SYSTEMATIC STUDIES OF THE SOUTHERN AFRICAN PSORALEOID LEGUMES

BY

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DECLARATION STATEMENT

I know the meaning of plagiarism and declare that all of the work in the document, save for that which is properly acknowledged, is my own.

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ABSTRACT

The Psoraleeae are of worldwide distribution, consisting of 185 species in nine genera. More than 60 % of the species are members of the genera, *Otholobium* C.H.Stirt and *Psoralea* L., both of which have a centre of diversity in the Cape Floristic Region of South Africa. This dissertation was aimed at conducting a systematic study of the southern African Psoraleeae. This involved reconstructing phylogenetic relationships, estimating dates of lineage divergence, investigating the role of edaphic factors in the distribution of the southern African Psoraleeae and revising the taxonomy of some species complexes in the genus *Psoralea*.

Phylogenetic relationships between and within the genera of the tribe Psoraleeae were reconstructed based on *trnL-F*, *rpoB-trnC* and *ITS* sequence data. The genera together formed a well supported clade, suggesting a monophyletic tribe Psoraleeae. However, its recognition at the tribal rank is questionable as some higher level molecular phylogenetic studies have shown that it is nested within the tribe Phaseoleae. Therefore, it is proposed that the Psoraleeae be recognised at sub-tribal rank.

A phylogeny of the southern African genera, *Otholobium* and *Psoralea* was reconstructed based on the three DNA loci mentioned above and a morphological and anatomical data set of 40 characters. The results indicated that the genus *Otholobium* is polyphyletic. Some South American species that are presently recognised as *Otholobium* were resolved in a clade distinct from the southern African ones, as sister to the American genus *Hoita*. Moreover, the genus *Psoralea* was embedded within the southern African *Otholobium*. This suggests that the current generic circumscriptions do not follow the Hennigian principle of monophyly, which requires that supraspecific taxa should be monophyletic. However, due to low resolution in most parts of the phylogeny, no taxonomic changes are made.

Estimation of divergence dates indicated that diversification of the southern African Psoraleeae started between 2.6 and 10 million years ago, with the genus *Psoralea* emerging about 2.4 million years ago. For such a species rich lineage (about 103 species) this indicates that speciation has been very rapid, and thus the Psoraleeae are one of the Cape lineages that have experienced recent rapid radiation possibly triggered by climate change in the late Miocene.

In terms of geographical distribution, it was found that 66 % of *Psoralea* species occur on sandstone-derived soils, with a few species occurring on limestone, granite, shale and sand soils.

On the other hand, *Otholobium* species are equally distributed on sandstone, granite and shale derived substrates. Furthermore, on the limestone and sand habitats, there are more species of the genus *Otholobium* than *Psoralea*. An analysis of the nutrient levels of soils occupied by species of both genera indicated that resprouters occupy soils with low nutrient levels. However, although some reseeders are associated with higher nutrient levels, some of them occur on low nutrient soils. This suggests that the evolution of either regeneration strategy might have been influenced by edaphic factors, with low nutrient levels favouring resprouting and high nutrient levels favouring the reseeding habit.

A revision of the taxonomic limits in the *Psoralea aphylla* and *Psoralea pinnata* complexes was carried out using morphometrics and multivariate statistical techniques. The results showed that there were several species embedded within each of the two complexes. Their full descriptions and keys are provided to aid in identification.

CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.0. Problem statement

The tribe Psoraleeae Benth. (Fabaceae, sub-family Papillionoideae) is of worldwide distribution, consisting of 185 species in nine genera (Table 1). About 60% of the diversity is endemic to southern Africa, where there are about 106 species in three genera (Stirton, 2005). These are *Otholobium* C.H.Stirt (53 species), *Psoralea* L. (50 species) and *Cullen* Medik. (three species). The rest of the diversity is widely distributed in various parts of the world, including Australia, North America, South America, Mediterranean Europe, Indonesia, Philippines, Malaysia, Turkey and Macaronesia, as indicated in Table 1.

Table 1. Genera of the tribe Psoraleeae and their distributions according to Stirton (2005).

Genus	Number of species	Distribution
Otholobium **C.H.Stirt.**	61	Southern Africa to East Africa (53 species), South America (8 species)
Psoralea **L.**	50	Southern Africa
Pediomelum **Rydb.**	21	Canada, USA and Mexico
Orbexillum **Raf.**	8	Eastern USA (Virginia to Florida)
Rupertia **J.W.Grimes**	3	Western USA (Vancouver Island to California) and northwest Mexico
Hoita **Rydb.**	3	Western USA (California)to northwest Mexico
Psoralidium **Rydb.**	3	Widespread in North America to Mexico
Cullen **Medik.**	34	Mainly Australia, one species in Philippines, Indonesia, Malaysia, Mediterranean, and 3 species in southern Africa
Bituminaria **Heist. ex Fabr.**	2	Macaronesia, Mediterranean, Turkey to Caucasus

The American and the Australian members of the tribe have been the focus of recent studies on taxonomy (Grimes, 1990 and Grimes, 1997), phylogenetics (Egan & Crandall, 2008a) and biogeography (Egan & Crandall, 2008b). However, although the greatest diversity of the Psoraleeae occur in southern Africa, no studies have been done on the phylogenetics, biogeography, macroevolution, dates of lineage divergence and drivers of speciation in the Psoraleeae endemic to this region. In terms of taxonomy, the position of the Psoraleeae as a tribe within the family Leguminosae is not yet fully resolved, and generic and species boundaries between and within the southern African Psoraleeae (i.e. the genera, *Otholobium* and *Psoralea*) are also not well resolved. The latest taxonomic treatment is a revision of the genus *Otholobium* by Stirton (1989) in an unpublished thesis. The genus *Psoralea* (51 species) on the other hand, has not been revised and because of this, species identification is difficult since nearly half of the species are known only by manuscript names (e.g. Goldblatt & Manning, 2000).

The overall goal of this dissertation was to carry out a systematic study of the southern African Psoraleeae. This involved reconstructing phylogenetic relationships, tracing the evolution of key diagnostic characters of the genera and estimating dates of lineage divergence. In addition, the study also sought to investigate species distributions in relation to edaphic factors, in order to explore whether edaphic factors have played a role in the diversification of lineage and finally, to revise the nomenclature and species delimitation within the genus *Psoralea*.

1.1. Some economic uses and ecological functions of the Psoraleeae

The Psoraleeae have for a long time received much attention from phytochemists because they produce large quantities of secondary metabolites, particularly furocoumarins (Maisch, 1889; Bourgaud *et al*., 1990; Innocenti *et al*., 1991; Pistelli *et al*., 2003; Maurich *et al*., 2006), which are widely used in the cosmetics and dermatology industry (Bourgaud *et al*., 1990; Bertoli *et al*., 2004). Studies on some Australian species of the genus *Cullen* showed that these species produce from 1000 to 8000 ppm of total furocoumarins per gram dry weight of leaf material (Innocenti *et al*., 1984; Innocenti *et al*., 1991). Such a high concentration of furocoumarins provides an alternative source for these compounds because their artificial synthesis is extremely expensive (Bourguad *et al*., 1990). Another study by Pistelli *et al*. (2003) showed that two species of the genus *Bituminaria* Heist ex. Fabr. produce large quantities of pterocarpans. These compounds are rare in plants and therefore the high yield obtained from these species implies a new and rich source of such compounds (Pistelli *et al*., 2003). Pterocarpans have a high agricultural potential as pest control agents (Ingham, 1973). Furthermore, a study by Maurich *et al*. (2006) showed that two such compounds isolated from *Bituminaria bituminosa* (L.) C.H.Stirt. have anti colon-cancer properties. Furthermore, other secondary metabolites from this species are of commercial interest in cosmetics and photo-chemotherapy (Tava *et al*., 2007).

Another species that has received extensive phytochemical study is *Cullen corylifolia* Medik. This is a very famous plant in Chinese traditional medicine. It has been used for several centuries to treat impotence, menstruation disorder and uterine haemorrhage (Ruan *et al*., 2007). However, although 60 % of the Psoraleeae occur in southern Africa, the only phytochemical study done on the species endemic to this region is that of Boardley *et al*. (1986), which confirmed the presence of furocoumarins and several flavonoids in species of *Otholobium* and *Psoralea*.

Besides the secondary metabolites, some species in the tribe play vital ecological functions in coastal sandy areas as they prevent soil erosion. Examples include *Psoralea repens* P.J.Bergius, which occurs along the coastline of the Western Cape of South Africa and *Bituminaria*

bituminosa, which plays the same role in Mediterranean region of Europe (Walker *et al.* (2006). *Bituminaria bituminosa* is also used as a fodder crop despite its high quantity of secondary metabolites (Ventura *et al.* 2000; Pecetti *et al*., 2007).

1.2. Taxonomic history and generic boundaries within the tribe Psoraleeae

The genus *Psoralea* was first described by Linnaeus (1753) based on two species from South Africa, *P. pinnata* L. and *P. aculeata* L. Later on, more species were described under *Psoralea* from North America, South America and South Africa. When Vail (1894) did a revision of the American members of the genus, he recognised 21 species. The genus was later revised by Rydberg (1928), who restricted the name *Psoralea* to the South African species (40 at the time) and re-classified the American species into several genera. These were *Amorpha* L., *Apoplanesia* C. Presl., *Aspalthium* Medik., *Cullen* Medik., *Eysenhardtia* Kunth., *Hoita* Rydb., *Kuhnistera* Lam., *Orbexilum* Raf., *Parosela* Cav., *Parryella* Torr. & A. Gray, *Pediomelum* Rydb., *Petalostemon* Michx., *Psoralidium* Rydb., *Psorodendron* Rydb., *Psorothamnus* Rydb., *Rhytidomene* Rydb. and *Thornbera* Rydb. However, many preferred to retain the name *Psoralea* for all species and never accepted his classification. Among those who rejected Rydbergôs classification are Guthrie (1939), Isely (1958) and Ockendon (1965). The latter noted that it was difficult to divide *Psoralea* in a very natural way, and the features used by Rydberg, most of which were features of the pod and arrangements of leaves, did not correlate well. It was only until Stirton (1981) proposed a new classification of the genus *Psoralea* based on flowers, fruits, seeds, leaf arrangement and leaf morphology that the splitting of *Psoralea* into various segregates became an accepted classification.

In his revision, Stirton (1981) split *Psoralea sensu lato* into six genera namely: *Psoralea, Hallia* Thunb., *Cullen, Bituminaria* Heist ex. Fabr., *Orbexillum* and *Otholobium.* The first five of these names had been used by previous workers, (described in sections to follow) while *Otholobium* was a new name established by Stirton (1981). He restricted the name *Psoralea* to about twenty species endemic to the Cape Floristic Region (CFR) of South Africa and a few others that extend their range as far north as Swaziland. He also established the new genus *Otholobium* for some twenty South African species. A brief description of each of the genera, with highlights of the generic limits and current taxonomic issues associated with each genus is provided in the following sections.

1.2.1. *Psoralea* L.

Stirton (1981) restricted the name *Psoralea* only to those species that match the original description of the genus *Psoralea* by Linneaus (1753), which was based on *Psoralea pinnata* and *P. aculeata*. These species differ from the rest of the Psoraleeae in that they have a cupulum. The term, cupulum was coined by Tucker & Stirton (1991) and it refers to a cup-shaped structure at the base of each flower pedicel formed by the fusion and intercalary growth of three to four successive bracts. The cupulum is a unique structure found only in *Psoralea* (including *Hallia*) among legumes (Tucker & Stirton, 1991). Although its function is not clear, Tucker & Stirton (1991) postulated that it is an extra protective sheath that protects the young flower bud by encapsulating the true sepals. According to Stirton (1995 and 2005), the genus *Psoralea* has about 51 species. However, a majority of the species are not formally published and this causes difficulty in identification, as species boundaries are unclear. The current study forms part of an ongoing revision of the genus.

1.2.2. *Otholobium* C.H.Stirt.

Otholobium differs from *Psoralea* by: the absence of the cupulum; the possession of entire recurved mucronate-obovate to oblanceolate leaflets; inflorescences characterised by bracteate triplets of flowers, with each triplet subtended by a single variously shaped bract (Stirton, 1981). At its establishment, the genus *Otholobium* contained twenty species of *Psoralea sensu lato*, occurring mainly in southern Africa, with a few extending their range into eastern Africa (Stirton, 1981). The type species for the genus is *Otholobium caffrum* (Eckl. & Zeyh.) C.H.Stirt. Subsequent work (Stirton, 1982; Stirton, 1983) led to the discovery of more species, while some species were a result of new combinations from the genus *Psoralea*. Stirton (1985) made 31 new combinations from the genus *Psoralea* and in a revision (Stirton, 1989) 53 species were recognised. Stirton (1991) described four species from Namaqualand (*O. arborescens* C.H.Stirt., *O. flexuosum* C.H.Stirt., *O. incanum* C.H.Stirt. and *O. pustulatum* C.H.Stirt.).

1.2.3. *Hallia* Thunb.

The genus *Hallia* was applied to nine species also endemic to the CFR (Stirton, 1981). These species are low ascending or trailing suffrutices with unifoliolate leaves and flowers that are subtended by the cupulum as in *Psoralea*. *Hallia* was originally established by Thunberg (1799) as a distinct genus from *Psoralea*, but Salter (1939) subsumed it into *Psoralea*, noting that there was no character distinguishing it from the latter. Tucker & Stirton (1991) supported the subsuming of *Hallia* into *Psoralea* based on the presence of the cupulum on all *Hallia* species. In addition, results of a cluster analysis of morphological and leaf anatomical characters showed no

distinct clustering pattern between the two genera (Crow *et al*., 1997). This was interpreted as an indication that *Hallia* species exhibit neotony because there were no differences in the morphology and anatomy of seedlings of either *Hallia* or *Psoralea* species. The validity of this hypothesis has not been tested so far, and hence the present study attempted to tackle it by including species of both genera in the phylogenetic analyses.

1.2.4. *Cullen* Medik.

Cullen, on the other hand, was applied to about 35 species whose centre of diversity is in Australia, with only one species occurring in each of the following countries: Indonesia, Saudi Arabia, Somalia, Philippines and China. Three species occur in the Nama-karoo biome of South Africa (Stirton, 1981). In a revision of the genus by Grimes (1997), *Cullen* was found to be a monophyletic assemblage characterised by a discontinuous floral vasculature; the possession of glandular non-beaked fruits and the presence of a small invagination of the pericarp just above the stalk of the fruit. Grimes (1997) recognised 37 species in the genus, while Stirton (2005) recognised 34 species.

1.2.5. *Bituminaria* Heist ex. Fabr.

This genus consists of three species, *Bituminaria morisiana* (Pignatti & Metlesics) Greuter, *B. bituminosa* (L.f) C.H. Stirt. and *B. acaulis* (Stev.) C.H. Stirt. The latter two are endemic to the Mediterranean area of Europe and North Africa, while *B. morisiana* is endemic to the inner parts of Sardinia Island (Pistelli *et al*., 2003). A cladistic analysis of 27 morphological characters, including several species of the genus *Cullen*, *B. acaulis* and *B. bituminosa* showed that the genus *Bituminaria* is paraphyletic (Grimes, 1997). Grimes (1997) further noted that there are several characters that separate these two species. For example, in *B. acaulis* the seed is free from the pericarp; the vasculature is continuous; the leaflets are palmate and the flowers are borne in triplets while, *B. bituminosa* lacks all these features. Moreover, in *B. acaulis* each triplet of flowers is subtended by a bract, with each flower in that triplet also subtended by a single bract. This latter feature is characteristic of the southern African *Otholobium*, but despite pointing out this similarity, Grimes (1997) did not make any taxonomic changes until the phylogeny of *Otholobium* is known. Furthermore, several studies have shown that *B. bituminosa* is highly polymorphic in both morphology and molecular features (Muñoz *et al*., 2000; Pistelli *et al.*, 2003; Juan *et al.*, 2005; Walker *et al*., 2006). However, the latest molecular phylogeny of the Psoraleeae by Egan & Crandall (2008a) did not shed light on the monophyly of *Bituminaria* because it included only *B. bituminosa.* Therefore, *Bituminaria* as a genus requires revision to evaluate its taxonomic status.

1.2.6. *Orbexillum* Raf.

When Rydberg (1928) revised the genus *Psoralea*, he applied the name *Orbexillum* to eight species, one native to Mexico and the rest native to the USA. The species were diagnosed by the following characters: perennial herbs with rootstocks, pinnately trifoliolate leaves and coriaceous, reticulate tubercled fruits. In an earlier study, Rydberg (1919) recognised two groups within the genus: *Euorbexillum*- with pod obliquely ovate, leaflets ovate to lanceolate and rootstalks; and *Poikadenia*- with pod suborbicular, leaflets linear or linear lanceolate and fusiform roots. These groups were maintained in the revision of 1928. Stirton (1981) did not commit himself on the position of the American members of Psoraleeae and tentatively lumped all of them into *Orbexillum*. He indicated that lumping together all the American members of *Psoralea* into *Orbexillum* was unsatisfactory given that there could be several natural groupings representing various taxonomic ranks. In a revision of the new world genera of the Psoraleeae, Grimes (1990) retained the classification of *Orbexillum* according to Rydberg (1919) recognising the other American species under the genera, *Pediomelum, Hoita, Psoralidium and Rupertia* as discussed below.

1.2.7. *Pediomelum* Rydb.

At its establishment by Rydberg (1919), this genus had 22 species, all of which were endemic to North America and were diagnosed by having dehiscent pods. Rydberg (1928) divided it into two sections: *Eupediomelum* (sixteen species, with truly digitate leaves and sessile leaflets) and *Geomelum* (six species, with a petiolate median leaflet). When Ockendon (1965) revised the genus (at that time it was a subgenus of *Psoralea*), he recognised nineteen species, two subspecies and two varieties and recognised two sections. Unlike Rydberg (1928), his sections were defined by whether the species had a well-developed main stem or not (i.e. acaulescent or with lateral stems). The genus has its centre of distribution in the southwestern part of the USA. Nine species occur in Texas; six occur in Arizona, California and Utah, while the other three species are of widespread distribution within the region (Ockendon, 1965). At present, the genus has 21 species (Stirton, 2005). In the phylogenetic study by Egan & Crandall (2008a), it was shown that embedded within this genus is *Psoralidium tenuiflorum*, which has morphological features similar to the rest of *Pediomelum* thus making the genus paraphyletic.

1.2.8. *Hoita* Rydb.

At the inception of the genus *Hoita*, Rydberg (1928) recognised 11 species, but subsequent work on the taxonomy of the American Psoraleeae has resulted in only three species being recognised as true *Hoita* (Stirton, 2005). These species differ from the rest of Psoraleeae in that they have

pinnately trifoliolate leaves with entire short petioled, conspicuously glandular-punctate leaflets (Rydberg, 1928) and the beak of the pod is completely lacking (Ockendon, 1965). These are native to California and Mexico (Stirton, 2005). Egan & Crandall (2008a) established the monophyly of the genus.

1.2.9. *Psoralidium* Rydb.

This is a genus of three species, which are widespread in North America in the Great Plains and cordilleran regions of southern Canada to northern Mexico (Stirton, 2005). Their main characteristics include 3-5 digitately foliolate leaves; indehiscent fruits; a single seed that is short beaked, somewhat compressed, ovate or orbicular in outline and usually copiously gland-dotted; and the pericarp is coriaceous and free from the kidney-shaped seed (Rydberg, 1928). They resemble the Old World (southern African) *Psoralea* more than any of the other genera, and hence the selection of the name *Psoralidium*. Differences between the two genera lie in their leaves and inflorescences. *Psoralidium* has digitate and blunt tipped leaves, while *Psoralea* has pinnate leaves that have spinulose tips. The inflorescence of *Psoralidium* is always axillary, interruptedly spicate or racemose, with the flowers fascicled at the nodes and the corolla never strongly striately veined, as in the typical *Psoralea*. They also lack the cupulum, which is unique to *Psoralea sensu stricto*. The current circumscription of this genus renders it polyphyletic as *Psoralidium tenuiflorum* is embedded within *Pediomelum* (Egan & Crandall, 2008a).

1.2.10. *Rupertia* J.W. Grimes

The genus *Rupertia* consists of three species native to Western USA (Vancouver Island to California) and northwest Mexico. It was established by Grimes (1990) to accommodate some species that Rydberg (1928) had included in the genus *Hoita*. They are characterised by non-accrescent calyces and a unique secondary internal wall of the fruit, which are not observed in *Hoita* (Stirton, 2005). The genus is monophyletic as currently circumscribed (Egan & Crandall, 2008a).

1.3. Phylogenetic position of the Psoraleeae

The position of the tribe Psoraleeae has been a subject of scrutiny for a long time. It was originally treated as a subtribe (Psoraliinae) of the Galegeae (Taubert, 1894). Rydberg (1919) elevated it to tribal status (Psoraleae), but it was later split into two (Psoralieae and Daleae) by Hutchinson (1964) based on the location of petal insertion. Lestern & Wemple (1966) classified the Psoraleeae (*sensu* Hutchinson) as sister to the tribe Amorpheae based on four shared characters. These were gland-dotted leaves, one-seeded fruits, similar adnation of stipules and

the presence of a discontinuity plate. The discontinuity plate refers to a condition in which the xylem of the gynoecium and that of the stamens and petals is discontinuous with that of the pedicel. At the base of the gynoecium, where the gynoecial traces merge, xylem proliferates and forms a mass of tracheary elements flaring out horizontally from the lower end of the merged gynoecial bundles to form a plate like structure (Grimes, 1986; Turner, 1986). Lestern & Wemple (1966) believed that the discontinuity plate in particular, was a synapomorphy of the Psoraleeae and the Amorpheae. However, Barneby (1977) alluded that the two tribes were phylogenetically distinct based on differences in branching patterns, petal insertion, foliage and geographical distribution. Stirton (1981) provided more characters that separate the two tribes, (e.g. arrangement of the embryo and radicle in the seed, seed shape, fruit structure, and pollen) thus supporting the hypothesis that the two tribes are not closely related. In addition, further studies of other tribes showed that the discontinuity plate was also present in the tribes Desmodieae and Tephrosieae (Grimes, 1986).

Nodule morphology and anatomy are useful in clarifying tribal delimitation in the Leguminosae (Sprent, 2007). For example, Corby (1971) studied 400 species of wild legumes indigenous to Zimbabwe and found that root nodule shape was related to the tribal classification of the host plant. Sprent (1980) did a comparative study of root nodules for the tribes Phaseoleae, Vicieae and Trifolieae and reported similar findings. She found that nodules of these tribes differ in shape, growth form and anatomy. She also found that the principal export products of nitrogen fixing nodules of the Phaseoleae are the ureides, allantoin and allantoic acid, whilst the Vicieae and Trifolieae export the amides glutamine and asparagin. Another study by Kanu *et al.* (2008) investigating nodule morphology and anatomy of eight *Psoralea* species showed that all the species had spherical nodules (a characteristic of the tribe Phaseoleae), thereby suggesting that the Psoraleeae have affinities with the tribe Phaseoleae.

Several molecular phylogenetic studies indicate that the Psoraleeae are nested within the Phaseoleae and are not closely related to the Amorpheae. A study of the Leguminosae by Doyle *et al*. (1997) based on the *rbcL* gene placed the tribe Psoraleeae within the tribe Phaseoleae, as sister to the sub-tribe Glycininae. Similarly, a study by McMahon & Hufford (2004) based on the plastid *trnK* intron, including *matK,* and the nuclear ribosomal ITS1, 5.8S, and ITS2 showed that the Amorpheae form a well supported clade, which is sister to the Dalbergioids. Wojciechowski *et al*. (2004) corroborated these findings in a study based on the *matK* region, which showed the Amorpheae to be sister to the remainder of the Dalbergioids *s.l.*, whereas the Psoraleeae were

nested within the Phaseoleae *sensu lato*. Stefanovií *et al.* (2009) further confirmed that the tribe Psoraleeae is embedded within the tribe Phaseoleae as sister to the sub-tribe Glycininae.

Although there is such strong evidence for the phylogenetic position of the Psoraleeae, this position renders the Phaseoleae polyphyletic (Kajita *et al*., 2001) because two independent tribes, the Psoraleeae and the Desmodieae are embedded within it (Doyle & Doyle 1993; Doyle *et al*., 1997; Kajita *et al*., 2001; Stefanovií *et al*., 2009). This makes delimiting a recircumscribed Phaseoleae *sensu stricto* problematic. Schrire (2005) suggested that a solution would be to recognize a broad tribe Phaseoleae, comprising the sub-tribes Kennediinae, Cajaninae, Phaseolinae and Glycininae, assorted basally branching genera, and tribes Desmodieae and Psoraleeae, both treated as sub-tribes. However, it is difficult to make that change unless the monophyly of these tribes themselves is well established.

1.4. Sources of data for phylogenetic inference

Systematists use several sources of information about organisms to reconstruct their phylogeny and/or to derive classification systems. These include morphology, anatomy, embryology, chromosomes, palynology, secondary metabolites, proteins and nucleic acids (RNA and DNA). The use of DNA for phylogenetic inference has become popular in the last two to three decades with the development of several molecular techniques such as the polymerase chain reaction (PCR), the automation of DNA sequencing and the availability of efficient computer software with which to analyse the sequence data. DNA sequence data used for phylogenetic inference in plants are from plastids (mainly the chloroplast), the mitochondrion and the nuclear genomes. The mitochondrial genome is inherited through the maternal lineage (Ankel-Simons & Cummins, 1996; Mogensen, 1996), while the nuclear genome is inherited biparentally (Petit *et al*., 2005). However, for the plastid genome it has been shown that although it is maternally inherited for the majority of angiosperms, it is biparentally inherited in some angiosperm species (Tilney-Bassett, 1976; Hu *et al*., 2008). This diversity in the mode of inheritance of the different genomes implies that the kind of hypotheses that can be tested using data from one genome may differ from the other genome. A brief overview of these three sources of phylogenetic information follows.

1.4.1. The chloroplast genome

The chloroplast genome has received massive exploration for phylogenetic inference because it is the smallest of the three plant genomes: ranging from 135 to 160-kilo base pairs (kbp), it is quite stable both within cells and within species; and rearrangements of the genome are rare

enough in evolution that they are useful in dermacating major groups (Palmer, 1987). For example, the supra-generic relationships of angiosperms were inferred using the *rbcL* gene (Chase *et al*., 1993) which encodes the large subunit of the photosynthetic enzyme Rubisco, a major carbon acceptor in all photosynthetic eukaryotes and cyanobacteria (Nabors, 2004). However, the utility of coding gene regions such as *rbcL* is limited by their slow rate of change, which renders them less informative in the inference of phylogenetic relationships between closely related taxa such as genera and species. As a result, much attention has been paid towards non-coding regions of the chloroplast genome. For example, the *rps16* intron, *trnL* intron and the *trnL-F* intergenic spacer are some of the most widely used non-coding regions in studies of algae, bryophytes and vascular plants (e.g. Pennington *et al*., 2001; Klak *et al*., 2004; Koch *et al*., 2005). A review on the utility of various chloroplast markers in phylogenetics is provided by Shaw *et al*. (2005) and Shaw *et al*. (2007) indicating use at generic and specific levels for various plant groups.

1.4.2. The mitochondrion

The use of mitochondrial DNA (mtDNA) for phylogenetics is not as common in plants as it is in animal studies such as birds (Sturmbauer, 1998), fish (Bargelloni, 2000) and baboons (Newman, 2004). This is partly because mitochondrial genes evolve slowly (Crochet & Desmarais, 2000) and therefore may only be more useful for assessing ancient events, yet most studies on plants tend to focus on recent speciation events. Other reasons include frequent genomic rearrangements, the incorporation of foreign DNA from the nuclear and chloroplast genomes, and the disruption of gene continuity in introns or exons (Knoop, 2004). Nevertheless, the slow sequence evolution and the variable occurrence of introns in plant mtDNA provide an attractive reservoir of phylogenetic information to trace the phylogeny of older land plant lineages, which is not yet fully resolved. For example, Qiu *et al*. (1999) used the mitochondrial genes, *atp1* and *matR* in combination with the plastid markers, *atpB* and *rbcL* as well as the 18S nuclear rDNA to infer the origin of angiosperms. Other studies where mtDNA was used for reconstructing the phylogeny of seed plants include Gugerli *et al*. (2001), Soltis *et al*. (2002), Barkman *et al*. (2004) and Qui *et al*. (2005).

1.4.3. The nuclear genome

The nuclear genes with a copy number high enough for easy study are those encoding ribosomal RNA (Baldwin *et al*., 1995). These genes are arranged in tandem arrays of several hundred to several thousand copies. They encode the small subunit 18S and the large subunit 26S of the ribosome and are separated by the small 5.8S gene (Fig. 1.1). Between the three genes there are

short internal transcribed spacers (*ITS*) and separating each set of three genes from the next set is a large spacer, the intergenic spacer- IGS (Judd *et al*., 2008). These highly repetitive sequences undergo a homogenization process called concerted evolution, i.e. if a mutation occurs in one copy of the sequence, it is generally corrected to match the other copies and the non-mutated copies may be corrected to match the mutated one, causing nucleotide changes to propagate throughout the array (Elder *et al*., 1995).

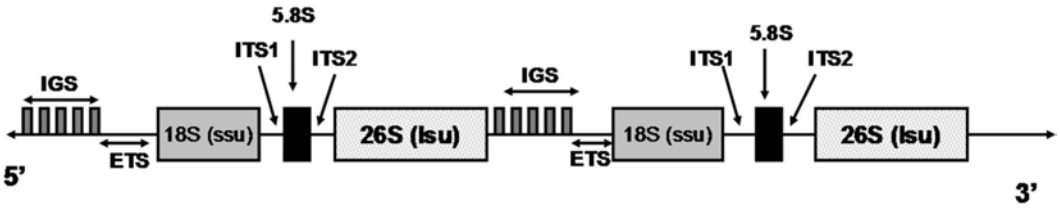

Figure 1.1 Structure of the ribosomal array. Coding regions of the 18S small subunit, 5.8S unit, and the 26S large subunit (LSU) are shown as rectangles marked 18S (ssu), 5.8S and 26S (lsu), respectively and the transcription unit is bracketed. Black lines represent spacers, and the small grey boxes represent short repeats in the intergenic spacer (IGS). ETS= External Transcribed Spacer and ITS= Internal Transcribed Spacer. Adapted from Judd *et al.* (2008).

The internal transcribed spacer has been used in phylogenetic studies of various plant groups including legumes (e.g. Wojciechowski *et al.*, 1999; Lavin *et al*., 2001; McMahon & Hufford, 2004; and Egan & Crandall, 2008a). It was recently used to reconstruct phylogenetic relationships within the genus *Indigofera*, which has about 750 species, most of which have diversified within the last 10 million years (Schrire *et al*., 2009). In combination with a morphological character data set, Schrire *et al*. (2009) were able to get well-supported trees, which reflect natural groupings within the genus. The *ITS* has the advantage that it evolves faster that the widely used chloroplast regions and therefore, it is useful in deducing relationships among closely related species (Álvarez & Wendel, 2003).

Although *ITS* has been and continues to be used extensively in phylogeny inference, there are some molecular genetic processes that affect *ITS* sequences in ways that may mislead phylogenetic inference (Álvarez & Wendel, 2003; Choi *et al*., 2006). For example, the high variability in the *ITS* [which may be due to the existence of multiple copies of varying size and location within the ribosomal DNA (Buckler *et al*., 1997)] may cause difficulty in amplification or sequence alignment (Choi *et al*., 2006). Another factor that reduces the utility of *ITS* is the

genomic harbouring of pseudo genes in various states of decay, and/or incomplete intra- or inter-array homogenization. These factors may separately or collectively create a network of paralogous sequence relationships, thus potentially confounding accurate phylogenetic reconstruction (Álvarez & Wendel, 2003).

Álvarez & Wendel (2003) suggested that single copy or low copy nuclear genes (LCNGs) may be better alternatives to *ITS*. However, even these regions are burdened with similar problems (Choi *et al*., 2006) and developing useful LCNGs requires time and effort (Feliner & Rosselló, 2007). For this reason, Feliner & Rosselló (2007) argue that *ITS* sequences, despite their drawbacks, can still produce insightful results in species-level phylogenetic studies or when non-anonymous nuclear markers are required, if a thoughtful use of them is made. They recommend that representative samplings following prospective pilot studies, careful lab protocols and mindful analysis can help minimize inaccurate phylogeny estimation with *ITS*. They further provide two guidelines in the form of flow charts, the first of which can help solve problems of amplification, detection of pseudo genes and paralogs, contamination and sequence artefacts. The second chart is to help one to find out causes for unresolved clades, to integrate gene phylogenies, to detect horizontal transfer and lineage sorting, and to reveal if *ITS* phylogeny is not a good estimate of organism phylogeny.

The external transcribed spacer (*ETS*) is another widely used marker in phylogenetic studies (e.g. Baldwin & Marcos, 1998; Jousselin *et al*., 2003; Sánchez-Baracaldo, 2004; Okuyama, 2005). It is relatively longer (Bena *et al*., 1998) and generally more variable (Kim & Mabry, 1991) than *ITS* and therefore may be suitable for use in interspecific and infraspecific phylogenetic studies. The *ETS* region has a rate of molecular evolution similar to that of *ITS*, and as part of the rDNA cistron, *ETS* is subject to the same molecular genetic processes as *ITS* (Soltis *et al*., 2008). For this reason, most of the advantages and disadvantages discussed for *ITS* also apply to *ETS*. Some studies where this marker has been used include Chandler *et al*. (2001), Chandler *et al*. (2003) and Choi *et al*. (2006).

1.4.4. Morphological data

Some authors have criticised the use of morphological data in phylogeny reconstruction and advocate for a purely molecular approach (e.g. Hebert *et al*., 2003; Scotland *et al*., 2003). The main criticisms are: most morphological characters are ambiguous, molecular and morphological phylogenies are sometimes incongruent, character coding and homology assessment is difficult and can be inaccurate. It is further argued that increased taxon sampling when there are fewer

characters does not strengthen the use of morphological data. However, ambiguities in morphological data can be resolved by treating morphological characters as continuous quantitative traits (Felsenstein, 1988; Wiens, 2001) and the problem of coding morphological data can be addressed by comparing how well different methods of analyzing morphological data recover clades that are strongly supported by independent, non morphological data sets (Wiens, 1998). Contrary to the argument that incresed taxon sampling does not strengthen the accuracy of morphological phylogenies, some simulation studies have shown that increased taxon sampling increases phylogenetic accuracy even when there are fewer characters (Huelsenbeck, 1991; Wiens, 1998).

It is also important to note that molecular phylogenies can be well resolved and strongly supported, and yet incorrect due to Long Branch attraction (Huelsenbeck, 1997), deviations between gene and species trees (Doyle, 1992; Maddison, 1997), sample contamination and specimen misidentification (Wiens, 2004a). In such cases, morphological phylogenies can act as a reality check for such phylogenies (Wiens, 2004a). Furthermore, macroevolutionary studies rely mainly on fossils as calibration points and since most fossils are not very similar to their extant relatives, their accurate placement on the phylogeny requires a morphology based phylogeny (Wiens, 2004a). Moreover, several studies have shown that combining morphological and molecular data improves phylogenetic resolution in several groups of organisms including plants, nematodes, insects, reptiles and mammals (e.g. Pennington, 1996; Renner, 1999; Giribet *et al*., 2001; Barker, *et al*., 2003; Hill, 2005; Wahlberg *et al*., 2005; Huys *et al*., 2007; Meldal *et al*., 2007; Schrire *et al*., 2009). Therefore, morphological data are useful in phylogenetics as long as they are properly coded and carefully integrated with the molecular data. Hence, the use of both molecular and morphological data in the present study considered the above-mentioned factors.

1.5. The Cape Floristic Region as a centre of diversity and endemism for the Psoraleeae

Although the Psoraleeae are of worldwide distribution, the greatest proportion of the species is in the genera *Otholobium* and *Psoralea*, both of which occur in southern Africa. Their centre of diversity and endemism is the Cape Floristic Region (CFR). The CFR is one of the richest floristic regions of the world. The next sections provide a review of the characteristics and evolutionary processes associated with this megadiverse region focusing on major drivers of diversification and speciation.

1.5.1. The physical environment of the Cape Floristic Region

The CFR is topographically complex, with ranges of mountains running parallel to the coast, occasionally interrupted by cross-valleys and separated from each other by deep and wide valleys (Linder, 1985). The landscapes can be divided into four broad categories, based on altitude and geographical position as follows: east montane, east lowland, west montane and west lowland (Cowling *et al*., 2009). The montane landscapes comprise a series of parallel ranges, which are sometimes massively folded quartzites and quartzitic sandstones of the Cape Supergroupôs Table Mountain and Witterberg Groups, with altitudes ranging from 1000-2000 m (Cowling *et al*., 2009). The western mountains are generally higher and steeper than those of the east. On the other hand, the lowlands vary from hilly to flat (Linder, 1985) and they include the coastal plains, which are less than 300 m high as well as the intermontane basins, which are 450-1000 m high (Cowling *et al*., 2009). These areas are underlain by softer sediments (mainly shales of the Cape Supergroupôs Bokkeveld Group and the Precambrian Malmesbury group), while the coastal margin is mantled almost everywhere by calcareous marine sediments, mainly aeolian sand and limestone (Cowling *et al*., 2009).

In terms of soils, the CFR consists of various edaphic types, which are sharply delimited, and thus forming into a mosaic of edaphic habitats (Linder, 1985). The montane landscapes are generally acidic, nutrient poor, coarse grained, rocky and shallow, and there is an increase in clay and silt content towards the east i.e. from sands and loamy sands in the west to sandy loams in the east (Campbell, 1986). The lowland soils are generally less rocky, deeper and fine textured. Clay-rich soils (e.g. sandy clay loams) are limited in extent, being mostly restricted to shales and granites, which are largely confined to the lower mountain slopes. Total exchangeable bases (S-value) and pH are best correlated with the fine soil fractions, the higher values being associated with fine textured soils (Campbell, 1986). Generally, there is a gradient from west to east, of increasing soil fertility across all sediment types in the Cape landscapes (Campbell, 1983).

1.5.2. Species richness and endemism

The Cape Floristic Region (CFR) is associated with a high level of endemism as well as high species richness, with about 9 000 species in an area of about 90 000 km^2 (Goldblatt & Manning, 2000; Cowling & Pressey, 2001). According to Linder (2003), such a high level of species richness is comparable to that of equatorial areas, while the endemism of almost 70 % (Goldblatt & Manning, 2002) is comparable to that found on islands. The level of endemism of the CFR is also comparable to that of the south-western region of Australia where out of the 18 000 vascular plant species of Australia, 5500 are endemic to that region. Such a phenomenon is not observed

in any other equivalent temperate region (Cowling *et al*., 1996; Linder *et al*., 2003). According to Linder (2003) the high level of endemism and high species richness in the CFR is partly a result of recent and massive diversification, associated with the onset of a seasonally arid climate during the late Miocene, about 10-14 million years ago (mya). For example, radiation in the genus *Ehrharta* (Poaceae) started around 9.8 mya (Verboom *et al*., 2003); *Moraea* (Iridaceae), 15 mya (Goldblatt *et al.,* 2002); the Ruschioideae between 3.8 and 8.7 mya (Klak *et al*., 2004); and *Phyllica* (Rhamnaceae), 7-8 mya (Richardson *et al*., 2001). Other indicators of recent diversification in Cape lineages include the restriction of localized endemics to very young sediments such as limestone areas, large clusters of closely related species resulting in poor phylogenetic resolution in clades and a very recent appearance (post Pleistocene) of species rich taxa (e.g. Mesembryanthemaceae) in the pollen record (Cowling & Pressey, 2001).

According to Linder (2003) radiation in a single clade may be explained by features unique to that clade, but where several lineages appear to have speciated extensively and possibly undergone a remarkable increase in the speciation rate, in the same geographical area and possibly at more or less the same time, then the explanation should be found in the environment. For a long time, climate change in the Miocene- Pliocene (10-14 mya) boundary was considered as the trigger of massive radiations in the CFR (Linder *et al*., 1992; Linder, 2003). However, as more molecular dating studies are published, it has become clear that not all lineages in the CFR started radiating after the late Miocene (Linder, 2005). For instance, the radiation of the Restionaceae is estimated to have started in the Oligocene, about 30.19 mya (Linder & Hardy, 2004; Linder *et al*., 2006), while the crown group of the Proteaceae date estimate was found to be 118.5 ± 8.2 million years old (Barker *et al*., 2007). Similarly, Edwards & Hawkins (2007) reported that the legume tribes Podalyrieae and Crotalarieae started diversifying between 44-46 mya, while the Cape Indigofereae crown node dates at 20.3 mya (Schrire *et al*., 2009).

A study by Verboom *et al.* (2009) showed that while most lineages identified as ancestrally endemic to the succulent karoo are consistent with the mid to late Miocene origin of this biome, the fynbos lineages show a different pattern. They reported that the fynbos biome has both lineages whose diversification is recent (less than 10 mya) as well as some which extend much further back in time, suggesting a much deeper history for the fynbos. They noted that the older fynbos elements are mostly endemic to the sandstones of the Cape Fold Mountains. This, coupled with the existence of a positive correlation between species richness (and rare species richness) and altitude in the western CFR, which could be a result of low extinction rates in montane habitats (Cowling & Lombard, 2002), led to the identification of the Cape Fold

Mountains as long term refugia on which the older fynbos elements have been able to persist. Therefore, Verboom *et al.* (2009) postulated that climate change in the Miocene to Pliocene could have affected the montane fynbos differently from lowland fynbos. They postulated that montane refugia could have suffered fragmentation, thus leading to allopatric speciation, while the lowland fynbos, which is associated with high substrate heterogeneity and landscape evolution (Verboom *et al*., 2004; Cowling *et al*., 2009) could have experienced massive extinction, which opened up new unoccupied niches for adaptive radiation.

1.5.3. Drivers of speciation in the CFR

Some of the main factors postulated to be playing a major role in driving speciation within lineages occurring in the CFR are pollinator specialization, fire, edaphic conditions (e.g. soil type and nutrient levels); phenology (e.g. flowering time); geographic isolation (e.g. habitat fragmentation); polyploidy or hybridization (Linder, 2003; Richardson *et al*., 2001). These factors have been explored by several researchers in the CFR as well as in many other floristic regions of the world. For example, studies focussing on the role of pollinators include Johnson (1996), Johnson *et al*. (1998), Vamosi *et al*. (2005) and van der Niet & Johnson (2009). There are also several papers discussing or providing evidence for the role of polyploidy and hybridization in driving diversification and speciation in several organisms. These include Ainouche *et al*. (2003), Barker & Bickham (1986), Buerkle *et al*. (2000), Bulini (1994), Burson & Voigt (1996), Chenuil *et al*. (1999), Goldblatt (1979), Grant (1981), Grant *et al*. (2006) Gross & Rieseberg (2005), Harrison *et al*. (2005), Lewis (1966), Lu *et al*. (2001), Mallet (2007), Rieseberg (1997), Rieseberg (2001), Rieseberg *et al*. (2003), Salzburger *et al*. (2002), Seehausen (2004), Soltis *et al*. (2009), Spies & Stirton (1982), Steiner & Cruz (2009), Templeton (1981) as well as White (1978).

On the other hand, edaphic factors have not received as much attention as some of the other drivers of diversification and speciation. This study focuses on edaphic heterogeneity in terms of soil types and nutrient concentrations as well as the evolution of post-fire regenerative strategy (i.e. reseeding and resprouting) to explore their role in diversification of the southern African Psoraleeae. Since the latter (persistence strategy) is also influenced by fire, a brief discussion on fire follows. A brief introduction of the discussion on soils was given in section 1.5.1, and it is continued further in Chapter 3 of this dissertation.

Fire

The fynbos biome (where a majority of the Psoraleeae occur) is known to burn regularly, with a fire cycle of between 5 and 50 years (Cowling, 1987). However, the fire history of the CFR is not well known, but palaeopalynological work in Australia suggests that fire was less frequent prior to the Miocene (Linder, 2003). Based on this, Linder (2003) postulated that the same might apply to the CFR. If this is the case, then those lineages whose onsets of radiation are recent may have been influenced by fire in some way. However, the lack of a comprehensive record of the fire history of the region compromises efforts to test this. Many members of *Psoralea* and *Otholobium* which occur in the fynbos, are predominantly resprouters (from field observations), a feature which is associated with adaptation for fire (Higgins *et al*., 2007; Barraclough, 2006). However, reseeders also occur in both genera, with some species forming dominant stands locally. This raises the question as to whether the evolution of the different survival strategies was influenced by fire alone or a combination of several forces such as edaphic heterogeneity as discussed later in Chapter 3.

The model explaining how fire would promote speciation is complex and the role of fire seems to be indirect, with geographical isolation ultimately driving speciation (Cowling *et al*., 1992; van der Niet & Johnson, 2009). Linder (2003) suggested that shifts in fire survival strategy could drive speciation associated with differences in growth form and phenological differences that could isolate populations from each other. Another model is based on the premise that fire-induced plant mortality increases generation turnover (i.e. for reseeders), thereby providing potential for more rapid evolution than resprouters (Schutte *et al*., 1995; Marzluff & Dial, 1991; Cowling & Pressey, 2001). Moreover, small and weakly persistent seed banks, in combination with fire sensitivity may result in non-overlapping generations, thereby increasing the probability of the manifestation of genetic novelties associated with each generation as well as increasing the probability of population fragmentation via fire-induced local extinction (Cowling, 1987). Finally, restricted gene flow, a consequence of short distance seed dispersal and insect pollination may promote isolation and hence diversification of populations in different habitats (Linder, 1985; Slingsby & Bond, 1985).

1.6. Testing speciation processes

Studies on speciation processes seek to answer the question, ñwhat causes a single ancestral species to split into two (or more) daughter species?ò The common approach to answering this question has been to study sister species to infer what processes might have led to the split of

their ancestor (Barraclough and Nee 2001). With the advances in molecular phylogenetics and the development of lineage dating methods that allow one to estimate the relative ages of nodes with confidence intervals from sequence data even in the absence of a molecular clock, species relationships can be accurately established and the timing of diversification can be estimated. This allows for accurate inference of speciation processes. However, this approach requires that preferably all species from the group in question should be sampled, otherwise there is the risk of falsely assigning sister species status to taxa included in the analysis (van der Niet & Johnson, 2009). In addition, the taxonomic status of those species must be well resolved (Barraclough & Nee, 2001). However, even with these conditions met, there may still be limitations to the inference of speciation processes. For example, changes can occur since speciation, and so patterns observed for even closely related species could be the incidental outcome of the independent evolutionary histories of those species, rather than indicative of the forces under which speciation occurred (Barraclough & Nee, 2001). Therefore, the interpretations of such results need to consider this factor.

1.7. Species concepts and species delimitation within the southern African Psoraleeae

Species are one of the fundamental units of comparison in virtually all subfields of biology such as anatomy, morphology, behaviour, development, ecology, evolution, genetics, molecular biology, paleontology, physiology, and systematics (de Queiroz, 2005a). However, biologists often have to deal with two fundamental questions: (i) what is a species? and (ii) how can one determine whether two or more individuals are members of the same species or not? Unfortunately, there are no simple answers to these questions because there are several definitions of species, otherwise called óspecies conceptsô. For example, Mayden (1997, 1999, and 2002) recognizes 24 species concepts, while de Queiroz (2007) recognizes 13 species concepts. The large number of species concepts is due to several reasons. Firstly, patterns of variation are so diverse and complex that no one concept can suffice (Sokal, 1973). Secondly, in terms of evolution, if descent with modification occurs, i.e. if new forms arise from older ones more or less gradually, then difficult cases should be expected (Donoghue, 1985). Another reason is that there are disagreements over basic philosophical issues, such as the argument that organisms are real but species are not (Donoghue, 1985), while others contend that species really exist and are not arbitrary (Mayr, 1963). Detailed descriptions of each of the various species concepts are provided in various literature sources including Wiley (1978); Templeton (1981); Mayden (1997); Wiens (2004b); De Queiroz (2005a, 2005b and 2007) and many others. In the next few paragraphs I briefly discuss some of the most commonly used concepts as well as a relatively new concept (the unified species concept) and then indicate which concept I adopt for

this thesis especially for Chapter 4. These include the biological species concept (BSC), evolutionary species concept (ESC), phylogenetic species concept (PSC), phenetic species concept (PhSC) and unified species concept (USC).

The biological species concept (BSC) defines species as populations that are distributed through time and space, interrelated through mutual interbreeding, and distinguished from others by reproductive barriers (Mayr, 1963; Sokal & Cravello, 1970; Mishler & Donoghue, 1982). This concept emphasizes reproductive isolation as a major determinant of species. The obvious weakness to such a definition is that it does not account for asexually reproducing organisms and perhaps hybridization. For example, in most plant groups the occurrence of gene flow may not mean that they are not distinct unless the hybridization is so pervasive that the species merge (Judd *et al*., 2008). In the genus *Psoralea* for instance, there are cases in which where two different species co-occur, some individuals exhibiting intermediate morphology are found, yet parent species remain distinct. The fertility of such putative hybrids has not been tested, but the occurrence of hybridization between two seemingly distinct lineages makes the BSC less reliable in delimiting species in this genus.

The evolutionary species concept defines a species as a lineage of ancestral descendant populations, which maintains its identity from other such lineages and has its own evolutionary tendencies and historical fate (Wiley, 1978). This is related to the phylogenetic species concept as described by Nixon & Wheeler (1990), who define a species as the smallest aggregation of populations or lineages diagnosable by a unique combination of character states in comparable individuals (which they term semaphoronts). They further define a character state as an inherited attribute distributed among all comparable individuals of the same historical population, clade, or terminal lineage. The difference between the two concepts is that the ESC emphasizes on unique evolutionary role, historical tendencies and fate, while the PSC emphasizes diagnosability and monophyly. These definitions sound simple and comprehensible, but there is no consensus among systematists as to what constitutes diagnosability, therefore making the application of the concepts subjective.

On the other hand, the phenetic species concept defines species as clusters of individuals in a multidimensional space, where each dimension marks a character axis and each cluster is separated from others by empty interspaces (Andersson, 1990). The PhSC depends on morphological differences considered by the taxonomist describing the new species to be of sufficient magnitude to warrant specific status i.e. gaps in the phenomes of different species

determine the species boundary (Sokal, 1973). The PhSC is widely used as a basis for identifying gaps in variation and hence identifying species boundaries. In many cases, the approach is easy to test, objective and applicable to many plant groups.

Finally, the unified species concept as proposed by De Queiroz (2007) recognizes that the common feature among all existing species concepts is that a species is a separately evolving metapopulation lineage. The USC treats this feature as the primary and sole defining property of a species. De Queiroz (2007) notes that existing species concepts disagree in adopting different properties acquired by lineages during the course of divergence (e.g. intrinsic reproductive isolation, diagnosability, monophyly) and he treats these as secondary properties (secondary species criteria). This is because they are properties that species may or may not acquire during the course of their existence (De Queiroz, 2007). Therefore, according to the USC, the presence of any such properties is evidence of lineage separation, but not the defining feature of a species. However, although the presence of a single secondary property provides evidence of lineage separation, a highly corroborated hypothesis of lineage separation (i.e. existence of separate species) requires multiple lines of evidence. De Queiroz (2007) further notes that the further along lineages are in the process of divergence, the larger the number of differences, while earlier lineages in the process of divergence may not show such differences. For the latter case, he suggests that new methods for testing hypotheses of lineage separation such as coalescent-based methods, as described by Knowles & Carstens (2007) can be used. Carstens & Dewey (2010) applied this approach on some North American bats (*Myotis*) and found that most of the currently described species within the *M. lucifugus*/western long-eared *Myotis* clades contain more than one evolutionarily independent lineage. According to the USC, these independently evolving lineages constitute distinct species. These findings indicate the potential utility of these methods in species delimitation for lineages that are in the early stages of divergence. However, in practice, it may be difficult to accept splitting a species based on DNA data alone. Therefore, such cases may limit the application and utility of the USC.

In this study, I adopt the phenetic species concept. The concept was favoured because it uses morphometrics and multivariate statistical analyses, which can help one to achieve repeatability and objectivity in classifications. Moreover, since the methods use quantitative data, they provide greater discrimination along the spectrum of taxonomic differences and are more sensitive in delimiting taxa (Sneath & Sokal, 1973). Furthermore, a study by Rieseberg *et al*. (2006) showed that 75 % of phenotypic clusters in plants correspond to reproductively independent lineages (as measured by post mating isolation), and thus represent biologically real

entities. This concept has been used to resolve species complexes in several plant groups such as the *Cullen patens* (Fabaceae, tribe Psoraleeae) complex (Grimes, 1997); the *Cimicifuga foetida* (Ranunculaceae) complex (Compton & Hedderson, 1997); the genus *Merciera* (Campanulaceae) by Cupido (2003); the *Amphilophus citrinellus* (Cichlidae) complex (Klingenberg *et al*., 2003); and the *Olinia rochetiana* (Olinaceae) complex (Sebola & Balkwill, 2009).

Species boundaries within the southern African Psoraleeae are still a major challenge especially within the genus *Psoralea*. There has not been any taxonomic revision of this genus after Stirton (1981) redefined it. As a result, several species are presently known by informal names in manuscripts that describe the flora of the region such as Goldblatt & Manning (2000). Since such species have no formal descriptions, the application of these names is always ambiguous and subjective due to lack of clarity on species boundaries.

1.8. Aim and objectives of the study

The aim of this study was to infer phylogenetic relationships and macroevolutionary patterns among the southern African Psoraleeae and to revise the taxonomy of selected species complexes in the genus *Psoralea*. The main objectives were:

1. to reconstruct phylogenetic relationships in the Psoraleeae and investigate the monophyly of *Psoralea* and *Otholobium*,

2. to carry out macro-evolutionary reconstruction of the key diagnostic characters for the genera *Otholobium* and *Psoralea*,

3. to estimate dates of lineage divergence in the Psoraleeae

4. to investigate the role of edaphic heterogeneity in the distribution of the southern African Psoraleeae, and

5. to revise species boundaries and nomenclature in the genus *Psoralea*.

The first three objectives are treated in Chapter 2, while the fourth and fifth objectives are treated in chapters three and four, respectively. While each chapter is complete with discussions and conclusions, a synoptic summary of the entire thesis is provided in Chapter 5.

CHAPTER 2

PHYLOGENETIC RELATIONSHIPS, MACROEVOLUTION AND ESTIMATION OF LINEAGE DIVERGENCE DATES IN THE PSORALEEAE

2.0 Introduction

2.0.1. Phylogenetic position and taxonomic status of the tribe Psoraleeae

The tribe Psoraleeae was first classified as a subtribe (Psoraliinae) of the tribe Galegeae by Taubert (1894) and later elevated to tribal status by Rydberg (1919). However, its phylogenetic position was controversial for a long time (as discussed in Chapter 1) until recently, when several molecular based phylogenies placed it within the Phaseoleae as sister to the subtribe Glycininae (Doyle *et al*., 1997; McMahon & Hufford, 2004; Wojciechowski *et al*., 2004; Stefanovíć *et al*., 2009). However, this position leaves the Phaseoleae as a polyphyletic tribe if the Psoraleeae and the Desmodieae, (both currently recognised as independent tribes) retain their tribal status. Schrire (2005) suggested that these two should be recognised as subtribes, but unless the monophyly of these is established, such a move could not be made. In this study, the monophyly of the tribe Psoraleeae is investigated, with the view to assessing whether Schrireôs proposal regarding its taxonomic status can be implemented.

2.0.2 Phylogenetic relationships within the tribe Psoraleeae

In Stirtonôs (1989) revision of the genus *Otholobium*, fifty-three species were recognized, of which sixteen were new. The revision was based on data from cytology, phytogeography, palynology, morphology, anatomy and phytochemistry. So far, this is the latest revision of the genus. However, phylogenetic relationships within *Otholobium* itself as well as between *Otholobium* and the other genera are not known. For example, *Otholobium* as currently circumscribed is polyphyletic because there are some eight South American species, which Grimes (1990) placed into *Otholobium* despite that they do not fit the description of *Otholobium* as provided by Stirton (1981).

The polyphyly of *Otholobium* was confirmed by a phylogenetic study of the American Psoraleeae by Egan & Crandall (2008a). They found that the South African *Otholobium* species formed a clade sister to the rest of the American Psoraleeae, while the South American *Otholobium* species were embedded within the American clade as sister to the genus *Orbexillum* (Fig. 2.1). Therefore, they suggested that *Otholobium* should be broken into two groups by geography, South America and South Africa.

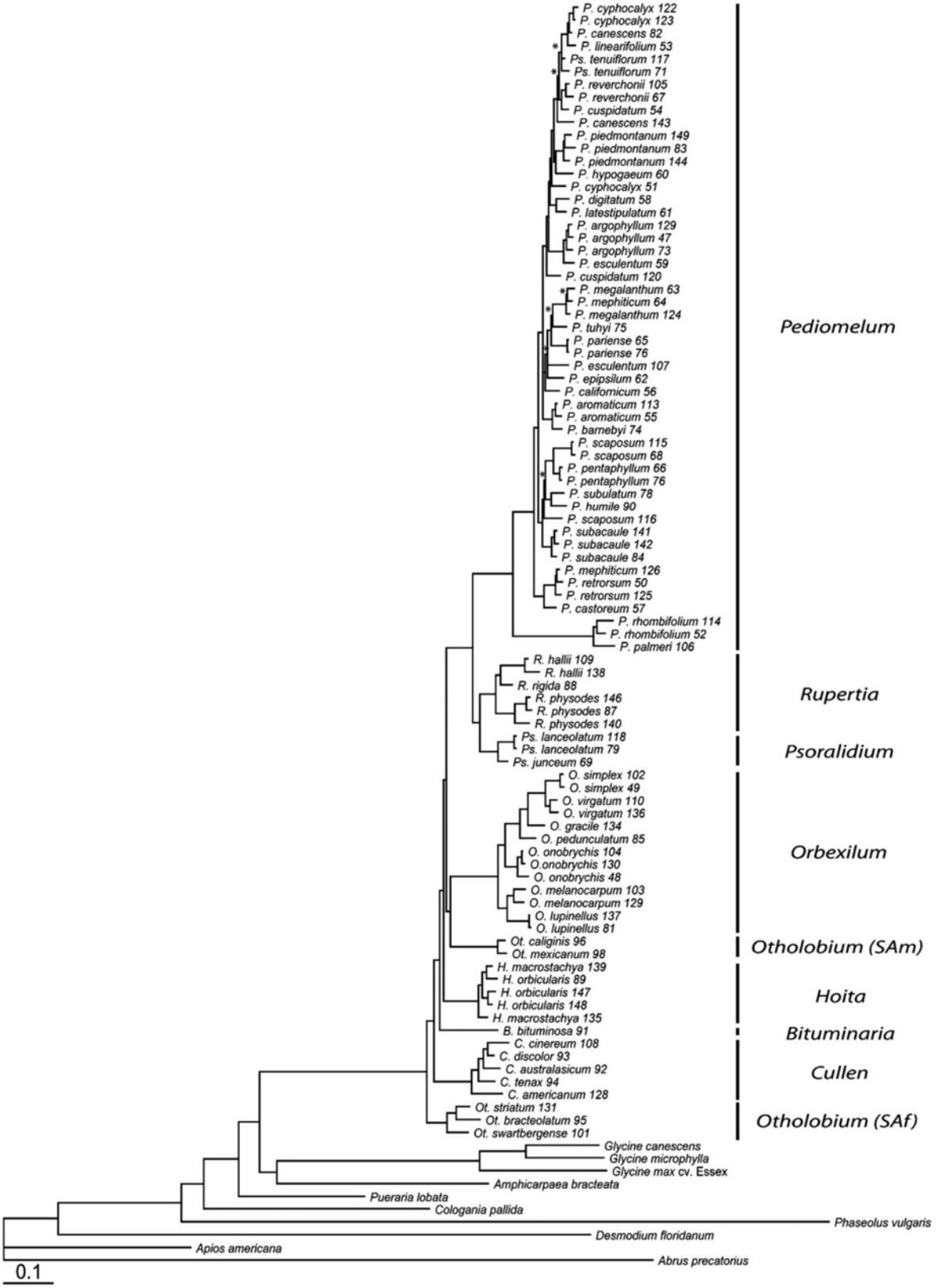

Figure 2.1 Bayesian Inference tree from Egan & Crandall (2008a). All nodes have posterior probability greater than 0.90 except those marked with * which have posterior probabilities between 0.50 and 0.90.

However, Egan & Crandallôs (2008a) analyses were not completely congruent with respect to the South American *Otholobium* species relationships relative to other Psoraleeae genera. For example, mixed models Bayesian inference with gaps as sic (simple indel coding) resolved South American species as sister to *Orbexilum* with good support (Fig. 2.1 pp=0.99). However, maximum parsimony analyses and Bayesian analyses without gaps placed the South American *Otholobium* species in a clade apart from, but in a basal polytomy with African *Otholobium* species or as sister to *Bituminaria*, but without support. Therefore, it was not clear whether these eight species should be included within *Orbexillum sensu Grimes* [*non Stirton*] or to recognize them as a new genus. As a result, Egan & Crandall (2008a) noted that greater sampling within both geographic areas might enable such a decision.

Besides the relationship between the southern African *Otholobium* and the American species, the phylogenetic position of *Psoralea sensu stricto* (African) is still unknown. A morphology-based cladogram by Grimes (1990) indicates that *Psoralea* and *Otholobium* together form a clade, sister to rest of the Psoraleeae (Fig. 2.2). However, the molecular phylogenetic study by Egan and Crandall (2008a) as shown in Fig. 2.1 does not support Grimesô (1990) hypothesis of relationships between the genera in Psoraleeae. For instance, the genus *Rupertia* is resolved as sister to *Pediomelum*, while *Cullen* and the South African *Otholobium* are basally branching as sister to the rest of the American Psoraleeae (Fig. 2.1). Therefore, there is a need to test the hypothesis of sister relationship between *Otholobium* and *Psoralea* as proposed by Grimes (1990) and to determine their phylogenetic position relative to the other genera in the tribe.

In addition, the taxonomic status of the genus *Hallia* is not yet clear. It was first subsumed into *Psoralea* by Salter (1939), was later recognised as a separate genus by Stirton (1981), but it was again subsumed into *Psoralea* by Crow *et al*. (1997). Besides the possession of the cupulum, which Crow *et al.* (1997) treated as a synapomorphy of *Psoralea*, *Hallia* species have some unique characters that distinguish them from *Psoralea* species. First, they have simple, broad leaves instead of the pinnate and filiform leaves found in *Psoralea*. Secondly, all *Hallia* species are creeping, multi-stemmed suffrutices, while *Psoralea* species are either shrubs or small trees. Furthermore, unlike in *Psoralea*, where the standard petal has a different colour from the wing petals, in flowers of *Hallia* species all the petals are uniformly coloured and have distinctive single coloured contrasting nectar guides. Crow *et al.* (1997) postulated that *Hallia* species might have arisen through neoteny from species of *Psoralea* that are shrubs or trees. This was because they found no differences in the morphology and anatomy of seedlings of both *Psoralea* and *Hallia* species. A molecular phylogeny may shed some light on the validity of this hypothesis.

Therefore, there is a need to reconstruct a phylogeny including both genera and to perform ancestral state reconstruction to determine whether the presence of the cupulum is a single evolutionary event or not and thus evaluate its taxonomic utility in defining *Psoralea*.

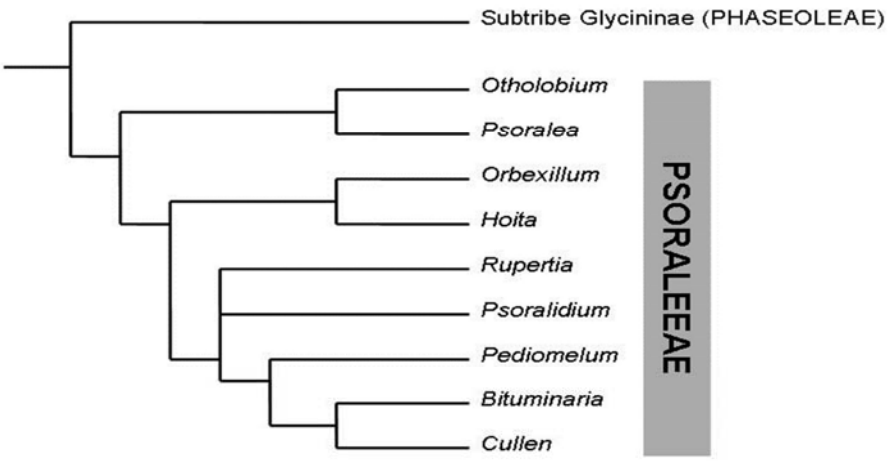

Figure 2.2 Phylogenetic relationships between the Psoraleeae genera as proposed by (Grimes, 1990). Adapted from Lewis *et al.* (2005).

2.0.3. Estimation of divergence dates

2.0.3. (a) Sources of error in date estimations

Molecular dating has become an important tool in sytematics and biogeographical research. However, the accuracy of molecular dates depends on a number of factors such as the dating method used, the molecule examined, the codon position examined, the rate smoothing method used, the calibration point used, the accuracy of the phylogeny, and the number of taxa sampled within a clade (Milne, 2009). Detailed discussions of each of these factors are found in several papers including Sanderson *et al*. (2004), Magallon & Sanderson (2005), Sanderson & Doyle (2001), Linder *et al*. (2005), Renner (2005) and many others. In this section, I briefly discuss only three of these factors i.e. sampling, calibration point and rate smoothing because they are interlinked in the way that they affect the accuracy of dates. However, this is not to say that the others are less important.

According to Linder *et al*. (2005), all date estimation methods are sensitive to under-sampling and this is more severe in methods that use extreme rate smoothing. They further indicate that error from under-sampling increases with distance from the calibration point, and this creates a problem when dating nodes that are not phylogenetically close to any fossil calibration points. On the contrary, a study by Hug & Roger (2009) showed that under-sampling might not affect the accuracy of the date estimate if as many fossil calibrations as possible are used and the correct methods for applying constraints to these nodes are used. They suggested that sampling should be adjusted to optimize the chances of obtaining the correct tree that will contain the appropriate nodes corresponding to the fossil calibration dates. However, most plant groups are not well represented in the fossil record and therefore it may not always be possible to find as many fossils as required. Therefore, in such cases under-sampling may still be a problem.

On another note, approaches that do not use fossil calibrations are problematic because a universal molecular clock cannot be assumed (Gaut, 1998; Kay *et al*., 2006), and a secondary calibration point introduces large errors into the results (Shaul & Graur, 2002). Using one or more phylogenetically distant fossils for calibration could be an effective approach, but only if the potential effects of taxon sampling are controlled for (Milne, 2009). Therefore, penalized likelihood and Bayesian methods are the most suitable when the molecular clock hypothesis has been rejected because they are more successful in finding optimal levels of smoothing to correct for rate heterogeneity and are less sensitive to undersampling (Linder *et al*., 2005).

2.0.3. (b) Age estimates for the Southern African Psoraleeae

The southern African Psoraleeae are a species rich lineage, (about 112 species, Stirton, 2005) and are one of 33 plant lineages known as Cape floral clades (Linder, 2003). Some of the species rich Cape lineages are products of recent rapid radiation, possibly triggered by climate change in the late Miocene. However, as indicated in section 1.5.2, the radiations of some Cape clades started much earlier than the late Miocene, suggesting that they have a deeper evolutionary history and there could be other triggers of radiation besides climate change in the late Miocene. For example, this could be due to high levels of species persistence (i.e. low extinction rates) and sustained diversification rates associated with climatic stability before the late Miocene (Valente *et al*., 2009). Therefore, this study sought to test whether the radiation of the southern African Psoraleeae occurred before or after the late Miocene and hence to determine whether the high species richness in the lineage is due to recent rapid radiation or not.

2.0.4. Objectives

The specific objectives of this chapter were:

- to test the monophyly of the tribe Psoraleeae and hence revise its tribal classification;
- to test Grimesô (1990) hypothesis on generic relationships within the tribe;
- to determine the phylogenetic position of the southern African genera: *Psoralea* and *Otholobium* with respect to the rest of the tribe and test their monophyly;
- to reconstruct the evolution of key diagnostic characters with emphasis on *Psoralea* and *Otholobium*; and
- to estimate the ages and patterns of diversification within the tribe.

2.1. Materials and methods

2.1.1. Taxon sampling

For the southern African genera, 40 species of *Otholobium* (representing 75% of the total number of species) and 36 species of *Psoralea* (representing 71 % of the total number of species in the genus) were sampled for the phylogenetic analysis. All the species were collected as part of this study. For each collection, a voucher specimen and tissue from young fresh leaves (or shoots in the case of leafless species) for DNA extraction were collected. The DNA tissue was dried in silica gel. For the other Psoraleeae genera outside southern Africa (i.e. *Pediomelum, Cullen, Hoita, Rupertia, Orbexillum* and *Bituminaria*) sequences were downloaded from GenBank, most of which were from the study by Egan & Crandall (2008a) and these are indicated in the Table 2.1, which summarises the information about the taxa used in the study. Out-group taxa were chosen based on the phylogenetic analysis of North American Psoraleeae by Egan & Crandall (2008a). These were *Abrus precatorius* (tribe Abreae), *Apios americana* (tribe Phaseoleae), *Amphicarpea bracteata*, *Cologania pallida*, *Glycine canascens, Glycine microphylla* (all from Phaseoleae, sub-tribe Glycininae) and *Desmodium floridanum* from the tribe Desmodieae.

2.1.2. Morphological, anatomical and phytochemical data scoring

A total of 40 characters, which include vegetative, inflorescence, floral, anatomical, and phytochemical attributes were scored for *Psoralea* and *Otholobium* species. The character set consisted of both binary and multi-state characters. Character states were not ordered. The primary sources of morphological data included: specimens collected as part of this study, herbarium specimens (BOL) and Stirtonôs (1989) revision of the genus *Otholobium*.

Table 2.1 Taxa studied. Areas marked with an asterisk indicate sequences not available in Genbank. For taxa sequenced in this study Y indicates that locus was sequenced, N indicates locus not sequenced.

					Genebank Accession Numbers		
Taxon name	**Collector**	**Number**	**Herbarium**	**Distribution**	**trnL-F**	**ITS**	**rpoB-trnC**
Abrus precatorius L.	Thorne et al	6971	BRY		EF543423	AF467015	*
Amphicarpaea bracteata (L.)Rickett &Stafleu	L.C. Anderson	20434	BRY	USA	EF543424	DQ006008	EF549828
Apios americana Medik.	R.D. Thomas	130655	BRY		EF543425	AF467019	EF549829
Bituminaria bituminosa (L.) C.H.Stirt.	Hobbs	1	*TEX*	Mediterranian	EF543418	EF517908	EF549820
Cologania pallida Rose	L.C. Higgins	17919	*BRY*	New Mexico	EF543427	EF517916	EF549831
Cullen americanum Rydb.		62476	Macb	Africa, Europe	EF543360	EF517848	EF549762
Cullen australasicum (Schltdl.) J.W.Grimes	Grimes	3188	*TEX*	Australia	EF543419	EF517909	EF549821
Cullen cinereum (Lindl.) J.W.Grimes	Henry	264	*TEX*	Western Australia	*	EF517832	EF549748
Cullen discolor (Domin) J.W.Grimes	Grimes	3213	*TEX*	Australia	EF543420	EF517910	EF549822
Cullen tenax (Lindl.) J.W.Grimes	Grimes	3159	*TEX*	Australia	EF543421	EF517911	EF549823
Desmodium floridanum Chapm.	no voucher			Florida	EF543408	EF517898	EF549811
Glycine canescens F.J. Herm.	Doyle	1075	*BH*	Australia	EF543426	EF517915	EF549830
Glycine microphylla(Benth.) Tindale	Doyle	1169	*BH*	Australia	EF543429	EF517918	EF549833
Hoita macrostachya Rydb.	Egan & Egan	276	*BRY*	California	EF543367	EF517853	EF549771
Hoita orbicularis Rydb.	Egan & Egan	269	*BRY*		EF543416	EF517906	EF549778
Orbexilum lupinellum (Michx.) Isely	Egan & Egan	257	*BRY*	SE USA	*	EF517899	EF549812
Orbexilum melanocarpum(Benth.) Rydb.	Grimes	2287	*TEX*	Mexico	EF543361	EF517849	EF549763
Orbexilum onobrychis (Nutt.) Rydb.	Raven & Raven	27603	*TEX*	N Midwest USA	EF543378	EF517850	EF549744
Orbexilum simplex (Nutt. ex Torr. & A.Gray) Rydb.	Thomas	65475	*TEX*	S Central USA	EF543379	EF517869	EF549782
Orbexilum virgatum (Nutt.) Rydb.	Egan & Egan	251	*BRY*	NE Florida	EF543349	EF517834	EF549768
Pediomelum argophyllum(Pursh) J.W.Grimes	Mcneilus	972	*TEX*	Midwest USA	EF543402	EF517847	EF549805
*Pediomelum aromaticum*var. *aromatucum* (Payson) S.L.Welsh	Egan & Egan	151	*BRY*	E Central Utah	*	EF517835	EF549788
Pediomelum aromaticum(Payson) W.A.Weber var. barnebyi S.L.Welsh	Egan & Egan	143	*BRY*	Kane co. Utah	EF543403	EF517893	EF549806
Pediomelum aromaticum var. *tuhyi* S.L.Welsh	Egan & Egan	157	*BRY*	Moab co. Utah	EF543404	EF517894	EF549807
Pediomelum canescens (Michx.) Rydb.	Egan & Egan	265	*BRY*	SE USA	EF543410	EF517900	EF549775
Pediomelum californicum(S.Watson) Rydb.	Egan & Egan	119	*BRY*	California	EF543386	EF517876	EF549789
Pediomelum castoreum(S.Watson) Rydb.	Egan & Egan	125	*BRY*	Virgin river, AZ, NV, CA	EF543387	EF517877	EF549790
Pediomelum cuspidatum(Pursh) Rydb.	Egan & Egan	193	*BRY*	Texas into Smidwest USA	EF543384	EF517841	EF549756
Pediomelum cyphocalyx(A.Gray) Rydb.	Egan & Egan	201	*BRY*	Central Texas	EF543381	EF517842	EF549784
Pediomelum digitatum(Nutt. Ex Torr & A.Gray) J.W. Grimes	Egan & Egan	190	*BRY*	Texas into Smidwest USA	EF543388	EF517878	EF549791
Pediomelum esculentum(Pursh) Rydb.	Egan & Egan	216	*BRY*	Midwest USA	EF543347	EF517831	EF549747
Pediomelum humile Rydb.	no voucher			Val Verde Co., Texas	EF543417	EF517907	EF549819
Pediomelum hypogaeum(Nutt.) Rydb.	Egan & Egan	209	*BRY*	Texas into Smidwest USA	EF543390	EF517880	EF549793
Pediomelum hypogaeum(Nutt.) Rydb. var. *scaposum* (A.Gray) Mahler	Egan & Egan	185	*BRY*	Central Texas	EF543398	EF517838	EF549801
Pediomelum hypogaeum(Nutt.) Rydb. var. *subulatum* (Bush) J.W.Grimes	Egan & Egan	190a	*BRY*	E Texas	EF543407	EF517897	EF549810
Pediomelum latestipulatum(Shinners) Mahler var.*appressum* (Ockendon) Gandhi & L	Egan & Egan	186	*BRY*	Edwards plateau, Texas	EF543391	EF517881	EF549794
Pediomelum linearifolium(Torr. & A. Gray) J.W. Grimes	Egan & Egan	206	*BRY*	Texas into Smidwest USA	EF543383	EF517873	EF549786
*Pediomelum megalanthum*var. *megalanthum* (Wooton & Standl.) Rydb.	Egan & Egan	158	*BRY*	Eastern Utah	EF543393	EF517883	EF549796
Pediomelum megalanthum var. *retrorsum* (Rydb.) J.W.Grimes	Egan & Egan	144	*BRY*	NW Arizona, SE Nevada	EF543380	EF517845	EF549783
Pediomelum mephiticum(S.Watson) Rydb.	Egan & Egan	126	*BRY*	Washington co. Utah	EF543394	EF517846	EF549760
Pediomelum pariense (S.L.Welsh & N.D. Atwood) J.W.Grimes	Egan & Egan	148	*BRY*	Kane co. Utah	EF543405	EF517885	EF549808
Pediomelum pentaphyllum(L.) Rydb.	Egan & Egan	172	*BRY*	Cochise co., Arizona	EF543406	EF517886	EF549809
*Pediomelum piedmontanum*J.R.Allison, M.W.Morris & A.N.Egan	Egan & Egan	263	*BRY*	Fall Line of GA, SC	EF543411	EF517862	EF549814
Pediomelum reverchoni(S.Watson) Rydb.	Orzell	55552	*TEX*	N Central Texas	EF543397	EF517829	EF549800
Pediomelum rhombifolium(Torr. & A.Gray) Rydb.	Egan & Egan	179	*BRY*	Texas, Mexico	EF543382	EF517836	EF549752
Pediomelum subacaule(Torr. & A.Gray) Rydb.	Egan & Egan	218	*BRY*	Cedar Glades of TN, GA	EF543412	EF517859	EF549773
Psoralidium junceum Rydb.	Egan & Egan	164	*BRY*	Kane/San Juan Co., Utah	EF543399	EF517889	EF549802

Table 2.1 continued

Taxon name	Collector	Number	Herbarium	Distribution	trnL-F	ITS	rpoB-trnC
Psoralidium lanceolatum Rydb.	Egan & Egan	153	*BRY*	W into Midwest USA	EF543401	EF517840	EF549804
Psoralidium tenuiflorum Rydb.	Egan & Egan	194	*BRY*	Midwest into SW USA	EF543400	EF517839	EF549803
Rupertia hallii (Rydb.) J.W.Grimes	Egan & Egan	278	*BRY*	N California	EF543366	EF517833	EF549770
Rupertia physodes (Douglas ex Hook.) J.W.Grimes	Egan & Egan	270	*BRY*	West Coast USA	EF543414	EF517858	EF549772
Rupertia rigida (Parish) J.W.Grimes	Egan & Egan	268	*BRY*	Baja California	EF543415	EF517905	EF549818
Otholobium swartbergense C.H.Stirt.	Taylor	8286	*TEX*	S. Africa	EF543342	EF517825	*
Otholobium striatum (Thunb.) C.H.Stirt.	Fellingham	37036	*TEX*	S. Africa	EF543362	EF517851	*
Otholobium mexicanum (L.f.) J.W.Grimes	Jorgenson	92018	*TEX*	NW S. America	*	EF517914	EF549826
Otholobium caliginis J.W. Grimes	Grimes	2513	*TEX*	N	EF543422	EF517913	EF549825
Otholobium bracteolatum (Eckl. & Zeyh.) C.H.Stirt.	Germ	4211	*TEX*	S. Africa	*	EF517912	*
Otholobium acuminatum (Lam.) C.H.Stirt.	Muasya, Stirton & Dludlu	3603	*BOL*	S. Africa	Y	N	Y
Otholobium arborescens C.H.Stirt.	Muasya, Stirton & Dludlu	3279	*BOL*	S. Africa	Y	Y	Y
Otholobium bolusii (Forbes) C.H.Stirt.	Dludlu, Muasya & Stirton	3	*BOL*	S. Africa	Y	N	N
Otholobium bracteolatum (Eckl. & Zeyh.) C.H.Stirt.	Muasya, Stirton & Dludlu	3164	*BOL*	S. Africa	N	Y	Y
Otholobium candicans (Eckl. & Zeyh.) C.H.Stirt.	Muasya, Stirton & Dludlu	3263	*BOL*	S. Africa	Y	Y	Y
Otholobium candicans (Eckl. & Zeyh.) C.H.Stirt.	Muasya, Stirton & Dludlu	3369	*BOL*	S. Africa	N	Y	N
Otholobium dreweae C.H. Stirt.	Dludlu, Muasya & Stirton	10	*BOL*	S. Africa	Y	N	Y
Otholobium flexuosum C.H.Stirt.	Muasya, Stirton & Dludlu	3276	*BOL*	S. Africa	Y	N	Y
Otholobium foliosum (Oliv.) C.H.Stirt.	Gehrke & Muasya	AF086	*EA*	Kenya	Y	Y	Y
Otholobium fruticans (L.) C.H.Stirt.	Muasya, Stirton & Dludlu	3480	*BOL*	S. Africa	N	Y	Y
Otholobium hamatum (Harv.) C.H.Stirt.	Muasya, Stirton & Dludlu	3310	*BOL*	S. Africa	N	Y	N
Otholobium hirtum (L.) C.H.Stirt.	Muasya, Stirton & Dludlu	3372	*BOL*	S. Africa	N	Y	N
Otholobium lanceolatum C.H. Stirt.	Dludlu, Muasya & Stirton	13	*BOL*	S. Africa	N	Y	N
Otholobium lucens C.H. Stirt. Ined.	Muasya, Stirton & Dludlu	3570	*BOL*	S. Africa	N	N	Y
Otholobium macradenium (Harv.) C.H.Stirt.	Muasya, Stirton & Dludlu	4452	*BOL*	S. Africa	Y	N	Y
Otholobium mundianum (Eckl. & Zeyh.) C.H.Stirt.	Muasya, Stirton & Dludlu	3885	*BOL*	S. Africa	Y	Y	Y
Otholobium nigricans C.H.Stirt.	Muasya, Stirton & Dludlu	3790b	*BOL*	S. Africa	Y	N	Y
Otholobium nitens C.H. Stirt. Ined.	Muasya, Stirton & Dludlu	3884	*BOL*	S. Africa	Y	Y	Y
Otholobium obliquum (E.Mey.) C.H.Stirt.	Muasya, Stirton & Dludlu	3198a	*BOL*	S. Africa	N	Y	Y
Otholobium obliquum (E.Mey.) C.H.Stirt.	Muasya, Stirton & Dludlu	3198b	*BOL*	S. Africa	N	Y	N
Otholobium parviflorum (E.Mey.) C.H.Stirt.	Muasya, Stirton & Dludlu	3199	*BOL*	S. Africa	N	Y	N
Otholobium parviflorum (E.Mey.) C.H.Stirt.	Muasya, Stirton & Dludlu	3199	*BOL*	S. Africa	N	Y	N
Otholobium polyphyllum (Eckl. & Zeyh.) C.H.Stirt.	Muasya, Stirton & Dludlu	3845	*BOL*	S. Africa	N	N	Y
Otholobium polyphyllum (Eckl. & Zeyh.) C.H.Stirt.	Muasya, Stirton & Dludlu	3678	*BOL*	S. Africa	N	N	Y
Otholobium polystictum (Benth. ex Harv.) C.H.Stirt.	Muasya, Stirton & Dludlu	3730	*BOL*	S. Africa	N	Y	N
Otholobium prodiens C.H. Stirt. Ined.	Muasya, Stirton & Dludlu	3854	*BOL*	S. Africa	N	Y	N
Otholobium prodiens C.H. Stirt. Ined.	Dludlu, Muasya & Stirton	92	*BOL*	S. Africa	Y	Y	N
Otholobium pungens C.H.Stirt.	Muasya, Stirton & Dludlu	3175	*BOL*	S. Africa	Y	Y	Y
Otholobium pustulatum C.H.Stirt.	Muasya, Stirton & Dludlu	3286	*BOL*	S. Africa	N	Y	Y
Otholobium rotundifolium (L.f.) C.H.Stirt.	Dludlu, Muasya & Stirton	4	*BOL*	S. Africa	Y	Y	Y
Otholobium saxosum C.H. Stirt.	Muasya, Stirton & Dludlu	102	*BOL*	S. Africa	Y	Y	Y
Otholobium sericeum (Poir.) C.H.Stirt.	Muasya, Stirton & Dludlu	3869	*BOL*	S. Africa	N	Y	Y
Otholobium spicatum (L.) C.H.Stirt.	Muasya, Stirton & Dludlu	3568	*BOL*	S. Africa	Y	Y	Y
Otholobium spicatum (L.) C.H.Stirt.	Muasya, Stirton & Dludlu	3445	*BOL*	S. Africa	N	Y	Y
Otholobium spissim C.H. Stirt. Ined.	Muasya, Stirton & Dludlu	4101	*BOL*	S. Africa	Y	N	Y
Otholobium stachydis Thunb.	Muasya, Stirton & Dludlu	3264	*BOL*	S. Africa	N	Y	N
Otholobium stachyerum (Eckl. & Zeyh.) C.H.Stirt.	Muasya, Stirton & Dludlu	3851	*BOL*	S. Africa	Y	Y	Y
Otholobium striatum (Thunb.) C.H.Stirt.	Muasya, Stirton & Dludlu	3339	*BOL*	S. Africa	Y	Y	Y

Table 2.1 continued

Taxon name	Collector	Number	Herbarium	Distribution	trnL-F	ITS	rpoB-trnC
Otholobium swartbergense C.H.Stirt.	Muasya, Stirton & Dludlu	3587	*BOL*	S. Africa	Y	Y	Y
Otholobium thomii (Harv.) C.H.Stirt.	Muasya, Stirton & Dludlu	3187	*BOL*	S. Africa	N	Y	N
Otholobium uncinatum (Eckl. & Zeyh.) C.H.Stirt.	Muasya, Stirton & Dludlu	3261	*BOL*	S. Africa	Y	Y	Y
Otholobium venustum (Eckl. & Zeyh.) C.H.Stirt.	Muasya, Stirton & Dludlu	4327	*BOL*	S. Africa	Y	N	Y
Otholobium virgatum (Burm. F.) C.H. Stirt.	Muasya, Stirton & Dludlu	3163	*BOL*	S. Africa	Y	Y	Y
Otholobium wilmsii (Harms) C.H.Stirt.	Muasya, Stirton & Dludlu	3782	*BOL*	S. Africa	Y	Y	Y
Otholobium zeyheri (Harv.) C.H.Stirt.	Muasya, Stirton & Dludlu	3173	*BOL*	S. Africa	Y	N	N
Psoralea aculeata L.	Muasya, Stirton & Dludlu	3170	*BOL*	S. Africa	Y	N	N
Psoralea aculeata L.	Muasya, Stirton & Dludlu	3185	*BOL*	S. Africa	Y	N	N
Psoralea affinis Eckl. & Zeyh.	Muasya, Stirton & Dludlu	3169	*BOL*	S. Africa	N	Y	Y
Psoralea affinis Eckl. & Zeyh.	Muasya, Stirton & Dludlu	3201b	*BOL*	S. Africa	N	Y	Y
Psoralea affinis Eckl. & Zeyh.	Muasya, Stirton & Dludlu	4074	*BOL*	S. Africa	N	Y	Y
Psoralea angustifolia Jacq.	Muasya, Stirton & Dludlu	3278	*BOL*	S. Africa	Y	N	Y
Psoralea aphylla L.	Muasya, Stirton & Dludlu	3203	*BOL*	S. Africa	Y	N	Y
Psoralea aphylla L.	Muasya, Stirton & Dludlu	3492	*BOL*	S. Africa	Y	Y	Y
Psoralea aphylla L.	Muasya, Stirton & Dludlu	4347	*BOL*	S. Africa	Y	N	N
Psoralea asarina (P.J.Bergius) Salter	Muasya, Stirton & Dludlu	4030	*BOL*	S. Africa	Y	N	Y
Psoralea azurea C.H. Stirt. Ined.	Muasya, Stirton & Dludlu	3612	*BOL*	S. Africa	N	Y	N
Psoralea elegans C.H. Stirt. Ined.	Dludlu, Muasya & Stirton	105	*BOL*	S. Africa	Y	Y	Y
Psoralea filifolia Eckl. & Zeyh.	Muasya, Stirton & Dludlu	4321	*BOL*	S. Africa	Y	N	Y
Psoralea fleta C.H. Stirt. Ined.	Muasya, Stirton & Dludlu	3385	*BOL*	S. Africa	Y	N	N
Psoralea fleta C.H. Stirt. Ined.	Muasya, Stirton & Dludlu	3342	*BOL*	S. Africa	Y	Y	N
Psoralea floccosa C.H. Stirt. Ined.	Muasya, Stirton & Dludlu	3273	*BOL*	S. Africa	N	Y	N
Psoralea gigantea M.N.Dludlu, A.M. Muasya & C.H. Stirton Ined.	Dludlu, Muasya & Stirton	57	*BOL*	S. Africa	Y	N	N
Psoralea glabra E.Mey.	Muasya, Stirton & Dludlu	3646	*BOL*	S. Africa	Y	Y	Y
Psoralea glabra E.Mey.	Muasya & Stirton in Abbott	8841.2	*BOL*	S. Africa	Y	N	N
Psoralea glaucescens Eckl. & Zeyh.	Muasya, Stirton & Dludlu	3289	*BOL*	S. Africa	Y	Y	Y
Psoralea imminens C.H. Stirt. Ined.	Muasya, Stirton & Dludlu	3596	*BOL*	S. Africa	N	Y	Y
Psoralea koudebergense M.N.Dludlu, C.H. Stirton & A.M. Muasya Ined.	Dludlu, Muasya & Stirton	64	*BOL*	S. Africa	Y	N	Y
Psoralea latifolia (Harv.) C.H. Stirton ined.	Muasya & Stirton in Abbott	8841.5	*BOL*	S. Africa	Y	Y	N
Psoralea laxa Salter	Muasya, Stirton & Dludlu	3611	*BOL*	S. Africa	N	Y	N
Psoralea monophylla (L.) C.H. Stirt.	Muasya, Stirton & Dludlu	3476	*BOL*	S. Africa	Y	Y	Y
Psoralea muirii C.H. Stirt. Ined.	Muasya, Stirton & Dludlu	3621	*BOL*	S. Africa	N	Y	N
Psoralea odoratissima Jacq.	Muasya, Stirton & Dludlu	3557	*BOL*	S. Africa	N	Y	N
Psoralea oligophylla Eckl. & Zeyh.	Muasya, Stirton & Dludlu	3798	*BOL*	S. Africa	N	Y	N
Psoralea oreophila Schltr.	Muasya, Stirton & Dludlu	3464	*BOL*	S. Africa	Y	Y	Y
Psoralea oreopola C.H. Stirt. Ined.	Muasya, Stirton & Dludlu	3271	*BOL*	S. Africa	Y	Y	Y
Psoralea peratica C.H. Stirt.	Dludlu, Muasya & Stirton	80	*BOL*	S. Africa	N	Y	Y
Psoralea pinnata L.	Muasya, Stirton & Dludlu	3165	*BOL*	S. Africa	N	Y	N
Psoralea pullata C.H. Stirt. Ined.	Muasya, Stirton & Dludlu	3178	*BOL*	S. Africa	Y	Y	Y
Psoralea repens P.J.Bergius	Muasya, Stirton & Dludlu	3168	*BOL*	S. Africa	N	Y	N
Psoralea repens P.J.Bergius	Muasya, Stirton & Dludlu	3168	*BOL*	S. Africa	N	Y	Y
Psoralea restioides Eckl. & Zeyh.	Muasya, Stirton & Dludlu	3216	*BOL*	S. Africa	N	Y	Y
Psoralea rhizotoma C.H. Stirton, M.N. Dludlu & A.M. Muasya ined.	Muasya, Stirton & Dludlu	3659	*BOL*	S. Africa	N	Y	Y
Psoralea rhizotoma C.H. Stirton, M.N. Dludlu & A.M. Muasya ined.	Muasya, Stirton & Dludlu	3677	*BOL*	S. Africa	N	Y	Y
Psoralea rigidula C.H. Stirt. Ined.	Dludlu, Muasya & Stirton	28	*BOL*	S. Africa	Y	Y	Y
Psoralea sordida C.H. Stirt. Ined.	Muasya, Stirton & Dludlu	3579	*BOL*	S. Africa	Y	Y	N

Table 2.1 continued

Taxon name	Collector	Number	Herbarium	Distribution	trnL-F	ITS	rpoB-trnC
Psoralea speciosa Eckl. & Zeyh.	Muasya, Stirton & Dludlu	3456	*BOL*	S. Africa	N	Y	N
Psoralea speciosa Eckl. & Zeyh.	Vlok	643	*BOL*	S. Africa	N	N	Y
Psoralea triflora Thunb.	Dludlu, Muasya & Stirton	89	*BOL*	S. Africa	Y	N	Y
Psoralea triflora Thunb.	Muasya, Stirton & Dludlu	3827	*BOL*	S. Africa	Y	N	Y
Psoralea usitataC.H. Stirt. Ined.	Muasya, Stirton & Dludlu	3414	*BOL*	S. Africa	Y	Y	Y
Psoralea vigilansC.H. Stirt. Ined.	Muasya, Stirton & Dludlu	3415	*BOL*	S. Africa	Y	N	Y
Psoralea verrucosa Willd. ex Spreng.	Muasya, Stirton & Dludlu	3269	*BOL*	S. Africa	Y	N	N
Psoralea verrucosa Willd. ex Spreng.	Muasya, Stirton & Dludlu	3592	*BOL*	S. Africa	Y	N	N

Secondary sources of data included published literature such as Trinder-Smith (2006), Bean & Johns (2005) and Goldblatt & Manning (2000). The anatomical and phytochemical data were from the following studies: Crow *et al.* (1997); Boardley *et al.* (1986); and Turner (1984). The characters and their corresponding character states are shown in Table 2.2.

2.1.3. DNA extraction, amplification and sequencing

2.1.3. (a) Extraction

DNA was extracted from the silica gel dried material using a modified Cetyltrimethylammonium Bromide (CTAB) technique from Doyle & Doyle (1987) and Gawel & Jarret (1991). The CTAB was mixed with mercapto-ethanol in the ratio 700:1 and incubated in a water bath at 65 °C. Twenty mg of the plant material was mixed with acid washed sand and polyvinylpolypyrrolidone (PVP) and ground while frozen in liquid nitrogen using a pestle and mortar into a fine powder.

700 µl of the pre-heated CTAB extraction buffer were added to each of the ground samples. These were then mixed thoroughly by vortexing and incubated at 65 °C for 60 minutes, with gentle shaking (by inversion) every 20 minutes. After incubation, 600 µl of 24:1 v/v chloroform: isoamyl alcohol were added to each sample, mixed by inversion for 5 minutes, and centrifuged at 12 000 rpm for 5 minutes. The supernatant was carefully pipetted out and placed into a clean 1.5 ml tube, to which an equal volume of ice-cold isopropanol was added and mixed briefly by inversion. At this stage, the samples were left in the freezer (-20 °C) for a minimum of 2 days to allow the DNA to precipitate.

The chilled samples were centrifuged at 12 000 rpm for 5 minutes to recover the DNA pellet, which was visible as a white or brownish pellet at the base of the tube. The isopropanol was carefully discarded and the open tubes were inverted onto tissue paper to allow residual liquid to drain out, but making sure not to lose the pellet. After about 10 minutes, any residual droplets were wiped off the rim of the tube and the DNA pellets were washed with 250 µl of 75 % ethanol. After discarding the ethanol, the tubes were left open on the bench top for the DNA pellets to dry. Once dry, the DNA pellet was suspended in 50 µl of sterile distilled water and stored in the fridge. Preliminary investigations showed that samples could not amplify unless the DNA was purified. Therefore, after every extraction, the DNA was purified using a GE Health Care DNA and Gel band purification kit according to the manufacturerôs protocols.

Table 2.2 Character list for *Psoralea* and *Otholobium* species used for phylogenetic analysis. n/a = not applicable.

1. Habit: 0= herbs (≤ 1 m) 1= small shrubs (>1 m but ≤ 2 m) 2= large shrubs (>2 m but ≤ 4 m) 3= trees (4 m or taller).
2. Regeneration strategy: 0= reseeder, 1= resprouter.
3. Stem habit: 0= decumbent, 1= semi-erect, 2= erect.
4. Number of stems: 0= one, 1= more than one.
5. Branch glandulosity: 0= pustulate, 1= non-pustulate.
6. Leaves: 0= present, 1= absent.
7. If present, type: 0= unifoliate (simple leaf), 1= unifoliolate (compound, but laterals lacking), 2= compound, ?= n/a.
8. If unifoliate, width: 0= narrow (<5 mm), 1= moderate (5-10 mm), 2= broad (>10 mm), ?= n/a
9. Length of unifoliate leaf: 0= short (0-5 mm), 1= moderate (>5-10 mm), 2= long (>10 mm), ?= n/a.
10. If unifoliate, leaf orientation: 0= clasping, 1= erect, ?= n/a
11. If compound, # leaflets: 0= three, 1= five, 2= seven, 3= nine, 4= eleven, 5= thirteen, 6= fifteen, ?= n/a.
12. If trifoliolate, leaf arrangement: 0= digitate, 1= pinnate, ?= n/a.
13. If compound, leaflet width: 0= filiform (<5 mm), 1= elliptic (5-10 mm), 2= broad (>10 mm), ?= n/a.
14. Apex of simple leaf : 0= acute, 1= mucronate, 2= emarginate, ?= n/a.
15. Length of terminal vs. basal laterals: 0= shorter, 1= equal, 2= longer, ?= n/a.
16. Fusion of stipules to petiole: 0= fused, 1= basally fused, 2= fused to the shaft, 3= free.
17. If leafless, type: 0= bare, 1= scales, 2= leafy on flowering shoots, ?= n/a.
18. Stipules persistence: 0= persistent, 1= caducous.
19. Stipule length vs. petiole length: 0= shorter, 1= equal, 2= longer, ?= n/a.
20. Stipule type: 0= woody, 1= cartilaginous, 2= chartaceous.

Table 2.2 continued

21. Curvature of mucro of terminal leaflet: 0= straight, 1= arching, 2= recurved, ?= n/a.

22. Inflorescence arrangements: 0= determinate (no shoot extension), 1= indeterminate (with shoot extension).

23. Flower congestion: 0= lax (inflorescence axis visible), 1= congested (inflorescence axis not visible).

24. Type of inflorescence: 0= spicate, 1= capitate.

25. Number of flowers per axil if inflorescence is axillary: 0= one, 1= three, 2= many, ?= n/a.

26. Cupulum: 0= present, 1= absent.

27. Number of cupulum lobes: 0= two, 1= two but one lobe having a cleft, 2= trifid, ?= n/a.

28. Position of cupulum on pedicel: 0= basal, 1= on the lower third, 2= central 3= upper third, ? n/a.

29. Calyx tube length vs. teeth: 0= shorter, 1= equal, 2= longer.

30. Glands on leaf and calyx: 0= absent, 1= present.

31. Glands colour if present: 0= orange, 1= black, ?= n/a.

32. Number of bracts subtending each flower: 0= single, 1= two.

33. Occurrence of callosities on standard petal: 0= absent, 1= present.

34. Presence of dark patch on keel petal: 0=present, 1= absent.

35. Leaf margins: 0= entire, 1= crinkled/undulate, ?= n/a.

36. Leaflet blade cross section: 0= flattened, 1= rounded & grooved, ?= n/a.

37. Standard petal reflexion: 0= not reflexed, 1= reflexed.

38. Occurrence of flowers in triplets, with each triplet subtended by a single bract and each flower subtended by its own bract: 0= present, 1= absent.

39. Presence of proanthocyanidins: 0= present, 1= absent.

40. Presence of trabeculae on epidermis of secretory cavity: 0= present, 1= absent.

2.1.3. (b) Screening of molecular markers

Several markers were screened for successful amplification, good sequencing and the amount of sequence variation between species. These were: *ETS* (Chandler *et al*., 2001); *ITS* (White *et al*., 1990); the intergenic spacer *psbA-trnH* (Sang *et al*., 1997); *trnL-F, ropB-trnC, trnQ-rps16*, *ndhF-rp132* and *trnD-trnT* (Shaw *et al*., 2005 and Shaw *et al*., 2007). The screening was done by performing PCR reactions (details are discussed below) with 6-8 samples (of *Psoralea* and *Otholobium* species) for each of the different markers, visualising the PCR products on agarose gel and taking a photo of the gel under UV light. Amplified products were then sent for sequencing. The sequences were aligned and variation was assessed by manual inspection. The DNA regions that were eventually used were *ITS*, *trnL-F*, and *rpoB-trnC*. The details of the primer sequences and the corresponding references are shown in Table 2.3. The PCR conditions used for each of the markers are described in the following sections.

2.1.3. (c) DNA amplification (PCR)

For the *ITS*, 50 µl total volumes of PCR reactions were prepared for each sample. These were made up of 5 µl buffer; 5 µl $MgCl_2$; 2 µl dNTP; 1.65 µl forward primer (*ITS*5); 1.65 µl reverse primer (*ITS*4); 0.33 µl *Taq* polymerase; 3.37 µl of template DNA and 31 µl of sterile distilled water. The PCR was run on an Applied Biosystems GeneAmp 2700 thermal cycler (Applied Biosystems, Foster City, CA, USA). The process involved an initial denaturation phase of 2 minutes at 94 ºC; followed by 33 cycles of 1 minute at 94 ºC; 1 minute at 52 ºC (annealing); 2 minutes at 72 ºC (extension) and a final extension phase of 7 minutes at 72 ºC.

The PCR products were loaded (3 µl of each sample) into wells on a 1 % agarose gel (that was stained with ethidium bromide) and ran in an electrophoresis tank containing 0.5 X TBE at 100 V for 15 minutes. They gel was then visualised under UV light and a photo of the gel was taken. For samples that showed multiple bands, the whole PCR product was loaded into 1 % agarose gel and run in the electrophoresis tank at 70 V for 30-40 minutes depending on how fast the bands were separating from each other. The DNA bands were visualised under UV light and when they had clearly separated, a scalpel was used to excise thin slices of each of the DNA bands. Each of these slices was placed in a labelled tube and purified using the GE Healthcare DNA purification kit according to the manufacturerôs protocols.

Table 2.3 Primers and their corresponding sequences that were used for amplification and sequencing of the different DNA regions. F/R= forward and reverse respectively.

Region	Name	F/R	Sequence (5'-3')	Reference
ITS	ITS5	F	GGAAGTAAAAGTCGTAACAAGG	White *et al.* (1990)
	ITS4	R	TCCTCCGCTTATTGATATGC	White *et al.* (1990)
TrnL-F	$trnL^{UAA}$	F	CGAAATCGGTAGACGCTACG	Tarbelet *et al.* (1991)
	$TrnF^{GAA}$	R	ATTTGAACTGGTGACACGAG	Tarbelet *et al.* (1991)
rpoB-trnC	$trnC^{GCA}$ R	F	CACCCRGATTYGAACTGGGG	Shaw *et al.* (2005)
	rpoB	R	CKACAAAAYCCYTCRAATTG	Shaw *et al.* (2005)

For the chloroplast regions, i.e. *trnL-F* and *rpoB-trnC*, 30 µl PCR volumes were made by mixing 3 µl buffer; 3 µl $MgCl_2$; 1.2 µl dNTP; 1 µl of forward primer; 1 µl of reverse primer; 0. 2 µl *Taq* polymerase; 2 µl of template DNA; and 18.6 µl of sterile distilled water. For *trnL-F* the PCR consisted of an initial denaturation step of 2 minutes at 94 °C followed by 33 cycles of 1 minute at 94 °C; 1 minute at 52 °C; 1 minute at 72 °C and a 7 minutes terminal elongation at 72 °C. On the other hand, the PCR for *rpoB-trnC* consisted of an initial denaturation phase at 80 °C for 5 minutes; followed by 33 cycles of 1 minute at 96 °C; 2 minutes at 52 °C; 3 minutes at 72 °C; and a final extension phase of 5 minutes at 72 °C. For both regions, 3 µl of the PCR product was loaded on a 1 % agarose gel, which was run on an electrophoresis tank with 0.5 X TBE at 100 V for 15 minutes. A photo of the gel was then taken under UV light at 0.200 seconds to visualise which samples had amplified successfully and this was determined by visual inspection of the DNA bands on the gel photo.

2.1.3. (d) DNA sequencing

Amplified PCR products were sent to MacroGen (http://www.macrogen.com) in Korea or the University of Stellenbosch DNA sequencing facility for sequencing using the same primers that were used in the PCR. For *ITS*, in the first batch of samples the purified products for all the isolated bands in each sample were sequenced, and then the sequences were aligned and visually inspected. After observing that all the sequences were identical, the product of only one band was sequenced in subsequent batches.

2.1.4. Sequence alignment and phylogenetic analysis

2.1.4. (a) Sequence alignment and gap coding

Sequences were assembled and edited using Staden package version 1.60 (Staden *et al*., 1998) and the consensus sequences were imported into Bioedit version 7.0 (Hall, 1999). They were first electronically aligned using the ClustalW multiple alignment, and then any remaining residues were aligned manually. Insertions and deletions were coded using simple indel coding in Gap Coder (Young & Healy, 2003).

2.1.4. (b) Phylogenetic reconstruction

The DNA data-sets were first analysed separately, and then in a second analysis the chloroplast data (i.e. *trnL-F* and *rpoB-trnC*) were combined. The third analysis involved combining data from all three DNA regions. Data for taxa absent from any of the separate partitions were coded as missing. The morphology data set was first analysed separately using the parsimony settings as described below. Multistate characters were treated as unordered. Since only the southern African Psoraleeae could be scored for morphology and anatomical characters, the matrix that combined morphology with DNA data excluded the rest of the Psoraleeae genera.

Phylogeny reconstruction was done using parsimony and Bayesian methods. Parsimony analyses were done in PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford, 2002). The searches were done heuristically, with 10 000 random addition replicates, tree bisection-reconnection (TBR) branch swapping and multrees in effect. For evaluating support, bootstrap analyses were done based on 1000 replicates, each involving a heuristic search, with simple addition sequence and TBR branch swapping. For the Bayesian analyses, model selection was done in Modeltest version 3.7 (Posada & Crandall, 1998). This program compares 56 different nested models of DNA substitution in a hierarchical hypothesis-testing framework and uses log likelihood scores to establish the model of DNA evolution that best fits the data (Creer *et al*., 2001). The Bayesian analyses were done in MrBayes Version 3.12 (Huelsenbeck & Ronquist, 2003). For each analysis, two simultaneous runs were done (starting from random trees). Each run had four chains (three heated and one cold chain) and the temperature was set at 0.4. Default priors of MrBayes were used. Markov chains were sampled every 100th generation and the whole analysis was run for ten million generations. To check for stationarity, the log-likelihoods were plotted against the generation time, and this gave an idea of the number of trees to discard (i.e. trees sampled during the burn in period). Trees sampled from the ôburn inô phase were discarded

from the analysis before calculating the posterior probabilities. Trees from all analyses were viewed in the program TreeView (Page, 1996).

2.1.4. (c) Ancestral state reconstructions

For the two genera, *Otholobium* and *Psoralea*, ancestral state reconstruction was done in Mesquite (Maddison & Maddison, 2006) on one of the trees from the combined DNA matrix using parsimony. This was done for the key diagnostic characters, used by Stirton (1981) to distinguish between the two genera, as well as between sections within the two genera as shown in Table 2.4 below. For those taxa in which a particular character was not applicable, it was scored as missing. For example, *Psoralea aphylla*, which has no leaves, was scored as ñ?ò for the characters ñleaf typeò and ñrecurved mucro on leavesò.

Table 2.4 Characters used for ancestral state reconstruction for *Otholobium* and *Psoralea*.

Character	state 0	state 1
Leaves	absent	present
Leaf type	simple	compound
Recurved mucro on leaves	absent	present
Cupulum	absent	present
Flowers borne in triplets subtended by a single bract	no	yes

2.1.5. Estimation of divergence dates

2.1.5. (a) Calibration points

Calibration points were derived from the family wide (Leguminosae) divergence dates analysis of Lavin *et al*. (2005) in which 13 fossil calibration points were used and the divergence dates obtained for North American Psoraleeae by Egan and Crandall (2008b). Although the use of such indirect secondary calibration has been criticised as being erroneous, especially if there are biases or errors in the prior analyses (Hedges & Kumar, 2004; Graur & Martin, 2004; Hug & Roger, 2007), it was the only point of reference in this study since there are no known fossils for the Psoraleeae. The dates obtained from these two studies should be reliable because they met the essential requirements for appropriate lineage dating as suggested by Benton & Donoghue (2007). These are: (i) the use of multiple calibration points and (ii) the use of several DNA loci. In the Lavin *et al*. (2005) study where a single gene region was used, the number of fossils used

(thirteen) is impressively large. Similarly, while Egan & Crandall (2008b) used just two calibration points, their analysis was based on eight DNA regions (nuclear and plastid).

When selecting a calibration point, it is important that the relationships of the selected group to other taxa should be well supported by the bootstrap/jacknife (Wikstrom *et al*., 2001). For this reason, only well supported groups (from the parsimony and Bayesian analyses of the combined data-set) were selected as calibration points. These were as follows: the most recent common ancestor (MRCA) of *Cullen* and *Rupertia* has a mean date of 6.3 mya with a standard deviation of 0.9 million years (Lavin *et al*., 2005); the MRCA of *Glycine* and Psoraleeae is 14.90 mya with a standard deviation of 2.18 million years and the MRCA of *Pediomelum* is 3.28 mya with a standard deviation of 1.483 million years (Egan & Crandall, 2008b). These dates were incorporated as calibration points into the BEAST analysis. They were modelled as a normal distribution whose mean is equal to the respective node age and the standard deviations as upper and lower bounds.

2.1.5. (b) Choice of method of date estimation

Literature sources (e.g. Magallón, 2004; Heads, 2005; Rutschmann, 2006) that describe and compare the various methods of lineage dating were consulted. A Bayesian MCMC sampling method implemented in the program BEAST (Bayesian Evolutionary Analysis Sampling Trees) was found to be more suitable. This is because BEAST has the following attractive features: (i) the parameters of the distributions can be estimated instead of being specified, (ii) it does not require a starting tree topology, thus accounting for phylogenetic uncertainty (it estimates the topology and node dates simultaneously, thus allowing sequence divergences to inform topology estimation), (iii) it permits for the definition of calibration distributions (e.g. normal, log-normal, exponential or gamma) to model calibration uncertainty instead of simple point estimates or age intervals, and (iv) it allows for simultaneous analysis of multiple data sets with different substitution models (Heads, 2005). On the other hand, the other methods such as non-parametric rate smoothing (NPRS: Sanderson, 1997); penalised likelihood (Sanderson, 2002) and the Bayesian approach applied in multidivtime (Thorne & Kishino, 2002) assume that substitution rates are auto-correlated among lineages from parent to daughter branches. Such an assumption may systematically distort branch lengths, leading to a reduction of the ratio of deep to shallow nodes (Martin *et al.*, 2004). BEAST on the other hand, allows each branch to draw its rate from a discretized log-normal distribution whose shape is estimated as part of the analysis (Drummond *et. al.*, 2006).

2.1.5. (c) Estimation of dates

Since the resolution of trees obtained from the analyses of individual gene regions was very poor, the dating exercise was performed only on the combined DNA data set which had given a better resolved tree. This was done on the program BEAST version 1.47 (Drommond & Rambaut, 2007) employing a relaxed clock model with log-normally distributed uncorrelated rates of substitution between branches. No topological constraints were employed, allowing topological uncertainty to be taken into account. The tree prior was modelled under the Yule speciation process. Other than the normally distributed priors on the calibration points, all other priors were set as default values in the program BEAUti version 1.4.7 (comes as part of the beast package) in which the input files for BEAST were created. The MCMC settings consisted of two separate runs of 20 million generations sampled every 1000 generations. On completion, the program Tracer version 1.4 (Rambaut & Drummond, 2007) was used to confirm likelihood stationarity, adequate mixing of the MCMC chains, whether the two separate runs had converged as well as the burnin. The posterior distribution for divergence date estimates across key nodes was summarised by specifying the burnin in TreeAnnotator version 1.4.7 (which also comes as part of the BEAST package) and the maximum clade credibility tree was computed. The tree was viewed in the program FigTree version 1.0 (Rambaut, 2007).

2.2. Results

2.2.1. Screening of molecular markers

Of the markers screened, *trnL-F*, *psbA-trnH*, *trnQ-rps16*, *ndhF-rp132, ITS and rpoB-trnC* amplified for more than 70% of the samples, *trnD-T* amplified for 20% of the samples while *ETS* did not amplify at all, despite several attempts at adjusting the annealing temperature and template DNA concentrations. However, for those markers which amplified well, sequence variation was very low (<3%) in all but *ITS*, *rpoB-trnC* and *trnL-F*. The latter were then used for amplification and sequencing for the rest of the samples. However, for the *ITS*, some PCR products had double bands and so did not sequence well. It was after such products had been run longer (30-40 minutes) at 70 V, instead of the usual 100 V, and then the individual bands excised from the gel, purified and sequenced separately that good sequences were obtained.

2.2.2. Data matrices

2.2.2. (a) Generic relationships within the tribe Psoraleeae

The aligned matrices for the three gene regions had 148 taxa, seven of which formed the out-group and the remainder constituted the in-group. Within the in-group, 64 % (95) of the taxa were the Southern African Psoraleeae (*Psoralea*: 45 taxa and *Otholobium*: 50 taxa) which were sequenced in this study. The remaining 36 % were from the Egan and Crandall (2008a) study representing the genera, *Pediomelum* (25 species: 17.6 %), *Cullen* (4 species: 2.7 %), *Hoita* (2 species: 0.14 %), *Rupertia* (3 species: 2.0 %), *Orbexillum* (5 species: 3.4 %), *Bituminaria* (1 species: 0.7 %) and the South American *Otholobium* (2 species: 1.35 %). The matrices each contained 943, 1618 and 1290 characters for ITS, *rpoB-trnC* and *trnL-F* respectively, and the aligned combined dataset consisting of all three regions was made up of 3851 characters (Table 2.5). Tree scores, number of variable and parsimony informative characters varied for each matrix between the original data set without gap coding and the matrix with simple indel coding (sic). In the parsimony analysis of the individual matrices, *ITS* had the highest proportion of parsimony informative characters (pic), followed by *rpoB-trnC*, and *trnL-F* had the lowest. Incorporation of gaps increased the proportion of parsimony informative characters for all three matrices (Table 2.5).

Table 2.5 Summary of DNA data matrices for alignment and Parsimony analysis: vc= variable parsimony un-informative characters, pic= parsimony informative characters, sic= simple indel coding, N/A= not applicable.

Matrix	Aligned length	No. (%) vc	No. (%) pic	No. indels	Tree length	CI	RI
ITS	943	156 (16.5)	353 (37.4)	N/A	1622	0.53	0.80
***ITS* sic**	1182	231 (19.5)	506 (42.8)	238	2054	0.53	0.83
trnL-F	1290	256 (19.8)	232 (18.0)	N/A	974	0.77	0.86
***trnL-F* sic**	1474	340 (23.1)	330 (22.4)	183	1087	0.73	0.85
rpoB-trnC	1618	666 (41.2)	358 (22.1)	N/A	1604	0.85	0.83
***rpoB-trnC* sic**	1862	801 (43.0)	464 (24.7)	243	1984	0.8	0.84
All combined	3851	1075 (27.9)	950 (24.7)	N/A	4196	0.67	0.79
All combined sic	4518	1372 (30.4)	1300 (28.8)	664	5406	0.64	0.81

2.2.2. (b) Data matrices for *Otholobium and Psoralea*

The morphological/anatomical data matrix contained 100 taxa and 40 characters. The character states for each taxon are shown in Appendix 1. Of the 100 taxa, three were out-groups, while 53 were *Otholobium* and 44 were *Psoralea* species. Out of the 40 characters, two characters were parsimony uninformative and the remaining 38 characters were parsimony informative. The

combined data matrix comprised a combination of the sequence data from the three gene regions (*ITS*, *trnL-F* and *rpoB-trnC*) as well as the morphological/anatomical data. It was composed of 105 taxa, seven of which were out-groups. The aligned matrix had 3634 characters. Of these characters, 2379 characters were constant, 661 were variable parsimony un-informative characters, and 594 characters were parsimony informative. Gaps were treated as missing data.

2.2.3. Models of DNA sequence evolution

The models of sequence evolution estimated by Modeltest for each of the DNA regions are as shown in Table 2.6. The *ITS* region was found to evolve according to the GTR + I + G model of DNA sequence evolution, while the individual chloroplast regions, *trnL-F* and *rpoB-trnC* and the combination of these two (indicated as ócp combinedô in the table) were found to follow the TVM + G model. On the other hand, the combined matrix consisting of the *ITS* and both chloroplast regions (indicated as óAll combinedô in the table) was best explained by the GTR + I + G model (Table 2.6).

Table 2.6 Models of evolution and likelihood scores for the various DNA sequences

Matrix	ln L scores	Model of evolution
ITS	-9438.9	GTR + I + G
trnL-F	-6434.87	TVM + G
rpoB-trnC	-8436.86	TVM + G
Cp combined	-15940.21	TVM + G
All combined	-28074.99	GTR + I + G

2.2.4. Phylogenetic reconstructions

2.2.4. (a) Generic relationships within the tribe Psoraleeae

The results of parsimony analyses of individual DNA regions were as follows: *ITS* gave 2630 trees with 1622 steps, consistency index (CI) =0.53 and retention index (RI) =0.80; the *trnL-F* matrix yielded 3426 trees with 974 steps, CI=0.77, RI=0.86; while *rpoB-trnC* had 4320 trees whose tree length was 1604, CI=0.85, RI=0.83. These and the results of incorporating gaps are as shown in Table 2.5. Trees from individual gene region analyses were poorly supported and a majority of the branches formed polytomies in the strict consensus trees. Incorporation of gaps slightly improved the resolution of genera for all three regions but the genera *Psoralea* and *Otholobium* formed a weakly supported clade, which formed a polytomy in the strict consensus

trees of all DNA regions. The combined analysis of the three gene regions without gap coding resulted in 140 most parsimonious trees, with a tree length of 4196, CI=0.67 and RI=0.79. The trees were better resolved than those from individual gene regions, but still most clades were poorly supported. The strict consensus tree for the 140 trees is shown in Fig. 2.3. There were no differences in topology and support between the results of the matrix without gap coding and the one where gaps were incorporated into the analysis. The results of the matrix without gap coding are presented.

The monophyly of the in-group (with all the genera of the tribe represented) was well supported (Bootstrap=100 %, PP=1). The genera *Psoralea* and the southern African *Otholobium* formed a well supported clade, (Bootstrap=94%, PP=0.9) sister to the genus *Hoita* (but this relationship was not well supported). Species of the southern African clade form a polytomy, such that there is no separation between *Otholobium* and *Psoralea*. Only a few clades are retained in the strict consensus tree (Fig. 2.3). The two South American species of *Otholobium* were resolved as a well supported clade (Bootstrap=100%, PP=1) sister to the genus *Bituminaria*, a relationship which had 54% bootstrap support and a posterior probability of 0.82. *Orbexillum*, *Cullen* and *Rupertia* were all monophyletic (Bootstrap=100%, 98%, and 100% respectively). *Pediomelum* and *Psoralidium* were paraphyletic. Two species of *Psoralidium* formed a clade (Bootstrap=100%, PP=1) sister to *Rupertia*, but one species, (*Psoralidium tenuiflorum*) was nested within *Pediomelum* (marked with an asterisk in Fig. 2.3).

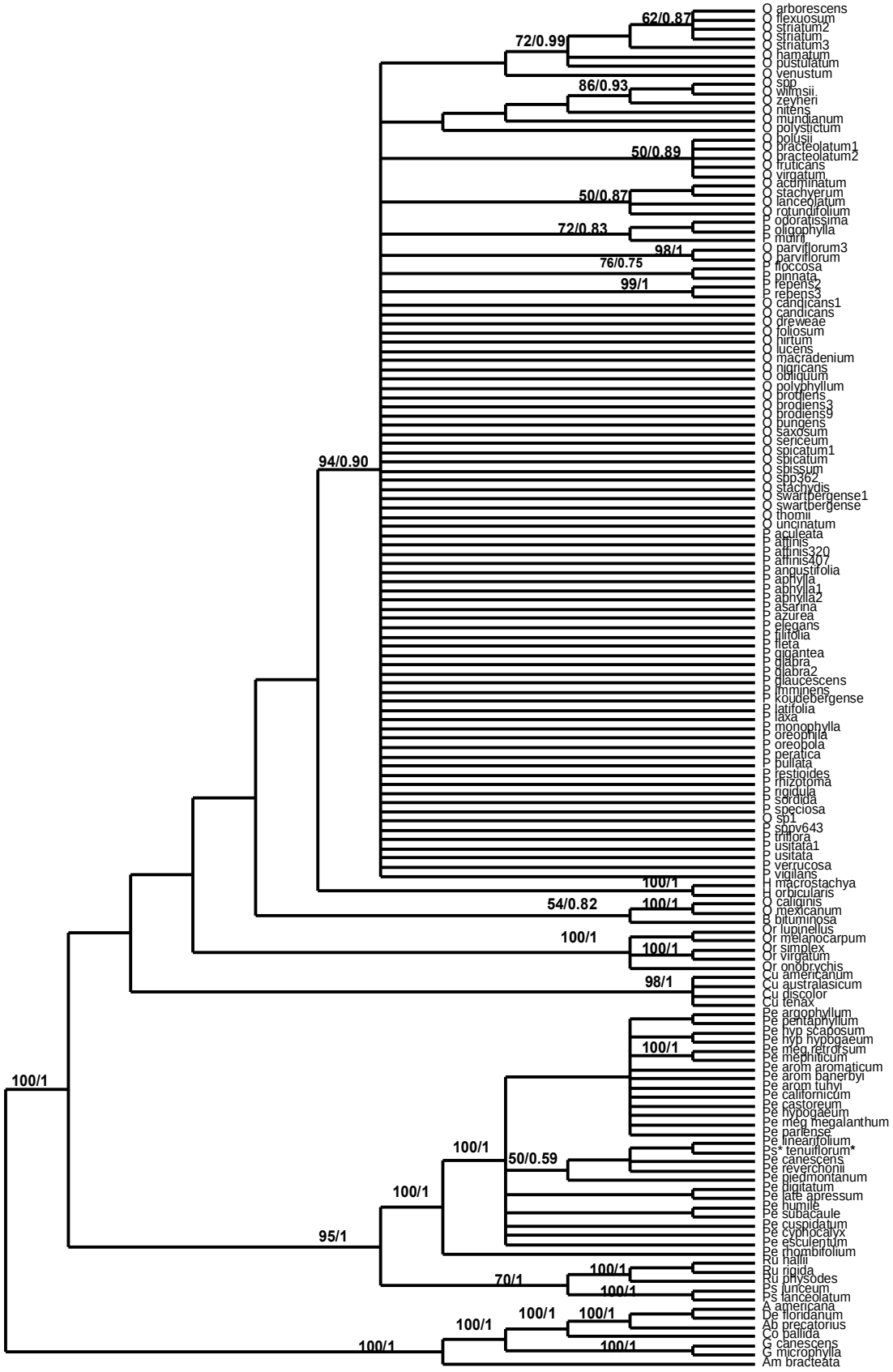
O arborescens
O flexuosum
O striatum2
O striatum
O striatum3
O hamatum
O pustulatum
O venustum
O spp
O wilmsii
O zeyheri
O nitens
O mundianum
O polysticum
O bolusii
O bracteolatum1
O bracteolatum2
O fruticans
O virgatum
O acuminatum
O stachyerum
O lanceolatum
O rotundifolium
P odoratissima
P oligophylla
P muirii
O parviflorum3
O parviflorum
P floccosa
P pinnata
P repens2
P repens3
O canicans1
O canicans
O drewiae
O foliosum
O hirtum
O lucens
O macradenium
O nigricans
O obliquum
O polyphyllum
O prodiens
O prodiens3
O prodiens9
O pungens
O saxosum
O sericeum
O spicatum1
O spicatum
O spissum
O spp362
O stachydis
O swartbergense1
O swartbergense
O thomii
O uncinatum
P aculeata
P affinis
P affinis320
P affinis407
P angustifolia
P aphylla
P abhylla1
P abhylla2
P asarina
P azurea
P elegans
P filifolia
P fleta
P gigantea
P glabra
P glabra2
P glaucescens
P imminens
P koudebergense
P latifolia
P laxa
P monophylla
P oreophila
P oreopola
P peratica
P pulilata
P restioides
P rhizotoma
P rigidula
P sordida
P speciosa
O sp1
P sppv643
P triflora
P usitata1
P usitata
P verrucosa
P vigilans
H macrostachya
H orbicularis
O caliginis
O mexicanum
B bituminosa
Or lupinellus
Or melanocarpum
Or simplex
Or virgatum
Or onobrychis
Cu americanum
Cu australasicum
Cu discolor
Cu tenax
Pe argophyllum
Pe pentaphyllum
Pe hyp scaposum
Pe hyp hypogaeum
Pe meg retrorsum
Pe meg meganthum
Pe arom aromaticum
Pe arom banerbyi
Pe arom tuhyi
Pe californicum
Pe castoreum
Pe hypogaeum
Pe meg megalanthum
Pe parlense
Pe linearifolium
Ps* tenuiflorum*
Pe canescens
Pe reverchonii
Pe piedmontanum
Pe digitatum
Pe late apressum
Pe humile
Pe subacaule
Pe cuspidatum
Pe cyphocalyx
Pe esculentum
Pe rhombifolium
Ru halii
Ru rigida
Ru physodes
Ps junceum
Ps lanceolatum
A americana
De floridanum
Ab precatorius
Co pallida
G canescens
G microphylla
Am bracteata
62/0.87
72/0.99
86/0.93
50/0.89
50/0.87
72/0.83
98/1
76/0.75
99/1
94/0.90
100/1
54/0.82
100/1
100/1
100/1
98/1
100/1
100/1
50/0.59
100/1
95/1
100/1
70/1
100/1
100/1
100/1
100/1
100/1

Figure 2.3. Strict consensus tree from the analysis of the combined DNA data set for the Psoraleeae. Numbers above branches are bootstrap percentages from parsimony and posterior probabilities from the Bayesian analysis. The names of the genera are abbreviated as follows: A=*Apios*, Ab=*Abrus*, Am=*Amphicarpea*, B=*Bituminaria*, Co=*Cologania*, Cu=*Cullen*, De=*Desmodium*, G=*Glycine*, H=*Hoita*, O=*Otholobium*, Or=*Orbexillum*, P=*Psoralea*, Pe=*Pediomelum*, Ps=*Psoralidium* and Ru=*Rupertia*.

2.2.4. (b) Phylogenetic relationships between *Otholobium* and *Psoralea*

The parsimony analysis of the morphological data set gave 40 most parsimonious trees, CI=0.22 and RI=0.78. One of these trees is shown in Fig. 2.4. Most of the branches had bootstrap support values much lower than 50 % and a majority of the nodes collapsed in the strict consensus tree. The analyses of the individual DNA data sets for *Psoralea* and *Otholobium* yielded poorly resolved trees, of which a majority of the branches collapsed in the strict consensus tree. However, the combined analysis, including all DNA regions and the morphological data gave a fairly resolved phylogeny, but still with many nodes being poorly supported (Bootstrap less than 50 %). It yielded 80 most parsimonious trees, with 2531 steps, CI=0.67 and RI=0.73. The strict consensus of these trees is shown in Fig. 2.5. The monophyly of the in-group is well supported [Bootstrap=95%, PP=1.00 (Fig. 2.5)]. The South American *Otholobium, O. caliginis* and *Hoita* formed a clade (Bootstrap=99%, PP=1.00) sister to the southern African Psoraleeae. *Otholobium* and *Psoralea* form a well-supported clade (Bootstrap=97%, PP=1.00), but the genus *Psoralea* is embedded within *Otholobium* (Bootstrap=72%, PP=0.61), with a majority of its species forming a terminal, but weakly supported clade (Bootstrap=67%, PP=0.61). However, the other basal nodes that contain *Otholobium* species lack support (i.e. Bootstrap and PP are less that 50% and 0.5, respectively) as shown in Fig. 2.5.

Figure 2.4. One of the most parsimonious trees from the morphological data set of *Psoralea* and *Otholobium*. Values above branches are the numbers of shared characters, while those below branches are bootstrap values greater than 50 %. Arrows indicate nodes that are retained in the strict consensus tree. C=*Cullen*, G=*Glycine*, O=*Otholobium*, P=*Psoralea* and R=*Rhynchosia*.

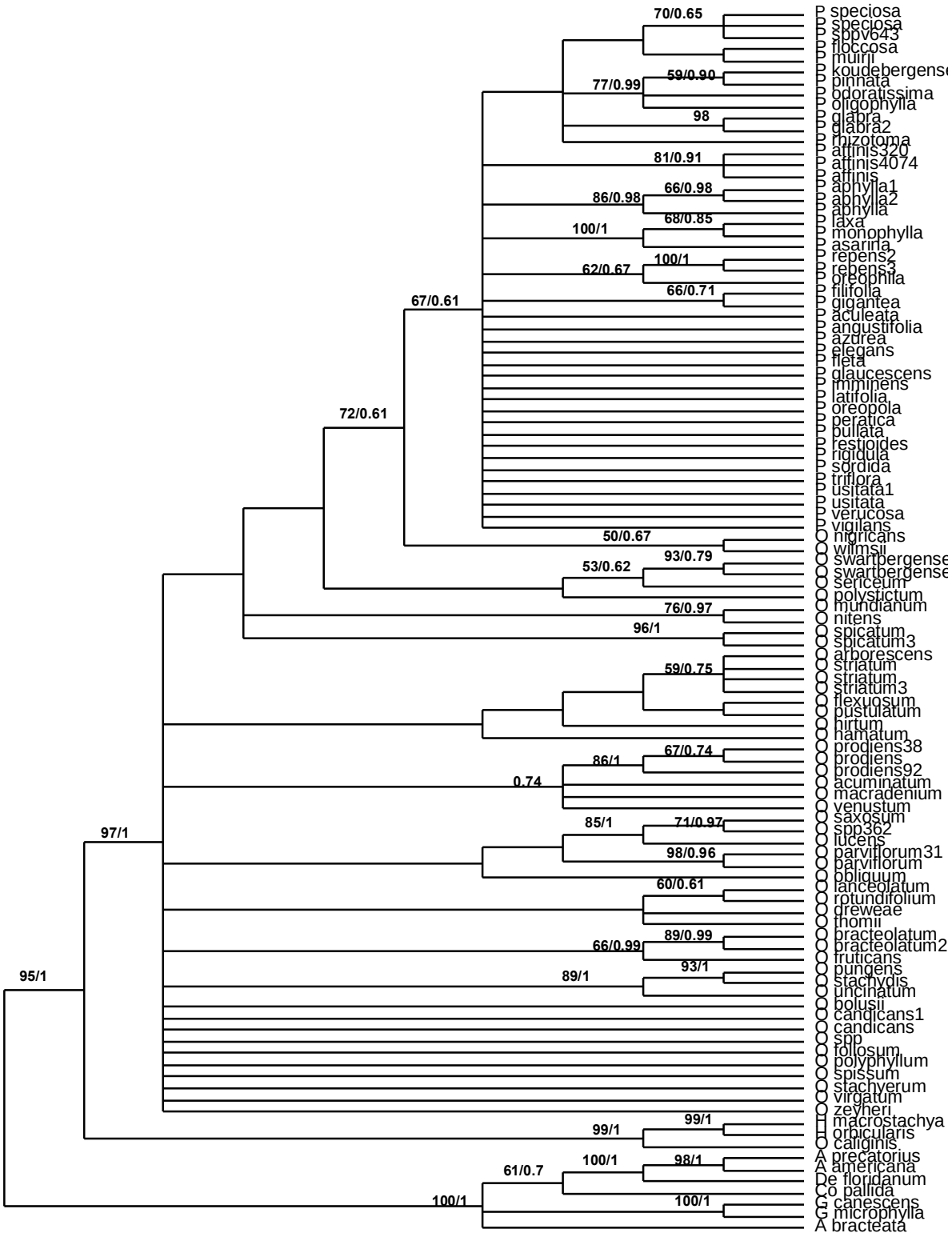

Figure 2.5. Strict consensus tree for the combined DNA and morphology data set for the southern African Psoraleeae. Numbers above branches are parsimony bootstrap percentages and Bayesian posterior probabilities. Genus abbreviations are the same as those of Fig. 2.3.

Three species which used to belong to the genus *Hallia*, i.e. *Psoralea laxa, P. monophylla* and *P. asarina* form a well supported clade (Bootstrap= 100%, PP=1.00), but its placement in the phylogeny relative to the rest of the *Psoralea* species is ambiguous (Fig. 2.5).

2.2.4. (c) Ancestral state reconstructions

The reconstructions were done on one of the parsimony trees obtained from the analysis of the combined DNA data set of the southern African Psoraleeae. Due to the poor resolution of the trees, the results of the reconstructions show that some species of *Psoralea* are embedded within *Otholobium* and vice versa. However, the phylogeny based on DNA and morphology indicates that at least *Psoralea* is monophyletic (Fig. 2.5). Therefore, the interpretation of these reconstructions considers the monophyly of *Psoralea* and other phylogenetic relationships shown in Fig. 2.5. The ancestral state reconstructions indicate that the presence of a cupulum is a derived state, which has evolved only in members of the genus *Psoralea*, while the absence of a cupulum is the ancestral state (Fig. 2.6).

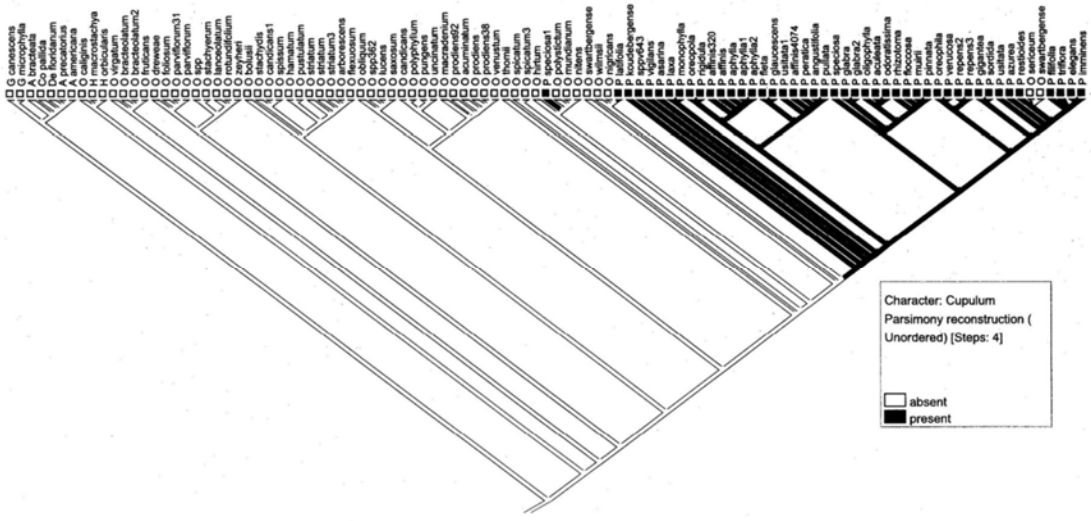

Figure 2.6. Ancestral state reconstruction for the presence of a cupulum

The secondary loss of leaves, which is characteristic of the *P. aphylla* complex, is reconstructed as a derived state that has arisen multiple independent times within the *Psoralea* clade (Fig. 2.7). On the other hand, the presence of leaves is the ancestral state of the lineage.

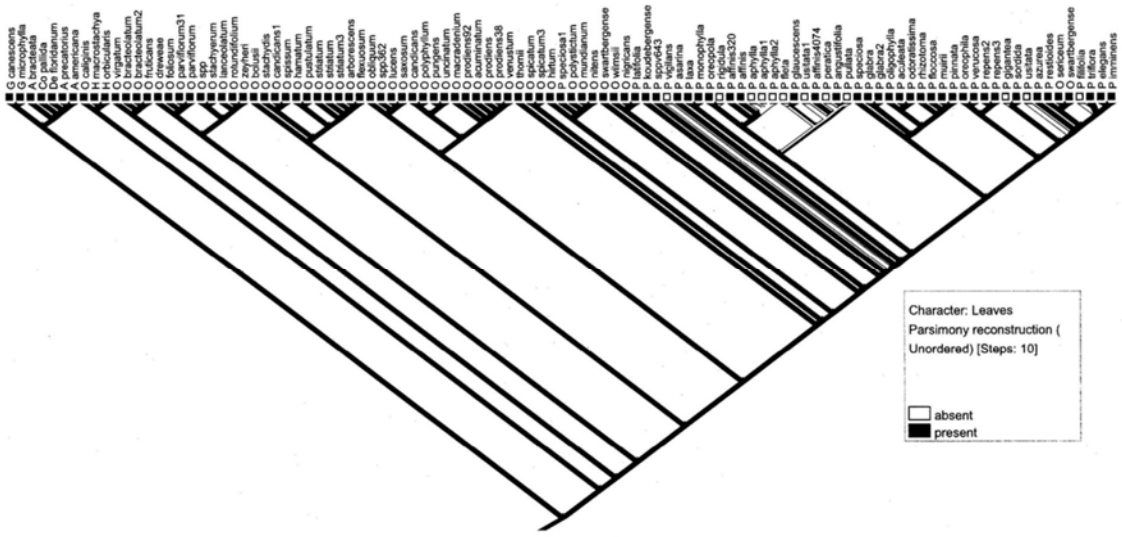

Figure 2.7 Ancestral state reconstructions for the presence of leaves.

In terms of leaf type, the possession of compound leaves is reconstructed as the ancestral state, while having a simple leaf is the derived state (Fig. 2.8). Note that in Fig. 2.5, the species that have simple leaves form a clade in both genera, suggesting that the loss of leaves may have occurred as a single event in each genus.

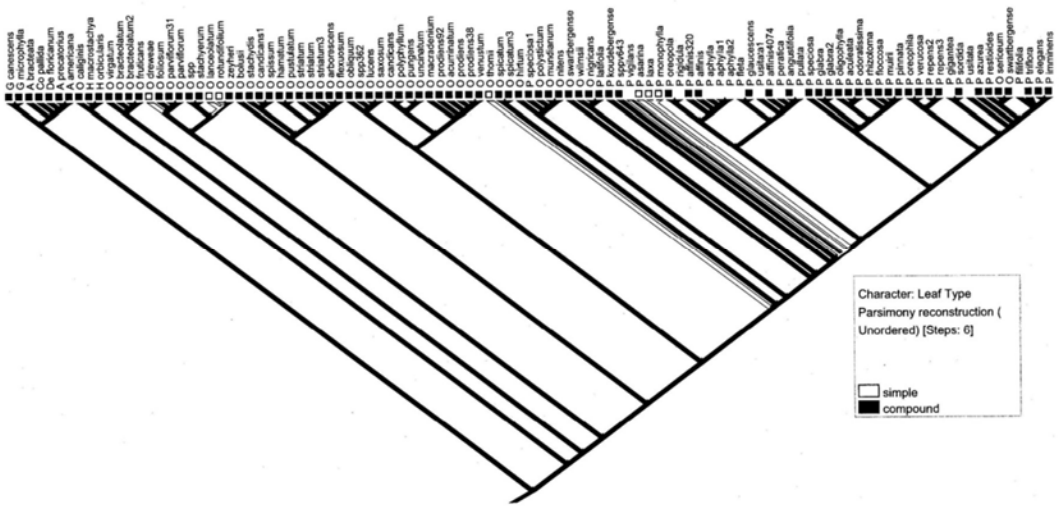

Figure 2.8. Ancestral state reconstructions for leaf type.

The occurrence of recurved mucronate leaf apices is reconstructed as the ancestral state for the southern African Psoraleeae clade, while the absence of these is a derived state (Fig. 2.9). The

occurrence of recurved mucronate leaf apices is reconstructed to have evolved multiple independent times in some species of *Otholobium*, but these are the species that form a clade in the tree from the DNA and morphology data set (Fig. 2.5). The reconstructions of the condition in which flowers are borne in triplets subtended by a single bract indicate this condition to be ancestral to the southern African clade and retained in *Otholobium*, but lost in *Psoralea* species (Fig. 2.10).

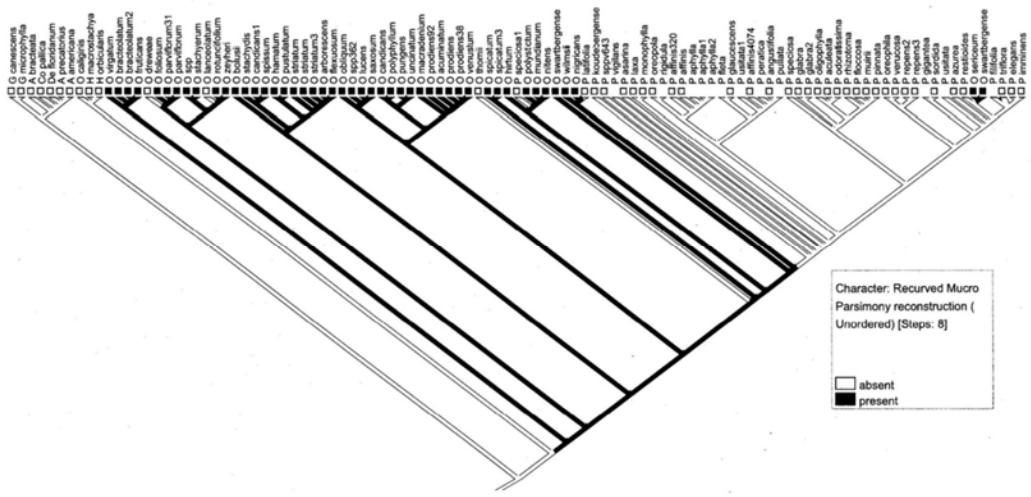

Figure 2.9. Ancestral state reconstructions for the presence of recurved mucro on the leaves.

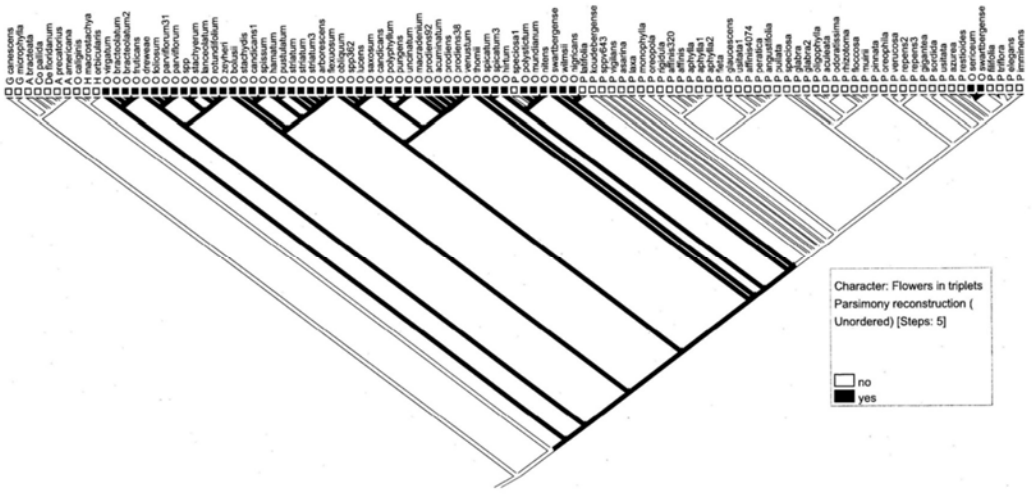

Figure 2.10. Ancestral state reconstructions for flowers borne in triplets.

2.2.5. Divergence dates estimates

The analysis of the combined nuclear and plastid data set yielded a maximum clade credibility tree similar to the phylogeny obtained from the parsimony and MrBayes analyses, suggesting a monophyletic tribe Psoraleeae (Fig. 2.11). Divergence date estimates of key nodes are shown in Table 2.7 along with the date estimates from the Egan & Crandall (2008b) study. The dates obtained from the present study are comparable to those of the Egan & Crandall (2008b) study. The mean age of the crown node of the Psoraleeae was estimated to be 15.84 million years (my), with a 95% confidence interval (CI) of 9.13-18.45 my (Fig. 2.11). According to the Egan & Crandall (2008b) study, it was estimated to be 14.90 my, with a 95 % confidence interval of 10.30-17.95 my (Table 2.7). The most recent common ancestor of *Orbexillum* was estimated to be 2.3 my, CI=1.04-4.89 my (Fig. 2.11), while Egan & Crandall (2008b) found it to be 3.45 my old (Table 2.7). The divergence dates for the southern African Psoraleeae, were determined for the first time in this study and the results showed that the age of the crown node of the southern African clade is 6.41 (CI=2.66-10.26) my old, and the most recent common ancestor of *Psoralea* is 2.44 (CI=1.30-4.59) my old (Fig. 2.11, Table 2.7).

Table 2.7 Divergence dates of key nodes estimated using BEAST. MRCA is most recent common ancestor. My is million years. The 95% upper and lower HPD is high posterior density credibility interval. E & C is divergence date estimate by Egan & Crandall (2008b).

MRCA of	Mean Age (my)	95% HPD Lower (my)	95% HPD Upper (my)	Mean Age Estimates [95 % HPD interval] by E & C (my)
Glycine & Psoraleeae	15.84	9.13	18.45	14.90 [10.39-17.95]
Pediomelum	3.31	1.76	4.92	3.28 [1.25-2.87]
Orbexillum	2.3	1.04	4.89	3.45 [1.72-4.06]
Rupertia and *Psoralidium*	3.62	0.46	4.42	4.09 [1.87-4.84]
Hoita	0.99	0.03	2.39	1.34 [0.15-1.21]
Cullen	1.7	0.47	4.27	1.50 [0.50-2.19]
Otholobium (southern African)	6.41	2.66	10.26	N/A
Psoralea	2.44	1.30	4.59	N/A
Bituminaria and the South American *Otholobium*	2.81	0.51	6.2	N/A

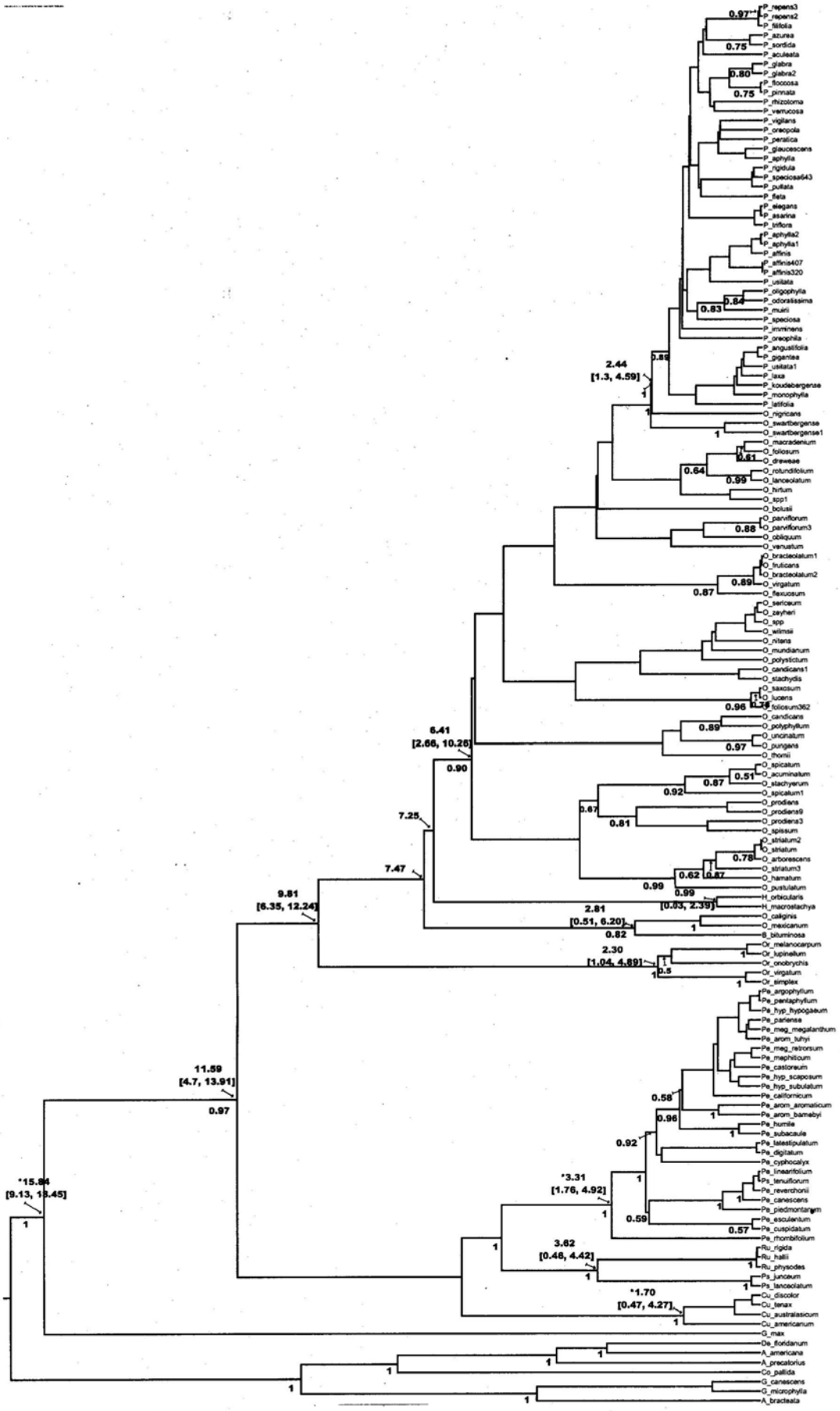
P_repens3
P_repens2
P_filifolia
P_azurea
P_sordida
P_aculeata
P_glabra
P_glabra2
P_floccosa
P_pinnata
P_rhizotoma
P_verrucosa
P_vigilans
P_oracopola
P_peratica
P_glaucescens
P_aphylla
P_rigidula
P_speciosa643
P_pullata
P_fleta
P_elegans
P_asarina
P_trifiora
P_aphylla2
P_aphylla1
P_affinis
P_affinis407
P_affinis320
P_usitata
P_oligophylla
P_odoratissima
P_muirii
P_speciosa
P_imminens
P_oreophila
P_angustifolia
P_gigantea
P_usitata1
P_laxa
P_koudebergense
P_monophylla
P_latifolia
O_nigricans
O_swartbergense
O_swartbergense1
O_macradenium
O_foliosum
O_drewiae
O_rotundifolium
O_lanceolatum
O_hirtum
O_spp1
O_bolusii
O_parviflorum
O_parviflorum3
O_obliquum
O_venustum
O_bracteolatum1
O_fruticans
O_bracteolatum2
O_virgatum
O_flexuosum
O_sericeum
O_zeyheri
O_spp
O_wilmsii
O_nitens
O_mundianum
O_polystictum
O_candicans1
O_stachydis
O_saxosum
O_lucens
O_foliosum362
O_candicans
O_polyphyllum
O_uncinatum
O_pungens
O_thomii
O_spicatum
O_acuminatum
O_stachyerum
O_spicatum1
O_prodiens
O_prodiens9
O_prodiens3
O_spissum
O_striatum2
O_striatum
O_arborescens
O_striatum3
O_hamatum
O_pustulatum
H_orbicularis
H_macrostachya
O_caliginis
O_mexicanum
B_biturninosa
Or_melanocarpum
Or_lupinellum
Or_onobrychis
Or_virgatum
Or_simplex
Pe_argophyllum
Pe_pentaphyllum
Pe_hyp_hypogaeum
Pe_parlensa
Pe_meg_megalanthum
Pe_arom_luhyi
Pe_meg_retrorsum
Pe_mephiticum
Pe_castoreum
Pe_hyp_scaposum
Pe_hyp_subulatum
Pe_californicum
Pe_arom_aromaticum
Pe_arom_bamebyi
Pe_humile
Pe_subacaule
Pe_latestipulatum
Pe_digitatum
Pe_cyphocalyx
Pe_linearifolium
Ps_tenuiflorum
Pe_reverchonii
Pe_canescens
Pe_piedmontanum
Pe_esculentum
Pe_cuspidatum
Pe_rhombifolium
Ru_rigida
Ru_hallii
Ru_physodes
Ps_junceum
Ps_lanceolatum
Cu_discolor
Cu_tenax
Cu_australasicum
Cu_americanum
G_max
De_floridanum
A_americana
A_precatorius
Co_pallida
G_canescens
G_microphylla
A_bracteata
2.44
[1.3, 4.59]
6.41
[2.66, 10.26]
7.25
7.47
9.81
[6.35, 12.24]
2.81
[0.51, 6.20]
2.30
[1.04, 4.89]
11.59
[4.7, 13.91]
*15.84
[9.13, 13.45]
*3.31
[1.76, 4.92]
3.62
[0.46, 4.42]
*1.70
[0.47, 4.27]
0.97
0.75
0.80
0.75
0.84
0.83
0.88
0.89
0.87
0.81
0.64
0.99
0.96
0.89
0.97
0.90
0.51
0.87
0.92
0.67
0.81
0.78
0.62
0.99
0.99
[0.03, 2.39]
0.82
0.5
0.58
0.96
0.92
0.59
0.57

Figure 2.11 Maximum clade credibility tree from the BEAST estimation of divergence dates for the Psoraleeae. Asterisks indicate nodes used as calibration points. The values on top of branches are average date estimates in millions of years, with the 95% high posterior density intervals in square brackets. Values below branches are posterior probabilities.

2.3. Discussion

2.3.1. Molecular markers

The low levels of sequence variation in the DNA loci sampled led to poor node support on trees and the collapse of several nodes (i.e. formation of polytomies) in the strict consensus trees (Fig. 2.3 and Fig. 2.5). This suggests that the radiation of the lineage (*Psoralea* and *Otholobium*) is of very recent origin, and therefore there has not been sufficient time for the accumulation of mutations that would lead to more variation in the sequences of the different species. Sequence variation was low even between the two genera, and hence the hypothesis of sister relationship (Grimes, 1990) was not supported by the present phylogeny. Therefore, more variable DNA loci, such as the *waxy* region that was used by Egan & Crandall (2008a) need to be explored. This marker was not used in this study because it requires cloning, which could not be done within the studyôs budget.

The amplification of DNA using *ETS* was unsuccessful despite that it had worked well in other legume studies [e.g. Chandler *et al*. (2001); Chandler *et al*. (2003); Choi *et al.* (2006)]. This could be due to primer mismatch given the high variability of *ETS*. Bena *et al*. (1998) also had difficulty sequencing the *ETS* region and according to Linder *et al.* (2000) this was because a conserved internal region could not be identified for priming a sequencing reaction throughout their taxa of interest. In this study the primers used were those used by Chandler *et al*. (2001) on the Australian genus *Gastrolobium*, and based on the explanation by Linder *et al.* (2000), perhaps specific primers would have to be designed for the Psoraleeae if *ETS* is to be used, but the success of that would still depend on whether a conserved region is found for the group.

Concerning the *ITS*, the amplification of multiple DNA fragments could be due to sample contamination or paralogous sequences (Feliner & Roselló, 2007). However, in this study, negative controls were used in all PCR experiments to ensure that contamination was detectable and blast searches were performed against other sequences on GenBank to confirm that sequences were indeed closely related to the group under study. Therefore, the multiple bands are more likely to have been due to paralogous sequences rather than DNA contamination. It is

generally known that nuclear ribosomal genes exist in several thousands of copies (Alvarez & Wendel, 2003). However, these copies evolve more or less in unison, i.e. instead of each copy acquiring a unique sequence variation due to the evolutionary accumulation of mutations, all repeat copies within an array may jointly share the same set of mutations because of concerted evolution (Elder & Turner, 1995; Alvarez & Wendel, 2003; Feliner & Roselló, 2007). Nevertheless, concerted evolution might not keep pace with variation generating processes, leading to the existence of multiple divergent copies (Alvarez & Wendel, 2003). Although this may explain the observation of multiple bands in some of the PCR products in this study, the observation that the sequences from these different bands were identical is a mystery. Attempts to find an explanation from literature were unsuccessful, perhaps because in theory the fact that the bands migrate at different rates in the gel indicates that they are of different molecular weight (Meyers *et al*., 1976) and thus the sequences should be different. Perhaps these paralogues differ in other structural features besides sequence length or nucleotide bases.

2.3.2. Monophyly of the tribe Psoraleeae and taxonomic implications

One of the objectives of the study was to revise the taxonomic rank of the Psoraleeae. The phylogeny of the present study, which includes all the genera of Psoraleeae and outgroups from different legume tribes shows that the Psoraleeae genera together form a well-supported clade (Fig. 2.3). Several molecular phylogenetic studies at tribal level have shown that the Psoraleeae are embedded within the tribe Phaseoleae as sister to the sub-tribe, Glycininae (Kajita *et al*., 2001; Wojciechowski *et al*., 2004; Stefanovic *et al*., 2009). Hence, the recognition of the Psoraleeae as a tribe rather than a sub-tribe has been questioned (Grimes, 1990; Schrire, 2005). However, since the majority of the Psoraleeae (about 60 %) had not been studied prior to this study, no taxonomic changes were made. Although the current study did not sample all the various tribes of the Phaseoleae, it is unlikely that doing so would change the monophyly of the Psoraleeae because the outgroups used here are from those tribes that are closely related to the Psoraleeae (based on Kajita *et al*., 2001; Wojciechowski *et al*., 2004 and Stefanovic *et al*., 2009). Therefore, I propose that the Psoraleeae should be recognised as a sub-tribe of the Phaseoleae. Since the Psoraleeae had once been recognised at sub-tribal level by Taubert (1894) under the name Psoraliinae, I propose that this name be revived. This lends support to the suggestion by Schrire (2005). Such a move will help resolve the polyphyly of the Phaseoleae as this leaves the Desmodieae as the only tribe embedded within the Phaseoleae, and a greater sampling within that tribe is needed to resolve its phylogenetic position and hence, its taxonomic status.

2.3.3. Generic relationships

The second objective of this study was to test whether the generic relationships proposed by Grimes (1990) as shown in Fig. 2.2, are supported by molecular data. Since Egan & Crandall (2008a) had already tested this for the North American Psoraleeae, the focus of this study was on the southern African genera. However, due to the poor support on the phylogeny obtained in this study, it is difficult to tell with great confidence whether the hypothesis of sister relationship between *Psoralea* and *Otholobium*, which was proposed by Grimes (1990) should be accepted or not. The current data only indicate that the two genera form a clade, and the genus *Otholobium* is polyphyletic (Fig. 2.3 and Fig. 2.5). The South American species are resolved as sister to the genus *Bituminaria* and the majority of *Psoralea* species are resolved as a clade within *Otholobium* (Fig. 2.3 and Fig. 2.5, respectively). Egan & Crandall (2008a) had proposed that *Otholobium* be broken into two by geography and the South American species be given a new name. Although the data from this study indicate that the South African *Otholobium* species do not form a clade with these species, I refrain from making any taxonomic changes in this regard until a more robust phylogeny including all the eight South American species is reconstructed. In addition, the phylogenetic position of the southern African clade relative to the rest of the Psoraleeae could not be established with the current data. The phylogeny indicated that its sister clade is the genus *Hoita*, but with no support (Fig. 2.3).

The inclusion of morphological data for the southern African Psoraleeae data slightly improved the resolution of the tree, but there were still several nodes collapsing in the strict consensus tree and support values were still low for most nodes (Fig. 2.5). The improved phylogenetic resolution after including morphological data is consistent with observations by several other authors such as Renner (1999), Huys *et al*. (2007) and Schrire *et al*. (2009). However, the genus *Psoralea* was still embedded within *Otholobium*, thus making the southern African *Otholobium* paraphyletic. The genus *Psoralea* has several unique characters. These include the presence of the cupulum, the absence of the triplet inflorescence arrangement that is characteristic of *Otholobium*, different floral structure, the lack of recurved mucronate leaves, and the presence of proanthocyanidines (Boardley *et al*., 1986). However, based on the studyôs findings that *Psoralea* is embedded within *Otholobium*, this implies that these characters can only be interpreted as synapomorphies (shared derived characters) of the clade that is presently recognised as *Psoralea* rather than characters that demarcate two different genera. By the same reasoning, the features that are unique to the *Otholobium* species can be interpreted as plesiomorphic (ancestral) features of the lineage.

In terms of classification, the present results would suggest that *Psoralea* should be subsumed into *Otholobium* in order for the genus to be monophyletic. This is because the Hennigian principle of monophyly, on which many classifications are based states that only monophyletic groups are natural and supraspecific taxa should refer only to such groups (Sosef, 1997). Nevertheless, there are some secondary principles that should guide a classification system in the case of limitations of the principle of monophyly. Although these principles do not necessarily permit the recognition of paraphyletic taxa, they indicate that in some cases paraphyly may be inevitable (Brummit, 1997; Brummit & Sosef, 1998). These principles are: maximising stability (reducing the danger that future research will reveal that those groups are not monophyletic); maximising ease of identification (there should be a suite of observable characters that define those groups); and maximising phylogenetic information, i.e. the classification should reflect the evolutionary relationships between groups (Sosef, 1997; Backlund & Bremer, 1998; Brummit, 2002; Brummit, 2003; Humphreys & Linder, 2009). However, since the present phylogeny is poorly resolved, it would not be appropriate to make taxonomic changes based on it. Therefore, the classification by Stirton (1981), which recognises *Otholobium* and *Psoralea* as distinct genera, is retained until more resolved phylogenetic relationships are available.

Grimes (1997) alluded that *Bituminaria* might be related to southern African *Otholobium*, but in this study, it was shown to be sister to the South American *Otholobium* (Fig. 2.3). However, this relationship is not strongly supported, and since the true phylogenetic position of the southern African clade could not be established with the current data, the validity of this hypothesis cannot be determined. There is no doubt that the genus *Bituminaria* needs a closer investigation to establish its monophyly and its phylogenetic position.

With the exception of the southern African clade, generic relationships between the rest of the Psoraleeae were similar to those found by Egan & Crandall (2008a). For example, the paraphyly of the genus *Psoralidium*, which was reported by Egan & Crandall (2008a) was found in the present study (Fig. 2.3). Egan & Crandall (2008a) proposed that *Psoralidium tenuiflorum* should be transferred to *Pediomelum* for the two genera to be monophyletic. They highlighted that the two species of *Psoralidium, Ps. lanceolatum* and *Ps. junceum* have deciduous bracts and a globose to subglobose fruit that is deciduous above the receptacle, while *Ps. tenuiflorum* has persistent bracts and an elliptical fruit that falls with the calyx, of which all three characters are more similar to *Pediomelum.* Nevertheless, taxonomic changes in this regard are beyond the scope of this study because of lack of thorough knowledge of the taxonomy of the North American Psoraleeae.

2.3.4. Ancestral state reconstructions

The goal of reconstructing ancestral states of the key diagnostic characters within the southern African Psoraleeae was to evaluate their taxonomic value in discriminating lineages, especially in separating the two genera (*Psoralea* and *Otholobium*). However, since the phylogeny on which these reconstructions were based is not well resolved, these reconstructions can only be viewed as indicators of what patterns are likely to be observed in a more robust phylogeny of the southern African Psoraleeae.

The main character that separates *Otholobium* from *Psoralea* is the presence of the cupulum in *Psoralea*. This is also the character upon which the sinking of *Hallia* into *Psoralea* (Salter 1939; Stirton, 1989; Crow *et al.*, 1997) was based. This is supported by the current results, as the reconstructions indicate that the cupulum has evolved only once in the Psoraleeae and thus a synapomorphy of the genus *Psoralea*. Another character that separates the two genera is the inflorescence structure of *Otholobium*, whereby flowers are borne in triplets, with each triplet subtended by a single bract and then each flower having a bract subtending it. The reconstruction shows that this is an plesiomorphic condition (Fig. 2.10) that has been retained exclusively by *Otholobium* and only a single state change has occurred i.e. this character has been lost in *Psoralea*. However, in light of the present phylogeny these two reconstructions do not support recognising *Otholobium* and *Psoralea* as distinct genera.

The third character that Stirton (1981) used to separate the two genera is the occurrence of recurved mucronate leaf apices. In the reconstruction (Fig. 2.9), this condition is ancestral, while the lack of the recurved mucro on leaf apices is the derived state. Although this character is unique to *Otholobium*, it is lacking in the species *O. dreweae, O. thomii, O. rotundifolium* and *O. lanceolatum*. Therefore, while it distinguishes some species of *Otholobium* from *Psoralea*, it is not a symplesiomorphy of the genus. Hence, if the character were to be used to demarcate genera, the four species of *Otholobium* that lack this character would have to be recognised as a different genus.

The loss of leaves, which is associated with the *Psoralea aphylla* complex, has arisen several times, independently (Fig. 2.7). However, given the lack of resolution in species level relationships, reconstructions for this character are not conclusive about its usefulness. In a more resolved phylogeny, the leafless members of *Psoralea* might form a clade, therefore making this condition a synapomorphy of the group. This group is studied further in Chapter 4 of this study.

In terms of leaf type, the results indicate that compound leaves are the ancestral state while simple leaves are the derived state (Fig. 2.8). This switch from compound to simple leaves has occurred once in either genera and in both cases, this character is associated with those species, which are suffrutices, multistemmed, and re-sprouting. In *Psoralea,* these are the species of the former genus *Hallia* (*P. asarina*, *P. laxa* and *P. monophylla*), and in *Otholobium,* these are the dwarf species that have capitate inflorescences held in long peduncles (i.e. *O. dreweae*, *O. thomii*, *O. rotundifolium* and *O. lanceolatum*). Therefore, leaf type may be a valuable diagnostic character within each genus but not between the two genera. However, due to the limitations of the current data, the hypothesis that *Hallia* species arose from *Psoralea* species that are shrubs or trees (by Crow *et al.*, 1997) could not be tested since the phylogenetic position of this clade may change with more data.

2.3.5. Divergence dates

The divergence date estimates obtained from this study indicate that the southern African Psoraleeae originated between 2.66 and 10.26 mya, with a mean estimate of 6.41 million years ago (Table 2.7). Such recent dates, combined with: (i) the large number of species in the lineage (about 103 species), (ii) the low sequence variation observed for all the DNA regions studied and (iii) the diverse morphology associated with the lineage, suggest that the southern African Psoraleeae are a product of recent rapid radiation. This is also true for the members of the tribe that occur outside southern Africa, as indicated by both the results of this study and those of Egan & Crandall (2008b). Although the present phylogeny is not fully resolved, the dates obtained were similar to those of Egan & Crandall (2008b), which was a resolved phylogeny. This suggests that if their dates are accurate, then the effect of the poor resolution in the current study was minimal. However, a more resolved phylogeny is still required in order to be more certain about the accuracy of the dates.

Divergence dates for other Cape legumes are very variable. For instance, the Crotalarieae are estimated to have radiated about 46.3 mya, while the Podalyrieae are estimated to have initiated radiation about 44.6 mya (Edwards & Hawkins, 2006). On the other hand, the Indigofereae are estimated to have initiated radiation from about 20 mya (Schrire *et al.*, 2009). These dates indicate that the Psoraleeae are younger than these other legumes and to have started radiating 6.41 mya suggests that the rapid radiation might have been triggered by the climate change in the late Miocene and speciation might have been driven by the edaphic heterogeneity associated with the CFR as postulated by Verboom *et al*. (2009). Distribution patterns of the Psoraleeae in relation to edaphic factors are explored in Chapter 3 of this dissertation.

2.4. Conclusions

This study has indicated that the Psoraleeae form a monophyletic entity and since several studies have established that the Psoraleeae are sister to the sub-tribe Glycininae of the Phaseoleae, the idea of recognising the Psoraleeae as a sub-tribe of the Phaseoleae (Schrire, 2005) is supported. However, the phylogenetic position of the southern African clade relative to the rest of the Psoraleeae has not been resolved. Furthermore, the current data do not support the hypothesis of sister relationship between *Otholobium* and *Psoralea* as proposed by Grimes (1990) because *Psoralea* is embedded within *Otholobium*. This suggests that the unique characters of the genus *Psoralea* that Stirton (1981) used to separate the two genera are only synapomorphies of the *Psoralea* clade. Nevertheless, due to the poor resolution of the phylogeny, further molecular data might support the hypothesis of sister relationship between the genera. Therefore, the classification of these two genera is better left unchanged for now.

Estimation of divergence dates for the southern African Psoraleeae indicated that they radiated after the onset of the Mediterranean type of climate in the late Miocene and have experienced massive rapid radiation within the last six million years, with the genus *Psoralea* emerging less than three million years ago. More work is required to obtain a more robust phylogeny and hence more accurate date estimates. These would allow for the determination of the place of origin of the whole tribe and to test biogeographical hypotheses in order to explain the worldwide distribution of the Psoraleeae.

CHAPTER 3

DISTRIBUTION PATTERNS IN THE SOUTHERN AFRICAN PSORALEEAE: EXPLORING THE ROLE OF EDAPHIC HETEROGENEITY

3.0. Introduction

3.0.1. General distribution patterns of *Otholobium* and *Psoralea*

About 96% of the species in *Otholobium* and *Psoralea* occur within southern Africa across a wide range of habitats. For *Otholobium*, the most common habitats are arid fynbos, renosterbos and eastern mountain grasslands (Stirton, 1989). Its centre of species richness is in the Cape Floristic Region (CFR), but a few species extend the range up to KwaZulu-Natal and Mpumalanga (Fig. 3.1). Several species of *Otholobium* are narrow endemics, occupying a few restricted localities or a group of isolated mountain tops (Stirton, 1989). Examples are *O. swartbergense* (Swartberg Mountain range); *O. accrescens* (Great Winterhoek Mountains); *O. bowieanum* (Langeberg Mountain range) and *O. lanceolatum*, which is endemic to Shawôs Pass in Caledon (Stirton & Schutte, 2000).

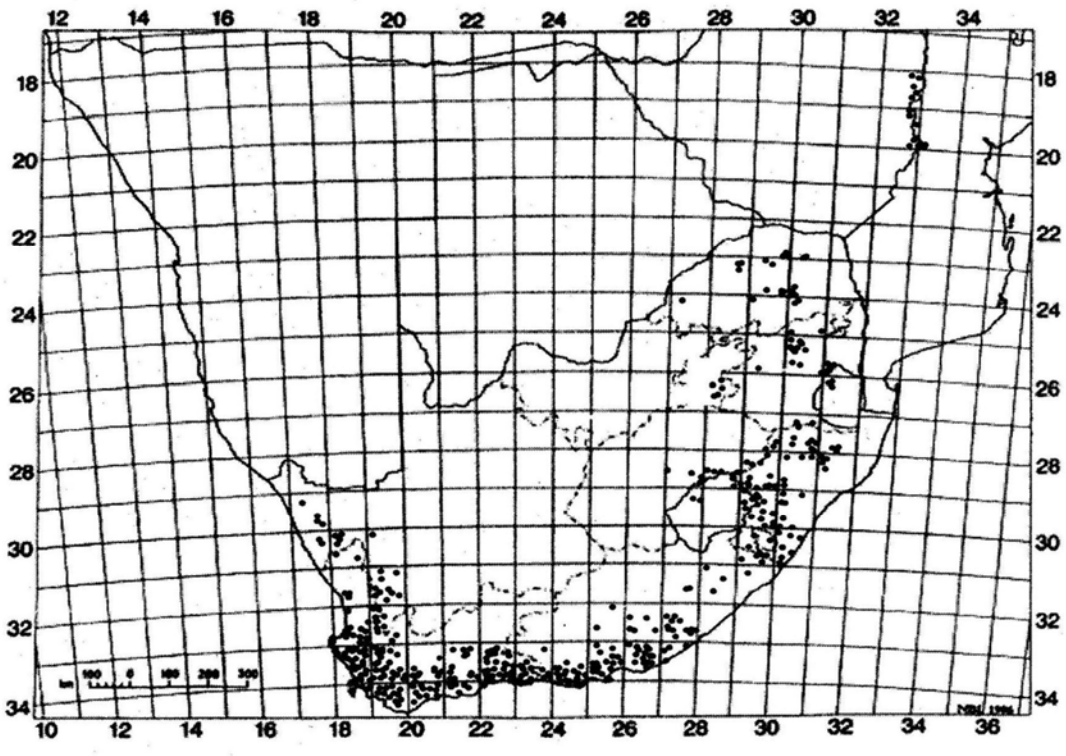

Figure 3.1. Distribution of *Otholobium* in southern Africa based on herbarium specimen data and collections made as part of the present study.

There are also some species that are of widespread distribution across various habitat types. These include *O. virgatum*, *O. bracteolatum* and *O. candicans. Otholobium virgatum* occurs in various habitats except the Karoo Mountain Centre and exhibits clinal variation (e.g. in the degree of hairiness and leaf shape) across its distribution range. *Otholobium candicans* also exhibits a number of clines of pubescence, leaf size, glandulosity, flower colour and growth form across its distribution range. Similarly, *Otholobium bracteolatum* is widely distributed throughout the Western Cape, extending to as far east as Port Elizabeth, growing on standveld at altitudes ranging from three to 400 m (Stirton, 1989) and exhibits great variation in growth form, leaf size and flower colour.

The genus *Psoralea*, like *Otholobium* has its centre of species richness in the CFR, with some species occurring along the east coast up to as far north as the Mpumalanga Province (Fig. 3.2). However, *Psoralea* species tend to occur predominantly on sandstone fynbos along stream banks, forest margins or rocky seepages at high altitudes.

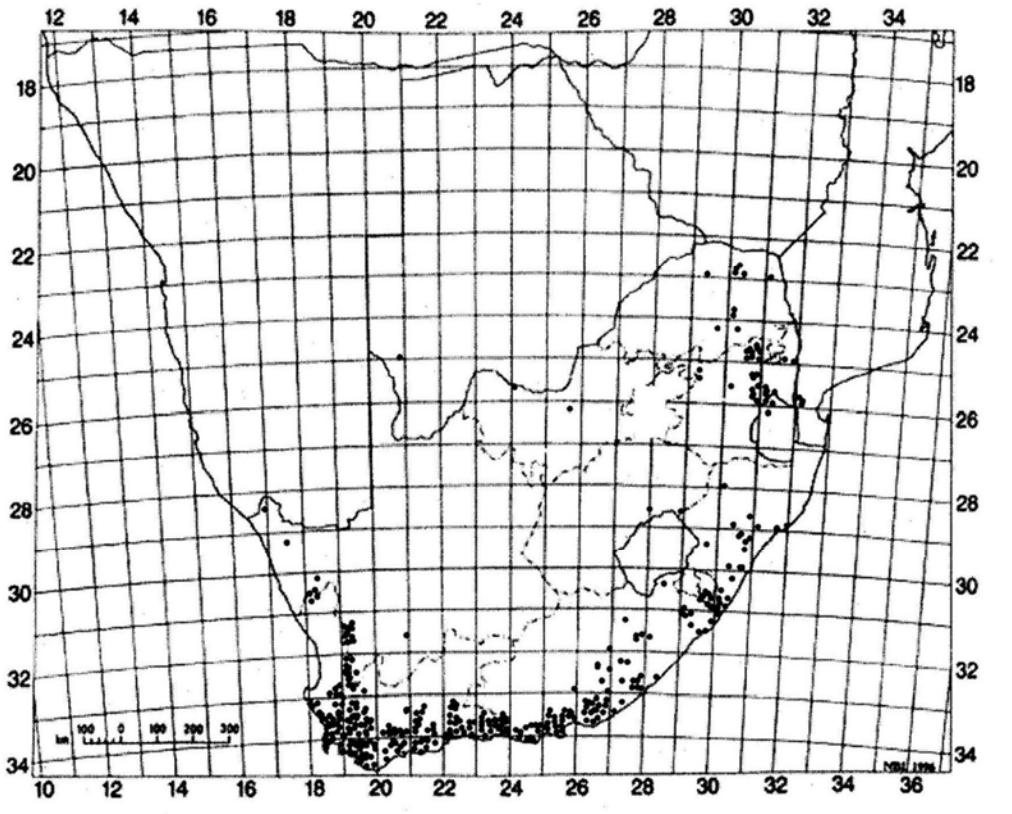

Figure 3.2. Distribution of *Psoralea* in southern Africa based on herbarium specimen data and collections made as part of the present study.

It also consists of species that exhibit widespread distribution as well as those with narrow distribution ranges (Stirton & Schutte, 2000). For example, *P. pinnata* (in the Linnean sense) is the most widespread species in the genus. Its distribution ranges from the Cape to KwaZulu-Natal and Swaziland. It also exhibits clinal variation across its distribution range so much that several taxa have been proposed under this species. A detailed treatment of the taxonomic issues around *P. pinnata* is presented in Chapter 4 of this dissertation. Other widespread species include *P. laxa*, which occurs from the Cape Peninsula to Albertinia; *P. repens*, which extends from the Cape Peninsula to the Eastern Cape and *P. alata*, occurring from the Cape Peninsula to Clanwilliam (Stirton & Schutte, 2000). Among the species with narrow distribution ranges are *P. rigidula* (Bainskloof Mountain range), *P. aphylla* (Cape Peninsula, mainly Table Mountain), *P. peratica* (Piketberg Mountain range) *and P. implexa,* which is restricted to Du Toitôs Kloof (Stirton & Schutte, 2000).

3.0.2. Factors driving diversification in CFR lineages

Several factors that play a role in driving diversification were discussed in Chapter 1 (section 1.5) and elsewhere (e.g. Linder, 2003; van der Niet & Johnson, 2009; Verboom *et al*., 2009). These factors include pollinator specialization, edaphic heterogeneity, climate, fire, polyploidy and hybridization. However, little work has been done in terms of testing these processes in the CFR lineages. The most recent work that tackled this question was that of van der Niet & Johnson (2009), which showed that most diversification in Cape plant lineages was associated with floral features, suggesting that there is strong selection for pollinator specializations. However, they also reported that shifts in general habitat were frequent among Cape lineages. This supports the view of Verboom *et al*. (2009) who indicated that speciation in the CFR, particularly the lowland fynbos, may be driven by edaphic heterogeneity, landscape evolution and microclimates.

3.0.2.1. Edaphic heterogeneity as a driver of diversification in the CFR

Ellis *et al*. (2006) did a study of adaptive radiation in the genus *Argyroderma* (Aizoaceae) and found that spatially isolated populations diverge phenotypically in response to divergent habitat selection, which in turn leads to the evolution of reproductive isolation through divergence of flowering times, perhaps as a correlated response to morphological divergence. A similar pattern was reported by Goldblatt (1979) for the genus *Galaxia* (Iridaceae), which exhibited species pairs separating on soil type (clay versus sand), which differ in pH (neutral vs. acidic), nutrient status (fertile vs. infertile) and particle size (fine vs. coarse). Goldblatt (1979) also observed hybrids in the genus *Freesia* (Iridaceae) occurring on the interface between the different soil

types. According to Linder (2003), this illustrates the absence of a pre- or post-zygotic isolating mechanism, thus reinforcing the interpretation that adaptation for the different soil types is the primary differentiating factor between the species. In another study by Verboom *et al.* (2004), in which the relative growth rates of resprouters were compared to those of reseeders in the genus *Ehrharta* (Poaceae), species growth rates were found to be correlated with their native substrates, indicating that edaphic heterogeneity has been central in directing the evolution of alternative persistence strategies and growth forms.

The edaphic heterogeneity of the CFR was comprehensively described by Goldblatt & Manning (2002) as follows:

> The Cape region consists of a mosaic of sandstone and shale substrata with local areas of limestone. It has a highly dissected, rugged topography and a diversity of climates with rainfall mostly falling in the winter months and varying from 2000 mm locally to less than 100 mm. Ecological gradients are steep because of abrupt differences in soil, altitude, aspect, and precipitation. These factors combine to form an unusually large number of local habitats for plants. Sandstone-derived soils have characteristically low nutrient status and many plants present on such soils have low seed dispersal capabilities, a factor promoting localized distribution.

The heterogeneity of substrates in the CFR and the variation in the vegetation types associated with these different substrates is comprehensively documented in Mucina & Rutherford (2006). Although there has not been a direct link between soil nutrient levels and speciation, some studies have shown that the distribution of some species in the CFR is related to chemical and physical characteristics in the soil types. For example, Richards *et al.* (1995) studied soils from 75 sites in a 30 hectare plot in the Soetanysberg hills, near the Cape Agulhas in the south western part of the Western Cape. They identified five communities associated with distinct soil types and two main compositional gradients. These gradients were associated with soil pH, soil depth and soil texture. In another study by Richards *et al*. (1997a) a comparison of the impact of competition and soil factors in determining the distribution of six fynbos Proteaceae species gave no evidence of a species competitively excluding another from its range. Instead, soil factors were found to play an important role in the species distributions.

Further evidence for the role of nutrients in shaping vegetation and species distributions in the CFR was provided by Richards *et al*. (1997b), who showed that soil nutrient content (total nitrogen, total phosphorus, organic carbon and various cations, including calcium, magnesium,

sodium and potassium) was significantly different among 18 sites of different soil and vegetation type in the Soetanysberg. Hence, they concluded that spatial variation in soil nutrient availability might be important in explaining landscape level species distribution and community composition of nutrient-poor Mediterranean-climate ecosystems.

In a study by Shane *et al*. (2008), the influence of phosphorus availability on plant uptake and growth was investigated using three species of the Proteaceae. The species were *Protea compacta* R.Br, which is endemic to the severely nutrient poor colluvial sands; *P. obtusifolia* Bueck ex. Meissner and *Leucadendron meridianum* I.J. Williams, which are both endemic to comparatively fertile limestone derived soils (Shane *et al*., 2008). They found that *P. compacta* was unable to down-regulate P uptake when supplied with high levels (5mM) of P, leading to toxic levels in the tissue and adversely affecting plant growth. This trait was associated with the limited distribution of *P. compacta* within the nutrient poor colluvial sands.

Some plant lineages in the CFR (e.g. Proteaceae, Fabaceae, and Cyperaceae) have evolved different nutrient acquisition strategies as a response to poor soil nutrition. For example, some plants produce cluster roots, which enhance plant P uptake from poorly available sources through the production of exudates that solubilize P bound to metal ions via ligand exchange, thus making it more available to the plants (Hawkins *et al*., 2005). Other strategies include mycorrhizal symbiotic relationships (e.g. in some Restionaceae and some Cyperaceae) and carnivory (Hawkins *et al*., 2005).

The evidence provided in the above mentioned studies together with studies from other Mediterranean regions [e.g. the Mediterranean coastline in western Galilee, Israel (Henkin *et al*., 2006) and South Western Australia (Foulds, 1993)] suggests that edaphic conditions may have an important role in driving lineage diversification. Therefore, studies of diversification processes need to include edaphic factors. The *Psoralea-Otholobium* clade is one of the 33 Cape floral clades as defined by Linder (2003) i.e. more than 50 % of the species are in the CFR and its basal elements are in the CFR. Species of both genera predominantly occur in the fynbos biome, which is generally associated with low nutrient soils and winter rainfall (Cowling *et al*., 1996). However, some species of the genus *Psoralea* occur in seepage areas of the succulent Karoo, while the *Otholobium* species occurring in this biome predominantly occur in the more arid habitats (Stirton, 1989). In general, these habitats are nutrient rich as compared to the fynbos (Cowling *et al*., 1996). However, the extent to which the distribution of the Psoraleeae is linked to edaphic factors is unknown.

3.0.2.2. Post-fire regenerative strategies

Post-fire regenerative mechanisms are a widespread phenomenon in fire and drought prone environments such as Mediterranean ecosystems (Paula & Pausas, 2006; Saura-Mas & Lloret, 2007). These include reseeding (through hard coated seeds or serotiny) and resprouting from stumps, lignotubers, rhizomes or adventitious buds (Lloret, 1999; Pausas & Verdú, 2005). Resprouters and reseeders tend to coexist in fire prone ecosystems, but they differ in their physiological and anatomical attributes. Generally, resprouters have slower growth rates, lower shoot: root dry weight ratios, and they develop larger areas of their root tissue for starch storage than do reseeder species (Pate *et al*., 1990; Bowen & Pate, 1993). In addition, resprouters tend to have deeper roots, which enable them to reach deeper water sources during dry months and therefore are able to resist drought (Clemente *et al*., 2005). However, some authors have shown that reseeders tend to outnumber resprouters on drier sites in most Mediterranean ecosystems (Ojeda, 1998; Pausas, 1999; Clarke & Knox, 2002). This indicates that they must possess other physiological, chemical or structural features to counteract their lower allocation to roots and hence confer drought resistance. Indeed, Paula & Pausas (2006) showed that reseeders are able to tolerate drought through their leaves, by having high water use efficiency and high leaf mass area ratio (LMA), which gives them high structural resistance to low leaf water content.

Since plants exhibiting either strategy are able to cope with water shortage, then the distribution of plants of either strategy must be influenced by a different factor. A study of post-fire regenerative strategies in the genus *Ehrharta* by Verboom *et al*. (2004) found that reseeders were associated with high growth rates and invested more resources to seed production, while resprouters were slow growers. Several other authors have reported a similar pattern, and have observed that nutrient rich environments are more likely to favour reseeders, while resprouters survive on nutrient poor environments (Midgely, 1996; Ojeda, 1998; Linder, 2003). Both resprouting and reseeding species have been reported in the southern African Psoraleeae (Stirton, 1989; Stirton & Schutte, 2000). However, there has been no investigation of the relationship between post-fire regenerative strategies and their distribution in the Psoraleeae.

3.0.3. Scope of this study

As pointed out earlier, the CFR is associated with highly heterogeneous edaphic conditions, (different soil types with different levels of fertility) and differences in rainfall availability and seasonality. These physical parameters create a large number of distinct niches, often in close proximity to each other (van der Niet & Johnson, 2009), thus providing a framework for the ecological divergence of species, and the consequent coexistence of numerous species (Linder,

1985). It has also been observed that closely related species often occur on different soils (Rourke, 1972; Goldblatt, 1982; Kurzweil *et al*., 1991) and closely related species occurring on different soil types are differentially adapted (Verboom *et al*., 2004). The initial aim of this study was to use phylogenetic relationships within *Otholobium* and *Psoralea* to investigate the role of edaphic conditions in driving diversification in the lineage. However, although the present phylogeny includes more than 70 % of the total species in both *Psoralea* and *Otholobium*, it is not sufficiently resolved at the species level to unambiguously reflect sister species relationships (Chapter 2), and therefore does not allow for the inference of speciation processes using sister species comparisons. Therefore, this study focuses on investigating whether there are any correlations between edaphic factors and species distributions in the Psoraleeae and tests the hypothesis that soils occupied by resprouters are nutrient poor than those of reseeders.

3.0.4. Objectives

The specific objectives of this chapter were:

1. to determine the kind of substrates (soil types) occupied by *Psoralea* and *Otholobium* species;
2. to test whether there is a nutritional difference between soils occupied by reseeders and those occupied by resprouters in the Psoraleeae; and
3. to investigate whether closely related species occupy soils with similar nutritional levels.

3.1. Materials and methods

3.1.1. Determination of soil types and post-fire regenarative strategy

The types of soils occupied by the various species of the two genera were determined using the Geological Survey maps of South Africa (1990) by plotting the GPS coordinates recorded during specimen collection as part of this study and grid references from herbarium sheets. Other sources of information about the locality of species were Stirton (1989), Stirton & Schutte (2000) and the PRECIS database of the South African National Biodiversity Institute (SANBI). Each species was scored for the soil types in which it occurs. To determine the proportions of species across the various soil types, the total number of species in each soil type was divided by the sum total of species in all the soil types. These values were plotted in separate pie charts for the two genera.

Species were also grouped based on their post-fire regeneration strategy. The determination of regeneration strategy was based on field observations, herbarium specimen information and

literature sources. This was recorded for all the species of both genera as documented in Stirton (1989), Stirton & Schutte (2000), and the PRECIS database.

3.1.2. Soil sampling and nutrient analysis

Soil samples were collected from sites occupied by representative species based on morphological types (i.e. suffrutices, small shrubs, and large shrubs) and regeneration strategy (i.e. reseeders and resprouters). Each species was sampled from a single site, except for *Psoralea aphylla* and *P. affinis* that were each sampled from two different sites. Wherever a soil sample was collected, a voucher specimen was also collected and these were deposited in the Bolus herbarium. From each site, two to four soil samples were taken. The soil was sampled using a soil corer or a trowel, taking a uniform slice of soil at a depth of 10-15 cm below ground level. The samples were placed into plastic bags and labelled according to the voucher specimen number, and adding letters of the alphabet for replicates. These were placed into cardboard boxes for transport to the laboratory.

In the laboratory, the samples were air-dried, cleaned off any plant residue and stones, and passed through a 2 mm sieve. The sieved soils were placed into 50 mm ziplock bags, labelled appropriately and sent to BemLab Private Laboratory, Somerset, where they were analysed for pH, concentration of phosphorus (P Bray II), nitrate (NO_3-N), ammonium (NH_4-N), potassium (K), carbon, sodium, potassium, calcium, and magnesium, exchangeable sodium, exchangeable potassium, exchangeable calcium, exchangeable magnesium and the T-value.

3.1.3. Data analysis

For the soil nutrient analysis, the data were normalised by the use of log transformation. Basic statistics (means and standard errors) and one-way ANOVA were performed in Statistica 8 (Statsoft, Tulsa, Oklahoma, USA) to test for differences in nutrient concentrations between sites. Tukeyôs pair-wise multiple comparison tests were used to determine which sites differed significantly with respect to each of the soil characteristics measured. Sites were then grouped according to soil type and differences in soil nutrient levels between the soil types were tested using nested ANOVA, in which sites were nested within the soil types. Similarly, species were grouped according to regeneration strategies and differences in nutrient levels between regeneration strategies were tested using nested ANOVA. In addition, Principal Components Analysis (PCA) was used to determine whether there was any separation in multivariate space between the soils occupied by reseeders and those occupied by resprouters, and to identify the elements influencing such separation if there is. To determine whether closely related species

occur on similar soils, the soil data for some species that form clades in the strict consensus tree (Fig. 2.5, Chapter 2) were also analysed using one way ANOVA and PCA.

3.2. Results

3.2.1 (a) Proportions of species on the various soil types

The distributions of the species in the two genera encompass several different soil types and in varying proportions. For the genus *Psoralea*, a majority (66 %) of the species occupy habitats with sandstone-derived soils. The next highest proportion (16 %) of the species occupies granitic soils, 8 % of the species occur on shale, 5 % occur on limestone soils and 5 % occurs on sandy soils, with 4 % on acidic sands and 1 % on coastal sands (Fig. 3.3). For the genus *Otholobium*, a majority of the species are almost equally distributed on shale, sandstone and granitic habitats (28 %, 27 % and 26 %, respectively). The remainder of the species occupy limestone (8 %), acidic sand (8 %), and coastal sand (3 %) habitats (Fig. 3.4).

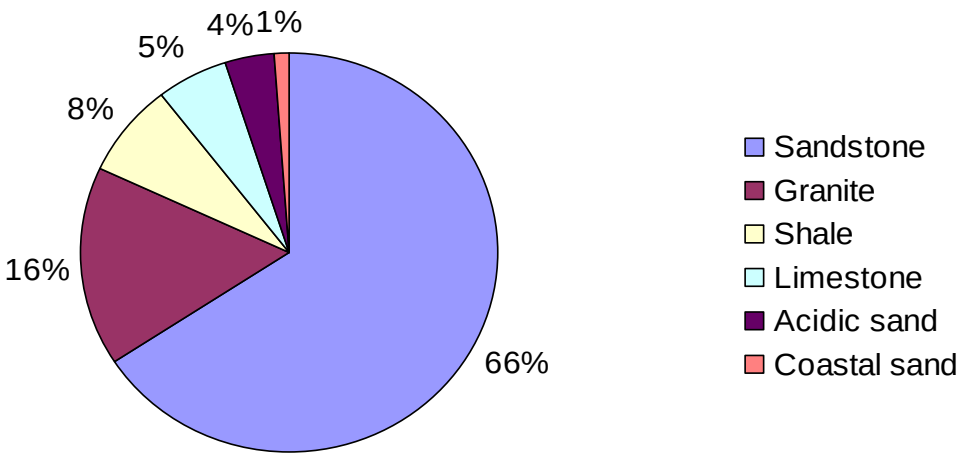

Figure 3.3 Proportions of *Psoralea* species across the different soil types in southern Africa.

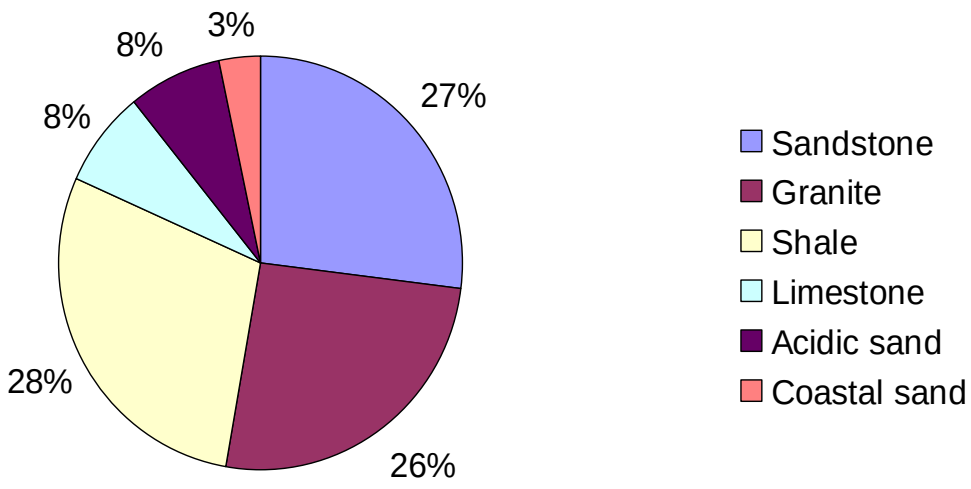

Figure 3.4 Proportions of *Otholobium* species across the different soil types in southern Africa.

3.2.1 (b) Proportions of reseeders and resprouters

Out of the 53 species of *Otholobium*, 23 species were identified as resprouters and the remaining 30 species were reseeders, while in *Psoralea*, out of the 56 total species, 25 of them were resprouters and 31 were reseeders (Fig. 3.5). A majority of the resprouters were species that are endemic to sandstone-derived soils for both genera. In contrast, reseeders had wide-ranging distributions across the various soil types, but for *Psoralea*, 62 % of the reseeding species were sandstone endemics while only 33 % of *Otholobium* reseeders were sandstone endemics.

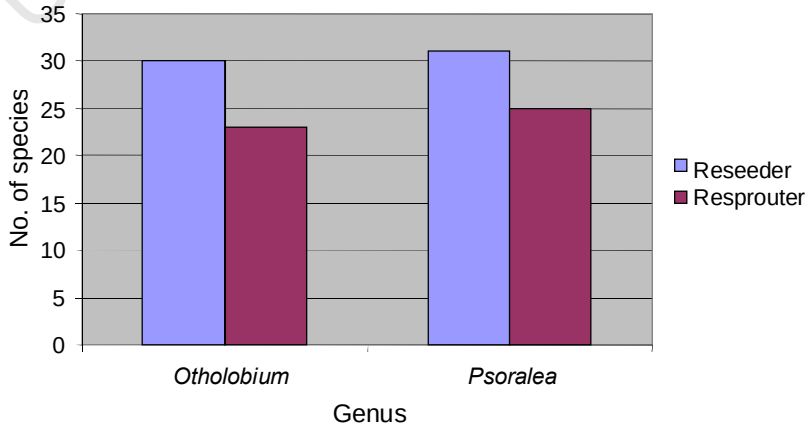

Figure 3.5 Numbers of reseeders and resprouter species in *Otholobium* and *Psoralea*

3.2.2. Soil nutrients in different sites

The soil nutrient data set included 29 sites, representing 29 species. Sites for which the soil samples were not replicated were excluded from all analyses. Of the 29 sites, thirteen sites were occupied by *Otholobium* species and the remaining sixteen sites were occupied by *Psoralea* species. The Mean±SE of the soil characteristics measured for the various sites are shown in Appendix 2.1. Univariate ANOVA for the soil characteristics measured indicated that all variables were significantly different ($p<0.05$) between sites (hence species) and the post hoc results showing which sites are different for the various soil characteristics are shown in Appendix 2.1.

3.2.2. (a) Soil nutrients in the different soil types

The nested ANOVA showed that from the sixteen soil characteristics analysed, six of them were significantly different ($p<0.05$) between soil types. These were pH, the concentration of potassium (K), the percentage potassium (% K), percentage calcium (% Ca), percentage magnesium (% Mg) and exchangeable potassium (Ex K) as shown in Table 3.1. Sandstones had the lowest pH (4.08), while the limestone and coastal sand habitats had the highest (6.85 and 8.58 respectively) pH. Potassium concentrations were highest in the shale (133.16 mg/kg) and granite (199.25 mg/kg) substrates, of which the latter was also associated with high % K and Ex K (Table 3.1). The lowest % Ca was from sandstones (37.15 %), while the highest percentages were recorded from limestone (90.49 %) and the coastal sand (91.83 %) habitats (Table 3.1). The percentage magesium was lowest on sandstone (20.05 %) and higher on the granite (24.06 %) and shale (29.71 %) substrates (Table 3.1).

3.2.2. (b) Principal components analysis of soil nutrients for reseeders and resprouters

When the soils data were analysed using principal components analysis (PCA), this resulted in sixteen principal components, of which 83 % of the total variance was explained by the first four components. These components and their corresponding eigenvectors for the different soil characteristics are shown in Table 3.2. The first principal component (PC1) accounted for 39.94 % of the total variance, and within this PC, the NH_4:NO_3 ratio had the highest contribution, followed by the NO_3-N concentration (Table 3.2). The second PC, which explained 17.00 % of the total variance, was largely influenced by the potassium concentration (K). In the third principal component (PC3), the variables that had the highest contribution in decreasing order were the NH_4-N concentration, the NO_3-N concentration and the percentage calcium (% Ca). Finally, the phosphorus concentration (P Bray II) was the soil characteristic contributing the most to variation in the fourth principal component (Table 3.2).

Table 3.1 Mean±SE and nested ANOVA results for the nutrient concentrations of the different soil types. Only the soil characteristics that were significantly different between soil types are shown. Values in brackets are the number of sites for the soil type. Different letters above the values indicate significant differences at $p<0.05$.

Soil parameter	Sandstone (14)	Limestone (1)	Shale (4)	Granite (9)	Coastal Sand (1)	p
	c	b	a	a	b	
pH KCl	4.08±0.09	6.85±0.33	5.06±0.16	4.53±0.11	8.58±0.33	<0.001
	a	a	b	b	a	
K mg/kg	79.71±10.49	34.67±37.47	133.16±18.74	199.25±12.49	32.00±37.47	<0.001
	a	ab	a	c	b	
%K	3.74±0.31	2.14±1.10	3.52±0.55	5.91±0.37	0.78±1.10	<0.001
	a	bc	ab	a	c	
%Ca	37.15±2.17	90.49±7.75	46.81±3.88	41.30±2.58	91.83±7.75	<0.01
	a	a	a	b	a	
Ex K mg/kg	0.20±0.03	0.09±0.10	0.32±0.05	0.51±0.03	0.08±0.10	<0.01

A scatterplot of the first two principal components, showing the ordination of reseeders and resprouters is shown in Fig. 3.6. The ordination indicates some separation of reseeders from resprouters, with a majority of the reseeders clustered separately from the resprouters on the positive side of principal component 2. However, some of the reseeders are nested within the resprouters (Fig. 3.6).

The nested ANOVA showed that indeed the concentrations of NO_3-N, P Bray II, Ex Ca, and the NH_4:NO_3 ratio were significantly different ($p<0.05$) between the soils occupied by species of the two strategies. For all these soil characteristics except for the ratio of NH_4:NO_3, the reseeders were associated with significantly ($p<0.05$) higher mean values compared to the resprouters (Table 3.3). The NH_4:NO_3 ratio was higher for resprouters (1.58) than that of the reseeders (0.76), suggesting that resprouter sites contain higher levels of NH_4 than the reseeder sites (Table 3.3).

Table 3.2 Eigenvectors for the first four components from the PCA of the soil nutrient data set. Eigenvectors that are in bold print are those corresponding to the soil parameters that contribute the most to the variance in the respective principal component (PC). The percentage contribution to total variance by each PC is indicated in brackets.

Soil parameter	PC1 (39.94 %)	PC2 (17.00 %)	PC3 (14.54 %)	PC4 (11.60 %)
pH KCl	0.037	0.000	-0.124	0.078
NO_3-N	**0.568**	-0.074	**0.440**	0.056
NH_4-N	0.015	0.033	**0.584**	0.202
P Bray II	0.263	0.014	0.127	**0.624**
K	0.277	**0.588**	-0.014	-0.186
%C	0.021	0.246	0.046	0.082
%Na	-0.029	0.107	0.067	-0.286
%K	0.099	0.136	0.061	-0.282
%Ca	0.069	0.068	**-0.422**	0.227
%Mg	0.061	0.250	0.106	-0.317
Ex Na	0.022	0.099	-0.002	-0.039
Ex K	0.060	0.107	-0.006	-0.032
Ex Ca	0.133	0.250	-0.363	0.314
Ex Mg	0.124	0.354	-0.009	-0.074
T-Value	0.120	0.322	-0.111	0.170
NH_4:NO_3	**-0.677**	**0.420**	0.300	0.263

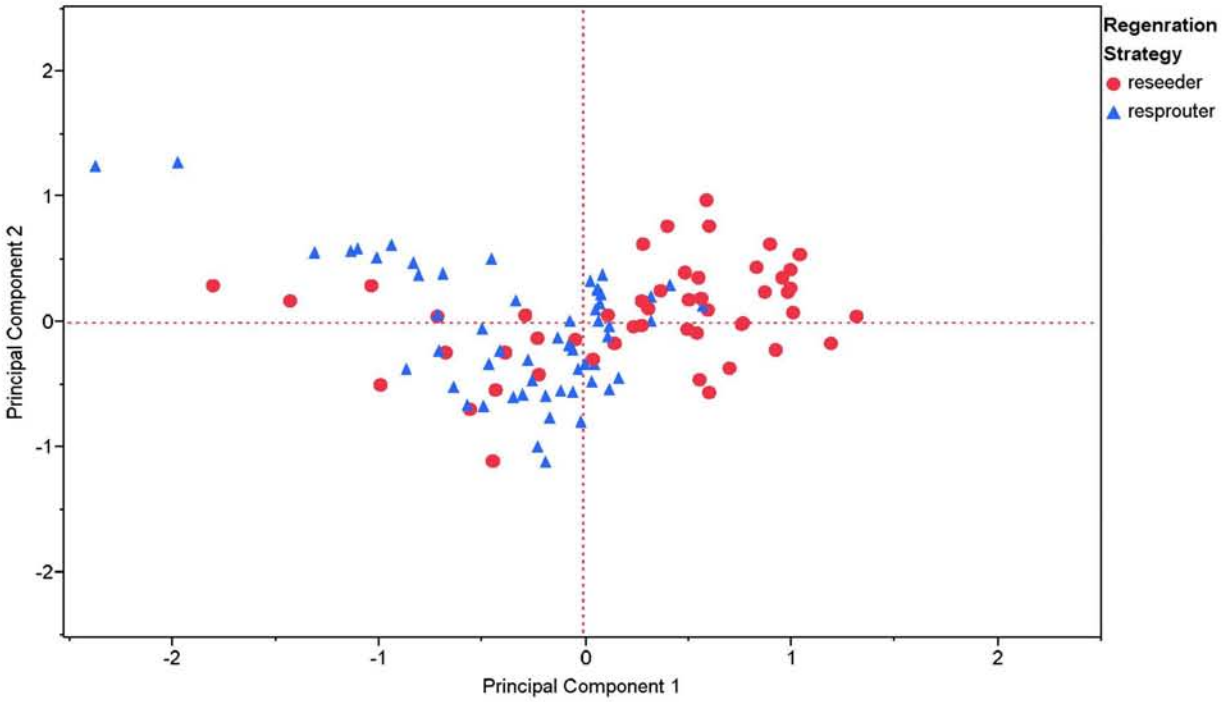

Figure 3.6. Ordination of reseeders and resprouters of *Otholobium* and *Psoralea* along the first two principal components of the soil data set.

Table 3.3 Mean±SE of the soil characteristics that were significantly different ($p<0.05$) between regeneration strategies. N= number of soil samples taken at the site.

Species	Site	N	Strategy	NO_3-N mg/kg	P Bray II mg/kg	Ex Ca mg/kg	NH_4:NO_3
O. acuminatum	Avontuur	2	Reseeder	10.37±1.23	3.50±2.50	8.91±8.69	0.59±0.08
O. bracteolatum	Heidehof Farm	4	Reseeder	3.18±0.37	16.50±1.65	5.87±1.27	1.19±0.09
O. flexuosum	Uitkyk Pass	4	Reseeder	8.45±0.45	7.75±1.55	2.04±0.38	0.82±0.21
O. hamatum	Welkom Farm	4	Reseeder	21.04±3.66	4.25±0.95	4.19±0.57	0.29±0.04
O. pustulatum	Grootberg	4	Reseeder	42.19±2.59	11.25±1.11	6.14±0.60	0.12±0.01
O. striatum	Van Rhyns Pass	4	Reseeder	22.95±3.79	21.67±1.03	5.33±0.70	0.24±0.08
P. affinis	Vogelgat Reserve	4	Reseeder	2.15±0.48	2.75±0.48	1.74±0.06	3.96±0.83
P. aphylla2	Jonkershoek	4	Reseeder	143.40±21.78	16.75±1.49	2.02±0.28	0.17±0.09
P. aphylla	Camps Bay	4	Reseeder	17.13±2.75	7.25±2.10	4.42±0.57	0.67±0.05
P. fleta	Mitchell's Pass	4	Reseeder	5.55±1.93	6.00±0.41	4.76±1.08	1.19±0.36
P. glaucescens	Brakputs (Grootberg)	4	Reseeder	42.19±2.59	11.25±1.11	6.14±0.60	0.12±0.01
P. pinnata	Dappat se Gat	4	Reseeder	40.79±5.41	9.75±2.87	5.93±0.83	0.35±0.1
P. affinis320	Tulbagh	4	Reseeder	76.60±4.94	7.50±1.26	2.22±0.86	0.17±0.04
O. dreweae	Fernkloof Nat. Reserve	4	Resprouter	1.70±0.45	1.00±0.35	3.52±0.50	2.66±1.51
O. lanceolatum	Shaw's Pass	4	Resprouter	1.42±0.04	1.00±0.34	2.15±0.12	2.57±0.25
O. candicans	Gydo Pass	4	Resprouter	9.23±2.77	14.67±2.32	6.34±0.72	0.62±0.17
O. fruticans	Constantia	4	Resprouter	8.01±1.03	1.33±0.24	3.58±0.62	0.57±0.06
O. obliquum	Jonkershoek	4	Resprouter	7.89±1.35	2.25±0.48	0.70±0.14	0.82±0.23
O. rotundifolium	Houwhoek Pass	4	Resprouter	13.23±5.02	1.50±0.20	1.52±0.32	0.41±0.04
O. thomii	Rotary Drive (Hermanus)	4	Resprouter	3.84±1.38	4.00±0.50	1.84±0.22	1.23±0.40
P. aculeata	Silvermine	4	Resprouter	3.32±0.85	3.00±0.71	2.59±0.31	7.38±3.37
P. asarina	Gouna	2	Resprouter	8.45±1.52	6.50±5.50	0.81±0.55	1.09±0.10
P. imbricata	Lion's Head	4	Resprouter	7.38±1.11	17.25±2.29	3.73±0.28	0.75±0.13
P. oreopola	Pakhuis pass	4	Resprouter	10.77±1.10	4.75±0.85	0.97±0.12	0.40±0.04
P. repens	Cape Hangklip	4	Resprouter	7.87±1.21	9.00±1.47	9.98±0.51	0.47±0.09
P. restioides	Tierfontein Farm	4	Resprouter	26.53±8.77	2.00±0.41	5.91±1.53	0.52±0.18
P. rigidula	Bainskloof Pass	4	Resprouter	4.83±0.95	2.50±0.29	1.46±1.56	1.55±0.20
P. triflora	Baaviankloof	3	Resprouter	8.48±1.17	3.67±1.45	0.85±0.55	1.04±0.22
P. verrucosa	Rosendall Farm	4	Resprouter	22.05±3.19	19.33±1.25	0.58±0.24	3.20±0.50
			Mean Reseeder	**33.54±4.00**	**9.71±1.42**	**4.59±1.27**	**0.76±0.15**
			Mean Resprouter	**9.06±1.79**	**5.86±1.34**	**2.91±0.43**	**1.58±0.47**
			p-value	**<0.01**	**<0.05**	**<0.05**	**<0.001**

3.2.3. Soil nutrients in relation to species' phylogenetic relationships

The species used for these analyses were those from the five clades shown in Fig. 3.7. For Clade 1, which consisted of *O. bracteolatum* and *O. fruticans*, PCA gave sixteen principal components, of which the first two accounted for 93.21 % of the total variance. PC1 accounted for 87.63 % of the total variance, and this was largely influenced by the concentration of P Bray II (Fig. 3.8A). PC2, which accounted for 5.58 % of the total variance, was mainly a component of the concentration of exchangeable calcium (Fig. 3.8A). The ordination of the soil data for these two species along the first two components indicates that the species form two distinct clusters (Fig. 3.8A.).

For Clade 2, PC1 accounted for 76.60 % of the total variance, while PC2 accounted for 10.87 %. These components were largely influenced by the NH_4:NO_3 ratio and the concentration of NO_3, respectively (Fig. 3.8B). There is no clear separation of the species along these first two components, except for the distinct separation of *O. rotundifolium* from *O. lanceolatum* (Fig. 3.8B). For the third clade, PC1, which accounts for 53.98 % of the total variance was largely

influenced by the NO_3 concentration, while PC2 (accounting for 16.24 % of the variance) was mainly a component of the P Bray II concentration (Fig. 3.8C). The species of this clade (*O. flexuosum*, *O. hamatum*, *O. pustulatum* and *O. striatum*) showed some distinct separation along these two components (Fig. 3.8C). The species of Clade 4 showed distinct separation along PC1 and PC2 (largely influenced by NO_3 and NH_4, respectively), but the soils of *P. aphylla*2 further separated into two distinct clusters (Fig. 3.8D). The soils of the two *P. affinis* sites showed distinct separation along the first two components (Fig. 3.8E). PC1 of these soils was a component of the NH_4:NO_3 ratio, while the PC2 was a component of the K concentration (Fig. 3.8E).

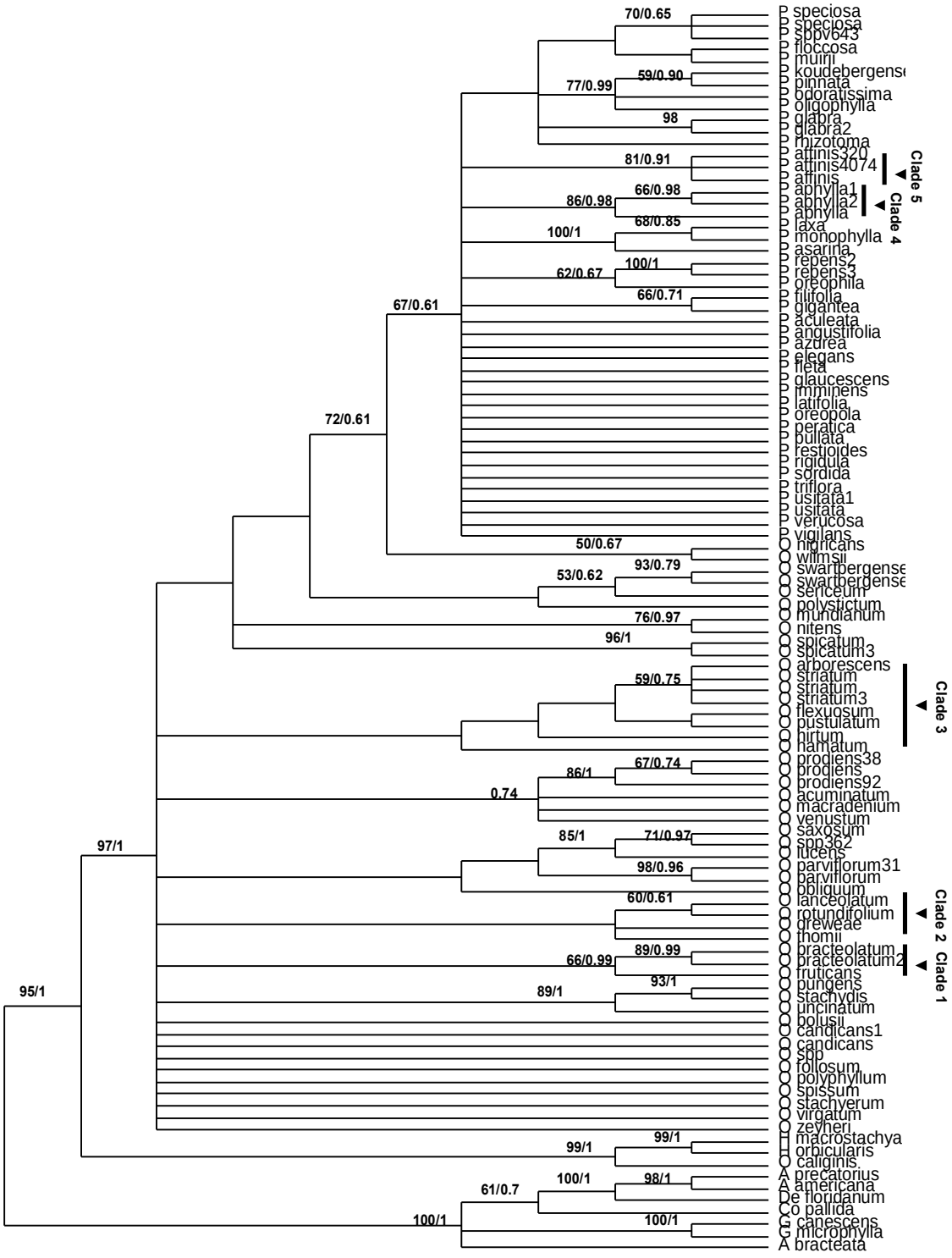

Figure 3.7. Strict consensus tree of the southern African Psoraleeae. Numbers above branches are parsimony bootstrap percentages and Bayesian posterior probabilities. Genus abbreviations are the same as those of Fig. 2.3. Solid bars indicate clades used for the comparison of soil data.

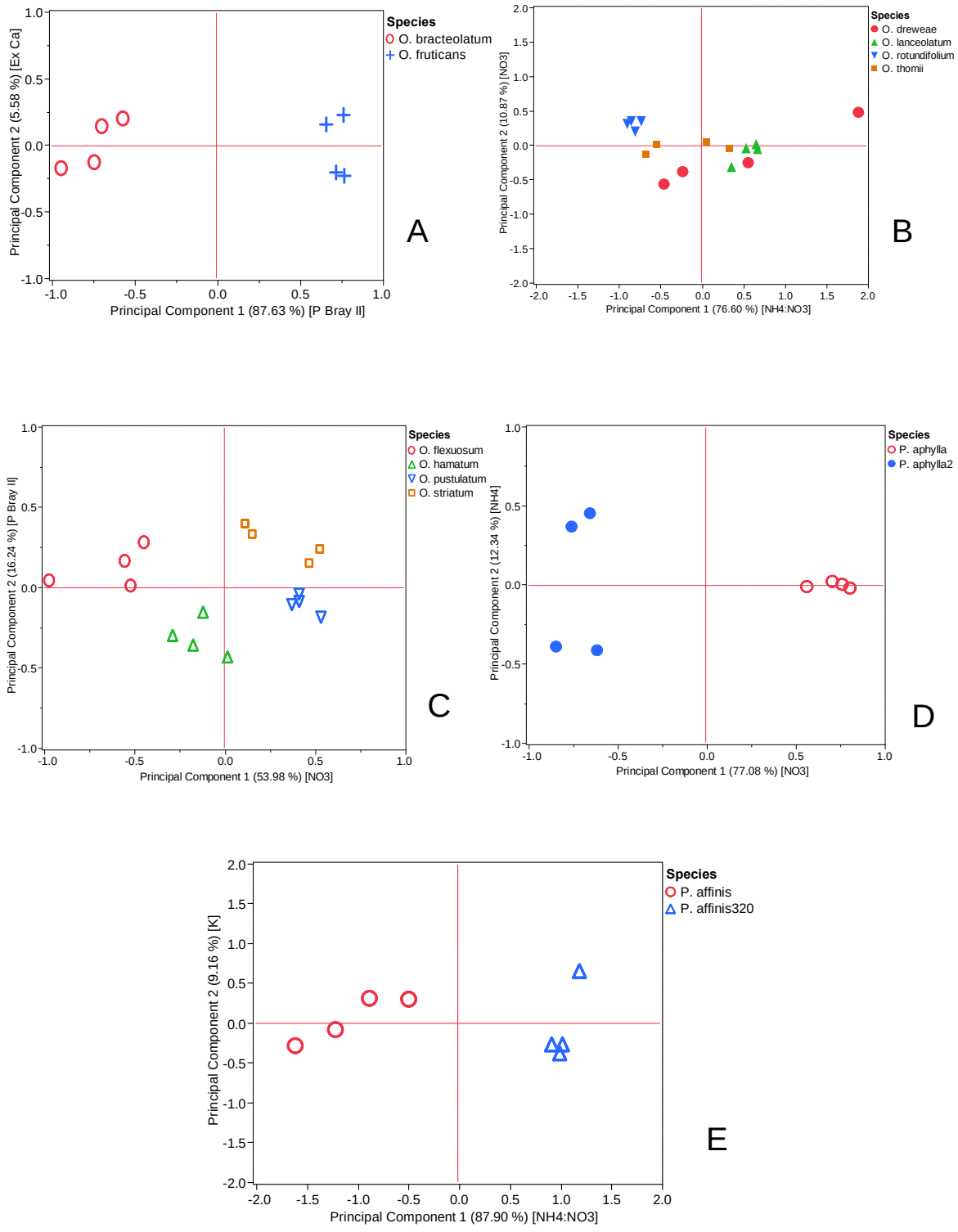

Figure 3.8 Ordination of species from selected clades (Fig. 3.7) along the first two principal components of the soil data set. A= Clade 1, B= Clade 2, C= Clade 3, D= Clade 4 and E= Clade 5. The percentage contribution of each PC is indicated in brackets and the soil characteristic with the highest contribution in the respective PC is indicated in square brackets.

3.3. Discussion

3.3.1. Soil types

The results showed that *Otholobium* species occur on the same soil types, as do *Psoralea* species, but in different proportions. While the *Psoralea* species are more concentrated on the sandstone habitats, the *Otholobium* species have equal distributions on sandstone, granite and shale habitats and there are more *Otholobium* species on limestone and sand habitats than *Psoralea* (Figs. 3.3 and 3.4). The occurrence of the greatest proportion of *Psoralea* species on sandstone habitats may be partly due to the fact that it is the most widespread soil type in the CFR, taking 310 km^2 (about a third) of the area of the fynbos biome (Mucina & Rutherford, 2006). Since the genus *Psoralea* was estimated to have arisen about 2.4 million years ago (Table 2.7, in Chapter 2), the sandstone habitats could be the ancestral habitats for *Psoralea*. Given that sandstone habitats are nutrient poor as compared to the other soil types, [as shown in this study (Table 3.1) and other previous studies e.g. Richards *et al*., (1995)] the occurrence of a large proportion of its species on these habitats suggests that it has not evolved the necessary adaptations for nutrient richer soils [e.g. the capacity to down-regulate nutrient uptake when exposed to high nutrient concentrations (Shane *et al*., 2008)]. On the other hand, the genus *Otholobium* is older than *Psoralea,* and so it might have had sufficient time to evolve the necessary adaptation for nutrient richer habitats, hence the wider distribution across the different soil types.

3.3.2. Regeneration strategy

The study showed that the soils occupied by reseeders are associated with high levels of phosphorus, nitrates and calcium, while the sites occupied by resprouters had lower levels of these elements, but higher NH_4:NO_3 ratios (Table 3.3). This indicates that the resprouter soils have higher NH_4 concentrations. According to Kleijn *et al*. (2008), soils that have high levels of NH_4 are more acidic, low in major nutrients and have low nitrification rates. Therefore, only species that are adapted to such conditions can survive such habitats. Resprouters have slow growth rates, high root: shoot ratios, and low reproductive capacity. On the other hand, reseeders have high growth rates, low root: shoot ratios and high reproductive capacity (Groom & Lamont, 1996; Lamont *et al*., 1998). Moreover, resprouters have a lower capacity to down-regulate nutrient uptake as compared to reseeders (Power *et al*., 2010). Therefore, the low nutrient habitats are conducive for resprouters, while the nutrient rich habitats are more suitable to reseeders because for them to grow quickly and reproduce, they need more nutrition.

Based on the above explanation, one would expect that since sandstone habitats are nutrient poor than the other soil types, a majority of the species occurring on such habitats should be resprouters. However, it was observed that in the genus *Psoralea*, 62 % of the reseeders occur on sandstone habitats, 33 % of the reseeders in *Otholobium* occur on sandstone habitats and for both genera, there were more reseeders than resprouters (Fig. 3.5). Nonetheless, the resprouters in both genera were predominantly on the sandstone habitats. In other words, while the distribution of the resprouters is consistent with the expected pattern, the distribution of reseeders in not. This is illustrated in Fig. 3.6, which shows that while some reseeders are restricted to the nutrient rich soils, there are some that occur on the nutrient poor soils. The occurrence of more reseeders than resprouters on nutrient poor habitats was observed by several other authors such as Bond & Wilgen (1996), Cruz *et al*. (2002) and Ojeda *et al*. (2005). This might be due to the predictable nature of the fire-return interval, making most plants to invest all their resources in reproduction at the expense of regeneration. The extra investment in underground organs may not be worth the cost since pulses of nutrients released by fire lie mostly on top of the soil, inaccessible to new growth sprouting from subterranean lignotubers or epicormic buds (Wisheu *et al*., 2000). This indicates that if the reseeders are able to grow fast enough and set seed before the next fire, they can still manage to survive in nutrient-poor environments. Some studies (e.g. Hester & Hobbs, 2003) have shown that fire increases levels of NH_4 in the soil. However, high levels of ammonia in the soil reduce the availability of nutrients (Kleijn *et al*., 2008) and thus favouring the resprouters. This suggests that the success of plants of either strategy in nutrient poor habitats is dependent on the interaction of fire and nutrients. Hence, the evolution of these strategies might have been influenced by tradeoffs between fire and edaphic factors.

3.3.3 Soil nutrients in relation to species' phylogenetic relationships

Out of the five clades that were selected, four of them contained species that occupied habitats with distinct nutrient levels (clades 1, 2, 4 and 5 in Fig. 3.7). This was indicated by the fact that the covariance of the soil characteristics showed distinct groupings, corresponding to each of the species in the respective clades (Fig. 3.8). This is consistent with the observations of several authors (Rourke, 1972; Goldblatt, 1982; Kurzweil *et al*., 1991; van der Niet & Johnson, 2009) who reported that for some lineages in the CFR, closely related species often occur on different soils, suggesting that adaptation for the different soils is the primary differentiating factor between the species (Linder, 2003). Therefore, if the soil nutrient levels of the species studied here represent the same conditions during speciation, then speciation in these clades may have been driven by edaphic factors. For the clades in which species did not show distinct separation (e.g. *O. thomii* and *O. dreweae* in clade 2 and the members of clade 3) this would indicate that

for them speciation was not influenced by edaphic factors. Perhaps geographical barriers (allopatry), pollinators or the formation of polyploids were responsible for speciation in these clades.

However, with the limited sampling of the current study, it is not possible to generalize about the other species of the two genera under study. Therefore, more sampling of species, covering their entire distribution ranges is essential. Analyzing such data on a robust phylogenetic framework should give insights about speciation processes in the southern African Psoraleeae. Sister species comparisons using phylogenetic independent contrasts (PICs) can be used to achieve this goal. PICs are calculated as differences in trait values between adjacent pairs of nodes or terminal taxa in a phylogenetic tree and since no two contrasts share the same branches of a tree, they are statistically independent samples of evolutionary change within a lineage (Felsenstein, 1985).

3.4. Conclusions

Although the current data represents a small sample of the lineage, it provides indicators concerning the role of edaphic heterogeneity in the distribution of the Psoraleeae. The data showed that the distribution of the Psoraleeae covers a wide range of soil types, with different soil nutrient levels. It showed that reseeders are associated with higher nutrient levels than resprouters and closely related species are occupying soils with different nutritional levels. Further studies, with comprehensive sampling, and incorporating a phylogenetic framework are required in order to test whether diversification in the Psoraleeae was driven by edaphic factors.

CHAPTER 4

MORPHOMETRIC STUDIES OF THE *PSORALEA APHYLLA* AND *PSORALEA PINNATA* SPECIES COMPLEXES

4.0. Introduction

At the establishment of the genus *Psoralea sensu stricto* by Stirton (1981), twenty species were recognised, all occurring in southern Africa, with a centre of diversity and endemism in the Cape Floristic Region (CFR). As Stirton did more fieldwork in an attempt to revise the genus, he discovered more species such that when he completed the revision of the genus *Otholobium* (Stirton, 1989) he pointed out that the genus *Psoralea* had at least 47 species. In a later publication, Stirton (1995) alluded that there could be about 51 *Psoralea* species. However, a majority of these species are presently known by informal names in manuscripts that describe the flora of the region such as Goldblatt & Manning (2000). Since such species have no formal descriptions, the application of these names is always ambiguous and subjective due to lack of clarity on species delimitation. Species delimitation is compounded by observed morphological heterogeneity, whereby a single species has many variant local forms across its distribution range.

The various species concepts used by taxonomists to define species were discussed in Chapter 1. It was pointed out that since there is no single, all encompassing definition of species, the delimitation of species is always difficult. The biological species concept (BSC), which defines species as groups of natural populations that are capable of interbreeding and are reproductively isolated from other such groups of populations (Mayr, 1982) could not be directly applied. This was because applying this concept would require a demonstration of the presence or lack of gene flow between populations, and such was beyond the scope of the current study. Instead, the phenetic species concept (PhSC) was more feasible for the present study and more applicable to the group under study. This concept considers the species level as that at which distinct phenetic clusters are observed (Sneath, 1976). However, morphological similarity or dissimilarity between organisms is often considered in the inference of underlying genetic differences, and thus morphological discontinuity may indicate a discontinuity in gene flow (Stace, 1989). This suggests that there exists an interrelationship between the BSC and the PhSC. Therefore, although the study relies mainly on the PhSC, the BSC is, to some extent, also accommodated. The study considers two species complexes, the *Psoralea aphylla* and the *P. pinnata* complexes. These are described in the sections that follow.

4.0.1. *Psoralea aphylla* complex

This complex includes all the members of the genus *Psoralea* that fall into the broad concept of *Psoralea aphylla* L., as described by Harvey & Sonder (1862). They describe *P. aphylla* as being either leafless, having a subulate scale instead of a leaf, or sparsely leafy and having a unifoliolate or rarely trifoliolate leaf. A modification of this description was made by Forbes (1930) and includes ñleaves unifoliate, 5-17 mm long, linear, acute, present only on very young stems, their place in older stems being taken by ovate acute bracts, up to 5 mm long.ò Based on these definitions, two other formally published species (*P. filifolia* Eckl. & Zeyh. and *P. peratica* C.H. Stirt.) form part of the complex, making a total of three accepted species. Nevertheless, several other variants exist. They do not match the type specimens nor the original descriptions of any of the three formally published names, and the tendency among botanists and collectors working with such material has been to lump all leafless *Psoralea* material into the name *P. aphylla* hence the name ñ*P. aphylla* complex*"*.

As part of an ongoing revision of the genus, Stirton has proposed at least 7 taxa that can be recognised besides the already published *P. filifolia, P. peratica* and *P. aphylla*. Although his putative taxa are not formally published, their names are already in use in various manuscripts such as Stirton & Schutte (2000). These names are *P. fleta, P. usitata, P. pullata, P. ramulosa, P. vigilans, P. congesta* and *P. rigidula*. Moreover, these names are already being applied to specimens of the *P. aphylla* complex as depicted in the Bolus, Pretoria and Compton herbaria (determined by Stirton). Nevertheless, in the absence of published descriptions and keys to these species, the use and application of these names remains ambiguous and presents a taxonomic difficulty.

Field observations made during the study show that at the seedling stage, members of the *P. aphylla* complex have large pinnate leaves, with up to 11 pinnae. The shape, size and orientation of these leaves are highly variable among members of the complex. For example, *P. filifolia* has tiny, needle like pinnae, an observation also made by Crow *et al*. (1997), while *P. aphylla* has very long and broad pinnae. Besides these differences, as the plants mature, they lose their leaves gradually until they are completely aphyllous. This leaf loss takes three different paths: (i) the leaves are lost completely, leaving bare branches and shoots, (ii) the leaves are reduced to scales that may be tightly packed in the upper portions of the shoots, and (iii) each leaf is reduced into a tiny, filiform leaflet, occurring in the upper axils of the shoots. In some cases, the younger stems may retain a few trifoliolate or 5-foliolate leaves.

Besides the variation in leaf morphology, differences in other aspects of the group, such as flower morphology, growth form, flowering time, and geographical distribution suggest that the complex might be containing several species. Variation in flower morphology includes several features. First, there is variation in the cupulum, a cup shaped structure at the base of each pedicel, formed by the fusion and intercalary growth of three to four successive bracts (Tucker & Stirton, 1991). The position of the cupulum; shape of the cupulum lobes; and the number of cupulum lobes differ from one form of *P. aphylla* to another. Secondly, the colour, size and shape of the standard petal are also highly variable. The standard petal could be long and thin, short and broad or of similar length and width. The apex of the standard petal is either round, emarginate or obtuse. There is also variation in the degree of hairiness of the calyx. For example, the calyx can be glabrous, sparsely covered with white or black hairs, densely covered in white hairs or densely covered in long black hairs. Some forms have long and flexuous inflorescences, while others have short and stout inflorescences. In addition, some populations have congested inflorescences, while others have very lax inflorescences. This variation in flower morphology may be indicative of pollinator adaptations, but the pollination biology of the genus as a whole is not well known.

More variation exists in terms of the general habit or growth form of plants in the *P. aphylla* complex. It ranges from suffrutices, small shrubs, and large shrubs up to fully developed trees. The branching pattern ranges from those that branch at ground level, forming dense clumps, to multi-stemmed shrubs that start branching at a height of about one metre, while the large shrubs and trees have long, bare stems that only start branching at heights above two metres. There is also variation in terms of post-fire regeneration strategy, with some forms being resprouters, while others are reseeders.

Other factors such as flowering time, geographical distribution and altitudinal variation suggest that various taxa exist within the complex. However, the extent of the variation and the actual number of taxa that can be recognised is not known. In view of the extent of the variation and the taxonomic problems associated with the *P. aphylla* complex, it was found necessary to investigate taxonomic limits within the complex. This was done by considering the already available manuscript names, to test if they reflect natural groupings and then using that information to generate a classification for the complex. It was hypothesized that several taxa were embedded within the *P. aphylla* complex. Two key questions were addressed: (i) is *Psoralea aphylla* a single polymorphic species or is it a complex of several entities that can be recognised as distinct taxa? and (ii) if there are more than one taxa, how can they be identified?

4.0.2. *Psoralea pinnata* complex

Included in this complex are all the *Psoralea* members that fall within the broad concept of *Psoralea pinnata* as described by Linnaeus (1753). The major features of Linnaeusô concept of *P. pinnata* are: arborescent or shrubby, densely branched, pubescent or glabrous, leaves imparipinnate, in 3-5 pairs, linear or lanceolate linear, acute, very narrow, pedicels axillary, long or short, bracteolate beyond the middle, calyx very variable in incision and pubescence. Several species which fall within the broad description of *P. pinnata* were described after Linnaeus. These include *P. arborea* Sims, *P. latifolia* Torr; *P. affinis* Eckl. & Zeyh., *P. speciosa* Eckl. & Zeyh., *P. glabra* Meyer and *P. azurea* Philippi. However, when Harvey and Sonder (1862) revised the genus *Psoralea*, they recognised only one species, *P. pinnata* and treated all the others as local varieties of *P. pinnata*.

In a later revision of the genus by Forbes (1930), *P. pinnata* and *P. affinis* were recognised as separate species, but without clearly pointing out the difference between the two. *P. pinnata* was described as a tall, much branched woody shrub, up to 3 metres high with striate, virgate stems, while *P. affinis* was described as a tall, virgate shrub, up to 1.8 metres tall. Forbes (1930) did not mention any of the other earlier published species within this complex, but some of the specimens cited under her *P. pinnata*, are specimens that belong to the varieties of *P. pinnata* as depicted in Harvey & Sonder (1862). This suggests that except for recognising *P. affinis* as a distinct species, her concept of *P. pinnata* was similar to that of Harvey & Sonder (1862). Forbes further described *P. pinnata* as having a wider distribution than any other native species and as being in bloom the whole year. She provided various localities of both species, but although *P. affinis* had fewer localities than *P. pinnata*, a majority of the *P. affinis* localities overlapped with the *P. pinnata* localities. In addition, a majority of the morphological characters of the two species overlapped greatly, therefore making it difficult to distinguish between the two species.

Since the revision by Forbes (1930) no other revision has been done besides the generic changes done by Stirton (1981), where *Psoralea* was split into six genera. Stirton & Schutte (2000) retained the names *P. arborea; P. latifolia; P. speciosa;* and *P. azurea*, which were rejected by Forbes (1930), but since theirs was not a taxonomic treatment, it has little to do with the taxonomic status of those names. Therefore, the present study sought to test whether the diversity that exists within the *P. pinnata* complex is a depiction of several local forms of a single variable species as proposed by Harvey and Sonder (1862), or distinct taxa that need to be recognised as separate species.

4.1. Materials and methods

4.1.1. Field collections, morphometric measurements and data sets

Specimens from the Bolus (BOL), Pretoria (PRE) and Compton (NBG) herbaria were studied. These herbaria have the largest collection of *Psoralea* material and so capture a substantial amount of the variation within the genus. Additional observations and field collections were done within the CFR. Specimen details and distribution are provided in the taxonomic treatment section of this chapter.

4.1.2. Characters studied

One limitation of using herbarium specimens in taxonomic studies is that they represent only a portion of the whole individual and therefore do not capture all the information about the actual plant. However, this was unavoidable because it was not possible to visit all populations in the field. As a result, only characters that could be scored from the herbarium specimens were selected. This implied taking all measurements on dried specimens, including current collections, to avoid inconsistency. For the *P. aphylla* complex, most of the characters were flower characters (because they have no leaves) while for the *P. pinnata* complex both floral and leaf characters were measured. To measure floral characters, the flowers were soaked in hot water containing dishwashing liquid soap for at least five minutes to soften them. They were then carefully dissected, spread out and callipers were used to measure several parts of the flowers. Some of the leaf and floral characters measured are illustrated in Fig. 4.1. To avoid having many missing data, which could affect the analyses, only specimens that contained all the information needed were selected (i.e. specimens that had vegetative and mature floral parts). For each specimen, measurements were taken on two mature plant parts (flowers, leaves, stipules, etc) and the mean of the two was recorded for each character. For the *P. aphylla* complex, 22 characters were measured for 60 specimens, while for the *P. pinnata* complex, 25 characters were measured for 75 specimens (Table 4.1).

It is important to note that numerical taxonomy methods have been criticized for not taking into consideration the correlation among characters (e.g. Mayr, 1965). Such characters are those that show some degree of association, i.e. a certain character is the logical consequence of another character (Sneath & Sokal, 1963). An example of such characters would be the length and half the length of an organ. The criticism against such characters is that they do not necessarily indicate evolutionary history and therefore, cannot be used for classification. Nonetheless, such characters may be the ones that reflect taxonomic differences between groups, thus making their

use to be inevitable. According to Rohlf (1967), one way to deal with correlated characters is to avoid characters that are constant within the groups being considered and to use methods that take into account the patterns of variation and correlation that exists within operational taxonomic units, such as discriminant function analysis (DFA). For this study, all the characters used were not constant within the various groups considered and the DFA was the main analysis used for discrimination between the groups, in order to account for character correlations, where such correlations could occur.

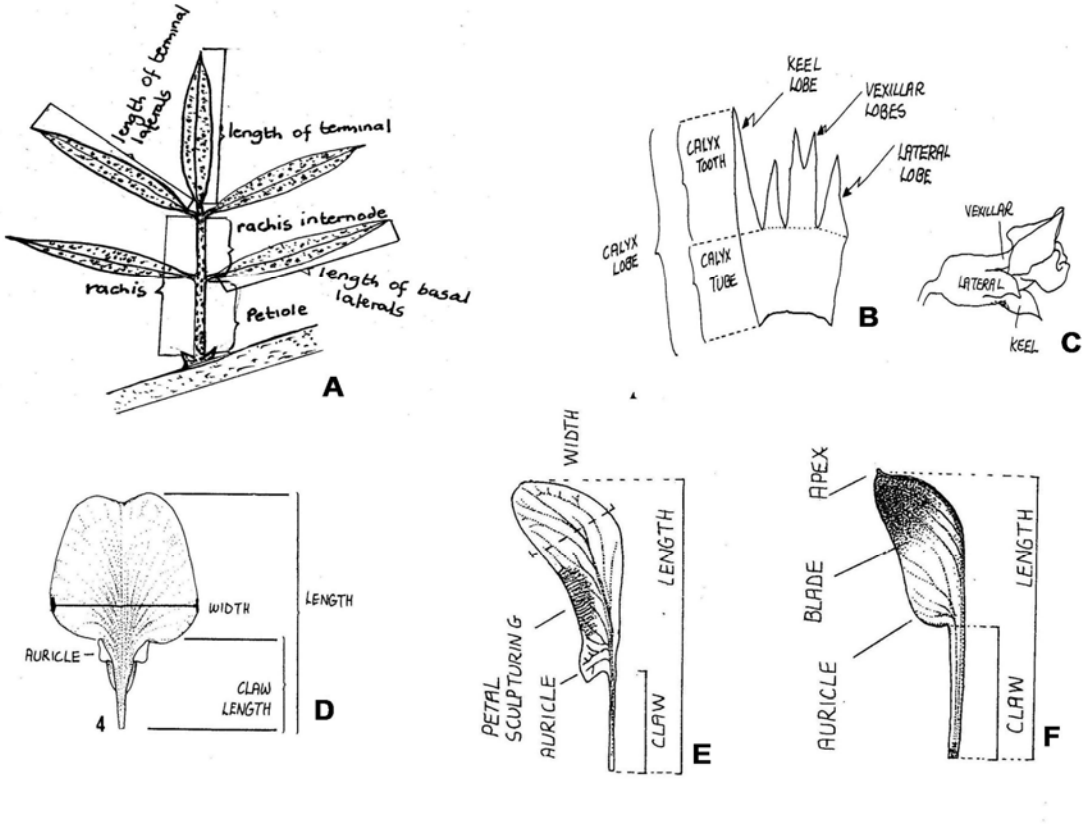

Figure 4.1 Illustrations of some of the characters measured for the *P. aphylla* and *P. pinnata* complexes. A= leaf, B and C= calyx, D= standard petal, E= wing petal F= keel petal. Drawings B-F were adapted from Stirton (1989).

4.1.3. Statistical analyses

4.1.3 (a) Data transformation

Statistical analyses were performed on Statistica version 8 (Statsoft, Tulsa, Oklahoma, USA). The characters were tested for normality and linearity [which are the assumptions of the discriminant function analysis that was used for the study (Sokal & Rohlf, 1981)]. Preliminary

investigations of the data showed that all the characters exhibited significant departure from normality (as indicated by the g_1 and g_2 statistics, $P<0.05$) and were non-linear. In order to meet these assumptions, each of the variables was Log_{10} transformed before performing the analyses. After transformation, all the variables were approximately normally distributed and bivariate relationships showed no significant departure from normality. Univariate analysis of variance (ANOVA) was performed to test the null hypothesis of equality of means among putative groups for each of the morphometric characters.

Table 4.1 Characters studied for the *P. aphylla* and *P. pinnata* complexes. Yes, indicates that the character was measured for the respective complex and no means the character was not.

Character	*P. aphylla* complex	*P. pinnata* complex
Plant height (m)	yes	no
Number of leaflets	yes	yes
Length of petiole (mm)	no	yes
Length of rachis (mm)	no	yes
Length of rachis internode (mm)	no	yes
Length of basal laterals (mm)	no	yes
Length of terminal laterals (mm)	no	yes
Length of stipules (mm)	yes	no
Width of stipules (mm)	yes	no
Length of terminal leaflet (mm)	yes	yes
Number of flowers per axil	yes	yes
Length of flower (mm)	yes	yes
Length of peduncle (mm)	yes	yes
Length of cupulum lobe (mm)	no	yes
Width of cupulum lobe (mm)	no	yes
Number of cupulum lobes	yes	yes
Length of pedicel (mm)	yes	yes
Length of calyx tube (mm)	yes	yes
Length of calyx tooth (mm)	yes	yes
Width of calyx lobe (mm)	yes	yes
Length of standard petal (mm)	yes	yes
Width of standard petal (mm)	yes	yes
Length of claw of standard petal (mm)	yes	no
Length of wing petal (mm)	yes	yes
Width of wing petal (mm)	yes	yes
Length of claw of wing petal (mm)	yes	yes
Length of keel petal (mm)	yes	yes
Width of keel petal (mm)	yes	yes
Length of claw of keel petal (mm)	yes	yes

4.1.3. (b) Cluster analysis

Before testing how well the data were able to discriminate between the proposed groups, cluster analysis (CA) was used to investigate if there were any clustering patterns in the data. This analysis is an exploratory tool for classifying objects, which has no statistical assumptions about the data (Henderson, 2006). This technique places similar objects in groups and these groups are in turn placed in groups that are more inclusive in a hierarchical manner. In other words, it brings together individuals or groups that are closely associated into a cluster (Blackith & Reyment, 1971). Such a cluster is then considered to be differentiated from other associations that form separate clusters, thus dividing a data set into *a priori* unknown subgroups (Flury & Riedwyl, 1988). The technique involves defining a clustering algorithm and a measure of distance between individuals. The Unweighted Pair Group Method with Arithmetic mean (UPGMA) was used as the clustering algorithm and Euclidean distances were used as a measure of distance. The UPGMA was favoured because it computes the average similarity or dissimilarity of a candidate operational taxonomic unit (OTU) to an extant cluster, weighting each OTU in that cluster equally, regardless of its structural subdivision (Sneath & Sokal, 1973). The Euclidean distance was chosen because unlike other distance measures, (e.g. squared Euclidean, Manhattan, power or percent disagreement distance) it represents the actual geometric distance in the multidimensional space and the distance between any two objects is not affected by the addition of new objects to the analysis (Sneath & Sokal, 1973). The existing published and manuscript names were used to putatively identify specimens and for cases in which specimens represented what was perceived to be new species, putative species names were assigned. For the *P. aphylla* complex, the given putative name was *P. gigantea*, while for the *P. pinnata* complex the putative names were *P. koudebergense, P. brilliantisssima* and *P. pedicellata*.

4.1.3. (c) Discriminant function analysis

According to Thorpe (1983), cluster analysis can impose a hierarchical structure on any data. Moreover, it often shows clusters that may not be recoverable in ordination analyses (Chandler & Crisp, 1988). Therefore, a robust taxonomic classification cannot be based on the results of a cluster analysis alone. Hence, in this study, discriminant function analysis (DFA) was used to examine multivariate morphometric differences among the putative groups. The DFA generates a linear combination of variables that maximizes the probability of correctly assigning observations to their pre-determined groups and can be used to classify new observations into one of the groups (Flury & Riedwyl, 1988; Quinn & Keough, 2002). The basic principle behind the discriminant function analysis is to find a suitable linear combination of several variables, i.e. canonical variates (CVs) in such a way as to maximise the correlations between the CVs and

group membership, and the ratio of between to within group variance (Sneath & Sokal, 1973; Krzanowski, 1990). Correlations between the original variables and the derived CVs, as well as the patterns of vector loadings for the original measurements allow reification of the CVs in terms of shape and size differences among groups (Compton & Hedderson, 1997). The DFA results in a classification matrix in which each specimen is classified according to the classification functions correctly, either according to the original grouping or into another group. The percentage of correct classifications is given and this gives an indication of the validity of the original grouping. DFA also has the advantage of being able to show which variables are the most discriminatory in classifying specimens and to identify unknown specimens (Henderson, 2006). This information is useful in the development of identification keys in taxonomy.

4.2. Results

4.2.1. *Psoralea aphylla* complex

4.2.1. (a) Basic statistics

A summary of the morphometric results for the 22 characters is shown in Table 4.2 with Mean±SE values for each character (the actual measurements for all the specimens measured are provided in appendix 3.1). There is considerable overlap in some of the characters between some of the groups for the 22 characters, but the univariate analysis of variance (ANOVA) showed that the means of 13 (marked with an asterisk) of the variables differ significantly ($p<0.05$) among the groups.

Some plots of the means and standard errors for some of the characters that were significantly different between the groups are presented in Fig. 4.2. Four groups (*P. aphylla*, *P. filifolia*, *P. pullata* and *P. usitata*) are not significantly different in plant height ($p>0.05$), but the remaining five groups are all significantly different (Fig. 4.2A). For flower length, most of the groups have overlapping means, but *P. peratica* is only similar to *P. rigidula*, and *P. filifolia* is only similar to *P. usitata* (Fig. 4.2B). While *P. gigantea*, *P. peratica* and *P. pullata* are associated with long calyx teeth, the rest of the groups have shorter calyx teeth, with minor variation between them (Fig. 4.2C). This also applies to the width of the calyx (Fig. 4.2D). Members of the *P. filifolia* group are associated with the shortest standard petals, a majority of the groups have intermediate standard petal lengths, while *P. peratica*, *P. gigantea* and *P. pullata* have the longest standard petals (Fig. 4.2E). The *P. rigidula* group has the shortest wing petals, while *P. peratica* and *P. pullata* have the longest wing petals (Fig. 4.2F).

Table 4.2. Summary of morphometric characters measured for the *P. aphylla* complex: Mean±SE. Characters marked with an asterisk are significantly different between groups at $p<0.05$.

Character	*aphylla n=4*	*gigantea n=3*	*filifolia n=4*	*fleta n=5*	*peratica n=6*	*pullata n=5*	*usitata n=16*	*rigidula n=6*	*ramulosa n=10*
plant height (m) *	2.88±0.33	8.00±0.39	3.00±0.33	4.5±0.30	2.67±0.27	3.00±0.30	2.53±0.17	0.60±0.27	1.20±0.21
length of leaflet (mm)*	4.35±1.77	19.93±2.04	16.78±1.77	9.95±1.45	N/A	N/A	N/A	N/A	N/A
Number of leaflets*	0.25±0.11	1.00±0.13	1.00±0.11	1.00±0.10	N/A	N/A	N/A	N/A	N/A
no. of florets*	5.00±1.24	2.00±1.43	11.00±1.24	5.20±1.11	6.67±1.01	3.20±1.11	2.75±0.62	4.00±1.01	4.80±0.78
length of stipules (mm)*	3.88±0.71	N/A	N/A	2.71±0.64	2.23±0.58	3.97±0.64	0.82±0.35	N/A	N/A
width of stipules (mm)*	0.96±0.21	N/A	N/A	0.67±0.18	0.87±0.17	1.48±0.18	0.28±0.10	N/A	N/A
length of flower (mm)*	20.58±1.35	21.12±1.57	17.12±1.36	20.34±1.21	20.90±1.10	23.94±1.21	19.70±0.68	22.26±1.11	21.60±0.86
length of peduncle (mm)	4.18±1.46	3.31±1.68	4.09±1.46	3.73±1.31	3.46±1.19	7.03±1.30	3.93±0.73	6.55±1.19	4.84±0.92
Number of cupulum lobes*	2.25±0.20	3±0.23	2.00±0.20	2.60±0.18	2.00±0.16	2.00±0.18	2.3±0.10	3.00±0.16	2.60±0.12
length of pedicel (mm)*	3.04±0.63	5.50±0.73	3.67±0.64	2.64±0.57	5.04±0.52	5.70±0.57	3.05±0.31	4.11±0.52	5.03±0.40
length of calyx tube (mm)*	3.14±0.34	3.93±0.39	3.65±.34	3.21±0.30	4.89±0.28	4.09±0.30	3.52±0.17	3.38±0.28	3.50±0.21
length of calyx tooth (mm)*	4.90±0.54	6.29±0.63	4.14±0.54	4.07±0.49	6.40±0.44	5.97±0.49	3.59±0.27	4.48±0.44	3.99±0.34
width of calyx tooth (mm)*	1.72±0.30	2.36±0.35	2.13±0.30	1.66±0.27	2.59±0.24	3.41±0.27	1.83±0.15	2.38±0.24	2.47±0.19
length of standard petal (mm)*	9.36±0.86	11.71±0.99	8.43±0.86	11.38±0.77	13.64±0.71	8.20±0.77	10.80±0.43	9.66±0.71	10.16±0.55
width of standard petal (mm)	10.71±1.11	11.11±1.28	11.23±1.11	11.97±0.99	13.80±0.91	9.64±0.99	12.45±0.55	10.76±0.91	12.05±0.71
length of claw of standard petal (mm)	4.2±0.51	4.27±0.59	5.56±0.51	4.10±0.45	4.95±0.41	3.91±0.45	3.53±0.25	4.72±0.41	5.08±0.32
length of wing petal (mm)*	12.21±1.04	13.14±1.21	11.98±1.04	13.53±0.94	15.99±0.86	11.21±0.94	13.58±0.52	11.55±0.86	13.60±0.66
width of wing petal (mm)	4.23±0.46	4.54±0.54	4.29±0.46	5.03±0.41	5.32±0.38	3.90±0.41	4.90±0.23	4.28±0.38	4.55±0.29
length of claw of wing petal (mm)	3.79±0.49	4.62±0.57	4.83±0.49	3.82±0.44	5.00±0.40	4.06±0.0.44	4.10±0.24	3.79±0.40	4.98±0.31
length of keel petal (mm)	10.22±0.88	11.25±1.02	10.87±0.88	11.44±0.79	13.69±0.72	9.36±0.79	10.95±0.44	9.58±0.72	11.63±0.56
width of keel petal (mm)	3.04±0.28	3.72±0.33	3.49±0.28	3.83±0.25	3.86±0.23	2.90±0.25	3.66±0.14	3.49±0.23	3.97±0.18
length of claw of keel petal (mm)	4.95±0.55	5.44±0.64	6.16±0.55	5.34±0.50	6.51±0.45	4.56±0.50	5.15±0.27	5.13±0.45	6.13±0.35

4.2.1. (b) Cluster analysis

The cluster analysis yielded the phenogram in Fig. 4.3. At Euclidean distance 1.5, the phenon line indicates two major clusters: i) the cluster containing *P. fleta, P. gigantea* and *P. filifolia* and ii) the cluster containing *P. rigidula, P. ramulosa, P. usitata*, *P. aphylla, P. peratica* and *P. pullata*. Within cluster i), taking a cut off at Euclidean distance 0.7, *P. gigantea* forms two clusters, while *P. filifolia* and *P. fleta* each forms a single cluster (Fig. 4.3). Within cluster ii) at Euclidean distance 0.7, five clusters can be recognised: cluster a), consisting of some members of *P. usitata*; cluster b), made up of *P. ramulosa*, *P. usitata* and *P. rigidula*, with no clear separation between these three putative species; cluster c), consisting of two members of *P. pullata*; cluster d), containing some members of *P. pullata*, *P. peratica* and *P. aphylla*; and cluster e), consisting of two *P. aphylla* specimens (Fig. 4.3).

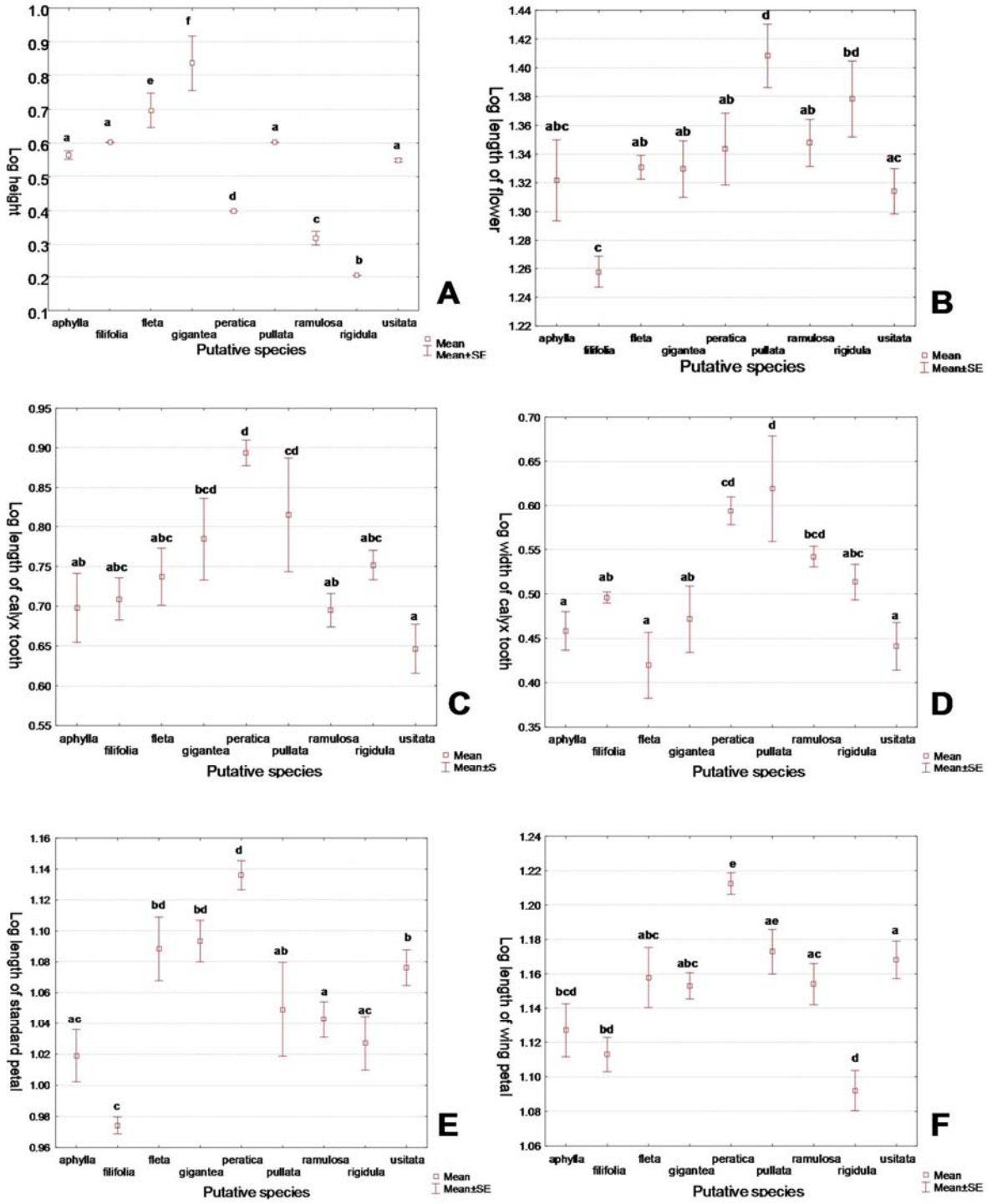

Figure 4.2. Plots of Mean±SE for some of the characters that were significantly different between putative species of the *Psoralea aphylla* complex. A=plant height, B= length of flower, C= length of calyx tooth, D=width of calyx tooth, E=length of standard petal and F= length of wing petal. Whiskers that have the same letter(s) above them are not significantly different (at $p<0.05$).

4.2.1. (c) Discriminant function analysis

In terms of the discriminant function analysis, the canonical analysis yielded eight canonical variates, with the first two jointly accounting for 98% of the total variation among the nine putative groups (species). The first CV was largely correlated with plant height (r=0.76), while the second CV was largely correlated with the length of the standard petal (r=0.42) and the length of the wing petal (r= 0.36). These two CVs were highly successful in their ability to assign specimens to the correct group as indicated by the posterior probabilities of the classification matrix. Of the 60 specimens examined in this study, all groups obtained 100% classification scores except only for *P. aphylla,* which had a classification score of 83%, as one of its specimens was misclassified. The discriminant function classified this specimen as belonging to *P. usitata* with a probability of 97% and when this specimen was re-visited, it was indeed confirmed to be *P. usitata*. In terms of distances between groups, all groups were significantly different from each other except for *P. aphylla* and *P. usitata* as well as the distance between *P. aphylla* and *P. pullata*: p> 0.05 (Table 4.3). A scatter-plot of the first CV against the second CV, showing how the different groups are distributed along the two axes is shown in Fig. 4.4. Eight distinct groups are unambiguously resolved in the DFA. With the exception of *P. aphylla* and *P. usitata*, which show some overlap, the rest of the distinct groups correspond to the different putative species (Fig. 4.4).

Table 4.3 p-values for distances between groups. Non-significant values are printed in bold.

	P. aphylla	*P. gigantea*	*P. filifolia*	*P. fleta*	*P. peratica*	*P. pullata*	*P. usitata*	*P. rigidula*	*P. ramulosa*
P. aphylla		0.000	0.000	0.040	0.030	**0.080**	**0.520**	0.000	0.000
P. gigantea	0.000		0.000	0.010	0.000	0.000	0.000	0.000	0.000
P. filifolia	0.000	0.000		0.010	0.000	0.000	0.000	0.000	0.000
P. fleta	0.040	0.010	0.010		0.000	0.000	0.000	0.000	0.000
P. peratica	0.030	0.000	0.000	0.000		0.000	0.000	0.000	0.000
P. pullata	**0.080**	0.000	0.000	0.000	0.000		0.000	0.000	0.000
P. usitata	**0.520**	0.000	0.000	0.000	0.000	0.000		0.000	0.000
P. rigidula	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.040
P. ramulosa	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.040	

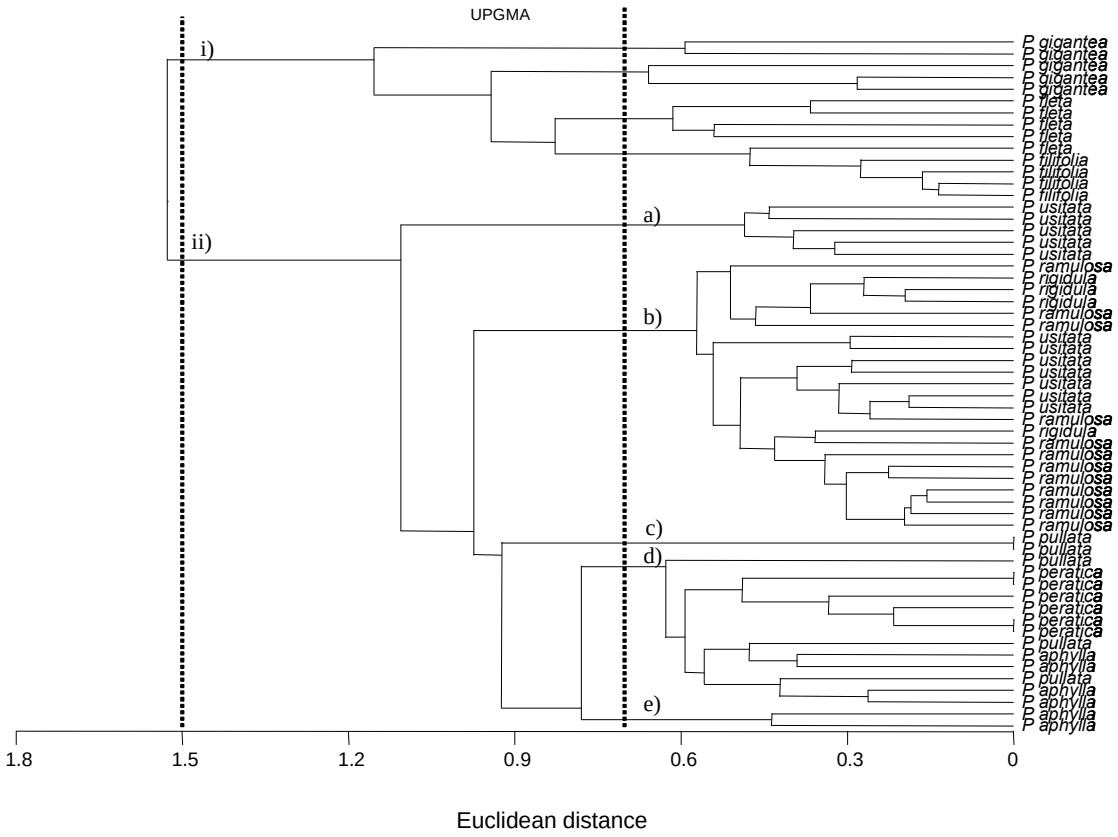

Figure 4.3 Phenogram of *Psoralea aphylla* complex based on UPGMA and Euclidean distances.

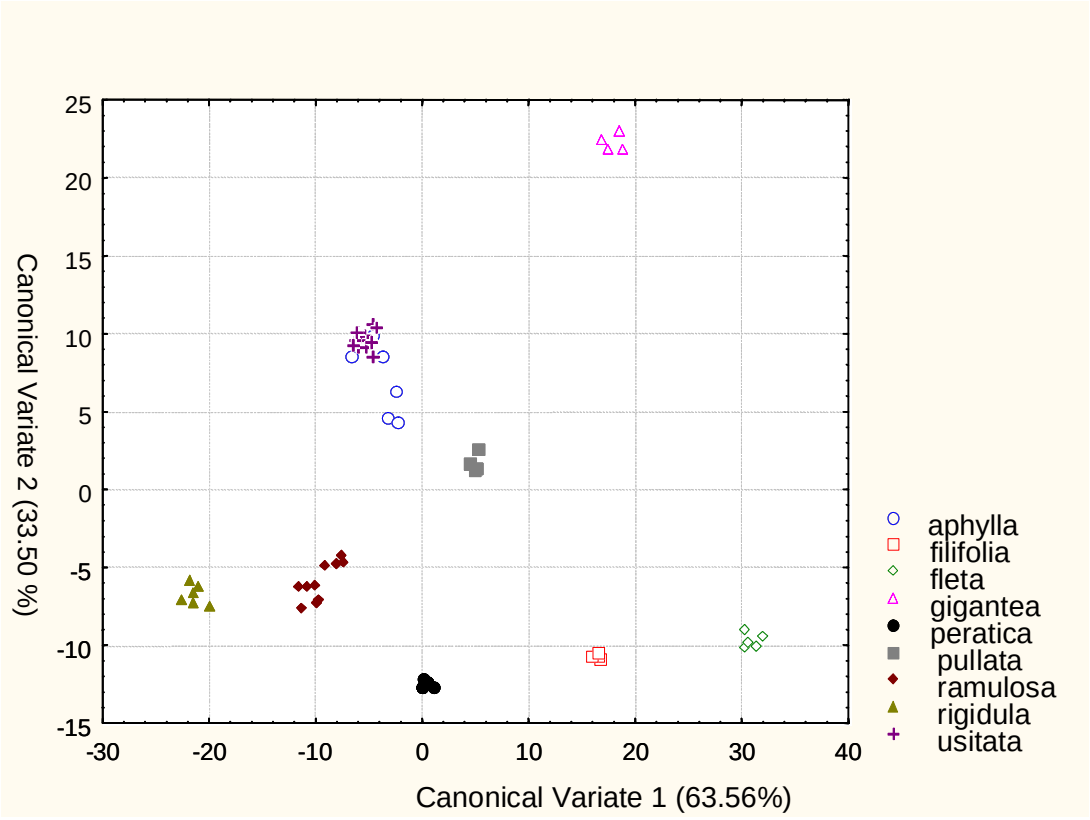

Figure 4.4. Ordination of specimens on the first and second canonical variates extracted in the discriminant analysis of the *Psoralea aphylla* complex.

4.2.2. *Psoralea pinnata* complex

4.2.2. (a) Basic statistics

A summary of the characters scored and their corresponding Mean±SE values calculated for each of the five putative species in the *Psoralea pinnata* complex is as shown in Table 4.4 (the actual measurements for all the specimens measured are provided in appendix 3.2). A majority of the characters differed significantly ($p<0.05$) between the groups, and these are marked with some asterisks in Table 4.4. Plots of the Mean±SE of some of these characters are presented in Fig. 4.5. The *P. pinnata*, *P. koudebergense* and *P. affinis* members have the longest petioles, while *P. brilliantissima*, *P. glabra* and *P. pedicellata* have shorter petioles (Fig. 4.5A). The longest basal lateral leaflets occur in *P. pinnata* and *P. koudebergense* specimens, while the other groups have comparably shorter basal lateral leaflets (Fig. 4.5B). The terminal lateral leaflets are longest in the *P. koudebergense* group and shortest in *P. glabra* and *P. pedicellata* specimens (Fig. 4.5C). The *P. koudebergense* group is also associated with the longest peduncles and claws of wing petals (Figs. 4.5D and E).

Table 4.4. Summary of morphometric characters measured for the *Psoralea pinnata* complex: Mean±SE. Characters marked with an asterisk are significantly different between groups at $p<0.05$. n=number of specimens measured.

Character	*brilliantissima* n=8	*pedicellata* n=23	*affinis* n=11	*koudebergense* n=5	*glabra* n=5	*pinnata* n=22
Number of leaflets	7.75±0.60	7.78±0.35	7.91±0.51	7.40±0.76	9.40±0.76	8.09±0.36
Length of petiole (mm)*	4.07±0.76	5.30±0.44	5.60±0.64	5.14±0.96	3.30±0.96	7.28±0.45
Length of rachis (mm)*	11.19±0.74	14.42±1.02	16.84±1.48	13.27±2.19	14.03±2.19	18.76±1.05
Length of rachis internode (mm)*	4.04±0.45	4.95±0.26	5.41±0.38	4.90±0.56	4.48±0.57	6.45±0.27
Length of basal laterals (mm)*	28.61±2.45	22.85±1.44	25.58±2.09	34.75±3.10	21.26±3.10	34.12±1.48
Length of terminal laterals (mm)*	24.29±2.17	18.42±1.28	20.33±1.85	29.87±2.74	16.67±2.74	25.11±1.31
Length of terminal leaflet (mm)*	20.96±2.38	18.68±1.40	20.11±2.02	30.78±3.00	15.68±3.00	25.47±1.43
Number of flowers per axil	2.63±0.15	3.00±0.09	3.00±0.13	3.00±0.18	3.00±0.18	2.91±0.88
Length of flower (mm)*	33.42±3.06	24.83±1.81	29.42±2.61	51.68±3.88	22.39±3.88	26.14±1.85
Length of peduncle (mm)*	16.99±2.79	9.81±1.64	13.68±2.68	31.71±3.53	9.58±3.53	12.83±1.68
Length of cupulum lobe (mm)*	4.97±0.27	3.32±0.16	0.27±0.23	5.56±0.35	3.23± 0.12	2.94±0.15
Width of cupulum lobe (mm)*	2.92±0.28	2.10±0.16	0.36±0.23	3.87±0.35	2.12±0.23	2.10±0.13
Number of cupulum lobes	2.25±0.25	2.22±0.09	2.18±0.13	2.20±0.20	3.00±0.20	2.36±0.09
Length of pedicel (mm)*	3.88±0.37	3.46±0.22	3.52±0.32	5.74±0.47	3.79±0.47	3.20±0.22
Length of calyx tube (mm)	4.65±0.28	4.18±0.16	4.44±0.24	4.76±0.36	4.56±0.36	4.28±0.17
Length of calyx tooth (mm)	3.75±0.36	4.28±0.21	4.53±0.30	3.48±0.45	3.42±0.45	4.05±0.21
Width of calyx lobe (mm)*	9.84±0.62	9.75±0.37	10.35±0.53	11.11±0.79	10.15±0.79	9.93±0.38
Length of standard petal (mm)*	11.51±0.83	13.05±0.49	11.12±0.71	14.15±1.05	9.72±1.05	9.99±0.50
Width of standard petal (mm)*	12.32±0.64	12.16±0.38	13.26±0.55	15.04±0.82	12.16±0.82	11.76±0.39
Length of wing petal (mm)*	10.89±0.58	12.55±0.34	14.28±0.50	13.34±0.74	13.04±0.74	12.41±0.35
Width of wing petal (mm)	5.19±0.32	5.02±0.19	5.26±0.27	6.24±0.40	4.90±0.40	4.44±0.19
Length of claw of wing petal (mm)	4.46±0.29	4.58±0.17	5.54±0.24	5.92±0.36	5.21±0.36	4.93±0.17
Length of keel petal (mm)*	8.96±0.67	9.92±0.40	12.04±0.57	10.33±0.85	11.18±0.85	11.26±0.40
Width of keel petal (mm)	3.94±0.14	3.95±0.08	3.95±0.12	4.53±0.18	3.81±0.18	3.48±0.08
Length of claw of keel petal (mm)	5.57±0.40	5.59±0.24	6.64±0.34	6.87±0.51	5.80±0.51	6.52±0.24

4.2.2. (b) Cluster analysis

The cluster analysis of the full data set for the *P. pinnata* complex yielded the phenogram in Fig. 4.6. With the exception of the two *P. pedicellata* specimens at the top of the figure, three main clusters are apparent at a Euclidian distance of 1.05 (Fig. 4.6). Cluster I, comprising *P. koudebergense*; cluster II, consisting of *P. pedicellata* and P. *brilliantissima*; and cluster III, comprising *P. pinnata*, *P. affinis* and *P. glabra*. Only cluster I (*P. koudebergense*) shows an exclusive clustering pattern with all the individuals belonging to one putative species. In cluster II although a majority of the *P. pedicellata* individuals cluster together, there are still four individuals that cluster together with *P. brilliantissima*. In the third main cluster, there is also no distinct clustering pattern among individuals of the same putative species.

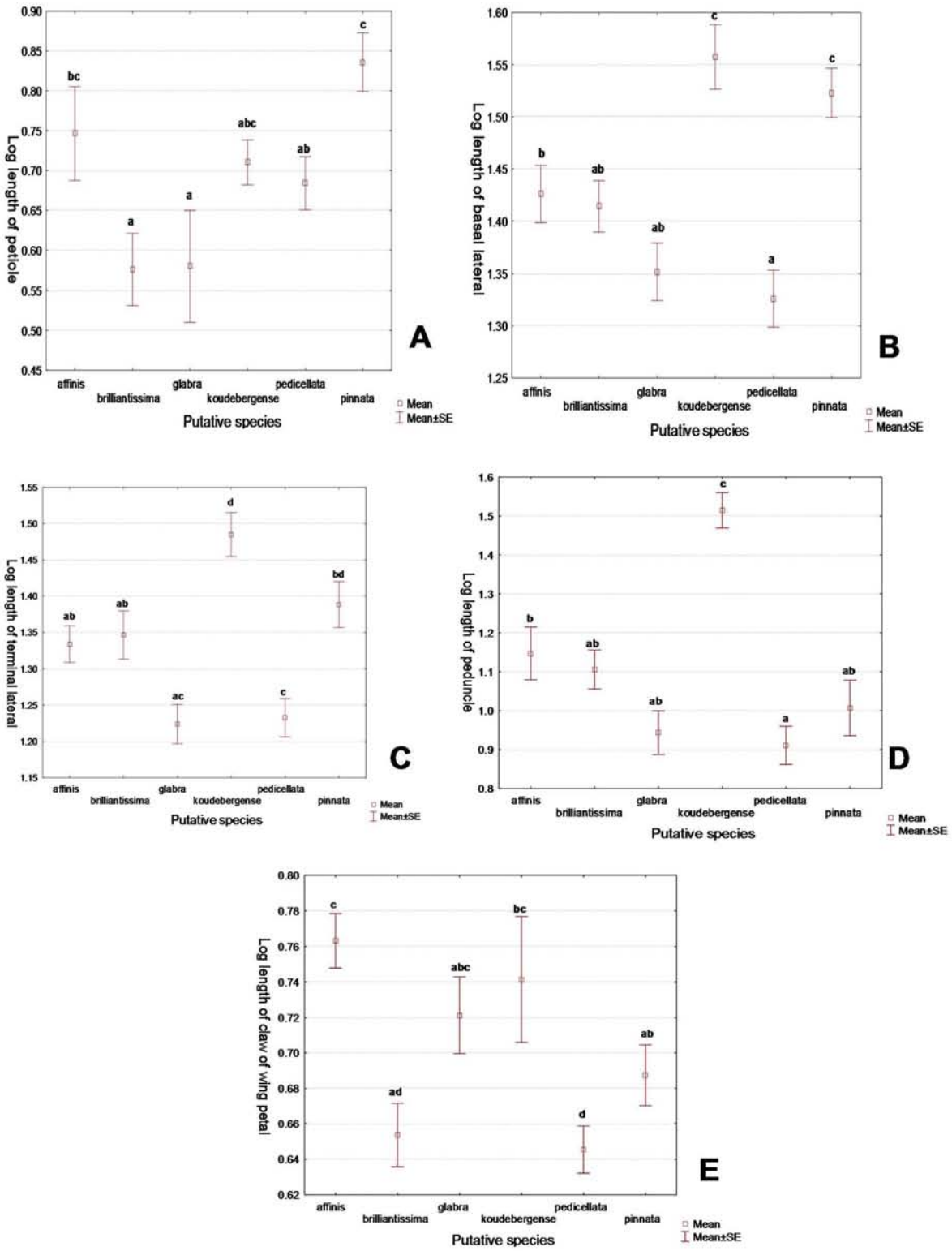

Figure 4.5. Plots of Mean±SE for some of the characters that were significantly different between the putative species in the *Psoralea pinnata* complex. A= length of petiole, B= length of basal lateral leaflet, C= length of terminal lateral leaflet, D= length of peduncle and E= length of claw of wing petal. Whiskers that have the same letter(s) above them are not significantly different at $p<0.05$.

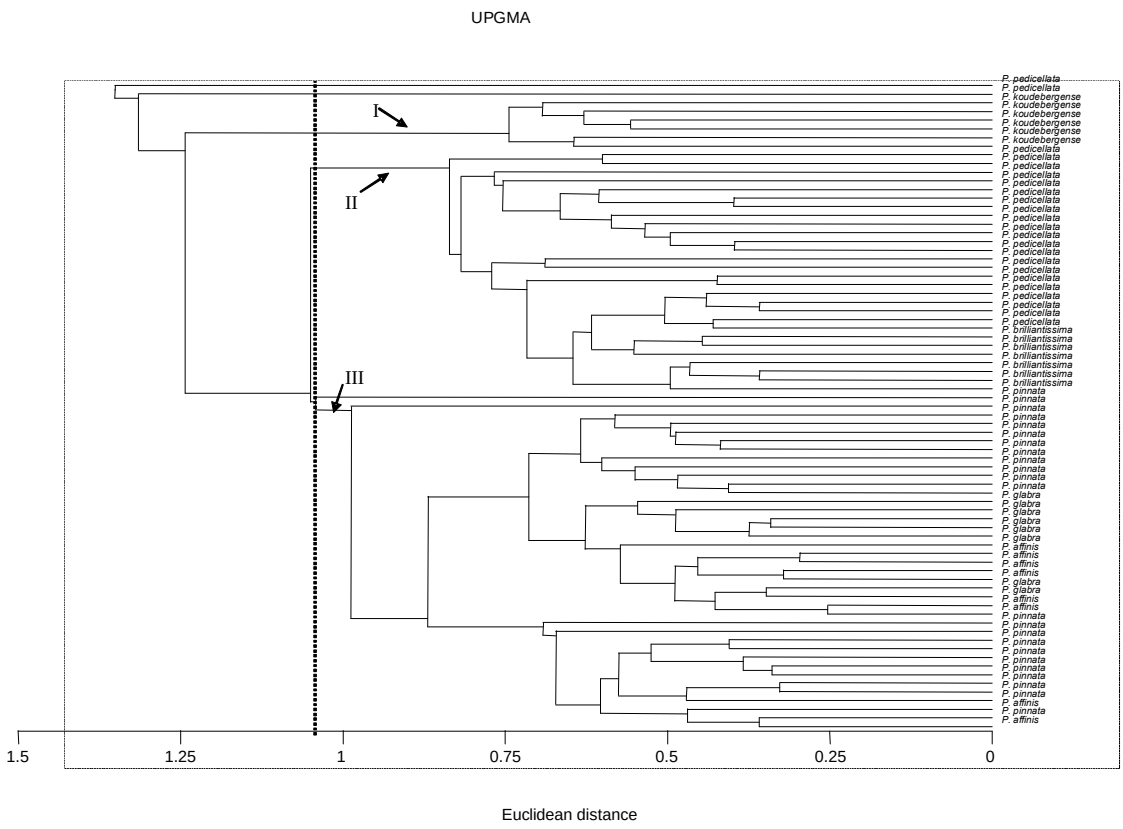

Figure 4.6 Phenogram of the *Psoralea pinnata* complex based on UPGMA as clustering algorithm and Euclidean distances.

4.2.2. (c) Discriminant function analysis

The DFA showed four canonical variates, with the first two jointly accounting for 95% of the total variation in the data. The first CV was largely correlated with the length of the rachis (r= 0. 40) and the length of the petiole (r= 0.26), while the second CV was largely correlated with the lengths of the basal and terminal lateral leaflets (r= 0.42 and 0.43, respectively). A scatter-plot of the first CV against the second CV, showing how the different groups are ordinated along the two axes is shown in Fig. 4.7. Distances between all groups were significantly greater than zero except for the distance between *P. affinis* and *P. glabra* (Table 4.5).

Table 4.5 p values for distances between groups. Non-significant values are printed in bold.

	P. affinis	*P. brilliantissima*	*P. glabra*	*P. koudebergense*	*P. pedicellata*	*P. pinnata*
P. affinis		0.00	**0.11**	0.00	0.00	0.04
P. brilliantissima	0.00		0.00	0.01	0.00	0.00
P. glabra	**0.11**	0.00		0.00	0.00	0.00
P. koudebergense	0.00	0.01	0.00		0.00	0.00
P. pedicellata	0.00	0.00	0.00	0.00		0.00
P. pinnata	0.04	0.00	0.00	0.00	0.00	

The first two canonical variates were highly successful in their ability to assign specimens to the correct group as indicated by the posterior probabilities of the classification matrix (Table 4.6). For the 75 specimens examined in this study, only one specimen was misclassified. This was a *P. affinis* specimen, which the DFA classified as *P. pinnata* with a probability of 0.53. Otherwise, all groups obtained 100% classification scores (Table 4.6).

Table 4.6 Classification scores matrix for the five putative species. Misclassifications are printed in bold.

	Percent	*P. affinis*	*P. brilliantissima*	*P. glabra*	*P. koudebergense*	*P. pedicellata*	*P. pinnata*
P. affinis	100	8	0	0	0	0	0
P. brilliantissima	100	0	7	0	0	0	0
P. glabra	100	0	0	8	0	0	0
P. koudebergense	100	0	0	0	6	0	0
P. pedicellata	100	0	0	0	0	23	0
P. pinnata	95.65	1	0	0	0	0	22
Total	98.67	9	7	8	6	23	22

The first canonical variate (CV) explained 91.1% of the total variance in the data. This CV was largely correlated with the length of the cupulum lobe (r= -0.6) and the length of the rachis (r= -0.39). This CV separates the complex into two distinct major groups: the group containing *P. pinnata*, *P. affinis* and *P. glabra*; and the group comprising of *P. pedicellata*, *P. koudebergense* and *P. briliantissima* (Fig. 4.7). Canonical variate 2, which carries the next highest variation (4.2 %) was largely correlated with the flower length and the length terminal lateral leaflet (r= -0.49, -0.43, respectively). There is no significant separation between *P. affinis* and *P. pinnata* along CV2. There is also one specimen of *P. affinis*, which overlaps with the otherwise distinct *P. glabra*. In the second group, CV2 resolves *P. pedicellata* as a distinct group (this includes the two *P. pedicellata* specimens which were outliers in the Cluster analysis), but shows no separation between *P. brilliantissima* and *P. koudebergense* (Fig. 4.7).

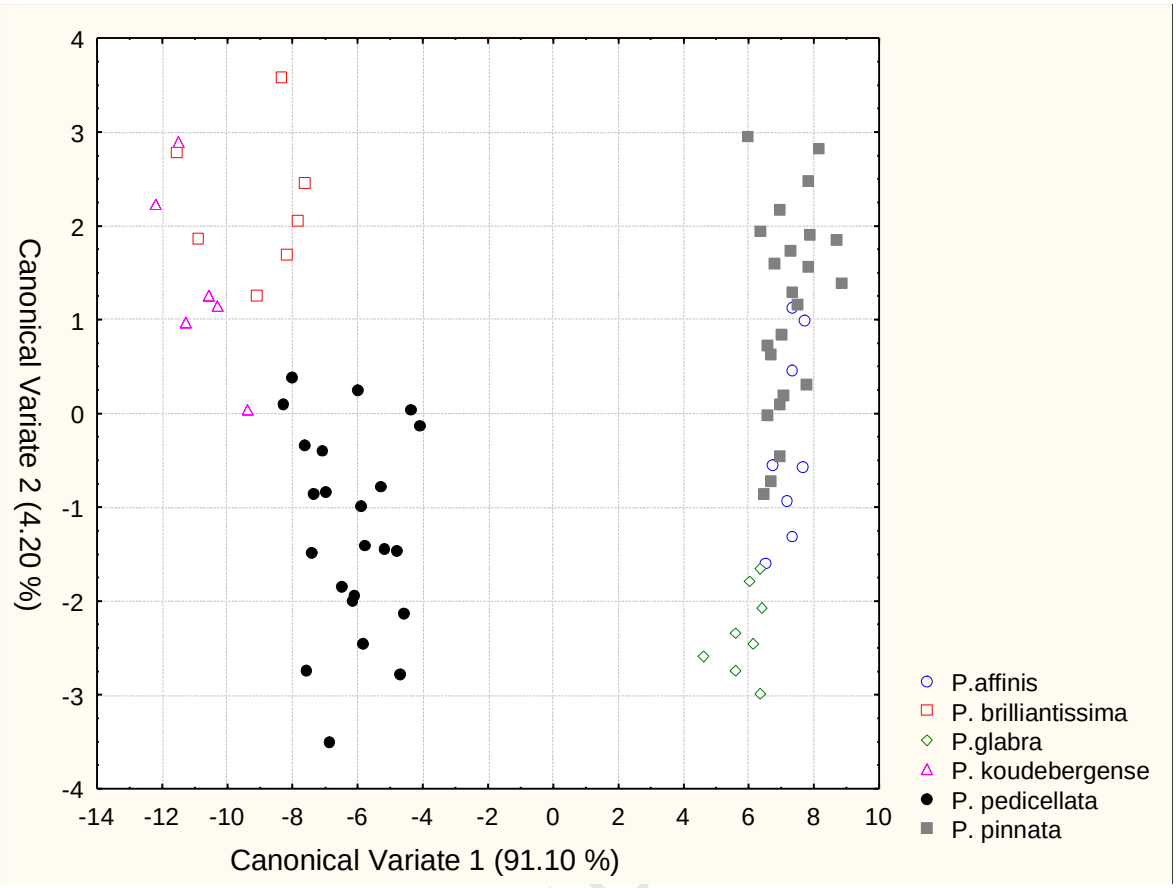

Figure 4.7 Ordination of specimens on the first and second canonical variates extracted in the discriminant function analysis of the *Psoralea pinnata* complex.

4.3. Discussions and conclusions

4.3.1. *Psoralea aphylla* complex

4.3.1 (a) Cluster analysis

The cluster analysis showed two main clusters. In both clusters, the putative species within each cluster share some common features that were not scored as part of this study due to either being unsuitable for the kind of analysis or the fact that they were not reflected on herbarium specimens. *P. aphylla*, *P. pullata* and *P. peratica*, which together formed cluster d) (Fig. 4.3) are characterised by leafless seasonal shoots that are covered with scales. Their flowers are hairy, but there is variation in the colour and degree of hairiness, with *P. aphylla* being lightly covered in black and white hairs; *P. peratica* sparsely covered in white hairs, while *P. pullata* is completely covered in long black hairs. In terms of distribution, *P. aphylla* is limited to the Cape

Peninsula; *P. peratica* is endemic to the Piketberg mountain range, while *P. pullata* is restricted to the Southern Cape, in the Hermanus to Potberg area. The species which formed cluster i) i.e. *P. filifolia*, *P. fleta* and *P. gigantea* are characterised by a tiny filiform leaflet on the seasonal shoots and congested inflorescences. They differ in their general habit, with *P. filifolia* being shrubs; *P. fleta* being tall and slender willowy shrubs while *P. gigantea* is a tree, with a trunk diameter of about 40 cm. *Psoralea ramulosa*, *P. rigidula* and *P. usitata*, which together formed clusters a) and b) have glabrous shoots, no scales, and axillary inflorescences. They differ in that *P. rigidula* is a small resprouting suffrutex with numerous stems that start branching at the base and is restricted to Bainskloof Mountains; *P. ramulosa*, a Cedarberg to Clanwilliam endemic is a multi-stemmed shrub, while the more widespread *P. usitata* is a large shrub with stiff branches that hang downwards when in flower. The fact that these groupings are reflected in the cluster analysis results suggests that the characters used in this study capture a substantial amount of information about the extent of variation between the groups.

4.3.1. (b) Discriminant function analysis

The results of the discriminant function analysis (DFA) demonstrate that there is significant morphometric distinction between the putative groups forming the *P. aphylla* complex. They support the hypothesis that *Psoralea aphylla* as currently circumscribed is an assemblage of distinct entities that can be recognised as different taxa. The DFA also indicates that although most of the univariate measures used in this study overlap, in multivariate space the groups are distinct. Furthermore, all the groups that were not well resolved in the cluster analysis were well separated in the DFA. This is not surprising because the two techniques operate in different approaches with different goals as discussed earlier. For example, in the cluster analysis, there were two main clusters; each having sub-clusters in it but with some overlap in placement of some specimens. *P. ramulosa* and *P. rigidula*, which had some overlap in the cluster analysis, separate well in the discriminant analysis. The same applies to *P. pullata* and *P. aphylla*, with *P. peratica* coming out very distant from these two. *P. fleta*, *P. filifolia* and *P. gigantea*, which form one cluster in Fig. 4.3, each having its own sub-cluster, are unambiguously separated in the DFA results (Fig. 4.4). However, *P. usitata*, which formed two separate clusters in the cluster analysis (Fig. 4.3), is not separated from *P. aphylla* in the DFA results (Fig. 4.4). This overlap is also supported by the distance comparison, which showed that these two are not significantly different (Table 4.3). This suggests that these two taxa are not distinct in multivariate space, and thus indicating that they may have to be merged into one species or one of them is to be recognised as a subspecies.

Stace (1989) suggested three main criteria for recognising species. First, individuals should bear a close resemblance to one another such that they are always readily recognisable as members of that group. Secondly, there should be gaps between the spectra of variation exhibited by related species and if there are no such gaps, then the taxa should be merged into a single species. Finally, each species must occupy a definable geographical area and be demonstrably suited to the environmental conditions that it encounters. For this study, these criteria, along with that of Sneath (1976), which defines species as distinct phenetic clusters, are used. At the infraspecific rank, the definition of sub-species by Mayr (1963) is applied. This defines a sub-species as an aggregate of populations in a geographic subdivision of the speciesô range that differs taxonomically from other populations. It is unfortunate that the phylogenetic reconstruction (Chapter 2 of this dissertation) was not well resolved at the specific level, yet it would have allowed for a comparison of the phylogenetic relationships between the various groups under these complexes and thus add value to the classification.

4.3.1. (c) Conclusions

The findings from this study indicate that the *P. aphylla* complex is an assemblage of several distinct taxa. Although the data set did not include all morphological characters, it is unlikely that additional characters or more sampling will change this conclusion, but rather would improve the power to discriminate between these groups. Based on the criteria of Stace (1989) and Sneath (1976) the data supports the recognition of *P. filifolia*, *P. peratica*, *P. rigidula*, *P. fleta*, *P. gigantea* and *P. ramulosa* as distinct species. *P. filifolia* and *P. peratica* are already validly published species, *P. gigantea* is a new species that was discovered as part of the current study and the rest of the species are the putative species names proposed by Stirton.

However, for *P. aphylla* and *P. usitata*, Staceôs (1989) second criterion would suggest merging the two into one species, while Mayr (1963) would suggest recognizing *P. usitata* as a sub-species of *P. aphylla*. *P. aphylla* is an erect shrub, with drooping branches, and dark green stems. Its leaves are reduced into long, congested scales, while *P. usitata* is an erect spreading shrub, with tan stems and no congested scales. *P. aphylla* has a completely bifid cupulum that overlaps with the calyx, while in *P. usitata* one of the lobes of the cupulum has a cleft (making it appear like a trifid cupulum) and never overlaps with the calyx. Furthermore, in *P. aphylla* the seasonal shoots are covered with white silvery hairs, while *P. usitata* is completely glabrous. The distribution ranges of the two are also different (see maps in the taxonomic treatment section).

Therefore, because of these differences, I choose to follow Mayrôs (1963) definition of subspecies and thus recognise *P. usitata* as a sub-species of *P. aphylla*.

4.3.2. *Psoralea pinnata* complex

4.3.2. (a) Cluster analysis

The three main clusters obtained from the phenogram (Fig. 4.6) reflect the levels of gross morphological similarity among the groups. Members of clusters I and II i.e. *P. pedicellata*, *P. koudebergense* and *P. brilliantissima* share the following characters: they have large flowers characterised by long pedicels and broad bifid cupulm lobes, and calyces which are covered by black hairs. Also, the inflorescences are longer than the subtending leaves and each axil may have three or more flowers borne in it. The opposite is true for the species that were in cluster III i.e. *P. pinnata*, *P. affinis* and *P. glabra*. The cluster analysis indicates that there is a high level of similarity among the members of cluster III, reflected by the overlap of several specimens from each of the three putative species, such that there is no clear separation between *P. pinnata*, *P. affinis* and *P. glabra*. For *P. pinnata* and *P. affinis*, this is not surprising because even if gross morphology of the two is examined, both living material and herbarium specimens, there are no characters that separate the two. *P. glabra* on the other hand differs from these two by both gross morphology and geographical distribution.

4.3.2. (b) Discriminant function analysis

The DFA results indicated that there are two distinct groups within the complex, one consisting of *P. pinnata*, specimens that are currently recognised as *P. affinis* and *P glabra*; and the other one consisting of the three putative species *P. pedicellata*, *P. koudebergense* and *P. brilliantissima* (Fig. 4.7). The overlap in morphometric space between what is currently recognised as *P. affinis* and *P. pinnata* was observed in both the cluster analysis and the DFA. The lack of separation between *P. affinis* and *P. pinnata* is evidence that there is no difference between the two and therefore they should be recognised as a single species. Besides the characters used in this study, the two overlap in many other features including habit, geographic distribution in the Cape Floristic region and flowering time. Even on herbarium sheets, several botanists have been applying the two names in such a way that the two names appear to be interchangeable.

An examination of the type specimens showed that there were no characters to separate *P. pinnata* from specimens that are currently recognised as *P. affinis*. However, the type specimen

of *P. affinis* was found to correspond with specimens that had been tentatively classified as *P. pedicellata* in this study. This suggests that the name *P. affinis* should be applied only to such specimens. On the other hand, *P. glabra*, which Harvey & Sonder (1862) also sunk into *P. pinnata*, emerged as distinct in the DFA (Figure 4.7). Its type specimen was also distinct from the other two, suggesting that *P. glabra* can be recognised as a distinct species. It differs from *P. pinnata* or *P. affinis* in its growth habit and many other features which are described in the taxonomic treatment (Section 4.4). Furthermore, its geographic range extends from the Eastern Cape all the way to KwaZulu-Natal and Swaziland and therefore does not overlap with either *P. affinis* or *P. pinnata*.

The DFA results showed an overlap in morphometric space between *P. koudebergense* and *P. brilliantissima*, which refutes my hypothesis that the two could be separate species. However, this is not surprising because the gross morphology of these two is quite similar; the main difference being that *P. koudebergense* is a more robust tree with a trunk diameter of nearly 50 cm while *P. brilliantissima* is a large shrub that branches profusely to form a rounded crown. However, the tree form of *P. koudebergense* is restricted to river valleys in Koudeberg Mountain. In such a sheltered habitat, it is less likely to be experiencing disturbance, particularly fire and therefore is able to grow into tall trees. Individuals with the same morphology (as the tree form of *P. koudebergense*) occurring outside the valley have the same general habit as *P. brilliantissima*. Therefore, these two (*P. koudebergense* and *P. brilliantissima*) can be recognised as one species.

4.3.2. (c) Conclusions

The discriminating power of the morphometric analyses and the correlation of morphometry and other morphological features of the groups as described in Section 4.4 provide evidence for classifying the *P. pinnata* complex as follows: *Psoralea pinnata* is to include all specimens that are presently recognised as *P. pinnata* and *P. affinis*. Since the type specimen of *P. affinis* is similar to those specimens that were tentatively classified as *P. pedicellata* in this study, the name *P. affinis* is to be applied to such. *Psoralea glabra* is recognised as a distinct species, with a distribution range from the Eastern Cape, KwaZulu-Natal up to Swaziland; and the new species, *Psoralea koudebergense* is described. This classification follows the criteria of both Stace (1989) and Sneath (1976).

4.4. Taxonomic treatment

4.4.1. *P. aphylla* complex

Key to species of the *Psoralea aphylla* complex

1a. General habit trees, trunk diameter greater than 30 cm.é é é ..é é é é ***P. gigantea sp nov.***

1b. General habit not trees, trunk diameter less than 30 cm..é é é é é é é é .é é é é é .é 2

2a. Growth form shrubs, more than 1m tall; 1-3 stems é é é é ..é é é é é é é é ....é é 3

2b.Growth form suffrutices, less than 1m tall; > 3 stemsé é é é é ***P. rigidula*** **C.H.Stirt. ined.**

3a. Flowering shoots 1-3 foliolateé é é é é é é é ...é é é é é é é é .é é é é é ..4

4a. Cupulum trifid, lobes not bilabiateé é é é é ..é é é é é .***P. fleta*** **C.H.Stirt. ined.**

4b. Cupulum bifid, with one of the lobes variously bilabiate.é .***P. filifolia*** **Eckl. & Zeyh.**

3b. Flowering shoots not leafyé é é é é é é é é é é é é é é é é é ..é é é é é ...5

5a. Seasonal shoots hairy; calyx tube hairyé é é é é é é é é é é é ...é é é ..é é é é 6

6a. Cupulum bifid with both lobes non-bilabiate, overlapping with the calyx tube; seasonal shoots with clasping, persistent scales.....é ***P. aphylla*** **subsp. *aphylla*.**

6b. Cupulum bifid with one of the lobes bilabiate, lobes not clasping; not overlapping with the calyx tube; seasonal shoots with patent, caducous scalesé é é é é é é ..8

5b. Seasonal shoots glabrous; calyx tube glabrousé é é .é é é é é é é é é é é é é é é 7

7a. Plants 1-1.5 m tall; resprouting; flowers borne in upper parts of seasonal shoots; peduncles long and flexuousé é é é é é é é é ...é é é é é é é é ..***P. ramulosa*** **C.H.Stirt. ined.**

7b. Plants 2-3 m tall; reseeding; flowers borne in most axils of seasonal shoots; peduncles short and stouté é é ..é .***P. aphylla*** **subsp. *usitata*.**

8a. Shoots canescent; calyx tube hairs long and black;.é é é é é é ***P. pullata*** **C.H.Stirt. ined.**

8b. Shoots pilose; calyx tube hairs short and silveryé é é ...é é ..é ....***P. peratica*** **C.H.Stirt.**

1. ***Psoralea aphylla* L.** Pl. Rar. Afr. 15 (1760); Amoen. Acad. 6: 93 (1763); Handl. Pl. Kruidk. 5: 552 (1776); Poir. In Lam. Encycl. 5: 681 (1804); Dietrich, Lex. Gart. Bot. 7: 607 (1807); Sims in Bot. Mag. 42: 1727 (1815); DC., Prodr. 2: 217 (1824) pro parte; E. Mey. In Linnea 7 :165 (1823); G. Don., Gen. Syst. 2: 202 (1832); Eckl. & Zeyh., Enum. 227 (1836); E. Mey., Comm. 84 (1936); Klinsman, Clav. Breyn. 9 (1855); Harv. in Harv. & Sond., Fl. Cap. 2: 145 (1862); Hamer, Wild Flowers Cape 23, t.71 (1926); Salter in Adamson & Salter, Fl. Cape Penins. 489 (1950); Forbes in Bothalia 3: 5 (1930); Kidd, Wild Flowers Cape Penins. T.(1973) non Reichenbach (1822); Stirton in S. Afr. J. Bot 64: (1998); Stirton & Schutte in Goldblatt & Manning, Strelitzia 9:505 (2000). **Type:** Cape of Good Hope, Oldenland, 685 (G) [Lectotype: designated by Stirton in Turland & Jarvis (ed.), *Taxon* 46: 479 (1997).] **Synonym:** *Psoralea decidua* Berg., Descr. Pl. Cap. 220 (1776) nom. illegit. non Thunb.

Psoralea aphylla* subsp. *aphylla

Small to large slender shrubs; 1.5- 3 m tall; erect; colonial and forming dense clumps; reseeder. **Stems** single to multiple; woody throughout and terete. **Branches** erect; commencing at a height of about 1m; flexuous and minutely glandular. **Seasonal shoots** densely covered in white to silvery pubescence; with a few randomly scattered glands. **Leaves** absent in mature plants, but seedlings have 5-foliolate leaves; some branches of young stems may have 3-foliolate (15-30mm long) leaves, otherwise seasonal shoots covered in scales. **Scales** 4-6 mm long, 1.5-3 mm wide; persistent; lanceolate; clasping the shoots; glabrous and tightly congested on the bare branches of seasonal shoots. **Inflorescences** axillary; lax, with one flower per axil. **Flowers** 16-20 mm long; dark purple; maturing more or less simultaneously; flower bracts inconspicuous, replaced by a tuft or ring of hairs. **Peduncles** 3-4 mm long; stout and rigid. **Cupulum** bilobed; lobes equally developed and overlapping with the calyx tube, lobes glabrous and minutely glandular, but hairy on teeth. **Pedicels** 2-4 mm long. **Calyx tube** 2.5-3.5 mm long; lightly covered in black and white hairs. **Calyx teeth** subequal, with keel tooth slightly longer; longer than calyx tube; carinal calyx tooth 4-6 mm long, 1-2.5 mm wide, acute; broader than other four teeth; vexillar calyx lobes free above the tube; calyx shorter than corolla; inner face of calyx teeth sparsely covered in small black stubby hairs; calyx glands constant in size but more dense on the tube. **Standard petal** 8-10 mm long, 9-11 mm wide; claw 3-5 mm long, elongated and narrow; auricles well-developed; apex emarginate. **Wing petals** 10-13 mm long, 3-5 mm wide; fused to but longer than keel petals; claw 3-4.5 mm long. **Keel petals** 9-12 mm long, 2-3.5 mm wide; claw 4-6 mm long.

Diagnostic characters: *P. aphylla* subsp. *aphylla* differs from all other members of the complex by the presence of large, clasping and persistent scales on its seasonal shoots. Its flowers have a bifid cupulum that clasps up to the base of the calyx and is covered in white or silvery hairs.

Note: There is variation between the lowland and montane forms of this species as indicated below, and more work might indicate that these are distinct taxa. See Plates 1a and 1b for illustrations.

Lowland form: *Stems* 1-2; greenish-tan; smooth; with numerous white lenticels. *Seasonal shoots* erect, becoming floppy when in flower; scales not overlapping and not tightly clasping. *Flowers* bluish mauve; wing petals white, horizontal; elongate nectar guide with intense purple vertical flash.

Montane form (congesta): *Stems* 1-3; greyish black; rough. *Seasonal shoots* clustered on branch ends; erect and stiff even when in flower; scales tightly clasping and overlapping, densely white pubescent below. *Flowers* purple; wing petals white, held vertically; nectar guide M shaped with white flash.

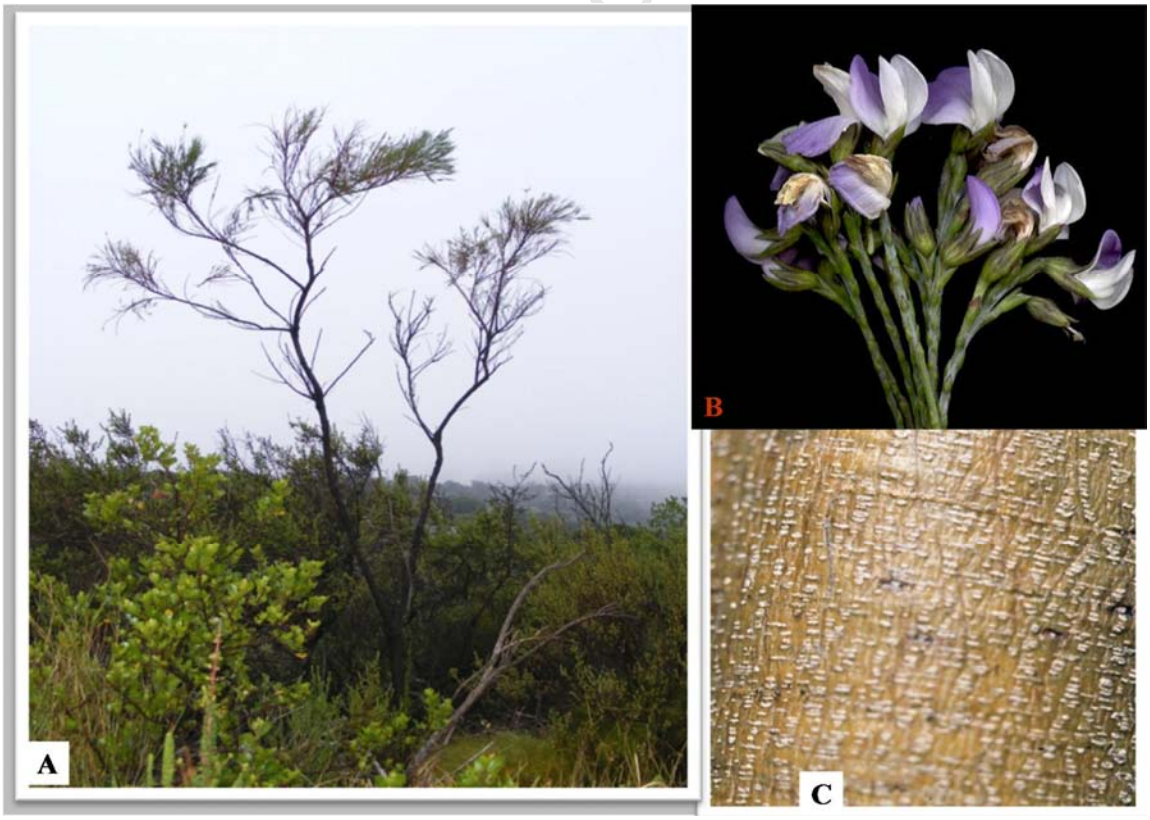

Plate 1a. *Psoralea aphylla* subsp. *aphylla*. A: general habit, B:flowering shoot, C: stem bark.

Plate 1b. *Psoralea aphylla* subsp. *aphylla*, montane form ócongestaô. A: general habit, B: flowering shoot, C: stem.

Habitat: Mountain and Lowland fynbos, stream banks and seepages.
Flowering time: October - May
Altitude: 80-1000 m
Distribution Cape Town: Table Mountain, Camps Bay, Silvermine Nature Reserve (Fig. 4.8).

Specimens examined

Western streams Table Mountain, 3318CD, Forbes, H.M.L., 140 (PRE); Western slopes of Table Mountain, 3318CD, Galpin, E.E., 3965 (PRE); Cape Point Reserve, 3418AB, Goldblatt, P., 1550 (PRE); No precise locality, 3318CD, Ecklon, C.F., 47 (PRE); 3318AB, Smuts, J.C., 1180 (PRE); Road Camps Bay, Hout Bay, 3418AB, Young, R.G.N., 230 (PRE); Tafelberg, Wetterstation, 3318CD, Werdemam, E. & Oberdieck, H., 42 (PRE); In prerupto mont. tabul, 3318CD, Ecklon, C.F., 660 (PRE); Camps Bay, Cassidy, 44 (BOL); Cape Flats, Ecklon, C.F., s.n, (PRE); Tafel & Duyvelsberg, Ecklon, C.F. & Zeyher, 1531 (BOL); Cap Bonae Spei, Forster, 1345 (BOL) (PRE); Cape Point Nature Reserve. Table Mountain, Macowan, 924 (BOL); near Sirkel Vlei, Salter,

7894 (BOL); Moddeerdam, Cape Peninsula, Salter, 7977b (BOL); Sieber, s.n, L; Behind Simonstown, Smuts 725 (PRE); Alongside Louwsrivier, Simonstown, 3418BD, Stirton, 9993 (PRE); Blousteenberge, 3418BD, Stirton, 10006, (PRE); Saddleback , Devil's Peak, Cape Town, Thode, 8347 (PRE); Saddleback, Cape Town, Thode, s.n, (PRE); Slope over Wynberg ranges, Wolley-Dod, 21 (BOL); Klawer Vley, Wolley-Dod, 298 (BOL); Vlakkenberg, Wolley-Dod, 2257 (BOL); Pariesvlei river valley, 3419DA, Stirton, C.H, 9739, (PRE).

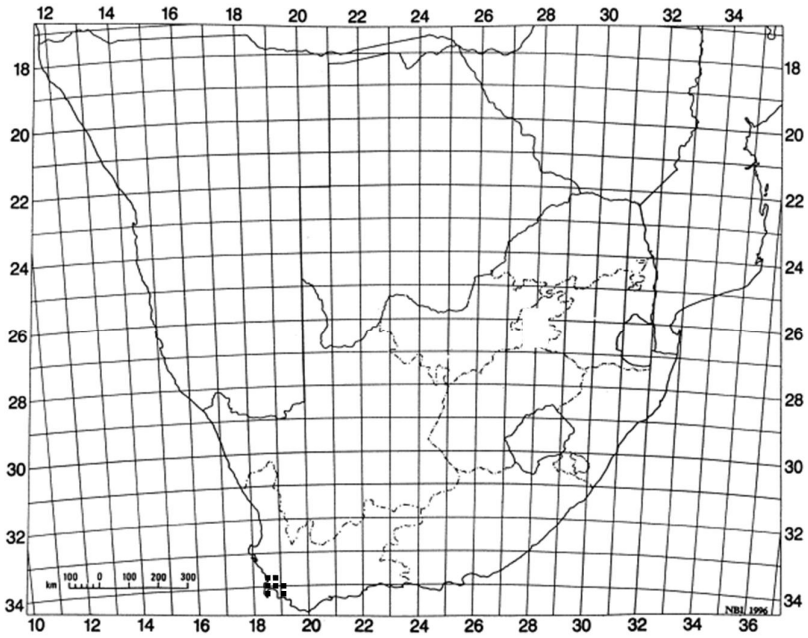

Figure 4.8. Known distribution of *Psoralea aphylla* subsp. *aphylla*.

Conservation status: Vulnerable, may be endangered in the future because of habitat conversion for human settlement.

2. ***Psoralea aphylla* subsp. *usitata*** (C.H. Stirt. ined.) Dludlu, Muasya & Stirton, **comb. & stat. nov. Type:** Klein Drakenstein, near Salem, Paarl. E.E. Galpin, 33213. (Holotype: BOL, Isotype: PRE, NBG).

Large shrubs; 2.5-3 m tall; reseeder, but other forms coppice after fires; usually solitary, but sometimes colonial and forming dense clumps. **Stems** greenish grey to tan; one to three but not more; woody throughout; terete; weakly furrowed. **Branches** erect; emerging in middle portions of plants. **Seasonal shoots** erect; smooth; glandular; with minute striations. **Leaves** sometimes

present, trifoliolate, otherwise aphyllous. **Stipules** 2.5-3.5 mm long, 0.5-1.7 mm wide; caducous; not tightly congested. **Inflorescences** axillary; pseudo-spicate; lax, with one flower per axil. **Flowers** 25-26 mm long; blue and white; maturing sequentially; bracts reduced to tuft or ring of hairs. **Peduncles** 5-12 mm long; stout and rigid, but may also be filiform and flexuous. **Cupulum** bilobed with one of the vexillar lobes variously bilabiate; glabrous; lobes equally developed, narrowly triangular. **Pedicels** 2-6 mm long. **Calyx tube** 2.5-4 mm long; glabrous or glabrescent; longer than the teeth. **Calyx teeth** subequal, the keel tooth slightly longer; the lateral and vexillar calyx teeth acute, falcate, narrowly triangular the carinal calyx tooth 2.5-5 mm long, 1.5-2.5 mm wide; acute, broader than the other four teeth; the vexillar calyx lobes fused for up to one third of their length above the tube; calyx shorter than corolla; ribs distinctly thickened; glands dense, constant in size and equally distributed across the teeth and tube. **Standard petal** 10-12 mm long, 11-13 mm wide; claw 2-5 mm long; elongated and narrow; apex emarginate. **Wing petals** 11-16 mm long, 4-6 mm wide; up-curving; auricles present; claw 3.5-5 mm long; fused to, but longer than keel petals. **Keel petals** 9-12 mm long, 3-4 mm wide; claw 4-6 mm long.

Diagnostic features: *P. aphylla* subsp. *usitata* differs from the other members of the complex in that its shoots and calyx tubes are glabrous and the leaves are reduced to appressed bright green glabrous scales. It also has tan stems with longitudinal fissures. See Plate 2 for some illustrations of *Psoralea aphylla* subsp. *usitata*.

Habitat: mountain and lowland fynbos on wet areas and seepages.
Flowering time: September to May
Altitude: 170-1600 m
Distribution: widespread within the Western Cape, particularly mountains, but does not overlap with *P. aphylla* subsp. *aphylla* (Fig. 4.9).

Specimens examined

Worcester, 3319DD, Smith, R.D, 101 (PRE); Langerberg, Gysmanshoek Pass, 3321CC, McDonald, D.J, 1488 (PRE); Jonkershoek, 3318DD, van De Merwe, P., 2119 (PRE); Jonkershoek, 3218DC, Strey, R.G., 20 (PRE); Hibertsdale top of Cloete Pass, 3321DD, Vlok, J.H.J., 1880 (PRE); Grabouw Viljoen Pass, 3419AA, Strey, R.G, 2901 (PRE); Dashung near Stoms Vlei, 3419BB, Stokoe, T.P., 55186 (PRE); Hermanus, 3419AC, Sutton, J.D, 447 (PRE); 3419BB, Stirton, C.H, 8219 (PRE); Waterkloof Farm, 3318DD, Nel, P & Boucher, C., 75 (PRE); Kogelberg State Forest, 3418BD, Kruger, I.J., 446 (PRE); Upper Zachariashoek

catchment, 3319CC, Haynes, R.A., H298 (PRE); Baviaanskloof, 3319CA, Taylor, H.C., 6603 (PRE); Mts above Corente river, Riversdale district, 3421AA, Muir, J.I., 80, (PRE); Stanford Road to Hermanus, 3419AD, Germishuizen, 4120 (PRE); Lebanon river bed, Grabouw, 3419AA, van Der Zel, D.W, 235, (PRE); Lebanon State Forest, Jakkalsrivier, 3419AA, Kruger, F.J., 1600 (PRE); Riviersonderend Mts, Vooruitzight, 3419BB, Rourke, J.P., 2077 (PRE); Jakkalsrivier, 3419AA, Kruger, F.J., 1098 (PRE); Bainskloof, 3319CA, Schlechter, F.R.R., 1246 (PRE); Riversdale, 3421AB, Schlechter, F.R.R., TRVF1246 (PRE); Poterville Mts, 3319AA, Thompson, M.F., 2030 (PRE); Tulbagh, 3319AC, Rogers, F.A, 17395 (PRE); Swellendam Zuurbraak, 3420BA, Thode, A2317 (PRE); Wemmershoek, 3319CC, Smuts, A.J.C., 1122, (PRE); Strawberry Hill, Heidelberg, 3420BB, Stokoe, T.P., SAM61583 (PRE); Nat Boutebok Park, 3420AB, Marais, J., 36 (PRE); Jonkershoek, 3318DD, Roalin, R.J., 3268 (PRE); Modderkloof West side of Paardeberg, 3318DB, Hugo, L., 2594 (PRE); Schoemaakers rivier Farm, Napier, 3419BD, Stirton, C.H., 8220 (PRE); Moerces river, 3322CC, Fourcade, H.G., 6281 (PRE); Jonkershoek, 3318DD, Bos, J.J., 1386 (PRE); Matroosberg MCA, Compt 3.4 Kanetvlei, 3319CD, Zeeman, H.T., 17 (PRE); Garcia's Pass near Toll house, 3321CC, Fellingham, A., 443 (PRE); Gymashoek pass, Riversdale, 3321CC, van Wyk, C.M., 724 (PRE); Riversdale, second stream flowing into Korente river, 3421AA, Bohenen, P., 5692 (PRE); Hottentot Holland Mts, 3418BB, Stokoe, T.P., PRE55184, (PRE); Eastern slopes of top of Mitchellôs Pass South of Ceres, 3319AD, Goldblatt, P., 1346 (PRE); Witterivier Valley, 3319CA, Esterhuysen, E.E., 28296, (BOL); Seven Sisters, Witte Valley, Esterhuysen, E.E., 28369 (BOL); Mt. Lebanon, Elgin, 3419AD, Esterhuysen, E.E., 35746 (BOL); Worcester, 3319CB, Fine, 30 (PRE); Moeras River, 8.3 m from George, 3323CC, Fourcade, H.G., 6281 (BOL, PRE, NBG); Kleindrakenstein, Salem, 3319CC, Galpin, E.E., s.n, (BOL); Paarl Mountain, Grant, 2209 (PRE); Groot Drakenstein, Gray, s.n, (BOL); Jonkershoek, 3418BB, Grobelaar, 393 (PRE); Mossel Rivier, 3419AD, 1.192, Guthrie, s.n, (PRE); Wellington, 3318DB, Knobel, s.n, (PRE).

Conservation status: Least concern, very abundant and widely distributed.

Plate 2. *Psoralea aphylla* subsp. *usitata*. A: general habit, B: flowering shoot, C: stem.

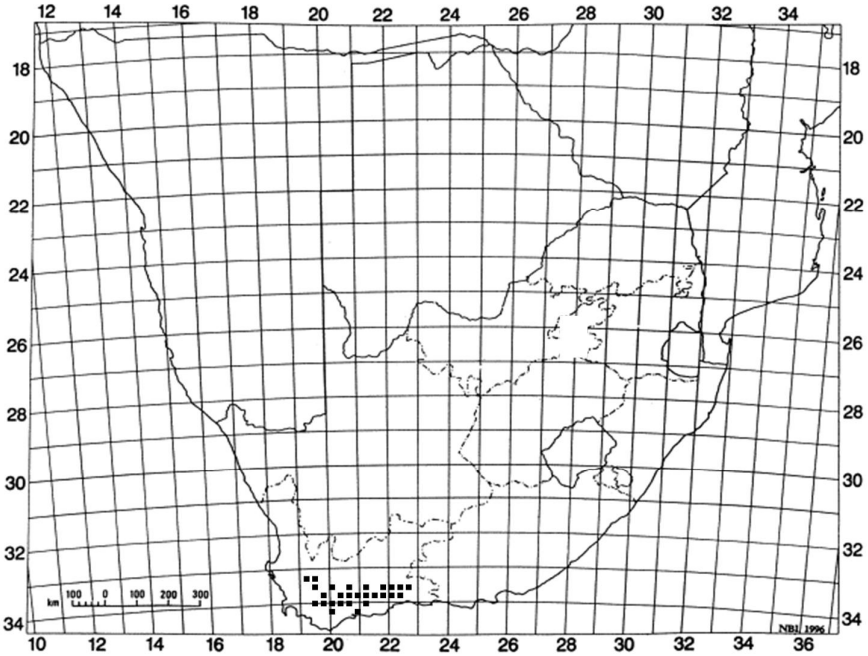

Figure 4.9. Known distribution of *Psoralea aphylla* subsp. *usitata.*

3. ***Psoralea filifolia*** **Eckl. & Zeyh.** Enum. Pl. Afric. Austral. 2: 227. (1836); Stirton & Schutte in Goldblatt & Manning Strelitzia 9: (2000). **Type:** Cape ñin humidis (Altit. II) fruticem prope Waterfall in valle Tulbagh (Worcester)ò (Lectotype: S, Isotype: L, MO, S).

Large shrubs; 3.5 -4 m tall; reseeder; may be colonial, but never forming dense clumps. **Stems** single; woody throughout; terete; not furrowed; greyish, with white lenticel. **Branches** stiff; emerging at a height of about 1.5 m. At each node several branches are produced giving the plants a somewhat ruffled or scruffy appearance. **Seasonal shoots** dark green; smooth; may be 1-foliolate, or 3-foliolate. Where leaf is present, it is 15-20 mm long, linear in cross-section with a straight, sharp and hard mucro at the apex. **Leaflet glands** on dry state visible with a 10X hand lens; black; impressed on surface; denser on upper surface. **Petioles** 2 mm long. **Stipules** 3.5-4 mm long, 1 mm wide; caducous; glabrous; not tightly congested. **Inflorescences** borne in most axils of seasonal shoots; pseudo-spicate; congested (up to 40 flowers per shoot); with one to three flower per axil. **Flowers** 16-20 mm long; mauve; maturing sequentially. **Peduncles** 3-4 mm long; stout and rigid. **Cupulum** bilobed with one of the vexillar lobes variously bilabiate; lobes equally developed, narrowly triangular, hairy on teeth; glands inconspicuous. **Pedicels** 3-4 mm long. **Calyx tube** 3-4 mm long; completely glabrous on the outside, but inner face of calyx teeth sparsely covered in small black stubby hairs. **Calyx teeth** equal; same length as the calyx tube; lateral and vexillar calyx teeth acute, straight, lanceolate; carinal calyx tooth 4 mm long, 2 mm wide, acute, broader than other four teeth; Calyx shorter than corolla; glands dense, constant in size and equally distributed across the calyx tube and teeth. **Standard petal** 8-9 mm long, 10-12 mm wide; claw 5 mm long; apex emarginate; auricles well developed. **Wing petals** 12-14 mm long, 4 mm wide; up-curving; auricles present; fused to, but longer than keel petals; claw 4-5 mm long. **Keel petals** 10-11 mm long, 4 mm wide; claw 6 mm long.

Diagnostic characters: This species is characterised by the presence of linear unifoliolate leaves (14-25 mm long), though sometimes trifoliolate in younger stems in which case the terminal leaflet is twice the length of the laterals. Leafy shoots are also found in *P. fleta*, but in that case the lateral leaflets are reduced to minute scales. In addition, *P. filifolia* is a large shrub with an untidy appearance due to its burst branching, while *P. fleta* lacks the burst branching and it branches in upper parts of tall slender stems. See Plate 3 for some illustrations.

Habitat: Lowland fynbos, stream banks and rocky seepages
Flowering time: September to February

Altitude: 88-975 m

Distribution: Tulbagh, Hottentots Holland, Rondeberg Private Nature Reserve (Fig. 4.10).

Specimens examined

Tulbagh Kloof, 3319AC, Pole Evans, I.B., 485 (PRE); Saron, Tulbagh, 3319AA, Stokoe, T.P., s.n., (PRE); Tulbagh, 3319AA, Stokoe, T.P., 55157 (PRE); Southern Hottentots Holland Mountain, Kogelberg State Forest, 3418BD, (PRE); Grootvadersbosch Estate, 3320DD, McDonald, D.J., 1514 (PRE); South of Pringle Bay, 3418BD, Boucher, C., 627 (PRE); No precise Locality, Stirton, C.H, 8219 (PRE). Rondeberg Private Nature Reserve, 3318AD, Muasya & Stirton, 4321 (BOL).

Conservation status: Vulnerable, mainly occurs in protected areas, but not very abundant.

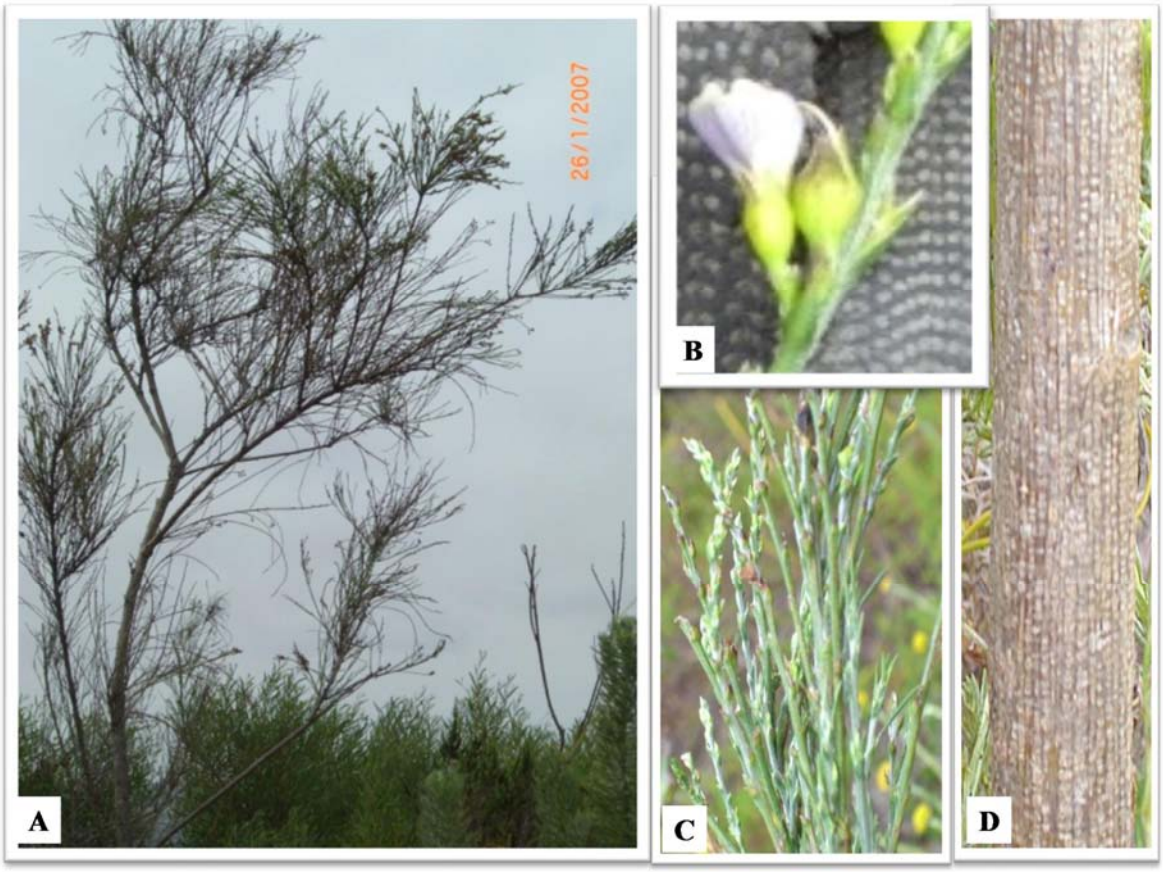

Plate 3. *Psoralea filifolia*. A: general habit, B: flowering shoot, C: shoot, D: stem.

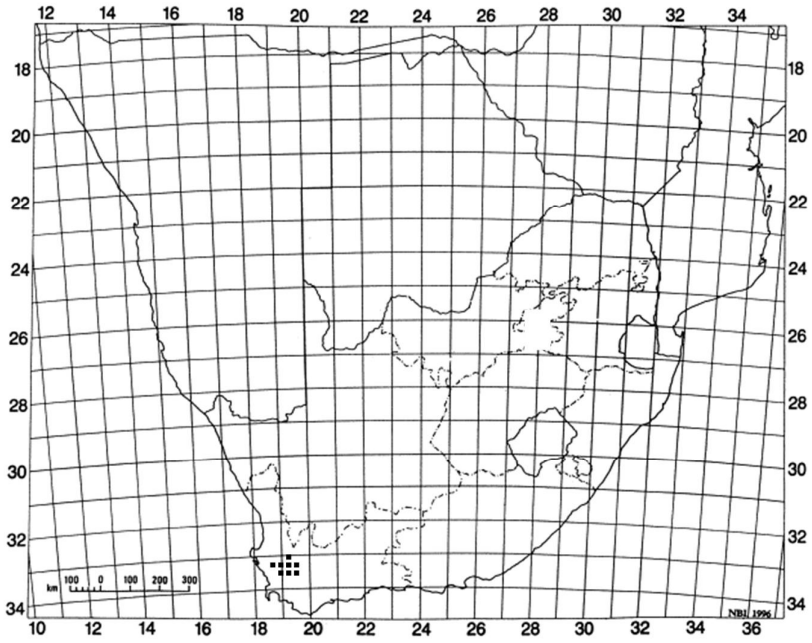

Figure 4.10. Known distribution of *Psoralea filifolia.*

4. ***Psoralea peratica* C.H. Stirt.** S. African J. Bot. 64(4): 244 (1998); Stirton & Schutte in Goldblatt & Manning Strelitzia 9: 506 (2000). **Type**: Cape, Piketberg Mountains, Goedverwacht. Rourke, J.P., 1863 (Holotype: NBG, Isotype: NBG, PRE, K).

Large shrubs to small trees; 3-5 m tall; reseeder; may be colonial, but never forming dense clumps. **Stems** erect; single to three; woody throughout; terete. **Branches** flexuous; emerging at a height of about 1-1.5 m; arching. **Seasonal shoots** densely pubescent; spreading or arching. **Stipules** 2.5-3 mm long; caducous; clasping; glabrous and not tightly congested. **Inflorescences** borne in uppermost axils of bare seasonal shoots; pseudo-capitate; congested, with up to three flowers per axil. **Flowers** 16-18 mm long; dark mauve; maturing sequentially. **Peduncles** 2 mm long; stout and rigid. **Cupulum** bilobed with one of the vexillar lobes variously bilabiate; hairy all over; lobes equally developed, glandular and narrowly triangular. **Pedicels** 5-8 mm long. **Calyx tube** 3 mm long; sparsely covered in white hairs and shorter than the teeth. **Calyx teeth** more or less equal; the lateral and vexillar calyx teeth acute, falcate and triangular; the carinal calyx tooth 6-7 mm long, 2.5-3 mm wide, acute, the same width as the other four teeth; the vexillar calyx lobes fused for up to one third of their length above the tube; calyx shorter than corolla; inner face of calyx teeth densely covered in small black stubby hairs; ribs distinctly

thickened; glands dense, constant in size and equally distributed across the calyx tube and teeth. **Standard petal** 14-16 mm long, 12-18 mm wide; claw 4-6 mm long, elongated and narrow; auricles well-developed, large and swollen; apex rounded. **Wing petals** 14-18 mm long, 5-7 mm wide; up-curving; longer than keel petals; claw 4-6 mm long; fused to, but longer than keel petals. **Keel petals** 12-15 mm long, 3-4 mm wide; claw 6-7 mm long.

Diagnostic characters: *P. peratica* is characterised by its dense erect habit with lax upper branches, densely velutinous branch tips and its large dark mauve flowers clustered at the ends of short seasonal shoots. See Plate 4 for some illustrations. This species is restricted to the Piketberg Mountains.

Habitat: Mountain fynbos, sandy seepages and streamsides
Flowering time: September to February
Altitude: 600-1000 m
Distribution Piketberg Mountain range (Fig. 4.11).

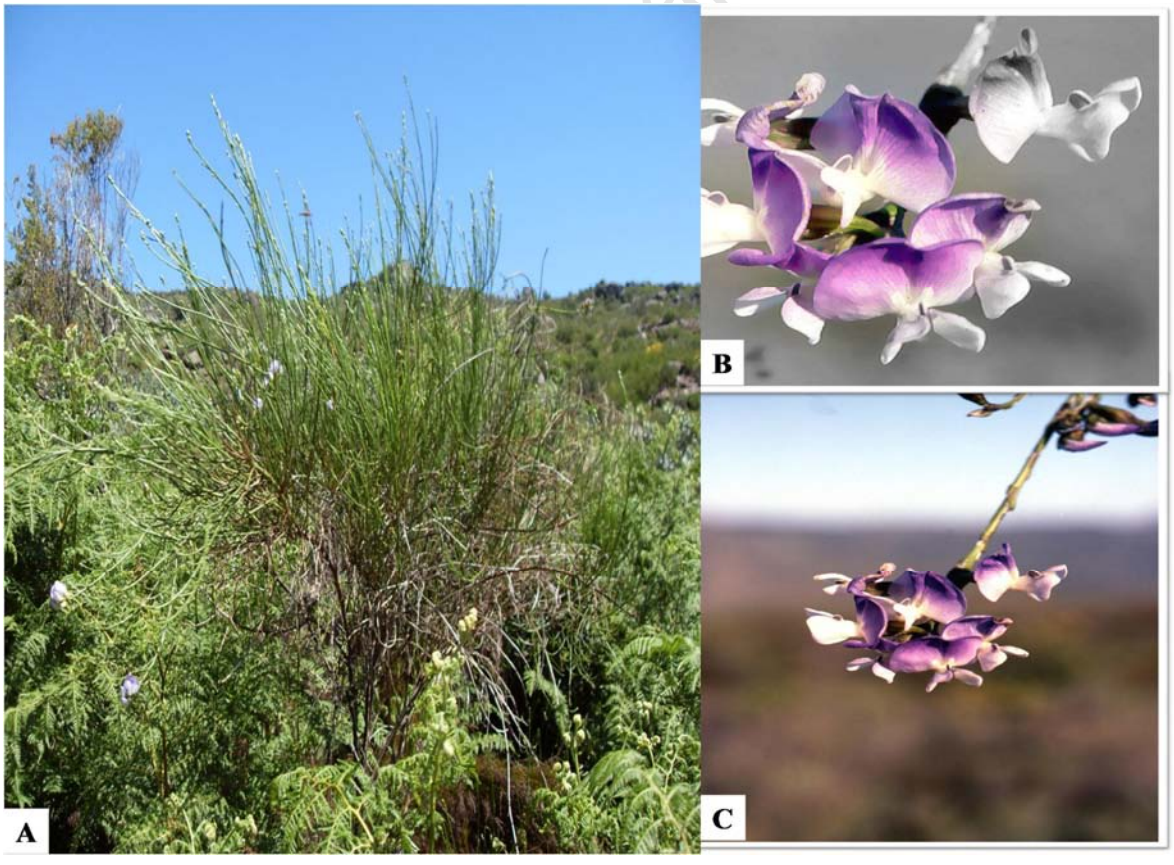

Plate 4. *Psoralea peratica*. A:general habit, B: flowers, C: flowering shoot.

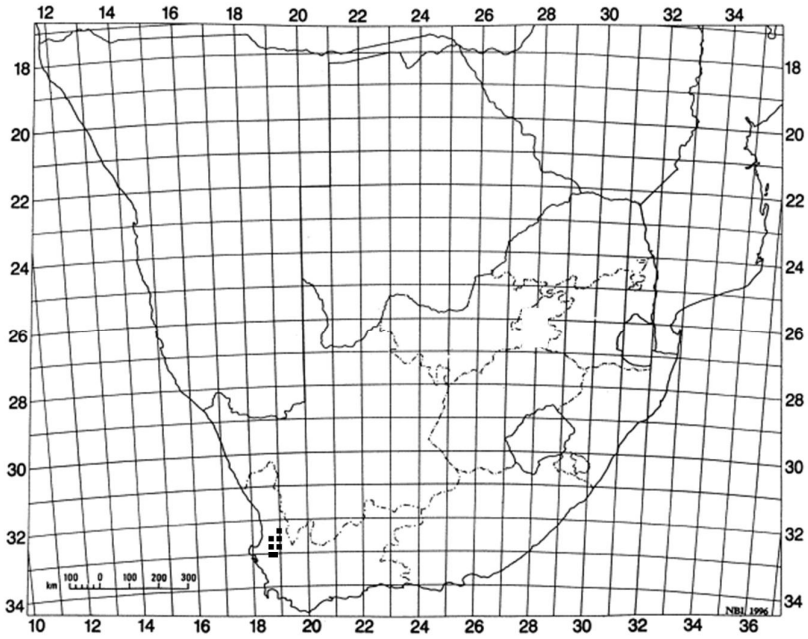

Figure 4.11. Known distribution of *Psoralea peratica.*

Specimens examined

Piketberg, 3318DC, Edwards, G.N., 248 (BOL, PRE); Platkloofrivier Mountain, 3218DC, Stirton, C.H., 9324 (PRE); Zuurvlakte, Zebrakop, Linder, 215 (BOL); Avontuur, 3218DA, Linder, 3142 (BOL); Gys-se-kraal, above Aurora, Western extremity of Piketberg, 3218DC, Rourke, J.P., 1863, (NBG); Goedverwacht, 3218DC, Stirton, 9324 (PRE); Kapteinskloof, Piketberg Mountains, 3218DA, Stirton, 10058 (PRE); Piketberg New Caledonia Farm, 3218DA, Dludlu, Muasya & Stirton, 74 (BOL); Piketberg, New Caledonia Farm, 3218DA, Dludlu, Muasya & Stirton, 80 (BOL).

Conservation status: Endangered, has a narrow distribution range and not very abundant.

5. ***Psoralea fleta* C.H. Stirt. ined.** Stirton & Schutte in Goldblatt & Manning Strelitzia 9: 506, (2000). **Type:** Cape, Bainskloof Pass, Stirton & Snijman, 11226 (Holotype: NBG, Isotype: K)

Tall, slender, willowy shrubs or treelets, 4-5 m tall; reseeder; usually forming dense clumps. **Stems** single; erect; woody throughout; may be weakly furrowed, but generally rounded. **Branches** pale green; lightly gland dotted; flexuous; emerging at upper parts of tall bare stems. **Seasonal shoots** covered in a close white pubescence; smooth; striate; non-pustulate; drooping

especially when in flower. **Leaves** absent, but may have a tiny filiform leaflet (9-11mm long) on some shoots. **Seedlings** have long and broad imparipinnate leaves (7-9 foliolate) which are secondarily lost as plants mature. On dry state, glands visible with a 10X hand lens, black and impressed on surface. Lateral leaflets suppressed or reduced to minute scales. Immature leaves hairy. **Petioles** 2.5 mm long. **Stipules** 1.5-5 mm long, 0.7-0.9 mm wide; caducous; longer than petioles; not fused to petioles; broadly obliquely ovate; clasping to the shoots; glabrous; not tightly congested. **Inflorescences** borne in uppermost axil of seasonal shoots; pseudo-spicate; congested, up to three flowers per axil. **Flowers** 19-22 mm long; light purple; maturing sequentially; bracts reduced to tuft or ring of hairs. **Peduncles** 4 mm long; stout and rigid; shorter than the subtending leaflet. **Cupulum** trilobed; with one of the lobes scarcely developed; broadly triangular; glabrous but hairy on teeth. **Pedicels** 3-4 mm long. **Calyx tube** 2-4.5 mm long; glabrous externally; inner face of calyx teeth sparsely covered in small black stubby hairs. **Calyx teeth** shorter than the calyx tube; unequal; the lateral and vexillar calyx teeth acute, straight and triangular; the carinal calyx tooth 6 mm long, 2-2.5 mm wide, acuminate and narrower than the other four teeth; the vexillar calyx lobes free above the tube; calyx shorter than corolla; ribs distinctly thickened; glands dense, constant in size and equally distributed across the calyx tube and teeth. **Standard petal** 12-14 mm long, 12-13 mm wide; claw 4 mm long, elongated and narrow; auricles present apex rounded or obtuse. **Wing petals** 12.5-15 mm long, 4.5-5.5 mm wide; up-curving; auricles present; fused to, but longer than keel petals; claw 4 mm long. **Keel petals** 11-13 mm long, 4 mm wide; claw 5-7 mm long.

Diagnostic features: *P. fleta* differs from the rest of the *P. aphylla* group by its filiform leaflets (9-11 mm long) borne on its flowering shoots. It is a tall slender willowy shrub, with drooping branches when in full flower. Its flower has a trifid cupulum. See Plate 5 for some illustrations.

Habitat: Mountain and lowland fynbos
Flowering time: September to March
Altitude: 250-2050 m
Distribution: Bainskloof Mountains, Ceres, Worcester (Fig. 4.12).

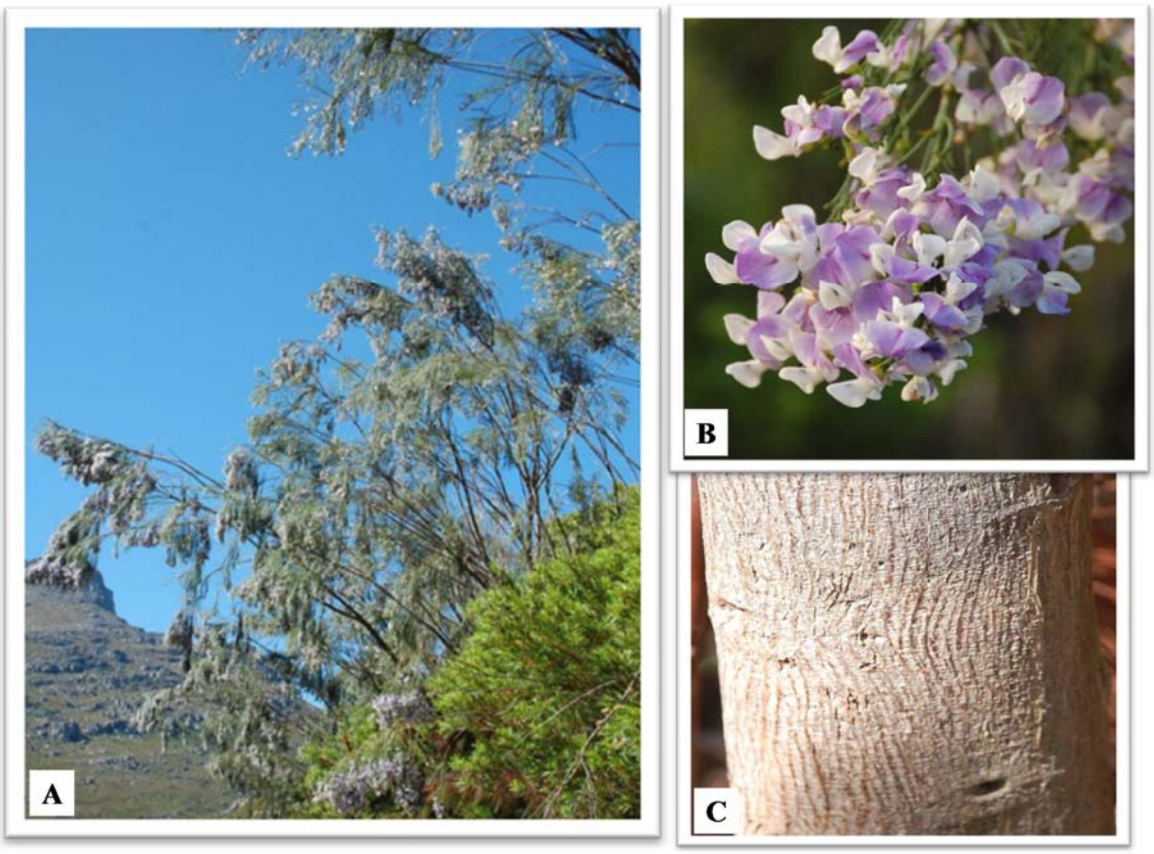

Plate 5. *Psoralea fleta*. A: general habit, B: flowering shoot, C: stem.

Specimens examined

Bainskloof, 3319CA, Taylor, H.C., 6987a (PRE); Worcester, Keeromsberg shale band, 3319DA, Esterhuysen, E.E., 9204 (PRE); Du Toit Kloof Pass Tunnel, 3319CA, Stirton, C.H., 9478 (PRE); Zachariashoek, Kasteelkloof, 3319CC, Viviers, M., 1140 (PRE); Bainskloof, 3319CA, Meyer, J.J., 1412 (PRE); Wolvekloof Forest Reserve, Bainskloof, 3319CA, Barker, 4244 (BOL); Bainskloof, Bainsberg, Bobbejaansrivier path on NW facing slope, 3319AD, Bean, 2129 (BOL); Berg River Hoek, Paarl District, Compton, 15637 (NBG); West Side of Keeromsberg, 3319DA, Esterhuysen, E.E., 9204 (BOL, PRE); Bainskloof, 3319CA, Hutchinson, 1065 (BOL); Bainskloof, Levyns, 9897 (BOL); Ceres, 4663 (BOL); Brandwagt, 3319CB, Van Breda, 347 (PRE); Mitchells Pass, 3319AD, Walgate, 379 (PRE). Bainskloof Pass, 3319CA, Muasya & Stirton, 3385 (BOL); Bainskloof, 3319CA Muasya & Stirton, 3883 (BOL); Bainskloof, Muasya & Stirton, 3960 (BOL); Bainskloof, Muasya & Stirton, 3961 (BOL); Bainskloof, 3319CA, Dludlu, Muasya & Stirton, 29 (BOL); Bottom of Mitchellôs Pass Tulbagh side, 3319AD, Muasya & Stirton, 3341 (BOL); Bottom of Mitchellôs Pass Tulbagh side, 3319AD, Muasya & Stirton, 3342 (BOL).

Conservation status: Vulnerable, although it has a wide distribution, it is not abundant.

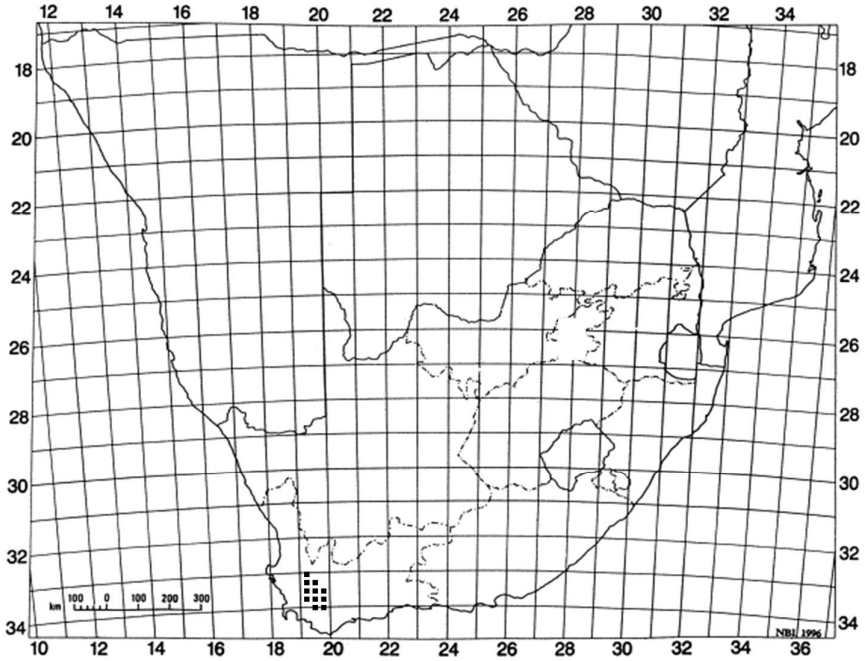

Figure 4.12. Known distribution of *Psoralea fleta.*

6. ***Psoralea pullata* C.H. Stirt. ined.** Stirton & Schutte in Goldblatt & Manning Strelitzia 9: 506, (2000). **Type:** Fernkloof Nature Reserve, P.A. Bean, 690 (Holotype: BOL).

Large shrubs, 2-3 m tall; reseeder; occasionally forming dense clumps. **Stems** two to three; woody throughout; weakly furrowed but generally rounded. **Branches** rigid; emerging in upper portions of plant; dense branching at each node. **Seasonal shoots** dark green; densely covered in black and white hairs; striate; with randomly scattered glands. **Stipules** 3.5-4 mm long, 3 mm wide; broadly obliquely ovate; clasping the shoots; glabrous; not tightly congested. **Inflorescences** borne in upper axils of seasonal shoots but without leafy extension; pseudo-spicate; lax, with one flower per axil. **Flowers** 15-16 mm long; mauve; maturing sequentially; bracts reduced to tuft or ring of hairs. **Peduncles** 1.5-2 mm long; stout and rigid. **Cupulum** bilobed with one of the lobes variously bilabiate; lobes clasping and overlapping with calyx tube; lobes equally developed and triangular; completely covered in long black hairs; glands of cupulum may be inconspicuous due to the dense pubescence. **Pedicels** 5-7 mm long. **Calyx tube**

4 mm long; completely covered in long black hairs. **Calyx teeth** equal, but longer than calyx tube; the lateral and vexillar calyx teeth acute, straight, and triangular; the carinal calyx tooth 5-7 mm long, 3-5 mm wide, acute and the same width as other four teeth; the vexillar calyx lobes fused for up to one third of their length above the tube; calyx shorter than corolla; inner face of calyx teeth densely covered in long black hairs; ribs distinctly thickened; glands dense, constant in size and equally distributed across the calyx teeth and tube. **Standard petal** 10-12 mm long, 11-15 mm wide; claw 4-5 mm long; auricles well-developed; apex emarginate. **Wing petals** 13.5-15 mm long, 4-5 mm wide; up-curving; longer than keel petals; claw 6 mm long; fused to, but longer than keel petals. **Keel petals** 11-13 mm long, 4 mm wide; claw 6-7 mm long.

Diagnostic characters: *P. pullata* is diagnosed by its densely hairy (black) flowers especially the calyx and the cupulum. Also, its calyx teeth are much longer than the calyx tube. See Plate 6 for some illustrations.

Habitat: Mountain fynbos, seepages.
Flowering time: June to December
Altitude: 95-1200 m
Distribution: Hermanus, Elim, Potberg, Stilbaai, Gouritz region (Fig. 4.13)

Specimens examined

Vogelgat, 3419AD, Williams, I., 2566 (PRE); Caledon Zwarteberg, 3419AB, 1847, Zeyher, C.L.P., 2383 (PRE); Kalkanberg, 3418AB, White, F., 5200 (PRE); Fernkloof Nature Reserve, upper Mossel river, 3419AD, Bean, 690 (BOL); Kleinrivier Mountain, Esterhuysen, E.E., 2929 (BOL); Swartberg, Caledon, 3418AB, Galpin, E.E., 3964 (PRE); Kalk Bay, 3418AB, Griffen, s.n., (PRE); Rooi Els, Leipoldt, 4189 (BOL); Fernkloof Nature Reserve, 3419AD, Orchard, 204 (PRE); Rooi Els, Parker, 4470 (BOL); South of Pringle Bay, Pillans, 8257 (BOL); West base of Potberg, Pillans, 9293 (BOL); Potberg, 3420BC, Pillans, 9393 (BOL); Vogelgat Nature Reserve, 3418AD, Stirton, 10768 (PRE); Hermanus, 3419AC, Stirton, 11179 (PRE); Kalk Bay, 3418AB, White, F., 5200 (PRE); Hermanus road, near Kleinmond turn to Caledon, Barker, 5881 (BOL, NBG); Platberg at head of Oudebos Forest, Kogelberg Forest Reserve, 3418BD, Boucher, 385 (PRE, NBG); Rooiels hut, 3418BD, Walsh, B.N, 22 (PRE); Hottentot Holland, Caledon, Hutchinson, 313 (BOL); Riviersonderend, Muasya & Stirton, 3903a (BOL); 500 m from Elim/Bredasdorp junction, 3419DA, Dludlu, Muasya & Stirton, 71 (BOL); 1 km from turn-off Sandies Glen towards Stanford, 3419BC, Dludlu, Muasya & Stirton, 72 (BOL).

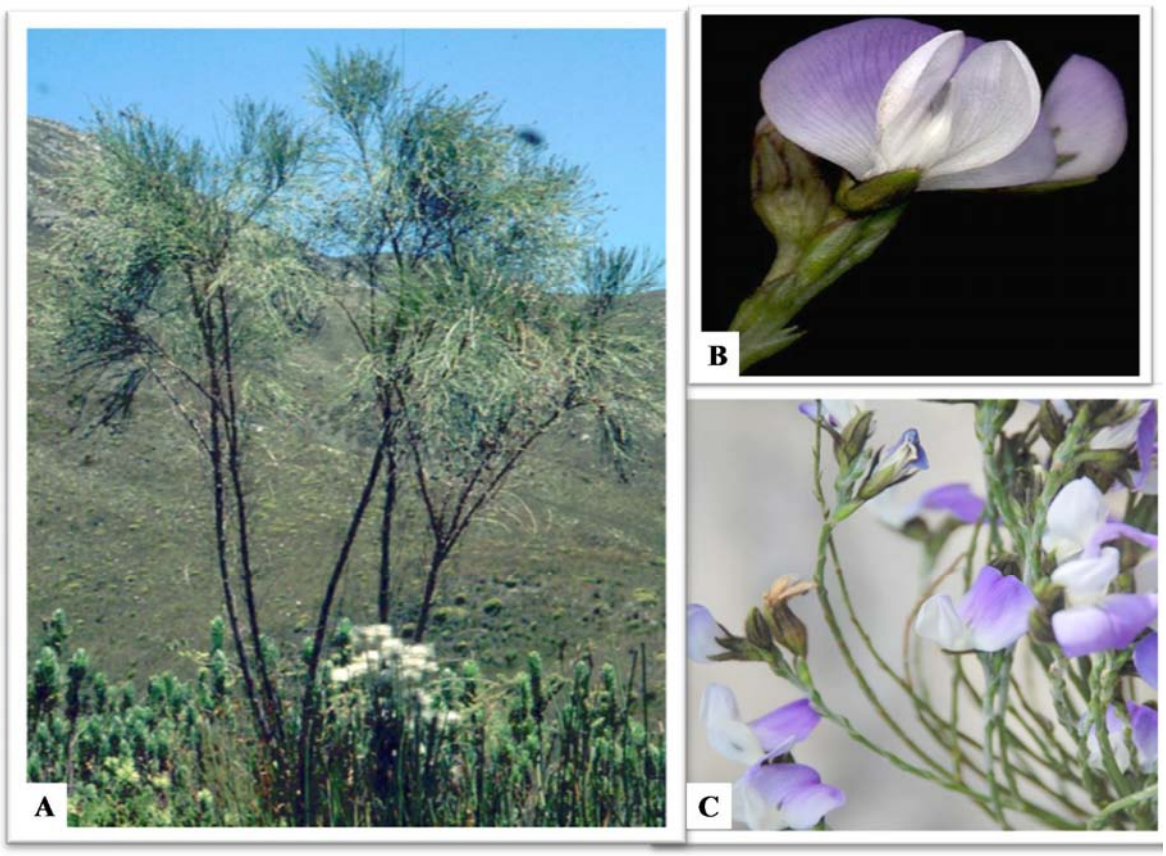

Plate 6. *Psoralea pullata*. A: general habit, B: flowering shoot, C: flowering shoots.

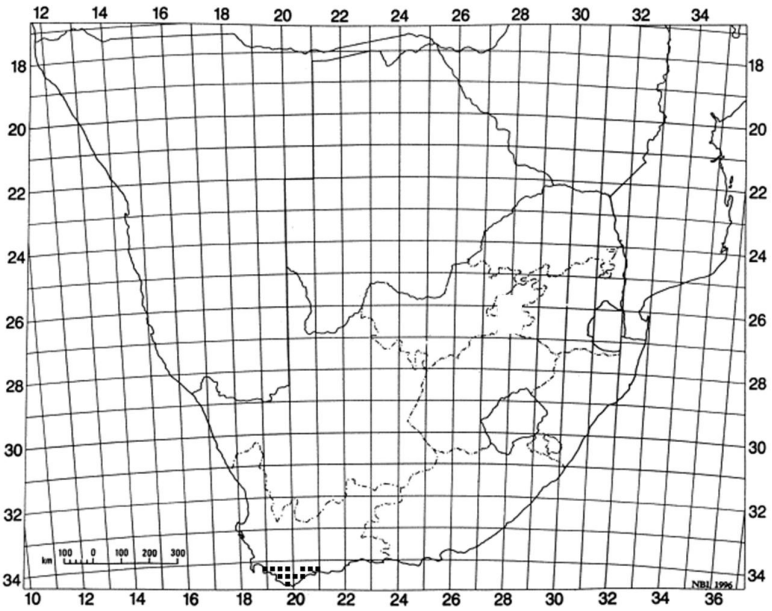

Figure 4.13. Known distribution of *Psoralea pullata.*

Conservation status: Vulnerable, it is widely distributed in its habitat, but may be in danger as most of it occurs outside protected areas on roadsides.

7. ***Psoralea rigidula* C.H. Stirt. ined. Type:** Cape, NE ridge of Du Toitôs Peak, above Delabat Ravine, Esterhuysen, 33766 (Holotype: BOL, Isotype: K, NBG)

Multi-stemmed suffrutices; up to 0.6 m tall; aphyllous; resproutes after fires; has a woody daucate rootstock; colonial and always forming dense clumps. **Stems** erect; numerous; woody at base, otherwise herbaceous; weakly furrowed. **Branches** yellowish-green; emerging at the base of plant**. Seasonal shoots** striate; gland dotted. **Stipules** 1.5 mm long, 1 mm wide; broadly obliquely ovate; glabrous; not tightly congested. **Inflorescences** borne in most axils of seasonal shoots; pseudo-spicate; lax, with one flower per axil. **Flowers** 19-24 mm long; blue; maturing sequentially; bracts reduced to tuft or ring of hairs. **Peduncles** 8-12 mm long; filiform and flexuous. **Cupulum** trilobed; glabrous; one of the lobes scarcely developed; lobes broadly triangular, gland dotted. **Pedicels** 3.5-6 mm long. **Calyx tube** 2-4 mm long; glabrous; longer than the teeth. **Calyx teeth** subequal, keel tooth slightly longer; the lateral and vexillar calyx teeth acute, straight, triangular; carinal calyx tooth 4-5 mm long, 2-3 mm wide, acute, broader than other the four teeth; the vexillar calyx lobes fused for up to one third of their length above the tube; calyx shorter than corolla; inner face of calyx teeth finely covered in white hairs with no stubby black hairs; ribs distinctly thickened; glands dense, constant in size more dense on the tube than the teeth. **Standard petal** 8-11 mm long, 9-10 mm wide; auricles absent; claw of standard petal 4-6 mm long; apex emarginate. **Wing petals** 10-13 mm long, 4-5 mm wide; up-curving; fused to, but longer than keel petals; claw 3-5 mm long. **Keel petals** 10-11 mm long, 3-4 mm wide; claw 4-6 mm long.

Diagnostic features: *P. rigidula* is a multistemmed, resprouting suffrutex with a woody daucate rootstock. Its flower has a trifid cupulum and a long (8-12 mm), flexuous peduncle. Its seasonal shoots are glabrous. See Plate 7 for some illustrations of this species.

Habitat: Mountain fynbos
Flowering time: November to April
Altitude: 560-1220 m
Distribution: Bainskloof Mountains to Du Toitôs Kloof (Fig. 4.14).

Specimens examined

Haelhoek Spitzkop, Esterhuysen, E.E., 14550 (BOL); Above Delabat Ravine, NE Du Toit Kloof, Esterhuysen, E.E., 28213 (BOL); Slopes of Winterberg, facing Haelhoek, Sneeukop, Esterhuysen, E.E., 28229 (BOL); NE ridge of Du Toitõs Peak, above Delabat Ravine, Esterhuysen, E.E., 33766 (BOL); Observation Point, Bainskloof Mountains, Esterhuysen, E.E., 35742 (BOL); Between Haelhoek Sneeukop and Winterberg, above stream flowing into Wemmershoek Valley, Esterhuysen, E.E., 28233a (BOL); Bainskloof Pass, 3319CA, Muasya & Stirton, 3390 (BOL); Bainskloof Pass, 3319CA, Muasya & Stirton, 3958 (BOL); Bainskloof Pass, 3319CA, Muasya & Stirton, 3962 (BOL); Bainskloof Pass, 3319CA, Muasya & Stirton, 3882 (BOL); Bainskloof hillside, Muasya & Stirton, 4333 (BOL).

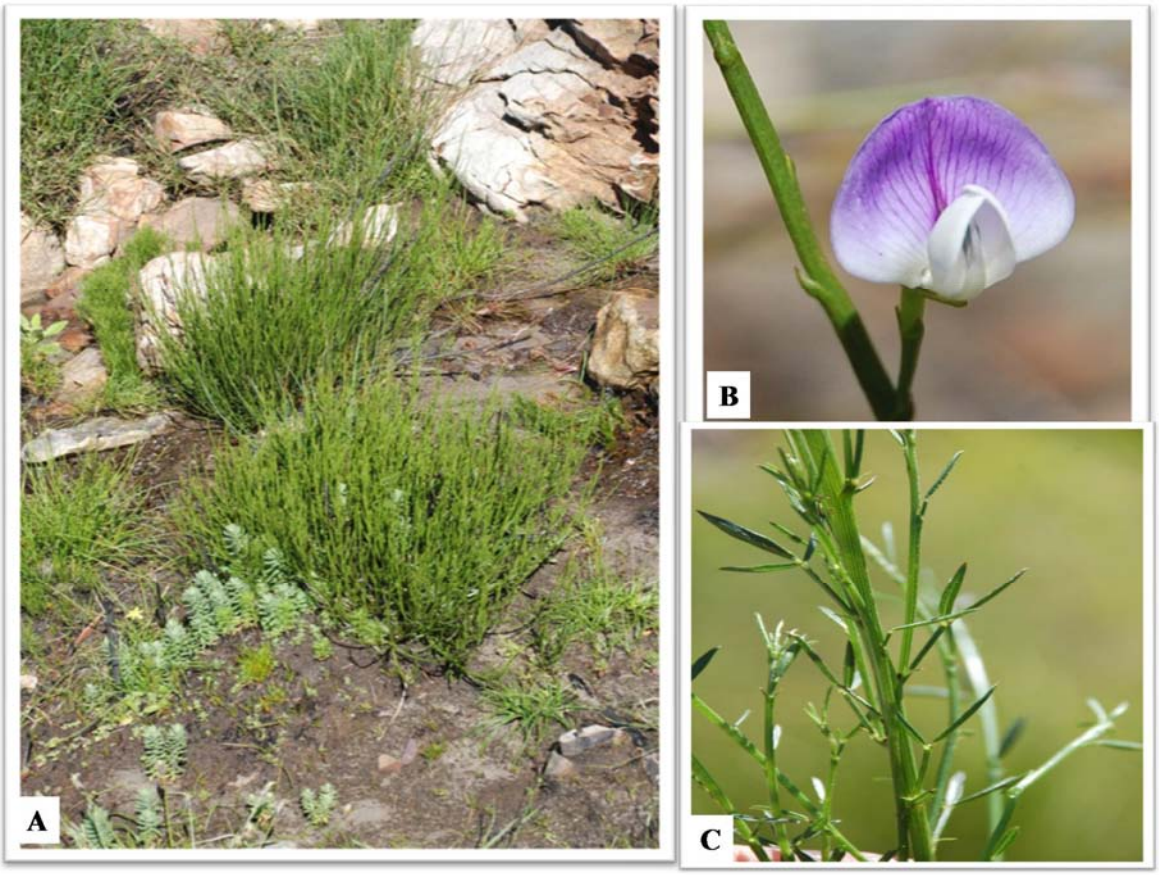

Plate 7. *Psoralea rigidula*. A: general habit, B: flowering shoot, C: young shoot.

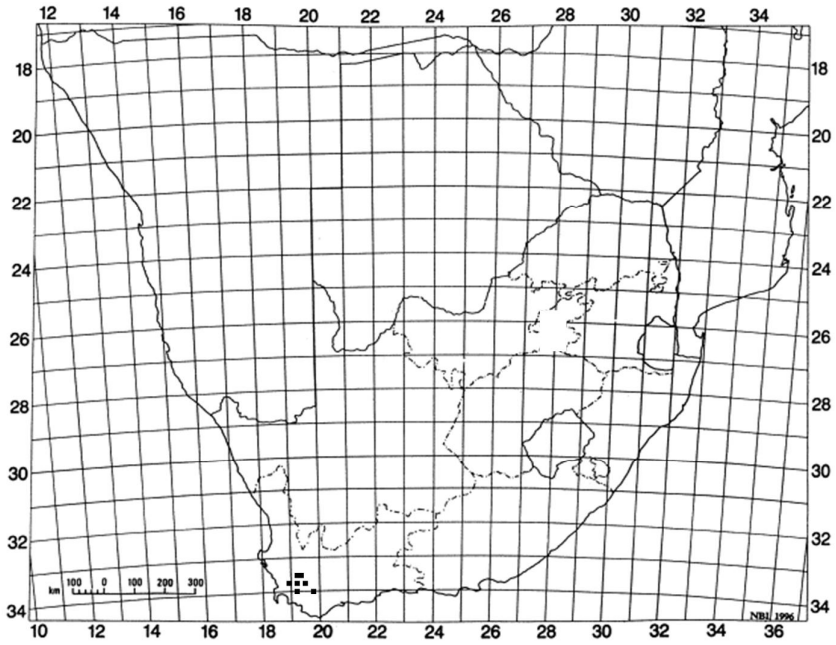

Figure 4.14. Known distribution of *Psoralea rigidula.*

Conservation status: Endangered, it has a narrow distribution range and not abundant.

8. ***Psoralea gigantea sp nov.*** M.N. Dludlu, A.M. Muasya & C.H. Stirton. ined.
   Type: Jonkershoek Nature Reserve, Stellenbosch. Dludlu, Muasya & Stirton, 57 (Holotype: BOL, Isotype: NBG, PRE).

Trees; 6-8 m tall; reseeder; colonial and forming dense clumps especially on valleys. **Stems** erect; with a trunk diameter of about 40cm; grey; woody throughout; not furrowed. Stems start branching in upper portions above 2 m. **Branches** robust; patent; with two or three branches at each node; culminating in drooping shoots. **Seasonal shoots** sparsely hairy; striate; lightly gland dotted; drooping; dark green; smooth. **Leaves** absent on seasonal shoots, though some young branches may have a filiform leaflet up to 30 mm long; glabrous. **Stipules** 1.5 mm long, 1.2 mm wide; narrowly triangular; patent; glabrous; not tightly congested. **Inflorescences** 33 mm long; borne in uppermost axil of seasonal shoots; pseudo-spicate; lax, but may also be congested. **Flowers** 24 mm long; reddish-violet; maturing sequentially. **Peduncles** 6 mm long; filiform and flexuous. **Cupulum** bilobed with one of the vexillar lobes variously bilabiate; glabrous, but hairy on teeth; lobes equally developed, glandular and narrowly triangular. **Pedicels** 3 mm long. **Calyx tube** 3.5 mm long; glabrous; longer than the teeth. **Calyx teeth** unequal, keel tooth much longer

than the other four teeth; the lateral and vexillar calyx teeth acuminate, falcate, and narrowly triangular; the carinal calyx tooth 8 mm long, 2.2 mm wide, broader than the other four teeth; the vexillar calyx lobes fused for up to one third of their length above the tube; calyx equal in length to the corolla; inner face of calyx teeth densely covered in small black stubby hairs; ribs distinctly thickened; glands dense, constant in size, more glands on the teeth than on the tube. **Standard petal** 10 mm long, 9 mm wide; claw 5 mm long; elongated and narrow; ovate; auricles well-developed; apex emarginate. **Wing petals** 12-14 mm long, 4 mm wide; up-curving; and longer than keel petals; fused to, but longer than keel petals; claw 3- 6 mm long **Keel petals** 11-12 mm long, 3-4 mm wide. Claw 5-7 mm long.

Diagnostic features: *P. gigantea* is the only aphyllous *Psoralea* which is a tree. Its flowering shoots have filiform unifoliolate leaves (up to 30 mm long) with one to two flowers per axil. See Plate 8 for some pictures of *Psoralea gigantea*.

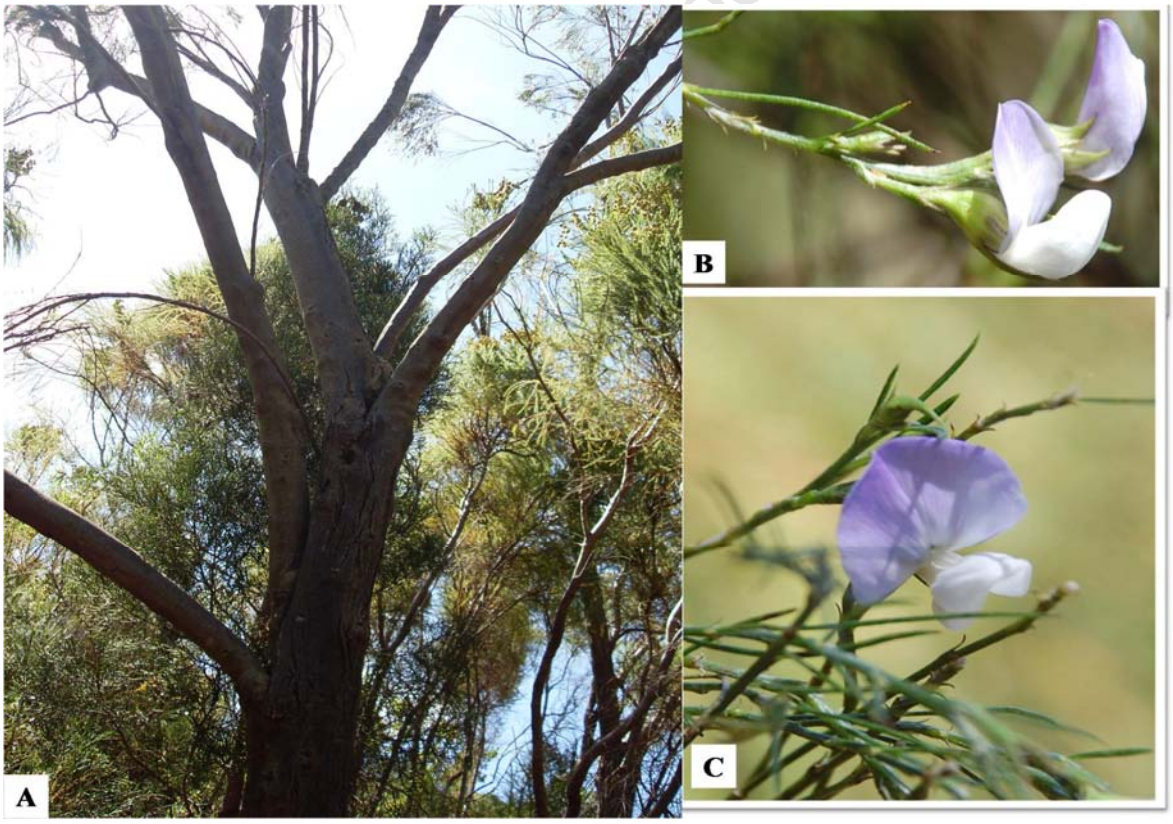

Plate 8. *Psoralea gigantea*. A: general habit, B: flowering shoot, C: flowering shoots.

Habitat: Mountain fynbos, streamsides.

Flowering time: September to December

Altitude: 300-700 m

Distribution: Stellenbosch and Mitchellôs Pass (Fig. 4.15).

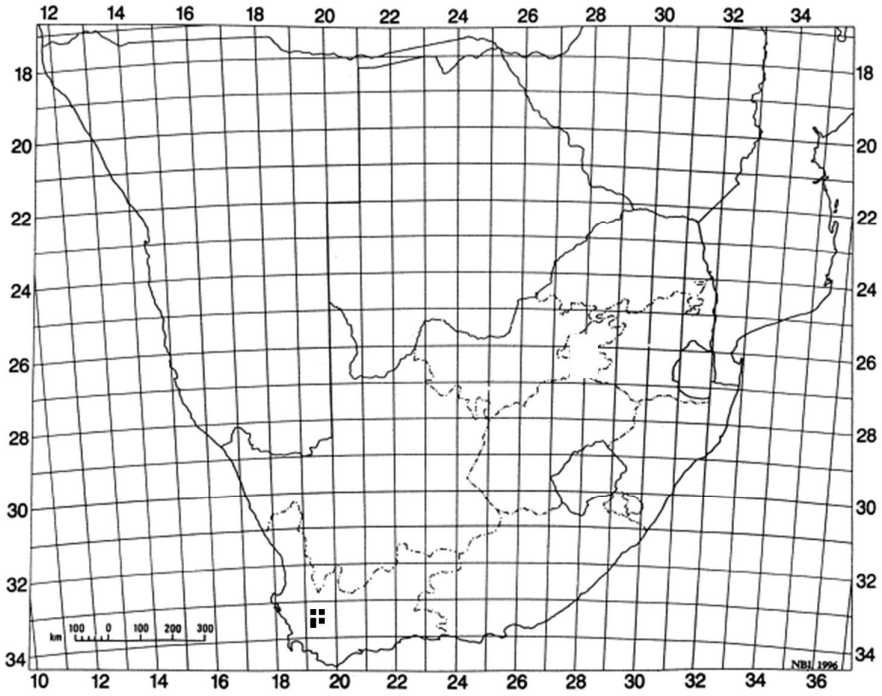

Figure 4.15. Known distribution of *Psoralea gigantea.*

Specimens examined

Mitchell's Pass, 3319AD, Esterhuysen, E.E., 6148 (BOL); Jonkershoek Nature Reserve, Dludlu, Muasya & Stirton, 57 (BOL); Mitchells Pass, Ceres, 3319AD, Goldblatt, P., 1346, (PRE); Mitchellôs pass junction of R46 & R43, 3319AD, Dludlu, Muasya & Stirton, 35 (BOL).

Conservation status: Vulnerable, only known from two localities and not abundant, but occurs in protected areas.

9. ***Psoralea ramulosa* C.H. Stirt. ined. Type:** Cederberg, 22km from Clanwilliam towards Algeria. C.H. Stirton, 10200 (Holotype: BOL, Isotype: PRE, NBG).

Small shrubs; 1-1.5 m tall; resprouter; occasionally forming dense clumps along stream banks or rocky seepages. **Stems** two to three; woody throughout; brown; terete; not furrowed. **Branches** flexuous; with glands scattered randomly; profuse at each node and non- uniform; giving the plant an indeterminate architecture. **Seasonal shoots** completely glabrous; sparsely gland-dotted; spreading or arching; yellowish green; smooth. **Leaves** absent, only has tiny caducous stipules. **Stipules** 1.2-2.5 mm long, 0.8-1 mm wide; lanceolate; clasping; glabrous; not tightly congested. **Inflorescences** borne in upper axils of seasonal shoots but without leafy extension; pseudo-spicate; lax, with one flower per axil. **Flowers** 17-28 mm long; maturing more or less simultaneously; bracts absent. **Peduncles** 5-15 mm long; stout and rigid. **Cupulum** bilobed with one of the vexillar lobes variously bilabiate; glabrous; lobes equally developed narrowly triangular; glands conspicuous. **Pedicels** 4-7 mm long. **Calyx tube** 3-5 mm long; glabrous; shorter than the teeth. **Calyx teeth** subequal, keel tooth slightly longer; the lateral and vexillar calyx teeth acute, falcate, lanceolate; the carinal calyx tooth 4-5 mm long, 2-3 mm wide, acute, broader than other four teeth; the vexillar calyx lobes free above the tube; calyx shorter than corolla; inner face of calyx teeth finely covered in white stubby hairs; ribs slender; glands dense, constant in size and denser on the tube than on the teeth. **Standard petal** 9-12 mm long, 10-13 mm wide; claw 4-5.5 mm long, elongated and narrow; apex emarginate. **Wing petals** 12-16 mm long, 3-5.5 mm wide; up-curving; claw 4-5.5 mm long; fused to, but longer than keel petals. **Keel petals** 11-13 mm long, 3-4 mm wide; claw 5-7 mm long.

Diagnostic characters: *P. ramulosa* is endemic to the Cedarberg Mountains. It differs from *P. usitata* by its brownish stems and long, flexuous peduncles as opposed to greenish- tan stems and short stout peduncles. On herbarium sheets *P. ramulosa* may resemble *P. rigidula*, but the growth form of these is very distinct, with *P. rigidula* having numerous herbaceous stems, and never growing taller than 0.6 m, while *P. ramulosa* has two to three stems, which are woody throughout and its height ranges from 1-1.5 m. Some illustrations of this species are shown in Plate 9.

Habitat: Mountain fynbos, seepages
Flowering time: December to April
Altitude: 180-1000 m

Distribution: Cedarberg (Fig. 4.16).

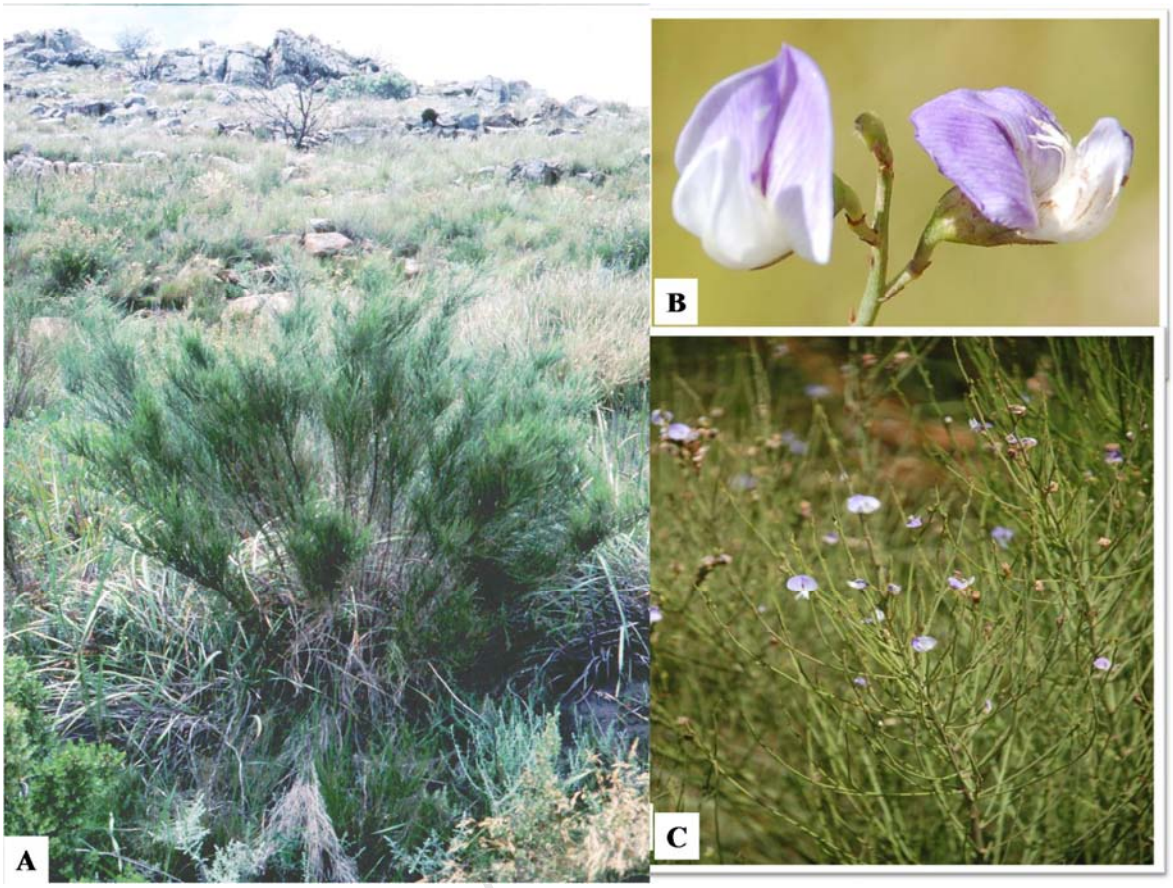

Plate 9. *Psoralea ramulosa*. A: general habit, B: flower, C: flowering shoots.

Specimens examined

Clanwilliam, 22km towards Algeria, 3218BD, Stirton, C.H., 10200 (PRE); 6km East of Citrusdal, 3219CA, Hardy, D.S., 1732 (PRE); Cedarberg, 3219AC, Dludlu, Muasya & Stirton, 24 (BOL); Kanje Farm opposite Geelberg, 3219CA, Muasya & Stirton, 4393 (BOL); Middelburg, 3219CA, Muasya & Stirton, 4395 (BOL). Rietvlei Farm near Keurbos between Algeria and Clanwilliam on riverbanks, 3218BD, Muasya & Stirton, 4357 (BOL); Top of Uitkykpas, 3219AC, Muasya & Stirton, 4363 (BOL); Top of Uitkykpas, 3219AC, Muasya & Stirton, 4368 (BOL); Top of Uitkykpas, 3219AC, Muasya & Stirton, 4369 (BOL); 1 km from Uitkykpas on road from Algeria to Cedarberg , 3219AC, Muasya & Stirton, 4362 (BOL).

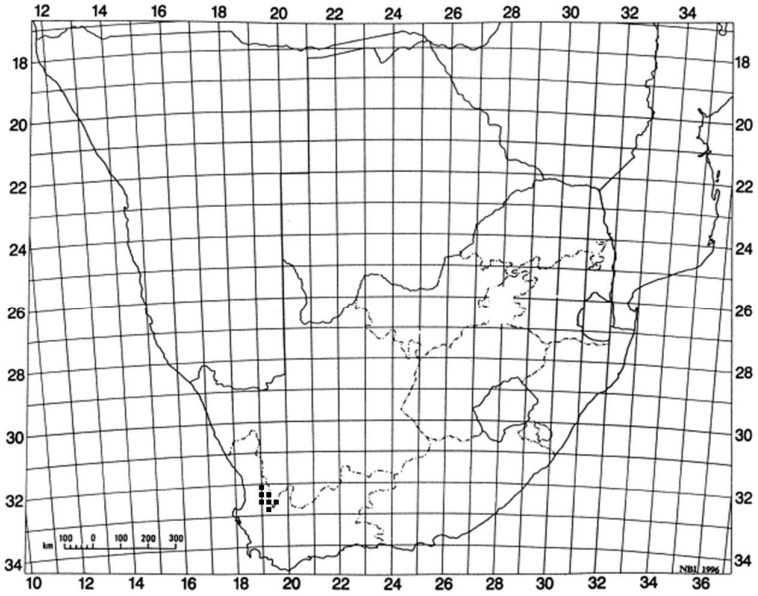

Figure 4.16. Known distribution of *Psoralea ramulosa.*

Conservation status: Vulnerable, not abundant in its habitat and has a narrow distribution range.

4.4.2. *Psoralea pinnata* complex

Key to the *Psoralea pinnata* complex

1a. Multi-stemmed; small shrubs (up to 1.5 m); resprouting; seasonal shoots glabrous; Eastern Cape, KwaZulu-Natal, Mpumalanga and Swazilandé é é é é é é ...é é .***P. glabra E. Mey.***

1b. Single-stemmed; large shrubs (2-4 m) or trees; reseeding; seasonal shoots pubescent; Western Capeé é é é é é é é é é é é é é é é ...é é é é é é é é é .é 2

2a. Inflorescence shorter than subtending leaves, calyces covered in white hairs.....***P. pinnata* L.**

2b. Inflorescence longer than subtending leaves, calyces not covered in white hairsé é é ...3

3a. Flowers white; calyx tube glabrous and shorter than the teeth, calyx longer than the petals; shrubs 3-5 m tall, with a trunk diameter of 25 cmé é é é é é é é ..***P. affinis Eckl. & Zeyh.***

3b. Flowers hyacinth blue; calyx tube densely covered in black hairs, calyx tube shorter than the teeth; calyx shorter than the petals, large shrubs and trees, tree form with trunk diameter of 50 cmé ***.P. koudebergense sp nov.***

1. ***Psoralea pinnata L.*** Pl. rar. Afr. (1770); Sp. Pl. 2:1074 (1752); Berg. Descr. Pl. Cap. 218 (1767); Mant. 225 (1767); Ait., Hort. Kew, ed. 2. 4: 374; Thunb., Prodr. 136 (1800); Thunb., Fl. Cap. 609 (1823); Poir. In Lam. Encycl. 5:690 (1804); Dietrich, Lex. Gart. Bot. 7: 612 (1807); DC., Prodr. 2:216 (1825); E. Mey. in Linnaeae 7:163 (1832); Eckl. & Zeyh., Enum. 224 (1836); E. Mey., Comm. 82 (1836); Richter, Codex 739 (1840); Walpers, Repert. 1: 655 (1842); Harv. In Harv. & Sond., 2: 144 (1862); Bews, Introdt. Fl. Natal Zulu. (1921); Forbes in Bothalia 3: 125 (1930); Salter in Adamson & Salter, Fl. Cape Penins. 485 (1950); Kidd, Wild Flowers Cape Penins. T. 81.12 (1972); Compton, J. S. Afr. Bot. suppl. Vol. 11 (1976); Moll, Trees Natal 485 (1981); Stirton & Schutte in Goldblatt & Manning Strelitzia 9:505 (2000); Schmidt, Mervyn & Lötter, Trees and shrubs of Mpumalanga and Kruger National Park. (2002); Loffler & Loffler, Swaziland Tree Atlas (2005). **Type:** Collector unknown (Hort. Cliff. 370.1 Lectotype designated by Stirton in *Taxon* 41: 568 (1992). **Synonyms:** *Ruteria pinnata* Medik.; *Lotodes pinnatum* Kuntze O.K., Pl. 3, 2: 65.

Large; slender shrubs to tree-lets; 2.5-4 m tall; with trunk diameter of 20-30 cm; reseeder; colonial and usually forming dense clumps. **Stems** single; erect; woody throughout; terete; not furrowed. **Branches** rigid; emerging on upper parts, at a height of about 1.5 m; with randomly scattered glands. **Seasonal shoots** dark green; densely covered in white hairs; striate; pustulate; arching. **Leaves** 5, 7, and 9- foliolate; imparipinnate; same number of leaflets produced at all stages of growth; crowded at the ends of bare branches; clasping the shoots. **Terminal leaflet** 10-30 mm long; shorter than basal pair of leaflets; mucro of terminal leaflets straight, fragile and soft; filiform; linear in cross-section; apex acute; base acute. **Lateral leaflets** always present;

symmetrical; about the same length as the terminal leaflet. Immature leaves hairy. Upper surface of mature leaflets dull. Lower surface of mature leaflets finely pubescent. **Leaflet glands** visible with a 10X hand lens; flush with the surface; dark brown or black; denser on upper surface. **Petioles** 3-12 mm long; shorter than terminal leaflets. **Stipules** 2-3 mm long, 1-1.5 mm wide; persistent; shorter than and free from petiole; narrowly triangular; glabrous; clasping the shoots; tightly congested. **Inflorescences** borne in upper axils of seasonal shoots but with terminal leafy extension; pseudo-spicate; lax, with two to three flowers per axil. **Flowers** 19-40 mm long; blue; maturing sequentially; shorter than the subtending leaf; bracts reduced to tuft or ring of hairs. **Peduncles** 5-30 mm long; filiform and flexuous. **Cupulum** bilobed with one of the vexillar lobes bilabiate; both lobes equally developed, glandular, broadly triangular; hairy on teeth. **Pedicels** 2-5 mm long. **Calyx tube** 3-5 mm long 7-11 mm wide; lightly covered in black hairs. **Calyx teeth** equal; the same length as the calyx tube; triangular; straight; apex acute; the carinal calyx tooth 2.5-5 mm long, broader than the other four teeth; the vexillar calyx lobes fused for up to one third of their length above the tube; calyx shorter than corolla; inner face of calyx teeth densely covered in small black stubby hairs; ribs slender; glands dense, constant in size and equally distributed across the calyx tube and teeth. **Standard petal** 8-12 mm long, 9-15 mm wide; apex emarginate; claw 5 mm long, elongated and narrow. **Wing petals** 10-15 mm long, 4-6 mm wide; up-curving; fused to, but longer than keel petals; claw 4-7 mm long. **Keel petals** 9-12 mm long, 2-4 mm wide; claw 5-7 mm long.

Diagnostic features: *P. pinnata* has no more than three flowers per axil, the inflorescence is shorter than the subtending leaves, and the leaves are born on tips of bare branches. Its seasonal shoots are densely covered in white hairs and have tightly congested stipules. Some illustrations of *P. pinnata* are shown in Plate 10.

Habitat: Mountain and lowland fynbos, stream banks and rocky seepages and forest margins
Flowering time: October to April
Altitude: 30-700 m
Distribution: Widespread in the South Western Cape (Fig. 4.17).

Plate 10. *Psoralea pinnata*. A: general habit, B: flowering shoot, C: flower, D: stem.

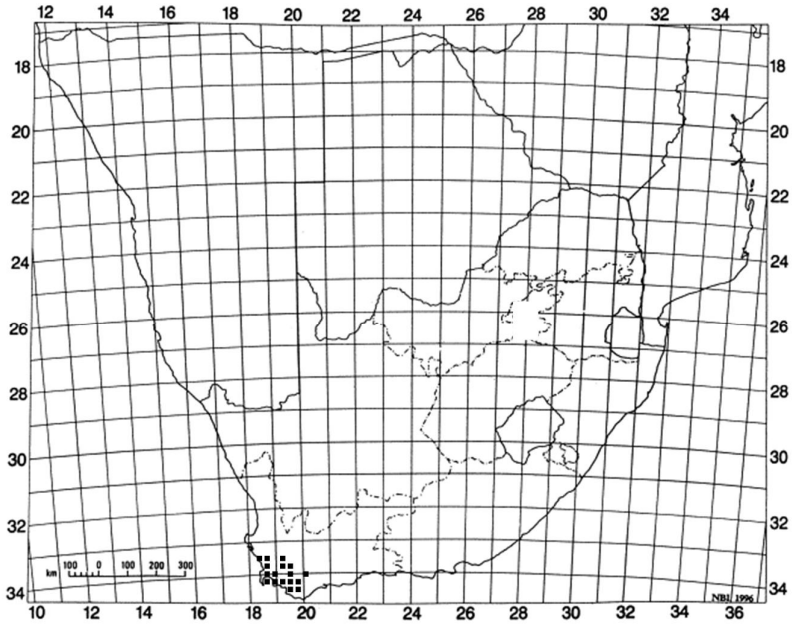

Figure 4.17. Known distribution of *Psoralea pinnata.*

Specimens examined

Dappet se gat, 3418BB, Muasya & Stirton, 3165 (BOL); Leopards Kloof, Harold Porter Botanic Garden, 3418BD, Muasya & Stirton 3171 (BOL); Rondebosch Common, Muasya & Stirton, 3394 (BOL); Somerset West, towards Stellenbosch, Muasya & Stirton, 3402 (BOL); Above Silvermine Reservoir, 3418BA, Muasya & Stirton, 3403 (BOL); Silvermine, Steenberg Plateau, 3318CD, Muasya & Stirton, 3407 (BOL); Rhodes Memorial, Muasya & Stirton, 4338 (BOL); Somerset West towards Stellenbosch, Muasya & Stirton, 3189 (BOL); Farm Murludi, Below Ager-Witzebberg, Tulbagh side, 3319AA, Muasya & Stirton, 3374 (BOL); South of Bainskloof Village, east of hut on Boland Hiking trail, Muasya & Stirton, 3444 (BOL); Valley below Jonaskop, 3319CD, Muasya & Stirton, 3340 (BOL); Wynberg Range, Wotley, A. H, 22 (BOL); Between Pringle Bay and Bettyôs Bay, 3418BD, Muasya & Stirton, 3169 (BOL); Leopardôs Kloof, Harold Porter Botanic Garden, 3418BD, Muasya & Stirton, 3172 (BOL); Kenilworth Race Course, Gray, A.S, (BOL); Constantiaberg, Pillans, N.S (BOL); Muizenberg, Pillans, N.S, 3460 (BOL); Leighton, J.M & Jagers, K, 1494 (BOL); Mosselbay, Linder, P, 4157 (BOL); Langeberg, Levyns, M.R.L, 2804 (BOL); Boschberg, Levyns, M.R.L 5572, (BOL); Palmiet River, Levyns, M.R.L, 7792 (BOL); Howieson Poort Grahamstown, J.R & B.R, 63 (BOL); Tradow Pass, Levyns, M.R.L, 678 (BOL); Fernkloof Nature Reserve, Burman,C, 1035 (BOL); Hill Slope North of Kogelbay, Gray, A.S, (BOL); Tierkloof Wemmershoek, Gray, A.S, (BOL); Entrance to Fernkloof Nature Reserve, 3419AD, Dludlu, Muasya & Stirton, 8 (BOL); Pilaarkloof, Esterhuysen, E, 31401 (BOL); Paardekop Near Knysna, Gray, A.S, (BOL); Bainskloof, Gray, A.S, (BOL); Kattery Pass, Levyns, M.R.L, 3703 (BOL).

Conservation Status: Least concern, widespread, abundant and mostly occurs in protected areas.

2. ***Psoralea glabra* E. Mey.** Comm. Pl. Afr. Austr. (1836); Walpers, Repert. 1: 656 (1842); Schmidt, Mervyn & Lötter, Trees and shrubs of Mpumalanga and Kruger National Park (2002); Loffler & Loffler, Swaziland Tree Atlas (2005). **Type:** ñWitbergen, am Fuss der Berge, bei Rietvlei, Bamboeshoek, Bamboosspruit en Wilgerboschspruitò, Drege s.n. (Lectotype: MO, Isolectotype: S). **Synonym:** *Psoralea pinnata* L. var. *glabra* (E. Mey) Harv. In Harv. & Sond., Fl. Cap. 2: 145 (1862).

Single to multi-stemmed shrub; 0.6-1.5 m tall; resprouting; colonial, but never forming dense clumps. **Stems** dark green; erect; woody throughout; terete; weakly furrowed. **Branches** stiff; emerging in lower portions; sparsely covered with randomly scattered glands. **Seasonal shoots** pale green; smooth; striate; spreading. **Leaves** 5 to 7-foliolate; linear; glabrous; patent; imparipinnate; number of leaflets variable at different stages of growth; evenly distributed along the branches. **Terminal leaflet** 10-25 mm long, 0.5-1.2 mm wide; lanceolate-linear; shorter than basal pair of lateral leaflets; apex and base acute; mucro straight, fragile, soft. **Lateral leaflets** always present; lanceolate-linear; symmetrical; longer than the terminal leaflet. **Leaflet glands** visible with a 10X hand lens; black; flush with the surface; denser on upper surface. **Petioles** 2-8 mm long; shorter than terminal leaflets. **Stipules** 1.5-2 mm long, 1.5 mm wide; recurved; persistent; broadly obliquely ovate; glabrous; shorter than petioles; not fused to petioles; minute; not tightly congested. **Inflorescences** borne in upper axils of seasonal shoots; pseudo-spicate; lax, bearing one or two flowers per axil. **Flowers** 20-30 mm long; pale blue; maturing more or less simultaneously; about the same length as the subtending leaves; bracts reduced to minute scales. **Peduncles** 7-16 mm long; filiform and flexuous. **Cupulum** bilobed with one of the vexillar lobes minutely bilabiate; lobes broadly triangular, equally developed; glandular; completely glabrous. **Pedicels** 2-5 mm long. **Calyx tube** 3.5-5.5 mm long, 7-11 mm wide; glabrous. **Calyx teeth** shorter than the calyx tube; unequal; the lateral and vexillar calyx teeth acute, triangular, falcate; the carinal calyx tooth 3-5.5 mm long, broader than other four teeth; the vexillar calyx lobes fused for up to one third of their length above the tube; calyx shorter than corolla; inner face of calyx teeth sparsely covered in small black stubby hairs; ribs distinctly thickened; glands dense, constant in size and mainly concentrated on the tube. **Standard petal** 9-10 mm long, 11-13 mm wide; claw 3-4 mm long; elongated and narrow; apex emarginate. **Wing petals** 12.5-14 mm long, 3.5-6 mm wide; up-curving; fused to, but longer than keel petals; claw 4.5-6.5 mm long. **Keel petals** 10.5-12 mm long, 3.8-4.5 mm wide; claw 5.5-6.5 mm long.

Diagnostic features: *P. glabra* is completely glabrous, leaflets are lanceolate-linear, and its inflorescences are about the same length as the subtending leaves. Some illustrations of *P. glabra* are shown in Plate 11.

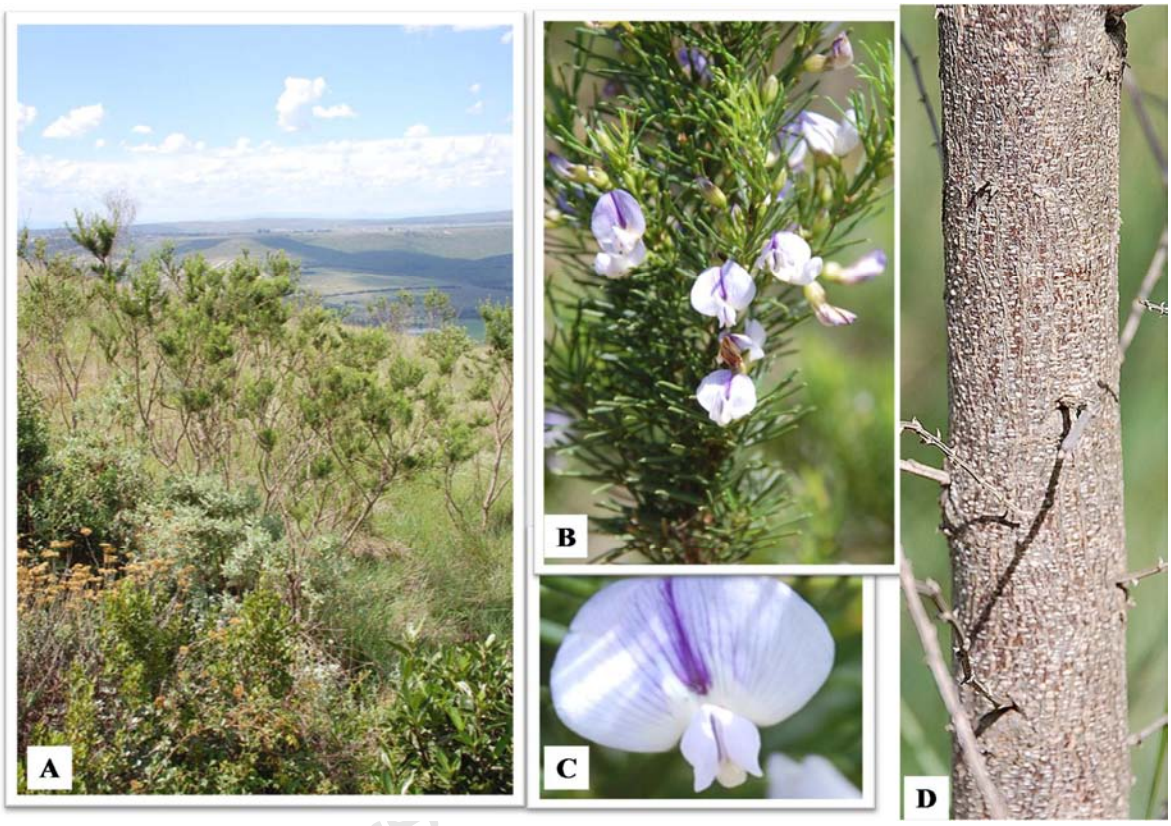

Plate 11. *Psoralea glabra*. A: general habit, B: flowering shoot, C: flower, D: stem.

Habitat: Forest margins, stream banks and seepage areas.

Flowering time: January - September

Altitude: 25-1400 m

Distribution: Eastern Cape to KwaZulu-Natal, Mpumalanga and Swaziland (Fig. 4.18).

Specimens examined

Palm Beach Malan Drive, 3030CD, Muasya & Stirton, 3646 (BOL); Umtavuma Reserve, Muasya & Stirton in Abbott, 8841.2 (BOL); Collington Kloof near Grahamstown, Isaac, W.E, 3079 (BOL); Karsten, M., 25100 (BOL); Grahamstown, Bulleni, G.V, 7 (BOL); Burrow, J.E & Burrows, S.M, 7368 (BOL); Port St. Johns, Bean, P.A, 2175 (BOL); Umtata, Bean, P.A & Viviers, 2301 (BOL); Grey Town, Natal, Wylie, J., (BOL); Goulimis, C., 32230 (BOL); Sand

Rivers Reservoir, Port Elizabeth, Holland, J.H., 36668 (BOL); Port Elizabeth, Bolus, H., 220 (BOL); Bulembu Mountain next to Bulembu Border Post, Swaziland, Dludlu, Muasya & Stirton, 107 (BOL); Mahamba Gorge, South western Swaziland, Dludlu, Muasya & Stirton 108 (BOL).

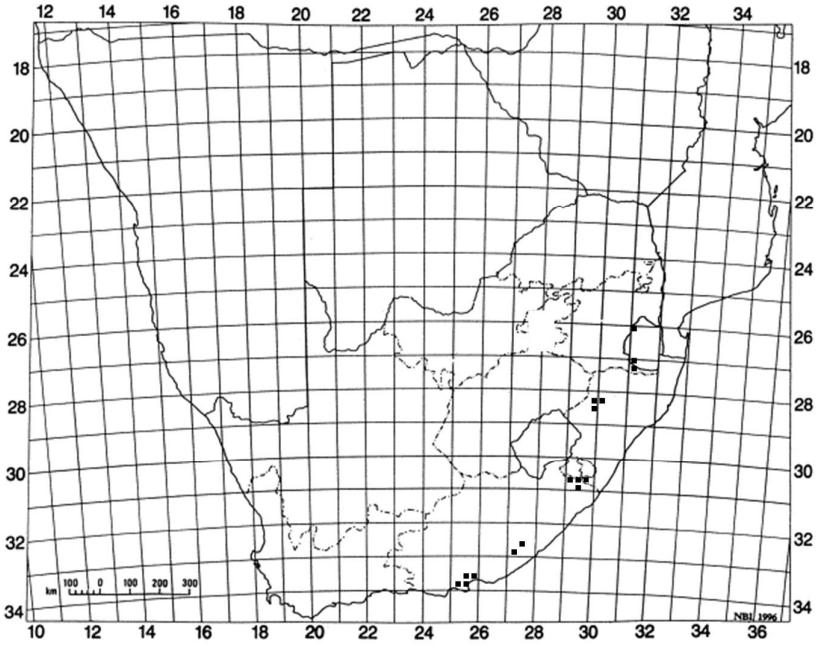

Figure 4.18. Known distribution of *Psoralea glabra.*

Conservation status: Least concern, widespread across its distribution range.

3. ***Psoralea affinis*** **Eckl. & Zeyh.** Enum 224 (1836); Walpers in Linnaea 13: 512 (1839); Walpers, Repert 1: 655 (1842); Forbes in Bothalia 3: 126 (1930); **Type:** inter frutices (altit. III) laterum montium Van Stadensrivierberge (Uitenhage), Ecklon & Zeyher s.n. (Lectotype: S, Isotype: K, L, MO)

Small trees; 3-5 m tall; robust; with a trunk diameter of 25 cm; reseeder; colonial and forming dense clumps. **Stems** single; brown-grey; erect; woody throughout; terete; weakly furrowed. **Branches** erect; stiff; emerging at a height of about 1m. **Seasonal shoots** dark green; striate; bearing randomly scattered glands; arching. **Leaves** 5, 7, 9, 11-foliolate; with minute silvery hairs; imparipinnate; number of leaflets variable at different stages of growth; patent; evenly distributed along the branches. **Terminal leaflet** 11-24 mm long; filiform-linear; apex acute; base acute; mucro arching, fragile; soft. **Lateral leaflets** always present; symmetrical; terminal

lateral leaflets about the same length as the terminal leaflet but basal laterals much longer than the terminal leaflet; lower surface of mature leaflets glabrous. **Leaflet glands** visible with a 10X hand lens; yellow to orange, densest on lower surface; distinctly raised above the surface. **Petioles** 4-8 mm long; shorter than terminal leaflets. **Stipules** 1.5 mm long, 1 mm wide; caducous; patent; narrowly triangular; glabrous; not tightly congested. **Inflorescences** borne in most axils of seasonal shoots; pseudo-spicate; congested, bearing three or more flowers per axil. **Flowers** 17-34 mm long; white; longer than subtending leaves; maturing sequentially; bracts reduced to tuft or ring of hairs. **Peduncles** 20-50 mm long, filiform and flexuous. **Cupulum** bilobed with one of the vexillar lobes variously bilabiate; lobes glabrous, distinctly glandular, equally developed. **Pedicels** 2.5-5.5 mm long. **Calyx tube** large: 4-6 mm long, 8-14 mm wide; densely covered in orange constant sized glands. **Calyx teeth** unequal; longer than calyx tube; the lateral and vexillar calyx teeth acute, falcate, triangular; the carinal calyx tooth 4-5 mm long, acute, much longer and broader than other four teeth; the vexillar calyx lobes fused for up to one third of their length above the tube; calyx longer than the corolla; inner face of calyx teeth sparsely covered in small black stubby hairs; ribs slender; glands constant sized; equally distributed on the teeth and tube. **Standard petal** large: 8-19 mm long, 9-16 mm wide; claw 4-6 mm long, elongated and narrow; apex emarginate. **Wing petals** 9-14 mm long, 4-6 mm wide; up-curving; fused to, but longer than keel petals; claw 4-6 mm long. **Keel petals** 6-13 mm long, 3-5 mm wide; claw 4-7 mm long.

Diagnostic features: *P. affinis* is a robust tree like shrub. The glands of the leaves and calyces are orange to yellow, and it has large white flowers with glabrous calyces. Its inflorescences consist of three or more flowers per axil which are longer than the subtending leaves. Peduncles may be up to 50 mm long and persist until the next flowering season. Some illustrations of this species are shown in Plate 12.

Habitat: Mountain and Lowland fynbos, stream banks
Flowering time: November to March
Altitude: 25-1047 m
Distribution: Riversdale, George, Port Elizabeth, Piketberg, Bainskloof, Vogelgat (Fig. 4.19).

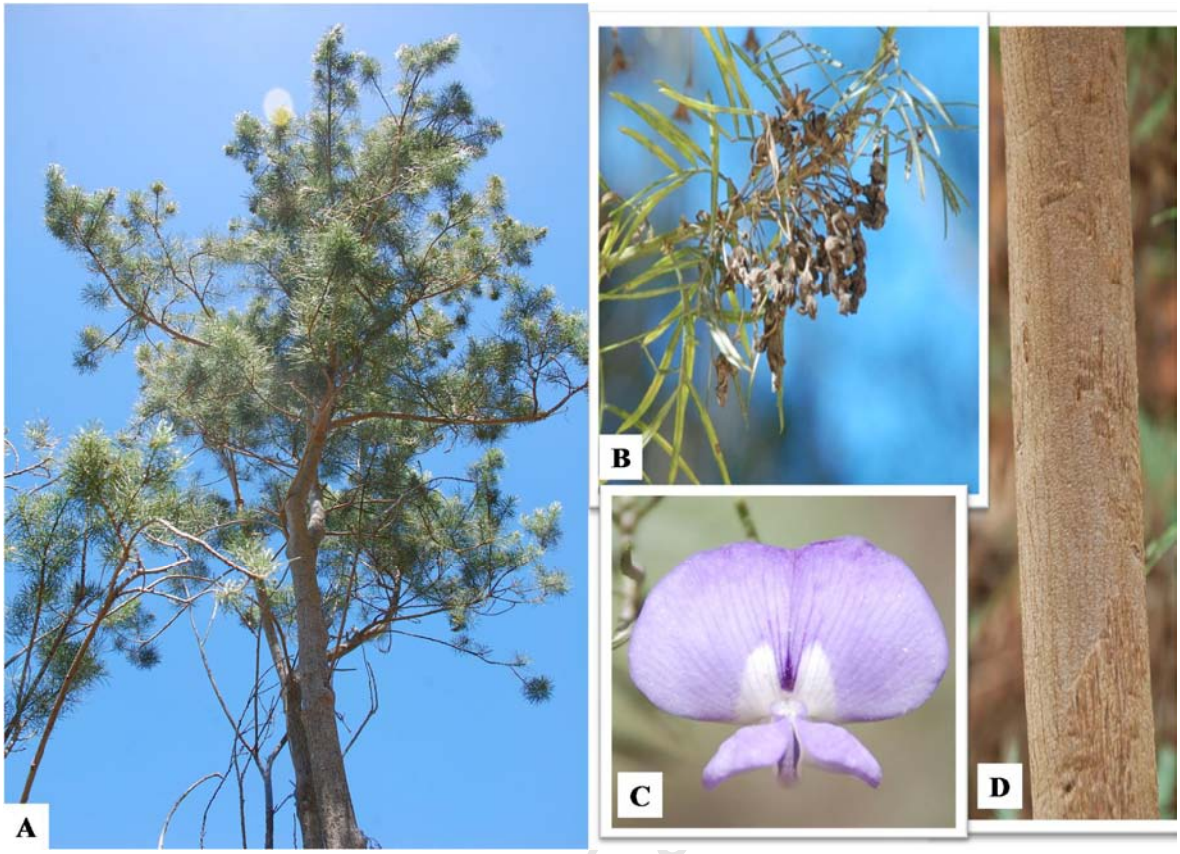

Plate 12. *Psoralea affinis*. A: general habit, B: flowering shoot, C: flower, D: stem.

Specimens examined

Vogelgat Centre, 3419AD, Muasya & Stirton, 3186 (BOL); Near crest of Robinson pass, Muasya & Stirton, 3614 (BOL); Bottom hills of Bainskloof towards Breedenkloof wines, Dludlu, Muasya & Stirton, 31 (BOL); Piketberg New Caledonia farm, 3218DA, Dludlu, Muasya & Stirton, 75 (BOL); Piketberg new Caledonia farm, 3218DA, Dludlu, Muasya & Stirton, 81 (BOL); Koudouberg, top of Montagu pass, 3322CD, Muasya & Stirton, 3609 (BOL); Below bridge next to causeway Kaaimans, 3322CD, Muasya & Stirton, 3868 (BOL); Riviersonderend, Muasya & Stirton, 3903b (BOL); Farm Dome Citrus valley, off Kanetolei, 3319AD, Muasya & Stirton, 4074 (BOL); Marloth Nature Reserve, 3320CD, Dludlu, Muasya & Stirton, 41 (BOL); Garciaôs pass, 3321CC, Dludlu, Muasya & Stirton, 48 (BOL); Slopes of the Dwarsberg, Boosmansbos Wilderness Area, 3320DD, Muasya & Stirton, 3468 (BOL); Farm Heidehof, 3419DA, Muasya & Stirton, 3212 (BOL); Knysna, Middlemost, A, BH 32221 (BOL); Fourcade, H.G, 892 (BOL); Swellendam Marloth Wild Flower Reserve, Warts, J.M., 373 (BOL); Kariedow Pass, Stirton. C.H., 10931 (BOL); Kloof below Somerset, Esterhuysen, E., 2634 (BOL);

Keishoek Forest Reserve, Esterhuysen, E., 32226 (BOL); Wellington, Grant, A.L., 2409 (BOL); South Slopes of Helderberg, Esterhuysen, E., 14634 (BOL); Bottom of Suruuranys Pass, Stirton, C.H., 10927 (BOL); Uitenhage Otterford Forest Reserve, Rodin R.J., 1130 (BOL); Formosa Peak at Uniondale, Esterhuysen, E., 4649 (BOL); Mosselbaai, Robinson Pass, Meyer, J.J., 340 (BOL); Fernkloof Nature Reserve, Bean, P.A., 700 (BOL); Joubertina, Uniondale, Esterhuysen, E., 6858 (BOL); Helderberg, Gillet, J.B., 1788 (BOL); George, Mitchell, M., 16090 (BOL); Navetyeiberg, Esterhuysen, E., 6450 (BOL); Blouberg, Loerie Plantation, Humansdorp, 1934, Dix, 19 (BOL).

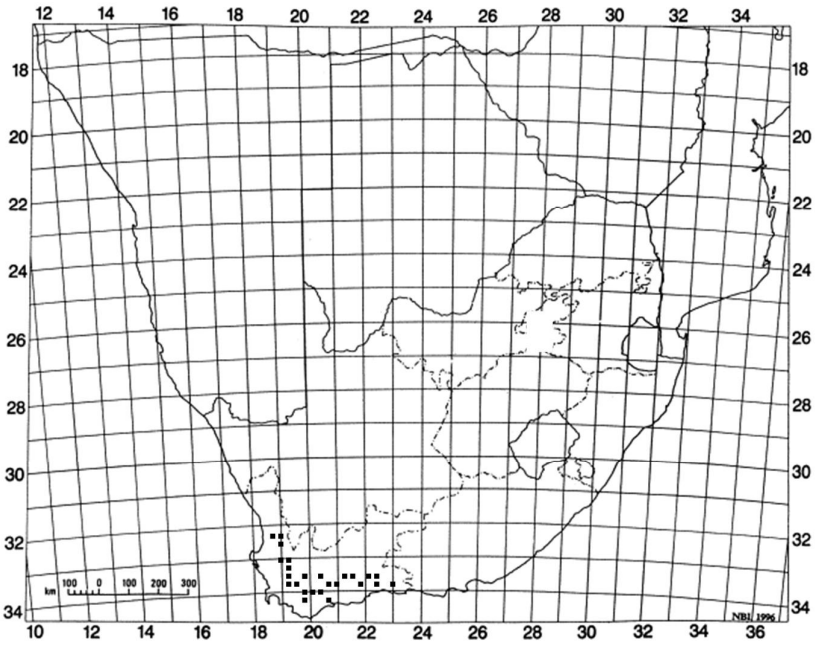

Figure 4.19. Known distribution of *Psoralea affinis.*

Conservation status: Least concern, it is widespread and abundant, occurring mainly in protected areas

4. ***Psoralea koudebergense sp nov.*** M.N. Dludlu, A.M. Muasya & C.H. Stirt. ined. **Type:** Tierfontein Farm, valley near Koudeberg Mountain, 1/11/2008 Dludlu, Muasya and Stirton 64 (Holotype: BOL, Isotype: NBG, PRE).

Shrubs, 2-3 m tall; to trees, 6-8 m tall, with trunk diameter of 40 cm; reseeder colonial and forming dense clumps. **Stems** single; cream white; erect; woody throughout; terete. **Branches**

erect; emerging in upper portions at a height of about 4m for the tree form; the shrub form branches profusely at height of 1 m forming a dome shaped crown. **Seasonal shoots** smooth; striate; with randomly scattered glands. **Leaves** 7- 9 foliolate; imparipinnate; glabrescent; erect; filiform; tightly packed; evenly distributed along the branches. **Terminal leaflet** 19-30 mm long; apex acute; mucro straight. **Lateral leaflets** always present; basal laterals much longer than the terminal laterals; upper surface of mature leaflets nitid. **Leaflet glands** visible with a 10X hand lens; black; flush with the surface; denser on upper surface. **Petioles** 5-7 mm long; shorter than terminal leaflets. **Stipules** 2-3 mm long, 1-1.5 mm wide; glabrous; persistent; recurved; shorter than petiole; free from petiole; not tightly congested. **Inflorescence borne in upper axils of seasonal shoots** with leafy extension; congested, with three or more flowers per axil. **Flowers** 18 mm long; hyacinth blue; longer than the subtending leaf; maturing sequentially. **Peduncles** 36-40 mm long; filiform and flexuous. **Cupulum** bilobed with one of the vexillar lobes variously bilabiate; lobes glandular, entirely covered with black hairs, equally developed; broadly triangular. **Pedicels** 3 mm long. **Calyx tube** 4-5 mm long 8-12 mm wide; densely covered with long black hairs. **Calyx teeth** equal; shorter than the calyx tube; the lateral and vexillar calyx teeth acuminate, straight, triangular; the carinal calyx tooth 2.5 mm long, acuminate, broader than the other four teeth; the vexillar calyx lobes fused for more than half their length above the tube; calyx shorter than corolla; inner face of calyx teeth densely covered in long black stubby hairs; glands dense, of constant size and more concentrated on the tube. **Standard petal** 9-10 mm long, 15-17 mm wide; apex emarginate; claw 4 mm long; elongated and narrow; auricles well-developed. **Wing petals** 12-14 mm long, 5 mm wide; up-curving; longer than keel petals; claw 6 mm long. **Keel petals** 12 mm long, 4 mm wide; claw 7 mm long.

Diagnostic features: *P. koudebergense* is the only species in this complex that has a tree form. It has hyacinth blue flowers, whose calyces are densely covered in long black hairs. Its inflorescences are longer than the subtending leaves and the peduncles may be up to 40 mm long. See Plate 13 for some illustrations.

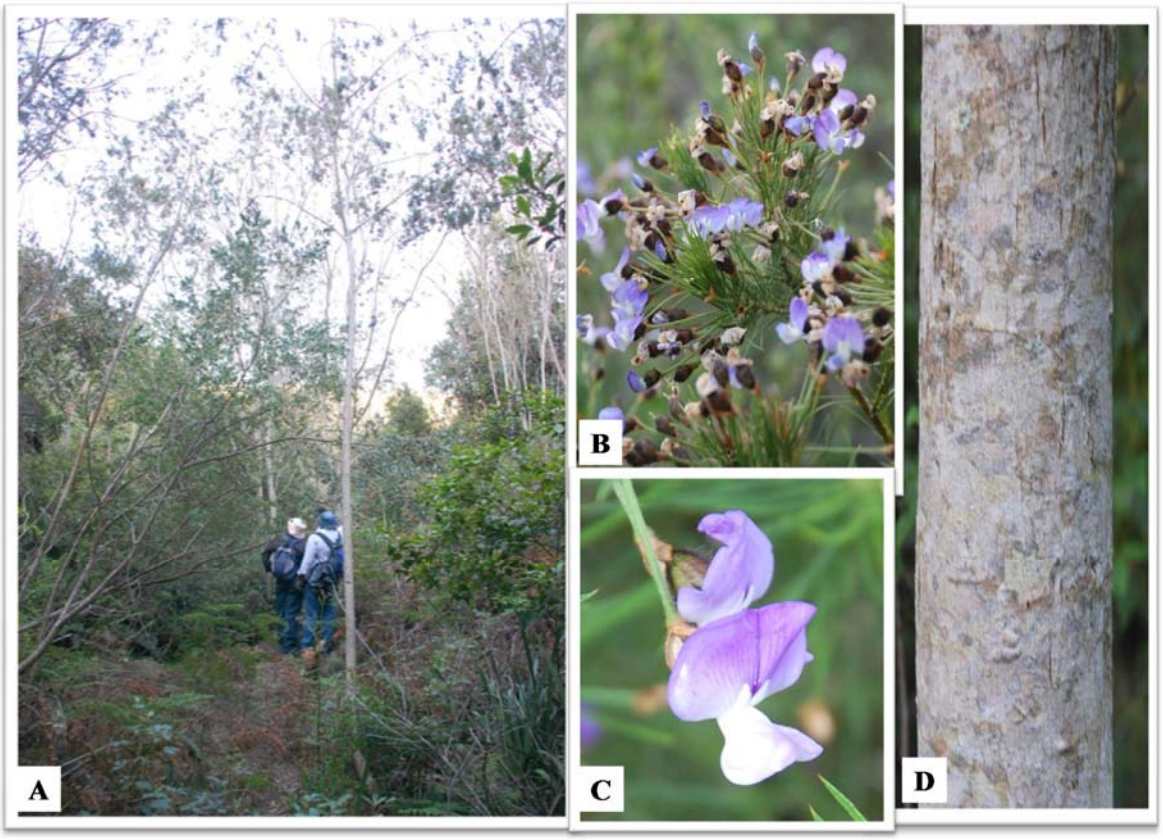

Plate 13. *Psoralea koudebergense*. A: general habit, B: flowering shoot, C: flower, D: stem.

Habitat: Mountain and coastal fynbos

Flowering time: July to December

Altitude: 50-300 m

Distribution: Southern Cape (Fig. 4.20).

Specimens Examined

Mosselbay Robinson Pass, Meyer, J.J., 340 (BOL); Bredasdorp, 3419CB, Beau, P.S &Viviers, M., 2036 (BOL). Stilbaai road from Albertinia, Dludlu, Muasya & Stirton, 50 (BOL); Tierfontein Farm, Koudeberg, 3419DA, Dludlu, Muasya & Stirton, 64 (BOL); Potberg Nature Reserve, 3420BC, Dludlu, Muasya & Stirton, 66 (BOL); Tierfontein Farm, Muasya & Stirton, 3241 (BOL); Tierfontein Farm, Muasya & Stirton, 3248 (BOL); Tierfontein Farm, 250m, Muasya & Stirton 3257 (BOL).

Conservation status: Least concern, it is abundant and occurs mainly in protected areas.

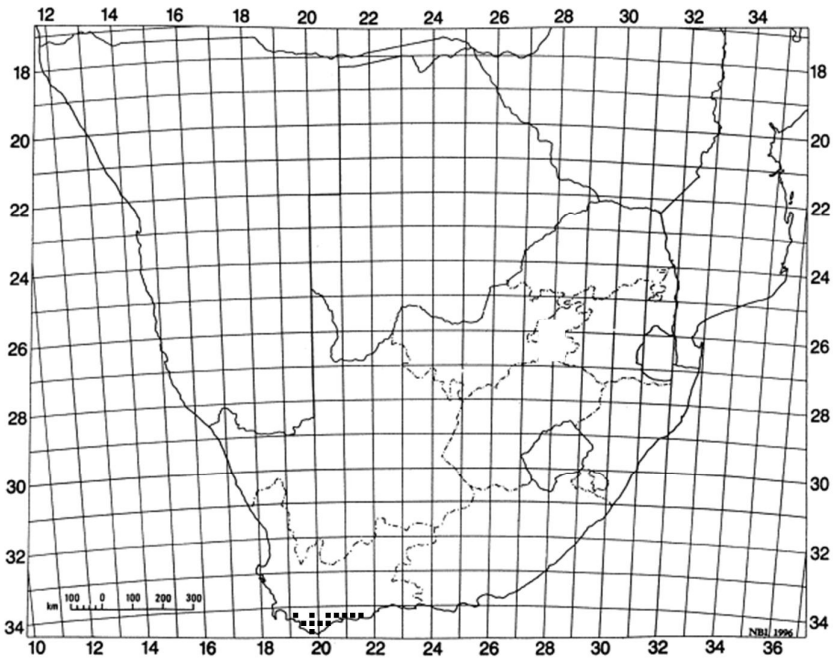

Figure 4.20. Known distribution of *Psoralea koudebergense.*

CHAPTER 5

SUMMARY AND SYNTHESIS

The study had four major objectives to achieve. Firstly, it was to reconstruct the phylogeny of the tribe Psoraleeae in order to evaluate its monophyly as well as to reconstruct phylogenetic relationships between the nine genera within the tribe and hence test their monophyly. Secondly, it was to estimate divergence dates within the Psoraleeae. Thirdly, it was to explore distribution patterns within the southern African members of the tribe, (the genera *Otholobium* and *Psoralea*) with a particular emphasis on the role of edaphic heterogeneity. Finally, the study sought to resolve nomenclature and define species boundaries within the genus *Psoralea*.

The purpose of evaluating the monophyly of the tribe itself was to address the long-standing question of the phylogenetic position and hence taxonomic status of the Psoraleeae. Evidence from morphology, phytochemistry and several molecular phylogenetic studies has shown that the tribe is embedded within the Phaseoleae, as sister to the sub-tribe Glycininae. Molecular evidence from this study further indicated that the taxon is a monophyletic entity and therefore it is proposed that Psoraleeae should be recognised as a sub-tribe of the Phaseoleae rather than a tribe. This study also established that the genus *Otholobium* is not monophyletic as currently circumscribed. Importantly, it indicated that the South American species that are currently classified under *Otholobium* (Grimes 1990) are not closely related to the Southern African *Otholobium* and therefore may need to be given a different name. On another note, the sister relationship between *Otholobium* and *Psoralea*, which was proposed by Grimes (1990) is not supported by the current data as *Psoralea* was resolved to be a clade nested within the genus *Otholobium*, making the South African *Otholobium* paraphyletic. However, since the resolution of the current phylogeny is poor, there remains some scope to expect that further molecular studies and perhaps more phytochemical and anatomical studies may prove the two genera to be monophyletic. Therefore, the taxonomic status of the two genera is better left unchanged for now. Similarly, the current data does not provide any evidence against the subsuming of *Hallia* into *Psoralea* and therefore, it is left unchanged.

Like some other Cape lineages, the Psoraleeae were estimated to have originated post the Miocene climate change (about 6.41 million years ago) and therefore the existence of such a large number of species within the lineage (about 103 species) indicates that they are a product of rapid radiation occurring within a short space of time.

Concerning distribution, the study showed that the distribution of species in the genus *Psoralea* is chiefly on sandstone derived substrates (66 % of the species), with a few species occurring on limestone, shale, granite and sand soils. On the other hand, *Otholobium* is ubiquitous, with equal proportions of its species occurring on sandstone, granite and shale substrates. In addition, for those species that occur on limestone and sand habitats, the *Otholobium* species are in larger proportions than the *Psoralea* species. Since *Otholobium* is relatively older than *Psoralea*, its wide distribution across the various soil types indicates that it has had more time to develop the necessary adaptations to exploit the various soil types. On the other hand, *Psoralea* species are still restricted to the nutrient poor sandstone habitats.

The study of the nutritional concentrations of the various soils occupied by both genera indicated that there is also considerable diversity in soil nutrition. It was also found that for the species that were studied for soil nutrient levels, there is a tendency of closely related species to occur on nutritionally diverse habitats. Furthermore, it was found that resprouters are associated with nutrient poor soils, while a majority of the reseeders occur on nutrient richer soils. These observations, as well as the observation that the distribution of species encompasses a wide diversity of soil types suggest that edaphic heterogeneity might have had an important role in driving diversification within the southern African Psoraleeae. Therefore, further studies, with greater sampling of soils and resolution of phylogenetic relationships are required in order to test whether the rapid radiation in the southern African Psoraleeae was mainly driven by edaphic factors.

In terms of the taxonomy, this study was able to show that both the *Psoralea aphylla* and *P. pinnata* complexes contain several entities that can be recognised as distinct species. These have been appropriately described and identification has been made easier by the provision of keys as well as diagnostic features for each of the species. The methods used here have proven useful in resolving species complexes and therefore can be applied to other species complexes to achieve a complete revision of the genus *Psoralea*.

Future directions

There is need to further resolve the phylogenetic relationships with regards to the southern African genera to ascertain their position, and then to test biogeographical hypotheses such as long distance dispersal to explain the worldwide distribution of the tribe. Finding robust phylogenies will also allow for inference of speciation processes by examining sister species pairs in relation to nutritional and niche requirements. This can also allow for testing whether co-

occurrence among species of the Psoraleeae reflects phylogenetic structure [a hypothesis explored in the Schoenoid sedges by Slingsby & Verboom (2006)]. More work on the southern African genera focusing on the cytogenetics of the group (chromosome counts), pollination biology and further analysis of soil nutrient data may yield useful information for the inference of drivers of speciation in the lineage. Finally, although taxonomic problems within the *P. aphylla* and *P. pinnata* complexes have been resolved in this study, there is still a need for a monograph of the genus *Psoralea* so that all taxonomic issues associated with it are fully resolved.

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Appendix 1. Morphological and ecological data set for the southern African Psoraleeae used for phylogenetic and ancestral trait reconstruction. Characters and character states are as defined in Table 2.2. G=*Glycine*, O=*Otholobium* and P=*Psoralea*.

Character #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
G. canescens	0	0	1	1	1	0	1	3	3	2	0	1	2	3	2	2	3	1	0	0	0	0	1	0	1	1	3	4	2	0
G. microphylla	0	0	1	1	1	0	1	3	3	2	0	1	2	3	2	2	3	1	0	0	0	0	1	0	1	1	3	4	2	0
O. acuminatum	1	0	1	0	1	0	1	3	3	2	0	0	1	3	2	0	3	0	2	1	1	1	1	3	1	1	3	4	2	1
O. arborescens	3	0	1	1	0	0	1	3	3	2	0	1	1	3	2	1	3	1	1	1	1	0	1	2	3	1	3	4	2	1
O. bolusii	0	1	1	1	1	0	1	3	3	2	0	1	1	3	2	1	3	0	2	1	0	0	1	0	0	1	3	4	2	1
O. bracteolatum	1	0	2	1	0	0	1	3	3	2	0	0	1	3	2	1	3	0	2	1	1	1	1	3	0	1	3	4	0	1
O. bracteolatum2	1	0	2	1	0	0	1	3	3	2	0	0	1	3	2	1	3	0	2	1	1	1	1	3	0	1	3	4	0	1
O. candicans1	1	0	1	1	0	0	1	3	3	2	0	0	1	3	2	1	3	0	0	1	0	0	1	2	1	1	3	4	0	1
O. candicans1	1	0	1	1	0	0	1	3	3	2	0	0	1	3	2	1	3	0	0	1	0	0	1	2	1	1	3	4	0	1
O. dreweae	0	1	1	1	1	0	0	2	2	0	7	2	3	1	3	3	3	0	0	2	2	1	1	3	3	1	3	4	0	1
O. flexuosum	2	0	1	0	1	0	1	3	3	2	0	0	1	3	2	0	3	1	0	1	1	0	1	3	1	1	3	4	1	1
O. foliosum	2	0	1	0	1	0	1	3	3	2	0	0	1	3	2	1	3	0	2	1	1	1	1	3	2	1	3	4	1	1
O. fruticans	0	1	2	1	1	0	1	3	3	2	0	0	1	3	2	1	3	0	2	1	1	1	1	3	0	1	3	4	0	1
O. hamatum	2	0	1	0	0	0	1	3	3	2	0	0	1	3	2	0	3	0	2	1	1	0	1	3	0	1	3	4	0	1
O. hirtum	2	0	1	1	1	0	1	3	3	2	0	0	1	3	2	0	3	1	2	1	1	0	1	3	0	1	3	4	0	1
O. lanceolatum	0	1	0	1	1	0	0	1	2	0	7	2	3	1	3	3	3	1	2	2	1	1	1	3	3	1	3	4	0	1
O. lucens	0	1	1	1	0	0	1	3	3	2	0	0	1	3	2	0	3	1	0	1	0	0	1	0	1	1	3	4	2	1
O. macradenium	1	1	1	1	0	0	1	3	3	2	0	0	1	3	2	0	3	0	0	1	0	0	1	0	0	1	3	4	2	1
O. mundianum	1	1	1	1	0	0	1	3	3	2	0	0	1	3	2	1	3	1	2	1	0	0	1	0	0	1	3	4	0	1
O. nigricans	1	0	1	1	1	0	1	3	3	2	0	0	1	3	2	2	3	1	2	1	0	1	1	1	2	1	3	4	2	1
O. nitens	1	0	1	1	1	0	1	3	3	2	0	0	1	3	2	1	3	1	2	1	0	0	1	0	1	1	3	4	0	1
O. obliquum	1	1	1	1	1	0	1	3	3	2	0	0	1	3	2	1	3	1	2	1	0	0	1	0	1	1	3	4	0	1
O. parviflorum31	1	1	1	1	0	0	1	3	3	2	0	0	1	3	2	2	3	1	2	1	1	1	1	3	1	1	3	4	0	1
O. parviflorum	1	1	1	1	0	0	1	3	3	2	0	0	1	3	2	2	3	1	2	1	1	1	1	3	1	1	3	4	0	1
O. polyphyllum	1	1	1	1	1	0	1	3	3	2	0	0	1	3	2	0	3	1	2	1	0	1	1	1	0	1	3	4	0	1
O. polystictum	2	0	1	1	0	0	1	3	3	2	0	0	1	3	2	2	3	0	0	1	0	0	1	0	0	1	3	4	2	1
O. prodiens92	2	0	1	0	1	0	1	3	3	2	0	0	1	3	2	1	3	1	0	1	1	1	1	3	0	1	3	4	0	1
O. prodiens38	2	0	1	0	1	0	1	3	3	2	0	0	1	3	2	1	3	1	0	1	1	1	1	3	0	1	3	4	0	1
O. prodiens	2	0	1	0	1	0	1	3	3	2	0	0	1	3	2	1	3	1	0	1	1	1	1	3	0	1	3	4	0	1
O. pungens	1	1	0	1	1	0	1	3	3	2	0	0	1	3	1	0	3	0	2	0	0	0	1	0	1	1	3	4	0	1
O. pustulatum	1	0	1	1	0	0	1	3	3	2	0	1	1	3	2	0	3	1	0	1	1	0	1	3	1	1	3	4	0	1
O. rotundifolium	0	1	1	1	1	0	0	2	2	0	7	2	3	1	3	3	3	1	2	1	0	1	1	2	3	1	3	4	0	1
O. saxosum	0	1	2	1	0	0	1	3	3	2	0	0	1	3	2	0	3	1	2	1	0	0	1	0	0	1	3	4	0	1
O. sericeum	2	0	1	1	1	0	1	3	3	2	0	1	2	3	2	2	3	1	2	1	2	1	1	3	3	1	3	4	2	1

Appendix 1. cont…

Character #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
O. spicatum3	2	0	1	1	1	0	1	3	3	2	0	0	1	3	2	1	3	1	2	1	1	1	1	3	0	1	3	4	2	1
O. spicatum3	2	0	1	1	1	0	1	3	3	2	0	0	1	3	2	1	3	1	2	1	1	1	1	3	0	1	3	4	2	1
O. spissum	1	0	1	1	1	0	1	3	3	2	0	0	1	3	2	0	3	0	0	1	0	0	1	0	0	1	3	4	0	1
O. stachydis	2	0	1	1	1	0	1	3	3	2	0	0	1	3	2	0	3	1	2	1	1	0	1	3	0	1	3	4	0	1
O. stachyerum	1	0	1	1	1	0	1	3	3	2	0	0	1	3	2	0	3	0	2	1	1	1	1	3	0	1	3	4	1	1
O. striatum	2	0	1	1	1	0	1	3	3	2	0	1	1	3	2	2	3	1	2	1	1	0	1	3	0	1	3	4	2	1
O. striatum3	2	0	1	1	1	0	1	3	3	2	0	1	1	3	2	2	3	1	2	1	1	0	1	3	0	1	3	4	2	1
O. striatum3	2	0	1	1	1	0	1	3	3	2	0	1	1	3	2	2	3	1	2	1	1	0	1	3	0	1	3	4	2	1
O. swartbergense	0	1	2	1	1	0	1	3	3	2	0	1	1	3	2	1	3	1	0	1	2	1	1	3	3	1	3	4	0	1
O. swartbergense	0	1	2	1	1	0	1	3	3	2	0	1	1	3	2	1	3	1	0	1	2	1	1	3	3	1	3	4	0	1
O. thomii	0	1	2	1	1	0	0	2	2	0	7	2	3	1	3	3	3	1	0	2	2	1	1	3	2	1	3	4	0	1
O. uncinatum	0	1	0	1	1	0	1	3	3	2	0	1	1	3	2	0	3	0	2	1	0	1	1	2	0	1	3	4	2	1
O. venustum	1	1	2	1	1	0	1	3	3	2	0	0	1	3	2	2	3	0	0	1	1	0	1	3	0	1	3	4	0	1
O. virgatum	0	1	0	1	1	0	1	3	3	2	0	0	1	3	2	0	3	1	2	1	0	1	1	0	0	1	3	4	2	1
O. wilmsii	1	0	1	1	1	0	1	3	3	2	0	0	1	3	2	2	3	0	0	1	0	0	1	2	3	1	3	4	2	1
O. zeyheri	0	1	2	1	1	0	1	3	3	2	0	0	1	3	2	1	3	1	2	1	0	1	1	2	3	1	3	4	2	1
P. aculeata	1	0	1	1	1	0	1	3	3	2	0	1	1	3	2	0	3	0	0	0	0	0	0	2	1	0	1	1	2	1
P. affinis320	2	0	1	0	1	0	1	3	3	2	2	2	0	3	2	2	3	0	0	0	1	1	2	0	1	0	1	1	2	1
P. affinis4074	2	0	1	0	1	0	1	3	3	2	2	2	0	3	2	2	3	0	0	0	1	1	2	0	1	0	1	1	2	1
P. affinis	2	0	1	0	1	0	1	3	3	2	2	2	0	3	2	2	3	0	0	0	1	1	2	0	1	0	1	1	2	1
P. angustifolia	1	0	1	1	1	0	1	3	3	2	0	1	1	3	2	2	3	1	0	0	0	0	2	0	2	0	1	2	2	1
P. aphylla1	2	0	1	1	1	1	2	3	3	2	7	2	3	3	3	3	1	0	3	2	1	0	0	3	1	0	0	3	0	1
P. aphylla2	2	0	1	1	1	1	2	3	3	2	7	2	3	3	3	3	1	0	3	2	1	0	0	3	1	0	0	3	0	1
P. aphylla	2	0	1	1	1	1	2	3	3	2	7	2	3	3	3	3	1	0	3	2	1	0	0	3	1	0	0	3	0	1
P. asarina	0	1	0	1	1	0	0	2	2	1	7	2	3	1	3	3	3	0	2	2	0	0	0	2	3	0	1	2	0	1
P. azurea	0	1	2	1	1	0	1	3	3	2	2	2	0	3	0	0	3	1	0	0	1	0	0	2	1	0	1	2	2	1
P. elegans	1	0	1	1	1	0	1	3	3	2	2	2	0	3	0	1	3	1	0	0	0	1	2	2	3	0	1	2	0	1
P. fleta	2	0	1	0	1	1	2	3	3	2	7	2	3	3	3	3	2	1	2	2	1	0	0	0	1	0	2	1	0	1
P. floccosa	1	0	1	1	1	0	1	3	3	2	1	2	0	3	1	1	3	0	0	0	1	1	1	0	0	0	1	0	2	1
P. filifolia	2	0	1	0	1	1	2	3	3	2	7	2	3	3	3	3	2	1	2	2	1	0	0	0	1	0	2	1	0	1
P. gigantea	3	0	1	0	1	1	2	3	3	2	7	2	3	3	3	3	2	0	2	2	1	0	0	0	1	0	1	1	0	1
P. glabra	2	0	1	1	1	0	1	3	3	2	1	2	0	3	0	0	3	1	0	0	0	1	1	1	1	0	1	2	2	1
P. glabra2	2	0	1	1	1	0	1	3	3	2	1	2	0	3	0	0	3	1	0	0	0	1	1	1	1	0	1	2	2	1
P.glaucescens	1	0	1	1	1	0	1	3	3	2	0	0	0	3	2	1	3	1	0	0	0	0	0	0	2	0	1	2	2	1

Appendix 1. cont…

Character #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
P. imminens	1	0	1	1	1	0	1	3	3	2	4	2	0	3	0	1	3	1	0	0	0	1	2	2	3	0	0	2	2	1
P. koudebergense	3	0	1	0	1	0	1	3	3	2	2	2	0	3	1	1	3	0	0	0	1	1	2	0	1	0	2	1	2	1
P. latifolia	1	1	1	1	1	0	1	3	3	2	3	2	3	3	0	1	3	1	0	0	0	0	0	2	3	0	0	2	2	1
P. laxa	0	1	0	1	1	0	0	1	2	1	7	2	3	1	3	3	3	0	2	2	0	0	0	2	3	0	1	2	0	1
P. monophylla	0	1	0	1	1	0	0	1	2	1	7	2	3	1	3	3	3	0	2	2	0	0	0	2	3	0	1	2	0	1
P. muirii	1	1	1	1	1	0	1	3	3	2	1	2	0	3	2	0	3	0	0	0	0	0	0	0	0	0	1	0	2	1
P. odoratissima	0	1	0	1	1	0	1	3	3	2	0	1	1	3	2	0	3	0	0	0	1	0	0	2	3	0	1	2	2	1
P. oligophylla	1	0	1	1	1	0	1	3	3	2	0	1	1	3	2	0	3	1	0	0	0	0	0	2	2	0	1	2	2	1
P. oreophila	0	1	0	1	1	0	1	3	3	2	0	0	1	3	1	0	3	1	0	0	0	0	0	2	3	0	0	2	0	1
P. oreopola	1	0	1	1	1	0	1	3	3	2	1	2	1	3	2	2	3	0	2	0	0	0	0	0	1	0	2	3	0	1
P. peratica	2	0	1	1	1	1	2	3	3	2	7	2	3	3	3	3	1	0	3	2	1	0	0	3	0	0	2	0	0	1
P. pinnata	3	0	1	0	1	0	1	3	3	2	2	2	0	3	1	1	3	0	0	0	1	1	2	0	1	0	2	1	2	1
P. pullata	2	0	1	1	1	1	2	3	3	2	7	2	3	3	3	3	1	0	3	2	1	0	0	3	1	0	1	3	2	1
P. repens2	0	1	0	1	1	0	1	3	3	2	0	1	1	3	1	1	3	1	0	2	0	0	2	0	3	0	2	1	0	1
P. repens3	0	1	0	1	1	0	1	3	3	2	0	1	1	3	1	1	3	1	0	2	0	0	2	0	3	0	2	1	0	1
P. restioides	0	1	2	1	1	0	1	3	3	2	0	0	1	3	2	1	3	0	2	0	0	0	0	0	1	0	0	1	0	1
P. rhizotoma	1	1	1	1	1	0	1	3	3	2	2	2	1	3	2	1	3	1	0	0	0	1	2	2	3	0	1	2	2	1
P. rigidula	1	1	1	1	1	1	2	3	3	2	7	2	3	3	3	3	0	1	3	2	0	0	0	3	2	0	1	2	0	1
P. sordida	1	1	1	1	1	0	1	3	3	2	0	0	0	3	0	1	3	0	0	0	0	1	1	0	1	0	1	1	2	1
P. speciosa	1	0	1	1	1	0	1	3	3	2	1	2	0	3	2	1	3	0	0	0	1	1	2	3	0	0	1	0	1	1
P. speciosa	1	0	1	1	1	0	1	3	3	2	1	2	0	3	2	1	3	0	0	0	1	1	2	3	0	0	1	0	1	1
P. triflora	1	0	1	1	1	0	1	3	3	2	0	1	1	3	2	0	3	1	0	0	0	0	0	2	2	0	1	2	2	1
P. usitata1	2	1	1	1	1	1	2	3	3	2	7	2	3	3	3	3	1	0	3	2	0	0	0	3	1	0	1	1	2	1
P. usitata	2	1	1	1	1	1	2	3	3	2	7	2	3	3	3	3	1	0	3	2	0	0	0	3	1	0	1	1	2	1
P. verrucosa	1	1	1	1	0	0	1	3	3	2	1	2	0	3	2	1	3	1	0	0	0	1	2	2	3	0	1	2	2	1
P. vigilans	2	1	1	1	1	1	2	3	3	2	7	2	3	3	3	3	1	0	3	2	0	0	0	3	1	0	1	1	2	1

Appendix 1. cont…

Character #	31	32	33	34	35	36	37	38	39	40
G. canescens	0	1	1	1	0	0	0	0	1	1
G. microphylla	0	1	1	1	0	0	0	0	1	1
O. acuminatum	1	0	1	0	0	0	0	0	1	1
O. arborescens	1	0	1	0	0	0	0	0	1	1
O. bolusii	1	0	1	0	0	0	0	0	1	1
O. bracteolatum	1	0	1	0	0	0	0	0	1	1
O. bracteolatum2	1	0	1	0	0	0	0	0	1	1
O. candicans1	1	0	1	0	0	0	0	0	1	1
O. candicans1	1	0	1	0	0	0	0	0	1	1
O. dreweae	1	0	1	0	0	0	0	0	1	1
O. flexuosum	1	0	1	0	0	0	0	0	1	1
O. foliosum	1	0	1	0	0	0	0	0	1	1
O. fruticans	1	0	1	0	0	0	0	0	1	1
O. hamatum	1	0	1	0	0	0	0	0	1	1
O. hirtum	1	0	1	0	0	0	0	0	1	1
O. lanceolatum	1	0	1	0	0	0	0	0	1	1
O. lucens	1	0	1	0	0	0	0	0	1	1
O. macradenium	1	0	1	0	0	0	0	0	1	1
O. mundianum	1	0	1	0	0	0	0	0	1	1
O. nigricans	1	0	1	0	0	0	0	0	1	1
O. nitens	1	0	1	0	0	0	0	0	1	1
O. obliquum	1	0	1	0	0	0	0	0	1	1
O. parviflorum31	1	0	1	0	0	0	0	0	1	1
O. parviflorum	1	0	1	0	0	0	0	0	1	1
O. polyphyllum	1	0	1	0	0	0	0	0	1	1
O. polystictum	1	0	1	0	0	0	0	0	1	1
O. prodiens92	1	0	1	0	0	0	0	0	1	1
O. prodiens38	1	0	1	0	0	0	0	0	1	1
O. prodiens	1	0	1	0	0	0	0	0	1	1
O. pungens	1	0	1	0	0	0	0	0	1	1
O. pustulatum	1	0	1	0	0	0	0	0	1	1
O. rotundifolium	1	0	1	0	0	0	0	0	1	1
O. saxosum	1	0	1	0	0	0	0	0	1	1
O. sericeum	1	0	1	0	0	0	0	0	1	1

Appendix 2.1 Mean±SE and ANOVA results for the *Otholobium* and *Psoralea* species.
Values in a row that do not share the same letter(s) above them differ significantly (p>0.05).

Soil parameter	*O. acuminatum*	*O. bracteolatum*	*O. candicans*	*O. dreweae*	*O. flexuosum*	*O. fruticans*	*O. hamatum*	*O. lanceolatum*	*O. obliquum*
# samples	2	4	4	4	4	4	4	4	4
	efgh	hi	eg	def	def	def	eg	def	abcdef
pH KCl	5.35±0.28	6.85±0.20	5.23±0.20	4.58±0.20	4.63±0.20	4.63±0.20	5.10±0.20	4.65±0.20	4.13±0.20
	bdefg	abc	bdefg	a	dbefg	bdefg	efgh	a	bde
NO3-N mg/kg	10.37±7.09	3.18±5.02	9.23±5.02	1.70±5.02	8.45±5.02	8.01±5.02	21.04±5.02	1.42±5.02	7.89±5.02
	abcdefgh	abc	abcd	a	abcdefg	abcde	abcdef	ab	abcdef
NH4-N mg/kg	6.01±2.57	3.69±1.82	4.33±1.82	2.97±1.82	6.87±1.82	4.70±1.82	5.67±1.82	3.65±1.82	5.54±1.82
	abcdef	ijkl	hijkl	a	efghijk	ab	abcdefg	a	abcd
P Bray II mg/kg	3.50±1.91	16.50±1.35	14.67±1.35	1.00±1.35	7.75±1.35	1.33±1.35	4.25±1.35	1.00±1.35	2.25±1.35
	abcd	ab	defghi	efghij	hijk	ghijk	fghijk	defghi	bcdefgh
K mg/kg	37.50±27.16	34.67±19.20	106.50±19.20	20	0	157.25±19.20	148.50±19.20	103.25±19.20	90.75±19.20
	abcde	ab	ghi	hi	abc	cdefgh	abcdef	defgh	abcdef
%C	1.69±0.67	0.93±0.47	4.53±0.47	5.05±0.47	1.28±0.47	2.75±0.47	2.14±0.47	3.09±0.47	2.16±0.47
	abcdeg	a	ab	bcdefgh	abcde	abcdeg	abcd	fhi	abcdeg
%Na	2.44±0.76	0.87±0.53	1.27±0.53	3.19±0.53	2.12±0.53	2.34±0.53	1.58±0.53	6.58±0.53	2.36±0.53
	ab	abcd	bcdefg	defghijk	l	hijkl	ghijk	cdefghij	kl
%K	1.16±0.82	2.14±0.58	2.60±0.58	4.00±0.58	10.76±0.58	5.81±0.58	5.38±0.58	3.92±0.58	8.41±0.58
	abcde	f	ef	cdef	cdef	cdef	ef	abcde	abcde
%Ca	52.17±7.21	90.49±5.10	59.82±5.10	44.85±5.10	46.34±5.10	48.91±5.10	56.92±5.10	31.38±5.10	26.82±5.10
	a	a	cdef	cdef	bcd	bcd	cdef	ef	b
%Mg	3.48±3.44	6.51±2.43	26.46±2.43	26.27±2.43	19.00±2.43	21.15±2.43	26.66±2.43	37.69±2.43	12.66±2.43
	ab	a	a	ab	a	ab	a	abc	a
Ex Na mg/kg	0.14±0.09	0.05±0.06	0.13±0.06	0.24±0.06	0.09±0.06	0.16±0.06	0.09±0.06	0.46±0.06	0.058±0.06
	abcd	ab	abcdefgh	bcdefgh	ghi	defgh	cdefgh	abcdefgh	abcdefgh
Ex K mg/kg	0.10±0.08	0.09±0.06	0.27±0.06	0.31±0.06	0.47±0.06	0.40±0.06	0.38±0.06	0.27±0.06	0.23±0.06
	abcdefgh	fgh	gh	bcdefgh	abcdefg	bcdefgh	cdefgh	abcdefg	ab
Ex Ca mg/kg	8.91±1.31	5.87±0.92	6.34±0.92	3.52±0.92	2.04±0.92	3.58±0.92	4.19±0.92	2.15±0.92	0.70±0.92
	ab	ab	ijk	ghij	abcdefgh	defghij	ghij	ijk	a
Ex Mg mg/kg	0.22±0.50	0.42±0.35	2.77±0.35	2.00±0.35	1.09±0.35	1.51±0.35	1.99±0.35	2.61±0.35	0.30±0.35
	abcdef	abcde	def	bcdef	abcd	bcdef	bcdef	bcdef	ab
T-Value	10.53±1.91	5.15±1.35	10.49±1.35	7.74±1.35	4.30±1.35	7.15±1.35	7.34±1.35	6.90±1.35	3.06±1.35
	abc	abcd	abc	bcde	abc	ab	a	cde	abc
NH4:NO3	0.59±1.06	1.19±0.75	0.62±0.75	2.66±0.75	0.82±0.75	0.57±0.75	0.29±0.75	2.57±0.75	0.82±0.75

Appendix 2.1 continued….

Soil parameter	*O. pustulatum*	*O. rotundifolium*	*O. striatum*	*O. thomii*	*P. aculeata*	*P. affinis*	*P. aphylla2*	*P. aphylla*	*P. asarina*
# samples	4	4	4	4	4	4	4	4	2
	eg	**abcdef**	**gh**	**cdef**	**a**	**ab**	**def**	**abcdef**	**abcdef**
pH KCl	5.00±0.20	4.08±0.20	6.18±0.20	4.40±0.20	3.20±0.20	3.25±0.20	4.05±0.20	4.70±0.20	4.00±0.28
	hi	**defg**	**gh**	**fgh**	**abc**	**ac**	**efgh**	**j**	**bcdefg**
NO3-N mg/kg	42.19±5.02	13.23±5.02	22.95±5.02	3.84±5.02	3.32±5.02	2.15±5.02	2	17.13±5.02	8.45±7.09
	abcdef	**abcdef**	**abcde**	**ab**	**h**	**bcdefgh**	**abcde**	**gh**	**abcdefg**
NH4-N mg/kg	5.20±1.82	5.42±1.82	4.82±1.82	3.09±1.82	16.66±1.82	7.40±1.82	19.04±1.82	11.12±1.82	9.03±2.57
	ghijkl	**ab**	**l**	**abcdefg**	**abcdef**	**abcde**	**defghij**	**ijkl**	**abcdefgh**
P Bray II mg/kg	11.25±1.35	1.50±1.35	21.67±1.35	4.00±1.35	3.00±1.35	2.75±1.35	16.75±1.35	7.25±1.35	6.50±1.91
	k	**bcdefg**	**ijk**	**efghij**	**cdefghi**	**bcdef**	**jk**	**ghijk**	**abcde**
K mg/kg	334.75±19.20	63.00±19.20	230.63±19.20	20	101.25±19.20	59.50±19.20	20	0	43.50±27.16
	defghi	**abcd**	**abcd**	**abcd**	**i**	**defghi**	**hi**	**hi**	**abcdefg**
%C	3.33±0.47	1.77±0.47	1.70±0.47	1.70±0.47	6.47±0.47	3.24±0.47	5.53±0.47	5.42±0.47	1.93±0.67
	abcde	**bcdefgh**	**abcde**	**defghi**	**abcde**	**abcdeg**	**hi**	**abcd**	**fghi**
%Na	2.16±0.53	3.31±0.53	2.50±0.53	4.11±0.53	2.07±0.53	2.73±0.53	1.55±0.53	7.35±0.53	6.78±0.76
	jkl	**fghijk**	**defghij**	**ijkl**	**bcdefgh**	**bcdef**	**fghijk**	**efghijk**	**abcdefgh**
%K	7.60±0.58	5.26±0.58	4.00±0.58	6.08±0.58	2.85±0.58	2.51±0.58	4.69±0.58	5.13±0.58	2.39±0.82
	def	**bcdef**	**ef**	**bcdef**	**abcde**	**abcde**	**abcde**	**abcd**	**ab**
%Ca	54.45±5.10	39.56±5.10	63.51±5.10	37.64±5.10	28.43±5.10	28.98±5.10	22.49±5.10	28.89±5.10	17.19±7.21
	cdef	**bcd**	**cdef**	**cde**	**bcd**	**bc**	**f**	**bc**	**bcdef**
%Mg	25.92±2.43	18.38±2.43	28.31±2.43	25.57±2.43	18.63±2.43	15.21±2.43	16.05±2.43	45.47±2.43	25.03±3.44
	ab	**a**	**abc**	**ab**	**ab**	**ab**	**d**	**a**	**abc**
Ex Na mg/kg	0.24±0.06	0.13±0.06	0.36±0.06	0.20±0.06	0.24±0.06	0.17±0.06	0.14±0.06	1.15±0.06	0.31±0.09
	j	**abcdefg**	**hi**	**abcdefgh**	**abcdefgh**	**abcde**	**ij**	**efgh**	**abcdef**
Ex K mg/kg	0.86±0.06	0.20±0.06	0.49±0.06	0.30±0.06	0.26±0.06	0.15±0.06	0.42±0.06	0.77±0.06	0.12±0.08
	gh	**abcde**	**efgh**	**abcdefg**	**abcdefg**	**abcdefg**	**defgh**	**abcdefg**	**abcd**
Ex Ca mg/kg	6.14±0.92	1.52±0.92	5.33±0.92	1.84±0.92	2.59±0.92	1.74±0.92	2.02±0.92	4.42±0.92	0.81±1.31
	jk	**abcdef**	**jk**	**bcdefghi**	**fghij**	**abcdefg**	**l**	**cdefghij**	**abcdefghij**
Ex Mg mg/kg	2.95±0.35	0.69±0.35	3.06±0.35	1.25±0.35	1.71±0.35	0.92±0.35	1.42±0.35	6.97±0.35	1.15±0.50
	def	**abc**	**def**	**abcde**	**cdef**	**abcdef**	**f**	**cdef**	**abcde**
T-Value cmol/kg	11.23±1.35	3.72±1.35	12.12±1.35	4.87±1.35	9.20±1.35	6.01±1.35	8.87±1.35	15.23±1.35	4.58±1.91
	a	**ab**	**a**	**abcd**	**e**	**de**	**abc**	**a**	**abcd**
NH4:NO3	0.12±0.75	0.41±0.75	0.24±0.75	1.23±0.75	7.38±0.75	3.96±0.75	0.17±0.75	0.67±0.75	1.09±1.06

Appendix 2.1 continued....

Soil param	*P. fleta*	*P. glaucescens*	*P. imbricata*	*P. oreopola*	*P. pinnata*	*P. affinis2*	*P. repens*	*P. restioides*	*P. rigidula*	*P. triflora*
# samples	4	4	4	4	4	4	4	4	4	3
	abcdef	**eg**	**eg**	**abcdef**	**bcdef**	**abcdef**	**i**	**abcdf**	**abc**	**def**
pH KCl	4.20±0.20	5.00±0.20	4.95±0.20	4.05±0.20	4.25±0.20	4.18±0.20	8.58±0.20	3.83±0.20	3.38±0.20	4.03±0.22
	abcd	**hi**	**bde**	**defg**	**hi**	**ij**	**bdef**	**fgh**	**abcd**	**dbefg**
NO3-N	5.55±5.02	42.19±5.02	7.38±5.02	10.77±5.02	40.79±5.02	76.60±5.02	7.87±5.02	26.53±5.02	4.83±5.02	8.48±5.80
	abcde	**abcdef**	**abcdef**	**abc**	**fgh**	**efgh**	**ab**	**cdefgh**	**abcdefgh**	**bcdefgh**
NH4-N	4.55±1.82	5.20±1.82	5.10±1.82	4.16±1.82	13.13±1.82	12.64±1.82	3.41±1.82	9.73±1.82	7.22±1.82	9.35±2.10
	cdefghi	**ghijkl**	**jkl**	**bcdefg**	**fghijkl**	**efghijk**	**fghijkl**	**abc**	**abcde**	**abcde**
P Bray II	6.00±1.35	11.25±1.35	17.25±1.35	4.75±1.35	9.75±1.35	7.50±1.35	9.00±1.35	2.00±1.35	2.50±1.35	3.67±1.56
	abc	**k**	**ghijk**	**a**	**ghijk**	**bcdefgh**	**ab**	**defghi**	**ab**	**abcde**
K mg/kg	43.00±19.20	334.75±19.20	163.75±19.20	20.75±19.20	0	92.25±19.20	32.00±19.20	0	33.25±19.20	17
	fghi	**defghi**	**efghi**	**a**	**hi**	**abcde**	**efghi**	**i**	**cdefg**	**bcdefg**
%C	4.47±0.47	3.33±0.47	4.11±0.47	0.84±0.47	5.40±0.47	2.18±0.47	4.09±0.47	6.45±0.47	2.43±0.47	2.62±0.55
	abc	**abcde**	**efghi**	**bcdefg**	**hi**	**ab**	**abcde**	**cdefgh**	**abc**	**i**
%Na	1.36±0.53	2.16±0.53	4.47±0.53	2.91±0.53	7.14±0.53	1.18±0.53	2.22±0.53	3.67±0.53	1.47±0.53	6.76±0.62
	abc	**jkl**	**hijkl**	**bcde**	**bcdefgh**	**cdefghi**	**a**	**abcde**	**abcd**	**bcdefg**
%K	1.71±0.58	7.60±0.58	5.73±0.58	2.33±0.58	2.82±0.58	3.57±0.58	0.78±0.58	2.14±0.58	1.92±0.58	3.53±0.67
	cdef	**def**	**cdef**	**cdef**	**abcde**	**abcde**	**f**	**cdef**	**abcde**	**abc**
%Ca	54.56±5.10	54.45±5.10	50.31±5.10	43.43±5.10	35.72±5.10	32.55±5.10	91.83±5.10	46.51±5.10	32.82±5.10	9
	b	**cdef**	**cde**	**bcd**	**cdef**	**cdef**	**a**	**bcd**	**bc**	**def**
%Mg	13.51±2.43	25.92±2.43	24.07±2.43	18.69±2.43	27.79±2.43	26.38±2.43	5.18±2.43	18.76±2.43	16.23±2.43	1
	a	**ab**	**abc**	**a**	**cd**	**a**	**ab**	**abc**	**a**	**bcd**
Ex Na	0.07±0.06	0.24±0.06	0.33±0.06	0.07±0.06	0.83±0.06	0.07±0.06	0.24±0.06	0.45±0.06	0.07±0.06	0.24±0.07
	ab	**j**	**fgh**	**a**	**bcdefgh**	**abcdefgh**	**ab**	**abcdefgh**	**ab**	**ab**
Ex K	0.09±0.06	0.86±0.06	0.42±0.06	0.05±0.06	0.33±0.06	0.24±0.06	0.08±0.06	0.27±0.06	0.08±0.06	0.11±0.06
	defgh	**gh**	**cdefgh**	**abc**	**gh**	**abcdefg**	**h**	**efgh**	**abcde**	**abcdef**
Ex Ca	4.76±0.92	6.14±0.92	3.73±0.92	0.97±0.92	5.93±0.92	2.22±0.92	9.98±0.92	5.91±0.92	1.46±0.92	0.85±1.07
	abcdefg	**jk**	**fghij**	**abc**	**kl**	**efghij**	**abcde**	**hij**	**abcdef**	**defghij**
Ex Mg	0.87±0.35	2.95±0.35	1.80±0.35	0.43±0.35	4.98±0.35	1.68±0.35	0.56±0.35	2.38±0.35	0.72±0.35	1.32±0.41
	abcde	**def**	**bcdef**	**a**	**def**	**abcdef**	**def**	**ef**	**abcd**	**abcde**
T-Value	6.26±1.35	11.23±1.35	7.42±1.35	2.23±1.35	12.25±1.35	6.215±1.35	10.86±1.35	12.46±1.35	4.44±1.35	5.11±1.56
	abcd	**a**	**abc**	**ab**	**ab**	**a**	**ab**	**abc**	**abcd**	**abc**
NH4:NO3	1.19±0.75	0.12±0.75	0.75±0.75	0.40±0.75	0.35±0.75	0.17±0.75	0.47±0.75	0.52±0.75	1.55±0.75	1.04±0.86

Appendix 2.1 continued....

Soil parameter	*P. verrucosa*	*F*	*p*
# samples	4		
pH KCl	**abcd** 3.73±0.20	19.60	<0.01
NO3-N mg/kg	**fgh** 22.05±5.02	32.32	<0.01
NH4-N mg/kg	**i** 66.05±1.82	14.78	<0.001
P Bray II mg/kg	**kl** 19.33±1.35	18.77	<.001
K mg/kg	**abcde** 47.75±19.20	17.49	<0.001
%C	**bcdefg** 2.33±0.47	14.42	<0.01
%Na	**abcdeg** 2.69±0.53	10.99	<0.01
%K	**cdefghij** 3.69±0.58	15.52	<0.01
%Ca	**a** 16.30±5.10	6.66	<0.01
%Mg	**bc** 15.58±2.43	21.89	<0.01
Ex Na mg/kg	**a** 0.08±0.06	6.43	<0.01
Ex K mg/kg	**abc** 0.12±0.06	16.61	<0.01
Ex Ca mg/kg	**a** 0.58±0.92	7.88	<0.01
Ex Mg mg/kg	**abcd** 0.52±0.35	20.04	<0.01
T-Value	**ab** 3.22±1.35	6.64	<0.001
NH4:NO3	**abcde** 3.20±0.75	6.61	<0.01

Appendix 3.1. Raw data for *Psoralea aphylla* complex. Characters are as follows: 1= plant height (m); 2= length of leaflet, if present; 3= # leaflets; 4= # flowers per shoot; 5=stipule length; 6= stipule width; 7= flower length; 8= peduncle length; 9= # cupulum lobes; 10= pedicel length; 11= calyx tube length; 12= carinal calyx tooth length; 13= carinal calyx tooth width; 14= standard petal length; 15= standard petal width; 16= standard petal claw length; 17= wing petal length; 18= wing petal width; 19= wing petal claw length; 20= keel petal length; 21= keel petal width; 22= keel petal claw length. All length measurements are in mm unless otherwise specified. Where a value is not applicable, it is recorded as 0.

Character #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
P aphylla 6281	3.00	0.00	0.00	3.00	1.50	0.80	24.67	8.92	2.00	4.21	3.50	5.60	2.38	8.97	11.32	3.76	12.77	5.08	4.81	10.70	3.31	5.83
P aphylla 3492	3.00	0.00	0.00	3.00	6.63	1.65	17.40	1.35	2.00	2.45	2.48	4.50	1.75	8.05	9.00	4.35	10.48	3.05	3.65	9.40	2.80	4.33
P aphylla 3452	2.50	0.00	0.00	2.00	5.90	0.70	18.75	2.80	3.00	1.95	2.60	3.50	1.35	9.73	12.18	4.20	13.05	4.50	3.20	10.65	3.35	4.95
P aphylla 3203	3.00	17.40	1.00	12.00	1.50	0.70	21.50	3.65	2.00	3.55	3.98	6.00	1.40	10.68	10.35	4.50	12.55	4.30	3.50	10.15	2.70	4.70
P filifolia 4321a	3.00	17.41	1.00	10.00	0.00	0.00	17.08	3.10	2.00	3.61	3.60	3.79	2.24	8.48	11.94	5.03	12.55	4.44	5.10	10.93	3.70	6.30
P filifolia 4321b	3.00	17.50	1.00	13.00	0.00	0.00	18.28	5.96	2.00	4.38	4.21	4.96	2.02	8.09	11.45	5.69	12.27	4.58	4.53	10.71	3.25	6.12
P filifolia 4321c	3.00	16.58	1.00	12.00	0.00	0.00	17.03	3.97	2.00	3.26	3.48	4.31	2.13	8.62	11.44	6.22	11.89	4.13	5.37	11.27	3.65	6.19
P filifolia 4321d	3.00	15.62	1.00	10.00	0.00	0.00	16.08	3.33	2.00	3.44	3.30	3.52	2.14	8.52	10.12	5.32	11.20	4.04	4.32	10.58	3.35	6.01
P fleta 054	2.50	11.42	1.00	5.00	0.00	0.00	20.68	3.15	2.00	4.37	3.78	4.14	2.69	13.24	12.90	5.08	15.50	5.12	4.23	12.86	4.38	6.90
P fleta 3341	5.00	9.40	1.00	8.00	4.50	0.70	19.60	1.83	2.00	3.05	2.38	3.60	1.45	9.95	12.65	3.20	12.20	5.75	3.65	11.40	3.65	4.50
P fleta 3342	5.00	10.00	1.00	5.00	1.50	0.90	21.00	4.15	3.00	2.25	3.15	3.68	1.38	12.10	12.00	4.50	13.95	4.35	3.45	10.85	3.75	4.70
P fleta 3383	5.00	9.45	1.00	0.00	1.65	0.85	21.00	7.55	3.00	2.05	3.48	3.70	1.35	11.00	11.00	3.70	13.05	4.45	2.58	10.65	3.60	4.73
P fleta 3385	5.00	9.53	1.00	8.00	5.90	0.90	19.40	1.95	3.00	1.50	3.25	5.25	1.43	10.60	11.30	4.00	12.95	5.50	5.20	11.43	3.75	5.90
P gigantea 035	8.00	21.28	1.00	0.00	0.00	0.00	20.20	2.53	3.00	5.05	3.50	4.93	1.88	12.00	11.53	4.43	13.50	4.40	4.50	11.15	3.50	5.53
P gigantea 034	8.00	16.80	1.00	3.00	0.00	0.00	23.80	3.89	3.00	5.22	4.89	6.42	2.66	12.62	12.13	3.85	13.58	4.91	4.25	12.15	4.31	5.25
P gigantea 057	8.00	21.70	1.00	3.00	0.00	0.00	19.35	3.52	3.00	6.24	3.40	7.54	2.54	10.50	9.68	4.52	12.35	4.32	5.10	10.44	3.36	5.54
P peratica 080	5.00	0.00	0.00	9.00	0.00	0.00	21.75	0.00	2.00	4.94	6.65	5.10	2.43	14.60	15.20	5.70	16.50	4.40	5.10	14.35	4.05	6.79
P peratica 510	5.00	0.00	0.00	4.00	0.00	0.00	18.00	1.35	2.00	2.30	3.20	6.20	1.60	16.10	18.02	4.05	18.20	7.02	4.01	12.02	3.90	4.02
P peratica Pl 3142	1.50	0.00	0.00	7.00	3.94	1.70	17.04	3.94	2.00	5.58	5.02	6.42	2.98	13.05	12.89	4.30	14.38	5.04	4.66	13.32	3.92	6.80
P peratica PL215	1.50	0.00	0.00	6.00	2.93	1.02	21.58	6.15	2.00	8.17	5.26	7.76	3.34	11.78	12.53	4.47	15.38	5.64	5.97	13.77	3.46	7.20
P peratica PL215	1.50	0.00	0.00	6.00	3.10	1.03	26.84	6.38	2.00	6.90	4.78	6.72	2.45	13.02	11.93	5.94	16.18	5.00	5.00	13.91	3.70	7.51
P peratica PL3142	1.50	0.00	0.00	8.00	3.44	1.52	20.20	2.92	2.00	2.40	4.40	6.23	2.76	13.27	12.25	5.23	15.30	4.80	5.24	14.80	4.11	6.75
P pullata 072	3.00	0.00	0.00	5.00	3.29	1.48	23.25	6.86	2.00	5.52	4.20	2.50	5.88	10.40	12.37	5.30	14.74	5.12	5.39	11.85	3.24	3.24
P pullata 3178	3.00	0.00	0.00	3.00	3.75	1.20	23.40	6.76	2.00	6.60	5.20	7.48	3.48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P pullata 4189	3.00	0.00	0.00	4.00	5.00	2.00	20.71	6.97	2.00	4.69	3.52	5.24	2.00	7.69	9.34	4.48	12.68	3.59	5.36	11.16	3.41	6.33
P pullata 690	3.00	0.00	0.00	4.00	3.80	1.02	24.95	7.47	2.00	4.95	4.04	7.70	2.61	12.40	15.02	4.38	15.12	5.69	3.52	12.31	4.28	6.34
P pullata 9293	3.00	0.00	0.00	0.00	4.00	1.70	27.38	7.10	2.00	6.75	3.50	6.92	3.08	10.52	11.45	5.38	13.52	5.10	6.04	11.50	3.57	6.90
P rigidula 33766	0.60	0.00	0.00	4.00	0.00	0.00	19.74	2.20	3.00	4.96	3.67	5.44	3.09	8.08	9.28	3.84	10.38	3.70	3.63	10.20	3.42	6.36
P rigidula 3390	0.60	0.00	0.00	3.00	0.00	0.00	21.50	3.56	3.00	6.09	3.44	4.01	2.74	8.68	10.28	2.82	11.20	4.12	3.63	5.69	3.34	4.93
P rigidula 35742	0.60	0.00	0.00	4.00	0.00	0.00	21.70	5.45	3.00	3.38	3.37	2.83	2.13	11.22	10.04	4.40	13.44	4.96	4.86	11.33	4.19	4.65
P rigidula 4333a	0.60	0.00	0.00	4.00	0.00	0.00	19.96	6.96	3.00	2.99	2.93	4.63	2.16	9.30	10.86	5.46	10.52	4.04	3.74	9.52	3.40	4.37
P rigidula 4333b	0.60	0.00	0.00	5.00	0.00	0.00	26.69	11.72	3.00	3.65	3.81	5.00	2.08	10.60	13.80	6.20	11.82	4.30	2.94	10.34	3.42	5.14
P rigidula 4333c	0.60	0.00	0.00	4.00	0.00	0.00	23.96	9.43	3.00	3.60	3.06	5.00	2.10	10.10	10.27	5.60	11.95	4.58	3.96	10.42	3.16	5.38
P ramulosa 4340	1.00	0.00	0.00	5.00	0.00	0.00	20.30	2.30	3.00	4.06	3.55	4.46	2.79	9.95	12.22	5.62	14.71	4.86	4.83	12.13	4.22	5.55
P ramulosa 4343	1.00	0.00	0.00	4.00	0.00	0.00	21.64	3.72	3.00	5.72	2.84	3.88	2.60	9.89	11.81	5.03	12.90	3.70	4.10	11.00	3.42	5.51
P ramulosa 4346	1.00	0.00	0.00	5.00	0.00	0.00	19.19	2.77	3.00	4.88	3.47	3.22	2.26	10.24	10.60	4.88	12.70	4.71	5.10	11.72	4.18	6.56

Appendix 3.1 continued

Character #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
P ramulosa 4347	1.00	0.00	0.00	7.00	0.00	0.00	23.46	2.35	3.00	6.94	4.11	4.31	2.65	10.17	11.58	5.62	15.82	4.77	6.42	11.94	4.05	6.81
P ramulosa 4349	1.00	0.00	0.00	5.00	0.00	0.00	17.46	2.16	3.00	4.97	2.92	3.24	2.63	9.40	11.81	5.82	13.65	4.60	5.12	11.06	3.59	5.80
P ramulosa 4352	1.00	0.00	0.00	4.00	0.00	0.00	18.15	2.05	3.00	4.07	2.86	2.96	2.39	9.43	13.80	5.59	12.68	5.09	4.61	11.37	4.18	5.74
P ramulosa 4362	1.50	16.53	1.00	8.00	0.00	0.00	24.73	7.85	2.00	4.72	2.98	4.56	2.70	10.74	13.04	4.32	13.93	4.93	5.07	12.78	4.31	6.54
P ramulosa 4363	1.50	0.00	0.00	3.00	0.00	0.00	28.71	15.47	2.00	5.42	4.38	3.94	1.88	12.03	13.05	4.05	13.96	5.45	4.95	11.34	4.41	6.19
P ramulosa 4393	1.50	0.00	0.00	4.00	0.00	0.00	21.70	4.14	2.00	4.59	3.10	4.34	2.42	9.28	10.74	4.88	12.40	3.37	4.90	11.75	3.73	6.82
P ramulosa 4395	1.50	0.00	0.00	3.00	0.00	0.00	20.66	5.64	2.00	4.95	4.84	5.00	2.42	10.42	11.86	5.00	13.22	4.04	4.71	11.22	3.64	5.81
P usitata 10200	2.50	0.00	0.00	4.00	5.00	1.50	16.12	3.30	2.00	2.54	4.82	2.37	1.80	9.10	10.27	4.80	12.34	4.22	4.31	10.78	3.83	5.59
P usitata 039	2.50	0.00	0.00	4.00	2.72	1.56	21.00	5.18	2.00	2.15	3.13	3.63	2.00	10.70	13.65	3.95	13.85	4.80	3.95	10.75	3.50	5.45
P usitata 040	2.50	0.00	0.00	3.00	0.00	0.00	22.08	6.00	2.00	3.84	3.78	4.83	2.60	10.62	13.08	4.36	13.67	5.80	3.85	11.91	3.55	5.43
P usitata 065	3.00	0.00	0.00	3.00	0.00	0.00	24.56	4.50	2.00	6.14	4.08	6.08	2.30	11.20	15.74	4.50	14.98	5.58	6.28	11.16	3.08	6.40
P usitata 32213	2.50	0.00	0.00	4.00	2.00	0.80	23.24	12.65	2.00	5.26	3.42	5.06	2.06	10.38	11.16	4.90	12.16	4.55	3.82	10.72	3.20	6.02
P usitata 3391	2.50	0.00	0.00	3.00	0.00	0.00	20.45	2.98	3.00	3.25	3.20	3.25	2.00	11.00	12.28	3.78	13.78	4.45	3.93	11.45	3.70	4.18
P usitata 3414	2.50	12.00	1.00	0.00	3.50	0.65	17.87	2.00	3.00	2.10	3.73	3.75	1.60	11.00	14.00	3.20	13.68	4.80	3.05	11.48	3.90	5.05
P usitata 3440	2.50	0.00	0.00	0.00	0.00	0.00	20.90	4.90	2.00	2.05	3.10	3.00	1.56	10.25	12.85	2.48	13.95	6.15	3.50	9.98	3.93	3.45
P usitata 3450	2.50	0.00	0.00	2.00	0.00	0.00	21.20	4.65	2.00	3.78	3.55	2.68	1.95	10.35	10.55	3.00	12.85	4.03	3.68	9.78	3.20	4.95
P usitata 3541	2.50	0.00	0.00	8.00	0.00	0.00	20.20	3.55	2.00	2.70	2.30	3.00	1.60	10.00	10.50	3.50	13.58	4.50	4.20	10.83	3.43	4.78
P usitata 4071	2.50	0.00	0.00	0.00	0.00	0.00	16.70	1.60	3.00	2.03	3.70	1.85	1.05	10.00	9.50	1.60	11.55	4.00	3.30	10.00	3.90	5.43
P usitata 4072	2.50	0.00	0.00	0.00	0.00	0.00	17.50	1.95	3.00	2.50	4.00	3.98	1.45	12.00	11.15	2.45	14.13	3.90	5.80	12.05	3.75	4.00
P usitata 4073	2.50	0.00	0.00	10.00	0.00	0.00	19.05	2.40	2.00	3.43	3.36	3.87	2.04	12.00	12.71	3.95	14.00	5.75	3.79	11.73	3.96	5.78
P usitata 4075	2.50	0.00	0.00	0.00	0.00	0.00	19.50	2.00	2.00	1.25	3.30	3.15	0.75	13.00	15.50	4.00	15.95	6.00	3.60	10.90	4.15	5.05
P usitata 4090	2.50	0.00	0.00	3.00	0.00	0.00	20.04	4.19	2.00	4.16	4.08	4.42	2.48	11.97	12.93	3.80	15.06	4.99	4.51	12.53	4.00	5.60
P usitata 3415	2.50	0.00	0.00	0.00	0.00	0.00	14.93	1.10	3.00	1.63	2.70	2.58	2.00	9.18	13.40	2.20	11.85	4.90	4.10	9.15	3.50	5.33

Appendix 3.2 Raw data for *Psoralea pinnata* complex. Characters are as follows: 1= # leaflets; 2= petiole length; 3= rachis length; 4= rachis internode length; 5= basal laterals length; 6= terminal laterals length; 7= terminal leaflet length; 8= # flowers per axil; 9= flower length; 10= peduncle length; 11= cupulum lobe length; 12= cupulum lobe width; 13= # cupulum lobes; 14= pedicell length; 15= calyx tube length; 16= calyx tooth length; 17= calyx lobe width; 18= standard petal length; 19= standard petal width; 20= wing petal length; 21= wing petal width; 22= wing petal claw length; 23= keel petal length; 24= keel petal width; 25= keel petal claw length. All length measurements are in mm unless otherwise specified.

Character #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
P affinis BG538	7.00	3.36	13.10	5.56	23.20	15.92	19.80	3.00	17.50	2.00	3.00	3.00	3.00	2.00	5.00	4.00	10.00	13.00	10.00	13.00	5.00	3.00	11.00	4.00	5.00
P affinis 1130	7.00	6.88	20.68	7.56	54.02	39.94	44.30	3.00	34.70	18.52	0.00	0.00	2.00	4.12	4.28	5.10	12.24	9.98	10.13	12.76	4.72	5.02	12.32	3.88	7.32
P affinis 3172	7.00	4.30	17.44	5.48	22.85	16.88	17.24	4.00	21.80	6.53	2.99	2.40	2.00	2.66	3.27	2.66	9.61	13.60	10.31	11.48	5.18	4.14	11.31	3.70	4.20
P affinis 3186	7.00	2.30	8.70	3.76	28.35	25.30	20.22	3.00	27.10	11.00	4.22	2.40	2.00	3.80	5.20	4.22	9.10	11.90	12.50	9.90	4.42	5.18	7.70	4.00	6.69
P affinis 3202	5.00	5.00	10.04	4.88	22.18	19.88	23.52	3.00	24.88	10.80	5.20	2.56	2.00	2.82	4.10	5.30	8.40	9.12	9.70	13.00	4.10	5.70	12.36	3.56	6.34
P affinis 3230	7.00	5.28	12.46	4.88	26.84	24.32	19.95	3.00	18.78	3.60	3.90	2.20	2.00	2.70	3.50	2.50	7.80	13.50	13.60	12.00	5.20	4.30	10.10	3.60	4.50
P affinis 3240	7.00	2.35	7.38	3.74	25.00	21.70	22.84	3.00	23.86	6.00	3.00	4.00	2.00	2.00	4.00	2.00	7.00	16.00	12.00	14.00	4.00	4.00	12.00	4.00	6.00
P affinis 3374	7.00	4.96	14.06	5.34	23.18	16.38	12.76	3.00	21.80	8.00	2.90	1.30	2.00	2.50	3.00	4.70	8.10	14.60	13.70	13.00	5.20	4.70	10.50	4.20	4.60
P affinis 3468	13.00	1.70	17.08	3.26	10.26	9.30	11.42	3.00	18.20	5.00	3.00	4.00	2.00	1.00	4.00	4.00	10.00	11.00	11.00	13.00	4.00	4.00	10.00	4.00	5.00
P affinis 3668	7.00	11.96	24.78	8.12	41.20	32.08	35.60	3.00	35.06	20.60	0.00	0.00	2.00	3.76	3.78	4.30	8.78	8.24	11.04	11.60	3.80	4.62	11.26	3.34	5.42
P affinis 4649	7.00	9.30	21.34	6.60	23.08	18.72	18.16	3.00	52.64	34.26	0.00	0.00	2.00	4.66	3.80	5.78	11.26	11.64	13.12	15.28	4.60	5.75	11.86	4.60	7.44
P brilliantissima 050a	7.00	3.30	11.84	4.90	23.02	19.60	19.30	2.00	22.98	12.12	4.10	2.70	2.00	4.60	4.80	4.80	9.12	9.74	11.94	8.60	4.64	4.62	6.90	3.74	5.26
P brilliantissima 050b	7.00	3.46	10.20	3.34	23.92	19.50	15.24	2.00	23.30	8.00	6.00	3.30	2.00	5.90	3.60	5.00	10.20	13.00	12.00	11.00	4.80	4.00	10.00	3.60	6.00
P brilliantissima 066a	7.00	6.22	8.92	3.76	27.20	25.10	26.90	3.00	19.98	5.00	3.00	2.00	2.00	2.00	3.00	4.00	6.00	12.00	11.00	12.00	4.00	4.00	11.00	4.00	5.00
P brilliantissima 066b	7.00	7.16	11.56	5.24	24.82	22.20	28.16	3.00	18.00	4.85	3.84	2.91	2.00	4.30	4.50	2.42	8.27	12.50	11.00	12.88	4.93	4.60	10.07	3.72	5.96
P brilliantissima 066c	7.00	6.25	9.32	3.72	17.70	17.02	19.24	3.00	19.20	5.80	4.00	2.10	2.00	2.80	4.80	3.80	10.00	14.10	18.00	13.10	9.00	3.90	10.20	3.70	5.50
P brilliantissima 3248	9.00	5.40	12.22	3.42	45.60	36.20	33.66	4.00	63.30	43.00	6.00	3.00	3.00	3.00	5.00	3.00	11.00	12.00	15.00	14.00	7.00	4.00	10.00	4.00	4.00
P brilliantissima TV	7.00	4.70	12.90	5.52	30.06	27.50	24.60	2.00	36.00	20.00	5.00	4.00	3.00	3.00	5.00	3.00	11.00	14.00	13.00	13.00	5.00	4.00	11.00	4.00	6.00
P brilliantissima 050c	7.00	4.70	10.60	3.48	20.20	15.72	14.38	2.00	28.00	11.30	3.46	2.42	2.00	5.34	3.42	4.32	9.20	9.40	11.18	8.32	5.16	5.00	6.36	4.00	5.58
P glabra 3646a	9.00	3.24	15.48	5.08	25.52	16.18	15.48	3.00	21.54	7.24	0.00	0.00	3.00	4.56	4.90	2.70	9.78	9.30	12.56	13.68	5.96	5.32	11.48	3.82	6.22
P glabra 3646b	9.00	1.94	9.03	3.03	16.84	13.72	13.73	3.00	21.45	8.88	0.00	0.00	3.00	4.00	4.23	2.50	10.70	8.88	12.90	12.46	4.76	5.20	11.20	3.78	6.26
P glabra 3792	7.00	5.98	11.72	4.54	20.54	16.18	14.44	3.00	20.60	16.40	0.00	0.00	3.00	3.56	3.66	2.90	7.94	10.24	10.86	12.82	3.72	4.50	10.68	4.14	5.76
P glabra 7	5.00	10.70	15.65	6.90	30.64	28.50	28.76	3.00	15.24	2.94	0.00	0.00	2.00	1.90	3.10	3.90	8.94	9.40	8.75	12.74	3.84	4.76	11.42	3.10	5.74
P glabra 869	7.00	8.08	26.28	9.98	31.02	28.70	27.96	3.00	31.06	11.70	0.00	0.00	2.00	1.98	4.36	4.54	8.10	10.00	11.20	13.92	4.08	4.62	11.54	3.76	6.22
P kodebergense 4028	9.00	6.40	24.45	6.90	43.00	37.70	39.32	3.00	64.38	43.00	6.42	3.60	2.00	6.88	4.72	4.78	15.00	11.20	16.60	12.00	7.10	7.30	8.53	4.30	7.10
P koudebergense 064a	7.00	4.64	7.04	2.54	33.10	31.34	29.30	3.00	52.40	31.00	5.00	4.00	3.00	4.00	6.00	2.00	10.00	21.00	15.00	16.00	6.00	6.00	13.00	5.00	7.00
P koudebergense 064b	7.00	4.00	10.60	5.02	28.94	28.70	25.20	3.00	49.92	28.00	5.70	3.07	2.00	5.85	4.10	3.55	9.97	9.77	14.73	9.21	4.04	5.62	6.88	4.37	7.08

Appendix 3.2 continued

Character #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
P koudebergense 064c	7.00	5.40	11.20	5.30	32.45	27.48	32.02	3.00	41.72	22.20	4.90	2.20	2.00	5.20	3.70	2.80	12.00	14.80	17.00	15.00	7.70	5.00	11.00	4.20	5.50
P koudebergense 064d	7.00	5.26	13.08	4.72	36.26	24.12	28.04	3.00	49.98	34.34	5.79	6.49	2.00	6.76	5.27	4.28	8.60	13.99	11.89	14.51	6.37	5.70	12.24	4.78	7.70
P pedicellata 892	7.00	5.34	16.84	7.12	26.46	20.74	21.06	3.00	23.82	17.76	0.00	0.00	2.00	2.26	4.72	4.12	11.44	11.26	15.78	15.06	6.78	6.06	13.40	4.30	6.86
P pedicellata 048a	11.00	2.98	17.72	4.80	22.08	17.70	13.50	3.00	26.22	8.10	0.00	0.00	3.00	3.20	5.34	5.28	11.60	10.20	11.82	14.06	5.40	6.34	11.78	3.82	5.60
P pedicellata 700	7.00	3.78	12.50	5.68	35.72	30.78	30.40	3.00	29.40	10.42	0.00	0.00	2.00	3.12	4.10	3.65	9.38	10.94	14.12	14.24	5.68	6.24	12.52	3.62	7.06
P pedicellata 048b	11.00	2.38	16.22	4.96	21.32	19.55	21.24	3.00	22.16	7.26	0.00	0.00	3.00	3.62	4.66	3.70	10.74	10.00	12.70	12.16	4.64	4.67	10.77	3.48	5.18
P pedicellata 10931	7.00	4.88	15.52	5.12	22.10	18.00	14.72	3.00	25.60	10.84	0.00	0.00	2.00	3.66	4.02	4.96	9.42	9.68	10.60	11.92	4.52	5.25	11.80	3.70	6.50
P pedicellata 045a	13.00	6.52	26.52	6.64	33.80	25.86	24.05	3.00	33.84	11.90	3.00	1.46	3.00	3.54	3.54	4.92	10.42	11.48	12.10	10.00	4.60	4.30	8.00	4.72	5.64
P pedicellata 045b	13.00	3.98	23.44	5.60	23.32	12.48	11.72	4.00	25.60	18.90	2.90	2.26	2.00	4.72	4.01	4.96	11.16	9.90	13.44	9.48	4.46	4.50	6.22	3.48	6.34
P pedicellata 075a	9.00	4.78	14.60	7.02	25.72	16.62	15.84	3.00	24.50	7.70	3.50	1.40	2.00	2.70	3.90	6.00	13.20	19.10	11.40	14.00	6.00	4.00	10.00	3.60	3.90
P pedicellata 6858	7.00	5.48	14.40	8.12	37.48	35.26	38.62	3.00	28.80	16.90	0.00	0.00	2.00	3.58	3.54	5.34	10.86	8.80	11.40	11.52	4.30	4.72	10.64	4.32	6.68
P pedicellata 14634	7.00	10.02	22.45	6.74	26.70	21.22	24.12	3.00	34.60	17.80	0.00	0.00	2.00	6.26	4.24	5.56	10.65	12.52	12.92	14.98	4.86	5.54	12.24	3.57	6.24
P pedicellata 075b	9.00	3.58	10.52	2.68	10.78	10.30	11.34	3.00	23.08	8.80	2.16	1.54	3.00	4.40	2.74	4.34	11.88	8.32	9.90	9.10	3.90	4.14	6.42	3.20	5.76
P pedicellata 075c	9.00	4.56	12.14	3.84	19.30	18.72	13.06	3.00	20.38	7.28	2.54	1.24	2.00	3.84	4.92	4.00	12.36	10.66	12.84	10.30	3.91	5.70	6.10	3.82	7.16
P pedicellata 081a	7.00	7.68	16.40	5.30	16.03	12.14	13.24	2.00	26.43	12.00	3.00	3.00	3.00	5.00	6.00	4.00	14.00	13.00	16.00	13.00	5.00	5.00	11.00	5.00	5.00
P pedicellata 081b	7.00	5.18	18.04	4.72	17.54	10.30	14.74	2.00	27.62	11.50	4.40	2.00	2.00	5.60	6.10	5.30	12.30	14.10	14.40	12.50	6.30	4.30	11.40	4.10	5.00
P pedicellata 140	7.00	5.23	14.03	4.34	22.50	17.43	19.20	3.00	25.30	13.00	3.00	3.00	2.00	4.00	4.00	4.00	8.00	16.00	11.00	14.00	5.00	4.00	1.00	4.00	6.00
P pedicellata 3200a	7.00	7.06	11.85	5.70	23.12	20.00	15.76	3.00	33.68	15.00	5.00	3.00	2.00	3.00	4.00	6.00	8.00	14.00	11.00	14.00	5.00	5.00	12.00	4.00	7.00
P pedicellata 3200b	7.00	7.28	13.34	4.98	25.88	22.19	20.40	3.00	32.44	12.00	3.20	1.50	3.00	3.30	4.60	4.20	10.40	11.10	11.90	14.30	5.10	5.82	11.60	4.32	5.40
P pedicellata 32226	7.00	6.35	14.14	5.32	32.60	21.62	16.66	3.00	30.70	12.62	0.00	0.00	2.00	2.46	4.92	4.12	10.18	13.42	16.16	15.58	6.60	5.04	11.70	4.40	7.60
P pedicellata 32221	7.00	3.72	15.54	5.62	27.88	23.38	24.98	3.00	28.10	11.68	0.00	0.00	2.00	3.72	5.52	3.12	12.04	10.45	13.82	14.36	5.50	6.90	13.12	3.44	7.42
P pedicellata 31	5.00	5.92	11.64	6.46	27.58	25.40	27.70	3.00	20.24	4.52	0.00	0.00	3.00	3.08	4.98	4.38	8.96	11.28	12.86	12.68	4.14	4.70	11.84	3.62	5.54
P pedicellata 3440	7.00	6.22	17.52	5.70	27.88	21.10	18.26	3.00	30.34	15.80	3.20	2.10	2.00	3.10	3.70	4.70	9.52	10.20	11.80	12.36	4.70	4.60	11.20	4.30	6.20
P pedicellata 4074	9.00	5.60	18.28	4.58	23.72	17.08	18.70	3.00	30.02	11.22	0.00	0.00	3.00	4.72	4.18	4.98	8.58	10.58	11.54	13.56	5.90	4.42	12.00	4.10	5.60
P pedicellata 41	7.00	5.36	15.28	4.25	23.80	17.86	17.50	3.00	23.50	14.90	4.70	1.40	2.00	4.70	3.90	3.90	7.50	13.50	12.10	13.00	4.70	4.30	10.90	4.20	5.10
P pedicellata 43	7.00	3.52	11.32	3.10	13.10	14.52	13.28	3.00	17.68	6.20	3.90	1.90	2.00	2.60	4.20	6.10	7.90	19.10	10.30	14.30	4.70	4.70	11.50	3.30	6.20
P pinnata 101050	7.00	3.30	10.60	3.58	34.40	31.86	28.78	3.00	20.28	4.24	0.00	0.00	2.00	2.64	3.68	4.80	10.62	11.00	14.26	11.68	4.90	4.90	11.50	3.02	5.92
P pinnata 1035	7.00	4.30	12.08	4.34	21.84	20.42	17.78	3.00	23.10	8.32	0.00	0.00	2.00	3.38	4.28	4.90	9.80	10.16	12.08	14.76	4.28	5.80	11.62	3.42	6.95
P pinnata 13751	9.00	10.35	26.24	8.88	42.08	23.98	26.30	3.00	41.88	25.00	0.00	0.00	2.00	4.88	4.55	3.15	8.31	8.62	10.31	10.94	4.02	3.84	10.10	3.25	5.92
P pinnata 22	7.00	12.32	18.14	5.52	37.32	27.26	31.16	3.00	19.70	5.00	0.00	0.00	2.00	3.08	4.28	5.00	9.96	11.40	11.28	13.74	5.23	5.32	11.26	3.16	6.85
P pinnata 3171	9.00	3.58	12.56	5.38	37.28	25.80	25.60	3.00	21.30	5.88	0.00	0.00	3.00	2.92	4.60	4.92	11.18	10.00	14.50	14.86	4.90	6.80	12.42	3.40	7.70
P pinnata 3189	9.00	4.30	17.03	5.52	41.40	25.54	26.80	3.00	19.10	4.26	0.00	0.00	3.00	2.10	5.92	2.70	9.30	10.40	13.46	12.76	5.10	5.54	11.28	4.20	7.18

Appendix 3.2 continued...

Character #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
P pinnata 3340	9.00	5.38	13.02	6.94	43.52	36.55	33.72	3.00	30.56	19.56	0.00	0.00	3.00	3.66	6.34	3.10	9.94	10.26	13.84	13.32	4.60	5.38	12.12	3.92	6.41
P pinnata 3394	9.00	5.40	18.00	7.26	34.68	28.48	16.20	3.00	17.12	4.52	0.00	0.00	3.00	3.06	3.80	4.42	9.88	8.70	10.86	12.32	4.22	5.00	11.50	3.50	5.70
P pinnata 3403	9.00	4.36	12.72	4.00	21.22	13.04	11.06	3.00	19.34	4.80	0.00	0.00	3.00	3.90	5.36	4.96	12.48	10.46	12.42	14.28	4.24	6.02	12.50	3.16	6.48
P pinnata 3407	9.00	5.64	15.04	4.64	30.09	14.38	19.32	3.00	19.80	4.55	0.00	0.00	3.00	3.17	3.82	3.88	10.60	10.22	13.48	15.22	4.70	6.47	12.92	3.56	13.08
P pinnata 3532	13.00	3.56	19.42	4.08	20.16	10.50	10.80	1.00	26.22	24.90	0.00	0.00	3.00	3.25	3.40	3.32	8.60	9.94	12.38	11.80	3.40	4.14	11.00	3.62	5.62
P pinnata 3547	9.00	6.70	19.56	5.10	31.06	22.34	23.24	3.00	27.45	10.58	0.00	0.00	3.00	3.54	5.12	4.65	11.10	10.65	14.68	14.34	5.18	6.00	12.24	4.34	6.40
P pinnata 3703	9.00	9.36	23.12	7.46	23.10	15.58	16.88	3.00	21.18	5.52	0.00	0.00	2.00	3.48	5.00	2.98	8.76	11.96	14.12	14.42	4.82	6.24	10.50	2.62	7.24
P pinnata 51150	7.00	6.52	18.76	6.45	33.74	23.10	23.98	3.00	39.65	26.05	0.00	0.00	2.00	2.82	3.42	5.20	10.10	8.92	10.34	11.34	5.14	4.52	9.92	3.84	5.80
P pinnata 5572	7.00	5.72	16.00	5.64	30.08	21.92	21.36	3.00	30.20	19.50	0.00	0.00	2.00	3.08	4.34	2.88	7.45	8.98	10.62	13.14	4.52	4.42	10.68	2.94	6.10
P pinnata 6912	7.00	8.15	18.50	6.46	16.94	11.32	9.64	3.00	15.58	4.94	0.00	0.00	2.00	1.84	4.08	2.10	7.72	8.36	9.90	10.68	3.66	3.74	9.90	3.20	5.78
P pinnata 9	9.00	11.72	28.10	7.02	36.02	22.84	26.66	3.00	26.98	17.90	0.00	0.00	2.00	3.40	3.65	4.28	8.44	8.42	9.00	10.02	3.65	3.10	9.76	3.38	4.40
P pinnata 008a	9.00	5.04	11.84	4.20	29.32	24.88	17.02	3.00	35.34	17.40	5.58	2.60	2.00	2.90	4.40	2.86	9.40	7.76	10.00	8.50	4.82	4.80	7.38	4.00	4.70
P pinnata 008b	9.00	3.68	11.24	3.74	28.44	25.62	23.22	3.00	31.30	13.10	5.40	2.90	2.00	2.50	5.80	2.80	9.70	14.30	12.90	13.80	5.70	4.10	12.30	4.20	6.30
P pinnata 10927	9.00	8.40	22.78	6.86	34.74	24.98	23.72	3.00	18.54	6.78	0.00	0.00	2.00	2.88	3.78	3.62	10.94	7.52	9.98	11.00	4.76	4.88	11.10	3.25	6.14
P pinnata 340	9.00	11.22	35.70	9.10	42.94	34.08	33.44	3.00	53.56	35.34	0.00	0.00	2.00	3.72	4.38	4.70	14.50	16.96	13.78	10.20	5.02	4.42	11.90	4.30	6.40
P pinnata 66	9.00	6.13	22.44	7.42	29.72	24.72	30.74	3.00	40.87	18.14	0.00	0.00	2.00	5.40	5.28	4.58	12.38	11.18	12.32	13.94	5.54	6.75	12.25	4.34	6.68