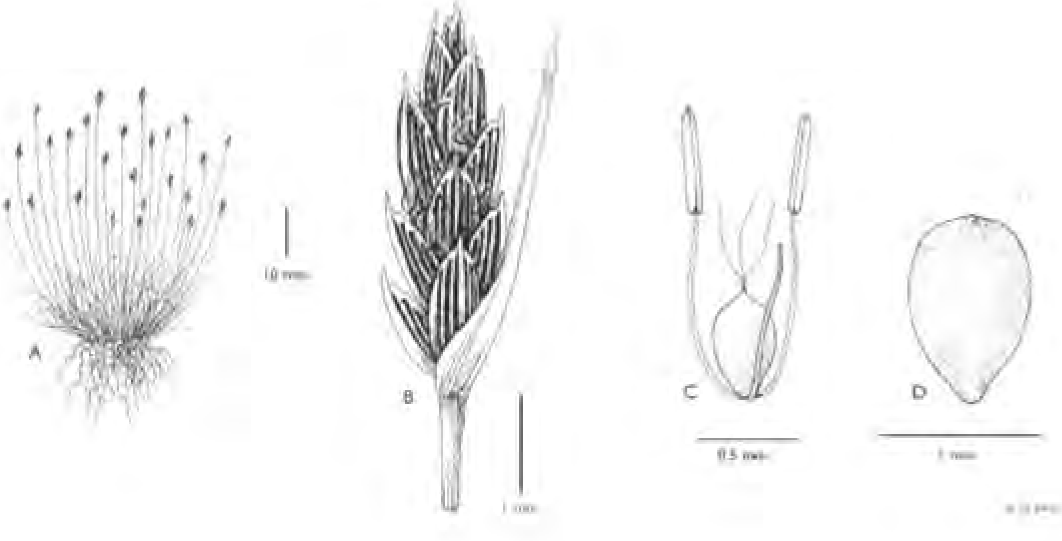
Duplicate

Isolepis R.Br. (*Cyperaceae*) Revisited:

A Phylogeny Based on Plastid and Nuclear Ribosomal DNA Data



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Abstract

The approximately 75 species of *Isolepis* R. Br. are currently described as annual or short lived perennials with predominantly terete spikelets, bisexual flowers with no perianth and a nutlet with no gynophore. It is unclear of the current morphological characteristics used to delimit the infrageneric groups is robust as some species have been observed to have a rudimentary, *Ficinia* Schrad. like gynophore. This study uses molecular plastid and nuclear sequence data to investigate the monophyly of *Isolepis* and its relationship to *Ficinia*. This study found that *Isolepis* is currently polyphyletic with a clade of isolepis sister to a clade containing both *Isolepis* and *Ficinia* species, which is sister to the rest of the *Isolepis*. The ancestral life-form, either annual or perennial, of the clades was re-constructed on the phylogeny and it was found that the common ancestor of both *Isolepis* and *Ficinia* is supported as being annual. Biogeographical interpretations of the data gives supporting evidence towards the hypothesised origin of the *Isolepis* in the Winter Rainfall region of South Africa. Incongruence between the nuclear and plastid data suggests that there may have been an ancient hybridisation event between Australian members of the subgenus *Fluitantes* (C.B. Clarke) Muasya and the Australian members of the subgenus *Isolepis* section *Proliferae* (C.B. Clarke) Muasya. Finally, a new classification is suggested based on the molecular data from this study.

Introduction

The predominantly Southern Hemisphere genus *Isolepis* R. Br., currently consists of approximately 75 species with the centre of diversity in Southern Africa and Australia/New Zealand. Species within the genus *Isolepis* can be described as being annual or short lived perennial herbaceous plants with minute roots or rhizomes. *Isolepis* are usually tufted but may also be mat forming (For example, *I. fluitans* (L.) R. Br.). The usually terete inflorescence consists of sessile spikelets made up of spirally arranged glumes containing bisexual flowers with no perianth segments. Two species (*Isolepis leucoloma*, (Nees) C. Archer and *Isolepis levynsiae* Muasya & Simpson) with flattened spikelets and distichous glumes have been recently transferred from *Cyperus* L. s.l. to *Isolepis* (Archer 1998; Muasya *et al*, 2006, in press; Muasya *et al*, 2006) based on re-interpretation of spikelet morphology and DNA sequence data. The inflorescence is subtended by an involucral bract, which can either be longer or shorter than the spikelet, thus resulting in either a pseudolateral or terminal spikelet respectively. The inflorescence may or may not be proliferating (Muasya *et* al, 2001; Muasya & Simpson, 2002). Along with the above mentioned characteristics, nutlet surface ornamentation has been widely used to delimit species as it shows high levels of variation. Nutlets generally lack a gynophore although some species (e.g. *Isolepis marginata* (Thunb.) A. Dietr. and *Isolepis antarctica* (L.) Roem. & Schult.) have been described as having a poorly developed gynophore and their taxonomic position is currently unclear (Muasya *et* al, 2001; Muasya & Simpson, 2002, Vrijdaghs *et al*, 2005). A typical example of an *Isolepis* can be seen in Figure 1.

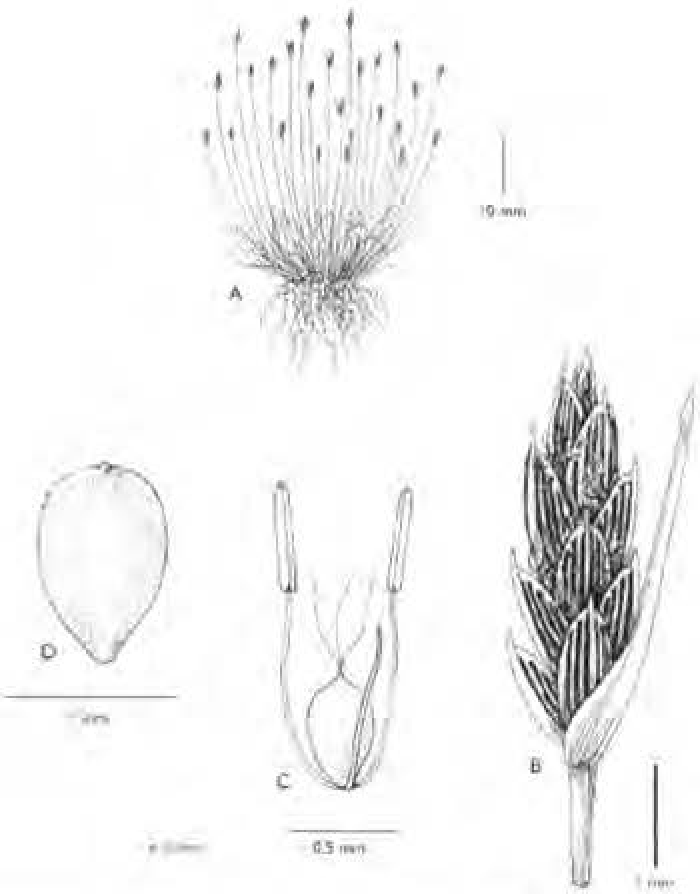

Figure 1: Diagram of *Isolepis capensis* showing a tufted growth form (**A**); spikelet with involucral bract (**B**); bisexual flower (**C**) and nutlet with no gynophore (**D**). Borrowed from Muasya & Simpson, 2002.

Figure 2 and Table 1 show the current taxonomic relationship of the *Isolepis* according to Muasya & Simpson (2002). *Isolepis* is currently classified into three subgenera (Table 1), namely subgenus *Micranthae* (C.B. Clarke) Muasya, subgenus *Fluitantes* (C.B. Clarke) Muasya and subgenus *Isolepis* (C.B. Clarke) Muasya with the entire genus being sister to *Ficinia* Schard. except for *I. humillima* (Benth.) K.L. Wilson. Subgenus *Isolepis* is further split into three sections, section *Proliferae* (C.B. Clarke) Muasya, section *Cernua* (C.B. Clarke) Muasya and section *Isolepis* (C.B. Clarke) Muasya. A summary of the morphological diagnoses of these infrageneric groups can be seen in Table 1. Each of the above clades is supported by both morphological (Table 1) and molecular (Figure 2) data.

Table 1: Morphological diagnosis of the infrageneric groups of *Isolepis* (Muasya & Simpson, 2002)

	Morphological Characteristics	**Example**
Subgen **Micranthea** (C.B. Clarke) Muasya	Plants tufted; inflorescence pseudolateral, occasionally proliferating; nutlet elliptic, papillose	*I. sepulcralis* *I. hystrix*
Subgen **Fluitantes** (C.B. Clarke) Muasya	Plants mat-forming; inflorescence terminal, not proliferating; nutlets oblong, reticulate	*I. fluitans* *I. crassiuscula*
Subgen **Isolepis** (C.B. Clarke) Muasya		
i) Sect. *Proliferae* (C.B. Clarke) Muasya	Plants tufted; inflorescence pseudolateral, proliferating; nutlets oblong, smooth, reticulated or striated	*I. prolifera* *I. inundata* *I. costata*
ii) Sect. *Cernua* (C.B. Clarke) Muasya	Plants tufted; inflorescence pseudolateral, not proliferating; nutlets oblong, tuberculate	*I. cernua* *I. platycarpa*
iii) Sect. *Isolepis* (C.B. Clarke) Muasya	Plants tufted; inflorescence pseudolateral, not proliferating; nutlets oblong, striated	*I. setacea* *I. diabolica*

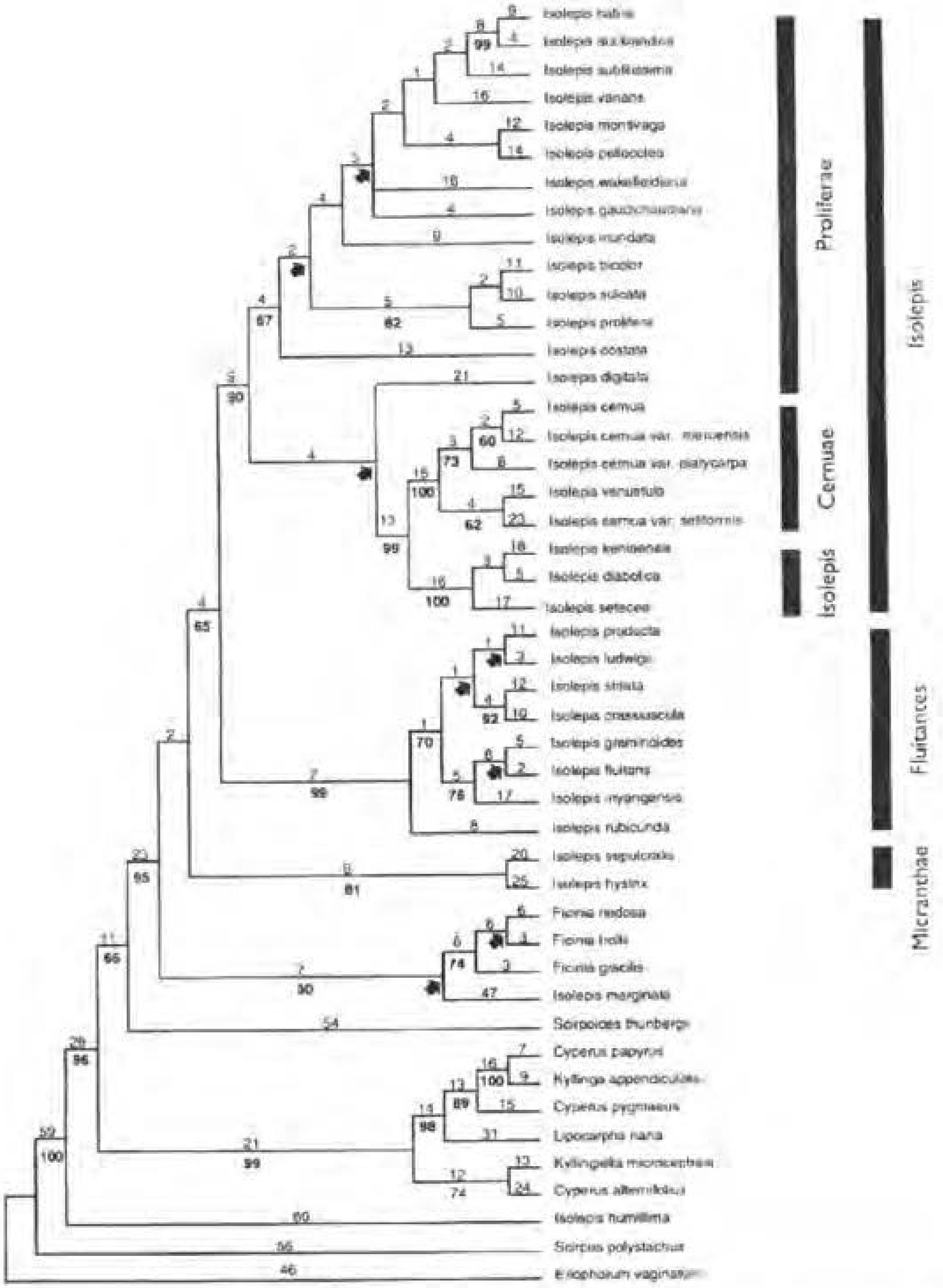

Figure 2: Current taxonomic relationship of the *Isolepis* according to Muasya & Simpson, 2002 shown on the single most parsimonious tree found with weighted, morphological data combined with DNA data. Arrows mark clades not present in the strict consensus. Numbers above branches are inferred substitutions (ACCTRAN optimisation). Bootstrap percentages are shown below branches (Muasya & Simpson, 2002).

The *Ficinia* are generally diagnosed by having a perennial, herbaceous growth form, usually with a woody rhizome. The papery leaf sheath often has a ligule, the presence, occasional absence and structure of which is of taxonomic importance. The bisexual flowers, lacking perianth segments, are aggregated into spikelets in a lateral inflorescence. The presence of a gynophore on the nutlet of *Ficinia* is one of the key characteristics defining the genus but this character is not robust as some *Ficinia* have no gynophore disc (Muasya *et al*, 2001; Muasya, 2005; Vrijdaghs *et al*, 2005). This makes the taxonomy of *Ficinia*, based on morphology, very difficult. Molecular DNA studies are making some headway into revising the taxonomy of the genus (Muasya, 2005). A typical example of a *Ficinia* can be seen in Figure 3.

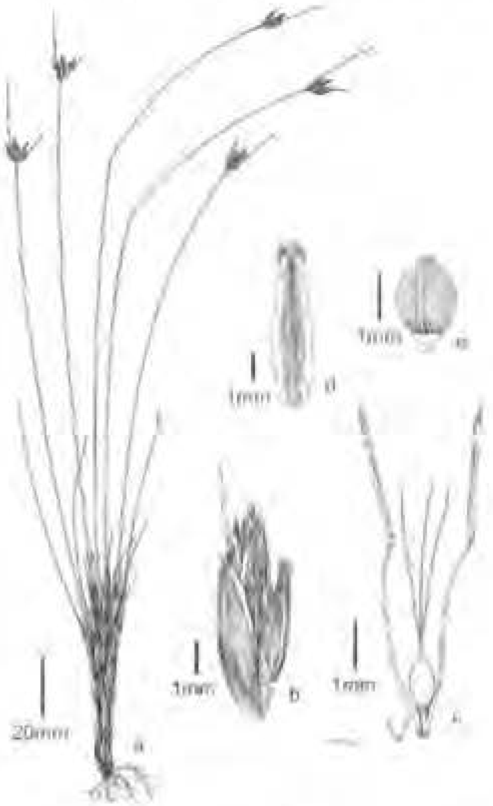

Figure 3: Diagram of *Ficinia anysbergensis* Muasya. showing the herbaceous growth form with a woody rhizome (**A**); inflorescence (**B**); bisexual flower (**C**); glume (**D**) and nutlet with gynophore (**E**). Borrowed from Muasya, 2005.

The aims of this study are to investigate the fine scale phylogeny of the *Isolepis* and whether or not *Isolepis* is monophyletic, with an emphasis on taxa from the winter rainfall region of South Africa. Plastid DNA sequences have been used in many studies to investigate relationships on many levels from the population level, (e.g. Schönswetter *et al*, 2006), through to the familial level (e.g. Chandler & Plunket, 2004). In this study, the *rps16* intron plastid marker was used as it was found to show a moderate amount of variation across the species being studied and thus was expected to give useful resolution. The Internally Transcribed Spacer (ITS), a nuclear ribosomal marker, was also used to investigate the phylogenetic relationships of the genus as it has been previously used in phylogenetic studies of *Cyperaceae* (e.g. Roalson & Friar, 2000 and Roalson *et al*, 2001). This marker was also found to also have a valuable level of variation and was also expected to provide resolution at the level needed for this study.

Using the DNA phylogeny obtained from the *ITS* and *rps16* sequence data, the current classification of the genus *Isolepis* is evaluated. Patterns of biogeography as well as the evolution of life form were also investigated. The relationship of *Ficinia* to *Isolepis* is also under investigation due to the morphological continuity between them in species such as *I. marginata* and *I. antarctica*. This study also aims to include molecular data for many species, which to date, have not yet been included in other molecular studies of the genus to establish their phylogenetic position.

In summary, this study will focus on the following questions:

1) Are all of the *Isolepis* species from the winter rainfall region of South Africa derived from a common ancestor?

2) Are species with intermediate morphology (i.e. *I. marginata* and *I. antarctica*) *Ficinia's* or *Isolepis*.

3) Does this expanded DNA study support or reject the classification in previous studies, which were based on limited sampling? (e.g. Muasya *et al*, 2002)~~?~~

4) Are there any biogeographical patterns present in this DNA ~~study~~ data?

Methods

A total of 106 taxa were included in this study. They consist~~s~~ of 4 outgroup species, 55 *Isolepis* species, 5 *Ficinia* species and 1 *Desmoschoenus* species. The outgroup taxa include two *Scirpoides* species and one species each from *Hellmuthia* and *Schoenoplectus*. The complete table of taxa, including voucher information, can be seen in Table 2. Samples labelled with an 'aff' (*affinis*) could not be positively identified to species level but were morphologically similar to the species, which are named after the 'aff' label. Samples labelled 'sp.' (species) could not be identified to species level. These samples were also included in this study to try and validate their phylogenetic position. Some of these taxa are also undescribed and the data provided here will be valuable in describing these species.

DNA extraction and PCR:

Total DNA was extracted from 0.02 – 0.08g silica dried samples or from 0.05 – 0.1g fresh plant material when available. The extraction protocol was slightly modified from that of Gawel and Jarret (1991). One plastid and one nuclear region were amplified using PCR. The *rps16* intron region of the plastid genome was amplified using the primers *rpsF* and *rpsR2* designed by Oxelman *et al.* (1997). *ITS* (Internally Transcribed Spacer) region of the nuclear genome was amplified using *ITS4* and *ITS5* primers designed by White *et al,* 1990. The PCR reactions were performed in 30µl volumes consisting of 18.6µl of sterile water, 3µl of 10x DNA polymerase buffer (Bioline, London, UK), 5µl of $MgCl_2$ (50mM), 1µl each of the forward and reverse primers (10 µM), 1.2µl dNTP (10mM), 0.2µl of *Taq* DNA polymerase (Bioline, London, UK) 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 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 then checked on a 1% agarose gel. The successful PCR products were then sent to MacroGen (http://www.macrogen.com) for sequencing using the same primers as were used in the PCR. Sequences were cleaned, assembled, edited and aligned using CodonCode Aligner (CodonCode corporation, MA, USA).

Data Analysis

In the analysis, indels were coded for as additional binary characters and a matrix of these characters was created and analysed together with the nucleotide data. The data matrices were analysed in three ways. Firstly, for the *ITS* (including indels) data; secondly, for only the *rps16* (including indels) data and finally for both data sets combined (including both *ITS* and *rps16* indel matrices). This was done to ensure that the separate data sets produced topologically similar results so that the data sets could be combined, which is similar to the methodology explained in Wiens, 1998. After the above analyses, the indel data was plotted by hand to ascertain, which phylogenetically important clades they supported.

Phylogenetic relationships were inferred using both parsimony and Bayesian approaches. All trees were rooted on *Schoenoplectus*, *Scirpoides holoschoenus* and *Scirpoides dioecus*. Parsimony search used a heuristic search was done of 10,000 random addition replicates, TBR branch swapping and MULTREES on. 500 Bootstrap (BS) replicates were used to asses the branch support using a heuristic search with 300 random addition replicates, TBR branch swapping and MULTREES on. PAUP* version 4.0b10 (Swofford, 2002) was also used for the above analysis.

Mr. Bayes version 3.12 (Huelsenbeck & Ronquist, 2003) was used to perform the Bayesian analysis, which was only carried out on the combined dataset. The most complex model was used in this analysis, which corresponds to the GTR+I+G model. This was done as it has been shown that the accuracy of a Bayesian model suffers more in response to under parameterisation than over parameterisation (Huelsenbeck & Rannala, 2004). In the analysis, parameters were estimated separately for each of the two 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 every 100^{th} 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 was also used to determine stationarity, and as they had dropped to $\leq$ 0.005 this indicated that the tree samples had become similar enough after 10^6 generations to be regarded as stationary. Trees sampled from the 'burn-in' period were discarded from the analysis prior to the calculation of the Bayesian posterior probabilities. A total of six runs were performed to make sure that the same –log likelihood score was reached for each run. This was done to ~~make~~ ensure that the tree space was being sampled efficiently.

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was used to investigate the surface structures of the seeds of some of the taxa used in the molecular analysis. Samples were mounted on stubs and then sputter coated with gold palladium before viewing with the SEM (LEO fully analytical S440 Scanning Electron Microscope, Cambridge, UK).

Table 2: Taxa analysed in the combined study of *ITS* and *rps16* genetic markers.

TAXON	COLLECTOR	VOUCHER & HERBARIA	PROVENANCE
Desmoschoenus spiralis *Hook.f.*	HUBG	2003-0699 GENT	New Zealand
Ficinia aff. **anceps** Nees	Muasya	2885 BOL	South Africa
F. esterhuyseniae *Muasya*	Muasya	2312 BOL	South Africa
F. indica *(Lam.) Pfeiff*	Muasya	2909a BOL	South Africa
F. indica *(Lam.) Pfeiff*	Muasya	2909b BOL	South Africa
F. pinguior *C.B. Clarke*	Muasya	1183 K	South Africa
F. secunda *(Vahl) Kunth*	Muasya	2908a BOL	South Africa
Hellmuthia membranacea *(Thunb.) R.W. Haines & Lye*	Muasya	3081 BOL	South Africa
Isolepis aff. **brevicaulis** (Levyns) J. Raynal	Muasya	3008 BOL	South Africa
I. aff. **brevicaulis** (Levyns) J. Raynal	Muasya	3023 BOL	South Africa
I. aff. **cernua** *(Vahl) Roem. & Schult.*	Muasya	2974 BOL	South Africa
I. aff. **incomtula** *Nees*	Muasya	3020 BOL	South Africa
I. aff. **trachysperma** *Nees*	Muasya	3003 BOL	South Africa
I. aff. **trachysperma** *Nees*	Muasya	3009 BOL	South Africa
I. antarctica (L.) Roem. & Schult.	Muasya	3007 BOL	South Africa
I. antarctica (L.) Roem. & Schult.	Muasya	3013 BOL	South Africa
I. auklandica *Hook. f.*	Wilson *et al*	9492 K	Australia
I. auklandica *Hook. f.*	Gardner *et al*	AK289102	New Zealand
I. basilaris *Hook. f.*	Gardner *et al*	AK289204	New Zealand
I. bicolor *Carmich.*	Richardson	105 K	Tristan da Cunha
I. brevicaulis *(Levyns) J. Raynal*	Muasya	2912 BOL	South Africa
I. brevicaulis *(Levyns) J. Raynal*	Muasya & Wiswedel	3074 BOL	South Africa
I. brevicaulis *(Levyns) J. Raynal*	Muasya & Wiswedel	3079 BOL	South Africa
I. caligenis *(V.J. Cook) Soják*	Gardner *et al*	AK289205	New Zealand
I. capensis *Muasya*	Muasya	3019 BOL	South Africa
I. capensis *Muasya*	Muasya & Wiswedel	3078 BOL	South Africa
I. cernua *(Vahl) Roem. & Schult. var.* **cernua**	Muasya	1058 K	United Kingdom
I. cernua *(Vahl) Roem. & Schult.*	Muasya	3002 BOL	South Africa
I. cernua *(Vahl) Roem. & Schult.*	Muasya & Wiswedel	3073 BOL	South Africa
I. cernua *(Vahl) Roem. & Schult.*	Gardner *et al*	AK288391	New Zealand
I. cernua *(Vahl) Roem. & Schult. var.* **meruensis** *(R.W. Haines & Lye) Muasya*	Muasya	1061 K	Tanzania
I. cernua *(Vahl) Roem. & Schult. var.* **platycarpa** *(S.T.Blake) Soják*	Coveny *et al*	17465 K	
I. costata *A. Rich*	Muasya	1049 K	Kenya
I. crassiuscula *Hook. f.*	Wilson	9487 K	Australia
I. crassiuscula *Hook. f.*	Coveny *et al*	17478 K	Australia
I. crassiuscula *Hook. f.*	Gardner *et al*	AK289564	Australia
I. crassiuscula *Hook. f.*	Gardner *et al*	AK289630	Australia
I. diabolica *(Steud.) Schrad.*	Muasya	1163 K	South Africa
I. digitata *Nees ex Schrad*	Muasya	84 K	South Africa
I. fluitans *(L.) R. Br.*	Muasya	961 K	Kenya
I. fluitans *(L.) R. Br.*	Muasya	1007 K	Kenya
I. fluitans *(L.) R. Br.*	Muasya	1057 K	Kenya
I. fluitans *(L.) R. Br.* var. **nervosa**	Muasya	2621 BOL	Ethiopia
I. fluitans *(L.) R. Br.*	Muasya	3135 BOL	Kenya
I. fluitans *(L.) R. Br.* var. **lenticularis**	Gardner *et al*	AK289561	New Zealand
I. fluitans *(L.) R. Br.* var. **nervosa**	Gardner *et al*	AK289724	New Zealand
I. gaudichaudiana *Kunth*	Wilson	9483 K	Australia
I. graminoides *(R.W. Haines & Lye) Lye*	Muasya	986 EA, K	Kenya
I. graminoides *(R.W. Haines & Lye) Lye*	Muasya	2597 BOL	Kenya
I. habra *(Edgar) Sojak.*	Coveny *et al*	17480 NSW	Australia
I. hemiuncialis *(C.B. Clarke) J. Raynal*	Muasya *et al*	2895 BOL	South Africa
I. hemiuncialis *(C.B. Clarke) J. Raynal*	Muasya	3001 BOL	South Africa

SPECIES	COLLECTOR	VOUCHER & HERBARIA	PROVENANCE
I. humillima *(Benth.) K.L. Wilson*	Thomas *et al*	622 BRI	Australia
I. hystrix *(Thunb.) Nees*	Muasya	2971 BOL	South Africa
I. incomtula *Nees*	Muasya	2897 BOL	South Africa
I. incomtula *Nees*	Muasya	3017 BOL	South Africa
I. inconspicua *(Levyns) J. Raynal*	Muasya	2972 BOL	South Africa
I. inundata *R. Br.*	Wilson *et al*	9493 NSW	Australia
I. inundata *R. Br.*	Gardner *et al*	AK285423	New Zealand
I. inundata *R. Br.*	Gardner *et al*	AK289482	New Zealand
I. inyangensis *Muasya & Goetgh*	Muasya *et al*	2025 NSW	Zimbabwe
I. karoica *(C.B. Clarke) J. Raynal*	Muasya	1146 K	South Africa
I. karoica *(C.B. Clarke) J. Raynal*	Muasya & Wiswedel	3064 BOL	South Africa
I. keniaensis *Lye*	Muasya	2552 BOL	Kenya
I. leucoloma *(Nees) C. Archer*	Muasya	2976 BOL	South Africa
I. levynsiae *Muasya & Simpson*	Muasya	2977 BOL	South Africa
I. ludwigii *(Steud.) Kunth*	Muasya	1138 K	South Africa
I. marginata (Thunb.) A. Dietr.	Muasya	2999 BOL	South Africa
I. marginata (Thunb.) A. Dietr.	Muasya	3012 BOL	South Africa
I. marginata (Thunb.) A. Dietr.	Muasya	3018 BOL	South Africa
I. marginata (Thunb.) A. Dietr.	Coveny *et al*	17452 K	Australia
I. minuta *(Turrill) J. Raynal*	Muasya	2334 BOL	South Africa
I. montivaga *(S.T. Blake) K.L. Wilson*	Wilson *et al*	9489 K	Australia
I. natans *(Thunb.) A. Dietr.*	Muasya	2891 BOL	South Africa
I. nigricans *Kunth*	Cazalet & Pennington	5357 K	Ecuador
I. pellocolea *B.L. Burtt*	Browning	694 NU	South Africa
I. potsii *(V.J. Cook) Soják*	Gardner *et al*	AK289620	New Zealand
I. producta *(C.B. Clarke) K.L. Wilson*	Wilson	9475 K	Australia
I. producta *(C.B. Clarke) K.L. Wilson*	Wilson *et al*	9510 K	Australia
I. prolifera *(Rottb.) R. Br.*	Muasya	1168 K	South Africa
I. prolifera *(Rottb.) R. Br*	Coveny *et al*	17487 K	Australia
I. prolifera *(Rottb.) R. Br.*	Gardner *et al*	AK288281	New Zealand
I. pusilla *Kunth*	Muasya	2975 BOL	South Africa
I. reticularis *Colenso*	Gardner *et al*	AK289481	New Zealand
I. rubicunda *(Nees) Kunth*	Muasya	1154 K	South Africa
I. rubicunda *(Nees) Kunth*	Muasya	1221 K	South Africa
I. sepulcralis *Steud.*	Muasya	1165 K	South Africa
I. sepulcralis *Steud*	Muasya	1211 K	South Africa
I. setacea *(L.) R. Br.*	Muasya	1059 K	Kenya
I. setacea *(L.) R. Br*	Faden *et al*	96/417 K	Tanzania
I. sp.	Muasya	2973 BOL	South Africa
I. sp.	Muasya & Wiswedel	3076 BOL	South Africa
I. sp. *(L.) R. Br.*	Muasya & Wiswedel	3067 BOL	South Africa
I. striata *(Nees) Kunth*	Muasya	1180 K	South Africa
I. striata *(Nees) Kunth*	Muasya	2906 BOL	South Africa
I. subtilissima *Boeck.*	Coveny *et al*	17475 K	Australia
I. sulcata *(Thouars) Carmich*	Richardson	80 K	Tristan da Cunha
I. tenuissima *(Nees) Kunth*	Muasya	2369 BOL	South Africa
I. trachysperma *Nees*	Muasya	2893 BOL	South Africa
I. trachysperma *Nees*	Muasya	2905 BOL	South Africa
I. trachysperma *Nees*	Muasya	3014 BOL	South Africa
I. venustula *Kunth*	Muasya	1189 K	South Africa
I. wakefieldiana *(S. T. Blake) K. L. Wilson*	Neish *et al*	110 K	Australia
Schoenoplectus leucanthus (Boeck.) J. Raynal	Muasya	2882 BOL	South Africa
Scirpoides dioecus *(Kunth) J. Browning*	Muasya & Wiswedel	3062 BOL	South Africa
Scirpoides holoschoenus (L.) Soják	Acocks	s.n.	South Africa

Results

The alignment of samples for both *ITS* and *rps16* required the insertion of numerous gaps due to sequence length variation. A total of five informative gaps were coded for *rps16* and two informative gaps were coded for *ITS* in an indel matrix. The aligned *ITS* sequence (including indels) consisted of 276 parsimony informative characters out of a total of 694 characters while the aligned *rps16* sequences (including indels) yielded a 164 parsimony informative characters out of a total of 906 characters. As the most parsimonious topologies of the two loci showed little mojor incongruences apart from a single clade (C4,1 in Figures 4 and 5) the two data sets were combined for further analysis. The combined *ITS* and *rps16* data set (including indels) had a total of 1599 characters, 439 of which were parsimony informative. A bootstrap tree of each of the three above mentioned data sets (*ITS*, *rps16* and combined *ITS* and *rps16*) can be seen in figures 4, 5 and 6 respectively as well as a phylogeny based on a GTR+G+I model Bayesian approach (Figure 7). I will refer to the combined *ITS* and *rps16* bootstrap (Figure 6) and Bayesian (Figure 7) phylogenies for descriptions of the main features and the support values will come from these two trees unless otherwise stated.

The main features present, starting from the base of the trees, are:

Isolepis humillima is strongly supported (BS = 100, PP = 1.00) as being sister to *Schoenoplectus*. This is not surprising as it has been previously been shown to be more closely related to other *Schoenoplectus* in previous studies (see Muasya *et al*, 2001 and Muasya & Simpson, 2002). The rest of *Isolepis* (including *Ficinia* and *Desmoschoenus*) form a clade (A-C) with strong support (BS=100, PP=1.00). Three clades are observed for this group, clades A, B and C.

Clade A is a clade of *Isolepis*, which is strongly supported (BS = 100, PP = 1.00) as being sister to the rest of *Isolepis* and *Ficinia*. This clade has not yet been described. The clade consists of *I. incomtula*, *I. hemiuncialis* and a new, unnamed *Isolepis sp.*, all species from this clade are from the Western Cape, South Africa.

Clade B is strongly supported (BS = 100, PP = 1.00) as being sister to the rest of the *Isolepis* (Clade C). With the rest of the *Isolepis* forming a strongly supported (BS = 93, PP = 1.00) monophyletic group. Clade B consists of two clades. Clade B_1 is made up of what are currently, the *Ficinia* and it has strong support as being monophyletic (BS = 98, PP = 1.00). Nested within this clade is *Desmoschoenus spiralis*. The second clade (B_2), which is strongly supported (BS = 89, PP = 1.00) to being sister to the *Ficinia*, is a clade of *Isolepis*. This clade is also strongly supported as being monophyletic (BS = 100, PP = 1.00) and consists of *I. capensis*, *I. leucoloma*, *I.marginata* and *I. antarctica*.

The rest of *Isolepis* ~~in~~ (clade C) (BS = 93, PP = 1.00) can be broken into 4 sub-clades (C_1 to C_4) with the following support values C_1 (BS = 100, PP = 1.00), C_2 (BS = 65, PP = 1.00), C_3 (BS = 72, PP = 1.00) and C_4 (BS < 50, PP = 1.00.

Within Clade C is a strongly supported Clade C_1 (BS = 100, PP = 1.00), which consists mainly of the previously described subgenus *Micranthae* excluding *I. natans*. It also contains two species currently included in subgenus *Isolepis* section *Cernua* (*I. minuta* and *I. pusilla*) as well as one species currently included in subgenus *Isolepis* section *Proliferae* (*I. tenuissima*).

The rest of Clade C_2, which has moderate to strong support (BS = 65, PP = 1.00), consists mainly of species from subgenus *Isolepis* section *Cernua*. Nested within this clade is a small clade ($C_{2,1}$), which consists of species from the subgenus *Isolepis* section *Isolepis* but also including *I. caligenis* from subgenus *Isolepis* section *Proliferae*. Subgenus *Isolepis* section *Isolepis* does have strong support as being monophyletic (BS = 76, PP = 1.00) with the inclusion of *I. caligenis*. The phylogenetic positions of *I. inconspicua*, *I. karoica*, *I. trachysperma* and *I. brevicaulis* have been reported here for the first time.

Clade C_3 has strong support (BS = 72, PP = 1.00) and consists of the African members of the subgenus *Fluitantes* with a Cape group (*I. striata* and *I. rubicunda*) being sister to a group consisting of other African species with individuals from the Cape (*I. ludwigii*) through to Tropical Africa (e.g. *I. fluitans*) and Ethiopia (*I. fluitans* var. *nervosa*).

Clade C_4 has strong Bayesian support ($PP = 1.00$) but weak Bootstrap support ($BS < 50$) and consists mainly of Australian taxa. Clade C_4 is strongly supported ($BS = 90$, $PP = 1.00$) as having Australian *Fluitantes* clade ($C_{4.1}$). The *Fluitantes, I. fluitans* var. *fluitans* and *I. fluitans* var. *lenticularis*, from New Zealand are not supported as being in the same clade as the Australian *Fluitantes* and their phylogenetic position within clade C_4 is unclear. Clade C_4 also has a weakly supported ($BS < 50$, $PP < 0.85$) Clade $C_{4.2}$ containing mainly taxa from the subgenus *Isolepis* section *Proliferae* (excluding *I. inundata* AK285423 from New Zealand). This grouping is most clear in the Bayesian phylogeny but, the support is weak ($PP < 0.85$).

The two indels present in the *ITS* data define the same taxa. All taxa within clades A and B as well as all outgroup taxa had a fourteen base sequence inserted at position 401, which has been lost in the common ancestor of Clade C, as well as a five base sequence deletion at position 581, which has been gained since the common ancestor of clade C. It is noted that when the data was analysed without the indel matrices, the same topology was found.

The *rps16* data set consisted of five indels, which were coded for. The first indel corresponded to a 5 base pair repeat at position 75, which was present in three of the four Australian *Fluitantes* (*I. crassiuscula* (9487), *I. crassiuscula* (17478) and *I. producta* (9510)) and thus would give support for the monophyletic nature of the clade. The second indel also consisted of a 5 base repeat, which was present in all taxa in clades A and B as well as all outgroup taxa and, like the *ITS* indels, was lost in the common ancestor of clade C. Two separate indels, one at position 537 (10 bases) and one at position 563 (5 bases) are missing in *Schoenoplectus leucanthus* and *Isolepis humillima* and supports these two taxa being sister to one another. The final *rps16* indel was a 5 base insertion found in all of the *I. cernua* as well as *I. venustula* and supports the clade containing these five taxa.

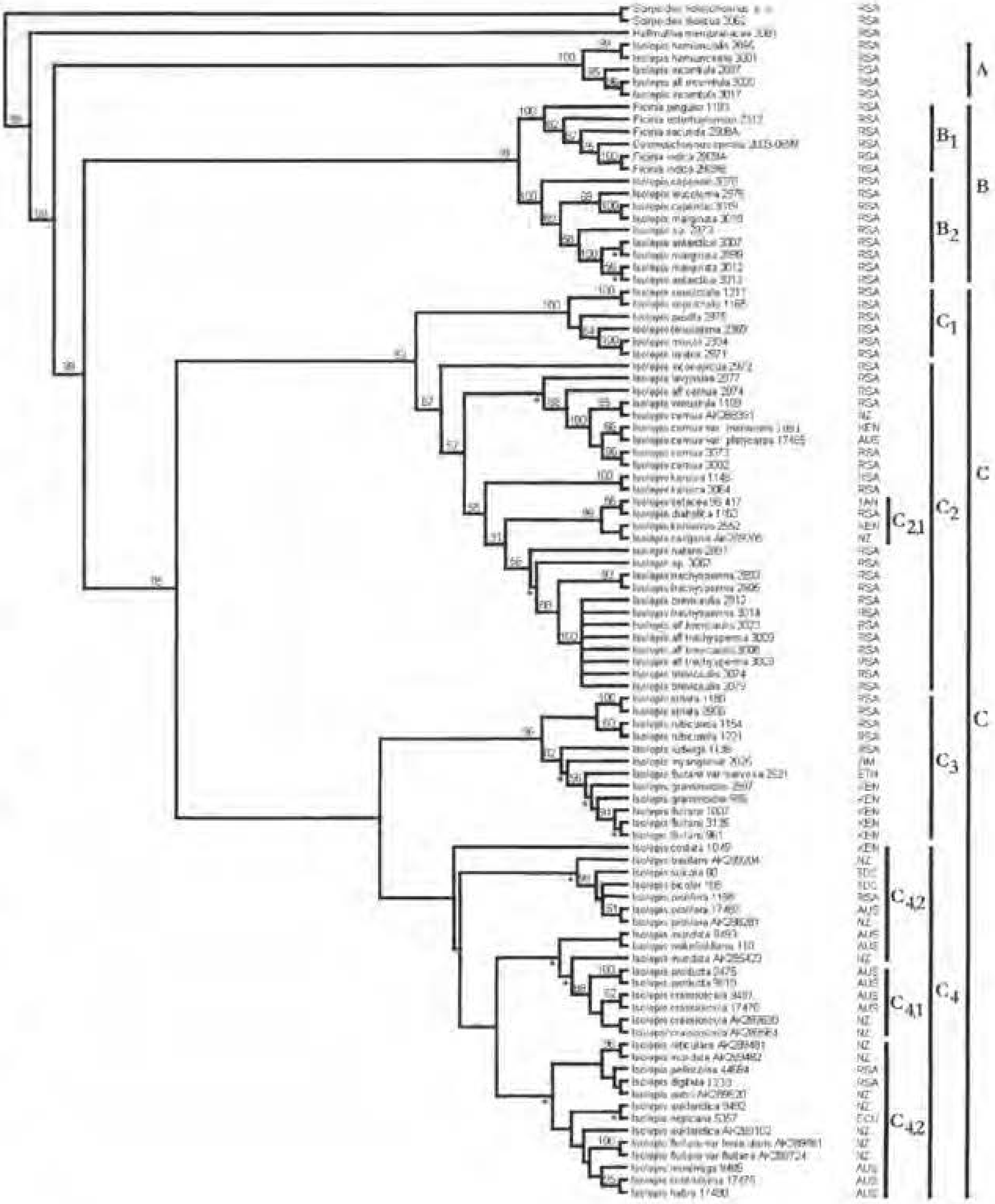

Figure 4: Bootstrap tree of the *ITS* data from 500 bootstrap replicates with the main clades that are described in the text labeled with letters. Bootstrap percentages (numbers above branches) are only shown on branches with support ≥ 50% (CI = 0.50, RI = 0.78). Nodes marked with a * are collapsed in the strict consensus parsimony tree. The localities of the sampled individuals used in the phylogeny are indicated by the following codes: AUS - Australia; ECU - Ecuador, ETH - Ethiopia; KEN - Kenya; NZ - New Zealand; RSA - South Africa; TAN - Tanzania; TDC - Tristan da Cuhna; UK - United Kingdom; ZIM - Zimbabwe

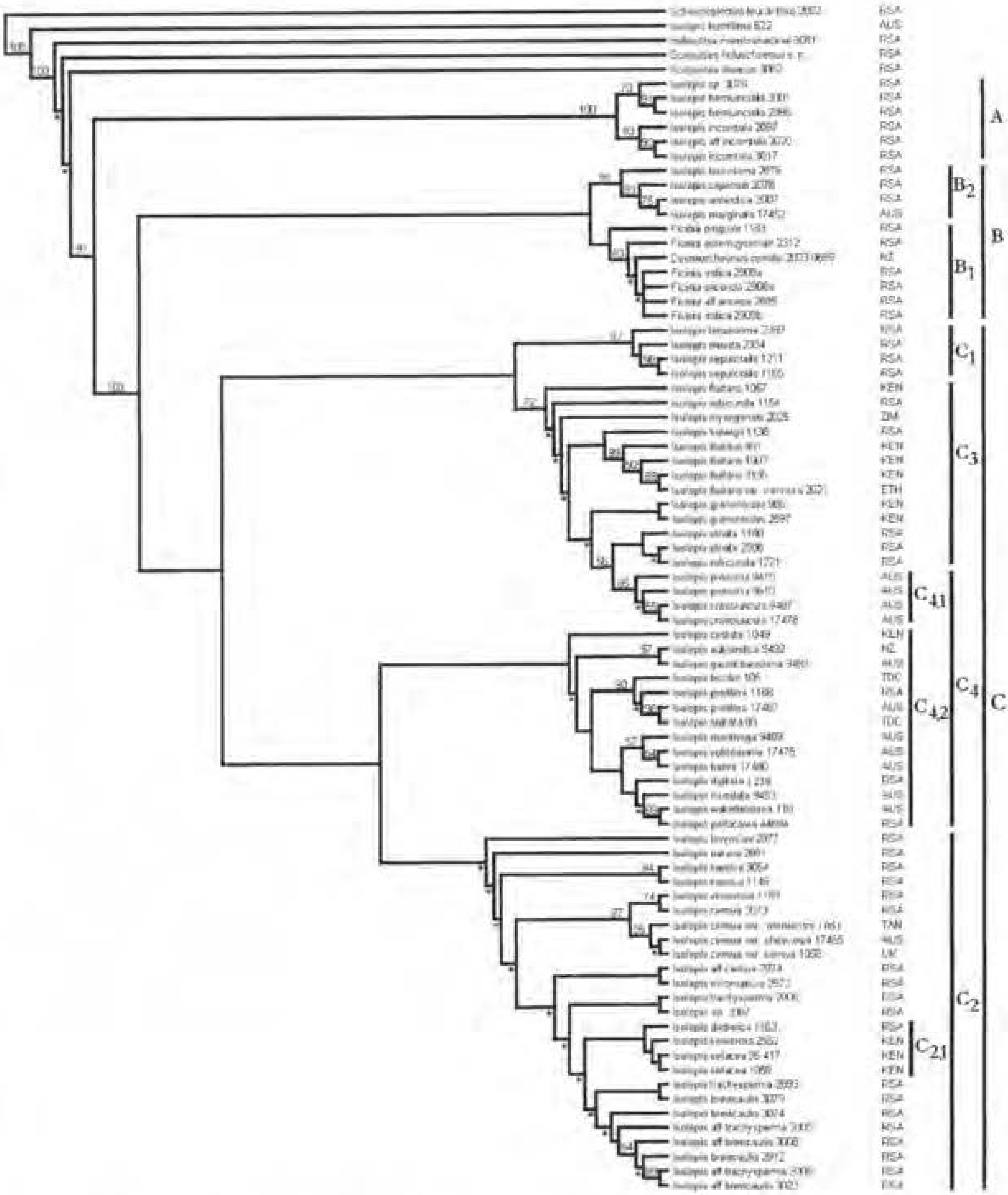

Figure 5: Bootstrap tree of the *rps16* data from 500 bootstrap replicates with the main clades that are described in the text labeled with letters. Bootstrap percentages (numbers above branches) are only shown on branches with support ≥ 50% (CI = 0.70, RI = 0.79). Nodes marked with a * are collapsed in the strict consensus parsimony tree. The localities of the sampled individuals used in the phylogeny are indicated by the following codes: AUS - Australia; ECU - Ecuador; ETH - Ethiopia; KEN - Kenya; NZ - New Zealand; RSA - South Africa; TAN - Tanzania; TDC - Tristan da Cuhna; UK - United Kingdom; ZIM - Zimbabwe

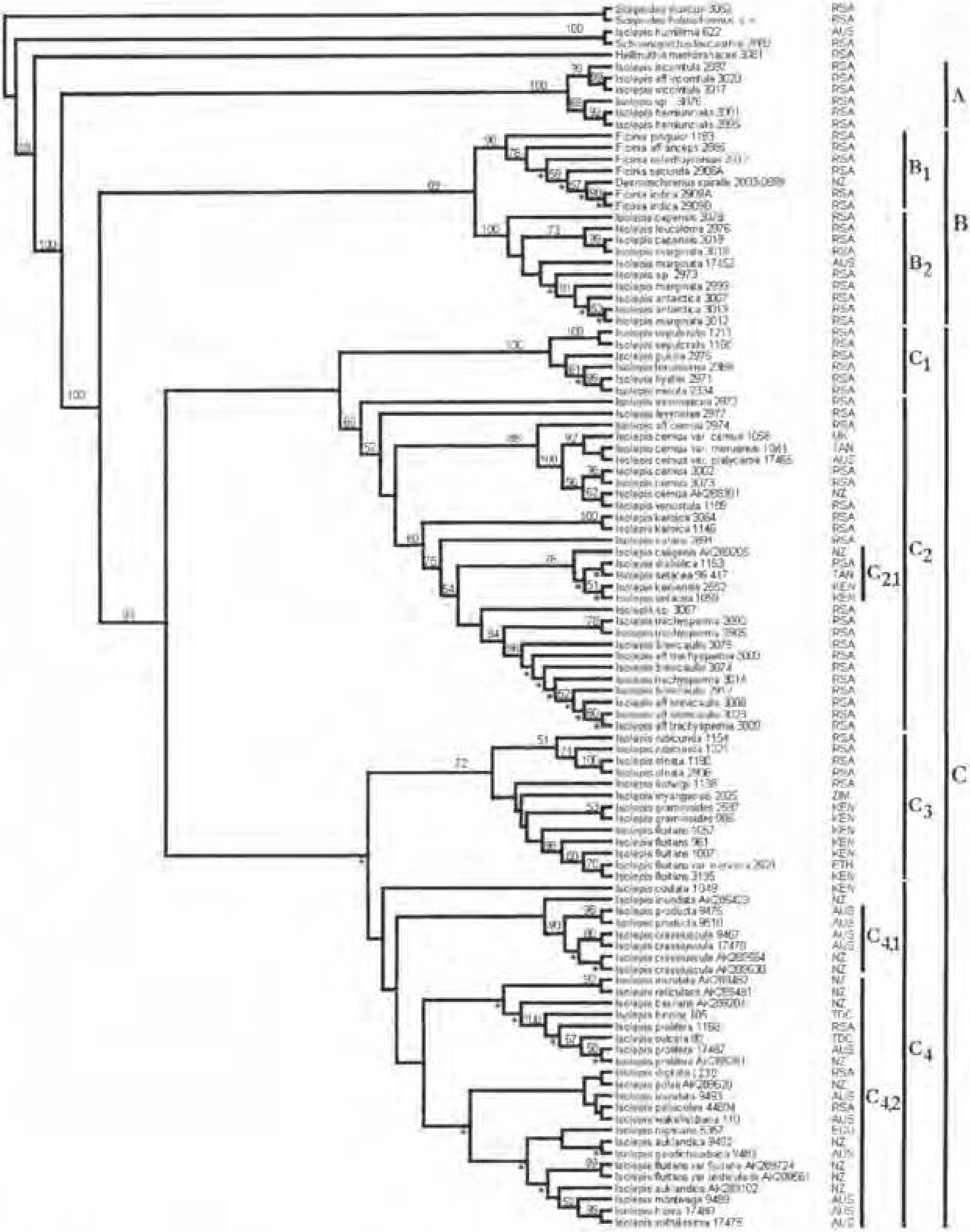

Figure 6: Bootstrap tree of the combined *rps16* and *ITS* data from 500 bootstrap replicates with the main clades that are described in the text labeled with letters. Bootstrap percentages (numbers above branches) are only shown on branches with support ≥ 50% (CI = 0.56, RI = 0.78). Nodes marked with a * are collapsed in the strict consensus parsimony tree. The localities of the sampled individuals used in the phylogeny are indicated by the following codes: AUS - Australia; ECU - Ecuador; ETH - Ethiopia; KEN - Kenya; NZ - New Zealand; RSA - South Africa; TAN - Tanzania; TDC - Tristan da Cuhna; UK - United Kingdom; ZIM - Zimbabwe

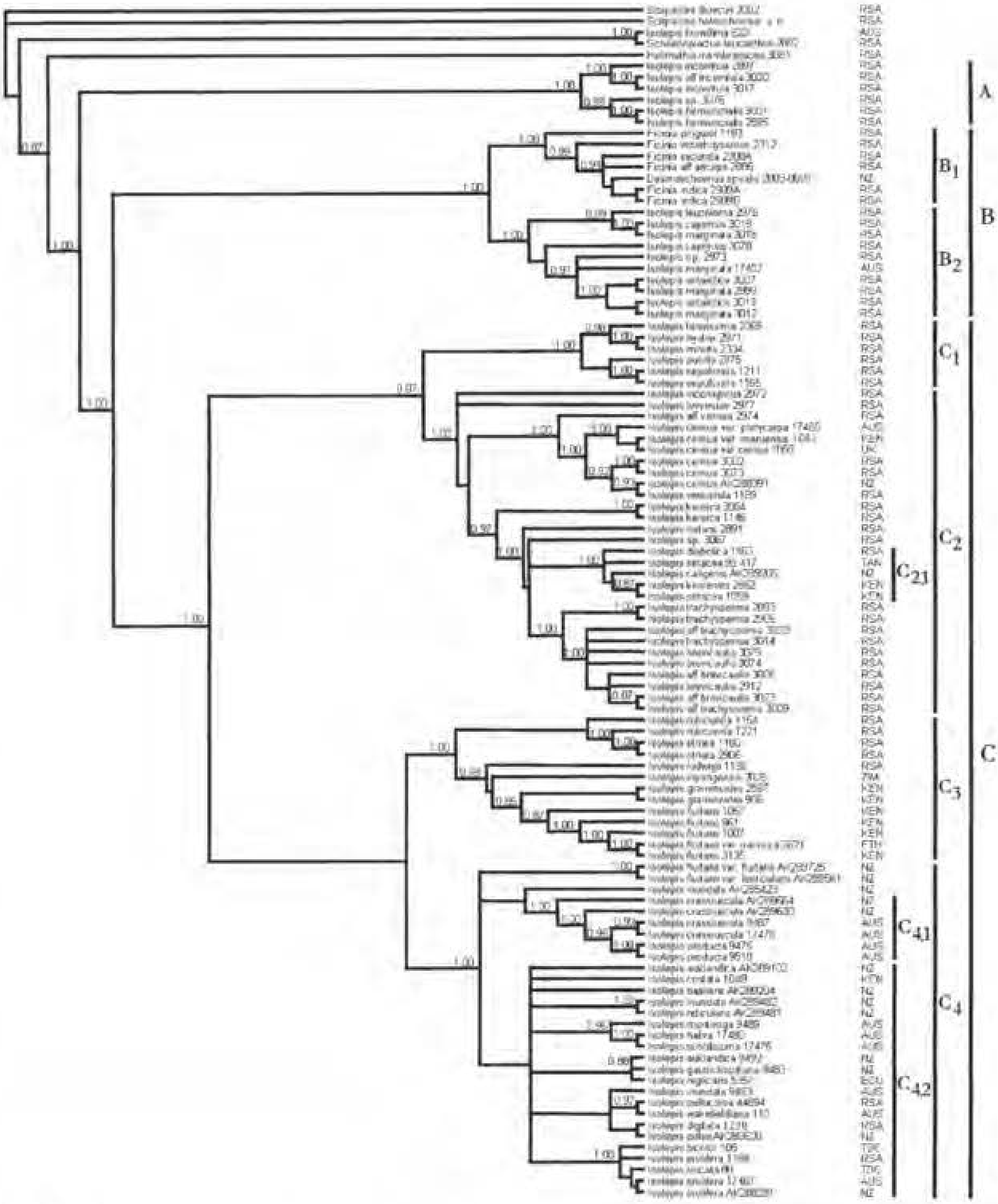

Figure 7: A phylogeny of *Isolepis* based on a GTR + G + I model Bayesian approach using a combined *ITS* and *rps16* data set with the main clades that are described in the text labeled with letters. All branches with a posterior probability (PP) ≥ 0.50 are shown but only branches with a (PP) ≥ 0.90 are labeled (label above the branch). The localities of the sampled individuals used in the phylogeny are indicated by the following codes: AUS - Australia; ECU - Ecuador; ETH - Ethiopia; KEN - Kenya; NZ - New Zealand; RSA - South Africa; TAN - Tanzania; TDC - Tristan da Cuhna; UK - United Kingdom; ZIM - Zimbabwe

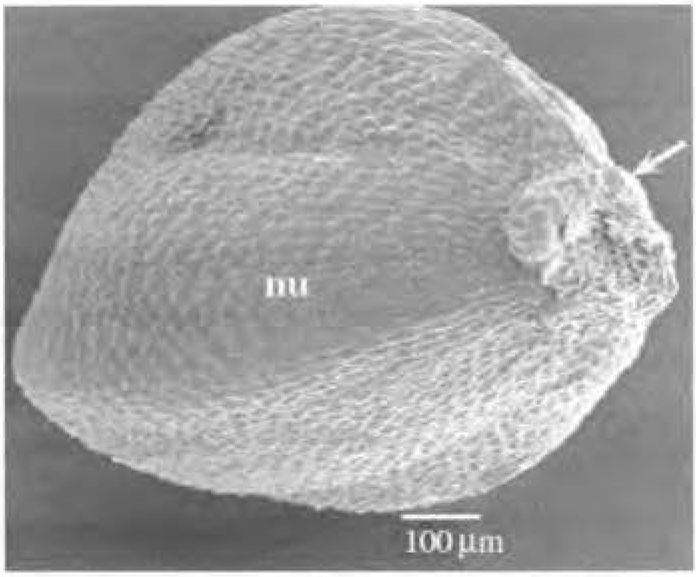

Figure 9: Scanning electron microscope image of the nutlet (nu) of *Isolepis antarctica*. The arrow shows the poorly developed gynophore. (From Vrijdaghs *et al*, 2005)

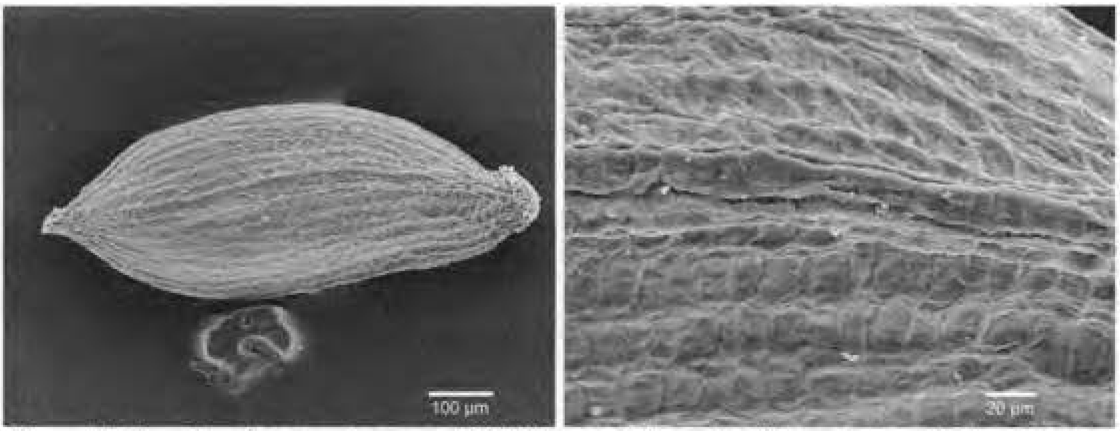

Figure 10: Scanning electron microscope (SEM) image of the nutlet of *I. tenuissima* showing the surface ornamentation

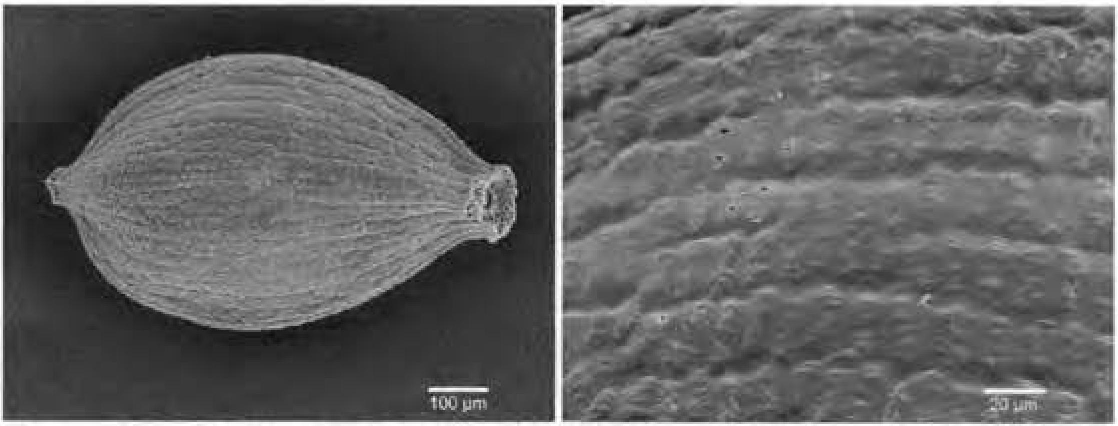

Figure 11: Scanning electron microscope (SEM) images of the nutlet of *I. hystrix* showing the minutely papillose surface ornamentation.

Discussion

Clades were first labelled according to the combined Bootstrap tree (Figure 6) and then all the clade labels were transferred to the other phylogenies (Figures 4, 5 and 7) so that the clades mentioned below, apply to all phylogenies reported in this study.

It may be argued that the two different topologies of the nuclear and plastid phyologenies are evidence of incomplete lineage sorting and that this could cause difficulties in inferring relationships from the combined phylogeny and that the two phylogenies cannot be combined. It recently has been shown however, despite incomplete lineage sorting, gene sequences retain enough signal to accurately estimate phylogeny as long as the number of individuals sampled is high and that accuracy is also increased with an increased number of gene regions in spite of incomplete lineage sorting (Maddison & Lacey Knowles, 2006).

Phylogenetic relationships

The position of *I. humillima*, being closer to *Schoenoplectus*, has been previously discussed (Muasya *et al*, 2001) and this taxonomic study does little to clarify its position. It does however confirm its being closer to *Schoenoplectus* than to the *Scirpoides* and not within the rest of *Isolepis*.

The *Isolepis* (not including *I. humillima*) are found to be resolved into three clades (Clades A, B and C). Clade A is comprised of *I. incomtula*, *I. hemiuncialis* and one of the *I. sp* (3076). Clade B is comprised of both *Ficinia* and *Isolepis* (*I. capensis*, *I. marginata*, *I. sp* (2973), *I. leucoloma* and *I. antarctica*) species while clade C contains the rest of the *Isolepis*. Due to limited taxon sampling, the pattern of clade A being sister to both the *Ficinia* and *Isolepis* has not yet been documented. In Muasya *et al* (2000) *I. marginata* was also found to be more closely related to *Ficinia* than to the rest of *Isolepis* but due to limited taxon sampling, the position of *I. marginata* was considered to be ambiguous. This study confirms the position of *I. marginata* as well as other *Isolepis* species (*I. capensis* and *I. antarctica*) as having strong support (BS = 89, PP = 1.00) as being sister to *Ficinia*.

Clade A is a small clade of *Isolepis*, which is strongly supported (BS = 100, PP = 1.00) as being sister to a combined clade containing both the *Ficinia* as well as all the other *Isolepis*. This clade is supported by both the plastid (BS = 100) and nuclear (BS = 100) markers. There are no obvious morphological characters defining these species. It was beyond the scope of this study to investigate the morphologies of these species in detail and more work is needed to identify the defining features of this clade.

Clades B has strong support (BS = 89, PP =1.00) as well as having strong support (BS = 100, PP = 1.00) as being sister to the rest of *Isolepis*, excluding those already mentioned in Clade A. It has been shown that some of the *Isolepis* within clade B_2 share some morphological similarities with *Ficinia*. Both *I. marginata* and *I. antarctica* have been described as having poorly developed gynophores (Figure 9) as well as cartilaginous glumes (Muasya & Simpson, 2002, Vrijdaghs *et al*, 2005) both of which are features, which are used to characterize the *Ficinia*. This clade also includes the recently described *I. capensis* and *I. leucoloma*, the phylogenies of which were previously unclear. Clades B_1, the *Ficinia*, and B_2, the above mentioned *Isolepis* have strong support (BS = 98, PP = 1.00 and BS = 100, PP = 1.00 respectively) as being separate monophyletic groups. Nested within the *Ficinia* clade, B_1, is *Desmoschoenus spiralis* but this was expected as it has been shown to resolve within the *Ficinia* in other molecular studies (Muasya *et al*, 2001) and it has also been shown to have a gynophore (Browning & Gordon-Gray, 1995). Sister to clade B is clade A, which contains 'true' *Isolepis* with no gynophore. Clade B is also sister to clade C, which is comprised of the rest of the 'true' isolepis, which also have no gynophores. Thus, clade B has evolved from a common ancestor with no gynophore and this structure has evolved after the divergence of this clade from the rest of the *Isolepis*. Interestingly, the annual species in clade B (*Isolepis*) only have a rudimentary gynophore while most of the perennial species (*Ficinia's*) are characterised by a well developed gynophore. Further investigation into the function of the gynophore would need to be done to test whether there is a real relationship between life form and the ← presence or absence of the gynophore.

Clade C contains two distinct smaller clades, C_1 and C_2, with strong (BS = 100, PP = 1.00) and moderate to strong (BS = 65, PP = 1.00) support respectively. Two (*I. sepulcralis* and *I. hystrix*) of the three species in the current subgenus *Micranthea* resolve in clade C_2 while the third species (*I. natans*) is found to resolve within the current subgenus *Isolepis* section *Cernua* and sister to section *Isolepis*. Clade C_2 also contains *I. pusilla*, *I. tenuissima* and *I. minuta*. Since these species, to date, have not yet been included in a molecular phylogeny and that the support for this clade is strong in all of the molecular phylogenies reported here ($BS \geq 92$, PP = 1.00), it suggests that this is a natural clade. The nutlet morphology of *I. pusilla* lends itself to inclusion within the *Micranthea* as it has a nutlet, which is also described as being minutely papillose (Muasya & Simpson, 2002), which is one of the defining feature of the subgenus. The nutlet of *I. tenuissima* (Figure 10) does not however share the minutely papillose surface ornamentation, which characterises the subgenus. An example of a nutlet with minutely papillose surface ornamentation can be seen in Figure 11. The nutlet surface ornamentation of *I. minuta* (Figure 2 in Muasya & Simpson, 2002) also resembles that of the above mentioned taxa. It also appears to be minutely papillose but is not described as such in the monograph of the *Isolepis* (Muasya & Simpson, 2002).

The moderate to strong support (BS = 62, PP = 100) for clade C_2 resembles the current grouping of the remaining species in the subgenus *Isolepis* section *Cernua*. Nested within this clade however is the moderately to strongly supported clade $C_{2.1}$, which corresponds to the subgenus *Isolepis* section *Isolepis* except that it contains *I. caligenis*, which is currently grouped into subgenus *Isolepis* section *proliferae*. *I. caligenis* was previously excluded from the subgenus *Isolepis* section *Isolepis* due to the nutlet not being striated (Muasya & Simpson, 2002.).

The rest of clade C contains a strongly supported clade C_3 (BS = 72, PP = 1.00) and a weak to strongly supported clade C_4 ($BS < 50$, PP = 1.00), which also contains clades $C_{4,1}$ and $C_{4.2}$. The subgenus *Fluitantes* is polyphyletic with taxa in clades C_3, [illegible] and C_4[illegible]. Clade C_3 consists of only of African *Fluitantes*. This clade (C_3) contains two smaller clades. One weakly supported (BS = 51, $PP < 0.85$) clade consisting of only *I. rubicunda* and *I. striata*, which are both endemic to the Western Cape of South Africa. The second clade has moderate to strong support ($BS < 50$, PP = 0.98) and contains a single Cape endemic, *I. ludwigii*, which is basal to the rest of the African *Fluitantes*.

The rest of the *Fluitantes* are split into two groups within clade C_4, which is a predominantly Australian clade of the subgenus *Isolepis* section *proliferae*. *Isolepis fluitans* var. *fluitans* and *Isolepis fluitans* var. *lenticularis* from New Zealand are strongly supported as being sister to each other (BS = 99, PP = 1.00) but their position within clade C_4 is uncertain. The second group of *Fluitantes* within clade C_4 is strongly supported (BS = 90, PP = 1.00) and is made up of *I. producta* from Australia and *I. crassiuscula* from both Australia and New Zealand. If one looks at the plastid marker (*rps16*, Figure 5) only, the Australian *Fluitantes* are nested within the African *Fluitantes* with the African taxa being sister to the Australian taxa. The support for the Australian *Fluitantes* clade itself is moderate (BS = 0.56) but there is strong support for its inclusion within the African *Fluitantes* (BS = 0.72). The nuclear marker (*ITS*, figure 4) on the other hand shows the Australian *Fluitantes* grouped with the Australian *Proliferae*. This demonstrates that there may be different maternal and paternal histories within the *Fluitantes*.

The rest clade C_4 ($C_{4.2}$) contains the rest of the current subgenus *Isolepis* section *proliferae* (excluding the afore mentioned *I. tenuissima* and *I. caligenis*) which is a section, which has diversified in the Southern Hemisphere (Muasya & Simpson, 2002).

Evolution of life form

Most of the *Isolepis* are described as being either mat forming or tufted 'annual or short live perennial' species. Only *Isolepis digitata* is described as being a truly perennial species of *Isolepis* (Muasya & Simpson, 2002). Some species are known to show a more perennial nature (e.g. *I. costata*, *I. inundata* and some of the *Fluitantes* such as *I. ludwigii*) but are still considered as long-lived annuals. The *Ficinia* however, are characterized as being a clade of usually perennial plants with woody rhizomes. If the growth habit is reconstructed on the phylogeny the following patterns can be seen. Clade A consists of only annual species of *Isolepis* so their common ancestor is reconstructed as also being an annual. Clade B contains a *Ficinia* clade (B_1), whos common ancestor is reconstructed to be a perennial while the ancestor of the *Isolepis* clade (B_2) is reconstructed to be an annual. The growth habit of the common ancestor of clade B is therefore uncertain. The common ancestors of clade C is found to be annuals or short lived perennials even though it contains species, which may be considered to have a more perennial nature. This appears to be a derived character, which has evolved on separate occasions (e.g. *I. digitata, I. costata, I. inundata* and some of the *Fluitantes* such as *I. ludwigii*) but as the majority of the species are annuals or short live perennials, the common ancestors of this clade is reconstructed as being annual or short lived perennial. The common ancestor of clade B and the rest of the *Isolepis* (clade C) is somewhat uncertain due to the common ancestor of clade B being ambiguous. As the common ancestor of clades C is an annual or short lived perennial and the common ancestor of clade A, which is sister to clades B and C is also an annual or short lived perennial, the common ancestor, which gave rise to all of the *Isolepis* and the *Ficinia* must have been an annual or short live perennial species.

Biogeography of the *Isolepis*

It has been suggested that the *Isolepis* originated and diversified in the winter rainfall region of South Africa (e.g. Muasya & Simpson, 2002, Vrijdaghs *et al*, 2005) but this relationship has not been described in terms of the phylogenetic relationships between different species found in different geographical areas.

Within clades C_1 and C_2, two separate but similar patterns can be seen. Clade C_2 contains two clades with individuals from outside of South Africa. The *setacea* clade ($C_{2.1}$) has species from tropical East Africa (*I. setacea* and *I. keniensis*) as well as one species from New Zealand (*I. caligenis*). The *cernua* clade contains species from the UK (*I. cernua* var. *cernua*), Tanzania (*I. cernua* var. *meruensis*) and from Australia (*I. cernua* var. *platycarpa*). Strongly supported as being sister to both these clades, are Cape endemic taxa. For example, the clade containing the two *I. karoicia* samples ~~are~~ is strongly supported (BS = 60, PP = 97) as being sister to the clade containing the *setacea*; and *I. inconspicua* and *I. levynsiae* (Cape endemics?) are strongly supported (BS = 65, PP = 1.00) as being sister to the *cernua* clade. This creates a strong case for this clade (C_2) originating in the ~~CFR~~ winter rainfall. Clade C_1 contains mainly Cape endemics (*I. pusilla, I. minuta, I. hystrix* and *I. tenuissima*) as well as *I. sepulcralis*, which is has an African as well as Austral distribution. With the common ancestor of clade C_2 reconstructed as being of Cape origin and with the abundance of Cape endemics within clade C_1, the common ancestor of clade C_1 and C_2 is supported as having originated and diversified in the Cape with subsequent dispersal from the Cape to other parts of the world.

The African *Fluitantes* (clade C_3) provide another example of a clade with Cape origin and the dispersal out of the Cape is clearly illustrated. The clade containing the Cape endemics, *I. rubicunda* and *I. striata*, is strongly supported (BS = 96, PP = 1.00) as being sister to the rest of the African *Fluitantes*. In the clade containing the rest of the African *Fluitantes*, the Cape endemic, *I. ludwigii*, is strongly supported (BS = 82, PP = 0.98) as being sister to all the more Northerly, African *Fluitantes* with *I. inyangensis* being weakly supported (BS < 50, PP < 0.85) as being sister to the Kenyan *I. graminoides* (and the Kenyan samples of *I. fluitans*), which has weak to strong support (BS < 50, PP < 0.85) as being sister to the Ethiopian *I. fluitans* var *nervosa*. If one looks at the data from the rps16 phylogeny, the African *Fluitantes* are seen to be sister to the Australian *Fluitantes* and, the afore mentioned Cape clade C_1, is sister to the *Fluitantes* as a whole. These relationships gives strong support for an origin of the *Fluitantes* within the CFR with dispersal North into Africa and also into Australia/New Zealand.

If one reconstructs the biogeography of clade B, the common ancestor is found to also be of Cape origin. The *Ficinia* (B_2) are known to be an African clade with the majority in the Cape Floristic Region (CFR) (Muasya, 2005) while the presence of *Desmoschoenus spiralis* can be explained by a rare, long-distance dispersal event. A similar circumpolar dispersal can be seen in *F. nodosa*, which has a distribution in Africa as well as Australia, New Zealand, Chile, St Paul and St Helena (Muasya *et al*, 2000) The common ancestor of the *Isolepis* in clade B_2 is found to also be of Cape origin even though this clade does contain the cosmopolitan *I. marginata*.

Due to the uncertainty of the origins of the common ancestor of clade D_2, the origin of clade D cannot be reconstructed with any certainty. In spite of this, the common ancestor of the *Isolepis* as well as the *Ficinia* is reconstructed to be of Cape origin as all sister clades to clade D (Clades A, B and C) are of Cape origin.

The outgroup taxa (*Scirpoides dioecus*, *Scirpoides holoschoenus*, *Schoenoplectus leucanthus* and *Hellmuthia membranacea*) are also all South African taxa. Thus, clades A, B and C all reconstruct to have an origin in the winter rainfall region of South Africa with multiple subsequent dispersals North, into Africa as well as into other parts of the world.

Hybridisation within the *Fluitantes*

To investigate the polyphyletic nature of the *Fluitantes*, one needs to investigate both the nuclear, *ITS* (Figure 4), and plastid, *rps16* (Figure 5), phylogenies seperately. The maternally inherited, plastid phylogeny shows the *Fluitantes* as being monophyletic with the African *Fluitantes* (C_3) having moderate support (BS = 56) as being sister to the Australian *Fluitantes* ($C_{4,1}$), which has strong support (BS = 72) as being nested within the African *Fluitantes* and forming a combined clade of C_3 and $C_{4,1}$. Weakly supported (BS < 50) as being sister to this clade is clade C_1. The paternally inherited, nuclear phylogeny shows the *Fluitantes* as being polyphyletic with the strongly supported (BS = 96) African *Fluitantes* (C_3) being sister to a clade containing both the subgenus *Isolepis* section *proliferae* ($C_{4,2}$) as well as the Australian *Fluitantes* ($C_{4,1}$), which form a strongly supported clade (BS = 88) nested within the subgenus *Isolepis* section *proliferae*.

The differing maternal and paternal histories of the *Fluitantes* may be explained by an ancient hybridisation event between the common ancestor of subgenus *Isolepis* section *proliferae* (clade $C_{4,2}$) and the common ancestor of the Australian *Fluitantes* (clade $C_{4,1}$). Following the diversification of the *Fluitantes* in Africa, they were able to disperse to Australia and New Zealand through at least one, rare, long distance dispersal event. Once in Australia the evidence suggests that there was at least one hybridisation event with pollen from an ancestor of the subgenus *Isolepis* section *proliferae* before the evolution of; *I. crassiuscula*, and *I. producta* in Australia, and *I. fluitans* var. *fluitans* and *I. fluitans* var. *lenticularis* in New Zealand. This would leave a signal in the nuclear phylogeny due to the gene flow through pollen but not in the plastid phylogeny as this is only maternally inherited. Even though there has been gene flow between the Australian *Fluitantes* and the subgenus *Isolepis* section *proliferae*, the Australian *Fluitantes* have kept their typical *Fluitantes* morphological characteristics thus showing that these are most probably also maternally inherited characteristics.

Proposed classification

The proposed classification can be seen in Figure 8. The plastid phylogeny was used as this phylogeny corresponds more closely to morphological characteristics than the nuclear phylogeny. This is described above in the section of the hybridisation of the *Fluitantes*.

The evidence provided in this study supports the recognition of clades A, B and C as genera. Clade A is strongly supported as being monophyletic and sister to *Ficinia* and the rest of *Isolepis* in all of the phylogenies presented here. Currently there is no proposed name for this genus as it was beyond the scope of this project to investigate the defining characters of the species within it. Nov derived from the Latin word *Nova* meaning 'new' will act as a place holder until the genus has been named. There are however two alternative interpretations of this data. Clades A, B and C could be a single genus in which case *Ficinia* would be merged into *Isolepis* or, the current taxonomy could be unaltered and *Isolepis* could simply be considered as a paraphyletic genus.

Both molecular and morphological data suggest renaming the *Isolepis* in clade B_2 as ~~belonging to~~ [illegible] *Ficinia*. Therefore, clade B would represent the genus *Ficinia* and contain two subgenera. Namely *Ficinia* subgenus *Ficinia*, which would contain all the current *Ficinia*, and *Ficinia* subgenus *pseudoisolepis* (a proposed generic name) which would contain all the *Isolepis* species in clade B_2. Species in *Ficinia* subgenus *pseudoisolepis* would be characterised as being annuals or short lived perennials resembling species of the genus *Isolepis* but having cartilaginous glumes and/or a poorly developed gynophore (e.g. *I. antarctica*, Figure 9). This transfer of the above *Isolepis* species into *Ficinia* has been previously suggested (Muasya & Simpson, 2002, Vrijdaghs *et al.* 2005) but was not carried out due to the lack of strong molecular evidence, which this treatment provides.

The remaining clade, C, is supported as comprising of the previously described genus *Isolepis*. From the morphological and molecular support described, the subgenus

Micranthea is suggested to be expanded to include all the taxa within clade C_1, while *I. natans* is suggested to be removed from this subgenus. The morphological character of an anther apex with no crest does not agree with the currently proposed subgenus *Micranthea* but the previously described nutlet characters (ellipsoid, nutlet surface minutely papillose) described by Muasya & Simpson, 2002, are robust (Except for in *I. tenuissima*).

This study does not propose any changes to *Isolepis* subgenus *Fluitantes* but it is important to note that the nuclear and plastid phylogenies give conflicting results. The fact that the morphological and plastid data display the same relationship supports no re-organisation of the subgenus *Fluitantes*.

The plastid and nuclear data suggests upgrading *Isolepis* subgenus *Isolepis* section *Proliferae* to *Isolepis* subgenus *Proliferae*, as it is in a clade sister to the clade containing the type species of the genus (*I. setacea*) and not imbedded within it. This clade corresponds to Clade $C_{4,1}$ in the phylogenies.

Finally, the data suggests the removal of *Isolepis* subgenus *Isolepis* section *Cernua* and section *Isolepis* and creating a single subgenus *Isolepis* (corresponding to clade C_2) with no sections.

It is noted however, that the duration of this study did not allow for a thorough investigation into the morphological characters, which define these infrageneric groupings. Much morphological work is still necessary to be able to define these infrageneric groupings upon any other bases than that described in this study.

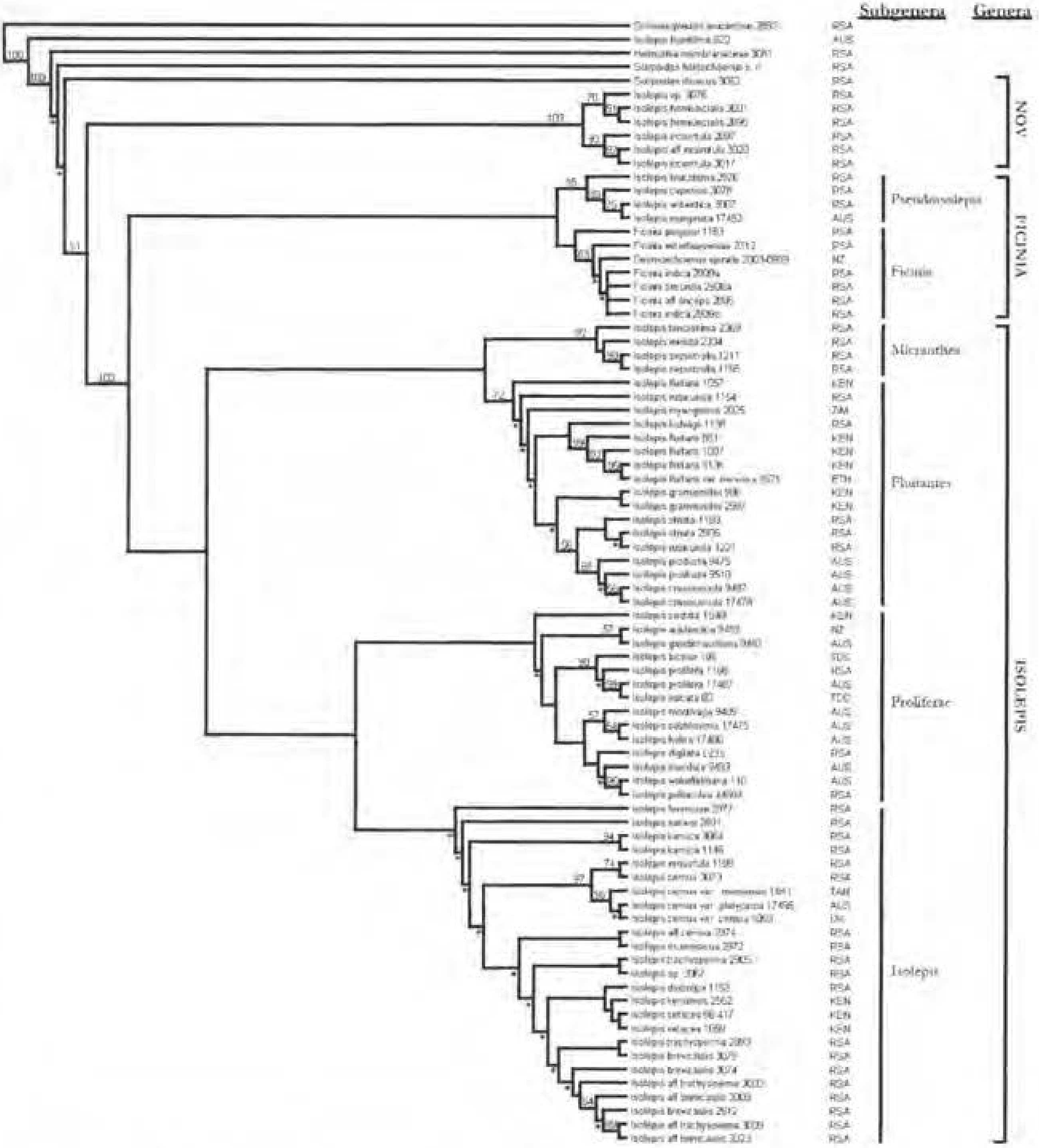

Figure 8: Bootstrap tree of the *rps16* data from 500 bootstrap replicates showing the suggested phylogeny of the *Isolepis*. Bootstrap percentages (numbers above branches) are only shown on branches with support ≥ 50% (CI = 0.56, RI = 0.78). Nodes marked with a * are collapsed in the strict consensus parsimony tree.

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References

BROWNING, J. & GORDON-GRAY, K.D. 1996. A gynophore in *Desmoschoenus* (*Cyperaceae*). *New Zealand Journal of Botany*, 34: 131-134

CHANDLER, G.T. & PLUNKETT, G.M. 2004. Evolution in Apiales: nuclear and chloroplast markers together in (almost) perfect harmony. *Botanical Journal of the Linnean Society*, 144(2): 123-147

HUELSENBECK, J.P. & RONQUIST, F. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19 (12): 1572-1574

MADDISON, W.P. & LACEY KNOWLES, L. 2006. Inferring Phylogeny Despite Incomplete Lineage Sorting. *Systematic Biology*, 55(1): 21-30

MUASYA, A.M. 2005. *Ficinia anysbergensis* and *F. esterhuyseniae* (Cyperaceae), two new species from the Cape Floristic Region of South Africa. *South African Journal of Botany*, 71(2): 197-200

MUASYA, A.M. & SIMPSON, D.A. & GOETGHEBEUR, P. 2000. New Combinations in *Trichophorum*, *Scirpoides*, and *Ficinia* (*Cyperaceae*). *Novon*, 10(2): 132-133

MUASYA, A.M. & SIMPSON, D.A. & CHASE, M.W. 2001. A Phylogeny of *Isolepis* (Cyperaceae) Inferred Using Plastid *rbcL* and *trnL-F* Sequence Data. *Syetematic Botany*, 26(2): 342-353

MUASYA, A.M. & SIMPSON, D.A. 2002. A monograph of the genus *Isolepis* R. Br. (*Cyperaceae*). *Kew Bulletin*, 57: 257-362

MUASYA, A.M. & SIMPSON, D.A. & SMETS, E. 2006. *Isolepis tenella*, a New Combination in *Cyperaceae*. *Novon*, 16: 89-90

MUASYA, A.M & VRIJDAGHS, A. & SIMPSON. D.A. & CHASE, M.W. & GOETGHEBEUR, P. & SMETS, E. 2006. What is a genus in Cypereae:

phylogeny, character homology assessment and generic circumscription. *In press.*

OXELMAN, B. & LIDEN, M. & BERGLUND, D. 1997. Chloroplast *rps16* intron phylogeny of the tribe Sileneae (Cayophyllaceae). *Plant Systematics and Evolution,* 206: 393-410

ROALSON, E.H. & FRIAR, E.A. 2000. Infrageneric Classification of *Eleocharis* (*Cyperaceae*) Revisited: Evidence from the Internal Transcribed Spacer (*ITS*) Region of Nuclear Ribosomal DNA. *Systematic Botany,* 25(2): 323-336

ROALSON, E.H. & COLUMBUS, J. T. & FRIAR, E. A. 2001. Phylogenetic Relationships in *Cariceae* (*Cyperaceae*) Based on *ITS* (nrDNA) and *trnT-L-F* (cpDNA) Region Sequences: Assessment of Subgeneric and Sectional Relationships in *Carex* with Emphasis on Section *Acrocystis*. *Systematic Botany,* 26(2): 318-341

SCHÖNSWETTER, P. & POPP, M. & BROCHMAN, C. 2006. Central Asian origin of and strong genetic differentiation among populations of the rare and disjunct *Carex atrofusca* (*Cyperaceae*) in the Alps. *Journal of Biogeography,* 33(5): 948-956

SWOFFORD, D. L. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts

VRIJDAGHS, A. & GOETGHEBEUR, P. & MUASYA, A.M. & CARIS, P. & SMETS, E. 2005. Floral Ontogeny in *Ficinia* and *Isolepis* (Cyperaceae), with Focus on the Nature and Origin of the Gynophore. *Annals of Botany,* 96: 1247-1264

WHITE, J.J. & BRUNS, T. & LEE, S. & TAYLOR, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics *In:* INNIS, M. & GELFAND, D. & SNINSKY, J. & WHITE, T. (*eds*). PCR Protocols: A guide to methods and applications. *Academic Press,* Orlando, *Fl, USA*: 315-322

WIENS, J.J. 1998. Combining Data Sets with Different Phylogenetic Histories. *Systematic Biology,* 47(4): 568-581