson et al. (2001)		AY725950	AF285074
Machaerina sp.	New Guinea: Johns 9195 (K)	**DQ058321**	DQ058340	DQ058300
Mesomelaena pseudostygia (Kük.) K.L.Wilson	Australia: Chase 2226 (K)	**DQ058322**	Y12959	DQ058301
Neesenbeckia punctoria (Vahl) Levyns	S. Africa: Muasya 1214 (K)	**DQ058327**	AY725952	DQ058306
Oreobolus kukenthalii Steenis	Malaysia: Simpson 2659 (K)		Y12972	
Oreobolus oligocephalus W.M.Curtis	Zhang et al.(2004)		-	EF178536 AY230031

Ptilothrix deusta (R. Br.) K.L. Wilson	Zhang et al. (2004)		-	
Schoenus nigricans L.	Saudi Arabia: Edmondson 3382 (K)	This study (Skelton, R.)	Y12983	AJ295814
Tetraria compar (L.) Lestib.	S. Africa: Verboom 549, (BOL)	This study (Skelton, R.)	DQ058350	DQ058312
Tricostularia pauciflora (R.Br.) Benth.	Australia: Coveny et al. 17484 (K);Zhang et al. (2004)	This study (Skelton, R.)	AY725954	AY230038
Scirpeae Kunth ex Dumort.				
	Malaysia: Simpson 2660 K			
Amphiscirpus grossus				
	Argentina: Charpin et al. 20575 (GENT)			
Amphiscirpus nevadensis (S. Watson) Oteng-Yeboa			DQ317926	DQ317925
Eriophorum angustifolium Honckney	Simpson et al. (2003)			AY344177
Eriophorum vaginatum L.	Poland: Beyer et al. 2 (K)	AF449553	Y12951	
				AJ295769
Oreobolopsis clementis (M.E.Jones) Dhooge & Goetgh.	Dhooge (2005)			
			AJ811011	Dhooge (2005)
Phylloscirpus acaulis (Phil.) Goetgh. & D.A.Simpson	Dhooge et al. 2003		AJ575926	
				AJ576029

Phylloscirpus deserticola (Phil.) Dhooge & Goetgh.	Ecuador: Laegaard et al. 21478 (GENT)	This study (Skelton, R.)	AJ704785	AJ704786
Scirpus polystachyus F. Muell.	Australia: Pullen 4091 (K)		Y12974	AJ295813
Trichophorum alpinum (L.) Pers.	CANADA: Waterway 2002.95 (GENT)		AJ810999	DQ317924
Zameioscirpus atacamensis (Phil.) Dhooge & Goetgh.	Bolivia: Ruthsatz & Budde 10328 (Trier)	This study (Skelton, R.)	AJ575929	AJ576032
Sclerieae Kunth ex Fenzl				
Scleria distans Poir.	Kenya: Muasya 1023 (EA, K)	DQ058320	Y12968	DQ058299
Scleria foliosa A.Rich.	Tanzania: Muasya 939 (EA, K)		Y12986	
Scleria terrestris (L.) Fassett	Malaysia: Simpson 2658 (K)		Y12947	
Trilepideae Goetgh.				
Afrotrilepis	Reynders *et al.* (2005)	This study (Skelton, R.)		
Coleochloa abyssinica (A.Rich.) Gilly	Ethiopia: Vollesen 80/2 (K)	This study (Skelton, R.)	Y12975	

Microdracoides squamosus Hua	Bonn Acc. 150	This study (Skelton, R.)	AY725951	
Trilepis lhotzkiana Nees	Bonn Acc. s.n.		AY725955	
II. Mapanioideae C.B.Clarke				
Chrysitricheae Lestib. ex Fenzl				
Capitularia foliata Uitt.	Indonesia: Johns 8725 (K)		EF178588	
Capitularina involucrata (J.V.Suringar) Kern	Simpson et al. (2003)			AY344168
Chorizandra cymbaria R.Br.	Bremer (2002)		AJ419940	
Chrysitrix capensis L.	S. Africa: Muasya 1242 (K)	AY344148	AJ419938	AY344171
Exocarya sclerioides (F.Muell.) Benth.	Simpson et al. (2003)	AY344145		AY344167
Lepironia articulata (Retz.) Domin.	Malaysia: Simpson 1236 (K)		Y12957	AY344169
Hypolytreae Presl ex Fenzl				
Diplasia karatifolia Rich. ex Pers.	Simpson et al. (2003)			AY344166
Hypolytrum nemorum (Vahl) Spreng.	Malaysia: Simpson 1379 (K)	AY344142	Y12958	AJ295816
Mapania cuspidata (Miq.) Uittien	Brunei: Marsh 4 (K)	DQ058318	Y12955	AJ295817

Scirpodendron bogneri S.S. Hooper	Malaysia: Simpson 2650 (K)	**AY344143**	Y12946	AY344164
Outgroups				
Juncus effusus L.	Simpson et al. (2003); Chase et al., 1993		L12681	AY344156
Prionium serratum Drège	S. Africa: Gettliffe Norris, s.n. (NBG)		U49223	AY344155