UNIVERSITY OF KWAZULU-NATAL

SYSTEMATIC STUDIES IN *GNIDIA* L.

(THYMELAEACEAE)

ANGELA JANE BEAUMONT

SYSTEMATIC STUDIES IN *GNIDIA* L.

(THYMELAEACEAE)

by

ANGELA JANE BEAUMONT

Submitted in fulfilment of the academic requirements for the degree of

DOCTOR OF PHILOSOPHY

in the

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Regular consultation took place between the student and ourselves throughout the investigation. We advised the student to the best of our ability and approved the final document for submission to the Faculty of Science and Agriculture Higher Degrees Office for examination by the University appointed Examiners.

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DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis (include publications in preparation, submitted, *in press* and published and give details of the contributions of each author to the experimental work and writing of each publication)

Publication 1

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Publication 2

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Publication 3

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Publication 4

EDWARDS, T.J., BEAUMONT, A.J. & STYLES, D. 2001. New records and distributional disjunctions from South Africa, Zimbabwe and Mozambique. *Bothalia* **31**: 199–201.

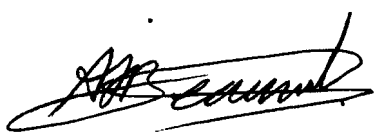
Publication 5

BEAUMONT, A.J., EDWARDS, T.J. & SMITH, F.R. 2006. The first record of gynodioecy in a species of *Gnidia* (Thymelaeaceae) from South Africa. *Botanical Journal of the Linnean Society* **152**: 219–233.

Publication 6

BEAUMONT, A.J., EDWARDS, T.J., MANNING, J., MAURIN, O., RAUTENBACH, M., MOTSI, M.C., FAY, M.F., CHASE, M.W. & VAN DER BANK, M. 2009. *Gnidia* (Thymelaeaceae) is not monophyletic: taxonomic implications for Thymelaeoideae and a partial new generic taxonomy for *Gnidia*. *Botanical Journal of the Linnean Society* **160**: 402–417.

I am the senior author of all the publications except for publication 4, in which I am a co-author. In publication 6, the molecular work was done by M. VAN DER BANK and M. RAUTENBACH (Molecular Systematics Laboratory, Department of Botany and Plant Biotechnology, University of Johannesburg, South Africa).



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ABSTRACT

SYSTEMATIC STUDIES IN *GNIDIA* L. (THYMELAEACEAE)

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Gnidia L., variously estimated to contain 100–160 species, is the largest genus in the sub-cosmopolitan family Thymelaeaceae. Most species are shrubby, and occur in tropical and southern Africa, with one species reaching southern India and Sri Lanka, and 14 species endemic to Madagascar. Assorted segregate genera have been established using characters considered by some as too few, too trivial or unreliable. Generic limits have been contentious with authors following either a narrower concept of *Gnidia* or a broader circumscription within which segregate genera are placed in synonymy under *Gnidia*. Regional treatments for African and Madagascan floras have been published over the last century until very recently, but the genus was last revised in its entirety 153 years ago. Today, a broad-based concept of *Gnidia* is generally recognised, but there is no modern infrageneric classification, and species relationships are poorly understood.

Homogenous groups of species are identified by their similarities of leaf length and width or bract length and width ratios. Species comprising the homogenous groups for leaf ratios differ to those comprising the homogenous groups based on bract ratios, and there is no correlation between leaf and bract length and width ratios. This suggests that the factors influencing leaf diversity differ from those influencing

bract diversity. Bracts differ most from leaves in species with capitate inflorescences, and involucres of several layers of bracts likely protect reproductive organs (flowers) in heads. Previously overlooked morphological and micromorphological details, and morphometric analyses of leaf, bract and floral dimension data help define individual species, and clades of species derived from phylogenetic analyses of molecular data. Evidence from a phylogenetic analysis of nuclear ribosomal and plastid DNA sequence data confirms the polyphyly of *Gnidia*. Three lineages contain *Gnidia* species and species of genera from southern Africa, southern South America or Australia, while another lineage corresponds largely to the previously recognized genus *Lasiosiphon*. The genus *Lasiosiphon* is reinstated characterised by flowers mostly in heads, bracts different from the leaves, and the presence of smooth hairs; it now includes species with tetramerous flowers as well as ones with pentamerous flowers. Gynodioecy is recorded for the first time in a single species and represents the first documented example of sexual polymorphism involving unisexual flowers in *Gnidia* and sub-Saharan Thymelaeaceae.

The findings of this thesis are discussed in terms of their phylogenetic value and contribution to our better understanding of the generic limits of *Gnidia* and its relationships with other southern hemisphere Thymelaeoideae. The circumscription and generic affinities of *Gnidia* as suggested by results presented in this thesis are compared to previous classification systems for congruence and dissimilarity.

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CHAPTER 1

INTRODUCTION

The genus *Gnidia* L. belongs to the Thymelaeaceae, a sub-cosmopolitan family found in temperate and tropical regions of both the northern and southern hemispheres, with Africa, Australia and South America especially rich in genera and species. Forty-five to 60 genera, with 500 to 800 species have been recognized (ROBYNS 1975, RYE 1990, BRUMMITT 1992, MABBERLEY 1997, TAKHTAJAN 1997, HERBER 2003, PETERSON 2006 and HEYWOOD ET AL. 2007). The family comprises mostly trees and shrubs with fewer herbs and lianas. Taxa commonly inhabit temperate and tropical forests, grasslands, alpine habitats and regions experiencing Mediterranean-type climates of seasonally hot, dry summers, and cold, wet winters. (HEYWOOD ET AL. 2007). *Pimelea halophila* Rye (subfamily Thymelaeoideae) from Western Australia is adapted to conditions of high salinity (RYE 1990), and the pneumatophores of *Gonystylus bancanus* (Miq.) Kurz. (subfamily Gonystyloideae sensu DOMKE 1934 or Octolepidoideae sensu HERBER 2003) are an adaptation to the low oxygen conditions of the peat swamp-forest habitat of this species.

Subfamilies of the Thymelaeaceae

GILG (1921) recognised seven subfamilies in the Thymelaeaceae, namely: Microsemmatoideae, Octolepidoideae, Aquilarioideae, Phalerioideae, Synandrodaphnoideae, Thymelaeoideae and Drapetoideae. DOMKE (1934)

presented a more popular classification that divided the Thymelaeaceae into four subfamilies, namely Gonystyloideae, Aquilarioideae, Gilgiodaphnoideae and Thymelaeoideae. ARCHANGELSKY (1971) accepted the Aquilarioideae, Gilgiodaphnoideae and Thymelaeoideae of DOMKE (1934), and added three more subfamilies, namely: Microsemmatoideae, Octolepidoideae and Synandrodaphnoideae to establish a total of six subfamilies in the Thymelaeaceae. Evidence from studies of molecular data sets (discussed below and in Chapter 2 of this thesis) has lent the most recent support to DOMKE's (1934) long-lasting classification into four subfamilies.

Using data from studies of pollen morphology, wood anatomy and flower morphology, HERBER (2002, 2003) and KUBITZKI & CHASE (2003) recognised only two subfamilies in the Thymelaeaceae, namely Octolepidoideae and Thymelaeoideae, later supported by ROGERS (2005) in his revision of *Octolepis* Oliv. HERBER (2002) conflated the Aquilarioideae and Synandrodaphnoideae with the Thymelaeoideae, and transferred *Octolepis* from the Aquilarioideae to the Gonystyloideae. The transfer of *Octolepis* made it necessary to change the name of this recircumscribed Gonystyloideae to Octolepidoideae, because as the older of the two names, Octolepidoideae (GILG 1901), has priority over Gonystyloideae (DOMKE 1934), (HERBER 2002, ROGERS 2005). The most recent molecular studies by VAN DER BANK ET AL. (2002) and BEAUMONT ET AL. (2009), however, support the four subfamilies sensu DOMKE (1934) (Table 1.1).

The trend within the family has thus been to recognise four subfamilies based on evidence from molecular-based studies; whereas evidence from non-molecular

studies specifically those drawing on features of pollen, wood and floral anatomy supports only two subfamilies, with the smallest of these subfamilies, the Gilgiodaphnoideae now known more correctly as the Synandrodaphnoideae (ROBYNS 1975, MABBERLEY 1997).

Taxonomic affinities of the Thymelaeaceae

Taxonomic affinities of the Thymelaeaceae have been determined in part, by whether the petal-like structures that sit in the sinuses of the sepal lobes are true petals, or whether flowers are apetalous. HEINIG (1951) reviewed the various interpretations of these structures, among which that they are petals, scales, stipules or stamens. GILG (1894a), HUTCHINSON (1926), LEANDRI (1930) and DOMKE (1934) took these structures to be true petals, whereas others, for example MEISNER, (1857), BAILLON (1880) and BENTHAM & HOOKER (1880-1883) called them scales. HEINIG (1951) considered them to be petaloid scales rather than petals and showed that their vascular systems are derived from side branches of the sepal vascular traces, instead of consisting of a separate vascular whorl, as in separate sepal and corolla whorls. Among the most recent treatments, BEYERS (2001) called these structures floral scales, whilst HERBER (2003) and PETERSON (2006) called them petals. ROGERS (2004, 2005 and 2009) has referred to them variously as petals, petaloid scales or scale-like appendages. Absent in some groups altogether, these structures nevertheless strongly influenced early thoughts on taxonomic affinities of the Thymelaeaceae.

DE JUSSIEU (1789) provided the root of the family name Thymelaeaceae when he established the order Thymelææ in his Class VI. DE JUSSIEU (1789) considered the Thymelææ apetalous and placed it between the orders Elæagni and Proteæ, together with the Lauri, Polygoneæ and Atriplices, all similarly lacking petals and with perigynous stamens. Today, these orders are broadly equivalent to the families Thymelaeaceae, Elaeagnaceae, Proteaceae, Lauraceae, Polygonaceae and Chenopodiaceae, respectively. ENDLICHER (1847), MEISNER (1857) and BENTHAM & HOOKER (1880-1883) considered the Thymelaeaceae apetalous and sought relatives among similarly petal-less families, whilst BAILLON (1880) who also considered the family apetalous nevertheless allied the Thymelaeaceae with petalous families. The Thymelaeaceae has also been linked to the petalous Myrtales and Rosaceae and the apetalous Oliniaceae and Penaeaceae (reviewed in HEINIG 1951).

BENTHAM & HOOKER (1880-1883) placed the five orders Thymelaeaceae, Laurineae, Proteaceae, Penaeaceae and Elaeagnaceae together in the series Daphnales. DE JUSSIEU (1789) and BENTHAM & HOOKER (1880-1883) both recognized a strong relationship among the orders Thymelaeaceae, Laurineae, Proteaceae and Eleagnaceae and BENTHAM & HOOKER's (1880) series Daphnales largely agrees with DE JUSSIEU's (1789) Class VI. Both the Daphnales and 'Class VI' were defined by the (usually) monocarpic ovary, hermaphrodite flowers with a well-developed calyx, and perigynous stamens usually as numerous as, or twice as numerous as the number of flower lobes among members. BENTHAM & HOOKER (1880) furthermore used vegetative features, and the usually uniovulate locules to help define the Daphnales. HUTCHINSON (1959) recognized the order

Thymelaeales comprising five families namely Gonystylaceae, Aquilariaceae, Geissolomataceae, Thymelaeaceae and Nyctaginaceae. All five families have flowers that usually lack petals, a hypanthium that forms the cup-shaped or tubular flower body, usually monocarpellate ovaries with few ovules, stipules that are minute and glandular or absent, and inflorescences in heads surrounded by an involucrum of leafy bracts. HUTCHINSON (1959) placed the Thymelaeales between the Bixales (petals present) and the Proteales (petals absent), furthermore stating his belief that the Proteales and Thymelaeaceae were “clearly related”. Altogether, the Bixales, Thymelaeales and Proteales comprised a distinctive group according to HUTCHINSON (1959), but he did not specify characters to distinguish this from other groups of orders. Today, the families Aquilariaceae, Gonystylaceae and Thymelaeaceae are commonly recognised as subfamilies of the Thymelaeaceae, *sensu* DOMKE (1934), whilst the Geissolomataceae is now placed in the Crossomatales, and the Nyctaginaceae in the Caryophyllales (HEYWOOD ET AL. 2007).

LEANDRI (1950) emphasized the homogeneity of the Thymelaeaceae and the difficulties in distinguishing it from associated families. He considered the Thymelaeaceae as having petals and best placed between the apetalous Elaeagnaceae and petalous Rosaceae (both in the order Rosales). Both the Elaeagnaceae and the subfamily Thymelaeoideae of the Thymelaeaceae have a tubular hypanthium, perigynous stamens near the flower mouth, often a two-locular ovary and both lack petals. Given these shared features, at first glance, flowers of *Elaeagnus* L. especially, look like flowers of many Thymelaeoideae. LEANDRI (1930) also believed that the sub-family Phalerioideae sensu GILG (1921), comprising

Phaleria Jack and *Peddiea* Harv., was close to the Proteaceae on account of both groups having a single carpel with two, more or less separate uniovulate locules. DOMKE (1934) also grouped together *Phaleria* and *Peddiea* in the tribe Phalerieae, under the Thymelaeoideae. The Phalerieae are exceptional in their two-locular ovaries in contrast to the rest of the Thymelaeoideae, in which the ovary is one-locular.

The circumscription of the Thymelaeaceae by HUTCHINSON (1959) largely corresponded with that of the subfamily Thymelaeoideae, being the largest of four subfamilies proposed by DOMKE (1934). HUTCHINSON (1959) divided the dicotyledons into two divisions: Lignosae for mostly woody groups (including Thymelaeaceae in Thymelaeales) and Herbaceae for mostly herbaceous groups. HUTCHINSON (1959) included five other families in the Thymelaeales namely: Gonystylaceae, Aquilariaceae, Geissolomataceae, Penaeaceae and Nyctaginaceae. HUTCHINSON (1959) considered the apetalous Thymelaeales closely related both to the Bixales (petals are present in six of the eight member families) and the apetalous Proteales represented by a single family, the Proteaceae. He also believed the mostly austral distributions of both the Thymelaeaceae and the Proteaceae also supported their close relationship, and suggested that the Thymelaeales was allied to the petalous family Flacourtiaceae in the order Bixales.

Evidence for and against including the Thymelaeaceae in the Myrtales was weighed up during the Myrtales symposium held during the thirteenth International Botanical Congress, in Sydney, Australia in 1981. RAVEN (1984) summarized the majority viewpoint among participants, namely that Thymelaeaceae should be excluded from

the Myrtales and instead grouped with the Euphorbiaceae of the Malpighiales. THORNE (1976) briefly included the Thymelaeaceae in the Myrtales, but later concurred with popular opinion that the family should be placed in its own order. DAHLGREN & THORNE (1984) reviewed historical opinion that showed mainstream support for excluding the Thymelaeaceae from the Myrtales and instead placing it in its own order, the Thymelaeales. Only CRONQUIST (1984) consistently argued for the inclusion of the Thymelaeaceae in the Myrtales.

DAHLGREN & THORNE (1984) excluded the Thymelaeaceae from the Myrtales based on the following characters that differ from most Myrtales: stipules absent (versus stipules mostly present); a pseuodomonomerous ovary in which the second carpel fails to develop, pollen grains pantoporate (also known as zonocolpate with many pores), pollen grains with crotonoid tectums; a seed wall structure altogether more resembling the condition in Euphorbiaceae; and pendulous ovules with an obturator (which guides the pollen tube to the egg cell) descending from the base of the stylar canal. Above all, DAHLGREN & THORNE (1984) considered that embryological and chemical evidence excluded the Thymelaeaceae from the Myrtales, although they also acknowledged their many other similarities. Instead, DAHLGREN & THORNE (1984) suggested the Thymelaeaceae was more closely related to the Euphorbiaceae, whilst both families were nevertheless allied with the Myrtales. Structurally unique diterpenes with daphnane, ingenane and tigliane-type skeletons are present only in members of the Thymelaeaceae and Euphorbiaceae which furthermore supports their close relationship (HE ET AL. 2002). DAHLGREN & THORNE (1984) argued that even if the most primitive subfamily, the Gonystyloideae was removed other discordant features among the remaining

members of the Thymelaeaceae would still exclude it from the Myrtales. Whilst CRONQUIST (1984) acknowledged some features of the Thymelaeaceae would make it somewhat atypical within the Myrtales, he nevertheless supported its inclusion within this order because he disliked the idea of establishing a new order for this single family as TAKHTAJAN (1980) had done.

Molecular studies using the plastid gene ribulose biphosphate carboxylase (*rbc*L), and combined chloroplast and adenosine triphosphate (*atp*B) sequence data support the inclusion of the Thymelaeaceae in the Malvales (reviewed in ROGERS 2005). Furthermore the ANGIOSPERM PHYLOGENY GROUP (APG 2003) adopted an expanded concept of the Thymelaeaceae, which included the monogeneric Tepuianthaceae Maguire & Steyerm., which WURDACK AND HORN (2001) placed as sister to the Thymelaeaceae based on a combined study of morphological features and nuclear and plastid genome analyses. NANDI ET AL. (1998) also used evidence from analysis of the *rbcL* gene and morphological data to support the inclusion of the Thymelaeaceae in a broad-based concept of the Malvales. CONTI ET AL. (1996), in a phylogenetic analysis of *rbcL* variation, found strong support for the monophyly of the Myrtales, with Thymelaeaceae, Malvales, Sapindales, together with an expanded Capparales most likely forming a sister clade. In a later study CONTI ET AL. (1997) included two species of the Thymelaeaceae among the outgroup taxa in an attempt to identify morphological synapomorphies that might lend support to the *rbcL*-based topology of the Myrtales in their previous work CONTI ET AL. (1996). Using molecular and other data KUBITZKI AND CHASE (2003) identified four clades within the Malvales, including the ‘thymelaean clade’ with two families, Thymelaeaceae and Tepuianthaceae.

Authors of plant names

Authors of plant names follow BRUMMITT & POWELL (1992).

Geographical distributions of the Thymelaeaceae

The Aquilarioideae and most of the Gonystyloideae (sensu DOMKE 1934) occur in Malaysia and East Asia (HOU 1960, HUTCHINSON 1967, TAWAN 1999). A few members of the Gonystyloideae also occur in Australia, New Caledonia and New Hebrides, with one genus, *Octolepis* Oliv., found in tropical West Africa and Madagascar (ROGERS 2009). The monotypic Synandrodaphnoideae is also found in tropical West Africa (HOU 1960, HUTCHINSON 1967, ROBYNS 1975, RYE 1990, MABBERLEY 1997). In contrast, the largest subfamily, the Thymelaeoideae, is almost cosmopolitan and comprises continental and island-based taxa (BEAUMONT ET AL. 2006).

Thymelaeaceae in sub-Saharan Africa

The Thymelaeoideae in sub-Saharan Africa is represented by nine genera (including *Gnidia* sensu lato), of which three are also represented in Madagascar. Madagascar is also home to the monotypic endemic genus *Atemnosiphon* Leandri, with eight species of *Stephanodaphne* Baill. endemic to Madagascar, and one species endemic to Mayotte in the Comoro Islands (ROGERS 2004, BEAUMONT ET AL. 2006).

Table 1.1. Characters of the subfamilies Gonystyloideae, Aquilarioideae, Synandrodaphnoideae and Thymelaeoideae according to DOMKE (1934). Compiled from HOU 1960, ROBYNS 1975, TAN 1980, RYE 1990, BRUMMITT 1992 and MABBERLEY 1997.

Gonystyloideae	**Aquilarioideae**	**Synandrodaphnoideae**	**Thymelaeoideae**
8 genera	6 genera	1 genus	c. 36 to 45 genera
Leaves pellucid-punctate	Leaves not pellucid-punctate	Leaves not pellucid-punctate	Leaves not pellucid-punctate
Flowers bisexual or unisexual	Flowers bisexual, rarely unisexual	Flowers bisexual	Flowers bisexual or unisexual
Flowers cup-shaped	Flowers bell- or tube-shaped	Flowers tube-shaped	Flowers tube-shaped
Flower tube not constricted	Flower tube not constricted	Flower tube not constricted	Flower tube constricted or not
Stamens 8 to 80	Stamens 10 to 8, or 5, rarely numerous	Stamens 4; staminodes 4	Stamens 4, 8 or 10, rarely 2 or 1; or reduced to staminodes
Filaments free	Filaments adnate to flower tube	Filaments adnate to flower tube	Filaments adnate to flower tube
Locules 8 to 2	Locules 12 to 2	Locules 2	Locules 2 or 1
Fruit a dehiscent or indehiscent capsule	Fruit a dehiscent capsule	Fruit a dehiscent capsule	Fruit an indehiscent, fleshy berries or dry and achene-like

Octolepis Oliv., placed either in Aquilarioideae (DOMKE 1934) or Octolepidoideae Gilg (HERBER 2002, 2003, ROGERS 2005), has one tropical African, and five Madagascan species. The monotypic *Synandrodaphne* Gilg, placed either in the Synandrodaphnoideae Gilg (= Gilgiodaphnoideae, DOMKE 1934) or included in the Thymelaeoideae (HERBER 2002, 2003) occurs in West Africa (ROBYNS 1975).

Gnidia (*ca*.100–160 species) is the largest genus of the Thymelaeoideae, distributed mostly in southern and tropical East Africa. *Gnidia kraussiana* Meisn. is the most widespread species, extending from South Africa throughout eastern tropical Africa, the Middle East, western India and Sri Lanka. Species diversity is highest in the

Cape Province, and 14 species are endemic to Madagasacar. *Gnidia* species inhabit forest margins, grasslands and Fynbos (South Africa), from sea level to alpine levels of mountain slopes (PETERSON 2006).

Gnidia species, in the broad, inclusive sense as advocated most recently by PETERSON (2006), comprise small- to medium-sized shrubs or trees or perennials with annual stems produced from thickened, woody rootstocks. Leaves are estipulate, simple, sessile or shortly petiolate, entire, usually dorsiventrally flattened, rarely needle-like and hairless to densely hairy. Inflorescence form is diverse from single, axillary flowers to many-flowered heads. One or more rows of leafy, to modified and non-leafy involucral bracts subtend inflorescences, or bracts are absent altogether. Flowers are bisexual (one species is gynodioecious, BEAUMONT ET AL. 2006), with a tubular expanded hypanthium, usually constricted above the ovary, and with four or five terminal lobes. HEINIG (1951) concluded that the tubular part of the flower consisted of the lower calyx and adnate parts of the androecium, with four or five calyx lobes. Petals or petal-like structures between the calyx lobes may be membranous or fleshy, show consistent to variable development or be absent. Stamens number 5 or 8 (rarely 4), filaments are usually shorter than the anthers, and the ovary pseudomonomerous by abortion, unilocular with a single ovule, a lateral style and stigmas round to oblong. The tiny annular nectary is found at the base of the ovary, and fruits are dry achenes.

BEAUMONT ET AL. (2009) recognised the genus *Lasiosiphon* based on evidence from molecular data. Their results supported an amended circumscription of *Lasiosiphon* which included species with tetramerous flowers as well as species with

pentamerous flowers and not exclusively taxa with pentamerous flowers as originally defined by FRESENIUS (1838).

Molecular evidence (BEAUMONT ET AL. 2009) placed two species, *Gnidia pinifolia* L. and *Gnidia racemosa* Thunb. in *Struthiola* L. LINNAEUS (1753) listed *Gnidia pinifolia* as the first of three species in his *Species plantarum*, and *G. pinifolia* is the type species for *Gnidia* (ROGERS & SPENCER 2006). The taxonomic implications of these results are discussed in Chapters 5 and 7 of this thesis.

Pollination of Thymelaeoideae

The Thymelaeoideae are primarily entomophilous, with wind- and bird-pollination uncommon. However, pollination studies in the Thymelaeoideae are limited, but include for example studies in *Thymelaea* Mill. (CORNARA ET AL. 2005, EL-KLEBLAWY ET AL. 1996, EL-KLEBAWY & FREEMAN 1999) and *Struthiola* (MAKHOLELA & MANNING 2006). HENNING (1984) recorded butterfly pollination in flowers of *Gnidia kraussiana* Meisn. (= Lasiosiphon kraussii Meisn.) and SOMANATHAN ET AL. (2004) reported beetle pollination of *Lasiosiphon eriocephalus* (Meisn.) Decne. (= *Gnidia glauca* (Fresen.) Gilg.

Passerina L. currently represents the only exclusively anemophilous genus in the Thymelaeaceae. Anthers are exserted on relatively long filaments and dehiscence is extrorse, which maximizes pollen dispersal in the windy, springtime conditions of the Cape Fynbos Biome, where most species are found (BREDENKAMP 2002). Among Thymelaeaceae extrorse anther dehiscence is known only in *Passerina* species

(BREDENKAMP & VAN WYK 1996, HERBER 2002). The pollen walls of *Passerina* species are unusual in that the supratectal subunits (forming the typical crotonoid pattern of most Thymelaeaceae) are fused completely to form a smoother, continuous secondary reticulum, and grains lack the sticky pollenkit, typical of entomophilous genera. BREDENKAMP & VAN WYK (1996) considered *Passerina* to be phylogenetically advanced among Thymelaeoideae because of these adaptations to anemophily and raised the sub-tribe Passerininae sensu DOMKE (1934), containing only *Passerina,* to the tribe Passerineae.

Thymelaeaceae in horticulture

The genus *Daphne* L. from Eurasia is popular in horticulture, with a number of species, cultivars and varieties grown as ornamentals. In particular, *Daphne mezereum* var. *mezereum* L. with its attractive pink flowers and red berries, and the variant *D. mezereum* L. var. *alba* Aiton with white flowers and yellow berries are commonly grown (BRICKELL & MATHEW 1976, BLAMEY & GREY-WILSON 1989).

Pimelea species are popular horticultural subjects in Australia, used for amenity and roadside planting and in private gardens. ELLIOT & JONES (1993, 1997) outlined the requirements for cultivating two species of *Kelleria* Endl. and at least 84 species and varieties of Australasian *Pimelea* Banks & Sol. ex Gaertner. *Pimelea* species also have potential for indoor and container planting (SLATER ET AL. 1994). Accordingly, several studies have investigated the conditions necessary for the optimal growth and flowering of *Pimelea* species, for example SLATER ET AL. (1994) and KING ET AL. (1995).

African Thymelaeaceae, are poorly known in horticulture. *Dais cotinifolia* L. is cultivated today as an ornamental in South Africa. Plants form large shrubs or medium-sized trees with attractive clusters of pink flowers, hence the English vernacular ‘”Pompom Tree”. BEYERS (2001) found *Lachnaea* L. species hard to cultivate. Among *Passerina* species, *Passerina filiformis* L. has been cultivated in Britain and Europe since the eighteenth Century and *Passerina obtusifolia* Thoday is gathered for the wild flower industry in the Cape Province (BREDENKAMP 2002). BREDENKAMP (2002) suggested *Passerina falcifolia* C.H.Wright had potential as an ornamental, and species could be used to bind soil after clearance of invasive alien plant species (BREDENKAMP & VAN WYK 2003). *Gnidia* and *Lasiosiphon* species are not well-known among the general public and not often cultivated. However, *Gnidia virescens* Wikstr., is cultivated in greenhouses in Europe (PETERSON 2006).

Techniques used in the cultivation of other Thymelaeaceae may be useful in promoting *Gnidia* and related taxa in horticulture. *Pimelea* species respond well to pruning (ELLIOT & JONES 1997), and tip-pruning of *Pimelea ciliata* Rye encourages compact growth and even flowering, thereby making this species suitable as a pot plant (SLATER ET AL. 1994). Many grassland species of *Gnidia* develop long, bare and unattractive stems with leaves and flowers clustered at the ends of branches. Tip-pruning of *Gnidia* and *Lasiosiphon* species may, like in *P. ciliata*, promote bushiness and increase their attractiveness as garden subjects.

Ethnopharmacological uses and phytochemical properties of Thymelaeaceae

The Thymelaeaceae comprises many genera with species documented to have toxic, irritant or cocarcinogenic principles affecting animals and humans. Polyfunctional diterpenoid esters of the daphnane, tigliane and 1-alkyldaphnane type are responsible for the toxic effects of members of this family (BORRIS ET AL. 1988). HE ET AL. (2002) reviewed the diversity and biological activities of the structurally unique daphnane-type diterpene derivatives common to the Thymelaeaceae and Euphorbiaceae. Daphnane derivatives are mostly ring C-orthoseters and are more common among Thymelaeaceae than Euphorbiaceae. Daphnetoxin (from several *Daphne* L. species) was the first daphnane-type derivative to be recognized. Mezerein, a derivative of daphnetoxin, was first isolated from *Daphne mezereum* L. Mezerein was identified as the poisonous principle in livestock poisoning by the South African species *Gnidia burchellii* (Meisn.) Gilg (= *Lasiosiphon burchellii* Meisn.). Other South African Thymelaeaceae with toxic properties include *Englerodaphne ovalifolia* (Meisn.) E.Phillips and *Peddiea africana* (VAN WYK ET AL. 2005). Toxicity of *Gnidia* species to livestock can vary with season and locality. For example flowering plants of *Gnidia polycephala* (C.A.Mey.) Gilg are apparently more toxic than non-flowering plants (KELLERMAN ET AL. 2005). *Gnidia* extracts have also shown antileukemic properties (WATT & BREYER-BRANDWIJK 1962, HUTCHINGS 1996, VAN WYK & GERICKE 2000, HE ET AL. 2002). *Gnidia* and other Thymelaeaceae have tremendous pharmacological potential, but the useful daphnane orthoesters occur in trace amounts and there still is no large-scale cultivation sufficient for commercial purposes (HE ET AL. 2002).

Vernacular names and etymology

Vernacular or common names of southern African species reflect their domestic and medicinal uses, morphological features and toxic properties. Names derived from Dutch or Afrikaans, their English equivalents and Latin binomials include the following: *Aandbossie* (*Lasiosiphon wilmsii* C.H.Wright = *Gnidia wilmsii* (C.H.Wright) Engl.) meaning “evening-bush”, in reference to the fragrance of flowers in the evening; *Baardbossie* (*Lasiosiphon hoepfnerianus* Vatke & Engl. = *Gnidia kraussiana* Meisn.) meaning “beard-bush”, pertaining to the hairiness of plants; *Balbossie* (*Lasiosiphon microphyllus* Meisn. = *Gnidia microphylla* Meisn.) meaning “ball-bush”, in reference to the round inflorescences; *Basbos* meaning “bark-bush”, and *Gonnabas* (*Gnidia oppositifolia* L.): “*gonna”*, being a collective name given by the Hottentots people to members of the Thymelaeaceae from which bark was used to make rope or twine; *Besembos*(*sie*) (Arthrosolen microcephalus (Meisn.) E.Phillips = *Gnidia microcephala* Meisn., and *Arthrosolen polycephalus* C.A.Mey. = *Gnidia polycephala* (C.A.Mey.) Gilg) meaning “broom-bush”. *Boegoekaroo* is a name used by the Hottentot people for various unrelated aromatic shrubs of the north-eastern Karoo areas including *Gnidia stricta* (Thunb.) Wikstr. (= *Gnidia wikstroemiana Meisn.*), *Brandbas*(*bossie*) (*Gnidia sericea* L.) meaning “fire-bark” and *Brandbossie* (Lasiosiphon anthylloides Meisn. = *Gnidia anthylloides* (L.f.) Gilg) meaning “fire-bush”: the Southern Sotho people sometimes used this plant for fuel, or the smoke for medicinal purposes (but see below); *Gifbos*(*sie*) (*Lasiosiphon capitatus* Burtt Davy = *Gnidia capitata* L.f.), and *Lasiosiphon kraussianus* Meisn. = *G. kraussiana*) meaning “poison-bush”, in reference to the toxicity of plants; *Koorsbossie* (*Lasiosiphon meisnerianus* Endl. = *Gnidia cuneata* Meisn.) meaning “fever-bush”: the

plant being used to treat fevers; *Hotnotsverfbossie* and *Saffraan* (*Lasiosiphon deserticola C.H.Wright* = *Gnidia deserticola* Gilg): both references to the pigments derived from this plant and used by the Hottentot people to dye leather, i.e. *verf* meaning paint, colour or dye, and *saffraan*, the yellow pigment saffron; *Harpuisbos* (*Lasiosiphon burchelli* Meisn. = *Gnidia burchellii* (Meisn.) Gilg) meaning "resin-bush", and *Kerrieblom* (*L. capitatus* = *G. capitata*) meaning "curry-flower", in reference most likely to the burning sensation in the mouth and throat when the plant is ingested; *Januariebos*(*sie*) and *Waaibos*(*sie*) refer to the January flowering time and windy South African Karoo Biome (MUCINA & RUTHERFORD 2006) habitat of *A. polycephalus* (= *G. polycephala*); and *Roemenaggie* or *juffertjie-roer-by-die-nag* which translates as "little-lady-gad-about-at-night", and refers to the diffuse, sweet, night-time fragrance of flowers of *Gnidia ornata* (Meisn.). These last two names are also applied to *Struthiola* species (SMITH 1966, MAKHOLELA & MANNING 2006).

Zulu vernacular names of *Gnidia* species include: *esimhlope* (*Gnidia calocephala* (C.A.Mey.) Gilg), meaning white, and likely refers to the white flowers; *empofu* (*Gnidia polyantha* Gilg) meaning pale, which may refer to the silvery hairy leaves and flowers giving this species a pale appearance; *umsilawengwe* (*Gnidia kraussiana* Meisn.) meaning cat's tail or leopard's tail, describes a long, tail-like peduncle with a tufted, hairy spent receptacle; *imfuzane* (also *G. kraussiana*) is a name for small burrowing animals like mice. This name may refer to these plants and animals living in close proximity to each other, or it may refer to the 'disappearance' (i.e. burning off) of vegetative parts after fire and regrowth from a woody, underground rootstock. *Esikhulu* (*Gnidia anthylloides* (L.f.) Gilg), is rooted in the Zulu word *khulu* which has several meanings including leader, as in an important leader of a community, a

political figure, an elder, ancestor or someone powerful or respected. It can also mean physically big or massive (Patience Magwaza, Eunice Ngcobo, personal communications). The derivation of this name is unclear, but it possibly acknowledges the importance of *G*. *anthylloides* in traditional African medicine.

Madagascan gnidias are known by the Malagasy vernacular *havoa*, meaning fibre, and the fibrous bark of some species is used to make paper (ROGERS 2009).

Gnidia and related genera in Traditional African Medicine and non-medicinal uses

Species of *Gnidia* and other African Thymelaeaceae have been used in the traditional treatments of a variety of medicinal complaints in humans and animals. In Africa *Gnidia* species have been used to treat a range of conditions in humans including conception and childbirth, asthma, backache, nightmares, boils, induce blistering, treat bruises and burns, constipation, coughs, earache, epilepsy, headache, influenza and fevers, insanity, malaria, measles, pulmonary tuberculosis, poor appetite, smallpox, snake bites, sprains and fractures, toothache, ulcers and yellow fever and as broad-spectrum purgatives. The Southern Sotho people believe the smoke from burning *Gnidia anthylloides* bewitches people and makes them quarrelsome. Nevertheless they will use smoke from this plant to treat fevers and bad dreams. In livestock, *Gnidia* species have been used in the treatment of anthrax and botulism (WATT & BREYER-BRANDWIJK 1962, VAN WYK ET AL. 2005). In Madagascar, leaves of *Gnidia gilbertae* Drake are used as a purge to induce vomiting (ROGERS 2009).

Crushed roots of *G. kraussiana* are used to make fish poison (PETERSON 2006), and in Madagascar the fibrous barks of *Gnidia* species are used to make rope and twine, paper and ceremonial clothing (ROGERS 2009.)

Taxonomic treatments of African Thymelaeaceae and relatives based on morphological and anatomical data.

MEISNER (1857) provided the last comprehensive treatment of *Gnidia* (with *Lasiosiphon* treated separately); and PEARSON (1910), WRIGHT (1915), STANER (1935), AYMONIN (1966a, 1966b), GASTALDO (1969), ROBYNS (1975), BEAUMONT (2000) and PETERSON (1978, 2006), all contributed regional accounts of African gnidias and kin. LEANDRI (1950) presented an account of Madagascan taxa, which included 15 species and three varieties of *Lasiosiphon*, and four species of *Gnidia*. In contrast, ROGERS (2009) conflated *Lasiosiphon* with *Gnidia* in his recent account of Malagasy *Gnidia*.

Recent monographic treatments of other southern African Thymelaeaceae include *Passerina* (BREDENKAMP & VAN WYK, 2003) and *Lachnaea* (BEYERS 2001). The last comprehensive treatment of the only other sizeable southern African genus, *Struthiola*, was compiled by WRIGHT in 1915. PETERSON (1958) intended to present a monograph of *Struthiola*, but this was never published. ROGERS (2005) included the sole African representative of *Octolepis* Oliv. from tropical Africa in his revision of this otherwise Madagascan genus, and he also revised the Malagasy endemic genus *Stephanodaphne* Baill. (ROGERS 2004).

Molecular studies of use in the taxonomy of the Thymelaeaceae.

VAN DER BANK ET AL. (2002) provided the first extensive survey of phylogenetic relationships among Thymelaeaceae using evidence from analyses of molecular sequence data. They performed separate and combined parsimony analyses of 41 *rbc*L (ribulose biphosphate carboxylase) nucleotide sequences and plastid *trn*L and *trn*L-F intergenic spacer sequences for selected African and Australian taxa. Their results confirmed the monophyly of the family Thymelaeaceae, supported the four sub-families sensu DOMKE (1934), and moreover determined that *Gnidia* was not monophyletic, with species variously linked to *Lachnaea*, *Struthiola* and *Passerina*, plus the geographically disjunct *Drapetes* Banks ex Lam. and *Pimelea* Banks & Sol. ex Gaertner.

GALICIA-HERBADA (2006) presented a phylogenetic analysis of the Mediterranean genus *Thymelaea* Mill. using ITS (*r*DNA) sequence data to investigate species relationships and timelines of diversification. Evidence from *rbc*L sequence data (ROBERTS 2007) supported HERBER'S (2003) inclusion of *Jedda* Clarkson (CLARKSON 1986) in the *Linostoma* group, together with African and Malaysian taxa. Elsewhere in the Thymelaeaceae, in the subfamily Aquilarioideae sensu DOMKE 1934 (or Thymelaeoideae sensu HERBER 2003), EURLINGS & GRAVENDEEL (2005) recommended that *Gyrinops* Gaertner should be conflated under the morphologically very similar *Aquilaria* Lam. Their suggestion was based on evidence from *trn*L-*trn*F sequence data, which indicated *Aquilaria* and *Gyrinops* are paraphyletic. Altogether, these studies demonstrate the increasing importance of molecular data in resolving phylogenetic relationships within the Thymelaeaceae.

Rationale for thesis and aims of the present study

To date, no recent, comprehensive phylogenetic assessment of *Gnidia* and kin has been produced. Currently, most treatments follow a broad-based concept of *Gnidia* within which opinions are divided on whether *Arthrosolen* (MEYER 1857), *Lasiosiphon* (FRESENIUS 1838) and *Englerodaphne* (GILG 1894b) should be recognized as separate genera or conflated within *Gnidia*. Within this large genus species relationships are poorly understood and there is no widely accepted infrageneric classification of taxa.

Much focus has rested on floral characters, specifically floral merosity, numbers of stamens, and aspects of the floral or petal-like scales among taxa in defining the generic limits of *Gnidia*. The slight variability among these floral features has been the root of the taxonomic disputes surrounding the generic circumscription of *Gnidia* and recognition or not of segregate genera.

The aim of the present study was to investigate *Gnidia* species using phenetics, morphology and micromorphology and morphometric analyses to reevaluate features traditionally used to delimit genera and to look for novel features of potential taxonomic and systematic value. Thereafter the findings from these investigations could be evaluated in the context of the phylogenetic framework among *Gnidia* and kin suggested by molecular sequence data. Molecular analyses by VAN DER BANK ET AL. (2002) first showed that *Gnidia* is not monophyletic, and the present study provided an opportunity to expand on these initial findings by analysing more taxa. Gynodioecy was discovered for the first time in *Gnidia* in the course of this study. The gynodioecious species was included in molecular analyses in order to gain some

insight as to its closest relatives within *Gnidia* and other southern hemisphere Thymelaeoideae, and how its phylogenetic position based on molecular evidence can be related to its classification in tribe Gnidieae. Breeding systems represent one aspect of systematics, and the discovery of the first species of Thymelaeoideae with unisexual flowers on the sub-Saharan African continent presented an opportunity to assess the distribution of sexual polymorphism elsewhere in the Thymelaeoideae.

Layout of this thesis.

Following the introduction (Chapter 1), an historical review of *Gnidia* and relatives is presented in Chapter 2. Chapter 3 describes the diversity and taxonomic value of leaves and involucral bracts using morphometric analyses to investigate the relationship among leaf and involucral bract length/width dimensions, with illustrations of micromorphological and anatomical features of selected taxa. Chapter 4 investigates patterns of diversity among involucral bracts, inflorescences and flowers using morphometric analyses. A phylogenetic analysis of selected genera and species of the Thymelaeoideae including 35 species of *Gnidia* using nuclear and plastid datasets is presented in Chapter 5, and Chapter 6 presents the first account of gynodioecy in a species of *Gnidia,* in which floral morphology, energy investment and fruit set is investigated among sexual morphs within and between two populations. Chapter 7 is a general discussion of the findings and value of this thesis with suggestions for future research.

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CHAPTER 2

TAXONOMIC HISTORY OF *GNIDIA*:

GENERIC CONCEPTS, CHARACTERS AND CONTROVERSY

Introduction

The generic circumscription of *Gnidia* L. has traditionally been contentious. This has largely stemmed from the lack of unique or stable morphological characters to define this and other Southern Hemisphere Thymelaeoideae. Generic limits of *Gnidia* have been recognised variously as broad to narrow with satellite genera established to reflect the diversity of species within this large group. Of these segregate genera, three in particular have remained contentious up to now, namely *Arthrosolen* C.A.Mey., *Lasiosiphon* Fresen. and *Englerodaphn*e Gilg., with opinions still divided as to whether these three should be treated as distinct genera or considered synonymous with *Gnidia*. Today, most treatments accept a broad-based concept of *Gnidia*, yet species relationships remain poorly understood, and there is no modern monograph for this group.

Early encounters with Gnidia

Published accounts of plants that would later belong to the genus *Gnidia* first appeared in the eighteenth century and featured taxa from the Western Cape Province, South Africa. This region is recognized as a centre of diversity for *Gnidia* and other southern

African Thymelaeoideae including *Lachnaea* L. (BEYERS 2001), *Passerina* L. (BREDENKAMP & VAN WYK 2003) and *Struthiola* L. (HERBER 2003, PETERSON 2006). European colonization and expansion into the southern African interior began in the mid-seventeenth century from the region that today supports the city of Cape Town, South Africa (GUNN & CODD 1981). Consequently species and genera from this southernmost region of southern Africa, including *Gnidia*, were among the first Thymelaeoideae from the southern African continent to be received by European herbaria.

Early taxonomic literature defined species using vegetative and floral features to establish short descriptions called phrase names. Phrase names often noted the similarity of the subject to other species or group, and included up to twelve words starting with the generic name (STACE 1985). BREYNE (1674) provided the earliest description of a *Gnidia* species as follows: *Thymelæa æthiopica Passerinae foliis.* This description indicates a plant belonging to *Thymelæa* from South Africa with leaves like those of *Passerina*. This species is known today as *Gnidia subulata* Lam. (WRIGHT 1915). Four other early descriptions of *Gnidia* species, also from the Western Cape, are as follows: "*Rapunculus foliis nervosis linearibus, floribus argenteis, non galeatis*" (BURMAN 1738); *Thymelæa capitata, lanuginosa, foliis creberrimis* (BURMAN 1739a); *Thymelæa sericea, foliis oblongis* (BURMAN 1739b) and *Thymelæa foliis planis acutis* (BURMAN 1739c). These species are more correctly known today as *Gnidia pinifolia* L., *Gnidia polystachya* Berg., *Gnidia oppositifolia* L. and *Gnidia sericea* L., respectively. Leaf characters were important in these phrase names, but later treatments valued reproductive characters more. This stemmed largely from Linnaeus's highly influential

sexual system of botanical classification introduced in his *Systema naturae* (LINNAEUS 1735).

LINNAEUS (1742) provided the first reference to the name *Gnidia* when he cited *Struthia* (a manuscript name of Royen), as a synonym of *Gnidia* in his second edition of *Genera plantarum.* LINNAEUS (1751) later included the name *Gnidia* in his *Philosophia botanica*. The name *Gnidia* is derived from the Latin geographical name *Gnidus* (in Greek, *Knidos*) in Caria, known today as Anatolia in Turkey (STEARN 1996).

The starting-point of the name *Gnidia* is accepted as 1753 when LINNAEUS established the genus with three species. He distinguished this genus by its flowers with a funnel-shaped calyx, a tube with four terminal limbs, a *corollâ* of four petals that are shorter than the lobes, eight stamens dehiscing inwardly to the centre of the flower, and a laterally placed style, a round stigma and dry fruits (LINNAEUS 1754). The term *corollâ* is derived from the Latin meaning ‘little crown or garland’ (STEARN 1987). These structures sit in the sinuses between the calyx lobes of *Gnidia* flowers and have been variously interpreted as petals, petal-like or floral scales, outgrowths, glands, or even stipules (reviewed by HEINIG 1951). HEINIG (1951) showed that the vasculature of these structures is derived from the lateral sepal traces instead of being organised in a separate petal whorl.

LINNAEUS (1753, 1754) embraced the relationships between the numbers of stamens and pistils among plants in his classification of genera. To define classes of higher plants he used primarily stamen number and the distribution of sexes within flowers and

within and among plants. Thereafter he subdivided each class into orders using features of the pistil or of fruits (STEARN 1960). He placed *Gnidia* in class Octandria, order Monogynia, a group comprising plants with eight stamens and a single style. His system organized genera into both 'natural' groups i.e. those that today are considered closely (phylogenetically) related, and 'artificial' groups that are not. For example, LINNAEUS (1753, 1754) included in his class Octandria, order Monogynia: *Daphne* L., Dirca L., *Gnidia*, *Stellera* L., *Passerina* L. and *Lachnaea* L.: all recognized today in the Thymelaeaceae, together with genera now in other families.

LINNAEUS (1753) did not specify the collections he used when he described the first three species of *Gnidia*, namely: *Gnidia pinifolia* L., *Gnidia tomentosa* L. and *Gnidia oppositifolia* L. The Linnaean Herbarium (LINN) contains 16 sheets of *Gnidia* specimens and ROGERS & SPENCER (2006) elected types for *Gnidia* species from this collection. Unaware of the better placement of *Thymelaea capitata lanuginosa* (BURMAN 1739a) in *Gnidia* (discussed previously), LINNAEUS (1753) transferred this plant to *Daphne* L.: a genus he established in 1735 in the first edition of his *Systema naturae*. He classified *Daphne* and *Gnidia* in class Octandria, order Monogynia, because both have flowers with four lobes, eight stamens and either a single style (*Gnidia*) or a single, sessile stigma (*Daphne*). He also reduced *Rapunculus foliis nervosis* (BURMAN 1738) to synonymy under *G. pinifolia*, this, the first species listed in his *Species plantarum*, and the type species for *Gnidia.*

DE JUSSIEU (1789) established the family name *Thymeleae* (*Les Thyméelés*) for genera lacking petals and with perigynous stamens. This gave rise to the modern

equivalent name Thymelaeaceae (LANJOUW ET AL. 1961). The name commemorates the type genus *Thymelaea* P.Mill. from the Mediterranean (HUTCHINSON 1967). Whilst LINNAEUS (1754) recognized true 'petals' (*petala*, *corollâ*) in *Gnidia*, in contrast DE JUSSIEU (1789) classified *Gnidia* and others in the *Thymeleae* as apetalous referring to the petal-like structures between the calyx lobes as *squamulae* (scales). DE JUSSIEU (1789) considered apetaly important in defining the Thymelaeaceae, omitting all genera with 'true' petals included by LINNAEUS (1753, 1754) in his class Octandria, order Monogynia. DE JUSSIEU (1789) nevertheless did retain one genus with true petals in the *Thymeleae*, namely *Quisqualis* L., because he thought it looked like *Daphne*. *Quisqualis* is now placed in Combretaceae.

Between the latter half of the 18th to the late 19th centuries eight genera were established that were soon reduced to synonymy under *Gnidia*. These were: *Dessenia* (ADANSON 1763), *Nectandra* (BERGIUS 1767), *Canalia* (SCHMIDT 1793), *Thymelina* (HOFFMANSEGG 1824), *Trimeiandra* (RAFINESQUE 1836), *Epichroxantha* (MEISNER 1857), *Gnidiopsis* (VAN TIEGHEM 1893a) and *Rhytidosolen* (VAN TIEGHEM 1893b).

ADANSON (1763) distinguished *Dessenia* by its alternate and opposite leaves, flowers in heads, bracteoles absent, tubular flowers with four lobes and four "teeth" (i.e. petal-like structures), eight stamens in two rows of four, and dry, ovoid fruits. RAFINESQUE (1836) added three species to *Dessenia*, but later HOOKER (1895) suggested that two of these, namely *Dessenia daphnefolia* Rafin. and *Dessenia hirsuta* Rafin., were in fact the Malagasy species *Lasiosiphon madagascariensis* (Lamk.) Decne ex Cambess. and *Lasiosiphon pubescens* (Lamk.) Decne. ex Cambess., respectively (LEANDRI 1950).

These last two were later reduced to synonyms of *Gnidia daphnifolia* L.f. by ROGERS (2009). ROGERS & SPENCER (2006) and ROGERS (2009) listed *Dessenia* as a superfluous name of *Gnidia*. This is because *Gnidia* was established before *Dessenia*, and the description of *Dessenia* above matches the original description of *Gnidia* (LINNAEUS 1753, JEFFREY 1973).

BERGIUS (1767) established *Nectandra* with three species: *Nectandra laevigata* Berg., *Nectandra sericea* Berg. and *Nectandra tetrandra* Berg. The name *Nectandra* is derived from the Latin *nec*, meaning 'not' or 'and not', and *andro*, meaning male. Altogether the name is likely a reference to the comparatively large, fleshy, column-like petal-like scales that resemble anthers, but are 'not anthers'. *Nectandra laevigata* is synonymous with *G. oppositifolia* L.*,* published 14 years previously when LINNAEUS (1753) established *Gnidia*, *N. sericea* is a synonym of *Gnidia sericea* L. and *N. tetrandra* is a synonym of *Struthiola erecta* L. *Nectandra* was published after both *Gnidia* (LINNAEUS 1753) and *Struthiola* (LINNAEUS 1767) and with two of its three species having already been described under as *Gnidia*, and one under *Struthiola*, *Nectandra* species necessarily became reduced to synonymy under *Gnidia* and *Struthiola.*

SCHMIDT (1793) established *Canalia* with a single species, *Canalia daphnoides* Schmidt (SCHMIDT 1830). *Canalia daphnoides* and *G. pinifolia* are one and the same species, and given that *G. pinifolia* predates *C. daphnoides*, the former name holds priority. The name *Canalia* is derived from the Latin *canalis*, meaning groove or channel. Flower tubes of *G. pinifolia* have distinctive longitudinal and parallel vascular traces, which may have inspired the generic name. In 1824, HOFFMANSEGG established

Thymelina, with two species, but these were synonymous with two *Gnidia* species published previously, namely: *Gnidia polystachya* Berg. and *Gnidia sericea* L. (WRIGHT 1915).

Trimeiandra Rafin. has been variously cited as a synonym of *Passerina* (ANGELY 1956; HUTCHINSON 1967) or *Gnidia* (DOMKE 1934) or both (DE DALLA TORRE & HARMS 1900-1907), plus the International Plant Names Index (IPNI) lists *Trimeiandra* as a synonym of *Arthrosolen* C.A.Mey. In fact, the plant to which the name *Trimeiandra* refers is a member of the genus *Lonchostoma* Wikstr. (Bruniaceae). THUNBERG (1794) originally placed this plant incorrectly in the Thymelaeaceae as *Passerina pentandra* Thunb. Thereafter, RAFINESQUE (1836) transferred it to his new genus *Trimeiandra* and established the type species *Trimeiandra spicata* Rafin., with *P. pentandra* as a synonym. The generic name is a combination of the Greek elements *tri*, meaning three, *mei*, meaning fewer and *andra* from *andro-* or *andro*, meaning male, and describes the flowers having five (i.e. eight less three), rather than eight stamens, which is the condition in *Passerina*. In the Thymelaeaceae, only *Gyrinops* Gaertner has flowers with five lobes and five stamens (HOU 1960, HUTCHINSON 1967). All other pentamerous genera have flowers with ten stamens. RAFINESQUE (1836) described *T. spicata* as native to South Africa, with ovate, hairy leaves, flowers in spikes (hence the species epithet, *spicata*) and flowers with five calyx lobes and five stamens. The type of *P. pentandra* is housed at UPS-LINN and examination of an electronic scan of this specimen confirmed it to be a member of the genus *Lonchostoma*. The genus *Lonchostoma* is restricted to the Cape Province, South Africa. With their sessile, estipulate leaves and flowers with tubular corollas with five lobes, *Lonchostoma* plants

bear a superficial resemblance to members of the Thymelaeaceae. IPNI lists *Trimeiandra* as a synonym of *Arthrosolen*, specifically citing *T. spicata* as a synonym of *Arthrosolen spicatus* C.A.Mey., known today as *Gnidia spicata* (L.f.) Gilg. Possibly their spike-like inflorescences contributed to the mistaken synonymy of these two species.

MEISNER (1857) established *Epichroxantha* Eckl. & Zeyh. ex Meisn. with six species. The generic name is derived from the Greek elements *epi,* meaning above or on top of, *chrom*- or *chromus*, meaning coloured and *xantho* meaning yellow and refers to the conspicuous, bright yellow flowers at the ends of branches. Species of *Epichroxantha* are similar: all are small, with herbaceous perennial stems, paired leaves with apiculate tips and small inflorescences of yellow flowers, each with large yellow scales and highly distinctive funnel-shaped floral tubes with broad mouths and narrow constriction point just above the ovary. Nearly 40 years later, VAN TIEGHEM (1893a, 1893b) established two genera, *Gnidiopsis* Van Tiegh. and the monotypic *Rhytidosolen* Van Tiegh. The name *Gnidiopsis* is a combination of the generic name *Gnidia* and the Greek *opsis* meaning resemblance, and refers to the similarity of plants of *Gnidia* and *Gnidiopsis* (STEARN 1987). VAN TIEGHEM (1893a) possibly did not realize that his concept of *Gnidiopsis* was more or less the same as that of *Epichroxantha*, because *Gnidiopsis* comprised most of the species included by MEISNER (1857) in *Epichroxantha.* Both ultimately became synonyms of *Gnidia*, on account of their tetramerous, eight-staminate flowers with petal-like scales: essentially a description of *Gnidia* species (DE DALLA TORRE & HARMS 1900-1907). *Rhytidosolen* is derived from the Greek elements *rhytidos* meaning wrinkled or puckered and *solen* meaning pipe, and refers to the conspicuous longitudinal vascular ribs of the flower tubes (STEARN 1987). The

basionym *Rhytidosolen laxus* Van Tiegh. and its synonym *Arthrosolen laxus* C.A.Mey. are now both recognized as synonyms of *Gnidia laxa* (L.f.) Gilg. MEYER (1843) transferred *R. laxus* to *Arthrosolen* because of the absence of petal-like scales in flowers, which he considered diagnostic of *Arthrosolen*.

Three genera of lasting taxonomic controversy: Arthrosolen, Lasiosiphon and Englerodaphne.

The taxonomic positions of three genera have remained controversial to the present day namely, *Arthrosolen* C.A.Mey., *Lasiosiphon* Fresen. and *Englerodaphne* Gilg. Authors have variously recognised these taxa as separate genera or synonymous with *Gnidia*.

FRESENIUS (1838) defined *Lasiosiphon* plants by their alternate leaves, flowers in capitate heads encircled by an involucrum of many leaves and a spherical, hairy receptacle. FRESENIUS (1838), however, also characterised *Lasiosiphon* by its pentamerous flowers with ten stamens in two rows, and five or ten petal-like glands or *squamæ* (scales), but he established no infrageneric ranks in the genus. The name *Lasiosiphon* is derived from the Greek elements *lasio*- meaning woolly and *siphon* meaning tube and describes the hairy floral tubes (STEARN 1987). The slightly unstable pentamerous floral plan has caused most of the controversy surrounding the generic status of *Lasiosiphon*. Occasional tetramerous and hexamerous flowers occur in heads in which by far the majority of flowers are pentamerous. Petaloid scales among *Lasiosiphon* species are always small, scarcely visible to the naked eye and flap- or tongue-like and although they may be irregular in outline, they are never fleshy.

Sometimes these petal-like scales are absent altogether from the calyx lobe sinuses, but more commonly only the odd one or two scales are missing from a flower. The genus *Lasiosiphon* was generally recognised by authors until about the mid 20th century, thereafter, it was largely included under *Gnidia* (Tables 2.1–2.2).

MEYER (1843) established the genus *Arthrosolen* for species lacking petal-like, floral scales (squamulae). The generic name is derived from the Latin *arthro*- meaning jointed and the Greek *solen* meaning pipe, and refers to the articulating floral tubes. MEYER (1843) included species with tetramerous as well as species with pentamerous flowers in *Arthrosolen* and recognised four sections, namely *Arthrosolenia*, *Gymnurus*, *Rhytidosperma* and *Calocephalus*, based on characters of flower merosity, inflorescence structure, presence or absence of involucral bracts and seed surface texture. Like *Lasiosiphon*, *Arthrosolen* also proved to be a well-supported genus among many authors (Tables 2.1–2.2).

PHILLIPS (1944) defined the genus *Arthrosolen*, not by an absence of petal-like or floral scales as MEYER (1843) had done, but by features of the inflorescence. Both STANER (1935) and PHILLIPS (1944) argued that petal development as a generic character would cause closely related species to be placed in different genera. Therefore, PHILLIPS (1944), defined the genus *Arthrosole*n primarily by the more stable characters of inflorescences in many-flowered heads surrounded by involucral bracts differing in one or more aspects of shape, size, colour and texture from the leaves, and he included one tetramerous species with, and three pentamerous species without petal-like scales

in this genus. Furthermore, unlike MEYER (1843), PHILLIPS (1944) recognized no infrageneric classification in *Arthrosolen*.

The third among the most controversial of genera segregated from *Gnidia* is *Englerodaphne*, established by GILG (1894). The name commemorates the German botanist A. Engler and the genus *Daphne*. *Englerodaphne* plants, however, bear scant resemblance to *Daphne* plants, being geographically disjunct, with *Englerodaphne* in the Southern Hemisphere and *Daphne* in the Northern Hemisphere and both differ in chemistry and morphology (HERBER 2003). *Englerodaphne* was defined primarily by its flowers in ebracteate spikes, in which the floral internodes lengthen in the infructescence, and the leaves arranged in opposite pairs and which are particularly thin and flimsy in texture compared with those of *Gnidia*. PHILLIPS (1944, 1951) upheld *Englerodaphne* citing the flat, membranous leaves and ebracteate inflorescences as significant characters to set this genus apart from *Gnidia*. He also pointed out that one of the species listed under *Gnidia* by WRIGHT (1915), namely *Gnidia ovalifolia* Meisn., would be better placed in *Englerodaphne*.

PHILLIPS (1944, 1951) maintained *Gnidia*, *Arthrosolen* and *Lasiosiphon* as separate genera largely because of their different distributions in South Africa. *Gnidia* species mostly occur in the Western Cape Province, in contrast to *Lasiosiphon* from more northerly regions of the country. PHILLIPS (1944, 1951) placed great emphasis on geographical distributions to support generic distinctions, but was presumably unaware

Table 2.1. Classification of *Gnidia* and associated genera (1753 – 1934).

Rank	Linnaeus 1753, 1754	De Jussieu 1789	Endlicher 1847	Meisner 1857	Bentham & Hooker 1880	Gilg 1894, 1921	Pearson 1910; Wright 1915; Burtt Davy 1926	Marloth 1925	Domke 1934
Tribe I: **Subtribe I:** **Division** **Genera:**	***Gnidia***	***Gnidia***	Thymelinae Daphneae Stellereae ***Gnidia*** ***Arthrosolen***	Daphneae Diplostemoneae ***Arthrosolen***	Euthymelaeeae ***Gnidia*** ***Arthrosolen*** ***Lasiosiphon***	Gnidieae Gnidiinae ***Gnidia*** (including *Lasiosiphon, Arthrosolen, Epichroxantha*)	Euthymelaeae ***Gnidia*** ***Lasiosiphon*** ***Englerodaphne*** ***Arthrosolen***	Gnidieae ***Gnidia*** ***Lasiosiphon***	Gnidieae Gnidiinae ***Gnidia*** (including *Arthrosolen, Englerodaphne, Epichroxantha),* *Craspedostoma,* ***Lasiosiphon***
Division **Genera**			Thymeleae ***Lasiosiphon***						
Tribe II: **Subtribe II:** **Genera:**				Gnidieae Diplostemoneae ***Gnidia*** (including *Epichroxantha*) ***Lasiosiphon***		Dicranolepideae Linostomatinae ***Englerodaphne***		Dicranolepideae ***Englerodaphne***	
Tribe III: **Genera:**								Daphneae ***Arthrosolen***	

Table 2.2 Classification of *Gnidia* and associated genera (1935 – 2009).

	Staner 1935	**Phillips 1944, 1951**	**Leandri 1950**	**Hutchinson 1967**	**Robyns 1975**	**Dyer 1975**	**Peterson 1959, 1978, 2006**	**Herber 2003**	**Rogers 2009**
Tribe I:	Gnidieae							Daphneae	
Subtribe I:									
Genera:	***Gnidia*** (including *Arthrosolen, Lasiosiphon*)	***Gnidia*** ***Lasiosiphon*** ***Arthrosolen*** ***Pseudognidia*** ***Basutica*** ***Struthiolopsis*** ***Englerodaphne***	***Gnidia*** ***Lasiosiphon*** ***Atemnosiphon***	***Gnidia*** (including *Pseudognidia Craspedostoma Epichroxantha*) ***Lasiosiphon***	***Gnidia*** (including *Lasiosiphon, Arthrosolen, Englerodaphne*)	***Gnidia*** (including *Lasiosiphon Arthrosolen Pseudoginidia Basutica Struthiolopsis*) ***Englerodaphne***	***Gnidia*** (including *Lasiosiphon Englerodaphne Arthrosolen Pseudognidia Basutica Struthiolopsis Craspedostoma*)	***Gnidia*** (including *Lasiosiphon, Arthrosolen, Englerodaphne, Craspedostoma, Basutica, Pseudognidia, Struthiolopsis, Atemnosiphon*)	***Gnidia*** (including *Arthrosolen, Lasiosiphon*)
Tribe II:									
Subtribe II:									
Genera:				***Englerodaphne***					
Tribe III:									
Genera:				***Arthrosolen*** ***Basutica*** ***Struthiolopsis***					

of the extent to which *Gnidia* and *Lasiosiphon* are sympatric in East Africa, Tropical Africa and Madagascar. Only six years later in 1950 would the French anatomist LEANDRI present his account of Madagascan Thymelaeaceae, showing clear sympatry between these genera.

PHILLIPS (1944), clearly more of a 'splitter' than a 'lumper', removed three more species from *Gnidia* to establish three new genera, namely, *Pseudognidia* Phillips, *Basutica* Phillips and *Struthiolopsis* Phillips. The name *Pseudognidia* is derived from the Greek *pseudo* meaning false, i.e. resembling but not equaling and the generic name *Gnidia,* altogether meaning a genus that resembles *Gnidia.* PHILLIPS (1944) primarily distinguished *Pseudognidia* from *Gnidia* by its four- versus eight-staminate flowers. PHILLIPS (1944) transferred *Gnidia anomala* Meisn. to *Pseudognidia* (the species epithet *anomala* refers to its unusual four-staminate flowers), but he presented no thoughts on another *Gnidia* species with four-staminate flowers namely, *Gnidia harveyana* Meisn. Possibly he had no access to material of this very rare species and therefore refrained from any assessment of its taxonomic position.

The name *Basutica* is derived from Basutoland, the former name for Lesotho within which is located a large part of the Drakensberg Mountain Range. Two species included previously in *Basutica*, namely *Gnidia aberrans* (C.H.Wright) E.Phillips and *Gnidia propinqua* (Hilliard) B.Peterson, inhabit the high-altitude eastern part of Lesotho. Although both *Basutica* and *Pseudognidia* species have four-staminate flowers, PHILLIPS (1944) again used their geographical separation to distinguish them, with

Pseudognidia from the Western Cape Province separated from *Basutica* further north (WRIGHT 1915, HILLIARD & BURTT 1987).

PHILLIPS (1944) transferred *Gnidia pulvinata* Bolus to his new genus *Struthiolopsis* and established a second species, *Struthiolopsis bolusii* E. Phillips. The name *Struthiolopsis* is a combination of the generic name *Struthiola* and the Greek, *opsis* meaning appearance (hence resemblance) and indicates the similarity of *Struthiolopsis* plants to *Struthiola*. PHILLIPS (1944) used the multiplicity of laciniae of the four petals interspersed with many stiff hairs in *Struthiolopsis* to distinguish it from *Gnidia.* PHILLIPS (1944) noted that the upper stamens of *Struthiolopsis* flowers are sometimes not developed, leaving only four, lower fertile stamens. Such plants with their four-staminate flowers with stiff hairs at the flower mouth looked very much like *Struthiola*, hence the name *Struthiolopsis* reflecting this likeness between these genera. All the while, however, PHILLIPS (1944) was apparently unaware that *Struthiolopsis* was, in fact, synonymous with *Craspedostoma* Domke, published earlier in 1934.

ENDLICHER (1847) maintained *Gnidia*, *Arthrosolen* and *Lasiosiphon* as separate genera. He separated *Gnidia* and *Arthrosolen* with constricted floral tubes from *Lasiosiphon* species with non-constricted floral tubes. This, however, is puzzling, because floral tubes are constricted in most *Lasiosiphon* species (PETERSON 1978, HERBER 2003, ROGERS 2009). Thereafter, ENDLICHER (1847) used the presence of petal-like scales in *Gnidia* to distinguish it from *Arthrosolen* in which they are absent. ENDLICHER (1847) also established two sections in *Gnidia* based on inflorescence structure: *Eugnidia*, for species with flowers in terminal, capitate heads and *Phidia*, for

species with flowers in spikes, lateral clusters or flowers axillary and solitary. He recognized *Arthrosolen* and the four infrageneric groups therein proposed by MEYER (1843), and recognised the genus *Lasiosipho*n. ENDLICHER (1847) valued the combination of inflorescence structure, presence or absence of involucral bracts and scales and flower merosity to help define genera and infrageneric groups. He valued an absence of scales before flower merosity to distinguish *Arthrosolen*, because he included both species with tetramerous and species with pentamerous flowers in *Arthrosolen*.

MEISNER (1840) initially included *Lasiosiphon* in *Gnidia*, placing species with tetramerous flowers in section *Tetramerae* and those with pentamerous flowers (including *Lasiosiphon* species) in section *Pentamerae*. Within section *Tetramerae* MEISNER (1840) divided species into two groups based on inflorescence structure. Later, however, MEISNER (1857) changed his mind and recognised *Lasiosiphon* as a genus proper. Again, he used floral merosity to distinguish *Gnidia* from *Lasiosiphon*. In short, Meisner (1840) first used floral merosity as a sectional character and then later (MEISNER 1857) as a generic character to distinguish *Gnidia* from *Lasiosiphon*. MEISNER (1857) maintained his adoption of ENDLICHER's (1847) two sections in *Gnidia* to separate groups of species based on inflorescence structure. Ultimately the classifications of ENDLICHER (1847) and MEISNER (1857) for *Gnidia* and *Lasiosiphon* and the characters used to distinguish them were very similar.

Both MEISNER (1857) and BENTHAM & HOOKER (1880-1883) distinguished *Arthrosolen*, lacking floral scales, from both *Gnidia* and *Lasiosiphon* in which floral

scales are present (Table 2.1). MEISNER (1857), however, had a simpler infrageneric classification for *Arthrosolen*, dividing species into two groups based on inflorescence structure, in contrast to the four groups identified by MEYER (1843) and adopted by ENDLICHER (1847), based on flower merosity, inflorescence structure, presence or absence of involucral bracts and seed surface texture. MEISNER (1857) was happy to include both species with tetramerous and species with pentamerous flowers in *Arthrosolen*. In his opinion the most important defining character of *Arthrosolen* was the absence of petal-like scales.

In contrast, GILG (1894) had a broader concept of *Gnidia* (Table 2.1). He separated diplostemonous *Gnidia* (including *Arthrosolen* and *Lasiosiphon*) from haplostemonous *Struthiola*. Thereafter he divided *Gnidia* species into two groups: *Involucratae* for species with flowers in heads with involucral bracts and *Exinvolucratae* for species with neither flowers in heads nor involucres of bracts. Unlike ENDLICHER (1847), MEISNER (1857) and BENTHAM & HOOKER (1880-1883), GILG (1894) did not use the by now familiar features of flower lobe and stamen numbers or characters of scales to distinguish small groups of *Gnidia* species within sections. Instead, he introduced some novel characters to distinguish clusters of *Gnidia* species including texture, shape and hairiness of leaves, inflorescence size and position, details of the receptacle and of involucral bracts and geographical distribution.

During the first three decades of the 20th century, three authors of African regional floras followed a segregated rather than inclusive concept of *Gnidia* (Table 2.1). PEARSON (1910), WRIGHT (1915) and BURTT DAVY (1926), like BENTHAM & HOOKER (1880-

1883) before them, used flower merosity to distinguish *Gnidia* from *Lasiosiphon*, and the absence of petals (petal-like structures) in *Arthrosolen* to distinguish it from *Gnidia*. PEARSON (1910) did, however, admit that the scales of some species of *Gnidia* are nevertheless “very minute”.

BURTT DAVY (1926) was mindful of the often fine lines set among genera based on such “meagre” characters as numbers of calyx lobes and “petals”. His farsighted commentary stated that to fully understand the limits and relationships among genera would require all-encompassing studies of taxa and that piece-meal provincial treatments alone contributed little to this task. That said he considered the floral characters above, used previously to distinguish *Gnidia*, *Arthrosolen* and *Lasiosiphon*, as of more than just specific importance, opting in the meantime to maintain these three as separate genera.

WRIGHT (1915), like MEISNER (1857), was happy to include both species with tetramerous and species with pentamerous flowers together in *Arthrosolen*, yet at the same time believed flower merosity justified separating tetramerous *Gnidia* from pentamerous *Lasiosiphon*. WRIGHT (1915) also recognized the genus *Englerodaphne*, using its ebracteate terminal fascicles of inflorescences to distinguish it from both *Gnidia* and *Lasiosiphon* with their bracteate heads of flowers.

ENGLER (1921) separated *Gnidi*a and *Struthiola* from *Englerodaphn*e using inflorescence structure and development of floral scales. He used stamen number to distinguish *Gnidia* from *Struthiola*, and considered *Lasiosiphon* and *Arthrosolen*

synonymous with *Gnidia*, these last three corresponding to the sub-genera: *Lasiosiphon* Fresen., *Phidia* Endl., and *Eugnidia* Endl., respectively. ENGLER (1921) used floral pentamery to distinguish subgenus *Lasiosiphon*, and differences in inflorescence structure to separate the sub-genera *Phidia* and *Eugnidia*. ENGLER (1921) placed all species with pentamerous flowers in sub-genus *Lasiosiphon*. This comprised mostly species previously classified in the genus *Lasiosiphon* plus three species included previously in the genus *Arthrosolen* namely *Gnidia polycephala* (C.A.Mey.) Gilg, *Gnidia sericocephala* (Meisn.) Gilg ex Engl. and *Gnidia calocephala* (C.A.Mey.) Gilg. GILG's (1921) visionary association of these three species with *Lasiosiphon* species was to foreshadow the results of a much later study by BEAUMONT ET AL. (2009) who, using evidence from molecular sequence data, supported the grouping of two species of *Arthrosolen* with pentamerous flowers now known as *G. sericocephala* and *G. calocephala* above, with similarly pentamerous *Lasiosiphon* species.

In his classification of South African Thymelaeaceae MARLOTH (1925) recognised *Gnidia*, *Lasiosiphon*, *Arthrosolen* and *Englerodaphne* as separate genera. He used floral merosity to distinguish pentamerous *Lasiosiphon* from tetramerous *Gnidia* and *Struthiola*, thereafter and like ENGLER (1921), separating diplostemonous *Gnidia* from haplostemonous *Struthiola*.

DOMKE (1934) presented a popular classification system that recognized four sub-families in the family Thymelaeaceae. The largest sub-family, the Thymelaeoideae contained four tribes. DOMKE (1934) considered the tribe Gnidieae as representing the "more highly developed" among genera of the Thymelaeoideae. Characters that

generally typified the Gnidieae, in DOMKE's (1934) opinion, included a well-developed floral tube, usually articulated above the ovary with the uppermost portion commonly dehiscing during fruit formation. The unarticulated floral tubes of *Thymelaea*, *Pimelea* and *Kelleria* are, however, exceptional. Most species in Gnidieae *sensu* Domke have tetramerous flowers with flowers pentamerous in *Dais* and *Lasiosiphon*; most genera have bisexual flowers, nearly all have a laterally-placed style, the pericarp is usually dry and thin and the seed coat is always hard. Within his tribe Gnidieae, DOMKE (1934) presented six sub-tribes. The largest of these, the sub-tribe Gnidiinae, included *Dais*, *Lasiosiphon*, *Gnidia*, *Craspedostoma* Domke, *Struthiola*, *Lachnaea* and *Cryptadenia*, distributed variously in tropical and southern Africa. Another four monogeneric tribes accommodated four more genera from the southern hemisphere, among them the tribe Passerininae containing *Passerina* L. Adaptations to anemophily tell apart *Passerina* from all other African Thymelaeoideae (BREDENKAMP & VAN WYK 2003). *Thymelaea* Mill., the sole member of the sub-tribe Thymelaeinae, however, is anomalous on account of its Mediterranean distribution to the north, in contrast to the rest of this otherwise austral tribe. DOMKE (1934) stated his belief in clear phylogenetic relationships between *Lasiosiphon* and *Gnidia*; between *Gnidia* and *Craspedostoma* and between *Lachnaea* and *Cryptadenia*. *Cryptadenia* is now included in *Lachnaea* based on the similarities of their floral scales (BEYERS 2001).

DOMKE (1934) postulated *Dais* as the most "original" genus in the sub-tribe Gnidiinae, i.e. it represented more primitive character states including pentamerous (as opposed to tetramerous) flowers, no articulation of floral tubes, a non-lateral style, a large stigma, scales in a continuous ring and broad flower bases. Here, his use of the word "petals" is

puzzling, because *Dais* has no petal-like (or scale-like) structures. DOMKE (1934) also remarked on the close phylogenetic relationship between *Dais* and *Lasiosiphon*, nonetheless he placed *Dais* in its own sub-tribe. He distinguished *Lasiosiphon* with pentamerous flowers from *Gnidia* with tetramerous flowers, while emphasizing that the two genera were nevertheless connected. DOMKE (1934) cited the presence of scales and a diplostemonous androecium to support the close relationship of *Gnidia* with *Craspedostoma*, while haplostemonous species in both genera indicated their affinities with the exclusively haplostemonous *Struthiola*.

DOMKE (1934) praised the diversity of characters used by GILG (1921) to achieve what he considered to be a more natural classification of the Thymelaeaceae. However, DOMKE (1934) felt that GILG (1921) did not adequately reflect what he believed to be the close (phylogenetic) relationships between *Dais* and *Lasiosiphon*; and between *Gnidia*, *Pimelea* Banks & Sol. ex Gaert. and *Kelleria* Endl.

STANER (1935) presented an account of 15 *Gnidia* species from East and West Africa. Like ENGLER (1921) he supported a broad generic concept of *Gnidia* and considered the traditional characters to distinguish *Gnidia*, *Arthrosolen* and *Lasiosiphon* (namely flower merosity and presence or absence of petals) artificial. He rejected the generic value of petals being present or absent by citing an example of two virtually identical species *Gnidia chrysantha* (Solms) Gilg and *Gnidia oliveriana* (Vatke.) Engl. & Gilg, distinguished only by the presence of tiny scales (petals) in the former and their absence in the latter. Using the presence or absence of petals or petal-like scales as a generic character in this instance, would he argued, place these otherwise obviously closely

related taxa in different genera. He furthermore acknowledged the variable development of these outgrowths among flowers in the same inflorescence, and that they were sometimes so tiny, as to have been overlooked by some authors. Instead he supported apetaly as a character of infrageneric rather than generic value. Accordingly he followed ENGLER (1921) in recognising two sub-genera in *Gnidia*, namely *Pergnidia* Engl. for species with scales, and *Arthrosolen* (C.A. Mey) Engl. for species without scales. Within *Pergnidia* he recognized two sections: *Eugnidia* Engl. and *Lasiosiphon* (Fresen.) Engl., distinguished by differences in the hairiness of floral tubes surrounding the ovary, and flower merosity. The infrageneric concept of *Arthrosolen* by STANER (1935) loosely recalled that of ENGLER (1921) who applied the name to a section in the subgenus *Eugnidia* Endl. for tetramerous species lacking petal-like structures. Unlike ENGLER (1921), however, STANER (1935) made no mention of flower merosity in defining his sub-genus *Arthrosolen*, because the three species he included in his treatment were all tetramerous.

The French anatomist LEANDRI (1950) included seven genera in his account of the Thymelaeaceae of Madagascar. Like BENTHAM & HOOKER (1880), PEARSON (1910) and WRIGHT (1915) before him, LEANDRI (1950) also thought the floral tetramery of *Gnidia* sufficiently distinct from the pentamerous flowers of *Lasiosiphon* to maintain these as separate genera. In his key to *Gnidia*, LEANDRI (1950) placed species with large numbers of flowers grouped in heads and surrounded by involucral bracts in *Eugnidia*, and species with few-flowered clusters or solitary flowers and lacking a distinct involucrum in *Phidia*.

Earlier, LEANDRI (1929) established the basionym *Lasiosiphon coriaceus* Leandri for a plant that he later transferred to his new genus *Atemnosiphon* Leandri (LEANDRI 1947). This monotypic genus from Madagascar resembles *Dais* in habit and in its terminal clusters of pink, pentamerous flowers with ten stamens. Leaves of *Atemnosiphon* are more leathery in texture than in *Dais*, commemorated in the species epithet *coriaceus*. Recently, however, HERBER (2003) included *Atemnosiphon* under *Gnidia*, although he did not give reasons for this synonymy.

HUTCHINSON (1967) maintained the following genera: *Arthrosolen*, *Struthiolopsis*, *Lasiosiphon, Gnidia*, *Englerodaphne* and *Basutica*. He reduced *Pseudognidia* to synonymy under *Gnidia*, because he found a full complement of eight stamens in two rows in flower tubes, unlike PHILLIPS (1944) who recorded only the four stamens of the upper row present and used this character to distinguish *Gnidia* from *Pseudognidia*. HUTCHINSON (1967), however, upheld *Lasiosiphon* on account of its usually pentamerous flowers. In his opinion the slightly variable pentamerous condition, with the occasional tetramerous flower, did not diminish the generic value of this character. HUTCHINSON (1967) also reduced *Atemnosiphon* to synonymy under *Lasiosiphon*, but offered no argument for doing so.

The 20th century Swedish botanist Bo Peterson worked on several projects in African Thymelaeaceae and provided the most recent regional accounts of *Gnidia* and relatives from continental Africa (ALMBORN 1991). His last Flora account for the family was published posthumously (PETERSON 2006). He intended to present a monograph of *Struthiola* (PETERSON 1958), although this was never realised. In his correspondence

with Ding Hou (who worked on south-east Asian and Malaysian Thymelaeaceae) Peterson stated his intention to merge several genera in a proposed monograph of *Gnidia*. Both remarked on their mutual difficulties in defining generic limits in the Thymelaeaceae because of the lack of robust and clearly delimited characters of taxonomic use (HOU 1960). Later, PETERSON (1959, 1978) included *Arthrosolen*, *Basutica*, *Englerodaphne*, *Lasiosiphon*, *Pseudognidia* and *Struthiolopsis* as synonyms of *Gnidia*. He also intended to place one (unnamed) *Gnidia* species in a separate section because of the unique whorl of hairs surrounding each petal-like scale in the flowers (HOU 1960). To date, there is no published comprehensive monograph or infrageneric classification of *Gnidia* by Bo Peterson.

PETERSON (1956) advocated a broad generic concept of *Gnidia* with 11 synonyms as follows: *Canalia* F.W.Schmidt (1793), *Trimeiandra* Raf. (1836), *Lasiosiphon* Fresenius (1838), *Arthrosolen* C.A.Mey. (1843), *Rhytidosolen* Van Tiegh. (1893), *Gnidiopsis* Van Tiegh. (1893), *Englerodaphne* Gilg (1894), *Craspedostoma* Domke (1934), *Pseudignidia* Phill. (1944), *Basutica* Phill. (1944) and *Struthiolopsis* Phill. (1944). Of these, *Trimeiandra* was a misapplied name, because the type material is a specimen of the genus *Lonchostoma* (family Bruniaceae), discussed previously.

PETERSON (1959) argued that the segregation of small genera from *Gnidia* based on one or two inconsistent characters would only add to the confusion surrounding this genus. He considered the characters used previously to distinguish *Arthrosolen*, *Gnidia* and *Lasiosiphon*, namely number of calyx lobes (i.e. flower merosity) and presence or absence of petal-like scales too variable to uphold these genera. He also combined

Pseudognidia, *Basutica* and *Struthiolopsis* with *Gnidia*. He rejected the generic value of the characters proposed by PHILLIPS (1944) for recognizing these three taxa, namely the number of calyx lobes in relation to the number of stamens, and the unusual multiplicity of petal segments and hairs (in *Struthiolopsis*), arguing this rationale was no more justifiable than to uphold *Gnidia*, *Arthrosolen* and *Lasiosiphon* using number of calyx lobes and presence or absence of scales. *Struthiolopsis* flowers have four petal-like scales, but they are extensively laciniate, giving the appearance of many petal-like scales present. PETERSON (1959) defended his standpoint by stating that there were a number of other species that differed in some characters from (in his words) "*true gnidias*", but to follow suit and split off single or small numbers of species into separate genera would cause greater confusion. Instead, PETERSON (1959) advocated placing divergent species in subgenera under *Gnidia*, which he evidently viewed as a 'natural' group.

PETERSON (1978) did not combine *Struthiola* and *Gnidia*, even though he acknowledged that only one character, namely the ratio of the number of stamens to calyx lobes separated them, yet all the while he accepted haplostemonous species in *Gnidia*. It seems an inconsistency therefore that PETERSON (1959) maintained *Gnidia* and *Struthiola* as separate genera on the basis of one less-than-absolute character (stamen number), yet combined *Lasiosiphon* with *Gnidia* because of the slight inconsistency of the pentamerous floral condition in *Lasiosiphon*. PETERSON (1978) possibly valued the absolute constancy of the isostemony in *Struthiola* species, which show no trace of staminodes, unlike some haplostemonous *Gnidia* species, for example *G. anomala* Meisn., *Gnidia linearifolia* (Wikstr.) B.Peterson and *G. nana* (L.f.) Wikstr. in

which the upper row of stamens is reduced to staminodes in some flowers, and absent altogether in others. However, this argument may also have little grounding, because *Gnidia aberrans* C.H.Wright and *Gnidia propinqua* (Hilliard) B.Peterson (both formerly included in *Basutica*), are like *Struthiola*, strictly haplostemonous, with no trace of staminodes, yet curiously, PETERSON (1959) combined these two within *Gnidia*. PETERSON (1959) proposed a broad-based concept of *Gnidia* with species divergent in one or other characters accommodated under different subgenera. Possibly he intended to place haplostemonous species in one of his proposed subgenera of *Gnidia*.

PETERSON (1959, 1978 and 2006) largely influenced the viewpoints of subsequent authors on the circumscription of *Gnidia*. Two contemporaries of Bo Peterson generally shared his broad-based view of *Gnidia*. Following PETERSON (1959, 1978), DYER (1975) combined *Lasiosiphon, Arthrosolen*, *Pseudognidia*, *Basutica* and *Struthiolopsis* with *Gnidia*, but maintained *Englerodaphne*, citing the comparatively membranous leaves, and ebracteate inflorescences and paired petal-like structures in each calyx sinus to distinguish it from *Gnidia*. A similarly inclusive view of *Gnidia* was supported by ROBYNS (1975) in his treatment of Central African Thymelaeaceae. Like STANER (1935), PETERSON (1958, 1959) and contemporaries, ROBYNS (1975) also combined *Lasiosiphon*, *Arthrosolen* and, (unlike DYER 1975), *Englerodaphne* in *Gnidia*, using the predictable argument that the instability of both flower merosity and presence or absence of “petals” were altogether not robust enough as generic characters (ROBYNS 1975, and references therein).

Monophyly and molecules

VAN DER BANK ET AL. (2002) using evidence from cladistic analyses of combined data sets from *rbc*L, *trn*L and *trn*L-F nucleotide sequence data, found strong support for the monophyly of *Passerina* and *Struthiola*, but their results also suggested that *Gnidia* was polyphyletic. This represented a significant change in thinking which had generally considered *Gnidia*, whether in a broad or narrow sense, as a 'natural', i.e. monophyletic group. Their analyses identified four main lineages for *Gnidia*, which suggested very close phylogenetic relationships of some *Gnidia* species variously with taxa from South Africa, South America and Madagascar. VAN DER BANK ET AL. (2002) presented the first evidence in support of significant phylogenetic links of selected *Gnidia* species with either *Drapetes* Banks ex Lam. (South America and Falkland Islands) or *Pimelea* Banks ex Sol. (Australia).

In his classification of the Thymelaeoideae, HERBER (2003) established four groups corresponding more or less to the four tribes recognised by DOMKE (1934). The 'Gnidia group' of HERBER (2003) corresponds on the whole, with tribe Gnidieae sensu DOMKE (1934), except for *Thymelaea* which HERBER (2003) placed in his 'Daphne group', comprising genera mostly from the northern hemisphere and none from sub-Saharan Africa. In contrast, the 'Gnidia group' comprises southern hemisphere genera namely, *Gnidia*, *Dais*, *Struthiola*, *Lachnaea* (including *Cryptadenia*), *Passerina*, *Drapetes*, *Kelleria* Endl. and *Pimelea*. Altogether the 'Gnidia group' represents a diversity of character traits, with habit, gender, floral tube articulation, floral merosity, numbers of stamens, lengths of filaments, the presence or absence of a nectariferous disc and

attachment of the style together with geographical distribution all variable among members. HERBER (2003) admitted that no obvious synapomorphy was apparent for any of his four groups and that they could not be considered as monophyletic entities. HERBER (2003) followed PETERSON (1959) in supporting a broad generic concept of *Gnidia* (Table 2.2). He also reduced the Malagasy monotypic endemic *Atemnosiphon* to synonymy under *Gnidia*, although he did not explain his reasons for doing so.

Based on evidence from a molecular study of the Thymelaeaceae using nuclear ribosomal DNA internal transcribed spacer (ITS), and plastid *rbcL*, *trn*L intron and *trnL*-F intergenic spacer regions, BEAUMONT ET AL. (2009, Chapter 5, this thesis) reinstated the genus *Lasiosiphon*, amended from its original concept by FRESENIUS (1838) to include both species with tetramerous and species with pentamerous flowers. Their results also supported *Dais* and *Phaleria* Jack as sister to *Lasiosiphon*, underlining the belief by DOMKE (1934) that *Dais* and *Phaleria* were closely related to *Lasiosiphon* and *Gnidia*, but differing from HERBER (2003) who placed *Dais* and *Phaleria* far apart in different ‘groups’.

ROGERS (2009) presented a revision of 14 Malagasy *Gnidia* species based on morphological characters. He combined *Gnidia*, *Lasiosiphon* and *Arthrosolen* because of the lack of novel morphological evidence to challenge the generally held opinion advanced by PETERSON (1959, 1978) of this synonymy. Also, a preliminary phylogenetic analysis of *Gnidia* and other Thymelaeaceae using molecular data (VAN DER BANK ET AL. 2002) did not resolve the generic status of these three taxa. ROGERS (2009) therefore followed the generally accepted norm and supported a broad

concept of *Gnidia*. However, unlike HERBER (2003), ROGERS (2009) did not include *Atemnosiphon* in *Gnidia*, citing the exserted filaments, leaf venation, unarticulated hypanthium, a comparatively large sub-gynoecial (nectariferous) disc and type of fruit dehiscence of this species as atypical within the circumscription of *Gnidia* advanced by HERBER (2003). Furthermore styles are hairy immediately below the stigma in *Atemnosiphon* and are not hairy elsewhere in *Gnidia* (Beaumont, personal observation). In his key to Malagasy *Gnidia*, ROGERS (2009) first distinguished species with tetramerous flowers from those with pentamerous flowers, finding flower merosity consistent in both flower types within species; but he presented no infra-generic classification of *Gnidia*.

BEAUMONT ET AL. (2009) investigated the generic limits of *Gnidia* using a phylogenetic analysis of nuclear ribosomal DNA internal transcribed spacer (ITS) and plastid *rbc*L, *trnL* intron and *trnL*-F intergenic spacer regions. This study expanded on the work of VAN DER BANK ET AL. (2002). The study by BEAUMONT ET AL. (2009) incorporated data for 36 *Gnidia* species plus selected species of the Thymelaeoideae drawn mostly from genera of southern and tropical Africa and Australia. Their results supported the initial findings of VAN DER BANK ET AL. (2002) namely that *Gnidia,* when treated in its broad inclusive sense (i.e. *sensu* PETERSON, 1959, 1978, 2006), is *not* monophyletic. Results of the study by VAN DER BANK ET AL. (2002) strongly supported the monophyly of both *Passerina* and *Struthiola*, but the results of BEAUMONT ET AL. (2009) suggested that *Struthiola*, as generally accepted, is not monophyletic. BEAUMONT ET AL. (2009) identified four, well-supported clades involving *Gnidia* species in both parsimony and Bayesian analyses, and showed that

selected *Gnidia* species are phylogenetically more closely related to various southern African, Australian and South American taxa. Furthermore, results in BEAUMONT ET AL. (2009) placed the type species for *Gnidia* within *Struthiola*; and showed clearly that *Gnidia penicillata* Licht. is embedded within the Cape endemic genus *Lachnaea*. BEAUMONT ET AL. (2009) also reinstated the genus *Lasiosiphon*: amended to include both tetramerous and pentamerous taxa. The taxonomic implications of these results and identification of morphological synapomorphies in support of these clades is discussed in detail in Chapter 7. Table 2.3 lists the chronological order of publication of selected Southern Hemisphere genera of Thymelaeoideae. The potential implications for the taxonomy of *Gnidia* and its relatives and the issue of priority of names are also discussed in Chapters 5 and 7.

In the pre-molecular era of plant taxonomy workers relied on morphological and anatomical features to help classify *Gnidia* and relatives. The Linnaean 'Sexual System' of classification, constructed above all, on reproductive characters, focused the attention of subsequent workers on such characters, with vegetative characters given scant regard.

Flower merosity, the development of the petal-like structures (*viz*. *côrolla sensu* Linnaeus, scales or *squamulae*) situated between the sepal lobe sinuses and number of stamens, have all been used at the generic level to distinguish segregate genera from *Gnidia*, but because these characters show some variation many workers rejected their usefulness at the generic level and accordingly reduced these segregate genera to synonymy under *Gnidia*. Alternatively, these and other characters such as the presence

Table 2.3. Geographical distributions, type species and authors and dates of publication of selected genera of Southern Hemisphere Thymelaeoideae. Genera are listed primarily in chronological order of publication, thereafter in alphabetical order.

Genus	Geographical distribution	Type species	Author and date of publication
Gnidia L.	Sub-Saharan Africa, Madagascar	*Gnidia pinifolia* L.	LINNAEUS 1753
Lachnaea L.	South Africa	*Lachnaea eriocephala* L.	LINNAEUS 1753
Passerina L.	Sub-Saharan Africa	*Passerina filiformis* L.	LINNAEUS 1753
Struthiola L.	Sub-Saharan Africa	*Struthiola virgata* L.	LINNAEUS 1767
Pimelea Banks & Sol. ex Gaertn.	Australia, New Zealand, and adjacent islands	*Pimelea laevigata* Gaertn.	BANKS & SOLANDER 1788
Drapetes Lam.	South America, Falkland Islands	**Drapetes muscosus* Lam. ***Drapetes muscosa* Lam.	LAMARCK 1792
Lasiosiphon Fresen.	Sub-Saharan Africa, Madagascar	*Lasiosiphon glaucus* Fresen.	FRESENIUS 1838
Arthrosolen C.A.Mey.	Sub-Saharan Africa	*Arthrosolen polycephalus* C.A.Mey.	MEYER 1843
Englerodaphne Gilg	Sub-Saharan Africa	*Englerodaphne leiosiphon* Gilg	GILG 1894

* As cited in GREUTER ET AL. (1993).

** As cited in HEADS (1990).

and degree of modification of involucral bracts, inflorescence structure and the degree of hairiness and distribution of hairs of receptacles and floral tubes have been used at infrageneric levels of classification within *Gnidia*. Such classifications with their infrageneric ranks, however, have been restricted to regional accounts of species, and

to date no comprehensive monograph of *Gnidia* has been published since the mid-19th century. Phylogenetic studies using molecular sequence data in the late 20th and early 21st centuries have further challenged our understanding of the generic limits of *Gnidia*. Two such studies strongly suggest that *Gnidia* is polyphyletic, and not monophyletic as generally assumed before. Furthermore, whilst workers have traditionally acknowledged the close relationships of *Gnidia* with other mostly South African genera such as *Struthiola* and *Lachnaea*, none have to date gone so far as to suggest that these genera should be combined. Recent molecular evidence presented in Chapter 5 of this thesis, however, suggests that at least two species of *Gnidia* are more closely related to *Struthiola* than to other *Gnidia* species; that one *Gnidia* species is better-placed in *Lachnaea*, that a fourth is tentatively considered phylogenetically closest to *Pimelea*, and that some *Gnidia* species are phylogenetically closer to *Drapetes* from southern South America. Furthermore, the genus *Lasiosiphon* was reinstated (Chapter 5, this thesis) based on compelling molecular evidence from the same study and backed up by morphological features that help distinguish this from *Gnidia* and relatives.

That preliminary molecular studies suggest *Gnidia* is polyphyletic with close phylogenetic associations to a number of both African and non-African genera, compels us to re-examine characters of morphology and anatomy to help us better distinguish *Gnidia* and relatives. The taxonomic implications for *Gnidia* and associated taxa stemming from these results are potentially vast and will not be received without some controversy. However, if a reappraisal of morphological characters can help make clear any proposed changes to the classification of *Gnidia* and kin, we may better understand this and related genera.

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CHAPTER 3

LEAF AND BRACT DIVERSITY IN *GNIDIA* (THYMELAEACEAE): PATTERNS AND TAXONOMIC VALUE

BEAUMONT, A.J., EDWARDS, T.J. & SMITH, F.R. 2001. Leaf and bract diversity in *Gnidia* (Thymelaeaceae): patterns and taxonomic value. *Systematics and Geography of Plants* **71:** 399-418.

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Leaf and bract diversity in *Gnidia* (Thymelaeaceae): patterns and taxonomic value

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Abstract. *– Morphological, anatomical and morphometric studies of leaf and bract diversity among selected species of* Gnidia *(Thymelaeaceae) were conducted. Surface details of fresh and herbarium-dried leaves and bracts were examined using Light Microscopy and Scanning Electron Microscopy. Intraspecific and interspecific variation of leaves and bracts based on logarithmic transformations of linear data, was performed using Univariate Nested Analyses of Variance and Univariate Analyses of Variance. A Correlation Analysis was performed to test the correlation between leaf and bract ratios within species. Interspecific variation was examined further using Discriminant Analysis to maximise the separation of species on the basis of leaf and bract dimension data. Newly observed features of leaves and bracts include: hair ornamentation; specialisation of stomata; aerenchymatous-like tissue and reduced mesophyll. Homogenous groups of species were identified on the basis of their similarity of leaf length and width or bract length and width ratios. The species comprising the homogenous groups for leaf ratios are different from the species comprising the homogenous groups for bract ratios. There is no correlation between leaf and bract ratios, and the factors influencing leaf diversity appear to differ to those influencing bract diversity. Results also suggest that inflorescence organisation strongly influences bract diversity.*

Key words: *analyses of variance, anatomy, bracts,* Gnidia, *leaves, morphology, numerical analyses, Thymelaeaceae.*

Résumé. **– Diversité des bractées et des feuilles de *Gnidia* (Thymelaeaceae): caractéristiques et valeur taxonomique.** *Des études morphologiques, anatomiques et morphométriques de la diversité des bractées et des feuilles dans une sélection d'espèces de* Gnidia *(Thymeleaceae) ont été menées. Les détails de la surface de bractées et feuilles fraîches ou d'herbier ont été examinés au microscope optique et électronique à balayage. La variation intraspécifique et interspécifique des feuilles et des bractées basée sur les transformations logarithmiques de données linéaires a été donnée en utilisant les analyses de variance univariées et univariées hiérarchisées. Une analyse de corrélation a été exécutée pour tester la corrélation entre les dimensions des feuilles et des bractées au sein de l'espèce.*

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La variation interspécifique a été examinée plus à fond en utilisant l'analyse discriminante afin d'optimaliser la séparation des espèces sur base des dimensions mesurées des feuilles et bractées. Les nouvelles caractéristiques observées de feuilles et des bractées comprennent: ornemention des poils, spécialisation des stomates, tissu semblable à l'aérenchyme et mésophylle réduit. Des groupes homogènes d'espèces ont été identifiés sur base de leur ressemblance quant au rapport de la longueur et de la largeur des feuilles et des bractées. Les espèces comprenant les groupes homogènes pour les rapports des feuilles sont différents des espèces comprenant les groupes homogènes pour les rapports des bractées. Il n'y a pas de corrélation entre les dimensions des feuilles et des bractées et les facteurs influençant la diversité des feuilles semblent différents de ceux qui influencent la diversité des bractées. Les résultats suggèrent aussi que l'organisation de l'inflorescence influence fortement la diversité des bractées. Traduit par le journal.

1 Introduction

Gnidia L. is the largest genus of the family Thymelaeaceae, comprising about 140 species that occur in southern and eastern tropical Africa, Madagascar and India (Heywood 1979). Southern Africa is home to more than 100 species and the southern Cape is the centre of taxonomic diversity. Species occupy a range of habitats: coastal and mountain forests and grasslands, to fynbos and semi-arid karoo. *Gnidia* plants are small to medium shrubs or rarely small trees. Leaves are simple and entire, and stipules are absent or vestigial. Bracts are modified or reduced leaves that subtend a flower or inflorescence (Heywood 1979). They are absent or poorly developed in some genera of the Thymelaeaceae. In other genera, bracts have undergone extensive modification and their diversity is useful for distinguishing levels of taxa. Bracts occur in most species of *Gnidia* and resemble leaves or are variously modified. Inflorescences vary from capitate, racemose, spicate and few-flowered clusters to flowers in pairs in leaf-like bract axils to solitary flowers. The floral tube ending in four or five free petal-like lobes is calycine (true petals are absent), and flowers are colourful and conspicuous, to dull-coloured and leaf-like in colour, making them not immediately discernible from the vegetative body.

The generic limits of *Gnidia* are controversial. Taxonomic limits of *Gnidia* and allied genera have relied mostly on evidence from studies of floral morphology. Floral features however, gained notoriety for their variability, and their taxonomic value needs reassessing. Current opinion favours the inclusion of *Arthrosolen* C.A.Mey., *Basutica* Phill., *Englerodaphne* Gilg, *Lasiosiphon* Fresen., *Pseudognidia* Phill. and *Struthiolopsis* Phill. within *Gnidia* (Peterson 1959, 1978). Within the broad circumscription of *Gnidia*, there is no sub-generic classification and species relationships are poorly understood. This work contributes to an investigation of the diversity among vegetative and floral organs of *Gnidia* species, and the results will be incorporated in to a cladistic analysis of this group. Other workers have realised the diversity and systematic value of leaves in the Thymelaeaceae. Rye (1990) used leaf arrangement; venation; presence, density and types of hairs; leaf colour dimorphism; and both whole leaf shape and leaf shape in transverse section, to distinguish species of *Pimelea* Banks & Sol. ex Gaertner. Beyers & Van der Walt (1995) found no macromorphological characters of leaves that distinguished *Lachnaea* L. from *Cryptadenia* Meisn. Anatomical similarity confirms their close relationship, and together with floral and fruit data, supports the inclusion of *Cryptadenia* in *Lachnaea* (Beyers & Van der Walt 1995). Bredenkamp & Van Wyk (1999) provided evidence to support the authenticity of mucilaginous cell walls in epidermal tissue of *Passerina* species. The work of Bredenkamp & Van Wyk (2000) detailed epidermal features of *Passerina* of taxonomic significance at the species level.

Preliminary observations show that leaves and bracts of *Gnidia* species are diverse and potentially useful in systematic studies. This work was inspired by the paucity of comparative morphological and anatomical studies in this genus, and together with morphometric analyses, forms part of a survey of the diversity of leaves and bracts in *Gnidia*.

2 Material and methods

2.1 Morphometric analyses

Eighteen species of *Gnidia* were examined (tab. 1) which represents approximately one eighth of the total number of species and encompassing the broad range of leaves and bracts for the genus.

Lengths and widths of leaves and bracts were measured using dried herbarium specimens. Five leaves and five bracts were measured from five specimens of each species. Leaves and outermost bracts were measured to within 0.25 mm accuracy, except for *G. polycephala*, in which the innermost and widest bracts were measured because these show greatest modification. Using these data, leaf length to width ratios, and bract length to width ratios were calculated. Length to width ratios were chosen because linear measurements of leaves and bracts often overlap among species and do not help to distinguish taxa. Length and width ratios however, appear to be more consistent within species, even if leaf and bract sizes vary within or among plants.

Table 1. Species and specimens of the statistical analysis.

Species and their abbreviated codes as illustrated in the results of the statistical analyses; specimens used in the statistical analyses.

ABE: *G. aberrans* C.H.Wr.
Bayliss 5526 (C), *Bruyns-Haylett sn* (NU), *Hilliard & Burtt 8758* (NU), *Nicholas, Priday & Keet 2046* (NH), *Wright 670* (NU).

ANT: *G. anthylloides* (L.f.) Gilg
Bayliss 5762 (C), *Cooper 1521* (PRE), Nielsen 1452 (C), *Scharf 1466* (PRE), *Schlechter 1904* (C).

BAU: *G. baurii* C.H.Wr.
Grice sn (NU), *Huntley 179* (NH), *Jordaan 564* (NH), *MacDevette 1362* (NH), *Wood 10610* (NH).

BUR: *G. burchellii* (Meisn.) Gilg
Ferreira F004 (PRE), *Joffe 374* (PRE), *Rogers 15900* (PRE), *Theron 1528* (PRE), *Van Wyk 4462* (PRE).

DAN: *G. danguyana* Leandri
"*Havoa Hafotran N. 118. M. Louvel- Madagascar*" (P), "*Herbier di Petit Thomars*" (P), "*Madagascar Exposition Coloniale de Marseille*" (P), "*Madagascar. M. Louvel. Foret de Tampiva No. 1970 Syntype*" (P), *Service des Eaux et Forets No. 9900*" (P).

DEN: *G. denudata* Lindl.
Dahlstrand 1296 (STE), "*Herb. US No. 14412, Knysna*" (STE), *Hugo 1300* (STE), *Meyer 10966* (STE), *Rogers 206802* (STE).

GEM: *G. geminiflora* E. Mey. ex Meisn.
"*Botanical Dept. Univ of Cape Town No. 2888*" (BOL), *Esterhuysen 16134* (BOL), *Esterhuysen 18031* (BOL), *Goldblatt 3799* (GB), *Schlechter sn*, "Elim 9.1 1867" (GB).

INV: *G. involucrata* A. Rich.
De Wilde & Gilbert 246 (EA), *Guillaume 5* (EA), *Leedal 4072* (EA), *Lindsay 10* (EA), *Milne-Redhead & Taylor 9257* (EA).

KRA: *G. kraussiana* Meisn.
Bayer & McClean 170 (PRE), *Galpin 9461* (PRE), *Ross 2158* (PRE), *Rudatis 1064* (PRE), *Theron 1533* (PRE).

LAX: *G. laxa* (L.f.) Gilg
Bolus 11371 (PRE), *Pillans 3789* (PRE), *Smith 4205* (PRE), *Thode A2384* (PRE), *Wolley-Dod 2108* (PRE).

LIN: *G. linoides* Wikstr.
Burchell 7042 (PRE), *Drège sn* (PRE no. 58504) (PRE), *Schlechter 2037* (C), *Schlechter 7243* (E), *Schlechter 9309* (E).

NOD: *G. nodiflora* Meisn.
Abbott 1538 (NH), *Abbott 21096b* (NH), "*Herbarium Natalensis 20238*" (NH), *Johnstone 564* (NU), *Van Wyk 8438* (NH).

PIN: *G. pinifolia* L.
Hugo 1662 (STE), *Oliver 3332* (STE), *Orchard 325* (STE), *Rycroft 2271* (STE), *Rycroft 2386* (STE).

POC: *G. polycephala* (C.A. Mey.) Gilg
Brink 560 (GRA), *Dyer 1010* (GRA), *Moran 19* (GRA), *Pearson 751* (GRA), *Sister Francis 43* (GRA).

POS: *G. polystachya* Berg.
Britten 671 (GRA), *Galpin 4524* (GRA), *Noel 323* (GRA), *Schlechter 9465* (GRA), *Schonland 794* (GRA).

SER: *G. sericocephala* (Meisn.) Gilg ex Engler
Codd 16181 (J), *Leendertz 2497* (J), *Moss sn* (J), *Ottley & Moss 2374* (J), *Van Rensburg sn* (J).

SPL: *G. splendens* Meisn.
Maguire sn (J), *Compton 32282* (PRE), *Germishuizen 3256* (PRE), *Stalmans 583* (PRE), *Tyson 1229* (J), *Venter 6216* (PRE).

VES: *G. vesiculosa* Eckl. & Zeyher ex Meisn.
Beyers 133 (PRE), *Compton 14761* (PRE), *Sidey 1846* (PRE), *Smith 5046* (PRE), *Van Breda 1746* (PRE).

Statistical analyses were performed using Statgraphics Plus 7.0. Logarithmic transformations of leaf and bract lengths, widths and ratios were used to normalise the data and stabilise their variances. Univariate Nested Analyses of Variance were performed separately on leaf and bract ratios to determine the variation between species compared to the variation within individual plants. In these analyses, the species effect was tested against the variation among specimens within species, while the specimen effect was tested against the residual error (Sokal & Rohlf 1995). Univariate Analyses of Variance were performed separately on leaf and bract ratios, using the means of specimens (n=5) for each species, to determine the extent to which species differ. Tukey's Multiple HSD multiple range tests were then used to test for differences in leaf ratios and bract ratios between pairs of species. A Correlation Analysis (using specimen means) was also performed to test the correlation between leaf and bract ratios. To further analyse interspecific variation, a discriminant analysis (Klecka 1980, Nybom *et al* 1997) was performed using leaf length, leaf width, bract length and bract width data. The aim of this analysis was to maximise separation among species using these four variables.

2.2 Morphology and anatomy

Comparative illustrations of leaves and bracts, representing the diversity of leaf and bract shape and size for the genus were prepared. Species illustrated include all species used in the morphometric analyses, together with poorly collected species that further show the diversity of these organs, but for which insufficient material was available for analyses. These species are: *G. compacta* (C.H. Wr.) J.H. Ross; *G. glauca* Steud.; *G. insignis* Compton; *G. macropetala* Meissn.; *G. madagascariensis* (Lam.) Decne. var. *baronii* (Bak.) Leandri and *G. usafuae* Gilg. Terminology follows that of Lawrence (1955) and Dilcher (1974).

Morphology of leaves and bracts was examined using a Wild Heerbrugg stereo light microscope. Fresh material was examined using an Electron Microscope SP-2000 "Sputter Cryo" low temperature system. Herbarium-dried material was mounted on brass stubs and coated with gold-palladium. A Hitachi S570 Scanning Electron Microscope, using accelerating voltages of 6-8 kv for Cryo preparations, and 15 kv for dried material was used for micromorphological observations.

Fresh material was fixed directly in 3% glutaraldehyde with 1% caffeine for a minimum of 8 hours and embedded in Epon/Araldite resin according to standard techniques. Herbarium-dried material was reconstituted by heating in warm water and also prepared following the above protocol for fresh material. Sections 1-1.5 µm thick were cut using a Reichert-Jung Ultracut microtome and stained using Ladd's Multiple Stain (consisting of 0.365g Toluidine Blue in 35ml H_20 and 0.135g Basic Fuchsin in 15 mls of 30% ethyl alcohol, stirred and filtered). Permanent mounts were made with fresh Epon/Araldite [Epon 812: 1 part; Araldite CY212: 1 part; Dodecanyl succinic anhydride (DDSA): 3 parts, and 1 drop 2,4,6-tridimethyl amino methyl phenyl (DMP) per 1 ml Epon] resin and baked overnight at 70 °C. Sections were photographed with an Olympus BH-2 photomicroscope using PAN F 50 ASA black and white film and a green filter.

Specimens examined in morphological and anatomical studies

G. anthylloides Beaumont & Smith *s.n.* (LD); *G. macropetala* Beaumont *s.n.* (NU); *G. nana* Bolus 9238 (LD); *G. nodiflora* Beaumont & Beckett *s.n.* (NU); *G. pinifolia* Beaumont & Beckett *s.n.* (NU); *G. polycephala* Nortier *s.n.* (GB); *G. scabrida* Ecklon & Zeyher "Stellenbosch, Houhoeksbergen, 1000'-3000' Juli." (S); *G. thesioides* Beaumont *s.n.* (NU); *G. tomentosa* McKinnon 298 (STE).

3 Results

3.1 Morphometric analyses

Results of the Univariate Nested Analyses of Variance (tab. 2) show that both leaf and bract ratios vary significantly among species ($P< 0.001$), with an added but smaller, significant variance component among specimens within species ($P < 0.001$).

Results of the Univariate Analysis of Variance of leaf ratios showed a significant difference among species ($P < 0.001$). The Multiple Range test of leaf ratios revealed seven homogenous groups of species at the 95% confidence interval (tab. 3). There was considerable overlap in leaf ratios over the range of species, with a continuum of increasing leaf ratios from Group 1 to Group 7. The Multiple Range test when applied to bract ratios also revealed seven homogenous groups of species at the 95% confidence interval (tab. 3). Again, there was considerable overlap in bract ratios over the range of species, with a continuum of increasing ratios occurring across Groups 2 to 6. However, the species compositions of the homogenous groups for bract ratios were not the same as the species compositions of the homogenous groups for leaf ratios. Finally, there was no correlation between leaf and bract ratios for the group of eighteen species.

Group means (centroids) for the eighteen species along with positions of their specimens, are plotted on the first two discriminant functions obtained from the Discriminant Analysis of the log-transformed data (fig. 1). The analysis yielded four significant discriminant functions ($P < 0.001$). The first two functions explained 83% of the variation among species. The first discriminant function accounted for

Table 2. Nested Analyses of Variance of leaf and bract ratios among 18 *Gnidia* species and among specimens within species.
(***= $P<0.001$, *df*= degrees of freedom).

Character	Source of variation	F-ratio	*df*	Significance level	Percent of variation
Leaf ratio	among species	22.11	17/72	***	74.8
	among specimens	9.47	72/360	***	15.9
Bract ratio	among species	21.30	17/72	***	73.1
	among specimens	8.05	72/360	***	15.8

52% of the variation and clearly separated *G. danguyana*, *G. polycephala* and *G. vesiculosa* from each other and from the remaining species. Leaf width and bract width were the most important variables that discriminated species in the first function (fig. 1). The second discriminant function accounted for another 31% of the variation among species, and separated the remaining fifteen species into two core groups of species. Here, bract width and leaf length were the most important variables that discriminated these species.

3.2 Leaves

3.2.1 Leaf morphology

In woody species, leaves occur on distal stems; herbaceous species bear leaves along most of their stems. Leaf arrangement alternate, or rarely decussate; spreading or rarely upright. Petiole usually short and linear-oblong, occasionally minutely (*G. aberrans*, fig. 2A1 and *G. baurii*, fig. 2C1), or rarely absent (*G. vesiculosa* (fig. 7C1); hairy or glabrous. Lamina needle-like, linear-lanceolate, elliptic to oblong, ovate or obovate (fig. 2-7); bases attenuate to broadly attenuate (*G. compacta*, fig. 3A1 and *G. danguyana* fig. 3B1) to cuneate (*G. linoides*, fig. 5C1), or rarely cordate (*G. insignis*, fig. 4B1); tips obtuse acute, rarely mucronate (*G. glauca*, fig. 4A1).

3.2.2 Leaf micromorphology and anatomy

Among mesic species, the lamina is flat and extends either side of the main vascular bundle, narrow, linear and flat in transverse section, whilst the leaves of smaller-leafed species are upwardly-curved crescent-shaped in transverse section. Leaves lens-shaped in transverse section rare (*G. pinifolia*, fig. 10A). Hairs unicellular; absent, or polymorphic in populations of G. *kraussiana*; abaxial and/or adaxial, usually of one type on a leaf, usually colourless, rarely yellow (*G. glauca*), exclusively adaxial in *G. geminiflora* (fig. 3D1 and 3D2), absent in *G. insignis* (fig. 4B1), *G. involucrata* (fig. 4C1) and *G. usafuae* (fig. 7B1), short to long; straight to tomentose, woolly, villous or pilose. Hairs smooth in formerly recognised species of *Lasiosiphon*, warty or pustulate in small-leaved species (*G. baurii* and *G. nodiflora*, fig. 8D) or barbed (*G. nana*, fig. 8E). Cuticle smooth (*G. macropetala*, fig. 8A) to rarely minutely papillate (*G. pinifolia*, fig. 8B). Surface-borne wax particles sparse to dense (fig. 8A), flake-like to granular, most abundant in mesic species. Stomata amphistomatic or epi- or hypostomatic; anisocytic (fig. 8A) or paracytic (fig. 8B); guard cells symmetrical, flush with lamina surface (fig. 8A), or sunken, sometimes overtopped by ridge-like peristomatal cuticular rims (fig. 8C). Epidermal cells, penta- and hexagonal in surface view, anticlinal cell wall outlines straight (fig. 8A), or cells tetragonal with straight and rounded anticlinal wall outlines (fig. 8B); slightly flattened dorsiventrally, oblong-elliptic (fig. 9A) or square (fig. 9B) in transverse section. Mucilagenous cells punctuate epidermal layers (fig. 10A), their frequency and distribution varying between species, but particularly abundant in *G. pinifolia* (fig. 10A). Multicellular projections of the epidermis around the hair bases in four species from the Western Cape Province, South Africa (fig. 8D). Mesophyll chlorenchymatous, palisade-like

Table 3. Seven homogenous groups of *Gnidia* species based on Tukey's 95 % HSD Multiple Range Test of leaf length/width ratios and bract length/width ratios.
(n = 5 specimens per species. Refer to tab. 1 for descriptions of species codes).

Species code	Average ratio	Homogenous groups						
		1	2	3	4	5	6	7
		Leaf ratios						
DAN	0.386	x						
VES	0.482	x	x					
DEN	0.519	x	x	x				
ANT	0.539	x	x	x				
BAU	0.552	x	x	x				
KRA	0.559	x	x	x				
ABE	0.599	x	x	x	x			
GEM	0.648		x	x	x	x		
SPL	0.672		x	x	x	x		
BUR	0.708			x	x	x		
LAX	0.721			x	x	x		
POS	0.815				x	x	x	
POC	0.816				x	x	x	
NOD	0.865					x	x	x
INV	0.974						x	x
SER	1.004						x	x
PIN	1.034						x	x
LIN	1.051							x
		Bract ratios						
INV	0.199	x						
POC	0.199	x						
SER	0.369	x	x					
SPL	0.441		x	x				
ANT	0.489		x	x	x			
BUR	0.507		x	x	x	x		
LAX	0.507		x	x	x	x	x	
PIN	0.516		x	x	x	x	x	
DEN	0.518		x	x	x	x	x	
POS	0.537		x	x	x	x	x	
BAU	0.542		x	x	x	x	x	
ABE	0.553		x	x	x	x	x	
KRA	0.600			x	x	x	x	
DAN	0.662				x	x	x	
GEM	0.680				x	x	x	
VES	0.696					x	x	
NOD	0.719						x	
LIN	0.979							x

(lengthened and perpendicular to the axis) to short and isodiametric. One or two layers of palisade mesophyll occurs in larger, mesic leaves; multiple palisade in small foliaceous leaves and narrowly oblong leaves, palisade isolateral in smaller-leaved species (fig. 10A), isolateral and bifacial palisade in mesic species. Venation usually of single midrib and less-well defined lateral to semi-lateral secondary brochidodromous veins. Leaves (and bracts) of *G. denudata* (fig. 3C) and species with small, narrowly foliaceous leaves and bracts, for example, *G. linoides* (fig. 5C) have acrodromous venation. Median vascular bundle resembles flattened ellipse in transverse section; intraxylary phloem absent; sclerenchyma sparse in larger-leaved species, abundant in species with small, foliaceous leaves (*G. nodiflora,* fig. 9A).

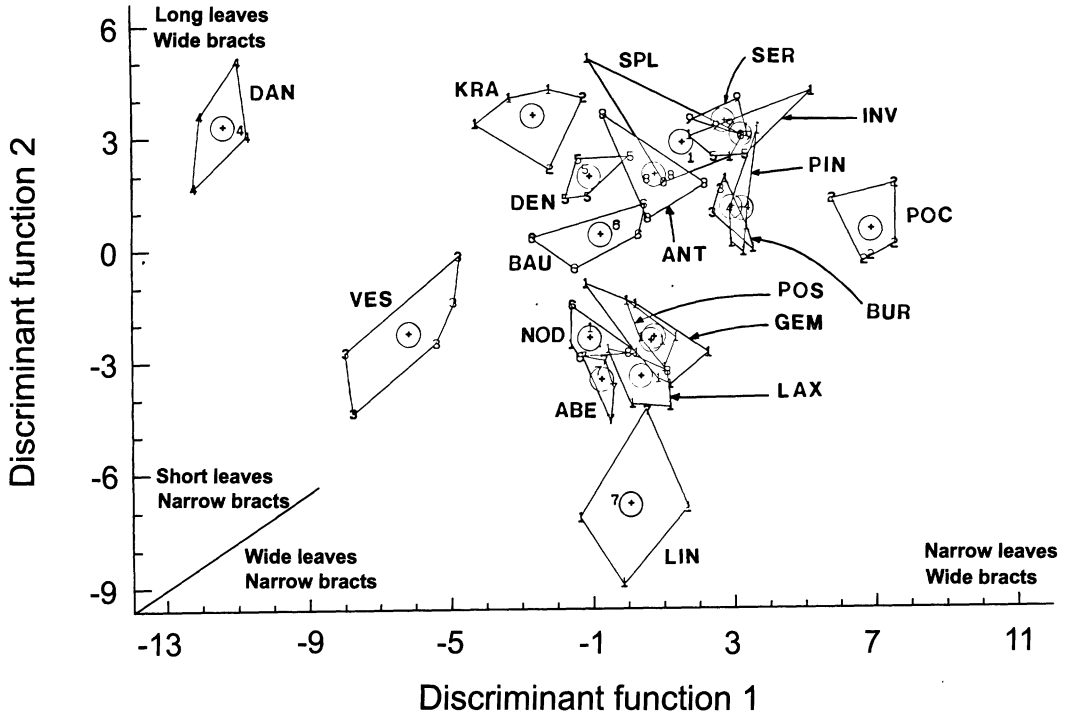

Figure 1.
Discriminant Analysis of species using transformed data for leaf and bract lengths and widths.
Group centroids (+) of the four variables for each species are circled.
Small numbers show the positions of the five specimens for each species.
Code letters indicate species (tab. 1).

3.3 Bracts

3.3.1 Bract morphology

Multiple or single whorls subtending many- to few-flowered inflorescences, to single units subtending individual flowers, reduced (*G. danguyana*, fig. 3B2) or absent. Whorls spreading in *G. pinifolia*; ascending in few-flowered inflorescences, to upright and vase-like in former species of *Lasiosiphon*. Petioles short or absent; resembling leaf petioles in small-leaved species, with single flowers or few-flowered clusters for example, *G. aberrans* (fig. 2A2) and *G. linoides* (fig. 5C2), broadened and flattened such that bracts almost sessile (*G. kraussiana*, fig. 5A2, *G. macropetala*, fig. 5D2 and *G. pinifolia*, fig. 6B2) or hardened and bulb-like (*G. sericocephala*, fig. 7B2). Lamina outline resembles that of leaves or differs. Bracts as broad as long (*G. glauca*, fig. 4A2 and *G. insignis*, fig. 4B2), to almost as broad as long in *G. involucrata* (fig. 4C2) and *G. usafuae* (fig. 7B2). Bases cuneate (*G. linoides*, fig. 5C2) to round (*G. anthylloides*, fig. 2B2, *G. denudata*, fig. 3C2 and *G. polycephala*, fig. 6C3). Tips acute to round, strongly caudate in *G. madagascariensis var. baronii* (fig. 5E2) and *G. sericocephala* (fig. 7A2).

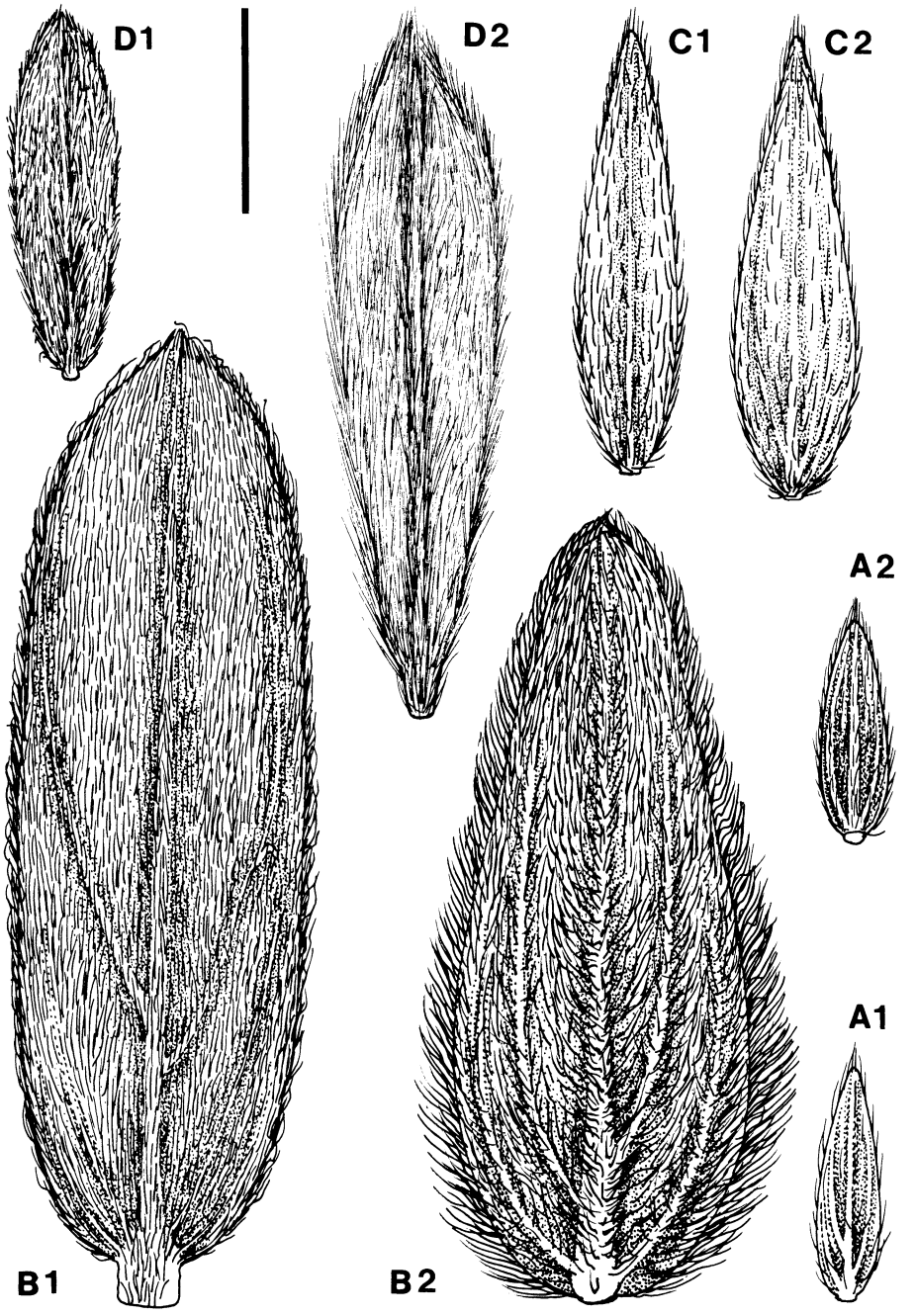

Figure 2. Leaves and bracts of Gnidia *species I. A* G. aberrans, *Bruyns-Huylett 64* (NU)*: A1 leaf, A2 bract. B.* G. anthylloides, *Ward 6114* (PRE)*: B1 leaf, B2 bract. C.* G. baurii, *Baur 732* (NH)*: C1 leaf, C2 bract. D.* G. burchellii, *Sidey 3856* (PRE)*: D1 leaf, D2 bract. Bar scale = 5 mm.*

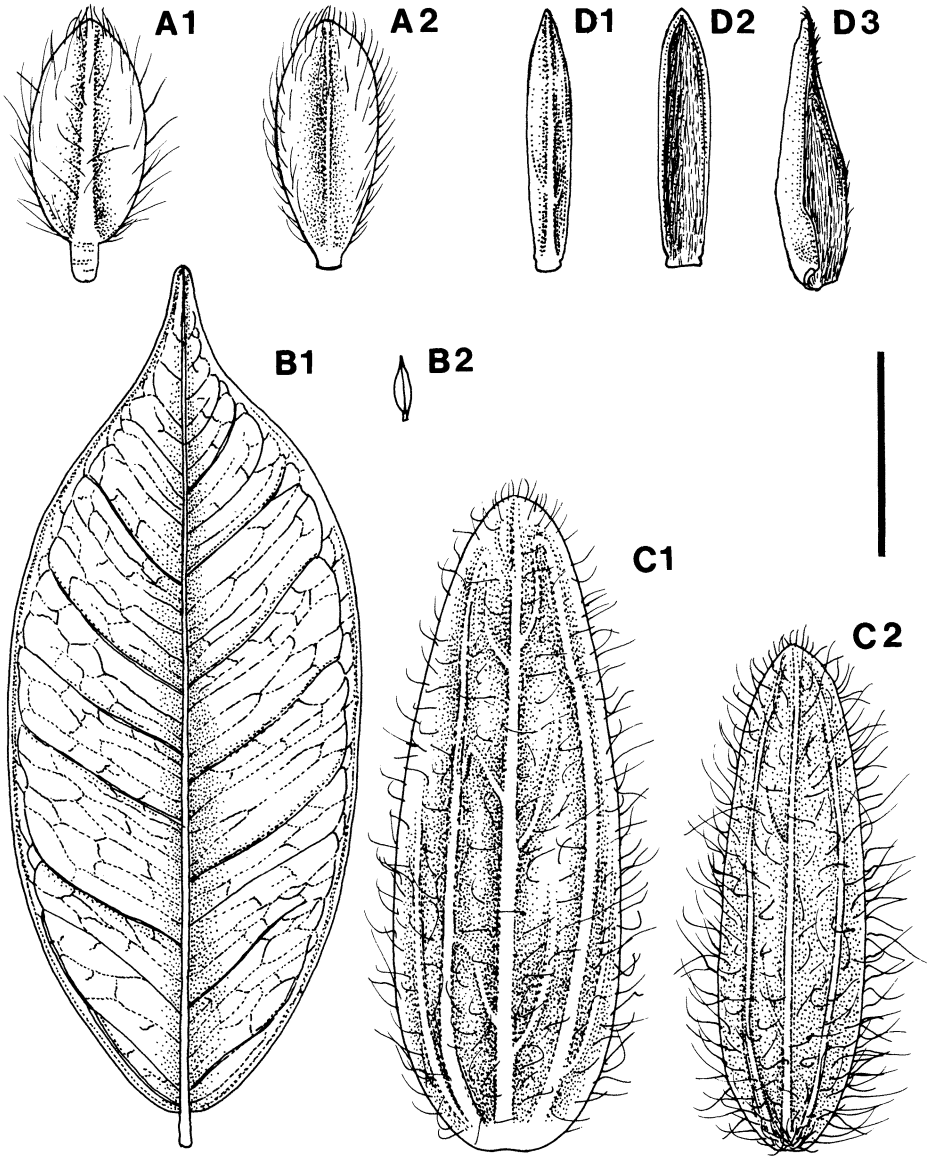

Figure 3. Leaves and bracts of Gnidia *species II. A.* G. compacta, *Hilliard & Burtt 9335* (NU)*: A1 leaf, A2 bract. B.* G. danguyana *Louvel 118* (P)*: B1 leaf, B2 bract. C.* G. denudata*: C1 leaf, C2 bract. D.* G. geminiflora, *Goldblatt 3799* (GB)*: D1 abaxial leaf surface, D2 adaxial leaf surface, D3 bract. Bar scale: A, C, D = 5 mm; B = 15 mm.*

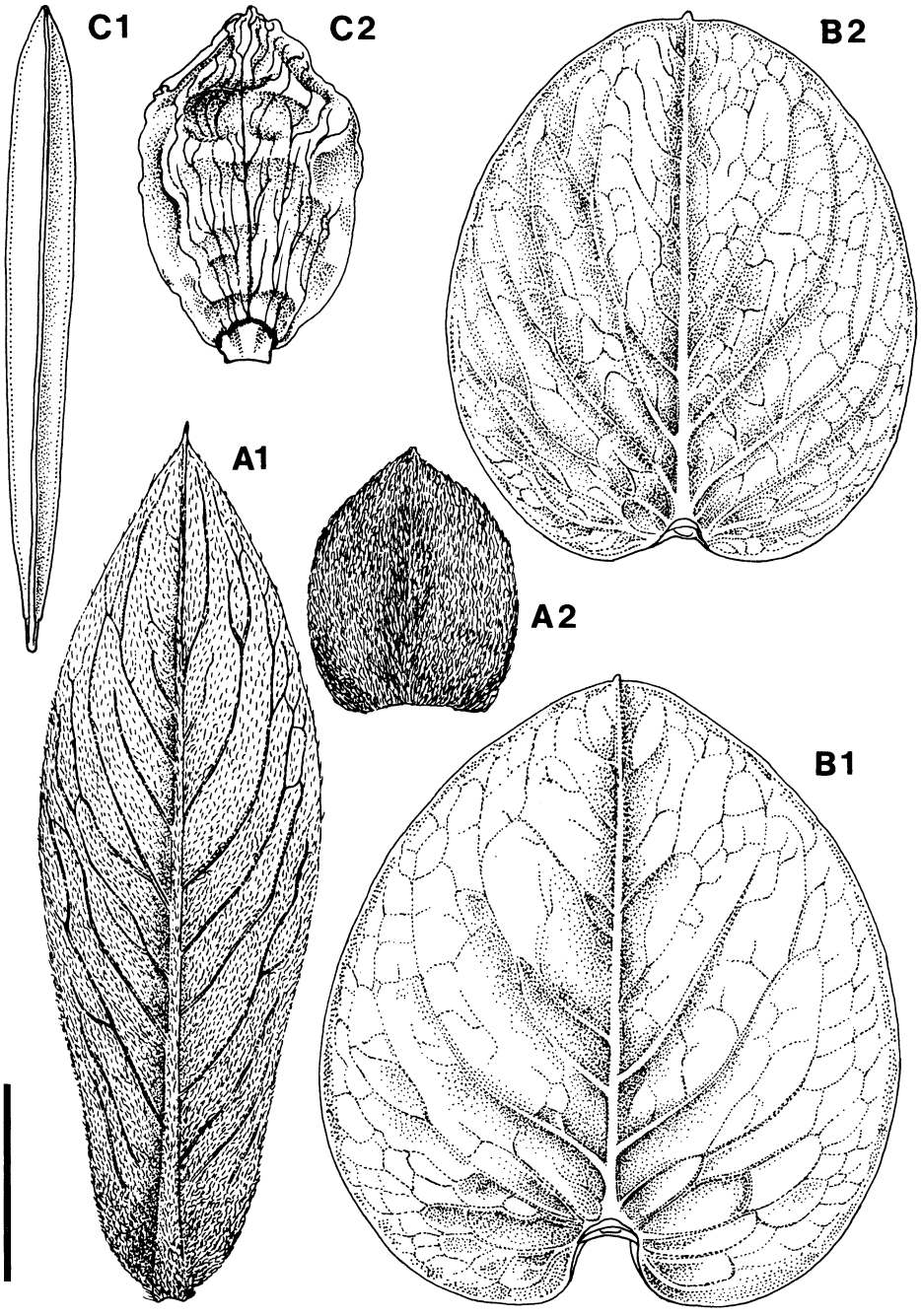

Figure 4. Leaves and bracts of Gnidia *species III. A.* G. glauca *Friis & al 1524* (EA)*: A1 leaf, A2 bract. B.* G. insignis, *Esterhusen 14060* (BOL)*: B1 leaf, B2 bract. C.* G. involucrata, *Guillaume 5* (EA)*: C1 leaf C2 bract. Bar scale: A, B = 10 mm; C = 5 mm.*

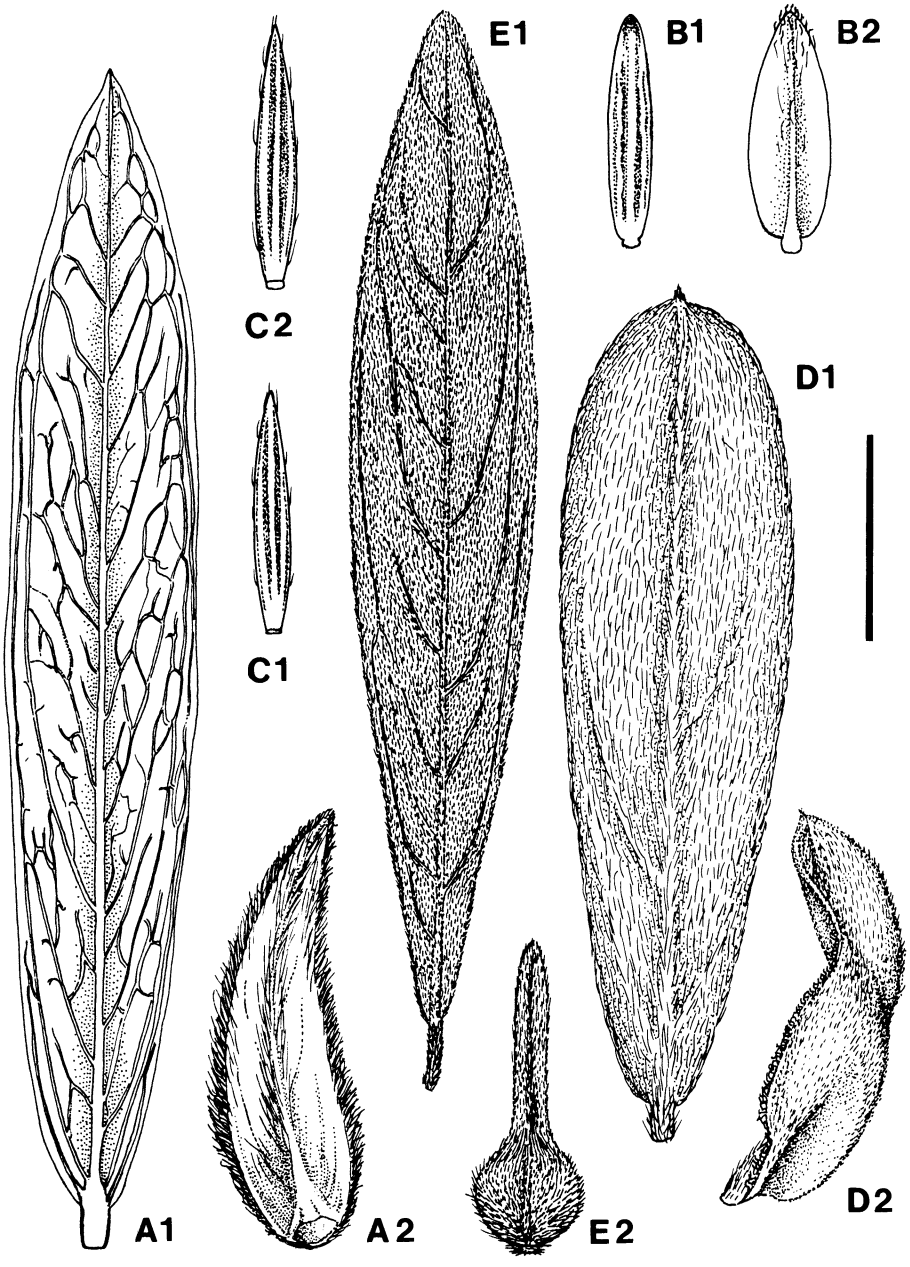

Figure 5. Leaves and bracts of Gnidia *species IV. A.* G. kraussiana, *Phelan 103,* (NU)*: A1 leaf, B2 bract. B.* G. laxa*: B1 leaf, B2 bract. C. G.* linoides, *Pica Survey 838* (BOL)*: C1 leaf, C2 bract. D.* G. macropetala *Beaumont 3/97* (NU)*: D1 leaf, D2 bract. E.* G. madagascariensis *var.* baronii, *Perrier 8552* (P)*: E1 leaf, E2 bract. Bar scale: A, B, C, D = 5 mm; E = 10 mm.*

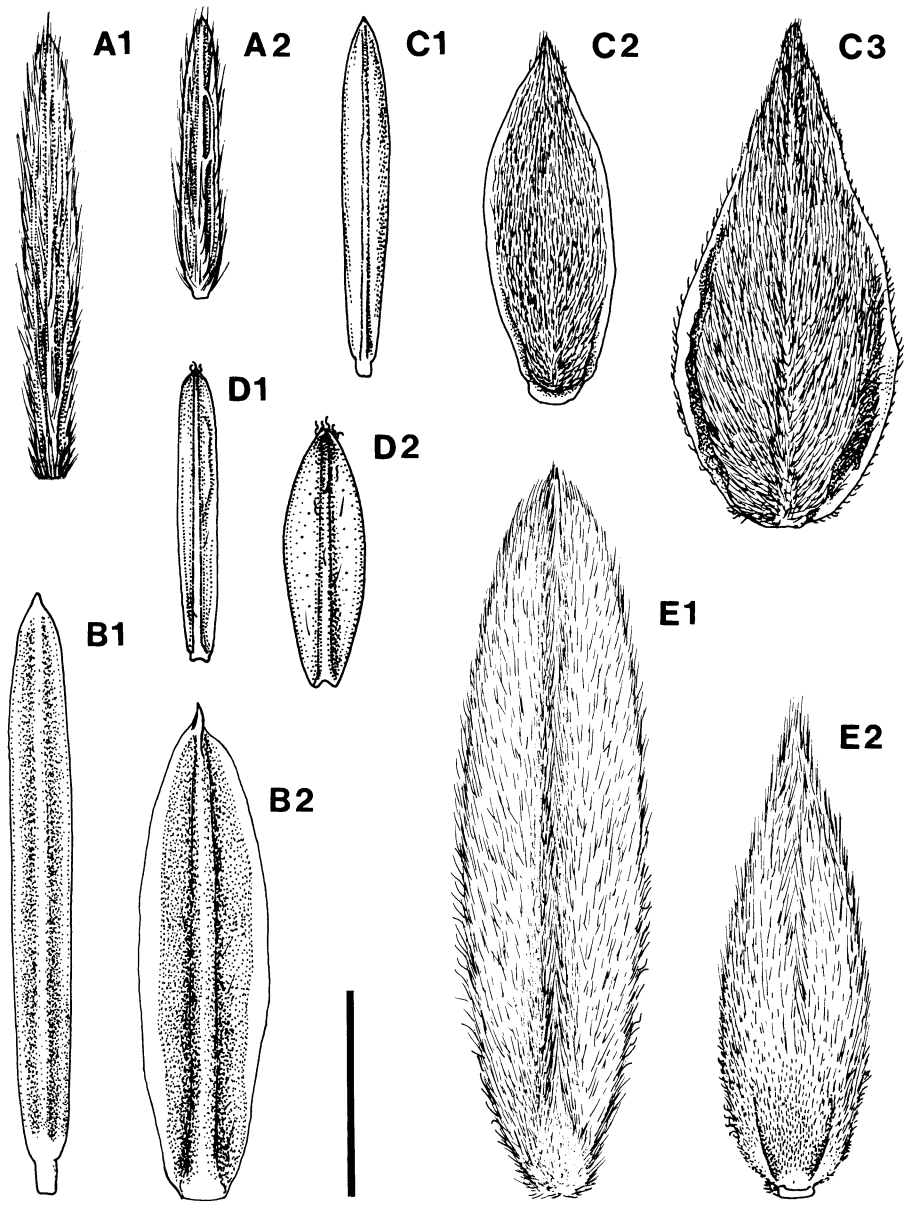

Figure 6. Leaves and bracts of Gnidia *species V. A* G. nodiflora, *Van Wyk 8438* ((NH)*: A1 leaf, A2 bract. B.* G. pinifolia, *Oliver 3332* (STE)*: B1 leaf, B2 bract. C.* G. polycephala, *Dinter 7707* (WIND)*: C1 leaf, C2 outer bract, C3 inner bract. D.* G. polystachya, *Bayliss 5700* (BOL)*: D1 leaf, D2 bract. E.* G. splendens, *Germishuizen 3256* (PRE)*: E1 leaf, E2 bract. Bar scale: A, B, C, D = 5 mm; E = 10 mm.*

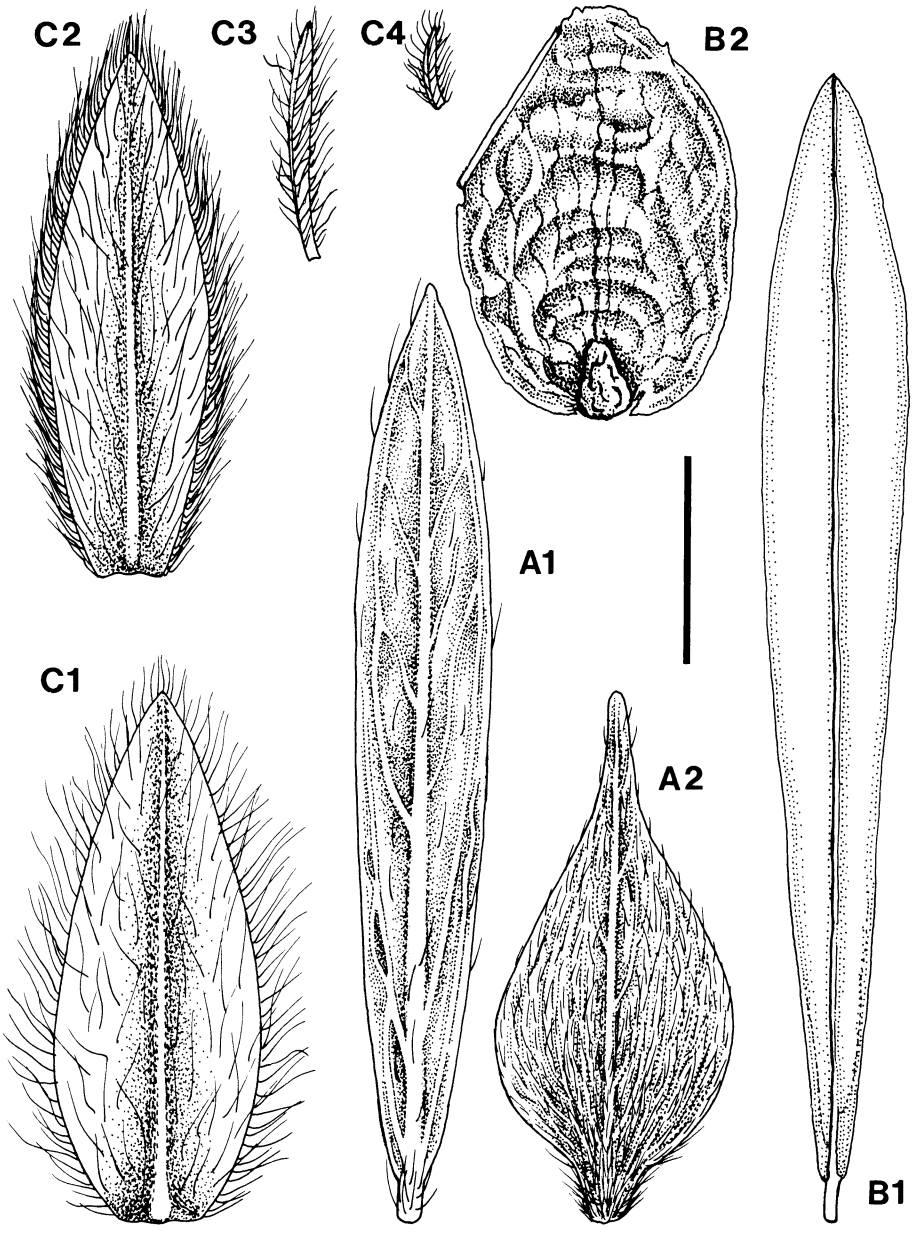

Figure 7. Leaves and bracts of Gnidia *species VI. A.* G. sericocephala, *Leendertz 2497* (J)*: A1 leaf, A2 bract. B.* G. usafuae, *Kerfoot 1688* (EA)*: B1 leaf, B2 bract. C.* G. vesiculosa, *Beyers 133* (PRE)*: C1 leaf, C2 outer bract, C3 and C4 bracteoles. Bar scale = 5 mm.*

3.3.2 Bract micromorphology and anatomy

Bract outlines in transverse section linear oblong about a central vascular bundle. Leaf-like bracts broadest, specialized bracts thinnest in transverse section (*G. polycephala,* fig. 10C). Hairs unicellular; absent or polymorphic, colourless or rarely yellow, bract hair density, distribution and length often identical with leaf hairs (*G. baurii* , fig. 2C1 and 2C2, *G. denudata,* fig. 3C1 and 3C2 and *G. pinifolia,* fig. 6B1 and 6B2), or different. *G. polycephala* (fig. 10C) bracts are hairy whilst leaves are glabrous. Bract vestiture polymorphic among plants of *G. kraussiana* and may or may not correlate with equally polymorphic leaf vestiture (fig. 5A1 and 5A2). Hair ornamentation correlates with leaf hair ornamentation among species. Stomata distribution and type identical with that of leaves in non-specialized bracts, or reduced to almost absent in specialized bracts (fig. 10C). Mucilagenous cells punctuate the epidermal layer in most species, most abundant in species with abundant mucilaginous cells in leaves, to least abundant in highly specialized, scarious bracts. Mesophyll of specialized bracts very reduced, scarious (fig. 10C). Bases leaf-like in unspecialised bracts, thin and scarious in specialized bracts and swollen and aerenchymatous in bracts of former *Lasiosiphon* species (fig. 9B). **Venation** of leaf-like bracts brochidodromous (*G. insignis,* fig. 4B2), or acrodromous in small-leaved species with similar leaves and bracts, (*G. linoides*, fig. 5C1 and 5C2, and *G. denudata,* fig. 3C1 and 3C2).

3.4 Bracteoles

Bracteoles present in *G. vesiculosa* (fig. 7C3 and 7C4).

Figure 11 illustrates eight groups representing the diversity of leaves, bracts and inflorescence structure identified among *Gnidia* species. By definition, bracts are associated with inflorescences and therefore are included in the schematic groups. Table 4 outlines some morphological features of the habit types in *Gnidia,* as illustrated in fig. 11, and outlined above.

Table 4. Eight generalised types of habit recognised in *Gnidia,* and comparisons of leaf, bract andinflorescence characters.
See fig. 11 for illustrations of groups.

Character	Group A	Group B	Group C	Group D	Group E	Group F	Group G	Group H
Leaves								
Form	foliaceous	foliaceous	needle-like	foliaceous	foliaceous	foliaceous	foliaceous	foliaceous
Bracts								
Bract modification	highly modified	modified	modified	highly modified	vestigial or absent	very slightly modified	very slightly modified	very slightly modified
Bract development	consistent	consistent	consistent	consistent	inconsistent, often absent	consistent	consistent	consistent
Bract texture	slightly leathery, dry	mesic	slightly mesic	very thin, dry, papery	mesic	mesic	mesic	mesic
Inflorescence								
Inflorescence structure	many-flowered	many-flowered	many-flowered	many-flowered	few-flowered	few-flowered	single, terminal	raceme

4 Discussion

4.1 Morphometric analyses

Species are discriminated mostly on the basis of their leaf and bract widths, and leaf lengths. Seven homogenous groups of species can be identified on the basis of their similarity of leaf length to width ratios, or bract length to width ratios. However, the species compositions of the homogenous groups for leaf ratios and the species compositions of the homogenous groups for bract ratios differ. Further there is no correlation between leaf and bract ratios and this indicates that leaves and bracts are under different selective criteria in *Gnidia.*

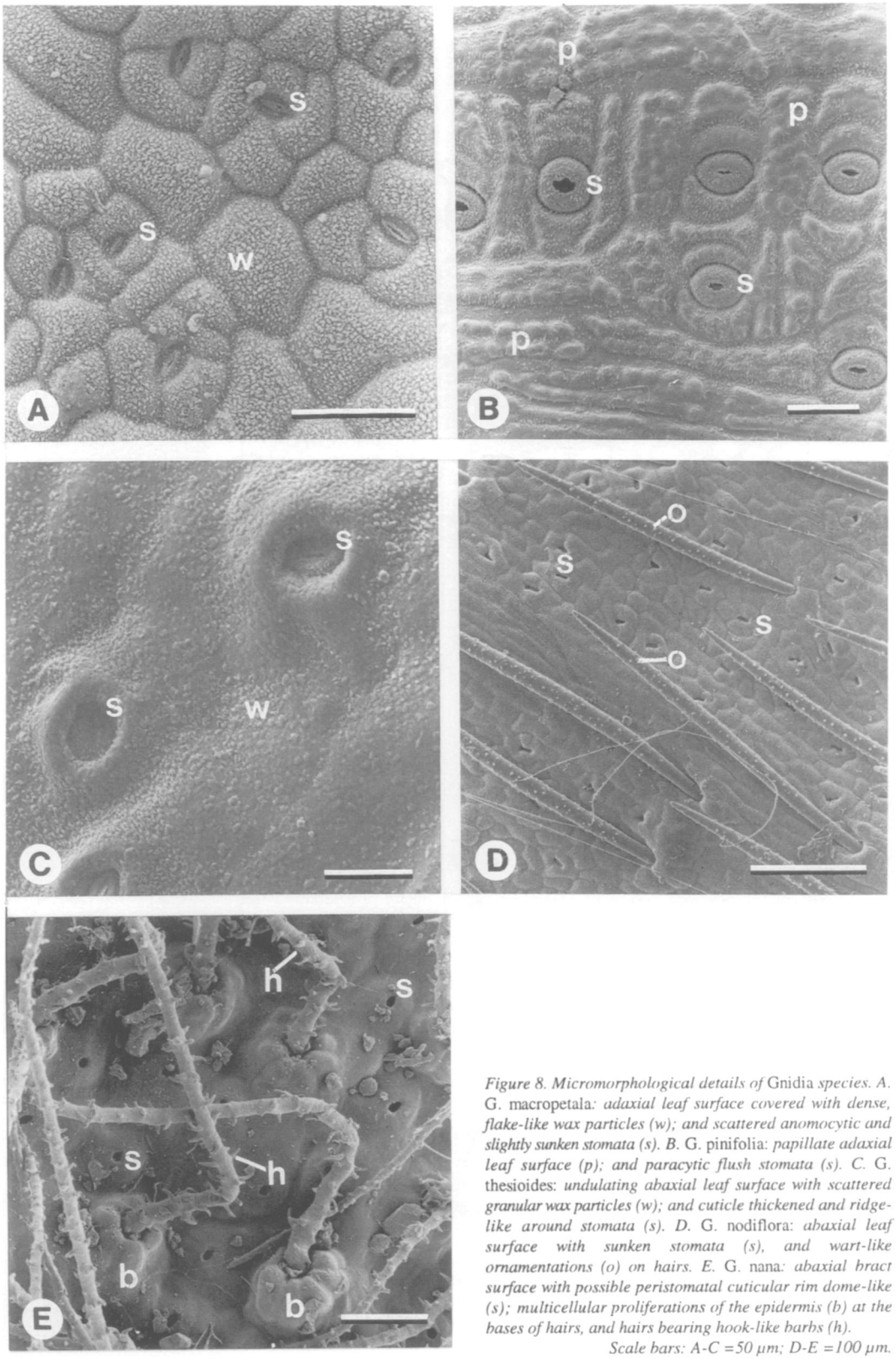

Figure 8. Micromorphological details of Gnidia *species. A.* G. macropetala*: adaxial leaf surface covered with dense, flake-like wax particles (w); and scattered anomocytic and slightly sunken stomata (s). B.* G. pinifolia*: papillate adaxial leaf surface (p); and paracytic flush stomata (s). C.* G. thesioides*: undulating abaxial leaf surface with scattered granular wax particles (w); and cuticle thickened and ridge-like around stomata (s). D.* G. nodiflora*: abaxial leaf surface with sunken stomata (s), and wart-like ornamentations (o) on hairs. E.* G. nana*: abaxial bract surface with possible peristomatal cuticular rim dome-like (s); multicellular proliferations of the epidermis (b) at the bases of hairs, and hairs bearing hook-like barbs (h).*

Scale bars: A-C =50 µm; D-E =100 µm.

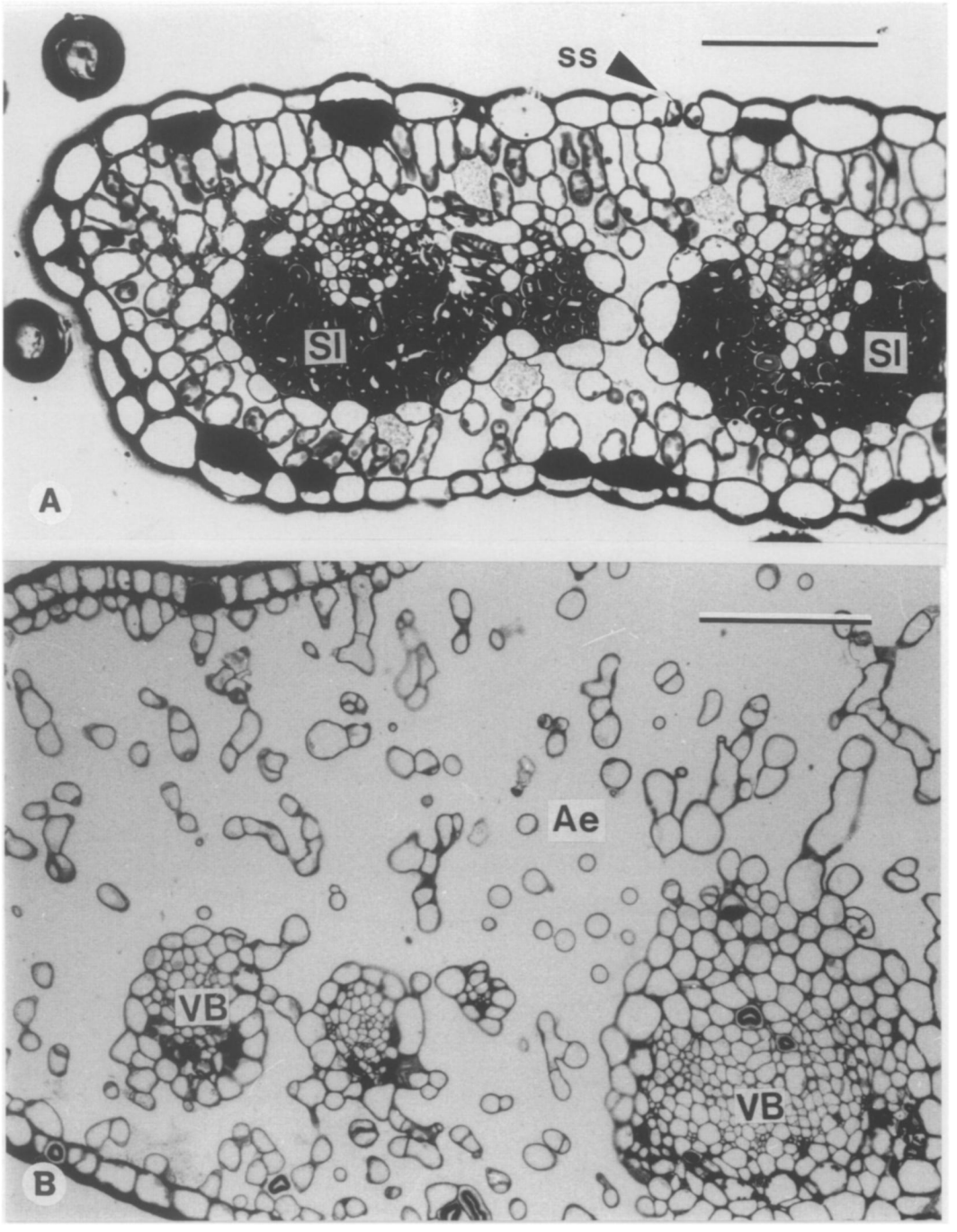

Figure 9. Anatomical details of Gnidia species. A. G. nodiflora*: transverse section of leaf with sunken stomata (ss) and well-developed sclerenchyma (Sl). B.* G. anthylloides*: transverse section of swollen bract base with abundant aerenchyma (Ae); VB = vascular bundle. Scale bars = 100 µm.*

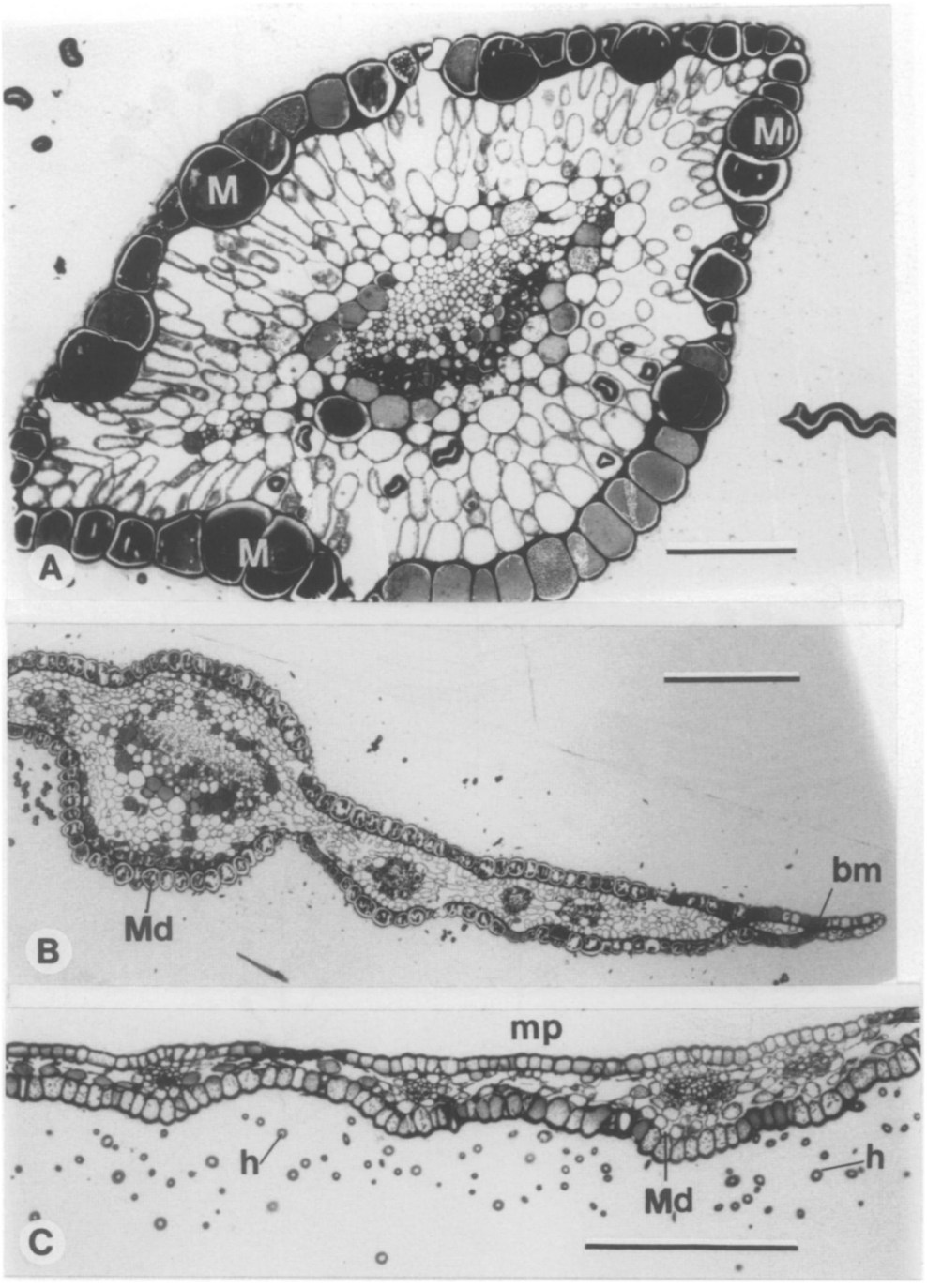

Figure 10. Anatomical details of Gnidia *species. A.* G. pinifolia*: transverse section of needle-like leaf with abundant mucilaginous cells (M). B.* G. pinifolia: *transverse section of semi- foliaceous bract with mesophyll diminishing towards the bract margin (bm); Md=midvein. C.* G. polycephala*: transverse section of scarious bract with very reduced mesophyll (mp); h=transverse sections through abaxial hairs; Md=vascular bundle; H=hairs. Scale bars: A,C = 250 µm; B= 500 µm.*

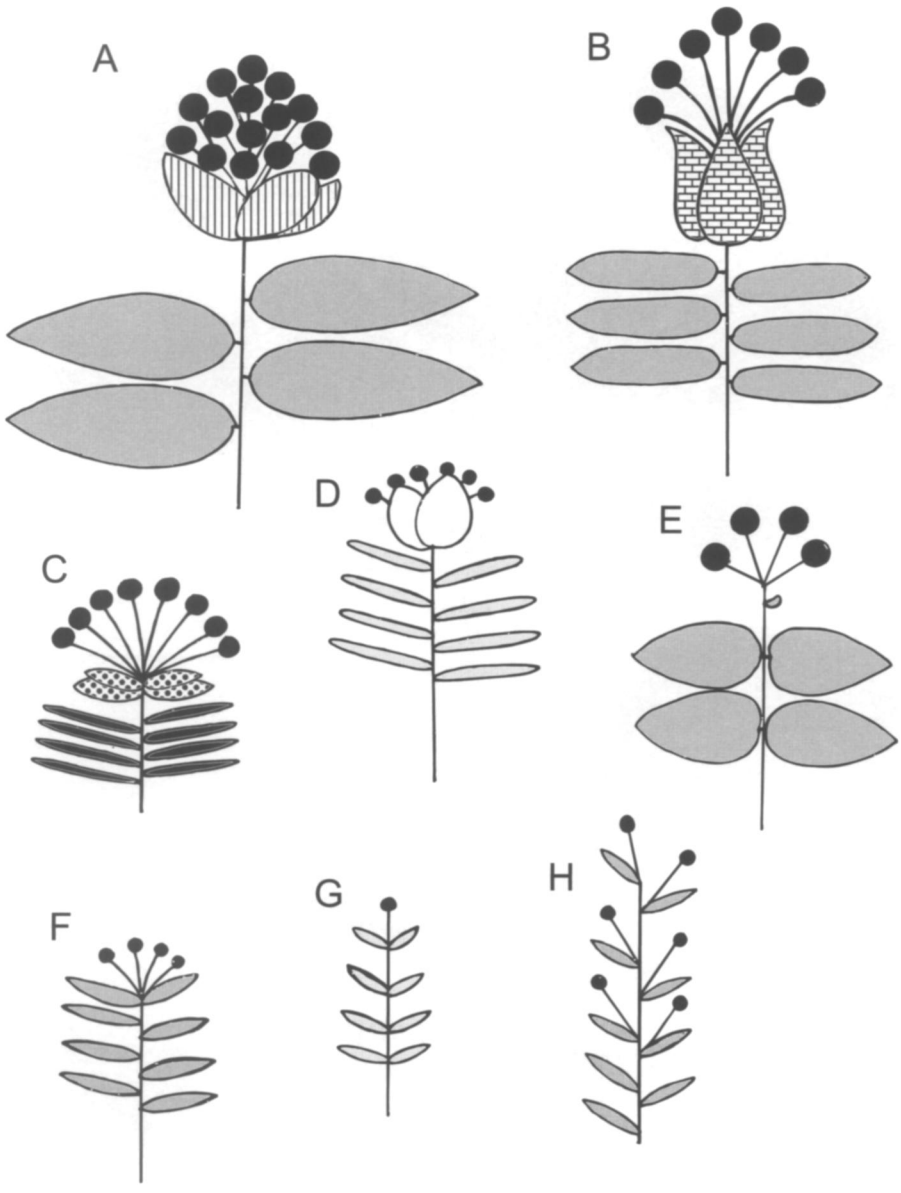

Figure 11. Schematic illustrations of eight groups representing the main trends in diversity among leaves, bracts and inflorescence structure in Gnidia. *Species examples representing groups: A.* G. glauca*; B.* G. kraussiana*; C.* G. pinifolia; *D.* G. involucrata*; E.* G. subcordata*; F.* G. racemosa*; G.* G. nodiflora*; H.* G. linoides. *Black circles indicate flowers; white bracts represent those that are scarious, vertical lines indicate modified and leathery bracts, brick patterning indicates partially modified, green, leafy bracts, and stippled bracts represent those that are part foliaceous and part needle-shaped. Black leaves represent those that are needle-shaped, medium-grey cauline and floral leaves are foliaceous, and pale grey cauline and floral leaves are narrowly foliaceous to needle-shaped.*

4.2 Morphology and anatomy

Preliminary findings here suggest that morphological and possibly anatomical characters in *Gnidia* are of taxonomic value at the infra-generic and species levels. Although there are no species-unique characters in bracts or leaves, combinations of character states could be used to distinguish individual and groups of species. Bracteoles are present in only two species: *G. spicata* and *G. vesiculosa*. Thereafter, leaf and bract dimensions alone (further underlined by floral differences) distinguish these species. Multicellular projections around the bases of hairs on the leaves and bracts of *G. linearifolia*; *G. nana*; *G. scabrida* and *G. tomentosa* from the Western Cape Province, South Africa, for example, are unique to this group. These multicellular proliferations of the epidermis correlate with barb- and hook-like ornamentations of leaf and bract hairs, together with floral characters, for example, stiff hairs borne on the rims of flower tubes such as those similarly found in *Struthiola* L. flowers, suggesting that these taxa comprise a closely related group.

Gnidia species collectively represent a diversity of leaf forms in the Thymelaeaceae. The diversity of habitats occupied by species make necessary different means of adaptation to variable environmental factors, particularly temperature, water loss and nutrient availability. Mucilaginous cells are variously well-represented among taxa. Within our sample of species, *G. pinifolia* leaves and bracts contain abundant mucilage-bearing cells. Plants growing in areas with Mediterranean climates often bear mucilaginous epidermal cell walls (Van der Merwe *et al* 1994). Most species of *Passerina* are endemic to the Cape Floristic Region and Bredenkamp & Van Wyk (1999) suggest that a mucilaginous epidermis assists plants of *Passerina* species to store water, and filter light density to avoid irradiation damage to palisade tissue. Plants of *G. pinifolia* may similarly benefit from the filtering and water storage functions that mucilaginous cells confer.

Pimelea is the second largest genus in the Thymelaeaceae, with some 108 species, mostly in Australasia (Rye 1990). Work by Rye (1988) suggests that leaf and bract diversity among *Pimelea* species generally resembles that among *Gnidia* species. Bracts of both genera are highly modified, leaf-like to almost identical to the leaves, or absent. Species with capitate inflorescences have highly modified bracts that protect flowers. Rye (1988) used the absence of involucral bracts to help distinguish *Pimelea* section *Epallage*. Similarly, Phillips (1944) distinguished *Englerodaphne* from *Gnidia* citing differences in leaf texture between the genera and the ebracteate inflorescences of *Englerodaphne* to support his opinion. Bract number and flower number within inflorescences vary independently within Thymelaeaceae. In *Gnidia* species with many-flowered capitate heads, bract number equals or exceeds that of flower number, and among species of *Pimelea,* flower number exceeds bract number among inflorescences. Species of *Dais* L. (from southern and tropical Africa) and *Thecanthes* Wikstrom (from northern Australia and southern Indonesia) with similarly large compound inflorescences, have fewer bracts. The bracts of *Dais* species are among the most highly modified and persistent in the Thymelaeaceae. These bracts are almost round, and initially green, but rapidly becoming thickened and woody. In contrast, bracts are never woody in *Gnidia.*

5 Conclusions

Gnidia species are discriminated by their leaf and bract widths and leaf lengths. There is no correlation between leaf length and width and bract length and width ratios. Leaves are foliaceous to narrow and needle-like, and bracts are more diverse. Within species, bracts are either scarcely distinguishable from the leaves or highly modified. Inflorescence structure influences bract modification, whereby highly modified bracts protect capitate, many-flowered inflorescences; and scarcely modified leaves subtend axillary flowers.

Morphological characters are conservative in *Gnidia*, although some characters distinguish groups of species. Preliminary results suggest that micromorphological and anatomical features are of

considerable value in assisting a better taxonomic understanding of this group, and current work is investigating these features further. We aim to incorporate such findings in a cladistic analysis of *Gnidia*.

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CHAPTER 4

PATTERNS OF DIVERSITY AMONG INVOLUCRAL BRACTS, INFLORESCENCES AND FLOWERS IN *GNIDIA* (THYMELAEACEAE)

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Patterns of diversity among involucral bracts, inflorescences and flowers in *Gnidia* (Thymelaeaceae)

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Abstract. *– A number of examples exist of* Gnidia *species closely resembling each another, and possibly being synonymous. Many such species have been established on the basis of other non quantitative differences in character variation, but no studies have tested the validity of morphometric data in distinguishing taxa in this genus. In addition, no studies in this group have tested the patterns in and relationships between character variation. Morphometric analyses of features of involucral bracts, inflorescences and flowers of* Gnidia *species were performed to investigate patterns of diversity among these organs. In addition, bracts were assigned to classes representing different levels of modification from the leaves, and inflorescences were classified according to flower number and whether capitate or spicate. Species were also scored as having either static infructescences or elongating infructescences. Floral tube lengths, lengths of tubes from flower bases to constrictions above the ovary, sepal lengths and numbers of flowers in inflorescences were measured using species representing the diversity of these characters in the genus. Chi-square analysis, discriminant analysis, univariate nested analysis of variance and correlations were used to test character variation and relationship.* Gnidia *species are discriminated by their floral tube lengths, lengths of tubes from flower bases to constrictions above the ovary, sepal lengths and flower number. Involucral bract modification is influenced by inflorescence type, and species with elongating infructescences have unmodified bracts. These findings suggest that bracts protect flowers, especially the floral tubes surrounding ovaries. Bracts are most different from leaves in species that concentrate reproductive investment in the production of many-flowered inflorescences. Total floral tube length and length of tube from flower base to constriction are positively correlated as are total tube length and sepal length. Sepal length and flower number per inflorescence are negatively correlated and there is no relationship between total tube length and flower number among species. These results demonstrate how morphometric analyses contribute to testing the validity of taxa, especially within pairs or small groups of similar species, and subspecific taxa.*

Key words: *bracts, flowers, inflorescences,* Gnidia, *numerical analyses, Thymelaeaceae.*

Résumé. **– Modèles de diversité des bractées involucrales, des inflorescences et des fleurs chez *Gnidia* (Thymelaeaceae).** *Nombreux sont les exemples de ressemblance étroite entre espèces de* Gnidia, *certaines d'ailleurs étant probablement synonymes. Beaucoup de ces espèces ont été établies sur base de différences non quantitatives relatives à certains caractères mais aucune étude n'a testé*

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la validité des données morphométriques qui ont permis la distinction de taxons au sein du genre. Par ailleurs, aucune étude menée dans ce groupe n'a envisagé de modéliser la variation des caractères et de leurs relations. Des analyses morphométriques des types de bractées involucrales, des inflorescences et des fleurs de différentes espèces de Gnidia *ont été réalisées de manière à étudier et à modéliser la diversité de ces organes. Les bractées ont également été classées en fonction de leur degré de modification par rapport aux feuilles et les inflorescences distinguées d'après leur caractère capité ou spiculé et le nombre de leurs fleurs. Des espèces ont aussi été différenciées d'après l'élongation ou non de leurs infrutescences. La longueur des tubes floraux, celle mesurée de la base de la fleur à la constriction surmontant l'ovaire, la longueur des sépales et le nombre de fleurs par inflorescence ont été calculées sur des espèces représentatives de la diversité de ces caractères au sein du genre. Un test Chi-carré, une analyse discriminante, une analyse de variance à un critère et de corrélations ont été utilisés pour tester la variation des caractères et leurs relations. Les espèces de* Gnidia *sont discriminées d'après la longueur de leur tube floral, celle mesurée de la base de la fleur à la constriction surmontant l'ovaire, de la longueur des sépales et du nombre de fleurs. La modification des bractées involucrales est influencée par le type d'inflorescence et les espèces possédant des infrutescences allongées présentent des bractées non modifiées. Ces résultats suggèrent que les bractées protègent les fleurs et, en particulier, les tubes floraux entourant les ovaires. Les bractées diffèrent le plus des feuilles chez les espèces qui consacrent majoritairement leur effort reproductif à la production d'inflorescences multiflores. La longueur totale du tube floral et celle mesurée de la base de la fleur à la constriction surmontant l'ovaire sont positivement corrélées. Il en va de même de la longueur totale du tube et de celle des sépales. La longueur des sépales et le nombre de fleurs par inflorescence sont corrélés négativement et aucune relation n'existe, au sein des espèces, entre la longueur totale du tube et le nombre de fleurs. Ces résultats démontrent comment des analyses morphométriques contribuent à tester la validité de taxons, particulièrement au sein de paires ou de petits groupes d'espèces similaires ou de taxons subspécifiques.* Traduit par le journal.

1 Introduction

1.1 General

Gnidia L. is the largest genus in the Thymelaeaceae, comprising about 140 species in southern and eastern Tropical Africa, Madagascar and India (Heywood 1979). Most species occur in southern Africa, where species diversity is highest in the Cape Province. Flowers are usually yellow, white, red, orange, pink, blue, lilac or green and vary from less than 10 mm long to about 25 mm long.

1.2 Terminology

Terminology follows that of Heinig (1951), Lawrence (1955) and Beyers & van der Walt (1995).

1.3 Description of plants

Woody or herbaceous, often ericoid. Leaves borne on ends of branches of woody species, along lengths of branches of herbaceous taxa; opposite or alternate; simple, entire; linear, lanceolate elliptic, oblong to obovate; glabrous to hairy; bases acute to rounded; tips acute to mucronate; pedicel short or absent. Flowers in capitate heads, few-flowered terminal and lateral clusters, racemes, spikes, paired or solitary in leaf-like bract axils. Bracts rarely absent, solitary subtending sparse lateral inflorescences or in one or two whorls subtending terminal capitulae; leaf-like or specialized; elliptic, oblong to broadly ovate; bases acute to rounded; tips acute, sometimes mucronate. Pedicel short or rarely absent. Hypanthium (an expanded torus) tubular, usually constricted just above ovary; tube below constriction barrel-shaped and round in transverse section; upper part of tube above constriction cylindrical to campanulate, or rarely (*G. poggei* Gilg) quadrangular in transverse section, hairy to glabrous outside, rarely sparsely hairy

inside. Sepals 4-5 -lobed; as long as, or shorter than tube; hairy or glabrous outside, rarely hairy inside. Petaloid glands often in sepal sinuses, entire or variously divided, large, fleshy without stomata or membranous with secretory stomata, rarely hairy. Stamens usually twice the number of calyx lobes, in two whorls: upper whorl antisepalous in mouth of tube, lower whorl below sepal sinuses in throat of tube, identical to slightly heteromorphic, rarely one row aborted; filaments very short, basifixed or dorsifixed; anthers bi-thecate with longitudinal dehiscence towards centre of tube. Nectariferous disc small, cup-shaped, membranous to absent. Ovary bi-carpellate with a single ovule; style lateral, included; stigma simple, capitate or oblong, penicillate. Fruit dry, oblong to conical, surrounded by remnants of lower tube; elaiosome sometimes present. Seed small, brown or black, smooth or with transverse ridges or pits.

1.4 Taxonomic history

The presently accepted circumscription of *Gnidia* advocated by Peterson (1959a) and followed by Dyer (1975) comprises *Gnidia* L. *sensu* Linnaeus (1753), together with *Lasiosiphon* Fresen., *Arthrosolen* C.A.Mey., *Pseudognidia* Phill., *Basutica* Phill. and *Struthiolopsis* Phill. Peterson (1959a) accepted the inclusion of *Lasiosiphon*, *Arthrosolen* and *Englerodaphne* in *Gnidia* because of the presence of intermediate species lacking characters that distinguish these genera. Similarly, in his studies of selected species from the Western Cape, South Africa, Peterson (1959b) also included within *Gnidia*, genera sharing floral characters of both *Gnidia* and *Struthiola* L. Presently, no sub-generic classification of *Gnidia* exists and species relationships are poorly understood.

A phylogenetic analysis of *Gnidia* is currently in progress. A number of taxa suggest they are conspecific: the only differences between taxa being different values of numeric characters. Peterson (1959a) noted the increasing similarity among five species of East Africa, with the accumulation of material for study. Peterson (1982) recognised *G. robusta* Peterson as a separate species that resembled (by his own admission) the very variable *G. caffra* (Meisn.) Gilg. He justified recognition of *G. robusta* on the basis of its more robust branching, dense leaves, and larger petals [petaloid scales] than *G. caffra*. There is however no quantitative analysis of the morphometric variation among these characters in *G. caffra* and *G. robusta* to support the distinction between these species. Peterson (1959a) cited (among other characters) petaloid scales as taxonomically unreliable for distinguishing between *Gnidia*, *Lasiosiphon* and *Arthrosolen*. Their development is unstable among species (including *G. caffra*) formerly classified under *Lasiosiphon*, and therefore they should be used with caution as sources of evidence to support taxonomic decisions. We recognise other examples of very similar species that differ only with respect to linear measurements of some characters, for example *G. canoargentea* (C.H.Wright) Gilg and *G. splendens* L.

Morphometric data have proved valuable for solving similar problems in other plant groups. Lefèbvre & Vekemans (1995) used numerical characters to distinguish three subspecies within the polymorphic and widespread species *Armeria maritima* (Mill.) Willd. (Plumbaginaceae). Feliner (1996) used Principal Component Analysis and Discriminant Analysis of vegetative and floral morphometric data to challenge the validity of *Daphne laureola* subsp. *latifolia* (Coss.) Rivas-Mart. (Thymelaeaceae). Nybom *et al.* (1997) used morphometric data to distinguish species of *Rosa* Tourn. (Rosaceae) for taxonomic and breeding purposes. These works prompted our first morphometric investigations of *Gnidia* species to analyse the relationship between leaf type and extent of bract modification among species, and the results showed that there is no correlation in leaf or bract ratios (Beaumont et al. 2002).

The aim of this work was to examine the amount of intra- and interspecific morphometric variation of selected floral features among selected *Gnidia* species. Prompted by our findings that leaf and bract length and width ratios are not correlated, we also investigated the relationship between bract modification, inflorescence organisation and floral morphometric characters.

Table 1. Categories of bracts among *Gnidia* species.
Categories as identified by comparing and contrasting three characters between leaves and bracts.
Category 1 represents bracts that are identical or least different to their leaves,
category 2 represents bracts that are leaf-like but differ in size to the cauline leaves and category 3 represents bracts that are highly modified and most different to their leaves.

Bract category	Character state relative to that of leaves		
	Colour	Shape	Texture
1 (unmodified)	same	same	same
2 (partly modified)	same	different	same
3 (highly modified)	different	different	different

Table 2. Classes of inflorescence type in *Gnidia* species.

Class of inflorescence	Type of inflorescence
1	Single or paired flowers in leaf axils
2	Few-flowered clusters
3	Many-flowered, capitate, pedunculate
4	Spikes

Table 3. Species and their codes.
As used in the correlation tests, nested analyses of variance and discriminant analysis.

Species	**Code**
G. aberrans C.H.Wright	aber
G. baurii C.H.Wright	baur
G. compacta (C.H.Wright) J.H.Ross	comp
G. denudata Lindl.	denu
G. geminiflora E.Mey. ex Meisn.	gemi
G. gymnostachya Gilg	gymn
G. involucrata A.Rich.	invo
G. juniperifolia Lam.	juni
G. kraussiana Meisn.	krau
G. lamprantha Gilg	lamp
G. linoides Wikstr.	lino
G. oppositifolia L.	oppo
G. pinifolia L.	pini
G. polycephala (C.A.Mey.) Gilg	poly
G. sericocephala (Meisn.) Gilg ex Engl.	seri

2 Methods

2.1 Data collection

Data from two groups of species were collected for morphometric analyses of floral features in *Gnidia*. Firstly, 115 species were scored separately for the degree of involucral bract modification (tab.1) and inflorescence type (tab. 2). Species were also scored as having either static infructescences or elongating infructescence axes.

Species selected for scoring: *G. aberrans* C.H.Wright; *G. acutifolia* Wikstr.;*G. albicans* Meisn.; *G. albosericea* M.Moss ex B.Peterson; *G. anomala* Meisn.; *G. anthylloides* Gilg; *G. apiculata* Gilg; *G. baurii* C.H.Wright; *G. bojeriana* Baill.; *G. buchananii* Gilg; *G. burchellii* Gilg; *G. burmanni* Eckl. & Zeyh. ex. Meisn.; *G. butayei* E.A.J.De Wilde.; *G. caduca* H.H.W.Pearson; *G. caffra* Meisn.; *G. calocephala* Gilg; *G. caniflora* Meisn.; *G. capitata* L.f.; *G. chapmanii* B.Peterson; *G. chrysantha* Gilg; *G. chrysophylla* Meisn.; *G. clutyoides* E.A.Bruce; *G. compacta* (C.H.Wright) J.H.Ross; *G. coriacea* Meisn.; *G. decurrens* Meisn.; *G. dekindtiana* Gilg; *G. denudata* Gilg; *G. deserticola* Gilg; *G. dregeana* Meisn.; *G. eminii* Engl. & Gilg; *G. eriocephala* Meisn.; *G. fastigiata* Rendle; *G. fischeri* Engl. & Gilg; *G. foliosa* (H.H.W.Pearson); *G. francisci* Bolus; *G. fraterna* (N.E.Brown) Phillips; *G. fruticulosa* Gilg; *G. galpini* C.H.Wright; *G. geminiflora* E.Mey. ex Meisn.; *G. glauca* Gilg; *G. goetzeana* Gilg; *G. gymnostachya* Gilg; *G. harveyana* Meisn.; *G. huillensis* Gilg; *G. humilis* Meisn.; *G. inconspicua* Meisn.; *G. insignis* Compton; *G. involucrata* Steud. ex A.Rich.; *G. juniperifolia* Lam.; *G. kerstingii* Gilg ex Engl.; *G. kraussiana* Meisn.; *G. lamprantha* Gilg; *G. latifolia* Gilg; *G. laxa* Gilg; *G. leiantha* Gilg; *G. linearifolia* (Wikstr.) B. Peterson; *G. linoides* Wikstr.; *G. macropetala* Meisn.; *G. macrorhiza* Gilg; *G. madagascariensis* Baill.; *G. meyeri* Meisn.; *G. microcephala* Meisn.; *G. mittuorum* Gilg; *G. mollis* C.H.Wright; *G. montana* H.H.W.Pearson; *G. nana* Wikstr.; *G. newtonii* Gilg; *G. nitida* Bolus ex C.H.Wright; *G. nodiflora* Meisn.; *G. nutans* H.H.W.Pearson; *G. oliveriana* Engl. & Gilg; *G. oppositifolia* L.; *G. orbiculata* C.H.Wright; *G. ovalifolia* Meisn.; *G. parviflora* Meisn.; *G. parvula* Dod; *G. penicillata* A.Licht. ex Meisn.; *G. pinifolia* L.; *G. poggei* Gilg; *G. polyantha* Gilg; *G. polycephala* Gilg ex Engl.; *G. polystachya* Berg.; *G. propinqua* (Hilliard) B.Peterson; *G. pulchella* Meisn.; *G. racemosa* Thunb.; *G. renniana* Hilliard & B.L.Burtt; *G. rivae* Gilg; *G. rubrocincta* Gilg; *G. schweinfurthii* Gilg; *G. sericea* L.; *G. sericocephala* Meisn. (Gilg ex Engl.); *G. setosa* Wikstr.; *G. simplex* L.; *G. singularis* Hilliard; *G. somalensis* Gilg; *G. spicata* Gilg; *G. splendens* Meisn.; *G. stenophylla* Gilg; *G. styphelioides* Meisn.; *G. suavissima* Dinter; *G. subcordata* Meisn.; *G. subulata* Lam.; *G. tenella* Meisn.; *G. thesioides* Meisn.; *G. thomsonii* H.H.W.Pearson; *G. tomentosa* L.; *G. triplinervis* Meisn.; *G. usafuae* Gilg; *G. variabilis* (C.H.Wright) Engl.; *G. virescens* Wikstr.; *G. welwitschii* Hiern.; and *G. woodii* C.H.Wright.

Fifteen species (tab. 3) from the group of 115 taxa were then chosen from which to measure additional floral characters. This smaller group comprises approximately one eighth of the total number of species sampled, and species were selected to represent the diversity of inflorescence organisation and flower shape and size within the genus. Five herbarium specimens per species were selected for morphometric flower characters for each of the fifteen species. For each specimen the numbers of flowers in each of five mature, re-hydrated inflorescences were recorded. A mature flower from each of the five inflorescences was selected and (i) the length of the tube from the base of the flower to the level where the sepal lobes diverge, (ii) the length of the lower part of the tube from the base of the flower to the constriction point, and (iii) the length of one of the outer (and longest) sepal lobes, were measured to within 0.25 mm accuracy (fig. 1). For *G. linoides*, five herbarium specimens were sampled, but only two to three inflorescences per specimen could be recorded because of the paucity of material.

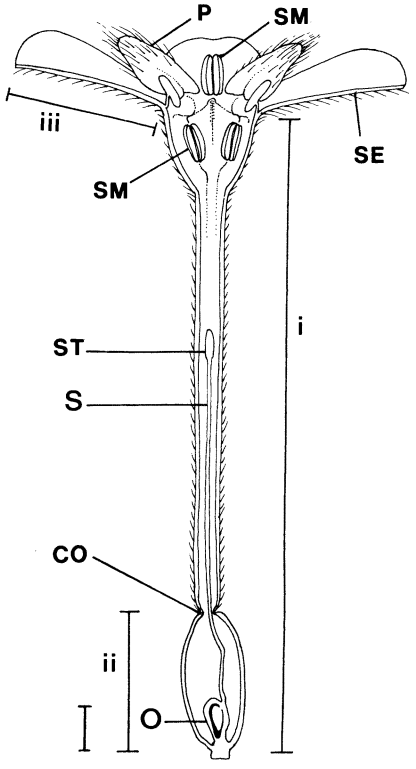

Figure 1. Longitudinal section through flower of G. pinifolia *L.*
CO = constriction in floral tube,
O = ovary,
P = petaloid gland,
S = style,
SE = sepal,
SM = stamen,
ST = stigma,
(i) = length of floral (hypanthial) tube,
(ii) = length of tube from base of flower to constriction,
(iii) = sepal lobe length.
Scale bar = 1 mm.

2.2 Morphometric analyses

Statistical analyses were performed using Statgraphics Plus 7.0 (Stastistical Graphics System 1993). Square root transformations of lengths of floral tubes, lengths of tubes from the bases of the flowers to their constriction points, and sepal lobe lengths, together with logarithmic transformations of flower number were made to improve normality of data and stabilise the variances.

Chi-square analyses (Siegel & Castellan 1988) were used to test the association between bract type and inflorescence type, and the association between bract type and infructescence elongation among 115 species.

Univariate Nested Analyses of Variance were performed separately on floral tube lengths, sepal lobe lengths, lower tube lengths from flower base to constriction and flower number to determine the variation among species as compared to the variation among specimens (individuals) within species. In these analyses, the species effect was tested against the variation among specimens within species, while the specimen effect was tested against the residual error (Sokal & Rohlf 1995). *G. linoides* was excluded from these analyses because of unequal sample sizes.

Associations between the different floral characters were analysed in pairwise correlation tests.

To further analyse interspecific variation, a discriminant analysis (Klecka 1980, Nybom *et al.* 1997) was performed using floral tube length, sepal lobe length, length of the tube from the base of the flower to the constriction and flower number. The distinctiveness of the original fifteen groups (i.e. species) was tested using a reassignment routine. For the reclassification test the specimens were allocated *a posteriori* to different groups as defined by the discriminant functions to indicate the ability of the chosen characters to separate the species studied.

3 Results

3.1 Morphometric analyses

In the Chi-square analysis of the association of bract class and inflorescence type among species, the four classes of inflorescence type were collapsed to two classes because the expected frequencies in two classes were too low for analysis (Siegel & Castellan 1988). Inflorescences with axillary or paired flowers (Type 1) and inflorescences of few-flowered clusters (Type 2) were combined, and inflorescences of capitate, many-flowered pedunculate heads (Type 3) retained. Spicate inflorescences (Type 4) were not included in this analysis because they lack bracts. The results (tab. 4) showed a highly significant association between bract type and inflorescence type ($\chi^2 = 87.3369$, $df = 4$, $P < 0.0001$).

In the Chi-square analysis of the association of bract type and infructescence elongation, bract type classes were reduced to two to eliminate low score values. Bract types 2 and 3 therefore were combined and tested against type 1. The results (tab. 5) showed a significant association between bract type and non-elongating or elongating infructescences ($\chi^2 = 6.44497$, $df = 1$, $P = 0.01$).

Results of the Univariate Nested Analyses of Variance (tab. 6) showed that floral tube length, length of tube from flower base to constriction, sepal lobe length and flower number per inflorescence differed significantly among species. For each variable, a significant but small contribution to the total variation was made by specimen differences within species.

Pairwise correlations of the four variables among fifteen species were significant in all comparisons except that between tube length and flower number (tab. 7). All character comparisons showed positive correlations except that sepal lobe length and flower number were negatively correlated.

Group centroids (means) for the fifteen species are plotted on the first two discriminant functions together with the 95% confidence limits for the location of these means (fig. 2). The Discriminant Analysis yielded four significant discriminant functions (tab. 8). The eigenvalues of the first two functions (29.65 and 13.81 respectively) and the canonical correlations of all four functions (0.81; 0.87; 0.96 and 0.98 for the fourth to the first functions respectively) were very high. The first function accounted for 61% of the variation where flower number was the most important variable contributing to the discrimination among species. The second discriminant function accounted for another 28% of the variation where flower tube length contributed most to the separation of the species (fig. 2). All specimens for ten out of the fifteen selected species were reclassified into their correct species (tab. 9). The successful reclassification of the remaining specimens varied from 84 to 96%.

Table 4. The distribution of 108 *Gnidia* species across combined bract type and inflorescence type classes.

'Axillary' = Axillary or paired and few-flowered clusters

	Bract type (categories 1-3 of table 1)		
Inflorescence type	Unmodified (1)	Partly m. (2)	Highly m. (3)
Axillary (1 + 2)	46	7	1
Capitate (3)	1	16	37

Table 5. The distribution of 114 *Gnidia* species across combined bract type and infructescence type classes.

Infructescence	Bract type	
	1 (unmodified)	2 + 3 (modified)
1 (static)	46	61
2 (elongating)	7	0

Table 6. Nested analyses of variance
of total tube length, length of tube from flower base to constriction, sepal length and flower number among 14 *Gnidia* species (*G. linoides* excluded) and among specimens within species.

S = source of variation (SP, among species; SM, among specimens);

significance level:
throughout $P < 0.001$;

df = degrees of freedom.

Character	S	F-ratio	*df*	Percent of variation
Floral tube length (i)	SP	73.37	13/56	91.4
	SM	11.01	56/280	5.7
Length from tube base to constriction (ii)	SP	45.21	13/56	83.6
	SM	5.41	56/280	7.7
Sepal length (iii)	SP	53.62	13/56	87.9
	SM	9.00	56/280	7.4
Flower number	SP	197.12	13/56	96.1
	SM	6.70	56/280	2.1

Table 7. Pairwise correlations
of total tube length, length of tube from flower base to constriction, sepal length, and flower number among 15 *Gnidia* species. Roman numerals in brackets refer to each variable as illustrated in fig. 1.

**** $P < 0.0001$,
n.s. = not significant

Pairwise comparison	Correlation coefficient	Sample size	Significance level
Floral tube length (i) vs Length from tube base to constriction (ii)	0.6540	362	****
Floral tube length (i) vs sepal length (iii)	0.7547	362	****
Floral tube length (i) vs. flower number	0.0632	362	n.s.
Length from tube base to constriction (ii) vs sepal length (iii)	0.4359	362	****
Length from tube base to constriction (ii) vs flower number	0.3375	362	****
Sepal length (iii) vs flower number	-0.2536	362	****

Table 8. Eigenvalues and measures of importance of the four discriminant functions in the discriminant analysis.

**** $P < 0.0001$

Discriminant function	Eigenvalue	Relative percentage	Canonical correlation	Significance level
1	29.66	60.91	0.98	****
2	13.81	28.37	0.97	****
3	3.24	6.65	0.87	****
4	1.99	4.07	0.82	****

Table 9. Percentage of material (specimens x measurements) of 15 species classified correctly into species groups defined by four discriminant functions using total tube length, length of tube from flower base to constriction, sepal length and flower number.

'Specimens' = Number of specimens.

'Measurements' = Number of measurements per character.

% = Material correctly classified (%).

Species	Specimens	Measurements	%
G. baurii	5	5	100
G. compacta	5	5	100
G. geminiflora	5	5	100
G. gymnostachya	5	5	100
G. juniperifolia	5	5	100
G. kraussiana	5	5	100
G. linoides	5	2 to 3	100
G. pinifolia	5	5	100
G. polycephala	5	5	100
G. sericocephala	5	5	100
G. denudata	5	5	96
G. lamprantha	5	5	96
G. aberrans	5	5	92
G. oppositifolia	5	5	92
G. involucrata	5	5	84

4. Discussion

4.1. Bract types and inflorescence types

There is a highly significant association between bract type and inflorescence type. Bracts show increasing modification as the numbers of flowers per inflorescence increases.

Species lacking bracts or bearing unmodified, leaf-like bracts. An absence of bracts was a diagnostic feature of the formerly recognised genus *Englerodaphne* (Gilg 1895, Dyer 1975). The inflorescences of members of this taxon comprise terminal, few- to many-flowered clusters. The floral axis expands longitudinally as the inflorescence matures and fruits develop. The fruits of these species

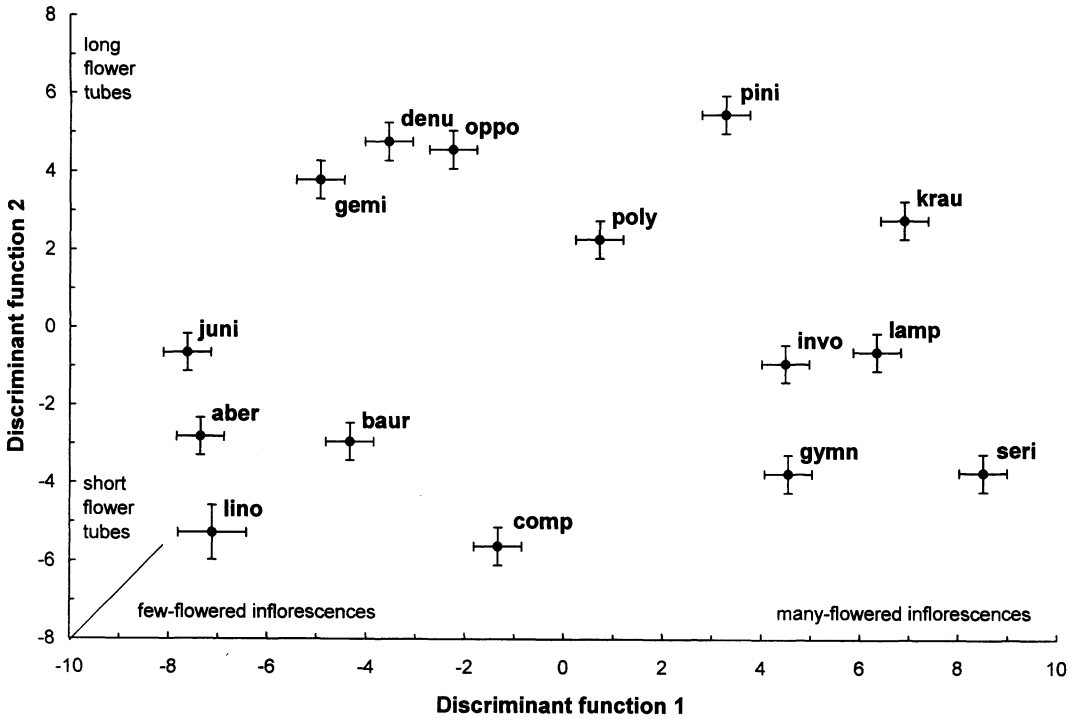

Figure 2. Discriminant Analysis of species using transformed data for floral tube lengths (i), lengths of tubes from bases of flowers to points of constriction (ii), sepal lengths (iii) and flower number per inflorescence.

are broadly conical and the lengthening of the rachis reduces competition for space among developing fruits. Flowers in this group are medium-sized, greenish yellow and white, and moderately conspicuous. Similarly, the inflorescence of *G. gymnostachya* is composed of a terminal cluster of sessile or subsessile, greenish and non-showy flowers. The flowering axis lengthens as the fruits develop and this, together with the absence of bracts, gives the inflorescence a spike-like appearance. This species was formerly ascribed to the somewhat diverse genus *Arthrosolen* which, unlike *Englerodaphne*, also included some species with highly modified bracts. Bracts are reduced in these species. By contrast other species have bracts that are attractive, protective or photosynthetic.

Leaf-like bracts, that are virtually identical to the cauline leaves, subtend single flowers and few-flowered inflorescences which do not lengthen as fruits develop. Beaumont et al. (2002) illustrates examples of virtually indistinguishable leaf-like bracts.

The flowers of *G. racemosa* are scattered along the stems, in the axils of leaves in clusters of one to four (rarely five) flowers. These flowers, like those of *G. gymnostachya*, are greenish-yellow, inconspicuous and comparatively small, rarely exceeding the leaf length. The single, terminal pale blue flowers of *G. linoides*, and the slightly larger, bright blue and conspicuous flowers of *G. penicillata*, are borne singly in the axils of unmodified terminal leaves. Among species with inflorescences comprising few-flowered clusters, leaf-like and unmodified bracts typically subtend smaller, non-showy flowers borne on non-

elongating rachises. The dull colouration of flowers, and their diffuse distribution on these plants might negate the need for protection by specialized bracts, at least in mature inflorescences. Comparatively few-flowered inflorescences represent a moderately valuable concentration of reproductive resources. In developing inflorescences bracts closely envelop buds and therefore provide some protection to young flowers. Workers have identified the protective role of bracts in the early stages of inflorescence development.

Partly modified bracts. In species with few-flowered clusters, flowers range from small and pale, for example *G. nodiflora,* to large and brightly coloured, for example *G. polycephala* and most of the species formerly classified under *Lasiosiphon*. Here, bracts associated with small and pale flowers are scarcely distinguishable from the leaves, whereas whorls of partly modified bracts subtend larger, more showy flowers in other species. Partly modified bracts are usually smaller than the leaves, but show considerable increase of the width and breadth of their bases which are often aerenchymatous (Beaumont et al. 2002), especially among species formerly classified under *Lasiosiphon*. They are otherwise predominantly green and leaf-like, often with red edges. These bracts are frequently imbricate, encapsulating the ovaries and hypanthial tubes and affording protection to nectar resources and developing ovaries.

Highly modified bracts. Highly modified bracts that are unlike the cauline leaves subtend many-flowered often capitate inflorescences. Aggregations of flowers in clusters or capitulae enhance their attractiveness to pollinators by creating a massed, visually attractive cue. In *Gnidia*, the flowers of such species are brightly coloured, (yellow, orange, red) for example *G. kraussiana, G. chrysantha* (Solms-Laub.) Gilg, and *G. rubescens* B.Peterson, or white, thereby contrasting with background foliage, and covered with dense, highly reflective hairs that maximise the visibility of flowers, for example *G. calocephala*. Among species bearing many-flowered capitulae, flowers are small, for example *G. goetzeana*, or comparatively large, for example *G. robusta*. Highly modified bracts are usually much broader than their leaves and mesophyll is very reduced (Beaumont et al. 2002). As a result, bracts are either thin, papery and translucent, or thinly leathery. They lack stomata or have reduced stomatal densities, which together with reduced chlorophyllous tissue and often showy colouration, suggests they no longer have a photosynthetic role, but rather help to protect flowers, or increase floral advertisement to pollinators. Leaves and highly modified bracts show marked colour contrasts. Among *Gnidia* species leaf laminas are uniformly sap- to medium green. Highly modified bracts vary from pale yellowish-green to clear yellow, brown, pinkish-brown to red-brown. Bracts contribute to the attractiveness of inflorescences to pollinators. Prior to identifying the protective role of bracts of *Dalechampia*, Armbruster (1997) presumed they attracted pollinating insects to the flowers. Herrera (1997) revealed that the colourful bracts of *Lavandula stoechas* L. (Lamiaceae) provide visual cues to pollinators from a long distance, but are less important at short pollination distances, concluding that they increase pollination distances in areas of low plant density. In *Pimelea* Banks & Sol. ex Gaertner (Thymelaeaceae), bracts are similarly diverse as in *Gnidia*, and colourful among species with capitate inflorescences, although one species is remarkable in its extent of specialization of bracts. Keighery (1975) suggests that *Pimelea physodes* Hook. is pollinated by birds. The bracts of this species are the largest and most showy of the genus, the outermost ones displaying bright red and purple portions, and concealing the elongate, terminal flowers in pendulous, bell-shaped whorls (Rye 1988). There is no evidence for bird pollination in *Gnidia*, and no species of this genus has bracts comparable in size and showiness to those of *P. physodes*.

Highly visible flowers are however, more vulnerable to predation in contrast to dispersed small, dull-coloured flowers. A localised incident of damage by insect or megaherbivore browsers might destroy a

greater proportion of the reproductive potential of a plant with densely-flowered inflorescences, than in a plant with scattered, solitary flowers. Workers in other families have identified the protective role of floral bracts. Armbruster (1997) identified the evolution of large bracts that close at night to protect the flowers of *Dalechampia* L.(Euphorbiaceae) species. In *Gnidia* species however, there is no evidence for the diurnal movements of bracts. The capitate heads of *Gnidia* superficially resemble those found in distantly related groups, most notably the Asteraceae and Dipsacaceae. In these families, taxa have very reduced or modified calyces and involucres commonly of one or more whorls of overlapping bracts (Heywood 1979). In *Gnidia* species, brightly coloured sepals comprising the calyces attract, and provide a platform for butterfly pollinators. Like genera in Asteraceae and Dipsacaceae, whorls of bracts replace the protective function of the calyx in *Gnidia.*

Bracts are often essential to the maintenance of an optimum microenvironment for the development of reproductive organs. The removal of bracts of *Rheum nobile* Hook.f. & Thomson (Polygonaceae), a species of the alpine zone of East Nepal, interferes with pollen development and produces grains lacking cytoplasm and with deformed exines. Omori and Ohba (1996) concluded that such pollen grains in their experiment were sterile and that the chilling effect induced by bract removal inhibited microsporogenesis in *Rheum* plants. The few *Gnidia* species that occupy high-altitude habitats do not have specialized bracts to perform a similar role of temperature regulation. However, species of lower altitudes with thin, membranous bracts, for example *G. polycephala*, might use these modified organs to protect developing flowers possibly by magnifying heat through the thin, semi-translucent bracts.

Bracts help to disperse fruits in other families. Light, papery bracteoles (reduced bracts) in *Atriplex sagittata* Borkh. (Chenopodiaceae) aid fruit dispersal (Mandák & Pyšek, 2001). In contrast, the bracts of *Gnidia* species do not appear to aid fruit dispersal. The dry fruits of *G. polycephala* however, are enveloped by the remnants of the lower portion of the hypanthial tube, from which arises long, spreading hairs which might aid help fruit dispersal. The delicate papery bracts are easily displaced as the fruits mature, and pose little hindrance to release of fruits. In *Gnidia* species, bracts senesce once fruits have dispersed.

4.2 Flower characters

Morphometric trends. Flower tube lengths, lengths of tubes below constrictions, sepal lobe lengths and flower number differed significantly among the fifteen species. Total tube lengths and lengths of tubes below constrictions were positively correlated. Among *Gnidia* species, larger flowers (with longer tubes) have longer, cylindrical ovaries, especially those formerly classified under *Lasiosiphon,* which mature into similarly-shaped fruits. Total tube and sepal lengths were also positively correlated. Longer-tubed flowers have longer (and broader) sepals which contribute to the showiness of flowers. In *G. juniperifolia* Lam., flowers are solitary and scattered, and sepal length almost equals that of floral tube length. This condition is however, exceptional, because sepal length rarely equals that of the hypanthium among other *Gnidia* species. Many-flowered capitate heads occur in more than half the species of *Gnidia* and most of the remaining species have inflorescences of few-flowered clusters. Large sepals that overlap neighbouring flowers in crowded inflorescences would hinder pollinator access to the reproductive organs, and reduce the collective visual impact of the inflorescences, therefore, reduced lobes have a selective reproductive advantage. Further, among species with many-flowered inflorescences, flower maturation is acropetal, with the tubes of older flowers bending downwards and away from the central axis so that they do not obstruct the opening of successive flowers.

Petaloid sepals of *Gnidia* species contribute to the visibility of flowers, but large and colourful (and non-glandular) or tiny secretory petaloid glands also attract pollinators. The negative correlation between

sepal length and flower number helps to explain the shorter lengths of sepals compared to those of tubes among species. Capitate inflorescences among *Gnidia* species accommodate short or long flowers. Total floral tube length, sepal length, length of the tube from the base of the flower to the constriction above the ovary, and flower number per inflorescence were all significantly important variables contributing to the separation of selected species.

5 Conclusion

This study confirms the value of morphometric data in resolving species limits. *Gnidia* species are discriminated by their floral tube lengths, lengths of tubes from the base of flowers to constrictions, sepal lengths, and numbers of flowers per inflorescence. Bract modification is influenced by inflorescence type and species with elongating rachises have unmodified bracts. Floral tube length and length of tubes from base to constriction are positively correlated, as are total tube length and sepal length. Sepal length and flower number per inflorescence are negatively correlated, and there is no relationship between total tube length and flower number among species. All four variables contribute significantly to the discrimination of species in which flower number followed by total tube length are the most important variables. The high percentage of specimens correctly reclassified to different groups (species) indicates the strong value of the four characters in separating the species. Morphometric data from additional floral and vegetative characters would help resolve intra- and interspecific variation. Specifically, such studies would help clarify the individual taxonomic identities of similar-looking taxa, by analysing the significance of morphometric character variations, hitherto only approximated or judged by eye.

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CHAPTER 5

GNIDIA (THYMELAEACEAE) IS NOT MONOPHYLETIC: TAXONOMIC IMPLICATIONS FOR THYMELAEOIDEAE AND A PARTIAL NEW GENERIC TAXONOMY FOR *GNIDIA*

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Gnidia (Thymelaeaceae) is not monophyletic: taxonomic implications for Thymelaeoideae and a partial new generic taxonomy for *Gnidia*

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We address the generic limits of *Gnidia* (Thymelaeaceae) through a phylogenetic analysis of nuclear ribosomal DNA internal transcribed spacer (ITS) and plastid *rbcL*, *trnL* intron and *trnL-F* intergenic spacer regions. Maximum parsimony and Bayesian inference were used to produce trees and assess internal support. The most significant conclusion drawn from the molecular analysis is that *Gnidia* is polyphyletic as currently circumscribed, comprising at least four distinct lineages that are each related to other genera within Thymelaeoideae. *Gnidia pinifolia* and *G. racemosa* are members of a clade within which *Struthiola* is embedded; a second group of species allies with *Drapetes* as sister to *Passerina*; and a third lineage corresponds to the previously recognized genus *Lasiosiphon*. The remaining species of *Gnidia* included in this study are allied with the Australian genus *Pimelea*. The taxonomic implications of these findings are discussed in relation to the principle of monophyly.

ADDITIONAL KEYWORDS: internal transcribed spacer (ITS) – *Lasiosiphon* – molecular systematics – *Passerina* – *Pimelea* – *rbcL* – *Struthiola* – *trnL-F*.

INTRODUCTION

Gnidia L. (Thymelaeaceae) is a genus of about 140 species of perennial herbs, shrubs and small trees. Species' diversity is greatest in tropical and southern Africa, with about 20 species endemic to Madagascar. Domke (1934) recognized four subfamilies in Thymelaeaceae: Aquilarioideae, Gonystyloideae, Synandrodaphnoideae (= Gilgiodaphnoideae; Robyns, 1975) and Thymelaeoideae. This has been a generally popular classification and one that is also supported by the molecular findings of Van der Bank, Fay & Chase (2002). Flowers with a single ovule are a distinguishing feature of the largest subfamily, Thymelaeoideae. *Peddiea* Harv. ex Hook., however, is the exception among Thymelaeoideae in having bilocular ovaries with a single ovule in each locule (Peterson, 1978). *Gnidia* and *Pimelea* Banks & Sol. ex Gaertn. (approximately 110 species from Australia and New Zealand) are the largest genera in the subfamily. Domke (1934) included *Gnidia* in his tribe Gnidieae, subtribe Gnidiinae, with other southern African genera, including *Dais* L., *Lasiosiphon* Fresen., *Craspedostoma* Domke, *Struthiola* L. and

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Lachnaea L. (including *Cryptadenia* Meisn.; Beyers, 2001), and the remaining members of tribe Gnidieae, namely *Thymelaea* Miller, *Passerina* L., *Kelleria* Endl., *Drapetes* Banks ex Lam. and *Pimelea*, in individual subtribes.

Linnaeus (1753) established *Gnidia* with three species, including the type species *Gnidia pinifolia* L. He distinguished *Gnidia* from related genera in the family by its tetramerous perianth and eight stamens, and remarked on the resemblance between *Gnidia* and *Passerina*, noting that only the presence of petal-like structures (his *corollâ*) in *Gnidia* and their absence in *Passerina* could distinguish them. The distinction between *Gnidia* and other African Thymelaeoideae has fluctuated considerably (Table 1). Floral characters previously used to distinguish genera are variable, resulting in the reduction of some groups to synonymy under *Gnidia*. For example, the pentamerous floral plan in *Lasiosiphon* is unstable, with inflorescences occasionally including flowers with parts in fours or sixes. In contrast, the tetramerous condition in flowers of *Gnidia s.s.* appears stable (A. J. Beaumont, pers. observ.). Peterson (1959) considered this slight instability of the pentamerous condition to justify the reduction of *Lasiosiphon* to synonymy under *Gnidia*. Ding Hou (1960), citing Peterson (1959), acknowledged the challenge of finding robust characters to separate genera in Thymelaeaceae. Peterson (1978) provided the most recent account of Thymelaeaceae in tropical and eastern Africa, and modern southern African flora accounts (for example, Bredenkamp & Beyers, 2000; Goldblatt & Manning, 2000) have followed his broad circumscription of *Gnidia*.

To assess the relationships among genera of Thymelaeoideae, we performed a combined phylogenetic analysis of nuclear and plastid molecular datasets: nuclear internal transcribed spacer (ITS) ribosomal DNA and plastid *rbcL*, *trnL* intron and *trnL-F* intergenic spacer regions. Representatives of 32 of the 45 genera accepted in Thymelaeaceae were included in the study, among which are 23 of the 33 genera of Thymelaeoideae.

MATERIAL AND METHODS

Taxon sampling

In total, we analysed 106 species, representing 32 genera of Thymelaeaceae and including 35 species of *Gnidia* that represent the full range of floral diversity and habit within the genus. Representatives of Sphaerosepalaceae [*Dialyceras coriaceum* (R.Cap.) J.-F.Leroy, *Rhopalocarpus* sp.] and Neuradaceae (*Grielum humifusum* E.Mey. ex Harv. & Sond.) were selected as outgroups, because of their close relationship to Thymelaeaceae (Fay *et al.*, 1998; Bayer *et al.*, 1999). An earlier study by Van der Bank *et al.* (2002) concluded that Thymelaeaceae are monophyletic with four subfamilies, and Synandrodaphnoideae and Gonystyloideae are successively sister to Aquilarioideae/Thymelaeoideae. Therefore, representatives of Aquilarioideae, Gonystyloideae and Synandrodaphnoideae were also included. Voucher specimens for the taxa used in this study and GenBank accession numbers are listed in Appendix 1.

DNA extraction, amplification and sequencing

DNA was extracted using the 2X cetyltrimethylammonium bromide (CTAB) method (Doyle & Doyle, 1987) from herbarium, fresh or silica-dried material. DNA was purified using either a QIAQuick polymerase chain reaction (PCR) purification kit (QIAgen, Inc., Hilden, Germany) or caesium chloride/ethidium bromide gradient centrifugation. PCR amplification and sequencing for *rbcL* and the *trnL-F* region (intron and spacer) were performed as in Van der Bank *et al.* (2002). The ITS nuclear ribosomal DNA region was amplified using the primers of White *et al.* (1990; ITS 2, 3, 4 and 5). For PCR amplification of the ITS region, the following programme was used: pre-melt at 94 °C for 120 s, denaturation at 94 °C for 60 s, annealing at 48 °C for 60 s, extension at 72 °C for 3 min, final extension at 72 °C for 7 min (30 cycles). For the editing and assembly of complementary electropherograms, Sequencher version 4.1 (Gene Codes Corporation, Ann Arbor, MI, USA) was used. Each base position was checked for agreement of the complementary strands, and most sequences had nearly 100% of both strands available. The aligned matrices are available from MVDB and MWC: mvdbank@uj.ac.za; m.chase@kew.org.

Phylogenetic analysis of molecular data

Molecular data were analysed using maximum parsimony and Bayesian methods employing PAUP* version 4.0b10 (Swofford, 2003) and MrBayes version 3.1b2 (Ronquist & Huelsenbeck, 2003), respectively. Prior to Bayesian analysis, the best-fit model of evolution was determined for each molecular marker via the Akaike information criterion (Akaike, 1979), as implemented in MODELTEST version 3.06 (Posada & Crandall, 1998), which uses log-likelihood scores to estimate the model of DNA evolution best suited to a specific dataset (Posada & Crandall, 1998).

Tree searches and branch support

We did not analyse each of the plastid regions separately because, individually, they exhibit low levels of

Table 1. Classification of *Gnidia* and associated genera

	Gilg (1894)	Pearson (1913); Wright (1915); Burtt Davy (1926)	Marloth (1925)	Domke (1934)	Phillips (1944, 1951)	Hutchinson (1967)	Dyer (1975)	Peterson (1959, 1982)
Tribe I	Gnidieae	Euthymelaeae	Gnidieae	Gnidieae				
Subtribe I	Gnidiinae			Gnidiinae				
Genus	*Gnidia* (including *Lasiosiphon*, *Arthrosolen*)	*Gnidia*	*Gnidia*	*Gnidia* (including *Arthrosolen*, *Englerodaphne*, *Epichroxantha*)	*Gnidia*	*Gnidia* (including *Pseudognidia*, *Craspedostoma*, *Epichroxantha*)	*Gnidia* (including *Lasiosiphon*, *Arthrosolen*, *Pseudoginidia*, *Basutica*, *Struthiolopsis*)	*Gnidia* (including *Lasiosiphon*, *Englerodaphne*, *Arthrosolen*, *Pseudognidia*, *Basutica*, *Struthiolopsis*, *Craspedostoma*)
Genus				Craspedostoma				
Genus		*Lasiosiphon*	*Lasiosiphon*	*Lasiosiphon*	*Lasiosiphon*	*Lasiosiphon*		
Tribe II	Dicranolepideae		Dicranolepideae					
Subtribe II	Linostomatinae							
Genus	*Englerodaphne*	*Englerodaphne*	*Englerodaphne*		*Englerodaphne*	*Englerodaphne*	*Englerodaphne*	
Tribe III			Dapneae					
Genus		*Arthrosolen*	*Arthrosolen*		*Arthrosolen*	*Arthrosolen*		
Genus					*Pseudognidia*			
Genus					*Basutica*	*Basutica*		
Genus					*Struthiolopsis*	*Struthiolopsis*		

Table 2. Statistics from maximum parsimony analyses obtained from separate and combined datasets

	rbcL	*trnL-F*	Combined plastid	Internal transcribed spacer (ITS)	Combined plastid + ITS
Number of taxa included	118	106	120	86	120
Number of included characters	1378	984	2362	651	3014
Number of constant characters	1074	620	1694	293	1988
Number of variable sites	304 (22.1%)	364 (37%)	668 (28.3%)	358 (55%)	1026 (34%)
Number of parsimony informative sites	193 (14%)	226 (23%)	419 (17.7%)	293 (45%)	712 (23.62%)
Number of trees (Fitch)	53	205	2610	651	5360
Number of steps (tree length)	668	679	1421	1599	3040
Consistency index	0.54	0.69	0.58	0.36	0.48
Retention index	0.83	0.84	0.81	0.72	0.75
Average number of changes per variable site (number of steps/ number of variable sites)	2.19	1.87		4.46	

sequence divergence; in addition, there is no reason to suspect incongruence among different regions of the plastid genome as they cannot assort independently or recombine. To evaluate congruence, we analysed the ITS and plastid matrices separately before combining them. All matrices were analysed using heuristic searches with 1000 random sequence additions, but keeping only ten trees per replicate to reduce the time spent on branch swapping in each replicate. Tree bisection-reconnection (TBR) was performed with MulTrees on (keeping multiple equally parsimonious trees) and all character transformations treated as equally likely (Fitch parsimony; Fitch, 1971). The trees collected in the 1000 replicates were then used as starting trees for another search without a tree limit. For the illustration of branch lengths, DELTRAN (delayed transformation) character optimization was used instead of ACCTRAN (accelerated transformation) because of reported errors with the latter in PAUP* 4.0b10. Internal support was estimated by the bootstrap as implemented by PAUP* using 1000 bootstrap replicates performed with equal weights employing TBR branch swapping with 10 trees held at each step and simple taxon addition.

As a result of the poor quality DNA of some taxa, we could not amplify all regions for all taxa, and thus the individual data matrices do not contain identical sets of taxa. We investigated the effects of these missing data on the patterns of relationships and support in the combined analysis by comparing results from matrices in which we included only taxa for which all data were available with results of the larger matrices with missing data. We found that neither was affected in any obvious way, and therefore illustrate the combined results with all taxa. Congruence between the ITS and plastid datasets was addressed by comparison of bootstrap percentages from the separate analyses. Bootstrap trees were considered incongruent only when 'hard' (i.e. with high bootstrap support) instead of 'soft' (with low bootstrap support) incongruence was displayed (Seelanan, Schnabel & Wendel, 1997; Wiens, 1998). No 'congruence tests', such as the incongruence length difference test, were used, because of their reported unreliability (Reeves *et al.*, 2001; Yoder, Irwin & Payseur, 2001). The following scale for support percentages was used: 50–74%, low; 75–84%, moderate; 85–100%, high.

A Bayesian approach for inferring phylogenies was also used. For each matrix, the best model was selected using MODELTEST version 3.06 (Posada & Crandall, 1998). For all regions, GTR + I + G was the resulting model, with substitutions = 6, rates = gamma, base frequency = empirical, clock = unconstrained. Four parallel Markov chain Monte Carlo estimations were run for 3 000 000 generations with trees sampled every 200 generations. The resulting trees were plotted against their likelihoods to determine the point at which likelihoods converged on a maximum value, and all the trees before convergence were discarded as the 'burn-in'. All remaining trees were imported into PAUP* 4.0b10, and a majority-rule consensus tree was produced showing frequencies (i.e. posterior probabilities or PP) of all observed bi-partitions. The following scale was used to evaluate PP: below 0.85, poor; 0.85–0.95, moderate; 0.95–1.0, high.

RESULTS

The characteristics of each partition and the statistics of each analysis are reported in Table 2. The alignment of ITS sequences between subfamilies of

Thymelaeaceae and the outgroup families was difficult, and there were many ambiguous regions. Thus, *Edgeworthia* Meisn., *Wikstroemia* Spreng., *Thymelaea* Mill. and *Daphne* were selected as outgroups for this data matrix.

The aligned plastid matrix included the *rbcL* gene with 1378 base pairs (bp) and the *trnL-F* region (intron and spacer) with 984 bp. The aligned ITS dataset consisted of 651 bp. As a result of ambiguous alignments, portions of the *trnL-F* region had to be excluded (three regions, 300 bp in total). Some taxa in the *trnL-F* region had deletions of 397 bp or more (for example, *G. coriacea* Meisn., *G. galpinii* C.H.Wright, *G. humilis* Meisn., *G. squarrosa* L., *G. subulata* Lam., *G.* aff. *viridis*, *Pimelea clavata* Labill., *P. decora* Domin, *P. gilgiana* E.Pritz., *P. graniticola* Rye, *P. haematostachya* F.Muell., *P. holroydii* F.Muell., *P. punicea* R.Br., *P. pygmaea* Meisn., *P. sanguinea* F.Muell., *P. spiculigera* F.Muell., *P. trichostachya* Lindl., *Thecanthes punicea* Wikstr. and *T. sanguinea* (F.Muell.) Rye). The aligned region of ITS contained the most variable sites: 358 (55%) compared with *trnL-F* with 364 (37%) and *rbcL* with 304 (22%). The number of potentially informative characters was also higher for ITS (293; 45%) than for *trnL-F* (226; 23%) or *rbcL* (193; 14%). Variable positions changed more rapidly for ITS: 4.46 vs. 2.19 (*rbcL*) and 1.87 (*trnL-F*). The length of the combined plastid regions (*rbcL* + *trnL-F*) included in the analysis was 2362 positions, 28.3% of which were variable and 17.7% potentially informative. Analysis resulted in 2610 equally parsimonious trees with a consistency index (CI) of 0.58 and retention index (RI) of 0.81.

The combined plastid analysis (Fig. 1) was largely congruent with the ITS analysis (Fig. 2), except for one moderately supported incongruence. In the plastid tree, *Lachnaea* was moderately supported as monophyletic (79 bp), whereas, in the ITS tree, monophyly received no support. For *Gnidia*, the phylogenetic trees did not conflict with each other, although many taxa were reduced to polytomies because of a lack of sufficient informative sequence variation. Where taxon placement differed slightly between the two topologies, bootstrap support was weak and did not provide credible evidence of conflict. We thus directly combined the plastid and nuclear datasets.

Combined molecular analysis

The parsimony analysis resulted in 5360 equally parsimonious trees (tree length, 3040 steps; CI = 0.48; RI = 0.75). Of the 3014 included characters, 1988 were constant, 1026 (34%) were variable and 712 (23.6%) were potentially parsimony informative. The combined maximum parsimony analysis is largely congruent with the Bayesian analysis, and therefore results can be displayed on the same tree (Fig. 3).

Thymelaeaceae are strongly supported as monophyletic (99 bp/1.0 PP). Aquilarioideae (100 bp/1.0 PP) and Synandrodaphnoideae are moderately supported as successively sister (98 bp/1.0 PP and 78 bp/0.70 PP, respectively) to Thymelaeoideae (89 bp/1.0 PP). Gonystyloideae are paraphyletic to the rest of Thymelaeaceae, comprising two clades: (I) three taxa of *Octolepis* Oliv., namely *O. dioica* Capuron, *O. dioica* forma *oblanceolata* Capuron and *Octolepis* sp. (97 bp/1.0 PP); (II) representatives of *Lethedon* Spreng., *Arnhemia* Airy Shaw, *Deltaria* Steenis, *Gonystylus* Teijsm. & Binn. and *Solmsia* Baill. (75 bp/1.0 PP).

Within Thymelaeoideae, three major clades are retrieved: clade I comprises tropical African and south-eastern Asian taxa; clade II includes exclusively non-African taxa; and clade III comprises southern and tropical African, south-eastern Asian, Australasian and New World taxa. Clades II and I are strongly supported as successively sister to clade III (89 bp/1.0 PP and 97 bp/1.0 PP, respectively). Clade I includes *Craterosiphon* Engl. & Gilg and *Synaptolepis* Oliv. grouped together, with *Enkleia* Griff. and *Dicranolepis* Planch. successively sister to them. Clade II includes two strongly supported sister clades comprising *Wikstroemia* plus *Stelleria* L. (98 bp/1.0 PP) and *Diarthron* Turcz. plus *Thymelaea* and *Daphne* (89 bp/1.0 PP). *Edgeworthia* Falc. is sister (97 bp/1.0 PP) to this pair of clades. Clade III includes all remaining genera in the analysis.

Within clade III, there is strong support for the clade comprising *Dais* L. and *Phaleria* Jack (99 bp/1.0 PP) and moderate support for *Ovidia* Raf. and *Dirca* L. (68 bp/0.99 PP). *Passerina* (99 bp/1.0 PP), *Struthiola* (95 bp/0.98 PP) and *Stephanodaphne* Baill. (100 bp/1.0 PP) are strongly supported as monophyletic assemblages. The largest genus in this clade, *Gnidia*, is shown to be highly polyphyletic. *Gnidia penicillata* Lichtenst. ex Meisn. is strongly supported (73 bp/1.0 PP) as being embedded within *Lachnaea*, but even with the exclusion of *G. penicillata*, the remaining species of the genus are dispersed among four clades: clade 1 positions *Drapetes muscosus* Lam. sister to six *Gnidia* taxa (69 bp/1.0 PP); clade 2 allies *Gnidia pinifolia* L. and *Gnidia racemosa* Thunb. with *Struthiola* (100 bp/1.0 PP); clade 3 allies 14 species of *Gnidia* with *Pimelea* and *Thecanthes* (99 bp/1.0 PP); and clade 4 retrieves as monophyletic those *Gnidia* taxa previously recognized as *Arthrosolen* or *Lasiosiphon* (100 bp/1.0 PP).

DISCUSSION

Thymelaeaceae are strongly supported as monophyletic in both parsimony and Bayesian analyses

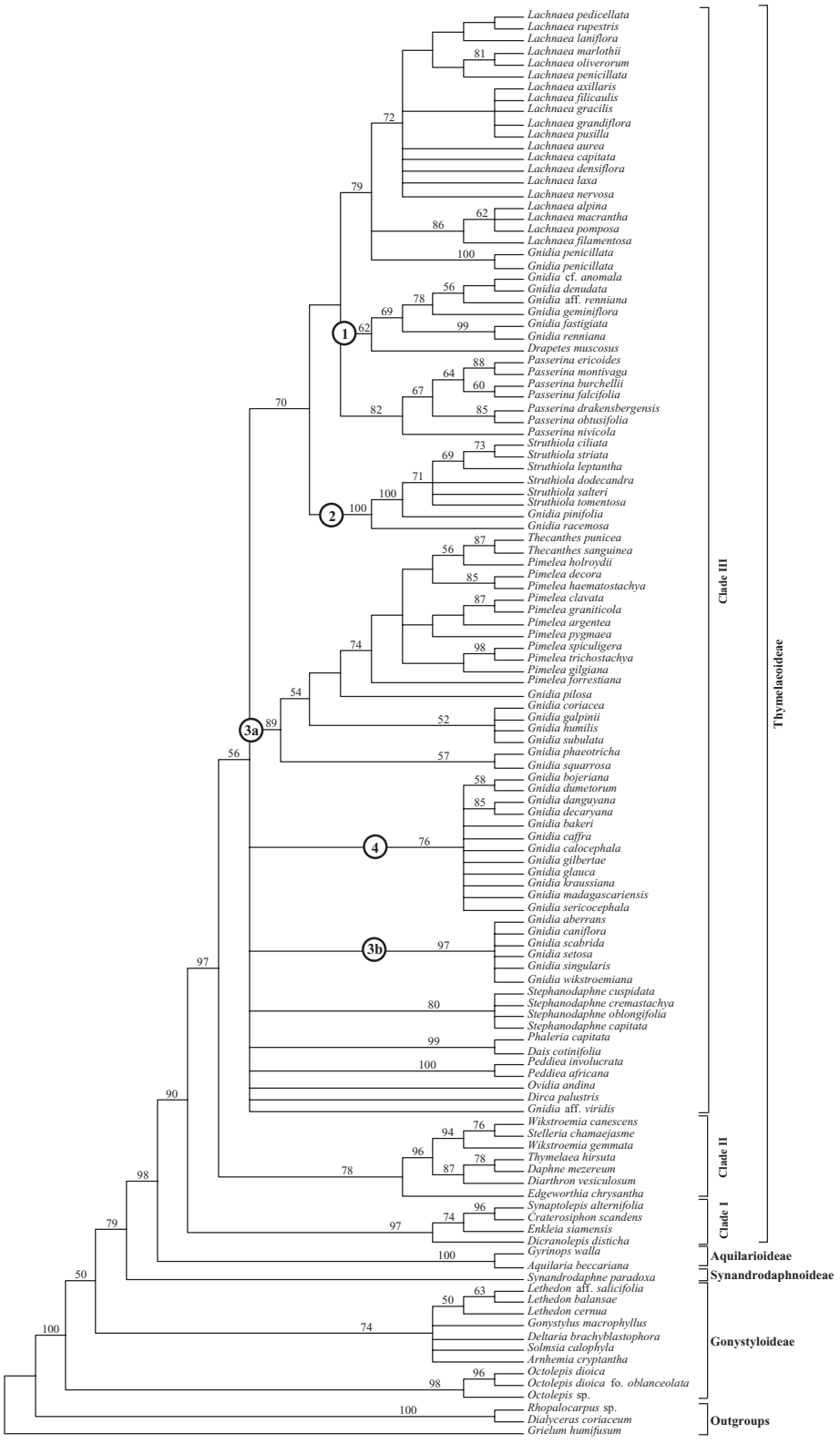

Figure 1. Strict consensus tree based on combined plastid data (*rbcL* and *trnL-F*). Bootstrap percentages above 50 are shown above the branches. The three clades indicated are as follows: (I) tropical African and south-eastern Asian taxa; (II) non-African taxa; and (III) southern and tropical African, south-eastern Asian and Australasian species plus two New World taxa. Lineages 1–4 indicate non-monophyletic *Gnidia*.

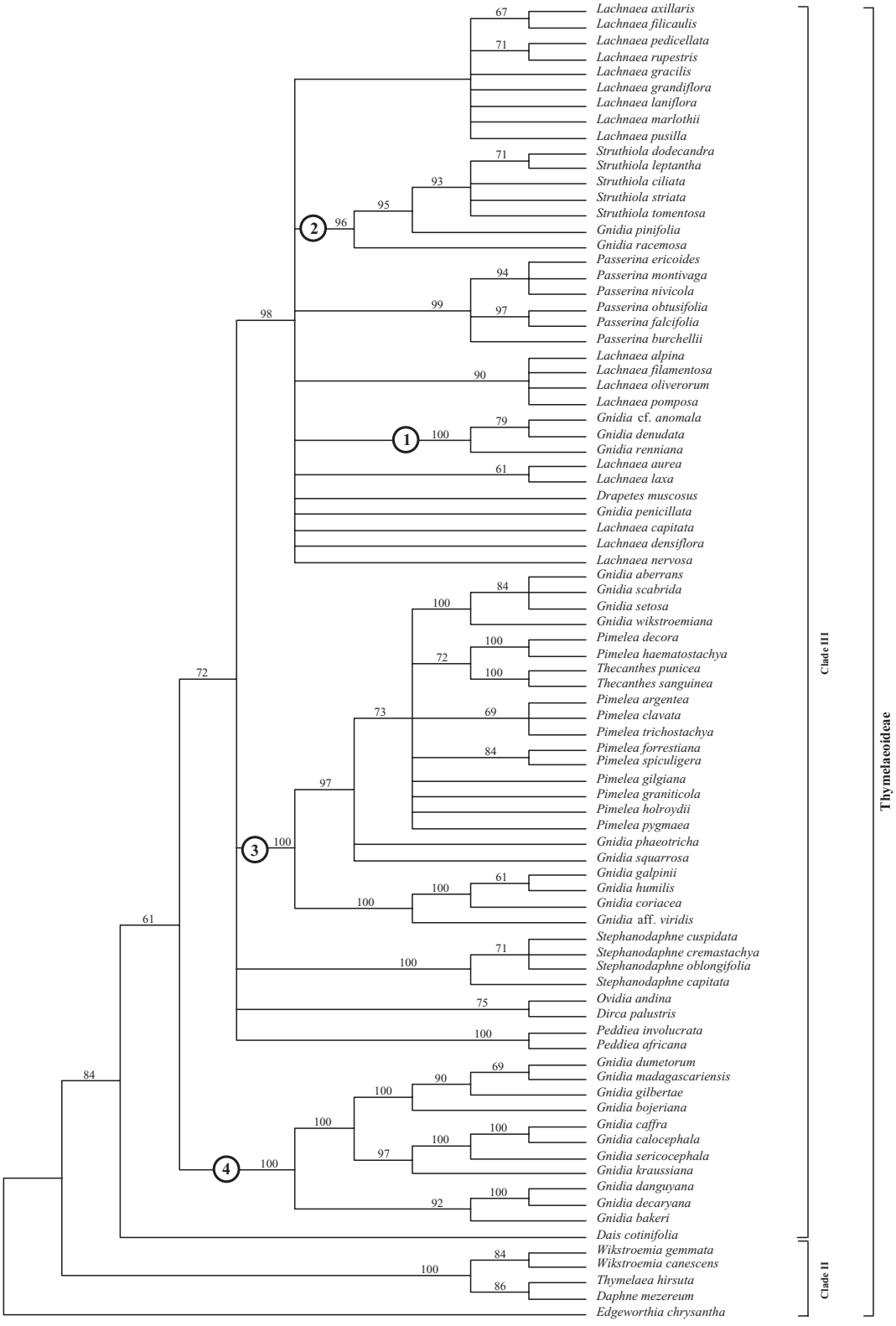

Figure 2. Strict consensus tree from the parsimony analysis of the nuclear ribosomal internal transcribed spacer (ITS) region. Bootstrap percentages above 50 are indicated above the branches. The two clades indicated are as follows: (II) the non-African taxa; and (III) the southern and tropical African, south-eastern Asian and Australasian species plus two New World taxa. Lineages 1–4 indicate non-monophyletic *Gnidia*.

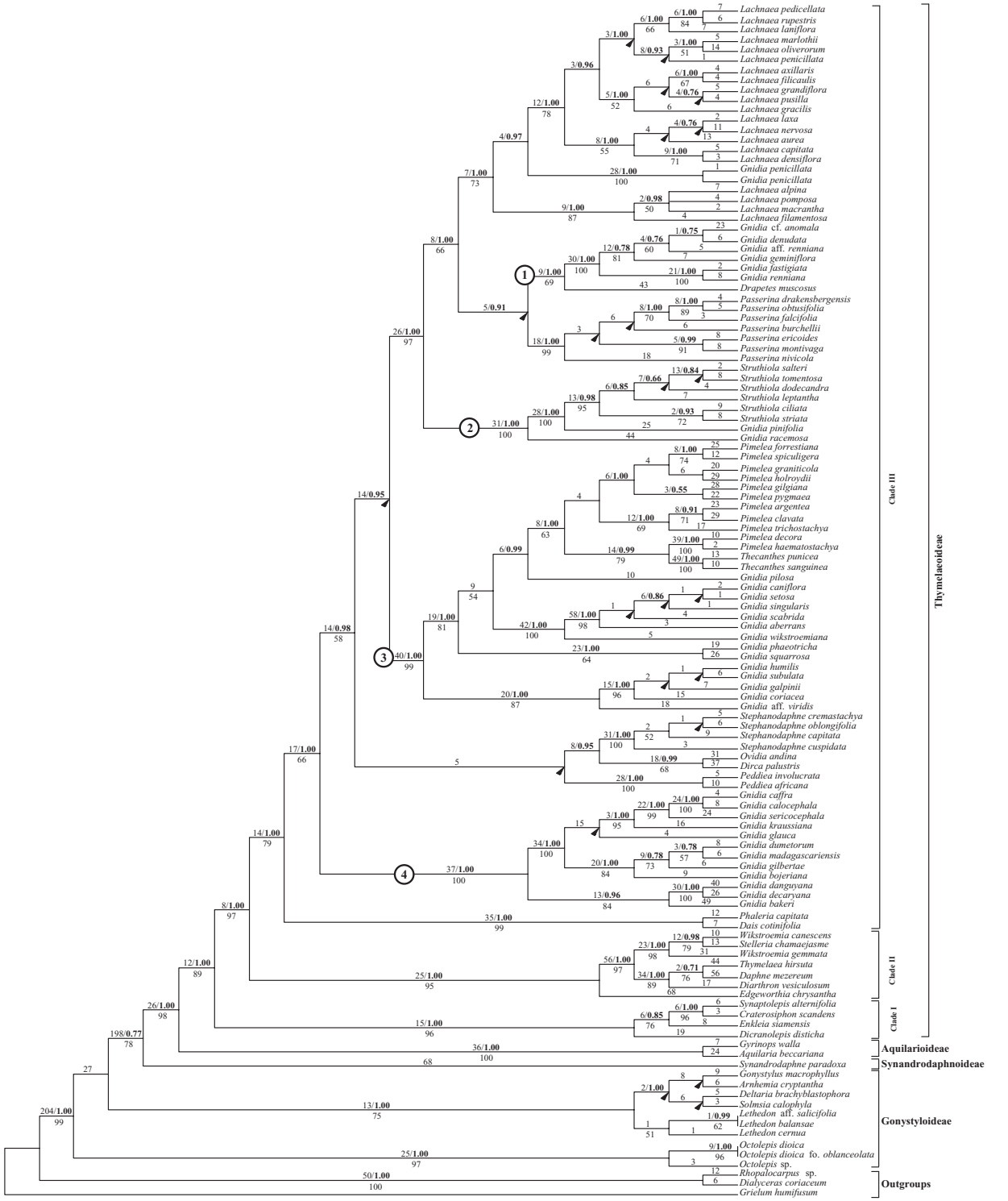

Figure 3. One of the equally most parsimonious trees from the combined *rbcL*, *trnL-F* region and internal transcribed spacer (ITS) analysis (consistency index, 0.48; retention index, 0.75; tree length, 3040 steps). Numbers displayed above each branch are Fitch lengths (DELTRAN optimization)/PP > 0.5 from Bayesian analysis (in bold). Percentages below the branches are bootstrap percentages equal to or greater than 50. Full arrows indicate groups not present in the Fitch strict consensus tree. The three clades indicated are as follows: (I) the tropical African and south-eastern Asian taxa; (II) the non-African taxa; and (III) the southern and tropical African, south-eastern Asian and Australasian species plus two New World taxa. Lineages 1–4 indicate non-monophyletic *Gnidia*.

(99 bp/1.0 PP). The major clades found in our analyses support those identified in the molecular analysis of Van der Bank *et al.* (2002) and are broadly compatible with the four subfamilies [Synandrodaphnoideae (= Gilgiodaphnoideae), Aquilarioideae, Thymelaeoideae and Gonystyloideae] recognized by Domke (1934), although the circumscription of Gonystyloideae should be re-examined. This study shows Gonystyloideae to be paraphyletic with the inclusion of *Octolepis*. Furthermore, although Domke (1934) included *Lethedon*, *Solmsia* and *Octolepis* in Aquilarioideae, our results support Rye (1990) who moved *Lethedon* to Gonystyloideae. The inclusion of *Solmsia* in Gonystyloideae corresponds to the findings of Domke (1934) and Van der Bank *et al.* (2002).

Relationships within Thymelaeoideae

Domke (1934) recognized four tribes in Thymelaeoideae, namely Dicranolepideae, Phalerieae, Daphneae and Gnidieae. A previous molecular study (Van der Bank *et al.*, 2002) supported the results obtained here: that Thymelaeoideae, as circumscribed by Domke (1934), are shown to be a monophyletic group that includes three highly supported clades. Clade I comprises tropical African taxa plus the tropical Asian genus *Enkleia* Griff., and partly corresponds to Domke's (1934) tribe Dicranolepideae and subtribes Linostomatinae and Dicranolepidinae. Clade II comprises seven taxa from Asia and the Mediterranean region, including northern Africa, and represents tribes Daphneae (subtribes Wikstroemiinae, Dendrostellerinae and Daphninae) and Gnidieae (subtribe Thymelaeinae; Domke, 1934). Clade III, the largest clade, includes southern and tropical African, southeastern Asian and Australasian species plus New World taxa. The taxa in clade III collectively represent, in part, the tribes Phaleriae (subtribe Phaleriinae), Daphneae (subtribe Daphnopsinae) and Gnidiinae (subtribes Drapetinae, Gnidiinae and Passerininae; Domke, 1934). Clade III received moderate support in the parsimony analysis and high support in the Bayesian analysis and comprises several lineages with low resolution because of low levels of genetic variation.

The southern African *Dais cotinifolia* L. and the southern Pacific *Phaleria capitata* Jack grouped together with high support in both the parsimony and Bayesian analyses (99 bp/1.0 PP) and are moderately supported as being sister to the rest of clade III (79 bp/1.00).

Two species of *Peddiea* Harv. from Africa and Madagascar are weakly supported in the Bayesian analysis (0.57 PP) as sister to clades 1, 2 and 3. In the parsimony analysis, however, they form an unsupported clade with *Dirca* and *Ovidia* from North and South America, respectively (Nevling, 1964; Heads, 1990) and *Stephanodaphne* from Madagascar and Mayotte (Rogers, 2004). The bilocular ovaries in *Peddiea* are anomalous among the otherwise unilocular condition in the rest of Thymelaeoideae. Furthermore, fruits are drupes in contrast with the dry fruits of *Gnidia* and other taxa in clades 1–3 (Peterson, 1978). There was strong support for the monophyly of *Stephanodaphne* (100 bp/1.0 PP), with *Ovidia* and *Dirca* sister to it (0.95 PP).

The molecular data presented here strongly indicate that *Gnidia*, in its broad, inclusive sense (i.e. that of Peterson, 1959), is not monophyletic, and comprises at least four moderately to strongly supported clades in the parsimony and Bayesian analyses (Fig. 3). Different groups of *Gnidia* species are embedded within various southern African and Australian genera. In addition, six *Gnidia* taxa are sister to the monotypic *Drapetes*.

One group of *Gnidia* spp. was shown to be sister to *Passerina*. The monophyly of *Passerina* was highly supported, corresponding to the findings of Van der Bank *et al.* (2002). Bredenkamp & Van Wyk (1996) suggested the placement of *Passerina* as the sole member of subtribe Passerininae on the basis of pollen morphology. *Passerina* is separated morphologically from the rest of Thymelaeaceae by the extrorse dehiscence of its anthers (Beyers & Marais, 1998; Bredenkamp & Beyers, 2000) and is also the only genus in Thymelaeaceae adapted to wind pollination. Our analysis provided clear evidence that *Passerina* is embedded within subtribe Gnidiinae as currently circumscribed, which includes *Lachnaea*, *Gnidia* and *Struthiola*.

Lachnaea is sister to the *Passerina*/*Gnidia* clade, a placement weakly supported in the parsimony analysis and strongly supported in the Bayesian analysis. Support was moderate in the parsimony analysis and high in the Bayesian analysis for the monophyly of a slightly expanded circumscription of *Lachnaea* to include *G. penicillata* (73 bp/1.0 PP). This placement was confirmed by the inclusion of two accessions of *G. penicillata* in the analysis. *Gnidia penicillata* is anomalous in *Gnidia* with several features more typical of *Lachnaea*: a slender, conical stigma (vs. the capitate stigma of *Gnidia*), clearly obconical style (vs. generally uniformly cylindrical styles or very slightly obconical styles of *Gnidia*) and royal blue calyx lobes. Flowers in shades of blue, mauve or pink occur in several species of *Lachnaea*, but are rare in *Gnidia*. In addition, the floral scales number four to eight per flower in *G. penicillata*, whereas the floral scales always number eight in *Lachnaea* (Beyers, 2001). *Gnidia penicillata* more closely resembles other *Gnidia* species in its floral disc, and its floral scales are inserted above the level of the lower series of

stamens. In *Lachnaea*, the floral scales all arise at the same level as the filaments or are all inserted below the stamens. The relative positions of the floral scales and stamens have traditionally been used to distinguish *Gnidia* from *Lachnaea* (Wright, 1915; Beyers, 2001). Our results challenge the usefulness of this character in delimiting these genera, and the position of *G. penicillata* in *Lachnaea* and its taxonomic implications will be considered in a separate paper.

Relationships among *Gnidia* species and other genera

Clade 1

In this clade, *Drapetes muscosus* is sister to six representatives of *Gnidia*: *G. renniana* Hilliard & B.L.Burtt, *G. fastigiata* Rendle, *G. geminiflora* E.Mey. ex Meisn., *G.* aff. *renniana*, *G. denudata* Lindl. and *G. anomala* Meisn. This grouping has weak support in the parsimony analysis and strong support in the Bayesian analysis. The position of *D. muscosus* in clade 1 supports Domke's (1934) classification of this New World genus in the tribe Gnidieae, together with African, European/North African (*Thymelaea*) and Australasian (*Pimelea*) taxa, rather than with other New World taxa that are representative of his tribes Dicranolepideae and Daphneae. The distribution of *D. muscosus* in southern South America and islands in the southern Atlantic and Pacific Oceans represents a geographical disjunction with *Gnidia*. Although the flowers of *Drapetes* resemble those of *Gnidia*, these genera differ in the terminal rather than the sublateral attachment of the style, and the absence of internal phloem in *Drapetes*. Furthermore, *Drapetes* lacks the tenacious (stripping) bark that otherwise typifies the family.

Readily identifiable morphological synapomorphies are lacking for clade 1. All taxa in this clade have eight stamens, although the flowers of *G. anomala* (syn. *Pseudognidia anomala*; Phillips, 1944) are often four staminate through the reduction or loss of the upper series of stamens. Elsewhere in *Gnidia*, four-staminate species include *G. aberrans* C.H.Wright [syn. *Basutica aberrans* (C.H.Wright) E.Phillips; Phillips, 1944] and *G. propinqua* Hilliard. *Gnidia aberrans* is not included in clade 1, but in clade 3 with the similarly four-staminate *G. singularis* Hilliard, plus eight-staminate *Gnidia* spp. and characteristically two-staminate Australian taxa. These placements suggest that a reduction in stamen number has occurred several times within *Gnidia* and that the number of stamens alone is not taxonomically or phylogenetically informative. *Gnidia singularis* and *D. muscosus* both have flowers with four stamens, and filaments longer than anthers. Generally, filaments are short in *Gnidia*. The topology suggests that both states are independently derived in these two species. A detailed analysis of morphological characters in this clade and sampling of more species may better define clade 1.

Clade 2

Gnidia pinifolia and *G. racemosa* form a grade with *Struthiola* in both the parsimony and Bayesian analyses. Resolution within *Struthiola* is low in the parsimony but moderate in the Bayesian analysis. These results correspond to the findings of Van der Bank *et al.* (2002), in which *G. racemosa* was well supported as sister to three representatives of *Struthiola*. Inflorescences in spikes, flowers each with four, not eight, stamens and bracteoles distinguish *Struthiola* from most *Gnidia* spp. (Pearson, 1913; Wright, 1915; Peterson, 1978; Hilliard, 1993). *Gnidia pinifolia* and *G. racemosa* have dissimilar features and, furthermore, scarcely resemble *Struthiola*. Inflorescences are not spicate, and bracteoles are lacking in both species; instead, we find many-flowered, terminal bracteate clusters in *G. pinifolia* and scattered, single flowers or few-flowered pseudobracteate clusters in *G. racemosa*. Furthermore, flowers of both taxa have eight, not four, stamens. Morphological synapomorphies are lacking for an expanded generic circumscription of *Struthiola* to include *G. pinifolia* and *G. racemosa*, and generic limits will have to be reconsidered for these taxa.

Clade 3

Gnidia pilosa from mainland Africa is placed as sister to 13 species of the Australasian genera *Pimelea* and *Thecanthes* included in our analyses. This result is similar to that obtained by Van der Bank *et al.* (2002), in which *G. pilosa* and *G. subulata* Lam. (as *G.* aff. *viridis*) were allied to *Pimelea*. The remainder of clade 3 comprises 13 *Gnidia* taxa. Our molecular findings support the conclusions of Gilg (1894), Bentham (1873), Threlfall (1982) and Motsi *et al.* (MC Mosti, University of Johannesburg, Auckland Park, South Africa, unpubl. data) that *Thecanthes* should be included within *Pimelea* and that subtribe Pimeleinae is therefore monogeneric. The position of *G. pilosa* as sister to *Pimelea* is morphologically incongruous. *Gnidia pilosa* instead resembles two other African species: *G. leiosiphon* Gilg (Domke) and *G. ovalifolia* Meisn. All three species have paired, flat, flimsy leaves, with long internodes and few-flowered, ebracteate umbels with primary floral axes that lengthen during fruit development. These features led Gilg (1894) to establish *Englerodaphne*; Phillips (1944) maintained *Englerodaphne* but conceded that there were no 'outstanding structural differences' between the flowers of *Gnidia* and *Englerodaphne*. Modern treatments, however (for example, Arnold & De Wet

(1993), follow Peterson (1959) and list *Englerodaphne* as a synonym of *Gnidia*.

Gnidia aberrans, *G. caniflora* Meisn., *G. setosa* Wikstr., *G. scabrida* Meisn., *G. singularis* and *G. wikstroemiana* Meisn. form a well-supported subclade within clade 3 in both the parsimony and Bayesian analyses. All of these species have local distributions in South Africa and tetramerous flowers. These six species represent different degrees of reduction of the androecium from eight-staminate (*G. caniflora* and *G. setosa*) to the upper series of stamens being smaller than the lower series (*G. scabrida*) to four-staminate (*G. aberrans* and *G. singularis*) or gynodioecious and eight-staminate (*G. wikstroemiana*; Beaumont, Edwards & Smith, 2006). A small subclade in clade 3 comprises two morphologically dissimilar species: *G. phaeotricha* Gilg and *G. squarrosa* Druce. *Gnidia phaeotricha* plants are cryptic within their grassland habitats, producing annual stems from a woody perennial rootstock, spike-like inflorescences with small, non-colourful flowers with very short tubes and floral axes that lengthen in fruit. In contrast, *G. squarrosa* is shrubby with capitate inflorescences and floral axes that do not lengthen in fruit (Wright, 1915; AJB, pers. obs.) making synapomorphies with which to define this subclade elusive. However, sister to the rest of clade 3 is a morphologically identifiable subclade comprising former members of *Epichroxantha* (Meissner, 1857), namely *G. galpinii*, *G. humilis*, *G. coriacea*, *G. subulata* and *G.* aff. *viridis*. These species resemble each other, sharing pungent, coriaceous leaves (except *G. humilis* in which leaves are more flimsy), tetramerous funnel-shaped flowers with four large membranous petals and eight stamens (Bond & Goldblatt, 1984; Levyns, 1950; A. J. Beaumont, pers. observ.).

Clade 4

This strongly supported clade comprises three subclades, containing southern and tropical African and Madagascan taxa. Our results expand on those obtained by Van der Bank *et al.* (2002), in which *G. kraussiana* Meisn. (= *Lasiosiphon kraussii* Meisn.) was separated from the rest of *Gnidia*. These initial results provided some support for reinstating the genus *Lasiosiphon*. Fresenius (1838) established *Lasiosiphon* for species with pentamerous flowers, ten stamens, small floral scales, hairy floral tubes with long, silky basal hairs (in all species hairs are smooth), involucral leafy bracts surrounding heads of many yellow flowers and alternate leaves. *Lasiosiphon* was recognized by Endlicher (1847), Leandri (1950), Meissner (1857), Wright (1915) and Phillips (1944), but not by Gilg (1894), Staner (1935), Peterson (1959, 1978) or Robyns (1975), who considered it a synonym of *Gnidia*.

The first subclade within clade 4 comprises four Madagascan endemics: *G. dumetorum* Leandri, *G. madagascariensis* (Lam.) Decne. ex Cambess., *G. gilbertae* Drake and *G. bojeriana* Baill. *Gnidia gilbertae* has tetramerous flowers, but the rest have pentamerous flowers and, as such, were previously classified under *Lasiosiphon* (Leandri, 1950). This subclade received high support in the Bayesian analysis (1.00 PP) and moderate support (BP 84) was obtained in parsimony analysis.

The second subclade includes the tropical and southern African species *G. caffra* (Meisn.) Gilg, *G. calocephala* Gilg, *G. sericocephala* (Meisn.) Gilg ex Engl. and *G. kraussiana*, with the position of *G. glauca* (Fresen.) Gilg unresolved. *Gnidia kraussiana* and *G. caffra* were previously included in *Lasiosiphon*. *Gnidia calocephala* and *G. sericocephala* also have pentamerous flowers and ten stamens, but were previously classified under *Arthrosolen* not *Lasiosiphon*. *Arthrosolen* included *Gnidia*-like species with no floral scales and represented a diverse collection of species, morphologically at odds with each other (Wright, 1915).

The last subclade of clade 4 is an exclusively Madagascan group, highly supported in both parsimony and Bayesian analyses, and comprising *G. bakeri* Gilg, *G. danguyana* Leandri and *G. decaryana* Leandri. All species in this subclade have tetramerous flowers. *Gnidia decaryana*, however, more closely resembles species once included in *Englerodaphne* with flowers arranged in dense terminal clusters on comparatively long, bare peduncles. As the fruits mature, the primary floral axis elongates and the internodes lengthen, which is also a feature of *Englerodaphne*. These morphological traits are thus interpreted as convergent. The third species in this subclade, *G. bakeri*, is morphologically more similar to both *G. calocephala* and *G. sericocephala* than to *G. danguyana* or *G. decaryana* with which it groups in our results. Again, morphological synapomorphies supporting the molecular association of *G. bakeri*, *G. danguyana* and *G. decaryana* are elusive.

CONCLUSIONS

Few morphological characters have previously been used to distinguish genera within Thymelaeaceae, and the literature is full of discussions on the relative merits of the various characters that have been employed to delimit genera. Recent phylogenetic studies, for example Van der Bank *et al.* (2002) and Galicia-Herbada (2006), have included representatives of Thymelaeaceae as place holders, in the hope that the resolution of relationships would aid in the development of an improved taxonomic scheme. This hope appears to be unfounded because of a lack of

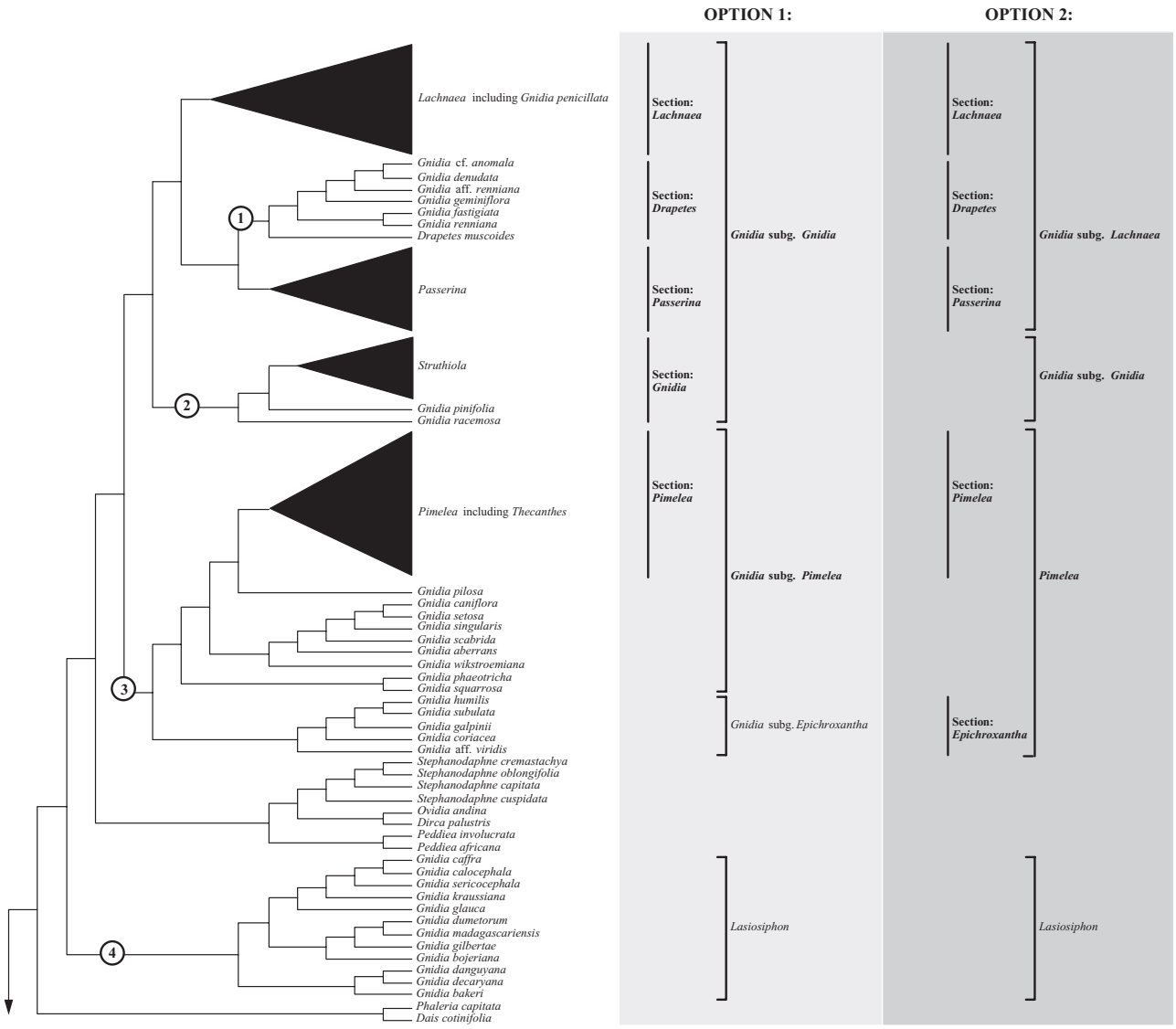

Figure 4. The two options proposed for a monophyletic circumscription of genera within Thymelaeoideae.

obvious morphological synapomorphies observed for the clades found in the molecular studies. Many of the characters previously used to delimit genera in the family represent parallel adaptations. The results from the DNA studies indicate that the current generic limits are untenable, yet the way forward is unclear. The lumping of separate clades of *Gnidia* into other genera to which they are related would require paying no attention to the characters that have been used as the basis for these other genera, and it is not clear which other characters could replace them. If lumping is untenable, then perhaps splitting is a better option.

Although additional species of this large genus should be included in the analysis pending a final classification of the subfamily, current results are sufficient to warrant a partial solution. We present two options towards a monophyletic circumscription of genera in the subfamily (Fig. 4).

Option 1 proposes a very broadly circumscribed *Gnidia*, comprising clades 1, 2 and 3 and inclusive of all taxa between *G.* cf. *anomala* and *G.* aff. *viridis*. Within this large genus, it is possible to recognize three subgenera: subgenus *Gnidia* to include all *Gnidia* spp. in clades 1 and 2 plus the genera *Lachnaea*, *Passerina* and *Struthiola*; subgenus *Pimelea* for the genus *Pimelea* plus associated species from *G. squarrosa* to *G. pilosa*; and subgenus *Epichroxantha* for all taxa inclusive of *G. galpinii* to *G.* aff. *viridis.*

The following monophyletic, morphologically diagnosable lineages within the large subgenus *Gnidia* can be recognized at sectional level: section *Drapetes* comprising *D. muscosus* plus associated species of *Gnidia*; section *Passerina* for species currently placed

in this genus; section *Lachnaea* for species of *Lachnaea* plus *G. penicillata* and allied species; and section *Gnidia* for the remaining species of *Gnidia* plus *Struthiola*.

The subdivision of subgenus *Pimelea* is less clear and should only be attempted following increased sampling within *Gnidia.*

The remaining species of *Gnidia* comprise a single lineage from Africa and Madagascar. Most of the species in this lineage were previously classified under *Lasiosiphon* or *Arthrosolen*, and we propose that the genus *Lasiosiphon* be reinstated for the members of clade 4 (Fig. 4).

Option 2 proposes a less extensive circumscription of the genus *Gnidia*, which should be restricted to include those taxa in clades 1 and 2, that is the genera *Lachnaea*, *Passerina* and *Struthiola*. Within this clade, two subgenera may be recognized: subgenus *Gnidia* to include *Struthiola* and related species of *Gnidia* (features that may help to define this group include anthers on short filaments, uniformly cylindrical styles and capitate stigmas); and subgenus *Lachnaea* for all remaining taxa, including *Lachnaea* and *Passerina* (features that may help to identify members in this subgenus include extrorse anthers, styles that widen towards the stigmas, often non-capitate stigmas and bracteoles). Within subgenus *Gnidia*, the eight lineages identified in option 1 are to be treated as sections, with an additional three sections in subgenus *Lachnaea*: section *Drapetes*, with the seven taxa outlined in option 1, section *Passerina* and section *Lachnaea*, all with the same taxa as in option 1.

The genus *Pimelea* should be retained for members of clade 3, with subdivision into subgenera following more comprehensive analysis. The genus *Lasiosiphon* should be reinstated for the members of clade 4.

Discussion of options

Option 1 proposes a synthetic circumscription of the genus *Gnidia*. As defined here, the genus encompasses well-known taxa from both Africa and Australia, which have clear monophyletic sublineages, including the African groups *Lachnaea*, *Passerina* and *Struthiola*, and the Australasian *Pimelea* (including *Thecanthes*). Such a circumscription is maximally stable. This is a distinct advantage given the weak correspondence between morphological discontinuities and monophyletic lineages.

A significant disadvantage is the large number of nomenclatural changes that will be required. Flowers with two stamens distinguish *Pimelea* from other members of Thymelaeoideae, although our analysis suggests a closer link with some species of *Gnidia* than previously realized. *Pimelea* is large with ±110 species, and its synonymy under *Gnidia* would require extensive and unpopular name changes at this stage. Sampling of more *Gnidia* and *Pimelea* spp. would undoubtedly help to clarify the relationships among these groups. However, although the outcome of this may indeed make necessary extensive species name changes in *Pimelea* in future, we feel it prudent at this time to maintain the genus *Pimelea* (although aware of the obvious phylogenetic links with *Gnidia*) until we can offer more evidence in support of a concept of *Gnidia* that embraces *Pimelea*, either in the whole or in part.

Some changes to the taxonomy of the family can be proposed without encountering too many problems (for example, *Thecanthes* should remain in *Pimelea*; MC Motsi, University of Johannesburg, Auckland Park, South Africa, submitted). We also formally propose the reinstatement of *Lasiosiphon.* Substantial changes in the circumscription of *Gnidia* are still required, but will have to await the results of much more detailed studies.

Arguments for reinstating *Lasiosiphon*

All pentamerous *Gnidia* species sampled here group together in clade 4. A well-supported clade comprising *Stephanodaphne*, *Peddiea*, *Dirca* and *Ovidia* separates clade 4 from other *Gnidia* clades. We argue that this separation indicates a distinctive evolutionary route of specialization, although morphological synapomorphies for clade 4 as a whole are elusive. Clade 4 also contains two pentamerous *Gnidia* spp., *G. calocephala* and *G. sericocephala*, included previously in *Arthrosolen* because they lack floral glands. However, these species share the following characters with African species formerly classified as *Lasiosiphon*: flowers grouped in heads with an involucre of leafy bracts, hairy floral tubes, capitate stigmas and fleshy pedicels. We argue that the absence of floral glands should not exclude *G. calocephala* and *G. sericocephala* from *Lasiosiphon* in the same way that *Struthiola anomala* Hilliard is not excluded from *Struthiola* despite its lack of floral scales (Hilliard, 1993).

The inclusion of tetramerous taxa and species with ebracteate few-flowered inflorescences expands on Fresenius' (1838) original idea of *Lasiosiphon* as a solely pentamerous group with flowers always in heads with bracts. The diversity of morphological features represented among members of clade 4 may make it necessary to recognize subgeneric groups within *Lasiosiphon* in future.

TAXONOMIC CHANGES

We reinstate the genus *Lasiosiphon* as follows: *Lasiosiphon* Fresen. emend. A.J.Beaumont, *Flora* 21: 603 (1838). – Type: *Lasiosiphon glaucus* Fresen., Ethiopia: *Rüppel s.n.* (FR, holotype!). Synonyms, *Gnidia* L. *pro parte*; *Arthrosolen* C.A.Mey. *pro parte*.

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APPENDIX 1

List of taxa with voucher information and GenBank accession numbers for each DNA region.

Species, voucher specimen, herbarium acronym, *trnL-F* GenBank accession, *rbcL* GenBank accession, ITS GenBank accession.

Neuradaceae:

***Grielum humifusum* Thunb.**, *Chase 5711*, (K), —, AJ402955†, —.

Sphaerosepalaceae:

Dialyceras coriaceum (Capuron) J.-F.Leroy, *Schatz & al. 3848*, MO, —, AJ29723†, —. ***Rhopalocarpus* sp.**, *Chase 906*, K, AJ30864†, Y15148*, —.

Thymelaeaceae:

Aquilaria beccariana* Tiegh.**, *Chase 1380*, K, AJ308643†, Y15149*, —. ***Arnhemia cryptantha Airy Shaw, *Lazarides 7870*, K, AJ308678†, AJ297236†, —. ***Craterosiphon scandens*** Engl. & Gilg, *Lock 84/84*, K, —, AJ297235†, —. ***Dais cotinifolia*** L., *Chase 1381*, K, AJ308644†, AJ297234†, AJ744928. ***Daphne mezereum*** L., *Chase 6357*, K, AJ308645†, AJ297233†, AJ744931. ***Deltaria brachyblastophora*** Steenis, *McPherson 4965*, K, AM404304, AM398174, —. ***Diarthron vesiculosum*** C.A.Mey, *Merton 3960*, K, AJ308646†, AM39818, —. ***Dicranolepis disticha*** Planch., *Gereau et al. 5626*, MO, AM40435, AM39818, —. ***Dirca palustris*** L., *Horn 12584*, NBYC, AJ308647†, U26322*, AM159528. ***Drapetes muscosus*** Banks ex Sol., *Kubitzki & Feuerer 99—34*, HBG, AJ308648†, AJ297237†, AM159529. ***Edgeworthia chrysantha*** Lindl., *Chase 6338*, K, AJ308649b, AJ297920b, AJ744932. ***Enkleia siamensis*** (Kurz) Nevling, *Von Beusekam 4060*, K, —, AJ297921b, —. ***Gnidia aberrans*** C.H.Wright, *Hilliard & Burtt 6898*, NU, AM404222, AM162523, AM159508. ***Gnidia*** cf. ***anomala*** Meisn., *Mark Johns s.n.*, Kogelberg Reserve Field Herbarium, AM400982, AM162539, AM158940. ***Gnidia bakeri*** Gilg, *Rogers et al. 126*, MO, —, AM162506, AM159510. ***Gnidia bojeriana*** (Decne.) Baill., *Rogers et al. 183*, MO, AM404224, AM162507, AM159511. ***Gnidia caffra*** (Meisn.) Gilg, *Burrows & Burrows 7754*, J, —, AM398170, AM396520. ***Gnidia calocephala*** (C.A.Mey.) Gilg, *Reid 885*, PRE, AM404225, —, AM396521. ***Gnidia caniflora*** Meisn., *Fourcade 5580*, PRE, AM404223, AM396993, —. ***Gnidia coriacea*** Meisn., *Mark Johns s.n.*, Kogelberg Reserve Field Herbarium, AM404227, AM162516, AM159512. ***Gnidia danguyana*** Leandri, *Rogers et al. 76*, MO, AM404226, AM162515, AM159513. ***Gnidia decaryana*** Leandri, *Rogers et al. 108*, MO, AJ745153, AJ745179, AJ744926. ***Gnidia denudata*** Lindl., *Beaumont s.n.*, NU, AJ308670†, AJ295266†, AM159514. ***Gnidia dumetorum*** Leandri, *Rogers et al. 109*, MO, AM404228, AM162514, AM159515. ***Gnidia fastigiata*** Rendle, *Hilliard & Burtt 6142*, NU, AJ308650†, AM162513, —. ***Gnidia galpinii*** C.H.Wright, *Mark Johns s.n.*, Kogelberg Reserve Field Herbarium, AM404230, AM396994, AM159516. ***Gnidia geminiflora*** E.Mey. ex Meisn., *Goldblatt 3799*, GB, AM404231, AM397275, —. ***Gnidia gilbertae*** Drake, *Randrianasolo 529*, MO, AJ745154, AJ745180, AJ744927. ***Gnidia glauca*** Gilg, *J. Adanson 6156*, K, AM404232, AM162511, —. ***Gnidia humilis*** Meisn., *Mark Johns s.n.*, Kogelberg Reserve Field Herbarium, AM404236, AM162510, AM159517. ***Gnidia kraussiana*** Meisn., *Beaumont s.n.*, NU, AJ308674†, AJ295267†, AM159518. ***Gnidia madagascariensis*** Baill., *Rogers et al. 133*, MO, AM404237, AM162509, AM159519. ***Gnidia penicillata*** —, —, —. ***Gnidia penicillata,*** —, —, —. ***Gnidia phaeotricha*** Gilg, *Balkwill 10316*, J, —, AM162517, AM159520. ***Gnidia pilosa*** Burtt Davy, *Beaumont s.n.*, NU, AJ308651†, AJ295264†, —. ***Gnidia pinifolia*** L., *I. Kruger 399*, NBG, AM404240, AM162518, AM159521. ***Gnidia racemosa*** Thunb., *Beaumont s.n.*, NU, AJ308665†, AJ295268†, AM159522. ***Gnidia renniana*** Hilliard & B.L.Burtt, *Beaumont s.n.*, NU, AM404233, AM162519, AM396522. ***Gnidia*** aff. ***renniana*** Hilliard & B.L.Burtt, *Edwards 1492*, NU, AJ308666†, AJ295265†, —.

APPENDIX 1 *Continued*

Gnidia scabrida Meisn., *Juli, Ecklon & Zeyher 53.7*, S, AM404238, AM397277, AM396987. ***Gnidia sericocephala*** (Meisn.) Gilg ex Engl., *Dehning & Dehning 108*, J, AM404241, AM408173, AM159523. ***Gnidia setosa*** Wickstr., *J. Hutchinson 519*, GRA, AM404296, AM162520, AM159524. ***Gnidia singularis*** Hilliard, *Manning 554*, NU, AM404297, AM162521, —. ***Gnidia squarrosa*** (L.) A.P.Druce, *Mark Johns s.n.*, Kogelberg Reserve Field Herbarium, AM404235, AM162522, AM159525. ***Gnidia subulata*** Lam., *Beaumont s.n.*, NU, AJ308652†, AM162508, AM159509. ***Gnidia wikstroemiana*** Meisn., *Beaumont & Smith SRFe9*, NU, AM404299, AM162524, AM159526. ***Gonystylus macrophyllus*** (Miq.) Airy Shaw, *Chase 1382*, K, AJ308653†, AJ308677†, Y15150*, —. ***Gnidia* aff. *viridis*** Berg., *Beaumont s.n.*, NU, AJ308652, AM162508, AM159509. ***Gyrinops walla*** Gaertn., *Chase 10511*, K, AM40430, AM39817, —. ***Lachnaea alpina*** (Eckl. & Zeyh.) Meisn., *Beyers 258*, NBG, AJ697829, AJ697771, AJ745754. ***Lachnaea aurea*** Eckl. & Zeyh., *Aggenbach s.n.*, NBG, AJ697828‡, AJ697781‡, AJ745737‡. ***Lachnaea axillaris*** Meisn., *Snijman 1871*, NBG, AJ308671†, AJ297219†, AJ745742‡. ***Lachnaea capitata*** (L.) Crantz., *Bean 2603*, NBG, AJ697811‡, AJ697798‡, AJ745744‡. ***Lachnaea densiflora*** Meisn., *Beyers 145*, NBG, AM404353‡, —, AJ745738‡. ***Lachnaea filamentosa*** Meisn., *Beyers 245*, NBG, AJ697833‡, AJ697801‡, AJ745755‡. ***Lachnaea filicaulis*** (Meisn.) Beyers, *Oliver 1108*, NBG, AJ308672‡, AJ297221‡, AJ745729‡. ***Lachnaea glomerata*** Fourc., *Beyers 192*; NBG, AJ697832‡, AJ697765‡, AJ745736‡. ***Lachnaea gracilis*** Meisn., *Beyers 254*, NBG, AJ697819‡, AJ697767‡, AJ745722‡. ***Lachnaea grandiflora*** (L.f.) Baill., *Handsford 7*, NBG, AJ697820‡, AJ697768‡, AJ745730‡. ***Lachnaea laniflora*** (C.H.Wright) Bond, *Oliver 10679*, NBG, AJ697831‡, AJ697802‡, AJ745739‡. ***Lachnaea laxa*** (C.H.Wright) Beyers, *Oliver & Oliver 11977*, NBG, AJ697821‡, AJ697769‡, AJ745733‡. ***Lachnaea macrantha*** Meisn., *Oliver 11017,* NBG, AJ697822, AJ697784/5, —. ***Lachnaea marlothii*** Schltr., *Oliver & Oliver 11304*, NBG, AJ697823‡, AJ697776‡, AJ745726‡. ***Lachnaea nervosa*** (Thunb.) Meisn., Hansford & Hansford 103, NBG, —, AJ697793/4, AJ745747. ***Lachnaea oliverorum*** Beyers, *Viviers & Vlok 430*, NBG, AJ697817‡, AJ697786‡, AJ745752‡. ***Lachnaea pedicellata*** Beyers, *Beyers 260*, NBG, AM404354‡, AJ697778‡, AJ745724‡. ***Lachnaea penicillata*** Meisn., *McDonald 1980*, NBG, AJ697826, AJ697791/2, —. ***Lachnaea pomposa*** (= ***Lachnaea buxifolia***) Beyers, *Oliver 10767*, NBG, AJ697835‡, AJ697796‡/AJ697797‡, AJ745753‡. ***Lachnaea pusilla*** Beyers, *de Villiers 45*, NBG, —, AJ697788, AJ745749. ***Lachnaea rupestris*** Beyers, *Oliver 11262*, NBG, AJ697807‡, AJ697779‡, AJ745731‡. ***Lethedon* aff. *salicifolia*** (Labill.) Aymonin, *McPherson & Munzinger 18055*, MO, AM404306‡, AM398175‡, —. ***Lethedon balansae*** (Baill.) Kosterm., *McPherson & Munzinger 610*, P/MO, AM404307‡, AM398176‡, —. ***Lethedon cernua*** (Baill.) Kosterm., *McPherson & Munzinger 18025*, MO, AM404305‡, AM398177‡, —. ***Octolepis dioica*** Capuron, *Rogers et al. 46,* MO, AM404350, AM398178, —. ***Octolepis dioica*** Capuron f. ***oblanceolata*** Capuron, *Rogers et al. 102*, MO, —, AM398179, —. ***Octolepis* sp.**, *Rogers et al. 165*, MO, AM404349, AM398180, —. ***Ovidia andina*** Meisn., *Kubitzki & Fewerer 99–42*, NBG, AJ308675, AJ297222, AM159530. ***Passerina burchellii*** Thoday, *Bredenkamp 1546*, PRE, AM404356, AM162526, AM158925. ***Passerina drakensbergensis*** Hilliard & B.L.Burtt, *Bredenkamp 1020*, PRE, AM404358, AM162528, —. ***Passerina ericoides*** L., *Bredenkamp 962*, PRE, AM404359, AM162529, AM158927. ***Passerina falcifolia*** C.H.Wright, *Bredenkamp 915*, PRE, AJ745150, AJ297224†, AJ744917. ***Passerina montivaga*** Bredenkamp & A.E.van Wyk, *P. van Wyk 2586*, PRE, AM404361, AM162531, AM158930. ***Passerina nivicola*** Bredenkamp, *Bredenkamp 1046*, PRE, AJ308655†, AJ297226†, AJ744916. ***Passerina obtusifolia*** Thoday, *Meyer 1505*, PRE, AM404367, AM162532, AM158931. ***Peddiea africana*** Harv., *Chase 6330*, K, AJ308662†, AJ297227†, AJ744921. ***Peddiea involucrata*** (Barker) Baill., *Rogers & al. 121*, MO, AJ745151, AJ745176, AJ744920. ***Phaleria capitata*** Jack, *Chase 1383*, K, AJ308661†, AJ297228†, —. ***Pimelea argentea*** R.Br., *M. Hislop & M. Griffiths WW 111.39*, PERTH, AM406675, AM167530, AM162490. ***Pimelea clavata*** Labill., *R. J. Cranfield 19510*, PERTH, AM407408, AM167532, AM162492. ***Pimelea decora*** Domin, *Purdie R.W. 5905,* CANB, FJ572694, FJ572826, FJ572732. ***Pimelea forrestiana*** F.Muell., *K. Coate 695*, PERTH, AM407407, AM167533, AM162493. ***Pimelea gilgiana*** E.Pritz, *I. B. Shepherd 269*, PERTH, AM406678, AM167534, —. ***Pimelea graniticola*** Rye, *B. Archer 1664*, PERTH, AM406679, —, —. ***Pimelea haematostachya*** F. Muell., *Lepschi BJ 1202*, CANB, FJ572695, FJ572827, FJ572733. ***Pimelea holroydii*** F.Muell., *S. van Leeuwen 3769*, PERTH, AM406687, AM167539, AM162496. ***Pimelea pygmaea*** F.Muell., *Chase 6360*, K, AJ308669†, AJ297230†, AJ744922. ***Pimelea spiculigera*** var. ***thesioides*** (S.Moore) Rye, *R. Davis 10390*, PERTH, —, AM398183, AM162499. ***Pimelea spiculigera*** var. ***thesioides*** (S.Moore) Rye, *J. Docherty 130*, PERTH, AM406681, —, AM162500. ***Pimelea trichostachya*** Lindl., *K. F. Kenneally 12623 & D. J. Edinger 3822*, PERTH, AM406682, AM167537, AM162501. ***Solmsia calophylla*** Baill., *Guillaumin s.n.*, K, AJ308656†, AJ295261†, —. ***Stellera chamaejasme*** L., *Chase 5530*, K, AJ308657†, AJ295262†, —. ***Stephanodaphne capitata*** (Leandri) Leandri, *Rogers et al. 139*, MO, AM407411, AM398184, AM159531. ***Stephanodaphne cremostachya*** Baill., *Tolaria 13.01.1990*, K, AJ308658, AJ295263, AM159532. ***Stephanodaphne cuspidata*** (Leandri) Leandri, *Rogers et al. 68*, MO, AM406683, AM398185, AM159533. ***Stephanodaphne oblongifolia*** Leandri, *Rogers et al. 127*, MO, AJ745152, AJ745177, AJ744924. ***Struthiola ciliata*** (L.) Lam., *Mark Johns s.n.*, Kogelberg Reserve Field Herbarium, AM404300, AM397279, AM396986. ***Struthiola dodecandra*** (L.) A.P.Druce, *Mark Johns 004*, Kogelberg Reserve Field Herbarium, AM404298, AM398171, AM396988/AM396989. ***Struthiola leptantha*** Bolus, *Beyers 265*, NBG, AJ308639†, AJ297243†, AJ745757. ***Struthiola salteri*** Levyns, *Mark Johns s.n.*, Kogelberg Reserve Field Herbarium, AM404301, AM397280, —. ***Struthiola striata*** Lam., *Mark Johns 005*, Kogelberg Reserve Field Herbarium, AM404302, AM398172, AM396990, AM396991. ***Struthiola tomentosa*** Andrews, *Mark Johns s.n.*, Kogelberg Reserve Field Herbarium, —, AM162540, AM158946. ***Synandrodaphne paradoxa*** Gilg, *Lisowski 46609*, K, AJ308676†, AJ297240†, —. ***Synaptolepis alternifolia*** Oliver, *Vollesen 4043*, K, AJ308663†, AJ297239†, —. ***Thecanthes punicea*** (R.Br.) Wickstr., *T. Handasyde TH99 488*, PERTH, AM406684, AM167540, AM162502. ***Thecanthes sanguinea*** (F.Muell.) Rye, *A. A. Mitchell 3945*, PERTH, AM406685, —, AM162503. ***Thymelaea hirsuta*** Endl., *Chase 1883*, K, AJ308640†, Y152151*, AJ744930. ***Wikstroemia canescens*** Meisn., E 82170, AM406686, AM398186, AJ549496. ***Wikstroemia gemmata*** (E.Pritz.) Domke, *Chase 3955*, K, AJ308641†, AJ295269†/AJ297223†, AJ744929.

*Fay *et al.* (1998).
†Van der Bank *et al.* (2002).
‡Van der Bank *et al.* (unpubl. data).

CHAPTER 6

THE FIRST RECORD OF GYNODIOECY IN A SPECIES OF *GNIDIA* (THYMELAEACEAE) FROM SOUTH AFRICA

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The first record of gynodioecy in a species of *Gnidia* (Thymelaeaceae) from South Africa

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Sexual polymorphism was studied in the shrub *Gnidia wikstroemiana* (Thunb.) Meisn. from the semiarid Nama Karoo Biome, South Africa. The populations comprised plants bearing either female flowers, or hermaphrodite flowers with variable female function. In two populations, female plants accounted for 36–37% of the flowering plants. Female flowers were smaller and their stamens were reduced to staminodes, but their styles were significantly longer than those of hermaphrodite flowers. Energy investment in flowers and fruits for females and hermaphrodites was measured using bomb calorimetry. Females produce a greater number of less costly flowers than hermaphrodites, and invest less energy per unit in production of flowers and inflorescences. In contrast, females invest more energy per unit in production of fruits and infructescences than hermaphrodites. Females overall invest 7.3% more energy in reproduction than hermaphrodites. Female flowers were obligate out-crossers (xenogamous), with 35% of nonmanipulated, open-pollinated flowers setting fruit, comparable with fruit set among selfed hermaphrodite flowers. The breeding strategy of *G. wikstroemiana* most closely resembles gynodioecy. This is the first report of sexual dimorphism in *Gnidia* L. and sub-Saharan Thymelaeaceae.

ADDITIONAL KEYWORDS: breeding systems – energy investment – floral morphometrics – flower micromorphology – fruit set – *Gnidia wikstroemiana* – Nama Karoo – semiarid vegetation – sex ratios – sexual dimorphism.

INTRODUCTION

Gender variation in the Thymelaeaceae

Domke (1934) recognized four subfamilies in the Thymelaeaceae, distinguished largely by characters of the fruits and seeds: Gonystyloideae, Aquilarioideae, Gilgiodaphnoideae and Thymelaeoideae. The smallest of these subfamilies, the Gilgiodaphnoideae, is now more correctly known as the Synandrodaphnoideae (Robyns, 1975; Mabberley, 1997). More recently, molecular evidence (Van der Bank, Fay & Chase, 2002) has upheld these groups. Most genera including *Gnidia* L. belong to the largest subfamily, the Thymelaeoideae (Table 1).

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Hermaphrodite flowers (functionally male and female) are ubiquitous in the Aquilarioideae and Synandrodaphnoideae. Within the Gonystyloideae unisexual flowers are found only in some species of *Lethedon* Sprengel (Rye, 1990). In contrast to the three smaller subfamilies, unisexual flowers are well represented in the Thymelaeoideae, being present in 34% of genera (Table 1). Unisexual flowers are found in both large and small genera, and among many of the larger genera all or most species are sexually dimorphic. For example, exclusively dioecious genera (species comprising separate male and female plants) are limited to Central and South America and the Caribbean, and include the large genus *Daphnopsis* Mart. & Zucc., together with the smaller genera *Funifera* Leandro ex C.A.Mey. and *Goodallia* Benth. (Table 1). *Thymelaea* Miller is a group primarily of the Mediterranean, and

Table 1. Geographical distributions and occurrence (+) of unisexual (U) and hermaphrodite (H) flowers among genera of the subfamily Thymelaeoideae

Genus by geographical region	Flower gender		References
	U	H	
Americas			
Daphnopsis Mart. & Zucc.	+		Nevling (1959); Barringer & Nevling (1994); Nevling & Barringer (1986, 1988, 1993)
Dirca L.		+	Nevling (1959)
Funifera Leandro ex C.A.Mey	+		Nevling (1959)
Goodallia Benth.	+		Nevling (1959)
Lagetta Juss.		+	Nevling (1959)
Lasiadenia Benth.		+	Nevling (1959)
Linodendron Griseb.		+	Nevling (1959)
Lophostoma Meisn.		+	Nevling (1959, 1963)
Ovidia Meisn.	+	+	Nevling (1959)
Schoenobiblus Mart. & Zucc.	+	+	Nevling (1959), Hutchinson (1967)
Asia			
Daphnimorpha T.Nakai		+	Mabberley (1997)
Diarthron Turcz.		+	Tan (1982)
Enkleia Griff.		+	Hou (1960)
Pentathymelaea Lecomte		+	Lecomte (1916); Mabberley (1997)
Stellera L.		+	Tan (1982)
Asia and Australasia			
Linostoma Wall.		+	Hou (1960)
Wikstroemia Endl.	+	+	Hou (1960); Mayer (1990)
Phaleria Jack.		+	Hou (1960); Rye (1990)
Asia, Australasia and South America			
Drapetes Banks ex Lam.		+	Hou (1960); Heads (1990a)
Australasia			
Jedda J.Clarkson		+	Clarkson (1986); Rye (1990)
Kelleria Endl.	+	+	Heads (1990a, b)
Oreodendron C.White		+	Rye (1990)
Pimelea Banks & Sol. ex Gaertner	+	+	Burrows (1960); Rye (1988, 1990, 1999); Threlfall (1982)
Eurasia			
Daphne L.	+	+	Tan (1980), Kikuzawa (1989)
Europe and North Africa			
Thymelaea Miller	+	+	Tan (1980); Dommée *et al*. (1990, 1995); El-Keblawy, Lovett-Doust & Lovett-Doust (1996)
Madagascar			
Atemnosiphon Leandri		+	Leandri (1950)
Stephanodaphne Baill.		+	Leandri (1950)
Madagascar and sub-Saharan Africa			
Dais L.		+	Leandri (1950); Peterson (1978)
Peddiea Harv.		+	Leandri (1950); Peterson (1978)
Synaptolepis Oliv.		+	Leandri (1950); Peterson (1978)
Sub-Saharan Africa			
Craterosiphon Engl. & Gilg		+	Peterson (1978)
Dicranolepis Planch.		+	Peterson (1978)
Lachnaea L.		+	Beyers & Van der Walt (1995)
Passerina L.		+	Bredenkamp & Van Wyk (2003)
Struthiola L.		+	Peterson (1978)
Sub-Saharan Africa, Madagascar, Middle East and Asia			
Gnidia L.		+	Leandri (1950); Phillips (1951); Peterson (1959, 1978)

includes only one hermaphrodite species: the other 29 are sexually dimorphic (Tan, 1980). The large Australasian genus *Pimelea* Banks & Sol. ex Gaertner (*c*. 108 spp.) has hermaphroditic, dioecious and gynodioecious taxa (female and hermaphrodite flowers on separate plants) (Rye, 1988, 1990, 1999). Burrows (1960) reported temporal variation in sex for *Pimelea traversii* Hook. *f.* All Hawaiian species of *Wikstroemia* Endl. are functionally dioecious, and non-Hawaiian taxa are largely hermaphroditic (Mayer, 1990). Thus, outside sub-Saharan Africa and Madagascar, unisexual flowers are well represented in the Thymelaeaceae, most commonly as dioecious and gynodioecious breeding systems in the Thymelaeoideae.

In contrast to the extensive occurrence of unisexual flowers among the Thymelaeaceae elsewhere in the world, within sub-Saharan Africa and Madagascar hermaphrodite flowers are highly conserved. This is surprising given that the Thymelaeoideae is well represented in tropical, central and southern Africa and Madagascar; altogether these regions are home to 11 genera (Table 1). Southern Africa in particular represents an important centre of radiation for the Thymelaeoideae. *Gnidia* (*c*. 140 spp.) occurs mostly in tropical and southern Africa with the Cape Province especially rich in species (Beaumont, 2000), and about 20 species are endemic to Madagascar (Peterson, 1978). *Struthiola* L. (*c*. 40 spp.) inhabits the Western Cape Province of South Africa (Beyers, 2000). Ten of the 20 species of *Passerina* are endemic, and four others are near endemic to the Cape Floristic Region (CFR) (Bredenkamp & Van Wyk, 2003) and the 40 species of *Lachnaea* L. are restricted to the CFR (Beyers, 2001). It is remarkable, therefore, that sub-Saharan Africa and Madagascar have, until now, yielded no sexually dimorphic representatives, considering the high species and generic diversity of the Thymelaeoideae in these regions, and the abundance of unisexual taxa elsewhere in the world.

Gnidia wikstrœmiana Meisn. [synonym *Gnidia stricta* (Thunb.) Wikstr.] is an uncommon, small, woody shrub from the semiarid Nama Karoo Biome, South Africa. Densely leafy, short side-branches support terminal clusters of two to eight flowers. Flowers are almost sessile, small and tubular, each with a narrow medial constriction and four terminal lobes. The last treatment of this species (Wright, 1915), based on the original description by Meisner (1840), reported eight perfect and oblong anthers and a style 1.25 lines (almost 2.8 mm) long in flowers. Furthermore, Wright (1915) recorded all species of *Gnidia* (including *G. wikstroemiana*) as having exclusively hermaphroditic flowers. As with other species in the group, the ovary contains a single ovule and the fruit is a dry achene that ripens inside the shrivelled remains of the lower part of the tube.

As part of a revision of the genus *Gnidia*, flowers of *G. wikstroemiana* were dissected and distinct sexual morphs (females and hermaphrodites) were found on separate specimens (see Specimens examined). Flowers from *Zietsman 170* (PRE) appeared to be uniformly functionally female because they were smaller than hermaphroditic flowers, with comparatively larger gynoecia (larger ovaries and styles about as long as the floral tubes), and their stamens reduced to staminodes that lacked pollen. In contrast, hermaphrodite flowers of other specimens had longer floral tubes and eight well-developed stamens containing pollen, but also showed variable development of gynoecia. These preliminary observations led us to postulate that *G. wikstroemiana* is gynodioecious.

Gynodioecious populations comprise plants with hermaphrodite flowers, which contribute genes through pollen and ovules to the next generation, and plants with female (male-sterile) flowers, which contribute genes only via their ovules (Ågren & Willson, 1991). Lloyd (1976) recognized constant females, which are functionally unisexual and inconstant males, or bisexuals (i.e. hermaphrodites), in which female function is variable among flowers for gynodioecious species. Gynodioecy therefore is represented by a continuum of relative maleness and femaleness in flowers, derived from the relative proportions of male genes transmitted by pollen and ovules. As such, gynodioecy merges into hermaphroditism at one extreme (in which genes for male-sterility are scarcely established in a population) and strict dioecy (constant females, and constant males with no female function) at the opposite extreme (Lloyd, 1976). Lloyd (1976) presented 12 species from different angiosperm families to illustrate the variable proportions of female flowers (0.15–0.51) in populations among gynodioecious taxa. For four *Pimelea* species, the proportion of female plants was 0.37–0.50. Among sexually polymorphic populations, the allele for female sterility will reach equilibrium within gynodioecious populations. The extent of female sterility within such populations depends on the relative female contributions from hermaphrodites and females, the amount of selfing among hermaphrodites and the extent of inbreeding depression from selfing (Silvertown & Charlesworth, 2001).

Bawa (1980) cited the occurrence of hermaphroditic, gynodioecious and dioecious species in genera (including *Pimelea*; Rye, 1988, 1990) as evidence to support the widely held view (for example, Charlesworth & Charlesworth, 1978; Silvertown & Charlesworth, 2001) that gynodieocy is an intermediate state in the transition from the hermaphrodite condition to dioecy.

Gynodioecy arises when genes for male sterility (femaleness) become established in a population.

Such mutations can occur in mitochondrial loci that are inherited maternally via ovules (called cytoplasmic male sterility or CMS factors), or in nuclear genes that are inherited paternally via pollen (Gouyon & Couvet, 1987; Silvertown & Charlesworth, 2001).

For unisexual morphs (i.e. genes for male or female sterility) to be maintained in populations, it is expected that one or more survival- or fecundity-related benefits are conferred on unisexual individuals that outweigh the disadvantages of loss of one or other sexual functions. Two hypotheses have been proposed to account for the maintenance of females in gynodioecious populations. Firstly, offspring borne on females have increased fitness or vigour derived from their guaranteed heterozygous (outcrossed) parentage, while the progeny of selfing among hermaphrodites could experience inbreeding depression arising from the accumulation of lethal or undesirable genes from their homozygous origins. Among gynodioecious taxa, sexual dimorphism is often but not always characterized by self-compatibility of hermaphrodites (Charlesworth & Charlesworth, 1978; Thomson & Barrett, 1981). Secondly, females, released from the burden of producing pollen, can instead divert resources to offspring development (see References in Marshall & Ganders, 2001). Marshall & Ganders (2001) furthermore suggested that ecological factors play an important role in the maintenance of breeding systems and demonstrated the first record of sex-biased predation of seeds in a gynodioecious species. Theoretical models predict that among gynodioecious species, female plants must incur some increased fecundity-related fitness that compensates for the loss of their male function. However, plenty of studies show that this is not always the case and other factors, such as inbreeding avoidance, over-dominance at loci of male-sterility (this last rejected by Charlesworth & Charlesworth, 1978), increased resource allocation to seeds borne on female plants while spatial structuring of populations along environmental gradients and population traits may also maintain females in populations (references in Alonso & Herrera, 2001).

Commonly in gynodioecious species, flowers on female plants are smaller than those on hermaphrodites for a number of floral traits. This floral size dimorphism can result either as a reduction in female flower size that allows reallocation to greater fruit and seed production, or as an increase in hermaphroditic flower size resulting from the increased importance of pollinator attraction and pollen export for hermaphroditic flowers (Miller & Venable, 2003).

This study investigated gender variation in *G. wikstroemiana* to answer the following questions: (1) How variable is gender dimorphism within and between plants and populations? (2) What are the sex ratios in populations? (3) What are the contributions of self- and cross-pollination to fruit set between putative sexual morphs? (4) Does reproductive resource allocation differ between putative sexual morphs?, and (5) What is the likely breeding system in *G. wikstroemiana*?

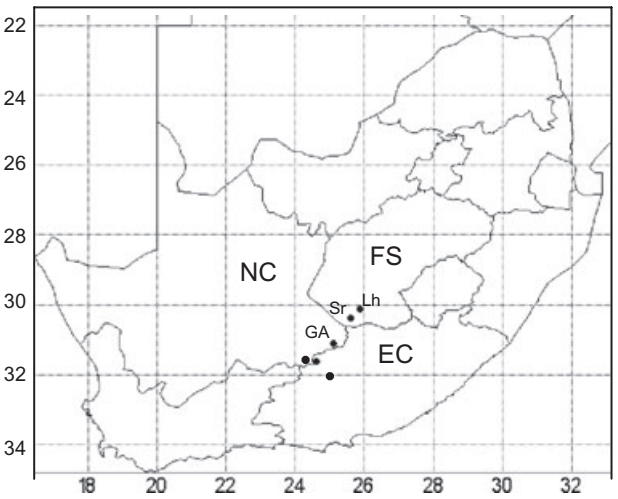


Figure 1. Distribution of populations (●) of *Gnidia wikstroemiana* in the Free State (FS), Northern Cape (NC) and Eastern Cape (EC) Provinces of South Africa, and locations of the Lockshoek (Lh), Smartryk (Sr) and Glen Alan (GA) study sites.

STUDY SITES

Three sites were surveyed (Fig. 1). The Lockshoek Farm population (30°3′S, 26°0′E) is 1500 m a.s.l. and occupies a rocky, flat-topped ridge with a mean annual precipitation (MAP) of 406 mm (Lillydale rainfall station). The Smartryk population (30°16′S, 25°56′E) is at 1600 m and occurs on a south-east slope with a MAP of 453 mm (Springfontein municipality rainfall station). Both sites lie within the False Upper Karoo vegetation type (Acocks, 1953), equivalent to Eastern Mixed Nama Karoo (Hoffman, 1996). female and hermaphrodite flowers for bomb calorimetry were sampled from the Glen Alan population (31°12′S, 25°4′E). This population occupies an area of South-eastern Mountain Grassland vegetation (Lubke, Bredenkamp & van Rooyen, 1996).

METHODS

VARIATION OF FLORAL CHARACTERS AMONG SEXUAL PHENOTYPES

We sampled three female plants and four hermaphrodite plants from Lockshoek, and seven female plants

and three hermaphrodite plants from Smartryk. For each plant, branches were randomly selected, pressed and dried. Flowers were removed and gently rehydrated before dehydration in an alcohol series from 70 to 100% ethanol and critical point-dried. Flowers were mounted on double-sided tape, attached to brass stubs and dissected lengthways. Flowers were then coated with gold-palladium and viewed using a FEI XL30 Environmental Scanning Electron Microscope at 10–15 kV. The following characters were measured: length of the lower part of the flower tube from the base of the flower to the point of constriction; length of the upper part of the flower tube from the point of constriction to the bases of the calyx lobes; circumference of the upper part of the tube; the lengths and widths of the outermost calyx lobes; the lengths and widths of the innermost calyx lobes; lengths of anthers of the upper row of stamens; lengths of anthers of the lower row of stamens; ovary length; style length (including stigma) and stigma width (Fig. 2). In total 5–12 female flowers and 6–18 hermaphrodite flowers were sampled from the Lockshoek population, and 6–15 female flowers and 5–6 hermaphrodite flowers from the Smartryk population. The petal-like glands in the sinuses of calyx lobes, and the receptacles of the flower tubes were examined for nectariferous tissue. A discriminant function analysis was performed on the flower characters. All statistical analyses were performed using Statgraphics Plus 7.0 (SGPLUS, 1993). Logarithmic ($\log_{10}$) transformations of floral characters were made to improve normality of data and to stabilize variances.

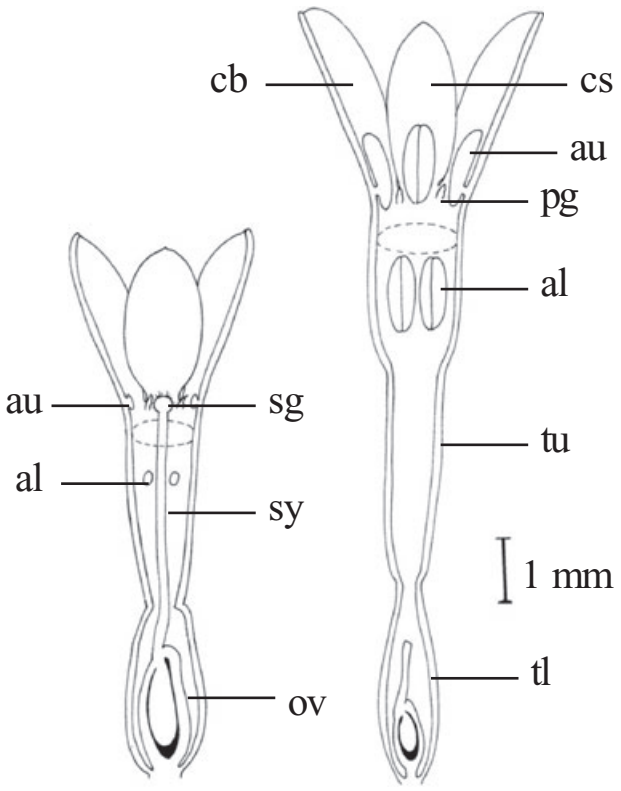


Figure 2. Half-flower illustrations of female (left) and hermaphrodite (right) flowers of *Gnidia wikstroemiana*. al, lower anther (nonfunctional staminode in female); au, upper anther (nonfunctional staminode in female); cb, big (outer) calyx lobe; cs, small (inner) calyx lobe; ov, ovary; pg, petal-like gland; sg, stigma; sy, style; tl, lower tube; tu, upper tube.

Proportions of female and hermaphrodite plants in populations

In order to assess sex ratios of the two populations, we classified all *G. wikstroemiana* individuals on Lockshoek and Smartryk as females or hermaphrodites during peak flowering time in September 2001.

Fruit set of female flowers

Four female plants were used to test for xenogamy under natural conditions. Lightweight net bags were secured over 97 open flowers, i.e. flowers potentially pollinated under natural conditions. Unopened buds were removed from these inflorescences before bagging. In order to test for seed set in the absence of pollen (agamospermy), exclusion bags were secured over 925 unopened, mature buds of 11 female plants. Open flowers were removed from these inflorescences before bagging.

Fruit set of hermaphrodite flowers

Eleven hermaphrodite plants were used to test for within-flower fertilization (autogamy). Exclusion bags were placed over 1504 unopened, intact buds, with open flowers and tiny buds removed before bagging. Five plants were used to test for fruit set among hermaphrodite flowers under natural conditions. Net bags were secured over 112 open flowers with unopened buds removed prior to bagging. This test did not discriminate between possible fruit set from selfing or outcrossing. In order to test for agamospermy, stamens were removed from 405 unopened, mature buds of 15 hermaphrodite plants and the buds covered with exclusion bags. Small buds and open flowers were removed from inflorescences before bagging.

For all treatments, plants were bagged in September 2001 and the bags collected in December 2001. The numbers of developed and undeveloped fruits were counted in each bag. Chi-square tests were used to determine differences in fruit set among the sexes and bagging treatments.

Sizes of inflorescences and infructescences

We estimated inflorescence size (numbers of flowers per inflorescence) of female and hermaphrodite plants using specimens collected for measurements of floral characters. We included 8–11 inflorescences from each plant and calculated the mean number of flowers per inflorescence per plant.

Unbagged shoots supporting infructescences were harvested from plants used in the bagging experiments. We sampled 50 infructescences from 11 female plants (9 from Lockshoek, 2 from Smartryk) and 109 infructescences from 20 hermaphrodite plants (16 from Lockshoek, 4 from Smartryk). Data from both sites were pooled for each gender type and differences in infructescence size between gender types were tested using Student's *t*-test for unequal sample sizes.

Energy investment in flowers and fruits

Twelve hundred hermaphrodite and 1800 female flowers were used to determine the sex-dependent net energy investment in flowers. Flowers were sampled from at least four plants of each morph. In order to generate sufficient mass for bomb calorimetry, flowers were pooled into samples of 100 hermaphrodite or 150 female flowers. Before combustion, material was air-dried at ambient temperature and massed using a Sartorius electronic balance. Twelve replicates of each sexual system were bombed using a DDS CP 500 Digital Oxygen Bomb Calorimeter.

In total, 415 fruits from 10 hermaphrodite plants and 189 fruits from 11 female plants were used to determine the net energy investment in fruits of different sexed plants. In order to generate sufficient mass for bomb calorimetry, fruits from hermaphrodite plants were pooled into three samples comprising 130, 131 and 154 fruits, and fruits from female plants were pooled into two samples of 91 and 98 fruits. Samples were ground to a fine powder in a mortar and pestle before bombing as described above for flower samples.

Energy investment in reproduction by females and hermaphrodites

We estimated investment in reproduction by females and hermaphrodites using data collected for inflorescence and infructescence sizes, flower and fruit masses, and energy costs of flowers and fruits. Energy invested in inflorescences for each sex was calculated from the product of inflorescence size, flower mass (mg) and energy cost (J mg^{-1}). Mean values of inflorescence size for each sex were obtained from data from both bagging sites. Similarly, energy investment in infructescences was calculated from the product of infructescence size, fruit mass (mg) and energy cost (J mg^{-1}).

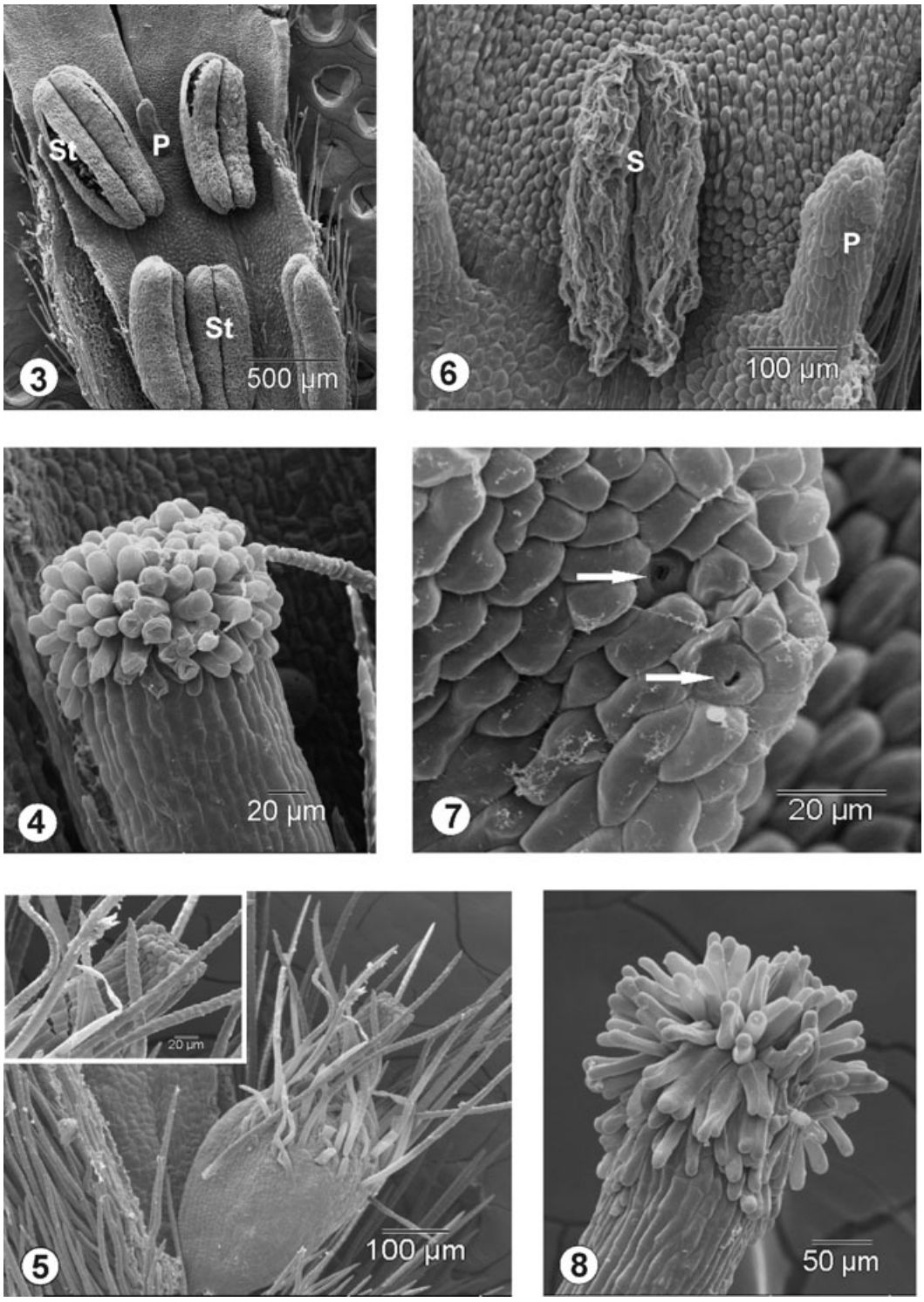
RESULTS

Micromorphology of flowers

Hermaphrodite flowers have 8 introrse, subsessile stamens in 2 rows, one row in the mouth and one in the throat (Fig. 3). Flowers are yellow with tiny petaloid glands. The nectary is small and annular around the base of the ovary but absent in flowers with pistillodes. Pistils vary in size and development and the stigmatic hairs may be dense, long and finger-like among larger gynoecia with longer styles, to absent in vestigial gynoecia with scarcely developed styles (Figs 4, 5). Female flowers are smaller than male flowers. In females, the stamens are reduced to eight nonfunctional staminodes (Fig. 6) and ovaries are comparatively larger, with longer styles and denser, longer stigmatic hairs (Fig. 8) than in hermaphrodite flowers. Petaloid scales are similar in both morphs, being finger-like or less often triangular-ovate in planar view (Figs 3, 6), and with terminal stomata in both hermaphrodite and female flowers (Fig. 7).

Variation of floral characters within and between female and hermaphrodite plants

The discriminant function analysis of 12 floral characters clearly separated our sample of plants into females and hermaphrodites (Fig. 9). The discriminant analysis yielded eight significant discriminant functions of which the first two were most important (Table 2). Converting eigenvalues into relative percentages allowed us to compare the total discriminating power of each function (Klecka, 1980). The first function in our results contained 93.01% of the total discriminating power in our system of 12 functions. The second function contained a further 3.23% of the total discriminating power in our system of equations. The canonical correlations of the first two discriminant functions were both very close to 1 (Table 2), indicating almost maximum association between the groups (plant sexes) and these discriminant functions (Klecka, 1980). The highest coefficient of 0.998 found for the first function shows that a very strong relationship exists between it and the discrimination of the plants into two sexes. Wilks' Lambda is a multivariate measure of plant differences over several discriminating flower variables. Our very low λ values for the first two derived functions (Table 2) denoted high discrimination between plants of different sex (Fig. 9). Furthermore, conversion of the Wilks' Lambda to an approximation of the Chi-square distribution as a test of significance also showed that floral characters between plants of different sex were significantly different before the derivation of any discriminant functions (Table 2). The significance level ($P < 0.0001$) indicated, therefore, that the 17 plants were highly



Figures 3–8. Micromorphological details of hermaphrodite (Figs 3–5) and female (Figs 6–8) flowers of *Gnidia wikstroemiana*. Fig. 3. Inner surface of upper flower tube with two of the four stamens of the upper row and three of the four stamens of the lower row of the androecium, the stamens of the upper row dehiscing before those of the lower row. Fig. 4. Stigma with dense, clavate to shortly finger-like papillae. Fig. 5. Reduced gynoecium with style scarcely developed, stigma (inset) smaller than in female flower, stigmatic papillae absent. Fig. 6. Antisepalous nonfunctional staminode and petal-like gland. Fig. 7. Stomata (indicated by arrows) at tip of petal-like gland. Fig. 8. Stigma with dense, long finger-like papillae. p, petal-like gland; s, nonfunctional staminode; st, stamen.

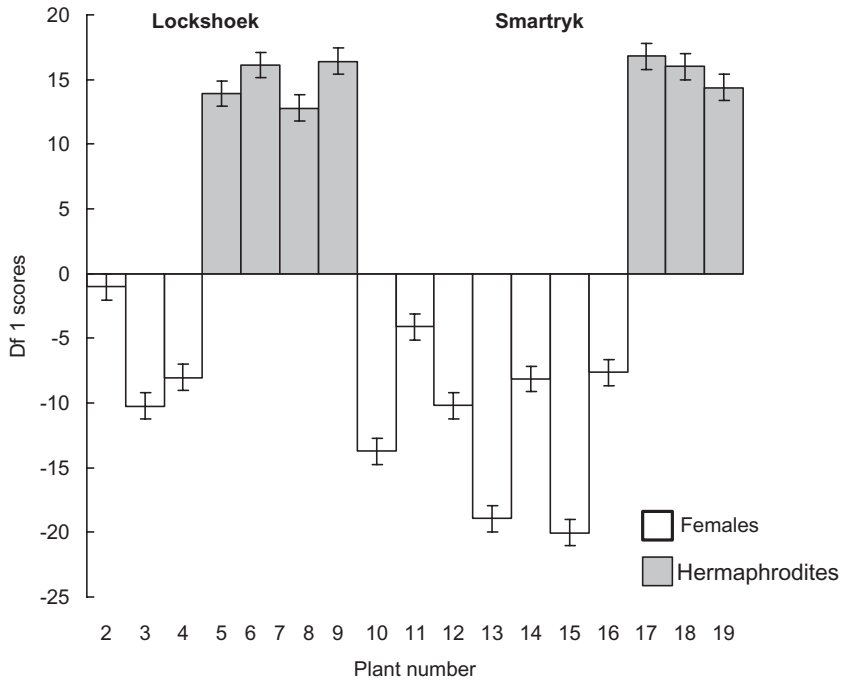


Figure 9. Group centroids and 95% confidence intervals of the first discriminant function scores (Df 1) of flower morphology determined for female and hermaphrodite plants of *Gnidia wikstroemiana* sampled from single populations on Lockshoek and Smartryk. Flower morphology is based on the dimensions of 12 flower characters with all data $\log_{10}$ transformed; 5–18 flowers were measured per plant.

Table 2. Discriminant function analysis of flower morphology of female and hermaphrodite plants of *Gnidia wikstroemiana* based on the dimensions of 12 flower characters. Only the results of the first two functions of the 12 discriminant functions are shown

Function	Eigenvalue	% variance	Canonical correlation
1	209.14	93.01	0.998
2	7.26	3.23	0.938

Functions derived	Wilks′ Lambda	χ^2	d.f.	*P*
0	< 0.0001	1502	192	< 0.0001
1	0.001	858	165	< 0.0001

variable with respect to the 12 floral characters. That is, variation of floral characters was high both among hermaphrodite plants and female plants, but character variation was strongest between the sexes (Fig. 9). The significant contribution of sex in the discrimination of plants into females and hermaphrodites was furthermore confirmed by the results of the two-way ANOVA of the first discriminant function scores of flower morphology between female plants ($N = 10$) and hermaphrodite plants ($N = 7$) sampled from single populations on Lockshoek ($N = 7$ plants) and Smartryk ($n = 10$ plants). Here, sex accounted for the maximum source of variation ($F = 107.05$, d.f. = 1, $P < 0.0001$) while sources of variation due to population and its interaction with sex were not significant.

Lengths of anthers of the upper row of stamens, followed by style length were the most important variables contributing to the determination of mean scores for plants on the first discriminant function. All floral parts except for characters of the gynoecia were significantly larger in hermaphrodites (Fig. 10, Table 3). Populations showed little divergence with respect to floral characters except for calyx lobe lengths (Table 3).

Proportions of female and hermaphrodite plants in populations

Female plants accounted for 0.35 and 0.37 of the total number of flowering plants in the Smartryk and Lockshoek populations, respectively.

Fruit set

There was no difference in natural fruit set between female and hermaphrodite flowers. Outcrossed

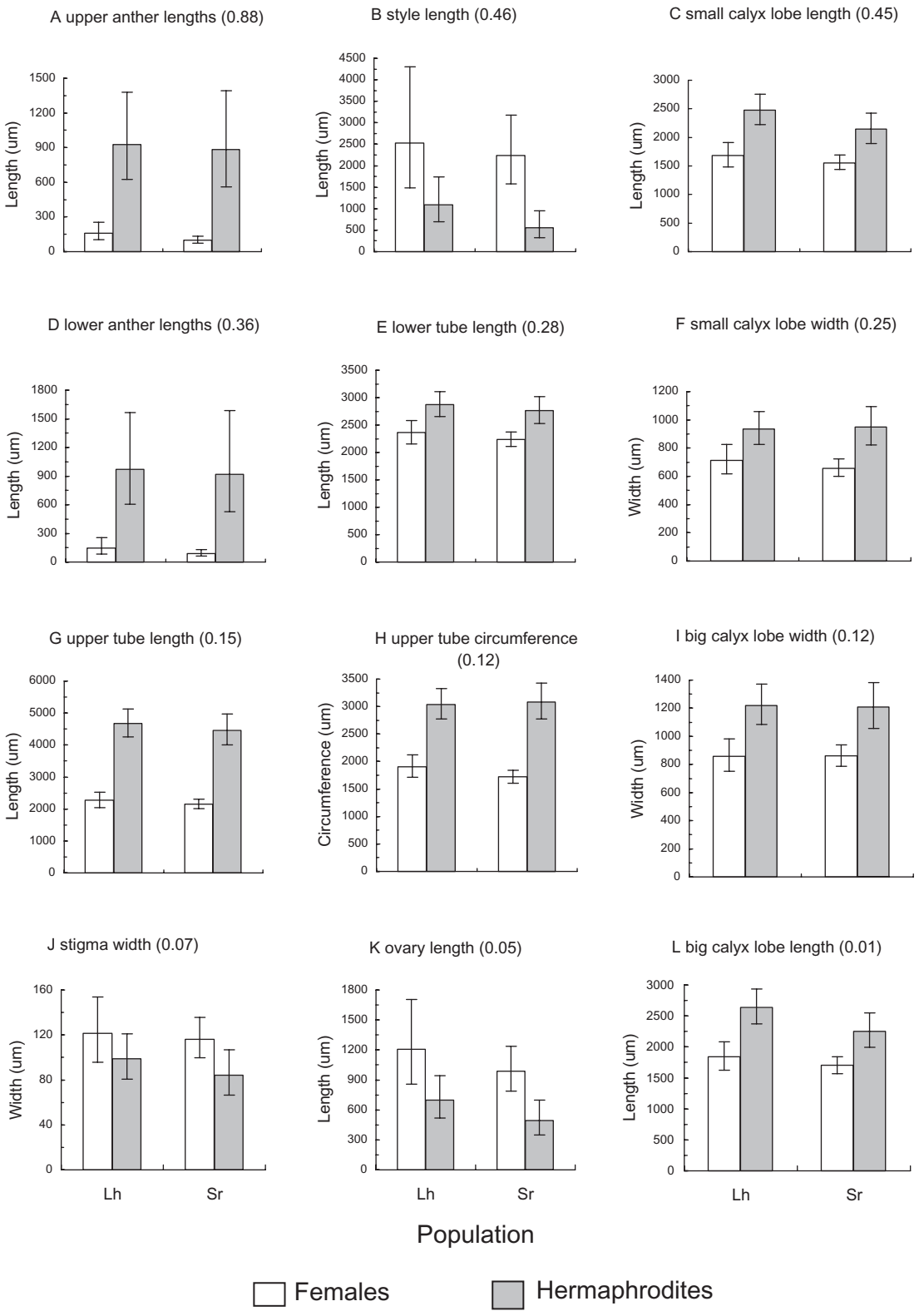


Figure 10. Detransformed $\log_{10}$ least-square means and 95% confidence intervals of flower character dimensions of female ($N = 3$–7) and hermaphrodite ($N = 3$–4) plants of *Gnidia wikstroemiana* sampled from single populations on Lockshoek (Lh) and Smartryk (Sr). Characters are arranged from most important (A) to least important (L) according to their contributions (standardized coefficients) to the first discriminant function scores.

Table 3. Significant effects of sex and population on flower-character dimensions ($\log_{10}$ transformed data) in two-way analyses of female ($N = 10$) and hermaphrodite ($N = 7$) plants of *Gnidia wikstroemiana* sampled from single populations on Lockshoek ($N = 7$ plants) and Smartryk ($N = 10$ plants). Interactions between sex and population were nonsignificant for all characters ($1 > P > 0.07$). Order of characters follows that in Figure 4

Character	Main effect	d.f.	MS	F	*P*
Upper anther length	Sex	1	2.77	109.9	< 0.0001
Style length	Sex	1	0.879	25.6	< 0.001
Small calyx lobe length	Sex	1	0.089	47.4	< 0.0001
	Population	1	0.009	4.7	< 0.05
Lower anther length	Sex	1	3.166	86.3	< 0.0001
Lower tube length	Sex	1	0.029	29.7	< 0.001
Small calyx lobe width	Sex	1	0.072	29.0	< 0.001
Upper tube length	Sex	1	0.376	267.5	< 0.0001
Upper tube circumference	Sex	1	0.196	144.4	< 0.0001
Big calyx lobe width	Sex	1	0.085	38.5	< 0.0001
Stigma width	Sex	1	0.050	7.3	< 0.05
Ovary length	Sex	1	0.274	19.2	< 0.001
Big calyx lobe length	Sex	1	0.073	39.9	< 0.0001
	Population	1	0.010	5.5	< 0.05

Table 4. Differences in fruit set (%) among sexes and bagging treatments ($\chi^2 = 236.16$, d.f. = 4, $P < 0.00001$) of *Gnidia wikstroemiana* plants based on combined data from Lockshoek and Smartryk. Fruit set values with different superscripts are significantly different at $P < 0.001$ for pair-wise comparisons of bagging treatments between and within sexes

Sex	Biological process	Bagging treatment	No. of			Fruit set (%)
			Plants	Developed fruits	Undeveloped fruits	
Female	Xenogamy	Open, pollinated flowers	4	34	63	35.1[a]
	Agamospermy	Unopened buds	11	31	894	3.4[c]
Hermaphrodite	Xenogamy	Open, pollinated flowers	5	34	78	30.4[a]
	Selfing	Unopened buds	11	395	1109	26.3[a]
	Agamospermy	Unopened buds, emasculated	15	59	346	14.6[b]

females set significantly more fruit than bagged, non-pollinated females ($\chi^2 = 142.86$, d.f. = 1, $P < 0.0001$). Among hermaphrodite flowers fruit set was similar for open-pollinated and selfing treatments, but was significantly lower in the treatment to test for agamospermy compared to the open-pollinated ($\chi^2 = 13.78$, d.f. = 1, $P < 0.001$) and selfing ($\chi^2 = 23.44$, d.f. = 1, $P < 0.0001$) treatments (Table 4).

Sizes of inflorescences and infructescences

Female inflorescences contained more flowers than those of hermaphrodite plants in both populations (Fig. 11; $F = 5.89$, d.f. = 1, $P < 0.05$) and flower number per inflorescence was significantly higher in the Lockshoek population than in the Smartryk population ($F = 5.98$, d.f. = 1, $P < 0.05$). However, no significant interaction was found between sex and population. The mean values of inflorescence size for each sex using data from both bagging sites were 3.9 for females and 3.1 for hermaphrodites. In contrast, we found no significant difference in infructescence size between gender types and therefore estimated an overall mean of 2.5 fruits per infructescence for both females and hermaphrodites.

Energy investment in reproduction by females and hermaphrodites

Hermaphrodite flowers were on average almost twice the mass of females and showed significantly higher levels of energy investment. Fruits from females were significantly heavier than those of hermaphrodites but the difference in energy investment between gen-

Table 5. Means (±SE) of mass (mg) and energy cost (J mg^{-1}) for female (F) and hermaphrodite (H) flowers and fruits of *Gnidia wikstroemiana* and results of *t*-tests for differences between means; NS, not significant

	Sex				
Variable	F	H	*t*	d.f.	P^1
Flower mass (mg)	0.79 ± 0.01	1.14 ± 0.01	38.94	17	< 0.0001
Flower energy cost (J mg^{-1})	16.38 ± 0.12	16.92 ± 0.13	3.12	22	< 0.01
Fruit mass (mg)	2.98 ± 0.09	2.53 ± 0.03	4.58	39	< 0.0001
Fruit energy cost (J mg^{-1})	21.05 ± 0.22	20.66 ± 0.11	1.59	2	NS

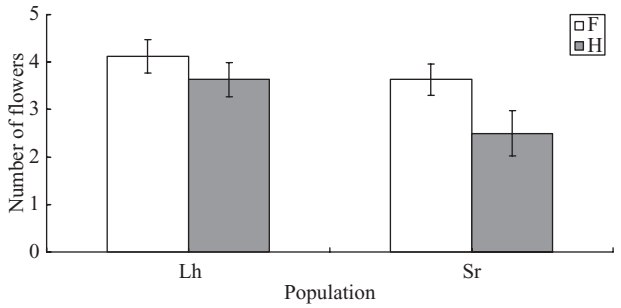


Figure 11. Means and 95% confidence intervals of numbers of flowers per inflorescence on female (F) plants ($N = 11$) and hermaphrodite (H) plants ($N = 8$) of *Gnidia wikstroemiana* sampled from single populations on Lockshoek (Lh) ($N = 10$ plants) and Smartryk (Sr) ($N = 9$ plants); 8–11 inflorescences were sampled per plant.

der types was not significant (Table 5). Energy investment in inflorescences was estimated as 50.5 J per inflorescence for females and 60 J per inflorescence for hermaphrodites. Energy investment in infructescences was estimated as 155.4 J per infructescence for females and 132 J per infructescence for hermaphrodites. Therefore, total energy invested in reproduction per inflorescence and infructescence combined is estimated as 206 J for females and 192 J for hermaphrodites.

DISCUSSION

Evidence for gynodioecy in *G. wikstroemiana*

Gnidia wikstroemiana comprises female plants and plants bearing hermaphrodite flowers with variable female function. Female flowers are smaller, their stamens are reduced to staminodes and they have larger gynoecia than hermaphrodites. Hermaphrodite flowers have longer floral tubes, eight polleniferous stamens, and gynoecia that either resemble those of female flowers or show a range of continuous reduction. At their most extreme, some outwardly hermaphroditic flowers are functionally male because their gynoecia are completely reduced. Female plants of *G. wikstroemiana* account for one third of the total number of plants in each of two populations. These values lie within the sex ratios expected under gynodioecy (Lloyd, 1976; Silvertown & Charlesworth, 2001). Furthermore *G. wikstroemiana* represents a unique deviation from the otherwise wholly hermaphroditic condition in *Gnidia* and other members of sub-Saharan Thymelaeaceae. It remains unclear why sexual dimorphism is so rare in sub-Saharan Africa, but so common in apparently similar habitats in other parts of the world.

Reproductive resource allocation

Females invest more energy in reproduction than hermaphrodites but allocation of resources to flowers and fruits differs between sexes. Females produce a greater number of less costly flowers per inflorescence than hermaphrodites. However, although females produce the same number of fruits per infructescence as hermaphrodites, each female fruit is slightly more costly to produce than each fruit developing from a hermaphrodite flower. Females invest an estimated 16% less energy per inflorescence but 18% more energy per infructescence than hermaphrodites. Therefore, females invest an estimated 7.3% more energy overall in the combined production of an inflorescence and an infructescence than hermaphrodites. Our results are consistent with the findings in other gynodioecious species in which females, freed from having to expend energy in pollen production, instead increase energy investment in fruit production (Silvertown & Charlesworth, 2001).

Female flowers are smaller and less colourful in *G. wikstroemiana* and this agrees with the trend for diminutive and less showy female flowers elsewhere in the family (Nevling, 1959; Burrows, 1960; Kikuzawa, 1989; Dommée *et al.*, 1990; Mayer, 1990; Rye, 1990; Barringer & Nevling, 1994). Female flowers must receive pollen from hermaphrodites to set seed and therefore must attract pollinators. Female inflorescences each have an estimated one flower more than hermaphrodites, and the massing of the smaller

and duller female flowers into slightly more floriferous heads may increase their attractiveness to insect pollinators.

Fruit set

Percentage fruit set is similar between outcrossed females and selfed hermaphrodites, with approximately two-thirds of flowers failing to develop in both morphs. The small proportion of females setting seed in our test for agamospermy likely resulted from the erroneous inclusion of closed, pollinated flowers in our bagging treatment. Similarly, the significantly lower percentage of fruit set recorded in our test for agamospermy among hermaphrodites was likely due to contamination of their stigmas by within-flower pollen during emasculations. There was no difference in fruit set between selfing and outcrossing treatments in hermaphrodite flowers, indicating that hermaphrodite flowers are self-pollinated. Fruit set resulting from self-pollination is strongly suspected in both treatments, because cross-pollination of hermaphrodite flowers of *G. wikstroemiana* is unlikely. This is because anthers dehisce before flowers open and the stigmas sit well below the stamens, often underneath the constriction points along floral tubes. Therefore, the stigmas are clogged with pollen falling from within. Pollen accumulation at these constriction points furthermore forms a considerable physical barrier to geitonogamy or xenogamy. Kikuzawa (1989) reported similar clogging of stigmas by within-flower pollen in hermaphrodite flowers of *Daphne kamtchatica* var. *jezoensis* Ohwi, but concluded that hermaphrodites were self-incompatible because nonemasculated, bagged flowers set no fruit. Flowers of *Daphne* species are, like those of *Gnidia*, uniovulate with eight stamens producing large amounts of pollen, and pollination and potential fruit set in such flowers requires only a single pollen grain. It is unlikely that hermaphrodite stigmas of both genera escape contamination by within-flower pollen. These results illustrate that reproduction and the genetic inheritance of offspring is different between two gynodioecious species. Furthermore, Kikuzawa (1989) also found that emasculation followed by hand-pollination with xenogamous pollen achieved the highest fruit set in a series of pollination treatments for hermaphrodites. He suggested that outcrossing may not be the primary pressure in maintaining females in *D. kamtchatica* var. *jezoensis*. In contrast, offspring from female plants of *G. wikstroemiana* are always outcrossed, and carry the inferred increased genetic fitness of their heterozygous parentage. Our results suggest that hermaphrodites are self-compatible in *G. wikstroemiana* and it is likely that most of their offspring are homozygous. Thus, outcrossing as a selective pressure may contribute to the maintenance of females in *G. wikstroemiana*. Inbreeding depression is not immediately apparent in hermaphrodites of *G. wikstroemiana*. If hermaphrodites are suffering inbreeding depression one might expect them to act as pure males in populations. Our biased sex ratios do not support this situation of cryptic dioecy in *G. wikstroemiana*. Cryptic dioecy is known in a number of angiosperm taxa (Mayer & Charlesworth, 1991). Mayer (1990) recognized two forms of functional dioecy in *Wikstroemia*: morphological dioecy and pseudohermaphroditism. In morphological dioecy, male flowers produce pollen but have reduced gynoecia, and female flowers have staminodes and functional gynoecia. Pseudohermaphrodite flowers appear outwardly hermaphroditic, but functional females produce nonviable pollen and the gynoecia of functional males lack ovules. Fruit set of hermaphrodites of *G. wikstroemiana* is comparable with that of open, pollinated hermaphrodite flowers and this suggests that hermaphrodites have dual male and female fertility.

Emasculation and cross-pollination of flowers is necessary to test for cross compatibility of hermaphrodites in *G. wikstroemiana*. Evidence, if any, of xenogamous fruit set among hermaphrodites of *G. wikstroemiana* may suggest that the ancestral breeding system of this species involved xenogamy among exclusively hermaphroditic flowers.

Ovaries of female flowers of *G. wikstroemiana* are larger than those of hermaphrodites, and this may help to explain why fruits from female plants are significantly heavier than those from hermaphrodite plants. Energy investment nevertheless is the same per fruit for females and hermaphrodites. Similarly in a species of *Daphne*, Alonso & Herrera (2001) found no differences in seed set and seed size between fruits from cross-pollinated female flowers and fruits from selfed hermaphrodite flowers. They did, however, find that seeds from female plants produced more seedlings than seeds of hermaphrodites. We need to investigate germination and seedling survivorship in *G. wikstroemiana* to better understand if the derivation of fruits, whether from female or hermaphrodite plants, confers any competitive advantages on offspring survivorship.

Sex ratios

Females account for one third of plants in two populations of *G. wikstroemiana*. Elsewhere in the family, sex morph ratios can vary greatly, both between dimorphic species and among different populations of the same species. For example, and in contrast to the condition in *G. wikstroemiana*, female plants number almost twice as many as hermaphrodites in populations of the gynodioecious shrub *D. kamtchatica* var.

jezoensis (Kikuzawa, 1989). Among gynodioecious species of *Pimelea* the female : hermaphrodite plant ratio varies from species to species, but hermaphrodites are usually more numerous (Burrows, 1960; Rye, 1988).

Environmental factors can skew the 1 : 1 ratio of females to males expected in dioecious species. Individuals among genetically diverse offspring will be variously suited to one or other suite of environmental factors. For example, female plants account for one fifth to half of the plants in populations of the gynodioecious species *D. laureola* L. (Alonso & Herrera, 2001). In contrast to other authors, such as Ashman (1999), Alonso & Herrera (2001) concluded that females with the advantages of increased vigour resulting from their heterozygous genotypes are better able to establish and survive in the harsher environments than can homozygous hermaphrodites. However, and in apparent contradiction to the findings of Alonso & Herrera (2001), Shaltout (1987) found that male plants of *Thymelaea hirsuta* L. were more vigorous in harsher habitats and concluded that female plants sacrifice some of their competitive ability by allocating more resources to reproductive effort. Our two populations of *G. wikstroemiana* inhabit the arid Eastern Mixed Nama Karoo Biome. Other populations of this species, for example that of Compassberg Farm (see Specimens examined), inhabit areas of vegetation corresponding to wetter South-eastern Mountain Grassland (Lubke *et al.*, 1996). Here grass and shrub growth is more vigorous than in the Nama Karoo (authors' observations). Future work might focus on measuring sex morph ratios in *G. wikstroemiana* within and between populations situated along gradients of wetter to drier conditions. Additional studies using genetic markers are needed to better understand the breeding system in *G. wikstroemiana*

Fruit set was comparable among open pollinated hermaphrodite flowers and unopened bagged hermaphrodite buds. These results show that hermaphrodite flowers are able to set seed via selfing with pollen falling onto stigmas below. As discussed above, the narrow floral tube, the positions of the stamens above the stigmas and the simultaneous maturation of the sexual whorls make cross-pollination unlikely but not impossible among hermaphrodite flowers. Therefore, we cannot conclude that hermaphrodite plants are fully selfed (autogamous). Further studies using genetic markers are needed to understand better what proportion, if any, of hermaphrodite ovules are selfed vs. outcrossed.

Self-pollination in hermaphrodites carries no risk of failure resulting from a lack of pollination vectors. By contrast, outcrossing of hermaphrodite and female ovules relies on insects for successful pollination. Insects are likely pollinators in *G. wikstroemiana* because flowers of this species show no obvious adaptations for wind pollination identified elsewhere in the family, specifically *Passerina* species (Bredenkamp & Van Wyk, 1996).

Hermaphrodites act as the sole pollen donors within the populations, so pollen is always outcrossed onto female morphs. This xenogamous seed set in the female morphs results in highly heterozygous offspring. For females to be maintained in populations they have to compensate for the fact that they can only transmit their genes through ovules, whereas hermaphrodites gain fitness through their pollen and ovules. It is unlikely that hermaphrodites suffer severe inbreeding depression through selfing. If they did, then we would expect more functionally male-only plants in our populations. Some smaller level of inbreeding depression occurs among hermaphrodites because the differential production, recruitment or mortality of different morphs will affect ratios of plant sex morphs. In years when pollinators are scarce, the amount of progeny from female flowers will be much lower and recruitment will consist of more offspring derived from hermaphrodites. Reproductive success in *G. wikstroemiana* is a balance between selfing in hermaphrodite plants, with possible concomitant inbreeding depression in homozygous offspring, and the heterozygous vigour of females whose reproductive success nevertheless depends on pollinator availability.

Investigations of reproductive resource allocation in relation to environmental factors, such as moisture, nutrient availability and access to pollinators, will help us to understand better the origin and maintenance of gynodioecy in *G. wikstroemiana*.

Specimens of *G. wikstroemiana* examined

Acocks 16572 (Grootfontein Herbarium Middelburg) Gordonville, Sneeuwberg; *Acocks 17523* (PRE) 3124BB (Hanover), Colesberg, 9 miles east by south of Naauwpoort; *Acocks 21597* (PRE) Compassberg Farm, Middelburg; *Bolus 13828* (LD) Vlakplaats, Richmond Division; *Drège 7369* (NY, holotype) Central Region, Graaff Reinet Division, Sneeuwberg Range, 1200–1500 m; *Smith 4467* (PRE) 3025BC (Colesberg); *Zietsman 170* (PRE) 3026AA (Aliwal North). *G. stricta*: *Denoon 56* (BOL, type) 3124BB (Hanover), near Naauwpoort, Hanover.

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CHAPTER 7

GENERAL DISCUSSION AND FUTURE RESEARCH

In this thesis, I have explored the applications of phenetics, morphology, micromorphology, morphometric and phylogenetic analyses using molecular sequence data in systematic studies in *Gnidia*. Furthermore, I have provided the first account of gynodioecy in a species of *Gnidia*, with a synopsis of sexual polymorphism among Thymelaeoideae. In this discussion I will consider the value of these findings to a better understanding of *Gnidia* systematics and phylogeny, and suggest areas of priority for future research.

In this discussion, references to clades 1, 2, 3 and 4 refer to the clades presented in Figure 3, Chapter 5 (BEAUMONT ET AL. 2009).

Generic delimitation of *Gnidia* and southern African relatives has been notoriously controversial with genera recognised on the basis of few and often unstable characters. Until now, debate has focussed mostly on whether *Arthrosolen*, *Englerodaphne* and *Lasiosiphon* should be combined with *Gnidia* or retained as separate genera. Evidence from molecular sequence data presented in Chapter 5 supports the reinstatement of an amended genus *Lasiosiphon*, which includes two species previously classified in *Arthrosolen*. The taxonomic status of *Englerodaphne* based on evidence from molecular studies is unclear at this stage. *Gnidia pilosa* (representing *Englerodaphne*) from southern Africa is sister to *Pimelea* (Chapter 5).

Pimelea species differ from other taxa in the Thymelaeoideae, including *G. pilosa*, by their two- or very rarely one-staminate flowers. However more extensive sampling of species is necessary to better understand the relationships among these geographically disjunct taxa.

Evidence from molecular sequence data has broken down generic distinctions between *Gnidia* and other southern hemisphere taxa, namely *Struthiola*, *Lachnaea*, *Drapetes* and *Pimelea*: none of which have ever been considered synonymous with *Gnidia* (Chapter 5). Elsewhere in the Thymelaeoideae, generic distinctions have collapsed in the light of evidence from more recent analyses of molecular sequence data. For example, RYE (1988) upheld the generic status of *Thecanthes* Wikstrom, whereas THRELFALL (1982) considered it a section of *Pimelea*. In spite of clear, unique morphological synapomorphies including funnel-shaped receptacles and dorsiventrally compressed pedicels common to all *Thecanthes* species, evidence from molecular sequence data (BEAUMONT ET AL. 2009) nevertheless placed *Thecanthes* in *Pimelea*. The same molecular analyses also confirmed the synonymy of *Cryptadenia* in the highly similar genus *Lachnaea* (Chapter 5). The realization that *Gnidia* is polyphyletic means that the current classification of *Gnidia* and relatives will need a complete reassessment after more sampling and analyses of taxa for molecular sequence studies (discussed in Chapter 5).

Circumscription of tribe Gnidieae

Molecular sequence data (BEAUMONT ET AL. 2009) supported the inclusion of the Southern Hemisphere genera *Gnidia*, *Struthiola*, *Lachnaea*, *Passerina*, *Drapetes* and

Pimelea in tribe Gnidieae (DOMKE 1934). DOMKE (1934) also placed the Mediterranean genus *Thymelaea* in this tribe, but in the subtribe Thymelaeinae. VAN DER BANK ET AL. (2002) identified a well-supported clade containing *Daphne*, *Thymelaea* and *Diarthron* Turcz. using *rbc*L and *trn*L-F sequence data, although the relationships among these three were unresolved. Using evidence from ITS (rDNA) sequence data, GALICIA-HERBADA (2006) confirmed the monophyly of *Thymelaea,* and sister position of *Daphne,* also from the Northern Hemisphere. These findings supported a closer phylogenetic link between *Thymelaea* and *Daphne* than suggested by DOMKE (1934) who placed these genera in different tribes, but perhaps more similar to HERBER (2003) who placed both genera in his 'Daphne group'. Results of GALICIA-HERBADA (2006) and BEAUMONT ET AL. (2009) placed *Thymelaea hirsuta* (L.) Endl. in lineages with taxa from the Northern Hemisphere, suggesting *Thymelaea* might be better placed in tribe Daphneae *sensu* DOMKE (1934). DOMKE (1934) placed *Dais* in subtribe Gnidiinae of tribe Gnidieae together with other southern African taxa except *Passerina*, which he placed in subtribe Passerininae. The sister position of *Dais cotinifolia* L. and *Phaleria capitata* Jack to clade 4 (Chapter 5) supports the inclusion of, or at least a close relationship of *Dais* to members of tribe Gnidieae. However DOMKE (1934) placed *P. capitata* in tribe Phalerieae, in contrast to the molecular evidence (Chapter 5), which supports a close phylogenetic relationship between these two species.

Close relations, far apart

The inclusion of six southern African *Gnidia* species and *Drapetes muscoides* auct. (more correctly known as *Drapetes muscosus* Lam.) in clade 1 (Chapter 5) supports

the hypothesis of a Gondwanan affinity between these taxa, as suggested previously by HEADS (1990, 1994). The monotypic genus *Drapetes* is distributed south of the 40^0 S latitude in Argentina, Chile and adjacent islands including the Falkland Islands and Tierra del Fuego. Only *Drapetes* represents the Thymelaeaceae on the Falkland Islands. Geological evidence points to a former land connection between the Falklands Islands and south-eastern South Africa. Taxa comprising clade 1 represent in part, what is now a remnant of the former Gondwanan biotic connection between South Africa and the Falkland Islands that diverged some 200 million years ago. As the Falkland Islands broke away from South Africa, they drifted across the expanding Atlantic Ocean towards South America, from where much of their extant biota is derived (MACDONALD 2003, MCDOWALL 2005).

The low, compact, moss-like habit of *Drapetes* in southern South America and adjacent islands is suited to its cold, wet and exposed, peaty, boggy habitats whereas *Gnidia* species rarely encounter such harsh conditions and are never moss-like. In habit, *Drapetes* plants more resemble plants of *Kelleria* Endlicher, from Australia, Malesia and New Zealand, although vegetative and floral features distinguish the two, and neither has ever been combined with *Gnidia*. Both Drapetes and New Zealand species of *Kelleria* (distributed around the 40^0S line of latitude) are closer to the southern Polar region than southern African *Gnidia* species (all north of the 40^0 S line of latitude) and as such, experience colder average temperatures than *Gnidia* species. Furthermore, both *Kelleria*, and to a lesser extent *Drapetes*, develop adventitious roots, not recorded in *Gnidia* (HEADS 1990). *Drapetes* therefore is anomalous among other members of clade 1 in its moss-like or cushion-like habit and adventitious roots.

Patterns of diversity among leaves and bracts in Gnidia and their potential phylogenetic value

As in other members of the Thymelaeaceae, leaves of *Gnidia* are simple, entire and estipulate. These unvarying traits limit the scope of vegetative morphological characters of potential phylogenetic value. Leaf shape (length to width ratio) is variable within and among clades 1–4, and therefore leaf shape does not contribute towards distinguishing lineages. Leaf and bract length to width ratios are not correlated in *Gnidia* and appear to be under different selection pressures (Tables 7.1–7.6).

Although leaf and bract length to width ratios are of limited value in defining *Gnidia* taxa above the rank of species, they are useful for species identification. Leaf and bract dimension data, leaf and bract lamina shapes and number of primary veins are diagnostic for some *Gnidia* species, even in the absence of floral features. A comprehensive, illustrated atlas of leaves and bracts, expanding on Figures 2–7 (Chapter 3) would help species identification in *Gnidia*.

Morphometric analyses have been used to distinguish taxa elsewhere in the Thymelaeaceae. Discriminant Analysis (DA) of leaf and bract length and width ratios can be used to distinguish *Gnidia* species (Chapter 3). ROGERS (2004) used Principal Components Analysis (PCA) of 19 leaf characters among the nine species of *Stephanodaphne* Baill. to distinguish a new species. Leaf length, leaf width, the number of secondary veins and the distance of the submarginal loop from the leaf edge all contributed significantly to the first principal component of his analysis.

Table 7.1. Selected morphological characters compared among the five *Gnidia* species and *Drapetes muscoides* auct. of Clade 1. (BEAUMONT ET AL. 2009, Figure 4: Chapter 5, this thesis). References: WRIGHT 1915; LEVYNS 1950; HILLIARD & BURTT 1987; HEADS 1990; BEAUMONT 2000.

	Species					
Character	*Gnidia anomala* Meisn.	*Gnidia denudata* Lindl.	*Gnidia fastigiata* Rendle	*Gnidia geminiflora* E.Mey. ex Meisn.	*Gnidia renniana* Hilliard & B.L.Burtt	*Drapetes muscoides* auct.
Habit	Subshrub	Shrub or small tree	Subshrub	Subshrub	Dwarf subshrub	Dwarf subshrubs: moss-like mats
Leaf shape	Elliptic, ovate-oblong	Ovate-oblong, oblong-elliptic	Lanceolate	Lanceolate	Linear-lanceolate	Elliptic-obovate
Inflorescence structure	Terminal clusters: flowers in pairs in clustered leaf axils	Terminal and lateral clusters of 4–9 flowers	Few-flowered clusters, terminal or sub-terminal on short axillary branches: flowers rarely solitary	Flowers in pairs in terminal and sub-terminal leaf axils	Solitary flowers in leaf axils along nearly whole branch lengths: flowers rarely in pairs	Few-flowered terminal umbels
Bracts	Leafy	Leafy	Leafy	Leafy	Leafy	Leafy
Calyx lobe no.	4	4	4	4	4	4
Stamen no.	4 (+ 4 staminodes)	8	8	8	8	4
Floral scale no.	8	8	8	4	8 – 10 or 0	0

Table 7.2. Morphological characters compared among selected species of *Struthiola and Gnidia* in Clade 2. (BEAUMONT ET AL. 2009, Figure 3, Chapter 5, this thesis). References: WRIGHT 1915; LEVYNS 1941, 1950; BEYERS 2000.

	Species							
Character	*Struthiola ciliata* (L.) Lam. *s.l.*	*Struthiola dodecandra* (L.) Druce	*Struthiola leptantha* Bolus	*Struthiola salteri* Levyns	*Struthiola striata* Lam.	*Struthiola tomentosa* Andrews	*Gnidia pinifolia* L.	*Gnidia racemosa* Thunb.
Leaf shape	Ovate, ovate-lanceolate, linear	Linear, linear-lanceolate	Oblong	Ovate	Ovate-oblong	Elliptic-oblong	Acerose	Ovate, obovate, obovate-lanceolate
Inflorescence structure	Spicate	Spicate	Spicate	Spicate	Spicate	Spicate	Capitate	Racemose to scattered
Bracts	Leafy	Leafy	Leafy	Leafy	Leafy	Leafy	Leafy (slightly wider than leaves)	Leafy
Bracteoles	Present	Present	Present	Present	Present	Present	Absent	Absent
Calyx lobe no.	4	4	4	4	4	4	4	4
Stamen no.	4	4	4	4	4	4	8	8
Floral scale no.	8	8	8	8	4	12	4	8

Table 7.3. Selected morphological characters compared among species of *Gnidia* comprising the "Haplostemonous subclade" of Clade 3 (BEAUMONT ET AL. 2009, Figure 3: Chapter 5, this thesis). References: WRIGHT (1915); HILLIARD & BURTT 1989; BEAUMONT ET AL. (2006).

	Species					
Character	*Gnidia aberrans* C.H.Wright	*Gnidia caniflora* Meisn.	*Gnidia scabrida* Meisn.	*Gnidia setosa* Wikstr.	*Gnidia singularis* Hilliard	*Gnidia wikstroemiana* Meisn.
Habit	Subshrub	Subshrub	Shrub	Shrub	Subshrub	Shrub or subshub
Leaf shape	Ovate-lanceolate	Oblong or lanceolate	Ovate-lanceolate, lanceolate	Lanceolate	Narrowly obovate	Oblong-lanceolate
Inflorescence structure	Flowers solitary and axillary in upper leaf axils	Terminal and sub-terminal clusters of 2-4 flowers	Flowers solitary in terminal and sub-terminal leaf axils	Terminal clusters, elongating in fruit and becoming spicate	Terminal and sub-terminal clusters of 2-4 flowers	Clusters of 2-8 flowers, terminal, and lateral on short, side shoots
Bracts	Leaf-like	Leaf-like	Leaf-like	Leaf-like	Leaf-like	Leaf-like
Calyx lobe no.	**4**	4	4	4	4	4
Stamen no.	0 (antesepalous) 4 (antepetalous	8	4 (antesepalous, reduced) 4 (antepetalous)	8	4 (antesepalous) 0 (antepetalous)	8 (hermaphrodite flowers) 0 (female flowers)
Floral scale no.	4	8	8	8	4	4

Table 7.4. Selected morphological characters compared among species of *Gnidia* comprising "*Epichroxantha* subclade" of Clade 3, (BEAUMONT ET AL. 2009, Figure 3, Chapter 5).

	Species				
Character	*Gnidia coriacea* Meisn.	*Gnidia galpinii* C.H.Wright	*Gnidia humilis* Meisn.	*Gnidia subulata* Lam.	*Gnidia* aff. *viridis* Lam.
Habit	Shrub	Shrub	Subshrub or shrub	Subshrub or shrub	Subshrub or shrub
Leaf shape	Ovate-oblong to sub-lanceolate	Oblong-lanceolate	Oblong	Linear-subulate	Linear-subulate
Inflorescence structure	Terminal clusters of 2-4 clusters	Flowers in pairs, terminal	Terminal flowers in pairs, lateral flowers solitary in uppermost leaf axils	2-3-flowered clusters, terminal	2-3-flowered clusters, terminal
Bracts	Leaf-like	Leaf-like	Leaf-like	Leaf-like	Leaf-like
Calyx lobe no.	4	4	4	4	4
Stamen no.	8	8	8	8	8
Floral scale no.	4	4	4	4	4

Table 7.5. Selected morphological characters compared among continental African species of *Gnidia* of the "*Lasiosipho*n" Clade 4, (BEAUMONT ET AL. 2009, Figure 3, Chapter 5).

	Species				
Character	*Gnidia caffra* Meisn. Gilg	*Gnidia calocephala* (C.A.Mey.) Gilg	*Gnidia glauca* (Fresen.) Gilg	*Gnidia kraussiana* Meisn.	*Gnidia sericocephala* (Meisn.) Gilg ex Engl.
Habit	Perennial herb or subshrub	Shrub	Large shrub or tree to 9 m tall	Perennial herb or subshrub	Shrub
Leaf shape	Linear	Oblong-lanceolate	Elliptic to obovate	Narrowly elliptic to ovate, obovate or sub-orbicular	Linear
Inflorescence structure	Terminal, few-flowered heads	Terminal, many-flowered heads	Terminal, many-flowered heads	Terminal, many-flowered heads	Terminal, many-flowered heads
Bracts	Foliaceous, smaller and broader than leaves	Foliaceous, similar to uppermost leaves	Coriaceous, smaller than leaves	Foliaceous, smaller than leaves	Foliaceous, smaller and broader than leaves
Calyx lobe no.	5, rarely 4	5, rarely 4	5, rarely 4	5, rarely 4	5, rarely 4
Stamen no.	10, rarely 8	10, rarely 8	10, rarely 8	10, rarely 8	10, rarely 8
Floral scale no.	5, sometimes fewer or 0 in a flower	0	5, sometimes fewer or 0 in a flower	5, sometimes fewer or 0 in a flower	0

Table 7.6. Selected morphological characters among Malagasy species of *Gnidia* of the "*Lasiosiphon*" Clade 4, (BEAUMONT ET AL. 2009, Figure 3: Chapter 5). **Gnidia bakeri* Glg is more correctly known as *Gnidia gnidioides* (Baker) Domke. (ROGERS 2009).

	Species						
Character	**Gnidia bakeri* Gilg	*Gnidia bojeriana* (Decne.) Gilg	*Gnidia danguyana* Leandri	*Gnidia decaryana* Leandri	*Gnidia dumetorum Leandri*	*Gnidia gilbertae* Drake	*Gnidia madagascariensis*
Habit	Shrub	Shrub	Shrub or small tree	Shrub	Shrub	Shrub or small tree	Shrub or subshrub
Leaf shape	Acerose, rarely very narrowly obovate or ovate	Narrowly elliptic or obovate	Broadly ovate or ovate-elliptic	Obovate to sub-orbicular	Elliptic to elliptic-obovate	Broadly ovate to nearly elliptic	Elliptic to obovate-lanceolate
Inflorescence structure	Dense clusters forming many-flowered globose heads	Axillary, pedunculate, many-flowered heads	6-23-flowered terminal racemes, elongating in fruit	Terminal, axillary few-flowered heads	Terminal, axillary heads of c. 12 flowers	Terminal, axillary many-flowered heads	Capitate heads of c. 15-20 flowers
Bracts	Foliaceous, differ from leaves in shape and smaller size	Foliaceous, differ from leaves in shape and smaller size	Foliaceous, very reduced and smaller than leaves	Foliaceous, very reduced and smaller than leaves	Foliaceous, smaller than leaves	Chartaceous, differ from leaves in shape and smaller size	Semi-chartaceous, smaller than leaves, base very swollen, tip rostrate.
Calyx lobe no.	4	5	4	4	5	4	5
Stamen number	8	10	8	8	10	8	10
Floral scale no.	0	5	0	0	5 or 0	4	5

Leaves and bracts of *Gnidia* species are glabrous to densely hairy. Hairs are non-glandular and uniseriate, short to long and smooth to curly. Hair ornamentation among selected *Gnidia* species was identified and illustrated for the first time in BEAUMONT ET AL. (2001a, Chapter 3). Hair surfaces may be smooth, warty or hook-like. Hairs are smooth among species previously classified in *Lasiosiphon*, and this character may prove synapomorphic for an amended *Lasiosiphon* as more taxa are included in the molecular analyses outlined in Chapter 5. Hair ornamentation, in combination with other characters may prove valuable in species identification and possibly in helping to characterize lineages.

The peristomatal region of the cuticle is raised and dome-like with multicellular proliferations of the epidermis around the bases of hairs of leaves and bracts, appearing as minute white dots to the unaided eye in *Gnidia nana* (L.fil.) Wikstr. (Figure 8E, Chapter 3). The leaves and bracts of *Gnidia linearifolia* (Wikstr.) B.Peterson and *Gnidia penicillata* are likewise punctulate. Evidence from molecular sequence data (Chapter 5), places *Gnidia penicillata* in *Lachnaea*, but punctulate leaf and bract surfaces are not recorded elsewhere in *Lachnaea* (BEYERS 2001). This character together with the relative positions of stamens and floral scales, make *G. penicillata* anomalous among *Lachnaea* species of clade 1. Unfortunately, neither material of *G. nana* nor *G. linearifolia* was available for inclusion in the molecular study described in Chapter 5. However, all three species were once placed in *Craspedostom*a. DOMKE (1934) derived the name *Craspedostoma* from the Greek *Craspedon*, meaning edge or border, and *stoma*, meaning mouth or opening, referring to the raised peristomatal region of the cuticle described above (STEARN 1987). Attempts to collect specimens of *G. nana* for study for this thesis were

unsuccessful, and PETERSON (1959) noted that this species had not been collected since the second decade of the 19th century.

Bracts differ most from leaves in shape, size and texture among species with many-flowered capitate inflorescences (BEAUMONT ET AL. 2001b, Chapter 4). Inflorescences and bracts are most similar in clades 1, 3 and African species of clade 4 and help to define these lineages (Tables 7.1 and 7.3–7.5). In contrast the inflorescences of *Gnidia pinifolia* L. and *Gnidia racemosa* Thunb. are unlike those of the rest of clade 2 comprising *Struthiola* species. Spicate inflorescences and paired bracteoles accompanying each flower have traditionally helped to distinguish *Struthiola*, although these characters are also found in *Gnidia ornata* (Meisn.) Gilg and *Gnidia spicata* (L.f.) Gilg. Material of both these species was unavailable for inclusion in the molecular analyse outlined in Chapter 5. Furthermore, the diplostemonous rather than haplostemonous androecia of *G. pinifolia* and *G. racemosa* distinguish them from *Struthiola* species. Inflorescence structure is variable in clade 2 and does not help to distinguish this lineage. Inflorescence structure varies among Malagasy members of clade 4 and does not help to define this lineage either, although all members from continental Africa share identifiably capitate heads of seven to many flowers, surrounded by involucres of bracts that are generally green and leafy, but smaller than the leaves. *Gnidia glauca* (Fresen.) Gilg is the only arborescent member of African species in clade 4. Like the other African members of clade 4, bracts of *G. glauca* differ in size and shape to the leaves, but in this species bracts are furthermore coriaceous not foliaceous, and pinkish-brown, not green (Figure 4A1–4A2, Chapter 3). Capitate inflorescences with modified bracts in involucres therefore help distinguish African members of clade 4.

Gnidia bracts protect buds in species with many-flowered inflorescences. Bract characters have been used elsewhere in the Thymelaeoideae to support infrageneric classifications. RYE (1988) distinguished two of the sections of *Pimelea* using bract characters: sections *Epallage* and *Macrostegia*. Section *Epallage* comprises 18 species lacking bracts and with flowers with comparatively longer floral tubes below the line of abscission, and small calyx lobes. Section *Macrostegia* has a single species, *Pimelea physodes* Hook. with relatively massive, red bracts that conceal flowers and are commemorated in the sectional name. *Macrostegia* is derived from the Greek *macros* meaning large, and *stege* or *stegos* meaning shelter (STEARN 1987). In contrast, bracts never conceal flowers in mature inflorescences of *Gnidia*.

Distribution of tetramerous and pentamerous flowers and their value in defining clades.

Flowers of species in clades 1–3 are tetramerous and this floral plan is stable. Clade 4 comprises both pentamerous species and tetramerous species from mainland Africa and Madagascar (Tables 7.5–7.6). These include species that have always been placed in *Gnidia,* plus others previously included in *Lasiosiphon* or *Arthrosolen*. BEAUMONT ET AL. (2009) reinstated the genus *Lasiosiphon,* amended to accommodate both tetramerous and pentamerous species. Elsewhere in the Thymelaeoideae, the genus *Daphne* includes both pentamerous and tetramerous species (HALDA 1998).

Tetramery is derived from pentamery (RONSE DECRAENE & SMETS 1994). As such, pentamery may be perceived as primitive relative to tetramery among

Thymelaeoideae. Both *Dais cotinifolia* and *Phaleria capitata* have pentamerous flowers and together are sister to clade 4 (a mix of pentamerous and tetramerous species), supporting the opinion of DOMKE (1934) who considered *Dais* as among the most “original” (i.e. primitive) of taxa. Pentamery may therefore represent a more primitive floral plan among Thymelaeoideae, with tetramery more advanced.

Species with predominantly pentamerous flowers in fact, show only slight instability of this floral arrangement, with the odd tetramerous or hexamerous flower infrequent among heads in which flowers are, by and large pentamerous. This slight instability of the pentamerous condition does not, in the opinion of BEAUMONT ET AL. (2009), justify conflating *Lasiosiphon* with *Gnidia* as PETERSON (1959, 1978) suggested, simply because these taxa with overwhelmingly pentamerous flowers produce the odd tetramerous flower. The pentamerous condition is a little unstable among many families with regular flowers. For example, in a single plant of *Cestrum laevigatum* Schlecht. (Solanaceae), 29 out of 365 flowers (8%) combined had three, four or six petals (personal observation) scattered among branches with no apparent correlation to position on plant. ELLSTRAND (1983) found floral inconstancy among plants and populations of the predominantly pentamerous species *Ipomopsis aggregata* (Pursh) V.Grant (Polemoniaceae). Thirty three percent of plants in 13 populations, and 10% of flowers deviated from the expected pentamerous condition, with atypical flowers usually showing more petals. Although reasons for floral instability in *I. aggregata* were elusive, environmental stress and genetic background have been identified as contributing factors in atypical flower production elsewhere in the Polemoniaceae. For example, among 34 populations of five species of *Linanthus* HUETHER (1969) found 1-4% of flowers deviated from the usual pentamerous condition with most

atypical flowers having fewer than five petals. HUETHER (1969) suggested that environmental stress promoted abnormal flower formation in *Linanthus*. Late-season flower primordia produced on new lateral shoots in response to herbivory experience longer daylight hours and greater extremes of temperature beyond the optimum ranges for these factors enjoyed by early-season primordia. HUETHER (1969) hypothesized that these sub-optimum conditions of daylight and temperature disrupted normal flower development. The low levels of variability in floral pentamery among Polemoniaceae are similar to those in normally pentamerous taxa in Thymelaeoideae. At this stage, the reasons behind the slightly variable pentamerous condition in Thymelaeoideae are unclear.

Sexual polymorphism in Gnidia and its taxonomic and systematic value

Gnidia wikstroemiana Meisn. represents both the first record of gynodioecy in *Gnidia* and the first documented account of sexual polymorphism involving unisexual flowers among sub-Saharan, continental African Thymelaeaceae (BEAUMONT ET AL. 2006, Chapter 6). Very recently, evidence from a study of five populations suggested that the breeding system in *G. wikstroemiana* involves both nuclear gynodioecy and subdioecy (SMITH 2009).

Flowers are hermaphroditic among the rest of sub-Saharan continental African Thymelaeoideae (Chapter 6). Flowers of *Dais cotinifolia* L., however, are heterostylous, comprising three distinctive morphs borne on separate plants, distinguished by differences in stamen, style and floral tube lengths (MARLOTH 1925, ZAVADA & LOWREY 1995).

Dais cotinifolia and *Phaleria capitata* together formed a strongly supported clade in the molecular analyses outlined in Chapter 5, and together are sister to clade 4. Overall *Dais* and *Phaleria* are similar and one of the synonyms of *Phaleria* is *Pseudais* Decne, derived from the Greek, *pseud*, meaning false, and *dais*, a reference to the genus *Dais*, indicating the resemblance of these two genera (STEARN 1987, RYE 1990, HERBER 2003). Like *D. cotinifolia*, some *Phaleria* species have different flower morphs. Reciprocal differences in the positions of anthers and stigma distinguish these morphs, which are confined to separate plants (RYE 1990). It is unclear, however, whether flowers of *P. capitata* are heteromorphic. Among Thymelaeoideae, heterostyly appears to be confined to *Dais* and *Phaleria*. If, like *D. cotinifolia*, flowers of *P. capitata* prove to be heterostylous, this unusual breeding strategy will be common to two phylogenetically and morphologically close, yet geographically distant taxa. Like *Drapetes* and selected *Gnidia* species, *Dais* and *Phaleria* are likely another example of taxa made geographically disjunct after the breakup of Gondwanaland.

Sexual polymorphism in conjunction with other characters (e.g. geographical distribution) has been used to help define infrageneric ranks in Thymelaeaceae. For example, in his revision of *Octolepis* Oliv. (subfamily Octolepidoideae), ROGERS (2005) used gender and geographical separation, together with vegetative and floral characters to distinguish two sections: the single continental African species with monomorphic, hermaphroditic flowers in section *Octolepi*s, and the five dioecious Malagasy species in section *Dioicae*. The two sections are further supported by molecular evidence using combined *rbc*L and *trn*-F sequence data (ROGERS 2005).

The sole example of gynodioecy among southern African Thymelaeoideae is, in itself, of little systematic value. *Gnidia wikstroemiana* shares with other species placed in the "Haplostemonous subclade" of clade 3 (Table 7.3), leaf-like bracts, tetramerous flowers and non-capitate inflorescences. There is no evidence to suggest that haplostemony is an early stage in the evolution of sexual polymorphism in the Thymelaeoideae. Therefore it is unlikely that sexual polymorphism in *G. wikstroemiana* is a factor linking this species with haplostemonous, or near-haplostemonous taxa in this subclade. Elsewhere, *Struthiola* species are exclusively and constantly haplostemonous, yet there is no evidence to suggest that any of these species are sexually polymorphic. Within this subclade, *G. aberrans* C.H.Wright, *G. singularis* Hilliard and *Gnidia wikstroemiana* Meisn. are similar overall and geographically relatively close, but not overlapping, in distributions. All inhabit montane grasslands and shrublands of the southern Drakensberg (*G. aberrans*, HILLIARD & BURTT 1987), Sani Top, Lesotho (*G. singularis*, HILLIARD 1989) or Eastern Cape (*G. wikstroemiana*, SMITH 2009). Nutrient resources are limiting in both montane and semi-arid regions where low temperatures and low rainfall slow the rate of nutrient acquisition by plants. Resource limitations in these environments may have selected for haplostemony above diplostemony, or sexual dimorphism above hermaphroditism. This needs experimental verification in future studies.

Synapomorphies defining clades 1–4

Considering eight morphological characters, among them some that have featured prominently in past classifications of *Gnidia*, namely habit; leaf shape; inflorescence

structure; bract form; number of calyx lobes (merosity); number of androecial whorls (diplostemony versus haplostemony) and number of floral scales (Tables 7.1–7.6), clades 1–4 show the following synapomorphies:

Clade 1 (Table 7.1):

Bracts leaf-like; flowers tetramerous

Clade 2 (Table 7.2):

Flowers tetramerous

Clade 3 ("Haplostemonous subclade") (Table 7.3):

Bracts leaf-like; flowers tetramerous

Clade 3 ("*Epichroxantha* subclade") (Table 7.4):

Bracts leaf-like; flowers tetramerous; androecium diplostemonous; floral scales 4.

Clade 4 (*Lasiosiphon* Fresen. emend. A.J.Beaumont) (Tables 7.5-7.6):

Many-flowered inflorescences; bracts leaf-like, smaller than leaves.

Taxon sampling and identifying polyphyly

Molecular studies are more and more revealing the polyphyletic nature of genera and higher ranks, previously assumed to be monophyletic (for example, ROBERTS & URBATSCH 2004, SWENSON ET AL. 2008). More extensive sampling of *Gnidia*

species and related genera for molecular sequence data analyses is needed to better understand the phylogenetic relationships among in particular the Thymelaeoideae.

LANYON (1994) and SOLTIS ET AL. (2004) highlighted the importance of comprehensive sampling of taxa and using many genes in molecular-based phylogenetic studies. However, they favoured increased sampling of taxa above sampling of complete or near-complete genomes in order to obtain phylogenetically more accurate topologies, because limited sampling of species may not necessarily include the most phylogenetically informative taxa. LANYON (1994) found that increased sampling for the ingroup improved topology accuracy in a phylogenetic study using molecular sequence data in the blackbird genus *Agelaius*. By expanding his original ingroup to include representatives of all blackbird genera and subgenera, LANYON (1994) revealed the polyphyletic nature of this genus. In his conclusions, LANYON (1994) emphasized the importance of comprehensive sampling of taxa, and cautioned against assuming *a priori* monophyly. Similarly, more extensive sampling of *Gnidia* and relatives for molecular sequence data, combined with a thorough assessment of morphological characters is needed to better identify phylogenetic relationships within and among *Gnidia* and its relatives.

A classification system should be useful to the wider professional community working in botany and related fields. Morphological characters have traditionally been the foundation for defining recognizable groups. Morphological synapomorphies that define clades of *Gnidia* are, however obscure. The task of identifying morphological synapomorphies for above-species ranks has become even more challenging given that *Gnidia* is polyphyletic.

SWENSON ET AL. (2008) briefly reviewed the proponents and antagonists for the inclusion of morphological data in molecular–based phylogenies. For example, SCOTLAND ET AL. (2003) favoured the use of fewer, but scrupulously assessed anatomical features mapped onto molecular phylogenies as the best approach towards integrating morphological and molecular evidence. This prompted WIENS (2004) and JENNER (2004) to reply in support of the value of morphological characters in phylogeny reconstructions. In their study of the subfamily Chrysophylloideae (Sapotaceae) SWENSON ET AL. (2008) concluded that unique combinations of characters would best define groups in the absence of unique distinguishing synapomorphies in this highly homoplasious subfamily. It appears that this could also be the case in *Gnidia*. To best understand *Gnidia* phylogeny now requires that more species of *Gnidia* and other southern hemisphere taxa need to be included in molecular analyses and their morphological and micromorphological characters scrutinized for their potential in helping to define clades and lineages: to study *Gnidia* in isolation will not suffice. Classification systems for *Gnidia* and kin have repeatedly evaluated floral merosity and the numbers of stamens and petal-like glands (i.e. floral scales) for their usefulness in defining genera, with little consensus among workers. In this thesis I have introduced the idea that vegetative characters, including leaf and bract morphometric data, and micromorphological details of the surfaces of leaves, bracts and hairs may prove useful, in combination with other characters, in delimiting lineages. At this stage more detailed studies of novel characters are necessary if we wish to delimit lineages of *Gnidia* using morphology. The vegetative characters outlined above together with floral features examined more recently such as stigma shape, presence or absence of stomata on petaloid scales and fruit shape and surface ornamentation (Beaumont, unpublished data) may well,

in combination with other characters, prove more useful in defining lineages of *Gnidia* and its relatives.

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ERRATA

Corrections to Chapters 2, 3, 4, 5 and 6 of this thesis are as follows:

Chapter 2 Taxonomic History of *Gnidia*: Generic Concepts, Characters and Controversy.

Addition to References:

GILG, E.F. 1921. Ũber die Phylogenese der Thymeleaceae. *Ber. Freie. Ver. Pflanzengeographische* 1919. 60–68.

Chapter 3. Leaf and bract diversity in *Gnidia* (Thymelaeaceae): patterns and taxonomic value.

Page 75, “smaller-leafed” should read “smaller-leaved”. “fig. 8D” on the last line of the page should read “fig. 8E”.

Page 81, A1 leaf, “B2 bract” in caption to Figure 5 should read “A2 bract”.

Page 84, “**Venation**” should be “Venation”.

Page 89, in *Gnidia* species with many-flowered capitate heads, bract number equals or exceeds flower number, whereas the flower heads of *Pimelea* species have fewer bracts than flowers.

Chapter 4. Patterns of diversity among involucral bracts, inflorescences and flowers in *Gnidia* (Thymelaeaceae).

Page 93, (3rd line of “1.3 Description of plants”) “pedicel” should read “petiole”.

Page 94, anthers dehisce towards the centre of the flower and are termed introrse. The ovary is functionally monocarpellate (psedomonomerous), with a single locule containing a single ovule. “Sepals 4-5-lobed” should read “Sepals 4 or 5”.

Page 96, 112, not 115 species sampled.

Page 101, reference for protective function of bracts:

WEBSTER, G.L. & WEBSTER, B.D. 1972. The Morphology and relationships of *Dalechampia Scandens* [sic] (Euphorbiaceae). *American Journal of Botany* **59**(6): 573–586.

Addition to References:

LEFÈBVRE, C. & VEKEMANS, X. 1995. A numerical taxonomic study of *Armeria maritima* (Plumbaginaceae) in North America and Greenland. *Canadian Journal of Botany* **73**(10): 1583–1595.

Chapter 5. *Gnidia* (Thymelaeaceae) is not monophyletic: taxonomic implications for Thymelaeaceae and a partial new generic taxonomy for *Gnidia*.

Page 115, “Morphological synapomorphies are lacking for an expanded generic circumscription of *Struthiola* to include *G. pinifolia* and *G. racemosa*, and generic limits will have to be reconsidered for these taxa”. This sentence was intended to remark only on the difficulties of recognising clear synapomorphies for a group comprising *Struthiola* species and two species of *Gnidia*. It was meant neither to imply that any changes have been made to the taxonomic status of either of the genera *Gnidia* or *Struthiola*, nor that *G. pinifolia* should be renamed as a species of *Struthiola*.

Page 116, “petals” should read “floral scales”.

Page 118, fleshy pedicels are a feature of many *Lasiosiphon* species but whether they are ever-present and exclusive to this group, has yet to be confirmed.

Chapter 6. The first record of gynodioecy in a species of *Gnidia* (Thymelaeaceae) from South Africa.

General

The term “non-functional staminodes” is used to emphasize the fact that these reduced structures have both ceased to produce pollen and also that they have not assumed any other function. It is also correct to say that these

structures are staminodes or non-functional stamens in female flowers of *Gnidia wikstroemiana* (= *Gnidia stricta*).

Addition to References:

DOMMÉE, B., BIASCAMANO, A., DENELLE, N., BOMPAR, J.-L. & THOMPSON, J.D. 1995. Sexual tetramorphism in *Thymelaea hirsuta* (Thymelaeaceae): morph ratios in open-pollinated progeny. *American Journal of Botany* **82**(6): 734–740.