Molecular systematics of the Western Cape genus *Serruria* Salisb. (Proteaceae L.) based on DNA sequence data

By Margaret J. de Villiers

A thesis presented in partial fulfilment
of the requirements for the degree
of Masters of Science
at the University of Stellenbosch

NATIONAL BOTANICAL INSTITUTE

UNIVERSITEIT STELLENBOSCH UNIVERSITY

Supervisors: Dr G Reeves,
Dr LL Dreyer and
Dr EM Marais

April 2004

Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously, in its entirety or in part, submitted it at any university for a degree.

Abstract

The Cape Floristic Region (CFR) is situated at the southern tip of Africa and possesses a flora that is unique amongst the floras of the rest of the world, both in terms of its incredibly high species richness, and its high levels of endemism. Proteaceae, the family to which *Serruria* belongs, is widely distributed amongst the landmasses of the southern hemisphere, with its centres of diversity occurring in Australia and southern Africa.

Previous molecular and morphological analyses performed on the South African subfamily Proteoideae have shown *Serruria,* a CFR endemic, to form a well-supported monophyletic group. Based upon the strong monophyly of *Serruria,* DNA sequence data were collected for 53 of the 55 species from the plastid (*rps16* intron, *atpB-rbcL* intergenic spacer, *trnL-F* region and *psbA-trnH* intergenic spacer) and nuclear (internal transcribed spacer region or ITS) genomes in order to investigate evolutionary relationships within the genus. *Spatalla* taxa were used as the outgroup.

Both parsimony and Bayesian analyses were carried out on each of these data sets. The resulting trees were reasonably well resolved. All the *Serruria* taxa grouped together in a well-supported clade, except for *S. flava*, which emerged well within the *Serruria* clade in the analyses of the nuclear genome, but outside the clade in the plastid analyses. It was therefore proposed that this taxon represents a hybrid. Apart from this case, there was widespread agreement between the trees reconstructed using data from the two genomes. The plastid and nuclear data were therefore combined in order to analyse the data sets together.

The molecular data does not support most of the groupings proposed by previous authors based on morphological data. Additionally, in some cases, multiple representatives of species do not group together. These specimens probably do not represent monophyletic taxa. Current ideas about relationships within *Serruria* are based predominantly on floral characters, and it is suggested that pollinator pressures have led to plasticity in the floral characters.

Consequently, it is evident from this study that relationships within *Serruria* need to be re-examined in order to determine the patterns of evolution within the genus.

Opsomming

Die Kaapse Floristiese Streek is aan die suiderpunt van Afrika geleë, en beskik oor 'n unieke flora relatief tot ander wêreldfloras, beide ten opsigte van die ongelooflike hoë spesie diversiteit en die hoë vlakke van endemisme. Proteaceae, die familie waaraan *Serruria* behoort, kom wydverspreid tussen die vastelande van die Suidelike Halfrond voor, en het diversiteitsentrums in Australië en suider Afrika.

Vorige molekulêre sowel as morfologiese analises wat op die Suid-Afrikaanse subfamilie Proteoideae uitgevoer is, dui aan dat *Serruria* (wat endemies is tot die Kaapse Floristiese Streek) 'n goed ondersteunde monofiletiese groep is. Gebaseer op die sterk monofilie van *Serruria,* is DNA-volgorde-data vir 53 van die 55 spesies vanuit die plastied (*rps16* intron, *atpB-rbcL* intergeniese spasie, *trnL-F* area en *psbA-trnH* intergeniese spasie) en kern (intern getranskribeerde spasie area, ook ITS genoem) ingewin om die evolusionêre verwantskappe binne die genus te ondersoek. *Spatalla* is as die buitegroep gebruik.

Beide parsimonie en Bayesian analises is op elk van hierdie datastelle uitgevoer. Die resulterende bome het redelike hoë resolusie getoon. Al die *Serruria*-taxa het in 'n goed ondersteunde klade saam gegroepeer, behalwe vir *S. flava*, wat binne die *Serruria* klade val vir die kern genoom, maar buite die klade vir die plastied analise. Dit is dus voorgestel dat hierdie taxon as 'n hibried beskou mag word. Behalwe vir hierdie geval, was daar wydverspreide ooreenstemming tussen die bome wat verkry is vanaf data van die twee genome. Die plastied- en kern-data is derhalwe gekombineer om die datastelle saam te kan analiseer.

Die molekulêre data ondersteun nie die meerderheid van morfologiese groeperings wat deur verskeie outeurs voorgestel is nie. Verder, in sommige gevalle, groepeer verskillende monsters van dieselfde spesie nie bymekaar nie. Dit is derhalwe voorgestel dat hierdia taxa nie monofileties is nie. Huidige idees omtrent die verwantskappe binne *Serruria* is grotendeels op blommorfologiese kenmerke gebaseer, en dit word voorgestel dat bestuiwing-druk gelei het tot plastisiteit van die blommorfologiese kenmerke. Verskille tussen die bome wat uit plastied- en kern-data gerekonstrueer is word aan vroeëre hibridisasie gebeure toegeskryf.

Op grond van hierdie studie is dit duidelik dat die verhoudings binne *Serruria* verder ondersoek moet word om die patrone van evolusie binne die genus te bepaal.

Acknowledgements

I am deeply grateful to my supervisors – Dr Gail Reeves, Dr Léanne Dreyer and Dr Elisabeth Marais – whose encouragement, support and advice were invaluable to the completion of this project.

This project would not have been possible without the grants provided by the University of Stellenbosch and the National Botanical Institute (NBI), which financed both my research and upkeep.

Many other people have also contributed to this project in significant ways:

- I am very grateful to Dr Tony Rebelo and Dr John Rourke, who collected most of the specimens included in this project and provided me with invaluable advice concerning the morphology and ecology of the various taxa. I would not have been able to complete this project without their imputs. Other field trip companions who were a great help to me include Ruth Smart, Christopher Cupido, Adrian Möhl and Nigel Forshaw.
- My fellow lab colleagues, Ferozah Conrad and Angeline Khunou, were always very helpful, congenial and supportive throughout my term at the NBI.
- Walter Smit helped me to construct the map depicting the distribution of *Serruria*.
- The rest of the staff at the NBI (Kirstenbosch) were friendly and always willing to provide me with help when it was required.
- Visitors to the Leslie Hill Molecular Systematics Lab gave me the opportunity to increase my understanding of molecular systematics by asking me numerous questions.
- Finally, my family, whose love of nature first stimulated my interest in the biological sciences, has always been there for me. Their help, support, proof-reading and advice have been invaluable to me over the past two years.

Contents

Figures

Tables

Chapter 1: Introduction

The Cape Floristic Region (CFR; Goldblatt 1978) is situated at the southern tip of Africa (Figure 1.1), stretching from the Bokkeveld escarpment in the north to Port Elizabeth in the east. Since European botanists first arrived in South Africa, the Cape has been recognised as possessing a truly unique flora. Although it comprises an area of only 90 000 km^2 (Goldblatt 1997), the CFR flora is so different from the floras of the rest of the world and the regions bordering it, that it is thought by some (e.g. Good 1974) to constitute one of six floral kingdoms of the world.
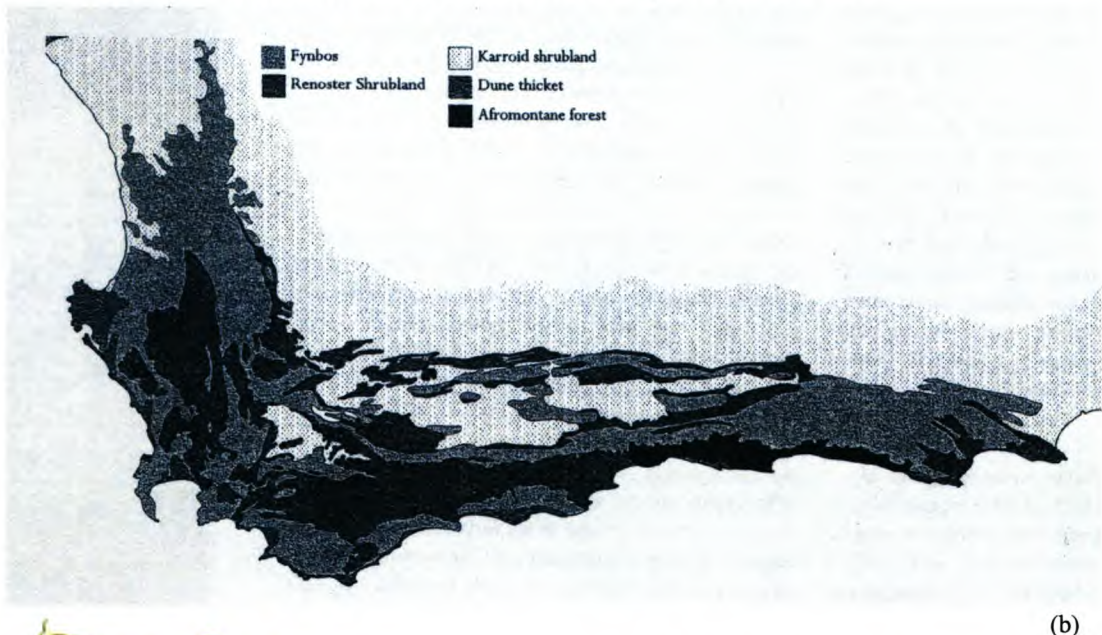
Figure 1.1: The Cape Floristic Region located at the tip of southern Africa. (a) The southern African subcontinent with the Cape Floristic Region demarcated and (b) the five vegetation communities that constitute the Cape Floristic Region (adapted from Louw & Rebelo 1996).

One of the factors contributing to the uniqueness of the CFR is its high levels of endemism, with approximately 69% of the 8 920 angiosperm species indigenous to the region being endemics (Goldblatt & Manning 2002). The CFR also possesses five distantly related endemic families (although proposed changes to the delimitation of Stilbaceae suggested by Olmstead *et al.* (2001) will negate its endemic status) and a few near endemics e.g. Bruniaceae. In contrast to this, the whole of southern Africa has only ten endemic families (Goldblatt & Manning 2002). In fact, the levels of endemism found in the CFR more closely mimic that of islands than other continental areas (Linder 2003).

The focus of this thesis is the CFR genus, *Serruria* Salisb. Belonging to the Proteaceae and comprising *ca.* 55 species, *Serruria* is the largest genus of this family endemic to the CFR. It has been chosen as the subject of this study for several reasons. Firstly, a strong taxonomic foundation for the genus exists, vital for evaluating current ideas of relationships in light of new molecular evidence. Secondly, in order to evaluate the role of ecological factors in diversification, both a comprehensive species-level phylogenetic hypothesis, and detailed ecological data relating to habitat preferences and distributional data are needed. Both geographical range and ecological attributes have been recorded extensively for the Proteaceae by the Protea Atlas Project (www.nbi.ac.za/protea), and it is the aim of this study to reconstruct a species-level phylogeny of *Serruria* using DNA sequence data. Although this thesis does not deal with causes of diversification within *Serruria*, this aspect will form the focus of further study and was influential in choosing this Cape genus.

The following sections provide an introduction to the family Proteaceae and its largest Cape endemic genus, *Serruria*. This is followed by a discussion of current hypotheses dealing with possible causes of speciation within *Serruria* and other taxa in the CFR, as well as the use of molecular systematics as a tool for phylogenetic reconstruction. The chapter finally highlights specific questions that will be addressed during this study, in addition to the layout of the rest of the thesis.

Proteaceae

Proteaceae, the family to which *Serruria* belongs, is one of the most prominent angiosperm families in the southern hemisphere and almost all of the *ca.*1 700 species (79 genera; Douglas 1995) of this group are confined to this region (Rebelo 2001). The family is widely distributed amongst the southern land masses (Vogts 1960), but its centres of greatest diversity occur in Australia and southern Africa (Douglas 1995).

Morphologically Proteaceae can be recognised by their flowers, which are always monochlamydeous (with the perianth composed of one whorl of accessory parts known as tepals) and perigynous (flowers in which a cuplike or tubular structure surrounds the gynoecium). The flowers possess four stamens that are either positioned opposite or attached to four tepals; longitudinally dehiscent anthers; a terminal, simple style and a superior ovary with a single carpel and parietal placentation (Rao 1971; Judd 1999). These shared floral characteristics, as well as the arrangement of the flowers in the inflorescence of the member taxa, have led to a general consensus that the family forms a monophyletic clade within the angiosperms (Hoot & Douglas 1998).

Owing to their distribution across the southern continents, Proteaceae are thought to represent an ancient Gondwanan lineage. The most recent classification (Douglas 1995) recognises seven sub-families, namely Persoonioideae (hypothesised by Johnson and Briggs (1975) to contain members

of the family possessing the most primitive characteristics), Bellendinoideae (hypothesised by Roa (1971) to contain members of the family possessing the most primitive characteristics), Eidotheoideae, Sphalmioideae, Carnarvonioideae, Grevilleoideae and Proteoideae. Of these sub-families, only Proteoideae and Grevilleoideae are represented on the African continent. The two monotypic genera, *Brabejum* L. (also spelt *Brabeium*; a Cape endemic) and *Malagasia* L. Johnson & B. Briggs (occurring in Madagascar), have been assigned to Grevilleoideae, while the remaining African species have been placed within Proteoideae (Rourke 1998).

Hoot and Douglas (1998) carried out a phylogenetic analysis of 46 taxa representing all currently recognised subfamilies and tribes of Proteaceae using DNA sequences from the *atpB* exon and the *atpB-rbcL* intergenic spacer. The combined analysis of sequences from both regions showed limited congruence with morphological characters and chromosome numbers. However, it did appear to agree with previously hypothesised chromosome doubling and multiple aneuploidy events. In the combined analysis, they found that Bellendinoideae formed a weakly-supported sister group to the rest of the family. This group was proposed by Rao (1971) as being the most primitive member of the family based on the absence of certain morphological characters. Of the three multigeneric subfamilies (Persoonioideae, Grevilleoideae and Proteoideae), only Persoonioideae was found to be monophyletic in the strict consensus tree. Eidotheoideae was found to be embedded within Proteoideae, and both Sphalmioideae and Carnarvonioideae were strongly supported as being embedded within the Grevilleoideae. Within Proteoideae, none of the tribes recognised by Douglas (1995) were found to be monophyletic.

Proteaceae in Africa

Of the approximately 400 Proteaceae species in 14 genera indigenous to Africa, 331 are endemic to the CFR (Rourke 1998). In 1912, Phillips and Hutchinson revised the Proteaceae occurring in the CFR. They divided the 296 known species amongst 14 genera. During subsequent years, morphological revisions (Levyns 1970; Rourke 1969, 1972, 1976, 1980, 1982a, 1984a, 1984b, 1987 & 1996; Williams 1972) have been compiled for all but two of the South African Proteaceae genera - *Serruria* and the South African members of *Faurea* Harv. Only two changes have been made at the generic level since the revision by Phillips and Hutchinson (1912), namely the members of *Spatallopsis* Phillips have been included in *Spatalla* Salisb. (Rourke 1969), and Rourke (1984b) established the new genus, *Vexatorella* Rourke, to accommodate some species that were previously placed in *Leucospermum* R.Br.

Currently, the 14 South African Proteaceae genera, *Protea* L., *Aulax* PJ Bergius, *Faurea*, *Serruria*, *Paranomus* Salisb., *Sorocephalus* R.Br., *Spatalla*, *Leucospermum*, *Diastella* Salisb., *Vexatorella*, *Mimetes* Salisb., *Orothamnus* Pappe, *Leucadendron* R.Br. and *Brabejum*, are delimited, primarily according to floral arrangements. Representatives from each of these genera are shown in Figure 1.2 (p. 22). *Hakea* Schrad. and *Grevillea* R.Br. *ex* Knight are also found in South Africa, but have both been introduced by man from Australia. Of the genera occurring naturally in Africa, all

excepting *Protea*, *Faurea* and *Leucospermum* are endemic to South Africa (Vogts 1960). There are no indigenous genera common to both Africa and Australia (Vogts 1982).

For a better understanding of the relationships of the African genera to the rest of the family, Reeves (2001) added *atpB-rbcL* intergenic spacer sequences from a further 20 African Proteaceae species representing each of the Cape Proteaceae genera (except *Sorocephalus*) to the sequences collected by Hoot & Douglas (1998). In this analysis, all the African Proteoideae genera formed a monophyletic group along with the Australasian Proteoideae genera *Isopogon* R.Br. *ex* Knight, *Adenanthos* Labill., *Petrophile* R.Br. *ex* Knight and *Cenarrhenes* Labill. However, because this tree was built using information from only one plastid non-coding region, relationships within this clade remained unresolved. Therefore, Reeves (2001) sequenced an additional three non-coding regions from the plastid genome (*trnL-F* intergenic spacer, *trnL* intron and *rps16* intron) for the African Proteoideae genera to provide a more accurate estimation of relationships. In this phylogenetic tree, *Protea* and *Faurea* were resolved as sister to one another, in turn sister to a clade containing the remaining African genera, along with *Adenanthos* and *Isopogon*. Within this latter clade, *Isopogon* and *Adenanthos* formed a paraphyletic grade, with the remainder of the African genera forming the terminal clade. The only strongly supported pattern within this African clade was the placement of *Leucadendron* as sister to the remainder of the group. These patterns of relationships were congruent with a similar investigation by Barker *et al.* (2002) using information from the internal transcribed spacer (ITS) region of nuclear DNA.

In both the plastid and nuclear analyses, many species of the same genus were not resolved as monophyletic groups. Thus, in order to investigate patterns of relationships within the African Proteaceae further, Reeves (pers. comm.) collected DNA sequence data from four non-coding plastid DNA regions (*trnL-F* intergenic spacer, *trnL* intron, *rps16* intron and *atpB-rbcL* intergenic spacer) for 230 African Proteaceae taxa. These results demonstrated *Serruria*, *Leucadendron*, *Protea*, *Faurea* and *Aulax* to form strongly supported monophyletic groups. However, generic delimitations for the remainder of the Proteaceae in South Africa were less clear, and warrant further sampling of both taxa and sequence data (Figure 1.3, p. 23).

Rourke (1998) conducted a cladistic analysis of 18 representatives of Proteoideae tribe *Proteea* using morphological characters. The analysis included representatives of three groupings within *Serruria*, which formed a monophyletic clade within a group also containing *Spatalla*, *Sorocephalus* and *Paranomus*, named the '*Serruria*' group. Within this group, *Serruria* formed a sister clade to *Paranomus*. However, Rourke (1998) did not include many of the Australian taxa that are believed to be closely related to the Cape genera in his analysis. He also assumed that the tribe to be monophyletic, which Hoot & Douglas (1998) found not to be the case according to sequence data from the *atpB* exon and the *atpB-rbcL* intergenic spacer. Additionally, many of the morphological characters were homoplasious in the strict consensus tree, and most of the nodes lacked homologous synapomorphies (Hoot & Douglas 1998).

in summary, both molecular and morphological cladistic analyses support *Serruria* as a monophyletic group within the Proteaceae.

Serruria

Also known informally as the spiderheads, *Serruria* is the largest Proteaceae genus endemic to the CFR (Rourke 1994; Figure 1.4, p. 24). It comprises approximately 55 species, all of which are characterised by a shrub growth habit; leaves that are usually pinnately dissected with terete segments, but occasionally entire (e.g. *S. heterophylla* Meisn.); an inflorescence surrounded by involucral bracts; bisexual, usually regular flowers with sessile anthers and ovary, and a pubescent achene with a beaked apex and pedicellate base (Rourke 2000a).

Morphological variation in *Serruria*

Within *Serruria*, a number of characters display significant variation (Figure 1.5, p. 25) of which the most notable is in the inflorescence. The inflorescence (or flowerhead) is in the form of a panicle (e.g. *S. adscendens* (Lam.) R. Br.), terminal capitulum (e.g. *S. gremialis* Rourke) or a panicle of capitula or headlets (e.g. *S. altiscapa* Rourke) (Rourke 2000a). The flower head stalk ranges in length from absent or hidden by dense leaves at the base of the inflorescence (as in *S. brownii* Meisn.) to 90 cm long (as in *S. altiscapa*). In some species e.g. *S. florida* (Thunb.) Salib. *ex* Knight, the involucral bracts form a conspicuous, often brightly coloured "skirt" around the flowerhead, while in others, like *S. aitonii* R.Br., the bracts are so small as to be inconspicuous. The number of flowers per headlet ranges between 2–7 in *S. meisneriana* Schltr. and 10–100 in *S. williamsii* Rourke. The flower orientation ranges from upright when in bud, in which case the flowers are often clustered in loose headlets on short stalks (e.g. *S. fasciflora* Salisb. *ex* Knight), to strongly curved when in bud (e.g. *S. incrassata* Meisn.). Other variable floral characters are floral bract size and flowerhead shape. The style is also occasionally hairy (Rebelo 2001).

Vegetative parts of *Serruria* plants that display significant variation include the stem and leaves. The stem is erect in most species, including *S. hirsuta* R.Br., but prostrate in a few species, with leaves arising vertically e.g. in *S. decumbens* (Thunb.) R.Br. The leaves are usually fine and thin, but in *S. triternata* (Thunb.) R.Br. the leaves are large and stout (Rebelo 2001). Leaf and stem size and indumentum are also variable characters that have been used to delimit species.

Unfortunately, morphologically diverse species complicate the classification of *Serruria*. For example *S. nervosa* Meisn. and *S. inconspicua* L. Guthrie & T.M. Salter each possess one to many flowerheads linked to a common basal stalk. Additionally, the length of the common stalk of *S. roxburghii* R. Br. and *S. nervosa* is variable, ranging from so reduced that it appears to be absent to about 5 cm in length. *S. aitonii* and *S. aemula* may appear to possess multiple flowerheads, but in fact bear many separate flowerheads at the apex of the branches. These latter two species are

considered to possess the most primitive characters of the Paw and Skirted spiderheads, respectively (Rebelo 2001).

The taxonomy of *Serruria*

Prior to 1807, Thunberg had placed all the South African Proteaceae into *Brabeium* and *Protea*. However, in 1807 R.A. Salisbury proposed the establishment of the genus *Serruria*, along with eight other Cape Proteaceae genera.

The last full revision of *Serruria* was published by Phillips and Hutchinson in 1912. They divided the genus into two sections, *Monocephalae* and *Pleiocephalae* (Table 1.1; p. 26). *Monocephalae* is characterised by taxa with flowerheads that are solitary and borne on a simple axillary or terminal peduncle, and *Pleiocephalae* by taxa with racemose, paniculate or corymbose flowerheads borne on a common stalk. However, the authors made no further suggestions regarding how the species within these two sections might be related to one another. This revision is now outdated. More modern ideas relating to the taxonomy and relationships among the species have been proposed, and many new species have since been described.

Due to the efforts of the Protea Atlas Project headed by Dr Tony Rebelo, the distribution of the South African members of Proteaceae, including *Serruria*, is well documented. The Protea Atlas project was established in 1991 in order to increase public awareness of the South African flora and to collect geographical and ecological data on Proteaceae throughout southern Africa for conservation purposes. The project aimed to improve the documentation of the diversity displayed within the family as a whole. Contributors to the project have discovered eight new Proteaceae species since its inception, including *Serruria rebeloi* Rourke.

Rebelo (2001), who based his classification predominantly on Rourke (2000b), also divided *Serruria* into two groups based on the structure of the inflorescence (Table 1.1). Within these two groups, he described eight sub-groups based upon both vegetative and floral characters. The first group, consisting of taxa possessing many headlets at the branch apices, contains the Pin, Curly, Tulbagh, Stalked and Whip-leaf spiderheads (Rebelo 2001). The Pin spiderheads are believed to be the most primitive members of the genus (Rebelo pers. comm.) and are characterised by fine, thin leaves and headlets having relatively few flowers. The flowers are clustered into loose headlets on short stalks, and are straight when in bud (Rebelo 2001) and scented when open (Rebelo pers. comm.).

The Curly spiderheads also possess relatively fine leaves and flowers that are clustered into loose headlets on short common stalks. However, their flowers are slightly to markedly curved in bud unlike that of the Pin spiderheads, to which they are believed to be closely related. The inflorescence can branch prolifically under ideal conditions and is usually more branched than is the case in the Pin spiderheads (Rebelo pers. comm.). There are three exceptions within these

groups. *S. roxburghii* and *S. nervosa* have crowded flowerheads united on a very short, inconspicuous stalk, and *S. collina* has long flowerhead stalks (Rebelo 2001).

The Tulbagh spiderhead group, which contains only one member (*S. triternata*), has dense, untidy and flat-topped flowerheads that are borne on very short common stalks. The plants have long, stout leaves, which are only comparable to those of the Stalked spiderheads, leading to the view that these two groups are closely related (Rebelo 2001).

The taxa within the Stalked spiderheads consist of erect plants with large, stout leaves and long, leafless flowerhead stalks (usually longer than 80 mm; rarely 20 mm long). However, *S. confragosa* has a relatively short and leafy flowerhead stalk. The leaves are among the most robust within the genus and are generally located in tufts at the base of the flowerhead. The flowerhead is either lax, containing many clusters of headlets, or it contains many simple headlets (Rebelo 2001).

The last group within the multiple headlet group, the Whip-leaf spiderheads, contains only two species – *S. decumbens* and *S. flagellifolia*. This group comprises species with red, creeping stems, leaves that arise vertically from the stems and has one to four tips (mostly three), small flowerheads with four to ten flowers each, and large floral bracts that are showy compared to the flowers. The Whip-leaf spiderheads are believed to be closely affiliated to *S. meisneriana* (in the Stalked spiderhead group), which also has showy floral bracts (Rebelo 2001).

The solitary flowerhead group includes three subgroups: the Paw, Stalkless and Skirted spiderheads. The Paw spiderheads are characterised by inconspicuous involucral bracts, conspicuous flowerhead stalks and flowers that are slightly to strongly curved in bud. Within this group, taxa occurring along the West Coast usually have glabrous styles, while in those closer to the South Coast the styles are partially or completely covered by trichomes (Rebelo 2001).

The flowerheads of the Stalkless spiderheads are usually solitary and the stalks are either absent or hidden by dense leaves. Their flowers are straight in bud, with white hairs at the tip of the perianth. The styles are glabrous (Rebelo 2001). Relationships within this group, as well as between this group and the rest of *Serruria,* are uncertain (Rebelo pers. comm.).

The last subgroup is the Skirted spiderheads, which possesses conspicuous flowerhead stalks, involucral bracts (usually brightly coloured) that form a distinct "skirt" around the flowerhead, and flowers that are straight or slightly kinked in bud. This group can be divided further into the Sprawling Skirted spiderheads and the Mountain Skirted spiderheads (Table 1.1). The Sprawling Skirted spiderheads occur in lowland areas and tend to have a sprawling habit. Although most of them possess conspicuous involucral bracts, these are not as noticeable once the flowers have opened. The Mountain Skirted spiderheads, on the other hand, are mostly erect plants with a conspicuous series of involucral bracts below the flowerheads (Rebelo 2001).

Dr John Rourke of the National Botanical Institute (Kirstenbosch, South Africa) has been working on a morphological revision of *Serruria* over the past 17 years. He has described 12 new *Serruria* species (1982, 1990, 1991, 1994, 1996 & 1999), which have not been included in any published classification system other than that of Rebelo (2001).

Although Rourke is yet to publish his revision, his interpretation of morphological variation in *Serruria* has led him to divide the genus into four main groups based on the nature of the inflorescence. The first, which contains those taxa possessing heterothetic double panicles (multiple order panicles composed of lateral panicles as well as a panicle at the tip of the main axis), contains the least reduced inflorescences and is therefore considered to constitute the most primitive group. The inflorescences of taxa belonging to his second group form double racemes of capitula with the capitula representing condensed axillary racemes. Taxa with single terminal capitula constitute the third group (Rourke 1998), while the fourth group contains highly reduced inflorescences and are therefore believed to represent the most advanced state within the genus (Rourke pers. comm.).

Rourke (pers. comm.) divided these four main groups into 12 subgroups based on the nature of the inflorescence and different floral characters (Table 1.1). For convenience, I have named his subgroups alphabetically. The first main group described above contains subgroups A, B and C. Group A is characterised by taxa that possess rather loose paniculate inflorescences. The capitula of taxa in group B are very condensed. Group C, which is equivalent to Rebelo's (2001) Pin spiderhead group, forms a well-defined group within *Serruria*. Taxa within this group possess compound inflorescences comprising panicles of numerous, few-flowered, fasciculate capitula.

The second group described above contains two subgroups: groups D and E. Group D possesses branched paniculate capitula. Members of group E are characterised by small capitula clustered into groups (Rourke pers. comm.).

Rourke's (pers. comm.) third main group described above has been further divided into five subgroups, namely groups F, G, H, I and J. Taxa within group F (similar to Rebelo's (2001) Skirted spiderhead group) possess simple inflorescences, with solitary capitula subtended by prominent involucral bracts. Groups G and I are considered to be closely related, both possessing pedunculate capitula. Members of group G possess glabrous styles, while group I contains taxa with hairy styles. Group H contains taxa with inflorescences consisting of a solitary headlet of sweetly scented flowers that are straight in bud and have straight, glabrous styles. Group J contains species with a prostrate habit, flowers arranged into capitula and glabrous styles (Rourke pers comm.).

The fourth group contains two subgroups: groups K and L. Group K contains taxa with very reduced flowerheads and leaves and large, showy floral bracts in comparison to the flowers, while members of group L have sessile, reduced inflorescences containing a solitary headlet (Rourke pers comm.).

The classification systems of Phillips and Hutchinson (1912), Rebelo (2001) and Rourke (pers. comm.) agree to a large extent, but there are some important differences. Most notably, Phillips and Hutchinson (1912) and Rebelo (2001) both divided *Serruria* into two main groups, namely those with simple and those with compound inflorescences, whereas Rourke (pers. comm.) divided the genus into four main groups, namely those with highly reduced inflorescences, those with simple inflorescences and two groups with compound inflorescences. The sub-groups are also delimited differently. While Rebelo (2001) grouped taxa mainly based on overall similarity (a phenetic approach), Rourke (pers. comm.) delimited his sub-groups mainly on the basis of the characters that he believed to be synapomorphies (a phylogenetic approach). However, neither of the treatments are based upon formal phylogenetic analysis.

In summary, incredible morphological diversity and high species-richness are present within *Serruria*. However, this is typical of many Cape genera, and a thorough understanding of *Serruria* will therefore provide an ideal case study for increasing our knowledge of speciation causes and patterns in the Cape flora.

Possible causes of species richness in CFR taxa

The CFR is one of the world's most botanically diverse regions. Although the region constitutes less than 5% of southern Africa (Goldblatt 1978; Figure 1.1, p. 1), it contains 44% of the 21 817 species occurring on the subcontinent (Germishuizen & Meyer 2003). At the generic level, half of the approximately 1 888 angiosperm genera indigenous to the subcontinent are found in the CFR (Goldblatt & Manning 2002). Species richness levels in the CFR exceed that found in all other temperate zones, including other areas with Mediterranean climates. Only floras in certain areas of the wet tropics rival species richness levels in the CFR (Goldblatt 1997).

The high species-richness present in the CFR is mostly due to the high ratio of species to genera, which is 9.1:1. This is especially high when compared with other floras of the world (Fenner *et al*. 1997). However, this high value is mostly the result of the large number of species present in a few large genera (Linder 2003). For example, approximately 1/16 (657) of all Cape angiosperm species are members of the genus *Erica* L., while *Aspalathus* L. and *Pelargonium* L'Hér. contain 272 and 148 species, respectively (Goldblatt & Manning 2002). However, the high species to genus ratio of the CFR is slightly lower than that of southern Africa as a whole, which has a ratio of 9.6:1 (Goldblatt 1997). These high levels are probably an indication of a recent rapid increase in net speciation rates. This suspected uneven radiation has also caused the ten most species-rich

families in the CFR (Proteaceae being the seventh largest family) to contain 59.2% of all the species (Goldblatt & Manning 2002).

Various hypotheses have been proposed to explain the incredible species diversity present in the CFR, including the 'classical theory' of restricted gene flow (Raven 1980) and the 'ecological theory' (Linder 1985). The age of the landscape has also been invoked to explain species-richness (Whittaker 1972), and, more recently, pollinator-driven speciation (Johnson 1994). These hypotheses are discussed below in more detail.

The Classical Theory

Various species concepts have been proposed over the years in an attempt to understand the nature of a species as a taxonomic group. One of these, the biological species concept (BSC; Mayr 1969; Grant 1971 & 1981), defines a species as a group of interfertile populations that are reproductively isolated from other groups. According to this species concept, speciation is believed to occur when reproductive isolation enables different groups to accumulate random mutations over time, which eventually results in the divergence of the groups from one another to form separate species. This species concept has been used to explain speciation patterns in many parts of the world, including the CFR (e.g. Goldblatt 1978), and was termed the 'classical theory' by Raven (1980).

If reproductive isolation has been the primary driving force behind speciation in the CFR, then the following assumptions would have to be made: (1) gene flow is effective over the entire distribution range of each species (otherwise speciation would have already occurred) and (2) that speciation can only occur when gene flow is interrupted by some factor (Linder 1985). Geographical isolation has been proposed as one of the main causes of interrupted gene flow, and allopatric speciation has therefore been proposed as one of the main modes of speciation in the CFR (Rourke 1972; Oliver 1980; Goldblatt 1971, 1978).

The classical theory has been severely criticized (Ehrlich & Raven 1969; Bradshaw 1972; Raven 1980) on the basis that gene flow between plants appears to be spatially very limited in most cases. Gene flow can occur in two ways: through pollination or through seed dispersal (Linder 1985). Species with effective dispersal mechanisms tend to have wide ranges, fewer species per genus and low levels of local endemism, which would result in a lower species diversity. The opposite is true for species with poor or no seed dispersal mechanisms (Goldblatt & Manning 2000).

Many Cape species are not well adapted for long distance seed dispersal (Linder 1985). This is partly due to the low-nutrient soils dominating the region (especially the sandstone-derived soils), which probably limit the production of protein-rich structures e.g. fruit, to attract seed dispersal vectors (Bond & Slingsby 1983). Seeds are dispersed by three main agents (Linder 1985). About

1/8 of Cape species are myrmecochorous (seeds dispersed by ants). According to Berg (1975), seeds are probably not carried beyond 6 meters. Myrmecochory is the main mode of seed dispersal in *Serruria* (www.nbi.ac.za/protea) and has probably evolved as a survival strategy against fire. Wind dispersal of seeds in Cape plants is believed to be as common as myrmecochory. The dispersal distance depends on the fall-rate of the seeds. Ornithochory (seed dispersal by birds) is very rare in the CFR (Le Maitre & Midgley 1992). Dispersal distances are also suspected to be quite limited for both wind and bird dispersal (Linder 1985). Recently, Midgley (2002) has observed mouse dispersal in a small group of taxa belonging to *Leucadendron*. This dispersal mechanism also does not appear to occur over more than a few meters. Consequently, from the available data, seed dispersal in most taxa does not appear to occur over large distances.

The other way in which gene flow occurs is by pollination. There are four main pollinating vectors in the Cape, namely wind, birds, insects and rodents. Insects are the main pollinating vectors in most Cape species (Linder 1985). Levin and Kerstner (1974) found that most insect-distributed pollen neighbourhoods are less than 10 square meters. About 1/8 of Cape species are anemophilous (pollen carried by the wind). Although the distance travelled depends on the physical characteristics of the pollen grains and wind speed, most pollen is not dispersed beyond 100 meters from the donor plant (Levin & Kerstner 1974). Approximately 500 species are ornithophilous (pollinated by birds). Despite many birds being strong fliers, most forage within stands, resulting in pollen not travelling further than 500 meters in most cases (Linder 1985). Some species of *Protea* are pollinated by rodents (Rourke & Wiens 1977). The distance that pollen travels usually does not exceed 100 meters. Consequently, none of these vectors appear to carry pollen much further than about 500 m away from the source plant (Linder 1985).

Thus, it appears that gene flow mostly occurs across short distance in the CFR, and is therefore not effective over the entire distribution ranges of many species. Linder (1985) proposed that species integrity is probably maintained by stabilising selection (selection for a phenotype within the norm of a population) rather than gene flow. The belief that allopatry is one of the main modes of speciation in the CFR might also be flawed. Closely related species can often be found growing side by side, suggesting that sympatric and parapatric speciation have probably occurred in many cases (Linder 1985). Examples can be found in *Freesia* Klatt (Goldblatt 1982); *Disa* P.J. Bergius section *Disa* (Linder 1981) and *Tritonia* Ker Gawl. section *Tritonia* (De Vos 1982). Allopatry has probably also played a role in speciation amongst certain taxa in the CFR, but is probably not one of the main modes of speciation. As a replacement of the 'classical theory', Linder (1985) proposed the 'ecological theory'.

The Ecological Theory

The CFR is characterised by incredible environmental heterogeneity. According to Linder (1985), speciation in the CFR has mostly been driven by adaptation to various environmental factors. The physical attributes of the CFR are discussed below.

Geology: The substrate of the CFR consists of a mosaic of sandstone (predominantly found in the mountains) and shale (occurring mainly in the valleys), each giving rise to distinct soil types. These soils are mostly depauperate in nutrients (Goldblatt & Manning 2002), including nitrogen and phosphorus, which are essential for protein synthesis, but high in aluminium (Campbell 1983). Local areas of limestone (especially along the south coast) and granite outcrops (found mostly along the west coast) also contribute to the edaphic diversity of the Cape (Goldblatt 1997). In addition, a wide band of sand of marine origin is present along much of the West coast (Linder 1985). *Serruria* taxa can be found on all these soil types, but most seem to prefer sandstone-derived soils (www.nbi.ac.za/protea). These variations in soil type occur in sharply delimited areas (Linder 1985), leading to steep edaphic gradients across the landscape. Additionally, Marloth (1908) found that the interface between soil types and vegetation types coincides precisely. The diversification of Ericaceae, Proteaceae, Restionaceae and Cyperaceae are closely associated with the nutrient-poor sandstone soils, and these families seldom occur on any other soil type (Goldblatt & Manning 2002).

Landscape: The Cape has a varied topography, with numerous rugged mountains separated by deep, wide valleys and plains. The mountains range in height from about 1 000 to 2 000 meters (Goldblatt 1997). *Serruria* taxa can be found at almost all altitudes, ranging from close to sea level (*S. decipiens*) to as high as 1 800 meters (*S. phylicoides*) above sea level (www.nbi.ac.za/protea). Although the Cape mountains are not high enough for alpine conditions to exist at their summits, winter freeze does occur, necessitating special adaptations in their floras. Additionally, the mountainsides are dissected, providing a high variety of micro-habitats (Goldblatt 1997).

Most of the present Cape mountains were formed during the Jurassic period, when Antarctica separated from the southern coast of Africa and South America drifted away from the western coast. The resulting folds all run parallel to the coast. Consequently, a series of east-west aligned mountains, occasionally interrupted by cross-valleys, line the southern coast, while north-south trending mountains are found along the west coast (Goldblatt & Manning 2002).

Climate: The larger part of the CFR is subject to a Mediterranean-type climate, which is characterised by hot, dry summers and cold, wet winters. Due to the mountainous topography and the proximity of the area to the sea, the climate is highly variable from one locality to the next, being influenced by both elevation and aspect (Linder 2003). For example, annual rainfall in the mountains varies from 2 000 mm on the windward slopes of high mountains close to the sea to less than 100 mm on the leeward slopes of inland mountains (McDonald 1995). Consequently, steep local precipitation gradients are present

in many parts of the CFR. The distribution of rainfall throughout the seasons also differs from locality to locality – precipitation is much more evenly distributed between summer and winter in the eastern parts of the CFR than in the west (Linder 2003). Cowling *et al.* (1992 & 1997) showed that western landscapes within the CFR have more than double the number of species found in eastern landscapes across all area sizes. This is probably linked to precipitation seasonality and reliability (Cowling & Lombard 2002).

Wind also plays a very important role in the climate of the Cape. North westerly and south easterly winds bring showers to the area; southerlies bring cold air from the southern oceans that can cause snow on the mountains and north easterlies (berg winds) bring warm air from the interior. During the summer, south easterly winds predominate, which can regularly reach gale-force speeds in areas where mountains or promontories extend into the ocean (Cowling & Richardson 1995). Consequently, vegetation in these areas has adapted to these conditions by being low-growing.

The inception of the Mediterranean climate in the CFR about five million years ago (Axelrod & Raven 1978) has had a dramatic effect on the flora of the region. However, the climate in the Cape has been undergoing periodic changes ever since the break up of Gondwanaland (Cowling & Richardson 1995) and probably before that time as well. This is evident in that the vegetation of the Cape has changed over geological time. For example, the area covered by evergreen forests has been decreasing since the mid Tertiary (Appendix A; Coetzee 1993). Additional evidence supporting a previously larger arboreal vegetation comes from early to mid Miocene deposits on the west coast, which indicate that a fauna, mostly adapted to life in forests, existed at that time (Hendey 1982). Floral taxa such as members of Arecaceae and Winteraceae, which are no longer found on the African continent, also disappeared from the CFR sometime after the middle of the Miocene (Coetzee & Muller 1985).

Fire: This is also a major, recurring environmental factor that can have dramatic effects on the flora of an area (Goldblatt & Manning 2000). However, fire has probably been occurring periodically for thousands of years in the CFR (Kruger 1979), and many species have therefore developed adaptations to cope with or even exploit fire to their advantage (Cowling & Richardson 1995). Indeed, many species are so dependent on fire as a signal to move on to the next stage of their life cycle, that they would soon become extinct if fire regimes were to be omitted from their environment.

Fires are most likely to occur in summer, during which winds can reach high speeds, temperatures are high and the vegetation has dried out significantly. The effect that fire has on vegetation can vary dramatically, depending on its frequency, season, intensity (relative rate of energy release of a fire) and the area it covers, collectively known as the fire

regime. The frequency at which fires occur is determined both by how quickly the fuel load accumulates after the previous fire and climatic conditions. Approximately 12 to 15 years elapse between fire in fynbos communities (the predominant vegetation type in the CFR and the vegetation type in which *Serruria* most frequently grows; Cowling & Richardson 1995). If a community burns too frequently, some of the plants might not have enough time to complete their life cycles. The season during which a fire burns influences the intensity of the fire. For example, a summer fire is usually hotter than a winter fire, because the vegetation is drier. Vegetation composition can also affect both the frequency and intensity of fires. Additionally, high winds are frequent in the summer, which aids in the spread of fire. The area destroyed by the fire influences how quickly the burnt area will be re-colonized. These aspects of the fire regime influence the composition of the vegetation after the fire (Cowling & Richardson 1995).

The evolution of different strategies for surviving, and even profiting from different fire regimes has probably also driven diversification in the CFR (Grubb 1977). Most *Serruria* taxa are reseeders (species that survive fire in the form of seeds). Examples include *S. aitonii* and *S. dodii*. Their seeds are stored underground by ants and germinate after fire. However, the resprouters i.e. taxa that sprout new growth from buds that are protected under bark or in underground lignotubers after fire, also produce seeds that are stored by ants underground. Consequently, the resprouters probably employ both strategies in order to survive fire. The *Serruria* resprouters regenerate after fire from an underground rootstock e.g. *S. collina* (www.nbi.ac.za/protea).

Fire might have also increased the number of species that co-exist. Hutchinson (1961) and Connell (1978) stated that a low level of disturbance may increase alpha diversity (the number of species per unit area in a community) by preventing one or a few taxa from becoming competitively dominant. Local extinction of taxa can be caused by fires following too closely on one another, preventing the resprouters from reaching a sufficient age and reseeders from being able to produce the next seed set on time (Linder 1985).

No specific set of ecological characteristics has been found to be common to all the species-rich genera in the CFR. Many large Cape genera, including the two largest genera in Proteaceae, *Protea* and *Leucadendron*, prefer sandy soils and are most diverse in montane habitats. In contrast, members of other prominent genera in the Cape e.g. *Oxalis* L., *Gladiolus* L. and *Crassula* L., prefer lowland habitats and seem to be equally frequent on nutrient-poor, nutrient-intermediate and nutrient-rich soils. As far as growth form is concerned, two of the largest 20 genera in the Cape (*Lampranthus* N.E.Br. and *Crassula*) consist of succulent plants; four genera (*Disa, Oxalis, Gladiolus* and *Moraea* Mill.) are geophytic and the rest of the 20 largest genera consist mostly of shrubs and small trees (Goldblatt & Manning 2002). Thus, specific soil preferences and growth

forms of a group do not seem to coincide with greater species diversity, although this has never been subjected to phylogenetic scrutiny.

Although most of the larger Cape genera do not share preferences for any specific set of environmental factors, the extensive physical heterogeneity found in the CFR (Goldblatt & Manning 2000) might still have contributed to speciation in the area by separating closely related individuals from each other along ecological gradients. Templeton (1981) observed that speciation driven by heterogeneity in environmental factors is capable of occurring faster than is allowed under the 'classical theory' discussed above.

The variation and sudden changes that occur in the above environmental factors (soil, topography, climate and fire) across the CFR result in the presence of steep ecological gradients. Consequently, a large number of different local habitats are present, each one supporting its own complement of species. These different habitat types exist in close proximity to one another and are repeated many times over the landscape (Goldblatt & Manning 2002).

Because of the high diversity of different habitats located close to one another, areas possessing similar vegetation types are often separated by large stretches of terrain that cannot support their growth (Goldblatt & Manning 2002). This could have provided a means for speciation to occur, provided that gene flow has been limited. Limited gene flow caused by significant environmental differences between the CFR and surrounding areas is probably also responsible for the high levels of endemism found in the region (Linder 2003).

Parapatric speciation caused by limited gene flow, a highly variable environment and nutrient depauperate soils has therefore probably played a significant role in driving the high diversity levels present in the Cape flora of today (Levin 1993). This is supported by the fact that the turnover of species along an environmental gradient (beta diversity) is exceptionally high in the fynbos biome, and that the change in species composition in the same environment but in different geographical locations (gamma diversity) is unparalleled elsewhere in the world (Cowling & Richardson 1995). Parapatric speciation, with differences in soil type and microclimate as the isolation factors, has been identified as an important mode of speciation in many Cape genera, including *Rhodocoma* Nees (Restionaceae; Linder & Vlok 1991) and *Lapeirousia* Pourr. (Iridaceae; Goldblatt & Manning 1996).

However, the CFR is not unique in its physical heterogeneity. Other Mediterranean areas, e.g. the California Floristic Province and the Mediterranean Basin, both have a variety of soil types, diverse climates and rugged topographies. Although these areas also have exceptionally rich floras for their latitude (Cowling *et al*. 1996), their species diversities do not approach those of the CFR. Consequently, although a diversity in abiotic environmental factors undoubtedly contributes to the species richness of this area (Goldblatt 1997), other factors are probably also involved.

History of the CFR

The geology and climate of the Cape have been relatively stable for millions of years (Linder 1985). Europe, North America and southern South America have all experienced extreme fluctuations in their climate due to glacier formation and melting during the Quarternary period (Villagran 1994), which made it impossible for their present floras to survive before that time. In contrast, the climate in the CFR has remained quite stable during the past five million years since the beginning of the Pliocene epoch (during the Tertiary period) (Goldblatt & Manning 2002). This probably only caused numerous extensions and contractions of the flora (Linder 1985). Additionally, the geology of the CFR has been reasonably stable since the Jurassic period, when Antarctica and South America separated from Africa, causing the formation of many of the present mountain ranges. Consequently, most of the factors responsible for the isolation of the Cape flora from the rest of southern Africa were already present by the beginning of the Tertiary period (Linder 2003). Whittaker (1972) therefore suggested that the high species diversity present in the CFR today is the result of the flora having a long time to evolve under relatively stable conditions.

Pollinator-driven speciation in the CFR

Although biological forces are not as influential in the CFR as in some other plant communities, e.g. savanna, animals have influenced the evolution of CFR taxa. Many birds, insects and mammals play a very important role in pollinating plants. Studies have shown that pollinator abundance in fynbos is usually quite low, especially after fires, and there is consequently great competition between plants for pollinators (Cowling & Richardson 1995). Thus, rivalry for pollinators has been a major driving force for evolution in the CFR, resulting in the diversity of floral structures present in fynbos today (Cowling & Richardson 1995).

Within many of the Cape genera, the structure of the flower varies greatly. This is probably caused by closely related species attracting different pollinators (Goldblatt & Manning 2002), thereby promoting speciation by minimizing gene flow between taxa that would otherwise be compatible with each other.

Pollination strategies that are more common in the Cape than in other African floras include those using sunbirds, long-proboscid flies, monkey beetles, rodents and the butterflies *Aeroptes* and *Meneris* (Goldblatt & Manning 1996; Goldblatt *et al*. 1998 & Goldblatt; Manning 1999). Each of these pollinators favours flowers with different characteristics. Differences in pollination strategies have consequently contributed to the extensive botanical diversity present in the Cape (Goldblatt & Manning 2002). For example, members of both Ericaceae and Iridaceae have developed a number of pollination strategies that are not present or only weakly expressed elsewhere across their range (Vogel 1954; Goldblatt & Manning 1996, 1998; Bernhardt & Goldblatt 2000).

All *Serruria* taxa are probably insect pollinated (www.nbi.ac.za/protea). However, different characteristics of various species indicate that at least some *Serruria* species might attract specific pollinators. For example, *S. glomerata* has white to cream flowers with a sweet scent, and therefore it is likely to be pollinated by moths at night. Other insects that have been observed pollinating *Serruria* taxa include scarab beetles, flies and bees (Rebelo and Rourke pers. comm.).

It has therefore been proposed that species richness in the CFR is the result of an interplay between a complex mosaic of steep ecological gradients causing diverse habitats and gene flow mostly occurring over short distances, against a background of relatively stable climatic and geological conditions after the establishment of the Mediterranean-type climate (Goldblatt 1997). Selection for different pollinators amongst closely related taxa may also have led to radiation in at least some Cape taxa. One approach to directly evaluate the past influence of these factors on speciation and extinction in the CFR over evolutionary time is through the use of robust and complete species-level phylogenetic trees.

Direct observation of speciation events is usually impossible and fossil records are usually inadequate to investigate modes and driving forces behind speciation (Panchen 1992). Phylogenetic trees, especially those including as many representatives of the diversity in a group as possible, provide an indirect record of the speciation events that have led to present-day species (Hennig 1966). Thus phylogenetics, and specifically molecular phylogenetics, has opened up a powerful new approach to the study of speciation questions, which will be discussed in the following section.

Molecular systematics as a tool for reconstructing phylogenies

DNA sequence data has been used successfully in many studies to reconstruct the phylogeny of a diverse spectrum of organisms. With respect to the flora of the CFR, species-level phylogenetic reconstruction using DNA sequence data has been attempted for a wide spectrum of genera and families. These include *Protea* (Proteaceae; Reeves 2001), *Pelargonium* (Geraniaceae; Bakker *et al.* 1999), *Phylica* L. (Rhamnaceae; Richardson *et al.* 2001), *Moraea* (Iridaceae; Goldblatt *et al.* 2002), *Cliffortia* L. (Rosaceae; Whitehouse 2002), *Disa* (Orchidaceae; Douzery *et al.* 1999; Bellstedt *et al.* 2001) and *Ehrharta* Thunb. (Poaceae; Verboom *et al.* 2003). Specifically, within Proteaceae, DNA sequence data has been applied to elucidate relationships both among genera (Barker *et al.* 2002; Hoot & Douglas 1998) and within genera e.g. *Protea* (Reeves 2001) and *Banksia* L. and *Dryandra* R.Br (Mast 1998).

Using DNA sequence data to infer phylogenetic trees has certain advantages over data obtained in other fields of systematics. It is easy to accumulate a large number of characters for each taxon, unlike in many other fields of systematics, and discrete character states can be scored unambiguously in most cases (Soltis *et al.* 1998). However, present technology only allows the

sequencing of a minute percentage of the total genome, which may lead to the inference of inaccurate phylogenies. In the case of other systematic fields, on the other hand, a more complete set of the characters can be examined. Additionally, our limited knowledge of how DNA evolves might result in some of the included characters not being independent of one another (Dixon & Hillis 1993). For example, indels that might have been formed on several separate occasions may be scored as one event (Lee 2001). Moreover, DNA regions will not necessarily reflect the correct phylogeny, especially if processes such as hybridisation and introgression have occurred. Consequently, data obtained ideally from different sources, e.g. different genomes, should be combined to obtain a more accurate evolutionary tree.

Useful information concerning relationships within a group can be deduced more accurately by comparing nuclear and plastid trees, especially at lower taxonomic levels. For example, pitfalls like chloroplast capture, which involves the transfer of the plastid genome of one taxon to another, and lineage sorting, can be detected if sequences from both genomes have been surveyed (Soltis *et al*. 1998). Sequence data from the mitochondrial genome is less informative at the specific level in plants, since the rate of base substitution is very low. This makes the investigation of mitochondrial sequences more valuable at higher taxonomic levels. Additionally, mitochondrial DNA is not as abundant in leaf material as is plastid DNA, and the structure of the mitochondrial genome is very unstable (Palmer 1992).

In contrast to the mitochondrial genome, the plastid genome evolves relatively slowly at the sequence level (Palmer 1985). Most of the sequences of the plastid genome are therefore surveyed for assessing relationships at higher taxonomic levels. However, because rates of evolution for specific DNA regions usually vary both between and within groups, it is usually impossible to decide *a priori* which DNA regions will be most suitable for the phylogenetic analysis of a particular group (Soltis *et al*. 1998). It is consequently advantageous to try a number of DNA regions before the most suitable ones are chosen for any particular investigation.

There are several advantages of using the plastid genome (Figure 1.6, p. 19) as a source of DNA sequence data. Firstly, it is small, usually between 120 and 200 kb long (Soltis *et al*. 1998), so a greater proportion of the total genome can be sequenced than is possible with the nuclear genome. Most of the genes are single-copy, whereas many of the genes in the nuclear genome belong to multigene families, possibly causing difficulties during the phylogenetic analysis (Soltis *et al*. 1998). Plastid genomes are transferred through the maternal line, making them a hierarchically inherited character, provided that hybridisation has not taken place. Finally, unlike the nuclear and mitochondrial genomes, the plastid genome does not contain many foreign sequences originating from horizontal gene transfer (Soltis *et al*. 1998).
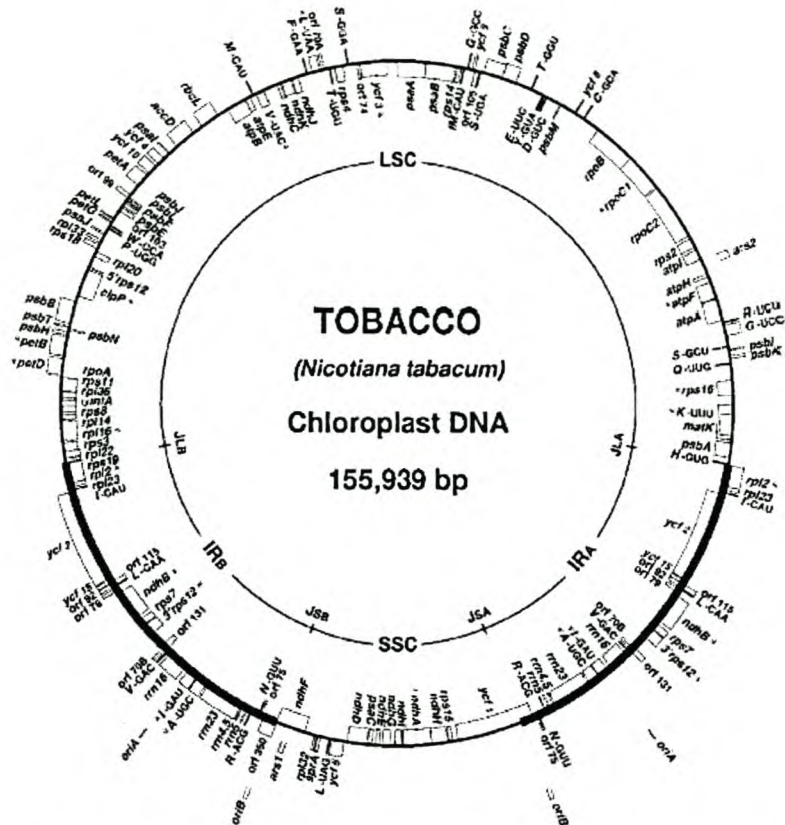
Figure 1.6: The plastid genome of the tobacco plant (*Nicotiana tabacum*), which is 155 939 bp in size. Genes shown on the inside of the circle are transcribed in a clockwise direction, while genes shown on the outside are transcribed in an anti-clockwise direction. Open reading frames only found in tobacco are denoted by orf and the codon number. Open reading frames shared by most plastid genomes but with unknown function are denoted by ycf and the designation number. Asterisks indicate split genes. LSC = large single copy region; SSC = small single copy region; IRA and IRB = the two inverted repeats. Obtained from Wakasugi *et al.* (1998).

A disadvantage of using the plastid genome in determining phylogenies at lower taxonomic levels is the potential for chloroplast transfer (or capture) to have occurred during the evolution of some of the taxa (Rieseberg & Soltis 1991; Rieseberg 1995). This involves the movement of the plastid genome from one species to another as a result of introgression, and can thus lead to erroneous inferences concerning the relationships between closely related species. However, this can usually be detected if there is a lack of congruence between the nuclear and plastid data sets. Another disadvantage is that plastid DNA sequences very often show too little variation between taxa to allow robust phylogenetic reconstruction at the specific level e.g. the *Lampranthus* group (Klak *et al.* 2003) and *Protea* (Reeves 2001).

Because the plastid genome does not undergo recombination (they are inherited as units), sequence data from various regions on the genome of plastids can be combined into a single data set (Soltis *et al.* 1998). There are several advantages in combining sequence data from many DNA regions, especially in the case of large data sets, including shorter run times for computer

analyses, better resolution in the final tree, greater internal support of clades and the presence of unique clades that do not appear when the data are analysed separately (Soltis *et al.* 1997).

Due to the susceptibility of the plastid genome to phenomena such as hybridisation and introgression, it is necessary to consider other data, e.g. sequence data from the nuclear genome, when attempting to infer evolutionary relationships between taxa. Largely due to a lack of nuclear regions that have proven to be useful for phylogenetic reconstruction, plant systematists have chiefly relied on plastid sequences as a source of DNA sequence data for inferring evolutionary relationships. Only a few nuclear regions have been used so far, including 18S, 26S and 5.8S rDNA, the internal transcribed spacer (ITS; Figure 1.7) and 5S rDNA spacer. Of these, only the last two have proven to be useful at the specific level (Soltis *et al.* 1998). A further disadvantage of using the nuclear genome is that, because of crossing over, it is possible that the region being sequenced does not have a single history (as is the case with the chloroplast genome). However, using sequences from the nuclear genome can provide valuable insights into the evolution in a specific plant group.
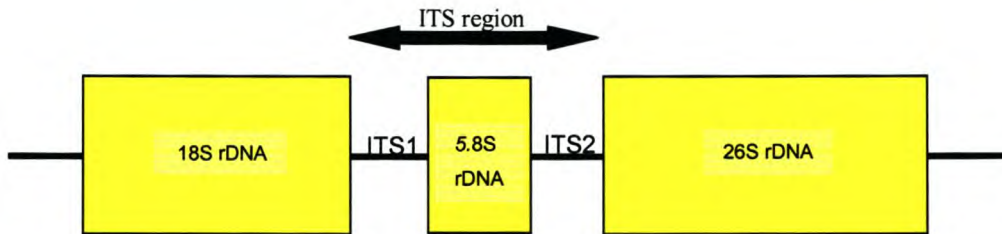
Figure 1.7: Part of a single repeat unit of ribosomal DNA (rDNA). Modified from Baldwin (1992).

The present study utilises DNA sequences from several non-coding plastid regions and the ITS region of the nuclear genome to elucidate species-level relationships in *Serruria*. It is evident that despite the considerable attention that *Serruria* has received from a taxonomic standpoint, there still remain many questions relating to species-level relationships within the genus. Therefore, the resulting phylogenetic tree presented here is then compared with existing ideas of relationships in an attempt to clarify some of the taxonomic problems within the group. The main focus of this discussion is:

- To what extent are the groups delimited by Phillips and Hutchinson (1912), Rebelo (2001) and Rourke (pers. comm.) upheld by molecular data?
    - Do the taxa with simple flowerheads and those with compound flowerheads represent separate lineages within *Serruria*, as both Phillips and Hutchinson (1912) and Rebelo (2001) have proposed?
    - Are taxa within the Whip-leaf spiderheads closely related to *S. meisneriana*, belonging to the Stalked spiderheads (Rebelo 2001)?

- Are the Curly and Pin spiderheads closely related (Rebelo 2001)?

- Did the taxa in the Pin spiderheads evolve in such a way that there is a progression from a flowerhead that contains many lax headlets to one that only contains a few headlets (two or three) (Rebelo 2001)?

- Rebelo (2001) placed *S. collina*, *S. roxburghii* and *S. nervosa* into the Curly spiderheads, along with five other species, but remarked that they are morphologically quite different from the other taxa placed within this group. Do these three species cluster with the rest of the taxa allocated to the Curly spiderheads?

- Are the Stalked spiderheads closely related to the Tulbagh spiderhead (Rebelo 2001)?

- Rebelo (2001) recognised two groups within the Skirted spiderheads, namely the Sprawling Skirted spiderheads and the Mountain Skirted spiderheads. Do taxa belonging to these two groups divide into separate lineages?

- Are the four main groups into which Rourke (pers. comm.) divided *Serruria* supported by molecular data?

- Rourke (pers. comm.) believes that the group with the least reduced capitula forms a sister clade to the rest of *Serruria*, while the group containing the most reduced floral characters contains the taxa that evolved most recently. Does molecular evidence support this?

- In light of the comparisons derived from the DNA trees and current taxonomy, the discussion then explores those morphological characters that appear to be phylogenetically informative within *Serruria*.

Layout of the Thesis

This thesis is set out in the following way:

Chapter 2: In paper format, this chapter presents a species-level phylogenetic hypothesis for *Serruria* based upon DNA sequence data, and discusses the agreement between the reconstructed phylogeny of *Serruria* and current taxonomic groupings.

Chapter 3 contains a general summary of the study.
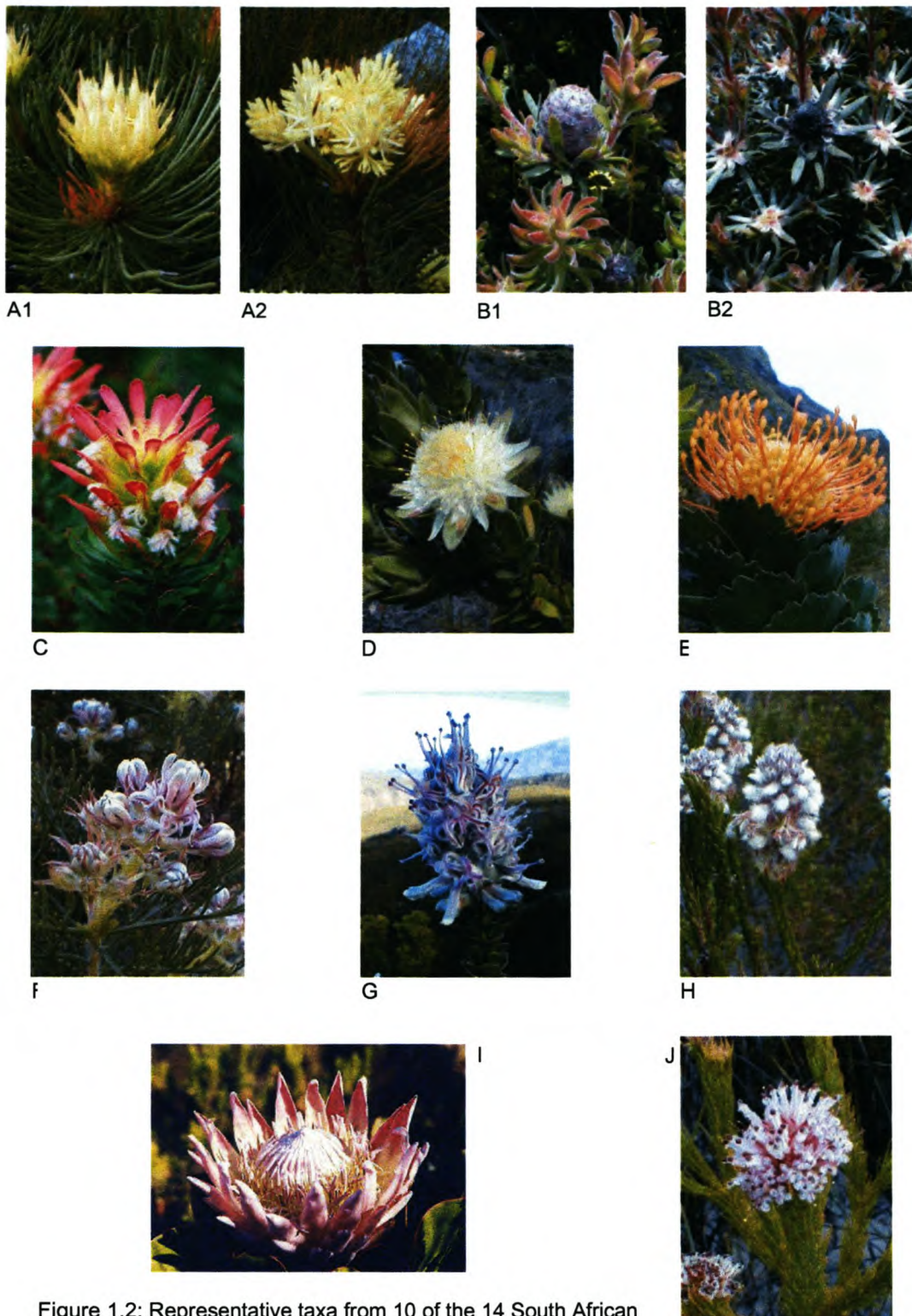
Figure 1.2: Representative taxa from 10 of the 14 South African genera of Proteaceae. (A1) female and (A2) male representatives of *Aulax cancellata*, (B1) female and (B2) male representatives of *Leucadendron radiatum*, (C) *Mimetes cucullatus*, (D) *Diastella thymelaeoides*, (E) *Leucospermum patersonii*, (F) *Serruria decipiens*, (G) *Paranomus adiantifolius*, (H) *Spatella ericoides*, (I) *Protea cynaroides* and (J) *Sorocephalus scabridus*. Photographs obtained from the Protea Atlas website (www.nbi.ac.za/protea) with kind permission from Dr Tony Rebelo.

Legend:
- ■ Australian taxa
- ■ *Diastella*
- ■ *Leucospermum*
- ■ *Mimetes*
- ■ *Orothamnus*
- ■ *Paranomus*
- ■ *Spatalla*
- ■ *Vexatorella*
- ■ *Sorocephalus*
- ■ *Serruria*
- ■ *Leucadendron*

Figure 1.3: (a) One of the equally most parsimonious trees obtained in an analysis of South African and Australian members of *Proteoideae* and *Grevilleoideae* using *Platanus occidentalis* (Platanaceae) as the outgroup. This tree was reconstructed using data from five plastid regions: *psbA-trnH* intergenic spacer, *atpB-rbcL* intergenic spacer, *rps16* intron and *trnL* intron and *trnL-F* intergenic spacer. Taxon names are not shown due to a lack of space. The clade labelled A, which contains all the Cape Proteoideae genera, except for *Protea*, *Faurea* and *Aulax*, is depicted in more detail in (b). (b) Clade A of the bootstrap consensus tree reconstructed using all the taxa included in (a). Taxon names are colour-coded according to the genus to which they belong. Bootstrap percentages are shown above and on the branches. All *Serruria* taxa included in this analysis, with the exception of *S. flava* and *S. lacunosa*, form a monophyletic group with bootstrap support of 99%. *Spatalla* taxa form two separate monophyletic clades in a polytomy containing the other Cape genera, except for *Leucadendron*. Reeves (pers. comm.)

Figure 1.4: Distribution of *Serruria* in the Western Cape, South Africa, indicating the number of species found 16 km^2. Constructed with the help of Walter Smith using data from the Protea Atlas Project data base.
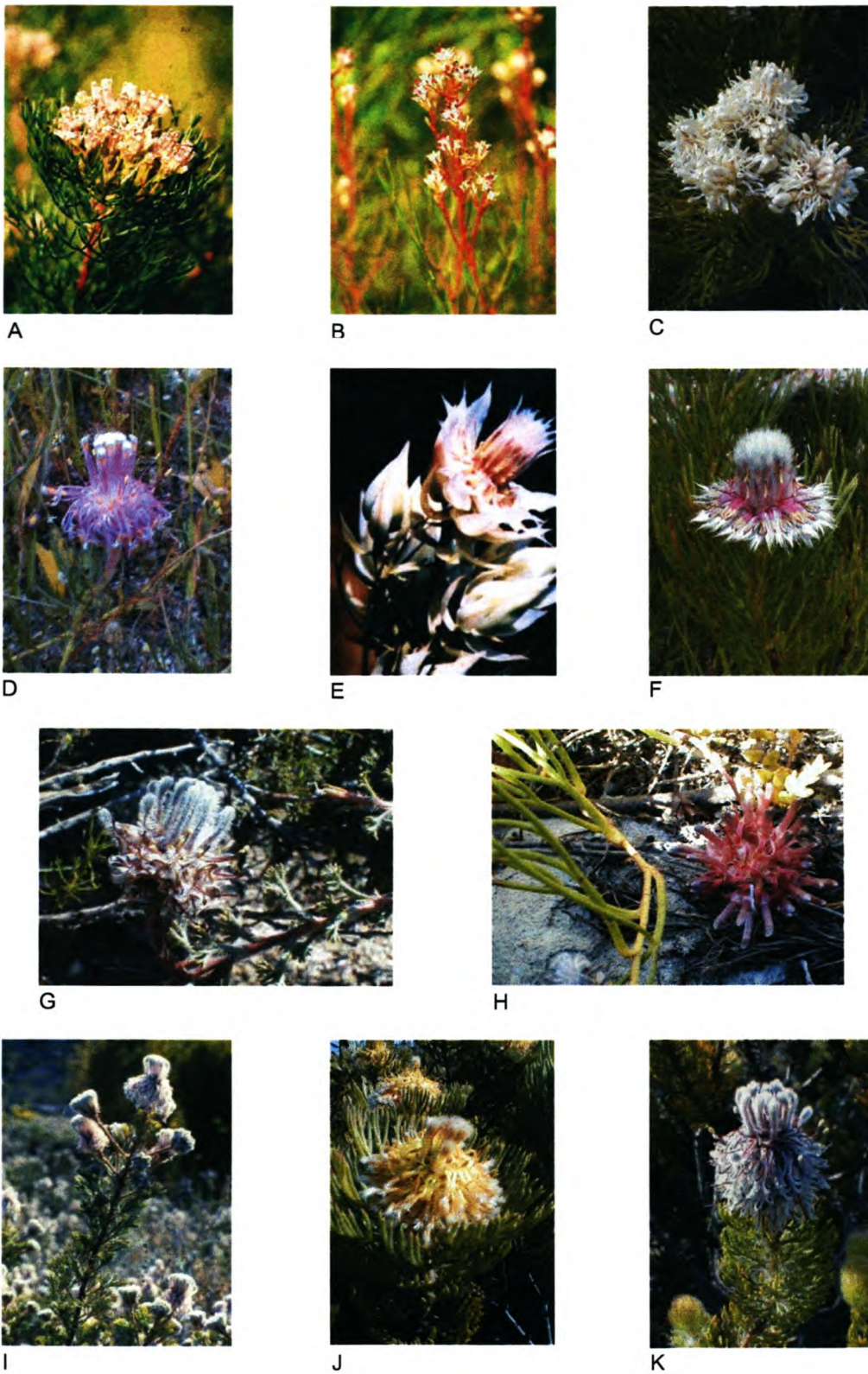
Figure 1.5: Representative taxa of *Serruria* to illustrate the morphological diversity in the genus. (A) *S. fasciflora*, (B) *S. meisneriana*, (C) *S. glomerata*, (D) *S. gracilis* ssp. *pinnata*, (E) *S. florida*, (F) *S. phylicoides*, (G) *S. incrassata*, (H) *S. decumbens*, (I) *S. aitonii*, (J) *S. villosa* and (K) *S. brownii*. Photographs obtained from the Protea Atlas website (www.nbi.ac.za/protea) with kind permission from Dr Tony Rebelo.

Table 1.1: Suggested classification systems for *Serruria*. 1, 2, 3 and 4 refer to Rourke's four main groupings described in the text, while S and C refer to Rebelo's two main groups: the simple flowerhead group and group containing taxa with many headlets per flowerhead, respectively. Within Rebelo's Skirted spiderheads, M refers to the Mountain Skirted spiderheads, whereas P refers to the Sprawling Skirted spiderheads. Nomenclature follows Rourke (2000b). - = unclassified; * = unpublished. Figure 1.6 contains photographs of selected *Serruria* taxa.

Taxon	Rourke (pers. comm.)	Rebelo (2001)	Phillips & Hutchinson (1912)
S. decipiens R. Br.	1, Group A	C, Curly	-
S. rubricaulis R. Br.	1, Group A	C, Curly	-
S. adscendens (Lam.) R. Br.	1, Group A	C, Curly	*Pleiocephalae*
S. bolusii E. Phillips & Hutch.	1, Group A	C, Curly	*Pleiocephalae*
S. collina Salisb. *ex* J. Knight	1, Group A	C, Curly	*Pleiocephalae*
S. glomerata (L.) R. Br.	1, Group A	C, Curly	*Pleiocephalae*
S. decumbens (Thunb.) R. Br.	1, Group A	C, Whip-leaf	*Pleiocephalae*
S. nervosa Meisn.	1, Group B	C, Curly	*Pleiocephalae*
S. roxburghii R. Br.	1, Group B	C, Curly	*Monocephalae*
S. aemula Salisb. *ex* J. Knight	1, Group B	S, Skirted, P	*Monocephalae*
S. inconspicua L. Guthrie & TM Salter	1, Group C	C, Pin	-
S. viridifolia Rourke	1, Group C	C, Pin	-
S. zeyheri Meisn.	1, Group C	C, Pin	-
S. candicans R. Br.	1, Group C	C, Pin	*Pleiocephalae*
S. fasciflora Salisb. *ex* J. Knight	1, Group C	C, Pin	*Pleiocephalae*
S. kraussii Meisn.	1, Group C	C, Pin	*Pleiocephalae*
S. altiscapa Rourke	2, Group D	C, Stalked	-
S. confragosa Rourke	2, Group D	C, Stalked	-
S. elongata (P.J. Bergius) R. Br.	2, Group D	C, Stalked	*Pleiocephalae*
S. leipoldtii E. Phillips & Hutch.	2, Group D	C, Stalked	*Pleiocephalae*
S. williamsii Rourke	2, Group D	C, Stalked	*Pleiocephalae*
S. lacunosa Rourke	2, Group E	C, Stalked	-
S. triternata (Thunb.) R. Br.	2, Group E	C, Tulbagh	*Pleiocephalae*
S. fucifolia Salisb. *ex* J. Knight	2, Group E	S, Paw	*Monocephalae*
S. rosea E. Phillips	3, Group F	S, Skirted, M	-
S. stellata Rourke	3, Group F	S, Skirted, M	-
S. florida (Thunb.) Salisb. *ex* J. Knight	3, Group F	S, Skirted, M	*Monocephalae*
S. heterophylla Meisn.	3, Group F	S, Skirted, M	*Monocephalae*
S. phylicoides (P.J. Bergius) R. Br.	3, Group F	S, Skirted, M	*Monocephalae*
S. cyanoides (L.) R. Br.	3, Group F	S, Skirted, P	*Monocephalae*
S. furcellata R. Br.	3, Group F	S, Skirted, P	*Monocephalae*
S. gracilis Salisb. *ex* J. Knight	3, Group F	S, Skirted, P	*Monocephalae*
S. linearis Salisb. *ex* J. Knight	3, Group F	S, Skirted, P	*Monocephalae*
S. trilopha Salisb. *ex* J. Knight	3, Group F	S, Skirted, P	*Monocephalae*
S. reflexa Rourke	3, Group G	S, Paw	-
S. aitonii R. Br.	3, Group G	S, Paw	*Monocephalae*

Taxon	Rourke (pers. comm.)	Rebelo (2001)	Phillips & Hutchinson (1912)
S. flava Meisn.	3, Group G	S, Paw	*Monocephalae*
S. incrassata Meisn.	3, Group G	S, Paw	*Monocephalae*
S. scoparia R. Br.	3, Group G	S, Skirted*	*Monocephalae*
S. brownii Meisn.	3, Group G	S, Stalkless	-
S. millefolia Salisb. *ex* J. Knight	3, Group G	S, Stalkless	*Monocephalae*
S. hirsuta R. Br.	3, Group H	S, Stalkless	*Monocephalae*
S. villosa (Lam.) R. Br.	3, Group H	S, Stalkless	*Monocephalae*
S. balanocephala Rourke	3, Group I	S, Paw	-
S. gremialis Rourke	3, Group I	S, Paw	-
S. acrocarpa R. Br.	3, Group I	S, Paw	*Monocephalae*
S. dodii E. Phillips & Hutch.	3, Group I	S, Paw	*Monocephalae*
S. pedunculata (Lam.) R. Br.	3, Group I	S, Paw	*Monocephalae*
S. effusa Rourke	3, Group J	S, Paw	-
S. cygnea R. Br.	3, Group J	S, Paw	*Monocephalae*
S. meisneriana Schltr.	4, Group K	C, Stalked	*Pleiocephalae*
S. flagellifolia Salisb. *ex* J. Knight	4, Group K	C, Whip-leaf	*Monocephalae*
S. deluvialis Rourke	4, Group L	S, Stalkless	-
S. rebeloi Rourke	4, Group L	S, Stalkless	-
S. rostellaris Salisb. *ex* J. Knight	4, Group L	S, Stalkless	*Monocephalae*

Chapter 2: A molecular phylogenetic analysis of the Western Cape genus *Serruria* Salisb. (Proteaceae L.) based on DNA sequence data

Introduction

Serruria Salisb. (Proteaceae), known informally as the spiderheads, is one of 13 genera in the subfamily Proteoideae indigenous to South Africa. The genus, consisting of 55 species, is endemic to the Cape Floristic Region (CFR) of the Western Cape, one of the most species-rich floral regions in the world. According to Rourke (2000a), members of *Serruria* are characterised by a shrub growth habit; leaves that are usually pinnately dissected with terete segments, but occasionally entire (e.g. *S. heterophylla* Meisn.); an inflorescence surrounded by involucral bracts; bisexual, usually regular flowers with sessile anthers and ovary; and a pubescent achene with a beaked apex and pedicellate base.

Recent cladistic analyses of South African Proteaceae have confirmed that *Serruria* represents a monophyletic group within the Proteoideae (Barker *et al.* 2002 & Reeves pers. comm.). Using morphological characters, Rourke (1998) conducted a cladistic analysis of the subtribe Proteeae (one of two tribes within the subfamily Proteoideae) using representatives from all the South African Proteoideae genera, including three specimens representing distinct sections within *Serruria*. In this analysis, *Serruria* formed a monophyletic clade within a group also containing members of *Spatalla* Salisb., *Sorocephalus* R. Br. and *Paranomus* Salisb., henceforth known as the '*Serruria*' group. Within this group, the three *Serruria* taxa formed a sister clade to *Paranomus*.

Barker *et al.* (2002) used DNA sequence data from the ITS region on the nuclear genome to reconstruct relationships between Cape Proteoideae genera. Although the relationship of *Serruria* to the other Cape genera differed depending on which algorithm was employed (parsimony or neighbour-joining) to analyze the data, the three *Serruria* species sampled (*S. adscendens* (Lam.) R. Br., *S. aemula* Salisb. *ex* Knight and *S. trilopha* Salisb. *ex* Knight) formed a monophyletic clade in each analysis.

Reeves (pers. comm.) used a combination of four plastid regions to reconstruct a phylogenetic tree of 230 Proteaceae taxa, including many representatives from all the Cape genera. Although little variation was found in the sampled DNA regions, the combined data set did provide some resolution and showed that the *Serruria* taxa sampled formed a well-supported monophyletic clade within Proteoideae.

However, subgeneric relationships are less certain. Phillips and Hutchinson last revised *Serruria* in 1912. They divided the genus into two sections, *Monocephalae* and *Pleiocephalae*, based on the structure of the flowerhead. A total of 12 new species have been described since then, and this revision is now outdated. In recent years, two classification systems have been proposed by Rebelo (2001; based on Rourke 2000) and Rourke (pers. comm.). Both these authors based their classifications on the nature of the inflorescences and divided *Serruria* into two and four main groups, respectively. Rebelo (2001) further divided his two main groups into eight subgroups, according to inflorescence, floral and vegetative characters, whereas Rourke (pers. comm.) divided his four main groups into 12 subgroups, based predominantly on floral characters.

Thus, based upon both molecular and morphological evidence, *Serruria* appears to be monophyletic. However, the analyses performed by Rourke (1998), Barker *et al.* (2002) and Reeves (pers. comm.) all included a limited number of taxa from *Serruria* and consequently were not able to clarify subgeneric relationships. It is therefore the aim of this study to reconstruct a species-level phylogeny of the genus using DNA sequence data from both nuclear and plastid regions. This reconstructed phylogeny will then be compared with existing ideas of relationships in an attempt to clarify taxonomic problems within *Serruria*.

Materials and Methods

Plant Collections

For each of the species included in this study, field collections were made from plants growing in their natural habitat. Voucher specimens were collected at the same time and identified by Dr Tony Rebelo and/or Dr John Rourke. Specimens will be housed at Compton Herbarium (NBG), Cape Town (South Africa).

Representatives from *Spatalla* were included in the analysis as outgroups, since cladistic analyses of morphological data by Rourke (1998) and preliminary results from DNA sequence data (Figure 1.4, p. 31) have demonstrated *Spatalla* to be one of the most closely related genera to *Serruria*. This is in accordance with the recommendation of Maddison *et al.* (1992) that taxa to be used as outgroups should not be too distantly related from those of the ingroup.

A total of 75 specimens were collected. Of these, 60 were obtained from *Serruria* taxa, representing 53 recognised species and one undescribed species (*S.* sp. from Ontongskop). The duplicates included three specimens each of *S. millefolia* Salisb. *ex* Knight and *S. acrocarpa* R. Br. and two specimens each of *S. meisneriana* Schltr. and *S. aitonii* R. Br. Only two described *Serruria* species – *S. confragosa* Rourke and *S. williamsii* Rourke – were

omitted from the analysis, due to a lack of material. Fifteen out of the twenty described *Spatalla* species were also represented in this study. Table 2.1 (p. 33) lists the taxa sampled.

DNA Extraction

Total genomic DNA was extracted using 0.5 – 1.0 g of fresh leaf material or 0.2 g of silica dried tissue and a modified version of the 2 X CTAB method (Doyle & Doyle 1987). The modification involved a final purification step using QIAquick silica columns (Qiagen Inc.) according to the manufacturer's protocol for cleaning polymerase chain reaction (PCR) products. Purified DNA samples were resuspended in $1 \times$ TE and stored at 4 °C.

PCR and DNA Sequencing

The following plastid DNA regions were sequenced: *psbA-trnH* intergenic spacer, *rps16* intron, *atpB-rbcL* intergenic spacer and *trnL-F* region (consisting of the adjacent *trnL* intron and *trnL-F* intergenic spacer). Sequences were also obtained from the nuclear ITS region (internal transcribed spacer). Appendix B provides a description of the DNA regions.

The following primers were used to amplify each region: *rps16* intron: *rps16 F* and *rps16 2R* (Oxelman *et al*. 1997); *trnL* intron: *'c'* and *'d'* and *trnL-F* intergenic spacer: *'e'* and *'f'* (Taberlet *et al*. 1991); *atpB-rbcL* intergenic spacer: *rbcL1R* (Savolainen *et al*. 1994) and *IGS2R* (Manen *et al*. 1994); *psbA-trnH* intergenic spacer: *psbAF* and *trnHR* (Sang *et al*. 1997) and ITS: *ab101F and ab102R* (Baldwin 1992). In the cases where the ITS region could not be amplified in one piece, primers *ab101F* and *ITS2* and *ITS3* (White *et al*. 1990) and *ab102R* were used.

All regions were amplified using 100 μl PCR reactions. The reaction mixture contained 2.5 U Taq polymerase; magnesium-free thermophilic buffer (50 mM KCl, 10 mM Tris-HCl, 0.1% Triton X-100); 3 mM $MgCl_2$; 0.004% bovine serum albumin (BSA; Salvolainen *et al*. 1995); 0.2 mM of each dNTP; 100 ng of each primer and 20 – 50 ng of total genomic DNA. PCR reactions were carried out in a GeneAmp® PCR System 9700 using the following PCR parameters: initial denaturation of double stranded DNA at 94.0°C for two minutes, 94.0°C denaturation for one minute, 48.0 °C annealing for one minute (52.0°C in the case of the *psbA-trnH* intergenic spacer and *ITS*), 72.0°C extention for one minute, followed by a final extension of 72.0°C for seven minutes. The *rps16* intron, *atpB-rbcL* intergenic spacer and *trnL-F* region were each amplified using 30 cycles, while the *psbA-trnH* intergenic spacer and ITS were amplified in 28 cycles. Amplified DNA was purified using QIAquick silica columns (Qiagen Inc.) or GFX™ PCR columns (Amersham Biosciences) according to the manufacturer's protocol.

Cycle sequencing reactions were carried out on both strands of the PCR products for 26 cycles in a GeneAmp® PCR System 9700 using the ABI PRISM Dye Terminator Cycle

Sequencing Ready Reaction Kit (Applied Biosystems). Each cycle consisted of 96ºC denaturation for ten seconds, 50ºC annealing for five seconds and 60ºC extension for four minutes. The same primers were used as for the initial PCR. Cycle sequencing products were then run on an Applied Biosystems 377 automated DNA sequencer using a 4.75% polyacrylamide denaturing gel. Table 2.1 contains a list of the DNA regions sequenced for each taxon.

Sequence Assembly and Phylogenetic Analyses

Sequence assembly and editing were performed using Sequencher 4.1 (Gene Codes Inc.). All matrices were aligned by eye; with two insertions or deletions (indels) each scored as discrete characters (A/T) and added to the end of the matrix (Appendix D). Both parsimony and Bayesian phylogenetic analyses were performed in PAUP* version 4.8b (Swofford 2000) and MrBayes version 3.0 (Huelsenbeck & Ronquist 2001), respectively. The following matrices, each containing 60 *Serruria* taxa and 15 *Spatalla* taxa, were analysed using parsimony and Bayesian inference: (1) *ITS*, (2) a combination of the five plastid regions (*trnL* intron, *trnL-F* intergenic spacer, *rps16* intron, *atpB-rbcL* intergenic spacer and *psbA-trnH* intergenic spacer), from here on referred to as the plastid data set, and (3) a combined data set of ITS and the plastid data.

Parsimony Analyses

Nucleotides were treated as unordered characters with equal weighting (Fitch parsimony; Fitch 1971). Heuristic searches used 1 000 replicates of random taxon addition and tree bisection-reconnection (TBR) branch swapping with a limit of five trees saved during each replicate to reduce the time spent swapping on islands of equally parsimonious trees. The number of steps contributed by each of the individual DNA regions was calculated for the plastid and combined analyses. The consistency (CI) and retention (RI) indices, which provide an indication of the measure of fit between the data and tree topologies, were calculated for each analysis.

Both jackknife resampling (Farris *et al*. 1996) and nonparametric bootstrap analyses (Felsenstein 1985) were performed in PAUP* to assess the internal support for each of the nodes. For both jackknife and bootstrap analyses, a heuristic search strategy with 1 000 replicates (with no more than five of the shortest trees saved per replicate), simple taxon addition and TBR branch swapping were employed. In addition, jackknife resampling was carried out using 33.3 % deletion. Only jackknife and bootstrap values of over 50 % were reported.

Bayesian Analyses

Bayesian analyses were carried out on the three data sets using the general time-reversal (GTR) model of DNA substitution (Tavare 1986) and a discrete gamma distribution model of

evolution (Yang 1993) with four rate classes. In order to explore the parameter space more thoroughly, Markov chain Monte Carlo (MCMC) simulations (Metropolis *et al.* 1953; Hastings 1970; Geyer 1991) were run with four incrementally heated chains. Parameter values were not defined *a priori*, but instead treated as unknown variables with uniform priors using the default values. Using a random starting tree, 500 000 generations were run. The Markov chain was sampled at intervals of 10 generations to obtain 50 000 sample points. In order to test for uniformity in the results, the Bayesian analyses were carried out multiple times.

Stationarity was determined by plotting the natural logarithm of the likelihood (lnL) against generation time for each analysis (Figure 2.2, p. 43). Samples collected prior to stationarity and convergence were discarded, as they contain no useful information concerning parameter values (Huelsenbeck & Ronquist 2001). The equilibrium samples were then used to generate 50% majority-rule consensus trees in PAUP*, with the percentage of samples containing a particular group representing that group's posterior probability. These are known as the P values, and P ≥ 95% was considered evidence of significant support for a group (Huelsenbeck & Ronquist 2001). Groups contained in 90% – 94% of all the sampled trees were considered to be strongly supported (Leache & Reader 2002).

Table 2.1: Taxa included in this study, with DNA regions sequenced for each taxon indicated. ✓ = whole region sequenced; partial = only part of the sequence included; - = sequence not included; sp. = species; s.s. = *sensu stricto*.

Taxon	Plastid regions					Nuclear region
	rps16 intron	*atpB-rbcL* intergenic spacer	*trnL* intron	*trnL-F* intergenic spacer	*psbA-trnH* intergenic spacer	*ITS*
Serruria acrocarpa R. Br. s.s. (A)	✓	✓	✓	✓	✓	✓
Serruria acrocarpa R. Br. s.s. (B)	✓	✓	✓	✓	✓	✓
Serruria acrocarpa R. Br. ssp. *ludwigiana* (undescribed)	✓	✓	✓	✓	✓	✓
Serruria adscendens (Lam.) R. Br.	✓	✓	✓	✓	✓	✓
Serruria aemula Salisb. *ex* Knight	✓	✓	✓	✓	✓	✓
Serruria aitonii R. Br. (A)	✓	✓	✓	✓	✓	✓
Serruria aitonii R. Br. (B)	✓	✓	✓	✓	✓	✓
Serruria altiscapa Rourke	✓	✓	✓	✓	✓	✓
Serruria balanocephala Rourke	✓	✓	✓	✓	✓	✓
Serruria bolusii E. Phillips & Hutch.	✓	✓	✓	✓	✓	✓
Serruria brownii Meisn.	✓	✓	✓	✓	✓	✓
Serruria candicans R. Br.	✓	✓	✓	✓	✓	✓
Serruria collina Salisb. *ex* Knight	✓	✓	✓	✓	✓	✓
Serruria cyanoides (L.) R. Br.	✓	✓	✓	✓	✓	✓
Serruria cygnea R. Br.	✓	✓	✓	✓	✓	✓
Serruria decipiens R. Br.	✓	✓	✓	✓	✓	✓
Serruria decumbens (Thunb.) R. Br.	✓	✓	✓	✓	✓	✓
Serruria deluvialis Rourke	partial	✓	✓	-	✓	✓
Serruria dodii E. Phillips & Hutch.	✓	✓	✓	✓	✓	✓
Serruria effusa Rourke	✓	✓	✓	✓	✓	✓
Serruria elongata (P.J. Bergius) R. Br.	✓	✓	✓	✓	✓	✓
Serruria fasciflora Salisb. *ex* Knight	✓	✓	✓	✓	✓	✓
Serruria flagellifolia Salisb. *ex* Knight	✓	✓	✓	✓	✓	✓
Serruria flava Meisn.	✓	✓	✓	✓	✓	✓
Serruria florida (Thunb.) Salisb. *ex* Knight	✓	✓	✓	✓	✓	✓
Serruria fucifolia Salisb. *ex* Knight	✓	✓	✓	✓	✓	✓

Taxon	Plastid regions					Nuclear region
	rps16 intron	*atpB-rbcL* intergenic spacer	*trnL* intron	*trnL-F* intergenic spacer	*psbA-trnH* intergenic spacer	*ITS*
Serruria furcellata R. Br.	✓	✓	✓	✓	✓	✓
Serruria glomerata (L.) R. Br.	✓	✓	✓	✓	✓	✓
Serruria gracilis (Salisb. *ex* Knight) ssp. *pinnata* (undescribed)	✓	✓	✓	✓	✓	✓
Serruria gremialis Rourke	✓	✓	✓	✓	✓	✓
Serruria heterophylla Meisn.	✓	✓	✓	✓	✓	✓
Serruria hirsuta R. Br.	-	✓	✓	✓	✓	✓
Serruria inconspicua L. Guthrie & TM Salter	✓	✓	✓	✓	✓	✓
Serruria incrassata Meisn.	✓	✓	✓	✓	✓	✓
Serruria kraussii Meisn.	✓	✓	✓	✓	✓	✓
Serruria lacunosa Rourke	✓	✓	✓	✓	✓	✓
Serruria leipoldtii E. Phillips & Hutch.	✓	✓	✓	✓	✓	✓
Serruria linearis Salisb. *ex* Knight	✓	✓	✓	✓	✓	✓
Serruria meisneriana Schltr. (A)	✓	✓	✓	✓	✓	✓
Serruria meisneriana Schltr. (B)	✓	✓	✓	✓	✓	✓
Serruria millefolia Salisb. *ex* Knight (A)	✓	✓	✓	✓	✓	✓
Serruria millefolia Salisb. *ex* Knight (B)	✓	✓	✓	✓	✓	✓
Serruria millefolia Salisb. *ex* Knight (C)	✓	✓	✓	✓	✓	✓
Serruria nervosa Meisn.	✓	partial	✓	✓	partial	✓
Serruria pedunculata (Lam.) R. Br.	✓	✓	✓	✓	✓	✓
Serruria phylicoides (P.J. Bergius) R. Br.	✓	✓	✓	✓	✓	✓
Serruria rebeloi Rourke	✓	✓	✓	✓	✓	✓
Serruria reflexa Rourke	✓	✓	✓	✓	✓	✓
Serruria rosea E. Phillips	✓	✓	✓	✓	✓	✓
Serruria rostellaris Salisb. *ex* Knight	✓	✓	✓	-	✓	✓
Serruria roxburghii R. Br.	✓	✓	✓	✓	✓	✓

Taxon	Plastid regions					Nuclear region
	rps16 intron	*atpB-rbcL* intergenic spacer	*trnL* intron	*trnL-F* intergenic spacer	*psbA-trnH* intergenic spacer	*ITS*
Serruria rubricaulis R. Br.	✓	✓	✓	✓	✓	✓
Serruria scoparia R. Br.	✓	✓	✓	✓	✓	✓
Serruria sp. (undescribed)	✓	✓	✓	✓	✓	✓
Serruria stellata Rourke	✓	✓	✓	✓	✓	✓
Serruria trilopha Salisb. *ex* Knight	✓	✓	✓	✓	✓	✓
Serruria triternata (Thunb.) R. Br.	✓	✓	✓	✓	✓	✓
Serruria villosa (Lam.) R. Br.	✓	✓	✓	✓	✓	✓
Serruria viridifolia Rourke	✓	✓	✓	✓	✓	✓
Serruria zeyheri Meisn.	partial	✓	✓	✓	✓	✓
Outgroups						
Spatalla argentea Rourke	✓	✓	✓	✓	✓	✓
Spatalla barbigera Salisb. *ex* Knight	partial	✓	✓	✓	-	✓
Spatalla caudate (Thunb.) R. Br.	✓	✓	✓	✓	✓	✓
Spatalla confusa (E. Phillips) Rourke	✓	✓	✓	✓	✓	✓
Spatalla curvifolia Salisb. *ex* Knight	✓	✓	✓	✓	✓	✓
Spatalla ericoides E. Phillips	✓	✓	✓	✓	✓	✓
Spatalla incurva (Thunb.) R. Br.	✓	✓	✓	✓	✓	✓
Spatalla longifolia Salisb. *ex* Knight	✓	✓	✓	✓	✓	✓
Spatalla mollis R. Br.	✓	-	✓	✓	partial	✓
Spatalla prolifera (Thunb.) Salisb. *ex* Knight	✓	✓	✓	✓	✓	✓
Spatalla racemosa (L.) Druce	✓	✓	✓	✓	✓	✓
Spatalla salsoloides (R. Br.) Rourke	✓	✓	✓	✓	✓	✓
Spatalla setacea (R. Br.) Rourke	✓	✓	✓	✓	✓	✓
Spatalla squamata Meisn.	✓	-	✓	✓	✓	✓
Spatalla tulbaghensis (E. Phillips) Rourke	✓	-	✓	✓	✓	✓

Results

The ITS Data Set

The aligned ITS data set included 75 taxa (comprising 60 *Serruria* and 15 *Spatalla* taxa; Table 2.1) and 752 characters. Of the 752 characters included in the analysis, 105 (14.0%) were variable and 56 (7.4%) were potentially parsimony informative. Analysis using the parsimony algorithm yielded 4 405 equally parsimonious trees, each 162 steps long (CI = 0.747 and RI = 0.931). Tree statistics are summarized in Table 2.2 (p. 41). The strict consensus tree of the 4 405 trees is shown in Figure 2.1 (p. 42). Jackknife (JK; in normal font) and bootstrap percentages (BS; in bold font) of over 50% are shown above and below the branches, respectively.

The log likelihood scores of the Bayesian analysis of the ITS data set reached stationarity just before 50 000 generations (Figure 2.2 A, p. 43). The 50% majority-rule consensus tree was constructed from the trees obtained during the last 320 000 generations, in order to ensure that only the best trees were included in the final consensus tree. The generations used to construct the final consensus tree had a mean lnL of - 2 445.3 with a variance of 68.8 (Table 2.3; p. 41). The 50% majority rule consensus tree constructed from the trees obtained after stationarity is shown in Figure 2.3 (p. 44). Posterior probability values (PP) are indicated above the branches. Eight of the 20 nodes present within the *Serruria* group displayed posterior probabilities of over 95%.

Most of the nodes within the consensus trees constructed using the parsimony and Bayesian algorithms were in agreement. These nodes are marked with black arrows in Figures 2.1 and 2.3. The monophyly of *Serruria* was well-supported (JK, BS and PP of 100%) by the ITS data. However, only limited resolution was obtained within the *Serruria* group, with the Bayesian algorithm producing a more resolved tree than the parsimony algorithm. The limited resolution is due to a lack of informative characters in the sequence matrix, which would also explain the generally low support values obtained for many of the nodes.

None of Rourke's (pers. comm.) or Rebelo's (2001) main groups or Phillips and Hutchinson's (1912) sections emerged as monophyletic in either analysis. In addition, only one of Rourke's (pers. comm.) and Rebelo's (2001) subgroups, the Pin spiderheads (Compound flowerhead group; Rebelo 2001) or subgroup C (Group 1; Rourke pers. comm.), formed a monophyletic group (Figures 2.1 & 2.3). Additionally, this group only contained members from section *Pleiocephalae* (Phillips and Hutchinson 1912). Within this group, both algorithms supported the division of the subgroup into two groups, one containing *S. kraussii* and *S. zeyheri*, and the other containing the remaining four taxa belonging to this group. In both consensus trees, *S. triternata*, which is the only member of the Tulbagh spiderheads (also a member of the Compound flowerhead group; Rebelo 2001), emerged as sister taxon to this group.

Although none of the other morphological groupings emerged as monophyletic groups, four more of Rourke's (pers. comm.) subgroups – B, H, J and L – formed polytomies with members of other groups (Figures 2.1 & 2.3). It is therefore possible that, given more data, these groups would resolve into monophyletic groups. The members of Rourke's (pers. comm.) and Rebelo's (2001) remaining subgroups were dispersed amongst different groups containing representatives from other subgroups.

Most of the species that are represented by more than one specimen grouped together (Figures 2.1 & 2.3). The two representatives of *S. meisneriana* grouped together with very high support values (JK = 95%, BS = 92%, PP = 100%), while the two representatives of *S. aitonii* and the three of *S. millefolia* all emerged in a polytomy with other taxa. It is therefore possible that if more information were available, the representatives of these two species would group together. The three representatives of *S. acrocarpa* were also positioned in a polytomy with other taxa. However, one of them, *S. acrocarpa* s.s. B, formed a weekly supported group with *S. pedunculata* in the Bayesian analysis (Figure 2.3).

The Plastid Data Set

Due to the lack of sequence variability evident in the individual sequence matrices obtained from the plastid genome, they were combined into a single analysis. Because of their uniparental mode of inheritance, we would expect these data sets to be congruent, and they can therefore be analysed in a combined matrix. Sequence data from the following taxa were included for each of the plastid DNA regions (Table 2.1): *rps16* intron (15 *Spatalla* taxa and 59 *Serruria* taxa), *atpB-rbcL* intergenic spacer (12 *Spatalla* taxa and 60 *Serruria* taxa), *trnL* intron (15 *Spatalla* taxa and 60 *Serruria* taxa), *trnL-F* intergenic spacer (15 *Spatalla* taxa and 58 *Serruria* taxa) and *psbA-trnH* intergenic spacer (14 *Spatalla* taxa and 60 *Serruria* taxa).

Of the 2 826 characters included in the analysis, 341 (12.1%) were variable and 153 (5.4%) were potentially parsimony informative. Analysis using the parsimony algorithm yielded 3 745 equally parsimonious trees with a tree length of 510 (CI of 0.727; RI of 0.867; Table 2.2). Of the 510 steps, 112 were contributed by the *trnL-F* region; 182 by the *rps16* intron; 87 by the *atpB-rbcL* intergenic spacer and 129 by the *psbA-trnH* intergenic spacer. The strict consensus tree of the 3 745 equally parsimonious trees is shown in Figure 2.4 (p. 45). Jackknife (in normal font) and bootstrap percentages (in bold font) of over 50% are shown above and below the branches, respectively.

The Bayesian analysis of the plastid data set was run for 500 000 generations and reached stationarity between 50 000 and 100 000 generations (Figure 2.2 B, p. 43). A 50% majority-rule consensus tree was constructed using trees from the last 350 000 generations (Figure 2.5, p. 46), in order to ensure that only the best trees were included in the final consensus tree. Posterior probability values (PP) are indicated above the branches. The generations used to construct the final consensus tree had a mean lnL of - 7 768.3 and a variance of 134.4 (Table 2.3). Of the 41

nodes present within the *Serruria* group, over half (33) displayed posterior probabilities of equal to or greater than 95%.

Serruria was supported as a monophyletic group by high support values (JK = 100%; BS = 99% and PP = 100%) in both trees. However, *S. flava* emerged amongst the *Spatalla* taxa in the parsimony strict consensus tree (Figure 2.4) and was placed in a trichotomy with the *Serruria* and *Spatalla* (excluding *Spatalla barbigera* and *S. setacae*) groups in the Bayesian majority-rule consensus tree (Figure 2.5). Although the spine of the *Serruria* clade in the parsimony strict consensus tree formed a polytomy, the nodes within the *Serruria* clade were generally highly resolved and in agreement between the two trees.

Within the *Serruria* group, none of Rebelo's (2001) or Phillips and Hutchinson's (1912) groups emerged as monophyletic. While none of Rourke's (pers. comm.) main groups formed monophyletic groups, members of one of his subgroups, Group K, grouped together in both the parsimony strict and Bayesian majority-rule consensus trees with very high support values (JK = 100%; BS = 99% and PP = 100%). The two members of Rourke's (pers. comm.) subgroup H, *S. hirsuta* and *S. villosa*, emerged separately in the Bayesian majority-rule consensus tree (Figure 2.5), but grouped together in the parsimony strict consensus tree (Figure 2.4). However, this grouping is not supported by very strong support values (JK = 74%; BS = 71%), so the plastid data only weakly supports this subgroup.

In most cases, the Bayesian and parsimony analyses yielded similar results concerning the monophyly of the species with two or more representatives included in the analyses. The two representatives of *S. meisneriana* formed a well-supported polytomy with *S. flagellifolia* in both cases, constituting Rourke's (pers. comm.) subgroup K. There is probably not enough information to separate the two *S. meisneriana* specimens from the *S. flagellifolia* representative. The three representatives of *S. millefolia* emerged in a polytomy with *S. reflexa* and one of the representatives of *S. aitonii* (*S. aitonii* A). The other member of *S. aitonii*, *S. aitonii* B, emerged as one of the sister lineages to this group. The three representatives of *S. acrocarpa* do not appear to constitute a monophyletic group. Although the two specimens that most resemble each other, *S. acrocarpa* s.s. A and B, formed a reasonably well-supported clade in the parsimony strict consensus tree (JK = 83% and BS = 74%), these two specimens emerged separately in the Bayesian majority-rule consensus tree. The third specimen, *S. acrocarpa* ssp. *ludwigiana*, emerged separately from the other two representatives in both trees, consistently grouping with *S. gremialis* and *S. balanocephala* (all belonging to Rebelo's (2001) Paw spiderheads and Rourke's (pers. comm.) subgroup I).

Although most of the well-supported clades in the ITS and plastid trees built using the parsimony algorithm were congruent with each other, this was not the case for the plastid and ITS Bayesian majority-rule consensus trees. In some places, the Bayesian majority-rule consensus tree

displayed high PP values that were not well supported by BS and JK values. This can be explained by the fact that MrBayes tends to over estimate posterior probability values for groups with short branch lengths (Lewis pers. comm.).

The Combined Data Set

Parsimony analysis of the combined plastid and ITS data sets yielded 980 equally parsimonious trees of 740 steps with a CI of 0.665 and RI of 0.849 (Table 2.2). Of the 740 steps, 112 were contributed by the *trnL-F* region; 195 by the *rps16* intron; 85 by the *atpB-rbcL* intergenic spacer; 142 by the *psbA-trnH* intergenic spacer and 206 by ITS. The strict consensus tree of the 980 trees is shown in Figure 2.6 (p. 47). Jackknife (in normal font) and bootstrap percentages (in bold font) of over 50% are shown above and below the branches, respectively.

The log likelihood scores of the Bayesian analysis of the combined data set reached stationarity just before 280 000 generations (Figure 2.2 C, p. 43). The burnin trees were discarded and the 50% majority-rule consensus tree was then constructed from the trees obtained during the last 220 000 generations. The generations used to construct the final consensus tree had a mean lnL of -10 521.9 with a variance of 117.3 (Table 2.3). The 50% majority rule consensus tree constructed from the trees obtained after stationarity is shown in Figure 2.7 (p. 48). Posterior probability values (PP) are indicated above the branches. Out of the 48 nodes present within the *Serruria* group, over half (32) displayed posterior probabilities of over 95%.

As is the case for the trees built from the plastid and ITS data, *Serruria*, this time including *S. flava*, was well-supported as a monophyletic group by both analyses (JK, BS, and PP of 100%). The position of *S. flava* at the base of the Serruria group is probably the result of conflict between the plastid and ITS data, and may therefore not reflect the true relationships between *S. flava* and the other taxa. Most of the well-supported nodes (PP > 95%; BS and JK ≥90) in the *Serruria* group of the Bayesian majority-rule consensus tree agreed with those in the parsimony strict consensus tree.

As in the ITS and plastid trees, none of Rourke's (pers. comm.), Rebelo's (2001) or Phillips and Hutchinson's (1912) main taxonomic groups emerged as monophyletic. However, a few of the subgroups grouped together. Rebelo's (2001) Pin spiderheads or Rourke's (pers. comm.) subgroup C formed a monophyletic group in the Bayesian majority-rule consensus tree (Figure 2.7), but emerged as two separate clades in the parsimony strict consensus tree (Figure 2.6). However, the nodes between these two clades in the parsimony strict consensus tree are not well-supported. Although members of Rourke's (pers. comm.) subgroup B formed part of a polytomy with members of his subgroup A in the parsimony strict consensus tree (Figure 2.6), the more highly resolved relationships in the Bayesian majority-rule consensus tree indicated that the members formed part of a grade with representatives of Rourke's (pers. comm.) subgroup A. In both the parsimony strict

and Bayesian majority-rule consensus trees, Rourke's (pers. comm.) subgroups H and K formed well-supported monophyletic groups.

In both trees, the representatives of *S. meisneriana* and two of the three representatives of *S. acrocarpa*, *S. acrocarpa* s.s. A and B, emerged as well-supported monophyletic groups (Figures 2.6 & 2.7). However, as was the case in the plastid analyses, the third *S. acrocarpa* specimen, *S. acrocarpa* ssp. *ludwigiana*, emerged separately, grouping with *S. gremialis* and *S. balanocephala*. The two representatives of *S. meisneriana* formed a well-supported sister group to *S. flagellifolia*, which together constitute Rourke's (pers. comm.) subgroup K. As is the case for the plastid data, the three representatives of *S. millefolia* emerged in a polytomy with *S. reflexa* and one of the representatives of *S. aitonii*. The other member of *S. aitonii* emerged as one of the sister lineages to this group.

Consequently, members of only three of Rourke's (pers. comm.) and one of Rebelo's (2001) subgroups grouped together. Rourke's (pers. comm.) subgroup C (equivalent to Rebelo's (2001) Pin spiderheads) grouped together in three (the two ITS analyses and the combined Bayesian analysis) of the six analyses. Rourke's (pers. comm.) subgroup H, consisting of *S. hirsuta* and *S. villosa*, grouped together in all but the Bayesian majority-rule consensus tree constructed from the plastid data set. The third of Rourke's (pers. comm.) subgroups that emerged as monophyletic is subgroup K. The monophyly of this group was only disputed in one of the analyses, namely that of the Bayesian analysis carried out on the ITS data set. However, apart from the above cases, none of the proposed taxonomic groups are supported by the molecular data, although the same relationships between the taxa tended to emerge during the analyses of all three data sets.

Table 2.2: Comparison of number of included (T), variable (V) and potentially parsimony informative (P) characters as well as the number of equally parsimonious trees, the length of the most parsimonious trees and the retention and consistency indices obtained in each parsimony analysis. No. = number

	Total number of characters (T)	Variable characters (V)		Parsimony uninformative characters (U)			Parsimony informative characters (P)			Number of equally parsimonious trees	Length of most parsimonious trees	Consistency index (CI)	Retention index (RI)
		No.	% V/T	No.	% U/T	% U/V	No.	% P/T	% P/V				
ITS	752	105	14.0	49	6.5	46.7	56	7.4	53.3	4 405	162	0.747	0.931
Plastid	2 826	341	12.1	188	6.7	55.1	153	5.4	44.9	3 745	510	0.727	0.867
Combined	3 578	446	12.5	237	6.6	53.1	209	5.8	46.9	980	740	0.665	0.849

Table 2.3: The generations used to build the 50% majority rule consensus trees, as well as the mean natural logarithm of the likelihood (lnL) and variance of the Bayesian analyses.

	Variance	Mean lnL	Generations used
ITS	68.8	- 2 445.3	180 000 – 500 000
Plastid	134.4	- 7 768.3	150 000 – 500 000
Combined	117.3	- 10 521.9	280 000 – 500 000

Figure 2.1: The strict consensus tree (CI = 0.747; RI = 0.931) of 4 405 equally parsimonious trees (length = 162) built using the ITS data set and the parsimony algorithm. Numbers attached to branches indicate support greater than 50%; bold font for bootstrap and regular font for jackknife percentages. *Spatalla* taxa are written in black font. Within *Serruria*, the colour-coding of the taxon names correspond to Rebelo's (2001) groupings, while the coloured bars on the right indicate Rourke's groupings (pers. comm.). The letters to the right of the coloured bars correspond to Phillips and Hutchinson's (1912) sections. Black arrows indicate nodes within the *Serruria* group that are present in the ITS trees built using both parsimony and Bayesian algorithms, and red arrows indicate groups that are congruent in the analyses of the plastid, ITS and combined data sets. sp. = species; ssp. = subspecies; s.s. = *sensu stricto*

Figure 2.2: Plots of the natural logarithm of the likelihood (lnL) plotted against the number of generations for the cold chains of the Bayesian analyses of the (A) ITS, (B) plastid and (C) combined data sets. For each analysis, (1) the lnL values for all the generations, as well as (2) an enlargement of the lnL values once they have plateaued are shown. The point at which stationarity was reached is indicated by an arrow in each of the graphs in the left column.

Figure 2.3: The 50% majority rule consensus tree built using the ITS data set and the Bayesian algorithm (mean lnL = - 2 445.3; variance = 68.8). Numbers above branches indicate Bayesian posterior probability values greater than 50%. *Spatalla* taxa are written in black font. Within *Serruria*, the colour-coding of the taxon names correspond to Rebelo's (2001) groupings, while the coloured bars on the right indicate Rourke's groupings (pers. comm.). The letters to the right of the coloured bars correspond to Phillips and Hutchinson's (1912) sections. Black arrows indicate nodes within the *Serruria* group that are present in the ITS trees built using both parsimony and Bayesian algorithms, and red arrows indicate groups that are congruent in the analyses of the plastid, ITS and combined data sets. sp. = species; ssp. = subspecies; s.s. = *sensu stricto*
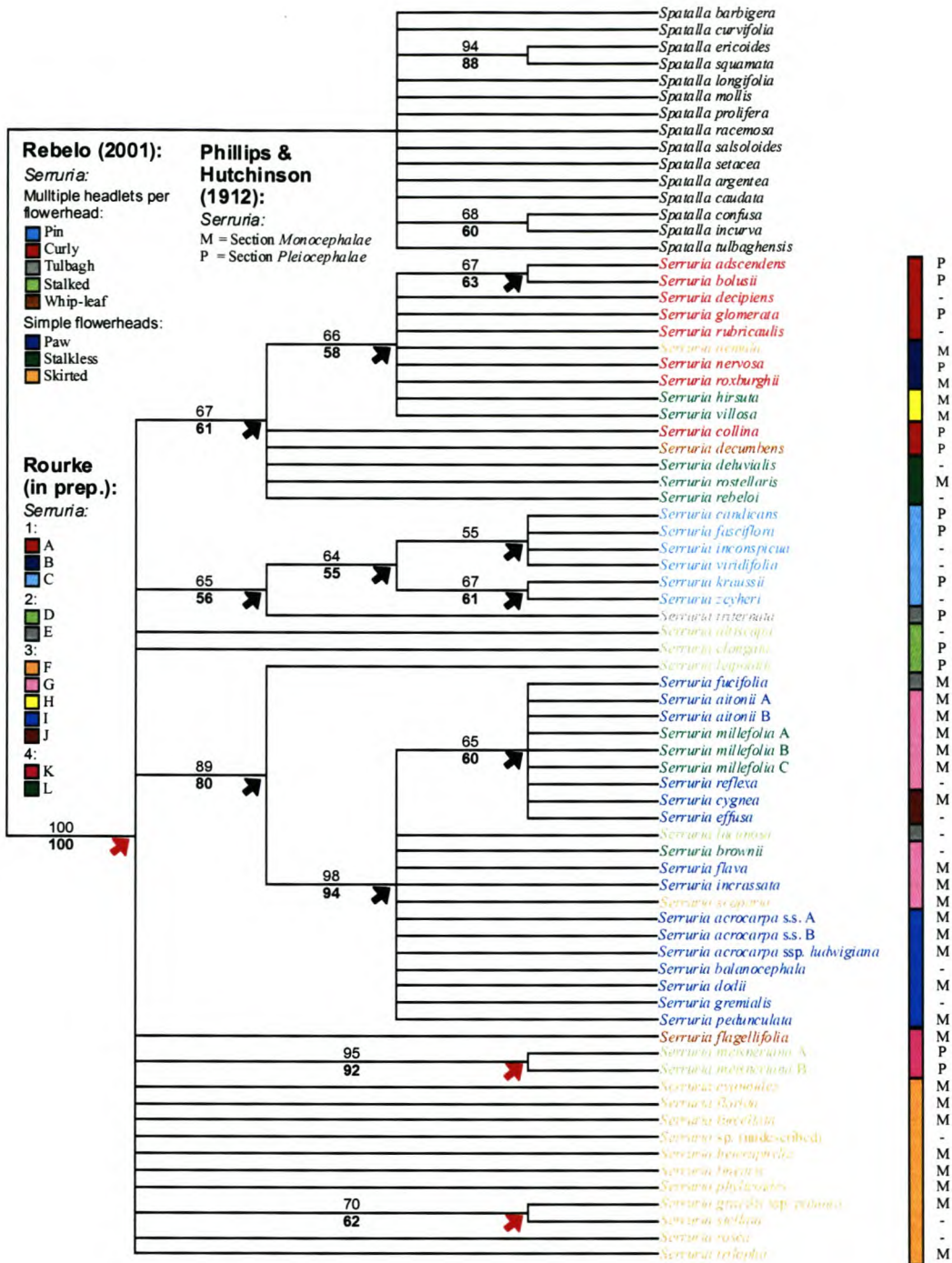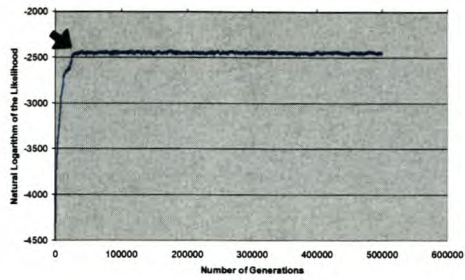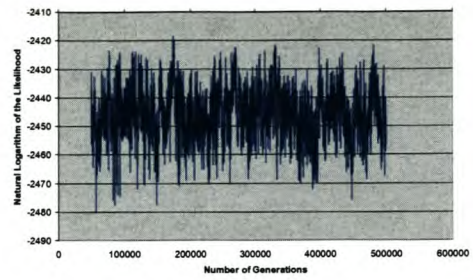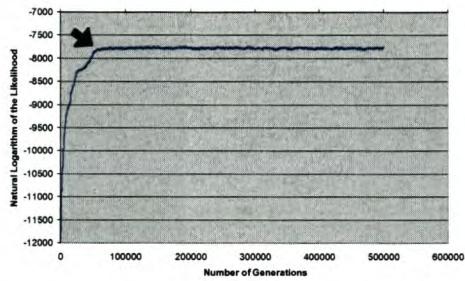
Figure 2.4: The strict consensus tree (CI = 0.727; RI = 0.867) of 3 745 equally parsimonious trees (length = 510) built using the plastid data set and the parsimony algorithm. Numbers attached to branches indicate support greater than 50%; bold font for bootstrap and regular font for jackknife percentages. *Spatalla* taxa are written in black font. Within *Serruria*, the colour-coding of the taxon names correspond to Rebelo's (2001) groupings, while the coloured bars on the right indicate Rourke's groupings (pers. comm.). The letters to the right of the coloured bars correspond to Phillips and Hutchinson's (1912) sections. Black arrows indicate nodes within the *Serruria* group that are present in the plastid trees built using both algorithms, and red arrows indicate groups that are congruent in the analyses of the plastid, ITS and combined data sets. sp. = species; ssp. = subspecies; s.s. = *sensu stricto*

Figure 2.5: The 50% majority rule consensus tree built using the plastid data set and the Bayesian algorithm (mean lnL = - 7 768.3; variance = 134.4). Numbers above branches indicate Bayesian posterior probability values greater than 50%. *Spatalla* taxa are written in black font. Within *Serruria*, the colour-coding of the taxon names correspond to Rebelo's (2001) groupings, while the coloured bars on the right indicate Rourke's groupings (pers. comm.). The letters to the right of the coloured bars correspond to Phillips and Hutchinson's (1912) sections. Black arrows indicate nodes within the *Serruria* group that are present in the plastid trees built using both algorithms, and red arrows indicate groups that are congruent in the analyses of the plastid, ITS and combined data sets. sp. = species; ssp. = subspecies; s.s. = *sensu stricto*
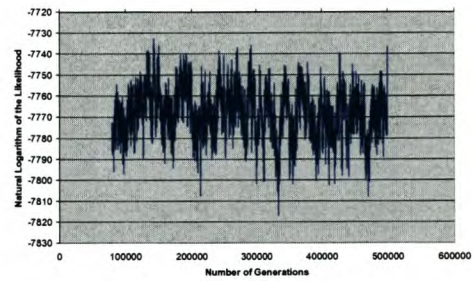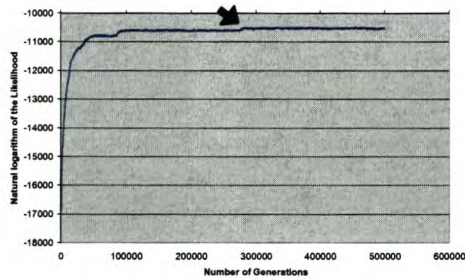
Figure 2.6: The strict consensus tree (CI = 0.665; RI = 0.849) of 980 equally parsimonious trees (length = 740) built using the combined data set and the parsimony algorithm. Numbers attached to branches indicate support greater than 50%; bold font for bootstrap and regular font for jackknife percentages. *Spatalla* taxa are written in black font. Within *Serruria*, the colour-coding of the taxon names correspond to Rebelo's (2001) groupings, while the coloured bars on the right indicate Rourke's groupings (pers. comm.). The letters to the right of the coloured bars correspond to Phillips and Hutchinson's (1912) sections. Black arrows indicate nodes within the *Serruria* group that are present in the combined trees built using both algorithms, and red arrows indicate groups that are congruent in the analyses of the plastid, ITS and combined data sets. sp. = species; ssp. = subspecies; s.s. = *sensu stricto*
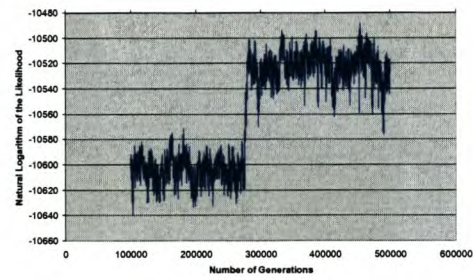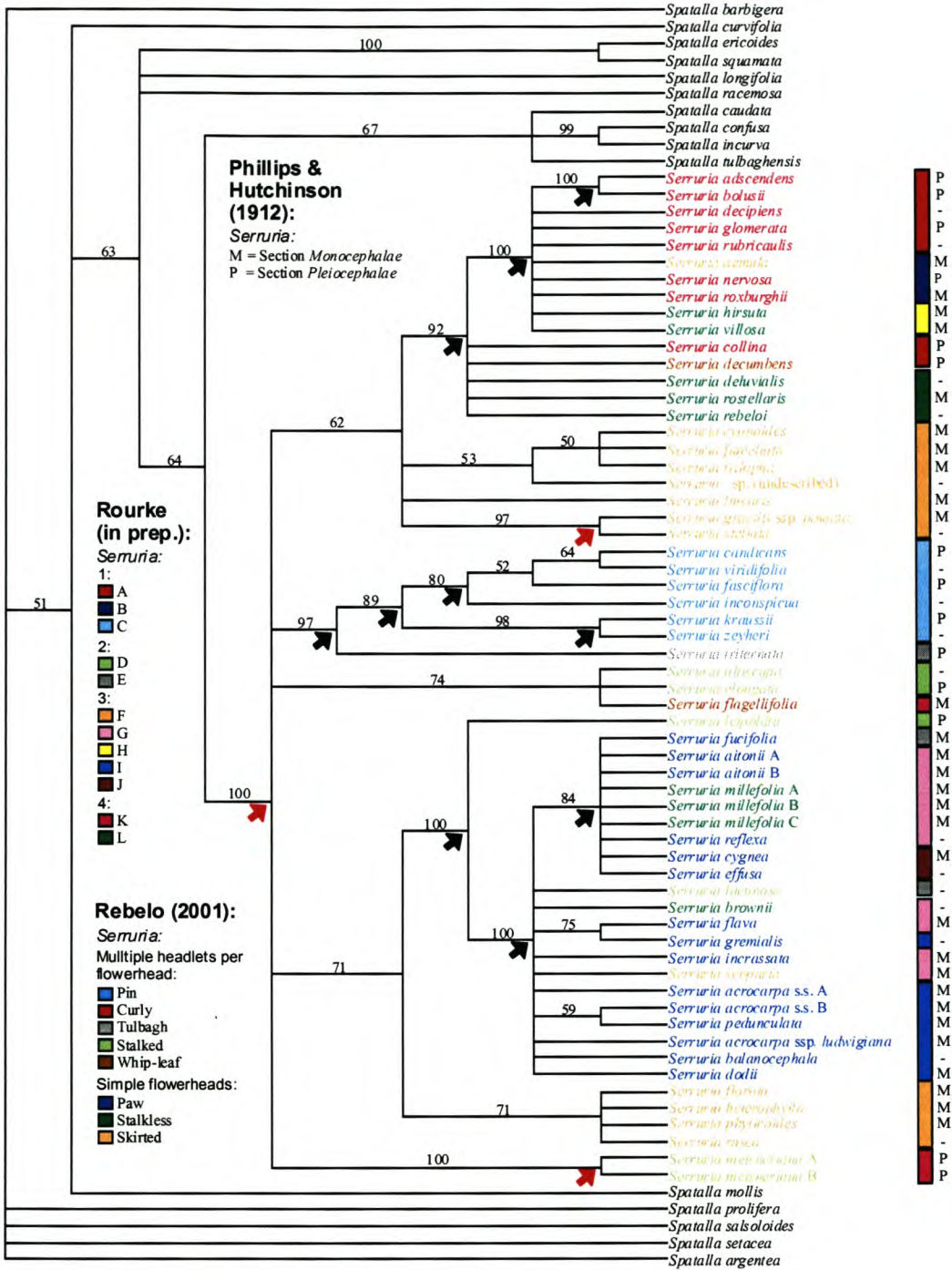
Figure 2.7: The 50% majority rule consensus tree built using the combined data set and the Bayesian algorithm (mean lnL = - 10 521.9; variance = 117.3). Numbers above branches indicate Bayesian posterior probability values greater than 50%. *Spatalla* taxa are written in black font. Within *Serruria*, the colour-coding of the taxon names correspond to Rebelo's (2001) groupings, while the coloured bars on the right indicate Rourke's groupings (pers. comm.). The letters to the right of the coloured bars correspond to Phillips and Hutchinson's (1912) sections. Black arrows indicate nodes within the *Serruria* group that are present in the combined trees built using both algorithms, and red arrows indicate groups that are congruent in the analyses of the plastid, ITS and combined data sets. sp. = species; ssp. = subspecies; s.s. = *sensu stricto*
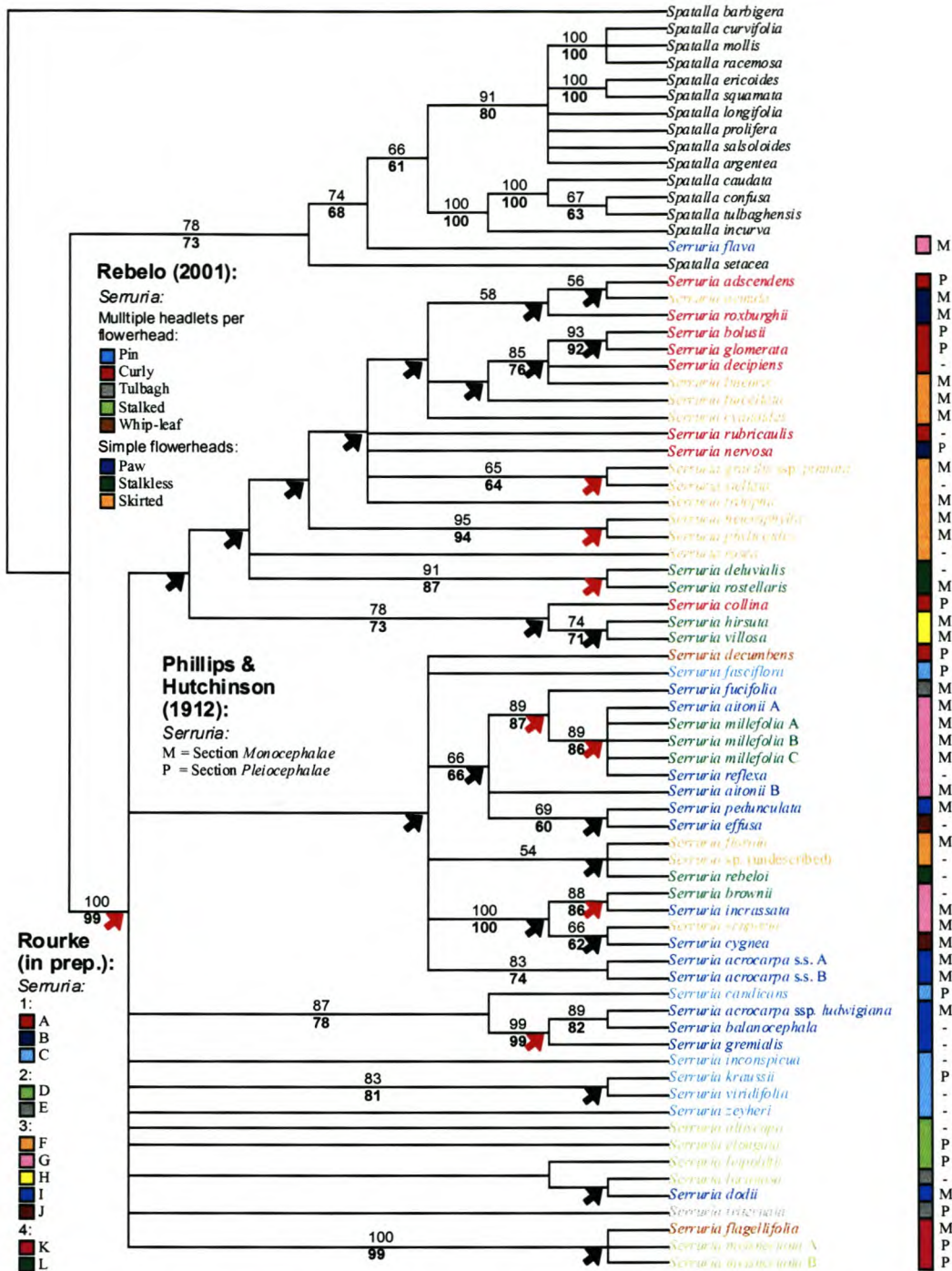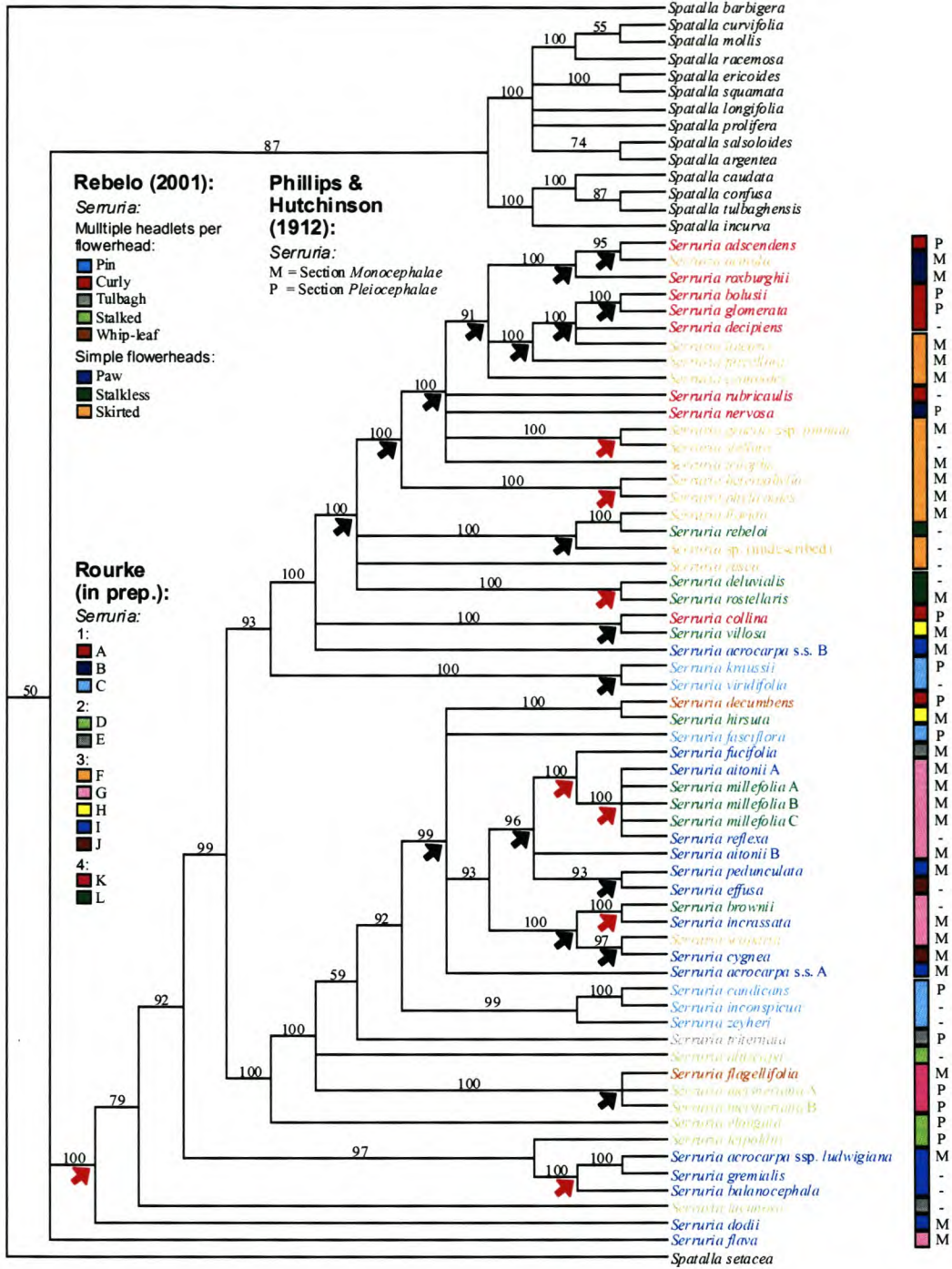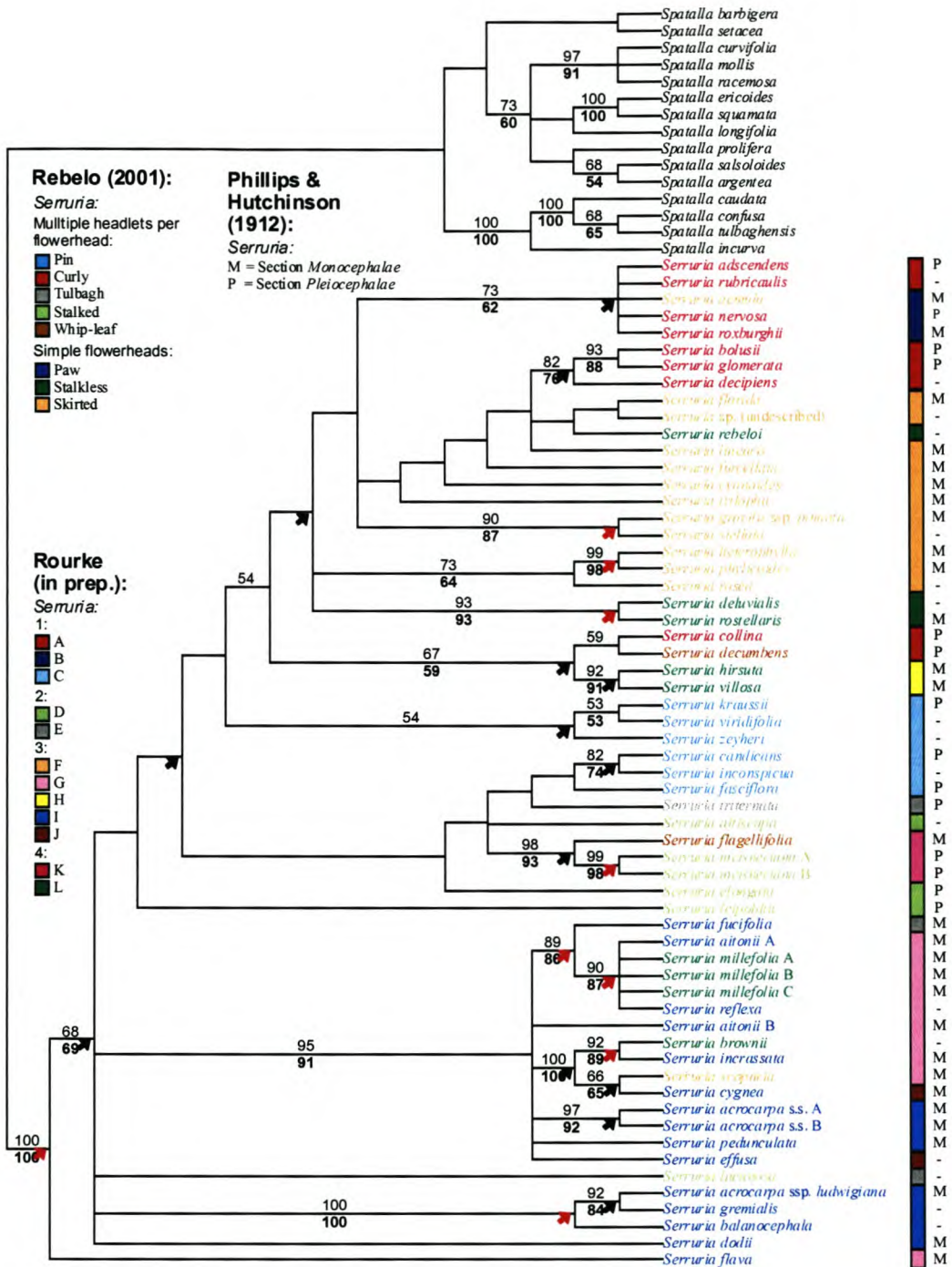
Discussion

Phylogenetic inference within *Serruria*

Previous molecular (Barker *et al.* 2002 & Reeves pers. comm.) and morphological (Rourke 1998) studies have supported the monophyly of *Serruria* within Proteoideae. In agreement with this, all *Serruria* taxa included in this study consistently emerged in a well-supported group with the exception of *S. flava*. In both ITS analyses, *S. flava* was deeply embedded within *Serruria*, with the genus including *S. flava* receiving high support (Figures 2.1 & 2.3; PP; BS & JK = 100%). However, in the parsimony strict consensus tree built using the plastid data set, *S. flava* was placed within *Spatalla*. Although the *Spatalla* clade only received moderate support (Figure 2.4; BS = 73%; JK = 78%), the clade defining *Serruria* excluding *S. flava* received high support (BS = 99%, JK= 100%). In the plastid Bayesian majority-rule consensus tree *S. flava* was placed in an unsupported position, outside both *Serruria* and *Spatalla* (Figure 2.5). In the combined parsimony and Bayesian analyses, *S. flava* was placed as sister to the rest of *Serruria* receiving high support for its placement within the genus (Figures 2.6 & 2.7; PP, BS & JK = 100%).

One possible explanation for the ambiguous position of *S. flava* may be that it represents an intergeneric hybrid between *Serruria* and *Spatalla*, or another Cape Proteoideae genus. This would lead to the plastid and nuclear genomes tracking different phylogenetic histories. The position of *S. flava* in the trees built using the combined data set (Figures 2.6 & 2.7) may therefore be the result of conflict between the nuclear and plastid data sets, rather than a true reflection of the taxon's relationship to the other taxa included in the analysis.

Hybridization events between taxa from different Proteoideae genera have been recorded. For example, contributors to the Protea Atlas Project recorded a specimen that appeared to be the product of hybridization between *Sorocephalus scabridus* Meisn. and *Spatalla caudata* (http://protea.worldonline.co.za/splr_ccg.htm). Additionally, hybridization causes polytomies between clades containing the parental lineages in consensus trees (Bremer & Wanntorp 1979; Funk 1985). In the present study, the spine of the *Serruria* group is largely unresolved or only weakly supported in most of the analyses, indicating that intra-generic hybridisation could be at play here. Many hybridisation events have been recorded on the Protea Atlas website, including *S. cygnea* X *S. dodii*, *S. cygnea* X *S. pedunculata*, *S. dodii* X *S. cygnea*, *S. glomerata* X *S. villosa*, *S. pedunculata* X *S. cygnea* and *S. villosa* X *S. glomerata* (http://protea.worldonline.co.za/p31hybrd.htm). However, a lack of resolution could also be caused by a lack of informative characters, which is definitely the case for the sequences collected for *Serruria*.

Hybridisation has been suspected to be the cause of discrepencies between gene trees in many other taxonomic groups apart from Proteaceae, including the genera *Gossypium* L. (Cron *et al.*

2002), *Helianthus* L. (Rieseberg & Morefield 1995), *Paeonia* L. (Sang *et al.* 1997), *Phlox* L. (Ferguson & Jansen 2002), *Populus* L. (Smith & Sytsma 1990) and *Rhododendron* L. (Milne *et al.* 1999). Additionally, evidence has been found that many of today's families and subfamilies might have arisen as a result of hybridisation (e.g. Phillips *et al.* 1991).

Another feature of the analyses presented here refers to the observation that some of the groups receiving high posterior probability values in the Bayesian majority-rule consensus trees are not well supported by either the bootstrap or jackknife in the parsimony trees. *A priori* we would expect strong phylogenetic signal in the data to be reflected in high bootstrap, jackknife and posterior probability values, and not to be present in one and not the others. However, it has been brought to our attention that posterior probability values may be inflated for groups that are defined by very short branches due to a bug in the current version of MrBayes (Lewis pers. comm.). In effect, MrBayes only includes nodes to calculate posterior probabilities that are bifurcating. Polytomies (caused by lack of nucleotide variation) are ignored, and therefore posterior probabilities are calculated as a proportion of only the small subset of trees that contain the node in question. This can lead to highly exaggerated posterior probability values, especially if the branches leading to these clades are short. For this reason, I have not discussed groups here that are not well supported by all methods of nodal support implemented.

Agreement between morphological and molecular data

To a large extent, the phylogenetic trees presented here based upon data from five plastid and one nuclear region, do not agree with past and present taxonomic groupings within *Serruria* (Table 1.1). None of Phillips and Hutchinson's (1912) sections, or Rebelo's (2001) and Rourke's (pers. comm.) main groups formed monophyletic groups. Additionally, Rourke (pers. comm.) has proposed that the group with the least reduced capitula (Group 1) is placed as sister to the rest of *Serruria*, while the group containing the most reduced floral characters (Group 4) contains taxa that have evolved most recently. In the combined trees presented here, neither Groups 1 or 4 are monophyletic, and thus these hypotheses are not supported by the molecular data. However, two of Rourke's (pers. comm.) subgroups – H and K – were consistent with the molecular data and were retrieved in the combined analyses. The only other proposed grouping to form a monophyletic group based on the molecular data is Rourke's subgroup C (equivalent to Rebelo's (2001) Pin spiderheads), which formed a monophyletic group in the ITS trees (Figures 2.1 & 2.3).

S. hirsuta and *S. villosa*, constituting Rourke's (pers. comm.) subgroup H and two of the members of Rebelo's (2001) Stalkless spiderheads, were well supported in the combined analysis (Figures 2.6 & 2.7; PP = 100%; BS = 91%; JK = 92%). They share a number of morphological characteristics. Both reach approximately the same heights at maturity (0.3 – 0.5 m) and have erect habits; dissected leaves covered in silky hairs; peduncles that are either absent or hidden by dense leaves; a flowerhead or inflorescence consisting of a solitary headlet or capitulum of sweetly

scented flowers that are straight in bud; straight glabrous styles; and club-shaped or clavate pollen presenters (Rebelo 2001).

Rourke's (pers. comm.) subgroup K emerged as monophyletic in most of the trees. Support values for the combined analyses are PP = 100%, BS = 93% and JK = 98% (Figures 2.6 & 2.7). This subgroup, comprising *S. meisneriana* (Stalked spiderheads) and *S. flagellifolia* (Whip-leaf spiderheads), is characterised by glabrous, petiolate leaves; ovate involucral bracts; large, showy floral bracts in comparison to the flowers; pink, glabrous perianths; and straight styles (Rebelo 2001). Rebelo (2001) proposed that taxa within the Whip-leaf spiderheads (*S. decumbens* and *S. flagellifolia*) are closely related to *S. meisneriana*, belonging to the Stalked spiderheads. Although the two representatives of *S. meisneriana* did not group with *S. decumbens* in any of the analyses, they emerged with *S. flagellifolia* in the combined analyses, thereby partially confirming this hypothesis.

The monophyly of members of Rourke's (pers. comm.) subgroup C was weakly supported by the ITS analyses (BS = 55%; JK = 64%; PP = 89%), and members of the subgroup did tend to group together in the other analyses. Morphologically, subgroup C forms a well-defined group of taxa. All the species are characterized by finely dissected leaves; compound inflorescences, consisting of panicles of numerous few-flowered, loosely clustered headlets or capitula (Rourke 1990) on short inflorescence stalks or peduncles; flowers that are straight in bud and straight styles (Rebelo 2001). Rebelo (2001) proposed that the taxa in the Pin spiderheads evolved in such a way that there is a progression from an inflorescence that contains many lax capitula to one that only contains a few capitula (two or three). Although the six analyses did not yield the same relationships between the Pin spiderheads, the taxa with inflorescences containing many lax capitula did tend to group together apart from the taxa with only two or three capitula. The Pin spiderheads showed no significant affiliations with the Curly spiderheads, as was hypothesised by Rebelo (2001).

S. triternata, the only member of Rebelo's (2001) Tulbagh spiderheads, was weakly supported as a sister lineage to the Pin spiderheads in both ITS analyses (Figures 2.1 & 2.3; PP = 97%, BS = 56%, JK = 65%). Characteristics that this species shares with members of the Pin spiderheads include dissected leaves; very short peduncles; compound inflorescences; and straight styles (Rourke 1990 & Rebelo 2001). Rebelo (2001) proposed that the Stalked spiderheads are closely related to the Tulbagh spiderhead. However, *S. triternata* was not well supported as being closely related to the Stalked spiderheads in any of the analyses.

The undescribed species included in the analysis, *Serruria* sp. (undescribed), was suspected by Rebelo (pers comm.) to be closely related to the Skirted spiderheads. However, the molecular data did not support any specific affiliations with any other taxon, as its position varies in the separate analyses, probably also as a result of a lack of informative characters.

Although not completely representative of described groupings, other groups consistent with current taxonomy were also retrieved in the combined trees. One such group contains *S. acrocarpa*, *S. balanocephala* and *S. gremialis* (PP, BS & JK = 100% in the combined analyses), all members of Rourke's (pers. comm.) subgroup I and Rebelo's (2001) Paw spiderheads. These three species share dissected, petiolate leaves that are mostly glabrous when mature; a conspicuous peduncle; inconspicuous, ovate involucral bracts; a globose inflorescence comprising a solitary, terminal capitulum, usually with a few axillary capitula lower down on the flowering shoot; conspicuous floral stalks; flowers that are slightly to strongly curved in bud; pubescent perianths; and adaxially curved styles that are pubescent on the lower third and are partially retained on the fruit (Rourke 1994 & Rebelo 2001).

S. brownii and *S. incrassata*, another two species that consistently emerged together in most of the analyses, including the combined analyses (PP = 100%; BS = 89%; JK = 92%), both have dissected, petiolate leaves that are pubescent at maturity; inflorescences comprising a single capitulum; sweetly scented flowers; perianths covered in silver, silky hairs; inwardly curving, glabrous styles of approximately the same length; and clavate pollen presenters of about 1 mm long (Rebelo 2001). Both these taxa are members of Rourke's (pers. comm.) subgroup G, while Rebelo (2001) placed *S. brownii* into the Stalkless spiderheads and *S. incrassata* into the Paw spiderheads (both belonging to the Simple flowerhead group).

The group containing *S. aitonii*, *S. millefolia*, *S. reflexa* and *S. fucifolia* consistently emerged as a well-supported group in the plastid (PP = 100%; BS = 86%; JK = 89%; Figures 2.4 & 2.5) and combined analyses (PP = 100%; BS = 87%; JK = 89%; Figures 2.6 & 2.7), and as part of a polytomy in the ITS analyses (Figures 2.1 & 2.3). The lack of resolution in the ITS analyses is probably the result of a limited number of informative characters or hybridisation events. These taxa share an erect habit; dissected, petiolate, pubescent leaves; globose inflorescences comprising a single capitulum; pubescent perianths, glabrous styles; and pollen presenters of about 1 mm long (Rourke 1990 & Rebelo 2001). Within this group, *S. aitonii*, *S. millefolia*, *S. reflexa*, the three species that always emerged as the most closely related, are all members of Rourke's (pers. comm.) subgroup G. However, *S. aitonii* and *S. reflexa* have been placed in the Paw spiderheads, while *S. millefolia* has been placed in the Stalkless spiderheads by Rebelo (2001). All three of these species have, in addition to the above-mentioned characteristics, ovate involucral bracts and clavate pollen presenters (Rourke 1990 & Rebelo 2001).

S. deluvialis and *S. rostellaris* are both members of Rourke's (pers. comm.) subgroup L and Rebelo's (2001) Stalkless spiderheads (PP = 100%; BS & JK = 93%; Figures 2.6 & 2.7). They both possess dissected, erect, petiolate, glabrous leaves; sessile, globose inflorescences comprising a single capitulum, occasionally with axillary capitula clustered beneath the terminal capitulum; lanceolate involucral bracts; flowers that are straight or only slightly curved in bud; pubescent

perianths; straight to slightly curved, glabrous styles and pollen presenters of about the same length (Rourke 1990 & Rebelo 2001).

S. heterophylla and *S. phylicoides* also grouped together in all, including the combined analyses (PP = 100%; BS = 98%; JK = 99%; Figures 2.6 & 2.7) and are both members of Rourke's (pers. comm.) subgroup F or Rebelo's (2001) Mountain Skirted spiderheads. They are both shrubs with an erect habit that grow to approximately the same height and have erect, petiolate, glabrous leaves; conspicuous inflorescences stalks of approximately the same length; a conspicuous series of involucral bracts below the globose inflorescence, which consists of a single capitulum; conspicuous floral stalks; flowers that are straight to slightly kinked in bud; pink to carmine perianths with white silky hairs and straight styles with clavate pollen presenters (Rebelo 2001).

Two other members of Rourke's (pers. comm.) subgroup F, *S. gracilis* ssp. *pinnata* (Sprawling Skirted spiderheads) and *S. stellata* (Mountain Skirted spiderheads), also consistently grouped together. Support values in the combined analyses are PP = 100%, BS = 87%, JK = 90% (Figures 2.6 & 2.7). They are both prostrate shrubs with creeping stems and have dissected, petiolate leaves that arise vertically from horizontal stems, have fine leaf tips and are glabrous at maturity; conspicuous peduncles; involucral bracts that are conspicuous just before anthesis; purple-pink involucral bracts; globose inflorescences comprising a solitary capitulum; conspicuous floral stalks; sweetly scented, slender flowers that are straight or slightly kinked in bud; pubescent perianths; straight, glabrous styles and clavate pollen presenters (Rourke 1991 & Rebelo 2001).

Rebelo (2001) recognised two groups within the Skirted spiderheads, namely the Sprawling Skirted spiderheads and the Mountain Skirted spiderheads. However, apart from the two groupings discussed above, the Skirted spiderheads did not group very closely together. Additionally, while *S. heterophylla* and *S. phylicoides* are both members of Rebelo's (2001) Mountain Skirted spiderheads, *S. gracilis* ssp. *pinnata* and *S. stellata* are members of the Sprawling and Mountain Skirted spiderheads, respectively. Consequently, these four taxa did not separate into the two groupings.

Members of Rebelo's (2001) Curly spiderheads tended to emerge as quite closely related in most of the analyses, but did not form a monophyletic clade. Rebelo (2001) placed *S. collina*, *S. roxburghii* and *S. nervosa* into the Curly spiderheads, along with five other species, but remarked that they are morphologically quite different from the other taxa placed within this group. While *S. collina* tended to emerge separately from the rest of the taxa in this group in the six analyses, *S. roxburghii* and *S. nervosa* emerged with the rest of the taxa allocated to the Curly spiderheads in most cases.

Only certain morphological characters have been scored for *Serruria*, so hypotheses concerning morphological characters that are potentially phylogenetically informative are only based on known

data. However, it is clear that certain morphological characters tend to remain constant within these groups, but differ between the groups. These include growth habit, leaf indumentum, peduncle visibility, involucral bract shape and visibility, flower orientation, floral scent, floral bract size and visibility, perianth indumentum, and style orientation and indumentum. Although many of these characters are presently viewed as informative (Rebelo 2001), they are currently mostly used to divide the genus up into hypothesised lineages. However, taxa with specific character states of the above-mentioned characters tend to be scattered amongst the groups rather than all grouping together, indicating that many of these characteristics probably evolved several times in *Serruria*, and are therefore only informative within specific lineages within the genus. It is difficult to determine which morphological characters show deeper relationships within the genus, because the trees lack resolution along the spine. Other aspects, e.g. palynology and anatomy, have not been examined thus far, but based upon the results presented here further investigation of potentially informative non-molecular characters appears warranted.

Apart from the above-mentioned groups, none of the other proposed taxonomic groups emerged as monophyletic. These taxonomic groupings are based predominantly on floral characters (characters within the flowers themselves as well as the arrangement of the flowers into inflorescences). Floral characters are viewed as relatively stable, because mutations could inhibit the reproductive fitness of the individual, which could reduce the individual's ability to produce offspring. Thus, floral mutations are usually "sieved" out of taxa. However, some mutations do confer advantages to the individual (e.g. attracts a more efficient pollinator), and these mutations might therefore become fixed in a population (if stochastic events do not wipe them out). Floral characters are consequently thought to evolve slowly compared to vegetative characters, which are much more sensitive to fluctuations in environmental conditions and therefore usually evolve faster. Floral characters usually indicate deeper relationships within a group than do vegetative characters, and are less likely to have undergone reversions (because of their relatively slow rate of evolution). They are therefore usually quite reliable in demarcating groups of taxa, and are consequently often used to delimit taxonomic groups (Moore *et al.* 1998).

However, within some plant groups, floral characters have been found to be unreliable. These include groups where floral characters have remained more or less unchanged, e.g. *Cliffortia* L. (Whitehouse 2002) and *Oxalis* L. (Dreyer pers. comm.). All the species within *Cliffortia* are wind-pollinated, and there is very little variation among the flowers and inflorescences of the species. Consequently, species differentiation within *Cliffortia* is based primarily on vegetative and fruit morphology (Whitehouse 2002). In *Oxalis*, a genus with a tristylous breeding system, floral characters are fixed into a syndrome and therefore do not vary much between species, as is the case in all other heterostylous taxa (Richard & Barrett 1992). Consequently, other characters such as vegetative morphology and palynology, are relied upon to a greater extent to determine evolutionary relationships within the group (Dreyer pers. comm.).

At the other extreme, there is little correlation between floral morphology in *Pelargonium* L'Hér, section *Hoarea* (Sweet) DC. (Geraniaceae) and DNA sequence data. Touloumenidou *et al.* (in press) collected ITS nuclear sequence data for 43 of approximately 85 species within the section. Six clades were identified in the monophyletic *Hoarea* clade. These groupings appear to correlate better with pollen morphology than with floral morphology and karyology. It was therefore proposed that changes in pollinator availability could have caused shifts and convergences in floral types (Goldblatt & Manning 2000).

It is consequently possible that floral characters, or at least the floral characters that are currently considered to be important within *Serruria*, do not accurately reflect relationships within the group for the same reason. Many authors have investigated pollinator-driven speciation in taxonomic groups (e.g. Steiner 1989; Manning & Linder 1992; Manning & Goldblatt 1996; Goldblatt *et al.* 2000), leading Johnson (1995) to suggest that pollinator selection could play a pivotal role in speciation in the CFR. Adaptation to different pollinators results in the evolution of reproductive barriers and different floral characteristics. Pollinator-driven speciation therefore usually results in daughter lineages that are easily distinguishable using floral characters, have different pollinators and are reproductively isolated from their sister lineage (Linder 2003).

Very little is known about pollinators in *Serruria*. *Diptera* and other small flying insects have been observed visiting certain *Serruria* taxa e.g. *S. stellata* (Rourke 1991). The species with inflorescences positioned close to the ground, e.g. *S. rebeloi*, might be pollinated by ants (Rourke 1999). Bees are suspected as the potential pollinators of *Serruria* flowers that are open during the day time, while moths might pollinate the taxa with fragrant white or cream flowers that open nocturnally (Rourke pers comm.). Yet another suspected pollinator is the scarab beetle (Rourke pers comm.). However, none of these insects have thus far been caught to verify whether they transfer pollen between plants (Rourke pers comm.), and much more research is needed in this field.

The lack of congruence between floral and molecular characters has also been found in other taxonomic groups e.g. Orchidaceae (Linder & Kurzweil 1994). Floral characters, especially anther configuration and pollinarium structure, have been used extensively in the classification of the family (Dodson 1962; Romero 1990). However, Dodson (1962) and Atwood (1986) hypothesized that these characters are especially prone to selective pressures of pollinators, and are therefore likely to display high levels of convergence and parallelism. Consequently, as is the case in *Serruria*, researchers have proposed different relationships within Orchidaceae based on morphological evidence e.g. Schlechter (1926); Garay (1960) and Dressler (1993). Additionally, molecular data collected from *rbcL* (Cameron *et al.* 1999) and ITS (Douzery *et al.* 1999) sequences display widespread incongruence with the floral characters currently used in the classification of the group (Kurzweil pers. comm.). However, trees built using trnL-F sequences mostly agree with current classifications based on morphology (Bellstedt *et al.* 2001).

In addition to species not grouping in a manner consistent with proposed ideas based on morphology, two of the four species that have two or more representatives included in the analysis (*S. aitonii* and *S. acrocarpa*) do not group together. It is therefore possible that these species do not represent coherent groups within the genus. This is supported by the fact that both of these species have disjunct distribution ranges (Kohli 2001). If populations are separated from one another for long periods of time, their gene pools will diverge from one another. If hybridization with a member of another species causes different genetic characters to become fixed within one of the populations, the relationship between representatives of different populations will not necessarily emerge as monophyletic. However, this does not mean that the representatives will necessarily look different morphologically. Many of the morphological characters that are viewed as diagnostic of the specific species might be dominant over the characters of the species with which it has hybridized. The hybrid would therefore retain many of the dominant morphological characters of the original species, while possessing a different genetic structure. Hybridization might also lead to introgression (defined by Anderson (Anderson & Hubricht 1938; Anderson 1949, 1953)). It is possible that the regions chosen for a molecular phylogenetic analysis might by chance be chosen from the "incorrect" or non-dominant species, leading to conflict between the phenotype and genotype. A third possibility of why specimens might look very similar, but might possess different histories, is that convergent evolution might have taken place. If two species are growing in similar environments, which is the case for many of the *Serruria* species (Figure 1.4), they might independently adopt similar survival strategies, without sharing a genetic basis to these adaptations. Additionally, many pollinators within the CFR e.g. the Mountain Pride butterfly (*Meneris tulbaghia*), sunbirds and bees, visit a wide variety of distantly related species (Johnson 1994). Naturally, the floral structures of species pollinated by the same vectors have to be quite similar to one another in order for the pollinator to carry out its function with the greatest amount of success. Paraphyletic species have been found to be common (e.g. Rieseberg & Brouillet 1994; Crisp & Chandler 1996) and polyphyletic (multiple-origin) species have also been detected in many groups (e.g. Soltis & Soltis 1999; Grant 2002).

Incorrect assumptions about the morphological characters that are phylogenetically informative are not the only explanation for disagreement between the molecular and morphological data. It is also possible that the molecular evidence might be misleading. Although molecular systematics has revolutionized phylogenetics, it is subject to the same problems as are characters from other sources, including morphological characters. Early molecular systematists believed DNA to be less subject to convergence and parallelisms than is the case for morphological data. This is because morphological characters are more exposed to environmental influences than DNA. They therefore proposed that molecular data is more likely to reflect the "true" phylogeny (Kellogg 1999). However, this early idea has proven to be incorrect under certain circumstances e.g. where hybridization or introgression have occurred.

However, the main cause of molecular data retrieving the incorrect history is in cases where either insufficient characters or taxa have been sampled. Although most of the species (53 of the 55 currently recognized species) are represented in this study, most of the DNA regions sequenced displayed only limited variation among taxa. In addition, strong nodal support was lacking in many cases and this would suggest that insufficient phylogenetically informative characters have been sampled (this despite the fact that over 3 000 characters were collected per taxon).

In summary, a thorough understanding of the evolutionary history of *Serruria* has remained somewhat elusive, despite sampling of a large number of molecular characters across the entire genus. However, although this study has shown that morphological groupings are largely incongruent with molecular data, it perhaps does point towards the need for a comprehensive revision of the genus.

Chapter 3: Summary

Serruria is a relatively large Proteaceae genus, comprising approximately 55 species, which are all endemic to the Western Cape (Rourke 1994). The genus provides an ideal case study for investigating speciation patterns within the CFR for several reasons. The genus has been supported as forming a monophyletic group within Proteoideae by both molecular (Barker *et al.* 2002 & Reeves pers. comm.) and morphological investigations (Rourke 1998), and proposals have been made in the past concerning relationships within the genus (Phillips & Hutchinson 1912; Rebelo 2001 and Rourke pers. comm.). *Serruria* therefore has a strong taxonomic foundation, which is very important for evaluating current ideas of relationships in light of new molecular evidence. Additionally, much is known about the distribution and the environments in which the species grow, making it possible for further investigations into speciation patterns within the genus to take place. Consequently the aim of this study was to reconstruct a species-level phylogeny of *Serruria* using DNA sequence data in order to evaluate proposed hypotheses concerning relationships within the genus. These species-level phylogenies can then be used for further investigations.

A background to the taxonomy and morphology of *Serruria* is outlined in Chapter 1, as well as discussing the current ideas concerning driving forces behind speciation in the CFR. In Chapter 2, these ideas were evaluated in light of the molecular evidence collected from five plastid and one nuclear region using both parsimony and Bayesian algorithms. Although sequence variation was found to be extremely low, resulting in a limited number of potentially informative characters (7.4% for the nuclear region; 5.4% for the combined plastid regions; and 5.8% for the combination of the plastid and nuclear regions), a reasonable degree of resolution was obtained in the final trees.

It was difficult to assess congruence between the data sets from the two genomes due to a lack of informative characters, but there is evidence that at least one intergeneric hybridisation event involving *S. flava* may have taken place. The limited resolution in the trees also prevented robust comparisons between the molecular and morphological data from being made. However, although some previously proposed groups were well supported by the molecular data, findings based on the molecular and morphological data were mostly incongruent with each other. The groupings proposed in light of morphological evidence have been mostly based on floral characters. I have proposed here that differences in pollinator availability may have led to certain floral characters evolving several times in the different lineages of *Serruria*. Little is known about pollinators of *Serruria* species, and this is potentially one of the avenues for further investigation.

In addition to many of the proposed morphological groupings not being supported by the molecular data, two of the four species that were represented by more than one specimen in the analyses did

not form monophyletic groups. It was therefore hypothesised that these taxa may not belong to the same species.

Consequently, this study has contributed to our understanding of evolutionary patterns within *Serruria*. While a lack of informative characters prevents alternative relationships from being proposed, it is clear that a thorough understanding of the evolutionary history of *Serruria* still requires further data. This study has shown that current morphological groupings are largely incongruent with molecular data, and a revision of the genus would therefore provide an ideal opportunity for investigations into alternative hypotheses regarding different modes of speciation. Speciation patterns within the genus could be assessed by mapping environmental factors onto the phylogenetic trees in order to determine the main driving forces of evolution in *Serruria*. Although it was not possible within the present study, other aspects of the taxa e.g. palynology, anatomy and karyology, should also be investigated to determine whether these fields can provide more reliable sources of informative characters. Finally, the numerous cases of species complexes and the paraphyletic species identified in this study could be further investigated by population level studies, e.g. amplified fragment length polymorphisms and microsatellites. Knowledge gained from these various aspects would contribute to many fields, including the conservation of our highly threatened flora.

Appendix A: Major divisions of geological time

Table A1: The eras, periods and epochs of the Earth's history.
BP = before present; mya = millions of years ago. Adapted from
Thompson (1995).

Era	Period	Epoch	Duration
Cenozoic	Quarternary	Holocene	100 000 BP to present
		Pleistocene	2 mya - 100 000 BP
	Tertiary	Pliocene	5 - 2 mya
		Miocene	24 - 5 mya
		Oligocene	38 - 24 mya
		Eocene	55 - 38 mya
		Palaeocene	65 - 55 mya
Mesozoic	Cretaceous		144 - 65 mya
	Jurassic		213 - 144 mya
	Triassic		248 - 213 mya
Palaeozoic	Permian		286 - 248 mya
	Carboniferous		360 - 286 mya
	Devonian		408 - 360 mya
	Silurian		438 - 408 mya
	Ordovician		505 - 438 mya
	Cambrian		590 - 505 mya
	Precambrian		4 600 - 590 mya

Appendix B: The DNA regions sequenced from the plastid and nuclear genomes

The choice of DNA regions to be surveyed depends on, amongst other things, the taxonomic level being investigated. Different parts of the plastid genome evolve at different rates, allowing a researcher to choose the regions that evolve at the rate most suitable for the project in question (Soltis *et al*. 1998). For example, regions that code for molecules (genic DNA) are under greater selection pressures than non-coding regions, and are therefore more likely to evolve at a slower rate. If one chooses a DNA region that evolves too slowly, then the sequences in the various taxa will be very similar and the analysis will yield very few informative characters with which to infer the evolutionary history. On the other hand, a region that is evolving too quickly will contain many sites that have undergone multiple changes among taxa. In the latter case, the phylogenetic signal will be very difficult to detect.

The following plastid regions, all non-coding DNA sequences, were surveyed in this project: *rps16* intron, *trnL-F* intergenic spacer, *trnL* intron, *atpB rbcL* intergenic spacer and *psbA-trnH* intergenic spacer. The internal transcribed spacer (ITS) was chosen as the region on the nuclear genome to be sequenced.

The *rps16* intron is situated between the exons of the ribosomal protein gene *rps16* on the plastid genome. The intron is one of the group II introns (Oxelman *et al*. 1997), which are characterised by a distinct secondary structure and a self-splicing mechanism during the preparation of the mRNA transcript for the translation of the protein (Brown 1999).

Oxelman *et al*. (1997) were the first to use sequences from the *rps16* intron for inferring evolutionary relationships between taxa. They compared *rps16* intron sequences from members of the tribe Sileneae (Caryophyllaceae) with previously assembled *ITS* sequences (Oxelman & Liden 1995) to determine evolutionary relationships within the tribe. They found that the *rps16* intron sequences are reasonably easy to amplify; sequence alignment is relatively straightforward; variability is more or less uniformly distributed throughout the region; sequence divergence is similar to that found in other plastid introns (Gielly *et al*. 1996, Downie *et al*. 1996) and that conserved areas correspond roughly with the stem regions in the secondary structure inferred for *Sinapis alba* (mustard) by Neuhaus *et al*. (1989). The trees built using the *rps16* intron sequences were roughly congruent with those built using the ITS sequences and worked well for sorting out many of the taxanomic problems within the tribe.

The *trnL* intron and *trnL-F* intergenic spacer were also sequenced in order to infer phylogenetic relationships in *Serruria*. These two regions are located in the large single-copy region close to the

rbcL and *atpB* genes, and appear to evolve at the same rate to three times faster than the *rbcL* gene (Soltis *et al*. 1998), a coding region that has been used to infer relationships among angiosperm families (e.g. Chase *et al*. 1993). The rate of evolution in the *trnL-F* intergenic spacer region is particularly variable amongst various taxonomic groups (Soltis *et al*. 1998).

The *trnL* intron and *trnL-F* intergenic spacer have been used to infer species-level relationships in many genera, including *Gentiana*. Gielly and Taberlet (1994 & 1996) used *trnL* sequences to resolve relationships between eight species in *Gentiana*, showing the potential importance of this sequence at the specific level. Other authors using this region successfully for inferring evolutionary relationships at the specific level include Sang *et al*. (1997) and Cros *et al*. (1998). An advantage of the *trnL* intron and *trnL-F* intergenic spacer is that they are easy to amplify and sequence (Taberlet *et al*. 1991). Both regions are relatively small – the *trnL* intron is between 350 and 600 bp long, while the *trnL-F* intergenic spacer appears to be 120-350 bp long in the taxa sampled thus far (Soltis *et al*. 1998).

The *atpB-rbcL* intergenic spacer region is located between the *rbcL* and *atpB* exons in the large single-copy region of the plastid genome. This region has been applied successfully to phylogenetic investigations of Celastrales (Savolainen *et al*. 1994), Rubiaceae (Manen & Natali 1995; Natali *et al*. 1995) and Poaceae (Golenberg *et al*. 1993). It is approximately 800 bp in length. The primer sites used for this region are highly conserved, since they are located in the *rbcL* and *atpB* genes, respectively. This makes the primers effective in a wide variety of plants (Soltis *et al*. 1998). These two genes have similarly slow rates of evolution (Ritland & Clegg 1987; Hoot *et al*. 1995) and are consequently usually used to determine phylogenies at the family level and above (Soltis *et al*. 1998).

Although the *atpB-rbcL* intergenic spacer region is primarily useful in determining relationships between and within genera (Soltis *et al*. 1998), it can also be used to resolve relationships at the specific level in some cases (Manen *et al*. 1994; Natali, *et al*. 1995). Indels (insertions/deletions) are frequent in this region and can be phylogenetically informative, as Natali *et al*. (1995) found in Rubiaceae. However, Golenberg *et al*. (1993) found that indels where homoplasious in the taxa that they investigated, as they occurred at a few labile sites, and warned against using them without caution in determine phylogenetic relationships.

The *psbA-trnH* intergenic spacer lies between the two genes *psbA* and *trnH* close to the end on the large single copy region in the plastid genome (Aldrich *et al*. 1988). This region is known to be a hotspot for length mutations and Aldrich *et al*. (1988) have shown the spacer to be no exception, containing indels between short, nearly perfect AT-rich direct repeats in taxa within Fabaceae and Solanaceae. However, deletions in certain taxa are not flanked by repeated sequences. Although this region has not been used extensively yet to infer evolutionary relationships, Sang *et al*. (1997) found it to be useful in inferring relationships between species of *Paeonia* (Paeoniaceae).

ITS was chosen as the region on the nuclear genome to be surveyed. This DNA sequence forms part of the repetitive region coding for ribosomal RNA (rRNA; Figure 1.7). Because ITS is still able to fold correctly even though it has experienced many nucleotide replacements, it can withstand reasonably high mutation rates and the region is therefore suitable for investigations at the specific level in many groups (Kellogg 1999).

The rRNA regions, including ITS, undergo homogenization in the nucleus – sequences that differ from the other sequences tend to be corrected. This mostly involves mutations being corrected, but because the enzyme responsible for the corrections is unable to distinguish between "correct" and mutated sequences, the original sequences are sometimes "corrected" to match the mutated sequences. Thus, mutations that start in one copy of the region tend to proliferate throughout the other copies. This is known as concerted evolution. However, if mutation rates are faster than correction rates, then the sequences of the different copies might not exactly match, creating polymorphisms (Kellogg 1999). The presence of polymorphisms in the ITS sequences of *Protea* and *Faurea* has caused problems in the utility of this region for inferring the phylogeny of these South African genera (Reeves 2001). However, Barker *et al*. (2002) has used the ITS DNA region successfully to build a tree of all the South African Proteaceae genera, so the polymorphisms occurring in *Protea* and *Faurea* do not appear to be widely spread within Proteoideae. ITS was therefore chosen as the region on the nuclear genome to be used to compare with the plastid data set in order to detect whether hybridisation has occurred in *Serruria*.

Appendix C: Abbreviations Used in the Text

ABI:	Applied Biosystems Inc.
bp:	base pairs
BSA:	bovine serum albumin
BSC:	biological species concept
ca.:	circa, approximately
CFR:	Cape Floristic Region
CI:	consistency index
CTAB:	cetyltrimethylammonium bromide
DNA:	deoxyribonucleic acid
dNTP:	deoxyribonucleotide triphosphate
ed(s).:	editor(s)
indels:	insertions and deletions
in prep.:	in preparation
ITS:	internal transcribed spacer
KCl:	potassium chloride
lnL:	natural logarithm of the likelihood
$MgCl_2$:	magnesium chloride
NBI:	National Botanical Institute
no.:	number
p(p).:	page(s)
PAUP*:	phylogenetic analysis using parsimony and other methods
PCR:	polymerase chain reaction
pers. comm.:	personal communication
rDNA:	ribosomal DNA
RI:	retention index
s.s.:	*sensu stricto*
sp.:	species
ssp.:	subspecies
Taq:	*Thermus aquaticus*
TBR:	tree bisection reconnection
vol.:	volume

References

ALDRICH, J; CHERNEY, BW; MERLIN, E & CHRISTOPHERSON, L. 1988. The role of insertions/deletions in the evolution of the intergenic region between *psbA* and *trnH* in the chloroplast genome. *Current Genetics* **14**: 137 – 146.

ANDERSON, E. 1949. Introgressive hybridisation. Wiley, New York.

ANDERSON, E. 1953. Introgressive hybridisation. *Biological Reviews* **28**: 280 – 307.

ANDERSON, E & HUBRICHT, L. 1938. Hybridisation in *Tradescantia*. III. The evidence for introgressive hybridisation. *American Journal of Botany* **25**: 396 – 402.

ATWOOD, JT. 1986. The size of the Orchidaceae and the systematic distribution of epiphytic orchids. *Selbyana* **9**: 171 – 186.

AXELROD, DI & RAVEN, PH. 1978. Late Cretaceous and Tertiary vegetation history of Africa. In *Biogeography and Ecology of Southern Africa* (eds. MJA Werger & AC van Bruggen). Dr. W. Junk Publishers, The Hague.

BAKKER, FT; CULHAM, A; DAUGHERTY, LC & GIBBY, M. 1999. A *trnL-F* based phylogeny for species of *Pelargonium* (Geraniaceae) with small chromosomes. *Plant Systematics and Evolution* **216**: 309 – 324.

BALDWIN, B.G. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: An example from the Compositae. *Molecular Phylogenetics and Evolution* **1** (1): 3 – 16.

BARKER, NP; WESTON, PH; ROURKE, JP & REEVES, G. 2002. The relationships of the southern African Proteaceae as elucidated by internal transcribed spacer (ITS) DNA sequence data. *Kew Bulletin* **57**: 867 – 883.

BELLSTEDT, DU; LINDER, HP & HARLEY, EH. 2001. Phylogenetic relationships in *Disa* based on non-coding *trnL-trnF* chloroplast sequences: evidence of numerous repeat regions. *American Journal of Botany* **88** (11): 2088-2100.

BERG, RY. 1975. Myrmecochorus plants in Australia and their dispersal by ants. *Australian Journal of Botany* **23** (3): 475 – 508.

BERNHARDT, P & GOLDBLATT, P. 2000. The diversity of pollination mechanisms in the Iridaceae of southern Africa. In *Systematics and Biology of the Monocots* (eds K Wilson & P Weston), pp. 301 – 308. Royal Botanic Gardens, Sydney, Australia.

BOND, WJ & SLINGSBY, P. 1983. Seed dispersal by ants in shrublands of the Cape Province and its evolutionary implications. *South African Journal of Science* **79**: 231 – 233.

BRADSHAW, A. 1972. Some of the evolutionary consequences of being a plant. *Evolutionary Biology* **5**: 25 – 48.

BREMER, K & WANNTORP, HE. 1979. Hierarchy and reticulation in systematics. *Systematic Zoology* **28**: 624 – 627.

BROWN, TA. 1999. *Genomes*. BIOS Scientific Publishers Ltd, Oxford.

CAMERON, KM; CHASE, MW; WHITTEN, WM; KORES, PJ; JARRELL, DC; ALBERT, VA; YUKAWA, T; HILLIS, HG & GOLDMAN, DH. 1999. A phylogenetic analysis of the Orchidacaeae: Evidence from *rbcL* nucleotide sequences. *American Journal of Botany* **86** (2): 208 – 224.

CAMPBELL, BM. 1983. Montane plant environments in the fynbos biome. *Bothalia* **14**: 283 – 298.

CHASE, MW; SOLTIS, DE; OLMSTEAD, RG; MORGAN, D; LES, DH; MISHLER, BD; DUVALL, MR; PRICE, RA; HILLS, HG; QIU, Y-L; KRON, KA; RETTIG, JH; CONTI, E; PALMER, JD; MANHART, JR; SYTSMA, KJ; MICHAELS, HJ; KRESS, WJ; KAROL, KG; CLARK, WD; HEDREN, M; GAUT, BS; JANSEN, RK; KIM, K-J; WIMPEE, CF; SMITH, JF; FURNIER, GR; STRAUSS, SH; XIANG, Q-Y; PLUNKETT, GM; SOLTIS, PS; SWENSEN, SM; WILLIAMS, SE; GADEK, PA; QUINN, CJ; EGUIARTE, LE; GOLENBERG, E; LEARN, GH; GRAHAM, SW; BARRETT, SCH; DAYANANDAN, S & ALBERT, VA. 1993. Phylogenetics of seed plants: An analysis of nucleotide sequences from the plastid gene *rbcL*. *Annals of the Missouri Botanical Gardens* **80**: 528 – 580.

COETZEE, JA. 1993. African flora since the terminal Jurassic. In *Biological Relationships Between Africa and South America* (ed. P. Goldblatt), pp. 37 – 61. Yale University Press, New Haven.

COETZEE, JA & MULLER, J. 1985. The phytogeographic significance of some extinct Gondwana pollen types from the Tertiary of the southwestern Cape (South Africa). *Annals of the Missouri Botanical Gardens* **71**: 1088 – 1099.

CONNELL, JH. 1978. Diversity in tropical forests and coral reefs. *Science* **199**: 1302.

COWLING, RM; HOLMES, PM & REBELO, AG. 1992. Plant diversity and endemism. In *The Ecology of Fynbos: Nutrients, fire and diversity* (ed. RM Cowling), pp. 62 – 112. Oxford University Press, Cape Town.

COWLING, RM; RUNDEL, PW; LAMONT, BB; ARROYO, MK & ARIANOUTSOU, M. 1996. Plant diversity in Mediterranean-climate regions. *Trends in Ecology and Evolution* **11**: 362 – 366.

COWLING, RM; RICHARDSON, DM & MUSTART, PJ. 1997. Fynbos. In: Vegetation of Southern Africa (eds. RM Cowling, DM Richardson & SM Pierce), pp. 99 – 130. Cambridge University Press, Cambridge.

COWLING, RM & LOMBARD, AT. 2002. Heterogeneity, speciation/ extinction history and climate: explaining regional plant diversity patterns in the Cape Floristic Region. *Diversity and Distribution* **8**: 163 – 179.

COWLING, RM & RICHARDSON, DM. 1995. Fynbos: South Africa's unique floral kingdom. Fernwood Press, Vlaeberg.

CRISP, MD & CHANDLER, GT. 1996. Paraphyletic species. *Telopea* **6**: 813 – 844.

CRON, RC; SMALL, RL, HASELKORN, T & WENDEL, JF. 2002. Rapid diversification of the cotton genus (*Gossypium*: Malvaceae) revealed by analysis of sixteen nuclear and chloroplast genes. *American Journal of Botany* **80**: 89 – 92.

CROS, J; COMBES, MC, TROUSLOT, P; ANTHONY, F; HAMON, S; CHARRIER, A & LASHERMES, P. 1998. Phylogenetic analysis of chloroplast DNA variation in *Coffea* L. *Molecular Phylogenetics and Evolution* **9**: 109 – 117.

References

DE VOS, MP 1982. The African genus *Tritonia* Ker-Gawler 1. *Journal of South African Botany* **48**: 105 – 163.

DIXON, MT & HILLIS, DM. 1993. Ribosomal RNA secondary structure: compensatory mutations and implications for phylogenetic analysis. *Molecular Biology and Evolution* **10**: 256 – 267.

DODSON, C. 1962. The importance of pollination in the evolution of the orchids of tropical America. *American Orchid Society Bulletin* **31**: 525 – 554.

DOUGLAS, AW. 1995. Proteaceae. In: *Flora of Australia* Volume 16, Elaeagnaceae, Proteaceae I (ed. P. McCarthy), pp. 4 – 14. Australian Nature Conservation Agency, Melbourne.

DOUZERY, EJP; PRIDGEON, AM; KORES, P; LINDER, HP; KURZWEIL, H & CHASE, MW. 1999. Molecular phylogenetics of Diseae (Orchidaceae): A contribution from nuclear ribosomal ITS sequences. *American Journal of Botany* **86** (6): 887 – 899.

DOWNIE, SR; KATZ-DOWNIE, DS & CHO, K-J. 1996. Phylogenetic analysis of Apiaceae subfamily Apioideae using nucleotide sequences from the chloroplast *rpoCI* intron. *Molecular Phylogenetics and Evolution* **6**: 1 – 18.

DOYLE, JJ & DOYLE, JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* **19**: 11 – 15.

DRESSLER, RL. 1993. Phylogeny and classification of the orchid family. Dioscorides Press, Portland, OR.

EHRLICH, PR & RAVEN, PH. 1969. Differentiation of populations. *Science* **165**: 1228 – 1232.

FARRIS, JS; ALBERT, VA; KALLERSJO, M; LIPSCOMB, D & KLUGE, AG. 1996. Parsimony jackknifing outperforms neighbour-joining. *Cladistics* **12**: 99 – 124.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using bootstrap. *Evolution* **46**: 159 – 173.

FENNER, M; LEE, WG & WILSON, JB. 1997. A comparative study of the distribution of genus size in twenty angiosperm floras. *Biological Journal of the Linnean Society* **62**: 225 – 237.

FERGUSON, CJ & JANSEN, RK. 2002. A chloroplast DNA phylogeny of eastern *Phlox* (Polemoniaceae): implications of congruence and incongruence with the ITS phylogeny. *American Journal of Botany* **89**: 1324 – 1335.

FITCH, WM. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Systematic Zoology* **18**: 374 – 385.

FUNK, VA. 1985. Phylogenetic patterns and hybridization. *Annals of the Missouri Botanical Gardens* **72**: 681 – 715.

GARAY, L. 1960. On the origin of the Orchidaceae. *Botanical Museum Leaflets* **19**: 57 – 88.

GERMISHUIZEN, G & MEYER, NL (eds). 2003. Plants of southern Africa: an annotated checklist. *Strelitzia 14*. National Botanical Institute, Pretoria.

GEYER, CJ. 1991. Markov chain Monte Carlo maximum likelihood. In *Computing Science and Statistics: Proceedings of the 23rd Symposium on the Interface* (ed. EM Keramidas), pp 156 – 163. Fairfax Station: Interface Foundation.

GIELLY, L & TABERLET, P. 1994. The use of chloroplast DNA to resolve plant phylogenies: non-coding versus *rbcL* sequences. *Molecular Biology and Evolution* **11**: 769 – 777.

GIELLY, L & TABERLET, P. 1996. A phylogeny of the European gentians inferred from chloroplast *trnL* (UAA) intron sequences. *Botanical Journal of the Linnean Society* **120**: 57 – 75.

GIELLY, L; YUAN, YM; KUPFER, P & TABERLET, P. 1996. Phylogenetic use of non-coding regions in the genus *Gentiana* L.: chloroplast *trnL* (UAA) intron versus nuclear ribosomal internal transcribed spacer sequences. *Molecular Phylogenetics and Evolution* **5**: 460 – 466.

GOLDBLATT, P. 1971. Cytological and morphological studies in the southern African Iridaceae. *Journal of South African Botany* **37**: 377 – 460.

GOLDBLATT, P. 1978. An analysis of the flora of southern Africa: its characteristics, relationships and origins. *Annals of the Missouri Botanical Gardens* **65**: 369 – 436.

GOLDBLATT, P. 1982. Systematics of *Freesia* Klatt (Iridaceae). *Journal of South African Botany* **48**: 39 – 91.

GOLDBLATT, P. 1997. Floristic diversity in the Cape flora of South Africa. *Biodiversity and Conservation* **6**: 359 – 377.

GOLDBLATT, P; BERNHARDT, P & MANNING, J. 1998. Pollination of petalloid geophytes by monkey beetles (Scarabaeidae: Rutelinae: *Hopliini*) in southern Africa. *Annals of the Missouri Botanical Gardens* **85**: 215 – 230.

GOLDBLATT, P & MANNING, JC. 1996. Phylogeny and speciation in *Lapeirousia* subgenus *Lapeirousia* (Iridaceae subfamily Ixioideae). *Annals of the Missouri Botanical Gardens* **83**: 346 – 361.

GOLDBLATT, P & MANNING, JC. 1998. *Gladiolus* in southern Africa. Fernwood Press, Vlaeberg.

GOLDBLATT, P & MANNING, JC. 1999. The long-proboscid fly pollination system in *Gladiolus* (Iridaceae). *Annals of the Missouri Botanical Gardens* **86**: 758 – 774.

GOLDBLATT, P & MANNING, J. 2000. The long-proboscid fly pollination system in southern Africa. *Annals of the Missouri Botanical Gardens* **87**: 146 – 170.

GOLDBLATT, P & MANNING, J. 2002. Plant diversity of the Cape region of southern Africa. *Annals of the Missouri Botanical Gardens* **89**: 281 – 302.

GOLDBLATT, P; MANNING, JC & BERNHARDT, P. 2000. Adaptive radiation of pollination mechanisms in *Sparaxis* (Iridaceae: Ixioideae). *Adansonia* **22**: 57 – 70.

GOLDBLATT, P; SAVOLAINEN, V; PORTEOUS, O; SOSTARIC, I; POWELL, M; REEVES, G; MANNING, JC; BARRACLOUGH, TG & CHASE, MW. 2002. Radiation in the Cape flora and the phylogeny of peacock irises *Moraea* (Iridaceae) based on four plastid DNA regions. *Molecular Phylogenetics and Evolution* **25**: 341 – 360.

GOLENBERG, EM; CLEGG, MT; DURBIN, ML; DOEBLEY, J & MA, DP. 1993. Evolution of a non-coding region of the chloroplast genome. *Molecular Phylogenetics and Evolution* **2**: 52 – 64.

GOOD, R. 1974. The geography of the flowering plants. Longmans, Green & Co., London.

GRANT, V. 1971. Plant speciation. Columbia University Press, New York and London.

GRANT, V. 1981. Plant speciation. Columbia University Press, New York.

GRANT, V. 2002. Frequency of spontaneous amphiploids in *Gilia* (Polemoniaceae) hybrids. *American Journal of Botany* **89**: 1197 – 1202.

GRUBB, P. 1977. The maintenance of species richness in plant communities: importance of the regeneration niche. *Biological Review* **52**: 107 – 145.

HASTINGS, WK. 1970. Monte Carlo sampling methods using Markov chains and their applications. *Biometrika* **57**: 97 – 109.

HENDEY, QB. 1982. Langebaanweg: a record of past life. South African Museum, Cape Town.

HENNIG, W. 1966. Phylogenetic systematics. University of Illinois Press, Illinois.

HOOT, SB; CULHAM, A & CRANE, PR. 1995. The utility of *atpB* gene sequences in resolving phylogenetic relationships: Comparison with *rbcL* and 18S ribosomal DNA sequences in the Lardizabalaceae. *Annals of the Missouri Botanical Garden* **82**: 194 – 208.

HOOT, SB & DOUGLAS, AW. 1998. Phylogeny of the Proteaceae based on *atpB* and *atpB-rbcL* intergenic spacer region sequences. *Australian Systematic Botany* **11** (3 – 4): 301 – 320.

HUELSENBECK, JP & RONQUIST, F. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17**: 754 – 755.

HUTCHINSON, GE. 1961. The paradox of the plankton. *American Naturalist* **95**: 137 – 145.

JOHNSON, LAS & BRIGGS, BG. 1975. On the Proteaceae: The evolution and classification of a Southern family. *Journal of the Linnean Society of Botany* **70**: 83 – 182.

JOHNSON, SD. 1994. Pollination and the evolution of floral traits: selected studies in the Cape flora. Ph.D. thesis, University of Cape Town, Cape Town.

JOHNSON, SD. 1995. Pollination, adaptation and speciation models in the Cape flora of South Africa. *Taxon* **45**: 59 – 66.

JUDD, WS. 1999. Chapter 8: Phylogenetic relationships of angiosperms. In *Plant systematics: A phylogenetic approach* (eds WS Judd, CS Campbell, EA Kellogg & PF Stevens), pp. 161 – 419. Sinauer Associates Inc., Massachusetts.

KELLOGG, EA. 1999. Chapter 5: Molecular systematics. In *Plant systematics: A phylogenetic approach* (eds WS Judd, CS Campbell, EA Kellogg & PF Stevens), pp. 93 – 106. Sinauer Associates Inc., Massachusetts.

KLAK, C; HEDDERSON, TA & LINDER, HP. 2003. A molecular systematic study of the *Lampranthus* group (Aizoaceae) based on the chloroplast *trnL-trnF* and nuclear ITS and 5S NTS sequence data. *Systematic Botany* **28** (1): 70 – 85.

KOHLI, N. 2001. Protea Atlas Project Interim distribution maps, Cape edition 3: Volume 4: Spiderheads and allies (ed. T. Rebelo).

KRUGER, FJ. 1979. Fire. In *Fynbos ecology: a preliminary synthesis* (ed. J Day, *et al.*). CSIR Report No. 40, Pretoria.

LEACHE, AD, & REEDER, TW. (2002). Molecular systematics of the Eastern Fence Lizard (*Sceloporus undulatus*): A comparison of parsimony, likelihood, and Bayesian approaches. *Systematic Biology* **51**: 44 – 68.

LEE, MSY. 2001. Unalignable sequences and molecular evolution. *Trends in Ecology and Evolution* **16**: 681 – 685.

LE MAITRE, DC & MIDGLEY, JJ. 1992. Plant reproductive ecology. In *The ecology of fynbos: nutrients, fire and diversity* (ed. R.M. Cowling), pp. 135 – 174. Oxford University Press, Cape Town.

LEVIN, DA. 1993. Local speciation in plants: The rule not the exception. *Systematic Botany* **18**: 197 – 208.

LEVIN, DA & KERSTNER, HW. 1974. Gene flow in seed plants. *Evolutionary Biology* **7**: 139 – 220.

LEVYNS, MR. 1970. A revision of the genus *Paranomus* (Proteaceae). *Contributions from the Bolus Herbarium* **2**: 3 – 48.

LINDER, HP. 1981. Taxonomic studies on the Disinae. III. A revision of *Disa* Berg. excluding sect. *Micranthae* Lindl. *Contributions from the Bolus Herbarium* **9**: 1 – 370.

LINDER, HP. 1985. Gene flow, speciation and species diversity patterns in a species-rich area: the Cape flora. In *Species and Speciation* 4 (ed. E.S. Vrba), pp. 53 – 57. Transvaal Museum, Pretoria.

LINDER, HP. 2003. The radiation of the Cape flora, southern Africa. *Biological review* **78**: 597 – 638.

LINDER, HP & KURZWEIL, H. 1994. The phylogeny and classification of the Diseae (Orchidoideae: Orchidaceae). *Annals of Missouri Botanical Gardens* **81**: 687 – 713.

LINDER, PH & VLOK, JH. 1991. The morphology, taxonomy and evolution of *Rhodocoma* (Restionaceae). *Plant Systematics and Evolution* **175**: 139 – 160.

LOUW, AB & REBELO, AG. 1996. Vegetation of South Africa, Lesotho and Swaziland. Department of Environmental Affairs and Tourism, Pretoria.

MADDISON, DR; RUVOLO, M & SWOFFORD, DL. 1992. Geographic origins of human mitochondrial DNA: Phylogenetic evidence from control region sequences. *Systematic Biology* **41**: 111 – 124.

MANEN, JF & NATALI, A. 1995. Comparison of the evolution of ribulose-1,5-biphosphate carboxylase (*rbcL*) and *atpB-rbcL* noncoding spacer sequences in a recent plant group, the tribe Rubiaea (Rubiaceae). *Journal of Molecular Evolution* **38**: 577 – 582.

MANEN, JF; NATALI, A & EHRENDORFER, F. 1994. Phylogeny of Rubiaceae-Rubieae inferred from the sequence of a cpDNA intergenic region. *Plant Systematics and Evolution* **190**: 195 – 211.

MANNING, JC & GOLDBLATT, P. 1996. The *Prosoeca peringueyi* (Diptera: Nemestrinidae) pollination guild in southern Africa: long-tongued flies and their tubular flowers. *Annals of the Missouri Botanical Garden* **83**: 67 – 86.

MANNING, JC & LINDER, HP. 1992. Pollinators and evolution in *Disperis* (Orchidaceae), or why are there so many species? *South African Journal of Science* **88**: 38 – 49.

MARLOTH, R. 1908. Das Kapland, insonderheit das Reich der Kapflora, das Waldgebiet und die Karroo, pflanzengeographisch dargestellt. In: *Wissenschaffliche Ergebnisse der deutschen Tiefsee-Expedition auf dem Dampfer 'Valdivia' 1898 – 1899*, Vol. 2, Part 3 (ed. C Chun), pp. 1 – 427. Gustav Fischer, Jena.

References

MAST, AR. 1998. Molecular Systematics of subtribe Banksiinae (*Banksia* and *Dryandra*; Proteaceae) based on cpDNA and nrDNA sequence data: Implications for taxonomy and biogeography. *Australian Systematic Bontany* **11**: 321 – 342.

MAYR, E. 1969. The biological meaning of species. *Biological Journal of the Linnean Society* **1**: 311 – 320.

MCDONALD, DJ. 1995. Phytogeography, endemism and diversity of the fynbos of the southern Langeberg. PhD thesis, University of Cape Town.

METROPOLIS, N; ROSENBLUTH, AW; ROSENBLUTH, MN; TELLER, AH & TELLER, E. 1953. Equations of state calculations by fast computing machines. *Journal of Chemistry and Physics* **21**: 1087 – 1091.

MIDGLEY, JJ. 2002. More fynbos mouse stories. *Veld & Flora* **88**: 55.

MILNE, RI; ABBOTT, RJ; WOLFF, K & CHAMBERLAIN, DF. 1999. Hybridisation among sympatric species of *Rhododendron* (Ericaceae) in Turkey: morphological and molecular evidence. *American Journal of Botany* **86**: 1776 – 1785.

MOORE, R; CLARK, WD & VODOPICH, DS. 1998. Botany. WCB/McGraw-Hill, Boston, Massachusetts.

NEUHAUS, H; SCHOLZ, A & LINK, G. 1989. Structure and organisation of a split chloroplast gene from mustard (*Sinapis alba*): ribosomal protein gene *rps16* reveals unusual transcriptional features and complex RNA maturation. *Current Genetics* **15**: 63 – 70.

OLIVER, EGH. 1980. Studies in the Ericoideae. III. The genus *Grisebachia*. *Bothalia* **13**: 65 – 93.

OLMSTEAD, RG; DEPAMPHILIS, CW; WOLFE, AD; NELSON, ND; ELISENS, WJ & REEVES, PA. 2001. Disintegration of the Scrophylariaceae. *American Journal of Botany* **88**: 348 – 361.

OXELMAN, B & LIDEN, M. 1995. Generic boundaries in the tribe Sileneae (Caryophyllaceae) as inferred from nuclear rDNA sequences. *Taxon* **44**: 525 – 542.

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

PALMER, JD. 1985. Comparative organisation of chloroplast genomes. *Annual Review of Genetics* **19**: 325 – 354.

PALMER, JD. 1992. Mitochondrial DNA in plant systematics: applications and limitations. In *Molecular Systematics of Plants* (eds. PS Soltis, DE Soltis and JJ Doyle), pp. 36 – 49. Chapman & Hall, New York.

PANCHEN, AL. 1992. Classification, evolution and the nature of biology. Cambridge University Press, Cambridge.

PHILLIPS, EP & HUTCHINSON, J. 1912. Proteaceae. In WT Thisleton-Dyer, *Flora capensis* **5**: 654 – 685.

PHILLIPS, JB; ROBERTSON, KR; ROHRER, JR & SMITH, PG. 1991. Origins and evolution of subfam. Maloideae (Rosaceae). *Systematic Botany* **16**: 303 – 332.

RAO, V. 1971. Proteaceae. Botanical Monograph no. 6. Council of Scientific & Industrial Research, New Delhi.

RAVEN, PH. 1980. Hybridisation and the nature of species in higher plants. *Canadian Botanical Association Bulletin*, Supplement to Vol. **13** (1): 3 – 10.

REBELO, T. 2001. Proteas: A field guide to the Proteas of southern Africa. Fernwood Press, Vlaeberg.

REEVES, G. 2001. Radiation and macroevolutionary ecology of the African genus *Protea* L. Ph.D. thesis, Imperial College of Science, Technology and Medicine, University of London, London.

RICHARDS, JH & BARRETT, SCH. 1992. The development of heterostyly. In: *Monographs on Theoretical and Applied Genetics 15: Evolution and function of heterostyly* (ed. SCH Barrett), pp. 85 – 127. Springer-Verlag, Berlin Heidelberg.

RICHARDSON, JE; WEITZ, FM; FAY, MF; CRONK, QCB; LINDER, HP; REEVES, G & CHASE, MW. 2001. Phylogenetic analysis of *Phylica* L. with an emphasis on island species: evidence from plastid *trnL-F* DNA and nuclear internal transcribed spacer (ribosomal DNA) sequences. *Taxon* **50**: 405 – 427.

RIESEBERG, LH. 1995. The role of hybridisation in evolution: old wine in new skins. *American Journal of Botany* **82**: 944 – 953.

RIESEBERG, LH & BROUILLET, L. 1994. Are many species paraphyletic? *Taxon* **43**: 21 – 32.

RIESEBERG, LH & MOREFIELD, JD. 1995. Character expression, phylogenetic reconstruction, and the detection of reticulate evolution. In *Experimental & Molecular Approaches to Plant Biosystematics* 53 (eds PC Hoch & AG Stephenson), pp. 333 – 353.

RIESEBERG, LH & SOLTIS, DE. 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. *Evolutionary Trends in Plants* **5**: 65 – 84.

RITLAND, K & CLEGG, MT. 1987. Evolutionary analysis of plant DNA sequences. *American Naturalist* **130**: S74 – S100.

ROMERO, GA. 1990. Phylogenetic relationships in subtribe Catasetinae (Orchidaceae). *Lindleyana* **5**: 160 – 181.

ROURKE, JP. 1969. Taxonomic studies of *Sorocephalus* R. Br. and *Spatalla* Salisb. *Journal of South African Botany* Supplement **7**: 1 – 124.

ROURKE, JP. 1972. Taxonomic studies on *Leucospermum* R. Br. *Journal of South African Botany* Supplementary Vol. **8**: 1 – 194.

ROURKE, JP. 1976. A revision of *Diastella* (Proteaceae). *Journal of South African Botany* **42**: 185 – 210.

ROURKE, JP. 1980. The proteas of southern Africa. Purnell Press, Cape Town.

ROURKE, JP. 1982a. *Mimetes*. An account of *Mimetes* Salisb. and *Orothamnus* Pappe. Tiyan Publishers, Cape Town.

ROURKE, JP. 1982b. Two new species of *Serruria* Salisb. (Proteaceae) from the South Western Cape. *Journal of South African Botany* **48** (3): 285 – 293.

ROURKE, JP. 1984a. A revision of the genus *Mimetes* Salisb. (Proteaceae). *Journal of South African Botany* **50**: 171 – 236.

ROURKE, JP. 1984b. *Vexetorella* Rourke, a new genus of the Proteaceae from Southern Africa. *Journal of South African Botany* **50**: 373 – 391.

ROURKE, JP. 1987. The inflorescence morphology and systematics of *Aulax* (Proteaceae). *Journal of South African Botany* **53**: 464 – 480.

ROURKE, JP. 1990. Four new species of *Serruria* (Proteaceae) from the south-western Cape. *South African Journal of Botany* **56** (4): 497 – 505.

ROURKE, JP. 1991. *Serruria stellata*. *Flowering Plants of Africa*: t. 2032.

ROURKE, JP. 1994. Three new species of *Serruria* (Proteaceae) from the southwestern Cape. *Bothalia* **24** (1): 1 – 7.

ROURKE, JP. 1996. A new species of *Serruria* from the northern part of the Western Cape. *Bothalia* **26** (2): 154 – 157.

ROURKE, JP. 1998. A review of the systematics and phylogeny of the African Proteaceae. *Australian Systematic Botany* **11**: 267 – 285.

ROURKE, JP. 1999. A new species of *Serruria* from the southern Cape, South Africa. *Bothalia* **29** (2): 263 – 266.

ROURKE, JP. 2000a. Proteaceae. In *Cape Plants: a conspectus of the Cape Flora of South Africa* (eds P Goldblatt & JC Manning), pp. 457 – 461. National Botanical Institute of South Africa & MBG Press, Missouri Botanical Garden, Cape Town.

ROURKE, JP. 2000b. *Serruria*. In *Cape Plants: a conspectus of the Cape Flora of South Africa* (eds P Goldblatt & JC Manning), pp. 592 – 595. National Botanical Institute of South Africa & MBG Press, Missouri Botanical Garden, Cape Town.

ROURKE, JP & WIENS, D. 1977. Convergent floral evolution in South African and Australian Proteaceae and its possible bearing on pollination by non-flying mammals. *Annals of the Missouri Botanical Gardens* **64** (1): 1 – 17.

SALISBURY, RA. 1807. Paradisus Londinensis Vol. 1. Williams Hooker, London.

SANG, T; CRAWFORD, DJ & STUESSY, TF. 1997. Chloroplast DNA phylogeny, reticulate evolution and biogeography of *Paeonia* (Paeoniaceae). *American Journal of Botany* **84** (8): 1120 – 1136.

SAVOLAINEN, V; CUÉNOUD, P; SPICHIER, R; MARTINEZ, MPP; CRÕVECOEUR, M & MANEN, JF. 1995. The use of herbarium specimens in DNA phylogenetics: evaluation and improvements. *Plant Systematics and Evolution* **197**: 87 – 98.

SAVOLAINEN, V, MANEN, JF, DOUZERY, E & SPICHIGER, R. 1994. Molecular phylogeny of families related to Celestrales based on *rbcL* 5' flanking sequences. *Molecular Phylogenetics and Evolution* **3**: 27 – 37.

SCHLECHTER, R. 1926. Das System der Orchidaceen. *Notizblatt des Botanischen Gartens und Museums zu Berlin-Dahlem* **9**: 563 – 591.

SMITH, RL & SYTSMA, KJ. 1990. Evolution of *Populus nigra* (sect. *Aigeiros*): introgressive hybridization and the chloroplast contribution of *Populus alba* (sect. *Populus*). *American Journal of Botany* **77**: 1176 – 1187.

SOLTIS, DE; HIBSCH-JETTER, C; SOLTIS, PS; CHASE, MW & FARRIS, JS. 1997. Molecular phylogenetic relationships among angiosperms: an overview based on *rbcL* and 18S rDNA sequences. In: Evolution and Diversification of Land Plants (eds. K Iwatsuki and PH Raven), pp. 157 – 178. Springer -Verlag, Tokyo.

SOLTIS, DE & SOLTIS, PS. 1999. Polyploidy: recurrent formation and genome evolution. *Trends in Ecology & Evolution* **14**: 348 – 352.

SOLTIS, DE; SOLTIS, PS & DOYLE, JJ. 1998. Molecular systematics of plants II: DNA sequencing. Kluwer Academic Publishers, Dordrecht.

STEINER, KM. 1989. The pollination of *Disperis* (Orchidaceae) by oil-collecting bees in southern Africa. *Lindleyana* **4**: 164 – 183.

SWOFFORD, DL. 2000. PAUP*. *Phylogenetic Analysis using Parsimony (*and other methods)*. Version 4. Sinauer Associates, Sunderland, Massachusettes.

TABERLET, P; GIELLY, L; PAUTOU, G & BOUVET, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* **17**: 1105 – 1109.

TAVARE, S. 1986. Some probabilistic and statistical problems on the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences* **17**: 57 – 86.

TEMPLETON, AR. 1981. Mechanisms of speciation – a population genetic approach. *Annual Review of Ecology and Systematics* **12**: 23 – 48.

THOMPSON, D (ed.). 1995. The concise Oxford Dictionary. Clarendon Press, Oxford.

THUNBERG, CP. 1781. Dissertatio Botanica de Protea. J Edman. Uppsala.

VERBOOM, AG; LINDER, HP & STOCK, WD. 2003. Phylogenetics of the grass genus *Ehrharta*: Evidence for radiation in the summer-arid zone of the South African Cape. *Evolution*: **57** (5): 1008 – 1021.

VILLAGRÁN, CM. 1994. Quarternary history of the mediterranean vegeation of Chile. In *Ecology and biogeography of Mediterranean ecosystems in Chile, California and Australia* (eds MT Kalin Aroyo, PH Zedler & MD Fox), pp. 3 – 20. *Ecological Studies* **108**. Springer-Verlag, New York.

VOGEL, S. 1954. Blütenbiologische Typen als Elemente der Sippengliederung. *Botanische Studien* **1**: 1 – 338.

VOGTS, M. 1960. The South African Proteaceae: The need for more research. *South African Journal of Science* **56** (12): 297 – 305.

VOGTS, M. 1982. South Africa's Proteaceae: Know them and grow them. C. Struik (Pty) Ltd Publishers, Cape Town.

WAKASUGI, T; SUGITA, M; TSUDZUKI, T & SUGIURA, M. 1998. Updated gene map of tobacco chloroplast DNA. *Plant Molecular Biology Reporter* **16**: 231 – 241.

WHITE, TJ; BRUNS, T; LEE, S & TAYLOR, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR protocols: a guide to methods and applications* (eds. MA Innis, D Gelfond, J Sninsky & T. White), pp 315 – 322. Academic Press, San Diego.

WHITEHOUSE, CM. 2002. Systematics of the genus *Cliffortia* L. (Rosaceae). Ph.D. thesis, University of Cape Town, Cape Town.

WHITTAKER, RH. 1972. Evolution and measurement of species diversity. *Taxon* **21**: 213 – 251.

WILLIAMS, IJM. 1972. A revision of the genus *Leucadendron* (Proteaceae). *Contributions from the Bolus Herbarium* **3**: 1 – 425.

YANG, Z. 1993. Maximum likelihood estimation of phylogeny from DNA sequences when substitution rates differ over sites. *Molecular Biology and Evolution* **10**: 1396 – 1401.