PHYLOGENETIC ANALYSES AND TAXONOMIC STUDIES OF SENECIONINAE: SOUTHERN AFRICAN SENECIO SECTION SENECIO

Joseph J. Milton

A Thesis Submitted for the Degree of PhD
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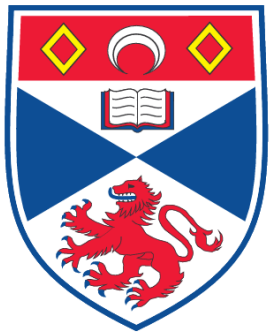

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PHYLOGENETIC ANALYSES AND TAXONOMIC STUDIES OF SENECIONINAE: SOUTHERN AFRICAN *SENECIO* SECTION *SENECIO*

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**A thesis submitted to the
University of St Andrews for
the degree of Doctor of Philosophy**

**School of Biology
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ABSTRACT

Molecular phylogenetic analyses of subtribe Senecioninae, based on combining sequenced *ITS* and *trn*L-F fragments from specimens collected in the field with sequences collected from GenBank, suggest the subtribe is monophyletic, as is *Senecio* s.str. (including *Robinsonia*), and suggest an expanded monophyletic section *Senecio*. Many *Senecio* species should be removed from the genus, as they are only distantly related to it, emphasising the para- or polyphyletic nature of *Senecio* as it is currently circumscribed.

Area optimisation suggests southern Africa as a possible geographical origin for the genus and section. Harvey's (1865) sectional classification of South African *Senecio* species (the only attempt to date to impose infrageneric groupings on these taxa), was tested for monophyly which, however, was not seen in the sections tested. A number of southern African species from Harvey's sections are suggested for inclusion in an expanded section *Senecio*.

A clade suggested as basal to sect. *Senecio,* consisting of *Senecio engleranus* and *Senecio flavus,* was found to be only distantly related to the section. Resolution of the two species within the clade was not evident; a comparative study was therefore made employing RAPDs, morphometrics and breeding experiments. The two proved to be distinct entities, both genetically and morphologically, although they remain interfertile, suggesting that intrinsic postzygotic barriers between them are weak, and that hybridisation – not found in the wild - is mainly prevented by prezygotic barriers. F1 hybrids created between the two were seen to have intermediate morphologies and RAPD profiles. A single F1 individual self-pollinated to produce a vigorous F2 generation, allowing preliminary surveys of pollen number, pollen fertility and pappus type. Pappus type is seen to be under the control of allelic variations in a single major gene, while pollen numbers and pollen fertility are seen to be under more compex genetic control.

DECLARATION

I, Joseph Milton, hereby certify that this thesis, which is approximately 39,000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

Joseph Milton

May, 2009

STATEMENT

I was admitted as a research student in October, 2003 and as a candidate for the degree of PhD in October, 2004; the higher study for which this is a record was carried out in the University of St Andrews between 2003 and 2009.

Joseph Milton

May, 2009

CERTIFICATE

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of PhD in the University of St Andrews and that the candidate is qualified to submit this thesis in application for that degree.

Richard J. Abbott

May, 2009

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Justice catches up with Professor Abbott: Richard in the Old Gaol Backpackers Hostel in Grahamstown, South Africa.

CONTENTS

CHAPTER 1:

GENERAL INTRODUCTION

1.1: The genus *Senecio*

Senecio L. (Asteraceae Bercht. & J. Presl.; Senecioneae Cass.) is one of the largest genera of flowering plants, containing somewhere between 1,000 and 3,000 species (Jeffrey et al., 1977; Nordenstam, 1978; Bremer, 1994; Vincent, 1996; Mabberley, 1997). The most recent and reliable estimate suggests that it comprises approximately 1,250 species (Nordenstam, 2007).

The name *Senecio* comes from the Latin “senex” which means “old” (an old man), in reference to the grey pappus (formed from modified calyces) found on the cypsela (the fruit) (Johnson & Smith, 1947). The genus is almost cosmopolitan in distribution, with the type species *Senecio vulgaris* L. (Figs. 1.1 and 1.2) being found throughout the world, with the exception of the Caribbean and Antarctica. *Senecio vulgaris,* and many other members of the genus, are weedy annual herbs which thrive in disturbed environments, and therefore enjoy considerable success in the modern world - thanks to the effects of human activities.

In the UK, the genus is best known for the weedy species it contains, such as *S. vulgaris* and the introduced and invasive *S. squalidus* L. These two species have hybridised, and the resulting hybrids have undergone an allopolyploid event, forming the sexual hexaploid, *S. cambrensis* Rosser. This event occurred independently in both North Wales and Scotland, and thus the allohexaploid originated on at least two occasions (Ashton & Abbott, 1992b), although the Scottish lineage became extinct after persisting for approximately 19 years (Abbott & Forbes, 2002). Similar hybridisation events resulting in the origin of allopolyploid species are thought to

have occurred in other parts of the world, leading, for example, to the origin of *S. teneriffae* Schultz. Bip. in the Canary Islands (Lowe & Abbott, 1996) and *S. mohavensis* A. Gray in North Africa (Coleman et al., 2001; Kadereit et al. 2006).

Figure 1.1: *Senecio vulgaris.*

Figure 1.2: Pappus on the cypsela of *S. vulgaris*

Although diagnostic morphological features in *Senecio* are lacking, in gross morphology the genus is generally characterised by capitulum inflorescences (which can be either heterogamous, made up of disc and ray florets, or homogamous with only disc florets), calyces modified into a pappus of hairs, and an involucrum of fused, uniseriate bracts. Most other features vary widely throughout the genus (Alexander, 1979).

Senecio is noted for its highly toxic members, such as *S. jacobaea* L., which contain pyrrolizidine alkaloids, and are responsible for the deaths of a large number of domestic livestock around the world every year. These toxic species may be found growing in disturbed pasture and as weeds of agricultural land; as a result, hay

containing *Senecio* species can end up being fed to livestock. Human deaths have also been recorded in South Africa, where toxic *Senecio* species can be a contaminant of flour used in bread making, and are sometimes used as constituents of unlicensed herbal medicines (Abbott, personal communication).

The genus is very diverse in life history, morphology, and growth form, containing succulents (e.g. *S. pyramidatus* DC.), annuals (e.g. *S. vulgaris*), perennials (e.g. *S. rigidus* L.), semi-aquatic forms (e.g. *S. aquaticus* Hill.), climbers and stragglers (e.g. *S. oxyodontus* DC.), and shrubs (e.g. *S. lyratus* Forssk.). This wide range of diversity would appear to be an example of adaptive radiation, brought about by natural selection favouring types that are adapted to different ecological niches. However, there is also great diversity amongst morphologically similar weedy species which occur sympatrically. These species are recognisable to taxonomists as distinct entities, although identifications can be very difficult with many of them. Some of these taxa exist in complexes of almost or completely indistinguishable species [for example, *S. madagascariensis* Poir., *S. burchellii* DC., *S. pellucidus* DC. and *S. inaequidens* DC. form such a complex in southern Africa (Hilliard, 1977)]. In these cases, the separation into distinct species may even be erroneous because, based on the observation of only a small number of specimens, overlap in characters that exhibit continuous variation has not been recognised. Herbaria contain a large number of misidentified specimens (personal observation), which may have led researchers to treat specimens as different taxa when they are not. Nevertheless, there is certainly unusually high diversity among similar weedy species. This might have been caused by allopatric distributions in the past leading to evolutionary divergence, followed by a return to sympatry casued by climatic change or human activitity. Many of these weedy species are found on roadsides and other disturbed areas, and it is possible that

they have expanded their distributions with the development of road and rail networks. For example, it is known that *S. squalidus* (the Oxford Ragwort) spread throughout the UK along railway lines after escaping from the botanical gardens at Oxford University in the late 18th Century (Ashton & Abbott, 1992b).

1.2: Classification of the genus *Senecio* and tribe Senecioneae

Historically the distinction between the genus *Senecio* and the tribe in which it resides (Senecioneae) has been confused. A lack of clarity in morphology, uncertain generic limits both of *Senecio* and other closely and less closely related genera, and inconsistency in approaches to classifying the genus or tribe around the world have all created problems for taxonomists working on the group (Jeffrey et al., 1977; Jeffrey, 1979; Vincent & Getliffe, 1992). These issues are discussed in more detail in the introduction to chapter 2, which summarises the taxonomic history of both genus and tribe.

1.3 Aims of research

The principal aims of the research reported in this thesis were to identify potential members of section *Senecio* in southern Africa and investigate the relationships between them and other taxa from the subtribe Senecioninae J. Presl. In addition, relationships in the wider subtribe were investigated. Southern African species were collected in the field, DNA extracted from them, and fragments of interest sequenced for inclusion in wider phylogenetic analysis of Senecioninae, with the object of identifying those taxa which are most closely related to the type species *S. vulgaris*, and which might therefore belong in section *Senecio*. A large number of sequences from the subtribe were collected from GenBank to enable the placing of

these taxa in a wider phylogeny and to investigate other relationships in Senecioninae. Results of the phylogenetic analyses are presented in chapter 2.

The secondary aim of the research was to investigate the sister species pair (*Senecio engleranus* O.Hoffm. and *Senecio flavus* (Decne.) Sch.Bip.) suggested as basal to section *Senecio* by previous phylogenetic analysis of *ITS* sequence variation at the University of St Andrews (Coleman, 2002; Coleman et al., 2003). The affinities of the pair were investigated as part of the wider phylogenetic study, and their status as separate species examined using randomly amplified polymorphic DNA (RAPD) analysis and analysis of morphological traits. The interfertility of the two species was investigated through breeding experiments, followed by pollen counts and the estimation of pollen fertility in the resulting progeny. Results of this comparative study are presented in chapter 3.

1.4: Phylogenetic analysis

1.4.1: An introduction to phylogenetics

Phylogenetics is an approach to biological classification concerned with reconstructing evolutionary history and recovering the history of speciation (Thain & Hickman, 1995). Molecular phylogenetic techniques now use sequenced fragments of DNA to build phylogenetic trees of species. Modern phylogenetic analyses are carried out using a variety of methods, including maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI). These methods are discussed in more detail below.

Classification of living organisms has always been a concern of man, and recorded systems of classification date back to the ancient Greeks. Pliny, Aristotle and Theophrastus all produced classifications of living organisms; the system devised

by Theophrastus was widely used for many centuries. It was only finally superseded in the 18th Century, by the work of Carl von Linné (1707-1778), who devised the Latin binomials which are still used to name organisms to this day (von Linné even renamed himself in Latin as Carolus Linnaeus). Linnaeus made what is probably the single greatest contribution to the classification of organisms in the history of mankind, basing his system on floral and body structure. The publication of his *Species Plantarum* in 1753 marked the beginning of a new era in plant taxonomy (Dobzansky et al., 1977).

Historically, classification systems were devised by developing an intuitive picture of relationships, often by one or a few researchers, through observation of specimens in herbaria. (e.g. de Candolle, 1838; Bentham & Hooker, 1862-1883). The main sources of data available historically were micro- and macromorphology, although taxonomists also looked at chemistry, anatomy and embryology. This intuitive approach came to be regarded as unscientific in the early 20th Century, as the resulting classification systems were not repeatable or objective in any way. Two different taxonomists looking at the same plant group often arrived at two different classification systems, because of differences of opinion with regard to which characters are useful for classification (Sokal & Sneath, 1963). Beginning in the 1940s, attempts were made by researchers to tackle this lack of objectivity by using numerical taxonomy. The idea was to add as many different morphological characters as possible to a data matrix, and then create trees based on overall similarity (Gilmour, 1940). There were, however, some serious problems with this method when it was used to look at relationships between taxa at, and above, the species level. Not all morphological characters are indicative of relationships, and any signal as to biologically meaningful groups from truly homologous morphological characters

tended to get lost in the noise created by similarity in other non-informative characters. Hennig (1950; 1966) attempted to overcome this problem with his own method of phylogeny reconstruction, phylogenetic systematics, which later became known as cladistics. He realised that not all morphological features were indicative of evolutionary relationships, and that only shared, advanced characters (synapomorphies) could be used to infer phylogeny. If two organisms shared a primitive character (plesiomorphy), this was no indication that they were closely related. Hennig suggested applying the principle of Occam's razor to analyses of characters, so as to find the simplest solution that explained the data. Hennig also recognised the existence of homoplasy, where characters appear the same through convergent evolution, rather than because of an evolutionary relationship (Hennig, 1950). Homoplasious characters remain a confounding factor in maximum parsimony analyses to this day (Felsenstein, 1978). When Hennig first published his ideas, a heated academic debate, which came to be known as the 'cladist wars', began between proponents of cladistics and those who believed numerical taxonomy was a more suitable way to infer evolutionary history (Ebach et al., 2008). The cladists won, and numerical methods are now generally used to investigate relationships below the species level, rather than to resolve species relationships.

Before the advent of the polymerase chain reaction (PCR) allowed rapid sequencing of DNA, cladistic theory was applied using mainly morphological characters, but it is perhaps more useful in reconstructing phylogenies from DNA data. In morphological terms, it is extremely difficult to be sure that one is looking only at truly synapomorphic characters, as identifying these involves decisions as to which morphological character states are primitive and which are advanced - a subject of heated debate among taxonomists (Sokal & Sneath, 1963). DNA data solved this

problem as there was now no need to decide subjectively which character states were primitive and which were advanced.

More recently, it has been suggested that maximum parsimony analyses of DNA data may not reproduce evolutionary history reliably, as parsimony always looks for the shortest route between sequences, and accepts the simplest explanation for the data. In other words, parsimony will choose trees requiring the fewest mutations to explain the data. This process is only valid if evolution itself always proceeds along the most parsimonious route, a situation which seems very unlikely to be the case. This use of a single mutational map is probably the biggest drawback of MP methods (Holder & Lewis, 2003). Parsimony also suffers from the problem of long branch attraction, where sequences at the end of long evolutionary branches may be similar because of convergent evolution, rather than because of close evolutionary relationship. This is homoplasy and is undetectable in MP analyses, which will group taxa with homoplasious sequences together as if closely related (Felsenstein, 1978).

In an attempt to recover true phylogenetic relationships more accurately, many researchers now use maximum likelihood or Bayesian inference methods to produce phylogenetic trees. Maximum likelihood uses probabilities of change from one character to another to calculate the likelihood that a given phylogenetic tree would lead to the dataset observed. Unlike maximum parsimony, ML takes the possibility of multiple mutational events at the same site into account. This is computationally very intensive and consequently demands a lot of computer time when large data matrices are involved. For this reason, with large datasets of more than 100 taxa, it is impractical to use ML methods (Holder & Lewis, 2003).

Bayesian inference is a recently developed method of phylogeny reconstruction based on the statistical work of Rev. Thomas Bayes (1702–1761)

(Heulsenbeck & Ronquist, 2001). BI methods use a set of prior assumptions about the data matrix to infer the probability that a hypothesis may be true. The posterior probability for a hypothesis is proportional to the likelihood multiplied by the prior probability of the hypothesis. Uninformative prior probability distributions are specified by researchers, with the result that that most of the observed differences in posterior probabilities are caused by differences in likelihood. Bayesian inference analyses allow complex models of molecular evolution to be included (these can cause problems in ML analyses) (Holder & Lewis, 2003). BI calculates posterior probabilities based on new evidence according to Bayes' theorem:

$$P(H|E) = \{P(E|H)P(H)\}/P(E)$$

- H is the hypothesis.
- P(H) is the prior probability of H that was inferred before new evidence, E, became available.
- P(E | H) is the conditional probability of seeing the evidence E if the hypothesis H is true
- P(E) is the marginal probability of E: the *a priori* probability of witnessing the new evidence E under all possible hypotheses.
- P(H | E) is the posterior probability of H given E.

Bayesian inference uses the Markov chain Monte Carlo (MCMC) algorithm, which forms a chain of locations in parameter space. Parameter space, in the case of phylogeny reconstruction, is a description of the tree and the parameters of the specified model of sequence evolution, so the chain moves through different trees and models of evolution. The next link in the chain is chosen by changing a few of the parameters in the present location. If the posterior probability of the new location is higher than the previous location, the new location is accepted as the next link in the chain. If the new location has a lower posterior probability, then it is only accepted as the new position in the chain a proportion (*p*) of the time. The proportion (*p*) is the ratio of the posterior probability of the proposed new location, compared with the posterior probability of the present location. Effectively, small moves downward in

probability are usually accepted, whereas large falls in probability are rejected. If the new position is rejected, the present position is used again as the new position. This process is repeated millions of times, leading to the creation of a chain of locations in parameter space, which tends to stay in areas of high posterior probability. The proportion of time that a chain spends in a region of parameter space is used as an estimate of the posterior probability of the region. At the end of the analysis the result is an estimate of the posterior probability of the given tree being accurate (Holder & Lewis, 2003). Unlike maximum parsimony, in which many equally parsimonious trees are likely to be produced, Bayesian inference produces just one final tree, and gives the posterior probability of each clade as a measure of statistical support for each grouping (Heulsenbeck & Ronquist, 2001).

In this phylogenetic study, two methods of phylogeny reconstruction have been adopted in the interest of comparing results. These were maximum parsimony using PAUP* 4.0b10 (Swofford, 2000) and Bayesian inference using Mr Bayes v.3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003; Ronquist et al., 2005)

1.4.2: Statistical support in phylogenetic analyses

Maximum Parsimony

In MP analyses, statistical support for clades is calculated using a number of different methods. The most commonly used is bootstrapping, in which characters (nucleotides) are randomly removed from the data matrix and replaced with duplications of other characters chosen randomly from the same matrix. The analysis is then run using the modified dataset. This procedure is repeated many times (generally between 10,000 and 100,000), removing and replacing different characters

in each bootstrap replicate, and a percentage figure is given for each clade, representing the proportion of retrieved trees in which the given clade is seen. The higher the bootstrap value, the more robust the clade (Swofford et al., 1996). The acronym MPB is used in the present study to indicate maximum parsimony bootstrap values.

Decay indices (DI) are another statistic used to gain an idea of how robust clades are. Programs like Autodecay (Eriksson, 1998) calculate how many steps would have to be added to the tree in order to collapse the clade in question, giving an integer value for each node. The higher the decay index, the more robust the group (Judd et al., 2002). As the study presented in chapter 2 of this thesis concentrates on Bayesian inference results, DI values have not been included here.

Bayesian Inference

Statistical support for BI analyses is given as a posterior probability for each clade (Holder & Lewis, 2003). How these posterior probabilities are calculated is discussed in detail above. The acronym BPP is used here to denote Bayesian posterior probabilities. BI methods have a tendency to produce very high support values when compared with equivalent maximum parsimony bootstrap values, and it is likely that Bayesian analyses overestimate the posterior probabilities of clades. For this reason, it is worth providing equivalent maximum parsimony bootstrap values for comparison (Alfaro et al., 2003).

1.5: Randomly amplified polymorphic DNA (RAPD) analysis

RAPD analysis (Williams et al., 1990) enables random sampling of the entire genome without the need for sequence data, using decamer oligonucleotide primers, with arbitrary sequences to randomly amplify anonymous regions of DNA. Primer binding sites are spread throughout both nuclear and cytoplasmic genomes in all DNA classes. Reaction products are mixed with ethidium bromide, run on agarose gels and visualised with UV transillumination, producing banding patterns, where each band is thought to represent a diallelic locus (band present or absent). RAPDs are dominant markers, making it impossible to distinguish between a band present-present homozygote and a present-absent heterozygote.

A relatively simple and cost effective way of assessing genetic variation, RAPDs were widely used in population genetic studies in the 1990's (e.g. Glover & Abbott, 1995), but more recently, the reproducibility of RAPD banding patterns has been questioned (Pérez et al, 1998). As a result, use of the technique has become less common, although it is still employed as a relatively cheap and easy way of screening for genome-wide molecular markers. Applications of RAPDs are widespread, including estimating genetic diversity (e.g. Hansen et al., 2000; Torres et al., 2003), investigating hybrid origins of species (e.g. Friesen et al., 1997; De Greef & Triest, 1999; James & Abbott, 2005; Saito et al., 2006), and investigating population structure (e.g. Sales et al., 2001; Chapman & Abbott, 2005). In cases where phylogenetic analyses of available sequence data fail to resolve the relationships between closely related taxa satisfactorily, such as in the case of *S. engleranus* and *S. flavus,* surveys of RAPD variation can be useful in determining whether they are distinct genetic entities, and therefore deserving of separate species status (Comes & Abbott, 2001).

1.6: Morphometric analysis

Morphometric analysis involves the multivariate analysis of a set of quantitative morphological characters of individual specimens of the taxa of interest (sometimes referred to as operational taxonomic units, or OTUs). This is often used to determine whether closely related species have discrete or overlapping morphologies, which may be important in the taxonomic revision of a group.

1.7: Hybridisation Experiments

There are no reports of hybrids between *Senecio engleranus* and *S. flavus* being found in the wild, and currently nothing is known about the nature of reproductive barriers – prezygotic and/or postzygotic - that exist between this pair of sister species. Such barriers often exist between closely related species. In the present study, an examination is made of whether the species may be crossed artificially, in addition to a comparative study of their morphology.

CHAPTER 2:

Molecular phylogenetic analyses of Senecioninae (Asteraceae) with an emphasis on southern African members of *Senecio* section *Senecio*.

2.1: INTRODUCTION

2.1.1: Taxonomy of *Senecio* and Senecioneae

The distinction between the genus *Senecio* and the tribe in which it resides (Senecioneae) has historically been blurred. This taxonomic problem stems from a lack of clarity in morphology, uncertain generic limits both of *Senecio* and other closely and less closely related genera, and inconsistency in approaches to classifying the genus or tribe around the world (Jeffrey et al., 1977; Jeffrey, 1979; Vincent & Getliffe, 1992). The genus has previously been estimated to contain around 3000 species, but it is likely that this estimate refers to much of the tribe rather than the core genus *Senecio.* A number of different concepts of the genus itself exist. Jeffrey (1979) suggested a distinction between *Senecio* sensu lato and *Senecio* sensu strictum. *Senecio* s.l. could perhaps be taken to encompass the entire tribe while *Senecio* s.str. represents a core group of species clustered around the type species *S. vulgaris.* This is still a very large group, containing somewhere in the region of 1000 - 1500 species. The biggest taxonomic problem in classifying the tribe is achieving a monophyletic delimitation of the genus *Senecio,* and because of the highly toxic nature of the genus, it is particularly important that we have a biologically meaningful group for study (Pelser & van der Meijden, 2002).

Because of this confusion it is important to include as many different species as possible from the tribe in any initial analysis or classification. Without reference to a wider phylogeny of the tribe, it would be impossible to determine if one were

working with a monophyletic group or not. Many genera such as *Aetheolaena* Cass., *Culcitium* Bonpl., *Hasteola* Raf., *Iocenes* R. Nordenstam, *Lasiocephalus* Willd. ex Schldl and *Robinsonia* DC. nest within clades of species ascribed to *Senecio*, while species with the generic name *Senecio* are scattered throughout phylogenetic trees of the tribe as a whole (Pelser et al., 2007). This makes the genus *Senecio*, as it is currently circumscribed, paraphyletic or polyphyletic (Knox & Palmer, 1995; Kadereit & Jeffrey, 1996; Vincent, 1996; Pelser et al., 2002). Because of this, smaller monophyletic groups for study must be chosen by referring to larger analyses of the tribe as a whole, in order to identify groups suitable for phylogenetic analysis. This is the approach adopted in this study.

2.1.2: Tribal taxonomic history

The taxonomy and evolutionary relationships of members of tribe Senecioneae have never been clear despite extensive investigation by previous researchers (e.g. De Candolle, 1838; Harvey, 1865; Bentham, 1873a, 1873b; Hoffman, 1890; Greenman, 1903; Muschler, 1909; Small, 1919; Nordenstam, 1977, 1978, 2007; Jeffrey & Chen, 1984; Jeffrey, 1986, 1992; Bremer, 1994). The tribe is composed of about 150 genera and around 3,000 species and has, historically, been split into assemblages of genera, the largest of which have been ascribed the formal rank of subtribes (Nordenstam, 1977; Bremer, 1994). The number of accepted subtribes, and their composition and limits, have changed over time, as different researchers have investigated the tribe. Bremer (1994) recognised three subtribes within the Senecioneae: Blennospermatinae Rydberg, Senecioninae and Tussilagininae (Cass.) Dumort. In his morphological cladistic study, monophyly was supported for the two larger tribes, Senecioninae and Tussilagininae, but more recent molecular phylogenetic studies of the subtribes have

suggested that Senecioninae at least is paraphyletic (Knox & Palmer, 1995) or have questioned the monophyly of both of these larger subtribes (Kadereit & Jeffrey, 1996). Smaller assemblages of genera have not been ascribed taxonomic rank and have remained informal groups, never having been assigned to a subtribe (Jeffrey, 1979; 1986; 1992). Relationships between genera within these subtribes have hardly been investigated, researchers instead tending to work on resolving phylogenies of particular genera or on resolving sections within *Senecio* itself. Amongst the genera of the Senecioneae studied using molecular phylogenetic techniques are *Robinsonia* (Sang et al., 1995), *Packera* Löve & D. Löve (Bain & Jansen, 1995; Bain & Golden, 2000), *Abrotanella* Cass. (Wagstaff et al., 2006), *Blennosperma* Less., *Crocidium* Hook., *Ischnea* F. Muell. (Swenson & Bremer, 1997), *Dendrosencio* (Hedb.) R. Nordenstam (Knox, 1996), *Pericallis* D. Don (Panero et al., 1999; Swenson & Manns, 2003), *Doronicum* L. (Álvarez Fernández et al., 2001), *Brachyglottis* Forster & Forster (Wagstaff & Breitwieser, 2004), and a complex of Himalayan genera, the *Ligularia* Cass. - *Cremanthodium* Benth. - *Parasenecio* W.W.Sm. & Small complex (Liu et al., 2006). Constant, binding morphological characters for these genera do not appear to exist or their identification has not been included in the aims of the study in question.

In order to improve understanding of the tribe, a phylogenetic study was required that included species representative of the whole tribe, rather than concentrating on particular genera or sections. A recent attempt to apply the techniques of molecular phylogenetics to the tribe as a whole, a very ambitious project, was led by Pieter Pelser (Pelser et al., 2007). The researchers collected nuclear *ITS* and plastid DNA sequences for as many species from the tribe as possible both from GenBank and from other researchers working on Senecioneae.

Morphological characters had in the past provided conflicting ideas about the relationships within the tribe and Pelser et al. hoped that applying molecular phylogenetic techniques to the problem would help obtain a more definitive classification. *ITS* and plastid fragments were analysed both separately and in a combined analysis, resulting in a new phylogeny for the tribe and a new delimitation for *Senecio*. In an attempt to create monophyletic subtribes, the authors suggest abolishing subtribes Adenostylinae, Blennospermatinae and Tephroseridinae, and instead recognise Abrotanellinae, Othonninae and Senecioninae as true monophyletic subtribes. In order to acheive monophyly, existing subtribe Tussilagininae could be split into three or four subtribes: Brachyglottidinae, Chersodominae, Tussilagininae, and possibly Doronicinae.

The only feature which Pelser et al. (2007) identified as a possible diagnostic morphological character for the tribe *Senecioneae* is the presence of uniseriate involucral bracts, but even this is a case of 'usually' as there are several species which at least appear to have more than one whorl of bracts [e.g. Dendrosenecio (Hauman ex Hedberg) B.Nord. species], although this may be an enlarged calyculus rather than more than one bracteate whorl (Knox & Palmer, 1995).

2.1.3: Generic taxonomic history

In his *Species Plantarum* of 1753, Linnaeus included 11 *Senecio* species, including *S. vulgaris*, the type species. Nine of these species are still recognised today. He formally circumscribed *Senecio* in his *Genera Plantarum* of 1754, but neglected to include any infra-generic groupings.

de Candolle (1838) wrote the most long-standing account of the genus, and included many species absent from Linnaeus' account. He split the genus at the infra-

generic level for the first time, but along biologically dubious lines. This remained the only comprehensive account of *Senecio* until very recently, and parts of the classification system were still being reproduced until the 1960s (e.g. Komarov, 1961). There are no modern worldwide monographic accounts of *Senecio*.

Drury and Watson (1965) were the first to suggest that de Candolle's system needed a complete overhaul when they studied some morphological characters in a number of species from de Candolle's series Caucasici DC. They noticed that, for example, yellow ray florets had been used as a diagnostic morphological character when this is a common feature throughout the genus and tribe. Further distinctions between species relied in some cases on differing shades of yellow ray florets as observed on herbarium specimens, hardly reliable diagnostic characters, as herbarium specimens tend to discolour over time. A new classification was created for the species studied, based on the limited number of characters they observed.

To try and clarify the circumscription of *Senecio* along more robust biological lines, attempts have been made to look at global sectional and generic limits of the genus. These have often resulted in proposals to reduce *Senecio* from the levels of 2000-3000 species, and, as mentioned above, a sense of *Senecio* s.l. and *Senecio* s.str. has developed. In fact, a number of different concepts, particularly of *Senecio* s.str., have arisen. Serious attempts to clarify the concept and limits of the genus were made by Jeffrey et al. (1977) in a review of the global limits of the genus and the sectional limits which should be adopted within it. The researchers estimated *Senecio* s.l. to contain about 3,000 species. The generic concept applied was so loose that the genus included species with a range of character variation overlapping or even exceeding the combined ranges of species placed in several other genera. They concluded that the 182 included species of *Senecio* s.l. could be split into 16 groups, to which they

did not ascribe formal taxonomic rank. Only group IX, they concluded, should be regarded as 'true' *Senecios*, as this group contained the type species, *S. vulgaris.* They suggested that the other members of *Senecio*, which fell outside group IX, should perhaps be assigned other generic names.

Jeffrey et al. (1977) recommended that a set of uniform criteria be applied to *Senecio* taxonomy the world over as a solution to the variation in treatment which had arisen as a result of the undertaking of regional accounts without reference to classifications from other regions, and because of the absence of an accepted world-wide monographic account of the genus. This was certainly a sensible idea, although whether their 'uniform criteria' would have been appropriate is unclear.

In a later paper, Jeffrey (1979) suggested that his informal groups could be used as operational taxonomic units or 'OTU's in phylogenetic studies, but his own phylogenetic study was never fully published. He also failed to elaborate on the characters which were important in defining the groups, quoting his 'some 15 years working experience of the group' as one of the justifications for his system. Lists of synapomorphic characters for proposed groups are usually presented with more recent taxonomic accounts, as a justification for the system adopted. Quoting personal and subjective experience without presenting some kind of evidence is no longer accepted as a way of justifying a classification system. Jeffrey further divided the informal group IX into two sections, which he termed 'eusenecioids' (containing the type species), and 'gynuroids' based on studies of the pappus, phytochemistry and succulence (Jeffrey, 1979). Jeffrey thought that studies of *Senecio* should start with the type species, *S. vulgaris* and work outward from there, in order to better define the cluster of species around the type. This cluster would represent what was really meant

by *Senecio* s.str., and he hoped would result in a biologically meaningful, monophyletic group.

As a result of this tightening of the generic concept of *Senecio,* Jeffrey reduced his estimation of some 3,000 species in 1977 to some 1,000 species in a paper published in 1984, the result of the application of his 'uniform criteria' in examining the group internationally (Jeffrey & Chen, 1984).

Taking the lead from Jeffrey et al. (1977), Vincent & Getliffe (1992) applied their own 'uniform criteria' based on six characters which they thought might be important, observed from 93 *Senecio* species from Natal, to other areas of the world besides southern Africa. These were:

1. style-arm apices,
2. anther apices,
3. cell wall configuration of the endothecial tissue,
4. length of the filament collars
5. shape of the filament collars
6. cypsela disc shape.

They concluded that many taxa could be removed from a more strictly defined generic concept and advanced a new concept of core *Senecios, Senecio* sensu strictum sensu Vincent. Vincent (1996) admitted though, that the 'core genus' created here is paraphyletic or possibly even polyphyletic, and suggested that it was unlikely that the situation would be resolved by further study of the characters which he had chosen in defining *Senecio* sensu strictum sensu Vincent. The pair had earlier produced a paper on the endothecium in *Senecio,* noting the inner anticlinal configuration of the endothecial tissue, corresponding to the radial endothecial tissue pointed out by Nordenstam (1978). By this they mean that the endothecial cells are tabular, with

thickenings restricted to the longitudinal wall nearest the connective [which they refer to as the inner anticlinal (radial) wall]. It should be noted that, although a character for *Senecio,* this endothecial configuration is not unique to the genus (Vincent & Getliffe, 1988).

Nordenstam (1978) agreed that not all of the taxa placed in *Senecio* s.l. should be so ascribed, and suggested that the genus be cut down to a core of around 1,500 species. He noted that, in his view, some important characters in defining the core *Senecios* or *Senecio* s.str. were 'senecioid' filament collars, radial endothecial tissue, truncate style branches, 'banded' discrete stigmatic areas and a haploid chromosome number of 10.

There are also several molecular phylogenetic studies of *Senecio,* which have tended to concentrate on established sections or species complexes within the genus, rather than attempting to sample the genus as a whole (e.g. Bain & Jansen, 1995; Pelser et al., 2002; Coleman et al., 2003). More recently, in a thorough molecular phylogenetic study of Senecioneae, an attempt was made by Pelser et al. (2007) to obtain a more solid monophyletic delimitation of *Senecio.* The authors concluded that to achieve monophyly several currently separate genera should be ascribed to *Senecio*: *Aetheolaena, Culcitium, Hasteola, Iocenes, Lasiocephalus* and *Robinsonia.* Conversely, there are several species groups currently ascribed to *Senecio* which are only distantly related to the core of the genus, such as *S. angulatus* L.f. and *S. repandus* Thunb. These should be removed from *Senecio* and ascribed to other genera. They include a description of the genus, reproduced below:

***Senecio* L.**

Herbs, subshrubs, shrubs or small trees with alternate (sometimes rosulate) leaves. Involucre campanulate or cup-shaped, calyculate; phyllaries uni- or bi- (rarely pluri-) seriate, free. Capitula radiate, disciform or discoid; florets often yellow, sometimes white, green, pink, purple, or rarely blue. Anthers ecaudate; endothecial tissue radial; filament collar balusterform. Disc floret style with separated stigmatic areas; tips truncate with short sweeping hairs, sometimes with a median hair pencil. Cypselas homomorphic, 8-12-ribbed, with papillate surface; carpopodium present. Pappus bristles numerous, slender, barbellate, white. Base chromosome number x=10. Distribution almost worldwide, but non-native in some regions, e.g., the West Indies (Pelser et al., 2007)

2.1.4: Infra-generic taxonomic history

There has often been a lack of reference by taxonomists to existing *Senecio* classification systems when producing accounts of the genus in a particular part of the world. Thus infra-generic groupings in *Senecio* have reached the point where there are now approximately 150 recognised sections within the genus, many of which have a very vague circumscription, and all of which lack solid diagnostic characters. Most are also specific to a particular geographic area. These sections tend to share an overall similarity in gross morphology, and should be regarded not as biologically or phylogenetically meaningful sections, but simply as informal groups constructed by taxonomists largely for the sake of convenience (Pelser et al., 2002).

As mentioned above, de Candolle (1838) was one of the only early authors to attempt to split the genus at the infra-generic level, although, according to the International Code of Botanical Nomenclature, his system cannot be accepted because

he used the series category above the sectional category in his taxonomic hierarchy. de Candolle's series followed purely geographical lines of distribution and are therefore very likely to be biologically dubious. His series Caucasici, for example, encompassed all species within the range inhabited by Caucasian man. This division was no doubt made in an attempt to make some sort of sense of such a large cosmopolitan genus. This series was then further subdivided into ten sections, based on morphological characters. Despite series divided along such arbitrary lines, and the confusion in rank, de Candolle's system of sections was widely used by taxonomists for many years, in the absence of anything better (Alexander, 1975). A reassessment of de Candolle's taxonomic treatment was suggested by Drury and Watson (1965) who thought his system flawed, and that a wide range of species should be included in a single study of morphological characters.

A revision of 23 of the Mediterranean species of sections *Senecio* and *Delphinifolius* Rchb. was undertaken at the Royal Botanic Garden, Edinburgh, as part of a PhD thesis and later published as a revision (Alexander, 1975; 1979). In the revision, Alexander based his study on groups proposed by Jeffrey et al. (1977), and examined morphological, micromorphological and cytological characters. Unsurprisingly, he concluded that distinctions between some of the sections proposed within *Senecio* are biologically dubious. The main distinction between sections *Senecio* and *Jacobaea* (Cass.) Dumort., for example, is the difference in annual versus perennial or biennial habit. He decided that sect. *Jacobaea* should be sunk into sect. *Senecio*, as members of the two sections are so similar, morphologically and genetically. Interestingly, more recent molecular phylogenetic work has instead suggested the converse, that sections *Senecio* and *Jacobaea* are indeed distinct. Pelser

et al. (2002; 2007) went as far as to declare the members of sect. *Jacobaea* a separate genus from those usually ascribed to sect. *Senecio.*

Alexander (1975; 1979) concluded that the genus itself and many of the accepted sections within it are probably paraphyletic or polyphyletic. His morphological description of sect. *Senecio* has been the main one in use by biologists since, but unfortunately contains no solid diagnostic morphological characters. A list of features of the section as defined by Alexander is included below. As the reader will notice, none of these characters is constant throughout the section.

Characters defining section *Senecio* (Alexander, 1975; 1979)

1) Decumbent or erect
2) Annual, biennial (or short lived perennial – n.b. may be sect. *Jacobaea*)
3) Glabrous to arachnoid or lanate
4) Occasionally glandular
5) Stems terete, ridged
6) Stem may be suffrutescent below
7) Stems often branched
8) Leaves linear, elliptic to oblong
9) Leaves usually pinnatifid to pinnatisect or lyrate-pinnatisect, sometimes unlobed
10) Leaf margins entire, toothed, crenate to denticulate
11) Leaf bases often auriculate-amplexicaul
12) Capitula urceolate, oblong or cup shaped
13) Capitula in lax or dense corymbs, occasionally solitary
14) Peduncles usually bracteate, sometimes plants more or less scapose
15) Calyculus of 1 – 25 bracts
16) Calyculus bracts linear, subulate or triangular, rarely lacerate, occasionally absent
17) Bracts often black-tipped
18) Involucre a single whorl of 8 – 30 phyllaries
19) Phyllaries often black-tipped
20) Ray flowers 5 -30, or often absent
21) Ray flowers with long or short, yellow, rarely lilac or purple ligules
22) Disc flowers numerous
23) Disc flowers tubular
24) Disc flowers yellow, rarely purple
25) Achenes sub-cylindrical
26) Achenes glabrous, strigulose or lanate
27) Pappus of shortly toothed hairs
28) Outer pappus whorl usually with a few fluked or clavate hairs

A molecular study of Mediterranean members of *Senecio* sect. *Senecio* was undertaken by Comes & Abbott (2001), who used the definition of sect. *Senecio* in Alexander (1979). A group of 26 diploid, tetraploid and hexaploid species, the Mediterranean species complex of *Senecio* sect. *Senecio,* was the focus of the study. This species complex was thought to be monophyletic, and was therefore deemed suitable for phylogenetic analysis. Comes & Abbott surveyed chloroplast DNA and *ITS* sequence variation for phylogenetic analysis. In addition, they used randomly amplified polymorphic DNAs (RAPDs) to provide greater resolution in parts of the phylogenetic analysis. Maximum parsimony analysis of 37 different accessions representing 18 different species produced 2 most parsimonious trees based on *ITS* sequences. *ITS* sequence divergence was generally low, suggesting some incidence of simultaneous and recent diversification. Most of the species investigated fell within two *ITS* subclades, but resolution within the subclades, particularly subclade A, containing many central and western Mediterranean diploid species, was very poor, as sequence divergence of *ITS* was particularly low. Subclade B consisted of *S. vulgaris*, *S. vernalis* Waldst. & Kit., and one accession of *S. rupestris* Waldst. & Kit., which may have been introgressed with an *ITS* sequence from *S. vernalis*. A third, less closely related subclade contained glandular tetraploids. Unfortunately, relationships suggested between the subclades were not well resolved or robust, although all three subclades were well supported in the maximum parsimony tree (Comes & Abbott, 2001).

A wider ranging study of sect. *Senecio* was carried out by Coleman et al. (2003). *ITS* sequences of 37 accessions, representing 18 species of both Old and New World sect. *Senecio* were analysed phylogenetically. The study concentrated on sect. *Senecio* from South Africa, the Mediterranean basin and North America, although

some South American and Macronesian taxa were also included. Again, the authors used Alexander's (1979) morphological definition of the section to choose species they thought appropriate to include in a sectional study. Maximum parsimony analysis produced eight most parsimonious trees. In the one tree reproduced in the paper, a clade which roughly corresponds to sect. *Senecio* can be seen. The authors have termed this the 'Groundsel clade', rather than attaching specific taxonomic rank. Statistical support for the result is very high, with a maximum parsimony bootstrap value of 100% and a decay index figure of 23. Despite the fact that this is not a strict consensus tree, combining all most parsimonious trees, the results are well supported, as maximum likelihood analysis produced a single tree with an identical topology to that of the most parsimonious tree described above. Relationships within this clade were less well resolved, but five subclades were suggested, three of which were predominantly South African. The other two were predominantly Mediterranean and predominantly North American. The South African taxa appeared to be basal and ancestral in the group, a finding which prompted the study undertaken here.

In some cases there was not enough differentiation between species in the *ITS* region to provide phylogenetic resolution, with five South African taxa from subclade V residing on a polytomy. An interesting geographical disjunction between North American and Mediterranean subspecies of *S. mohavensis* was also revealed by the results, which the authors invoke long distance epizoochory to explain (Coleman et al., 2001, 2003).

A detailed molecular study was carried out by Pelser et al. (2002), who concentrated on members of sect. *Jacobaea,* although they also included some species not ascribed to sect. *Jacobaea*, and others generally ascribed to separate genera. Sixty species of the tribe Senecioneae, representing 23 genera were sequenced for the *trn*T-

L intergenic spacer, *trn*L intron and parts of the *trn*K intron from the chloroplast genome, and *ITS1, 5.8S,* and *ITS2* genes and spacers from the nuclear genome. A combined analysis of all data produced a monophyletic sect. *Jacobaea,* including three species consistently assigned to the section and twelve which were not present in all previous accounts, with strong statistical support for the grouping, a bootstrap (BS) value of 99%, and a decay index (DI) of 9 (Pelser et al., 2002). It is interesting to note that these results conflict with the intuitive sinking of sect. *Jacobaea* within sect. *Senecio* proposed by Alexander (1979), and seem to imply that there may be some biological meaning in the distinction between the annual and perennial members of the genus. It is however worth noting that sect. *Jacobaea,* as defined by the results of Pelser's study, does include at least one annual species, the southern and central Spanish *Senecio minutus* (Cav.) DC., a species previously placed in sect. *Senecio* by de Candolle (1838), and in sect. *Delphinifolius* by Chater & Walters (1976). The species is now known as *Jacobaea minuta* (Cav.) Pelser & Veldkamp (Pelser et al., 2002).

Pelser et al. (2004) also carried out a morphological phylogenetic study on the members of Section *Jacobaea* to compare the results with phylogenies produced using DNA sequences. The morphological results were very poorly supported and conflicted with molecular results. No strong constant diagnostic morphological characters were identified for the section.

Other recent work includes a study of the mainly North American aureoid complex, which contains about fifty species, by Bain & Jansen (1995). This complex has been segregated as the genus *Packera* Löve & D. Löve. by Jeffrey (1979), and is now usually regarded as a separate monophyletic genus. It is defined by the haploid chromosome number of 22 or 23, and by a particular pollen wall morphology rarely

seen outside the complex. The investigators produced a maximum parsimony analysis of *ITS* sequence data and found that their results conflicted with accepted sectional delimitations within the complex - perhaps unsurprising when so many sections within *Senecio* s.l. are poorly defined (Pelser et al., 2002).

2.1.5: Specific and infra-specific taxa

For more than 100 years after Linnaeus, many new species of *Senecio* were described. The fashion of the time was to ascribe species rank to any new variation seen; this was often done with a little too much aplomb, with the result that many taxa proposed during this period are no longer accepted. Some infra-specific names were also published by de Candolle (1838) and Boissier (1875) for instance, although Boissier did not assign rank in all cases, and grouped some taxa together under binomials without specifying rank (Alexander, 1975). Towards the end of the 19th century, infra-specific categories became popular, and there was a reduction in the number of accepted species. The *Flora de l'Algerie* combined many local 'species' with more widespread 'species' to create subspecies, varieties and forms within species (Battendier & Trabut, 1888). A new trend in invoking varieties, forms and subspecies had been adopted, which was taxonomically helpful in some cases, but in others was almost certainly a hindrance. Long lists of varieties within species can be seen in Fiori & Paoletti (1903) for Europe and in Jahandiez & Maire (1934), for N.W.Africa.

Given the large, unwieldy and uncertain nature of *Senecio,* several molecular studies have concentrated on small species groups to clarify relationships between particular closely related species, or to determine the origins of species. These include studies of the relationships of British *Senecios,* particularly *S. squalidus* (Abbott et al.,

2000; James & Abbott 2005), *S. vulgaris* (Ashton & Abbott, 1992a), *S. nebrodensis* L. and *S. viscosus* L. (Kadereit et al., 1995). These studies have helped to clarify relationships within these species complexes somewhat. Other studies have served to investigate the validity of unusual disjunct distributions of single species, such as in the case of *S. madagascariensis* (Scott et al., 1998) and *S. flavus* (Coleman et al., 2003).

2.1.6: Taxonomy of *Senecio* in southern Africa

Senecio in southern Africa comprises somewhere between 350 and 500 species (Hilliard, 1977). Southern Africa is one of the centres of diversity for the genus (Nordenstam, 1977; Bremer, 1994). The taxonomy in this part of the world is particularly neglected, with no attempts at infra-generic circumscription since Harvey's *Flora Capensis* of 1865. Twelve sections within *Senecio* found in the Cape regions of southern Africa were proposed by Harvey, which included 174 species. This seems to be one of the only serious early attempts to split the genus along biological lines rather than arbitrarily through geographic distribution, as was the case in de Candolle's treatment. However, Harvey (1865) included only species from southern Africa and was further limited, by the difficulties of collecting specimens, to mainly the Western Cape region of South Africa. No attempt seems to have been made to fit these southern African *Senecios* into the existing taxonomic framework of de Candolle. Harvey's proposed sections were:

1. *Annui* Harvey
2. *Sinuosi* Harvey
3. *Plantaginei* Harvey
4. *Paucifolii* Harvey
5. *Rigidi* Harvey
6. *Microlobi* Harvey

7. *Leptolobi* Harvey
8. *Leptophylli* Harvey
9. *Pinifolii* Harvey
10. *Scandentes* Harvey
11. *Kleinoidei* Harvey
12. *Aphylii* Harvey

Characters which Harvey used in splitting the genus and defining sections included rootstock, habit, and branching patterns of both sterile and fertile stems (Harvey, 1865).

Another account exists in Adamson and Salter's *Flora of the Cape Peninsula* (1950), including 48 species, although no attempt at infra-generic circumscription has been made, and the scope of the account is limited to a very small geographical area. One of the most thorough and enduring accounts of *Senecio* in southern Africa is in Hilliard's *Compositae in Natal* (1977). She includes detailed descriptions of 124 Natalese members of the genus. However, she does not split the group at the infra-generic level, or ascribe the described species to existing sections. The species are simply listed under the genus heading. Merxmüller's *Prodromus einer Flora von Südwestafrika* (1976) is of interest because it includes *S. flavus* and *S. engleranus*, a sister species pair which appeared to be basal to sect. *Senecio* in the phylogenetic analysis of Coleman et al. (2003). The account includes 24 species, again listed rather than ascribed to sections.

Vincent & Getliffe (1992) carried out extensive phenetic studies of 93 *Senecio* species from Natal, examining 122 morphological and micromorphological characters, and producing principal component analysis (PCA) plots. The characters which they thought were important in classifying *Senecio* are listed in the generic history section. However, they did not ascribe the southern African taxa to sections.

The most recent southern African species descriptions are found in Goldblatt and Manning's *Cape Plants* (2000). They include 111 species grouped under brief lists of binding characters, avoiding ascribing formal taxonomic rank at the infra-generic level. The species descriptions are very brief, and of limited use in identifying *Senecio* species reliably. This is presumably because the enormous scope of the book limited the time that could be spent on any particular group. Mirroring Harvey's approach in adopting a sect. *Annui* based on annuality, they include a group of 17 species based on annual habit.

The reader may notice that there is no mention of sect. *Senecio* in the above account of southern African taxonomic history. Sect. *Senecio* has never been a concept in southern African *Senecio* taxonomy, as there has been no serious attempt to split the southern African members of the genus at the infra-generic level since Harvey's system of 1865. Despite the fact that Harvey's treatment is far from comprehensive, in the absence of anything better, his key is still used by South African taxonomists who wish to identify *Senecio* specimens from the Cape regions. As mentioned above, Harvey was based in the Western Cape, and consequently there appears to be a more thorough sampling from this area than from the other Cape provinces - or from any other South African province. As a result there are many known southern African taxa missing from his account.

2.1.7: Molecular phylogenetic analysis of *Senecio* in Southern Africa

For the molecular phylogenetic analysis of *Senecio* from South Africa reported in this chapter, it was originally intended to sequence multiple DNA fragments for use in tree construction. Use of mutiple fragments for phylogenetic analyses is becoming more and more common, as the resulting tree topologies are

thought to be more robust than those obtained using a single fragment (Pelser et al, 2002). In the event, because financial constraints limited the number of fragments which could be sequenced, and because of the need to include a large number of taxa from GenBank for which *ITS* was the only sequence available, only nuclear *ITS* DNA and the plastid DNA fragment *trn*L-F were used.

ITS Nuclear DNA

The internal transcribed spacer region of rDNA (*ITS*) has been used successfully to achieve phylogenetic resolution at around the species level in many angiosperm genera (Hillis & Dixon, 1991; Baldwin, 1992; Baldwin et al., 1995). *ITS* is found in the part of the nuclear genome coding for ribosomal RNA. The RNA genes are made up of repeat units, each consisting of an *IGS* (inter-genomic spacer), 18S gene, *ITS*1 spacer, 5.8S gene, *ITS*2 spacer and 26S gene. Within the *IGS* are two regions, the *NTS* (non-transcribed spacer) and *ETS* (external transcribed spacer). The structure of *ITS* is illustrated in Fig. 2.1.

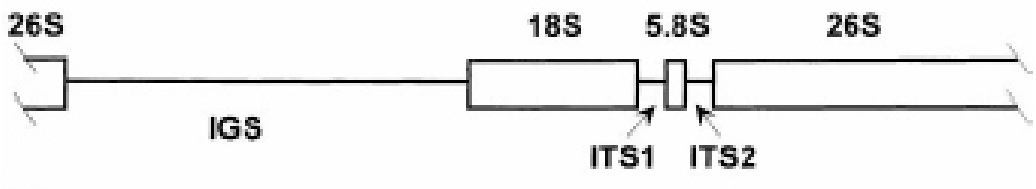


Figure 2.1: rDNA *ITS* structure (Linder et al., 2000).

_trn_L-F plastid DNA

Although chloroplast DNA generally evolves more slowly than nuclear DNA, the chloroplast genome contains a number of spacers which evolve relatively rapidly, and can even show intraspecific variation (Taberlet et al., 1991). The structure of the *trn*L-F region is illustrated in Fig. 2.2. Unfortunately, it was not possible to obtain complete *trn*L-F sequences for more than a few of the species for which *ITS* data were collected from GenBank, so phylogenetic analyses of *trn*L-F were carried out on a reduced Dataset. For the purposes of comparison and possible combination of Datasets, a small matrix of corresponding *ITS* sequences was also constructed and analysed.

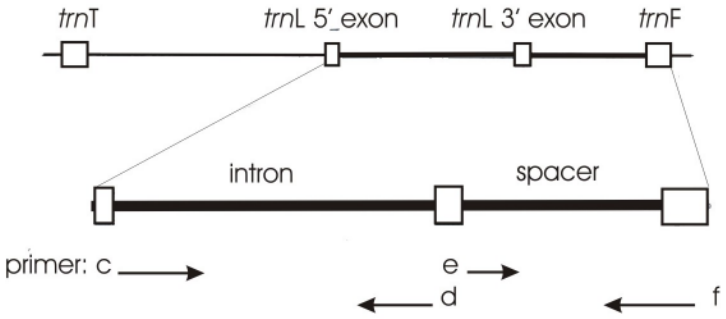


Figure 2.2: The *trn*L-F region of chloroplast DNA. Primer annealing sites are marked (adapted from Taberlet et al., 1991)

2.1.8: Aims of the study

The aims of the project undertaken here were to identify potential members of section *Senecio* in southern Africa and investigate the relationships between them and other taxa from the subtribe Senecioninae. In addition, relationships in the wider subtribe were investigated. Southern African species were collected in the field, DNA extracted from them, and fragments of interest sequenced for inclusion in wider phylogenetic analysis of Senecioninae, with the object of identifying those taxa which

are most closely related to the type species *S. vulgaris*, and which might therefore belong in section *Senecio*. A large number of sequences from the subtribe were collected from GenBank to enable the placing of these taxa in a wider phylogeny, and to investigate other relationships in Senecioninae.

Another aim of the project was to investigate geographic structure in *Senecio* s.str. in the trees resulting from phylogenetic analyses, to see if there was any support for the idea suggested in Coleman et al. (2003), that the section may have originated in southern Africa, and to infer routes of colonisation in the genus. The evolution of flower colour in the group was also investigated.

2.2: MATERIALS AND METHODS

2.2.1: Choice of study group and fieldwork

On inspection of herbarium specimens and descriptions in Harvey (1865) and other works, the existing southern African section which appears to be morphologically closest to sect. *Senecio* is Harvey's sect. *Annui*, so this section was chosen as the basis for a phylogenetic study group. It seemed very unlikely that Sect *Annui* would represent a monophyletic group for study as the only unique binding character given for the section by Harvey is annuality. Harvey's short sectional description is reproduced below:

Section *Annui*: Root annual. Stem herbaceous, mostly branched, erect or diffuse. Inflorescence diffusely panicled or subcorymbose. Heads rarely discoid; mostly radiate, the rays yellow or purple.

Lifespan is generally thought to be a relatively plastic character in plant groups. However, in choosing a suitable study group, Sect. *Annui* seemed a reasonable place to start. Southern African annuals identified from other accounts of the genus were also included in the study group. These were mainly taken from Hilliard (1977), Goldblatt and Manning (2000), and in the case of *S. flavus* and *S. engleranus*, Merxmüller (1976). Study group members are listed below:

Study group (annual and biennial members of *Senecio* in southern Africa):

From Harvey (1865):

S. abruptus Thunb. (= *S. diffusus* Thunb.)
S. arenarius Thunb.
S. cakilefolius DC.
S. cardaminifolius DC.
S. consanguineus DC.
S. diffusus Thunb.
S. elegans L.
S. erysimoides DC.
S. glutinarius DC.

S. glutinosus Thunb.
S. laxus DC.
S. littoreus Thunb.
S. lobelioides DC.
S. maritimus L.
S. paarlensis Thunb.
S. puberulus DC.
S. repandus
S. sophioides DC.
S. vulgaris

From Goldblatt & Manning (2000):

S. carroensis DC.
S. pinnulatus Thunb.
S. pterophorus DC.

From Hilliard (1977):

S. chrysocoma Meerb.
S. decurrens DC.
S. digitalifolius DC.
S. juniperinus L.f.
S. lanceus Aiton
S. madagascariensis
S. panduriformis Hilliard
S. polyanthemoides Sch. Bip.
S. poseideonis Hilliard & B.L. Burtt
S. skirrhodon DC.

From Merxmüller (1976):

S. engleranus
S. flavus

Annuals for which locality information was unavailable:

S. agapetes C. Jeffrey (= *S. amabilis* DC.)
S. laevigatus Thunb.
S. lessingii Harv.
S. matricariaefolius DC.
S. multibracteatus Harv.
S. ruderalis Harv.
S. sisymbrifolius DC.
S. tenellus DC.
S. trachylaenus Harv.

Representatives of Harvey's 11 other sections were also chosen, with the intention of investigating whether phylogenetic analyses would support Harvey's intuitive classification system.

Representatives of Harvey's sections other than Sect. *Annui*

Section 2: *Sinuosi*

S. polyodon DC. (= *S. concolor* DC., = *S. speciosus* Willd.)
S. erubescens Aiton
S. macrocephalus DC.

Section 3: *Plantaginei*

S. coronatus (Thunb.) Harv.
S. discodregeanus Hilliard & B.L. Burtt

Section 4: *Paucifolii*

S. isatideus DC.
S. latifolius DC.

Section 5: *Rigidi*

S. gerrardii Harv.
S. oxyodontus

Section 6: *Microlobi*

S. lineatus (L.f.) DC.

Section 7: *Leptolobi*

S. achilleifolius DC.
S. rhyncholaenus DC.

Section 8: *Leptophylii*

S. inaequidens
S. harveianus MacOwan (= *S. vimineus* (DC.) Harvey)

Section 9: *Pinifolii*

S. pinifolius (L.) Lam.
S. triqueter Less.

Section 10: *Scandentes*

S. deltoideus Less.
S. mikanioides Walp.

Section 11: *Kleiniodei*

S. corymbiferus DC.
S. pyramidatus

Section 12: *Aphylii*

S. junceus (DC.) Harv.

Locality information for the species of interest was noted from herbarium collections at the Royal Botanic Gardens, Edinburgh, the University of Cape Town, Kirstenbosch Botanic Gardens in Cape Town, and from the works mentioned above. This information was mapped (see Fig. 2.3) and a route around the three Cape provinces of South Africa planned (i.e. Western, Northern and Eastern Cape provinces). Two one-month long visits to South Africa were undertaken during September of 2004 and September of 2005. Fieldwork was based at Kirstenbosch Botanic Gardens in Cape Town, travelling east as far as East London (E Cape) and north as far as Springbok (N Cape).

Representatives of Harvey's other sections were included in addition to the study group, to test Harvey's intuitive system against the rigours of molecular phylogenetic investigation. *Senecio engleranus*, believed, along with its sister species, *S. flavus*, to be basal to sect. *Senecio* was collected during a two week field trip to Namibia in April 2005. In the case of *S. engleranus* and *S. flavus*, locality information was collected from specimens provided by Bertil Nordenstam (personal communication). These localities were again mapped, and are shown in Fig. 2.4.

Herbarium specimens were collected, and identified by reference to herbarium collections at RBGE and the relevant literature. Specimens were also lodged in the

herbarium at RBGE. Leaf samples for subsequent DNA extraction and sequencing were collected in silica gel (Chase & Hills, 1991), and where possible seed was also gathered so that plants could be raised in the glasshouse at the University of St Andrews.

Figure 2.3: Localities of southern African annual *Senecio* species and representatives of other sections gathered from herbarium collections. Localities are marked with red dots.

Figure 2.4: Locality information for *S. flavus* and *S. engleranus* in Namibia. Red dots represent *S. engleranus* localities, while green dots represent *S. flavus* localities.

2.2.2: Laboratory work

DNA extraction

DNA was extracted from all specimens using approximately the same method, derived from Doyle & Doyle (1987). Dried leaf material [either preserved in silica gel (Chase & Hills, 1991) or from herbarium specimens] was frozen using liquid nitrogen and macerated using a pestle, first dry and then with 0.5 ml CTAB (hexadecyl-trimethyl-ammonium bromide) solution (2% CTAB, 20 mM EDTA, 100mM Tris-HCl pH8.0, 1.4M NaCl), preheated to 65° C, containing 2µl 2β-mercaptoethanol per ml CTAB. A further 0.5 ml CTAB solution was added, and a pinch of PVPP (polyvinyl-polypyrrolidone). Samples were placed in a heated block and left for half an hour in the case of silica dried specimens or for an hour in the case of herbarium specimens. 0.5ml chloroform isoamyl alcohol (24:1) was then added to precipitate proteins, tubes were placed on an orbital shaker for 20 minutes, and centrifuged at 13000 rpm for 10 minutes. The supernatant was removed to a clean 1.5ml eppendorf tube and the chloroform extraction outlined above was repeated, again retaining the supernatant. Two thirds volume freezer cold isopropanol was added, and silica dried samples left in a freezer overnight. Material derived from herbarium specimens was left in the freezer for up to a week, in an attempt to precipitate as much DNA as possible. The DNA was then pelleted by centrifugation at 13000 rpm for 10 minutes, and the supernatant removed. The pellet was washed in 1ml of wash buffer (76% ethanol and 10mM NH_4Ac) and left for 30 minutes. After centrifugation for a further 5 minutes, the supernatant was removed, before drying the pellet in a vacuum centrifuge and diluting in 75µl of TE (tris-ethylenediaminetetraacetic acid).

Polymerase Chain Reaction (PCR)

The mixture used in PCR was the same for both amplified fragments (*ITS* and trnL-F). For each specimen, 2.5µl of Bioline 10x NH_4 reaction buffer (160mM $(NH_4)_2SO_4$, 670mM Tris HCl, 0.1% Tween 20, pH 8.8), 2.5µl of 2mM dNTPs, 1.25µl of 50 mM Bioline $MgCl_2$, 0.75µl of each primer, 0.125µl of Bioline Taq polymerase, 16.25µl of deionised water and 1µl of template DNA were used, giving a total of 25µl per reaction. Reactions were run on an MJ Research PTC-200 Peltier Thermal Cycler. A list of primers used to amplify the fragments of interest is given in Table 2.1, together with their sequences.

Table 2.1: Primers used in PCR reactions

Fragment to be Amplified	**Primer Name (and Direction)**	**Primer Sequence**
*trn*L-F(Chloroplast)	c (forward)	5'-CGA AAT CGG TAG ACG CTA CG-3' (Taberlet et al., 1991)
*trn*L-F(Chloroplast)	f (reverse)	5'-ATT TGA ACT GGT GAC ACG AG-3' (Taberlet et al., 1991)
ITS (Nuclear)	ITS5 (forward)	5'-GGA AGT AAA AGT CGT AAC AAG G-3' (White et al., 1990)
ITS (Nuclear)	ITS4 (reverse)	5'-TCC TCC GCT TAT TGA TAT GC -3' (White et al., 1990)

***trn*L-F PCR**

The *trn*L-F PCR cycle parameters were as follows: 4 minutes of initial template DNA denaturing, 35 cycles consisting of: denaturing at 94°C for 45 seconds, primer annealing at 55°C for 45 seconds, and primer extension at 72°C for 3 minutes. This was followed by a final extension step of 10 minutes at 72°C.

ITS PCR

ITS PCR cycle parameters were as follows: 3 minutes of initial template DNA denaturing, 35 cycles consisting of: denaturing at 94°C for 30 seconds, primer annealing at 55°C for 30 seconds, and primer extension for 1 minute and 30 seconds at 72°C. This was followed by a final step of extension for 1 minute at 72°C.

All PCR products were run out on agarose gels to check for successful reactions, before being purified using Qiagen, QIAquick™ PCR purification kits, according to the protocols supplied by the manufacturer. Where there were detectable differences in size between fragments, or banding patterns created by the presence of more than one size of fragment in a specimen, this was detected quickly and easily using gel electrophoresis.

Agarose gel electrophoresis

Agarose gels were made by mixing 0.4g agarose with 150ml of 0.5x TBE (Tris-HCl Borate EDTA) buffer, heating until dissolved, and adding 25µl of 1mg/ml ethidium bromide, once the solution had cooled sufficiently. The gel was then poured into a tray with an appropriately sized comb and left to set for about 30 minutes. DNA extracts were run on agarose gels to indicate approximate concentration, using 3 µl of extracted DNA mixed with 5 µl of loading solution. PCR products were also loaded onto gels in 3 µl quantities, mixed with 5 µl of loading solution. Size of PCR fragments was detected using a Bioline Hyperladder I, 1kb size ladder. Gels were run at approximately 80V in an electrophoresis tank for approximately 90 minutes, and visualised using UV transillumination.

Cloning

For *ITS* PCR products that showed double banding when run on an agarose gel, or which suggested the presence of more than one product when sequencing electropherograms were examined, cloning was employed to obtain usable sequences in the phylogeny.

PCR products were ligated into pGEM-TEasy vectors (Promega) using the manufacturer's protocol. Ligation reactions were set up as follows: 5μl 2x rapid ligation buffer, 1μl T4 DNA ligase and 1μl vector (50ng), all supplied with the kit. To each reaction, 3 μl of PCR product was added, mixed gently using a pipette and left overnight at 4°C.

JM109 competent cells (Promega) were placed on ice for 5 minutes until just thawed. 50 μl of cells were gently pipetted into 1.5 ml microfuge tubes, one per ligation. 7.5μl of the ligation mix was added gently to the side of the tube and tapped down into the cells. Tubes were left on ice for 20 minutes, heat shocked at exactly 42°C for 90 seconds and placed back on ice for 2 minutes. 500μl LB growth medium were added and each tube was then placed at 37°C for 1 hour with shaking. Three agar plates per reaction were plated with 50μl, 200μl and the rest of the mix, left for 10 minutes then incubated upside down overnight at 37°C.

The pGEM-TEasy vector contains a gene conferring ampicillin resistance, so only bacteria which have taken up the vector will grow on the plate. The vector also contains the β-galactosidase gene, which turns the colony blue in the presence of X-GAL. The insert in the vector interrupts the β-galactosidase gene, so colonies containing the vector with the insert do not turn blue (they remain white). Individual colonies were selected using a sterile toothpick, plated out in duplicate, and then the toothpick was dipped into PCR mix. Twelve colonies were screened per reaction,

plated in duplicate and then incubated overnight. PCR products were resolved on 1.2% agarose gels to ensure the insert size was the same as the initial PCR product.

Colonies to be sequenced were removed from agar plates using sterile toothpicks and placed in 5ml LB (containing 2.5µl Ampicillin (100mg/ml) in a 30ml universal glass tube. Cultures were grown up overnight at 37°C with rotation. Plasmids were extracted from 2.5ml of the overnight culture using the Perfectprep mini kit (Eppendorf), following the manufacturer's protocol. Cultures (1.25ml) were centrifuged for 20s at 13,000rpm in 1.5ml microfuge tubes to pellet the cells. The liquid was removed and another 1.25ml culture added, and the step repeated. Cell pellets were re-suspended in 100µl of solution 1 by vigorous vortexing. To this was added 100µl of solution 2, mixed gently, then 100µl of solution 3 was added and mixed vigorously. Tubes were centrifuged at 13,000rpm for 30s and the supernatant placed in a spin column in a collection tube. To this was added 450µl DNA binding matrix, mixed and centrifuged at 13,000rpm for 30s. The filtrate was decanted, and the spin column placed back in the collection tube. To the column was added 400µl of diluted purification solution, before shaking briefly. Columns were centrifuged twice at 13,000rpm for 1 minute, with the filtrate removed between spins. The column was then placed in a fresh collection tube, and the plasmid was eluted by adding 50µl ddH_20, vortexing and centrifuging for 1 minute. Plasmid concentration was estimated using 1.2% agarose gels and Bioline Hyperladder 1 as a size and concentration standard.

Cycle Sequencing

Sequencing was outsourced to the University of Dundee DNA Sequencing Service and carried out on an ABI 3730 capillary DNA sequencer.

2.2.3: Phylogenetic Analyses

Sequences were edited using GeneDoc Multiple Sequence Alignment Editor and Shading Utility V.2.6.002 (Nicholas et al., 1997) and Chromas v.2.3 (McCarthy, 1996). Sequences were aligned manually in PAUP* 4.0b10 (Swofford, 2000). Gap matrices were coded using Gapcoder (Young & Healy, 2003)

Four Datasets were analysed: Dataset 1 was an *ITS* matrix of taxa from throughout the subtribes Senecioninae and Othonninae. Dataset 2 was an *ITS* matrix of *Senecio* s.str. Dataset 3 was an *ITS* matrix including species from subtribes Senecioninae and Othonninae, but only including those species for which complete *trn*L-F data were available. Dataset 4 was a *trn*L-F matrix of species from subtribes Senecioninae and Othonninae.

In the interest of creating a more complete phylogeny, sequences derived from taxa collected during fieldwork were combined with sequence data collected from GenBank. While there were a large number of complete *ITS* sequences available from GenBank, eqivalent *trn*L-F sequenes were rare or often partial, many consisting of the *trn*L gene and intron alone. Exploratory matrices were built which either included or excluded the partial *trn*L-F sequences. The larger *trn*L-F matrix, which included the partial sequences, was analysed in PAUP*4.0b10 (Swofford, 2000) and Mr Bayes v.3.1.2 (Huelsenbeck and Ronquist, 2003; Ronquist and Huelsenbeck, 2005), both including and excluding the missing data in order to investigate whether it had an effect on the resulting tree topologies. Obvious differences between the topologies were observed, and the analysis of the larger of the two *trn*L-F Datasets has not been included in this thesis as a result. Analysis of an *ITS* matrix, which corresponded to this larger *trn*L-F matrix for the purposes of comparison, has also been excluded from the thesis.

With the exception of the larger *trn*L-F matrix (for which analyses were run using six different character exclusion sets), each matrix was analysed in PAUP* 4.0b10 and Mr Bayes 3.1.1 using four different character exclusion sets as detailed in Table 2.2.

Table 2.2: Exploratory analyses carried out using MP and BI methods

Dataset	No. of characters in matrix	Exclusion Set	No. of excluded sites	No. of included sites	No. of MPTs obtained	Tree Length	No. of included taxa
1	1046	No ambiguous areas	164	882	3625	2735	222
1	1046	No ambiguous areas, no 5.8S gene	317	729	2686	2619	222
1	1046	No ambiguous areas, 5.8S gene or gap matrix	496	550	4863	2216	222
1	1046	No ambiguous areas or gap matrix	343	703	4182	2332	222
2	868	No ambiguous areas	156	712	324	722	135
2	868	No ambiguous areas, no 5.8S gene	309	559	324	701	135
2	868	No ambiguous areas, 5.8S gene or gap matrix	367	501	2052	603	135
2	868	No ambiguous areas or gap matrix	214	654	540	624	135
3	850	No ambiguous areas	140	710	210	854	80
3	850	No ambiguous areas, no 5.8S gene	293	557	210	809	80
3	850	No ambiguous areas, 5.8S gene or gap matrix	346	504	60	720	80
3	850	No ambiguous areas or gap matrix	193	657	60	765	80
4	991	No ambiguous areas	111	880	3199	248	69
4	991	No ambiguous areas, no exon	163	828	2400	237	69
4	991	No ambiguous areas, exon or gap matrix	198	793	3254	166	69
4	991	No ambiguous areas or gap matrix	148	843	3213	174	69

After these exploratory analyses were completed, exclusion sets were chosen for the final analyses. In the case of the three *ITS* Datasets (Datasets 1, 2 and 3) the 5.8S gene and areas of ambiguous alignment were excluded from the final analyses. In the case of the *trn*L-F Dataset (Dataset 4), the exon and areas of ambiguous alignment were excluded.

A full list of all taxa included in the analyses, together with information regarding the source of the sequence, the accession number or collector number (in

the case of taxa collected during field work), locality information and associated publications (in the case of GenBank accessions) are provided in Appendix 1. Final aligned matrices for Datsets 1, 2, 3 and 4 are included in electronic format on the CD which accompanies this thesis.

Maximum parsimony (MP) analyses

MP analyses were performed using PAUP* 4.0b10 (Swofford, 2000). Parsimony settings were set to collapse branches if minimum length = 0, outgroups were unspecified and set as monophyletic sister groups to ingroups. Heuristic searches were performed using a two-step search strategy, consisting of an initial round of 10,000 random addition replicates, with multrees and steepest descent options switched off, and no branch swapping, followed by a round with multrees and steepest descent on and TBR (tree bi-section and reconnection) performed on the trees held in memory from the first round. In the case of larger matrices, a maxtrees limit of 10,000 trees was set to enable the analyses to be completed. Resulting trees were filtered to ensure that only the most parsimonious solutions were saved and included in strict consensus trees. In all cases, strict consensus trees and phylograms of the most parsimonious trees were generated. Matrix and tree statistics were also derived from PAUP* 4.0b10 (Swofford, 2000) and MacClade v. 4.06 (Maddison & Maddison, 2003). Distribution of variation graphs were also produced using MaClade v. 4.06.

For branch support, bootstrap values were calculated using 100,000 bootstrap replicates in PAUP*, employing the same search algorithm as used in the retrieval of most parsimonious trees.

Bayesian inference (BI) Analyses

BI analyses were carried out using Mr Bayes v.3.1.2 (Huelsenbeck and Ronquist, 2003; Ronquist and Huelsenbeck, 2005). Suitable model parameters were obtained by running the various matrices in Modeltest v.3.7 (Posada & Crandall, 1998) with various exclusion sets (see Table 2.2 for details of exclusion sets). For each analysis, an initial 500,000 generation 'burn-in' run of four chains was conducted. The probabilities of the resulting trees were then graphed using Microsoft Excel, and a number of trees to discard from the analyses calculated using the number of generations taken to reach a plateau of posterior probability divided by the sample frequency. One run of four chains was then conducted for $2x10^6$ generations in the case of exploratory analyses. Two independent runs of four chains were conducted for $5x10^6$ generations in the final analyses. After discarding the number of trees suggested by the 'burn-in' run, the consensus of the final trees was computed using PAUP* 4.0b10, along with posterior probabilities of the clades and average branch lengths.

Character Optimisation

To study geographic structure in the trees produced from Dataset 2, the geographic distribution of the species was optimised onto the cladograms resulting from the BI analysis. Area optimisation was carried out using the 'trace' function in MacClade v. 4.06 (Maddison & Maddison, 2003). Eight areas were defined for this analysis: South Africa, North Africa, Eurasia, Asia, China, Australasia, North America and South America. To study the evolution of flower colour in the trees produced from Dataset 2, flower colour was optimised onto the cladogram resulting

from the BI analysis. Flower colour optimisation was also carried out using the ‘trace’ function in MacClade v. 4.06 (Maddison & Maddison, 2003).

2.3: RESULTS

2.3.1: Field Work

Silica dried leaf and herbarium specimen collections were made in South Africa from a total of 16 annual species of *Senecio*. These were:

S. abruptus, S. arenarius, S. cakilefolius, S. elegans, S. engleranus, S. erysimoides, S. glutinarius, S. glutinosus, S. hastatus L., *S. littoreus, S. madagascariensis, S. maritimus, S. pterophorus, S. repandus, S. sisymbrifolius, S. sophioides.*

A further 22 biennial or perennial *Senecio* species were also collected for inclusion in phylogenetic analyses. These were:

S. erosus L.f., *S. erubescens S. macrocephalus* and *S. speciosus* from Harvey's sect. *Sinuosi; S. latifolius* from sect. *Paucifolii; S. glastifolius* L.f., *S. lyratus*, *S. oxyodontus, S. pellucidus, S. pubigerus* L. and *S. rigidus* from sect. *Rigidi; S. paniculatus* P.J.Bergius and *S. parvifolius* DC. from sect. *Leptolobi; S. longifolius* L. and *S. burchellii* from sect. *Leptophylli*; *S. angulatus, S. deltoideus* Less. and *S. tamoides* DC. from sect. *Scandentes; and S. coronatus* and *S. inaequidens*, which are not in Harvey's account. One *Kleinia* Mill. species, *Kleinia crassulaefolia* DC. was also collected as *Kleinia* is also a genus of the subtribe Senecioninae. *Curio articulatus* (L.f.) P.V. Heath was also collected as the taxon is synonymous with *S. articulatus* (L.f.) Sch. Bip.

A full list of all taxa included in the analyses, together with information regarding the source of the sequence, the accession number or collector number (in the case of taxa collected during field work), locality information and associated publications (in the case of GenBank accessions) is provided in Appendix 1.

2.3.2: Sequence Analysis

Sequence characteristics of the four Datasets are presented in Table 2.3.

Table 2.3: Sequence characteristics derived from PAUP* 4.0b10 (Swofford, 2000) for Datasets 1-4.

Parameter	*ITS* (Dataset 1[a])	*ITS* (Dataset 2[a])	*ITS* (Dataset 3[a])	*trn*L-F (Dataset 4[b])
Unaligned Length Range (bp) [c d]	440 (*S. oxyodontus*) – -494 (*Kleinia galpinii*)	479 (*Synotis nagesium*) - 493	440 (*S. oxyodontus*) - 494 (*Kleinia galpinii*)	734 (*S. cakilefolius* & *S. glutinarius*)- 757 (*Euryops brownei*)
Unaligned Length Mean (bp)	486.41	489.33	486.38	743.11
Aligned Length (*ITS*1 &*ITS*2)	573	524	523	793
G+C Content Mean (all taxa) (%) [c d]	48.7	49.82	49.50	34.00
Number of Excluded Ambiguous Sites	23	22	19	0
Length After Exclusion (bp)[a]	550	502	504	793
Number of Variable Sites[a b]	423/550 (76.9%)	290/502 (57.8%)	292/504 (57.9%)	128/793 (16.1%)
Number of Constant Sites[a b]	127/550 (23.1%)	212/502 (42.2%)	212/504 (42.1%)	665/793 (83.9%)
Number of Informative Sites (%)[a b]	356/550 (64.7%)	182/502 (36.3%)	206/504 (40.9%)	53/793 (6.7%) refer to 1 below)
Number of Autapomorphic Sites (uninformative variable sites) (%)[a b]	67/550 (12.2%)	108/502(21.5%)	86/504 (17.1%)	75/793 (9.5%)
Total Sequence Divergence (%)	0- 40.3% (*Packera aurea* & *Othonna parvifolia*)	0-19.0% (*S. pseudo-arnica* & *S. engleranus* pop 2)	0 - 25.9% (*S. thinaschanicus* & *Emilia discifolia*)	0 – 5.8% (*S. pinnulatus* & *Emilia discifolia*)
Ingroup Sequence Divergence (%)	0- 37.4% (*Packera aurea* & *Senecio pseudo-arnica*)	0-19.0% (*S. pseudo-arnica* & *S. engleranus* pop 2)	0 - 25.9% (*S. thinaschanicus* & *Emilia discifolia*)	0 – 5.8% (*S. pinnulatus* & *Emilia discifolia*)
Outgroup Sequence Divergence (%)	0.4- 24.2% (*Othonna parvifolia* & *Erechtites hieraciifolius*1)	7.4% (*Synotis nagesium* and *Synotis lucorum*)	One outgroup taxon only	One outgroup taxon only
In-outgroup Sequence Divergence (%)	12.3 (*Euryops pectinatus* & *Oresbia heterocarpa*)- 40.3% (*Packera aurea* & *Othonna parvifolia*)	10.6 (*S. engleranus* pop 3 & *Synotis* nagesium) - 18.3% (*Synotis nagesium* & *S. pseudo-arnica*)	12.6% (*Euryops brownei* & *Oresbia heterocarpa*) – 25.4% (*Euryops brownei* and *Emilia discifolia*)	1.6% (*Euryops brownei* & *Phaneroglossa bolusii*)- 5.1% (*Euryops brownei* & *S. pinnulatus*)
Number of gap characters	179	87	60	44
Size of Indels (bp)	1-51	1-9	1-51	1-12
Transitions (minimum)	908	316	313	36
Transversions (minimum)	649	193	220	35
Transitions / Transversions	1.4	1.6	1.4	1.0
Number of MPTs	2284	10000	210	60
Tree Length	2618	859	809	235
Consistency Index (CI)	0.3325	0.5460	0.6143	0.7447
Retention Index (RI)	0.8199	0.8376	0.8719	0.8819
Rescaled Consistency Index (RC)	0.3075	0.4573	0.5356	0.6567
Steps per character	4.76	1.71	1.61	0.30

[a] –Based on alignment excluding ambiguous sequence sites, 5.8S and gap matrix. [b]-Based on alignment excluding ambiguous sequence sites and exon. [c]-excluding 5.8S gene in the case of *ITS* . [d]-excluding exon in the case of *trn*L-F.

Sequence Characteristics

Dataset 1 included 222 taxa and had a total *ITS* length of 1046 bp. The aligned length, after exclusion of ambiguous data and the 5.8S gene, was 550bp of which 423bp (76.9%) were variable and 356bp (64.7%) were parsimony informative. A total of 179 indels were recorded, ranging in size from 1-51bp. MP analysis resulted in the retrieval of 2284 most parsimonious trees of length 2618. The consistency index (CI) was 0.3325, and the retention index (RI) 0.8199. Mean G+C content of *ITS* sequences was 48.7%.

Dataset 2 included 135 taxa and had a total *ITS* length of 868 bp. The aligned length, after exclusion of ambiguous data and the 5.8S gene, was 502bp, of which 292 (57.8%) were variable, and 182 (36.3%) were parsimony informative. A total of 87 indels were recorded, ranging in size from 1-9bp. MP analysis resulted in the retrieval of 10,000 most parsimonious trees of length 859. The consistency index (CI) was 0.5460 and the retention index (RI) was 0.8376. Mean G+C content of *ITS* sequences was 49.82%.

Dataset 3 included *ITS* sequences of 80 taxa, and had a total length of 850 bp. Aligned length, after exclusion of ambiguous areas and 5.8S gene, was 504 bp, of which 292 (57.9%) were variable and 206 (40.9%) were parsimony informative. A total of 60 indels were coded ranging in size from 1-51bp. MP analysis resulted in the retrieval of 210 most parsimonious trees of length 809. The consistency index (CI) was 0.6143 and the retention index (RI) 0.8719. Mean G+C content of *ITS* sequences was 49.5%.

Dataset 4 included *trn*L-F sequences of 69 taxa, in which total length of sequence was 991bp. The aligned sequence length, after exclusion of ambiguously aligned areas and the exon, was 793bp, of which 128 (16.1%) were variable and 53

(6.7%) were parsimony informative. A total of 44 indels were coded ranging in size from 1-12bp. MP analysis resulted in the retrieval of 60 most parsimonious trees of length 235. The consistency index (CI) was 0.7447 and the retention index (RI) was 0.8819. Mean G+C content of *trn*L-F sequences was 34.0%.

2.3.3: Sequence Variation

Figures for sequence variation in each of the four data sets (Figs. 2.5 – 2.8) were generated using MacClade v. 4.06 (Maddison & Maddison, 2003).

ITS Sequence Variation

Most *ITS* sequence variation (figs. 2.5 – 2.7) is present in *ITS*1 and *ITS*2, particularly in the middle sections of each spacer. Very low sequence variation is present in the 5.8S gene, i.e. in the middle section of each figure.

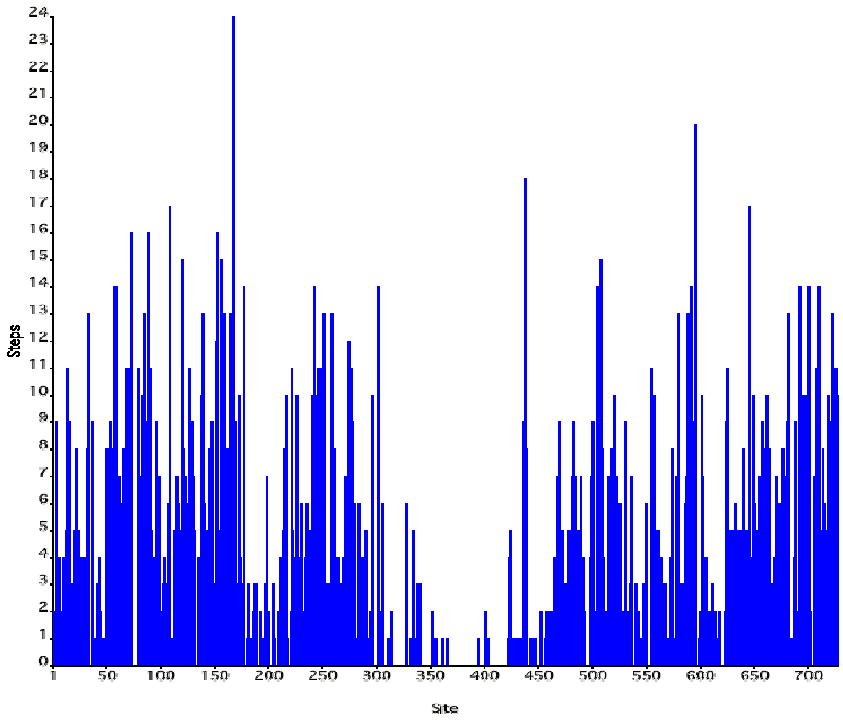


Figure 2.5: Sequence variation in Dataset 1.

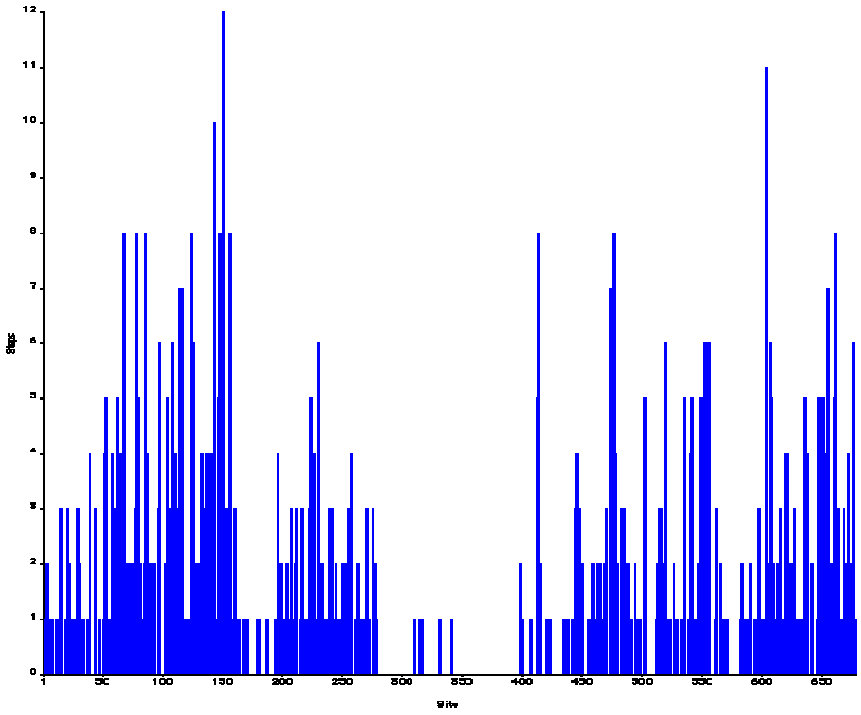


Figure 2.6: Sequence variation in Dataset 2.

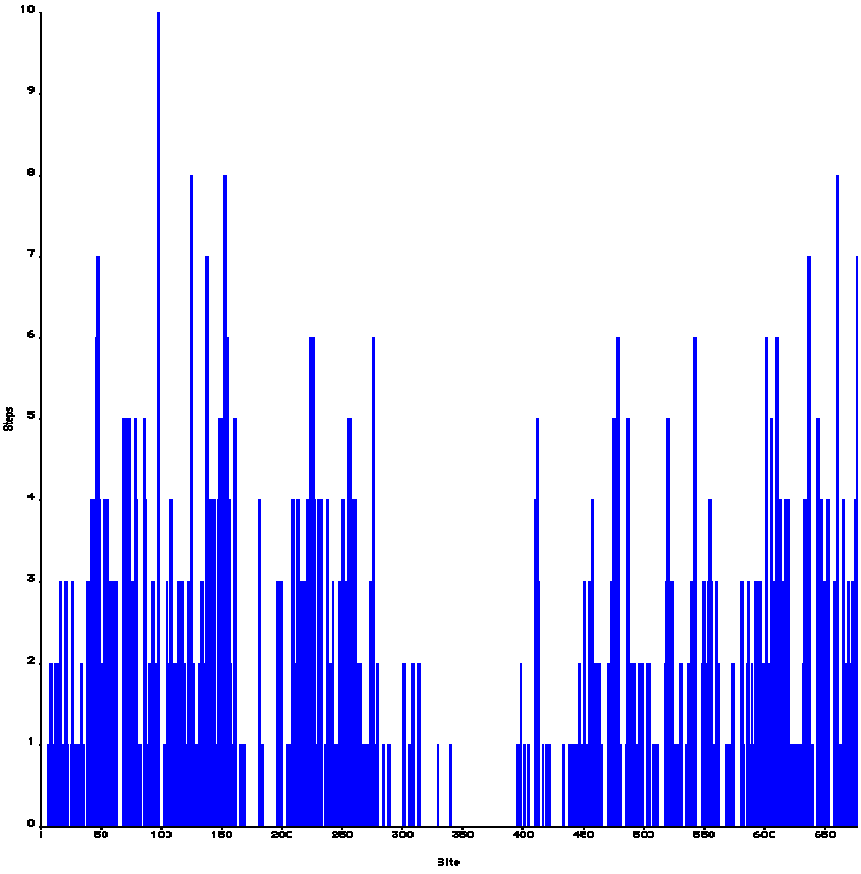


Figure 2.7: Sequence variation in Dataset 3.

_trn_L-F Sequence Variation

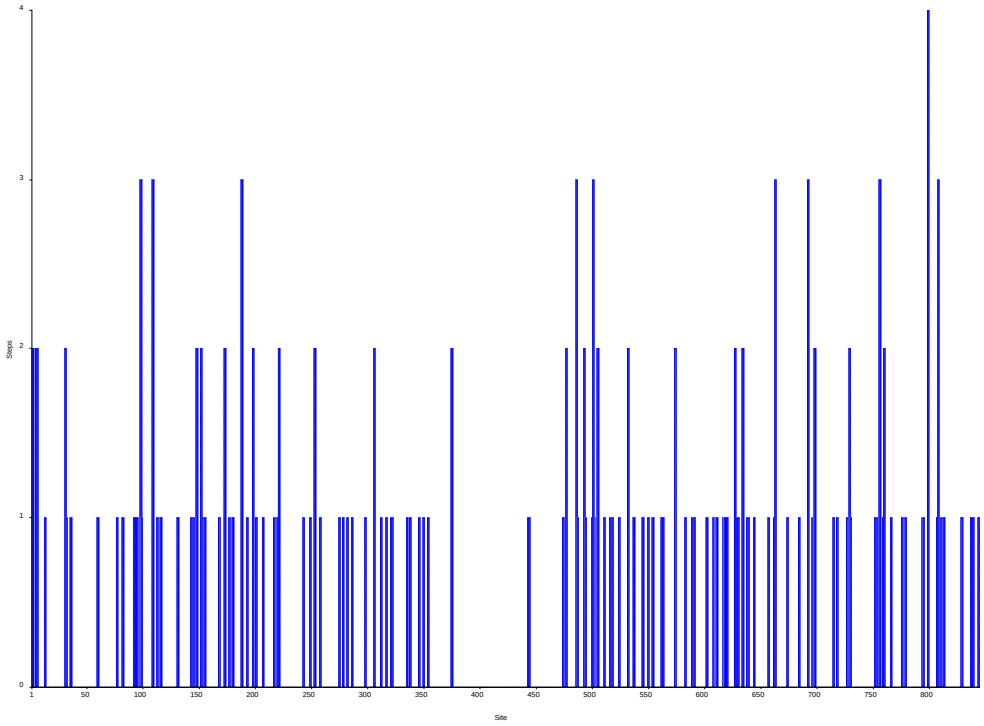


Figure 2.8: Sequence variation in Dataset 4.

As expected, sequence divergence in the plastid *trn*L-F fragment is much lower than that present in the *ITS* sequence. Chloroplast DNA tends to be more highly conserved than nuclear DNA (Taberlet et al., 1991). The area in the middle section of Fig. 2.8 showing very low divergence corresponds to the exon.

2.3.4: Phylogenetic Analyses

Selection of sequence evolution model for BI analyses

For all four final matrices, the model of sequence evolution chosen by Modeltest v.3.7 (Posada & Crandall, 1998) was the general time reversible model, with a gamma-shaped distribution of rates across sites.

Dataset 1: Phylogenetic trees generated from BI and MP analyses of Dataset 1 are presented in Figs. 2.9 – 2.14.

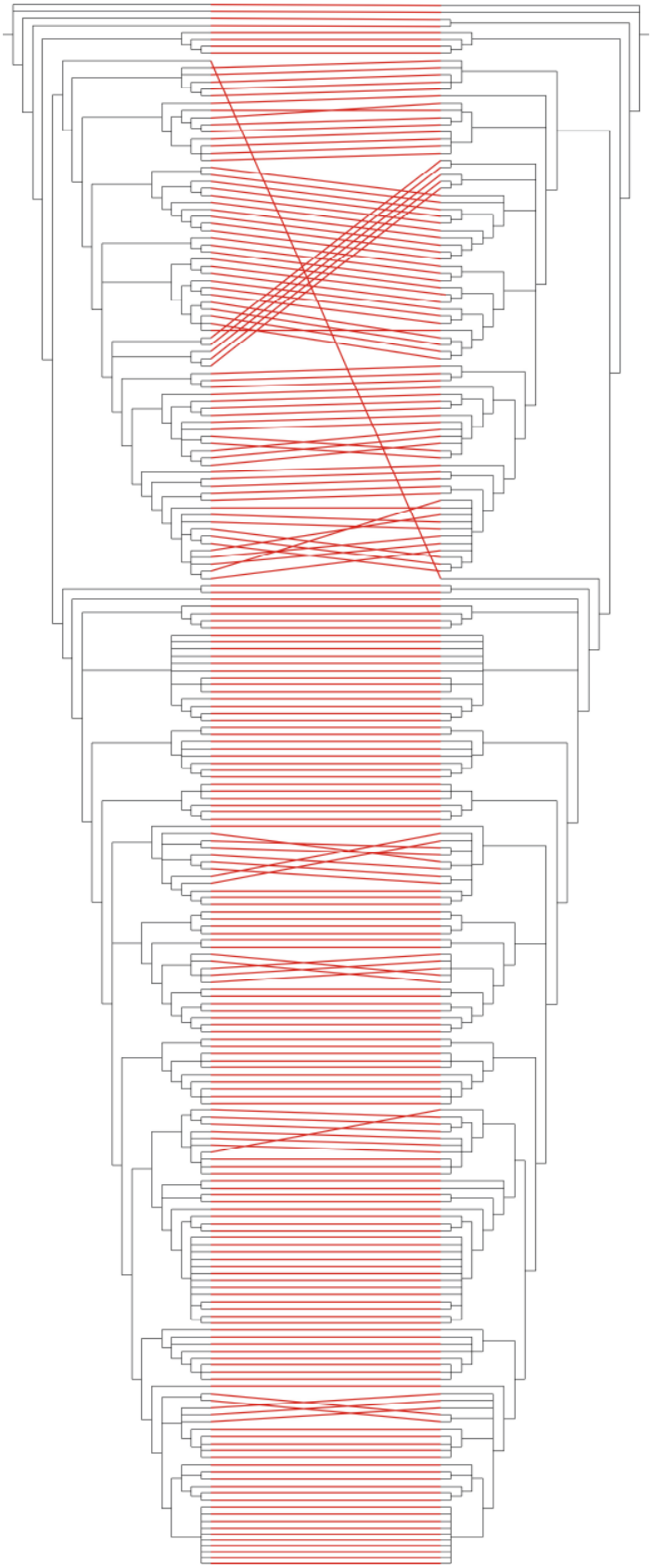
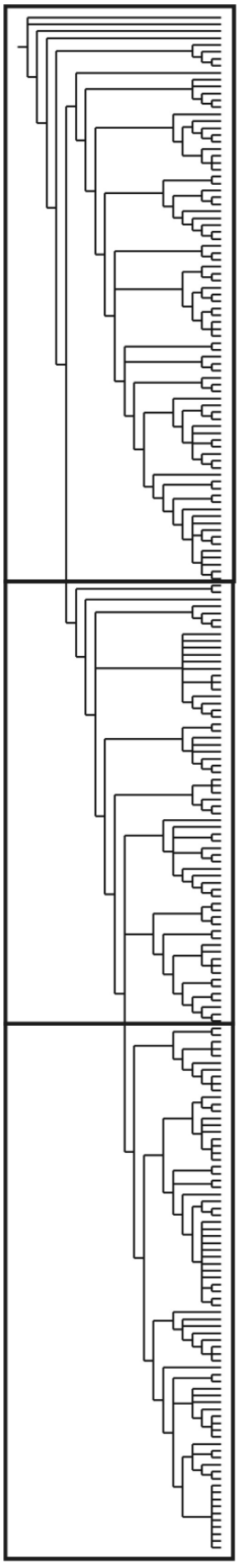


Figure 2.9: Congruence between 50% majority rule consensus cladogram of BI analysis and strict consensus cladogram of MP analysis for Dataset 1. BI tree on the left. Fig 2.9 including taxon labels and support values is presented on the CD which accompanies this thesis.

As seen in Fig 2.9 there are some minor differences in topology between trees generated by BI and MP. Although overall topology is similar in both trees, there are a few taxa which alter position when comparisons are made between trees. Most of these taxa occupy the same clades in both analyses, and are only slightly repositioned within these clades, e.g., members of *Jacobaea* (see fig. 2.9 on the CD which accompanies this thesis for taxon labels and support values). However, there are a few taxa which occupy more fundamentally different locations in the tree. *Dauresia alliariifolia* (O. Hoffm.) B. Nord. & Pelser (syn: *Senecio alliariifolius* O. Hoffm.) is an example of a taxon which occurs in completely different major clades of the BI and MP trees. Support for the position of this species is very weak in each tree, so it is difficult to make a choice as to which clade this *Dauresia* species should be assigned.

Senecio seminiveus J.M.Wood & M.S. Evans, *S. achilleifolius, S. deltoideus, S. speciosus* and *S. tamoides* remain in the same major clade, but appear in a more derived position in the BI analysis. The other differences revealed in topology are more minor. The 50% majority rule tree produced by the BI analysis are those reproduced below.

Figure 2.9 including taxon labels and both Bayesian and maximum parsimony support values is provided in electronic format on the CD which accompanies this thesis.



See p. 62 (Fig. 2.12) for enlarged graphic of this part of the tree

See p. 63 (Fig. 2.13) for enlarged graphic of this part of the tree

See p. 64 (Fig. 2.14) for enlarged graphic of this part of the tree

Fig. 2.10: Overall 50% majority rule consensus cladogram of BI analysis for Dataset 1.

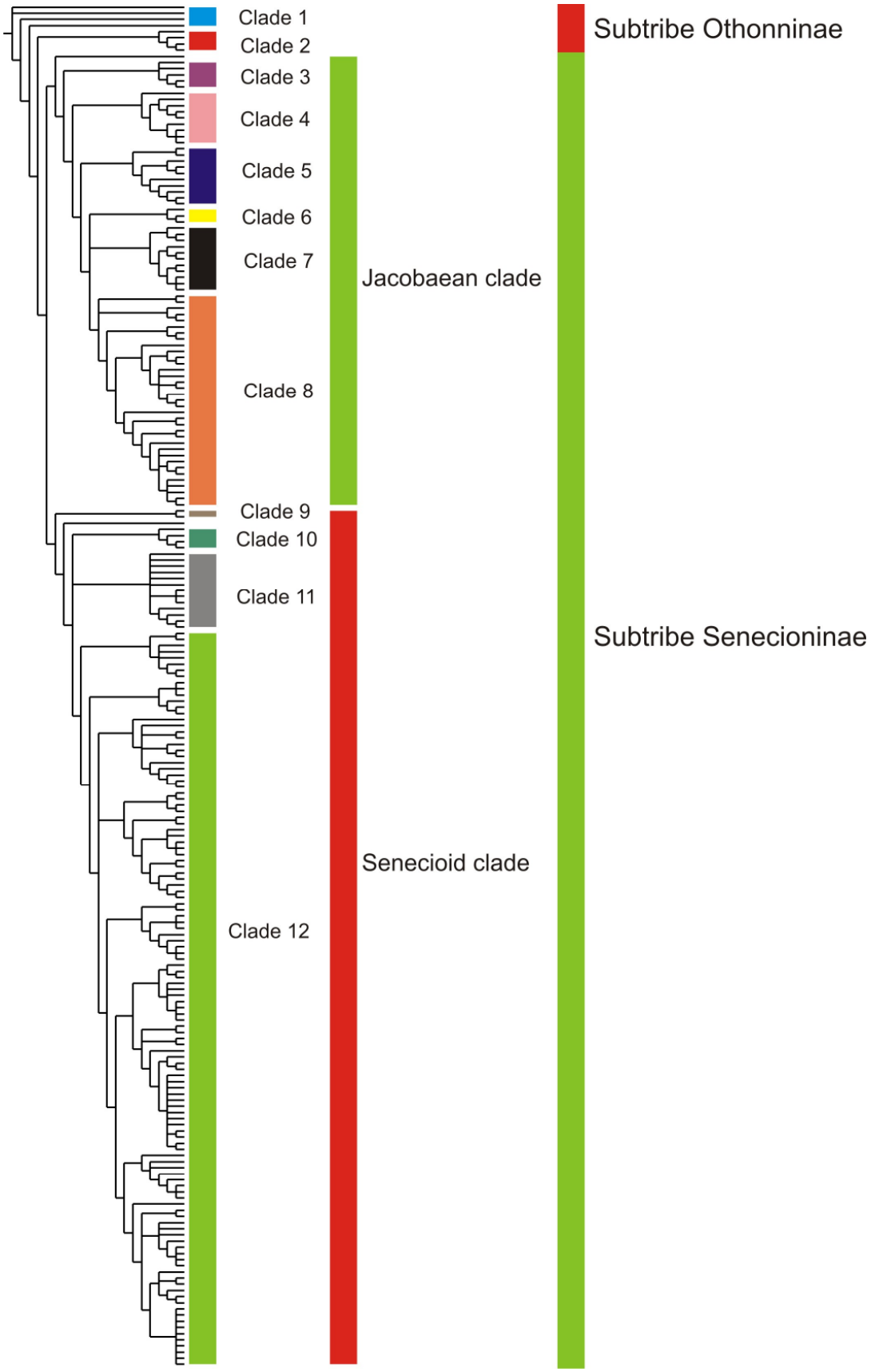
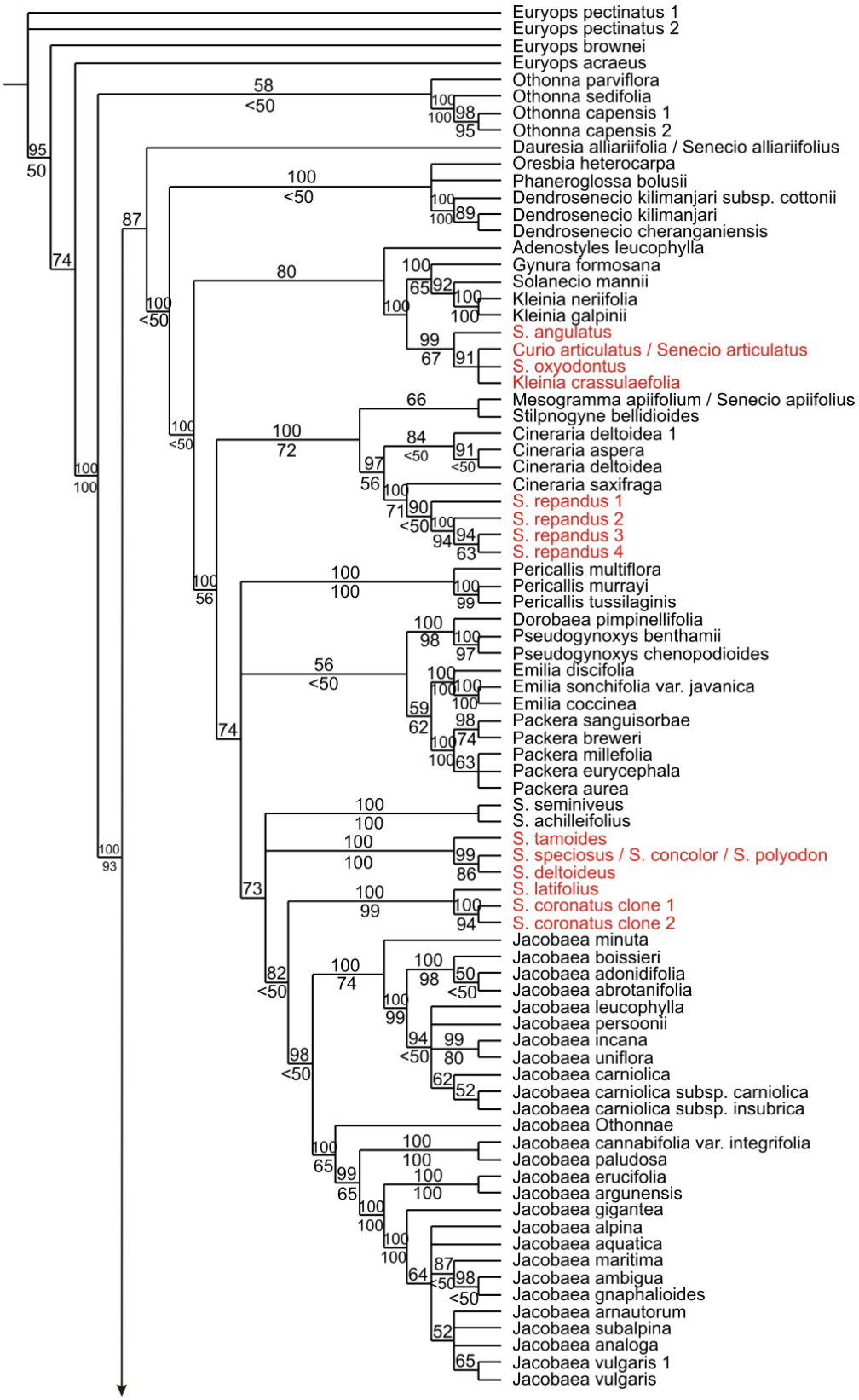


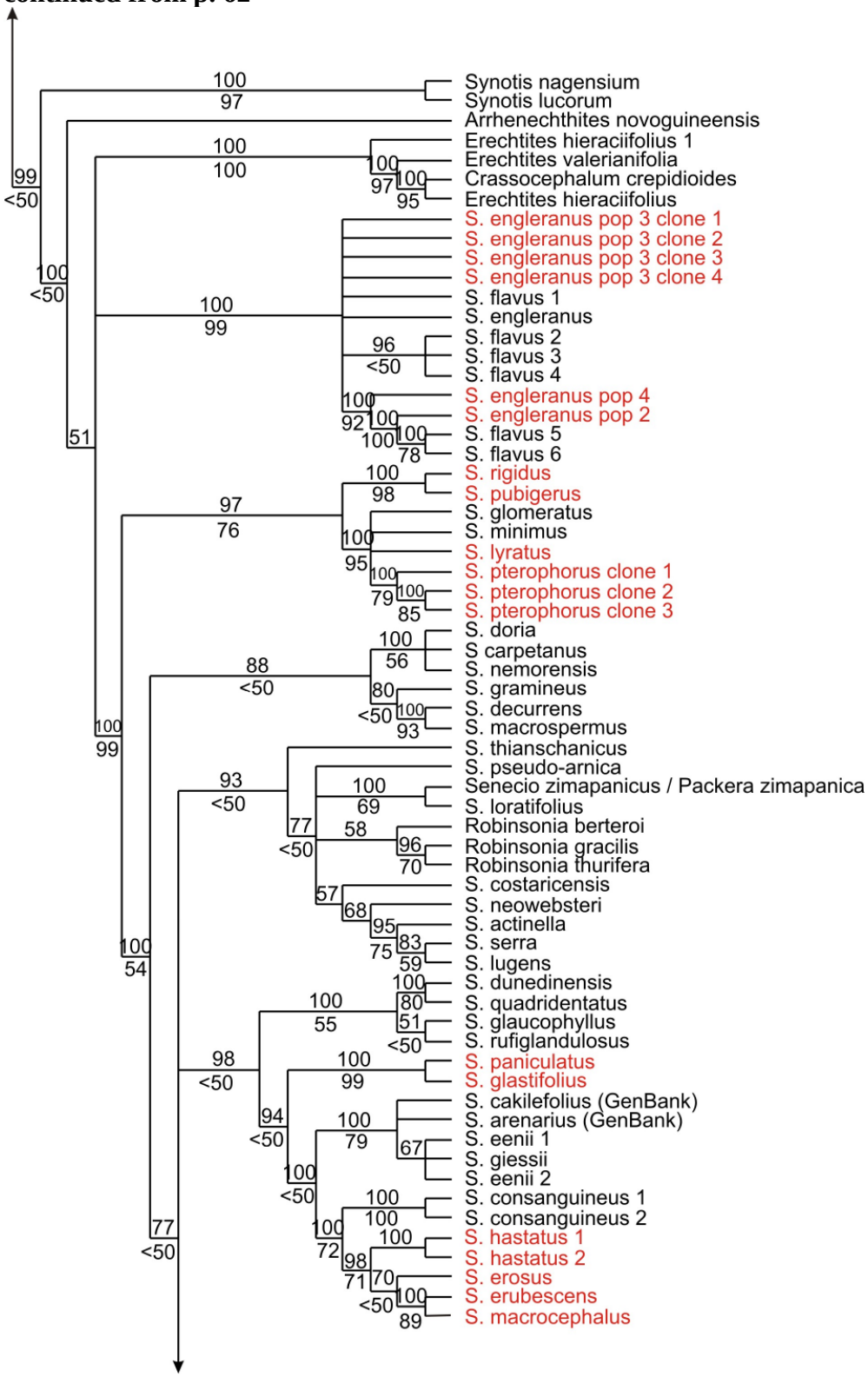
Figure 2.11: Structure of clades in 50% majority rule consensus cladogram of BI analysis for Dataset 1.



continued on p. 63

Figure 2.12: Part 1 of 50% majority rule consensus cladogram of BI analysis for Dataset 1. Bayesian consensus percentages (posterior probabilities x 100) are above the branches, while corresponding parsimony bootstrap percentages are below them. Taxa collected in southern Africa are coloured red.

continued from p. 62



continued on p. 64

Figure 2.13: Part 2 of 50% majority rule consensus cladogram of BI analysis for Dataset 1. Bayesian consensus percentages (posterior probabilities x 100) are above the branches, while corresponding parsimony bootstrap percentages are below them. Taxa collected in southern Africa are coloured red.

Continued from p. 63

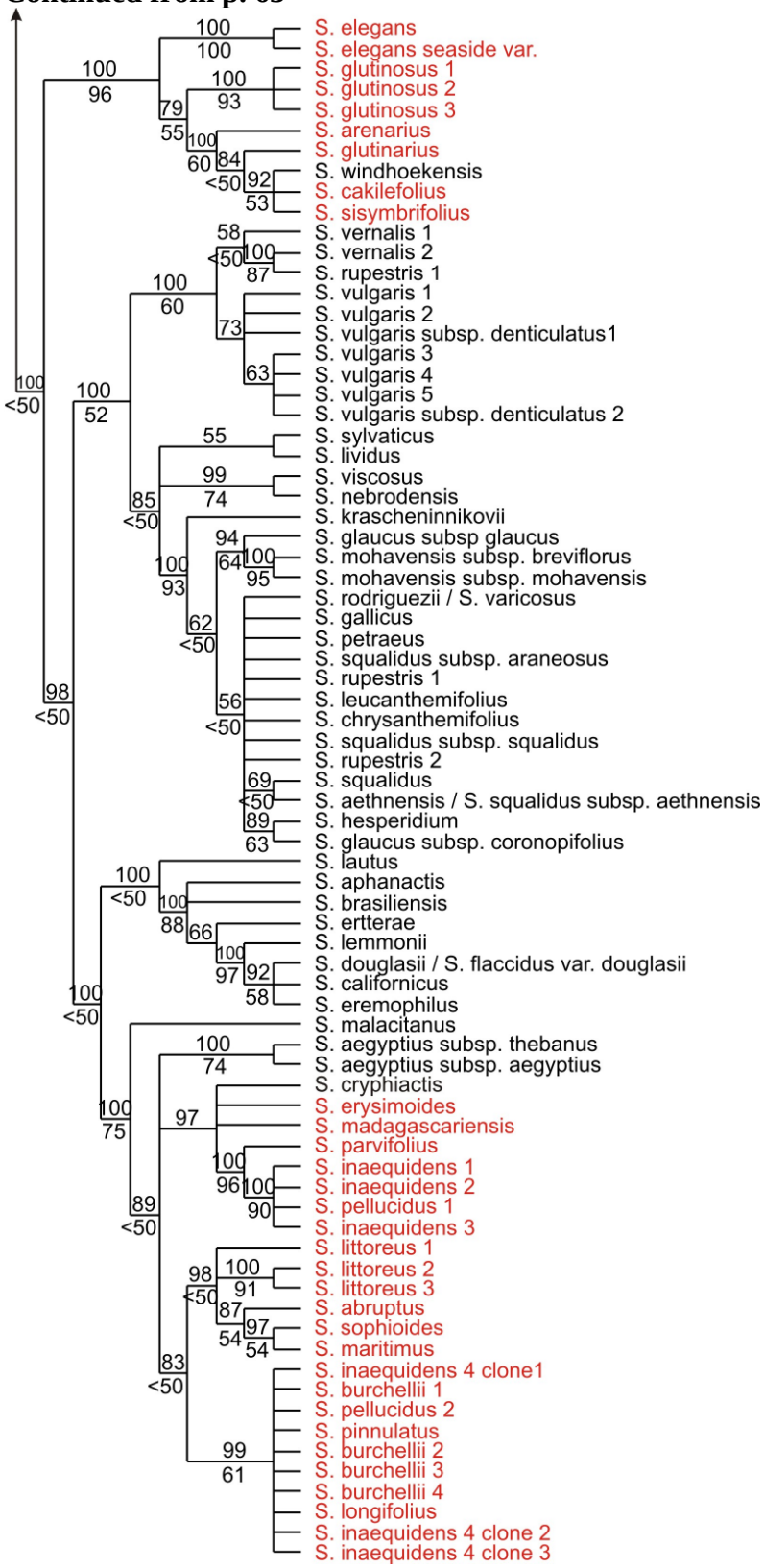


Figure 2.14: Part 3 of 50% majority rule consensus cladogram of BI analysis for Dataset 1. Bayesian consensus percentages (posterior probabilities x 100) are above the branches, while corresponding parsimony bootstrap percentages are below them. Taxa collected in southern Africa are coloured red.

Dataset 1: Subtribes Senecioninae and Othonninae

Dataset 1 includes taxa from throughout the subtribes Senecioninae and Othonninae. Suitable taxa were chosen by referring to preliminary trees of tribe Senecioneae produced by Pieter Pelser (Pelser, personal communication). Pelser's tree was also used to determine a suitable outgroup for the analysis (*Euryops* Cass.). The topology of the resulting tree broadly matches that of Pelser et al. (2007). Clades suggested by the analyses are illustrated in Fig. 2.11.

They are:

Clade 1: (Outgroup): *Euryops acraeus* M.D. Hend., *Euryops brownei* S. Moore, *Euryops pectinatus* (L.) Cass.

Clade 2: *Othonna capensis* L.H. Bailey, *Othonna parviflora* P.J. Bergius, *Othonna sedifolia* DC.

Clade 3: *Dendrosenecio cheranganiensis* (Cotton & Blakelock) E.B. Knox, *Dendrosenecio kilimanjari* (Mildbr.) E.B. Knox, *Dendrosenecio kilimanjari* subsp. *cottonii* (Hutch. & G.Taylor) E.B. Knox, *Oresbia heterocarpa* Cron. & B. Nord., *Phaneroglossa bolusii* (Oliv.) B. Nord.

Clade 4: *Adenostyles leucophylla* (Willd.) Rchb., *Curio articulatus* (syn: *Senecio* articulatus), *Gynura formosana* Kitam., *Kleinia crassulaefolia, Kleinia galpinii* Hook.f., *Kleinia neriifolia* Haw., *Senecio angulatus, Senecio oxyodontus, Solanecio mannii* (Hook.f.) C.Jeffrey.

Clade 5: *Cineraria aspera* Thunb., *Cineraria deltoidea* Sond., *Cineraria saxifraga* DC., *Mesogramma apiifolium* DC. [syn: *Senecio apiifolius* (DC.) Benth. & Hook.f. ex O.Hoffm.], *Senecio repandus, Stilpnogyne bellidioides* DC.

Clade 6: *Pericallis multiflora* (L'Hér.) B.Nord., *Pericallis murrayi* (Bornm.) B.Nord., *Pericallis tussilaginis* (L'Hér.) D.Don.

Clade 7: *Dorobaea pimpinellifolia* (Kunth) B.Nord., *Emilia coccinea* (Sims) G.Don, *Emilia discifolia* (Oliv.) C.Jeffrey, *Emilia sonchifolia* (L.) DC. var. *javanica* (Burm. f.) Mattf., *Packera aurea* (L.) Löve.&D.Löve., *Packera breweri* (Burtt Davy) W.A.Weber & A.Löve, *Packera eurycephala* (Torr. & Gray ex Gray) W.A. Weber & A. Löve, *Packera millefolia* (Torr. & Gray) T.M. Barkl., *Packera sanguisorbae* (DC.) C. Jeffrey, *Pseudogynoxys benthamii* (Baker) Cabrera, *Pseudogynoxys chenopodioides* (Kunth.) Cabrera.

Clade 8: *Jacobaea abrotanifolia* (L.) Moench, *Jacobaea adonidifolia* (Loisel) Pelser & Veldkamp, *Jacobaea alpina* (L.) Moench, *Jacobaea ambigua* (Biv.) Pelser & Veldkamp, *Jacobaea analoga* (DC.) Veldkamp, *Jacobaea aquatica* (Hill) P.Gaertn., B. Mey. & Scherb., *Jacobaea argunensis* (Turez.) Veldkamp, *Jacobaea arnautorum* (Velen.) Pelser, *Jacobaea boissieri* (DC.) Pelser, *Jacobaea cannabifolia* (Less.) E. Wiebe var. *integrifolia* (Koidz.) ined., *Jacobaea carniolica* (Willd.) Schrank, *Jacobaea carniolica* subsp. *carniolica*, *Jacobaea carniolica* subsp. *insubrica* (Chenevard) Pelser, *Jacobaea erucifolia* (L.) P. Gaertn., B. Mey. & Scherb., *Jacobaea gigantean* (Desf.) Pelser, *Jacobaea gnaphaloides* (Sieber ex Spreng.) Veldkamp, *Jacobaea incana* (L.) Veldkamp, *Jacobaea leucophylla* (DC.) Pelser, *Jacobaea maritime* (L.) Pelser & Meijden, *Jacobaea minuta*, *Jacobaea othonnae* (M. Bieb) Spreng. ex. C.A. Mey., *Jacobaea paludosa* (L.) P. Gaertn., B. Mey. & Scherb., *Jacobaea persoonii* (De Not.) Pelser, *Jacobaea subalpina* (W.D.J. Koch) Pelser & Veldkamp, *Jacobaea uniflora* (All.) Veldkamp, *Jacobaea vulgaris* Gaertn., *Senecio achilleifolius* DC., *Senecio coronatus*, *Senecio deltoideus*, *Senecio latifolius*, *Senecio seminiveus*, *Senecio speciosus* (syn: *Senecio polyodon*, *Senecio concolor*), *Senecio tamoides.*

Clade 9: *Synotis leucorum* (Franch.) C. Jeffrey & Y.L. Chen, *Synotis nagesium* (C.B. Clarke) C. Jeffrey & Y.L. Chen.

Clade 10: *Crassocephalum crepidioides* (Benth.) S. Moore, *Erechtites hieraciifolius* (L.) Raf. ex DC., *Erechtites valerianifolius* (Wolf) DC.

Clade 11: *Senecio engleranus, Senecio flavus.*

Clade 12: *Robinsonia gracilis* Decne., *Robinsonia thurifera* Decne., *Robinsonia berteroi* (DC.) R.W. Sanders, Stuessy & Martic., *Senecio abruptus, Senecio actinella* Greene, *Senecio aegyptius* (L.) subsp. *aegyptius, Senecio aegyptius* subsp. *thebanus* Kadereit, *Senecio aethnensis* DC. [syn: *Senecio squalidus* subsp. *aethnensis* (DC.) Greuter], *Senecio aphanactis* Greene, *Senecio arenarius, Senecio brasiliensis* (spreng.) Less., *Senecio burchellii, Senecio cakilefolius, Senecio californicus* DC., *Senecio carpetanus* Boiss. & Reut., *Senecio chrysanthemifolius* (Poir.) Greuter, *Senecio consanguineus, Senecio costaricensis* R.M. King, *Senecio cryphiactis* O.Hoffm., *Senecio decurrens, Senecio doria* L., *Senecio douglasii* DC. [syn: *Senecio flaccidus* Less. var. *douglasii* (DC) B. Turner & T. Barkley], *Senecio dunedinensis* Belcher, *Senecio eenii* (S. Moore) Merxm., *Senecio elegans, Senecio eremophilus* Richardson, *Senecio erosus, Senecio ertterae* T.M. Barkley, *Senecio erubescens, Senecio erysimoides, Senecio gallicus* Vill., *Senecio giessii* Merxm., *Senecio glastifolius, Senecio glaucophyllus* Cheesem., *Senecio glaucus* (L.) subsp. *coronopifolius* (Maire) C. Alexander, *Senecio glaucus* subsp. *glaucus, Senecio glomeratus* Desf. ex Poir., *Senecio glutinarius, Senecio glutinosus, Senecio gramineus* Harv., *Senecio hastatus, Senecio hesperidium* Jahand., Maire & Weiller, *Senecio inaequidens, Senecio kraschenninikovii* Schischk., *Senecio lautus* G. Forst. ex Willd., *Senecio*

lemmonii A. Gray, *Senecio leucanthemifolius* Poir., *Senecio littoreus*, *Senecio lividus* L., *Senecio longifolius, Senecio loratifolius* Greenm., *Senecio lugens* Richardson ex Hook., *Senecio lyratus, Senecio macrocephalus, Senecio macrospermus* DC., *Senecio madagascariensis, Senecio malacitanus* (Huter) Greuter, *Senecio maritimus*, *Senecio minimus* Poir., *Senecio mohavensis* subsp. *breviflorus* (Kadereit) M. Coleman, *Senecio mohavensis* subsp. *mohavensis, Senecio nebrodensis, Senecio nemorensis* L., *Senecio neowebsteri* S.F. Blake, *Senecio paniculatus, Senecio parvifolius, Senecio pellucidus, Senecio petraeus* Boiss. & Reut., *Senecio pseudo-arnica* Less., *Senecio pterophorus, Senecio pubigerus, Senecio quadridentatus* Labill., *Senecio rigidus, Senecio rodriguezii* Willk. ex Rod. (syn: *Senecio varicosus* L.f.), *Senecio rufiglandulosus* Colenso, *Senecio rupestris, Senecio serra* Hook., *Senecio sisymbrifolius, Senecio sophioides, Senecio squalidus* subsp. *araneosus* (Emb. & Maire) C. Alexander, *Senecio squalidus* subsp. *squalidus, Senecio sylvaticus* L., *Senecio thianchanicus* Regel. & Schmalh., *Senecio vernalis, Senecio viscosus, Senecio vulgaris, Senecio vulgaris* subsp. *denticulatus* (O.F. Müll.) P.D. Sell, *Senecio windhoekensis* Merxm., *Senecio zimapanicus* [syn: *Packera zimapanica* (Hemsl.) Freeman & Barkley].

Monophyly of Senecioninae (marked in Fig. 2.11) is strongly supported by this analysis (BPP = 1.00, MPB = 93%). Clade 12 is strongly supported and represents *Senecio* s.str. (BPP = 1.00, MPB = 99%). The clade includes almost all species ascribed to *Senecio.* The group of mainly *Senecio* species exists on a polytomy with the *Senecio engleranus / Senecio flavus* clade (clade 11), and *Erechtites* Raf. and *Crassocephalum* Moench. (clade 10). The polytomy is very weakly supported (BPP = 0.51, MPB = <50%). If *Arrhenechthites* Mattf. is added to

the group, BI analysis suggests strong support (BPP = 1.00). However, the corresponding parsimony bootstrap value is <50%, suggesting only very weak support for the group, even with *Arrhenechthites* added. Clades 11 and 12 were studied in more detail as Dataset 2.

Genera of the Senecioninae and Othonninae which appear to be monophyletic in this analysis are *Othonna* L. (BPP = 0.58, MPB = <50%, only very weakly supported), *Pericallis* D. Don (BPP = 1.00, MPB = 100%, very strongly supported), *Emilia* Cass. (BPP = 1.00, MPB = 100%, very strongly supported), *Jacobaea* (BPP = 0.98, MPB = <50%, only weakly supported), and *Synotis* (C. B. Clarke) C. Jeffrey & Y. L. Chen (BPP = 1.00, MPB = 97%, strongly supported). Excluding *Packera zimapanica*, a synonym for *Senecio zimapanicus*, *Packera* is also monophyletic (BPP = 1.00, MPB = 100%, very strongly supported).

Dataset 2: *Senecio* s.str.

Phylogenetic trees generated from BI and MP analyses of Dataset 2 are presented in Figs. 2.15 – 2.19.

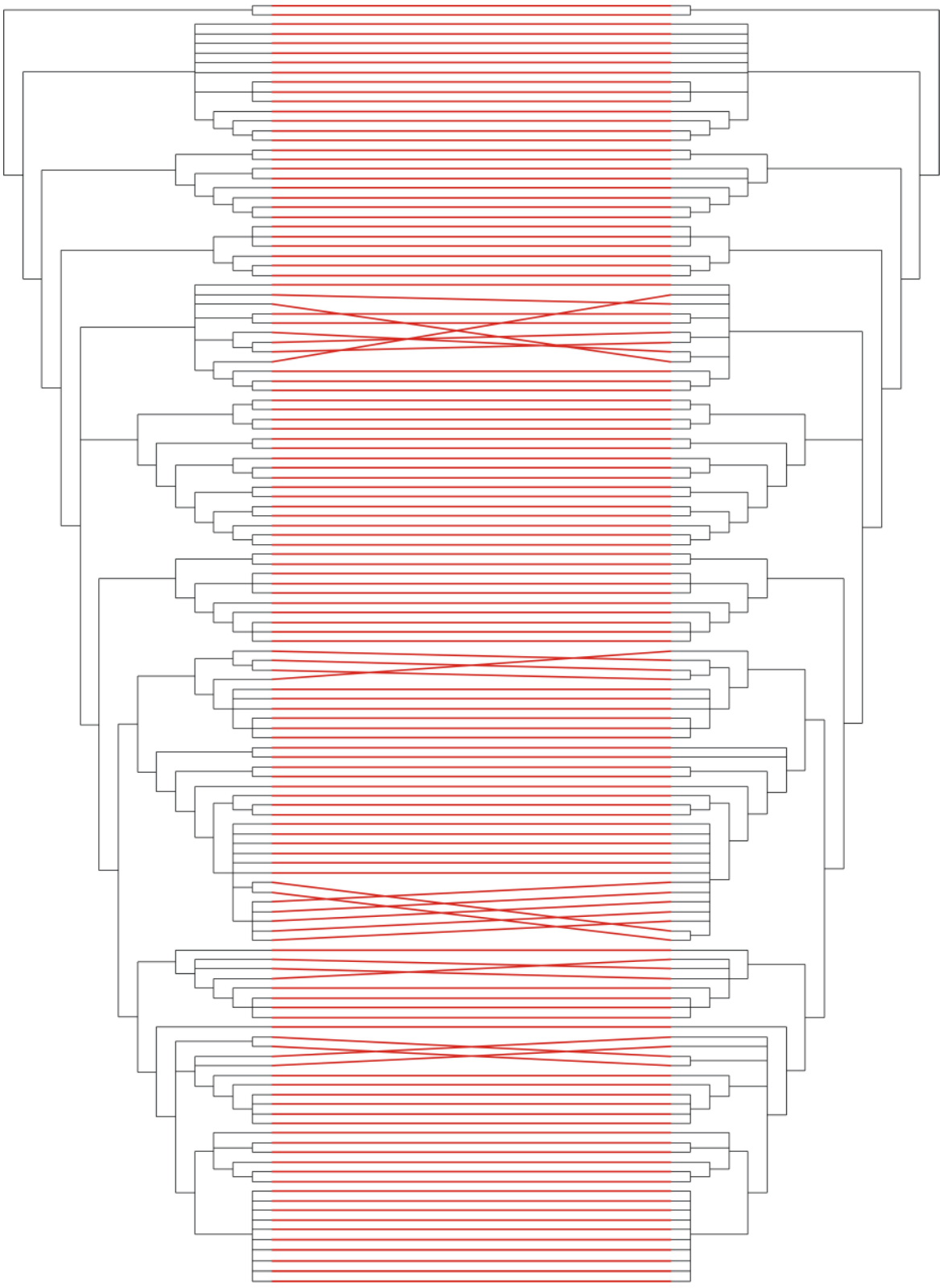
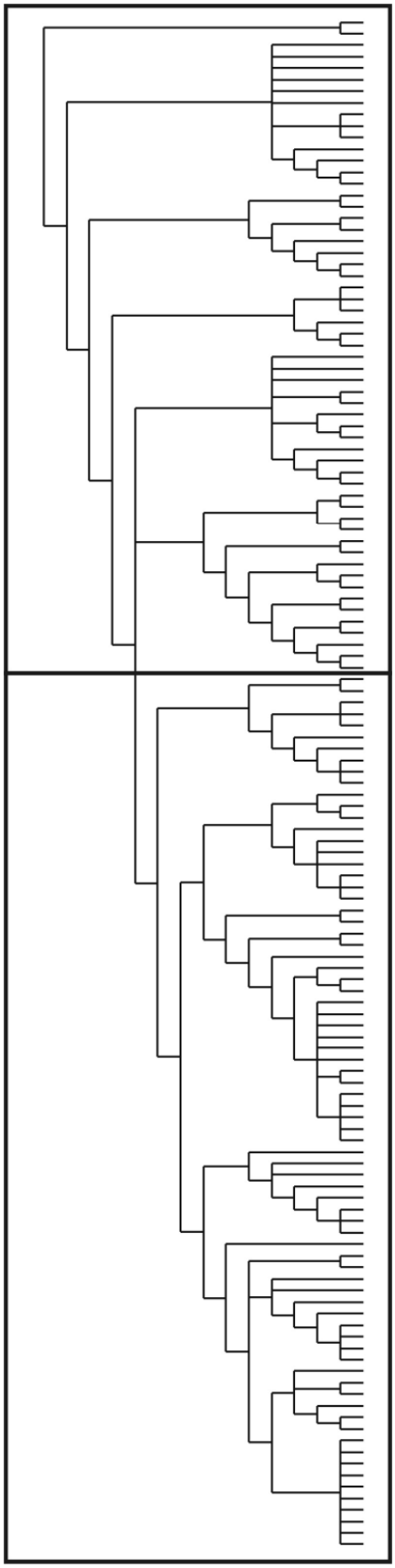


Figure 2.15: Congruence between 50% majority rule consensus cladogram of BI analysis and strict consensus cladogram of MP analysis for Dataset 2. Fig 2.15 including taxon labels and support values is presented on the CD which accompanies this thesis.

There are only minor topological differences between the 50% majority rule tree produced by BI analysis and the MP strict consensus tree (Fig. 2.15). Some species occupy slightly different positions within small clades, but overall the structure is very similar. The 50 % majority rule trees produced by the BI analysis are reproduced below (Figs. 2.16 – 2.19). Fig. 2.15 including taxon labels, and both BI and MP support values is provided in electronic format on the CD which accompanies this thesis.



See p. 75 (Fig. 2.18) for enlarged graphic of this part of the tree

See p. 76 (Fig. 2.19) for enlarged graphic of this part of the tree

Figure 2.16: 50% majority rule consensus cladogram of BI analysis for Dataset 2.

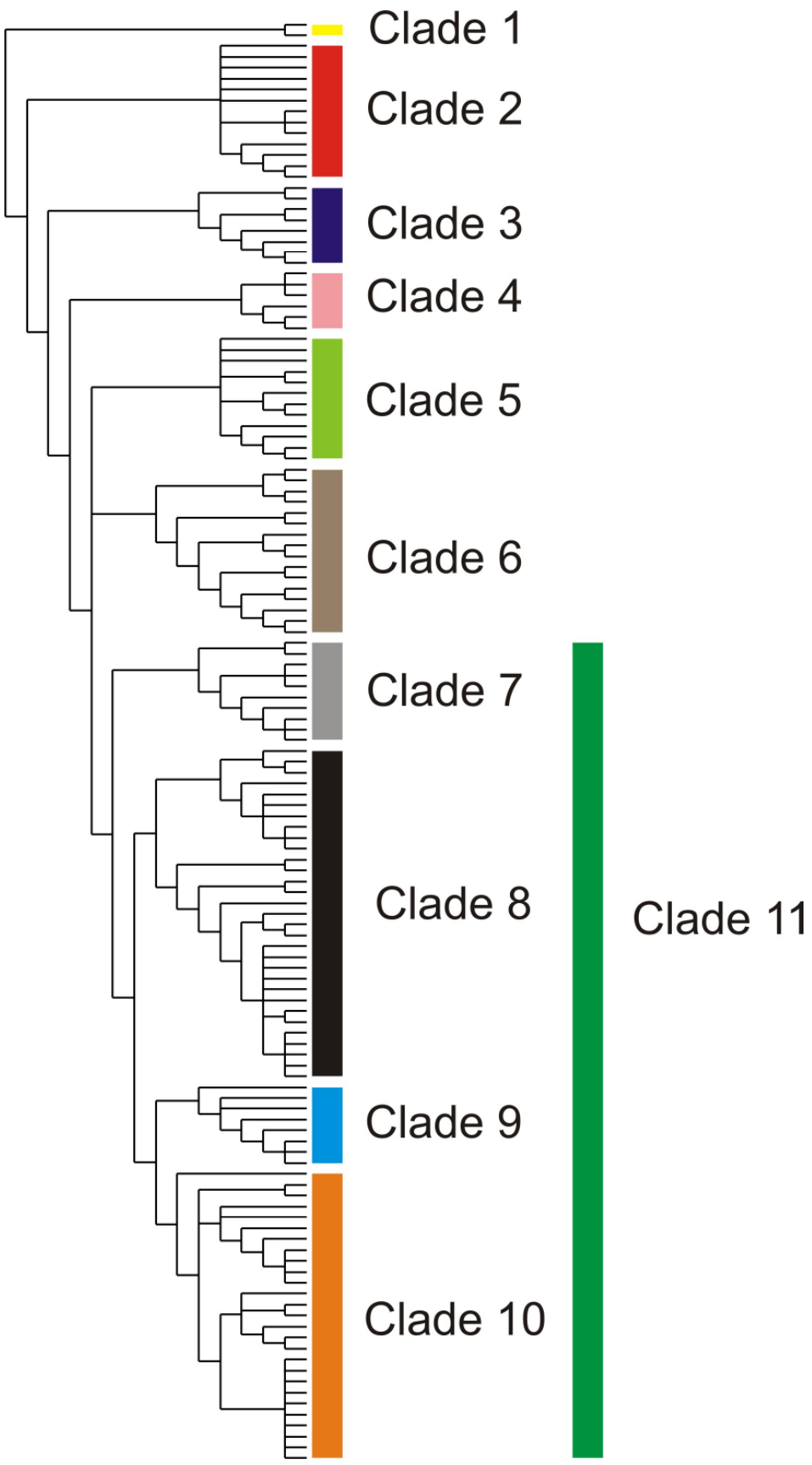
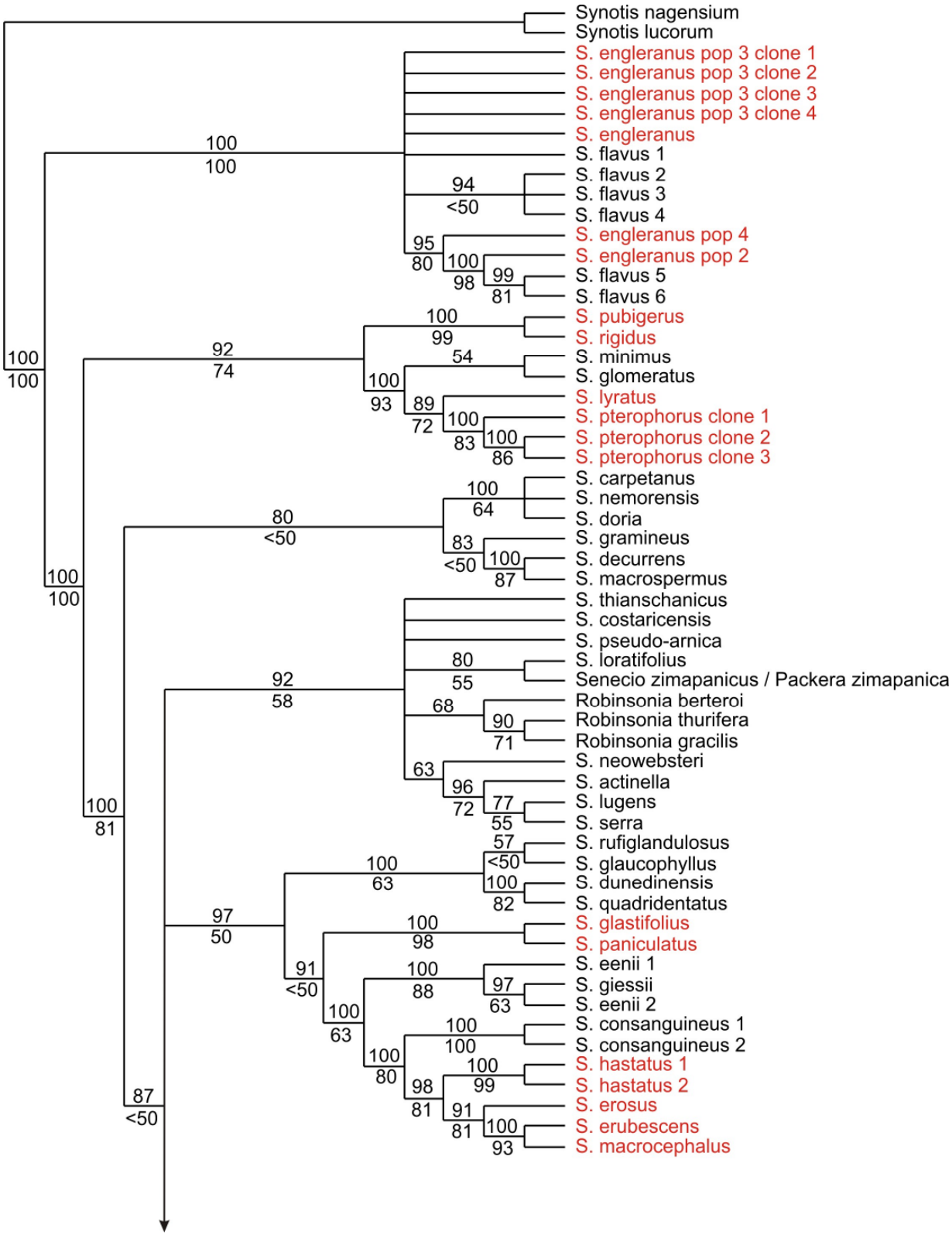


Figure 2.17: Structure of clades in 50% majority rule consensus cladogram of BI analysis for Dataset 2.



continued on p. 76

Figure 2.18: Part 1 of 50% majority rule consensus cladogram of BI analysis for Dataset 2. Bayesian consensus percentages (posterior probabilities x 100) are above the branches, while corresponding parsimony bootstrap percentages are below them. Taxa collected in southern Africa are coloured red.

Continued from p. 75

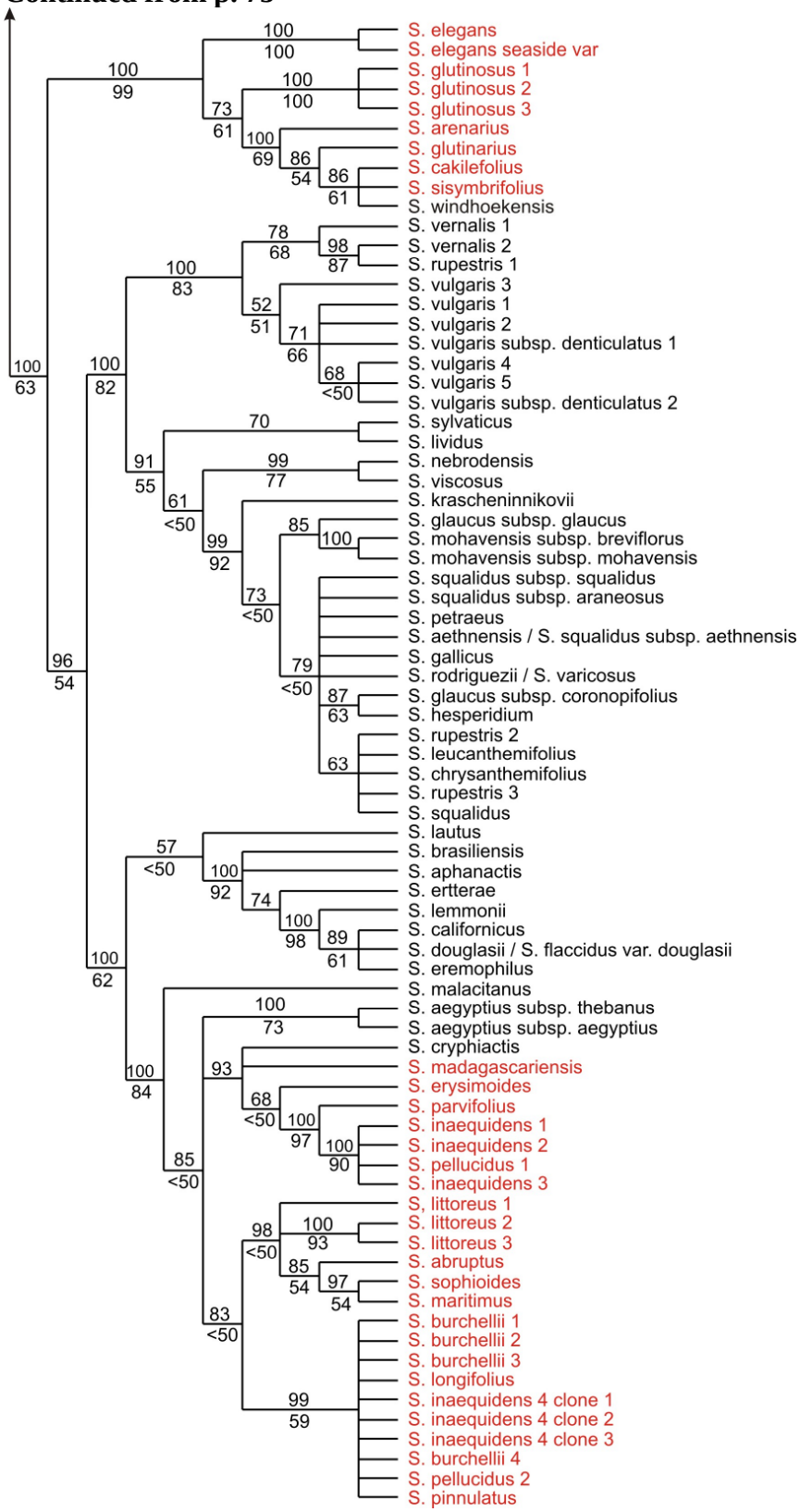


Figure 2.19: Part 2 of 50% majority rule consensus cladogram of BI analysis for Dataset 2. Bayesian consensus percentages (posterior probabilities x 100) are above the branches, while corresponding parsimony bootstrap percentages are below them. Taxa collected in southern Africa are coloured red

Dataset 2: *Senecio* s.str.

Dataset 2 includes taxa from *Senecio* s.str., as well as *S. engleranus* and *S. flavus*. *Synotis* was chosen as a suitable outgroup, also based on the wider analyses of Dataset 1. Clades suggested by the analyses (Fig. 2.17) are:

Clade 1: (Outgroup) *Synotis leucorum, Synotis nagesium.*

Clade 2: *Senecio engleranus, Senecio flavus.*

Clade 3: *Senecio glomeratus, Senecio lyratus, Senecio minimus, Senecio pterophorus, Senecio pubigerus, Senecio rigidus.*

Clade 4: *Senecio carpetanus, Senecio decurrens, Senecio doria, Senecio gramineus, Senecio macrospermus, Senecio nemorensis.*

Clade 5: *Robinsonia berteroi, Robinsonia gracilis, Robinsonia thurifera, Senecio actinella, Senecio costaricensis, Senecio loratifolius, Senecio lugens, Senecio neowebsteri, Senecio pseudo-arnica, Senecio serra, Senecio thianschanicus, Senecio zimapanicus* (syn: *Packera zimapanica*)

Clade 6: *Senecio consanguineus, Senecio dunedinensis, Senecio eenii, Senecio erosus, Senecio erubescens, Senecio giessii, Senecio glastifolius, Senecio glaucophyllus, Senecio hastatus, Senecio macrocephalus, Senecio paniculatus, Senecio quadridentatus, Senecio rufiglandulosus.*

Clade 7: *Senecio arenarius, Senecio cakilefolius, Senecio elegans, Senecio glutinarius, Senecio glutinosus, Senecio sisymbrifolius, Senecio windhoekensis.*

Clade 8: *Senecio aethnensis* (syn: *Senecio squalidus* subsp. *aethnensis*), *Senecio chrysanthemifolius, Senecio gallicus, Senecio glaucus* subsp. *coronopifolius, Senecio glaucus* subsp. *glaucus, Senecio hesperidium, Senecio kraschenninikovii, Senecio leucanthemifolius, Senecio lividus, Senecio*

mohavensis subsp. *breviflorus*, *Senecio mohavensis* subsp. *mohavensis*, *Senecio nebrodensis*, *Senecio petraeus*, *Senecio rodriguezii* (syn: *Senecio varicosus*), *Senecio rupestris*, *Senecio squalidus* subsp. *araneosus*, *Senecio squalidus* subsp. *squalidus*, *Senecio sylvaticus*, *Senecio vernalis*, *Senecio viscosus*, *Senecio vulgaris*, *Senecio vulgaris* subsp. *denticulatus.*

Clade 9: *Senecio aphanactis*, *Senecio brasiliensis*, *Senecio californicus*, *Senecio douglasii* (syn: *Senecio flaccidus* var. *douglasii*), *Senecio eremophilus*, *Senecio ertterae*, *Senecio lautus*, *Senecio lemmonii.*

Clade 10: *Senecio abruptus*, *Senecio aegyptius* subsp. *aegyptius*, *Senecio aegyptius* subsp. *thebanus*, *Senecio burchellii*, *Senecio cryphiactis*, *Senecio erysimoides*, *Senecio inaequidens*, *Senecio littoreus*, *Senecio longifolius*, *Senecio madagascariensis*, *Senecio malacitanus*, *Senecio maritimus*, *Senecio parvifolius*, *Senecio pellucidus*, *Senecio sophioides.*

Clade 11: Clade 11 represents what may be sect. *Senecio* on this tree of *Senecio* s.str. and consists of clades 7, 8, 9 and 10.

Dataset 3: Reduced Senecioninae *ITS* matrix

Phylogenetic trees generated from BI and MP analyses of Dataset 3 are presented in Figs. 2.20 – 2.23.

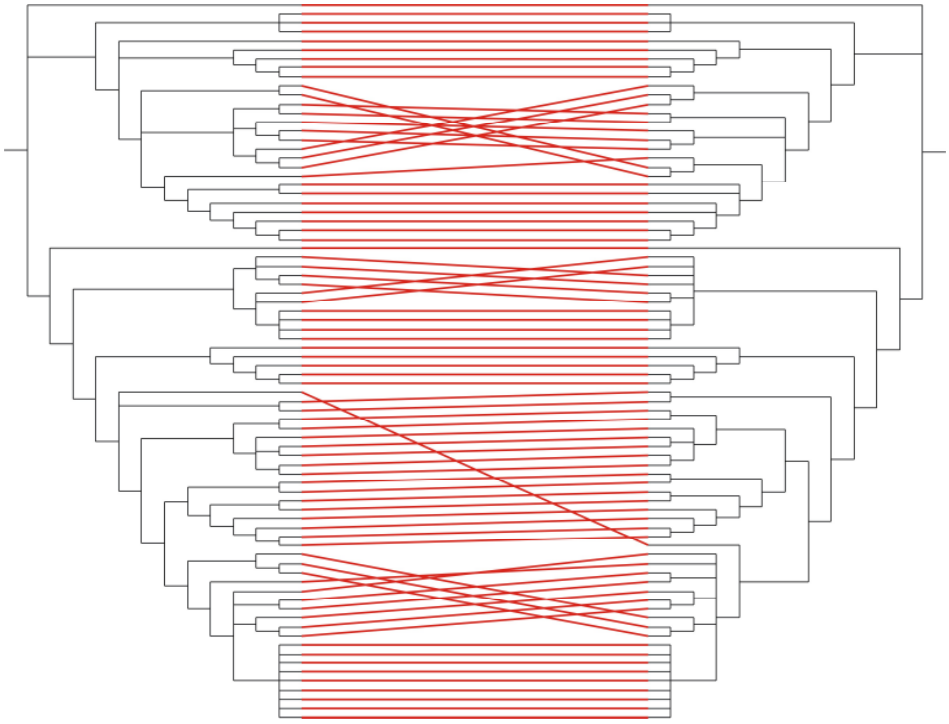


Figure 2.20: Congruence between 50% majority rule consensus cladogram of BI analysis and strict consensus cladogram of MP analysis for Dataset 3. BI tree on the left. Fig 2.20 including taxon labels and support values is presented on the CD which accompanies this thesis.

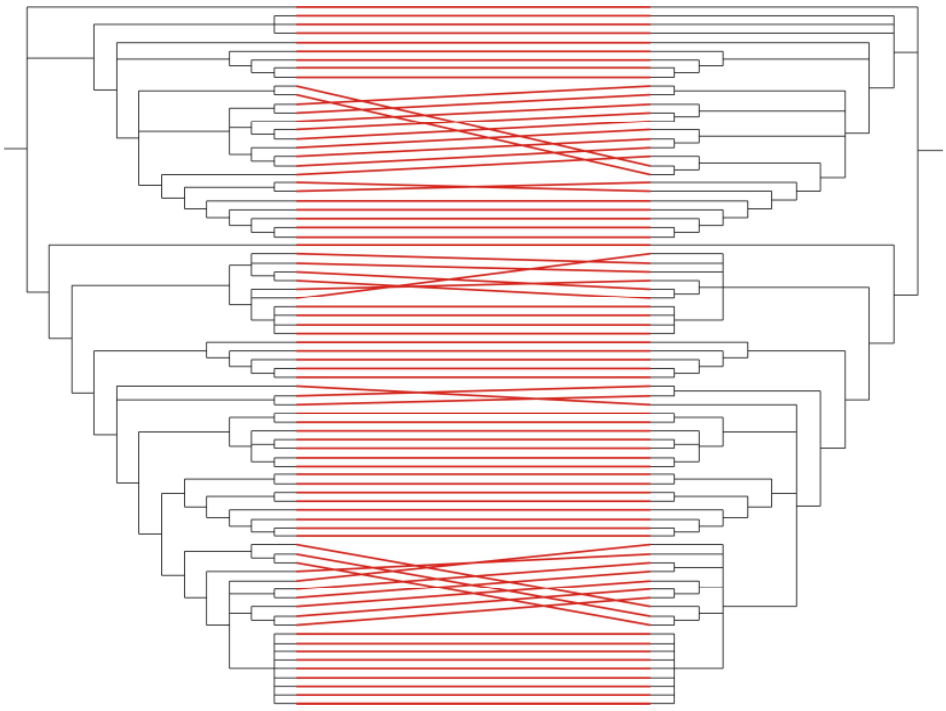


Figure 2.21: Congruence between 50% majority rule consensus cladogram of BI analysis and 50% majority rule bootstrap cladogram of MP analysis for Dataset 3. BI tree on the left. Fig 2.21 including taxon labels and support values is presented on the CD which accompanies this thesis.

There are mainly only minor differences between the topologies of the 50% majority rule tree produced by BI analysis and the MP strict consensus tree for Dataset 3 (Fig. 2.20). However, *Senecio thianschanicus* is positioned in different clades of the two trees, although in each case its position is poorly supported (see Fig. 2.20 on the CD which accompanies this thesis for taxon labels and support values).

Congruence is actually higher between the BI tree and the 50% majority rule MP bootstrap tree (Fig. 2.21). In the bootsrap tree, *S. thianschanicus* occupies a similar position as in the BI tree. The 50 % majority rule trees produced by the BI anlaysis are illustrated below (see Fig. 2.21 on the CD which accompanies this thesis for taxon labels and support values).

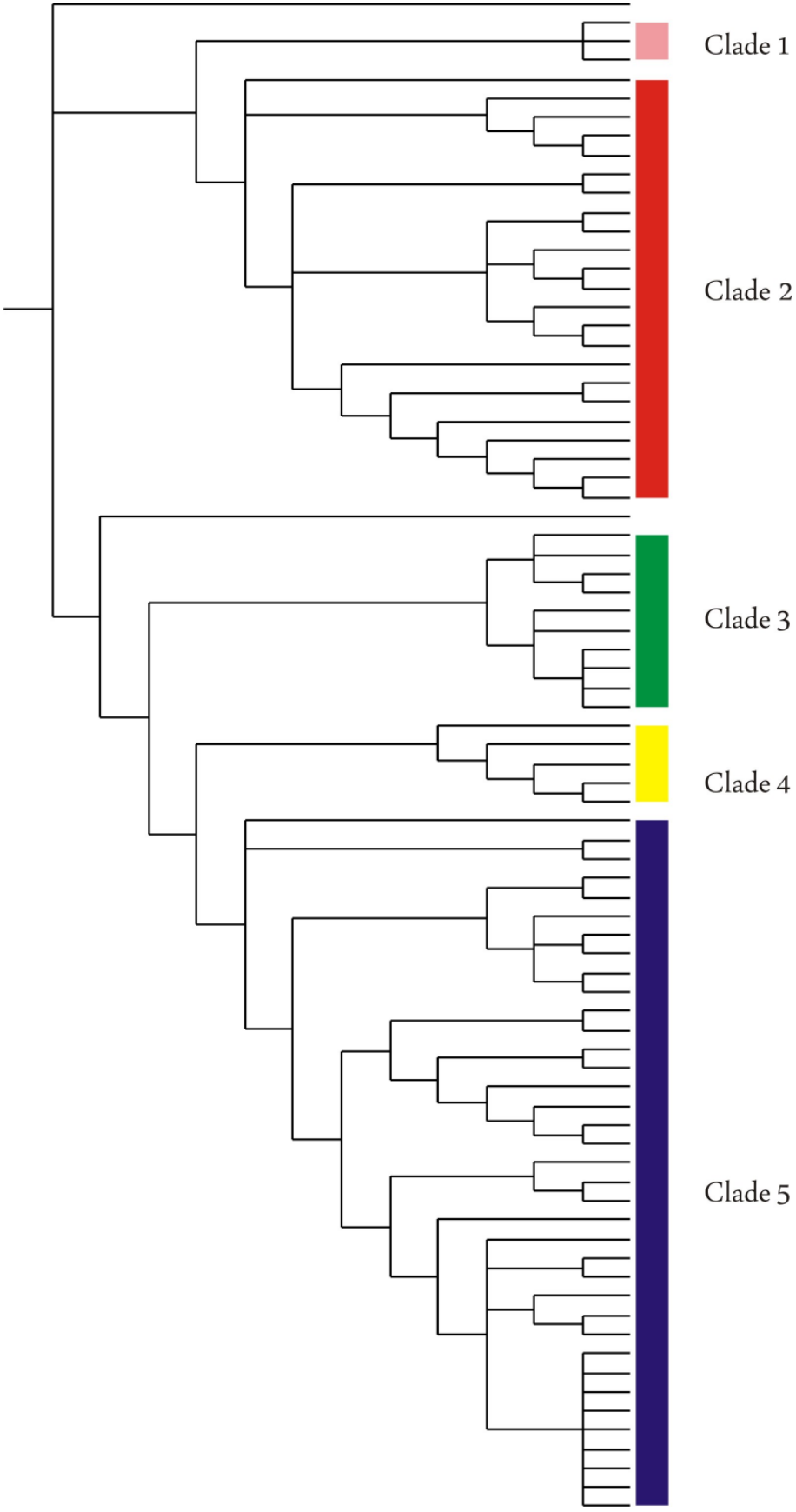


Figure 2.22: Structure of clades in 50% majority rule consensus cladogram of BI analysis for Dataset 3.

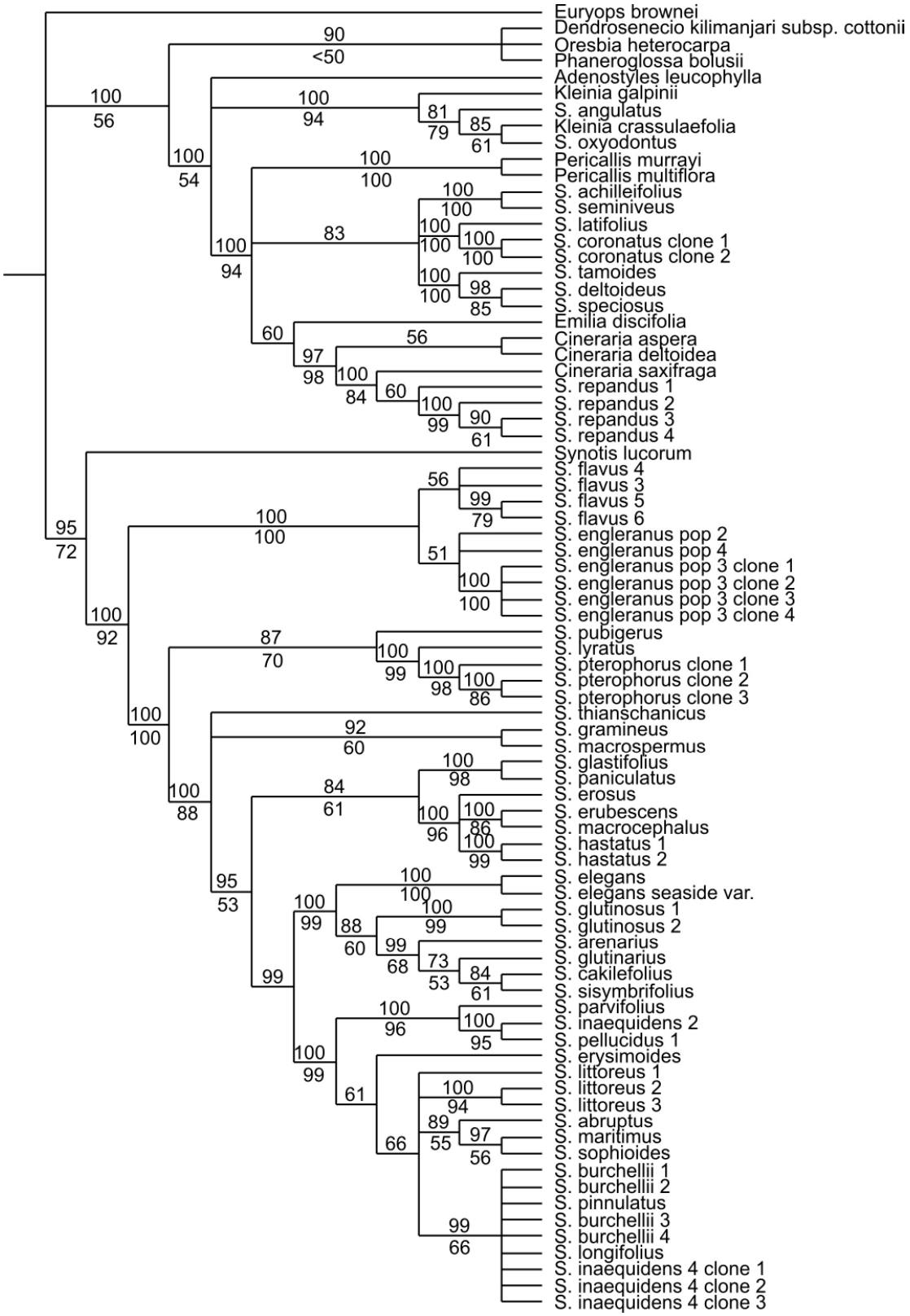


Figure 2.23: 50% majority rule consensus cladogram of BI analysis for Dataset 3. Bayesian consensus percentages (posterior probabilities x 100) are above the branches, while corresponding parsimony bootstrap percentages are below them.

Dataset 3 includes taxa from throughout subtribe Senecioninae. Clades suggested by the analysis (Fig. 2.22) are:

Clade 1: *Dendrosenecio kilimanjari* subsp. *cottonii, Oresbia heterocarpa, Phaneroglossa bolusii.*

Clade 2: *Adenostyles leucophylla, Cineraria aspera, Cineraria deltoidea, Cineraria saxifraga, Emilia discifolia, Kleinia crassulaefolia, Kleinia galpinii, Pericallis multiflora, Pericallis murrayi, Senecio achilleifolius, Senecio angulatus, Senecio coronatus, Senecio deltoideus, Senecio latifolius, Senecio oxyodontus, Senecio repandus, Senecio seminiveus, Senecio speciosus, Senecio tamoides.*

Clade 3: *Senecio engleranus, Senecio flavus.*

Clade 4: *Senecio lyratus, Senecio pterophorus, Senecio pubigerus.*

Clade 5: *Senecio abruptus, Senecio arenarius, Senecio burchellii, Senecio cakilefolius, Senecio elegans, Senecio erosus, Senecio erubescens, Senecio erysimoides, Senecio glastifolius, Senecio glutinarius, Senecio glutinosus, Senecio gramineus, Senecio hastatus, Senecio inaequidens, Senecio littoreus, Senecio longifolius, Senecio macrocephalus, Senecio macrospermus, Senecio maritimus, Senecio paniculatus, Senecio parvifolius, Senecio pellucidus, Senecio sisymbrifolius, Senecio sophioides, Senecio thianschanicus.*

Dataset 4: Senecioninae *trn*L-F matrix

Phylogenetic trees generated from BI and MP analyses of Dataset 4 are presented in Figs. 2.24 – 2.26.

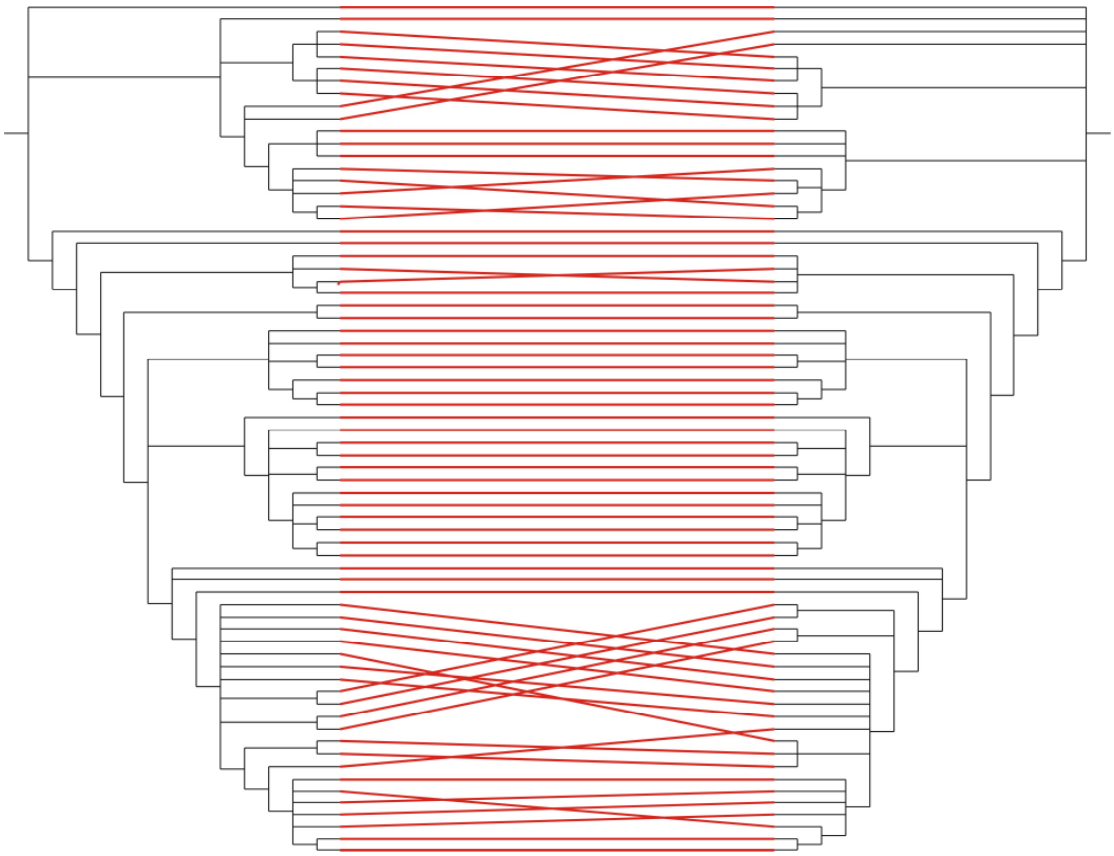


Figure 2.24: Congruence between 50% majority rule consensus cladogram of BI analysis and strict consensus cladogram of MP results for Dataset 4 (*trn*L-F data from members of Senecioninae). BI tree on the left. Fig 2.24 including taxon labels and support values is presented on the CD which accompanies this thesis.

There are only minor differences between the topologies of the 50% majority rule tree produced by BI analysis and the MP strict consensus tree (Fig. 2.24). Figure 2.24 including taxon labels and support values is provided in electronic format on the CD which accompanies this thesis.

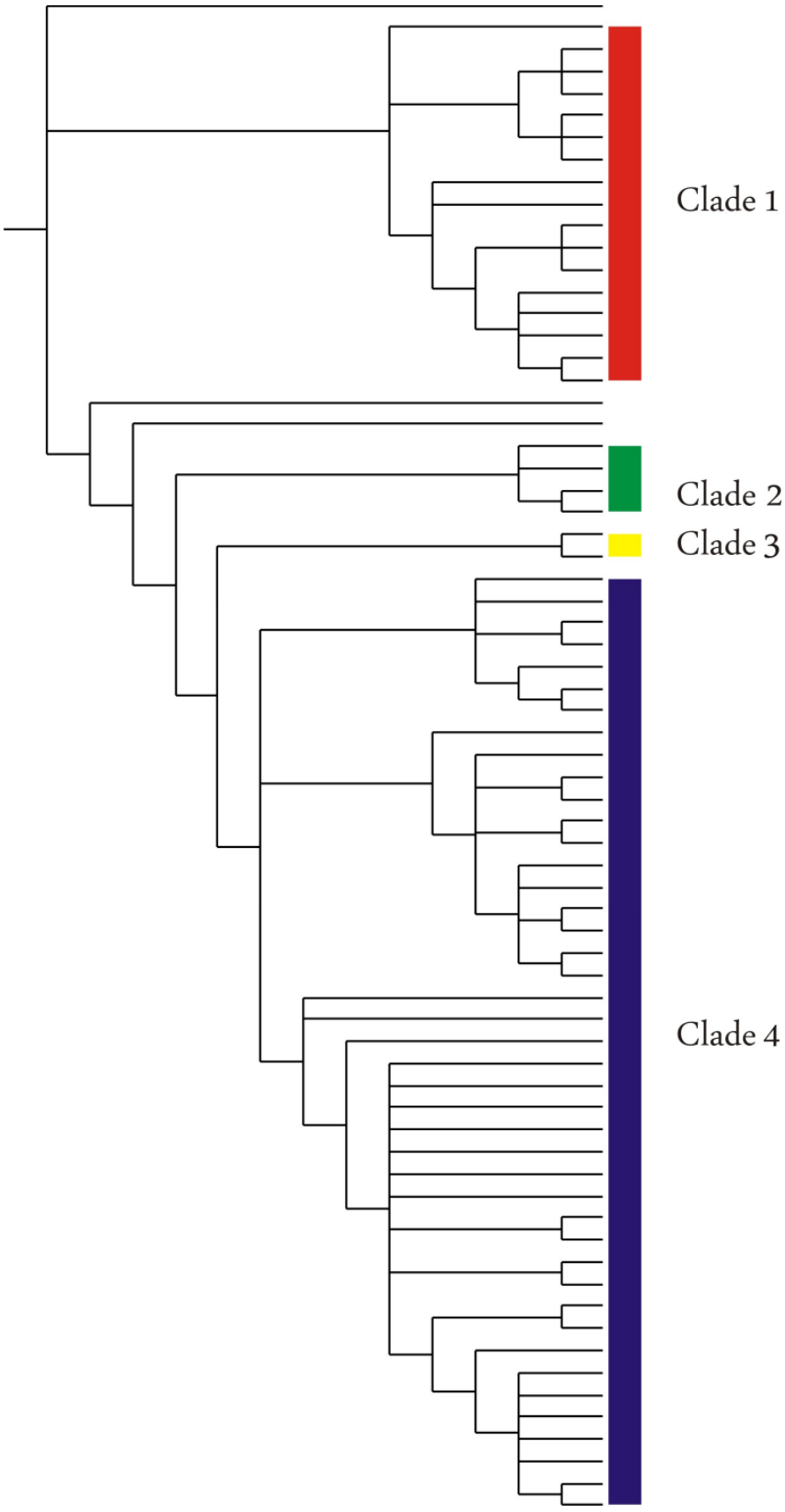


Figure 2.25: Structure of clades in 50% majority rule consensus cladogram of BI analysis for Dataset 4.

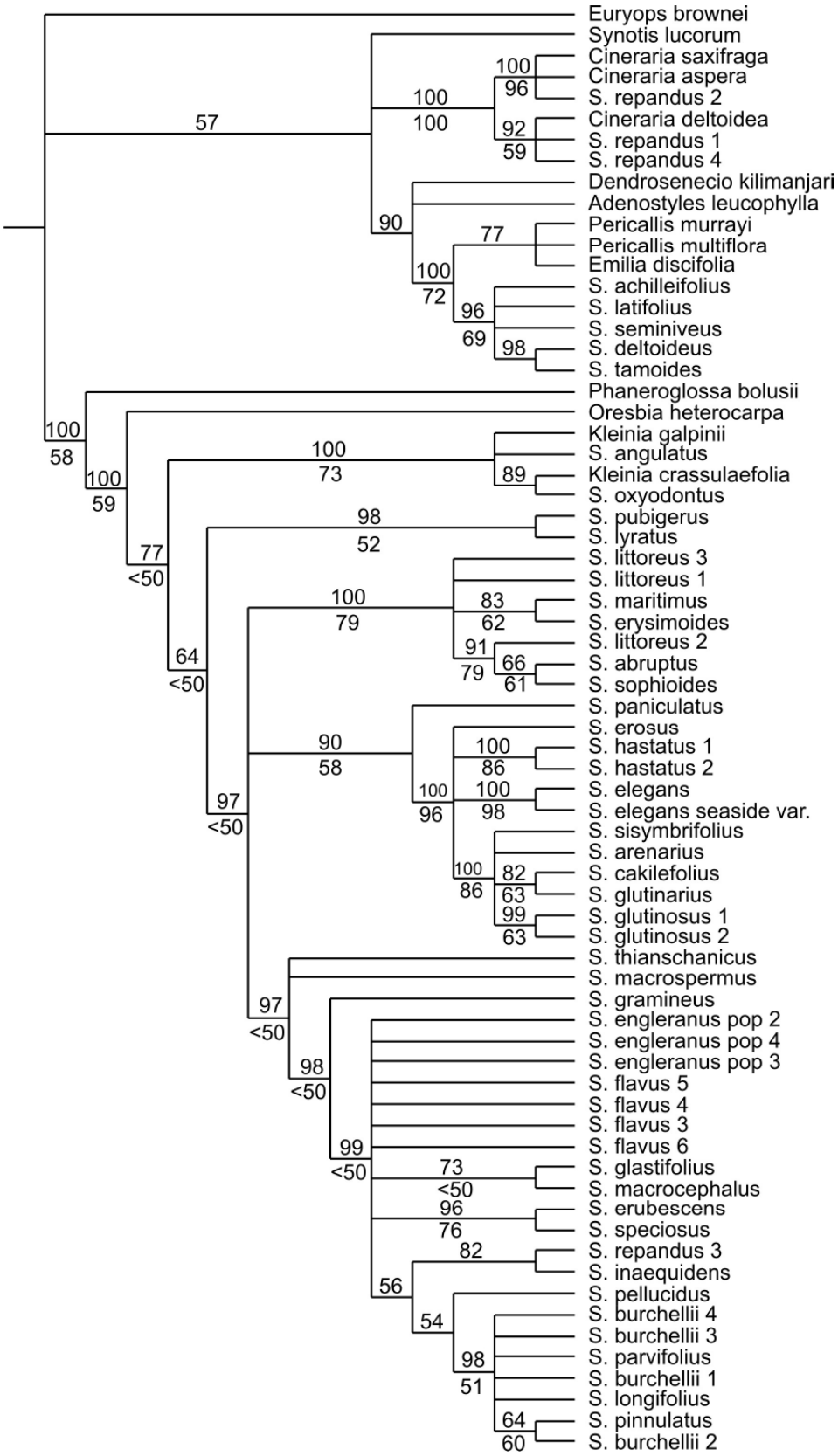


Figure 2.26: 50% majority rule consensus cladogram of BI analysis for Dataset 4. Bayesian consensus percentages (posterior probabilities x 100) are above the branches, while corresponding MP bootstrap percentages are below them.

Dataset 4 includes taxa from throughout the subtribe Senecioninae. Clades suggested by the analysis (Fig. 2.25) are:

Clade 1: *Adenostyles leucophylla, Cineraria aspera, Cineraria deltoidea, Cineraria saxifraga, Dendrosenecio kilimanjari, Emilia discifolia, Pericallis multiflora, Pericallis murrayi, Senecio achilleifolius, Senecio deltoideus, Senecio latifolius, Senecio repandus, Senecio seminiveus, Senecio tamoides, Synotis leucorum.*

Clade 2: *Kleinia crassulaefolia, Kleinia galpinii, Senecio angulatus, Senecio oxyodontus.*

Clade 3: *Senecio lyratus, Senecio pubigerus.*

Clade 4: *Senecio abruptus, Senecio arenarius, Senecio burchellii, Senecio cakilefolius, Senecio elegans, Senecio engleranus, Senecio erosus, Senecio erubescens, Senecio erysimoides, Senecio flavus, Senecio glastifolius, Senecio glutinarius, Senecio glutinosus, Senecio gramineus, Senecio hastatus, Senecio inaequidens, Senecio littoreus, Senecio longifolius, Senecio macrocephalus, Senecio macrospermus, Senecio maritimus, Senecio paniculatus, Senecio parvifolius, Senecio pellucidus, Senecio repandus, Senecio sisymbrifolius, Senecio sophioides, Senecio speciosus, Senecio thianschanicus.*

2.3.5: Congruence Between *ITS* and *trn*L-F phylogenies

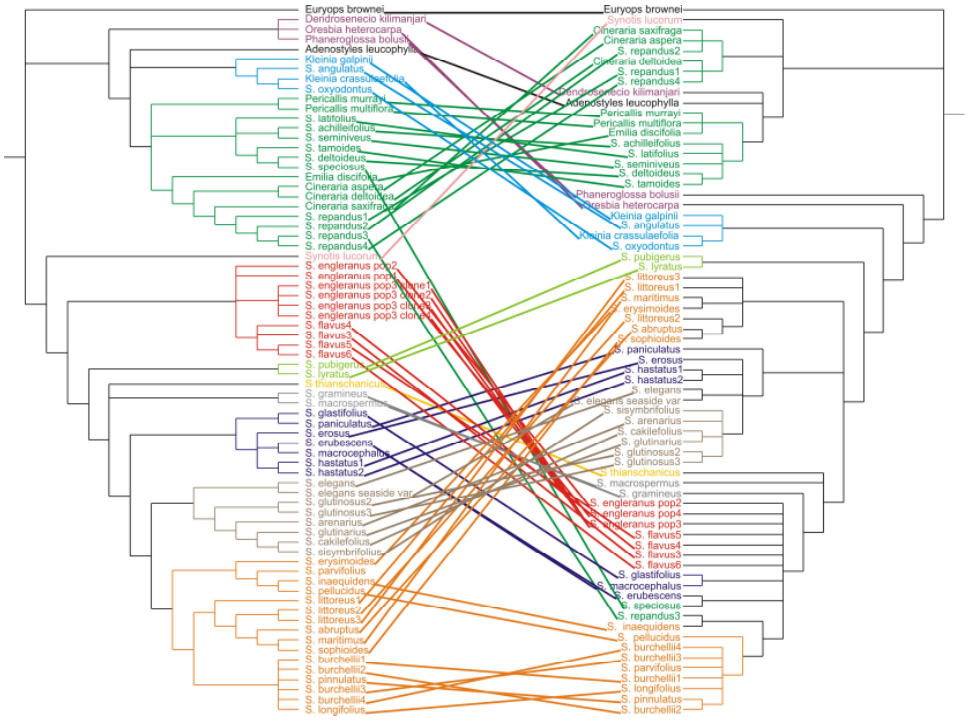


Figure 2.27: Congruence between BI 50% majority rule consensus cladograms of Dataset 3 (*ITS*) and Dataset 4 (*trn*L-F). *ITS* tree on the left.

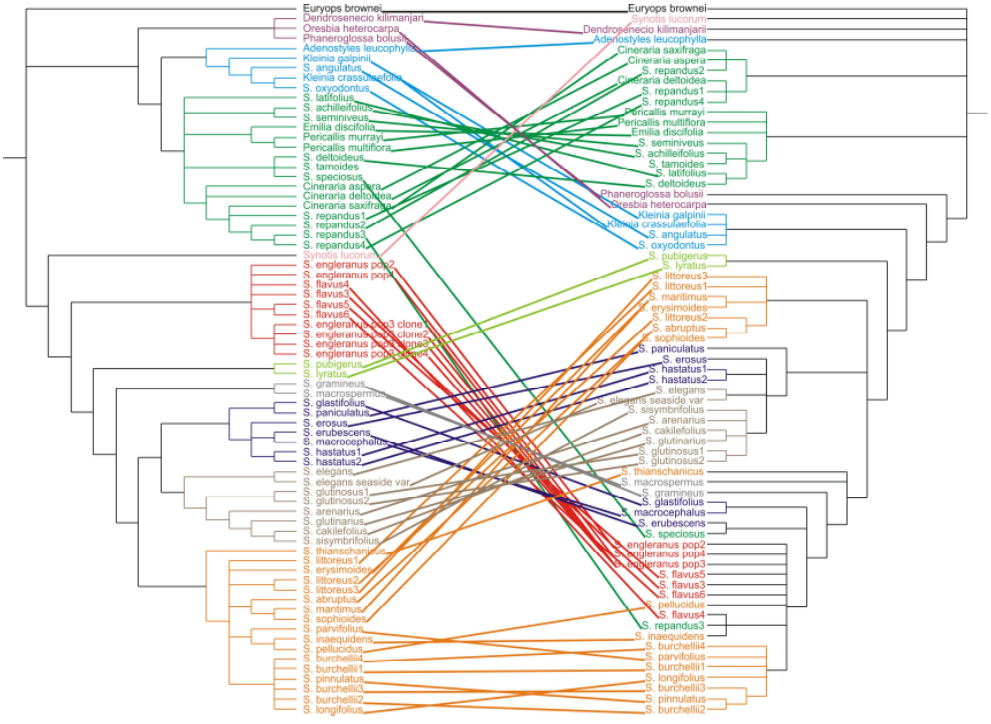
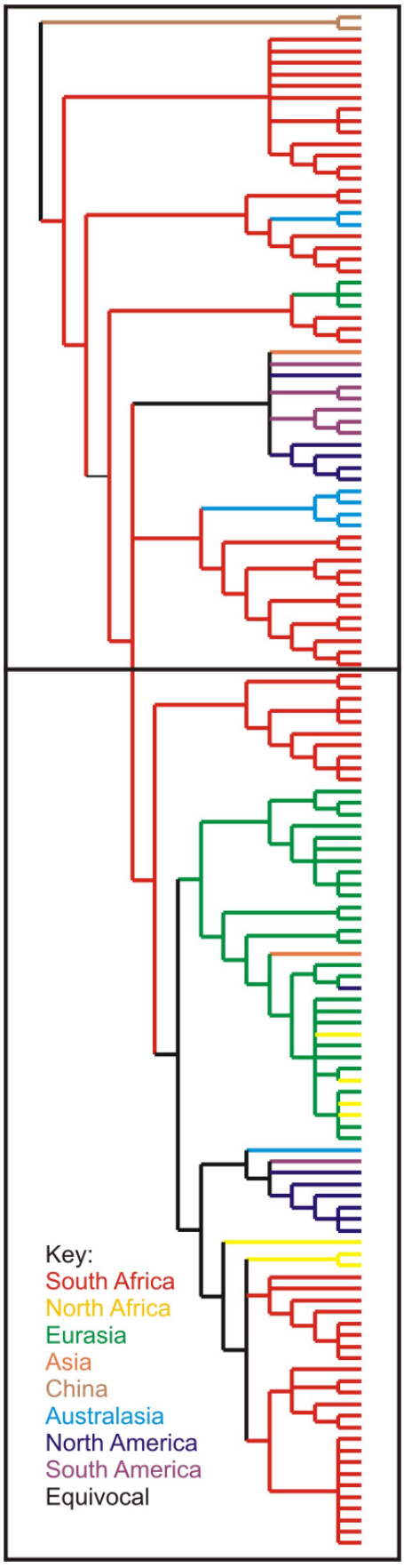


Figure 2.28: Congruence between MP strict consensus cladograms of Dataset 3 (*ITS*) and Dataset 4 (*trn*L-F). *ITS* tree on the left.

Congruence between *ITS* and *trn*L-F phylogenetic trees

Congruence between the *ITS* and *trn*L-F phylogenetic trees generated from both BI and MP approaches was very low (Figs. 2.27, 2.28). Topologies varied considerably. Partition homogeneity analysis in PAUP* 4.0b10 (Swofford, 2000) showed significant incongruence between the plastid and nuclear datasets (P = < 0.05). As a result, the two datasets were not combined into a single matrix for analysis.

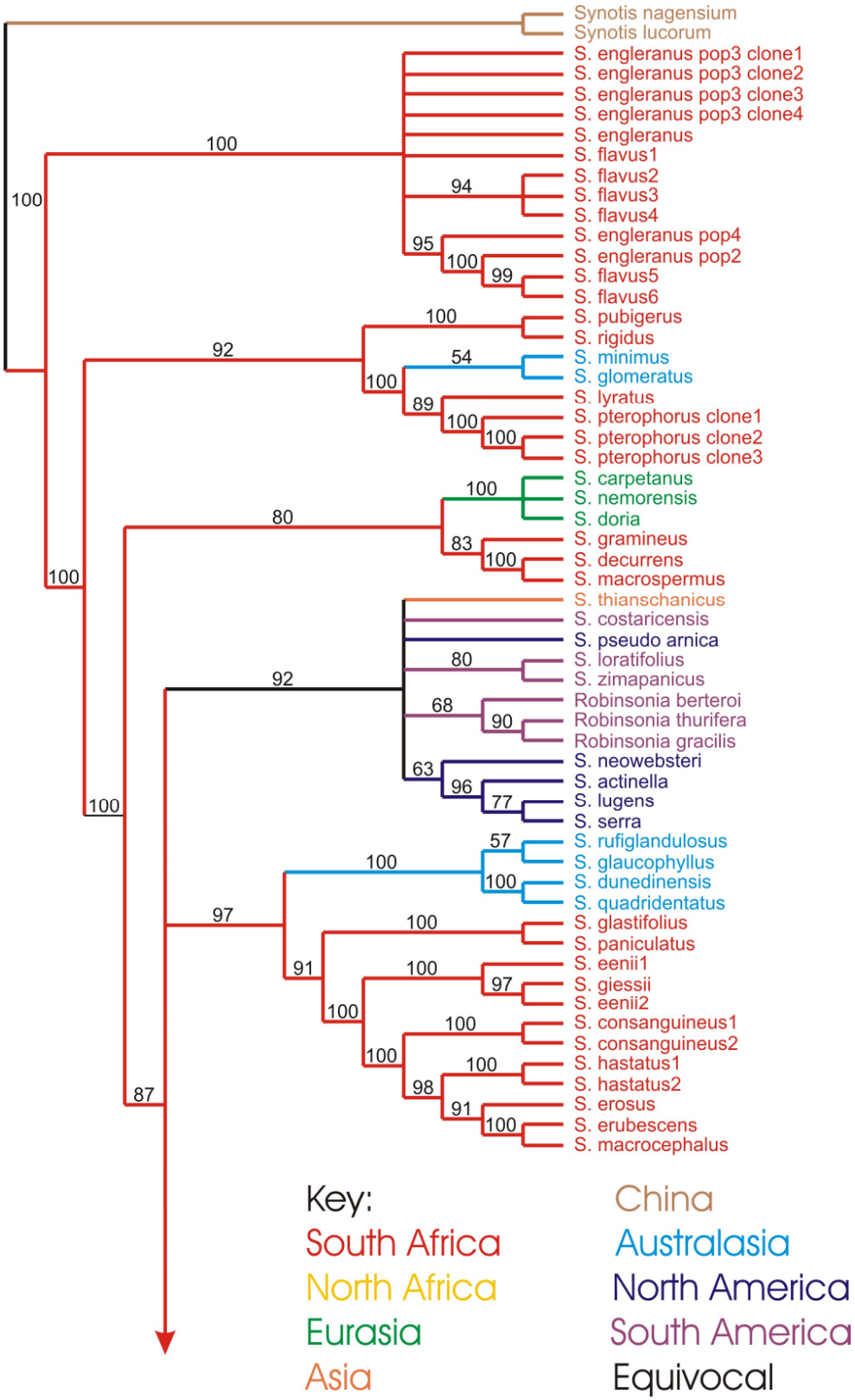
2.3.6: Biogeographic Results



See p. 91 (Fig. 2.30) for enlarged graphic of this part of the tree.

See p. 92 (Fig. 2.31) for enlarged graphic of this part of the tree.

Figure 2.29: Area optimisation on BI 50% majority rule consensus cladogram of Dataset 2. Adapted from MacClade v. 4.06 (Maddison & Maddison, 2003).



Continued on p. 92

Figure 2.30: Part 1 of area optimised BI 50% majority rule consensus cladogram of Dataset 2. Adapted from MacClade v. 4.06 (Maddison & Maddison, 2003). Bayesian consensus percentages (posterior probabilities x 100) are above the branches.

Continued from p. 91

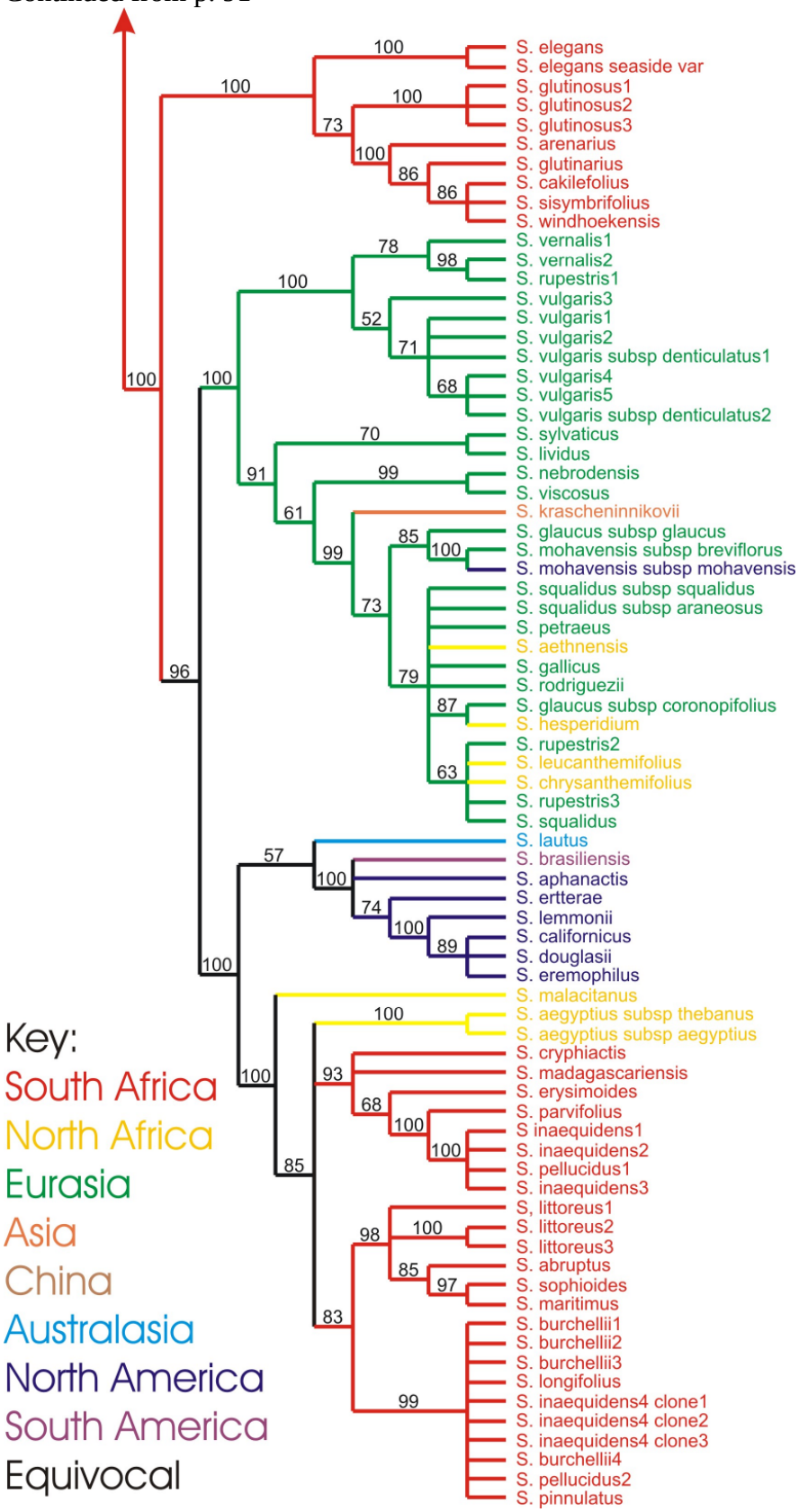


Figure 2.31: Part 2 of area optimised BI 50% majority rule consensus cladogram of Dataset 2. Adapted from MacClade v. 4.06 (Maddison & Maddison, 2003). Bayesian consensus percentages (posterior probabilities x 100) are above the branches.

Biogeographic Results

Strong geographic structure is evident from the area-optimised BI tree (Figs. 2.29 – 2.31). The backbone of the ingroup is southern African, indicating a strong southern African influence throughout the evolutionary history of the genus. This is also the case for the section *Senecio* clade (clade 11, Fig. 2.17)

2.3.7: Evolution of Flower Colour

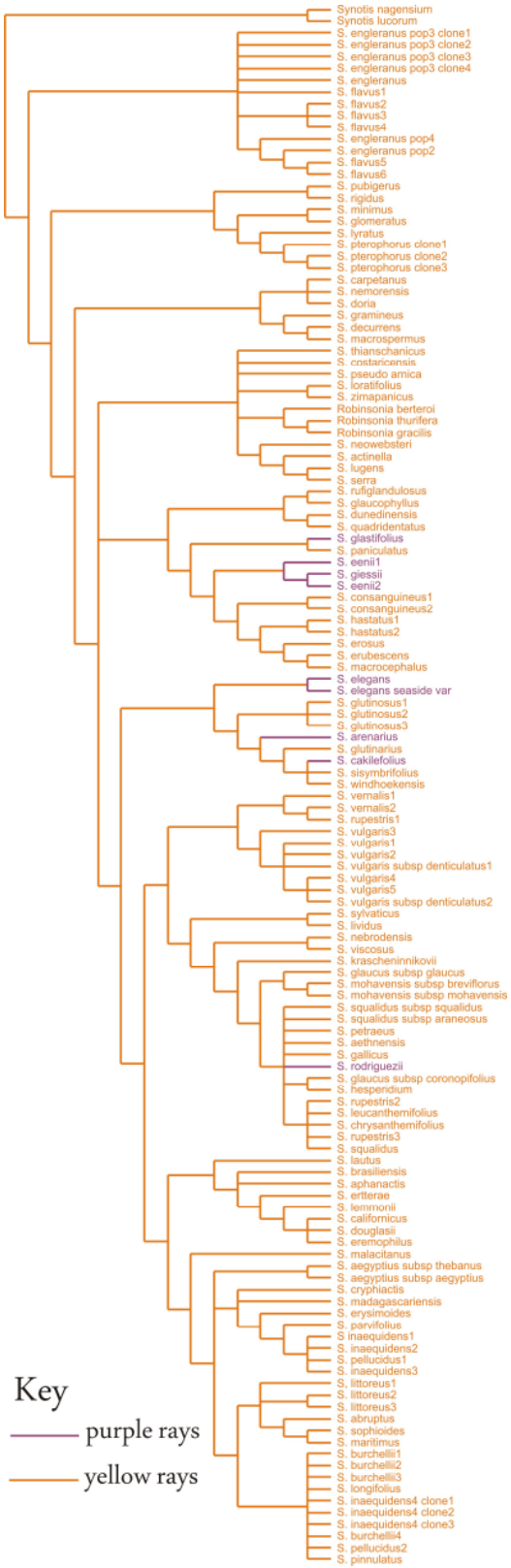


Figure 2.32: Flower colour optimised onto BI 50% majority rule consensus cladogram of Dataset 2. Adapted from MacClade v. 4.06 (Maddison & Maddison, 2003).

Yellow ray florets appear to be the ancestral state in *Senecio* s.str. (Fig. 2.32), with purple ray florets evolving independently at least six times in the genus. To determine exactly how many times purple ray florets have evolved in the genus, the genus will need to be sampled more thoroughly.

2.4: DISCUSSION

2.4.1: Harvey's classification system

Members of section *Annui*

Members of sect. *Annui* in Harvey's *Flora Capensis* (1865) found during fieldwork and included in this study are *S. arenarius, S. cakilefolius, S. elegans, S. erysimoides, S. glutinarius, S. glutinosus, S. littoreus, S. maritimus, S. repandus, S. sisymbrifolius* and *S. sophioides.* In addition, *ITS* sequences for two *S. consanguineus* accessions were collected from GenBank and included in the analysis. It proved impossible in the limited time available to collect all species assigned to the section by Harvey, although this section was the main focus of the present study. A major problem was the lack of locality information of high quality. Many specimens from which locality information was gathered were very old, some dating back to the 18th Century, and relevant information was often vague to the point of being useless, or simply out of date. The age of herbarium specimens, and the tendency of collectors to preserve specimens in alcohol, to avoid rotting, meant that attempts to extract DNA from the herbarium specimens themselves were fruitless. Because of these factors, the following species assigned to the section by Harvey were not available for inclusion in the phylogenetic analyses: *S. cardaminifolius, S. diffusus, S. laevigatus, S. laxus, S. lessingii, S. lobelioides, S. matricariaefolius, S. multibracteatus, S. paarlensis, S. puberulus, S. ruderalis, S. tenellus* and *S. trachylaenus.* Harvey also included *S. vulgaris* in sect. *Annui*, although it is likely that this is an introduced species, as the only specimen of *S. vulgaris* seen during fieldwork was in a cultivated garden.

Referring to Figs. 2.17 – 2.19, of the species included, *S. consanguineus* falls in clade 6, *S. arenarius, S. cakilefolius, S. elegans, S. glutinarius, S. glutinosus* and *S. sisymbrifolius* fall in clade 7, while *S. erysimoides, S. littoreus S. maritimus* and *S.*

sophioides fall in clade 10. *Senecio repandus* falls far from the core of *Senecio* s.str., in clade 5 in Fig. 2.11. Sect. *Annui* is therefore not a monophyletic group.

Clade 6 in Fig. 2.11 is weakly supported (BPP = 0.97, MPB = 50%) and includes only a single member of sect. *Annui, S. consanguineus,* which forms a reasonably well supported clade with members of sect. *Sinuosi* (BPP = 1.00, MPB = 80%).

Clade 7 in Fig. 2.11 is robustly supported (BPP = 1.00, MPB = 96%) and exclusively southern African. All species in the clade were assigned to sect. *Annui* in Harvey's account, with the exception of *S. windhoekensis,* which has never been assigned to a section.

Clade 10 in Fig. 2.11 is reasonably well supported (BPP = 1.00, MPB = 84%) and consists entirely of African species. The clade also includes species assigned to sects. *Rigidi, Leptolobi* and *Leptophylli* by Harvey.

The southern African species found in clades 7 and 10 in fig 2.11 are the most likely candidates from the region for inclusion in an expanded section *Senecio,* represented by clade 11, and discussed in more detail below.

As mentioned above, *S. repandus* falls far from the core of *Senecio* s.str., within clade 5 in the 'Jacobaean' clade (see Fig. 2.11). Its closest relatives appear to be members of *Cineraria,* with which *S. repandus* forms a weakly supported clade (BPP = 0.97, MPB = 71%). Clade 5 (BPP = 1.00, MPB = 72%) in Fig. 2.11 also includes *Stilpnogyne bellidioides* and *Mesogramma apiifolia* (syn: *Senecio apiifolius*). Upon further investigation, it may prove necessary to remove *S. repandus* from *Senecio* altogether, as it appears to be only distantly related to *Senecio* s.str.

Members of section *Sinuosi*

Members of sect. *Sinuosi* collected during fieldwork were *S. erosus, S. erubescens, S. hastatus* (syn: *S. hastulatus*) *S. macrocephalus* and *S. speciosus* (syn: *S. concolor, S. polyodon*). The other members of the section, not included in the study are *S. albifolius* DC., *S. barbatus* DC., *S. bellis* Harv., *S. eriobasis* DC., *S. glabrifolius* DC., *S. hieracioides* DC., *S. hypochoerideus* DC., *S. incomptus* DC., *S. odontopterus* DC., *S. purpureus* L., *S. reptans* Turcz., *S. robertiaefolius* DC., *S. sandersoni* Harv., *S. serratus* Sond., *S. spiraeifolius* Thunb. and *S. thyrsoides* DC.

Referring to Fig. 2.17, *S. erubescens, S. hastatus, S. erosus* and *S. macrocephalus* are found together in a reasonably supported sub-clade (BPP = 0.98, MPB = 81%) within clade 6. These species form a larger clade with *S. consanguineus*, placed by Harvey in section *Annui*, which also has reasonable support (BPP = 1.00 MPB = 80%). This clade is part of a larger clade containing two Namibian species, *S. eenii* and *S. giessii*, which have never been assigned to a section, and two more South African taxa, *S. glastifolius* and *S. paniculatus*, which were placed by Harvey in sects. *Rigidi* and *Leptolobi* respectively. Support for including all of these southern African species in a single clade is weak (BPP = 0.91 MPB = <50%). Sister to the southern African group is an exclusively Australian clade of four species, completing a weakly supported clade 6 (BPP = 0.97, MPB = 50%).

Of the included members of sect. *Sinuosi*, only *S. speciosus* does not fall in clade 6 in Fig. 2.17, and is found instead in the 'Jacobaean' clade in Fig. 2.11, in a clade with *S. deltoideus* and *S. tamoides*, close to members of *Jacobaea*. This is surprising, as *trn*L-F analysis places S. *speciosus* within *Senecio* s.str, as sister to another member of *Sinuosi*, *S. erubescens*, and the species is morphologically very

similar to another member of sect. *Sinuosi, S. macrocephalus,* differing in Harvey's account only in petioles.

Members of section *Plantaginei*

Members of sect. *Plantaginei* collected during fieldwork were *S. coronatus, S. decurrens, S. gramineus* and *S. macrospermus.* The other members of the section, which could not be included in the analyses, are *S. albanensis* DC., *S. caudatus* DC., *S. crenulatus* DC., *S. crispus* Thunb., *S. dregeanus* Hilliard & B.L.Burtt, *S. inornatus* DC., *S. monticolus* DC., *S. othonnaeflorus* DC., *S. petiolaris* DC., *S. polyodon* (syn: *S. concolor, S. speciosus*), *S. striatifolius* DC. and *S. digitalifolius.*

Of the species included, *S. decurrens, S. gramineus* and *S. macrospermus* form a weakly supported monophyletic group (BPP = 0.83, MPB = <50%) within clade 4 in Fig. 2.11. Sister to this subclade is a weakly supported clade of European species (BPP = 1.00, but MPB = 64%), consisting of *S. carpetanus, S. doria* and *S. nemorensis.* When the southern African and European clades are taken together as clade 4 in Fig. 2.11, support is even weaker (BPP = 0.80, MPB = <50%), suggesting that any lumping of these species together into a single section should be resisted for the time being.

Senecio coronatus appears to be only distantly related to the other members of sect. *Plantaginei* appearing close to *Jacobaea* in clade 8 in Fig. 2.11, in a strongly supported clade with *S. latifolius,* assigned to sect. *Paucifolii* by Harvey (BPP = 1.00, MPB = 99%). These species should almost certainly be removed from *Senecio.*

Members of section *Paucifolii*

Only a single member of sect. *Paucifolii* was collected during fieldwork, *S. latifolius,* which, as mentioned above, forms a subclade with *S. coronatus* close to members of *Jacobaea,* within clade 8 in Fig. 2.11. Members of the section unavailable for inclusion in the study are *S. adnatus* DC., *S. anthemifolius* Harv., *S. bupleuroides* DC., *S. cordifolius* L.f., *S. cymbalarifolius* Less., *S. diversifolius* Harv., *S. glaberrimus* DC., *S. isatideus S. orbicularis* Sond., *S. oxyrieafolius* DC., *S. paucifolius* DC., *S. rhomboideus* Harv., *S. tuberosus* Harv. and *S. venosus* Harv.

With just a single representative of the section available for inclusion in the study, nothing can be said about the potential monophyly or otherwise of the group, although *S. latifolius* should probably be removed from *Senecio.*

Members of section *Rigidi*

Members of sect. *Rigidi* collected during fieldwork were *S. glastifolius, S. lyratus, S. oxyodontus, S. pellucidus, S. pterophorus, S. pubigerus* and *S. rigidus*. The remaining species in the section which were not available for inclusion in the study are *S. amabilis* DC., *S. aquifoliaceus* DC., *S. arnicaeflorus* DC., *S. blattarioides* DC., *S. caulopterus* DC., *S. cinerascens* Ait., *S. coleophyllus* Turcz., *S. crenatus* Thunb., *S. expansus* Harv., *S. gerardi* Harv., *S. halimifolius* L., *S. hirtifolius* DC., *S. ilicifolius* Thunb., *S. incisus* Thunb., *S. juniperinus* L.f., *S. lanceus* Ait., *S. microglossus* DC., *S. microspermus* DC., *S. oederiaefolius* DC., *S. pandurifolius* Harv., *S. picridifolius* DC., *S. scoparius* Harv., *S. serra* Sond., *S. serratuloides* DC., *S. thunbergii* Harv., *S. tortuosus* DC., *S. verbascifolius* Burm., *S. vestitus* Berg. and *S. zeyheri* Turcz.

Of the included species from the section, *S. lyratus, S. pterophorus, S. pubigerus* and *S. rigidus* appear together in a monophyletic group with two Australian

species, *S. glomeratus* and *S. minimus.* This is clade 3 in Fig. 2.17, which has weak support (BPP = 0.92, MPB = 74%). Within this clade *S. lyratus* and *S. pterophorus* appear to be more closely related to the Australian species than to *S. pubigerus* and *S. rigidus.* The grouping of the Australian species with *S. lyratus* and *S. pterophorus* is strongly supported (BPP = 1.00, MPB = 93%).

S. glastifolius is found outside the clade mentioned above, appearing instead as sister to *S. paniculatus* (assigned to sect. *Leptolobi* by Harvey), as part of clade 6 in Fig. 2.17. A weakly supported, exclusively southern African subclade within clade 6 consists of members of Harvey's sects. *Annui*, *Sinuosi* and *Leptolobi*, as well as *S. eenii* and *S. giessii*, which have never been assigned to a section (BPP = 0.91, MPB = <50%). The subclade is sister to a clade consisting of four Australian species, as mentioned above. *S. glastifolius* should not be regarded as part of sect. *Rigidi*, although weak support values suggest further research would be required to establish its affinities firmly.

Senecio oxyodontus falls in a clade with *S. angulatus* (placed in sect. *Scandentes* by Harvey), *Curio articulatus* (syn: *S. articulatus*) and *Kleinia crassulaefolia.* This is part of clade 4, within the 'Jacobaean' clade in Fig. 2.11, which also contains *Adenostyles, Solanecio,* and two other *Kleinia* species. Overall support for clade 4 is very weak if *Adenostyles* is included (BPP = 0.80), and the inclusion of *Adenostyles* collapses in the MP results. Excluding *Adenostyles,* support for the grouping is more robust (BPP = 1.00, MPB = 78%). The results suggest that *S. oxyodontus* is only distantly related to *Senecio* s.str. and should be removed from the genus. It may be more appropriately placed in *Curio* or *Kleinia,* along with *S. angulatus,* although further research is required before any taxonomic decisions are made.

Two different accessions of *S. pellucidus* are found in different positions in the results of this study. One is present in clade 10 in Fig. 2.17, on a weakly supported polytomy with *S. burchelii, S. longifolius, S. pinnulatus* and one accession of *S. inaequidens* (BPP = 0.99, MPB = 59%), while the other is also found in clade 10, but grouped separately, on a more robustly supported polytomy with three other accessions of *S. inaequidens* (BPP = 1.00, MPB = 90%).

Senecio pellucidus does not group with the other members of sect. *Rigidi*, and should not be considered part of the section. It would perhaps be more fittingly placed in an expanded sect. *Senecio.*

Members of section *Microlobi*

Unfortunately no members of sect. *Microlobi* were found during fieldwork, and no *ITS* sequences from any of its representatives were available on GenBank. This small section consists of five species, *S. lineatus* DC., *S. oliganthus* DC., *S. penninervius* DC., *S. quinquenervius* Sond. and *S. triplinervius* DC. The section should be included in any further research on the southern African members of the genus to establish its monophyly or otherwise, and its affinities. *Senecio lineatus*, included in the phylogeny of Senecioneae produced by Pelser et al. (2007), appears to be closely related to *S. deltoideus*, assigned to sect. *Scandentes* by Harvey, and *S. scandens*, which is absent from Harvey's account. Support for the grouping of these three species is very weak (BPP = 0.62, MPB = <50%). The clade containing them falls far from the core of *Senecio* in Pelser et al.'s work, and they appear to be more closely related to *Jacobaea* and *Bethencourtia* Choisy, suggesting that *S. lineatus* should be removed from *Senecio*, although further work would be required to confirm

this. As only one species from the section is included in Pelser et al.'s study, conclusions about the monophyly of this section cannot be drawn.

Members of section *Leptolobi*

Members of sect. *Leptolobi* collected during fieldwork were *S. achilleifolius S. paniculatus, S. parvifolius* and *S. pinnulatus.* Other members of the section, unavailable for inclusion in the study are *S. bipinnatus* Less., *S. carroensis, S. euryopoides* DC., *S. foeniculoides* Harv., *S. grandiflorus* Berg., *S. leucoglossus* Sond., *S. multicaulis* DC., *S. muricatus* Thunb., *S. pinnatifidus* Less., *S. rhyncholaenus* DC., *S. serrurioides* Turcz., *S. tanacetoides* Sond., *S. umbellatus* L.

Senecio parvifolius and *S. pinnulatus* both appear in clade 10 in Fig. 2.17, but not in a monophyletic group. *Senecio pinnulatus* is found on a polytomy with *S. burchellii, S. longifolius,* one accession of *S. pellucidus* and one accession of *S. inaequidens.* The other species in the polytomy are virtually indistinguishable morphologically, but the presence of *S. pinnulatus* is surprising, as it is morphologically distinct from the others, having an unusual pinnati-partite leaf form. *Senecio pinnulatus* may be more appropriately placed in sect. *Annui* or in an expanded sect. *Senecio,* than in sect. *Leptolobi.* Further research is required to confirm this.

Senecio parvifolius is also found in clade 10 in Fig. 2.17, in a strongly supported subclade with other accessions of *S. inaequidens* and *S. pellucidus* (BPP = 1.00, MPB = 97%). The closest relative of the subclade appears to be *S. erysimoides,* placed by Harvey in sect. *Annui.* These species are part of a weakly supported polytomy with *S. madagascariensis* and *S. cryphiactis,* not included in Harvey's account (BPP = 0.93). The grouping collapses in the MP results. *Senecio parvifolius*

would perhaps be better assigned to sect. *Annui* or to an expanded sect. *Senecio,* than to sect. *Leptolobi.*

Senecio achilleifolius falls within clade 8, as part of the 'Jacobaean' clade in Fig. 2.11. Its closest relative included in the analysis appears to be *S. seminiveus,* which is not included in Harvey's account. Support for the grouping of these two species is strong (BPP = 1.00, MPB = 100%). The affinities of the pair are less clear as they appear on a polytomy with *Jacobaea* and some other southern African *Senecio* species in the BI results, but appear further from *Jacobaea* in the MP results. *Senecio achilleifolius* probably does not belong in *Senecio* as, in both sets of results, this taxon falls far from the core of *Senecio.* However, further research is required to determine where this species would be best placed, particularly as these positions conflict with that suggested by Pelser et al. (2007), who place it in *Senecio* s.str.

Senecio paniculatus is found grouped with *S. glastifolius,* assigned to sect. *Rigidi* by Harvey. The species form part of clade 6 in Fig. 2.17, which is discussed above in reference to *S. glastifolius* as part of sect. *Rigidi.*

None of the included representatives of sect. *Leptolobi* group together, and their affinities with other sections are varied, suggesting that this section should perhaps be adandoned altogether. However, further research, including denser taxon sampling of the section is required to confirm this, and to establish more appropriate sectional positions for the taxa placed therein by Harvey.

Members of section *Leptophylli*

Members of sect. *Leptophylli* collected during fieldwork were *S. burchellii, S. inaequidens* and *S. longifolius.* Members of the section unavailable for inclusion in the study are *S. angustifolius* Willd., *S. debilis* Harv., *S. diodon* DC., *S.*

dracunculoides DC., *S. filifolius* Harv. non Berg., *S. hirtellus* DC., *S. leptophyllus* DC., *S. mucronatus* Willd., *S. niveus* Less., *S. persicifolius* L., *S. rosmarinifolius* L.f., *S. serrulatus* DC., *S. skirrhodon* DC. and *S. vimineus* DC..

All members of the section included fall within clade 10 in Fig. 2.17, which also includes species assigned by Harvey to sects. *Annui*, *Rigidi*, and *Leptolobi*, as well as four species not included in Harvey's account.

All four included accessions of *S. burchellii* fall on a reasonably well supported polytomy with *S. longifolius*, *S. pinnulatus*, one accession of *S.inaequidens*, and one accession of *S. pellucidus* (BPP = 0.99, MPB = 79%). Three other accessions of *S. inaequidens* do not fall on this polytomy, instead forming a strongly supported polytomy with the other accession of *S. pellucidus* (BPP = 1.00, MPB = 90%). Members of section *Leptophylli* do not form a monophyletic group, although all the included species do seem to be quite closely related to one another. Although further research is required, the included species could perhaps be added to sect. *Annui*, or an expanded sect. *Senecio*. As mentioned above, the species in these polytomies are virtually indistinguishable morphologically, and further work is currently underway at the University of St Andrews to investigate the morphological species complex composed of *S. burchellii, S. inaequidens, S. longifolius, S. madagascariensis* and *S. pellucidus* (Coyle, personal communication). Looking at the results presented here, one would also wish to include a number of other species which appear to be genetically close to the complex: *S. abruptus, S. aegyptius, S. cryphiactis, S. erysimoides, S. littoreus, S. malacitanus, S. maritimus, S. parvifolius, S. pinnulatus, S. sophioides.*

Members of section *Pinifolii*

Unfortunately, no members of sect. *Pinifolii* were found during fieldwork and no *ITS* sequences were available from GenBank. This section consists of just two species, *S. pinifolius* and *S. triqueter.* These two species are included in the study of Senecioneae by Pelser et al. (2007), where they form a strongly supported monophyletic group (BPP = 1.00, MPB = 100%), which appears to be more closely related to *Jacobaea* then to *Senecio* s.str. This suggests that members of sect. *Pinifolii* should be removed from *Senecio.*

Members of section *Scandentes*

Members of sect. *Scandentes* collected during fieldwork were *S. angulatus, S. deltoideus* and *S. tamoides.* The other members of the section are *S. brachypodus* DC., *S. bryoniaefolius* Harv., *S. canalipes* DC., *S. macroglossus* DC., *S. mikanioides* Walp. and *S. quinquelobus* DC.

Senecio deltoideus and *S. tamoides* fall together in a strongly supported clade with *S. speciosus* (syn: *S. concolor, S. polyodon*) (BPP = 1.00, MPB = 100%), within clade 8 in Fig. 2.11. However, the affinities of the clade are less clear, as it appears close to *Jacobaea* in the BI results, but on a much wider ranging polytomy in the MP results. In both cases the species fall far from the core of *Senecio*, and should probably be removed from the genus. Where they would be best placed should be the subject of further research.

Senecio angulatus also falls far from the core of *Senecio* in the 'Jacobaean' clade in Fig. 2.11, appearing as basal to a polytomy consisting of *Curio articulatus* (syn: *Senecio articulatus*), *S. oxyodontus* (assigned to section *Rigidi* by Harvey), and *Kleinia crassulaefolia.* Support for this grouping is fairly weak (BPP = 0.99, MPB =

67%). The clade is part of a larger group consisting of other members of *Gynura* Cass., *Kleinia* and *Solanecio* (Sch.Bip.) Walp. *Senecio angulatus* should probably be removed from *Senecio,* and may be more appropriately placed in *Kleinia* or *Curio.* However, further research is required to confirm this.

Members of section *Kleinoidei*

No members of the succulent stemmed sect. *Kleinoidei* were found during fieldwork and, at the time of anlaysis, no *ITS* sequences from members of the section were available on GenBank. The section consists of *S. acutifolius* DC., *S. aloides* DC., *S. bubinefolius* DC., *S. corymbiferus, S. cotyledonis* DC., *S. crassiusculus* DC., *S. pyramidatus, S. scaposus* DC., *S. subsinuatus* DC., *S. succulentus* DC. Two species from the section are included in the phylogeny of tribe Senecioneae produced by Pelser et al. (2007): *S. pyramidatus* and *S. scaposus.* The species fall together in a strongly supported clade with *S. medley-woodii* Hutch. (BPP = 1.00, MPB = 100%). This clade is particularly distant from the core of *Senecio,* falling in subtribe Tussilagininae, suggesting the species which make up the clade should be removed from *Senecio.* Further research including all members of the section would clarify the monophyly of the section and might more clearly indicate the affinities of its members.

Member of section *Aphylli*

Monotypic sect. *Aphylli* consists of *S. junceus,* which was not available for inclusion in this study. The species is included in Pelser et al.'s (2007) phylogeny of tribe Senecioneae. Monophyly of a monotypic section is, of course, assured. Pelser et al. (2007) find that the closest relative of *S. junceus* as suggested by MP analysis, is

the African *S. oxyriifolius* DC., while their BI results conflict with this, suggesting a closer relationship with *S. crassissimus* Humb., *S. melastomifolius* Baker, *S. meuselii* Rauh., *Solanecio, Kleinia* and *Gynura.* Whichever of these results one looks at, it seems likely that *S. junceus* is only distantly related to the core of *Senecio,* as it falls in the '*Austrosynotis – Cineraria'* clade of Pelser et al. (2007), equivalent to the 'Jacobaean' clade in Fig. 2.11.

Monophyly of Harvey's sectional classification

None of Harvey's sections appears to be a monophyletic group when molecular phylogenetic techniques are applied to his classification system, although many of the species which Harvey thought had affinities with one another do appear to be closely related. It is notable that all included members of section *Sinuosi* (with the exception of *S. speciosus*) form a monophyletic group, all included members of sect. *Leptophylli* are closely related, and sect. *Pinifolii* appears to be a monophyletic group based on Pelser et al.'s analysis (2007). However, even clades representing the most phylogenetically accurate sections devised by Harvey tend to contain species which Harvey had assigned to other sections, or do not contain all the species which Harvey had assigned to a particular section. There are also, of course, many species which appear in clades with members of his sections, to which Harvey could not have had access, based as he was in the Cape regions of South Africa in the 19th Century - as they come from distant corners of the globe.

2.4.2: A comparison of the phylogeny produced from Dataset 1 (Subtribes Senecioninae and Othonninae) with the phylogenetic results of Pelser et al. (2007)

As mentioned in the introducton to this chapter, a recent paper by Pelser et al. (2007) reported an *ITS* phylogeny for the entire tribe Senecioneae, allowing comparison with the phylogenetic results produced here from Datasets 1 and 2.

There is broad agreement between the results reported by Pelser et al. (2007) and those produced here in Figs. 2.9 – 2.14. In particular, both show that members of subtribe Senecioninae form a monophyletic group composed of two large sister clades. The composition of the two clades is almost identical in the two studies, and the composition of individual smaller clades within them in the present study is very similar to that in Pelser et al. (2007), although the taxa included in the respective studies vary greatly. I have presented the results in terms of Bayesian inference methods, whereas Pelser et al. (2007) used tree topologies suggested by maximum parsimony. Congruence between BI trees and MP trees is indicated in Figs. 2.9, 2.15, 2.20, 2.21, and 2.24. These figures, including taxon labels and support values, are included on the CD which accompanies this thesis. The rationale for emphasising the Bayesian results in the present study is the fact that BI methods are a more recent addition to the array of tools available to the phylogeneticist or taxonomist. As discussed in Chapter 1, MP methods assume that evolution will always take the most parsimonious route between two sequences, although it is unlikely that this is always the case. In contrast, BI allows for greater complexity, taking into account the most fitting model of molecular evolution for the data, and a set of prior information about the matrix being analysed (Holder & Lewis, 2003).

Subtribe Othonninae

Members of subtribe Othonninae are placed in clades 1 (*Euryops*) and 2 (*Othonna*) in the Dataset 1 phylogeny (Figs. 2.9 – 2.14). Separation from members of subtribe Senecioninae is evident in both this phylogeny and the one of Pelser et al. (2007). A detailed investigation of subtribe Othonninae was beyond the scope of this study, but Pelser et al. (2007) looked more extensively at the subtribe and noted that *Othonna* itself appears to be a non-monophyletic genus, with species currently ascribed to *Othonna* falling in two separate clades. A taxonomic revision of *Othonna* s.l. is currently being undertaken in an attempt to clarify the generic and species limits within the subtribe (Nordenstam, in prep.).

Subtribe Senecioninae

In the present study, monophyly of subtribe Senecioninae is strongly supported (BPP= 1.00, MPB = 93%), whereas in Pelser et al.'s phylogeny, monophyly of subtribe Senecioninae is less robustly supported (BPP = 1.00, MPB = <50%). There are some differences between Pelser et al.'s results and those presented here in Figs. 2.9 – 2.19. However, although the results of the two studies appear to differ substantially in places, this is mainly because of the different methods of phylogeny reconstruction emphasised. Despite these differences, two main sister clades within Senecioninae are evident from both studies. In both cases, one of the sister clades contains members of *Senecio* s.str. (as well as other genera) and the other contains the closest relatives of *Senecio jacobaea*, designated separate genus status as *Jacobaea* by Pelser et al. (2002) (as well as other genera). Pelser et al. (2007) termed these clades the *Cissampelopsis-Crassocephalum* clade (containing members of *Senecio* s.str.) and the *Austrosynotis-Cineraria* clade (containing members of

Jacobaea). For the purposes of this study, the sister clades have been termed the 'Senecioid' clade (equivalent to the *Cissampelopsis-Crassocephalum* clade) and 'Jacobaean' clade (equivalent to the *Austrosynotis-Cineraria* clade) in Fig. 2.11. The composition of these two sister clades is almost identical in the two studies, although the scope of Pelser et al.'s study was much wider, and included representatives of Senecioneae from around the world - and thus many genera and species for which sequences were unavailable for inclusion in the present study.

Position of *Dauresia alliariifolia*

Dauresia alliariifolia (syn: *Senecio alliariifolius*) occupies a different position in the phylogeny reported here and the MP tree of Pelser et al. (2007). This species is the lone member of a monotypic genus, and is endemic to Namibia. In Pelser et al. (2007) the taxon was positioned as sister to the *Cissampelopsis-Crassocephalum* clade according to MP analysis, but sister to the *Austrosynotis-Cineraria* clade using BI methods. The latter position mirrored that found in the present study, where the taxon was sister to the 'Jacobaean' clade (Figs. 2.9 – 2.14).

An identical effect of the different methods of phylogeny reconstruction is evident in the present study. Fig. 2.9 shows the congruence between the two trees produced using the different methods, and it is noticed that *D. alliariifolia* changes its position from sister to the 'Jacobaean' clade in the BI tree to sister position to the 'Senecioid' clade in the MP tree (for clade labels refer to Fig. 2.11). Although it was thought initially that this change of position could be a result of errors in the matrices used for analysis, it was established that the change is due to the different methods of phylogeny reconstruction, as confirmed by Pelser et al.'s results. Support for *D. alliariifolia* occupying either of these positions is weak. In both Pelser et al.'s study

and the present study, the MPB value was <50% for the positioning of the taxon as sister to the *Cissampelopsis*-*Crassocephalum* clade. Similarly, the BPP for the sister relationship to the '*Jacobaean* clade' was 0.87 in the present study and 0.58 in the study by Pelser et al. (2007). Pelser et al. (2007) pointed out that the taxon possesses caudate anthers and palmately veined leaves, which are features of Jeffrey's 'synotoid' group (Jeffrey, 1979). These features might suggest that the taxon is more related to the '*Senecioid* clade', although historically, morphology has been generally unhelpful in classifying *Senecioneae*. The best course of action at present is probably to collapse this branch and make no assumptions about the affinities of this taxon until a more comprehensive phylogenetic analysis, including a greater number of DNA fragments, has been performed.

Apart from this example, and a different position for *Senecio achilleifolius* (discussed below), the composition of the two sister clades is identical in the two studies, although many species and genera included in Pelser et al.'s (2007) study could not be included in this study.

Relative positions of the 'Jacobaean' clade and the 'Senecioid' clade

Although the two major sister clades (the *Cissampelopsis*-*Crassocephalum* and *Austrosynotis*-*Cineraria* clades in Pelser et al.'s study, and the 'Senecioid' and 'Jacobaean' clades in this study) within subtribe Senecioninae are placed in different parts of the phylogenetic trees reported in the two studies, sister status means that these two clades could be placed in either position correctly - so the results of the two studies do not contradict one another. Pelser et al.'s results show a tree with the *Austrosynotis – Cineraria* clade (the 'Jacobaean' clade in the present study) at the bottom of the tree and the *Cissampelopsis – Crassocephalum* clade (the 'Senecioid'

clade in the present study) at the top. These positions are reversed in the BI tree derived from Dataset 1 (Figs. 2.9 – 2.14).

The 'Jacobaean' clade

In Fig. 2.11, clade 3 is uppermost within the 'Jacobaean' clade, and contains *Dendrosenecio cheranganiensis, Dendrosenecio kilimanjari, Dendrosenecio kilimanjari* subsp. *cottonii* and *Oresbia heterocarpa* and *Phaneroglossa bollusii,* (BPP = 1.00, but MPB = <50%). A similar clade, which also includes *Austrosynotis* (unavailable for inclusion in the present study), appears in the same position in Pelser et al.'s work, at the top of the *Austrosynotis-Cineraria* clade (BPP = 0.55, MPB = <50%). The low support values suggest this group of genera requires further phylogenetic investigation.

Clade 4 contains *Adenostyles leucophylla, Curio articulatus* (syn: *Senecio articulatus*), *Kleinia crassulaefolia, Kleinia galpinii, Kleinia neriifolia, Gynura formosana, Solanecio mannii* and two *Senecio* species: *S. angulatus* and *S. oxyodontus.* Support for clade 4 is low (BPP = 0.80). In the MP tree of the present study, the grouping of *Adenostyles leucophylla* with the other members of clade 4 collapses, although excluding *A. leucophylla,* an identical group in the same position is seen (BPP = 1.00, MPB = 78%), suggesting good support for the grouping of the other taxa listed above. A corresponding clade can be seen in Pelser et al.'s tree, termed the 'Gynuroid' clade, and occupying a similar position (BPP = 1.00, MPB = 72%). These support values, as well as those seen here, lend some support to the 'Gynuroid' clade, but suggest the grouping probably requires further investigation before any firm conclusions can be drawn.

Mirroring the BI results presented here, Pelser et al. (2007) find that a clade containing *Adenostyles leucophylla* and *Adenostyles alpina* (L.) Bluff & Fingerh. [as well as *Caucasalia* B. Nord., *Dolichorrhiza* (Pojark.) Galushko, *Iranecio* B. Nord. and *Pojarkovia* Askerova] is sister to the 'Gynuroid' clade. Within Pelser et al.'s 'Gynuroid' clade, the succulent genus *Kleinia* appears as a monophyletic group with strong support (BPP = 1.00, MPB = 100%), but the results of the present study place *Kleinia crassulaefolia* in a weakly supported clade (BPP = 0.99, MPB = 67%) with *Curio articulatus* and two species currently ascribed to *Senecio*: *S. oxyodontus* and *S. angulatus* (these two species and *Kleinia crassulaefolia* are absent from Pelser et al.'s analysis). This clade is sister to a clade containing the remaining *Kleinia* species, *Solanecio mannii* and *Gynura formosana.* The results of the present study therefore suggest monophyly of *Kleinia* may require further investigation, with a more thorough taxon sample.

Clade 5 contains *Cineraria aspera, Cineraria deltoidea, Cineraria saxifraga, Mesogramma apiifolia* (syn: *Senecio apiifolius*), *Stilpnogyne bellidioides* and *Senecio repandus* (BPP = 1.00, MPB = 72%). This grouping is not seen in the study by Pelser et al. (2007), which does not include *Senecio repandus* or *Stilpnogyne bellidioides*. Their clade containing *Cineraria* species is part of a larger clade including *Bolandia* Cron., *Emilia, Packera, Pericallis* and *Steirodiscus* Less., a combination of the '*Pericallis – Emilia* clade' and the '*Messogramma – Cineraria* clade'. This larger clade is sister to a clade containing *Jacobaea*, termed the *Faujasia – Bethencourtia* clade. In the present study, *Pericallis, Packera* and *Emilia* do not fall in a clade with *Cineraria*; instead they appear in clade 6 (*Packera*) and clade 7 (*Pericallis*), as part of a polytomy with clade 8 in Fig. 2.11 (which includes *Jacobaea*). The polytomy is sister to clade 5. In Pelser et al.'s study, *Cineraria* does group with *Mesogramma*

apiifolia, forming the *Messogramma-Cineraria* clade, along with members of *Bolandia* (unavailable for inclusion here) (BPP = 1.00, MPB = 68%). Again, the MPB value is relatively low, suggesting further work is required to investigate the positions and validity of these clades.

Clade 6, which contains members of *Pericallis*, is very robustly supported (BPP = 1.00, MPB = 100%), and falls on a polytomy with clades 7 and 8 in the BI results presented in Fig. 2.11. Greater resolution is seen in the MP results of the present study, where *Pericallis* groups with clade 7, forming a larger, very weakly supported clade (MPB = <50%). MP analysis places this larger clade on a polytomy with clades 5 - 8, so at this higher level, the MP results are less well resolved than the BI results. Members of *Pericallis* also form a clade in Pelser et al.'s work (BPP = 1.00, MPB = 100%), which is part of a larger clade including *Emilia* and *Packera* from clade 7, termed the *Pericallis-Emilia* clade, but this does not include *Dorobaea* or *Pseudogynoxys* from clade 7. In both studies monophyly of *Pericallis* is very strongly supported.

Clade 7 contains *Dorobaea, Emilia*, *Packera* and *Pseudogynoxys,* and is very weakly suported (BPP = 0.56, MPB = <50%), suggesting the affinities of these genera require further investigation. The clade exists on a polytomy with clades 6 and 8 in the BI tree, while MP results show less resolution, and place the clade on a larger polytomy with clades 4 – 8. A corresponding clade cannot be found in Pelser et al.'s study, although *Emilia* and *Packera* do fall together, as part of the '*Pericallis* - *Emilia* clade'. In this study *Emilia* and *Packera* also fall together in a very weakly supported subclade within clade 7 (BPP = 0.59, MPB = <50%). In Pelser et al.'s work, *Dorobaea* and *Pseudogynoxys* appear in another clade, termed the '*Faujasia-Oldfeltia* clade', a different clade altogether to their '*Pericallis* - *Emilia* clade'. The '*Faujasia-*

Oldfeltia clade' in their work also includes several other genera unavailable for inclusion in this study: *Antillanthus* B. Nord., *Charadranaetes* Janovec & H. Rob., *Dendrophorbium* (Cautrec.) C. Jeffrey, *Ekmaniopappus* Borhidi., *Elekmania* B. Nord., *Eriothrix* Cass., *Faujasia* Cass., *Garcibarrigoa* Cautrec., *Graphistylis* B. Nord., *Herodotia* Urb. & Ekman, *Hubertia* Bory, *Jessea* H. Rob. & Cautrec, *Leonis* B. Nord., *Lundinia* B. Nord., *Mattfeldia* Urb., *Misbrookea* V.A. Funk, *Monticalia* C. Jeffrey, *Nesampelos* B. Nord., *Oldfeltia* B. Nord. & Lundin, *Pentacalia* Cass., *Scrobicaria* Cass., *Talamancalia* H. Rob. & Cautrec, *Werneria* Kunth., *Xenophyllum* V.A. Funk and *Zemisia* B. Nord. Differences in tree topology seen between the two studies in this area may owe something to the absence of these genera in the analysis presented here.

Clade 8 includes members of *Jacobaea* and seven species still ascribed to *Senecio*: *S. achilleifolius, S. coronatus, S. deltoideus, S. latifolius, S. seminiveus, S. speciosus* and *S. tamoides*. This clade is similar to Pelser et al.'s weakly supported '*Faujasia-Bethencourtia* clade' (BPP = 0.95, MPB = <50%), although members of *Bethencourtia,* and some of the *Senecio* species in the clade were not available for inclusion in the present study. However, a number of additional southern African species currently ascribed to *Senecio*, absent from Pelser et al.'s analysis, are included in this group. They are: *S. coronatus, S. seminiveus, S. speciosus* and *S. tamoides.* Support for clade 8 including these *Senecio* species is weak (BPP = 0.73), and in the MP results of the present study *S. achilleifolius, S. seminiveus, S. speciosus* and *S. tamoides* are found on a much more wide-ranging polytomy with clades 5-8. Because of the discrepancy between results obtained using different methods of phylogeny reconstruction, and the weak Bayesian support for the group, these *Senecio* species cannot be definitively added to *Jacobaea,* although they should almost certainly be

removed from *Senecio.* Looking at Pelser et al.'s results, *Jacobaea* appears to be more closely related to *Bethencourtia* than to the *Senecio* species in the group, so any inclusion of these *Senecios* in *Jacobaea* would also require *Bethencourtia* to be lumped in with *Jacobaea.* However, support for *Bethencourtia* as a monophyletic genus is high in Pelser et al.'s work (BPP = 1.00, MPB = 99%), suggesting it may make more sense to retain the separate generic status of *Bethencourtia*, although this excludes the possibility of lumping these *Senecio* species in with *Jacobaea.* This part of the phylogeny would therefore benefit from further research, including a more thorough taxon sample and more informative markers, before any taxonomic decisions are made regarding the appropriate genus in which to place these *Senecio* species. It should be noted that Pelser et al.'s work places *S. achilleifolius* in a completely different position - within *Senecio* s.str. This may be a case of misidentification, or a consequence of an erroneous sequence having been used in one of the studies. *Senecio achilleifolius* was obtained from RBGE living collections as part of the present study, while the origin of the sequence used by Pelser et al. is unknown. However, it is easy to see that with such a volume of sequence data in the Pelser et al. study, combined with a tendency to work with sequences derived by other researchers rather than with plants collected from the field, mix-ups or misidentifications might occur.

Within clade 8, species reassigned to *Jacobaea* by Pelser et al. (2002) form a weakly supported monophyletic group in both the BI and MP results of the present study (BPP = 0.98, MPB = <50%). Monophyly of *Jacobaea* is more robustly supported in Pelser et al. (2007) (BPP = 1.00, MPB = 74%).

2.4.3: Comparing the 'Senecioid' clade with the *Cissampelopsis* – *Crassocephalum* clade of Pelser et al. (2007)

Clade 9 in Fig. 2.11 contains members of *Synotis*, and is strongly supported (BPP = 1.00, MPB = 97%). In the BI results of this study, this clade is basal in the 'Senecioid' clade, while MP analysis places *Dauresia alliariifolia* in this basal position. In Pelser et al.'s study, a less robustly supported clade of *Synotis* species (BPP = 1.00, MPB = 70%) is found in a similar position, basal in the clade containing *Senecio* s.str. and its closest relatives.

Clade 10 consists of *Crassocephalum* and *Erechtites.* This strongly supported clade (BPP = 1.00, MPB = 100%) exists on a polytomy with clades 11 and 12 (*S. engleranus, S. flavus* and *Senecio* s.str.). The same clade is seen in Pelser et al.'s results, as sister to *Senecio* s.str.

Clade 11 is the strongly supported *Senecio engleranus / Senecio flavus* clade (BPP = 1.00, MPB = 99%), which is seen on a polytomy with clades 10 and 12-14. These two species also form a clade in Pelser et al.'s work, with strong support (BPP = 1.00, MPB = 100%), although the affinities of the clade are unclear. Structure within clade 11 is also unclear, and resolution of the two species within is not evident. These two species were subjected to further investigation as reported in Chapter 3 of this thesis.

Clade 12 is discussed in more detail below in relation to the analysis of Dataset 2.

2.4.4: The Closest Relatives of *Senecio* s.str.

The BI results of the present study place *Crassocephalum, Erechtites*, *S.engleranus* and *S. flavus* on a very poorly supported polytomy with *Senecio* s.str. (BPP = 0.51), making the closest genus to *Senecio* s.str. unclear here. *Arrhenechtites*

is basal to the polytomy, although with such low support, it could almost certainly be added to the polytomy, as is seen in the MP results, which place *Arrhenechtites, Erechtites, Crassocephalum, S.engleranus* and *S. flavus* on a very weakly supported polytomy with *Senecio* s.str. (MPB = <50%).

The results of Pelser et al.'s study suggest several possibilities for the closest genus to *Senecio* s.str. Their MP analysis suggests that *Crassocephalum* and *Erechtites* may be the closest relatives of the genus, although support is very low (MPB = <50%), while their BI results point to a closer relationship with the *Senecio engleranus* / *Senecio flavus* clade, although again, support is very low (BPP = 0.58). Their MP results place the *S. engleranus* / *S. flavus* clade as basal in a clade containing *Arrhenechtites, Crassocephalum, Dendrocacalia, Erechtites* and *Senecio* s.str. (Pelser et al., 2007).

The poorly supported polytomies in the present study, and the conflicting results between BI and MP methods in Pelser et al.'s work, show that it is still unclear which are the closest relatives of *Senecio* s.str. This could perhaps be clarified by more thorough phylogenetic investigation, including a larger number of suitable DNA fragments. Ideally one would wish to sequence more rapidly evolving areas of the genome, although selection of suitable fragments is likely to prove problematic.

2.4.5: Other genera that nest within *Senecio* s.str.

Robinsonia is found nested within the *Senecio* s.str. clade in both BI and MP results of this study (Figs. 2.15 – 2.19). The BI results suggest *Robinsonia* may be a monophyletic group, within clade 5 in Fig. 2.17, although support for monophyly is very weak (BPP = 0.58), and the MP tree suggests *R. berteroi* is more closely related to *Senecio pseudo-arnica* than to the other included members of *Robinsonia, a*

relationship also seen in Pelser et al.'s MP results. In the present study, the clade consisting of *R. berteroi* and *S. pseudo-arnica* is very weakly supported (MPB = <50%), and is found on a polytomy with other included members of *Robinsonia*, as well as several *Senecio* species. *Robinsonia berteroi* has a floral morphology distinct from other members of the genus, which has led some researchers to place it in a monotypic genus, *Rhetinodendron* Meisn., although it shares the tree-like habit and dioecious nature which characterise *Robinsonia* (Pelser et al., 2007).

Pelser et al. (2007) note five other genera which also nest within *Senecio* s.str: *Aetheolaena, Culcitium, Hasteola, Iocenes* and *Lasiocephalus.* These six genera differ from the rest of the genus in features which have in the past been considered important in splitting the tribe Senecioneae at the sub-tribal or generic level, such as flower colour, the presence or absence of stylar appendages, life history, habit, and radiate versus discoid capitula. It would appear that homoplasious evolution may have confounded taxonomic decisions, as DNA evidence from both nuclear and plastid DNA, as well as karyological data, point to these genera belonging in *Senecio* s.str. (Pelser et al., 2007)

2.4.6: A comparison of the results of Dataset 2 (*Senecio* s.str.) with Pelser et al. (2007)

Clades 3 -10 in Fig. 2.17 together make up *Senecio* s.str.

Clade 3 in Fig. 2.17 is moderately well supported (BPP = 0.97, MPB = 76%), consists of *S. glomeratus, S. lyratus, S. minimus, S. pterophorus, S. pubigerus* and *S. rigidus,* and is sister to clades 4 – 10 combined. In Pelser et al.'s results, *S. glomeratus* and *S. minimus* are seen in the strongly supported 'Australian *Senecio* clade 1' (BPP = 1.00, MPB = 94%), while *S. pubigerus* is sister to the clade. Support for the clade

including *S. pubigerus* is much lower (BPP = 0.66, MPB = 60%). In the present study, *S. pubigerus* forms a strongly supported subclade with *S. rigidus* (absent from Pelser et al.'s analyses) (BPP = 1.00, MPB = 98%), which is sister to a strongly supported subclade consisting of *S. glomeratus, S. lyratus, S. minimus* and *S. pterophorus* (BPP = 1.00, MPB = 95%). *Senecio lyratus* and *S. pterophorus* (absent from Pelser et al.'s analysis) are South African taxa which group with the Australian species, *S. minimus* and *S. glomeratus.* Strong support values suggest the 'Australian *Senecio* clade 1' of Pelser et al. is not exclusively Australian (Pelser et al., 2007).

Clade 4 is composed of *S. carpetanus, S. decurrens, S. doria, S. gramineus, S. macrospermus* and *S. nemorensis,* and is poorly supported (BPP = 0.88, MPB = <50%). In Pelser et al.'s work, *S. doria, S. carpetanus, S. nemorensis* and *S. decurrens* fall together in a poorly supported, unlabelled clade (BPP = 0.97, MPB = <50%) along with *S. coriaceous* Aiton., *S. doronicum* L., *S. dregeanus, S. franchetii* C. Winkl., *S. panduriformis, S. perralderianus* Coss., *S pyrenaicus* L. and *S. sarracenicus* L. (unavailable for inclusion here). *Senecio doria, S. nemorensis* and *S. carpetanus* appear to be more closely related to one another than to *S. decurrens* in both studies, although support is again weak, (BPP = 1.00, MPB = 56% in the present study). Pelser et al. (2007) also find *S. doria, S. nemorensis* and *S. carpetanus* group together within larger clades (BPP = 1.00, MPB = <50%). Their clade containing *S. decurrens, S. dregeanus* and *S. panduriformis* is weakly supported (BPP = 1.00, MPB = <50%), while the clade containing *S. decurrens, S. gramineus* and *S. macrospermus* in the present study is also very weakly supported (BPP = 0.80, MPB = <50%). *Senecio gramineus* and *S. macrospermus* are absent from Pelser et al.'s study.

Clade 5 in Fig. 2.17 is a weakly supported polytomy consisting of *Robinsonia berteroi, Robinsonia gracilis, Robinsonia thurifera, S. actinella, S. costaricensis, S.*

loratifolius, S. lugens, S. neowebsteri, S. pseudo-arnica, S. serra, S. thianschanicus and *S. zimapanicus* (BPP = 0.92, MPB = 58%), which resides on a trichotomy with clade 6 and clades 7-10. These are all New World species, with the exception of *S. thianschanicus*, which is native to the Indian subcontinent. The presence of *Robinsonia* species, nested within *Senecio* s.str., and the relationships between them are discussed above. All of these species (including *S. thianschanicus*) are found together in a weakly supported clade in Pelser et al's work termed the 'New World *Senecio* Clade 1', which also includes five other genera, as well as *Robinsonia* and *Senecio* species as discussed above (BPP = 1.00, MPB = <50%).

Within the polytomy, *S. actinella, S. lugens, S. neowebsteri* and *S. serra* appear to be more closely related, forming a weakly supported clade in the results of the present study (BPP = 0.63). These taxa are also found together as part of a larger clade in Pelser et al.'s work, although support for their clade is also weak (BPP = 0.75, MPB = <50%). Mirroring the BI results presented here, Pelser et al.'s work suggests *S. neowebsteri* may be basal to the other three taxa. However, in the MP results of the present study, the basal position of *S. neowebsteri* collapses, and the taxon is found on the clade 5 polytomy. Support for the grouping of *S. actinella, S lugens* and *S. serra* is higher if *S. neowebsteri* is excluded from the group, (in the present study BPP = 0.98, MPB = 72%, in Pelser et al.'s work BPP = 1.00, MPB = 62%).

Clade 6 is weakly supported (BPP = 0.97, MPB = 50%), and consists of a subclade containing the Australian *S. dunedinensis, S. glaucophyllus, S. quadridentatus* and *S. rufiglandulosus*, sister to a southern African subclade containing *S. consanguineus, S. eenii, S. erosus, S. erubescens, S. giessii, S. glastifolius, S. hastatus, S. macrocephalus* and *S. paniculatus.* Pelser et al. (2007)

place the Australian taxa in their ‘Australian *Senecio* Clade 2’, which is weakly supported (BPP = 0.98, MPB = 52%). A few Australian taxa, unavailable for inclusion in this study, have been included in their work: *S. macranthus* A. Rich., *S. gunnii* (Hook.f.) Belcher and *S. wairauensis* Belcher. ‘Australian *Senecio* Clade 2’ is also sister to a southern African clade, although the composition of this differs from the composition of the sister clade in this study. Pelser et al.’s southern African sister clade, termed the ‘*Senecio consanguineus* - *S. sisymbrifolius* clade’, consists of *S. consanguineus, S. eenii* and *S. giessii*, in common with the present study, but also includes *S. arenarius, S. cakilefolius* and *S. sisymbrifolius,* which appear in clade 7 in Fig. 2.17. *ITS* Sequences for *S. arenarius* and *S. cakilefolius* found on GenBank differed from sequences derived from field-collected specimens used in the present study. These are distinctive species, for which identifications were relatively easy and it is therefore puzzling that the available GenBank sequences for these taxa appear to be derived from misidentified specimens. Pelser et al. (2007) have used these possibly erroneous GenBank sequences in their analyses, which explains why these two taxa occupy different positions in the respective studies. As part of the present study, the GenBank sequences were included in the analyses of Dataset 1, and appear in a clade with *S. consanguineus, S. eenii, S. erosus, S. erubescens, S. giessii, S. hastatus* and *S. macrocephalus* (Fig. 2.13), in the same group as they are found in Pelser et al.’s tree, and separately from the accessions of these taxa collected in the field as part of the present study.

Support for the ‘*Senecio consanguineus* - *S. sisymbrifolius* clade’ in Pelser et al.’s analysis is weak (BPP = 1.00, MPB = 57%). The larger clade made up of the ‘*Senecio consanguineus* - *S. sisymbrifolius* clade’ and ‘Australian *Senecio* Clade 2’ is sister to a large clade which includes the type species, *S. vulgaris,* the most closely

related *Senecios* and *Culcitium niveo-aureum* Cautrec. In Pelser et al.'s study, *S. glastifolius* groups with *S. achilleifolius,* with strong support, (BPP = 1.00, MPB = 99%). However, this may have been a case of misidentification, as in the present study *S. achilleifolius* is found in the 'Jacobaean' clade, equivalent to their '*Austrosynotis-Cineraria* clade', grouped with *S. seminiveus*, also with strong support (BPP = 1.00, MPB = 100%).

Clade 7 is well supported (BPP = 1.00, MPB = 99%), and consists of southern African species, *S. arenarius, S. cakilefolius, S. elegans, S. glutinarius, S. glutinosus, S. sisymbrifolius* and *S. windhoekensis.* This clade is seen in the same position in both the BI and MP trees. However, this clade is not seen in Pelser et al.'s work, in which *S. glutinosus* and *S. windhoekensis* fall in a clade with *S. hastatus,* seen in clade 6 in the present study. Pelser et al. have found strong support for this clade (BPP = 1.00, MPB = 100%), which is sister to a large clade containing *S. vulgaris.* It is likely that erroneous GenBank accessions of *S. arenarius* and *S. cakilefolius* appear in their poorly supported '*Senecio consanguineus - S. sisymbrifolius* clade', which also includes *S. sisymbrifolius*, as mentioned above. *S. elegans* and *S. glutinarius* are not included in Pelser et al.'s work.

Clade 8 is quite well supported (BPP = 1.00, MPB = 82%), and consists of the closest included relatives of the type species *S. vulgaris.* As well as *S. vulgaris*, the clade contains *S. aethnensis* (syn: *S. squalidus* subsp. *aethnensis*), *S. chrysanthemifolius, S. gallicus, S. glaucus, S. hesperidium, S. krascheninnikovii, S. leucanthemifolius, S. mohavensis, S. nebrodensis, S. petraeus, S. rodriguezii* (syn: *S. varicosus*), *S. rupestris, S. squalidus, S. sylvaticus, S. vernalis* and *S. viscosus.* Within clade 8 are two sister clades, one consisting of multiple accessions of *S. vernalis* and *S. vulgaris,* the other consisting of the remaining taxa listed above. Support for the *S.*

vulgaris-containing sister clade is strong (BPP = 1.00, MPB = 83%), while the other sister clade is less robustly supported (BPP = 0.91, MPB = 55%). Pelser et al.'s results show a similar pattern with the grouping of *S. vulgaris* and *S. vernalis* forming a clade sister to a clade containing the other species mentioned above. Pelser has included '*Senecio* clade A' to represent the complex seen in the present study between accessions of *S. gallicus, S. glaucus, S. hesperidium, S. leucanthemifolius, S. mohavensis, S. petraeus, S. squalidus* and *S. rodriguezii.* Some structure is seen in this group in the BI results of the present study, but support is very low, and much of this structure collapses into a polytomy in the MP results, which suggest only a closer relationship between *S. glaucus* subsp. *glaucus* and *S. mohavensis* (BPP = 0.85, MPB = 63%), and a closer relationship between *S. glaucus* subsp. *coronopifolius* and *S. hesperidium* (BPP = 0.87, MPB = 63%). In the BI results, a closer relationship is also seen between *S. chrysanthemifolius, S. leucanthemifolius, S. rupestris* and *S. squalidus,* but this clade is very weakly supported (BPP = 0.63).

Clade 9 consists of the Australian *S. lautus*, which occupies a basal position in relation to the other taxa in the clade, the New World *S. aphanactis, S. brasiliensis, S. erterrrae, S. lemmonii, S. californicus, S. douglasii and S. eremophilus.* Overall support for clade 9 is very weak (BPP = 0.57, MPB = <50%). Pelser et al.'s work includes *S. lautus*, which appears with two other Australian taxa, *S. spanomerus* I. Thomps. and *S. pinnatifolius* A. Rich. (absent from the present study), in a weakly supported clade (BPP = 1.00, MPB = 53%) termed 'Australian *Senecio* clade 3'. This clade also appears in a sister position relative to the New World taxa from clade 9 included in their study: *S. aphanactis, S. brasiliensis, S. californicus, S. ertterae* and *S. lemmonii.* These taxa are found together in a larger clade termed 'New World *Senecio* clade 2' (weakly supported including only the taxa listed above, with BPP =

0.74, MPB = <50%, slightly more robustly supported with BPP = 1.00, MPB = 64% if another taxon, *S. deferens* Griseb. is included, completing 'New World *Senecio* clade 2'). If a single southern African taxon, *S. meyeri-johannis* Engl. is included in this clade of otherwise New World taxa, the enlarged clade is sister to a clade containing the taxa found in clade 10 in the present study, discussed below.

Clade 10 (BPP = 1.00, MPB = 62%) consists of the African taxa, *S. abruptus, S. aegyptius, S. burchellii, S. cryphiactis, S. erysimoides, S. inaequidens, S. littoreus, S. longifolius, S. madagascariensis, S. malacitanus, S. maritimus, S. parvifolius, S. pellucidus, S. pinnulatus* and *S. sophioides.* Pelser et al.'s study includes *S. abruptus, S. aegyptius, S. burchellii, S. inaequidens* and *S. madagascariensis,* all of which are found together in a weakly supported (BPP = 0.64, MPB = <50%), exclusively African clade. Pelser et al.'s MP results suggest the exclusively African clade is sister to a clade containing the type species of the genus, *S. vulgaris* and its closest relatives. The relationship suggested by both MP and BI results of the present study differs from that suggested by Pelser et al.'s MP results. Clades 9 and 10 are sister to one another. Clades 9 and 10 together form a clade (BPP = 1.00, MPB =62%) sister to clade 8, which contains *S. vulgaris* and its closest relatives. A similar pattern of relationships is also seen in Pelser et al.'s BI results.

2.4.7: Species which should be removed from *Senecio*

There are a number of species currently ascribed to *Senecio* which appear to be only distantly related to the core of *Senecio* s.str. Species which may need to be removed from *Senecio* and assigned to other genera include:

1) *Senecio angulatus* and *S. oxyodontus*, which are only distantly related to *Senecio* s.str., and group with *Adenostyles, Curio, Gynura, Kleinia* and *Solanecio* in

clade 4, within the 'Jacobaean' clade in Fig 2.11. These species should be reassigned to other genera. Other *Senecio* species which may belong in this group are *S. abbreviatus* S. Moore, *S. bulbinefolius* DC., *S. corymbiferus, S. crassissimus* Humb., *S. junceus, S. limifolius* L., *S. macroglossus, S. melastomifolius* Baker, *S. milanjianus* S. Moore, *S. mueselii, S. muirii* L. Bolus, *S. oxyriifolius* and *S. spiculosus* (sheph.) Rowley (Pelser et al., 2007).

2) *Senecio repandus* is found in clade 5 in Fig 2.11 as part of the 'Jacobaean' clade, grouped with *Cineraria* species. Further research would be required before adding this species to *Cineraria,* as support values for the clade consisting of *S. repandus* and *Cineraria* species are low (BPP = 0.97, MPB = 56%).

3) *Senecio achilleifolius, S. coronatus, S. deltoideus, S. latifolius, S. seminiveus, S. speciosus* and *S. tamoides* appear to be more closely related to *Jacobaea* than to *Senecio* s.str. and should be removed from *Senecio.* However, it is unclear where they would be best placed. It should be noted that in Pelser et al.'s work, *S. achilleifolius* falls within *Senecio* s.str., so further research would be required to confirm that this species does not belong in *Senecio. Senecio glaberrimus, S. lineatus, S. pinifolius, S. retrorsus* DC., *S. scandens* Buch. –Ham. ex D. Don and *S. triqueter* may also belong in this group and should be considered for removal from *Senecio* (Pelser et al., 2007).

Several other *Senecio* species are suggested for removal from the genus by Pelser et al. (2007): *S. adamantinus* Bong., *S. arnaldii* Cabr., *S. hemmendorffii* Malme., *S. medley-woodii, S. otites* Kunze. ex DC., *S. pyramidatus, S. saxatilis* Wall. ex DC., *S. scaposus, S. stigophlebius* Baker and *S. thapsoides* DC. *Senecio ayopayensis* Cautrec. and *S. subnemoralis* Dusén are currently being formally transferred to *Dendrophorbium* (Pelser et al., 2007).

2.4.8: Possible southern African members of Section *Senecio*

As mentioned in the introduction, sect. *Senecio* has never been defined with respect to southern African species, although a few species ascribed to the section are found in southern Africa. For example, the type species of the section, *Senecio vulgaris* is found there, although it may well have been introduced by human activity. A single specimen of *S. vulgaris* was collected during fieldwork in Namibia, but was found in a cultivated garden, while no specimens of *S. vulgaris* were found in the wild during extensive searches in both Namibia and the Cape regions of South Africa.

The results presented here, whilst highlighting the complexity and inadequacy of the currently accepted system of around 150 sections within the genus, suggest that there are some southern African species, as well as some from other areas of the world, not currently assigned to section *Senecio,* which could perhaps belong in the section. Although the composition of a definitive clade representing the section remains unclear, there are several species which appear to be closely related to the clade containing the type species, *Senecio vulgaris.* Referring to Fig. 2.17, in which section *Senecio* is marked as clade 11, of the taxa collected as part of this study in southern Africa, the following could be tentatively placed in section *Senecio*:

S. abruptus, S. arenarius, S. burchellii, S. cakilefolius, S. elegans, S. erysimoides, S. glutinarius, S. glutinosus, S. inaequidens, S. littoreus, S. longifolius, S. madagascariensis, S. maritimus, S. parvifolius, S. pellucidus, S. pinnulatus, S. sisymbrifolius, S. sophioides, S. windhoekensis.

Species from other areas of the world which may belong in the section are:

S. aegyptius subsp. *aegyptius, S. aegyptius* subsp. *thebanus, S. aethnensis, S. aphanactis, S. brasiliensis, S. californicus, S. chrysanthemifolius, S. cryphiactis, S. douglasii, S. eremophilus, S. ertterae, S. gallicus, S. glaucus* subsp. *coronopifolius, S.*

glaucus subsp. *glaucus*, *S. hesperidium*, *S. kraschenninnikovii*, *S. lautus*, *S. lemmonii*, *S. leucanthemifolius*, *S. lividus*, *S. malacitanus*, *S. mohavensis* subsp. *breviflorus*, *S. mohavensis* subsp. *mohavensis*, *S. nebrodensis*, *S. petraeus*, *S. rodriguezii*, *S. rupestris*, *S. squalidus* subsp. *araneosus*, *S. squalidus* subsp. *squalidus*, *S. sylvaticus*, *S. vernalis*, *S. viscosus*, *S. vulgaris*, *S. vulgaris* subsp. *denticulatus.*

Support for the group taken here to represent section *Senecio* is strong in the BI analysis (BPP = 1.00), but much weaker in the MP analysis (MPB = 63%).
In all cases, further research is required to confirm the placing of the taxa within the section. Ideally, a complete taxon sample would be used, but this would be very difficult to achieve. More informative DNA sequences are also required.

2.4.9: Incongruence of results obtained from nuclear and plastid datasets

As is evident in Figs. 2.27 and 2.28, congruence between the phylogenetic results based on *ITS* nuclear DNA and *trn*L-F plastid DNA sequences is very low. This high level of incongruence is unexpected, and is unhelpful in clarifying evolutionary relationships between clades within Senecioneae. A similarly high level of incongruence between results of nuclear and plastid data analyses was evident from the work of Pelser et al. (2007), suggesting that the incongruence is not a result of human error such as incorrect sequence alignment.

Many of the same clades are present in both the *ITS* and *trn*L-F trees, but the relationships between them are strikingly different, as many subsidiary clades switch positions between the major clades. The incongruence may be caused, at least in part, by taxon sampling effects. As noted above, *trn*L-F data for Senecioneae were much more sparse than *ITS* data, and most of the taxa for which complete *trn*L-F sequences were available were those collected during fieldwork as part of the present study. As a

result only relatively few taxa represented a very large and phylogenetically diverse group. Dataset 4 (the *trn*L-F matrix) included far fewer taxa than Datasets 1 and 2. Dataset 3 was constructed to include *ITS* sequences of only those taxa for which a full *trn*L-F sequence was available, so that congruence between trees based on nuclear and plastid data could be more easily investigated. A wider ranging analysis including incomplete *trn*L-F sequences available on GenBank was attempted, but was abandoned, as both the resolution and support values in the resulting trees were too low to be of any real use.

Another problem may be the lack of a strong phylogenetic signal in both Datasets, but this could not be the case where strongly supported but incongruent clades are found on analysis of Datasets 3 and 4.

Sequencing of paralogous *ITS* copies could also be a source of incongruence. *ITS* is present in multiple copies in the genome, and recently the efficiency of homogenisation of *ITS* sequences in individuals by concerted evolution (Hillis & Dixon, 1991) has been questioned (Möller, 2000). It has been shown that *ITS* variation in an individual can exceed interspecific *ITS* variation (Karvonen et al., 1994; Smith & Klein, 1994; Oxelman & Liden, 1995). If non-homologous *ITS* copies have been used in phylogenetic analysis, incorrect tree topologies may be retrieved (Möller, 2000).

Alternatively, because nuclear DNA is biparentally inherited, while plastid DNA is maternally inherited, another possible cause of incongruence is hybridisation. Some *Senecio* species undergo interspecific hybridisation, which can be followed by introgression. These phenomena can confound phylogenetic inference, as phylogenetic analyses can only represent hierarchical structure, not the reticulating structure of hybridising species. It also means that certain gene sequences within an

individual may give a misleading impression of its evolutionary history (Abbott et al., 2000).

A number of species of hybrid origin are well known in *Senecio* including *S. cambrensis* in the UK (a hexaploid hybrid species, created by the crossing of *S. squalidus* and *S. vulgaris*) **(**Lowe & Abbott, 1996), *S. teneriffae* Schultz. Bip. (Kadereit, 1984) in the Canary Islands (a hexaploid derivative of *S. vulgaris* and *S. glaucus*) (Lowe & Abbott, 1996) and *S. mohavensis* in North Africa (a hybrid derivative of *S. glaucus* and *S. flavus*) (Coleman et al., 2001; Comes & Abbott, 2001; Kadereit et al. 2006). Although hybrid origins of new taxa are well documented in *Senecio*, it is unclear whether the origin of new species through hybridisation is common enough in the tribe to account for the major and extensive differences in tree topologies seen between nuclear and plastid Datasets.

The incongruence could also indicate lineage sorting effects, or rapid diversification (Wendel & Doyle, 1998).

This range of possible explanations for the incongruity of the two Datasets suggests that further investigation should be carried out. In particular, in order to eliminate taxon sampling effects as a main cause of incongruity, a larger matrix of plastid data should be constructed and analysed. A project with this aim in mind is currently being carried out by Pelser et al. (in prep.).

2.4.10: Biogeographic Patterns in *Senecio* s.str.

The results optimised for geographic locality on the BI tree of *Senecio* s.str. (Figs. 2.29 – 2.31) suggest a strong southern African influence in the evolution of both the *Senecio* s.str. and section *Senecio* itself. Strong geographic structure is seen, lending support to the clades seen in the analyses.

Early diversification in the genus appears to have taken place in southern Africa, resulting in the major groups seen within *Senecio* s.str., a conclusion supported by area optimisation trees in Pelser et al. (2007) and by the fact that southern Africa is a known centre of diversity for the genus (Nordenstam, 1977; Bremer, 1994).

There have been at least two independent colonisations of Australasia from southern Africa, in the case of a clade consisting of *S. glomeratus* and *S. minimus*, within clade 3 in Fig. 2.17, and a clade consisting of *S. dunedinensis, S. glaucophyllus, S. quadridentatus* and *S. rufiglandulosus* within clade 6 in Fig. 2.17. Another clear colonisation event is seen from southern Africa into Eurasia, in the case of a clade consisting of *S. carpetanus, S. doria* and *S. nemorensis*, within clade 4 in Fig. 2.17.

Other major colonisations of geographical areas are less straightforward. There are two clades for which colonisation events appear to have begun from southern Africa, although both nodes are equivocal, making it impossible to tell which area was colonised first. Clade 5 in Fig. 2.17 consists of New World taxa and a single Asian species. This would suggest that there might have been a colonisation of the New World from southern Africa, followed by colonisation of Asia. The other clade is composed of clades 8, 9 and 10 in Fig. 2.17, part of the section *Senecio* clade, and consists of Eurasian, New World, northern African, southern African and a single Asian species. It is less clear here which route colonisations may have taken.

Coleman et al. (2003) included some species from these clades in an area-optimised phylogeny, and suggested that there may have been a colonisation of the Mediterranean from southern Africa, resulting in the evolution of a group which contains the type species, *S. vulgaris*, followed by a colonisation of North America by

S. mohavensis subsp. *mohavensis*. A similar pattern of the colonisation of North America by *S. mohavensis* subsp. *mohavensis* is seen in clade 8 in Fig. 2.17 in the present study. There appears to have been an additional colonisation of Asia from the Mediterranean within this group, in the case of *S. kraschenninikovii* [not included in Coleman et al. (2003)]. Coleman et al. (2003) also suggest a colonisation of South America from southern Africa, followed by a move into North America. Denser taxon sampling in the present study shows that the Australian *S. lautus* is, in fact, the most basal taxon in this predominantly New World group (see clade 9 in Fig. 2.17), suggesting that Australia was first colonised from southern Africa, followed by subsequent colonisations of South America and North America.

2.4.11: Evolution of Flower Colour in *Senecio* s.str.

It is clear from Fig. 2.32, that yellow ray florets are the ancestral state for *Senecio* s.str. It is also evident that purple ray florets have evolved at least six times independently. Because the analysis does not include all taxa in *Senecio* s.str., it is likely that there have been more incidents of purple ray florets evolving independently in the evolutionary history of the genus. A complete taxon sample of the genus would allow determination of the number of separate incidents of purple ray floret evolution.

2.4.12: Conclusions

The results of the molecular phylogenetic anlaysis of *ITS* data presented here largely agree with the phylogeny of Senecioneae by Pelser et al. (2007) and support a monophyletic subtribe Senecioninae, a monophyletic *Senecio* s.str. which includes *Robinsonia,* and a newly expanded monophyletic sect. *Senecio,* including South

African taxa which have never before been placed in the section. Several other *Senecio* species are only distantly related to the core of the genus, and should be assigned to other genera.

The closest relatives of *Senecio* s.str. remain unclear according to the results of the *ITS* phylogenetic analysis. The clade representing the genus is found on a polytomy with *Erechtites, Crassocephalum* and the *Senecio engleranus / Senecio flavus* clade. *Senecio* s.str. also appears to be closely related to *Arrhenechtites.* Further research, including a greater number of DNA fragments could perhaps provide a solution as to which species or genus represents the closest relatives of *Senecio* s.str. The *Senecio engleranus / Seneco flavus* clade was shown to be only distantly related to sect. *Senecio,* rather than basal in the clade as had been suggested by Coleman et al. (2003). The affinities of the clade remain unclear, although it is certainly closely related to *Senecio* s.str.

Harvey's (1865) classification of southern African *Senecio* species was tested for the monophyly of sections. Of the sections tested, none were seen to be monophyletic groups. Future studies should include a more complete taxon sample from his sections, which would involve a long period of fieldwork. Biogeographic results suggest southern Africa as an important geographical area for diversification in the genus. The genus itself, and major groups within *Senecio* s.str., may well have originated there, including sect. *Senecio.* This emphasises the need for a clearer understanding of the relationships of southern African *Senecio* species. Biogeographic results also suggest that there have been at least five intercontinental colonisations originating in southern Africa in the history of the genus, underlining the need to include species from different geographical areas together in analyses.

Surprisingly high incongruence was seen between results obtained on analysis of nuclear and plastid data. A more thorough sample of plastid data would be a first step towards understanding the causes of this high level of incongruence.

In both BI and MP analyses, support values for many clades were weak, suggesting that more informative markers need to be identified for use in future analyses of closely related members of Senecioninae, although identification of suitable markers is likely to prove problematic. Ideally, future work should also include as complete a taxon sample as possible, although dealing with such a speciose group with a cosmopolitan distribution would represent an enormous task.

CHAPTER 3:

A comparative study of *Senecio engleranus* and *Senecio flavus*: evidence from RAPDs, morphometric analysis and breeding experiments

3.1.1: INTRODUCTION

Senecio engleranus O. Hoffm. and *Senecio flavus* (Decne.) Sch.Bip. (Fig. 3.1) are a little-known pair of diploid sister species (2n=20), previously investigated at the University of St Andrews as part of a wider *ITS* phylogenetic analysis of sect. *Senecio* (Coleman, 2003; Coleman et al., 2003). The *ITS* phylogeny reported by Coleman et al. (2003) pointed to a basal position in sect. *Senecio* for a clade composed of the pair, suggesting that they might represent ancestral taxa for the section, and that their relationship to sect. *Senecio* warranted further research. On early observation of herbarium material, it remained unclear whether these two sister species were genuinely distinct entities, or if the differences seen represented a continuum of intraspecific variation.

Figure 3.1: *S. engleranus* (left) and *S. flavus* (right), showing the larger capitula and more succulent leaves of *S. engleranus*.

An observed difference in pappus morphology, however, suggested they might represent separate entities. *Senecio flavus* specimens have a modified pappus, termed a ‘connate fluked’ pappus by Coleman et al. (2003), which has an extra set of hairs with grappling-hook-like appendages fused to the cypsela (Fig. 3.2). Coleman et al.

(2003) suggested that this pappus type might account for the disjunct distribution of *S. flavus,* which occurs in Namibia and northern Africa / the Mediterranean basin. The connate fluked pappus would allow for distribution by ectozoochory (external animal dispersal) in addition to the distribution by anemochory (wind dispersal) afforded by the ordinary pappus. Migratory birds travelling from southern to northern Africa may have carried cypselas of *S. flavus,* causing the observed disjunct distribution. *Senecio engleranus,* in contrast, lacks a connate fluked pappus and is known only from Namibia, although very few accounts of the distribution of this species are available. In fact, only one such account appears to exist, in Merxmüller's, *Prodromus einer Flora von Südwestafrica* (1976). The worldwide distributions of *S. engleranus* and *S. flavus* are shown in Fig. 3.3.

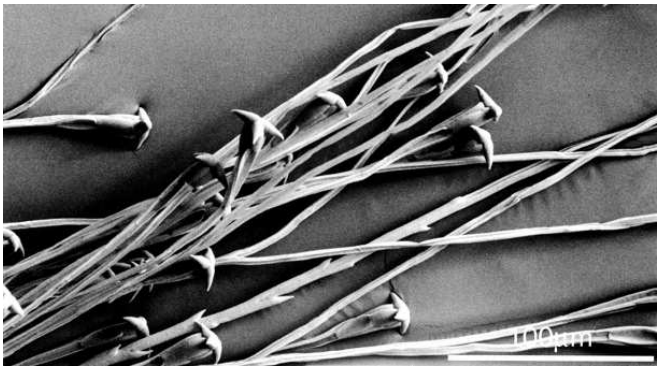


Figure 3.2: Connate fluked pappus in *S. flavus* (from Coleman et al., 2003)

The analysis by Coleman et al., (2003) revealed some minor variation between *ITS* sequences of the two species, but it remained unclear whether there was genome-wide sequence variation between the two. It was also unclear whether there were any consistent morphological differences between the two taxa, apart from the observed differences in pappus morphology.

With the intention of collecting *S. engleranus* and *S. flavus* and possible hybrids between the two in areas of sympatry, fieldwork in Namibia, where the

distributions of these species are reported to overlap (Merxmüller, 1976), was carried out in April 2005. Unfortunately, because of phenological differences, it was only possible to collect *S. engleranus* from Namibia, as the flowering season of *S. flavus* appeared to have passed. A week was spent fruitlessly searching the south of the country, in areas where *S. flavus* had previously been collected by Bertil Nordenstam (personal communication), who kindly supplied herbarium material with locality information of a high quality for both species. Hybrids between the two species were not found during fieldwork, and it is likely that the two species do not hybridize in the wild as hybrids have not been reported previously.

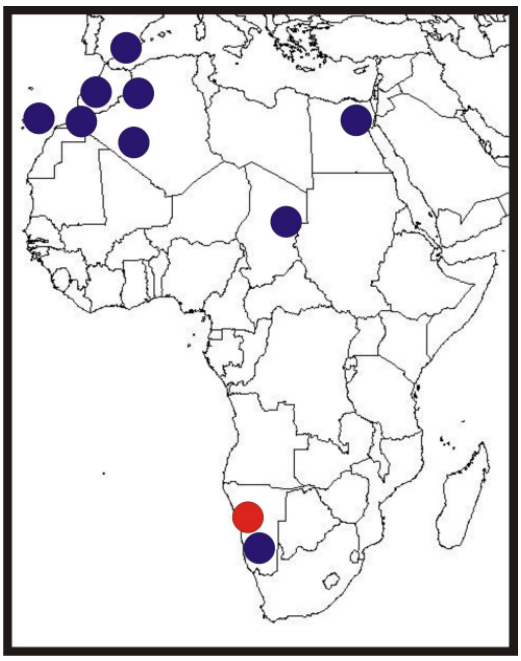


Figure 3.3: Geographical distribution of *S. engleranus* (red dots) and *S. flavus* (blue dots). *S. engleranus* locality information from Merxmüller (1976), *S. flavus* locality information from Chapman (2004).

Fortunately, *S. flavus* plants grown from seed were available for study, having previously been collected in North Africa and the Canary Islands by other researchers at the University of St Andrews.

As mentioned above, Coleman et al. (2003) suggested that the clade composed

of *S. engleranus* and *S. flavus* was basal to sect. *Senecio.* However, the position of the clade in their phylogeny might be an artefact caused by taxon sampling effects. Although increased taxon sampling makes phylogenetic analysis more computationally demanding, introducing additional taxa can result in more accurate estimates of evolutionary relationships - whereas inadequate taxon sampling can lead to low resolution between taxa, or incorrect inference of phylogenetic relationships (Heath et al., 2008). Only a very limited number of taxa from *Senecio* were included in Coleman et al.'s (2003) analyses, and the more densely sampled *ITS* phylogenies presented in Chapter 2 of this thesis and in a study of Senecioneae by Pelser et al. (2007) indicate that the clade comprising *S. engleranus* and *S. flavus* is only distantly related to sect. *Senecio* (see Figs. 2.11 – 2.21). Indeed, Pelser et al. (2007) suggest that the species pair may not even be part of *Senecio* s.str, although they might represent the closest relatives of the genus. The BI results of the phylogenetic study presented as part of this thesis placed the '*S. engleranus* / *S. flavus* clade' on a polytomy with *Senecio* s.str., *Erechtites* and *Crassocephalum*, while the MP results placed it on a polytomy with the above genera and *Arrhenechtites.*

3.1.2: Structure within the '*S. engleranus* / *S. flavus* clade'

Four accessions of *S. engleranus* (representing three of four populations sampled in Namibia and a single Genbank accession, also from Namibia (accession no. AF457417) and six accessions of *S. flavus* sampled by others from Namibia, Egypt, Morocco and the Canary Islands were included in Bayesian inference and maximum parsimony analyses of nuclear *ITS* and plastid *trn*L-F DNA as part of the phylogenetic study presented in Chapter 2 of this thesis. However, the structure seen within the clade composed of these accessions was not clearly resolved (Fig. 3.4).

Moreover, analyses of plastid *trn*L-F data, including three *S. engleranus* and four *S. flavus* accessions, showed no resolution at all between the species (Fig. 2.28).

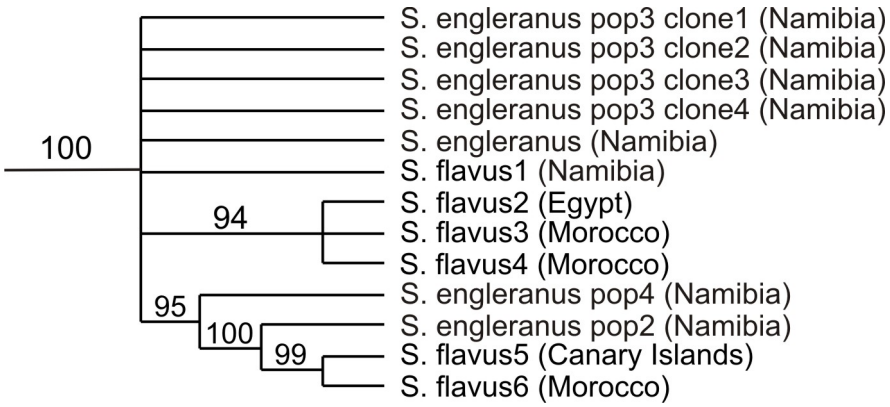


Figure 3.4 Structure of the '*S. engleranus / S. flavus* clade' based on BI analysis of *ITS* nuclear DNA. Countries of origin of each accession are given in brackets. Figures above nodes indicate probabilities of clades x100.

The ITS tree presented in Fig. 3.4 shows that accessions representing two *S. engleranus* populations (populations 2 and 4) are more closely related to *S. flavus* accessions (*S. flavus* 5 and 6) than to the other accessions of *S. engleranus* included (*S. engleranus* pop 3 and the GenBank accession of *S. engleranus*). This is despite the fact that the three populations of *S. engleranus* collected in Namibia were geographically close to one another. *Senecio flavus* 1 (a Namibian accession) also appears to be more closely related to *S engleranus* population 3 and the GenBank accession of *S. engleranus* than to the other accessions of *S. flavus*. This puzzling result, which suggests that *S. engleranus* and *S. flavus* may not, in fact, be two distinct species, led to the more thorough study of the taxa which is reported in this chapter. In this study, surveys of randomly amplified polymorphic DNA (RAPD) and morphometric variation were conducted to investigate genetic and morphological differences between the two taxa.

In the course of conducting this work, it was established that the sister species pair were interfertile, suggesting that intrinsic postzygotic barriers between them were

weak. It therefore proved possible to create F1 hybrids between the two species by crossing them in the glasshouse. These F1 plants were included in the morphometric and RAPD analyses that compared *S. engleranus* and *S. flavus*.

Casual observations further indicated that the two species differed in mating system, with *S. engleranus* failing to set seed when left to self, whereas *S. flavus* produced seed readily on selfing. Because species that reproduce by self-fertilisation normally produce much less pollen per ovule than do outcrossing species (Cruden 1977), a comparison of pollen production and fertility was made between *S. flavus* and *S. engleranus*, their F1 hybrids, and also among F2 plants raised from one self-fertile F1 plant.

Finally, a genetic analysis of one particular morphological trait that distinguishes *S. flavus* and *S. engleranus* – the presence/absence of connate fluked pappus - was conducted by examining segregation of the trait in an F2 family produced from a cross between the two species. This F2 was the same as the one described above in connection with the analysis of pollen number and fertility.

3.2: MATERIALS AND METHODS

3.2.1: Fieldwork

Locality information for *S. engleranus* and *S. flavus* in Namibia was noted from herbarium collections supplied by Bertil Nordenstam, mapped (Fig. 3.5) and a suitable time chosen for fieldwork (April, 2005) based on dates of collections and the available literature (Merxmüller, 1976). Herbarium specimens of *S. engleranus* were collected, as well as leaf samples in silica gel for subsequent DNA extraction and sequencing. Population level seed collections were also gathered, so that plants could be raised in the glassouse at the University of St Andrews. Plants of *S. flavus* used in analyses were derived from seed sampled previously by others from different parts of North Africa. Details of collections made, and of *S. flavus* plants are in Table 3.5 in the results section.

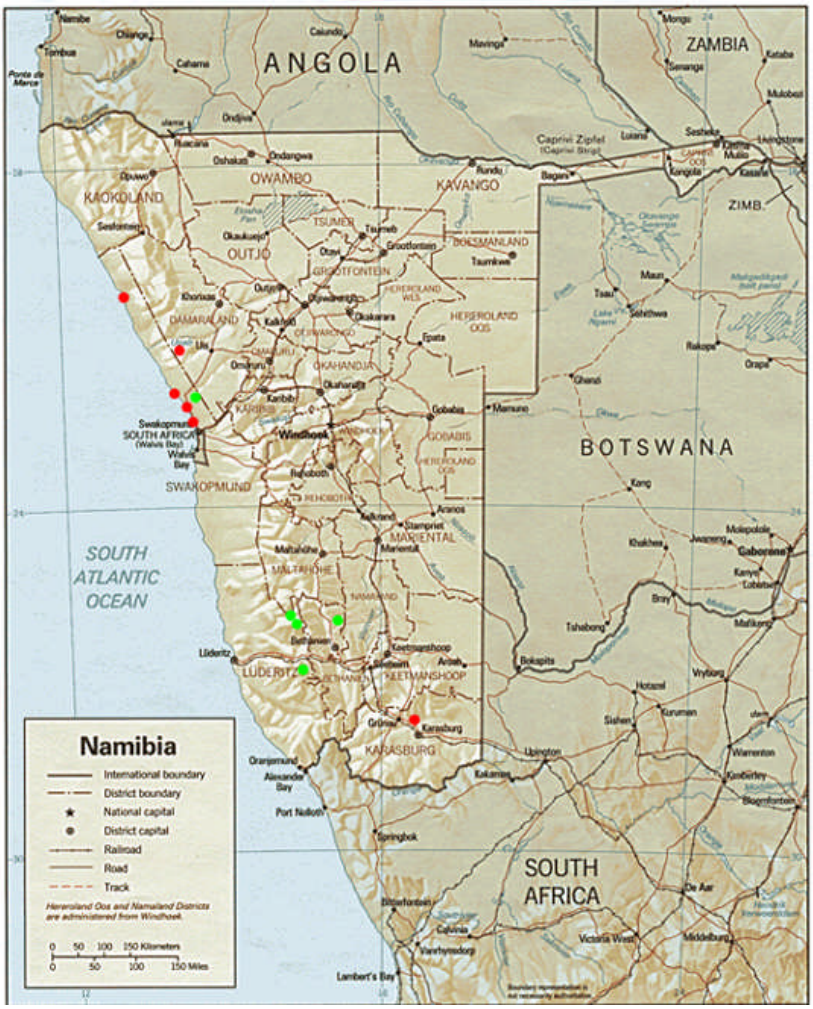


Figure 3.5: Locality information for *S. engleranus* and *S. flavus* in Namíbia. Red dots represent *S. engleranus* localities, while green dots represent *S. flavus* localities. Locality information from B. Nordenstam (personal communication).

3.2.2: RAPD PCR

Initially six DNA extracts; three of *S. engleranus*, and three of *S. flavus* (made using a protocol adapted from Doyle & Doyle, 1987, see Chapter 2 Materials and Methods) were amplified using 60 random decamer oligonucleotides (Operon technologies sets A, B and C), following the conditions set out below. Of the 60 primers screened, 12 that gave strong and easily scored bands were selected. The primer sequences for these primers are shown in Table 3.2.

DNA extracts from twenty individuals of *S. engleranus*, ten of *S. flavus* and four F1 hybrids created by crossing the two species (see Table 3.1 for details) were

amplified using the 12 selected primers listed in Table 3.2, resulting in 48 bands scored. Each primer reaction was carried out in triplicate. Details of accessions of taxa included in the analysis are listed in Table 3.1.

Table 3.1: Accessions of taxa and synthetic F1 hybrids included in RAPD analysis

Taxon	**Code**	**Source**	**Locality**
S. engleranus	eng 2/2	Namibia pop.2	See Table 3.5
S. engleranus	eng 2/4	Namibia pop.2	See Table 3.5
S. engleranus	eng 2/9	Namibia pop.2	See Table 3.5
S. engleranus	eng 2/11	Namibia pop.2	See Table 3.5
S. engleranus	eng 2/13	Namibia pop.2	See Table 3.5
S. engleranus	eng 2/24	Namibia pop.2	See Table 3.5
S. engleranus	eng 3/5	Namibia pop.3	See Table 3.5
S. engleranus	eng 3/7	Namibia pop.3	See Table 3.5
S. engleranus	eng 3/21	Namibia pop.3	See Table 3.5
S. engleranus	eng 3/25	Namibia pop.3	See Table 3.5
S. engleranus	eng 3/29	Namibia pop.3	See Table 3.5
S. engleranus	eng 3/31	Namibia pop.3	See Table 3.5
S. engleranus	eng 3/43	Namibia pop.3	See Table 3.5
S. engleranus	eng 3/44	Namibia pop.3	See Table 3.5
S. engleranus	eng 3/45	Namibia pop.3	See Table 3.5
S. engleranus	eng 3/49	Namibia pop.3	See Table 3.5
S. engleranus	eng 3/60	Namibia pop.3	See Table 3.5
S. engleranus	eng 4/1	Namibia pop.4	See Table 3.5
S. engleranus	eng 4/2	Namibia pop.4	See Table 3.5
S. engleranus	eng 4/3	Namibia pop.4	See Table 3.5
S. engleranus	eng 4/4	Namibia pop.4	See Table 3.5
S. flavus	SF3	Seed collections	Morocco
S. flavus	SF5	Seed collections	Morocco
S. flavus	SF7	Seed collections	Morocco
S. flavus	SF15	Seed collections	Morocco
S. flavus	SF16	Seed collections	Morocco
S. flavus	SF22	Seed collections	Morocco
S. flavus	SF75	Seed collections	Morocco
S. flavus	fl14388	Seed collections	Canary Islands
S. flavus	fl 26145	Seed collections	Canary Islands
S. flavus	fl – Sinai	Seed collections	Egypt
S. flavus	fl14454	Seed collections	Morocco
S. flavus x *S. engleranus*	SF75 x eng 3/29 (1)	F1 hybrid	St Andrews
S. flavus x *S. engleranus*	SF75 x eng 3/29 (2)	F1 hybrid	St Andrews
S. flavus x *S. engleranus*	SF5 x eng 2/2 (1)	F1 hybrid	St Andrews
S. flavus x *S. engleranus*	SF5 x eng 2/2 (2)	F1 hybrid	St Andrews

Table 3.2: RAPD primers with number of bands scored / primer and primer sequences (Operon technologies).

Primer Name	No. of Bands Scored	Sequence (5' – 3')
A02	4	TGCCGAGCTG
A07	4	GAAACGGGTG
A09	4	GGGTAACGCC
A13	3	CAGCACCCAC
B08	4	GTCCACACGG
B09	5	TGGGGGACTC
B12	5	CCTTGACGCA
B15	4	GGAGGGTGTT
B17	7	AGGGAACGAG
C08	2	TGGACCGGTG
C09	3	CTCACCGTCC
C18	3	TGAGTGGGTG

The RAPD amplification procedure was as follows. For each RAPD reaction: 2.5µl of Bioline 10x NH_4 reaction buffer (160mM $(NH_4)_2SO_4$, 670mM Tris HCl, 0.1% Tween 20, pH 8.8), 2.5µl of 2mM dNTPs, 1.25µl of 50 mM Bioline $MgCl_2$, 0.75µl of each primer, 0.125µl of Bioline Taq polymerase, 16.25µl of deionised water and 1µl of template DNA, giving a total of 25µl per reaction. Thermal cycling began with 3 minutes of denaturing at 94°C, followed by 45 cycles of 30s at 94°C, 45s at 35°C and 90s at 72°C, and a final elogation step of 4 minutes at 72°C. Amplification products were resolved in 1.4% agarose gels for ~ 4 hours at 100V and visualised using UV transillumination.

Homology testing

In order to tackle the reproducibility issues of RAPD profiles referred to in Chapter 1, some form of homology testing is usually carried out on the bands scored. This can be achieved by RFLP analysis, hybridisation of cloned products, or genome mapping (Lowe et al., 2004). RFLP analysis was attempted, extracting the bands of

interest using gel extraction kits, digesting the bands with restriction enzymes and comparing the resulting banding patterns on polyacrylamide (PAGE) gels. In this case homology testing was attempted, but after sustained failure to get single banded products when extracted bands were used in PCR, time ran out, and homology testing was abandoned. Although homology testing using RFLP analysis failed, each primer was used in three different iterations of the same reaction, as mentioned above, to see if banding patterns were reproduced reliably. Banding patterns that were not reproduced in all three of the replicates were not scored.

3.2.3: RAPD data analysis

Bands were scored manually as either present (1) or absent (0), and the resulting data were inputted into a matrix within Microsoft Excel. Within Excel, GenAlEx 6.1 (Peakall & Smouse, 2006) was used to output a pairwise genetic distance matrix, using the binary genetic distance calculation suitable for dominant markers, following the method of Huff et al. (1993). GenAlEx was then employed to perform a principal coordinates analysis (PCoA) on the genetic distance matrix, based on an algorithm published by Orloci (1978) (Peakall & Smouse, 2006).

An analysis of molecular variance (AMOVA) was also carried out on the *S. engleranus* RAPD data to determine how genetic diversity was partitioned between and within the three populations of *S. engleranus* sampled from Namibia. In GenAlEx 6.1, AMOVA follows the methods of Excoffier et al. (1992), Huff et al. (1993), Peakall et al. (1995), and Michalakis and Excoffier (1996). AMOVA was conducted on the same genetic distance matrix employed in PCoA. AMOVA outputs a table indicating the proportions of total molecular variance attributable to within and between group variation (Peakall & Smouse, 2006).

3.2.4: Plant propagation

Seed of *S. engleranus* sampled from populations in Namibia, seed of *S. flavus* available at the University of St Andrews, and seed generated by experimental crosses between the two species, was sown onto damp filter paper and, following germination, seedlings with a root length of approximately 1cm were transplanted to 3 inch pots containing a 3:1 mix of Levington M2 compost and gravel. Plants were raised at ambient temperature in the glasshouse under 400W mercury vapour lamps, with the photoperiod set at 16 hours. Plants were arranged randomly by numbering each plant and generating a random grid.

3.2.5: Morphometric analysis

A character set consisting of 18 characters for morphometric analysis of *S. flavus*, *S. engleranus* and F1 hybrids was adapted from a character set used previously by Lowe (1996) and Lowe & Abbott (2000) for morphometric analysis of *Senecio* taxa. Nineteen individuals of *Senecio engleranus*, eleven individuals of *S. flavus*, and four F1 hybrids were measured. Details of the taxa included in the analysis are given in Table 3.3. The morphometric character set used is shown in Table 3.4. Definitions of the characters are given below.

Table 3.3: Details of taxa included in morphometric analysis.

Taxon	Code	Source	Locality
S. engleranus	eng 2/4	Namibia pop.2	See Table 3.5
S. engleranus	eng 2/4	Namibia pop.2	See Table 3.5
S. engleranus	eng 2/6	Namibia pop.2	See Table 3.5
S. engleranus	eng 2/8	Namibia pop.2	See Table 3.5
S. engleranus	eng 2/11	Namibia pop.2	See Table 3.5
S. engleranus	eng 2/13	Namibia pop.2	See Table 3.5
S. engleranus	eng 2/14	Namibia pop.2	See Table 3.5
S. engleranus	eng 2/15	Namibia pop.2	See Table 3.5
S. engleranus	eng 2/16	Namibia pop.2	See Table 3.5
S. engleranus	eng 3/5	Namibia pop.3	See Table 3.5
S. engleranus	eng 3/7	Namibia pop.3	See Table 3.5
S. engleranus	eng 3/21	Namibia pop.3	See Table 3.5
S. engleranus	eng 3/25	Namibia pop.3	See Table 3.5
S. engleranus	eng 3/31	Namibia pop.3	See Table 3.5
S. engleranus	eng 3/43	Namibia pop.3	See Table 3.5
S. engleranus	eng 3/44	Namibia pop.3	See Table 3.5
S. engleranus	eng 3/45	Namibia pop.3	See Table 3.5
S. engleranus	eng 3/49	Namibia pop.3	See Table 3.5
S. engleranus	eng 3/60	Namibia pop.3	See Table 3.5
S. flavus	SF3	Seed collections	Morocco
S. flavus	SF4	Seed collections	Morocco
S. flavus	SF7	Seed collections	Morocco
S. flavus	SF12	Seed collections	Morocco
S. flavus	SF13	Seed collections	Morocco
S. flavus	SF15	Seed collections	Morocco
S. flavus	SF19	Seed collections	Morocco
S. flavus	SF20	Seed collections	Morocco
S. flavus	SF22	Seed collections	Morocco
S. flavus	SF26	Seed collections	Morocco
S. flavus	fl26145	Seed collections	Canary Islands
S. flavus	fl14454	Seed collections	Morocco
S. flavus x *S. engleranus*	SF75 x eng 3/29	F1 hybrid	St Andrews
S. flavus x *S. engleranus*	SF75 x eng 3/29	F1 hybrid	St Andrews
S. flavus x *S. engleranus*	SF5 x eng 2/2	F1 hybrid	St Andrews
S. flavus x *S. engleranus*	SF5 x eng 2/2	F1 hybrid	St Andrews

Table 3.4: Characters measured on individuals of *S. engleranus* and *S. flavus* and F1 hybrids created by crossing the two.

C1 Plant Height	**C10** Longest Leaf Length
C2 Inflorescence Length	**C11** Midleaf Length
C3 Peduncle Length	**C12** Number of Midleaf Lobes
C4 Capitulum Length	**C13** Midleaf Apical Angle
C5 Capitulum Width	**C14** Mid-lobe Secondary Vein Angle
C6 Number of Phyllaries	**C15** Standardised Leaf Perimeter
C7 Proportion of black tipped phyllaries	**C16** Standardised Square of Leaf Area
C8 Number of Calyculus Bracts	**C17** Number of Peduncle Bracts
C9 Mean Calyculus Bract Length	**C18** Percentage water in leaf

The Character Set

C1 Plant Height (mm)
Length from the base of the stem, defined as the cotyledon node, to the level of the stigma of the apical capitulum at anthesis.

C2 Inflorescence Length (mm)
Length of the apical stem node, defined as the node subtending the apical capitulum, to the level of the stigma of the apical capitulum at anthesis.

C3 Peduncle Length (mm)
Length of the peduncle from the apical stem node to the point at which the peduncle widens into the receptacle.

C4 Capitulum Length (mm)
Length from the point at which the peduncle widens into the receptacle to the end of the stigma of the central ray floret of the apical capitulum.

C5 Capitulum Width (mm)
Diameter of the apical capitulum, measured at the end of the capitulum.

C6 Number of Phyllaries

C7 Proportion of black tipped phyllaries (%)
Defined as the number of phyllaries with black or brown tips divided by the total number of phyllaries.

C8 Number of Calyculus Bracts
Total number of bracts which are attached to the receptacle above the point at which the peduncle widens.

C9 Mean Calyculus Bract Length (mm)
Defined as the sum of the length of the calyculus bracts divided by the total number of calyculus bracts.

C10 Longest Leaf Length (mm)
Length of the longest leaf measured parallel to the primary vein.

C11 Midleaf Length (mm)
Maximum length of the midleaf, defined as the leaf attached to the stem nearest to the midpoint of plant height (C1). Measured parallel to the primary vein.

C12 Number of Midleaf Lobes
The number of secondary veins which supply defined lobes plus the apical lobe. The apical lobe is defined as originating at the point at which the secondary veins are of equal thickness to the primary vein.

C13 Midleaf Apical Angle
Defined as the angle between the apex of the primary vein and the apices of the adjacent marginal tooth sinuses.

C14 Mid-lobe Secondary Vein Angle
Defined as the angle between the secondary vein of the lobe closest to the midpoint of the midleaf and the primary vein.

C15 Standardised Leaf Perimeter
Defined as the perimeter of the midleaf divided by the midleaf length (C11).

C16 Standardised Square of Leaf Area
Defined as the square root of the area of the midleaf, divided by the midleaf length (C11).

C17 Number of Peduncle Bracts
Total number of bracts which are attached to the peduncle, below the point at which the peduncle widens into the receptacle.

C18 Percentage of water in midleaf (%)
Defined as the weight of the fresh midleaf minus the weight of the dried midleaf, divided by the weight of the fresh midleaf, x 100.

Character definitions C1 – C17 were taken from Lowe (1996). C18 was measured by weighing midleaves, drying them overnight in an oven and then reweighing.

3.2.6: Character comparison (one-way ANOVA and multiple range tests)

After transformation of the data (non-normally distributed measurements were $\log^e$ transformed, percentages and proportions were arcsine transformed, and perimeter and square of area measures were divided by midleaf length to standardise),

one-way analysis of variance (ANOVA) was performed to detect significant differences between mean values of each character in turn between both species and the F1s. Multiple range tests were then conducted to detect which means differed significantly from each other.

3.2.7: Principal components analysis (PCA) of morphometric data

Within NTSYSpc V.2.0 (Rohlf, 1997), a matrix was constructed for 15 of the 18 characters in the dataset detailed above. Excluded characters were C7 (proportion of black-tipped phyllaries), C8 (number of calyculus bracts) and C17 (number of peduncle bracts). Data were standardised, and a matrix of correlations among variables was computed. Three eigenvectors were extracted from the correlation matrix, and eigenvector scores for each individual were projected onto these eigenvectors. The objects were then visualised as a three-dimensional plot, each axis representing an eigenvector (PCA plot).

3.2.8: Creation of artificial hybrids

Hybrids were created between individuals of *S. flavus* and *S. engleranus* in the glasshouse at the University of St Andrews, using the emasculation techniques of Ornduff (1964). The terminal 2-3 mm of several unopened capitula of *S. flavus* individuals were sliced off with a razor blade prior to anthesis, removing the anthers but leaving the stigmas intact. The capitula were covered with a bag made of lens tissue and left to mature for 2-3 days, after which developing stigmas were checked for the presence of pollen. If no pollen was detected, pollen collected from individuals of *S. engleranus* was carefully brushed onto the stigmas of emasculated *S. flavus* capitula. These capitula were then re-bagged, to stop any extraneous pollen from

reaching the stigmas. The fruit was collected when mature, and sown out in the glasshouse at the University of St Andrews.

3.2.9: Pollen counts and pollen fertility estimates

Pollen fertility of plants was estimated by employing aceto-carmine to stain viable pollen grains. Slides for pollen counts and fertility estimates were made by slicing florets open, placing them on slides, adding a drop of aceto-carmine stain, and then pressing down gently on them with cover slips. Aceto-carmine stain allows distinction between viable and inviable pollen grains. Viable pollen grains stain pink to red, while inviable grains remain unstained (McClintock, 1929).

Counts were made using a light microscope. Using between two and five florets per capitulum, and between one and two capitula per individual (depending on the amount of available material), total pollen counts were made for a single *S. engleranus* individual (eng 3/29), a single *S. flavus* individual (SF751), the self-fertile F1 hybrid [SF751 x eng3/29 (1)] and thirty-two F2 hybrids, offspring produced after self-pollination of the F1 hybrid SF751 x eng3/29(1). The data collected are presented in Appendix 2. Pollen fertilities were noted at the same time that pollen counts were recorded.

Mean pollen numbers and mean percentage pollen fertilities were calculated for parental species, the F1 hybrid and F2 offspring.

Figures of pollen counts and pollen fertility in the F2 generation were constructed in Microsoft Excel.

3.2.10: Genetic control of pappus type

Parental specimens representing the two species, the single self-fertile F1 hybrid, and eighty-seven F2 hybrids, were checked for presence/absence of connate fluked pappus using a light microscope.

3.3: RESULTS

3.3.1: Fieldwork

Seed, silica dried leaf material and herbarium specimens were successfully collected from four coastal populations of *S. engleranus* located near Swakopmund, in the west of Namibia (Fig. 3.5). As no Namibian populations of *S. flavus* were found during fieldwork, *S. flavus* individuals grown in the glasshouse at the University of St Andrews from previously collected seed were used in the comparative studies presented here. Details of Namibian collections and of *S. flavus* accessions used in the comparative studies are presented in Table 3.5.

Table 3.5: Collections of *Senecio engleranus* made in Namibia (April, 2005) and *Senecio flavus* accessions used in analyses.

Taxon	Collector Number	Locality Information	Seed Collected Y /N
***S. engleranus* population 1**	JJM109.1	Namibia, Swakopmund, east bank of dry Swakop River bed, about 2 hrs. walk from river mouth. 22° 40' 491" S, 14° 33' 103"E; alt: 27m	**N**
***S. engleranus* population 2**	JJM110.1	Namibia, Swakopmund, road between Walvis Bay and Rooikop Airport, next to quarry on the D road off the C14 road. 22° 40' 491"S, 14° 33' 103"E; alt: 60m	**Y**
***S. engleranus* population 3**	JJM111.1	Namibia, Swakopmund, lichen desert between Swakopmund and Henties Bay, about 40 km from Swakopmund. 22° 21' 565"S, 14° 26' 191"E; alt: 24m	**Y**
***S. engleranus* population 4**	JJM113.1	Namibia, Swakopmund, Henties Bay, in dried up mouth of Omaruru River. 22° 07' 00"S, 14° 17' 00"E; alt: 3m	**Y**
S. flavus	SF3	Morocco, Tafraoute	N/A
S. flavus	SF4	Morocco, Tafraoute	N/A
S. flavus	SF5	Morocco, Tafraoute	N/A
S. flavus	SF7	Morocco, Tafraoute	N/A
S. flavus	SF12	Morocco, Tafraoute	N/A
S. flavus	SF13	Morocco, Tafraoute	N/A
S. flavus	SF15	Morocco, Tafraoute	N/A
S. flavus	SF16	Morocco, Tafraoute	N/A
S. flavus	SF19	Morocco, Tafraoute	N/A
S. flavus	SF20	Morocco, Tafraoute	N/A
S. flavus	SF22	Morocco, Tafraoute	N/A
S. flavus	SF26	Morocco, Tafraoute	N/A
S. flavus	SF751	Morocco, Tafraoute	N/A
S. flavus	fl14388	Spain, Canary Islands	N/A
S. flavus	fl14454	Morocco, Tata	N/A
S. flavus	fl 26145	Spain, Canary Islands	N/A
S. flavus	fl – Sinai	Egypt, Sinai, Dahab	N/A

3.3.2: RAPD Variation

A total of 48 polymorphic RAPD bands were amplified using 12 primers. The number of RAPD fragments scored per primer ranged from two (C08) to seven (B17). Ten bands were specific to *S. engleranus* and 15 specific to *S. flavus*, with 23 bands recorded in both species. All 33 bands recorded in *S. engleranus* were present in the F1 hybrids, while 34 out of 38 bands recorded in *S. flavus* were also present in the hybrids. The four *S. flavus* bands not found among the F1 hybrids were private to *S. flavus*. Identical RAPD phenotypes shared between two individuals were recorded in four cases within species, and in one case between two hybrid offspring of the same parents. The pairings were: eng 3/25 and eng 4/3, eng 2/2 and eng 3/29, SF 5 and SF751, SF16 and flSinai, and two F1 hybrids produced from the cross SF751 x eng 3/29. The mean percentage of polymorphic loci over all the samples was 31.25% ± (SE=13.66), while the percentage of polymorphic loci was 47.92% in *S. engleranus*, 41.67% in *S. flavus* and 4.17% among the F1 hybrids. RAPD variation is summarised in Table 3.6.

Table 3.6: Summary of RAPD variation in *S. engleranus*, *S. flavus* and F1 hybrids

Taxon	No. of bands	No. of private bands	Mean number of alleles per locus	Mean no. of effective alleles per locus	Expected hetero-zygosity	Expected hetero-zygosity (unbiased)	Percentage of polymorphic loci	Shannon's Index of Diversity
S. engleranus	33	0	1.167 ± 0.127	1.358 ± 0.060	0.198 ± 0.032	0.203 ± 0.033	47.92	0.286 ± 0.045
S. flavus	38	4	1.208 ± 0.111	1.325 ± 0.059	0.180 ± 0.032	0.188 ± 0.033	41.67	0.258 ± 0.045
F1 hybrids	44	0	0.958 ± 0.051	1.036 ± 0.025	0.019 ± 0.013	0.022 ± 0.015	4.17	0.027 ± 0.019
Total	-	4	1.111 ± 0.059	1.240 ± 0.031	0.132 ± 0.017	0.138 ± 0.018	31.25 ± 13.66	0.191 ± 0.024

The complete matrix of RAPD data is presented in Appendix 3.

3.3.3: Principal Coordinates Analysis (PCoA) of RAPD data

The first three principal coordinates accounted for 59.35%, 24.34% and 6.50% of the total variance, respectively. Plots of the scores for each individual on principal coordinate 1 against those on principal coordinate 2 revealed two discrete clusters corresponding to *S. engleranus* and *S. flavus*, as well as a third cluster positioned between the two species clusters, corresponding to the F1 hybrids. (Fig. 3.6). These results indicate that the two species are distinct genetic entities.

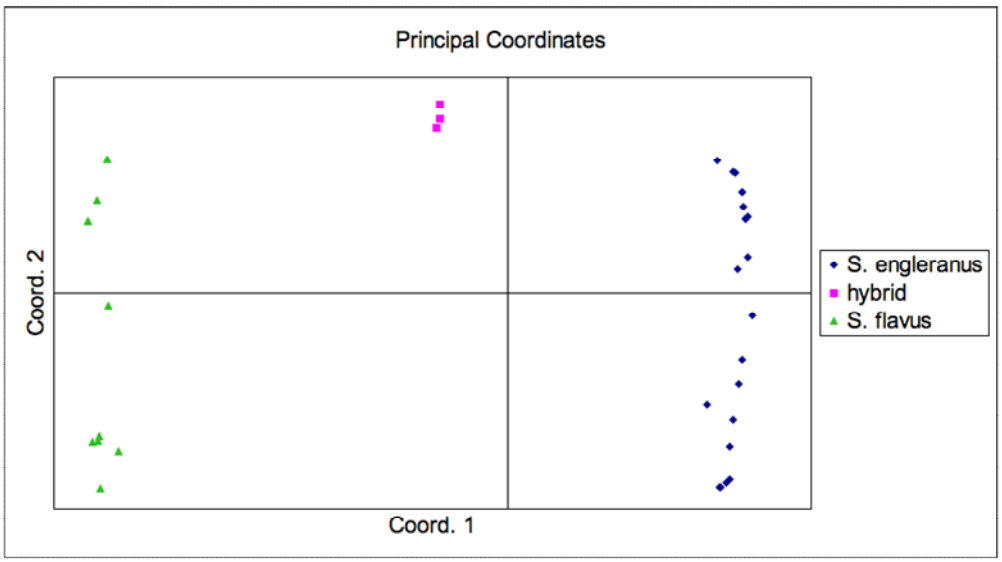


Figure 3.6: Results of principal coordinates analysis (PCoA) of RAPD data for *S. engleranus*, *S. flavus* and three F1 hybrids created by crossing the two species.

3.3.4: Analysis of Molecular Variance (AMOVA) of RAPD data for *S. engleranus*

The AMOVA conducted on RAPD data for *S. engleranus* revealed that 98% of the total estimated variance was due to differences within populations while only 2% was caused by differences between populations (Table 3.7). Such partitioning of genetic variance might be expected for a set of geographically close populations of an obligate outcrossing species (Huff et al., 1993), and would reflect a high level of gene flow between populations. The analysis shows that variance among populations is not significant ($\Phi_{PT} = 0.022$, $P = 0.30$).

Table 3.7: AMOVA table

Source of variation	df	Sum of squares	Mean square	Estimated Variance	% of variance	Stat	Value	p
Among Pops	2	21.150	10.575	0.209	2%	ΦPT	0.022	0.300
Within Pops	17	157.700	9.276	9.276	98%			
Total	19	178.850	19.851	9.486				

The data for *S. flavus* were not subjected to AMOVA, as all but one population examined consisted of a single individual.

3.3.5: Morphometric results

A total of 18 morphometric characters (Table 3.4) were scored for 35 individual plants (Table 3.3). Means, standard errors, and the results of statistical tests performed on the individual characters are summarised in table 3.8. The data matrix constructed from the collected morphometric data is presented in appendix 4.

3.3.6: Morphometric variation

Differences in character means between *S. engleranus, S. flavus* and F1 hybrids

One-way ANOVA revealed significant differences in means between *S. engleranus*, *S. flavus* and their F1s for 13 of the 18 characters recorded (Table 3.8). Multiple range tests showed that there were significant differences between the means of *S. engleranus* and *S. flavus* for all of these characters except C15 (standardised leaf perimeter) and C17 (number of peduncle bracts). Thus relative to *S. flavus*, *S. engleranus* was shorter in height, had wider capitula with a greater number of shorter calyculus bracts, and produced smaller, more succulent leaves with fewer midleaf lobes. For most of these characters, the means of F1 plants were significantly different from those of each parent species (Table 3.8). Interestingly, for four characters

(height, capitulum width, calyculus bract length, and number of peduncle bracts), the means of F1s were significantly greater than either parent species.

Table 3.8: Mean (± S.E.) for eighteen morphological characters measured on *S. engleranus* and *S. flavus* and F1 hybrids. Shared letters in superscript imply non-significant ($P > 0.05$) difference in character mean (multiple range test).

Character and units	*S. engleranus* mean value & S.E.	*S. flavus* Mean value & S.E.	F1 hybrids Mean & S.E.	*P* value
C1 Plant height (mm)*	166.79 ± 7.28^{a}	231.25 ± 13.46^{b}	327.5 ± 24.02^{c}	< 0.05
C2 Inflorescence Length (mm)*	19.11 ± 1.18^{ab}	15.92 ± 0.84^{a}	19.50 ± 1.66^{b}	0.156
C3 Peduncle Length (mm)*	10.16 ± 1.00^{ab}	8.00 ± 0.84^{a}	11.50 ± 1.66^{b}	0.178
C4 Capitulum Length (mm)*	8.95 ± 0.39^{a}	8.00 ± 0.43^{a}	8.00 ± 0.00^{a}	0.286
C5 Capitulum Width (mm)*	4.45 ± 0.14^{a}	2.78 ± 0.09^{b}	5.50 ± 0.29^{c}	< 0.05
C6 Number of Phyllaries	11.53 ± 0.41^{a}	11.92 ± 0.23^{a}	13.00 ± 0.00^{b}	0.175
C7 Proportion of black tipped phyllaries**	0.96 ± 0.02^{a}	1.00 ± 0.00^{a}	1.00 ± 0.00^{a}	0.413
C8 Number of Calyculus Bracts	6.21 ± 0.28^{a}	4.92 ± 0.15^{b}	7.25 ± 0.48^{c}	< 0.05
C9 Mean Calyculus Bract Length (mm)*	0.77 ± 0.12^{a}	0.97 ± 0.07^{b}	1.18 ± 0.06^{b}	< 0.05
C10 Longest Leaf Length (mm)*	22.58 ± 1.13^{a}	40.67 ± 1.22^{b}	27.75 ± 1.65^{c}	< 0.05
C11 Midleaf Length (mm)*	14.11 ± 1.21^{a}	30.67 ± 2.05^{b}	21.25 ± 1.80^{c}	< 0.05
C12 Number of Midleaf Lobes	9.44 ± 0.54^{a}	17.44 ± 1.29^{b}	15.75 ± 0.50^{b}	< 0.05
C13 Midleaf Apical Angle (°)	46.36 ± 1.76^{a}	57.64 ± 3.15^{b}	57.50 ± 3.23^{b}	< 0.05
C14 Mid-lobe Secondary Vein Angle (°)	39.13 ± 2.99^{a}	50.82 ± 3.43^{b}	46.25 ± 3.75^{ab}	< 0.05
C15 Standardised Leaf Perimeter***	5.81 ± 0.56^{a}	14.86 ± 0.86^{a}	5.50 ± 0.50^{b}	< 0.05
C16 Standardised Square of Leaf Area***	1.56 ± 0.20^{a}	7.00 ± 1.07^{b}	1.50 ± 0.23^{c}	< 0.05
C17 Number of Peduncle Bracts	1.42 ± 0.14^{a}	1.73 ± 0.24^{a}	3.00 ± 0.00^{b}	< 0.05
C18 Percentage water in midleaf (%)**	91.42 ± 0.78^{a}	79.45 ± 3.76^{b}	87.48 ± 1.60^{c}	< 0.05

* = $\log^{e}$ transformed, **=arcsine transformed, ***=divided by midleaf length

3.3.7: Principal Component Analysis (PCA) of Morphometric Data

The first, second and third principal components accounted for 37.38%, 16.1% and 10.1% of the total variation respectively. Eigen vector loadings for the first three principal components for each morphological character are in Table 3.9, providing a measure of the contribution of each character to each component.

Two fairly distinct clusters are evident in the three-dimensional PCA plot (Fig 3.7), representing the two different species, while F1s are positioned between these two clusters.

Table 3.9: Eigen vector loadings for the first three principal components from PCA of specimens of *Senecio engleranus*, *Senecio flavus* and four F1 hybrids *S. flavus* x *S. engleranus.*

Character / Principal Component	**1**	**2**	**3**
C1 Plant Height	0.447	0.488	0.339
C2 Inflorescence Length	-0.452	0.690	-0.534
C3 Peduncle Length	-0.329	0.780	-0.369
C4 Capitulum Length	-0.490	0.000266	-0.567
C5 Capitulum Width	-0.696	0.219	0.156
C6 Number of Phyllaries	0.134	0.422	0.152
C9 Mean Calyculus Bract Length	0.291	0.575	0.0724
C10 Longest Leaf Length	0.853	0.0000862	0.0192
C11 Midleaf Length	0.795	0.183	-0.0212
C12 Number of Midleaf Lobes	0.852	0.0999	0.00246
C13 Midleaf Apical Angle	0.458	0.516	0.172
C14 Mid-lobe Secondary Vein Angle	0.403	0.378	0.292
C15 Standardised Leaf Perimeter	0.648	-0.0146	-0.420
C16 Standardised Square of Leaf Area	0.898	-0.266	-0.377
C18 Percentage water in leaf	-0.763	0.121	0.410

Contribution of characters to principal components

The variables with the highest component loadings on PC1 are, in descending order, variables C16 (standardised square of leaf area), C10 (longest leaf length), C12 (number of midleaf lobes), C11 (midleaf length) and C18 (percentage water in leaf). The highest component loadings on PC2 are, in descending order, C3 (peduncle

length), C2 (inflorescence length) and C9 (mean calyculus bract length), while the highest component loadings on PC3 are, in descending order, C4 (capitulum length), C2 (inflorescence length) and C15 (standardised leaf perimeter).

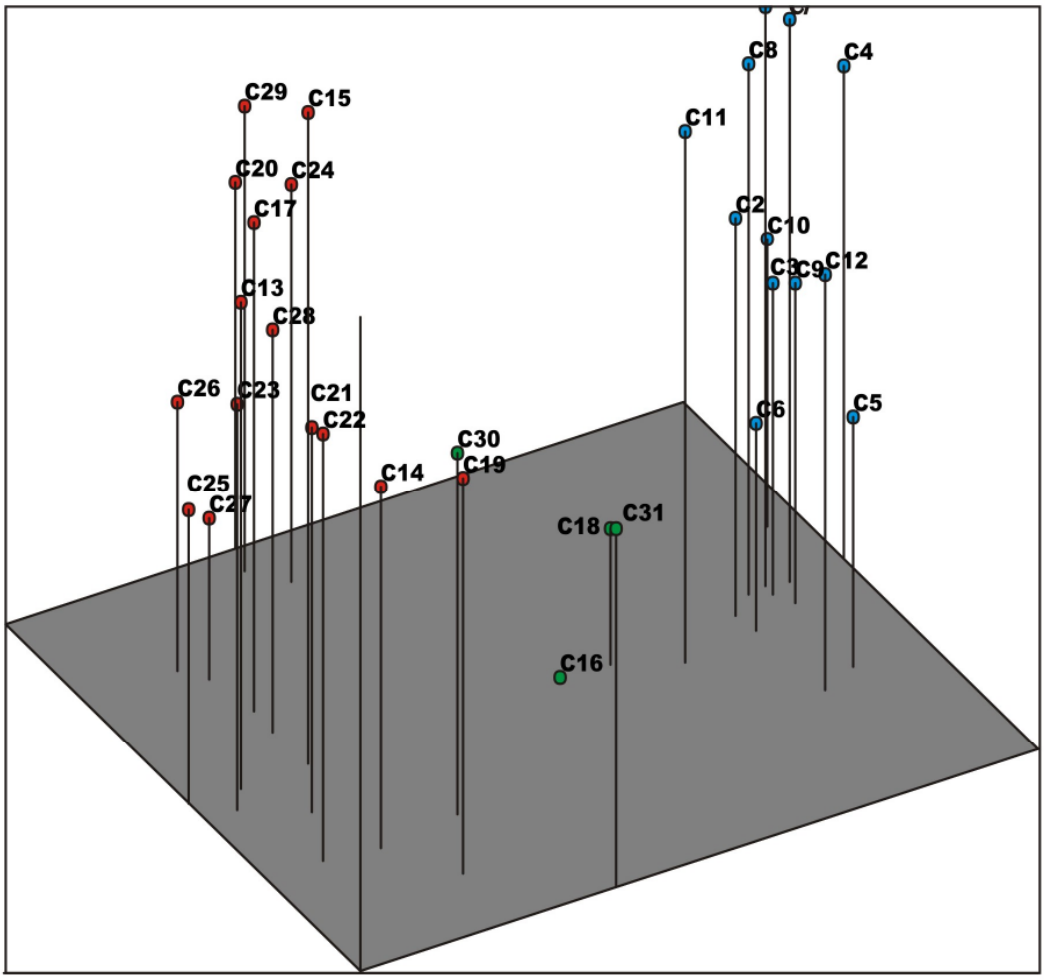


Figure 3.7: Principal components analysis **(**PCA) plot of eigenvector scores for *S. engleranus* (red dots), *S. flavus* (blue dots) and F1 hybrids (green dots). The three axes represent the first three eigenvectors extracted from the standardised data. The cluster of points on the far-left represent *S. engleranus* individuals, while those on the far-right represent *S. flavus* individuals. The points clustered in the middle are representative of the F1 hybrids.

3.3.8: Pollen Count and Pollen Fertility Results

Table 3.10 summarises the mean pollen count and mean pollen fertility data for the parent species, *S. engleranus* and *S. flavus,* the self-fertile F1 hybrid and F2 generation. A full list of taxa for which pollen counts and pollen fertility estimates were made, together with the data, is given in Appendix 2.

Table 3.10: Mean pollen counts (± S.E.) and mean pollen fertilities (± S.E.) of *S. engleranus, S. flavus,* F1 hybrids and F2 offspring.

Taxon	No. of individuals (pollen count)	No. of florets	Mean pollen count (± S.E.) (grains/floret)	No. of individuals (pollen fertility)	No. of florets	Mean pollen fertility (% ± S.E)
***S. engleranus eng* 3/29** (parent)	1	5	3394.40 ± 153.15	1	10	99.00 (±0.23)
***S. flavus* SF751** (parent)	1	10	495.20 ± 46.95	1	10	89.68 (± 4.36)
F1 hybrid SF751 x *eng* 3/29(1) (parent of F2)	1	5	409.00 ± 31.52	1	5	59.30 (± 2.00)
F2 hybrids	32	110	1825.35 ± 48.14	32	110	85.24 (± 1.20)

Pollen counts and fertility estimates for *S. engleranus, S. flavus,* the F1 hybrid generation created between the two, and the F2 generation

Senecio engleranus produced more than six times the amount of pollen per floret than *S. flavus* based on a comparison of the two parent individuals examined (Table 3.10). However, there was no difference between these two individuals in pollen fertility, which was very high in both, but particularly in the *S. engleranus* individual.

The mean pollen count of the self-fertile F1 plant produced from these two parent plants was approximately the same as the *S. flavus* parent (409 ± 31.52 grains/floret),

while pollen fertility was significantly reduced (59.3 ± 2.00). The mean pollen count of the F2 generation was intermediate to that of the parents (1825.35 ± 48.14 grains/floret), and pollen fertility was greater than that recorded for the F1.

3.3.9: Distribution of pollen count and pollen fertility data in the F2 generation

Mean pollen counts and fertilities for 32 individuals of the F2 generation were calculated, based on counts of between two and four florets per individual. A histogram of pollen counts is shown in Fig. 3.8 and of pollen fertility in Fig. 3.9.

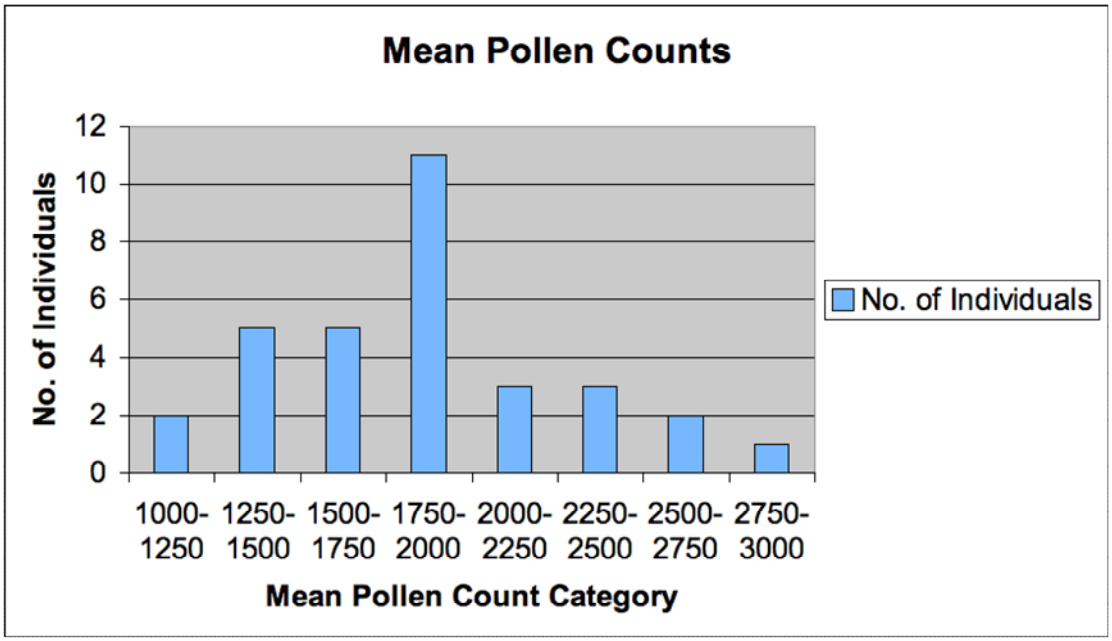


Figure 3.8: Distribution of mean pollen counts for plants of the F2 generation

Fig. 3.8 shows that 11 individuals had mean pollen counts between 1750 and 2000 grains/floret, an intermediate value between the mean pollen count values of the two parents (*S. engleranus*: 3394.40 ± 153.15 grains/floret; and *S. flavus*: 495.20 ± 46.95). This pollen count class contains more individuals than any other, and the distribution of the data is unimodal and appears normal. Thus, the number of individuals in each class decreases as values reach the extremes of high or low pollen

numbers recorded. Rather surprisingly, none of the F2 individuals have mean pollen counts as low as either the *S. flavus* parent or the F1 generation, with the lowest values falling in the 1000-1250 grains/floret class.

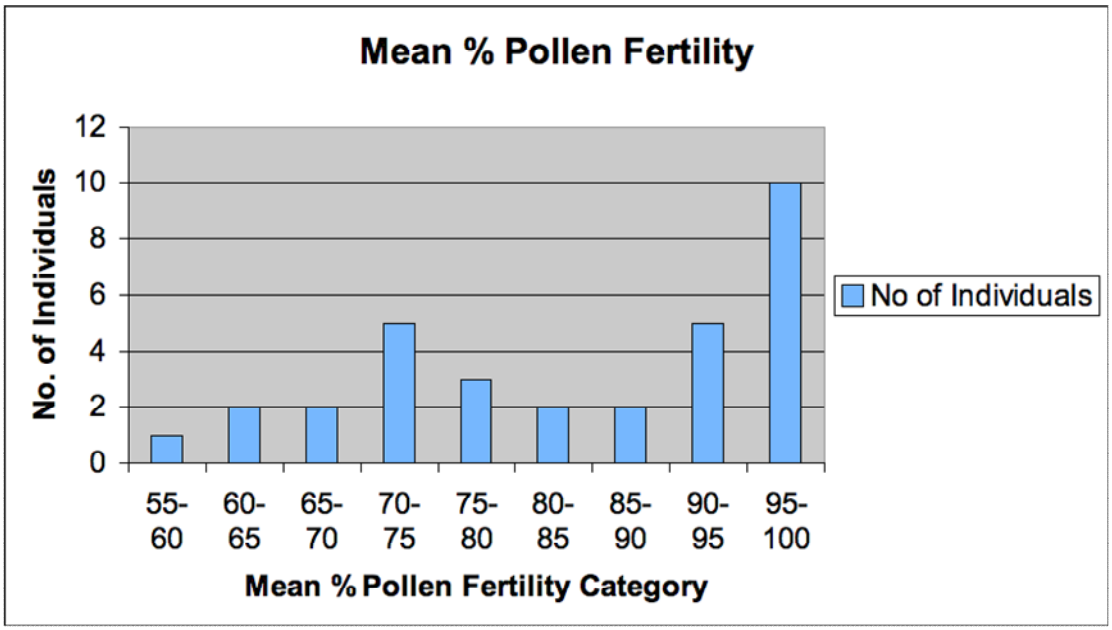


Figure 3.9: Distribution of mean percentage pollen fertility in the F2 generation

Fig. 3.9 indicates a bimodal distribution for pollen fertility in the F2 generation with 5 individuals having a mean fertility of 70-75% and 10 individuals having a 95-100% fertility. Other categories contain fewer individuals. Only one individual had a fertility as low as that of the F1 (55-60%).

3.3.10: Genetics of pappus type variation

The *S. flavus* individual (SF751) used as the parent of the F1 and F2 generations produced cypsela with connate fluked pappus, while the *S. engleranus* parent (eng 3/29) did not. The F1 hybrid resulting from crossing these two individuals also produced cypselas with a connate fluked pappus. Of eighty-four individuals from the F2 hybrid generation, 68 were seen to have a connate fluked pappus, while the

remaining 19 produced only a normal pappus. This represents a ratio of 3.58:1 - a Chi-square test returned $\chi^2 = 0.571$ ($p = 0.450$), showing that the observed ratio does not deviate significantly from the 3:1 ratio expected for a trait controlled by a single major gene.

3.4: DISCUSSION

3.4.1: Phylogenetic position of the *S. engleranus* / *S. flavus* clade

Coleman et al. (2003) produced a phylogeny of *Senecio* sect. *Senecio* based on *ITS* sequence variation and, from their tree, suggested that a clade composed of *S. engleranus* and *S. flavus* may be basal to sect. *Senecio.* However, the phylogenetic position of this clade would appear to be an artefact caused by limited taxon sampling (Heath et al., 2008). Coleman et al. (2003) included only a very limited number of taxa from *Senecio* in their analysis, whereas the more densely sampled *ITS* phylogeny presented in Chapter 2 of this thesis indicates that the clade comprising *S. engleranus* and *S. flavus* is only distantly related to sect. *Senecio,* suggesting instead that the species pair may be closely related to *Senecio* s.str. This conclusion, that *S. engleranus* and *S. flavus* are not basal to sect. *Senecio,* is also supported by the phylogenetic results obtained by Pelser et al. (2007).

3.4.2: Differences between *S. engleranus* and *S. flavus*

Although a lack of resolution, and some unexpected affinities of individual accessions, are evident within the *S. engleranus / S. flavus* clade in phylogenetic analyses of *ITS* DNA sequences (Fig. 3.4), the results presented in this chapter show that the species are clearly separated both genetically, as demonstrated by the analysis

of RAPD variation, and morphologically, as demonstrated by analysis of morphometric characters.

RAPD variation between *S. engleranus* and *S. flavus*

Of 48 RAPD bands scored, 10 were specific to *S. engleranus* and 15 were specific to *S. flavus.* The remaining 23 bands were present in both species. Although there were five cases of two individuals sharing identical RAPD profiles, no phenotypes were shared between the two species. *Senecio engleranus* contained more diversity than *S. flavus* as reflected by measures of unbiased expected heterozygosity (0.203 ± 0.033 in *S. engleranus* vs. 0.188 ± 0.033 in *S. flavus*), percentage of polymorphic loci (47.92% vs. 41.67%) and Shannon's diversity index (0.286 ± 0.045 vs. 0.258 ± 0.045). Principal Coordinate Analysis showed a clear separation between the two species when scores for the first two principal coordinates were plotted against one another. The conclusion to be drawn from this evidence is that these two species have diverged in sequence at many loci across their genomes.

A direct comparison of amounts and proportions of RAPD variation contained within populations of each species was not possible, as this was only examined in *S. engleranus*. In this species, a very high proportion of total variance was attributed to that within populations, as is often found for species that reproduce by outcrossing (Huff et al., 1993).

Morphological Differences Between *S. engleranus* and *S. flavus*

Analysis of individual morphometric characters showed that *Senecio engleranus* and *S. flavus* differ greatly in morphology. For plants raised under glass at St Andrews, *S. engleranus* was shown to be shorter in height, with wider capitula and

a greater number of shorter calyculus bracts. *Senecio engleranus* also produced smaller, more succulent leaves with fewer midleaf lobes. However, the two species were similar in the number of peduncle bracts and their length, inflorescence and capitulum length, number of phyllaries, and proportion of black tipped phyllaries. The wider capitula of *S. engleranus* might help to attract pollinators, which may promote outcrossing. Moreover, the species shows greater leaf succulence which may be related to habitat preference, as succulent leaves help to conserve water in arid desert environments, such as those on the west coast of Namibia. Here the main source of available water is mist carried inland by the Benguela current, the eastern boundary current of the South Atlantic subtropical gyre (Peterson and Stramma 1991, Wedepohl et al. 2000). Areas visited in Namibia while looking for *S. flavus*, in contrast, were at higher altitude, more sheltered and less arid, with higher levels of plant cover. Two localities, one of *S. engleranus* and one of *S. flavus* are shown in Fig. 3.10, which illustrates these habitat differences. Although *S. flavus* was not found at this particular locality at the time of visiting, from collections of *S. flavus* made in North Africa it appears that the species is normally found in more mesic conditions than those that characterise the habitats of *S. engleranus* in Namibia (Abbott personal communication).

Figure 3.10: Habitat of *S. engleranus* (population 4) near Swakopmund (left) and an *S. flavus* habitat near Keetmanshoop (right).

Another interesting morphological difference between the two sister species is the morphology of the pappus (Coleman et al., 2003). The pappus is an appendage of hairs on the cypsela that aids dispersal of fruits by wind. In *S. engleranus*, the pappus is simple, consisting of hair-like structures which detach easily from the fruit, whereas *S. flavus* has a connate-fluked pappus in addition to the normal pappus. Coleman et al. (2003) suggested that the connate fluked pappus might enable ectozoochory, and that this could account for differences in the geographical distribution between the two species (Fig. 3.3).

Taken overall, the two species differ for numerous morphological traits, some of which may relate to differences in breeding system (selfing in *S. flavus* vs. outcrossing in *S. engleranus*), habitat (more arid in *S. engleranus*), and fruit dispersal (ectozoochory in addition to anemochory in *S. engleranus*).

3.4.3: Differences in pollen number

Outcrossing species normally produce much more pollen per ovule than do self-fertilising species (Cruden 1977). In the comparison made here, *S. engleranus* produced more than six times the amount of pollen seen in *S. flavus. Senecio engleranus* always failed to set seed when left to self, whereas *S. flavus* produced seed readily on selfing. Thus, together with the finding that *S. engleranus* has wider capitula, making it possibly more attractive to pollinators, these results suggest stongly that whereas *S. flavus* is predominantly a self-pollinator, *S. engleranus* is most likely an obligately outcrossing species.

3.4.4: Habit

During fieldwork in Namibia, several *S. engleranus* plants were observed which clearly had more than a single year's growth, although the species is reported as being annual (Merxmüller, 1976). Some individuals were large, with dead, brown areas, while other areas on the same individual were green and healthy (Fig. 3.11). *S. flavus*, in contrast, appears to be annual. Although *S. flavus* plants for this study were grown in the glasshouse and wild material was not observed personally, naturally occurring material does not exhibit this sign of bienniality/perenniality, and the plants remain small (Abbott, personal communication). Observed herbarium specimens did not show more than a single year's growth, suggesting the two species also differ in habit.

Figure 3.11: *S. engleranus* individual from population 4 showing more than a single year's growth (details of this population are in Table 3.5).

To summarise: there are considerable genetic and morphological differences between *S. engleranus* and *S. flavus* as shown by the surveys of RAPD and morphometric variation reported here. In addition, the two species appear to differ in habitat preference, breeding system, habit, and mechanism of fruit dispersal. The

evidence suggests that *S. engleranus* and *S. flavus* should retain their separate species status, despite a lack of clear separation between them in phylogenetic analyses of nuclear *ITS* and plastid *trn*L-F sequences. Although they appear to be clear-cut and distinct taxonomic species, the two were found to be interfertile, as shown by the creation of artificial F1 hybrids, one of which produced vigorous F2 offspring through self-pollination. Despite the observed interfertility of *S. engleranus* and *S. flavus*, which suggests that intrinsic postzygotic barriers to hybridisation between them are weak, there are no records of naturally occurring hybrids in the wild, suggesting that interbreeding in Namibia is prevented by prezygotic barriers.

3.4.5: Why are there no hybrids in the wild?

Hybridisation between *S. engleranus* and *S. flavus* appears to be prevented in areas where they occur sympatrically (northern Namibia) by habitat isolation (the two species appear to be adapted to different habitats), temporal isolation (phenological differences meant flowering *S. flavus* plants were not found during fieldwork in Namibia, while *S. engleranus* was in flower) and, to some extent, geographical isolation (all *S. engleranus* localities were in the north of the country, whereas, with one exception, all known localities of *S. flavus* were in the south; Fig. 3.5). A difference in breeding systems is also likely to reduce the probability of interspecific hybridisation. *Senecio engleranus* appears to be an obligate outcrosser, whereas *S. flavus* reproduces by self-fertilisation. Because *S. flavus* produces much smaller amounts of pollen relative to *S. engleranus*, pollen of *S. flavus* is likely to comprise only a small proportion of the pollen pool sampled by *S. engleranus* plants during outcrossing. The probability of hybridization would therefore be reduced, relative to a situation where both species contribute equivalent amounts of pollen to the

outcrossing pollen pool, assuming all other things are equal. Despite the high proportion of *S. engleranus* pollen in the outcross pollen pool, it is also expected that *S. flavus* will produce few hybrids as a mother plant, mainly because it is likely to self-fertilise shortly after anthesis, before *S. engleranus* pollen lands on its stigmas. The discoid capitulum of *S. flavus* is also small and therefore unlikely to attract insects that might preferentially visit the larger capitula of *S. engleranus.* Even if hybrids form, they are likely to be ill-adapted to either of the parent species' habitats and therefore will be quickly lost, perhaps before reproducing.

Thus, the combination of prezygotic barriers to interbreeding between the two species would seem to make hybridisation in the wild very unlikely. However, artificial hybrids were created in the greenhouse at the University of St Andrews producing an F1 generation which was at least partly self-fertile, suggesting that there are only weak intrinsic postzygotic barriers to hybridisation between the two species. The combination of prezygotic barriers may be so effective in preventing interbreeding that there has been no reason for postzygotic barriers to evolve (Cozzolino & Scopece, 2008)

3.4.6: Genetics of differences between species in pappus type, pollen number and pollen fertility

The ability to raise an F2 generation from one of the F1 hybrids produced between *S. engleranus* and *S. flavus* provided the opportunity to determine whether some trait differences between the species were under simple major gene control. In this way, a preliminary analysis was conducted on the genetics of differences in pappus type, mean pollen number per individual, and mean pollen fertility per individual.

In regard to pappus type, all F1 plants examined produced cypsela with connate fluked pappus. In the F2, the ratio of offspring with connate fluked pappus versus those which lacked it was not significantly different from a 3:1 ratio. It is therefore concluded that this very important character difference between the two species, which may account in part for their very different geographical distributions, is caused by an allelic difference in a single major gene.

In contrast, the segregation of pollen number and fertility in the F2 appeared to be more complex, and a larger family size would have to be examined before a meaningful analysis of the minimum number of genes controlling the differences between species for these particular traits could be made. That said, the bimodality shown in the distribution of pollen fertility in the F2 indicates that the genetic control of this character may not be too complex, and might involve some major genes. Clearly, it would be extremely valuable to conduct a detailed analysis of the genetics of all the traits that distinguish this pair of sister species of *Senecio*. Coleman et al (2003) estimated that the two species diverged from their most recent common ancestor within the last 15,000 years, so the evolution of genetic differences between them has occurred over a relatively short period of time. Whether allelic substitutions at a few major genes of large effect have been mainly responsible for the genetic divergence that has occurred, rather than substitutions at many loci of individual minor effect, remains to be established.

CHAPTER 4:

GENERAL CONCLUSION

4.1: Chapter 2

Molecular phylogenetic analysis of *ITS* rDNA showed that subtribe Senecioninae is monophyletic and that members of *Senecio* s.str. form a strongly supported monophyletic group, which corresponds with the monophyletic group representing the genus seen in the study of tribe Senecioneae by Pelser et al. (2007). The analysis in Chapter 2 also showed that the New World genus *Robinsonia* is part of *Senecio* s.str., and species of this genus should be renamed accordingly. In contrast, a number of southern African species with the generic name *Senecio* are found outside this clade: *S. articulatus, S. angulatus, S. oxyodontus, S. seminiveus, S. achilleifolius, S. tamoides, S. speciosus, S. deltoideus, S. latifolius, S. coronatus, S. repandus.* These should probably be reassigned to other genera, reflecting the para- or polyphyletic nature of *Senecio* as it is currently recognised. The study has helped to establish more firmly the species composition of *Senecio* s.str., a taxonomic problem as old as de Candolle.

In seeking to identify which South African *Senecio* species might belong in section *Senecio,* the choice of Harvey's section *Annui* as a study group proved to be appropriate, as ten of eleven members of the section collected in South Africa were shown to have close affinities with established members of section *Senecio.* This is despite the fact that the composition of Harvey's section *Annui* is based solely on the annual habit. Only a single included member of the section - *S. repandus* - was shown to be distantly related to them. *ITS* analysis suggested that several South African species, assigned to various sections by Harvey (1865), could be tentatively placed in section *Senecio*: *S. abruptus, S. arenarius, S. cakilefolius, S. elegans, S. erysimoides,*

S. glutinarius, S. glutinosus, S. littoreus, S. maritimus, S. sisymbrifolius, and *S. sophioides,* from section *Annui*; *S. pellucidus,* from section *Rigidi*; *S. parvifolius* and *S. pinnulatus,* from section *Leptolobi*; *S. burchellii* and *S. inaequidens* from section *Leptophylli* (Harvey, 1865). Section *Senecio,* including these taxa, was well supported in Bayesian inference analysis, but support was less robust in maximum parsimony analysis. The phylogenetic analysis provides a more complete account of section *Senecio,* which has now been shown to include a number of species never before considered part of the section. Improved understanding of the species composition of section *Senecio* will aid future studies of both genus and section.

All Harvey's sections which were tested for monophyly by including them in the *ITS* phylogenetic analysis proved non-monophyletic. Nevertheless his recognition of affinities between particular taxa, although intuitive, was often correct. The degree of accuracy seen is surprising because his classification system was based almost entirely on morphological characters - notoriously misleading as a basis for inferring evolutionary relationships within *Senecio* and closely related genera. In particular, all of the included members of section *Sinuosi,* with a single exception, were found together in a monophyletic group. However, several of Harvey's sections do not include any species found in the *Senecio* s.str. clade, probably because the classification system was devised at a time when the definition of the genus *Senecio* was considerably looser.

Clear identification of the genus or species group with the closest ties to *Senecio* could not be made because there was no resolution in this part of the *ITS* tree. Candidates for the title of closest genus or species group are *Arrhenechtites, Crassocephalum, Erechtites,* and the *Senecio engleranus* / *Senecio flavus* clade, all of which appear on a polytomy with *Senecio* s.str. Although *Senecio engleranus* and

Senecio flavus should remain in the genus *Senecio* for the time being, future studies may reveal that they belong in a separate genus.

Incongruence between tree topologies retrieved on analysis of nuclear and plastid DNA was unusually high, suggesting that there may be factors confounding phylogenetic inference in *Senecio.* Possible explanations include hybrid origins for many *Senecio* species, rapid diversification, and lineage sorting effects.

It is likely that *Senecio* s.str. originated in southern Africa. Early diversification in the genus appears to have taken place there, a conclusion supported by area optimisation trees in this thesis (figs. 2.29 - 2.31), in Pelser et al. (2007), and by the fact that southern Africa is a known centre of diversity for the genus (Nordenstam, 1977; Bremer, 1994). However, determining which area of southern Africa is the likely origin for the genus is more problematic, as there are three possible candidates.

The first of these is Namibia, as the most basal clade in the phylogeny of *Senecio* s.str. produced here (figs. 2.15 - 2.19 and 2.29 – 2.31) consists of the Namibian species pair, *Senecio engleranus* and *Senecio flavus.* From here the genus may have spread to the Cape Floristic Region (CFR), followed by an expansion east into Kwa-Zulu Natal. However, species numbers in Namibia are low compared with both the CFR and Kwa-Zulu Natal, with just 24 species listed by Merxmüller (1976), suggesting this may not be the origin. It is unclear how exhaustive this account is, although Namibia is generally floristically depauperate compared with South Africa. A phylogeny based on a complete taxon sample of southern African *Senecio* might reveal a more basal clade from another area.

The second possibility is that *Senecio* originated in the CFR itself. With around 9,000 species in 90,000km^2 the CFR is remarkably diverse, and recognised as

one of six floristic kingdoms (Takhtajan, 1986), with species richness comparable to that of the most diverse equatorial regions. Endemism levels are more comparable with island floras, with about 70% endemism at the species level, and about 16% at the generic level (Goldblatt & Manning, 2002). While high levels of endemism are likely the result of ecological and geographical isolation, reasons for the unusually high levels of species richness at this latitude are less clear. Much of this species richness is attributable to relatively few plant groups, with 33 'Cape Floral Clades' (CFCs) accounting for approximately 50% of the diversity (Linder, 2003). A possible explanation for the current richness of the flora and its composition is that the Mid-Miocene tropical vegetation of the area was wiped out by the failure of summer rainfall, associated with the upwelling of cold waters along the Atlantic seaboard of southern Africa, caused by the glaciation of Antarctica around 8 – 10 Mya. This led to the current pattern of winter rainfall, and allowed the ancestral lineages of the Cape flora to radiate from restricted mountainous areas into newly opened up habitats, creating the rich diversity seen today (Linder, 2004). Although the genus *Senecio* cannot be considered a CFC in the sense of Linder (2003), as more than 50% of known species occur outside the CFR, approximately 75% - 85% of the species found there appear to be endemic. Recent, rapid speciation, as seen in the CFR (Linder et al., 1992), could account for the large number of morphologically similar *Senecio* species found in the CFR, as not enough time has passed for substantial morphological differentiation between taxa or for the extinction of intermediate forms (Linder, 2003). However, morphological similarity of species in *Senecio* is not limited to the Cape, with complexes of similar species known from Europe (Alexander, 1975; 1979), Australia (Ali, 1969), and Kwa-Zulu Natal (Hilliard, 1977). The number of *Senecio* species in the CFR is approximately 111 (Goldblatt & Manning, 2000) - 174

(Harvey, 1865), roughly four to seven times that of Namibia. Although not entirely reliable, centres of diversity can indicate centres of origin. *Senecio* may have originated in the CFR and spread east to Kwa-Zulu Natal, where new radiations took place, and north to Namibia, where only a few taxa were able to adapt to harsh desert conditions.

The third possibility is that *Senecio* originated in Kwa-Zulu Natal. There may be as many *Senecio* species here as are found in the CFR, although reliable numbers are not available for either area. Hilliard (1977) describes 124 species found in the area, compared with 174 described from the Cape by Harvey (1865), and 111 described more recently from the Cape by Goldblatt and Manning (2000). The generic concept applied by Hilliard was looser than that accepted for *Senecio* s.str. here, making it very difficult to estimate how many of these 124 species might belong in the core genus. Several species from Hilliard's account of the genus were included in the phylogeny in chapter 2, and appeared outside the *Senecio* s.str. clade, suggesting the number of species attributed to *Senecio* would reduce if molecular phylogenetic techniques were applied to this group. Interestingly, species composition of the genus in these two areas differs significantly, despite their geographical proximity. Between about 85-150 species (≈ 75% – 85%) appear to be endemic to the CFR, while approximately 100 species (≈ 80%) appear to be endemic to Kwa-Zulu Natal. Only about 25 species are common to both areas. *Senecio* might have originated in Kwa-Zulu Natal, and moved from there into the CFR, where it diversified and moved north to Namibia. Kwa-Zulu Natal is ecologically and floristically distinct from the CFR, having richer soils and a different climate. High diversity and endemism seen there could alternatively reflect a pattern seen in other genera, such as *Ehrharta* Thunb., where increased speciation rates are seen in association with the colonisation of less

oligotrophic soils in seasonally dry habitats (Verboom, 2000). Kwa-Zulu Natal may have been colonised from the nutrient-poor soils of the CFR, followed by rapid radiation. Our understanding of *Senecio* in Kwa-Zulu Natal would benefit greatly from further study, particularly phylogenetic analyses, which would help to shed further light on the origins of *Senecio* s.str.

Many of the problems encountered during the present phylogenetic study of *Senecio* are common to studies of large genera, which have historically been extremely difficult to study in their entirety. As a result, few comprehensive monographic accounts of such genera exist, a major problem in the case of *Senecio*, where large numbers of independently created regional accounts have led to a great deal of confusion about overall species numbers and sectional delimitations. More recently, as in this thesis, molecular phylogenetic techniques have enabled more measured and less subjective estimates of generic and sectional limits to be made in troublesome and unwieldy plant genera, hopefully leading to more stable and biologically meaningful classification systems. *Senecio* represents a case in which phylogenetic reconstruction has led to a reduction in species numbers, leading to a more manageable generic delimitation, and to the abandonment of many previously recognised, but weakly defined, sections. Complete taxon sampling remains an issue in very large genera because of the time and money involved in collecting a large number of different species in the field and the cost of sequencing DNA fragments. However, it is hoped that an improved understanding of the evolution of large plant genera may help to understand broader patterns of plant evolution (Frodin, 2004).

A thorough reclassification of *Senecio* and tribe Senecioneae is long overdue, but would represent a huge undertaking, as it would be desirable to sample all the known taxa in the genus, and identify molecular markers which are more informative

than *ITS* to improve resolution between closely related species. The collection of the taxa, in particular, would likely represent a lifetime's work.

4.2: Chapter 3

Phylogenetic analysis of *ITS* and *trn*L-F DNA showed that the species pair *Senecio engleranus* and *Senecio flavus* is only distantly related to section *Senecio.* Thus they do not represent ancestral taxa for the section as suggested by Coleman et al. (2003). A lack of resolution between the taxa in the trees generated, together with early morphological observations, suggested they might not be separate entities. However, analyses of RAPD variation, morphology, and pollen number and fertility confirmed that *Senecio engleranus* and *Senecio flavus* are clearly separable genetically and morphologically, and should therefore retain separate species status.

The creation of artificial F1 hybrids between the two, resulting in a vigorous F2 generation showed that, despite being clearly separable, they retain high levels of interfertility, suggesting that intrinsic postzygotic barriers between them are weak, and that hybridisation in the wild is mainly prevented by prezygotic barriers. However, only a single F1 hybrid went on to produce progeny, and pollen fertility in the F1 was greatly reduced, suggesting that, although weak, intrinsic postzygotic barriers to hybridisation do exist.

A difference in pappus morphology between *S. engleranus* and *S. flavus*, which may explain the very different recorded distributions of the two species, was seen to be the result of allelic differences in a single major gene, whereas pollen number and pollen fertility appear to be under more complex genetic control. The successful production of an F2 generation suggests the species pair would be useful in future studies investigating the genetics of traits which differ between the two species.

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Appendix 1: Details of taxa included in phylogenetic analyses

Name on Trees with Authorities	Source	Accession /Collector Number	Sequenced *ITS* / *trn*L-F (in present study)	Available Locality Information	Associated Publication
Adenostyles leucophylla	Genbank	AY176130	*ITS* – N *trn*L-F - N	N/A	Liu, J. (Unpublished) Karyological and molecular phylogeny of *Ligularia* and its related genera.
Arrhenechthites novoguineensis	Genbank	AF459972	*ITS* – N *trn*L-F - N	New Guinea	Pelser et al. (2002)
Cineraria aspera	Genbank	AY275656	*ITS* – N *trn*L-F - N	South Africa, Western Cape.	Cron, G.V., Balkwill, K. and Knox, E.B. (Unpublished) Generic circumscription of *Cineraria* L. (Senecioneae, Asteraceae) based on molecular evidence.
*Cineraria deltoidea*1	Genbank	AY953907	*ITS* – N *trn*L-F - N	South Africa, Western Cape.	Cron, G.V., Balkwill, K. and Knox, E.B. (Unpublished) Generic circumscription of *Cineraria* L. (Senecioneae, Asteraceae) based on molecular evidence.
*Cineraria deltoidea*2	Genbank	AY953905	*ITS* – N *trn*L-F - N	Kenya.	Cron, G.V., Balkwill, K. and Knox, E.B. (Unpublished) Generic circumscription of *Cineraria* L. (Senecioneae, Asteraceae) based on molecular evidence.
Cineraria saxifraga	Genbank	AY953916	*ITS* – N *trn*L-F - N	South Africa, Western Cape.	Cron, G.V., Balkwill, K. and Knox, E.B. (Unpublished) Generic circumscription of *Cineraria* L. (Senecioneae, Asteraceae) based on molecular evidence.
Crassocephalum crepidioides	Genbank	AF459968	*ITS* – N *trn*L-F - N	Africa.	Pelser et al. (2002)
Curio articulatus (syn: *Senecio articulatus*)	Genbank	DQ915882	*ITS* – N *trn*L-F - N	N/A	Sombra Staeheli, D., Eggli, U. and Nyffeler, R. (Unpublished) Molecular phylogenetics and comparative anatomy of succulent species of *Senecio*
Dauresia alliariifolia (syn: *Senecio alliariifolius*)	Genbank	AF457413	*ITS* – N *trn*L-F - N	Namibia, Lemoenputs.	Coleman et al. (2003)
Dendrosenecio cheranganiensis	Genbank	AF155962/AF155995	*ITS* – N *trn*L-F - N	Africa.	Panero et al. (1999)
Dendrosenecio kilimanjari	Genbank	AF155963/AF155996	*ITS* – N *trn*L-F - N	Africa.	Panero et al. (1999)
Dendrosenecio kilimanjari subsp. *cottonii*	Genbank	AF155963/AF155996	*ITS* – N *trn*L-F - N	Africa.	Panero et al. (1999)
Dorobaea pimpinellifolia	Genbank	AF155964/AF155997	*ITS* – N *trn*L-F - N	Americas.	Panero et al. (1999)
Emilia coccinea	Genbank	AF459966	*ITS* – N *trn*L-F - N	Africa.	Pelser et al. (2002)
Emilia discifolia	Genbank	AY953930	*ITS* – N *trn*L-F - N	Zimbabwe.	Cron, G.V., Balkwill, K. and Knox, E.B. (Unpublished) Generic circumscription of *Cineraria* L. (Senecioneae, Asteraceae) based on molecular evidence.
Emilia sonchifolia var. *javanica*	Genbank	EF108405	*ITS* – N *trn*L-F - N	N/A	Hsieh, C.C., Chang, Y.S., Kuo, C.L., Liu, S.L., Chiu, T.H., Chen, F.J., Shih, W.C. and Chao, J.J. (Unpublished) The genomic ITS sequence of nuclear ribosomal DNA identification and construction of the bioinformatics database of pharmaceutical botany in Taiwan.
*Erechtites hieraciifolius*1	Genbank	AF459965	*ITS* – N *trn*L-F - N	America.	Pelser et al. (2002)

*Erechtites hieraciifolius*2	Genbank	EF107652	*ITS* – N *trn*L-F - N	N/A	Hsieh, C.C., Chang, Y.S., Kuo, C.L., Liu, S.L., Chiu, T.H., Chen, F.J., Shih, W.C. and Chao, J.J. (Unpublished) The genomic ITS sequence of nuclear ribosomal DNA identification and construction of the bioinformatics database of pharmaceutical botany in Taiwan.
Erechtites valerianifolia	Genbank	EF108401	*ITS* – N *trn*L-F - N	N/A	Hsieh, C.C., Chang, Y.S., Kuo, C.L., Liu, S.L., Chiu, T.H., Chen, F.J., Shih, W.C. and Chao, J.J. (Unpublished) The genomic ITS sequence of nuclear ribosomal DNA identification and construction of the bioinformatics database of pharmaceutical botany in Taiwan.
Euryops acraeus	Genbank	AF457410	*ITS* – N *trn*L-F - N	South Africa.	Coleman et al. (2003)
Euryops brownei	Genbank	AY953936	*ITS* – N *trn*L-F - N	Kenya.	Cron, G.V., Balkwill, K. and Knox, E.B. (Unpublished) Generic circumscription of *Cineraria* L. (Senecioneae, Asteraceae) based on molecular evidence.
*Euryops pectinatus*1	Genbank	AF459964	*ITS* – N *trn*L-F - N	Africa.	Pelser et al. (2002)
*Euryops pectinatus*2	Genbank	AF155965/AF155998	*ITS* – N *trn*L-F - N	Africa.	Panero et al. (1999)
Gynura formosana	Genbank	AF155966/AF155999	*ITS* – N *trn*L-F - N	Taiwan	Panero et al. (1999)
Jacobaea abrotanifolia	Genbank	AF459956	*ITS* – N *trn*L-F - N	Europe.	Pelser et al. (2002)
Jacobaea alpina	Genbank	AF459954	*ITS* – N *trn*L-F - N	Europe.	Pelser et al. (2002)
Jacobaea ambigua	Genbank	AF459927	*ITS* – N *trn*L-F - N	Europe	Pelser et al. (2002)
Jacobaea analoga	Genbank	AF459947	*ITS* – N *trn*L-F - N	Central Asia , Himalaya.	Pelser et al. (2002)
Jacobaea aquatica	Genbank	AF459952	*ITS* – N *trn*L-F - N	Europe.	Pelser et al. (2002)
Jacobaea arnautorum	Genbank	AF459934	*ITS* – N *trn*L-F - N	Europe.	Pelser et al. (2002)
Jacobaea boissieri	Genbank	AY155603	*ITS* – N *trn*L-F - N	Spain.	Pelser et al. (2003)
Jacobaea cannabifolia var. *integrifolia*	Genbank	AF459949	*ITS* – N *trn*L-F - N	North-east Asia.	Pelser et al. (2002)
Jacobaea carniolica	Genbank	AF459942	*ITS* – N *trn*L-F - N	Europe.	Pelser et al. (2002)
Jacobaea carniolica subsp. *carniolica*	Genbank	AY155604	*ITS* – N *trn*L-F - N	Italy.	Pelser et al. (2003)
Jacobaea carniolica subsp. *insubrica*	Genbank	AY155605	*ITS* – N *trn*L-F - N	Switzerland.	Pelser et al. (2003)
Jacobaea erucifolia	Genbank	AF459944	*ITS* – N *trn*L-F - N	Europe and N and C Asia.	Pelser et al. (2002)
Jacobaea gigantea	Genbank	AY155606	*ITS* – N *trn*L-F - N	N Africa, Algeria.	Pelser et al. (2003)
Jacobaea gnaphalioides	Genbank	AY155607	*ITS* – N *trn*L-F - N	Greece.	Pelser et al. (2003)
Jacobaea incana	Genbank	AY155609	*ITS* – N *trn*L-F - N	France.	Pelser et al. (2003)
Jacobaea leucophylla	Genbank	AY155611	*ITS* – N *trn*L-F - N	Spain.	Pelser et al. (2003)
Jacobaea maritima	Genbank	AF459950	*ITS* – N *trn*L-F - N	Europe.	Pelser et al. (2002)
Jacobaea minuta	Genbank	AF459938	*ITS* – N *trn*L-F - N	Europe.	Pelser et al. (2002)
Jacobaea othonnae	Genbank	AY155612	*ITS* – N *trn*L-F - N	Georgia.	Pelser et al. (2003)
Jacobaea paludosa	Genbank	AF459935	*ITS* – N *trn*L-F - N	Europe and N Asia.	Pelser et al. (2002)
Jacobaea persoonii	Genbank	AY155613	*ITS* – N *trn*L-F - N	Italy.	Pelser et al. (2003)

Jacobaea subalpina	Genbank	AF459929	*ITS* – N *trn*L-F - N	Europe.	Pelser et al. (2002)
Jacobaea uniflora	Genbank	AY155608	*ITS* – N *trn*L-F - N	Italy.	Pelser et al. (2003)
*Jacobaea vulgaris*1	Genbank	AF459941	*ITS* – N *trn*L-F - N	Europe and north Asia	Pelser et al. (2002)
Jacobaea vulgaris2	Genbank	AY155610	*ITS* – N *trn*L-F - N	N/A	Pelser et al. (2003)
Jacobaea adonidifolia	Genbank	AF459955	*ITS* – N *trn*L-F - N	Europe.	Pelser et al. (2002)
Kleinia crassulaefolia	South African Collection	JJM104.1	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, on road form Riversdale to Barrydale (Tradouw Pass) 33° 59' 055"S 20° 42' 390"E alt: 289m	
Kleinia neriifolia	Genbank	AF459962	*ITS* – N *trn*L-F - N	Spain, Canary Islands.	Pelser et al. (2002)
Kleinia_galpinii	Genbank	AY953934	*ITS* – N *trn*L-F - N	South Africa, Johannesburg.	Cron, G.V., Balkwill, K. and Knox, E.B. (Unpublished) Generic circumscription of *Cineraria* L. (Senecioneae, Asteraceae) based on molecular evidence.
Mesogramma apiifolia (syn: *Senecio apiifolius*)	Genbank	AF457412	*ITS* – N *trn*L-F - N	Namibia, Okaukuejo.	Coleman et al. (2003)
Oresbia heterocarpa	Genbank	AY953935	*ITS* – N *trn*L-F - N	South Africa, Western Cape.	Cron, G.V., Balkwill, K. and Knox, E.B. (Unpublished) Generic circumscription of *Cineraria* L. (Senecioneae, Asteraceae) based on molecular evidence.
*Othonna capensis*1	Genbank	AF459960	*ITS* – N *trn*L-F - N	Africa.	Pelser et al. (2002)
*Othonna capensis*2	Genbank	DQ915865	*ITS* – N *trn*L-F - N	South Africa.	Sombra Staeheli, D., Eggli, U. and Nyffeler, R. (Unpublished) Molecular phylogenetics and comparative anatomy of succulent species of *Senecio*
Othonna parviflora	Genbank	AF155967/AF156000	*ITS* – N *trn*L-F - N	Africa.	Panero et al. (1999)
Othonna sedifolia	Genbank	DQ915866	*ITS* – N *trn*L-F - N	South Africa.	Sombra Staeheli, D., Eggli, U. and Nyffeler, R. (Unpublished) Molecular phylogenetics and comparative anatomy of succulent species of *Senecio*
Packera aurea	Genbank	AF459959	*ITS* – N *trn*L-F - N	North America.	Pelser et al. (2002)
Packera breweri	Genbank	AF161613/AF161663	*ITS* – N *trn*L-F - N	North America.	Bain & Golden (2000)
Packera eurycephala	Genbank	AF161616/AF161666	*ITS* – N *trn*L-F - N	North America.	Bain & Golden (2000)
Packera millefolia	Genbank	AF161623/AF161673	*ITS* – N *trn*L-F - N	North America.	Bain & Golden (2000)
Packera sanguisorbae	Genbank	AF161633/AF161683	*ITS* – N *trn*L-F - N	North America.	Bain & Golden (2000)
Pericallis multiflora	Genbank	AY953931	*ITS* – N *trn*L-F - N	Spain, Canary Islands.	Cron, G.V., Balkwill, K. and Knox, E.B. (Unpublished) Generic circumscription of *Cineraria* L. (Senecioneae, Asteraceae) based on molecular evidence.
Pericallis murrayi	Genbank	AY953932	*ITS* – N *trn*L-F - N	Spain, Canary Islands.	Cron, G.V., Balkwill, K. and Knox, E.B. (Unpublished) Generic circumscription of *Cineraria* L. (Senecioneae, Asteraceae) based on molecular evidence.
Pericallis tussilaginis	Genbank	AJ563924	*ITS* – N *trn*L-F - N	Spain, Canary Islands.	Swenson & Manns (2003)
Phaneroglossa bolusii	Genbank	AF155991/AF156024	*ITS* – N *trn*L-F - N	Africa.	Panero et al. (1999)
Pseudogynoxys benthamii	Genbank	AF459958	*ITS* – N *trn*L-F - N	America.	Pelser et al. (2002)

Pseudogynoxys chenopodioides	Genbank	AF155992/AF156025	*ITS* – N *trn*L-F - N	Americas.	Panero et al. (1999)
Robinsonia berteroi	Genbank	EF028712/EF028719	*ITS* – N *trn*L-F - N	Juan Fernández Islands	Sang et al. (1995)
Robinsonia gracilis	Genbank	EF028709/EF028716	*ITS* – N *trn*L-F - N	Juan Fernández Islands	Sang et al. (1995)
Robinsonia thurifera	Genbank	EF028711/EF028718	*ITS* – N *trn*L-F - N	Juan Fernández Islands	Sang et al. (1995)
Senecio abruptus	South African Collection	JJM50.1	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, Cederberg Reserve, Matjies River Cape Nature Station, gravel pile near station. 32° 29' 556"S 19° 20' 051"E alt:742m	
Senecio achilleifolius	RBGE living collections	1997 2286A	*ITS* – Y *trn*L-F - Y	Lesotho, Sani Top. 29°S 29°E	
Senecio actinella	Genbank	L33183/L33213	*ITS* – N *trn*L-F - N	USA, New Mexico, Catron Co.	Bain & Jansen (1995)
Senecio aegyptius subsp. *aegyptius*	Genbank	AJ400777	*ITS* – N *trn*L-F - N	Egypt or Sudan	Comes & Abbott (2001)
Senecio aegyptius subsp. *thebanus*	Genbank	AJ400778	*ITS* – N *trn*L-F - N	Egypt, Damanhur.	Comes & Abbott (2001)
Senecio aethnensis (syn: *Senecio squalidus* subsp. *aethnensis*)	Genbank	AJ400779	*ITS* – N *trn*L-F - N	Italy, Mt Etna.	Comes & Abbott (2001)
Senecio angulatus	South African Collection	JJM102.1	*ITS* – Y *trn*L-F - Y	South Africa, East Cape, Wilderness, car parking area at the beach 33° 59' 413"S 22° 34' 135"E alt: 7m	
Senecio aphanactis	Genbank	AF457430	*ITS* – N *trn*L-F - N	USA, CA, Alameda Co.	Coleman et al. (2003)
Senecio arenarius	Genbank	AF457421	*ITS* – N *trn*L-F - N	Namibia, LU 70.	Coleman et al. (2003)
Senecio arenarius	South African Collection	JJM60.1	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, Cape Town, above Kalk Bay on trail off main road from Cape Town. 34° 08' 000"S 18° 27' 000"E	
Senecio argunensis	Genbank	AY176154	*ITS* – N *trn*L-F - N	N/A	Liu, J. (Unpublished) Karyological and molecular phylogeny of *Ligularia* and its related genera.
Senecio brasiliensis	Genbank	AF457434	*ITS* – N *trn*L-F - N	Brazil, Campos do Jordão.	Coleman et al. (2003)

*Senecio burchellii*1	South African Collection	JJM49.1	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, Cederberg Reserve, Matjies River, next to long house at Cape Nature Station. 32° 29’ 556”S 19° 20’ 051”E alt: 742m	
*Senecio burchellii*2	South African Collection	JJM40.1	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, Cape Town, Kirstenbosch Botanic Garden, Research Centre, roadside. 33° 58’ 593”S 18° 26’ 123”E	
*Senecio burchellii*3	South African Collection	JJM75.1	*ITS* – Y *trn*L-F - Y	South Africa, Northern Cape, Kamiesberg Range, Groenkloof, 3km north of Lieliefontein,. 30° 19’ 528”S 18° 05’ 344”E alt: 1378m	
*Senecio burchellii*4	South African Collection	JJM34.2	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, Gouritsmond, bridge just west of Gouritsmond. 34°16’ 938”S 21° 49’ 624”E alt: 59m	
Senecio cakilefolius	Genbank	AF457423	*ITS* – N *trn*L-F - N	South Africa, Karee Bergen.	Coleman et al. (2003)
Senecio cakilefolius	South African Collection	JJM62.2	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, Clanwilliam, Hoek se Berg, next to big solar panel. 32° 07’ 126”S 19° 10’ 485”E alt: 710m	
Senecio californicus	Genbank	AF457431	*ITS* – N *trn*L-F - N	USA, CA, Monterey Co.	Coleman et al. (2003)
Senecio carpetanus	Genbank	AF459948	*ITS* – N *trn*L-F - N	S W Europe.	Pelser et al. (2002)
Senecio chrysanthemifolius	Genbank	AJ400780	*ITS* – N *trn*L-F - N	Italy, Mt Etna.	Comes & Abbott (2001)
*Senecio consanguineus*1	Genbank	AF457420	*ITS* – N *trn*L-F - N	Namibia, WIN 361.	Coleman et al. (2003)
*Senecio consanguineus*2	Genbank	AF457419	*ITS* – N *trn*L-F - N	South Africa, Ficksburg.	Coleman et al. (2003)

Senecio coronatus clone1	South African Collection, Möller.	MM1192	*ITS* – Y *trn*L-F - N	South Africa, Natal.	
Senecio coronatus clone2	South African Collection, Möller.	MM1192	*ITS* – Y *trn*L-F - N	South Africa, Natal.	
Senecio costaricensis	Genbank	AF161639/AF161689	*ITS* – N *trn*L-F - N	North America.	Bain & Golden (2000)
Senecio cryphiactis	Genbank	AF457429	*ITS* – N *trn*L-F - N	Namibia, Porto Vehlo.	Coleman et al. (2003)
Senecio decurrens	Genbank	EF538324	*ITS* – N *trn*L-F - N	Lesotho.	Pelser et al. (2007)
Senecio deltoideus	South African Collection	JJM7.2	*ITS* – Y *trn*L-F - Y	South Africa, Eastern Cape, Gonubie, Estuary Drive, roadside. 32° 55’ 846”S 27° 59’ 656”E	
Senecio doria	Genbank	AF459946	*ITS* – N *trn*L-F - N	Europe and N W Asia.	Pelser et al. (2002)
Senecio douglasii (syn: *Senecio flaccidus* var. *douglasii*)	Genbank	AF161640/AF161690	*ITS* – N *trn*L-F - N	North America.	Bain & Golden (2000)
Senecio dunedinensis	Genbank	AY554109	*ITS* – N *trn*L-F - N	New Zealand, SI, Canterbury, Two Thumb Range.	Wagstaff & Breitwieser (2004)
*Senecio eenii*1	Genbank	AF457425	*ITS* – N *trn*L-F - N	Namibia, Rosh Pinah.	Coleman et al. (2003)
*Senecio eenii*2	Genbank	AF457424	*ITS* – N *trn*L-F - N	Namibia, Kwang Pan.	Coleman et al. (2003)
Senecio elegans	South African Collection	JJM62.1	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, Cape Town, Dyunefontein, near nuclear power station. 33° 39’ 033”S 18° 27’ 042”E	
Senecio elegans seaside var.	South African Collection	JJM9.2	*ITS* – Y *trn*L-F - Y	South Africa, Eastern Cape, Haga-Haga, on the beach. 32° 45’ 753”S 28° 15’ 154”S	
Senecio engleranus	Genbank	AF457417	*ITS* – N *trn*L-F - N	Namibia, River Huab.	Coleman et al. (2003)
Senecio engleranus pop2	Namibian Collection	JJM110.1	*ITS* – Y *trn*L-F - Y	Namibia, Swakopmund, road between Walvis Bay and Rooikop Airport, next to quarry on the D road off the C14. 22° 40’ 491”S 14° 33’ 103”E alt: 60m	

Senecio engleranus pop3 clone1	Namibian Collection	JJM111.1	*ITS* – Y *trn*L-F - Y	Namibia, Swakopmund, lichen desert between Swakopmund and Henties Bay, about 40 km from Swakopmund. 22° 21' 565"S 14° 26' 191"E alt: 24m	
Senecio engleranus pop3 clone2	Namibian Collection	JJM111.1	*ITS* – Y *trn*L-F - Y	Namibia, Swakopmund, lichen desert between Swakopmund and Henties Bay, about 40 km from Swakopmund. 22° 21' 565"S 14° 26' 191"E alt: 24m	
Senecio engleranus pop3 clone3	Namibian Collection	JJM111.1	*ITS* – Y *trn*L-F - Y	Namibia, Swakopmund, lichen desert between Swakopmund and Henties Bay, about 40 km from Swakopmund. 22° 21' 565"S 14° 26' 191"E alt: 24m	
Senecio engleranus pop3 clone4	Namibian Collection	JJM111.1	*ITS* – Y *trn*L-F - Y	Namibia, Swakopmund, lichen desert between Swakopmund and Henties Bay, about 40 km from Swakopmund. 22° 21' 565"S 14° 26' 191"E alt: 24m	
Senecio engleranus pop4	Namibian Collection	JJM113.1	*ITS* – Y *trn*L-F - Y	Namibia, Swakopmund, Henties Bay, in dried up mouth of Omaruru River. 22° 07' 00"S 14° 17' 00"E alt: 3m	
Senecio eremophilus	Genbank	AF459945	*ITS* – N *trn*L-F - N	USA.	Pelser et al. (2002)

Senecio erosus	South African Collection	JJM54.1	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, Cederberg Reserve, Matjies River 32° 29' 556"S 19° 20' 051"E Alt: 742m	
Senecio ertterae	Genbank	AF457433	*ITS* – N *trn*L-F - N	USA, OR, Malheur Co.	Coleman et al. (2003)
Senecio erubescens	South African Collection	JJM49.2	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, Cape Town, Table Mountain, on Skeleton Gorge route from Maclear's Beacon 33° 58' 865"S 18° 24' 981"E alt: 748m	
Senecio erysimoides	South African Collection	JJM37.2	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, Cape Infanta, in village, garden weed. 34° 25' 276"S 20° 51' 280" alt: 22m	
*Senecio flavus*1	Genbank	AF457416	*ITS* – N *trn*L-F - N	Namibia, MAL 5	Coleman et al. (2003)
*Senecio flavus*2	Genbank	AJ400782	*ITS* – N *trn*L-F - N	Egypt, Sharm El-Sheikh/Dahab	Comes & Abbott (2001)
*Senecio flavus*3	St Andrews Seed Collections	SF751	*ITS* – Y *trn*L-F - Y	Morocco, Tafraoute.	
*Senecio flavus*4	St Andrews Seed Collection	S. flavus St A	*ITS* – Y *trn*L-F - Y	Morocco, Tafraoute.	
*Senecio flavus*5	St Andrews Seed Collections	Flavus26145	*ITS* – Y *trn*L-F - Y	Spain, Canary Islands.	
*Senecio flavus*6	St Andrews Seed Collections	SF3	*ITS* – Y *trn*L-F - Y	Tafraoute, Morocco	
Senecio gallicus	Genbank	AJ400784	*ITS* – N *trn*L-F - N	Spain, Montuenga.	Comes & Abbott (2001)
Senecio giessii	Genbank	AF457418	*ITS* – N *trn*L-F - N	Namibia, Aurusberge.	Coleman et al. (2003)
Senecio glastifolius	South African Collection	JJM94.1	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, Tsitsikama Toll road, east of bridge	
Senecio glaucophyllus	Genbank	AY554110	*ITS* – N *trn*L-F - N	New Zealand, NI, Nelson, Mt. Arthur.	Wagstaff & Breitwieser (2004)
Senecio glaucus subsp. *coronopifolius*	Genbank	AF457439	*ITS* – N *trn*L-F - N	Morocco, Tizi Mlil.	Coleman et al. (2003)
Senecio glaucus subsp. *glaucus*	Genbank	AF457440	*ITS* – N *trn*L-F - N	Israel, Nof Yam.	Coleman et al. (2003)

Senecio glomeratus	Genbank	AY554111	*ITS* – N *trn*L-F - N	New Zealand, SI, Canterbury, Hurunui River.	Wagstaff & Breitwieser (2004)
Senecio glutinarius	South African Collection	JJM73.1	*ITS* – Y *trn*L-F - Y	South Africa, Northern Cape, Kamiesberg Range, end of Kamiesberg Pass, on route to Lieliefontein, roadside. 30° 10' 463"S 18° 01' 156"E alt: 1042m	
*Senecio glutinosus*1	Genbank	AF457427	*ITS* – Y *trn*L-F - Y	South Africa, Port Alfred.	Coleman et al. (2003)
*Senecio glutinosus*2	South African Collection	JJM23.2	*ITS* – Y *trn*L-F - Y	South Africa, East Cape, R343 from Salem to Kenton-on-Sea, roadside. 33° 36' 201"S 26° 36' 521"E alt: 126m	
*Senecio glutinosus*3	South African Collection	JJM91.1	*ITS* – Y *trn*L-F - N	South Africa, Western Cape, east of entrance to Plettenberg Bay, on N2, roadside. 34° 01' 581"S 23° 22' 293"E alt:27m	
Senecio gramineus	RBGE Living Collections	1997 2300	*ITS* – Y *trn*L-F - Y	Lesotho, Hodgson's Peaks 29°S 29°E	
*Senecio hastatus*1	South African Collection	JJM55.1	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, Cape Town, Signal Hill, near car park. 33° 55' 009"S 18° 24' 104"E	
*Senecio hastatus*2	South African Collection	JJM40.2	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, De Hoop Nature Reserve, Klipspringer Trail, near end of trail. 34° 22' 617"S 20° 32' 059"E alt: 177m	
Senecio hesperidium	Genbank	AJ400789	*ITS* – N *trn*L-F - N	Morocco, Sidi Rbat.	Comes & Abbott (2001)

*Senecio inaequidens*1	Genbank	AF097537	*ITS* – N *trn*L-F - N	South Africa, Transvaal.	Vincent, P.L.D. and Holtsford, T.P. (Unpublished) Elucidative studies on the generic concept of *Senecio* (Asteraceae) based on ITS sequences of nuclear ribosomal DNA
*Senecio inaequidens*2	South African Collection	JJM100.1	*ITS* – Y *trn*L-F - Y	South Africa, Eastern Cape, Port Elizabeth, car park of 'Silver Cloud Spur' restaurant just inside west side of Port Elizabeth. 33° 56' 525"S 25° 33' 208"E alt: 182m	
*Senecio inaequidens*3	Genbank	AF459943	*ITS* – N *trn*L-F - N	South Africa	Pelser et al. (2002)
*Senecio inaequidens*4 clone1	South African Collection	JJM99.1	*ITS* – Y *trn*L-F - N	South Africa, Eastern Cape, Plettenberg Bay, on N2 20 km east of Stormsrivier bridge. 34° 02' 318"S 24° 24' 075"E alt: 216m	
*Senecio inaequidens*4 clone2	South African Collection	JJM99.1	*ITS* – Y *trn*L-F - N	South Africa, Eastern Cape, Plettenberg Bay, on N2 20 km east of Stormsrivier bridge. 34° 02' 318"S 24° 24' 075"E alt: 216m	
*Senecio inaequidens*4 clone3	South African Collection	JJM99.1	*ITS* – Y *trn*L-F - N	South Africa, Eastern Cape, Plettenberg Bay, on N2 20 km east of Stormsrivier bridge. 34° 02' 318"S 24° 24' 075"E alt: 216m	
Senecio krascheninnikovii	Genbank	AF457437	*ITS* – N *trn*L-F - N	India, Himachal Pradesh.	Coleman et al. (2003)

Senecio latifolius	South African Collection	JJM13.2	*ITS* – Y *trn*L-F - Y	South Africa, Eastern Cape, N2 between King William's Town and East London, just before King William's Town, roadside. 32° 55' 163"S 27° 43' 646"E alt: 331m	
Senecio lautus	Genbank	AF459940	*ITS* – N *trn*L-F - N	Australia	Pelser et al. (2002)
Senecio lemmonii	Genbank	AF457432	*ITS* – N *trn*L-F - N	USA, AZ, Pima Co.	Coleman et al. (2003)
Senecio leucanthemifolius	Genbank	AJ400790	*ITS* – N *trn*L-F - N	France, Calvi.	Comes & Abbott (2001)
*Senecio littoreus*1	South African Collection	JJM45.2	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, Kommetjie, disturbed grassy area in the town. 34° 08' 465"S 18° 19' 557"E alt: 77m	
*Senecio littoreus*2	South African Collection	JJM41.1	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, Cape Town, Newlands, near entrance to Kirstenbosch Botanic Garden, roadside. 33° 59' 198"S 18° 25' 312"E	
*Senecio littoreus*3	South African Collection	JJM44.1	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, Cape Town, Cape Flats, Edith Stephens Wetlands Reserve. 34° 00' 020"S 18° 33' 125"E	
Senecio lividus	Genbank	AJ400795	*ITS* – N *trn*L-F - N	Spain, Florez, Puente de Domingo.	Comes & Abbott (2001)
Senecio longifolius	South African Collection	JJM61.1	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, Cape Town, Dyunefontein, near nuclear power station. 33° 39' 033"S 18° 27' 042"E	
Senecio loratifolius	Genbank	AF161643/AF161693	*ITS* – N *trn*L-F - N	North America.	Bain & Golden (2000)
Senecio lugens	Genbank	L33196/L33226	*ITS* – N *trn*L-F - N	USA, Yukon, Demster Hwy.	Bain & Jansen (1995)

Senecio lyratus	South African Collection	JJM79.1	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, Cape Town, N2, Kogelberg Nature Reserve. 34° 09' 029"S 18° 57' 245"E alt: 414m	
Senecio macrocephalus	South African Collection	JJM11.2	*ITS* – Y *trn*L-F - Y	South Africa, Eastern Cape, 50km from King William's Town, on road from East London (N2), roadside. 32° 55' 757"S 27° 47' 036"E alt:312m	
Senecio macrospermus	RBGE Living Collections	1997 2276C	*ITS* – Y *trn*L-F - Y	Lesotho, Sani Pass 29°S 29°E	
Senecio madagascariensis	South African Collection	JJM26.2	*ITS* – Y *trn*L-F - N	South Africa, Eastern Cape, on R67 between Grahamstown and Port Alfred, roadside. 33° 27' 872"S 26° 48' 800"E alt: 182m	
Senecio malacitanus	Genbank	AJ400813	*ITS* – N *trn*L-F - N	Morocco.	Comes & Abbott (2001)
Senecio maritimus	South African Collection	JJM42.2	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, Cape Town, Hout Bay, at old canon placements by the sea. 34° 03' 324"S 18° 20' 858"E alt: 8m	
Senecio minimus	Genbank	AY554114	*ITS* – N *trn*L-F - N	New Zealand, SI, Otago, Dunedin City.	Wagstaff & Breitwieser (2004)
Senecio mohavensis subsp. *breviflorus*	Genbank	AF457435	*ITS* – N *trn*L-F - N	Israel, Khirbet Mezin.	Coleman et al. (2003)
Senecio mohavensis subsp. *mohavensis*	Genbank	AF457436	*ITS* – N *trn*L-F - N	USA, AZ, Maricopa Co.	Coleman et al. (2003)
Senecio nebrodensis	Genbank	AJ400797	*ITS* – N *trn*L-F - N	Spain, Capileira/Mt Mulhacen.	Comes & Abbott (2001)
Senecio nemorensis	Genbank	AF459937	*ITS* – N *trn*L-F - N	Europe and Asia.	Pelser et al. (2002)
Senecio neowebsteri	Genbank	AF161644/AF161694	*ITS* – N *trn*L-F - N	North America.	Bain & Golden (2000)

Senecio oxyodontus	South African Collection	JJM17.2	*ITS* – Y *trn*L-F - Y	South Africa, Eastern Cape, R345 from King Willian's Town, towards Hogsback, between 1 and 4 km before Hogsback 32° 36' 428"S 26° 55' 931"E alt: 1068m	
Senecio paniculatus	South African Collection	JJM106.1	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, on road from Riversdale to Barrydale (Tradouw Pass). 33° 59' 055"S 20° 42' 390"E alt: 289m	
Senecio parvifolius	South African Collection	JJM74.1	*ITS* – Y *trn*L-F - Y	South Africa, Northern Cape, Kamiesberg Range, Groenkloof, 5km north of Lieliefontein, past telecom tower. 30° 20' 372"S 18° 06' 524"E alt: 1341m	
*Senecio pellucidus*1	South African Collection	JJM92.1	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, east of entrance to Plettenberg Bay, on N2, roadside. 34° 01' 581"S 23° 22' 293"E alt:27m	
*Senecio pellucidus*2	South African Collection	JJM85.1	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, Wilderness, picnic spot overlooking Wilderness Lake, just east of village. 34° 00' 268"S 22° 44' 530"E alt:57m	
Senecio petraeus	Genbank	AJ400798	*ITS* – N *trn*L-F - N	Spain, Ronda	Comes & Abbott (2001)

Senecio pinnulatus	South African Collection	JJM53.1	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, Cederberg Reserve, Ceres, R303, Michell's Pass, 33° 24' 0" S 19° 17' 0"E alt: 966m	
Senecio pseudo-arnica	Genbank	AF161645/AF161695	*ITS* – N *trn*L-F - N	North America.	Bain & Golden (2000)
Senecio pterophorus clone1	South African Collection	JJM8.2	*ITS* – Y *trn*L-F - N	South Africa, Eastern Cape, Gonubie, Estuary Drive, roadside, 32° 55' 846"S 27° 59' 656"E alt:54m	
Senecio pterophorus clone2	South African Collection	JJM8.2	*ITS* – Y *trn*L-F - N	South Africa, Eastern Cape, Gonubie, Estuary Drive, roadside, 32° 55' 846"S 27° 59' 656"E alt:54m	
Senecio pterophorus clone3	South African Collection	JJM8.2	*ITS* – Y *trn*L-F - N	South Africa, Eastern Cape, Gonubie, Estuary Drive, roadside, 32° 55' 846"S 27° 59' 656"E alt:54m	
Senecio pubigerus	South African Collection	JJM78.1	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, Cape Town, N2, Kogelberg Nature Reserve. 34° 09' 029"S 18° 57' 245"E alt: 414m	
Senecio quadridentatus	Genbank	AF422134	*ITS* – N *trn*L-F - N	New Zealand, SI, Canterbury, Hurunui River	Wagstaff & Breitwieser (2002)
*Senecio repandus*1	South African Collection	JJM24.2	*ITS* – Y *trn*L-F - Y	South Africa, Eastern Cape, R343 between Salem and Kenton-on-Sea, roadside. 33° 38' 991"S 26° 37' 937"E alt: 83m	

*Senecio repandus*2	South African Collection	JJM57.1	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, Cape Town, Signal Hill, near car park. 33° 55’ 108”S 18° 23’ 574”E	
*Senecio repandus*3	South African Collection	JJM46.2	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, Cape Town, Cape Peninsula National Park, Olifantsbos, next to parking area. 34° 15’ 537”S 18° 22’ 938”S alt: 12m	
*Senecio repandus*4	South African Collection	JJM38.2	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, Cape Infanta, at end of road through the village, next to ‘Wild West Whale Chalet’ sign. 34° 25’ 445”S 20° 51’ 419”E	
Senecio rigidus	South African Collection	JJM95.1	*ITS* – Y *trn*L-F - N	South Africa, Western Cape, Tsitsikamma toll road, on East side of bridge, roadside. 33° 58’ 014”S 23° 55’ 495”E alt: 246m	
Senecio rodriguezii (syn: *Senecio varicosus*)	Genbank	AJ400799	*ITS* – N *trn*L-F - N	Spain, Majorca, Formentor.	Comes & Abbott (2001)
Senecio rufiglandulosus	Genbank	AF422135	*ITS* – N *trn*L-F - N	New Zealand, NI, Ruahine Range, Whanahuia Range	Wagstaff & Breitwieser (2002)
*Senecio rupestris*1	Genbank	AJ400802	*ITS* – N *trn*L-F - N	Greece, Mistras.	Comes & Abbott (2001)
*Senecio rupestris*2	Genbank	AJ400801	*ITS* – N *trn*L-F - N	Italy, Sulmona.	Comes & Abbott (2001)
*Senecio rupestris*3	Genbank	AJ400800	*ITS* – N *trn*L-F - N	Germany, Bleiwaesche.	Comes & Abbott (2001)
Senecio seminiveus	RBGE living collections	1996 1926A	*ITS* – Y *trn*L-F - Y	Lesotho, Sani Lodge. 29° 34’ 40”S 29° 17’ 35”E	
Senecio serra	Genbank	AF161641/AF161696	*ITS* – N *trn*L-F - N	North America.	Bain & Golden (2000)

Senecio sisymbrifolius	South African Collection	JJM66.2	*ITS* – Y *trn*L-F - Y	South Africa, W Cape, on road from Clanwilliam to Calvinia. 32° 06' 932"S 19° 03' 129"E Alt: 479m	
Senecio sophioides	South African Collection	JJM105.1	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, midway between Barrydale and Montagu, fruit tree plantation. 33° 55' 462"S 20° 36' 464"E alt: 425m	
Senecio speciosus (syn: *Senecio concolor*, *Senecio polyodon*)	South African Collection	JJM21.2	*ITS* – Y *trn*L-F - Y	South Africa, Eastern Cape, just outside Grahamstown on N2 towards Port Elizabeth, roadside. 33° 19' 440"S 26° 30' 937"E alt: 617m	
Senecio squalidus	Genbank	AF459926	*ITS* – N *trn*L-F - N	Europe, north Africa and west Asia	Pelser et al. (2002)
Senecio squalidus subsp. *araneosus*	Genbank	AJ400804	*ITS* – N *trn*L-F - N	Morocco, Djebel Tazaote.	Comes & Abbott (2001)
Senecio squalidus subsp. *squalidus*	Genbank	AJ400803	*ITS* – N *trn*L-F - N	United Kingdom, Ainsdale	Comes & Abbott (2001)
Senecio sylvaticus	Genbank	AF459928	*ITS* – N *trn*L-F - N	Europe and north Asia	Pelser et al. (2002)
Senecio tamoides	South African Collection	JJM108.1	*ITS* – Y *trn*L-F - Y	South Africa, Western Cape, George, in forest near 'George East Caravan Park' 33° 58' 000"S 22° 27' 000"E	
Senecio thianschanicus	Genbank	AY176156	*ITS* – N *trn*L-F - N	N/A	Liu, J. (Unpublished) Karyological and molecular phylogeny of *Ligularia* and its related genera.
*Senecio vernalis*1	Genbank	AJ400807	*ITS* – N *trn*L-F - N	Israel, Zomet El Rom.	Comes & Abbott (2001)
*Senecio vernalis*2	Genbank	AJ400806	*ITS* – N *trn*L-F - N	Germany, Eppelheim.	Comes & Abbott (2001)
Senecio viscosus	Genbank	AF459925	*ITS* – N *trn*L-F - N	Europe and W Asia.	Pelser et al. (2002)
Senecio vulgaris subsp. *denticulatus*1	Genbank	AJ400812	*ITS* – N *trn*L-F - N	Italy, Monti Nebrodi/Cesaro.	Comes & Abbott (2001)
Senecio vulgaris subsp. *denticulatus*2	Genbank	AJ400811	*ITS* – N *trn*L-F - N	United Kingdom, Jersey.	Comes & Abbott (2001)

*Senecio vulgaris*1	Genbank	AF422136	*ITS* – N *trn*L-F - N	New Zealand, SI, Canterbury, Lincoln.	Wagstaff & Breitwieser (2002)
*Senecio vulgaris*2	Genbank	AF097541	*ITS* – N *trn*L-F - N	USA, CA.	Vincent, P.L.D. and Holtsford, T.P. (Unpublished) Elucidative studies on the generic concept of *Senecio* (Asteraceae) based on ITS sequences of nuclear ribosomal DNA
*Senecio vulgaris*3	Genbank	AF459924	*ITS* – N *trn*L-F - N	Europe.	Pelser et al. (2002)
*Senecio vulgaris*4	Genbank	AJ400809	*ITS* – N *trn*L-F - N	Spain, Matalascanas.	Comes & Abbott (2001)
*Senecio vulgaris*5	Genbank	AF097538	*ITS* – N *trn*L-F - N	Bolivia.	Vincent, P.L.D. and Holtsford, T.P. (Unpublished) Elucidative studies on the generic concept of *Senecio* (Asteraceae) based on ITS sequences of nuclear ribosomal DNA
Senecio windhoekensis	Genbank	AF457426	*ITS* – N *trn*L-F - N	Namibia, WIN 134.	Coleman et al. (2003)
Senecio zimapanicus (syn: *Packera zimapanica*)	Genbank	AF161636/AF161686	*ITS* – N *trn*L-F - N	North America	Bain & Golden (2000)
Solanecio mannii	Genbank	AF459923	*ITS* – N *trn*L-F - N	Africa.	Pelser et al. (2002)
Stilpnogyne bellidioides	Genbank	AF457411	*ITS* – N *trn*L-F - N	South Africa, Moordkuil.	Coleman et al. (2003)
Synotis lucorum	Genbank	AY723255	*ITS* – N *trn*L-F - N	China.	Liu et al. (2006)
Synotis nagensium	Genbank	AF459922	*ITS* – N *trn*L-F - N	Asia.	Pelser et al. (2002)

Appendix 2: Pollen count and pollen fertility data.

Taxon	Specimen	Floret	Pollen count (grains/floret)	Pollen fertility (%)
S. engleranus	*eng* 3/29	a	3060	99.64
S. engleranus	*eng* 3/29	b	3691	98.63
S. engleranus	*eng* 3/29	c	3046	99.2
S. engleranus	*eng* 3/29	d	3779	98.44
S. engleranus	*eng* 3/29	e	3396	100
S. engleranus	*eng* 3/29	f	-	99.64
S. engleranus	*eng* 3/29	g	-	97.83
S. engleranus	*eng* 3/29	h	-	99.24
S. engleranus	*eng* 3/29	i	-	99.39
S. engleranus	*eng* 3/29	j	-	98.03
S. flavus	SF751	a	651	99.08
S. flavus	SF751	b	531	97.62
S. flavus	SF751	c	388	68.57
S. flavus	SF751	d	655	94.32
S. flavus	SF751	e	251	59.77
S. flavus	SF751	f	-	99.08
S. flavus	SF751	g	-	91.52
S. flavus	SF751	h	-	94.47
S. flavus	SF751	i	-	97.1
S. flavus	SF751	j	-	95.22
F1 hybrid	SF751 x *eng* 3/29 (1)	a	358	65
F1 hybrid	SF751 x *eng* 3/29 (1)	b	360	60.5
F1 hybrid	SF751 x *eng* 3/29 (1)	c	398	60
F1 hybrid	SF751 x *eng* 3/29 (1)	d	399	58.4
F1 hybrid	SF751 x *eng* 3/29 (1)	e	530	52.6
F2 hybrid	01	a	2107	97.7
F2 hybrid	01	b	2278	99.5
F2 hybrid	01	c	1441	96.9
F2 hybrid	01	d	2132	99
F2 hybrid	02	a	2224	92.5
F2 hybrid	02	b	2121	86.9
F2 hybrid	02	c	2255	83.6
F2 hybrid	02	d	2151	86.9
F2 hybrid	03	a	2147	88.6
F2 hybrid	03	b	2267	83.4
F2 hybrid	03	c	2079	94.8
F2 hybrid	05	a	762	82.4
F2 hybrid	05	b	1050	81.1
F2 hybrid	05	c	1784	82.6
F2 hybrid	05	d	482	82
F2 hybrid	06	a	1589	81.6
F2 hybrid	06	b	1007	78.2
F2 hybrid	06	c	1084	78.1
F2 hybrid	06	d	1340	80.1
F2 hybrid	13	a	2134	74.6
F2 hybrid	13	b	2784	72.5
F2 hybrid	13	c	1734	79.7
F2 hybrid	13	d	2057	76.2
F2 hybrid	14	a	1848	96.5
F2 hybrid	14	b	2222	95
F2 hybrid	14	c	2543	92.9
F2 hybrid	14	d	2700	92.8
F2 hybrid	19	a	1559	98.7
F2 hybrid	19	b	1684	99.1
F2 hybrid	19	c	1013	97.8
F2 hybrid	19	d	2014	99.2
F2 hybrid	23	a	1520	95.1
F2 hybrid	23	b	1370	91.2
F2 hybrid	23	c	1345	88.3
F2 hybrid	23	d	1605	92
F2 hybrid	27	a	727	69.1
F2 hybrid	27	b	1057	72
F2 hybrid	27	c	1964	74.2
F2 hybrid	27	d	984	84.2
F2 hybrid	28	a	1893	95.9
F2 hybrid	28	b	1490	95.5
F2 hybrid	28	c	1918	94.6

Taxon	Specimen	Floret	Pollen count (grains/floret)	Pollen fertility (%)
F2 hybrid	28	d	1837	96.4
F2 hybrid	31	a	1932	96.2
F2 hybrid	31	b	1822	95.4
F2 hybrid	31	c	1116	97.2
F2 hybrid	31	d	1762	96.4
F2 hybrid	32	a	2259	94.9
F2 hybrid	32	b	1918	85
F2 hybrid	32	c	1807	88.6
F2 hybrid	32	d	1693	93.9
F2 hybrid	33	a	2323	77
F2 hybrid	33	b	2208	74.9
F2 hybrid	34	a	1610	97.1
F2 hybrid	34	b	1804	97.7
F2 hybrid	34	c	2377	94.9
F2 hybrid	34	d	1532	94.2
F2 hybrid	35	a	1710	69.9
F2 hybrid	35	b	1850	64.7
F2 hybrid	35	c	1783	70.8
F2 hybrid	35	d	1802	74.2
F2 hybrid	36	a	1809	73.2
F2 hybrid	36	b	2188	70.7
F2 hybrid	36	c	1922	65.9
F2 hybrid	36	d	1000	71.6
F2 hybrid	38	a	1408	69.5
F2 hybrid	38	b	1551	69.9
F2 hybrid	38	c	1474	64.9
F2 hybrid	38	d	1416	70
F2 hybrid	39	a	1978	95.8
F2 hybrid	39	b	1487	98.6
F2 hybrid	39	c	2070	96.7
F2 hybrid	39	d	1662	98.1
F2 hybrid	40	a	1788	87.1
F2 hybrid	40	b	2083	96.3
F2 hybrid	40	c	2212	96.6
F2 hybrid	40	d	1651	92.8
F2 hybrid	42	a	929	91.6
F2 hybrid	42	b	1849	92.7
F2 hybrid	42	c	2496	92.6
F2 hybrid	42	d	1629	92.9
F2 hybrid	44	a	1583	96.8
F2 hybrid	44	b	2212	99.3
F2 hybrid	44	c	1955	94.6
F2 hybrid	44	d	1456	98.8
F2 hybrid	46	a	3053	99.1
F2 hybrid	46	b	2975	99.6
F2 hybrid	46	c	2857	99.3
F2 hybrid	46	d	2585	98.8
F2 hybrid	48	a	1689	84.4
F2 hybrid	48	b	1616	85.3
F2 hybrid	56	a	2677	99
F2 hybrid	56	b	2440	99.2
F2 hybrid	58	a	1419	74.7
F2 hybrid	58	b	1382	67.3
F2 hybrid	59	a	2124	60.6
F2 hybrid	59	b	2608	63.5
F2 hybrid	62	a	2814	98.9
F2 hybrid	62	b	2666	96.3
F2 hybrid	67	a	1878	67.7
F2 hybrid	67	b	1943	70.7
F2 hybrid	69	a	2053	64
F2 hybrid	69	b	1731	64.6
F2 hybrid	70	a	2008	58.7
F2 hybrid	70	b	1584	54.7
F2 hybrid	70	c	1969	59.3
F2 hybrid	82	a	1072	62.3
F2 hybrid	82	b	1475	82.1
F2 hybrid	82	c	1233	70.5
F2 hybrid	82	d	1520	76.2

Appendix 3: RAPD Data Matrix

taxon / band	A02 (1)	A02 (2)	A02 (3)	A02 (4)	A07 (1)	A07 (2)	A07 (3)	A07 (4)	A09 (1)	A09 (2)	A09 (3)	A09 (4)	A13 (1)	A13 (2)	A13 (3)	B08 (1)	B08 (2)	B08 (3)	B08 (4)	B09 (1)	B09 (2)	B09 (3)	B09 (4)	B09 (5)
eng2/4	0	1	1	1	0	1	1	1	1	1	0	0	1	1	1	1	1	0	1	0	0	1	0	0
eng2/9	0	1	1	0	0	1	1	1	0	1	0	0	0	0	1	1	1	0	1	0	0	0	0	0
eng2/11	0	1	1	0	0	0	1	1	0	1	0	0	0	0	0	1	1	0	1	0	0	1	0	0
eng2/24	0	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	0	1	0	0	1	0	0
eng2/13	0	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	0	1	0	0	1	0	0
eng3/5	0	1	1	1	0	1	1	1	1	1	0	0	1	1	1	1	1	0	1	0	0	0	0	0
eng3/7	0	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	0	1	0	0	1	0	0
eng3/21	0	1	1	0	0	0	1	1	0	1	0	0	0	0	1	1	1	0	1	0	0	1	0	0
eng3/25	0	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	0	1	0	0	1	0	0
eng3/31	0	1	1	1	0	1	1	1	1	0	0	0	0	1	1	1	1	0	1	0	0	1	0	0
eng3/43	0	1	1	0	0	1	1	1	0	1	0	0	0	0	1	1	1	0	1	0	0	1	0	0
eng3/44	0	1	1	1	0	1	1	1	0	0	0	0	0	1	1	1	1	0	1	0	0	1	0	0
eng3/45	0	1	1	0	0	1	1	1	0	1	0	0	0	0	1	1	1	0	0	0	0	0	0	0
eng3/49	0	1	1	0	0	0	1	1	0	1	0	0	0	0	1	1	1	0	1	0	0	1	0	0
eng3/60	0	1	1	1	0	1	1	1	1	0	0	0	0	1	1	1	1	0	1	0	0	1	0	0
eng4/1	0	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	0	0	0	0	1	0	0
eng4/2	0	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	0	1	0	0	1	0	0
eng4/3	0	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	0	1	0	0	1	0	0
eng4/4	0	1	1	1	0	1	1	1	1	1	0	0	0	0	1	1	1	0	1	0	0	0	0	0
eng2/2	0	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	0	1	0	0	1	0	0
eng3/29	0	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	0	1	0	0	1	0	0
SF751xeng3/29(1)	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
SF751xeng3/29(2)	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
SF5xeng2/2(1)	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1
SF5xeng2/2(3)	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
SF5	1	0	0	1	1	1	0	0	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1
SF751	1	0	0	1	1	1	0	0	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1
SF3	1	0	0	1	0	0	0	0	0	1	1	1	0	0	1	0	0	1	1	1	1	0	1	1
SF7	1	0	0	1	0	1	0	0	0	1	1	1	0	0	1	0	0	1	1	1	1	1	1	1
SF15	1	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	1	1	1	1	0	1	1
SF16	1	0	0	1	1	1	0	0	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1
SF22	1	0	0	0	1	1	0	0	0	1	1	1	1	0	1	0	0	1	1	1	1	0	1	1
fl14588	1	0	0	1	0	1	0	0	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1
fl26145	1	0	0	1	0	1	0	0	0	1	1	1	0	0	1	0	0	1	1	1	1	0	1	1
flSinai	1	0	0	1	1	1	0	0	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1
fl14454	1	0	0	1	1	1	0	0	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1

taxon / band	B12 (1)	B12 (2)	B12 (3)	B12 (4)	B12 (5)	B15 (1)	B15 (2)	B15 (3)	B15 (4)	B17 (1)	B17 (2)	B17 (3)	B17 (5)	B17 (6)	B17 (7)	B17 (8)	C08 (1)	C08 (2)	C09 (1)	C09 (2)	C09 (3)	C18 (1)	C18 (2)	C18 (3)
eng2/4	0	1	1	1	0	1	1	0	1	1	0	1	0	1	0	0	1	0	0	1	0	0	1	1
eng2/9	0	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0	1	0	0	0	1
eng2/11	0	0	1	1	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1
eng2/24	1	1	1	1	0	1	1	0	1	1	1	0	0	1	0	0	1	0	0	1	0	0	1	1
eng2/13	1	1	1	1	0	1	1	0	1	1	1	1	0	1	0	0	1	0	0	1	0	1	1	1
eng3/5	1	1	0	1	0	1	1	0	1	1	1	0	0	1	0	0	1	0	0	1	0	0	1	1
eng3/7	1	1	0	1	0	1	1	0	1	1	1	0	0	1	0	0	1	0	0	1	0	0	1	1
eng3/21	0	1	1	1	0	0	1	0	1	0	0	1	0	1	0	0	1	0	0	1	0	0	1	1
eng3/25	1	1	1	1	0	1	1	0	1	1	1	1	0	1	0	0	1	0	0	1	0	0	1	1
eng3/31	0	1	1	1	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0	1	0	0	1	1
eng3/43	0	0	0	1	0	1	1	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1
eng3/44	0	1	1	1	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0	1	0	0	1	1
eng3/45	0	0	0	1	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
eng3/49	0	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0	1	0	0	0	1
eng3/60	1	1	1	1	0	1	1	0	1	0	0	0	0	1	0	0	1	0	0	1	0	0	1	1
eng4/1	1	1	1	1	0	1	1	0	1	1	1	1	0	1	0	0	1	0	0	1	0	1	1	1
eng4/2	1	1	1	1	0	1	1	0	1	1	1	1	0	1	0	0	1	1	0	1	0	1	1	1
eng4/3	1	1	1	1	0	1	1	0	1	1	1	1	0	1	0	0	1	0	0	1	0	0	1	1
eng4/4	0	1	1	1	0	0	1	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0
eng2/2	1	1	0	1	0	1	1	0	1	1	1	1	0	1	0	0	1	0	0	1	0	0	0	1
eng3/29	1	1	0	1	0	1	1	0	1	1	1	1	0	1	0	0	1	0	0	1	0	0	0	1
SF751xeng3/29(1)	1	1	1	1	0	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
SF751xeng3/29(2)	1	1	1	1	0	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
SF5xeng2/2(1)	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SF5xeng2/2(3)	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SF5	0	0	1	0	1	1	0	1	1	1	1	0	1	0	1	1	1	1	1	0	1	1	1	0
SF751	0	0	1	0	1	1	0	1	1	1	1	0	1	0	1	1	1	1	1	0	1	1	1	0
SF3	0	1	0	0	1	1	0	1	1	0	0	0	1	0	1	1	0	0	1	0	1	0	0	1
SF7	0	0	0	0	1	0	0	1	1	0	0	0	1	0	1	1	0	1	1	0	1	0	1	1
SF15	0	0	1	0	1	0	0	1	1	0	0	1	1	0	1	1	0	0	1	0	1	0	0	0
SF16	1	1	1	0	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1
SF22	0	0	1	0	1	0	0	1	0	0	0	0	1	0	1	1	0	0	1	0	1	0	0	1
fl14588	0	1	1	0	1	0	0	1	1	0	0	1	1	0	1	1	1	1	1	0	1	0	1	1
fl26145	0	0	1	0	1	0	0	1	1	0	0	0	1	0	1	1	1	1	1	0	1	0	0	1
flSinai	1	1	1	0	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1
fl14454	0	1	1	0	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	0	1

Appendix 4: Morphometric Data

Character / specimen	SF751 x *eng* 3/29 (1)	SF751 x *eng* 3/29 (2)	SF5 x *eng* 2/2 (1)	SF5 x *eng* 2/2 (3)	SF20	SF3	SF4	SF26	SF7	SF12	SF15	SF19	fl26145
C1 Plant height (mm)	340	390	285	295	190	200	280	240	250	265	310	225	135
C2 Infl. l*eng*th (mm)	16	24	19	19	16	17	16	15	19	16	14	10	21
C3 Peduncle l*eng*th (mm)	8	16	11	11	6	7	10	6	12	9	9	3	11
C4 Capitulum l*eng*th (mm)	8	8	8	8	9	10	8	9	7	7	5	7	10
C5 Capitulum width (mm)	6	5	5	6	2.5	3	3	2	3	2.8	2.8	3	3
C6 No. of phyllaries	13	13	13	13	13	12	12	12	12	12	12	12	13
C7 Prop. of black tipped phyllaries	1	1	1	1	1	1	1	1	1	1	1	1	1
C8 No. of calyculus bracts	6	8	7	8	6	5	5	5	5	5	5	4	5
C9 Mean calyculus bract l*eng*th (mm)	1.2	1.3	1.2	1	1	1.5	1.3	1.1	0.9	0.7	0.9	0.8	0.9
C10 Longest leaf l*eng*th (mm)	32	27	24	28	43	42	45	39	35	40	38	34	46
C11 Midleaf l*eng*th (mm)	22	19	26	18	34	21	18	34	36	32	30	29	46
C12 Number of midleaf lobes	15	16	16	16	19	15	13	-			23	15	19
C13 Midleaf apical angle (º)	50	55	65	60	58	68	56	-	69	65	43	50	65
C14 Midleaf secondary vein angle (º)	55	40	40	50	40	50	54	-	63	70	63	56	46
C15 Standardised leaf perimeter	5	5	7	5	18	-	-	-	-	-	12	16	13
C16 Standardised square of leaf area	2	1	2	1	13						6	5	6
C17 Number of peduncle bracts	3	3	3	3	1	1	1	1	1	3	2	3	2
C18 Pecentage of water in midleaf	84.2	88.6	85.7	91.4	69.7	-	-	-	-	-	83.9	83.7	90.5

Character / specimen	fl14454	*eng*2 (9)	*eng*2 (4)	*eng*2 (6)	*eng*2 (8)	*eng*2 (11)	*eng*2 (13)	*eng*2 (14)	*eng*2 (15)	*eng*2 (16)	*eng*3 (5)	*eng*3 (60)	*eng*3 (45)	*eng*3 (43)
C1 Plant height (mm)	215	210	125	190	130	150	170	175	200	150	189	180	220	95
C2 Infl. l*eng*th (mm)	19	24	18	22	10	19	11	21	14	20	31	25	17	19
C3 Peduncle l*eng*th (mm)	12	13	9	11	5	7	5	13	6	10	21	15	9	10
C4 Capitulum l*eng*th (mm)	7	11	9	11	5	12	6	8	8	10	10	10	8	9
C5 Capitulum width (mm)	2.8	4.5	4	4	4	4	4	3.5	4.5	6	5	5	4	5
C6 No. of phyllaries	11	12	10	12	10	12	11	8	14	9	12	13	13	11
C7 Prop. of black tipped phyllaries	1	1	1	1		1	1	1	1	1	1	1	0.67	1
C8 No. of calyculus bracts	5	8	7	6	4	6	7	5	6	8	5	6	8	8
C9 Mean calyculus bract l*eng*th (mm)	0.7	0.6	0.8	0.8	0.7	0.7	0.8	0.7	0.4	0.5	0.8	0.6	0.7	0.4
C10 Longest leaf l*eng*th (mm)	37	26	15	20	25	24	27	13	30	22	28	20	27	17
C11 Midleaf l*eng*th (mm)	30	5	11	21	10	11	17	6	9	17	16	20	11	7
C12 Number of midleaf lobes	22	10	10	14	11	10		10	10	13	7	9		7
C13 Midleaf apical angle (º)	70	50	35	50	50	25		45	43	45	49	55	47	45
C14 Midleaf secondary vein angle (º)	43		47	28	60	40		23	22	33	50	50	35	
C15 Standardised leaf perimeter	13	2	4	10	4	6		4	4	7	6	6		4
C16 Standardised square of leaf area	5	1	1	3	1	1		1	1	2	1	2		1
C17 Number of peduncle bracts		2	2	1	1	0	2	1	2	1	1	2	2	2
C18 Pecentage of water in midleaf		89.3	82.4	88.9	92.9	91.8	96	92	92.6	92	93.1	90.7		92.9

Character / specimen	*eng*3(31)	*eng*3(49)	*eng*3(25)	*eng*3(21)	*eng*3(7)	*eng*3(44)
C1 Plant height (mm)	145	140	170	190	185	155
C2 Infl. l*eng*th (mm)	15	16	21	15	25	20
C3 Peduncle l*eng*th (mm)	6	7	13	5	16	12
C4 Capitulum l*eng*th (mm)	9	9	8	10	9	8
C5 Capitulum width (mm)	5	5	4	5	4	4
C6 No. of phyllaries	12	13	13	13	13	8
C7 Prop. of black tipped phyllaries	1	0.69	1	1	1	1
C8 No. of calyculus bracts	6	7	5	5	5	6
C9 Mean calyculus bract l*eng*th (mm)	0.3	0.7	0.4	0.3	2.3	2.1
C10 Longest leaf l*eng*th (mm)	18	22	24	29	17	25
C11 Midleaf l*eng*th (mm)	16	15	22	17	16	21
C12 Number of midleaf lobes	8	7	6		9	10
C13 Midleaf apical angle (º)	50	50	38	55	50	52.5
C14 Midleaf secondary vein angle (º)	36	53	37	22	52.5	37.5
C15 Standardised leaf perimeter	5	6	10		7	8
C16 Standardised square of leaf area	1	1	3		2	3
C17 Number of peduncle bracts	1	2	2	1	1	1
C18 Pecentage of water in midleaf	92.5	97.7	93.6	89.9	88.7	88.5