Phylogeny reconstruction of the Schoeneae (Cyperaceae) with a focus on southern-African genera

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Abstract

In this study both plastid and nuclear DNA sequences (*rbc*L, *trn*L-*trn*F, *rps*16, *ITS* and *ETS*) were analysed. New sequences were added to the matrix from Verboom (2006). Parsimony method was used for phylogeny reconstruction. Morphological characters were then optimised on the parsimony tree using both maximum likelihood and parsimony reconstruction. The Schoeneae is not monophyletic. *Scleria* may be included in the Schoeneae. *Schoenus* is probably not monophyletic as was suggested by morphological heterogeneity. It seems as though the Schoeneae ancestor probably had present leaf blades, leaves spread out wide along the stem and an open inflorescence. Further research needs to be done including the sampling of more taxa and more sequences. The monophyly of this tribe is still yet to be resolved. Lower taxonmic relationships need to be resolved further.

Introduction

The generic and specific composition of the Schoeneae has changed over time (previous classifications by Nees von Esenbeck 1834, Clarke 1908 and Bruhl 1995). These classifications were based solely on morphological data. The Schoeneae as described by Goetghebeur (1998) contains 29 genera, which will be investigated in this project. The Schoeneae tribe is difficult to classify based on morphology (Verboom 2006), so the use of molecular data may help to resolve these classification problems. Schoeneae have not been well studied in the past. This new molecular work will hopefully provide more resolution to the phylogeny. In a previous study, the Cyperaceae have been shown to be monophyletic based on *rbc*L sequences (Muasya et al. 2000). This paper by Muasya et al. (2000) also provided weak support for the monophyly of the Schoeneae (65%).

The monophyly of the Schoeneae will be investigated by reconstructing the phylogeny from Verboom (2005). This phylogeny reconstruction will include the use of additional genetic markers, ITS and ETS, as well as additional taxon sampling. This will hopefully improve the structure and support for the Schoeneae phylogeny.
ITS and ETS will be sequenced for those species from Verboom (2005) that have all of *trn*LF, *rbc*L and *rps*16 sequences already. Included as well are some genera that were previously classified (as per Nees von Esenbeck 1834, Clarke 1908 and Bruhl 1995 in Verboom 2005) as part of the Schoeneae and are no longer (as per Goetghebeur 1998) e.g. *Baumea*. Verboom (2006) demonstrated the monophyly of the Schoeneae, whereas Bruhl (1995) found the Schoeneae to be paraphyletic.

Sclerieae is closely related tribe included in the Cyperaceae, it includes the genus *Scleria*. It has been proposed that the Sclerieae may be included in the Schoeneae. The inclusion of more Scleria species in this analysis may help to answer this question. *Schoenus* is a large genus, containing many morphologically diverse species. Previously with these species belonging to different morphological Series as per Bentham and Von Meuller (1878). The inclusion of these species allows the monophyly of this genus to be investigated. This genus appears that it may not be monophyletic based on morphology but the use of molecular markers in this study should help resolve this question.

This phylogeny reconstruction will also include morphological characters. Including morphology in this analysis should allow us to get an idea of the ancestral morphology of the Schoeneae. This is interesting because the Schoeneae is a very morphologically diverse group and is hard to characterise based on morphology. This look at morphology will mostly be focused leafiness, culm nodes and inflorescence form. Looking at the phylogeny, we will be able to tell how many times the morphology of this tribe has changed. It is hypothesised that the Schoeneae ancestor mostly likely had all its leaves at the base, a closed leaf sheath and a capitate inflorescence (Verboom 2008 pers comm.). And then the morphology has moved towards having an open inflorescence with leaves spread out wide. The ancestral areas of the Schoeneae will be scored on the phylogeny, this hopefully start to answer the question of how these species migrated from their point of origin to become more widespread.

Table 1: Classification of the Schoeneae by Goetghebeur (1998)

Family Cyperaceae				
Subfamily Cyperoideae				
Tribe Schoeneae	Genus	Number of species	Species (if there is only one)	Areas in which the genus is found
	Arthrostylis	1	*A. aphylla*	NE Australia
	Actinoschoenus	3		Gabon, SE Zaire, Zambia, Madagascar, Sri Lanka, SE Asia, Phillippines, New Caledonia
	Trichoschoenus	1	*T. bosseri*	Madagascar
	Trachystylis	1	*T. stradbrokensis*	E Australia
	Rhynchospora	±250		Subcosmopolitan, concentrated in (sub)tropical America
	Pleurostachys	±30		Tropical and subtropical S America
	Schoenus	±100		Concentrated Australia and Malesia. S. nigrican is subcosmopolitan
	Gymnoschoenus	2		Australia
	Mesomelaena	5		SW Australia
	Ptilothrix	1	*P. deusta*	E Australia
	Cyathochaeta	4		SW and E Australia
	Oreobolus	±15		Malesia, SE Australia, Tasmania, New Zealand, Tahiti, Hawaii, Juan Fernandez Is., Falkland Is., W South America, Central America
	Carpha	±15		S Africa, Central African Mountains, Madagascar, Mascarenes, New Guinea, S Japan, SE Australia, SE Australia, New Zealand, Chile
	Trianoptiles	3		South Africa (SW Cape)
	Tetraria	±50		Most in S Africa (ca. 45), a few in the mountains of SE and Central Africa (2), SW Australia (6), New Zealand (1). T. borneensis is

				found in Borneo
	Cyathocoma	±3		S Africa (S Cape and Natal)
	Neesenbeckia	1	*N. punctoria*	South Africa (SW Cape)
	Epischoenus	±8		South Africa (SW and S Cape)
	Costularia	±20		New Caledonia (12), Malesia (1), Seychelles, Madagascar to Southern Africa (2)
	Gahnia	±30		SE Asia, Malesia, Australia, New Zealand, Pacific Islands
	Morelotia	2		Hawaii, New Zealand
	Reedia	1	*R. spathacea*	SW and W Australia
	Evandra	2		SW and W Australia
	Caustis	±6		Australia
	Cladium	±4		C. mariscus is subcosmopolitan
	Rhynchocladium	1	*R. steyermarkii*	S Venezuela
	Machaerina	±50		Malesia, Madagascar, the Mascarenes, SE Asia, SE Australia, New Zealand, New Caledonia, Pacific Islands, tropical South America, West Indies
	Lepidosperma	±55		SE Asia + Malesia (1), Australia (ca. 50), New Zealand (3), New Caledonia (4)
	Tricostularia	6		Sri Lanka + SE Asia + Malesia (1), Australia (5), New Caledonia (2)
Total	29 genera	±670 species		

Materials and Methods

DNA samples

The matrix from Verboom (2006) was downloaded from TreeBase. More sequences were added to this matrix, these sequences were downloaded from GenBank. More taxa and more regions, ITS and ETS from existing taxa were also sequenced. The total DNA of these samples was extracted using the modified CTAB extraction method. For a complete list of all sequences used in this project, see Appendix 1.

PCR

The Internal Transcribed Spacer (ITS) region was amplified using the forward and reverse primers, ITSL and ITS4, while the External Transcribed Spacer was amplified using the primers, ETS1F and 18SR as given in Starr et al. (2003). Polymerase Chain Reaction (PCR) was used to amplify the selected gene regions. 30μl reaction volumes were prepared on ice, using 2μl of DNA template, 16.4μl of PCR water, 3μl of 10x DNA polymerase buffer, 4.2μl of $Mg2+Cl2$ (50mM), 0.2μl of Taq DNA polymerase enzyme, 1.2μl of dNTP (10mM), 1μl of forward primer (10μM) and 1μl of reverse primer (10μM). An Applied Biosystems GeneAmp 2700 thermal cycler (Applied Biosystems, Foster City, CA, USA) was used for the amplification. The PCR programme used had an initial denaturation phase of 2 minutes at 94°C, followed by 33 cycyles of 1 minute at 94°C, 1 minute at 52°C and 2 minutes at 72°C, then the final extension phase at 72°C for 7 minutes. A 1% agarose gel was used to see whether the PCR product contained successfully amplified DNA. Successful PCR products were then sent to Macrogen (http://www.macrogen.com) laboratories in Korea for cycle sequencing using the same primer used for amplification.

Sequences were compiled using Staden 1.7.0 for Windows. Sequences were aligned in BioEdit using the Clustal W alignment and then were aligned by eye. New sequences were added to the matrix from Verboom (2006) in MacClade. The methods for phylogeny reconstruction given in Harrison and Langdale (2006) and Verboom (2006) were followed.

Parsimony

Parsimony analysis was done on PAUP* version 4.0b10 (Swofford 2002) using a Heuristic search. 10 000 random addition sequences and TBR branch swapping was used. COLLAPSE and MULTREES options were in effect. 500 bootstrap replicates were done with simple addition sequence and TBR branching. The tree was rooted using *Luzula sylvatica*.

Bayesian

The Bayesian analysis was performed using MrBayes (Huelsenbeck and Ronquist 2001). An evolutionary model with 6 different rates for each type of transition and transversion was used in this analysis. The complex GTR+I+G model was used because under parametrisation is a greater problem with Bayesian analysis than overparameterisation (Huelsenbeck and Rannala 2004). The Markov Chain Monte Carlo (MCMC) algorithm was used. Each run had four Markov chains, of which one was cold while the others were heated. Every Markov chain was run for a million generations (10^6) and sampled every 100^{th} generation.

Morphological characters

The parsimony tree was used for the ancestral character state reconstruction. This was done using Mesquite (Maddison and Maddison 2007). Morphological characters were optimised on the tree using both Parsimony method and Maximum likelihood (MK1 model).

Morphological characteristics evaluated:

1) leaf position-all basal (0) or spread out wide (1)
2) leaf blade presence- present (>5mm) (0) or absent (<5mm) (1)
3) inflorescence type- capitate (0) or open (1)
4) ligule- presence (0) or absence (1)
5) leaf sheath- open (>5mm) (0) or closed (1)
6) leaf sheath- reticulate (0) or not reticulate (1)

7) amphicarphy- presence (0) or absence (1)
8) ancestral area- Africa (0) or Australasia (1) or South America (2) or Northern Hemisphere (3)

Results

The matrix of molecular sequence data had 88 taxa and 6475 characters, with 832 of those excluded from the analysis and 5643 characters included in the analysis. All characters are weighted equally. 2471 of those characters were constant. 955 variable characters were parsimony-uninformative, with a total of 2217 parsimony-informative characters. Gaps were treated as missing data. The parsimony analysis produced 314 trees of equal length.

Phylogeny: Parsimony

The parsimony strict consensus tree (Appendix 2) was very poorly resolved with a large polytomy including most of the study species. So one of the parsimony trees is shown (Figure 1). The Schoeneae is not monophyletic. A number of the outgroups such as: *Hypolytrum, Calyptrocarya, Lagenocarpus, Isolepsis, Ficinia,* etc. are included with the ingroup.

The large genus *Tetraria* is also not monophyletic. Most of the species are divided into two seperate clades. *Schoenus* was also found to not be monophyletic.
All the *Scleria* species make up a well supported clade (BS=98%), excluding *Scleria distans.* There is strong support for *Baumea rubignosa* being sister to *Schoenus efoliatus* (BS=98%). Strong support for the clade containing all the *Gahnia* species (BS=96%) and that it is sister to the clade made up of *Mesomelaena* and *Ptilothrix* (BS=91%).

Bayesian

The Bayesian analysis did not work. After a million generations, the 2 runs had not converged. The analysis is being run again for 5 million generations and sampled

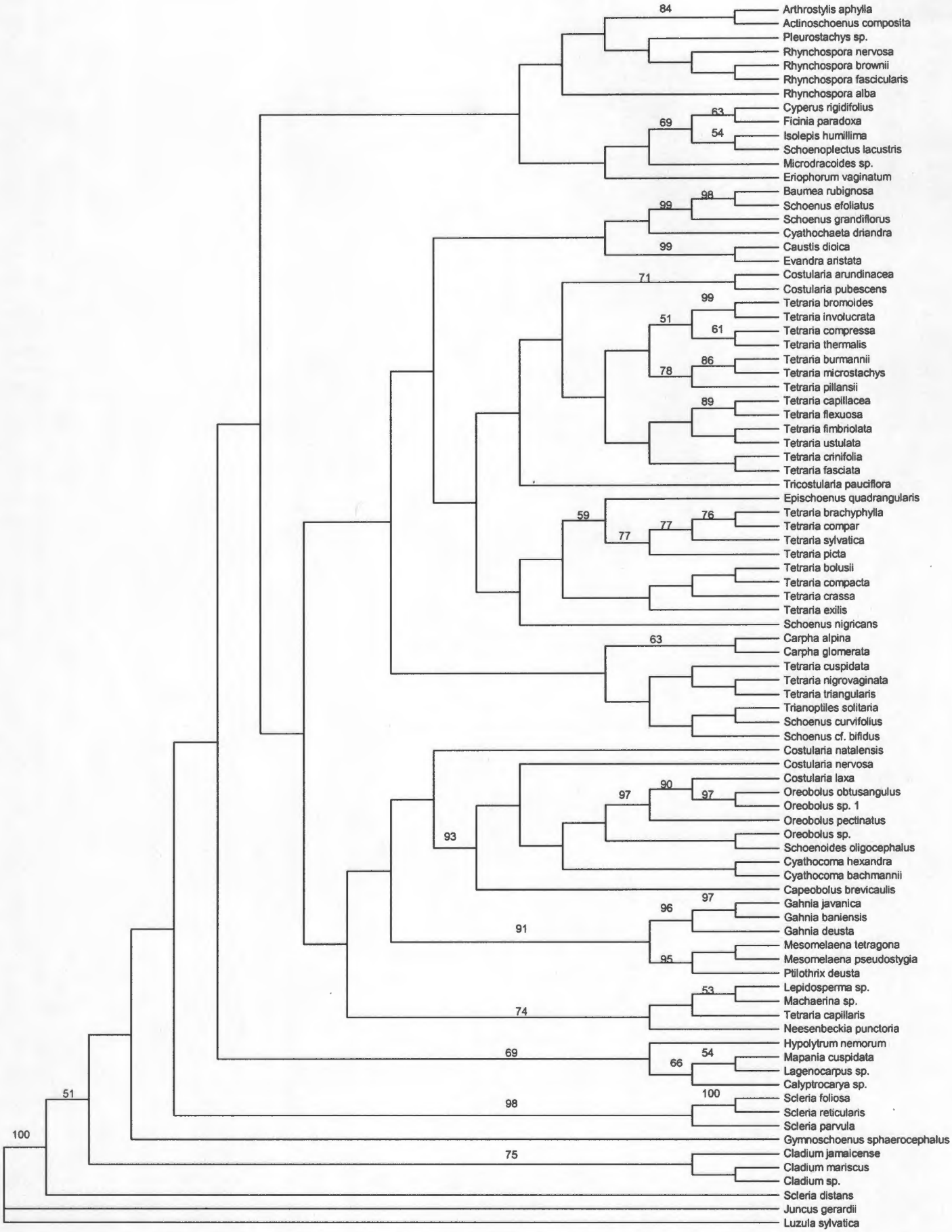

Figure 1: One of the 314 trees obtained from the analysis of the molecular data, showing relationships within the Schoeneae with bootstrap values

every 1000th generation using 2 Markov chains. This analysis is still running and therefore the results will not be included in this write-up.

Morphology- ancestral character state reconstruction

The maximum likelihood character state optimisations are given for most of the characters but for a few of them such as: leaf sheath open or closed and ancestral area, the parsimony reconstruction is shown as well. This is because the model used for the maximum likelihood reconstruction can not deal with missing data and polymorphisms but parsimony can.

In Figure 2, it was reconstructed that the Schoeneae ancestor mostly likely had leaves spread out wide along the stem (PL=0.66). Having all leaves at the base appears to be more of a derived character, evolving later with multiple independent evolutions. There appears to be no distinct pattern, this character does not appear to be found in particular genera. The Schoeneae ancestor had leaf blades, this extremely well supported with a significant probability likelihood of 0.99 (Figure 3). Leaf blade absence is derived and has evolved as many as 4 separate times in different genera e.g. *Epischoenus, Schoenus*, *Actinoschoenus* and *Lepidosperma.* A capitate inflorescence appears to have evolved multiple independent times (Figure 4). The Schoeneae ancestor probably had an open inflorescence (PL=0.63). Very few species sampled had a capitate inflorescence.

Figure 5 shows that the presence or absence of a ligule is quite variable as there seems to be no distinct pattern in the distribution of this character. The Schoeneae ancestor probably had a ligule (PL=0.92). The clade containing *Gahnia, Ptilothrix* and *Mesomelaena* shows the presence of a ligule (PL=0.94). The large genus *Tetraria* is variable for this character, with some member having a ligule and other members not. Maximum likelihood failed to reconstruct leaf sheath open or closed as a character because there was a lot of missing data and some polymorphisms (Figure 6). In Figure 7, parsimony analysis shows that the Schoeneae ancestor probably had a closed leaf sheath, with an open leaf sheath evolving later. The parsimony character state reconstruction took 17 steps to get the tree. The clade containing *Neesenbeckia, Lepidosperma, Gahnia, Mesomelaena, Costularia, Cyathocoma, Oreobolus* all have

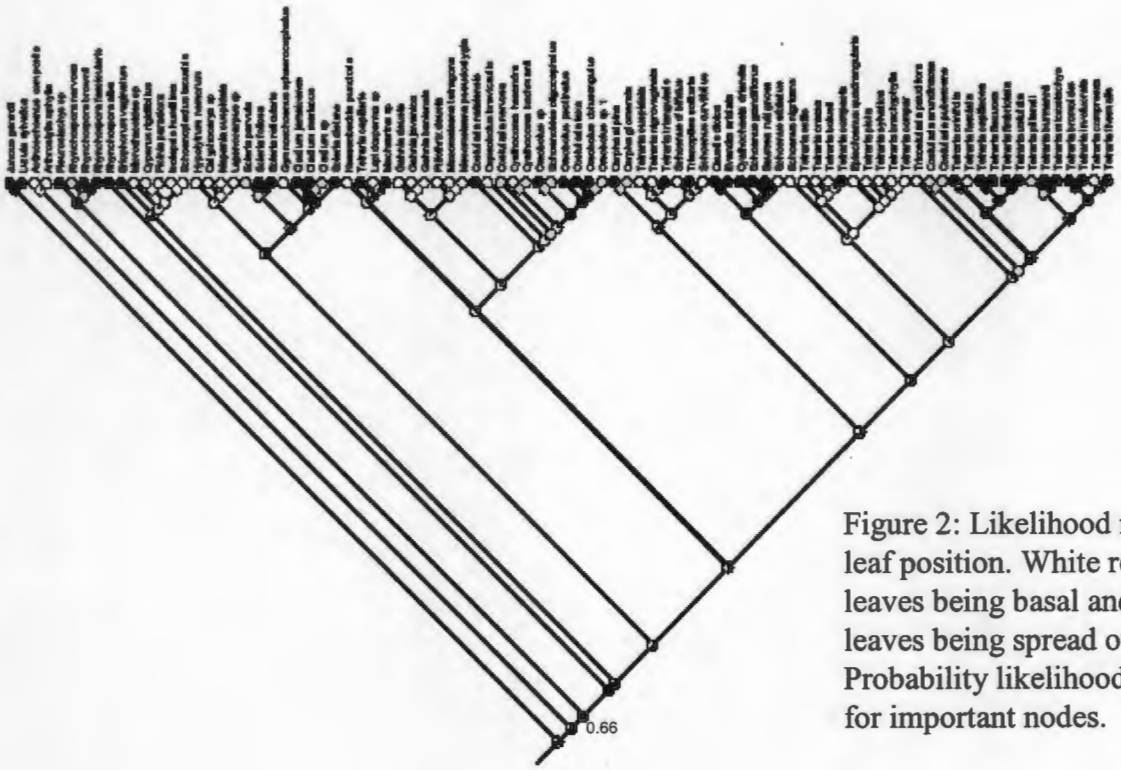

Figure 2: Likelihood reconstruction for leaf position. White represents all leaves being basal and black represents leaves being spread out wide. Probability likelihood values are given for important nodes.

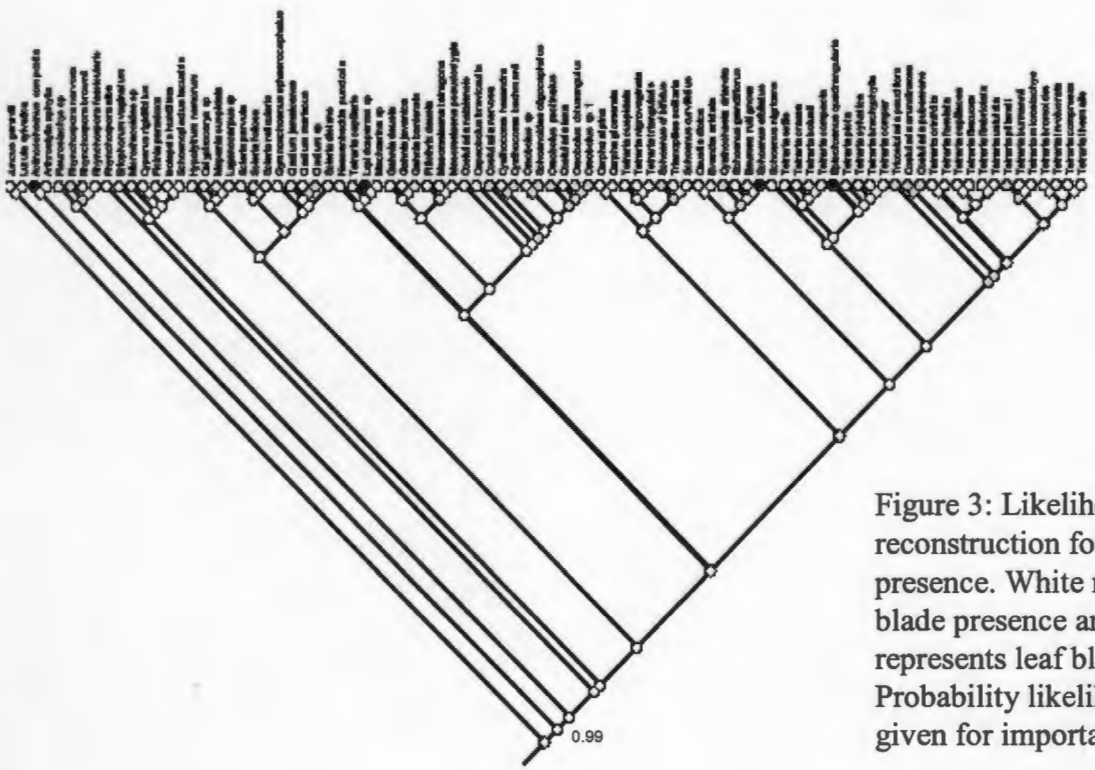

Figure 3: Likelihood reconstruction for leaf blade presence. White represents leaf blade presence and black represents leaf blade absence. Probability likelihood values are given for important nodes.

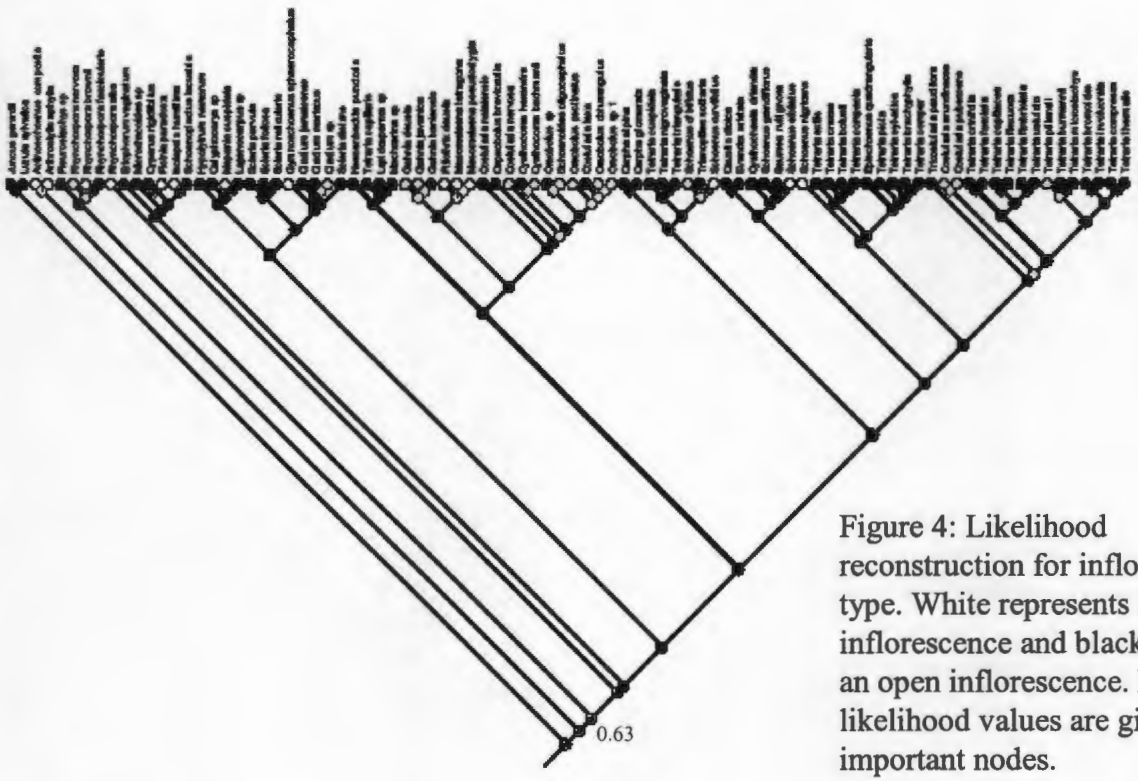

Figure 4: Likelihood reconstruction for inflorescence type. White represents a capitate inflorescence and black represents an open inflorescence. Probability likelihood values are given for important nodes.

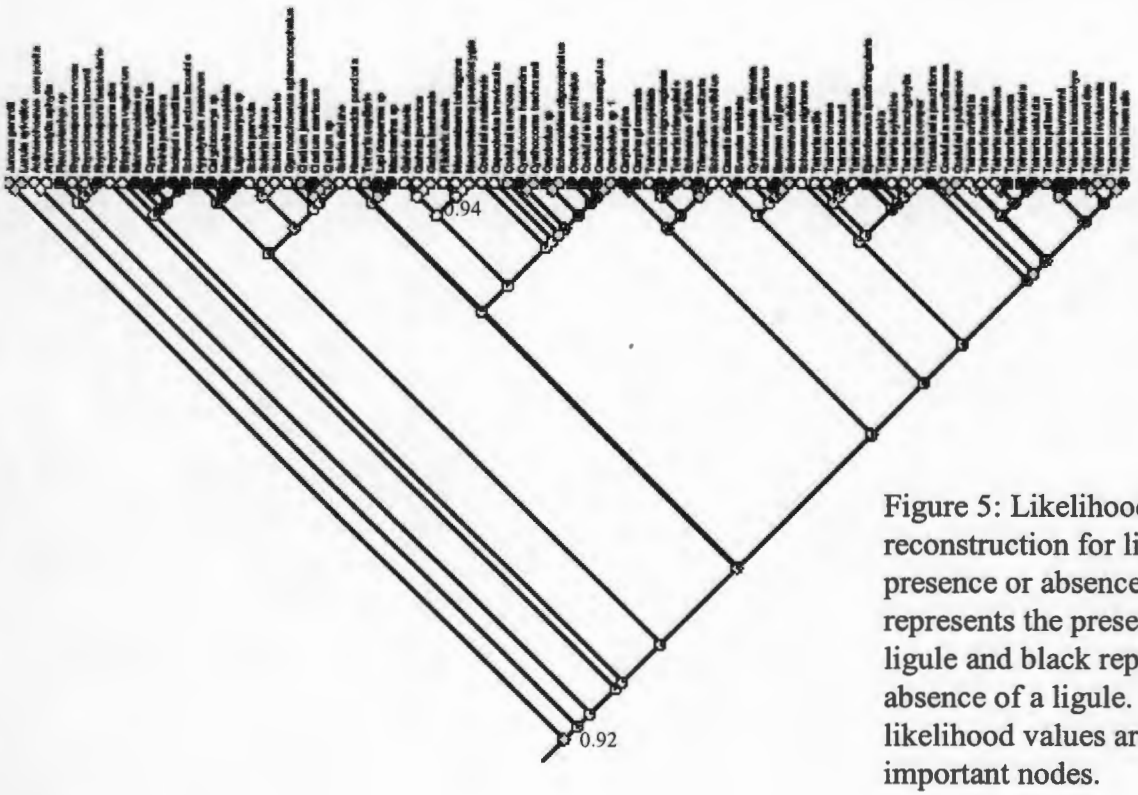

Figure 5: Likelihood reconstruction for ligule presence or absence. White represents the presence of a ligule and black represents the absence of a ligule. Probability likelihood values are given for important nodes.

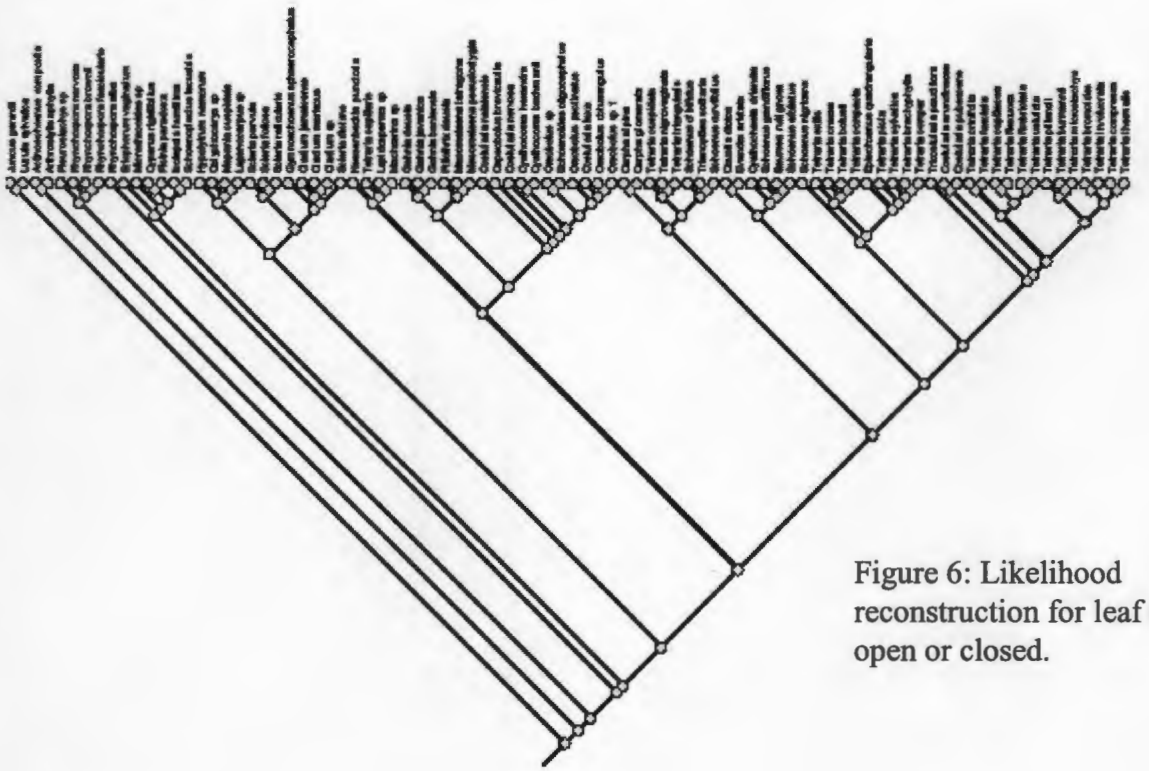

Figure 6: Likelihood reconstruction for leaf sheath open or closed.

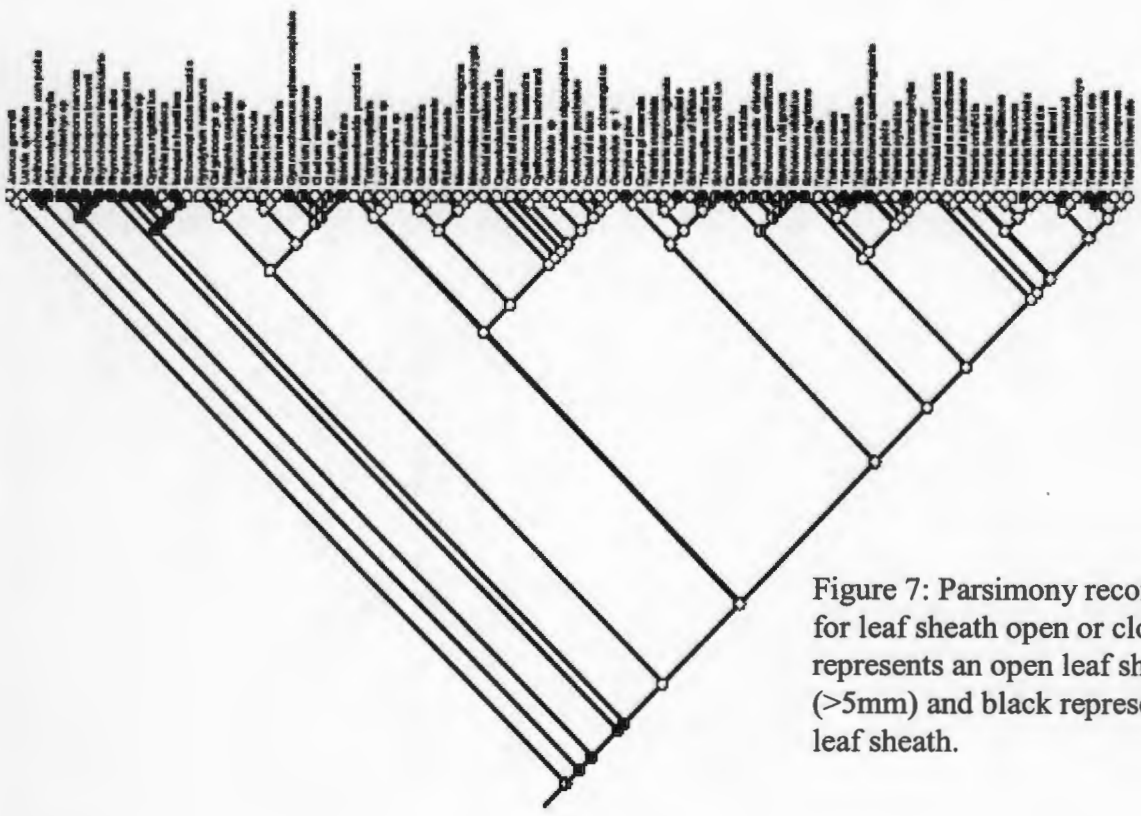

Figure 7: Parsimony reconstruction for leaf sheath open or closed. White represents an open leaf sheath (>5mm) and black represents a closed leaf sheath.

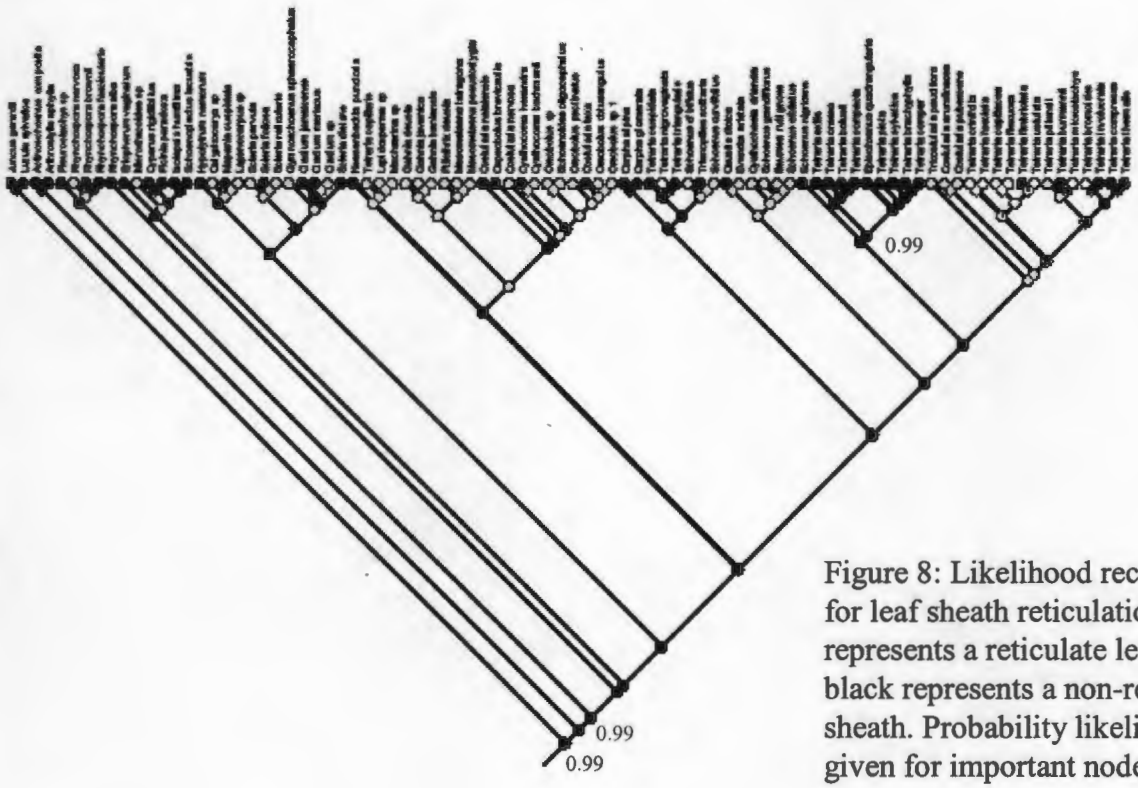

Figure 8: Likelihood reconstruction for leaf sheath reticulation. White represents a reticulate leaf sheath and black represents a non-reticulate leaf sheath. Probability likelihoods are given for important nodes.

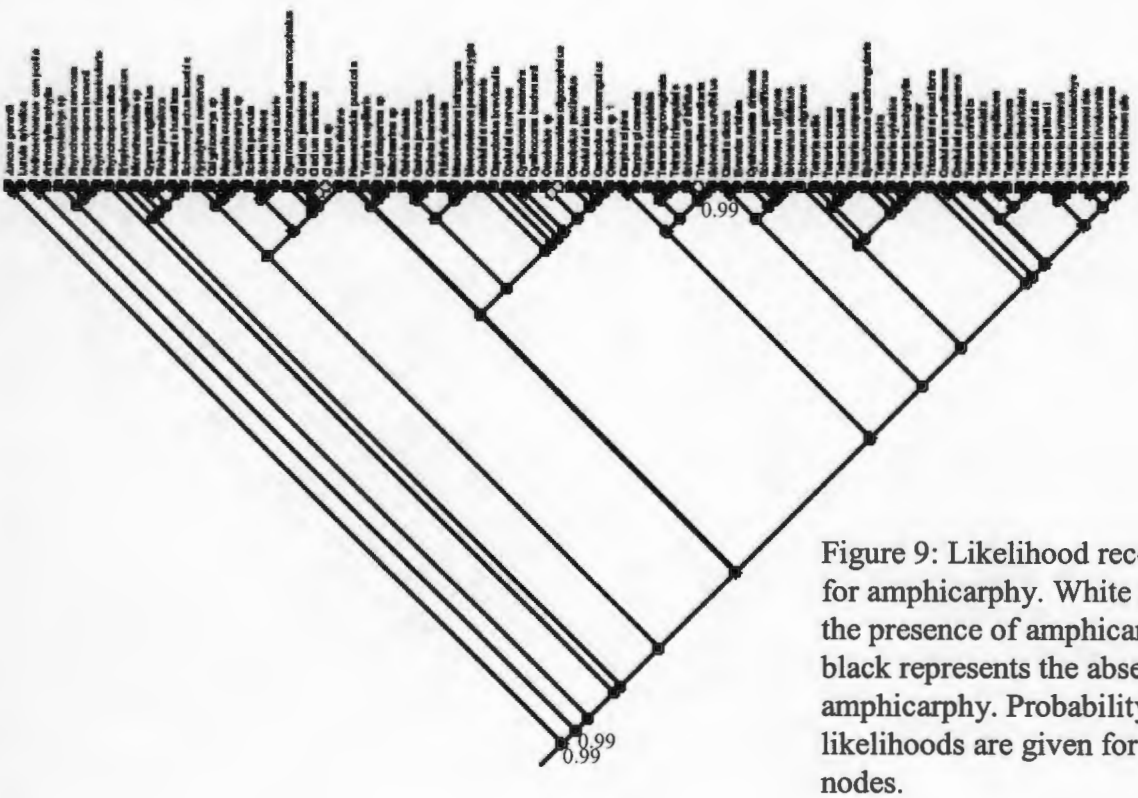

Figure 9: Likelihood reconstruction for amphicarphy. White represents the presence of amphicarphy and black represents the absence of amphicarphy. Probability likelihoods are given for important nodes.

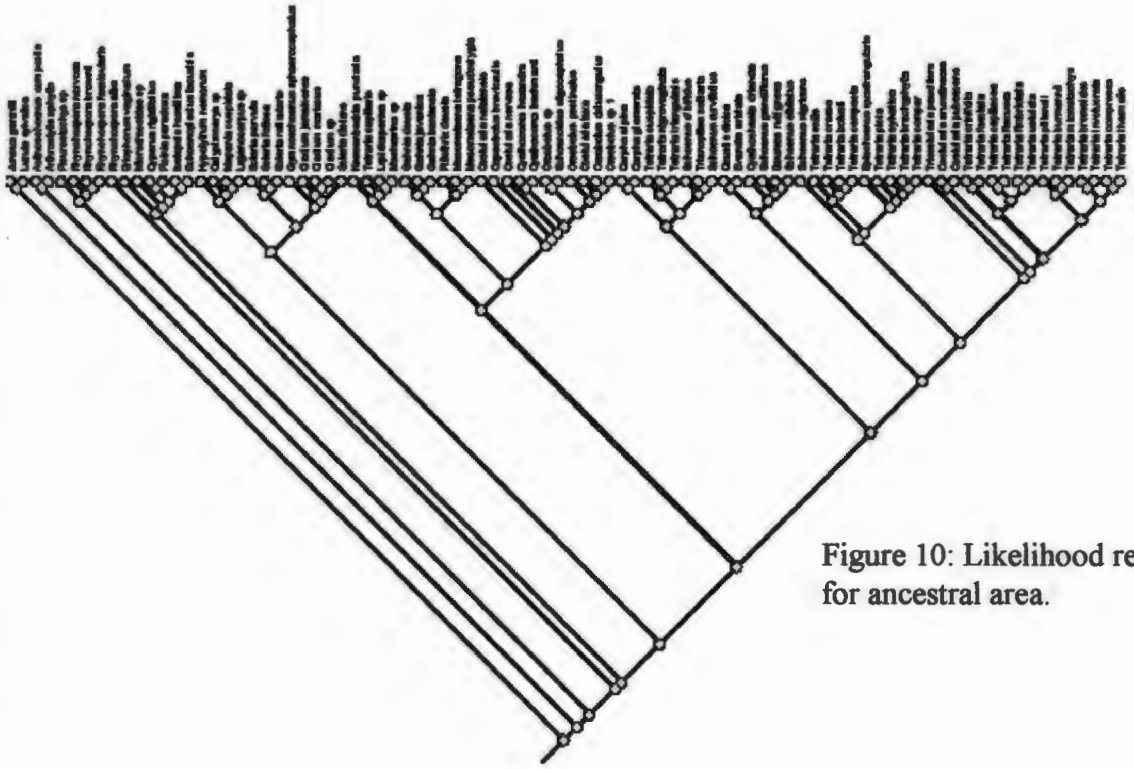

Figure 10: Likelihood reconstruction for ancestral area.

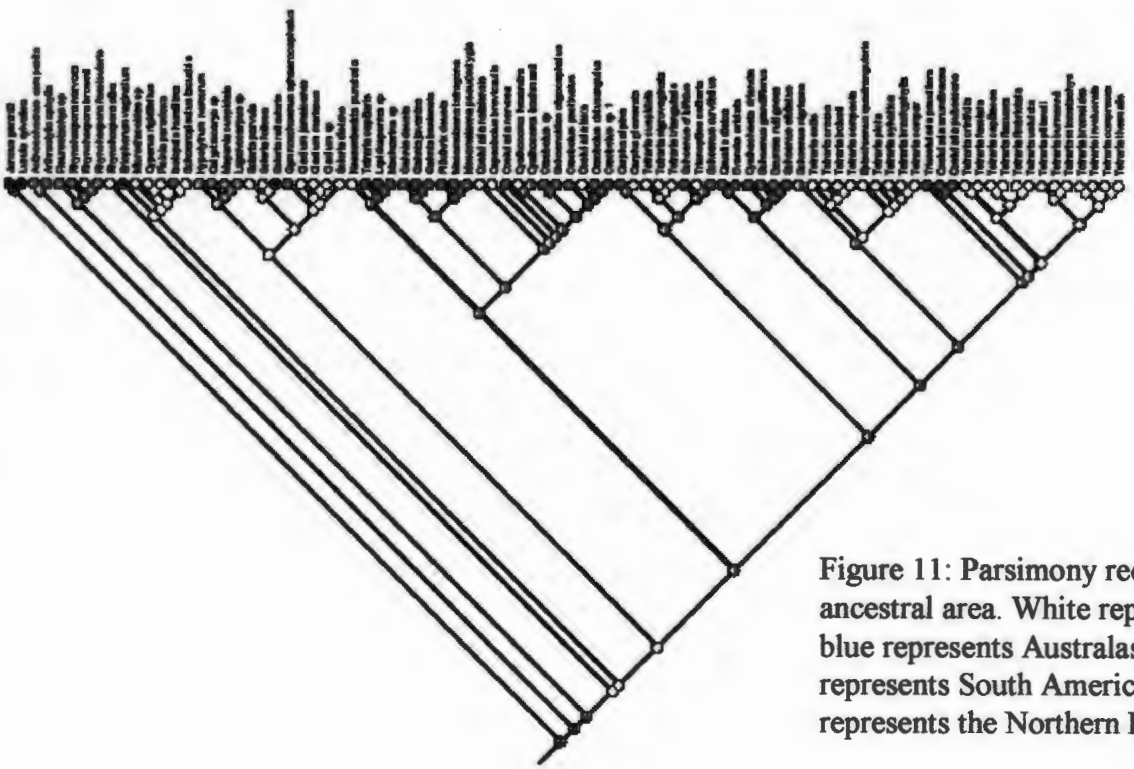

Figure 11: Parsimony reconstruction for ancestral area. White represents Africa, blue represents Australasia, black represents South America and green represents the Northern Hemisphere.

open leaf sheaths. Figure 8 shows that a lot of species were coded as unknown (grey) for leaf sheath reticulation. The clade containing some of the *Tetraria* species, *Schoenus nigricans* and *Epischoenus quadrangularis* all have leaf sheaths that are not reticulate (PL= 0.99).

In Figure 9, amphicarphy has only evolved once in the genus *Trianoptiles* (PL=0.99). Almost all other species did not have amphicarphy (PL=0.99). So the Schoeneae ancestor mostly likely did not have amphicarphy (PL=0.99). Maximum likelihood was unable to properly reconstruct geographical origin of the Schoeneae because of the presence of polymorphisms (Figure 10). Parsimony reconstruction showed that the species were likely to have originated in all of the areas, from Africa, Australasia, South America and the Northern Hemisphere (Figure 11).

Discussion

Phylogenetic relationships

The Schoeneae is not monophyletic. This is true if we use the classification of the Schoeneae as described by Goetghebeur (1998). Verboom (2006) showed that the Schoeneae was monophyletic. Clade containing *Costularia, Oreobolus, Cyathocoma, Schoenoides* and *Capeobolus* (BS=93%) is also found in Verboom (2006) with a BS=100%.

Schoenus curvifolius is sister to *Schoenus cf. bifidus*, while *Scoenus efoliatus* is sister to *Schoenus grandiflorus* and *Schoenus nigricans* is not sister to any other *Schoenus* species.Of the 5 *Schoenus* species included, they were all from different morphological Series (Bentham and Von Meuller 1878). *Schoenus* is probably not a monophyletic genus. It is not monophyletic in this analysis because of morphological heterogeneity within the genus. *Scleria* is most likely part of the Schoeneae, it forms a well supported clade within the Schoeneae (BS=98%). This would need to be confirmed by including more *Scleria* species and sequences in the analysis.

Morphological character evolution

Leaf position

Leaves spread out wide is the ancestral condition with all leaves at the base evolving later.

Leaf blade presence

Most of the species in this analysis had a leaf blade (>5mm). *Epishoenus quadrangularis, Schoenus efoliatus, Lepidosperma* sp. and *Actinoschoenus composita* are the only species without a leaf blade (Figure 3). the Schoeneae ancestor most likely had present leaf blades (PL=0.99).

Inflorescence type

This character seems to have no evolutionary pattern and has evolved independantly multiple times. Most species sampled have an open inflorescence. Capitate inflorescence is not the ancestral character state of the Schoeneae.

Ligule

Ligule is a very variable character. It has evolved multiple independent times.T*etraria,Schoenus, Scleria, Gahnia*, etc. are some of the genera in which species have a ligule (Figure 5).

Leaf sheath reticulation

The ancestral character state is very likely to have been a leaf sheath that is not reticulate (PL=0.99). In the large genus, *Tetraria* both character states are present. *Tetraria* is also the only genus in which species have a reticulate leaf sheath (Figure 8). A reticulated leaf sheath may have only evolved once. Many of the outgroups such as: *Luzula sylvatica* and *Juncas gerardii* have a non-reticulate leaf sheath (PL=0.99).

Amphicarphy

Amphicarphy has only evolved once as can be seen in Figure 9. Amphicarphy is a trait that Amphicarphy has also evolved in the genus, *Carpha* (Goetghebeur 1998),

but the *Carpha* species analysed in this project did not have amphicarphy. None of the other genera within the Schoeneae have amphicarphy.

Ancestral area

Bremer (2002) found that the Cyperaceae and sister families originated in South America and/or Africa. Most of the Schoeneae are from Africa or Australasia (Figure 10). This does support Bremer's (2002) findings. Although the Schoeneae ancestor could not be reconstructed with any more certainty. It appears that the Schoeneae ancestor could have come from any region of the world. The *Rhynchospora* clade is found in the Northern Hemisphere.

Morphology results were not what was expected. It now seems as though the Schoeneae ancestor probably had leaf spread out wide along the stem and an open iflorescence. Ancestral trait reconstruction results may also be what they are because of what species were sampled. This may have affected the reconstruction of some of the traits and would be better resolved if more species were included in the analysis. The maximum likelihood reconstructions were better than the parsimony reconstructions because parsimony was able to reconstruct the character states for all of the morphological characters. This is because parsimony is very conservative. Also maximum likelihood gives probability likelihoods which are useful for trying to understand how things have changed.

Conclusion

The Schoeneae is not a monophyletic tribe. Further study needs to be done on the specific and generic composition of the tribe. Further research should include sampling of more taxa and more sequences. The monophyly of this tribe is still yet to be resolved. Also, lower taxonomic relationships are not well resolved and need further analysis.

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Appendix 1: Complete list of all sequences used in this analysis, showing GenBank accession number and references

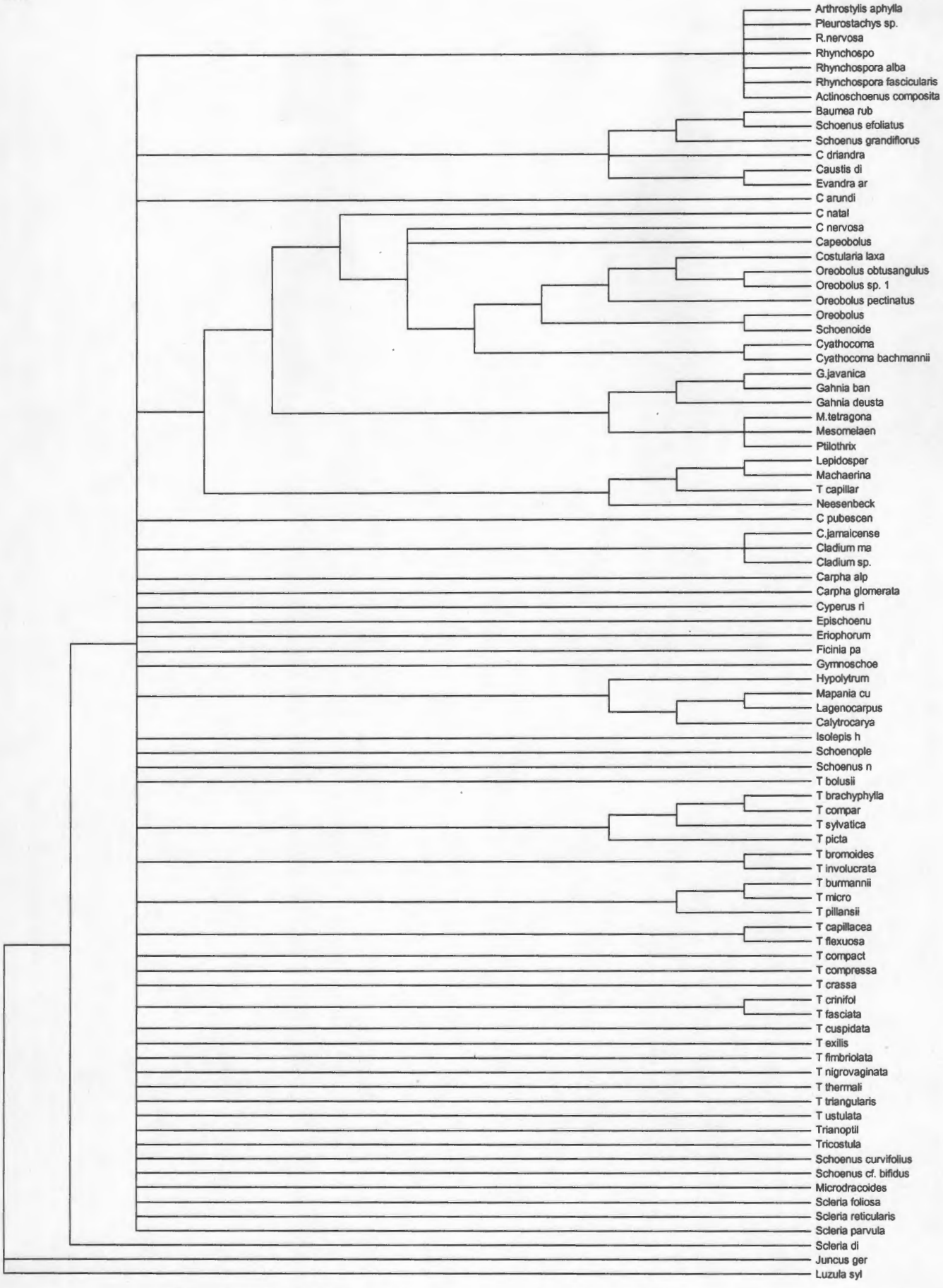
taxon	*trn* LF	*rbc* L	*rps* 16	*ITS*	*ETS*
Ingroup					
Actinoschoenus composita	AY506703 (Ghamkhar et al. 2007)			AY506755 (voucher K. L. Clarke 213 (NE)	
Arthrostylis aphylla R.Br.	AY506700 (Ghamkhar et al. 2007)	AY725939 (Simpson et al. unpub)		AY506757 (Ghamkhar et al. 2007)	
Baumea rubiginosa (Spreng.) Boeck.		AY725940 (Muasya et al. 2000)			JLH
Carpha alpina	AY230012 (Zhang et al. 2004)	AF307910 (Wardle et al. 2001)		DQ385557 (Gardner et al. unpub)	
Carpha glomerata Nees	AY230026 (Zhang et al. 2004)	AY725941 (Simpson et al. unpub)			
Caustis dioica R. Br.		Y12976 (Muasya et al. 1998)		JLH	JLH
Cladium jamaicense Crantz		Y12988 (Muasya et al. unpub)			
Cladium mariscus (L.) R. Br.	DQ058298 (Locality unknown: MJC 292, K)	DQ058338 (Locality unknown: MJC 292, K)	DQ058319 (Locality unknown: MJC 292, K)	JLH	Matthew Britton
Cladium sp.		AY123234 (Michelangeli et al. 2003)			
Costularia arundinacea (Sol. Ex Vahl) Kuek.	AY230036 (Zhang et al. 2004)				
Costularia laxa Cherm.	DQ456955 (Madrinan et al. 2006)			DQ450465 (Madrinan et al. 2006)	
Costularia natalensis C.B. Cl.	DQ058305 (S. Africa: Verboom 773, BOL)	DQ058345 (S. Africa: Verboom 773, BOL)	DQ058326 (S. Africa: Verboom 773, BOL)		
Costularia nervosa Raynal	AY230032 (Zhang et al. 2004)				
Costularia pubescens Raynal	AY230037 (Zhang et al. 2004)				
Cyathochaeta diandra (R. Br.) Nees	AY230042 (Zhang et al. 2004)			JLH	JLH
Cyathocoma bachmannii (Kuk.) C.Archer					
Cyathocoma hexandra (Nees) J. Browning	DQ058304 (S. Africa: Verboom 648, BOL)	DQ058344 (S. Africa: Verboom 648, BOL)	DQ058325 (S. Africa: Verboom 648, BOL)	JLH	
Epischoenus quadrangularis (Boeck.) C. B. Cl.	DQ058311 (S. Africa: Verboom 636, BOL)	DQ058349 (S. Africa: Verboom 636, BOL)	DQ058332 (S. Africa: Verboom 636, BOL)		JLH
Eriophorum vaginatum L.	AJ295769 (Muasya et al. 2001) and AJ577322 (Dhooge et al. 2003'	Y12951 (Muasya et al. 1998)	AF449553 (Muasya et al. 2002)	AY242008 (Starr et al. 2004)	AY242009 (Starr et al. 2004)
Evandra aristata R. Br.		AY725944 (Muasya et al. 2000)			JLH
Ficinia paradoxa (Schrad.) Nees	DQ058317 (S. Africa: Verboom 534, BOL)	DQ058354 (S. Africa: Verboom 534, BOL)		JLH	JLH
Gahnia baniensis Beni.	DQ058302 (Malaysia: Simpson 2737, K)	DQ058342 (Malaysia: Simpson 2737, K)	DQ058323 (Malaysia: Simpson 2737, K)		JLH
Gahnia deusta (R.Br.) Benth.		U49231 (Plunkett et al. 1995)			
Gahnia javanica Mor.		Y12973 (Muasya et al. unpub)			
Gymnaschoenus sphaerocephalus Nees	AY230033 (Zhang et al. 2004)	AY725945 (Muasya et al. 2000)			
Lepidosperma sp.	AF285074 (Roalson et al. 2001)	AY725950 (Muasya et al. 2000)			
Machaerina sp.	DQ058300 (New Guinea: Johns 9195, K)	DQ058340 (New Guinea: Johns 9195, K)	DQ058321 (New Guinea: Johns 9195, K)		JLH
Mesomelaena pseudostygia (Kuek.) K.L. Wilson	DQ058301 (Australia: Chase 2226, K)	DQ058341 (Australia: Chase 2226, K)	DQ058322 (Australia: Chase 2226, K)	JLH	
Mesomelaena tetragona (R. Br.) Benth.		Y12949 (Muasya et al. unpub)			
Neesenbeckia punctoria (Vahl) Levyns	DQ058306 (S. Africa: Verboom 650, BOL)	DQ058346 (S. Africa: Verboom 650, BOL)	DQ058327 (S. Africa: Verboom 650, BOL)		JLH
Oreobolus obtusangulus Gaudich.	DQ456962 (Madrinan et al. 2006)	AF307926 (Wardle et al. 2001)		DQ450472 (Madrin et al. 2006)	
Oreobolus pectinatus Hook.f.	DQ456965 (Madrinan et al. 2006)	AF307927 (Wardle et al. 2001)		DQ450475 (Madrin et al. 2006)	
Oreobolus sp.	AY230030 (Zhang et al. 2004)	Y12972 (Muasya et al. 1998)		AY242047 (Starr et al. 2004)	
Oreobolus sp. 1	DQ456967 (Madrinan et al. 2006)			DQ450477 (Madrin et al. 2006)	
Pleurostachys sp.		Y12989 (Muasya et al. unpub)			
Ptilothrix deusta (R. Br.) K. L. Wilson	AY230041 (Zhang et al. 2004)				
Rhynchospora alba (L.) Vahl	AY344174 (Simpson et al. 2003)		AY344151 (Simpson et al. 2003)		
Rhynchospora brownii Roem. et Schultes	DQ058316 (S. Africa: Verboom 616, BOL)	DQ058353 (S. Africa: Verboom 616, BOL)	DQ058336 (S. Africa: Verboom 616, BOL)		Matthew Britton
Rhynchospora fascicularis Vahl		U49233 (Plunkett et al. 1995)			
Rhynchospora nervosa (Vahl.) Boeck.		AF206818 (Soltis et al. unpub)		AY242050 (Starr et al. 2004)	
Schoenoplectus lacustris (L.) Palla	AJ295809 (Muasya et al. 2001b) and AJ577331 (Dhooge et al. 2003'	Y12943 (Muasya et al. 1998)	AF449554 (Muasya et al. 2002)		
Schoenus cf. *bifidus* (Nees) Boeck.				JLH	JLH
Schoenus curvifolius (R. Br.) Benth.					JLH
Schoenus efoliatus F. Muell.				JLH	JLH
Schoenus grandiflorus (Nees) F. Muell.				JLH	JLH
Schoenus nigricans L.	DQ058310 (Saudi Arabia: Edmonson 3382, K)	Y12983 (Muasya et al. 1998)	DQ058331 (Saudi Arabia: Edmonson 3382, K)		
Tetraria bolusii C. B. Clarke	DQ058315 (S. Africa: Verboom 606, BOL)		DQ058335 (S. Africa: Verboom 606, BOL)	JLH	JLH
Tetraria brachyphylla Levyns	DQ419863 (Slingsby and Verboom 2006)		DQ419895 (Slingsby and Verboom 2006)		
Tetraria bromoides (Lam.) Pfeiffer	DQ419881 (Slingsby and Verboom 2006)		DQ419883 (Slingsby and Verboom 2006)		
Tetraria burmanii (Schrad.) C. B. Cl.	DQ419856 (Slingsby and Verboom 2006)		DQ419888 (Slingsby and Verboom 2006)		
Tetraria capillacea (Thunb.) C. B. Cl.	DQ419860 (Slingsby and Verboom 2006)		DQ419892 (Slingsby and Verboom 2006)		
Tetraria capillaris (F. Muell.) J.M. Black		No GenBank Accession (Muasya et al. 2000)		DQ385604 (Gardner et al. unpub)	JLH
Tetraria compacta Levyns	DQ058313 (S. Africa: Verboom 614, BOL)	DQ058351 (S. Africa: Verboom 614, BOL)		JLH	JLH
Tetraria compar (L.) Lestib.	DQ058312 (S. Africa: Verboom 549, BOL)	DQ058350 (S. Africa: Verboom 549, BOL)	DQ058333 (S. Africa: Verboom 549, BOL)		JLH
Tetraria compressa Turill			DQ419886 (Slingsby and Verboom 2006)		
Tetraria crassa Levyns	DQ058314 (S. Africa: Verboom 507, BOL)	DQ058352 (S. Africa: Verboom 507, BOL)	DQ058334 (S. Africa: Verboom 507, BOL)	JLH	JLH
Tetraria crinifolia (Nees) C. B. Cl.	DQ058309 (S. Africa: Verboom 638, BOL)	DQ058348 (S. Africa: Verboom 638, BOL)	DQ058330 (S. Africa: Verboom 638, BOL)	JLH	JLH
Tetraria cuspidata (Rottb.) C. B. Cl.	DQ419865 (Slingsby and Verboom 2006)		DQ419897 (Slingsby and Verboom 2006)		
Tetraria exilis Levyns	DQ419866 (Slingsby and Verboom 2006)		DQ419898 (Slingsby and Verboom 2006)		
Tetraria fasciata (Rottb.) C. B. Cl.	DQ419858 (Slingsby and Verboom 2006)		DQ419890 (Slingsby and Verboom 2006)		
Tetraria fimbriolata (Nees) C. B. Cl.	DQ419862 (Slingsby and Verboom 2006)		DQ419894 (Slingsby and Verboom 2006)		
Tetraria flexuosa C. B. Cl.	DQ419859 (Slingsby and Verboom 2006)		DQ419891 (Slingsby and Verboom 2006)		
Tetraria involucrata (Rottb.) C. B. Cl.	DQ419852 (Slingsby and Verboom 2006)	AM234973 (Forest et al. 2007)	DQ419884 (Slingsby and Verboom 2006)		
Tetraria microstachys (Vahl.) Pfeiffer	DQ058307 (S. Africa: Verboom 640, BOL)	DQ058347 (S. Africa: Verboom 640, BOL)	DQ058328 (S. Africa: Verboom 640, BOL)	JLH	Matthew Britton
Tetraria nigrovaginata (Nees) C.B. Cl.	DQ419857 (Slingsby and Verboom 2006)		DQ419889 (Slingsby and Verboom 2006)		
Tetraria picta C. B. Cl.	DQ419867 (Slingsby and Verboom 2006)		DQ419899 (Slingsby and Verboom 2006)		

Tetraria pillansii Levyns			DQ419887 (Slingsby and Verboom 2006)		
Tetraria sylvatica (Nees) C. B. Cl.	DQ419864 (Slingsby and Verboom 2006)		DQ419896 (Slingsby and Verboom 2006)		
Tetraria thermalis (L.) C. B. Cl.	DQ058308 (S. Africa: Verboom 643, BOL)		DQ058329 (S. Africa: Verboom 643, BOL)		Matthew Britton
Tetraria triangularis (Boeck.) C. B. Cl.	DQ419853 (Slingsby and Verboom 2006)		DQ419885 (Slingsby and Verboom 2006)		
Tetraria ustulata (L.) C. B. Cl.	DQ419861 (Slingsby and Verboom 2006)		DQ419893 (Slingsby and Verboom 2006)		
Trianoptiles solitaria (C. B. Cl.) Levyns	AY230027 (Zhang et al. 2004)			JLH	JLH
Tricostularia pauciflora (F. Muell.) Benth.	AY230038 (Zhang et al. 2004)	AY725954 (Muasya et al. 2000)		JLH	

outgroups

Calyptrocarya sp.				JLH	JLH
Capeobolus brevicaulis (C.B. Cl.) J. Browning	DQ058303 (S. Africa: Verboom 646, BOL)	DQ058343 (S. Africa: Verboom 646, BOL)	DQ058324 (S. Africa: Verboom 646, BOL)	JLH	JLH
Cyperus rigidifolius Steud.	AY040600 (Muasya et al 2001)	Y13016 (Muasya et al. 1998)	AF449535 (Muasya et al. 2002)		
Hypolytrum nemorum (Vahl) Spreng.	AJ295816 (Muasya et al. 2001b)and AJ577325 (Dhooge et al. 2003)	Y12958 (Muasya et al. 1998)	AY344142 (Simpson et al. 2003)	AY242046 (Starr et al. 2004)	
Isolepsis humillima (Benth.) K.L. Wilson	AJ295784 (Muasya et al. 2001b)	AJ404728 (Muasya et al. 2001b)	AF449539 (Muasya et al. 2002)		
Juncus gerardii Loisel.	AY344157 (Simpson et al. 2003)	AY216613 (Drabkova et al. 2003)	AY344134 (Simpson et al. 2003)		
Lagenocarpus sp.				JLH	JLH
Luzula sylvatica (Huds.) Gaud.	AY344159 (Simpson et al. 2003)	AY216637 (Drabkova et al. 2003)	AY344136 (Simpson et al. 2003)		
Mapania cuspidata (Miq.) Uittien	DQ058297 (Brunei: Marsh 4, K)	DQ058337 (Brunei: Marsh 4, K)	DQ058318 (Brunei: Marsh 4, K)	JLH	Matthew Britton
Microdracoides sp.				JLH	
Schoenoides oligocephalus (W. M. Curtis) Seberg	AY230031 (Zhang et al. 2004)				
Scleria distans Poir. Schoeneae	DQ058299 (Kenya: Muasya 1023, EA, K)	DQ058339 (Kenya: Muasya 1023, EA, K)	DQ058320 (Kenya: Muasya 1023, EA, K)	JLH	JLH
Scleria foliosa A.Rich.				AY242049 (Starr et al. 2004)	
Scleria parvula Steud.				AB261689 (Hirhara et al. 2007)	
Scleria reticularis Michx.				AY728806 (Roalson unpub)	

JLH= sequenced done by the author in the present study
Highlighted species were used in Verboom (2006)



Appendix 2: Parsimony strict consensus tree from the analysis of the molecular data