Systematics of subtribes Athanasiinae and Phymasperminae (Anthemideae, Asteraceae)

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Student number……**3767105**……..declare that the attached dissertation entitled…..**Systematics of subtribes Athanasiinae and Phymasperminae (Anthemideae, Asteraceae)** ……is my own work and that all the sources I have quoted have been indicated and acknowledged by means of complete references.

Signed this day ….**18**….. of ……**November**……. **2020**….at ……**Bellville**…….

Signature

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ABSTRACT

The tribe Anthemideae is a large tribe of the family Asteraceae comprising 111 genera and 1 800 species distributed in Africa, Europe, Asia, and the Northern temperate region, with southern Africa as one of its main centers of diversity, together with Central Asia and the Mediterranean. Ongoing studies have focused on analysing relationships among the southern African subtribes of Anthemideae and the most recent classification recognised fourteen subtribes in which there is a clear biogeographical gradient, with the six southern African subtribes comprising the earliest diverging lineages. The present study focuses on two of these southern African-centred lineages (viz. subtribes Athanasiinae and Phymasperminae). Previous phylogenetic studies of the tribe were based on nuclear nrDNA ITS and plastid cpDNA *ndhF* sequence data and included only a single representative from each of the genera within the subtribes Athanasiinae and Phymasperminae. The phylogenetic relationships and circumscriptions of the two subtribes have been uncertain and highlighted as being in need of re-assessment, as the monophyly of Athanasiinae is in question and the placement of Phymasperminae differed substantially between nuclear and plastid datasets. The present study aimed at expanding the datasets by including 78 of the 107 (73%) species for these two subtribes based on two nuclear (ITS and ETS) and two plastid (*rpl32-trnL* and *3'rps16-5'trnK*) regions. The resultant nuclear and plastid data were analysed using Maximum Parsimony and Bayesian Inference and the phylogenetic trees used in the reconstruction of morphological characters to assess generic and subtribal relationships of Athanasiinae and Phymasperminae. The phylogenetic analyses of all datasets resolved the previous incongruent position of the subtribe Phymasperminae, consistently recovering the subtribe as sister to Athanasiinae to form a strongly supported monophyletic clade. This relationship between Athanasiinae and Phymasperminae was supported by the following synapomorphies: anthers with polarized endothecial tissues, leaves with secretory cavities and the presence of furanosesquiterpenes. While Phymasperminae could be circumscribed by the cypselas with more than ten ribs and the papillose pericarp, the circumscription of Athanasiinae would be problematic as there were no synapomorphies identified to circumscribe it. Phymasperminae is therefore here subsumed into an expanded Athanasiinae s.l. supported by the anthers with polarized endothecial tissues, leaves with secretory cavities and the presence of furanosesquiterpenes. Generic

circumscriptions within the expanded Athanasiinae were also re-assessed and refined based on morphological and phylogenetic data. The circumscription of *Phymaspermum* Less. is here expanded to include the closely related *Gymnopentzia* Benth. and *Eumorphia* DC, while the monotypic genera *Asaemia* Harv. ex Benth. and *Adenoglossa* B.Nord. are subsumed within *Athanasia* L. and *Leucoptera* B.Nord., respectively. The expanded *Phymaspermum* s.l. can be recognized by the synapomorphic papillose fruit (either short or long and sometimes also with glandular trichomes) with multiple ribs (10 or more ribs).

The previously unplaced genus *Inulanthera* Källersjö was shown to have a fairly isolated position in all analyses. The nuclear analyses recovered *Inulanthera* as sister to a broader Athanasiinae - Phymasperminae clade, while in the plastid analyses it was recovered with the Penztiinae - Ursiniinae clade. We therefore describe a new subtribe Inulantherinae S.Akimana, Boatwr. & Magee to accommodate the genus, which can be distinguished from the other subtribes by the tailed anthers, fruits lacking secretory cavities, elongated cells in the ribs and its pappus present as an extension of the cypselas ribs, with each rib extended as a small horn or as a scale.

Several taxonomic and nomenclatural changes are implemented here based on the results of the expanded phylogenetic analyses and the morphological reconstructions. New combinations are provided for 9 taxa, two genera are reduced to sectional rank, one new subtribe described and the circumscription of one subtribe expanded.

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CHAPTER 1

INTRODUCTION AND OBJECTIVES OF THE STUDY

1.1. Introduction

The Asteraceae is recognised as one of the largest families of flowering plants in the world, comprising ca. 1 600 -1 700 genera and 24 000 species (Funk et al. 2009, Fu et al. 2016) distributed worldwide, except in Antarctica (Bohm et al. 2001, Kaderiet and Jeffrey 2007, Funk et al. 2009). The family is recognised as the largest and most diverse in the core Cape Flora, as well as in the Greater Cape Floristic Region of southern Africa, with more than 125 genera and 1077 species (Manning and Goldblatt 2012, Snijman 2013). Asteraceae can easily be distinguished from other families solely by the arrangement of the individual florets into the characteristic flower head or capitulum typical of the family (Herman et al. 2001, Adedeji et al. 2008, Funk et al. 2009, Paul et al. 2017). Members of the Asteraceae are well known and commercially cultivated. They are used as ornamental plants, for medicine and as commercial crops, with some of the latter becoming important weeds in several countries (Rahman et al. 2008, Van Wyk et al. 2008, Lakshman et al. 2014, Paul et al. 2017).

The most recent classification of the Asteraceae, summarized in Funk et al. (2009), recognizes 12 subfamilies and 43 tribes. Among these tribes, the Anthemideae is one of the largest of the family Asteraceae (Bremer and Humphries 1993, Funk et al. 2009), comprising ca. 111 genera and 1 800 species (Oberprieler et al. 2005, Kaderiet and Jeffrey 2007). Members of the tribe are largely distributed in Africa, Europe, Asia, and the northern temperate region, with southern Africa as one of the three main centers of diversity, together with Central Asia and the Mediterranean (Heywood et al. 1977; Bremer and Humphries 1993; Himmelreich et al. 2008). It is most likely that the Anthemideae originated in southern Africa and apparently dispersed and diversified into Asia and the Mediterranean region (Watson et al. 2000; Oberprieler et al. 2005 and Himmelreich et al. 2008). This is further supported by the fact that the basal grade of the Anthemideae is mostly composed of southern African genera from six subtribes comprising the earliest diverging lineages (Oberprieler et al. 2007) of which 27 genera and 290 species are either endemic to or have their distributional centers within the region (Herman et al. 2001; Himmelreich et al. 2008).

Some members of Anthemideae are known to be aromatic and used as medicinal plants, e.g. *Artemisia afra* Jacq. Ex Willd., *Achillea colina* L. and *Santolina chamaecyparissus* L. (Da Silva 2004, Van Wyk et al. 2008, Oberprieler et al. 2009, Paul et al. 2017). *Tanacetum cinerarifolium* Sch.Bip. is used as a natural source of the insecticide "pyrethrum" (Oberprieler et al. 2009). Many species are cultivated as ornamentals, notably several members from the southern African genera *Athanasia* L., *Eriocephalus* L., *Oncosiphon* Källersjö. and *Ursinia* Gaertn. (Oberprieler et al. 2009).

The circumscription of the Anthemideae has remained largely unchanged since the treatment of Bentham and Hooker (1873). However, the classification of the tribe into natural subtribal units has been more problematic. Bremer and Humphries (1993) presented a detailed study of the Anthemideae using morphological, anatomical and chemical characters to determine inter-generic relationships and recognized 12 subtribes.Watson et al. (2000) re-evaluated the traditional subtribal classification of the Anthemideae, as well as the phylogeny and subtribal groupings proposed by Bremer and Humphries (1993). The study of Watson et al. (2000) aimed to construct a phylogeny based on sequence data from the chloroplast gene *ndh*F for representatives of 50 genera of the Anthemideae in order to examine the evolutionary and biogeographic trends within the tribe. The non-monophyly of the subtribes proposed by Bremer and Humphries (1993) was clearly demonstrated by the findings of Watson et al. (2000) and many South African genera fell into a basal grade of lineages contains genera previously placed in subtribes Ursiniinae (*Ursinia, Lasiospermum* Lag., *Hymenolepis* Cass., *Athanasia*), Thaminophyllinae (*Osmitopsis* Cass. and *Lidbeckia* P.J. Bergius) and Matricariinae (*Soliva* Ruiz & Pav., *Hippia* L., *Schistostephium* Less., *Cotula* L., *Eriocephalus* L.), indicating a possible southern African origin for the Anthemideae with subsequent dispersal into Asia and the Mediterranean region (Watson et al. 2000).

Oberprieler et al. (2007) expanded the analyses of Watson et al. (2000) to include representatives of 103 genera of the Anthemideae utilizing sequence data of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (nrDNA) and it was in this study that the current subtribal circumscriptions of Anthemideae were proposed. In the classification of Oberprieler et al. (2007), three southern African genera, *Eumorphia* DC., *Gymnopenztia* Benth. and *Phymaspermum* Less., formed a strongly supported monophyletic clade. This clade was described as the subtribe Phymasperminae based on the presence of papillose cypselas with more than 10 ribs (Källersjö 1986, Bremer and Humphries 1993, Ruiters 2014, Ruiters et

al. 2016). The phylogenetic analyses of Oberprieler et al. (2007) based on nrDNA ITS, placed this subtribe within subtribe Athanasiinae. In the nuclear analyses the subtribe Athanasiinae was recovered as a paraphyletic assemblage of seven southern African genera (*Adenoglossa* B.Nord., *Asaemia* Harv. ex Benth., *Athanasia* L., *Eriocephalus L.*, *Hymenolepis* Cass., *Lasiospermum* Lag. and *Leucoptera* B.Nord). As acknowledged by Oberprieler et al. (2007), the subtribe Athanasiinae is not supported by any morphological synapomorphies nor is it recovered as monophyletic in any of the molecular analyses performed thus far. It does, however, with the exception of *Eriocephalus*, share the presence of polarized endothecial tissue in the anthers with the subtribe Phymasperminae (Källersjö 1986, Bremer and Humphries 1993, Oberprieler et al. 2007).

Himmelreich et al. (2008) included 61 genera of the Anthemideae, 29 of which were from the southern hemisphere, 10 from Asia as well as 22 Eurasian/Mediterranean genera. The study was based on nrDNA ITS and cpDNA *ndhF* sequence data and aimed to reconstruct the evolutionary history of the basal groups within the Anthemideae, as well as to clarify the position of unplaced southern hemisphere genera. Himmelreich et al. (2008) found that even though the results mostly agreed with those from the aforementioned study by Oberprieler et al. (2007), there were some notable incongruences. The most important difference was the placement of Phymasperminae in the two phylogenies. According to the analysis based on nrDNA ITS the subtribe Phymasperminae was placed within a paraphyletic Athanasiinae, while in the analysis based on cpDNA *ndh*F sequences, the paraphyletic Athanasiinae formed part of a basal grade to the Eurasian clade while Phymasperminae was within the Pentziinae.

From these aforementioned phylogenetic studies, the molecular evidence was equivocal with respect to the phylogenetic relationship between subtribes Athanasiinae and Phymasperminae and the monophyly of Athanasiinae (no morphological characters appeared to support the concept of subtribe Athanasiinae). In addition, there was notable incongruence in the position of Phymasperminae in phylogenies derived from nuclear and chloroplast markers. Thus, Oberprieler et al. (2007) chose to recognize the two subtribes Athanasiinae and Phymasperminae as distinct until better resolution within the phylogeny could be obtained. If the sister relationship found in nrDNA ITS analyses is correct, then the anthers with polarized endothecial tissue (Källersjö 1986) could be considered as a synapomorphy for the broader clade including both subtribes (Oberprieler et al. 2007, 2009).

In the study by Himmelreich et al. (2008), the position of the southern African Anthemideae genus *Inulanthera* Källersjö was unresolved. *Inulanthera* was described by Källersjö (1986) as a genus having tailed anthers, with the cypselas lacking ellipsoid, secretory cavities and elongated cells in the fruit ribs. The closest relatives of *Inulanthera* were two genera from the Canary Islands, *Gonospermum* Less. and *Lugoa* DC., with which they shared glandular-punctate leaves with broad and rounded leaf lobes (Källersjö 1986). The study of Bremer and Humphries (1993) treated *Inulanthera* as a member of the polyphyletic subtribe Gonosperminae based on pappus of scales or teeth projected from the ribs. The analyses of Himmelreich et al. (2008) based on cpDNA *ndhF* sequences were placed *Inulanthera* in a well-supported clade sister to *Ursinia* Gaertn., while the analyses based on ITS sequence data the two genera *Inulanthera* and *Ursinia* were not consistently associated with each other. The same pattern was also evident in the analyses of Oberprieler et al. (2009), who refrained from including *Inulanthera* in Ursiniinae, underlining the isolated phylogenetic position of the genus. The phylogenetic analyses of Magoswana et al. (2016) based on the ITS region, showed that *Inulanthera* was sister to Athanasiinae. This was incongruent with the molecular reconstructions of Oberprieler et al. (2007, 2009) and Himmelreich et al. (2008), but *Inulanthera* was found to be a monophyletic assemblage (Magoswana et al. 2016). The incongruence mentioned between nuclear and plastid placement prevents the inclusion of *Inulanthera* in either in the subtribes Athanasiinae or Ursiniinae (Magoswana et al. 2016).

Phylogenetic resolution requires an adequate amount of data sampling and additional gene regions (Pick et al. 2010). Studies by Graybeal (1998), Denis et al. (2007), Pick et al. (2010) and Philippe et al. (2011) clarified the importance of increasing sampling and additional gene regions to improve resolution in phylogenies. Graybeal (1998), Denis et al. (2007), Pick et al. (2010) and Philippe et al. (2011) demonstrated that inadequate sampling could affect congruence between datasets and exacerbate the lack of resolution, due to the mutual cancellation of the phylogenetic signal (historical) and the non-phylogenetic signal (due to systematic errors).

The present study aims to expand the sampling of the subtribes Athanasiinae and Phymasperminae for two nuclear (ITS and ETS) and two plastid (*rpl32-trnL* and *3'rps16-5'trnK*) regions, with a representative sampling from other early-diverging subtribes within Anthemideae in order to elucidate phylogenetic relationships within the two subtribes, to

resolve the phylogenetic position of Phymasperminae as well as the apparent paraphyly of the subtribe Athanasiinae. In addition, the subtribal placement of *Inulanthera* was also explored.

1.2. The explicit objectives of this study were to:

1. Assess the subtribal circumscriptions and phylogenetic relationships between Athanasiinae and Phymasperminae and other subtribes through phylogenetic analyses of molecular sequence data and morphological studies.
2. Assess the generic relationships within the subtribe Athanasiinae and Phymasperminae.
3. Assess the generic status and relationships of *Adenoglossa*, *Asaemia* and *Leucoptera*.
4. Assess the phylogenetic placement of *Inulanthera* in tribe Anthemideae.

CHAPTER 2

MATERIALS AND METHODS

2.1. Taxon sampling

Species of Athanasiinae and Phymasperminae were observed in the field and material collected. Fresh leaf material was placed on silica gel (Sass 1958) to be used for molecular studies. All material collected was pressed and dried, and herbarium specimens housed at the Compton Herbarium (NBG) at Kirstenbosch. In selected cases, leaf material was sampled from herbarium specimens. Table 1 provides voucher information for the material used in the present study and the sequences generated and used in this study will be submitted to GenBank.

2.2. DNA extraction, amplification, and sequencing

Samples were disrupted and ground with a microtube pestle by adding liquid nitrogen to a 2 ml microcentrifuge tube with 0.2-0.3g of dried plant leaf material. DNA extractions were performed using the DNeasy Plant Minikit (Qiagen Inc.) following the manufacturer's instructions, except for increasing the incubation in RNAse and extraction buffer to 60 mins. Sources of plant material and voucher specimen information of material used during the study as well as those obtained from previous studies and Genbank are listed in Tables 1 and 2.

Amplification of the selected DNA regions was carried out using Polymerase Chain Reactions (PCR) in 25 µl reactions containing 12.5 µl PCR EmeraldAmp Master Mix [2X PCR Master Mix which consisted of DNA polymerase, optimized reaction buffer, dNTPd, density reagent, and green dye] (Takara, Ohtsu, Japan), 0.3 µl of both reverse and forward primers (Table 3), 0.5 µl Bovine Serum Albumin (BSA), 0.5 µl of dimethyl sulfoxide (DMSO), 0.5-1.0 µl of DNA template and sterile distilled water added to make up a total volume of 25 µl. However, to amplify problematic samples, dilutions of the DNA were done, ranging from 1:10 to 1:50. The Asteraceae are known to contain various compounds in their plant tissues, such as phenolics and polysaccharides that may inhibit PCR amplification (Baldwin et al. 1995; Wilson 1997). The presence of such inhibitors was problematic for some samples, particularly those from the genera *Athanasia* L., *Leucoptera* B.Nord., *Adenoglossa* B.Nord. and *Hymenolepis* Cass. The DNA of these species was either diluted with sterile water or cleaned using the Zymo

OneStep™ PCR Inhibitor Removal Kit (Clancy et al. 2001) following the manufacturer's instructions.

Two nuclear regions were amplified using the following primer combinations: ETS-18S, ASTIF (Baldwin and Markos, 2001); ITS-ITS5A and ITS4 (Funk et al. 2004). The plastid regions *rpl32-trnL* and *trnk-rps16* were amplified using the primers of Shaw et al. (2007). The primer sequences are listed in Table 3.

The PCR reactions were carried out using the following thermal conditions: initial denaturation at 94 °C for 2 min, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 53 °C for 1 min, extension at 72 °C for 1 min, with final extension at 72 °C for 8 min. For samples that did not amplify successfully, the protocol was adjusted to include a temperature ramp following Shaw et al. (2007). PCR products were visualized on 1% agarose gels containing Ethidium Bromide using an ENDURO$_{TM}$ GDS Gel Documentation system. The ExoSAP procedure was used for PCR clean-up following Werle et al. (1994). The procedure removes contaminating primers and unconsumed dNTPs, resulting in 100% recovery of both short and long PCR products. The procedure uses two hydrolytic enzymes, Exonuclease I (Exo) and Shrimp Alkaline Phosphatase (SAP). The first removes residual single-stranded primers and any extraneous single-stranded DNA produced in the PCR; the second removes the remaining dNTPs from the PCR mixture. For a single 25 ul PCR product 2.25 ul of distilled water (H_2O), 0.25 ul Exo and 0.5 ul SAP were mixed in one tube and 3 ul of the mixture was added directly to PCR tube and incubated at 37 ^{o}C for 30 minutes. The enzymes were removed through heat inactivation 80 ^{o}C for 15 minutes. The cleaned PCR products were sent to Macrogen Inc. (Seoul, Korea) for sequencing using the same primers as were used in the PCR reactions.

2.3. Sequence alignments and phylogenetic analysis

Electropherograms obtained from the sequenced gene regions were edited and aligned manually using MEGA version 6.06 (Tamura et al. 2013) and adjusted where required, positioning gaps to minimize nucleotides mismatches. Phylogenetic analyses on the combined nuclear, combined plastid and combined nuclear and plastid datasets were conducted using the maximum parsimony (MP) algorithm of PAUP* version 4.0b1 (Swofford 2002) with character transformations treated as unordered and equally weighted (1971), treating gaps as missing data. The matrices were analysed using a heuristic search with 1000 random sequence additions, TBR (tree bisection reconnection) branch swapping, with the MULTREES option

turned on. A limit of 10 trees per replicate was set to reduce the time spent on swapping in each replicate. Internal support was assessed with 1000 bootstrap replicates (Farris et al. 1996) using TBR swapping but holding 10 trees per replicate. Bootstrap percentage (BP) values greater than 50% are reported in the results using the following scale to evaluate support percentages: 50%–74%, weak; 75%–84%, moderate; and 85%–100%, strong.

Bayesian inference (BI) was conducted using MrBayes v. 3.2.3 (Ronquist and Huelsenbeck, 2003). The combined nuclear, combined plastid, and combined nuclear and plastid datasets were analyzed, respectively, for 5 000 000 generations with a sample frequency of 1000. The analyses were performed using the CIPRES Portal version 3.3 (Miller et al. 2011). The standard deviation of split frequencies stabilized below 0.01 for all analyses, providing evidence that a sufficient number of generations had been completed. Using Tracer v.1.5 (Rambaut and Drummond 2009), suboptimal trees were discarded as the "burn-in" phase. Only support values greater than 0.5 were retained, and the following scale was used to evaluate support values: 0.50–0.94 weak; and 0.95–1.0 strong. Appropriate models of nucleotide evolution were selected for each gene region based on the Akaike information criterion (AIC) of jModelTest (Posada 2008). The following models of evolution were selected: TIM3ef+G was applied in ITS, TIM2+G was applied in ETS, TPM2uf+G for *(rpl32-trnL)* and TrN+G was applied in *trnk-rps16*. Before combining the nuclear and plastid datasets, support values from the separate analyses were visually examined and compared for well-supported discrepancies (hard incongruence: Seelanan et al. 1997; Wiens 1998). Taxa showing hard incongruence in their placement (defined as those supported by BS values ≥ 80% and/or PP ≥ 0.95) were excluded from the combined analyses (Pelser et al. 2010).

2.4. Morphological character reconstruction

Generic and species relationships within Athanasiinae and Phymasperminae were explored using *Cotula thunbergii* Harv. and *Cotula barbata* DC. from the early diverging subtribe Cotuliinae to root the tree. The information of the characters and their states were sourced from literature and some characters were examined from floral dissections (receptacle in *Eumorphia* species). Vegetative and reproductive character states were polarized using the method of outgroup comparison and reconstructed onto the BI majority rule consensus trees, using the parsimony trace character history function in Mesquite version 3.04 (Maddison & Maddison 2015). Intermediate taxa (taxa with more than one-character state) were coded as polymorphic

(i.e. 0/1) following the Mesquite manual (Maddison & Maddison 2015). Coded morphological characters and character states are listed in Appendix A.

Table 1. Voucher information of material used for DNA extraction and sequencing in this study. The sequences used in this study will be submitted to GenBank.

Taxon	Voucher specimen
Athanasia rugulosa E.Mey. ex DC.	*Holmes s.n* (NBG)
Athanasia flexuosa Thunb.	*Mucina 090905/19* (NBG)
Athanasia linifolia Burm.	*Magee & Boatwright 378* (NBG)
Athanasia linifolia Burm.	*Pienaar T74B* (NBG)
Athanasia pubescens L.	*Mucina 191008/06* (NBG)
Athanasia spathulata (DC.) D.Dietr.	*Magee & Boatwright 398* (NBG)
Athanasia trifurcata L.	*Magee & Boatwright 412* (NBG) *Magee & Boatwright 395* (NBG) *Magee & Boatwright 396* (NBG) *Magee 464* (NBG)
Athanasia vestita (Thunb.) Druce	*Magee & Boatwright 398* (NBG)
Athanasia virgata Jacq.	*Magee 367* (NBG)
Athanasia calophyla Källersjö	*Akimana 10* (NBG)
Athanasia bremeri Källersjö	*Akimana 11* (NBG) *Akimana 4* (NBG)
Athanasia sertulifera DC.	*Helme 4013* (NBG)
Athanasia pectinata L.	*Helme 3875* (NBG)
Athanasia gyrosa Powell & Magee	*Jardine & Jardine 1054* (NBG)
Athanasia scabra Thunb.	*Helme 5165* (NBG)
Athanasia cuneifolia Lam.	*Helme 5343* (NBG)

Athanasia oocephala (DC.) Källersjö	*Helme 4537* (NBG)
Athanasia quiquedentata Thunb.	*Helme 7257* (NBG)
Athanasia argentea Powell&Magee	*Helme 1767* (NBG)
Athanasia pachycephala DC.	*Helme 4128* (NBG)
Hymenolepis cynopus Bremer & Källersjö	*Rourke 2017* (NBG)
Hymenolepis dentata (DC.) Källersjö	*Helme 4447* (NBG)
Hymenolepis gnidioides (S.Moore) Källersjö	*Magee & Boatwright 368* (NBG)
Hymenolepis incisa DC.	*Helme 7056* (BOL)
Hymenolepis parviflora Källersjö	*Mucina 021106/06* (NBG)
Hymenolepis speciosa (Hutch.) Källersjö	*Helme 6099* (NBG)
Hymenolepis calva Magoswana &Magee	*Magee & Boatwright 366* (NBG)
Hymenolepis crithmifolia (L.) Greuter et.al.	*Akimana 16* (NBG)
Leucoptera nodosa (Thunb.) B.Nord.	*Helme 4685* (NBG)
Leucoptera subcarnosa B.Nord.	*Magee & Boatwright 332* (NBG) *Magee & Boatwright 324* (NBG) *Boatwright 679* (NBG) *Akimana 3* (NBG)
Leucoptera oppositofolia B.Nord.	*Akimana 4* (NBG)
Adenoglossa decurrens B.Nord.	*Mucina 030906/24* (NBG) *Mucina 290806/30* (NBG)
Eriocephalus brevifolius (DC.) M.A.N.Müll.	*Magee & Boatwright 340* (NBG)
Eriocephalus ericoides subsp. *ericoides* Druce	*Magee & Boatwright 204* (NBG)
Eriocephalus eximius DC.	*Magee & Boatwright 386* (NBG)
Eriocephalus microphyllus DC.	*Magee & Boatwright 321* (NBG) *Magee & Boatwright 317* (NBG)

Eriocephalus namaquensis M.A.N.Müll.	*Magee &Boatwright 314* (NBG)
Eriocephalus paniculatus DC.	*Magee & Boatwright 336* (NBG)
Eriocephalus purpureus (G.Don.) Burch.	*Magee & Boatwright 318* (NBG)
Eriocephalus racemosus var. *affinis* L.	*Magee & Boatwright 363* (NBG)
Eriocephalus sp.	*Magee & Boatwright 364* (NBG)
Eriocephalus sp.	*Magee & Boatwright 365* (NBG)
Eriocephalus sp.	*Magee & Boatwright 384* (NBG)
Eriocephalus sp.	*Magee & Boatwright 385* (NBG)
Eriocephalus sp.	*Magee & Boatwright 387* (NBG)
Eriocephalus sp.	*Magee & Boatwright 388* (NBG)
Eriocephalus sp.	*Magee & Boatwright 409* (NBG)
Eriocephalus sp.	*Magee & Boatwright 410* (NBG)
Eriocephalus sp.	*Boatwright 719 (11/2/2015)* (NBG)
Lasiospermum bipinnatum (Thunb.) Druce	*Mucina 300805/05* (NBG) *Magee 429* (NBG) *Magee 432* (NBG)
Lasiospermum brachyglossum DC.	*Magee & Boatwright 328* (NBG)
Lasiospermum penduculare Lag.	*Magee & Boatwright 315* (NBG)
Lasiospermum poterioides Hutch.	*Magee & Boatwright 389* (NBG)
Lasiospermum poterioides Hutch.	*Magee 401* (NBG)
Asaemia minuta (Benth. & Hook.) Harv.	*Boatwright 691* (NBG)
Phymaspermum athanasioides (S.Moore) Källersjö	*Magee 394* (NBG) *Magee et al. 303* (NBG) *Loffler 1182* (NBG)

Phymaspermum acerosum (DC.) Källersjö	*Magee & Swelankomo 306* (NBG) *Magee & Swelankomo 309* (NBG) *Magee 460* (NBG)
Phymaspermum appresum Bolus	*Magee & Boatwright 369* (NBG)
Phyamaspermum leptophyllum (DC.) Benth. & Hook.f.	*Magee & Boatwright 372* (NBG)
Phyamaspermum parvifolium (DC.) Benth. & Hook.f.	*Magee & Boatwright 203* (NBG)
Phyamspermum pinnatifidum (Oliv.) Källersjö	*Young 1441* (NBG)
Phymaspermum thymelaeoides (DC.) Magee & Ruiters	*Magee & Boatwright 381* (NBG)
Phymaspermum erubescens (Hutch) Källersjö	*Magee 436* (NBG)
Phymaspermum woodii (Thell.) Källersjö	*Magee 447* (NBG) *Magee 457* (NBG)
Phymaspermum argenteum Brusse	*Buitow & Manning 9004* (NBG)
Phymaspermum aciculare (DC.) Benth. & Hook.f.	*Germishuizen 8586* (PRE)
Eumorphia corymbosa E. Phillips	*Shearing K391* (NBG)
Eumorphia davyii Bolus	*Mucina 200706/21* (NBG) *Magee & Swelankomo 307* (NBG)
Eumorphia prostrata Bolus	*Magee & Boatwright 312* (NBG)
Eumorphia swaziensis Compton	*Magee et al. 300* (NBG)
Eumorphia sericea subsp. sericea J.M.Wood & M.S.Evans	*Mucina 190207/08* (NBG) *Mucina 200207/23* (NBG) *Magee 1086* (NBG)
Gymnopentzia bifurcata Benth.	*Magee 445* (NBG) *Magee 1085* (NBG) *Magee 462* (NBG) *Breytenbach* (NBG)

Inulanthera tridens (Oliv.) Källersjö	*Cloete 989* (NH)
Inulanthera leucoclada (DC.) Källersjö	*Magoswana 15* (NBG)
Inulanthera dregeana (DC.) Källersjö	*Ebrahim 86* (NBG) *Magee 1077* (NBG)
Inulanthera thodei (Bolus) Källersjö	*Magee 1087* (NBG)
Ursinia nana DC.	*Magee & Boatwright 377* (NBG)
Ursinia anthemoides (L.) Poiret	*Magee & Boatwright 376* (NBG)
Cotula thunbergii Harv.	*Jakoet, Magee, Boatwright & Poovan 19* (NBG)
Cotula barbata DC.	*Jakoet, Magee, Boatwright & Poovan 28* (NBG)
Cymbopappus piliferus (Theil.) B.Nord.	*Magee & Boatwright 175* (NBG)
Pentzia incanna (Thunb.) Kuntze	*Magee & Boatwright 182* (NBG)
Marasmodes schlechteri Magee & J.C.Manning	*Magee et al. 145* (NBG)
Faveolina tenella (DC.) Källersjö	*Magee et al. 163* (NBG) *Manning 3439* (NBG)
Faveolina burchellii (Theil.) Källersjö	*Magee & Boatwright 390* (NBG)
Felicia brevifolia (DC.) Grau	*Manning 3436* (NBG)
Osmitopsis asteriscoides Cass.	*Mucina 141207/15* (NBG)

Table 2. Voucher specimens of material obtained from Genbank.

Taxon	**Accession no**			
	ITS	ETS	*trnK-rps16*	*rpl32-trnL*
Artemisia santolina Turcz. ex Besser	AM774460	JN456789	AB25678	EU567490
Chrysanthemum indicum L.	AH011699	JN315964	AB234858	EU747088

Table 3. Primers sequences and references for the regions used in this study.

Regions	Primer sequence (5'-3')	Reference
cpDNA *trnK-rps16*	trnK $^{(UUU)}$X1: TTA AAA GCC GAG TAC TCT ACC rpS16 x2F2: AAA GTG GGT TTT TAT GAT CC	Shaw et al. (2007)
cpDNA *rpl32-trnL*	rpl32: CAG TTC CAA AAA AACGTA CTT C trnL$^{(UAG)}$: CTG CTT CCT AAG AGC AGC GT	Shaw et al. (2007)
nrDNA 18S-ETS ASTIF	ACT TAC ACA TGC ATG GCT TAA TCT CGT AAG GTG CAT GAG TGG TGT	Baldwin and Markos (1998) Baldwin and Markos (2001)
NrDNA ITS5A ITS4	GGA AGG AGA AGT CGT AAC AAG G TCC TCC GCT TAT TGA TAT GC	Funk et al. (2004)

CHAPTER 3

GENERIC RELATIONSHIPS WITHIN THE SUBTRIBE PHYMASPERMINAE

3.1. Introduction

The subtribe Phymasperminae was established by Oberprieler et al. (2007) to include three southern African endemic genera, *Eumorphia* DC. (6 spp., 2 subsp.); *Gymnopentzia* Benth. (1 spp.); *Phymaspermum* Less. (17 spp.). The Phymasperminae is one of the six earliest diverging subtribes within the tribe Anthemideae (Oberprieler et al. 2007). The genera *Phymaspermum, Eumorphia, Gymnopentzia* are distinguished from the other southern African genera by having papillose cypselas with more than 10 primary ribs each containing vascular bundles (Källersjö 1986). Subsequently, these characters were used by Oberprieler et al. (2007) to define subtribe Phymasperminae.

The nominate genus *Phymaspermum* is regarded as the largest and most complex within the subtribe Phymasperminae with 17 species all endemic to southern Africa (Ruiters 2014, Ruiters et al. 2016). The genus is distinguished from *Eumorphia* and *Gymnopentzia* by the presence of stalked myxogenic trichomes on the cypselas (Källersjö 1986, Bremer and Humphries 1993, Ruiters 2014, Ruiters et al. 2016) which plays a significant role in decreasing the rate of water loss taken up by the seeds, facilitating the germination during the cool season and for seedling survival in the desert environment (Jannathan et al. 2014). Within *Phymaspermum* the myxogenic trichomes are absent in two species, *P. acerosum* DC. and *P. pinnatifidum* Oliv. Källersjö (1986) remarked that these two species should perhaps be recognised as a separate genus. Ruiters (2014) showed that *P. acerosum* and *P. pinnatifidum* should be maintained within *Phymaspermum* and recovered the stalked myxogenic trichomes as a synapomorphy for genus *Phymaspermum* although these were subsequently lost and replaced by resin canals in *P. acerosum* and *P. pinnatifidum* (Ruiters 2014).

Eumorphia consists of six species and two subspecies, which are also endemic to southern Africa (Källersjö 1986; Bremer and Humphries 1993; Swelankomo 2011). The genus is currently recognized by the opposite leaves (although alternate in *E. davyi* Bolus and *E. swaziensis* Compton) and radiate heads with paleate receptacles (although paleae restricted only to marginal florets in *E. prostrata* Bolus). As such, there is currently no unique character

to support the circumscription of the genus with the above-mentioned characters also shared with either *Gymnopentzia* or *Phymaspermum*, viz. the opposite leaves are present in *Gymnopentzia* (Bremer and Humphries 1993, Källersjö 1986), radiate heads found in several species of *Phymaspermum* (Ruiters 2014 and Ruiters et al. 2016) and the presence of at least marginal paleae in both *Gymnopenztia* and *Phymaspermum* (Kadereit and Jeffrey 2007, Ruiters 2014).

Gymnopentzia is a monotypic genus restricted to southern Africa. The genus is unique in that it has distinctive bifurcate leaves and the cypselas with long papillose hairs (Källersjö 1986, Bremer and Humphries 1993, Ruiters 2014, Ruiters et al. 2016).

Bremer and Humphries (1993) made use of morphological, cytological and phytochemical characters to define inter-generic relationships within the subtribes of Anthemideae. Bremer and Humphries (1993) recognized the sister relationship between the genera *Eumorphia* and *Gymnopentzia* based on the shared opposite leaves and noted that in alternative equally parsimony cladograms, *Gymnopentzia* and *Phymaspermum* were found to be sister genera since both shared a loss of receptacle paleae.

Recent molecular phylogenetic analyses of Watson et al. (2000), Oberprieler et al. (2007) and Himmelreich et al. (2008) recovered the monophyly of subtribe Phymasperminae, but the interrelationships of the genera *Eumorphia*, *Gymnopentzia* and *Phymaspermum* have not been thoroughly investigated and the generic circumscriptions within Phymasperminae was still uncertain. Only a single accession per genus of Phymasperminae was included in the studies of Watson et al. (2000), Oberprieler et al. (2007) and Himmelreich et al. (2008). Even the more densely sampled ITS phylogeny of Ruiters (2014) showed a sister relationship between *Gymnopentzia* and *Eumorphia prostrata* Bolus, with the putatively closely related *Eumorphia sericea* J.M.Wood & M.S.Evans placed outside of this clade with *Eumorphia corymbosa* E.Phillips which is often confused with *Phymaspermum* species (Shearing 1994). The *Gymnopentzia* and *Eumorphia prostrata* clade was sister to *P. athanasioides* Källersjö, and successively sister to a strongly supported clade comprising *P. leptophyllum* DC. and *P. parvifolium* DC. The phylogenetic results of Ruiters (2014) showed that the generic relationship within Phymasperminae were unresolved. As such none of the genera (*Eumorphia*; *Gymnopenztia* and *Phymaspermum*) were found to be monophyletic. In the morphological phylogeny of Ruiters (2014), the results showed the three genera to be monophyletic, although

without bootstrap support. Despite the shared presence of opposite leaves *Eumorphia* and *Gymnopentzia* were not recovered as sister genera. Rather *Gymnopenztia* was recovered together with *Phymaspermum* based on the synapomorphic loss of receptacular paleae.

The limited resolution within Phymasperminae found in previous studies (Watson et al. 2000, Oberprieler et al. 2007, Himmelreich et al. 2008, Ruiters 2014) warrants a more extensive sampling of species of Phymasperminae and sampling of additional gene regions to the existing matrices. The present study therefore aims to:

- Assess the generic circumscriptions of subtribe Phymasperminae through comparisons of morphology and DNA sequence data.
- Explore the relationships of the genera within subtribe Phymasperminae.
- Test the monophyly of the genera within subtribe Phymasperminae.

3.2 Materials and methods

Details of the methods and materials used can be found in Chapter 2.

3.3. Results

3.3.1. Phylogenetic analyses

3.3.1.1. Nuclear datasets

The combined nuclear (ETS and ITS) DNA matrix consisted of 35 taxa, 1128 characters of which 575 were constant, 202 variable and 351 parsimony-informative characters. Maximum parsimony (MP) analysis retrieved 6670 trees with a tree length of 834 steps, a consistency index (CI) of 0.82 and retention index (RI) of 0.83. The topology of the MP strict consensus tree was similar to that of the Bayesian Inference (BI) majority rule consensus tree and the support values are summarized on the BI tree (Fig. 3.1).

Pentziinae was strongly supported as a monophyletic clade (PP 1.0, BP 100) and sister to the Athanasiinae and Phymasperminae clades (PP 1.0, BP 78). Both Athanasiinae (PP 0.7) and Phymasperminae (PP 1.0, BP 100) were recovered as monophyletic, and sister to each other (PP 1.0, BP 88).

Within the Phymasperminae (*Phymaspermum*-*Gymnopentzia*) three weekly to well-supported clades were recovered. The *Phymaspermum* clade was supported as monophyletic (PP 0.8) and sister to *Eumorphia* (PP 0.6). This contrasts with the analyses of Ruiters (2014) where *Phymaspermum* was not monophyletic and the sister relationship between *Phymaspermum* and *Eumorphia* was uncertain. Within the *Phymaspermum* clade three weakly to strongly supported subclades were found. In the first subclade the discoid species *P. woodii* (PP 0.5, BP 56) and *P. argenteum* (PP 1.0, BP 55) were recovered as sister. In the second subclade *P. athanasioides* (PP 1.0, BP 86) was recovered as sister to the *P. appressum* group (PP 0.7). The latter group (PP 0.7) comprised of *P. acerosum* (PP 1.0, BP 50), *P. appresum* and *P. pinnatifidum*. In Ruiters (2014), *P. athanasioides* was rather recovered in a clade with *Eumorphia* and *Gymnopentzia*. In the third subclade the radiate species *P. leptophyllum* and *P. parvifolium* were supported as sister (PP 1.0, BP 50) and this relationship between *P. leptophyllum* and *P.*

parvifolium was also recovered in Ruiters (2014). The positions of *P. erubescens* and *P. thymelaeoides* were unresolved.

The *Eumorphia* clade was recovered as monophyletic (PP 0.9), whilst in Ruiters (2014) *Eumorphia* was not monophyletic. Within the *Eumorphia* clade one sample of *E. sericea* was recovered as sister to the other species (PP 0.9), the other two (PP 0.9) formed a clade that was unresolved in its relationship with other species (PP 0.9, BP. 84) and *E.corymbosa* was recovered as sister to all of these (PP 0.9), while in Ruiters (2014) *E. corymbosa* was recovered with *E. sericea* outside of the *Gymnopentzia-Eumorphia* clade.

The *Gymnopentzia* clade was recovered as a monophyletic group (PP 0.98, BP 81) and formed a strongly supported monophyletic clade with *Eumorphia* and *Phymaspermum* (PP 1.0, BP 100), whilst in Ruiters (2014) *Gymnopentzia* was not monophyletic and a strongly supported clade with *Eumorphia* and *Phymaspermum* was not recovered.

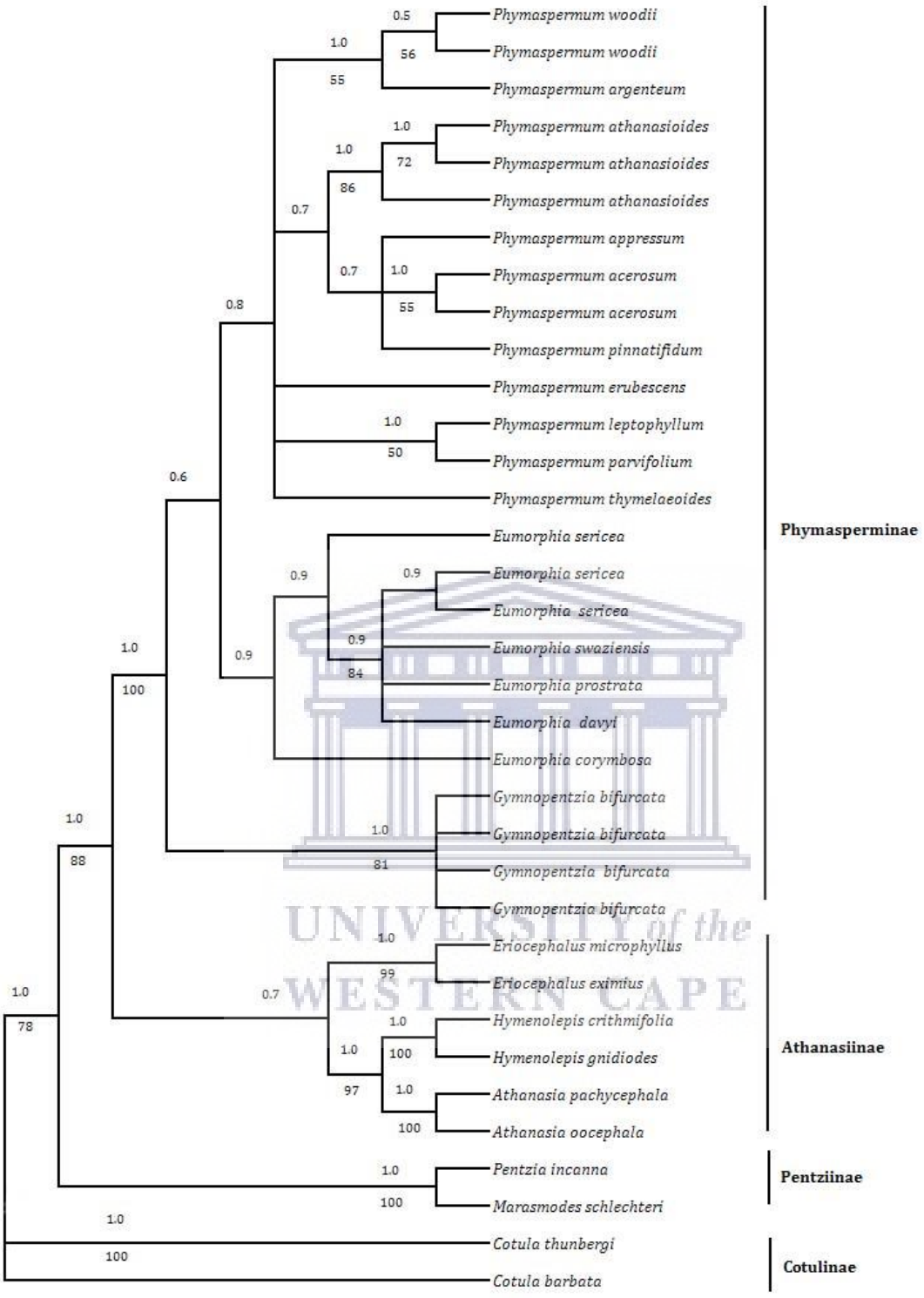


Figure 3.1. Bayesian inference (BI) majority rule consensus tree of the combined nuclear (ETS and ITS) sequence dataset. Posterior probability (PP) values are presented above the branches and bootstrap percentages (BP) below the branches. BP and PP values <50% and 0.50, respectively, are not indicated.

3.3.1.2. Plastid datasets

The combined plastid (*rps16* and *rpl32*) DNA matrix consisted of 35 taxa, 2155 characters of which 1631 were constant, 163 variable and 357 parsimony-informative characters. Maximum parsimony (MP) analysis retrieved 7080 trees with a tree length of 619 steps, a consistency index (CI) of 0.91 and retention index (RI) of 0.94. The topology from the MP strict consensus tree was similar to that of the Bayesian Inference (BI) majority rule consensus tree and the support values are summarized on the BI tree (Fig. 3.2).

Pentziinae was strongly supported as a monophyletic clade (PP 0.9, BP 100) and sister to the Athanasiinae and Phymasperminae clades (PP 1.0, BP 100). The Athanasiinae was unresolved, with *Athanasia* and *Hymenolepis* strongly supported as sister (PP 1.0, BP 100). The species of *Eriocephalus* included in the plastid analysis are not both the same as in the nuclear analysis. The Phymasperminae was strongly supported as a monophyletic clade (PP 1.0, BP 100) and strongly supported in a clade with Athanasiinae (PP 1.0, BP 100). Within the Phymasperminae clade (*Phymaspermum-Gymnopentzia*) three supported clades were recovered. The *Phymaspermum* clade was supported as a monophyletic (PP 1.0) and this relationship was also recovered in nuclear analyses.

Within *Phymaspermum* three weekly to strongly supported subclades were found. In the first subclade all accessions of *P. athanasioides* were recovered as monophyletic (PP 1.0, BP 97). In the second subclade the discoid species *P. erubescens* and *P. pinnatifidum* were recovered as sister (PP 0.8, BP 58), while in the nuclear analyses *P. erubescens* was unresolved and *P. pinnatifidum* was recovered with *P. acerosum* and *P. appresum*. In the third subclade all accessions of *P. acerosum* were recovered as monophyletic (PP 1.0, BP 77). The positions of *P. woodii*, *P. appresum*, *P. leptophyllum*, *P. thymelaeoides*, *P. parvifolium* and *P. argenteum* were unresolved, while in the nuclear analyses, *P. woodii* was recovered with *P. argenteum* and *P. leptophyllum* was sister to *P. parvifolium*.

The resolution within *Eumorphia* was weak. In the nuclear analyses *Eumorphia* was recovered as monophyletic and the sister relationship between *Eumorphia* and *Phymaspermum* recovered in nuclear analyses was unresolved in the plastid analyses.

The *Gymnopentzia* clade was recovered as a monophyletic group (PP 0.7) and formed a monophyletic group with *Eumorphia* and *Phymaspermum* (PP 1.0, BP 100). This relationship was recovered in the nuclear analyses.

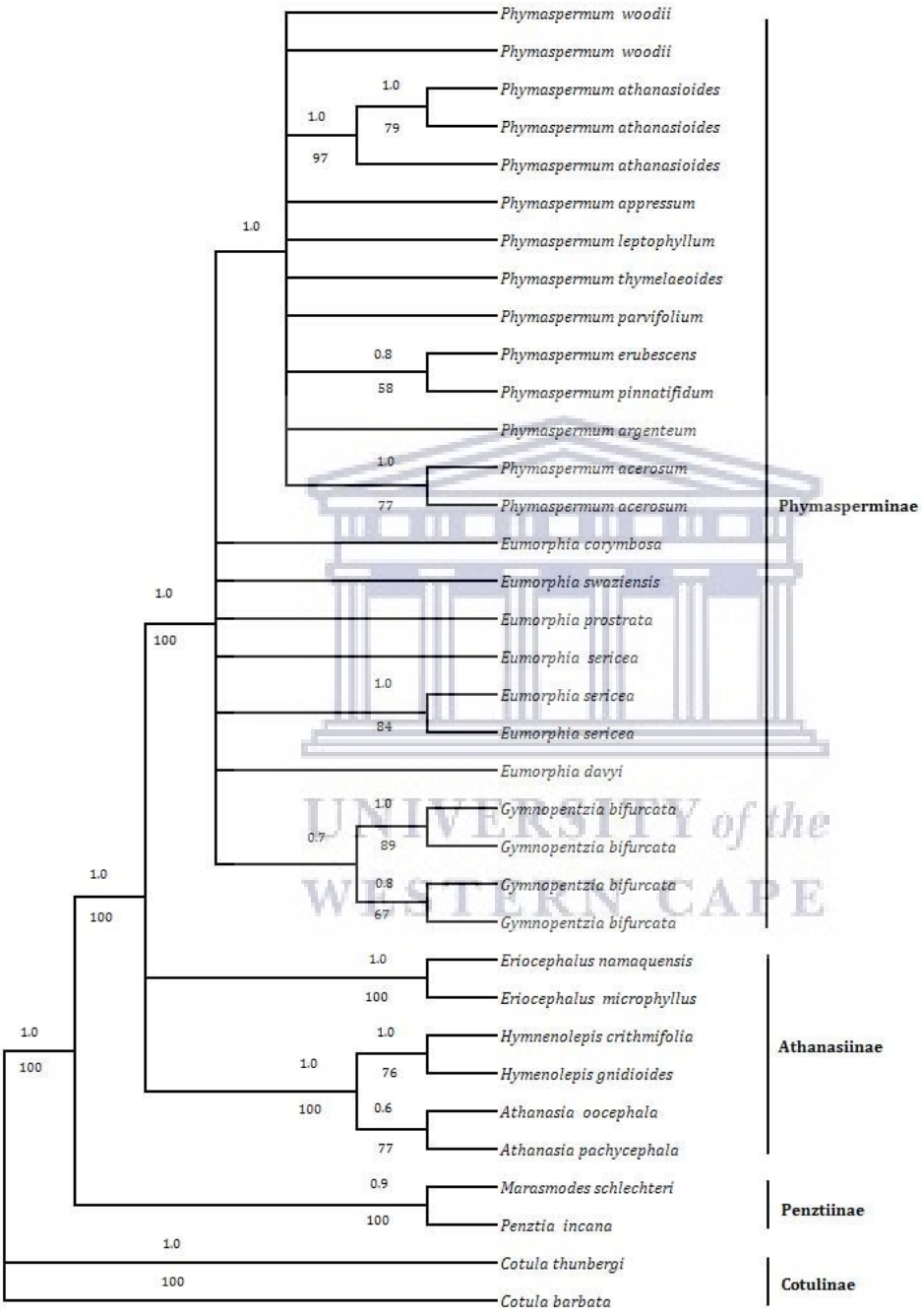


Figure 3.2. Bayesian inference (BI) majority rule consensus tree of the combined plastid (*rps16* and *rpl32*) sequence dataset. Posterior probability (PP) values are presented above the branches and Bootstrap percentage (BP) values below the branches. BP and PP values <50% and 0.50, respectively are not indicated.

3.3.1.3. Combined Nuclear/plastid datasets

No strongly supported incongruent patterns were found between the nuclear and plastid datasets and the two datasets were combined directly. The combined nuclear and plastid datasets included 35 taxa consisting of a total of 3279 characters of which 365 were variable, 708 parsimony-informative retrieved 3771 trees with a tree length of 1463 steps, a consistency index (CI) of 0.85 and retention index (RI) of 0.89. The topology from the MP strict consensus tree was similar to that of the Bayesian Inference (BI) majority rule consensus tree and the support values are summarized on the BI tree (Fig. 3.3).

Pentziinae was strongly supported as a monophyletic clade (PP 1.0, BP 100) and sister to the Athanasiinae and Phymasperminae clades (PP 1.0, BP 100). The Athanasiinae was recovered as a monophyletic clade (PP 0.8, BP 100) and sister to Phymasperminae (PP 1.0, BP 86). This relationship was recovered in separate nuclear analyses and unresolved in the plastid analyses.

The Phymasperminae was strongly supported as monophyletic (PP 1.0, BP 100) and sister to Athanasiinae (PP 1.0, BP 86). Within the Phymasperminae clade (*Phymaspermum-Gymnopentzia*) three weakly to strongly supported clades were recovered. The *Phymaspermum* clade was supported as monophyletic (PP 1.0) and this relationship was also recovered in the separate nuclear and plastid analyses. *Phymaspermum* was recovered as sister to *Eumorphia* (PP 0.9), similar to the nuclear analyses, but unresolved in the plastid analyses.

Within *Phymaspermum* clade three weakly to strongly supported subclades were found. In the first subclade *P. thymelaeoides* and *P. pinnatifidum* (PP 0.9, BP 100) were recovered as sister, with *P. woodii* (PP 0.5) as sister to them (PP 0.5), while *P. parvifolium* (PP 0.5, BP 53) successively sister to this clade of species. The position of *P. thymelaeoides* was unresolved in the separate nuclear and plastid analyses. In the second subclade *P. acerosum* and *P. appresum* (PP 1.0) were recovered as sister, with *P. athanasioides* (PP 1.0) and *P. leptophyllum* (PP 0.8) successively sister. The *P. leptophyllum* clade was supported as sister to the *P. parvifolium* clade (PP 0.6). The discoid species *P. argenteum* and *P. athanasioides* (PP 1.0, BP 65) were supported as sister. The positions of *P. erubescens* and *P. acerosum* were unresolved, while in plastid analyses *P. erubescens* was recovered as sister to *P. pinnatifidum* and was unresolved in the nuclear analyses. *P. acerosum* was recovered with *P. appresum* and *P. pinnatifidum* in the nuclear analyses and *P. acerosum* was recovered as monophyletic in plastid analyses.

The *Eumorphia* clade was recovered as monophyletic (PP 0.9). This relationship was recovered in the separate nuclear analyses, whilst in plastid analyses *Eumorphia* was not unresolved. Within *Eumorphia*, *E. sericea* was recovered as sister to *E. prostrata* (PP 1.0, BP. 84) and they were recovered in a clade with *E. sericea*, *E. swanziesis* and *E. davyi* (PP1.0). The positions of *E. corymbosa* and an accession of *E. sericea* were unresolved.

The *Gymnopentzia* clade was strongly supported as a monophyletic (PP 1.0, BP100) and formed a strongly supported clade with *Eumorphia* and *Phymaspermum* (PP 1.0, BP 100). This relationship was recovered in the separate nuclear and plastid analyses.

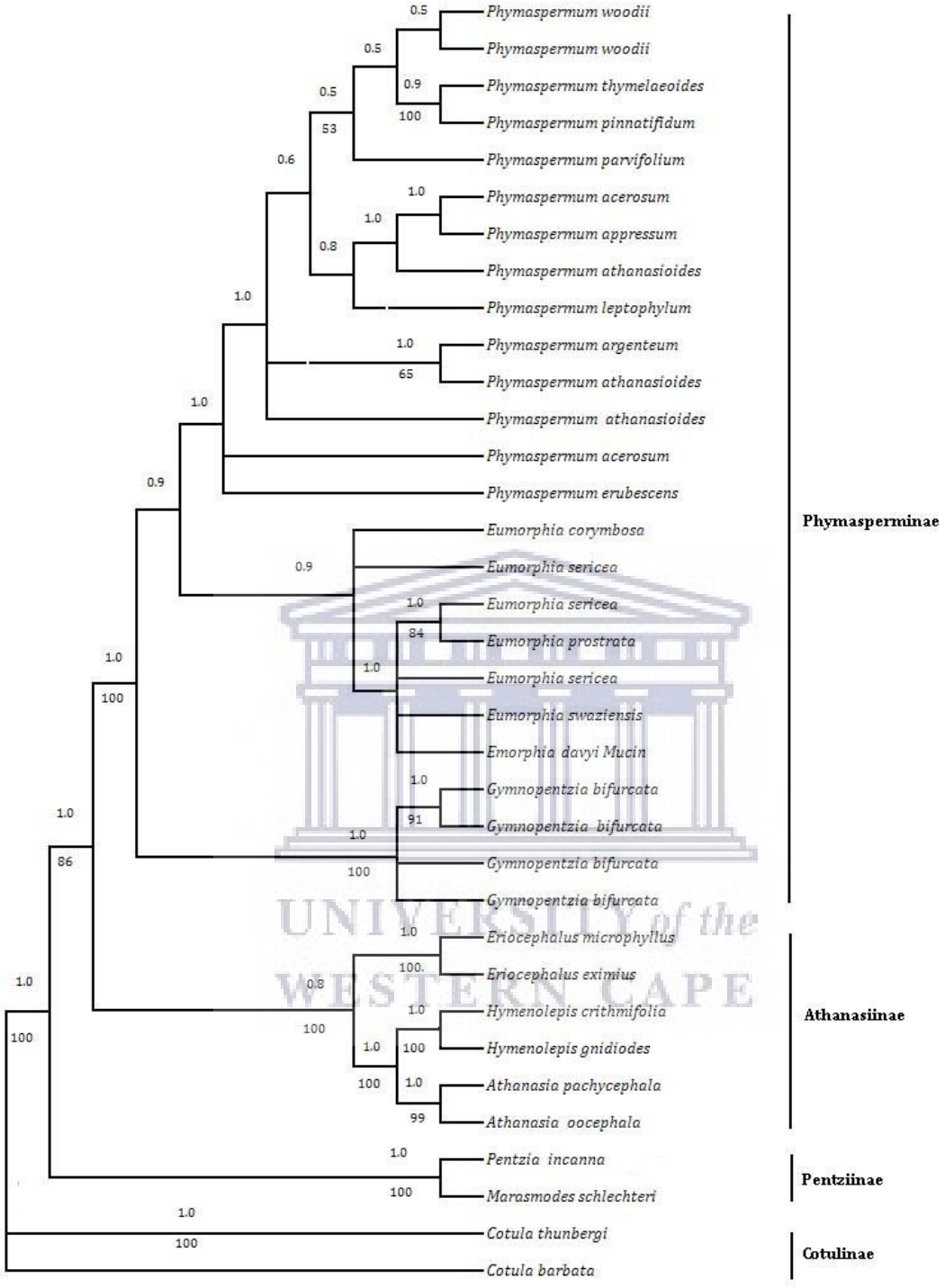


Figure 3.3. Majority rule consensus tree from the Bayesian inference (BI) of the combined nuclear (ETS and ITS) and plastid (*rps16* and *rpl32*) dataset. Posterior probability (PP) values are presented above the branches and bootstrap percentage (BP) values below the branches. BP and PP values <50% and 0.50, respectively, are not indicated

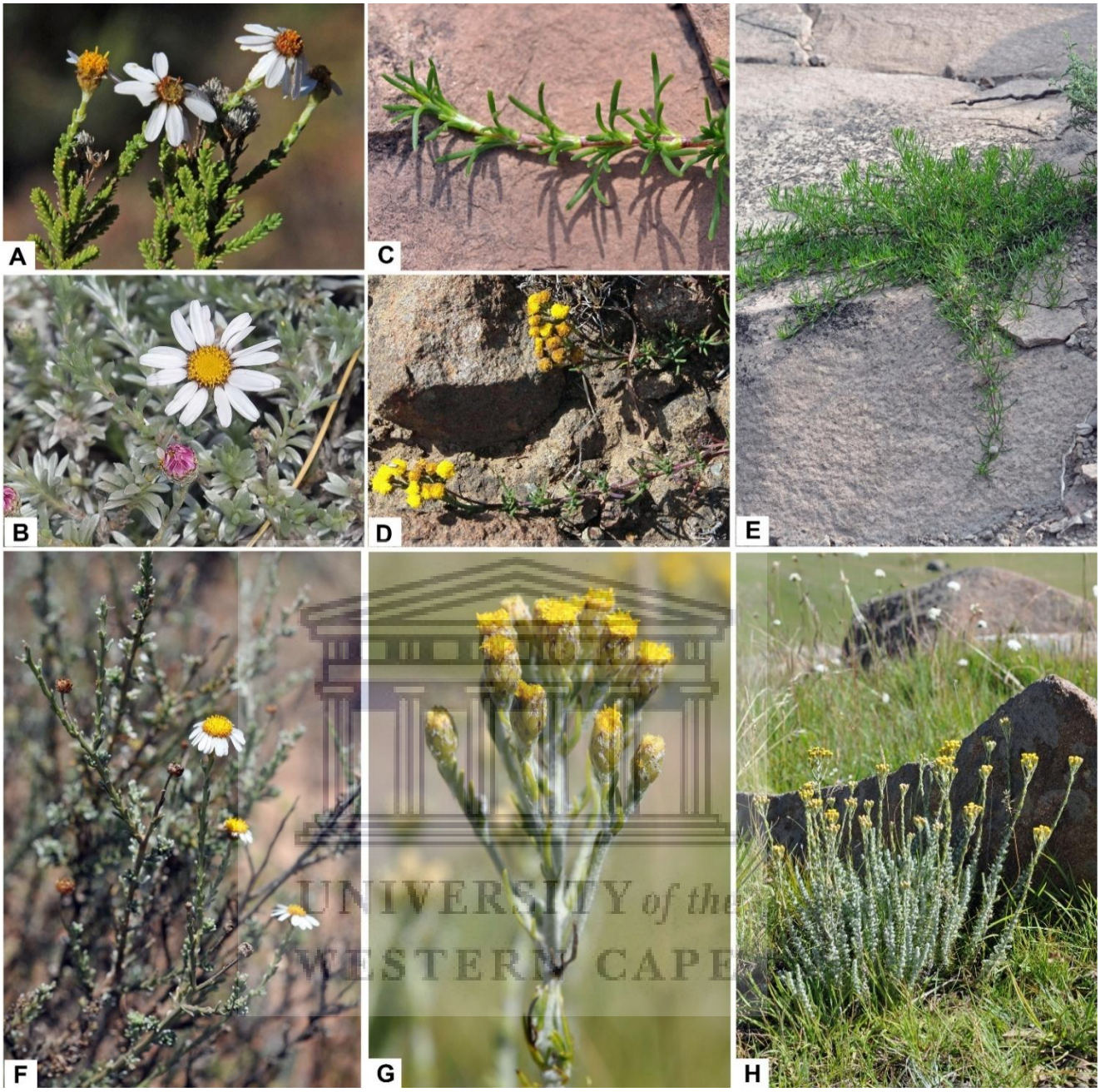


Figure 3.4. General morphology of *Eumorphia, Gymnopentzia* and *Phymaspermum.* A-B subshrub habit with the opposite leaves, solitary and radiate capitula of *E. dregeana* and *E. prostrata*. C-E. Single stemmed habit with the discoid capitula, leaves bifurcate and opposite in *G. bifurcata*. F. The shrubby habit, silvery sericeous, alternate leaves and the radiate capitula of *P. trifidum.*G-H. Multistimmed habit, corymbose synflorecence, the discoid capitula and the alternate leaves of *P. woodii*. Photographs: A by T. Rebelo; B by B. Ncole; C & E by A.R.Magee; D by T. Rebelo; F-H by A.R. Magee.

3.3.2. Morphological character reconstruction

Two main growth forms (life history), i.e. single-stemmed and multi-stemmed, are found in the genera *Eumorphia*, *Gymnopentzia* and *Phymaspermum* and this character is most variable in *Phymaspermum* (Ruiters 2014, Ruiters et al. 2016). A single-stemmed growth form was mostly recovered in *Eumorphia*, *Gymnopenztia* and some species of *Phymaspermum*, while a multi-stemmed growth form was found in *Phymaspermum* and *Eumorphia swaziensis* (Fig. 3.5A, Fig. 3.4).

An alternate leaf arrangement is the plesiomorphic state within the tribe Anthemideae, with opposite leaves a derived character state (Bremer and Humphries 1993, Ruiters 2014). Similarly, in the present reconstructions, alternate leaves were recovered as the plesiomorphic character with the opposite leaves found in most *Eumorphia* species and all *Gymnopentzia* species (Fig. 3.5B, Fig. 3.4). Although not included in the current sampling, opposite leaves are also present in *Phymaspermum oppositifolium* Magee and Ruiters (Ruiters et al. 2016).
The presence of completely paleate receptacles were recovered in most species of *Eumorphia*, with marginal paleae in *Phymaspermum*, *Gymnopenztia* and *Eumorphia prostrata* (Fig. 3.6A). Although the fully paleate receptacles were occasionally present in *Phymaspermum* and *Gymnopenztia* (Fig. 3.6B).

The capitula of Anthemideae can be radiate or discoid and this character is variable in the genera of Anthemideae (Bremer and Humphries 1993). In the present reconstruction, discoid heads were recovered as the plesiomorphic state in the tribe, with radiate heads a derived character in *Eumorphia* and several species of *Phymaspermum* (Fig. 3.7A, Fig. 3.4).

The cypselas of Anthemideae generally have a smooth or glabrous pericarp and some members have eglandular hairs on the pericarp (Bremer and Humphries 1993, Oberprieler et al. 2009). A papillose pericarp was recovered as a synapomorphic character for *Phymaspermum*, *Eumorphia* and *Gymnopentzia* (Ruiters 2014, Ruiters et al. 2016). Short papillose cypselas were recovered in *Phymaspermum* and *Eumorphia*, while long papillae are present in *Gymnopentzia* (Fig. 3.7B).

Few ribbed (four or five evenly arranged ribs) cypselas are common in most genera of the tribe Anthemideae and may represent the plesiomorphic state (Bremer and Humphries 1993,

Oberprieler et al. 2009). In the present reconstruction, multi-ribbed (10 or more ribs) cypselas were recovered as a synapomorphic character for *Phymaspermum*, *Eumorphia* and *Gymnopentzia*, i.e. the subtribe Phymasperminae (Fig. 3.8A).

Glandular trichomes on the cypselas are absent in most genera of the Anthemideae (plesiomorphic state), while the presence of glandular trichomes was reconstructed as a synapomorphy for *Phymaspermum*, although there are reversals in some species (Fig. 3.8B).

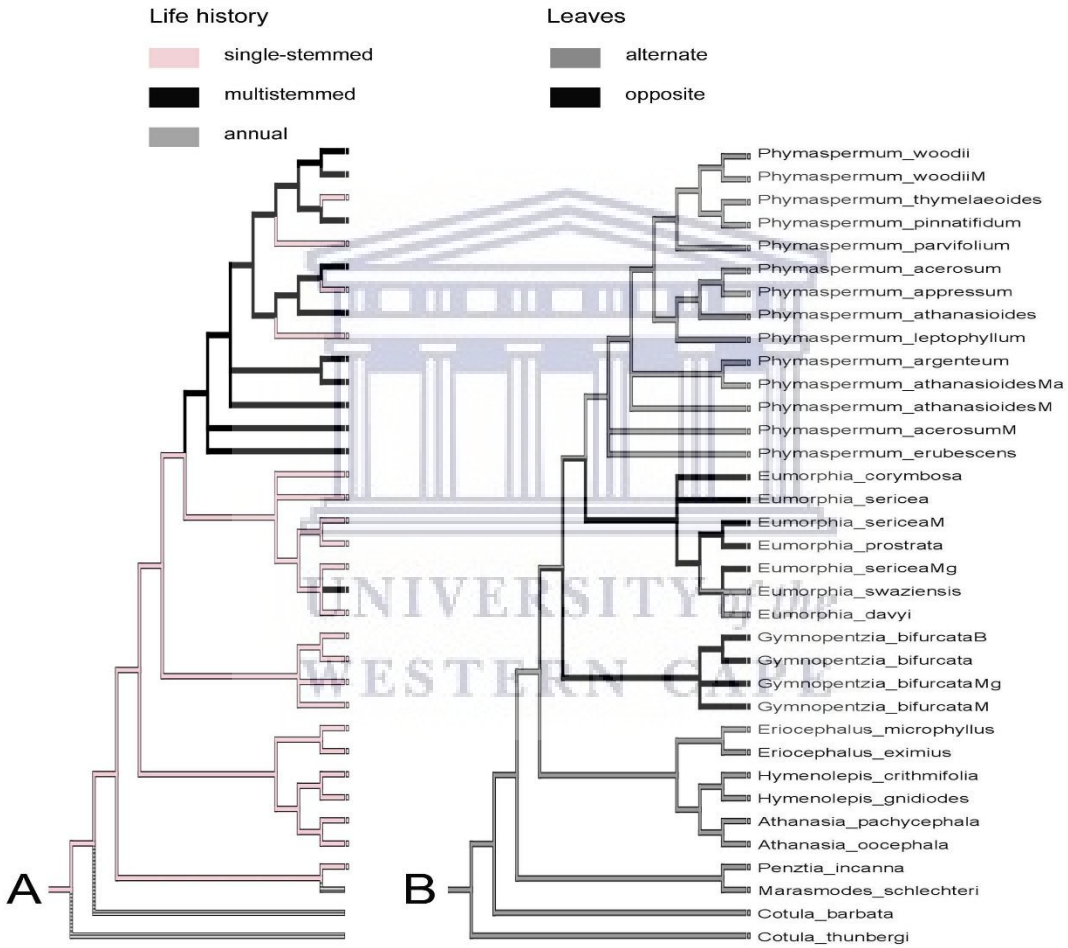


Figure 3.5. Parsimony-based character reconstruction of life history (A) and leaf arrangement (B) on the majority rule consensus tree from the Bayesian Inference (BI) analysis of the combined nuclear and plastid dataset.

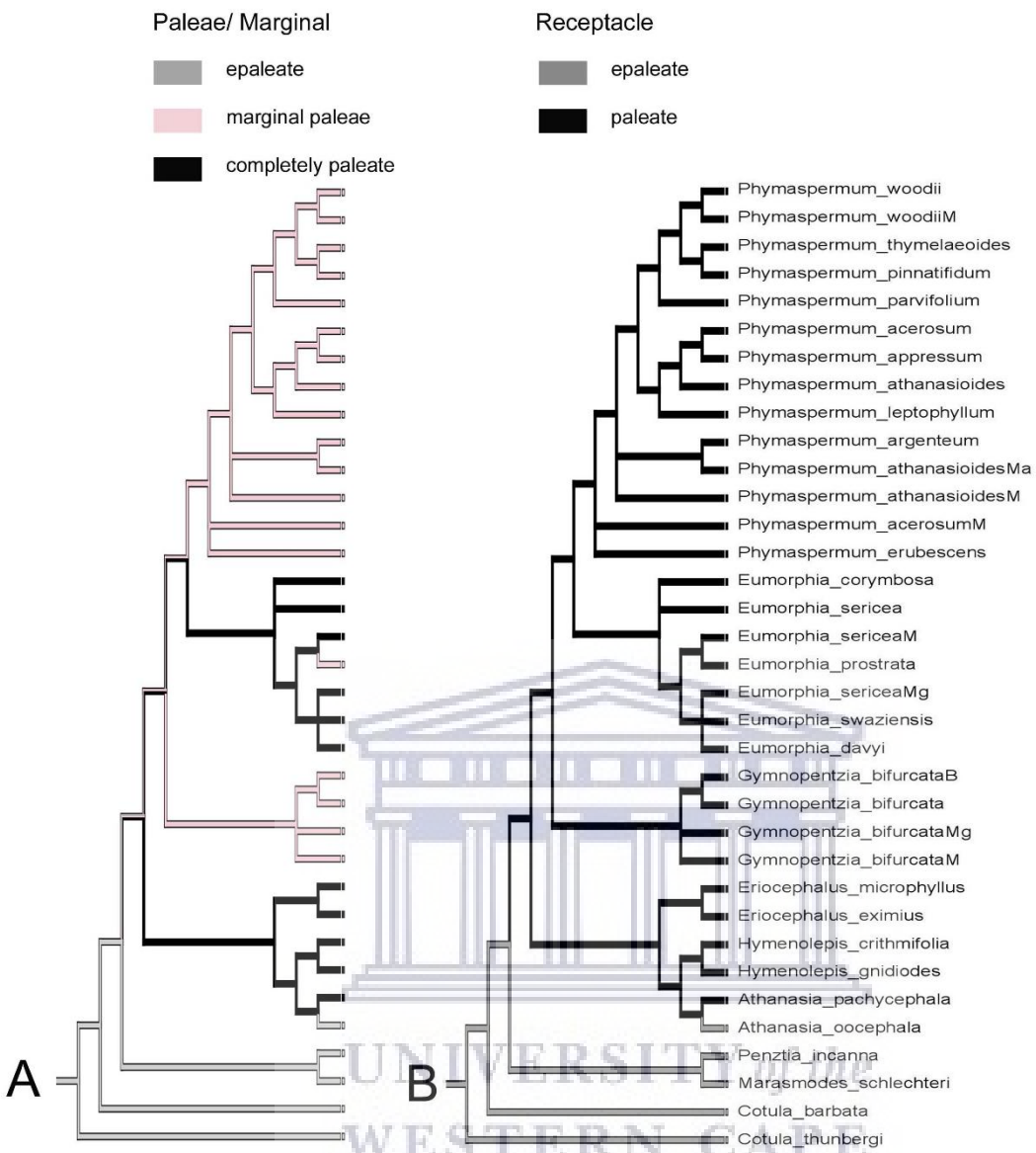


Figure 3.6. Parsimony-based character reconstruction of the receptacle being completely paleate, with marginal paleate (A) or totally epaleate (B) on the majority rule consensus tree from the Bayesian Inference (BI) analysis of the combined nuclear and plastid dataset.

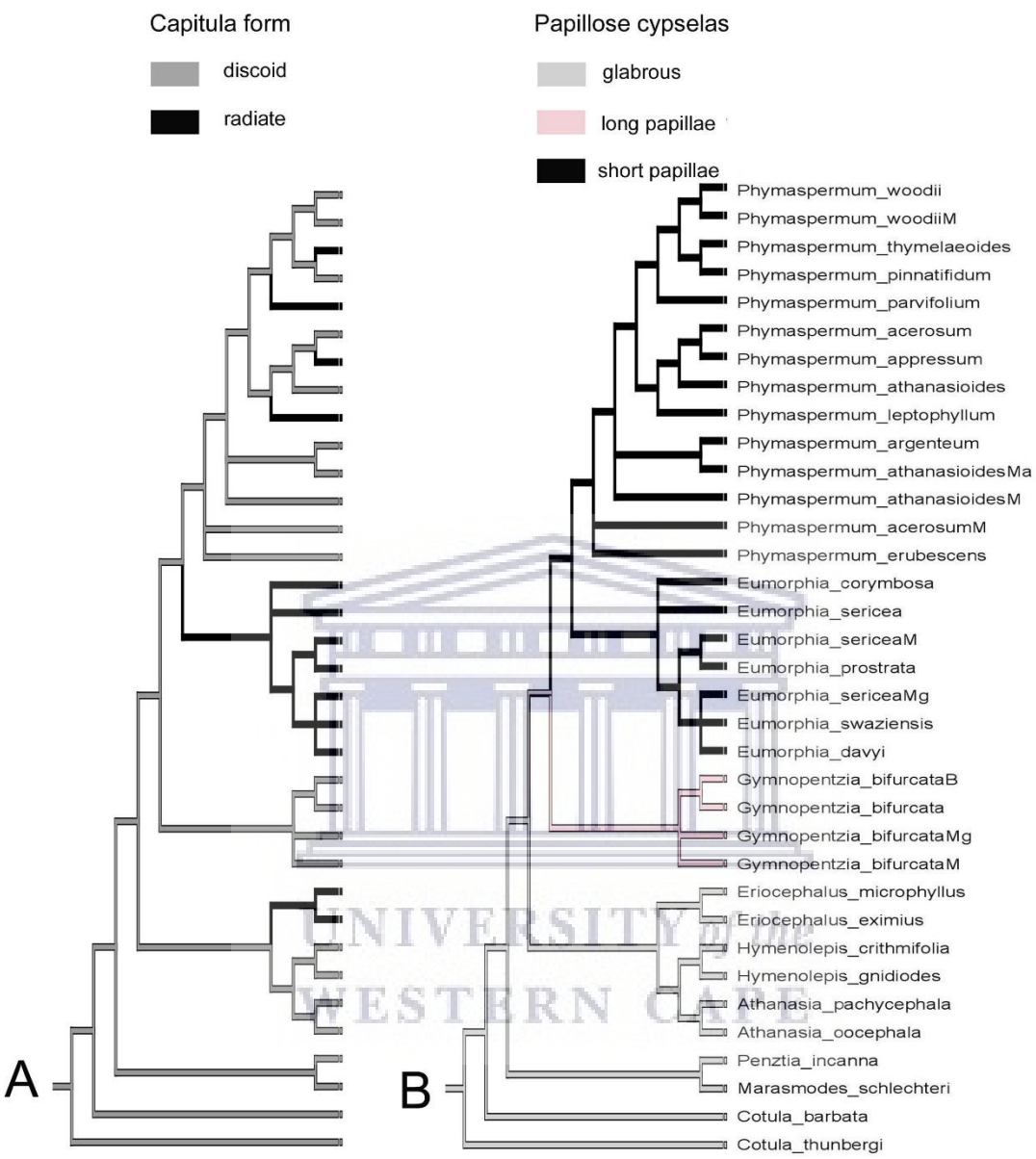


Figure 3.7. Parsimony-based character reconstruction of capitula form (A) and papillose cypselas (B) present with short or long papillose on the majority rule consensus tree from the Bayesian Inference (BI) analysis of the combined nuclear and plastid dataset.

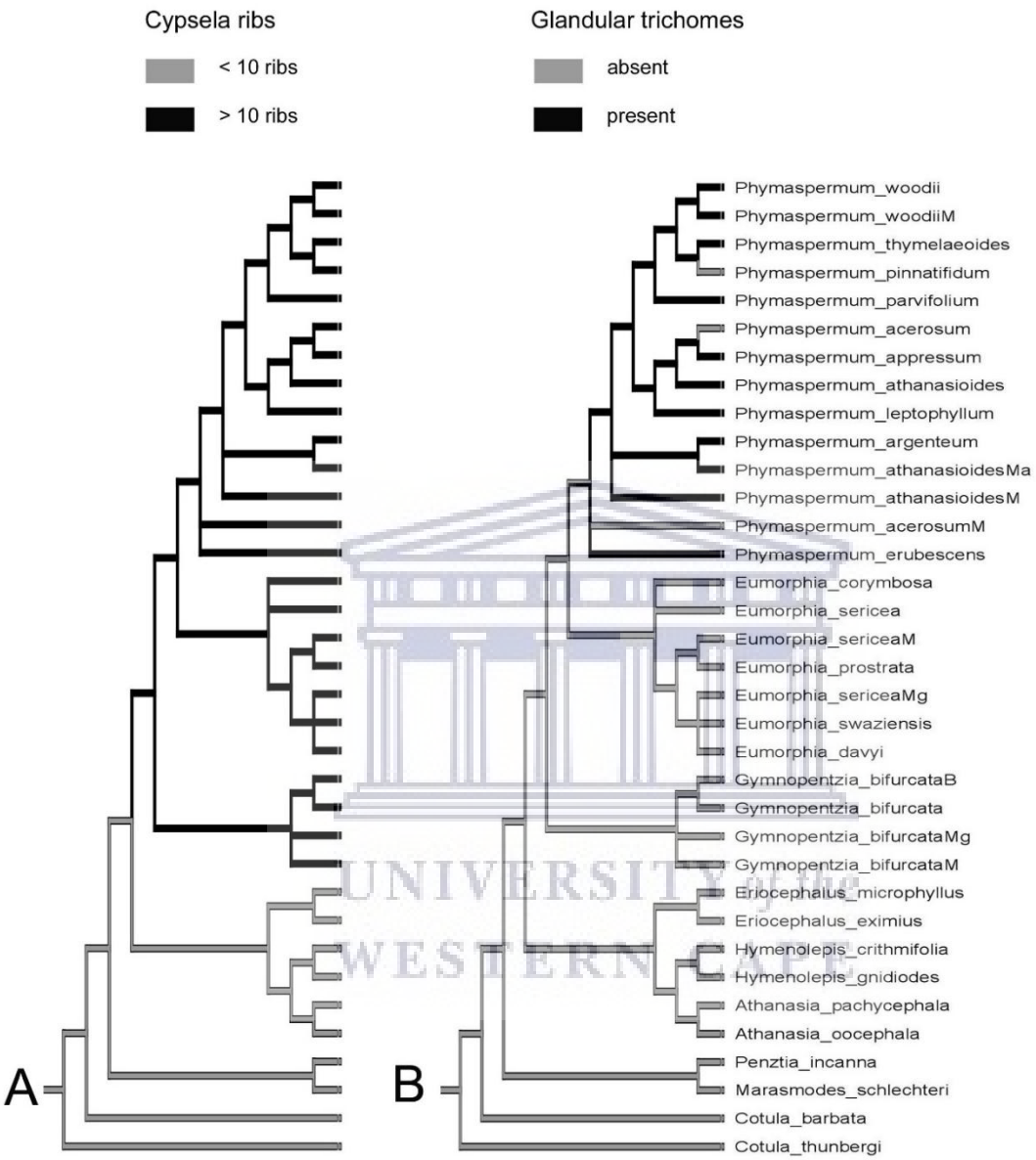


Figure 3.8. Parsimony-based character reconstruction of cypselas ribs (A) and glandular trichomes (B) on the majority rule consensus tree from the Bayesian Inference (BI) analysis of the combined nuclear and plastid dataset.

3. 4. Discussion

3. 4. 1. Generic circumscriptions within subtribe Phymasperminae

Expanded phylogenetic sampling, both additional markers as well as more extensive taxon sampling often improves phylogenetic signal, providing comprehensive results from which to assess generic circumscription (Pick et al. 2010; Echternacht et al. 2014). This was observed in the present study, where expanded sampling of subtribe Phymasperminae and additional DNA regions were included. The phylogenetic analyses presented here have, for the first time, resolved the relationships within Phymasperminae. Three clades within Phymasperminae were recovered, each corresponding to the current circumscriptions of *Eumorphia*, *Gymnopenztia* and *Phymaspermum*, although the *Eumorphia* clade was unresolved in the plastid analyses (Fig. 3.1- Fig. 3.3).

The phylogenetic analyses based on separate nuclear and combined nuclear and plastid analyses suggested that *Phymaspermum* is sister to *Eumorphia* (Fig. 3.1 and Fig. 3.3), supported by a synapomorphy of the cypselas with short papillae (Fig. 3.7B; Källersjö 1986, Kadereit and Jeffrey 2007, Ruiters 2014). *Gymnopenztia* was recovered as successively sister to the *Phymaspermum*-*Eumorphia* clade (Fig. 3.1- Fig. 3.3) and this relationship is supported by the morphological synapomorphies of cypselas with a papillose pericarp and multi-ribbed (10 or more ribs) (Fig. 3.7B, 3.8A, Källersjö 1986, Ruiters 2014).

The current strict circumscription of *Phymaspermum* was supported by the synapomorphy of the presence of myxogenic trichomes on the achenes, although this character was secondarily and independently lost in two species *P. acerosum* and *P. pinnatifidum* (Fig. 3.8B; Källersjö 1986; Ruiters 2014; Ruiters et al. 2016). These results supported the decision by Ruiters et al. (2016) not to recognise these two species as separate genus outside of *Phymaspermum*.

Two main clades (a discoid and radiate clade) of *Phymaspermum* were recovered in the morphological phylogeny of Ruiters et. al. (2016). The two clades were not recovered in the present DNA analyses although the resolution within the genus is poorly resolved at the moment. For example the two radiate species *P. leptophyllum* and *P. parvifolium* were suggested to be sister species by Ruiters et al. (2016). In the separate nuclear analyses (Fig. 3.1), the sister relationship was supported but in the combined nuclear and plastid analyses (Fig 3.3) they were not recovered together. The discoid *P. woodii* and *P. argenteum* were recovered

as sister in nuclear analyses (Fig.3.1), but their position were unresolved in plastid analyses (Fig.3.2), whilst in the combined nuclear and plastid analyses (Fig. 3.3) *P. woodii* was recovered as sister to radiate *P. thymelaeoides* and discoid *P. pinnatifidum* while *P. argenteum* was recovered as sister to discoid *P. athanasioides.* The discoid *P.acerosum*, *P.pinnatifidum* were recovered in the same subclade with the radiate *P.appressum* (Fig. 3.1). Although in Ruiters et al. (2016), *P. appressum* was recovered as sister to the radiate *P. erubescens*.

The circumscription of *Gymnopenztia* was supported by the synapomorphy of cypselas with long papillae (Fig. 3.7B, Källersjö 1986; Bremer and Humphries 1993; Ruiters 2014) and the distinct bifurcate leaves (Fig. 3.4 E). In the nuclear analyses of Ruiters (2014) *Gymnopenztia bifurcata* was sister to *Eumorphia prostrata* and they were sister to *Phymaspermum athanasioides* (Ruiters (2014). In the present analyses, *Gymnopenztia* is strongly supported as sister to *Phymaspermum* and *Eumorphia* (Fig. 3.1- Fig. 3.3).

There are no clear synapomorphies to support the circumscription of *Eumorphia*, as most characters were shared with either *Phymaspermum* or *Gymnopentzia* viz. the radiate capitula in *Eumorphia* and in several species of *Phymaspermum* (Fig. 3.7A; Ruiters 2014; Ruiters et al. 2016). The opposite leaves in most *Eumorphia* species as well as *Gymnopentzia* (Fig.3.5B; Ruiters 2014) and in *Phymaspermum oppositifolium* Magee and Ruiters (Ruiters et al. 2016), although the latter species not included in the current phylogenetic analyses; the receptacles are usually completely paleate in *Eumorphia* (Fig. 3.6A) but this character is likely to be the plesiomorphic state within the tribe. Within *Eumorphia*, *E. corymbosa* is often confused with *Phymaspermum* species (Shearing 1994) and was recovered within *Phymaspermum* in the morphological analyses of Ruiters (2014). In the current analyses *E. corymbosa* was recovered in the *Eumorphia* clade (Fig. 3.1- Fig. 3.3). Similarly *Eumorphia prostrata* which has marginal paleae (Fig. 3.6A, Fig. 3.6 B) was recovered in our analyses with it congeners (Fig. 3.1- Fig.3.3), although with *Gymnopentzia* in the morphological analyses of Ruiters (2014). The multistemmed *E. swaziensis* is incorporated in molecular phylogenetic analyses for the first time and its position within *Eumorphia* confirmed (Fig. 3.1- Fig. 3.3).

The Phymasperminae clade including *Gymnopentzia*, *Eumorphia* and *Phymaspermum* was strongly supported as monophyletic in all analyses performed (separate nuclear, plastid, and combined nuclear and plastid analyses; Fig. 3.1- Fig. 3.3). The monophyly of the broader clade

was supported by the following morphological synapomorphies: cypselas multi-ribbed (10 or more ribs) with a papillose pericarp (Fig. 3.6B, 3.7A, Källersjö 1986, Ruiters 2014, Ruiters et al. 2016).

Although the three genera as currently circumscribed were recovered as monophyletic clades in the analyses presented, two options were assessed for circumscribing the genera. The first option is that the three genera of Phymasperminae be retained in their current circumscriptions. While *Phymaspermum* and *Gymnopentzia* could be readily circumscribed (stalked glandular trichomes in *Phymaspermum*; long papillae and bifurcate leaves in *Gymnopentiza*), the circumscription of *Eumorphia* would remain problematic as the fully paleate receptacles, absent in *E. prostrata*, are likely to be the plesiomorphic state and shared with members of the Athanasiinae. While radiate heads were recovered as a synapomorphy for *Eumorphia*, this character state was also recovered in several species of *Phymaspermum* (all species of the radiate clade of Ruiters 2014) and often shown to be a very labile state throughout the family (sometimes even within species, i.e. *P. acerosum*, Ruiters 2016). As such there were no strong synapomorphies to support the monophyly of *Eumorphia* and the genus would be distinguished from *Phymaspermum* by the usually opposite leaves and mostly completely paleate receptacles and from *Gymnopentzia* by the radiate heads and mostly completely paleate receptacles.

In the second option, the circumscription of *Phymaspermum* could be expanded to include *Eumorphia* and *Gymnopentzia* and these three main lineages reduced to sectional ranks. In this option, the expanded *Phymaspermum* s.l. could be recognized by the following synapomorphies: multi-ribbed cypselas (10 or more ribs) with a papillose pericarp (either short or long and sometimes also with glandular trichomes). With the information available the second option is favoured here. The expanded generic concept is morphologically easily circumscribable and by recognizing the previous genera as sections this retains the recognition of these lineages but better reflects the limited synapomorphies and numerous shared characters between them and highlights their phylogenetic affinities. The required nomenclatural changes are made below.

Phymaspermum Less., Syn. Gen. Comp. 253 (1832). Type: *Phymaspermum leptophyllum* (DC.) Benth. & Hook.f. ex B.D.Jacks.

Eumorphia DC., Prod. 6: 2 (1838), **syn. nov.** Type: *Eumorphia dregeana* DC.

Gymnopentzia Benth. & Hook.f., Gen. Pl. 2(1): 537 (1873)., **syn. nov.** Type: *G. bifurcata* Benth.

Key to the sections of *Phymaspermum*

1a. Fruit with glandular trichomes (if rarely absent then leaves alternate and heads discoid) ……...……Sect. *Phymaspermum*

1b. Fruit without glandular trichomes; leaves opposite (alternate in *E. davyii, E.swaziensis*):

2a. Heads discoid; fruit long hairy; leaves bifurcate; paleae restricted to marginal florets …… ….....……Sect. *Gymnopentiza*

2b. Heads radiate; fruit shortly hairy; leaves entire to pinnate, paleae present throughout receptacle (absent in *E. prostrata*) ……Sect. *Eumorphia*

Section *Phymaspermum*

17 species endemic to southern Africa with one species extending into Zimbabwe. Six of the species are endemic to the Greater Cape Floristic Region. Complete synonymies and key to the species can be found in Ruiters et al. (2016).

List of species

Phymaspermum acerosum Källersjö, *P. aciculare* Källersjö, *Phymaspermum aphyllum* Magee and Ruiters, *Phymaspermum appressum* Källersjö, *Phymaspermum argenteum* Brusse, *Phymaspermum athanasioides* Källersjö , *Phymaspermum comptoni* Magee and Ruiters, *Phymaspermum erubescens* Källersjö, *Phymaspermum leptophyllum* DC., *Phymaspermum oppositifolium* Magee and Ruiters, *Phymaspermum parvifolium* DC., *Phymaspermum peglerae* Hutch., *Phymaspermum pinnatifidum* Källersjö, *Phymaspermum scoparium* Källersjö , *Phymaspermum thymelaeoides* DC., *Phymaspermum trifidum* Magee and Ruiters, *Phymaspermum woodii* Källersjö.

Section *Eumorphia* (DC.) Akimana, Boatwr. & Magee **stat. nov.** *Eumorphia* DC., Prod. 62 (1838). Type: *Eumorphia dregeana* DC.

Six species and two subspecies endemic to southern Africa. It occurs widely in southern Africa in Limpopo, Mpumalanga, Swaziland, Free State, Kwazulu-Natal, Lesotho, Western and Eastern Cape. Complete synonymies and key to the species can be found in Swelankomo (2011).

List of species and new combinations:

Phymaspermum corymbosa (E.Phillips) S.Akimana, Boatwr. & Magee, **comb. nov.** *Eumorphia corymbosa* E.Phillips, Journal of South African Botany 16:20 (1950). Type: Western Cape, 3222 (Beaufort West): on top of Molteno Pass [1804 m], May, *J.P.H. Acocks 14340*, (PRE, holo1.!).

Phymaspermum davyi (Bolus) S.Akimana, Boatwr. & Magee, **comb. nov.** *Eumorphia davyi* Bolus, Transactions of the South African Philosophical Society 16: 387 (1906). Type: Eastern Transvaal [Mpumalanga], 2430 (Pilgrim's Rest): Graskop, near Pilgrim's Rest (-DD), 29 Jan. 1906, *J. Burtt Davy* 1474 (BOL, holo.e!, PRE!, K e! iso).

Phymaspermum dregeana (DC.) S.Akimana, Boatwr. & Magee, **comb. nov.** *Eumorphia dregeana* DC., Prodromus 6:3 (1838). Type: Eastern Cape, 3124 (Hanover): Middelburg, Sneeuweberg between Kompasberg and Rhenosterberg, 5000-6000' [1 640-1 964 m], (-DC), Sept., *Drège* s.n, (G-DC,holo.e!, MO e!, M e!, PRE !, SAM e!, P e!, K e!, NBG e! iso.).

Phymaspermum prostrata (Bolus) S.Akimana, Boatwr. & Magee, **comb. nov.** *Eumorphia prostrata* Bolus, Transactions of the South African Philosophical Society 16: 388 (1906). Type: Eastern Cape, 3028 (Matatiele): Barkly East, Summit Doodman's Krans Mtn, (-CA), 8 Mar 1904, *E.E. Galpin* 6700, (BOL, holo.e!, K e!, PRE, two sheets!, SAM e! iso.).

Phymaspermum sericea (J.M.Wood & M.S.Evans) S.Akimana, Boatwr. & Magee, **comb. nov.** *Eumorphia sericea* J.M.Wood & M.S.Evans, Journal of Botany 35: 488 (1897). Type: Natal, Summit of Drakensberg, near Bushman's River Pass, 8000-10000', Apr.1896. (NH, holo, PRE, K, iso.).

Phymaspermum sericea (J.M.Wood & M.S.Evans) S.Akimana, Boatwr. & Magee subsp. *sericea*. Type: as above.

Phymaspermum sericea (J.M Wood & M.S. Evans) S.Akimana, Boatwr. & Magee subsp. *robustior* (Hilliard & B.L.Burtt) S.Akimana, Boatwr. & Magee, **comb.nov.** *Eumorphia sericea* J.M Wood & M.S.Evans subsp. *robustior* Hilliard & B.L.Burtt in Notes from the Royal Botanic Garden Edinburg 40: 248 (1982). Type: Eastern Cape, 3127(Lady Frere): Elliot, Fetcani Pass, common bush up to 5'[1.64 m], on slopes and especially in rock-fall scrub, (BB), 7500' [2460M],22 Jan .1979, O.M. Hilliard & B.L. Burtt 12331 (NU, holo, E, PRE, K, M, S, NBG, iso).

Phymaspermum swaziensis (Compton) S.Akimana, Boatwr. & Magee, **comb. nov.** *Eumorphia swaziensis* Compton, Journal of South African Botany 33: 300 (1967). Type: Swaziland, 2631 (Mbabane): Hill northeast of Mbabane, (-AC), 4000' [1 312 m], 15 Mar. 1960 (SDNH, holo.).

Section *Gymnopentzia* (Benth. & Hook.f.) Akimana, Boatwr. & Magee **stat. nov.** *Gymnopentzia* Benth & Hook *f., Gen. Pl.* 2(1):537 (1873). Type*: Gymnopentzia bifurcata* Benth.

A monotypic section endemic to southern Africa. It occurs widely in the Eastern Cape, Natal, and in Lesotho. Treatments of this single species can be found in Källersjö (1986) and Bremer and Humpries (1993).

List of species and new combinations:

Phymaspermum bifurcatum (Benth.) S.Akimana, Boatwr. & Magee. c**omb. nov.** *Gymnopentzia bifurcata* Benth in Benth & Hook.f, Gen Pl.2 (1): 537(1873). Type: Eastern Cape, 3225 (Sommerset East): damp rocks to the west of Mt.Boschberg, [1463 m], (-DA), 1877, MacOwan.

CHAPTER 4

PHYLOGENETIC RELATIONSHIPS WITHIN THE SUBTRIBE ATHANASIINAE

4.1. Introduction

The subtribe Athanasiinae was described by Oberprieler et al. (2007) to accommodate seven genera and about 86 species. Oberprieler et al. (2009) acknowledged the problematic circumscription of the subtribe, due to a lack of morphological synapomorphies and molecular phylogenetic evidence suggesting that the current assemblage was paraphyletic. Previously, in the classification of Bremer and Humphries (1993), these genera had been placed in subtribes Matricariinae (*Adenoglossa* B. Nord., *Eriocephalus* L., and *Leucoptera* B. Nord.) and Ursiniinae (*Asaemia* Harv., *Athanasia* L., *Hymenolepis* Cass., and *Lasiospermum* Lag.). The subtribe Ursiniinae in the sense of Bremer and Humphries (1993) included the mentioned genera as well as *Ursinia* Gaertn. and was based on the shared the presence of furanosesquiterpenes (Bentham et al.1873, Heywood et al. 1977), as well as morphological and anatomical characters, viz. paleate receptacles, tubular epidermal cells on the ray floret limbs and polarized endothecial cells on the anthers (Källersjö 1986, Bremer and Humphries 1993). However, the phylogenetic evidence shows *Ursinia* to be isolated from the other genera and so the subtribe was retained by Oberprieler et al. (2007) as monogeneric. The Matricariinae *sensu* Oberprieler et al. (2007) now includes only the northern hemisphere/Eurasian genera (Oberprieler et al. 2009). The species of Athanasiinae are largely all endemic to southern Africa, with a single species, *Lasiospermum brachyglossum* DC. occurring also in the Sinai desert of Egypt (Müller et al. 2001).

The monophyly of Athanasiinae has been uncertain in all molecular analyses performed thus far (Oberprieler et al. 2007, 2009, Himmelreich et al. 2008, Ruiters 2014). The analyses based on nuclear ITS sequence data showed that the Athanasiinae formed a paraphyletic clade with *Adenoglossa, Eriocephalus* and *Leucoptera* being sister to the group comprising the genera *Athanasia*, *Hymenolepis*, *Lasiospermum* and Phymasperminae, as well as the Pentziinae-Eurasian clade. Analyses based on plastid *ndhF* data (Himmelreich et al. 2008, Oberprieler et al. 2009) again recovered Athanasiinae as paraphyletic and forming a basal grade to the

Pentziinae-Eurasian clade. In contrast, the subtribe Phymasperminae was placed within the Pentziinae-Eurasian clade rather than within Athanasiinae as suggested by the nuclear analyses. Despite the clear non-monophyly of the subtribe Athanasiinae, Oberprieler et al. (2007) recognized the two subtribes Athanasiinae and Phymasperminae separately until the incongruence between the nuclear and plastid phylogenies and the placement of Phymasperminae could be resolved.

The Phymasperminae, following the results presented in Chapter 3, is a monogeneric subtribe (with *Eumorphia* DC. and *Gymnopentzia* Benth. included in *Phymaspermum* Less). The members of the subtribe share the presence of polarized endothecial tissue with the subtribe Athanasiinae (Källersjö 1986) and as a result Oberprieler et al. (2009) suggest that the possibility of including Phymasperminae within Athanasiinae should be considered. Such a circumscription is supported by the current nuclear analyses, although the chloroplast data placed Phymasperminae rather with members of the Pentziinae-Eurasian clade (Oberprieler et al. 2009).

The phylogenetic position of the genus *Inulanthera* Källersjö was unresolved in the recent classification of Oberprieler et al. (2009) and Magoswana et al. (2016). The molecular reconstruction of Oberprieler et al. (2009) suggested a close relationship with *Ursinia* but *Inulanthera* species are morphologically distinct from *Ursinia* by its habit (shrubs) with 8 to 10-ribbed cypselas, with a pappus of scales terminating each rib, while *Ursinia* are shrublets, cypselas usually 5-ribbed with a basal tuft of hairs or they are sometimes smooth (Oberprieler et al. 2009). Magoswana et al. (2016) revised the genus *Inulanthera* and their analyses placed *Inulanthera* with Athanasiinae which was incongruent with the reconstruction of Oberprieler et al. (2009). Thus, the uncertainty in the position of *Inulanthera* needs to be assessed to improve the resolution of its placement in the tribe Anthemideae. Within the subtribe Athanasiinae, the phylogenetic analyses thus far sampled only a single species per genus. Ruiters (2014) included a more representative sampling of species for the subtribe Phymasperminae for her ITS analysis. The sampling for this subtribe was further expanded in Chapter 3.

The detailed sampling of Athanasiinae and Phymasperminae presented in the present study is aimed at unravelling the true relationships between these subtribes and with the other subtribes in Anthemideae. If Phymasperminae is indeed embedded within Athanasiinae, as suggested by

the previous studies based on ITS data and data presented in Chapter 3, the polarized endothecial tissue of the anthers present in these taxa could be considered as a synapomorphy for the broader clade and may provide an argument for the expansion of Athanasiinae to include Phymasperminae (Oberprieler et al. 2009).

The aims of this chapter are to:

- Expand the phylogenetic sampling within subtribe Athanasiinae.
- Test the monophyly of Athanasiinae and its relationship to Phymasperminae.
- Assess generic relationships within subtribe Athanasiinae.
- Resolve the subtribal placement of *Inulanthera*.

4.2 Materials and methods

Details of the methods and materials used can be found in Chapter 2.

4.3. Results

4.3.1. Phylogenetic analyses

4.3.1.1. Nuclear datasets

The combined nuclear (ETS and ITS) DNA matrix consisted of 92 taxa and 1129 characters of which 411 were constant, 229 variable and 489 parsimony informative. Maximum parsimony (MP) analysis retrieved 67152 trees with a tree length of 1805 steps, a consistency index (CI) of 0.60 and retention index (RI) of 0.84. The topology of the MP strict consensus tree was similar to that of the Bayesian Inference (BI) majority rule consensus trees and the support values are summarized on the BI tree (Fig. 4.1).

As in Oberprieler (2007), the Osmitopsidinae and Cotulinae were recovered as the earliest diverging lineages of the tribe Anthemideae. *Inulanthera* was strongly supported to be monophyletic (PP 1.0, BP 100) and recovered as sister to the broader Athanasiinae-Phymasperminae clade (PP 1.0). This contrasted with previous molecular reconstructions of Oberprieler et al. (2007, 2009) which recovered *Inulanthera* as sister to *Ursinia*, or unresolved

in the analyses of Himmelreich et al. (2008). The subtribe Penztiinae were recovered as monophyletic (PP 1.0, BP 99) and sister to Artemisiinae (PP 1.0, BP 62).

The Athanasiinae with the exception of *Lasiospermum*, was strongly recovered as monophyletic (PP 1.0) and recovered within a broader and weakly supported Athanasiinae-Phymasperminae clade (PP 0.7, BP 60). The position of *Lasiospermum* in relation to Phymasperminae and Athanasiinae was unresolved although the genus was strongly supported as monophyletic (PP 1.0, BP 100). The Phymasperminae were strongly supported to be monophyletic (PP 1.0, BP 100). Previous analyses did not recover a broader Athanasiinae-Phymasperminae clade but rather a basal grade within Athanasiinae *Leucoptera*, *Adenoglossa* and *Eriocephalus* was sister to *Athanasia*, *Hymenolepis*, *Lasiospermum* and Phymasperminae plus Pentziinae-Eurasian clade.

Within the core Athanasiinae clade (*Leucoptera-Athanasia*) four strong to moderately well-supported subclades were recovered. The *Leucoptera* subclade was moderately supported (PP 0.9, BP 72) and includes only *Leucoptera* and *Adenoglossa*. Most of the accessions of *Leucoptera* were recovered in a monophyletic group (PP 1.0, BP 82), with the exception of one accession of *L. subcarnosa* which was unresolved. The non-succulent species *L. nodosa* from the sandveld was sister to the succulent quartz endemic species *L. subcarnosa* (the two accession) and *L. oppositifolia*. The *Eriocephalus* subclade (PP 1.0, BP 100) was sister to the *Leucoptera* subclade (PP 1.0) and this relationship with *Leucoptera* and *Adenoglossa* was previously recovered by Oberprieler et al. (2007, 2009) and Himmelreich et al. (2008). The *Athanasia* subclade was strongly supported as monophyletic (PP1.0, BP 96) and included *Athanasia* and *Asaemia*. *Asaemia* was not included in previous molecular analyses of Oberprieler et al. (2007, 2009) and Himmelreich (2008). The *Hymenolepis* subclade (PP 0.8, BP 75) was sister to *Athanasia* subclade (PP1.0, BP 96) and this relationship with *Athanasia* was previously recovered in nuclear (ITS) analyses of Oberprieler et al. (2007, 2009) and Himmelreich et al. (2008).

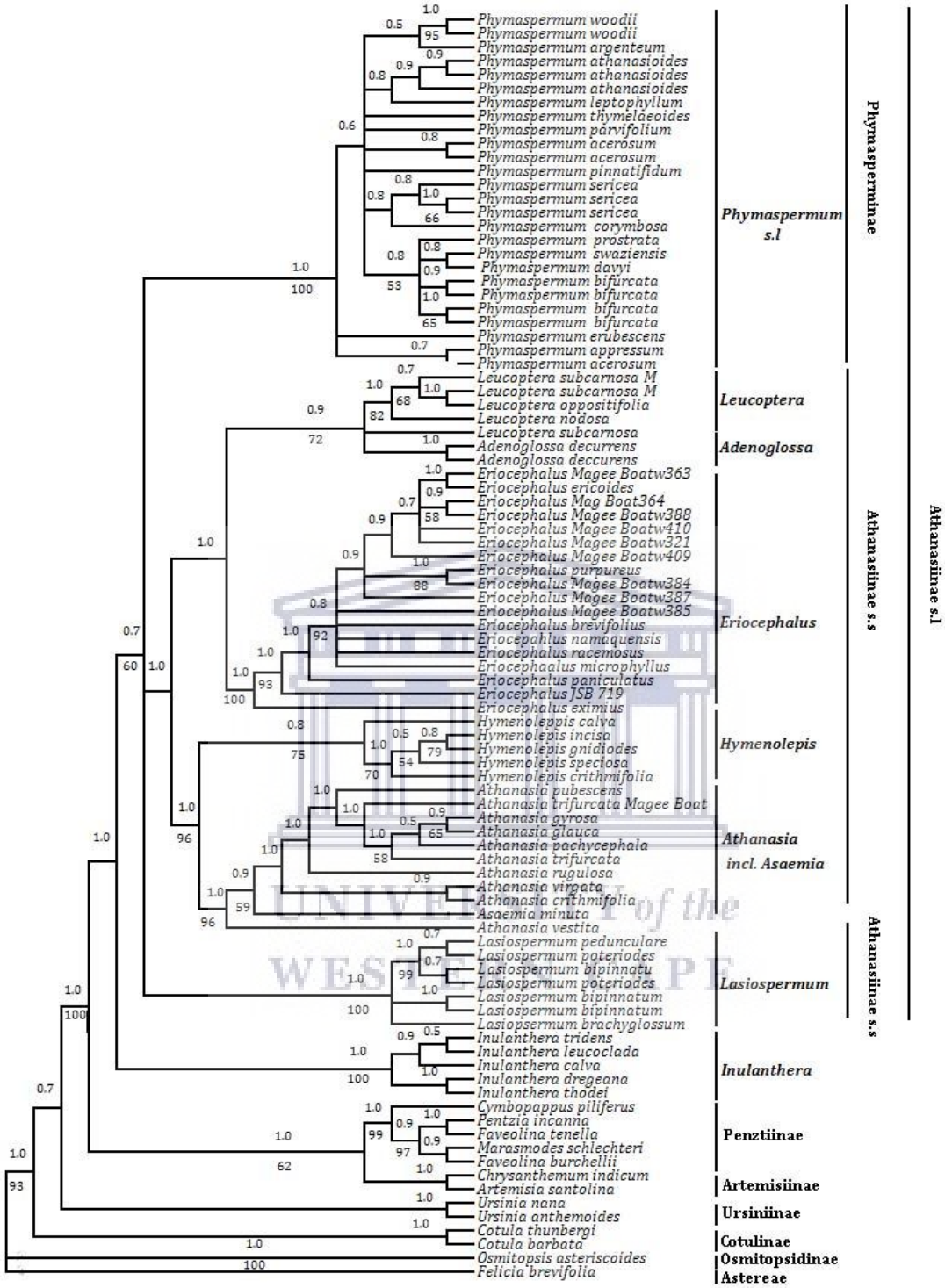


Figure 4.1. Bayesian inference (BI) majority rule consensus tree of the combined nuclear (ETS and ITS) sequence dataset. Posterior probability (PP) values are presented above the branches and bootstrap percentages (BP) below the branches. BP and PP values <50% and 0.50, respectively, are not indicated.

4.3.1.2. Plastid datasets

The combined plastid (*rps16* and *rpl32*) DNA matrix consisted of 93 taxa with 2240 characters of which 1258 were constant, 380 variable and 602 parsimony informative. Maximum parsimony (MP) analysis retrieved 181112 trees with a tree length of 1682 steps, a consistency index (CI) of 0.74 and retention index (RI) of 0.89. The topology of the MP strict consensus tree was similar to that of the Bayesian Inference (BI) majority rule consensus trees and the support values are summarized on the BI tree (Fig. 4.2).

The subtribe Cotulinae and Ursiniinae were recovered as sister (PP1.0, BP 92). The subtribe Penztiinae was recovered as monophyletic (PP 1.0) and sister to Artemisiinae (PP 1.0, BP 100). *Inulanthera* was strongly supported to be monophyletic (PP 1.0, BP 100) and recovered as sister to the Pentziinae-Cotulinae clade (PP 1.0, BP 99). This contrasted with the nuclear analyses in which *Inulanthera* was sister to the broader Athanasiinae-Phymasperminae clade.

Athanasiinae was not recovered as monophyletic as *Leucoptera* and *Adenoglossa* were weakly supported as sister to Phymasperminae (PP 0.6), and the position of *Lasiospermum* was unresolved, although the genus was moderately supported as monophyletic (PP 1.0, BP 93). The *Leucoptera* subclade was strongly supported (PP 1.0, BP 96) and included *Leucoptera* and *Adenoglossa*. The accessions of *Leucoptera* were recovered in a monophyletic group (PP 1.0) with the succulent quartz endemic species *L. subcarnosa* (two accessions) sister to the non-succulent species *L. nodosa* from the sandveld and *L. oppositifolia* (PP 1.0). As in the nuclear analyses, the Phymasperminae was strongly supported to be monophyletic (PP 1.0, BP 100) and recovered within a broader strongly supported Athanasiinae-Phymasperminae clade (PP 1.0, BP 100). This contrasted with previous plastid (*ndh*F) analyses of Himmelreich et al. (2008) and Oberprieler et al. (2009) in which a broad Phymasperminae-Athanasiinae clade was not recovered, but rather Phymasperminae was placed within the Penztiinae-Eurasian clade.
Within the core Athanasiinae clade (*Eriocephalus-Athanasia*) three strong to moderately well-supported subclades were recovered. The *Eriocephalus* subclade was strongly supported as monophyletic (PP 1.0, BP 100) and sister to *Hymenolepis* and *Athanasia* subclades (PP 0.9, BP 87). This contrasted with nuclear analyses in which *Eriocephalus* was rather sister to *Leucoptera* subclade. *Hymenolepis* was embedded within *Athanasia* (PP1.0, BP 81), although all its species grouped together (PP 1.0, BP 95). This is in contrast to the nuclear analyses where these two genera were strongly supported as sister.

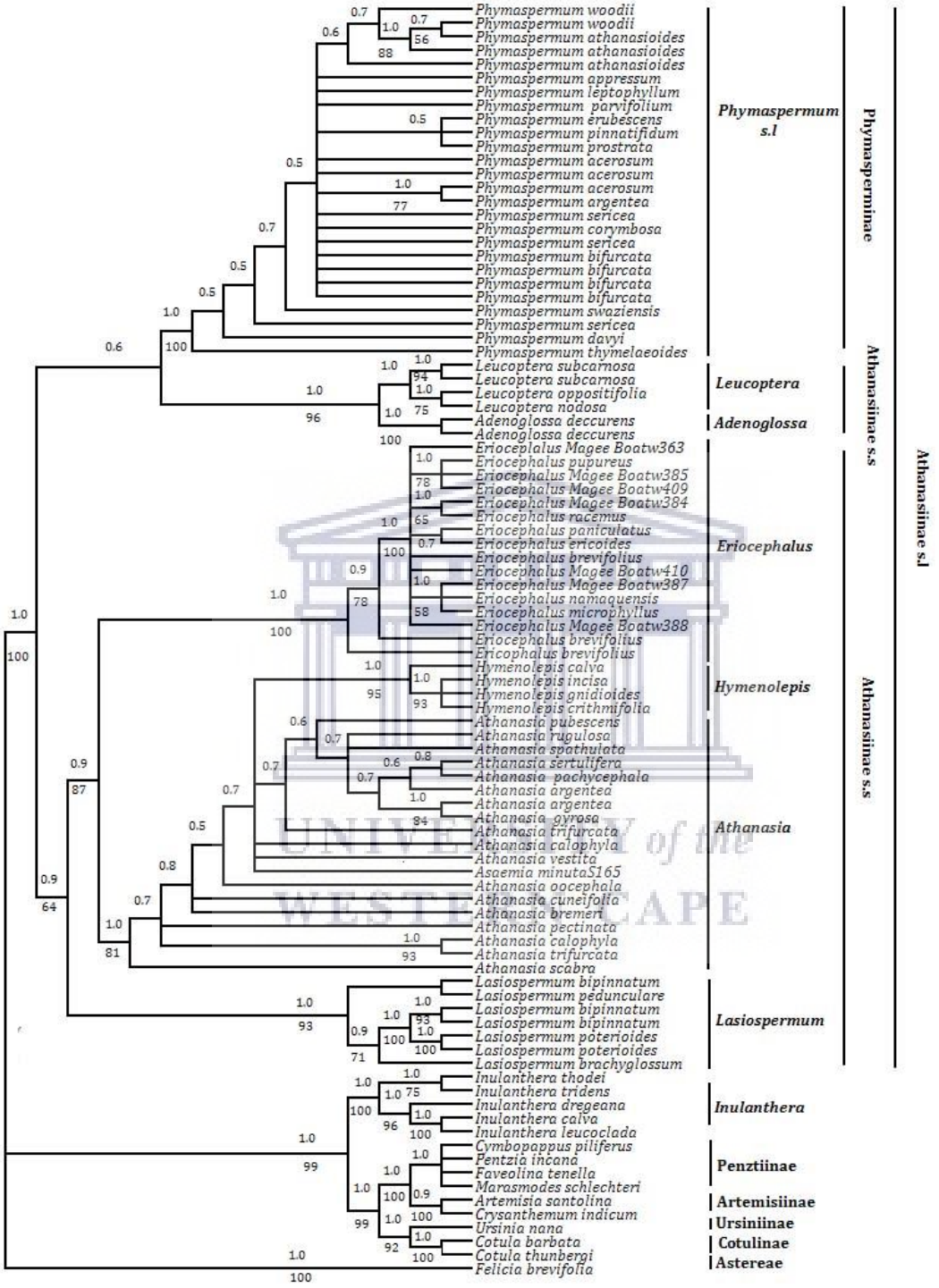


Figure 4.2. Bayesian inference (BI) majority rule consensus tree of the combined plastid (*rps16* and *rpl32*) sequence dataset. Posterior probability (PP) values are presented above the branches and Bootstrap percentage (BP) values below the branches. BP and PP values <50% and 0.50, respectively, are not indicated

4.3.1.3. Combined Nuclear/plastid datasets

Hard incongruence between the nuclear and plastid datasets was observed for the positions of *Inulanthera*, Ursiniinae and Cotulinae. In the nuclear analyses, *Inulanthera* was recovered as sister to a broader Athanasiinae-Phymasperminae clade. This contrasted with the plastid analyses that recovered *Inulanthera* with Penztiinae-Cotulinae clade. Ursiniinae was recovered as sister to Cotulinae in the plastid analyses, although their relationship was unresolved in the nuclear analyses. These taxa (*Inulanthera*, Ursiniinae and Cotulinae) were therefore excluded from the combined nuclear and plastid matrix.

The reduced dataset comprising 83 taxa consisted of 3369 characters, of which 1924 were constant, 569 were variable and 876 parsimony informative. Maximum Parsimony (MP) analysis of the combined molecular data yielded 9990 with a tree length of 2629 steps, a consistency index (CI) = 0.70 and retention index (RI) = 0.88. Trees obtained from both the MP and the BI analyses yielded the same overall topologies and (Fig. 4.3) and the support values are summarized on the BI tree (Fig. 4.3).

As in the separate nuclear and plastid analyses, the subtribe Penztiinae was recovered as monophyletic (PP 1.0, BP 62) and sister to Artemisiinae (PP 1.0, BP 97). The Athanasiinae clade was strongly supported as monophyletic (PP 1.0, BP 100) and recovered as sister to the Phymasperminae clade (PP 1.0, BP 100). This contrasts with the separate nuclear and plastid analyses where all members of Athanasiinae were not recovered as a monophyletic clade. The broader Athanasiinae-Phymasperminae clade was recovered also in the separate nuclear and plastid analyses. The Phymasperminae clade was strongly supported as monophyletic (PP 1.0, BP 100), as found in the other analyses.

Within the Athanasiinae clade (*Leucoptera-Lasiospermum*) four strong to moderately well-supported subclades were recovered. The *Leucoptera* subclade was strongly supported (PP 1.0, BP 99) and included *Leucoptera* and *Adenoglossa*. The accessions of *Leucoptera* were recovered in a monophyletic group (PP 1.0, BP 100). The monophyly of *Leucoptera* was recovered in the plastid analyses, while in the nuclear analyses the position of one accession of *Leucoptera* was unresolved. The *Eriocephalus* subclade (PP 1.0, BP 92) was recovered as sister to the *Leucoptera* subclade (PP 1.0) as also recovered in the separate nuclear analyses, whilst in plastid analyses *Eriocephalus* was recovered as sister to *Hymenolepis* and *Athanasia*.

Hymenolepis (PP 1.0, BP 81) was strongly supported as sister to *Athanasia* (PP1.0, BP 100). *Athanasia* (PP1.0, BP 73) included *Athanasia* and *Asaemia*. *Lasiospermum* was strongly supported as monophyletic (PP 1.0, BP 100) and sister to *Athanasia* and *Hymenolepis* (PP 0.8, BP 96), while in the separate nuclear and plastid analyses the position of *Lasiospermum* in relation to Phymasperminae and Athanasiinae was unresolved.

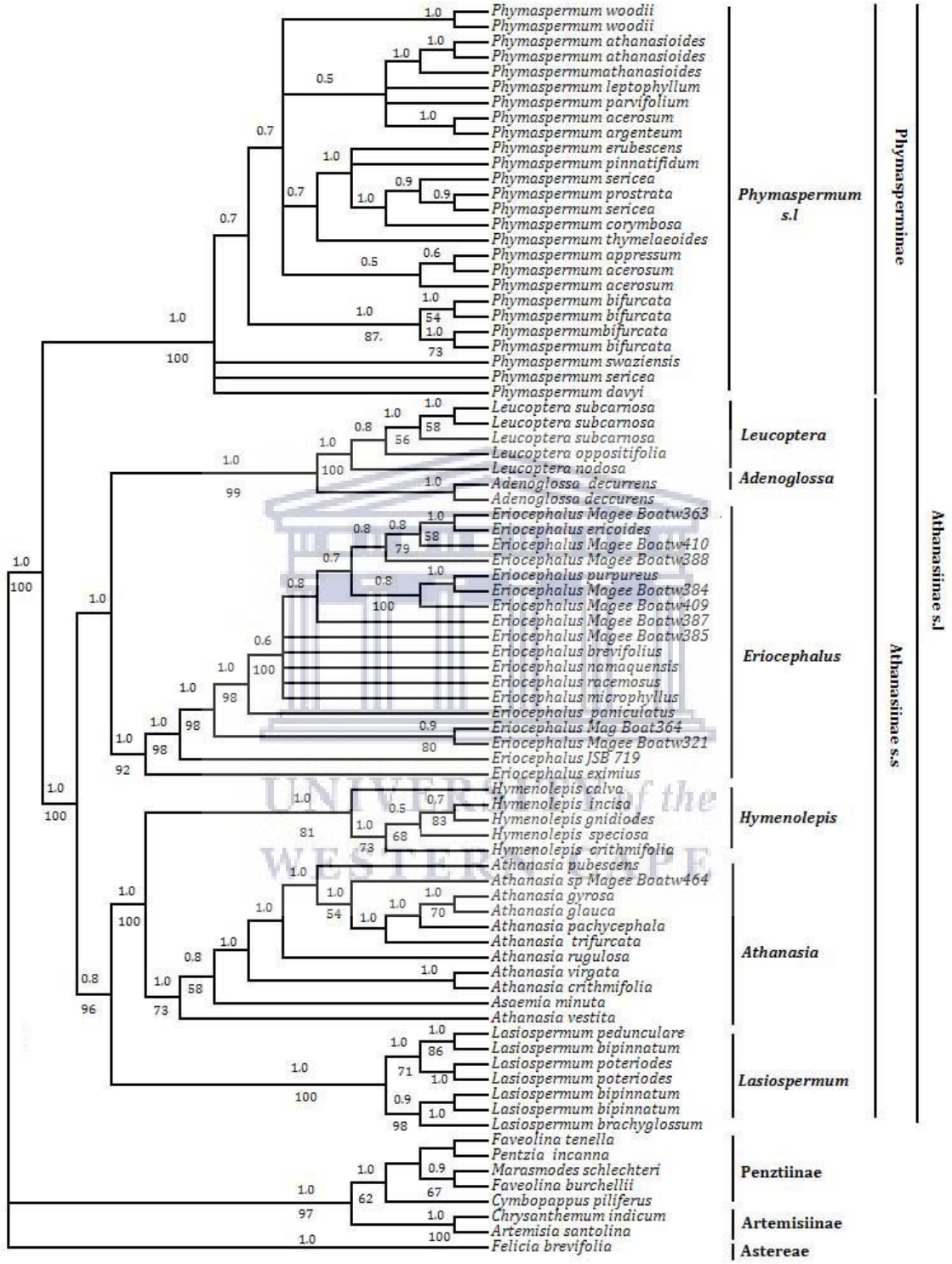


Figure 4.3. Majority rule consensus tree from the Bayesian inference (BI) of the combined nuclear (ETS and ITS) and plastid (*rps16* and *rpl32*) dataset. Posterior probability (PP) values are presented above the branches and Bootstrap percentage (BP) values below the branches. BP and PP values <50% and 0.50, respectively, are not indicated.

4.3.2. Morphological character reconstruction

The subtribes of Anthemideae show transitions between perennial and annual longevity (Oberprieler et al. 2009). Bremer and Humphries (1993) considered the annual type to have independently derived several times within Anthemideae as most of the members are perennial. Similarly, in the present reconstructions, the perennial life history was recovered as a plesiomorphic character with annuality a derived character for the monotypic *Adenoglossa* and one species of *Lasiospermum* (*L. brachyglossum* DC., Fig. 4.4A). This character is more variable in other subtribes.

Leaves without ellipsoid secretory cavities was recovered as the plesiomorphic state in Anthemideae, while leaves with ellipsoid secretory cavities was reconstructed as a synapomorphic character for Athanasiinae and Phymasperminae, with the exception of *Lasiospermum* (Fig. 4.4B).

Bremer and Humphries (1993) and Oberprieler et al. (2009) considered the presence of paleae to be the plesiomorphic state in the Anthemideae. In the present reconstruction, the presence of paleae enclosing the fruit was recovered as a derived character for Ursiniinae (Fig. 4.4C). A paleate receptacle is present in most genera of the Athanasiinae and Phymasperminae, with epaleate receptacles found in *Leucoptera*, *Adenoglossa* and *Asaemia* (Fig. 4.5A). Bisexual disc florets were recovered as the plesiomorphic state in Anthemideae, with the presence of male disc florets a derived character for *Eriocephalus* (Fig. 4.5B).

The pericarp with a continuous ring of sclerenchyma was recovered as a derived character for *Athanasia* and *Asaemia* (Fig. 4.5C), supporting the inclusion of the latter in the former. Isodiometric cypselas are found in most genera of Anthemideae, as well as in Athanasiinae and Phymasperminae, with the exception of *Adenoglossa*, *Leucoptera* and *Eriocephalus* that have dorsiventrally flattened cypselas, also found *Faveolina* and Cotulinae (Fig. 4.5D).

The absence of the cypselas with myxogenic cells were recovered as the plesiomorphic state in Anthemideae, with myxogenic epidermal cells a derived character for Penztiinae and the stalked myxogenic trichomes present on the cypselas recovered as a synapomorphic character for section *Phymaspermum* (Fig. 4.6A).

Few ribbed (2- 4 ribs) cypselas were reconstructed as a plesiomorphic state, and are also found in the *Leucoptera* - *Eriocephalus* subclades of Athanasiinae. Most of the Anthemideae have cypselas with 5-10 ribs, while more than 10 ribs was reconstructed as a synapomorphic character for the Phymasperminae (Fig. 4.6B).

Two main types of indumentum hairs are found on the cypselas in the Anthemideae: basifixed or unbranched hairs and medifixed or branched hairs (Bremer and Humphries 1993). The members of the early diverging subtribes all have basifixed hairs (Bremer and Humphries 1993; Oberprieler et al. 2009). Similarly, in the present reconstruction, basifixed hairs were recovered as the plesiomorphic state with the presence of medifixed hairs a derived character for the *Athanasia* - *Hymenolepis* subclades (Fig. 4.6C).

Cypsela vestiture varies from glabrous to densely hairy (Bremer and Humphries 1993). In the present reconstruction, glabrous cypselas were recovered in most genera of the Athanasiinae, with hairy fruit found in Phymasperminae, *Eriocephalus* and *Lasiospermum* (Fig. 4.6D).

The absence of anthers with baluster-shaped filament collars was recovered as the plesiomorphic state in Anthemideae with the presence of baluster-shaped filament collars as a derived character for Ursiniinae (Fig. 4.7A). The endothecial tissue of the anthers are usually not polarized in the tribe Anthemideae (Bremer and Humphries 1993). In present reconstruction, the anthers with polarized endothecial tissues were recovered as a synapomorphic character for Athanasiinae and Phyamaspermiane, but not in *Eriocephalus* (Fig. 4.7B). The absence of tailed anthers was recovered as the plesiomorphic state in Anthemideae, with the presence of tailed anthers a derived character for *Inulanthera* and Osmitospidinae (Fig. 4.7C). Anthers with triangular-linear-lanceolate apical appendages were recovered as the plesiomorphic state, while those with broad ovate apical appendages a derived character for Ursiniinae (Fig. 4.7D). Anthers with secretory cavities are a derived character for *Athanasia* and *Asaemia* (Fig. 4.8A), supporting the inclusion of the latter in *Athanasia*.

The cypselas of Anthemideae can have a pappus, which is an extension of the cypselas apex or a scaly pappus (Källersjö 1986, Bremer and Humphries 1993). In the present reconstruction, the presence of a pappus was recovered as the plesiomorphic state with the absence of a pappus a derived character for most of the genera within Athanasiinae and Phymasperminae (Fig. 4.8B). A pappus formed by an extension of the cypselas ribs into small horns or scales was

recovered as a synapomorphic character for *Inulanthera* (Fig. 4.8C). Enlarged pappus scales in the fruits was recovered as a unique character for Ursiniinae (Fig. 4.8D). The development of an apically papillate style was recovered as the plesiomorphic state in Anthemideae, with a penicillate style unique in Osmitopsidinae (Fig. 4.9A).

The pollen asteroid with a columnar structure was recovered as the plesiomorphic state for Anthemideae, and pollen without a columnar structure recovered as a derived character for Ursiniinae (Fig. 4.9B). The absence of furanosesquiterpenes was recovered as the plesiomorphic state in Anthemideae, with the presence of furanosesquiterpenes a synapomorphic character for Athanasiinae and Phymasperminae (Fig. 4.9C). According to Heywood and Humphries (1977) and Bremer and Humphries (1993), the most common base chromosome number in Anthemideae is x=9. Similarly, in the present reconstruction the chromosome number x=9 was recovered in most of the Athanasiinae with a reduction to x=8 in *Athanasia*, *Hymenolepis*, *Inulanthera* and Pentziinae, while the chromosome numbers are as yet unknown for the Phymasperminae (Fig. 4.9D).

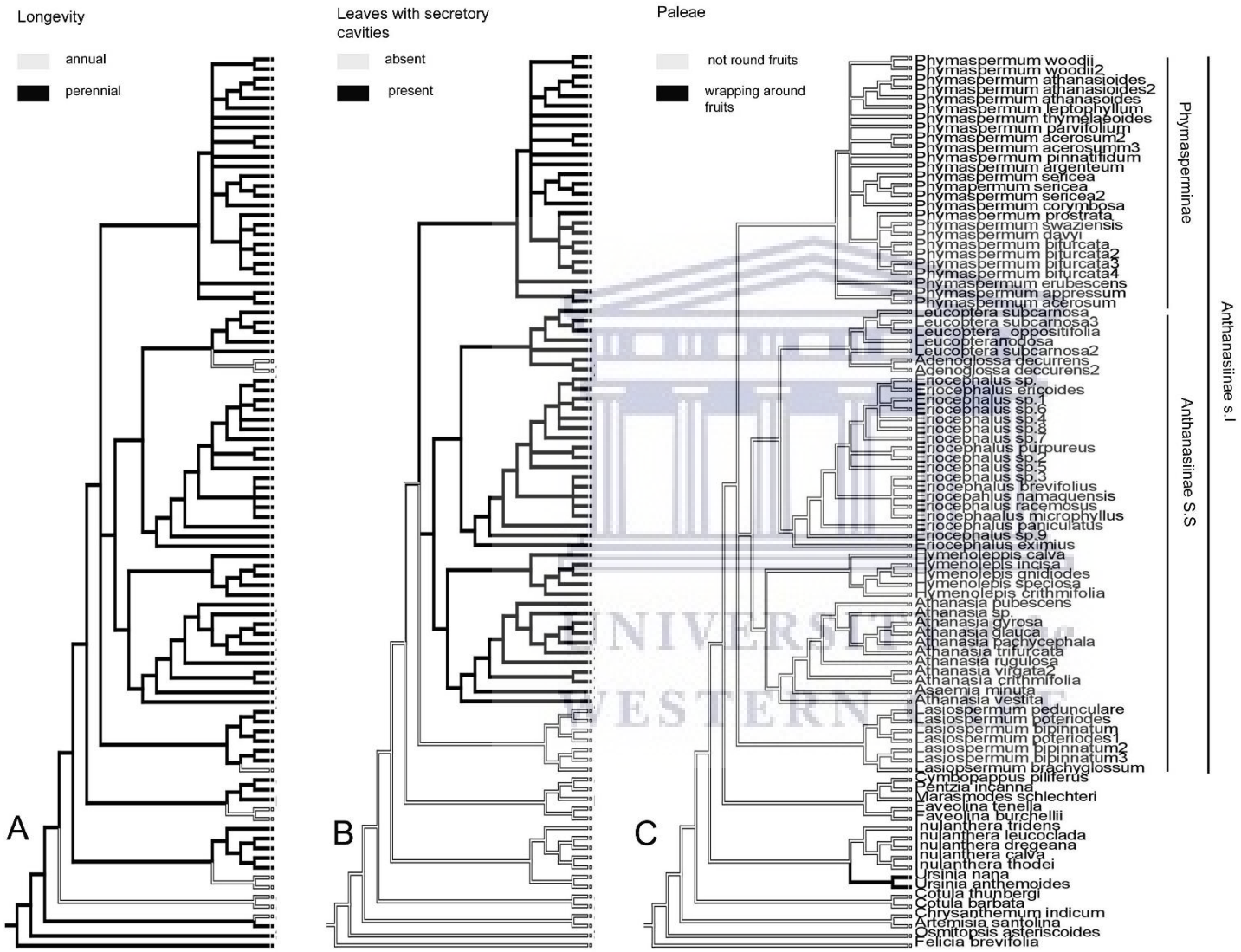


Figure 4.4: Parsimony-based character reconstruction of longevity, leaf and paleae on the Majority- Rule consensus tree from the Bayesian Inference (BI) analysis of the combined nuclear dataset.

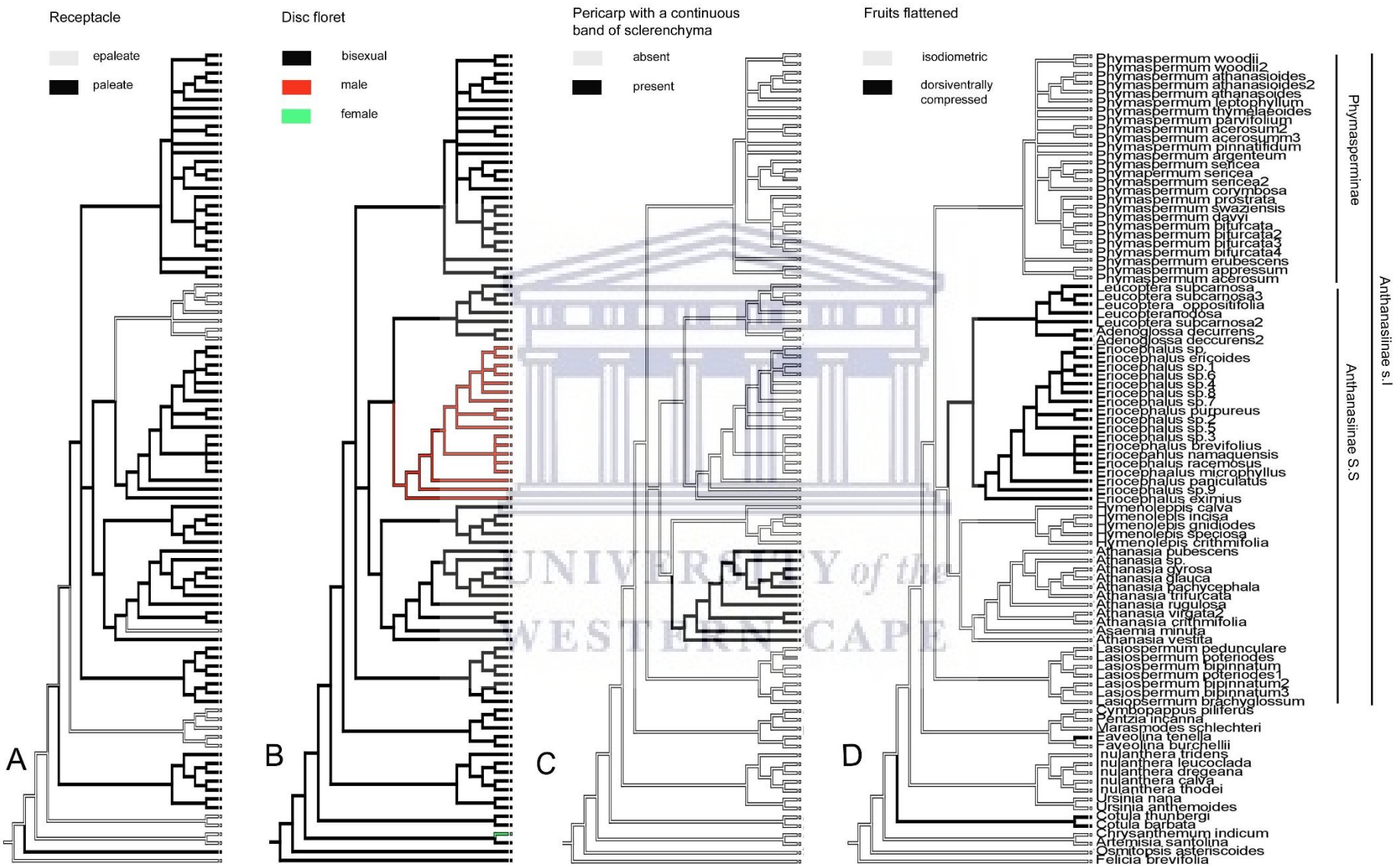


Figure 4.5: Parsimony-based character reconstruction of the receptacle, disc florets, pericarp and cypselas on the Majority -Rule consensus tree from the Bayesian Inference (BI) analysis of the combined nuclear dataset.

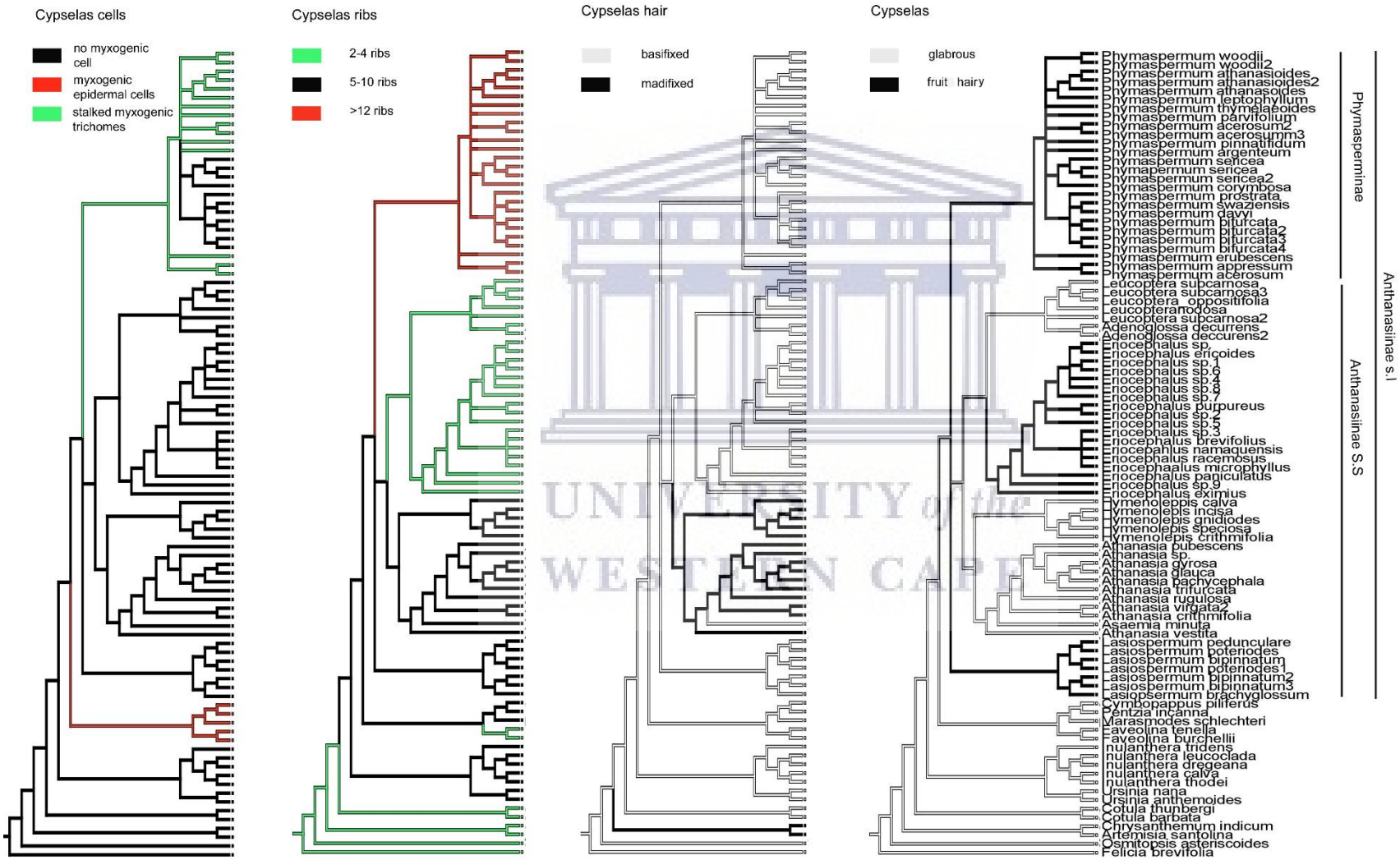


Figure 4.6: Parsimony-based character reconstruction of cypsela characters on the Majority-Rule consensus tree from the Bayesian Inference (BI) analysis of the combined nuclear dataset.

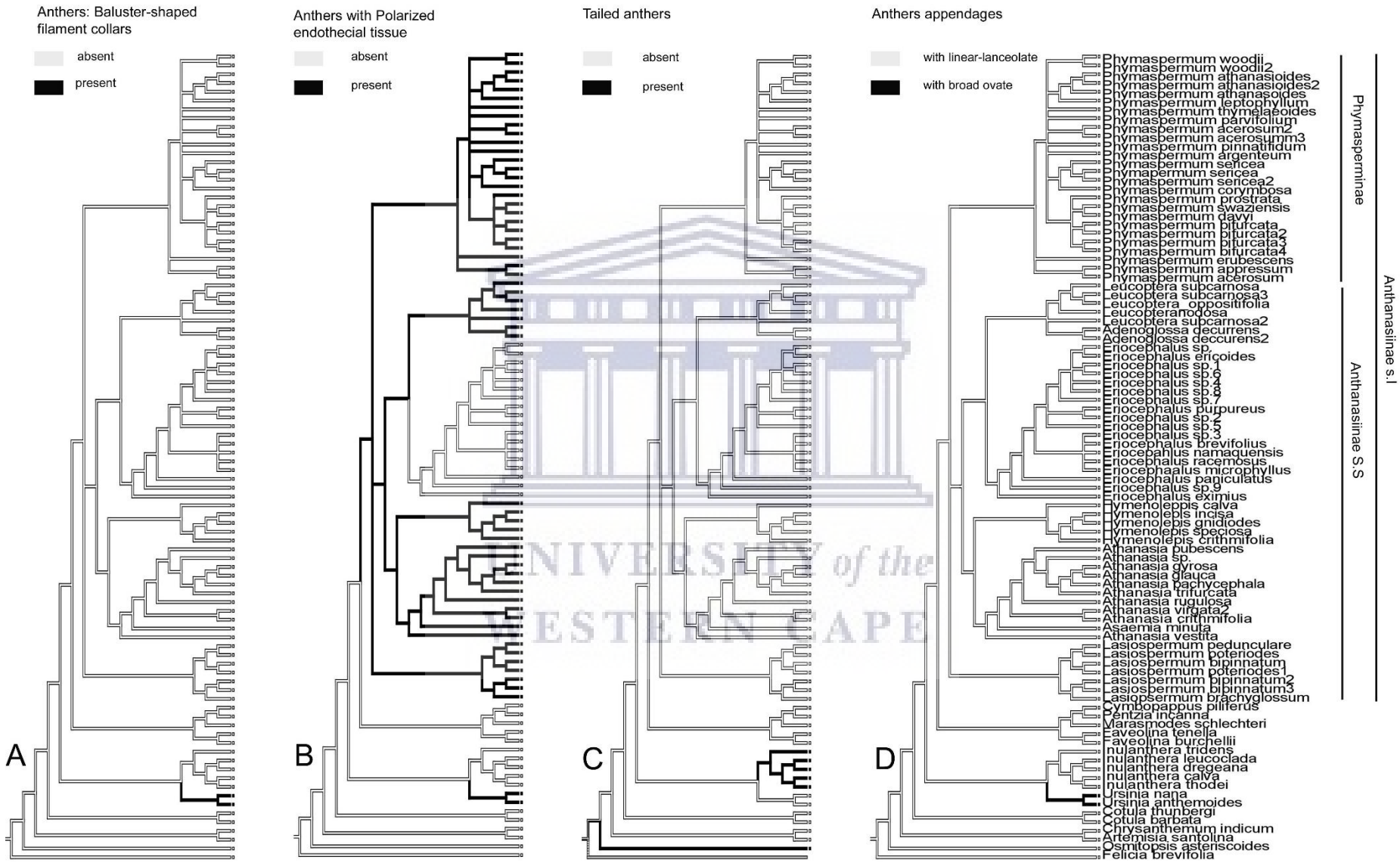


Figure 4.7: Parsimony-based character reconstruction of anther characters on the Majority-Rule consensus tree from the Bayesian Inference (BI) analysis of the combined nuclear dataset.

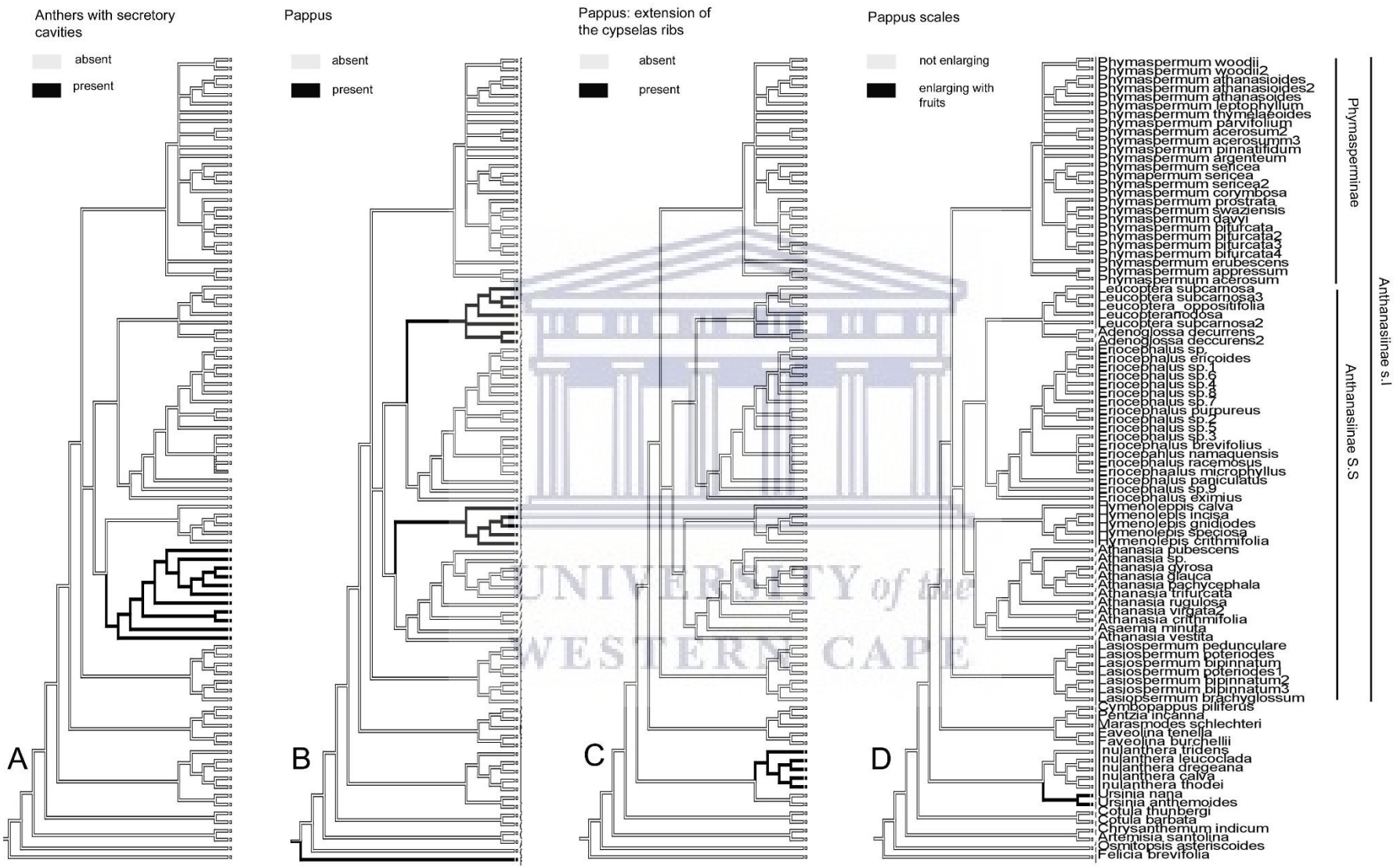


Figure 4.8: Parsimony-based character reconstruction of anther and pappus characters on the Majority-Rule consensus tree from the Bayesian Inference (BI) analysis of the combined nuclear dataset.

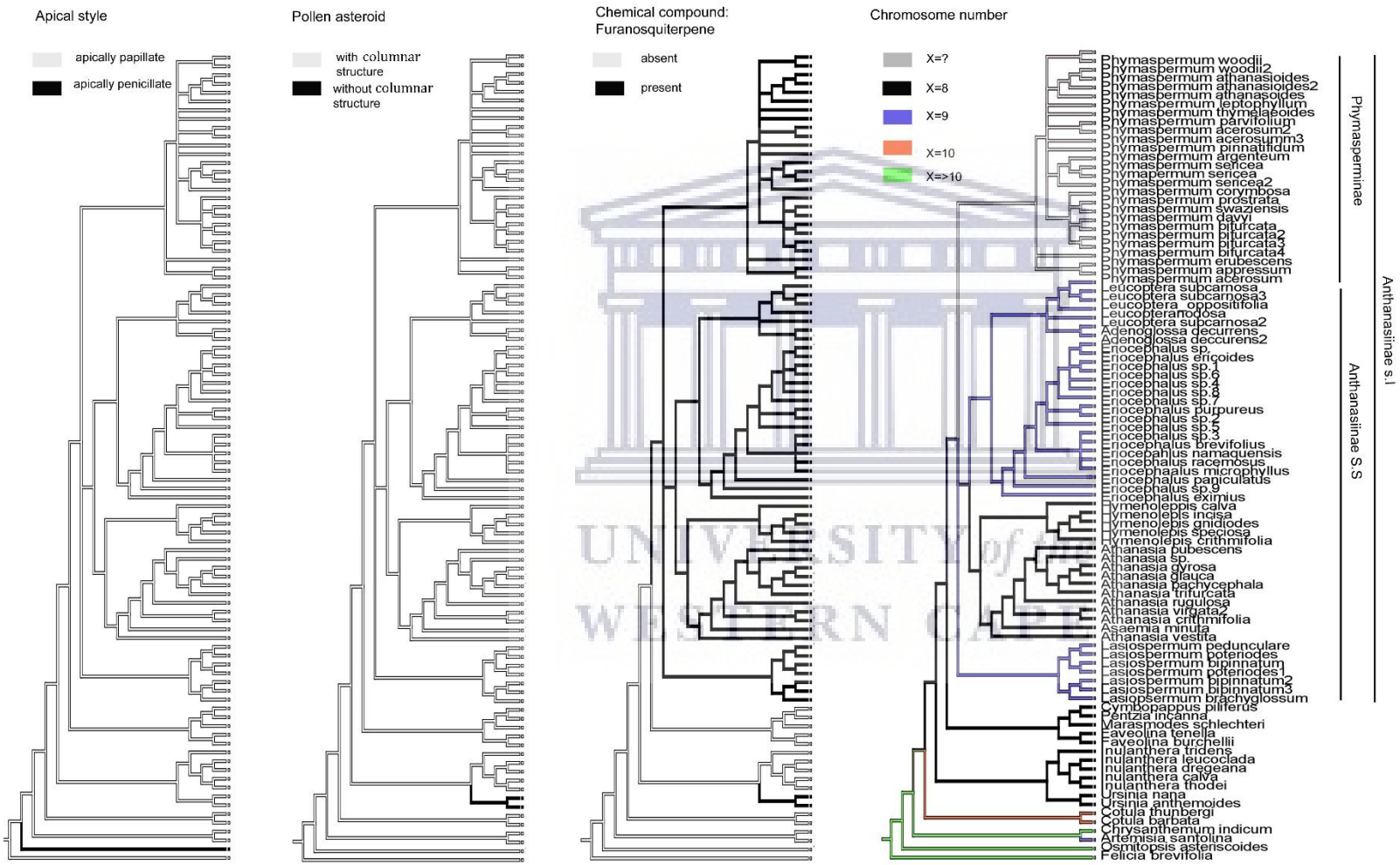


Figure 4.9: Parsimony-based character reconstruction of apical style, pollen asteroid, chemical compounds and chromosome number on the Majority-Rule consensus tree from the Bayesian Inference (BI) analysis of the combined nuclear dataset.

4. 4. Discussion

4. 4. 1. Monophyly of the subtribes Athanasiinae and Phymaspeminae

Expanded sampling in phylogenetic studies has been shown to decrease the amount of non-phylogenetic signal, while preserving the phylogenetic signal from which to assess subtribal and generic circumscriptions (Pick et al. 2010, Philippe et al. 2011; Echternacht et al. 2014). This was found in the present study, where the expanded sampling of Athanasiinae and Phymaspeminae, which included 78 of the 107 species (73%), revealed the phylogenetic relationship between the subtribes Athanasiinae and Phymaspeminae for the first time. These results differed from those shown by Watson (2000), Oberprieler et al. (2007, 2009) and Himmelreich et al. (2008), most notably in the position of Phymasperminae and the monophyly of Athanasiinae.

In the present study, the position of Phymasperminae within Athanasiinae was consistently supported in the nuclear, plastid as well as in combined analyses (Fig. 4.1- Fig. 4.3). The previous incongruence between the nuclear and plastid regions (Watson 2000, Oberprieler et al. 2007, 2009, Himmelreich et al. 2008) was here resolved with the result that these datasets could be combined. The difference in the topologies was largely due to the denser sampling within taxa of Athanasiinae and Phymasperminae. The relationship between Phymasperminae and Athanasiinae was strongly supported in the plastid and combined nuclear-plastid analyses (PP 1.0, BP 100%). Additionally this relationship is supported by the following synapomorphies: anthers with polarized endothecial tissue, the presence of furanosesquiterpenes, and the leaves with secretory cavities (Fig. 4.4B, 4.7B and 4.9C, Källersjö 1986).

The subtribe Athanasiinae is for the first time recovered as monophyletic, although only in the combined nuclear and plastid analyses (Fig. 4.3). In the nuclear analyses (Fig. 4.1) the position of *Lasiospemum* was unresolved while in the chloroplast analyses (Fig.4.2) *Lasiospemum* was recovered in the Athanasiinae clade but with *Leucoptera* and *Adenoglossa* weakly supported as sister to Phymasperminae. In both the nuclear (Fig.4.1) and combined nuclear and plastid analyses (Fig.4.3) the *Leucoptera* subclade was recovered as sister to *Eriocephalus*. The latter grouping is supported by the shared few ribbed and dorsiventrally flattened cypselas (Fig. 4.5D, Fig. 4.6B, Bremer and Humphries 1993). In all previous analyses (Watson 2000, Oberprieler et al. 2007, 2009, Himmelreich et al. 2008) Athanasiinae as currently circumscribed has no

morphological synapomorphies and has consistently been recovered as paraphyletic in previous molecular phylogenetic studies (Oberprieler et al. 2007, 2009) and Himmelreich et al. 2008).The previous nuclear analyses of Oberprieler et al. (2007, 2009) and Himmelreich et al. (2008) recovered Athanasiinae and Phymasperminae as sister, while the plastid analyses recovered Athanasiinae as sister to a broader that included Pentziinae, Phymasperminae, Artemisiinae as well as the Eurasian grade-Mediterranean clade. The present analyses (Fig. 4.1- Fig. 4.3) revealed that Athanasiinae and Phymasperminae form a monophyletic clade, although with both subtribes recovered as monophyletic only in the combined nuclear and plastid analyses (Fig.4.3). This clade is strongly supported by the following morphological synapomorphies: anthers with polarized endothecial tissue, presence of furanosesquiterpenes and leaves with secretory cavities (Fig. 4.7B, 4.9C and 4.4B).

Two options for the circumscription of Athanasiinae and Phymasperminae were considered based on these results. In the first option the two subtribes are retained, despite uncertainty about the monophyly of Athanasiinae in the separate plastid and nuclear analyses. While Phymasperminae could be circumscribed by the cypselas with more than 10 ribs and the papillose pericarp, the circumscription of Athanasiinae would be problematic. No synapomorphies were found to circumscribe Athanasiinae s.s., as the characters previously used to circumscribe it are shared with the closely related Phymasperminae. In the second option Athanasiinae is expanded to include Phymasperminae. The monophyly of an expanded Athanasiinae (hereafter referred to as Athanasiinae s.l.) is supported in all analyses (nuclear, plastid as well as combined; Fig. 4.1- Fig. 4.3). Furthermore, Athanasiinae s.l. could be readily circumscribed by at least three synapomorphies (viz. the anthers with polarized endothecial tissues, the leaves with secretory cavities and the presence of furanosesquiterpenes).

As an expanded Athanasiinae s.l. (including Phymasperminae) is readily and more convincingly circumscribed morphologically and supported in all analyses, the second option is favoured here and the necessary changes made below.

Athanasiinae (Less) Lindl. ex Pfeiff.; emend. S.Akimana, Boatwr. & Magee, **emend. nov.**

Type: *Athanasia* L. *Athanasia crithmifolia* (L) L.

Phymasperminae Oberpr. & Himmelreich, **syn. nov**.

Type: *Phymaspermum* Less. [*Phymaspermum junceum* Less.].

Athanasiinae s.l. can be distinguished from other subtribes in the Anthemideae by the presence of furanosequiterpenes, the anthers with polarized endothecial tissue (secondarily lost in genus *Eriocephalus*) and the leaves with ellipsoid secretory cavities. The expanded Athanasiinae s.l. comprises of six genera distributed in southern Africa with a single species, *Lasiospermum brachyglossum* DC. occurring also in the Sinai desert of Egypt.

4.4.2. Generic status and relationships of *Asaemia*

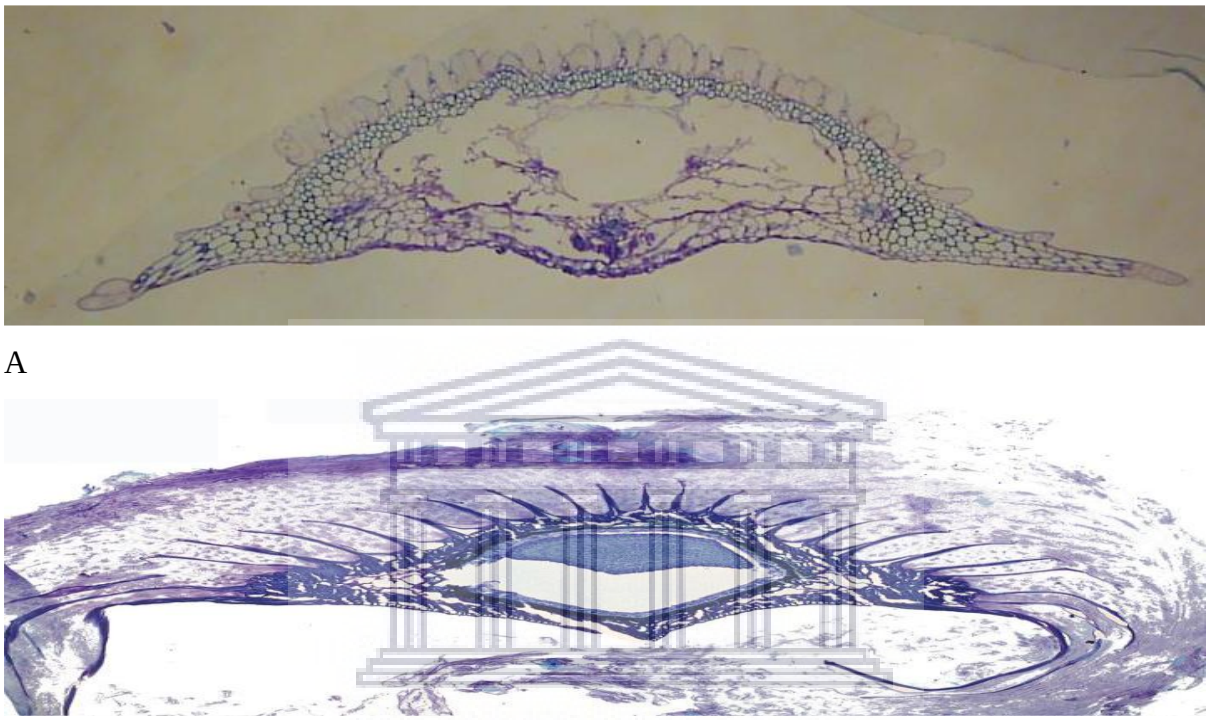
When described by Bremer (1983), the monotypic genus *Asaemia* was shown to share morphological characters with *Athanasia*. *Asaemia* was distinguished from *Athanasia* by the opposite leaves and the dorsiventrally flattened cypselas usually with one ventral and two lateral ribs (Bremer 1983). Källersjö (1991) as part of her taxonomic revision subsequently included *Asaemia* in her concept of *Athanasia*. This decision was based on the shared presence of secretory cavities in the apical parts of anthers (Fig. 4.8A, Källersjö 1986) as well as the well- developed pericarp with a continuous ring of sclerenchyma (Fig. 4.5C, Källersjö 1986), both characters were recovered as synapomorphies in the present reconstructions. The inclusion of *Asaemia* in *Athanasia* has, however, not been widely accepted (Bremer and Humphries 1993; Goldblatt and Manning 2002, Germishuizen and Mayer 2003, Manning and Goldblatt 2012, and Snijman 2013).

Asaemia was included in molecular phylogenetic analyses for the first time in this study. The results from separate nuclear, plastid and combined nuclear and plastid analyses clearly show that *Asaemia* is embedded within *Athanasia* (Fig. 4.1- Fig. 4.3) and supports Källersjö's (1991) broader generic concept.

4.4.3. Inclusion of the monotypic genus *Adenoglossa* in *Leucoptera*

The genus *Leucoptera* is an erect perennial, slender, shrublets with alternate or opposite somewhat fleshy leaves, with the capitula solitary, long-pedunculate and radiate. It is endemic to the Western Cape Province of South Africa (Nordenstam 1976, Bremer and Humphries 1993). The closely related monotypic genus *Adenoglossa* is an erect annual herb with alternate or opposite leaves, narrowly linear, entire and fleshy with the capitula solitary, long-pedunculate and radiate. It is endemic to in the Northern Cape Province (Nordenstam 1976, Bremer and Humphries 1993). Nordenstam (1976) described these two closely related genera with the only distinction being the annual or perennial life histories (Fig. 4.4A).

Bremer and Humphries (1993) retained *Leucoptera* and *Adenoglossa* as distinct sister genera with the following morphological synapomorphies supporting their close relationship: the cypselas with dorsiventrally compressed, 3-ribbed lateral wings, the presence of an epaleate receptacle and the presence of a pappus (Fig. 4.5A, 4.5D, 4.8B, Nordenstam 1976 and Bremer and Humphries 1993).



A

B

Figure 4.10: Dorsiventrally compressed, 3-ribbed cypselas with lateral wings of (A) *Leucoptera subcarnosa* Magee & Boatwright 332 (NBG) and (B) *Adenoglossa decurrens* Mucina 030906/24 (NBG). Sectioned by Prof P.M.Tilney.

The phylogenetic results from the separate nuclear, plastid, and combined nuclear and plastid analyses presented here confirmed the sister relationship between *Leucoptera* and *Adenoglossa* (Fig. 4.1- Fig. 4.3).

Annual and perennial species are found in several other genera within the tribe Anthemideae, e.g. *Lasiospermum*, *Cotula*, and *Ursinia*. As *Adenoglossa* and *Leucoptera* differ only in their annual vs perennial life histories (Fig. 4.4A, Nordenstam 1976), we here reduce *Adenoglossa*

into synonymy with *Leucoptera*. This is warranted by the numerous synapomorphies shared by the two. The nomenclatural changes for the genus *Leucoptera* are made below:

Leucoptera B. Nord.in Bot. Notiser 129: 141 (1976). Type: *Leucoptera nodosa* (Thunb.) B.Nord.
Adenoglossa B.Nord.in Bot. Notiser 129: 137 (1976), **syn. nov.** Type: *Adenoglossa decurrens* (Hutch) B.Nord.

The expanded genus *Leucoptera* includes four species endemic to the Greater Cape Floristic Region of South Africa. Taxonomic descriptions and complete synonymies can be found in Nordenstam (1976).

List of species:

1. *Leucoptera decurrens* (Hutch.) S.Akimana, Boatwr. & Magee, **comb. nov**. *Adenoglossa decurrens* (Hutch.) B. Nord., Bot.Notiser 129: 137 (1976). *Chrysanthemum decurrens* Hutch., Bull. Misc. Inform. Kew 1917(3): 116 (1917). Type: Bolus 9571, L.Namaqualand Div., in dry places between Port Nolloth and Oograbies, ca.300ft, VIII 1883 (K holotype, BOL isotype).
2. *Leucoptera nodosa* B. Nord.
3. *Leucoptera oppositifolia* B. Nord.
4. *Leucoptera subcarnosa* B. Nord.

4.4.4. Phylogenetic position of *Inulanthera*

Inulanthera was described by Källersjö (1986) as a genus having tailed anthers, cypselas lacking ellipsoid, secretory cavities and elongated cells in the fruit ribs (Magoswana et al .2016). The tailed anther is a generic synapomorphy for *Inulanthera*, but within tribe Anthemideae are also found in *Osmitopsis* Cass., the earliest diverging lineage of the tribe Anthemideae (Bremer and Humpries 1993; Oberprieler et al. 2009).

The phylogenetic position of *Inulanthera* was unresolved in the subtribal classification of Oberprieler et al. (2007). Källersjö (1986) suggested that the closest relatives of *Inulanthera* are two genera from the Canary Islands, *Gonospermum* Less. and *Lugoa* DC., with which it shared glandular-punctate leaves with broad and rounded leaf lobes in some species of *Inulanthera* (*I. brownii* Källersjö, *I. nuda* Källersjö and *I. schistostephioides* Källersjö). The study of Bremer and Humphries (1993) treated *Inulanthera* as a member of the polyphyletic subtribe Gonosperminae, but the molecular reconstructions of Oberprieler et al. (2007, 2009) suggested a closer relationship with *Ursinia*. The phylogenetic analyses of Himmelreich et al. (2008) based on cpDNA *ndh*F sequences placed *Inulanthera* in a well-supported clade sister to *Ursinia*, while the analysis based on ITS sequence data revealed that *Inulanthera* and *Ursinia* were not associated with one other, rather the position of *Inulanthera* was unresolved. Oberprieler et al. (2009), refrained from including *Inulanthera* in Ursiniinae as *Inulanthera* is morphologically very different from *Ursinia*. Due to the unresolved and potentially isolated phylogenetic position of *Inulanthera*, Oberprieler et al. (2009) deemed it premature to describe an independent subtribe for *Inulanthera* until further investigations with a broader tribal sampling were conducted.

The phylogenetic study of Magoswana et al. (2016) based on the ITS region revealed the monophyly of *Inulanthera* and that it was sister to Athanasiinae, which was in contrast with the molecular reconstructions of Oberprieler et al. (2007, 2009) and those of Himmelreich et al. (2008). The phylogenetic analyses performed in the present study (Fig. 4.1- Fig. 4.3) confirm the monophyly of *Inulanthera* which is supported by the following synapomorphies: A pappus is an extension of the cypselas ribs with each rib extended as a small horn or as a scale (Fig.4.8C, Källersjö 1986, Magoswana et al. 2016) and the tailed anthers shared with *Osmitopsis* (Fig. 4.7C, Magoswana et al. 2016).

Inulanthera was recovered as sister to Athanasiinae s.l. in the nuclear analyses (Fig 4.1), and with a Penztiinae-Ursiniinae clade in plastid analyses (Fig. 4.2). *Inulanthera* is morphologically distinct from Ursiniinae by having pollen asteroids with a columnar structure while Ursiniinae has pollen asteroids without a columnar structure of the exine (Fig. 4.9B, Oberprieler et al. 2007, Magoswana et al. 2016). Furthermore, Ursiniinae has the anthers with broad ovate apical appendages (Fig. 4.7D, Bremer and Humpries 1993) and baluster-shaped filament collars (Fig. 4.7A, Oberprieler et al. 2007), while *Inulanthera* has conspicuously tailed anthers (Fig. 4.7C, Källersjö 1986). Athanasiinae is also morphologically distinct from *Inulanthera* by its anthers with polarized endothecial tissue, the presence of furanosesquiterpenes and the leaves with secretory cavities (Fig. 4.4B, 4.7B and 4.9C, Källersjö 1986). These results and morphological characters demonstrate that *Inulanthera* can not be accommodated within either Ursiniinae or Athanasiinae and as a result we here describe a new subtribe to accommodate it.

Inulantherinae S.Akimana, Boatwr. & Magee, **subtrib. nov.**
Type: *Inulanthera* Källersjö.

Inulantherinae is a monogeneric, southern African subtribe that shares the tailed anthers with Osmitopsiinae, but distinguished by its discoid capitula (generally in corymbs), apically papillate fruit and linear styles. The subtribe differs further from Athanasiinae by the lack of polarized endothecial cells, secretory cavities and furanosequiterpenes.

CHAPTER 5

GENERAL CONCLUSIONS

The broader sampling of taxa, additional gene regions and reconstruction of morphological characters have provided several novel insights into the relationship and generic circumscriptions within the subtribes Athanasiinae and Phymasperminae.

The clade including *Phymaspermum*, *Eumorphia* and *Gymnopentzia* (previously subtribe Phymaperminae) was confirmed to be monophyletic and strongly supported by the synapomorphic papillose pericarp and ten or more ribs of the cypselas. While *Phymaspermum* and *Gymnopentzia* could be easily circumscribed (stalked glandular trichomes in *Phymaspermum*, long papillose cypselas and bifurcate leaves in *Gymnopentzia*), the circumscription of *Eumorphia* was shown to be problematic with most characters shared with either *Phymaspermum* or *Gymnopentzia* (ie. opposite leaves, radiate heads). *Phymaspermum* was therefore expanded to include *Eumorphia* and *Gymnopentzia* and these three main lineages recognised at sectional levels. The expanded circumscription is supported by the following morphological synapomorphies: the cypselas with more than ten ribs and the papillose pericarp

An expanded phylogenetic sampling of Athanasiinae and Phymasperminae recovered Athanasiinae as sister to Phymasperminae and supported the monophyly of Athanasiinae (although in some analyses only). No morphological synapomorphies were identified to support the current circumscription of Athanasiinae. The phylogenetic position of Phymasperminae sister to (or within in some analyses) Athanasiinae was consistently supported in all analyses performed in Chapter 3 and Chapter 4. The Phymasperminae-Athanasiinae clade was supported by the anthers with polarized endothecial tissue, the presence of furanosesquiterpenes and leaves with secretory cavities. While Phymasperminae could be circumscribed by the cypselas with more than ten ribs and the papillose pericarp, the circumscription of Athanasiinae would be problematic as there were no synapomorphies to circumscribe it. It was noted that Athanasiinae shares several synapomorphic characters with Phymasperminae and as a result the circumscription of Athanasiinae s.l. was expanded to include Phymasperminae.

Generic circumscriptions within the Athanasiinae s.l. were also explored. The two small and closely related genera *Leucoptera* and *Adenoglossa* were shown to be sister lineages in all phylogenetic analyses. These two closely related genera differ however only in their annual vs perennial life histories and share several synapomorphies. Annual and perennial species are found in several other genera within the tribe Anthemideae, e.g. *Lasiospermum*, *Cotula,* and *Ursinia*. *Adenoglossa* was therefore reduced into synonymy with *Leucoptera* and the expanded genus is recognised by the cypselas with dorsiventrally compressed, 3-ribbed lateral wings, the presence of an epaleate receptacle and the presence of a pappus.

The monotypic genus *Asaemia* was included in molecular phylogenetic analyses here for the first time and clearly shown to be embedded within *Athanasia* in all analyses. These results support Källersjö's (1991) broader generic concept of *Athanasia* which includes *Asaemia* as a synonymy. Although subsequent authors have been hesitant to follow this broader generic concept (Bremer and Humphries 1993; Goldblatt and Manning 2002, Germishuizen and Mayer 2003, Manning and Goldblatt 2012, and Snijman 2013), the result presented here finally provide clarity on this question.

The position of the southern African genus *Inulanthera* in the tribe Anthemideae is here resolved. The genus has previously been unplaced in the new subtribal classification of the tribe. While the exact phylogenetic position of the genus is still unresolved due to incongruence between the nuclear and plastid analyses, despite the expanded sampling, the phylogenetic results consistently support the fairly isolated position of the genus in Anthemideae. As a result, we describe here a new independent subtribe to accommodate the genus, viz. Inulantherinae S.Akimana, Boatwr. & Magee, differing from other subtribes by the tailed anthers, fruits lacking secretory cavities, elongated cells in the ribs and its pappus is an extension of the cypselas ribs, with each rib extended as a small horn or as a scale.

Table 5.1: Summary of generic and subtribal circumscriptions within the subtribes Athanasiinae s.l. and Inulantherinae s.l. Genera and subtribes altered in this study are indicated in bold.

BEFORE STUDY		**AFTER STUDY**	
GENUS	SUBTRIBE	GENUS	SUBTRIBE
***Athanasia* s.s**	**Athanasiinae s.s.**	***Athanasia* s.l** (including *Asaemia*)	**Athanasiinae s.l.**(including Phymasperminae)
Asaemia			
Hymenolepis		*Hymenolepis*	
Adenoglossa		***Leucoptera* s.l.** (including *Adenoglossa*)	
***Leucoptera* s.s.**			
Eriocephalus		*Eriocephalus*	
Lasiospermum		*Lasiospermum*	
Gymnopentzia	**Phymasperminae**	***Phymaspermum* s.l.** (including *Gymnopentzia* and *Eumorphia*)	
Eumorphia			
***Phymaspermum* s.s.**			
Inulanthera	**Unplaced**	*Inulanthera*	**Inulantherinae**

CHAPTER 6

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APPENDIX

Appendix A. Coded morphological characters and character states.

Appendix A1. Morphological characters and character states coded in morphological cladistics analysis for expanded genus *phymaspermum.*

1. Life history: single-stemmed = 1; multistemmed = 2, **2. Leaf arrangement:** alternate =0, opposite = 1, **3. Receptacle:** epeleate =0, Marginal paleate=1, completely paleate=2, **4. Receptacle:** Epaleate=0, paleate=1, **5.Capitula form:** Discoid =0, radiate =1. **6. Papillose cypselas:** Globose=0, Fruit hairs with long papillose=1, Short papillose=2, **7. Cypsela ribs:** >10-ribbed=0, < 10-ribbed=1, **8. Glandular trichomes:** absent=0, present=1.

Appendix A2. Morphological characters and characters state codes in morphological cladistics analysis for species of subtribe Athanasiinae.

1. Longevity: annual=0, perennial=1, **2. Leaves with secretory cavities:** absent=0, present=1, **3. Paleae:** not around fruits=0,rapping around fruits=1, **4. Receptacle :** epaleate=0, paleate=1, **5. Disc florets:** bisexual=0,male=1,female=2, **6. Pericarp with a continuous band of sclerenchyma:**absent=0,present=1,**7. Cypselas:**isodiometric=0,dorsiventrally compressed=1, **8. Cypselas cells:** no myxogenic cells=0,myxogenic with epidermal cells =1,myxogenic with stalked trichomes=2, **9. Cypselas ribs:** 5-ribs=0, 8-10 ribs=1, >10ribs=2, 2-4 ribs=3, **10. Cypsela Hairs:** basifxed =o, medifixed=1, **11. Cypselas:** glabrous=0, hair=1, **12. Anthers: baluster shaped filament collars:** absent=0, present=1, **13. Anthers with polarized endothecial tissue:** absent=0, present=1, **14. Tailed anthers:** obtuse anthers at the base=0, conspicuous tailed anthers =1, absent=2, **15. Anthers appendages:** with triangular-linear-lanceolate=0, broadly ovate=1, **16. Anthers with secretory cavities:** absent=0, present=1, **17. Pappus:** absent =0, present=1, **18. Pappus:** normal=0, an extension of the cypsela ribs=1, **19. Pappus scales:** not enlarging=0, enlarging with fruits=1, **20. Apical style:** apically papillate=0, apically penicillate=1, **21. Pollen asteroid:** with columnar structure=0, without columnar structure=1, **22. Chemical compound:** furanosquiterpenes: absent=0, present=1, **23. Chromosomes numbers:** (x=8)=0,(x=9)=1,(x=10)=2,(x>10)=3,Unkown=?.