Origin and diversification of the Australasian genera *Pimelea* and *Thecanthes* (Thymelaeaceae)

by

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Abstract

Pimelea Banks & Sol. Ex Gaertn. nom. cons. is a large genus consisting of 110 species, of which 90 species are endemic to Australia, 19 to New Zealand and one to Lord Howe Island. The genus has a great diversity of life forms, breeding systems and habitat. Its closest related genus is *Thecanthes* Wikstr. *Thecanthes* comprises five species of annual herbs occurring in the Philippines, New Ireland and northern Australia. Australasian *Thecanthes* and *Pimelea* are the only genera within sub-tribe Pimeleinae (angiosperm family Thymelaeaceae) and are characterised by the reduction to two stamens. Here I present the most comprehensive molecular phylogenetic study for *Pimelea* and *Thecanthes.* Sequences data from nuclear ITS rDNA and plastid *rbcL, rps16, matK* and *trnL-F* intergeneric spacer were used to reconstruct a phylogeny for these genera. I have produced 457 new DNA sequences (five genes and 150 taxa) for the present analyses. The resulting phylogeny was used to assess the taxonomic status of *Thecanthes* and to evaluate the relationships with Pimeleinae since previous studies indicated a close relationship between *Pimelea*, *Thecanthes* and species of *Gnidia* L. from tropical Africa. The morphological delimitation of sections within *Pimelea,* the biogeography and the radiation of the genus have been revaluated. *Pimelea* was found to be monophyletic. It was concluded that *Pimelea* and *Thecanthes* are congeneric; consequently a paper has been submitted transferring all species of *Thecanthes* into *Pimelea* and making the new combination *Pimelea filifolia* (Rye) Motsi & Rye. Data analysis revealed very low sequence variation within the subtribe Pimeleinae. This suggested a rapid radiation of the genera, which was confirmed by my molecular dating analyses. Based on molecular clock techniques, I calculated the following ages for the origin of *Pimelea*: 4.1 mya for New Zealand *Pimelea spp.* and 13.38 mya for other *Pimelea spp.* The molecular data also indicated that *Pimelea* and South Africa *Gnidia* have a direct common ancestor. I also show that the New Zealand *Pimelea* are derived and dispersed from Australian.

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Dedication

This thesis is dedicated to my family for their love and support during my study.

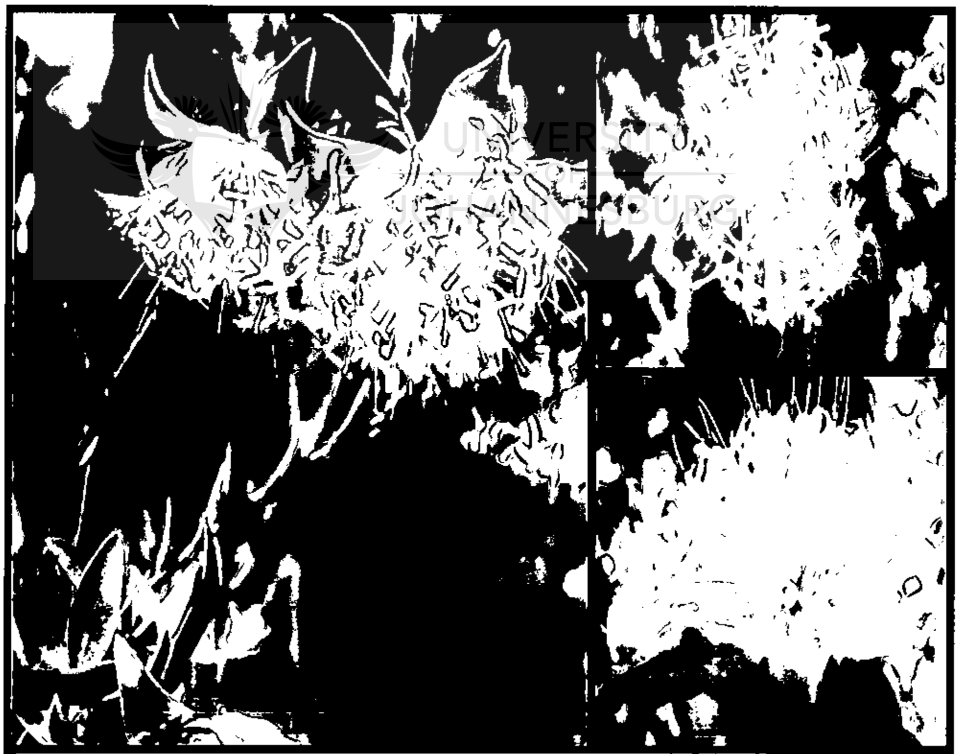
List of abbreviations

ACCTRAN	accelerated transformation
AIC	Akaike Information Criterion
BEAST	Bayesian Evolutionary Analysis Sampling Trees
bp	base pair
BP	bootstrap percentage
BSA	Bovine Serum Albumin
CI	consistency index
cp	chloroplast
DELTRAN	delayed transformation
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
ESS	effective sample size
g	gram(s)
HPD	high posterior density interval
ILD	Incongruence length difference test
ITS	Internal Transcribed Spacer
MCMC	Markov Chain Monte Carlo
min	minute(s)
ML	Maximum likelihood
MP	Maximum parsimony
MYA	Million years ago
nst	number of substitutions
^{0}C	degrees Celsius

PAUP	phylogenetic analysis using parsimony
PCR	polymerase chain reaction
pers comm.	personal communication
PP	posterior probability
PVP	polyvinylpyrrolidone
rDNA	ribosomal deoxyribonucleic acid
RI	retention index
SD	Standard Deviation
sec	second(s)
TBR	tree bisection and reconnection
TL	tree length
UCLN	uncorrelated lognormal
v	version

CHAPTER ONE

LITERATURE OVERVIEW AND OBJECTIVES



Pimelea suaveolens Photos:MC Motsi

1.1. General Introduction

De Jussieu in 1789 first established Thymelaeaceae and currently 45 genera and 800 species are recognised within the family (Heywood *et al.,* 2007). Shrubs, trees and climbers are common while perennial and annual herbs are less common. Thymelaeaceae has a cosmopolitan distribution and its main centres of diversity are Africa and Australia (Wright, 1915; Peterson, 1978; Heywood, 1993; Schmidt, 1994; Takhtajan, 1997). Some members of the family occur in the Mediterranean region, Pacific Islands, with a few members in western, eastern and south east Asia as well as north and south America. South Africa has a good representation with eight genera (*Dais* Royen ex L., *Englerodaphne* Gilg, *Gnidia* L., *Lachnaea* L., *Passerina* L., *Peddiea* Harv., *Struthiola* L., and *Synaptolepis* Oliv.) and about 200 species while in Australia there are eight native genera (*Arnhemia* Airy Shaw, *Jedda* J.R.Clarkson, *Kelleria* Endl., *Lethedon* Spreng., *Phaleria* Jack, *Pimelea* Banks & Sol. ex Gaertn. nom. cons., *Thecanthes* Wikstr. and *Wikstroemia* Endl.; Rye, 1990; Pooley, 2003) and about 130 species.

The family is characterised by a strong fibrous bark, which is difficult to break when stripped from the stems (Wright, 1915; Peterson, 1978; Pooley, 2003). Besides this character, the leaves are alternate or opposite, simple, and without stipules (Rye, 1990; Herber, 2002; Heywood *et al.,* 2007). The inflorescences are terminal or axillary racemes, often condensed into dense head-like clusters or flowers in smaller clusters or sometimes solitary (Rye, 1990). Plants are commonly hermaphrodite, dioecious or gynodioecious and less commonly polygamous (Rye, 1990; Herber, 2002; Heywood *et al.,* 2007).

The flowers are actinomorphic and usually have a floral tube. Sepals are borne at the summit of the tube or rarely free and white, red, pink, yellow, greenish or brown and are (3)4-5(6) and petaloid (Rye, 1988; Herber, 2002; Pooley, 2003; Heywood *et al.,* 2007). Petals are usually smaller than the sepals; they are twice or even more than twice as many as the sepals or are of the same number or are reduced to scales or absent (Herber, 2003; Heywood *et al.,* 2007). Stamens range from two in *Pimelea* (reduced to one in the Tasmanian species *Pimelea filiformis* Hook.f.) or 3–5 or 8–10 or up to 100 (Rye, 1988; Heywood *et al.,* 2007). Endosperm is rare in the family and the embryo is straight and oily (Rye, 1990; Heywood *et al.,* 2007). The fruit is variable, often a drupe, an achene-like berry, or occasionally a capsule (*Aquilaria* Lam.; Heywood *et al.,* 2007). It has a probable base chromosome number of x=9 (Federov, 1974) with 2n=2x=18 reported for all genera (except *Pimelea*) and also with limited polyploidy in *Daphne* L., *Edgeworthia* Falc., and *Wikstroemia* (Darlington and Wylie, 1955; Bolkhovskikh *et al.,* 1969; Moore, 1973). In *Pimelea* all known chromosome numbers are polyploid, mostly tetraploid (n=18, 2n=36), but the octoploid number of 2n=72 is also recorded (Cruickshank, 1953; Rattenburg, 1957; Burrows, 1958; Blaise, 1959).

A number of species are economically useful, for example *Gonostylus* Teijsm. & Binn., *Daphne, Wikstroemia, Lagetta lagetto* (Sw.) Nash, *Dais, Dirca* L. *Gonostylus bancanus* (Miq.) Kurz and *Gonystylus macrophyllus* (Miq.) Airy Shaw provide timber (Herber, 2002). The bark of genera such as *Wikstroemia* and *Edgeworthia* yields fibres that are useful for paper manufacture (Herber, 2002). *Lagetta lagetto*'s inner bark is stretched to yield an ornamental textile in Jamaica (Heywood *et al.*, 2007). *Daphne* is usually cultivated as an ornamental shrub, for its fragrant flowers (Heywood *et al.*, 2007) and *Aquilaria malaccensis* Lam., *Wikstroemia tenuiramis* Miq. and *Gonystylys macrophyllus* (Herber, 2002) have useful essential oils.

1.2. Taxonomy of Thymelaeaceae

Contributions from morphological, anatomical, palynological, chemical and embryological studies have presented conflicting views among authors working on Thymelaeaceae. Two hypotheses of relationships have been proposed for Thymelaeaceae. The first supported the inclusion of Thymelaeaceae in Myrtales based on the co-occurrence of internal phloem, vestured pits, a prominent hypanthium, and elongated crystals in the wood (Van Vliet and Baas, 1984). The second favoured the exclusion of Thymelaeaceae from the order because of the absence of tannins and the presence of pseudomonomerous gynoecium (Conti *et al.*, 1996). Embryological studies (Fuchs, 1938; Davis, 1966; Corner, 1976; Tobe and Raven, 1983) showed some distinctiveness between Myrtales and Thymelaeaceae. In 1952, Erdtman indicated an affinity of Thymelaeaceae with crotonoid members of Euphorbiaceae. The seed wall structure of Thymelaeaceae and Euphorbiaceae has some resemblance (Corner, 1976) and differ from myrtaceous families. The flavonoid pattern for Thymelaeaceae (Gornall *et al.*, 1979) and differs from myrtaceous. As mentioned in Dahlgren and Thorne (1984), Thymelaeaceae should be disbanded from Myrtales due to possession of poisonous compounds, coumarins of daphnetic and daphnoretin type, and the lack of tannins and ellagic acid but may be closely related with Euphorbiaceae. The pollen of Thymelaeaceae is distinct from that of Myrtales but shows similarities to most genera of Euphorbiaceae (Dahlgren and Thorne, 1984).

There are some authors who have recognised Thymelaeaceae with other families within different orders such as Thymelaeales, Myrtales and Euphorbiales (Table 1.1). In Hutchison (1959) and Soó (1967; 1975) Penaeaceae was placed in Thymelaeales while Crypteroniaceae and Oliniaceae were placed in Cunoniales (Table 1.1). Emberge (1960) and Melchior (1964) included Geissolomataceae, Dichapetalaceae, Elaeagnaceae, Oliniacea, Penaeaceae and Thymelaeaceae in Thymelaeales. Hutchinson (1967) listed five families for the Thymelaeales. However Cronquist (1968) proposed that the Thymelaeaceae were embedded within the Myrtales, with ancestry within Rosidae. The unstable position of Thymelaeaceae continued with Thorne (1968; 1976; 1981) placing it in Euphorbiales or Myrtales, while Dahlgren and Thorne (1984) excluded Thymelaeaceae from Myrtales. Dahlgren and Thorne (1984) disagreed with this classification and rather placed the family with Euphorbiaceae within order Malvales. Cronquist (1988) placed Thymelaeales alongside Myrtales. Thorne (1992) considered Thymelaeaceae to belong in the Euphorbiales. Heywood (1993) included Thymelaeaceae in Myrtales as in Thorne (1976). Two families Gonystylaceae and Thymelaeaceae were recognised within Thymelaeales by Takhtajan (1997).

Table 1.1. Proposed ordinal affinities of Thymelaeaceae

Authors	Families included with Thymelaeaceae	Order
Hutchison (1959)	Penaeaceae	Thymelaeales
Emberge (1960)	Penaeaceae, Oliniaceae, Thymelaeaceae, Geissolomataceae and Eleagnaceae	Thymelaeales
Melchior (1964)	Penaeaceae, Geissolomataceae, Dichapetalaceae and Elaeagnaceae	Thymelaeales
Hutchinson (1967)	Gonystylaceae, Aquilariaceae, Geissolomataceae, Penaeaceae, Thymelaeaceae and Nyctaginaceae	Thymelaeales
Cronquist (1968)	Thymelaeaceae is embedded with Myrtales	Myrtales
Thorne (1968)	Thymelaeaceae placed in Euphorbiales	Euphorbiales
Soó (1967; 1975)	Penaeaceae included in Thymelaeales	Thymelaeales
Thorne (1976)	Included Thymelaeaceae in Myrtales	Myrtales
Thorne (1981)	Returned Thymelaeaceae to Euphorbiales	Euphorbiales
Cronquist (1988)	Thymelaeales consisting solely of Thymelaeaceae and placed alongside Myrtales	Thymelaeales
Thorne (1992)	Accepted the superorder Malvane, but included Thymelaeaceae in the Euphorbiales	Euphorbiales
Heywood	Included Thymelaeaceae in Myrtales	Myrtales

(1993)		
Takhtajan (1997)	Recognised two families within Thymelaeales: Gonystylaceae and Thymelaeaceae (with two subfamilies Aquilarioideae, Thymelaeoideae)	Thymelaeales

There are some authors who have recognised Thymelaeaceae as a family on its own in the Thymelaeales, such as Takhtajan (1959), but most (Table 1.2) placed a number of families with it. According to Takhtajan (1959; 1966; 1969) Thymelaeales have a common origin with Euphorbiales and Malvales rising from a Flacourtiaceae-type ancestor and are not related to Myrtales. According to Archangelsky (1971) Thymelaeales together with Euphorbiales belong to the subclass Dilleniialae. Dahlgren (1975a; 1975b) placed Thymelaeales between Euphorbiales and Myrtales. Some evidence suggested that the Gonystyloideae were out of place in this family, and may not even be closely allied to it (Dahlgren and Thorne, 1984). Dahlgren (1989) considered it to have an affinity with Malvales rather than Myrtales.

Table 1.2. Proposed taxonomic affinities of Thymelaeales

Authors	Taxonomic position of Thymelaeales
Takhtajan (1969)	Thymelaeales have common origin with Euphorbiales and Malvales all rising from a Flacourtiaceae-type ancestor
Takhtajan (1959; 1966; 1969)	Thymelaeales placed in sequence with Euphorbiales and not considered as related to Myrtales
Archangelsky (1971)	Both Euphorbiales and Thymelaeales belong to the subclass Dilleniialae and originated from an ancestral line of Dilleniales, Violales, Malvales
Dahlgren (1975a ; 1975b)	Thymelaeales placed between Euphorbiales and Myrtales
Dahlgren (1980)	Maintained Thymelaeales, Malvales, Euphorbiales and Urticales together as members of superorder Malvanae
Dahlgren and Thorne (1984)	Lecythidaceae, Haloragaceae, Rhizophoraceae and Thymelaeaceae excluded from Myrtales
Dahlgren (1989)	Maintained close affinity between Malvales, Euphorbiales, Urticales and Thymelaeales (members of the superorder Malvanae)

In addition to the controversies over the affinities of the Thymelaeaceae, the number of subfamilies recognised has also been contentious. The family has been variously divided into subfamilies, some of which are also recognised by some authors as separate families, and there is currently no agreement on what to include or exclude (Table 1.3). Gilg (1894) recognised four subfamilies namely, Aquilarioideae, Phalerioideae, Thymelaeoideae and Drapetoideae. He later (Gilg 1921) further divided the family into seven subfamilies. Domke (1934) proposed these four subfamilies: Aquilarioideae, Gilgiodaphnoideae, Gonystyloideae and Thymelaeoideae, predicting a genetic relationship with Malvaceae and Euphorbiaceae. Based on new morphological and palynological data, the limits of subfamilial groups within Thymelaeaceae were adjusted so that only two subfamilies, Octolepidoideae and Thymelaeoideae, were recognised (Herber, 2002; 2003).

Table 1.3. Proposed subfamilies within the Thymelaeaceae

Authors (date)	Subfamilies
Gilg (1894)	Aquilarioideae, Drapetoideae, Phalerioideae and Thymelaeoideae
Gilg (1921)	Aquilarioideae, Drapetoideae, Microsemmatoideae, Octolepidoideae, Phalerioideae, Synandrodaphnoideae and Thymelaeoideae
Domke (1934)	Aquilarioideae, Gilgiodaphnoideae, Gonystyloideae and Thymelaeoideae
Herber (2002; 2003)	Octolepidoideae and Thymelaeoideae

Due to the taxonomic confusion within this family, Thymelaeaceae has also recently been the focus of several molecular studies, eg. Conti *et al.* (1996), APG (1998), Fay *et al.* (1998), Bayer *et al.* (1999), Van der Bank *et al.* (2002), and Galicia-Herbada (2006), in the hope of resolving the relationships with the family (Table 1.4). Bayer *et al.* (1999) performed a molecular study on the recircumscribed Malvales, resulting in highly supported lineages including the Thymelaeaceae with strong support for the subfamilies Aquilarioideae Gonostyloideae and Thymelaeoideae. According to the Angiosperm Phylogeny Group, the current classification for the Thymelaeaceae follows that of Herber (2002; 2003), where the family is divided into only two subfamilies, namely Octolepidoideae and Thymelaeoideae. Van der Bank *et al.* (2002) found Thymelaeaceae to be monophyletic and divided into the four subfamilies proposed by Conquist (1981), Heywood (1993) and Heywood *et al.* (2007), which are delimited as outlined below:

Table 1.4. Proposed taxonomic position of Thymelaeaceae based on molecular data

Authors (date)	Taxonomic position
Martin and Dowd (1986)	Connected Thymelaeaceae to other families within Myrtales
Fay *et al.* (1998)	Five clades were strongly supported but no bootstrap support was observed for the relationship within Malvales
APG (1998)	Included Thymelaeaceae in Malvales
Nandi *et al.* (1998)	Used *rbcL* and non-molecular data, and found Thymelaeaceae to be nested within the Malvales sensu lato clade
Bayers *et al.* (1999)	Performed a molecular study on the recircumscribed Malvales resulting in highly supported lineages including Thymelaeaceae with strong support for the subfamilies Thymelaeoideae, Aquilarioideae, and Gonystyloideae
Van der Bank *et al.* (2002)	Thymelaeae was found to be monophyletic and was divide into four subfamilies: Thymelaeoideae, Aquilarioideae, Gonystyloideae, Synandrodanoideae as proposed or supported by Heywood (1993) that follows that of Cronquist (1981)

Subfamily Aquilarioideae is differentiated by its unique fruit type. The flowers are with a short to cylindric tube or sepals free. Stamens (in the Malaysian species) are mostly 10 and either diplostemonous or haplostemonous (Ding Hou, 1960; Hutchison, 1967). The ovary is 2–12-locular while the fruits are loculicidal capsules (Hutchison, 1967). Seeds are large, often hanging out by one end on a thin and stringlike funicle. Endosperm is rare (Ding Hou, 1960). This subfamily comprises seven genera and is distributed from the Pacific area and Africa (Heywood *et al.,* 2007).

Subfamily Gonystyloideae is characterised by leaves that are pellucid-punctate unlike Aquilarioideae and Thymelaeoideae (Ding Hou, 1960). The flowers are with a short or inconspicuous tube. There are about 8–80 stamens (Ding Hou, 1960; Rye, 1990) and the ovary has from 2 to 8 cells but is usually 3–5-celled (Ding Hou, 1960). The fruit is loculicidal capsule and is woody (Ding Hou, 1960; Hutchinson, 1967; Rye, 1990). Seeds are without a chalazal fold and endosperm is absent (Ding Hou, 1960; Hutchinson, 1967). The subfamily contains three small genera (*Amyxa* Tiegh., *Aetoxylon* (Airy Shaw) Airy Shaw, and *Gonystylus* Teijsm. & Binn.) located in Malaya and the Pacific Islands (Ding Hou, 1960; Hutchinson, 1967; Heywood, 1993).

Subfamily Synandrodaphnoideae (=Gilgiodaphnoideae) with one species, *Synandrodaphne paradoxa* Gilg, from tropical West Africa (Robyns, 1975) distinguished by having four stamens and four staminodes that are united in a tube (Heywood, 1993).

Subfamily Thymelaeoideae is the largest (45 genera and 500 species) with most of the members in Thymelaeaceae belonging to it, and is characterised by a uniloculate ovary with a solitary ovule (Herber, 2002). *Peddiea* species from eastern tropical Africa, southern Africa and Madagascar are exceptional: the ovary is bilocular with a single ovule in each locule (Hutchinson, 1967). In Thymelaeoideae the floral tube is funnel-shaped or cylindric. Stamens are up to twice as many as sepals (Rye, 1990). The fruit is a drupe or drupaceous (Ding Hou, 1960). Seeds are mostly without or rarely with a small chalazal fold, and endosperm is present or absent (Ding Hou, 1960).

Although Thymelaeoideae has a worldwide distribution it mainly comprises African and Australasian genera (Heywood *et al.*, 2007). *Gnidia* and *Pimelea*, with about 140 and 110 species respectively, are the largest genera in this subfamily. Other large genera include *Daphne* with 70 species, from Australia extending across Asia to Europe and North Africa, *Wikstroemia* also with approximately 70 species from Australia to southern China, and *Daphnopsis* Mart. (± 65 spp.) in the neotropics. African genera include *Struthiola*, endemic to the Cape of South Africa with 42 species (Beyers and Marais, 1998); *Lachnaea* endemic to the Cape with 40 species (Bredenkamp and Beyers, 2000; Beyers, 2001); and *Passerina* with 20 species (Bredenkamp and Beyers, 2000), mostly distributed in southern Africa, mainly western and eastern Cape extending into Zimbabwe along the eastern escarpment. Other genera, in order of decreasing size, are: *Thymelaea* (30 spp.) in Mediterranean region; *Phaleria* (30 spp.) in Indomal; *Lethedon* Spreng (15 spp.) in Australia; *Kelleria* (11 spp.) in Borneo, New Guinea, Australia and New Zealand; *Peddiea* (± 10 spp.) in tropical (Tanzania), South Africa and Madagascar; *Synaptolepsis* (± 5 spp.) occurring in tropical Africa and Madagascar, Europe and North Africa; *Thecanthes* consisting of five species occurring in northern Australia and extending to the Philippines; *Dais* (2 spp.) in Africa and Madagascar; and two monotypic genera, *Arnhemia* and *Jedda,* endemic to northern Australia (Rye, 1990; Galicia-Herbada, 2006; Heywood *et al.,* 2007; Mabberley, 2008).

1.3. The Australasian genera *Pimelea* and *Thecanthes* as a case study

Pimelea is a relatively large genus consisting of 110 species, of which 90 species are endemic to Australia, 19 to New Zealand and one to Lord Howe Island (Allan, 1961; Burrows, 1962; Rye, 1988; 1990; Wilson and Galloway, 1993). The genus has a great diversity of life forms, breeding systems and habitats. Its species are all perennials, except for the arid-zone species *Pimelea trichostachya* Lindl., and are always at least partially woody (Rye, 1988). They range from dwarf shrubs less than 10cm high (*P. pygmaea* F.Muell. & C.Stuart ex Meisn.) to small trees up to at least 5m high and with a trunk up to at least 12cm diameter in *P. clavata* Labill. Most species are erect and single-stemmed but a few are prostrate with adventitious roots (e.g. *P. spicata* R.Br.) or lignotuberous, with multiple stems arising from ground level (e.g. *P. suaveolens* Meisn.). Flower colour varies mainly from white to deep pink or to bright yellow but is red or greenish in a few species.

Closely related to *Pimelea* is a small genus *Thecanthes*, which was reinstated as a separate genus (Rye, 1988). *Thecanthes* contains five species of annual herbs occurring in the Philippines, New Ireland and northern Australia (Rye, 1988; 1990). It is distinguished by its specialised inflorescence with four involucral bracts united into a funnel-shaped or obconic structure and with glabrous flattened pedicels. Flower colour also varies mainly from white to deep pink or to bright yellow but is red or greenish in a few species.

Pimelea and *Thecanthes* have flowers with the most reduced numbers of parts in the Thymelaeaceae, having four sepals, no petals, two (rarely only one) stamens and a uniloculate 1-ovulate ovary and have been placed in the subtribe Pimeleinae. Most *Pimelea* species are dioecious, hermaphrodite or gynodioecious, while *Thecanthes* is always hermaphrodite (Burrows, 1960; Rye, 1988; 1990). Together, the two genera occupy all of the main habitat types that exist in Australia for terrestrial plants, including coastal, alpine, tropical, temperate, arid and saline ones, in varied rock and soil types in a great variety of vegetation types. One thing most of the species seem to have in common is a tendency to be favoured by disturbance, which can result in very large populations of plants.

The classification system of sections within *Pimelea* has changed since Bentham 1873 (see Table 1.5). Bentham (1873) recognised seven sections within *Pimelea*. He further divided section *Calyptrostegia* into three subsections namely *Calyptridium*, *Phyllolaena* and *Choristachys*. These sectional divisions within section *Calyptrostegia* were based on the following characters: subsect. *Calyptridium* shrubs with opposite leaves and flower-heads terminal with 4-6 broad persistent involucral bracts; subsect. *Phyllolaena* having flower-heads with numerous involucral bracts not broader than the leaves; and subsect. *Choristachys* leaves flat or slightly recurved margins with flowers are in cluster spikes or racemes, without involucres, or the bracts not broader than the leaves and very deciduous. In 1894, Gilg differed with other authors in that he recognised six sections and two subgenera *Thecanthes* and *Eupimelea* within the genus *Pimelea*. He also had three divisions within sect. *Calyptrostegia* like Bentham (1873). Threlfall (1983) followed the same classification of Bentham (1873) except for raising subsection *Choristachys* to the sectional level and she did not mention section *Malistachys*. Rye (1988) divided the genus *Pimelea* into two genera *Pimelea* and *Thecanthes* recognising seven sections within *Pimelea*, namely *Heterantheros*, *Pimelea*, *Epallage*, *Calyptrostegia*, *Macrostegia*, *Stipostachys* and *Heterolaena*. Section *Macrostegia* was a new combination while sect. *Heterantheros* and *Stipostachys* were established in 1988 (Rye, 1988). Their distributions and the distinctive characters are as follows (Rye, 1988; 1990):

Section *Heterantheros* is a monotypic section occurring in south Western Australia. It is characterised by an unusual concave receptacle. Its habitat is close to the coast, associated with outcropping limestone.

Section *Pimelea* comprises 38 species, of which 18 are endemic to Australia, one to Lord Howe Island and 19 to New Zealand and Chatham Island. It is the only group in which the fruit is sometimes succulent. Australian species are widespread, occurring in all states and in habitats ranging from sand and rock, coastal limestone, around salt-lakes, mallee, shrub lands, forest and alpine.

Section *Epallage*, with 19 species, is widespread in Australia. This section is distinguished by a combination of characters including a lack of sessile bracts and having the stamens usually inserted below the base of the sepals. Its species occur in sand, clay, soils, rocky areas, woodland, shrubland and alpine habitats.

Section *Calyptrostegia*, with 33 species, occurs mainly in temperate areas of Australia. This section comprises species that have compact or slightly elongated inflorescences with sessile involucral bracts and usually an elongate and regularly circumscissile floral tube. The habitat ranges from sand, clay, rocks, coastal limestone, plains, dunes, mallee and other shrublands, woodlands, seasonally waterlogged depressions, and alpine.

Section *Macrostegia* is a monotypic section occurring in south-west Australia on sand or rocky habitats. Its single species, P. physodes Hook., is the only bird-pollinated member of the genus (Keighery, 1975) and is distinguished by its highly differentiated bracts and very long sepals and stamens.

Section *Stipostachys* has three species, one occurring in the Pilbara region of Western Australia and other two in Queensland. These species are characterised by stem nodes not protruding abaxially and by elongated inflorescences. The plants grow in grasslands, in red clay or other heavy-textured soil.

Section *Heterolaena* has 14 species endemic to south-western Australia on sand, clay, laterite, granite, seasonally waterlogged flats on coastal dunes, plains and limestones. This section has compact flower heads with well-defined involucral bracts. Its elongate floral tube is unusual is usually having short and long hairs intermixed in its lower half and inusually being fully persistent (rather than being circumscissile or splitting irregularly) in fruit.

Table 1.5. Comparison of different classification systems within *Pimelea* (modification of Rye, 1988)

Bentham (1873)	Gilg (1894)	Threfall (1983)	Rye (1988)
Pimelea	*Pimelea*	*Pimelea*	
sect. *Thecanthes*	subgen. *Thecanthes*	sect. *Thecanthes*	*Thecanthes*
	subgen. *Eupimelea*		*Pimelea*
			sect. *Heterantheros*
sect. *Pimelea*	sect. *Autopimelea*	sect. *Pimelea*	sect. *Pimelea*
sect. *Dithalamia*	sect. *Dithalamia*	sect. *Dithalamia*	
sect. *Heterolaena*	sect. *Heterolaena*	sect. *Heterolaena*	sect. *Heterolaena*
			sect. *Macrostegia*
sect. *Calyptrostegia*	sect. *Calyptrostegia*	sect. *Calyptrostegia*	sect. *Calyptrostegia*
subsect. *Calyptridium*	*Calyptridium*		
subsect. *Phyllolaena*	*Phyllolaena*		
subsect. *Choristachys*	*Choristachys*	sect. *Choristachys*	
			sect. *Stipostachys*
sect. *Malistachys*	sect. *Malistachys*	sect. not	

		mentioned	
sect. *Epallage*	sect. *Epallage*	sect. *Epallage*	sect. *Epallage*

1.4. Objectives of the study

The complicated taxonomic history presented above illustrates the confusion of delimiting the Thymelaeaceae and other taxonomic categories and the difficulty in resolving the phylogeny of the family. The taxonomic history of *Pimelea* and *Thecanthes* illustrates the problem of delimiting genera and sectional boundaries. Rye (1999) even suggested that section *Heterolaena* could be combined with section *Calyptrostegia*. Recent molecular studies (Van der Bank *et al.,* 2002; Rautenbach, 2006) further exposed the need for a more thorough molecular investigation of the family and genera. From these studies, it was vividly clear that the genus *Pimelea* needed much attention. Recently Rautenbach (2006) also confirmed the findings of Van der Bank *et al.* (2002) that *Pimelea* is embedded within *Gnidia*.

The overall purpose of this study is to elucidate the phylogeny of *Pimelea* and *Thecanthes* using a robust sampling of DNA sequence data. For this purpose I produced 457 DNA sequences from the plastid genes *rbcL*, *matK*, *rps 16*, *trnL* intron and 3' exon, the intergenic spacer between *trnL* and *trnF* (*trnL-trnF*) and the nuclear ITS region (Table 1.6).

Specific objectives were as follows:

- To re-evaluate the reinstatement of *Thecanthes* as a separate genus and the generic delimitation of *Thecanthes* and *Pimelea*.

- To assess the morphological delimitation of the sections within *Pimelea*, as well as the relationship of *Pimelea* with other genera within Thymelaeoideae.

- To present the biogeographical history and radiation of the genus *Pimelea*.

In Chapter 2, I reconstruct a phylogeny to address the generic delimitation of *Thecanthes* and *Pimelea.* Maximum parsimony (MP) and Bayesian analysis were applied to four plastid genes (*trnL-F, rbcL, rps 16, matK*) and one nuclear marker (ITS). In all the analyses *Thecanthes* is embedded within *Pimelea*. The results indicate that the subtribe Pimeleinae is monotypic and that *Pimelea* and *Thecanthes* are congeneric. All species of *Thecanthes* are transfer into *Pimelea* and a new combination is made.

In Chapter 3, I address the shortcoming of constructing a robust species-level phylogeney for *Pimelea.* Maximum parsimony analyses of three genes (*trnL-F, rbcL,* ITS) was conducted to generate a phylogenetic tree and study the biogeography of *Pimelea.*

In Chapter 4, I dated the phylogeny of family Thymelaeaceae and subfamily Thymelaeoideae with emphasis on the Australasian genera *Pimelea* and *Thecanthes.* Three genes (*trnL-F, rbcL,* ITS) are used to reconstruct the phylogeny of this group. Sampling within the the family includes species with Australasian, Sub-Saharan Africa, Madagascarian, Eurasia, Meditarreanean, South, Central and North American distribution. A chronogram is generated by using BEAST. The resulting chronogram is used to address the biogeography and timing of radiation of the family. The chronogram indicates a common origin for South African *Gnidia* and Australasian genera *Pimelea* and *Thecanthes.* Also, a recent rapid origin for *Pimelea* is observed and with New Zealand species being derived from Australian *Pimelea.*

Finally, in Chapter 5 I will present a general conclusion.

Chapters 2, 3 and 4 have been written as draft papers for submission to scientific journals. Chapter 2 has already been submitted to *Australian Systematic Botany* and is currently under review.

Table 1.6. Sequences samples for the thesis with indication of numbers of sequences downloaded from GenBank versus those produced by myself

Chapter	*trnL-F*		*rbcL*		ITS		*rps 16*		*matK*	
	This thesis	GenBank	This thesis	GenBank	This thesis	GenBank	This thesis	GenBank	This thesis	GenBank
2	37	66	50	53	50	42	51	22	60	-
3	67	30	71	31	71	24	-	-	-	-
4	17	96	28	90	30	69	-	-	-	-
Total	109		121		121		51		60	

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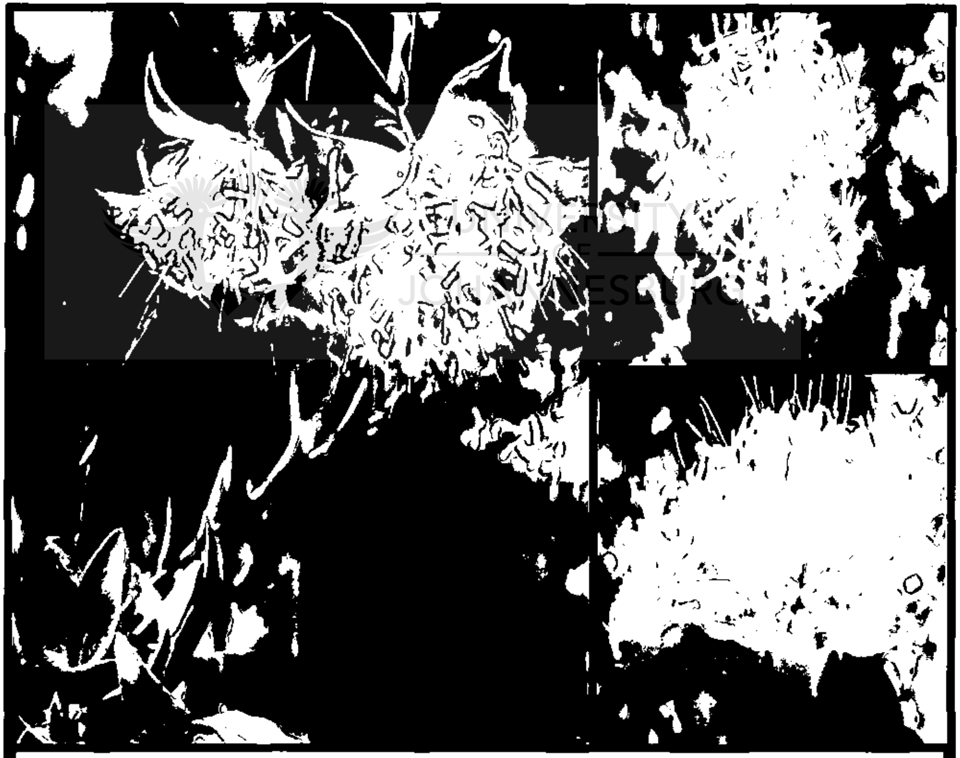
CHAPTER TWO

THE GENERIC DELIMITATION OF *PIMELEA* AND *THECANTHES* (THYMELAEACEAE): EVIDENCE FROM PLASTID AND NUCLEAR RIBOSOMAL SEQUENCE DATA

This chapter has been submitted to Australian Systematic Botany and is in review



Pimelea suaveolens Photos:MC Motsi

2.1. Introduction

The family Thymelaeaceae occurs in southern Africa, tropical Africa, Madagascar, Australia, New Zealand, the Mediterranean region, South and North America and the steppes of Asia. Heywood (1993) divided this large cosmopolitan family into four sub-families namely Gonystyloideae, Aquilarioideae, Synandrodaphnoideae (= Gilgiodaphnoideae; Robyns, 1975) and Thymelaeoideae. Wurdack and Horn (2001) proposed three sub-families Tepuianthaceae, Octolepidioideae and Thymelaeoideae within Thymelaeaceae. Currently, the family is divided into two sub-families, namely Octolepidoideae and Thymelaeoideae (Stevens, 2001; Herber, 2002; 2003). The latter has been divided into three tribes, namely Synandrodaphneae, Aquilarieae and Daphneae, of which Daphneae correspond to Thymelaeoideae sensu Domke (1934). Within Thymelaeoideae, four groups are recognised; Dicranolepideae, Phalerieae, Daphneae and Gnidieae sensu Domke (1934). Domke further classified the tribe Gnidieae into five the sub-tribes Thymelaeinae, Gnidiinae, Kelleriinae, Drapetinae and Pimeleinae. Beyers and Marais (1998), however, suggested the placement of *Passerina* L. in a monogeneric sub-tribe Passerininae, thus recognising six sub-tribes within Gnidieae. Gnidiinae includes genera such as *Struthiola* L., *Lachnaea* L. and *Gnidia* L. The sub-tribe Pimeleinae consists of two genera, *Pimelea* Banks & Sol. ex Gaertn. and *Thecanthes* Wikstr. (Domke, 1934).

Pimelea and *Thecanthes* have an Australasian distribution. *Pimelea* consists of about 110 species, widespread in Australia, but absent from much of the tropical northern region, and extending south-east to New Zealand, while *Thecanthes* consists of five species in northern Australia and extending north to the Philippines. According to Heads (1994), a reduction to two stamens is the only constant character separating *Pimelea* and *Thecanthes* from the rest of Gnidieae. Wikström established *Thecanthes* in 1818, but Bentham (1873) treated *Thecanthes* as one of a number of sections within *Pimelea*. Gilg (1894) treated *Thecanthes* as a sub-genus of *Pimelea* and Threlfall (1983; 1984) followed Bentham in treating the group as a section within *Pimelea*. More recently, Rye (1988) reinstated the genus *Thecanthes* based on its annual habit and specialised inflorescence. She recognised seven sections within *Pimelea*, namely *Heterantheros*, *Pimelea*, *Calyprostegia*, *Macrostegia*, *Stipostachya*, *Heterolaena* and *Epallage*.

In this study, I performed a combined phylogenetic analysis of molecular datasets: nuclear ITS rDNA (internal transcribed spacer of ribosomal DNA) and plastid *rbcL*, *rps*16, *matK* and *trnL-F* intergeneric spacer. The choice of genes was based on previous studies of the family (Van der Bank *et al.,* 2002; Robinson, 2004; Schrader and Graves, 2004; Eurlings and Gravendeel, 2005; Van Niekerk, 2005; Galicia-Herbada, 2006; Rautenbach, 2006). The aim of this chapter is to assess the taxonomic status of *Thecanthes* and to evaluate the relationships within Pimeleinae since previous studies indicated a close relationship between *Pimelea*, *Thecanthes*, *Gnidia pilosa* Burtt Davy (= *Englerodaphne pilosa*) Burtt Davy and other *Gnidia* species from tropical Africa (Van der Bank, pers. comm.).

2.2. Materials and Methods

2.2.1. Taxon sampling

In total, I have analysed 23 genera, representing 102 species of Thymelaeoideae (Table 2.1). Within the sub-tribe Pimeleinae, I included 45 species of *Pimelea* (45/110) and all species of *Thecanthes* (5/5). Sampling of *Pimelea* included both Australian and New Zealand species with representatives from all seven sections recognised by Rye (1988).

The *rbcL*, *trnL-F*, *rps 16* and ITS regions were chosen as two recent studies using these genes (Van der Bank *et al.*, 2002; Robinson, 2004) enabled us to take advantage of the database of in- and outgroups already available. The *matK* region was also sequenced since it has proven to be variable and useful at the species level (Lahaye *et al.*, 2008). Voucher information, literature citations and GenBank accession numbers are listed in Table 2.1.

2.2.2. DNA extraction and amplification

Genomic DNA was extracted from 0.1 – 0.3g fresh and herbarium materials using the 2X CTAB methods of Doyle and Doyle (1987) with addition of PVP (polyvinylpyrrolidone) to bind tannins. The precipitation period and the precipitator were different in terms of fresh and herbarium material. After precipitating the DNA with ethanol or isopropanol (for herbarium material), it was left in a -20^{0}C freezer for a minimum of three days or two weeks (Fay *et al.*, 1998), respectively. DNA extract was further purified and concentrated using QIAquick silica columns (Qiagen Inc.), according to the manufactures protocol for cleaning PCR products.

PCR amplification and sequencing of *rbcL* and *trnL-F* (intron and spacer) regions were performed as in Van der Bank *et al.* (2002). The *rps 16* was amplified using the 1F and 2R primers (Oxelman *et al.,* 1997) and a portion of the *matK* gene was amplified using the primers provided by Kim Ki-Joong, 3F (5´CGTACAGTACTTTTGTGTTTACGAG-3´) and 1R (5´ACCCAGTCCATCTGGAAATCTTGGTTC-3´). The ITS region was amplified in two overlapping pieces using two internal primers and a forward and reverse primer (White *et al.,* 1990; Sun *et al.,* 1994). All polymerase chain reactions (PCR) were performed using Ready Master mix (Advanced Biotechnologies, Epsom Surrey, UK). Bovine serum albumin (BSA; 3.2%) was added to both nuclear and plastid reactions whereas dimethyl sulfoxide (DMSO; 4.5%) was only added to nuclear reactions. These additives serve as stabilisers for enzymes, reduce secondary structure problems and favour precise annealing (Palumbi, 1996).

PCR amplification was performed using the following programs: for *rbcL, trnL-F* and *rps-16*: pre-melt at 94°C for 120sec, denaturation at 94°C for 60sec, annealing at 48°C for 60sec, extension 72°C for 60sec, final extension 72°C for 7min (30 cycles). For ITS, I used the same program just with a longer extension time (3min instead of one). For *matK* the protocol consisted of 60sec at 94°C followed by 35 cycles of 30sec at 94°C for 30sec, 30sec at 53°C and 40sec followed by a final 5min extension (72°C). The PCR products were purified using QIAquick (Qiagen, Inc.) according to the manufacturer's protocol. Each strand was sequenced on an Applied Biosystem 3031*xl* DNA Analyser using the version 3.1 Big Dye Terminator following the manufacturer's protocol (Applied Biosystems, Inc.). For editing and assembly of complimentary strands "Sequencher v.3.1.2" (Gene Codes Corporation) were used.

2.2.3. Phylogenetic analyses

Maximum parsimony (MP) was performed for separated and combined analysis and Bayesian methods for the combined dataset only. The incongruence length difference test (ILD; Farris *et al.,* 1994) and visual comparisons of topologies were employed to detect incongruences among the data sets. The partition homogeneity test was performed with PAUP 4.0b1 using a heuristic search with 1000 replications, simple stepwise addition, and one tree held at each step, MULPARS on (Swofford, 2003) For visually assessment of the datasets the bootstrap trees were considered incongruent only if they displayed "hard" (i.e., with high bootstrap support) rather than "soft" (with low bootstrap support) incongruence (Seelanan *et al.,* 1997; Wiens, 1998).

Due to degraded DNA in a few samples, I could not amplify all of *rbcL, trnL-F, rps-16, matK* and ITS, thus the individual data matrices do not contain identical sets of taxa (Table 2.2). Sequences were aligned manually. Each insertion/deletion was characterised assigning gaps. Prior to the use of the gap-coding program a MP analysis was done. There was a significant difference in terms of excluded characters before and after the gap-coding program. The data characters were reduced. The align data matrix (combined plastids and ITS) had 5664 characters and 102 taxa. Prior to gap-coding characters was manually scored and excluded in MacClade 4.08. Gaps introduced into the alignment are by default treated as missing data, but to exploit the potential utility of gapped positions, all parsimony-informative gaps were scored with a gap-coding program (Borchsenius, 2007; FastGap1.0.8) using the simple indel coding method of Simmons and Ochoterena (2000). The final data matrix analyses used MP via the heuristic search option in PAUP* for Macintosh version 4.0b1 (Swofford, 2003) with uninformative characters excluded. Data matrices were analysed using a heuristic search with 1000 random taxon additions, TBR (tree bisection reconnection) branch swapping with MULPARS on, and all transformations treated as equally likely (Fitch parsimony; Fitch, 1971). A limit of one tree per replicate was set so that less time was spent on each replicate. Internal support was accessed with 1000 bootstrap replicates (Felsenstein, 1985) using simple stepwise addition, but only holding one tree per replicate. Only groups of greater than 50% frequency were reported. DELTRAN (Delayed transformation) character optimisation was used instead of (ACCTRAN) acceleration of transformation due to reported errors with the version of PAUP 4.0b1.The following arbitrary scale for describing bootstrap support was applied: 50 – 74% weak, 75 – 84% moderate and 85 – 100% high.

Bayesian analysis was done using MrBayes version 3.1b2 (Ronquist and Huelsenbeck, 2003). Prior to Bayesian analysis, a model test was run to determine which evolutionary model best suits the data sets (Posada and Crandall, 1998). All five regions had the same model test. Settings for the model were lset nst=6, rates=gamma, and 5,000,000 for every 200 generation. Resulting trees were plotted against their likelihoods. The point where the likelihoods converged on a maximum value was determined and all trees before this convergence were discarded as the “burn-in” phase 7500 trees. A majority consensus tree was produced showing the posterior probabilities (PP). The following scale was used to evaluate the PP's: 0.5<0.8 low, 0.8<09 moderate 0.9<1 high.

Table 2.1. Sources of plant materials used ([1]Robinson, 2004; [2]Van der Bank *et al.*, 2002; [3]Rautenbach, unpublished; [4]Van Niekerk, 2005; [5]Fay *et al.*, 1998; [6]Van der Bank, pers. comm.)

Species	**Location/collector/herbarium**	**Genbank accession number**				
		ITS	***rbcL***	***trnL-F***	***rps 16***	***matK***
***Pimelea* Banks & Sol. ex Gaertn. Section *Calyprostegia* (C.A.Mey.) Benth.**						
P. aeruginosa F. Muell.	White Wells Station, *D. J. Edinger 3969 & G. Marsh* (PERTH)	FJ 572729	FJ 572823	FJ 572691	FJ 572865	FJ 572771
P. alpina Meisn.	Kosciusko National Park, along track from Rawson Pass	FJ 572728	FJ 572822	FJ 572690	FJ 572864	FJ 572770

	towards Lake Cootapatamba, *Taylor J. M. 1263* (CBG)					
P. ammocharis F.Muell.	Ca. 800m N pf Well 13 on Canning Stock Route, Little Sandy Desert, *R. Davis 11172* (PERTH)	FJ 605463	FJ 605469	FJ 605475	FJ 605472	FJ 605466
P. *angustifolia* R.Br.	Lake Grace Shire, *M & J. Steward 79* (PERTH)	FJ 572726	FJ 572820	FJ 572688	FJ 572862	FJ 572768
P. calcicola Rye	Beaconsfield, *J. W. Horn 2574* (PERTH)	FJ 572722	FJ 572816	FJ 572684	FJ 572858	FJ 572764
P. erecta Rye	Kundip, *C. F. Craig 5994* (PERTH)	FJ 572721	FJ 572815	FJ 572683	FJ 572857	FJ 572763
P. graniticola Rye	South-west of Norseman, *B.*	FJ 572718	FJ 572812	AM 406679	FJ 572854	FJ 572760

	Archer 1664 (PERTH)					
P. imbricata var. *imbricata* R.Br.	Deep River, *R. J. Cranfield 17565* (PERTH)	FJ 572719	FJ 572813	FJ 572681	FJ 572855	FJ 572761
P. longiflora subsp. *longiflora* R.Br.	Marbellup Hill, *Read & Low 15* (PERTH)	FJ 572724	FJ 572818	FJ 572686	FJ 572860	FJ 572766
P. octophylla R.Br.	South Coast Rd, 1.7 km W of Mt Stockdale Rd, *West J. G. 5372* (CANB)	FJ 572717	FJ 572811	FJ 572680	FJ 572853	FJ 572759
P. phylicoides Meisn.	Little Desert National Park central block. Lawloit Track, ca 0.2 km north of Sister Mills Track, *Walsh N. G. 3539* (CANB)	FJ 572723	FJ 572817	FJ 572685	FJ 572859	FJ 572765

P. preissii Meisn.	North of Collie, *B. G. Ward & R. J. Cranfield 18582* (PERTH)	FJ 572725	FJ 572819	FJ 572687	FJ 572861	FJ 572767
P. suaveolens subp. *suaveolens* Meisn.	Brookton Highway, *R. J. Cranfield FC 524* (PERTH)	FJ 572731	FJ 572825	FJ 572693	FJ 572867	FJ 572773
P. sulphurea Meisn.	Beverley, *F. Hort 1256* (PERTH)	FJ 572727	FJ 572821	FJ 572689	FJ 572863	FJ 572769
P. tinctoria Meisn.	Steward Rd, *R. J. Cranfield 17360* (PERTH)	FJ 572730	FJ 572824	FJ 572692	FJ 572866	FJ 572772
P. villifera Meisn.	Between Bolgart and Calingiri, *F. & B. Hort 2110* (PERTH)	FJ 572720	FJ 572814	FJ 572682	FJ 572856	FJ 572762
Section *Epallage* (Endl.) Benth.						
P. argentea R.Br.	Near Beverley, *M. Hislop & M.*	AM 162490	AM 167530	AM 406675[3]	FJ 572850	FJ 572756

	Griffiths WW 111.39 (PERTH)					
P. micrantha Meisn.	Cape Arid National Park, *G. J. Keighery 11545* (PERTH)	FJ 572715	FJ 572809	AM 406688[3]	FJ 572851	FJ 572757
P. sericostachya subsp. *sericostachya* F.Muell.	On road to Yarama (in Undara Volcanic National Park), *c.* 6 km from Gulf Development Road, before park entrance, *Purdie R. W 5783* (CANB)	FJ 572714	FJ 572808	FJ 572678	FJ 572848	FJ 572754
P. strigosa Gand.	Severn River, *c.* 4km upstream of confluence with MacIntyre River, turnoff to river opposite “Lavender Vale”. ca. 23 km (direct) NNW of Ashford,	FJ 572716	FJ 572810	FJ 572679	FJ 572852	FJ 572758

	Coveny RG 14395 (CBG)					
P. trichostachya Lindl.	Earaheedy Station, *K. F. Kenneally 12623 & D. J. Edinger 3822* (PERTH)	AM 162501	AM 167537	AM 406682[3]	FJ 572849	FJ 572755
Section *Heterolaena* (Endl.) Benth.						
P. avonensis Rye	East of Pithara, *M. Hislop, B. Smith & M. Griffiths WW 116.10* (PERTH)	FJ 572735	FJ 572829	FJ 572697	FJ 572873	FJ 572779
P brevistyla subsp. *brevistyla* Rye	Serpentine, *B. Nyanatusia 130* (PERTH)	FJ 572736	FJ 572830	FJ 572698	FJ 572874	FJ 572780
P. ciliata subsp.	Westdale, *P. Clynk & T.*	FJ 572737	FJ 572831	FJ 572699	FJ 572875	FJ 572781

ciliata Rye	*Watson 35* (PERTH)					
P. ferruginea Labill.	Torndirrup National Park, on walk trail below Sharp Point Lookout, *B. L. Rye & M. C. Motsi BLR279053* (PERTH)	FJ 605464	FJ 605470	FJ 605476	FJ 605474	FJ 605468
P. hispida R.Br.	Northcliffe Park, *J. Smith 6* (PERTH)	FJ 572738	FJ 572832	FJ 572700	FJ 572876	FJ 572782
P. lanata R.Br.	Kooljerrenup Nature Reserve, *P. Foreman & J. Kelly KO 332* (PERTH)	FJ 572739	FJ 572833	FJ 572701	FJ 572877	FJ 572783
P. lehmanniana subsp. *lehmanniana* Meisn.	Near gravel pit on Millbrook Rd, 0.5 km NW of Kingswood Rd and 1.4 km from Chester Pass	FJ 605465	FJ 605471	FJ 605477	FJ 605473	FJ 605467

	Rd, *B. L. Rye & M. C. Motsi BLR279056* (PERTH)					
P. lehmanniana subsp. *nervosa* (Meisn.) Rye	Balingup, *E. M. Sandiford 590* (PERTH)	FJ 572734	FJ 572828	FJ 572696	FJ 572871	FJ 572777
P. rara Rye	Kalamunda, Darling Range, *F. & J. Hort 2510* (PERTH)	AM 162498	AM 167538	AM 407412[3]	FJ 572872	FJ 572778
Section *Heterantheros* Rye						
P. gilgiana E.Pritz.	Brand Highway, *I. B. Shepherd 269* (PERTH)	FJ 572710	AM 167534	AM 406678[3]	FJ 572842	FJ 572747
Section *Macrostegia*						

(Turcz.) Rye						
P. physodes Hook.	CULTIVATED :Gosford Primary Industries Institute, Narara as NSW781572, *Lidbetter J s.n.* (NSW)	FJ 687339	FJ 687343	FJ 687345	-	FJ 687341
Section *Pimelea* Gaertn.						
P. buxifolia Hoof.f.	N. Island volcanoes, Ruahine Ranges, *J. M. Ward 22620* (CANU)	FJ 572740	FJ 572835	FJ 572703	FJ 572879	FJ 572785
P. clavata Labill.	Mondrain Island, *S. Comer 26* (PERTH)	AM 162491	AM 167531	AM 406689[3]	FJ 572845	FJ 572751
P. concinna Allan	N. Canterbury Marlborough, *L.*	FJ 572742	FJ 572837	FJ 572705	FJ 572881	FJ 572787

	Burrows 38865 (CANU)					
P. forrestiana F.Muell.	Milgun-Meekatharra road, *K. Coate 695* (PERTH)	AM 162493	AM 167533	AM 407407[3]	FJ 572846	FJ 572752
P. halophila Rye	Lake King area, *D. Papenfus 714 & K. Macey* (PERTH)	FJ 572711	FJ 572805	AM 406679[3]	-	FJ 572748
P. milliganii Meisn.	Mt Curly, *Jarman S. J. 240* (CANB)	FJ 572712	FJ 572806	FJ 572676	FJ 572844	FJ 572750
P. "orongo" (C.J. Burrows, in preparation, 2009)	Coastal Wellington, *C. J. Burrows 38838* (CANU)	FJ 572741	FJ 572836	FJ 572704	FJ 572880	FJ 572786
P. pelinos Rye	Near Pingrup, *B. J. Lepschi 3448, T. R. Lally & K. L. Brown* (PERTH)	AM 162497	AM 167535	AM 406680[3] AM 407410[3]	FJ 572843	FJ 572749

P. spicata R.Br.	CULTIVATED: Australian National Botanic Gardens, Canberra, Nursery, as 8602385C, *Beesley P. 1112* (CBG)	FJ 572713	FJ 572807	FJ 572677	FJ 572847	FJ 572753
P. suteri Kirk	Nelson, W. Southland, *C. J. Burrows 38867* (CANU)	-	FJ 572834	FJ 572702	FJ 572878	FJ 572784
Section *Stipostachys* Rye						
P. decora Domin	Hughenden-The Lynd Road, c. 10km N from turnoff to Pretty Plains homestead, *Purdie R. W. 5905* (CANB)	FJ 572732	FJ 572826	FJ 572694	FJ 572868	FJ 572774

P. haematostachya F. Muell.	25 km SE of Baralaba on road to Banana, *Lepschi B. J. 1202* (CANB)	FJ 572733	FJ 572827	FJ 572695	FJ 572869	FJ 572775
P. holroydii F. Muell.	Mt Sheila, Hamersley Range, *S. van Leeuwen 3769* (PERTH)	AM 162496	AM 167539	AM 406687[3]	FJ 572870	FJ 572776
***Thecanthes* Wikstr.**						
T. concreta (F.Muell.) Rye	*c.* 16 km E of Myra Falls, Western Arnhem Land, *Cowie I. D. 8563* (MEL)	FJ 572743	FJ 572839	FJ 572708	FJ 572884	FJ 572790
T. cornucopiae (M. Vahl) Wikstr.	W of Turrel Hill, Macrossan Range, Silver Plains, *Forster P. I. 23116* (MEL)	FJ 572744	FJ 572840	FJ 572706	FJ 572885	FJ 572791
T. filifolia Rye	Arnhem Land, headwaters of	FJ 572745	FJ 572841	FJ 572709	FJ 572886	FJ 572792

	the Cadell River, *c.* 90 km S of Maningrida, *Cowie I. D. 8702* (MEL)					
T. punicea (R.Br.) Wikstr.	Cockburn Range, *T. Handasyde TH99 488* (PERTH)	AM 162502	AM 167540	AM406684[3]	FJ 572882	FJ 572788
T. sanguinea (F.Muell.) Rye	Kalumburu area, *A. A. Mitchell 3945* (PERTH)	AM162503	FJ 572838	AM 406685[3]	FJ 572883	FJ 572789
***Aquilaria* Lam.**						
Aquilaria beccariana Tiegh.	Indomalesia, *Chase 1380* (K)	-	Y 15149[5]	AJ 308642[2]	-	FJ 572802
***Dais* Royen ex L.**						
Dais continifolia L.	N. South Africa to King Williamstown District, Malawi,	AJ 744928[3]	AJ 297234[2]	AJ 308644[2]	-	FJ 572801

	Tanzania, Zimbwabwe, Swaziland and Lesotho, *Chase 1381* (K)					
***Daphne* L.**						
Daphne mezereum L.	Europe, *Chase 6357* (K)	AJ 744931[3]	AJ 297233[2]	AJ 3038645[2]	-	-
***Dicranolepis* Planch.**						
Dicranolepis disticha Planch.	Cameroon South, *Gereau et al. 5626* (MO)	-	AM 39818[3]	AM 40435[3]	-	-
***Dirca* L.**						
Dirca palustris L.	Central North America, *Horn 12584* (NBYC)	AM 159528[6]	U 26322[6]	AJ 308647[2]	-	-

***Drapetes* Lam.**						
Drapetes muscoides Pers.	South America, Borneo, New Guinea, Fuegia and Falkland Islands, *Kubitzki & Feuerer 99-34* (HBG)	AM 159529[6]	AJ 297237[2]	AJ 308648[2]	-	-
***Edgeworthia* Falc.**						
Edgeworthia chrysantha Lindl.	China and Japan, *Chase 6338* (K)	AJ 744932[2]	AJ 297920[2]	AJ 308649[2]	-	-
***Gnidia* L.**						
G. anomala cf Meisn.	Distributed in Kogelberg, *Mark Jonhs s. n.* (Kogelberg Reserve Field Herbarium)	AM 159510[3]	AM 162539[3]	AM 400982[3]	FJ 572887	-
G. calocephala Gilg	Camperdown District, c. 2km	AM 396521[3]	-	AM 404225[3]	-	-

	west of 1000 Hills Hotel, Drummond, KZN, *Reid 885* (PRE)					
G. decaryana Leandri	Madagascar, *Rogers et al. 108* (MO)	AJ 744926^{6}	AJ 745179^{6}	AJ 745153^{6}	-	-
G. denudata Lindl.	South Africa: Swellendam t Humansdorp, *Beaumont s.n.* (NU)	AM 159514^{3}	AJ 295266^{2}	AJ 308670^{2}	-	FJ 572795
G. fastigiata Rendle	Uganda, Kenya, Tanzania, Zimbabwe and Malawi, *Hilliard & Burt 6142* (NU)	-	AM 162513^{3}	AJ 308650^{2}	-	-
G. geminiflora E.Mey. ex Meisn.	Top and upper slopes of Witteberg, *Goldblatt 3799* (GB)	-	AM 397275^{3}	AM 404231^{3}	-	-

G. gilbertae Drake	Madagascar: Mahajanga, *Randrianasolo 529* (MO)	AJ 744927^{6}	AJ 745180^{6}	AJ 745154^{6}	-	-
G. humilis Meisn.	Kogelberg Reserve Field Herbarium, *Mark Johns s.n.* (Kogelberg Reserve Field Herbarium)	AM 159517^{3}	AM 162510^{3}	AM 404236^{3}	-	-
G. juniperifolia cf. Lam.	Kogelberg Reserve Field Herbarium, *Mark Johns s.n.* (Kogelberg Reserve Field Herbarium)	-	AM 397276^{3}	AM 404239^{3}	-	-
G. kraussiana Meisn. (=*Lasiosiphon*	Uganda, Kenya, Tanzania, Guinée, Togo, North Nigeria eastwards to Sudan and	AM 159518^{3}	AJ 295267^{2}	AJ 308674^{2}	-	-

krausii Meisn.)	southwards to Angola, South Africa and Lesotho, *Beaumont s.n.* (NU)					
G. phaeotricha Gilg	Umzinto District, Vernon Crookes Nature Reserve, *Balkwill 10316* (J)	AM 159520^3	AM 162517^3	-	-	FJ 572798
G. pilosa Burtt Davy (=*Englerodaphne pilosa* Burtt Davy)	South Africa: Cape region, *Beaumont s.n.* (NU)	-	AJ 295264^6	AJ 308651^3	-	FJ 572797
G. pinifolia L.	Kogelberg Reserve, *I. Kruger 339* (NBG)	AM 159521^3	AM 162518^3	AM 404240^3	-	FJ 572794
G. racemosa Thunb.	South Africa: Riversdale to Uitenhage- eastern Cape,	AM 159522^3	AJ 295268^2	AJ 308665^2	-	-

	Beaumont s.n. (NU)					
G. renniana Hilliard & B. L. Burtt	South Africa: Natal, *Beaumont s.n.* (NU)	AM 396522^{3}	AM 162519^{3}	AM 404233^{3}	-	-
G. squarrosa (L.) Druce	Kogelberg Reserve Field Herbarium, *Mark Johns s.n.* (Kogelberg Reserve Field Herbarium).	AM 159525^{3}	AM 162522^{3}	AM 404235^{3}	-	FJ 572799
G. wistroemiana Meisn.	Smartryl Farm, road between Trompsburg and Philippolis, *Beaumont & Smith SRFe9* (NU)	AM 159526^{3}	AM 162524^{3}	AM 404299^{3}	FJ 572888	FJ 572796
G. aff viridis Berg.	South Africa: Cape Peninsula, *Beaumont s.n.* (NU)	AM 159509^{3}	AM 162508^{3}	AJ 308652^{6}	-	-

***Gyrinops* Gaertn.**						
Gyrinops walla Gaertn.	Madagascar, *Chase 10511* (K)	FJ 605462	AM 39817[3]	AM40430[3]	-	-
***Kelleria* Endl.**						
Kelleria dieffenbachii (Hook.)Endl.	Ben Lomond National park, Hamilton Crags, 1.5 km E of Legges Tor, *Davies, F.E. 1263* (CBG)	FJ 572746	-	FJ 572707	-	FJ 572793
***Lachnaea* L.**						
L. aurea Eckl. & Zeyh. ex Meisn.	Range from Hermanus to Bredasdorp and southwards to Agulhas, *Aggenbash s.n.* (NBG)	AJ 745737[1]	AJ 697781[1]	AJ 697828[1]	AJ 697638[1]	-

L. axillaris Meisn.	Occurs in the Hopefield and Darling Districts in the southwestern area and re-apears along the southern coast from Elimto the Gourits, *Snijman 1871* (NBG)	AJ 745742[2]	AJ 297129[1]	AJ 308671[2]	AJ 697642[1]	-
L. capitata Crantz	Restricted to the Western Cape from Clanwilliam, southwards to the Cape Peninsula and inland to Franschhoek and Goudini, *Bean 2603* (NBG)	AJ 745744[1]	AJ 697798[1]	AJ 697811[1]	AJ 697641[1]	-
L. diosmoides Meisn.	Slopes of the Attakwas Mountains southeast of	AJ 745745[1]	AJ 697800[1]	AJ 697814[1]	AJ 697620[1]	-

	Oudtshoorn, across Outeniqua and Tsitsikamma Mountains, to the Storms River Forest Reserve, *Beyers 181* (COMP)					
L. ericoides Meisn.	Lower slopes from Cloak Peaks in the Swellendam Districts to Garcia Forest Reserve in the Riverdale District, *McDonald 1842* (NBG)	AJ 745727[1]	AJ 297220[2]	AJ 308673[2]	AJ 697634[1]	-
L. eriocephala L.	Range from Roodezand, Obiekwa and Voëlvlei Mountains near Tulbagh, to Dassenberg near Malmesbury	AJ 745728[1]	AJ 697780[1]	AJ 697834[1]	AJ 697625[1]	-

	and southwards to Betty' Bay, *Beyers 54* (NBG)					
L. filamentosa Meisn.	Endemic to the Western Cape, from Pakhuis Mountains in Clanwilliam District along to the Cederberg range, through the Groot Winterhoek and Roodesand Mountains near Tulbagh to Mostertshoek mountains, *Beyers 245* (COMP)	AJ 745755[1]	AJ 697801[1]	AJ 697833[1]	AJ 697635[1]	-
***Ovidia* Raf.**						
Ovidia andina	Temperate South America,	AM 159530[3]	AJ 297222[3]	AJ 308675[3]	-	-

Meisn.	*Kubitzi & Fewerer 99-42* (HBG)					
***Passerina* L.**						
P. burchellii Thoday	Species endemic to the Southwestern and Langeberg Centres and is common on the mountain summits of Villiersdorp and Genadendal Districts, *Bredenkamp 1546* (PRE)	AM 158925[4]	AM 162526	AM404356	AM 159159[4]	-
P. drankensbergensis Hilliard & B.L.Burtt	Restricted to the Bergville District in northern KwaZulu-Natal Drankensberg, *Bredenkamp 1020* (PRE)	AM 158926[4]	AM 162528	AM 404358	AM 159160[4]	-

P. ericoides L.	Distributed from the Melkbosstrand along the coast of Cape Peninsula to De Mond in Bredasdorp District, *Bredenkamp 962* (PRE)	AM 158927[4]	AM 162529	AM 404359	AM 159161[4]	-
P. falcifolia (Meisn.) C.H.Wright	Near-endemic in Karoo Mountains, southwestern and southeastern Centre. From Zuurberg, Blaaukrantz and Alexandria Forests in Eastern Cape, *Bredenkamp 915* (PRE)	AJ 744917[4]	AJ 297224[2]	AJ 745150[4]	AM 159162[4]	-
P. montivaga Bredenk. & A.E.van	Mossel Bay and Oudtshoorn, northwards along to KwaZulu-	AM 158930[4]	AM 162531	AM 404361	AM 159164[4]	-

Wyk	Natal, Swaziland, Mpumalanga, Northern Province, Mozambique and Zimbabwe, *Van Wyk 2586* (PRE)					
P. nivicola Bredenk. & A.E. van Wyk	Ranges from Ceres and Worcester Districts, *Bredenkamp 1046* (PRE)	AJ 744916[4]	AJ 297226[2]	AJ 308655[2]	AM 159165[4]	-
P. obtusifolia Thoday	Endemic to Northern, Western and Eastern Cape, from Worcester to Grahamstown in the Eastern Cape, *Meyer 1505* (PRE)	AM 158931[4]	AM 162532[3]	AM 404367[3]	AM 159166[4]	-
Peddiea **Harv.**						

P. africa Plant ex Meisn.	Tanzania: Morogoro district, *Chase 6330* (K)	AJ 744921^{6}	AJ 297227^{2}	AJ 308662^{2}	-	FJ 572800
P. involucrata Baker	Madagascar: Antananarivo Ambohitantely, *Rogers et al. 121* (MO)	AJ 744920^{6}	AJ 745176^{6}	AJ 745151^{6}	-	-
***Phaleria* Jack**						
Phaleria capitata Jack	Indomalesia and Western Pacific, *Chase 1383* (K)	-	AJ 297228^{2}	AJ 308661^{2}	-	-
***Stephanodaphne* Baill.**						
S. cuspidata (Leandri) Leandri	Madagascar: Fianarantsoa Manombo, *Roger et al. 68* (MO)	AM 159533^{6}	AM 398185^{3}	AM 406683^{3}	-	-

S. oblongifolia Leandri	Madagascar, *Roger et al. 127* (MO)	AJ 744924[6]	AJ 745177[6]	AJ 745152[6]	-	-
Struthiola **L.**						
S. ciliata Lam.	Ranges from Namaqualand south and towards Albertinia, including the small Karoo, *Chase 6445* (K)	AJ 745756[6]	AJ 297241[2]	AJ 308637[2]	AJ 697645[6]	FJ 572803
S. dodecandra Druece	South Africa: from Cape Peninsula to Bredasdorp, Knysna, *Chase 6354* (K)	-	AJ 297246[2]	AJ 308639[2]	FJ 572889	FJ 572804
S. leptantha Bolus	From Nieuwoudtsville, Cederberg to Malmesburg (Namaqualand), *Beyers 265*	AJ 745757[6]	AJ 297242[2]	AJ 308638[6]	AJ 697646[6]	-

	(NBG)					
***Synaptolepis* Oliver**						
Synaptolepis alternifolia Oliv.	Tanzania, Malawi, Mozambique and Zimbabwe, *Vollesen 4043* (K)	-	AJ 297239[2]	AJ 308663[2]	-	-
***Thymelaea* Mill.**						
Thymelaea hirsuta Endl.	Spain, *Chase 1883* (K)	AJ 744930[6]	Y 152151[5]	AJ 308640[2]		
***Wikstroemia* Spreng.**						
Wikstroemia gemmata (E.Pritz.)	Southern China to Australia and Pacific, *Chase 3955* (K)	AJ 744929[3]	AJ 295269[2] AJ 297223[2]	AJ 308641[2]	-	-

Domke						
Synandrodaphne						
Meisn.						
Synandrodaphne paradoxa Gilg	Tropical West Africa, *Lisowski 46609* (K)	-	AJ 297240[2]	AJ 308676[2]	-	-

Table 2.2. Sampling in data matrix.

	Number of taxa	Number of sequences completed > 80%				
		ITS	*rbcL*	*trnL-F*	*rps 16*	*matK*
Pimelea	45	39	41	44	37	43
Thecanthes	5	4	5	5	4	5
Other genera	54	39	34	46	14	10

2.3. Results

For each separate and combined analysis, Table 2.3 shows the number of included aligned positions in the matrix, number of variable sites, number of phylogenetically informative sites, and percentage of sites that are variable. For each analysis I also report number of trees, number of steps, consistency index (CI), retention index (RI), and average changes per variable site. Alignment of ITS sequences between Thymelaeoideae and *Synandrodaphne* was difficult due to several ambiguous regions. Thus, *Gyrinops*, *Edgeworthia*, *Wikstroemia, Thymelaea* and *Daphne* were selected as outgroups for this data matrix. Individual plastid sequence analyses were topologically consistent (results not shown) and I therefore combined them and treated it as a single dataset. Results from three of the analyses are presented: Fitch parsimony tree for combined plastid regions (*rbcL* + *trnL-F* + *rps-16* + *matK*; Fig. 2.1), Fitch parsimony tree for ITS (Fig. 2.2), and one of the equally parsimonious trees from the combined molecular data set (Fig. 2.3).

The aligned region of ITS contained the most variable sites namely 411 (58%) compared to *trnL-F* with 362 (35%), *matK* with 220 (30%), *rps*16 with 193 (23%), and *rbcL* with 266 (19%). The number of potentially informative characters were also higher for ITS (334; 47%) than for *trnL-F* (196; 19%), *matK* (96; 13%), *rps*16 (93; 11%) and *rbcL* (157; 11%). Variable positions changed more rapidly for ITS, 4.6 vs. 2.1 (*rbcL*), 1.7 (*trnL-F*) and 1.5 (*rps16* and *matK*).

2.3.1. Congruence of data sets

Results from the ILD test were significant (P=0.01), indicating that incongruences may be present in the data (Farris *et al.*, 1994). However, when the individual results were compared on a node-by-node basis from the separate analyses, specifically with respect to levels of resolution and bootstrap support (Wiens, 1998) no strong incongruences were evident (clades > 85 BP are similar in ITS and plastid analyses; Fig. 2.1 and 2.2). I thus did not take into account the results from the ILD test because of reported unreliability of these congruency tests (Reeves *et al.*, 2001; Yoder *et al.*, 2001) and went ahead combining the different data sets.

2.3.2. Combined plastid analysis

Analysis of the combined plastid data set included 4362 base pairs (bp) of which 1169 (26%) were variable and 606 (14%) parsimony informative (CI=0.65; RI=0.78; TL=2152; Table 2.3; Fig. 2.1). *Aquilaria* and *Gyrinops* (Aquilarieae; 100 BP) grouped together. There was high support for the monophyly of Thymelaeoideae (97 BP). Within this sub-family, three main clades are present: Gondwana taxa (I), non-African taxa (II), and Tropical African and southeast Asian taxa (III). Clades II and III received a high support of 92 BP and 99 BP respectively. In Clade I, bootstrap support was weak (58 BP). The monophyly of *Passerina*, *Lachnaea, Peddiea* and *Stephanodaphne* were highly supported (97 BP; 100 BP; 100 BP; 100 BP respectively). There was strong support for the clade *Dais* and *Phaleria* (98 BP) and for *Kelleria* and *Drapetes* (95 BP). *Gnidia* was polyphyletic with at least four lineages: 1) *Gnidia pilosa* (previously *Englerodaphne pilosa*), together with other *Gnidia* species, as sister to the genera *Pimelea* and

Thecanthes (63 BP); 2) *Gnidia pinifolia* and *G. racemosa* grouped with *Struthiola* (100 BP); 3) a clade containing some *Gnidia* species with *Drapetes muscoides and Kelleria dieffenbachii* sister (51 BP) to this clade; 4) taxa previously recognised as *Lasiosiphon* grouped together with another set of *Gnidia* species (94 BP). Pimeleinae is strongly supported as monophyletic (97 BP) with *Thecanthes* embedded within *Pimelea*. Although relationships within *Pimelea* are not resolved, several groupings were observed: 1) Three of the four New Zealand species *P. orongo*, *P. concinna* and *P. suteri* formed a moderately supported clade (80 BP), 2) a clade containing all five species of *Thecanthes* received 100 BP, and 3) weak support (55 BP) for the grouping *P. rara*, *P. hispida*, *P. ciliata*, *P. ferruginea*, *P. villifera* and *P. lanata.*

2.3.3. ITS analysis

The ITS region comprises 708 aligned base pairs of which 297 characters were constant, 411 (58%) characters were variable and 334 (47%) were parsimony-informative. The heuristic search found 40 equally parsimonious trees with a tree length (TL) of 1875 steps (CI=0.38; RI=0.69; Table 2.3; Fig. 2.2). Sub-family Thymelaeoideae is partly resolved. The overall topology of the ITS tree (Fig. 2.2) was congruent with that of the combined plastid results (Fig. 2.1) in that the same groupings in Thymelaeoideae found in the plastid tree were also recovered in the ITS tree. Clade I is present but not supported. Clade II is strongly supported with 100 BP and again there was strong support for *Gnidia* being polyphyletic. Within Thymelaeoideae two main clades are recognised (Clade A, 100 BP and Clade B, 98 BP). In Clade A, *Gnidia phaeotricha* and *G. squarrosa* are sister to sub-tribe Pimeleinae with a high support of 96 BP. The root node of Pimeleinae is weakly supported (67 BP) with *Thecanthes* nested within *Pimelea*. Other groupings within Clade A include: 1) *Pimelea rara*, *P. hispida*, and *P. lanata* form a polytomy supported with 93 BP, 2) The New Zealand species, *Pimelea orongo*, *P. concinna* and *P. buxifolia* form a weakly supported clade of 62 BP, and 3) *Pimelea decora* is sister to *P. haematostachya* with a high support of 100 BP.

Clade B includes: 1) the monophyletic genera *Passerina* (100 BP) and *Lachnaea* (62 BP), 2) '*Struthiola ciliata- Gnidia racemosa*' clade with a high support of 98 BP, and 3) the grouping of *Kelleria dieffenbachii* and *Drapetes muscoides,* which is highly supported with 96 BP. The '*Gnidia kraussiana-G. decaryana*' clade and the monophyly of *Peddiea* and *Stephanodaphne* are highly supported (100 BP).

2.3.4. Combined molecular analysis

Though the ILD test was not significant, I decided to combine the two data sets since the test is too conservative. No strongly incongruent patterns were observed, therefore allowing the direct combination of plastid data and nuclear data. Analysis of the combined molecular data set included 102 base pairs of which 2153 (38%) were variable and 1154 (20%) parsimony informative, Fitch analysis produced two equally most parsimonious trees (TL=5018; CI=0.54; RI=0.72; Fig. 2.3). The combined MP analysis is largely congruent with the Bayesian analysis, but as their topology differs slightly they are displayed on separate trees (Figs. 2.3 and 2.4).

Thymelaeoideae are highly supported as monophyletic (99 BP, 1.0 PP). Within Thymelaeoideae, three major clades are present, comprising

Gondwanan taxa (I; 59 BP, 0.99 PP), all non-African taxa (II; 98 BP, 1.0 PP) and tropical African and southeast Asian taxa (**III**; 99 BP, 1.0 PP). Clades II, and III are moderately supported in the MP analysis and strongly supported in BP analysis as being successively sister to the Gondwana taxa (clade **I**; 59 BP, 0.99 PP; 67 BP, 1.0 PP, respectively).

The *Lachnaea, Passerina* and *Stephanodaphne* clades were highly supported (98 BP, 1.0 PP; 100 BP, 1.0 PP; 100 BP, 1.0 PP respectively). There was strong support for the clade *Dais* and *Phaleria* (100 BP, 1.0 PP) and moderate support for *Ovidia* and *Dirca* (83 BP, 1.0 PP). *Gnidia*, was again indicated as polyphyletic, with the same four lineages found in the combined plastid and ITS analysis.

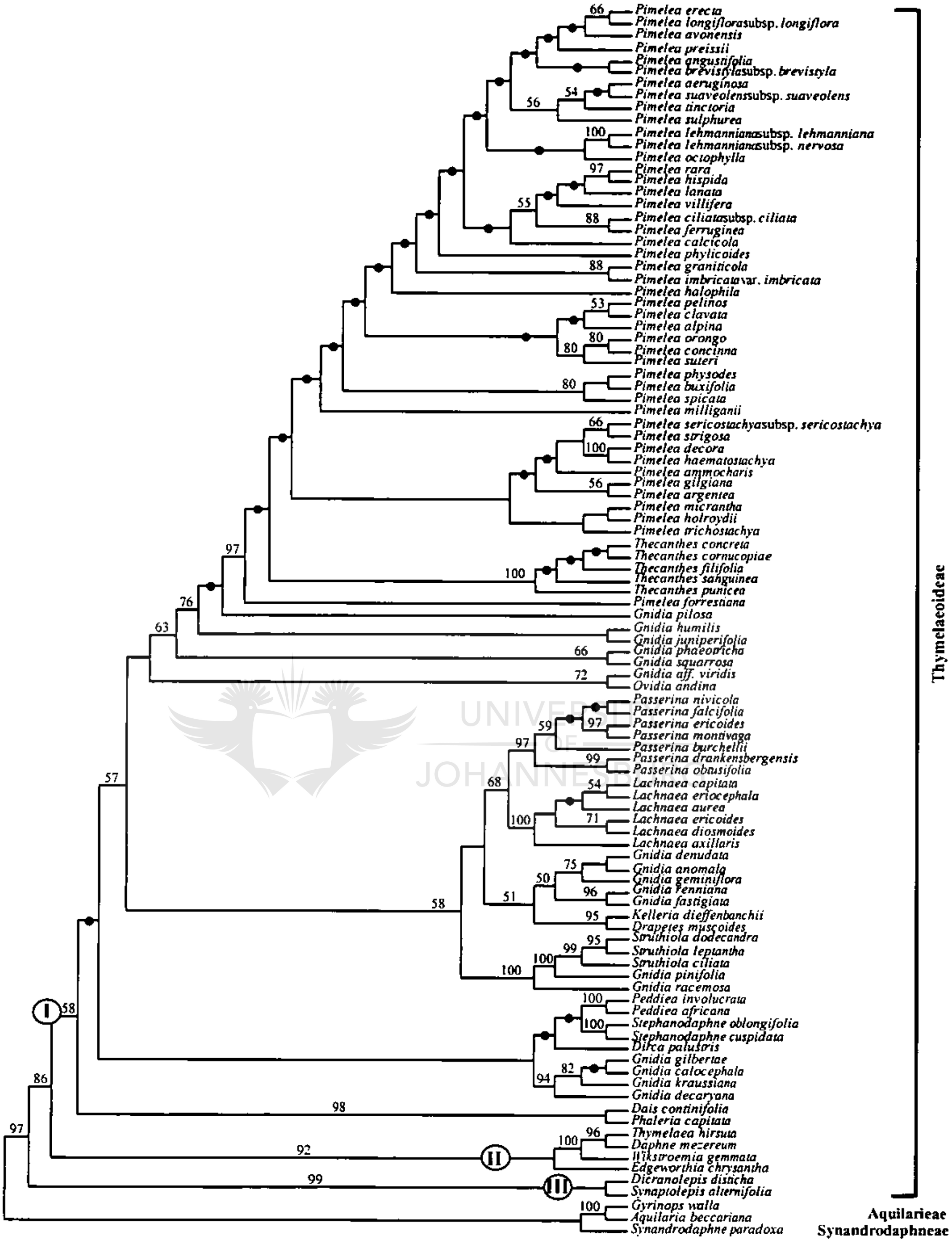
The root node of sub-tribe Pimeleinae is highly supported (99 BP, 1.0 PP) with *Thecanthes* nested within *Pimelea*. Although relationships within Pimeleinae are generally poorly resolved a few clades/groups are supported in the MP and BP analysis: 1) All five species of *Thecanthes* grouped together (100 BP, 1.0 PP) with *P. decora* and *P. haematostachya* sister to it (52 BP, 0.99 PP), 2) the New Zealand species *P. orongo*, *P. concinna*, *P. suteri* and *P. buxifolia* formed a weakly supported clade in MP and strongly supported clade in BP analysis (60 BP, 0.99 PP), 3) *Pimelea rara* and *P. hispida* grouped together (98 BP, 1.0 PP) with *P. lanata* sister to this grouping (87 BP, 1.0 PP), 4) *Pimelea lehmanniana* subsp. *lehmanniana* and *P. lehmanniana* subsp. *nervosa* are highly supported (100 BP, 1.0 PP), and 5) '*Pimelea tinctoria- P. sulphurea*' clade is moderately supported in MP and strongly supported in BP analysis (76 BP, 1.0 PP). The sectional classification proposed by Rye (1988) for *Pimelea* is not upheld.

Table 2.3. Paup statistics of analyses obtained from separate and combined datasets.

	rbcL	*trnL-F*	*rps-16*	*matK*	**Combined plastids**	**ITS**	**Combined plastids + ITS**
No. of taxa	100	97	65	61	102	91	102
No. of included characters	1378	1026	831	739	4362	708	5664
No. of constant characters	1112	664	638	519	3193	297	3511
No. of variable sites	266 19%	362 35%	193 23%	220 30%	1169 26%	411 58%	2153 38%
No. of parsimony informative sites	157 11%	196 19%	93 11%	96 13%	606 14%	334 47%	1154 20%
No. of trees (Fitch)	2740	2280	110	3780	1700	40	2

No. of steps (Tree length)	551	647	298	334	2152	1875	5018
CI	0.55	0.72	0.77	0.75	0.65	0.38	0.54
RI	0.81	0.82	0.87	0.75	0.78	0.69	0.72
Average number of changes per variable site (number of steps / number of variable sites)	2.1	1.9	1.5	1.5	1.8	4.6	2.3

Fig. 2.1 (next page). One of the equally parsimonious trees from the combined plastid dataset (*rbcL*, *trnL-F*, *rps 16*, *matK*). Numbers displayed above each branch are bootstraps equal to or greater than 50%. Closed circles on branches indicate groups not present in the Fitch strict consensus tree. The three clades indicated are: I) the Gondwanan taxa, II) the non-African taxa and III) the tropical African and southeast Asian taxa.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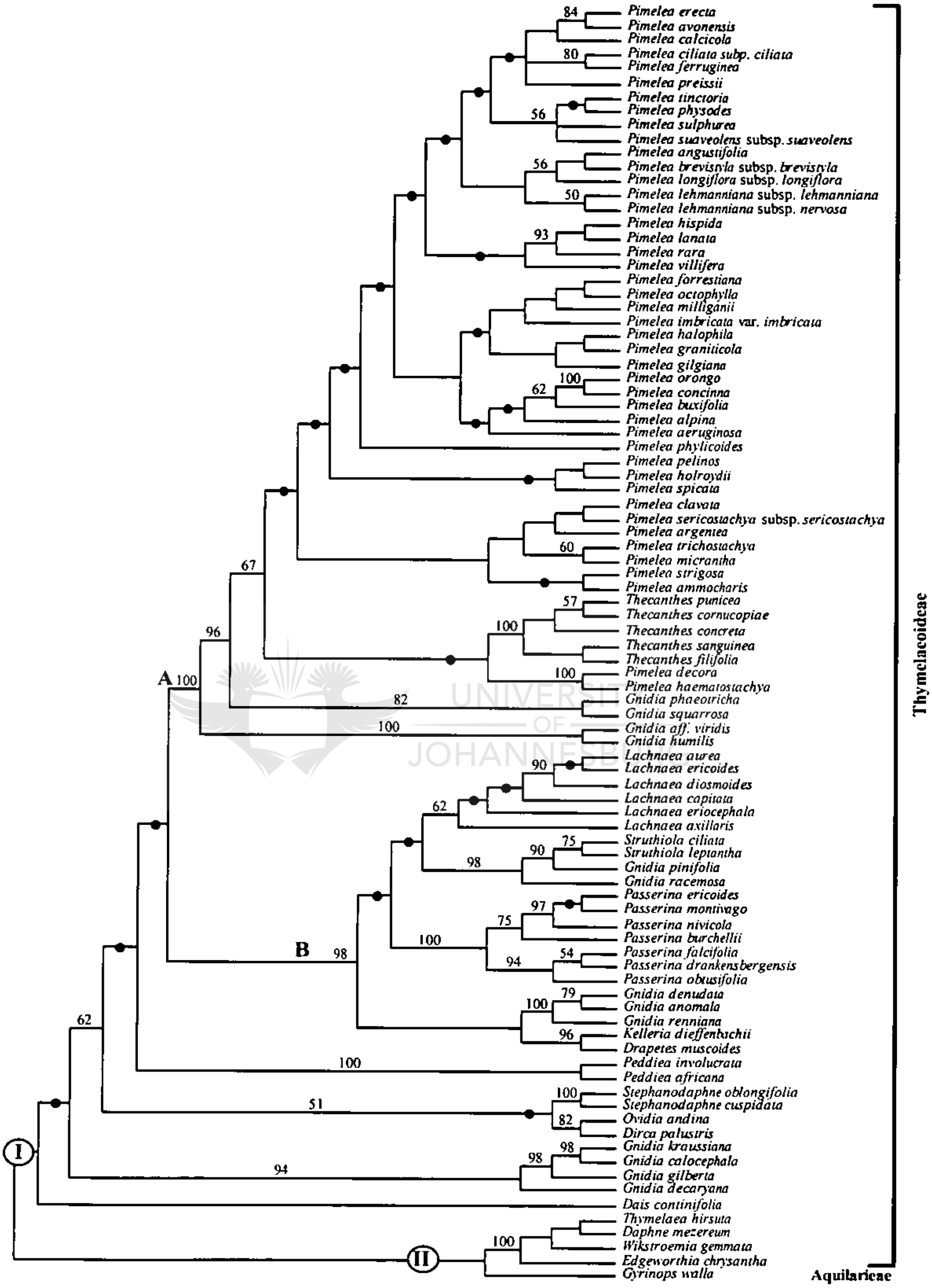
Pimelea avonensis
Pimelea preissii
Pimelea angustifolia
Pimelea brevistylasubsp. brevistyla
Pimelea aeruginosa
Pimelea suaveolenssubsp. suaveolens
Pimelea tinctoria
Pimelea sulphurea
Pimelea lehmannianasubsp. lehmanniana
Pimelea lehmannianasubsp. nervosa
Pimelea octophylla
Pimelea rara
Pimelea hispida
Pimelea lanata
Pimelea villifera
Pimelea ciliatasubsp. ciliata
Pimelea ferruginea
Pimelea calcicola
Pimelea phylicoides
Pimelea graniticola
Pimelea imbricatavar. imbricata
Pimelea halophila
Pimelea pelinos
Pimelea clavata
Pimelea alpina
Pimelea orongo
Pimelea concinna
Pimelea suteri
Pimelea physodes
Pimelea buxifolia
Pimelea spicata
Pimelea milliganii
Pimelea sericostachyasubsp. sericostachya
Pimelea strigosa
Pimelea decora
Pimelea haematostachya
Pimelea ammocharis
Pimelea gilgiana
Pimelea argentea
Pimelea micrantha
Pimelea holroydii
Pimelea trichostachya
Thecanthes concreta
Thecanthes cornucopiae
Thecanthes filifolia
Thecanthes sanguinea
Thecanthes punicea
Pimelea forrestiana
Gnidia pilosa
Gnidia humilis
Gnidia juniperifolia
Gnidia phaeotricha
Gnidia squarrosa
Gnidia aff. viridis
Ovidia andina
Passerina nivicola
Passerina falcifolia
Passerina ericoides
Passerina montivaga
Passerina burchellii
Passerina drankensbergensis
Passerina obtusifolia
Lachnaea capitata
Lachnaea eriocephala
Lachnaea aurea
Lachnaea ericoides
Lachnaea diosmoides
Lachnaea axillaris
Gnidia denudata
Gnidia anomala
Gnidia geminiflora
Gnidia renniana
Gnidia fastigiata
Kelleria dieffenbanchii
Drapetes muscoides
Struthiola dodecandra
Struthiola leptantha
Struthiola ciliata
Gnidia pinifolia
Gnidia racemosa
Peddiea involucrata
Peddiea africana
Stephanodaphne oblongifolia
Stephanodaphne cuspidata
Dirca palustris
Gnidia gilbertae
Gnidia calocephala
Gnidia kraussiana
Gnidia decaryana
Dais continifolia
Phaleria capitata
Thymelaea hirsuta
Daphne mezereum
Wikstroemia gemmata
Edgeworthia chrysantha
Dicranolepis disticha
Synaptolepis alternifolia
Gyrinops walla
Aquilaria beccariana
Synandrodaphne paradoxa
I
II
III
Thymelaeoideae
Aquilarieae
Synandrodaphneae

Fig. 2.2 (next page). One of the equally parsimonious trees from the ITS dataset. Numbers displayed above each branch are bootstraps equal to or greater than 50%. Closed circles on branches indicate groups not present in the Fitch strict consensus tree. The two clades indicated are: I) the Gondwanan taxa, II) the non-African taxa.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57
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A 100
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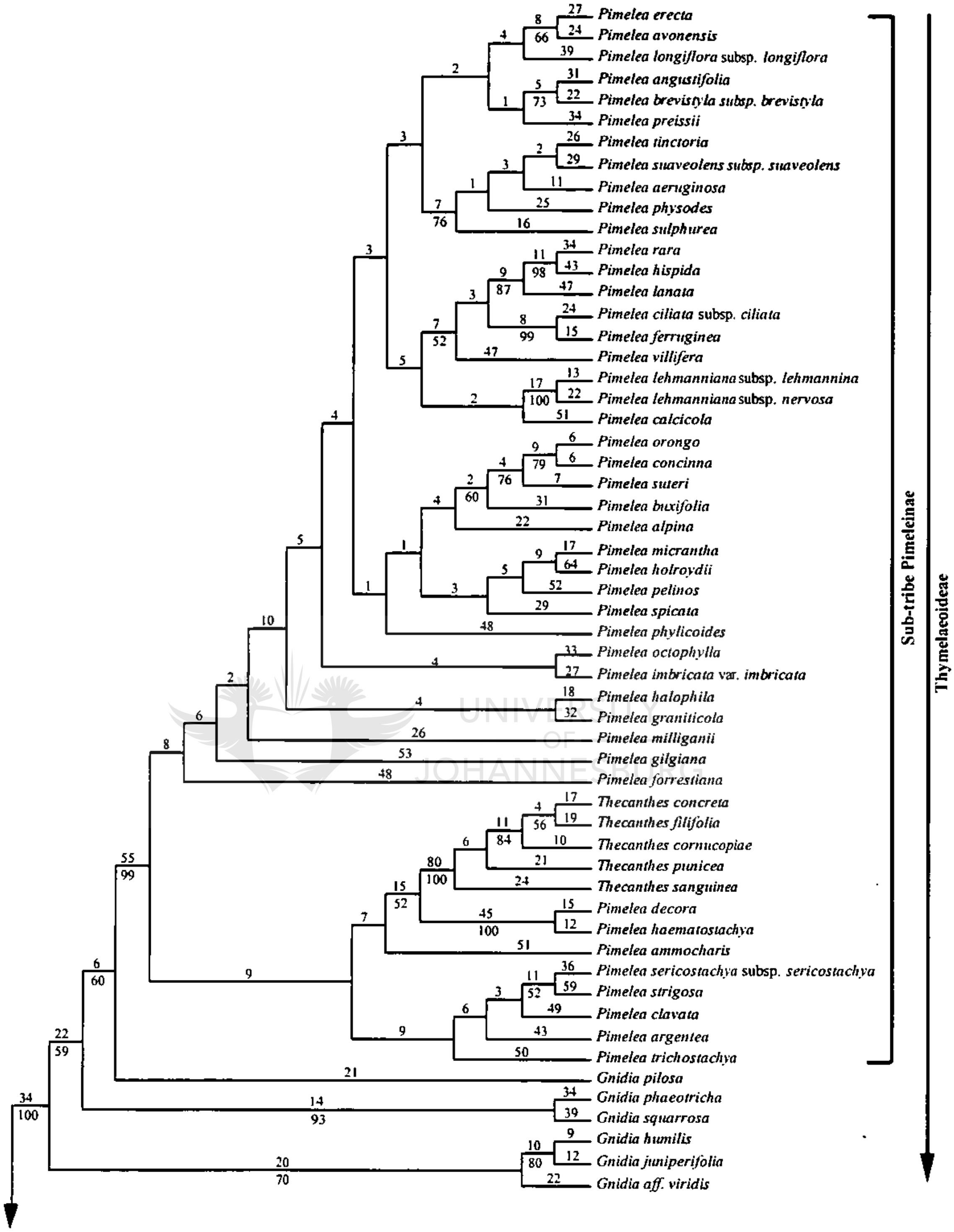
98
97
75
100
54
94
B 98
79
100
96
100
51
100
82
98
98
62
94
100
I
II
Pimelea erecta
Pimelea avonensis
Pimelea calcicola
Pimelea ciliata subp. ciliata
Pimelea ferruginea
Pimelea preissii
Pimelea tinctoria
Pimelea physodes
Pimelea sulphurea
Pimelea suaveolens subsp. suaveolens
Pimelea angustifolia
Pimelea brevistyla subsp. brevistyla
Pimelea longiflora subsp. longiflora
Pimelea lehmanniana subsp. lehmanniana
Pimelea lehmanniana subsp. nervosa
Pimelea hispida
Pimelea lanata
Pimelea rara
Pimelea villifera
Pimelea forrestiana
Pimelea octophylla
Pimelea milliganii
Pimelea imbricata var. imbricata
Pimelea halophila
Pimelea graniticola
Pimelea gilgiana
Pimelea orongo
Pimelea concinna
Pimelea buxifolia
Pimelea alpina
Pimelea aeruginosa
Pimelea phylicoides
Pimelea pelinos
Pimelea holroydii
Pimelea spicata
Pimelea clavata
Pimelea sericostachya subsp. sericostachya
Pimelea argentea
Pimelea trichostachya
Pimelea micrantha
Pimelea strigosa
Pimelea ammocharis
Thecanthes punicea
Thecanthes cornucopiae
Thecanthes concreta
Thecanthes sanguinea
Thecanthes filifolia
Pimelea decora
Pimelea haematostachya
Gnidia phaeotricha
Gnidia squarrosa
Gnidia aff. viridis
Gnidia humilis
Lachnaea aurea
Lachnaea ericoides
Lachnaea diosmoides
Lachnaea capitata
Lachnaea eriocephala
Lachnaea axillaris
Struthiola ciliata
Struthiola leptantha
Gnidia pinifolia
Gnidia racemosa
Passerina ericoides
Passerina montivago
Passerina nivicola
Passerina burchellii
Passerina falcifolia
Passerina drankensbergensis
Passerina obtusifolia
Gnidia denudata
Gnidia anomala
Gnidia renniana
Kelleria dieffenbachii
Drapetes muscoides
Peddiea involucrata
Peddiea africana
Stephanodaphne oblongifolia
Stephanodaphne cuspidata
Ovidia andina
Dirca palustris
Gnidia kraussiana
Gnidia calocephala
Gnidia gilberta
Gnidia decaryana
Dais continifolia
Thymelaea hirsuta
Daphne mezereum
Wikstroemia gemmata
Edgeworthia chrysantha
Gyrinops walla
Thymelaeoideae
Aquilarieae

Fig. 2.3 (next two pages). One of the two equally parsimonious trees from the combined molecular data sets. Numbers displayed above each branch are Fitch lengths (DELTRAN optimisation). Values below the branches are bootstrap percentages equal to or higher than 50%. Closed circles on branches indicate groups not present in Fitch strict consensus tree. The three clades indicated are: I) the Gondwanan taxa, II) the non-African taxa and III) the tropical African and southeast Asian taxa.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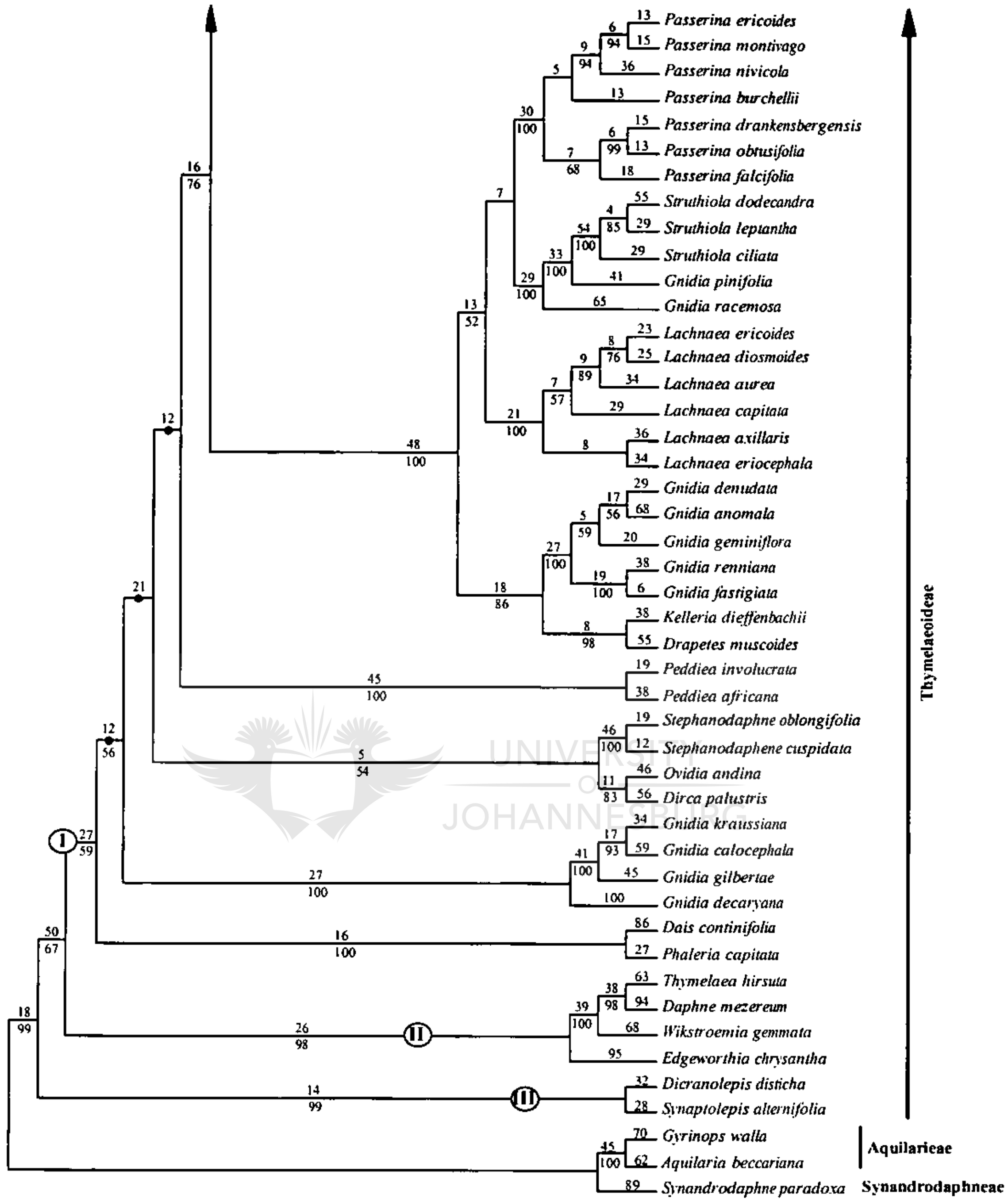
22 Pimelea brevistyla subsp. brevistyla
34 Pimelea preissii
26 Pimelea tinctoria
29 Pimelea suaveolens subsp. suaveolens
11 Pimelea aeruginosa
25 Pimelea physodes
16 Pimelea sulphurea
34 Pimelea rara
43 Pimelea hispida
47 Pimelea lanata
24 Pimelea ciliata subsp. ciliata
15 Pimelea ferruginea
47 Pimelea villifera
13 Pimelea lehmanniana subsp. lehmannina
22 Pimelea lehmanniana subsp. nervosa
51 Pimelea calcicola
6 Pimelea orongo
6 Pimelea concinna
7 Pimelea suteri
31 Pimelea buxifolia
22 Pimelea alpina
17 Pimelea micrantha
64 Pimelea holroydii
52 Pimelea pelinos
29 Pimelea spicata
48 Pimelea phylicoides
33 Pimelea octophylla
27 Pimelea imbricata var. imbricata
18 Pimelea halophila
32 Pimelea graniticola
26 Pimelea milliganii
53 Pimelea gilgiana
48 Pimelea forrestiana
17 Thecanthes concreta
19 Thecanthes filifolia
10 Thecanthes cornucopiae
21 Thecanthes punicea
24 Thecanthes sanguinea
15 Pimelea decora
12 Pimelea haematostachya
51 Pimelea ammocharis
36 Pimelea sericostachya subsp. sericostachya
59 Pimelea strigosa
49 Pimelea clavata
43 Pimelea argentea
50 Pimelea trichostachya
21 Gnidia pilosa
34 Gnidia phaeotricha
39 Gnidia squarrosa
9 Gnidia humilis
12 Gnidia juniperifolia
22 Gnidia aff. viridis
Sub-tribe Pimeleinae
Thymelaeoideae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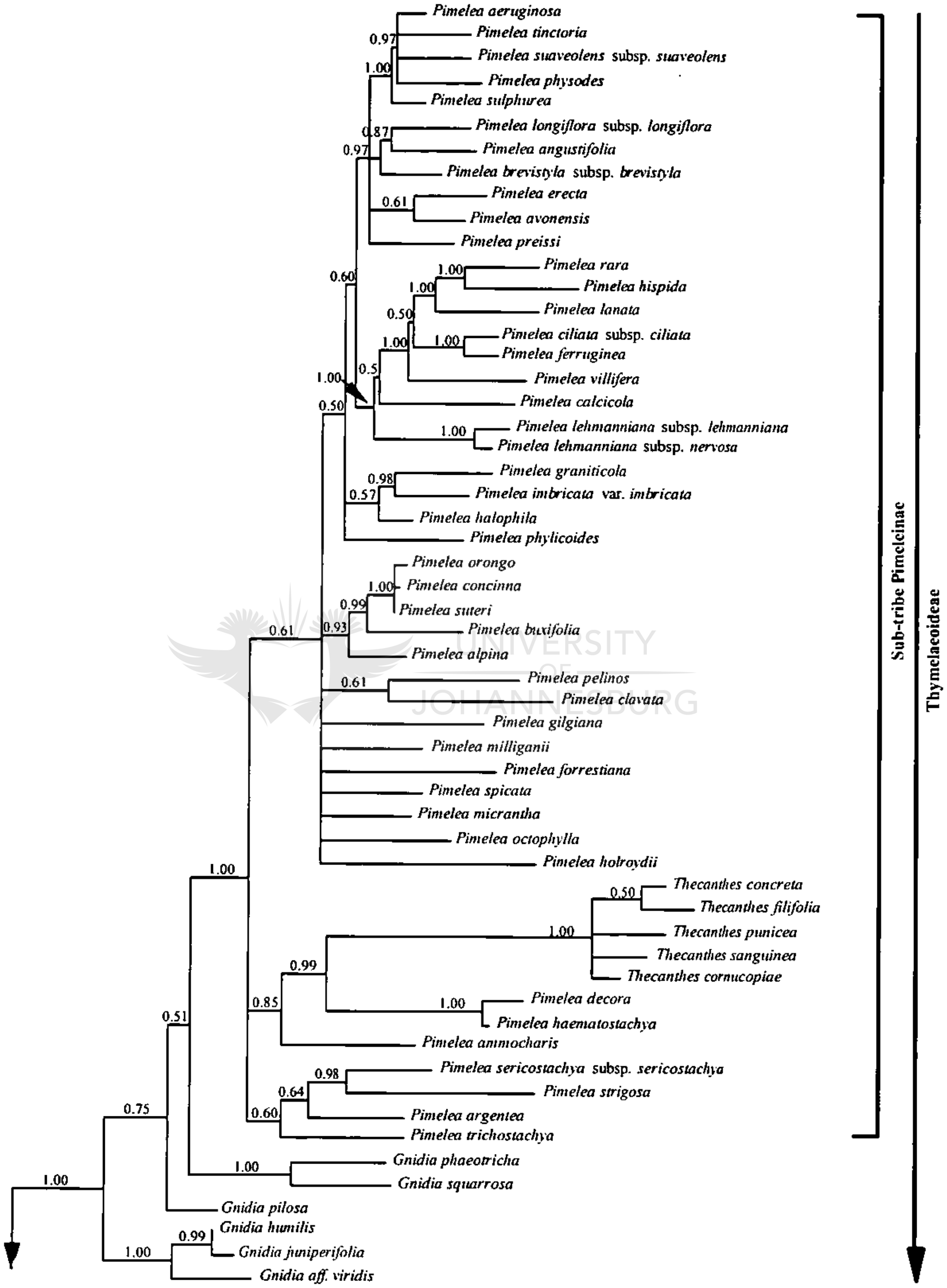
Passerina montivago
Passerina nivicola
Passerina burchellii
Passerina drankensbergensis
Passerina obtusifolia
Passerina falcifolia
Struthiola dodecandra
Struthiola leptantha
Struthiola ciliata
Gnidia pinifolia
Gnidia racemosa
Lachnaea ericoides
Lachnaea diosmoides
Lachnaea aurea
Lachnaea capitata
Lachnaea axillaris
Lachnaea eriocephala
Gnidia denudata
Gnidia anomala
Gnidia geminiflora
Gnidia renniana
Gnidia fastigiata
Kelleria dieffenbachii
Drapetes muscoides
Peddiea involucrata
Peddiea africana
Stephanodaphne oblongifolia
Stephanodaphene cuspidata
Ovidia andina
Dirca palustris
Gnidia kraussiana
Gnidia calocephala
Gnidia gilbertae
Gnidia decaryana
Dais continifolia
Phaleria capitata
Thymelaea hirsuta
Daphne mezereum
Wikstroemia gemmata
Edgeworthia chrysantha
Dicranolepis disticha
Synaptolepis alternifolia
Gyrinops walla
Aquilaria beccariana
Synandrodaphne paradoxa
Thymelaeoideae
Aquilarieae
Synandrodaphneae
I
II
III

Fig. 2.4 (next two pages). Bayesian analysis of combined ITS and plastid dataset. PPs > 0.5 are shown above the branches. The three clades indicated are: I) the Gondwanan taxa, II) the non-African taxa and III) the tropical African and southeast Asian taxa.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Pimelea longiflora subsp. longiflora
0.97
Pimelea angustifolia
Pimelea brevistyla subsp. brevistyla
0.61
Pimelea erecta
Pimelea avonensis
Pimelea preissi
1.00
Pimelea rara
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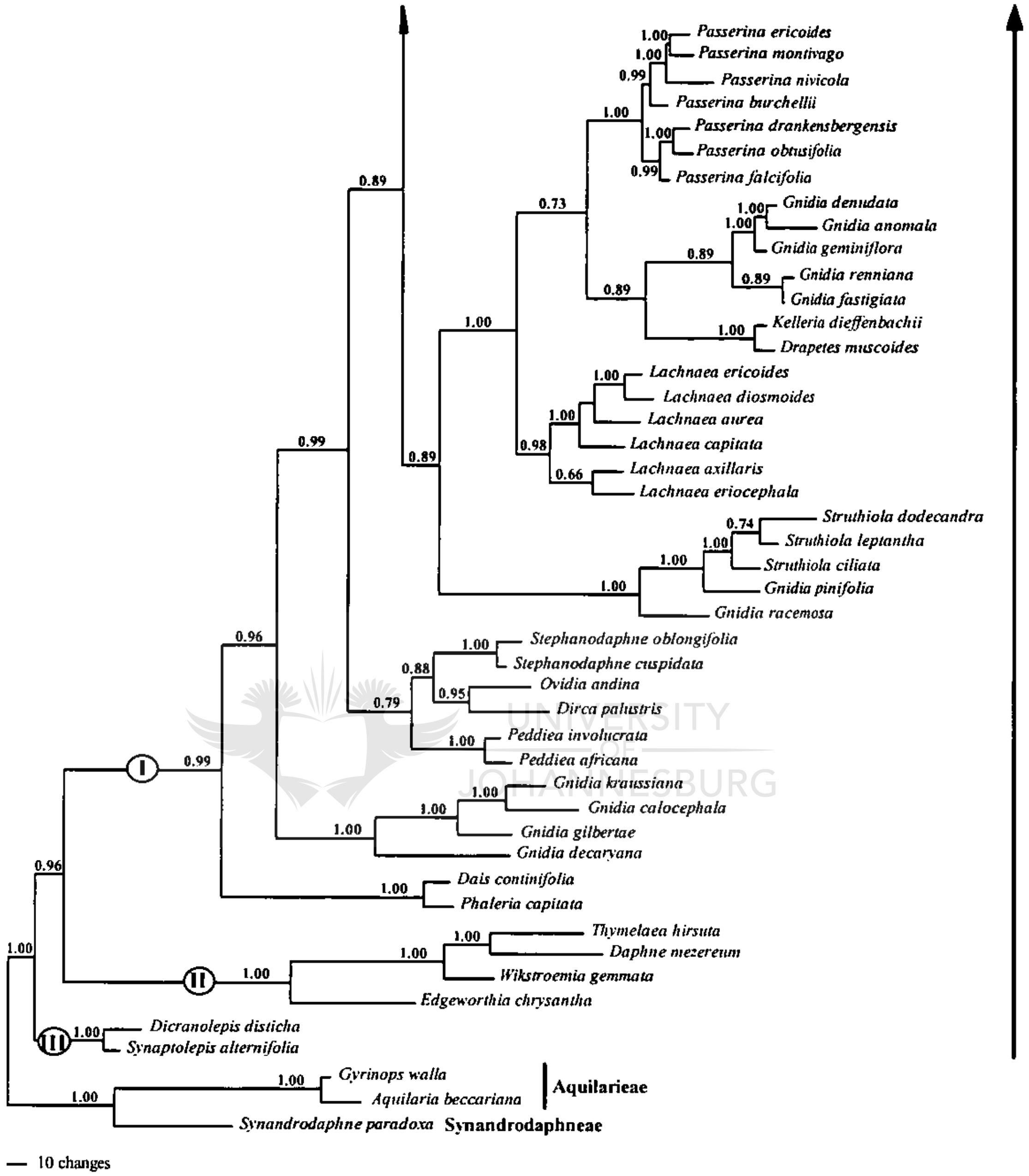
Pimelea hispida
1.00
Pimelea lanata
0.50
1.00
1.00
Pimelea ciliata subsp. ciliata
Pimelea ferruginea
0.5
Pimelea villifera
1.00
Pimelea calcicola
0.50
1.00
Pimelea lehmanniana subsp. lehmanniana
Pimelea lehmanniana subsp. nervosa
Pimelea graniticola
0.98
Pimelea imbricata var. imbricata
0.57
Pimelea halophila
Pimelea phylicoides
Pimelea orongo
1.00
Pimelea concinna
0.99
Pimelea suteri
0.61
0.93
Pimelea buxifolia
Pimelea alpina
0.61
Pimelea pelinos
Pimelea clavata
Pimelea gilgiana
Pimelea milliganii
Pimelea forrestiana
Pimelea spicata
Pimelea micrantha
Pimelea octophylla
Pimelea holroydii
1.00
0.50
Thecanthes concreta
Thecanthes filifolia
1.00
Thecanthes punicea
0.99
Thecanthes sanguinea
Thecanthes cornucopiae
0.85
1.00
Pimelea decora
Pimelea haematostachya
0.51
Pimelea ammocharis
0.98
Pimelea sericostachya subsp. sericostachya
0.64
Pimelea strigosa
0.60
Pimelea argentea
0.75
Pimelea trichostachya
1.00
Gnidia phaeotricha
1.00
Gnidia squarrosa
Gnidia pilosa
0.99
Gnidia humilis
1.00
Gnidia juniperifolia
Gnidia aff. viridis
Sub-tribe Pimeleinae
Thymelaeoideae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1.00 Passerina montivago
0.99 Passerina nivicola
Passerina burchellii
1.00
1.00 Passerina drankensbergensis
Passerina obtusifolia
0.99 Passerina falcifolia
0.89
0.73
1.00 Gnidia denudata
1.00 Gnidia anomala
Gnidia geminiflora
0.89
0.89 Gnidia renniana
Gnidia fastigiata
0.89
1.00 Kelleria dieffenbachii
Drapetes muscoides
1.00
1.00 Lachnaea ericoides
Lachnaea diosmoides
1.00 Lachnaea aurea
Lachnaea capitata
0.99
0.89
0.98
0.66 Lachnaea axillaris
Lachnaea eriocephala
0.74 Struthiola dodecandra
1.00 Struthiola leptantha
1.00 Struthiola ciliata
Gnidia pinifolia
1.00
Gnidia racemosa
0.96
1.00 Stephanodaphne oblongifolia
Stephanodaphne cuspidata
0.88
0.95 Ovidia andina
0.79 Dirca palustris
1.00 Peddiea involucrata
Peddiea africana
I
0.99
1.00 Gnidia kraussiana
1.00 Gnidia calocephala
Gnidia gilbertae
1.00 Gnidia decaryana
0.96
1.00 Dais continifolia
Phaleria capitata
1.00
1.00 Thymelaea hirsuta
1.00 Daphne mezereum
II 1.00 Wikstroemia gemmata
Edgeworthia chrysantha
III 1.00 Dicranolepis disticha
Synaptolepis alternifolia
1.00 Gyrinops walla
1.00 Aquilaria beccariana
Aquilarieae
Synandrodaphne paradoxa
Synandrodaphneae
Thymelaeoideae
10 changes

2.4. Discussion and conclusion

In this study Thymelaeoideae have been found to be monophyletic and contain three major clades, one Gondwanan, another all non-African and the third tropical African and southeast Asian. An important conclusion drawn from this molecular analysis is that *Gnidia* is not monophyletic and comprises at least four distinct clades. The changes to the classification of *Gnidia* and the new combinations proposed will be treated in forthcoming publications (Beaumont and Van der Bank pers. comm).

The current paper provides new phylogenetic evidence into the taxonomic status of *Thecanthes*, a group that has variously been treated as a section, subgenus or distinct genus. The relationships within Pimeleinae were evaluated to assess the taxonomic status of *Thecanthes*. The sub-tribe Pimeleinae was found to be monophyletic (100 BP, Fig. 2.1; 100 BP, Fig. 2.2; 100 BP, Fig. 2.3; 1.0 PP, Fig. 2.4). Molecular data evidently support a monophyletic genus *Pimelea* and *Thecanthes* (nested within *Pimelea*). *Thecanthes* is strongly supported as a monophyletic group, but all molecular data sets indicated that it is nested within *Pimelea*; hence it is not retained as a distinct genus.

According to Peterson (1959), Thymelaeaceae is a family in which it is very difficult to find sound characters for generic delimitation. *Thecanthes* is separated from *Pimelea* in being a glabrous annual and having a more specialised inflorescence (Rye, 1988). In *Thecanthes* the inflorescence is terminal, erect and head-like with a concave receptacle and flowers born on long dorsiventrally-compressed pedicels and surrounded by four united involucral bracts, while the inflorescence of *Pimelea* varies from racemose and occasionally reduced to one or two flowers to head-like but with a convex to flat receptacle, with involucral bracts free or sometimes absent. The similar floral morphology of the two genera, both with four sepals, no corolla lobes and two stamens, supports the current molecular topology.

With the view of establishing natural genera and on the basis of the findings of this molecular analysis, I support the viewpoints of Gilg (1894), Bentham, (1873) and Threlfall, (1983; 1984) who classified *Thecanthes* either as a subgenus or section within *Pimelea*. Therefore, *Thecanthes* is no longer recognised as a distinct genus and sub-tribe Pimeleinae is restored to being monogeneric, comprising the single genus *Pimelea*.

Nomenclature

Based on results of this paper *Thecanthes* now should be considered a synonym of the genus *Pimelea*. Accordingly, one new species combination is made to transfer a species of *Thecanthes* to *Pimelea* and the other four species of *Thecanthes* are restored to their original names in *Pimelea*.

Pimelea Banks & Sol. ex Gaertner *nom. cons.*, *Fruct. Sem. Pl.* 1:186 (1788). Type species: *Pimelea laevigata* Gaertner *nom. illeg.* [= *Pimelea prostrata* (Forster & G. Forster) Willd.]

Thecanthes Wikström., *Kongl. Vetensk. Acad. Handl*: 271 (1818); *Pimelea* a. *Thecanthes* (Wikström) Endl., *Gen. Pl*: 331 (1837): *Calyptrostegia* A.

Calyptridium Endl. *nom. illeg.* a. *Thecanthes* (Wikström) Endl., *Gen. Pl.* Suppl. 4: 62 (1848); *Pimelea* sect. *Thecanthes* (Wikström) Meissner *in* A. P. de Candolle., *Prodr.* 14: 496 (1857); *Pimelea* subgen. *Thecanthes* (Wikström) Gilg, *in* A. Engler & K. Prantl, *Nat. Pflanzenfam.*111, 6a: 243 (1894); *Banksia* sect. *Thecanthes* (Wikström) Kuntze, *in* T. E. von Post & C. E. O. Kuntze, *Lex. Gen. Phan.* 59 (1903). Type species: *Thecanthes punicea* (R. Br.) Wikström (lecto, *fide* S. Threlfall, *Brunonia* 5: 118 (1983)).

Pimelea sanguinea F. Muell., *Fragm.* 1(4): 84 (1859). *Banksia sanguinea* (F.Muell.) Kuntze, *Revis. Gen. Pl.* 2: 583 (1891); *Thecanthes sanguinea* (F.Muell.) Rye, *Nuytsia* 6: 267 (1988). Type: Australia, Pandanus Springs, upper Roper River, Northern Territory, 20 July 1856, *F. Mueller* s.n (holo: MEL)

Pimelea punicea R.Br., *Prodr.* 359 (1810); *Thecanthes punicea* (R.Br.) Wikström, *Kongl. Vetensk. Acad. Handl.* 272 (1818); *Calyprostegia punicea* (R.Br.) Endl., *Gen. Pl.* Suppl. 4: 60 (1848); *Banksia punicea* (R.Br.) Kuntze, *Revis. Gen.* 2: 583 (1891). Types: Australia, North Bay, Arnhem Land, Northern Territory, *R. Brown* s.n. (lecto: BM, *fide* B. L. Rye, *Nuytsia* 6: 264 (1988); isolecto: MEL).

Pimelea punicea var. *breviloba* F.Muell. ex Benth., *Fl. Austral.* 6: 6 (1873). Types: Australia, Purdie's Ponds, Northern Territory, *J. McDouall Stuart s.n.* (lecto: K, *fide* B. L. Rye, Nuytsia 6: 264 (1988); isolecto: MEL).

Pimelea concreta F.Muell., *Fragm.* 5: 73-74 (1865); *Banksia concreta* (F.Muell.) Kuntze, *Revis. Gen. Pl.* 2: 583 (1891); *Thecanthes concreta* (F.Muell.) Rye, *Nuytsia* 6: 267 (1988). Type: Australia, Camden Harbour, Western Australia, *J.S. Roe s.n.* (holo: MEL).

Pimelea brevituba Fawc. *in* H. O. Forbes, *A Naturalist's Wanderings*: 516 (1885). Type: East Timor, Mt Sobale, Samoro, 28 Apr. –3 May 1883, H.O. *Forbes* 3828 (holo: BM).

Pimelea cornucopiae Vahl, *Enum. Pl.* 1: 305 (1804); *Thecanthes cornucopiae* (Vahl) Wikström, *Kongl. Vetensk. Acad. Handl.* 271 (1818); *Calyptrostegia cornucopiae* (Vahl) Endl., *Gen. Pl.* Suppl. 4(2): 60 (1848); *Banksia cornucopiae* (Vahl) Kuntze, *Revis. Gen. Pl.* 2: 583 (1891). Type: Australia, *D. Montin s.n.*; (holo: C *n. v.*, *fide* S. Threlfall, *Brunonia* 5: 123 (1983)).

Pimelea ramosissima Schumann *in* K. Schumann & K. Lauterbach. *Nachtr Fl. Schutzgeb. Südsee* 324 (1905).Type: Papua New Guinea, New Britain, Bismarck Archipelago, Jan. 1902, *R. Schlechter* 13979 (*n. v.*).

Pimelea philippinensis C. Robinson, *Philipp. J. Sci.* C. 6: 345 (1911).Type: Philippines, Sanchez Mira, Province of Cagayan, Luzon, *Ramos* 7410 (*n. v.*).

Pimelea filifolia (Rye) Motsi & Rye *ms; Thecanthes filifolia* Rye, *Fl. Australia* 18: 325 (1990). Type: Australia, Magela Creek, Northern Territory, 25 Feb. 1973, *C.R. Dunlop* 3357 (holo: CANB; iso: BRI, DNA, NSW).

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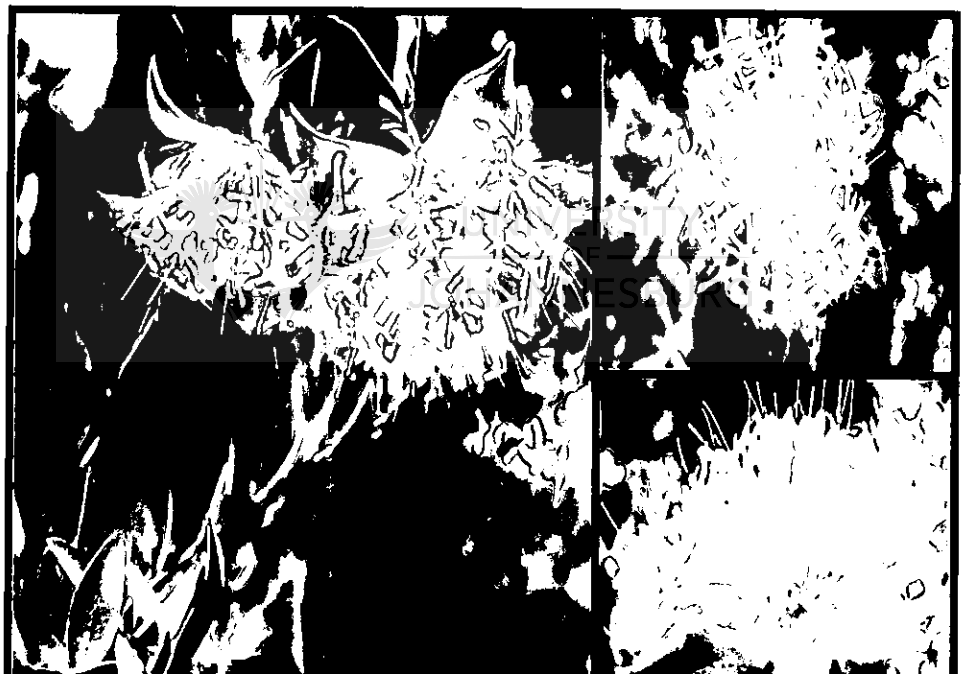
CHAPTER THREE

TOWARDS A COMPLETE SPECIES-LEVEL MOLECULAR PHYLOGENY OF THE GENUS *PIMELEA* (THYMELAEACEAE)



Pimelea suaveolens Photos:MC Motsi

3.1. Introduction

This chapter concentrates on the species relationships and infra-generic classification of *Pimelea* Banks & Sol. Ex Gaertn. *Pimelea* is a relatively large genus consisting of 110 species, of which 90 species are endemic to Australia, 19 to New Zealand and one to Lord Howe Island (Allan, 1961; Burrows, 1962; Rye, 1988; Wilson and Galloway, 1993; Burrows, 2008). *Thecanthes*, which we consider as part of *Pimelea* (Motsi *et al.,* submitted; and see also Chapter 2), contains five species occurring in the Philippines, Indonesia, Timor and northern Australia.

The genus *Pimelea* was first described in 1769/70 by Solander who wrote about four species from an unpublished manuscript prepared following James Cook's first voyage to New Zealand (Burrows, 2008). These species are currently known as *Pimelea arenaria* A. Cunn., *P. prostrata* (J.R. Forst. et G. Forst.) Willd. *pro parte, P. tomentosa* and *P. longifolia* Sol. ex Wickstr (Burrows, 2008). The generic name *Pimelea* was ignored by J. R. and J. G. Forster in their publication in 1776 when describing two of the four species referred to above and the genus was described as *Banksia* L.f. rather than *Pimelea.* In 1782 Linnaeus used *Passerina* L. rather than *Banksia,* which he also used for some Proteaceae. *Passerina* is currently used for one of the southern Africa endemic genera within Thymelaeaceae. Linnaeus' species of *Passerina* were published in 1786 by Foster. It was only in 1788 that Gaertner used the generic name *Pimelea* and published it according to the rules of the Code of Nomenclature.

Subsequently, different authors gave different generic names to species within *Pimelea*: (*Gymnococca*, *Heterolaena* and *Calyptrostegia*; Meyer, 1845); (*Gymnococca*, *Calyptrostegia*; Endlicher, 1848); (*Macrostegia*; Turczaninow, 1852). Some of these names have been used by various authors including Rye (1988; 1990) for the sectional classification, however Kuntze's reinstatement of the genus *Banksia* in 1891 and 1903 was invalid. In New Zealand different authors (Hooker, 1853, 1867; Kirk, 1880, 1894; Petrie, 1912; 1917; Cockayne, 1921; Allan, 1961) contributed to the description of species within *Pimelea*. Many of the names published by Colenso (1886; 1888; 1889; 1890; 1896; 1899) were rejected as they were considered to be of hybrids or to be synonyms rather than new species and others were referred to as unresolved by Allan (1961).

Pimelea is commonly known as "rice flower" or "banjine" by Australians and "New Zealand daphne" or "native daphne" by New Zealanders and is the second largest genus (110 species) within the family Thymelaeaceae (Rye, 2002; Burrows, 2008). The genus *Pimelea* is well characterised by its great diversity of life forms (see Chapters 1 and 2). The name *Pimelea* comes from "*pimele*"- meaning fatty, referring to the oily seeds or the fleshy cotyledons (Rye, 1988; 1990; Burrows, 2008). The name *Thecanthes* is derived from "*theca*", an envelope or sac and "*anthos*" a flower, in reference to the sac-like concave receptacle and attached bracts enclosing the flower (Rye, 1988).

The current sections show some geographic structure (Table 3.1), with most sections centered in southern Australia (Threlfall, 1983). Section *Heterolaena* and the monotypic sections *Macrostegia* and *Heterantheros* occur only in the south-western part of Western Australia. The temperate area of Australia is home to 33 species of section *Calyptrostegia.* Section *Pimelea* has a diverse distribution, with 19 species in New Zealand and one species on Lord Howe Island and the rest in Australia.

The varied life forms and breeding systems found in Pimelea were described in Chapter 1. The flowers within sub-tribe Pimeleinae range from very deep red (*Thecanthes*) to white, pink, yellow, or greenish (Fig. 3.1). *Pimelea* occupies a great variety of vegetation types (Fig. 3.2). *Pimelea lanata* R.Br. can grow as tall as 4 m and is closely associated with wetlands (Fig. 3.3). Many species thrive in disturbed areas (Fig. 3.4).

According to Keighery (1975) and Burrows (1960), bees, flies, beetles and other small insects act as pollinators. Butterflies and moths are also common visitors and appear to be the primary pollinators of some species (Fig. 3.5). *Pimelea physodes* Hook., commonly known as Qualup Bell, is the only species that is bird pollinated (Fig. 3.6) and is thought to mimic *Darwinia* Rudge (Myrtaceae; Keighery, 1975).

Some species, e.g. *Pimelea pauciflora* R.Br. and *P. simplex* F. Muell., are poisonous to cattle (Lazarides and Hince, 1993; Wiersema and León, 1999). Others such as *Pimelea ferruginea* Labill., *P. glauca* R. Br., *P. humilis* R.Br., *P. spectabilis* Lindl. and *P. nivea* Labill., are horticultural plants. *Pimelea trichostachya* Lindl., *P. microcephala* R.Br. and *P. linifolia* Sm. can be used as fodder, ornaments or shelter (Lazarides and Hince, 1993). Berries of

Pimelea microcephala can be eaten and a drink can be brewed from its roots for relieving throat and chest pains (Rye, 2002).

As the genus *Pimelea* is so diverse, the objective of this chapter is to reconstruct a comprehensive molecular phylogeny of *Pimelea s.l.*, ideally encompassing all species within the genus. Here I also assess the morphological delimitation of the sections within *Pimelea*, as well as the relationship of *Pimelea* with some other genera within Thymelaeoideae. The biogeographical patterns within the genus are finally discussed.

Table 3.1. Size and distribution of sections of *Pimelea*

Sections	**Distributions and number of species**
Heterantheros	South-western Australia with one species
Pimelea	Australia with 18 species; Lord Howe Island with one species; New Zealand with 19 species
Epallage	All Australian states with 19 species
Calyptrostegia	All Australian state, mainly temperate areas with 33 species
Macrostegia	south-western Australia with one species
Stipostachya	Western Australia mainly Pilbara with one species; Queensland two with species
Heterolaena	south-western Australia with 15 species

3.2 Materials and Methods

3.2.1 Taxon sampling and outgroups

A total of 169 taxa were sampled for this chapter, of which 137 belong to sub-tribe Pimeleinae (Table 3.1). The number of DNA regions for which 80% of the sequence length has been completed, per taxon, are also indicated in Table 3.1. The choice of outgroups was based on previous studies by Van der Bank *et al.* (2002) and Motsi *et al.* (submitted). Alignment of ITS sequences was difficult due to several ambiguous regions. Thus, *Edgeworthia*, *Daphne, Thymelaea* and two species of *Wikstroemia* were selected as outgroups specifically for the ITS data matrix. Sampling within sub-tribe Pimeleinae includes a wide range of species from Australia and New Zealand. Some of the New Zealand species are as yet undescribed.

3.2.2 DNA extraction, amplification and sequencing

Total DNA was extracted using CTAB as described in Doyle and Doyle (1997) from 0.1 - 0.3 g of fresh, silica-dried leaf, or herbarium material. A list of all samples, their voucher information and GenBank accession numbers are listed in Table 3.2. PCR amplification and sequencing of *rbcL* and *trnL-F* (intron and spacer regions) were performed as in Van der Bank *et al.* (2002) and ITS was amplified into two overlapping pieces as in Motsi *et al.* (submitted; see Chapter 2).

3.2.3. Phylogenetic analyses

The Maximum parsimony (MP) optimality criterion was used for individual and combined phylogenetic analyses. Visual comparisons of topologies were employed to detect incongruence among the data sets. For visual assessment of the datasets the bootstrap trees were considered incongruent only if they displayed "hard" (i.e., with high bootstrap support, > 85%) rather than "soft" (with low bootstrap support, < 85%) incongruence (Seelanan *et al.,* 1997; Wiens, 1998).

Due to degraded DNA in a few samples, I could not amplify all of *trnL-F, rbcL* and ITS, thus the individual data matrices do not contain identical sets of taxa (Table 3.3). Sequences were aligned manually. Each insertion/deletion was characterised by assigning gaps. The data matrix analyses used MP via the heuristic search option in PAUP* for Macintosh version 4.0b1 (Swofford, 2003) with uninformative characters excluded. Data matrices were analysed using 1000 random taxon additions, TBR (tree bisection reconnection) branch swapping with MULPARS on and all transformations treated as equally likely (Fitch parsimony; Fitch, 1971). A limit of one tree per replicate was set so that less time was spent on each replicate. Internal support was accessed with 1000 bootstrap replicates (Felsenstein, 1985) using simple stepwise addition, but only holding one tree per replicate. Only groups with bootstrap percentage (BP) greater than 50% are reported. DELTRAN (Delayed transformation) character optimisation was used instead of ACCTRAN (Acceleration of transformation) due to reported errors with the version of PAUP 4.0b1. The following arbitrary scale for describing bootstrap support was applied: 50 – 74% weak, 75 – 84% moderate and 85 – 100% high.

Table 3.2. Sources of plant material used ([1]Robinson, 2004; [2]Van der Bank *et al.*, 2002; [3]Rautenbach, 2006; [4]Van Niekerk, 2005; [5]Fay *et al.*, 1998; Asterisk* = send to GenBank)

Species	Location/ voucher specimen/ herbarium	Genbank accession numbers		
		trnL-F	*rbcL*	ITS
***Pimelea* Banks & Sol. ex Gaertn.**				
Section *Calyptrostegia* (C.A.Mey.) Benth.				
P. aeruginosa F.Muell.	Australia: Western Australia, *D. J. Edinger 3969 & G. Marsh* (PERTH)	FJ 572691	FJ 572823	FJ 572729
P. alpina Meisn.	Australia: New South Wales, *Taylor J. M. 1263* (CBG)	FJ 572690	FJ 572822	FJ 572728
P. ammocharis F.Muell.	Australia: Western Australia, *R. Davis 11172* (PERTH)	FJ 605475	FJ 605469	FJ 605463
P. angustifolia R.Br.	Australia: Western Australia, *P. Foreman*	FJ 572688	FJ 572820	FJ 572726

	& J. Kelly KO 365 (PERTH)			
P. bracteata Threlfall	Australia: New South Wales, *Crawford I. 7268* (CANB)	GQ 205323	GQ 205261	GQ 205201
P. calcicola Rye	Australia: Western Australia, *J. W. Horn 2574* (PERTH)	FJ 572684	FJ 572816	FJ 572722
P. ciliolaris (Threlfall) Rye	Australia: New South Wales, *Streimann H.616* (CBG)	-	GQ 205252	GQ 205191
P. cracens subsp. *cracens* Rye	Australia: Western Australia, *M. Bennett 214* (PERTH)	-	GQ 205263	GQ 205203
P. cracens subsp. *glabra* Rye	Australia: Western Australia, *S. Clarke 161 16/11/2004* (PERTH)	GQ 205325	GQ 205264	GQ 205204
P. drummondii (Turcz.) Rye	Australia: Western Australia, *M. Paxman 22* (PERTH)	GQ 205326	GQ 205265	GQ 205205

P. erecta Rye	Australia: Western Australia, *C.F. Craig 5994* (PERTH)	FJ 572683	FJ 572815	FJ 572721
P. floribunda Meisn.	Australia: Western Australia, *J. & P. Foss 361* (PERTH)	-	GQ 205285	-
P. graniticola Rye	Australia: Western Australia, *B. Archer 1664* (PERTH)	AM 406679^{3}	FJ 572812	FJ 572718
P. humilis R.Br.	Australia: New South Wales, *Cayzer L.W. 915* (CANB)	GQ 205321	GQ 205259	GQ 205198
P. imbricata var. *imbricata* R.Br.	Australia: Western Australia, *R. J. Cranfield 17565* (PERTH)	FJ 572681	FJ 572813	FJ 572719
P. imbricata var. *major* (Meisn.)Rye	Australia: Western Australia, *L. W. Sage 779 3/10/1996* (PERTH)	GQ 205317	GQ 205253	GQ 205192
P. imbricata var. *petraea* (Meisn. Rye	Australia: South Australia, *Briggs J. D.*	-	GQ 205255	GQ 205194

	1417 (CBG)			
P. imbricata var. *piligera* (Benth.) Diels	Australia: Western Australia, *G. Byrne 585* (PERTH)	GQ 205318	GQ 205254	GQ 205193
P. ligustrina Labill.	Australia: New South Wales, *Lidbetter J. s.n.* (NSW)	*	*	*
P. ligustrina subsp. *ciliata* Threlfall	Australia: Victoria, *Forbes S.J. 803* (MEL)	*	-	*
P. linifolia Sm.	Australia: New South Wales, *Lidbetter J. s.n.* (NSW)	*	*	*
P. linifolia subsp. *caesia* Threlfall	Australia: Victoria, *Walsh N.G. 5270* (MEL)	-	-	GQ 205200
P. linifolia subsp. *collina* (R.Br.) Threlfall	Australia: Queensland, *Forster P. I. 20016* (MEL)	GQ 205322	GQ 205260	GQ 205199
P. linifolia subsp. *linifolia* Sm.	Australia: Queensland, *Forster P. I. 1752*	*	GQ 205288	*

	(BRI)			
P. longiflora subsp. *eyrei* (F.Muell.) Rye	Australia: Western Australia, *J. E. Wajon 238* (PERTH)	GQ 205319	GQ 205257	GQ 205196
P. longiflora subsp. *longiflora* R.Br.	Australia: Western Australia, *Read & Low 15* (PERTH)	FJ 572686	FJ 572818	FJ 572724
P. macrostegia (Benth.) J.M.Black	Australia: South Australia, *Jackson I. 7* (CBG)	GQ 205324	GQ 205262	GQ 205202
P. octophylla R.Br.	Australia: South Australia, *West J.G. 5372* (CANB)	FJ 572680	FJ 572811	FJ 572717
P. penicillaris F.Muell.	Australia: New South Wales, *Lidbetter J. s.n.* (NSW)	-	-	-
P. phylicoides Meisn.	Australia: Victoria, *Walsh N.G. 3539* (CANB)	FJ 572685	FJ 572817	FJ 572723

P. preissii Meisn.	Australia: Western Australia, *B. G. Ward & R. J. Cranfield 18582* (PERTH)	FJ 572687	FJ 572819	FJ 572725
P. stricta Meisn.	Australia: South Australia, *Crisp, M.D.7221* (CBG)	FJ 649638	FJ 649633	FJ 649628
P. suaveolens subsp. *suaveolens* Meisn.	Australia: Western Australia, *R. J. Cranfield FC 524* (PERTH)	FJ 572693	FJ 572825	FJ 572731
P. subvillifera (Threlfall) Rye	Australia: Western Australia, *B Archer 1645* (PERTH)	-	GQ 205256	GQ 205195
P. sulphurea Meisn.	Australia: Western Australia, *F. Hort 1256* (PERTH)	FJ 572689	FJ 572821	FJ 572727
P. sylvestris R.Br.	Australia: Western Australia, *B. Salter 94, C. Warburton & H. Green* (PERTH)	FJ 687346	FJ687344	FJ687340
P. tinctoria Meisn.	Australia: Western Australia, *R. J.*	FJ 572692	FJ 572824	FJ 572730

	Cranfield 17360 (PERTH)			
P. treyvaudii F.Muell.ex Ewart & B.Rees	Australia: New South Wales, *Purdie R.W. 3049* (CBG)	GQ 205320	GQ 205258	GQ 205197
P. villifera Meisn.	Australia: Western Australia, *F. & B. Hort 2110* (PERTH)	FJ 572682	FJ 572814	FJ 572720
Section *Epallage* (Endl.) Benth.				
P. argentea R.Br.	Australia: Western Australia, *M. Hislop & M. Griffiths WW 111.39* (PERTH)	AM 406675[3]	AM 167530	AM 162490
P. biflora N.A. Wakef.	Australia: New South Wales, *Carroll E. J. 352* (CBG)	GQ 205312	GQ 205247	GQ 205186
P. curviflora var. *curviflora* R.Br.	Australia: New South Wales, *Brown R. s.n.* (MEL)	GQ 205305	GQ 205241	-
P. curviflora var. *divergens* Threlfall	Australia: New South Wales, *Telford I. R.*	GQ 205309	GQ 205245	GQ 205183

	10593 (CBG)			
P. curviflora var. *gracilis* (R.Br.) Threlfall	Australia: Victoria, *Rehwinkel R. 238* (CANB)	GQ 205307	GQ 205243	GQ 205181
P. curviflora var. *sericea* Benth.	Australia: New South Wales, *Beesley P. 468* (CBG)	GQ 205308	GQ 205244	GQ 205182
P. curviflora var. *subglabrata* Threlfall	Australia: New South Wales, *Albrecht D. E. 3205* (MEL)	GQ 205306	GQ 205242	GQ 205180
P. interioris Rye	Australia: Northern Territory, *Willis J. H. s.n.* (MEL)	GQ 205304	GQ 205240	GQ 205179
P. latifolia subsp. *elliptifolia* Threlfall	Australia: New South Wales, *Wiecek B. M. 496* (MEL)	GQ 205311	GQ 205246	GQ 205185
P. latifolia subsp. *latifolia* R.Br.	Australia: Queensland, *Batianoff G. N. 940538* (BRI)	GQ 205310	-	GQ 205184

P. leptospermoides F.Muell.	Australia: Queensland, *Craven L. A. 9985* (CANB)	GQ 205314	GQ 205249	GQ 205188
P. micrantha Meisn.	Australia: Western Australia, *G. J. Keighery 11545* (PERTH)	AM 406688	FJ 572809	FJ 572715
P. sericea R.Br.	Australia: Tasmania, *Burns R. 345* (CBG)	GQ 205316	GQ 205251	GQ 205190
P. sericostachya subsp. *amabilis* (Domin) Threlfall	Australia: Queensland, *Elsol J. A. 771* (BRI)	GQ 205302	-	GQ 205177
P. sericostachya subsp. *sericostachya* F.Muell.	Australia: Queensland, *Purdie R. W. 5783* (CANB)	FJ 522678	FJ 572808	FJ 572714
P. simplex subsp. *continua* (J.M.Black) Threlfall	Australia: South Australia, *Vonow H. P. 618* (CBG)	-	GQ 205239	-
P. simplex subsp. *simplex* F.Muell.	Australia: South Australia, *Mulham W. E. 1125* (CANB)	GQ 205301	GQ 205238	GQ 205190

P. strigosa Gand.	Australia: New South Wales, *Coveny R. G. 14395* (CBG)	FJ 572679	FJ 572810	FJ 572716
P. trichostachya Lindl.	Australia: Western Australia, *K. F. Kenneally 12623 & D. J. Edinger 3822* (PERTH)	AM 406682[3]	AM 167537	AM 162501
P. umbratica Meisn.	Australia: New South Wales, *Floyd A. G. 1698* (CANB)	GQ 205313	GQ 205248	GQ 205187
P. venosa Threlfall	Australia: New South Wales, *Beesley P. 721A* (CBG)	GQ 205315	GQ 205250	GQ 205189
P. williamsonii J.M.Black	Australia: New South Wales, *Symon D. E. 4217* (CANB)	GQ 205303	-	GQ 205178
Section *Heterantheros* Rye				
P. gilgiana E.Pritz	Australia: Western Australia, *I. B.*	AM 406678[3]	AM 167534	FJ 572710

	Shepherd 269 (PERTH)			
Section *Heterolaena* (Endl.)Benth.				
P. avonensis Rye	Australia: Western Australia, *M. Hislop, B. Smith & M. Griffiths WW 116.10* (PERTH)	FJ 572697	FJ 572829	FJ 572735
P. brachyphylla Benth.	Australia: Western Australia, *E. Tink 252, 2/8/1998* (PERTH)	-	-	*
P. brevifolia subsp. *brevifolia* R.Br.	Australia: Western Australia, *R. Davis 10487* (PERTH)	GQ 205350	GQ 205287	*
P. brevifolia subsp. *modesta* (Meisn.) Rye	Australia: Western Australia, *J. M. Flint 187* (PERTH)	GQ 205329	GQ 205268	GQ 205208
P. brevistyla subsp. *brevistyla* Rye	Australia: Western Australia, *B. Nyanatusita 130* (PERTH)	FJ 572698	FJ 572830	FJ 572736
P. ciliata subsp. *ciliata* Rye	Australia: Western Australia, *P. Clynk &*	FJ 572699	FJ 572831	FJ 572731

	T. Watson 35 (PERTH)			
P. ferruginea Labill.	Australia: Western Australia, *B. L. Rye & M. C. Motsi BLR 279053* (PERTH)	FJ 605476	FJ 605470	FJ 605464
P. hispida R.Br.	Australia: Western Australia, *J. Smith 6* (PERTH)	FJ 572700	FJ 572832	FJ 572738
P. lanata R.Br.	Australia: Western Australia, *P. Foreman & J. Kelly KO 332* (PERTH)	FJ 572701	FJ 572833	FJ 572739
P. lehmanniana subsp. *lehmanniana* Meisn.	Australia: Western Australia, *B. L. Rye & M. C. Motsi BLR 279056* (PERTH)	FJ 605477	FJ 605471	FJ 605465
P. lehmanniana subsp. *nervosa* (Meisn.) Rye	Australia: Western Australia, *Balingup, E. M. Sandiford 590* (PERTH)	FJ 572696	FJ 572828	FJ 572734
P. leucantha Diels	Australia: Western Australia, *F. Littleton 31/10/2003* (PERTH)	GQ 205328	GQ 205267	GQ 205207

P. rara Rye	Australia: Western Australia, *F and J. Hort 2510* (PERTH)	AM 407412[3]	AM 167538	AM 162498
P. *rosea* R.Br.	Australia: Western Australia, *Fox J.M. 88/226* (CANB)	FJ 649637	FJ 649632	FJ 649627
P. rosea R.Br. subsp. *annelsii* Rye	Australia: Western Australia, *B. L. Rye & M. C. Motsi 279055* (PERTH)	GQ 205349	GQ 205286	*
P. sessilis Rye	Australia: Western Australia, *G. Flowers 218 & S. Donaldson* (PERTH)	GQ 205327	GQ 205266	GQ 205206
P. spectabilis Lindl.	Australia: Western Australia, *Chase 2198* (K)	AJ 308659[2]	AJ 297231[2]	AJ 744923[2]
Section *Macrostegia* (Turcz.) Rye				
P. physodes Hook.	Australia: Western Australia, *Lidbetter J. s.n.* (NSW)	FJ 687345	FJ 687343	FJ 687339

Section *Pimelea* Gaertn.				
P. “ahipara” (C.J.Burrows, in preparation, 2009)	New Zealand, *C. J. Burrows 38843* (CANU)	GQ 205342	GQ 205280	GQ 205217
P. axiflora subsp. *alpina* (Benth.) Threlfall	Australia: New South Wales, *Whalen A. J. 274* (CBG)	GQ 205297	GQ 205233	GQ 205171
P. axiflora subsp. *axiflora* Meisn.	Australia: New South Wales, *Purdie R. W. 4917* (CANB)	GQ 205298	GQ 205234	GQ 205172
P. axiflora subsp. *pubescens* Rye	Australia: New South Wales, *Beesley P. 431* (CBG)	GQ 205299	GQ 205235	GQ 205173
P. buxifolia Hook.f.	New Zealand, *J. M. Ward 22620* (CANU)	FJ 572703	FJ 572835	FJ 572740
P. cinerea R.Br.	Australia: Tasmania *Moscal A.12736* (CANB)	GQ 205291	GQ 205227	-
P. clavata Labill.	Australia: Western Australia, *S. Comer 26*	AM 406689[3]	AM 167531	AM 162491

	(PERTH)			
P. concinna Allan	New Zealand, *L. Burrows 38865* (CANU)	FJ 572705	FJ 572837	FJ 572742
P. drupacea Labill.	Australia: Victoria, *Eichler J. 98* (CANB)	GQ 205300		
P. forrestiana F.Muell.	Australia: Western Australia, *K. Coate 695* (PERTH)	AM 407407[3]	AM 167533	AM 162493
P. flava subsp. *dichotoma* (Schldl.)Threlfall	Australia: South Australia, *Whibley D. J. E. 9664* (CANB)	GQ 205290	GQ 205225	GQ 205164
P. flava subsp. *flava* R.Br.	Australia: Tasmania, *Davies F.E. 1008* (CBG)	GQ 205289	GQ 205224	GQ 205163
P. gnidia Willd.	New Zealand, *D. A. Norton 33757* (CANU)	FJ 649634	FJ 649629	FJ 649624
P. "cf gnidia" (C.J.Burrows, in preparation, 2009)	New Zealand, *C. J. Burrows 10864* (CANU)	GQ 205331	GQ 205270	GQ 205210

P. halophila Rye	Australia: Western Australia, *D. Papenfus 714 & K. Macey* (PERTH)	AM 406679	FJ 572805	FJ 572711
P. hewardiana Meisn.	Australia: Victoria, *Phillips M. E 131* (CBG)	FJ 649635	FJ 649630	FJ 649625
P. longifolia (Thunb.) Meisn.	New Zealand: Nelson/ Marlborough, *C. J. Burrows 26070* (CANU)	FJ 649636	FJ 649631	FJ 649626
P. lyallii Hook.f.	New Zealand, *B. A. Fineran 8755* (CANU)	GQ 205343	GQ 205281	GQ 205218
P. “manaia” (C.J.Burrows, in preparation, 2009)	New Zealand, *C. J.Burrows 38797* (CANU)	GQ 205345	-	GQ 205220
P. “maunga” (C.J.Burrows, in preparation, 2009)	New Zealand, *S. Courtney 38868* (CANU)	GQ 205337	GQ 205275	-
P. microcephala subsp. *microcephala* R.Br.	Australia: Western Australia, *M. Hislop & R. Davis WW91.39* (PERTH)	GQ 205292	GQ 205228	GQ 205166

P. microphylla Colenso	New Zealand, *C. J. Burrows 38861* (CANU)	GQ 205341	GQ 205279	GQ 205116
P. milliganii Meisn.	Australia: Tasmania, *Jarman S. J. 240* (CANB)	FJ 572676	FJ 572806	FJ 572712
P. neoanglica Threlfall	Australia: New South Wales, *Lyne A. M. 2134* (CBG)	GQ 205293	GQ 205229	GQ 205167
P. oreophila Burrows	New Zealand, *C. J. Burrows 6927* (CANU)	GQ 205332	-	-
P. "cf oreophila" (C.J.Burrows, in preparation, 2009)	New Zealand, *D. A. Norton 36852* (CANU)	GQ 205338	GQ 205276	GQ 205215
P. "orongo"(C.J.Burrows, in preparation, 2009)	New Zealand, *C.J. Burrows 38838* (CANU)	FJ 572704	FJ 572836	FJ 572741
P. pauciflora R.Br.	Australia: New South Wales, *Lyne A. M.*	GQ 205294	GQ 205230	GQ 205168

	2035 (CBG)			
P. pelinos Rye	Australia: Western Australia, *B. J. Lepschi 3448, T. R. Lally and K. L. Brown* (PERTH)	AM 406680[3]/ AM 407410[3]	AM 167535	AM 162497
P. petrophila Meisn.	Australia: South Australia, *Hill K. 2130* (CANB)	-	GQ 205223	-
P. “pipinui” (C.J.Burrows, in preparation, 2009)	New Zealand, *P. J. de Lange 38523(=AK230901)* (CANU)	GQ 205336	GQ 205274	GQ 205214
P. poppelwellii Petrie	New Zealand, *D. R. Given, J. Anderson 38866* (CANU)	GQ 205340	GQ 205278	-
P. prostrata Willd.	New Zealand, *C. J. Burrows 38853* (CANU)	GQ 205347	GQ 205284	-
P. pseudolyallii Allan	New Zealand, *C. J. Burrows 32660*	GQ 205334	GQ 205272	GQ 205212

	(CANU)			
P. pygmaea Meisn.	Australia: Tasmania *Chase 6360* (K)	AJ 308669[2]	AJ 297230[2]	AJ 744922[2]
P. serpyllifolia subsp. *occidentalis* Rye	Australia: Western Australia, *Wilson P. G.7697* (CANB)	-	GQ 205226	GQ 205165
P. spicata R.Br.	Australia: New South Wales, *Beesley P. 1112* (CBG)	FJ 572677	FJ 572807	FJ 572713
P. spiculigera var. *spiculigera* F.Muell.	Australia: Western Australia, *Wilson P. G.10026* (MEL)	-	GQ 205237	GQ 205175
P. spiculigera var. *thesioides* (S.Moore) Rye	Australia: Western Australia, *J. Docherty 130* (PERTH)	-	AM 167536	AM 162500
P. spinescens subsp. *pubiflora* Rye	Australia: Victoria, *Walsh N. G.6348* (MEL)	GQ 205296	GQ 205232	GQ 205170
P. spinescens subsp. *spinescens* Rye	Australia: Victoria, *Stajsic V.3343* (CANB)	GQ 205295	GQ 205231	GQ 205169

P. suteri Kirk	New Zealand, *C. J. Burrows 38867* (CANU)	FJ 572702	FJ 572834	-
P. “tapo” (C.J.Burrows, in preparation, 2009)	New Zealand, *C. J. Burrows 38842* (CANU)	GQ 205330	GQ 205269	GQ 205209
P. “te mata” (C.J.Burrows, in preparation, 2009)	New Zealand, *C. J. Burrows 38808* (CANU)	GQ 205346	GQ 205283	GQ 205221
P. “tongariro” (C.J.Burrows, in preparation, 2009)	New Zealand, *C. J. Burrows 38841* (CANU)	GQ 205333	GQ 205271	GQ 205211
P. traversii Hook.f.	New Zealand, *C. J. Burrows 38835* (CANU)	*	-	GQ 205222
P. “turakina” (C.J.Burrows, in preparation, 2009)	New Zealand, *C. J. Burrows 38863* (CANU)	GQ 205339	GQ 205277	*
P. “waipara” (C.J.Burrows, in preparation,	New Zealand, *C. J. Burrows 38839*	GQ 205344	GQ 205282	GQ 205219

2009)	(CANU)			
P. “waitaha” (C.J.Burrows, in preparation, 2009)	New Zealand, *C. J. Burrows 38830* (CANU)	GQ 205335	GQ 205273	GQ 205213
Section *Stipostachys* Rye				
P. decora Domin	Australia: Queensland, *Purdie R. W. 5905* (CANB)	FJ 572695	FJ 572827	FJ 572733
P. haematostachya F.Muell.	Australia: Queensland, *Lepschi B. J. 1202* (CANB)	FJ 572695	FJ 572827	FJ 572733
P. holroydii F.Muell.	Australia: Western Australia, *S. van Leeuwen 3769* (PERTH)	AM 406687	AM 167539	AM 162496
Thecanthes Wikstr.				
T. concreta (F.Muell.) Rye	Australia: Western Australia, *Cowie I. D. 8563* (MEL)	FJ 572708	FJ 572839	FJ 572743

T. cornucopiae (M.Vahl) Wikstr.	Australia: Queensland, *Forster P. I. 23116* (MEL)	FJ 572706	FJ 572840	FJ 572744
T. filifolia Rye	Australia: Northern Territory, *Cowie I. D. 8702* (MEL)	FJ 572709	FJ 572841	FJ 572745
T. punicea (R.Br.) Wikstr.	Australia: Western Australia, *T. Handasyde TH99 488* (PERTH)	AM 406684[3]	AM 167540	AM 162502
T. sanguinea (F.Muell.) Rye	Australia: Western Australia, *A. A. Mitchell 3945* (PERTH)	AM 406685[3]	FJ 572838	AM 162503
Others				
***Dais* Royen ex L.**				
D. cotinifolia L.	Africa: Southern Africa, *Chase 1381* (K)	AJ 308644[2]	AJ 297234[2]	AJ 744928[3]
***Daphne* L.**				
D. mezereum L.	Europe, *Chase 6357* (K)	AJ 308645[2]	AJ 297233[2]	AJ 744931[3]

***Diarthron* Turcz.**				
D. vesiculosum Endl.	Europe, Asia, *Merton 3960* (K)	AJ 308646[2]	AM 398181[3]	-
***Dirca* L.**				
D. palustris L.	Central North America, *Horn 12584* (NBYC)	-	U 26322[5]	AM 159528[3]
***Drapetes* Lam.**				
D. muscoides Pers. (=*Drapetes muscosus* Lam.)	South America, Borneo, New Guinea, Fuegia and Falkland Islands, *Kubitzki & Feuerer 99-34* (HBG)	-	AJ 297237[2]	AM 159529[3]
***Edgeworthia* Meisn.**				
E. chrysantha Lindl.	China, Japan, *Chase 6338* (K)	AJ 308649[2]	AJ 297920[2]	AJ 744932[3]
***Gnidia* L.**				
G. bojeriana Baill.	Madagascar: Antananarivo, *Roger et al.*	AM 404224[3]	AM 162507[3]	AM 159511[3]

	183 (MO)			
G. caniflora Meisn.	South Africa, *Fourcade 5580* (PRE)	AM 404223[3]	AM 396993[3]	-
G. decaryana Leandri	Madagascar, *Roger et al. 108* (MO)	AJ 745153[3]	AJ 745179[3]	AJ 744926[3]
G. denudata Lindl.	South Africa: Western Cape, *Beaumont s.n.* (NU)	AJ 308670[2]	AJ 295266[2]	AM 159514[3]
G. fastigiata Rendle	Africa: Uganda, Kenya, Tanzania, Zimbabwe and Malawi, *Hilliard and Burt 6142* (NU)	AJ 308650[2]	AM 162513[3]	-
G. humilis Meisn.	South Africa: Western Cape, *Mark Johns s.n.* (Kogelberg Reserve Field Herbarium)	AM 404236[3]	AM 162510[3]	AM 159517[3]
G. juniperifolia cf. Lam.	South Africa: Western Cape, *Mark Johns s.n.* (Kogelberg Reserve Field Herbarium)	AM 404239[3]	AM 397276[3]	-
G. pilosa Burtt Davy (=*Englerodaphne*	South Africa: Cape region, *Beaumont s.n.*	AJ 308651[2]	AJ 295264[2]	-

pilosa Burtt Davy)	(NU)			
G. setosa Wikstr.	South Africa: Western Cape, *J. Hutchinson 519* (GRA)	AM 404296[3]	AM 162520[3]	AM 159524[3]
***Kelleria* Endl.**				
Kelleria dieffenbachii (Hook.) Endl.	Tasmania: Ben Lomond National Park, *Davies, F.E. 1263* (CBG)	FJ 57270	-	FJ 572746
***Lachnaea* L.**				
L. aurea Eckl. & Zeyh. ex Meisn.	South Africa: Western Cape, *Aggenbash s.n.* (NBG)	AJ 697828[1]	AJ 697781[1]	AJ 745737[1]
L. axillaris Meisn.	South Africa: Western Cape, *Snijman 1871* (NBG)	AJ 308671[1]	AJ 297129[1]	AJ 745742[1]
***Ovidia* Raf.**				
O. andina Meisn.	Temperate South America, *Kubitzki and*	-	AJ 297222[1]	AM 159530[1]

	Feuerer 99-42 (HBG)			
Passerina **L.**				
P. ericoides L.	South Africa: Western Cape, *Bredenkamp 962* (PRE)	AM 404359[4]	AM 162529[4]	AM 158927[4]
P. montivaga Bredenk. & A.E.van Wyk	Southern Africa: South Africa, Swaziland, Mozambique and Zimbabwe, *Van Wyk 2586* (PRE)	AM 404361[4]	AM 162531[4]	AM 158930[4]
Peddiea **Harv.**				
P. africana Harv.	Tanzania, Morogoro district, *Chase 6330* (K)	AJ 308662[2]	AJ 297227[2]	AJ 744921[3]
P. involucrata Baker	Madagascar: Antananarivo Ambohitantely, *Roger et al. 121* (MO)	AJ 745151[3]	AJ 745176[3]	AJ 744920[3]
Phaleria **Jack**				

P. capitata Jack	Indomalesia and Western Pacific (Eurasia), *Chase 1383* (K)	AM 308661[2]	AJ 297228[2]	-
***Stellera* Turcz.**				
S. chamaejasme L.	Nepal to China, *Chase 5530* (K)	AJ 308657[2]	AJ 295262[2]	-
***Stephanodaphne* Baill.**				
S. cuspidata (Leandri) Leandri	Madagascar: Prov. Fianarantsoa, *Roger et al. 68* (MO)	AM 406683[3]	AM 398185[3]	AM 159533[3]
S. oblongifolia Leandri	Madagascar: Prov. Antananarivo, *Roger et al. 127* (MO)	AJ 745152[3]	AJ 745177[3]	AJ 744924[3]
***Struthiola* L.**				
S. ciliata Lam.	South Africa: Western Cape, *Mark Johns s.n.* (Kogelberg Reserve Field Herbarium)	AM 404300[3]	AM 397279[3]	AM 396986[3]
S. stricta Donn	South Africa: Western Cape, *Mark Johns*	AM 404302[3]	AM 398172[3]	AM 396990[3]/

	s.n. (Kogelberg Reserve Field Herbarium)			AM 396991[3]
***Thymelaea* Mill.**				
T. hirsuta Endl.	Spain, *Chase 1883* (K)	AJ 308640[2]	Y 152151[5]	AJ 744930[3]
***Wikstroemia* Spreng.**				
W. gemmata (E.Pritz.) Domke	Southern China to Australia and Pacific, *Chase 3955* (K)	AJ 308641[2]	AJ 295269[2]/ AJ 297223[2]	AJ 744929[3]
W. indica (L.)C.A.Mey.	*Cowie I. 6755* (CANB)	-	*	*

Table 3.3. Sampling in data matrix

	Number of taxa	Number of sequences completed >80%		
		trnL-F	*rbcL*	ITS
Pimelea	132	64	99	98
Thecanthes	5	5	5	4
Other genera	32	22	28	21

3.3 Results

For each separate and combined analysis, Table 3.4 shows the number of aligned positions included in the matrix, number of variable sites and number of phylogenetically informative sites. For each analysis the number of trees, number of steps, consistency index (CI), retention index (RI) and average changes per variable site are also reported. Individual plastid sequence analyses were topologically consistent (results not shown) and were therefore combined into a single 'plastid' dataset. I present the results from the combined plastid analysis (*trnL-F* + *rbcL*) in Fig. 3.7, from ITS analysis in Fig. 3.8 and from the combined molecular data set with all DNA sequences in Fig. 3.9. Because some sequences were completed with uncertainties, my analyses were restricted to the subset of sequences that have been completed for at least 80% of the sequence length for all three DNA regions; these results are presented in Fig. 3.10. For all the trees produced clades II and III follow that of Van der Bank *et al.* (2002) and Beaumont *et al.*, (submitted).

The aligned region of *trnL-F* contained the most variable sites, namely 184 (8.2%) compared to *rbcL* with 107 (7.9%) and ITS with 86 (13%). The number of potentially informative characters were higher for ITS (301; 46%) than for *rbcL* (207; 15%) and *trnL-F* (147; 6.6%). Variable positions changed more rapidly for ITS, 22.8 versus 6.9 (*rbcL*) and 2.8 (*trnL-F;* Table 3.4).

3.3.1. Congruence of data sets

Examination of the individual trees on a node-by-node basis from the separate analyses, specifically with respect to levels of resolution and bootstrap support (Wiens, 1998), did not show any strong incongruences (clades > 85 BP are similar in ITS and plastid analyses or not recovered in one of the analyses; Fig. 3.7 and 3.8). Therefore, I went ahead and combined data sets, both plastid regions and all regions together.

3.3.2. Combined plastid analysis

Analysis of the combined plastid data set included 2445 characters of which 291 (12%) were variable and 354 (14%) parsimony informative (CI=0.61; RI=0.77; TL=1322; Table 3.4; Fig. 3.7). There was high support for the monophyly of Thymelaeoideae minus the outgroups (85 BP). The monophyly of *Passerina*, *Peddiea* and *Stephanodaphne* were highly supported (100 BP; 100 BP; 100 BP respectively), whereas the monophyly of *Lachnaea* was only weakly supported (61 BP). There is a strongly supported sister relationship for *Dais* and *Phaleria* (99 BP). The '*Kelleria* and *Drapetes*' clade, including *Passerina* as its sister, is weakly supported by 51 BP. *Thecanthes* and *Wikstroemia* are embedded within *Pimelea*, but without support (< 50 BP). Although relationships within *Pimelea* are not well resolved, several groupings were observed, for example: 1) *P. lehmanniana* subsp. *lehmanniana* and *P. lehamanniana* subsp. *nervosa* have 84 BP; 2) the clade containing all five species of *Thecanthes* received 88 BP; and 3) *Pimelea brevifolia* subsp. *modesta* and *P. temata* have 90 BP (Fig. 3.7).

3.3.3. ITS analysis

The ITS region comprises 652 characters of which 265 characters were constant, 86 (13%) characters were variable and 301 (46%) were parsimony-informative. The heuristic search found 20 equally parsimonious trees with a tree length of 1958 steps (CI=0.3; RI=0.64; Table 3.4; Fig. 3.8). The root node of Pimeleinae is highly supported (94 BP) with *Thecanthes* nested within *Pimelea* (Fig. 3.8). Other groupings within sub-tribe Pimeleinae include: 1) Some New Zealand species within the clade *'Pimelea orongo-P. buxifolia'* with 56 BP; 2) *'Pimelea cililaris- P. imbricata* var. *petraea'* clade weakly supported with 61 BP; and 3) *Pimelea decora* is sister to *P. haematostachya* with a high support of 100 BP, which in turn are sister to the *Thecanthes* clade (Fig. 3.8). Within Thymelaeoideae moderate to high support was recovered among: 1) the monophyletic genera *Passerina, Lachnaea* and *Struthiola* clades (with 100 BP, 77 BP, 100 BP, respectively); 2) the grouping of *Kelleria dieffenbachii* and *Drapetes muscoides,* which is highly supported with 87 BP; and 3) the monophyly of *Peddiea* and *Stephanodaphne* are also highly supported (both 100 BP; Fig. 3.8).

3.3.4. Combined molecular analysis

As explained above, two analyses were performed for the combined molecular data. The first analysis includes all DNA sequences in spite of uncertainties resulting in the length of some the sequences being less than 80% complete (Fig. 3.9); whereas the second analysis includes only the DNA sequences with 80 - 100% completed sequences for all three regions (Fig. 3.10). The latter will be used for discussion.

3.3.4.1. Molecular analysis including all sequences

Analysis of the combined molecular data set included 3097 characters of which 377 (12%) were variable and 655 (21%) parsimony informative, Fitch analysis produced 50 equally most parsimonious trees (TL=3501; CI=0.41; RI=0.65; Table 3.4; Fig. 3.9).

The root node of sub-tribe Pimeleinae is moderately supported (71 BP), with *Thecanthes* nested within *Pimelea*. Although relationships within Pimeleinae are generally poorly resolved a few clades/groups receive high levels of support (Fig. 3.9): 1) All five species of *Thecanthes* are recovered in a highly supported clade (98 BP), with the strongly supported sister pair of *P. decora* and *P. haematostachya* (99 BP) sister to the *Thecanthes* clade, (80 BP); 2) *Pimelea microcephala* subsp. *microcephala* and *P. spiculigera* var. *spiculigera* are grouped together (97 BP), sister to *P. forrestiana* (87 BP); 3) *Pimelea rara* and *P. hispida* are grouped together (91 BP) with *P. lanata* sister to this grouping (82 BP); and 4) the two sub-species of *Pimelea lehmanniana*, *P. lehmanniana* subsp. *lehmanniana* and *P. lehmanniana* subsp. *nervosa* are highly supported as a sister pair (89 BP) and are recovered as sister to *P. subvillifera* (81 BP). Other sister species which received a high level of support are: *P. ligustrina* subsp. *ligustrina* and *P. ligustrina* sp. (90 BP); *P. rosea* and *P. rosea* subsp *annelsii* (92 BP); *P. sericostachya* subsp. *serichostachya* and *P. serichostachya* subsp. *amabilis* (92 BP); *P. flava* subsp. *flava* and *P. flava* subsp. *dichotoma* (93 BP). The sectional classification proposed by Rye (1988) for *Pimelea* is not upheld.

The monophyly of Thymelaeoideae minus the outgroups is highly supported with 85 BP. The *Lachnaea, Passerina* and *Stephanodaphne* clades are highly supported (97 BP; 100 BP; 100 BP, respectively). There was strong support for the clade *Dais* and *Phaleria* (99 BP) and moderate support for *Ovidia* and *Dirca* (79 BP).

3.3.4.2. Molecular analysis including sequences for 80% and more successfully sequenced

Analysis of the combined molecular data set included 3097 characters of which 381 (12%) were variable and 627 (20%) parsimony informative, Fitch analysis produced 370 equally most parsimonious trees (TL=3265; CI=0.43; RI=0.65; Table 3.4; Fig. 3.10). The figure is divided into three clades named A and B, which comprise sub-tribe Pimeleinae and C, comprising other ingroups and outgroups. In comparison to chapter 2 (Table 2.2) there are more taxa included in this study (Table 3.2).

Clade A is poorly resolved with only a few internal groups receiving low to high support (Fig. 3.10). These groups include: 1) *Pimelea rosea* and *P. rosea* subsp. *annelsii with* 94 BP and sister to this pair is *P. leucantha* with 51 BP; 2) *Pimelea rara* and *P. hispida* are grouped together with 88 BP and *P. lanata* is sister to this grouping with 90 BP; 3) *Pimelea lehmanniana* subsp. *lehmanniana* and *P. lehmanniana* subsp. *nervosa* are highly supported with 90 BP and sister to this group is *P. subvillifera* with 85 BP; 4) *Pimelea erecta* and *P. avonensis* are supported with 87 BP; 5) New Zealand species form a weakly supported clade with 56 BP and within this clade there is high support for the sister relationship between *P. gnidia* and *P. longifolia* (96 BP); and 6) *Pimelea flava* subsp. *flava* and *P. flava* subsp. *dichotoma* are also highly supported with 93 BP (Fig. 3.10).

Clade B is also poorly supported with a few exceptions (see Fig. 3.10). Sister relationships, which receive support of 100 BP are: 1) *Pimelea neoanglica* and *P. pauciflora*; 2) *P. linifolia* subsp. *collina* and *P. linifolia sp.*; 3) *P. spinescens* subsp. *spinescens* and *P. spinescens* subsp. *pubiflora*; and 4) within the *Thecanthes* clade; *Pimelea decora* and *P. haematostachya.* Those with > 90 BP are: 1) *Pimelea axiflora* subsp. *alpina* and *P. axiflora* subsp. *axiflora*; 2) *Pimelea microcephala* subsp. *microcephala* and *P. spiculigera* subsp. *spiculigera*; 3) *Pimelea sericostachya* subsp. *sericostachya* and *Pimelea sericostachya* subsp. *amabilis*; 4) *Pimelea latifolia* subsp. *elliptifolia* and *P. strigosa*. The root node of sub-tribe Pimeleinae is highly supported (85 BP) with *Thecanthes* nested within *Pimelea*. The five species of *Thecanthes* are again recovered as a highly supported clade (100 BP). Once again, the sectional classification proposed by Rye (1988) for *Pimelea* is not upheld.

Clade C shows high support for the sub-family Thymelaeoideae minus the outgroups taxa with 88 BP. The *Lachnaea, Passerina* and *Stephanodaphne* clades were highly supported (98 BP; 100 BP; 100 BP, respectively). There was strong support for the clade *Dais* and *Phaleria* (99 BP) and moderate support for *Ovidia* and *Dirca* (78 BP).

Table 3.4. Paup statistics of analyses obtained from separate and combined datasets

	trnL-F	***rbcL***	**Combined plastids**	**ITS**	**Combined Plastid + ITS (all sequence)**	**Combined Plastid + ITS (>80% sequence)**
No. of taxa	151	157	165	151	167	134
No. of included characters	2241	1347	2445	652	3097	3097
No. of constant characters	767	1033	1800	265	2065	2089
No. of variable sites	184	107	291	86	377	381
	8.2%	7.9%	12%	13%	12%	12%
No. of parsimony informative sites	147	207	354	301	655	627
	6.6%	15%	14%	46%	21%	20%
No. of trees (Fitch)	1130	590	170	20	2	370

No. of steps (Tree length)	521	741	1322	1958	5018	3265
CI	0.79	0.52	0.61	0.3	0.54	0.43
RI	0.84	0.78	0.77	0.64	0.72	0.65
Average number of changes per variable site (number of steps / number of variable sites)	2.8	6.9	4.5	22.8	9.3	8.6

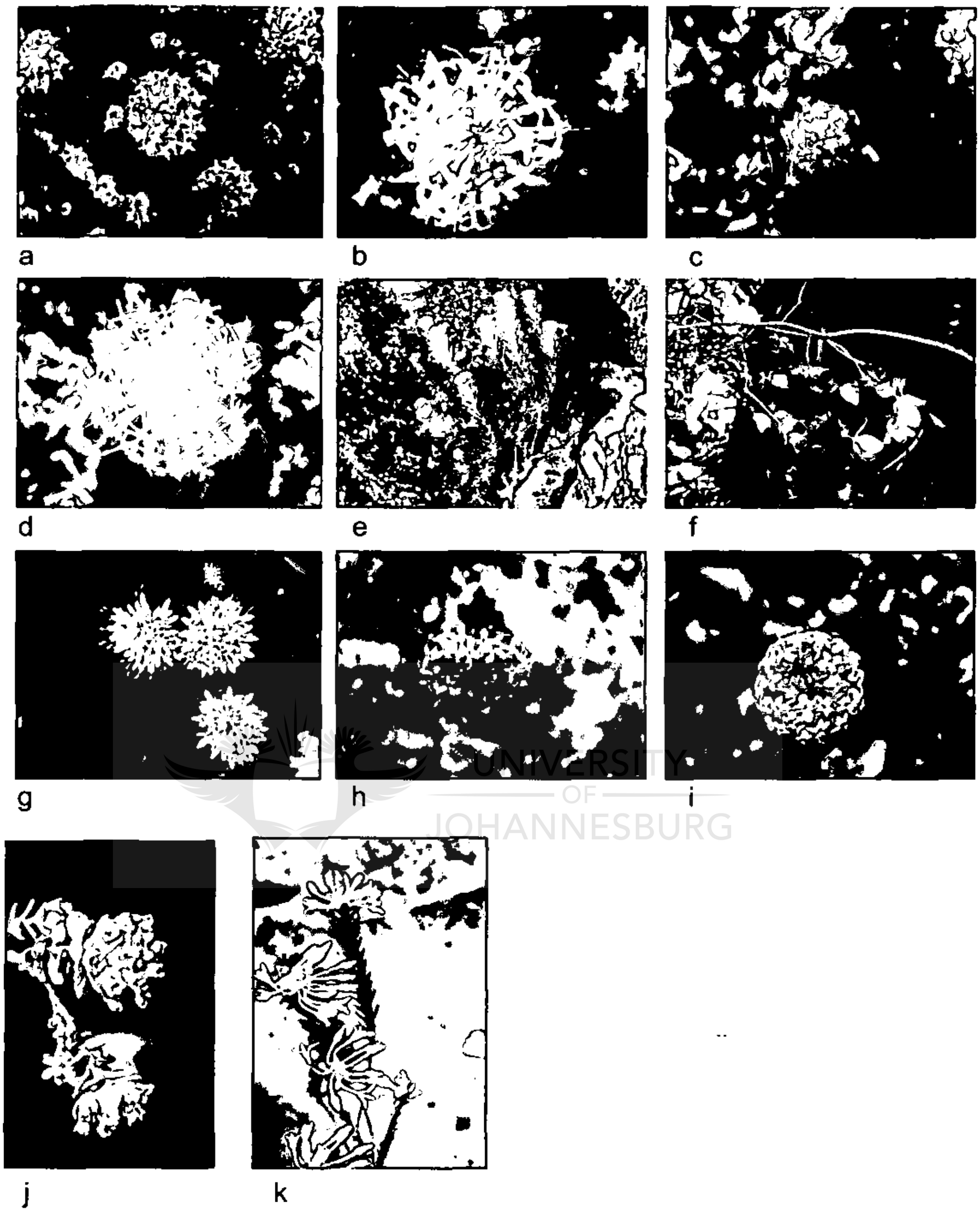


Fig. 3.1. Floral diversity within *Pimelea*: a) *Pimelea ferruginea*; b) *P. ciliata* subsp. *ciliata*; c) *P. argentea*; d) *P. suaveolens* subsp. *suaveolens*; e) *P. graniticola*; f) *P. cracens*; g) *P. erecta*; h) *P. rosea* subsp. *rosea*; i) *P. rosea* subsp. *annelsii*; j) *P. halophila*; k) *P. longiflora* subsp. *eyrei*. (Photos taken by MC Motsi).

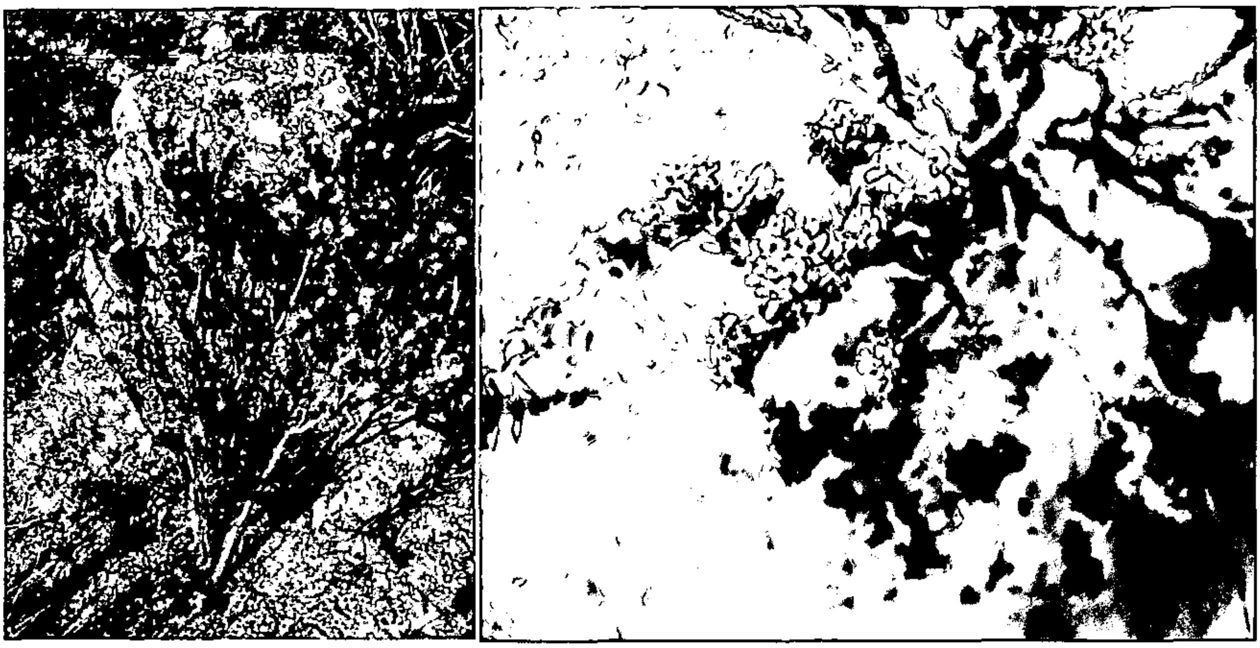


Fig. 3.2. Examples of habitat types for *Pimelea*: a) *P. graniticola* on a granite outcrop; b) *P. halophila* on a salt lake. (Photos taken by MC Motsi).

Fig. 3.3. *Pimelea lanata* is restricted to swamps. (Photo taken by MC Motsi).

Fig. 3.4. A large populations of *Pimelea brevistyla* subsp. *brevistyla*. Following disturbance (in an area cleared of forest). (Photo taken by MC Motsi).

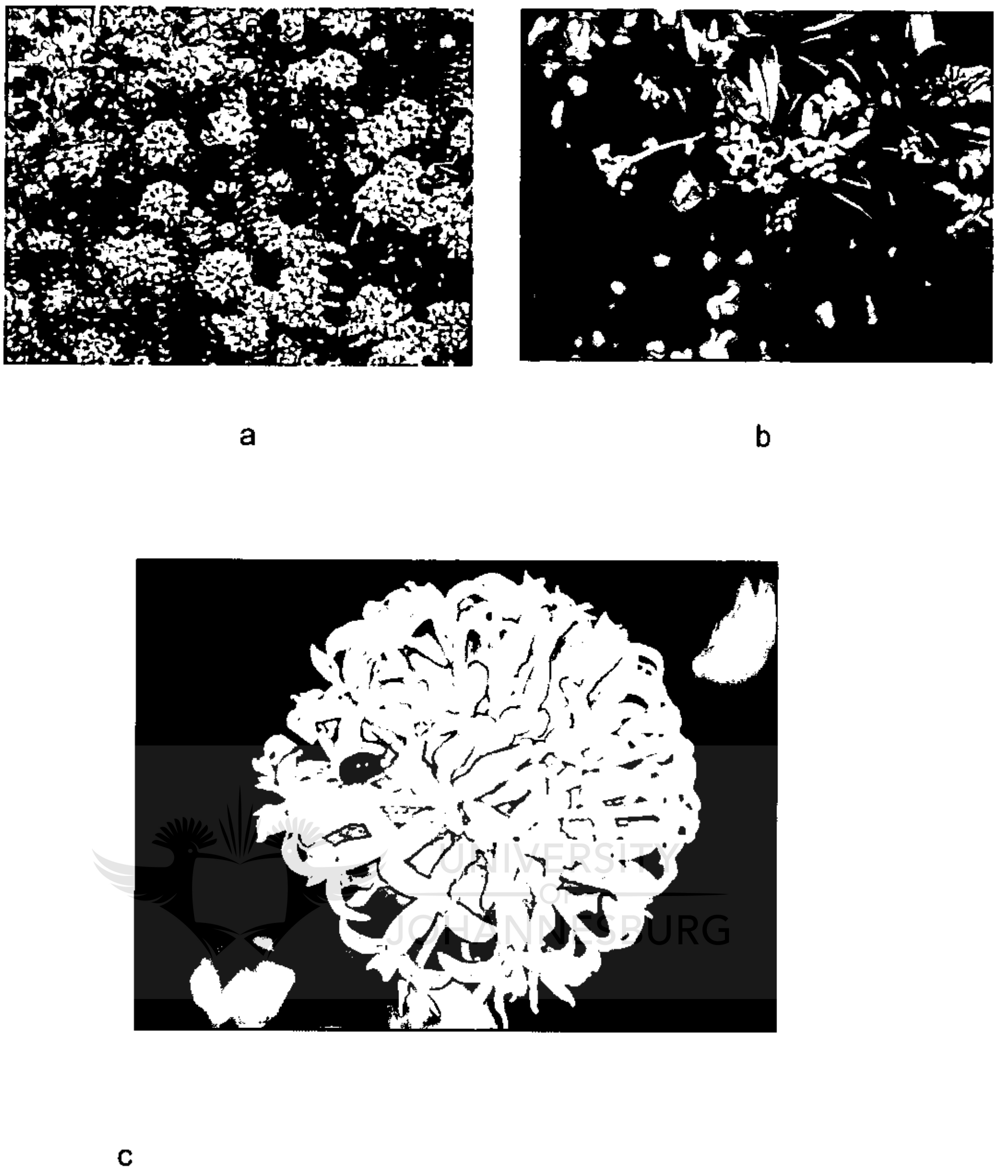
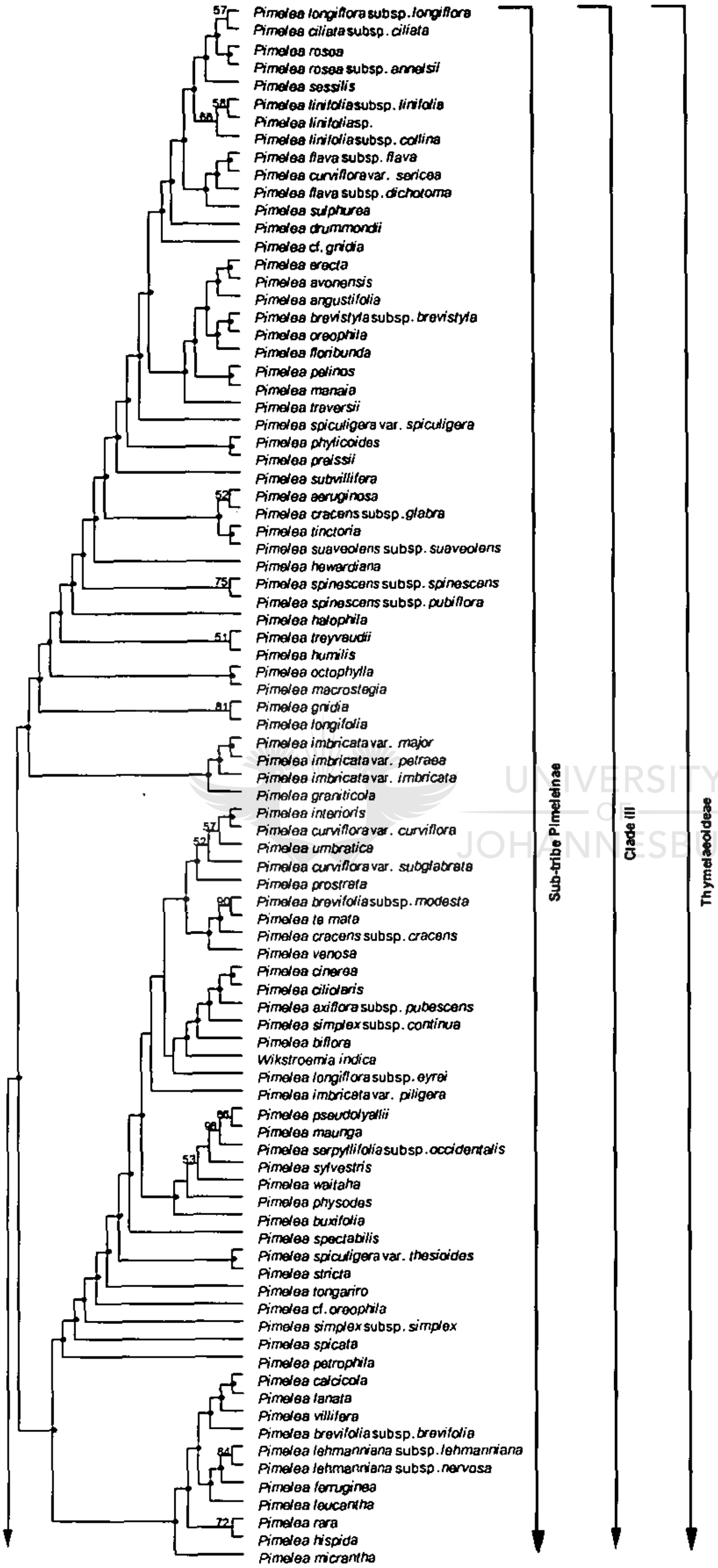


Fig. 3.5. Pollinators in *Pimelea*. e.g. a) butterfly, the Australian Painted Lady (*Vanessa kershawi*), on *P. ferruginea*; b) ant on *P. clavata*; c) beetle on *P. preissii*. (Photos taken by MC Motsi).

Fig. 3.6. *Pimelea physodes* with inflorescences adaptive to bird-pollination. (Photos taken by MC Motsi).

Fig. 3.7 (next two pages). One of the equally parsimonious trees from the combined plastid dataset (*trnLF, rbcL*). Numbers displayed above each branch are bootstraps equal to or greater than 50%. Closed circles on branches indicate groups not present in the Fitch strict consensus tree. The two clades indicated are: II) the non-African taxa and III) the southern and tropical African, southeast Asian and Australasian species plus two New World taxa.


57
Pimelea longiflora subsp. longiflora
Pimelea ciliata subsp. ciliata
Pimelea rosea
Pimelea rosea subsp. annelsii
Pimelea sessilis
58
68
Pimelea linifolia subsp. linifolia
Pimelea linifolia sp.
Pimelea linifolia subsp. collina
Pimelea flava subsp. flava
Pimelea curviflora var. sericea
Pimelea flava subsp. dichotoma
Pimelea sulphurea
Pimelea drummondii
Pimelea cf. gnidia
Pimelea erecta
Pimelea avonensis
Pimelea angustifolia
Pimelea brevistyla subsp. brevistyla
Pimelea oreophila
Pimelea floribunda
Pimelea pelinos
Pimelea manaia
Pimelea traversii
Pimelea spiculigera var. spiculigera
Pimelea phylicoides
Pimelea preissii
Pimelea subvillifera
52
Pimelea aeruginosa
Pimelea cracens subsp. glabra
Pimelea tinctoria
Pimelea suaveolens subsp. suaveolens
Pimelea hewardiana
75
Pimelea spinescens subsp. spinescens
Pimelea spinescens subsp. pubiflora
Pimelea halophila
51
Pimelea treyvaudii
Pimelea humilis
Pimelea octophylla
Pimelea macrostegia
81
Pimelea gnidia
Pimelea longifolia
Pimelea imbricata var. major
Pimelea imbricata var. petraea
Pimelea imbricata var. imbricata
Pimelea graniticola
57
52
Pimelea interioris
Pimelea curviflora var. curviflora
Pimelea umbratica
Pimelea curviflora var. subglabrata
Pimelea prostrata
90
Pimelea brevifolia subsp. modesta
Pimelea te mata
Pimelea cracens subsp. cracens
Pimelea venosa
Pimelea cinerea
Pimelea ciliolaris
Pimelea axiflora subsp. pubescens
Pimelea simplex subsp. continua
Pimelea biflora
Wikstroemia indica
Pimelea longiflora subsp. eyrei
Pimelea imbricata var. piligera
86
98
53
Pimelea pseudolyallii
Pimelea maunga
Pimelea serpyllifolia subsp. occidentalis
Pimelea sylvestris
Pimelea waitaha
Pimelea physodes
Pimelea buxifolia
Pimelea spectabilis
Pimelea spiculigera var. thesioides
Pimelea stricta
Pimelea tongariro
Pimelea cf. oreophila
Pimelea simplex subsp. simplex
Pimelea spicata
Pimelea petrophila
Pimelea calcicola
Pimelea lanata
Pimelea villifera
Pimelea brevifolia subsp. brevifolia
84
Pimelea lehmanniana subsp. lehmanniana
Pimelea lehmanniana subsp. nervosa
Pimelea ferruginea
Pimelea leucantha
72
Pimelea rara
Pimelea hispida
Pimelea micrantha
Sub-tribe Pimeleinae
Clade III
Thymelaeoideae

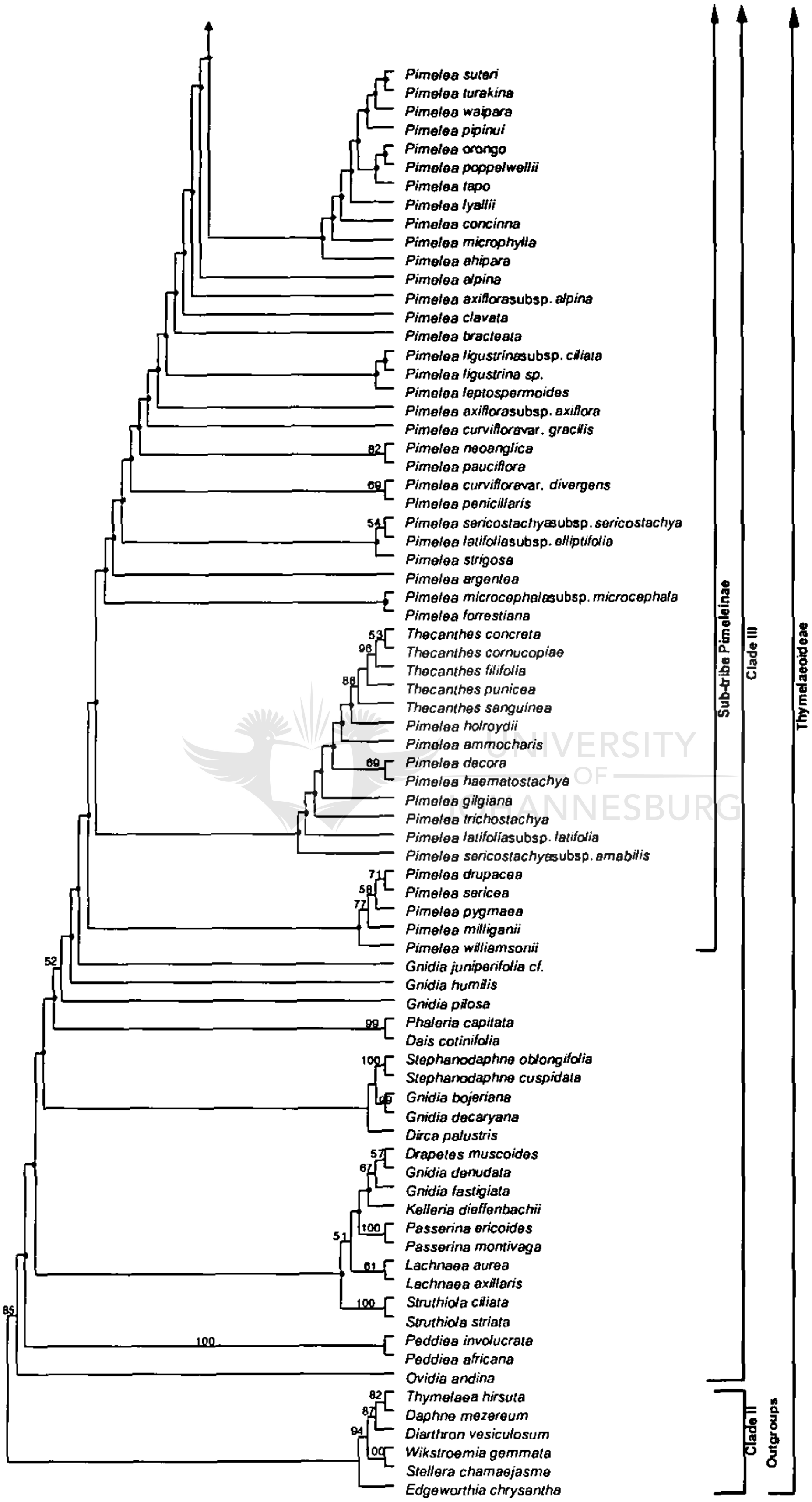
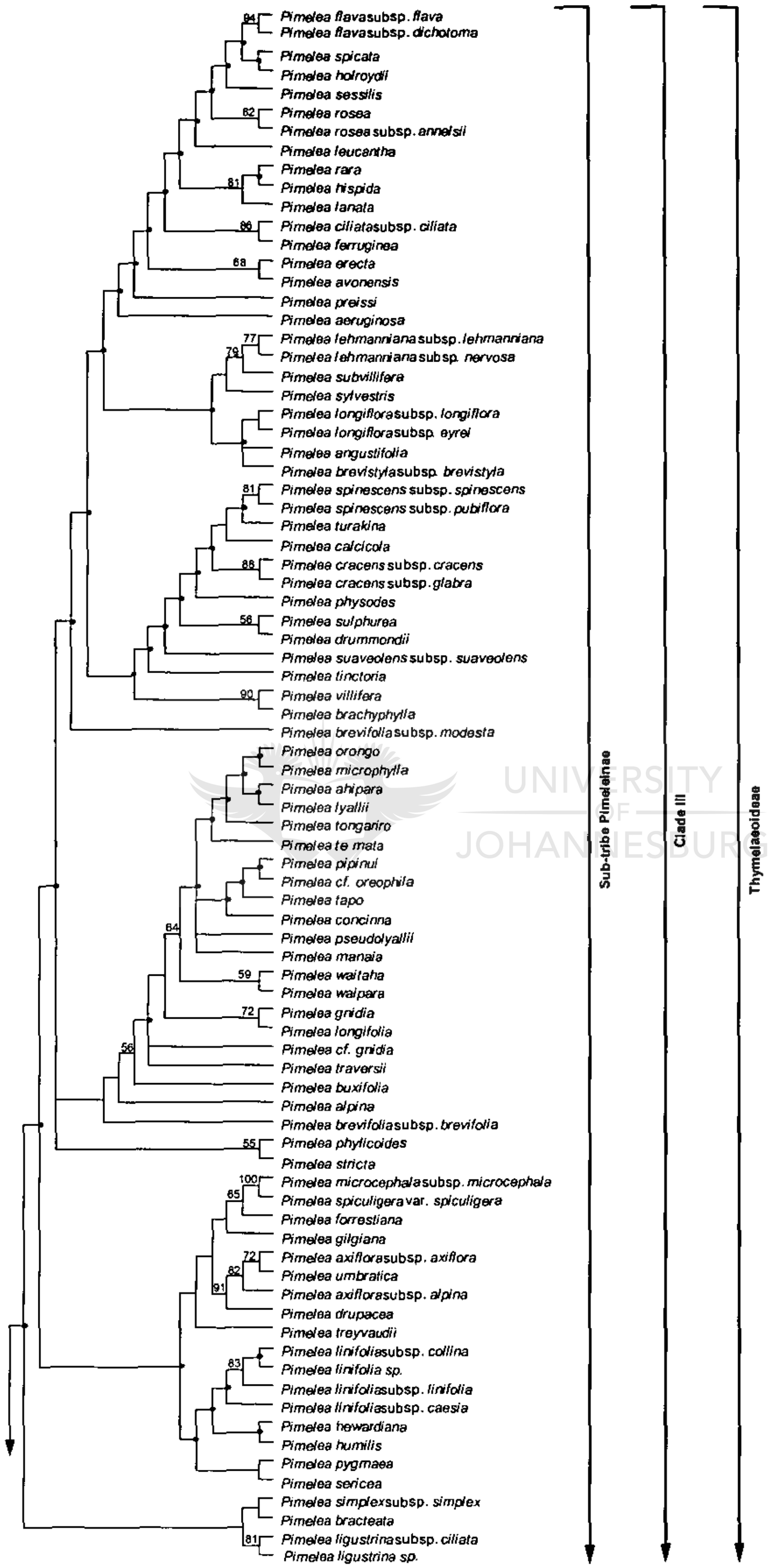

Pimelea suteri
Pimelea turakina
Pimelea waipara
Pimelea pipinui
Pimelea orongo
Pimelea poppelwellii
Pimelea tapo
Pimelea lyallii
Pimelea concinna
Pimelea microphylla
Pimelea ahipara
Pimelea alpina
Pimelea axiflorasubsp. alpina
Pimelea clavata
Pimelea bracteata
Pimelea ligustrinasubsp. ciliata
Pimelea ligustrina sp.
Pimelea leptospermoides
Pimelea axiflorasubsp. axiflora
Pimelea curvifloravar. gracilis
82
Pimelea neoanglica
Pimelea pauciflora
69
Pimelea curvifloravar. divergens
Pimelea penicillaris
54
Pimelea sericostachyasubsp. sericostachya
Pimelea latifoliasubsp. elliptifolia
Pimelea strigosa
Pimelea argentea
Pimelea microcephalasubsp. microcephala
Pimelea forrestiana
53
Thecanthes concreta
96
Thecanthes cornucopiae
Thecanthes filifolia
88
Thecanthes punicea
Thecanthes sanguinea
Pimelea holroydii
Pimelea ammocharis
69
Pimelea decora
Pimelea haematostachya
Pimelea gilgiana
Pimelea trichostachya
Pimelea latifoliasubsp. latifolia
Pimelea sericostachyasubsp. amabilis
71
Pimelea drupacea
58
Pimelea sericea
77
Pimelea pygmaea
Pimelea milliganii
Pimelea williamsonii
52
Gnidia juniperifolia cf.
Gnidia humilis
Gnidia pilosa
99
Phaleria capitata
Dais cotinifolia
100
Stephanodaphne oblongifolia
Stephanodaphne cuspidata
96
Gnidia bojeriana
Gnidia decaryana
Dirca palustris
57
Drapetes muscoides
67
Gnidia denudata
Gnidia fastigiata
Kelleria dieffenbachii
100
Passerina ericoides
51
Passerina montivaga
61
Lachnaea aurea
Lachnaea axillaris
100
Struthiola ciliata
Struthiola striata
85
100
Peddiea involucrata
Peddiea africana
Ovidia andina
82
Thymelaea hirsuta
87
Daphne mezereum
94
Diarthron vesiculosum
100
Wikstroemia gemmata
Stellera chamaejasme
Edgeworthia chrysantha
Sub-tribe Pimeleinae
Clade III
Thymelaeoideae
Clade II
Outgroups

Fig. 3.8 (next two pages). One of the equally parsimonious trees from the ITS dataset. Numbers displayed above each branch are bootstraps equal to or greater than 50%. Closed circles on branches indicate groups not present in the Fitch strict consensus tree. The two clades indicated are: II) the non-African taxa and III) the southern and tropical African, southeast Asian and Australasian species plus two New World taxa.


94
Pimelea flava subsp. flava
Pimelea flava subsp. dichotoma
Pimelea spicata
Pimelea holroydii
Pimelea sessilis
62
Pimelea rosea
Pimelea rosea subsp. annelsii
Pimelea leucantha
Pimelea rara
81
Pimelea hispida
Pimelea lanata
86
Pimelea ciliata subsp. ciliata
Pimelea ferruginea
68
Pimelea erecta
Pimelea avonensis
Pimelea preissi
Pimelea aeruginosa
77
Pimelea lehmanniana subsp. lehmanniana
79
Pimelea lehmanniana subsp. nervosa
Pimelea subvillifera
Pimelea sylvestris
Pimelea longiflora subsp. longiflora
Pimelea longiflora subsp. eyrei
Pimelea angustifolia
Pimelea brevistyla subsp. brevistyla
81
Pimelea spinescens subsp. spinescens
Pimelea spinescens subsp. pubiflora
Pimelea turakina
Pimelea calcicola
88
Pimelea cracens subsp. cracens
Pimelea cracens subsp. glabra
Pimelea physodes
56
Pimelea sulphurea
Pimelea drummondii
Pimelea suaveolens subsp. suaveolens
Pimelea tinctoria
90
Pimelea villifera
Pimelea brachyphylla
Pimelea brevifolia subsp. modesta
Pimelea orongo
Pimelea microphylla
Pimelea ahipara
Pimelea lyallii
Pimelea tongariro
Pimelea te mata
Pimelea pipinui
Pimelea cf. oreophila
Pimelea tapo
Pimelea concinna
64
Pimelea pseudolyallii
Pimelea manaia
59
Pimelea waitaha
Pimelea waipara
72
Pimelea gnidia
Pimelea longifolia
56
Pimelea cf. gnidia
Pimelea traversii
Pimelea buxifolia
Pimelea alpina
Pimelea brevifolia subsp. brevifolia
55
Pimelea phylicoides
Pimelea stricta
100
Pimelea microcephala subsp. microcephala
65
Pimelea spiculigera var. spiculigera
Pimelea forrestiana
Pimelea gilgiana
72
Pimelea axiflora subsp. axiflora
82
Pimelea umbratica
91
Pimelea axiflora subsp. alpina
Pimelea drupacea
Pimelea treyvaudii
Pimelea linifolia subsp. collina
83
Pimelea linifolia sp.
Pimelea linifolia subsp. linifolia
Pimelea linifolia subsp. caesia
Pimelea hewardiana
Pimelea humilis
Pimelea pygmaea
Pimelea sericea
Pimelea simplex subsp. simplex
Pimelea bracteata
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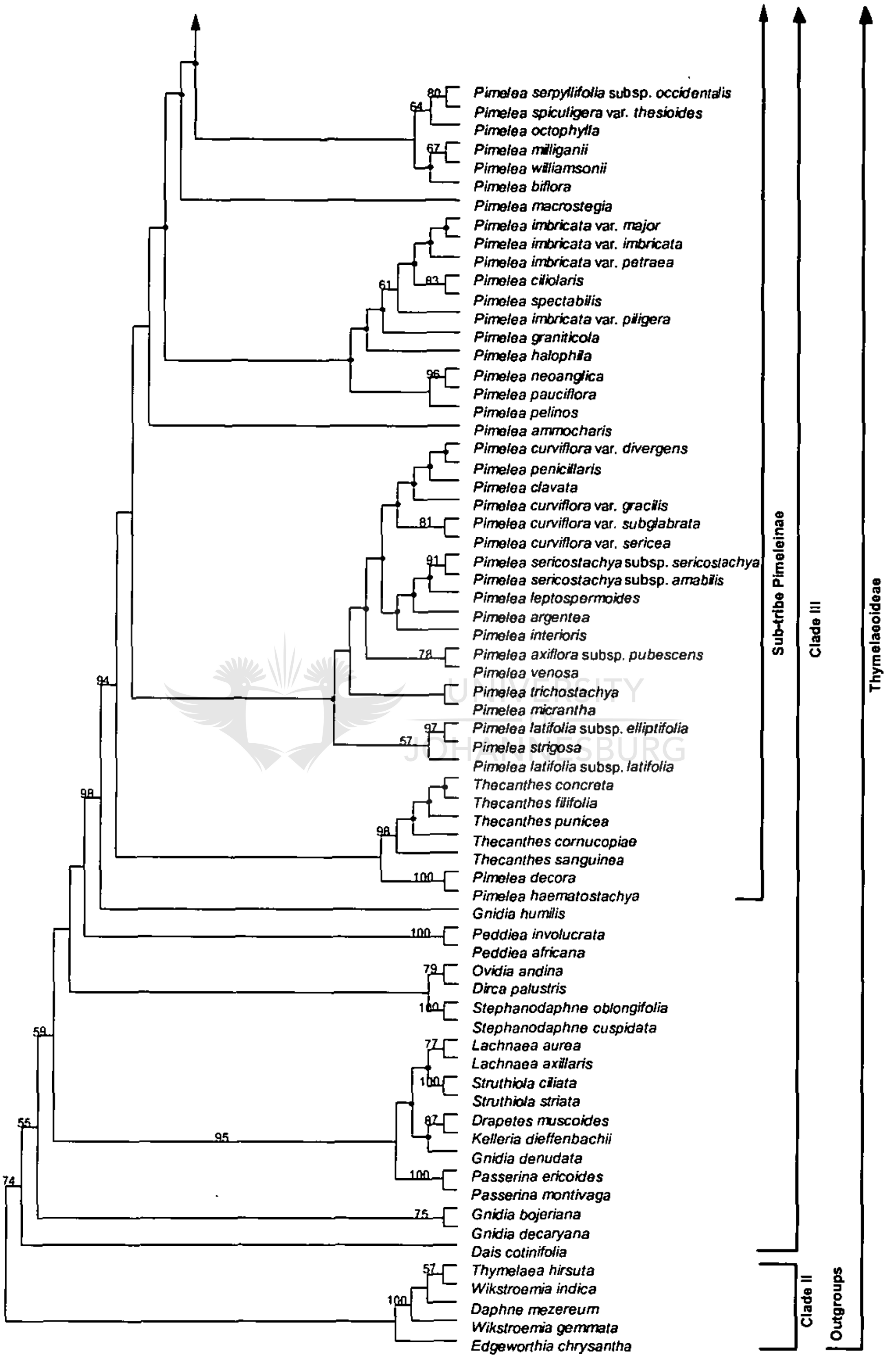
Pimelea ligustrina subsp. ciliata
Pimelea ligustrina sp.
Sub-tribe Pimeleinae
Clade III
Thymelaeoideae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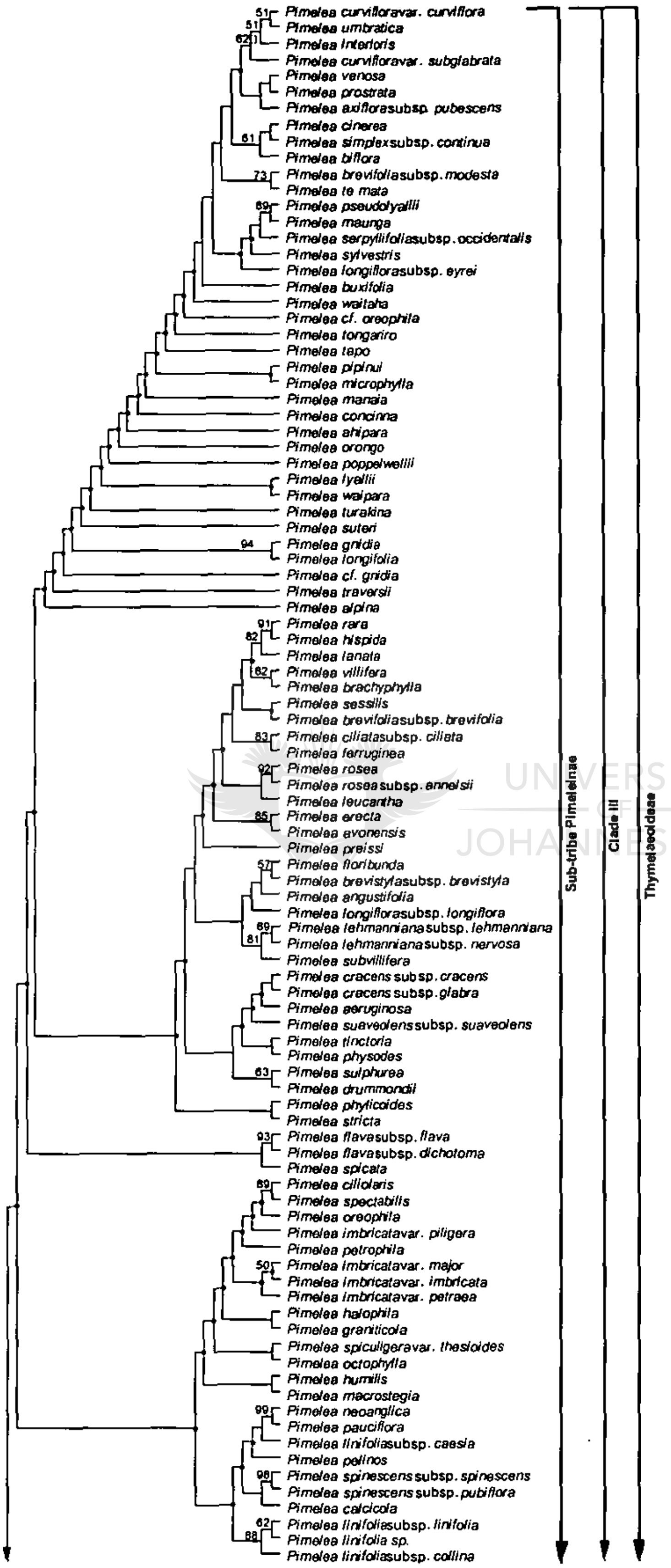
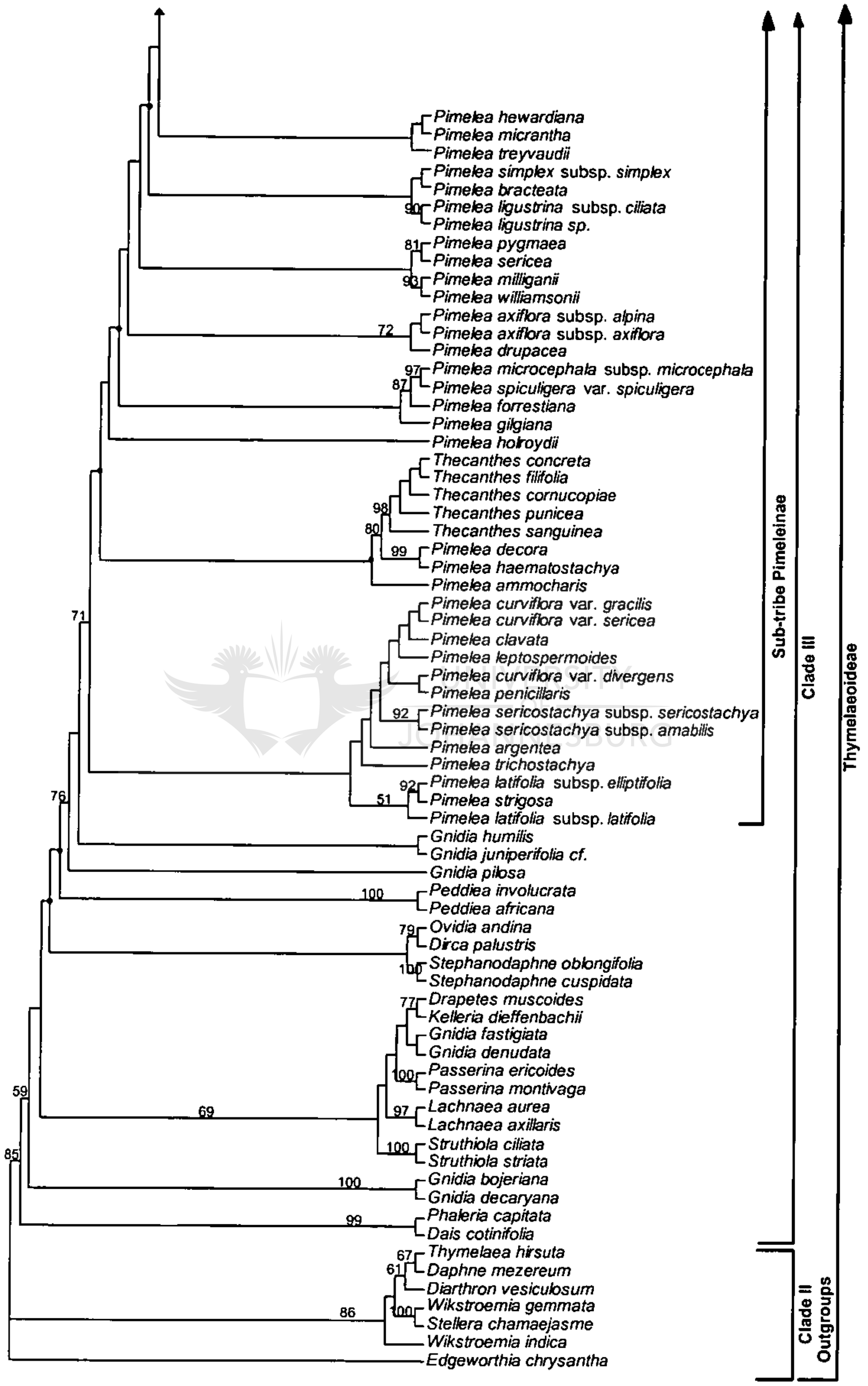
Pimelea williamsonii
Pimelea biflora
Pimelea macrostegia
Pimelea imbricata var. *major*
Pimelea imbricata var. *imbricata*
Pimelea imbricata var. *petraea*
61
83
Pimelea ciliolaris
Pimelea spectabilis
Pimelea imbricata var. *piligera*
Pimelea graniticola
Pimelea halophila
96
Pimelea neoanglica
Pimelea pauciflora
Pimelea pelinos
Pimelea ammocharis
Pimelea curviflora var. *divergens*
Pimelea penicillaris
Pimelea clavata
Pimelea curviflora var. *gracilis*
81
Pimelea curviflora var. *subglabrata*
Pimelea curviflora var. *sericea*
91
Pimelea sericostachya subsp. *sericostachya*
Pimelea sericostachya subsp. *amabilis*
Pimelea leptospermoides
Pimelea argentea
Pimelea interioris
78
Pimelea axiflora subsp. *pubescens*
Pimelea venosa
94
Pimelea trichostachya
Pimelea micrantha
97
Pimelea latifolia subsp. *elliptifolia*
57
Pimelea strigosa
Pimelea latifolia subsp. *latifolia*
Thecanthes concreta
Thecanthes filifolia
98
Thecanthes punicea
98
Thecanthes cornucopiae
Thecanthes sanguinea
100
Pimelea decora
Pimelea haematostachya
Gnidia humilis
100
Peddiea involucrata
Peddiea africana
79
Ovidia andina
Dirca palustris
100
Stephanodaphne oblongifolia
Stephanodaphne cuspidata
59
77
Lachnaea aurea
Lachnaea axillaris
100
Struthiola ciliata
Struthiola striata
55
87
Drapetes muscoides
95
Kelleria dieffenbachii
Gnidia denudata
100
Passerina ericoides
74
Passerina montivaga
75
Gnidia bojeriana
Gnidia decaryana
Dais cotinifolia
57
Thymelaea hirsuta
Wikstroemia indica
100
Daphne mezereum
Wikstroemia gemmata
Edgeworthia chrysantha
Sub-tribe Pimeleinae
Clade III
Thymelaeoideae
Clade II
Outgroups

Fig. 3.9 (next two pages). One of equally parsimonious trees from the combined molecular data sets. Numbers displayed above each branch are Fitch lengths (DELTRAN optimisation). Values below the branches are bootstrap percentages equal to or higher than 50%. Closed circles on branches indicate groups not present in Fitch strict consensus tree. The two clades indicated are: II) the non-African taxa and III) the southern and tropical African, southeast Asian and Australasian species plus two New World taxa.


51
Pimelea curvifloravar. curviflora
51
Pimelea umbratica
62
Pimelea interioris
Pimelea curvifloravar. subglabrata
Pimelea venosa
Pimelea prostrata
Pimelea axiflorasubsp. pubescens
Pimelea cinerea
61
Pimelea simplexsubsp. continua
Pimelea biflora
73
Pimelea brevifoliasubsp. modesta
Pimelea te mata
69
Pimelea pseudolyallii
Pimelea maunga
Pimelea serpyllifoliasubsp. occidentalis
Pimelea sylvestris
Pimelea longiflorasubsp. eyrei
Pimelea buxifolia
Pimelea waitaha
Pimelea cf. oreophila
Pimelea tongariro
Pimelea tapo
Pimelea pipinui
Pimelea microphylla
Pimelea manaia
Pimelea concinna
Pimelea ahipara
Pimelea orongo
Pimelea poppelwellii
Pimelea lyallii
Pimelea waipara
Pimelea turakina
Pimelea suteri
94
Pimelea gnidia
Pimelea longifolia
Pimelea cf. gnidia
Pimelea traversii
Pimelea alpina
91
Pimelea rara
82
Pimelea hispida
Pimelea lanata
62
Pimelea villifera
Pimelea brachyphylla
Pimelea sessilis
Pimelea brevifoliasubsp. brevifolia
83
Pimelea ciliatasubsp. ciliata
Pimelea ferruginea
92
Pimelea rosea
Pimelea roseasubsp. annelsii
Pimelea leucantha
85
Pimelea erecta
Pimelea avonensis
Pimelea preissi
57
Pimelea floribunda
Pimelea brevistylasubsp. brevistyla
Pimelea angustifolia
Pimelea longiflorasubsp. longiflora
69
Pimelea lehmannianasubsp. lehmanniana
81
Pimelea lehmannianasubsp. nervosa
Pimelea subvillifera
Pimelea cracens subsp. cracens
Pimelea cracens subsp. glabra
Pimelea aeruginosa
Pimelea suaveolenssubsp. suaveolens
Pimelea tinctoria
Pimelea physodes
63
Pimelea sulphurea
Pimelea drummondii
Pimelea phylicoides
Pimelea stricta
93
Pimelea flavasubsp. flava
Pimelea flavasubsp. dichotoma
Pimelea spicata
69
Pimelea ciliolaris
Pimelea spectabilis
Pimelea oreophila
Pimelea imbricatavar. piligera
Pimelea petrophila
50
Pimelea imbricatavar. major
Pimelea imbricatavar. imbricata
Pimelea imbricatavar. petraea
Pimelea halophila
Pimelea graniticola
Pimelea spiculigeravar. thesioides
Pimelea octophylla
Pimelea humilis
Pimelea macrostegia
99
Pimelea neoanglica
Pimelea pauciflora
Pimelea linifoliasubsp. caesia
Pimelea pelinos
98
Pimelea spinescenssubsp. spinescens
Pimelea spinescenssubsp. pubiflora
Pimelea calcicola
62
Pimelea linifoliasubsp. linifolia
88
Pimelea linifolia sp.
Pimelea linifoliasubsp. collina
Sub-tribe Pimeleinae
Clade III
Thymelaeoideae


Pimelea hewardiana
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Pimelea treyvaudii
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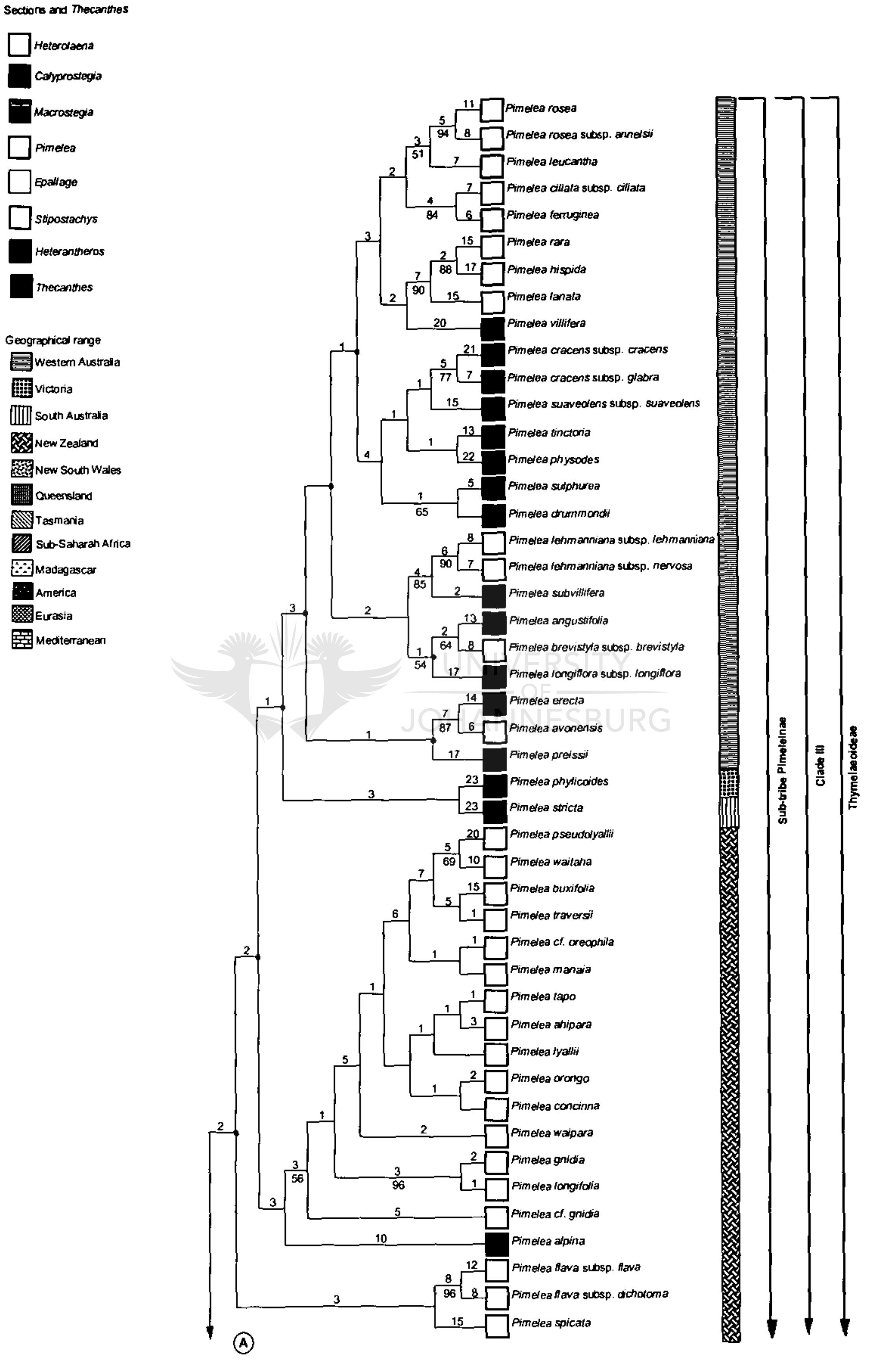
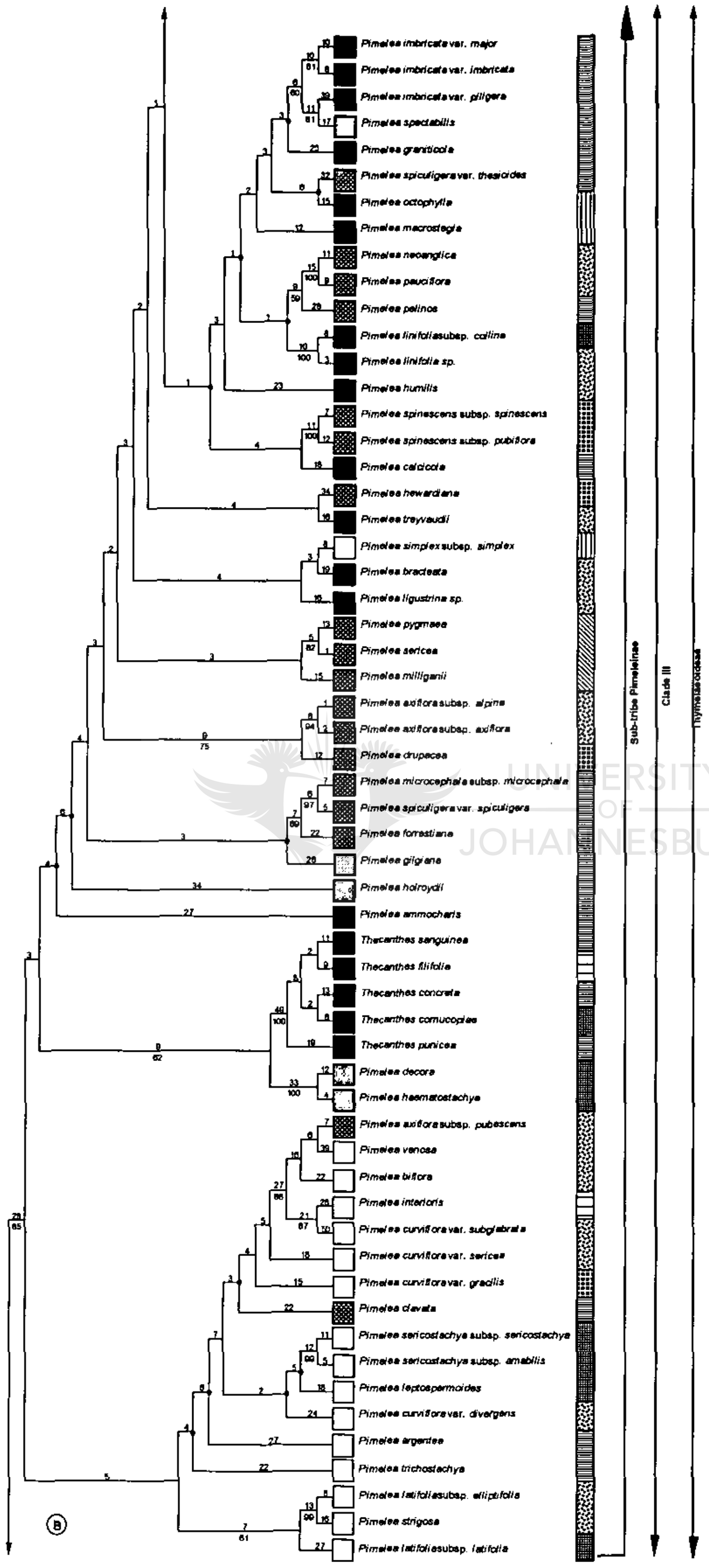
Pimelea bracteata
90
Pimelea ligustrina subsp. ciliata
Pimelea ligustrina sp.
81
Pimelea pygmaea
Pimelea sericea
93
Pimelea milliganii
Pimelea williamsonii
Pimelea axiflora subsp. alpina
72
Pimelea axiflora subsp. axiflora
Pimelea drupacea
97
Pimelea microcephala subsp. microcephala
87
Pimelea spiculigera var. spiculigera
Pimelea forrestiana
Pimelea gilgiana
Pimelea holroydii
Thecanthes concreta
Thecanthes filifolia
Thecanthes cornucopiae
98
Thecanthes punicea
80
Thecanthes sanguinea
99
Pimelea decora
Pimelea haematostachya
Pimelea ammocharis
71
Pimelea curviflora var. gracilis
Pimelea curviflora var. sericea
Pimelea clavata
Pimelea leptospermoides
Pimelea curviflora var. divergens
Pimelea penicillaris
92
Pimelea sericostachya subsp. sericostachya
Pimelea sericostachya subsp. amabilis
Pimelea argentea
Pimelea trichostachya
92
Pimelea latifolia subsp. elliptifolia
76
51
Pimelea strigosa
Pimelea latifolia subsp. latifolia
Gnidia humilis
Gnidia juniperifolia cf.
Gnidia pilosa
100
Peddiea involucrata
Peddiea africana
79
Ovidia andina
Dirca palustris
100
Stephanodaphne oblongifolia
Stephanodaphne cuspidata
77
Drapetes muscoides
Kelleria dieffenbachii
Gnidia fastigiata
Gnidia denudata
100
Passerina ericoides
Passerina montivaga
59
69
97
Lachnaea aurea
Lachnaea axillaris
100
Struthiola ciliata
Struthiola striata
85
100
Gnidia bojeriana
Gnidia decaryana
99
Phaleria capitata
Dais cotinifolia
67
Thymelaea hirsuta
61
Daphne mezereum
Diarthron vesiculosum
100
Wikstroemia gemmata
86
Stellera chamaejasme
Wikstroemia indica
Edgeworthia chrysantha
Sub-tribe Pimeleinae
Clade III
Thymelaeoideae
Clade II
Outgroups

Fig. 3.10 (next three pages). One of 370 equally parsimonious trees from the combined molecular data sets for number of sequences completed at 80%. Numbers displayed above each branch are Fitch lengths (DELTRAN optimisation). Values below the branches are bootstrap percentages equal to or higher than 50%. Closed circles on branches indicate groups not present in Fitch strict consensus tree. The two clades indicated are: II) the non-African taxa and III) the southern and tropical African, southeast Asian and Australasian species plus two New World taxa.


Sections and *Thecanthes*
Heterolaena
Calyptrostegia
Macrostegia
Pimelea
Epallage
Stipostachys
Heterantheros
Thecanthes
Geographical range
Western Australia
Victoria
South Australia
New Zealand
New South Wales
Queensland
Tasmania
Sub-Saharah Africa
Madagascar
America
Eurasia
Mediterranean
Pimelea rosea
Pimelea rosea subsp. *annelsii*
Pimelea leucantha
Pimelea ciliata subsp. *ciliata*
Pimelea ferruginea
Pimelea rara
Pimelea hispida
Pimelea lanata
Pimelea villifera
Pimelea cracens subsp. *cracens*
Pimelea cracens subsp. *glabra*
Pimelea suaveolens subsp. *suaveolens*
Pimelea tinctoria
Pimelea physodes
Pimelea sulphurea
Pimelea drummondii
Pimelea lehmanniana subsp. *lehmanniana*
Pimelea lehmanniana subsp. *nervosa*
Pimelea subvillifera
Pimelea angustifolia
Pimelea brevistyla subsp. *brevistyla*
Pimelea longiflora subsp. *longiflora*
Pimelea erecta
Pimelea avonensis
Pimelea preissii
Pimelea phylicoides
Pimelea stricta
Pimelea pseudolyallii
Pimelea waitaha
Pimelea buxifolia
Pimelea traversii
Pimelea cf. *oreophila*
Pimelea manaia
Pimelea tapo
Pimelea ahipara
Pimelea lyallii
Pimelea orongo
Pimelea concinna
Pimelea waipara
Pimelea gnidia
Pimelea longifolia
Pimelea cf. *gnidia*
Pimelea alpina
Pimelea flava subsp. *flava*
Pimelea flava subsp. *dichotoma*
Pimelea spicata
Sub-tribe Pimeleinae
Clade III
Thymelaeoideae
A


Pimelea imbricata var. major
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Pimelea imbricata var. piligera
Pimelea spectabilis
Pimelea graniticola
Pimelea spiculigera var. thesioides
Pimelea octophylla
Pimelea macrostegia
Pimelea neoanglica
Pimelea pauciflora
Pimelea pelinos
Pimelea linifolia subsp. collina
Pimelea linifolia sp.
Pimelea humilis
Pimelea spinescens subsp. spinescens
Pimelea spinescens subsp. pubiflora
Pimelea calcicola
Pimelea hewardiana
Pimelea treyvaudii
Pimelea simplex subsp. simplex
Pimelea bracteata
Pimelea ligustrina sp.
Pimelea pygmaea
Pimelea sericea
Pimelea milliganii
Pimelea axiflora subsp. alpina
Pimelea axiflora subsp. axiflora
Pimelea drupacea
Pimelea microcephala subsp. microcephala
Pimelea spiculigera var. spiculigera
Pimelea forrestiana
Pimelea gilgiana
Pimelea holroydii
Pimelea ammocharis
Thecanthes sanguinea
Thecanthes filifolia
Thecanthes concreta
Thecanthes cornucopiae
Thecanthes punicea
Pimelea decora
Pimelea haematostachya
Pimelea axiflora subsp. pubescens
Pimelea venosa
Pimelea biflora
Pimelea interioris
Pimelea curviflora var. subglabrata
Pimelea curviflora var. sericea
Pimelea curviflora var. gracilis
Pimelea clavata
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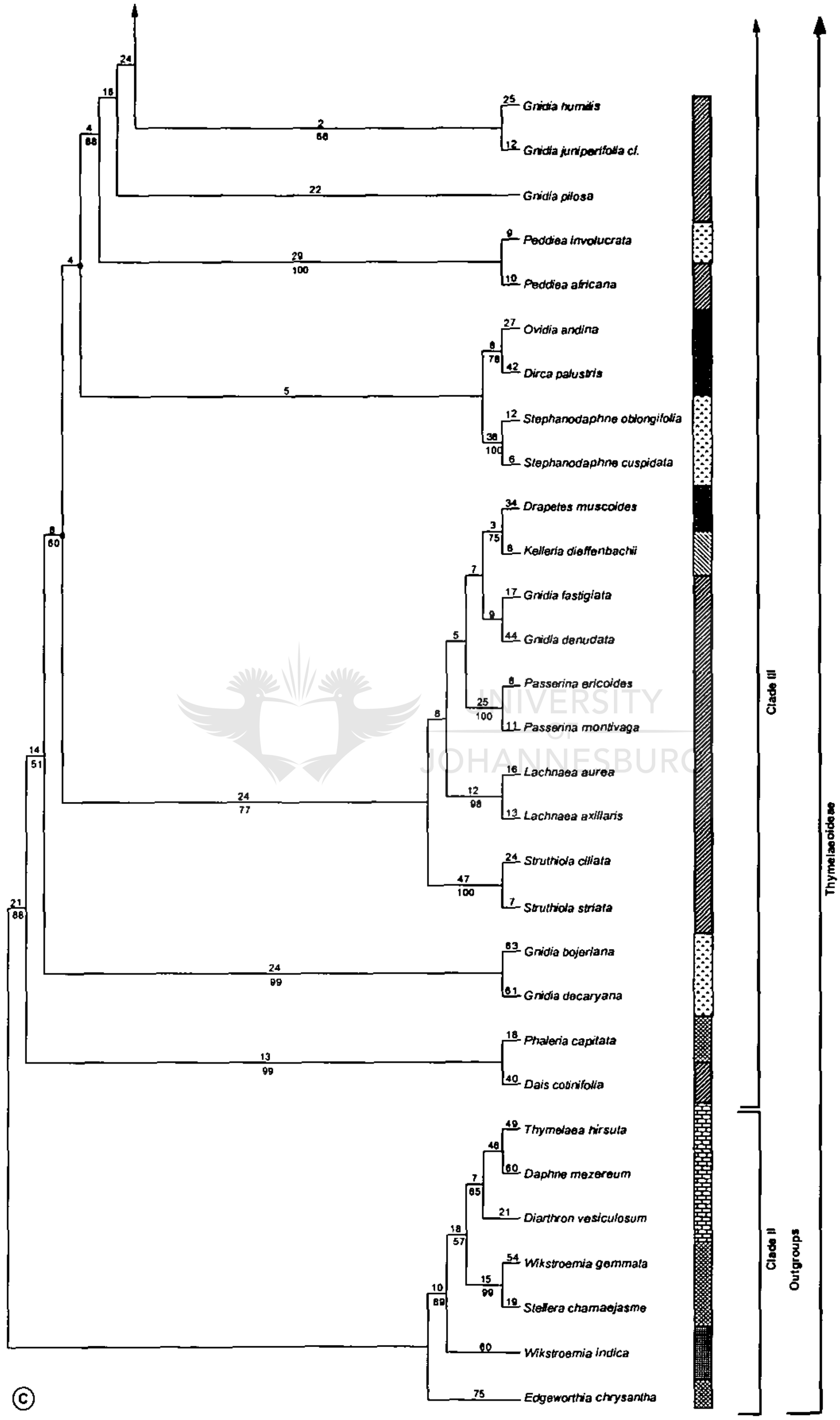
Pimelea sericostachya subsp. amabilis
Pimelea leptospermoides
Pimelea curviflora var. divergens
Pimelea argentea
Pimelea trichostachya
Pimelea latifolia subsp. elliptifolia
Pimelea strigosa
Pimelea latifolia subsp. latifolia
Sub-tribe Pimeleinae
Clade III
Thymelaeoideae
B


Gnidia humilis
Gnidia juniperifolia cf.
Gnidia pilosa
Peddiea involucrata
Peddiea africana
Ovidia andina
Dirca palustris
Stephanodaphne oblongifolia
Stephanodaphne cuspidata
Drapetes muscoides
Kelleria dieffenbachii
Gnidia fastigiata
Gnidia denudata
Passerina ericoides
Passerina montivaga
Lachnaea aurea
Lachnaea axillaris
Struthiola ciliata
Struthiola striata
Gnidia bojeriana
Gnidia decaryana
Phaleria capitata
Dais cotinifolia
Thymelaea hirsuta
Daphne mezereum
Diarthron vesiculosum
Wikstroemia gemmata
Stellera chamaejasme
Wikstroemia indica
Edgeworthia chrysantha
Clade III
Clade II
Outgroups
Thymelaeoideae
c

3.4. Discussion and conclusion

This study has brought a new insight on the sub-tribe Pimeleinea, although there were also problems encountered in attempting to reconstruct this phylogeny to species-level.

Taxa sampling within *Pimelea* was difficult and I regularly had to extract DNA from herbarium material. Though I had access to most of the herbarium material, the problem was how the DNA was preserved and what kinds of chemicals were present in the samples. The method of plant collection and duration of drying the material is important for the survival of the DNA (Savolainen *et al.*, 1995). Some of my materials were oven-dried and the old material had been exposed to pesticides, both of which could have degraded the quality of the DNA. According to Savolainen *et al.* (1995), the presence of certain chemicals on particular species, the development stage and duration of the drying method could hamper the quality of the DNA. Nevertheless, I was able to successfully extract 200 (including doubles) and amplify 137 taxa, but more than 30 failed to produce sequence data.

In spite of all these technical drawbacks and compared to the previous Chapter, a phylogeny was reconstructed with more species sampled across all seven sections and also including more of the New Zealand *Pimelea* species. The results show a relationship between species of sections *Heterolaena* and *Calyprostegia*, as well as *Calyptrostegia* and *Epallage*. As mentioned by Rye (1999) some sections might need to redefined. The molecular data indicate that some species from section *Calyptrostegia* need to be incorporated with either section *Heterolaena* or section *Epallage*. The monotypic section *Macrostegia* would also need to be included within an expanded section *Heterolaena*. New Zealand species apparently all belong in section Pimelea, since it is clear that they form a monophyletic group (supported by 56 BP) but Australian species previously placed in this section may all need to be excluded.

My results using two plastid regions show a general lack of bootstrap support for many branches of the tree. The ITS tree shows better support. When data are combined (Fig. 3.10) there is better resolution in comparison to the other plastid versus nuclear individual trees. For example the monophyly of Thymelaeoideae (minus the outgroups taxa) is highly supported with 85 BP; the same level of support was received in the plastid tree, but in the ITS tree the sub-family was only moderately supported (75 BP). Also, *Ovidia* and *Dirca* are recovered as sisters in the combined and ITS trees, but not in the plastid tree.

However, in the combined dataset the trees still display very short branches. Similar cases of poorly resolved species-level trees were observed in *Brachyglottis* (Asteraceae; Wagstaff and Breitwieser, 2004); *Rheum* (Polygonaceae; Wang *et al.,* 2005); Salicornioideae (Chenopodiaceae; Shepherd *et al.,* 2005); and *Aquilegia* (Ranunculaceae, Hodge, 1997). They indicated recent and rapid radiation, maybe also linked to hybridisation. Hybridisation is known to occur between some pairs of Pimelea species in New Zealand (Burrows, 2008, 2009). In other plant groups there are cases reported of species with low genetic variation (and hence lack resolution in the phylogeny) due to hybridisation, such as in the genera *Afromomum* (Zingiberaceae, Harris *et al.*, 2000; Rangsiruji *et al.*, 2000); *Illicium* (Illiciaceae, Hao *et al.*, 2000); *Lepidium* (Brassicaceae, Mummenhoff *et al.*, 2004); and *Viguiera* (Asteraceae, Schilling *et al.*, 2000).

Nevertheless, the phylogenetic trees (especially the one better supported and resolved in Fig. 3.10) show some interesting phylogeographic patterns. For example, it shows that *Pimelea* (section *Heterolaena*) has radiated extensively in Western Australia (WA), a renowned biodiversity hotspot for Conservation International. It also seems that this WA clade may have radiated from Victoria (V) or South Australia (SA) because the basal lineage comprises *Pimelea phylicoides* (V) and *P. stricta* (SA). Another striking pattern is that all New Zealand species form a single clade. They have reached New Zealand once, most likely from New South Wales (NSW) and then radiated. By contrast, the NSW and V species seem to have multiple origins. Tasmania (T) species have only two origins both from NSW. *Thecanthes* species have originated in Queensland (QLD) and expanded to WA, Northern Territory (NT). Northern Territory seems to have been colonised twice.

In conclusion, the level of genetic variation (at least in the markers used here) has been exceeded by the amount of morphological diversity within the genus *Pimelea*. This would indicate that the genus represents a recent rapid radiation (which I explore further in the next chapter). Nevertheless, some phylogeographic pattern is already observed here. For further research, it would be desirable to have a more complete sampling of the species, including the species from Lord Howe Island and the mountainous parts of New Zealand. To explore cases of hybridisation, I would also suggest the use of other molecular techniques such as microsatellite and Amplified Fragment Length Polymorphism, which will be appropriate as a complement to DNA sequences as used here for the genus *Pimelea*.

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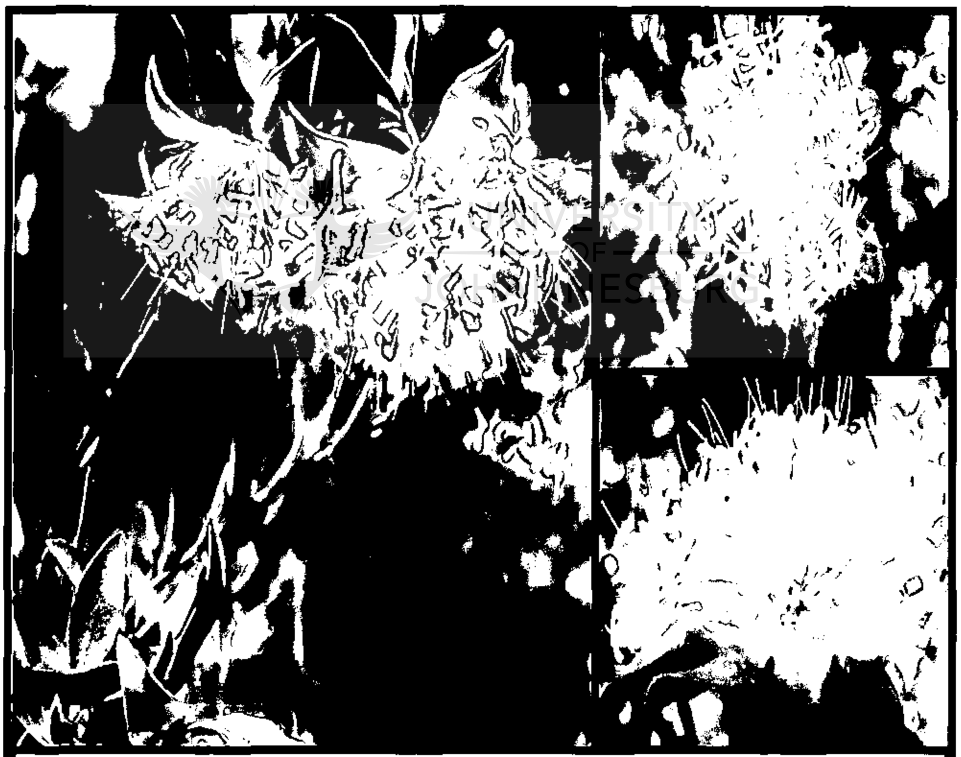
CHAPTER FOUR

BIOGEOGRAPHY OF THYMELAEOIDEAE WITH EMPHASES ON THE AUSTRALIAN GENERA *PIMELEA* AND *THECANTHES* (THYMELAEACEAE)



Pimelea suaveolens Photos:MC Motsi

4.1. Introduction

The distribution pattern and implied biogeographical events of organisms in the southern hemisphere have attracted a lot of attention. The question is how numerous plant groups, with geographical ranges interrupted by thousands of kilometres of open ocean, have reached their current fragmented distributions (de Queiroz, 2005). Since Darwin, and until the 1960s, the predominant answer was via "oceanic dispersal" (Nelson, 1979). Oceanic dispersal begun to lose its appeal during the 1960's and 1970's when evidence of plate-tectonics emerged and offered vicariance as an alternative explanation for plant disjunctions from fragments of Gondwana (Nelson and Platnick, 1981; Wiley, 1988). The use of plate-tectonics has recently been surpassed by the inception of molecular systematics and molecular dating techniques (de Queiroz, 2005).

Oceanic dispersal has progressively overtaken vicariance as the main theory explaining scenarios where sister taxa are separated by an ocean. The genus *Sophora* L. (formerly *Edwardsia*, Leguminosae) serves as a good example. The Gondwanan distribution of *Sophora* suggested vicariance. However, Charles Darwin thought it could have reached its current range by dispersal and wrote in a letter to Joseph Hooker "I believe you are afraid to send me a ripe *Edwardsia* pod for fear I shall float it from N. Zealand to Chile!!!" (Darwin, 1857). The recent molecular data of Hurr *et al.* (1999) support long distance dispersal of *Sophora* rather than vicariance. There are numerous other similar examples. For example, African *Adansonia* L. spp. diverged 5–23 million years ago from other baobabs in Madagascar and Australia (Baum *et al.,* 1998). The oldest estimates of tectonic separation of

Africa, Madagascar and Australia is around 120 million years ago, which implies these baobabs must have dispersed across oceans (Wilford and Brown, 1994). Oceanic dispersal rather than vicariance was also recorded in Malpighiaceae (Davis *et al.,* 2002), Rapateaceae (Givnish *et al.,* 2000), and Atherospermataceae (Renner *et al.,* 2000).

The primary Gondwanan distribution of the current study group within the Thymelaeaceae provides a further opportunity to test these hypotheses. According to Craw *et al.* (1999) the distribution of the genera of the tribe Gnidiea provides a classical example of transoceanic patterns (Indian, Atlantic, or a combination of Indian/Atlantic). Occurring mainly at both ends of the Indian Ocean basin, this tribe comprises *Gnidia* in Africa (especially southern Africa) and India, and three more genera (*Pimelea* Banks & Sol. ex Gaertn., *Thecanthes* Wikstr. and *Kelleria* Endl.) in Borneo, the Philippines, Australasia, and the southwest Pacific (Heads, 1990; 1994). Altogether, the family Thymelaeaceae consists of approximately 45 genera and 800 species widely distributed in temperate and tropical regions (Fig. 4.1). It is however more diverse in the southern than in the northern Hemisphere, and is mainly concentrated in Africa and Australia (Wright, 1915; Peterson, 1978; Heywood, 1993; Schmidt, 1994; Takhtajan, 1997). The family is also represented in the Mediterranean region and on Pacific Islands, with a few members occurring in western, eastern and Southeast Asia as well as North and South America.

According to Raven and Axelrod (1974), the high representation of the family in Australasia, South America, and Africa implies isolation in the Upper Cretaceous. They believe that Thymelaeaceae is among the groups that migrated between Africa and South America during or prior to the Paleocene. The hypothesis that long-distance dispersal may have been far more frequent than previously supposed has led to plant biogeographers using modern molecular tools to re-examine the relative importance of vicariance and dispersal in explaining the classic patterns of worldwide plant disjunction (Givnish and Renner, 2004; Renner, 2005). Hence, the robust estimates of phylogenetic relationships, ages of relevant clade formation, and the geological time sequence of barrier formation are key issues (Sytsma *et al.*, 2004). Thus to test the hypothesis of Raven and Axelrod (1974), we estimated the age of Thymelaeaceae and of clades within Thymelaeoideae with emphases on the Australasian genera *Pimelea* and *Thecanthes* using fossil-calibrated molecular data. It is well known that the fossil record of Thymelaeaceae is poor although fossils are known from the beginning of the Eocene (Krutzsch, 1966; Muller, 1981; Venkatachala *et al.*, 1988).

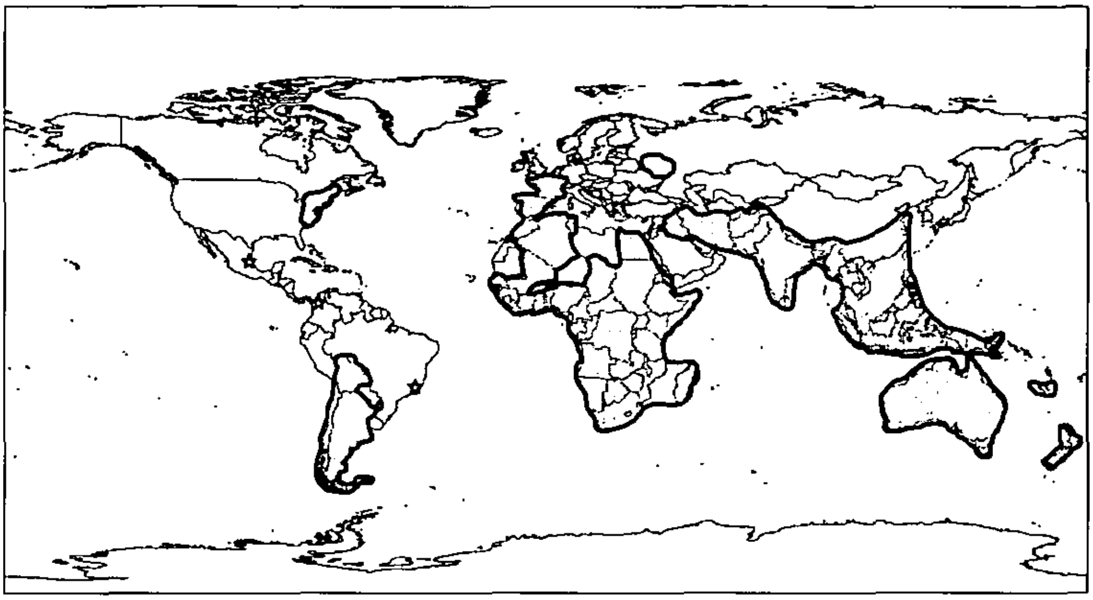


Fig. 4.1. Geographical distribution of the sampled genera and species of Thymelaeaceae. Asterisks represents genera or species not included in the study.

4.2 Materials and Methods

4.2.1 Sampling

A total of 132 taxa were used in the data sets. The family Thymelaeaceae is represented by 129 taxa and three taxa from the families Neuradaceae and Sphaerosepalaceae are used as outgroups. The choice of outgroups was based on previous studies by Van der Bank *et al.* (2002). For the Thymelaeaceae a wide range of Australasian, Sub-Saharan Africa, Madagascarian, Eurasian, Mediterranean, North, Central and South American species was sampled (Fig. 4.1), including 31 taxa belonging to the sub-tribe Pimeleinae.

4.2.2 DNA extraction, amplification, sequencing and phylogenetic analyses

Total DNA was extracted using CTAB as described in Doyle and Doyle (1997) from 0.1-0.3g of fresh or silica-dried leaf and herbarium material. The list of species sampled as well as their location, voucher information and GenBank accession numbers are presented in Table 4.1. Most sequences were obtained from GenBank but several were also generated and have been used in chapter two and three and this current chapter (see Table 4.1). PCR amplification and sequencing of *rbcL* and *trnL-F* (intron and spacer regions) were performed as in Van der Bank *et al.* (2002) and ITS was amplified into two overlapping pieces as in Motsi *et al.* (submitted). The posterior probability (PP) of chronogram scale was used to evaluate the PP's: 0.5<0.8 low, 0.8<0.9 moderate and 0.9<1 high.

4.2.3 Divergence time estimation

Divergence times were estimated using a Bayesian MCMC approach implemented in BEAST (v. 1.4.8; Drummond and Rambaut, 2007), which allows to simultaneous estimation of the topology, substitution rates and node ages (Drummond and Rambaut, 2007). The dataset was divided into three partitions according to the gene regions used in this study (*trnL-F*, *rbcL* and ITS). We implemented the GTR+Γ+I model of sequence evolution for each partition based on the Akaike Information Criterion (AIC) scores for substitution models evaluated using MrModeltest (v. 2.3; Nylander, 2004) with a gamma-distribution with four rate categories. A speciation model following a Yule process was selected as the tree prior, with an uncorrelated lognormal (UCLN) model for the rate variation among branches.

A uniform prior with a lower bound of 11.2 Mya was used as a minimum age constraint for the *Daphne-Thymelaea* clade based on fossil information (Galicia-Herbada, 2006). This calibrating point was based on the oldest fossil that is found on the *Daphne-Thymelaea* evolutionary lineage from the Miocene. Barrón (1995) has identified pollen grains relating to *Daphne* and *Thymelaea* in Tortonian sediments while Médus and Pons (1980) made mention of *Daphne's* macrofossils from the Miocene and the Pliocene of Europe (Palamarev, 1989; Gregor, 1990). Additionally, the crown-group age of *Thymelaea* was set to a normal distribution with a mean of 9.2 Mya (SD=1). Seven independent runs of 5,000,000 generations, sampling every 2500 generations were performed. The adequacy of sampling was assessed using the Effective Sample Size (ESS) diagnostic with Tracer (v.1.4; Rambaut and Drummond, 2007). Between 500,000 and 1,500,000 generations were removed as burn-in before combining all runs to build the maximum clade credibility tree using TreeAnnotator (v. 1.4.8; Drummond and Rambaut, 2007). The geological time scale used here is provided in Table 4.2 (Walker and Geissman, 2009).

Table 4.1. List of taxa included in this study and their locality, with corresponding reference or voucher specimen ([1]Robinson, 2004; [2]Van der Bank *et al.*, 2002; [3]Rautenbach, 2006; [4]Van der Niekerk, 2005; [5] Fay *et al.*, 1998; [6]Van der Bank, pers. comm.; [7]Galacia-Herbada, 2006)

Species	**Location/ voucher specimen/herbarium acronym**	**Genbank accession number**		
		trnL-F	***rbcL***	**ITS**
Thymelaeaceae				
Aquilarioideae				
***Aquilaria* Lam.**				
A. beccariana Tiegh.	Indonalesia, *Chase 1380* (K)	AJ 308642[2]	Y 15149[5]	-
***Gyrinops* Gaertn.**				
G. walla Gaertn.	Sri Lanka and India, *Chase 10511* (K)	AM 404303[3]	AM 398173[3]	FJ 605462
Gonystyloideae				

***Arnhemia* Airy Shaw**				
A. cryptantha Airy Shaw	Australia: Northern Territory, *Lazarides 7870* (K)	AJ 308678[2]	AJ 297236[2]	-
***Deltaria* Steenis**				
D. brachyblastophora Steenis	New Caledonia, *McPherson 4965* (K)	AM 404304[3]	AM 398174[3]	-
***Gonystylus* Teijsm. & Binn.**				
G. macrophyllus (Miq.) Airy Shaw	Southeast Asia, *Chase 1382* (K)	AJ 308653[2]/ AJ 308677[2]	Y 15150[5]	-
***Lethedon* Spreng.**				
L. balansae (Baill.) Kosterm.	New Caledonia, *McPherson and Munzinger 610* (P/MO)	AM 404307[6]	AM 398176[6]	-
L. cernua (baill.) Kosterm.	New Caledonia, *McPherson and Munzinger 18025* (MO)	AM 404305[6]	AM 398177[6]	-

L. salicifolia aff. (Labill.)	New Caledonia, Aymonin, *McPherson and Munzinger 18055* (MO)	AM 404306[6]	AM 398175[6]	-
***Octolepis* Oliv.**				
O. dioica Capuron	Madagascar, *Roger et al. 46* (MO)	AM 404350[3]	AM 398178[3]	-
O. dioica Capuron f. *oblanceolata* Capuron(=*O. blanceolata* (Capuron)Z.S.Roger	Madagascar, *Roger et al. 102* (MO)	-	AM 398179[3]	-
Octolepis sp.	Madagascar, *Roger et al. 165* (MO)	AM 404349[3]	AM 398180[3]	-
***Solmsia* Baill.**				
S. calophylla Baill.	New Caledonia, *Guillaumin s.n.* (K)	AJ 308656[2]	AJ 295261[2]	-
Synandrodaphnoideae				
***Synandrodaphne* Meisn.**				
S. paradoxa Gilg	Tropical West Africa, *Lisowski 46609*	AJ 308676[2]	AJ 297240[2]	-

	(K)			
Thymelaeoideae				
***Craterosiphon* Engl. & Gilg**				
C. scandens Engl. & Gilg	Western Africa, Lock 84/84 (K)	-	AJ 297235[2]	-
***Dais* Royen ex L.**				
D. cotinifolia L.	Africa: southern Africa, *Chase 1381* (K)	AJ 308644[2]	AJ 297234[2]	AJ 744928[3]
***Daphne* L.**				
D. cneorum L.	Andorra: Port de Cabris, Pyrenees, D. *Galicia et al. s.n.*	-	-	AJ 549490[7]
D. gnidium L.	Spain, Paradela del Río, *León, Galicia Herbada 96183* (DGH)	-	-	AJ 549491[7]
D. mezereum L.	Europe, *Chase 6357* (K)	AJ 308645[2]	AJ 297233[2]	AJ 744931[3]
D. oleoides Schreb.	Spain: Sierra Mágina, *Jaén, Galicia*	-	-	AJ 549492[7]

	Herbada 97215 (DGH)			
***Diarthron* (Pobed.) Kit Tan (=*Stelleropsis* Pobed.)**				
D. antoninae (Pobed.) Kit Tan	Iran, Teheran, *Galicia Herbada 81243* (DGH)	-	-	AJ 549495[7]
***Diarthron* (=*Dendrostellera* Tiegh.)**				
D. lessertii (Wikstr.)*Kit Tan* **(=*Dendrostellera lessertii*)**	Iran, Hamadan, Ganjnameh, *Galicia Herbada 81242* (DGH)	-	-	AJ 549494[7]
***Diarthron* Turcz.**				
D. vesiculosum Endl.	Europe, Asia, *Merton 3960* (K)	AJ 308646[2]	AM 398181[3]	-
***Dicranolepis* Planch.**				
D. disticha Planch.	Cameroon: south, *Gereau et al. 5626*	AM 404352[3]	AM 398182[3]	-

	(MO)			
***Dirca* L.**				
D. palustris L.	Central North America, *Horn 12584* (NBYC)	AJ 308647[2]	U 26322[5]	AM 159528[3]
***Drapetes* Lam.**				
D. muscoides Pers. (=*Drapetes muscosus* Lam.)	South America, Borneo, New Guinea, Fuegia and Falkland Islands, *Kubitzki & Feuerer 99-34* (HBG)	AJ 308648[2]	AJ 297237[2]	AM 159529[3]
***Edgeworthia* Meisn.**				
E. chrysantha Lindl.	China, Japan, *Chase 6338* (K)	AJ 308649[2]	AJ 297920[2]	AJ 744932[3]
***Enkleia* Griff.**				
E. siamensis (Kurz) Nevling	southeast Asia, *von Beusekam 4060* (K)	AJ 308664[2]	AJ 297921[2]	-
***Gnidia* L.**				

G. *aberrans* C.H.Wright	South Africa: KwaZulu Natal, *Hilliard and Burtt 6898* (NU)	AM 404222[3]	AM 162523[3]	AM 159508[3]
G. *anomala cf.* Meisn.	South Africa: Western Cape, *Mark Johns s.n.*(Kogelberg Reserve Field Herbarium)	AM 400982[3]	AM 162539[3]	AM 159510[3]
G. *bakeri* Gilg	Madagascar, *Roger et al. 126* (MO)	-	AM 162506[3]	AM 159510[3]
G. *bojeriana* Baill.	Madagascar: Antananarivo, *Roger et al. 183* (MO)	AM 404224[3]	AM 162507[3]	AM 159511[3]
G. *caffra* Meisn.	southern Africa, *Burrows & Burrows 7754* (J)	-	AM 398170[3]	AM 396520[3]
G. *calocephala* Gilg	South Africa: KwaZulu Natal, *Reid 885* (PRE)	AM 404225[3]	-	AM 396521[3]
G. *caniflora* Meisn.	South Africa, *Fourcade 5580* (PRE)	AM 404223[3]	AM 396993[3]	-

G. coriacea Meisn.	South Africa: Western Cape, *Mark Johns s.n.* (Kogelberg Reserve Field Herbarium)	AM 404227[3]	AM 162516[3]	AM 159512[3]
G. danguyana Leandri	Madagascar: Fianatantsoa, *Roger et al. 76* (MO)	AM 404226[3]	AM 162515[3]	AM 159513[3]
G. decaryana Leandri	Madagascar, *Roger et al. 108* (MO)	AJ 745153[3]	AJ 745179[3]	AJ 744926[3]
G. denudata Lindl.	South Africa: Western Cape, *Beaumont s.n.* (NU)	AJ 308670[3]	AJ 295266[3]	AM 159514[3]
G. dumetorum Leandri	Madagascar, *Roger et al. 109* (MO)	AM 404228[3]	AM162514[3]	AM 159515[3]
G. fastigiata Rendle	Africa: Uganda, Kenya, Tanzania, Zimbabwe and Malawi, *Hilliard and Burt 6142* (NU)	AJ 308650[3]	AM 162513[3]	-
G. galpinii C.H.Wright	South Africa: Western Cape, *I. Kruger*	AM 404229[3]	AM 162512[3]	AM 396992[3]

	325 (NBG)			
G. *geminiflora* E.Mey ex Meisn.	South Africa: Western Cape, *Goldblatt 3799* (GB)	AM 404231[3]	AM 397275[3]	-
G. *gilbertae* Drake	Madagascar: Mahajanga, *Randrianasolo 529* (MO)	AJ 745154[3]	AJ 745180[3]	AJ 744927[3]
G. *glauca* Gilg	Tropical Africa, *J. Adanson 6156* (K)	AM 404232[3]	AM 162511[3]	-
G. *humilis* Meisn.	South Africa: Western Cape, *Mark Johns s.n.* (Kogelberg Reserve Field Herbarium)	AM 404236[3]	AM 162510[3]	AM 159517[3]
G. *juniperifolia cf.* Lam.	South Africa: Western Cape, *Mark Johns s.n.* (Kogelberg Reserve Field Herbarium)	AM 404239[3]	AM 397276[3]	-
G. *kraussiana*	Africa: Uganda, Kenya, Tanzania,	AJ 308674[3]	AJ 295267[3]	AM 159518[3]

Meisn.(=*Lasiosiphon krausii* Meisn.)	Guineé, Togo, Nigeria, Sudan, Angola, South Africa and Lesotho, *Beaumont s.n.* (NU)			
G. madagascariensis Baill.	Madagascar, *Roger & al. 133* (MO)	AM 404237[3]	AM 162509[3]	AM 159519[3]
G. phaeotricha Gilg	South Africa: KwaZulu Natal, *Balkwill 10316* (J)	-	AM 162517[3]	AM 159520[3]
G. pilosa Burtt Davy (=*Englerodaphne pilosa* Burtt Davy)	South Africa: Cape region, *Beaumont s.n.* (NU)	AJ 308651[3]	AJ 295264[3]	-
G. pinifolia L.	South Africa: Western Cape, *I Kruger 339* (NBG)	AM 404240[3]	AM 162518[3]	AM 159521[3]
G. racemosa Thunb.	South Africa: Western and Eastern Cape, *Beaumont s.n.* (NU)	AJ 308665[3]	AJ 295268[3]	AM 159522[3]

G. *renniana aff.* Hilliard & B.L.Burtt	South Africa: KwaZulu Natal, *Edwards 1492* (NU)	AJ 308666[3]	AJ 295265[3]	-
G. *renniana* Hilliard & B.L.Burtt	South Africa: KwaZulu Natal, *Beaumont s.n.* (NU)	AM 404233[3]	AM 162519[3]	AM 396522[3]
G. *scabrida* Meisn.	South Africa, *Juli, Ecklon & Zeyher 53.7* (S)	AM 404238[3]	AM 397277[3]	AM 396987[3]
G. *sericocephala* (Meisn.) Gilg ex Engl.	South Africa, *Dehning & Dehning 108* (J)	AM 404241[3]	AM 408173[3]	AM 159523[3]
G. *setosa* Wikstr.	South Africa: Western Cape, *J. Hutchinson 519* (GRA)	AM 404296[3]	AM 162520[3]	AM 159524[3]
G. *singularis* Hilliard	Southern Africa: Lesotho, *Manning 554* (NU)	AM 404297[3]	AM 162521[3]	-
G. *squarrosa* Druce	South Africa: Western Cape, *Mark*	AM 404235[3]	AM 162522[3]	AM 159525[3]

	Johns s.n. (Kogelberg Reserved Field Herbarium)			
G. viridis aff. Berg.	South Africa: Western Cape, *Beaumont s.n.* (NU)	AJ 308652^{3}	AM 162508^{3}	AM 159509^{3}
G. wikstroemiana Meisn.	South Africa: Free State, *Beaumont and Smith SRTe9* (NU)	AM 404299^{3}	AM 162524^{3}	AM 159526^{3}
***Kelleria* Endl.**				
Kelleria dieffenbachii (Hook.) Endl.	Tasmania: Ben Lomond National Park, *Davies, F.E. 1263* (CBG)	FJ 57270	-	FJ 572746
***Lachnaea* L.**				
L. aurea Eckl. & Zeyh. ex Meisn.	South Africa: Western Cape, *Aggenbash s.n.* (NBG)	AJ 697828^{1}	AJ 697781^{1}	AJ 745737^{1}
L. axillaris Meisn.	South Africa: Western Cape, *Snijman*	AJ 308671^{1}	AJ 297129^{1}	AJ 745742^{1}

	1871 (NBG)			
L. capitata Crantz	South Africa: Western Cape, *Bean 2603* (NBG)	AJ 697811[1]	AJ 697798[1]	AJ 745744[1]
L. diosmoides Meisn.	South Africa: Western Cape, *Beyers 181* (COMP)	AJ 697814[1]	AJ 697800[1]	AJ 745745[1]
L. ericoides Meisn.	South Africa: Western Cape, *McDonald 1842* (NBG)	AJ 308673[1]	AJ 297220[1]	AJ 745727[1]
L. eriocephala L.	South Africa: Western Cape, *Beyers 54* (NBG)	AJ 697834[1]	AJ 697780[1]	AJ 745728[1]
***Ovidia* Raf.**				
O. andina Meisn.	Temperate South America, *Kubitzki and Feuerer 99-42* (HBG)	AJ 308675[1]	AJ 297222[1]	AM 159530[1]
***Passerina* L.**				

P. burchellii Thoday	South Africa: Western Cape, *Bredenkamp 1546* (PRE)	AM 404356[4]	AM 162526[4]	AM 158925[4]
P. drankensbergensis Hilliard & B.L.Burtt	South Africa: KwaZulu Natal, *Bredenkamp 1020* (PRE)	AM 404358[4]	AM 162528[4]	AM 158926[4]
P. ericoides L.	South Africa: Western Cape, *Bredenkamp 962* (PRE)	AM 404359[4]	AM 162529[4]	AM 158927[4]
P. falcifolia C.H.Wright	South Africa: Eastern Cape, *Bredenkamp 915* (PRE)	AJ 745150[4]	AJ 297224[4]	AJ 744917[4]
P. montivaga Bredenk. & A.E.van Wyk	southern Africa: South Africa, Swaziland, Mozambique and Zimbabwe, *Van Wyk 2586* (PRE)	AM 404361[4]	AM 162531[4]	AM 158930[4]
P. nivicola Bredenk. & A.E.van Wyk	South Africa: Western Cape, *Bredenkamp 1046* (PRE)	AJ 308655[4]	AJ 297226[4]	AJ 744916[4]

P. obtusifolia Thoday	South Africa: Cape region, *Meyer 1505* (PRE)	AM 404367[4]	AM 162532[4]	AM 158931[4]
***Peddiea* Harv.**				
P. africana Harv.	Tanzania, Morogoro district, *Chase 6330* (K)	AJ 308662[2]	AJ 297227[2]	AJ 744921[3]
P. involucrata Baker	Madagascar: Antananarivo Ambohitantely, *Roger et al. 121* (MO)	AJ 745151[3]	AJ 745176[3]	AJ 744920[3]
***Phaleria* Jack**				
P. capitata Jack	Indomalesia and Western Pacific (Eurasia), *Chase 1383* (K)	AM 308661[2]	AJ 297228[2]	-
***Pimelea* Banks & Sol. ex Gaertn.**				
P. argentea R.Br.	Australia: Western Australia, *M. Hislop*	AM 406675[3]	AM 167530	AM 162490

	& M. Griffiths WW 111.39 (PERTH)			
P. buxifolia Hook.f.	New Zealand: Central North Island, *J.M. Ward 22620* (CANU)	FJ 572703	FJ 572835	FJ 572740
P. calcicola Rye	Australia: Victoria, *J.W.Horn 2574* (PERTH)	FJ 572684	FJ 572816	FJ 572722
P. clavata Labill.	Australia: Mondrain Island, *S. Comer 26* (PERTH)	AM 406689[3]	AM 167531	AM 162491
P. concinna Allan	New Zealand: N. Canterbury, *L. Burrows 38865* (CANU)	FJ 572705	FJ 572837	FJ 572742
P. decora Domin	Australia: Queensland, *Purdie R.W. 5905* (CANB)	FJ 572695	FJ 572827	FJ 572733
P. forrestiana F.Muell.	Australia: Western Australia, *K. Coate 695* (PERTH)	AM 407407[3]	AM 167533	AM 162493

P. gilgiana E.Pritz	Australia: Western Australia, *I. B. Shepherd 269* (PERTH)	AM 406678[3]	AM 167534	FJ 572710
P. gnidia Willd.	New Zealand: West Coast region, *D. A. Norton 33757* (CANU)	FJ 649634	FJ 649629	FJ 649624
P. graniticola Rye	Australia: Western Australia, B. Archer 1664 (PERTH)	AM 406679[3]	FJ 572812	FJ 572718
P. haematostachya F.Muell.	Australia: Queensland, *Lepschi B. J. 1202* (CANB)	FJ 572695	FJ 572827	FJ 572733
P. hewardiana Meisn.	Australia: Victoria, *Phillips M. E. 131* (CBG)	FJ 649635	FJ 649630	FJ 649625
P. holroydii F.Muell.	Australia: Western Australia, *S. van Leeuwen 3769* (PERTH)	AM 406687[3]	AM 167539	AM 162496
P. longifolia (Thunb.) Meisn.	New Zealand: Nelson/ Marlborough, *C.*	FJ 649636	FJ 649631	FJ 649626

	J. Burrows 26070 (CANU)			
P. "orongo"(C.J.Burrows, in preparation, 2009)	New Zealand: Coastal Wellington, *C. J. Burrows 38838* (CANU)	FJ 572704	FJ 572836	FJ 572741
P. pelinos Rye	Australia: Western Australia, *B. J. Lepschi 3448, T. R. Lally and K. L. Brown* (PERTH)	AM 406680[3]/ AM 407410[3]	AM 167535	AM 162497
P. physodes Hook.	CULTIVATED: Gosford Primary Industries Institute, Narara as NSW781572, *Lidbetter J. s.n.* (NSW)	FJ 687345	FJ 687343	FJ 687339
P. pygmaea Meisn.	Australia: central Tasmania, *Chase 6360* (K)	AJ 308669[2]	AJ 297230[2]	AJ 744922[2]
P. rara Rye	Australia: Western Australia, *F & J. Hort 2510* (PERTH)	AM 407412[3]	AM 167538	AM 162498

P. *rosea* R.Br.	Australia: Western Australia, *Fox J. M. 88/226* (CANB)	FJ 649637	FJ 649632	FJ 649627
P. spectabilis Lindl.	Australia: Western Australia, *Chase 2198* (K)	AJ 308659[2]	AJ 297231[2]	AJ 744923[2]
P. spicata R.Br.	CULTIVATED, Australian National Botanic Gardens, Canberra, Nursery, as 8602385C, *Beesley P. 1112* (CBG)	FJ 572677	FJ 572807	FJ 572713
P. spiculigera var. *thesioides* (S.Moore) Rye	Australia: Western Australia, *J. Docherty 130* (PERTH)	AM 406681[3]	AM 167536	AM 162500
P. stricta Meisn.	Australia: South Australia, *Crisp, M. D.7221* (CBG)	FJ 649638	FJ 649633	FJ 649628
P. trichostachya Lindl.	Australia: Western Australia, *K. F. Kenneally 12623 & D. J. Edinger 3822*	AM 406682[3]	AM 167537	AM 162501

	(PERTH)			
***Stellera* Turcz.**				
S. chamaejasme L.	Nepal to China, *Chase 5530* (K)	AJ 308657^{2}	AJ 295262^{2}	-
***Stephanodaphne* Baill.**				
S. capitata (Leandri) Leandri	Madagascar: Prov. Antsiranana, *Rogers et al. 139* (MO)	AM 407411^{3}	AM 398184^{3}	AM 159531^{3}
S. cremostachya Baill.	Madagascar: Comores, *Toliara 13.01.1990* (K)	AJ 308658^{3}	AJ 295263^{3}	AM 159532^{3}
S. cuspidata (Leandri) Leandri	Madagascar: Prov. Fianarantsoa, *Roger et al. 68* (MO)	AM 406683^{3}	AM 398185^{3}	AM 159533^{3}
S. oblongifolia Leandri	Madagascar: Prov. Antananarivo, *Roger et al. 127* (MO)	AJ 745152^{3}	AJ 745177^{3}	AJ 744924^{3}
***Struthiola* L.**				

S. *ciliata* Lam.	South Africa: Western Cape, *Mark Johns s.n.* (Kogelberg Reserve Field Herbarium)	AM 404300[3]	AM 397279[3]	AM 396986[3]
S. *dodecandra* Druce	South Africa: Western Cape, *Mark Johns s.n.* (Kogelberg Reserve Field Herbarium)	AM 404298[3]	AM 398171[3]	AM 396988[3] / AM 396989[3]
S. *leptantha* Bolus	South Africa: Western Cape, *Beyers 265* (NGB)	AJ 308639[2]	AJ 297243[2]	AJ 745757[3]
S. *salteri* Levyns	South Africa: Western Cape, *Mark Johns s.n.* (Kogelberg Reserve Field Herbarium)	AM 404301[3]	AM 397280[3]	-
S. *stricta* Donn	South Africa: Western Cape, *Mark Johns s.n.* (Kogelberg Reserve Field	AM 404302[3]	AM 398172[3]	AM 396990[3] /

	Herbarium)			AM 396991^3
S. tomentosa Andrews	South Africa: Western Cape, *Mark Johns s.n.* (Kogelberg Reserve Field Herbarium)	-	AM 162540^3	AM 158946^3
***Synaptolepis* Oliv.**				
S. alternifolia Oliv.	Africa: Tanzania, Malawi, Mozambique, Zimbabwe, *Vollesen 4043* (K)	AJ 308663^2	AJ 297239^2	-
***Thecanthes* Wikstr.**				
T. concreta (F.Muell.) Rye	Australia: Western Arnhem Land, *Cowie I. D. 8563* (MEL)	FJ 572708	FJ 572839	FJ 572743
T. cornucopiae (M.Vahl) Wikstr.	Australia: W of Turrel Hill, *Forster P. I. 23116* (MEL)	FJ 572706	FJ 572840	FJ 572744
T. filifolia Rye	Australia: Arnhem Land, *Cowie I. D.*	FJ 572709	FJ 572841	FJ 572745

	8702 (MEL)			
T. punicea (R.Br.) Wikstr.	Australia: Cockburn Range, *T. Handasyde TH99 488* (PERTH)	AM 406684[3]	AM 167540	AM 162502
T. sanguinea (F.Muell.) Rye	Australia: Kalumburu area, *A. A. Mitchell 3945* (PERTH)	AM 406685[3]	FJ 572838	AM 162503
***Thymelaea* Mill.**				
T. argentata (Lam.) Pau	Spain, La Nucía, Alicante, *Galicia Herbada 93029* (DGH)	-	-	AJ 549489[7]
T. hirsuta Endl.	Spain, *Chase 1883* (K)	AJ 308640[2]	Y 152151[5]	AJ 744930[3]
T. microphylla Coss. & Durieu	Tunisia, Moulares, *E86689*	-	-	AJ 549482[7]
T. putorioides Emb. & Maire	Morocco, oued Tessaout, High Atlas, *Galicia Herbada 00271*(DGH)	-	-	AJ 549459[7]
T. villosa Endl.	Spain, Los Barrios, Cádiz, *Galicia*	-	-	AJ 549483[7]

	Herbada and L. Moreno 97204 (DGH)			
***Wikstroemia* Spreng.**				
W. alberti (Regel) Domke	Tajikistan, Hissar range, *E86737*	-	-	AJ 549497[7]
W. canescens Meisn.	Nepal, Ramechhap, E *82170*	AM 406686[3]	AM 398186[3]	AM 549496[3]
W. gemmata (E.Pritz.) Domke	southern China to Australia and Pacific, *Chase 3955* (K)	AJ 308641[2]	AJ 295269[2]/ AJ 297223[2]	AJ 744929[3]
Outgroup				
Neuradaceae				
***Grielum* L.**				
G. humifusum Thunb.	South Africa, *Chase 5711* (K)	-	AJ 402955[2]	-
Sphaerosepalaceae				
***Dialyceras* Capuron**				
D. coriaceum (R.Cap.) J.-F.Leroy	Madagascar, *Schatz et al. 3848* (MO)	-	AJ 297232[2]	-

***Rhopalocarpus* Teijsm. & Binn.ex Miq.**				
Rhopalocarpus sp.	Madagascar, *Chase 906* (K)	-	Y 15148[5]	-

Table 4.2. Geological time scale [modified from Walker and Geissman 2009]

PERIOD		EPOCH	AGE	Million Years
QUATERNARY		HOLOCENE		
		PLEISTOCENE	CALABRIAN	1.8-0.01
			GELASIAN	2.6-1.8
TERTIARY		PLIOCENE	PIACENZIAN	3.6-2.6
			ZANCLEAN	5.3-3.6
	NEOGENE	MIOCENE	MESSINIAN (Late Miocene)	7.2-5.3
			TORTONIAN (Late Miocene)	11.6-7.2
			SERRAVALLIAN (Middle Miocene)	13.8-11.6
			LANGHIAN (Middle Miocene)	16.0-13.8
			BURDIGALIAN (Early Miocene)	20.4-16.0
			AQUITANIAN (Early Miocene)	23.0-20.4
	PALEOCENE	OLIGOCENE	CHATTAIN (Late Oligocene)	28.4-23.0
			RUPELIAN (Early Oligocene)	33.9-28.4
		EOCENE	PRIABONIAN (Late Eocene)	37.2-33.9

			BARTONIAN (Middle Eocene)	40.4-37.2
			LUTETIAN (Middle Eocene)	48.6-40.4
			YPRESIAN (Early Eocene)	55.8-48.6

4.3. Results

The results provide a molecular phylogeny and estimated absolute ages for all subfamilies, major clades and lineages recognised within Thymelaeaceae. The phylogeny of Thymelaeaceae with posterior probability is presented in Fig. 4.2. The chronogram is presented in Fig. 4.3, whilst Fig. 4.4 presents a detailed sub-tree derived from node 2 in Fig. 4.3. The stem age of divergence for Thymelaeaceae is Late (Lutetian) Eocene at 50.72 Mya (Fig. 4.3; HPD: 29.81-74.91; Table 4.3), with a crown group age of 44.34 Mya (Fig. 4.3; node 24; HPD: 27.51-64.56; Table 4.3). The family Thymelaeaceae is divided into four subfamilies: Thymelaeoideae and Aquilarieae whose origins derive from a split 40.06 Mya (Fig. 4.3; node 22, HPD: 25.30-58.20), and Gonystyloideae and Synandrodaphneae which split 27.78 Mya (Fig. 4.3; node 25, HPD: 11.52-47.9; Table 4.3).

4.3.1. Subfamily Thymelaeoideae

The subfamily Thymelaeoideae has a crown age of 35.81 Mya (Fig. 4.3; node 20; HPD 22.43-51.45) and comprises three major clades (Fig. 4.3; numbered I, II, III), four lineages of *Gnidia*, three endemic South Africa genera (*Passerina*, *Lachnaea* and *Struthiola*) and the group comprising *Stephanodaphne*, *Peddiea*, *Dirca, Ovidia*, *Dais*, and *Phaleria*. The stem age of Thymelaeoideae is dated in the Bartonian (Mid-Late) Eocene. The split for clades I (i.e. the tropical African, south-east Asia, Australasian and New World taxa) and II (Asian and Mediterranean including northern Africa), is 33.39 Mya (Fig. 4.3; node16; HPD 21.4-48.63; Table 4.3), and for clade III (tropical Africa plus tropical Asian taxa) is 35.81 Mya (Fig. 4.3; node 20; HPD 22.43-51.45; Table 4.3). All three of the clades split during the Rupelian (Early) Oligocene.

The respective crown ages of the three clades are: Clade I 31.54 Mya (Fig. 4.3; node 15; HPD: 19.79-45.37; Table 4.3); clade II 29.31 Mya (Fig. 4.3; node 19; HPD: 18.86-42.74; Table 4.3); and clade III 14.74 Mya (Fig. 4.3; node 21; HPD: 5.79-25.69; Table 4.3).

The four lineages of *Gnidia* occurred during the Miocene with lineages 1 and 2 in the Serravallian (Middle Miocene) and lineages 3 and 4 in the Burdigalian (Early Miocene). The crown ages of these lineages are as follows: lineage 1, 14.86 Mya (Fig. 4.3; node 6; HPD: 8.27-22.63; Table 4.3); lineage 2, 13.73 Mya (Fig. 4.3; node 8; HPD: 6.97-20.86; Table 4.3); lineage 3, 21.14 (Fig. 4.3; node 2; HPD: 13.03-30.77; Table 4.3) and lastly lineage 4, 20.86 Mya (Fig. 4.3; node 14; HPD: 11.56-31.05; Table 4.3).

Lineage 1 comprises two sister clades (the *Gnidia* clade and the (*'Drapetes-Kelleria'* clade) from the Serravallian (Middle Miocene), whereas lineage 2 (which comprises *Gnidia pinifolia*, *G. racemosa* and *Struthiola* species) originated in the Messinian (Late Miocene). Lineage 3 comprises *Thecanthes* embedded within *Pimelea* and some Southern African and tropical African *Gnidia* species. *Pimelea* and *Thecanthes* split in the Serravallian (Middle Miocene) aged 13.38 Mya (node 1, Fig. 4.3.; node 1, Fig. 4.4; HPD: 9.79-23.51). The New Zealand species of *Pimelea* and also the *Thecanthes* clade originated in the Zanclean (Early Pliocene), the former 4.1 Mya (Fig. 4.4; HPD: 1.72-6.91; Table 4.3) and the latter 5.13 Mya (Fig. 4.4; HPD: 2.65-8.25; Table 4.3). The clades of *Gnidia* originated in the Tortonian (Late Miocene) between 9.14 (Fig. 4.4; HPD: 4.65-14.48; Table 4.3) and 9.62 Mya (Fig. 4.4; HPD: 3.99-16.76; Table 4.3) respectively. Lastly lineage 4 comprises *Gnidia* species from South Africa and Madagascar. The split between South African and Madagascar species of *Gnidia* is 14.42 Mya (node 13; HPD: 8-22.19; Table 4.3). The split of *Passerina* and *Lachnaea* is in the Serravallian (Middle Miocene) 13.28 Mya (node 4; HPD: 6.83-20.2; Table 4.3). The split between *Stephanodaphne* and *Peddiea* is much older than between any other genera within the subfamily Thymelaeoideae, occurring 23.02 Mya (node 10; HPD: 11.54-35.37; Table 4.3). The split between *Thymelaea* and *Daphne* occurred 15.41 Mya (node 17; HPD: 11.2-21.88; Table 4.3).

4.3.2. Subfamily Aquilarioideae

The subfamily Aquilarioideae is dated in the Bartonian (Middle Eocene) 40.06 Mya (Fig. 4.3; node 22; HPD: 25.3-58.2; Table 4.3), with a crown age of 8.13 Mya (Fig. 4.3; node 23; HPD: 2.29-15.63; Table 4.3). Within Aquilarioideae, *Gyrinops* and *Aquilaria* split in the Tortonian (Late Miocene) 8.13 Mya (Fig. 4.2; node 23; HPD: 2.29-15.63; Table 4.3).

4.3.3. Subfamily Gonystyloideae

The subfamily Gonystyloideae originated in the Chattian (Late Oligocene) 27.78 Mya following the split from subfamily Synandrodaphnoideae (Fig. 4.3; node 25; HPD: 11.52-47.9; Table 4.3) and has a crown age of 13.19 Mya (Fig. 4.3; node 26; HPD: 6.03-22.41). Within Gonystyloideae two clades are recognised. The *Octolepis* clade is dated in the Serravallian (Middle Miocene) at 13.19 Mya (Fig. 4.3; node 26; HPD: 6.03-22.41), and within the second clade there is a further split between the

'*Deltaria-Gonystylus*' clade and the *Lethedon* clade dated at 7.76 Mya (node 27; HPD: 3.49-13; Table 4.3).

4.3.4. Subfamily Synandrodaphnoideae

The subfamily Synandrodaphnoideae originated in the Chattian (Late Oligocene) 27.78 Mya (Fig. 4.3; node 25; HPD: 11.52-47.9; Table 4.3), which is its crown age.

Fig. 4.2 (next page). Phylogeny of Thymelaeaceae with posterior probability obtained from BEAST.

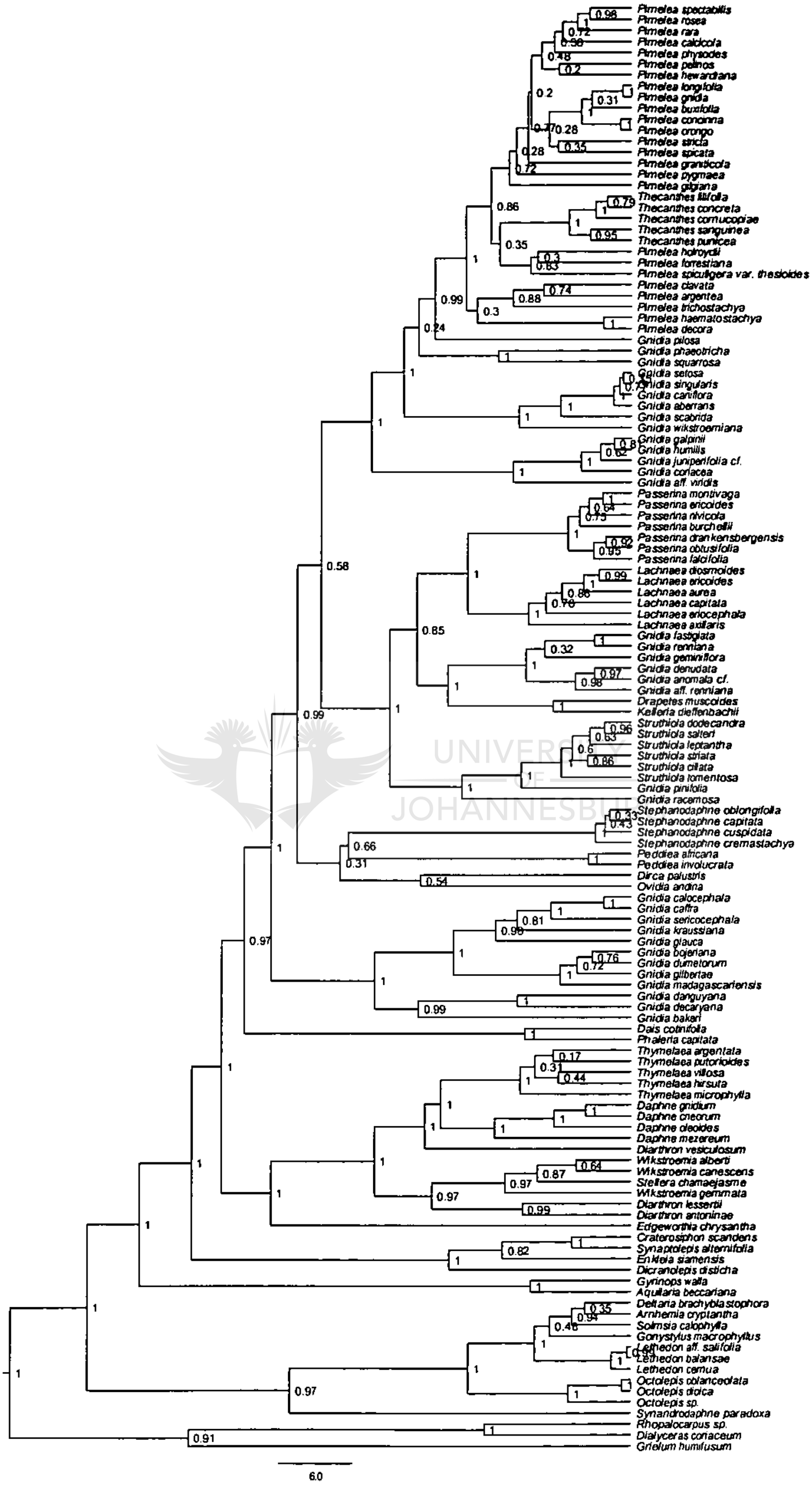

Pimelea spectabilis
Pimelea rosea
Pimelea rara
Pimelea calcicola
Pimelea physodes
Pimelea pelinos
Pimelea hewardiana
Pimelea longifolia
Pimelea gnidia
Pimelea buxifolia
Pimelea concinna
Pimelea orongo
Pimelea stricta
Pimelea spicata
Pimelea graniticola
Pimelea pygmaea
Pimelea gilgiana
Thecanthes filifolia
Thecanthes concreta
Thecanthes cornucopiae
Thecanthes sanguinea
Thecanthes punicea
Pimelea holroydii
Pimelea forrestiana
Pimelea spiculigera var. thesioides
Pimelea clavata
Pimelea argentea
Pimelea trichostachya
Pimelea haematostachya
Pimelea decora
Gnidia pilosa
Gnidia phaeotricha
Gnidia squarrosa
Gnidia setosa
Gnidia singularis
Gnidia caniflora
Gnidia aberrans
Gnidia scabrida
Gnidia wikstroemiana
Gnidia galpinii
Gnidia humilis
Gnidia juniperifolia cf.
Gnidia coriacea
Gnidia aff. viridis
Passerina montivaga
Passerina ericoides
Passerina nivicola
Passerina burchellii
Passerina drakensbergensis
Passerina obtusifolia
Passerina falcifolia
Lachnaea diosmoides
Lachnaea ericoides
Lachnaea aurea
Lachnaea capitata
Lachnaea eriocephala
Lachnaea axillaris
Gnidia fastigiata
Gnidia renniana
Gnidia geminiflora
Gnidia denudata
Gnidia anomala cf.
Gnidia aff. renniana
Drapetes muscoides
Kelleria dieffenbachii
Struthiola dodecandra
Struthiola salteri
Struthiola leptantha
Struthiola striata
Struthiola ciliata
Struthiola tomentosa
Gnidia pinifolia
Gnidia racemosa
Stephanodaphne oblongifolia
Stephanodaphne capitata
Stephanodaphne cuspidata
Stephanodaphne cremastachya
Peddiea africana
Peddiea involucrata
Dirca palustris
Ovidia andina
Gnidia calocephala
Gnidia caffra
Gnidia sericocephala
Gnidia kraussiana
Gnidia glauca
Gnidia bojeriana
Gnidia dumetorum
Gnidia gilbertae
Gnidia madagascariensis
Gnidia danguyana
Gnidia decaryana
Gnidia bakeri
Dais cotinifolia
Phaleria capitata
Thymelaea argentata
Thymelaea putorioides
Thymelaea villosa
Thymelaea hirsuta
Thymelaea microphylla
Daphne gnidium
Daphne cneorum
Daphne oleoides
Daphne mezereum
Diarthron vesiculosum
Wikstroemia alberti
Wikstroemia canescens
Stellera chamaejasme
Wikstroemia gemmata
Diarthron lessertii
Diarthron antoninae
Edgeworthia chrysantha
Craterosiphon scandens
Synaptolepis alternifolia
Enkleia siamensis
Dicranolepis disticha
Gyrinops walla
Aquilaria beccariana
Deltaria brachyblastophora
Arnhemia cryptantha
Solmsia calophylla
Gonystylus macrophyllus
Lethedon aff. salicifolia
Lethedon balansae
Lethedon cernua
Octolepis oblanceolata
Octolepis dioica
Octolepis sp.
Synandrodaphne paradoxa
Rhopalocarpus sp.
Dialyceras coriaceum
Grielum humifusum
6.0

Table 4.3. Node ages in millions of years. For each node the estimated age along with the 95% high posterior density interval (95%HPD) are presented. Node numbers correspond to those in Fig. 4.2.

Node	**Description**	**Age**	**95% (HPD)**
1	Subtribe Pimeleinae	13.38 Serravallian (Middle) Miocene	[8.23,19.96]
2	Africa and southern Africa *Gnidia* spp.	21.14 Burdigalian (Early) Miocene	[13.03, 30.77]
3		25.24 Chattian (Late) Oligocene	[15.8, 36.26]
4	Split between *Passerina* and *Lachnaea*	13.28 Serravallian (Middle) Miocene	[6.83, 20.2]
5		17.42 Burdigalian (Early) Miocene	[10.61, 26.31]
6		14.86 Serravallian (Middle) Miocene	[8.27, 22.63]
7		19.64 Burdigalian (Early) Miocene	[11.73, 29.1]
8	Africa and southern Africa *Gnidia* spp.	13.73 Serravallian (Middle) Miocene	[6.97, 20.86]
9		27.23 Chattian (Late) Oligocene	[16.89, 39.06]

10	Split between *Stephanodaphne* and *Peddiea*	23.02 Aquitanian (Early) Miocene	[11.54, 35.37]
11		23.69 Chattian (Late) Oligocene	-
12		29.32 Chattian (Late) Oligocene	[18.4, 42.13]
13	Split between African and southern African and Madagascar *Gnidia* spp.	14.42 Serravallian (Middle) Miocene	[8, 22.19]
14		20.86 Aquitanian (Early) Miocene	[11.56, 31.05]
15	Crown age of clade I	31.54 Serravalian (Middle) Miocene	[19.79, 45.37]
16		33.39 Rupelian (Early) Oligocene	[21.4, 48.63]
17	Split between *Thymelaea* and *Daphne*	15.41 Langhian (Middle) Miocene	[11.2, 21.88]
18		20.87 Burdigalian (Early) Miocene	[13.66, 30.13]
19	Crown age of clade II	29.31 Chattian (Late) Oligocene	[18.68, 42.74]
20	Crown age of subfamily Thymelaeoideae	35.81 Rupelian (Early) Oligocene	[22.43, 51.45]

21	Crown age of clade III	14.74 Serravallian (Middle) Miocene	[5.79, 25.69]
22	Split between subfamilies Thymelaeoideae and Aquilarieae	40.06 Bartonian (Middle) Eocene	[25.30, 58.20]
23	Crown age of subfamily Aquilarieae	8.31 Tortonian (Late) Miocene	[2.29, 15.63]
24	Crown age of family Thymelaeaceae	44.34 Lutetian (Middle) Eocene	[27.51, 64.56]
25	Split between subfamilies Synandrodaphneae and Gonystyloideae ; Crown age of subfamily Synandrodaphneae	27.78 Chattian (Late) Oligocene	[11.52, 47.9]
26	Crown age of subfamily Gonystyloideae	13.19 Serravallian (Middle) Miocene	[6.03, 22.41]
27		7.76 Tortonian (Late) Miocene	[3.49, 13]
28		6.6 Tortonian (Late) Miocene	-
29	Stem age of Thymelaeaceae	50.72 Ypresian (Early) Eocene	[29.81,74.91]

subtree	New Zealand *Pimelea* spp.	4.1 Zanclean (Early) Pliocene	[1.72,6.91]
subtree	*Thecanthes* clade	5.13 Zanclean (Early) Pliocene	[2.65,8.25]
subtree	Southern Africa *Gnidia* spp.	9.14 Tortonian (Late) Miocene	[4.65, 14.48]
subtree	Southern Africa *Gnidia* spp.	9.62 Tortonian (Late) Miocene	[3.99, 16.76]

Fig. 4.3 (next page). Chronogram of the family Thymelaeaceae based on *trnL-F*, *rbcL* and ITS regions. Clades indicated are: I, the tropical African, South-East Asia, Australasian and New World taxa; II, Asian and Mediterranean including northern Africa; and III, tropical Africa plus tropical Asian taxa. Absolute ages are in million years. Geological timescale is illustrated at the bottom.

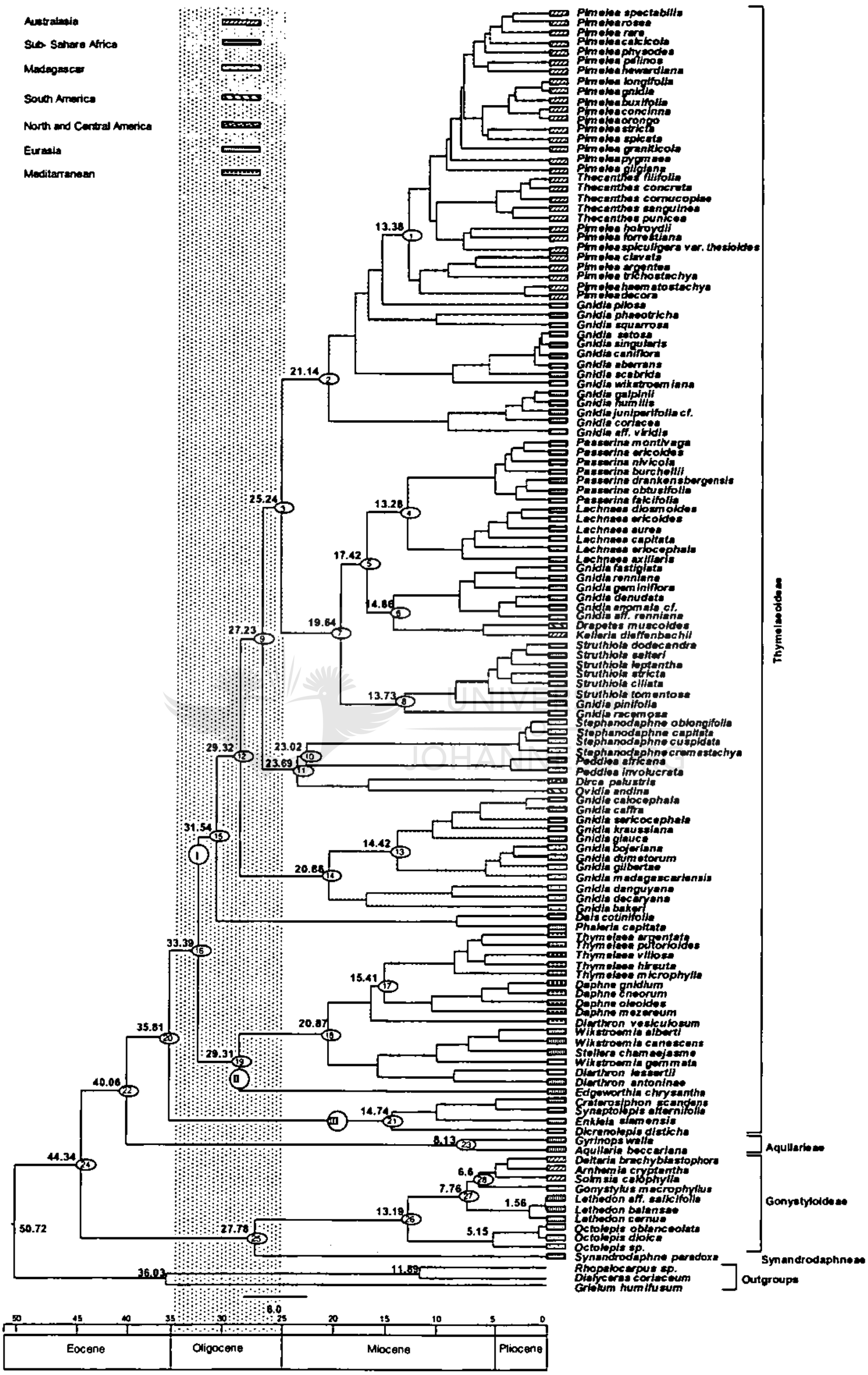
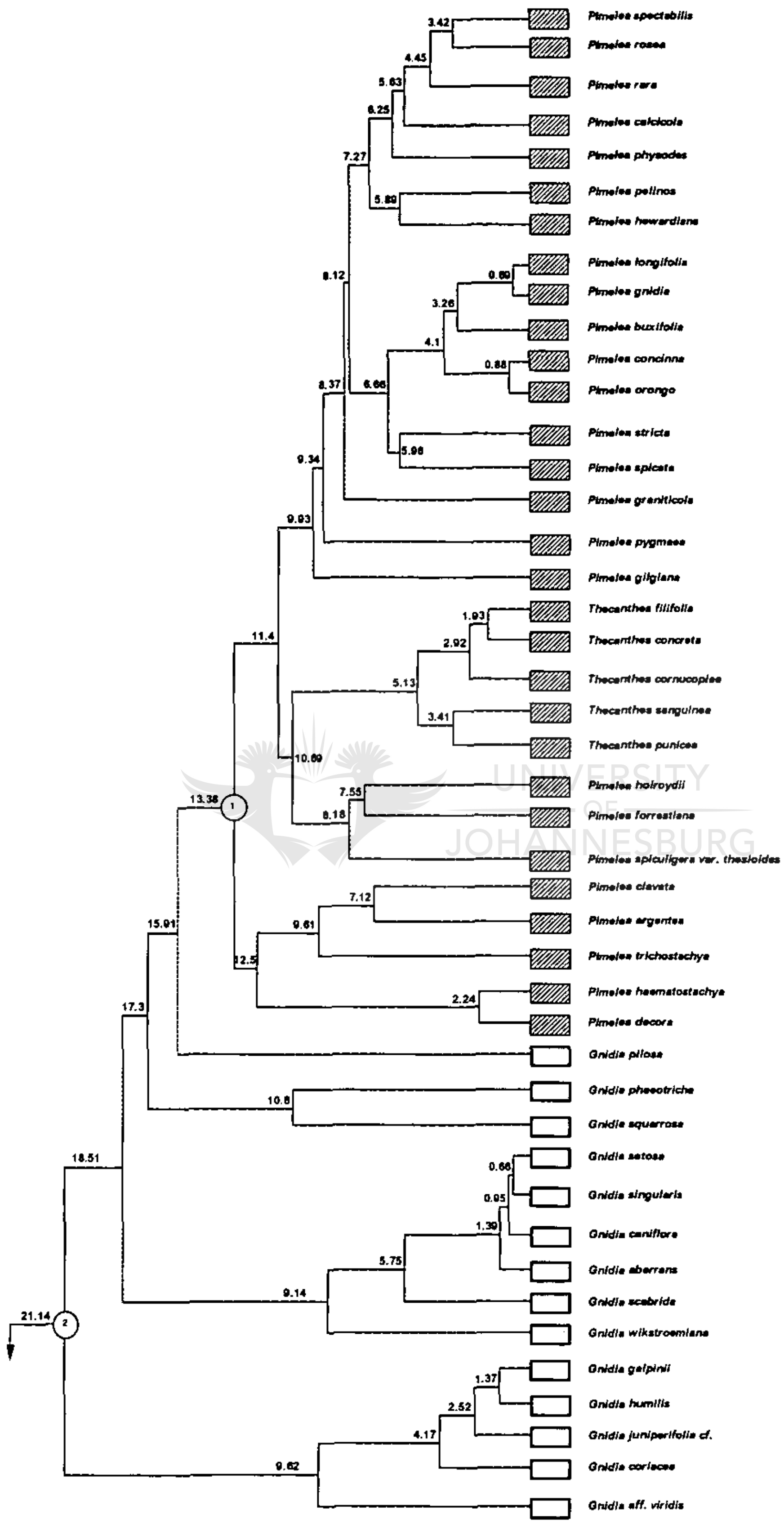

Australasia
Sub- Sahara Africa
Madagascar
South America
North and Central America
Eurasia
Mediterranean
Pimelea spectabilis
Pimelea rosea
Pimelea rara
Pimelea calcicola
Pimelea physodes
Pimelea pelinos
Pimelea hewardiana
Pimelea longifolia
Pimelea gnidia
Pimelea buxifolia
Pimelea concinna
Pimelea orongo
Pimelea stricta
Pimelea spicata
Pimelea graniticola
Pimelea pygmaea
Pimelea gilgiana
Thecanthes filifolia
Thecanthes concreta
Thecanthes cornucopiae
Thecanthes sanguinea
Thecanthes punicea
Pimelea holroydii
Pimelea forrestiana
Pimelea spiculigera var. thesioides
Pimelea clavata
Pimelea argentea
Pimelea trichostachya
Pimelea haematostachya
Pimelea decora
Gnidia pilosa
Gnidia phaeotricha
Gnidia squarrosa
Gnidia setosa
Gnidia singularis
Gnidia caniflora
Gnidia aberrans
Gnidia scabrida
Gnidia wikstroemiana
Gnidia galpinii
Gnidia humilis
Gnidia juniperifolia cf.
Gnidia coriacea
Gnidia aff. viridis
Passerina montivaga
Passerina ericoides
Passerina nivicola
Passerina burchellii
Passerina drankensbergensis
Passerina obtusifolia
Passerina falcifolia
Lachnaea diosmoides
Lachnaea ericoides
Lachnaea aurea
Lachnaea capitata
Lachnaea eriocephala
Lachnaea axillaris
Gnidia fastigiata
Gnidia renniana
Gnidia geminiflora
Gnidia denudata
Gnidia anomala cf.
Gnidia aff. renniana
Drapetes muscoides
Kelleria dieffenbachii
Struthiola dodecandra
Struthiola salteri
Struthiola leptantha
Struthiola stricta
Struthiola ciliata
Struthiola tomentosa
Gnidia pinifolia
Gnidia racemosa
Stephanodaphne oblongifolia
Stephanodaphne capitata
Stephanodaphne cuspidata
Stephanodaphne cremostachya
Peddiea africana
Peddiea involucrata
Dirca palustris
Ovidia andina
Gnidia calocephala
Gnidia caffra
Gnidia sericocephala
Gnidia kraussiana
Gnidia glauca
Gnidia bojeriana
Gnidia dumetorum
Gnidia gilbertae
Gnidia madagascariensis
Gnidia danguyana
Gnidia decaryana
Gnidia bakeri
Dais cotinifolia
Phaleria capitata
Thymelaea argentata
Thymelaea putorioides
Thymelaea villosa
Thymelaea hirsuta
Thymelaea microphylla
Daphne gnidium
Daphne cneorum
Daphne oleoides
Daphne mezereum
Diarthron vesiculosum
Wikstroemia alberti
Wikstroemia canescens
Stellera chamaejasme
Wikstroemia gemmata
Diarthron lessertii
Diarthron antoninae
Edgeworthia chrysantha
Craterosiphon scandens
Synaptolepis alternifolia
Enkleia siamensis
Dicranolepis disticha
Gyrinops walla
Aquilaria beccariana
Deltaria brachyblastophora
Arnhemia cryptantha
Solmsia calophylla
Gonystylus macrophyllus
Lethedon aff. salicifolia
Lethedon balansae
Lethedon cernua
Octolepis oblanceolata
Octolepis dioica
Octolepis sp.
Synandrodaphne paradoxa
Rhopalocarpus sp.
Dialyceras coriaceum
Grielum humifusum
Thymelaeoideae
Aquilarieae
Gonystyloideae
Synandrodaphneae
Outgroups
13.38 1
21.14 2
25.24 3
13.28 4
17.42 5
14.86 6
19.64 7
13.73 8
27.23 9
23.02 10
23.69 11
29.32 12
14.42 13
20.66 14
31.54 15
I
33.39 16
15.41 17
20.87 18
29.31 19
II
35.81 20
14.74 21
III
40.06 22
8.13 23
44.34 24
27.78 25
13.19 26
7.76 27
6.6 28
1.56
5.15
50.72
36.03
11.89
8.0
50 45 40 35 30 25 20 15 10 5 0
Eocene
Oligocene
Miocene
Pliocene

Fig. 4.4 (next page). Lineage 3 is the sub-tree of my study-group derived from node 2 in the Thymelaeaceae chronogram presented in Fig. 4.3.


3.42
4.45
5.63
6.25
7.27
5.89
8.12
0.69
3.26
4.1
0.88
6.66
8.37
5.96
9.34
9.93
1.93
2.92
5.13
3.41
11.4
10.69
7.55
8.16
13.38
1
7.12
9.61
15.91
12.5
2.24
17.3
10.8
18.51
0.66
0.95
1.39
5.75
9.14
21.14
2
1.37
2.52
4.17
9.62
Pimelea spectabilis
Pimelea rosea
Pimelea rara
Pimelea calcicola
Pimelea physodes
Pimelea pelinos
Pimelea hewardiana
Pimelea longifolia
Pimelea gnidia
Pimelea buxifolia
Pimelea concinna
Pimelea orongo
Pimelea stricta
Pimelea spicata
Pimelea graniticola
Pimelea pygmaea
Pimelea gilgiana
Thecanthes filifolia
Thecanthes concreta
Thecanthes cornucopiae
Thecanthes sanguinea
Thecanthes punicea
Pimelea holroydii
Pimelea forrestiana
Pimelea spiculigera var. thesioides
Pimelea clavata
Pimelea argentea
Pimelea trichostachya
Pimelea haematostachya
Pimelea decora
Gnidia pilosa
Gnidia phaeotricha
Gnidia squarrosa
Gnidia setosa
Gnidia singularis
Gnidia caniflora
Gnidia aberrans
Gnidia scabrida
Gnidia wikstroemiana
Gnidia galpinii
Gnidia humilis
Gnidia juniperifolia cf.
Gnidia coriacea
Gnidia aff. viridis

4.4. Discussion and conclusion

A knowledge of past diversification and distribution of plants is important when testing the correspondence between geological and biological history (Rutschmann *et al.*, 2004), given that it is alleged that geological events left a mark on the origin of biotic elements and evolution among various groups (Lieberman, 2000). Divergence time estimation of Thymelaeaceae links the fossil-calibrated molecular phylogenies with major events in historical continental evolution.

The break-up of super-continent Pangaea is surrounded by conflicting theories (Scotese *et al.*, 1988); the expanding earth theory (Shield, 1979) and the lost super-continent of Pacifica (Kamp, 1980), and has been summarised in geological area cladograms (Scotese *et al.*, 1988; Veevers, 1991; Veevers *et al.*, 1991; Lawver *et al.*, 1992; McLoughlin, 2001; Sanmartín and Ronquist, 2004). Gondwana formed the southern portion of the Pangaea super-continent during the Triassic period with two climatically different biotic provinces: a Northern Tropical Gondwana province (northern South America, Africa, Madagascar, India, northern Australia, New Guinea), and a Southern Temperate Gondwana province (southern South America, Australia, Antarctica, New Zealand, New Caledonia, southern temperate Africa). During the Jurassic (165-150 Mya), Gondwana fragmented and simultaneously there was drift between India and Australia-east Antarctica. Madagascar and India drifted away from Africa during the early Cretaceous (121 Mya) and Madagascar drifted southeast, to where it is now in front of Mozambique (Rabinowitz *et al.*, 1983). During the middle

Paleogene (60 Mya) Africa was moving northwards to collide with Eurasia, and southern South America moved to the southwest into contact with Antarctica (McLoughlin, 2001). The separation between Australia and Antarctica was completed in the late Eocene with the opening of the South Tasman Sea and followed by the break-up of southern South America and the Antarctic (Woodburne and Case, 1996; McLoughlin, 2001). During this period of Antarctic glaciations the Drake Passage opened between the continents, allowing the establishment of the Antarctic Circumpolar Current. It was only until the early Tertiary that Antarctica enjoyed an occupied climate with widespread *Nothofagus* Blume forests (Anderson *et al.,* 1999; Dingle and Lavelle, 2000).

The current phylogenetic resolution within the Thymelaeaceae reveals similar patterns to those found previously (Van der Bank *et al.,* 2002; Robinson, 2004; Van Niekerk, 2005; Rautenbach, 2006; Beaumont *et al.,* submitted and Motsi *et al.,* submitted), with several important stipulations for phylogenetic relationships and estimated divergence times. The family is a monophyletic group with four distinct, well-supported, subfamilies being upheld (Thymelaeoideae, Aquilarieae, Gonystyloideae, and Synandrodaphnoideae). The stem age (50.72 Mya) of the family is consistent with the report of pollen in Europe in the Eocene (Krutzsch, 1996; Muller, 1981), as well as evidence from Raven and Axelrod (1974) who speculated that the family is older than the Oligocene. Continental plates moving northward in Africa during the Eocene (Behrensmeyer *et al.,* 1992) could have triggered the dispersal or migration of Thymelaeaceae from Europe to other continents, although the geographic origin of the family is unclear from the phylogenetic tree. As Africa was gradually drifting apart from other Gondwanan land masses (e.g. Madagascar, South America) the filter for diversification was gradually narrowing down. By the time the Thymelaeaceae stem group diversified, the former Gondwana area and other continents had already separated and southern continents where remote from each other. Divergence within the family spread during the Eocene to the Oligocene and colonisation of genera is more concentrated in Miocene. Following their hypothesised southward migration during the Oligocene, the majority of genera are colonised in the southern hemisphere, with the exception of a few (e.g. *Thymelaea* and *Daphne,* which occur in the Mediterranean/Euro-Siberian region). For instance, the subfamilies Synandrodaphnoideae and Gonystyloideae split during the Oligocene and were colonised in South Africa, South America, Eurasia and Madagascar during the Miocene epoch, and diverged from each other 44.34 Mya. Colonisation in Eurasia by subfamily Aquilarieae took place during the Late Miocene. A definitive age for each subfamily could not be made as denser sampling is required.

Sub-family Thymelaeoideae split from Aquilarieae in the Mid-Eocene (40.06 Mya). The origin of this subfamily, and that of clade I within the subfamily, is not clear from the phylogenetic reconstruction; despite this there are several interesting dispersal events within clade I that can be discussed. *Gnidia* species seem to have arrived in separate events, as indicated by the four separate lineages recovered in the phylogenetic tree. The oldest lineage is lineage 3 (crown age 21.14 Mya), followed by lineage 4 (crown age 20.86 Mya), then by lineage1 (crown age 14.86 Mya) and the youngest is lineage 2 (crown age 13.73 Mya). Lineage 3 is marked by transoceanic long-distance dispersal; the southern

African *Gnidia* species in this lineage are colonised in southern Africa, whilst *Pimelea* (including *Thecanthes*) dispersed to Australasia. The southern Africa *Gnidia* seem to have colonised Africa from 9-10 Mya, a date that is concurrent with the radiation of the Cape Flora (Levyns, 1964; Linder *et al.,* 1992, Goldblatt and Manning, 2000).

Based on my results and those of previous studies, it has been shown that *Pimelea* is nested within South African *Gnidia* (Van der Bank *et al.,* 2002; Rautenbach, 2006; Beaumont *et al.,* submitted and Motsi *et al.,* submitted), and reached Australia via dispersal across the Indian Ocean. Upon arrival in Australia they then could have adapted to varied environmental conditions. The advanced floral structure, with a reduced number of stamens, and no corolla lobes, could have influenced the ability of *Pimelea* to diversify and be adaptive to Australia.

There are 90 endemic species of *Pimelea* with seven sections, distributed in Australia. These species are very diverse in terms of their morphology and their habitat. *Thecanthes* which is reinstated as *Pimelea* (Motsi *et al.,* submitted) is dispersed in the Philippines and North Australia. Australia experienced a drier climate and seasonal weather between 25 and 10 Mya which resulted in a decline of lineages such as *Nothofagus*, whilst in other groups increased speciation rates occurred, for example in pea-flowered legumes (Crisp *et al.,* 2004). Restionaceae show a further example of divergence from an African lineage (Linder *et al.,* 2003). Before these climatic changes it is known that *Adansonia* dispersed from Africa to Australia (Baum *et al.,* 1998), as well as Gnaphalieae (Asteraceae; Bayer *et al.,* 2002; Bergh and Linder, 2009). There are a few instances where the dispersal is from Australia northward or to New Zealand such as *Flindersia* R.Br. (Rutaceae; Scott *et al.,* 2000), *Alectryron* Gaertn. (Sapindaceae; Edwards and Gadek, 2001), and Gnaphalieae (Asteraceae; Breitwieser *et al.,* 1999).

New Zealand species of *Pimelea* are derived from Australian stock. The estimated time of divergence of New Zealand from Australian *Pimelea* (6.6 Mya) is consistent with the fossil assigned to *Pimelea* in New Zealand that dates back to the Pliocene (Macphail and Cantrill, 2006). There are 19 endemic New Zealand species. According to Burrows (2008), there is an enormous floral and vegetative diversity in Australian *Pimelea* species in comparison with that in New Zealand species which are often difficult to distinguish. He also mentioned that the New Zealand species did not conform to the infrageneric classification of Rye (1990). The New Zealand *Pimelea* are mostly recognised as gynodioecious, bisexual flowers capable of producing seeds, development of ovaries into succulent, drupe-like fruits after fertilisation, these characters could have motivated the capacity to diversify in New Zealand (Burrows, 1960; 2008). The fleshy fruits of New Zealand *Pimelea*, are likely to be swallowed by birds (including seabirds for coastal dispersal) although this has not been observed (Burrows, 1958; Dawson *et al.,* 2005).

It could be concluded that the dispersal of *Pimelea* to New Zealand is the result of the prevailing winds and bird migrations from west to east Australia. In a previous study, which showing this route of dispersal, Wagastaff and Wege (2002) suggested that Stylidiaceae has Australian origins and that the species diversified in New Zealand. Pole (1994) and Macphail (1997) mentioned that the extant flora of New Zealand must have arrived from Australia during the renewed uplift in the Miocene. Though *Pimelea* was diversifying in the Middle Miocene most of diversification happened during Late Miocene to Pliocene within Australia, making this argument plausible.

Lineage 2, which comprises some of the Sub-Sahara Africa species, split from lineage 1 around 19.64 Mya, and has the youngest crown age of the four lineages (13.73 Mya). According to Beaumont *et al.* (submitted) there are no synapomorphic characters between species of *Struthiola* and *Gnidia pinifolia* and *G. racemosa* (which make up this lineage), and so generic limits will need to be reconsidered. Despite that, the age estimates are in accordance with the events that correspond to the radiation of the Cape flora (Levyns, 1964; Linder *et al.*, 1992, Goldblatt and Manning, 2000) inferred by the Benguela Upwelling. Amongst other genera which diversified in the Cape flora during Middle to Late Miocene are *Phylica* L. (8-7 Mya; Richardson *et al.*, 2001), *Ehrharta* Thunb. (9.8 Mya; Verboom *et al.*, 2003), and *Indigofera* L. (6-17 Mya; Schrire *et al.*, 2003).

In lineage 4, our estimate of the minimum divergence of Madagascan species of the genus *Gnidia* therefore shows a colonised Madagascar species of *Gnidia* and dispersal to Sub-Sahara Africa by other members of the genus. This is consistent with the theory of Yoder and Nowak (2006), which claims that numerous Malagasy taxa are the closest sister groups to African taxa, and dispersal occurred during the Cenozoic. This is indicated by a high posterior probability (one) and the impending reinstatement of the generic name

Lasiosiphon for lineage 4 (Beaumont *et al.,* submitted). The date estimation assumes that *Gnidia* originated in Madagascar then dispersed in Sub-Sahara Africa. Aymonin (1965) noted that there are surprising morphological similarities between Madagascan members of the tribe Thymelaeeae and East African *Gnidia*.

Lineage 1 is of Sub-Saharan African origin, and consists of the *"Gnidia-Kelleria'* clade, which split from the exclusively Sub-Sahara Africa *"Passerina-Lachnaea"* clade 17.42 Mya. The *"Gnidia- Kelleria'* clade was colonised in Sub-Sahara Africa and subsequently *Kelleria* and *Drapetes* dispersed to Australasia and South America respectively. The close relationship between *Drapetes* and *Kelleria* has been mentioned previously (Head, 1990), along with the fact that the flowers of *Gnidia s.l.* are very similar to those of *Kelleria* and *Pimelea*.

Elsewhere in the phylogeny, clade II is shown to have a Mediterranean/Euro-Siberian origin. Within the clade, the Mediterranean genera *Thymelaea* and *Daphne* are recovered as a sister relationship with strong support. It is worth mentioning that the age estimate for the colonisation of the two genera in the Meditarranean (15.41 Mya) is within the minimum age of 11.2 Mya estimated by Galacia-Herbada in 2006. Of the Eurasian genera, *Diarthron* is paraphyletic while *Stellera* is embedded within *Wikstroemia*. However, a wider sampling for the '*Wikstroemia-Diarthron'* clade should be examined before more definitive claims on its detailed relationships and estimated divergence times are made. Although Huang and Zhang (1999) indicated that it is problematic to separate *Wikstroemia* from *Daphne* from a morphological perspective, my phylogeny clearly shows the two are separated, having split 20.87 Mya. Clade III has a Sub-Sahara African origin and dispersed to Eurasia.

In summary, the family has had a dynamic biogeographic history. Although its geographic origin is unclear, several events can be inferred from the phylogenetic reconstruction of the group. For example node 3 (Fig. 4.3) has a Sub-Sahara African origin and underwent inter-continental dispersal to Australasia and South America. The divergence time estimates show that the family Thymelaeaceae is considerably younger than the break up of Gondwana, implying that dispersal is a more viable method of diversification than vicariance. Further studies should conduct reconstruction of biogeography using character optimisation methods in order to investigate in greater detail the complex history of the family.

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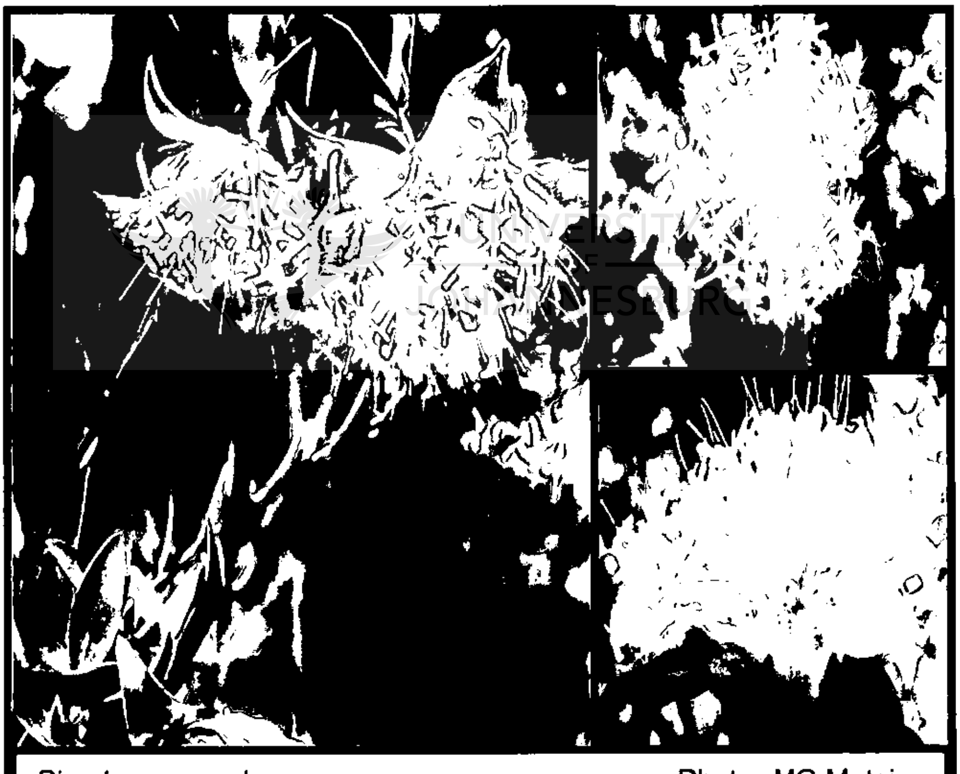
CHAPTER FIVE

GENERAL CONCLUSIONS



Pimelea suaveolens Photos:MC Motsi

5.1. General conclusions

The aim of this thesis was to elucidate the phylogeny of *Pimelea* and *Thecanthes* using a robust sampling of DNA sequence data. This is the first time that molecular data were used to reconstruct the phylogeny of the subtribe Pimeleinae. Molecular phylogenetic analyses of plastid and nuclear DNA sequences were used to reassess the relationship of the Australasian genera *Thecanthes* and *Pimelea* (Chapter 2), reconstruct a detailed phylogeny of *Pimelea* (including *Thecanthes*; Chapter 3), and estimate the dates of divergence within Thymelaeoideae with emphasis on *Pimelea* and *Thecanthes* (Chapter 4). The key findings of the study will be outlined below.

Using multiple DNA markers, I found that both the family Thymelaeaceae and subfamily Thymelaeoideae are monophyletic, as reported in other studies (Van der Bank *et al.*, 2002; Robinson, 2004; Van Niekerk, 2005; Rautenbach, 2006; Beaumont *et al.*, submitted). Further, I found the subtribe Pimeleinae to be monophyletic with all of the molecular data presented in this study. Phylogenetic trees based on plastid and nuclear data sets all concur in indicating that *Thecanthes* is nested within *Pimelea*; and therefore it has been reduced to a synonym and a new combination made (in Motsi *et al.*, submitted) for one species transferred to *Pimelea*. *Pimelea* would then be the only genus within subtribe Pimeleinae. This finding sheds light on what has been a matter of taxonomic confusion for many years (Bentham, 1873; Gilg, 1894; Threlfall, 1983; Rye 1988). The placement of *Thecanthes* within *Pimelea* fits the morphological description of these two genera in that they both have a reduced number of stamens to two (rarely one stamen - in one Tasmanian species of *Pimelea*), possess four sepals and no corolla lobes (Rye, 1988).

The close relationship of *Pimelea* and southern African *Gnidia* has always been questioned and remains of interest (Van der Bank *et al.,* 2002; Rautenbach, 2006; Beaumont *et al.,* submitted). It is clear that the sister relationship of *Pimelea* with one clade of the polyphyletic *Gnidia* is still maintained as suggested by Van der Bank *et al.* (2002); Rautenbach, (2006); Beaumont *et al.* (submitted). This sister relationship is surprising, as there is a high degree of morphological divergence between *Pimelea* and *Gnidia*. Examination of the biogeographical history (in chapter 4), shows southern African *Gnidia* and *Pimelea* to have had the same direct ancestor, having split 21.14 Mya. It is shown that *Pimelea* is derived from *Gnidia* and subsequently dispersed to Australia, whereupon it developed adaptive traits which enabled it to colonise new niches within Australia. Beaumont *et al.* (submitted) suggested sectional changes within polyphyletic *Gnidia* to include *Pimelea*. In view of their morphological differences, I suggest that the genus *Pimelea* be maintained until there is enough evidence to determine more conclusively how many genera should be recognised.

The status of sections within subtribe Pimeleinae has changed since Bentham's initial proposal (1873). Based on the topologies presented here (Chapter 3), none of Rye's sections (1988) are upheld in their current delimitation although she suspected several of the results I found based on molecular data (Rye, 1999). For example, sections *Heterolaena* and *Epallage* should be expanded to include some species previously in section *Calyptrostegia*. New placements need to be found for those Australian species that have been included in section *Pimelea*. The results do show, however, that the New Zealand *Pimelea* species (section *Pimelea*) form a weakly supported clade, which is sister to *Pimelea alpina* (currently in section *Calyptrostegia*) (chapter 3). Low levels of genetic variation suggest that *Pimelea* is the product of a recent and rapid radiation. Precise relationships between species were only recovered within the limitations imposed from the lack of DNA variation. Nevertheless some phylogeographical patterns were found, such as the single colonisation of New Zealand from New South Wales, as far as my sampling is concerned.

My dating exercise using Bayesian statistics shows that the origin of the family Thymelaeaceae is in the Eocene (60.72 Mya). I also determined that sub-family Thymelaeoideae split from Aquilarieae in the Mid-Eocene (40.06 Mya), although the geographic origin of this sub-family is not clear from the phylogenetic reconstruction. The sub-families Synandrodaphnoideae and Gonystyloideae split during the Oligocene and were colonised in South Africa, South America, Eurasia and Madagascar during the Miocene epoch, and diverged from each other 44.34 Mya. Colonisation in Eurasia by sub-family Aquilarieae took place during the Late Miocene. The phylogeny indicated a Sub-Sahara African origin of the clade comprising *Pimelea*, which subsequently dispersed to Australasia. The derived morphological characters within subtribe Pimeleinae (e.g. those relating to diverse breeding systems and life forms) could have facilitated morphological and species diversification, as they may have aided adaptation to a new environment, particularly with respect to colonising disturbed areas more efficiently. This phylogeny also confirmed that the genus is relatively young, having colonised Australia 13.38 Mya and New Zealand 4.1 Mya. This substantiates the assumption in chapter 3 that section *Epallage* is the oldest lineage which gave rise to the rest of the genus, and that New Zealand species were derived at a later stage from Australian species following a single dispersal event from Australia to New Zealand.

The level of genetic variation in *Pimelea* has been exceeded by the amount of morphological diversity within this genus. The sectional delimitations of the genus are supported by geography more than morphology. Thus, I suggested that the number of sections should be reduced and changes made to the circumscription of all of the four main sections (*Heterolaena*, *Calyprostegia*, *Epallage* and *Pimelea*), as proposed in chapter 3 with section *Pimelea* restricted (or largely restricted) to the New Zealand *Pimelea* species.

5.2. Future research

Finally, I would like to make a few suggestions for future research following this thesis. I suggest increasing sampling for *Pimelea*, if possible from fresh material given the difficulties encountered in obtaining high-quality DNA from herbarium samples, and including the species from Lord Howe Island and the mountainous parts of New Zealand. Using this increased sampling, phylogenetic analyses should be conducted. The type species, *Pimelea prostrata,* is also crucial for future DNA studies. Since attempts to amplify 80% of the sequence or other regions failed, further attempts should also be made to generate DNA sequence data for all the geographic regions studied here. This would enable the production of a complete species-level phylogenetic reconstruction of *Pimelea*, from which we would be better placed to address questions regarding the rate of evolutionary diversification of the genus, and to elucidate the precise relationships between all extant taxa.

The use of complete species-level phylogenies, especially when combined with geographical and ecological data, allows for causes of diversification to be evaluated more fully (Barraclough and Nee, 2001). As phylogenetic reconstruction provides an indirect record of the speciation events that have resulted in the extant taxa observed in a given group, to maximise the efficiency and accuracy of such methods all species of a given group should be included. Missing species from a group reduces the sample size of reconstructed speciation events available, and can introduce bias into the observed pattern, for example the removal of the most recent speciation events and introduction of the effects of other evolutionary processes such as extinction (Nee *et al.*, 1994; Barraclough *et al.*, 1998; Barraclough and Nee, 2001).

Because *Pimelea* displays low genetic variation in gene sequences, it makes it also difficult to explore potential cases of hybridisation. To evaluate hybridisation more fully, I would suggest the use of other molecular techniques such as microsatellites and Amplified Fragment Length Polymorphism (AFLP), which will be appropriate to complement the DNA sequence data used here for the genus *Pimelea.*

For the complete reconstruction of biogeography within the family, and for the genus *Pimelea* in particular, character optimisation methods should be used.

Character optimisation methods can also be employed to look at causes of diversification within the group, and to see which traits have promoted diversification events.

So far, I have reconstructed the molecular phylogeny of the second largest genus in the family. I have simplified the taxonomy by transferring the genus *Thecanthes* back into *Pimelea*; this will be helpful for future research when adding more samples to the phylogeny or using different molecular techniques. My study has also provided new insight into the radiation of this genus. The estimated time of divergence of the genus *Pimelea* will also be useful for determining the age of radiation for other genera within the family.

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